

Supplemental Material for the Submission

Cell cycle dependent transcription factors control the expression of yeast telomerase RNA.

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Supplemental Figure Legends

Supplemental Figure S1. Alignment of the *TLC1* promoter region obtained from different *Saccharomyces* “sensu stricto” species. The sequence from *S. cerevisiae* S288C (Scec), *S. cerevisiae* SK1 (Scek), *S. paradoxus* (Spar), *S. cariocanus* (Scar), *S. mikatae* (Smik), *S. kudriavzevii* (Skud), *S. bayanus* (Sbay) and *S. pastorianus* (Spas) were aligned according to the major 5'-end of Scec as indicated by +1. The numbering is indicated by vertical lines each 10 nucleotides according to Scec and dashes (-) denote gaps introduced to improve the alignment. Dashed underline nucleotides represent the coding sequence of *TLC1* (from +1 and downstream), *snR161* (-252 to -412) and *PDX3* (from -642 and upstream). The positions of the different TATA box-like sequence are indicated in italic bold (-75, -171 and -221). The positions of the MCB1/SCB1 (-108) and MCB2 (-120) are indicated in bold underlined. The consensus sequence (Cons) is shown at bottom with the uppercase, lower case and dots representing universally conserved, conserved (~ 75%) or variable nucleotides respectively. The conserved regions examined in this study are indicated by letters (A to G). The percent conservation is indicated below each region in brackets.

Supplemental Figure S2. (A) Schematic representation of the *TLC1* chromosomal locus and the flanking upstream genes. The start codon of *PDX3* as well as the 5'-ends of the mature *snR161* and *TLC1* RNAs are represented by arrows indicating the transcription direction. The identity of the conserved sequence motifs studied is indicated by letters. Some regions between the *PDX3* and *TLC1* genes are magnified at bottom. The position of the different 5'-ends of *TLC1* detected by primer extension performed in B is indicated by arrowheads and the name of the deletion is indicated on the left. Stars (*) represent 5'-ends detected for specific deletions and the numbers indicated are relative to each deletion respectively and not to *TLC1* WT 5'-end. **(B)** Primer extension analysis of *TLC1* RNA derived from strains carrying different promoter deletions. The 5'-end was mapped using primer specific to *TLC1* sequence. DNA sequencing of a cloned *TLC1* gene was performed with the same primer and included on the left as a marker. Black arrowhead (+1) represents the major *TLC1* 5'-end while white arrowheads represent minor 5'-ends.

Supplemental Figure S3. RNA samples from strain BY4741 (WT), BY4741-*swi4*Δ and BY4741-*swi6*Δ were analyzed by qRT-PCR. The *TLC1* RNA levels were measured and normalized against Act1. The WT value is arbitrarily set to 1.

Supplemental Figure S4. qRT-PCR analysis of *Cln2* RNA levels at indicated time points for the same experiments as shown in Figure 5D. *CLN2* is a well-known cell cycle-regulated gene by SBF and serves here as a positive control for the synchronization and activation of the transcription. Average values of three independent biological replicates normalized against Act1 with standard deviation are shown as fold change over t = 0 (G1).

