CALCULATIONS AND CONVERSIONS IN PREPARATION FOR Illumina Miseq RUN

The average size (bp) from the Bioanalyzer is used together with your Qubit quantitation reading to convert your sample concentration reading from the Qubit to the “Illumina concentration” of a sample to be used for your MiSeq run:

1. **Illumina’s Concentration conversion:** the average size of fragments in your library, as indicated by the Bioanalyzer is assigned a concentration conversion value for every 1ng/µl as follows:
	* 300bp  5 nM
	* 350bp  4.375 nM
	* 400bp  3.75 nM
	* 450bp  3.33 nM
	* 500bp  3nM
	* 550bp  2.73nM
	* 600bp  2.5nM
	* 1000bp  1.5nM
2. **Illumina Miseq concentration calculation:**

Multiply the **nM** indicator above for your average size (from the Bioanalyzer) by the Qubit concentration reading (ng/ul) to generate the Illumina converted concentration value for each sample.

1. **Dilution:**

The Illumina protocol for preparing your library for a MiSeq run begins with 5µl of a 4nM library so you need to dilute all samples to be run, to 4nM converted concentration. Thus, divide the Illumina converted concentration by 4 to determine the dilution required to bring your sample concentration to 4nM. \*Recommended: When possible, make your dilution with 2ul of sample and the rest with nuclease free water in order to limit pipetting errors.

**Sample calculation:**

Bioanalyzer average size reading: **533bp**

Qubit reading: **48.6 ng/ul**

1. Concentration conversion: 533bp  550bp  2.73nM
2. Illumina concentration calculation: (2.73nM)(48.6 ng/ul)=145.8nM
3. Dilution: 145.8nM/4nM=36.5

⇒ 1ul Sample + 35.5ul nuclease free ddH2O= 36.5ul total volume

\* Recommended 2ul Sample where possible:

Dilution x 2

⇒ 36.5 x 2 = 73

⇒ 2ul cDNA + 71ul dH2O= 73ul total volume

**For notebook, use table format:**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample # | Sample name | Bioanalyzer average size (bp) | Size approx.(bp) | Illumina Conversion (nM) | Qubit Concentration (ng/ul) | Illumina Concentration calc. (nM) | Dilution to 4nM | Multiply dilution by 2 for accuracy |
| 1 | B.B | 533 | 550 | 2.73per 1ng/µl | 48.6 | 3x48.6=145.8 | 145.8/4 = 36.5 | 2ul Sample+71ul dH2O |
|  |  |  |  |  |  |  | 1µl sample to 35.5µl ddH20 |  |