Earth Microbiome Project

16S rRNA Amplification Protocol

Primers for paired-end 16s community sequencing on the Illumina HiSeq platform using bacteria/archaeal primer 515F/806R. Please see this article:

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. **ISME J.**

For running these libraries in the MISeq and HiSeq please make sure you read the supplementary methods of the above manuscript very well – you will need to make your sample more complex by adding 50% PhiX to your run.

515F (forward primer) PCR primer sequence:

Field number (space-delimited), description:

1. 5′ Illumina adapter
2. Forward primer pad
3. Forward primer linker
4. Forward primer

AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCG GTAA

806R (reverse primer) PCR primer sequence (each sequence contains different barcode):

2168 GoLay barcoded reverse PCR primers. Each primer is followed by a barcode identifier generated specifically for this set of primers.

Field number (space-delimited), description:

1. Reverse complement of 3′ Illumina adapter
2. Golay barcode
3. Reverse primer pad
4. 4, Reverse primer linker 5, Reverse primer

CAAGCAGAAGACGGCATACGAGAT TCCCTTGTCTCC AGTCAGTCAG CC

GGACTACHVGGGTWTCTAAT 806rcbc0

The complete set of barcodes is available for download from this link.

Sample details/prep here (eg method of prep,etc) -

final primer concentration: 0.2 μM (micromolar) -pooling: see Caporaso et al PNAS 2010

Illumina PCR Conditions: 515-806 region of the 16S rRNA gene (Caporaso et al PNAS 2010)

Reagent	1X Vol
H20 (a)	13 5
Prime Hot MM (b)	10.0
Forward (10 uM) (c)	0.5
Reverse (10 uM) (c)	0.5
Template	1.0

(a) PCR grade water was purchased from MoBio Laboratories (b) This is the older version of the 5 Prime Hot Master Mix with the 'self adjusting' MgCl2. The new version has 1.5 mM MgCl2, hence you may need to add MgCl2 for your specific application. (C) Primer concentrations are for the working stock.

Thermocyler Temp

Time		
94°C	3 min	
94°C	45 sec	
50°C	1 min	35 cycles
72°C	1.5 min	
72°C	10 min	
4°C	hold	

Protocol

1. Amplify samples in triplicate
2. Pool replicate reactions
3. Run samples on agarose gel
4. Quantify amplicons with Picogreen

5. Combine equal amounts of amplicons into a single tube

6. Amplicon pool is cleaned using Mobio UltraClean PCR Clean-Up Kit #12500 according to manufacturer's instructions.

8. Measure concentration and 260/280 of final cleaned, pooled sample. 260/280 must be between 1.8-2.0

9. Send for sequencing