Dec 17, 2019 Version 1

Total DNA extraction from plant tissue using CTAB method V.1

Forked from a private protocol

GigaByte

In 1 collection

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Protocol status: In development
We are still developing and optimizing this protocol

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Materials

2X CTAB Buffer: 100 Mm Tris pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 1% PVP40, and 0.5% (v/v) beta-mercaptoethanol (added daily before the extraction)

Troubleshooting

1 Using a pre-chilled mortar and pestle, grind frozen plant tissue (1-5g grams) into a fine powder

2 Add 500 ul of pre-heated 2 x CTAB buffer (with beta-mercaptoethanol added) to the pelleted cells. Vortex and re-suspend by gently pipetti

ng up and down. 60 °C

3 Incubate at 60 C for 30 min. Periodically, mix gently during the incubation. 60 °C 00:30:00

4 Add an equal volume of chloroform:isoamyl alcohol (24:1), mix very well at RT for 5 min to generate a monophasic solution.

5 Centrifuge for 10 min in 5,000 x g

6 Transfer aqueous phase (upper layer) to new tube.

7 (Optional) Repeat chloroform:isoamyl alcohol extraction (steps 3 and 4) until the interface is clean.

8 Add 2/3 volumes of isopropanol, mix gently for 5 min at RT.

9 Centrifuge for 15 min at 10,000 x g. Discard supernatant.

10 Add 500 uL of 70% ethanol to the DNA pellet, and briefly vortex to dislodge the pellet.

11 Air dry pellet for 30 min. Resuspend in 100ul of TE Buffer.

12 Add 1 ug/mL DNase-free RNase (final concentration) and incubate at 37C for 30 min.

13 Extract the DNA once with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). Centrifuge at 10,000x g for 10 min. Transfer the aqueous phase to a clean tube.

14 Precipitate DNA with 1/3 volume of 7.5 M ammonium acetate and 2.5 volumes of 100% ethanol.

15 Centrifuge at 10,000x g for 10 min, wash with 70% ethanol, and air dry for 30 min at RT. Resuspend in 50 ul of TE Buffer. Allow the DNA to dissolve overnight at 4C