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Title of resource
DNA Quality Control Check
Authored by
When using this protocol, the following should be referenced: Samuel Armoo[1], Romain Derelle[2], Daniel Boakye[3], Mike Y. Osei-Atweneboana[1], Charles Brockhouse[4], John Colbourne[2.]. Adapted from NanoDrop spectrophotometer (Thermo Scientific), and Qubit fluorometer (ThermoFisher Scientific) manufacturer's manuals. [1]Council for Scientific and Industrial Research – Water Research Institute, Accra, Ghana. [2]University of Birmingham, Birmingham, UK [3]Noguchi Memorial Institute for Medical Research, Accra, Ghana [4]Creighton University, Omaha, NE, USA
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Description
This protocol is for assessing DNA quality and yield prior to sequencing library preparation. This is a very important step given the low affinity of sequencing adapter ligases when it comes to poor DNA quality. Adapted from NanoDrop spectrophotometer (Thermo Scientific), and Qubit fluorometer (ThermoFisher Scientific) manufacturer's manuals.
Intended use
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Resource history

GN 25 DNA Quality Control Check

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1.0 Introduction

Residual ethanol, or other impurities such as pigments from blackflies can inhibit downstream applications of DNA. Adapter ligation, which is a key step in the sequence library preparation process, is greatly affected by residual ethanol from DNA extraction since the T4 ligase is inhibited by ethanol. Therefore, before library preparation, the quality, as well as quantity of DNA is checked.

2.0 DNA Quality Control Check

The NanoDrop spectrophotometer (Thermo Scientific) was used to check for DNA quality following the steps below:

- Launch NanoDrop software to start application and adjust setting for DNA quantification. Select "Nucleic Acid".
- Clean the top and bottom pedestal with a Kimwipe.
- Blank with 1 uL of the same elution buffer used for DNA extraction.
- Check whether instrument is calibrated properly by applying 1 uL of elution buffer. DNA concentration should be close to zero for a properly quantified spectrophotometer.
- Apply 1uL of DNA on the probe to measure DNA quality.
- As a general rule of thumb, the accepted 260/280 wavelength ratio for DNA should range from 1.8 to 2.0 (Figure 2.1). Any significantly varying values, will be indicative of low-quality DNA with residues from the extraction process.

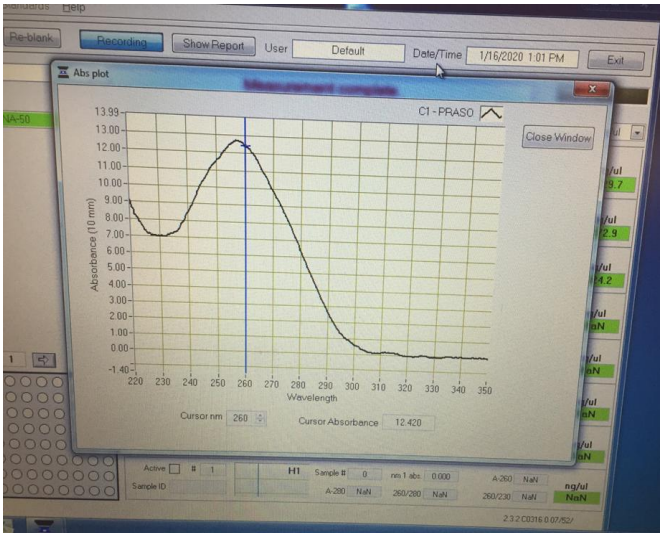

Figure 2.1: Expected 260 wavelength absorbance curve for DNA with acceptability quality.

3.0 Purification of Low-Quality DNA

In cases of low-quality DNA, an Ampure XP beads -based protocol is used for purification. Details below:

- Warm Ampure beads to room temperature for 30 minutes.
- Add nuclease-free water to the DNA to bring the reaction volume to 100 µL. It is important to ensure the final volume is 100 µL, prior to adding AMPure XP Beads.
- Add 100 µL (1X) of resuspended AMPure XP Beads and mix well by pipetting up and down at least 10 times.
- Incubate for 5 minutes at room temperature.
- Quickly spin the tube in a microcentrifuge and place the tube on an appropriate magnetic rack to separate beads from the supernatant. After the solution is clear (about 5 minutes), discard the supernatant that contain impurities.
- Add 200 µl of freshly prepared 80% ethanol to the tube while on the magnetic rack. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant.
- Repeat above-step one more time (making it a total of 2 washes).
- Briefly spin the tube, and put the tube back in the magnetic rack.
- Completely remove the residual ethanol, and air-dry beads for 5 minutes while the tube is on the magnetic rack with the lid open. ***Caution:*** *Do not over dry the beads. This may result in lower recovery of DNA target.*
- Remove the tube from the rack, and then elute DNA from the beads with 52 µL of elution buffer. Mix well by pipetting up and down, and incubate for 2 minutes at room temperature.
- Put the tube in the magnetic rack until the solution is clear.
- Transfer 50 µL of supernatant to a clean PCR tube, and discard beads.

4.0 Quantification of DNA

DNA that pass the quality control check are quantified using the Qubit fluorometer (ThermoFisher Scientific), prior to library preparation. This is to ensure that the recommended amount of DNA is used for library preparation for optimal sequence output. Qubit quantification was done as follows:

- Prepare a (1/200) Qubit dye-buffer solution. E.g. 4µL dye to 796 µL of buffer.
- To calibrate the fluorometer, 5 µL of standards 1 and 2 where added to 95 µL of dye-buffer solution in two different and well labelled Qubit assay tubes. Incubate tubes at room temperature for 2 mins.
- T quantify DNA, 1 uL of a DNA sample was transferred into 99 uL of dye-buffer solution. DNA quantity was measured as ng/ uL.