

# DNA MISMATCH REPAIR DEFICIENCY AND TELOMERE DYNAMICS

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#### Abstract

DNA mismatch repair (MMR) deficiency is the hallmark of Lynch syndrome (LS), an autosomal inherited predisposition for an early onset of colorectal cancer (CRC) characterized by the presence of high levels of microsatellite instability. CRC cell lines carrying a MMR gene mutation show higher mutation rates than MMR-proficient cells not only at microsatellites but also at telomeres.

Here, the MMR protein MSH2 was downregulated in WI38 cells using an shRNA and stable clones were grown in a 5%  $O_2$  environment to reduce the effect of oxidative stress on the telomere shortening rate (TSR). It was found that clones with a greater than 60% protein downregulation had a higher TSR with an exponential relationship between protein content and TSR for the B but not the  $\Delta$  allele for 12q what might be related with the telomeric variant repeat structure. Additionally, oxidative stress was found to have a synergistic effect on TSR together with MMR deficiency. Mutant XpYp telomeres generated from LoVo (*MSH2*<sup>-/-</sup>) were studied by telomere variant repeat (TVR) analysis to understand the mutation process and a tendency towards deletions was suggested. Furthermore, a novel 3<sup>rd</sup> next generation sequencing technology was tested to obtain full information of the telomere repeat array establishing the bases for future experiments. Finally, telomere length was measured in saliva DNA from 37 controls and 91 LS patients. It was found that telomeres shortened with age at similar rates in both cohorts and no significant relationship was found between telomere length and age for  $MLH1^{+/-}$ patients. Besides,  $MSH2^{+/-}$  patients had significantly shorter age-adjusted telomere length for XpYp but not 12q than MMR<sup>+/+</sup> and parent-children pair comparisons for age-adjusted telomere lengths showed that MMR<sup>+/+</sup> children had longer telomeres than their MMR<sup>+/-</sup> siblings and their MMR<sup>+/-</sup> parent but shorter than their MMR<sup>+/+</sup> parent.

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#### Abbreviations

ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
BER	Base Excision Repair
bp	base pair
CEPH	Human Polymorphism Study Center
CIN	Chromosomal Instability
CRC	Colorectal Cancer
DAPI	4,6-Diamidino-2-phenylindole dihydrochloride
DDR	DNA Damage Response
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
<b>DN-hTERT</b>	Dominant Negative human Telomerase Reverse Transcriptase
DSB	Double Strand Break
dsDNA	double stranded DNA
EDTA	Ethylenediaminetetraacetic acid
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
HR	Homologous Recombination
FAC	Fluorescence-Activated Cell sorting
FAP	Familial Adapamentaria Polynosis
FISH	Fluoroscont In Situ Hybridisation
C4	G-quadruplay
	Insertions/Deletions
IDL5 lzb	kilohagag
	Lungh gundromo
	Lynch Synuronie
mTI	Loss of fileterozygosity
	Migmatch Danain
MMININ	Mismatch Repair Mignogotollito Ingtobility
MED	Microsatemite Instability
NEK	Nucleotide Excision Repair
NHEJ	Non-nomologous End Joining
nt	
PBS	Phosphate-Buffered Saline
P/C	Parent/Children pair
PCNA	Proliferating Cell Nuclear Antigen
PCR	Polymerase Chain Reaction
PD	Population Doubling
PI	Proliferation Index
POL	DNA Polymerase
RFC	Replication Factor C
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RPA	Replication Protein A
shRNA	short hairpin Ribonucleic Acid
siRNA	small interfering Ribonucleic Acid
SSB	Single Stranded DNA Binding protein
ssDNA	single stranded DNA
STELA	Single Telomere Length Analysis
STR	Short Tandem Repeat
TERC	RNA component of telomerase
TERT	Telomerase Reverse Transcriptase
TRAP	Telomerase Repeat Amplification Protocol
TSR	Telomere Shortening Rate
TVR	Telomere Variant Repeat
UV	Ultraviolet light

### 1.1 DNA mismatch repair

#### 1.1.1 Origin of mismatches in the DNA

The DNA mismatch repair (MMR) pathway detects and corrects mismatches in the DNA that can be either **single-base**, and there are eight different types, producing transition (A/C and G/T) or transversion (G/G, C/C, A/G, C/T, A/A and T/T) mispairs or **insertion/deletion** loops (IDLs) that provoke gains or losses of nucleotides. The main causes involved in the formation of these mismatches are summarised in figure 1.1:

**Physical or chemical damage** of the DNA and its precursors. Some of the genotoxic agents that produce mismatches are reviewed in Hoeijmakers (2001), Lord & Ashworth (2012) and Iyama & Wilson (2013):

- <u>Ionising radiation</u> such as ultraviolet (UV) light produces covalent bonds that crosslink adjacent pyrimidines (cytosine and thymine) in the DNA strand.
- <u>X-rays</u> generate free radicals within the cell resulting in single-strand and double-strand breaks.
- <u>Alkylating agents</u> such as MNNG (1-methyl-3-nitro-1-nitrosoguanidine) that adds alkyl groups to the  $O^6$  of guanine and  $O^4$  of thymine producing  $O^6$ -methylguanine and  $O^4$ -methylthymine base modifications that can lead to transitions between AT and GT.
- <u>Oxidative stress</u>: elevated concentrations of ROS (Reactive Oxygen Species) at the extra and/or intra cellular lever produce oxidative DNA damage. ROS can interact with all four DNA bases and the sugar-phosphate backbone producing many oxidative products (reviewed in Dizdaroglu

(2012)). Recently, Cilli *et al.* (2015) showed that incorrect incorporation of ribonucleotides during replication, might facilitate formation of complex lesions involving oxidized nucleotides. The three main mechanisms that repair this type of lesion are: base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR), depending on the specific nucleotide modification (reviewed in Bridge *et al.* (2014)).

**Replication.** The DNA polymerases that synthesise nuclear DNA are:  $\alpha$ and  $\delta$  (mainly involved in the lagging strand replication) and  $\epsilon$  (for the leading strand) (Lujan *et al.*, 2012) and together with POL $\gamma$  (for mitochondrial DNA replication) are highly accurate polymerases, generating on average less than one base substitution or IDL for every 10,000 of correct incorporation events (McCulloch & Kunkel, 2008). The error rate is different for each polymerase and is directly dependent on the sequence context and size, being higher in long repetitive regions than in short non-repetitive ones (Lujan et al., 2015). Because of the need to accurately replicate the genome, polymerases have developed mechanisms that favour the incorporation of the correct dNTP; besides, POL $\delta$ ,  $\epsilon$  and  $\gamma$  show 3' to 5' exonuclease activity which acting as proofreading control against incorrect base incorporation and decreasing the error rates compared to pol $\alpha$  that lacks this editing activity (McCulloch & Kunkel, 2008). To increase replication fidelity, different post-replication repair pathways co-operate with polymerases: BER (Krokan et al., 2000), NER (Marteijn et al., 2014) and MMR (Modrich, 1991). It has been demonstrated in Saccharomyces cerevisiae that the efficiency of the MMR pathway to repair POL $\alpha$ ,  $\delta$ and  $\epsilon$  replication errors is different, correcting more efficiently potential deleterious errors to assure a high-fidelity replication (Lujan et al., 2012). The polymerase selectivity and the proofreading function of DNA polymerases result in an error rate of approximately  $10^{-7}$  bp per cycle of replication and including the repair mechanisms preventing that mutations arise during replication, the mutation rate in vivo has been estimated to be lower than  $10^{-9}$  bp per cell division (McCulloch & Kunkel, 2008).

**Genetic recombination**. Two mechanisms occurring during genetic recombination affect the formation and processing of mispaired intermediates (Modrich & Lahue, 1996):

- <u>Gene conversion</u>: This kind of homologous recombination involves the unidirectional transfer of genetic material from a "donor" sequence to a homologous "acceptor" with a very high sequence identity (Chen *et al.*, 2007). Heteroduplex DNA structures are formed during the repair process, which are called Holliday junctions (Holliday, 2007).
- Homeologous recombination: This kind of recombination occurs between DNA sequences that are similar but not identical and generates mismatches in the DNA increasing genome instability (reviewed in Li (2008)).

#### **1.1.2 MMR mechanism in prokaryotes**

The first organism in which the MMR mechanism was studied was Salmonella pneumoniae and two genes, HexA (Balganesh & Lacks, 1985) and HexB (Prats et al., 1985) were discovered. It was found that  $Hex^-$  (HexA<sup>-</sup> and HexB<sup>-</sup>) mutants displayed a 5 to 30-fold increase in spontaneous mutation rates, indicating that the Hex system played a role in mutation avoidance by correcting errors during replication (Tiraby & Fox, 1973). Furthermore, transfection studies using  $\lambda$  heteroduplexes demonstrated direct evidence for a mismatch repair system in *Escherichia coli* (reviewed in Claverys & Lacks (1986)). Subsequently, the effect of mutator alleles on mismatch repair genes was studied and mutations in *UvrD*, MutH, MutS or MutL were found to cause high mutability; in addition, DNA methylation was studied as a target for correct strand discrimination (Modrich, 1987).

#### 1.1.2.1 MMR in *E. coli*

There are many pathways that repair DNA damage in *E. coli* but two main mechanisms can be distinguished on the basis of mispair specificity and size of the region that needs to be repaired (Modrich, 1991). The **long-patch mis-match correction** also called MutHLS mismatch repair pathway or *E. coli* 



Figure 1.1: **DNA damage response.** Figure adapted from figure 1 in Hoeijmakers (2001). The main DNA damaging agents are shown on the left, the DNA damage that they produce is shown in the middle and the main DNA damage response pathway activated per DNA lesion is shown on the right. Abbreviations are: BER: base excision repair; NER: nucleotide excision repair; HR: homologous recombination; NHEJ: non-homologous end joining; MMR: mismatch repair.

methyl-directed system ((Modrich, 1991); (Fishel & Kolodner, 1995); (Kolodner, 1996)) repairs single-base mispairs (except C-C), and the signal directing the strand-specificity is secondary and can be located very far away from the mismatch. The **short-patch mismatch repair** repairs up to 10 nucleotides IDLs, and the signal triggering the outcome of correction resides within the mismatch or very close to it. In this section, we will focus on the long-patch MMR, and the main proteins involved in it: MutS, MutL, MutH, DNA helicase II (MutU/UvrD), exonucleases (ExoI, ExoVII, ExoX and RecJ), SSB (Single Stranded DNA Binding protein), DNA polymerase III and DNA ligase. Figure 1.2 shows a diagram of the repair pathway that can be divided into three main steps:

a) Initiation: The mismatch is recognised by the **MutS** homodimer. The *mutS* gene was first isolated from Salmonella typhimurium by Pang et al. (1985) and it was shown to have homology to hexA. The crystal structure of the MutS dimer in *E.coli* was discovered by Lamers *et al.* (2000) and it was shown that the two MutS monomers have different conformations and only one of them recognizes the mismatch in an ATP dependent way (Junop et al., 2001). The asymmetry was described by Jiricny (2000) as "two praying hands with the thumbs coming close and the fingers slightly touching". Once the mismatch has been recognised, the **MutH** protein identifies the newly synthesized strand by binding to a hemi-methylated d(GATC) site (the daughter strand is transiently unmethylated) and because of its endonuclease function, it makes a nick in the unmodified strand, serving as a signal for direct excision repair (reviewed in Iyer et al. (2006)). MutH was first described by Welsh et al. (1987) in E.coli as a 28 KDa monomeric endonuclease dependent on Mg<sup>+</sup><sub>2</sub> that discriminated between strands. Evolutionarily, this protein was related to type-II restriction endonucleases (reviewed in Yang (2000)) but homologues of this protein have only been found in gram-negative bacteria suggesting that other mechanisms are used for strand specificity in other organisms. Junop et al. (2003) found that the active site of MutH in E. coli was composed of two separate structural domains and that the C-terminal 5' residues of MutH affected DNA binding and cleavage.

- b) Excision: In a next step, the MutL homodimer binds to MutS and MutH and it has been demonstrated by Ban & Yang (1998) that ATP-binding and not hydrolysis by MutL is essential for activating MutH. The *mutL* gene was also identified in S. typhimurium and E. coli (Pang et al., 1985) and it showed homology with the S. pneumoniae hexB gene (Prats et al., 1985) and (Claverys & Lacks, 1986). The crystal structure of a 40-kDa Nterminal fragment of E. coli MutL (LN40) was described in Ban & Yang (1998) and Ban et al. (1999). They also demonstrated that MutL was an ATPase and that there was a structural similarity among MutL, DNA gyrase and Hsp90. The C-terminal domain of this protein included a  $\beta$  binding motif that mediated a weak but specific interaction between MutS and MutL (Pillon et al., 2011). When the heterodimer between MutS and MutL is formed around the heteroduplex complex of DNA, the methyl-directed excision system is activated. Then, helicase II is recruited and it generates single-stranded DNA (ssDNA) around the nick made by MutH. This ssDNA is protected by single-strand binding proteins (SSB) from nuclease attack. MMR is bidirectional (Cooper et al., 1993), meaning that mistakes can be corrected from 3' or 5' direction and depending on the position of the strand break, different exonucleases are needed. ExoI and ExoX are 3' to 5' exonucleases while ExoVII and RecJ are 5' to 3' exonucleases. The first experiment that demonstrated a role of **ExoI** in MMR was in S. pombe (Szankasi & Smith, 1995).
- c) <u>Resynthesis</u>: It has been demonstrated that MutL physically interacts with the clamp subunits of **DNA polymerase III** (DiFrancesco *et al.*, 1984) and this interaction is sufficient to support the repair synthesis step in *E. coli*. Finally, **DNA ligase** (Lahue *et al.*, 1989) restores the DNA integrity by sealing the gap.



Figure 1.2: **MMR mechanism in** *E.coli*. Schematic representation of the DNA mismatch repair mechanism in the prokaryote *E. coli*. The square at the bottom contains the names and figures used to represent each protein involved in this pathway and a brief summary of the actions occurring in each step is reported on the right part of the figure.

#### **1.1.3 MMR mechanism in eukaryotes**

The first study in eukaryotes identifying MutHLS homologous was done in *Saccharomyces cerevisiae*. Williamson *et al.* (1985) demonstrated that these genes were required for MMR and later, biochemical studies showed that MMR in eukaryotes depended on homologous proteins to the bacterial *MutL* and *MutS* (Reenan & Kolodner, 1992). The MMR pathway is evolutionarily very conserved and some similarities such as substrate, bidirectionality and specificity are common between prokaryotes and eukaryotes. Nevertheless, the core proteins in humans, although homologous to the *E. coli* ones, are more numerous and the protein interactions more complex. In addition, the strand discrimination and excision mechanisms are different. Table 1.1 compares the bacterial and human proteins with their functions and interactions.

#### 1.1.3.1 MMR mechanism in humans

Figure 1.3 shows a diagram of the MMR pathway in humans. Likewise in *E*. *coli*, the MMR pathway in humans can be divided into three main steps:

- a) <u>Initiation</u>: The human homologous of the bacterial *MutS* is a group of proteins called MSH proteins (from Mut S Homologous) and the repair is initiated when either **MutS** $\alpha$  (MSH2-MSH6) which repairs single base-base and 1to 2 IDLs mismatches, or **MutS** $\beta$  (MSH2-MSH3) which repairs larger IDLs, heterodimers bind to a mismatch (Kunkel & Erie, 2005). In humans, there are three different types of MSH proteins:
  - hMSH2 (\$P43246): the gene was found to be near a locus implicated in hereditary non-polyposis colon cancer (HNPCC) (Peltomäki et al., 1993). The hMSH2 gene is located in the human chromosome 2p22-21, has 16 exons and encodes a 105 kDa protein that selectively binds to DNA containing base-base mispairs and IDLs mismatches (reviewed in Kunkel & Erie (2005)). It was shown by Fishel et al. (1994) and Umar et al. (1994) that cells containing mutant hMSH2 genes exhibit microsatellite instability (MSI) and are defective in MMR (for more information refer to section 1.1.4). Repair activity can be restored in

these extracts by a protein preparation designated hMutS $\alpha$  ((Drummond *et al.*, 1995), (Umar *et al.*, 1997) and (Watanabe *et al.*, 2000)) consisting of a heterodimer of hMSH2 and hMSH6. Most of the germline and somatic mutations affecting hMSH2 and other MMR genes were reviewed in Papadopoulos & Lindblom (1997) and Peltomäki & Vasen (1997). Missense mutations account for 17% of all identified alterations of this gene and Ollila *et al.* (2008) analysed some of these mutations located in the amino-terminal domain to demonstrate the pathogenicity of those mutations. Studies *in vivo* by Mastrocola & Heinen (2010) generated human cell lines stably expressing some missense mutations and tested their effect on DNA repair and checkpoint response function. They found that those MSH2-deficient cells had lost their normal DNA checkpoint response to alkylating agents and also lost or decreased their MMR activity *in vivo*.

- hMSH6 (#P52701): it was demonstrated by Drummond et al. (1995) and Palombo et al. (1995) that the hMutS homologue was a heterodimer (called MutSα) of approximately 100 KDa (corresponding to hMSH2) and 160-KDa (firstly called GTBP; now known as hMSH6). This gene was localized by Papadopoulos et al. (1995) in 2p16 and they proposed that hMSH2 and hMSH6 might have been produced by duplication of a primordial mutS repair gene. Bowers et al. (1999) showed that mutations in a very conserved residue involved in binding to the mismatched base in S. cerevisiae conferred loss of MMR in vivo.
- hMSH3 (#P20585): one mutS homologue located upstream of the human dihydrofolate reductase gene (DHFR) was identified by Fujii & Shimada (1989) and located at 5q11.2-q13.2. In the mouse, a mutS homologue was also found and it was called Rep3 (mouse repair gene 3). When later on, in S. cerevisiae MSH3 was identified by New et al. (1993) as an open reading frame near the DHFR gene, a relationship was thought to exist between these three genes. Three years later, a frameshift mutation in hMSH3 was observed in an endometrial carcinoma (Risinger et al., 1996) showing MSI that was partially

reduced when introducing human chromosome 5, so they proposed that hMSH3 acts in the repair of some but not all mismatches.

All MutS homologous have highly conserved domains: one of them of approximately 150 amino acids forms an helix-turn-helix domain associated with an adenine nucleotide and a magnesium binding motif (Walker-A motif). It has been demonstrated that this domain has ATPase activity (Haber & Walker, 1991). The MSH2-MSH6 complex has two different conformations: the ADP-bound form, called ON, that binds to mismatched nucleotides, and the ATP-bound form, called OFF, that, on the contrary, cannot bind to mismatches (Gradia *et al.*, 1997). In addition, domains I and IV bind to DNA, domain V contains the dimerization interface and nucleotide-binding site and the N-terminal region contains a motif interacting with PCNA ( $\beta$ -clamp homologue).

- b) Excision: Equivalent to what happens in E. coli, the next complex that takes part in the repair pathway is a MutL homologue. In yeast, Prolla et al. (1994) identified two genes, MLH1 and PMS1 with homology to E. coli mutL and S. pneumoniae hexB. They also demonstrated that disruption of MLH1 resulted in high spontaneous mutation rates. Subsequently, three human mutL homologous were described by Papadopoulos et al. (1994):
  - *hMLH1* (*#P40692*): is located in chromosome 3p21.3 and Hemminki et al. (1994) showed that loss of heterozygosity (LOH) of markers within or adjacent to this gene occur in some HNPCC tumours. Some mutations in *hMLH1* disrupt *h*MLH1 protein forming complexes with *h*EXO1 and PMS2 *in vivo* (Jäger *et al.*, 2001). Somatic promoter hypermethylation has also been found in sporadic colon tumours.
  - PMS1 (#P54277): the name of PMS proteins comes from post-meiotic segregation as they were isolated by Williamson *et al.* (1985) after meiosis in *S. cerevisiae*. In humans, PMS1 has three isoforms (a, b and c) and mutations of this gene have been identified in some HNPCC patients (Nicolaides *et al.*, 1994). PMS1 and *h*MLH1 form a heterodimer called MutLβ with unknown function in humans.

PMS2 (#P54278): is a yPMS1 homologue endonuclease that has also been found mutated in HNPCC patients (Nicolaides *et al.*, 1994). It forms a heterodimer with hMLH1 called MutLα (MLH1-PMS1 in yeast) coordinating events after mismatch binding by MutS homologue (reviewed in Hsieh & Yamane (2008)).

The C-terminal domain of MutL in *B. subtilis* and *E. coli* specifically interacts with  $\beta$  sliding clamp (Pillon *et al.*, 2011), stimulating MutL endonuclease activity. **PCNA** is the human homologue of the  $\beta$  subunit of *E. coli* polymerase III and interactions between MutL and PCNA enhance polymerase  $\delta$  processivity by enabling its sliding during the elongation process (O'Donnell *et al.*, 1992) through its interaction with MutS $\alpha$ , MutL $\alpha$  and *h*EXO1 (Fiorentini *et al.*, 1997). MutL $\alpha$  has a PCNA/RFC - dependent endonuclease activity that plays a critical role in 3' nick-directed MMR involving *h*EXO1 (Kadyrov *et al.*, 2006). It is not clear yet how strand discrimination occurs in eukaryotes as no MutH homologue has been found; however, it was shown that small gaps of 4 to 18 nt might highlight the newly synthesised strand (Iams *et al.*, 2002) and later, Ghodgaonkar *et al.* (2013) demonstrated that incorrect addition of ribonucleotides by DNA polymerases can initiate the MMR pathway.

c) Resynthesis: In eukaryotes, the MMR is bidirectional as in bacteria but in this case, *h*EXO1 has both 5' and 3' exonucleolytic activities (Genschel *et al.*, 2002) excluding the necessity for different exonucleases. PCNA is essential during 3' nick-directed MMR but not for 5' nick-directed MMR (Li, 2008). It has been demonstrated that PCNA binds preferentially to the 3' terminus of a strand break whereas **RFC** (replication factor C) binds at the 5' terminus (Jiricny, 2006). **RPA** (replication protein A) stimulates *h***EXO1** activity in the presence of a MutS $\alpha$  complex bounded to a mismatch, stabilizes the single-stranded DNA and inhibits *h*EXO1 activity in conjunction with MutL $\alpha$  when the mismatch is removed. Finally, polymerase  $\delta$  fills the gap and DNA ligase 1 seals the remaining nick.

E.coli proteins	Function	Eukaryote homologous	Function
MutS (Su & Modrich, 1986)	Recognises and binds mismatches	$hMSH2$ - $hMSH6$ (MutS $\alpha$ ) (Fishel <i>et al.</i> , 1994) $hMSH2$ - $hMSH3$ (MutS $\beta$ ) (Fishel <i>et al.</i> , 1994)	Recognises single base and 1-2 base IDL mismatches Recognises some single base IDLs and IDLs $\geq 2$ bases
		<i>hMLH1-h</i> PMS2 ( $\gamma$ PMS1) (MutL $\alpha$ ) (Hemminki <i>et al.</i> , 1994); (Papadopoulos <i>et al.</i> , 1994) and (Prolla <i>et al.</i> , 1994)	Intermediates the coordination of MMR by binding MutS homologues
MutL (Pang <i>et al.</i> , 1985)	Intermediates the coordination of MMR	MLH1-MLH2 ( $h$ PMS1) (MutL $\beta$ ) (Nicolaides <i>et al.</i> , 1994)	Unknown function in humans but it suppresses some IDL mutagenesis in yeast
		MLH1-MLH3 (MutL $\gamma$ ) (Kolas $et~al.,2005$ )	Suppresses some IDL mutagenesis and participates in meiosis
MutH (Welsh et al., 1987)	Recognises hemimethylated GATC sites and nicks the unmethylated strand	None	
$\gamma$ - $\delta$ Complex	Loads $eta$ -clamp onto DNA	RFC complex	Loads PCNA Modulates excision polarity
	Interacts with MutS		Interacts with MutS and MutL homologues Recruits MMR proteins to mismatches
$\beta$ -Clamp	Enhances processivity of DNA pol III	PCNA (O'Donnell et al., 1992)	Increases MM binding specificity of MSH2-MSH6 Participates in excision and probably in signalling
			Participates in DNA repair synthesis Participates in DNA re-synthesis
Helicase II	Loads onto DNA at nick by MutS and MutL	None	
	Unwinds DNA to allow excision of ss- DNA		
ExoI (Szankasi & Smith, 1995), ExoX	Performs 3'-5' excision of ssDNA	hEXOI (Tishkoff <i>et al.</i> , 1997a)	Performs excision of dsDNA
RecJ	Performs 5'-3' excision of ssDNA	3' exo of polδ	Performs excision of ssDNA
ExoVII	Performs 5'-3' and 3'-5' excision of ssDNA	3' exo of pol $\epsilon$	Synergistic mutator with Exo1 mutant
DNA pol III	Performs re-synthesis of DNA	DNA polô	Performs repair synthesis
SSB	Protects ssDNA from nucleases attack	RPA	Participates in excision and in DNA synthesis by stabilizing excision intermediates
DNA ligase	Seals nicks after DNA synthesis	DNA ligase I	Seals nicks after DNA synthesis

Table 1.1: Identity and functions of *E. coli* and eukaryotic homologous proteins involved in the MMR pathway. Adaptation from table 1 in Kunkel & Erie (2005), table 1 in Hsieh & Yamane (2008) and table 1 in Li (2008).



Figure 1.3: **MMR mechanism in eukaryotes.** Schematic representation of the DNA mismatch repair mechanism in humans. Adapted from figure 3 in Jiricny (2006) and figure 1 in Hsieh & Yamane (2008). The square at the bottom contains the names and figures used to represent each protein involved in this pathway and a brief summary of the actions occurring in each step is reported on the right part of the figure.

#### 1.1.3.2 Models for MMR mechanism

Three models have been proposed for MutS-MutL interaction during the MMR pathway. Two models are referred as "*cis*" or "moving" models because MutS-MutL complexes are loaded at a mismatch site and then moved away from the site to search for the strand break that serves as strand discrimination signal. The third model is called "*trans*" or stationary model because the complex binds to the mismatch and only communicates with more distant signals by complex protein interactions. A diagram comparing the three models is shown in figure 1.4.

- A. Hydrolysis-dependent translocation model:Allen *et al.* (1997) and Dao & Modrich (1998) proposed that in the presence of MutL and ATP, the MutS protein covers the mismatch and the endonuclease activity of MutH (in *E. coli*) or MutL $\alpha$  (in humans) produce an incision, triggering the degradation of the newly synthesised strand. In this model, ATP hydrolysis is needed for bidirectional translocation of the MutS complex along the helix (reviewed in Kunkel & Erie (2005)). DNA runs through the protein complex until MutS and MutL complexes reach a strand discrimination signal, forming a DNA loop (reviewed in Li (2008)). This model is not currently favored because there are not known molecular controls able to regulate ATP hydrolysis and/or ADP to ATP exchange in this process (Acharya *et al.*, 2003).
- **B.** Molecular switch model: Fishel (1998) and Ban & Yang (1998) described this model based on the observation that hMSH heterodimeric complexes showed significant mismatch-dependent ATPase activity (reviewed in (Gradia *et al.*, 1997) and (Gradia *et al.*, 2000)). In this model, supported by the crystaline structure of MSH2 described by Obmolova *et al.* (2000), ATP binding to hMSH proteins produces the formation of a DNA sliding clamp. The term "sliding clamp" was first used to describe the  $\beta$ -subunit of *E. coli* DNA polymerase III and PCNA and refers to a protein complex that encircles DNA allowing it to pass through the hole

in its centre. MutS initially binds to mismatched DNA in an ADP bound state triggering a tridimensional change that allows an ADP to ATP exchange. This results in conformational changes allowing the clamp to slide through the DNA (reviewed in Li (2008)). It was suggested that the role of this switch is to control the timing of the downstream excision repair event Gradia *et al.* (1997). Later experiments refined this model proposing that MutS heterodimers act as a mismatch sensor and that a threshold number of localized ATP-bound MutS sliding clamps are required to initiate MMR (Acharya *et al.*, 2003), introducing a redundancy into the model which is necessary to ensure that the MMR reaction can be restored from the last end-point if any dissociation happens during the process.

C. Transactivation model: Junop *et al.* (2001) proposed that MutS-MutL complexes can communicate with the mismatch and downstream proteins using protein-protein interactions and DNA bending. In this model, MutS heterodimers scan the DNA looking for a mismatch using the ATP-ase activity which acts as a proofreading enzyme to verify mismatch binding and authorizes the downstream excision (Junop *et al.*, 2003). It was postulated that MutS heterodimers bind ATP after recognition of a putative mismatch and this binding is sufficient for recruitment of MutL $\alpha$  and downstream repair proteins (reviewed in Iyer *et al.* (2006)).

Those three models are suitable for the prokaryotes and eukaryotes MMR pathway and no consensus has been reached towards which one is the most likely. However, Haye & Gammie (2015) showed that the MutS heterodimers are linked to the replisome possibly via their interaction with PCNA. They proposed a moving model in which MutS $\alpha$  or MutS $\beta$  load into the DNA as it is being replicated scanning for mismatches or small IDLs. Furthermore, Supek & Lehner (2015) demonstrated that the MMR is more efficient repairing early replicating errors in euchromatic regions of the genome, suggesting that either the accessibility to the DNA and/or the coupling with the replication machinery are needed for correct repair.



Figure 1.4: **Models proposed for the MMR mechanism.** Adapted from figure 1 in Li (2008). The two "cis" or moving models are the TRANSLOCATION and MOLECULAR SWITCH and the "trans" is the STATIONARY model (reviewed in Acharya *et al.* (2003)).

#### 1.1.4 MMR, MSI and cancer predisposition

Microsatellites are short tandem DNA repeat sequences (STRs) of 1 to 6 base pairs distributed throughout the genome which are prone to suffer from slippage errors during replication, producing microsatellite instability (MSI) (reviewed in Li *et al.* (2004)). This instability changes the length of the microsatellite, resulting in gain or loss of one or more repeat units and it can be used as diagnostic tool when comparing microsatellite profiles between tumours and normal tissue for the same patient (reviewed in Umar & Kunkel (1996) and Shah *et al.* (2010)). See figure 1.5 for a schematic representation.

Knock-out mice for MMR genes showed overall higher mutation frequencies than MMR-proficient mice, with the highest mutuation rates in  $Mlh1^{-/-}$ and  $Msh2^{-/-}$  mice (Hegan *et al.*, 2006). Mutation rates in tandem repeats were assessed by transfection experiments using neomycin (neo) gene disrupted with a microsatellite. Spontaneous mutation rates of microsatellites in normal human cells have been proposed to vary between  $3.1 \times 10^{-9}$  to  $4.5 \times 10^{-9}$  mutations per cell per generation (Boyer & Farber, 1998) whereas the mutation rate in MMR-defective cancer cells was reported to be approximately two orders of magnitude higher than in repair-proficient cancer cells by Boyer et al. (1995) and Hanford et al. (1998). Studies in tumour cell lines mutants for MSH2 (eg. LoVo or HEC59), MSH6 (HCT15), MLH1 (HCT116) or PMS2 (DU145) have shown MSI and high mutation rates in endogenous genes (Boyer et al., 1995) compared to MMR-proficient cancer cells (HT1080). It was demonstrated by Peltomäki et al. (1993) that microsatellites are highly unstable in most tumours from HNPCC (hereditary non-polyposis colon cancer, see section 1.3.1 for more details) patients and in some sporadic colon cancers (SCC). MSI is present in 15% of sporadic CRC (Liu et al., 1995) and in almost all hereditary CRC (2-5% of all CRC) (Buecher et al., 2012). The risk of suffering cancer may be increased by the inheritance of different mutations called variants of uncertain significance (VUS) (Kantelinen et al., 2012). Inheriting different MMR VUS, which individually may not have any effect in the development of cancer, produce a synergistic effect contributing to the development of CRC. Diagnosis of HNPCC can be performed using 5 microsatellites markers (BAT26, BAT25, D5S346, D2S123 and D17S250) and tumours are classified as **MSI-High** (MSI-H) when two of more markers show MSI, **MSI-Low** (MSI-L) with one unstable marker or **MSS** (microsatellite stable) when no microsatellite loci are unstable compared to the normal tissue (reviewed in Vasen *et al.* (2007) and Lynch *et al.* (2009)). Interestingly, MSI has been shown to be reduced during treatment with aspirin in colorectal but not endometrial MMR-deficient tumours (Rüschoff *et al.*, 1998) and thus, treatment with aspirin has been proposed as prophylactic treatment for families with an hereditary colon cancer predisposition.



Figure 1.5: **Diagram showing replication slippage in a**  $(CA)_4$  **microsatellite.** The template strand is shown in blue and the new strand in red. Black dots indicate Watson-Crick bounding. Adapted from figure 2 in Umar & Kunkel (1996).

#### **1.1.5 Other functions for MMR**

Regardless its main role detecting and correcting replication errors, the MMR pathway controls several non-canonical functions reviewed in Iyer *et al.* (2006), Bak *et al.* (2014) and Crouse (2016).

- Sensor for genetic damage: in normal (MMR-proficient) cells, treatment with cytotoxic agents such as 6-thioguanine or MNNG leads to an accumulation in G2 phase of the cell cycle and activation of the p53 apoptosis pathway that protects cells against genetic damage acting as a mechanism to avoid cancer development. Treatment of MMR-deficient cells with the same cytotoxic agents, produces no accumulation in G2 phase nor an activation of the apoptosis pathway (reviewed in Iyer et al. (2006), Jiricny (2006) and Hsieh & Yamane (2008)). This effect can be reversed by introducing the human chromosome 2 containing the wildtype of *hMSH2* and *hMSH6* into HEC59, LoVo or HCT15 ((Umar et al., 1997) and (Watanabe et al., 2000)). DNA methylators such as 5-fluoro-2'deoxyuridine (FdU) and cisplatin which are mutagenic (promoting nucleotide mispairs) and cytotoxic agents, have been used as antitumour drugs because they accumulate in proliferative cells promoting apoptosis. The effectiveness of this kind of chemotherapy is sometimes reduced in MMR-defective tumours as deficiency in the MMR pathway impedes to recognise the mismatch generated by FdU incorporation, resulting in poor response against cytotoxic agents in MMR-deficient patients ((Iyer et al., 2006) and (Liu et al., 2008)). Interestingly, it was found that selective inhibition of caspase 3 (a key protease involved in cellular degradation during apoptosis) in HCT116 (*MLH1<sup>-/-</sup>*) cancer cells improved the response to chemotherapy with FdU (Flanagan et al., 2016), suggesting that in MMR-defective cancer cells, caspase 3 has a greater impact on cell proliferation than in apoptosis promotion.
- **Triplet repeat instability**: some neurological, neurodegenerative and neuromuscular diseases such as myotonic dystrophy type 1 and Huntington's disease are caused by expansions of CTG and CAG trinucleotide re-

peats (TNRs) (reviewed in Iyer *et al.* (2015)). It was shown that MutL $\alpha$ and MutS $\beta$  are required for the repair of CTG slippage replication errors in vitro (Panigrahi et al., 2012), but a transcriptional process might be involved in CAG tracks stabilization in vivo (Lin et al., 2006). Therefore, MSH2 deficiency in transgenic mice containing (CAG)<sub>n</sub> repeats was shown to prevent somatic instability (Manley *et al.*, 1999) and to delay the age onset of the disease (Wheeler et al., 2003); additionally, MSH2 deficient transgenic mice containing (CTG)<sub>n</sub> repeats had a higher proportion of contractions in spermatogonia (Savouret et al., 2004) while depletion of MSH3 but not MSH6 reduced (CTG)<sub>n</sub> expansions in DM1 mice (van den Broek *et al.*, 2002) and recently, a polymorphism in *hMSH3* has been found to be associated to the variation in somatic instability in a group of DM1 patients (Morales et al., 2016). Furthermore, Msh2<sup>-/-</sup> transgenic mice for X-fragile syndrome showed an intergenerational reduction in CGG repeat expansions (Lokanga et al., 2014). Interestingly, it was found that some triplet repeat sequences, such as  $(ATT)_3$ , can form non-B-DNA structures that bind MMR proteins suppressing their activity what was suggested to be a natural *cis*-acting suppressor of the normal MSH2-dependent MMR machinery (Lujan et al., 2012).

Anti-recombination activity: some studies revealed that MutS and MutL were able to access mismatched base pairs within early strand transfer intermediates blocking the recombination process (reviewed in (Iyer *et al.*, 2006)). In MMR-deficient cells, the homeologous recombination frequency was dramatically elevated, suggesting that MMR suppresses this kind of recombination. This might be illustrated by the fact that, in human cells, MutS $\alpha$  or MutL $\alpha$  deficiency increased the rate of gene duplication 50 to 100-fold, what may contribute to cancer predisposition (Hsieh & Yamane, 2008). The anti-recombination activity of MMR proteins (mainly MSH2 and MSH6) inhibiting interactions between homeologous sequences was likely to have a role promoting genome stability and preventing genome rearrangements ((de Wind *et al.*, 1999) and (Harfe & Jinks-Robertson, 2000a)). Role in meiosis: MSH4-MSH5 and MLH1-MLH3 heterodimers constitute in mammals the meiotic MMR complex promoting meiotic recombination. Loss of any of those four proteins results in a decreased level of crossing-over events during meiosis (see Kirkpatrick (1999), Harfe & Jinks-Robertson (2000a) and Clark et al. (2013)) having an effect on both, male and female fertility as summarised in table 1.2. Many human diseases are related with mutations in either hMSH4 or hMSH5 such as neoplasias (breast cancer, myeloma, lung cancer, ovarian cancer, glioma and colorectal cancer) or immune diseases (systemic lupus erythematosus, Kawasaki disease and type 1 diabetes) but also with reproductive disorders as azoospermia and premature ovarian failure, both caused by a defect on hMSH5 (reviewed in Clark et al. (2013)). Baker et al. (1995) demonstrated that male but not female mice mutants for *Pms2* were infertile because they produced only abnormal spermatozoa but this effect was not present when the mutated gene was Msh2. Furthermore, the localization of MLH3 protein in mice, associated to repetitive regions -centromeres- in late pachytene spermatocytes, was upregulated in  $Pms2^{-/-}$  but downregulated in  $Mlh1^{-/-}$  because in the absence of PMS2, MLH1 associates with MLH3 (Kolas et al., 2005).

Genotype	Male Fertility	Female Fertility	References
Mlh1 <sup>_/_</sup>	-	-	Baker <i>et al.</i> (1995)
$Pms1^{-/-}$	+	+	Prolla <i>et al.</i> (1998)
$Pms2^{-/-}$	-	+	Prolla <i>et al.</i> (1998)
$Mlh3^{-\!/\!-}$	-	-	reviewed in Hsieh & Yamane (2008)
Msh2 <sup>_/_</sup>	+	+	Kirkpatrick (1999)
$Msh3^{-\!/-}$	+	+	de Wind <i>et al.</i> (1999)
$Msh4^{-\!/-}$	-	-	reviewed in Neyton et al. (2004)
$Msh5^{-\!/\!-}$	-	-	reviewed in Clark et al. (2013)
Msh6 <sup>_/_</sup>	+	+	reviewed in Hsieh & Yamane (2008)
Exol <sup>-/-</sup>	-	-	reviewed in Hsieh & Yamane (2008)

Table 1.2: Mouse lines with MMR defects and their relationship with fertility. Adapted from table 2 in Hsieh & Yamane (2008). "+" indicates fertile and "-" indicates infertile.

Generation of immunoglobulin diversity: immunoglobulin class switching and somatic hypermutation are mechanisms for increasing antibody diversity during antigen-stimulated B-cell differentiation (reviewed in Bak et al. (2014)). During this process G:U mispairs, that can be recognized and processed by the MMR pathway, are generated by activationinduced cytidine deaminase (AID) which deaminates cytosine residues to uracil (Iyer et al., 2006). It has been proposed that those residues are processed by error-prone DNA polymerases such as DNA polymerase  $\eta$  resulting in a mutagenic process. It was demonstrated that  $Mlh3^{-/-}$ mice had an increased mutation frequency in immunoglobulin (Ig) variable regions (reviewed in Hsieh & Yamane (2008)) suggesting that this gene usually reduces the accumulation of mutations in this region. Experiments with knockout mice have demonstrated that the presence of MutS $\alpha$  promotes mutagenesis rather than prevents it because B cells from MMR-deficient mice display a low level of somatic hypermutation and reduced class switch recombination ((Jiricny, 2006) and (Li, 2008)).
# **1.2 Telomeres**

# **1.2.1** Function and structure of human telomeres

Telomeres, the natural end of linear chromosomes in eukaryotes, are short tandem repeat (STRs) arrays (Blackburn, 1991). Human telomeres are organized as a double-stranded (dsDNA) tandem repeats that start after a chromosome specific subtelomeric region (Baird et al., 2003). The non-canonical repeats ((TGAGGG)<sub>n</sub>, (TCAGGG)<sub>n</sub> or (TTGGGGG)<sub>n</sub> among others) are called telomere variant repeats (TVR) and are located closer to the subtelomeric region (Baird *et al.*, 1995) and the canonical, (TTAGGG)<sub>n</sub> repeats, that follow the TVR region are followed by a terminal 3' G-rich single-stranded (ssDNA) extension (called G-overhang) of approximately 100 nucleotides (reviewed in Garrido-Ramos (2012), Stewart et al. (2012) and Lu et al. (2012)). The number of repeats per telomere varies widely among species; while in humans it encloses 10-15 kb (in germline cells) in mouse and rats telomere length might be longer than 50 kb (Nandakumar & Cech, 2013). Their G-rich composition makes them prone to form G-quadruplexes (G4) (Zahler et al., 1991) or alternative structures such as **T-loops** as shown in figure 1.6 (reviewed in Lipps & Rhodes (2009) and Nandakumar & Cech (2013)):

**T-loops:** are lariat-like structures resulting from the dsDNA invasion by ss-DNA. They were discovered by Griffith *et al.* (1999) who also correlated the size of the t-loop circle with the length of the telomeric repeat array. It is not known if all telomeres have this structure or if it is formed during the cell cycle to allow replication, but it was proposed by Luke-Glaser *et al.* (2012) that there should be a balance between t-loop resolving and forming activities constituting a precise temporal regulation. This control should be mediated by rapid and reversible post-translational protein modifications; furthermore they proposed that the RTEL helicase is able to resolve t-loops when present. The technique called STORM, developed by Rust *et al.* (2006), allows to see t-loops with a better resolution and might resolve some unsolved questions about them. **G-quadruplexes:** are G-quartets which are formed by the association of four guanines, in a square, planar structure and that is hydrogen-bounded by Hoogsteen-base pairing (non-Watson-Crick pairing that involves N7 atom of a purine) and stabilized by K<sup>+</sup> and Na<sup>+</sup> ions (reviewed in Lipps & Rhodes (2009)). Interestingly, Zahler *et al.* (1991) demonstrated that these structures inhibit telomere elongation mediated by telomerase (see **section 1.2.2.3**). Taking this feature into account, later studies have designed small organic ligands that stabilize G-quadruplexes as anticancer therapies (an extensive review of those experiments together with the most recent studies *in vivo* about G-quadruplexes presence and function can be found in Lipps & Rhodes (2009) and table 1.6).



Figure 1.6: **Diagram of the telomere structure and its possible conformations.** Purple line indicates the Grich strand and green line, the C-rich strand. The top part of the figure shows the telomere double stranded (dsDNA) and single stranded (ssDNA) regions, indicating the location of the canonical (TTAGGG<sub>n</sub>) and non canonical repeat variants. The bottom part shows the two possible conformations in T-loops and G-quadruplexes as reviewed in Lipps & Rhodes (2009) and Nandakumar & Cech (2013).

The ends of linear chromosomes are targets for DNA damage response pathways and in addition, are strongly exposed to genotoxic stress and other mechanisms involved in telomeric loss; therefore, the two main problems of telomeres are: **End-protection problem:** was first described by Muller & Altenburg (1930) when chromosome end fusions were observed in the fruit fly (*Drosophila melanogaster*). Because of their structure, telomeres look like DNA double strand breaks (DSBs) that need to be repaired, activating the DNA damage response (DDR) pathway or cell cyle arrest (reviewed in de Lange (2009)):

- <u>DNA repair</u>: DSBs are potentially lethal for mammalian cells and they usually stimulate homologous recombination (HR), a DNA repair mechanism that uses sister chromatids as a repair template (Liang *et al.*, 1998). When that template is not available, another repair mechanism is favored: non-homologous end joining (NHEJ). This mechanism is errorprone in contrast with HR, but both constitute the two major pathways for DSBs repair. The consequences of DSBs repair in telomeres are endto-end chromosomal fusions that are highly unstable as demonstrated by Muller & Altenburg (1930) because dicentric chromosomes are formed and they can initiate multiple bridge-breakage-fusion cycles.
- <u>Cell cycle arrest</u>: is activated as a response against DNA damage and the major regulators of this response are ATM (ataxia telangiectasia mutated kinase) and ATR (ataxia telangiectasia and Rad3 kinase) (for a review see Cimprich & Cortez (2008)). Both kinases phosphorylate a group of substrates promoting cell-cycle arrest and DNA repair even though their mechanism of action and the effects of mutations in those genes are different. Activation of the ATM kinase pathway leads to up-regulation of p53 and induction of senescence (see section 1.2.2.2 for more details), as it can be demonstrated by the presence of telomere dysfunction-induced foci (Verdun & Karlseder, 2007). It has been demonstrated that senescence occurs when the unprotected chromosome ends are recognized as DSBs because in those foci, DSB repair complexes co-localise with telomeres (Muraki *et al.*, 2012).

**End-replication problem:** was predicted by James Watson in 1972 and one year later, Olovnikov proposed the "marginotomy theory of ageing" (reviewed in Verdun & Karlseder (2007)). In this theory, the "telogenes" (ends of DNA molecules) were ramdomly shortened in each mitosis cycle, providing a mechanism for ageing that agreed with the Hayflick limit (Hayflick & Moorhead, 1961). The end-replication problem is caused by the inability of DNA polymerases to regenerate the RNA primer of the most distal Okazaki fragment, resulting in the loss of some nucleotides each time a cell divides. In S. cerevisiae the telomere erosion rate in normal cells has been reported as 3 to 4 bp per generation while the erosion rate in human cells is estimated between 50 to 200 bp/cell division ((Baird, 2008), (Palm & de Lange, 2008) and (Muraki *et al.*, 2012)) and in mice  $\approx$  7 kb/cell division (Vera *et al.*, 2012), indicating the great variability in telomere shortening rates among eukaryotes. The progressive loss of telomeric DNA has been suggested to be a molecular clock reflecting the number of cell divisions that have undergone and contributing as a signal for the entrance into senescence (Harley et al., 1990) and (Chadeneau et al., 1995). It has been estimated that human sperm telomeres are about 10 to 14 kb long, whereas somatic cell telomeres are only 5 to 10 kb long, reflecting the number of divisions that have undergone in somatic cells (Pickett et al., 2011).

#### 1.2.1.1 Shelterin complex

The shelterin complex caps the most distal part of chromosomes, preventing genomic instability (reviewed in Palm & de Lange (2008), O'Sullivan & Karlseder (2010) and Stewart *et al.* (2012)) as it a) enables cells to distinguish their natural chromosome ends from dsDNA or ssDNA strand breaks, b) represses DNA repair reactions by compacting telomeric chromatin (Bandaria *et al.*, 2016), c) regulates telomerase-based telomere maintenance (Xin *et al.*, 2008), d) is proposed to be implicated in the formation of t-loops and e) controls the synthesis of telomeric DNA by telomerase (de Lange, 2005). It is composed of six telomere-specific proteins (TRF1, TRF2, TIN2, Rap1, TPP1 and POT1) that can be found together in a single complex or forming different subcomplexes (see below for further information of each protein). The common characteristics of the shelterin complex compared with other telomere-associated proteins are that a) it is abundant at chromosome ends but does not accumulate elsewhere, b) it is present at telomeres throughout the cell cycle and c) its function is limited to telomeres (de Lange, 2005).

- **TRF1** (*Telomeric repeat-binding factor 1*) (*P54274*): first described by Zhong et al. (1992) is a 50 kDa protein binding to dsDNA and preferentially recognising the telomeric repeat sequence present at mammalian chromosome ends (as it does not bind well to other tandem repeat arrays such as TAGGG, TTTAGGG, TTTTAGGG, TTGGGG and TTAGGC). It is proposed that TRF1 negatively regulates telomere length in telomerase-positive human cells as its interaction with PINX1, an inhibitor of telomerase, decreases telomere length (reviewed in Xin et al. (2008)). Besides, diminished TRF1 loading onto telomeres has been found in ageing endothelial cells with very short telomeres (Hohensinner et al., 2016) suggesting that deprotection of telomeres by loss of TRF1 might increase telomere attrition. TRF1 also promotes replication throughout the telomere duplex as its removal from telomeres leads to replication fork stalling, defects in the telomeric tract packaging and fusions of sister telomeres (Stewart et al., 2012). In addition, TRF1 binding to the C-terminus of ATM constitutes a negative regulatory feedback that diminishes the ability of TRF1 to interact with DNA, delaying mitotic entry and apoptosis (reviewed in Xin et al. (2008)).
- TRF2 (*Telomeric repeat-binding factor 2*) (\$Q15554): described by Bilaud et al. (1996) is known to homodimerize and it has been proposed to stabilize t-loop formation and to protect the telomeres by binding ds-DNA, since: a) it preferentially binds to the end of a dsDNA TTAGGG repeat array if it contains a G-strand overhang of at least 6 nt, b) the multimeric binding mode of TRF2 introduces positive supercoils that promote unwinding and strand invasion and c) its ability to bind to Holliday junctions (reviewed in de Lange (2002) and Palm & de Lange (2008)). Therefore, TRF2 primarily acts in chromosome end protection by promoting topological changes in telomeric DNA, t-loop and chromatin assembly and the suppression of ATM-dependent DNA damage response and

NHEJ (reviewed in O'Sullivan & Karlseder (2010)).  $Trf2^{-/-}$  mice, which die early in embryogenesis (de Lange, 2005), demonstrated that TRF2 is an essential protein. The dominant negative TRF2  $\Delta B \Delta M$  is a truncated version that forms inactive heterodimers with the endogenous protein and effectively removes TRF2 from telomeres (van Steensel *et al.*, 1998), resulting in end-to-end chromosome fusions. Conversely, overexpression of TRF2 has been found to increase telomere shortening (Karlseder *et al.*, 2002) in an oxidative stress-independent way (Richter *et al.*, 2007) that relies on the formation of telomeric ultrafine anaphase bridges (Nera *et al.*, 2015).

**TIN2** (*TRF1-interacting nuclear protein 2*) (#*Q9BSI4*): was identified by its interaction with TRF1, in a two-hybrid screen, and it was localized at telomeres (Kim et al., 1999). Although both TRFH domains in TRF1 and TRF2 are very similar, they cannot heterodimerize, so TIN2 is necessary to bring them together. Additionally, TIN2 binds TPP1, thus providing a bridge between the shelterin components that bind to dsDNA and ssDNA (see figure 1.7). The subcomplex formed by TRF1-TIN2-TRF2-TPP1 stabilizes the shelterin complex but whether other subcomplexes of shelterin coexist in cells remains unknown (reviewed in Nandakumar & Cech (2013)). However, it is known that TIN2 contains mitochondria targeting signals and knock down of TIN2 by siRNA enhanced oxygen consumption and mitochondrial ATP synthesis (Lu et al., 2012), suggesting that TIN2 may play a role regulating aerobic glycolysis and oxidative phosphorylation. Furthermore, mutations in TIN2 have been found in some dyskeratosis congenita (DC) patients and knock-in mice carrying this mutation had accelerated telomere shortening over generations (Frescas & de Lange, 2014).

- Rap1 (*Repressor and activator protein 1*) (#*Q9NYB0*): is a 399 amino acids protein of approximately 47 KDa that interacts with TRF2 by its C-terminal domain (Li *et al.*, 2000) and shares its structure with *Rap1p* (its yeast homologue) although in contrast to it, human Rap1 only has one Myb-like domain, lacking the one that directly binds to telomeric DNA (Palm & de Lange, 2008). The Rap1 C-terminus also contains a putative nuclear localization signal (NLS) (de Lange, 2005) and inhibition of Rap1 by dominant-negative expression leads to elongated telomeres and loss of telomere heterogeneity (Li *et al.*, 2000).
- **TPP1** (*POT1 and TIN2-interacting protein*) (#*Q96AP0*): similarly to TIN2, TPP1 also acts as a bridge between different shelterin components. This protein has a C terminus of 60 aminoacids that binds to the N-terminal of TIN2 and a central 100-amino-acid region which binds to the C terminus of POT1 (Liu et al., 2004). The N terminus of TPP1 has an OB-fold (oligonucleotid/oligosaccharide-binding) domain that interacts with telomerase raising the possibility that TPP1 is involved in the recruitment or regulation of telomerase (Palm & de Lange, 2008). TPP1 increases the affinity of POT1 for DNA by 10-fold and it also recruits telomerase in vivo (reviewed in Hwang et al. (2012)). Furthermore, depletion of TPP1 by shRNA resulted in inappropriate telomere elongation (Liu et al., 2004) which is a phenotype shown when POT1 is diminished. TPP1 is also required to localize POT1 to the telomeres due to the lack of a NLS in POT1 (reviewed in Stewart et al. (2012)). The interactions between TIN2-TPP1 and TPP1-POT1 occur in both the cytoplasm and the nucleus, contrasting to TIN2-TRF2 interaction (that exclusively takes place in the nucleus), what suggests that there should be a nuclear export signal (NES) in TPP1 that has been localized next to the POT1-recruitment domain on TPP1 (Xin et al., 2008).
- POT1 (*Protection of telomeres protein 1*) (<sup>#</sup>Q9NUX5): was identified by Baumann & Cech (2001) due to its homology with the DNA-binding domain of TEBPα, a ciliate telomere protein. The two OB-fold domains in POT1 allow it to specifically bind the ssDNA overhang, preventing

inappropriate ATM activation (reviewed in Palm & de Lange (2008)). It was suggested that POT1 binds to G4 in a sequential manner due to the two OB folds and that two POT1 monomers are needed to unfold the G4 (reviewed in Hwang *et al.* (2012)); furthermore, the POT1-TPP1 complex shows a highly dynamic sliding movement on the telomeric overhang, inducing continuous unfolding and refolding of the G4 (Hwang *et al.*, 2012) and determining telomere length by competing with telomerase for access to the G-overhang (reviewed in O'Sullivan & Karlseder (2010)).



Figure 1.7: Shelterin proteins and interactions. A shows the secondary structure of the six shelterin proteins indicating the domains in different colours. The TRFH homology domain acts as a dimerization domain in both TRF1 and TRF2. The SANT/Myb DNA-binding domain in C-terminal of TRF1 and TRF2, but not in Rap1, binds to double stranded DNA (dsDNA). The D/E rich domain (acidic aminoacids) in TRF1 is a binding site for tankyrase 1 and 2. The GAR domain (Gly/Arg-rich) is a basic domain with a sequence-independent affinity for DNA junctions, including Holliday junctions. The FxLxP motif in TIN2 binds to the TRFH domain of TRF1 and the N-terminal of TRI2 binds not only to TRF2 but also to TPP1 (Palm & de Lange, 2008). The BRCT domain in the N-terminal of Rap1 is a central Myb-type helix-turn-helix motif and the acidic C-terminal binds to TRF2 (reviewed in Palm & de Lange (2008)). Dotted coloured lines indicate protein-protein interaction colour-codded based on the domains involved in it. Dashed black lines indicate interactions of some of these domains with either DNA or telomerase (adapted from figure 2 in Palm & de Lange (2008)). B 3D representation (not based on crystalographyc structure) of the shelterin complex at telomeres (adapted from figure 2 in de Lange (2005)) and figure 1 in Stewart *et al.* (2012)).

#### 1.2.1.2 Other telomere-associated proteins

There are lots of proteins with a function at telomeres and most of them do not form part of the shelterin complex. These non-shelterin factors are typically much less abundant at telomeres than the shelterin proteins and some are only transiently associated, whereas the shelterin complex is present at telomeres throughout the cell cycle (reviewed in de Lange (2005)). Besides, most of these non-shelterin proteins have non-telomeric functions, mainly related with DNA repair (Ku70/80, XPF/ERCC1, Apollo, the MRN complex, RAD51D, tankyrases 1 and 2), DNA damage signaling (the MRN complex), DNA replication (ORC, RecQ helicases) or chromatin structure (HP1 proteins) (reviewed in Palm & de Lange (2008)). The interactions between all those proteins and the shelterin complex are very complicated and an example of the main protein-protein interactions on telomeres is shown on figure 1 in Xin *et al.* (2008) showing the telomere interactome. Here, I will enumerate the main proteins and their main interaction and function at telomeres:

- **Tankyrases** (TRF1-interacting, ankyrin-related ADP-ribose polymerase): Smith *et al.* (1998) discovered tankyrase 1 and 2 in a yeast two-hybrid screen with TRF1 and showed that their central domain (ankyrin) interacted with the acidic domain of TRF1 but not with TRF2 because the homologous domain is basic. In addition, its function *in vitro* was described as a negative regulator of TRF1 because ADP-ribosylation inhibited the ability of TRF1 to bind to telomeric DNA. Later, *in vivo* experiments demonstrated that tankyrase 1 is highly expressed in telomerasenegative cells such as WI38 and that overexpression of this protein in the nucleus of telomerase-positive cells induced loss of TRF1 and promoted telomere lengthening (Cook *et al.*, 2002).
- WRN and BLM: those are RecQ helicases responsible for Werner syndrome and Bloom syndrome respectively, when mutated. Both were demonstrated to bind to TRF2 by ChIP analysis (Opresko *et al.*, 2002) and although the function for BLM on telomeres is still unknown, WRN is likely to resolve G4 (reviewed in de Lange (2005)).

- MRN Complex: Mre11, Rad50 and Nbs1 form this complex that recognizes and accumulates in DNA DSB sites having multiple functions for maintenance of the genome integrity. Its presence at telomeres increases when they are deprotected and its role is usually related to HR and NHEJ (reviewed in de Lange (2005)).
- **ERCC1/XPF:** is a complex that functions as a structure-specific endonuclease that cuts DNA duplexes adjacent to a 3' ssDNA. Mutations in those genes have been found in mice but not in humans and ERCC1/XPF-deficient cells develop a novel telomere phenotype called telomeric DNA-containing double minute chromosomes (for a review see de Lange (2005)). It was shown that this complex associates with TRF2, is present at telomeres, and is involved in overhang processing (Zhu *et al.*, 2003).

	Proteins	Main functions on telomeres	Main Interactions	
	TRF1	Negative regulation of TL	dsDNA, TRF2, TIN2, ATM, BLM, PINX1, Tankyrase	
	TRF2	Promotes T-loop formation Prevents recognition of dsDNA breaks Inhibits DSB repair	dsDNA, TRF1, TIN2, ATM/ATR, Rap1, WRN, PARP1, Apollo Nbs1/Mre11/Rad50, ERCC1/XPF	
	TIN2	Bridge between dsDNA and ssDNA binding proteins	TRF1, TRF2 and TPP1	
Shelterin complex	Rap1	Improves affinity and selectivity of TRF2 for dsDNA	TRF2	
1	TPP1	Increases POT1 affinity for ssDNA Regulates TL by recruiting telomerase	POT1 and telomerase	
	POT1	Prevents ssDNA degradation Maintains G4 Regulates TL by competing with telomerase	ssDNA, TPP1	
	Tankyrases	Regulates TL through inhibition of TRF1	TRF1	
Non- shelterin proteins	WRN	Resolves G4	TRF2, BLM, p53, Ku70/80, PCNA	
	BLM	Unknown function	TRF2, WRN, ATM, Rad51, MLH1	
	Mre11, Rad50, Nbs1	DNA damage sensor	TRF2, ATM	
	ERCC1/XPF	Overhang processing	TRF2	

Table 1.3: **Summary of the main proteins at human telomeres.** Proteins belonging to the shelterin complex are shown at the top and non-shelterin proteins at the bottom. The main telomeric function and interactions are shown. Abbreviations are: TL: telomere length; dsDNA: double-stranded DNA; ssDNA: single-stranded DNA; G4: G-quadruplexes. For more information about the proteins refer to section 1.2.1.1 for shelterins and 1.2.1.2 for non-shelterins.

#### 1.2.1.3 Telomere length measurement

The knowledge of telomere dynamics mainly arises as a result of telomere length (TL) measurements under different conditions. It was shown that TL varies depending on the DNA extraction method (Cunningham *et al.*, 2013), the method used to measure TL and even between laboratories (Martin-Ruiz *et al.*, 2015). Therefore, it is important to explain here the main available and well established methods for TL measurement including their advantages and disadvantages (see table 1.4 for a summary).

Terminal Restriction Fragments (TRF) was the first technique used to measure telomere length (Harley et al., 1990). In this method, genomic DNA is digested by frequent cutter restriction enzymes that do not recognize telomeric and subtelomeric sequences. Then, the undigested subtelomeric and telomeric regions are resolved by size in an agarose gel, Southern blotted and hybridized. TL is measured by the labeled DNA smear that can be sized using a DNA ladder comparison; although extensively used, considerations such as probe intensity (greater for longer sequences) have to be taken into account before measurement. The next method to size TL (qPCR) was developed by Cawthon (2002) and lately modified for multiplexing purposes (Cawthon, 2009). This PCR method measures TL as a ratio between the telomere (T) and a single copy gene (S) amplification. More recently, an adaptation of the original qPCR method has been described (SCT-pqPCR) to measure TL in single cells after a pre-amplification step for T and S template. Baird et al. (2003) developed single telomere length analysis (STELA), a PCR based method that uses a subtelomeric-specific primer and a linker-specific primer that is ligated to the 5' of the telomere using the 3' G-overhang as a template. In a small-pool PCR, the subtelomeric primer and a reverse primer sharing the 5' sequence with the linker-primer amplify single telomeres (as shown in figure 2.2 in chapter 2) at sub-visible level. Southern blot and hybridization with a labeled-telomere probe is needed before individual telomeres are sized by a DNA size ladder comparison (a more detailed description of the method is shown in chapter 2 section 2.2.2.8). Finally, two methods based on fluorescence in situ hybridisation (FISH) are available: Q-FISH (Lansdorp et al.,

1996) and Flow-FISH (Rufer *et al.*, 1998) in which a fluorescently labeled-PNA probe is hybridised to the telomeres and detected and quantified by software. In both cases, viable cells are needed but while in Q-FISH cell spreads (metaphase or interphase) are made in slides and detection is performed using a microscope, in Flow-FISH, labeled-cells are separated and analysed using flow cytometry (Baerlocher *et al.*, 2006).

Method	Measures	Advantages	Disadvantages
TRF Kimura <i>et al.</i> (2010)	Average TL	Well established method Mean TL for total cell population Not specialized equipment re- quired Optional overhang measurement	Large amounts of DNA (0.5-5µg) Time-consuming (3-5 days) 1kb resolution (overestimation of TL) TVR sequence and length might affect DNA digestion
qPCR Cawthon (2002) MMQ-PCR Cawthon (2009)	Average TL	Adapted for high throughput Small amount of DNA (20ng)	TL referenced to standard single copy gene Reference standards lacking Need for replicate testing and standard samples Should be used only for diploid and karyotypic stable cells
SCT-pqPCR Wang et al. (2013)	Single-cell average TL	High throughput Small amount of DNA Compares TL between single cells Lower CV than qPCR	Based on qPCR
STELA Baird <i>et al.</i> (2003)	Chromosome- specific TL	Measures single telomeres Resolution 0.1 kb High sensitivity Small amounts of DNA (250 pg) Allows to size critically short telo- meres	Time-consuming (3-5 days) Need of unique primers for each telomere Lack of primers for all ends Underestimation of TL due to poor detection of long telomeres
Q-FISH Lansdorp <i>et al.</i> (1996)	Average TL and chromosome- specific (metaphase)	Detects single telomeres Low cell numbers Strong signal and easily quantifi- able Allows to see telomere fusion events and telomere-free ends	For chromosome spreads cell via- bility is needed TL cannot be measured in senes- cent cells or slow dividing cells High intra-assay variation Requires digital microscopy
Flow-FISH Rufer <i>et al.</i> (1998)	Average TL	Adapted for high throughput Allows to compare between cell subpopulations First TL method validated for clinical purposes	Limited to blood samples Calibration needed to measure very small telomeres (weak fluo- rescence)

Table 1.4: **Summary of the main methods to measure telomere length.** Table adapted from table 1 in Aubert *et al.* (2012b), table 1 in Ozturk *et al.* (2014) and tables 1 and 2 in Montpetit *et al.* (2015). Abbreviations are: TRF: terminal restriction fragments; TL: telomere length; TVR: telomere variant repeat; qPCR: quantitative PCR; MMQ-PCR: monochrome multiples qPCR; SCT-pqPCR: single-cell telomere length qPCR; CV: coeficient of variation; STELA: single telomere length analysis; Q-FISH: quantitative fluorescence *in situ* hybridisation.

# **1.2.2 Regulation of telomere length**

#### **1.2.2.1** Telomere replication and processing

Replication of telomeric DNA is a multi-step process that involves not only the passage of a replication fork along the DNA duplex but also processing of the DNA terminus to generate the 3' overhang (Stewart et al., 2012). The formation of the 3' overhang is important due to its two main functions: a) serving as a template for telomere elongation by telomerase (see section 1.2.2.3 and b) allowing the formation of T-loop as a protective structure against telomere degradation (see section 1.2.1) (reviewed in Bonetti et al. (2013)). Replication of human telomeres, like other regions in the human genome, occurs in a semiconservative way and due to the end replication problem, the lagging strand automatically loses a few nucleotides, generating a small 3' overhang. However, 3' overhangs are observed at both, lagging and leading strands, suggesting that a more complex, other than replication, process controls its formation (reviewed in Arnoult & Karlseder (2015)). In fact, Chow et al. (2012) demonstrated that generation of the overhangs occurs in a different manner (timely and mechanistically wise) for lagging and leading strands: while the former has mature 3' overhangs rapidly after replication, the latter needs a longer processing time that includes 5' C-strand resection.

It has been proposed that regulation of TL is controlled by a homeostatic pathway that is achieved through a negative feedback loop established by the shelterin complex ((Cong *et al.*, 2002) and (Bandaria *et al.*, 2016)). In long telomeres, telomerase (see section 1.2.2.3) might be inhibited because lots of shelterin complexes bind to telomeres whereas for short telomeres, there are only few shelterin complexes, what cannot inhibit telomerase (de Lange, 2005). All proteins forming the shelterin complex seem to be implicated in this negative regulation but POT1, which is the only able to bind ssDNA, is the one proposed to block telomerase through competing for the 3' overhang with telomerase.



Figure 1.8: **Telomere replication and processing.** A is a schematic drawing showing that telomeres (black) shorten with every cell division. B shows the three main mechanisms involved in telomere shortening. Purple is the G-rich, leading strand and green, the C-rich lagging strand. Red zigzag line indicates RNA primer for DNA replication. Dashed line indicates the newly replicated strands. End processing nucleases are shown in orange.

## 1.2.2.2 Telomere shortening and ageing

Telomere length can be maintained by the telomerase enzyme that is active in germline, immortal cells and some stages during early embryogenesis, between the morula and blastocyst stages (see section 1.2.2.3). However, in most tissues telomeres shorten with every cell division (Harley *et al.*, 1990) and consequently, during human life. Hence, telomeres have been proposed to be a molecular clock that controls ageing (Bodnar *et al.*, 1998). The natural state of cell proliferation arrest due to accumulation of DNA damage response (DDR) signals (Fumagalli *et al.*, 2014) including short or damaged telomeres, is called senescence and it is a protective mechanism preventing the accumulation of DNA lesions and hence considered as a tumour suppressor mechanism (reviewed in Suram & Herbig (2014) and Muñoz-Espín & Serrano (2014)). Interestingly, telomeres have been found shorter but less variable in length in healthy older than younger people (Halaschek-Wiener *et al.*, 2008), suggesting that disease resistance and healthy ageing might be related with an optimal telomere length maintenance.

Analysing single cells in *S. cerevisiae*, Xu *et al.* (2015) showed that there are two very different types of lineages leading to senescence; on the one hand, the commonly called "senescent cells" (type A cells) that suffer a unique severe event triggering cell proliferation arrest and on the other hand, the "cryptic cells" (type B cells) that suffer some reversible cell cycle arrest earlier in time before senescence takes place. Those two types of lineages correlate with the two distinct types of senescence that can be distinguished in human cells (Nelson *et al.*, 2014):

Replicative Senescence (RS) is the consequence of several consecutive rounds of cell divisions and is linked to the "Hayflick limit", telomere shortening being the main trigger (this would be equivalent to type A cells in *S. cerevisiae*), although many other factors have also been described (reviewed in Zeman & Cimprich (2014)). Interestingly, protection of telomeres and not necessarily their relative length has been described to be involved in RS, considering that over expression of TRF2 prevented end-to-end fusions of critically short telomeres thus, delaying RS (Karlseder *et al.*, 2002). In addition, as telomere length at senescence can vary between cell types (von Zglinicki *et al.*, 2000a) it has been proposed that RS is directly related with age more than with telomere length itself (Serra & von Zglinicki, 2002). However, telomere shortening rates with age have been shown to vary between different primary cell types, human populations and age ranges (see table 1.5). In addition, cells with mutant p53 can escape RS and will undergo crisis triggered by telomere fusions produced as a consequence of the uninterrupted telomere short-ening (Hayashi *et al.*, 2015).

Induced Senescence (IS) or premature senescence on the contrary, is not necessarily linked to cell divisions since it has been shown that primary fibroblasts can undergo senescence after a long period of quiescence (Marthandan et al., 2014). IS can be initiated either by DNA damage or by oncogene activation such as Ras, Raf or MEK resulting in a cancer-preventing mechanism that can be reverted. Wei et al. (2001) demonstrated that in normal human fibroblasts, the growth arrest that precludes senescence occurs in the p53-p21-RB pathway at the level of p53. Premature senescence is characterized by the formation of senescence-associated heterochromatin foci ((Schulz & Tyler, 2005) and (Zhang et al., 2007)) that seems to be absent in RS (Kosar et al., 2011), as well as the presence of different protein expression profiles compared to RS. The higher expression of  $\beta$  galactosidase activity is shared for both RS and IS (Gary & Kindell, 2005), making it a standard assay for detecting senescence although new membrane markers such as DEP1 or BM2G have recently being proposed as another suitable detection method in aged cells (Althubiti et al., 2014).

	Method	Cohort size [P] (T)	Type of study	Age range	$\mathbf{Age} \pm \mathbf{SD}$	Telomere shortening rate $\pm$ error (bp/year)
(	TKF.	47 (blood, PBL)	cross-sectional	20-85	52.5	33
2)	$\mathbf{TRF}$	31 (skin and fibroblasts)	cross-sectional	0-93	46.5	$15\pm 6$ (donor age)
	$\operatorname{TRF}$	119 (blood, PBL)	cross-sectional	0-107	53.5	$40\pm3.6$ (women) and $50\pm4.2$ (men)
94)	TRF	123 [MZ and DZ] (blood, PBMC)	cross-sectional	2-95	48.5	31.0
		30 (blood, PBMC)	cross-sectional	4-39	21.5	84
	TKF	50 (blood, PBMC)	cross-sectional	40-95	67.5	41
	TRF	85 (blood, PBL)	cross-sectional	0-82	41	$12 \pm 1.2$
6)	TRF	26 (blood, PBMC)	cross-sectional	26-104	65	30
			cross-sectional	06-0	45	39 (granulocytes) and 59 (lymphocytes)
	Flow		bisegmented	0-1	0.5	3052 (granulocytes) and 1088 (lymphocytes)
	FISH	>əuu [36 MID-DZ] (D1000, FBL) -	subset	0-4	2	483 (granulocytes) and 457(lymphocytes)
			subset	4-90	47	36 (granulocytes) and 52 (lymphocytes)
			cross-sectional	29-97	62	44 (normal mucosa)
())(	TKF	124 (normal + CKC tissue)	cross-sectional	29-97	62	50 (cancer tissue)
2000b)	TRF	37 (blood, PBMC)	cross-sectional	18-98	58	20
	трг	9 (blood, PBL)	more contional	73-95	84	50
6	.1111	7 (skin)	INTIMPAG-SCO IN	73-91	82	78
	TRF	193 (blood, WBC)	cross-sectional		$56{\pm}11$	36 (women) and 38 (men)
	TRF	191 (PM liver) 137 (PM renal cortex)	cross-sectional	0-104	52	60 46
	Flow- FISH	51 (blood, PBL)	cross-sectional	0-92	49.0	$15\pm3$ (T cells) and $33\pm3$ (B cells)
02)	TRF	52 (PM epidermis) 48 (PM lingual epithelium)	cross-sectional	0-101	50.5	$37.5 \pm 1.5$ $31 \pm 1$
	qPCR +TRF	143 (blood, PBMC)	cross-sectional	60-97	78.5	14
	$\operatorname{TRF}$	327 [P/C pairs] (blood, WBC)	cross-sectional			19 (women) and 24 (men)
	TRF	70 [50 whites, 20 blacks] (blood, WBC)	longitudinal	21-44	32.5	31.3 (tel.attrition in 64; tel. gain in 5; no change in 1)
005)	qPCR	598 (blood, PBMC)	longitudinal	85-101	93	$5\pm13$ (no significant)
(	qPCR	132 [P/C pairs] (blood, PBMC)	cross-sectional	32-86	59	16 (women) and 25 (men)
			cross-sectional	30-80	55	31 (women) and 14 (men)
	TRF	125 (blood, PBMC)	subset	30-49	39.5	51.3
		I	subset	50-80	65	19.8
	TRF	1122 [women] (blood,WBC)	cross-sectional	18-76	$47.8\pm12.1$	27
		1517 [women TwinsUK] (blood	cross-sectional	18-79	$48\pm12.7$	$20.5\pm1.1$
	$\mathbf{TRF}$		subset	18-50	34	26
		WBC)	subset	51-79	65	25
	TRF	1552 [women TwinsUK] (blood, WBC)	cross-sectional	18-75	46.5	$19.8 \pm 1.7$
		(0 <b>7</b>				

Author	Method	Cohort size [P] (T)	Type of study	Age range	$\mathbf{Age} \pm \mathbf{SD}$	Telomere shortening rate $\pm$ error (bp/year)
Do Morrow of al (9007)	твр	2433 [Asklepios study popula-	moce-contional	37_75	16 9 F E Q	90.7 (momon) and 31.0 (mon)
The threader of me. (2001)	. 191 1	tion] (bood, PBMC)	101101101101101	00-00	0.0 T 7.0F	
Nicion at al (2007)	qPCR	907 [Amish families; P/C pairs]	anore contionel	18 09	ц ц	
Majou et ut. (2001)	+TRF	(blood, PBL)	CI NSS-SECTIONIAL	76-0T	00	40
Steer et al. (2007)	TRF	1151 (blood, WBC)	cross-sectional		48.2	38
Bataille et al. (2007)	TRF	1897 (blood, PBL)	cross-sectional	18-79	46.0	$27 \pm 1.5$
		1696 [Twin Res.Unit, St.			0 0 1	
Barwell <i>et al.</i> (2007)	TRF	Thomas' Hospital] (blood, WBC)	cross-sectional	45-17-d	56.3	22
Cherkas <i>et al.</i> (2008)	$\operatorname{TRF}$	2401 [Twins UK] (blood, PBL)	cross-sectional	18-81	$48.8\pm12.9$	$21 \pm 1.3$
Richards et al. (2008)	TRF	1319 [Twins UK, 92% women]	cross-sectional	1	$40.9\pm12.5$	18.5
		(blood, PBL)				
$H_{1111} \rightarrow \alpha I (9008)$	TRF +	1395 [NHLBI and BHS cohorts]	mose-sectional	19-03	дe	90 (whites) and 90 (hlacks)
	qPCR	(blood, PBL)		00-0T	00	20 (WITTGES) and 20 (DIARNE)
Aviv et al. (2009)	TRF	635 [BHS; 450 whites and 185	longitudinal	20-40(+6)	31.4	37.8 (whites) and 40.7 (blacks)
		black] (blood, PBL)	0			(tel. attrition in 561; tel. gain in 68; no change in 6)
			longitudinal	45-85 (+10)	58	45.5 (tel. attrition in 84%; tel. gain in 15.9%)
Ehrlenbach <i>et al.</i>	aPCR +	510 [Bruneck Study]	$\mathbf{subset}$	< 51		39
	-		subset	52-58	55	32
(2009)	TKF.	(blood, PMBC)	subset	59-66	62.5	48
			$\mathbf{subset}$	> 67		28
Epel <i>et al.</i> (2009)	qPCR	236 [MacArthur Health Aging Study] (blood, PBL)	longitudinal	70-79 (+2.5)	73.7	Tel. attrition in $30\%$ ; tel. gain in $24\%$ ; no change in $46\%$
Farzaneh-Far <i>et al.</i> (2010)	qPCR +TRF	608 (blood, PBL)	longitudinal	1	$65\pm11$	42 (tel. attrition in 276; tel. gain in 140; no change in 192)
Houben <i>et al.</i> (2011)	qPCR	75 [Zutphen Elderly Study] (blood, WBC)	longitudinal	73-91 (+7)	78-83	40.2 (tel. attrition in 84%; tel. gain in 16%)
				S1:19.9-41.5	30.7	24.6
			cross-sectional	S2:25.7-46.3	36	25.4
$O_{12} = \frac{1}{2} (9011)$	TDF			S3:31.5-49.9	40.7	23.6
Chen et al. (2011)	TUL	ZII [DH2] (D1000, FDL)		S1-S2 (+5.8)	I	31.4 (tel. attrition in 86%; tel. gain in 14%)
			longitudinal	S2-S3 (+6.6)		33.5 (tel. attrition in 86%; tel. gain in 14%)
				S1-S3 (+12.4)		32.2 (tel. attrition in 98.5%; tel. gain in 1.5%)
Svenson et al. (2011)	STELA	50 (blood,PBL)	longitudinal			tel. attrition in 50%; tel. gain in 50%
Fitzpatrick et al. (2011)	TRF	1136 [CHS] (blood, PBL)	longitudinal	>65	1	26
Daniali et al. (2013)	TRF	87 (blood PBL, muscle, skin, fat)	cross-sectional	19-77	44	26 (leukocytes), 24 (muscle), 23 (skin) and 25 (fat)

1.2. TELOMERES

## CHAPTER 1. INTRODUCTION

Author	Method	Cohort size [P] (T)	Type of study	Age range	$\mathbf{Age} \pm \mathbf{SD}$	Telomere shortening rate $\pm$ error (bp/year)
(1001 of 21 0010)	מטמ״	105 (human fetal tissues) and 13	lonoto contono	11 CW	0 E CW	Cham chontoning her CW 7 hert soughted TT After CW
(et nz) .im ia Ritatio	dr cn	(cord blood cells)	ci oss-secuoiiai	MD 11-0	0.0 GW	Sharp shortenning by GW / Dut constant 11 alver GW
Steenstrun et al		476 [LSADT twins] (blood, PBL)	cross-sectional	73-94	83.5	29 (women) and 18 (men)
	$\mathbf{TRF}$	80 IT SA DW truit of Alaced DBL)	امتناب مناب الم	(01.) 10 67		34 (women) and 24 (men)
(20102)		ou [LOAD1 [WIIIS] (BIOUU, F DL)	Iongruaniai	(01+) 10-01		tel. attrition in 92.5% ; tel. gain in 7.5%
Hidmhans at al (9015)	ШБ	889 [GEMINAKAR Study and	anoto continuol	10 64	1 F	00.00
(etoz) <i>'m 1a</i> gloonnafu	INF	TwinsUK] (blood, PBL)	CL 0SS-Sectional	+0-61	41.0	07-77
	-		» E	L "	LT	

Abbreviations are: **TRF**: Terminal Restriction Fragments; **FISH**: fluorescent *In Situ* Hybridisation; **PBL**: Peripheral Blood Leukocytes; **MZ**: Monozygotic Twins; **DZ**: Dizygotic Twins; **PBMC**: Peripheral Blood Mononucleated Cells; **CRC**: colorectal; **PM**: Post Mortem; **WBC**: White Blood Cells; **P/C**: Parent/Children; **NHLBI**: National Heart, Lung and Blood Institute; **BHS**: Bogalusa Heart Study; **STELA**: Single Telomere Length Analysis; **CHS**: Cardiovascular Health Study; **GW**: Gestational Week; **LSADT**: Longitudinal Study of Aging Danish Twins.

#### 1.2.2.3 Telomere elongation

Very short telomeres have been proposed to activate TERRA (telomeric repeat containing RNA) transcription thus promoting recruitment and nucleation of telomerase (Cusanelli *et al.*, 2013). Telomerase is a eukaryotic ribonucleoprotein complex that adds TTAGGG repeats to the human telomeres by using an RNA template ((Greider & Blackburn, 1985) and reviewed in Blackburn *et al.* (1989)) and it is mainly active in germline and immortal cells.

The two main components of the catalytic core of telomerase are: TERT (also called TRT or Est2), that has homology with the catalytic motifs of the reverse transcriptase family and which main function on telomeres is to synthesise telomere DNA repeats, and TERC (also called TR or TLC1), that constitutes an RNA template of  $\approx 450$  bp with sequence CUAACCCUAAC (reviewed in Greider (1996)). See figure 1.9 for further details on telomerase structure. In addition to its telomeric lengthening function, telomerase also protects against double strand breaks in a telomere synthesis independent manner (Fleisig *et al.*, 2016), and has other non-canonical functions reviewed in Low & Tergaonkar (2013).

As a mechanism compensating for the DNA erosion during replication, after C-strand resection, telomere elongation by telomerase produces extension of the 3' overhang and later, the C-strand can be filled in by  $Pol\alpha$ -primase (reviewed in Stewart *et al.* (2012), Nandakumar & Cech (2013) and Bonetti *et al.* (2013)). Another non-telomerase-based mechanism for telomere maintenance called ALT (alternative lengthening of telomeres) has been described (Bryan *et al.*, 1997) and is almost exclusively found in some tumours such as soft tissue sarcomas. Its mechanism involves recombination using sister chromatids as template and it has been detected in anomalous situations such as human cancer cells (10-15%) or telomerase-null mouse cell lines. Nevertheless, some evidence suggest that this mechanism might also occur in normal cells under certain situations (reviewed in de Lange (2005) and Podlevsky & Chen (2012)).



Figure 1.9: Structure of telomerase. Adapted from Kim et al. (2008) and Podlevsky & Chen (2012). A represents TERT motifs and domains. TEN domain: is a basic amino-terminal domain containing DNA-binding domains in a single-stranded way, it is involved in TERT localization to the nucleolus and it contains RNA interacting domain 1 (RID1) with affinity by the pseudoknot domain in TERC and a DAT motif implicated in telomerase recruitment. TRBD domain: is implicated in RNA recognition and binding (Rouda & Skordalakes, 2007) and the interaction between TRBD and TERC through the CR4/5 domain is required for the proper assembly and enzymatic activity of the holoenzyme, both in vitro and in vivo. RT domain: is the reverse transcriptase domain containing seven conserved motifs (1,2, A, B', C, D, E), which tertiary structure remains a right hand, with two domains (1 and 2) binding to the incoming nucleotides and five (A, B', C, D and E) constituting the catalytic site (reviewed in de Lange (2005)). CTE domain: shares functionality with the HIV RT C-terminus binding to the RNA template/DNA primer duplex. Mutations in this domain affect telomerase processivity and a DAT domain has also been identified, suggesting that this region may also be involved in telomerase recruitment to the telomere. B represents TERC motifs and domains. Template/pseudoknot: contains a triple helix and a template sequence that can be divided into a 5' region encoding for telomeric DNA repeats and a 3' region annealing to the DNA primer after template translocation (reviewed in Podlevsky & Chen (2012)). CR4/5: is the other domain required for enzymatic activity. It is distal to the pseudoknot domain and composed of a three-way junction of helices. H/ACA: is located at the 3' end of TERC and contains two stem-loops separated by the box H/ACA moieties acting as binding sites for dyskerin, NOP10, NHP2 and GAR1. There is another domain, located in the 3' stem-loop, called Cajal body localization (CAB box) that serves as a signal for TERC localization in Cajal bodies, where the RNA component of the telomerase is packaged (reviewed in O'Sullivan & Karlseder (2010)). The H/ACA domain is essential for TERC biogenesis (reviewed in Podlevsky & Chen (2012)).

## 1.2.2.3.1 Telomerase inhibitors as therapeutic agents

Telomerase is active in 80-90% of human cancers ((Kim *et al.*, 1994) and (Shay & Bacchetti, 1997)), thus, it has been extensively studied as a therapeutic approach against cancer. Different targets have been proposed and table 1.6 summarises the most important ones (Phatak & Burger, 2007):

	Inhibitor	Description and effects	
	PNAs: Peptide Nucleic Acids (Fleisig et al., 2016)	First telomerase inhibitors used Trigger telomere loss Very high cytotoxic effects The drug GRN163 has been used to reduce tumour volume in xenograft mice and later in phase II clinical trials	
hTERC inhibition	As-ODNs: Antisense oligo- deoxynucleotides Jiang et al. (2003)	Short ssDNA sequences complementary to TERC Initiate antiviral pathway mediated by RNase-L, breaking down TERC Telomere loss has not being studied High cytotoxic effects	
	GDEPT: Gene-directed enzyme prodrug therapy Plumb et al. (2001)	TERC promoter activity is 300-fold higher in cancer cells than in normal cells Combination of the bacterial NTR gene (under TERC pro- moter) and the pro-drug CB1954 sensitizes cancer cells	
	Nucleoside analogs (Olivero <i>et al.</i> , 1997)	AZT has being tested in cell lines showing inhibition of telo- merase, telomere shortening and cytotoxic effects High drug concentrations are required	
hTERT inhibition	BIBR1532 (Damm <i>et al.</i> , 2001)	High concentrations induce acute cytotoxic effecs The most specific synthetic hTERT inhibitor to date Produced telomere shortening in several cancer cell lines Requires lag-time (dependent on TL) before cell death or senescence	
	DN-hTERT (Dominant negative TERT) (Hahn <i>et al.</i> , 1999) and (Zhang <i>et al.</i> , 1999)	Is reversible Is non-cytotoxic Produces telomere shortening and proliferative defects ending in growth arrest Mechanism: cytoplasmic exportation of endogenous TERT and degradation via ubiquitination Might affect non-telomeric functions	
Telomere	G4 ligands (Hurley, 2002)	Examples: BRACO19 or telomestatin Produce disruption of the T-loop Effectively inhibit telomerase and POT1 binding Reduces telomere elongation Some cytotoxic effects are observed Used in xenograft mice to reduce tumour volume	
targeting agents (TTA)	T-oligos Wojdyla <i>et al</i> . (2014)	Homologous to the 3' overhang May mediate DDR via p53/p73 pathway Inhibit proliferation by inducing apoptosis and/or senescence No telomere length effect	

Table 1.6: **Main telomerase inhibitors.** Table adapted from tables 1 and 2 in Rankin *et al.* (2008) and data found in Phatak & Burger (2007). Abbreviations are: ssDNA: single stranded DNA; NTR: bacterial nitroreductase; AZT: azydothymidine; TL: telomere length. DDR: DNA damage response.

