Amplicon Visualization and Quantification Protocols

Gel Electrophoresis

1. Prepare a 1% agarose gel by combining 1g of agarose with 100ml 1xTBE in an 250ml Erlenmeyer flask (add agarose first then liquid).
   Note: The volume of the gel should be adjusted to fit the cassette you are working with. I only use 100mls here as an example.
2. Microwave the mixture for ~2m (time is dependent on microwave and size of gel) or until the mixture is completely clear.
   Note: During this step watch the mixture as it microwaves, when it begins to bubble pause the microwave, allow the bubbling to stop, and carefully mix by gently swirling the flask. Use hot hands or an autoclave glove and be very careful to ensure that the mixture does not boil over.
3. Allow mixture to cool on the bench for several minutes. It is cool enough when, while using a nitrile-gloved hand, you can hold your hand to the flask for 5s. Alternatively, place the flask in a 55˚C water bath for ~30m, use thermometer to measure temp before proceeding.
4. Add 10 ul of 10,000X sybr gold nucleic acid stain (ThermoFisher #S11494) to the flask and mix by gently swirling (1:10,000 dilution).
   Note: ethidium bromide (EtBR) can also be used, but EtBR requires special disposal and sybr gold does not so I do not use here.
5. Pour gel into gel cassette. Be sure to add the comb to the gel.
6. Allow to cool at 4˚C for ~20m.
7. Combine 5ul of each PCR product with 5ul of ddH2O and 2ul of loading dye (1:6 dilution of dye).
8. Place solidified gel into gel box in the correct orientation (DNA is negatively charged and will migrate toward the red positive post).
9. Fill gel box to fill line with 1xTBE.
10. Load gel starting with prepared 100bp ladder in the first lane. **Note: the concentration of the ladder varies by manufacturer. Sybr gold is very sensitive and DNA in ladders tends to be very concentrated, if you ladder appears deformed or as a bright smear, reduce the concentration of the ladder. I use a 1:8 dilution.**
11. Load PCR samples.
12. Connect the gel box to the power supply.
13. Run gel at 80v - 100v for ~45m.
14. Turn off power and disconnect the gel box.
15. Remove gel and visualize.
    Note: Expected band size for 515f/806r is roughly 300 – 350 bp.

DNA Quantification with Qubit dsDNA HS Assay Kit (adapted from manufacturer's protocol*).

	Standard **Assay tubes**	***User Sample*** **Assay tubes**
Volume of **Working solution** (see step 2 below)	190µL	199
Volume of **DNA Standard**	10µL	-
Volume **user DNA**	-	1µL
Total Volume	200µL	200µL

1. Set up two tubes for the DNA standards
2. Prepare the **Working Solution** by diluting the Qubit reagent 1:200 in Qubit buffer.

3. Prepare tubes as above for each pooled PCR reaction.
4. Vortex all tubes 2-3 seconds.
5. Incubate for 2m at room temp in the dark.
6. Insert tubes into Qubit and press DNA, then hsDNA
7. Read standards if appropriate (standards should be read at the very minimum weekly, but preferably daily)
8. Read sample
9. Calculate the ng/ul using the Qubit calculate function after reading each sample (note ng/ul must be selected from the drop down screen).

* http://www.thermofisher.com/us/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/fluorometers/qubit.html