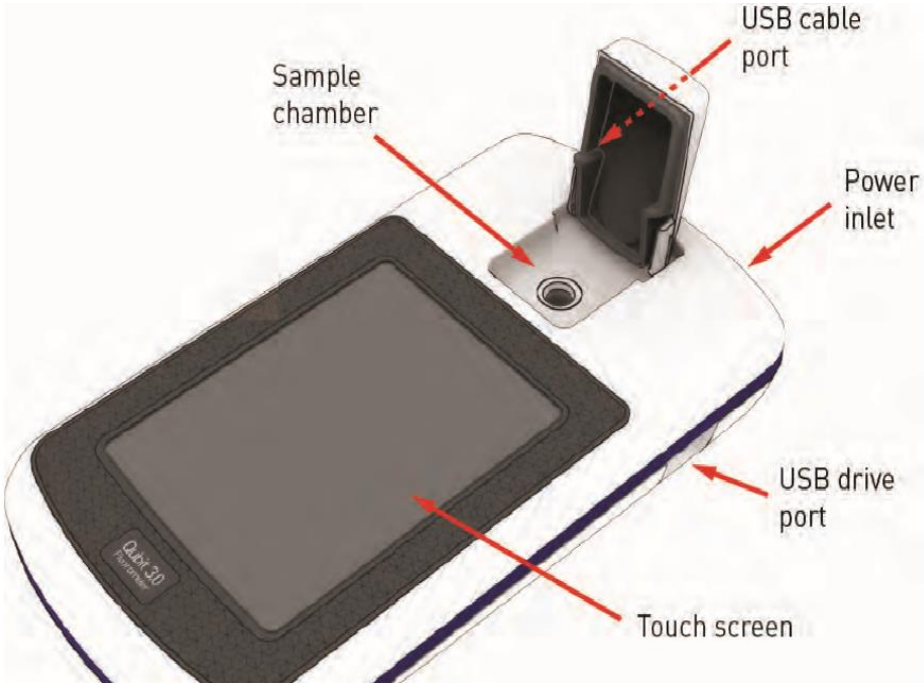
Qubit 3.0 Fluorometer Protocol

Note: For best results, the kits will be stored in the 4 degree Celsius biotechnology fridge in the back room, however ensure that all the assay reagents are at room temperature before beginning. **DO NOT EXPOSE QUBIT REAGENT TO LIGHT**!!

Qubit Fluorometer manual-



Marterials

- A 1.5 mL sterile microcentrifuge tube (working solution)
- Qubit Buffer
- Qubit reagent (photodegradable)
- Qubit centrifuge tubes (# depends on individuals needs)
- Standards (two total)
- Sharpie (for labeling)
- Micropipettes and tips (0.5-10ul and 100-1000ul)
- Qubit

Step 1- **READ BEFORE YOU PROCEED**. Please do not do this procedure before reading all the steps as it could mess up your results

**Note: make sure to verify on the outside of the kit package that you are using the correct range for what you want to quantify (High sensitivity vs. Broad range). In terms of plasmid DNA, high sensitivity is used for low-copy number plasmids, while broad range is used for High-copy number plasmid.

Step 2- Begin by calculating your n value. N= the number of standards plus the number of samples. For example, If you will use two standards (which everyone one will) and you want to analyze 2 samples, your n value will be 4 (2+2=4).

Step 3- To creating the working solution, you will need the 1.5 microcentrifuge tube, the Qubit buffer and the Qubit reagent. To calculate how much Qubit Buffer you will need to add to create the working solution, multiply your n value by 199. Therefore, If your n value is 4, then you would pipette 796 ul of Qubit Buffer into the 1.5 microcentrifuge tube using a 100-1000 ul micropipette. The next step is to add Qubit reagent. **THIS REAGENT IS PHOTOSENSITIVE**, meaning it **WILL DEGRADE** if exposed to light. Keep inside box unless you are using it. The amount of Qubit reagent you will need for the working solution is equal to your n value. For example, if your n value is equal to 4, then you will pipette 4 ul of Qubit reagent into the 1.5 microcentrifuge tube using the 0.5-10ul micropipette. Once you have added the reagent, label on the side of the working solution R+, in order to indicate that the action has been done. Remember to not expose the tube to direct light as the reagent that is in the tube is photodegradable. Vortex spin working before proceeding to next step for 3 seconds.

Step 4- The next step is to prepare your standards. Obtain 2 Qubit assay tubes (1 for each standard) and label them standard 1 and standard 2. Add 190 ul of the Qubit Working Solution you created during the previous step to each labeled Qubit centrifuge tube using the 100-1000 ul micropipette. Now add 10 ul of Standard 1 from the kit into your Qubit centrifuge tube labeled standard 1. Do the same for standard 2, pipetting 10 ul of standard 2 from the kit into the labeled standard 2 Qubit centrifuge tube.

Step 5- The last step now that the standards are complete, are to make the user samples. In the example the protocol has been following, the n value is 4; 2 standards are being analyzed in the fluorometer, while 2 user samples are being analyzed. The user samples will contain the DNA you will be quantifying. To prepare the user sample, add anywhere from 180-199 ul of the Qubit Working Solution into each assay tube labeled user sample using a 100-1000ul micropipette.Then add the DNA being used to where the total volume of the each user sample is 200ul. For example, if 2 samples are going to be analyzed, if 180ul of working solution are added to the sample, then 20 ul of DNA will be added to complete the user sample assay.

***Keep all assays prepared out of direct light until needed to run.

Step 6- Now it is time to use the fluorometer. Once the fluorometer has been plugged in, the first screen shown says to choose an assay, and gives various options. Choose the assay that corresponds to the sample you are running. For example, if your sample consists of plasmids DNA, the you would click dsDNA.

Step 7- Choose the range that your plasmid is going to be run at during the quantification (High sensitivity vs. Broad range). Note that this should have already been determined before doing the procedure.

***Note: Vortex all assays that will be run before proceeding to the next step for optimal quantification.

Step 8- Next step is to click read standards, and insert standard 1 into the sample chamber. Click read standard. Next insert standard 2 into the sample chamber and click read sample. A fluorescence (RFU's) vs. Qubit tube concentration (ng/mL) will appear, graphing the standard values accordingly. These values refer to the actual fluorescence values. The purpose of the standards is to account for troubleshooting the quantity of molecules found in your sample.

Step 9- Once calibration is complete, it is time to run the sample assays. When you click run samples, a screen will appear asking for the amount from your original sample that you added into the sample assay (Between 1 to 20ul). Adjust accordingly. Next you will need to select the output sample units (common units used are ng/mL).

Step 10- Click read sample.

Step 11- Once the instrument has calculated the amount of DNA in the original sample tube, a next screen will appear list all vital information the Fluorometer calculated. Take a picture of this information or write down the graphs and tables given in your lab composition notebook.

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