Telomere quantitative PCR Protocol (Zheng lab)

1. High quality genomic DNA should be used. The DNA is diluted to 6 ng/ul using dd water on the day of the real-time PCR experiments.

2. Telomere (T) PCRs and single copy gene (S) PCRs are performed in separate 384-well plates. Repeated measures of the T/S ratio in the same DNA sample give the lowest the variability when the sample well position for the T PCR on the first plate matched its well position for S PCR on the second plate. Each DNA sample is assayed three times (triplicate) on the same plate.

3. Two master mixes of PCR reagents are prepared (see master mixture sheet), one with the T primer pair, the other with the S primer pair. Primer sequence (WRITTEN 5'—3'): Tel-FP, CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT; Tel-RP, GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT; 36B4u, CAG CAA GTG GGA AGG TGT AAT CC; 36B4d, CCC ATT CTA TCA TCA ACG GGT ACA A. 36B4 is 60S acidic ribosomal phosphoprotein P0 gene (RPLP0). Product = 76 bp.

Telomere PCR mixes:

Reagents in ul	1x	400x
10x PCR buffer	2	800
10 mM dNPTs	0.4	160
100 mM DTT	0.48	192
50 mM Mgcl2	1.0	400
5 M Betaine	4.0	1600
10x SYBR	0.40	160
dd water	5.96	2384
10 uM Tel-FP	0.3	120
10 uM Tel-RP	0.3	120
iTaq DNA polymerase, 5U/ul	0.16	64
DNA, 6 ng/ul	5.0	
Total, ul	20	8000

SC PCR mixes:

Reagents in ul	1x	400x
10x PCR buffer	2	800
10 mM dNPTs	0.4	160

100 mM DTT	0.48	192
50 mM Mgcl2	1.0	400
5 M Betaine	4.0	1600
10x SYBR	0.40	800
dd water	5.76	2304
10 uM Tel-FP	0.4	160
10 uM Tel-RP	0.4	160
iTaq DNA polymerase, 5U/ul	0.16	64
DNA, 6 ng/ul	5.0	
Total, ul	20	8000

4. The final concentrations of reagents in the PCR are: 0.2X SYBR green I, 15 mM Tris-HCl pH 8.0, 50 mM KCl, 2 mM MgCl2, 0.2 mM each dNTP, 5 mM DTT, 1M Betaine and 0.8 U iTaq polymerase (Bio-Rad).

5. Primer concentration: Tel-FP&Tel-RP 150 nM, 36B4u and d 200 nM.

6. Cycling: The PCR is performed on a QuantStudio 12K flex real-time PCR machine (Thermo Fisher) using the 384-well PCR plates.

   Telomere PCR: 95^{o} C for 10 min, followed by 2 cycles of 95^{o} C for 15 s, 49^{o} C for 30 s, and followed by 40 cycles of 95^{o} C for 15 s, 62^{o} C for 10 s, 74 ^{o} C for 15s.

   36B4 PCR: 95^{o} C for 15 min, followed by 2 cycles of 95^{o} C for 15 s, 56^{o} C for 20 s, 72^{o} C for 30s, and followed by 40 cycles of 95^{o} C for 15 s, 56^{o} C for 20 s, 72^{o} C for 30s.

7. Results and Quality control measures:

   The q-PCR data are analyzed using QuantStudio^{TM} 12K flex software.

   DNA concentration was measured using a Nanodrop spectrum meter. DNA samples with 260/280 ratio > 1.70 and < 2.10 are accepted for telomere analysis.

   Standard DNA used is pooled DNA from the test set extracted using the same method as the samples. The 2:1 series dilution is used to generate the standard curve containing 120, 60, 30, 15, 7.5, 3.75, 1.875 and 0.94 ng DNA per well.

   Criteria to accept results: the amplification efficiencies of both telomere PCR and single copy gene PCR of the standard curve are > 90% and < 110%. The co-efficiency of variation of the triple repeats of each sample is < 15%. The mean Ct of triplicates are used as final data for analysis.

The T/S ratio is calculated following the formula $[2^{C_t(Tel)}/2^{C_t(36B4)}]^{-1} = 2^{-deltaCt}$ in the original method paper published by Cawthon R, 2002.

Reagents:

Reagent	Cat #	Source	Price	amount	totally Price
100 mM dNTP mix, 1.0 ml	201913	qiagen	241.00	2	482.00
Ultra clear cap 8-strip, 120 strips	AB-0866	ABgene/Fisher	23.23		
Fluorescein Cal. dye, 1mM, 150ul	170-8780	Bio-Rad			
FG, 384-well plate pack of 50	4309849	thermofisher	367.00	2	734.00
SYBR Green I, 10,000x, 500 ul	S7563	fisher scientific	335.00	1	335.00
DTT	43816-10ml	sigma	26.57	1	26.57
iTaq polymerase, 5000U (5u/µl), 1ml	170-8875	Bio-Rad	4152.00	1	4152.00
PicoGreen dsDNA Quantitation Kit	P11496	Invitrogen	309.00		
DMSO, DNase RNase free, 250 ml	327182500	ACROS Organics	126.93	1	126.93
water, molecular biology grade 100ml	248700-100	fisher scientific	33.80	1	33.80
Betain, 5M	B0300-5vl	sigma	52.08	2	104.16