Qubit Quantification

Two kits, both kept in the fridge: Broad Range (BR) and High Sensitivity (HS)

- Broad Range kit is for DNA with concentration of ~100-1000 ng/uL *
- High Sensitivity kit is for DNA with concentration of ~1-100 ng/uL*
  * DNA concentration can be estimated using the NanoDrop first

NOTE: The dye is LIGHT SENSITIVE and should be kept in the dark as much as possible! Keep the tube of dye wrapped in foil, and thaw in the dark. Work quickly when making up mixtures, and use a cover to block out light if desired.

1. Bring reagents to room temperature (dye, buffer, two standards*) – the dye can sometimes take a long time to thaw!
   - The two standards to use are the 0 and the [10 for HS, 100 for BR]

2. Obtain and label qubit tubes (one per sample, plus two – one for each standard). Do not make any marks on the sides of the qubit tubes – only write on the cap.

3. Make a master mix of the dye and the buffer: 1uL of dye and 199uL of buffer per sample – make enough for each of your samples, plus the two standards, plus some extra for pipetting error. Be sure to vortex the mixture thoroughly and spin down.

4. For each sample: combine 1-10uL DNA with dye/buffer mix to make a total volume of 200uL.

5. For each standard, combine 10uL of standard with 190uL dye/buffer mixture.

6. Make sure all mixtures are thoroughly homogenized by vortexing for several seconds, then spinning down tubes in benchtop mini centrifuge. Ensure there are no bubbles in the tubes.

7. Get the key for the van Valkenburgh lab (found in the drawer under the printers). Use a layer of foil to cover samples and block out light while transporting samples down to the 2nd floor lab.

8. Enter the 2nd floor lab through 2162. The Qubit reader is on a bench in the Alfaro lab space (middle of the lab).

9. Turn on the Qubit reader touch screen by tapping. Choose dsDNA for either BR or HS.

10. Lift the lid, add the tube for the 0 standard first, close the lid, and press the button for “read standard #1”, then repeat with the second standard.

11. Follow with your sample tubes. Load your tube then press the button to “read next sample”. Press the button for “calculate stock concentration” after getting the reading. Make sure the appropriate sample volume is selected, depending on how much DNA you added to the tube. You may also change the units to give a reading in ng/uL.

12. You may measure the concentrations of the standards at any time to ensure that the readings are correct and there is proper calibration. This is more important when doing a lot of measurements. Recalibration can be done at any time.