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We use this protocol and it's working

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🌐 16S Metagenomics in a Field Setting V.3

📁 In 1 collection

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ABSTRACT

This protocol is was used to conduct DNA 16S metagenomics on FPI's Genomics in the Jungle - 2018 field course at the Green Lab, located and Inkaterra Guides Field Station, Madre de Dios, Peru.

GUIDELINES

This protocol starts from already extracted DNA

MATERIALS

STEP MATERIALS

⊗ Agencourt Ampure XP Beckman Coulter Catalog #A63880

Keywords: 16S, metagenomics, microbiome, wildlife, fieldwork, nanopore, minion

Amplification

1 Remove samples and the following reagents and let thaw, once thawed keep on ice block

- 10mM DNTPs
- 25 mMgCl
- 5x Go Taq Buffer
- Forward primer 16S-27F (10uM)
- Reverse primer 16S-1429R (10uM)
- GoTaq Hotstart Polymerase 5u/ul

Make PCR cocktail for # of samples * 1.1 (10% extra). Don't forget to include 1 PCR negative control for each separate PCR

Run PCR according to the following cycle conditions:

- Initial denaturation 95 °C for 120s
- 25 cycles of 95 °C for 30s, 51 °C for 30s, 72 °C for 30s
- Final extension 72 °C for 420s

Upon completion remove, label, and store at 4 °C, or take directly to electrophoresis

Electrophoresis

2 Equipment

- BlueGel system
- MiniOne system

Create .8 - 1.0% agarose 1 gel with 13 combs

- Measure 1 g of agarose
- Mix agarose with 100 mL of 1xTBE
- Microwave the mixture until agarose is completely dissolved (1-3 min)
- Pour the agarose gel into the tray with the comb in place.
- Allow the agarose gel to harden (20-30 min)

Insert the agarose gel into electrophoresis equipment and add 1xTBE buffer until the agarose gel is submerged

Spot check with 2 μL of each sample

Mix 1 μL of loading dye to 2 μL of each sample and load the gel. (If Green Taq buffer with built in loading dye was used, skip this step).

Load 5 μL of 100bp ladder into the agarose gel.

Turn on the electrode and let the DNA run until the band is identifiable (

Barcoding PCR

- 3
 - A barcoding PCR was run to attach barcodes from the 96-barcode kit for the MinION to each sample
 - We did not use special PCR mastermix at this stage, using instead a mix similar to that of the PCRs above
 - We used 1 μL of each barcode primer and 2 μL of every positive PCR amplicon in a total volume of 25 μL

We ran the PCR at the following conditions:

- Initial denaturation of hotstart taq at 95 °C for 00:02:00
- Denaturation at 95 °C for 00:00:30
- Annealing at 62 °C for 00:00:30
- Extension at 72 °C for 00:00:45
- Total number of cycles - 18
- Final Extension at 72 °C for 00:05:00

Quantification

4

Equipment

Equipment

Quantus Fluorometer

NAME

DNA and RNA Quantifier

TYPE

Promega

BRAND

E6150

SKU

<https://www.promega.com/products/fluorometers-luminometers-multimode-readers/fluorometers/quantus-fluorometer/?catNum=E6150>

LINK

- dsDNA dye
- Qubit Assay Tubes
- Your DNA sample

Pulse vortex your sample and spin down.

Add 200 µL of dsDNA dye into the qubit assay tube.

- Make sure to cover up the tube from light

Transfer 1 µL of DNA sample into qubit assay tube

Pulse vortex and spin down

Let it sit for 00:05:00 in room temperature

- Covered from the light

Gently vortex and spindown the sample

Calibrate the Quantus Fluormeter by standard and reference testing.

- Follow the same process but use 1 µL ddH₂O and Lambda DNA.

Insert the qubit assay tube into the Quantus Flurometer

Normalisation and Pooling

5 Dilute each sample to 50 nM

- Mix 5 uL of the sample with calculated amount of ddH₂O to make each sample the same concentration of 50 nM
- Then pool 5 uL of each dilution into a single tube.
- This is now the library.

SPRI Bead Cleanup

- 6 Run a SPRI cleanup of the library using your choice of bead purification systems/kits in a 1:1 ratio. Resuspend in the same volume.

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