Conditions for quantitative PCR of telomere length Richard M. Cawthon, M.D., Ph.D.

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1) Latest primer set for telomere PCR (written 5' to 3'):

tel 1b CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT
tel 2b GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT

2) Latest reaction conditions for telomere PCR (final concentrations are given):

Composition shared by the T (telomere) PCR and the S (single copy gene) PCR:
- 15 mM Tris-HCl, pH 8.0
- 50 mM KCl
- 200 micromolar each dNTP
- 1% DMSO
- 2.5 mM DTT
- 0.4x Sybr Green I
- 12.5 nanograms of subject's genomic DNA

Composition specific to the T PCR:
- 1.5 mM $MgCl_2$
- 1.0 Units of AmpliTaq Gold DNA polymerase (per 20 microliter PCR reaction)
- 450 nM tel 1b primer
- 450 nM tel 2b primer

Composition specific to the S PCR:
- 3.5 mM $MgCl_2$
- 0.5 Units of AmpliTaq Gold DNA polymerase (per 20 microliter PCR reaction)
- 300 nM 36B4u primer
- 500 nM 36B4d primer

Final volume: 20 microliters per reaction. (Note: <u>no</u> ROX dye or any other reference dye.)

3) Thermal cycling profile on the ABI Prism 7700:

95°C x 10 min. to activate the polymerase
Then 95°C, 15 seconds; 56°C, 1 minute; data collection during the 56°C step.
Repeat the previous line 17 more times if T PCR; 26 more times if S PCR.

Note: if using a different thermocycler, set it to emulate a Perkin Elmer 9600 machine (i.e. one degree per second temperature ramps).

4) Standard curves: serial dilution over a 24-fold range for telomere PCR, from 3.75 ng per well to 90 ng per well; over a 16-fold range for single copy gene PCR, from 3.75 ng per well to 60 ng per well.

5) SDS v. 1.7 software settings

<u>Baseline</u>: cycles 1-5 for T PCR; cycles 3-15 for S PCR.
<u>Threshold</u>: between the $50^{th}$ and $75^{th}$ percentiles of the deltaRn range over which the plot of log fluorescence (y axis) vs. cycle number (x axis) is linear.