

NGS Library Dilution and QC Analysis

CRI Next-Gen Sequencing Core

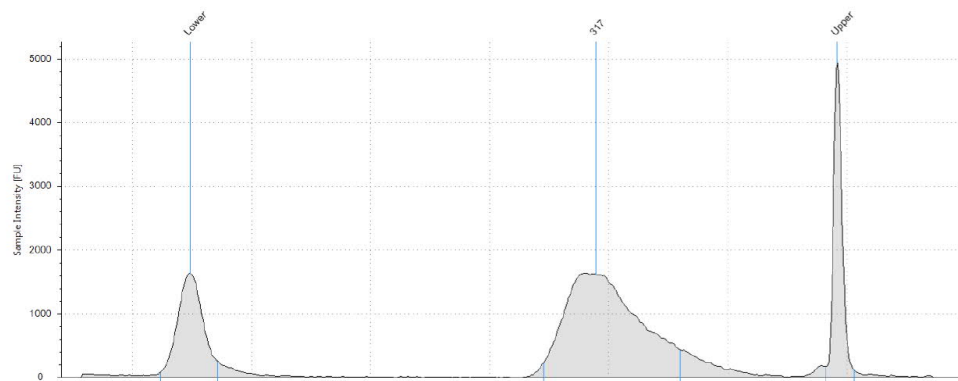
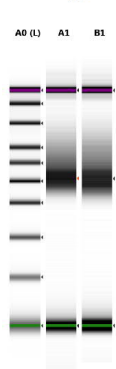
The following procedure is highly recommended to obtain an accurate library quantification and quality check:

1. Take BioAnalyzer (or TapeStation) trace of every library to find average library size. Type in the library size in the QC form “BioAnalyzer Size (bp)”.

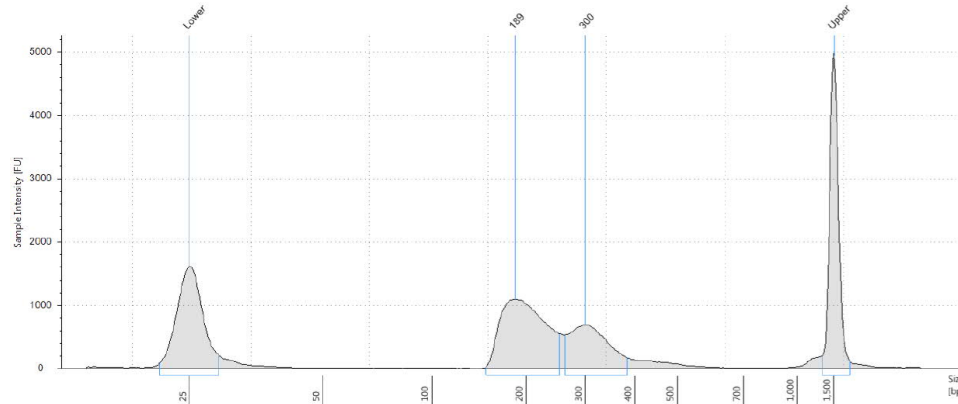
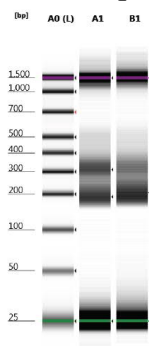
Sample gel images and peak distribution are shown below:

ChIP-seq or RNA-seq

Gel Images



ATAC-seq



2. Quantify each library using Qubit by at least duplicate. Calculate the mean value, and type in the value in QC form “Qubit Conc. (ng/ul)”. The Qubit nM concentration will be automatically calculated in the QC form.
3. Dilute each library to 10nM (or 4nM) based on the calculation in the QC form. The targeted conc. for the 4nM dilution library is also shown on the QC form.
4. Measure the concentration of the diluted 10nM (or 4nM) library by Qubit. Record the actual concentration in the QC form.

*Note: the actual concentration should be similar to the target concentration (e.g. within +/- 10% of the target concentration). **If the actual concentration is significantly higher or lower, please repeat***

steps #2 and #3 (e.g. re-measure the qubit concentration of the original library and re-dilute to 4nM).

5. Pool the diluted libraries to a single tube by pipetting 5ul of each diluted library.
6. Measure the concentration of the pooled 10nM (or 4nM) library by Qubit. The concentration should be close to the average value of all 10nM libraries before dilution. Record the actual concentration of the pooled library in the QC form.
7. Complete the QC form and Sample Drop-off form, and email to YoonJung.Kim@UTSouthwestern.edu and Sihan.Wu@UTSouthwestern.edu.

NGS Library Sample QC Reagents and Information

Qubit™ dsDNA HS Assay Kit

<https://www.thermofisher.com/order/catalog/product/Q32851#/Q32851>

Invitrogen™ Qubit™ Assay Tubes

<https://www.fishersci.com/shop/products/qubit-assay-tubes-set-of-500/Q32856>

TapeStation D1000 ScreenTape (For the analysis of DNA from 35 to 1000 bp)

https://www.chem.agilent.com/store/productDetail.jsp?catalogId=5067-5582&catId=SubCat2ECS_227720

TapeStation D1000 Reagent (ladder and sample buffer)

https://www.chem.agilent.com/store/productDetail.jsp?catalogId=5067-5583&catId=SubCat2ECS_227720

If the library size is larger than 1kb, use the following reagents:

TapeStation D5000 ScreenTape (For the analysis of DNA from 100 to 5000 bp)

https://www.chem.agilent.com/store/productDetail.jsp?catalogId=5067-5588&catId=SubCat2ECS_227720

TapeStation D5000 Reagent (ladder and sample buffer)

https://www.chem.agilent.com/store/productDetail.jsp?catalogId=5067-5589&catId=SubCat2ECS_227720