Qubit 4.0 Fluorometer Protocol
Qubit 1X dsDNA HS Assay Kit (Q33231)

Introduction

- *The Qubit 4.0 fluorometer is used to measure DNA concentrations and is preferred over the Nanodrop for genomic applications because of its greater reliability. Nanodrop tends to give artificially higher concentrations of DNA.*
- *The 1X HS Assay kit is a high sensitivity kit that can detect DNA at concentrations from 0.1 to 120 ng/µl.*
- *This 1X kit comes with a pre-prepared Working Solution that is ready to use. No need to prepare a working solution.*
- *For measuring concentrations of PCR products or samples that are likely highly concentrated, it is best to dilute the DNA 1:10 in water to avoid going over the upper limit of detectability.*
- *More info on the kit at: https://www.thermofisher.com/order/catalog/product/Q33231*

Set up

1. Obtain the Qubit 1X dsDNA HS Assay Kit.
    - **Standards #1 and #2** are kept in the kit box in the small lab refrigerator. The **Working Solution** is at room temperature in the drawer with the Qubit and Nanopore sequencers.
2. Obtain 0.5 mL **Qubit tubes** to use for each standard and each DNA sample to be quantified. Label the lids of the tubes STD 1 and STD 2.
    - Make sure to use Qubit tubes, not regular tubes.
    - Do not label the sides!

Preparation of standards

The Qubit requires standardization only once per work session.

1. Take a 0.5 mL Qubit tube and label it STD 1, add 190 µL of working solution and 10 µL of the Standard #1 from the assay kit. Set aside and keep at room temperature for 2 min.
2. Take a 0.5 mL Qubit tube and label it STD 2, add 190 µL of working solution and 10 µL of the Standard #2 from the assay kit. Set aside and keep at room temperature for 2 min.
3. Mix each sample vigorously by vortexing for 3–5 seconds, then quick spin.
4. Calibrate the Qubit using the standards. From the initial home screen, select “DNA”, then select the assay type “dsDNA 1X High Specificity protocol”. Select the “Read Standards” option.
5. Insert the tube STD 1 into the chamber. Close the lid and select “Read standard.” When reading is complete, remove the tube from the chamber and set aside.
6. Insert the tube STD 2 into the chamber. Close the lid and select “Read standard.” When the reading is complete, remove the tube from the chamber and set aside.

Preparation of samples

We will use a High Sensitivity kit designed to measure DNA concentrations when there may be little DNA in the sample. If you are not sure whether your samples will be above 120ng/ul, the first thing to do is make a 1:10 dilution of the DNA in water. So, take 1ul of DNA and add it to 9ul

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of water. The reason is that if the DNA concentration is too high, the Qubit may not be able to read the concentration with this kit.

1. To each 0.5 mL Qubit sample tube, add 198 µL of working solution and 2 µL of the diluted DNA sample (if you conducted the 1:10 dilution). Close the lid and vortex 2-3 sec and quick spin. Set aside and keep at room temperature for 2 min.
    a. *Note: Total volume in sample tube must be 200 µL.*
    b. *Note: Any amount of DNA between 1 and 20 µl is OK, just adjust water to a final volume of 200ul.*

Reading Concentrations with Qubit

1. After the 2 min incubation, read the DNA samples. From the current screen (post calibration), select “Run samples”. Select the sample DNA volume (should be 2 µL) using the +/- button and the units (ng/µL) from the dropdown menu.
2. Insert the first DNA sample tube into the chamber. Close the lid and select “Read sample.” When reading is complete, remove the sample tube from the chamber and set aside.
3. If you diluted the DNA, **multiply the number you get by 10** to account for the fact that you used DNA that was diluted by a factor of 10. Record the concentration in ng/µL.
4. Repeat for the remaining DNA sample tubes.