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Working

## 16S Metagenomics in a Field Setting

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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** A63880 **Beckman Coulter** Agencourt Ampure XP

SAFETY WARNINGS

## Amplification

- 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 1,72 °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  $\frac{1}{2}$   $\frac{1}{\mu}$  of each sample

Mix  $\frac{1}{2}$   $\mu$ I of loading dye to  $\frac{1}{2}$   $\mu$ I of each sample and load the geI. (If Green Taq buffer with built in loading dye was used, skip this step).

Load 5 µl of 100kp ladder into the agarose gel.

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

## Barcoding PCR

- 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 11 µl of each barcode primer and 12 µl of every postive PCR amplicon in a total volume of 25 uL

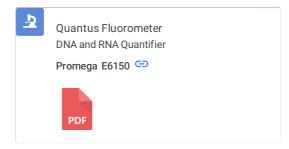
We ran the PCR at the following conditions:

- Initil denaturation of hotstart taq at 8 95 °C for ⑤ 00:02:00
- Denaturation at § 95 °C for ⑤ 00:00:30
- Annealing at 8 62 °C for ( 00:00:30
- Extension at **§ 72 °C** for **⑤ 00:00:45**
- Total number of cycles 18
- Final Extension at ↑ 72 °C for ♦ 00:05:00

## Quantification

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## **Equipment**



- dsDNA dye
- Qubit Assay Tubes
- Your DNA sample

Pulse vortex your sample and spin down.

Add  $200 \mu$  of dsDNA dye into the qubit assay tube.

Make sure to cover up the tube from light

Transfer 1 ul of DNA sample into qubit assay tube

Pulse vortex and spin down

Let it sit for (00005:00) 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  $\boxed{1}$   $\mu$  ddH<sub>2</sub>O 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<sub>2</sub>O 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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