ABSTRACT

This protocol is 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.

GUIDELINES

This protocol starts from already extracted DNA

MATERIALS

STEP MATERIALS

PROTOCOL MATERIALS

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Protocol status: Working
We use this protocol and it's working
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 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 \( \text{2 mL} \) of each sample

Mix \( \text{1 mL} \) of loading dye to \( \text{2 mL} \) of each sample and load the gel. (If Green Taq buffer with built in loading dye was used, skip this step).

Load \( \text{5 mL} \) of 100 bp ladder into the agarose gel.

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

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### Quantification

#### Equipment

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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 ddH₂O and Lambda DNA.

Insert the qubit assay tube into the Quantus Flurometer

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### Normalization and Pooling

4 Dilute each sample to 50 nM
  - Mix 5 µL of the sample with calculated amount of ddH₂O to make each sample the same concentration of 50 nM
  - Then pool 5 µL of each dilution into a single tube.
  - This is now the library.

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### SPRI Clean-up

5 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.