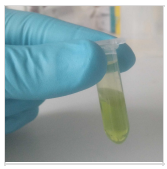

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CTAB genomic DNA-extraction

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ABSTRACT

Protocol for the isolation of genomic DNA for a wide range of plant species.
This protocol is based on a paper published by Aboul-Maaty et al. 2019 (https://doi.org/10.1186/s42269-019-0066-1).
Comments have been added in bold in brackets.
CTAB is used here to filter out phenolic compounds usually found in plant samples that cannot be removed otherwise. We tried extracting DNA using established protocols for *C.reinhardtii* although this doesn't work that well and the quality of the sample is very poor.

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PROTOCOL CITATION

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KEYWORDS

null, genomic DNA Extraction, CTAB, plant, phototroph, iGEM

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MATERIALS TEXT

CTAB (cetyltrimethylammonium bromide), NaCl, Tris-HCl, EDTA (Ethylenediaminetetraacetic acid), 2-β Mercaptoethanol, Chloroform:isoamyl alcohol (24:1 *v/v*), potassium acetate, 100% isopropyl alcohol, 70% Ethanol.

SAFETY WARNINGS

Be careful when working with 2-β Mercaptoethanol, Chloroform:isoamyl alcohol and liquid nitrogen. Wearing gloves and working under the fume hood is advised. (Do not wear rubber gloves when handling liquid nitrogen, but proper ones).

BEFORE STARTING

Make sure to prepare enough of the buffers beforehand and make sure to work with extra care after centrifugation. Every little carryover lowers the purity of your sample.

Buffer preparation

1
- 3X Extraction buffer (autoclaved): 3% CTAB (*w/ v*), 1.4M NaCl, 0.8M Tris-HCl pH 8.0, 0.5M EDTA pH 8.0
- 3M Potassium acetate
- 6M NaCl
- 1x TE Buffer (autoclaved): 10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0

Grinding plant sample

2 Preheat 3x Extraction buffer in water bath at 65°C. Add 0.3% 2-β-mercaptoethanol to the 3× CTAB extraction buffer immediately before use.

3 Grind 50 mg of plant samples into powder in liquid nitrogen using pre chilled mortar and pestle. While still in the mortar, add 800 µl of the preheated 3× CTAB extraction buffer to the grinded plant samples and swirl gently to mix using the pestle. **(This can be scaled depending on how much plant sample is used)**

4 Transfer the sample mixture to a 2-ml microcentrifuge tube, incubate in water bath at 60–65 °C for 1 h, mix gently every 20 min by inverting the tube for 20 times each, then cool down to the room temperature. **(If using greater volumes it is recommended to switch to either 15ml or 50ml falcon tubes)**

Chloroform washing

5 Add an equal volume of chloroform:isoamyl alcohol (24:1 *v/ v*) and mix by slight inversion.

6 Centrifuge at 13,000 rpm for 15 min at room temperature (RT).

7 Using a wide-bore pipet, carefully transfer the upper aqueous phase, which contains the DNA, to a new 1.5-ml Eppendorf tube.

8 Repeat steps 5-8 until the upper aqueous phase is clear.

Precipitation + Washing of DNA pellet

9 Estimate the volume of the aqueous phase (approximately 700 µl) then add half this volume (350 µl) of 6 M NaCl and mix well. Successively, add 1/10 the volume (70 µl) 3 M potassium acetate and simultaneously mix with 500 µl ice-cold 100% isopropyl alcohol (approximately two-thirds the volume of the aqueous phase). Invert gently to precipitate DNA until the formation of DNA threads.

10 Incubate at − 20 °C for 30 min.

11 Centrifuge at 13,000 rpm for 5 min, discard the supernatant.

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12 Invert the tube containing the DNA pellet on tissue paper to complete draining off the supernatant.

13 Wash the DNA pellet with 500 µl of 70% ethanol and invert once (to dissolve residual salts and to increase the purity of the DNA).

14 Centrifuge at 13,000 rpm for 5 min.

15 Discard 70% alcohol from tubes. invert the on filter paper, and allow tubes containing pellet to air dry at room temperature for 15 min. **(Incubating at 60°C worked fine and saves time)**

Dissolving and Analysis of DNA

16 Resuspend the DNA pellet in 50 µl 1× TE buffer. Incubate the DNA at 50 °C for 1 to 2 h to ensure complete re-suspension. **(I usually did this overnight to increase dissolving time)**

17 Measure the quality and quantity of your DNA using a NanoDrop. **(Usually, I get around 25µg of genomic DNA from 50mg of plant sample)**

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