Table S1. Primers used in this study

Name	Sequence (5'→ 3')
SPE-931TLC1FWD ^a	GGGTACACTAGTAGCCTTTCTAGAGGTTCC (SpeI)
SPE-298TLC1FWD ^a	GGGTACACTAGTGGTAGAGAAAGACACGG (SpeI)
SPE+1TLC1FWD ^a	GGGTACACTAGTAGAGAGGAAGATAGGTACC (SpeI)
-6TLC1FWD ^a	GAGAGGAAGATAGGTACC
-35TLC1FWD ^a	GTGATTTTTCTGGTTTGAG
-91TLC1FWD ^a	GGTGACATATAGATCTCAAGG
-150TLC1FWD ^a	GGAAATGGGAGAAAAGTTTTTC
-248TLC1FWD ^a	GTTTATAAATAAATTTTATATCAC
-305-224TLC1REV ^a	GTGATATAAAATTTATTTATAAAC ACGGGTAGTCTCTCTAAG
-298+20TLC1REV ^a	GGTACCTATCTTCCTCTCT ACGGGTAGTCTCTCTAAG
-241-122TLC1REV ^a	AACTTTTCTCCCATTTC CTAACACAAGTAAATATACAACACCC
-150-70TLC1REV ^a	CCTTGAGATCTATATGTCACC TTTTTTTTTTATTTTTTTGGCC
-87-7TLC1REV ^a	TTCTCAAACCGAGAAAATCAC ATTGCTAAAGAGGAAGTTTTTTTCGCG
-28+20TLC1REV ^a	GGGTACCTATCTTCCTCTCT ACTAAAAGCTACAAAGAAGGTC
+1502TLC1ECOREV ^a	AACAGAATTCGGGAAGGTAAATACCACC (EcoRI)
ADH1 TERM FWD ^a	CCGGAATTCGAGCTCAAGCTTTGGACTTCTTCGCC
ADH1 TERM REV ^a	ATCCGCGGACTAGTGGCCGGTAGAGGTGTGG
TLC1-A-FOR ^b	CATTTTCAGTGCAGATCCGTTGA
TLC1-B-REV ^b	CAT ACGGGTAGTCTCTCTAAGCTGG
TLC1-C-FOR ^b	GGTAGAGAAAGACACGGGCAGC
TLC1-G-REV ^b	CATT AGTTTTATTCTCAAACCGAGAA
TLC1-D-FOR ^b	GTTTATAAATAAATTTTATATC
TLC1-E-FOR ^b	GGAAATGGGAGAAAAGTTTTCA
TLC1-F-FOR ^b	GGTGACATATAGATCTCAAGGT
TLC1-EFA ^{b, d}	GGAAATGGGAGAAAAGTTTTTCAGCGCGCGAACTCGCGAAAAAACTTCC TCTTTAGCAATATG
TLC1-ERA ^{b, d}	CAT ATTGCTAAAGAGGAAGTTTTTTTCGCGAGTTCGCGCGCTGAAAACCT TTCTCCATTTC
ADH1-FORW ^{b, d}	ACACAGAATTCAGAAGCTTTGGACTTCTTC
ADH1-REV ^{b, d}	ATGACGAATTCGGGCATGCCGGTAGAG
TLC1-33-49 ^c	CAGCCATTGACATTTTC
LACZ2 ^c	GCAAGGCGATTAAGTTGGG
CYC1-FOR ^d	CCTACCTGAATCTAAAATTCCC
CYC1-REV ^d	CATTATTAATTTAGTGTGTATTTGTG
CAR1-FA ^d	TTAGCGGTAGCCGCCGAGGGGTCTAAAGAGTA
CAR1-RA ^d	TACTCTTTAGACCCCTCGGCGGCTACCGCTAA
ACT1 coding For ^d	TCCGGTGATGGTGTACTCA
ACT1 coding Rev ^d	ATTCTCAAAATGGCGTGAGG
TLC1-DFA ^d	GTTTATAAATAAATTTTATATCACTATATGTGTGGTAAAAGGAAGAGCA ATCCTGCTAAAGCTTTTATATCTAAACGCCAAAAAAATAAAAAA TTTTTTTTTTATTTTTTTGGCGTTTAGATATAAAAGCTTTAGCAGGATTGC TCTTCCTTTTACCACACATATAGTGATATAAAATTTATTTATAAAC
MCB1/2b-Rev ^e	GGTCTTTTAATTGAGAACC TGAGATCT ATATGTCACCATTGCTAAAGAG GAAGTTTTTCCAGAGTTCCCGAGCTGAAAACTTTTCTCCATTTC
MCB1mutF ^e	GTTTTCAGCGCGCGAACT <u>GGCT</u> AAAAAACTTCTCTTTAG
MCB1mutR ^e	CTAAAGAGGAAGTTTTTT <u>AGCC</u> AGTTCGCGCGCTGAAAAC
MCB2mutF-2 ^e	GGGAGAAAAGTTTTTCAGC <u>TCGG</u> GAACT <u>GGCT</u> AAAAAACTTC
MCB2mutR-2 ^e	GAAGTTTTTT <u>AGCC</u> AGTTCCCGAGCTGAAAACTTTTCTCC

^aOligonucleotides used for PCR deletion strategy. Underlined sequences are restriction sites added for subsequent cloning (corresponding restriction enzyme is in parenthesis), bold nucleotides include sequences located on both sides of the deleted region.

^bOligonucleotides used for PCR amplification of *TLC1* promoter region, bold nucleotides in the reverse primers are added sequences corresponding to the “start codon ATG”.

