PCR PROTOCOL
Purpose: Bacterial microbiome sequencing of 16S-V4 rRNA
Original Source: Schloss lab / Kozich et al., 2013
Document By: BRPB (1/13/2020); edited TRK (10/29/2020)

Notes: This is the standard PCR protocol for 16S rRNA sequencing in the lab. It amplifies the V4 region of bacterial 16S rRNA. These primers have been modified to include Nextera-XT overhangs. These overhangs permit Illumina library preps using Nextera protocols.

Target size ~291 bp

Primers:

515_overhang	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTAA
806r_overhang	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT

Reaction
Accuprime (21 µl master mix)

Reagents	Final Concentration	Volume for 1 Reaction (µL)
Primer 1	10 µM	2
Primer 2	10 µM	2
Accuprime mastermix	1x	17
DNA Template		1

Total Master Mix Volume (for 1 Rxn): 21.0 µL
DNA Template: 1 µL (1-500ng, ideally about 25)
Total Reaction Volume: 22.0 µL

***Make extra master mix and include a negative and positive control. Use extraction blanks and 'mock' mixes.**

Program: Accuprime_16s_Amp on Eppendorf Mastercycler Nexus GSX1

Process	Temperature (°C)	Time
Initial denaturing	95	2 min
35 cycles: Denaturing	95	20 sec
35 cycles: Annealing	55	15 sec
35 cycles: Extension	72	5 min
Final Extension	72	10 min
Hold	4	Forever

Footnotes

Locus specific primer sequences without the overhangs:
515f: GTGCCAGCMGCCGCGGTAA
806r: GGACTACHVGGGTWTCTAAT