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Working

## 18S Metagenomics in a Field Setting

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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.

### 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 5'-CAGCAGCCGCGTAATTCC-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 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



Quantus Fluorometer  
DNA and RNA Quantifier  
Promega E6150 



- 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<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

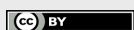
- Mix 5 uL of the sample with calculated amount of ddH<sub>2</sub>O (based on DNA concentration above) to make each sample the same concentration of 50 nM
- Then pool 5 uL of each dilution into a single tube.
- This is now your library.

## 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  
by Beckman Coulter  
Catalog #: [A63880](#)



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