^cOligonucleotides used for primer extension and Northern blot analysis.

^dOligonucleotides used for PCR amplification of *CYC1* promoter regulated by different *TLC1* promoter regions.

^eOligonucleotides used for site-directed mutagenesis; underlined nucleotides correspond to the mutations introduced in the MCB sequence.

-640 -630 -620 -610 -600 -590 -580 -570 -560 -550 -540 -530 -520 -510 -500 -490 -480 -470 -460
 Scec AGTCATTTTCAGTGCAGATCCGTG--AAGTGTACTACTGGTCTAGGTAGAGTATCTTTAAAATATCTAATCATGCAAATTAGTCATT---TCTTCTTTCTTATAAGTTGCGGCCCTTC-TAGTATTTACAATTTACCCGGGCTGG-TTCTGGTGGCATCTATAAGAGCTCTATCTGGCTGCTAAGCAGACTTGCTGTT
 SceK AGTCATTTTCAGTGCAGATCCGTG--AAGTGTACTACTGGTCTAGGTAGAGTATCTTTAAAATATCTAATCATGCAAATTAGTCATT---CTTCTTTCTTATAAGTTGCGGCCCTTC-TAGTATTTACAATTTACCCGGGCTGG-TTCTGGTGGCATCTATAAGAGCTCTATCTGTCTGCTAAGCAGACTTGCTGTT
 Spar AGTCATTGTCCGGTGCAGATCTGTG--AAGTGTACTACTAGTTPATGTACAGTATCTTTCAAAGCCTTAATCATGCAGATTAGTCATT---TCTTCTTTCTTCCAAGTTGCGGCCCTTC-TAGTACTTACAATTTACCCGGGCTAGCTTCCGGTGGCATCTATAAGAGCACTATTTGGGTTGGTGTAGTACCCGCTCAT
 Scar AGTCATTGTCCGGTGCAGATCTGTG--AAGTATCTACTAGTTTACGTTTCAGTATCCTTCAAAGCTTTAATCATGCAGATTAGTCATT---TCTTCTTTCTTCCAAGTTGCGGCCCTTC-TAGTACTTACAATTTACCCGGGCTTGGTCCGGTGGCATCTACAAGAGCACTTTTGGAGCTGGTGTAGTACCCGCTCAT
 Smik AGTCATTGTAGGTGCAGATGCTAC--GAGTGTGCTCTTAGTATGGTGTGCTGATTCTCTAATGCGTTAACTATGTAGATTAGTCATT---TCTTCTTTGTT-CATGTTGCGGCCCTTCACAGTACTTACAAATTTACCCGGTTCCTGTCCCTCGCGGAATTTATTAGAGCACTTTTGAATGTTAAGCAGACTTAGTCAT
 Skud AGTCATTGTCCGGTGCAGATATGTG--AAGTGTGCTTCTGGTTTGG-TAC-GTTTCTTGCAAAACATAAATCATATACATTAGTCATT---TCTTCTTTCTT-TAAGTTGCGGCCSCKGC--AGCCCTTATGGTTTACCCGGCACCCACTTTCAGTGGCAGCTATAAGAGCATCATTTAGCTGTTGAGCAATCCAGCAGT
 Sbay AGTCATTGTCCGGTGAATATATGT---AGTGTGCTATTCGTTTGGACCAG-TTTTCTTATAGAGCATTAGTCATACAGATTAGTCTTTGCTTCTTCTTCTT-CAGGTTACGACCTCTTCTGACCTGCTAAATTTACCCGGC-CCCACTCCAATTTGGCAGCCACAAGAGGGCCACTGGGTTGTTGCGCAGC-CTTAAGAT
 Spas AGTCATTGTCCGGTGCAGATATGTGTAGAGTGTGGTAGTGTGTTGGCTAC-GTTTCTTGCAGAGATTAAATCATACAGATTAGTCATTCTTCTTCTTCTT-CAAGTTGCGAGCCCTTCAAACCCGCTGGTTTACCCGGC-CTGCGCAAAATGGCAGCCATAAGAGAGTCTTTGGGTTGATGTGCGAGGCCAAGGT
 Cons AGTCATTgTc.GTgcAgAT..gt....AgTgTgcTa.T.GT.T...ta..gT.TctT..Aaa....TAatcAT.cAgATTAGTCaTT....CTTCTTTcTtT..AaGTTgCG.cCcc.tc..ag...Tta.aatTTACCCGG..c..g.t.c..gtGGcA.ctAtaAGAgc.c.attg.g.TG.T..GcAga.....T

