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③ 18S Metagenomics in a Field Setting



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Protocol status: Working

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

Protocol materials

X Agencourt Ampure XP Beckman Coulter Catalog #A63880

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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 \$\mathbb{\math
- Final extension **§** 72 °C for **ⓑ** 00:05:00

Upon completion remove, label, and store at $\$ 4 $^{\circ}\text{C}$, 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 $\perp 2 \mu$ of each sample

Mix $\perp \!\!\! \perp 1 \, \mu L$ of loading dye to $\perp \!\!\! \perp 2 \, \mu L$ of each sample and load the gel. (If Green

Tag buffer with built in loading dye was used, skip this step).

Load Δ 5 μ L 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 \$\mathbb{\perp}\$ 1 \mu L of each barcode primer and \$\mathbb{\perp}\$ 2 \mu L of every postive PCR amplicon in a total volume of 25 uL

We ran the PCR at the following conditions:

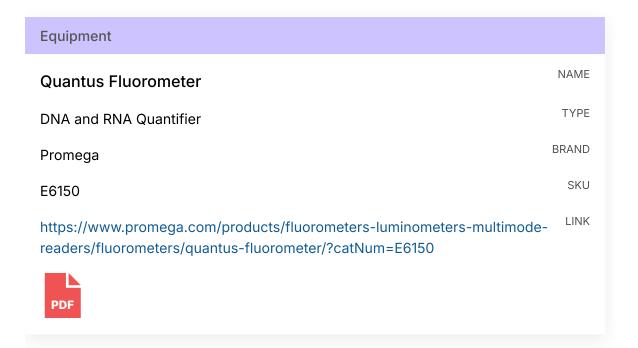
- Initil denaturation of hotstart taq at \$\\$\\$\\$\$ 95 °C for (5) 00:02:00
- Denaturation at **\$** 95 °C for **(*)** 00:00:30
- Annealing at **\$** 62 °C for **(**) 00:00:30
- Extension at **3** 72 °C for **6** 00:00:45
- Total number of cycles 18
- Final Extension at # 72 °C for (00:05:00

Quantification

4



Equipment



- dsDNA dye
- Qubit Assay Tubes
- Your DNA sample

Pulse vortex your sample and spin down.

Add $\perp 200 \mu$ 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 \bot 1 \bot L 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 amound of ddH₂O (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.
 - Agencourt Ampure XP Beckman Coulter Catalog #A63880