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

Version: 2018

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/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:

<table>
<thead>
<tr>
<th>Reagent/Material</th>
<th>Vendor</th>
<th>Stock Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eppendorf LoBind tubes</td>
<td>Fisher Scientific</td>
<td>13-698-791</td>
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Protocol:

1. In a clean hood, add 20 µL of DNA to each of the LoBind tubes. Submit samples with concentrations between 1 to 5 ng/uL. Clearly label tubes with PI, Reference ID, sample number and date.

2. Fill out submission form.

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

4. 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 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