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Modified CTAB procedure for extraction of plant DNA from bird feces

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Metabarcoding of Plant ...

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We use this protocol and it's working

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Abstract

Modified CTAB procedure to extract plant DNA from bird feces stored in alcohol. Final concentration of the 2% CTAB solution include the following: [M] 2 % volume CTAB, [M] 1.4 Molarity (M) NaCl, [M] 20 millimolar (mM) EDTA, [M] 100 millimolar (mM) Tris-HCl (pH 8.0), [M] 2 % volume PVP (40). We recommend making a new batch of CTAB solution before each extraction. Modifications include an increased lysis time (up to 12 hours), as well as the use of sodium acetate ([M] 3 Molarity (M)) and NaCl ([M] 6 Molarity (M)) in the wash step. DNA acquired is suitable for PCR.

Guidelines

A fume hood is required to carry out this protocol due to the use of chloroform (CIA 1:24).

Materials

Find a list of materials required per sample below:

Equipment
Analytical balance
Dry bath
Centrifuge
Mini Beadbeater-96
10 beads
1000 µL pipette
100 µL pipette
10 µL pipette

Solutions
800 µL extraction buffer (2% CTAB)
1200 µL of CIA (chloroform-isoamyl alcohol) 24:1
300 µL of 6M NaCl
60 µL of 3M sodium acetate
400 µL cold isopropanol (-20°C)
2 mL of 70% ethanol
1 mL of 95% (or absolute) ethanol
49.5 µL TE
0.5 µL RNase
Distilled water

Disposables
2 tubes (2 mL)
1 tube (1.5 mL)
Tips

Sample
50 to 100 mg of fecal material
200 µL of the alcohol in which the feces were preserved

Safety warnings

Chloroform toxic if swallowed or inhaled. It can cause severe and irreversible health effects, including death. Short-term exposure to high levels of chloroform can damage the central nervous system, liver, and kidneys. Long-term exposure to lower levels of chloroform can damage the liver and kidneys. Use appropriate PPE when applying this protocol.

Before start

This protocol is intended to be carried out over the course of two days. We recommend preparing your samples in the afternoon/evening and carrying out the extraction the following day. Samples will need to stay an additional night in the refrigerator to elute, although you can use a dry bath to accelerate this process.

Prepare materials and samples for extraction (Day 1)

1 **Preparation of Materials**

- Verify that all solutions are prepared and autoclaved material is available
- Turn on the dry bath to 65°C
- Prepare three identical sets of numbered tubes (2 sets of 2 mL tubes and 1 set of 1.5 mL tubes)
- Record in the lab notebook the correspondence between the tube numbers and sample IDs

2 **Preparation of Samples**
Weigh 50 to 100 mg (~80 mg) of fecal material for each sample directly into the tube. Add 200 µL of the sample alcohol to the separated feces.

2.1 Place the open tubes in the dry bath (65°C) for ~2 hours until the alcohol evaporates.

2.2 Add 800 µL of CTAB buffer and 20 µL of Proteinase K with 10 porcelain beads. Place in the Minibeater for 5 minutes.

2.3 Incubate the tubes in a water bath at 60°C overnight (~12 hours)

Extraction (Day 2)

3 **Extraction with CIA I**
Let samples come to room temperature. In a fume hood, perform the first extraction with an organic solvent by adding 600 µL of CIA (chloroform-isoamyl alcohol 24:1).

3.1 Vortex the tubes and then invert them for 5 minutes (at least 20 times) or until a homogeneous emulsion forms.

4 Centrifuge the tubes in a microcentrifuge at maximum speed (13,000 rpm) for 10 minutes.

5 Carefully remove the tubes from the centrifuge, avoiding disturbance of the interface between the two phases.

5.1 Pipette the upper (aqueous) phase into a new tube.

- To expedite this step, set the pipette to 180 µL and withdraw four fixed aliquots (~720 µL). Even if some volume is left behind, this helps avoid contamination with the lower

organic phase.

6 **Extraction with CIA II**
Repeat the extraction with 600 µL of CIA (steps 3/4/5).

6.1 In a fume hood, perform the second extraction with an organic solvent by adding 600 µL of CIA (chloroform-isoamyl alcohol 24:1).

6.2 Vortex the tubes and then invert them for 5 minutes (at least 20 times) or until a homogeneous emulsion forms.

7 Centrifuge the tubes in a microcentrifuge at maximum speed (13,000 rpm) for 10 minutes.

8 Carefully remove the tubes from the centrifuge, avoiding disturbance of the interface between the two phases.

9 Pipette the upper (aqueous) phase into a new tube
- To expedite this step, set the pipette to 180 µL and withdraw three fixed aliquots (~540 µL). Even if some volume is left behind, this helps avoid contamination with the lower organic phase

10 **Precipitate DNA**
Add ½ volume of 6M NaCl (~270 µL) and 1/10 volume (54 µL) of 3M sodium acetate. Then add 2/3 of the volume of the aqueous solution (~400 µL) of cold isopropanol (-20°C).
- Mix gently to precipitate the nucleic acids.

11 Place the tubes in a -20°C freezer for 2 hours.

12 **Wash DNA**
Centrifuge the tubes at 13,000 rpm in a microcentrifuge for 10 minutes to form a pellet.
- Don’t worry if you can't see the pellet—it’s likely there, but it may be small and hard to see.

13 Carefully pour off as much supernatant as possible without losing the pellet.

14 Wash the pellet with 500 µL of 70% ethanol.

14.1 Let the pellet sit immersed for 5 minutes.

14.2 Centrifuge for 5 minutes at 13,000 rpm.

15 Wash the pellet with 500 µL of 100% ethanol.

15.1 Let the pellet sit immersed for 2 minutes.

15.2 Centrifuge for 2 minutes at 13,000 rpm.

16 **Elute DNA**
Resuspend the pellet in 50 µL of TE with RNase.

16.1 Mix 49.5 µL of TE with 0.5 µL of RNase, and multiply by the number of samples.

17 Store in freezer at -20°C