



Dec 17, 2019

## Total DNA extraction from plant tissue using CTAB method

Forked from [Total DNA extraction from microalgae using CTAB method](#)Robert Auber<sup>1</sup><sup>1</sup>Purdue University

In Development

[dx.doi.org/10.17504/protocols.io.bamnic5e](https://doi.org/10.17504/protocols.io.bamnic5e)

Wisecaver Lab



Robert Auber



### MATERIALS TEXT

**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)

- 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



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited