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Pleurozium schreberi (moss) DNA extraction

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Works for me

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ABSTRACT

This is a detailed protocol concerning the DNA extraction from the genome paper titled "**Genome Sequencing of *Pleurozium schreberi*: The Assembled and Annotated Draft Genome of a Pleurocarpous Feather Moss**" (<https://doi.org/10.1534/g3.119.400279>).

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

"Genome Sequencing of *Pleurozium schreberi*: The Assembled and Annotated Draft Genome of a Pleurocarpous Feather Moss" (<https://doi.org/10.1534/g3.119.400279>).

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"Genome Sequencing of *Pleurozium schreberi*: The Assembled and Annotated Draft Genome of a Pleurocarpous Feather Moss" (<https://doi.org/10.1534/g3.119.400279>).

KEYWORDS

Pleurozium schreberi, DNA extraction

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MATERIALS TEXT

- Axenic *Pleurozium schreberi*
- CTAB Buffer; 10% CTAB, 100 mM Tris, 1.4 M NaCl, 20 mM Na2EDTA (pH 8), autoclaved (store at 4°C)
- autoclaved milli-Q water
- N-lauroylsarcosine buffer: 10% N-lauroylsarcosine, 100 mM Tris, 20 mM Na2EDTA (pH 8), autoclaved (store at 4°C)
- Proteinase K stock: 10 µg/µL
- Pronase stock: 10 µg/µL

- RNase A stock: 10 µg/µL
- β-Mercaptoethanol
- ethanol for cleaning
- sterile bench
- water bath that can be set to 60°C and can shake.
- 5 M ammonium acetate
- 70% ethanol
- centrifuge
- chloro-form-isoamylalcohol
- isopropanol
- TE buffer
- Liquid nitrogen
- mortar and pestel
- 2 ml eppendorf tubes
- scale

SAFETY WARNINGS

This protocol involves the usage of 10% CTAB, which is highly volatile.

BEFORE STARTING

Ensure you have obtained the axenic *Pleurozium schreberi* line from the International moss stock centre in Freiburg, Germany, or from one of the laboratories that currently have it. To ensure that you do not contaminate your DNA take the precaution of taking the tissue while in a sterile hood.

Also it is important to have a tissue blender to make young tissue, which is easier to extract DNA from.

Have isopropanol stored at -20°C

Pre-labelled tubes always helps.

Tissue destruction

- 1 Pre-warm the CTAB buffer and N-lauroylsarcosine buffer.
- 2 Ensure that the sterile bench is clean off and all the lab plastic and equipment is sprayed and placed in the sterile bench for at least 15 minutes.
- 3 Clean forceps with ethanol and burn until red.

Proceed to take the tissue and place in 2 ml tubes for storage or to use immediately.
- 4 Grind the tissue using mortar and pestle in the presence of liquid nitrogen until finely ground. Transfer frozen ground tissue to a 2 ml tubes.
- 5 Weigh the tissue by subtracting the tube.

DNA extraction

- 6 For every gram fresh weight add;
- 1 mL CTAB buffer

- 300 µL N-lauroylsarcosine buffer
- 60 µL Proteinase K stock
- 90 µL Pronase stock
- 120 µL β-Mercaptoethanol.

- 7 Mix by inversion and incubate in a water bath in 60°C for 60 min.
- 8 Cool to room temperature.
- 9 Add 1 vol of sterile 5 M ammonium acetate, mix, and incubate for 5 min.
- 10 Centrifuge for 10 min at 15,000 g at room temperature.
- 11 Precipitate the DNA from the supernatant with 1 vol of isopropanol at -20°C for 30 min.
- 12 Centrifuge for 30 min at 12,000 g at 4°C.
- 13 Wash pellet with 70% ethanol, dry, and redissolve in 1 mL TE buffer.
 - 13.1 If the DNA will be used for sequencing, use water instead (milli-Q water)
- 14 Add 100 µL of RNase stock. Incubate at 37°C for 15 min.
- 15 Perform 2 extractions with 1 vol of chloro-form-isoamylalcohol (24:1).
 - 15.1 Vortex for 1 minute and centrifuge for 10 min at 12,000 g at 4°C.
 - 15.2 Transfer the upper, aqueous phase to a fresh tube and add 1 volume of chloroform:isoamyl alcohol (24:1).

15.3 Vortex for 1 minute and centrifuge for 10 min at 12,000 g at 4°C.

15.4 Transfer the upper, aqueous phase to a fresh tube.

15.5 Remove any transferred chloroform by centrifugation 10 seconds at top speed followed by removal of the bottom phase.

16 Precipitate the DNA from the aqueous phase with 1 volume of isopropanol.

17 Centrifuge for 30 min at 12,000 g at 4°C.

18 Wash pellet with 70% ethanol

18.1 Use pipette to discard the ethanol

19 Air dry to ensure ethanol is gone.

Do not overdry

20 Redissolve the pellet in 500 µL TE buffer.

20.1 If the DNA will be used for sequencing, use water instead (milli-Q water)