# **1.2.3** Telomere length inheritance

It has largely been discussed how telomeres are inherited since a high heritability (h<sup>2</sup>) index has been described (70-78%) based on twin studies ((Slagboom et al., 1994), (Graakjaer et al., 2004) and (Broer et al., 2013)) and on linkage disequilibrium (81.9  $\pm$  11.8%) for 12q telomere (Vasa-Nicotera *et al.*, 2005). However, other studies have suggested lower  $h^2$  values (36 and 44%) based on quantitative-trait linkage analysis (Njajou et al., 2007) and statistical analysis (Andrew et al., 2006). Moreover, the distinct contribution of the progenitors to their offspring's telomere length is still under discussion (Eisenberg, 2014), resulting in more studies suggesting that fathers have a stronger impact on their offspring's TL ((Nawrot et al., 2004), (Nordfjäll et al., 2005), (Unryn et al., 2005), (De Meyer et al., 2007), (Nordfjäll et al., 2010) and (Holohan et al., 2015)) than mothers (Broer et al., 2013). Those studies concluded that, as a positive correlation between sperm TL and donor-age has been found ranging from 71 bp/year (Allsopp et al., 1992) to 135 bp/year (Baird et al., 2006), older parents might transmit longer telomeres to their offspring ((Kimura et al., 2008) and (Eisenberg et al., 2012)). Nevertheless, other studies have suggested that this "lengthening" of sperm TL with age might be due to a decrease of the TL over generations, that therefore, distorts the data ((Holohan et al., 2015) and (Stindl, 2016)). It is important here to describe telomere dynamics in the germ line and during gametogenesis, to understand the parental contribution to their offspring's TL:

**Oogenesis:** Liu & Li (2010) and Ozturk *et al.* (2014) reviewed several studies that have analysed the presence of telomerase in the ovary. It has been described that telomerase is necessary to maintain TL in the ovarian surface epitelium (stem cells). Therefore, telomerase activity would contribute to folliculogenesis and ovulation as short telomeres in the ovary and lack of telomerase in granulosa cells was related to ovarian insufficiency and female infertility (Keefe & Liu, 2009). During oocyte maturation, telomerase activity is reduced and an important telomere shortening (from  $11.41 \pm 0.81$  kb to  $8.9 \pm 0.86$  kb) is observed despite

the absence of cell divisions (Turner & Hartshorne, 2013). Furthermore, telomerase is known to be activated by estrogen (either by a direct activation of *TERT* gene transcription or by an indirect mechanism involving *MYC*). Other factors, such as oxidative stress or accumulation of unrepaired DNA damage during meiotic arrest, might contribute to this telomere shortening observed in humans, as it has been demonstrated to occur in mice (Yamada-Fukunaga *et al.*, 2013).

Spermiogenesis: telomerase is mainly active in primary spermatocytes and it disappears in mature differentiated spermatozoa (reviewed in Ozturk (2015)) suggesting that mature sperm TL is defined very early during maturation. Furthermore, short telomeres in sperm have been related to male infertility (Ferlin *et al.*, 2013) although telomerase activity levels were not different between fertile and infertile testis (Fujisawa *et al.*, 1998), suggesting that its presence in infertile testis does not restore TL. Finally, it is also important to consider that the male pronuclei has shorter telomeres than the female one (Turner & Hartshorne, 2013), what might be related with the greater number of cell divisions occurring in the former compared to the later. Interestingly, Reig-Viader *et al.* (2014) found higher levels of TERRA colocalising with telomeres in spermatocytes I than in occytes I along all phases of gametogenesis, what suggests that TL in sperm cells might be related to the abundance of these transcripts at telomeres.

A minimum TL in the germline is likely to be needed to produce a competent embryo (reviewed in Kalmbach *et al.* (2014)) and a model is proposed in which after fertilisation, telomere lengthening occurs during the earliest pre-implantation development (morula-blastocyst), correlating with the high levels of telomerase activity detected in this stage ((Wright *et al.*, 1996) and (Wright *et al.*, 2001)). This telomere elongation was proposed to be needed to reach a set point after which the zygote genome can be activated (Schaetzlein *et al.*, 2004). Then, telomeres would be maintained (possibly due to ALT in addition to telomerase) and telomere shortening might start together with adult cell differentiation and the progressive loss of telomerase activity that was found to be different among distinct tissues (Wright *et al.*, 1996). In adulthood, telomere shortening rates have been described to be two fold higher in longitudinal versus cross-sectional studies (reviewed in Unryn *et al.* (2005) and see table 1.5 for more details) possibly due to changes in the TL over generations; however, Verhulst *et al.* (2013) suggested that baseline TL might have an impact overestimating shortening rates. To overcome this issue, although more expensive and time consuming, longitudinal studies should be preferred versus cross-sectional as they represent a direct observation of a biological process (during the follow-up time) that might be masked when performing cross-sectional analysis.

## **1.2.4** Telomeres and MSI: role in cancer

Development of human cancer has been associated with genetic instability and in the case of colorectal cancer (see section 1.3), at least two forms of this instability have been described (reviewed in Fodde *et al.* (2001b) and Centelles (2012)). On the one hand, microsatellite instability (MSI) caused by MMR defects (present in 10-15% of sporadic colorectal cancers (SCC) and  $\approx$ 90% of familial cancer), results in a mutator phenotype characterized by changes in the number of repetitive sequences of microsatellites (see figure 1.5). On the other hand, chromosomal instability (CIN) (in 65-70% of SCC) produces an accelerated rate of gains or losses of whole or large portions of chromosomes resulting in karyotypic variability from cell to cell and as a consequence, aneuplody, subchromosomal genomic amplifications and a high frequency of loss of heterozygosity (LOH) (Pino & Chung, 2010).

Telomere length and telomerase activity are not only implicated in the control of the proliferative capacity of normal cells but also in malignant progression. Takagi *et al.* (2000) showed that high MSI (MSI-H) colorectal tumours showed shortened telomeres suggesting that MSI may be associated with telomere shortening in colorectal carcinogenesis despite the presence of telomerase (Hiyama *et al.*, 1995). Furthermore, Pickett *et al.* (2004) and Mendez-Bermudez & Royle (2011) showed a higher telomere mutation frequency (defined by the appearance of mutations that changed the sequence of the assayable proximal variant repeat region of the telomere) in MMRdefective tumours (specially  $MSH2^{+/-}$ ) compared to MMR-proficient and hypothesised that a tendency to losses of repeats might be triggering an increase rate of telomere shortening, contributing to the profile of short telomeres in colorectal tumours. In addition, the telomeric varian repeat (CTAGGG)<sub>n</sub> was found to be associated with an exceptional mutation rate in the male germline that was only transmitted to the offspring via male but not female germline (Mendez-Bermudez et al., 2009). Cells with dysfunctional, very short uncapped telomeres, can a) enter replicative senescence as a tumour suppressing mechanism (reviewed in Martínez & Blasco (2011), Muraki et al. (2012) and Suram & Herbig (2014)) or b) continue dividing and escape cellular crisis by a p53 mutation and enter breakage-fusion-bridge cycles that can continue for multiple cell generations leading to a dramatic genome reorganization (Pino & Chung, 2010). Interestingly, MSH2 deficiency in  $Terc^{-/-}$  mice was shown to abolish the protecting mechanism of dysfunctional telomeres, thus increasing lifespam but without an increase in telomere recombination (Martinez et al., 2009). Besides, Jones *et al.* (2014) showed in an  $MLH1^{-/-}$  cancer cell line that ligase 3 (specially the BRCT domain) was required for cells with very short telomeres to escape cellular crisis.

#### 1.2.4.1 Cancer risk and genetic anticipation

Genetic anticipation is the phenomenon whereby the symptoms of a genetic disorder become apparent and gradually more severe at an earlier age on successive generations (see more information in the MedGen database). The mechanism for anticipation in many neurological disorders has been related with triplet repeat expansions (reviewed in Iyer *et al.* (2015)) suggesting that repetitive structures such as telomeres might as well being involved in the earlier age onset of certain types of tumours. Dyskeratosis congenita (DC), an inherited predisposition for a bone marrow failure that might result in malignancy, is caused by germline mutations in genes directly affecting telomerase (reviewed in  $Gu \ et \ al.$  (2009)); hence, it is not surprising that DC patients have very short telomeres and that genetic anticipation has been related with

progressive telomere shortening over the generations (Vulliamy et al., 2004). Interestingly, telomeres in DC patients were predicted to have a reduced telomere attrition rate with age than controls (cross-sectional study in Alter et al. (2007)) due to its very short length although, when some of those patients were followed up over time, telomere seemed to shorten at similar rate than controls for some and even to lengthen for others (Alter et al., 2012). Furthermore, despite shorter age-adjusted telomere lengths in aplastic anemia (a subtype of bone marrow failure) compared to controls, patients with longer telomeres responded better to immunosuppressive therapy (Brümmendorf et al., 2001). Progressive shortening of telomeres has also been correlated with disease progression in chronic myelogenous leukaemia (reviewed in Bronner et al. (1994). Martinez-Delgado et al. (2011) showed that telomeres shortened in following generations of hereditary breast cancer but later, Pooley et al. (2014) found no association between telomere length and cancer risk in BRCA mutation carriers, although telomeres were found longer in those compared to their mutation non-carrier relatives. However, there might still be a relationship between telomere stability and cancer risk in hereditary breast cancer as Benitez-Buelga et al. (2016) showed that a specific mutation in OGG1, that repairs oxidative damage and has been described to maintain telomere stability (Wang et al., 2010), was found to be related with higher cancer risk in combination with BRCA1 or BRCA2 mutations.

The existence of genetic anticipation in Lynch syndrome (see section 1.3) is still under debate, as contradictory data has been published (reviewed in Bozzao *et al.* (2011b)); furthermore, significant results in the presence (or absence) of anticipation in LS highly depend on the statistical method used (reviewed in Boonstra *et al.* (2010)). Recently, Valls-Bautista *et al.* (2015) showed that in colorectal cancer patients, telomere lengths were altered in blood and normal mucosa, and Qin *et al.* (2014) found a significant association between short telomeres and colorectal cancer risk (especially in younger than 60 years patients). Conversely, long telomeres were related with cancer risk in familial colorectal type X patients that fulfill the Amsterdam criteria (see section 1.3.1) but do not show MMR deficiency or MSI (Seguí *et al.*, 2014).

# 1.3 Colorectal cancer

Colorectal cancer (CRC) is the 4<sup>th</sup> most common cancer in the UK and the 3<sup>rd</sup> worldwide. A model for colorectal tumorigenesis was described by Fearon & Vogelstein (1990) and more updated reviews ((Rustgi, 2007), (Pino & Chung, 2010), (Kanthan et al., 2012) and (Centelles, 2012)) explain in detail the molecular mechanisms involved in colorectal carcinogenesis. Most of the CRC cases (95%) are sporadic while only a 5% are inherited predispositions (Lynch & de la Chapelle, 2003); of those sporadic CRC, the vast majority (60-80%) arise as a MMR defect caused by somatic mutations (usually, promoter hypermethylation of *MLH1*). In addition, approximately 4% of the inherited CRC predisposition corresponds to Lynch syndrome (see details below) that is characterised by an inherited germline MMR mutation. Ahmed et al. (2013) genetically characterised 24 CRC cell lines describing the great heterogeneity in mutations and epigenetic regulation that can be found in this type of tumours (reviewed in Kim & Kang (2014)). Furthemore, an extensive molecular description of the somatic mutations found in 224 CRC samples by next generation sequencing was published by Cancer Genome Atlas Network (2012) suggesting that the mutation rate might be a good prognostic signal as MSI tumours had a better survival. In addition to MSI, there are many other prognostic markers to study CRC (reviewed in Deschoolmeester et al. (2010)) and to identify the specific type.

# 1.3.1 Hereditary colon cancer

The hereditary predisposition for CRC is classified in: Lynch syndrome (LS) (previously known as hereditary non-polyposis colorectal cancer, HNPCC), accounting for  $\approx 3$  to 4% of CRC and familial adenomatous polyposis (FAP) that accounts for nearly 1% of the hereditary CRC (reviewed in Lynch & de la Chapelle (2003), Rustgi (2007) and Garre *et al.* (2014)).

Familial adenomatous polyposis cancer ( $\ddagger 175100$ ), is an autosomal inherited disorder caused by mutations in the tumour suppressor gene

*APC* and is characterised by adenomatous polyps in the colon and rectum. Fodde *et al.* (2001a) suggested that mutations in the C-terminus of *APC* produce the chromosomal instability that characterises non-LS colorectal tumours. In addition, three autosomal recessive variants also account for this type of inherited CRC predisposition: MYH-associated polyposis (MAP) associated to MUTYH gene mutations (#608456), Peutz-Jeghers syndrome (PJS) associated to STK11 gene mutations (#175200) and juvenile polyposis syndrome (JPS) associated to BMPRIA or SMAD4 gene mutations (#174900) (reviewed in (Centelles, 2012).

Lynch syndrome (*120435*), is an autosomal dominant inherited disorder caused by a germline mutation in an MMR gene. It is characterized by an early-onset of CRC, often with a bias for the right colon and the presence of other extra colonic tumours such as urologic, upper gastrointestinal or gynaecologic cancer. The clinical hallmarks of this inherited disease resulted in a classification scheme, first designated Amsterdam Criteria I and II and later as Bethesda criteria and Revised Bethesda guidelines (reviewed in (Rustgi, 2007) and (Serrano et al., 2012)) that classify potential families for further screening. Genetic linkage studies identified two loci reporting 90% of HNPCC (Fishel & Kolodner, 1995); one of them was described by Peltomäki et al. (1993) and mapped to 2p15-16 (corresponding to MSH2 gene) and accounts for 60% of LS; the other, was located in 3p21 (corresponding to MLH1 gene) and causing 30% of LS (for a review of MLH1 and MSH2 mutations refer to (Peltomäki & Vasen, 1997)). Human tumour cell lines have been identified having *MSH2* or *MLH1* mutations and presenting mutator phenotypes and microsatellite instability (MSI) ((Bronner et al., 1994) and (Fishel & Kolodner, 1995)). MLH6 and MLH3 germline mutations have also been found in LS patients although at lower frequencies than for MSH2 and MLH1 ((Kolodner et al., 1999) and (Wu et al., 2001)) and it was proposed that mutations in MSH6 predispose individuals to late onset familial colorectal carcinomas compared with the early onset familial colorectal carcinomas. Later, some MSH6 germline mutations were studied in pu-

tative HNPCC patients although the repair function was not affected by these mutations because they did not abolish MSH2-MSH6 interaction (Kariola et al., 2002). Very few mutations have been reported in other genes related to the MMR pathway such as *EXO1* (Jagmohan-Changur et al., 2003) or PMS2 (reviewed in Peltomäki (2016)) suggesting that if related to LS, they might be involved in low penetrance cancer susceptibility. In addition, germline mutations in genes not directly related with the MMR pathway such as *POLE* (DNA polymerase  $\epsilon$ ) have also been found to predispose to CRC (Elsayed et al., 2015). A genetic screen approach has been found useful for detecting the pathogenicity of mutations in MMR genes called VUS (variants of unclear significance), allowing to design diagnostic tools for LS patients (Drost et al., 2013). Nevertheless, inheriting a germline mutation in these genes or others related to the MMR pathway is not sufficient for tumour initiation, so heterozygosity in MMR genes is just a prerequisite for the development of cancer ((Hemminki et al., 1994) and (Chang et al., 2005)). The cytoplasmic location of the MSH2 protein in many HNPCC tumours, suggested that instead of an absence of the protein, the germline gene mutation produced an abnormal translocation of the protein to the nucleus (Fujiwara et al., 1998). Currently, it is accepted that LOH of tumour suppressor genes is one of the key steps to carcinogenesis in colorectal cancer (reviewed in Ozaslan & Aytekin (2009)) and the loss of one allele at a constitutionally heterozygous locus indicates the probability of loosing a tumour suppressor gene, what might promote neoplastic progression. In addition, it has been found that miR-155 overexpression can downregulate MLH1, MSH2 and MSH6 protein expression (reviewed in Yamamoto et al. (2012)) possibly contributing to LOH. Furthermore, telomerase activity in normal mucosa and lymphocytes in HNPCC patients has been found at higher levels compared to non-HNPCC (Cheng et al., 1998), suggesting that the MMR defect directly or indirectly might contribute to telomerase reactivation in normal tissues thus, increasing the cancer risk.

# **1.4 Hypothesis and main aims**

Taking into account the preliminary data discussed above, in particular that MSH2 deficiency increases the telomere mutation frequency (measured as changes in the length of the telomere variant repeats), we hypothesised that defective mismatch repair could produce a trend towards losses of repeats from telomeres, so contributing to an increase in the telomere shortening rate. If 50% reduction in mismatch repair protein expression is sufficient to limit repair of tandem repeats during replication then, Lynch syndrome individuals carrying an MSH2 germline mutation, may show an increase in the telomere shortening rate compared with healthy individuals. This might be an explanation for the proposed genetic anticipation in this disease. To test our hypothesis, the main aims of this Thesis were:

- 1. To down-regulate the MSH2 protein in human primary cells grown in  $5\% O_2$  and to compare the telomere shortening rates between clones with different MSH2 protein levels and control clones.
- To analyse mutant telomeres in a colon cancer cell line with mutated MSH2 gene to determine whether MSH2 deficiency results in a bias towards loss of telomeric repeats, contributing to telomere shortening.
- 3. To test a telomerase inhibitor in the same colon cancer cell line, with mutated *MSH2*, to understand the impact of DNA mismatch repair deficiency on telomere length maintenance.
- 4. To compare telomere shortening with age in a cohort of healthy and Lynch syndrome individuals and to determine whether the inheritance of telomere length is affected in Lynch syndrome families.

# 2 Materials and methods

# 2.1 Materials

- Antibodies: Mouse monoclonal (clone 3A2B8C) anti-MSH2 IgG (Abcam, UK); mouse monoclonal (clone 6C5) anti-GAPDH (Life Technologies, UK); sheep horseradish peroxidase (HRP) conjugated secondary anti-mouse (GE healthcare, UK).
- **Cells:** Table 2.1 shows general information for the cell lines used in this Thesis.

	SW-480	LoVo	WI38
Organism	Homo sapiens	Homo sapiens	Homo sapiens
Tissue	Colon	Colon	Lung
Morphology	Epithelial	Epithelial	Fibroblast
Properties	Adherent	Adherent	Adherent
Donor age	50 years	56 years	3 GM fetus
Ethnicity	Caucasian	Caucasian	Caucasian
Sex	Male	Male	Female
Disease	Duke's type B, colorectal adenocarcinoma	Duke's type C, grade IV, colorectal adenocarcinoma	Normal
MMR	proficient	msh2 -	proficient
TSG	apc-	_	_
Oncogenes	myc+, myb+, ras+, fos+, p53+, sis+, abl-, ros-, src-	myc+, myb+, ras+, fos+, p53+, sis-, abl-, ros-, src-,	none
Reference	(Witty <i>et al.</i> , 1994)	(Umar <i>et al.</i> , 1994)	(Hayflick & Moor- head, 1961)

Table 2.1: **Cell lines information.** Data acquired and adjusted from the American Type Culture Collection (ATCC) website. "GM" gestation months; "TSG" tumour suppressor gene.

- **Cell staining:** β-galactosidase staining kit (Cell signalling, UK); Propidium iodide (Sigma-Aldrich, UK); DAPI (Sigma-Aldrich, UK) and Trypan blue solution, 0.4% (Life Technologies, UK).
- Chemicals and others: Deionized formamide (purity > 99.5%) (Merck, UK); AccuGel (40% Acrylamide:Bis-Acrylamide, 19:1) (Fisher Scientific, UK); ProtoGel (30%) 37.5:1 acrylamide to bisacrylamide (Fisher Scientific, UK); Cloning discs (Sigma-Aldrich, UK); BLUeye prestained protein ladder (GeneFlow, UK); Hi-Di<sup>™</sup> formamide (Applied Biosystems, UK); MapMarker<sup>®</sup> X-Rhodamine labeled 1000 bp (ROX-1000XL) ladder (BioVentures, US); minimum essential medium (Thermo Scientific, UK); Dulbecco's modified Eagle medium (Thermo Scientific, UK); 100X non-essential amino acids (Thermo Scientific, UK); fetal bovine serum (Pan-Biotech, Germany); EDTA (Sigma-Aldrich, UK).
- **Drugs:** Puromycin dihydrocloride (Sigma, UK); ampicillin (Sigma, UK) and BIBR1532 -telomerase inhibitor- (Damm *et al.*, 2001) (Stratech Scientific, UK).
- Enzymes: All enzymes were bought from New England Bio labs (NEB, UK).
- **Hybridization:** MAGNA nylon transfer membrane, 0.45 microns (Fisher Scientific, US) for Southern and dot blots and Amersham Hybond ECL membrane (Fisher Scientific, UK) for western blots.
- Kits: E.Z.N.A.<sup>®</sup> Plasmid Maxi Kit (Omega, UK); MaXtract High Density (Qiagen, UK); Zymoclean<sup>™</sup> Gel DNA Recovery Kit (ZymoResearch, UK); Oragene<sup>™</sup> DNA saliva kit (Genotek, US); Enhanced chemiluminescence western blotting detection system (ECL prime) (GE healthcare, UK); TRAPeze<sup>®</sup> telomerase tetection kit (Millipore, UK); Ingenio<sup>®</sup> electroporation kit for Lonza-Amaxa<sup>®</sup> Nucleofector<sup>®</sup> II/2b devices (Mirus, UK); quick start<sup>™</sup> Bradford protein assay kit (BioRad, UK); Pellet Paint<sup>®</sup> (Millipore, UK); AmPure beads (Beckman Coulter, UK) and Dynabeads<sup>®</sup> His-Tag (Thermo Scientific, UK).

- Oligonucleotides and plasmids: all primers were ordered from Sigma, UK, except for NED labeled TagTelX primer, that was ordered from Thermo Scientific, UK. pSuperior.puro and pSuperior.puro.shMSH2 plasmids were ordered from Oligoengine, US.
- Saliva samples: Ethics approval was originally granted by Birmingham Women's Hospital and transferred to University Hospitals of Leicester NHS Trust for Lynch syndrome samples and granted by the University of Leicester for control samples. Written informed consent was obtained from all participants. Saliva samples were collected using the Oragene<sup>™</sup> DNA saliva kit (Genotek, US) that was sent by post to each participant together with all the documentation accepted by the Ethical committees.
  - <u>Control samples</u>: Volunteers and their families were mostly identified from the Department of Genetics in the University of Leicester, UK. The recruitment process was set up by Carmen Garrido and Dr. Nicola Royle and samples were collected and processed by Carmen Garrido.
  - Lynch syndrome (LS) samples: LS families were recruited from the Birmingham Women's Hospital (BWH) and from the Leicester Royal Infirmary (LRI). The recruitment process was set up by Dr. Frances Tippins (Univ. of Leicester iB.Sc. student 2013-2014) and Dr. Nicola Royle, in conjunction with Prof. Eamonn Maher (BWH) and Dr. Julian Barwell (LRI). Samples were collected by Dr. Frances Tippins (Univ. of Leicester iB.Sc. student 2013-2014) and Carmen Garrido (Univ. of Leicester, 2014-2016) with the collaboration of Jonathan Hoffmann and Wayne Glover (BWH). Only ten families were fully analysed by Dr. Frances Tippins (FT) and the remainder were processed and analysed by Carmen Garrido (CG), including the recruitment of 5 new BWH families. For a breakdown of the samples collected by FT or CG refer to appendix F.

# 2.2 Methods

# 2.2.1 Cell culture

Tissue culture was performed in a class II laminar flow hood in a designated tissue culture area. SW480 and LoVo adherent cells were grown in Dulbecco's modified Eagle medium (Thermo Scientific, UK) with 10% fetal calf serum (Sigma, UK). WI38 primary fibroblast cells were grown in modified Eagle medium (Thermo Scientific, UK) supplemented with 1X non-essential amino acids (Thermo Scientific, UK) and 20% fetal bovine serum (PAN Biotech, DE). Cells were grown in a 37°C incubator with ambient  $O_2$  (21%), 5%  $CO_2$  and high humidity with the exception of the WI38 primary cells transfected with the shMSH2 construct that were grown at  $37^{\circ}$ C in a 5% O<sub>2</sub> and 5% CO<sub>2</sub> environment (Sanyo, UK). Subculture of adherent cells was performed when 80% - 90% confluence was reached. Media was aspirated, and Trypsin-EDTA (Gibco, UK) added (1 ml or 2 ml for T25 or T75 cm flasks -Greiner bio-onerespectively). Fresh media was added when cells were completely detached. Cell counts or Trypan blue assays were made using a Neubauer hemocytometer as described below and cells were seeded in new flasks with fresh media using 1:2 to 1:8 splits depending on the cell line.

Cell pellets for DNA or protein extractions were snap-frozen at -80°C after a five minutes centrifugation step at 420x g, a PBS (Phosphate-Buffered Saline) wash and a five minutes spin at 420x g (all tissue culture centrifugation steps were done using a Sorvall<sup>™</sup> Legend RT centrifuge).

#### 2.2.1.1 Cell counts and population doubling calculation

Cell counts were performed in a Neubauer hemocytometer as recommended by the manufacturer. Population doublings (PD) were calculated using the following equation as described in ATCC (2014):

$$PD = \frac{\log 10 \text{ Final Count} - \log 10 \text{ Starting Count}}{0.301}$$
(2.1)

#### 2.2.1.2 Trypan blue exclusion

Trypan blue is a staining solution that penetrates the cellular membranes of dead cells, therefore it can be used to calculate percentage of viability in a cell culture. Cells resuspended in PBS were incubated with 1:1 (v/v) 0.4% Trypan blue for three minutes and cell counts were performed in a Neubauer hemocytometer. Cell viability was calculated using the following equation:

%Viability = 
$$1 - \frac{\text{Number of dead (blue) cells}}{\text{Total No. cells}} * 100$$
 (2.2)

#### **2.2.1.3** $\beta$ -galactosidase

The  $\beta$ -galactosidase assay is widely used to assess the percentage of senescent cells in a cell culture. This assay indirectly detects the intrinsic  $\beta$ galactosidase activity through a colorless substrate (X-gal) that turns blue when is degraded by this enzyme that is present and active at pH 4.0 in all cells. This assay uses a buffer at pH 6.0 at which none of the X-gal should become blue, but due to the higher proportion of  $\beta$ -galactosidase enzyme in senescent compared to non-senescent cells, some of the X-gal will be degraded by senescent cells producing a blue color. The protocol used was described in Debacq-Chainiaux et al. (2009). Cells  $(5x10^4)$  were seeded in 24 well plates and cultured for 24 or 48 hours before the assay. Cells were fixed with 2% formaldehyde and 0.02% glutaraldehyde in 1X PBS for three minutes and washed three times with 1X PBS. The staining solution (0.2 M citric acid/sodium phosphate pH 6.0, 0.15 M NaCl, 2 mM MgCl<sub>2</sub>, 50 mM potassium ferrocyanide, 50 mM potassium ferricyanide and 20 µg of X-gal in DMF -dimethylformamide-) was added to each well and the plate was incubated in a dry 37°C incubator for 15 to 16 hours. The staining solution was removed and three washes with 1X PBS were done. Fixation was done for 30 seconds with 100 % methanol and air dry were followed by DAPI (1 µg/ml in 1X PBS) staining for five minutes. DAPI was removed and fresh 1X PBS was added to
each well. Subsequently, the plate was analyzed by scanR software (version 2.4.0.11) to quantify the total number of cells (fluorescent DAPI staining) and senescent cells (brightfield blue-staining). The percentage of senescent cells was calculated as shown in the following equation:

%Senescence = 
$$\frac{\text{Senescent cells}}{\text{Total No. cells}} * 100$$
 (2.3)

## 2.2.1.4 Transfection

Plasmids for transfection were propagated in E. coli XL-blue (Mutant alleles are: recA1, endA1, gyrA96, thi-1, hsdR17, supE44, relA1. Wild type alleles are: lac [F' proAB lacI<sup>q</sup>Z $\Delta$ M15 Tn10 (Tet<sup>r</sup>)]. Bacteria were streaked on agar plates with 100 µg/ml ampicillin and incubated at 37°C in an air incubator over night. The following day, single colonies were grown in 5 ml of Luria broth (LB) media with 100 µg/ml ampicillin at 37°C in a shaking incubator for 6 hours as a starter culture and 1 ml of the starter culture was inoculated into 100 ml of LB with 100 µg/ml ampicillin that was grown at 37°C in a shaking incubator overnight. The next day, plasmid DNA was extracted by using the E.Z.N.A.<sup>®</sup> Plasmid Maxi Kit (Omega, UK). Plasmid quantification was assessed by Nanodrop (Thermo Scientific, UK) and plasmid quality was assessed by electrophoresis in a 0.8% LE agarose gel in 0.5X TBE (89 mM Tris, 89 mM Boric Acid, 2 mM EDTA) with 0.5 µg/ml ethidium bromide. Plasmids were linearized by digesting 20 µg of DNA with 10 units of BamHI at 37°C for a minimum of one hour. Linearization was assessed by electrophoresis in a 0.8% LE agarose gel as described before. Linear plasmids were precipitated by adding 0.25 mM sodium acetate at pH 5.6 and 2.5 volumes of 100% ethanol and eluted in pure water (SIGMA, UK).

Prior to transfection, WI38 cells were expanded to get the desired number of cells for parallel transfection reactions and conditioned media from each passage was collected, purified from debris by spinning five minutes at 420x g and stored at 4°C until use. On the day of transfection, cells were counted and  $1x10^{6}$  cells were pelleted at 90x g for ten minutes. Each cell pellet was mixed with 100 µl of Ingenio<sup>®</sup> transfection solution (Mirus, UK) and 2 µg of plasmid (either transient pmaxGFP for transfection efficiency or pSuperior.puro and pSuperior.puro-shMSH2 for stable clones). Electroporation was performed in 0.2 cm cuvettes in an Amaxa<sup>®</sup> Nucleofector<sup>®</sup> II/2b device using program V-001 as it has been an efficient delivery method for other primary fibroblasts (Nakayama et al., 2007). After electroporation, cells were incubated in 500 µl of pre-warmed media (1:1 ratio of normal:conditioned media) for ten minutes and transferred into 10 cm<sup>2</sup> Petri dishes (Sarstedt, UK) adding 7.5 ml of media. For cells transfected with pmaxGFP plasmid for transfection efficiency, cells were diluted into 24 well plates with acid-treated #1 coverslips for subsequent cell screening in an Olympus fluorescence microscope. For the remaining cells transfected with pSuperior.puro or pSuperior.puro-shMSH2, three days after transfection, media was removed and selection with 0.75 µg/ml of puromycin was added and media changed every three or four days. Clones were isolated using cloning discs (Sigma, UK) between one to two weeks after selection and transferred to 24-well plates (Sarstedt, UK). Media was added every four days while checking for confluency. When  $\approx 90\%$  confluency was reached for a clone, it was transferred to 6-well plate prior to taking a cell count as a starting PD and subsequent expansions were performed in T25 flasks. During approximately the next three months, sub-culturing and cell pellets for the dividing clones were performed alongside Trypan blue counts and  $\beta$ -galactosidase assays.

#### 2.2.1.5 Telomerase inhibition

A stock solution of 10 mM of the telomerase inhibitor BIBR1532 (Stratech Scientific, UK) was diluted in 100% dimethyl sulfoxide (DMSO). For telomerase inhibition directly on crude cell extracts, a final concentration of 30 µM was prepared using CHAPS buffer (3-[(3-cholamidopropyl)dimethylammonio]-1propanesulfonate) and the cell lysate was incubated on ice for fifteen minutes before preforming telomerase repeat amplification protocol (TRAP assay) as described in section 2.2.2.10. For telomerase inhibition on cell culture, a final concentration of 10 and 30 µM were used (El-Daly *et al.*, 2005) and (El Daly & Martens, 2007). Aliquots of Dulbecco's modified Eagle medium (Thermo Scientific, UK) with the indicated concentration of BIBR1532 were prepared once a week and the stock solution of drug was stored at -80°C. DMSO in an equivalent and non-cytotoxic concentration (0.3%) to drug dilution was used as negative control. Every three-four days, cells were counted, harvested for FAC (Flow-cytometry analysis) and/or pelleted for DNA extraction and sub-cultured in fresh Dulbecco's modified Eagle medium (Thermo Scientific, UK) with 10 or 30  $\mu$ M of BIBR1532 or 0.3% DMSO.

## 2.2.1.6 Flow-cytometry analysis (FAC)

To perform FAC analysis, cells and supernatant were collected at each passage at a concentration of approximately  $1 \times 10^5$  cells/ml, centrifuged 0.4x g during five minutes at 4°C, resuspended in 500 µl of precooled 70% ethanol and stored at -20°C until use. Cells were stained with 10 µg/ml propidium iodide dissolved in 1X PBS and 5 µg/ml of RNase for one hour at 37°C. The FAC reading was conducted using a BD FACSCanto<sup>TM</sup> II, the analysis was performed using the BD FACSDiva software and statistical analysis of the results and graphs were done using GraphPad Prism software, version 6.0a.

# 2.2.2 Molecular biology

# 2.2.2.1 DNA extraction from cell pellet

Cell pellets were resuspended in 1X filtered SSC (15 mM sodium citrate, 150 mM sodium chloride) buffer. For small ( $10^4$  to  $10^5$  cells), 250 µl of 1X SSC, and for bigger (>  $10^6$  cells) pellets, 500 µl of 1X SSC were added and cell suspension split into two 2 mL eppendorf tubes. Cells were lysed by adding 250 µl of lysis solution (100 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM EDTA, 1% sarkosyl) and RNA was digested by adding 5 µl of RNase (10 mg/µl) at room temperature for twenty minutes. Proteins were digested by adding 100 µg/ml of proteinase K and incubating at 55°C for 5 to 6 hours in a water bath. DNA extraction was made by adding 500 µl of phenol:chloroform:isoamyl alcohol (25:24:1) and mixing. The organic and aqueous phases were separated by centrifugation at 15,600x g for seven minutes at room temperature using

MaXtract High Density tubes (Qiagen, UK). The aqueous phase was transferred to a fresh eppendorf and DNA was precipitated using 0.2 M NaOAc (pH 5.6) and 2.5 volumes of 100% ethanol (for very small pellets, 2 µl of Pellet Paint<sup>®</sup> (Millipore, UK) was added to facilitate the visibility of the DNA pellet). The DNA pellet was transferred to a new tube and washed with 800 µl of 80% ethanol. The pellet was dried at room temperature and DNA dissolved in the appropiate volume of pure water (SIGMA, UK). The DNA concentration was quantified using Nanodrop (Thermo Scientific, UK) and DNA quality was assessed by electrophoretic size separation in a 0.8% LE agarose in 0.5X TBE (89 mM Tris, 89 mM Boric Acid, 2 mM EDTA) with 0.5 µg/ml ethidium bromide.

## 2.2.2.2 DNA extraction from saliva

DNA from Lynch syndrome patients and control families was extracted from saliva using the Oragene<sup>™</sup> DNA saliva Kit (Genotek, US). Each sample was weighed after arrival and heated for 3 hours at 50°C in an air incubator to assure homogeneity. Only half of the sample ( $\sim 2$  ml) was extracted and the other half was stored at -80°C as a backup. A volume of 1/25<sup>th</sup> of prepITL2P DNA extraction solution (Genotek, US) was added to the sample, the mix was incubated on ice for ten minutes and centrifuged for ten minutes at 2,500x g at room temperature in an Eppendorf 5804 centrifuge. Supernatant was transferred to a new tube and DNA was precipitated by mixing 2.5 volumes of 100% ethanol. After three minutes incubation at room temperature the sample was swirled gently to precipitate the DNA and incubated for ten additional minutes at room temperature. After a ten minutes centrifugation step at 2,500x g at room temperature, supernatant was discarded and DNA pellet washed with 80% ethanol. Finally, the DNA pellet was dissolved in 500 ul of 5 mM PCR-clean Tris (pH 7.5) and vortexed. DNA solution was incubated over night at room temperature to ensure complete DNA rehydration and concentration was estimated using a Nanodrop (Thermo Scientific, UK). Measurements for absorbances at 230, 260, 280 and 320 nm were recorded as well as 260/280 and 260/230 ratios. Corrected  $A_{260}/A_{280}$  was calculated using the following formula as recommended by the manufacturer:

Corrected 
$$A_{260}/A_{280} = \frac{A_{260} - A_{280}}{A_{260} - A_{320}}$$
 (2.4)

High molecular DNA quality was assessed by size resolution in a 0.8% LE agarose in 0.5X TBE with 0.5 µg/ml ethidium bromide gel. DNA dilutions (10 ng/µl) were prepared using 5 mM PCR-clean Tris (pH 7.5) and stored at -20°C for subsequent STELA analysis; prior to the analysis, DNA dilutions were anonimysed using letters in alphabetic order to eliminate any bias on the analysis related with the known age or pedigree location for each sample.

#### 2.2.2.3 Sanger sequencing

For sequencing reactions, the Sanger method (Sanger *et al.*, 1977) was used. PCR was performed to amplify the DNA fragment of interest and agarose gel electrophoresis was used to resolve the fragment by size. Gel extraction of the amplicon was performed using a Zymoclean<sup>TM</sup> Gel DNA Recovery Kit (ZymoResearch, UK) following the manufacturer's protocol. For the sequencing reaction, 1 µl of the Big Dye<sup>TM</sup> Terminator and 3.5 µl of 5x Big Dye<sup>TM</sup> Terminator buffer were mixed with the amplicon (20 ng of purified amplicon per kb of sequence) and 3.2 µM of sequencing primer for a 20 µl reaction volume. The reaction was cycled 28 times at 50°C annealing temperature for twenty seconds and 70°C extension time for two minutes. A cleaning step with filtered 2X SSC was followed by column purification and samples were sent to the protein nucleic acid chemistry laboratory in University of Leicester for sequencing. Traces analysis was performed using FinchTV.

#### 2.2.2.4 DNA nanopore sequencing

1. **DNA sample preparation:** Amplicons to be sequenced were generated by PCR (for control sequences) or by STELA (for single telomeres), using the primers on table 2.2. After amplification, PCR products were purified using 1.2X of AmPure beads following the manufacturer protocol and eluted in pure water (SIGMA, UK) for a final concentration of  $\approx$ 500 ng/µl. The eluted product was measured by Nanodrop (Thermo Scientific, UK) and resolved by size using 1% agarose gel electrophoresis to confirm concentration and DNA quality.

Library	Primer pair
Tel1	XpYpE2 + Telorette2 + Teltail
Tel2	XpYp427G/415C + Telorette2 + Teltail
OL A31	HHV6 probe 31 F + HHV6 probe 31 R
KK A49	HHV6A probe 49 F + HHV6A probe 49 R

Table 2.2: Amplicons and primers for nanopore sequencing

- 2. **Pre-library:** amplicons were prepared for sequencing using the Genomic DNA kit SQK-MAP004 from Oxford Nanopore as follows:
  - (a) <u>End-repair</u> using NEBNext<sup>®</sup> End Repair Module (NEB, UK) was performed for thirty minutes at 20°C in a Veriti<sup>®</sup> PCR device following the manufacturer's protocol for a 50 µl reaction. Blunted DNA was again cleaned up using 1.2X of AmPure beads and eluted in 13 µl of pure water (SIGMA, UK) in DNA LoBind eppendorfs.
  - (b) <u>A-tailing</u> using NEBNext<sup>®</sup> dA-Tailing Module (NEB, UK) was performed for thirty minutes at 37°C in a Veriti<sup>®</sup> PCR device following the manufacturer protocol for a 15 µl reaction.
  - (c) Ligation of 15 µl of d-A tailed DNA with 5 µl of adapter mix, 1 µl of HP adapter (Oxford Nanopore, UK) and 25 µl of Blunt/TA Ligase Master Mix (NEB, UK) was performed at room temperature for ten minutes. After ligation, the pre-library was cleaned up using 400 µg of Dynabeads<sup>®</sup> His-Tag (Thermo Scientific, UK) following the manufacturer's protocol and eluted in 13 µl of pure water (SIGMA, UK) in protein LoBind eppendorfs.
- 3. Library: half of the pre-library (6 µl) was mixed with 140 µl of EP buffer and 4 µl of Fuel (Oxford Nanopore, UK) and loaded onto the minION<sup>™</sup> device (Oxford Nanopore, UK) after running platform QC (Quallity Control) for the device and washing twice with 150 µl of EP buffer. Analysis of the results was conducted using SPECTRE (Special Computational Teaching and Research Environment) as described in chapter 4.



Figure 2.1: **Diagram of the library preparation steps for minION<sup>TM</sup> sequencing.** This diagram summarises the main steps involving library preparation. The sample preparation for genomic DNA (gDNA) consists on DNA fragmentation, but as we were only interested in one single telomere, we amplified it by STELA and all different telomere lengths behaved as fragmented gDNA.

## 2.2.2.5 Dot blotting

The dot blot was performed during the optimization of the telomere variant repeat (TVR) PCR to study primer labeling efficiency (see section 4.3.1). TS30T is a forward primer that, after being 5' end labeled with  $\gamma^{32}$ P-dATP, will amplify the A allele of the XpYp telomere together with variant repeat primers (see figure 2.3). Using different labeling conditions for the TS30T primer and blotting it against a template for the XpYp subtelomeric region, we predicted the best labeling conditions for the TVR. An amplicon template from the subtelomeric region of XpYp was prepared by PCR using primers XpYpB2 and XpYpE2 at 0.3 µM final concentration and 10 ng of DNA extracted from the  $MSH2^{-/-}$  cancer cell line LoVo. The PCR was cycled 30 times as follows: 10 seconds at 96°C, 30 seconds at 66.5°C and 50 seconds at 68°C with a final extension step of 10 minutes at 68°C. The amplicon was resolved by size in 2.5%NuSieve agarose gel electrophoresis and a band of the expected size, 500 bp, was observed. The XpYp subtelomeric amplicon was diluted 1:2 and 1:4 in water and used as triplicates. Denaturing solution (0.5 M NaOH, 2 M NaCl and 25 mM EDTA) mixed with bromophenol blue was added to each amplicon (5 volumes per tube, 250 µl). After five minutes incubation at room temperature, the samples were loaded onto a nylon transfer membrane (Fisher, UK) using a dot blot apparatus Hybri-Dot manifold (Life Technologies, UK) previously assembled. Each individual well was washed with 150 µl of 2X SSC. The dot blot was dismantled, the membrane dried at 80°C for five minutes and UV cross-linked (UV 700 kJ/cm<sup>2</sup>). The membranes were soaked in 3X SSC and introduced in the hybridisation bottles. The pre-hybridisation step was performed in the oven at 58°C for ten minutes with 2 ml of pre-warmed TMAC solution (3 M tetramethyl ammonium chloride, 0.6% sodium dodecyl sulfate -SDS-, 1 mM diNaEDTA, 10 mM sodium phosphate pH 6.8, 5X Dehnardts solution and 4 µg/µl yeast RNA). The TMAC solution was discarded and 2.5 ml of TMAC solution were added to each tube for the hybridisation step. To stop the labeling, 20 µl of kinase stop solution (25 mM diNa EDTA, 0.1% SDS and 10 µM ATP) were added to each probe prior to denaturation in a hot block at 100°C for five minutes. After denaturation, the probe (TS30T primer end labeled under different conditions) was added to the bottles to hybridise for one hour at 53°C. The washing solution (3 M TMAC, 0.6% SDS, 1 mM diNaEDTA and 10 mM sodium phosphate pH 6.8) was prewarmed at 50°C. Three 2.5 ml washes with the washing solution for ten minutes at 55°C and one extra of 3.5 ml washing solution were performed. Membranes were scanned using the Typhoon phosphoimager and quantified using ImageQuant software, version 7.0 (GE Healthcare, UK).

## 2.2.2.6 Western blotting

**Cell lysates**. Cell pellets (from  $7x10^4$  to  $5x10^5$  cells) were lysed by the addition of 25 µl of lysis buffer (0.05 M HEPES pH 7.4, 1% Triton-X-100, 0.1 M NaCl, 1 mM PMSF -phenylmethylsulfonyl flouride- and 1X protease inhibitor cocktail) and incubated for twenty minutes on ice. Lysates were centrifuged at 15,600x g for ten minutes to remove debris and supernatant was quantified using quick start Bradford protein assay (BioRad, UK). Lysates were diluted in PBS and Laemmli loading buffer (10% glycerol, 0.02% bromophenol blue, 0.05%  $\beta$ -mercaptoethanol, 2% SDS, 60 mM Tris pH 6.8) at a final concentration of 1 µg/µl of protein. Samples were boiled at 95°C for five minutes and quickly spun prior to gel loading. Lysates were aliquoted and stored at -20°C for subsequent use.

**SDS-PAGE**. Stacking gel (4% acrylamide, 0.1% bis-acrylamide, 0.1% SDS and 1.6 M Tris, pH 6.8) and running gel (10% acrylamide, 0.3% bis-acrylamide, 0.1% SDS and 2 M Tris, pH 8.8) were polymerised by the addition of APS -ammonium persulfate- (0.3% or 0.8% for running and stacking gel respectively) and TEMED (0.08% final concentration). Each sample (10 ng) was loaded into each well and 2  $\mu$ l of BLUeye Prestained Protein Ladder were used as a size marker. The gel was run in an electrophoresis chamber (Bio-Rad, UK) at 100 V for stacking gel and 150 V for running gel (until the bromophenol blue dye reached the bottom of the gel).

**Western blot preparation**. The membrane and the gel were incubated for ten minutes in transfer buffer (25 mM Tris, 192 mM glycine, 20% ethanol) and the protein transfer was carried out in semi-dry conditions using the Trans-Blot<sup>®</sup> BioRad, UK) onto a nitrocellulose membrane (Amersham-ECL), (GE Healthcare, UK) at 1 mAmp per cm<sup>2</sup> for one hour. Ponceau staining solution 0.1% (Sigma, UK) was used to check the transfer efficiency, the homogeneity of protein concentration and the evenness of loading between tracks.

**Protein detection**. The blot was cut in two small blots, each containing the size range for MSH2 (98 KDa) or GAPDH (20 KDa) proteins. As blocking agent, 5% milk powder (Marvel), dissolved in PBST (Phosphate-Buffered Saline with 0.1% Tween 20) was used for 45 minutes. Blots were then incubated with the respective primary antibody dissolved in 5% milk in PBST at specific dilutions (1:1,000 for MSH2 and 1:10,000 for GAPDH) for one hour at room temperature. Following binding of the primary antibody, the membrane was washed three times in PBST, for ten minutes each. Incubation with the secondary anti-mouse (to detect MSH2 and GAPDH) antibody conjugated to horseradish peroxidise (GE healthcare, UK) in a 1:10,000 dilution was for 45 minutes. The membrane was washed with PBST as before. The blot was developed using ECL prime western blotting detection system (GE healthcare, UK) during five minutes, the blot dried with 3 mm Whatman filter paper and covered with plastic wrap. The signal was visualized by exposing the membrane to an X-ray film in a cassette. Quantitative analysis of the resulting bands was performed using the ImageJ software, version 1.46.

#### 2.2.2.7 Southern blotting

The 0.8% LE agarose gels were trimmed to the desired size with a scalpel and washed, shaking in depurinating (0.25 M HCl) solution for seven minutes, rinsed in distilled water, incubated twice in denaturing (0.5 M NaOH, 1 M NaCl) solution for ten minutes and twice in neutralising (0.5 M Tris, 3 M NaCl) solution for ten minutes. MAGNA nylon transfer membrane, 0.45 microns was incubated in 6X SSC for ten minutes before setting up the blot. The blotting apparatus was set up with 20X SSC in a tray with a glass plate and a piece of 3 mm Whatman paper. After 4 to 5 hours, the apparatus was dismantled and the membrane dried at 80°C in an air incubator. DNA was UV crosslinked at 700 kJ/cm<sup>2</sup> in a UVP UV crosslinker.

## 2.2.2.8 Single telomere length analysis (STELA)

This technique was used to amplify individual telomeres and to measure their length using a modification of the original protocol in (Baird et al., 2003). Extracted DNA as show in sections 2.2.2.1 or 2.2.2.2 was diluted at a final concentration of 250 pg/µl in a solution containing 225 ng/µl of Telorette 2 and 0.75 ng/µl of carrier yeast tRNA (for primer sequences refer to appendix). Telorette 2 consists of 20 nucleotides, which complement the 3' overhang of the telomere (and share the same sequence as Teltail), and 7 nucleotides of unique sequence. Long-range PCR was carried out using Teltail primer and a chromosome-specific primer located in the subtelomeric region. The cycling conditions were as follow: 24 cycles of 96°C for 10 seconds, with primerspecific annealing temperatures for 30 seconds and extension at 68°C for 12 minutes. DNA fragments were resolved in 0.8% LE agarose in 0.5X TBE, 0.5 µg/ml ethidium bromide gel and Southern blotted as described before. Prehybridisation and hybridisation was carried out in modified Church buffer (0.5 M sodium phosphate buffer, 1 mM EDTA, 7% SDS) at 65°C and a randomprimed  $\alpha^{32}$ P-dCTP-labeled telomere and ladder (1 kb and HR) probes added to the hybridisation. Membranes were washed in high stringency buffer (0.5X SSC, 0.1% SDS) and hybridized fragments were detected using a Typhoon 9400 PhosphorImager (GE Healthcare, UK). The results were analysed using ImageQuant software, version 7.0 (GE Healthcare, UK) for sizing and Graph-Pad Prism software, version 6.0a for statistical analyses.



Figure 2.2: Diagram of single telomere length analysis (STELA). Adapted from Baird et al. (2003).

## 2.2.2.9 Telomere variant repeat PCR (TVR)

This method described in (Baird *et al.*, 1995) allows determination of the interspersion of the consensus telomere repeat (TTAGGG) with sequence variant repeats (such as TCAGGG or TGAGGG among others), present at the start of some telomere alleles. Here, single molecule STELA products (smSTELA) were generated to identify telomere molecules with mutations in those repeat variants. Genomic DNA (70 to 150 pg per reaction) was amplified by STELA (as described before 2.2.2.8) in 20  $\mu$ l PCRs. PCR products (5 to 6  $\mu$ l) were resolved by size using 0.8% LE agarose gel electrophoresis, Southern blotted and hybridized to the telomeric probe to assess smSTELA that would be subsequently analysed by TVR.

- 1. Denaturing acrylamide gels: in the original protocol (Baird et al., 1995), 0.1  $\mu$ M of forward primer was 5' end labeled with  $\gamma^{32}$ P-dATP and the PCR performed with 0.4 µM of each variant repeat primer. PCRs reactions were cycled 19 times for 40 seconds for the annealing step and 2 minutes for the extension step at 70°C. After the PCR, formamide loading dye (95% formamide, 10 mM EDTA pH 8.0, 0.1% bromophenol blue and 0.1% xylene cyanol) was added to each sample and a five minute denaturation step at 96°C was performed in a thermocycler. The PCR products were resolved in denaturing acrylamide gels (6% acrylamide, 7.6 M urea in 1X TBE) polymerised with TEMED and APS. Gels were pre-run using 1X TBE as running buffer at constant 60 W for twenty minutes before loading the samples. When the gel electrophoresis was finished (different times depending on telomere and number of repeats), acrylamide gels were stuck to two pieces of 3 mm Whatman paper and were dried at 80°C in a gel drier (BioRad, UK) for two hours. The image of the radioactive TVR gels was detected using a Typhoon 9400 PhosphorImager (GE Healthcare, UK) and manually analysed.
- 2. **Capillary electrophoresis:** the original protocol has several limitations (see table 2.3) that we tried to overcome modifying it to make it suitable for capillary electrophoresis. Using the same primer sequences,

the variant repeat detector primers were fluorescently labeled. Different fluorophores were used for each variant repeat primer, so allowing all TVR products to be separated in the same capillary gel if required.

Primer concentrations were as in the original protocol (0.4  $\mu$ M for each reverse primer and 0.1  $\mu$ M of forward primer) and PCRs were cycled 24 times for 40 seconds for the annealing step and 2 minutes for the extension step at 70°C. After the PCR, 1  $\mu$ l of PCR product was added to 9  $\mu$ l of Hi-Di<sup>TM</sup> formamide (Applied Biosystems, UK) with ROX-1000XL ladder (BioVentures, US) into a 96-well plate. PCR products were denatured for five minutes at 96°C in a termocycler and run into the ABI 3100 Genetic Analyzer. Settings and conditions for the run are shown in table 2.4 and figure 2.3 graphically compares the two methods. Results were analysed using GeneMapper v4.1 (Applied Biosystems, UK).

	Sequencing gels	ABI 3130
Samples per run	< 50	up to 192
PCR volume per run	$> 5 \ \mu l$	1µl
Time per experiment	2 to 3 days	4 to 5 hours
Hazards	Formamide Radiation Leaking Voltage	Formamide
Sizing	None	Size ladder
Analysis	Manual	Gene Mapper software
Labeling stability	< 2  m ~weeks	several months at -20°C
Variable factors	Radiation activity Probe labeling efficiency Gel mixture Gel electrophoresis	Polymer Ladder

Table 2.3: TVR methods comparison. The table compares the performance of TVR using sequencing gels or the ABI 3130 Genetic Analyzer.

Condition	Setting
Injection voltage	1.2 kVolts
Run voltage	15 kVolts
Pre run time	180 sec
Injection time	40 sec
Voltage step interval	15 sec
Run time	1200 sec
Polymer	POP-7 <sup>™</sup> (Thermo Scientific, UK)

Table 2.4: Conditions for the ABI 3100 genetic analyzer.



# CHAPTER 2. MATERIALS AND METHODS

## 2.2.2.10 Telomerase repeat amplification protocol (TRAP)

Adaptation of the original protocols in Kim et al. (1994) and Piatyszek et al. (1995) was made to measure telomerase activity. Cell pellets stored at -80°C were lysed with 1X CHAPS lysis buffer for twenty minutes on ice, centrifuged at 12,000x g for twenty minutes and supernatant analysed by quick start Bradford protein assay (BioRad, UK) to measure protein concentration (Bradford, 1976). The TS primer was end-labeled in the presence of  $\gamma$ -<sup>32</sup>P-ATP and T4 polynucleotide kinase (0.5 units/ul) for twenty minutes. The T4 polynucleotide kinase was inactivated by five minutes heat shock at 95°C. The reaction was performed with a final protein concentration of 100 to 500 µg/µl using TRAPeze<sup>®</sup> telomerase detection kit (Millipore, UK). Heat inactivated control lysates were prepared by incubation in a water bath at 95°C for ten minutes. The extension of the TS primer by the telomerase present in the cell lysates was at 30°C for thirty minutes and the PCR cycling was: 28 cycles of 94°C during 30 seconds and 55°C during 33 seconds for amplification (see figure 2.4 for further information). DNA fragments were resolved in 6% denaturing (7.7 M of urea) acrylamide gel. After drying the gel for two hours at 80°C in a gel dryer (BioRad, UK), the radioactive bands were detected using a Typhoon 9400 PhosphorImager (GE Healthcare) and the image visualized using ImageQuant version 7.0 (GE Healthcare, UK).

# 2.2.3 Statistical analyses

All the statistical tests were performed using GraphPad Prism software, version 6.0a and a p value below 0.5 was considered significant. For each purpose, distinct tests were used; briefly:

1. Linear regression: was used to compare slopes between two or more groups, producing an ANCOVA (analysis of covariance) p value for the slopes and the Y intercepts (if slopes are not different). In chapter 3 it was used to compare cell growth at 5 and 21% O<sub>2</sub>, rate of cell division in control and shMSH2 clones and the relationship between telomere shortening rate and initial telomere length. In chapter 5 it was used to

compare cell growth curves between treated and untreated cancer cells. In chapter 6 it was used to establish the telomere shortening rate with age in control and Lynch syndrome individuals and to compare between the different groups.

- 2. Non-linear regression: was used in chapter 3 to draw a best-fit curve in those XY graphs that did not adjust to a linear curve.
- 3. Groups comparison: the non-parametric two tailed Mann-Whitney t-test was used to compare two groups while one-way ANOVA was used for more than two groups. When the comparison focused on the distribution (rank) of the data, non-parametric Kruskal-Wallis ranking test was performed, for example for comparisons of senescence and viability between control and shMSH2 clones (chapter 3), for comparisons of the age distribution of the saliva samples among the groups (chapter 6) and before pooling data from replicates for STELA. Finally, when more than two groups wanted to be compared, but each comparison stood alone, the ANOVA was uncorrected for multiple comparisons and Fisher test was used.



Figure 2.4: **Diagram of TRAP assay.** This diagram summarises the main steps in the telomerase repeat amplification protocol (TRAP). As the reverse primer 1 can anneal in any repeat, the PCR products would be shown as a 6 bp ladder as described by Kim *et al.* (1994).

# 3 Telomere dynamics in a human primary cell under MSH2 deficiency

# 3.1 Background

Telomeres of human fibroblast cells grown in culture shorten with each cell division between 30-200 base pairs (bp) ((Harley *et al.*, 1990) and reviewed in Verdun & Karlseder (2007)). Therefore, a negative relationship between telomere length and replicative capacity of human primary fibroblasts was predicted (Allsopp *et al.*, 1992). The maximum number of cell divisions that a primary cell line can undergo before entering replicative senescence, the natural state of cell proliferation arrest, is known as the *"Hayflick limit"* (Hayflick & Moorhead, 1961) and telomere length, specially short telomeres, and not the number of cell divisions, regulate the onset of telomere driven replicative senescence ((Sitte *et al.*, 1998) and (Munro *et al.*, 2001)). On the contrary, cancer cells maintain their telomere length by activating either the telomerase enzyme (Blackburn *et al.*, 1989) or the Alternative Lengthening of Telomeres (ALT) pathway (reviewed in de Lange (2009) and Takubo *et al.* (2010)).

Telomeres are very susceptible to oxidative damage because of their high guanine content, being 8-oxo-7,8-dihydroguanine (8-oxoG) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) the two main DNA lesions produced in the presence of reactive oxygen species (ROS) (Wang *et al.*, 2010). During replication, 8-oxoG might bind either to cytosine or adenine; in the first case, the lesion remains silent but in the second case, a G:C to A:T transversion might occur (Bridge *et al.*, 2014). In addition, oxidative stress increases the frequency of single-stranded breaks (SSB) throughout the genome but preferentially on telomeres (Petersen *et al.*, 1998) and it has been shown that the SSB repair on telomeres is not as efficient as in other regions such as microsatellites (Coluzzi *et al.*, 2014). Previously, it was demonstrated that hyperoxic (40% O<sub>2</sub>) conditions increased telomere attrition rate (from 90 to 500 bp per

population doubling) and impeded cell proliferation after a few cell divisions (von Zglinicki *et al.*, 1995). Besides, it was demonstrated that low oxygen conditions (Balin *et al.*, 2010) and/ or high antioxidant levels (Lorenz *et al.*, 2001) delayed the onset of replicative senescence and reduced the TSR (Serra *et al.*, 2003) possibly due to the decline in the number of SSBs (von Zglinicki *et al.*, 2000a).

Oxidative stress is related with both, replicative (RS) and induced senescence (IS) (see section 1.2.2.2). On the one hand, elevated ROS concentrations increase telomere shortening as demonstrated by von Zglinicki et al. (1995), blocking cell proliferation after few cell divisions. On the other hand, the use of antioxidants can revert senescence phenotypes such as endothelial dysfunction (Bhayadia et al., 2016), what is only possible in reversible IS but not RS. Interestingly, it was demonstrated by Britt-Compton et al. (2009b) that under low (3%), but not normobaric (21%) oxygen conditions, senescence was triggered by telomere shortening (overall mean of 83 bp/PD  $\pm$  23 SD) in IMR90 fibroblasts while in MRC5 fibroblasts, senescence was triggered by telomere attrition at both oxygen conditions. This suggested that different cell lines might have distinct responses under oxygen stress as it was shown in ex vivo cultures of human CD34<sup>+</sup> cells (Fan et al., 2008) and more recently by transcriptional assays in two human cancer cell lines (the hepatocellular carcinoma, Hep G2 and the colorectal adenocarcinoma, Caco2) (Deferme et al., 2015). In addition, primary fibroblasts (MRC5) growth-arrested by confluency exhibited a higher telomere shortening rate (TSR) when the proliferation block was released and accumulated SSB on the telomeres (Sitte et al., 1998). Therefore, it was suggested that accumulation of SSB on telomeres was the main trigger for the increased TSR observed under hyperoxia ((von Zglinicki et al., 2000a) and (Honda et al., 2001)).

The DNA MMR pathway repairs single base mismatches and insertion/deletion loops as well oxidative lesions through interaction of the MSH2-MSH6 complex with monoubiquitinated PCNA and DNA polymerase  $\eta$  (Zlatanou *et al.*, 2011) and it has been described that colorectal cancer cells deficient for the MSH2 protein have a higher telomeric mutation frequency in the variant repeat region of the telomere (Pickett *et al.*, 2004). Interestingly, a synthetic lethality has been described between MMR gene mutations and DNA polymerases:  $MSH2^{-/-}$  combined with  $POLB^{-/-}$  or  $MLH1^{-/-}$  combined with  $POLG^{-/-}$  leading to the accumulation of oxidative lesions (Martin *et al.*, 2010).

To understand the effect that MSH2 depletion had on the telomeres of a primary cell line, Mendez-Bermudez & Royle (2011) firstly transfected the human primary lung fibroblast cell line (CCD34-Lu) with the catalytic subunit of telomerase (hTERT) (as it is known that telomerase can extend the life span of primary fibroblasts in culture by lengthening their telomeres (Bodnar *et al.*, 1998) specially the shortest ones (Ouellette *et al.* (2000) and Britt-Compton *et al.* (2009a)). Unexpectedly, despite detectable telomerase activity, telomere length continued to shorten in the CCD34-Lu-hTERT cells cultured at 21% O<sub>2</sub>. Secondly, the MSH2 protein was depleted using an shRNA and it was found that telomeres in CCD34-Lu-hTERT clones with depleted MSH2 shortened at a significantly higher rate  $242 \pm 13$  bp per Population Doubling (PD) compared to control clones ( $51 \pm 6$  bp/PD) suggesting that the MMR pathway was involved in the telomere length maintenance during replication.

Nevertheless, there were some unanswered questions arising from that experiment: a) was the effect cell line specific or do other primary cell lines show increased telomere shortening rate (TSR) in upon depletion of MSH2?, b) why were telomeres shortening even when telomerase was expressed?, c) was the TSR affected by the presence of telomerase?, and d) was the increased TSR in MSH2 depleted clones caused by the oxidative stress imposed by growing the cells at normobaric (21%  $O_2$ ) conditions?

# **3.2** Aims

To answer the previous questions, stable clones expressing the same shMSH2 construct described in Mendez-Bermudez & Royle (2011) were generated but using a different telomerase negative human primary lung fibroblast cell line (WI38) that is known to undergo many cell divisions before entering senescence. This eliminated the need to transfect the WI38 cells with hTERT prior to depletion of MSH2. To dismiss oxidative stress as a contributory factor, the WI38 cell line and clones generated after transfection were grown under 5%  $O_2$  conditions. The objectives for this aim were:

- To transfect WI38 cells with a plasmid expressing shMSH2 to generate stable clones.
- To grow independent MSH2-depleted stable clones and to follow their growth dynamics.
- To measure the level of MSH2 expression by western blot and to select the clones with the highest MSH2 downregulation for further analysis.
- To measure telomere shortening rates (TSR) using single telomere length analysis (STELA) on clones with reduced MSH2 expression and control clones.
- To compare data with that obtained in a different cell line (Mendez-Bermudez & Royle, 2011) to overcome the limitations from the previous experiment.

# 3.3 Results

# 3.3.1 Experiment design

Normoxic (5%) oxygen conditions were used to confirm that the higher TSR described in (Mendez-Bermudez & Royle, 2011), when the cells were grown under 21%  $\mathrm{O}_2$  conditions, was solely due to depletion of the MSH2 protein and not to the high levels of oxidative stress. As previously described (von Zglinicki et al., 1995), the untransfected WI38 cell line grew faster and underwent a larger number of population doublings (PD) at 5%  $O_2$  compared to  $21\% O_2$  conditions (figure 3.1). Interestingly, growth curves comparisons between both oxygen conditions were not statistically significant (p = 0.05) for "younger" cells (PD = 25, figure 3.1.A) but were highly significant (p < 0.0001) for "older" cells (PD = 32, figure 3.1.B), confirming that low oxygen conditions delay the onset of senescence in WI38 cells (Balin et al., 2010). In addition, the slopes for the growth curves at PD = 25 and PD = 32 grown under the same oxygen conditions were statistically (p < 0.0001) different for both, 5% and 21% O<sub>2</sub> conditions suggesting that "older" primary cells divided more slowly than "younger" ones despite the oxygen concentration (due to the higher proportion of senescent cells in the former). Those results were consistent with a) experiments performed by Betts et al. (2008) showing that senescence can be delayed under low oxygen conditions even when telomeres are very short and b) the positive relationship between production of antioxidant enzymes such as glutathione (GST) and oxygen tension that occurs in log-phase ("younger") cells but is reduced in senescent ("older") cells (Balin et al., 2010).



Figure 3.1: **WI38 growth curves under different oxygen conditions.** A shows the growth curve for a WI38 "young" passage (P 25). B shows the growth curve for a WI38 "old" passage (PD 32). For both graphs, blue colour refers to normoxic (biological) oxygen conditions (5%  $O_2$ ) and green colour refers to normobaric (ambient) oxygen conditions (21%  $O_2$ ). Best fit curves for each oxygen condition are shown on grey colour. "PD" = Population Doubling.

Figure 3.2 shows dose-response curves for WI38 cells grown in two different puromycin concentrations (0.5  $\mu$ g/ml and 1  $\mu$ g/ml) and untreated cells as a control, to asses the best conditions for puromycin selection after transfection with the pSuperior.puro and pSuperior.puro.shMSH2 plasmids. The optimal concentration would be the lowest that kills most of the cells after one day. Following this assessment, colony selection was conducted with puromycin at 0.75  $\mu$ g/ml in subsequent experiments.



Figure 3.2: **Puromycin dose-response curve for WI38 cells.** The control curve shown in purple represents WI38 cells grown in media (MEM + 20 % FBS + 1X non essential amino acids) without antibiotic. Red and green curves represent WI38 cells treated with 0.5 µg/ml and 1 µg/ml of puromycin respectively.

The plasmids used for transfection (pSuperior.puro and pSuperior.puroshMSH2), taken from Mendez-Bermudez & Royle (2011) are shown in figure 3.3.A. As previously described in section 2.2.1.4, the plasmids were propagated in bacteria (*E. coli* XL-blue) and extracted using maxi prep extraction kits. The sequence across the shMSH2 insert was confirmed by Sanger sequencing as shown in figure 3.3.B. The transfection was performed in an Amaxa<sup>®</sup> Nucleofector<sup>®</sup> II/2b device as described in section 2.2.1.4. Puromycin selection was added three days after transfection and the delivery efficiency was measured as the total number of colonies formed per million of transfected cells (figure 3.4.A). Two transfection experiments were performed, the first of which was not successful in terms of surviving clones due to contamination but the number of colonies was scored for subsequent comparisons. In figure 3.4.A, blue bars represent the first, unsuccessful experiment and green bars the second. The significant increase in the number of colonies was obtained by diluting each transfection reaction into three 10 cm Petri dishes (second experiment) instead of one. For transfections with the shMSH2 plasmid, an increase from 20 to 134 colonies per million of transfected cells was obtained and for the control plasmid, the increase was from 19 to 151 colonies per million cells. In addition, co-transfection with pmaxGFP plasmid with either pSuperior.puro control or pSuperior.puro-shMSH2 plasmids was used to estimate delivery efficiency by counting the percentage of green cells in reference to the total cell number (figure 3.4.C).



Figure 3.3: **Plasmid and shRNA used for transfection.** A pSuperior.puro-shMSH2 plasmid map. It contains an ampicillin resistance (AmpR) gene that will be expressed in *E. coli* cells for plasmid amplification and a puromycin resistance (PuroR) gene that will be expressed in mammalian cells for selection of transfected cells. The control plasmid (pSuperior.puro) does not contain the shMHS2 sequence. **B** shows the location of the shMSH2 under the H1 promoter was confirmed by Sanger sequencing using pSup1primer (see table in appendix B). The sequence chromatogram of 60 nucleotides corresponding to the shMSH2 is shown. **C** is the structure of the shMSH2, showing the complementarity between 19 nucleotides (small letters), the loop (capital letters) and both, 5' and 3' tails (capital letters).



Figure 3.4: **Transfection efficiency.** A is a bar graph showing the transfection efficiency measured as number of colonies per million transfected cells between two independent experiments (blue and green bars) for both, shMSH2 (\*\*\*p = 0.0005) and control (\*\*p = 0.0091) plasmid. Ranks were compared by Mann Whitney test. B is an example of the green fluorescence signal produced by the co-transfection of shMSH2 and pmaxGFP plasmids in one positive cell compared with two non-positive cells which nuclei were stained with 1 µg/mL DAPI. C shows quantification of the transfection efficiency as a measurement of green-positive cells in relation to green-negative cells expressed as a percentage. Green bars represent values for transfection with only pmaxGFP plasmid; purple, for pSuperior.puro-shMSH2 co-transfected with pmaxGFP plasmid and blue for pSuperior.puro control vector co-transfected with pmaxGFP plasmid. Table below the graph shows the number of cells with green signal over the total number of scored cells per day. Day 1 and 3 represent one and three days after transfection respectively. Selection was added on day 3 after scoring cells for that day. Day 4, 6 and 11 represent one, three and seven days after puromycin selection respectively.

# 3.3.2 WI38 *MSH2*<sup>+/-</sup> clones growth

Due to the elevated mutation rate reported in MMR-deficient cells across the genome and in particular at microsatellite regions (Coolbaugh-Murphy *et al.*, 2010) and at telomeres (Pickett *et al.*, 2004), it was anticipated that fewer shMSH2 clones would survive or that they may grow more slowly and so more of these clones were isolated compared to control clones. In total, 33 shMSH2 clones were isolated using cloning discs, 23 of which grew successfully, giving a recovery efficiency of 70%. For the control clones, 18 were isolated, 7 of which were subsequently followed up for cell growth. In addition, 10 shMSH2 and 5 control populations were grown in parallel to clones to study the effect that clonal variation had on growth curves. To draw growth curves, Population Doublings (PDs) were calculated following the equation 2.1.

Each clone was considered to be derived from a single cell (as sufficient dilution after transfection should allow colony formation from a single cell progenitor) and a 100% recovery was assumed following trypsinization using cloning discs to isolate independent clones. The first cell count was made at the trypsinization step in an 80% confluent 24 well plate and it corresponded to the Final Count (FC) of the first passage after transfection. Subsequent cell counts were used to plot growth curves for each clone, as displayed in figure 3.5.B. Simultaneously to the isolation of clones, the remaining cells derived from transfection were pooled together and considered as different populations (figure 3.5.A). As WI38 cells were thawed at  $\approx$  PD 19 and 3 PDs were needed to expand the cells prior to transfection, in all graphs on figure 3.5, the Y intersection on the X axis corresponds to PD 20, indicating the total number of PD that WI38 had undergone before transfection. The best fit curves (sigmoidal) show that the log-phase (where cells were actively dividing) lasted until, approximately 30 cumulative days and was followed by the plateau phase (where cells divided more slowly as the percentage of senescence increased). The non-linear regression  $\mathbb{R}^2$  values for populations are 0.96 and 0.98 for shMSH2 and control populations respectively and Kolmogorov-Smirnov test indicated that the curves were not significantly different to each other (p = 0.96). The best fit curves for clones had an  $\mathbb{R}^2$  of 0.92 and 0.88

for shMSH2 and control clones respectively and Kolmogorov-Smirnov test also indicated that the curves were not significantly different to each other (p = 0.60), suggesting that depletion of the MSH2 protein in WI38 cells did not have a dramatic effect on cell growth. Furthermore, as clones behaved very similar to populations, it was assumed that clonal variation did not significantly impact on cell growth.

Figure 3.6.A shows the growth curves for the clones that were subsequently analysed for telomere length accounting for their MSH2 protein level (described on section 3.3.3 and shown on figure 3.9), and figure 3.6.B shows the decrease in the rate of cell division per day of those clones from approximately 0.8 PD/day after transfection to 0.2 PD/day at the end of the experiment. Linear regression curves in figure 3.6.B (with  $R^2$  0.92 and 0.86 for control and MSH2 depleted clones respectively) exhibit significantly (p = 0.0001) different intercepts between both curves, indicating that MSH2 depleted clones divided more slowly than control clones; nevertheless, it was expected that senescence was reached at similar time points as slopes were not significantly different (p = 0.6) between controls and shMSH2 clones.



Figure 3.5: Clones and population growth curves. A summarises growth curves for populations and B for clones. The top graphs show individual growth curves and bottom graphs show the best fit curves (sigmoidal) for each counterpart on the top. For all graphs, red colours represent shMSH2-transfected cells and blue colours, controls.



Figure 3.6: Growth curves for clones analysed for telomere length. A plots the growth curves for the clones that were analysed for telomere shortening rates by STELA. The green points indicate the final PD at which cell pellets were taken for DNA extraction (the starting time points are not indicated because all fall within the same exponential region). B plots the rate of cell division as population doubling (PD) per day of the clones shown on graph A, grouped into controls (blue colour) and MSH2 depleted (red colour).

Cell viability was measured using Trypan blue staining during consecutive days and calculated by the equation 2.2. The percentage of viable cells was constant during the whole experiment (approx. 90%) among all the samples (figure 3.7.A). The percentage of senescent cells was determined using the  $\beta$ -galactosidase assay as described in section 2.2.1.3. The senescence assay was conducted in duplicate and for some populations (e.g. shA.3 or psupA.2), two different time points were assayed. As the percentage of senescent cells would increase with successive population doublings, the PD at which senescence was measured is indicated in the table below figure 3.7.B to allow comparison between clones. Figure 3.7.C summarises the percentage of viable (pink bars) and senescent (cyan bars) cells for the transfected WI38 clones and populations. Kruskal-Wallis tests showed no significant difference in viability and senescence (neither for the mean cumulative PD at which senescence was assayed) between control and shMSH2 clones or between control and shMSH2 populations. However, the percentage of senescent cells was significantly higher in shMSH2 clones than shMSH2 population, reflecting the effect of clonal variation.

Combining this data with the cell growth curves on figure 3.6 it was hypothesised that the lower cell division rate described in shMSH2 clones compared to controls might be a consequence of replication fork stoppage due to MSH2 depletion although no effect on viability was shown, suggesting that cells continued dividing and therefore, accumulating mutations that were not repaired by the MMR pathway. In addition, the fact that senescence was reached at similar time points for controls and shMSH2 clones suggests that less cell divisions occurred in the shMSH2 before entering senescence and therefore, an increase in telomere shortening rate might be anticipated in those clones.



Figure 3.7: Senescence and viability. A shows means  $\pm$  standard errors (SE) for viability assay measured by Trypan blue staining. B shows means  $\pm$  SE for  $\beta$  galactosidase assay. The number of cumulative PDs at which senescence was assayed and the number of cells scored is indicated in the table below the graph. The colour coding for A and B is: grey for non-transfected WI38 cells grown in parallel, red for shMSH2 and blue for controls; clear colour bars for clones and striped bars for populations. C summarises the percentages of viable cells (pink) and senescent cells (cyan) plotted on the left axis. Means and  $\pm$  SE are shown. Green dots represent the mean and SE of the cumulative PDs, plotted on the right axis, used for  $\beta$  galactosidase assay. \*p = 0.015 for one-way ANOVA uncorrected for multiple comparisons.

## 3.3.3 MSH2 downregulation in WI38 clones

To asses the level of MSH2 protein downregulation, western blots (WB) were optimised for protein concentration and primary antibody dilution using nontransfected WI38 cell pellets, as shown in figure 3.8, and reproducibility was assessed among independent protein lysates. Figure 3.8.A shows a linear relationship between the relative amount of MSH2 protein and the amount of primary antibody for 5 and 10 µg protein concentration that was lost for 20 µg protein, as the increased protein concentration produced oversaturation of the control antibody. To avoid inaccuracies due to overexposure, 10 µg of protein and a dilution of 1/1,000 for the primary anti-MSH2 antibody in 5% milk PBST (Phosphate Buffered Saline Tween-20) was used for subsequent experiments. To assess biological reproducibility, three different cell lysates (A, B and C) were assayed for the level of MSH2 protein expression. One-way ANOVA analysis with Tukey test correction for multiple comparisons showed that the protein content in lysates A (p = 0.03) and C (p = 0.002) was significantly different from B. Nevertheless, when using a centrifugation step of 10 minutes at 13,000 rpm (in a 5415R Eppendorf Centrifuge) to remove cell debris (for cell lysates D, E and F), the variability of the relative MSH2 expression among biological replicates decreased to non significant levels (figure 3.8). Therefore, this centrifugation step was included before protein quantification by the Bradford assay (Bradford, 1976).

Figure 3.9 represents the relative expression of MSH2 to GAPDH (control protein), calculated using the normalisation method *by the sum* described in Degasperi *et al.* (2014), for clones and populations of WI38 cells transfected with either psuperior.puro control or psuperior.puro-shMSH2 plasmids. Briefly, the intensity of each band was divided by the sum of the total intensity for the same protein (MSH2 or GAPDH respectively) within a blot to obtain the relative amount of protein per blot, and subsequently, the relative amount of MSH2 for each lysate was divided by the total amount of GAPDH for the same lysate and blot, what allowed to compare replicates from different gels.

Clones sh143, sh123, sh110, sh101, sh112 and sh104 with a percentage of MSH2 downregulation of 99, 94, 93, 63, 54 and 31 respectively were chosen



Figure 3.8: **Preliminary western blots.** A shows the relative amount of MSH2 measured by western blot using three different final protein concentrations (5, 10 and 20 µg) and three primary antibody dilutions (1/2,000, 1/1,000 and 1/500) were assessed. B shows the relative amount of MSH2 was quantified in six lysates (A to F) from WI38 cell pellets collected at different time points and it is plotted as mean  $\pm$  SE. For lysates A, B and C no centrifugation step was performed after the lysis whereas for D, E and F a centrifugation step of 10 minutes at 13,000 rpm was performed. \*p = 0.03 and \*\*p = 0.002.

for subsequent telomere length analysis. Unfortunately, clones sh116, sh107 and sh109 with a greater than 50% protein downregulation were not suitable for telomere analysis as too small cell pellets at the end of the cell culture were obtained. In addition, clone sh100, having an 80% downregulation in a cell pellet corresponding to PD = 48.6, was not analysed for telomere shortening as another WB from PD = 43.5 showed a greater MSH2 expression, suggesting that the MSH2 downregulation might have occurred as a later event. After transfection, the populations were heterogeneous for a) integration sites, b) copy number and c) plasmid expression, therefore, the level of the MSH2 protein was not significantly different from populations transfected with either control or shMSH2 plasmids and due to that heterogeneity, populations were not included in the telomere length analysis.



Figure 3.9: **MSH2 downregulation in WI38 clones**. The relative MSH2 protein expression in WI38 populations (A) or clones (**B**, **C** and **D**) transfected with either control or shMSH2 plasmids is shown. Also WI38 non-transfected cells were assayed as a controls (shown in **B**). Each bar represents the mean of at least 4 independent measurements (up to 6-7 when sample was available)  $\pm$  SE. Red refers to shMSH2 (labeled "sh") and blue to controls (labeled "cont."). For clones control 110, control 128 and sh 100, two cell pellets corresponding to two different time points were assessed, represented as -s and -m for start and middle time points respectively. **E** summarises the relative MSH2 protein levels for all the clones. Data from all control clones was pooled into one data set (blue) and the percentage of MSH2 downregulation was calculated comparing the mean of each clone to the mean of the pooled control clones. pvalues for one-way ANOVA uncorrected for multiple comparisons are shown for each clone. Clones selected for telomere analysis are indicated with a green arrow.

# 3.3.4 Telomere shortening in WI38 *MSH2*<sup>+/-</sup> clones

Telomere length was measured using single telomere length analysis (STELA) (Baird *et al.*, 2003) as described in section 2.2.2.8. When the telomere length is measured at two different time points with a known number of cell divisions (PDs) between them, the Telomere Shortening Rate (TSR) can be calculated as shown in equation 3.1, where mTL is the median telomere length for each time point and PDs is the number of population doublings separating the two time points.

$$TSR = \frac{mTL_1 - mTL_2}{PDs}$$
(3.1)

Four control clones (control100, control110, control112 and control128) and six MSH2 downregulated clones (sh143, sh123, sh110, sh101, sh112 and sh104) were analysed for three different telomeres (XpYp, 12q and 17p) using STELA. An example of four STELA gels for clones sh123, sh143 and control112 is shown in figure 3.10.

It has been demonstrated using FISH (Londoño-Vallejo *et al.*, 2001) and STELA (Baird *et al.*, 2003) that different telomere alleles might have distinct mean lengths and even distinct attrition rates ((Britt-Compton *et al.*, 2006) and (Graakjaer *et al.*, 2006)). Based on SNPs in the flanking (subterminal) sequences (Baird *et al.*, 2000), the WI38 cell line is heterozygous for the XpYp (haplotypes A and B) and 12q (haplotypes B and  $\Delta$ ) telomeres. Furthermore, it contains an allele in 17p that can be amplified using the 17p6 primer designed in Britt-Compton *et al.* (2006). Therefore, haplotype specific primers were used for 12q and XpYp to investigate allele-specific telomere shortening rates (for primer sequences refer to appendix B).



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for XpYpE2 and 434 bp for both allele specific XpYp primers). Scatter plots shown below each STELA blot were plotted using GraphPad Prism6. Median values and interquartile ranges are

shown. For the DNA dilutions that produced no PCR bands (e.g. control 112 end), the DNA concentration was increased in subsequent STELA reactions.

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Figure 3.11 shows four scatter plots for the telomere length of four clones (two controls and two shMSH2) where the two different alleles for the XpYp telomere were amplified. When the universal XpYpE2 primer (that amplifies A and B alleles) was used, the mTL at the last time point was greater than the mTL at the first time point, impeding to accurately calculate TSR and indicating the presence of two different length alleles that were amplified at distinct efficiencies. Therefore, the use of haplospecific primers allowed us to study allele A and B independently and all four graphs show the presence of both telomere length subpopulations when the universal primer was used. Figure 3.12 shows the scatter plots for all clones analysed for each telomere in all 10 clones and table 3.1 summarises the median telomere lengths (mTLs) and telomere shortening rates (TSRs). Due to the limited amount of DNA, the haplotype-specific primers for XpYp were only assayed when the presence of two subpopulations with very different telomere lengths made the TSR estimate inaccurate.



Figure 3.11: Scatter plots for the XpYp telomere. In all graphs, blue indicates first and orange second, time points. When both XpYp alleles are shown, universal primer XpYpE2 was used for STELA. For A allele (Hap.A), the XpYp-427G/415C and for B allele (Hap.B), the XpYp-427A/415T primers were used.



