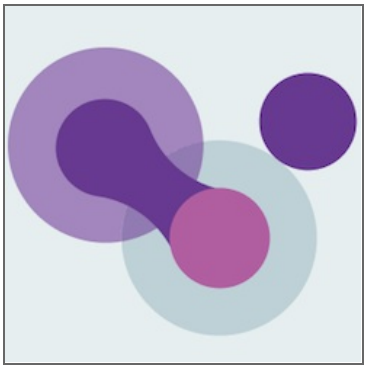

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We use this protocol and it's working

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# C-SOP-401: Quality Control (QC) of DNA Libraries for Whole Genome Sequencing

Forked from C-SOP-401: Quality Control (QC) of DNA Libraries for Whole Genome Sequencing

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## DISCLAIMER

The steps described within this protocol have been adapted from the technology and methods developed by Agilent Technologies and New England BioLabs (NEB).

## ABSTRACT

The success of fragmentation and size selection is best confirmed using an electrophoretic instrument designed for automated and high throughput use, suitable for applications such as NGS. Unlike gel electrophoresis, the output of electropherograms provides qualitative measurements of 1. median fragment size, 2. distributions of fragments sizes within the sample alongside quantitative estimates like sample concentration.

An ideal electropherogram for bacterial whole genome sequencing (WGS) libraries should reveal a single peak/band of desired size with no tailing and excessive broadening. Prior to the sequencing run, all WGS libraries are checked on a fragment analyser (the most popular of which are Agilent's Bioanalyzer and Tapestation instruments). The assays runs on these instruments are capable of verifying if library size distributions are as expected as well as the unwanted presence of any contaminating adapter-dimers or single-stranded material. These can be a major issue with exclusion-amplification clustering chemistry on Illumina platforms.

Alongside electropherograms, it is recommended that libraries be quantified to specifically estimate the amount of double-stranded usable library for equal representation within a pool when loaded onto the sequencer. This can be done using fluorometric dye-based quantitation using either a fluorometer (such as Qubit) or real-time quantitative PCR (qPCR). Between fluorometry and qPCR, only the latter can specifically target the adaptor-ligated amplifiable molecules by using primers complementary to the adaptor sequences. Quantifying only these viable sequencing templates gives you the best chance at normalising libraries accurately downstream.

**Keywords:** library quant, library qc, bioanalyzer 2100, tapestation, qpcr, SYBR, NGS library QC, genome sequencing, D1000 screentape, high sensitivity DNA, NEBNext Library Quant Kit, illumina, E7630

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This protocol describes methods to estimate:
A. library fragment size and concentration using both the Bioanalyzer 2100 and Tapestation 4200 workstations.
B. library and pool concentrations using an adaptor-ligating primer-based qPCR assay.

The steps described have been adapted from the technology and methods developed by Agilent Technologies and New England BioLabs (NEB).



## GUIDELINES

**Storage of Kit Components:**

**1. Agilent D1000 ScreenTape Assay** and **Agilent High Sensitivity DNA Assay:**
See instructions on packaging.

**2. NEBNext Library Quant Kit for Illumina:**

All of the individual components of the NEBNext Library Quant Kit for Illumina are stable at –20°C for two years. When not in use, kit components should be stored at –20°C. All components of the kit, including the combined NEBNext Library Quant Master Mix and Primer Mix, are stable for at least 30 freeze/thaws.

Once the Primer Mix has been added to the NEBNext Library Quant Master Mix (see Protocol on page 6 of manual), the resulting mix can be stored at –20°C for seven months. For short-term storage, this mix may be stored at 4°C for up to six weeks.

Reactions can be prepared at room temperature, but unused portions of the kit reagents should be kept on ice after thawing them for use.

## MATERIALS

1. Adaptor-ligated, size-selected, indexed double-stranded DNA libraries

**Specifications:**

- NGS sheared DNA or libraries: The total DNA in the samples must be between 100 pg/µL to 10 ng/µL.
- PCR samples: The total DNA in the sample must be between 5 – 500 pg/µL.

