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Seagrass Microbiome Sample Collection and Preservation

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1 *Works for me* dx.doi.org/10.17504/protocols.io.fxzbpp6

EisenLab Seagrass Microbiomes

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ABSTRACT

This collection protocol was developed as part of the Seagrass Microbiome Project. This was originally a collaboration among **Jonathan Eisen** and **Jay Stachowicz** at the University of California, Davis and **Jessica Green** at the University of Oregon, with funding from the Gordon and Betty Moore Foundation.

EXTERNAL LINK

https://seagrassmicrobiome.org/

GUIDELINES

Collection: Seagrass Microbiome Tissue Samples

1 ***Collection***: If possible, wear gloves whenever you handle the plants. Pull up one plant including the roots. Gently swish the plant in the water to remove loose sediment from the roots. Process these samples in the field according to steps 2 and 3.

If you cannot process them in the field, cut the plant into two pieces (above-ground leaves and below-ground roots/rhizomes) and place in two separately-labelled clean bags on dry ice to transport to shore/lab; these samples must be processed as soon as possible and no longer than 5 hours from the time of collection.

2 ***Processing Roots:*** Pull off root hairs with tweezers and place them in a labelled microcentrifuge tube. If you are preserving the samples in a buffer, make sure the roots are fully submerged.

3 ***Processing Leaves***:. Cut a 2 cm section from a healthy, green section of an outer leaf blade. Put the leaf section into a labelled microcentrifuge tube. If you are preserving the samples in buffer, make sure the leaves are fully submerged.

4 ***Tools***: Wipe down all tools with ethanol between samples.

Collection: Sediment Microbiome Samples

5 ***Collection***: Near where you collect your seagrass tissue sample, collect a sediment sample. Insert the barrel of the syringe into the sediment and, at the same time, carefully and slowly pull back on the plunger so that the sediment surface remains intact and in place while the syringe barrel is pushed into the sediment.

Each sediment sample should be collected using one 6cc syringe with the bottom removed (see a picture of the ZEN kit for an example).

6 ***Collection***: Remove the syringe from the sediment and extrude the sediment until the base of the plunger is at the 3cc mark.

7 Use a plastic spatula to transfer approximately 0.25 grams of sediment in the syringe into a labelled microcentrifuge tube. If you are using buffer, the sediment should be fully submerged in the buffer solution.

8 ***Tools:*** Wipe down all tools with ethanol between samples.

Preservation

9 We recommend storing samples on dry ice in the field no more than 5 hours after you collect them. Subsequently, you should store samples at -20 °C or -80 °C as soon as possible.

If you do not have access to a freezer or dry ice at or near your field site, we recommend using Zymo's Xpedition Lysis/Stabilization Solution™ since it is stable at room temperature for at least a month.

Note: If you choose to use the Zymo Xpedition buffer be aware that it works optimally when extraction happens within a month of collection. Additionally, it forms a precipitate when interacting with the MoBio PowerSoil Extraction Kit C1 solution. See the DNA extraction protocols for the ZEN project for advice to deal with this issue.