Protocol for the Quantification of Telomere Length Using qPCR
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Creating TE Buffer (skip this step if you have stock solutions)

TRIS-HCL (100ml, 1M)

1. Weigh 12.11g tris-base. Add to 100ml beaker.
2. Measure 80ml distilled water.
3. Mix with magnetic flea.
4. Add a pH meter.
5. Slowly add concentrated HCL to pH to 8.0.
6. Once desired pH has been reached, top solution off with distilled water to 100ml total.

EDTA (100ml, 0.5M)

1. Weigh 14.69g EDTA free acid.
2. Measure 80ml distilled water.
3. Mix with magnetic flea on a hot plate (this will take appox. 10 minutes)
4. Add pH meter.
5. Slowly add NaOH pellets to pH to 8.0 (reducing the pH will assist with mixing).
6. Once desired pH has been reached, top solution off with distilled water to 100ml total.

100ml TE Buffer

1. Add 1ml (1000ul) Tris-HCL (1M, pH 8).
2. Add 0.2 ml (200ul) EDTA (0.5M, pH 8).
3. Add 98.8ml distilled water.
4. Invert to mix. Confirm pH 8.0.

DNA Isolation from Saliva Samples

Protocol based off: DNA Genotek
Sampling Kt: DNA Genotek ORAcollect OCD-100 (DNA Genotek Inc., Ottawa, ON)

1. Mix DNA Genotek kit sample by inversion for a few second.
2. Incubate in water at 50°C for 1 hour or in air incubator for 2 hours.
3. Transfer 500ul of sample of 1.5ml microcentrifuge tube.
4. Add 20ul PT-L2P and mix by vortex.
5. Incubate on ice for 10 minutes.
6. Place in centrifuge in a known orientation.
7. Centrifuge for 5 minutes at 15,000 x g.
8. Transfer clear supernatant to a fresh microcentrifuge tube. Do not disturb the pellet containing impurities. Discard the pellet.
9. Transfer 500ul supernatant to a new microcentrifuge tube. Store remaining supernatant for future use if required.
10. Add 600ul room temperature 95% to 100% ethanol to the 500ul supernatant.
11. Mix by inversion 10 times.
12. Incubate at room temperature for 10 minutes to allow DNA to precipitate.

13. Place tube in a centrifuge in a known orientation.
14. Centrifuge for 2 minutes at 15,00 x g.
15. You will most likely not be able to visualize the pellet, however you can locate it based on the orientation it was in, in the centrifuge.
16. CAREFULLY remove all supernatant and discard the supernatant. Do not disturb the pellet.
17. Ethanol wash: add 250ul 70% ethanol to pellet. Let stand 1 minute. Pulse spin in centrifuge. Completely remove ethanol without disturbing pellet.
18. Optional: place microcentrifuge tube on air incubator at 37°C for 10 minutes to allow ethanol to evaporate out of tube.
19. Rehydrate DNA: Add 100ul TE buffer to DNA pellet. Vortex for at least 5 seconds.
20. Incubate at 50°C for 1 hour, vortex every 10 minutes to ensure full rehydration of DNA pellet OR overnight.
21. Storage: 1 month at 4°C or long term at -20°C.

Nanodrop

Nanodrop: Nanodrop 1 (ThermoFisher Scientific, Waltham, MA)

1. Place 1.3ul of DNA in TE solution on Nanodrop.
2. 260/230 ratio is expected to be low due to the presence of carbohydrates (0.8-1.2).
3. Dilute samples to 20 ng/ul with Dep-C water.

qPCR Analysis

Create Master Mix for Telomere Plate – volumes given are for ONE PCR well. Multiply as needed for number of wells required.

PCR Plate Volumes from: Cawthon (2003)

1. 0.54ul forward primer (GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGG)
2. 1.8ul reverse primer (TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCT)
3. 10ul SYBR Green Master Mix
4. 6.66ul Dep-C water

Create Master Mix for 364B Plate – volumes given are for ONE PCR well. Multiply as needed for number of wells required.

PCR Plate Volumes from: Cawthon (2003)

1. 0.6 ul forward primer (CAGCAAGTGGGAAGGTGTAATCC)
2. 1.0ul reverse primer (CCCATTCTATCATCAACGGGTACAA)
3. 10ul SYBR Green Master Mix
4. 7.4ul Dep-C water

Prepare PCR Plate

1. Blueprint location of each sample. Run each sample in duplicate or triplicate.
2. Allocate one well for NTC.
3. Dispense 19ul Master Mix into each well.
4. Dispense 1ul template into each well. Use Dep-C water in place of template in NTC.
5. Place film over PCR plate. Ensure a proper seal with no air bubbles.
6. Remove edges of the film.
7. Place plate in prepared PCR machine.

Setting Cycling Parameters
PCR Cycling Protocols from: Cawthon (2002)
PCR Machine: QIAquant 96 5plex (QIAGEN, Germantown, WA)

1. Open a new protocol by selecting "file → new".
2. Set a title.
3. Set cycling parameters, melt curve and which steps require measurement.
4. Select dye "FAM" (if using SYBR Green Master Mix).
5. Assign each sample to the proper well.
6. Assign the NTC.
7. Start. A countdown will be given once the protocol has start.

Telomere Cycling Parameters

	Temperature	Time	Cycles	Measurement
Enzyme Activation	95°C	10min		
Denaturation	95°C	15s	40X	X
Annealing and Extension	60°C	2min		X
Melt Curve	50°C to 90°C	15s		

364B Cycling Parameters

	Temperature	Time	Cycles	Measurement
Enzyme Activation	95°C	10min		
Denaturation	95°C	15s	40X	X
Annealing and Extension	58°C	2min		X
Melt Curve	50°C to 90°C	15s		

Export PCR Results

1. Right click on the sample you would like to export.
2. Select export to Excel.
3. Name the file.
4. Results will open in excel.

Calculate Telomere Length

T/S Ratio: $[2^{Ct(\text{telomeres})}/2^{Ct(36B4)}]^{-1} = 2^{-\Delta C_t}$