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EMP 16S rRNA PCR

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protocol .

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The 16S protocol detailed here is designed to amplify prokaryotes (bacteria and archaea) using paired-end 16S community sequencing on the Illumina platform. Primers 515F–806R target the V4 region of the 16S SSU rRNA.

Allyson Hirsch, George Testo 2022. EMP 16S rRNA PCR. **protocols.io** https://protocols.io/view/emp-16s-rrna-pcr-cakescte

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Reagents

- PCR Water
- PCR Reagents (buffer, Forward Primer, dNTPs, BSA, Taq)

Supplies

- 50mL conical(s)
- Unskirted plate(s)
- Green temporary seal(s)
- PCR seal(s)
- Foil seal(s)
- Sharpie

Equipment

- Plate sealer
- Conical rack
- Plate holder(s)
- Boat & covers
- 200uL pipette & tips
- 1000uL pipette & tips
- 10uL 12-channel pipette & tips
- 200uL 12-channel pipette & tips

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The primer sequences without linker, pad, barcode, or adapter are as follows:

Updated sequences: 515F (Parada)–806R (Apprill), forward-barcoded: FWD:GTGYCAGCMGCCGCGGTAA; REV:GGACTACNVGGGTWTCTAAT

Original sequences: 515F (Caporaso)–806R (Caporaso), reverse-barcoded: FWD:GTGCCAGCMGCCGCGGTAA; REV:GGACTACHVGGGTWTCTAAT

Note: Primers used for PCR reaction(s) are determined by project and or lab.

1 Prepare PCR cocktail calculations and a plate map for all PCR reactions using barcoded primers.

The following calculations must be made based on the number of samples to be amplified:

1	**PCR Cocktail for 16S ribosomal amplification**				
2	16S Illumina amplicons				
3	**Final Volume**	**Number of samples (incl controls)**			
4	25	300			
5	**Component**	**Conc**	**Volume/ reaction**	**[final]**	**Volume in MM**
6	**PCR buffer**	10X	2.5	1X	**787.50**
7	**Forward primer**	10uM	1	.4uM	**315**
8	**Reverse primer**	10uM	1	.4uM	~~**315**~~
9	**BSA**	20mg/ml	1	0.56mg/ul	**315.00**
10	**dNTP mix**	2.5mM each	2	200 uM each	**630**
11	**Hot Start ExTaq**	5U/ul	0.125	0.625	**39.38**
12	**Template**	10-100 ng	2	n/a	~~**630**~~
13	**Total Reagents**		9.625		
14	**H2O**		15.375		**4843.13**
15	**Total volume**		25		**7875**
16	**Volume per well/tube**		**23**		
17					
18	**Date**				
19	**Name**				

Note: The numbers used above are only for demonstration purposes. If calculating the amount of PCR reagents necessary for a set of PCR reactions, calculate using the demonstrated concentrations and volumes per reaction. Typically, every 6 triplicates (or 6 samples + NTCs) requires an input of 25 samples. Therefore, 1 unskirted plate of PCR reactions equates to 100 samples (as the required input to the PCR cocktail calculator as shown above).

Preparing PCR Hood 30m

2 Decontaminate a PCR hood with DNA Away and Ethanol.

3 Place materials inside of the PCR Hood (refer to materials section).

30m

4 Turn on the UV setting for at least **00:30:00** .

Preparing Master Mix 30m

5 Label unskirted plate(s) with the following: Project, Extraction Name, Initials, Date, & Plate Number

6 Pour PCR water into a 50mL conical and make the master mix (specific to project and or number of samples).

7 Using a 200uL 12-channel pipette, pipette **22 µL** of master mix into each well on each unskirted plate.

> **Note:** Samples will be amplified in triplicate. This means that each sample will be amplified in 3 replicate 25uL PCR reactions. This ensures that enough amplicons are created for subsequent preparations. Triplicate PCR reactions for each sample will be pooled together during EGels.

Adding Reverse Primer 30m

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Once finished, retrieve EMP **10 micromolar (µM)** Reverse Primer Plate and DNA plate. Let thaw and spin down.

9 Add **1 µL** of Reverse Primer according to the plate map specific for the project and sample(s).

Adding Sample(s) 30m

10 Add **2 µL** of sample according to the plate map specific for the project and sample(s).

11 Seal everything with PCR and or freezer seals and clean the PCR hood.

11.1 Return sample(s), reagents, and EMP Reverse Primer plate to the appropriate freezer.

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Spin down PCR plate(s).

Running Thermocycler 30m

13 Place plate(s) on the thermalcycler and start an amplification protocol.

13.1 Use the following thermocycler conditions as a part of this earth microbiome project (EMP) protocol:

A	B	C
98C	3 mins	1x
98C	45 secs	30x
50C	60 secs	30x
72C	90 secs	30x
72C	10 mins	1x
4C	hold	

14 Once the PCR has finished on the thermalcycler, place PCR plate(s) in a **-20 °C** freezer or run a gel.