DEL [<-----A----->] (35.8)

-450 -440 -430 -420 -410 -400 -390 -380 -370 -360 -350 -340 -330 -320 -310 -300 -290 -280 -270
 Scec ACTTGTATGTGCTGTTTATCAAGATT-GGGGCAT----TACGCATGTGTATGTGTGAACACTACAGTAGTGTATGAAATACAGCCTGAA-AGAC-GAAAACCTTCACAACGGAACTTAAGCGGTTCAATAAA-CATCTATCTGCCAGCTTAGAGAGACTACCCGTTGGTAGAGAAAAGACACGGGCAGCCAAAAATACTGGGTG
 SceK ACTTGTATGTGCTGTTTATCAAGATT-GGGGCAT----TGCGCATGTGTATGTGTGAACACTACAGTAGTGTATGAAATACAGCCTGAA-AGAC-GAAAACCTTCACAACGGAACTTAAGCGGTTCAATAAA-CATCTATCTACCAGCTTAGAGAGACTACCCGTTGGTAGAGAAAAGACACGGGCAGCCAAAAATACTGGGTG
 Spar ATTTGCATATACTGCTTACCAAGATT-AGGTGTG----TACGCGTGTGTATGTGTGAACACTACAGTAGTGTATGAAATACAGCCTGAA-AGACGAAAAACTTCACAACGGAACTTAAGTGGTTCATTAAA-CATCTATCTGCCAGCTTAGAGAGACTACCCGTTGGTAGAGAAAATACGCGGGCAGCCAAAAATACTGGGTG
 Scar ATTTGCATATACTGCTTACCAAGATT-AGATGTG----TACGTATGTGTATGTGTGAACACTACAGTAGTGTATGAAATACAGCCTGAA-AGGCTAAAAACTTCACAACGGAACTTAAGTGGTTCATTAAA-CATCTATCCGCCAGCTTAGAGAGACTAACCGTAGGTGAGAAAATACGCGGGCAGCCAAAAATACTGGGTG
 Smik ATTTGGCGTGATTGTTTACTAAGATCCGAGTATT----TACGTATCTATATGTGTGAACACTACAGTAGTGTATGAAATACAGCCTAAAGAGACGAGAACTTCACAACGGAACTTAAGTGGTTCATTAAAATATCTATCTACCAGCTTAGAGAGACTACCCATGAGTGGGAGATACGCGGAGCAGCCAAAAATACTGGGTG
 Skud CCTGGTGCATGCTGCCTATCAAGATTGGTGTACGATGGTGTGCGGTGTGTGCGAACTACAGTAGTGTATGAAATACAGTCTTTAAAGACTAACAACTTCACAACGGAACTTAAGTGGTTCATTGA-ACGCTATCTGCCAGCTTAGAGAGGCCACCAACGGGTGAGTGTATACGCGGGTGGCCGAAAAATACTGGATG
 Sbay GCCAGTGTGTTGTTCTTGTCAAGNC----TAC-----TGTGTGTCGGGTGTGTGAACACTACAGTAGTGTATGAAATACAGCCT--GAAGGG-AAAAACTTCACAACGGAACTTAAGTGGTTCATCGAGCTGTTTATCTGCCAGCTTAGAGTAAACCATCCGCGGTGAGTGTATGCGCGGGCAGTCAATGATACTGG--G
 Spas ACCAGAGTATGGTCTTGTCAAGGTT-----TAC-----TGTGTGTGAACACTACAGTAGTGTATGAAATACAACT--AAATGGCAAAACTTCACAACGGAACTTAAGTGGTTCATCGAGTGTGTTTATCTGCCAGCTTAGAGTAAACCATCCGCGGTGAGTGTATGCGCGGGCAGTCAATAACTG--G
 Cons a.t.G..t.T..Tg.tTa.caAGatt.....T.c.....t.TGTgtGAACACTACAGTAGTGT.ATGAAATACA.cCT..a.Ag.c.aaaAACTTCACAACGGAaCTTAAGTGGTTCAtt.A..c.TcTATctgCCAGCTTAGAGagaC.A.Ccg.gggtGag..ataCgCgGcAaGcCaAaaATACTGG..G

