DNA Extraction Protocol for Plant and Lichen Tissues Stored in CTAB

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Abstract

This protocol is a modified version of the MoBio PowerPlant Pro DNA extraction kit for plant and lichen tissues that have been stored in CTAB buffer. Prior to being placed in CTAB, photosynthetic tissues from plants and lichens were surface-sterilized with sequential washes in 95% EtOH, 0.5% sodium hypochlorite, and 70% EtOH and air dried under sterile conditions. Twenty-four tissue pieces per host were placed in 750 uL of CTAB buffer. Four tubes per host individual were sampled for a total of 96 tissue pieces.

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Guidelines

Guidelines:

- *Gloves must be worn at all times. Change gloves frequently and decontaminate often with DNA Away.*
- *Pipetting must be done extremely carefully to minimize the risk of aerosols that can easily cause contamination between samples.*
- *Use only unopened, sterile, aerosol-resistant pipette tips (filter tips) to minimize contamination of the pipette shaft and your samples. Clean the body of the pipettes regularly with DNA Away. Discard pipette tips after each use to avoid cross-contamination. Eject pipette tips carefully to prevent aerosol formation or other splashing.*
- *Remember to always clean the hood and any equipment with DNA Away and decontaminate where possible with UV for 30 minutes prior to use to avoid cross-contamination.*
- *The pipettes used need to be calibrated annually.*
- *Be careful not to touch the inside lid of tubes as it will cross-contaminate samples.*

Before start

You will need the following reagents and equipment for the protocol.

Reagents:

-DNase Away

-Stainless Steel Beads, 3.2 mm (Next Advance Cat # SSB32)

-Stainless Steel Bead blend, 0.9-2.0mm (NextAdvance Cat # SSB14B)

-Zirconium Oxide Beads, 2.0 mm (Next Advance Cat # ZRoB20)

-Liquid Nitrogen

-MoBio PowerPlant Pro DNA extraction kit (MoBio Cat # 13400-50)

Equipment

-Laminar flow hood

-Lyophilizer

-Centrifuge capable of handling 24 1.5-2.0 mL tubes

-Optional: PowerVac™ and 40 autoclaved Mini Spin Filter Adapters

-Next Advance Bullet Blender Storm or comparable bead beater

-Heat block and water bath at 65C

-Parafilm

-Sterile needle

-1000 uL filter tips

-200 uL filter tips

-20 uL filter tips

-10 uL filter tips

-Freezer block (-20C)

Protocol

LYOPHILIZATION

Step 1.

For each host individual, remove 4 CTAB tubes (each containing 24 tissue pieces) from -80 freezer and thaw at room temperature.

LYOPHILIZATION

Step 2.

Clean the laminar flow hood, pipettes, and tip boxes with EtOH and DNase Away. In the laminar flow hood, remove as much CTAB buffer as possible using a 200 ul pipette with filter tips. Discard CTAB buffer.

LYOPHILIZATION

Step 3.

After removing CTAB buffer from the tissue (don't let samples sit at room temperature for very long because the DNA will degrade) quickly parafilm the opening of tube so that the lid remains open.

Use a sterile needle to poke 1-3 holes in the top of the parafilm so that the water can escape during lyophilization. Place tubes in the -20 or -80 freezer.

LYOPHILIZATION

Step 4.

Once all of your sample are cold, turn on the refrigerator for the lyophilizer and place the glass plate on the front (make sure there is no water in the chamber or drain tube).

LYOPHILIZATION

Step 5.

Once the lyophIlizer temperature is at -60C, turn on the vacuum pump. Make sure that there is a vacuum seal on the lid and keep an eye on the pressure gauge. It must be below 150 miliTorr to work properly. If the pressure doesn't drop within an hour do not use the machine.

LYOPHILIZATION

Step 6.

Once pressure is at the appropriate level, release the pressure by turning the white port 180 degrees Quickly take frozen tubes out of freezer and place inside the lyophilizer chamber. Close the lid and close the port. Make sure there is a vacuum seal and the pressure returns to <150 miliTorr.

LYOPHILIZATION

Step 7.

DNA EXTRACTION PREP

Step 8.

In a sterile laminar flow hood, remove the parafilm from the tubes (being careful not to touch the inside lip of the cap) and add beads for homogenization.

For all plant samples except mosses, add 100 ul (3-4 beads) of the autoclaved 3.2 mm stainless steel beads and 100 ul of the autoclaved stainless steel bead blend using a sterile scoop.

For mosses and lichens, add 100 ul of the autoclaved 2 mm zirconium oxide beads to each tube with a sterile scoop. Close the lids securely.

DNA EXTRACTION PREP

Step 9.

DNA EXTRACTION

Step 10.

