Field sampling of root-associated microbes for DNA/RNA extraction

Version 2

Roey Angel[1]

[1]Soil and Water Research Inrastructure

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SoWa Anaerobic and Molecular Microbiology

Roey Angel
Soil and Water Research Inrast...

ABSTRACT

This protocol describes a procedure for sampling plant roots in the field for future DNA and RNA extraction for microbiome analysis. The protocol is deliberately designed to be simple and requires no electronic equipment. Root samples are preserved in LifeGuard Soil Preservation Solution for protecting against nucleic acid degradation.

TAGS

dna

plant

Show tags

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Micro-spatula set	AT16.1	Carl Roth
LifeGuard Soil Preservation Solution	12868-100	Qiagen
Scissors	HCT7.1	Carl Roth
Technical-grade ethanol (70%)	T913.1	Carl Roth
Paper towels	Y03.1	Carl Roth
Microcentrifuge tubes 2 ml	CK06.1	Carl Roth
Garden trowel	View	Amazon
Disposable pasteur pipettes	EA61.1	Carl Roth
Tweezers set	PX40.1	Carl Roth
Cooling box	AA46.1	Carl Roth
Cooling packs	E447.1	Carl Roth

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Garden trowel	View	Amazon
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BEFORE STARTING

- Clean spatulas using 70% ethanol

1 **Sample a triplicate of plant individuals spaced out a few metres apart from each otherMake sure you are reall sampling individual plants and not offshoots of the same plant.**

2 **Using a garden trowel, carefully dig out the plants while keeping the root system intact** (as much as possible, of course).

Garden trowel
by Amazon
View

3 **While holding the plant by the shoot, shake the root system hard enough so that all loose soil is removed from it.** Take care to damage the plant as little as possibleYou can use a spatula to remove large soil aggregates that are attached to the roots.

Micro-spatula set
by Carl Roth
Catalog #: AT16.1

4 **From the remaining root system (plus soil particles plus attached to the roots), trim a 'representative sample' of roots using scissors or scalpel.** It is usually best to trim the roots onto a piece of paper towel.

Scissors
by Carl Roth
Catalog #: HCT7.1

Paper towels
by Carl Roth
Catalog #: Y03.1

5 **Cut the trimmed out roots a little so that they fit into a 2.0 ml tube.**

Scissors
by Carl Roth
Catalog #: HCT7.1

Paper towels
by Carl Roth
Catalog #: Y03.1

6 **Place about 2-3 g of that cut out sample into a 2.0 ml tube.**

Tweezers set
by Carl Roth
Catalog #: PX40.1

Microcentrifuge tubes 2 ml
by Carl Roth
Catalog #: CK06.1

2 g

NOTE
The root tissue should make up at least half or more of the mass, while the remaining attached soil should make up the rest

7 **Press the sample a little into the bottom of the tube to decrease its volume.**

Micro-spatula set
by Carl Roth
Catalog #: AT16.1

NOTE
Make sure the roots do not take up more than 1/2-2/3 of the volume
It is of course possible to split each sample into several separate tubes, depending on the specific type of roots, and submerge each with LifeGuard solution

8 **Add as much LifeGuard solution so that the sample is submerged in about twice of its volume (about 1.0 – 1.5 ml).**
Best is to use a disposable Pasteur pipette for dispensing the solution.

LifeGuard Soil Preservation Solution
by Qiagen
Catalog #: 12868-100

Disposable pasteur pipettes
by Carl Roth
Catalog #: EA61.1

1.5 ml

9 **Place the tubes in cooling (around 4 °C) and keep them cooled until you reach the lab. The solution will protect nucleic acids even at room temperature for several days, but cooling is preferred.**

Cooling box
by Carl Roth
Catalog #: AA46.1

Cooling packs
by Carl Roth
Catalog #: E447.1

4 °C

10 **In the lab, store the samples in a freezer (-20 – -80 °C).**

-20 °C or -80 °C