Method for Sequencing the 16S region, Argonne National Labs

Genomic DNA was amplified using the Earth Microbiome Project barcoded primer set, adapted for the Illumina HiSeq2000 and MiSeq by adding nine extra bases in the adapter region of the forward amplification primer that support paired-end sequencing. The V4 region of the 16S rRNA gene (515F-806R) was amplified with region-specific primers that included the Illumina flowcell adapter sequences. The forward amplification primer also contains a twelve base barcode sequence that supports pooling of up to 2,167 different samples in each lane (Caporaso 2010, Caporaso 2012). Each 25ul PCR reaction contains 12ul of MoBio PCR Water (Certified DNA-Free), 10ul of 5 Prime HotMasterMix (1x), 1ul of Forward Primer (5uM concentration, 200pM final), 1ul Golay Barcode Tagged Reverse Primer (5uM concentration, 200pM final), and 1ul of template DNA. The conditions for PCR are also follows: 94°C for 3 minutes to denature the DNA, with 35 cycles at 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; with a final extension of 10 min at 72 °C to ensure complete amplification. Amplicons were quantified using PicoGreen (Invitrogen) and a plate reader. Once quantified, different volumes of each of the products are pooled into a single tube so that each amplicon is represented equally. This pool is then cleaned up using the UltraClean® PCR Clean-Up Kit (MoBIO), and then quantified using the Qubit (Invitrogen). After quantification, the molarity of the pool is determined and diluted down to 2nM, denatured, and then diluted to a final concentration of 6.75pM with a 10% PhiX spike for sequencing on the Illumina MiSeq.

Method for Sequencing the ITS region, Argonne National Labs

Genomic DNA was amplified using the Earth Microbiome Project accepted ITS barcoded primer set, adapted for the Illumina HiSeq2000 and MiSeq. These primers were designed by Kabir Peay's lab at Stanford University (Plos one; Smith, Peay 2014). The reverse amplification primer also contained a twelve base barcode sequence that supports pooling of up to 2,167 different samples in each lane (Caporaso 2010, Caporaso 2012). Each 25ul PCR reaction contains 12ul of MoBio PCR Water (Certified DNA-Free), 10ul of 5 Prime HotMasterMix (1x), 1ul of Forward Primer (5uM concentration, 200pM final), 1ul Golay

Barcode Tagged Reverse Primer (5uM concentration, 200pM final), and 1ul of template DNA. The conditions for PCR are also follows: 94°C for 3 minutes to denature the DNA, with 35 cycles at 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; with a final extension of 10 min at 72 °C to ensure complete amplification. Amplicons were quantified using PicoGreen (Invitrogen) and a plate reader. Once quantified, different volumes of each of the products are pooled into a single tube so that each amplicon is represented equally. This pool is then cleaned up using the UltraClean® PCR Clean-Up Kit (MoBIO), and then quantified using the Qubit (Invitrogen). After quantification, the molarity of the pool is determined and diluted down to 2nM, denatured, and then diluted to a final concentration of 6.75pM with a 10% PhiX spike for sequencing on the Illumina MiSeq.