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:
 - o 300bp → 5 nM
 - o 350bp → 4.375 nM
 - o 400bp → 3.75 nM
 - o 450bp → 3.33 nM
 - o 500bp → 3nM
 - o 550bp **→** 2.73nM
 - o 600bp **→** 2.5nM
 - o 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.

3. 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- <u>Dilution</u>: 145.8nM/4nM=36.5
- ⇒ 1ul Sample + 35.5ul nuclease free ddH20= 36.5ul total volume
- * Recommended 2ul Sample where possible:

Dilution x 2

- \Rightarrow 36.5 x 2 = 73
- ⇒ 2ul cDNA + 71ul dH20= 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 dH20