Protocol for submitting DNA for Illumina sequencing genomes Nextera XT library prep kit

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/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 and the “Preparing Libraries for Sequencing on the MiSeq” (part 15039740, Rev. D) protocol was used to prepare libraries with a final load concentration of 12-20 pM, spiked with 2% PhiX. Clients choose 500-cycle or 600-cycle kit for sequencing and FASTQ files are distributed upon completion.

Reagents and Materials:

<table>
<thead>
<tr>
<th>Reagent/Material</th>
<th>Vendor</th>
<th>Stock Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminum foil, sterile</td>
<td>VWR</td>
<td>89049-034</td>
</tr>
<tr>
<td>Full-skirted PCR plate (twin.tec)</td>
<td>Eppendorf</td>
<td>951020401</td>
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Protocol:

1. In a clean hood, add 20 µL of DNA to each well of the twin.tec plates. Samples should have a concentration between 1 to 5 ng/uL. Seal with sterile foil seal. Clearly label plates with PI, Reference ID, Plate number and date.

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

2. Fill out submission form.

3. Send electronic plate map and shipment tracking information (if apply) 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