Quantifying Nucleic Acids Using Qubit

Adapted from manufacturer's protocol.
Updated 29-Feb-2024 by Katie Murphy

BACKGROUND:

For many molecular projects, you will need to quantify DNA/RNA using fluorometry rather than relying on spectrophotometry (such as a nanodrop). We stock three types of fluorometric Qubit kits at LAB that are free to use for small projects*. Please note for larger projects, you are expected to buy your own Qubit kits.*

- 1X dsDNA High Sensitivity (HS) assay kit
- dsDNA Broad Range (BR) assay kit
- RNA HS assay kit

Other types of Qubit kits such as for ssDNA are available directly from Invitrogen. Please order these as needed.

HELPFUL HINTS:

- If your kit includes separate dye and buffer, allow the kit to come to room temperature before use (the dye needs to be thawed). This will take at least 30 minutes.

- We recommend running standards every time you quantify samples, since the dye integrity and resulting standard curve could vary between different kits of the same type.

- Run the assay at room temperature.

- Choose High Sensitivity (HS) or Broad Range (BR) based on your expected concentration range:
    - The quantitation range of the HS DNA kit is 0.2 – 100 ng total DNA in each assay tube. If using 1 µL DNA, this means you can run samples up to 100 ng/µL.
    - The quantitation range of the BR DNA kit is 2 – 1000 ng total in each assay tube. If using 1 µL DNA, this means you can run samples up to 1 µg/µL.
    - The quantitation range of the HS RNA kit is 5 – 100 ng total RNA in each assay tube.

- If you are running more than ~24 samples, you may want to use the Quant-iT assay instead of Qubit, as it allows you to quantify samples in plate format.

MATERIALS & REAGENTS:

- DNA or RNA to be quantified
- DNA or RNA Qubit kit (HS or BR)
- 0.5 mL Agilent or GeneMate tubes (or comparable thin-walled tubes)

PROTOCOL:

Step 1 – Setting up your assay tubes

1.1 Ensure your DNA/RNA samples are fully thawed and homogenous.

1.2 If using a kit that includes separate dye and buffer (not a 1X kit), allow the kit to come to room temperature for 30 minutes before use. Make sure that the tube of dye is thawed, then spin it briefly.

1.3 *If you don't have a 1X kit*: If you need to dilute your dye, make a 1X working stock in a plastic tube. Calculate the number of reactions needed for your samples and two standards, making sure to include a few extra reactions to allow for pipetting error.

a. For each reaction, you'll need 1 µL of dye and 200 µL of buffer.
b. Do not dilute the dye in a glass container, because the dye will bind to the glass.
c. Once you dilute the dye, it's stable at room temp for 3 hours as long as it's protected from light.

1.4 Get out enough 0.5 mL tubes for all samples and standards, and label the caps as needed.

1.5 Add 190 µL of diluted dye to each of your two standard assay tubes.

1.6 Add 10 µL of each standard to their corresponding assay tubes.

1.7 Determine what volume of DNA/RNA you want to use for your samples. The assay accepts anything from 1 – 20 µL.

a. We recommend 2 µL instead of 1 µL if possible, as pipetting is more accurate with 2 µL.

1.8 Each assay tube needs a final volume of 200 µL, so calculate what volume of buffer to add to each tube (200 – n, where n is the volume of DNA).

1.9 Add the appropriate volume of buffer to each assay tube.

1.10 Add 1-20 µL of each DNA sample to its designated tube, such that the final volume is 200 µL.

a. For your calculations, it is simplest if you use the same volume of DNA for all samples. But this isn't strictly necessary, as long as you record what volume of DNA you used for each sample.

1.11 Vortex to mix all tubes, then spin briefly. Ensure there are no bubbles present.

1.12 Incubate all assay tubes at room temperature for at least 2 minutes before reading on the Qubit.

a. Your assay will be stable for up to two hours at room temperature, as long as you store the tubes in a dark location.

1.13 Return your Qubit kit to the fridge (or room temp drawer for BR DNA buffer and dye; standards at 4°C).

Step 2 – Running your quantification assay on the Qubit

You can use either the newer Qubit 4.0 (Step 2A) or one of the older Qubit models (2B).

2A. Using the Qubit 4.0

2A.1 On the Qubit device, select the assay you wish to run.

2A.2 Select **Read Standards**.

2A.3 Insert standard 1, close the Qubit lid, then click **Read**. After the read finishes, remove the tube.

2A.4 Insert standard 2, close the Qubit lid, then click **Read**. After the read finishes, remove the tube.
2A.5 Click **Run Samples**.
2A.6 Select the volume of DNA or RNA used in your assay and adjust the units if desired.
2A.7 Insert your first sample, close the lid, and click **Read Tube**.
2A.8 Record the concentration of your sample. On the Qubit 4.0, the large value in the center of the screen is your original DNA or RNA stock concentration – this is the value to record.
    a. The small value shown underneath is the concentration of your diluted assay tube.
2A.9 Repeats steps 7-8 for all samples.
2A.10 If desired, you can export the data to a USB flashdrive (excel format).
2A.11 Once done, press the **Home** icon to return to the start screen.
2A.12 Make sure to dispose of your tubes.

2B. Using the Qubit 1.0 or 2.0

2B.1 On the Qubit device, select the assay you wish to run.
2B.2 The Standards screen will be displayed. Press **Yes** to read your standards.
2B.3 Insert standard 1, close the Qubit lid, then click **Read**. After the read finishes, remove the tube.
2B.4 Insert standard 2, close the Qubit lid, then click **Read**. After the read finishes, remove the tube.
    a. After reading standard 2, the instrument should display the Sample screen.
2B.5 Insert your first sample, close the lid, and click **Read**.
2B.6 The value initially displayed is the concentration in your diluted assay tube, NOT the original concentration of your DNA or RNA stock. You must calculate the stock concentration by clicking **Calculate Stock Conc**.
    a. You could also calculate by hand by multiplying the value by your dilution factor (skip steps 7-10 if so).
2B.7 Scroll to select the volume of stock sample used in your assay.
2B.8 To change the units displayed, press **ng/mL** and select your desired units (likely **ng/µL**) in the popup.
2B.9 Click on the screen outside of the popup to close the popup menu.
2B.10 Record the stock concentration of your sample.
2B.11 Insert your second sample, close the lid, and press **Read Next Sample**. Follow steps 6-11 for any additional samples, making sure to record all stock concentrations.
2B.12 Once done, press the **Home** icon to return to the start screen.
2B.13 Make sure to dispose of your tubes.