16S rRNA Amplicon PCR (Adapted from EMP 16S amplification protocol*)

PCR reagent Master Mix

Reagent	Volume
PCR Grade H2O (ddH2O)	13.0 µL
5 Primer Hot MM	10.0 µL
Forward primer (10µM)	0.5 µL
Reverse primer (10µM)	0.5 µL
Template DNA	1.0 µL
Total reaction volume	**25.0 µL**

Thermocycler Conditions for 96 well thermocyclers:

Step	Temp	Time	# cycles
Hot Start	94˚C	3m	1
Denature	94˚C	45s	35
Anneal	50˚C	60s	
Extend	72˚C	90s	
Final Extension	72˚C	10m	1
Hold	4˚C	Hold	Hold

Note: Samples can be prepared in strip tubes or plates provided that the plastic is compatible with the thermocycler.

1. Prepare master mix in the order above for each sample in triplicate (*i.e.,* each sample is amplified in 3 individual replicate 25µL reactions).
   Note: Each individual sample must have a unique barcoded primer, but all replicate PCR reactions of that sample will share that barcoded primer.
2. Place tubes into the thermocycler and start the program above.
3. At the end of the PCR program remove the tubes and place the samples on ice for 2m.
4. Spin down the samples briefly (~3-5sec).
5. Combine the triplicate PCR reactions for each sample into a single tube. Combination will result in a total of 75 µL of amplicon for each sample. **Do NOT combine amplicons from different samples at this point!**
6. Proceed to amplicon visualization and quantification protocols.

*Earth microbiome project(EMP; http://www.earthmicrobiome.org)