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18S Metagenomics in a Field Setting

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We use this protocol and it's working

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Abstract

This protocol is was used to conduct DNA 18S 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

Protocol materials

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Troubleshooting

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 5′-CAGCAGCCGCGGTAATTCC-3′ (10uM)
- Reverse primer 5′-CCCGTGTTGAGTCAAATTAAGC-3′ (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 98 °C for 00:00:30 , 51 °C for 00:00:30 , 72 °C for 00:00:45
- Final extension 72 °C for 00:05:00

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

2 **Equipment**

- BlueGel system
- MiniOne system

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

- Measure 1 g of agarose
- Mix agraose 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 postive PCR amplicon in a total volume of 25 uL

We ran the PCR at the following conditions:

- Initil 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 ddH_2O 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 amound of ddH_2O (based on DNA concentration above) to make each sample the same concentration of 50 nM
- Then pool 5 uL of each dilution into a single tube.
- This is now your library.

SPRI 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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