Protocol for Qubit assays of DNA extraction samples

The following protocol is designed for use with a Qubit 2 Fluorometer using the Qubit dsDNA HS (high sensitivity) assay kit. This protocol will provide volumes for 1, 20 and 100 samples.

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Before you start

- Read the important notes on DNA work below.
- Always use filter tips
- In the laboratory: wipe down the surfaces, oven, centrifuge, pipettes, door handles, everything with an EBE clean method.
  - Ensure you have sufficient gloves, towels, tips, waste containers, tubes etc.
  - Collect all consumables
- Bring samples and Qubit kit to room temperature
- Once mixed the Qubit-sample solution is stable at room temperature for 3 hours. For processing large numbers of samples (50+), it is therefore advisable to split the work into two halves, mixing and measuring 50 samples, before continuing with the remaining samples.
- Always calibrate the machine before measuring samples and update the calibration if it has been 3+ hours since calibration.
- The Qubit 2.0 provides the concentration in ng/mL - the concentration in the tube. You will need to calculate the concentration of your sample with the known ratio of sample to solution. See the section on this for more.

Important notes on DNA work:

- It is important to do the ethanol-bleach-ethanol "EBE" clean to prevent chemicals unintentionally interacting and to prevent sample contamination from external sources.
- Photograph your lab book and field data sheet at the end of every day as a backup.

Consumables

- Gloves
- 200µL and 10µL pipette filter tips
- Thin-walled, plastic, single 0.2mL PCR tubes with caps
- 1.5mL microcentrifuge tubes with caps
- Qubit dsDNA HS Assay kit with standards 1 & 2

Equipment

- Rack for 0.2mL PCR tubes
- 200µL and 10µL pipettes
- Qubit 2.0 Fluorometer
- Desktop vortex
- Desktop mini-spinner

Qubit Assays

Preparing the Qubit solutions

1. In the rack, set up the required number of 0.2mL tubes for standards and samples - the dsDNA HS assay requires two standards. Label the tube lids *NOT the sides as this could interfere with the sample read.*
2. In a second rack, set up a 50mL conical tube for the working solution if your sample size is ≥70; prepare a 5mL tube if your sample is <70. Label it with the date and 'Qubit working sx'. NEVER mix this solution in glass.
3. Prepare the Qubit working solution by diluting Qubit dsDNA HS Reagent 1:200 in Qubit dsDNA HS Buffer. The final volume of every reaction must be 200µL. Every standard requires 190µL of working solution, and each sample requires 195µL. Prepare sufficient working solution to account for the number of standards (2) plus the number of samples (*N*), plus 5% for pipetting error. You can use Table 1 to identify volumes required for standard sample sizes.

Table 1. Working solution components volumes based on sample size, 2 standards and 5% for error.

Number of Samples	Qubit dsDNA HS Reagent	Pipette volume (iterations)	Qubit dsDNA HS Buffer	Pipette volume (iterations)	Total Volume
1	3µL	3µL (1)	600µL	600µL (1)	603µL
15	17.35µL	17.35µL (1)	3462µL	577µL (6)	3469.35µL
100	104.4µL	104.4µL (1)	20,769µL	903µL (23)	20,873.4µL
115	120µL	120µL (1)	23,825µL	953µL (25)	23,945µL

All volumes include the amount for 2 standards plus 5% error, i.e. 1 sample is enough for 1 sample and 2 standards. Calculations also assume you will use 5µL of sample with 195µL of Qubit working solution.

4. Into each of the standards' tubes add 190µL of working solution.
5. Add 10µL of each Qubit standard to the appropriate tube, then vortex 2-3 seconds. *Be careful not to create bubbles.*
6. Allow the standards to incubate at room temperature for 3 minutes.

Calibrating the Qubit 2.0 Fluorometer

7. You'll now calibrate the Qubit machine using the prepared standards.
8. On the Home Screen press **DNA** then select **dsDNA High Sensitivity**. The screen displayed will be Standards. If prompted select **Yes** to read new standards. *If you want to use the previous calibration, select No and proceed to 'Measuring samples with Qubit'.*
9. Insert the tube containing Standard #1 into the chamber. Close the lid, then press read. When the reading is complete (~3 seconds), remove Standard #1.
10. Insert the tube containing Standard #2 into the chamber. Close the lid, then press read. When the reading is complete (~3 seconds), remove Standard #2.

11. When the calibration is complete, the instrument will display the Sample screen.

Measuring samples with Qubit

12. Into each of the samples' tubes add 195µL of working solution. *Remember once combined the sample-working solution is only stable for 3 hours.*
13. Add 5µL of sample to its corresponding tube, then vortex 2-3 second. Be careful not to create bubbles.
14. Allow all tubes to incubate at room temperature for 2 minutes.
15. Now you'll read samples through the Qubit. The Qubit will display the concentration value, in ng/mL, as the sample is in the tube. In the next section you will convert these value to the concentration of the sample based on the known ratio of sample to working solution (e.g. 5:195µL). Write this value down on the data sheet. The Qubit does save values however you may wish to re-read samples, or end up doing them in a non-sequential order, so always write down the displayed value.
16. If the machine has gone to sleep in the time since calibrating thats okay: from the Home page press DNA **then select** dsDNA. Then press **No** to read new samples and this will bring you to the Samples page.
17. Insert a sample tube into the chamber, close the lid, then press **Read**. When the reading is complete (~3 seconds), record the value displayed, and remove the sample.
18. Repeat until all samples are processed.

Calculating sample concentrations

19. To calculate the concentration of your sample using the ng/µL value read by the Qubit 2.0, use the following equation:

    Concentration of your sample (ng/µL) = QF value x 200/x,

    where QF = the value given by the Qubit,

    and x = the number of microliters of sample added to the assay tube (e.g. 5).

    You can calculate these values in the digital data sheet after entering the samples Qubit read values.