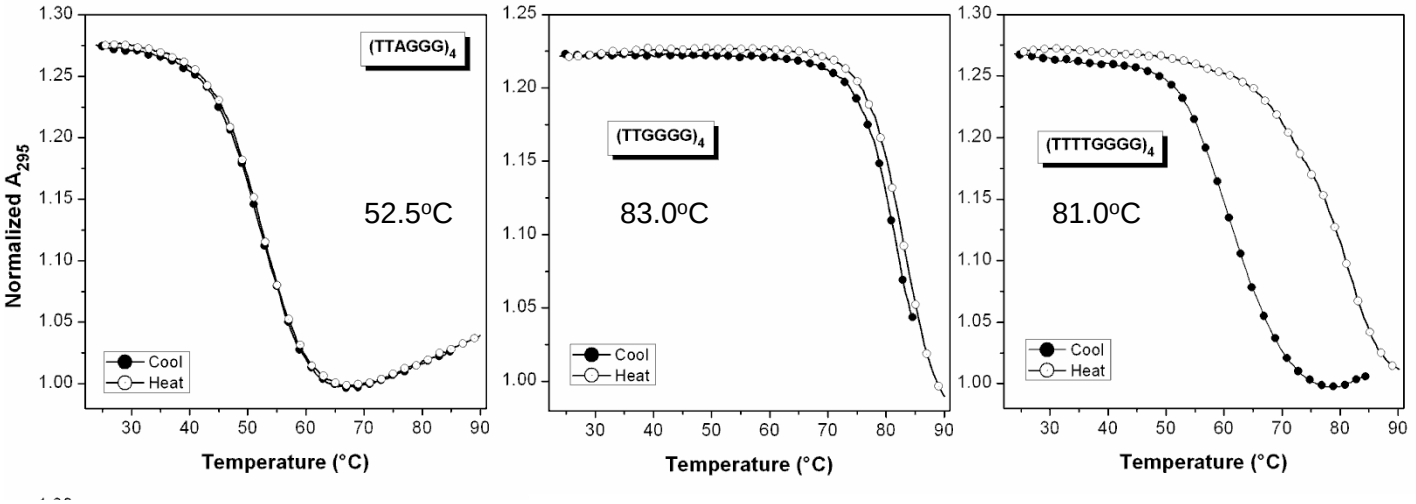
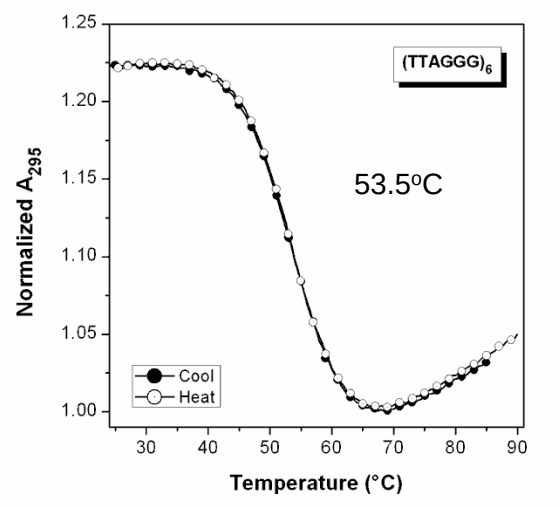
Supplemental Figure Legends

Figure S1. UV melting curves for ciliate and human telomeric repeats. Melting curves for oligonucleotides GT-$(TTAGGG)_4$-TC, GT-$(TTAGGG)_6$-TC, GT-$(TTGGGG)_4$-TC and GT-$(TTTTGGGG)_4$-TC were recorded at 295 nm in solutions containing 100 µM KCl. The overlap of heating (open circle) and cooling (closed circle) curves show reversible transitions for each, except oligonucleotide GT-$(TTTTGGGG)_4$-TC which shows a hysteresis.

