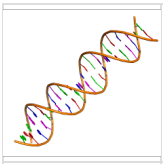

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DNA Quantification using the Qubit Fluorometer

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1 Works for me dx.doi.org/10.17504/protocols.io.bi8dkhs6

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ABSTRACT

This procedure outlines the protocol for quantitation of gDNA for subsequent WGS.

This document applies to all laboratory personnel in the Division of Microbiology (DM) as well as laboratories in the GenomeTrakr Network.

Complete in order:

1. DNA Extraction (Manual DNA Extraction *or* Automated DNA Extraction using the Qiacube)
- Step-by-step procedures to obtain high quality DNA from isolates in TSB for whole genome sequencing

2. DNA Quantitation (included SOP)
- Quantitation of extracted DNA using the Qubit Flourometer

3. Library Preparation for WGS (Library preparation using Illumina DNA Prep *or* Library Preparation using Illumina Nextera XT)
- Library preparation using NexteraXT *or* Illumina DNA Prep (previously Nextera DNA Flex)

4. Sequencing using Illumina MiSeq

5. Data Quality Checks and NCBI Submission

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PROTOCOL CITATION

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KEYWORDS

DNA Quantitation, Qubit dsDNA, Whole Genome Sequencing, GenomeTrakr

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GUIDELINES

Abbreviations:
BR: Broad Range
DM: Division of Microbiology
HS: High Sensitivity
PI: Principal Investigator
ST1: Standard 1
ST2: Standard 2

MATERIALS

NAME	CATALOG #	VENDOR
Qubit dsDNA HS Assay Kit	Q32851	Thermo Fisher Scientific
Qubit assay tubes	Q32856	Thermo Fisher Scientific
Qubit™ dsDNA BR Assay Kit	Q32853	Thermo Fisher

MATERIALS TEXT

Supplies:
- Pipette tips, sterile, filtered (assorted volumes)
- 5ml round-bottom polystyrene tubes (Fisher Sci Cat# 14-959-2A or equivalent)

Equipment:
- Qubit 3.0 or 4.0 Fluorometer
- Vortex
- Micropipettes, various volumes
- Microcentrifuge

SAFETY WARNINGS

Take proper precautions and wear appropriate PPE when handling potentially hazardous chemicals. Ensure that chemicals, spent containers, and unused contents are disposed of in accordance with governmental safety standards.

Qubit dsDNA High Sensitivity and Broad Range Reagent Kit: See ThermoFisher Scientifics SDSs for additional information

• **HS/BR Reagent 200x in DMSO**: GHS Category 4 for flammability, no data is available on the mutagenicity or toxicity of the reagent, it is known to bind nucleic acid so the same safety precautions should be used as when handling other potential mutagens.

• **HS/BR Buffer**: No GHS classification but may cause irritation with susceptible persons.

BEFORE STARTING

The buffer and reagent should be stored at room temperature while the standards must be stored at 4oC. The reagent is light and humidity sensitive, it should be stored in the dark and kept in the Ziploc bag with the desiccate pack. When stored as directed, kits are stable for 6 months.

Qubit Working Solution and Sample preparation

1 Set up the required number of Qubit tubes for standards and samples. The Qubit dsDNA assays require two standards for calibration. Although, calibrations do not have to be performed each time it is recommended to perform prior to quantifying samples with critical input values.

2 Label the tube lids. (Do not label the side of the tube as this could interfere with the sample reading), label tubes for standards as S1 and S2.

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3 Prepare the Qubit working solution by diluting the Reagent in a 1:200 ratio in Buffer. It is recommended to add an additional sample for overage.

	Total Volume per Sample	10 Samples	20 Samples
Reagent	1 µl	10 µl	20 µl
Buffer	199 µl	1,990 µl	3,980 µl

4 Add 190 µl of working solution to each of the standard tubes.

5 Add 10 µl of each standard to the appropriate tube, then mix by vortexing.

6 Add 198 µl of working solution to each sample tube.

7 Add 2 µl of each sample to the appropriate tube, then mix by vortexing. The final volume should be 200 µl.

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

9 Proceed to Reading Standards and Samples for the appropriate instrument.

Reading Standards and Samples with the Qubit 3.0 Fluorometer

10 On the home screen of the Qubit, press **dsDNA**, then select **dsDNA: High Sensitivity** or **dsDNA: Broad Range** depending on the kit being used.

11 If calibration is being performed, press **Read Standards** to proceed. (If a calibration is not necessary, you can omit the following steps)

11.1 Insert the tube containing Standard #1 into the sample chamber, close the lid, and press **Read Standard**. When reading is complete, remove the tube.

Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read**

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11.2 **Standard**. When reading is complete, remove the tube.

11.3 The instrument displays the results of the calibration, for further information on interpretation of the results, refer to the Qubit 3.0 Fluorometer User Guide.

12 Press **Read Samples** to proceed

13 In the **Sample Volume** screen, select the volume of sample added to the tube and the output concentration units (i.e. ng/µl)

14 Insert the first sample tube into the sample chamber, close the lid, and then press **Read Tube**.

15 The software displays the results of the sample. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration. Record the concentration of the original sample, remove the tube, and repeat readings and results recording for each additional sample.

Reading Standards and Samples with the Qubit 4.0 Fluorometer

16 The Qubit 4.0 has an on-board Reagent calculator that can be used to quickly determine the correct amount of reagent and buffer required to prepare the working solution.

16.1 On the **Home** screen, press **Reagent Calculato**r

16.2 Enter the total number of samples and standards that you will be running.

*Optional: Select **Include overage** if you want to include an additional tube in the total calculated volume.*

16.3 Press **Done** to return to the **Home** screen to run your Qubit Assay

17 On the **Home** screen, select **dsDNA**, then **dsDNA: High Sensitivity** or **dsDNA:Broad Range** depending on the kit being used.

18 If calibration is being performed, press **Read Standards** to proceed. (If a calibration is not necessary, you can omit the following steps)

18.1 Insert the tube containing Standard #1 into the sample chamber, close the lid, and press **Read Standard**. When reading is complete, remove the tube.

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18.2 Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read Standard**. When reading is complete, remove the tube.

18.3 The instrument displays the results of the calibration, for further information on interpretation of the results, refer to the Qubit 4.0 Fluorometer User Guide.

19 Press **Read Samples** to proceed.

20 In the **Sample Volume** screen, select the volume of sample added to the tube and the output concentration units (i.e. ng/µl)

21 Insert the first sample tube into the sample chamber, close the lid, and then press **Read Tube**.

22 The software displays the results of the sample. The top value (in large font) is the concentration of the original sample. The bottom value is the dilution concentration. Record the concentration of the original sample, remove the tube and repeat readings and results recording for each additional sample.

Reference Documents

23 Qubit 3.0 Fluorometer User Guide

Qubit 4.0 Fluorometer User Guide

Quick Reference Qubit Assays

Qubit dsDNA HS Assay Guide

Qubit dsDNA BR Assay Guide

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