**qPCR**

1. Make a plate layout in excel and calculate master mix recipes

I place NTC wells on the top of the plate

1. Place SYBR Green, primers and water in hood to thaw
2. Prepare computer with plate layout
   1. Run housekeeping gene protocol
      1. 95C for 1min
      2. 95C for 20sec
      3. 60.5C for 20sec
      4. 72C for 30sec
         1. Plus plate read after
      5. GO TO 2 for 39 more times
      6. Melt curve 72 to 95C, increments of 0.5C for 10 sec
         1. Plus plate read
3. Wipe down bench top thoroughly with 70% ethanol
4. Get ice bucket filled and on your bench
5. Open a fresh box of 10ul pipette tips and leave on bench top
6. Return to qPCR hood
7. Make master mix

Make sure the eppie tubes in the hood have been autoclaved to prevent contamination.

* 1. 1X (for one well)

10ul SYBR Green

3ul H20

1ul Primer F

1ul Primer R

1. Add 15ul per well of master mix
2. Remove PCR plate from hood and keep on ice on lab bench
   1. Add 5ul Ultrapure water to NTC wells
   2. Add 5ul of cDNA or RNA to corresponding wells

I start with the water for the NTC wells, then RNA, then finally cDNA. I also only removed the cDNA/RNA from the freezer once I have pipetted the NTC wells with water.

1. Change gloves
2. Cover PCR plate and centrifuge at 1000xg for 2 minutes
3. Run PCR