1.PCR for 16S rRNA amplicon sequencing

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ABSTRACT

This protocol follows the emp guidelines for 16S rRNA sequencing. For more details refere to http://press.igsb.anl.gov/earthmicrobiome/protocols-and-standards/16s/

MATERIALS TEXT

- 515F forward primer, barcoded

Field descriptions (space-delimited):

1. 5′ Illumina adapter
2. Golay barcode
3. Forward primer pad
4. Forward primer linker
5. Forward primer (515F)

AATGATACGGCGACCACCGAGATCTACACGCT XXXXXXXXXXXX TATGGTAATT GT GTGYCAGCMGCCGCGGTAA

- 806R reverse primer

Field descriptions (space-delimited):

1. Reverse complement of 3′ Illumina adapter
2. Reverse primer pad
3. Reverse primer linker
4. Reverse primer (806R)

CAAGCAGAAGACGGCATACGAGAT AGTCAGCCAG CC GGACTACNVGGGTWTCTAAT

- PCR-grade water
- Platinum Hot Start PCR Master Mix (2x) from ThermoFisher (cat. no. 13000014)
- 5mL tube for master mix (this size depends on the total master-mix volume)
- Long 10ul pipette tips (these are necessary to reach the deep wells of the primer plate)
- reagent reservoir
- Thermocycler
- PCR hood

Prepare for PCR

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- Remove PCR reagents from -20 °C and allow reagents to thaw on ice or at room temperature.
- Remove PCR primers from -80 °C to thaw out in 4 °C and keep cool during the entire process to avoid primer degradation.
- Wipe down PCR hood with bleach and ethanol.
- Place disposables such as tubes, plates, plate sealers, and water in PCR hood and turn on UV light for 00:20:00
- Once everything is thawed vortex PCR reagents and spin down.
- Very** gently vortex primers and then spin down plate in a plate centrifuge (this will avoid primers from cross contaminating).
- Keep reagents cool or on ice during the duration of the protocol.

PCR thermocycler settings

2 ▪ Thermocycler should be set to a program with the below settings:

94 °C	3 min	3 min	
94 °C	45 s	60 s	x35
50 °C	60 s	60 s	x35
72 °C	90 s	105 s	x35
72 °C	10 min	10 min	
4 °C	hold	hold	

PCR master-mix

3 ▪ PCR reaction mixture for 50ul rxn

NOTE

This reaction can be for 25ul, but it may be diffcult to pipette 0.5ul of primer, especially with a multichannel.

NOTE

- Add DNA last to each well and do not add the DNA or barcoded primers (i.e. 515 F) to the mastermix, but instead add to each individual tube or well.
- Add a negative control since this is esstential in order to check for false positives. Make enough mastermix for all your samples, negative control, and extra reactions for pipetting error (ex. add 10% more of reagent)

Component	volume per Rxn	Order to add	*rxn# + 10%
PCR-grade water	26.0 µL	1	
PCR master mix (2x)	20.0 µL	2	
Reverse primer (10 µM)	1 µL	3	
Forward primer (10 µM)	1 µL	4	NA
Template DNA	2 µL	5	NA
Total reaction volume	50.0 µL		NA

- Once the PCR master-mix reagents are combined, mix the master-mix gently and spin down to collect mixture and to remove bubbles that may interfere with downstream PCR amplifications.
- For a 50ul reaction, aliquot 47ul of master-mix into each well. Then add one unique barcode to each well, and finally add DNA to each well. Gently mix and spin down -- examine plate or PCR tubes for any bubbles and try to remove them by spinning.
- Place samples in thermocycler.

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