

CTAB Plant Genomic DNA Extraction (modified by Rahul Patharkar)

Reagents:

- 2X CTAB buffer (2% (w/v) cetyl-trimethyl-ammonium bromide (CTAB), 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA)
- Chloroform
- 2-Propanol
- 70% ethanol
- TE buffer (10 mM Tris HCl pH 8.0, 1 mM EDTA)
- Linear Polyacrylamide (LPA) 20 μ g/ μ l (acts as a carrier for DNA precipitation; produces a visible pellet regardless of DNA quantity).

40 mL 2x CTAB buffer	
0.8 g	CTAB
3.27 g	NaCl
4.0 mL	1.0 M Tris-HCl (pH 8.0)
1.6 mL	0.5 M EDTA (pH 8.0)
adjust volume to 40 mL	

Procedure:

- 1.) Add 100 μ L 2X CTAB buffer (w/ 1 μ L LPA) to a 1.5 mL tube (Genemate C32621 tubes fit blue pestles perfectly for optimal grinding).
- 2.) Grind a small amount to plant tissue in the buffer.
- 3.) Rinse the pestle with 200 μ L 2X CTAB buffer into 1.5 mL tube to make a total of 300 μ L.
- 4.) Incubate at 65 degrees Celsius for 10 minutes or longer (up to several hours)
- 5.) Allow to cool
- 6.) Add 300 microliters chloroform and shake hard by hand or vortex thoroughly (should look cloudy)
- 7.) Spin at top speed for 5 minutes (or 1 minute if in a hurry) in a microfuge to separate phases
- 8.) Transfer the upper, aqueous phase to a fresh reaction tube
- 9.) Add 180 microliters 2-Propanol and mix well
- 10.) Spin in a microfuge for 5 minutes to pellet the DNA (the pellet will be clear)
- 11.) Remove supernatant and wash pellet with 500 microliters 70% ethanol (the pellet will become white)
- 12.) Spin briefly in a microfuge
- 13.) Carefully remove the ethanol by pipeting
- 14.) Spin for 5-10 seconds to get all residual ethanol to the bottom
- 15.) Remove ethanol by pipeting again being careful to get everything without removing the pellet
- 16.) Add 100 microliters TE buffer and allow the pellet to dissolve
- 17.) Mix or vortex before use. Use 1 microliter in a PCR reaction

Note: This protocol can easily be scaled down to one third the size and use 0.5 mL tubes instead (RPI #145505 tubes fit RPI 0.5 mL pestles perfectly)

For video protocol see: <https://rahulpatharkar.000webhostapp.com/2018/10/plant-genomic-dna-extraction>