Mar 11, 2019 Version 2

16S Metagenomics in a Field Setting V.2

DOI
dx.doi.org/10.17504/protocols.io.y2hfyb6

Gideon Erkenswick[1,2], Stefan Prost[3,4], Mrinalini Watsa[1,5,2], Aaron Pomerantz[6,7]

[1]University of Missouri - Saint Louis; [2]Field Projects International;
[3]LOEWE-Center for Translational Biodiversity Genomics, Senckenberg Museum, 60325 Frankfurt, Germany;
[4]South African National Biodiversity Institute, National Zoological Garden, Pretoria 0184, South Africa;
[5]Washington University in Saint Louis;
[6]University of California, Berkeley, CA, USA, Department of Integrative Biology;
[7]Marine Biological Laboratory, Woods Hole, MA - USA

Mrinalini Watsa
San Diego Zoo Wildlife Alliance

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.y2hfyb6

Protocol Citation: Gideon Erkenswick, Stefan Prost, Mrinalini Watsa, Aaron Pomerantz 2019. 16S Metagenomics in a Field Setting. **protocols.io https://dx.doi.org/10.17504/protocols.io.y2hfyb6**

Protocol status: Working
We use this protocol and it's working

Created: March 11, 2019

Last Modified: March 11, 2019

Protocol Integer ID: 21289

Keywords: 16S, metagenomics, microbiome, wildlife, fieldwork, nanopore, minion

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.

Guidelines

This protocol starts from already extracted DNA

Materials

STEP MATERIALS

Agencourt Ampure XP **Beckman Coulter Catalog #**A63880

Protocol materials

Agencourt Ampure XP **Beckman Coulter Catalog #**A63880

Agencourt Ampure XP **Beckman Coulter Catalog #**A63880

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 (

Quantification

3

Equipment

Equipment	
Quantus Fluorometer	NAME
DNA and RNA Quantifier	TYPE
Promega	BRAND
E6150	SKU
https://www.promega.com/products/fluorometers-luminometers-multimode-readers/fluorometers/quantus-fluorometer/?catNum=E6150	LINK

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

Normalization and Pooling

4 **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 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.

Agencourt Ampure XP **Beckman Coulter Catalog #**A63880