PCR for 16S Sequencing

Materials:

Milli-Q Water

GoTaq Flexi (Promega, 5 units per µL)

5x **Colorless** GoTaq Flexi Buffer

$MgCl_2$ (25 mM)

dNTPs [10 mM mix (2.5 mM each)]

Fwd Primer (515F FlxB, 10µM)

Rev Primer (806r w/Barcode, 10 µM)

Sample DNA

Notes & Protocol:

Amplification of all 16S is very sensitive to contamination. Work in the hood and use freshly filtered water for every batch, using a Falcon tube and the Milli-Q machine. Also use individually wrapped Eppendorf tubes for master mixes.

1. For 15 minutes, irradiate in the hood both the water and the tubes to be used for master mixes, with caps off.
2. Make a master mix for each sample, mix and spin down, and irradiate in hood with caps off for 15 minutes. Master mix should include the following reagents in the given proportions and should account for pipetting error by mixing more than will be needed; for example, with triplicates and a negative control:

	Single Reaction (25 µL) (µL)	MM for 4 reactions (4x+1x) (µL)	Final Conc.
Water	6.875	34.375	-
Buffer	5	25	1x
$MgCl_2$	5	25	5 mM
dNTPs	2	10	1 mM
Taq	0.125	0.675	0.625 units

3. Complete master mix by adding primers, then mix and spin down:

	Single Reaction (µL)	MM for 4 reactions (µL)	Final Conc.
515F FLXB	2.5	12.5	1 µM
806r (Barcode differs by sample)	2.5	12.5	1 µM

4. Aliquot master mix into PCR tubes (24 µL for this 25µL reaction).
5. Add one µL of sample DNA to each experimental PCR tube. Use Milli-Q water for negative controls.
6. Mix and spin down, and run the following thermocycler protocol:

Temperature (°C)	Time	
94	3 min.	
94	45 s	} repeat 35x
50	60 s	
72	90s	
72	10 min	

7. Run on gel to verify amplification & purity, and pool replicates.