Figure 3.12: Scatter plots for telomere length of WI38 transfected clones. A plots medians and interquartile ranges of three telomeres (XpYp, 12q and 17p) for four controls clones. B plots medians and interquartile ranges of three telomeres for six MSH2 downregulated clones. For all graphs each telomere is measured at the beginning of the cell culture (blue colour) and at the end (orange colour).

	control 100	control 110	control 112	control 128	sh 143	sh~123	sh 110	sh 101	sh 112	sh 104
MSH2 downregulation	ı	I		·	<i>%</i> 66	94~%	93~%	63~%	54~%	31~%
Population Doubling <sup>†</sup>	5.12	9.66	6.00	13.77	2.94	3.55	5.23	4.52	8.77	6.98
mTL-1 17p (# values)	2,461 (67)	3,135(31)	2,073 (56)	1,852 (22)	2,176 (70)	1,781 (27)	2,528 (22)	1,584(29)	2,580 (30)	2,382 (51)
mTL-2 17p (# values)	2,388~(5)	2,268~(42)	2,005(25)	854.3 (26)	1,742~(28)	1,608~(44)	2,365~(23)	1,296(36)	2,059~(30)	1,653~(33)
TSR 17p	14.26	89.75	11.33	72.45	147.62	48.73	31.17	63.72	59.41	104.44
mTL-1 12qB (# values)	5,976 (62)	5,666~(40)	4,876 (56)	5,525~(98)	5,261~(65)	6,055(29)	5,997~(49)	5,008(81)	5,557~(32)	4,639~(68)
mTL-2 12qB (‡ values)	5,579~(17)	4,817 (107)	4,414 (17)	3,456~(125)	3,988~(24)	4,665 (47)	4,874(20)	4,456~(55)	5,041~(18)	4,270~(90)
TSR 12qB	77.54	87.89	77.00	150.25	432.99	391.55	214.72	122.12	58.84	52.87
mTL-1 12q $\Delta$ ( $\ddagger$ values)	5,630 (30)	6,678~(30)	5,462~(54)	5,127~(24)	4,891 (51)	4,393~(12)	5,082 (28)	2,113(13)	5,282 (28)	6,648 (28)
mTL-2 12q $\Delta$ ( $\ddagger$ values)	5,311(7)	5,744~(17)	4,750 (5)	3,954~(27)	4,793~(30)	4,212~(31)	4,796 (18)	1,704(16)	4,381 (46)	6,132~(12)
TSR 12q $\Delta$	62.31	96.69	118.67	85.19	33.33	50.99	54.69	90.49	102.74	73.93
mTL-1 XpYpE2 (‡ values)	9,643~(52)	2,443~(85)	835.9~(192)	1,202~(171)	8,904~(44)	2,838 (37)	10,382 $(43)$	5,474~(97)	6,105~(54)	5,002~(216)
mTL-2 XpYpE2 (‡ values)	8,967 (24)	2,071 (137)	7,722 (18)	2,308~(183)	8,472 (27)	2,902~(51)	10,334 (31)	4,676 (87)	5,652~(47)	7,623 (32)
TSR XpYpE2 (bp/PD)	132.03	38.51	*	*	146.94	*	9.20	176.55	51.65	*
mTL-1 XpYp Hap.A(\$ values)	ı		698.3(144)	6,094~(25)	ı	12,451 (11)	ı		ı	5,202~(114)
mTL-2 XpYp Hap.A (# values)		I	570.9 (11)	5,038~(47)		11,517 (11)		ı	ı	4,392~(12)
TSR XpYp Hap.A		I	21.23	76.69		263.10				116.05
mTL-1 XpYp Hap.B (# values)	ı	I	8,938 (22)	1,038(38)	ı	2,568~(6)	I	I	ı	7,469 (27)
mTL-2 XpYp Hap.B (# values)		I	8,581 (11)	739.7 (4)		2,543 (8)		·		4,910~(26)
TSR XpYp Hap. B			59.50	21.66		7.04				366.6
Combined TSR (bp/PD)	71.5	78.1	57.6	81.9	190.2	152.3	77.5	113.2	68.2	86.8
$\pm$ SEM	$\pm 24.3$	$\pm 13.4$	$\pm$ 19.5	$\pm 20.5$	$\pm 85.3$	$\pm$ 74.7	$\pm 46.7$	$\pm 24.3$	$\pm$ 11.7	$\pm$ 14.4
Table 3.1: <b>Summary of STELA re</b> obtained from the scatter plots on f using the equation 3.1. The number no TSR was calculated based on the TSR represents the mean for all tel	sults. Median tel àgure 3.12. † indi r of population do t universal primer omeres measured	omere lengths (m cates the numbe ublings used per and allele-specif i n each clone $\pm$	iTL) expressed in r of population d clone is also sho ic primers were u SE. and will be u	abase pairs (bp), oublings between wn in the table a used instead. "2" i sed in figure 3.1	at two differen n the two time nd was calcula indicates that 7.	t time points (n e points. The te ated using the e the allele-speci	nTL-1 represen lomere shorten squation 2.1. An fic primer was r	ts first time pc iing rate (TSR) a asterisk (*) r not used for th	vint and mTL- <sup>2</sup> ) per telomere lext to the TSH e specific clone	, second), were was calculated t indicates that . The combined

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Figure 3.14.A shows the mean for the TSR  $\pm$  SE per clone. ANOVA analysis showed no significant differences between control and shMSH2 clones due to the great error. Nevertheless, as the percentage of MSH2 downregulation decreased, the TSR variation between different telomeres was smaller as shown in figure 3.14.B, implying that downregulation of the MSH2 protein might have an impact on TSR, but it might be different for each telomere. To address this question, the TSR between controls and shMSH2 clones was plotted per telomere in figure 3.14.C and with the exception of the  $12\Delta$  allele, all telomeres in shMSH2 clones shortened at a higher, although not significant, rate compared to control clones, suggesting that some ends might be more vulnerable than others to the loss of the MSH2 protein. Therefore, the TSR was plotted per telomere accounting for the relative amount of MSH2 protein (figure 3.13) and it was found that the B allele for the 12q telomere had an exponential correlation (r = 0.86) with the level of MSH2 protein downregulation while the A allele of the XpYp telomere had a linear (r = 0.96) correlation. Conversely, the other allele for the XpYp telomere had almost no relationship (r = 0.07) with the level of MSH2 protein downregulation. Interestingly, the  $12q\Delta$  allele showed a mild significant (r = -0.63) correlation between the TSR and the protein level although in the opposite direction than 12qB allele.

To try to explain the different behaviour observed between the two alleles of the 12q telomere, the telomere variant repeat region was studied into detail. As shown in figure 3.15.A, 17p and XpYp telomeres had a very homogeneous array of TTAGGG repeats. Conversely, both alleles for the 12q telomere had many more interspersed repeats. In the telomere maps showed in figure 3.15.A, each letter refers to a hexanucleotide (*e.g* T=TTAGGG). Specific hexanucleotide repeat combinations that were present in both alleles or only in one of them were studied and private repeat combinations for 12q $\Delta$ (CG, TTT, CTT, GTTG, TTGT, GGTT, TTTG and TGTT) and for 12qB (CC, TC, CT, CCC, TCC, CCCC and TCCC) were found. Those repeat combinations might be responsible for the different behaviour of both telomeres but in addition, shared repeats were more or less abundant in different alleles (*e.g.* GT, TT, TTG, GGT, GGTG and GTG were much more abundant in the  $12q\Delta$  allele, compared to GG, GGG, TGT or TGTG that were more abundant in the 12qB allele). It is also important to mention that there were 5 different di-hexanucleotide repeat combinations for the  $12q\Delta$  allele and 7 for the 12qB allele. Taking into account that short repeats have a higher mutation rate than longer ones (reviewed in Ellegren (2004) and Shah *et al.* (2010)), the mutation likelihood of the 12qB allele was expected to be higher confirming our results on telomere shortening rates, if a tendency towards deletions *vs* insertions occurred as a result of MMR deficiency (as suggested in chapter 4).



Figure 3.13: **Relationship between TSR and the level of MSH2 downregulation**. The TSR for control clones with no MSH2 downregulation is plotted in X = 0 and each shMSH2 clone, with their respective MSH2 protein level on X-axis. The shortening rate for each telomere is colour coded (red for 17p, green for  $12q\Delta$  allele, pink for both alleles of XpYp -assayed with the universal primer-, brown for A allele of XpYp and purple for B allele). Exponential lines are plotted per telomere with R<sup>2</sup> values of 0.74, 0.40, 0.08, 0.002, 0.93 and 0.005 for 12qB,  $12q\Delta$ , 17p, XpYp, XpYp A and XpYp B respectively.



Figure 3.14: **Telomere shortening rates in WI38 transfected clones.** A plots the mean TSR and SE for all 10 clones analysed. A summary table for these values can be seen in table 3.1. **B** represents the TSR for all telomeres analysed (with a different colour code). The TSR grand mean for each clone is represented as an horizontal line. **C** is a bar graph with mean  $\pm$  SE that compares the TSR for each telomere between controls (solid colour) and shMSH2 clones (stripped bars).





Δ

ХрҮр А

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Telomere length, specially the length of the shortest and longest telomere, has been associated to replicative senescence and chromosome stability (Hemann et al., 2001) and it has been demonstrated that telomerase preferentially elongates the shortest telomeres in a primary cell line transfected with hTERT (Britt-Compton et al., 2009a). The initial mTL for the clones characterized here varied between 698 to 12,451 bp among different telomeres and clones (see table 3.1 for more information). Due to that variability and taking into account the previous studies, I wanted to asses whether the initial telomere length had an effect on the TSR. Figure 3.16 is a plot of the starting mTL of each telomere against the TSR for control and shMSH2 clones. The graph also shows that telomeres with a longer starting mTL had a higher TSR compared to telomeres with a shorter starting mTL independently of the level of MSH2 protein. The linear regression analysis showed a slope of  $0.01\pm$ 0.003 and 0.02  $\pm$  0.009 for controls and shMSH2 respectively but not significantly different to each other (pvalue = 0.67), suggesting that downregulation of MSH2 did not alter the linear relationship between the starting mTL and the TSR. However, the  $R^2$  values for the linear regression were 0.4 and 0.1 for controls and shMSH2 respectively, indicating that the heterogeneity in the TSR found in shMSH2 clones was greater than for controls, possibly due to the different levels of MSH2 downregulation.

### 3.3.5 MSH2 deficiency, TSR and oxidative stress

Free radicals produced during oxidative stress react with the purinic and pirimidinic bases within the DNA producing several types of DNA damage (Dizdaroglu, 2012). To protect against genomic instability, these lesions are usually repaired by any of the following DNA repair mechanisms: base excision repair (BER), nucleotide excision repair (NER) or MMR (reviewed in Bridge *et al.* (2014)). Within the genome, there are regions that are more vulnerable than others to oxidative stress depending on their nucleotide composition. von Zglinicki *et al.* (1995) and von Zglinicki (2002) showed that growing fibroblasts under hyperoxic (40% oxygen) conditions accelerates TSR from 90 bp to 500 bp per PD compared to fibroblasts grown in normobaric (21%) oxy-



Figure 3.16: **Effect of starting mTL on TSRs**. The graph shows TSR in for four control (circles) and six shMSH2 (stars) clones for all different starting mTL. Each telomere is presented with a different colour: 17p in red, 12q B allele in blue,  $12q \Delta$  allele in green, XpYp in pink (both alleles), A allele in brown and B allele in purple. Regression lines are performed for controls (blue) and shMSH2 (red) clones with  $R^2$  of 0.5 and 0.1 respectively. The dotted circles indicate that each telomere was clustered around a specific starting median length equally for controls and shMSH2 clones; the red circle highlights 17p telomere clustered around 2,000 bp and the other circle clusters both alleles for 12q telomere around 5,000 bp.

gen conditions; this might be due to the fact that single strand DNA (ssDNA) breaks are induced at telomeres under oxidative stress ((Petersen *et al.*, 1998) and (Honda *et al.*, 2001)). The presence of extracellular superoxide dismutase in human fibroblasts slows the process of telomere shortening, extending the life span (Serra *et al.*, 2003). In fact, BJ fibroblasts (normal human foreskin fibroblasts), which have a very low level of peroxide content, have a longer lifespan and a lower TSR of 15 to 20 bp/PD (Lorenz *et al.*, 2001) compared to other fibroblasts with a higher peroxide content.

To confirm whether the reduction of oxidative stress impacts on the TSR when MSH2 is depleted, results from a previous study in which a primary cell line with depleted MSH2 was grown at 21%  $O_2$  were compared to results obtained here. Figure 3.17 shows a comparison of the combined TSR for controls and shMSH2 clones at two different oxygen concentrations. Taking into account that oxidative stress increases the TSR (von Zglinicki *et al.*, 1995), when comparing results from the current experiment (at 5%  $O_2$ ) with that carried out by Mendez-Bermudez & Royle (2011) (at 21%  $O_2$ ), it would be expected to find a lower TSR for the control clones, due to the reduction of oxidative

stress in the present study. Surprisingly, one-way ANOVA analysis showed no significant differences between the TSR for controls under different oxygen conditions what might be due to a) primary cell specific telomere attrition rates or b) no changes on TSR between 21 and 5%  $O_2$ . Notwithstanding, the TSR obtained under 5%  $O_2$  condition for WI38 cell line agreed with previous studies on the same cell line and oxygen conditions ((von Zglinicki et al., 1995) and (Dumont et al., 2001)). Two-tailed t-test showed significant differences (p = 0.005) between controls (51  $\pm$  6 bp/PD) and all shMSH2 clones (201  $\pm$  34 bp/PD) for Mendez-Bermudez & Royle (2011), contrasting with no significant differences (p = 0.13) between controls (72  $\pm$  5 bp/PD) and shMSH2 clones  $(115 \pm 20 \text{ bp/PD})$  analysed in this chapter. However, the variances between both groups were significantly different (p = 0.03) as predicted by the greater heterogeneity in the TSR for shMSH2 clones. In addition, when clones with a greater than 60% downregulation level were compared with control clones, a moderate significance (p = 0.05) was shown. Finally, the significant difference found between shMSH2 clones grown at 21 and 5%  $O_2$  might indicate that telomeres in MMR-deficient cells are more vulnerable to oxidative stress and hence, some of the high shortening found previously might be a consequence of accumulation of incorrectly repaired oxidative damage at telomeres.



Figure 3.17: Telomere shortening rates under different oxygen conditions. Comparison of the combined telomere shortening rates between this study and the one performed in Mendez-Bermudez & Royle (2011). A shows mean  $\pm$  SE per clone from Mendez-Bermudez & Royle (2011) in grey (control) and black (shMSH2) bars and data from table 3.1 in blue (control) and red (shMSH2) bars. The level of MSH2 downregulation is shown below each bar for shMSH2 clones. B shows mean  $\pm$  SE for all controls and shMSH2 clones from graph A. C shows same controls as B but for shMSH2 only clones with a greater than 60% protein downregulation are shown. One-way ANOVA p values are: \*\*p = 0.01, \*\*\*p = 0.001 and \*\*\*\*p < 0.0001.

## 3.4 Discussion

In this experiment, stable clones with depleted MSH2 protein were generated and grew under low oxygen conditions  $(5\% O_2)$  to study the effect of MSH2 downregulation on telomere shortening rate (TSR) with reduced levels of oxidative stress. In total, 21 clones for the WI38 primary cell line stably expressing the shRNA shown on figure 3.3 were isolated, 9 of which had a protein downregulation level higher than 50% demonstrated by western blot. In addition, 7 control clones expressing the plasmid without the shRNA were isolated in parallel. Kansikas *et al.* (2014) assayed the repair efficiency of human cells with variable proteins levels (25 to 75%) for the most common MMR genes to mimic Lynch syndrome (LS) heterozygous patients and found that for MLH1 and MSH2 genes, the total loss of repair was reached when less than 25% of the protein was expressed. This suggests that only clones with a greater than 75% protein downregulation (in this study clones sh143, sh123 and sh110) might be expected to have completely depleted DNA mismatch repair function.

The telomere length was analysed by STELA (single telomere length analysis) and the TSR for three telomeres was calculated using the equation 3.1. The difference in TSR between control and all shMSH2 clones grown in 5%  $O_2$  was not statistically different although a greater heterogeneity in TSR in MSH2 downregulated compared to control clones was observed. There was a trend towards an increase in telomere attrition rate for some telomeres (17p, 12qB and XpYp A and B alleles) but not for the 12q $\Delta$  allele and specific repeats in the proximal telomeric region of this allele, that might be more vulnerable to deletions (CC, TC, CT, CCC, TCC, CCCC and TCCC) than others (CG, TTT, CTT, GTTG, TTGT, GGTT, TTTG and TGTT) were identified. Furthermore, although the linear relationship between the TSR and the starting telomere length was weaker for MSH2 than control clones, the linear trend was maintained, suggesting that shorter telomeres shortened at a slower rate than longer ones independently on the MSH2 protein level. Then, results shown in this chapter (under 5%  $O_2$ ) were compared with those performed under normoxic (21%  $O_2$ ) oxygen conditions (Mendez-Bermudez & Royle, 2011) and surprisingly it was found that the telomeres for control clones grown at different oxygen concentrations did not shorten at different rates. This might be explained by any of the following hypothesis:

- 1. Telomeres for the WI38 cell line might shorten at a higher rate than for the CCD34-Lu cell line at the same oxygen condition and therefore, under different conditions, telomeres might shorten at similar rates. This possibility is supported by the fact that the TSR for the WI38 cell line compared to other primary fibroblast strains (such as MRC5, IMR91 or HCA2) is greater (Britt-Compton *et al.*, 2006), being 132 bp/PD the rate at 21%  $O_2$  and 72 bp/PD at 5%  $O_2$ .
- 2. The effect on telomeres between cells grown at 21% or 5% O<sub>2</sub> is not significant to show differences between the TSR for controls. This possibility is unlikely as previous studies showed differences on shortening rates for other cell lines comparing those two oxygen concentrations (von Zglinicki, 2002), but as some primary cell lines are less vulnerable than others to oxidative stress (due to their high antioxidant content) such as BJ fibroblasts (Lorenz *et al.*, 2001), it might be possible that the differences between the two cells lines in different oxygen environment is too low to be detected (or more telomere ends should have been tested to see an effect).
- 3. The TSR for the CCD34-Lu cell line might be underestimated if telomerase was elongating the shortest telomeres. In Mendez-Bermudez & Royle (2011), transfection with hTERT was done to allow the primary cell line undergo a higher number of cell divisions before entering senescence. Although telomere shortening was observed despite the presence of telomerase, it might have occurred that the TSR reported was an underestimation because of the presence of telomerase. Being that the case, it would also be anticipated that the TSR for shMHS2 clones may also have been underestimated.

TSR comparisons between control and MSH2 downregulated clones presented in this chapter were only significantly different when clones with a greater than 60% downregulation were included. Furthermore, the TSR between MSH2 clones were significantly different between the two oxygen conditions, suggesting that the significant differences found at  $21\% O_2$  arose, at least partially, as a consequence of an incorrect repair of oxidative damage at telomeres. ATR is known to suppress telomere fragility (reviewed in Verdun & Karlseder (2007)) and is activated by replication stress (reviewed in Yang et al. (2004) and Zeman & Cimprich (2014)) or different DNA damages, including oxidative stress ((Wang & Qin, 2003) and reviewed in Yan et al. (2014)). In addition, MSH2 has been shown to recruit ATR after exposure to different damaging agents such as MNNG (Methylnitronitrosoguanidine) or cisplatin (Pabla et al., 2011) suggesting that the resistance to some chemotherapeutic drugs such as 5' FU (5-fluorouracil) in MMR-deficient colorectal patients might be due to the inability to activate the DNA damage Response (DDR) by MSH2-ATR interaction. Besides, reduced amounts of MSH2 might interfere with the correct repair of replication errors that are more prone in repetitive regions such as telomeres, increasing the telomere attrition rate if there is a tendency to accumulation of losses against gains of repeats. It has been demonstrated in mice that loss of MSH2 leads to telomere capping defects (Campbell et al., 2006) contributing therefore to telomere shortening and our results suggest that depletion of MSH2 contributes to an accelerate TSR partially due to a) an incorrect repair of oxidative damage at telomeres and b) an accumulation of uncorrected deletion loops that might be telomere-variant repeat specific.

# 4 Telomere instability in a cancer cell line deficient for MSH2

## 4.1 Background

The DNA mismatch repair pathway (MMR) is known to play a major role in correcting replication errors (single base or small insertion/deletion loops) that arise despite the selectivity and proofreading functions of DNA polymerases (reviewed in McCulloch & Kunkel (2008) and Flood et al. (2015)). The first report of spontaneous mutation rates of a dinucleotide in normal human cells suggested that they occurred ranging from  $3.1 \times 10^{-8}$  to  $44.8 \times 10^{-8}$ mutations/nucleotide/generation (Boyer & Farber, 1998) and more recently, a background rate of  $5 \times 10^{-5}$  slippage mutations/locus/cell division was reported (Lever & Sheer, 2010). As previously explained in section 1.1.4, mutation rates significantly increase in MMR deficient cancer cells (specially MSH2<sup>-/-</sup> and MLH1<sup>-/-</sup>) compared to MMR proficient (Hanford *et al.*, 1998). This results in microsatellite instability (MSI), which has been proposed to be an early event in tumorigenesis in MMR defective cancers and persists after transformation (Shibata et al., 1994). Recently, Supek & Lehner (2015) showed that mutations arising after MMR inactivation are spread homogeneously along euchromatic and heterochromatic regions in comparison with mutations in MMR-proficient tumours (that accumulate preferentially in heterochromatic regions), confirming that MMR defects increase genomic instability. Furthermore, Tomasetti et al. (2015) showed that the number of mutations in colorectal cancers (CRC) with MMR deficiency were about ten times higher than in MMR-proficient CRC, although he suggested that only three driver mutations were sufficient to trigger carcinogenesis using a mathematical model that considered the cancer incidence and the number of somatic mutations in MMR-deficient patients. Interestingly, higher mutation rates were also found in blood from LS patients (heterozygous carriers for a germline MMR gene mutation) compared to blood from age-matched sporadic CRC patients ((Parsons *et al.*, 1995) and (Coolbaugh-Murphy *et al.*, 2010)), suggesting that the instability driven by the MMR defect might also occur, at lower levels, in normal tissues such as the germline, bone marrow or intestinal crypts (all tissues with a high mitotic index). If those mutant alleles were transmitted to the next generation through the germline, they might contribute to genetic anticipation as they might increase the cancer risk for an earlier onset (Seguí *et al.*, 2013).

There are two types of mutations occurring in short tandem repeat (STRs) regions or microsatellites: point mutations and frameshift mutations, being the latter the most frequent (reviewed in Li *et al.* (2004) and Pumpernik *et al.* (2008)). The MMR pathway detects the single-base changes and the insertion/deletion loops correcting both types of mutations (Kolodner, 1996); however, under MMR-deficiency the frameshift mutations, occurring due to replication slippage, contribute to MSI. It has been shown that the genomic location, length of the array and sequence context affect the mutation rate of microsatellites (Harfe & Jinks-Robertson, 2000b). The repeat unit size (mononucleotide, dinucleotide, trinucleotide, *etc.*) is inversely correlated to mutation frequency while motif length (number of units) is directly related (reviewed in Shah *et al.* (2010)).

Mutations in microsatellites have been proposed to affect tumour progression by three mechanisms: gene activation, gene inactivation or gene expression levels change (reviewed in Shah *et al.* (2010)). Actually, many protooncogenes such as *KRAS* or *EGFR* and some tumour supressor genes such as *RB* or *TP53*, have repetitive regions within their sequence and in addition, cancer predisposing genes such as *MSH2*, *MLH1*, *MSH6*, *PMS2*, *BRCA1*, *BRCA2*, *NF1*, *NF2*, *APC*, *PTCH1*, and *VHL* also contain repetitive structures within their sequence (Centelles, 2012), (Schmidt & Pearson, 2016) and (Smith *et al.*, 2016). Besides, Ahuja *et al.* (1997) showed that MSI tumours had a higher promoter methylation frequency with a more extensive pattern due to an increase in the frequency of *de novo* CpG methylation, suggesting an interaction between the MMR pathway and chromatin remodeling activities.

Telomeres, due to their repetitive nature, might be expected to show high instability under MMR deficiency; in fact, Pickett et al. (2004) showed a higher telomere mutation frequency telomere mutation frequency (defined by the appearance of mutations that changed the sequence of the assayable proximal variant repeat region of the telomere) in MMR-defective colon tumours than in sporadic colon cancers and Mendez-Bermudez & Royle (2011) suggested that this instability might be biased towards deletions, contributing to an increase in telomere shortening rate (as previously discussed in section 3). Furthermore, Shah et al. (2010) reviewed the mutational bias depending on the DNA structure and MMR defect and suggested that MSH2 and MLH1 mutants might have a bias to deletions. Among all MMR-defective colon cancer cell lines analysed in Bermudez (2007), LoVo (MSH2<sup>+/-</sup>) had the highest telomeric mutation frequency 16%, compared to  $MLH1^{+/-}$  (3.9-6.4%) or  $MSH6^{+/-}$  (5.6%) cancer cells. A deeper look into the mutant molecules from the previous study also suggested that there might be a tendency towards deletions against insertions (5:1) although it was not statistically significant. Conversely, mutations in the *hPMS2* gene have been involved in tetranucleotide repeat expansions ((TTTC/AAAG) and (TTCC/AAGG) sequences) suggesting that microsatellite instability due to different MMR gene mutations might have distinct impacts on telomeres (Shah & Eckert, 2009).

The traditional methods to measure MSI were based on PCR, allowing to amplify some mini or microsatellite markers in tumour and normal tissue and to distinguish between the mutated version and the non mutated based on their length ((Jeffreys *et al.*, 1991) and reviewed in Geiersbach & Samowitz (2011) and Kim & Park (2014)). This has been used to discriminate between microsatellite stable (MSS) and unstable (MSI) tumours, contributing to the prognosis; however, due to the high frequency of mutated *versus* progenitor alleles in the tumour compared to the normal tissue, the frequency of the mutated allele for a specific loci cannot be estimated. The use of small pool PCR analysis can be used to detect the progenitor and the low-frequency mutant alleles thus, providing a more accurate estimate of the mutation frequencies (Coolbaugh-Murphy *et al.*, 2004) and single molecule PCR has been used to study germline mutations in microsatellites and expanded simple tandem repeats (Beal *et al.*, 2015). However, MSI analysis based on PCR are usually restricted to the specific markers used for clinical diagnosis and give poor information about MSI across the genome. Nishant *et al.* (2009), Kim & Park (2014) and Zavodna *et al.* (2014) reviewed how can the new sequencing technologies contribute to explore this issue as they allow to a) identify somatic alterations, b) estimate mutation rates, c) compare mutation rate variation between individuals and d) detect new rare microsatellite variants.

Baird *et al.* (1995) developed a PCR-based method to study the telomere variant repeats (TVR, described in section 2) and it has been used to estimate telomere mutation frequencies as mutational analysis on the canonical telomere repeat array cannot be performed ((Coleman *et al.*, 1999), (Baird *et al.*, 2000), (Pickett *et al.*, 2004), (Mendez-Bermudez *et al.*, 2009) and (Mendez-Bermudez & Royle, 2011)). However, it is important to note that a) pure repeat arrays are expected to have a higher slippage mutation frequency than short interspersed repeats and b) there is a limit in the type of mutations that can be scored by TVR, as only mutants that change the pattern (one single event) can be detected while mutations that restore the original pattern (more than one mutational event) will remain masked.

In this chapter, I present a XpYp telomere mutation analysis in the LoVo colon cancer cell line based on TVR analysis on single molecule STELA products to amplify individual telomeres. I compared the results with those shown in Bermudez (2007) to increase statistical power and to look for shared mutations. In addition, I exploited the potential of capillary electrophoresis to resolve TVR products to improve accuracy, resolution and throughput. Finally, I examined the use of a third generation sequencing technology (based on nanopores) to sequence the XpYp telomere in LoVo as a new approach to get a full map of the interspersion pattern.

## **4.2 Aims**

Single molecule STELA (smSTELA) products have been previously used to detect somatic mutation frequency in normal and colon carcinomas (Jeyapalan *et al.*, 2008), (Mendez-Bermudez *et al.*, 2009) and (Mendez-Bermudez *et al.*, 2012). Therefore, to understand whether MSH2 deficiency leads to gains or losses of repeats smSTELA products were generated from the LoVo (MSH2<sup>-/-</sup>) cell line to analyse telomere mutant molecules (defined by the appearance of mutations that changed the sequence of the assayable proximal variant repeat region of the telomere). The objectives for the aim described above were:

- To generate more than 100 smSTELA that will be further analysed by Telomere Variant Repeat (TVR) PCR.
- To resolve TVR products by capillary electrophoresis to increase throughput.
- To compare data with previous mutation analysis on the same cell line to look for shared mutants and to increase the sample size.
- To use a new third generation sequencing method (MinION<sup>™</sup> sequencing) to study STELA products from the LoVo cell line to explore the more distant telomere variant repeat region.

## 4.3 Results

#### 4.3.1 Mutant telomeres analysed by traditional TVR

Generation of 303 smSTELA products was performed in 96 well plates by diluting LoVo DNA to 90-170 pg per PCR. To confirm the presence or absence of PCR products and to measure the telomere length of the amplicon, size separation by agarose gel electrophoresis in a 0.8% LE agarose gel and subsequent Southern blot hybridisation to a random-primed  $\alpha^{32}$ P-dCTP-labeled telomere and ladder (1kb and HR) probes as described in section 2.2.2.8 was performed. Figure 4.1 shows an example of a blot with some single and double molecules and a diagram indicating primer annealing sites. Single telomeres generated by STELA were amplified in a final 20 µl volume, and 5 µl were run by agarose gel electrophoresis as shown in figure 4.1; therefore, less than 15 µl were available for subsequent TVR-PCR (performed as described in section 2.2.2.9). Only 1 µl was needed per PCR but different primer pairs and conditions were tested, and the abundance of negative results even for the positive controls, resulted in the final analysis of only 130 smSTELA products.



Figure 4.1: **Single XpYp telomere molecules generated by smSTELA.** The blot on the left shows some single and double bands that were run in a 0.8% agarose gel to screen for single bands. The LoVo cell line is heterozygous (haplotypes A and B) for the XpYp telomere and as only the A allele is informative for TVR, the allele-specific primer (XpYp 427G/415C) for this haplotype was used to generate smSTELA. The diagram on the right, shows the structure of the smSTELA products (green), indicating with a blue arrow the position of the primers used for STELA. The nested PCR that involves the analysis of the telomere variant repeats (TVR) is shown in red. The position of the forward primer (also haplotype A-specific) is shown with a blue arrow and all the potential binding sites for variant repeat-specific primers with grey arrows.

Application of the original protocol described by Baird *et al.* (1995) resulted in the detection of some (33) variant repeats maps within the smSTELA products but the abundance of negative results suggested that our conditions were suboptimal. To control for PCR independent variables, *e.g.* gel mix and electrophoresis conditions, large amounts of 10X TBE and 6% acrylamide-urea gel mix were produced for exclusive TVR use. In addition, annealing temperatures tested by gradient PCR (shown in figure 4.2) and labeling conditions of the TS30T primer by dot blot (shown in figure 4.3) were assessed. A range of annealing temperatures (from 61 to  $67^{\circ}$ C) was assayed with no significant change in the amplification efficiency from  $63-66^{\circ}$ C (figure 4.2). We decided to use the highest temperature ( $66^{\circ}$ C) as it seemed to be more stringent for the TagTelW primer that, despite being specific for TTAGGG repeats amplification, it can also amplify TGAGGG repeats under suboptimal PCR conditions. For the denaturing acrylamide gel electrophoresis conditions, 60W were used and depending on the track to be resolved, longer (>3 hours) or shorter (2 h  $30^{\circ}$ ) time were employed.

In addition, the 5' end labeling conditions for the flanking primer (TS30T) were optimised as described in section 2.2.2.5. Briefly, a template for the subtelomeric XpYp telomere was dot blotted and the TS30T primer labeled using different conditions was used as probe. The method described in Baird *et al.* (1995) uses 0.5  $\mu$ M for the allele-specific forward primer (TS30T in our experiment) so we tested concentrations ranging from 0.2 to 1  $\mu$ M to discover if the primer concentration had an effect on labeling efficiency. The top part of figure 4.3 shows that concentrations above 0.6  $\mu$ M of TS30T primer gave similar and higher relative intensities than concentrations below 0.6  $\mu$ M, suggesting that we could use the concentration recommended by Baird *et al.* (1995). Nevertheless, we further used different amounts of  $\gamma^{32}$ ATP and found that reducing the amount of radiation (from 37 to 26 Bq/reaction) and increasing the primer concentration (from 0.5 to 1.0  $\mu$ M) we could reach higher labeling efficiency (bottom part of figure 4.3).



Figure 4.2: **TVR optimisation conditions. A** is a denaturing acrylamide gel electrophoresed at 60 Watts (W) for 3 hours, 2 hours and 30 minutes and 2 hours to show the time effect on TVR product resolution. Two different smSTELA products (B7 and B8) were run and three annealing temperatures were used (61, 62 and 63°C). **B** shows two acrylamide sequencing gels comparing the effect of power and time. The one on the left, containing the same products that on figure A, was run at 55 W for 2 hours and 20 minutes and the one on the right, containing two new products (E2 and E7) was run at 60 W for 2 hours and 35 minutes. **G** represents TGAGGG repeats (amplified using TagTelX primer), **T** represents TTAGGG repeats (amplified using TagTelV primer) and J represents TTGGGG (amplified using TagTelJ primer) repeats.

Using the conditions described before (annealing temperature 66°C, 26 Bq/reaction and 0.1  $\mu$ M of TS30T primer) and in the methods (section 2.2.2.9), 33 molecules were analysed by 5'  $\gamma^{32}$ P-dATP end labeled flanking primer, two of which were the same deletion mutant (mutant A) involving the deletion of 6 T repeats and giving a mutation frequency of 7.7%. Figure 4.4.A shows an example of a gel including one mutant molecule and 8 non mutants. Their



Figure 4.3: **Primer labeling efficiency using dot blots.** A shows quantification of dot blots (on the left) using different TS30T primer concentrations (ranging from 0.2  $\mu$ M to 1.0  $\mu$ M, A to E respectively). B shows quantification of dot blots (on the left) using combinations of primer concentrations (1.0 and 0.6  $\mu$ M) and  $\gamma^{32}$ ATP concentrations (26 and 37 Becquerels/reaction). Mean and standard error of the mean from triplicate experiments is shown. \*p = 0.01 and \*\*\*p = 0.0003 using Fisher's test.

corresponding telomere variant map is also indicated in figure 4.4.B and compared with that previously generated by Mendez-Bermudez & Royle (2011). Although a very similar telomere map was obtained, the two more distant blocks of T repeats that were previously described could not be amplified; furthermore, many negative results (including positive controls) were generated and the repetition of experiments reduced the amount of smSTELA available for further analysis. In addition to those reasons, the method described in Baird *et al.* (1995) is low-throughput and labor-intensive and mutants are scored manually; therefore, the same principle was applied to capillary electrophoresis as described in section 2.2.2.9 to improve throughput.



Figure 4.4: **Telomere variant maps from LoVo DNA. A** shows details for an example TVR gel and their related variant map. Each vertical lane represents amplification of a particular variant repeat and 10 different sm-STELA are shown. Key for the map: **G** represents TGAGGG repeats (amplified using TagTelX primer), T represents TTAGGG repeats (amplified using TagTelW primer) and J represents TTGGGG (amplified using TagTelJ primer) repeats. **B** shows a comparison of the maps generated in this experiment with previous published data from the same MMR-deficient cell line. Black boxes indicate inconsistencies between the two maps.

#### 4.3.2 TVR by capillary electrophoresis

We first validated the suitability of capillary electrophoresis for our purpose using PCR products on the DRr (right direct repeat) region of the Human Herpesvirus-6A (HHV-6A). This region was chosen because a) the HHV-6A genome contains telomere-like repeats allowing it to integrate into the human telomeres (Huang *et al.*, 2014) and b) large amount of amplicons of a determined size could be generated. Amplicons were generated by Dr. Victoria Cotton with U100F and TJ1R primers on different cell lines and when this amplicon was assayed by TVR with TagTelW primer it produced different TVR patterns depending on the cell line of origin. Figure 4.5 compares the patterns obtained either using a 5'  $\gamma^{32}$ P-dATP end labeled flanking primer or a 5' FAM-labeled TagTelW primer and resolving the PCR products in an ABI 3100 Genetic Analyzer (see section 2.2.2.9 for a detailed comparison of the two methods), indicating that capillary electrophoresis is not only capable of detecting TVR products and distinguishing between different patterns but it also gives a greater resolution as products longer than 1 kb were detected.

Subsequently, smTELA products for LoVo DNA were assayed using FAM-TagTelW, NED-TagTelX and HEX-TagTelJ primers. To improve the signal intensity we increased the number of PCR cycles from 19 to 24 for all three primers and annealing temperatures were maintained as 66°C. The use of fluorescence and capillary electrophoresis allowed us to detect the distant T block that was not detected using 5' end labeled flanking primer and no other additional repeats were observed using capillary electrophoresis suggesting a) generation of the same map and b) absence of PCR artifacts due to the primer modifications. Chromatograms were analysed using the GeneMapper v4.1 software. A set of bins was created with a mean size for each bin corresponding to the location of the first nucleotide of each repeat  $\pm 1$  nt, allowing comparisons to the progenitor allele. Figure 4.6 shows a comparison of the progenitor allele map generated using 5'  $\gamma^{32}$ P-dATP end labeled flanking primer and 5' fluorescent end labeled variant repeat-specific primers, indicating the position and correlation of each peak within the map.



Figure 4.5: Validation of TVR analysis by capillary electrophoresis. The top part of the figure shows five telomere maps generated by Dr. Victoria Cotton using the 5'  $\gamma^{32}$ P-dATP end labeled flanking primer (TJ1R) and TagTelW reverse primer as described in Baird *et al.* (1995). The bottom part of the figure shows five chromatograms from the same amplicons using FAM-labeled TagTelW primer and the ABI 3100 Genetic Analyzer for resolution of the PCR products. Red lines show the comparison for each block. The chromatogram presents all amplification products following a linear relationship compared to the size ladder; however, the mobility of the products in a denaturing acrylamide gel follows an exponential, non-linear, function and therefore, to allow comparison of the results between both methods, the red lines shown on the figure are not straight. The codes BAN59, 7022, COR264, KK and 813 refer to each cell line.



Figure 4.6: **Telomere variant map details of the progenitor XpYp A haplotype. A** is a screenshot of the GeneMapper v4.1 software used to screen TVR fragments. Vertical grey, blue and green lines are bins for G (TGAGGG), T (TTAGGG) and J (TTGGGG) repeats respectively. **B** shows a detail of the progenitor chromatogram generated by capillary electrophoresis. The top part of the figure shows three superimposed chromatograms (**black** for TGAGGG, blue for TTAGGG and green for TTGGGG repeats). Letters below each chromatogram indicate the letter and colour used to generate the telomere map. Colours for the map are different to those in the chromatogram to be able to compare the telomere map with previous versions in (Bermudez, 2007). **C** compares the map generated using either fluorescence or  $\gamma^{32}$ P-dATP with the reference allele from the previous study. Black boxes indicate inconsistencies between the maps.

In total, 130 smSTELA were analysed by TVR (33 using  $\gamma^{32}$ P-dATP and 97 using fluorescence and capillary electrophoresis), 25 of which contained mutations (19.2 % mutation frequency) corresponding to 7 different types of mutations (see figures 4.7 and 4.9 for a detailed comparison of the mutant molecules with the progenitor allele):

- <u>Mutant A:</u> this mutant was present two times and showed a large deletion of the most distal block of J repeats. It was estimated that, at least, 18 repeats were lost, thus around 108 bp were lost per molecule due to this mutant.
- <u>Mutant B</u>: this mutant was present two times and contained the same mutation as clone 54 in <u>Bermudez</u> (2007). Two J repeats were lost in the first block of 15 J repeats, resulting in the detection of only 13 J repeats; this was equivalent to 12 bp lost per molecule.
- <u>Mutant C</u>: this mutant was present four times and contained two repeat deletions. As the 15 J block of repeats started one repeat earlier compared to the progenitor allele but no T repeat lost was found, we estimated that one of the repeat loss corresponded to an N repeat between the third T block and the first J block. In addition, there was one T repeat loss in the 9 T block repeat, resulting in the displacement of the last J block of repeats. In total, 12 bp were lost per molecule as in mutant B.
- <u>Mutant D</u>: this mutant was found only once and it showed lost of 2 J repeats, one in the first block of 15 J, resulting in a track of just 14 J, and the other in the second block of 7 J, resulting in a track of 6 J. As total number of base pair loss, this mutant was equivalent to B and C and 12 bp were loss.
- <u>Mutant E:</u> this mutant was found only once and it showed a gain of 1 J repeat in the 7 J repeats block, resulting in a block containing 8 J repeats and gain of 1 T repeat in the 9 T block. In total, 12 bp were gained in this molecule.

- <u>Mutant F:</u> this mutant was the most frequent and it was found 14 times. It contained a complex semi-duplication in the most distant portion of the 500 bp variant repeat array that could have arisen as a result of single strand annealing recombination (as shown by Tishkoff *et al.* (1997b) in *S. cerevisiae* when the *RAD27* gene was mutated). The complex insertion included: 6 G repeats, between the 7 J and the 6 J blocks, and following the last J block, 4 T, 2 N, 10 T and 15 J, resembling the variant map of the progenitor allele but in a more distant position. We estimated that, at least, 150 bp were gained in those mutants and due to its complexity and the abundance of molecules carrying this mutation, it was expected that it had occurred very early during the cell expansion. There was one insertion mutation in Bermudez (2007) that could not be described (clone 79) as the gain occurred further in the array. As this mutant was the most frequent, it might be anticipated that it might contained the same mutation as clone 79.
- <u>Mutant G:</u> this mutant was found only once and it contained a 4 repeat deletion. One of them, occurred in the first block of 5 T, where only 4 where detected; the next one, containing two J repeat deletion occurred in the 15 J block, what would be equivalent to mutant B, and the last one, occurred in the 9 T block where one T repeat resulted in the detection of only 8 T repeats. In total, 24 bp were lost in this molecule.

To calculate the mutation rate (probability of a mutation occurring per cell division) based on the Luria and Delbrück method (reviewed in Pope *et al.* (2008)), mutation data is needed at different time points to estimate the likelihood of a mutation event to occur earlier or later in the cell growth. However, the calculation of the mutation frequency (proportion of mutants present in a population), although less informative, only requires number of mutants. If the number of cells per tube is known, the fluctuation test would allow to calculate the mutation rate as the number of cells in the population approximately equals the number of cell divisions. In the present experiment, no data was obtained based on the total cell number and no estimates could be done on the number of cell divisions, thus, only mutation frequencies are reported. The mutation frequency of 19.2% corresponded to the 25 mutant molecules, described above, from the total of 130 molecules analysed. However, if we considered it in a more conservative way, each different type of mutant (7 types) would represent a distinct mutation event and the abundance of one type compared to another, might only reflect how early or late it occurred during cell expansion and not how likely is this event to happen. Being that the case, the mutation frequency would be 5.4% as shown in table 4.1 and this hypothesis would explain why we found mutant F 14 times. As it is a very complex insertion in which, part of the variant map was semi-duplicated without interruption of the J repeats pattern, it might be expected to occur only once as an early event and not 14 independent times. Thus, considering that we found 5 different types of deletion mutants (all corresponding to an overall loss of 168 bp) and only 2 different types of insertions (corresponding to an overall gain of 162 bp), our results suggested that deletions were 2.5 times more frequent than insertions, confirming previous results in Bermudez (2007).



Figure 4.7: Details of the mutant XpYpA telomeres found in LoVo DNA. The progenitor allele is shown at the top of the figure. Four deletions found by capillary electrophoresis are shown below (the fifth mutant -A- was detected using  $\gamma^{32}$ P-dATP). The two insertion mutants are shown at the bottom of the figure. The number of molecules scored per mutant are shown below the mutant name.

In addition, to prove that the same molecule was not analysed more than once (and therefore biasing the data), smSTELA products were sized using ImageQuant software, version 7.0. All smSTELA had different lengths indicating that distinct molecules were analysed and that the mutations found more than once (*e.g.* mutant F) reflected their frequency. Figure 4.8 shows the size for all progenitor allele in purple, with a median  $\pm$  interquartile range telomere length of  $2873 \pm 831$  bp. In addition, the size of the molecules containing insertions or deletion is plotted in green and orange respectively. The median length  $\pm$  interquartile range for the F mutants was  $2868 \pm 758$  bp, not significantly different from the progenitor allele, suggesting that we were analysing the same population of molecules.



Figure 4.8: **Size of the smSTELA analysed by TVR.** The scatter plot shows the size of all 130 smSTELA analysed by TVR, differentiating between progenitor allele (in purple), deletion (in orange) and insertion (in green) molecules. For the progenitor allele and mutant F, medians and interquartile ranges are shown.

Subsequently, we compared telomere maps generated here (figure 4.9.A) with those from Bermudez (2007) (map comparison shown in figure 4.9) to a) increase the population size of mutant molecules and b) detect shared mutations. Only mutant B described here was shared between both studies (being the same mutation found in clone 54). Interestingly, as the same mutation was found in mutant G, accompanied with 2 T repeats deletions, we could hypothesise that the mutation B arose earlier in time, and that, for mutant G, in successive S phase cycles, it would have accumulated the additional loss of the 2 T repeats. Furthermore, in both studies it was found a deletion of one

J repeat in the largest block of J repeats that was either accompanied by loss of 1 or 2 T repeats (clones 28 and 33 respectively) or a loss of an additional J repeat in the next J repeats block (mutant D), suggesting that the loss of the first J repeat might have been another early and shared event in both studies. Additionally, one large deletion (mutant A) and one complex insertion (mutant F) were found in the present study but were not previously reported. We could hypothesise that mutant A originated relatively late during cell expansion, as only two molecules were found containing the same mutations; however, as discussed previously, mutant F was likely to have originated as an early event and therefore might have been expected to be detected in the previous study. Interestingly, one of the mutations found in Bermudez (2007) (clone 79) could not be characterised as the location of the mutation in the molecule impeded gel resolution, and as mutant F was very frequent and contained a duplication increasing the size of the array, we could hypothesise that clone 79 contained the same mutation.

The mutation frequency for the combined data based on the number of mutant molecules scored (40/223) was 17.9% with no significant differences between the two studies (Exact Fisher's test). Table 4.1 summarises the mutation frequency accounting for the number of molecules and the type of mutation observed in both studies and figure 4.10 shows a bar graph comparing the results. In both mutation analyses, a greater variation of deletion types was found in comparison to insertions (9:3); however, more insertion molecules were found in total in the present study compared to the earlier one. Therefore, the lack of significance between deletions (23/223) and insertions (16/223) in the combined data (1.4:1 ratio) might be due to the fact that the same mutant molecules were detected more than once. Those mutants, specially mutant F, might be more frequent and were randomly analysed according to their frequency, thus, artificially increasing the mutation frequency. When only accounting for the type of mutant (13/223), the mutation frequency decreased to 5.4% in the combined data; furthermore, there were 9 different types of deletions versus 3 insertions resulting in a 3:1 ratio.

A Progenitor allele:	) N	bp/mut	
"JILLIJJJJJJJJJJJCCCCCNNJJJJJJCCCCCCNNNNCCCCCC	(105)		
Deletions: Mutant A:NNNGGGGGGGGNTTTTTTNTTTTTTNTTTTNNJJJJJJJJ	(2)	-108	
Mutant B:NNNGGGGGGGGGNTTTTTTTTTTTTTTTTTUJJJJJJJJJJ	(2)	-12	
Mutant C:NNNGGGGGGGGGNTTTTTTTTTTTTTTTTTTU IN repeat delation II repeat delation II repeat delation	(4)	-12	
Mutant D:NNNGGGGGGGGGNTTTTTTTTTTTTTTTTTTTTTTTTT	(1)	-12	
Mutant G:NNNGGGGGGGGGNTTTTTTTTTTTTTTTTTTTTTTTT	(1)	-24	
	tal (10)	-168	
LIBET CLUDS: Mutant E:NNNGGGGGGGGNTTTTTTNNTTTTTNNJJJJJJJJJJJJ	(1)	+12	
Mutant F:NNNGGGGGGGGGNTTTTTNNTTTTTTNNJJJJJJJJJJJ	<u> </u>	+150	
	tal (15)	+162	
В			
Progenitor allele:	<u>N</u>	bp/mut	
NNGGGGGGGGTTTTTTNNTTTTTNNJJJJJJJJJJJJJJ	(28)		
Deletions: Clone 28: NNGGGGGGGGGTTTTTTNNTTTTTTNNJJJJJJJJJJJJ	(1)	-12	
Clone 33: NNGGGGGGGGGGGTTTTTTTTTTTTTTTTTTTTTTTTT	(2)	-18	
Clone 54: NNGGGGGGGGGTTTTTTTNNTTTTTTNTTTTTNNJJJJJJJJ	(1)	-12	
Clone 60: NNGGGGGGGGGTTTTTTTTTTTTTTTTTTTTTTJJJJJJJJ	(9)	- 6	
Clone 64: NNGGGGGGGGGGTTTTTTTTTTTTTTTTTTTTTTTJJJJJJJ	(3)	-12	
	tal (13)	-60	
LINSETTION: CLORE 80: NNGGGGGGGGGTTTTTTNNTTTTTTNNJJJJJJJJJJJJ	(1)	+30	
Tot	tal (1)	+30	

Figure 4.9: **Telomere variant repeat maps comparison for the LoVo** (MSH2<sup>-/-</sup>) **cell line. A** shows the progenitor allele map at the top. Each letter, G, N, T and J, represents a 6 base pair telomere repeat. Key for the map: **G** represents TGAGGG repeats, T represents and J represents TTGGGG repeats and J represents TTGGGG repeats. In brackets, the amount of molecules analysed. Mutant molecules are grouped by deletions or insertions. **B** shows data from (Mendez-Bermudez & Royle, 2011) carried out in the same cell line as comparison. Maps for 6 of the 7 mutants found are shown.

	Garrido, 2016	<b>Mendez, 2007</b>	Combined
Molecules analysed: n	130	93	223
Deletion molecules: n (%)	10 (7.7)	13 (14.0)	23(10.3)
Insertion molecules: n (%)	15(11.5)	1(1.1)	16(7.2)
Not defined: n (%)	0 (0)	1(1.1)	1(0.5)
Total mutant molecules: n (%)	25 (19.2)	15 (16.1)	40 (17.9)
Types of del.mutants: n (%)	5 (3.8)	5 (5.4)	9* (4.0)
Types of ins.mutants: n (%)	2(1.5)	1(1.1)	3(1.4)
Not defined: n (%)	0 (0)	1(1.1)	1(0.5)
Total types of mutants: n (%)	7 (5.4)	7 (7.5)	$12^{\dagger}$ (5.4)
Deletions: bp involved	-168	-60	-216**
Insertions: bp involved	+162	+30	+192
Total balance of bp	-6	-60	-24

Table 4.1: **Mutation frequencies for LoVo.** n: number of molecules or mutant types; %: percentage; del: deletions; ins: insertions. \* as mutant B is same mutant as clone 54, nine different types of mutants instead of ten were considered; \*\* to avoid duplication, the 12 bp loss corresponding to mutant B and clone 54 were only considered once; <sup>†</sup> as discussed in the text, the not defined mutant might be mutant F so to avoid duplication, it has only being included once in the total type of mutants.



Figure 4.10: **Graphical comparison of the mutation analyses in LoVo. A** shows the total number of molecules analysed in brown and how many of them were deletions (purple) or insertions (orange). **B** shows in blue the total number of mutant molecules scored, splitting them in deletions (red) and insertions (green).

#### 4.3.3 Sequencing telomeres using the MinION<sup>™</sup> device

The use of the TVR method allowed us to study the telomeric mutation frequencies based on the first 600 bp of the telomere variant repeat array but gave no further information about the telomeric structure following this repeat array. Due to their repetitive nature, telomeres are challenging structures to sequence and although Sanger sequencing was performed on reamplified smSTELA products using either XpYpE2 or Telorette2 primers, the presence of overlapped reads impeded to interpret results. I wanted to study the LoVo XpYp A allele, analysed in the previous section, in further detail to discover if more distant variant repeats were present in the telomeric sequence, so I could characterised this allele better for future analysis.

Telomeres are not suitable for short reads sequencing platforms as their repetitive structure impedes accurate assembly, but the development of third next generation sequencing technologies, that allow long reads sequencing, has become an exciting opportunity to explore the telomeric structure. There are only two commercially available platforms for long reads sequencing at the moment: single-molecule sequencing in real time (SMRT) developed by Pacific Biosciences<sup>®</sup> and nanopore sequencing using the MinION<sup>TM</sup> device developed by Oxford Nanopore<sup>TM</sup> Technologies. The SMRT technology has recently been used to sequence mice telomeres (Tong *et al.*, 2015), demonstrating its suitability for sequencing long repetitive molecules, but, to our knowledge, the nanopore sequencing technology has not yet been explored for telomere sequencing.

The MinION<sup>™</sup> device only started to be commercially available in May 2015 but we had the opportunity to access it earlier through the MinION<sup>™</sup> Access Programme (MAP). The MinION<sup>™</sup> device is a portable sequencer (87 g of weight and dimensions: width 105, height 23 and depth 33 mm) for long molecules, providing integral reads of single molecules loaded onto its flow-cell. It contains a USB 3.0 port that will transfer the sequencing information from the consumable flow cell, connected to the device, to a host computer, where the MinKNOW software is run. Each flow cell contains up to 512 nanopores embedded in a polymer membrane and it integrates an ASIC
(Application-Specific Integrated Circuit) and a sensor chip. The former applies an electric potential across each nanopore and the latter measures the resulting ionic current flow. Each nanopore has four sensors (although only one is active at a time) detecting changes in the electric flow due to the interaction with the analyte (in our case, a deoxyribonucleotide but it can be adapted to detect ribonucleotides or amino acids). The sensor chip collects the information (at a rate of tens of thousand electric signals per second) from each of the active sensor and sends the information to the MinKNOW software that: a) receives the data, b) analyses it and sends feedback in real-time, c) controls the device selecting the run parameters, d) identifies and tracks the sample and e) ensures that the platform chemistry is performing correctly. Figure 4.11 shows a brief description of the device and its components.



Figure 4.11: **MinION<sup>TM</sup> device description.** Figures obtained from Oxford Nanopore<sup>TM</sup> Technologies and composition generated for this Thesis for explanatory purposes. The MinION<sup>TM</sup> device and the flowcell are shown on the left side of the figure. On the right side of the figure, a schematic representation shows a detail of the disruption in the current when a molecule pass through the nanopore.

The DNA library loaded onto the flowcell is double stranded, containing an anchorage molecule (a processive enzyme) in 5' called tether (shown in brown in figure 2.1). This enzyme a) binds to the nanopore, b) allows the DNA sequence to pass through the nanopore one base at a time in a single stranded manner and c) recognises a single stranded overhang (called leader) located at the 3' position of the DNA sequence, detecting when the complementary strand of the DNA molecule is being sequenced. Nucleotides are read as a combination called K-mer generating a specific disruption in the current flow that can be used to determine the order of bases in a particular DNA molecule.

There are two workflows that can be used to base call the MinION<sup>™</sup> reads in Metrichor<sup>™</sup> : 1D and 2D workflows. The former, generate reads from only one strand, and therefore are expected to be less accurate whereas the latter, generates three types of reads: "Template", "Complement" and "Two directions". The first two correspond to the forward and reverse strands while the latter consists of a consensus sequence created by the base calling software for each pair of template/complement (within the same molecule). Here, I will refer to 1D reads as those generated by the 1D workflow and I will include all three types of reads generated by the 2D workflow in the 2D reads. The output data from the MinION™ device are .fast5 files (an application of the HDF5 standard files) that needed to be submitted to the base calling software Metrichor<sup>™</sup>, a cloud-based data analysis service (available in: https://metrichor.com/s/) provided by Oxford Nanopore™ Technologies prior to its analysis. The sequencing analysis were performed in SPECTRE (Special Computational Teaching and Research Environment) that provides a Linux environment enabling large data-set analysis. To convert .fast5 to .fastq files, poretools was used. This toolkit is under development by Nick Loman and Aaron Quinlan and is publicly available in the public repository GitHub (https://github.com/arq5x/poretools). LAST (Local Alignment Search Tool) was used to align the reads to a consensus sequence. The options for the alignment were: -Q1 -r1 -q1 -a1 -b1, indicating that the input was .fastq(-Q1), the match score was 1(-r1), the cost of a mismatch was 1(-q1) and the cost of a gap was set at 1+1x gap length (-a1 -b1) (for more information about this tool go to the manual page: http://last.cbrc.jp/doc/lastal.html). The aligned .maf file was converted to .sam using a python script developed by Martin C. Frith (maf-convert.py). The use of the package samtools allowed us to convert the .sam file into .bam and to get some information about the alignment (for more details about this tool go to the manual page: http://samtools.sourceforge.net) and subsequently, the alignment was visualized using the Integrative Genomic Viewer (IGV) that can be freely downloaded in https://www.broadinstitute.org/igv/ (from the Broad Institute). Finally, to verify the alignment, we retrieved the consensus sequence generated by IGV and performed a ClustalW alignment to the reference using the MacVector software, V11.1.1.



Figure 4.12: **Pipeline for MinION™ reads analysis.** Blue boxed rectangles indicate bioinformatic packages and green ones scripts used. Names in italics are files that resulted from data analysis. Dotted lines indicate a filtering step that was only performed for the telomere libraries sequencing analysis as a first alignment was done on the telomere flanking region and a second was performed only on the reads that aligned to the reference.

As no previous experiments had been carried out in our laboratory, we tried to sequence two well-characterised amplicons that contained some homopolymeric interspersed repeats as controls together with each telomere sequencing experiment. Libraries for each amplicon were generated as described in methods (section 2.2.2.4) and the sequencing analysis was performed as summarised in figure 4.12.

Four sequencing libraries were prepared, two for the control sequences OL A31 and KUK A49 (donated by Enjie Zhang) and two for the XpYp telomere from the LoVo (MSH2<sup>+/-</sup>) cancer cell line (generated by STELA as described in section 2.2.2.4). The OL A31 control amplicon is 4558 bp long, containing 40% G+C content while the KUK A49 control amplicon is 4439 bp long, containing 39% G+C content. Both amplicons contain homopolymeric regions and for both, a concentration of  $\approx 500$  ng was used to prepare the sequencing library. The first telomere library (Tel1) was amplified using the universal XpYpE2 primer that amplified haplotypes A and B and the second telomere library (Tel2) was amplified using the allele-specific XpYp427G/415C primer that amplifies A allele (the same allele that I previously used for mutation analysis). The expected size for the STELA products for both telomere libraries was around 4 kb. The DNA concentration was higher for both control libraries compared to the telomere libraries as for the latter, the STELA products were generated using 250 pg genomic DNA and 25 cycles (as described in section 2.2.2.4) to avoid generating collapsed PCR products compared to the amplification carried out for the controls, that was performed using 10 ng of genomic DNA and 35 cycles as one size single amplicon was obtained.

I used the OL A31 library as internal control for Tel1 library, thus, I first loaded the Tel1 library onto the flowcell and sequenced it for 24 hours. As flowcells can be active up to 72 hours and different experiment can be run in the same flowcell if necessary, I added the OL A31 library on the day after loading Tel1. This allowed me to test the flowcell and to compare the performance of the two different types of libraries (one composed of one single size amplicon -control- and the other, containing different size STELA products). Subsequently, I used the KUK A49 library as an external control, therefore, it was loaded onto a different flowcell that was only used to sequence this control library. This allowed me to test a) the sequencing accuracy without interference of other DNA molecules not related to the amplicon and b) to increase the sequencing time (up to 48 hours). The Tel2 library was then loaded onto an independent flowcell (like KUK A49) and performance of both sequencing experiments was compared.

Figures 4.13 and 4.14 show screen shots of the sequencing runs from the minKNOW (software used to sequence and process the reads from the Min-ION<sup>™</sup> device). When the first telomere library was loaded onto the flowcell, 495/512 pores were active, in comparison with 293/512 that were active after 24 hours when the control OL A31 sequence was loaded. The abundance of pores in salmon colour (figure 4.13.B) contrasts with the shortage of it in figure 4.14.B. Those are unavailable pores that can be activated in the future in the presence of DNA and will turn into green while sequencing; the fact that they were more abundant when the Tel1 library was loaded suggests that not enough DNA was available to activate them. However, when the OL A31 library was loaded, the percentage of unavailable (salmon) pores decreased, and the percentage of reading pores (green) increased compared to the Tel1 library. In addition, the histograms show that reads for the telomere library (Tel1) had a median size around 3 kb (according to agarose gel electrophoresis, the median length is approximately 4 kb) while for OL A31 reads the median was greater ( $\approx 15$  kb), not agreeing with the expected product size (4558 bp) and suggesting that some concatenated artifacts might being sequenced.

Table 4.2 shows the result of the stats script from poretools for the first sequencing experiment in which OL A31 and the first telomere library were run. This script was run on the raw .fast5 files, retrieved from Metrichor<sup>TM</sup>, before the alignment was performed. More 1D reads were obtained for the control sequence than the telomere library but conversely, more 2D reads were obtained for the latter. However, the N50 values were similar between 1D and 2D reads for each library (256 and 532 bp longer in 2D than 1D for OL A31 and telomere library respectively) and agreeing with the estimate product size for OL A31 (4558 bp). As different length products were present in

Tel1 library, the N50 values only suggested that the median of the sequenced products had an estimated length of  $\approx 2000$  bp. The minimum values (shortest read length) for the 1D reads from OLA A31 (23 bp) and the 1D and 2D reads from Tel1 library (21 and 6 bp respectively) were very low compared to the 2D reads for the OL A31 (2273 bp), suggesting that either the sequence started to pass through the pore but quickly detached from it or that some artifacts produced a disruption of the current through the pore, creating a false positive. Either way, the quality values for those reads are expected to be very low compared to expected size reads, and thus filtration of the data for quality scores might be useful for eliminating them.

	OL A31 1D	OL A31 2D	Tell 1D	Tell 2D
total reads	1234	204	262	4305
total base pairs	4376014	874549	187796	3733073
mean (bp)	3546.20	4287.00	716.78	867.15
median (bp)	3948	4353	247	231
minimum (bp)	23	2273	21	6
maximum (bp)	15101	8982	5458	139998
N25 (bp)	4400	4472	2814	4190
N50 (bp)	4106	4362	1911	2443
N75 (bp)	3724	4220	771	1136

Table 4.2: Poretools stats output on the first sequencing experiment in the MinION<sup>TM</sup> device. N25, N50 and N75 refer to the length for which the 25, 50 and 75 % respectively of all bases in the sequences are in a sequence of length L < N (definition from: https://www.broad.harvard.edu/crd/wiki/index.php/N50).

For the sequencing analysis, reads generated from the control OL A31 library were firstly aligned to the reference sequence (4558 bp) resulting in a .bam file with 180 and 571 reads for 1D and 2D respectively. The consensus sequence of the alignment was retrieved from IGV and aligned again to the reference using ClustalW and a 95% identity was reached for the 2D reads (see alignment in appendix C and details of the alignment in table 4.4); however, the 1D reads alignment resulted in a very low (8%) percentage of identity (alignment not shown). In addition, as the control library was run after the Tel1 library, we wanted to discard any interference from the telomere sequences with the alignment and in fact, no alignment was produced when either the telomere or flanking sequence (see below for further explanation) were used as consensus, suggesting that the reads produced when the control sequence was loaded corresponded exclusively to the OL A31 amplicon (possibly due to the higher concentration of this library in comparison with the

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Figure 4.13: **Screen shots for Tell library sequencing run.** A shows the profile of accumulated reads after 24 hours. The top part shows data (such as ID, Asic and heat sink temperatures, or voltage) in reference to the flow cell and in the bottom part there is an histogram indicating the frequency of reads of indicated sizes. B shows the channels panel after a 24 hours run. The top part of the figure is shared with A, and the bottom shows each nanopore channel (identified by a number in white). Colours are: black=saturated (unable to make further reads), cyan= zero (no strand is being read at the moment but it is active for future reads), green=single pore or strand (pores that are currently reading) and salmon=unavailable (not reading or available at the moment but can be active in the future). The green triangle in the corner of some pores indicates the active sensor (out of the four that has each pore) that is active.



Figure 4.14: **Screen shots for the control OL A31 library sequencing run.** A shows the profile of accumulated reads after 24 hours. The top part shows data (such as ID, Asic and heat sink temperatures, or voltage) in reference to the flow cell and in the bottom part there is an histogram indicating the frequency of reads of indicated sizes. B shows the channels panel after a 24 hours run. The top part of the figure is shared with A, and the bottom shows each nanopore channel (identified by a number in white). Colours are: black=saturated (unable to make further reads), cyan= zero (no strand is being read at the moment but it is active for future reads), green=single pore or strand (pores that are currently reading) and salmon=unavailable (not reading or available at the moment but can be active in the future). The green triangle in the corner of some pores indicates the active sensor (out of the four that has each pore) that is active.

telomere one).

The telomere library was aligned with a consensus telomeric sequence of the XpYp (haplotype A) telomere containing up to 9 kb of telomeric repeats  $(TTAGGG)_n$  that commenced with 407 bp of subtelomeric sequence (corresponding to the A allele) followed by a block of variant repeats generated based on TVR analysis. However, and due to the high number of repeats, the alignment generated a high number of false positives, resulting in 249 and 6777 reads (for 1D and 2D respectively) aligned to this telomeric consensus, producing very low identity (11 and 3% respectively) and suggesting that the alignment was artificially created by the amount of repeats and not by real identity. Therefore in a next step, the flanking, subtelomeric region (407 bp) was used as a first consensus to filter data (as shown in figure 4.12). Figure 4.15 shows the pairwise alignment generated with ClustalW. Highlighted in yellow are the known SNPs that differed between A and B alleles. We used a consensus for the A allele sequence but our sequences corresponded to both alleles; therefore, if the highlighted SNPs in the consensus were not enough for allele discrimination during the alignment, a mismatch might be expected in those SNP positions when a B allele read aligned to the reference. The red circles (in figure 4.15) indicate the mismatched positions over the SNPs and the blue circles, mismatched positions in locations other than the SNPs. As expected, the identity was greater (98 vs 93%) for 2D than 1D reads and the location of mismatches in the former was restricted to SNPs.

Finally, using the .bam files generated aligning against the flanking reference, a list of read identifiers (corresponding to the sequences aligned with the flanking sequence) was generated for the 1D and 2D reads and were used to filter the original .fastq file creating a new one that only contained the reads aligning to the flanking region. Subsequently, the consensus for the telomeric sequence described above was used again on the filtered .fastq files. Using this additional step, we: a) reduced the number of sequences that aligned to the consensus telomeric reference: from 249 to 123 for 1D reads and from 6777 to 592 for 2D reads and b) improved the accuracy of the alignment: from 11 and 3% identity (for 1D and 2D reads respectively) to 88% (for both 1D and 2D reads). In addition, the pairwise analysis comparing 1D and 2D consensus was high (93.5%) suggesting that both types of reads might be useful for sequencing. Unexpectedly, in this second alignment, the accuracy in the flanking region decreased as more mismatches (103/407 *vs* 26/407 and 105/407 *vs* 5/407 for 1D and 2D reads respectively) were generated. However, the alignment of 4800 bp reproducing large regions of TTAGGG repeats suggested that this technology might be explored for telomere sequencing. Unfortunately, none of the non-canonical variant repeats studied by TVR (TGAGGG and TTGGGG) were reproduced by this library, although the consensus generated indicated either T or G (K) for the positions where the TGAGGG variant was expected and either A or G (R) for the positions where the TTGGGGG variant the correct nucleotide (visualised on IGV with an allele frequency threshold of 0.5). However, this low accuracy impeded precise analysis of the telomere sequence.

The XpYp B allele in the LoVo cell line has been reported to have a homogeneous TTAGGG telomere with lack of variant repeats (Bermudez, 2007), hence, to avoid misinterpretation of our results during the alignment, we decided to create a pure A allele library by using the XpYp427G/415C primer. In addition, we used a different control library (KUK A49) that was run in a different flowcell to check for sequencing accuracy between experiments, to analyse sequencing performance of the MinION<sup>TM</sup> in homopolymeric repeats and to extend the sequencing time for each library (up to 48 hours) in order to get the maximum number of reads (specially for the telomere library).

	KUK 49 1D	KUK 49 2D	Tel2 1D	Tel2 2D
total reads	15943	12612	2322	2589
total base pairs	39358603	23737063	3354462	3667959
mean (bp)	2468.71	1882.10	1444.64	1416.75
median (bp)	790	660	423	633
minimum (bp)	9	119	9	5
maximum (bp)	363496	8553	69710	64935
N25 (bp)	8880	4408	7693	4406
N50 (bp)	7467	4305	4270	3031
N75 (bp)	3503	3907	2023	1825

Table 4.3: **Poretools** stats **output on the second sequencing experiment in the MinION<sup>TM</sup> device.** N25, N50 and N75 refer to the length for which the 25, 50 and 75 % respectively of all bases in the sequences are in a sequence of length L < N (definition from: https://www.broad.harvard.edu/crd/wiki/index.php/N50).

```
1. Reference vs. 2d.LoVo.E2
  Aligned Length = 407 Gaps = 0
  Identities = 402 (98%)
           1 TTGTCTCAGGGTCCTAGTGTGTGTGTGGAATTGGTGGGTTCTTGGTCTCACTGACTTCAAGA 60
Reference
2d.LoVo.E2
         1 TTGTCTCAGGGTCCTAGTGTGTCTGGAATTGGTGGGTTCTTGGTCTCACTGACTTCAAGA
                                                              60
             61 ATGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAGGTGGCATGTCCGGAGTT 120
Reference
        61 ATGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAGGTGGC<mark>RY</mark>GTCCGGAGTT 120
2d. LoVo. E2
             ********
2d.LoVo.E2 121 TGTTTCTTCTGATGTTCAGATGTGTTCTTGAGGTTTCTTCTTCTGGTGGGGGTTGTGGGTCTC 180
             Reference
         181 ACTGGCTCAGGAGTGAAGCTGCAGACCTTTGCGGTGAGTGTCACAGCTCA<mark>S</mark>AAAGGCAGT 240
2d.lovo.e2 181 actggctcaggagtgaagctgcagacctttgcggtgagtgtcacagctca<mark>k</mark>aaaggcagt 240
             241 GTGGACCCAAAGAGTGAGCAGTAGCAAGATTTATTGCAAAGAGTGAAAGAACGAAGCTTC 300
Reference
2d.lovo.e2 241 gtggacccaaagagtgagc<mark>ar</mark>tagcaagatttattgcaaagagtgaaagaacgaagcttc 300
                             *******************
Reference
         301 CACAGTATGGAAAGGGACCCCATTGGGTTGCCACTGCTGGCTCAGGCAGTCTGCTTTTAT 360
2d.LoVo.E2 301 CACAGTATGGAAAGGGACCCCATTGGGTTGCCACTGCTGGCTCAGGCAGTCTGCTTTTAT 360
             361 TCTCTAATCTGCTCCC
Reference
2d.LoVo.E2 361 TCTCTAATCTGCTCCCWCCCCACATCCTGCTGATTGGTCCACTTTCAG 407
             2. Reference vs. 1d.LoVo.E2
  Aligned Length = 407 Gaps = 0
  Identities = 379 (93%)
           1 TTGTCTCAGGGTCCTAGTGTGTGTGTGGAATTGGTGGGTTCTTGGTCTCACTGACTTCAAGA
Reference
                                                              60
1d.LoVo.E2
         1 TTGTCTCAGGGTCTINAGTGTGTCTGGAATTGGTTNGTTCTTCMTCTCACTGACTTCAAGA
                                                               60
                                         *****
             ******
                         *****
          61 ATGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAGGTGGCATGTCCGGAGTT 120
Reference
1d.Lovo.E2 61 ATGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAAGTGGSGCGTCCGGAGTT 120
             /*********
         121 TGTTTCTTCTGATGTTCAGATGTGTTCTGAGTTTCTTCTTCTGGTGGGGTTGTGGGTCTC 180
Reference
1d.Lovo.E2 121 TGTTTCTTCTGATGTTCAGATGTGTTCTGAGTTTCTTCTTCTTCTGYCGYGGTTGTGGTCTY 180
         181 ACTGGCTCAGGAGTGAAGCTGCAGACCTTTGCGGTGAGTGTCACAGCTCAGAAAGGCAGT 240
         181 ACTGGQYCAGGAGTGAAGCTGCAGAAMCTTTGCGGTGAGTGTCACAGCTCATAAAGGCAGT 240
1d.LoVo.E2
         241 Grepacccaaagagtgagc<mark>ag</mark>ragcaagatttattgcaaagagtgaaagaacgaagcttc 300
Reference
1d.lovo.e2 241 GWKGACCCAAAGAGTGAGCAAGAGATTTATTGCAAAGAGTGAAAGAACGAAGCTTC 300
               301 CACAGTATGGAAAGGCACCCATTGGGTTGCCACTGCTCGGCCAGGCAGTCTGCTTTTAT 360
Reference
1d.Lovo.E2 301 CACAGTATGGAAAGGSACYNSATTGGGTTGCCACTGCTGGCTCAGGCAGTCTGCTTTTAT 360
         361 TCTCTAATCTGCTCCCTCCTACATCCTGCTGATTGGTCCACTTTCAG 407
Reference
1d.Lovo.E2 361 TCTCTAATCTGCTCCCANNCACATCCTGCTGATNGNTCCACTTTCAG 407
```

Figure 4.15: ClustalW (v1.83) multiple sequence alignment for Tell library. 1d.LoVo.E2 and 2d.LoVo.E2 indicate 1D and 2D reads respectively from the first telomere library in which the E2 primer was used to amplify A and B alleles of the XpYp telomere in LoVo. Reference refers to the A allele flanking, subtelomeric region of the XpYp telomere. Yellow colour indicates SNP positions, red circles show mismatches within the SNP positions and blue circles mismatches outside the SNP positions. Gaps inserted=0; conserved identities=402 and 379 for 2D and 1D respectively; Pairwise alignment mode: slow; pairwise alignment parameters: open gap penalty=10.0; extend gap penalty=0.1.

Table 4.3 shows the stats results for the external control and the second telomere library (Tel2), indicating that many fewer reads were generated for the STELA products than for the control sequence. However, the N50 and N75 indicated that for both libraries, the average length of most of the reads was within the estimated product size (specially for 2D reads). Figure 4.16.A shows screenshots for the flowcell used to sequence KUK A49 library and 4.16.B, for the flowcell used to sequence the second telomere library. The greater number of green pores in the first figure compared to the second, indicates that a higher number of pores were actively sequencing the KUK A49 but not the telomere library. As a consequence, fewer reads were obtained for the STELA products as shown in table 4.3. In addition, more black (dead) pores at the end of the KUK A49 sequencing suggest that this flowcell was more active. This might be influenced by the concentration and purity of the input DNA but also by batch to batch variations in the quality of the flowcells (Ip *et al.*, 2015).

The KUK A49 library generated two .bam files containing 8540 and 128 sequences (for 1D and 2D reads respectively) aligning to the reference. When the consensus sequence generated in IGV was retrieved and aligned to the reference using ClustalW, a very high (99%) identity for both types of reads was observed (full alignment shown in appendix D). The location of the mismatches was different in 1D and 2D alignments suggesting that the combined use of 1D and 2D reads when possible might increase accuracy.

Reads from the second telomere library were filtered using the flanking region (as previously described for Tel1 library) to remove alignment artifacts. The .bam files produced contained 62 and 44 sequences (for 1D and 2D respectively) and figure 4.17 shows the pairwise alignment of the consensus generated to the flanking region. The percentage of identity obtained was the same as for Tel1 library (93 an 99% for 1D and 2D respectively), although in this case, the three mismatches for the 2D alignment were not located in the SNPs but in other positions suggesting the presence of only one haplotype sequences. Besides, the mismatches in the 1D alignment were independently located in or out the SNP positions and were shared with those for the first te-



Figure 4.16: Screen shots of the channels panel for two flowcells. A is the flowcell used to run the control KUK A49 library. B is the flowcell used to run the second telomere library. In both figures, the pictures on the left show the flowcells 10 minutes after loading the library and the pictures on the right, the same flowcell after a 48 hours run (before stopping the run). Colours are: black=saturated (unable to make further reads), cyan= zero (no strand is being read at the moment but it is active for future reads), green=single pore or strand (pores that are currently reading), salmon=unavailable (not reading or available at the moment but can be active in the future) and yellow=multiple sequences around the pore (none of them is sequenced at the moment but the pore is active). The green triangle in the corner of some pores indicates the active sensor (out of the four that has each pore) that is active.

lomere library suggesting similar accuracy. After the filtration step, a second alignment was generated using the telomeric consensus sequence resulting in 493 and 924 1D and 2D reads respectively aligning to it. The same identity as for the telomere library 1 was reached (88%), but a greater difference was observed between 1D and 2D reads (84.7 vs 93.5%) suggesting that the mismatches generated in the alignment were different between the two experiments. Similarly to the alignment for Tel1 library, the accuracy in the flanking region decreased when the telomeric repeat consensus was used for the alignment although the 2D alignment for Tel2 library had less mismatches than for Tell library (87/407 vs 105/407). Despite, the non-canonical variants being poorly recognised, one TGAGGG (position 496 in the alignment) and one TTGGGG (position 856 in the alignment) repeats were sequenced in the 2D alignment (see appendix E). To review all the telomere alignments, the four consensus sequences (1D and 2D for telomere libraries 1 and 2) were aligned together with the reference telomeric sequence using ClustalW to see whether the mismatches were shared and whether the accuracy increased or not. Appendix E shows the full alignment. A large region of TTAGGG repeats (from position 890 to  $\approx$  4350) aligned to the consensus without quality lost with increasing length (in contrast with Sanger sequencing that loses resolution with longer fragments) suggesting that this technology might be suitable for telomere sequencing. Interestingly, in a more distant position (from 4550 bp until the end) the alignment decreased in accuracy and the presence of blocks of mismatches suggests that in this region non-canonical variants might be present. Besides, TACGGG (position 4547), CTAGGG (position 4564) or TCAGGG (position 4582 and 4786) repeats were sequenced despite not being present in the reference sequence, suggesting the presence of a more distant variant repeat region not previously described.

Finally, we used the Java based application Qualimap v2.2 developed by García-Alcalde *et al.* (2012) to extract statistical and graphical information from the .bam files (table 4.4 shows a summary). The duplicated reads rate had an average of 26.7 and 39.9% for the two control sequences (OL A31 and KUK A49 respectively). Unexpectedly, the average of duplicated reads for

the two telomere libraries was higher in the flanking compared to the telomere alignments (44.7 vs 35.8%) in spite of the higher repeat content of the latter. In addition, the high G+C content in all our alignments (>40%) was not underrepresented in the sequencing as the G+C content in the mapped reads was very similar to each reference, suggesting a comparable performance for G+C and T+A regions. With the exception of the alignments for the 1D reads from Tel1 library, a good mean coverage (over 60X) was reached for all the alignments, although a greater than the mean standard deviation in the alignments for the telomere sequence indicates that the coverage was lower in some regions. Interestingly, the high percentage of identity reported by ClustalW multiple alignment (with the exception of 1D reads from the OL A31 library) imply that less than 12% error was due to the mismatches (as ClustalW alignment did not insert any gap). However, when using the Qualimap software to look into detail at the .bam files generated using LAST alignment, we observed a much higher error rate (especially for all 1D compared to 2D reads and greater when using the telomere consensus compared to the flanking), suggesting that gaps were firstly introduced to increase the alignment accuracy by LAST and that they have a greater contribution to the error rate than the mismatches generated by ClustalW later on. This might be due to the score options chosen for the alignment as mismatches were scored equally as gaps; furthermore, the frequency of gaps in homopolymeric regions was below 29% in all alignments suggesting that they occurred more frequently in non-homopolymeric regions.

1.2d.hapA vs. Reference

Aligned Length = 407Gaps = 0Identities = 404 (99%) 1 TTGTCTCAGGGTCCTAGTGTGTGTCTGGAATTGGTGGGTTCTTGGTCTCACTGACTTCAAGA 2d.hapA 60 Reference 1 TTGTCTCAGGGTCCTAGTGTGTCTGGAATTGGTGGGTTCTTGGTCTCACTGACTTCAAGA 60 61 ATGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAGGTGGC<mark>AT</mark>GTCCGGAGTT 120 2d.hapA 61 ATGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAGGTGGC<mark>AT</mark>GTCCGGAGTT 120 Reference 2d.hapA 121 TGTTTCTTCTGATGTTCAGATGTGTTCTGAGTTTCTTCTTCTGGTGGGGGTTGTGGGCTCC 180 Reference 121 TGTTTCTTCTGATGTTCAGATGTGTTCTGAGTTTCTTCTTCTGGTGGGGGTTGTGGGTCTC 180 2d.hapa 181 ACTGGCTCAGGAGTGAAGCTGCAGACCTTTGCGGTGAGTGTCACAGCTCA<mark>G</mark>AAAGGCART 240 Reference 181 ACTGGCTCAGGAGTGAAGCTGCAGACCTTTGCGGTGAGTGTCACAGCTCA<mark>G</mark>AAAGGCAGT 240 2d.hapa 241 gtrgacqsaaagagtgagcagtagcaagatttattgcaaagagtgaaagaacgaagcttc 300 Reference 241 GTGGACCAAAGAGTGAGCAGTAGCAAGATTTATTGCAAAGAGTGAAAGAACGAAGCTTC 300 2d.hapA 301 CACAGTATGGAAAGGGACCCCATTGGGTTGCCACTGCTGGCTCAGGCAGTCTGCTTTTAT 360 Reference 301 CACAGTATGGAAAGGGACCCCATTGGGTTGCCACTGCTGGCTCAGGCAGTCTGCTTTTAT 360 2d.hapA 361 TCTCTAATCTGCTCCCCTCCCACATCCTGCTGATTGGTCCACTTTCAG 407 Reference 361 TCTCTAATCTGCTCCCTCCCACATCCTGCTGATTGGTCCACTTTCAG 407 

2. 1d.hapA vs. Reference

Align Ident	ed Le ities	ength = 407 Gaps = 0 s = 381 (93%)	
ld.hapA Reference	1 1	TTGTCTCAGGGTCTNAGTGTGTCTGGAATTGGTTNGTTCTTGNFCTCACTGACTTCAAGA TTGTCTCAGGGTCCTAGTGTGTCTCGGAATTGGTGGGGTTCTTCGGTCTCACTGACTTCAAGA	60 60
ld.hapA Reference	61 61	AYGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAMGTGGS <mark>GC</mark> GTCCGGAGTT 1 ATGAAGACGCGGAACCTCGCGGTGAGTGTTACAGTTCTTAAAGGTGGCATGTCCGGAGTT 1 ***********************************	.20 .20
ld.hapA Reference	121 121	TGTTTCTTCTGATGTTCAGATGTGTTCTGAGTTTCTGAGTTTCTTCTGNYGYGGTTGTGGTCTC 1 TGTTTCTTCTGATGTTCAGATGTGTTCTGAGTTTCTTCTTCTGGTGGGGTTGTGGTCTC 1 ******	-80 -80
ld.hapA Reference	181 181	actgggycaggagtgaagctgcagamctttgcggtgagtgtcacagctcataaaggcagt 2 actgggtcaggagtgaagctgcagacctttgcggtgagtgtcacagctcagaaaggcagt 2 ******	240 240
ld.hapA Reference	241 241	GTGGACCSAAAGAGTGAGCAAATAGCAAGATTTATTGCAAAGAGTGAAAGAACGAAGCTTC 3 GTGGACCCAAAGAGTGAGCAGIAGCAAGATTTATTGCAAAGAGTGAAAGAACGAAGCTTC 3 *******	300 300
ld.hapA Reference	301 301	CACAGTATGGAAASGSAMYNSATTGGGTTGCCACTGCTGGCTCAGGCAGTCTGCTTTTAT 3 CACAGTATGGAAAGGGACCCCATTGGGTTGCCACTGCTGGCTCAGGCAGTCTGCTTTTAT 3 ****************	360 360
ld.hapA Reference	361 361	TCTCTAATCTGCTCCCACCACCATCCTGCTGATAGGTCCACTTTCAG 407 TCTCTAATCTGCTCCCTCCCACATCCTGCTGATTGGTCCACTTTCAG 407	

Figure 4.17: ClustalW (v1.83) multiple sequence alignment for Tel2 library. 1d.hapA and 2d.hapA indicate 1D and 2D reads respectively from the second telomere library in which the XpYp427G/415C was used to amplify the A haplotype of the XpYp telomere in LoVo. Reference is the same sequence in figure 4.15. Yellow colour indicates SNP positions, red circles show mismatches within the SNP positions and blue circles mismatches outside the SNP positions. Gaps inserted=0; conserved identities=404 and 381for 2D and 1D respectively; Pairwise alignment mode: slow; pairwise alignment parameters: open gap penalty=10.0; extend gap penalty=0.1.

		10	A31	KUK	. A49			lel 1			L	el2	
		1D	2D	1D	2D	1D	2D	1D filtered	2D filtered	1D	2D	1D filtered	2D filtered
Deference	Name	0L-A31	<b>OL-A31</b>	KUK-A49	KUK-A49	Flanking	Flanking	Telomere	Telomere	Flanking	Flanking	Telomere	Telomere
stats	Length (bp)	4558	4558	4493	4493	407	407	9003	9003	407	407	9003	9003
	GC %	40	40	39	39	49	49	50	50	49	49	50	50
	Mapped bp	693383	2307166	35112966	483815	1577	31261	363593	1073682	24232	17547	920033	1821836
	N.of reads	180	571	8540	128	4	80	123	592	62	44	493	924
	Mapped reads	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
	Paired reads	0%0	0%0	%0	%0	0%0	0%0	0%0	0%0	0%0	0%0	%0	0%0
.bam	Read min	2519	1957	1071	1731	362	323	641	371	327	348	375	417
stats	Read max	4708	4779	4893	4412	423	427	4800	5030	438	427	5078	5395
	Read $\overline{\mathbf{x}}$ length	3866	3953	4148	3724	401	386	3036	1865	392	383	1930	2038
	Dup. reads	53	319	7410	43	0	58	51	373	40	31	258	640
	Dup. %*	29.4	55.9	86.8	33.6	0	72.5	41.5	63.0	64.5	70.5	52.3	69.3
	Dup. rate (% $^{\dagger}$ )	22.8	30.6	59.9	20.0	0	50.0	31.9	37.9	45.5	38.5	34.0	39.4
	N.of A's	169280	546151	8465570	110976	344	6899	71916	210883	5233	3754	181683	349916
	%  of $As$	25.3	25.3	25.0	24.4	22.9	22.7	20.3	20.6	22.8	23.0	20.7	20.0
	N.of C's	157421	451599	7961103	90240	328	6409	67430	133174	5026	3463	116756	261583
	% of $C's$	23.6	21.0	23.5	19.8	21.9	21.8	19.0	13.0	21.9	21.3	13.3	14.9
Nucleotide	$\rm N.ofTs$	179037	643645	9289873	143983	446	8733	103063	314000	6753	4809	26946	523354
Content	$\% { m of } { m Ts}$	26.8	29.8	27.4	32.0	29.7	29.7	29.0	30.7	29.4	29.5	30.7	29.9
	N.of G's	162264	515626	8216887	110529	383	7603	112757	364148	5935	4265	308637	61691
	%  of $G's$	24.3	23.9	24.2	24.3	25.5	25.8	31.8	35.6	25.9	26.2	35.2	35.2
	N.of Ns	0	0	0	0	0	0	0	0	0	0	0	0
	GC %	47.9	44.8	47.7	44.1	47.4	47.6	50.7	48.7	47.8	47.4	48.5	50.2
	Mean	152.1	506.2	7815.0	107.7	3.9	76.8	40.4	119.3	59.5	43.1	102.2	202.4
Coverage	SD	30.0	77.5	942.8	20.9	0.6	10.8	46.6	169.4	8.1	5.1	140.9	291.3
	$MQ(\bar{x})$	245.8	238.4	246.5	240.3	243.1	240.2	135.3	130.2	241.6	236.8	132.6	141.5
	Error rate (%)	63.6	48.6	64.5	48.9	23.4	20.7	60.6	42.9	21.9	20.6	43.5	44.7
JAT: 2.1A	Identity (%)	8.0	95.0	99.0	99.0	93.0	99.0	88.0	88.0	93.0	99.0	88.0	88.7
MISMATCH and indels	Insertions	17493	61873	913774	12832	62	577	11596	5229	897	421	47389	83979
	Deletions	15061	83303	715664	16527	52	1296	5629	34428	932	851	28887	48806
	$N_n$ IDls (%)	24.5	29.0	25.3	29.2	22.8	24.7	20.5	21.8	22.5	28.5	20.4	23.4
Table 4.4: Su	mmary of the al	ignment s	tatistics fo	or the four l	ibraries seq	uenced by	the MinIO	N <sup>TM</sup> device u	Ising differe	nt consensu "" +"	ls. Data was	obtained usi	ig the Qualimap
Soltware. ADC "C's"=cytosine:	s; "T's"=thymines;	mbols are: "G's"=guai	nines: "SD"=	aarrs; 'N =nu =standard de	mber; x =m( viation; "MQ	ean; Dup =0 "=mapping (	auplicated; quality; "N <sub>n</sub> "	% =esumate "=homopolym	er. The perce	mtage of iden	ercentage; 7 tity is based	% =percentage I on pairwise	; As =adenines; alignment using
ClustalW and	takes into accour	it only mis.	matches. T	he error rate	however, no	ot only accou	ints for misi	matches but a	also insertions	s/deletions ar	nd quality sc	ores. Note: q	uality scores for
MinION <sup>TM</sup> are	not Phred based	so error calc	culation by t	the Qualimap	might be ina	ccurate.							

