qPCR Reaction Preperation Protocol

**Keep consumables in ice at all times**

1. If chosen primers are not resuspended, follow the resuspension protocol
2. Irradiate the Laminar flow hood by UV exposure for 15 minutes
3. Wipe all consumables (Mastermix, Template DNA, RNAse-Free Water, and Primers) with RNAse Away
4. Allot 36 0.2mL PCR tubes and label each one
5. Carefully pipette 12.5uL of MasterMix in each tube (You can stop here for the day)
6. Make a working solution of all primers by diluting the stock (100uM) solution to 10uM.
7. Take 5uL of primer stock solution and dilute in 45uL of TE Buffer for each primer to make this working 10uM solution (50uL final volume per primer)
8. Combine both reverse and forward primer by pipetting 48uL of each into a new tube.
9. Repeatedly pipette in and out to ensure proper homogenization of primer set solution; alternatively, vortexing is a possibility if careful with aseptic techniques and by wiping the tube with RNAse Away upon re-entry to Laminar flow hood
10. Repeat steps 7-9 for next primer set
11. Pipette 5uL of one primer set solution into PCR tubes 1-18 and 5uL of second primer set solution into PCR tubes 19-36
12. Tubes 17-18 and 35-36 will serve as blanks per targeted gene and will not contain template DNA
13. Add desired amount of template DNA (<100ng) per sample in a duplicate fashion (i.e. tubes 1-2 are Sample 1, tubes 5-6 are Sample 3, tubes 19-20 are Sample 1). Do not cross contaminate pipette tips between samples
14. Add remaining volume of Nuclease-Free Water into each tube with the use of a new pipette tip per tube as to not contaminate the water.