**Universal Bacterial (515f/ 806r) and Fungal (FF390f/ FR1r) qPCR protocol**

Order primers for the region/gene you’re interested in testing for.

\*Ensure that there’s no chance of primer dimers or hairpins- SYBR green will bind and fluoresce if these are present.

\*Here’s a good tool for checking: http://www.premierbiosoft.com/netprimer/

\*We use 515f/806r (16S) for bacteria and FF390f/FR1r (18S) for fungal

Make standards - we’ve been using genomic *E.coli* DNA and genomic *A. fumigatus* DNA

\*We ordered the *A. fumigatus* gDNA and just extracted the *E. coli* gDNA from environmental samples we had using MOBIO DNA isolation kits

Get the concentration of your gDNA with a pico green assay - do multiple replicates, etc

Make standard dilutions of gDNA to use in the qPCR reaction. I use 10-3 to 10-7 ng/uL for environmental samples.

\*Do this by making 1:10 dilutions and heating each tube @ 60°C for 3min before use. Heat between each dilution!

\*Thermal mixing ensures accurate standards - DO NOT submerge tips from more      concentrated standards into less concentrated. Hover over tube and expel.

\*Ensure tubes you use are nice and clean – can leave under UV for ~15min

Convert the ng/uL units of concentration to copy number. There’s a good tool for that here:

http://cels.uri.edu/gsc/cndna.html

\*We use 29.0 Mb as the *A. fumigatus* genome size and 4.95 Mb as the *E. coli*

\* You can leave measurements in fungal or bacterial equivalent genomes or convert to rRNA gene count

Run qPCR with standards and hope everything works!

\*Most programs will automatically set-up your standards curve, just make sure you select SYBR green as the dye.

\*Make sure to check directions for your qPCR mix; ours has a mandatory 95°C 15min initial step

**qPCR recipe (1 reaction worth)**

1.25uL primer 1 (10uM)

1.25uL primer 2 (10uM)

12.5uL 2X qPCR mix

5uL water

5uL template DNA

         25uL total

**qPCR program**

         95°C 15min

          [94°C 45 sec, 50°C 1 min, 72°C 1:30 min] x40 cycles

72°C 10 min

(Add a 4°C hold if you plan to use DNA later)

25nmol Primers - 16S\_515F\_qPCR5’ –GTG CCA GCM GCC GCG GTA A -3’ 16S\_806R\_qPCR 5’ –GGA CTA CHV GGG TWT CTA AT-3’ FF390\_fungal\_qPCR\_F 5’ –CGA TAA CGA ACG AGA CCT -3’ FR1\_fungal\_qPCR\_R 5’ –ANC CAT TCA ATC GGT ANT -3’

Plates: Agilent technologies catalog #: 410088

qPCR mix: Thermo AB-1159/A (ABsolute QPCR SYBR Green Mix, no Rox, 400 x 25 ul rxns)