4.3. RESULTS

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#### 4.4 Discussion

In this chapter, mutation analysis on the A allele of the XpYp telomere in the LoVo (MSH2<sup>+/-</sup>) colon cancer cell line has been performed and it was compared with results in Bermudez (2007) to understand the telomere instability driven by MSH2 deficiency. Furthermore, it was demonstrated that flourescently labeled primers can be used to perform TVR analysis and that the use of capillary electrophoresis improved the resolution as well as introduced the possibility of accurately measure each PCR product using a size standard ladder. In addition, the use of bins for the location of each variant repeat block accelerated the analysis process allowing a higher throughput.

Combining data from radioactive and fluorescent TVR, a similar mutation frequency to the previous study (19.2 and 16.1% respectively) was obtained although more insertion molecules (15/130 vs 1/93) were scored, reducing the percentage of deletions from 14 to 7.7%. This was due to somatic mosaicism (reviewed in Campbell et al. (2015)) as the same mutation molecules were found more than once (especially an insertion mutation called mutant F, that was found 14 times). Nevertheless, the diversity and trend of the mutations was similar, as in both studies 5 different types of deletions were found but only three different types of insertions were found across both studies. Furthermore, one of the deletions found in both studies was shared (mutant B and clone 54) and the most frequent insertion mutant found here (mutant F) might represent the mutation that could not previously be mapped. Interestingly, a final balance of 9:3 (loss:gain) for the combined data suggests that deletions were three times more common than insertions; additionally, a total of 216 bp were lost due to all 9 deletion mutants while only 192 bp were gained due to the 3 insertion mutants, suggesting that despite less nucleotides lost per deletion (mean of 24 vs 64 bp), the greater variety of deletion mutants compared to insertions would have contributed to the increase in the telomere shortening rate of human primary cells with depleted MSH2 protein as shown by Mendez-Bermudez & Royle (2011) and in chapter 3 of this Thesis.

All MSI tumours have shorter telomeres than MSS tumours and in colo-

rectal cancer (CRC) cell lines, MSI has been strongly correlated with telomere shortening but not with telomerase activity (Takagi et al., 2000). Taking into account that telomeres in MMR-defective CRC cell lines shorten despite the presence of telomerase activity, it might be expected that inability of repairing slippage errors during replication would produce deletion mutants contributing to telomere shortening. However, as the cell divisions progress, accumulation of deletions, due to defective MMR, might lead to a significant decrease in the median telomere length and thus, very short telomeres might be more vulnerable to insertions. Then, there might be a balance between gains and losses of repeats in cancer cells while in primary cells, that cannot escape senescence, accumulation of deletions will increase the telomere shortening rate although the influence of senescence will impede that insertions occur as a late event. Some mathematical models reviewed in Ellegren (2004) called stepwise mutation models would agree with this hypothesis as they demonstrated that short microsatellites are more prone to suffer insertions whereas long microsatellite tend to accumulate deletions. Additionally, Conomos et al. (2012) found, in cells using the ALT mechanism for telomere length maintenance, that telomere variant repeats (especially TCAGGG) recruited nuclear receptors such as TR4 with greater affinity than TRF2 at telomeres, suggesting that the TVR region of the telomeres is essential for shelterin complex binding. Therefore, it might be anticipated that deletions in this region, reducing the number of variant repeats, would contribute to an accelerated telomere attrition rate due to unprotected, uncapped, telomeres. To demonstrate that MSI in CRC cells drives to losses of repeats when telomeres are long enough to keep shortening but to gains when telomeres reach a size limit, it might be interesting to analyse changes in the mutation profiles between two different time points as well as studying telomere length and shortening rates.

Subsequently, the same XpYp telomere allele analysed by TVR was sequenced using a third next generation sequencing device called MinION<sup>TM</sup> from Oxford Nanopore<sup>TM</sup> Technologies, that allows to sequence single long molecules, in an attempt to obtain full information from the whole telomere array. This new technology has already been used to sequence a phage genome (Mikheyev & Tin, 2014) and several bacterial genomes ((Quick *et al.*, 2015), (Karlsson *et al.*, 2015) and (Laver *et al.*, 2015)). Although the error rate has been described to be very high compared to other sequencing technologies ( $\approx$  38%) those rates were calculated using the R6 chemistry and as the technology is evolving, lower error rates are being expected. Karlsson *et al.* (2015) reported a consensus accuracy, after mapping to the reference genome the most common nucleotide at each position, of 99.8% with a coverage of 60X.

We used LAST as alignment tool and a consensus sequence generated based on the TVR profile (for two telomere libraries) and two reference sequences generated by Enjie Zhang using the Ion Torrent technology<sup>™</sup> (for two control libraries). We reached a high percentage of identity (95 and 99%) for the two control sequences validating the sequencing method for accuracy. In addition, and after pre-aligning reads to a flanking region of 407 bp, we reached also high percentages of identity in the two telomere libraries. The first one, containing A and B alleles for the XpYp telomere, had a lower (12%)but identical error rate, based on mismatches, than the 1D reads for the second library (containing only A allele). Furthermore, the 2D reads for this library reached an even lower error rate ( $\approx 10\%$ ) based on mismatches, suggesting that the sequencing accuracy was very high. However, the error rate reported by Qualimap v2.2 was greater than the calculated based on ClustalW (that only accounts for mismatches), suggesting that insertion/deletions (IDLs) had an important impact on the sequencing error rate. This might be due to the score options chosen for the alignment but also to the different scale (not Phred) used as quality scores given by the MinION<sup>™</sup>. Moreover, as the error based on IDLs decreased significantly when the two telomere libraries were aligned to the flanking region compared to the telomere, for future analysis it would be desirable to use the flanking region as an anchorage and not only as a filter for alignments against a tandem repeat reference. Alternatively, a more stringent score might be used for the gap penalty, trying to impede certain degree of misalignment. Interestingly, the percentage of IDLs in the two control sequences, containing homopolymeric repeat regions, was far less

frequent ( $\approx 27\%$ ) than in non-homopolymeric sequences, suggesting that generation of gaps by the alignment software was not only restricted to tandem repeats.

The main aim of using the MinION<sup>™</sup> to sequence the XpYp telomere in LoVo was to map the whole array of telomere variant repeats (TVR) and to be able to sequence through the canonical TTAGGG tandem repeat array in its entirety. Unfortunately, the variant repeats analysed by TVR (TGAGGG and TTGGGG) were poorly sequenced suggesting that using this technology to sequence variant repeats of unknown telomeric ends might be challenging. However, the lack of mismatches in a wide region of  $\approx$  4 kb of TTAGGG repeats in comparison with a block of mismatches near the end of the sequence suggested that our consensus sequence might be incomplete and thus, a de novo assembler might improve the sequencing results. The high error rate of *de novo* assemblers (particularly in repetitive regions) makes these tools highly inappropriate; however, some authors have recently used "hybrid" approaches to overcome this issue ((Madoui et al., 2015) and (Goodwin et al., 2015)). In those experiments, long reads from nanopore sequencing have also been sequenced using short reads technologies, what has been used to correct errors in the long reads, increasing the accuracy of *de novo* assemblers. Unfortunately, we could not use these approaches as telomeres are not suitable for short read sequencing technologies, but with the application of those experiments, improvements in the accuracy might be made in de novo assemblers for future applications over long repetitive sequences. Finally, the G+C content is known to affect sequencing performance in other platforms and it is still unclear whether the MinION<sup>™</sup> is affected by high G+C content, although Laver et al. (2015) showed that high G+C sequences were underrepresented using R6 chemistry. We used a newer chemistry (R7.3 with the genomic DNA kit SQK-MAP004) and found that the G+C content of the mapped reads was very similar to the reference, suggesting that in our libraries the sequencing performance did not depend on the nucleotide composition. This, is a developing technology and many bioinformatic tools need to be developed in order to analyse complex regions such as telomeres or other repetitive sequences.

### 5.1 Background

Human telomerase is a ribonucleoprotein that elongates telomeres in germline and immortal cells (Blackburn et al., 1989) and it is active in 80-90% of the tumours ((Kim et al., 1994) and (Shay & Bacchetti, 1997)) allowing telomere maintenance despite the high cell turnover. In addition, tissues with a high proliferation index (male germ cells, activated lymphocytes and some stem cell populations) also have some levels of telomerase activity (reviewed in Cong et al. (2002)). In the case of the normal mucosa in the human colon, three stem cell compartments have been described to be involved in the regeneration of this tissue (reviewed in Clatworthy & Subramanian (2001) and Sipos et al. (2012)) and telomerase activity has been found in normal as well as tumourous colon tissue (Kim et al., 1994). The main function of telomerase is to elongate telomeres to allow progressive cell divisions without loss of the proliferative capacity (Allsopp et al., 1992), although other non-canonical activities related with transcriptional regulation and metabolic reprogramming also impact on cancer cell survival (Low & Tergaonkar, 2013). In general, telomeres in colorectal tumours shorten (Hastie *et al.*, 1990), despite telomerase being active in most of the cases (Li *et al.*, 1996) and (Shay & Bacchetti, 1997), and a relationship between telomere shortening and microsatellite instability (MSI) was found in a group of 55 colorectal carcinomas (Takagi et al., 2000). However, no relationship was observed between telomerase activity and MSI (Vidaurreta et al., 2007), suggesting that in mismatch repair deficient cancer cells the predominant telomerase function might be other than telomere maintenance.

The study of telomerase inhibitors as potential therapeutical agents has resulted in several drugs targeting either the reverse transcriptase (hTERT) or the RNA component (hTERC) of telomerase in an attempt to increase senescence and/or apoptosis in the tumour cells (reviewed in Rankin et al. (2008) and Phatak & Burger (2007)). Damm et al. (2001) described a group of small chemical compounds that were able to inhibit telomerase activity in vitro, and to reduce tumorigenicity using *xenograft* mouse models. BIBR1532 is a small non-nucleosidic molecule that is able to inhibit endogenous or recombinant telomerase by affecting the processivity of, mainly, the long reaction products (Pascolo et al., 2002) and not by directly competing with the binding efficiency to the DNA. It was shown by Damm et al. (2001) that treatment of cancer cells with the telomerase inhibitor BIBR1532 lead to a cell proliferation arrest that was reverted if the treatment was removed. In addition, they suggested that this proliferation arrest might be due to the induction of a senescent phenotype that was shown to have an increased telomere erosion rate measured by TRF and dysfuctional telomeres measured by Q-FISH. In addition, Nakashima et al. (2013) proposed that cell death via the apoptosis mechanism might also be occurring as a consequence of telomerase inhibition in cancer cell lines.

Distinct concentrations of BIBR1532 have been found to be needed to inhibit telomerase activity;  $IC_{50}$  values for Damm *et al.* (2001) were 0.1 µM whereas for Barma *et al.* (2003) were 5 µM (50 fold higher). This difference was attributed to the purity of the telomerase enzyme to be inhibited; while the former used purified telomerase, the latter used crude cell extracts that might contain inhibitory substances reducing the effect of the BIBR1532 drug.  $IC_{50}$  values for El-Daly *et al.* (2005) on JVM13 cells (immortalized B-cell line from a patient with prolymphocytic leukemia) were 52 µM and  $IC_{50}$  values for Pascolo *et al.* (2002) were in the order of 0.1 µM for HeLa cell extracts and in the order of 100 µM for recombinant telomerase indicating the wide range at which this drug has been tested. El-Daly *et al.* (2005) demonstrated that concentrations higher than 30 µM of BIBR1532 had acute cytotoxic effects, within 72 hours after the treatment, on leukemia cells but not in normal hematopoietic stem cells. In addition, the antiproliferative effect of BIBR1532 was not dependent on telomerase activity or telomere length itself but on the increase of discrete dysfunctional telomeres and the associated loss of TRF2.

The suitability of telomerase inhibitors for treating MMR-defective tumours is not confirmed yet. On the one hand, it has been hypothesised that the efficiency of the treatment might be related to the initial telomere length (as cells with longer telomeres will undergo more cell divisions before senescence or apoptosis triggered by short telomeres occurs), and thus, tumours with shorter telomeres (MMR<sup>-/-</sup>) would be benefited from a telomerase inhibition treatment as they will reach senescence earlier. On the other hand, it has been shown that after telomerase inhibition of a MSH6<sup>-/-</sup> cancer cell, telomere elongation occurred in a telomerase-independent, ALT-like mechanism (Bechter et al., 2004), therefore, further studies on telomerase inhibition in MMR-deficient cancer cells are needed. Additionally, synthetic lethality studies have been performed in MMR-deficient cancer cell lines to explore its potential as therapeutic approaches. Martin et al. (2010) showed that the inhibition of POLB and POLG genes by siRNA in MSH2<sup>-/-</sup> and MLH1<sup>-/-</sup> cancer cells respectively, produced an increase in oxidative lesions, thus the presence of an additive effect for telomerase inhibition in MMR deficient cancer cells has been explored here.

## **5.2** Aims

To study the suitability of telomerase inhibition as a potential treatment in MMR-defective tumours by understanding the effect of telomerase inhibition on a human colon cancer cell line defective for the DNA mismatch repair (MMR) pathway compared to a MMR-proficient colon cancer cell line. The main objectives for this aim were:

- To treat two colon cancer cell lines, MMR-deficient (LoVo) and MMR-proficient (SW480) with the telomerase inhibitor BIBR1532.
- To assess telomerase activity after the treatment with the telomerase inhibitor BIBR1532 compared to untreated controls.
- To study cell cycle dynamics of the cancer cells treated with the telomerase inhibitor using flow-cytometry analysis (FAC).
- To measure telomere length by single telomere length analysis (STELA) to study telomere attrition rates with and without the telomerase inhibitor treatment.

## 5.3 Results

#### 5.3.1 Partial inhibition of telomerase using BIBR1532

The ability of BIBR1532 to inhibit telomerase was first tested in crude cell extracts from the two cancer cell lines described in table 5.1. Parsch *et al.* (2008) demonstrated a reduction in telomerase activity of  $\approx$  70% incubating cell lysates from the chondrosarcoma cell line SW1353 with 10 µM of BIBR15 for 15 minutes on ice. Here, the cell extracts were incubated on ice with 30 µM of BIBR1532 to try to get a higher reduction of telomerase activity measured by the telomerase repeat amplification protocol (TRAP assay).

	SW-480	LoVo
Tissue	Colon	Colon
Sex	Male	Male
Donor age (years)	50	56
Tumour type	Duke's type B	Duke's type C
	adenocarcinoma	adenocarcinoma
MMR	Proficient	MSH2 <sup>-/-</sup>
Tumour suppressor gene	$APC^{-/-}$	

Table 5.1: **Cell lines information**. Data acquired from the American Type Culture Collection (ATCC) website. For more information refer to chapter 2.

Telomerase activity was measured by the telomerase repeat amplification protocol (TRAP assay) as described in section 2.2.2.10 and semi-quantification was performed by measuring the relative intensity of the first PCR band compared to the internal control. Figure 5.1 shows that in the two adenocarcinoma cell lines a telomerase inhibition of 92.6 and 58.5% (for SW480 and LoVo respectively) was observed. This reduction was significantly different compared to untreated lysates (p < 0.0001 and p = 0.0186 for Exact Fisher's test respectively) although being more modest for LoVo than SW480.

To examine whether treatment of cells in culture with similar concentration of BIBR1532 had an effect on short term cell growth dynamics, the two cancer cell lines were grown during approximately 10 days in Dulbecco's modified Eagle medium containing either 10 or 30  $\mu$ M of BIBR1532, measured cell number and viability using Trypan blue and compared them with the same cells grown in Dulbecco's modified Eagle medium and 0.3% DMSO (the



Figure 5.1: **Telomerase downregulation in cell extracts.** A is an example for a TRAP assay gel. The cell lysate loaded onto each lane is indicated at the top. "hi"= heat inactivated. **B** is a bar graph indicating the relative telomerase activity as a measurement of the relative intensity of the first PCR band (1<sup>st</sup> PCR) to the internal control (IC) on figure A (measured using ImageQuant v. 7.0). Means and SE are shown for two replicate experiments. \*\*\*\*p < 0.0001 and \*p = 0.0186.

equivalent and non-cytotoxic concentration to drug dilution) as control. No significant differences (Exact Fisher's test) were found for either of the cell lines between the controls and telomerase inhibitor treated cells in terms of cell growth dynamics suggesting that none of the concentrations had cytotoxic or cytostatic effects. Unexpectedly, as shown in figure 5.2, SW480 had very low percentage (0-2%) of cell death for all three conditions (control and two BIBR1532 concentrations) but conversely, the LoVo cell line had a very high percentage of cell death including the non-treated control cells (40-60%), what was also confirmed by FAC analysis as described in section 5.3.2. As no significant differences between the treatments and control for viability or cell growth were observed, it could be concluded either that the use of BIBR1532, up to 30  $\mu$ M, in cell culture seemed to have no effect on short term growth for the two adenocarcinoma cells, independently of the effect on telomerase activity or that the drug was not effectively delivered to the cells.



Figure 5.2: Cell growth curves and cell death for short-term treatment with 10 and 30  $\mu$ M of BIBR1532. The graphs represent growth curves (triangles and straight lines) for SW480 (left) and LoVo (right) treated with two different BIBR1532 concentrations, 10 (dark green) and 30 (red)  $\mu$ M or with 0.3% DMSO as control (light green). Growth curves are plotted in the left axis and percentage of cell death measured by Trypan blue (circles and dashed lines) plotted in the right axis. When two measurements were available, mean  $\pm$  standard error are plotted.

The extraordinary proportion of dead cells shown in figure 5.2 for LoVo has not previously been reported in this cell line and as it was observed for all conditions, it could not be concluded that it resulted as a consequence of the treatment. Furthermore, the cell was checked by western blot for MSH2 protein levels to validate its identity (see figure 5.3), the telomere variant map was reproduced as shown in chapter 4 and previous experiments showed the presence of high levels of microsatellite instability (Bermudez, 2007), confirming its authenticity. Although there was no explanation for the very high percentage of cell death, it was a very significant feature distinguishing both cancer cell lines and it arose an important consideration about cell division and the calculation of cumulative population doublings (PD); as the mean of cell death was  $\approx 50\%$  for LoVo, half of the population should have divided twice for each passage (and not just once) to counteract the cell death before reaching confluency, and therefore, it might be expected that the number of PD would have been underestimated (see figure 5.5.B for an schematic representation).



Figure 5.3: Western blot for LoVo. The left part of the figure shows an example western blot result for the expression of MSH2 protein in LoVo compared with three different cell lysates from WI38. The right part of the figure is a bar graph quantifying the relative amount of MSH2 compared to GAPDH as described previously. Means and standard deviations are shown. WI38 1, 2 and 3 are three different cell pellets that were used for the WB optimization (see explanation in section 3.3.3).

# 5.3.2 Cell cycle dynamics of colon cancer cells treated with BIBR1532

Figure 5.2 shows that neither 10 nor 30  $\mu$ M of BIBR1532 had a detrimental impact on short-term cell growth and as I aimed to get the maximum telomerase inhibition but without cytotoxic effects (El-Daly *et al.*, 2005), the two cell lines were cultured with 30  $\mu$ M for approximately 60 days to study telomere length and cell cycle dynamics. Growth curves are shown on figure 5.4 and linear regression analysis showed very high R<sup>2</sup> values (> 0.9), indicating that, despite the treatment with the telomerase inhibitor, senescence was not reached as no stationary phase was detected. For treated SW480 cells, a slower growth rate was observed after passage 4 that was even more pronounced after passage 8, resulting in significantly (p = 0.0001) different slopes between control and treated cells. Contrarily, in the LoVo cell line, treated and control cells grew at similar rate during 60 days, suggesting that BIBR1532 might have a cytostatic effect for long term treatment in SW480 but not in LoVo.



Figure 5.4: Cell growth dynamics of cancer cell lines treated with 30  $\mu$ M of BIBR1532. The graphs represent growth curves for SW480 (left) and LoVo (right) for control (light green) and 30  $\mu$ M BIBR1532 (red) treated cells.

To study cell cycle dynamics, cells were collected for each passage and assayed for DNA content using propidium iodide staining and flow-cytometry analysis (FAC), as described in section 2.2.1.6. All samples (from different passages, cells and treatments) were analysed by FAC at the same time to reduce batch and setting variations and duplicate samples were collected for



Figure 5.5: Cell cycle description. A represents the phases of the cell cycle in different colours. G1 phase refers to the first stage in the interphase, when cells grow and increase the amount of cellular contents (except for DNA) in preparation for cellular division. S phase refers to the moment when DNA is duplicated. G2 phase occurs after the DNA synthesis and constitutes a checking point for DNA damage that would be repaired in this phase prior entering mitosis. In this phase, telomeres might be elongated by telomerase or processed by exonucleases (C-strand resection). M phase refers to mitosis. G0 phase is a quiescence stage in which cells do not divide. Cells only enter G1 phase when receiving the signals to start cell division. B is a schematic representation of the effect that the high percentage of cell death in LoVo might have in the estimation of the population doublings compared to SW480. Blue cells represent viable cells and salmon ones, non-viable ones. A 50% viability is represented for LoVo cell line to show that the viable cells would have undergone an extra population doubling to reach the same final number of cells. C is a graph relating the cell cycles with the amount of DNA (colour code is same as in figure A). 1 refers to a diploid karyotype and 2, a duplicated diploid karyotype before cell division. D is an example for two FAC profiles. The one on the left, from SW480, and the one on the right from LoVo. Each phase of the cell cycle is indicated with a skull symbol.

each measurement. Figure 5.5 shows a schematic representation of the cell cycle and the amount of DNA for each phase, relating it with a FAC profile for each cell line.

The proliferation index (PI) was calculated using the equation 5.1 described in Liu *et al.* (2014) to confirm whether the reduction in growth rates observed for SW480 as a result of cell counts correlated with the proliferation index calculated based on cell cycle and to understand the lack of effect on the growth rate for LoVo. In equation 5.1, "S" refers to the percentage of cells in the DNA synthesis phase with >N and 2N< DNA content; "G2/M" to cellular growth and mitosis with 2N DNA content and "G1" to stage after cell division with N DNA content.

$$PI = \frac{S + (G2/M)}{G1 + S + (G2/M)} \times 100$$
(5.1)

When performing FAC analysis, the percentage of cell death was assayed as sub-G1 peak (Death) that includes fragmented DNA arising as a consequence of apoptosis and/or necrosis. Therefore, the sub-G1 peak was considered as measurement of total death. I confirmed our previous results using Trypan blue for cell viability and found that control LoVo cells had a significantly (p < 0.0001) greater percentage of cell death compared to SW480. Accordingly, figure 5.6.A shows that in control cells, the proliferation index (PI) was usually greater (except for passages 7 and 8) in LoVo than in SW480 as cells needed to divide more actively to counteract the effect of the high death rate. However, under BIBR1532 treatment, the PI was very similar in both cell lines, although when compared with non treated cells, SW480 had greater PI under the treatment, what might reflect a counteracting mechanism against the slower growth rate observed in figure 5.4.

Furthermore, in primary cell lines that reach senescence after a certain number of replication cycles, the cell division rate measured as population doubling per day (PD/day) decreases with cumulative passages (as shown in figure 3.6.B). On the contrary, in cancer cells that can divide indefinitely this rate is independent on the number of passages and it can be very variable. Figure 5.6.B compares the cell division rate between both cells under each treatment. A large range (from 0.09 to 0.97 PD/days) was observed for different passages, but in control SW480 cells the rate seemed to be pretty constant, around 0.6 PD/day from passages 5 to 13, while under treatment it was more variable, usually with a lower value, confirming the results obtained with the growth curves. Interestingly, the cell division rate in control LoVo cells increased over 0.8 PD/day usually after two passages with a lower rate ( $\approx 0.4$  PD/day) but in BIBR1532 treated cells, this increase occurred in alternate passages after a very low (< 0.3 PD/day) cell division rate, what might be related with the higher percentage of cell death in passages 9, 11, 13, 15 and 17 shown in figure 5.7 that summarises the cell cycle distribution for each cell line under both conditions (control and drug-treated) for all the passages analysed.

The most relevant feature from the FAC profiles (figure 5.7) was the significant increase (p < 0.0001 estimated by paired t-test) in the proportion of S phase for SW480 treated cells compared to controls suggesting that cell divisions might have been promoted in this cell line upon the treatment to counteract the potential cytostatic effect of the drug. However, a slight increase in cell death for control SW480 cells that was not apparent in BIB1532 treated cells was also observed, thus having no relation with the treatment. Furthermore, as LoVo cell growth seemed not to be affected by the treatment, no significant changes were observed among the cell cycle, and due to the variability of the FAC data, no conclusions were drawn.



Figure 5.6: **Proliferation index and cell division rate for cancer cells treated with BIBR1532.** A shows the proliferation index (PI) in percentage, calculated as described in equation 5.1. **B** shows the cell division rate in number of population doubling (PD) per day for each passage. In both **A** and **B** graphs, SW480 is represented in orange colour and LoVo in purple. Graphs on the left refer to control and graphs on the right to treated cells.



Figure 5.7: **Cell cycle analysis by FACs.** The graphs on the left represent percentages of cells in each phase of the cell cycle for control cells (SW480 at the top and LoVo at the bottom). The graphs on the right represent percentages of cells in each phase of the cell cycle for each counterpart on the left but BIBR1532 treated. Blue colour refers to cell death measured by sub G1; red to G1 with DNA content = N; purple to G2/M with DNA content = 2N and green to S phase with DNA content >N and 2N<.

#### 5.3.3 Telomere dynamics and telomerase activity

To study the relative percentage of telomerase inhibition reached after long term treatment with 30 µM of BIBR1532, the relative telomerase activity was measured by TRAP assay at passage 13 for SW480 and LoVo for both, control and treated cells. This corresponds to PD 27.4 and 24.9 for SW480 (control and treated respectively) and PD 26.5 and 25.8 for LoVo (control and treated respectively). Graph on figure 5.8.B indicates that there was not a significant reduction in telomerase activity for any of the treated cell lines compared to their respective controls, suggesting, a) inefficient drug delivery, b) telomerase activity reactivation after a prolonged inhibition period (Delhommeau *et al.*, 2002) or c) underestimation due to the semi-quantitative nature of the method for measuring telomerase activity. However, telomerase has been proposed to have other functions than elongating telomeres (reviewed in Martínez & Blasco (2011) and Chiodi & Mondello (2012)) and as telomere shortening usually occurs in colonic tumour cells that are telomerase positive (Takagi et al., 2000), telomere shortening rates were studied to evaluate if a direct effect on telomere length was observed after long-term treatment with BIBR1532.

To study telomere length dynamics, two telomeres, 12q and XpYp were measured by STELA at two time points, from beginning to end of the cell culture (3.5 to 31.2 and 4.0 to 30.6 PDs for SW480 and LoVo respectively) for controls and (2.3 to 28.3 and 3.6 to 29.7 PDs for SW480 and LoVo respectively) for BIBR1532 treated cells. Figure 5.9 shows that both colorectal cancer cell lines had very short 12q and XpYp telomeres compared to other cancer cell lines (data not shown) agreeing with previous studies reviewed in Bertorelle *et al.* (2014). In addition, even with the presence of telomerase activity shown on figure 5.8, telomeres shortened in SW480 comparing start and end time points for both conditions as previously described by (Takagi *et al.*, 2000) and the telomere shortening rate (calculated using equation 1 in chapter 3) was significantly greater in treated than in control cells (Fisher's test).

Surprisingly, telomere length seemed to be more strongly maintained by telomerase in the LoVo cell line as even in the control, telomeres did not shorten (as previously described in Kuranaga *et al.* (2001) after long-term

treatment with anticancer drugs). The XpYp telomere was significantly shorter at the end of the cell culture in treated than control cells for SW480 but not for LoVo (despite of the cumulative PDs reached were lower for treated than controls). Conversely, the 12q telomere was not different at the end of the cell culture for any cell line independently on the condition, suggesting that the XpYp might be more strongly maintained than 12q telomere (see table 5.2). Interestingly, the telomere elongation in LoVo seemed to be reduced in treated cells compared to controls for the XpYp telomere (from 42.3 to 18.4 bp/PD) and not for 12q, suggesting that despite the absence of telomere shortening, the elongation process in LoVo was somehow interrupted more strongly for XpYp.
Β			SW480 + DMS0	SW480 + DMS0 hi	SWH480+BIBR1532	SWH480+BIBR1532 hi	LoV0 + DMS0	LoVo + DMSO hi	LoV0+BIBR1532	LoVo+BIBR1532 hi	Chaps buffer	1 <sup>st</sup> F 1C
В	Relative telomerase activity	1.5- 1.0- 0.5-		4480	SW	480						IC

Figure 5.8: **TRAP assay on cell pellets from cancer cells treated with 30 \muM of BIBR1532. A is an example for a TRAP assay gel.** The cell lysate loaded to each lane is indicated at the top. hi: heat inactivated. **B** is a bar graph indicating the relative telomerase activity as a measurement of the relative intensity of the first PCR band (1<sup>st</sup> PCR) to the internal control (IC) on figure A. Means and SE are shown for two replicate experiments.



Figure 5.9: Scatter plots for telomere length assayed by STELA. A shows XpYp (left) and 12q (right) median telomere lengths for SW480 at two time points. B shows XpYp (left) and 12q (right) median telomere lengths for LoVo at two time points. In all graphs, yellow colour indicates control (0.3%DMSO) and red colour indicates cells treated with 30 µM of BIBR1532. Medians and interquartile ranges are shown. Interquartile ranges and P values from Exact Fisher's test are shown.

	X	ГрҮр			12q	
	Control	BIBR1532	Diff	Control	BIBR1532	Diff
PD	27.7	26.0		27.7	26.0	
mTL Start (bp)	1943	2226		523.1	1261	
mTL End (bp)	1881	1558		329.3	305.3	
TSR (bp/PD)	2.2	25.7	-23.5	7.0	36.8	-29.8
PD	26.6	26.1		26.6	26.1	
mTL Start (bp)	1799	2151		207.9	300.8	
mTL End (bp)	2924	2632		289.9	385.7	
TLR (bp/PD)	42.3	18.4	-23.9	3.1	3.3	-0.2
	PD mTL Start (bp) mTL End (bp) TSR (bp/PD) PD mTL Start (bp) mTL End (bp) TLR (bp/PD)	X           PD         27.7           mTL Start (bp)         1943           mTL End (bp)         1881           TSR (bp/PD)         2.2           PD         26.6           mTL Start (bp)         1799           mTL End (bp)         2924           TLR (bp/PD)         42.3	K         K           PD         27.7         26.0           mTL Start (bp)         1943         2226           mTL End (bp)         1881         1558           TSR (bp/PD)         22.2         25.7           PD         26.6         26.1           mTL Start (bp)         1799         2151           mTL Start (bp)         2924         2632           TLR (bp/PD)         42.3         18.4	XP         Diff           Control         BIBR1532         Diff           PD         27.7         26.0            mTL Start (bp)         1943         2226            mTL End (bp)         1881         1558            TSR (bp/PD) <b>26.6</b> 26.1            mTL Start (bp)         1799         2151            mTL Start (bp)         2924         2632            mTL Kop/PD) <b>42.3 18.4</b> -23.9	KP         Control         BIBR1532         Diff         Control           PD         27.7         26.0         27.7           mTL Start (bp)         1943         2226         523.1           mTL End (bp)         1881         1558         329.3           TSR (bp/PD)         22.2         25.7         -23.5         7.0           PD         26.6         26.1         26.6         207.9           mTL Start (bp)         1799         2151         207.9           mTL End (bp)         2924         2632         289.9           TLR (bp/PD)         42.3         18.4         -23.9         3.1	Image: Key P         Image: Leg P           Control         BIBR1532         Diff         Control         BIBR1532           PD         27.7         26.0         27.7         26.0           mTL Start (bp)         1943         2226         523.1         1261           mTL End (bp)         1881         1558         329.3         305.3           TSR (bp/PD)         22.2         25.7         -23.5         7.0         36.8           PD         26.6         26.1         26.6         26.1         300.8           mTL Start (bp)         1799         2151         207.9         300.8           mTL End (bp)         2924         2632         289.9         385.7           TLR (bp/PD)         42.3         18.4         -23.9         3.1         3.3

Table 5.2: **Telomere lengths and telomere shortening rates for SW480 and LoVo cell lines.** PDs: Population Doublings; bp: base pairs; mTL: median telomere length; TSR: telomere shortening rate. TLR: telomere lengthening rate. Diff: difference of TSR or TLR between control and treated cells for each telomere.

### 5.4 Discussion

Telomerase is present in a high proportion of human cancer cells ((Kim *et al.*, 1994) and (Shay & Bacchetti, 1997)) and thus its activity has been proposed to be related with the acquisition of malignancy during tumorigenesis (Chadeneau *et al.*, 1995) as it is needed for replicative immortality. Accordingly, there are many studies trying to inhibit telomerase using different drugs in an attempt to discover a cancer treatment (reviewed in Rankin *et al.* (2008) and Phatak & Burger (2007)).

In this chapter, the telomerase inhibitor BIBR1532 has been used in two different cancer cell lines: LoVo (MMR-deficient, MSH2<sup>-/-</sup>) and SW480 (MMRproficient, APC<sup>-/-</sup>). The microsatellite instability (MSI) in LoVo was described to be two fold higher compared to HT1080 (a MMR proficient fibrosarcoma cell line) Hanford et al. (1998) and as explained in chapter 4, the MMR-deficient cell line (LoVo) had a high telomere instability driven by the MMR defect (Mendez-Bermudez & Royle, 2011). This might impact on telomere shortening and therefore, have an additive effect under telomerase deficiency. Using the BIBR1532 directly onto cell lysates a higher level of telomerase inhibition was reached for SW480 (92.6%) than for LoVo (58.5%); however, when the drug was used in cell culture for  $\approx 60$  days, no telomerase downregulation was measured using the semiquantitative method TRAP assay. Interestingly, cell growth curves indicated a slower cell growth for SW480 treated cells compared to controls that correlated with an increase in S phase arrest for treated cells shown by flow cytometry analysis (FAC) but no effect was shown for the LoVo cell line, suggesting that either this cell line was more resistant to the antiproliferative effect of BIBR1532 than SW480 or that the drug was not efficiently delivered to this cell line.

Subsequently, the proliferation index (PI) based on the FAC profile was estimated and it was found that the MMR-deficient cancer cell had a greater PI than the MMR-proficient one, specially in the controls, what was due to a very high percentage of cell death in LoVo calculated as subG1 peak compared to SW480. Previous experiments performed by Liu *et al.* (2014) for telomerase downregulation in the SW480 cancer cell line using an shTERT showed a reduction in cell growth with an increase in the proportion of apoptotic cells (by Annexin V staining, TUNEL assay and transmission electron microscopy) that reduced the tumour formation *in vivo*. Jiang *et al.* (2003) also showed an increase in apoptosis after inhibition of telomerase in SW480 using antisense oligodeoxynucleotides (As-ODN). Unfortunately, only a mild reduction in the cell growth that did not occur with an increase of cell death (as sub-G1 peak) was observed, showing that our drug conditions were sub-optimal.

Subsequently, the telomere length was analysed by STELA and the telomere shortening rates (TSR) were estimated. Despite the lack of evidence for telomerase inhibition measured by TRAP assay, it was found that in SW480 treated cells telomeres shortened at a significantly higher rate than in controls (23.5 and 29.8 bp/PD faster for XpYp and 12q respectively), suggesting that the slower cell growth described before might be a consequence of the increased shortening rate. However, this increment in the TSR was not enough to show an effect on cell death. Interestingly, telomeres were more strongly maintained in the MMR-deficient than in the MMR-proficient cell as telomeres in LoVo lengthened during the cell culture even in the absence of the drug. Accordingly, the high percentage of cell death, the very short median telomere lengths and the strong maintenance of them in LoVo, suggests that treatment of this cell line with a suitable telomerase inhibitor might be an interesting approach for tumour inhibition. Furthermore, it was observed that the XpYp telomere lengthened at a slower rate (23.9 bp/PD less) in treated than control LoVo cells but this effect was not observed for 12q, and no effect on cell growth dynamics was observed.

Treatment of the LoVo cell line with anticancer drugs such as CDDP (cisdiamminedichloroplatinum) and 5FU (5-fluorouracil) resulted in an increase in telomerase activity during the first 15 cumulative PDs and a continuous telomere elongation with increasing PDs (up to 39 cumulative PDs); however, the telomere lengthening was different depending on the initial telomere length (+93 bp with 2.80 kb length and +45 bp with 4.19 kb length) (Kuranaga *et al.*, 2001). In the present experiment, the starting telomere lengths for LoVo were shorter than for SW480 in controls and treated cells, suggesting that telomere elongation in this cell line might be needed to continue cell proliferation. Interestingly, El-Daly et al. (2005) showed that the gain of telomeric repeats in immortalized hTERT-HK1 cells was substantially reduced with increasing concentrations of BIBR1532, suggesting a similar effect of that found here in LoVo for the XpYp telomere. Furthermore, it was shown that a telomerase-independent telomere maintenance mechanism might occur in MMR deficient cells (Rizki & Lundblad, 2001) sharing characteristics with the recombination-based ALT mechanism (Bechter et al., 2004); this might explain the continuous telomere length maintenance in controls and treated LoVo cells in comparison with MMR-proficient cells if the BIBR1532 treatment would have reduced the telomere elongation by telomerase. In addition, MMR deficiency is known to promote G2/M cell cycle arrest after UV DNA damage (reviewed in Conde-Pérezprina et al. (2012)), although if not exposed to genotoxic agents, MMR-deficient cancer cells can escape apoptosis and restoration of the MMR defect in those cells increases the apoptosis rate (reviewed in Hassen et al. (2016)). Surprisingly, a significantly higher percentage of control LoVo cells in sub-G1 (that mostly accounted for dead, apoptotic cells) compared to SW480 was found and the percentage of dead cell did not increased upon treatment (possibly due to not efficient drug delivery as no evidences were found for telomerase activity reduction) and cell growth curves were not different from control and treated LoVo cells. Conversely, when telomerase was previously inhibited in the LoVo cell line using a dominant negative (DN-hTERT), cell proliferation was completely stopped (Hahn et al., 1999). The proposed mechanism by which BIBR1532 inhibits telomerase is due to its direct interaction with the enzyme and/or the telomere, directly affecting the enzyme translocation through the telomere or the dissociation between the enzyme and the DNA template (Pascolo et al., 2002); therefore it does not block the catalytic activity of telomerase but the elongation of the DNA template. In addition, BIBR1532 has been shown to interact with the TEL domain in TPP1, competing with telomerase for its binding to the telomere (Nakashima et al., 2013). Hence, very high concentrations of

BIBR1532 might not only affect the enzymatic activity of telomerase but also impact on the binding of other telomeric proteins such as TRF2 (El-Daly et al., 2005). Furthermore, the telomerase inhibition mechanism by the DN-hTERT depends on the removal of the endogenous hTERT from the telomeres and the cytoplasmic degradation via ubiquitination (Nguyen et al., 2009) having a more detrimental effect in cells where the non-canonical functions of telomerase prevail over the elongation process. In fact, in neuroblastoma cells where telomere elongation is not the most important function of telomerase, the use of DN-hTERT does not impact on telomere length but increases the vulnerability to apoptotic drugs (Samy et al., 2012). In this experiment, an increase in S phase for treated *vs* control was only found in SW480 but not in LoVo cells, while other telomerase inhibitors such as T-oligos have shown this arrest, cell growth inhibition and expression of DNA damage response proteins, with an increase in senescence that was not accompanied by telomere shortening (Wojdyla et al., 2014), suggesting that for LoVo, drug delivery was not as efficient as in other studies. Taking into account the outcome of this experiment, it was concluded that treatment of the LoVo cell line with BIBR1532 was not as efficient as for the MMR-proficient (SW480) cells. The contradictory results shown in this chapter might be due to several aspects that should be modified in future experiments:

- <u>Control</u>: SW480 might not have been the best control for this experiment as, although it is a similar type of cancer cell than LoVo, it contains other mutations (*e.g.* APC<sup>-/-</sup>) that might differently impact on cell cycle dynamics. For future experiments inhibiting telomerase in a MMR-deficient colorectal cancer cell line, the best control would be the same cancer cell line with the restored MMR-mutation (*e.g.* LoVo and LoVo+chromosome2 Watanabe *et al.* (2000)).
- <u>Drug</u>: The BIBR1532 drug was used, as it is the most specific synthetic telomerase inhibitor impacting exclusively on the elongation function of telomerase and therefore not affecting other non-canonical functions; however, there are two negative aspects of using this drug: a) high concentrations produce cytotoxic effects and b) the concentrations used in

this experiment are not in the nanomolar range used for clinical treatments, thus the clinical significance is reduced. The PNA drug GRN163 is being used in clinical trials to reduce tumour size by inhibiting telomerase activity and as it has been shown that it reduces telomere length, it might be interesting to see its effect on MMR-deficient cells.

- <u>Drug concentration</u>: The concentration of BIBR1532 used in this experiment was based on previous published work in the same cell lines but no titration experiments were performed. The assumption that the purity of the drug was the same between the published data and our batch might be risky and the performance of titration experiments should be the first step for future experiments.
- <u>Cell proliferation</u>: The use of FAC analysis to analyse cell proliferation by propidium iodide staining might be unreasonable, as other more simple proliferation assays (such as MTT or Ki69) could being used. However, the use of FAC might have been complemented with an additional fluorescent antibody marker, for example a telomere-associated protein such as TRF2 or an apoptosis marker such as Annexin V to increase the information and to give more details about the effect of the drug.
- <u>Other analysis:</u> The telomerase assay used here is a semi-quantitative assay and it would be more reliable if used in conjunction with other assays such as RT-PCR. Besides, comparisons of the relative telome-rase activity for the same treatment at different time points might show changes over time that are also important to consider. In addition, other complementary assays might be performed for future experiments such as western blot or qPCR to study protein expression changes in cell cycle-related proteins (such as p21 or p16 among others) between control and treated cells.

### 6.1 Background

Telomere length (TL) is known to shorten with every cell division in normal cells in culture ((Harley *et al.*, 1990), (Allsopp *et al.*, 1992) and reviewed in Blackburn (1991)) and also it has been shown in humans ((Mondello *et al.*, 1999), (Friedrich *et al.*, 2000) and (Chen *et al.*, 2011)), thus limiting the ability for indefinite cell divisions. Telomere length becomes less variable in healthy seniors (Halaschek-Wiener *et al.*, 2008) and the relationship between TL and disease susceptibility has been extensively studied. Diseases like chronic lymphocytic leukaemia (CLL) (Lin *et al.*, 2010) and other types of cancer such as colorectal cancer (CRC) ((Rampazzo *et al.*, 2011) have been associated with short telomeres compared to normal tissue. Furthermore, mutations in the telomerase gene or other related genes are found in dyskeratosis congenita (DC) patients (Vulliamy *et al.*, 2004), showing a positive association between very short telomeres, disease severity and earlier age of onset (Alter *et al.*, 2012).

Telomerase is known to be active during all the oogenesis stages in the human oocyte (Wright *et al.*, 2001) although in lower levels as the maturation takes place (Turner & Hartshorne, 2013). In the male germline, telomerase activity also decreases during spermatogenesis and it is inversely correlated to TL (Achi *et al.*, 2000). Sperm TL has been shown to increase during male lifetime ((Baird *et al.*, 2006), (De Meyer *et al.*, 2007) and (Aston *et al.*, 2012)). However, TL in oocytes has been found to be significantly longer than in sperm (Turner & Hartshorne, 2013) as a result of the fewer cell divisions that occur in the oocyte progenitor cells compared to the sperm. In addition, TERRA and telomerase have been found to be localized at telomeres during mammalian gametogenesis (Reig-Viader *et al.*, 2014), suggesting a very complex regulation of the TL during the development and maturation of human

sex cells.

Results shown in Graakjaer *et al.* (2006) suggested that despite the presence of telomerase activity in the germline, there was an inherited allelespecific telomere length from the parents that resulted in a fix starting point for each individual's TL, suggesting that the telomere length of the offspring is highly determined by the progenitor's TL in the germline. In addition, other authors have supported the idea that telomere length is not entirely reset in the zygote (De Meyer *et al.*, 2007). Studies in mouse models, where  $Tert^{+/+}$ mice were generated after 17 generations of  $Tert^{+/-}$  crosses, also support the idea that offspring's TL is determined by the parent's TL in the germline (Chiang *et al.*, 2010). Therefore, it is not surprising that offspring's TL has been associated to paternal age at birth ((Unryn *et al.*, 2005), (De Meyer *et al.*, 2007) and (Kimura *et al.*, 2008)). Furthermore, Graakjaer *et al.* (2004) suggested that the inherited TL pattern at conception is more important for determining TL during adulthood than any other factor occurring later in life (such as environmental or epigenetic changes affecting telomere length).

Lynch syndrome (LS) is an autosomal inherited disorder caused by a germ line mutation in a MMR gene, usually hMSH2 or hMLH1 (Peltomäki *et al.*, 1993). It is characterized by an early-onset of colorectal cancer (CRC), often with a bias for the right colon and the presence of other extra colonic tumours such as urologic, upper gastrointestinal or gynaecological (reviewed in Lynch *et al.* (2009) and Boland & Lynch (2013)).

The study of telomeres in LS families follows two main reasons: on the one hand, the microsatellite instability (MSI) that arises as a consequence of impaired MMR, increases the mutation frequency in repetitive DNA sequences including telomeres (Pickett *et al.*, 2004) with a suggested bias towards losses of repeats as shown by an increase in the telomere shortening rate when the MSH2 protein was downregulated in a primary human cell (Mendez-Bermudez & Royle, 2011) and in chapter 3. On the other hand, short telomeres have been related to disease anticipation in hereditary syndromes characterised by telomerase gene mutations such as dyskeratosis congenita (DC) (Vulliamy *et al.*, 2004) or p53 mutations like Li-Fraumeni syndrome (Ta-

bori et al., 2007), as well as suggested to be factors explaining other diseases such as vascular dementia (von Zglinicki et al., 2000b). In addition several meta-analysis have shown a positive relationship between short telomeres and cancer risk ((Ma et al., 2011) and (Zhu et al., 2016)) including colorectal cancer Qin et al. (2014) or hereditary breast cancer ((Martinez-Delgado et al., 2011) and (Duggan et al., 2014)). However, no mechanism has been described yet and controversy still remains towards the existence of genetic anticipation in LS. While some studies showed evidence of an earlier onset of cancer in successive generations ((Westphalen et al., 2005), (Nilbert et al., 2009) and (Timshel et al., 2009)), other studies suggested there might be an ascertainment bias generating artificial anticipation (Voskuil et al., 1997), (Tsai et al., 1997), (Stupart et al., 2013) and (Stupart et al., 2014). Interestingly, Stella et al. (2007) found the first evidence of three large deletions in MSH2 having an effect on the age onset of the disease; in particular, the 23 parent/children pair analysed showed a median anticipation of 12 years with no evidence for a birth cohort effect, and all were mutation carriers for any of three large deletions in MSH2, suggesting that genetic anticipation might occur in families carrying only specific germline mutations.

In a pilot study carried out by Dr. Frances Tippins (Univ. of Leicester iB.Sc. student 2013-2014), saliva samples from two LS cohorts were recruited. Results from some of the families analysed showed a lack of significance between telomere length and age for 12q but not XpYp, although only 39/91 (43%) samples were analysed. Interestingly, parent/children pair comparisons for the XpYp telomere showed a trend for longer telomeres in wild type children compared to their MMR<sup>+/-</sup> parents although the age-adjusted telomere length ( $\Delta$ Tel) was calculated based on the same cohort using 15 wild type samples (including children). Due to the lack of a control cohort establishing a standard curve for telomere attrition with age, no further conclusions were drawn.

# 6.2 Aims

To understand whether the inheritance of a germline MMR gene mutation in Lynch syndrome (LS) families had an impact on telomere length (TL), and if so, whether a mechanism based on TL might be hypothesised to explain the age of onset of cancer. This aim involved the following objectives:

- To recruit LS and control families with the desired parent/children pair combinations for the TL inheritance study.
- To use single telomere length analysis (STELA) to measure TL in DNA from saliva.
- To study TL in control and LS families to compare TL inheritance between MMR proficient and deficient individuals.
- To study TL inheritance in LS by parent/children pair comparisons to try testing an hypothesis for a mechanism describing genetic anticipation.

## 6.3 Results

#### 6.3.1 Recruitment and samples description

Saliva samples from three cohorts were collected, two of which included Lynch syndrome families belonging to either Leicester Royal Infirmary (LRI) or Birmingham Women's Hospital (BWH) and a third one including control families mostly from the Genetics Department in the University of Leicester. The sample collection for the LS families was set up by Dr. Frances Tippins and Dr. Nicola Royle in conjunction with Professor Eamonn Maher and Jonathan Hoffman (BWH) and Dr. Julian Barwell (LRI) as described in section 2.1. Table 6.1 shows a summary of the samples and their main features in terms of sex, age distribution and genetic characteristics. For a more detailed information about the mutations that each family carries, refer to table 6.2. The breakdown of samples collected by Dr. Frances Tippins (FT) or Carmen Garrido (CG) is shown in appendix F.

Figure 6.1 shows that most of the DNA extracted from the saliva samples was high molecular weight (HMW), and therefore, suitable for STELA. Nevertheless, there were two control families (TEL F5 and TEL F10) marked with a red asterisk in figure 6.1.A where the DNA quality was not good enough for STELA and were excluded from the study. In addition, two individuals (TELF6G1P2 and TELF7G2P2) had very degraded DNA and did not generated STELA products, so they were not included in the study. The Oragene<sup>TM</sup> DNA saliva kit contains a DNA stabilising agent to control bacterial growth and maintain DNA integrity (Theall *et al.*, 2013). We used the same reagents and procedure to extract HMW DNA as for the samples that gave degraded DNA, so it was hypothesised that in the samples we had to exclude, there was some source of DNA degradation at sample donation, not during DNA extraction.

Saliva samples were grouped into four main groups: LS MMR<sup>+/-</sup> cancerfree, LS MMR<sup>+/-</sup> cancer-affected, LS MMR <sup>+/+</sup> cancer-free and controls (figure 6.2.A). There were 5 samples that did not fit into any of these four groups: 2 of



Figure 6.1: **DNA quality for control families.** A shows a 1% agarose gel electrophoresis for DNA extracted from saliva in the control families. Red asterisks refer to families that were excluded as no individual generated any STELA products. Green asterisks indicate two additional individuals that were excluded because they did not produce STELA products either. B shows two 1% agarose gel electrophoresis for DNA extracted from saliva in the two LS cohort. All (91) individuals were included in the study. Codes for the samples are: TEL, control families; LRI, Leicester Royal Infirmary and BWH, Birmingham Women's Hospital. The "F" before a number indicates the family's number. "G" indicates generation and "P" indicates position on the pedigree.  $\Lambda$ -HindIII was the size marker used at two different concentrations: 10 and 20 ng/µl. "kb" indicates kilobases.

them were cancer-affected LS  $MMR^{+/+}$  patients (thyroid cancer and basal cell carcinoma) and 3 were controls with cancer (acute myeloid leukaemia, breast and testicular cancers). Nevertheless, none of the 5 samples had any LS-related tumour so they were considered as sporadic tumours, and therefore they were treated as controls. Figure 6.2.B shows the mean age at which the samples were collected for each group. Kruskal-Wallis ranking tests for the age distributions showed significant (p = 0.007) differences in the age at sampling between cancer-affected and cancer-free LS  $MMR^{+/-}$  individuals. This is due to the fact that  $MMR^{+/-}$  in the second generation (children) have not developed cancer yet.

For all families, a pedigree indicating: code, age, mutation status and cancer information is plotted in appendix I for controls and J for LS, together with a scatter plot showing the median telomere length (mTL) for XpYp and 12q telomeres and the interquartile (IQ) range.



Figure 6.2: Cohort description. A is a pie graph that summarises the number of samples within each group. B is a scatter plot with bar indicating the mean and standard deviation of the age at which samples were taken for each group. \*\*\*p = 0.007.

	1	ynch syndrom(	e tamilies (n=	24)		<b>Control fami</b>	lies (n=10)	
1	MMR+/	- (N=60)	MMR <sup>+/</sup>	( <b>N=31</b> )		Unrelated con	trols (N=37)	
1	Cancer	<b>Cancer-free</b>	Cancer	<b>Cancer-free</b>	Total	Non LS cancer	<b>Cancer-free</b>	Total
N	32	28	2	29	91	3	34	37
Parent: N (%) [P/C pairs]	24 (75) [38]	3* (10.7) [7*]	2(100)[3]	14 (48.3) [23]	43 (47.3) [72]	3 (100) [5]	15 (44.1) [29]	18 (48.7) [34]
Children: N (%) [P/C pairs]	8 (25) [12]	25(89.3)[40]	0 (0) [0]	15 (51.7) [19]	48 (52.7) [72]	0 (0) [0]	19 (55.9) [29]	19 (51.4) [29]
$MSH2^{+/-}$ ; N (%)	15 (46.9)	18 (64.3)			33 (36.3)			
MLH1 <sup>+/-</sup> : N (%)	17 (53.1)	10 (35.7)			27 (29.7)			
MMR <sup>+/+</sup> : N (%)			2(100)	29 (100)	31 (34.1)	3 (100)	34 (100)	37 (100)
Sex: N (%)	F: 14 (43.8)	F: 17 (60.7)	F: 2 (100)	F: 16 (55.2)	F: 49 (53.8)	F: 2 (66.7)	F: 21 (62.2)	23 (62.2)
	M: 18 (56.2)	M: 11 (39.3)	M: 0 (0)	M: 13 (44.8)	M: 42 (46.2)	M: 1 (33.3)	M: 13 (37.8)	14 (37.8)
Mean age at sample $\pm$ SD	$53.7\pm13.5$	$36.4\pm12.4$	$65.3\pm10.9$	$48.5\pm15.9$	${\bf 47.0 \pm 15.8}$	$55.5\pm2.8$	$44.4\pm19.0$	${\bf 45.3 \pm 18.4}$
Mean age at sample $\pm$ SD parents	$60.0\pm7.7$	$66.3\pm2.0$	$65.3\pm10.9$	$62.7\pm7.3$	$61.6 \pm 7.5$	$55.5\pm2.8$	$62.1\pm10.4$	$60.9 \pm 9.8$
Mean age at sample $\pm$ SD children	$34.8\pm8.2$	$32.9\pm7.0$		$35.1\pm 8.0$	$33.9 \pm 7.4$		$30.5\pm10.6$	${f 30.5\pm 10.6}$
Mean age 1st cancer $\pm$ SD	$39.4\pm13.4$	ı	$58.5\pm9.3$	ı	${\bf 40.5}\pm{\bf 14.2}$	$43.7\pm7.6$	ı	$\textbf{43.7} \pm \textbf{7.6}$
Mean age 1st cancer $\pm$ SD parents	$44.5\pm11.4$	ı	$58.5\pm9.3$	ı	ı	$43.7\pm7.6$	ı	ı
Mean age 1st cancer $\pm$ SD children	$23.9\pm7.1$	ı	ı	ı	ı		ı	

Table 6.1: Features of the studied groups. The two Lynch syndrome (LS) cohorts from BWH and LRI are grouped in the LS families group. n: number of families; N: number of subjects per group.  $MSH^{2+t-}$  and  $MLH^{1+t-}$ : heterozygous for a mismatch repair (MMR) gene mutation;  $MMR^{+t+}$ : homozygous for the wild type of a MMR gene; N: number of subjects; %: relative percentage to the total of each group; P/C: parent-children; SD: standard deviation; F: female; M: male. \*Indicates that the number is effectively +1 (individual/pair) as sample BWH F105 G4P9 was treated as children and as parent (see pedigrees in appendix I for control and J for LS families for more information).

#### 6.3.2 Telomere length measurement and reproducibility

All saliva samples had a unique code for identification; however, two different types of codes were used per sample. On the one hand, for sample recruitment and DNA extraction, the unique identifier used per individual contained a prefix with the family code (either BWH or LRI for LS families or TEL for control families), that was followed by a number referring to the family number. Next, a "G" indicated the generation and was followed by a number referring to the generation the individual had on the pedigree and finally, a number after a "P" indicated the position in the pedigree. For example, an individual coded as: BWH F105 G4P2, belonged to a LS family recruited in Birmingham Women's Hospital, was included in the pedigree for family 105, referred to the 4<sup>th</sup> generation and occupied the position 2 in the pedigree. On the other hand, for telomere length analysis a blind code was generated to avoid bias in the measurements based on the known age of the individual. This code contained the first part of the unique code, indicating cohort (BWH, LRI or TEL) and family number, but the "G-" and "P-" were substituted by a letter in alphabetical order depending on the number of individuals per family.

Reproducibility of the method was first assayed by comparing telomere length measurements for the same coded family (BWH F100) between the two students (FT and CG); the letters A, B and C in figure 6.3 anonymised each of the three members for this family. No significant differences were obtained when comparing data generated by either student, confirming that our results were highly reproducible and comparable.

In addition, the same control DNA, extracted from the lymphoblastoid cell line KK, was used in each STELA gel amplified with the flanking primer XpYpE2 (see table in appendix B for primer sequence). Analysis of this control sample allowed the data from saliva samples in different gels to be combined for the same individual and to compare between gels and families. For each sample, at least a duplicate experiment was performed and when possible, members from the same family were analysed in the same Southern blot. Kruskal-Wallis tests, corrected for multiple comparisons, were performed on all the control DNA experiments before pooling data. Only two replicate gels

Family	Type of mutation/location	C.Significance	Link
LRI-F1	Missense mutation (c.1447C>T; p.Glu483X) in exon 9 of the $hMSH2$ gene	Risk factor	rs587777421
LRI-F4	Deletion of exons 9 to 16 of the $hMSH2$ gene	Pathogenic	DOI 10.1002/humu.10291
LRI-F5	Missense mutation (c.1861C>T; p.Arg621X) in exon 12 of the $hMSH2$ gene	Pathogenic	rs63750508
LRI-F7	Missense mutation (c.1373T>G; p.Leu458X) in exon 8 of the $hMSH2$ gene	Pathogenic	rs63750521
LRI-F14	Deletion of exon 1 of the $hMSH2$ gene	Pathogenic	OMIM: 609309.0015
BWH-F5a	Missense mutation (c.2422G>T; pGlu808X) in exon 14 of the $hMSH2$ gene	Pathogenic	rs34986638
BWH-F11a	Frameshift mutation (c.1035-1036delGAinsT; p.Trp345Cysfs $\times$ 12) in exon 6 of the $hMSH2$ gene	Untested	-
BWH-F55	Nonsense mutation (c.2126T>A; p.L709X) in exon 13 of the $hMSH2$ gene	Untested	-
BWH-F71	Deletion of exon 1 of the $hMSH2$ gene	Pathogenic	OMIM: 609309.0015
BWH-F100	Splice-site mutation (c.2211-1G>C) in exon 13 of the $hMSH2$ gene	Untested	-
BWH-F105	Missense mutation (c.1030C>T; p.Gln344X) in exon 6 of the $hMSH2$ gene	Pathogenic	rs63750245
BWH-F108	Frameshift mutation (c.2562delT; p.Gln855SerfsX37) in exon 15 of the <i>hMSH2</i> gene	Untested	-
BWH-125	$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Untested	-
LRI-F12	Deletion of the entire coding region of the $hMLH1$ gene	Untested	-
BWH-F1a	Deletion of exons 1 to 19 in the $hMLH1$ gene	Untested	-
BWH-F3a	Missense mutation (c.200 G>A; p.Gly67Glu) in exon 2 of the $hMLH1$ gene	Pathogenic	rs63749939
BWH-F6a	Deletion (c.1852-1854 delAAG; p.Lys618 del) in exon 16 of the $hMLH1$ gene	Pathogenic	rs63751247
BWH-F2	$ \begin{array}{llllllllllllllllllllllllllllllllllll$	Pathogenic	rs587778906
BWH-F42	Deletion of exon 6 in the $hMLH1$ gene	Untested	-
BWH-F102	Missense mutation (c.380G>A; p.Arg127Lys) in exon 4 of the $hMLH1$ gene	Likely pathogenic	rs63751595
BWH-F106	Splice-site mutation (c.1668-1G>A) in intron 15 of the $hMLH1$ gene	Likely pathogenic	rs267607845
BWH-F107	Missense mutation (c.199G>A; p.Gly67Arg) in exon 2 of the $hMLH1$ gene	Pathogenic	rs63750206
BWH-F126	Frameshift mutation (c.94delA; p.Ile32SerfsX4) in exon 1 of the <i>hMLH1</i> gene	Untested	-
BWH-F130	Missense mutation (c.306G>T; p.Glu102Asp) in exon 3 of the <i>hMLH1</i> gene	Likely pathogenic	rs63751665

Table 6.2: **Mutation details per family.** The top part of the table shows the specific germline mutation that each family carries for the human MSH2 gene (hMSH2). The bottom part of the table shows the specific germline mutation that each family carries for the human MLH1 gene (hMLH1). For all mutations: "c." indicates location in the coding sequence; "p." location in the protein sequence; ">" refers to a point mutation; "-" between two numbers indicates frameshift mutation; "X" indicates stop codon; "del" indicates deletion and "ins" indicates insertion.

out of 128 gels were significantly different from the rest (representing a 1.6% error in the method) and therefore, data from those replicate gels were excluded from further analysis (see appendix G and H). Figure 6.5 shows four STELA gels from two different LS families and the quantification, as an example.



Figure 6.3: **Technical reproducibility between students.** The scatter plots show median telomere lengths (mTL) and interquartile ranges for XpYp (left) and 12q (right) telomeres in family BWH 100. Individuals were coded as A, B and C to avoid bias in the interpretation. Data in purple was generated by Frances Tippins and data in green generated by Carmen Garrido. Medians and interquartile ranges are shown.

Telomere length for XpYp and 12q telomeres were measured for all 128 individuals and to decide whether means or medians would describe our samples better, D'Agostino & Pearson omnibus normality test was run for each individual and telomere. Surprisingly, the percentage of individuals with normally distributed telomeres was greater for control individuals than LS for XpYp (40% -21/52- vs 33% -25/76-) and 12q (56% -29/52- vs 41% -31/76-). Nevertheless, there was no difference between parent/children percentages in normality between both cohorts, suggesting that the telomere length distribution per individual was not affected by age. Interestingly, when identifying outliers using the ROUT method (Q=1%), a greater percentage of LS individuals with outliers for XpYp (19% -14/76- vs 16% -6/52-) and especially for 12q (33% -25/76- vs 17% -9/52-) was observed. This might explain why the percentage of normality decreased in the LS cohort compared to controls but it also suggested that the presence of a MMR deficiency might have an impact on telomere length dynamics. In addition, correlation analysis between XpYp and 12q telomere lengths for each individual showed that both telomeres were significantly correlated (p < 0.0001 and p = 0.002 for controls and LS respectively). However, the r values of the linear regressions were smaller in LS individuals (r = 0.41) than in controls (r = 0.63), suggesting a greater TL heterogeneity in the LS cohort (figure 6.4), although the 95% confidence intervals overlapped somehow (0.20 to 0.59 for LS and 0.42 to 0.77 for controls).



Figure 6.4: **Correlation of XpYp and 12 telomere lengths.** Red colour indicates 52 control individuals (37 from the control cohort and 15 LS MMR<sup>+/+</sup> parents) and purple, 76 LS individuals (MMR<sup>+/-</sup> or MMR<sup>+/+</sup> children).



#### 6.3.3 Telomere shortening with age

To analyse the relationship between the age (at sample donation) and the telomere length (TL) in normal families, 10 healthy families were mostly recruited from the Genetics Department in the University of Leicester, comprising 37 individuals with a mean age of 45.3 ( $\pm$  18.4 SD) years. In addition, 15 samples from the LS cohort that were MMR<sup>+/+</sup> and spouses of the MMR<sup>+/-</sup> parents (therefore, from LS-non related families) were included as controls to increase the sample size of the control cohort. In total, the control cohort contained 52 samples that were used to draw a standard curve for the telomere attrition rate with age for subsequent comparisons with the samples from the LS families.

Two telomeres, 12q and XpYp were measured by STELA as described in section 2.2.2.8 and telomere length (TL) data was plotted against age for all the individuals. The linear regression was not strong (r < -0.3) and not significant (p = 0.0642 for XpYp and p = 0.0426 for 12q), suggesting weak relationship between TL and age (figure 6.6.A). As discussed in section 1.2.1.3 (view table 1.4), the main disadvantage of STELA is the underestimation of TL due to the inability of measuring very long telomeres. This is, to our knowledge, the first time that STELA has been used to measure individual's TL from DNA extracted from saliva in a cohort with such a large age-distribution cohort (18.2 to 82.6 years). To address this issue, we reduced the number of STELA products per track up to a maximum of 10, and therefore it was assumed that the lack of significance between age and TL was due to underestimation of the TL in younger samples and not to a bias for shorter telomeres. Thus, we performed a piecewise regression as described in Wagner et al. (2002). This is a segmented linear regression in which the dependent variable (TL) has different linear relationships with the independent variable (age) for at least two different intervals. We used the 25% percentile for age (35 years) as a breaking point and two linear regression curves were generated per telomere, one comprising the age interval 18 to 35 years and another for individuals older than 35 years. As shown in figure 6.6.B, the two age intervals had different slopes and samples over 35 years had a regression analysis slope that was significantly different from zero (with r = -0.3 and r = -0.4for XpYp and 12q respectively) in contrast to samples from individuals below 35 years. Human telomeres shorten with age at different rates during embryogenesis (Cheng et al., 2013) and in childhood (Frenck et al., 1998) but it has been largely agreed that the rate is constant during adulthood (Slagboom et al. (1994) and Chen et al. (2011)), even in different tissues (Daniali et al., 2013). Since regression results for the 75% of the individuals, including those older than 35 years, showed a significant negative linear relationship between telomere length and age that was not shown for individuals younger than 35 years, we assumed that a certain level of telomere length underestimation occurred in the 25% of our samples. However, we could not exclude those individuals because parent/children pairs were needed for subsequent analyses, therefore, we assumed that telomere length in younger than 35 years shortened with age at similar rate than in older individuals and we used the regression equations 6.1 and 6.2 obtained for the 75% of the data (including older than 35 years individuals) to transform the telomere length for the remaining 25% of the samples.

$$XpYp \ expected \ TL = (-35.12 \times Age) + 6870 \tag{6.1}$$

$$12q \ expected \ TL = (-31.14 \times Age) + 6905$$
 (6.2)

The mean expected TL (mTL<sub>exp</sub>) for all individuals with <35 years was 6017 and 6149 bp for XpYp and 12q respectively, nevertheless, the observed TL mean (mTL<sub>obs</sub>) was 4990 and 5202 bp for XpYp and 12q respectively. Using the observed and the expected values, we estimated that the telomere length for this group of individuals had been underestimated by 17.1% and 15.4% for XpYp and 12q respectively using the following equation:

% of underestimation = 
$$100 - \frac{100 \times mTL_{obs}}{mTL_{exp}}$$
 (6.3)

Therefore, we transformed the observed telomere length for each individual younger than 35 years multiplying by 1.171 and 1.154 for XpYp and 12q respectively. When the transformed data (for younger than 35 years) and the raw data (for older than 35 years) for telomere length was plotted against age, there was a very significant (p = 0.0002 for XpYp and p < 0.0001 for 12q) association for both telomeres with age, and the linear regression model significantly improved (r=-0.5 for XpYp and r=-0.6 for 12q) and allowed to include all 52 individuals belonging to the control cohort as shown in figure 6.6.C.

To study telomere shortening with age in the Lynch syndrome cohort only data for  $MMR^{+/-}$  (parents and children) and  $MMR^{+/+}$  children was included, as the  $MMR^{+/+}$  parents were non-LS related and were included in the control cohort. Regression analysis for both telomeres showed a slightly significant negative relationship between age and median telomere length (figure 6.7.A. However, as the age range of this cohort was not significantly different (p = 0.4237 for a rank Mann Whitney test) to the age range in the control cohort, we expected that telomere length might have been underestimated due to our method for individuals younger than 35 years. Thus, a piecewise regression using the same breaking point (35 years) as in the control cohort was also performed.

Contrary to what happened in the piecewise regression for the controls, in this cohort the regression line for younger (< 35 years) individuals had a positive slope, suggesting that the telomere length for the oldest individuals in this age interval was possibly more accurately measured. This effect might be explained if telomeres in LS individuals were shorter than in controls, and thus for the same age, a smaller degree of underestimation would be made. In addition, for the second age interval (> 35 years), the regression lines showed higher r values suggesting a better fit of the model and the significance of the curves was greater (figure 6.7.B). The data was transformed as previously described for the control cohort, but in this case, the equations used to calculate the estimated telomere length for the first age interval (< 35 years) were:



Figure 6.6: **Telomere shortening with age in control individuals.** For all graphs, blue is XpYp and green, 12q telomeres. **A** is a regression analysis of the raw data for control (non-LS related) individuals (52). Table on the right shows curves equations, r and  $r^2$  values as a measurement of the goodness of fit and p values for the slope confidence. **B** is a piecewise regression with breaking point at 35 years for the same data in A. Here, yellow is XpYp for younger than 35 years and brown, 12q for the same age interval. The table on the right shows same information as in A for all four curves. **C** is the final regression curve after data transformation.

$$XpYp \ expected \ TL = (-24.50 \times Age) + 6247 \tag{6.4}$$

$$12q \text{ expected } TL = (-21.51 \times \text{Age}) + 6353$$
 (6.5)

In this cohort, the mean expected telomere length (mTL<sub>exp</sub>) for the < 35 years group was 5539 and 5732 bp for XpYp and 12q respectively, while the mean for the observed telomere length (mTL<sub>obs</sub>) was: 5259 (for XpYp) and 5361 (for 12q) bp. As predicted before when describing the piecewise regression, the underestimation in the telomere length measurements was lower than in the control cohort: 5.06% and 6.47% for XpYp and 12q respectively. Subsequently, data for the observed median telomere length (in the first age interval individuals, <35 years) was multiplied by 1.051 and 1.065 for XpYp and 12q respectively and when transformed data for individuals < 35 years was plotted together with raw data for the second age interval against age, the model fit improved significantly as shown in figure 6.7.C.

Curve comparisons performing ANCOVA analysis (see table 6.3) between controls and MMR<sup>+/-</sup> individuals showed no significantly different slopes or Y-intercepts for any telomere as shown in figure 6.8.A (confirming previous results from Seguí et al. (2013)). Nevertheless, telomeres seemed to shorten at a slower rate in MMR<sup>+/-</sup> compared to controls (17 *vs* 34 bp/year for XpYp and 15 vs 31 bp/year for 12q), suggesting they might be shorter over generations in MMR<sup>+/-</sup>, what might result in an underestimation of the telomere attrition rate in cross-sectional studies as hypothesised by Holohan et al. (2015). Furthermore, the Y-intercepts (predicted telomere length at birth) were smaller in  $MMR^{+/-}$  than in controls (5824 vs 6807 bp for XpYp and 6011 vs 6840 bp for 12q), suggesting that the telomere attrition rate found might be explained, at least partially, by the baseline telomere length at birth. Comparisons within the LS cohort between MMR proficient (MMR<sup>+/+</sup>) and deficient  $(MMR^{+/-})$  samples showed that telomeres shortened at a slower (but not significantly different) rate for MMR<sup>+/-</sup> than MMR<sup>+/+</sup> (17 vs 30 bp/year for XpYp and 15 vs 27 bp/year for 12q) and that MMR<sup>+/-</sup> had smaller (but not signif-



Figure 6.7: **Telomere shortening with age in Lynch syndrome patients.** For all graphs, blue is XpYp and green, 12q telomeres. **A** is a regression analysis of the raw data for LS individuals (76). Table on the right shows curves equations, r and  $r^2$  values as a measurement of the goodness of fit and p values for the slope confidence. **B** is a piecewise regression with breaking point at 35 years for the same data in A. Here, yellow is XpYp for younger than 35 years and brown, 12q for the same age interval. The table on the right shows same information as in A for all four curves. **C** is the final regression curve after data transformation.

icantly different) Y-intercepts than MMR<sup>+/+</sup> (5824 vs 6933 bp for XpYp and 6011 vs 6866 bp for 12q) (figure 6.8.B). Interestingly, no significant correlation was found between telomere length and age for  $MLH1^{+/-}$  samples (see table 6.3), confirming previous results in Bozzao *et al.* (2011a). In addition,  $MSH2^{+/-}$  samples had the smallest Y-intercept (5688 and 5960 bp for XpYp and 12q respectively; figure 6.8.C), suggesting that  $MSH2^{+/-}$  carriers either inherit shorter telomeres than  $MSH2^{+/+}$  or suffer a higher telomere shortening rate during development.



Figure 6.8: **Regression curves comparison for telomere length with age.** A compares the telomere shortening with age between controls in red (52 individuals) and LS in purple (76 individuals) for XpYp (left graph) and 12q (right graph). **B** compares the telomere length with age between  $MMR^{+/-}$  in light green (60 individuals) and wild type ( $MMR^{+/+}$ ) children with a  $MMR^{+/-}$  parent in blue (16 individuals). **C** compares the telomere length with age between  $MLH1^{+/-}$  in yellow (27 individuals) and  $MSH2^{+/-}$  in green (33 individuals).

		ХрҮр				12q		
	Slope	Y- intercept	r	р	Slope	Y- intercept	r	р
	$\pm\mathrm{error}$	$\pm  \mathrm{error}$			$\pm  \mathrm{error}$	$\pm  \mathrm{error}$		
Control	$\textbf{-34}\pm 8$	$6807 \pm 448$	-0.5	0.0002	$\textbf{-31}\pm7$	$6840\pm349$	-0.6	<0.0001
$MMR^{+/-}$	$\textbf{-17}\pm7$	$5824 \pm 333$	-0.3	0.0204	$\textbf{-15}\pm6$	$6011\pm302$	-0.3	0.0187
ANCOVA p	0.1035	0.4786			0.0843	0.6320		
MMR <sup>+/-</sup>	$-17\pm7$	$5824 \pm 333$	-0.3	0.0204	$-15\pm 6$	$6011\pm 302$	-0.3	0.0187
MMR <sup>+/+</sup>	$\textbf{-30}\pm22$	$6933 \pm 875$	-0.3	0.2047	$\textbf{-}27\pm16$	$6866 \pm 636$	-0.4	0.1179
ANCOVA p	0.5117	0.0192			0.4967	0.0693		
MSH2	$-18\pm9$	$5688 \pm 418$	-0.4	0.0480	$-17\pm8$	$5960 \pm 382$	-0.4	0.0451
MLH1	$\textbf{-15}\pm11$	$6023\pm516$	-0.3	0.1604	$\textbf{-14}\pm \textbf{10}$	$6087 \pm 491$	-0.3	0.1872
ANCOVA p	0.8543	0.0337			0.8205	0.1828		

Table 6.3: **Regression curves comparison for telomere length attrition with age in saliva samples.** The data refers to the linear regression curves shown in figure 6.8. "ANCOVA p" indicates the p value for the analysis of covariance in linear regression curve comparison. "MMR<sup>+/+</sup>" refers to wild type children belonging to the LS cohorts and thus, having a mutation carrier progenitor."r" shows the goodness of the fit; "p" indicates the significance of the linear regression. Significant values are shown in italics.

It still remains unknown whether there is a sex bias in telomere length and/or shortening with age, as some authors have described women with longer telomeres than males ((Benetos et al., 2001), (Cawthon et al., 2003), (Steenstrup et al., 2013) and (Steenstrup et al., 2013) among others), whereas other authors have found men having longer telomeres than women (De Meyer et al., 2007) and in fact, shortening at a faster rate in males than females ((Vaziri et al., 1993) and (Gardner et al., 2014)). When telomere attrition with age was studied accounting for sex in the control cohort, no significant differences were observed, although Y-intercepts were slightly greater in females (7282 and 6915 bp for XpYp and 12q respectively) than in males (5956 and 6695 bp for XpYp and 12q respectively). No differences were observed either for the telomere shortening with age, but again, a tendency to greater shortening rate in females (43 and 32 bp/year for XpYp and 12q respectively) than in males (20 and 29 bp/year for XpYp and 12q respectively) was observed (figure 6.9.A). Likewise, no differences were observed in telomere length or attrition rates between males and females for the LS cohort, having a similar shortening for XpYp (22 and 21 bp/year) and 12q (20 and 19 bp/year) (figure 6.9.B).



Figure 6.9: **Regression curves comparison between sexes for telomere length with age.** A compares the telomere shortening with age between males (blue) and females (pink) in the control cohort. **B** compares the telomere length with age between males (blue) and females (pink) in the LS cohort.

#### 6.3.4 Telomere length inheritance

As telomeres shorten with age, all children might be expected to have longer telomeres than their parents. Therefore, the median telomere length was plotted for each family indicating children with circles and parents with triangles (figure 6.10.B). It was found that 42% (8/19) and 26% (5/19), for XpYp and 12q telomeres respectively, of the children had at least one telomere shorter than, at least, one parent (in the control cohort). Nevertheless, this percentage was greater when plotting data before transformation (figure 6.10.A), supporting again the idea of telomere length underestimation in the younger section of our cohort.



Figure 6.10: **Median telomere length in control families.** A is a scatter plot showing the raw median telomere length (mTL) of each control individual per family (before data transformation). **B** is a scatter plot showing the mTL for each control individual per family after data transformation of the < 35 years. For all graphs: blue colour refers to XpYp, green to 12q, circles are children, triangles parents, black triangles parents with cancer and red circles around children highlight the ones with shorter mTL than, at least, one parent. See pedigrees in appendix I for further family information.

The percentage of children with shorter telomeres than their parents was also studied in the LS families and compared them with controls. A smaller percentage of children with shorter telomeres than, at least, one parent was found compared to the control families (figure 6.11) for XpYp (36% -17/47-) and for 12q (23% -11/47-). Interestingly, of the children with shorter telomeres than at least one parent, all except one (LRI F4 G5P4), were mutation carriers in MSH2 families. However, there were similar number of children with shorter telomeres than, at least one parent, comparing MMR<sup>+/+</sup> and MMR<sup>+/-</sup> children in MLH1 families, suggesting different effects on telomere length depending on the mutated gene. In addition, only one of the four  $MSH2^{+/-}$ cancer-affected children had both telomeres longer than their parents (although it was a difficult family as the mother carried a BRCA2 mutation and the mutation status for this gene was not known for the daughter). However, in the MLH1 families there were three out of four  $MLH1^{+/-}$  cancer-affected children (BWH F42 G6P1, BWH F126 G4P3 and BWHF1a G4P3) with both telomeres longer than their parents, suggesting there might be a tendency for shorter telomeres in MSH2 and for longer in MLH1 families.

To study the relationship between telomere length and genetic anticipation of the disease, Vulliamy *et al.* (2004) measured the telomere length by TRF in 8 dyskeratosis congenita (DC) families carrying a mutation in the RNA component of the telomerase (*hTERC*). After adjusting the telomere length to age, comparisons between parent/children (P/C) pairs showed that children had shorter age-adjusted telomere lengths ( $\Delta$ Tel) than their parents compared to 87 unrelated control pairs, suggesting that the significant decrease in the telomere length over generations might be related to anticipation. Here, we hypothesised that if telomere length had an effect on the age of cancer onset and thus contributing to anticipation, we might observe a similar effect to that previously described in DC patients. Therefore, we calculated the estimated telomere length for all the LS and control individuals, as previously described, using the linear regression equations 6.6 and 6.7 for XpYp and 12q respectively. Subsequently, we estimated the age-adjusted telomere length ( $\Delta$ Tel) for each individual using equations 6.8 and 6.9 for XpYp and



Figure 6.11: **Median telomere length in Lynch syndrome families.** A shows the median telomere length (mTL) in LS families carrying an *MSH2* germline mutation. B shows the mTL in LS families carrying an *MLH1* germline mutation. For both graphs, left part shows data for the XpYp telomere (blue) and right part, for 12q (green). **Graph code:** circles are children and triangles parents. Grey colour indicates MMR<sup>+/+</sup> while blue or green indicate MMR<sup>+/-</sup> for either XpYp or 12q respectively; yellow indicates a MMR<sup>+/+</sup> parent that carries a BRCA2 germline mutation. Triangles or circles with a black border indicate cancer-affected individuals. Red circles around some children highlight children with shorter telomeres than, at least, one parent. When several branches can be studied independently within one family (see pedigrees on appendix J for more details), a letter A, B or C next to the family indicates the branch. For branch B in family BWH105, three generations can be studied, therefore: triangle shows first generation, hexagon the second and star the third.)

12q respectively and finally, the change in telomere length over generations was calculated as in Vulliamy *et al.* (2004) using equation 6.10.

$$eTL_{XpYp} = (-34.31 \times X) + 6870$$
 (6.6)

$$eTL_{12a} = (-31.01 \times X) + 6840 \tag{6.7}$$

$$\Delta X p Y p = o T L_{X p Y p} - e T L_{X p Y p}$$
(6.8)

$$\Delta 12q = oTL_{12q} - eTL_{12q} \tag{6.9}$$

Change in telomere length = 
$$\Delta \text{Tel}_{\text{children}} - \Delta \text{Tel}_{\text{parent}}$$
 (6.10)

where:

 $eTL_{XpYp} = expected telomere length for XpYp$   $eTL_{12q} = expected telomere length for 12q$  X = age  $oTL_{XpYp} = observed telomere length for XpYp measured by STELA$   $oTL_{12q} = observed telomere length for 12q measured by STELA$   $\Delta Tel_{children} = age-adjusted telomere length for children$  $\Delta Tel_{parent} = age-adjusted telomere length for parent$ 

Tables 6.4 (for controls) and 6.5 (for LS) show a summary for sex, age, age-adjusted telomere length ( $\Delta$ TL) for XpYp and 12q and parent/children pair (P/C) comparisons that were calculated by subtracting the  $\Delta$ TL for each parent to the  $\Delta$ TL of their offspring as described in equation 6.10.