DNA EXTRACTION (all steps except bead beating and centrifugation are done in sterile laminar flow hood)

**Always write down product #s for each kit used and perform a minimum of one extraction blank per kit.

Before beginning, turn on the water bath to 65C; clean the laminar flow hood, pipettes, and tip boxes, with EtOH and DNase Away; place tubes, reagents, and pipettes in hood with appropriate filter tips in hood; and to dissolve precipitates, heat solution PD2 at 60C on heating block prior to use.

DNA EXTRACTION

Step 11.

In sterile hood, label 3 sets of 40 2.0 mL tubes and 1 set of 40 tubes with the filter column from the MoBio PowerPlant Pro kit.

DNA EXTRACTION

Step 12.

Pour liquid N into small dewer. Remove 20 tubes from the -20 freezer when ready to proceed.

DNA EXTRACTION

Step 13.

Drop 20 of the tubes into the liquid N for a minimum of 15 seconds (until liquid N stops bubbling). Remove the tubes with a forceps and quickly place in the bead beater.

At level 12, bead beat lichens for 30 sec and plants for 1 minute (FYI: some plants require > 1 min to grind tissue. If this is necessary, refreeze tubes liquid N and beat for an additional 1 minute. Repeat

until tissue is ground). *Make sure that the tissue has been homogenized to a fine powder.* ***Do not allow tubes to thaw.***

Place tubes in -20 freezer block.

DNA EXTRACTION

Step 14.

In the laminar flow hood, quickly add 410 uL of solution PD1 and 40 uL of PSS solution to each tube. Then, to each tube add 50 uL of PD2 and 3 uL of RNase solution to each tube. Close the lids securely.

(Note: if you are doing a lot of samples, you can premix the RNase and with PD1.)

DNA EXTRACTION

Step 15.

Tubes might be frozen from -20 freezer block. Allow to thaw prior to bead beating an additional 15-30 sec to completely mix the tissue and reagents and remove the clump of tissue from the bottom of the tubes.

DNA EXTRACTION

Step 16.

Store tubes in refrigerator while you repeat steps 13 through 15 for another 20 tubes.

DNA EXTRACTION

Step 17.

Place all 40 tubes in water bath at 65C for 15 minutes, inverting every 5 minutes during incubation.

DNA EXTRACTION

Step 18.

Centrifuge tubes at 13.2 rpm (13,000 x g) for 2 minutes.

DNA EXTRACTION

Step 19.

Transfer the supernatant to a clean 2 mL tube (pre-labeled). (Expect around 400 uL of supernatant.)

DNA EXTRACTION

Step 20.

Add 200 uL of PD3. Vortex tubes for 5 seconds and then incubate tubes at 4 C for 5 min.

***This is the amount of PD3 that worked well for my samples. MoBio recommends adding 175-250 uL PD3 during this step. Test to see what works best for your samples.

DNA EXTRACTION

Step 21.

Centrifuge tubes at 13.2 rpm (13,000 x g) for 2 minutes.

DNA EXTRACTION

Step 22.

Avoiding the pellet, transfer up to 600 uL of supernatant to a clean 2 mL tube.

DNA EXTRACTION

Step 23.

Add 600 uL of PD4 and 600 uL of PD6 to tube. Vortex for 5 seconds to mix.

DNA EXTRACTION

Step 24.

Load 575 uL of lysate onto spin column in 2 ml tube. Centrifuge at 11.6 rpm (10,000 x g) for 30 seconds. Discard flow-through. Repeat 3x.

DNA EXTRACTION

Step 25.

Add 500 uL of PD5 to spin column. Centrifuge at 11.6 rpm (10,000 x g) for 30 seconds. Discard flow-through.

DNA EXTRACTION

Step 26.

Add 500 uL of PD6 to spin column. Centrifuge at 11.6 rpm (10,000 x g) for 30 seconds. Discard flow-through and centrifuge again for 2 min at maximum speed to remove EtOH.

DNA EXTRACTION

Step 27.

Place spin column in new, labeled 2.0 mL tube. Add 50-75 uL of solution PD7 (10 mM Tris, pH 8.0) to the center of the white filter membrane and incubate for 3 minutes at room temperature. Centrifuge at 11.6 rpm (10,000 x g) for 30 seconds. (Note: you can add 100 ul of PD7 if you want less concentrated DNA.)

DNA EXTRACTION

Step 28.

DNA EXTRACTION

Step 29.

Store DNA at -20 or -80 until ready for PCR.

**Note, some samples may still need an additional processing with MoBio PowerClean Pro to remove PCR inhibitors.

DNA EXTRACTION

Step 30.

Optional: Once all 4 tubes from a single host individual have been extracted, pool into a single tube (total volume per host individual will be 200 uL).