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U Michigan - DNA Extraction for Illumina 16S rRNA Extraction V.2

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Protocol status: Working
We use this protocol and it's working

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Abstract

Summary:

This protocol is for the submission of tissue/fecal samples for DNA extraction and subsequent processing to generate libraries for 16S rRNA sequencing, which can be used for bacterial community analysis and detect variations in the microbiota under differing conditions.

Up to 250 uL or 0.25 g of sample is added to each well of the Bead plate provided by the MMPC. Plates are properly packaged and shipped to the MMPC and processed using the Qiagen MagAttract PowerMicrobiome kit DNA/RNA kit (Qiagen, catalog no. 27500-4-EP) on the EpMotion 5075 (Eppendorf) liquid handler. DNA is lysed using mechanical bead beating and extracted using magnetic bead technology. Extracted DNA is then used to generate 16S rRNA libraries for community analysis. The process used for library generation has been 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 × 250 bp sequencing completes.

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.

Materials

MATERIALS

PowerMag Glass Bead plate **Qiagen**

Reagent/Material	Vendor	Stock Number
PowerMag Glass Bead plate	Qiagen	Contact msmblcore@umich.edu for shipment of bead plates. We will provide you with the dimensions and weight of package containing the bead plates and you provide us with a PDF shipping label with the shipping provider of your choice.

Note:

QIAGEN, RRID:SCR_008539

Troubleshooting

1 Briefly centrifuge the **PowerMag Glass Bead plate** to collect beads at the bottom of the well. In a clean hood, add a maximum of 0.25g or 250 uL of sample to the **PowerMag Glass Bead plate** and seal with mat. Reserve at least three wells in the bead plate for controls. Maximum number of samples per plate is 93. Clearly label plates with PI, Reference ID, and date.

> *IMPORTANT: Always wear appropriate PPE when handing biological samples. Ensure lab coat and gloves are clean to reduce contamination.*

2 Use these **shipping directions** to prepare the Bead plates for shipping (use the same instructions as PCR plates).

3 Fill out submission **form**.

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

5 Ship on dry ice to:

Attention: April Cockburn
Mouse Metabolic Phenotyping Centers
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.