	ц	arent				Children				ΔC	$\Delta P$
Code	age	Sex	$\Delta X p Y p$	$\Delta 12q$	Code	age	$\mathbf{Sex}$	$\Delta X p Y p$	$\Delta 12q$	XpYp	12q
TEL-F1 G1P1	81.6	Ŀ	-1476	-807	TEL-F1 G2P1	47.1	ы	528	-417	2004	390
TEL-F1 G1P1	81.6	ы	-1476	-807	TEL-F1 G2P2	44.5	Μ	-1764	-221	-289	586
TEL-F1 G1P1	81.6	Ŀ	-1476	-807	TEL-F1 G2P3	43.0	Ŀ	1028	-32	2504	774
TEL-F1 G1P2	82.6	Μ	-245	-436	TEL-F1 G2P1	47.1	ы	528	-417	773	19
TEL-F1 G1P2	82.6	Μ	-245	-436	TEL-F1 G2P2	44.5	Μ	-1764	-221	-1519	214
TEL-F1 G1P2	82.6	Μ	-245	-436	TEL-F1 G2P3	43.0	Ъ	1028	-32	1273	403
TEL-F2 G1P1	54.3	W	170	514	TEL-F2 G2P1	19.3	Μ	-1207	285	-1376	-229
TEL-F2 G1P2	55.2	Ч	-2418	-965	TEL-F2 G2P1	19.3	Μ	-1207	285	1211	1250
TEL-F3 G1P1	59.8	M	-216	53	TEL-F3 G2P1	22.9	ы	595	066	811	936
TEL-F3 G1P1	59.8	Μ	-216	53	TEL-F3 G2P2	18.2	ы	1663	-745	1879	-799
TEL-F3 G1P2	58.4	ы	3243	749	TEL-F3 G2P1	22.9	ы	595	066	-2648	240
TEL-F3 G1P2	58.4	ы	3243	749	TEL-F3 G2P2	18.2	ы	1663	-745	-1580	-1495
TEL-F4 G1P1	52.9	M	247	1151	TEL-F4 G2P1	19.4	Μ	-80	421	-327	-730
TEL-F4 G1P2	48.0	ы	264	-140	TEL-F4 G2P1	19.4	Μ	-80	421	-344	561
TEL-F6 G1P2	57.5	ξ	119	-1863	TEL-F6 G2P1	34.4	ы	-1575	-197	-1694	1666
TEL-F7 G1P1	63.8	W	612	1213	TEL-F7 G2P1	31.4	Μ	-380	-1005	-991	-2217
TEL-F7 $G1P2$	62.4	ы	-555	-785	TEL-F7 G2P1	31.4	Μ	-380	-1005	176	-220
TEL-F8 G1P1	53.6	ы	-1305	-1114	TEL-F8 G2P1	19.9	ы	-1399	-1613	-95	-499
TEL-F8 G1P1	53.6	ĿЧ	-1305	-1114	TEL-F8 G2P2	23.6	ч	368	655	1673	1769
TEL-F8 G1P1	53.6	ы	-1305	-1114	TEL-F8 G2P3	27.7	Μ	-169	-183	1136	931
TEL-F9 G1P1	69.4	Ŀч	1164	2041	TEL-F9 G2P1	37.0	ы	-171	440	-1335	-1600
TEL-F9 G1P1	69.4	ы	1164	2041	TEL-F9 G2P2	45.5	ы	359	697	-805	-1344
TEL-F9 G1P1	69.4	ы	1164	2041	TEL-F9 G2P3	47.9	ч	-470	-1026	-1634	-3067
TEL-F9 G1P2	72.2	Μ	1065	066	TEL-F9 $G2P1$	37.0	ы	-171	440	-1236	-549
TEL-F9 G1P2	72.2	Μ	1065	066	TEL-F9 G2P2	45.5	ы	359	697	-705	-293
TEL-F9 G1P2	72.2	Μ	1065	066	TEL-F9 G2P3	47.9	ξų	-470	-1026	-1534	-2016
TEL-F11 G1P1	62.1	W	1120	-974	TEL-F11 G2P1	29.6	Μ	-199	-678	-1319	296
TEL-F11 G1P1	62.1	Μ	1120	-974	TEL- $F11$ G2P2	25.6	ы	1113	-269	9-	705
TEL-F11 G1P2	57.7	Ŀ	285	4	TEL- $F11$ G2P1	29.6	Μ	-199	-678	-484	-682
TEL-F11 G1P2	57.7	Ы	285	4	TEL-F11 G2P2	25.6	ξų	1113	-269	828	-273
TEL-F12 G1P1	47.2	ы	-150	826	TEL-F12 G2P2	19.8	н	179	1042	330	216
TEL-F12 G1P1	47.2	Ŀ	-150	826	TEL-F12 G2P4	23.0	Ŀ	373	839	524	13
TEL-F12 G1P2	53.0	Μ	793	217	TEL-F12 G2P2	19.8	۲	179	1042	-614	825
TEL-F12 G1P2	53.0	Μ	793	217	TEL-F12 G2P4	23.0	Ъ	373	839	-420	622
Table 6.4: <b>Age-adjust</b> ¢ Red colour indicates P/(	<b>d telomere l</b> C comparisons	ength (∆Te where chile	el) and paren dren have shor	t/children pair ter age-adjusted	(P/C) comparisons for co telomere length than their ]	<b>ntrol famili</b> parents. Tabl	l <b>es.</b> le code: Sex:	F=female; M=	male.		
	•			,	,						

			Pare	ent							Childre	en				$\Delta \mathbf{C}_{-1}$	∆P
Code	Age	Gene	Sex	Cancer	Age C.	∆XpYp	$\Delta 12q$	Code	Age	Gene	Sex	Cancer	Age C.	$\Delta X p Y p$	$\Delta 12q$	XpYp	12q
LRIF1 G2P3	68.0	2	Μ	ON	N/A	-381	189	LRIF1 G3P3	43.0	2	Γų	CRC	31	-151	-863	230	-1051
LRIF1 G2P3	68.0	2	Μ	NO	N/A	-381	189	LRIF1 G3P5	41.0	2	ы	ON	33	-1449	-180	-1068	-368
LRIF1 G2P3	68.0	2	Μ	NO	N/A	-381	189	LRIF1 G3P7	38.3	2	ы	NO	N/A	-682	760	-301	571
LRIF1 G2P3	68.0	2	Μ	NO	N/A	-381	189	LRIF1 G3P8	34.7	0	Μ	ON	N/A	495	927	876	738
LRIF1 G2P3	68.0	2	Μ	NO	N/A	-381	189	LRIF1 G3P9	33.9	0	Μ	ON	N/A	323	-192	703	-380
LRIF1 G2P4	66.5	0	Ъ	NO	N/A	767	494	LRIF1 G3P3	43.0	2	ы	CRC	31	-151	-863	-917	-1357
LRIF1 G2P4	66.5	0	Ъ	NO	N/A	767	494	LRIF1 G3P5	41.0	2	ы	ON	33	-1449	-180	-2216	-674
LRIF1 G2P4	66.5	0	Γų	NO	N/A	767	494	LRIF1 G3P7	38.3	2	ы	NO	N/A	-682	760	-1449	266
LRIF1 G2P4	66.5	0	Ъ	NO	N/A	767	494	LRIF1 G3P8	34.7	0	Μ	ON	N/A	495	927	-272	432
LRIF1 G2P4	66.5	0	ы	NO	N/A	767	494	LRIF1 G3P9	33.9	0	Μ	NO	N/A	323	-192	-444	-686
LRIF4 G4P8	66.7	0	Μ	NO	N/A	1388	324	LRIF4 G5P4	41.8	0	ы	NO	N/A	164	-419	-1224	-743
LRIF4 G4P9	64.1	2	Ъ	NO	N/A	-612	-149	LRIF4 G5P4	41.8	0	ы	NO	N/A	164	-419	776	-270
LRIF5 G2P3	57.2	2	Μ	CRC	47	-375	-374	LRIF5 G3P1	27.1	2	Ŀι	NO	N/A	-287	-592	89	-218
LRIF5 G2P3	57.2	2	Μ	CRC	47	-375	-374	LRIF5 G3P2	29.4	0	ы	ON	N/A	66	-756	475	-382
LRIF7 G4P2	58.5	2	Μ	CRC	36	71	850	LRIF7 G5P2	26.4	2	Γų	MM	26	437	557	366	-293
LRIF7 G4P3	71.5	0	Ъ	NO	N/A	895	623	LRIF7 G5P2	26.4	2	ы	MM	26	437	557	-458	-66
LRIF12 G3P1	61.9	0	М	NO	N/A	-311	3	LRIF12 G4P1	31.2	1	۶	NO	N/A	-685	100	-373	98
LRIF12 G3P1	61.9	0	М	NO	N/A	-311	3	LRIF12 G4P2	32.6	1	ы	NO	N/A	-411	-485	66-	-488
LRIF12 G3P2	63.4	1	ы	EC	52	116	355	LRIF12 G4P1	31.2	1	ы	NO	N/A	-685	100	-801	-255
LRIF12 G3P2	63.4	1	ы	EC	52	116	355	LRIF12 G4P2	32.6	1	Ŀч	NO	N/A	-411	-485	-527	-840
LRIF14 G $3P2$	60.69	0	М	NO	N/A	-966	-532	LRIF14 G4P2	45.7	7	Γų	NO	N/A	-812	-360	154	173
LRIF14 G3P3	66.9	2	ы	ON	N/A	336	-162	LRIF14 G4P2	45.7	2	ы	ON	N/A	-812	-360	-1148	-197
BWHF1a G3P1	64.4	1	М	SCC	45	-286	535	BWHF1a G4P3	34.6	1	Έų	CRC	14	-127	695	160	160
BWHF1a G3P2	63.6	0	ы	NO	N/A	-128	153	BWHF1a G4P3	34.6	1	ы	CRC	14	-127	695	1	541
BWHF3a G3P2	46.9	1	ы	CRC	33	563	-65	BWHF3a G4P1	28.8	1	Μ	NO	N/A	182	1656	-381	1721
BWHF3a G3P2	46.9	1	н	CRC	33	563	-65	BWHF3a G4P3	27.6	0	Ъ	NO	N/A	2727	480	2164	544
BWHF5a G3P1	57.6	0	Εų	BCC	52	388	-640	BWHF5a G4P6	23.3	0	Εų	NO	N/A	-369	-1003	-757	-363
			Pare	int							Childre	en				$\Delta \mathbf{C}_{-1}$	$\Delta \mathbf{P}$
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Code	Age	Gene	Sex	Cancer	Age C.	$\Delta X p Y p$	$\Delta 12q$	Code	Age	Gene	Sex	Cancer	Age C.	$\Delta X p Y p$	$\Delta 12q$	XpYp	12q
HF6a G3P1	63.3	1	伍	EC	52	1231	-402	BWHF6a G4P2	31.9	1	Бщ	NO	N/A	-56	66-	-1286	303
HF6a G3P1	63.3	1	ч	EC	52	1231	-402	BWHF6a G4P3	36.4	1	Μ	NO	N/A	669-	-1550	-1930	-1148
F11a G3P1	62.4	2	Μ	CRC	60	795	-488	BWHF11a G4P4	38.2	2	Μ	NO	N/A	-463	-173	-1258	315
VHF2 G3P7	6.99	1	Μ	CRC	38	1504	1443	BWHF2 G4P11	39.3	1	Μ	CRC	23	-1054	-417	-2558	-1860
/HF2 G3P8	66.4	0	۲	NO	N/A	-1443	-706	BWHF2 G4P11	39.3	1	Μ	CRC	23	-1054	-417	389	289
HF42 G5P2	64.8	1	伍	CRC	51	363	1009	BWHF42 G6P1	34.9	1	Μ	CRC	20	246	166	-117	-843
HF42 $G5P2$	64.8	1	Έų	CRC	51	363	1009	BWHF42 G6P2	32.8	0	Μ	ON	N/A	1697	348	1333	-661
HF42 G5P2	64.8	1	۲	CRC	51	363	1009	BWHF42 G6P3	28.3	0	۲ų	ON	N/A	-1279	132	-1643	-878
HF55 G3P1	58.2	0	M	NO	N/A	-1044	-1048	BWHF55 G4P1	35.1	7	M	NO	N/A	-856	-614	188	435
HF55 G3P1	58.2	0	Μ	NO	N/A	-1044	-1048	BWHF55 G4P2	31.8	2	ы	NO	N/A	116	-309	1161	739
HF55 G3P2	58.8	7	伍	CRC	31	-358	86	BWHF55 G4P1	35.1	7	Μ	NO	N/A	-856	-614	-498	-700
HF55 G3P2	58.8	7	伍	CRC	31	-358	86	BWHF55 G4P2	31.8	7	ы	NO	N/A	116	-309	474	-395
HF55 G3P3	55.5	7	Μ	CRC	31	272	337	BWHF55 G4P4	19.2	7	Μ	CRC	15	-3029	-2296	-3301	-2633
HF71 G3P1	66.2	0	Μ	NO	N/A	-504	-147	BWHF71 G4P1	36.1	0	Μ	NO	N/A	413	-32	916	116
HF71 G3P1	66.2	0	Μ	ON	N/A	-504	-147	BWHF71 G4P2	34.4	7	μ	NO	N/A	-157	579	347	726
HF71 G3P2	65.9	7	ч	EC	50	-788	-75	BWHF71 G4P1	36.1	0	Μ	NO	N/A	413	-32	1201	44
HF71 G3P2	65.9	2	F	EC	50	-788	-75	BWHF71 G4P2	34.4	2	F	NO	N/A	-157	579	631	654
F100 G4P1	53.6	7	Μ	CRC	44	-679	700	<b>BWHF100 G5P1</b>	26.9	7	Έų	NO	N/A	-1925	361	-1246	-339
F100 G4P2	52.6	0	F	NO	N/A	-606	-467	BWHF100 G5P1	26.9	2	F	NO	N/A	-1925	361	-1319	828
F102 G3P3	61.8	1	Μ	CRC	40	882	1260	BWHF102 G4P4	30.9	1	F	NO	N/A	512	-1359	-370	-2619
F105 G3P5	64.1	2	Ъ	EC	31	459	127	BWHF105 G4P2	41.0	2	Ы	UBC	33	-326	-837	-786	-963
F105 G3P5	64.1	7	ч	EC	31	459	127	<b>BWHF105 G4P4</b>	38.8	0	μ	NO	N/A	1083	352	624	225
F105 G3P6	67.6	7	ч	CRC	60	412	-751	BWHF105 G4P9	42.0	7	μ	NO	N/A	6	-319	-403	432
F105 G3P6	67.6	7	μ	CRC	60	412	-751	BWHF105 G4P11	37.1	0	Μ	NO	N/A	4	856	-408	1607
F105 G4P9	42.0	2	н	ON	N/A	6	-319	BWHF105 G5P4	20.6	2	F	ON	N/A	447	-930	438	-611
F106 G4P2	51.0	0	Ε	NO	N/A	-1644	532	<b>BWHF106 G5P1</b>	27.8	1	Μ	NO	N/A	-599	-914	1046	-1446
F106 G4P1	58.2	1	Μ	CRC	44	-873	845	BWHF106 G5P1	27.8	1	Μ	NO	N/A	-599	-914	275	-1759

∆P	12q	7	-481	-1244	-1728	1341	671	379	-290	-434	-1612	-516	-1694	666	92	-42	-240	-2020	1970			=female; cancer;
$\Delta \mathbf{C}^{-7}$	XpYp	-864	2-	-1806	-949	1383	773	879	268	-142	-1899	609	-1148	-492	13	503	-3185	-3624	-1532			. Sex: F= / bladder
	$\Delta 12q$	-473	-956	-473	-956	1039	369	1039	369	-1041	-2219	-1041	-2219	979	72	-62	-475	-475	1662			wild type J=urinary
	$\Delta X p Y p$	-1449	-592	-1449	-592	871	261	871	261	-258	-2015	-258	-2015	64	568	1059	-1262	-1262	-747			<i>MSH2</i> ; 0=v noma; UBC
	Age C.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	29	N/A	N/A	N/A	N/A	N/A			<i>dLH1</i> ; 2=, cell carcii
u	Cancer	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	NO	CRC	NO	NO	NO	NO	NO			<b>ss.</b> Jenes: 1= <i>l</i> CC=basal
Childre	$\mathbf{Sex}$	M	ы	Μ	ы	Ŀι	Μ	ы	Μ	M	ы	Μ	Ч	Μ	ы	Μ	Μ	Μ	Μ			<b>familic</b> code: ( oma; B
•	Gene	1	0	1	0	2	2	2	2	7	2	2	2	1	0	1	1	1	0			<b>adrome</b> s. Table Il carcin
	Age	48.0	46.3	48.0	46.3	37.7	30.7	37.7	30.7	25.7	22.9	25.7	22.9	40.1	42.7	32.3	23.4	23.4	23.7			<b>nch sy</b> r ¢ parent mous ce
	Code	BWHF107 G4P1	BWHF107 G4P2	BWHF107 G4P1	<b>BWHF107 G4P2</b>	<b>BWHF108 G4P7</b>	<b>BWHF108 G4P8</b>	<b>BWHF108 G4P7</b>	<b>BWHF108 G4P8</b>	BWHF125 G5P2	<b>BWHF125 G5P3</b>	<b>BWHF125 G5P2</b>	<b>BWHF125 G5P3</b>	BWHF126 G4P3	<b>BWHF126 G4P5</b>	<b>BWHF126 G4P6</b>	BWHF130 G5P3	BWHF130 G5P3	BWHF130 G5P9			<b>comparisons for Ly</b> nere length than thei ial cancer; SCC=squa
	$\Delta 12q$	-475	-475	772	772	-302	-302	660	660	-607	-607	-526	-526	-20	-20	-20	-234	1545	-308	673	-491	pair (P/C) lusted telon =endometr
	$\Delta X p Y p$	-585	-585	357	357	-512	-512	2-	2-	-115	-115	-867	-867	555	555	555	1923	2362	785	-732	506	t <b>/children</b> ter age-adj anoma; EC plicable.
t	Age C.	57	57	65	65	64	64	N/A	N/A	N/A	N/A	34	34	64	64	64	46	N/A	26	N/A	33	<b>nd paren</b> have shoi gnant mel //A=not aț
	Cancer	CRC	CRC	TC	TC	UBC	UBC	ON	ON	NO	ON	CRC	CRC	EC	EC	EC	CRC	ON	CRC	NO	CRC	(∆ <b>Tel) a</b> ı ∋ children MM=mali <u></u> er onset. N
Parer	$\mathbf{Sex}$	M	Μ	ы	ы	Μ	Μ	ы	ы	M	Μ	ы	Ч	Γų	ы	н	Μ	ы	Μ	Μ	Μ	<b>engths</b> is wher ancer; ] st cance
	Gene		1	0	0	7	2	0	0	0	0	2	2	-	1	1	1	0	1	0	1	<b>omere l</b> 1parison rectal c ige at fir
	Age	72.4	72.4	73.0	73.0	73.1	73.1	71.9	71.9	62.1	62.1	49.0	49.0	65.3	65.3	65.3	53.2	49.9	49.3	50.4	44.8	sted tele P/C con RC=colo Mge C.= ε
	Code	BWHF107 G3P3	BWHF107 G3P3	BWHF107 G3P4	BWHF107 G3P4	BWHF108 G3P3	BWHF108 G3P3	BWHF108 G3P4	BWHF108 G3P4	BWHF125 G4P10	BWHF125 G4P10	BWHF125 G4P9	BWHF125 G4P9	BWHF126 G3P6	BWHF126 G3P6	BWHF126 G3P6	BWHF130 G4P1	BWHF130 G4P2	BWHF130 G4P5	BWHF130 G4P4	BWHF130 G4P7	Table 6.5: <b>Age-adju</b> Red colour indicates M=male. Cancer: C TC=thyroid cancer: A

Figure 6.12.A shows the age-adjusted telomere length ( $\Delta$ Tel) for all controls (52 samples) and LS (76 samples). No differences were observed in the distribution of the age-adjusted telomere lengths for either XpYp or 12q between the control and the LS cohort individuals. However, when dividing by the genotype, it was found that  $MSH2^{+/-}$  individuals had significantly shorter  $\Delta XpYp$  than the MMR<sup>+/+</sup> children in the LS cohort, suggesting that MSH2<sup>+/-</sup> patients might have shorter XpYp but not 12q telomere than MMR proficient patients, although not very different from controls. Additionally, when comparing the rank between parents and children for both cohorts using two-tailed Mann-Whitney test we found no differences in controls for XpYp (p = 0.6) or 12q (p = 0.8) but significantly shorter XpYp (p = 0.0169) and 12q (p = 0.0485) telomeres in the LS children compared to the LS parents (figure 6.12.B. Subsequently, parent/children pair comparisons were performed to calculate the change in telomere length as explained in equation 6.10 and no differences were observed between the two cohorts. However, and despite the very small sample size (7 pairs), in the MMR deficient pairs that have developed cancer in both generations, children seemed to have shorter telomeres than their parents (figure 6.12.C).

Interestingly, when the P/C pair comparison within the LS were studied accounting for the genotype in both generations, a similar trend for both telomeres (figure 6.13) was found, suggesting that a) MMR<sup>+/-</sup> children had shorter mean age-adjusted telomere length ( $\Delta$ Tel) than their parents (independent of the genotype); b) the MMR<sup>+/+</sup> children in the LS cohort had longer mean  $\Delta$ Tel than their MMR<sup>+/-</sup> parents and siblings and c) MMR<sup>+/+</sup> children in the LS cohort had shorter mean  $\Delta$ Tel than their MMR<sup>+/-</sup> parents and siblings and c) MMR<sup>+/+</sup> children in the LS cohort had shorter mean  $\Delta$ Tel than their MMR<sup>+/-</sup> parent their MMR<sup>+/+</sup> parent. Due to this final unexpected finding, it was concluded that despite not inheriting the germline mutation, having a MMR<sup>+/-</sup> parent might have an effect on the WT offspring's telomeres.

Telomere length was very heterogeneous among individuals with the same age, specially in the LS cohort as previously shown by lower r values for the regression analysis compared to the control cohort. Thus, parent/children pair comparisons might be very different due to that variability even between



Figure 6.12: **Age-adjusted telomere length.** A shows the mean age-adjusted telomere length ( $\Delta$ Tel) for controls (red) and LS (blue for XpYp and green for 12q telomeres). **B** compares the age-adjusted telomere lengths ( $\Delta$ Tel) between parent and children within the two cohorts. The XpYp telomere is shown in blue colours and the 12q telomere is shown in green colours (for both, darker and lighter colours are used for parents and children respectively). **C** shows the parent/children pair comparisons for  $\Delta$ Tel in controls (red) and LS (purple). In addition, 7 pairs are shown separately for MMR<sup>+/-</sup> parents and children that have both developed cancer (yellow). "n" indicates the number of pairs analysed per group. Means are shown as horizontal black lines and p values for two tailed-Mann-Whitney test are shown for A and B.



Figure 6.13: Change in telomere length over generations. The scatter plots show child/parent comparisons for XpYp (A) and 12q (B) telomeres. Controls are shown in red colour;  $MMR^{+/-}$  in both generations are shown in purple;  $MMR^{+/+}$  in both generations in blue;  $MMR^{+/-}$  children compared to their  $MMR^{+/+}$  parent in yellow;  $MMR^{+/+}$  children compared to their  $MMR^{+/-}$  siblings in light green. Means are shown as horizontal black lines. "n" indicates the number of pairs analysed per group.

pairs belonging to the same group (see large distribution in figure 6.13). Consequently, we decided to study the P/C pair comparisons within each family to find a difference in the pattern of telomere length inheritance. In addition, other factors affecting TL like environmental were also removed (as it would be expected that members from the same family would live under similar environmental conditions, at least during childhood when the telomere shortening rates have been reported to be greater Frenck et al. (1998) and Rufer et al. (1999)). Data from tables 6.4 and 6.5 were used to plot graphs in figure 6.14. Considering that the number of comparison per family were a limitation, finding a trend grouping the change in TL over the generations for the control families was difficult; an example of that great heterogeneity was shown by two families exhibiting the same number of comparisons (TELF1 and TELF9) that nevertheless behaved very differently. On the one hand, in TELF9 all three daughters had shorter XpYp and 12q telomeres compared to both parents while in TELF1, both daughters had longer telomeres than their parents and only the son (TELF1G2P2) had shorter XpYp than both parents. Interestingly, there were three sons (TELF2G2P1, TELF4G2P1 and TELF7G2P1) with shorter telomeres than their respective fathers but not their mothers. Conversely, son TELF11G2P1 had both telomeres shorter than his mother and only shorter XpYp than his father. In family TELF3, both daughters had shorter XpYp telomere than their mother but longer than their father; on the contrary, for family TELF12, both daughters had shorter XpYp telomere than their father but not their mother (it is noteworthy that in those two families, the mother -for TELF3- and the father -for TELF12were cancer-affected -breast and testicular cancer respectively-). Additionally, daughter TELF3G2P2 had shorter 12q than both parents whereas her sister had longer 12q than both parents. For family TELF8, only the youngest child had shorter telomeres than her mother compared to her two siblings. Finally, both children in family TELF11 had longer 12q telomere than their father but not their mother.

For the LS cohort, parent/children pair comparisons per family are shown in figure 6.14.B. Contrary to the control families (figure 6.14.A), there seemed to be a tendency to smaller variations between parent/children pairs, as there were several families (LRIF5, LRIF7, LRIF14, BWHF55-A, BWHF100, BWHF 105A, BWHF105C, BWHF5a; for MSH2 and LRIF12, BWHF126 and BWHF1a; for MLH1) having values for  $\triangle$ Children- $\triangle$ Parent very close to zero, suggesting that in those families, telomeres did not change in length between the generations. Conversely, there were some P/C comparisons that showed very negative  $\Delta$ Children- $\Delta$ Parent values (children with smaller age-adjusted telomeres than their parents) and only three comparisons (in BWF130-B and BWHF3a MLH1 families) that showed greater values (children with longer telomeres than their parents). Interestingly, individual BWHF55G4P4 had the shortest age-adjusted telomeres (both XpYp and 12q) and an earlier age onset of CRC (15 years) compared to his father (31 years). Also in family BWHF2, the individual G4P11 that had developed CRC at 23 years had much shorter telomeres than his father (who developed CRC at 38 years). Nevertheless, telomeres in this individual were slightly longer than his MMR<sup>+/+</sup> mother. Other P/C pair comparisons between cancer-affected pairs (in families LRIF7, BWHF42, BWHF126 and BWHF1a) did not show very negative  $\Delta$ Children- $\Delta$ Parent values, suggesting that cancer in the second generation might not be driven by very short telomeres.



Figure 6.14: **Comparison of median telomere lengths within families.** A shows a bar graph for control families. Blue colour indicates XpYp telomere and green, 12q. All parent-children comparisons are shown indicating the pairs in the X axis. **B** shows two bar graphs for the LS families. The top graph shows only MSH2 families and the bottom graph shows only MLH1 families. Red asterisks indicate MMR-MMR pairs with both cancer-affected generations. The colour code is indicated below the second graph.

#### 6.4 Discussion

Saliva samples have been reported to contain approximately  $4.3 \times 10^5$  cells/ml, consisting of either epithelial cells or leukocytes (Chiappin et al., 2007) although in different concentrations depending on the health status of the donor (Thiede et al., 2000). In addition, saliva contains hormones and a large amount of proteins that varies accounting for the glandular activity (reviewed in Chiappin et al. (2007)), and also other contaminants such as bacterial DNA ( $\approx$  $1.7 imes 10^7$  bacteria/ml) or food debris that might contribute to the variability between samples (Sun & Reichenberger, 2014). Nevertheless, this type of biological sample is increasing its popularity as diagnostic tool as it is a non-invasive, cheap way of obtaining reasonable amounts of DNA compared to blood samples (reviewed in Sun & Reichenberger (2014)). Telomere length has been measured in saliva samples using Q-PCR in several cross-sectional studies ((Hewakapuge et al., 2008), (Theall et al., 2013), and (Chen et al., 2015)) and it has been reported that telomeres tend to be longer in saliva samples than in blood as the cell turnover is lower in the former ((Theall et al., 2013) and (Mitchell et al., 2014)).

Since its development in 2003, STELA (single telomere length analysis) has been used to measure telomere length in a variety of human primary cell lines, mainly fibroblasts ((Baird *et al.*, 2003), (Britt-Compton *et al.*, 2006) and (Mendez-Bermudez & Royle, 2011)) and blood cells ((Hills *et al.*, 2009) and (Britt-Compton *et al.*, 2009a)) and a strong correlation between telomere length measured by STELA and TRF (Terminal Restriction Fragments) has been described ((Baird *et al.*, 2003) and (Baird *et al.*, 2006)). Nevertheless, it has not being used, at least to our knowledge, to measure telomere length in saliva samples. In Lin *et al.* (2010), STELA was used to measure telomere length in 41 blood samples from chronic lymphocytic leukaemia (CLL) patients (that are characterised by very short telomeres) with an age-range of 50 to 89 years (median = 71 years). Later in Lin *et al.* (2014), STELA was used in two bigger cohorts of CLL blood samples: one with an age range of 27 to 95 years (median = 64 years) and another with an age-range of 35 to 90

years (median = 66 years). Taking into account that the age-range in our cohorts was 18 to 83 years (for controls) and 19 to 73 years (for Lynch syndrome) and that we used a tissue for DNA extraction with a reported longer telomere length compared to blood, an underestimation of the TL in the younger individuals was not unexpected.

Telomere length for XpYp and 12q telomeres was measured by STELA in 128 saliva samples from control (52) and Lynch syndrome (76) individuals to study whether inheritance of a germline mutation in a DNA mismatch repair (MMR) gene (either hMSH2 or hMLH1) had any impact on telomere length dynamics. A significant relationship between telomere length and age was only found when data for individuals younger than 35 years was transformed, possibly due to an underestimation of TL as explained before. After data transformation, XpYp and 12q telomeres shortened at similar, although slightly higher, rates in controls (34 and 31 bp/year for XpYp and 12q respectively) than in LS patients (22 and 19 bp/year for XpYp and 12q respectively).

We found that some children in control families had shorter telomeres than their parents (42% and 26% for XpYp and 12q respectively), although the percentage decreased after data transformation. On the one hand, this might be explained if telomeres were shortening at a faster rate between 18 to 35 years than after 35 years, what might be supported by Frenck et al. (1998) who demonstrated that after a rapid decline in telomere length from birth until the age of 4 years, there was a stabilisation in the telomere length between the ages of 10 and 30 in human leukocytes that was followed by a more modest telomere attrition rate with age. Being that the case in our cohort, the constant used to transform the data should had been greater. On the other hand, the assumption that children have longer telomeres than their parents comes from cross-sectional studies where the telomere length of all individuals from different ages fits to a linear regression according to the telomere attrition rate estimated. Nevertheless, some longitudinal studies available at the moment (in which telomere length is measured for the same individual at least in two time points separated by a certain time period, either months or years), suggest that there is not only telomere loss at different rates between

individuals but also either no change or even gain of telomeric repeats during the follow-up period ((Aviv *et al.*, 2009), (Chen *et al.*, 2011), (Steenstrup *et al.*, 2013)). In addition, the strong paternal age effect on the offspring's TL, suggesting that children from older fathers have longer telomeres possibly due to the elongation of the telomere length in sperm with age ((De Meyer *et al.*, 2007), (Kimura *et al.*, 2008) and (Broer *et al.*, 2013)), might contribute to changes in the TL over generations as suggested by Holohan *et al.* (2015).

Another surprising finding was the fact that the percentage of normality (individuals with either XpYp or 12q normally distributed telomere length) was lower in LS than controls, which was explained by the presence of many more individuals showing outliers (single telomere lengths measured at low frequency) in the LS cohort. Besides, those outliers were, without exception, long molecules, suggesting that shorter telomeres were more abundant in LS than longer ones. As the telomere attrition rate with age was not different between both cohorts (confirming results in Seguí et al. (2013) using Q-PCR and a bigger cohort), the abundance of short telomeres in LS individuals might be explained by: a) inheritance of shorter telomeres compared to controls or b) faster shortening rate earlier in life or even during embryogenesis. In addition, contrary to what was described by Seguí et al. (2013), our results did not suggest shorter telomeres and faster telomere shortening rate with age in cancer-affected MMR<sup>+/-</sup> compared to controls and MMR<sup>+/-</sup> cancer-free patients. In addition, telomeres in MSH2 families were shorter than in MLH1 families in which no significant relationship between TL and age was found, confirming previous results in Bozzao et al. (2011a). Finally, parent/children (P/C) pair comparisons for age-adjusted telomere lengths ( $\Delta$ Tel) showed a similar trend for both telomeres suggesting that MMR<sup>+/-</sup> children had shorter telomeres than both their  $MMR^{+/-}$  and  $MMR^{+/+}$  parent. Besides,  $MMR^{+/+}$ children seemed to have longer telomeres than their MMR<sup>+/-</sup> parent and siblings but not their MMR<sup>+/+</sup> parent, suggesting that a shorter telomere length might have been inherited from the MMR<sup>+/-</sup> parent in agreement with Aubert et al. (2012a) who showed that hTERT or hTERC gene mutation non-carrier relatives had shorter than expected telomeres in blood.

### 7 Final remarks

The effect of DNA mismatch repair (MMR) deficiency on telomere dynamics has been explored in this Thesis. Particularly, I focused on the loss of the MSH2 protein by shRNA downregulation in a human primary cell line (WI38), in a colorectal cancer cell line (LoVo) with mutated *MSH2* gene and in germline mutations for either *MSH2* or *MLH1* genes in Lynch syndrome patients.

We found that MSH2 downregulation in WI38 cells grown under 5%  $O_2$  increased the heterogeneity in telomere shortening rates and that telomeres, in clones with protein concentrations levels below 40% had a significantly faster shortening rate than in control clones. Interestingly, the telomere associated with the 12qB haplotype in the telomere adjacent DNA shortened exponentially faster with decreasing protein levels but this linear relationship was not significant for the other 12q allele ( $\Delta$ ). The interspersion pattern of the telomere variant repeat (TVR) repeat was compared between different telomeres and we found that the 12q had more variant repeats compared to XpYp or 17p telomeres; additionally, the interspersed repeat pattern between the 12q alleles showed private repeat combinations that might be responsible for the different behaviour of the alleles although experiments on the mutation frequency on those repeats are needed to confirm it. Comparisons of the findings in this Thesis with preliminary experiments suggested that the effect of MMR deficiency might be reinforced by high oxygen concentrations, as cells with depleted MSH2 grown under 21% O<sub>2</sub> had a greater difference in telomere shortening rates compared to controls (Mendez-Bermudez & Royle, 2011) than in the present experiment performed under biological, 5%, O<sub>2</sub> conditions.

It was previously hypothesised that the telomere instability found in MMRdeficient cancer cells might also contribute to the increase in telomere shortening observed before. Hence, mutant telomeres in LoVo were analysed and a 5:2 ratio for deletion:insertion types was found; however, more molecules (15) were analysed containing any of the two insertion types compared to the molecules carrying a deletion (10) due to somatic mosaicism. Comparison with previous mutation analysis on the same cell line showed one shared mutation and the combined mutation frequency (5.8%) indicated a 9:3 ratio for deletions:insertions, suggesting that the former were three times more common than the latter. Therefore, the accumulation of deletions might contribute to the increase in telomere shortening rates described before but also, the loss of some variant repeats involved in the recruitment of the shelterin component TRF2, might be involved in telomere deprotection, although it needs to be confirmed in the future. Additionally, the TVR protocol was optimised to make it suitable for capillary electrophoresis increasing the resolution and throughput of the method.

Furthermore, a new third next generation sequencing technology (min-ION<sup>TM</sup>) for long reads was tested to explore its applicability for telomere sequencing. Two control amplicons (previously characterised by Enjie Zhang using Ion Torrent<sup>TM</sup>) carrying interspersed homopolymeric repeats were sequenced with a greater than 95% identity. The subtelomeric region of the XpYp was also sequenced with a more than 93% identity, with the possibility of distinguishing between alleles in the highest quality reads. Moreover, a telomeric reference based on the TVR map was used to align the reads against it. The variant repeats pattern was not accurately sequenced but a lack of identity to the reference was found in the most distant position (from 4550 bp to the end) in which some variant repeats such as TACGGG, CTAGG or TCAGGG were sequenced, suggesting that a not previously described, more distant, variant repeat region might be present in this telomere. However, further analyses are needed to confirm this and the use of the latest chemistry and device for the nanopore sequencing might improve these results.

Besides, the effect of telomerase inhibition by a small non-nucleosidic drug called BIBR1532 in the LoVo cell line was tested as it has been hypothesised that tumours with very short telomeres might benefit more from this therapy. The drug reduced significantly the cell growth in the control (MMR-proficient) colon cancer cell line but not in LoVo. The cell cycle was not significantly

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affected either in LoVo, although it showed an increase in S-phase for the other cell line. Correspondingly, the telomere shortening rate increased significantly in the MMR-proficient cell line treated with the drug but not in LoVo. However, those results were not conclusive as no telomerase activity reduction was observed after the treatment measured by TRAP assay. Ultimately, we hypothesised that, as the elongation function of telomerase is targeted by this chemical, telomerase activity might be intact, therefore regulating transcription and metabolic reprogramming that, despite the effect on telomere shortening rate, might still drive tumorigenesis. Therefore, the use of the DN-hTERT plasmid, that completely degrades the endogenous telomerase, or the site-directed mutagenesis of the hTERC or hTERT genes using CRISPR-Cas9 approaches might give a better insight into the effect of telomerase inhibition on MMR-deficient cells.

Finally, telomere length in saliva samples from control and Lynch syndrome families was studied by STELA and it was found that: a) telomeres shortened with age at a similar rate in both cohorts with no effect based on sex, b) there was no relationship between telomere length and age in MLH1<sup>+/-</sup> patients, c) the age-adjusted telomere length for XpYp but not 12q was significantly shorter in  $MSH2^{+/-}$  carriers compared to  $MMR^{+/+}$  children within the LS cohort, d) the MMR<sup>+/-</sup> children had shorter age-adjusted telomere lengths than both parents and e) the MMR<sup>+/+</sup> children had longer age-adjusted telomeres than their MMR<sup>+/-</sup> parents and siblings but shorter than their MMR<sup>+/+</sup> parent. The lack of difference in the telomere shortening rate with age between the two cohorts might be due different initial telomere lengths over generations what has been proposed an explanation for the distinct attrition rates found between cross-sectional and longitudinal studies; hence, it might be interesting to consider following up some of the most interesting families over time, in order to understand the direct effect of MMR-deficiency on the telomeres of specific individuals.

A Materials summary

List of the main materials used in this Thesis. The catalog number and the supplier is also indicated for an easier identification.

-	Product	Supplier, Country	Catalog number
	Anti-MSH2	Abcam, UK	ab52266
Antibodies	Anti-GAPDH	Life Technologies, UK	AM4300
	ECL Mouse IgG from sheep	GE Healthcare, UK	NA931
	$\beta$ -galactosidase staining kit	Cell Signalling, UK	9860
Cell	Propidium Iodide	Sigma-Aldrich, UK	P4170
staining	Trypan blue solution 0.4%	Life-Technologies, UK	15250-061
	4',6-Diamidino-2-phenylindole dihydrochloride, DAPI	Sigma-Aldrich, UK	32670
	Formamide	Merck, UK	S4117
	AccuGel (40%) 19:1 (acrylamide:Bis acrylamide)	Fisher Scientific, UK	12301469
	ProtoGel (30%) 37.5:1 (acrylamide:Bis acrylamide)	Fisher Scientific, UK	12381469
	Cloning discs	Sigma-Aldrich, UK	Z374466100EA
	BLUeye Prestained Protein Ladder	GeneFlow	S6-0024
Chemicals	Hi-Di <sup>™</sup> formamide	Applied Biosystems	4311320
and	MapMarker <sup>®</sup> X-Rhodamine Labeled 1000 bp	BioVentures, US	MM-1000XL-ROX
others	MAGNA nylon transfer membrane 0.45 microns	Fisher Scientific, US	1213403
	Minimum Essential Medium (MEM)	Thermo Scientific, UK	11095-080
	Dulbecco's Modified Eagle Medium (D-MEM )	Thermo Scientific, UK	41965-062
	100X Non-Essential AminoAcids (NEAA)	Thermo Scientific, UK	11140-050
	Fetal Bovine Serum (FBS) South African	Pan-Biotech, Germany	P30-1502
	Protran Premium Nitrocellulose membrane	Fisher Scientific, UK	15269794
	Puromycin dihydrocloride	Sigma-Aldrich, UK	P9620
Drugs	Ampicillin	Sigma-Aldrich, UK	A0166
	BIBR1532	Stratech Scientific, UK	S1186-SEL
	End-repair using NEBNext® End Repair Module	NEB, UK	E6050S
Enzymes	A-tailing using NEBNext <sup>®</sup> dA-Tailing Module	NEB, UK	E6053S
	Blunt/TA Ligase Master Mix	NEB, UK	M0367S
	E.Z.N.A. <sup>®</sup> Plasmid Maxi Kit	Omega	D6922-04
	MaXtract High Density	Qiagen, UK	129056
	Zymoclean <sup>™</sup> Gel DNA Recovery Kit	ZymoResearch, UK	D4002
	Oragene <sup>™</sup> DNA saliva kit	Genotek, US	OG-500
	ECL prime	GE healthcare	RPN2236
	TRAPeze®	Millipore, UK	S7700
Kits	Ingenio <sup>®</sup> Electroporation Kit	Mirus, UK	MIR 50115
	Quick Start <sup>™</sup> Bradford Protein Assay Kit	BioRad, UK	500-0201
	Pellet Paint <sup>®</sup>	Millipore, UK	69049
	Genomic DNA kit	Oxford Nanopore, UK	SQK-MAP004
	AmPure beads	Beckman Coulter, UK	A63880
	Dynabeads <sup>®</sup> His-Tag	Thermo Scientific, UK	10103D

# **B Primers summary**

List of the primers used in this Thesis. "T" refers to annealing temperature and "V" indicates variable depending on the forward optimum annealing temperature. Letters in bold indicate the allele-specific nucleotide.

	Name	Sequence 5' to 3'	Т
	Telorette2	TGCTCCGTGCATCTGGCATCTAACCCT	V
	(Baird <i>et al.</i> , 2003) Teltail	TGCTCCGTGCATCTGGCATC	V
	(Baird <i>et al.</i> , 2003) XpYp427G/415C	GGTTATCGACCAGGTGCTCC	66.5°C
¥'	(Baird et al., 2003) XpYp427A/415T	GGTTATCAACCAGGTGCTCT	$66.5^{\circ}\mathrm{C}$
STEI	(Baird <i>et al.</i> , 2003) XpYpE2	TTGTCTCAGGGTCCTAGTG	63°C
	(Baird et al., 2003) XpYpB2	TCTGAAAGTGGACCWATCAG	66°C
	(Baird <i>et al</i> ., 2003) 12qSTELA	CGAAGCAGCATTCTCCTCAG	$65^{\circ}\mathrm{C}$
	(Baird <i>et al.</i> , 2000) 12qnull3	GATGTCTGAGTGGATTCAGACATG	$67^{\circ}\mathrm{C}$
	(Baird <i>et al.</i> , 2000) 17p6 (Baird <i>et al.</i> , 2006)	GGCTGAACTATAGCCTCTGC	63°C
	TS30T	CTGCTTTTATTCTCTAATCTGCTCCCT	$66.5^{\circ}\mathrm{C}$
	(Baird <i>et al.</i> , 1995) TS30A	GTGCTTTTATTCTCTAATAATCTCCCA	$66.5^{\circ}\mathrm{C}$
	(Baird <i>et al.</i> , 1995) 12q-197A	GGGAGATCCACACCGTAGCA	$66^{\circ}C$
	(Baird <i>et al.</i> , 2000) 12q-197G	GGGAGATCCACACCGTAGCG	$66^{\circ}C$
CR	(Baird <i>et al.</i> , 2000) 12qdeletionSTELA	TGAGCATTCATGAGCATTACAGG	$67^{\circ}\mathrm{C}$
VR-F	(Baird et al., 2000) $12qB$ $(Baird et al., 2000)$	ATTTTCATTGCTGTCTTAGCACTGCAC	61°C
Έ	(Baird <i>et al.</i> , 2000) TAG TelX	TCATGCGTCCATGGTCCGGACCCTTACCCTTACCCTNACCCTC	$66^{\circ}C$
	(Baird <i>et al.</i> , 1995) TAG TelW	TCATGCGTCCATGGTCCGGACCCTTACCCTTACCCTNACCCTA	$66^{\circ}C$
	(Baird <i>et al.</i> , 1995) TAG TelJ	TCATGCGTCCATGGTCCGGAACCCCAACCCCAACCCCAACCCC	$66^{\circ}C$
	TAG TelX-NED TAG TelW-FAM TAG TelJ-HEX TAG TelCTA2-FAM TAG TelY-HEX	(NED)-TCATGCGTCCATGGTCCGGACCCTTACCCTTACCCTNACCCTC (FAM)-TCATGCGTCCATGGTCCGGACCCTTACCCTTACCCTNACCCTA (HEX)-TCATGCGTCCATGGTCCGGAACCCCAACCCCAACCCCAACCCC (FAM)-TCATGCGTCCATGGTCCGGACCCTTACCCTTRCCCTARCCCTAG (HEX)-TCATGCGTCCATGGTCCGGACCCTTACCCTTACCCTNACCCTG	66°C 66°C 66°C 66°C 66°C
	TS primer	AATCCGTCGAGCAGAGTT	$59^{\circ}\mathrm{C}$
TRA	(Kim <i>et al.</i> , 1994) RP primer (Kim <i>et al.</i> , 1994)	GCGCGG(CTTACC) <sub>3</sub> CTAACC	59°C
	TS-30ARev	AATCAGCAGGATGTGGGA	68°C
r	(Hills, 2004) TS-30TRev	TATCAGCAGGATGTGGGT	68°C
Othe	(Hills, 2004) 17p2	GAGTCAATGATTCCATTCCTAGC	$60^{\circ}\mathrm{C}$
	(Baird <i>et al.</i> , 2006) pSup1 (Mendez- Bermudez <i>et al.</i> ,	CAGAAAGCGAAGGAGCAAAG	$50^{\circ}\mathrm{C}$
	2009) HHV6 probe 31 F	ͲϹϾϾͽϫϾϾϾϾϭϾϫϾϫͲͲϹͲϫϫ	19°C
	HHV6 probe 31 R	CCACCTTCGCAACAACAATA	49°C
	HHV6A probe 49 F HHV6A probe 49 R	CAGGAAAGGGACGGTGATAA ATCGAAAGCACCACCTTCAC	50°C 50°C

## **C** Multiple alignment for OL A31

The control amplicon for OL A31 was sequenced using the minION<sup>™</sup> device. In the next pages, a multiple alignment generated using ClustalW in the MacVector software, V11.1.1 is shown. The reference sequence obtained by Enjie Zhang using Ion Torrent<sup>™</sup> sequencing is named: "OL A31 Ref"; the consensus generated by IGV from the alignment of the 1D reads is called "1d.OL A31" and for the 2D reads, "1d.OL A31". Each nucleotide is colour coded and the position of each nucleotide is indicated at the top of the alignment in red numbers. Letters are: A: adenine; T: thymine; C: cytosine; G: guanine; R: purine (A or G); Y: pirimidine (T or C); N: any nucleotide; W: Weak (A or T); S: Strong (C or G) and K: Keto (G or T).

	10	20	30	40	50	60	70
1d.OL A31	<b>T</b> Y        G        N        M        A        N	N N <mark>T</mark> N N N N N N N N N N	I <mark>C</mark> N N N N N N N N N	N N N N N N K N N	N <mark>G</mark> N N N N N N N <mark>A</mark>	ΝΝΝΟΝΝΥΥ	NNGNNNN
2d.OL A31	T C G G A A G C G G A G	A T T T <mark>C</mark> T A A T A A T <mark>C</mark>	C T T G T <mark>A A T C</mark>	C T G G T T T T A C	TGGAGAAAAA	AGACCTTCT	C C G T T T T
OL A31 Ref.	TCGGAAGCGGAG	A T T T <mark>C</mark> T A A T A A T <mark>C</mark>	C T T G T <mark>A A</mark> T C	<mark>C</mark> T G G T T T T <mark>A</mark> C	TGGAGAAAAA	AGACCTTCT	C C G T T T T
consensus	TCGGAAGCGGAG	ATTTCTAATAAT	CTTGTAATC	CTGGTTTTAC		AGACCTTCT	
	80	90	100	110	120	130	140
1d.OL A31	N N N N N N N N N N <mark>C</mark> N	M	I N N N N N N N N C	N N <mark>A</mark> N N N N N N N	N N N N N N N N N N	<u>NNNTC</u> NNNN	<u>N N N N N N</u>
2d.OL A31	T <mark>A</mark> TT <mark>AC</mark> ATTTCG	<mark>C</mark> N N <mark>T</mark> G G <mark>C A C</mark> G <mark>C</mark> G T	T <mark>A</mark> T T T <mark>G</mark> T T <mark>C</mark>	G G <mark>A A </mark> G T T <mark>A A</mark> T	G <mark>CTT</mark> GG <mark>AT</mark> GG	Τ <mark>Ζ</mark> ΤΤ <mark>Ζ</mark> ΤΤΤΤΙ	N <mark>A G A G G C</mark>
OL A31 Ref.	T <mark>A</mark> TT <mark>AC</mark> ATTTCG	C T A T G G C A C G C G T	T <mark>A</mark> T T T <mark>G</mark> T T <mark>C</mark>	G G <mark>A A </mark> G T T <mark>A A</mark> T	G <mark>C</mark> TTGG <mark>A</mark> TGG	Т <mark>С</mark> ТТ <mark>С</mark> ТТТТТ	T <mark>A</mark> G <mark>A</mark> G G <mark>C</mark>
consensus	TATTACATTTCG	C A T G G C A C G C G T	TATTTGTTC	GGAAGTTAAT	GCTTGGATGG	ТСТТСТТТТ	AGAGGC
	150	160	170	180	190	200	210
1d.OL A31	N N N N N N N N N N N N	N N <mark>A</mark> N N <mark>C</mark> N N N N N N N	I N N T N N N N N T	N N <mark>A</mark> N <b>T</b> Y N N N N	GNNNNNNNN	<u>N N N G W N G N N I</u>	N N N N N N N
2d.OL A31	C A G A G C A T A C T C	G T A T T <mark>C</mark> T G T A A <mark>C</mark> G	БТТТ <mark>А</mark> G <mark>АТ</mark> СТ	G G <mark>A</mark> T T T T N <mark>C</mark> A	G T <mark>C</mark> T T T T N N T	A T <mark>C</mark> G A T G T A <sup>-</sup>	T <mark>G</mark> T T T N <mark>A</mark>
OL A31 Ref.	C <mark>AGAGCATAC</mark> TC	G T A T T C T G T A A C G	T T T <mark>A G A</mark> T <mark>C</mark> T	G G <mark>A</mark> T T T T T <mark>C</mark> A	G T C T T T T T <mark>A</mark> T	A T <mark>C</mark> G A T G T A <sup>-</sup>	T <mark>G</mark> T T T T <mark>A</mark>
consensus	CAGAGCATACTC	G T A T T C T G T A A C G	; T T T <mark>A G A T C</mark> T	G G A T T T T C A	GTCTTTT	ATCGATGTA	T G T T T A
	220	230	240	250	260	270	280
1d.OL A31	<mark>A</mark> NNNNNNNNNNN	N N <mark>A</mark> N Y S N N N N N W N	I N N N N N M N N N	N N N N N N N N N N	ΑΤΝΝΝΟΝΝΟ	и <mark>лии а</mark> ииии	<u>N N N N N A</u>
2d.OL A31	<mark>AAGTAACAATC</mark> C	G <mark>C A T T </mark> G <mark>A A </mark> G T A T T	G <mark>C</mark> TT <mark>C</mark> ATGA	T <mark>A C A C</mark> A G T A A	<mark>A</mark> T T T T <mark>C</mark> G T G <mark>C</mark>	AGGGAATTT	C <mark>A A</mark> G <mark>A A</mark>
OL A31 Ref.	<mark>AAGTAACAATCC</mark>	G <mark>C</mark> ATTGAAGTATT	G <mark>C</mark> TT <mark>C</mark> ATGA	ΤΑ <mark>ϹΑ</mark> ϹΑΓΑΑ	<mark>A</mark> TTTT <mark>C</mark> GTG <mark>C</mark>	<mark>Α</mark> GGG <mark>ΑΑ</mark> ΤΤΤΤ	CAAGAA
consensus	A A G T A A C A A T C C	G C A T T G A A G T A T T	G <mark>C</mark> TT <mark>C</mark> ATGA	ΤΑ <mark>ϹΑ</mark> ϹΑΓΑΑ	ΑΤΤΤΤ <mark>Ϲ</mark> GΤ <mark>Ο</mark>	AGGGAATTT	
	290	300	310	320	330	340	350
1d.OL A31	N N N N N N <mark>A</mark> N N N <mark>C</mark>	N N N N N C N N N N N N N	I N <mark>C</mark> N N N N N N N	N N N N <mark>T</mark> N N N N N	<b>T</b> N N N N N N N N N	N N N T N N N G C /	<mark>a</mark> n n n n n n
2d.OL A31	T G T G G <mark>A C A</mark> T T T <mark>C</mark>	T C T G G C A T T G T T G	i <mark>T C C</mark> N <mark>A T T</mark> G G	TAAATTNGAG	TCGTAAGAAG	T G <mark>A T A T T </mark> G <mark>C</mark> /	A G T A A T T
OL A31 Ref.	T G T G G <mark>A C</mark> A T T T C	Τ <mark>Ϲ</mark> Τ <mark>G<mark>GϹ</mark>ΑΤΤ<mark>G</mark>ΤΤ Ο</mark>	T <mark>CCTA</mark> TTGG	TAAATTAGAG	Τ <mark>Ϲ</mark> Ϭ <mark>ͳ</mark> ΑΑ <mark>Ϭ</mark> ΑΑ	T G <mark>A</mark> T <mark>A</mark> T T G <mark>C</mark> /	A <mark>G T A A</mark> T T
consensus	TGTGGACATTTC					TGATATTGC	AGTAATT

	360	370	380	390	400	410	420
1d.OL A31	NNNNANTNT	N N N N N N N N N N N	NNMCNTNNN	N N N N N C N N N N	N N N N N G N N N N	N N N N N N N <mark>A</mark> M	тиииииии
2d.OL A31	G <mark>AC</mark> GAATTT	G T C G T C T C N N G	N G C C G T G T T	G <mark>T A A </mark> G <mark>C</mark> G A T A	T <mark>A T A T G T A A T</mark>	N N <mark>A C A C G G A A</mark>	т <mark>G T G T T G G T</mark> Т
OL A31 Ref.	G <mark>AC</mark> GAATTT	G T C G T C T C T A G	G G <mark>C C</mark> G T G T T	G <mark>T A A </mark> G <mark>C</mark> G A T A	T <mark>A T A T G T A A T</mark>	G G <mark>A C A C</mark> G G <mark>A A</mark>	T <mark>G T G T T G G T</mark> T
consensus	GACGAATTTT	GTCGTCTC G	GCCGTGTT	G T A A G C G A T A	ΤΑΤΑΤΓΑΑΤ	ACACGGAA	ТĠТĠТТĠĠТТ
	430	440	450	460	470	480	490
1d.OL A31	N	T C N N N N N N M N N	N N N N N N M N N	N N N N N N N N N N	N M N N N N N N N N	N N N N <mark>C</mark> N N N N	N N N N N N N N N
2d.OL A31	A G A T G T G A A C	T C T T T C N A A A N	N <mark>G A</mark> T T <mark>G A</mark> T T	T <mark>G </mark> A G T G T <mark>C</mark> T A	<mark>G A T A T T T A A A</mark>	ΤΤ <mark>Ο</mark> ΤΤ <mark>ΟΟ</mark> ΤΤΑ	T <mark>C </mark> G <mark>C</mark> G G A G A T
OL A31 Ref.	A G A T G T G A A C	T C T T T C T A A A T	AGATTGATT	T G <mark>A </mark> G T G T <mark>C</mark> T A	<mark>G A T A</mark> T T T <mark>A A A</mark>	Т Т <mark>С</mark> Т Т <mark>С С</mark> Т Т <mark>А</mark>	T <mark>C </mark> G <mark>C</mark> G G A G A T
consensus	AGATGTGAAC	TCTTTCAAAA	G A T T G A T T	T G <mark>A G T G T C T A</mark>	GATATTTAAA	ΤΤ <mark>Ϲ</mark> ΤΤ <mark>ϹϹ</mark> ΤΤΑ	TCGCGGAGAT
	500	510	520	530	540	550	560
1d.OL A31	N N N N N N <mark>G</mark> N N N	N N N N N N N N N N N	N	N N N N N N N N N N	N <mark>C</mark> N N N <mark>A</mark> N N N N	N N N N N N N N N N	N N <mark>A</mark> N S N N N N N
2d.OL A31	T T T N N T <mark>G T C</mark> T	G G <mark>C</mark> G <mark>T A</mark> C G T T G	C G <mark>A A</mark> G <mark>T C T</mark> G	<mark>C A A A T A G</mark> T T T I	T <mark>C</mark> T <mark>A A A</mark> T T T T	G <mark>ATTTC</mark> AAAG	T <mark>A A T G</mark> T T T <mark>A</mark> T
OL A31 Ref.	Т <mark>ТТТТТС</mark> Т	G G <mark>C</mark> G <mark>T A</mark> C G T T G	C G <mark>A A</mark> G <mark>T C T</mark> G	<mark>C</mark> AAATAGTTT	T <mark>C</mark> T <mark>A A A</mark> T T T T	G <mark>ATTTC</mark> AAAG	T <mark>AATG</mark> TTT <mark>A</mark> T
consensus	Т Т Т <mark>Т </mark> <b>Т <mark>С</mark> Т</b>	G G C G T <mark>A C</mark> G T T G	C G <mark>A A</mark> G T C T G	C A A A T A G T T T	T <mark>C</mark> T <mark>A A A</mark> T T T T	G <mark>ATTTC</mark> AAAG	T <mark>AATG</mark> TTT <mark>A</mark> T
	570	580	590	600	610	620	630
1d.OL A31	N N <mark>A</mark> N N N N N N N	N N N N N N N N N N N	NNNNNNNN	N N N N <mark>C</mark> N N N N N	N N N N N N N N N N	N N N N N <mark>A</mark> N N N	N N N N N N N N N N
2d.OL A31	A A A T T C N G A C	ATGGAAGAATA	C G T G <mark>A A</mark> C <mark>A</mark> C	C <mark>ATGCTTCCT</mark>	G <mark>T </mark> G <mark>T T T T <mark>C C</mark> G</mark>	T <mark>A A A G C A T T G</mark>	A A <mark>C </mark> A T A A T T T
OL A31 Ref.	AAATTCGGAC	ATGGAAGAATA	CGTGAACAC		G <mark>T G T T T T C C</mark> G	T <mark>A A A G C</mark> A T T G	ΑΑ <mark>Ϲ</mark> ΑΤΑΑΤΤΤ
consensus	AAATTCGAC	ATGGAAGAATA	C G T G <mark>A A C A</mark> C	C <mark>A</mark> TGCTTCCT	G <mark>T </mark> G <mark>T T T T <mark>C C</mark> G</mark>	T <mark>A A A G C</mark> A T T G	A A <mark>C</mark> A T A A T T T
	640	650	660	670	680	690	700
1d.OL A31	N N N N N N N N N N	N N <mark>A</mark> N N N N N N N N	N	N N <mark>G</mark> N N N N N N N	N N N <mark>A</mark> N N <mark>C A</mark> N	<mark>C</mark> N N N N N N N N N	N N <mark>C</mark> N N M N N N N
2d.OL A31	T T G G T A G T T G	T G <mark>A T</mark> G G T G <mark>C</mark> T <mark>C</mark>	Т Т Т N G Т Т <mark>С С</mark>	N <mark>C G A A C A A A</mark> N <mark>/</mark>	A G T A A T A <mark>C</mark> A T	<mark>C G T T A A T A A A</mark>	A G <mark>C </mark> A G <mark>A C C T T</mark>
OL A31 Ref.	TTGGTAGTTG	T G <mark>A T</mark> G G T G <mark>C</mark> T <mark>C</mark>	TTTTGTCC	T <mark>C</mark> GA <mark>AC</mark> AAAT	AGTAATACAT	CGTTAATAAA	AGCAGACCTT
consensus	TTGGTAGTTG	TGATGGTGCTC	TTT GTTCC	CGAACAAA	AGTAATACAT		AGCAGACCTT

	710	720	730	740	750	760	770
1d.OL A31	<mark>С</mark>	I N N N <mark>G</mark> N N N N N N N	1 N N N N N N N N	I N N N N N N N N N	I N <mark>A</mark> N N N N N N N N	N N N N N N N N N N	
2d.OL A31		IN <mark>TCGATAGAA</mark> A	CCAAAAGGG	5	AATAGGAATT	T <mark>ACC</mark> GGTGAG	A T A T T T
OL A31 Ref.	<b>C</b>	G T C G A T A G A A A		G G <mark>A A</mark> G <mark>T</mark> G G G <mark>C</mark>	AATAGGAATT	Τ <mark>Α</mark> Ϲ Ϲ ໑ Ϛ Ϛ Α ໑	A T A T T T
consensus							
	780	790	800	810	820	830	840
1d.OL A31		<u>INNNNNNNNNN</u>	<u>1 N N N N N N N N N N N N N N N N N N N</u>	I N N N <mark>A</mark> N N N A	<u>,                                    </u>	N N N N N N N N N N	NNNNN
2d.OL A31	G <mark>ATAA</mark> NG <mark>AGC</mark> GAT1	T G T G G <mark>A A A T A</mark> G	5	T T <mark>A A A</mark> T T <mark>C </mark> A		Т <mark>ИСТТТТССА</mark>	GTTTGA
OL A31 Ref.	G <mark>ATAATGAGC</mark> GAT1	T G T G G <mark>A A A T A</mark> G	Б <mark>С С А</mark> Т <mark>Б Т А</mark> Т	T T <mark>A A A T T C G</mark> A	<mark>С G Т С Т С Т Т Т</mark> Т	ттсттсса	G T T T <mark>G</mark> A
consensus	GATAATGAGCGATI	TGTGGAAATAG	GCCATGTAT	TTAAATTCGA			GTTTGA
	850	860	870	880	890	900	910
1d.OL A31	N N N N N N N N N N N N N N N		I N N <mark>N С</mark> N N N N N	I N N N N N N N N N N	I N <mark>A</mark> N N N N N N N N	N N N N <mark>A T</mark> N N N	
2d.OL A31	G <mark>ATAATAT</mark> CATGT	G <mark>ACATAT</mark> NA <mark>C</mark> A	<mark>\                                    </mark>	T <mark>C</mark> T T T G T <mark>C</mark> G T T	G <mark>A G G C</mark> T G T T G	G C A A C A T C T T	C A G C C T
OL A31 Ref.	G <mark>ATAATATC</mark> ATGT	G <mark>ACATATAAC</mark> A	A T T <mark>C</mark> G G T T T	T <mark>C</mark> T T T G T <mark>C</mark> G T T	G <mark>AGGC</mark> TGTTG	GCAACATCTT	C <mark>AGCC</mark> T
consensus	GATAATATCATGT	GACATAT ACA	ATTCGGTTT	- <mark>C</mark> T T T <mark>G T C G</mark> T T	GAGGCTGTTG	GCAACATCTT	
	920	930	940	950	960	970	980
1d.OL A31	N N N N N N N N N N N N N	มุ่ม <mark>ม ม ม ม ม ม ม ม</mark> ม ม ม ม ม	<u> </u>	I N N N N N N N N N	I N N N N N T N N N	N <mark>G</mark> N N N N N N N N	NNNNN
2d.OL A31	T T A T <mark>C</mark> G G <mark>A C T T </mark> G <mark>C</mark> /	G T T T G T <mark>A</mark> T G T <mark>A</mark>	N T G G <mark>C T</mark> G <mark>A</mark> G	6 <mark> </mark>	TCGGCGTGGC	AGACGAATAC	C G T G T C
OL A31 Ref.	TT <mark>ATC</mark> GG <mark>ACTTGC</mark>	G T T T G T <mark>A</mark> T G T <mark>A</mark>	G T G G C T G A G	<mark>A</mark> TTT <mark>AA</mark> TTT	ασσοτοία	AGACGAATAC	C G T G T C
consensus	TTATCGGACTTGC	G T T T G T <mark>A</mark> T G T <mark>A</mark>		<mark>A T T T A A T T T C</mark>	тссссстссс	AGACGAATAC	
	990	1000	1010	1020	1030	1040	1050
1d.OL A31	N <mark>A</mark> N N N N N N N N N A N	<u>                                      </u>	<u>1 N N N N N N N N N N N N N N N N N N N</u>	I N N N N N N N N N	N N N <mark>C</mark> N N N N N N	<u>N N <mark>A</mark> N N N N N N N</u>	
2d.OL A31	G <mark>ATTCGCTTT</mark> G <mark>AA</mark> 1	T T T N <mark>G C A</mark> T T T T	NGAGTTTGA	AGG <mark>C</mark> AGAAG	T G <mark>A C</mark> G T T <mark>A</mark> T T	T G A T G G T T T A	T A T T T A
OL A31 Ref.	GATTCGCTTTGAA	T T T T <mark>G <mark>C</mark> A T T T T</mark>	TGAGTTTG	AGG <mark>C</mark> AGAAG	T G <mark>A C</mark> G T T <mark>A</mark> T T	TGATGGTTTA	TATTA
consensus	GATTCGCTTTGAA		GAGTTTGA		TGACGTTATT	TGATGGTTTA	

	1060	1070	1080	1090	1100	1110	1120
1d.OL A31	N <mark>C</mark> N N N N N N N N N N N	N N <mark>G</mark> N N N N N <mark>A</mark> N N	N N N N N N N N <mark>A</mark> N	N N N N <mark>C</mark> N N N I	N N N N N N N N N N	и <mark>т</mark> и и <mark>с</mark> и и и и и	N N N N <mark>A</mark> N
2d.OL A31	A C A A T A A T C G G A	AAGATTAAATG	T T <mark>C A C T</mark> G G G <mark>A C</mark>	TT <mark>C</mark> T <mark>ACATA</mark>	G <mark>ATAC</mark> TNAA <i>A</i>	A T G A <mark>C</mark> G T T T (	G <mark>C</mark> GG <mark>C</mark> AT
OL A31 Ref.	A C A A T A A T C G G A	AAGATTAAATG	T T <mark>C A C T</mark> G G G <mark>A C</mark>	T T C T A C A T A (	G <mark>ATAC</mark> TAAAA	A T G A <mark>C</mark> G T T T (	G <mark>C</mark> GG <mark>CA</mark> T
consensus	ACAATAATCGGA	AAGATTAAATG	T T C A C T G G G A C	ΤΤΖΤΑΖΑΤΑ	GATACT AAA	ATGACGTTT	G <mark>C</mark> GG <mark>CA</mark> T
	1130	1140	1150	1160	1170	1180	1190
1d.OL A31	<u>N N N N N N N N N N N N </u>	N N N N N N N N N N N	ุ่ทุ <mark>ทุทุทุ</mark> ษไท่ทุท		<u>N N N N N N N N N N N N N N N N N N N </u>	1 N N N N N N N N N N	<u>N N N N N N N</u>
2d.OL A31	<mark>C</mark> GN <mark>ATTCAGTC</mark> G	C A A A C A C T A C G A	<mark>A T </mark> G <mark>T <mark>C A</mark> T T G T</mark>	G T A G A G C N C	T <mark>G T T G A T T A </mark> C /	A G A T G A A T T T C	G G <mark>C</mark> T T T A
OL A31 Ref.	<mark>C</mark> G <mark>T A T T C A </mark> G <mark>T C</mark> G	CAAACACTACG	A T G G <mark>T C A T T</mark> G T	GTAGAGCTC	T G T T G A T T A <mark>C</mark> A	GATGAATTT	G G <mark>C T T T</mark> A
consensus	C G A T T C A G T C G	C A A A C A C T A C G A	A T G G <mark>T C A</mark> T T G T	G T A G A G C C	T G T T G <mark>A T T A C</mark> /	A G A T G A A T T T (	G <mark>C T T T A</mark>
	1200	1210	1220	1230	1240	1250	1260
1d.OL A31	N N N N N N N N N N N N	N N <mark>A</mark> N N N N N N N N	N N N N N N N N N N N	N N N N N <mark>C</mark> N N I	<u>N N N N N N N N N N N N N N N N N N N </u>	1 N N N N N N N N N I	<u>N N N N N N </u>
2d.OL A31	<mark>A A T G C C N T T A</mark> G G	GTATTGNGGTG	T <mark>T A</mark> T G G G A A A T	T G T T T N <mark>C</mark> G <mark>A</mark> (	C G A T T A T C T C 1	G T T T G G <mark>A C</mark> A	G <mark>C</mark> NG <mark>A</mark> NT
OL A31 Ref.	A <mark>ATGCCCTTA</mark> GG	G T A T T G G G G T G	T <mark>TA</mark> TGGGAAA	T G T T T T C G A		G <mark>TTT</mark> GG <mark>AC</mark> A	G <mark>CCGAC</mark> T
consensus	A A T G <mark>C C</mark> T T A G G	G T A T T G G G T G	T T <mark>A T G G G A A A</mark> T	TGTTT CGA	C G A T T A T C T C T	G T T T G G <mark>A C</mark> A	G <mark>C</mark> G <mark>A</mark> T
	1270	1280	1290	1300	1310	1320	1330
1d.OL A31	ุ่ <mark>ผ</mark> ุ <sub>่</sub> ทุ่ม ทุ่ม ทุ่ม ทุ่ม ทุ่ม ท	N N N N N N N N N N N	N N N N N N N N N N N		<u>N N N N N N N N N N N N N N N N N N N </u>	1 N N N N N N N N N	<u>N N N N N N </u>
2d.OL A31	C A G G C A A A T T N T	G <mark>A G A T G T T G T G</mark>	N <mark>T C T T C </mark> N G <mark>A C</mark> G	ATTTAGAAA	G T G A A G A G C T (	6 <mark>A C </mark> G T <mark>A</mark> T T T T C	T G G <mark>A T A</mark>
OL A31 Ref.	CAGGCAAATTAT	G <mark>AGATGTTG</mark> TG	С <mark>ТСТТСС</mark> С <mark>АС</mark> С		G <mark>T</mark> G <mark>A</mark> AG <mark>A</mark> G <mark>C</mark> T	<mark>ACGTATTTT</mark>	T G G <mark>A</mark> T <mark>A</mark>
consensus	C <mark>AGGCAAAT</mark> T	G <mark>A</mark> G <mark>A T</mark> G T T G T G	TCTTC GACG	ATTTAGAAA	G T G <mark>A </mark> A G <mark>A </mark> G <mark>C</mark> T (	6 <mark>A C</mark> G T <mark>A</mark> T T T T C	T G G <mark>A T A</mark>
	1340	1350	1360	1370	1380	1390	1400
1d.OL A31	N N N N N N N N N N N N	N N N N N N N N N N N	N N N N N N N N N N N		N N N N N N N N N N N	<u>5 N N N N N N N N N N N N N N N N N N N</u>	งทุ่งทุ่งทุ่งท
2d.OL A31	T G <mark>A C G T A T A A A</mark> G	ATTTTGGAGTT	T A T C N G A A T G A	T A T T A T N A G	T <mark>CAT</mark> CNAANNO	A C A C G G A A A C	TATGAA
OL A31 Ref.	Τ <mark>ΓΑΖΓΓΑΤΑΑΑ</mark> Α	ATTTTGGAGTT	T <mark>ATC</mark> AGAATGA	TATTATAAG	Τ <mark>ΓΑΤ</mark> ΓΑΑΑΑΑ	ACACGGAAA	TATGAA
consensus	T G <mark>A C</mark> G T A T A A A G	A T T T T G G A G T T	T <mark>ATC</mark> G <mark>AAT</mark> GA	TATTAT AG	Τ <mark>ΓΑΤ</mark> Γ ΑΑ (	A C A C G G A A A C	T A T G A A

	1410	1420	1430	1440	1450	1460	1470
1d.OL A31	N N N N N N <mark>G</mark> N N N N	N N N N N N N N N N N N	N N N N N N N N N N	N N N N N N N N N	N N N N N N N N N N	<u>N N N N N N N N N N N </u>	N N N N N N
2d.OL A31	A A N T C T G T T A G G	TTTATAACCAAT	G T A C N N N A N N	ACTAAGCTC	A G G <mark>C</mark> A T N <mark>C C</mark> A T	T T A T G G A G N G A	Г <mark>С</mark> ТТТ <mark>А</mark>
OL A31 Ref.	A A C T C T G T T A G G	TTTATAACCAA	G T A C A A A A A A	A C T A A G C T C I	A G G <mark>C</mark> A T A <mark>C C</mark> A	T T <mark>A T </mark> G G <mark>A </mark> G <mark>C</mark> G A	Г <mark>G</mark> ТТ <mark>Т</mark> А
consensus	A A T C T G T T A G G	ТТТАТТ <mark>АСС</mark> ААТ	GTACA	ACTAAGCTC	AGGCAT CCA	TTATGGAG GA	Г <mark>Б</mark> ТТТ <mark>А</mark>
	1480	1490	1500	1510	1520	1530	1540
1d.OL A31	<u>N N N <mark>A A</mark> N N N N N N N</u>	N N N N <mark>A N G T</mark> N N N N	N N T N N N N N N N	N N N N N N N N N	N N N N N N N N <mark>C</mark> I	<u>N N N N N N N N N N N N N N N N N N N </u>	<u>N N N N N N</u>
2d.OL A31	T <mark>CCAA</mark> TT <mark>G</mark> TNNT	N <mark>A C C A C</mark> G T T A A A	GATGCANTCT	G T G T <mark>A</mark> G <mark>A</mark> G N	T T <mark>A T G A A A G C</mark> <sup>-</sup>	T <mark>GAAAA</mark> NNTAT	T <mark>AC</mark> AAA
OL A31 Ref.	Т <mark>ССААТТС</mark> ТССТ	AACCACGTTAAA	GATGCACTCT	G T G T A G A G C	T T <mark>A T G A A A G C</mark> <sup>-</sup>	T <mark>GAAAAA</mark> A <mark>A</mark> TAT	T <mark>AC</mark> AAA
consensus	Т <mark>ССААТТС</mark> Т Т	ACCACGTTAAA	GATGCATCT	G T G T A G A G	T T <mark>A T G A A A G C</mark> <sup>-</sup>	T <mark>GAAAA TAT</mark>	ΓΑΓΑΑΑ
			<u> </u>				
	1550	1560	1570	1580	1590	1600	1610
1d.OL A31	<u>N N N N N N N N N N N N N N N N N N N </u>		N N N N N N N N N N		N N N N N N N N N N I	<u>1                                      </u>	งทุ่งทุ่งทุ่ง
2d.OL A31	C G A T G G A T G T C G	T T T T T <mark>A T G A A A</mark> A	C T T T <mark>A A T T </mark> G G	C G A G T T T T C	T <mark>A T G T G C A</mark> N <mark>C</mark> (	G <mark>A T A A T C</mark> T T A A <sup>-</sup>	Г <mark>С</mark> А <mark>СТТ</mark>
OL A31 Ref.	C G A T G G A T G T C G	TTTTT <mark>T</mark> A <mark>TGAAAA</mark>	<mark>΄ ΄ Τ΄ Τ΄ Α΄ Α΄ Τ΄ Τ΄ </mark> ΄ ΄ ΄ ΄	C G A G T T T T C	Τ <mark>Α</mark> Τ <mark>ΓΓΓΟ</mark> ΟΟ	G <mark>ATAATC</mark> TTAA	Г <mark>С</mark> А <mark>С</mark> ТТ
consensus	C G A T G G A T G T C G	TTTTTATGAAAA	CTTTAATTGG			G <mark>ATAATC</mark> TT <mark>AA</mark> T	Г <mark>С</mark> А <mark>Б</mark> ТТ
				- <u>1 5 41 8 5 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7</u>			
	1620	1630	1640	1650	1660	1670	1680
1d.OL A31		N <mark>G C</mark> N N N N N N N N N	N N N N N N N N N N		N N N N N N N N N N I	<u>N N G N N N N N N N I</u>	ΝΝΝΤΝ
2d.OL A31	G <mark>C</mark> TTA <mark>AC</mark> AAATT	<mark>C</mark> GNTNN <mark>AGATC</mark> A	GTCAACACTT	AGCGATGTC	G A G A A A A T A A /	A <mark>C </mark> A G <mark>T C T C A </mark> T N (	G <mark>AGA</mark> TT
OL A31 Ref.	G <mark>CTTAACAAAT</mark> T	C G C T A C A G A T C A	GTCAACTT	AGCGATGTC	G <mark>AGAAAA</mark> TAA/	A C A G T C T C A T A G	G <mark>AGA</mark> TT
consensus	G C T T A A C A A A T T	CGCT AGATCA	GTCAACACTT	A G C G A T G T C	GAGAAATAA	A C A G T C T C A T	G <mark>AGA</mark> TT
	1690	1700	1710	1720	1730	1740	1750
1d.OL A31				N N N N T N N N N		<u>N N N N N N N N N N N N N N N N N N N </u>	<u>N N N N N </u>
2d.OL A31	G A T G G C G A A A A C	AGCAAGCGTCTT	TNAGTAGAGT	TGGATNCGA	ΤΤϹΤϹϹΑΤGΑ	G G A G A <mark>C</mark> A G G <mark>A T</mark>	T <mark>ATAT</mark> C
OL A31 Ref.	GATGGCGAAAA	AGCAAGCGTCTT	TTAGTAGAGT	TGGATCCGA	ΤΤ <mark>Ϲ</mark> Τ <mark>ϹϹ</mark> ΑΤ <mark>Ͼ</mark> Α	GGAGA <mark>C</mark> AGG <mark>A</mark> T	T <mark>ATAT</mark> C
consensus	GATGGCGAAAA	A G C A A G C G T C T T	TAGTAGAGT	TGGAT CGA	T T <mark>C T C C A T</mark> G <mark>A</mark> (	G G <mark>A </mark> G <mark>A </mark> C A G G <mark>A T</mark>	Г <mark>А</mark> Т <mark>АТ</mark> С

		1760	1770	1780	1790	1800	1810	1820
1d.OL A31	NNNNN		N N N N N N N <mark>A</mark> N N N	N N N N N N N N N N	N N N N N N N N N	N N N N N N N N N N	<u>N N N N N N N N N N N </u>	NNNNN
2d.OL A31		I G C C A A	<mark>A T G T T G T G <mark>A C</mark> G G</mark>	A A G C T N N G A G	TGAAAAGGT1	ΓΑΑΑΤ <mark>Ϲ</mark> GΑΤΑ	C <mark>ACGTCGAGTC</mark>	T G <mark>A A</mark> G G
OL A31 Ref.	A A G C G C	CTGCCAA	A T G T T G T G <mark>A C</mark> G G	AAGCTCCGAG	T G <mark>A A A A G G T</mark> 1	T <mark>AAATCGAT</mark> A	C <mark>ACGTCGAGT</mark> C	T G <mark>A </mark> A G G
consensus	A A G G	TGCCAA	ATGTTGTGACGG		TGAAAAGGTI			TGAAGG
		1830	1840	1850	1860	1870	1880	1890
1d.OL A31	ΝΝΝΝΝ	I N N N N N N	N N N N N N N <mark>T</mark> N N N	N N N N N N N N N N	N N N <b>T</b> N N N N	N N N N N N N N N N	<u>N N N N N N N N N C</u>	NNNNN
2d.OL A31	T G <mark>A</mark> G <mark>A</mark> G	G T G T <mark>A</mark> T G	G T <mark>C </mark> A N G <mark>C</mark> G T T A C	T G A A G G C G G C	A T A A T G A A A C	AAGAAAAG	G <mark>AACT</mark> GG <mark>C</mark> GT <mark>C</mark>	N N G T A C
OL A31 Ref.	T G <mark>A</mark> G <mark>A</mark> G	G T <mark>G T </mark> A T G	G T C A A G C G T T A C	TGAAGGCGGC	ATAATGAAA		G <mark>AACT</mark> GG <mark>CGTC</mark>	T <mark>A G T A C</mark>
consensus		G T G T <mark>A</mark> T G	GTCA GCGTTAC	TGAAGGCGGC	ATAATGAAA		G <mark>AACT</mark> GG <mark>CGTC</mark>	G T A C
		1900	1910	1920	1930	1940	1950	1960
1d.OL A31		ואאאא	<u>ทุ่ม ทุ่ม ทุ่ม ทุ่ม ท</u> ุ่ม ท	N N N N <mark>A</mark> N N N N N	N N N N N N N N N	NNNYNNNNN	<u>N N N N N N N N N N N </u>	NNNNN
2d.OL A31	C T G T A G	G <mark>CC</mark> GGTT	T <mark>A</mark> TTG <mark>C</mark> G <mark>C</mark> GTTC	AGGCATTAAA	C T G A G G C A G A	A A T G T N N G C /	A A G T A A A A A T T	<mark>a</mark> tt <mark>c</mark> tg
OL A31 Ref.	C T G T A G	G <mark>CC</mark> GG <mark>TT</mark>	T <mark>A</mark> TTG <mark>C</mark> G <mark>C</mark> GTTC	AGGCATTAAA	CTGAGGCAGA	AAATGTCCGC	A A <mark>G T A A A A A T T</mark>	<mark>A</mark> TT <mark>C</mark> TG
consensus	C T G T A G	G <mark>CC</mark> GGTT	T <mark>A</mark> TT <mark>GCGC</mark> GTT	A G G <mark>C A T T A A A</mark>	C T G A G G C A G A	A A T G T G C /	A <mark>AGTAAAAT</mark> T	<mark>a</mark> tt <mark>c</mark> tg
		1970	1980	1990	2000	2010	2020	2030
1d.OL A31	ΝΝΝΝΝ	1 <mark>C</mark> N N N N N	N N N N N N N N <mark>A</mark> N N	N N N N N N N N N N	N N N N N N N N N	N N N N N N N N N N	N N N N <mark>A</mark> N N N N N N	NNNNN
2d.OL A31	G <mark>C</mark> G <mark>AT</mark> G	G <mark>C</mark> AGATG	<mark>C C T </mark> G <mark>T A</mark> G G <mark>A T</mark> G	T A G A T A G T T T	T G C G T G T A G A	A G T T G A A G A T G	<mark>C T T T A T</mark> G G <mark>A</mark> G G	G <mark>A A T T</mark> G
OL A31 Ref.	GCGATO	G <mark>C</mark> AG <mark>AT</mark> G	CCTGTAGGGATG	TAGATAGTTT	T G <mark>C</mark> G T G T A G A	AGTTGAAGAT		G <mark>AATT</mark> G
consensus	G <mark>C</mark> G <mark>AT</mark> O	G <mark>C</mark> AG <mark>A</mark> TG	C <mark>C</mark> TGTAGGGATG	T <mark>A G A T A G T T</mark> T	T G <mark>C</mark> G T G T A G <mark>/</mark>	A G T T G A A G A T (	<mark>C T T T A T</mark> G <mark>G A</mark> G G	G <mark>A A</mark> T T G
		2040	2050	2060	2070	2080	2090	2100
1d.OL A31	NNNNN	I N N N N N N	N N N T N N N N N N N N	N N N N N N N N N N	N N N N N N N N N N	N N N N N N N N N N	<u>N N N N N N N N A T</u>	NNNNN
2d.OL A31	TGTCTT	TGTTCC	AGATGTAGGTTT		GCCGAGTTT	<mark>A T A A T T T C</mark> T	T T G G <mark>A</mark> G G G <mark>A</mark> A T	C A T G A G
OL A31 Ref.	TGTCTT	TGTTCC.	AGATGTAGGTT		GCCGAGTTT	<mark>A T A A T T T C</mark> T	<b>FT</b> GG <mark>A</mark> GGG <mark>A</mark> AT	CATGAG
consensus	Т <mark>С</mark> ТТ	ТСС	A G A T G T A G G T T T		GCCGAGTTT	G <mark>ATAATTTC</mark> T	T T G G A G G G A A T	C A T G A G

	2110	2120	2130	2140	2150	2160	2170
1d.OL A31	N N N N N N N N N N N N N	N N N N N N N N N N N	N N N N N N N N N	N N <mark>C</mark> N N N N N <mark>A</mark> N N	NNNNNNNN	<u> </u>	N N N <mark>A</mark> N N
2d.OL A31	A G C C T T T A G T T C A	T C G G C G N A A A C	G <mark>TACAT</mark> GG <mark>C</mark>	G T C G A A G C A T T	G T <mark>A T C</mark> G T <mark>A</mark> T <mark>/</mark>	A G <mark>C T</mark> G T T <mark>C</mark> G G T	T T <mark>A A</mark> T T
OL A31 Ref.	<mark>A G C C T T T A G T T C</mark> A	T C G G C G C A A A C	G <mark>TACAT</mark> GG <mark>C</mark>	G T C G A A G C A T T	G T <mark>A T C</mark> G T <mark>A</mark> T <mark>/</mark>	A G <mark>C T </mark> G T T <mark>C</mark> G G T	T T <mark>A A</mark> T T
consensus	AGCCTTTAGTTCA		GTACATGGC	GTCGAAGCATT	GTATCGTAT		ТТААТТ
	2180	2190	2200	2210	2220	2230	2240
1d.OL A31		N N N N N N N N N N N	N N N N N N N N N	N N N <mark>G</mark> N <mark>T</mark> N N N N N	N N N <mark>A</mark> N N N N N	<u> </u>	NNNNN
2d.OL A31	<mark>A T A A A T T C T C T G C</mark>	A T G T C T T T A G A	T G G A A A <mark>A C</mark> T	G <mark>CAGATTC</mark> GGN	G G N <mark>A G A C A</mark> G A	A T T <mark>G A T C</mark> A T <mark>C</mark> T	CCCATG
OL A31 Ref.	<mark>A T A A A T T C T C T G C</mark>	A T G T <mark>C</mark> T T T <mark>A G</mark> A	T G G A A A <mark>A C</mark> T	G <mark>C A</mark> G <mark>A T T C</mark> G G G	G G G <mark>A G A C A</mark> G A	A T T <mark>G A T C</mark> A T <mark>C</mark> T	CCCATG
consensus	ATAAATTCTCTGC	ATGTCTTTAGA	TGGAAAACT	GCAGATTCGG	GG AGACAGA	A T T G A T C A T C T	CCCATG
	2250	2260	2270	2280	2290	2300	2310
1d.OL A31	N N N N N N N <mark>A T</mark> N N N	N N N N N N N N N N N	N N <b>T T</b> N N N N N	N N N N <mark>T</mark> N N N N N	N N N N N N N N N	<u>N N N N N <mark>A</mark> N N N N</u>	NNNNN
2d.OL A31	N T T <mark>C C</mark> G G N <mark>A T</mark> N T <mark>A</mark>	G <mark>C</mark> AAGGGAGAA	A G T T G A T T G	A G A A T T <mark>C</mark> G A <mark>C</mark> A	<mark>Т <mark>С Т С С</mark> Т G G G /</mark>	A G A A G <mark>C</mark> A T T A G	G G <mark>A</mark> N <mark>T</mark> G
OL A31 Ref.	G T T <mark>C C</mark> G G <mark>A T G T </mark> A	G <mark>C</mark> AAGGGAGAA	A G T T <mark>G A</mark> T T G	A G A A T T <mark>C G A C</mark> A	TCTCCTGGG	A G A A G <mark>C A</mark> T T A G	G G <mark>A</mark> G <mark>T</mark> G
consensus	TTCCGG AT TA	GCAAGGGAGAA	AGTTGATTG	AGAATTCGACA	TCTCCTGGG	AGAAGCATTAG	GGATG
	2320	2330	2340	2350	2360	2370	2380
1d.OL A31	N N N N N N N N N N N N N	N N N N N N N N N <mark>C</mark>	N	<u>ท ท ท ท ท ท ท ท ท ท ท</u>	Т N N N <mark>A</mark> N N N <mark>T</mark>	<u>ง                                    </u>	ΝΝΝΝΝΝ
2d.OL A31	<mark>C T C</mark> G G <mark>A T A A G T T C</mark>	A T T N G N A A A A <mark>C</mark>	T G A A A T G T <mark>C</mark>	<mark>аатттс</mark> а <mark>тс</mark> аа	TNATAATTT	<mark>A G T G A T G C A</mark> T	TCGCAG
OL A31 Ref.	<mark>C T C</mark> G G <mark>A T A A G T T C</mark>	A T T A G A A A A A C	T G <mark>A A A T</mark> G T <mark>C</mark>	<mark>сстттссстс</mark> сс	ΤΤ <mark>Α</mark> Τ <mark>Α</mark> ΑΤΤΤ	C <mark>AGTGA</mark> TG <mark>C</mark> AT	TCGCAG
consensus	C T C G G A T A A G T T C	ATT G AAAAC	TGAAATGTC	<mark>сстттссстс</mark> сс	ΤΑΤΑΑΤΤΤ	AGTGATGCAT	TCGCAG
	2390	2400	2410	2420	2430	2440	2450
1d.OL A31	N N N N N N N N T N N <mark>C</mark>	N N N N N N N N N N N	N N N N N N N N N	ทุ่มพุ่มพุ่มพุ่มพุ่ม	N N N N N N N N N	งทุ่ทุ่ทุ่ทุ่ทุ่ทุ่ทุ่ทุ่ทุ่	NNNNN
2d.OL A31	A T A C G T C G T T G C	N <mark>A T G N C C A A T A</mark>	A T G A N T C C G	AAGAAACCATC	N	A G G G <mark>A A A</mark> T A T	C N A G T T
OL A31 Ref.	A A T A C G T C G T T G C	T A T G G C C A A T A	ATGACTCCG	AAGAAACCATC	CGGG <mark>AT</mark> G <mark>C</mark> A	AGGGAAATAT	CTAGTT
consensus	A A T A C G T C G T T G C	ATG CCAATA	ATGA TCCG	AAGAAACCATC	GGG <mark>AT</mark> G <mark>C</mark> A	<mark>A G G G A A A T A T</mark>	







	3510	3520	3530	3540	3550	3560	3570
1d.OL A31	<mark>G</mark> N N N N N N N N N N <mark>G</mark> N	N N N N N N N N N N N	N T T N N N N T	N N N N N N N N N N N	ุ่ <mark>ทุ่มหว</mark> ุ่มหมุ่มห	N N T N N N N T N	NNNNNN
2d.OL A31	G T G T <mark>C</mark> G T T <mark>C</mark> G T T G T	A T G T G T A A T G C	G T T G <mark>C</mark> A T T	ΤΤ <mark>Γ<mark>Γ</mark>Ο<mark>ΓΓΤΓΟΑ</mark>ΓΤΙ</mark>	A T G <mark>C</mark> T T T N G T	A G T G A N N T T	A T <mark>C</mark> T G G A
OL A31 Ref.	G T G T <mark>C</mark> G T T <mark>C</mark> G T T G T	A T G T G T A A T G C	G T T G <mark>C</mark> A T T	TTGGGGTTG <mark>A</mark> GT	A T G <mark>C</mark> T T T G G T	AGTGAGGTT	A T <mark>C</mark> T G G A
consensus	G T G T C G T T C G T T G T	ATGTGTAATGC	GTTGCATT	TTGGGTTGAGT	A T G C T T T G T	AGTGATT	A T C T G G A
	3580	3590	3600	3610	3620	3630	3640
1d.OL A31	<u>N N N N N N N N N A N N N</u>	N N N N N N G N N <mark>A</mark>	N N N N N N N		<u>ุ</u> ทุท <sub>ุ</sub> ทุท <sub>ุ</sub> ทุท <sub>ุ</sub> ทุท	N N N <mark>G</mark> N N N N N	NGNNNNN
2d.OL A31	Τ <mark>G G Τ Τ G Τ G Τ </mark> G <mark>Α</mark> G Τ Τ	TTTATCGNWGA	C G C G G T A G	TCGTTTCCAGG	C <mark>AGA</mark> NTTTTG	TTAGCTTCC	C G <mark>A C</mark> T T T
OL A31 Ref.	Τ <mark>ϾϾͳͳϾͳϾͳϾ</mark> ΑϾͳΤ	TTTATCGGTGA	C G C G G T A G	TCGTTTCCAGG	<mark>C A </mark> G <mark>A </mark> G T T T T G		C G <mark>A C</mark> T T T
consensus	ΤĠĠΤΤĠΤĠΤĠΑĠΤΤ	TTTATCGGTGA		TCGTTTCCAGG	<mark>ϲ</mark> Α <mark>Α Τ Τ Τ Τ </mark> Γ	TTAGCTTCC	CGACTTT
	3650	3660	3670	3680	3690	3700	3710
1d.OL A31	N N N N N N N N N N N N N N N	N N N N N N N G N N	NGNNNNN	G N N N N N N <mark>A</mark> N N	<mark>G</mark> N N N N N N N N N	N G N T N T N N N	N N N N N N N
2d.OL A31	<mark>С Т </mark>	N N T T T A A C G T C	C G T A G A T C	G T T C N G T G A T T	G <mark>TTT</mark> GGGGNT	AGATGTTAG	T C G T T T G
OL A31 Ref.	CTGGGGAACTTTTT	T T T T T <mark>A A C</mark> G T C	CGTAGATC	G T T C C G T G A T T	<mark>с т т т</mark> с с с с с с т	AGATGTTAG	TCGTTTG
consensus	C T G T G G <mark>A A C</mark> T T T	T T T <mark>A A C G T C</mark>	C G T A G A T C	G T T C G T G A T T	GTTGGGG	AGATGTTAG	T <mark>C</mark> G T T T G
	3720	3730	3740	3750	3760	3770	3780
1d.OL A31	N N N N G N N N N N N N N N	N N N N N N N N N N N	N N N N N N A		N N N N N N N N N N	N N N N N N N N N N	N N N G N N N
2d.OL A31	N G <mark>A C</mark> G <mark>A </mark> G <mark>A C</mark> G <mark>T</mark> G <mark>A</mark> G	C T T T T G T T <mark>A</mark> G T	ΤΤΓΟΟΤΟΑ	TTGAGTTCTGT	T G C N A G T C G T	T A G C T G T G T	T G T G <mark>A</mark> T A
OL A31 Ref.	TG <mark>AC</mark> GAGACGTGAG		ΤΤΓΟΟΤΟΑ	TTGAGTTCTGT	T G C T A G T C G T	TAGTGTGT	TGTGATA
consensus	G <mark>AC</mark> GAGA <mark>C</mark> G <mark>T</mark> GAG	C T T T T <mark>G</mark> T T <mark>A</mark> G T	T T G G <mark>C</mark> T G A	TTG <mark>AGTTC</mark> TGT	T G C A G T C G T	TAGTGTGT	T G T G <mark>A</mark> T A
	3790	3800	3810	3820	3830	3840	3850
1d.OL A31		NNGNNNNNNN	N N N N N N N N		NGNNNNNNNN	NNNNNGNN	NNNNTNN
2d.OL A31	T G A T T T G T C N A N N T	TAGACTTGTAT	T C G T T A A T	GGTTCCTTTGGG	C G T T G T T A G T	AAGTTTGAC	NAGNTTA
OL A31 Ref.	T G A T T T G T C G A C C T	TAGACTTGTAT		GGTTCCTTTGG	C G T T G T T A G T	AAGTTTGAC	
consensus	T G A T T T G T C A T	T A G A C T T G T A T	Τ <mark>Ϲ</mark> GΤΤΑΑΤ	G G T T C C T T T G G	<mark>C</mark> G T T G T T <mark>A</mark> G T	AAGTTTGAC	AG TTA

<u> </u>	3910	3920
1d.OL A31	N N N N N N N N	I N N N N N N
2d.OL A31 GAATGGCAGACTTTTGAAGATGTCGTTAAGGGACGTTTCAGGCGGTGAANNGNNATTG	A G N T G T N G T	G <mark>A</mark> T T T
OL A31 Ref. GAATGGCAGACTTTGAAGATGTCGTTAAGGACGTTTCAGGCGGTGAAGGGCCATTG	A G G T G T A G T	G <mark>A</mark> TTT
consensus GAATGGCAGACTTTTGAAGATGTCGTTAAGGGACGTTTCAGCGGTGAA	AG TGT GT	
<i>3930 3940 3950 3960 3970</i>	3980	3990
1d.OL A31 NNNNNNGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	ANNNNNNN	I N N N N N N
2d.OL A31 TTCCCATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG	ΑΤΟΤΟΟΘ	G T G A N T N
OL A31 Ref. TTCCCATGTGTGTGTGAGCGTTAAGAATTGGTGTGTGTGT	ATCTCTCC	G <mark>T</mark> G <mark>A</mark> G T G
consensus TTCCCATGTGTGTGTGTGGTGGTGAGCGTTAAGGAATTG TGTCATTCGTGGTGGTATTTCTGTGTGGTGGTGG	ATCTCTCC	
4000 4010 4020 4030 4040	4050	4060
1d.OL A31	N N N N N N N N	INNGNNN
2d.OL A31 NACGCCGACATTCTANTTGCTANTTTGCTTTGGTTCTTCGGTGACTTTANTGATTGAGTGTGTG	ATGAATTAC	T <mark>A </mark> G <mark>A T</mark> G
OL A31 Ref. GACCCGACATTCTATTCAGTTCTATTCGCTTCCTCGGTGACTTTAGTGAGTG	ATGAATTAC	T <mark>A </mark> G <mark>A</mark> T G
consensus ACGCCGACATTCTA TTTGCTTTGGTTCTTCGGTGACTTTA TGATTGAGTGTGTG	ATGAATTAC	TAGATG
<i>4070 4080 4090 4100 4110</i>	4120	4130
1d.OL A31 GNNNNGNATNNNNGTNNNNGNNNNNNNNNNNNNNNNNNN	N N N N N N N N	I N N N N N N
2d.OL A31 GTAAAGCATTGTTAGTTAGTTACTTGTACTTCAGTTTTGGCAGTTACATTTGACATTGACGGAG	ΤΤΝΝΝΤΤΤΤ	T G G <mark>A</mark> G T
OL A31 Ref. GTAAAGCATTGTTAGTTAGTTAGTTACTTGTACTTCAGTTTTGGCAGTTACATTTGACACGGTAG	ТТТТТТТТ	T G G <mark>A</mark> G T
consensus		
	т т  Т т т т	TGGAGT
	ТТТ ТТТТ	T G G <mark>A</mark> G T
4140 4150 4160 4170 4180	<b>T T T T T T</b> 4190	T G G A G T 4200
4140         4150         4160         4170         4180           1d.OL A31         NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	T     T     T     T     T       4190       N     N     A     N     N	4200 N C N N N N
4140         4150         4160         4170         4180           1d.OL A31         NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	T     T     T     T     T     T       4190       N     N     A     N     T     N     N       T     G     A     A     T     C     T	T         G         A         G         T           4200         N         N         N         N           N         C         N         N         N         N           T         C         C         T         C         T         C         T
4140       4150       4160       4170       4180         1d.OL A31       NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	4190       N     A       G     A       T     G       G     A       T     G       A     T       C     T	Image: Top of the second state         Image: Top of top



## **D** Multiple alignment for KUK A49

The control amplicon for KUK A49 was sequenced using the minION<sup>™</sup> device. In the next pages, a multiple alignment generated using ClustalW in the MacVector software, V11.1.1 is shown. The reference sequence obtained by Enjie Zhang using Ion Torrent<sup>™</sup> sequencing is named: "KUK49\_final "; the consensus generated by IGV from the alignment of the 1D reads is called "kuk.1d" and for the 2D reads, "kuk.2d". Each nucleotide is colour coded and the position of each nucleotide is indicated at the top of the alignment in red numbers. Letters are: A: adenine; T: thymine; C: cytosine; G: guanine; R: purine (A or G); Y: pirimidine (T or C); N: any nucleotide; W: Weak (A or T); S: Strong (C or G) and K: Keto (G or T).

