SI APPENDIX

Mutagenesis Primers

Mutation	Forward Primer Sequence	Reverse Primer Sequence
V299M	CCACCCATCCATGGGCCGCCAGCAC	GTGCTGGCGGCCCATGGATGGGTGG
R522K	CTTGGCTGCGCAAGAGCCCAGGTGAGGAGGTGGTGGC	GCCACCACCTCCTCACCTGGGCTCTTGCGCAGCCAAG
K570N	GAGACCACGTTTCAAAACAACAGGCTCTTTTTCTACCG	CGGTAGAAAAAGAGCCTGTTGTTTTGAAACGTGGTCTC
A1062T	CAAGGGCGCCACCGGCCCTCTG	CAGAGGGCCGGTGGCGCCCTTG

Mutagenesis PCR conditions: 95 °C for 2 min, followed by 12 cycles of 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 10 min, and concluded at 72 °C for 10 min.

Amplification and Sequencing of FLAG-TERT, FLAG-TERC, and FLAG Inserts

Amplification	
Forward	CTAACTAGAGAACCCACTGCTTAC
Reverse	GATGGCTGGCAACTAGAAGGCACAG

Sequencing	
427F	CAACACGGTGACCGACGCAC
514R	CAGCAGGTGAACCAGCACGTCG
843F	GTGGACCGAGTGACCGTGGTTTC
887R	GTCTGGCAGGTGACACCACACAG
1228F	CCTGTTTCTGGAGCTGCTTGG
1411R	GAGCAGCTGCACCAGGCGACGG
1697F	CTGATGAGTGTGTACGTCGTCG
1760R	GAAACGTGGTCTCCGTGAC
2176F	CTTTGTCAAGGTGGATGTGACG
2258R	GTTTGATGATGCTGGCGATGAC
2566F	CTCCACGCTGCTCTGCAGCCTGTG
2660R	CATCCACCAAACGCAGGAGC
2959F	GAACATGCGTCGCAAACTCTTTG
3040R	CTGGAGGCTGTTCACCTGCAAATC

FLAG-TERT amplification: 95° C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 50 s, and 72 °C for 4 min, and concluded at 72 °C for 5 min.

FLAG-TERC and FLAG amplification: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 50 s, and 72 °C for 1 min, and concluded at 72 °C for 3 min.

Assessment of TERC Expression by Real-Time Polymerase Chain Reaction

TERC Real-Time Primers

TERC R	AAG AGT TGG GCT CTG TCA GC
TERC L	ATG TGT GAG CCG AGT CCT G
TERC Probe	5’ 6-FAM/TCC GTT CCT CTT CCT GCG GC/3’-TAMSp

ASO Hybridization for High-Throughput A1062T Mutation Screen

Amplification

Exon 15TERT-Fwd	tgtaaaacgacggccagtGGTCAGAAGGCTCCCAAGCG
Exon 15TERT-Rev	caggaaacagctatgaccTCGTGACTCCTGCGGTGCTT

Allele Specific Oligonucleotides

1509 (wild type)	GGGCGCC**G**CCGGCCCTCT
1510 (A1062T variant)	GGGCGCC**A**CCGGCCCTCT