Protocol for submitting gDNA for Illumina 16S rRNA gene sequencing, V4 region

Version: 2018

Summary:

This protocol is for the submission of gDNA to generate libraries for 16S rRNA sequencing, which can be used for bacterial community analysis and detect variations in the microbiota under differing conditions.

DNA is aliquoted into 96 well plates, which are properly packaged and shipped to the HMI and libraries are prepared for community analysis as previously described by Seekatz et al. (1). Briefly, barcoded dual-index primers specific to the V4 region of the 16S rRNA gene amplify the DNA (2). PCR reactions are composed of 5 µL of 4 µM equimolar primer set, 0.15 µL of AccuPrime Taq DNA High Fidelity Polymerase, 2 µL of 10x AccuPrime PCR Buffer II (Thermo Fisher Scientific, catalog no. 12346094), 11.85 µL of PCR-grade water, and 1 µL of DNA template. The PCR conditions used consisted of 2 min at 95°C, followed by 30 cycles of 95°C for 20 s, 55°C for 15 s, and 72°C for 5 min, followed by 72°C for 10 min. Each PCR reaction is normalized using the SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, catalog no. A1051001). The normalized reactions are pooled and quantified using the Kapa Biosystems Library qPCR MasterMix (ROX Low) Quantification kit for Illumina platforms (catalog no. KK4873). The Agilent Bioanalyzer is used to confirm the size of the amplicon library (~399 bp) using a high-sensitive DNA analysis kit (catalog no. 5067-4626). Pooled amplicon library is then sequenced on the Illumina MiSeq platform using the 500 cycle MiSeq V2 Reagent kit (catalog no. MS-102-2003) according to the manufacturer's instructions with modifications of the primer set with custom read 1/read 2 and index primers added to the reagent cartridge. 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 15% PhiX to create diversity within the run. 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 20 uL of DNA to each well of the twin.tec plates. Submit samples with concentration between 1-5 ng/uL. DNA should be quantified by fluorescence based detection such as the Qubit or Picogreen from Life Technologies. DO NOT USE NANO DROP. Additional charges might apply if we need to dilute your samples to this concentration. *We can quantify your samples for an additional charge if you can't quantify them.*

2. Reserve at least two wells (H11 and H12) in the full-skirted plate for PCR controls. Maximum number of samples per plate is 94. Use our plate map template to keep track of your samples. 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.*

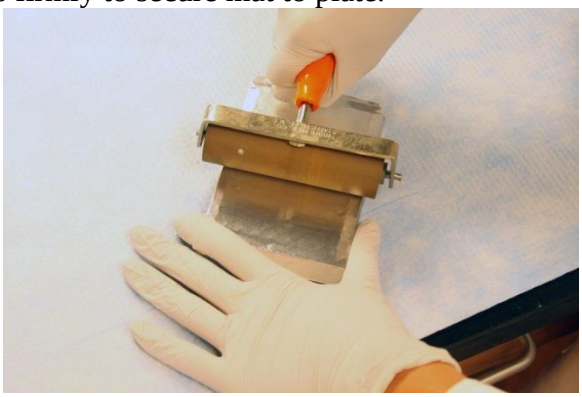
3. Fill out submission form.

4. Send electronic plate map and shipment tracking information to msmblcore@umich.edu.

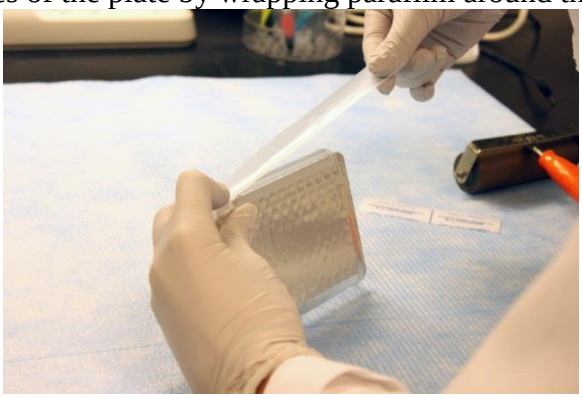
5. Please use the following packing directions to prepare plates for shipping. *If you are bringing samples to the lab you don't need to place the bead plate between cardboards.*

Step 1: Make sure the plates are labeled with PI, Reference ID, Plate number and date.

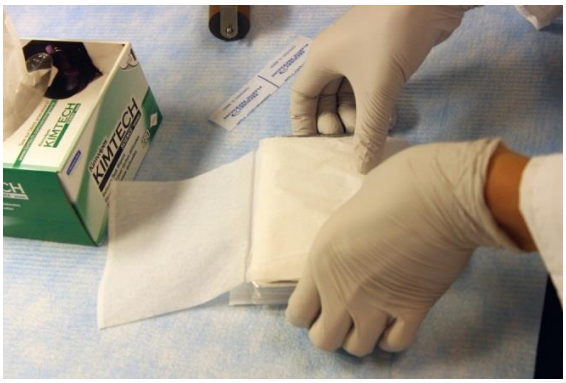
Step 2: Use a roller and press firmly to secure mat to plate.



Step 3: Secure the outer edges of the plate by wrapping parafilm around the perimeter of the plate.



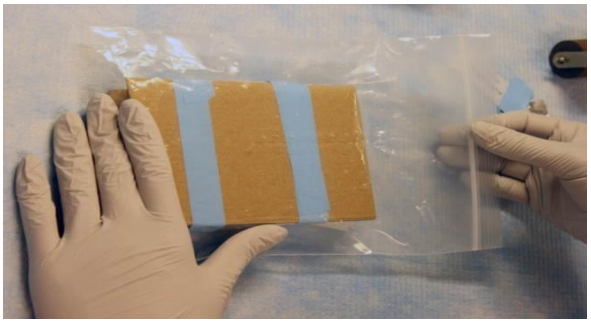
Step 4: Wrap plate with kimwipes to absorb any spillage or condensation from the plate during transport.



Step 5: Cut two pieces of cardboard the approximate size of the plate. Place the plate between the two pieces. This will help prevent damage during transport. Plates must be sandwiched between two pieces of cardboard prior to transport, not doing this may result in damage to the plates or seals.

Step 6: Use tape to secure the plate between the cardboard.

Step 7: Each plate must be secured in its own cardboard sandwich and sealed in individual bags.



6. Sample submission:
    - **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.

    - **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

References:

1. **Seekatz AM, Theriot CM, Molloy CT, Wozniak KL, Bergin IL, Young VB.** 2015. Fecal Microbiota Transplantation Eliminates Clostridium difficile in a Murine Model of Relapsing Disease. Infect Immun **83**:3838-3846. 10.1128/IAI.00459-15.

2. **Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD.** 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol **79**:5112–5120. 10.1128/AEM.01043-13.