	-	10	20	30	40	50	60	70
kuk.2d	<mark>C</mark> AGGAAAGG	G <mark>AC</mark> GG <mark>T</mark> G	A T A A T <mark>C</mark> T	T T G T G T A G G	T <mark>G T T </mark> G <mark>T C</mark> T <mark>C</mark> T <mark>C</mark> T	G T <mark>A G A T G C G C</mark> 1	ГТ <mark>АТ<mark>С</mark>ТТ<mark>G</mark>ТАТС</mark>	ΤΓΑΑΟ
kuk.1d	<mark>C</mark> AGGAAAGG	G <mark>AC</mark> GGTG	A T A A T <mark>C</mark> T	T T G T G T <mark>A</mark> G G	т <mark>G T T G T C</mark> T <mark>T C</mark> T <mark>G</mark> T <mark>G</mark> T <mark>G</mark> T	5 T <mark>A </mark> G <mark>A T</mark> G <mark>C</mark> G <mark>C</mark> 1	ГТ <mark>АТС</mark> ТТ <mark>G</mark> ТАТ	C T G <mark>A A C</mark>
KUK49_final	<mark>C</mark> AGGAAAGG	G <mark>AC</mark> GG <mark>T</mark> G	A T <mark>A A</mark> T <mark>C</mark> T	T T G T G T A G G	т <mark>сттстстсст с</mark> т	5 <mark>T A G A T G C G C 1</mark>	ГТ <mark>АТ<mark>С</mark>ТТ<mark>G</mark>ТАТС</mark>	C T G A A C
consensus	CAGGAAAGG	GACGGTG	ΑΤΑΑΤ <mark>Ο</mark> Τ	TTGTGTAGG	Т <mark>СТТСТСТС</mark> Т	G T A G A T G C G C T	ГТ <mark>АТС</mark> ТТ <mark>СТАТ</mark>	
	6	30	90	100	110	120	130	140
kuk.2d	G T <mark>A T C</mark> T A T <mark>C</mark>	G G <mark>A A </mark> G <mark>C</mark> G (	G <mark>AGA</mark> TT	C T <mark>A A T A A T C</mark>	C T T G T <mark>A A T C C T</mark> G	G <mark>GTTTTTA<mark>C</mark>TG0</mark>	G <mark>AGAAAAAGA</mark>	C T T C T
kuk.1d	G T A T <mark>C</mark> T A T <mark>C</mark>	G G <mark>A A </mark> G <mark>C</mark> G (	G <mark>A </mark> G <mark>A</mark> T T T	C T A A T A A T C	C T T G T <mark>A A T C C</mark> T G	5 <mark>6 T T T T A <mark>C</mark> T 6 0</mark>	G A G A A A A A G A G	
KUK49_final	G T <mark>A T C</mark> T <mark>A</mark> T <mark>C</mark>	G G <mark>A A </mark> G <mark>C</mark> G (	G <mark>A</mark> G <mark>A</mark> TTT	C T <mark>A A T A A T C</mark>	C T T G T <mark>A A T C C T</mark> G	G <mark>TTTTTAC</mark> TG0	G <mark>AGAAAAAGA</mark>	
consensus	GTATCTATC	GG <mark>A</mark> AG <mark>C</mark> G	G <mark>AGA</mark> TTT		C T T G T <mark>A A T C C</mark> T G	G <mark>T T T T <mark>A C</mark> T G (</mark>	G <mark>AGAAAAAGA</mark>	
	1	50	160	170	180	190	200	210
kuk.2d	<mark>ϹϹ</mark> ႺͳͳͳͳΚ <mark>Α</mark>	TT <mark>AC</mark> ATT	Г <mark>С</mark> С <mark>ТА</mark> Т	G G <mark>C A C</mark> G <mark>C</mark> G <mark>T</mark>	T <mark>A</mark> TTTGTT <mark>C</mark> GG <mark>A</mark>	A G T T A A T G <mark>C</mark> 1	ГТ <mark>GGATGG</mark> T <mark>C</mark> Т	ГСТТТК
kuk.1d	<mark>ϹϹ</mark> ႺͳͳͳͳΚ <mark>Α</mark>	TT <mark>AC</mark> ATT	T <mark>C </mark> G <mark>C T A</mark> T	G G <mark>C A C</mark> G <mark>C</mark> G <mark>T</mark>	T <mark>A</mark> TTTGTT <mark>C</mark> GG <mark>A</mark>	A G T T A A T G C 1	ГТ <mark>GGA</mark> ТGGT <mark>C</mark> Т	ГСТТТК
KUK49_final	<mark>Ϲ</mark> Ϲ <mark>Ϛ</mark> ΤΤΤΤΤΑ	TTACATT	T <mark>C</mark> G <mark>C</mark> T <mark>A</mark> T	G <mark>GCAC</mark> G <mark>C</mark> GT	T <mark>A</mark> TTTGTT <mark>C</mark> GG <mark>A</mark>	A G T T A A T G C 1	F T G G <mark> A</mark>  T G G <mark> T C</mark>  T T	T T T T
consensus	<mark>Ϲ</mark> Ϲ <mark>Ⴚ</mark> ΤΤΤΤΤΑ	TT <mark>AC</mark> ATT	Т <mark>С</mark>	G G <mark>C A C G C</mark> G T	T <mark>A T T T G T T <mark>C</mark> G G <mark>A</mark></mark>	A G T T A A T G C 1	Г <mark>Т </mark>	r <mark>c</mark> t t t t
	22	20	230	240	250	260	270	280
kuk.2d	NAGAGACCA	GAGCATA	C T C G T A T	T C T G T A A C G	T T T <mark>A G A</mark> T T T G G A	<mark>A</mark> T T T T T T <mark>A A</mark> T C	T T T T T <mark>A T G T C</mark> T	A T G T A
kuk.1d	NAGAGACCA	GAGCATA	C T C G T A T	T C T G T A A C G	T T T <mark>A G A</mark> T T T G G <mark>A</mark>	<mark>A</mark> T T T T T T <mark>A A</mark> T C	T T T T T <mark>A T G T C</mark> T	A T G T A
KUK49_final		GAGCATA	C T <mark>C</mark> G T <mark>A</mark> T		TTT <mark>AGA</mark> TTTGG <mark>A</mark>	<mark>\</mark> T T T T T T <mark>A A T (</mark>	TTTTTATGTC	T <mark>ATGTA</mark>
consensus	AGAGACCA	GAGCATA	C T <mark>C</mark> G T A T	T <mark>C T G T A A C</mark> G	T T T <mark>A G A</mark> T T T <mark>G G </mark> A	<mark>A</mark> T T T T T <mark>A A T</mark>	T T T T T <mark>A T </mark> G T <mark>C</mark> 1	r <mark>a</mark> t <mark>g</mark> t <mark>a</mark>
	29	90	300	310	320	330	340	350
kuk.2d	TGTTTTAAA	GTAACAA		TAAAGTATT	G C T T C A T G A T A C	A C A G T A A A T I	TTCGTGCAGG	A A T T T
kuk.1d	TGTTTTAAA	GTAACAA	T C C G C A T	TAAAGTATT	G <mark>CTTCATGATA</mark>	ACAGTAAAT	TTCGTGCAGG	A A T T T
KUK49_final	TGTTTTAAAA	GTAACAA	TCCGCAT	TAAAGTATT	G <mark>CTTCAT</mark> G <mark>ATA</mark> C	ACAGTAAAT		A A T T T
consensus	TGTTTTAAAA	GTAACAA		TAAAGTATT	GCTTCATGATAC			

		360		370		380		39	90		400		4	410		420
kuk.2d	CCAAGAA	T G T G G A	G <mark>A</mark> T T	тстст	G <mark>A C</mark> A	T T <mark>G</mark> T T	GCCC	T <mark>A</mark> T T	G <mark>G T</mark> A	AAT	TMGA	G <mark>T C</mark> G	T <mark>A A</mark> G	A A G T	G <mark>A</mark> G <mark>A</mark>	T T G C
kuk.1d	C C A A G A A	T G T G G <mark>A</mark>	G <mark>A</mark> T T	тстст	G <mark>A C</mark> A	T T <mark>G</mark> T T	GCCC	T <mark>A</mark> T T	G <mark>T</mark> A	A A T	TMGA	G <mark>T C</mark> G	T <mark>A</mark> A G	A A G T	G <mark>A</mark> G <mark>A</mark>	T T G <mark>C</mark>
KUK49_final	CCAAGAA	T G T G G <mark>A</mark>	G <mark>A</mark> T T	ТСТСТ	G <mark>A C</mark> A	T T <mark>G</mark> T T	GCCC	T <mark>A</mark> T T	G <mark>T</mark> A	A A T	T <mark>A</mark> G A	G <mark>T C</mark> G	T <mark>A </mark> A G	A A G T	G <mark>A</mark> G <mark>A</mark>	T T G C
consensus	CCAAGAA	TGTGGA	G A T T	тстст	GACA	T T G T T	GCCC	T <mark>A</mark> T T	G G T A	AAT		GTCG	T <mark>A A</mark> G		GAGA	TTGC
		430		440		450		46	50		470		4	180		490
kuk.2d	<mark>A G T A A </mark> T T	G <mark>A C</mark> G A A	ТТТТ	G T T G T	СТСТ	AGGG <mark>C</mark>	CGTG	T T <mark>G</mark> T	A A G C	G <mark>A</mark> T	A T A T A	A T G T	<mark>A A</mark> T G	G <mark>A</mark> CG	GGA	A T G T
kuk.1d	<mark>A G T A A </mark> T T	G <mark>A C</mark> G A A	ТТТТ	G T T G T	СТСТ	AGGG <mark>C</mark>	CGTG	T T <mark>G</mark> T	A A G C	G <mark>A</mark> T	A T A T A	A T G T	<mark>A A</mark> T G	G <mark>A</mark> CG	GGA	A T G T
KUK49_final	AGTAATT	G <mark>A</mark> CGAA	ТТТТ	GTTGT	CTCT	A G G G <mark>C</mark>	C G T G	T T <mark>G</mark> T	A A G C	G <mark>A</mark> T	<mark>A T A</mark> T <mark>/</mark>	A T <mark>G</mark> T	A <mark>A</mark> T G	G <mark>A</mark> CG	C G G <mark>A</mark>	A T <mark>G</mark> T
consensus	A <mark>G</mark> TAATT	G <mark>A C</mark> G <mark>A</mark> A	T T T	G T T G T	СТСТ	A G G G <mark>C</mark>	C G T G	T T <mark>G</mark> T	<mark>A</mark> AGC	G <mark>A</mark> T	<mark>a</mark> t <mark>a</mark> t,	<mark>a</mark> t <mark>g</mark> t	<mark>A A</mark> T G	G <mark>A</mark> CG	GGA	<mark>a</mark> t <mark>g</mark> t
		500		510		520		53	80		540			550		560
kuk.2d	GTTGGTT		GAAC	ТСТТТ	СКАА	ATAGA	TTGA	TTTG	AGTG	ТСТ		GTTT		TCTT		ATCG
kuk.1d	GTTGGTT		GAAC	ТСТТТ		ATAGA	TTGA	TTTG	AGTG	ТСТ		GTTT		TCTT		
KUK49_final	GTTGGTT	AGATGT	GAAC	ТСТТТ		ATAGA	TTGA	TITITG	AGTG	TCT		GTTT		TCTT		ATCG
consensus	GTTGGTT	A G <mark>A T</mark> G T	G <mark>A A C</mark>	т с т т т	C T A A	ATAGA	T T G A	T T T G	<mark>a</mark> g <mark>t</mark> g	T <mark>C</mark> T	A G A T	GTTT	A <mark>A</mark> A T	ТСТТ	C C T T ,	A <mark>T C</mark> G
		570		500		500		<i></i>			610					630
						590					610					630
KUK.20																
KUK.IO																
KUK49_IIIIai	CGAAGA		GICI		ACGI	GAGA	AGTC									GIGA
consensus	C G A A G A T	ТТТТТТ	GTCT	GGCGT	ACGT	TGAGA	AGTC	T G <mark>C</mark> A	A A T A	GTT	ТТСТ	A <mark>A</mark> AT	TTTG	ATTT	T <mark>AAA</mark>	G <mark>T</mark> G <mark>A</mark>
		640		650		660		67	70		680		4	590		700
kuk 2d						TACCT			ТСТТ			┍│┯│┯│┯				
kuk 1d									тстт							
KLIK49 final									ТСТТ			·····				
consensus																
00110011000			GGAC			ACG	GAAC	ACCA	6		6616			GAAG		

		710	720	730	740	750	760	770
kuk.2d	A T A A T T T	T T G G T <mark>A</mark> G T <mark>C</mark> (	G T G A T G G T A <mark>C</mark>	Т <mark>С</mark> ТТТТ <mark>С</mark> ТТ	CCTCGAACAAA	Y A G T A A A A C A	T <mark>G T T A A T</mark> A	AAAGC
kuk.1d	<mark>a t a a</mark> t t t	Т Т G G <mark>Т А</mark> G <mark>Т С</mark> (	G T G <mark>A T</mark> G G T <mark>A C</mark>	Т <mark>С</mark> Т Т Т Т <mark>С</mark> Т Т		Y A G T A A A A C A <sup>-</sup>	T T G T T A A T A	A A A G <mark>C</mark>
KUK49_final	ATAATTT	TTGGT <mark>A</mark> GT <mark>C</mark>	G T G A T G G T <mark>A C</mark>	T <mark>C</mark> T T T T <mark>G</mark> T T		TAGTAAAACA <sup>-</sup>	T G T T A A T A	AAAGC
consensus	A T A A T T T	T T G G T <mark>A</mark> G T <mark>C</mark> (	6 T G A T G G T <mark>A C</mark>	T <mark>C</mark> T T T T <mark>G</mark> T T	CCTCGAACAAA	T A G T A A A A C A	T <mark>G T T A A T</mark> A	AAAGC
		780	790	800	810	820	830	840
kuk.2d								
kuk.1d								
KUK49_final								GGGGA
consensus	ATACCTT		GTAGGTCGA	T <mark>AAAAAACC</mark> A		GTGGG <mark>CAAT</mark> AG		GGTGA
		850	860	870	880	890	900	910
kuk 2d	GATATTT							
kuk.1d		GATAATGAG						ТТТСС
KUK49 final	GATATTT	GATAATGAG						ТТТСС
consensus								
		920	930	940	950	960	970	980
kuk.2d	AGTTTGA	G <mark>A T A A T A T C</mark> /	T G T C G A C A T	<mark>A T A A C G A T T</mark>	<b>C</b> GGTTTCTC	TCGTTGAGGC	G T T G G <mark>C</mark> G <mark>A</mark>	САТСТ
kuk.1d	AGTTTGA	G <mark>A T A A T A T C</mark> /	A T G T C G A C A T	<mark>A T A A <mark>C</mark> G A T T</mark>	<mark>С</mark> GGTTT <mark>С</mark> ТТТ	TCGTTGAGGC	G T T G G <mark>C</mark> G <mark>A</mark>	C A T C T
KUK49_final	AGTTTGA	G <mark>ATAATATC</mark>	A T G T <mark>C G A C</mark> A T	A T A A <mark>C</mark> G A T T	CGGTTTCTC	TCGTTGAGGC	G T T G G <mark>C</mark> G <mark>A</mark>	C A T C T
consensus	AGTTTGA	G <mark>A T A A T A T C</mark> /	A T G T <mark>C G A C A</mark> T	<mark>A T A A C G A</mark> T T	С	TCGTTGAGGC	G T T G G <mark>C</mark> G A	C A T C T
		990	1000	1010	1020	1030	1040	1050
kuk.2d	TCAGCCT	TT <mark>ATC</mark> GG <mark>AC</mark>		TATGTAGTG	GCTGAGATTT	ATTTTTCGGC		AAATA
kuk.1d		TTATCGGAC		TATGTAGTG	GCTGAGATTT	ATTTTTCGGC		AAATA
KUK49_final			I G C A G T T T G			ATTTTTCGGC		AAATA
consensus	TCAGCCT	ΤΤΑΤ <mark>Ο</mark> ΘΘΑ <mark>Ο</mark> Ι	T G <mark>C A</mark> G T T T G	ΤΑΤGΤΑGΤG	GCTGAGATTTA	ATTTTTCGGC/	ATGGCAGAC	ΑΑΤΑ

		1060	1070	1080	1090	1100	1110	1120
kuk.2d	СССТСТС	C G <mark>A T T C G C</mark> T T T	G <mark>AA</mark> TTTTT	5 <mark>C</mark> A T T T T T G <mark>A</mark>	<b>G T T T G A A G G <mark>C</mark></b>	AGAA <mark>C</mark> TAATG	T T <mark>A T T T G A T G</mark>	G T T T
kuk.1d	СССТСТС	G <mark>A T T C G C</mark> T T T	G <mark>AA</mark> TTTTT	5 <mark>C</mark> A T T T T T G A	G T T T G A A G G <mark>C</mark>	A G A A <mark>C</mark> T A A T G	T T <mark>A T T T G A T G</mark>	G T T T
KUK49_final	CCGTGTC	G <mark>A T T C G C</mark> T T T	G <mark>AA</mark> TTTTT	5 <mark>C</mark> A T T T T T G A	G T T T G A A G G <mark>C</mark>	AGAAACTAATG	T T <mark>A T T T G A T G</mark>	G T T T
consensus			GAATTTTT	G <mark>C</mark> ATTTTGA	GTTTGAAGGC	AGAAACTAATG	TTATTTGATG	GTTT
		1130	1140	1150	1160	1170	1180	1190
kuk.2d	<mark>a</mark> t <mark>a</mark> ttt <mark>a</mark>	A <mark>C A A T A A T C G</mark>	G A A A G A T T A	A A T G T T <mark>C A</mark> C	T G G G <mark>A C T T C</mark> T	A <mark>C</mark> ATAGGATAC	T A A A A A T G A <mark>C</mark>	G T T G
kuk.1d	<mark>a</mark> tatta	A <mark>C</mark> A A T A A T <mark>C</mark> G	G A A A G A T T A	A A T G T T <mark>C A</mark> C	T G G G <mark>A C T T C</mark> T	A <mark>C</mark> A T A G G A T A C	ΤΑΑΑΑΑΤGΑC	G <mark>T T</mark> G
KUK49_final	<mark>a</mark> tatta	A <mark>C A A T A A T C</mark> G	GAAAGATT	A A T G T T <mark>C A</mark> C	T G G G <mark>A C T T C T</mark>	A <mark>CATA</mark> GGA <mark>TAC</mark>	T <mark>AAAAATGAC</mark>	G T T G
consensus	A T A T T T A	A <mark>C A A T A A T C </mark> G	GAAAGATT	AATGTTCAC	T G G G <mark>A C T T C T</mark>	A C A T A G G A T A C	ΤΑΑΑΑΑΤGΑC	G T T G
		1200	1210	1220	1230	1240	1250	1260
kuk.2d	G C G G C A T	F <mark>C</mark> GTATTCAGT	CGCAAACAC	<mark>T A C</mark> G <mark>A T</mark> G G T	C <mark>A</mark> TTGTGTAG	A G <mark>C T C</mark> T G T T G A	T T <mark>A C</mark> A G A T G A	A T T T
kuk.1d	G C G G C A T	「 <mark>С</mark> G T <mark>A T T C A </mark> G T	CGCAAACAC	T <mark>ACGAT</mark> GGT	C A T T G T G T A G	AGCTCTGTTGA	T T A C A G A T G A	A T T T
KUK49_final	G <mark>C</mark> G <mark>GC</mark> AT	C <mark>GTATTCA</mark> GT	CGCAAACA	T <mark>ACGAT</mark> GGT		AGCTCTGTTGA	TTACAGATGA	ATTT
consensus	G <mark>C</mark> G G <mark>C A</mark> T	T <mark>C</mark> GTATT <mark>C</mark> AGT	CGCAAACA	<mark>T A C</mark> G <mark>A T</mark> G G T	C <mark>A</mark> TTGTGTAG	A G <mark>C T C</mark> T G T T G A	T T <mark>A C</mark> A G A T G A	<mark>a</mark> tt T
		1270	1280	1290	1300	1310	1320	1330
kuk.2d	AGCTTT	AAATGCCCTT		G G G T G T T A T G	GGGAAATTGTT	TTCGACGATTA		G <mark>A C A</mark>
kuk.1d	AGCTTT			G G T G T T A T G	G G A A A T T G T T	T T C G A C G A T T A	ΤĊΤΤΓĠΤΤΓΓĠ	G A C A
KUK49_final	AGCITIA			GGG I G I I <mark>A</mark> I G	GGAAA			GACA
consensus	AGCTTT	A A T <mark>G C C C T T </mark> A		GGGTGTAT	GG <mark>AAATT</mark> G <mark>TT</mark>	TTCGACGATTA	T <mark>C</mark> TTTGTG	G <mark>A C</mark> A
		1240	1250	1200	1270	1200	1300	1400
kuk 0d								
kuk 1d								· · · ·
KUK49 final								
consensus								
00113011303	GCCGACI		AIGAAAIG			AGAGACCGAAG		

APPENDIX D. MULTIPLE ALIGNMENT FOR KUK A49
	1410	1420	1430	1440	1450	1460	1470
kuk.2d	<mark>C</mark> T G G <mark>A T A T G A C</mark> G T	ATAAAGATTT	T <mark>G G A G T T T A</mark> T	C A G A A T G A T A	T T <mark>A T A A G T C</mark> A '	F <mark>C</mark> AAAAAGAC <i>A</i>	A <mark>C</mark> G G <mark>A A A</mark>
kuk.1d	<mark>C</mark> T G G <mark>A T A T G A C</mark> G T	ATAAAGATTT	T <mark>G G A G T T T A</mark> T	CAGAATGATA	T T <mark>A T A A G T C</mark> A '	T <mark>C</mark> AAAAAGAC <i>A</i>	A <mark>C</mark> G G <mark>A A A</mark>
KUK49_final	<mark>C T</mark> G G <mark>A T A T</mark> G <mark>A C</mark> G T	ATAAAGATTT	T <mark>G G A G T T T A</mark> T	C A G A A T G A T A	T T <mark>A T A A G T C</mark> A '	T <mark>C</mark> AAAAAGACA	A <mark>C</mark> G G <mark>A A A</mark>
consensus	C T G G A T A T G A C G T	ATAAAGATTT	TGGAGTTTAT	CAGAATGATA	ΤΤΑΤΑΑΓΤΟΑ		
	1480	1490	1500	1510	1520	1530	1540
kuk.2d	C T A T G A A A C T C T	G T T A G G C T T A	T T <mark>A C C </mark> A A T G T	A C A A A A A A A C	T <mark>AAGCTC</mark> AGG	<mark>A T A C</mark> GG T T A T	G G <mark>A</mark> G <mark>C</mark> G
kuk.1d	C T A T G A A A C T C T	G T T A G G C T T A	T T <mark>A C C</mark> A A T G T	ACAAAAAAA	T <mark>A A G C T C</mark> A G G (	<mark>A T A C</mark> GG T T A T	G G <mark>A</mark> G <mark>C</mark> G
KUK49_final	C T A T G A A A C T C T	G T T A G G C T T A	TT <mark>ACCAA</mark> TGT	A C A A A A A A A C	T <mark>AAGCTC</mark> AGG	C <mark>ATAC</mark> GGTTA	G G <mark>A</mark> G <mark>C</mark> G
consensus	C T A T G A A A C T C T	G T T A G G C T T A	T T <mark>A C C A A T G</mark> T	ACAAAAAA	TAAGCTCAGG	C <mark>ATAC</mark> GGTTA	G G <mark>A </mark> G <mark>C</mark> G
	1550	1560	1570	1580	1590	1600	1610
kuk.2d	T T <mark>G T T T A T C</mark> T <mark>A A</mark> T	Τ <mark>ΟΤΟΓΑΑ</mark> ΟΟ	A C G T T M A A G A	T G C G C T T T G T	G <mark>T A G A G C</mark> T T A T	T G A A A G <mark>C T</mark> G A A	ΑΑΑΑΤΑ
kuk.1d	T T <mark>G T T T A T C</mark> T A <mark>A</mark> T	T G T C C T A A C C	A C G T T M A A G A	T G C G C T T T G T	G T A G A G <mark>C</mark> T T A	T G A A A G <mark>C T</mark> G A A	AAAATA
KUK49_final	T T G T T T A T C T A A T	T G T C C T A A C C	ACGTTAAAGA		G T A G A G <mark>C</mark> T T A	TGAAAG <mark>C</mark> TGAA	AAAAA
consensus	T T <mark>G</mark> T T T <mark>A T C</mark> T <mark>A A</mark> T	TGTCCTAACC	ACGTTAAAGA	T G C G C T T T G T	G T A G A G <mark>C</mark> T T A <sup>-</sup>	T G A A A G <mark>C T</mark> G A A	A A A A T A
	1620	1630	1640	1650	1660	1670	1680
kuk.2d	TTACAAACGATGG		T A T G A A A A C T	TTAATTGGCCG	AATTTTCCAT	G T G <mark>C A C C</mark> G A T A	
kuk.1d	TTACAAACGATGG	ATGTCGTTTT	T A T G A A A A C T	TTTAATTGGCCG	AATTTTCCAT	G T G <mark>C A C C</mark> G A T A	
KUK49_final		ATGTCGTTTT				G T G C A C C G A T A	
consensus	TTACAAACGATGG	A T G T C G T T T T	ΤΑΤΓΑΑΑΑΤ	TT <mark>AATT</mark> G <mark>GC</mark> G	AATTTT <mark>CC</mark> AT	G <mark>TGCACC</mark> GATA	
	1600	1700	1710	1770	1720	1740	1750
KUK.20							
KUK. IQ							
KUK49_TINAI							
consensus	ATCAGTTGCTTAA	CAAATTCGCT	ACAGATCAGT		C G <mark>A T</mark> G <mark>T C</mark> G <mark>A</mark> G	AAAATAAACAC	TCTCAT

		1760	1770	1780	1790	1800	1810	1820
kuk.2d	AGAGAT	T G <mark>A T</mark> G G <mark>C</mark> G ,	ΑΑΑΖΑΓΑΑ	A C G T C T T T T	<mark>A G T C</mark> G <mark>A G T T</mark> G G	ΑΤ <mark>ϹϹ<mark>Ͼ</mark>ΑΤΤϹΤ</mark>	CCATGAGGAG	A C G G G A
kuk.1d	AGAGAT	T G <mark>A T</mark> G G <mark>C</mark> G	ΑΑΑΖΑΓΑΑ	A C G T C T T T T	<mark>A G T C</mark> G <mark>A </mark> G T T G G	<mark>A T C C G A T T C</mark> T	CCATGAGGAG	A C G G G A
KUK49_final	AGAGAT	T	ΑΑΑΖΑΓΑΑ	4 <mark>C G T C</mark> T T T T	<mark>A G T C</mark> G <mark>A</mark> G T T G G	<mark>A T C C G A T T C</mark> T	CCATGAGGAG	A C G G G A
consensus	AGAGAT				AGTCGAGTTGG	ATCCGATTCT		ACGGGA
		1830	1840	1850	1860	1870	1880	1890
kuk.2d	T T <mark>A</mark> T <mark>A</mark> T	C <mark>AAGCGCT</mark>	G <mark>CCAAATGTT</mark>	5 T G <mark>A C</mark> G G A A	G <mark>C T C C</mark> A A G T G A	AAAGGTTAAA	T C G A T A C G C G	T T G <mark>A</mark> G T
kuk.1d	T T <mark>A</mark> T <mark>A</mark> T		G <mark>CCAAATGTT</mark>	5 T G <mark>A C</mark> G G <mark>A A</mark>	G <mark>C T C C </mark> A A G T G A	AAAGGTTAAA	TCGATACGCG	T T <mark>G A</mark> G T
KUK49_final	T T <mark>A</mark> T <mark>A</mark> T	C <mark>AAGCGCT</mark>	G <mark>CCAAA</mark> TGTT	5 <mark>T</mark> G <mark>A C</mark> G G <mark>A A</mark>	G <mark>CTCC</mark> AAGTGA	AAGGTTAAA	TCGATACGCG	T T G <mark>A</mark> G T
consensus	ΤΤΑΤΑΤ		GCCAAATGTT	GTGACGGAA	GCTCCAAGTGA	AAGGTTAAA	TCGATACGCG	TTGAGT
		1900	1910	1920	1930	1940	1950	1960
kuk.2d	C T G A A G (	G T G <mark>A</mark> G <mark>A</mark> G T (	G T <mark>A T A G</mark> T T <mark>A A</mark> G	5 <mark>C</mark> G T T <mark>A</mark> T T G	G <mark>AGGC</mark> GG <mark>CAT</mark> A	A T G A A A <mark>C</mark> A A G	AAGAAGAAT	T G G <mark>C</mark> G T
kuk.1d	C T G A A G	G T G <mark>A</mark> G <mark>A</mark> G T (	5 T <mark>A T A G T T A A</mark> G	5 <mark>C</mark> G T T <mark>A</mark> T T G	G <mark>A</mark> GG <mark>C</mark> GG <mark>CAT</mark> A	A T G A A A <mark>C</mark> A A G	AAGAAGAAT	T G G <mark>C</mark> G T
KUK49_final		G T G <mark>A</mark> G <mark>A</mark> G T (	G T <mark>A T A G</mark> T T <mark>A A</mark> G	5 <mark>C</mark> G T T <mark>A</mark> T T G	G <mark>A</mark> GG <mark>C</mark> GG <mark>CATA</mark>	A T G A A A C A A G	AAGAAGAAT	T G G <mark>C</mark> G T
consensus		GTGAGAGT	G T A T A G T T A A C	G C G T T A T T G	GAGGCGGCATA	ATGAAACAAG	AAGAAGAAT	TGGCGT
		1970	1980	1990	2000	2010	2020	2030
kuk.2d	C T A G T A	CCTGTAGC	<mark>C</mark> GGTTT <mark>A</mark> TTG	C G T T C A G	G <mark>CATTAAAC</mark> TG	A G G <mark>C</mark> A A A A A T	G T C C G C A A G T	ΑΑΑΑΤ
kuk.1d	CTAGTA	<mark>С С Т</mark> G <mark>Т А</mark> G <mark>С</mark> (	<mark>C</mark> G G T T T <mark>A</mark> T T G G	<mark>ACGTTCA</mark> G	G <mark>CATTAAACT</mark> G	A G G <mark>C</mark> A A A A A T	G T C C G C A A G T	ΑΑΑΑΤ
KUK49_final	C T A G T A		GGTTTATTG		G <mark>CATT</mark> AAA <mark>C</mark> TG	A G G <mark>C</mark> A A A A A T	G <mark>TCCGCAA</mark> GT	AAAAT
consensus	C T A G T A	CCTGTAGC	<mark>C</mark> GGTTTATGG	C G T T C A G	G <mark>CATTAAAC</mark> TG	AGGCAAAAAT	G T C C G C <mark>A A</mark> G T	
		2040	2050	2060	2070	2080	2090	2100
kuk.2d	TATCCT	S G <mark>C</mark> G <mark>A T</mark> G <mark>C</mark> /	A G A T G <mark>C C</mark> T G T A	<mark>\</mark> GGG <mark>ATGTA</mark>	G <mark>ATAGTTTTG</mark> C	G T G T G G <mark>A</mark> G T T	GAAGATCTTT	<mark>A</mark> TGGAG
kuk.1d	T <mark>A T C C T</mark> (	G <mark>C</mark> G <mark>A T</mark> G <mark>C</mark> /	A G A T G <mark>C C</mark> T G T A	<mark>\</mark> GGG <mark>AT</mark> G <mark>TA</mark>	G <mark>ATAGTTTTG</mark> C	G T G T G G <mark>A</mark> G T T	GAAGATCTTT	<mark>A</mark> TGG <mark>A</mark> G
KUK49_final	T <mark>A T C C</mark> T (	G <mark>C</mark> G <mark>AT</mark> G <mark>C</mark> /	A G A T G <mark>C C T</mark> G T A	AGGG <mark>ATGT</mark> A	GATAGTTTTGC	G <mark>T</mark> G <mark>T</mark> G <mark>A </mark> G T T	GAAGATCTTT	<mark>A</mark> TGGAG
consensus	TATCCT		AGATGCCTGT		GATAGTTTTGC	GTGTGGAGTT	GAAGATCTTT	ATGGAG

	2110	2120	2130	2140	2150	2160	2170
kuk.2d	G G <mark>A A T T </mark> G T <mark>G T </mark> C T T T G T	T <mark>C C A</mark> G <mark>A</mark> T G <sup>-</sup>	T <mark>A</mark> GGTTT <mark>C</mark> GAT	CAATAAGCC	G <mark>A</mark> GTTTGG <mark>C</mark> AA <sup>-</sup>	ГТТ <mark>С</mark> ТТТТ	G <mark>A</mark> GG <mark>AA</mark>
kuk.1d	G G <mark>A A T T G T G T C</mark> T T T G T	T <mark>C C A</mark> G <mark>A</mark> T G <sup>-</sup>	T <mark>A</mark> G G T T T <mark>C</mark> G A T	C A A T A A G C C	G <mark>A</mark> G T T T G G <mark>C</mark> A A <sup>-</sup>	T T T <mark>C</mark> T T T T	G <mark>A </mark> G G <mark>A A</mark>
KUK49_final	G G <mark>A A T T G T G T C</mark> T T T G T	T <mark>C C A</mark> G <mark>A</mark> T G	T <mark>A </mark> G <mark>G T T T <mark>C</mark> G <mark>A</mark> T</mark>	CAATAAGCC	G <mark>A</mark> G T T T G G <mark>C</mark> A A <sup>-</sup>	Г Т Т <mark>С</mark> Т Т Т Т	G <mark>A</mark> GG <mark>AA</mark>
consensus	G G <mark>A A T T G T G T C</mark> T T T G T	ΤϹϹΑGΑΤG	T <mark>A </mark> G <mark>G T T T C</mark> G A T	CAATAAGCC	G <mark>A </mark> G T T T G G <mark>C</mark> A A <sup>-</sup>	Г Т Т <mark>С</mark> Т Т Т Т	GAGGGAA
	2180	2190	2200	2210	2220	2230	2240
kuk.2d	T C A T G A G A G C C T T C A G	T T <mark>C A</mark> T <mark>C</mark> G G	C G <mark>C A A A C G T A</mark> C	A T G G <mark>C</mark> G <mark>T C</mark> G	A A G T A T T G T A T C	G T <mark>A T A G C</mark>	T G T T <mark>C</mark> G G
kuk.1d	T C A T G A G A G C C T T C A G	T T <mark>C A</mark> T <mark>C</mark> G G G	C G <mark>C A A A C G T A</mark> C	<mark>. A T </mark> G G <mark>C </mark> G <mark>T C</mark> G	A A G T A T T G T A T C	G T <mark>A T A G C</mark>	T G T T <mark>C</mark> G G
KUK49_final	TCATGAGAGCCTTCAG	TTCATCGG	CIGI <mark>CIAIAICIGIT</mark> AIC	<mark>ΑΤ</mark> G <mark>G</mark> C <mark>GΤC</mark> G	AAGTATTGTAT	GTATAGC	T G T T <mark>C</mark> G G
consensus	T C A T G A G A G C C T T C A G	T T <mark>C A</mark> T <mark>C</mark> G G	C G <mark>C A A A C G T A</mark> C	<mark>ATGGCGTC</mark> G	A A G T A T T G T A T C	G T A T A G C	T G T T <mark>C</mark> G G
	2250	2260	2270	2280	2290	2300	2310
kuk.2d	TTTAGCTATAAAATTCC	CTGCATGC	T T T T <mark>A G A T G G A</mark>			ACAGATT	GATCATC
kuk.1d							GATCATC
KUK49_final							GATCATC
consensus	TTTT <mark>AGCTAT</mark> AAATTCC		TTTT <mark>AGAT</mark> GG <mark>A</mark>		A <mark>TTC</mark> GGGGGGGGG	G <mark>AC</mark> AGATT	G <mark>ATCAT</mark> C
	7270	7220	7240	7750	7760	2270	2200
kuk 2d							
kuk 1d							
KUK49 final							
consensus							
conconcuc				GATTGAGAA			
	2390	2400	2410	2420	2430	2440	2450
kuk.2d	GAGAGTGCTCGGATAM	GTTCATTM	G A A A A A C T G A A	ATGTCGGTT	T C G T C G G T T A T	ATTTCAG	T G <mark>A C G C A</mark>
kuk.1d	GAGAGTGCTCGGATAM	G T T C A T T M (	G A A A A C T G A A	ATGTCGGTT	Т <mark>С </mark>		T G <mark>A C G C A</mark>
KUK49_final	GAGAGTGCTCGGATAA	GTTCATTA	GAAAAACTGAA	ATGTCGGTT	Т <mark>С </mark>		T G <mark>A C G C A</mark>
consensus	GAGAGTGCTCGGATAA	GTTCATTA	GAAAAACTGAA	ATGTCGGTT			TGACGCA

	2460	2470	2480	2490	2500	2510	2520
kuk.2d	TTCGCAGAAKACGT	с с т с с т с т с	G <mark>CC</mark> AG <mark>T</mark> AATA	ACTCCGAAGA	ΑΑϹϹΑΤΝΤΓΟ	G <mark>AT</mark> G <mark>TAC</mark> AGG	GAAATA
kuk.1d	ΤΤϹΓΟΑΓΑΑΚΑΟΓ	С	G C C A G T A A T A	ACTCCGAAGA	A A C C A T N T G G	G <mark>AT</mark> G <mark>TAC</mark> AGG	GAAATA
KUK49_final	T T C G C A G A A T A C G T (	<mark>с                                    </mark>	G <mark>C C A</mark> G <mark>T A A T A</mark>	A C T C C G A A G A A	A A C C A T C T G G	G <mark>AT</mark> G <mark>TAC</mark> AGG	GAAATA
consensus	TTCGCAGAATACGT					GATGTACAGG	GAAATA
	2530	2540	2550	2560	2570	2580	2590
kuk.2d	T <mark>C</mark> T <mark>A G</mark> T T T T <mark>A C</mark> G A T	T <mark>C G A A C T T</mark> G A	TCGTGCGGCA	CCCATATGAG'	ТТ <mark>СТС</mark> Т <mark>А</mark> ТТТ	ΑϹGAGTGTGA	T T T <mark>T </mark> G T
kuk.1d	T C T A G T T T T A C G A T	T <mark>C </mark> G <mark>A A C T T</mark> G <mark>A</mark>	TCGTGCGGCA	CCCATATGAG	Т <mark>Т <mark>С</mark> Т <mark>С</mark> Т <mark>А</mark> Т Т Т</mark>	A C G A G T G T G A	T T T T <mark>G</mark> T
KUK49_final	T <mark>C</mark> T <mark>AG</mark> TTTT <mark>AC</mark> GAT	T <mark>C</mark> G <mark>AAC</mark> TTGA	TCGTGCGGCA		Τ <mark>Τ<mark>Ϲ</mark>Τ<mark>Ϲ</mark>Τ<mark>Α</mark>ΤΤΤ</mark>	ACGAGTGTGA	TTTTGT
consensus	T C T A G T T T T A C G A T	T <mark>C</mark> G <mark>AAC</mark> TTGA	<b>TCGTGCGGC</b> A	CCCATATGAG	Т <mark>ТСТС</mark> Т <mark>А</mark> ТТТ	ACGAGTGTGA	T T T <mark>T </mark> G T
	2600	2610	2620	2630	2640	2650	2660
kuk.2d	TGGTGTCCATAAAA	ACGTCCATTT	T A C T A T T C C T	G T G <mark>A</mark> T G G <mark>C</mark> T T A	A C T A A A A T A G		
kuk.1d	T G G T G T C C A T A A A A	ACGTCCATTT		G T G <mark>A</mark> T G G <mark>C</mark> T T <i>I</i>	ACTAAAATAG		
KUK49_final	TGGTGTCCATAAAA	ACGTCCATTT		GTGATGGCTT	ACTAAAATAG		CGAAA
consensus	T G G T G T C C A T A A A A	Α <mark>Ϲ<mark>Ϭ</mark>Τ<mark>ϹϹ</mark>ΑΤΤΤ</mark>	Τ <mark>Α</mark> ϹΤΑΤΤ <mark>ϹϹ</mark> Τ	G T G A T G G <mark>C</mark> T T A	A <mark>CTAAAAT</mark> AG		CGAAAT
	2670	2680	2690	2700	2710	2720	2730
kuk.2d					AGAAAGTTAC		
KUK.10					A G A A A G T T A C		
KUK49_final							
consensus	ACAAGTGTCCGTAT	ΤΤΓ <mark>Γ</mark> ΑΤΤΤΓΓ			A G A A A G <mark> T T </mark> A <mark> C</mark>	<b>T</b> G G <b>T</b> T G <mark>C C</mark> G	TGTTTC
	2740	2750	2760	2770	2780	2700	2800
kuk 2d							
kuk 1d							
KUK49 final							
consensus							
00110011000							

		2810	2820	2830	2840	2850	2860	2870
kuk.2d	G <mark>C</mark> GG <mark>AA</mark> T	A C C N A G A A G C	G G G <mark>A T T</mark> G G G	C G T T G A T A A G	A G G T T A G T T	<mark>C                                    </mark>	<mark>, a a </mark> t t t t t t <mark>a a</mark>	ATGA
kuk.1d	G <mark>C</mark> GG <mark>AA</mark> T	ACCNAGAAG	<b>.</b>	C G T T G A T A A O	A G G T T A G T T	<mark>C   G   T   T   T   T   C</mark> K <mark>A   G</mark> A	<mark>, A A</mark> T T T T T T A A	A T G A
KUK49_final	G <mark>C</mark> GG <mark>AA</mark> T	ACCTAGAAGO	<b>_</b>	C G T T G A T A A G	<mark>A G G T T A G T T</mark>	<mark>C</mark> G T T T T <mark>C</mark> T <mark>A</mark> G <mark>A</mark>	A A T T T T T T A A	A T G A
consensus	G C G G A A T	ACC AGAAGO	G G G <mark>A T T</mark> G G G	CGTTGATAA	AGGTTAGTT	<mark>C G T T T T C T A G</mark> A		ATGA
		2880	2890	2900	2910	2920	2930	2940
kuk.2d	T T T C G C A	TTTACAGCC	G C C A C A C G T		G T C T T C A G A	T <mark>A T G T C</mark> T G T <mark>A</mark> G	TCCAAAGTTC	A T T G
kuk.1d	T T T C G C A	TTTACAGCC	G C C A C A C G T		G T C T T C A G A	T <mark>A T G T C</mark> T G T <mark>A</mark> G		A T T G
KUK49_final			GCCACACGT		GTCTTCAGA	TATGTCTGTAG		ATTG
consensus	T T T C G C A	TTTACAGCC	G <mark>CCACAC</mark> GT	<mark>Ϲ</mark> Ⴚ <mark>Ⴀ</mark> ႺႠႨႨႨ	GTCTTCAGA	T <mark>ATGTC</mark> TGTAG	TCCAAAGTTC	<mark>A</mark> TTG
		2050	2000	2070	2000	2000	2000	2010
								3010
KUK.20								
KUK40 final								
COnsensus								
consensus			AIGIA <mark>ICI</mark> GIAIAIAIAIA					GIIII
		3020	3030	3040	3050	3060	3070	3080
kuk.2d	CGGAGAT		ATTGCGCTG			GAGAGCTGTCT	GCTGTTAATC	ACGT
kuk.1d	C G G A G A T		A T T G C G C T G	TACATTTT		G <mark>A G A G C T G T C</mark> T	GCTGTTAATC	ACGT
KUK49_final	C G G A G A T	C T T T T C T G A A	A T T G C G C T G	ТАСАТТТТТ	C A G G T G A C A	G <mark>AGAGC</mark> TGTCT	GCTGTTAATC	ACGT
consensus			ATTGCGCTG			GAGAGCTGTCT	GCTGTTAATC	ACGT
		3090	3100	3110	3120	3130	3140	3150
kuk.2d	C G <mark>A C </mark> G C G	A A G <mark>A C T</mark> A G T 1	GAAAATAAC	G <mark>CTTAAGA</mark> T	AGTTGTAAG	A A A G C G C A A G	G <mark>C</mark> AAAG <mark>C</mark> TTA	T <mark>A</mark> T G
kuk.1d	C G <mark>A C </mark> G C G	A A G A C T A G T 1	GAAAATAAC	G <mark>CTTAAGA</mark> T	AGTTGTAAG	AAAGCGCAA	G <mark>C</mark> AAAGCTTA	T <mark>A</mark> T G
KUK49_final		AAGA <mark>CT</mark> AG <mark>T</mark> 1	GAAAATAAC	GCTTAAGAT	AGTTGTAAG	AAAGCGCAA	GCAAAGCTTA	T <mark>A</mark> T G
consensus	C G <mark>A C </mark> G C G	i <mark>A A G A C T A </mark> G T 1	GAAAATAAC	G <mark>C</mark> TT <mark>AA</mark> GAT0	A G T T G T A A G	AAAG <mark>C</mark> G <mark>C</mark> AAG	G <mark>C</mark> AAAG <mark>C</mark> TTA	T <mark>A</mark> T G

	3160	3170	3180	3190	3200	3210	3220
kuk.2d	ТСАСТСТССССССССС	Т Т Т Т <mark>А С</mark> А Т Т	<mark>C</mark> T T G T T T T T G	<mark>Ϲ Ͼ Ͼ Ϲ Ͼ Ϲ ͳ Α Ϲ</mark> Ͼ	G A A G T G G A C T	ТТТ <mark>А</mark> ТТ <mark>С</mark> Т <mark>С</mark>	C A G A G T G
kuk.1d	ТСАСТСТСССТС	Т Т Т Т <mark>А С</mark> <mark>А</mark> Т Т	<mark>C</mark> T T <mark>G T T T T T G</mark>	C G G C C G C T A C G	G A A G T G G <mark>A C</mark> T	ТТТ <mark>А</mark> ТТ <mark>С</mark> Т <mark>С</mark>	C A G A G T G
KUK49_final	Т <mark>С </mark> А	Т Т Т Т <mark>А С</mark> А Т Т	<mark>C</mark> T T <mark>G T T T T T G</mark>	<mark>C</mark> G G <mark>C C G C T A C</mark> G	G <mark>A A</mark> G <mark>T</mark> G G <mark>A C</mark> T	ТТТ <mark>А</mark> ТТ <mark>С</mark> Т <mark>С</mark>	<mark>C A</mark> G <mark>A</mark> G <mark>T</mark> G
consensus	TCAGTCTCTGCCTC	ΤΤΤΤΑ <mark>Α</mark> ΟΑΤΤ	сттсттс		GAAGTGGACT	ттт <mark>а</mark> ттстс	
	3230	3240	3250	3260	3270	3280	3290
kuk.2d	C C A T T C G C A K A C C T	A T G A G A T C G	T <mark>C</mark> T T <mark>G A A</mark> T T <mark>C</mark>	A T T T T <mark>C</mark> T T <mark>C</mark> G A	Т <mark>С</mark> Т <mark>СС</mark> ТТТТ	G <mark>ATAAATC</mark> K	K N T T T T <mark>A</mark>
kuk.1d	C C A T T C G C A K A C C T	A T G A G A T C G	T <mark>C</mark> T T <mark>G A A</mark> T T <mark>C</mark>	<mark>A</mark> TTTT <mark>C</mark> TT <mark>C</mark> GA	ͳ <mark>Ϲ</mark> ͳႺ <mark>Ⴚ</mark> ͳͳͳͳ	G <mark>ATAAATC</mark> K	K N T T T T <mark>A</mark>
KUK49_final		A T G A G A T C G	T <mark>C</mark> TT <mark>GAA</mark> TT <mark>C</mark>	<mark>A</mark> TTTT <mark>C</mark> TT <mark>C</mark> GA	ͳ <mark>Ϲ</mark> ͳ <mark>Ⴚ<mark>Ⴚ</mark>ͳͳͳͳ</mark>	G <mark>ATAAATC</mark> T	ΤΤΤΤΤΑ
consensus	C C <mark>A T T C G C A T A</mark> C C T	A T G A G A T C G	T <mark>C</mark> TT <mark>GAA</mark> TT <mark>C</mark>	<mark>A</mark> TTTT <mark>C</mark> TT <mark>C</mark> GA	T <mark>C</mark> TGG <mark>C</mark> TTTT	G <mark>ATAAATC</mark> T	T T T T A
	3300	3310	3320	3330	3340	3350	3360
kuk.2d	T T G T T A T G T T C T T T	<b>C</b> G <mark>C</mark> G <mark>A</mark> T T T T	C T T G A A A T A C	T G G T G T T A T A A	GACTTTTGCT	TCAGAGACG	G T G <mark>A A</mark> G G
kuk.1d	T T G T T A T G T T C T T T		CTTGAAATAC	T G G T G T T <mark>A T A </mark> A	GACTTTTGCT		G T G <mark>A A</mark> G G
KUK49_final	ΤΤΓΓΤΑΤΓΟΤΤΤ		CTTGAAATAC	TGGTGTTATAA	GACTTTTGCT		G <mark>T</mark> G <mark>AA</mark> GG
consensus	T T G T T A T G T T C T T T		<mark>C T T G A A A T A C</mark>	T G G T G T T A T A A	G <mark>ACTTTTGC</mark> T	T C A G A G A C G	G <mark>T</mark> G <mark>AA</mark> GG
	22.70	2200	2200	2400	2410	2420	2420
KUK.20			T <mark>A T T T T A A T T</mark>				
KUK.IO			T A T T T T A A T T				
KUK49_IIIIai							
consensus	G T T A T T A G A T <mark>C</mark> G <mark>C</mark> G		TATTTTAATT				TTTTGT
	3440	3450	3460	3470	3480	3490	3500
kuk 2d							
kuk 1d							
KUK49 final							
consensus							

		3510	3520	3530	3540	3550	3560	3570
kuk.2d	GAGTTT	T K R T T G A A G A	A <mark>C T</mark> G <mark>A</mark> G G <mark>A T</mark>	G <mark>AGCAGC</mark> TT	G G <mark>C </mark> A A <mark>C</mark> G T A	T G <mark>C </mark> A G T T <mark>A T</mark> G T	G <mark>C</mark> TGTGTTG	T T T <mark>A A A</mark>
kuk.1d	GAGTTT	T K R T T G A A G A	A <mark>C</mark> T G A G G A T	G <mark>A G C A G C T T T</mark>	G G <mark>C</mark> A A A <mark>C</mark> G T A	T G <mark>C</mark> A G T T A T G T	G <mark>C</mark> TGTGTTG	T T T <mark>A A A</mark>
KUK49_final	GAGTTT	T T <mark>A T T G A A G A</mark>	C T G A G G A T	G <mark>AGCAGC</mark> TT	Г G G <mark>C</mark> A A <mark>C</mark> G T A	T G <mark>C</mark> A G T T A T G T	<mark>стстстс</mark> т	T T T <mark>A A A</mark>
consensus	GAGTTT	T T <mark>A</mark> T T <mark>G A A G</mark> A		GAGCAGCTT	G G C A A A C G T A	TGCAGTTATGT	GCTGTGTTG	ΤΤΤΑΑΑ
		3580	3590	3600	3610	3620	3630	3640
kuk.2d	TCGCGT	<mark>C   G   T   T   C   G   T   T   G</mark> T	A T G T G T G A	T G C G T T G C A	ГТТТ <mark>СССТТСА</mark>	G <mark>T A T G C</mark> T T T G C	5 T T T <mark>C A</mark> G T A G	T G <mark>A</mark> G G T
kuk.1d	TCGCGT	<mark>C   G   T   T   C   G   T   T   G</mark> T	A T G T G T G A	T G C G T T G C A	ГТТТ <mark>СССТТСА</mark>	G <mark>T A T G <mark>C</mark> T T T G O</mark>	5 T T T <mark>C A</mark> G T A G	T G <mark>A</mark> G G T
KUK49_final	TCGCGT		A T G T G T G A	Τ <mark>ΓΓΓΓΓΓΓΑ</mark>	TTTGGGGTTG <mark>A</mark>	G <mark>TAT</mark> G <mark>C</mark> TTTG	TTT <mark>C</mark> AGTAG	T G <mark>A</mark> G G T
consensus	TCGCGT	<mark>C</mark> G T T <mark>C</mark> G T T G T	T <mark>A</mark> TGTGTGA	Т <mark>G <mark>C</mark> G Т Т <mark>G <mark>C</mark> А <sup>-</sup></mark></mark>	ГТТТGGGTTG <mark>A</mark>	G <mark>T</mark> ATG <mark>C</mark> TTTG	; T T T <mark>C </mark> A G T <mark>A</mark> G	T G <mark>A</mark> G G T
		3650	3660	3670	3680	3690	3700	3710
kuk.2d		G <mark>ATGGTTGT</mark> G						GTTAGC
kuk.1d								
KUK49_final					GCGGTAGTCG			GIIAGC
consensus	TATCTG	G <mark>AT</mark> GGTTGT	G T G <mark>A</mark> G T T T T	TATCGGTGA		Τ <mark>Τ</mark> G <mark>ΤΤΤΓ<mark>C</mark>C<mark>A</mark>G</mark>	; T <mark>AGAGTTG</mark> T	G <mark>TTA</mark> G <mark>C</mark>
		2770	2720	2740	2750	2760	2770	2700
kuk 2d								
kuk 1d								
KUK49 final								
consensus								
conconcue								
		3790	3800	3810	3820	3830	3840	3850
kuk.2d	GTCGTT	T G T G <mark>A C</mark> G <mark>A </mark> G	T G T T A G C T	Т Т Т <mark>G</mark> Т Т <mark>А </mark> G Т Т	ГТ G <mark>G C</mark> T <mark>G A T T G</mark> /	A G T T C T A T T G C		G <mark>CT</mark> GTG
kuk.1d	G T C G T T	TGTGACGAG	TGTTAGCT	ттт <mark>с</mark> тт <mark>ас</mark> т	ГТ G <mark>G C</mark> T G A T T G <mark>/</mark>	A G T T <mark>C</mark> T A T T G C		G <mark>CT</mark> GTG
KUK49_final	GTCGTT	T G T G <mark>A C</mark> G <mark>A </mark> G	T G T T A G C T	T T T <mark>G</mark> T T <mark>A G</mark> T T	TTGG <mark>C</mark> TGATTG	A G T T <mark>C</mark> T A T T G C		G <mark>CT</mark> GTG
consensus	GTCGTT					AGTTCTATTG		GCTGTG

		3860	3870	3880	3890	3900	3910	3920
kuk.2d	T T G T G A T	<mark>A</mark> T G <mark>A</mark> T <mark>C</mark> T G T	C C A N C T T A G	<mark>A C</mark> T T <mark>G T A</mark> T T <mark>C</mark> 0	TTAATSGTTC	C T T A G G C G T T	G T T G T T <mark>A </mark> G T <mark>A</mark>	A G T T
kuk.1d	ТТ <mark>БТБА</mark> Т	<mark>A T G A T C</mark> T G T	C C A N C T T A G	<mark>A C</mark> T T <mark>G T A T T C</mark> G	TT <mark>AA</mark> TSGTT	C T T A G G <mark>C</mark> G T T	G T T G T T <mark>A </mark> G T <mark>A</mark>	AGTT
KUK49_final	ТТ <mark>Б</mark> Т <mark>БА</mark> Т	<mark>A</mark> T G <mark>A</mark> T <mark>C</mark> T G T	C C <mark>A C C T T A</mark> G	<mark>A                                    </mark>	T T <mark>A A </mark> T G G T T C	C T T A G G <mark>C</mark> G T T	G T T G T T <mark>A</mark> G T <mark>A</mark>	AGTT
consensus	TTGTGAT	ATGATCTGT		ACTTGTATTC			GTTGTTAGTA	AGTT
		3930	3940	3950	3960	3970	3980	3990
kuk.2d	T G <mark>A C C</mark> A G	G T T <mark>A G G A T</mark> S	G T <mark>A</mark> G G <mark>C</mark> T T T	G <mark>AAGATGTC</mark> GT	T A A G A A <mark>C</mark> G T T	T C A G C G G T G A	A G G A N C G T T G	A G G T
kuk.1d	T G <mark>A C C</mark> A G	G T T <mark>A G G A T</mark> S	G T <mark>A</mark> G G <mark>C</mark> T T T	G <mark>AAGAT</mark> G <mark>TC</mark> GT	T A A G A A <mark>C</mark> G T T	T C A G C G G T G A	A G G A N C G T T G	A G G T
KUK49_final	T G <mark>A C C A</mark> G	G T T <mark>A</mark> G G <mark>A T</mark> G	G <mark>T </mark> A G G <mark>C</mark> T T T	G <mark>AAGAT</mark> G <mark>TC</mark> GT	T <mark>AAGAAC</mark> GTT	Τ <mark>Ϲ</mark> Α <mark>Ϛ</mark> ϚϚϚͳϚΑ	A G G A <mark>C C</mark> G T T G	A G G T
consensus	T G <mark>A C C A</mark> G	G <mark>T T A</mark> G G <mark>A T</mark> G	G T <mark>A</mark> G G <mark>C</mark> T T T	G <mark>AAGAT</mark> G <mark>TC</mark> GT	T <mark>A A G A A C </mark> G T T	T <mark>C</mark> AGCGGTGA	AGGA CGTTG	A G G T
		4000	4010	4020	4030	4040	4050	4060
kuk.2d	G T A G T T G	ТТТТТТССС	A T G T G T G A G	C G T T M A G A A T T	GGTGT <mark>C</mark> ATTC	G T G <mark>C</mark> T <mark>A</mark> T T T C	ΤĠΤĠΤĠĠΤΤΤ	A T C T
kuk.1d	G T <mark>A</mark> G T T G	ТТТТТТССС	A T G T G T G A G	C G T T M A G A A T T	GGTGT <mark>C</mark> ATTC	G T G C T A T T T C		
KUK49_final	GTAGTTG	TTTTTTCCG	ATGTGTGAG	CGTTAAGAATT	GGTGTCATTC	GTGCTATTTC	ТСТСТССТТТ	ATCT
consensus	GTAGTTG	TTTTTTT <mark>CC</mark> G	A T G T G T G A G	<mark>C G T T A A G A A T</mark> T	G G T G T <mark>C A T T C</mark>	G T G <mark>C</mark> T <mark>A</mark> T T T <mark>C</mark>	Τ <mark>ΓΓΓΓΓΓ</mark>	A T <mark>C</mark> T
		1070	1000	4000	1100	1110	44.2.0	4420
								4130
KUK.20								
KUK. IU								
consensus			T <mark>GAC</mark> ATTCT	<mark>A G T T T G C</mark> T T T A		GACTITITAGTG		AGA
		4140	4150	4160	4170	4180	4190	4700
kuk.2d	ATTACTA		GCATTGTTA	GTTATGTGTAC				TTTT
kuk 1d				G T T A T G T G T A C				ТТТТТ
KUK49 final		GATGGTAAAA	GCATTGTTA	G T T A T G T G T A C				ТТТТТ
consensus	ATTACTA		GCATTGTTA	GTTATGTGTA				ΤΤΤΤΤ

		4210	4220	4230	4240	4250	4260	4270
kuk.2d	TGTTT	G G <mark>A G T</mark> G <mark>A C A</mark> G	A C A A C G T C T	G <mark>A A T</mark> G G <mark>T A</mark> G G	T <mark>A G C T C</mark> G T T <sup>-</sup>	T G <mark>A</mark> G G <mark>A T T T C</mark>	A A G G T T G T C C T	T <mark>C</mark> TGGA
kuk.1d	TGTTTT	G G <mark>A G T</mark> G <mark>A C</mark> A G	A C A A C G T C T	G <mark>A A T</mark> G G <mark>T A</mark> G G	T <mark>A G C</mark> T <mark>C</mark> G T T <sup>-</sup>	T G <mark>A </mark> G G <mark>A T T T </mark> C	A A G G T T G T C C T	T <mark>C</mark> TGGA
KUK49_final	TGTTT	G G <mark>A G T</mark> G <mark>A C</mark> A G	A C A A C G T C T	G <mark>AA</mark> TGG <mark>T</mark> AGG	T <mark>A G C</mark> T <mark>C </mark> G T T <sup>-</sup>	T <mark>G A G G A</mark> T T T <mark>C</mark>	A A G G T T G T C C T	Г <mark>С</mark> Т <mark>GG</mark> А
consensus	TGTTT	G G <mark>A G T G A C </mark> A G	ACAACGTCT	GAATGGTAGG	TAGCTCGTT	T G A G G A T T T C	AAGGTTGTCC	
		4280	4290	4300	4310	4320	4330	4340
kuk.2d	A T T T G <mark>C</mark>	C R K T C A T G T T	ТТ <mark>G</mark> Т <mark>АС</mark> ТТ <mark>С</mark>	тотото	C T C A T G T A T (	G T G <mark>C</mark> G T G <mark>C</mark> T G	G T G T T T T G <mark>C C</mark> A A	A G T G G T
kuk.1d	ATTTGC	C R K T C A T G T T	T T G T A C T T C	тотото	CTCATGTAT	G T G C G T G C T G	G T G T T T T G <mark>C C</mark> A A	A G T G G T
KUK49_final			TTGTACTTC	ТСТСТССС		GTGCGTGCTG	GTTTTTGCCA/	A G T G G T
consensus	A T T T G C	<mark>C G G T C A</mark> T G T T	T T G T <mark>A C</mark> T T <mark>C</mark>	<mark>Т</mark> G <mark>Т</mark> G <mark>Т Т</mark> G <mark>Т</mark> G G	<mark>C T C A T</mark> G T A T (	G T G <mark>C</mark> G T G <mark>C T</mark> G	G <mark>TGTTTT</mark> G <mark>CCA</mark>	A G T G G T
		(250	12.50	(270	(200	(200		
KUK.20								
KUK. IU								
consensus		I AIAIAIA I <mark>CI</mark> GIGIA	GIGGIGIGI		GAAIIIIG			
		4420	4430	4440	4450	4460	4470	4480
kuk.2d	AGATAA			AATAGGTATT	GCCGCTAAT			
kuk.1d	AGATAA	ATAGAGTTTT	ΤΤΓΟΟΤΟΑΟ	A A T A G G T A T T	GCCGCTAAT	Г Т <mark>С С</mark> Т Т <mark>С А</mark> Т Т		
KUK49_final	AGATAA	A T A G A G T T T T	T T G G T G A A G	A A T A G G T A T T	G C C G C T A A T	Г Т <mark>С С</mark> Т Т <mark>Б А</mark> Т Т	ACTATTGTTG	T G C G A
consensus	AGATAA	ATAGAGTITIT	TTGGTGAAG	AATAGGTATT	GCCGCTAAT	TTCCTTGATT	ACTATTGTTG	
		4490						
kuk.2d	<mark>A</mark> GG <mark>T</mark> GG	T G <mark>C</mark> T T T <mark>C</mark> G <mark>A</mark> G						
kuk.1d	<mark>A</mark> GG <mark>T</mark> GG	T G <mark>C</mark> T T T <mark>C</mark> G <mark>A</mark> G						
KUK49_final	AGGTGG	T G <mark>C T T T C </mark> G <mark>A</mark> G						
consensus	A G G T G G	T G <mark>C T T T C </mark> G <mark>A</mark> G						

XXX

## E | Multiple alignment for XpYp telomere

The A allele of the XpYp telomere from the LoVo cell line  $(MSH2^{-/-})$  was sequenced using the minION<sup>TM</sup> device. Two libraries (one containing both XpYp alleles, and another containing only A allele) generated 1D and 2D reads that were first aligned to the flanking region of the A allele to filter the reads (as explained in section 4.3.3). Later, the filtered reads were aligned to a telomere consensus generated based on the TVR maps (described in section 4.9) called "Telomere.reference" for ClustalW multiple alignment. The consensus generated by IGV from the alignment of the 1D and 2D reads for both alleles are called: "1d.E2.filtered" and "1d.E2.filtered" respectively. The consensus generated by IGV from the alignment of 1D and 2D reads for A allele are called: "1d.hapA.filtered" and "1d.hapA.filtered" respectively. Each nucleotide is colour coded and the position of each nucleotide is indicated at the top of the alignment in red numbers. Letters are: A: adenine; T: thymine; C: cytosine; G: guanine; R: purine (A or G); Y: pirimidine (T or C); N: any nucleotide; W: Weak (A or T); S: Strong (C or G) and K: Keto (G or T).



