\*This protocol is modified from qPCR protocols from Kim Rosvall, Sara Wolf, and Britt Heidinger.

**Supplies:**

1. Multichannel pipettes
2. 10% Bleach in spray bottle
3. 70% Ethanol in spray bottle
4. Perfecta SYBR green SuperMix (Low ROX)
5. F & R telo primers
6. F & R GAPDH primers
7. Nuclease free water
8. 96-well or 384-well plates
9. Adhesive plate films
10. Ice packs

**DNA Dilutions (up to 24 hours prior to qPCR):**

1. For a 384-well plate, retrieve 121 DNA extracts from the freezer. Work off an ice pack.
2. Dilute DNA samples to 3.33ng/μL using sterile water using the “Loading\_telomere\_plates” excel sheet in the “2020\_TRES\_Telomere\_Labwork” Dropbox folder.
3. Under the “dilute samples“ tab, fill out the sample ID and nanodrop quantification for each sample under the “nano\_con” column. Low concentration samples are mixed at smaller total volumes and high concentration samples are mixed at higher total volumes. Those can also be adjusted manually.
4. The excel sheet will automatically calculate the amount of stock DNA and the amount of water you need for each dilution under the “add\_stock” and “add\_h2o” columns.
5. Store the extracts in the fridge until use (you will use 3μL of this dilution per well).

**qPCR Plate Set Up:**

1. Every sample will be run twice: once with telomere primers and once with GAPDH primers. Grab the appropriate supplies depending on which primer set you are using.

telomere F: 5'CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT3′

telomere R: 5'GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT3′

GAPDH F: 5'AACCAGCCAAGTACGATGACAT3′

GAPDH R: 5'CCATCAGCAGCAGCCTTCA3′

1. Retrieve the primers from the -30C freezer on shelf 3C in a box labeled "TRES Telomere qPCR Primers/Supplies" and the SYBR green from the -20C top freezer portion of the lab fridge.
2. Make a master solution by multiplying the following formula by 1.1\*# of samples. Reagent volumes are automatically calculated under the “master mix calculation” tab in the “Loading\_telomere\_plates” excel sheet (always round up):

1.96μL water

0.02μL F primer (from 100X stock, not diluted)

0.02μL R primer (from 100X stock, not diluted)

5μL Perfecta SYBR green SuperMix (Low ROX)

1. Vortex the master mix and spin all the liquid to the bottom.
2. For a 384-well plate, use two 96-well plates for pre-load setup. Follow the plate setup under the “Plate Layout” tab.
3. Load two rows of MM wells on one of the 96-well plates with master mix that has primers, water & mm in right ratio (~175μL per well).
4. Add 7μL of master mix to every well on 384-well plate using the multichannel pipette.
5. Add 15μL of diluted DNA sample, water control, golden sample, and standards to their designated wells on the 96-well plates.
6. Add 3μL of diluted DNA sample, water control, golden sample, and standards to their designated wells on 384-well plate using the multichannel pipette.
7. Cover the 384-well plate with an adhesive plate film and keep on ice while you transport it to the BRC.

**Run qPCR (in BRC):**

1. Once inside the BRC, spin the plate down in a plate centrifuge.
2. Start up the ViiA7 qPCR machine and make sure the 384-well plate block is installed.
3. Open the Design and Analysis Software 2.2.0 and double check the correct cycling conditions are selected under "Run Method" (depending on if you are doing telomere or GAPDH primer sets).

A) Telomere qPCR conditions:

* + 1. 10 min 95C
    2. 15s 95C
    3. 30s 58C
    4. 30s 72C
    5. repeat 2-4 27x
    6. 60s 95C
    7. 30s 58C
    8. 30s 95C

B) GAPDH qPCR conditions

* + 1. 10 min 95C
    2. 30s 95C
    3. 30s 60C
    4. repeat 2-3 40x
    5. 60s 95C
    6. 30s 55C
    7. 30s 95C

1. Double-check that the reaction volume is set to 10μL and the cooling/heating rate between cycling steps is set to 1.6C/s.