The influence of agricultural tillage practices on soil biodiversity: Soil metagenomic methods, microbial community

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Abstract

This protocol provides the sampling and molecular biology lab methods used to prepare microbial amplicons for MiSeq sequencing.

Citation: Jeff Strohm, Robert Hanner, Richard J Heck The influence of agricultural tillage practices on soil biodiversity: Soil metagenomic methods, microbial community. **protocols.io**
dx.doi.org/10.17504/protocols.io.efjbbkn
Published: 28 Mar 2018

Protocol

Sample collection

Step 1.
Two sterile 15mL Falcon tubes were used for collecting each sample (A and B). The first tube was hammered into the soil until fully submerged. It was then removed by digging down next to it with a trowel.

Sample collection

Step 2.
A small wall of soil was left between the trowel and the sample tube hole to avoid contamination between sites from the trowel. The first tube was removed and capped.

Sample collection

Step 3.
The second tube was then inserted into the hole made by the first tube, and the process was repeated.

Sample collection

Step 4.
All samples were isolated in plastic bags to avoid cross contamination and placed in a cooler kept in the shade.

Sample collection

Step 5.
Samples were frozen at -80 °C until DNA extraction.

DNA extraction

Step 6.
Sub-sampling was performed in a fume hood. (For detailed decontamination, sub-sampling and extraction protocols, see the Supplementary Methods.)

DNA extraction

Step 7.

The MO BIO PowerMax Soil extraction kit was chosen and the manufacturer's extraction protocols were strictly adhered to.

REAGENTS

PowerMax® Soil DNA Isolation Kit 12988-10 by Mobio

DNA extraction

Step 8.

DNA extract was stored at -80 °C until PCR

PCR

Step 9.

The high fidelity polymerase, KAPA HiFi HotStart in ReadyMix format was chosen. Annealing temperature gradients were performed for each primer set and were used to select "low, medium and high" annealing temperatures to be used for all samples and genes (16S, 18S, AMF(18S), ITS2

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HotStart ReadyMix (KAPA HiFi PCR kit) KK2601 by Kapa Biosystems

PCR

Step 10.

For PCR setup and cycling conditions see supplementary methods

PCR

Step 11.

Reaction success was confirmed with an Invitrogen E-gel and reactions run at the three different annealing temperatures were then pooled.

PCR

Step 12.

Amplicons were stored at 4 °C to be purified the following day.

Purification

Step 13.

Aline PCRClean DX was used to purify all amplicons and a size selection protocol was adopted from Ampure beads in order to help reduce primer carryover.

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PCRClean DX C-1003-5 by Aline Biosciences

Purification

Step 14.

For 16S, ITS2 and AMF- 18S, a ratio of 0.55 (beads):1 (sample) was used. For 18S, a ratio of 0.9:1 was used due to its shorter length.

Purification

Step 15.

Amplicons were eluted in 10mM Tris-HCL (pH 7.6).

Purification

Step 16.

An Invitrogen E-gel was used to confirm purification success.

Purification

Step 17.

Amplicons were stored at 4 °C for index PCR the following day.

Index PCR

Step 18.
A master mix tube was prepared for each index primer. Half the total volume of master mix was added to each reaction well from either master mix tube containing the forward or reverse index primer

Index PCR

Step 19.
For PCR setup and cycling conditions see supplementary methods

Purification

Step 20.
Aline PCRClean DX was used for purification, but this time, at the full concentration of 1.8X (beads): 1(sample).

REAGENTS

PCRClean DX C-1003-5 by Aline Biosciences

Purification

Step 21.
Amplicons were stored at 4 °C to be quantified and normalized the following day.

Quantification and Normalization

Step 22.
An Invitrogen Q-Bit Fluorometer was used to measure all amplicon concentrations

Quantification and Normalization

Step 23.
All samples were normalized to 4nmol with the addition of 10mM Tris-HCL (pH 7.6).

Quantification and Normalization

Step 24.
Amplicons were then pooled and submitted to the Advanced Analysis Center Genomics Facility at The University of Guelph for Illumina MiSeq sequencing using the 250bp paired end read chemistry.

Sequencing

Step 25.
Amplicons were loaded into the MiSeq as a 6pM denatured library which was 15% PhiX.

Sequencing

Step 26.
For the microbial samples two MiSeq runs were performed each amplicons from all four genes for 10 samples.