	290	300	310	320	330	340	350
1d.E2.filtered	R G T G <mark>A G C</mark> A K T A G C	NAGATTTAKTGC	ΝΨΑ <mark>ΓΑΓΓ</mark> ΟΝ	INNGWN <mark>C</mark> G <mark>A</mark> AGS	T T <mark>C C A</mark> S <mark>A G</mark> T	<mark>A T</mark> GGWW <mark>A</mark> GGG	√ N N S S <mark>A</mark>
2d.E2.filtered	R G T G A G C A K T A G C	NAGATTTAKTG	ΝΨΑĠΑĠΤĠΝ	INNGWN <mark>C</mark> G <mark>A</mark> AGS	T T <mark>C C A</mark> S <mark>A G</mark> T	<mark>A T</mark> GGWW <mark>A</mark> GGG	√ N N S S <mark>A</mark>
1d.hapA.filtered	R G T G A G C A K T A G C	N <mark>A G A T T T A K T G C</mark>	ΝΨΑ <mark>ΓΑΓΓ</mark> ΟΝ	I N N G W N <mark>C G A A G</mark> S	T T C C A S <mark>A G</mark> T	<mark>A T</mark> G G W W <mark>A</mark> G G G V	√ N N S S <mark>A</mark>
2d.hapA.filtered	A G T G A G C A G T A G C	N <mark>A G A T T T A T T G C</mark>	ΝΨΑ <mark>ΓΑΓΓ</mark> Α	N N G W N <mark>C</mark> G A A G C	T T C C A S <mark>A G</mark> T	<mark>A T</mark> G G W W <mark>A</mark> G G G V	v y n s s <mark>A</mark>
Telomere.reference	A G T G A G C A G T A G C	AAGATTTATTG	AAAGAGTGA	AAGAACGAAGC	T T <mark>C C A C A G T</mark>	<mark>a t</mark> gg <mark>aaa</mark> ggga	ACCCCA
consensus	A G T G A G C A G T A G C	AGATTTATTG		GA CGAAGC	T T C C A C A G T	A T G G A A A G G G A	AY CCA
			380				420
1d.E2.filtered							
20.E2.filtered							
Id.napA.filtered							
Zo.napA.nitered							
reiomere.reference							GCIGA
consensus					T T G T C		GCTGA
	430	440	450	460	470	480	490
1d.E2.filtered	<i>430</i> TTTGGTCCASTTWS	<i>440</i>	450 GGTKAGGG	<i>460</i> K <mark>agggt</mark> kaggg	470 TKAGGGTKA	480 GGG <mark>T</mark> K <mark>A</mark> GGG <mark>T</mark> K	490
1d.E2.filtered 2d.E2.filtered	<i>430</i> T T G G T C C A S T T W S T T G G T C C A S T T W S	<i>440</i> A G T T A G G G T T A C A G T T A G G G T T A C	<i>450</i> 5 G G T K <mark>A </mark> G G G T 5 G G T K <mark>A </mark> G G G T	<i>460</i> K <mark>A G G G T K A G G G K A G G G T K A G G G</mark>	<i>470</i> T K A G G G T K A T K A G G G T K A	<i>480</i> G G G T K A G G G T H G G G T K A G G G T H	<i>490</i> < <mark>A G G G T</mark> < <mark>A G G G T</mark>
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	<i>430</i> T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S	440 A G T T A G G G T T A G A G T T A G G G G T T A G A G T T A G G G G T T A G	<i>450</i> G G T K A G G G T G G T K A G G G T G G T K A G G G T	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A	<i>480</i> G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H	<i>490</i> < <mark>A G G G T</mark> < <mark>A G G G T &lt; <mark>A G G G T</mark></mark>
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	<i>430</i> T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T W S	440 A G T T A G G G T T A C A G T T A G G G T T A C A G T T A G G G T T A C A G T T A G G G T T A C	450 G G T K A G G G T G G T K A G G G T	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T N A G G G T K A	480 G G G T K A G G G T F G G G T K A G G G T F G G G T K A G G G T F G G G T K A G G G T F	<i>490</i> (
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	430 T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T C T T G G T C C A C T T T C	A       G       T       T       A       G       G       G       T       T       A       G         A       G       T       T       A       G       G       T       T       A       G         A       G       T       T       A       G       G       G       T       T       A       G         A       G       T       T       A       G       G       G       T       T       A       G         A       G       T       T       A       G       G       G       T       A       G         A       G       T       T       A       G       G       G       T       A       G         A       G       T       T       A       G       G       G       T       A       G	450 G G T K A G G G T G G T G A G G G T	460 K A G G G T K A G G G K A G G G G T K A G G G K A G G G G T K A G G G K A G G G G T K A G G G G A G G G T K A G G G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T N A G G G T K A T G A G G G T G A	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G	490 (AGGGGT AGGGGT (AGGGGT AGGGGT AGGGGT
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	430 T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T C T T G G T C C A C T T T C	440 A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G	450 G G T K A G G G T G G T G A G G G T G G T G A G G G T	460         K       A       G       G       T       K       A       G       G       G         K       A       G       G       G       T       K       A       G       G       G         K       A       G       G       G       T       K       A       G       G       G         K       A       G       G       G       T       K       A       G       G       G         K       A       G       G       G       T       K       A       G       G       G         K       A       G       G       G       T       K       A       G       G       G         K       A       G       G       G       T       K       A       G       G       G         G       A       G       G       G       T       G       A       G       G       G         G       A       G       G       G       T       G       A       G       G       G         G       A       G       G       G       T       G       A       G <td>470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T N A G G G T K A T G A G G G T G A</td> <td>480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G G G G T G A G G G T G</td> <td>490 (AGGGT AGGGT (AGGGT (AGGGT AGGGT AGGGT</td>	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T N A G G G T K A T G A G G G T G A	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G G G G T G A G G G T G	490 (AGGGT AGGGT (AGGGT (AGGGT AGGGT AGGGT
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	430 T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T C T T G G T C C A S T T T C T T G G T C C A C T T T C	440 A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G	450 G G T K A G G G T G G T G A G G G T G G T G A G G G T	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G G A G G G T G A G G G G A G G G T G A G G G	470 T K A G G G T K A T K A G G G T K A T N A G G G T K A T N A G G G T K A T G A G G G T G A	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G G G G T G A G G G T G	490 A G G G T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	430 T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T C T T G G T C C A C T T T C T T G G T C C A C T T T C 500	440 A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G	450 G G T K A G G G T G G T G A G G G T G G T G A G G G T 5 G G T G A G G G T 5 20	460 K A G G G T K A G G G K A G G G G T K A G G G K A G G G G T K A G G G K A G G G T K A G G G G A G G G T G A G G G G A G G G T G A G G G 530	470 T K A G G G T K A T K A G G G T K A T A G G G T K A T A G G G T K A T G A G G G T G A T G A G G G T G A 540	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G S550	490 ( A G G G T ( A G G G G T ( A G G G G T 5 A G G G G T 5 A G G G G T 5 5 0 5 0 5 0 5 0 5 0 5 0 5 0 5
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	430 T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A C T T T C T T G G T C C A C T T T C S00 K A G G G T K A G G G T T	440 A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G 510 A G G G T T A G G G T T	450 G G T K A G G G T G G T G A G G G T 5 G G T G A G G G T 5 C G T A G A G G G T 5 20 A G G G T T A G G	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G G A G G G T G A G G G G A G G G T G A G G G 530 G T T A G G G T T A G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T A G G G T K A T G A G G G T K A T G A G G G T A A T G A G G G T A A T G A G G G T C A	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G G G G T G A G G G T G 550 T A G G G T T A G G G	490 (AGGGGT (AGGGGT (AGGGGT AGGGGT 5AGGGGT 5AGGGGT 5AGGGGT 560 577AGG
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	<i>430</i> T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T W T G G T C C A C T T T C T T G G T C C A C T T T C <i>T T G G T C C A C T T T C</i> <i>S00</i> K A G G G T K A G G G T T K A G G G T K A G G G T T	440 A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G G T T A G G G T T A G A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G G T T A G G G T T	<i>450</i> G G T K A G G G T G G T G A G G G T G G T G A G G G T <i>520</i> T A G G G T T A G G A G G G T T A G G	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G G A G G G T G A G G G G A G G G T G A G G G G T T A G G G T T A G G T T A G G G G T T A G G T T A G G G G T T A G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T G A G G G T K A T G A G G G T G A T G A G G G T G A 540 G G T T A G G G T G G T T A G G G T	480 G G G T K A G G G T F G G G T K A G G G T F G G G T K A G G G T F G G G T K A G G G T F G G G T G A G G G T G G G G T G A G G G T G 550 T A G G G T T A G G G T A G G G T T A G G G	490 A G G G T A G G G G T 5 A G G G G T 5
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	<i>430</i> T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T C T T G G T C C A C T T T C T T G G T C C A C T T T C <i>500</i> K A G G G T K A G G G T T K A G G G T K A G G G T T K A G G G T K A G G G T T K A G G G T K A G G G T T K A G G G T K A G G G T T	A       G       T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A	<i>450</i> G G T K A G G G T G G T G A G G G T G G T G A G G G T <i>520</i> A G G G T T A G G A G G G T T A G G A G G G T T A G G	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G G A G G G T G A G G G G A G G G T G A G G G G T T A G G G T T A G G T T A G G G G T T A G G T T A G G G G T T A G G T T A G G G G T T A G G T T A G G G G T T A G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T A G G G T K A T G A G G G T G A T G A G G G T G A T G A G G G T G A 540 G G T T A G G G T G G T T A G G G T G G T T A G G G T	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G G G G T G A G G G T G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G	490 A G G G T A G G G G G G T A G G G G G G G G G T A G G G G G G G G T A G G G G G G G G T A G G G G G G G G T A G G G G G G G G G G G T A G G G G G G G G G G G G G G G G G G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	<i>430</i> T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A C T T T C T T G G T C C A C T T T C T T G G T C C A C T T T C <i>500</i> K A G G G T K A G G G T T K A G G G T K A G G G T K A G G G T T K A G G G T K A G G G T K A G G G T T K A G G G T K A G G G T K A G G G T T K A G G G T K A G G G T K A G G G T T K A G G G T K C C C T T C C C C T T T T C C C C T T T T C C C C T T T T C C C C C T T T T T C C C C C T T T T C C C C C T T T T C C C C C T T T T C C C C C T T T T C C C C C C C T T T T C C C C C C T T T T C C C C C C T T T T C C C C C C C C T T T T C C C C C C T T T T C	440 A G T T A G G G T T A G A G T T A G G G T T A G A G T T A G G G T T A G A G T A A G G G T T A G A G T A A G G G T T A G A G T A A G G G T T A G A G T A A G G G T T A G A G T T A G G G T T A G A G G G T T A G G G G T T A G T A G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G T A G T T A G G G T T A G T A G T T A G T T A G T T T A G T T T T	450 G G T K A G G G T G G T G A G G G T G G T G A G G G T 5 G G T G A G G G T 5 C G T A G G G T T A G G A G G G T T A G G	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G G A G G G T G A G G G G A G G G T A G A G G G G T T A G G G T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T A G G G T K A T G A G G G T G A T G A G G G T G A T G T T A G G G T G G T T A G G G T	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T H G G G T G A G G G T G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T A G G G G T T A G G G T T A G G G G T T A G G G G	490 A G G G T A G G G G T A G G G G T A G G G G G G T A G G G G G G T A G G G G G G T A G G G G G T A G G G G G T A G G G G G G G G T A G G G G G G G G T A G G G G G G G G G G G G G G G G G G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	<i>430</i> T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T W S T T G G T C C A S T T T W T G G T C C A C T T T C T T G G T C C A C T T T C T T G G T C C A C T T T C S 500 K A G G G T K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T K K A G G G T T K A G G G T T K A G G G T K K A G G G T T K A G G G T T K A G G G T K K A G G G T T K A C C C A C T T K A C C C C T T K A C C C C C C C C C C C C C C C C C C	A       G       T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T	450 G G T K A G G G T G G T G A G G G T 5 G G T G A G G G T 5 G G T G A G G G T 6 A G G G T T A G G A G G G T T A G G G A G G G G T T T A G G A G G G G T T T A G G G A G G G G T T T A G G G A G G G G T T T A G G G A G G G G T T T A G G G A G G G G T T T A G G G A G G G G T T T A G G G G T T T A G G G A G G G G T T T A G G G G T T T A G G G G	460 K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G K A G G G T K A G G G G A G G G T G A G G G G A G G G T A G A G G G G T T A G G G T A A G G T T A G G G T T A G G T T A G G G G T T A G G G G T T A G G T T A G G G G T T A G G G T T A G G T T A G G G G T T A G G G G T T A G G G T T A G G T T A G G G G T T A G G G G T T T A G G G G	470 T K A G G G T K A T K A G G G T K A T K A G G G T K A T A G G G T K A T G A G G G T G A T G A G G G T G A T G T T A G G G T G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T T A G G G G T T T A G G G T T T A G G G G T T T A G G G G	480 G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T K A G G G T H G G G T G A G G G T G T A G G G T T A G G G T T A G G G T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T A A G G G T T A G G G T A A G G G T T A G G G T T A G G G G	490 A G G G T A G G G G G T A G G G G T A G G G G T A G G G G G G T A G G G G G G T A G G G G G G G T A G G G G G G T A G G G G G G G G T A G G G G G G G G T A G G G G G G G G G T A G G G G G G G G G G G G G G G T A G G G G G G G G G G G G G G G G G G T A G G G G G G G G G G G G G G G G G G G

	570	580	590	600	610	620	630
1d.E2.filtered	G T T <mark>A</mark> G G G T T <mark>A</mark> G G G	T T A G G G T T A G G	G T T <mark>A</mark> G G G T T .	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TT</mark> AGG	G T T <mark>A G G G T T</mark> R	G G G T T R
2d.E2.filtered	G T T A G G G T T A G G G	T T A G G G T T A G G	G <mark>TTA</mark> GGG <mark>TT</mark>	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GGG	G T T <mark>A</mark> G G G T T R	G G G T T R
1d.hapA.filtered	G T T A G G G T T A G G G	T T A G G G T T A G G	G <mark>TTA</mark> GGG <mark>T</mark> T	ΑĠĠĠ <mark>Ŧ</mark> Ŧ <mark></mark> ゟĠĠ <mark></mark> Ŧ	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T R	G G G T T R
2d.hapA.filtered	G T T A G G G T T A G G G	T T A G G G T T A G G	G <mark>TTA</mark> GGG <mark>T</mark> T	ΑĠĠĠ <mark>Ŧ</mark> Ŧ <mark></mark> ゟĠĠ <mark></mark> Ŧ	T <mark>A</mark> GGG <mark>TTA</mark> GGG	G T T <mark>A</mark> G G G T T R	G G G T T R
Telomere.reference	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	Τ <mark>ΤΑ</mark> GGG <mark>ΤΤΑ</mark> GG	G <mark>TTA</mark> GGG <mark>TT</mark>	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GGG	G <mark>TT</mark> A <mark>GGGTT</mark> G	G G G T T G
consensus	G T T <mark>A</mark> G G G T T <mark>A</mark> G G G	T T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G G T T .	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T G	G G G <mark>T T</mark> G
	640	650	660	670	680	690	700
1d.E2.filtered	N G G T T R G G G T T R G	G G T T R G G G T T R	GGGTTRGGG	T T R G G G T T R G G	GTTRGGGTTR	GGG <mark>TT</mark> NGGGT	TRGGGT
2d.E2.filtered	N G G T T R G G G T T R G	G G T T R G G G T T R	GGGTTRGGG	T T R G G G T T R G G	GTTRGGGTTR	GGG <mark>TT</mark> NGGGT	TRGGGT
1d.hapA.filtered	N G G T T R G G G T T R G	G G T T R G G G T T R	GGGTTRGGG	T T R G G G T T R G G	GTTRGGGTTR	GGG <mark>TT</mark> NGGGT	TRGGGT
2d.hapA.filtered	GGG <mark>TT</mark> RGGG <mark>TT</mark> RG	G G T T R G G G T T R	GGGTTRGGG	Τ <mark>ΤΝGGGΤΤ</mark> ΝGG	GTTRGGGTTR	G G G T T R G G G T	TRGGGT
Telomere.reference	GGG <mark>TT</mark> GGGGG <mark>TT</mark> GG	<mark>ͼͼͺϫͺϫͺ</mark> ͼͺͼͺͼͺ <mark>ϫͺϫͺ</mark> ͼ	GGG <mark>TT</mark> GGGGG	ттсссаттссс	G <mark>TT</mark> GGGG <mark>TT</mark> G	GGG <mark>TT</mark> GGGGG <mark>T</mark>	T G G G G T
consensus	G G G T T G G G G T T G G	G G T T G G G G T T G	G G G T T G G G G	Τ <mark>Τ</mark> G G G G <mark>Τ Τ</mark> G G G	G T T G G G G T T G (	G G <mark>G T T</mark> R G G G T	T G G G <mark>T</mark>
	710	720	730	740	750	760	770
1d.E2.filtered	T R G G G T T R G G G T T	A G G G T T <mark>A</mark> G G G T		G G T T A G G G T T R	GGGTTNGGGT	TNGGGTTRGG	GTTRGG
1d.E2.filtered 2d.E2.filtered	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T	A G G G T T A G G G T A G G G T T A G G G T	T A G G G T T A G T A G G G T T A G	G G T T A G G G T T R G G T T A G G G T T R	G G G T T N G G G T G G G T T N G G G T	T N G G G T T R G G T N G G G T T R G G	G T T R G G G T T R G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T	A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	T A G G G T T A G T A G G G T T A G T A G G G T T A G	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R	G G G T T N G G G T G G G T G G G G T T N G G G T T N G G G T T N G G G T G G T G G G G T G G G T G G G T G G G T G G G T G G G G T G G G G T G G G G T G G G T G G G G G T G G G G G T G	T N G G G T T R G G T N G G G T T R G G T N G G G T T R G G	G T T R G G G T T R G G G T T R G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T	A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R	G G G T T N G G G T G G G G T T N G G G T G G G G T T N G G G T G G G G T T R G G G T G	T N G G G T T R G G T N G G G T T R G G T N G G G T T R G G T R G G G T T R G G	G T T R G G G T T R G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       G       G       G       G       T       T       G       G       T       T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R	G G G T T N G G G T G G G G T T N G G G T G G G G T T N G G G T G G G G T T R G G G T G G G G T T G G G G T G	N         G         G         T         T         R         G         G           T         N         G         G         T         T         R         G         G           T         N         G         G         T         T         R         G         G           T         N         G         G         T         T         R         G         G           T         N         G         G         T         T         R         G         G           T         N         G         G         T         T         R         G         G           T         N         G         G         T         T         R         G         G           T         N         G         G         T         T         R         G         G           T         R         G         G         T         T         R         G         G           T         R         G         G         T         T         R         G         G           T         G         G         G         T         T         G         G	G T T R G G G T T G G G
1d.E2.tiltered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       G       G       G       G       T       T       G       G       G       T       T         G       G       G       G       T       T       G       G       G       T       T         G       G       G       G       T       T       G       G       G       T       T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G	G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       G       T       T       R         G       G       T       T       A       G       G       G       T       T       R         G       G       T       T       A       G       G       G       T       T       G         G       G       T       T       A       G       G       G       T       T       G         G       G       T       T       A       G       G       G       T       T       G	G G G T T N G G G T T G G G T T N G G G T T G G G T T N G G G T T G G G T T R G G G T T G G G T T R G G G T T G G G T T R G G G T T	I         N         G         G         T         T         R         G         G           I         N         G         G         T         T         R         G         G           I         N         G         G         T         T         R         G         G           I         N         G         G         T         T         R         G         G           I         N         G         G         T         T         R         G         G           I         N         G         G         T         T         R         G         G           I         R         G         G         T         T         R         G         G           I         R         G         G         T         T         G         G         G         T         T         G         G         G         G         G         G         G         G         T         T         G         G         G         G         T         T         G         G         G         G         G         G         G         G         G         G	G T T R G G G T T G G G G T T G G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       G       T       T       R       G       G       T       T         T       G       G       G       G       T       T       G       G       G       T       T         T       G       G       G       G       T       T       G       G       G       T       T         G       G       G       G       T       T       G       G       G       T       T         G       G       G       G       T       T       G       G       G       T       T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T G G G T T A G G G T T G	G G G T T N G G G T G G G T T N G G G T T G G G T T N G G G T T G G G G	N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       R       G       G       T       T       G       <	G T T R G G G T T G G G G T T G G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         S000       G       T       T       A       G       G       T       T	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T G G G T T A G G G T T G 810 810	G G G T T N G G G T G G G T T N G G G T T G G G T T N G G G T T G G G G	$\begin{bmatrix} \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{R} & \mathbf{S} & \mathbf{S} & \mathbf{S} & \mathbf{S} & \mathbf{S} \\ \mathbf{S} & \mathbf{T} & \mathbf{T} & \mathbf{S} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \end{bmatrix}$	G T T R G G G T T G G G G T T G G G G T T G G G 840
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d E2 filtered	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         T       G       G	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         790       T       T       A       G       G       G       T       A <td>A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         8000       G       T       T       A       G       G       T       T         G       T       T       A       G       G       T       T       A</td> <td>G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T G B G T T A G G G T T G 810 A G G G T T A G G G T</td> <td>G G G T T N G G G T G G G T T N G G G T T G G G T T G G G T T G G G T T G G G T G G G T G G G G T G G G G T T G G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G G T G</td> <td>N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       R       G       G       T       T       G       <td< td=""><td>G T T R G G G T T G G G T 840</td></td<></td>	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         8000       G       T       T       A       G       G       T       T         G       T       T       A       G       G       T       T       A	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T G B G T T A G G G T T G 810 A G G G T T A G G G T	G G G T T N G G G T G G G T T N G G G T T G G G T T G G G T T G G G T T G G G T G G G T G G G G T G G G G T T G G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G T G G G G G T G	N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       N       G       G       T       T       R       G       G         T       R       G       G       T       T       G <td< td=""><td>G T T R G G G T T G G G T 840</td></td<>	G T T R G G G T T G G G T 840
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d hapA filtered	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         T       G       G       G       T       T       G       G       G       G       G       G       G	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         T       T       A       G       G       G       T       T       A	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         B       O       G       T       T       A       G       T       T         B       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T G B G T T A G G G T T G 810 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	G       G       T       T       N       G       G       T         G       G       G       T       T       N       G       G       T         G       G       G       T       T       N       G       G       T         G       G       G       T       T       N       G       G       T         G       G       G       T       T       R       G       G       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         B       G       G       T       T       R       G       G       T       T         B       G       G       T       T       A       G       G       T       A       G       G       G       T       A       G       G       G       T       A       G       G       G       G       G	$\begin{bmatrix} \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{I} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{T} $	G T T R G G G T T G G G G T T G G G G T T A G G G T T A G G G T T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d hapA filtered	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       R       G       G       T       T       R       G	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         T       T       A       G       G       G       T       T       A	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G<	G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T R G G T T A G G G T T G G G T T A G G G T T G 810 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	G       G       G       T       N       G       G       T         G       G       G       T       N       G       G       T         G       G       G       T       N       G       G       T         G       G       G       T       N       G       G       T         G       G       G       T       T       N       G       G       T         G       G       G       T       T       R       G       G       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         B       G       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A <td><math display="block">\begin{bmatrix} \mathbf{N} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{T} &amp; \mathbf{N} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{T} &amp; \mathbf{N} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{C} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{A} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} </math></td> <td><math display="block">\begin{bmatrix} \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{R} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} \\ \mathbf{G} &amp; \mathbf{T} &amp; \mathbf{T} &amp; \mathbf{G} &amp; \mathbf{G} &amp; \mathbf{G} \\ \end{bmatrix}</math></td>	$\begin{bmatrix} \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{C} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} $	$\begin{bmatrix} \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \end{bmatrix}$
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere reference	T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       R       G       G       T       T       R       G       G       T       T         T       G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       G       G       G       T       T         G       G       G       T       T       R       G       G       G       G       G       G       G	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         T       T       A       G       G       G       T       T       A	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G<	G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       R         G       G       T       T       A       G       G       T       T       G       G       T       T       G       G       T       T       G       G       T       T       G       G       T       T       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A	G       G       T       T       N       G       G       T         G       G       G       T       T       N       G       G       T         G       G       G       T       T       N       G       G       T         G       G       G       T       T       N       G       G       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         G       G       G       T       T       R       G       G       T       T         S20       T       A       G       G       G       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G       T     <	$ \begin{bmatrix} \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{N} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} \\ \mathbf{T} & \mathbf{R} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{A} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{G} \\ \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{T} & \mathbf{G} & \mathbf{G} & \mathbf{G} & \mathbf{T} & \mathbf{T} & \mathbf{T} \\ \mathbf{G} & \mathbf{T} \\ \mathbf{G} & \mathbf{T} & \mathbf{T}$	G T T R G G G T T G G G G T T G G G G T T G G G G T T A G G G T T A

	850	860	870	880	890	900	910
1d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> G	G G T T R G G G T T	N N <mark>G G T T</mark> R N <mark>G G T</mark>	TRGGGTTRGGG	T T <mark>A</mark> GGGTT	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG T
2d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TT</mark> A	G G T T R G G G T T	N N <mark>G G T T</mark> R N <mark>G G T</mark>	T R G G G T T R G G G	T T <mark>A</mark> GGG T T	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG T
1d.hapA.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> G	G G T T R G G G T T	N N <mark>G G T T</mark> R N <mark>G G T</mark>	TRGGGTTRGGG	T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> G G <mark>T</mark>
2d.hapA.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> G	<mark>σοσττ</mark> οοσο <mark>ττ</mark>	R G G G T T R G G G T	TRGGGTTNGGG	T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> G G G T
Telomere.reference	GGG <mark>TTA</mark> GGG <mark>TTA</mark> O	ајаја <mark>т т</mark> ајаја <mark>т т</mark> аја	<mark>ајаја т т</mark> ајаја <mark>т</mark>	твеветтевее	T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> GG <mark>G</mark> T
consensus	G G G T T <mark>A</mark> G G G T T <mark>A</mark> (	G G G T T G G G G T T	R G G G T T G G G G T	ΤĠĠĠĠŢŢĠĠĠĠ	T T <mark>A</mark> G G G T T	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> G G G T
	920	930	940	950	960	970	980
1d.E2.filtered	T <mark>A</mark> GGG T T A GGG T T	T <mark>A</mark> GGG <mark>TTA</mark> GGG	ͳͳ <mark></mark> ΑĠĠĠͳͳ <mark></mark> ΑĠĠ	G T T <mark>A</mark> G G G T T A G	G G T T A G G G	TTAGGGTTAGG	G <mark>TTA</mark> GG
2d.E2.filtered	T <mark>A</mark> GGG T T A GGG T T	T <mark>A</mark> GGG <mark>TTA</mark> GGG	ͳͳ <mark></mark> ΑĠĠĠͳͳ <mark></mark> ΑĠĠ	G T T <mark>A</mark> G G G T T A G	G G T T <mark>A</mark> G G G	TTAGGGTTAGG	G <mark>TTA</mark> GG
1d.hapA.filtered	T <mark>A</mark> GGG <mark>TTA</mark> GGGTT	T <mark>A</mark> GGG <mark>TTA</mark> GGG	Τ <mark>ΤΑ</mark> GGG <mark>ΤΤΑ</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A G G G	TTAGGGTTAGG	G <mark>TTA</mark> GG
2d.hapA.filtered	T <mark>A</mark> GGG <mark>TTA</mark> GGGTT	T <mark>A</mark> GGG <mark>TTA</mark> GGG	ͳ <mark>ͳͺΑ</mark> ͺϾͺϾͺ <mark>ͳͺͳͺ</mark> ΑͺϾͺϾ	G T T <mark>A</mark> G G G T T A G	G G T T A G G G	TTAGGGTTAGG	G <mark>TTA</mark> GG
Telomere.reference	T <mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark> T	<mark>A</mark> GGG <mark>TTA</mark> GGG	Τ <mark>ΤΑ</mark> GGG <mark>ΤΤΑ</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A G G G	TTAGGGTTAGG	G <mark>TT A</mark> GG
consensus	T <mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark> T	T <mark>A</mark> GGG <mark>TTA</mark> GGG	T <mark>T</mark> AGGG <mark>TT</mark> AGG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G <mark>T T A</mark> G G G	T T <mark>A</mark> GGG <mark>T T A</mark> GG	G <mark>T T </mark> A G G
	990	1000	1010	1020	1030	1040	1050
1d.E2.filtered	990 GTTAGGGTTAGGG	1000 TTAGGGTTAG	1010 G G T T A G G G T T A	1020 GGGTTAGGGTT	1030 AGGGTTAG	1040 GGTTAGGGTTA	1050 GGG <mark>TT</mark> A
1d.E2.filtered 2d.E2.filtered	990 G T T A G G G T T A G G G G T T A G G G T T A G G G	1000 TTTAGGGGTTAG TTAGGGGTTAG	1010 G G T T A G G G T T A G G T T A G G G T T A	1020 G G G T T A G G G T T G G G T T A G G G T T	1030 AGGGGTTAG AGGGGTTAG	1040 G G T T A G G G T T A G G T T A G G G T T A	1050 G G G T T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	990 G T T A G G G T T A G G C G T T A G G G T T A G G C G T T A G G G T T A G G C	1000 T T A G G G T T A G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T	1030 A G G G T T A G A G G G T T A G A G G G T T A G	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1050 G G G T T A G G G T T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G	1000 T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T	1030 A G G G T T A G A G G G T T A G	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1050 G G G T T A G G G T T A G G G T T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G	1000 T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G	1010         G       T       A       G       G       T       A         G       T       T       A       G       G       T       T       A         G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	1020         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T	1030 A G G G T T A G A G G G T T A G	Image: constraint of the stress of the st	1050 G G G T T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G	1000         T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G	1010         G       T       A       G       G       T       A         G       T       T       A       G       G       T       T       A         G       T       T       A       G       G       T       T       A         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         G       G       T       T       A       G       G       G       T       A	1020         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	1030 A G G G T T A G A G G G T T A G	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1050         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	990 G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G	1000         T       A       G       G       T       T       A       G         T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G	1010         G       T       T       A       G       G       T       T       A         G       T       T       A       G       G       T       T       A         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	1020         G G G       T       T       A G G G       T       T         G G G       T       T       A G G G       T       T         G G G       T       T       A G G G       T       T         G G G       T       T       A G G G       T       T         G G G       T       T       A G G G       T       T         G G G       T       T       A G G G       T       T         G G G       T       T       A G G G       T       T	1030 A G G G T T A G A G G G T T A G	Image: Constraint of the constraint	Image: Constraint of the constraint
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G 1060	1000 T T A G G G T T A G T T A G G G T T A G T A G G G T T A G	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A H A G G G T T A H A G G G T T A	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T A G G G T T G G G T A A G G G T T A G G G T T A G G G T T 1090	1030 A G G G T T A G A G G G T T A G 1100	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1110	1050 G G G T T A G G G T T A H G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G 1060 G G G T T A G G G T T A G G G	1000 T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1080 A G G G G T T A G G G T	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T 1090 T A G G G T T A G G G G	1030 A G G G T T A G A G G G T T A G 1100 T T A G G G T T	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A I 110 A G G G T T A G G G T	1050 G G G T T A G G G T T A 1120 T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G 1060 G G G T T A G G G T T A G	1000 T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1080 A G G G T T A G G G T A G G G T T A G G G T	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T A G G G T T G G G T A G G G T T G G G T T A G G G T T 1090 T A G G G T T A G G G G T A G G G T T A G G G	1030 A G G G T T A G A G G G T T A G 1100 T T A G G G T T T A G G G T T	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A H G G G T T A G G G T T A 1110 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	1050 G G G T T A G G G T T A H G G G T T A 1120 T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G 1060 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G	1000 T T A G G G T T A G T A G G G T T A G G G T A G G G T T A G G G G T A A G G G T T A G G G T T A G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1080 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T A A G G G T T G G G T A A G G G T T G G G T T A G G G T T 1090 T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G	1030 A G G G T T A G A G G G T T A G 1100 T T A G G G T T T A G G G T T T A G G G T T	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	1050 G G G T T A G G G T T A A G G G G T T A 1120 T A G G G G T T A G G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G G G T T A G G G T T A G G G G G T T A G G G G T T A G G G G T T A G G G G G T T A G G G G T T A G G G G T T A G G G G G T T A G G G G T T A G G G G T T A G G G G G T T A G G G G T T A G G G G T T A G G G G G T T A G G G G T T A G G G G T T A G G G G G T T A G G G G T T A G G G G T T A G G G G G T T A G G G G T T A G G G G T T A G G G G	1000 T T A G G G T T A G T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G G T T G G G T T A G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G G G T T A G G G G T T G T T A G T T G G G T T T A G G G G T T G G G T T T A G T G G G T T T A G G G T T T A G T T T T T T T T T T T T T T	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G G T	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G G T T A G G G T T 1090 T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G	1030 A G G G T T A G A G G G T T A G T T A G G G T T T A G G G T T T A G G G T T	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	1050 G G G T T A G G G T T A T A G G G T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	990 G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G G G T T A G G G T T A G G G G T T A G G G G	1000 T T A G G G T T A G T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T G G G T T T A G G G G T T T G G G T T T A G G G G T T T G G G T T T A G G G G T T T G G G T T T A G G G G T T T A G G G G T T T G G G T T T A G G G T T T A G G G G T T T G G G T T T A G G G T T T A G G G G T T T G G G T T T A G G G T T T A G G G G T T T G G G T T T A G G G T T T A G G G G T T T A G G G G	1010 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T	1020 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G	1030 A G G G T T A G A G G G T T A G T T A G G G T T T A G G G T T	1040 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	1050 G G G T T A G G G T T A T A G G G T T A G G G T

	1130	1140	1150	1160	1170	1180	1190
1d.E2.filtered	T <mark>A G G G T T A G G G T</mark> T	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> GGGTTA	G G <mark>T T </mark> A G G
2d.E2.filtered	T <mark>AGGGTTAGGGT</mark> T	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> GGG T T	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> G	G G <mark>T T </mark> A G G
1d.hapA.filtered	T <mark>A G G G T T A G G G T</mark> T	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T A G G G T T A G	G G <mark>T T A</mark> G G
2d.hapA.filtered	T <mark>A G G G T T A G G G T</mark> T	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>T T A</mark> GG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T A G G G T T A G	G G <mark>T T </mark> A G G
Telomere.reference	T <mark>A</mark> GG <mark>G</mark> TTAGGGGTT	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T	<mark>Α</mark> GGG <mark>TT</mark> ΑGGG	T A G G G T T A G	G G <mark>T T </mark> A G G
consensus	T A G G G T T A G G G T T	AGGGTTAGGGT	T <mark>A G G G T T A </mark> G G	G T T A G G G T T	AGGGTTAGGGT	TAGGGTTAG	G G T T A G G
	1200	1210	1220	1230	1240	1250	1260
1d.E2.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G T T A G G G T T A	G G G <mark>T T A</mark> G G G	TTAGGGTTAGG	G T T <mark>A</mark> G G G T T	A G G G <mark>T T</mark> A
2d.E2.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G T T A G G G T T A	G G G T T A G G G	Τ Τ <mark>Α</mark> G G G Τ Τ <mark>Α</mark> G G	G T T <mark>A</mark> G G G T T	A G G G T T A
1d.hapA.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G T T A G G G T T A	G G G T T A G G G	Τ Τ <mark>Α</mark> G G G Τ Τ <mark>Α</mark> G G	G T T <mark>A</mark> G G G T T	A G G G T T A
2d.hapA.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G T T <mark>A</mark> GGG T T A	GGG <mark>TTA</mark> GGG	Τ <mark>ΤΑ</mark> GGG <mark>ΤΤΑ</mark> GG	G T T <mark>A</mark> GGG T T	A G G G T T A
Telomere.reference	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	ΤΤΑ <mark>GGGΤΤΑ</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G T T <mark>A</mark> G G G T T	AGGG <mark>TT</mark> A
consensus	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	T T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G G T T	A G G G <mark>T T</mark> A
	1270	1280	1290	1300	1310	1320	1330
1d.E2.filtered	1270 GGGTTAGGGTTAG	1280 GGTTAGGGTTA	<i>1290</i> G G G T T A G G G T	1300 TAGGGTTAG	1310 G G T T A G G G T T A	<i>1320</i> GGG <mark>TTA</mark> GGG	1330 T T A G G G T
1d.E2.filtered 2d.E2.filtered	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G	1280 G G T T A G G G T T A G G T T A G G G T T A	1290 G G G T T A G G G T G G G T T A G G G T	1300 T A G G G T T A G T A G G G T T A G	1310 G G T T A G G G T T A G G T T A G G G G T T A	1320 G G G T T A G G G G G G T T A G G G	1330 T T A G G G T T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G	1280 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1290 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T	1300 T A G G G T T A G T A G G G T T A G T A G G G T T A G	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1320 G G G T T A G G G G G G T T A G G G G G G T T A G G G G G G T T A G G G	1330 T T A G G G T T T A G G G T T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G	1280 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1290 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T	1300 T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G G T T A G	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1320         G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G	1330 T T A G G G T T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G	1280         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A       A       A       A </td <td>Image: Second state sta</td> <td>1300 T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G G T A A G T A G G G T T A G</td> <td>1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A</td> <td>1320         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G</td> <td>1330 T T A G G G T T T A G G G T</td>	Image: Second state sta	1300 T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G G T A A G T A G G G T T A G	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1320         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G	1330 T T A G G G T T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	Image: Constraint of the constraint	1280         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A	1290         G       G       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T	1 3 00         1 A G G G G T T A G         A G G G G T T A G         T A G G G G T T A G         A G G G G T T A G         A G G G G T A A G         A G G G G T A A G         A G G G G T A A G         A G G G G T A A G         A G G G G T A A G         A A G G G G T A A G         T A G G G G T A A G         T A G G G G T A A G	1310         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	1320         G       G       T       T       A       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G	1330         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T A G G G T T A G G G G T A G G G T T A G G G G T A G G G T T A G G G G T A G G G T T A G G G G T A G G G T T A G G G G T A G G G T T A G G G G T A G G G T T A G	1280 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A H A G G G T T A A H A G G G T T A H A G G G G T T A H A G G G T T T A H A G G G	1290 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T 1260	1300 T A G G G T T A G T A G G G T A G	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	1320         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G	1330 T T A G G G T T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G H G G G T T A G G G T T A G 1340	1280 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1350 A G G G T T A G G G T T A	1290 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T 1360 T A G C T T A G G G	1300 T A G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G T T A G	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1380 A G G G T T A G G G T T A	1320         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G G T T A G G G         G G G G T T A G G G         I G G G T T A G G G         I G G G T T A G G G	1330 T T A G G G T T T A G G G T 1400
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d E2 filtered	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G 1340 T A G G G T T A G G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G	1280 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1350 A G G G T T A G G G T T A A G G G T T A G G G T T A A G G G T T A G G G T T A A G G G T T A G G G T T A A G G G T T A G G G T T A A G G G T T A G G G T T A A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T	1290 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G G T T A G G G T 1360 T A G G G T T A G G T A G G G T T A G G T A G G G T T A G G T A G G G T T A G G	1300 T A G G G T T A G T A G G G T T A G	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T T A 1380 A G G G T T A G G G G T	1320 G G G T T A G G G G G G T T A G G G T A G G G T T A G G G 1390 T A G G G T T A G T A G G G T T A G	1330 T T A G G G T T T A G G G G T 1400 G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G 1340 T A G G G T T A G G G T T T A G G G G T T A G G G T T T A G G G G T T A G G G T T T A G G G G T T A G G G T T T A G G G G T T A G G G T T T A G G G G T T T A G G G T T T	1280 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 1350 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	1290 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T 1360 T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T	1300 T A G G G T T A G T T A G G G T T G T T A G G G T T	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T	1320         G       G       T       A       G       G         G       G       G       T       T       A       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         J       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T <td< td=""><td>1330         T       A       G       G       T         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G       G         G       G       T       T       A       G       &lt;</td></td<>	1330         T       A       G       G       T         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G       G         G       G       T       T       A       G       <
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T T A G G G T T A G G G T T A G G G T T T T A G G G G T T A G G G T T A G G G T T A G G G T T T A G G	1280         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G	1290 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T 1360 T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T A G G G G T T A G G G T T A G G G T T A G G G T A G G G G T T T A G G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G T T T A G G T T T A G G T T T A G G G T T T A G G T T T A G G T T T A G	1300 A G G G T T A G A G G G T T A G T A G G G T T G T T A G G G T T	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T	1320         G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G       T       A       G       G       G	1330 T T A G G G T T T A G G G T 1400 G G T T A G G G G T T A G G G G T T A G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T T T A G G G T T A G G G T T A G G G T T A G G G T T T T A G G G G T T A G G G T T A G G G T T A G G G T T T T A G G G G T T A G G G T T A G G G T T A G G G T T T T A G G G G T T A G G G T T A G G G T T A G G G T T T A G G G G	1280         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         G       G       T       T       A       G       G       T       A         G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A	1290         G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         T       A       G       G       G       T       T       A       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T <td< td=""><td>1 300         1 A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         G T A A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T</td><td>1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T</td><td>1320         G       G       T       A       G       G         G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G       T       A       G       G       G       T       A       G</td><td>1330         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       T       T         T       T       A       G       G       T       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G</td></td<>	1 300         1 A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         A G G G G T T A G         G T A A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T	1310 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T	1320         G       G       T       A       G       G         G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G       T       A       G       G       G       T       A       G	1330         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       T       T         T       T       A       G       G       T       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	1270 G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T A A G G G T T A G G G G T A A G G G T T A G G G G T A A G G G T T A G G G G T A A G G G T T A G T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T T T A G G G G T T A G G G T T A G G G T T A G G G T T T T A G G G G T T A G G G T T A G G G T T A G G G G	I I I I A       G       G       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A<	1290         G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       G       T       T       A       G       G       T         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       T       T <td< td=""><td>1 300         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G</td><td>1310         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G</td><td>1320         G       G       T       A       G       G         G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       <td< td=""><td>1330         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G</td></td<></td></td<>	1 300         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G         1 T A G G G G T T A G	1310         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G	1320         G       G       T       A       G       G         G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T <td< td=""><td>1330         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G</td></td<>	1330         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G

	1410	1420	1430	1440	1450	1460	1470
1d.E2.filtered	G T T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T	A G G G T T <mark>A </mark> G G G T	T <mark>A</mark> GGGTTAGG	G T T <mark>A</mark> G G G T T	A G G G T T A
2d.E2.filtered	G T T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T <mark>A</mark> GGG <mark>TT</mark> AGG	G T T A G G G T T	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGGTTAGG	G T T <mark>A</mark> GGGTT	A G G G T T A
1d.hapA.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G G T T	A G G G <mark>T T</mark> A
2d.hapA.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G G T T	A G G G <mark>T T</mark> A
Telomere.reference	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T	<mark>A</mark> GGG <mark>TT A</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TT</mark> AGG	G T T <mark>A</mark> G G G T T	A G G G <mark>T T</mark> A
consensus	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	T <mark>A</mark> G G G T T <mark>A</mark> G G	GTTAGGGTT	A G G G T T A G G G T	ΤΑΓΟΓ	G T T <mark>A</mark> G G G T T	A G G G T T A
	1480	1490	1500	1510	1520	1530	1540
1d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	T T <mark>A G G G T T A </mark> G G	GTTAGGGTTA	G G G <mark>T T A</mark> G G G	T T <mark>A</mark> G G G T
2d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	T T <mark>A G G G T T A </mark> G G	GTTAGGGTTA	G G G <mark>T T A</mark> G G G	T T <mark>A</mark> G G G T
1d.hapA.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	T T <mark>A G G G T T A </mark> G G	GTTAGGGTTA	G G G <mark>T T A</mark> G G G	T T <mark>A</mark> G G G T
2d.hapA.filtered	G G G T T A G G R T T A G G	G T T A G G G T T A	GGG <mark>TTA</mark> GGR	TTAGGGTTAGG	GTTAGGGTTA	GGG <mark>TTA</mark> GGG	T T <mark>A</mark> G G G T
Telomere.reference	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A	GGG <mark>TT</mark> AGGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A G G G T T A</mark>	GGG <mark>TTA</mark> GGG	T T <mark>A</mark> G G G T
consensus	G G G <mark>T T A</mark> G G G <mark>T T A</mark> G G	G T T <mark>A</mark> G G G T T A	G G G <mark>T T </mark> A G G G	T T <mark>A</mark> GGG <mark>T T A</mark> GG	G T T <mark>A G G G T T </mark> A	G G G <mark>T T A</mark> G G G	T T <mark>A</mark> G G G T
	1550	1560	1.5.7.0	1500	4500	1.600	1.51.0
1d.E2.filtered							
2d.E2.filtered				G G T T A G G G T T A			G G T T A G G
2d.E2.filtered 1d.hapA.filtered	T A G G G T T A G G G T T A         T A G G G T T A G G G T T A         T A G G G T T A G G G T T A         T A G G G T T A		A     G     G     A     A     G       T     A     G     G     T     T     A     G       T     A     G     G     G     T     T     A     G       T     A     G     G     G     T     T     A     G       T     A     G     G     G     T     T     A     G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A		A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G	
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	T     A     G     G     T     T     A     G     G     T     T       T     A     G     G     G     T     T     A     G     G     T     T       T     A     G     G     G     T     T     A     G     G     T     T       A     G     G     G     T     T     A     G     G     G     T       T     A     G     G     G     T     T     A     G     G     T     T		T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T	A       G       G       G       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G	
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	A       G       G       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       G       T       T       A       G       G       T       A         T       A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       G       T       T         A       G       G       G       T       T       A       G       G       G       T       A         T       A       G       G       G       T       T       A       G       G       G       T       A	G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T	A         G         G         G         T         A         G           T         A         G         G         T         T         A         G           T         A         G         G         G         T         T         A         G           T         A         G         G         G         T         T         A         G           T         A         G         G         G         T         T         A         G           T         A         G         G         G         T         T         A         G           T         A         G         G         G         T         T         A         G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T	A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G	G G T T A G G G G T T A G G
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       G       T       T       A       G       G       T       T         T       A       G       G       G       T       T       A       G       G       T       T         T       A       G       G       G       T       T       A       G       G       T       T       A         T       A       G       G       G       T       T       A       G       G       T       T       A         T       A       G       G       G       T       T       A       G       G       T       T       A         T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       <	G G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T	A     G     G     A     A     G       T     A     G     G     T     T     A     G       T     A     G     G     T     T     A     G       T     A     G     G     T     T     A     G       T     A     G     G     T     T     A     G       T     A     G     G     T     T     A     G       T     A     G     G     G     T     T     A	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T	A       G       G       G       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G	G G T T A G G G G T T A G G
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	A       G       G       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       G       T       T       A       G       G       T       T         T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       A       G       G       G       T       A       G       G       G       T       A       G       G       G       G       T       A       G       G       G       T       A       G       G       G       G       G       T       A	G G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T	T A G G G T       T A G         T A G G G T       T A G         T A G G G T       T A G         T A G G G T       T A G         T A G G G T       T A G         T A G G G T       T A G         T A G G G T       T A G         T A G G G G T       T A G         T A G G G G       T T A G         T A G G G G       T A G         T A G G G G       T A G         1640	G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A       G     G     T     T     A     G     G     G     T     T     A    1650     T     T     A	G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         1660	A G G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         1670	G G T T A G G           G G T T A G G           G G T T A G G           G G T T A G G           G G T T A G G           G G T T A G G           G G T T A G G           G G T T A G G           G G T T A G G
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	A       G       G       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       T       T       A       G       G       T       T       A         T       A       G       G       T       A       G       G       T       A         T       A       G       G       T       A       G       G       T       A         T       A       G       G       T       A       G       G       T       A         T       A       G       G       T       A       G       G       T       A       A         T       A	G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         I       A       G       G       G       T       T       A       G       G       T	T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         1640       G       G       T       T       A       G       G       T       T	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A <i>I 650</i> A G G G <b>T T A</b> G G G <b>T</b>	G G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         1660         T A G G G T T A G G G	A G G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         G T A G G G T T A G	G         T         T         A         G           G         G         T         T         A         G           G         G         T         T         A         G         G           G         G         T         T         A         G         G         G         T         T         A         G         G           G         G         T         T         A         G         G         G         T         T         A         G         G         G         T         T         A         G         G         G         T         T         A         G         G         G         T         T         A         G         G         G         T         T         A         G         G         T         T         A         G         G         T         T         A         G         G         T         T         A         G         G         T         T         A         G         G         T         T         A         G         G         T         T         A         G         G         T         T         A         G         G
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	T       A       G       G       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       T       T       A       G       G       T       T       A         G       G       G       T       A       G       G       G       T       A         G       G       T       A       G       G       T       A       G       G       T       A         G       G       T       A       G       G       T       A       G       G       T       A       G       G       T       A       G       G       T       A       G       G	G G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         A G G G T T A G G G T         A G G G T T A G G G T         A G G G T T A G G G T	A       G       G       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T         G       T       T       A       G       G       T       T	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A 1650 A G G G T T A G G G T	G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         1660       T       A       G       G       G       T       A       G       G         T       A       G       G       G       T       T       A       G       G	A G G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         G T A G G G T T A G         G T A G G G T T A G         G T A G G G T T A G G         G T A G G G G T T A G G G T T	G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     G     G     T     T       A     G     G     G     T     T
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	A       G       G       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       T         A       G       G       T       T       A       G       G       T       T         A       G       G       T       T       A       G       G       T       T         A       G       G       T       T       A       G       G       T       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         G       T       A       G       G       T       A       G       G       T       A         G       T       A       G       G       T       A       G       G       T       A         G       T	G       G       G       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         1630       T       A       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T	$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       T       A         G       G       G       T       T       A       G       G       G       T       A         A       G	G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         I       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G <td>A G G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         G T A G G G T T A G         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T</td> <td>G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       A     G     G     G     T     T       A     G     G     G     T     T</td>	A G G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         G T A G G G T T A G         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T         G T T A G G G G T T	G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       G     G     T     T     A     G       A     G     G     G     T     T       A     G     G     G     T     T
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	A       G       G       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         T       A       G       G       T       T       A       G       G       T       T       A         T       A       G       G       T       T       A       G       G       T       T       A         T       A       G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A       G       G       T       A       G       G       T       A       G       G       T       A       G       G       T       A       G       G       T       A       G       G       T	G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       G       T         G       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G <td>A       G       G       G       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G&lt;</td> <td>G       T       T       A       G       G       T       A         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T</td> <td>G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G<td>A       G       G       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       A       G       G       G       T       T         G       T       A       G       G&lt;</td><td>G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A</td></td>	A       G       G       G       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G<	G       T       T       A       G       G       T       A         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T	G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G <td>A       G       G       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       A       G       G       G       T       T         G       T       A       G       G&lt;</td> <td>G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A</td>	A       G       G       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       A       G       G       G       T       T         G       T       A       G       G<	G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A
2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	A = G = G = G = T + A = G = G = G = T + A = G = G = T = G = T + A = G = G = T + A = G = G = T = G = T + A = G = G = T + A = G = G = T = G = T + A = G = G = T + A = G = G = T = G = T = G = T = G = T = G = G	G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       G       T         G       G       G       T       T       A       G       G       G       T         G       G       G       G       T       T       A       G       G       G     <	A       G       G       A       A         A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T	G       G       T       A       G       G       T       A         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A	G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         T       A       G       G       G       T       T       A       G       G         T       A       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       G       T       A       G       G       G       G       G       G       G	A       G       G       G       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T	G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A         A       G       G       G       T       T       A

	1690	1700	1710	1720	1730	1740	1750
1d.E2.filtered	GGG <mark>TT</mark> AGGG <mark>TT</mark> /	<b>A</b> G G G T T <mark>A</mark> G G G T T	A G G G T T <mark>A G G G T</mark>	T <mark>A</mark> GGGTT <mark>A</mark> GG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T T	A G G G T
2d.E2.filtered	GGG <mark>TT</mark> AGGG <mark>TT</mark> /	A G G G T T <mark>A</mark> G G G T T	A G G G T T <mark>A G G G T</mark>	T <mark>AGGGTTA</mark> GG	G T T <mark>A</mark> G G G T T	AGGGTTAGGGTT	A G G G T
1d.hapA.filtered	G G G T T <mark>A</mark> G G G T T /	A G G G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> GGGTT <mark>A</mark> GG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T T	A G G G T
2d.hapA.filtered	G G G T T <mark>A</mark> G G G T T /	A G G G T T <mark>A</mark> G G G T T	A G G G T T <mark>A G G G</mark> T	T <mark>A </mark> G G G T T <mark>A</mark> G G (	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T T	A G G G T
Telomere.reference	GGG <mark>TT</mark> AGGG <mark>TT</mark> /	A G G G T T A G G G T T	A G G G T T <mark>A</mark> G G G T	T A G G G T T <mark>A</mark> G G G	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T T	A G G G T
consensus	GGGTTAGGGTT/	A G G G T T A G G G T T	AGGGTTAGGGT		G T T <mark>A</mark> G G G T T	AGGGTTAGGGTT	AGGGT
	1760	1770	1780	1790	1800	1810	1820
1d.E2.filtered	<mark>Τ</mark> Α <mark>ΘΘ<mark>ΕΤΤΑ</mark>ΘΘΘ</mark>	TT <mark>A</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G <mark>TTA</mark> GGG <mark>TTA</mark>	G G G T T <mark>A</mark> G G G	TTAGGGTTAGGG	i <mark>T T A</mark> G G
2d.E2.filtered	<mark>Τ</mark> Α <mark>ΘΘ<mark>ΟΤΤΑ</mark>ΘΘΘ</mark>	T T <mark>A</mark> G G G T T <mark>A</mark> G G G	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T <mark>A</mark> G G G	TTAGGGTTAGGG	T T <mark>A</mark> G G
1d.hapA.filtered	<mark>Τ</mark> Α <mark>ΘΘ<mark>ΟΤΤΑ</mark>ΘΘΘΤ</mark>	TT <mark>A</mark> GGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark>	GGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GGG	T T <mark>A</mark> G G
2d.hapA.filtered	Τ <mark>Α</mark> ΘΘ <mark>ΑΤΤΑ</mark> ΘΘΘ	TT <mark>A</mark> GGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> GGG T T <mark>A</mark> G	GGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TT</mark> AGGG	TT <mark>A</mark> GG
Telomere.reference	T <mark>A</mark> GG <mark>GTTA</mark> GGG	<mark>TTA</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTAGG	G T T <mark>A</mark> GGG T T <mark>A</mark> G	G G G <mark>T T A</mark> G G G	TTAGGGTTAGGG	i <mark>tt</mark> AGG
consensus	TAGGGTTAGGG		TTAGGGTTAGG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG		TTAGG
					· · · · · · · · · · · ·		
	1830	1840	1850	1860	1870	1880	1890
1d.E2.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> G	G <mark>TTA</mark> GGG <mark>TTA</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark>	GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GG <mark>TTA</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark> G	i G G <mark>T T A</mark>
2d.E2.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> G	G <mark>TTA</mark> GGG <mark>TTA</mark> G	GGTTAGGGTTA	GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TTA</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark> G	i G G <mark>T T A</mark>
1d.hapA.filtered	G T T <mark>A</mark> G G G T T <mark>A</mark> G G	G <mark>T T A</mark> G G G <mark>T T A</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark>	GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TTA</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark> G	i G G <mark>T T A</mark>
2d.hapA.filtered	G T T <mark>A</mark> G G G T T <mark>A</mark> G G	G <mark>T T A</mark> G G G <mark>T T A</mark> G	G R T T A G G G T T A	GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TTA</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark> G	i G G <mark>T T A</mark>
Telomere.reference	G <mark>TTA</mark> GGG <mark>TTA</mark> G	G <mark>TTA</mark> GGG <mark>TTA</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark>	GGG <mark>TTA</mark> GGG <mark>T</mark> T	T <mark>A</mark> GGG <mark>TTA</mark> G	GG <mark>TTA</mark> GGG <mark>TTA</mark> G	i G G <mark>T T A</mark>
consensus	G T T A G G G T T A G G	G G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A G G G T T A	GGG <mark>TTA</mark> GGG <mark>T</mark>		GGTTAGGGTTA	IGG <mark>TT</mark> A
	1900	1910	1920	1930	1940	1950	1960
1d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TT</mark>	Α <mark>G G G T T A</mark> G G G <mark>T </mark> T	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G <mark>TTA</mark> GGGTT	A G G G T T A G G G T T	A G G G T
2d.E2.filtered	ϾϾϾͳͳ <mark>Ϸ</mark> ϾϾϾͳͳ	<mark>Α</mark> G G G <mark>Τ Τ </mark> Α G G G <mark>Τ </mark> Τ	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G <mark>TTA</mark> GGGTT	A G G G T T A G G G T T	A G G G T
1d.hapA.filtered	GGGTTA	A G G G <mark>T T A</mark> G G G <mark>T </mark> T	A G G G T T A G G G T		GTTAGGGTT		AGGG
1d.hapA.filtered 2d.hapA.filtered	G G G T T <mark>A</mark> G G G T T <mark>/</mark> G G G T T <mark>A</mark> G G G T T <mark>/</mark>	A     G     G     T     T     A     G     G     T     T       A     G     G     G     T     T     A     G     G     G     T     T	A G G G T T A G G G T A G G G T T A G G G T		G T T A G G G T T G T T <mark>A</mark> G G G T T		AGGGT AGGGT
1d.hapA.filtered 2d.hapA.filtered Telomere.reference	G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A	A         G         G         T         T         A         G         G         T         T           A         G         G         G         T         T         A         G         G         T         T           A         G         G         G         T         T         A         G         G         G         T         T           A         G         G         G         T         T         A         G         G         G         T         T	A     G     G     T     T     A     G     G     T       A     G     G     G     T     T     A     G     G     T       A     G     G     G     T     T     A     G     G     G       A     G     G     G     T     T     A     G     G     G	IT     A     G     G     T     T     A     G     G       IT     A     G     G     T     T     A     G     G       IT     A     G     G     G     T     T     A     G     G       IT     A     G     G     G     T     T     A     G     G	G T T A G G G T T G T T A G G G T T G T T A G G G T T A G G G T T	A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T	AGGGT AGGGT

	1970	1980	1990	2000	2010	2020	2030
1d.E2.filtered	T <mark>A G G G T T A G G G T T</mark>	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>TT</mark> AGG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GGR	T T <mark>A</mark> G G
2d.E2.filtered	T <mark>A</mark> GGGTTA <mark>G</mark> GGTT	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>TT</mark> AGGG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> GGGTTAGGR	T T <mark>A</mark> G G
1d.hapA.filtered	T <mark>A</mark> G G G T T <mark>A</mark> G G G T T	A G G G <mark>T T A</mark> G G G T	T <mark>A</mark> G G G T T <mark>A</mark> G G G	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GGR	T T <mark>A</mark> G G
2d.hapA.filtered	T <mark>A</mark> G G G T T <mark>A</mark> G G G T T	A G G G <mark>T T A</mark> G G G T	T <mark>A</mark> G G G T T <mark>A</mark> G G G	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T	T T <mark>A</mark> G G
Telomere.reference	T <mark>A</mark> G G G T T <mark>A</mark> G G G T T	A G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GGG	G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GGG	T T <mark>A</mark> G G
consensus	T A G G G T T A G G G T T	A G G G T T A G G G T	T A G G G T T A G G G	G T T <mark>A</mark> G G G T T	AGGGTTAGGGT	T A G G G T T A G G G	T T <mark>A</mark> G G
	2040	2050	2060	2070	2080	2090	2100
1d.E2.filtered	R T T A G G G T T A G G G	T T <mark>A</mark> GGG T T <mark>A</mark> GG	G T T A G G G T T A	G G G <mark>T T A</mark> G G G	T T <mark>A</mark> G G G <mark>T T A</mark> G G	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G <mark>T T A</mark>
2d.E2.filtered	R T T A G G G T T A G G G	T T <mark>A</mark> GGG T T <mark>A</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	T T <mark>A</mark> G G G <mark>T T A</mark> G G	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A
1d.hapA.filtered	R T T A G G G T T A G G G	TTAGGGTTAGG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	Τ <mark>ΤΑ</mark> GGG <mark>ΤΤΑ</mark> GG	G T T <mark>A</mark> GGG T T <mark>A</mark> G	G G T T A
2d.hapA.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG	<mark>Τ Τ Α</mark> G G G <mark>Τ Τ Α</mark> G G	G T T A G G G T T A	GIGIGI <mark>TITIA</mark> IGIGIG	TT <mark>A</mark> GGG <mark>TT</mark> AGG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A
Telomere.reference	GTTAGGGTTAGGG	<mark>Τ Τ Α</mark> GGG <mark>Τ Τ Α</mark> GG	GTTAGGGTTA	GGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T A G	G <mark>G T T </mark> A
consensus	G T T A G G G T T A G G G	ΤΤΑΓΟΟΓΑΟ	GTTAGGGTTA	G G G T T <mark>A</mark> G G G	T T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A
	2110	2120	2130	2140	2150	2160	2170
1d.E2.filtered	GGGTTAGGGTTAG	G G T T A G G G T T A	GGG <mark>TT</mark> AGGGGT	T <mark>A</mark> GGG <mark>TTA</mark> G	G G T T A G G G T T A	GGG <mark>TTA</mark> GGG <mark>TT</mark>	<mark>A</mark> GGG <mark>T</mark>
2d.E2.filtered							
1d hon A filtorod		GIGITIT <mark>A</mark> IGIGIGITITA	G G G T T <mark>A</mark> G G G T T	T <mark>A</mark> GGG <mark>TTA</mark> G	G G T T <mark>A</mark> G G G T T A	G G G T T A G G G T T	AGGG
ru.napA.nitereu	G G G T T A G G G T T A G	G G T T A G G G T T A G G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T A G G G T T G G G T T A G G G T T	T A G G G T T A G T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T T G G G T T A G G G T T	A G G G T A G G G T
2d.hapA.filtered	G G G T T A G G G T T A G G G G T T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G R T	T A G G G T T A G T A G G G T T A G T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T	A G G G T A G G G T A G G G T
2d.hapA.filtered Telomere.reference	G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T C G G G T T A G G G T C G G G T T A G G R T C G G G T T A G G R T C G G G T T A G G G T C	A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G	G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T
2d.hapA.filtered Telomere.reference consensus	G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G R T G G G T T A G G G T G G G T T A G G G T	T A G G G T T A G T A G G G T T A G	G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T
2d.hapA.filtered Telomere.reference consensus	G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	G G G T T A G G G T G G G T T A G G G T G G G T T A G G R T G G G T T A G G R T G G G T T A G G G T G G G T T A G G G T	T A G G G T T A G T A G G G T T A G A G G G T T A G T A G G G T T A G T A G G G T T A G	G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T	A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T
2d.hapA.filtered Telomere.reference <i>consensus</i>	G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G <i>G</i> G G T T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 2190	G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T C C G C T T A C C C C C C C C C C C C C C C C	T A G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G T T A G T A G G G T T A G T A G G G T T A G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A C G G T T A A G G G T T A	G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T C 2230	A G G G T A G G G G T A G G G G T A G G G T A G G G T 22240
2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       A       G       G       G       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       G       G       T       T       G       G       G       T       T       G       G       T       T       G       G       G       T       T       G       G       G       T       T       G       G       G       T       T       G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A <i>2190</i> A G G R T T A G G G T	G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T C G G G T T A G G G T T C G G G T T A G G G T T C C C C C C C C C C C C C C C C C C	T A G G G T T A G T A G G G T T A G 2210 G T A G G G T T	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G G G T T A G G G T T A 2220 A G G G T T A G G G T T A	G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T 2230 T A G G G T T A G G G G	A G G G T A G G G T A G G G T A G G G T A G G G T 2240 T T A G G
2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       G       T       T       A       G         G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A C G G T T A G G G T T A C C C C C C C C C C C C C C C C C C C	G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     G     T	I     A     G     G     T     T     A     A       I     A     G     G     T     T     A     G       I     A     G     G     G     T     T     A     G       I     A     G     G     G     T     T     A     G       I     A     G     G     G     T     T     A     G       I     A     G     G     G     T     T     A     G       I     A     G     G     G     T     T     A     G       I     A     G     G     G     T     T     A     G       I     A     G     G     G     T     T     A     G       I     T     A     G     G     G     T     T	G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         22220       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G	G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       G       G       T       T       A       G       G       G       G	A       G       G       G         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         Z2240       T       T       A       G         T       T       A       G       G
1d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered 2d.exercised 1d.E2.filtered 1d.hapA.filtered	G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       G       T       T       A       G         G       G       G       T       T       A       G       G       G       T       T       A       G         G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T <td< td=""><td>G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A <i>2190</i> A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T</td><td>G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     G     T     T     A     G     G       T     A     G     G     G     T     T     A     G     G       T     A     G     G     G     T     T     A     G     G       G     G     G     T     T     A     G     G     G</td><td>I       A       G       G       T       T       A       G         I       A       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G&lt;</td><td>G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T</td><td>G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       T       T       A       G       &lt;</td><td>A       G       G       G         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         Z2240       T       T       A       G       G         T       T       A       G       G       T         T       T       A       G       G       G         T       T       A       G       G       G</td></td<>	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A <i>2190</i> A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T	G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     G     T     T     A     G     G       T     A     G     G     G     T     T     A     G     G       T     A     G     G     G     T     T     A     G     G       G     G     G     T     T     A     G     G     G	I       A       G       G       T       T       A       G         I       A       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G<	G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T	G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       T       T       A       G       <	A       G       G       G         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         Z2240       T       T       A       G       G         T       T       A       G       G       T         T       T       A       G       G       G         T       T       A       G       G       G
1d.E2.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G G G G T T A G G G T T A G C G G G T T A G G G T T A G C G G G T T A G G G T T A G T A G G G G T T A G G G T T T A G G G T T A G G G T T T A G G G T T A G G G T T T A G G G T T A G G G T T	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T	G       G       T       T       A       G       G       T         G       G       T       T       A       G       G       T       T         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       G       T       T       A       G       G       G       T         G       G       G       G       T       T       A       G	I       A       G       G       T       T       A       G         I       A       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       G       T       T       A         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G       G       G       T       T         I       T       A       G<	G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       T         A       G	G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       G       T       T       A       G       G       G       G       T         G       G       G       G       T       T       A       G       G       G       G       G       G       G       G       G	A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         Z2240       Z2240       Z       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G
2d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered 7elomere.reference	G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G         G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       <	G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T A G G R T T A G G G T A G G G T T A G G G T	G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     T       G     G     G     T     T     A     G     G     G       T     A     G     G     G     T     T     A     G     G       T     A     G     G     G     T     T     A     G     G       G     G     G     T     T     A     G     G     G       G     G     G     T     T     A     G     G     G       T     A     G     G     G     T     T     A <td>I       A       G       G       T       T       A       G         I       A       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       T       A       G       G       G       T       T       A         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T     &lt;</td> <td>G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       T         A       G</td> <td>G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       G       T       T       A       G       G       G       G       T       T       A       G       G       G       G       G       G       G       G       G       G       G       G       G       G       G       G</td> <td>A       G       G       G         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         Z       Z       Z       Z       Z         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G</td>	I       A       G       G       T       T       A       G         I       A       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       A       G       G       G       T       T       A       G         I       T       A       G       G       G       T       T       A         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T     <	G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       T         A       G	G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       G       T       T       A       G       G       G       G       T       T       A       G       G       G       G       G       G       G       G       G       G       G       G       G       G       G       G	A       G       G       G         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         Z       Z       Z       Z       Z         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G

	2250	2260	2270	2280	2290	2300	2310
1d.E2.filtered	G T T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G <mark>T T </mark> A G G G T T A	G G G T T A G G R T	T <mark>A</mark> GGG T T A GGG	TTAGGGTTA	G G G T T A
2d.E2.filtered	G T T A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> AGG	G <mark>TTA</mark> GGG <mark>TT</mark> A		T <mark>A</mark> GGG T T A GGG	TTAGGGTTA	G G G <mark>T T</mark> A
1d.hapA.filtered	G T T A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TT A</mark> GG	G <mark>T T A</mark> G G G <mark>T T </mark> A	GGGTTAGGRT	T <mark>A</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTA	G G G <mark>T T </mark> A
2d.hapA.filtered	G T T A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TT A</mark> GG	G <mark>T T A</mark> G G G <mark>T T </mark> A	GGRTTAGGRT	T <mark>A</mark> GGG <mark>TTA</mark> GGG	TTAGGGTTA	G G G <mark>T T</mark> A
Telomere.reference	G <mark>TTA</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GG <mark>GTTA</mark> GG	G <mark>TTA</mark> GGG <mark>TT</mark> A	GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TT</mark> AGGG	TTAGGGTTA	G G G T T A
consensus	G T T <mark>A</mark> G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>T T A</mark> GG	G <mark>T T A</mark> G G G <mark>T T </mark> A	G G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>TT</mark> AGGG	TT <mark>A</mark> GGGTTA	G G G T T A
	2320	2330	2340	2350	2360	2370	2380
1d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	GTTAGGGTTA	GGG <mark>TTA</mark> GGG		GTTAGGGTTAG	GGTTAGGGT	
2d.E2.filtered	GGG <b>TTA</b> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T A	GGG <mark>TT</mark> AGGG		GTTAGGGTTAG	GGTTAGGGT	
1d.hapA.filtered	GGG <b>TTA</b> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T A			GTTAGGGTTAG	GGTTAGGGT	
2d.hapA.filtered	GGG <b>TTA</b> GGG <b>TTA</b> GGG	G T T <mark>A</mark> G G G T T A			GTTAGGGTTAG		T <mark>A</mark> GGG T
Telomere.reference	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GGG	G <mark>TTT</mark> AGGG <mark>TTT</mark> A	GGG <mark>TTA</mark> GGGG		G T T <mark>A</mark> G G G T T <mark>A</mark> G	GG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGGG T
consensus	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T A	GGG <mark>TT</mark> AGGG	T <mark>A</mark> GGG T T <mark>A</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG T
	2200	2400	2410	2420	2420	2440	2450
1d E2 filtorod							
Id.E2.Ilitered							
20.E2.IIItered							
2d han A filtored							
consensus							
consensus		GGG <mark>GTT A</mark> GGGGT		IG <mark>TTTA</mark> GGGGTTTA	GGGG <mark>TTTA</mark> GGGGTTT		GIIIAGG
	2460	2470	2480	2490	2500	2510	2520
1d.E2.filtered	G T T A G G G T T A G G G T	T A G G G T T A G G	G T T A G G G T T A		TAGGGTTAGGG	TTAGGGTTA	GGGTTA
2d.E2.filtered	G T T A G G G T T A G G G T	ΤΑΓΟΓΟΤΤΑΓΟ	G T T <mark>A</mark> G G G T T A	GGGTTAGGGT	TAGGGTTAGGG	T T A G G G T T A	GGGTTA
1d.hapA.filtered	G T T A G G G T T A G G G T	T <mark>A G G G T T A </mark> G G	G T T A G G G T T A	GGGTTAGGGT	TAGGGTTAGGG	TTAGGGTTA	GGGTTA
2d.hapA.filtered	G T T A G G G T T A G G G T	T A G G G T T A G G	G T T A G G G T T A		TAGGGTTAGGG	TTAGGGTTA	GGRTTA
Telomere reference	G T T A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> GG	<mark>σ Τ Τ Α</mark> σ σ σ Τ Τ <i>Α</i>		TAGGGTTAGGG	TTAGGGTTA	GGGTTA
000000000							

	2530	2540	2550	2560	2570	2580	2590
1d.E2.filtered	G G G T T <mark>A</mark> G G G T T	<mark>A</mark> GGG <mark>TTA</mark> GGGT	T <mark>A G G G T T A </mark> G G G	T T <mark>A G G G T T A</mark> G G	G T T <mark>A</mark> G G G T T	T <mark>A</mark> GG <mark>GTTA</mark> GGGT	T <mark>A</mark> GGG T
2d.E2.filtered	GGG <mark>TT</mark> AGGG <mark>T</mark> T	A G G G T T A G G G T	Τ <mark>Α</mark> ΘΘ <mark>ΤΤ</mark> ΑΘΘΘ	T T <mark>A G G G T T A G G</mark>	G T T <mark>A</mark> G G G T T	T <mark>A</mark> GGG <mark>TT</mark> AGGGT	T <mark>A</mark> GGG T
1d.hapA.filtered	G G G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T A G G G T T A G G G	T T <mark>A G G G T T A G G</mark>	G T T <mark>A</mark> G G G T T	Г <mark>А</mark> G G G <mark>T T </mark> A G G G T	T <mark>A</mark> GGG T
2d.hapA.filtered	G G G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A G G G T T A G G G</mark>	T T <mark>A G G G T T A G G</mark>	G T T <mark>A</mark> G G G T T	Г <mark>А</mark> G G G <mark>T T </mark> А G G G T	T <mark>A</mark> GGG T
Telomere.reference	G G G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A G G G T T A</mark> G G G	T T <mark>A</mark> GGG T T <mark>A</mark> GG	G T T <mark>A</mark> G G G T T	T <mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG T
consensus	GGGTTAGGGTT	AGGGTTAGGGT	T A G G G T T A G G G	T T <mark>A G G G T T A G G</mark>	G T T A G G G T T	T <mark>A</mark> GGG <mark>TTA</mark> GGGT	T <mark>A</mark> GGG T
	2600	2610	2620	2630	2640	2650	2660
1d.E2.filtered	<mark>T</mark> AGGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	R T T A G G G T T A G	G R T T A G G G T T A	GGG <mark>TTA</mark> GGC	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G <mark>T T A</mark> G G
2d.E2.filtered	<mark>T</mark> AGGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	R T T A G G G T T A G	G R T T A G G G T T A	GGG <mark>TTA</mark> GGC	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G <mark>T T A</mark> G G
1d.hapA.filtered	<mark>T</mark> AGGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	R T T A G G G T T A G	G R T T A G G G T T A	GGG <mark>TTA</mark> GGC	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G <mark>T T A</mark> G G
2d.hapA.filtered	<mark>Τ</mark> Α <mark>GGG</mark> ΤΤΑGGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A S	S G T T A G G G T T A	G G R T T A G G C	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G
Telomere.reference	<mark>Τ</mark> Α <mark>G</mark> G <mark>G</mark> ΤΤΑGGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G <mark>TTA</mark> GGG <mark>TTA</mark> G	G G T T A G G G T T A	GGG <mark>TTA</mark> GGG	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G <mark>T T A</mark> G G
consensus	<mark>Τ</mark> Α <mark>G</mark> G <mark>ΤΤΑ</mark> GGG	T T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A G G G T T A	GGG <mark>TTA</mark> GGG	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G
	2670	2680	2690	2700	2710	2720	2730
1d.E2.filtered	2670 GTTAGGGTTAG	2680 G G T T A G G G T T A	2690 GGGTTAGGGTT	2700 A G G G T T A G G G T	2710 <mark>T A G G G T T A</mark> G	2720 5 G G T T A G G G T T A	2730 GGGTTA
1d.E2.filtered 2d.E2.filtered	<i>2670</i> G T T A G G G T T A G G T T A G G G T T A G	2680 G G T T A G G G T T A G G T T A G G G T T A	2690 G G G T T A G G G T T G G G T T A G G G T T	2700 A G G G T T A G G G T A G G G T T A G G G T	2710 T A G G G T T A C T A G G G T T A C	2720 G G G T T A G G G T T A G G G T T A G G G T T A	2730 G G G T T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	2670 G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G	2680 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	2690 G G G T T A G G G T T G G G G T T A G G G T T G G G T T A G G G T T	2700 A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T	2710 T A G G G T T A C T A G G G T T A C T A G G G T T A C	2720 G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A	2730 G G G T T A G G G T T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	2690         G G G T T A G G G T T         G G G T T A G G G T T         G G G T T A G G G T T         G G G R T T A G G G T T         G G G R T T A G G G T T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	2710 T A G G G T T A C T A G G G T T A C T A G G G T T A C T A G G G T T A C	2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A	2730         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T       A       G	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	2690         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       R       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	2710         A       G       G       T       A       A         A       G       G       T       T       A       C         T       A       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C	2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A	2730         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	G       T       A       G       G       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	2690         G       G       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	2710         A       G       G       T       A       A         T       A       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C         T       A       G       G       G       T       T       A       C	2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       A         G       G       G       T       T </td <td>2730         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A</td>	2730         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       A       G	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         G       G       T       T       A       G       G       G       T       A	2690         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	2710 T A G G G T T A G T A G G G T T A G	2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G </td <td>2730         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A</td>	2730         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	2670 G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         C       G       T       T       A       G       G       G       T       T       A         C       G       T       T       A       G       G       G       T       T       A         C       C       F       G       G       G       T       T       A       C       C </td <td>2690 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T 2760 2760</td> <td>A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T</td> <td>2710 T A G G G T T A G T A G G G T T A G</td> <td>2720 G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A C T A G G G T T A G G G G T T T A C T A G G G T T A G G G G T T T A C T A G G G T T T A G G G G T T T A C T A G G G T T T A G G G G T T T A C T A G G G T T T A G G G G T T T A C T A G G G T T T A G G G G T T T A G G G G</td> <td>2730 G G G T T A G G G T T A C S C G T T A C S C C T T A</td>	2690 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T 2760 2760	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	2710 T A G G G T T A G T A G G G T T A G	2720 G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A C T A G G G T T A G G G G T T T A C T A G G G T T A G G G G T T T A C T A G G G T T T A G G G G T T T A C T A G G G T T T A G G G G T T T A C T A G G G T T T A G G G G T T T A C T A G G G T T T A G G G G T T T A G G G G	2730 G G G T T A G G G T T A C S C G T T A C S C C T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	2670 G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G C T T A G G G T T A G G G T T	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         G       G       T       T       A       G       G       G       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G	2690         G       G       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	2710         A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         Z7780       G       T       A       G       G       G       T       A       G	2720 G G T T A G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A C T A G G G T T A G G G T T A 2790 T A G G G T T A G G G T T A	2730 G G G T T A G G G T T A C B G G T T A C B G G T T A 2800 T A G G G T T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	2670 G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G 2740 G G G T T A G G G T T G G G T T A G G G T T	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         G       G       G       T       T       A       G       G       G       T       A         G       G       G       T       T       A       G       G       G       G       T       A	2690         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       G       G       G       G       G </td <td>A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G</td> <td>2710 T A G G G T T A G T A G G G G T T A G T A G G G G T T T A G G G G T T T A G T A G G G G T T T A G G G G T T T A G T A G G G G T T T A G G G G T T T A G G G G</td> <td>2720 G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A C A G G G T T A G G G T T A 2790 A G G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G T A G G G T A G G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G</td> <td>2730 G G G T T A G G G T T A C C G G T T A C C G G G G G T T A C C G G G G G G T T A C C G G G G G G G T T A C C G G G G G G G G G G G G G G G G G G</td>	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G	2710 T A G G G T T A G T A G G G G T T A G T A G G G G T T T A G G G G T T T A G T A G G G G T T T A G G G G T T T A G T A G G G G T T T A G G G G T T T A G G G G	2720 G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A C A G G G T T A G G G T T A 2790 A G G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G T A G G G T A G G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G T T A G G G G	2730 G G G T T A G G G T T A C C G G T T A C C G G G G G T T A C C G G G G G G T T A C C G G G G G G G T T A C C G G G G G G G G G G G G G G G G G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	2670 G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T A G G G T T A G G T A G G G T T A G G T A G G G T T A G G T A G G G T T A G G G T A G G G T T A G G G G T T A G G G T T G G G G T T A G G G G T T A G G G G G T T G G G G T T A G G G G T T A G G G G G T T G G G G T T A G G G G T T A G G G G G T T G G G G T T A G G G G T T A G G G G G T T G G G G T T A G G G G T T A G G G G G T T T G G G G T T A G G G G T T A G G G G G T T A G G G G	G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         A       G       G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       G	2690         G       G       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       G       G       G       G       G       G       G       G       G </td <td>A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G</td> <td>2710         A       G       G       T       T       A         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       &lt;</td> <td>2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A       A       A       T       A       G       G       T       T       A       A<td>2730         G       G       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G</td></td>	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G	2710         A       G       G       T       T       A         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       <	2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A       A       A       T       A       G       G       T       T       A       A <td>2730         G       G       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G</td>	2730         G       G       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G         T       A       G       G       G       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	2670 G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G G T A G G G T A G G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G G G T A G A G G G T A G G G T A G A G G G T A G G G T A G A G G G T A G G G T A G A G A G G T A G A G A C A G A G A C A C A G A C A C A G A C A C A G A C A C A C A C A C A C A C A C A C A C A C A C A	2680         G       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         G       G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T	2690         G       G       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       G       G       G       G       G       G       G       G       G </td <td>A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G       G         T       T       A       G       G       R       T       T       A</td> <td>2710 T A G G G T T A G T T A G G G T T A G G T T A G G G T T G T A G G G T T A G G G G T T A G G G T T A G G G G G T T A G G G G G G T T A G G G G G G T T A G G G G</td> <td>2720         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       A         G       G       G       T       T       A       G       G       G       T       A<!--</td--><td>2730         G       G       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T</td></td>	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G       G         T       T       A       G       G       R       T       T       A	2710 T A G G G T T A G T T A G G G T T A G G T T A G G G T T G T A G G G T T A G G G G T T A G G G T T A G G G G G T T A G G G G G G T T A G G G G G G T T A G G G G	2720         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       A         G       G       G       T       T       A       G       G       G       T       A </td <td>2730         G       G       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T</td>	2730         G       G       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	2670 G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A G G G G T T A G G G T T G G G T T A G G G G T T A G G G G T T G G G T T A G G G T T A G G G G T T G G G T T A G G G G T T A G G G G T T G G G T T A G G G T T A G G G G T T A G G G G	2680         G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G	2690         G       G       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       G       G       G       G       G       G       G       G       G       G       G       G       G <td>A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G       G         T       T       A       G       G       R       T       T       A</td> <td>2710         A       G       G       T       A       A         A       G       G       T       T       A       C         A       G       G       T       T       A       C         A       G       G       T       T       A       C         T       A       G       G       T       T       A       C         T       A       G       G       T       T       A       C       C         T       A       G       G       G       T       T       A       C       C       T       A       C         G       T       T       A       G       G       G       T       T       A       C       C       T       A       C       C       T       T       A       C       C       T       A       C       C       T       T       A       C       C       T       A       C       C       T       A       C       C       T       A       C       C       T       T       A       C       C       T       T       A       C       C</td> <td>2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A       A       G       T       T       A       A       A       A       A       G       T       T       A       A       T       A       A       A       T       A       G       G</td> <td>2730         G       G       T       A         G       G       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       G       T         T       A       G       G       G       G       T         T       A       G       G       G       G       G         T       A       G       G       G       G       G</td>	A       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       R       T       T       A       G       G         T       T       A       G       G       R       T       T       A	2710         A       G       G       T       A       A         A       G       G       T       T       A       C         A       G       G       T       T       A       C         A       G       G       T       T       A       C         T       A       G       G       T       T       A       C         T       A       G       G       T       T       A       C       C         T       A       G       G       G       T       T       A       C       C       T       A       C         G       T       T       A       G       G       G       T       T       A       C       C       T       A       C       C       T       T       A       C       C       T       A       C       C       T       T       A       C       C       T       A       C       C       T       A       C       C       T       A       C       C       T       T       A       C       C       T       T       A       C       C	2720         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A       A       G       T       T       A       A       A       A       A       G       T       T       A       A       T       A       A       A       T       A       G       G	2730         G       G       T       A         G       G       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         G       G       G       T       T       A         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       T         T       A       G       G       G       G       T         T       A       G       G       G       G       T         T       A       G       G       G       G       G         T       A       G       G       G       G       G

	2810	2820	2830	2840	2850	2860	2870
1d.E2.filtered	T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T <mark>A</mark> GGG <mark>TTA</mark> GG	R T T <mark>A</mark> GGGTT	A G G G T T <mark>A</mark> G G G T	T T <mark>A</mark> G G G T T <mark>A</mark> G	G G <mark>T T A</mark> G G
2d.E2.filtered	T <mark>A</mark> GGG <mark>TTA</mark> GGGT	T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T <mark>A</mark> GGG <mark>TT</mark> AGG	R T T <mark>A</mark> G G G T T	A G G G T T <mark>A</mark> G G G T	TT <mark>A</mark> GGG <mark>TTA</mark> G	G G T T <mark>A</mark> G G
1d.hapA.filtered	T <mark>A</mark> GGG <mark>TTA</mark> GGGT	T <mark>A G G G T T A G G G T</mark>	T <mark>A</mark> GGG <mark>T T A</mark> GG	R T T A G G G T T	A G G G T T <mark>A</mark> G G G T	T T <mark>A</mark> G G G T T <mark>A</mark> G	G G <mark>T T A</mark> G G
2d.hapA.filtered	T <mark>A</mark> GGG <mark>TTA</mark> GGGT	T <mark>A G G G T T A G G G T</mark>	T <mark>A</mark> GGG <mark>T T A</mark> GG	G T T <mark>A</mark> G G R T T	A G G G T T <mark>A</mark> G G G T	T T <mark>A</mark> S <mark>G G T T A</mark> G	G G <mark>T T A</mark> G G
Telomere.reference	T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T <mark>A</mark> GG <mark>G</mark> T T <mark>A</mark> GG <mark>G</mark> T	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T	A G G G T T <mark>A</mark> G G G T	T T <mark>A</mark> G G G T T <mark>A</mark> G	G G <mark>T T A</mark> G G
consensus	T A G G G T T A G G G T	T <mark>A G G G T T A G G G T</mark>	T <mark>A G G G T T A </mark> G G	G T T A G G G T T	AGGGTTAGGG	TTAGGGTTAG	G G T T A G G
	2880	2890	2900	2910	2920	2930	2940
1d.E2.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A S S G T T A G G	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	TTAGGGTTAG	G G <mark>T T A</mark> G G G <mark>T T</mark>	A G G G T T A
2d.E2.filtered	G T T A G G G T T A G G	G T T A S S G T T A G G	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	T T A G G G T T A G C	G G T T <mark>A</mark> G G G T T	A G G G T T A
1d.hapA.filtered	G T T A G G G T T A G G	G T T <mark>A</mark> S S <mark>G T T A</mark> G G	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	T T A G G G T T A G C	G G T T A G G G T T	AGGGTTA
2d.hapA.filtered	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G <mark>T T A</mark> G G	G T T <mark>A</mark> GGG T T A	GGG <mark>TTA</mark> GGG	T T A G G R T T A G C	G G T T A G G G T T	AGGGTTA
Telomere.reference	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG		G <mark>TTA</mark> GGG <mark>TT</mark>	A G G G T T A
consensus	G T T A G G G T T A G G	G <mark>T T </mark> A G G G <mark>T T </mark> A G G	G T T A G G G T T A	G G G <mark>T T </mark> A G G G	T T A G G G T T A G C	G G T T <mark>A</mark> G G G T T	A G G G <mark>T T</mark> A
	2950	2960	2970	2980	2990	3000	3010
1d.E2.filtered	<i>2950</i> G G G T T A G G G T T A	2960 G G G T T A G G G T T A	<i>2970</i> G G G T T A G G G T	2980 T A G G G T T A G	2990 G G T T A G S G T T A	<i>3000</i> Agggg <mark>tttaggg</mark>	<i>3010</i> <b>T T A G G G T</b>
1d.E2.filtered 2d.E2.filtered	<i>2950</i> G G G T T A G G G T T A G G G T T A G G G T T A	<i>2960</i> G G G T T A G G G T T A G G G T T A G G G T T A	2970 G G G T T A G G G T G G G T T A G G G T	2980 T A G G G T T A G T A G G G T T A G	2990 G G T T A G S G T T A G G T T A G S G T T A	<i>3000</i> A G G G T T A G G G A G G G T T A G G G	<i>3010</i> T T A G G G T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	2950 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A	2970 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T	2980 T A G G G T T A G T A G G G T T A G T A G G G T T A G	2990 G G T T A G S G T T A G G T T A G S G T T A G G T T A G S G T T A	<i>3000</i> A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G	<i>3010</i> T T A G G G T T T A G G G T T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       N       G       T       T       A       G       G       G       T       T       A         G       N       G       T       T       A       G       G       G       T       T       A         G       N       G       T       T       A       G       G       G       T       T       A	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G R T T A	2970 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G R T	2980 T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G R T A G T A G G R T T A G	2990         G       T       A       G       G       T       A         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       R       T       T       A       G       G       G       T       T       A         G       R       T       T       A       G       G       G       T       T       A         G       R       T       T       A       G       G       G       T       T       A	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A S G R T T A G G G A S G R T T A G G G	3010 T T A G G G T T T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	2950         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       N       G       T       T       A       G       G       G       T       T       A         G       N       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       A       A       A       A       A       A <td>2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G R T T A G G G T T A G G G T T A</td> <td>2970         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T</td> <td>2980 T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G R T A A G T A G G G R T A A G T A G G G T T A G</td> <td>2990         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       R       T       T       A       G       G       G       T       T       A         G       R       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A       A       G       G       G       T       T       A       A       A       A       G       G       G       T<td>3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G</td><td>T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T</td></td>	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G R T T A G G G T T A G G G T T A	2970         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T         G G G T T A G G G T	2980 T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G R T A A G T A G G G R T A A G T A G G G T T A G	2990         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       R       T       T       A       G       G       G       T       T       A         G       R       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A       A       G       G       G       T       T       A       A       A       A       G       G       G       T <td>3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G</td> <td>T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T</td>	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G	T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A	2960         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A	2970         G       G       T       A       G       G       T         G       G       G       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T	2980         A G G G G T T A G         A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G T T A G         T A G G G G T T A G         T A G G G G T T A G         T A G G G G T T A G	2990         G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G	T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	2950 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G N G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A 3020	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A 3030	2970 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T 3040	2980 T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G R T T A G T A G G G T T A G T A G G G T T A G T A G G G T T A G	2990 G G T T A G S G T T A G G T T A G S G T T A G G T T A G S G T T A G R T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A 3060	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G	3010 T T A G G G T T T A G G G T 3080
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	2950 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G N G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A 3020 T A G G G T T A G G G T	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A T A G G G T T A G G G T T A	2970 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G G T T A G G G T 3040 T A G G G T T A G G	2980 T A G G G T T A G T A G G G T T A G	2990 G G T T A G S G T T A G G T T A G S G T T A G G T T A G S G T T A G G T T A G S G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G G T T A 3060 A G G G T T A G G G G	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G G T T A G G G A G G G G T T A G G G 3070 T A G G G T T A G	T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	2950 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A 3020 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A G G G T T A T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T	2970 G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G R T G G G T T A G G G T G G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G	2980 T A G G G T T A G T A G G G T T A G 3050 G T T A G G G T T G T A G G G T T	2990         G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       G       T       A         J       G       G       G       T       T       A       G       G       G       G       G </td <td>3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G T A G G G T T A G 1 T A G G G T T A G 1 T A G G G T T A G</td> <td>T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         M       G       G       T       T       A       G       G         G       G       T       T       A       G       G       G       G       G       G</td>	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G T A G G G T T A G 1 T A G G G T T A G 1 T A G G G T T A G	T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         M       G       G       T       T       A       G       G         G       G       T       T       A       G       G       G       G       G       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	2950         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A T A G G G T G G G T T A G G G G T T A G G G T T	2970         G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         J       A       G       G       G       T       T       A       G       G         J       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T <td< td=""><td>2980 A G G G T T A G A G G G T T A G 3050 G T T A G G G T T G T A G G G T T G T A G G G T T</td><td>2990         G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T</td><td>3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G G T T A G G G T A G G G T T A G</td><td>T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G</td></td<>	2980 A G G G T T A G A G G G T T A G 3050 G T T A G G G T T G T A G G G T T G T A G G G T T	2990         G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G G T T A G G G T A G G G T T A G	T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	2950 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G N G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A T A G G G T G G G T T A G G G G T T A G G G T T	2960 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A T A G G G T G G G T T A G G G T T	2970         G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       G       T         G       G       G       G       T       T       A <td< td=""><td>2980         A       G       G       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G       T       T         G       T       T       A       G       G       G       T<!--</td--><td>2990         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T       A         G       R       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         G       G       T       T       A       G       G       G       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G</td><td>3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G G T T A G G G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G</td><td>3010         T       A       G       G       T         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G</td></td></td<>	2980         A       G       G       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G       T       T         G       T       T       A       G       G       G       T </td <td>2990         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T       A         G       R       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         G       G       T       T       A       G       G       G       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G</td> <td>3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G G T T A G G G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G</td> <td>3010         T       A       G       G       T         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G</td>	2990         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T       A         G       R       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         G       G       T       T       A       G       G       G       T       A         G       G       T       T       A       G       G       G       T       A         A       G       G       G       T       T       A       G       G       G	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G G T T A G G G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G T A G G G G T T A G	3010         T       A       G       G       T         T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	2950 G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A T A G G G T T A G G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T A G G G T T A G G G T T A G G G T T T A G G G T T	2960         G       T       A       G       G       T       A         G       G       T       T       A       G       G       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       A         G       G       G       T       T       A       G       G       G       T       A         G       G	2970         G       G       T       A       G       G       T         G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T <td< td=""><td>2980 A G G G T T A G A G G G T T A G A G G G T T A G T A G G G T T A G A G G G T T A G T A G G G G T T G T T A G G G G T T G T T A G G G G T T G T T A G G G T T</td><td>2990         G       T       T       A       G       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T       A         A       G       G       G</td><td>3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G T A G G G T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G G G T T T A G T A G G G G T T T A G G G T T T A G T A G G G G G T T T A G G G T T T</td><td>T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       <t< td=""></t<></td></td<>	2980 A G G G T T A G A G G G T T A G A G G G T T A G T A G G G T T A G A G G G T T A G T A G G G G T T G T T A G G G G T T G T T A G G G G T T G T T A G G G T T	2990         G       T       T       A       G       G       T       T         G       G       T       T       A       G       S       G       T       T         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       S       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       T       A         A       G       G       G       T       T       A       G       G       G       T       A         A       G       G       G	3000 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G T A G G G T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G T A G G G G T T T A G G G T T T A G T A G G G G T T T A G G G T T T A G T A G G G G G T T T A G G G T T T	T       T       A       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         T       T       A       G       G       G       T         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G <t< td=""></t<>

