Heidinger Lab qPCR protocol (updated 12/6/2017)

1. Dilute DNA samples up to 24 hours prior to qPCR
   1. Be consistent - all samples included in a study should be diluted around the same amount of time prior to qPCR
   2. Samples should be diluted to 3.33 ng/μL (total 20ng)and store in fridge until use
2. Prepare supplies at hood: pipettes, tips, plates, plate holders, well caps, cooler with ice, two 5 or 15mL tubes (for Master Mix) wrapped in foil
3. Vortex all samples and standard curve dilutions
4. Pipet 6 μL of sample or standard into each well, using a new tip for each well
5. Prepare master mix (MM) for the appropriate plate (telo or GAPDH)
   1. Calculate the volume of each ingredient by multiplying the list below by the number of wells plus a few extra for safety
   2. For EACH WELL/Rxn: MM
      1. 6 μL ultrapure H2O
      2. 0.25 μL F primer
      3. 0.25 μL R primer
      4. 12.5 μL SybrGreen
   3. Mix in a 5 or 15 mL tube wrapped in foil. First add water to the tube, then primers, then half of the Sybr. Mix gently by inverting the tube, add remaining Sybr, and mix again. Keep on ice during mixing and at all times. Do not mix vigorously or it might foam.
6. Take MM back to hood on ice, and gently mix again by inverting.
7. Pipet 19 μL MM into each well. Only expel to the 1st stop to prevent bubbles. Add 6ul of Std or diluted samples and mix by pipetting several times before removing pipet.
8. Cover plate with well caps. Shake the plate on a shaker, covered with foil, at 900-1000rpm for 3 minutes.
   1. If running plate immediately, continue to the next step
   2. If running later, place the plate in a dark box in the fridge until ready
   3. Try to standardize wait times as much as possible (ex. Every plate waits in the fridge for 1 hour prior to running)
9. Spin plate in the big centrifuge at ~2000 rpm for 1 minute.
10. Run in qPCR machine with appropriate template and thermal cycle

Other info:

-Always warm up the lamp for at least 30 minutes prior to running the first plate of the day.

-Run samples in duplicate, and run the standard curve in triplicate. Each plate should contain a “golden sample” that is run on every plate.

-When the run ends, first set the threshold using the 20 ng standard curve wells only. Lock the threshold by clicking the small padlock button next to the threshold number. This applies the threshold to the whole plate.

-Do not use any sample with Ct % > 0.25 between wells. Re-run those samples on later plates.

-Turn off the lamp at the end of your last plate!

Standard Curve: Read the papers

40, 20, 10, 5 and 2.5 ng.

7ng/ ul

2 + 1 + 0.5 + 0.25 + 0.17 = 4 ul

96: 4 standards, golden sample, neg control, = 24 standards, 72 samples, 36 individuals / plate

384 wells at Institute of Biotechnology

260/280 > 1.7

260/230 > 1.8