**2. For Agilent High Sensitivity DNA Assay (Bioanalyzer 2100):**

2a. Agilent High Sensitivity DNA Kit (Agilent, Cat no. 5067-4626)

- For reorder: Agilent High Sensitivity DNA Reagents (Agilent, Cat no. 5067-4627)

2b. Agilent 2100 Bioanalyzer System incl. Chip Priming Station (Agilent, Cat no. 5065-4401) and IKA vortex mixer

2c. Single-channel pipettes (P2, P10, P200 and P1000) with compatible, sterile tips

2d. 0.5 mL low-bind microcentrifuge tubes

2e. Microcentrifuge (with speeds of >13000g)

**3. For Agilent D1000 ScreenTape Assay (Tapestation 4150/4200):**

3a. D1000 ScreenTape (Agilent, Cat no. 5067-5582)

3b. D1000 Reagents (Agilent, Cat no. 5067-5583)

- For reorder: D1000 Sample Buffer (5067-5602), D1000 Ladder (5067-5586)

3c. Loading tips (Agilent, Cat no. 5067-5598, 1pk or 5067-5599, 10pk)

3d. 96-well Plates (Cat no. 5042-8502 or equivalent) and 96-well Plate Foil Seal (Cat no. 5067-5154 or equivalent)

Optical Tube 8x Strip (Cat no. 401428) and Optical Cap 8x Strip (Cat no. 401425)

3e. Vortex mixer IKA MS3 with adapter

3f. Single-channel pipettes (P2, P10, P200 and P1000) with compatible, sterile tips

**4. For New England BioLabs NEBNext Library Quant Assay:**

4a. NEBNext® Library Quant Kit for Illumina® (NEB, Cat no. E7630)

4b. Nuclease-free water

4c. qPCR machine

4d. Compatible qPCR plates and seals

4e. PCR strip tubes or microcentrifuge tubes

4f. Conical centrifuge tubes

## SAFETY WARNINGS

**1. Agilent D1000 ScreenTape Assay**
Kit components contain toxic agents
➔ Refer to product material safety data sheets for further information.
➔ When working with the ScreenTape assay follow the appropriate safety procedures such as wearing goggles, safety gloves and protective clothing.

D1000 ScreenTape Safety Sheet
D1000 Reagents Safety Sheet

**2. Agilent High Sensitivity DNA Assay:**
Kit components contain DMSO.
Because the dye binds to nucleic acids, it should be treated as a potential mutagen and used with appropriate care.
➔ Wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.
➔ Handle solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

High Sensitivity DNA Safety Sheet

**3. NEBNext Library Quant Kit for Illumina:**

Dilution Buffer (10X) Safety Sheet
DNA Standard 1 Safety Sheet
DNA Standard 2 Safety Sheet
DNA Standard 3 Safety Sheet
DNA Standard 4 Safety Sheet
ROX (High) Safety Sheet
ROX (Low) Safety Sheet

## Before Starting

1 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 according to local GLP guidelines for molecular methods.

Create an organised bench space by clearing away all clutter in order to maximize work efficiency and to avoid unnecessary movements that will minimise exposure of sterile materials to airborne and liquid contaminants.

Prepare a large bucket of ice to store reagents and samples temporarily during use.

## Assays

2 Perform the following assays based on the step-wise guides detailed below.

**Fragment analysis (choose one):**
- High Sensitivity DNA Assay using Bioanalyzer 2100
- D1000 ScreenTape Assay using Tapestation 4150 / 4200

**Quantitative PCR of double-stranded sequenceable library molecules:**
Adaptor-ligating primer based qPCR assay (NEBNext® Library Quant Kit for Illumina®)

STEP CASE

### High Sensitivity DNA Assay using Bioanalyzer 2100 1 step

3 Agilent High Sensitivity DNA Kit Quick Start Guide (Run 2-3 replicates per library sample to obtain a robust mean library fragment size)

Agilent High Sensitivity DNA Kit Guide

Agilent 2100 Bioanalyzer System (Maintenance and Troubleshooting Guide)

Agilent 2100 Bioanalyzer (Installation and Safety Guide)

Converting ng/µl to nM when calculating dsDNA library concentration

> **Note**
>
> Ensure that the mean fragment sizes of the replicates do not vary by >5% of each other.

If using Illumina Library Prep protocols, refer to the following Library quantification and quality control quick reference guide