

Supplemental Information

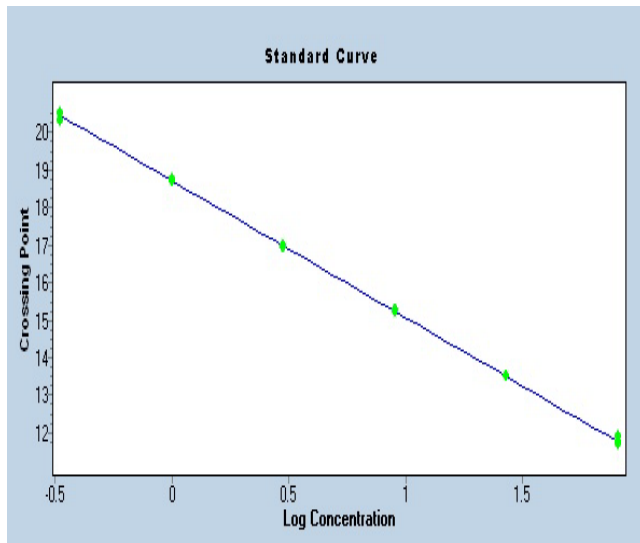
Additional information of the method of analysis of leukocyte telomere length

The telomere thermal cycling profile consisted of: Cycling for T(telomeric) PCR: denature at 96°C for 1 second, anneal/extend at 54°C for 60 seconds, with fluorescence data collection, 30 cycles. Cycling for S (single copy gene) PCR: denature at 95°C for 15 seconds, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, 8 cycles; followed by denature at 96°C for 1 second, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, hold at 83°C for 5 seconds with data collection, 35 cycles.

The primers for the telomere PCR were *tel1b* [5'-CGGTTT(GTTTGG)₅GTT-3'], used at a final concentration of 100 nM, and *tel2b* [5'-GGCTTG(CCTTAC)₅CCT-3'], used at a final concentration of 900 nM. The primers for the single-copy gene (human beta-globin) PCR were *hbg1* [5' GCTTCTGACACAACACTGTGTTCACTAGC-3'], used at a final concentration of 300 nM, and *hbg2* [5'-CACCAACTTCATCCACGTTCCACC-3'], used at a final concentration of 700 nM. The final reaction mix contained 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 200 μM each dNTP; 1% DMSO; 0.4x Syber Green I; 22 ng E. coli DNA per reaction; 0.4 Units of Platinum Taq DNA polymerase (Invitrogen Inc., Carlsbad, CA) per 11 microliter reaction; 0.5 - 10 ng of genomic DNA. Tubes containing 26, 8.75, 2.9, 0.97, 0.324 and 0.108 ng of a reference DNA (from HeLa cancer cells) were included in each PCR run so that the quantity of targeted templates in each research sample could be determined relative to the reference DNA sample by the standard curve method. The standard curves for both the T run (telomeric sequence) and S run (single copy gene) are show below. The same reference DNA was used for all PCR runs. All samples were run in triplicate wells and the average values for the triplicates were used for calculating T/S ratio.

To control for inter-assay variability, 8 control DNA samples were included in each run. In each batch, the T/S ratio of each control DNA was divided by the average T/S for the same DNA from 10 runs to get a normalizing factor. This was done for all 8 samples and the average normalizing factor for all 8 samples was used to correct the participant DNA samples to get the final T/S ratio. The T/S ratio for each sample was measured twice. When the duplicate T/S value and the initial value varied by more than 7%, the sample was run the third time and the two closest values were reported. Typically, about 15% of samples needed to be assayed the third time. Using this method, the CV for telomere length measurement is 3%.

Standard curve for T run



Standard curve for S run

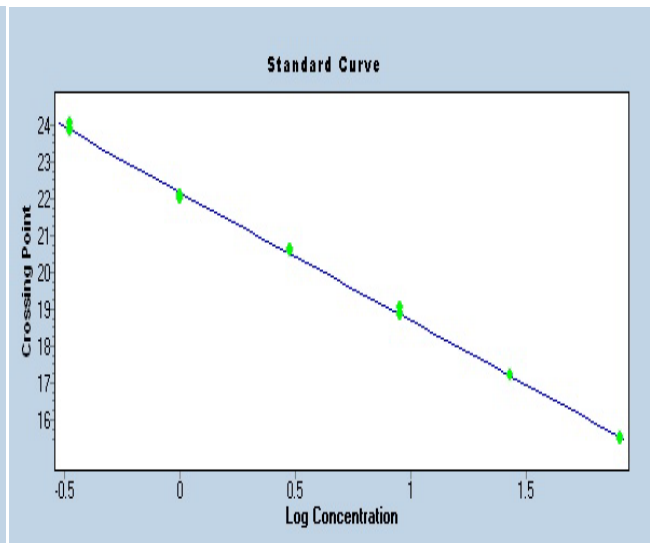


Figure S1. Standard curves for both the T run (telomeric sequence) and S run (single copy gene).