Mar 11, 2019

Working

16S Metagenomics in a Field Setting

Version 3

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dx.doi.org/10.17504/protocols.io.y2pfydn

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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.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

This protocol starts from already extracted DNA

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Agencourt Ampure XP	A63880	Beckman Coulter

SAFETY WARNINGS

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 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 of100kp ladder into the agarose gel.

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

Barcoding PCR

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- 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

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Equipment

Quantus Fluorometer
DNA and RNA Quantifier
Promega E6150

PDF

- 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 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.

Agencourt Ampure XP
by Beckman Coulter
Catalog #: A63880