

Materials List for:

Efficient Nucleic Acid Extraction and 16S rRNA Gene Sequencing for Bacterial Community Characterization

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Materials

Name	Company	Catalog Number	Comments
Equipment:			
Mini-Beadbeater-16	BioSpec	607	
PCR workstation			Any PCR hood can be used, e.g., the AirClean 600.
Thermocycler			Any thermal cycler with a heated lid can be used, e.g., MJ Research PTC-200.
Electrophoresis system			Any electrophoresis system can be used, e.g. the Thermo Scientific Owl EasyCast B1 Mini Gel Electrophoresis system.
Nanodrop	Thermo Scientific	2000C	Any other DNA quantification method will be sufficient
Bioanalyzer	Agilent	2100	An alternative is the Agilent 2200 TapeStation Instrument. Not absolutely necessary but very helpful.
MiSeq or HiSeq	Illumina		
Materials:			
Catch-All Sample Collection swab	Epibio	QEC89100	Other swabs can be used but the Catch-All swab is recommended by the Human Microbiome Project.
ELIMINase	Fisher	04-355-31	
SteriFlip 50 ml filtration device (0.22 µm)	EMD Millipore	SCGP00525	
0.1 mm glass beads	BioSpec	11079101	
2 ml screw-cap tubes	Sarstedt	72.694.006	For bead beating
UltraPure 5M NaCl	Life Technologies	24740-011	Molecular Biology Grade
1 M Tris-HCl	Ambion (Invitrogen)	AM9856	Molecular Biology Grade
0.5 M EDTA	Ambion (Invitrogen)	AM9260G	Molecular Biology Grade
Sodium Dodecyl Sulfate, 20% Solution	Fisher	BP1311-200	Molecular Biology Grade
UltraPure DNase/RNase-free distilled water	Ambion	10977-015	Molecular Biology Grade, for buffer preparation
2-Propanol BioReagent, for molecular biology, ≥99.5%	Sigma	I9516-500ML	Molecular Biology Grade
Phenol:Chloroform:IAA, 25:24:1	Invitrogen	AM9730	Warning: Toxic
3 M Sodium Acetate, pH 5.5	Life Technologies	AM9740	Molecular Biology Grade

Disposable sterile polystyrene forceps, PS	Cole Parmer	EW-06443-20	
1.5 ml, clear, PCR clean tubes	Eppendorf	22364120	
PCR grade water	MoBio	17000-11	For PCR
Phusion High-Fidelity DNA Polymerase	New England Biolabs	M0530S	
dNTP mix	Sigma	D7295-0.5mL	
0.2 ml PCR 8-tube with attached clear flat caps, natural	USA Scientific	1492-3900	Any 8-tube strips that are DNase, RNase, DNA, and PCR inhibitor free will work
Agarose	BioExpress	E-3121-25	
50x TAE buffer	Lonza	51216	
DNA gel stain	Invitrogen	S33102	
6x DNA Loading Dye	Thermo (Fisher)	R0611	
50 bp GeneRuler Ladder	Thermo (Fisher)	SM0373	
AllPrep DNA/RNA kit	Qiagen	80284	
UltraClean PCR Clean-up Kit	MoBio	12500-100	
Quant-iT PicoGreen dsDNA Assay Kit	Thermo Fisher Scientific	P11496	An alternative is Qubit Fluorometric Quantification (Life Technologies)
Name	Company	Catalog Number	Comments
Primers:			
515F (forward primer) 5'-AATGATACGGCGACCACCGAG ATCTACACTATGGTAATTGT GTGCCAGCMGCCGCGGTAA-3'			Order at 100 nmole; Purification: Standard Desalting. Resuspend at 100 µM. **Critical: primers must be resuspended with MoBio PCR Grade Water (see above) in a hood to avoid contamination.**
Reverse primers, see the Supplemental Code File and: ftp://ftp.metagenomics.anl.gov/data/misc/EMP/SupplementaryFile1_barcodeed_primers_515F_806R.txt	IDT is recommended		If ordering large sets of primers, order as a 96-well plate at the 100 nmole scale. Resuspend at 100 µM. Full directions for primer ordering and resuspension at http://www.earthmicrobiome.org/files/2013/04/EMP_primer_ordering_and_resuspension.doc . **Critical: primers must be resuspended with MoBio PCR Grade Water (see above) in a hood to avoid contamination.**
Read 1 Sequencing Primer 5'-TAT GGT AAT TGT GTG CCA GCM GCC GCG GTA A-3'			25 nmole; Purification: Standard Desalting. Resuspend at 100 µM.
Read 2 Sequencing Primer 5'-AGT CAG TCA GCC GGA CTA CHV GGG TWT CTA AT-3'			26 nmole; Purification: Standard Desalting. Resuspend at 100 µM.
Index Sequencing Primer 5'-ATT AGA WAC CCB DGT AGT CCG GCT GAC TGA CT-3'			27 nmole; Purification: Standard Desalting. Resuspend at 100 µM.
PhiX Control v3	Illumina	FC-110-3001	Required if performing the sequencing in-house. If the sequencing will be performed by a third-party sequencing center, they will already have PhiX.