DEL ----->] [<-----B----->] [<-----C----->] (69.6) (52.6)

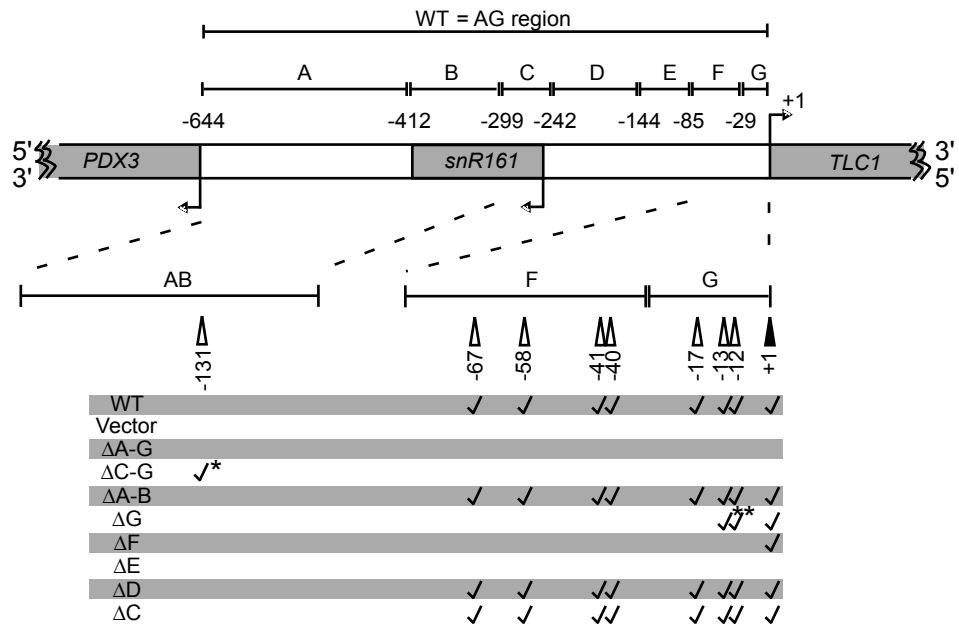
-260 -250 -240 -230 -220 -210 -200 -190 -180 -170 -160 -150 -140 -130 -120 -110 -100 -90
 Scec -TTGTATATTTACTTG----TGT-TAGTTTATAAATAA--ATTTTATACTACT--ATATGTGTGGTGAA-AAGGAAGAGCAATCCTGCTAAAGCTTTT-ATATCTAAACGCCAAAAAATAAAAAA-----GGAAATGGGAGAAAA-GTTTTCAAGCGCGGAAC-TCGCGAAAAAACTTCCTCTTTAGCAATGGTG-
 SceK -TTGTATATTTACTTG----TGT-TAGTTTATAAATAA--ATTTTATATCACT--ATATGTGTGGTGAA-AAGGAAGAGCAATCCTGCTAAAGCTTTT-ATATCTAAACGCCAAAAAATAAAAAA-----GGAAATAGGAGAAAA-GTTTTCAGCGCGCGAAC-TCGCGAAAAAACTTCCTCTTTAGCAATGGTG-
 Spar -TTGTATTTTACTTG----GGT-TAGTAGGAAAAGG-ATTTTATATATAAC--AAAATATATGGTGAAGAAAAGAGCAATTCGCCAAAGCTTTTTATATCTAAATGCCAGAAAAAATAAAAA-----GGAAATAGGAGAAAA-GTTTTCAGCGCGCGAAG-TC-SGAAAAAC-GTCTACTTCAGTAATGGCG-
 Scar -TTGTATTTTACTTG----GGT-TAGTAGGAAAAGACAT-TATATATAAC--AAAGTATATGGGGAAGAAAAGAGGAGCAATTCGCCAAAGCTTTTTATATCTAAATGCCAGAAAAAATAAAAA-----GAAATAGGAGAAAA-GTTTTCAAGCGCGGAAC-TCGCGAAAAAAGTCTACTTTCAGTAATGAGC-
 Smik -TTGTAT-TTTACTTGCTTTTATATTGCAAGCAAGCAAGATGTATCTATATATGCAATGCATGATTA-GAAAAAGAGCAGGCCGTC-AAAGATGTCTATATTTAAATAGCAAAAAAAGAA-----GTATAGGAGAAAA-GTTTTCAGCGCGCGCAAT-TCGCGAAAAACCGTTCTCTTTTCCAACAGCAT
 Skud TTTGTAT-TTTACTTGATTTTATGTGTCAAGAGAACACAG-----TATA-CAAACAATAGTGGTAAAGAATGAGAACAAGACTTGC-ATTGTTCCCTGTATTTAAATGCCA-----GAAGAAAA-GTTTTCAGCGCGTAAAAATCGCAAAAACTGCCAATTCGCGCAATG-A-
 Sbay GTTATAC-TTTACTTGGTTTTTATATTGCAAGGAAACAGAG-----TATA-CTAAGAACAATGGCAGAAAA-GAGCTCCGATTTTGA-AATGCTTCTTATATCTAAATGGAAAAAATA-----CAATAGAGAAAAAGTTTTCCAGCGCGCGAAT-TCGCAAAAAAGTACCTATTTCAATACCGGTG-
 Spas GTT-TAC-TTTACTTGGTTTTAT-TTGAAAGGAAGCAAG-----TATA-CAAGAGAGTATTTGGTGGAAAA-GAGCACCAGAAATTTGA-AATGTTTCTTATATCTAAATGAAAAAATAAAAA-----CGACAAAGAAAAAATTTTTCAGCGCGCGAAT-TCGCGAAAAACTGCCTATTTTCATCATCGATA-
 Cons .TT.TAt.TTTACTTG...t.T.T.g.a.g.aAa.a.....TAta.c....A...TGgtgaa.aA.gA..a.caa...Tgc.Aa.G.Tt...aTAtcTAAAcg..A.....g.A.AAAA..TTTtCagCGCgGAA..TC.cgAAAAA...c...TT.A..Aa.gg...

