Non Controlled Version

**Note: Controlled Versions of this document are subjected to change without notice*

Quantifying DNA samples using the Qubit Fluorometer

I. Purpose

To provide specific guidelines for quantifying DNA samples using the Qubit fluorometer in conjunction with the Qubit dsDNA High Sensitivity (HS) Assay Kit or Broad Range (BR) 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 QA 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 Assurance team to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
Qubit Fluorometer Instruction Manual	IM.0194

VI. Related Documents

Document Title	Document Number
N/A	N/A

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 material safety data sheet (MSDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number:	Model #	Catalogue #
Medicom Safetouch Nitrile gloves-large	Ultident	1137-D		✓
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	Fisher Scientific	05-090-100	✓	
Vortex Mixer	Labnet	S0200	✓	
Eppendorf Safe-Lock Tubes, 2ml	VWR	054027		✓
Falcon 2096 conical tubes, 15 ml	VWR	CA21008-918		✓
Qubit dsDNA HS Assay Kit 500 assays	Invitrogen	Q32854		✓
Qubit fluorometer	Invitrogen	Q32857		✓
Qubit assay tubes (500)	Invitrogen	Q32856		✓
Black ink permanent marker pen	VWR	52877-310		✓
Diamond Filter tips DF1000 (10 tip packs of 96 racked filter tips)	Mandel Scientific	GF-F171703		✓
Diamond Filter tips DFL10	Mandel	GF-F171203		✓
Diamond Filter tips DF200 (10 tip packs of 96 racked filter tips)	Mandel Scientific	GF-F171503		✓
Qubit dsDNA BR Assay Kit 500	Invitrogen	Q32853		✓

IX. Qubit assay guide

Qubit assay kit	Assay Range	Sample starting concentration
dsDNA Broad Range Assay (BR)	2 - 1000ng	100 pg/µL - 1 µg/µL
dsDNA High Sensitivity Assay (HS)*	0.2 - 70 ng	10 pg/µL - 70 ng/µL

*** The assay range of the High Sensitivity Assay has been modified for internal use due to indications that the upper limit is unreliable (Figure A1).**

X. Procedure

At Qubit workstation:

1. Retrieval of reagents and equipment preparation

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

1.2. Retrieve Standard #1 and Standard #2 from 4°C fridge and allow the standards to come to room temperature at the Qubit station.

1.3. Retrieve DNA samples and equilibrate to room temperature.

2. Setting up the assay

2.1. Set up the number of 0.5 mL tubes you will need 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).

2.2. In an Eppendorf tube or a Falcon tube, prepare the Qubit working solution by diluting 1µL of Qubit dsDNA HS reagent into 199µL Qubit dsDNA HS Buffer or BR reagent to create a 1:200 solution. Adjust the HS reagent and HS buffer volume according to the number of samples being quanted. Allow 200 µL of working solution for each test sample plus 400 µL (total) for the standards.

2.3. To prepare the standards, mix in a Qubit assay tube 190µL of Qubit working solution with 10 µL of standard. Vortex 2-3 seconds and pulse spin to remove bubbles.

Note: Careful pipetting is critical to ensure that exactly 10µL of each Qubit dsDNA HS or BR 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 right order.

2.4. To prepare the test samples, determine the volume of sample to be used in the assay (Generally, 1 µL of sample is required. Samples that are expected to be more dilute might need 2-5µL. If in doubt, discuss with supervisor). For each assay, pipet a volume of Qubit working solution into the assay tube that will result in a final volume of 200µL after the sample is added. For example, a sample volume of 5 µL requires 195 µL of Qubit working solution. After sample and Qubit working solution are combined, mix by vortexing 2-3 seconds. Pulse spin to remove bubbles.

2.5. Allow all tubes to incubate at room temperature for 2 minutes.

3. 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. Allow the sample tube to equilibrate at room temperature for 30 seconds prior to measurement if repeat measurements are required.

Concentrated samples will require multiple dilutions, minimum 3, 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.

3.1. Log onto the "Agilent" computer.

3.2. Open the Qubit Data Logger Program. Make sure the "Port Open" screen at the bottom left corner of the spreadsheet is green.

3.3. In the far left column, enter the names of the samples to be quantified in the same order as they will be tested.

3.4. Turn on the Qubit machine, press **Home** and key up or down to highlight Qubit dsDNA HS Assay or the Quant-iT dsDNA BR assay. Press **Go** to initiate the assay.

