**MiSeq Sequencing Submission Guidelines**

MiSeq sequencing is a next-generation sequencing instrument that is one branch of technologies available within the NEB DNA Sequencing Core. All sequencing submissions are to be made after approval from Rich Roberts.

Miseq runs average around **25 million** reads per run and lengths vary from 50-cycles to 600 cycles.

The order in which your samples will be sequenced depends on a variety of factors including: When your samples were first submitted and your place in the queue. If you are submitting multiple samples, please reflect the priority of your samples in the order you place them in your submission box. If applicable, list the individual completion date under the sample name on the bioanalyzer trace. We will do our best to accommodate your deadlines and ask for your patience in return. If you have any questions please contact members of the sequencing core.

**Template Preparation**

* Please run an aliquot of your completed library on the Agilent Bioanalyzer to make sure your sample is free of excess primers, primer dimer, and that the reactions worked. Staple a copy of the bioanalyzer traces to the submission form along with the calculations you made for the dilutions to of your sample(s).
* Samples must be submitted at a concentration of **4nM** in sterile Low TE in 1.5mL LoBind tubes. We may be able to work with lower concentrations if you discuss in advance.

**Template Information**

Sender name: Samantha Fossa

Lab Group: Taron Lab

Extension number: 7328

Email: sfossa@neb.com

Date Submitted: 7.01.2021

Requested Date of Completion: At your earliest convenience, no rush

Project Name: Dixie\_Metagenomic\_Library\_ plexWell384

Sample Name: SF\_seqWell\_Dixie-13-B1-14-B1-Redo11-Redo12\_07022021

Requested read lengths: 50 or 75 or 150 or 300: is it possible to do 250? If not 300

Paired-End or Single Read: Paired-End

Is this a Small RNA library? No

Is your sample Low Diversity? No but seqWell recommends 1% PhiX

Low Diversity means that the ratio of bases G, C, T, and A is not balanced equally.

Is your sample barcoded? Yes

Please indicate which indexes were used: 4 libraries with 96 samples were multiplexed for a total of 384 samples. Each set of 96 has a unique i7 barcode. After pooling a set of 96, each multiplexed library was given an i5 barcode. The library submitted has four different i5 indices used to distinguish each of set of 96 samples.

I have attached in my submission email the sample sheet that contains all of the barcodes for each of the 384 samples. Please email me for further clarification.