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Field sampling of root-associated microbes for DNA/RNA extraction V.2

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

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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.

Materials

MATERIALS

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

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Cooling box **Carl Roth** **Catalog #**AA46.1

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Before start

- 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 **Amazon**

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 **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 **Carl Roth Catalog** #HCT7.1

Paper towels **Carl Roth Catalog** #Y03.1

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

Scissors **Carl Roth Catalog** #HCT7.1

Paper towels **Carl Roth Catalog** #Y03.1

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

Tweezers set **Carl Roth Catalog** #PX40.1

Microcentrifuge tubes 2 ml **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 **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 **Qiagen** **Catalog** #12868-100

Disposable pasteur pipettes **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 **Carl Roth** **Catalog** #AA46.1

Cooling packs **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