DEL ----->] [<-----D----->] [<-----E----->] (21.4) (42.4)

-80 -70 -60 -50 -40 -30 -20 -10 +1 TLC1
 Scec AC-ATA-TAGATCTCAAGGTTTC----TCAA-----TTAAAAGACTTC--TTTGTAGCTTTTAGTGTGATTTTTCTGGTTTGA-----GAATA----A--AACTA--GAGAGGAA
 SceK AC-ATA-TAGATCTCAAGGTTTC----TCAA-----TTAAAAGACTTC--TTTGTAGCTTTTAGTGTGATTTTTCTGGTTTGA-----GAATA----A--AAATA--GAGAGGAA
 Spar GC-ATA-CAGATCTCGAGCTTC----TCGG-----TTTAAAGGCTTTC--TTTATAGCTTTCAATACGACTTATCCGGTTCGA-----GAATA----CA----CTA--CAAAGGAA
 Scar GC-GTA-CAGATCTCGAGCTTC----TCAG-----TTTAAAGGCTTTC--TTTATAGCTTTTCAGTGCAGCTTATCCGGTTTGA-----CAATA----CA----CTA--TAGAGGAA
 Smik ACTACAAAGCTCTCGACATA----TCAG-----TTAAAAGGCTTCT--TTTATGGTTTCCAGCTGAGTCCCTCAATAGTGTCTTCCAATAGGCGCATGCGCTA--ATAGAGAA
 Skud -C-ATA-CAGATCTCGAACATC----TCAC-----TTCAGATGTATTCT--TTCGTANNTTTCAAAACAGACCATCC----GATCG--AA----GCATGCGCTT--ATGAGGAA
 Sbay -C-ATA-TGATCTCGAGCATTTAAATTCAGCCTTGTGTCAGCAATGCAGATACCTTCCGTTGGCAGTTTTCAATACGGATCATCCG-ATTG-TC-----GCATGTGCTCATGAGAGGAA
 Spas -C-ACA-TAGATCTCGAGCATCAAAATCACGCTTGTGTCAGCAACACATTCGGATACCTTTTGTTCAGCTTCAATACAGATCATCCG-ATCG-T-----GGCATGTGCTTATGAGAGGAA
 Cons .C.atA..aGaTCTCgAgc.Tc....TCa.....Tt.aaA..c.Ttc..TT..tag.tTtca.t....t.c.....g.....A....cT....gAGGAA

DEL ----->] [<-----F----->] [<-----G----->] (32.1) (14.3)

A



B

