CENTER FOR MICROBIAL SYSTEMS
UNIVERSITY OF MICHIGAN

External
Protocol for submitting DNA for Illumina sequencing genomes

Version: 1

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 flourometric-based method and diluted to 0.2 ng/µL. 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
Aluminum foil, sterile	VWR	89049-034
Full-skirted PCR plate (twin.tec)	Eppendorf	951020401

Protocol:

1. In a clean hood, add at least 15 µL of DNA to each well of the twin.tec plates. Seal with sterile foil seal. Clearly label plates with PI, Reference ID, and date.

> ***IMPORTANT:*** *Seal plates very well to reduce evaporation and cross contamination between wells.*

2. Use these shipping directions to prepare the PCR plates for shipping.

3. Fill out submission form.

4. Send a list of the genome names and shipment tracking information to msmblcore@umich.edu.

5. Ship on dry ice or with ice packs 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

Please include reference ID on package documentation.