

Quantifying DNA Samples using the Qubit 4 Fluorometer	
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Quantifying DNA Samples Using the Qubit™ 4 Fluorometer

I. Purpose

To provide specific guidelines for quantifying DNA samples using the Qubit™ 4 Fluorometer in conjunction with the Qubit™ 1X dsDNA HS Assay Kit; Qubit™ Broad Range (BR) Assay Kit; and Qubit™ ssDNA Assay Kit.

II. Scope

All procedures are applicable to the BCGSC Library Core and Library TechD Groups.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a Quality Systems associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Systems to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
Qubit™ 4 Fluorometer User Guide	IM.0362

VI. Related Documents

Document Title	Document Number
Qubit® dsDNA BR Assay Kits	AM.0057
Qubit™ 1X dsDNA HS Assay Kits	AM.0056
Qubit® ssDNA Assay Kit	AM.0098

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the safety data sheet (SDS) for additional information.

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VIII. Materials and Equipment

Name	Supplier	Number:	Model #	Catalogue #
Fisherbrand Nitrile gloves- medium	Thermo Fisher	27-058-52		✓
Gilson P2 pipetman	Mandel Scientific	GF-44801		✓
Gilson P10 pipetman	Mandel Scientific	GF-44802		✓
Gilson P20 pipetman	Mandel Scientific	GF-23600		✓
Gilson P200 pipetman	Mandel Scientific	GF-23601		✓
Gilson P1000 pipetman	Mandel Scientific	GF-23602		✓
Mini-centrifuge	Thermo Fisher	05-090-100	✓	
Vortex Mixer	Labnet	S0200	✓	
Eppendorf Safe-Lock Tubes, 2ml	VWR	054027		✓
Falcon 2096 conical tubes, 15 ml	VWR	CA21008-918		✓
Qubit™ 1X dsDNA HS Assay Kit	Invitrogen	Q33230		✓
Qubit™ 4 Fluorometer	Invitrogen	Q33226		✓
Qubit™ assay tubes (500)	Invitrogen	Q32856		✓
Black ink permanent marker pen	VWR	52877-310		✓
Diamond Filter tips DF1000	Mandel	GF-F171703		✓
Diamond Filter tips DFL10ST	Mandel Scientific	GF-F171203		✓
Diamond Filter tips DFL30ST	Mandel Scientific	GF-F171303		✓
Diamond Filter tips DF200ST	Mandel Scientific	GF-F171503		✓
Qubit™ dsDNA BR Assay Kit 500	Invitrogen	Q32853		✓
Qubit™ ssDNA Assay Kit 100	Invitrogen	Q10212		✓

IX. Qubit™ assay guide

Qubit™ assay kit	Assay Range	Sample starting concentration
dsDNA Broad Range Assay (BR)	2 – 1000ng	100pg/μL - 1μg/μL
1X dsDNA High Sensitivity Assay (HS)	0.2 - 100ng	10pg/μL - 100ng/μL
ssDNA Assay	1 - 200ng	50pg/μL - 200ng/μL

X. Procedure

At Qubit™ workstation:

Note: Use only authorized accessories (adaptor, power cord, USB cable, etc)

1. Retrieval of reagents and equipment preparation

1.1. Put on a clean pair of gloves and lab coat.

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- 1.2. Retrieve appropriate pre-aliquoted Standard #1 and Standard #2 from 4°C fridge and allow the standards to come to room temperature for a minimum of two minutes.
- 1.3. Retrieve DNA samples and equilibrate to room temperature. Mix well and pulse spin.
- 1.4. Ensure there is communication with the Qubit™ Fluorometer and the instrument computer. If not, re-establish the connection by unplugging and re-plugging the microUSB port at the rear of the Qubit™ instrument.
- 1.5. Instrument touch screen is the user interface containing the buttons for all the functions needed and displays data from the assays.