3.5. On the calibration screen highlight **Run new calibration**.

3.6. Insert the tube containing Standard #1 in the Qubit fluorometer, close the lid and press GO. Remove Standard #1.

3.7. Insert the tube containing Standard #2 in the Qubit fluorometer, close the lid, and press GO. Press GO again. The sample should read 500ng/µL if the Qubit dsDNA HS assay was run, and 5ng/µL if the Qubit dsDNA BR assay was run (+/- 10% allowed). Remove Standard#2.

3.8. Insert a sample tube into the Qubit fluorometer, close the lid and press GO. The concentration is given in ng/mL. This is recorded on the Qubit Logger. To calculate the actual concentration of your sample on the Qubit, choose the calculate concentration option on the instrument, enter in the volume of sample used and the concentration (µg/mL) will be recorded on the Logger.

3.9. Test remaining samples. Requant Standard 2 after all the samples to ensure it is 500ng/uL for the Qubit dsDNA HS and 5ng/µL for the Qubit dsDNA BR (+/- 10% allowed).

3.10. Calculate the final concentration for all samples as described in Step 3.8. Copy all data into a new excel spreadsheet and save it in **R:\LibraryCore \QC\Qubit\.** The file name must be in the following format**:** Q, followed by the date, followed by your initials.

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

3.12. Discard tubes when finished. Use up or down key to return to the **Home** position on the Qubit. Select power off. Log off the Qubit Logger. Log off the computer.

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

Appendix

Linear Range of the Qubit HS DNA assay

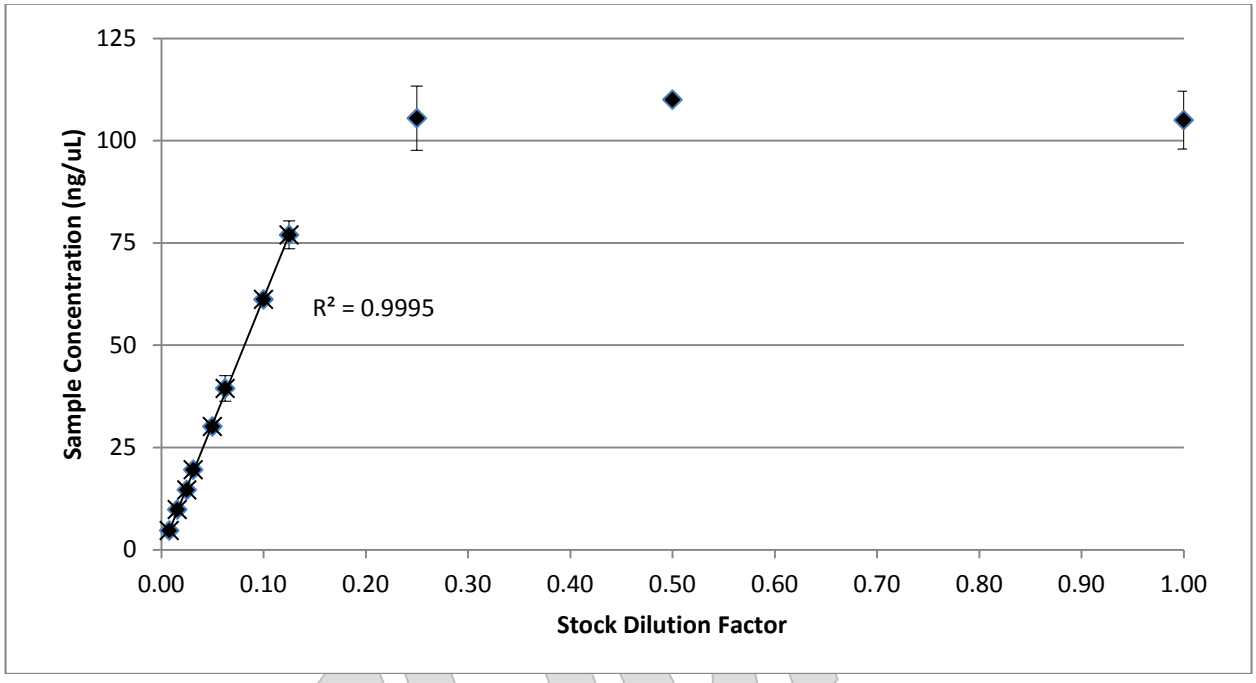

Figure A1. The linear range of the HSDNA assay does not extend to 100ng/uL. Intact gDNA was quantified after 2, 5 and 10 minutes after vortexing (average reported here). Repeat the assay using more than one dilution if the sample concentration is reported as 70ng/uL or greater. Bars are 1 SD.