**MiSeq Sequencing Submission Guidelines**

Section 1: YOU

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 after a request is made through Laurence Ettwiller.

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. Available slots are first given to the NEBNext group for product development and validation. We have an agreement with them where Research samples are allotted Friday sequencing spots. We ask that you please consider all the factors that go in to sequencing your samples when communicating with your collaborators. We will do our best to accommodate your deadlines and ask for your patience in return. If you have any questions please contact Laurie Mazzola. Thank you, again.

Section 2: Your Template Preparation

Library construction of MiSeq libraries is unique compared to other next-generation technologies. We ask that you read the protocol to have an understanding for the quality of starting material necessary for a successful run. TE buffer must not be used at any stage of the process as mentioned in the protocol and instead recommend Low TE. 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) to 4nM concentration. When diluting your libraries, dilute them in sterile **Low TE** only. The formulation for this buffer is 10mM Tris pH 8 0.1mM EDTA.

*We ask that you use NEBNext kits and reagents when possible and please mention them in your papers, presentations, and posters!!*

***If the DNA Sequencing Core sequences any samples please acknowledge us in your papers and posters by our names: Laurie Mazzola, Joanna Bybee, and Danielle Rivizzigno.***

Section 3: Your Template Information

Please, one project per submission form and one project per box. Upon completion, samples will be placed in the out box in the MiSeq fridge and it is your responsibility to retrieve within one week after completion date or they will be thrown out. Any runs that need to be repeated must be requested as a new submission and will be treated as a new project.

**MiSeq Sequencing Submission Form**

*\*\*All sections must be filled and the bioanalyzer traces attached (2 per page, 1-sided) to this form with your samples clearly labeled to match the correct trace, before template will be accepted\*\**

Sender name: Madalee Wulf

Lab Group: Ivan Correa

Extension number: 8963

Sender email: mwulf@neb.com

Date Submitted: 6/15/2021

Purpose of Sequencing: Assess template switching-based sequencing workflow in miRNA libraries

Requested Date of Completion:

*\*\*We will do our best to accommodate your deadline\*\**

Section 2: Your Template Preparation

Templates need to be at a concentration of 4nM in at least 50uL sterile, Low TE in 1.5mL LoBind Tubes or 0.2mL PCR tubes. If you cannot meet this requirement we will accept lower concentrations of samples but you need to communicate this with us upon or before sample submission. Samples should be in your own sample submission box and placed in the DNA Sequencing Core refrigerator in the red inbox bin and labeled appropriately. Please drop forms off in the appropriate basket over Laurie’s Desk.

*\*\*Templates diluted in anything but water will not sequence correctly\*\**

Section 3: Your Template Information

Project Name: SMARTer seq

Sample Name: MW-VI-450

Sample Insert Size: 25-30

Is this a DNA-Seq library? N

Is this a RNA-Seq library? Y

Is this a Small RNA library? N

Is your sample Low Diversity? Y (50% phi-x please)

Low Diversity means that the ratio of bases G, C, T, and A is not balanced equally. **Please come see us if you don’t know!**

Is your sample barcoded? Y

If you answered yes, please indicate which indexes per sample were used. Balanced indexes are important when pooling your samples, please be aware of this when planning your run.

Index/Samples in attached spreadsheet

1x50

![Chart

Description automatically generated]()