2. Setting up the Qubit™ 1X dsDNA High Sensitivity assay

- 2.1. Calculate the volume of Qubit™ 1X dsDNA HS working solution that will be needed to accommodate all standards and samples. The final volume in each tube must be 200µL. Each standard tube requires 190µL of Qubit working solution and each sample tube requires anywhere from 180–199µL. The reagent calculator in section 3.3 can be used to determine the amount of Qubit™ working solution required.
- 2.2. Retrieve the Qubit™ working solution from the 4°C fridge and mix well. To avoid cross-contamination, aliquot out the required volume of Qubit™ working solution from the main bottle into an appropriate sized sterile tube. After aliquoting, immediately return the stock bottle to 4°C fridge. Allow the aliquoted working solution to reach room temperature for at least 10 minutes.
- 2.3. Set up the number of 0.5mL tubes needed for all of the samples plus a tube for each of the standards. For best results, use clear, thin-walled Qubit™ assay tubes. Label the lid of each assay tube (do not write on the side wall).
- 2.4. Dispense 190µL Qubit™ working solution into tubes for Standard #1 and Standard #2.
- 2.5. Dispense 180–199µL Qubit™ working solution into tubes for the samples.
- 2.6. Add 10µL of each Qubit™ standard to the appropriate tube.

Note: Accurate pipetting is critical to ensure that exactly 10µL of each Qubit™ dsDNA HS standard is added to 190µL of Qubit™ working solution. Proper calibration of the Qubit™ Fluorometer requires that the standards be introduced to the instrument in the correct order.

- 2.7. Add 1–20µL of each user sample to the appropriate tube.

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2.8. Mix the standards and samples vigorously by vortexing for 3–5 seconds and pulse spin to remove bubbles.

2.9. Allow all tubes to incubate at room temperature for a minimum of 2 minutes.

3. Setting up the Qubit™ dsDNA Broad Range assay

3.1. Calculate the volume of Qubit™ dsDNA BR working solution that will be needed to accommodate all standards and samples. The final volume in each tube must be 200µL. Each standard tube requires 190µL of Qubit™ working solution and each sample tube requires anywhere from 180–199µL. The reagent calculator in section 3.3 can be used to determine the amount of Qubit™ working solution required.

3.2. Set up the number of 0.5mL tubes needed for all of the test samples plus a tube for each of the standards. For best results use clear, thin-walled Qubit™ assay tubes. Label the lid of each assay tube (do not write on the side wall).

3.3. On the Home screen, select onboard Reagent Calculator (Figures 1a,b,c) to determine the correct amount of Qubit™ Broad Range dye and buffer required to prepare for the samples and standards. In an appropriate sized tube, aliquot the appropriate volumes and vortex well.



Figure 1a



Figure 1b

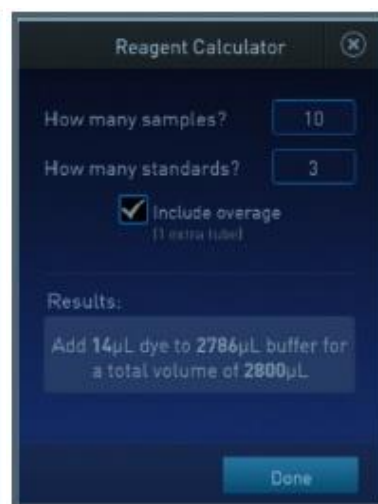


Figure 1c

3.4. Dispense 190µL Qubit™ working solution into tubes for Standard #1 and Standard #2.

3.5. Dispense 180–199µL Qubit™ working solution into tubes for the samples.

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3.6. Add 10 μ L of each QubitTM standard to the appropriate tube.

Note: Accurate pipetting is critical to ensure that exactly 10 μ L of each QubitTM dsDNA BR standard is added to 190 μ L of QubitTM working solution. Proper calibration of the QubitTM Fluorometer requires that the standards be introduced to the instrument in the right order.

