Soltis Lab CTAB DNA Extraction Protocol

(Reference: Doyle & Doyle, 1987; and Cullings 1992)

Revised November 14, 2002

1. Prepare CTAB buffer, use within 2-3 days, store capped: Add polyvinylpyrrolidone (Fisher Cat#: BP431-500) and β-mercaptoethanol (Fisher Cat#: BP176-100) and stir to dissolve right before starting extractions:

CTAB	PVP	β-merc
0.5 ml	0.02g	2.5μl
5 ml	0.2g	25μl
20ml	0.8g	100μl

2. Weigh out **10-20 mg** of silica-dried plant tissue.
3. **Grind** tissue with blue pestles
   a. Pestles can be reused, store in 10% bleach solution and rinse well with DI water before using them
   b. Grinding can be done with or without the aide of liquid nitrogen and/or washed and autoclaved sand.
4. Add **500μl of CTAB buffer** and grind samples a bit more.
5. Incubate samples at **55˚C for 1hr t**o overnight.
6. Add **500μl of 24:1 Chloroform :Iso Amyl Alcohol** and mix well by shaking tubes.
7. Centrifuge for **5-10 minutes** at maximum speed.
   a. Following centrifugation, you should have three layers: top: aqueous phase, middle: debris and proteins, bottom: chloroform.
   b. Go on to the next step quickly so the phases do not remix
8. Pipette off the **aqueous phase** taking care not to suck up any of the middle or chloroform phases. Pipetting slowly helps with this.
9. Place the aqueous phase into a new labeled eppendorf tube.
10. **Estimate the volume** of the aqueous phase.
11. Add 0.08 volumes of cold **7.5 M ammonium acetate**—see attached table.
12. Add 0.54 volumes (using the combined volume of aqueous phase and added AmAc) of cold **isopropanol** (=2-propanol)—see attached table.
13. Mix well.
14. Let sit in freezer for 15 min to overnight.
    a. Longer times will tend to yield more DNA, but also more contaminants.
15. Centrifuge for **3 min** at maximum speed.
16. Pour or pipette off the liquid, being careful not to lose the pellet with your DNA.
17. Add **700μl of cold 70% Ethanol** and mix
18. Centrifuge for **1 min** at maximum speed.
19. Pour or pipette off the liquid, being careful not to lose the pellet with your DNA.
20. Add **700μl of cold 95% Ethanol** and mix
21. Centrifuge for **1 min** at maximum speed.
22. Pour or pipette off the liquid, being careful not to lose the pellet with your DNA.
23. **Dry** the pellet:
    a. Place samples in the speed vac for 20 min.

or

b. Invert samples on a Kim-wipe and let stand for 1 hr or until dry.

24. **Resuspend** samples with **100μl of TE buffe**r. Allow to resuspend for 1hr at 55˚C or overnight in refrigerator before running a test gel using 5μl of the DNA.

Stocks:

CTAB: for 1L of CTAB buffer

100 ml of 1 M Tris, pH 8.0
280 ml of 5 M NaCl
40 ml of 0.5 M EDTA
20 g of CTAB (Cetyltrimethyl ammonium bromide, Amresco cat#:0833-1Kg)

TE buffer:

[Final]	for 1L use:
10 mM	10 ml of 1 M Tris, pH 8.0
1 mM	2 ml of 0.5 M EDTA

1 M Tris, pH 8.0: for 1 L

121.1 g Tris (Fisher Cat#: BP152-5)
700 ml ddH_2O
Dissolve tris and bring to 900 ml.
pH to 8.0 with concentrated HCl (will need ~50ml)
Bring to 1 L.

0.5 M EDTA pH 8.0: for 1 L

186.12 g of EDTA (Fisher Cat#: BP120-1)
750 ml ddH_2O
Add about 20 g of NaOH pellets
Slowly add more NaOH until pH is 8.0, EDTA will not dissolve until the pH is near 8.0.

5 M NaCl: for 1 L

292.2 g of NaCl (Fisher Cat#: BP358-10)
700 ml ddH_2O
Dissolve and bring to 1 L.

Cullings, K.W. 1992. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology* **1:**233-240.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* **19:**11-15.