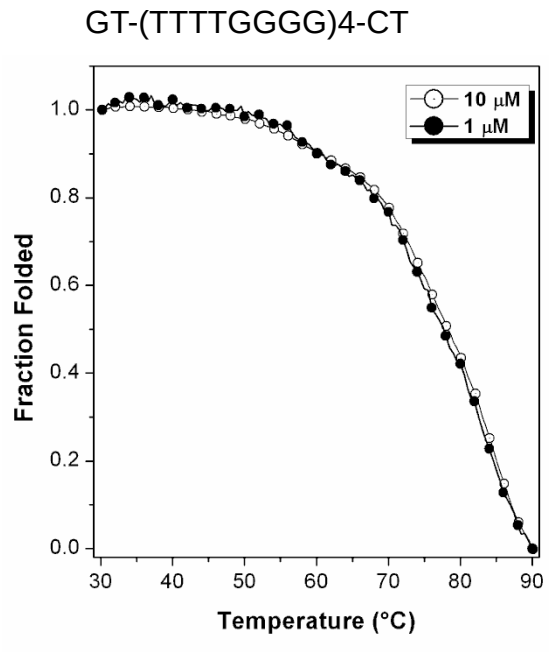
Figure S2. UV melting curves for *O. nova* telomeric repeats is not dependent on oligonucleotide concentration. Melting curves for oligonucleotide GT-$(TTTTGGGG)_4$-TC recorded at 295 nm in solutions containing 100 µM KCl. The oligonucleotide concentrations were 10 µM (open circle) and 1 µM (closed circle).

Supplemental Figure S1





Supplemental Fig. S2 Concentration dependence of UV melting



Supplemental Table 1. Oligonucleotides used for construction of telomeric vectors

Vector Name	Sequence (5' to 3') for the antisense strand of the plasmid
[TTAGGG]6[a]	caacgccgtacgacggaagctatggcctcgagaGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGT TAGG acgcgcagcctggtcgaacgcagacgcgtcaa
[CCCTAA]6	caacgccgtacgacggaagctatggcctcgagaCCCTAACCCTAACCCTAACCCTAACCCTAACCCT AA acgcgcagcctggtcgaacgcagacgcgtcaa
[TTAGGG]10[a]	caacgccgtacgacggaagctatggcctcgagaGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGT TAGGGTTAGGGTTAGGGTTAGGGTTAGGacgcgcagcctggtcgaacgcagacgcgtcaa
[CCCTAA]10	caacgccgtacgacggaagctatggcctcgagaCCCTAACCCTAACCCTAACCCTAACCCTAACCCT AACCCTAACCCTAACCCTAACCCTAAacgcgcagcctggtcgaacgcagacgcgtcaa
[TTGGGG]10[a]	caacgccgtacgacggaagctatggcctcgagaTGGGGTTGGGGTTGGGGTTGGGGTTGGGGTTG GGGTTGGGGTTGGGGTTGGGGTTGGGGTacgcgcagcctggtcgaacgcagacgcgtcaa
[CCCCAA]10	caacgccgtacgacggaagctatggcctcgagaCCCCAACCCCAACCCCAACCCCAACCCCAACCCC AACCCCAACCCCAACCCCAACCCCAAacgcgcagcctggtcgaacgcagacgcgtcaa
[GGGGTTTT]5[b]	caacgccgtacgacggaagctatggcctcgagaCGGGGTTTTGGGGTTTTGGGGTTTTGGGGTTTT GGGGTTTTGacgcgcagcctggtcgaacgcagacgcgtcaa
[CCCCAAAA]5	caacgccgtacgacggaagctatggcctcgagaCCCCAAAACCCCAAAACCCCAAAACCCCAAAAC CCCAAAACCacgcgcagcctggtcgaacgcagacgcgtcaa

Insert fragments were constructed by annealing to the complimentary strand for each (not shown).
[a]The interruption of one telomeric repeat was necessary to avoid codons that code for rare tRNAs in *E. coli* that cause a significant reduction in HSV-*tk* gene product.
[b]The insert began with a run of Gs, rather than Cs, to avoid codons that code for rare tRNAs in *E. coli*.

Table S2. Melting temperatures ^{o}C determined for G-4 oligonucleotides in 100 mM NaCl and 100 mM KCl.

