



# External Protocol for submitting DNA for Illumina 16S rRNA gene sequencing, V4 region

Version: 1

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## Summary:

This protocol is for the submission of DNA 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  $\mu$ L of 4  $\mu$ M equimolar primer set, 0.15  $\mu$ L of AccuPrime Taq DNA High Fidelity Polymerase, 2  $\mu$ L of 10x AccuPrime PCR Buffer II (Thermo Fisher Scientific, catalog no. 12346094), 11.85  $\mu$ L of PCR-grade water, and 1  $\mu$ L 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 at least 15 uL of DNA to each well of the twin.tec plates. Reserve at least two wells in the full-skirted PCR plate for controls. Maximum number of samples per plate is 94. 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 [electronic plate map](#) and shipment tracking information to [msmblcore@umich.edu](mailto: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.

## 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.