Illumina DNA Prep Kit Library Preparation Worksheet

For use with the Illumina DNA Prep kit (formerly Nextera Flex kit).

1. Calculate the volume of each sample to use

- Add 2-30µL of DNA to each well of a 96-well PCR plate so that the total input amount for each sample is 10-24ng. Ensure that the same amount of DNA is added for each sample.
- If the volume for any sample is <30µL, add nuclease-free water to DNA to bring the total volume to 30µL.
  - $\text{DNA volume to add } (\mu\text{L}) = \frac{\text{Target DNA amount (ng)}}{\text{DNA conc. } (\text{ng}/\mu\text{L})}$
  - $\text{Water volume to add } (\mu\text{L}) = 30 - \text{DNA volume to add } (\mu\text{L})$
  - $\text{Final amount (ng)} = \text{DNA conc. } (\text{ng}/\mu\text{L}) \times \text{DNA volume added } (\mu\text{L})$

2. Record barcode to use for each sample

Sample ID	Well	Qubit (ng/uL)	Volume to add	Water to add	Final amount	Barcode

3. Record number of PCR cycles used during DNA amplification: _____

4. Record library concentration and fragment size.

- Calculate average fragment size and assess library quality using a TapeStation or BioAnalyzer. Alternatively, assume an average fragment size of ~600 bp.

  Molecular weight [nM] = (Library concentration [ng/µL] * 1,000,000)/ (average library size [bp] x 660).

  Note: Do not pool libraries that are primarily primer dimers. Primer dimers will show up on a BioAnalyzer or TapeStation as shorter fragments, approximately 200bp in length.

- Dilute all libraries to 5nM in nuclease-free water.
- Pool individual libraries in equimolar amounts by adding 5 µL of each 5 nM library. Only pool libraries <5 nM if the library is relatively clear of primer dimers. In this case, add 5 µL without diluting.

Sample ID	Library (ng/uL)	Library (nM)	Volume to add	Water to add

5. Record final pool concentration: _____