	$(T_2AG_3)_4$	$(T_2AG_3)_6$	$(T_2G_4)_4$	$(T_4G_4)_4$	
NaCl	39.0	41.0	52.1	52.6	
KCl	52.5	53.5	83.0	81.0	

Supplemental Table S3. Mutant rates of shuttle vectors with human telomeric repeats in LCL721 clones

	Mutant frequencies per cell generation x 10^{-6a}						
Clone	**No insert**		**$(TTAGGG)_{10}$**		**$(CCCTAA)_{10}$**		**$(CCCTAA)_6$**
Experiment	1	2	1	2	1	2	1
A	21	3.2	1.4	1.8	2.2	3.4	2.4
B	4.1	27	2.0	2.3	3.1	2.8	3.1
C	4.8	3.3	3.8	2.1	4.4	71	4.5
D	3.0	2.7	1.6	5.8	11	3.4	5.0
E	5.6	2.4	3.0	2.7	14	3.7	
F	1.2	3.4	4.4		6.3	3.8	
G					4.8		
Median	3.3		2.3		3.8		3.8
N	12		11		13		4

[a]HSV-*tk* mutant frequency was determined by isolating shuttle vectors from LCL721 clones after 24-35 cell generations, followed by electroporation in reporter *E. coli* and selective plating. Clones A-G represent independent clones, and experiments 1 and 2 represent independent experiments.

Supplemental Table.S4. Mutant rates of shuttle vectors with ciliate telomeric repeats in LCL721 clones

	Mutant frequencies per cell generation x 10^{-6}[a]				
Clone	**$(TTGGGG)_{10}$**		**$(CCCCAA)_{10}$**	**$(GGGGTTTT)_5$**	**$(CCCCAAAA)_5$**
Electroporation	1	2	1	1	1
A	3.6	4.4	4.6	5.4	5.0
B	9.3	13	4.5	7.7	7.0
C	16	6.1	3.2	3.1	38
D	12	3.6	4.0	4.8	7.2
E			7.9		
F			4.4		
Median	7.7		4.4	5.1	7.1
N	8		6	4	4

[a]HSV-*tk* mutant frequency was determined by isolating shuttle vectors from LCL721 clones after 24-35 cell generations, followed by electroporation in reporter *E. coli* and selective plating. Clones A-F represent independent clones, and experiments 1 and 2 represent independent experiments.

Supplemental Table S5. Statistical comparison of telomeric shuttle vectors mutant rates in LCL721 human cells.

	No Insert	[TTAGGG]10	[CCCTAA]10	[TTGGGG]10	[CCCCAA]10	[GGGGTTTT]5	[CCCCAAAA]5
No Insert	-	0.1309	0.3557	**0.0203**	0.2277	0.2398	0.0604
[TTAGGG]10		-	**0.0178**	**0.003**	**0.0159**	**0.0264**	**0.011**
[CCCTAA]10			-	0.0896	0.512	0.5045	0.1363
[TTGGGG]10				-	0.2451	0.3677	0.9212
[CCCCAA]10					-	0.6095	0.1667
[GGGGTTTT]5						-	0.6286
[CCCCAAAA]5							**-**

[a]p values are derived from pair-wise comparisons of mutant rates reported in Figure 4 and Supplemental Tables 1-2 for each shuttle vector using the Mann-Whitney rank sum test (two-sided).

Supplemental Table S6. Mutational events in HSV-tk gene of no insert control shuttle vector after replication in LCL721 clones

5' mutations[a]	Clone[b]	3' mutations[a]	Clone[b]
Deletion 159 bp at -141	**2B**[c]	- G:C 116	D
Deletion 1808 bp at -1	A	T:A to C:G 176	A
G:C to A:T 24	C	C:G to A:T 178	A
Deletion 1565 bp at 52	**B**	C:G to G:C 180	A
-A:T 48	B	G:C to C:G 182	D
-G:C 49	B	G:C to T:A 183	A
T:A to A:T 59	C	Deletion 92 bp at 146	A
Deletion 284 bp upstream to 91	**A**	Deletion 954 bp at 301	2E[c]
C:G to T:A 115	C	G:C to T:A 304	A
Deletion 118 bp at 111	E	C:G to A:T 367	F
Deletion 630 bp at 111	E	C:G to T:A 370	F
Deletion 214 bp upstream to 112	F	-C:G 372-373	F
862 bp deletion upstream to position 501	D	Rearrangement after position 387	A
		+G:C 487- 493	E
		C:G to A:T 543	A
		-C:G 605-610	**B**
		G:C to T:A 716	**A**
		-G:C 846-849	**A**
		Deletion 327 bp at 975	F
		Deletion 115 bp at 991	E

All sequences represent the sense strand.

[a]5' mutations represent mutational events upstream of position 112 in the HSV-tk gene and 3' mutations represent mutational events downstream of position 112.

[b]Clones A-F represent the clones in Supplemental Table S3 from which the mutant was isolated and sequenced. Multiple independent mutants were characterized for each clone. Bolded letters denotes that the clone was independent experiment #2 (see Supplemental Table S3).

