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

Roey Angel

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.

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Before start

Clean spatulas using 70% ethanol

Materials

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

Protocol

Step 1.

Sample a triplicate of plant individuals spaced out a few metres apart from each other

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Make sure you are reall sampling individual plants and not offshoots of the same plant

Step 2.

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

REAGENTS
Garden trowel View by Amazon

Step 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 possible
You can use a spatula to remove large soil aggregates that are attached to the roots

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M
T

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REAGENTS
Micro-spatula set AT16.1 by Carl Roth

Step 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

REAGENTS
Scissors HCT7.1 by Carl Roth
Paper towels Y03.1 by Carl Roth

Step 5.

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

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REAGENTS

Scissors HCT7.1 by Carl Roth

Paper towels Y03.1 by Carl Roth

Step 6.

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

AMOUNT

2 g Additional info:

REAGENTS

Tweezers set PX40.1 by Carl Roth

Microcentrifuge tubes 2 ml CK06.1 by Carl Roth

NOTES

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

Step 7.

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

REAGENTS

Micro-spatula set AT16.1 by Carl Roth

NOTES

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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

Step 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

AMOUNT

1.5 ml Additional info:

REAGENTS

LifeGuard Soil Preservation Solution 12868-100 by Qiagen

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Disposable pasteur pipettes EA61.1 by Carl Roth

Step 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

TEMPERATURE

4 °C Additional info:

REAGENTS

Cooling box AA46.1 by Carl Roth

Cooling packs E447.1 by Carl Roth

Step 10.

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

TEMPERATURE

-20 °C Additional info: or -80 °C

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