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Protocol for preparing seagrass-microbiome samples for DNA extraction

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1 Works for me dx.doi.org/10.17504/protocols.io.butqnwmw

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ABSTRACT

This protocol details methods for preparing seagrass-asscociated microbiome samples for DNA extraction.

ATTACHMENTS

Protocol for preparing samples for DNA extraction.docx

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MATERIALS TEXT

Materials for Epiphyte washes:

Redford buffer solution:

A	B
Tris-HCL	1 M
NaEDTA	0.5 M
Triton-X	1.2%

Mo Bio solution C1

Materials for Endophyte sterilization:

95% EtOH
0.5% NaOCl
70% EtOH
Autoclaved H20

Weighing samples:

1

Before proceeding through wash steps (if necessary), samples should be weighed (this is important if you are considering quantitative PCR or want to standardize sample amounts); prepare balance for samples by cleaning with 70% ethanol solution and obtain sterile weigh boats.

Place weigh boat on balance and tare.

2 Place sample in weigh boat using sterile forceps or scoopulas.

Flame sterilize between samples; procedure: immerse in 1) 10% bleach (0.5% NaOCl) 2) water 3) ethanol 4) flame) – attempt to get approx. constant mass across different sample types – e.g. for sediment ~ **250 mg** .

3 Record sample weight.

4 Place sample in appropriate tube to proceed-sediment directly into DNA extraction tubes, other tissues into tubes to be washed.

Plant tissues (roots, leaves, rhizomes) can now be directly placed in tube for DNA extraction or can be further processed to get epiphyte washes and/or then further surface-sterilized in the following steps.

Epiphyte washes 25m

5 5m

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Using a 1:50 dilution (in autoclaved H20) of Redford buffer solution [[M]**1 Molarity (M)** Tris-HCL, [M]**0.5 Molarity (M)** NaEDTA, 1.2% Ctab] from Kembel et al 2014; I used 1.2% Triton-X instead of Ctab as in Kadivar & Stapleton 2003

Add **500 µl** of diluted Redford buffer to samples for **00:05:00** and vortex/agitate.

6  20m

Pellet in centrifuge @ **4000 x g** for **00:20:00** .

7 Resuspend pellet in MoBio solution C1 (or equivalent) and proceed with DNA extraction.

Endophyte sterilization 5m 5s

8 

Using the surface cleaning method described in Arnold et al 2007 (Mycologica); complete after washing for epiphytes – or – if epiphytes are not wanted, you can proceed here

Rinse tissue with autoclaved H20.

9 Immerse tissue in 95% EtOH (**00:00:05**). 5s

10 Immerse tissue in 0.5% NaOCl (~10% bleach) (**00:02:00**). 2m

11 Immerse tissue in 70% EtOH (**00:02:00**). 2m

12  1m

Rinse by with autoclaved H20 (**00:01:00**).

13 Repeat step 5 until tissue has been rinsed three times in H20.

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We can stop after this step if not proceeding directly to DNA extraction and can freeze tissues.

14 Crush tissue (using sterile centrifuge pestle) & proceed with DNA extraction.

For hardy plant tissues, you can sterilize an electric drill and use the centrifuge pestle as a drill bit to crush tissues – just make sure to clean drill in between samples.

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