[c]Multiple isolates from a single clone. Indicates the number of mutants from the designated clone that exhibited the mutation.

Supplemental Table S7. Mutational events in HSV-tk gene of $(TTAGGG)_{10}$ containing shuttle vector after replication in LCL721 clones

5' and telomeric mutations[a]	Clone[b]	3' mutations[a]	Clone[b]
Deletion 1401 bp at -81	A	Multiple base substitutions 178 – 183	C
Deletion 193 bp upstream to 1[d]	**C**		
C:G to T:A 37	**C**	CGCCGG:GCGGCC to AGGCCT:TCCGGA	
Deletion of all 10 telomeric repeats[d]	**C**	T:A to G:C 218	**9C**[c]
-C from (AATCCC) in the first telomeric repeat	B	Small deletion 12 bp at 280	E
Deletion 1099 bp upstream to 161	A	G:C to A:T 298	C
Deletion 1188 bp upstream to 247	B	T:A to C:G 299	C
Deletion 1178 bp upstream to 362	B	+G:C 301-303	D
		G:C to A:T 320	D
		+TT:AA between 372 and 373	B
		C:G to A:T 448	C
		-T:A 450-451	A
		-C:G 461-462	7A[c]
		Small deletion 8 bp at 470 (TTCTGGCT)	C
		+G:C 487-493	**C**
		Deletion 719 bp at 502	A
		G:C to A:T 541	**C**
		T:A to G:C 638	C
		G:C to T:A 674	**B**
		Deletion 63 bp at 696	**B**
		Deletion 268 bp at 722	D
		-G:C 824-826	**C**
		T:A to C:G 920	D
		Deletion 551 bp at 1035	A

All sequences represent the sense strand

[a]5' mutations represent mutational events upstream of and including the (TTAGGG)10 region at position 112 in the HSV-tk gene and 3' mutations represent mutational events downstream of the repeats and position 112.

[b]Clones A-F represent the clones in Supplemental Table S3 from which the mutant was isolated and sequenced. Multiple independent mutants were characterized for each clone. Bolded letters denotes that the clone was independent experiment #2 (see Supplemental Table S3).

[c]Multiple isolates from a single clone. Indicates the number of mutants from the designated clone that exhibited the mutation.

[d]Occurred together in the identical mutant from clone **C**.

Supplemental Table S8. Mutational events in HSV-tk gene of $(CCCTAA)_{10}$ containing shuttle vector after replication in LCL721 clones

5‘ and telomeric mutations[a]	Clone[b]	3' mutations[a]	Clone[b]
C:G to G:C 4	F	G:C to T:A 172	B
C:G to A:T 69	B	C:G to A:T 202[d]	F
C:G to G:C 89[d]	F	Deletion 47 bp at 206	G
+(TTAGGG)3 in telomeric region[d]	F	G:C to C:G 209	A
Deletion 184 bp upstream to 118	**A**	G:C to T:A 220	**A**
Rearrangement upstream to 437	**D**	G:C to C:G 223	**F**
Deletion 810 bp upstream to 462	**D**	Deletion 65 bp at 266	B
		T:A to A:T 277	F
		C:G to T:A 280	F
		G:C to A:T 281	F
		-C:G 282	F
		G:C to T:A 309	**A**
		T:A to A:T 316	**A**
		-C:G 461-462	**F**
		-G:C 487-493	**D**
		+G:C 487-493	A
		C:G to T:A 610	**4C**[c]
		C:G to A:T 632	B
		A:T to T:A 637	B
		+A:T between 638 and 639	7
		C:G to G:C 694	2F[c]
		G:C to A:T 739	**D**
		A:T to G:C 740	F
		C:G to T:A 763	E
		G:C to C:G 822	F
		Deletion 266 bp at 837	B
		-C:G 877-880	G
		T:A to A:T 972	B
		T:A to C:G 1042	F
		G:C to C:G 1045	F
		G:C to A:T 1134	F
		G:C to A:T 1150	F
		G:C to A:T 1178	F

All sequences represent the sense strand

[a]5’ mutations represent mutational events upstream of and including the (CCCTAA)10 region at position 112 in the HSV-tk gene and 3’ mutations represent mutational events downstream of the repeats and position 112.

