

NGS Library Quality and Quantity Control

Library quantification is one of the crucial steps and should be as accurate as possible. Check the fragment size and concentration of the libraries using a capillary electrophoresis system (e.g. Agilent TapeStation) and quantitative assay (e.g. Qubit dsDNA HS) respectively.

Workflow

Quantity measurement (Qubit)

Measure the concentration of the samples on a Qubit using the Qubit dsDNA HS assay kit. Check if the sample concentration is in the optimal range of the measurements for TapeStation measurement (for example ~1 ng/μl for High Sensitivity D5000).

If the sample is too concentrated, dilute the sample with Elution Buffer (EB) to 1 ng/μl. Make sure to have at least a total volume of 4 μl of the diluted sample. Measure the concentration of the diluted sample on the Qubit to check for pipetting errors. Run the diluted sample on the TapeStation. Please do not discard the dilutions after measurements.

Quality measurement (TapeStation)

Run 2 μl of the (diluted) library on the HS D5000 of the TapeStation for quality measurement. Please consider the following points:

- Keep the sample concentration in the advised range of kit.
- Check if the ladder runs correctly.
- Make sure that the baseline runs flat and that the curve of each sample is in between the lower and upper marker.
- Samples suitable for further sequencing should not contain a lot of high molecular weight contaminants or adapter/primer dimers. If the sample has many additional peaks, consider cleaning the sample once again. A maximum molecular weight of dimers of 10% is allowed.

Determining molarity

The Qubit is more accurate in measuring the DNA concentration compared to TapeStation. In order to take that into account in the molarity estimation, calculate the molarity of the sample using the concentration (ng/μl) obtained with the Qubit measurement and the molarity (nM) obtained by region analysis on the TapeStation:

1. Compare the concentration measured for each sample on Qubit and on TapeStation using region analysis.
If the concentrations differ considerably, it is advisable to re-measure the samples.
2. If the concentrations are in range – calculate a correction factor:
[Qubit] / [TapeStation].
3. Multiply the molarity obtained by the TapeStation region analysis with this correction factor to correct the molarity (nM) of the library.

Preparing master library pool for sequencing

Dilute the library shortly before sequencing to a 4 nM solution using EBT (EB with 0.02% Tween). Measure the diluted library with the Qubit and adjust the molarity for accordingly. Pool multiple libraries (each with different library index) equal molar to achieve similar number of reads per library. Store (master) library pool at -20°C until sequencing, no longer than 1 week.

Please let us know if you need help with the quality assessment, quantification or pooling. We can help or teach you if necessary.

Kind regards,

The Sequencing Facility team

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