DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30329

DNA Quantification with Qubit ds DNA High Sensitivity Kit

Virology, Surveillance and Diagnosis Branch, Genomics and Diagnostics Team (GDT)

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1. Purpose

1.1. The purpose of this procedure is to describe the process of quantifying double stranded DNA using the Invitrogen Qubit dsDNA HS Fluorescence Kit with the Qubit 4 Fluorometer.

2. Definitions

2.1. None

3. Critical Equipment

3.1. Qubit 4 Fluorometer

3.2. Qubit assay tubes, 0.5-mL (Invitrogen 500 tubes, Cat. No. Q32856)

3.3. Vortex

3.4. Pipettes (10 µl, 20 µl, 200 µl, and 1000 µl)

3.4.1. Corresponding aerosol barrier pipette tips

4. Reagents

4.1. Invitrogen Qubit dsDNA High-Sensitivity Assay Kit (Cat # Q32851 or Q32854)

4.1.1. Qubit dsDNA HS Reagent (Component A)

4.1.2. Qubit dsDNA HS Buffer (Component B)

4.1.3. Qubit dsDNA HS Standard #1 (Component C)

4.1.4. Qubit dsDNA HS Standard #2 (Component D)

4.2. Illumina DNA Prep pooled normalized library

5. Safety Precautions

5.1. Adhere to the safety guidelines provided in the Biosafety in Microbiological and Biomedical Laboratories and follow all established site-specific safety procedures, including wearing proper personal protective equipment (PPE).

6. Procedure

6.1. Assay Procedure

6.1.1. Equilibrate assay components to room temperature.

6.1.2. Set up the required number of Qubit tubes for standards and samples. Qubit dsDNA HS Assay requires 2 standards.

6.1.3. Label the tube lids. Labeling on the side could interfere with the sample read.

6.1.4. Make a working solution by diluting Qubit dsDNA HS Reagent 1:200 in Qubit dsDNA HS Buffer.

6.1.5. Prepare 2 tubes for standards, load 190 µl of diluted working solution into 2 Qubit tubes.

6.1.6. Add 10 µl of each λ DNA standard, (Component C and D), to separate tubes and mix well.

6.1.7. For each sample, load 198 µl of diluted working solution to Qubit tube according to the number of samples to be analyzed.

6.1.8. Add 2 µl of each unknown DNA amplicon or library sample to separate tubes and mix well.

6.1.9. Incubate at room temperature, protected from light, for a minimum of 2 minutes.

6.2. **Read Standards and Samples**

6.2.1. On the Home screen, touch **dsDNA**, then select **dsDNA High Sensitivity** as the assay type.

6.2.2. Touch **Read standards** to proceed.

6.2.3. Insert tube containing Standard #1 into the sample chamber, close the lid, then touch **Read standard**.

6.2.4. When reading is complete, remove Standard #1.

6.2.5. Insert tube containing Standard #2 into the sample chamber, close the lid, then touch **Read standard**.

6.2.6. When reading is complete, remove Standard #2.

6.2.7. Touch **Run samples**

6.2.8. On the assay screen, select Sample volume (**2 µl**) and Unit (**ng/ µl**).

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

6.2.10. When reading is complete remove sample tube. The top value is the concentration of the original input sample.

6.2.11. Repeat steps 8.2.9-8.2.10 until all samples have been read.

6.3. Export Data

6.3.1. Option 1: USB Drive

6.3.1.1. Insert USB drive into the Qubit 4 Fluorometer

6.3.1.2. Touch **Data**

6.3.1.3. On the **Export data** screen, press the **data set of interest**, and check the **selection box** to the left of the samples you want to export.

6.3.1.4. Select CSV and press Export.

6.3.1.5. Transfer the USB drive to the USB drive using the appropriate laboratory computer.

6.3.2. Option 2: Directly to computer vis USB cable

6.3.2.1. Download and install drivers from Qubit Technical Resources at thermofisher.com/qubit

6.3.2.2. Connect the Qubit instrument to the computer using a USB cable.

6.3.2.3. From the computer, access the Qubit 4 Fluorometer as an external device.

6.3.2.4. Access the files saved on the Qubit instrument using the operating system's normal file browsing menus.

6.3.2.5. Copy the desired files to the computer.

7. Related Procedures

7.1. LP-519 Illumina DNA Library Preparation - Manual Procedure

8. References

8.1. Qubit dsDNA HS Assay Kit Pub. No. MAN0002326 Rev C.0 https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FLSG%2Fmanuals%2FQubit_dsDNA_HS_Assay_UG.pdf

8.2. Qubit 4 Fluorometer Pub. No. MAN0017209 Rev D.0 https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017209_Qubit_4_Fluorometer_UG.pdf

8.3. Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition.

9. Attachments

9.1. N/A