3.7. Add 1–20 μ L of each user sample to the appropriate tube.

3.8. Mix the standards and samples vigorously by vortexing for 3–5 seconds and pulse spin to remove bubbles.

3.9. Allow all tubes to incubate at room temperature for a minimum of 2 minutes.

4. Setting up the QubitTM ssDNA assay

4.1. Calculate the volume of QubitTM ssDNA working solution that will be needed to accommodate all standards and samples. The final volume in each tube must be 200 μ L. Each standard tube requires 190 μ L of QubitTM working solution and each sample tube requires anywhere from 180–199 μ L. The reagent calculator in **Section 3.3** can be used to determine the amount of QubitTM working solution required.

4.2. Set up the number of 0.5mL tubes needed for all of the test samples plus a tube for each of the standards. For best results use clear, thin-walled QubitTM assay tubes. Label the lid of each assay tube (do not write on the side wall).

4.3. On the Home screen, select onboard Reagent Calculator (Figures 1a,b,c) to determine the correct amount of QubitTM ssDNA dye and buffer required to prepare for the samples and standards. In an appropriate sized tube, aliquot the appropriate volumes and vortex well.

4.4. Dispense 190 μ L QubitTM working solution into tubes for Standard #1 and Standard #2.

4.5. Dispense 180–199 μ L QubitTM working solution into tubes for the samples.

4.6. Add 10 μ L of each QubitTM standard to the appropriate tube.

Note: Accurate pipetting is critical to ensure that exactly 10 μ L of each QubitTM ssDNA standard is added to 190 μ L of QubitTM working solution. Proper calibration of the QubitTM Fluorometer requires that the standards be introduced to the instrument in the correct order.

4.7. Add 1–20 μ L of each user sample to the appropriate tube.

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- 4.8. Mix the standards and samples vigorously by vortexing for 3–5 seconds and pulse spin to remove bubbles.
- 4.9. Allow all tubes to incubate at room temperature for a minimum of 2 minutes.
- 4.10. Signal is stable for 30 minutes.

5. Using the Qubit™ Fluorometer

Important considerations: The outside of tubes should be dry before loading in Qubit™ chamber (inaccurate concentration will result if the chamber becomes wet). This assay is extremely temperature sensitive and must be performed at room temperature; therefore do not chill the tubes and avoid warming the samples by handling tubes near the top. The Qubit™ Fluorometer can be as much as 3°C higher than room temperature. If repeat measurements are required, allow the sample tube to equilibrate at room temperature for 30 seconds prior to measurement.

Concentrated samples may require multiple dilutions so that at least one measurement lies within the linear range of the assay. Repeat the assay with an appropriate dilution series if the measurements do not fall within the linear range of the assay.

- 5.1. Log onto the instrument computer. Double click or open the Qubit™4 Data icon. If there is no response, unplug and replug the microUSB cable at the rear of the Qubit instrument.
- 5.2. Use the touch screen to wake the Qubit™ instrument to the **Home Screen** (Figure 2a). Choose **dsDNA** Assay and then select the specific dsDNA assay (Figure 2b).

For ssDNA Assay, choose **Oligo** from the **Home Screen** (Figure 2a).

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Figure 2a

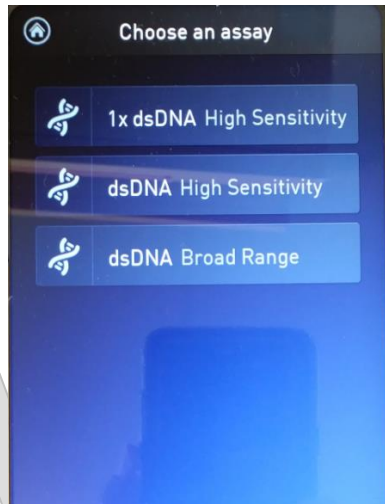
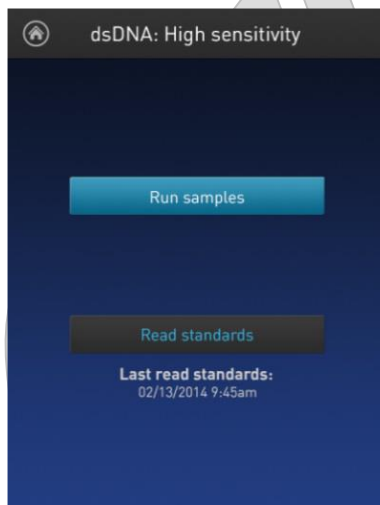


