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# 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 <u>E447.1</u> by <u>Carl Roth</u>

## **Protocol**

#### Step 1.

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

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)



Garden trowel View by Amazon

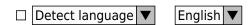
#### Step 3.

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

G M T



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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



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

REAGENTS

Scissors HCT7.1 by Carl Roth

Paper towels <u>Y03.1</u> by <u>Carl Roth</u>

# 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

Disposable pasteur pipettes **EA61.1** by **Carl Roth** 

# Step 9.

Place the tubes in cooling (around 4  $^{\circ}$ 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:



Cooling box <u>AA46.1</u> by <u>Carl Roth</u>
Cooling packs <u>E447.1</u> by <u>Carl Roth</u>

## Step 10.

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

**■ TEMPERATURE** 

-20 °C Additional info: or -80 °C