	3090	3100	3110	3120	3130	3140	3150
1d.E2.filtered	G T T <mark>A</mark> G S R T T <mark>A G G G T</mark>	T <mark>A</mark> GGG <mark>TTA</mark> GGG	T T <mark>A G G G T</mark> T	A G G G T T A G G G T	T A G G G T T A G G	G T T <mark>A</mark> G G G T T	AGGGTTA
2d.E2.filtered	G T T <mark>A</mark> G S R <mark>T T A</mark> G G G T	T <mark>A</mark> GGG <mark>TT</mark> AGGG	ТТ <mark>А</mark> СССТТ	AGGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> GGG T T	AGGGTTA
1d.hapA.filtered	G T T A G S R T T A G G G T	T <mark>A</mark> G G G <mark>T T A</mark> G G G	T T <mark>A G G G T</mark> T	AGGGTTAGGGT	T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G G T T	AGGGTTA
2d.hapA.filtered	G T T <mark>A</mark> G G G T T <mark>A</mark> G G G T	T <mark>A</mark> G G R <mark>T T A</mark> G G G	T T <mark>A G G G T</mark> T	AGGGTTAGGGT	T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T A S G R T T	AGGGTTA
Telomere.reference	G T T <mark>A</mark> G G G T T <mark>A</mark> G G G T	T <mark>A</mark> G G G <mark>T T A</mark> G G G	T T <mark>A G G G T</mark> T	A G G G T T A G G G T	T <mark>A</mark> GGGT T AGG	G T T <mark>A</mark> G G G T T	AGGGTTA
consensus	G T T <mark>A</mark> GGG T T <mark>A</mark> GGG T	T A G G G T T A G G G	TTAGGGTT	AGGGTTAGGGT	TAGGGTTAGG	G T T A G G G T T	AGGGTTA
	3160	3170	3180	3190	3200	3210	3220
1d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T <mark>A</mark> G G G	<b>TTA</b> GGG <mark>TTA</mark> GG	G T T A G G G T T A	G G G <mark>T T A</mark> G G G	T T <mark>A</mark> G G G T
2d.E2.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T <mark>A</mark> G G G	<b>TTA</b> GGG <mark>TTA</mark> GG	G T T A G G G T T A	G G G <mark>T T A</mark> G G G	T T <mark>A</mark> G G G T
1d.hapA.filtered	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G <mark>TTA</mark> GGG <mark>TTA</mark> G	G G T T A G G G	TTAGGGTTAGG	G T T A G G G T T A	GGG <mark>TT</mark> AGGG	T T <mark>A</mark> G G G T
2d.hapA.filtered	G G G T T <mark>A</mark> G G R T T <mark>A</mark> G G	G <mark>TT</mark> AGGG <mark>TT</mark> AG	G <mark>GTT</mark> AGGG	<mark>TTASGGTTA</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark>	GGG <mark>TT</mark> AGGG	T T <mark>A</mark> G G G T
Telomere.reference	G G G T T A G G G T T A G G	G <mark>TTA</mark> GGG <mark>TTA</mark> G	G G T T A G G G	TTAGGGTTAGG	G T T A G G G T T A	G G G T T A G G G	T T <mark>A</mark> G G G T
consensus	GGG <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A G		TTAGGGTTAGG	GTTAGGGTTA	G G G T T A G G G	TTAGGGT
	3230	3240	3250	3260	3270	3280	3290
1d.E2.filtered	<i>3230</i> T <mark>A G G G T T A G G G T T A</mark>	<i>3240</i> G G G T T A G G G T T	3250 AGGGTTAG	3260	<i>3270</i> GGGTTAGGNT	3280 T <mark>AGGGTTA</mark> G	3290
1d.E2.filtered 2d.E2.filtered	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A	3240 G G G T T A G G G T T G G G T T A G G G T T	<i>3250</i> A G G G T T A G A G G G T T A G	<i>3260</i> G G T T A G G G T T A G G T T A G G G T T A	<i>3270</i> G G G <mark>T T A</mark> G G N T G G G <mark>T T A</mark> G G N T	<i>3280</i> T A G G G T T A G T A G G G T T A G	<i>3290</i> G G T T A G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A	3240 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T	3250 A G G G T T A G A G G G T T A G A G G G T T A G	<i>3260</i> G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	<i>3270</i> G G G T T A G G N T G G G T T A G G N T G G G T T A G G N T	<i>3280</i> T A G G G T T A G T A G G G T T A G T A G G G T T A G	3290 G G T T A G G G G T T A G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	<i>3230</i> T A G G G T T A G G G T T A A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A	3240 G G G T T A G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T	3250 A G G G T T A G A G G G T T A G A G G G T T A G A G G G T T A G	<i>3260</i> G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	3270 G G G T T A G G N T G G G T T A G G N T G G G T T A G G N T G G G T T A G G N T	<i>3280</i> T A G G G T T A G T A G G G T T A G T A G G G T T A G T A G G G T A A	<i>3290</i> G G T T A G G G G T T A G G G G T T A G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	J       A       G       G       G       T       A       G       G       T       A       G       G       T       A       A       G       G       T       A       G       G       T       A       A       G       G       T       A       A       G       G       G       T       A       A       G       G       G       T       A       A       G       G       G       T       A	3240         G       G       T       A       G       G       T       T         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T	3250         A       G       G       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G	3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A	3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T	<i>3280</i> T A G G G T T A G T A G G G T T A G	3290 G G T T A G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A	3240         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T	3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G	3260         G G T T A G G G T T A         G G T T A G G G T T A         G G T T A G G G T T A         G G T T A G G G T T A         G G T T A G G G T T A         G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A         G G G T T A G G G T T A	3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T	3280         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G	3290 G G T T A G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	3230         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       A         T       A       G       G       T       T       A       G       G       T       T         T       A       G       G       G       T       T       A       G       G       T       T         T       A       G       G       G       T       T       A       G       G       T       T       A         T       A       G       G       G       T       T       A       G       G       T       T       A         T       A       G       G       G       T       T       A       G       G       T       T       A         T       A       G       G       G       T       T       A       G       G       T       T       A         T       A       G       G       G       T       T       A       G       G       T <td>3240         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T</td> <td>3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G</td> <td>3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A    &lt;</td> <td>3270         G       G       T       A       G       N       T         G       G       G       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T</td> <td>3280         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G</td> <td>3290         G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G</td>	3240         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T	3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G	3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       G       T       T       A    <	3270         G       G       T       A       G       N       T         G       G       G       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T	3280         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G	3290         G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	3230 T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A	3240 G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T	3250 A G G G T T A G A G G G T T A G	3260 G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A	3270 G G G T T A G G N T G G G T T A G G N T G G G T T A G G N T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T G G G T T A G G G T	3280 T A G G G T T A G T A G G G T T A G	3290 G G T T A G G G G T T A G G 3360
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A	3240         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T       A       G </td <td>3250 A G G G T T A G A G G G G T T A G A G G G G T A A G A G G G G T T A G 3320 T T A G G G T T</td> <td>3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G</td> <td>3270 G G G T T A G G N T G G G T T A G G N T G G G T T A G G N T G G G T T A G G T G G G T T A G G G T G G G T T A G G G T 3340 T A G G G T T A G G G</td> <td>3280 T A G G G T T A G T A G G G T A A G T A G G G T A A G T A G G G T A A G 3350 G T A G G G G T T</td> <td>3290 G G T T A G G G G T T A G G A G N G T A A</td>	3250 A G G G T T A G A G G G G T T A G A G G G G T A A G A G G G G T T A G 3320 T T A G G G T T	3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G       G       T       T       A       G       G       T       T       A         G       G	3270 G G G T T A G G N T G G G T T A G G N T G G G T T A G G N T G G G T T A G G T G G G T T A G G G T G G G T T A G G G T 3340 T A G G G T T A G G G	3280 T A G G G T T A G T A G G G T A A G T A G G G T A A G T A G G G T A A G 3350 G T A G G G G T T	3290 G G T T A G G G G T T A G G A G N G T A A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A G G G T T A <i>A</i> G T T A G G G T T A G G G T T A G G G T T A <i>G</i> T T A G G G G T T A G G G T T A G G G T T A <i>G</i> T T A G G G G T T A G G T T A G G G T T A G G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A G G T T A G G T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G G T T A G G T T A G G T T A G G G T T A G G T T A G G T T A G G T T A G G G T T A G G T T A G G T T A G G T T A G T A G T T A G G T T A G T A G T T A G G T T A G T T A G T T A G G G T T T A G	3240         G       G       T       A       G       G       T       T         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       G       T       T       A	3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         3320       T       T       A       G       G       G       T       T	3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         3330       T       T       A       G       G <t< td=""><td>3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       G       T         G       G       G       <td< td=""><td>3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         3350       G       T       T       A       G       G       T       T</td><td>3290         G       T       A       G         G       G       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         J       G       G       T       T       A       G       G         A       G       N       G       T       T       A</td></td<></td></t<>	3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       G       T         G       G       G <td< td=""><td>3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         3350       G       T       T       A       G       G       T       T</td><td>3290         G       T       A       G         G       G       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         J       G       G       T       T       A       G       G         A       G       N       G       T       T       A</td></td<>	3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         3350       G       T       T       A       G       G       T       T	3290         G       T       A       G         G       G       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         J       G       G       T       T       A       G       G         A       G       N       G       T       T       A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T A G G G T T <i>G</i> T T A G G G T T A G G G T T A G G G T T	3240         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       G       T       T       A       G       G       G       G </td <td>3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T         T       T       A       G       G       G       T       T</td> <td>3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         A       N       G       G       T       T       A</td> <td>3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       <td< td=""><td>3280         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T         G       T       T       A       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T</td><td>3290         G       T       A       G         G       T       T       A       G         G       T       T       A       G         G       T       T       A       G         G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         A       G       N       G       T       A         A       G       N       G       T       T         A       G       N       G       T       T         A       G       N       G       T       T</td></td<></td>	3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T         T       T       A       G       G       G       T       T	3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       A         A       N       G       G       T       T       A	3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G <td< td=""><td>3280         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T         G       T       T       A       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T</td><td>3290         G       T       A       G         G       T       T       A       G         G       T       T       A       G         G       T       T       A       G         G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         A       G       N       G       T       A         A       G       N       G       T       T         A       G       N       G       T       T         A       G       N       G       T       T</td></td<>	3280         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         G       T       T       A       G       G       T       T         G       T       T       A       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T	3290         G       T       A       G         G       T       T       A       G         G       T       T       A       G         G       T       T       A       G         G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G         A       G       N       G       T       A         A       G       N       G       T       T         A       G       N       G       T       T         A       G       N       G       T       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A G G G T 3300 G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T G T T A G G G G T T A G G G T T A G G G T G T T A G G G G T T A G G G T T A G G G T G T T A G G G G T T A G G G T T A G G G T G T T A G G G G T T A G G G T T A G G G T G T T A G G G G T T A G G G T T A G G G T G T T A G G G G T T A G G G T T A G G G T T G T T A G G G T T A G G G T T A G G G T T A G G G T G T T A G G G T T A G G G T T A G G G T T A G G G T T G T T A G G G T T A G G G T T A G G G T T A G G G T T	3240         G       G       T       A       G       G       T       T         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       T         G       G       G       G       T       T       A       G	3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G         J       T       A       G       G       G       T       T         T       T       A       G       G       G       T       T         T       T       A       G       G       G       T       T	3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A       A       A       N       G       G       T       T </td <td>3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       G       T       T       A       G       G       T         G       G       G       G       T       T       A       G       G       T         G       G       G       G       G       T       T       A       G       G         T       <td< td=""><td>3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       &lt;</td><td>3290         G       T       A       G         G       T       T       A       G         G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A</td></td<></td>	3270         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       N       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       G       T       T       A       G       G       T         G       G       G       G       T       T       A       G       G       T         G       G       G       G       G       T       T       A       G       G         T <td< td=""><td>3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       &lt;</td><td>3290         G       T       A       G         G       T       T       A       G         G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A</td></td<>	3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       <	3290         G       T       A       G         G       T       T       A       G         G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	<i>3230</i> T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A T A G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T A <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G T T A G G G T T <i>A</i> G G G T T A G G G T T A G G G T T <i>A</i> G G G T T A G G G T T A G G G T T <i>A</i> G G G T T A G G G T T A G G G T T <i>A</i> G G G T T A G G G T T A G G G T T <i>A</i> G G G T T A G G G T T A G G G T T A G G G T T <i>A</i> G T T A G G G G T T A G G G T T A G G G T T <i>A</i> G T T A G G G G T T A G G G T T A G G G T T <i>A</i> G T T A G G G T T A G G G T T A G G G T T A G G G T T <i>G</i> T T A G G G G T T A G G G T T A G G G T T A G G G T T <i>G</i> T T A G G G G T T A G T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A G G G T T A G G T T A G G T T A G G G T T A	3240         G       G       T       A       G       G       T       T         G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       G       T       T         G       G       G       T       T       A       G       G       G       G       G       G       G       G       G       G       G       G       G </td <td>3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G       G       T       T       A       G         A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       T       A       G       G       G       T       T       T       A       G       G       G       T       T       T       A       G       G       G       T       T       T       T       T       A       G       G       G       T</td> <td>3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       R       T         A       N       G       G       T       T       A</td> <td>3270         G       G       T       A       G       N       T         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T       T       A       G       G         G       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       G       G       G       G       G<!--</td--><td>3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       &lt;</td><td>3290         G       T       A       G         G       G       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       G       G       T       T       A         A       G       N       G       T       T       A         A       G       G       G       T       T       A         A</td></td>	3250         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       T       T       A       G         A       G       G       G       T       T       A       G         A       G       G       G       T       T       A       G       G       T       T       A       G         A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       T       A       G       G       G       T       T       T       A       G       G       G       T       T       T       A       G       G       G       T       T       T       T       T       A       G       G       G       T	3260         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       T       T       A         G       G       T       T       A       G       G       R       T         A       N       G       G       T       T       A	3270         G       G       T       A       G       N       T         G       G       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       T       T       A       G       N       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T         G       G       G       T       T       A       G       G       T       T       A       G       G         G       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       G       G       G       G       G </td <td>3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       &lt;</td> <td>3290         G       T       A       G         G       G       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       G       G       T       T       A         A       G       N       G       T       T       A         A       G       G       G       T       T       A         A</td>	3280         T       A       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       A       G       G       G       T       T         G       T       T       <	3290         G       T       A       G         G       G       T       A       G         G       G       T       T       A       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         G       G       T       T       A       G       G         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       N       G       T       T       A         A       G       G       G       T       T       A         A       G       N       G       T       T       A         A       G       G       G       T       T       A         A

	3376	3380	3390	3400	3410	3420	3430
1d.E2.filtered	GGG <mark>TT</mark> AGGG	T <mark>T </mark> A G G G T T <mark>A</mark> G G R	T T <mark>A</mark> GGGTTAG	G G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T <mark>A</mark> G G G T T	A N G G T T A G G G T	T <mark>A</mark> S <mark>G</mark> R T
2d.E2.filtered	GGG <mark>TT</mark> AGGG	T T <mark>A G G G T T A G G</mark> R	T T <mark>A G G G T T A G</mark>	G G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T <mark>A</mark> G G G T T	A N G G T T <mark>A</mark> G G G T	T <mark>A</mark> S <mark>G</mark> R T
1d.hapA.filtered	GGG <mark>TT</mark> AGGG	T <mark>T A</mark> GG <mark>G TT A</mark> GG R	T T <mark>A</mark> G G G T T A G	G <mark>GTTA</mark> GGG <mark>TTA</mark>	G G G T T <mark>A</mark> G G G T T	A N G G T T A G G G T	T <mark>A</mark> S <mark>G</mark> R T
2d.hapA.filtered	G G G T T A G G R T	T <mark>T</mark> ASGGTTAGGR	T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T <mark>A</mark> G G G T T	A G G G T T A G G G T	T <mark>A</mark> N <mark>G G T</mark>
Telomere.reference	GGG <mark>TTA</mark> GGG	r <mark>it a</mark> ggg <mark>tit a</mark> ggg	ΤΤ <mark>Α</mark> GGGΤΤΑG	G <mark>GTTA</mark> GGG <mark>TTA</mark>	<mark>ϾϾϾͳͳϪ</mark> ϾϾϾͳͳ	A G G G T T A G G G T	T <mark>A</mark> G G G T
consensus		TT <mark>A</mark> GGG <mark>TTA</mark> GGG	ΤΤΑΓΟΓΤΑΟ	G G T T <mark>A</mark> G G G T T A	GGGTTAGGGTT	AGGGTTAGGGT	T <mark>A</mark> GGGT
	3446	3450	3460	3470	3480	3490	3500
1d.E2.filtered	TAGSRTTASS	5 G T T A G G G T T A G	G G T T <mark>A</mark> G G G T T	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TT A</mark> GGG	ΤΤ <mark>Α</mark> GGGΤΤΑΚG	G <mark>TTA</mark> GG
2d.E2.filtered	TAGSRTTASS	5 G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T <mark>A</mark> G G G T T	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TT A</mark> GGG	Τ Τ <mark>Α</mark> G G G Τ Τ <mark>Α</mark> Κ G	G <mark>TTA</mark> GG
1d.hapA.filtered	T A G S R T T A S S	5 <mark>G T T A</mark> G G G T T A G	G G T T <mark>A</mark> G G G T T	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TTA</mark> GGG	ΤΤΑΓΟΓΑΚΟ	GTTAGG
2d.hapA.filtered	TAGGRTTANO	G R T T A G G G T T A G	G G T T A S G N T T	A S G G T T A G S G T	T <mark>A</mark> GGG <mark>TTA</mark> GGGG	TTAGGGTTAGG	GTTAGG
Telomere.reference		G <mark>TTA</mark> GGG <mark>TTA</mark> G	GGTTAGGGGTT	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TT A</mark> GGG	TTAGGGTTAGG	G <mark>TT A</mark> GG
consensus	T <mark>A</mark> GGG <mark>TTA</mark> GO	G G T T <mark>A</mark> G G G <mark>T T A</mark> G	G G T T <mark>A</mark> G G G T T	A G G G T T <mark>A</mark> G G G T '	T <mark>A</mark> GG <mark>G T T A</mark> GGG	T T <mark>A</mark> GGG T T <mark>A</mark> GG	G <mark>T T </mark> A G G
1d.E2.filtered							
2d.E2.filtered		A S G R T T A G G N T K			G T T A G G R T T A G		
1d.hapA.filtered		A SGRIIAGGNIK					
20.napA.tiltered							
reiomere.reference					GTTAGGGTTAG		GGGTTA
consensus	GTTAGGGTTA	A G G G T T A G G R T T	AGGGGTTAGGGG	TTAGGGGTTAGGG	G <mark>TTT</mark> A <mark>GGGTTT</mark> A <mark>G</mark>	GGTT AGGGGTTA	G G G <mark> T T </mark> A
	3586	3590	3600	3610	3620	3630	3640
1d.E2.filtered	G G G T T A G G R T	T A G G G T T A G N G	TTAGGGTTAG	G G T T <mark>A </mark> G G G T T A	GGGTTAGGGTT	AGGGTTAGGGT	T <mark>A</mark> GGGT
2d.E2.filtered	G G G T T A G G R T	T T A G G G T T A G N G	T T <mark>A G G G T T A G</mark>	G G T T <mark>A</mark> G G G T T A	G G G T T <mark>A</mark> G G G T T	AGGGTTAGGGT	T <mark>A</mark> G G G T
1d.hapA.filtered	GGGTTAGGR	T T A G G G T T A G N G	T T <mark>A G G G T T A G</mark>	G G T T A G G G T T A	GGGTTAGGGTT	AGGGTTAGGGT	TAGGGT
2d.hapA.filtered	G G G T T A S S G T	TASGRTTAGNN	T T A G G G T T A G	G G T T A G G G T T A	GSGTTAGGNTT	AGGGTTAGGGT	
Telomere reference	G G G T T <mark>A</mark> G G G T	T T A G G G T T A G G G	TTAGGGTTAG	G G T T A G G G T T A	G G G T T <mark>A</mark> G G G T T	AGGGTTAGGGT	T <mark>A</mark> G G G T
consensus		T <mark>A</mark> GGG <mark>TTA</mark> G G		G G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T A G G G T T		T <mark>A</mark> GGG T

	3650	3660	3670	3680	3690	3700	3710
1d.E2.filtered	T <mark>A</mark> GG <mark>GTTA</mark> GGG	ITT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A G	G G T T <mark>A</mark> G S G T T	A G G G T T <mark>A</mark> G S G	T T A G G G T T A G G G	T T <mark>A</mark> G G
2d.E2.filtered	<mark>Τ</mark> Α <mark>GGGΤΤΑ</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T <mark>A</mark> G S G T T	A G G G T T <mark>A</mark> G S G	T T <mark>A</mark> GGG T T A GGG	T T <mark>A</mark> G G
1d.hapA.filtered	T <mark>A</mark> GGG <mark>TTA</mark> GGG	i <mark>TTA</mark> GGG <mark>TTA</mark> GG	IGTTAGGGTTA	G G T T <mark>A</mark> G S G T T	A G G G T T <mark>A</mark> G S G	T T A G G G T T A G G G	T T <mark>A</mark> G G
2d.hapA.filtered	T A G G G T T A G G R	TTAGGRTTAGG	G T T A S G N T T A S	G N T T <mark>A</mark> G G N T T	A S N G T T A G G R	T T A G G R T T A G S R	T T <mark>A</mark> G G
Telomere.reference	T <mark>A</mark> GGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A G	G G T T <mark>A</mark> G G G T T	A G G G T T A G G G	TTAGGGTTAGGG	T T <mark>A</mark> G G
consensus		TTAGGGTTAGG	G T T A G G G T T A G	GGTTAGGGTT	ΑĠĠĠŢŢΑĠĠĠ	T T A G G G T T A G G G	T T A G G
	3720	3730	3740	3750	3760	3770	3780
1d.E2.filtered	G T T A G G G T T A G	GGTTAGGGTTA	GNNTTAGGGTT	A G G G T T A G G G	TTAGGGTTAG	G R T T A G G G T T A G	GGTTA
2d.E2.filtered	G T T A G G G T T A G	GGTTAGGGTTA	GNNTTAGGGTT	AGGG <mark>TTA</mark> GGG	TTAGGGTTAG	G R T T <mark>A</mark> G G G T T A G	GGTTA
1d.hapA.filtered	G T T A G G G T T A G	GGTTAGGGTTA	GNNTTAGGGTT		TTAGGGTTAG	G R T T <mark>A</mark> G G G T T A G	GGTTA
2d.hapA.filtered	R T T <mark>A</mark> G N G T T A G	NGTTAGGGTTA	GGGTTAGGRTT	AGSRTTAGSG	TTASGGTTAG	G G T T <mark>A</mark> G G G T T A G	GGTTA
Telomere.reference	G T T A G G G T T A G	GG <mark>TTA</mark> GGG <mark>TTA</mark>	GGGGTT AGGGGTT	AGGG <mark>TTA</mark> GGG	TTAGGGGTTAG	GG <mark>TTA</mark> GGG <mark>TTA</mark> G	GGTTA
consensus	G T T <mark>A</mark> G G G T T <mark>A</mark> G	GG <mark>TTA</mark> GGG <mark>TT</mark> A	GGG <mark>TTA</mark> GGG <mark>TT</mark>	A G G G T T A G G G	TTAGGGTTAG	G G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T A
	2700	2000	2010	2020	2020	2010	2050
	3790	3800	3810	3820			3850
1d.E2.filtered	<i>3790</i> GGGTTANGGTT	<i>3800</i> A G N G T T A G N G T	3810 TAGGGTTAGGG	3820 T T A G G G T T A G	3830	3840 A G N G T T A G G G T T	3850 AGGGT
1d.E2.filtered 2d.E2.filtered	<i>3790</i> GGGTTANGGTT GGGTTANGGTT	3800 A G N G T T A G N G T A G N G T T A G N G T	<i>3810</i> <b>T</b> A G G G T T A G G G <b>T</b> A G G G T T A G G G <b>T</b> A C C C T T A C C C	<i>3820</i> T T A G G G T T A G T T A G G G G T T A G	<i>3830</i> G G T T A G G G T T G G T T A G G G T T	<i>3840</i> A G N G T T A G G G T T A G N G T T A G G G T T	<i>3850</i> AGGGT AGGGT
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	<i>3790</i> G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T	3800 A G N G T T A G N G T A G N G T T A G N G T A G N G T T A G N G T	<i>3810</i> <b>T</b> A G G G <b>T T</b> A G G G <b>T</b> A G G G <b>T T</b> A G G G <b>T</b> A G G G <b>T T</b> A G G G <b>T</b> A G G G <b>T T</b> A G G G	<i>3820</i> T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G	<i>3830</i> G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T H G G G T T A G G G T T	3840 A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T	3850 A G G G T A G G G T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	<i>3790</i> G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T G G G T T A G G G T T	3800         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T	<i>3810</i> <b>T</b> A G G G <b>T T</b> A G G G <b>T</b> A G G G <b>T T</b> A G G G <b>T</b> A G G G <b>T T</b> A G G G <b>T</b> A G G R <b>T T</b> A G G G <b>T</b> A G G R <b>T T</b> A G G G	3820 T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G G G T T A G T T A G S G T T A G	<i>3830</i> G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T N G T T A G G G T T N G T T A G G G T T	3840 A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A S G G T T	3850 A G G G T A G G G T A G G G T A G G G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	3790         G       G       T       A       N       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       G       T       A       G       G       G       G       G       T       A       G       G       G       G       G       G       G       G       G       G       G       G       G       G	3810         T       A       G       G       T       T       A       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       R       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G	T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       S       G       T       T       A       G         T       T       A       G       S       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       G	3830         G G T T A G G G T T         G G T A G G G T T         G G T A G G G T T         G G T A G G G T T         N G T A G G G T T         A G G G T T         A G G G T T         A G G G T T         A G G G T T         A G G G T T         A G G G T T         A G G G T T         A G G G T T         A G G G T T         A G G G T T	3840         A       G       N       G       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       S       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T	3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	3790         G       G       T       A       N       G       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       N       G       G       T       T         G       G       G       T       T       A       G       G       T       T         G       G       G       T       T       A       G       G       T       T	A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T         A       G       G       G       T       T       A       G       G       T	3810         1       A       G       G       T       T       A       G       G         1       A       G       G       T       T       A       G       G       G         1       A       G       G       G       T       T       A       G       G         1       A       G       G       G       T       T       A       G       G         1       A       G       G       R       T       T       A       G       G         1       A       G       G       G       T       T       A       G       G         1       A       G       G       G       T       T       A       G       G         1       A       G       G       G       T       T       A       G       G       G	T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       S       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G	3830         G       G       T       T       A       G       G       T       T         G       G       T       T       A       G       G       T       T         G       G       T       T       A       G       G       T       T         G       G       T       T       A       G       G       G       T       T         N       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T         G       G       T       T       A       G       G       G       T       T	3840         A       A       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T	3850         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	3790 G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T 3860	A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G	3810         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       R       T       T       A       G       G       G         T       A       G       G       R       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G </td <td>T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       S       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G</td> <td>3830 G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T N G T T A G G G T T G G T T A G G G T T G G T T A G G G T T 3900</td> <td>3840 A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T</td> <td>3850 A G G G T A G G G T 3920</td>	T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       S       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       A       G       G       G       T       T       A       G	3830 G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T N G T T A G G G T T G G T T A G G G T T G G T T A G G G T T 3900	3840 A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T	3850 A G G G T A G G G T 3920
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	<i>3790</i> G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T G G G T T A G G G T T G G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T	3800 A G N G T T A G N G T A G N G T T A G N G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T	J       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G	3820         T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         S       S       G       G       T       T       A       G       G       G       T       T       A       G         S       S       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       G       G       G       G <td>3830 G G T T A G G G T T G G T T A G G G T T N G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G G T T A G G G G T T 3900 A G G G T T A G G G G G</td> <td>3840         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         3910       T       T       A       G       G       G</td> <td>3850 A G G G T A G G G T 3920 T T A G G</td>	3830 G G T T A G G G T T G G T T A G G G T T N G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G G T T A G G G G T T 3900 A G G G T T A G G G G G	3840         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         3910       T       T       A       G       G       G	3850 A G G G T A G G G T 3920 T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	<i>3790</i> G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T G G G T T A G G G T T G G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G G T T A G G G T T <i>A</i> G G G T T A G G G	A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G	3810         1       A       G       G       T       T       A       G       G         1       A       G       G       T       T       A       G       G       G         1       A       G       G       T       T       A       G       G       G         1       A       G       G       R       T       T       A       G       G       G         1       A       G       G       R       T       T       A       G       G       G         1       A       G       G       R       T       T       A       G       G       G         1       A       G       G       G       T       T       A       G       G       G         1       A       G       G       T       T       A       G       G       G         1       A       G       G       T       T       A       G       G       G         1       A       G       G       T       T       A       G       G       G         1       T       A	T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       S       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G       G       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T	3830 G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T N G T T A G G G T T G G T T A G G G T T G G T T A G G G T T 3900 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G	3840         A       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       G       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G </td <td>3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G</td>	3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	<i>3790</i> G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T G G G T T A G G G T T G G G T T A G G G T T G G G T T A G G G T T <i>A</i> G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G	A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G	3810         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       R       T       T       A       G       G       G         T       A       G       G       R       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       N       R       T       T       A       G       G       G       N       R       T </td <td>T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G</td> <td><i>3830</i> G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>3900</i> A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G</td> <td>3840         A       N       G       T       A       G       G       T       T         A       N       G       T       T       A       G       G       T       T         A       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       G       T       T         A       G       N       G       T       T       A       G       G       G       G<!--</td--><td>3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       A       G       G       G</td></td>	T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G	<i>3830</i> G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T <i>A</i> G G G T T A G G G T T <i>3900</i> A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G	3840         A       N       G       T       A       G       G       T       T         A       N       G       T       T       A       G       G       T       T         A       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       G       T       T         A       G       N       G       T       T       A       G       G       G       G </td <td>3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       A       G       G       G</td>	3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       T       A       G       G         T       A       G       G       G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	<i>3790</i> G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T G G G T T A G G G T T G G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G G T T A G G G T T <i>G</i> G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G <i>G</i> G G T T A G G G G <i>G</i> G G T T A G G G G <i>G</i> G G T T A G G G G <i>G</i> G G T T A G G G G T T A G G G <i>G</i> G G G T T A G G G G T T A G G G G <i>G</i> G G G T T A G G G G T T A G G G G <i>G</i> G G T T A G G G G T T A G G G G <i>G</i> G G G T T A G G G G T T A G G G G T T A G G G G	A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       N       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G       T       T       A       G       G       G	3810         T       A       G       G       T       A       G       G         T       A       G       G       T       T       A       G       G         T       A       G       G       T       T       A       G       G         T       A       G       G       R       T       T       A       G       G         T       A       G       G       R       T       T       A       G       G         T       A       G       G       R       T       T       A       G       G         T       A       G       G       G       T       T       A       G       G         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G       G       G       G         T       A       G       N       R       T       T       A       G       G       G       G       N       R       T       A       G       G       G       T </td <td>T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G</td> <td><i>3830</i> G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T <i>A</i> G G G T A G G G T <i>A</i> G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G G</td> <td>3840         A       A       G       A       A       A       G       A<td>3850 A G G G T A G G G G T T A G G T T A G G T T A G G T T A G G</td></td>	T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G	<i>3830</i> G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T <i>A</i> G G G T A G G G T <i>A</i> G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G G	3840         A       A       G       A       A       A       G       A <td>3850 A G G G T A G G G G T T A G G T T A G G T T A G G T T A G G</td>	3850 A G G G T A G G G G T T A G G T T A G G T T A G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	<i>3790</i> G G G T T A N G G T T G G G T T A N G G T T G G G T T A N G G T T G G G T T A G G G T T G G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G T T A G G G T T <i>G</i> G G G T T A G G G T A G G G T T A G G G T T A G G G T A G G G T T A G G G T T A G G G T A G G G T T A G G G T T A G G G G T A G G G T T A G G G T T A G G G G T A G G G T T A G G G G T T A G G G G T A G G G T T A G G G T T A G G G G T T A G G G G	A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       N       G       T       T       A       G       N       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T         A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G       T       T       A       G       G       G	3810         1       A       G       G       T       A       G       G       G         1       A       G       G       T       T       A       G       G       G         1       A       G       G       G       T       T       A       G       G       G         1       A       G       G       G       T       T       A       G       G       G         1       A       G       G       R       T       T       A       G       G       G         1       A       G       G       G       T       T       A       G       G       G         1       A       G       G       G       T       T       A       G       G       G         1       A       G       G       G       T       T       A       G	T       T       A       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G         T       T       A       G       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       T       T       A       G       G       G	<i>3830</i> G G T T A G G G T T G G T T A G G G T T G G T T A G G G T T N G T T A G G G T T G G T T A G G G T T G G T T A G G G T T <i>G</i> G T T A G G G T T <i>G</i> G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G A G G G T T A G G G G G T T A G G G G G A G G G T T A G G G G G T T A G G G G G	3840         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       N       G       T       T       A       G       G       T       T         A       G       G       T       T       A       G       G       T       T         A       G       G       T       T       A       G       G       T       T         A       G       G       T       T       A       G       G       T       T         A       G       G       N       G       T       T       A       G       G       G </td <td>3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       G       T         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A&lt;</td>	3850         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       T         A       G       G       G       G       T         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A       G       G       G         T       T       A<

	3930	3940	3950	3960	3970	3980	3990
1d.E2.filtered	G T T A G S G T T A S S	G T T <mark>A</mark> G G G T T <mark>A</mark> G G	G <mark>T T </mark> A G G G <mark>T T</mark> /	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TT</mark> AG	G G T T <mark>A</mark> G G G T	T <mark>A</mark> G G <mark>T T </mark> A
2d.E2.filtered	G T T A G S G T T A S S	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G <mark>GTTA</mark> GGG <mark>TT</mark>	A G G G T T A G G G T	T <mark>AGGGTTA</mark> G	G <mark>GTT</mark> T <mark>A</mark> GGGT	T <mark>AGGGTTA</mark>
1d.hapA.filtered	G T T A G S G T T A S S	G T T <mark>A</mark> G G G T T <mark>A</mark> G	G G T T <mark>A</mark> G G G T T /	A G G G T T A G G G T	T A G G G T T A G G	G G T T <mark>A</mark> G G G T <sup>-</sup>	T <mark>A G G G T T </mark> A
2d.hapA.filtered	G T T A G G G T T A G G	G T T <mark>A</mark> G G G T T <mark>A</mark> G	S G T T <mark>A G G G T T </mark> /	A G G G T T A S G G T	K A N G N T K N G (	G G T T <mark>A</mark> G G G T <sup>-</sup>	T <mark>A G</mark> N N T T <mark>A</mark>
Telomere.reference	G T T A G G G T T A G G	G T T <mark>A</mark> G G G T T A G	G G T T <mark>A</mark> G G G T T <mark>/</mark>	A G G G T T A G G G T	T <mark>A</mark> GGG <mark>TTA</mark> G	G G T T <mark>A</mark> G G G T	T <mark>A</mark> GGG <mark>TT</mark> A
consensus	G T T A G G G T T A G G	G T T <mark>A</mark> G G G T T A G	G G T T <mark>A G G G T T</mark> /	AGGGTTAGGGT	T A G G G T T A G G	G G T T <mark>A</mark> G G G T	ΤΑΓΟΓΑ
	4000	4010	4020	4030	4040	4050	4060
1d.E2.filtered	S G G T T A G G G T T A	G G G <mark>T T A</mark> G G G <mark>T T</mark> A	A G N G T T A G G G	T T <mark>A</mark> GGG <mark>TT A</mark> GG	G T T A G G G T T /	A G G N <mark>T T A</mark> G G (	G T T <mark>A</mark> G G G T
2d.E2.filtered	S G G T T A G G G T T A	G G G T T <mark>A</mark> G G G T T A	A G N G T T A G G G	T T <mark>A</mark> GGG <mark>TT A</mark> GG	G T T <mark>A</mark> G G G T T /	A G G N <mark>T T A</mark> G G (	G T T <mark>A</mark> G G G T
1d.hapA.filtered	S G G T T A G G G T T A	GGG <mark>TTA</mark> GGG <mark>TT</mark>	A G N G T T A G G G	T T <mark>A</mark> GGG <mark>TT A</mark> GG	G T T A G G G T T /	A G G N T T A G G (	G T T <mark>A</mark> G G G T
2d.hapA.filtered	G S S T T A G G G T T M	G N N T T <mark>A</mark> G N G T T A	A G G R T T A G G G	T T <mark>A</mark> GGG <mark>TT A</mark> GG	G T Y A G N S Y T /	A N G R T T A G G (	G T T <mark>A</mark> G G G T
Telomere.reference	GGG <mark>TTA</mark> GGG <mark>TTA</mark>	GGG <mark>TTA</mark> GGG <mark>TT</mark>	AGGG <mark>TTA</mark> GGG	TT <mark>A</mark> GGG <mark>TT</mark> AGG	6 <mark>G T T A</mark> G G G <mark>T T </mark> /	<mark>A</mark> GGG <mark>TTA</mark> GG	G <mark>TTA</mark> GGGT
consensus	GGG <mark>TT</mark> AGGG <mark>TT</mark> A	G G G T T <mark>A</mark> G G G T T <mark>A</mark>	A G G G T T A G G G T	T T <mark>A</mark> GGG <mark>T T </mark> AGG	G T T <mark>A</mark> G G G T T /	A G G R T T A G G (	G T T <mark>A</mark> G G G T
		4080	4090	4100	4110	4120	4130
1d.E2.filtered	4070 TAGGGTTAGGGT	4080 TAGGGTTAGGR	4090 TYANGNTTAN	4100 N N T T A G G R T T A		4120 KAGGSTTA	<i>4130</i> G G G T T A N R
1d.E2.filtered 2d.E2.filtered	<i>4070</i> T A G G G T T A G G G T T A G G G T T A G G G T	<i>4080</i> T A G G G T T A G G R T A G G G T T A G G R	4090 T Y A N G N T T A N I T Y A N G N T T A N I	4100 N N T T A G G R T T A N N T T A G G R T T A	<i>4110</i>	4120 I K A G G S T T A I I K A G G S T T A I	<i>4130</i> G G G T T A N R G G G T T A N R
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	<i>4070</i> T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T	<i>4080</i> T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R	4090 I Y A N G N T T A N I Y A N G N T T A N I Y A N G N T T A N	4100 N N T T A G G R T T A N N T T A G G R T T A N N T T A G G R T T A	<i>4110</i> <b>GGGTTAGGG</b> <b>GGGGTTAGGG</b> <b>GGGTTAGGG</b>	<i>4120</i> [ K A G G S T T A G [ K A G S T T T A G [ K A G S	<i>4130</i> G G G T T A N R G G G T T A N R G G G T T A N R
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	4070 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A S S G T T A G G G T	<i>4080</i> T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R A G S G T T A S S G	4090 T Y A N G N T T A N T Y A N G N T T A N T Y A N G N T T A N T Y A S G G T T A G	4100 N N T T A G G R T T A N N T T A G G R T T A N N T T A G G R T T A G S T A G G G T T A	<i>4110</i> G G G T T A G G G G G G T T A G G G	4120 K A G G S T T A K A G G S T T A K A G G S T T A N A G G G T T A	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G R T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	4070 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A S S G T T M S G G T T A G G G T T A G G G T	4       4       6       6       7       7       4       6       6       7       8       6       7       8       7       7       4       6       6       7       7       4       6       6       7       7       4       6       6       7       7       4       6       6       7       7       4       6       6       7       7       4       6       6       7       7       4       6       6       7       7       4       6       6       7       7       4       6       6       7       7       4       5       5       6       7       7       4       6       6       6       6       7       7       4       6       6       6       6       7       7       4       6       6       6       6       7       7       4       6       6       6       6       6       6       6       6       6       6       6       6       6       6       6       7       7       6       6       6       6       6       6       6       6       6       6       6       6       6	4090 T Y A N G N T T A N T Y A N G N T T A N T Y A S G G T T A S T A G G G T T A G T A G G G T T A G	4100 N N T T A G G R T T A N N T T A G G R T T A G S T T A G G G R T T A G G T T A G G G T T A	4110 G G G T T A G G G G G G T T A G G G G G G G T T A G G G G G G G T T A G G G G G G G T T A G G G G A G G G T T A G G G G	4120 K A G G S T T A K A G G S T T A K A G G S T T A N A G G G T T A T A G G G T T A	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G R T T A G G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	4070 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A S S G T T M S G G T T A G G G T T A G G G T T A G G G T T A G G G T	4080         A       G       G       T       T       A       G       R         A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G	4090 T Y A N G N T T A N Y A N G N T T A N Y A S G G T T A G T A S G G T T A G T A S G G T T A G	4100 N N T T A G G R T T A N N T T A G G R T T A G S T T A G G G T T A G S T T A G G G T T A G S T T A G G G T T A	4110         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G         G G G T T A G G G G         G G G T T A G G G G         G G G T T A G G G G	4120 T K A G G S T T A T K A G G S T T A T K A G G S T T A T A G G G T T A T A G G G T T A	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G R T T A G G G G G T T A G G G G G T T A G G
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	4070 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T	4080 T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G	4090 T Y A N G N T T A N T Y A N G N T T A N T Y A N G N T T A N T A S G G T T A G T A S G G T T A G	4100 N N T T A G G R T T A N N T T A G G R T T A G S T T A G G R T T A G G T T A G G G T T A G S T T A G G G T T A G S T T A G G G T T A	4110 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G	4120 T K A G G S T T A G T K A G G S T T A G T K A G G G S T T A G T N A G G G T T A G T A G G G T T A G 4190	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G R T T A G G G G G T T A G G G G G T T A G G A2200
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	4070 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T 4140 R T A G S R T A G N	4080 T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G G	4090 T Y A N G N T T A N T Y A N G N T T A N T Y A N G N T T A N T A S G G T T A G T T A G G G T T A G T T A S G G T T A G T T A S G G T T A G T T A S G G T T A G 4160 S G T T A G G R T T	4100 N N T T A G G R T T A N N T T A G G R T T A G S T T A G G G T T A G G T T A G G G T T A G S T T A G G G T T A A G N G T A G G G T T A	4110 A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G G A G G G G T T A G G G G G G A G G G G T T A G G G G G T T A G G G G	4120 T K A G G S T T A T K A G G S T T A T K A G G S T T A T A G G G T T A T A G G G T T A T A G G G T T A 4190 4190	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G R T T A G G G G G T T A G G G G G T T A G G G G G T T A G G 4200 T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered	4070 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A S S G T T A G G G T T	4080 T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G T T A G N G T T A G	4090 I Y A N G N T T A N I Y A N G N T T A N Y A N G N T T A N T A S G G T T A G T A S G G T T A G T A S G G T T A G G T T A G G R T T 4160 G G T T A G G R T T	4100 N N T T A G G R T T A N N T T A G G R T T A G S T T A G G G T T A G S T T A G G G T T A G S T T A G G G T T A A G N G T T A G G G T T A G N G T T A G G G G	4110 G G G T T A G G G G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G A G G G G T T A G G G G G G T T A G G G G	4120 K A G G S T T A K A G G S T T A K A G G S T T A T A G G G T T A T A G G G T T A T A G G G T T A 4190 G T K A G G G T G T K A G G G T	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G G T T A G G G G G T T A G G G G G T T A G G G G G T T A G G H A G G G T T A T A G G G T T A T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	4070 T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T A G G G T T A G G G T 4140 R T T A G S R T T A G N R T T A G S R T T A G N R T T A G S R T T A G N	4080 T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G R T A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T A G G G G T T A G G G T T A G N G T T A G G G T T A G N G T T A G G T T A G N G T T A G G T T A G N G T T A G	4090 I Y A N G N T T A N I Y A N G N T T A N I Y A N G N T T A N T A S G G T T A G T A S G G T T A G G T T A G G R T T A G G T T A G G R T T A G G T T A G G R T T A G G T T A G G R T T A	4100 N N T T A G G R T T A N N T T A G G R T T A G S T T A G G G T T A G G T T A G G G T T A G G T T A G G G T T A A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T	<i>4110</i> G G G T T A G G G G G G T T A G G G <i>A</i> G G G T T A G G G <i>A</i> G G G T T A G G G <i>A</i> G G G T T A G G G <i>A</i> G G G T T A G G G <i>A</i> G G G T T A G G G <i>A</i> G G G N T T A G G <i>A</i> A G G N T T A G G T A G G N T T A G G T A G G N T T A G G <i>A</i> G G N T T A G C <i>A</i> G G N T T A G G <i>A</i> G G N T T A G G C <i>A</i> G G N T T A G G C <i>A</i> G G N T T A G G C <i>A</i> G G N T T A G G C <i>A</i> G G N T T A G G C <i>A</i> G G N T T A G G C <i>A</i> G G A T T A G G C <i>A</i> G G A T T A G G C <i>A</i> G G A T T A G C C <i>A</i> G G A T T A G C C <i>A</i> G G A T T A G C C <i>A</i> G G A T T A G C C <i>A</i> G G A T T A G C C <i>A</i> G C A T T A G C C <i>A</i> G C A T T A C C C <i>A</i> G C C A T T A C C C C <i>A</i> G C C A T T A C C C C C C C C C C C C C C	4120 K A G G S T T A K A G G S T T A K A G G S T T A T A G G G T T A T A G G G T T A T A G G G T T A 4190 G T K A G G G T G T K A G G G T G T K A G G G T G T K A G G G T	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G G T T A G G G G G T T A G G G G G T T A G G G G G T T A G G 4200 T A G G G T T A I A G G G T T A I A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	4070 T A G G G T T A G G T T A G T T A G S R T T A G N G T T A G S R T T A G N	4080         A       G       G       T       T       A       G       R         T       A       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       S       G       T       T       A       S       S       G         T       A       G       G       T       T       A       G       G       R         T       A       G       G       T       T       A       G       G       G         T       A       G       G       T       T       A       G	4090 I Y A N G N T T A N I Y A N G N T T A N I Y A N G N T T A N T A S G G T T A G I T A S G G T T A G G T T A G G R T T A G G T T A G G G R T T A G G T T A G G R T T A G G R T T A G G T T A G G R T T A G G R T T A G G T T A G G R T T A G G R T T A G G T T A G G R T T A G G R T T A G G T T A G G R T T A G G R T T A G G R T T A G G T T A G G R T T A G	4100 N N T T A G G R T T A N N T T A G G R T T A N N T T A G G R T T A G S T T A G G G T T A G S T T A G G G T T A G S T T A G G G T T A A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T	<i>4110</i> G G G T T A G G G G G G T A A G G G G G G T A A G G G <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i> <i>4180</i>	4120 F K A G G S T T A K A G G S T T A K A G G S T T A K A G G G S T T A T A G G G T T A T A G G G T T A T A G G G T T A 4190 G T K A G G G T G T K A G G G T G T K A G G G T G T K A G G G T S G T K A G G G T S G T K A G G G T S N T K A G G G T	4130 G G G T T A N R G G G T T A N R G G G T T A N R G G G T T A G G G G G T T A G G G G G T T A G G G G G T T A G G T A G G G T T A
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	<i>4070</i> T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T <i>A</i> G G G T T A G G G T T A G G N <i>A</i> T T A G G G T T A G G T <i>A</i> G G G T T A G G G T T A G G G T <i>A</i> G G G T T A G G G T T A G G N <i>A</i> T T A G G G T T A G C T <i>A</i> G G G T T A G G T T A G G C T <i>A</i> G T T A G G G T T T A G G C T <i>A</i> T A G G G T T A G G T T A G G N <i>A</i> T T A G G G T T A G C T T A G G C T <i>A</i> G T T A G G G T T T A G G C T T T A G G C T <i>A</i> G T T A G G G T T T A G G T T T A G G C T <i>A</i> T A G G G T T A G G T T T A G G C T T T A G G C T T T A G G T T T A G T T A G G T T T A G T T T A G G T T T A G G T T T A G G T T T A G G T T T	4080         A       G       G       T       A       G       R         A       G       G       T       T       A       G       R         A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       R         T       A       G       G       G       T       T       A       G       G       G         T       A       G       G       G       T       T       A       G	4090 T Y A N G N T T A N Y A N G N T T A N Y A N G N T T A N T A S G G T T A G T A S G G T T A G T A G G G T T A G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G R T T G G T T A G G G R T T G G T T A G G R T T G G T T A G G G R T T G G T T A G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G G T T A G G G R T T G T T A G G G T T T A G G G T T T G T T T A G G G T T T A G G G T T T A G G G T T T G T T T A G G G T T T A G G G T T T A G G T T T A G G G T T T A G G T T T A G G T T T A G G T T T A G T T	4100 N N T T A G G R T T A N N T T A G G R T T A N N T T A G G R T T A G S T T A G G G T T A G S T T A G G G T T A G S T T A G G G T T A A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G N G T T A G G G T A G G G T T A G G G T	<i>4110</i>	4120 T K A G G S T T A T K A G G S T T A T K A G G S T T A T A G G G T T A 4190 4190 G T K A G G G T G T K A G G G T C T A G G G G T C T A G G G T C T A G G G T C T A G G G G T T	<i>4130</i> G G G T T A N R G G G T T A N R G G G T T A N R G G G T T A G G G G G T T A G G G G G T T A G G G G G T T A G G T A G G G T T A T A G G G T T A

	4210	4220	4230	4240	4250	4260	4270
1d.E2.filtered	G G G T T A G G G T Y N	G G G T T <mark>A</mark> G G G T T <mark>A</mark>	<mark>\</mark> G N G T T <mark>A</mark> G G G T	TNSGRTTAGG	G T T <mark>A G G G T T</mark> /	A G G G T T <mark>A</mark> G G G T	ΤΜΚΝ <mark>Γ</mark> Τ
2d.E2.filtered	G G G T T <mark>A</mark> G G G T Y N	<mark>ϾϾϾͳͳ</mark> ΑϾϾϾͳͳ <mark>Α</mark>	GNGTT <mark>A</mark> GGGT	TNSGRTTAGG	G T T <mark>A</mark> G G G T T <mark>/</mark>	<mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	ΤΜΚΝΟΤ
1d.hapA.filtered	GGGTTAGGGTYN	G G G <mark>T T A</mark> G G G <b>T T </b> A	G N G T T <mark>A</mark> G G G T	TNSGRTTAGG	G T T <mark>A</mark> G G G T T /	A G G G T T <mark>A</mark> G G G T	ΤΜΚΝΟΤ
2d.hapA.filtered	G G N T T A G G G T T A	G G G T T A S N G T T A	G N G T T <mark>A</mark> G G G T	Τ <mark>Α</mark> G S G Τ Τ <mark>Α</mark> G Κ (	G T T <mark>A G K G T T /</mark>	A G G G T Y <mark>A </mark> G G R T	TRGGGT
Telomere.reference	G G G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T <mark>A</mark> G G G T T <mark>A</mark>	<mark>A G G G T T A </mark> G G <mark>G</mark> T	T <mark>A G G G T T A</mark> G G (	G T T <mark>A G G G T T</mark> /	A G G G <mark>T T A</mark> G G G <mark>T</mark>	T <mark>A</mark> G G G T
consensus	G G G T T A G G G T T A	G G G T T A G G G T T A		TAGGGTTAGG	GTTAGGGTT	A G G G T T A G G G T	T <mark>A</mark> GGG T
	4280	4290	4300	4310	4320	4330	4340
1d.E2.filtered	<mark>Τ</mark> Α <mark>ΘΘΘΤΤΑ</mark> ΘΘ <mark>Ο</mark> Τ	T <mark>A</mark> GGG <mark>TT A</mark> GGG	T W G G G T T A G G	G T T A G G G T N A	G N G T T A G G R <sup>-</sup>	ͳ <mark>ͳͺΑ</mark> ͺϬͺϬͺ <mark>ͳͺͳͺΑ</mark> ͺϬͺϬ	G <mark>T T A</mark> G G
2d.E2.filtered	<mark>Τ</mark> Α <mark>ΘΘΘΤΤΑ</mark> ΘΘ <mark>Ο</mark> Τ	T <mark>A</mark> GGG <mark>TTA</mark> GGG	T W G G G T T A G G	G T T A G G G T N A	G N G T T A G G R <sup>-</sup>	ͳ <mark>ͳͺΑ</mark> ͺϬͺϬͺ <mark>ͳͺͳͺ</mark> ΑͺϬͺϬ	G <mark>T T A</mark> G G
1d.hapA.filtered	T A G G G T T A G G G T	Τ <mark>Α</mark> GGG <mark>ΤΤΑ</mark> GGG	<mark>Τ</mark> ΨGGG <mark>ΤΤΑ</mark> GG		G N G T T A G G R T	T T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T <mark>A</mark> G G
2d.hapA.filtered	T <mark>AGGGTTA</mark> GSRT	<mark>Τ</mark> Α <mark>SGGΤΤΑ</mark> GΝG	Υ <mark>Μ<mark>G</mark>GR<mark>ΤΤΑ</mark>GR</mark>	GTKAGNGTTA	5 G N T T A G G G T	<mark>ΓΤΑ</mark> GGG <mark>ΤΤΑ</mark> GG	G T T R G G
Telomere.reference	T <mark>A</mark> GGG <mark>TTA</mark> GGG <mark>T</mark>	T <mark>A</mark> GGG <mark>TT A</mark> GGGG	T <mark>A</mark> GGG <mark>T T A</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	<mark>ΓΤΑ</mark> GGG <mark>ΤΤΑ</mark> GG	G T T <mark>A</mark> G G
consensus	T <mark>A</mark> GGGTTAGGGT	Τ <mark>Α</mark> GGG <mark>ΤΤΑ</mark> GGG	T <mark>A</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A	GGG <mark>TTA</mark> GGG	Γ <mark>Τ Α</mark> G G <mark>Τ Τ Α</mark> G G	G T T <mark>A</mark> G G
	4350	4360	4370	4380	4390	4400	4410
1d.E2.filtered	GTTWGGNTTAGG	GTTAGGGTTAG		GSATTANNGT		NGTKMGNGTTA	G G G T T A
2d.E2.filtered	G T T W G G N T T A G G	G T T A G G G T T A G N	IGTTAGKGTTA	GSATTANNGT		N G T K M G N G T T A	G G G T T A
1d.hapA.filtered	G T T W G G N T T A G G	G T T A G G G T T A G N	IGTTAGKGTTA	GSATTANNGT		N G T K M G N G T T A	G G G T T A
2d.hapA.filtered	G T T <mark>A</mark> G G G T T <mark>A</mark> G G	G T T A K R G T T A G C	S T T A N G R T T A	GSSTTAGGRT	< <mark>A</mark> SGS <mark>TTA</mark> G	G G T T <mark>A</mark> G G G T T <mark>A</mark>	GRGTYA
Telomere.reference	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T A G G G T T A	GGGTTAGGGGT	<mark>A</mark> GGG <mark>TTA</mark> G	G <mark>TTA</mark> GGG <mark>TTA</mark>	GGG <mark>TT</mark> A
consensus	G T T <mark>A</mark> G G G T T <mark>A</mark> G G	G <mark>TTA</mark> GGG <mark>TTA</mark> GG	G T T <mark>A</mark> G G G T T <mark>A</mark>	G G <mark>A T T A</mark> G G G T	T <mark>A</mark> GGG <mark>TTA</mark> G	G G T T <mark>A</mark> G G G T T <mark>A</mark>	G G G T T A
	4420	4430	4440	4450	4460	4470	4480
1d.E2.filtered	4420 G G G T T A G R G T T A	4430 K N <mark>G T</mark> N <mark>A N G G W T A</mark>	4440	4450 K <mark>A G N G T T A</mark> S N (	4460 5 T T M G G R T T /	4470 A G G G T T A G G G T	4480 T A G G N T
1d.E2.filtered 2d.E2.filtered	4420 G G G T T A G R G T T A G G G T T A G R G T T A	4430 K N G T N A N G G W T A K N G T N A N G G W T A	4440 N G A T T A R G N T N G A T T A R G N T	4450 K A G N G T T A S N ( K A G N G T T A S N (	4460 TTTMGGRTTT GTTMGGRTTT	4470 A G G G T T A G G G T A G G G T T A G G G T	<i>4480</i> T A G G N T T A G G N T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	4420 G G G T T A G R G T T A G G G T T A G R G T T A G G G T T A G R G T T A	4430 K N G T N A N G G W T A K N G T N A N G G W T A K N G T N A N G G W T A	4440 N G A T T A R G N T N G A T T A R G N T N G A T T A R G N T	4450 K A G N G T T A S N G K A G N G T T A S N G K A G N G T T A S N G	4460 T T M G G R T T A T T M G G R T T A T T M G G R T T A	4470 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T	4480 T A G G N T T A G G N T T A G G N T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A	4430 K N G T N A N G G W T A K N G T N A N G G W T A K N G T N A N G G W T A N S W T T A G G R T K Y	4440 N G A T T A R G N T N G A T T A R G N T N G A T T A R G N T	4450         K       G       N       G       T       A       S       N         K       A       G       N       G       T       T       A       S       N         K       A       G       N       G       T       T       A       S       N         K       A       G       N       G       T       T       A       S       N         K       A       G       N       G       T       T       A       S       N         K       A       G       N       G       T       T       A       S       N         K       S       G       G       T       T       A       G       N	4460 5 T T M G G R T T / 5 T T M G G R T T / 5 T T M G G R T T / 5 T T A G S R T T /	4470 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T Y A G G R T	4480         T       A       G       N       T         T       A       G       N       T         T       A       G       G       N       T         T       A       G       G       N       T         T       A       G       C       N       T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       R       G       T       T       A         G       G       G       T       T       A       G       G       N       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A         G       G       G       T       T       A       G       G       G       T       T       A	4430 K N G T N A N G G W T A K N G T N A N G G W T A K N G T N A N G G W T A N S W T T A G G R T K N G G G T T A G G G T T A	4440         N       G       A       T       A       R       G       N       T         N       G       A       T       T       A       R       G       N       T         N       G       A       T       T       A       R       G       N       T         N       G       A       T       T       A       R       G       N       T         N       G       A       T       T       A       R       G       N       T         N       G       A       T       T       A       R       G       N       T         N       S       R       W       T       A       N       N       G       W         N       G       G       T       T       A       G       G       T       T       A       G       G       T	4450         K       G       N       G       T       T       A       S       N         K       A       G       N       G       T       T       A       S       N       G         K       A       G       N       G       T       T       A       S       N       G         K       A       G       N       G       T       T       A       S       N       G         K       A       G       N       G       T       T       A       S       N       G         K       A       G       G       G       T       T       A       G       G       G         T       R       S       G       G       T       T       A       G       G       G	4460 T T M G G R T T A T T M G G R T T A T T M G G R T T A T A G S R T T A T A G G G T T A	4470 A G G G T T A G G G T A G G G T T A G G G T A G G G T T A G G G T A G G G T Y A G G R T A G G G T T A G G G T	I       A       G       G       N       T         I       A       G       G       N       T         I       A       G       G       N       T         I       A       G       G       N       T         I       A       G       G       N       T         I       A       G       G       N       T

	4	1490	450	0	451	)	4520		453	80		4540		4	550
1d.E2.filtered	T <mark>A</mark> K <mark>G G T T</mark> A		MKNRTT	A G S G T	W <mark>A</mark> GGG	T <mark>A</mark> G G N	N <mark>T T </mark> A G G	GTNM	S <mark>G G T T</mark>	A K G C	G T T A G	G G T	T <mark>A</mark> S S I	NWTA	G
2d.E2.filtered	T <mark>A</mark> KGGTT		MKNRTT	A G S G T	WAGGG	T <mark>A</mark> G G N	N T T <mark>A</mark> G G	GTNM	S <mark>G G T T</mark>	A K G C	G T T A G	G G T	T <mark>A</mark> S S I	NWTA	G
1d.hapA.filtered	T <mark>A</mark> K <mark>G G T T</mark> A		MKNRTT	A G S G T	W <mark>A</mark> G G G	T <mark>A</mark> G G N	N <mark>T T A</mark> G G	G T N M	S <mark>G G T T</mark>	A K G C	G T T A G	G G T	T <mark>A</mark> S S I	N W T A	G
2d.hapA.filtered	Y <mark>A</mark> YGRYT <i>A</i>	N N T K	K <mark>A</mark> K K <mark>G T</mark> N	<mark>a</mark> ggg <mark>t</mark>	T <mark>A</mark> S R <mark>G</mark>	T <mark>T A</mark> G G O	G <mark>T</mark> K <mark>A</mark> G G	<mark>a</mark> k <mark>t a</mark>	S S <mark>G</mark> N T	r <mark>g</mark> r o	G T T A G	NGW	T <mark>A</mark> G G	g t t <mark>a</mark>	GK
Telomere.reference	T <mark>A</mark> GGGTT		A G G G T T	<mark>a</mark> ggg <mark>t</mark>	T <mark>A</mark> G G G	T <mark>A</mark> G G (	G <mark>TTA</mark> GG	G T T A	G G G T T	A G G G	G T T <mark>A</mark> G	G G T	T <mark>A</mark> GG	G T T <mark>A</mark>	GG
consensus	TAKGGTTA		AGKGTT	AGGG		T <mark>A</mark> GG	G <mark>TTA</mark> GG	GTTA	G G G T T	AGGO	GTTAG	GGT		G T T A	G
	4	1560	457	0	458	)	4590		460	00		4610		4	620
1d.E2.filtered	G T T N N G A T	G <mark>A</mark> G N G	<mark>C Τ Α</mark> Κ Ν Ν	G G <mark>A</mark> G G	G T T T G	G <mark>T C A</mark> (	S N <mark>C</mark> T T A	NGGN	TNGGG	TTN	A A T T	<mark>A</mark> G N	GNTC	GNGT	ΤN
2d.E2.filtered	G T T N N G A T	GAGNO	στακνν	GG <mark>A</mark> GG	G T T T G	G G T C A O	S N <mark>C T T A</mark>	NGGN	TNGGG	TTN	A A T T	<mark>A</mark> G N	GNTC	GNGT	ΤN
1d.hapA.filtered	G T T N N G A T	GAGNO	C T A K N N	GG <mark>A</mark> GG	G T T T G	G G T C A O	S N C T T A	NGGN	T N G G G	TTN	A A T T	<mark>A</mark> GN	GNTC	GNGT	ΤN
2d.hapA.filtered	R T T A G G R T	NAGGO	6 K <mark>T A A</mark> G G	T T <mark>A</mark> S G	RTTAN	G <mark>T T A</mark> (	G K <mark>G T T</mark> M	S <mark>G G T</mark>	T <mark>A</mark> G G G	T T <mark>A</mark> (	G G G T T	AGG	GTKN	C C G T	T <mark>A</mark>
Telomere.reference	G T T A G G G T	T <mark>T</mark> AGGG	G <mark>TTA</mark> GGG	T T <mark>A</mark> GG	G T T <mark>A</mark> G	G <mark>T T A</mark> (	G G G T T A	GGGT	T <mark>A</mark> GGG	T T <mark>A</mark> (	G G <mark>T T</mark>	AGG	G T T A	G G G T	T <mark>A</mark>
consensus	G T T <mark>A</mark> G G <mark>A</mark> T	G <mark>A</mark> G G G	G <mark>CTA</mark> KGG	GGAGO	G T T T G	G <mark>T C A</mark> (	G K <mark>C T T A</mark>	S <mark>G G T</mark>	T <mark>A</mark> G G G	T T <mark>A</mark> T	T <mark>A</mark> A T	<mark>a</mark> gg	GTTC	G S G T	T <mark>A</mark>
		1630	464	0	465	)	4660		467	70		4680		4	690
1d.E2.filtered	N <mark>G A T T A G</mark> A	1630 A <mark>GTTA</mark>	464 G G T T N G	0 NGTTA			4660 AGGGK	T M C Y	467 R K T A S	7 <i>0</i> R R T H	(MSS <mark>A</mark>	4680 T T A	<mark>G</mark> ΜΚΚ	4 Y <mark>A</mark> KG	690 G Y
1d.E2.filtered 2d.E2.filtered	N G A T T A G A N G A T T A G A	1630 G T T A G G T T A G	464 6 G G T T N G 6 G G T T N G	0 NGTTA NGTTA	465 GCNTT	)	<i>4660</i> A G G G K A G G G K	T M <mark>C</mark> Y T M <mark>C</mark> Y	467 R K T A S R K T A S	7 <i>0</i> R R T K R R T K	(mss <mark>a</mark>	<i>4680</i> T T A T T A	<mark>G</mark> M K K '	4 Y <mark>A</mark> KG Y <mark>A</mark> KG	690 G Y
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered	N G A T T A G A N G A T T A G A N G A T T A G A	4630 A G T T A G A G T T A G A G T T A G	464 G G T T N G G G T T N G G G T T N G	0 N G T T A N G T T A N G T T A	465 G C N T T G C N T T	A     G     G     T     T       A     G     G     G     T     T       A     G     G     G     T     T	4660 A G G G K A G G G K A G G G K	T M C Y T M C Y T M C Y	467 R K T A S R K T A S R K T A S	7 <i>0</i> R R T H R R T H R R T H	(	4680 T T A T A A A A	G M K K G M K K G M K K	4 Y A K G Y A K G Y A K G	690 G Y G Y
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	N G A T T A G A N G A T T A G A N G A T T A G A G G G T T A G F	4     6     T     T     A     6       4     6     T     T     A     6       4     6     T     T     A     6       8     6     T     T     A     6       8     5     T     T     A     6	464 G G G T T N G G G T T N G G G T T N G G G T N A G A G T N A G	0 N G T T A N G T T A N G T T A G G T T A	4650 G C N T T G C N T T G C N T T N G C T G	A G G G T 1 A G G G T 1 A G G G T 1 A N G N T 1	4660 A G G G K A G G G K A G G K A G G N T	T M C Y T M C Y T M C Y T C N G	467 R K T A S R K T A S R K T A S A T T A A	70 R R T H R R T H R R T H G A T 1	(	4680 T T A T T A T T A T T A N T N	G M K K G M K K G M K K G N T	<i>4</i> Y A K G Y A K G Y A K G T A G G	690 G Y G Y G Y
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	N G A T T A G A N G A T T A G A N G A T T A G A G G G T T A G A G G G T T A G A	4 G J T A G A G T T A G A G T T A G A G T T A G R S T T A G S G T T A G	464 G G T T N G G G T T N G G G T T N G A G T N A G G G T T A G	0 N G T T A N G T T A N G T T A G G T T A G G T T A	465 G C N T T G C N T T G C N T T N G C T G G G G T T	A         G         G         T         T           A         G         G         G         T         T           A         G         G         G         T         T           A         G         G         G         T         T           A         G         G         N         T         T           A         G         G         G         T         T	4660 A G G G K A G G G K A G G G K A G G N T A G G N T A G G N T	T     M     C     Y       T     M     C     Y       T     M     C     Y       T     M     C     Y       T     M     C     Y       T     A     G     G	467 R K T A S R K T A S R K T A S A T T A A G T T A G	70 R R T H R R T H R R T H G A T 1 G G T 1	( M S S A ( M S S A ( M S S A ( M S S A A G G N	4680 T T A T A A A A N T A N T A	G M K K G M K K G M K K G G N T G G G T	4 Y A K G Y A K G Y A K G T A G G T A G G	690 G Y G Y N T G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	N     G     A     T     T     A     G     A       N     G     A     T     T     A     G     A       N     G     A     T     T     A     G     A       N     G     A     T     T     A     G     A       G     G     G     T     T     A     G     A       G     G     G     T     T     A     G     C       G     G     G     T     T     A     G     C	4       G       T       T       A       G         4       G       T       T       A       G         4       G       T       T       A       G         4       G       T       T       A       G         4       G       T       T       A       G         5       G       T       T       A       G         6       G       T       T       A       G         4       G       T       T       A       G	464 G G T T N G G G T T N G G G T T N G A G T N A G G G T T A G G G T T A G	0 N G T T A N G T T A N G T T A G G T T A G G T T A G G T T A	4       5         4       5       7         4       6       0       7       7         4       6       0       7       7         4       6       0       7       7         4       6       0       7       7         4       6       6       7       7         4       6       6       7       7         4       6       6       7       7         4       6       6       7       7	A G G G T T A G G G T T A G G G T T A N G N T T A N G G G T T A G G G T T	4660 A G G G K A G G G K A G G G K A G G G K A G G G T A G G G T	T       M       C       Y         T       M       C       Y         T       M       C       Y         T       M       C       Y         T       M       C       Y         T       A       G       G         T       M       C       B	467 R K T A S R K T A S R K T A S R K T A S A T T A A G T T A S	70 R R T F R R T F R R T F G A T 1 G G T 1 G R T 1	( M S S A ( M S S A ( M S S A A G G N A G G A	4680 T T A T T A T T A N T N T A T A	G M K K G M K K G M K K G G N T G G G T	4 Y A K G Y A K G Y A K G T A G G T A G G	690 G Y G Y G Y N T G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A	4630 A G T T A G A G T T A G A G T T A G C T T A G G T T A G A G T T A G A G T T A G	464 G G T T N G G G T T N G G G T T N G A G T N A G G G T T A G G G T T A G	Ø       N     G     T     T     A       N     G     T     T     A       N     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A	465 G C N T T G C N T T G C N T T N G C T G G G G T T G C S T T	A G G G T T A G G G T T	4660 A G G G K A G G G K A G G G K A G G G N T A G G G T	T M C Y T M C Y T M C Y T C N G T A G G T M C B	467 R K T A S R K T A S R K T A S A T T A A G T T A G R T T A S	70 R R T H R R T H G A T T G G T T G R T T	( M S S A ( M S S A ( M S S A A G G N A G G A A G G A	4680 T T A T T A T A N T A N T N T A	G M K K G M K K G M K K G G N T G G G T G V G T	4 Y A K G Y A K G Y A K G T A G G T A G G T A G G	690 G Y G Y G Y N T G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i>	N G A T T A G A N G A T T A G A N G A T T A G A G G G T T A G F G G G T T A G A G G A T T A G A	4630 A G T T A G A G T T A G 700	464 G G T T N G G G T T N G G G T T N G G G T T A G G G T T A G G G T T A G 471 4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Ø       N     G     T     T       N     G     T     T       Q     G     T     T       G     G     T     T       G     G     T     T       G     G     T     T       G     G     T     T	465 G C N T T G C N T T G C N T T N G C T G G G G T T G C S T T 472		4660 A G G G K A G G G K A G G G K A G G G N A G G G T A G G G T 4730	T M C Y T M C Y T M C Y T C N G T A G G T M C B	467 R K T A S R K T A S R K T A S R K T A S A T T A A G T T A S R T T A S	70 R R T H R R T H G A T H G A T 1 G R T 1 G R T 1	( M S S A ( M	4680 T T A T T A T T A N T N T A T A 4750	G M K K G M K K G M K K G G N T G G G T G V G T	4 Y A K G Y A K G Y A K G T A G G T A G G T A G G A G G	690 G Y G Y N T G T G T
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered	N G A T T A G A N G A T T A G A N G A T T A G A G G G T T A G A G G G T T A G A G G G T T A G A G G A T T A G A G G A T T A G A G G A T T A G A	4       G       T       T       A       G         4       G       T       T       A       G         4       G       T       T       A       G         4       G       T       T       A       G         5       T       T       A       G       G         6       G       T       T       A       G         6       G       T       T       A       G         4       G       T       T       A       G         17700       S       S       Y       Y       Y	464 G G T T N G G G T T N G G G T T N G G G T T A G G G T T A G G G T T A G G G G T T A G 471 (A G G G T Y	Ø       N     G     T     T       N     G     T     T       Q     G     T     T       Q     G     T     T       Q     G     T     T       Q     G     T     T       Q     G     T     T	465 G C N T T G C N T T G C N T T N G C T G G G G T T G C S T T 4720 4770 4770 4770 4770 4770 4770 4770 4770 4770 4770 4		4660 A G G G K A G G G K A G G G K A G G G K A G G G T A G G G C A G G G K A G K K S A G K K S A G K K S K S K S K S K S K S K S K S K S	T M C Y T M C Y T M C Y T C N G T A G G T M C B	467 R K T A S R K T A S R K T A S A T T A A G T T A G R T T A S 472 G S S T T G S S T T	70 R R T H R R T H G A T T G G T T G R T T 70 4 K W S	( M S S A ( M S S A ( M S S A A G G N A G G A A G G A	4680 T T A T T A T T A N T N T T A T T A 4750 W S Y V S Y	G M K K G M K K G M K K G G N T G G G T G V G T	4 Y A K G Y A K G Y A K G T A G G	690 G Y G Y G Y G T G T 760 G S
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.bapA.filtered	N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         G       G       G       T       T       A       G       A       G         G       G       G       T       T       A       G       A       G         G       G       G       T       T       A       G       A       G         G       G       G       T       T       A       G       A       G         G       G       G       T       T       A       G       A       A       A         T       W       K       R       T       G       G       A       A       A         T       W       K       R       T       G       G       A       A       A       A         T       W       K       R       T       G       G       C <td>4 G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       B     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G</td> <td>464 G G T T N G G G T T N G G G T T N G G G T T A G G G G T T A G 471 (A G G G G T Y (A G G G G T Y</td> <td>Ø       N     G     T     T     A       N     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A       Ø     G     G     T     T       Ø     G     K     R     Y       R     G     K     R     Y</td> <td>465 G C N T T G C N T T G C N T T N G C T G G G G T T G C S T T 472 472 472 472 472 472 472 472</td> <td></td> <td>4660 A G G G K A G G G K A G G G K A G G G T A G G G G T A G G T A G G T A G G T A G T</td> <td>T M C Y T M C Y T M C Y T C N G T C N G T A G G T A G G T T A G T T A</td> <td>467 R K T A S R K T A S R K T A S A T T A A G T T A G R T T A S 472 G S S T T G S S T T G S S T T</td> <td>70 R R T H R R T H G A T T G A T T G R T T G R T T 70 70 70 70 70 70 70 70 70 70</td> <td>(M S S A (M S S A (M S S A A G G N A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G A G G G G G A G G G G G A G G G G G A G G G G G G A G G G G G A G G G G G G A G G G G G G G A G G G G G G G G A G G G G G G G G A G G G G G G G G G G G G G G G G G G G</td> <td>4680 T T A T T A T T A N T N T A T A T A T A A T A A T A A V S Y W S Y</td> <td>G M K K G M K K G M K K G G N T G G G T G V G T Y A S G Y A S G</td> <td>4 Y A K G Y A K G Y A K G T A G G G T A G G T A G G G G G T A G G G G T A G G G G G G G G G G G G G G G G G G</td> <td>690 G Y G Y G Y G Y N T G T 760 C S S G S</td>	4 G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       B     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G       A     G     T     T     A     G	464 G G T T N G G G T T N G G G T T N G G G T T A G G G G T T A G 471 (A G G G G T Y (A G G G G T Y	Ø       N     G     T     T     A       N     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A       G     G     T     T     A       Ø     G     G     T     T       Ø     G     K     R     Y       R     G     K     R     Y	465 G C N T T G C N T T G C N T T N G C T G G G G T T G C S T T 472 472 472 472 472 472 472 472		4660 A G G G K A G G G K A G G G K A G G G T A G G G G T A G G T A G G T A G G T A G T	T M C Y T M C Y T M C Y T C N G T C N G T A G G T A G G T T A G T T A	467 R K T A S R K T A S R K T A S A T T A A G T T A G R T T A S 472 G S S T T G S S T T G S S T T	70 R R T H R R T H G A T T G A T T G R T T G R T T 70 70 70 70 70 70 70 70 70 70	(M S S A (M S S A (M S S A A G G N A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G G A G G G G A G G G G G A G G G G G A G G G G G A G G G G G G A G G G G G A G G G G G G A G G G G G G G A G G G G G G G G A G G G G G G G G A G G G G G G G G G G G G G G G G G G G	4680 T T A T T A T T A N T N T A T A T A T A A T A A T A A V S Y W S Y	G M K K G M K K G M K K G G N T G G G T G V G T Y A S G Y A S G	4 Y A K G Y A K G Y A K G T A G G G T A G G T A G G G G G T A G G G G T A G G G G G G G G G G G G G G G G G G	690 G Y G Y G Y G Y N T G T 760 C S S G S
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1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered	N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         T       W       K       R       T       G       A       A         T       W       K       R       T       G       A	4 G       T       A       G         A G       T       T       A       G         A G       T       T       A       G         A G       T       T       A       G         A G       T       T       A       G         G       T       T       A       G         G       T       T       A       G         A G       T       T       A       G         A G       T       T       A       G         A S       Y       R       Y       Y         S S       Y       R       Y       Y         A G       N       G       T       T         A G       Y       R       Y       Y         A G       N       G       T       T	464 G G T T N G G G T T N G G G T T N G G G T T A G G G T T A G G G T T A G G G G T T A G G G G T T A G 471 A G G G G T Y A G G A N T T	0           N         G         T         T         A           N         G         T         T         A           G         G         T         T         A           G         G         T         T         A           G         G         T         T         A           G         G         T         T         A           G         G         T         T         A           G         G         T         T         A           Q         G         T         T         A           Q         G         K         R         Y           R         G         K         R         Y           N         N         G         A         T	465 G C N T T G C N T T G C N T T G C N T T G G G T T G G G G G T G G G G G T H G S G G K M G S G G G G G G G G G G G G G G G G G	A G G G T T A G G G G G G G T T A G G G G G G G T T A G G G G G G G T T A G G G G G G G T T A G G G G G G G T T A G G G G G G G T T A G G G G G G G G G G T T A G G G G G G G G G T T A G G G G G G G G G	4660 A G G G K A G G G K A G G G K A G G G T A G G G T A G G G T A G G G T A S K S T A S K S T A S K S T A S K S T A S K S	T M C Y T M C Y T M C Y T C N G T A G G T A G G T A G G T T A G T T A G T T A G A K W	467 R K T A S R K T A S R K T A S R T T A A G T T A S 472 G S S T T G S S T T G S S T T G S S T T G S S T T	0       R     R       R     R       R     R       G     A       G     G       T     T       G     G       T     T       G     R       T     T       G     R       T     T       G     R       T     T       G     R       T     T       G     R       T     T       G     R       T     T       G     R       T     T       T     T	<ul> <li>M S S A</li> <li>M S S A</li> <li>M S S A</li> <li>A G G N</li> <li>A G G A</li> <li>A G A A A</li> </ul>	4680 T T A T T A N T N T T A T T A T T A 4750 W S Y W S Y W S Y W S Y W S Y W S Y	G M K K G M K K G M K K G G N T G G G T G G G T G V G T Y A S G Y A S G Y A S G Y A S G Y A S G	4 Y A K G Y A K G Y A K G T A G K Y A G K Y A G K Y A G K Y A G K Y A	690 G Y G Y G Y N T G T 760 C S G S G S G S
1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference <i>consensus</i> 1d.E2.filtered 2d.E2.filtered 1d.hapA.filtered 2d.hapA.filtered Telomere.reference	N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         N       G       A       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         G       G       G       T       T       A       G       A         T       W       K       R       T       G       A       A         T       W       K       R       T       G       A	4       G       T       T       A       G         4       G       T       T       A       G         4       G       T       T       A       G         4       G       T       T       A       G         5       G       T       T       A       G         5       G       T       T       A       G         7       O       C       T       T       A         7       O       C       T       T       A         7       O       C       T       T       A       G         7       O       C       T       T       A       G         7       O       C       T       T       A       G         7       O       C       T       T       A       G         7       O       C       T       T       A       G         8       S       Y       R       Y       Y       A       G       G       T       T         8       S       Y       R       Y       Y       Y       A       G <t< td=""><td>464 G G T T N G G G T T N G G G T T N G G G T T A G G G T T A G G G G T T A G C T T A G G G G T T A G C T T T A G C</td><td>Ø         N       G       T       T       A         N       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         Ø       G       T       T       A         Ø       G       K       R       Y         R       G       K       R       Y         N       N       G       A       T         A       G       G       G       T</td><td>465 G C N T T G C N T T G C N T T G C N T T G C T G G G G T T G G C S T T 4 G C S T T 4 4 5 6 7 7 7 7 7 7 7 7 7 7 7 7 7</td><td>A G G G T T A G G G G G G T T A G G G G G T T A G G G G G G G T T A G G G G G G G G G G T T A G G G G G G G G T T A G G G G G G G G T T A G G G G G G G T T A G G G G G G G G T T A G G G G G G G G G G T T A G G G G G G G G T T A G G G G G G G G G G T T A G G G G G G G G G G G G G G G G G G G</td><td>4660 A G G G K A G G G K A G G G K A G G G K A G G G T A G G G T A G G G T A S K S T A S K S</td><td>T         M         C         Y           T         M         C         Y           T         C         N         G           T         C         N         G           T         A         G         G           T         T         A         G           G         T         T         A           G         T         T         A           G         A         K         W           G         T         T         A           G         A         K         W           G         T         T         A</td><td>467         R       T       A       S         R       K       T       A       S         R       K       T       A       S         A       T       T       A       A         G       T       T       A       A         G       T       T       A       S         R       T       T       A       A         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       G       G       C       Y         G       G       G       T       T</td><td>Ø           R         R           R         R           R         R           G         A           G         A           G         R           G         R           R         R           G         R           G         R           G         R           G         R           G         R           G         R           G         R           A         K           A         K           A         K           A         G           G         G</td><td>( M S S A ( M S S A ( M S S A ( M S S A A G G N A G G A A G G A</td><td>4680 T T A T T A T T A N T N T T A T T A 4750 W S Y W S Y</td><td>G M K K G M K K G M K K G G N T G G G T G G G T Y A S G Y A S G G Y A S G G S S S S S S S S S S S S S S S S</td><td>4 Y A K G Y A K G Y A K G T A G G K Y A G K Y A G K Y A G K Y A G K Y A G T T A</td><td>690 G Y G Y G Y G Y G Y G T 760 C G S G S G S G S G R G R</td></t<>	464 G G T T N G G G T T N G G G T T N G G G T T A G G G T T A G G G G T T A G C T T A G G G G T T A G C T T T A G C	Ø         N       G       T       T       A         N       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         G       G       T       T       A         Ø       G       T       T       A         Ø       G       K       R       Y         R       G       K       R       Y         N       N       G       A       T         A       G       G       G       T	465 G C N T T G C N T T G C N T T G C N T T G C T G G G G T T G G C S T T 4 G C S T T 4 4 5 6 7 7 7 7 7 7 7 7 7 7 7 7 7	A G G G T T A G G G G G G T T A G G G G G T T A G G G G G G G T T A G G G G G G G G G G T T A G G G G G G G G T T A G G G G G G G G T T A G G G G G G G T T A G G G G G G G G T T A G G G G G G G G G G T T A G G G G G G G G T T A G G G G G G G G G G T T A G G G G G G G G G G G G G G G G G G G	4660 A G G G K A G G G K A G G G K A G G G K A G G G T A G G G T A G G G T A S K S T A S K S	T         M         C         Y           T         M         C         Y           T         C         N         G           T         C         N         G           T         A         G         G           T         T         A         G           G         T         T         A           G         T         T         A           G         A         K         W           G         T         T         A           G         A         K         W           G         T         T         A	467         R       T       A       S         R       K       T       A       S         R       K       T       A       S         A       T       T       A       A         G       T       T       A       A         G       T       T       A       S         R       T       T       A       A         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       S       S       T       T         G       G       G       C       Y         G       G       G       T       T	Ø           R         R           R         R           R         R           G         A           G         A           G         R           G         R           R         R           G         R           G         R           G         R           G         R           G         R           G         R           G         R           A         K           A         K           A         K           A         G           G         G	( M S S A ( M S S A ( M S S A ( M S S A A G G N A G G A A G G A	4680 T T A T T A T T A N T N T T A T T A 4750 W S Y W S Y	G M K K G M K K G M K K G G N T G G G T G G G T Y A S G Y A S G G Y A S G G S S S S S S S S S S S S S S S S	4 Y A K G Y A K G Y A K G T A G G K Y A G K Y A G K Y A G K Y A G K Y A G T T A	690 G Y G Y G Y G Y G Y G T 760 C G S G S G S G S G R G R



1d.E2.filtered
2d.E2.filtered
1d.hapA.filtered
2d.hapA.filtered
Telomere.reference
consensus

							4	98	0								4	99	90										
Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	N
Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	N
Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν
Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν	Ν
G	Т	Т	A	G	G	G	т	т	A	G	G	G	т	т	A	G	G	G	т	Т	Α	G	G	G	т	т	Α	G	G

consensus

XLIX

# F Students' contribution to the families study

The table below shows the contribution of each student to the project. Abbreviations are: TEL: code for control families; LRI: code for LS samples from Leicester's Royal Infirmary; BWH: code for LS samples from Birmingham Women's Hospital; FT: Frances Tippins; CG: Carmen Garrido.

*Recruitment* refers to: ethical approval (for BWH families recruited by CG, documents generated by FT were used. For TEL families, new documents were generated), family selection based on pedigree, family contact (for BWH families recruited by CG, this was done by J. Hoffman in BWH), saliva kit preparation (including documents) and shipment. *Sample processing* refers to: aliquoting, stock storage, DNA extraction and STELA analysis (from PCR to telomere sizing). *Analysis* refers to: statistical analysis.

Family	Recruitment	Sample processing	Analysis
TEL F1	CG	CG	CG
TEL F2	CG	CG	CG
TEL F3	CG	CG	CG
TEL F4	CG	CG	CG
TEL F6	CG	CG	CG
TEL F7	CG	CG	CG
TEL F8	CG	CG	CG
TEL F9	CG	CG	CG
TEL F11	CG	CG	CG
TEL F12	CG	CG	CG
LRI F1	$\mathbf{FT}$	$\mathbf{FT}$	CG
LRI F4	$\mathbf{FT}$	CG	CG
LRI F5	$\mathbf{FT}$	$\mathbf{FT}$	CG
LRI F7	$\mathbf{FT}$	$\mathbf{FT}$	CG
LRI F12	$\mathbf{FT}$	CG	CG
LRI F14	$\mathbf{FT}$	CG	CG
BWH F1a	CG	CG	CG
BWH F3a	CG	CG	CG
BWH F5a	CG	CG	CG
BWH F6a	CG	CG	CG
BWH F11a	CG	CG	CG
BWH F2	$\mathbf{FT}$	$\mathbf{FT}$	CG
BWH F42	$\mathbf{FT}$	$\mathbf{FT}$	CG
BWH F55	$\mathbf{FT}$	CG	CG
BWH F71	$\mathbf{FT}$	$\mathbf{FT}$	CG
BWH F100	$\mathbf{FT}$	$\mathbf{FT}$	CG
BWH F102	$\mathbf{FT}$	CG	CG
<b>BWH F105</b>	$\mathbf{FT}$	CG	CG
BWH F106	$\mathbf{FT}$	CG	CG
<b>BWH F107</b>	$\mathbf{FT}$	$\mathbf{FT}$	CG
BWH F108	$\mathbf{FT}$	CG	CG
BWH F125	$\mathbf{FT}$	$\mathbf{FT}$	CG
BWH F126	$\mathbf{FT}$	$\mathbf{FT}$	CG
BWH F130	$\mathbf{FT}$	CG	CG

### G | Technical reproducibility for control families

The scatter plots in  $\mathbf{A}$  show telomere length assayed by single telomere length analysis (STELA) using the XpYpE2 primer for the control DNA extracted from the lymphoblastoid cell line KK and run in every gel to assess technical reproducibility. The legend in the X-axis refers to the family and the corresponding gel number. **B** shows the median and interquartile for A and Kruskal Wallis test showed no differences between any gel.



# H | Technical reproducibility for Lynch syndrome families

The same control DNA than in appendix G was used to assess technical reproducibility in STELA gels for the Lynch syndrome families. **A** and **C** show medians and interquartile ranges for 12q and XpYp gels respectively. Light green scatter plots show data generated by Frances Tippins (FT) and dark green plots show data generated by Carmen Garrido (CG). **B** and **D** show the mean values for A and C respectively. Purple colour represents the gels that were excluded as the control was significantly different by Kruskal-Wallis ranking test.



#### I Control families pedigrees

The next five pages contain two control families per page. For each family, the code is shown in bold letters after the prefix "TEL" next to the pedigree. Males are indicated with squares and females with circles. The code for each individual is shown under their respective symbol preceded by the "TEL + family code" prefix. A "G" in the code indicates generation and a "P", position in the pedigree. The age of each individual in shown below the code in brackets. Below each pedigree, scatter plots showing medians and interquartile ranges (IQ) for 12q (cyan) and XpYp (purple) telomeres measured by single telomere length analysis (STELA) is shown. Details of the medians and IQ are shown in the table below each scatter plot.



TEL F2











1429

**TEL F4** 

Median Telomere Length (bp)

2047

± IQ



1515

1768

1687

1140






TEL F9 TEL F9 G1P2 TEL F9 G1P1 (69.4)(72.2)TEL F9 G2P3 TEL F9 G2P2 TEL F9 G2P1 (47.9)(45.5)(37.0)(dq) 20000-Median Telomere Length 15000 10000 5000 0 TEL-F9 G1P2 G1P1 G2P3 G2P2 G2P1 G1P2 G1P1 G2P3 G2P2 G2P1 (72.2) (69.4) (47.9) (45.5) (37.0) (72.2) (69.4) (47.9) (45.5)(37.0) Median 5601 6736 4315 6110 6109 5400 5594 4684 5594 5350 1582 1257 1901 2728 2360 1601 1025 2343 3604 1984 ± IQ





# J Lynch syndrome families pedigrees

The next twelve pages contain two Lynch syndrome (LS) families per page. For each family, the code is shown in bold letters after the prefix "LRI" for families collected from Leicester Royal Infirmary or "BWH" for families collected form Birmingham Women's Hospital next to the pedigree. Males are indicated with squares and females with circles. The code for each individual is shown under their respective symbol preceded by the "LRI or BWH + family code" prefix. A "G" in the code indicates generation and a "P", position in the pedigree. The age of each individual in shown below the code in brackets. In addition, the genotype for the mismatch repair gene (either MSH2 or MLH1) is shown below the age and "WT" refers to the wild types, non-mutation carriers. Furthermore, cancer-affected patients are shown with coloured symbols and the age of onset is also indicated after "@". Below each pedigree, scatter plots showing medians and interquartile ranges (IQ) for 12q (cyan) and XpYp (purple) telomeres measured by single telomere length analysis (STELA) is shown. Details of the medians and IQ are shown in the table below each scatter plot. Abbreviations for the tumours and colour code is: purple for colorectal cancer (CRC), green for endometrial cancer (EC), orange for urothelial carcinoma (UBC), blue for malignant melanoma (MM) and red for basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and thyroid cancer (TC).



































ledian Telomere Length (t 0000 00001 0000								· · · · · · · · · · · · · · · · · · ·						
≥ 0	BWH F105 G3P6 (67.6) <i>MSH2</i> +/-	BWH F105 G4P9 (42.0) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G4P11 (37.1) WT	BWH F105 G5P4 (20.6) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G3P5 (64.1) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G4P2 (41.0) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G4P4 (38.8) WT	BWH F105 G3P6 (67.6) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G4P9 (42.0) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G4P11 (37.1) WT	BWH F105 G5P4 (20.6) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G3P5 (64.1) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G4P2 (41.0) <i>MSH2</i> <sup>+/-</sup>	BWH F105 G4P4 (38.8) WT
Median	3993	5219	6546	4951	4979	4732	5989	4900	5375	5538	6200	5067	5074	6559
± IQ	1567	1858	2269	2778	2365	1559	2411	1177	1800	1359	2654	2350	1537	1767











#### LXXIII

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