Figure 2b

5.3. Press **Read standards** (Figure 3a). Then follow the prompts for reading Standards #1 and #2 (Figure 3b).



Figures 3a

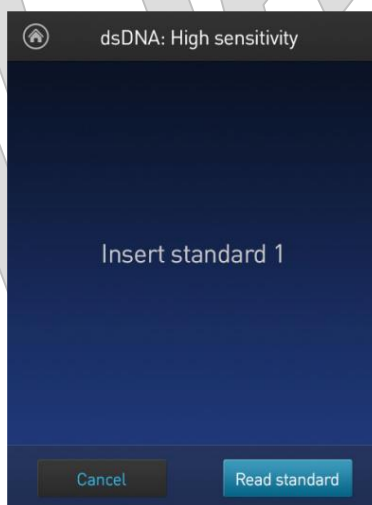


Figure 3b

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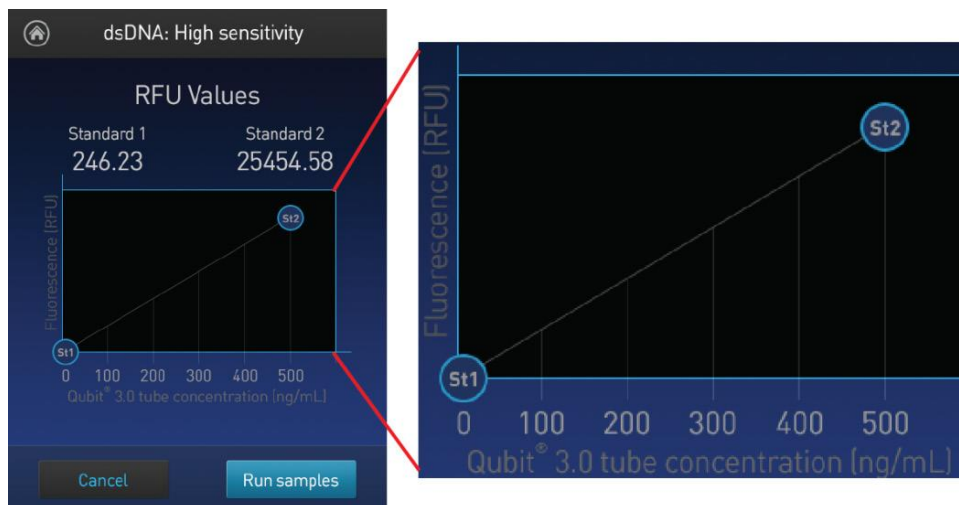


Figure 3c

A graph will be plotted for the Standards and subsequently for the samples (Figure 3c).

- 5.4. Wait 30 seconds and re-read Standard #2. The Qubit™ tube concentration is 500ng/mL for the Qubit™ dsDNA HS (acceptable range is from 475ng/mL to 510ng/mL). The dilution reading on the exported data table of 1 in 200 should read 100ng/μL (acceptable range is 95ng/μL to 102ng/μL). Alternatively, 10 in 200 should read 10ng/μL.

For the dsDNA BR Qubit™, the Standard #2 should read 5μg/mL (acceptable range from 4.75μg/mL to 5.1μg/mL).

For the ssDNA Qubit™, the Standard #2 should read 200ng/μL (acceptable range from 190ng/μL to 210ng/μL).

- 5.5. Insert a sample tube into the Qubit™ Fluorometer, close the lid.
- 5.6. Enter the original sample volume used in the Qubit™ working solution (e.g. **1 μL**). Select the volume by pressing the + or – buttons on the wheel or anywhere on the wheel itself (Figure 4a).
- 5.7. Select the Output sample units (e.g. **ng/μL**) from the dropdown menu (Figure 4a and b), and press **Read tube**.

NOTE: Wait at least 30 seconds for any subsequent re-reading of samples to allow the temperature within sample assay tube to reach back down to ambient temperature.

