

Protocol for submitting DNA for Illumina sequencing genomes in the background of low-diversity runs

Version: 2019

Summary:

This protocol is for the submission of DNA for the generation of paired-end libraries for sequencing on the Illumina MiSeq platform. DNA is quantified using a fluorometric-based method and diluted to 0.2 ng/ μ L. Libraries are prepared using the [NexteraXT Library prep kit](#), which uses an enzymatic reaction called tagmentation to fragment the DNA and add adapter sequences. After libraries are prepared they are quantified with another low-diversity library and run together on the MiSeq. The “Preparing Libraries for Sequencing on the MiSeq” (part 15039740, Rev. D) protocol was used to prepare libraries with a final load concentration of 5.5 pM, spiked with 85% low diversity library, 13% genomic library and 2% PhiX. FASTQ files are distributed to the client when the 2 x 250 bp sequencing completes.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Eppendorf LoBind tubes	Fisher Scientific	13-698-791

Protocol:

- In a clean hood, add 20 μ L of DNA to each of the LoBind tubes. Submit samples with concentrations between 1 to 5 ng/ μ L. Clearly label tubes with PI, Reference ID, sample number and date.
- Fill out submission [form](#).
- Send a list of the genome names and shipment tracking information to msmblcore@umich.edu.
- Sample submission:
 - Tubes/Plates can be drop off to our lab.** We are located in MSRB1, room 1500. Lab hours: 8am-3:30pm (Monday to Friday). Let us know when we should expect your samples.
 - Tubes/Plates can be shipped on dry ice.** Please fill empty space within the Styrofoam shipping container with packing material to prevent contents from shifting

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during transport. International shipping should be done through a courier service. Please refer to federal regulations regarding the shipping of biological specimens on dry ice. ***Please include reference ID on package documentation.***

Deliver on dry ice to:

Host Microbiome Initiative
University of Michigan Medical School
Internal Medicine/Infectious Diseases
1500 MSRB1
1150 W. Medical Center Drive
Ann Arbor, MI 48109-5666