SOP

10.3.4 DNA quantification using Qubit

Objectives and scope

This SOP describes the various steps to follow to measure the concentration of double-stranded nucleic acid (dsDNA) after extraction from pure bacterial cultures, using the Qubit™ dsDNA HS Assay Kit (Invitrogen).

This SOP is intended for Mini-Lab laboratory technicians.

Principle

The Qubit Fluorometer measures fluorescent dyes that emit light only when bound to target molecules, in this case double-strand DNA (dsDNA), which represents the genome of bacteria. The dsDNA HS Assay Kit is highly selective for double stranded DNA (dsDNA) over RNA, and common contaminants such as salts, free nucleotides, solvents, detergents, or protein are well tolerated.

Depending on sample volume, the assay is accurate for initial sample concentrations from 5 pg/µL to 120 ng/µL providing an assay range of 0.1–120 ng of DNA.

Note: Qubit™ assays deliver optimal performance when all solutions are at room temperature; temperature fluctuations can influence the accuracy of the assay.
To minimize temperature fluctuations, insert all assay tubes into the Qubit™ Fluorometer only for as much time as it takes for the instrument to measure the fluorescence. Qubit™ Fluorometers can raise the temperature of the assay solution significantly, even over a period of a few minutes. Do not hold the assay tubes in your hand before reading because this warms the solution and results in a different reading.

The HS reagent is light and humidity sensitive, therefore 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.

Safety and environment

- **Wear your PPE** for the duration of the protocol: lab coat, gloves;
- **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 use safety precautions for handling potential mutagens (avoid inhalation and spillage, always wear gloves, wash hands after handling, decontaminate surfaces after)
- **HS/BR Buffer:** No GHS classification but may cause irritation with susceptible persons.
- See ThermoFisher Scientifics SDSs for additional information
- Refer to the document "6.8 Internal waste management", if you have questions about how to handle any waste product.

Sample

- Type of material:
  - Nucleic Acid: Double stranded DNA

Equipment

Common Name	*Associated SOP*
Qubit 3.0	SOP TBD
Vortex	N/A

Commenté [1]: In red are the equipment SOPs to be done (we need to decide on the equipment)

Consumables

*Common Name**	*Storage conditions*
Gloves	*N/A*
Transfer pipettes (0.5-10 µl; 50–200 µl; 100–1000 µl)	*N/A*
5ml round-bottom polystyrene tubes	*N/A*
Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Catalog #Q32851)	*15–25°C*
Qubit assay tubes (Thermo Fisher Scientific Catalog #Q32856)	*N/A*
Pipette filtertips, various sizes	*N/A*

Commenté [2]: I added the pipette tips in the tabel. You are free to change the description of the tips.

Quality Control

No quality control, use of internal standards

Important points before starting

Check the solutions are at room temperature. The previously prepared standards should be kept in the fridge at 4°C.

Preparation of samples and standards

1. The Qubit dsDNA assays require two standards for calibration.
2. Take as many tubes as many samples* and standards to process and label the tube lids. (Do not label the side of the tube as this could interfere with the sample reading).

   ***Note:** always make an excess of 3 more reactions (tot n of samples + 3)

3. Label tubes for standards as S1 and S2.
4. Prepare the Qubit working solution by diluting the Reagent in a 1:200 ratio in Buffer. It is recommended to add an additional sample.

	Volume per sample	**x10 samples**	**x20 samples**
Reagent	1 µl	10 µl	20 µl
Buffer	199 µl	1999 µl	3980 µl
Final volume	200 µl	2000 µl	4000 µl

Commenté [3]: You have to be aware that you have to make an excess of the Qubit reaction mix.
I always make an excess of 3 more reactions.
When you have a shortage of Qubit reaction mix you can make a new batch but you have to test the 2 standards again and not use the standards from the mix before.

5. Add 190 µl of working solution to each of the standard tubes (S1 and S2).
6. Add 10 µl of each standard to the appropriate tube, then mix by vortexing.
7. Add 199 µl of working solution to each sample tube.

8. Add 1 µl of each sample to the appropriate tube, then mix by vortexing. The final volume should be 200 µl.

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

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

Reading Standards and Samples with the Qubit Fluorometer

11. On the Home screen, touch **dsDNA**, then select dsDNA High Sensitivity as the assay type.
12. If a calibration is performed, touch Read standards to proceed. (If a calibration is not necessary, you can skip to step 15)

Calibration

13. Insert the tube containing Standard #1 into the sample chamber, close the lid, and press Read Standard. When reading is complete, remove the tube.
14. Insert the tube containing Standard #2 into the sample chamber, close the lid, then press Read Standard. When reading is complete, remove the tube.
15. The instrument displays the results of the calibration, for further information on interpretation of the results, refer to the Qubit Fluorometer User Guide.

Samples reading

16. Press **Read Samples** to proceed.

On Qubit 3.0.

17. In the Sample Volume screen, select the Sample volume and units.

    a. Touch the + or – buttons on the wheel, or anywhere on the wheel itself, to select the sample volume added to the assay tube (1–20 µL).

    b. From the Unit dropdown menu, select the units for the output sample concentration.

18. Insert the first sample tube into the sample chamber, close the lid, and then press **Read Tube.**
19. 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.
20. Record the concentration of the original sample, remove the tube.
21. Repeat readings and results recording for each additional sample.

On Qubit 4.0

16. Touch **Run samples**

17. On the assay screen, select the Sample volume and units.

    a. Touch the + or – buttons on the wheel, or anywhere on the wheel itself, to select the sample volume added to the assay tube (1–20 µL).

    b. From the Unit dropdown menu, select the units for the output sample concentration.

18. Insert a sample tube into the sample chamber, close the lid, then touch **Read tube.**

19. When the reading is complete (~3 seconds), remove the sample tube. The top value (in large font) is the concentration of the original sample and the bottom value is the dilution concentration.

20. Record the concentration of the original sample, remove the tube.

22. Repeat readings and results recording for each additional sample.

23. Discard tubes and tips in the red benchtop bin.

Commenté [4]: @Marjan The qubit protocol says 1–20 µL per each sample, here i put 1ul as per your excel file. is this correct?

Commenté [5]: that is correct

Commenté [6]: @Marjan which Qubit do you use?

Commenté [7]: We us ethe Qubit 3,0

Commenté [8]: But you can also change the volume of the DNA with the wheel or + and - on the qubit -3

To calculate the ul per sample and the dilutions needed to be done to obtain the appropriate concentrations, and to store your samples, you can use the tool below:

20251118_WorkfileDNA.csv

Commenté [9]: @Marjan, do you thin this would be OK? I have adapted to the Mini-LIMS

Commenté [10]: Yes this was an example to register the sample and the data which belongs to. You can easily calculate the amount of DNA you have to use in the library-prep

CALCULATION OF DNA CONCENTRATION FOR WHOLE GENOME SEQUENCING

For Whole Genome Sequencing, 200 ng high molecular weight genomic DNA per sample is needed.

Related documents

- Qubit dsDNA HS Assay Kit User Guide https://documents.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_dsDNA_HS_Assay_UG.pdf
- Haendiges et al.. DNA Quantification using the Qubit Fluorometer. US FDA. Protocols.io, Aug 2020. dx.doi.org/10.17504/protocols.io.bi8dkhs6
- SOP-10.3.3. DNA Extraction for Sequencing
- SOP Qubit v3.0
- DOC-6.8-DECHINT: 6.8 Internal waste management