[b]Clones A-G represent the clones in Supplemental Table S3 from which the mutant was isolated and sequenced. Multiple independent mutants were characterized for each clone. Bolded letters denotes that the clone was independent experiment #2 (see Supplemental Table S3).

[c]Multiple isolates from a single clone. Indicates the number of mutants from the designated clone that exhibited the mutation.

[d]Occurred together in the identical mutant from clone F.

Supplemental Table S9. Mutational events in HSV-tk gene of $(TTGGGG)_{10}$ containing shuttle vector after replication in LCL721 clones

5' and telomeric mutations[a]	Clone[b]	3' mutations[a]	Clone[b]
Deletion 1993 bp -151	**B**	C:G to T:A 130	A
C:G to A:T 109	A	C:G to A:T 245	D
A:C to T:A first telomeric repeat	A	CGC:GCG to AA:TT 280 to 282	B
+(CCCCAA)2	6A, 2B[c]	C:G to A:T 344	B,C,D
Deletion of 8 telomeric repeats followed by 1515 bp deletion from 112	6C,1D[c]	Deletion 1091 bp at 414	D
+C:G in the first telomeric repeat	C	C:G to T:A 448	**A**
Deletion of 6 telomeric repeats followed by 1621 bp deletion from 112	A	-C:G 478-479	**C**
		+G:C 487-493	C
		A:T to G:C 494	C
		T:A to G:C 510	C,D
		G:C to A:T 541	C
		A:T to T:A 664	D
		G:C to T:A 674	C
		A:T to G:C 731	**D**
		Small deletion 4bp 778	**8A[c]**
		T:A to A:T 783	**8A[c]**
		Deletion 93 bp 803	C,D
		G:C to T:A 821	D
		-G:C 824-826	C
		-C:G 938-942	C
		Deletion 64 bp 991	C
		T:A to A:T 1051	D
		Deletion 643 bp 1107	**C**
		T:A to C:G 1130	C,D

All sequences represent the sense strand

[a]5' mutations represent mutational events upstream of and including the (TTGGGG)10 region at position 112 in the HSV-tk gene and 3' mutations represent mutational events downstream of the repeats and position 112.

[b]Clones A-D represent the clones in Supplemental Table S4 from which the mutant was isolated and sequenced. Multiple independent mutants were characterized for each clone. Bolded letters denotes that the clone was independent experiment #2 (see Supplemental Table S4). Some mutations occurred in multiple clones as indicated by distinct letters.

[c]Multiple isolates from a single clone. Indicates the number of mutants from the designated clone that exhibited the mutation.

Supplemental Table S10. Mutational events in HSV-tk gene of $(GGGGTTTT)_5$ containing shuttle vector after replication in LCL721 clones

5' and telomeric mutations[a]	Clone[b]	3' mutations[a]	Clone[b]
G:C to A:T 49	A, B	C:G to A:T 133	B
Deletion of 1 telomeric repeat	1A,2B[c]	G:C to A:T 166	B
Addition of 1 telomeric repeat	2A,1C[c]	G:C to C:G 189	B
Deletion of 2 telomeric repeats	A,B	G:C to A:T 220	B
Deletion 26 bp at 56	C	G:C to T:A 233	A
		T:A to A:T 236	B
		A:T to T:A 241	C
		C:G to A:T 245	B
		T:A to A:T 316	C
		C:G to A:T 344	C
		+G:C 400-403	C
		G:C to A:T 402	C
		A:T to G:C 494	C
		C:G to G:C 497	C
		T:A to G:C 498	C
		+G:C 499-501	A
		+GGGG:CCCC 499-501	C
		+G:C 511	C
		+G:C 522-523	C
		+G:C 567	C
		T:A to A:T 571	B
		-G:C 655-656	B
		+T:A 1159-1161	C
		+G:C 1173-1177	C

All sequences represent the sense strand.

[a]5' mutations represent mutational events upstream of and including the (TTTTGGGG)5 region at position 112 in the HSV-tk gene and 3' mutations represent mutational events downstream of the repeats and position 112.

[b]Clones A-D represent the clones in Supplemental Table S4 from which the mutant was isolated and sequenced. Multiple independent mutants were characterized for each clone. Bolded letters denotes that the clone was independent experiment #2 (see Supplemental Table S4). Some mutations occurred in multiple clones as indicated by distinct letters.

[c]Multiple isolates from a single clone. Indicates the number of mutants from the designated clone that exhibited the mutation.