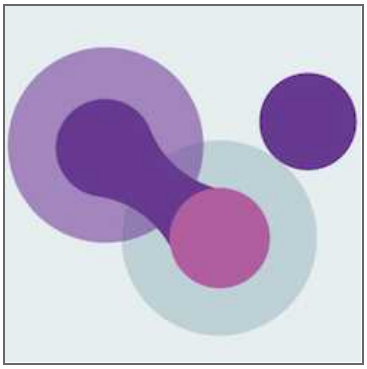

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Protocol status: Working
We use this protocol and it's working

Created: Nov 06, 2023

C-SOP-201: Genomic DNA Quantification using a Qubit Fluorometer

Forked from C-SOP-201: Genomic DNA Quantification using a Qubit Fluorometer

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DISCLAIMER

This protocol has been adapted from dsDNA quantification assays developed by ThermoFisher Scientific.

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ABSTRACT

In order to prepare high quality double-stranded DNA libraries for Illumina WGS, extracted genomic DNA needs to be accurately quantified. Qubit Fluorometers detect fluorescent dyes that are specific to the target of interest. These fluorescent dyes emit only when bound to specific target molecules, even at low concentrations. This provides obvious advantages over UV absorbance quantitation, which measures anything absorbing at 260 nm — DNA, RNA, protein, free nucleotides, or excess salts. Moreover, UV spectrophotometry often does not have the sensitivity to accurately measure low concentrations of DNA and RNA. The Qubit dsDNA broad-range (BR) and high-sensitivity (HS) assays are highly selective for double-stranded DNA (dsDNA) over RNA and single-stranded DNA (ssDNA).

This protocol has been adapted from dsDNA quantification assays developed by ThermoFisher Scientific.

Variations of this method include quantification assays from Promega:
DNA quantification using the Quantus fluorometer

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GUIDELINES

Storage (Qubit BR/HR kit):
When not in use, the buffer and BR/HS reagent (dye) should be stored at Room temperature while the standards must be stored at 4 °C.
The reagent is light and humidity sensitive, it should be stored in the dark and exposed minimally during assay and sample preparation. When stored as directed, the reagent has a shelf-life of about 6 months.

MATERIALS

1. Qubit Assay Tubes (ThermoFisher Scientific, Cat no. Q32856)

2. Qubit dsDNA BR (Broad Range) or High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, Cat no. Q32853 or Q32854)

3. Single or multi-channel pipettes (P2, P10, P200 and P1000) and compatible sterile, filtered tips

4. 5.0 or 15.0 ml Falcon tubes (BD Falcon, 5 ml: Cat no. 352196, 15 ml: Cat no. 352070 or equivalent)

5. Qubit 2.0/3.0/4.0/Flex Fluorometer
(Different versions of the Qubit fluorometer may be used - check that the kit is compatible with your version. Set up instructions may differ slightly)

6. Vortex

7. 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 local safety standards.

Qubit dsDNA Broad Range (BR) Reagent Kit: Refer to ThermoFisher Scientific's SDSs for additional information.

• 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.

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

Before starting

1 The buffer and BR/HS reagent (dye) should be stored at Room temperature while the standards must be stored at 4 °C.

The reagent is light and humidity sensitive, it should be stored in the dark and exposed minimally during assay and sample preparation. When stored as directed, the reagent has a shelf-life of about 6 months.

2 Prior to initiating the protocol, ensure that all active workbenches are cleaned with 80% ethanol, all relevant personal protective clothing is worn and the work area is prepared for DNA quantification according to local GLP guidelines.

Create an organised bench space by clearing away all clutter in order to maximize work efficiency.

Qubit Mastermix and Standard/Sample Preparation

3 Set up the required number of Qubit tubes for standards and samples.

> **Note**
>
> i. The Qubit dsDNA assays require two standards (#1 and #2) for calibration.
>
> ii. If you have already performed a calibration on the Qubit machine for the selected assay you can use the previous calibration stored on the machine. It is recommended to perform a new calibration for every sample batch but a same-day calibration would be fine to use for multiple batches.

4 Label the sample assay tube lids with sample IDs and the standard tubes lids as S1 and S2.

> **Note**
>
> i. Use only thin-wall, clear, 0.5mL PCR tubes. The recommended consumables are listed under Materials (Cat. No. Q32856).
>
> ii. Do not label the side of the tube as this could interfere with the sample reading

5 Prepare a Qubit Mastermix for each sample and standard by diluting the Reagent in a 1:200 ratio with Buffer.

Per sample:

Qubit® dsDNA BR/HS Reagent: 1 µL
Qubit® dsDNA BR/HS Buffer: 199 µL

To avoid pipetting errors, it is recommended to add some overage as indicated below in Table 1.

	A	B	C	D	E	F
		Volume per sample (µL)	For 10 samples (x 11)	For 20 samples (x 21)	For 30 samples (x 31)	For N samples
	Qubit Reagent	1	11	21	31	N+1
	Qubit Buffer	199	2189	4179	6169	199 x (N+1)

Table 1

Note

To avoid any cross-contamination, it is recommended that the total amount of working solution required for samples and standards is removed from the working solution bottle and then added to the mastermix container instead of pipetting directly from the stock bottle to each tube.

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Aliquot the appropriate volume of Qubit Mastermix into each tube:

- Standard tubes: 190 µL
- Sample tubes 198 µL

Next, to the appropriate tube:

- Add 10 µL of standards (S1 and S2).
- Add 2 µL of each sample.

The final volume in each tube must be 200 µL once the samples/standards are added.

Note

i. The Qubit dsDNA assays require 2 standards for every round of calibration.

ii. For samples that are suspected to have low yields of DNA, the sample tube volume can be decreased to anywhere between 180-198µL in order to supplement the final volume with a greater quantity of input DNA.

iii. When adding 1–2µL of sample input, use a P-2 pipette for best results.

7 Cap each tube and then mix vigorously by vortexing for 00:00:05. 2m 5s

Allow all tubes to incubate at Room temperature for 00:02:00.

Reading Standards and Samples with the Qubit 4.0

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

9 **INSTRUMENT CALIBRATION:**
If calibration is being performed, press **Read Standards** to proceed. (If a calibration is not necessary, you can omit steps 8.1 to 8.3).

9.1 Insert the tube containing Standard #1 into the sample chamber, close the lid, and press **Read Standard**. When reading is complete, remove the tube and place back in the rack.

9.2 Insert the tube containing Standard #2 into the sample chamber, close the lid, then press **Read Standard**. When reading is complete, remove the tube and place back in the rack.

9.3 The instrument displays the results of the calibration immediately after S1 and S2 have been read. For further information on interpretation of the results, refer to the Qubit 4.0 Fluorometer User Guide.

10 Next, press **Read Samples** to proceed to the assay.

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

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

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Note

i. The software displays the results of each 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 steps 9.1 and 9.2 to record results for each additional sample.

ii. To manage and import assay readings directly from the instrument, refer to the relevant **Fluorometer User Guide** in the next section.

Additional Information & Troubleshooting

12 For more information, refer to the following links depending on the model of instrument and type of assay being used.

Quick Reference Qubit Assays

Qubit dsDNA BR Assay Guide

Qubit dsDNA HS Assay Guide

Qubit 3.0 Fluorometer User Guide

Qubit 4.0 Fluorometer User Guide

Qubit Flex Fluorometer User Guide