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Figure 4a




Figure 4b

5.8. Swipe left or press the **Right facing caret** to view the Fluorescence versus Concentration graph (Figure 5).



Figure 5

5.9. For more in depth view of results, press **Data**, and then select the Data point of interest. To continue with data reading, press the arrow icon  located near top left corner of the screen.

5.10. Remove the current sample and insert a new sample. To change sample volume or unit output, swipe right or press the **Left facing caret**. Select appropriate values as described in step 5.6 and 5.7, then press **Read tube**.

NOTE: When one encounters samples out of reading range of too **high** (for example, **>600ng/mL**), make new dilutions of sample for subsequent reading. Occasionally, one

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will encounter samples out of reading of too **low** (<**0.50ng/mL**), for example, the negative controls.

5.11. Standard #2 can be re-measured after all the samples have been read to ensure output measurement is still within acceptable range. Refer to 5.4.

If the re-measuring of Standard #2 is going to be read soon after its initial reading, wait at least 30 seconds prior to the second reading.

6. Exporting Qubit™ Data

6.1. On the **Concentration, Graph, RNA IQ** results or **Home** screen, press **Data**. On the **Export data** screen, check the **selection box** to the left of each data set you wish to export (Figure 6).



Figure 6

6.2. To save only individual data entries from a data set, press the **data set of interest** and then check the **selection box** to the left of the samples you wish to export (Figure 7).

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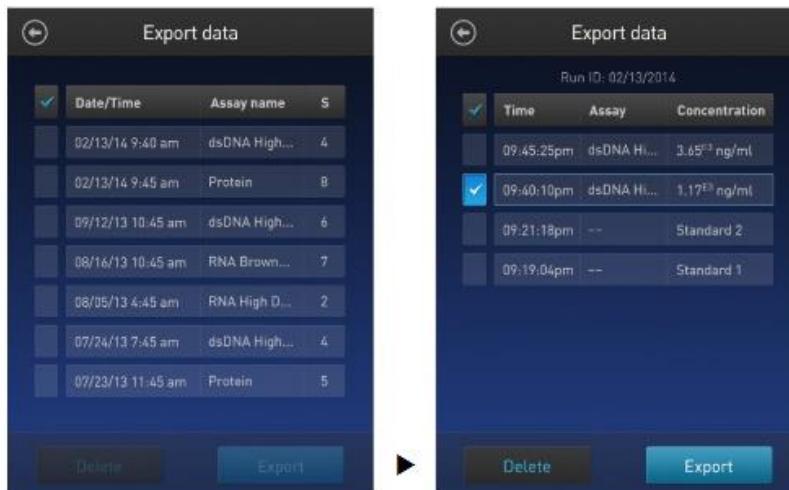


Figure 7

6.3. Press **Export** to export the data. The numeric data is automatically saved as a CSV file. Press **Done**.

6.4. On the desktop, double click the **Qubit4 - Data icon** to open the Qubit4-Data Internal Storage Drive. Open **Qubit4 File folder**. Select and open the most current **QubitData file folder**. Select and open the most current **QubitData - date.csv** file. Save and rename the raw date file as an Excel Workbook in the following format: **Q** followed by the date and the operator's initials in **R:\LibraryCore\QC\Qubit**, for example Q180807dm.

NOTE: At some point, the data folders on the Qubit4 internal drive should be deleted to allow more storage space.

6.5. Open the Excel file and copy the raw data onto new Excel sheet. Edit the file, as needed. For example, re-order the data by sorting the date via **Test Name** or **Test Date**. Save again.

6.6. When finished, discard tubes and other SYBR waste accordingly.

NOTE: Tubes can be stored in the dark temporarily while calculating the concentrations of all samples and re-assayed up to 3 hours later.

6.7. Press the **Home** icon on the Qubit™ 4 Fluorometer. It will go into auto-sleep mode. Log off the computer.

6.8. If one has tested samples for others in the group, notify them that their data can be found in the spreadsheet you have posted.