

DNA isolation was carried out according to the CTAB method, with minor modifications required to separate the secondary metabolites from the DNA.

Protocol:

- 1) Add 750 µl 2x CTAB buffer and 3.0 µl β-mercaptoethanol to Eppendorf tubes.
- 2) Grind 0.5–1.0 g of tissue using a homogenizer and small balls;
- 3) Add shredded tissue to each tube and mix well.
- 4) Incubate in a water bath at 55-60 ° C for 1-5 hours, stirring every 15 minutes.
- 5) Add 700 µl SEVAG (24: 1) to each tube and mix thoroughly. Centrifuge at 9240 rpm for 10-15 minutes. Transfer the supernatant to a new Eppendorf tube.
- 6) Add 0.33 volumes of ice-cold isopropanol and store at -30 ° C for at least 1 hour.
- 7) Centrifuge 9 240-13305 GPM for 10 minutes at room temperature. Remove supernatant. Dry. Repeat steps 6 and 7 two times;
- 8) Resuspend the pellet in 100-200 µl TE. Add 1-2 µl RNase 10 mg / ml.
Mix well and incubate for 30 min at 37 ° C.
- 9) Add 20 µl (0.1 volume) 2.5 M NaOAc and 500 µl (2-2.5 volumes) ice-cold 95% ethanol and store at -20 ° C ≥ 30 min. Centrifuge 9 240-13305 rpm for 5 min.
Remove supernatant;
- 10) Wash the precipitate with 1 ml of 70% ethanol. Do not touch the sediment. Unscrew on a centrifuge 9,204 rpm 4 min and drain off ethanol. Dry the sediment;
- 11) Add Mili Q water, resuspend, Store at -20 ° C.

Required Reagents:

- CTAB buffer (2 × CTAB: 50 ml of 1.0 Tris-HCl, pH 8, 140 ml of 5 M NaCl, 50 ml of 0.25 M EDTA pH 8, 10 g of CTAB, bring to 500 ml with distilled water)
- 2-mercaptoethanol
- chloroform solution to isoamyl alcohol (SEVAG), 24: 1
- Isopropanol
- Tris-EDTA (TE)
- RNase, 10 mg / ml
- Soda oxaloacetate (NaOAc), 2.5 M
- Ethanol, 70% and 95%