**Sorbitol extraction of plant DNA**

Adapted by Andrew Krohn (alk224@nau.edu) from: Storchova et al. (2000). An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. Taxon (49):79-84.

**EXTRACTION BUFFER:**
- 0.35 M sorbitol
- 0.1 M Tris-Cl, pH 7.6
- 0.005 M EDTA
- 10 mM 2-mercaptoethanol (0.1% v/v)

**LYSIS BUFFER:**
- 0.2 M Tris-Cl pH 7.6
- 0.05 M EDTA
- 2 M NaCl
- 2% (w/v) CTAB

**1X TER, pH 8.0:**
1X TE containing 10 ul RNase (100 mg/ml) per 100 ml

**CHLOROFORM:ISOAMYL ALCOHOL (24:1)**

**ISOPROPANOL**

**70% ETHANOL**

* Just prior to beginning, make fresh extraction buffer in a falcon tube. You will need 1.6 ml for every sample (times 8 = 12.8 ml). Account for possible excess needs and prepare 14 ml for every 8 samples you are preparing. To make 14 ml extraction buffer, add 14 ml pre-made extraction buffer to a falcon tube, and add 14 µl 2-mercaptoethanol. Close tube tightly and invert to mix. KEEP 2-MERCAPTOETHANOL IN THE HOOD! Leave 2-mercaptoethanol in the hood for at least 20 minutes to allow any spillage on the bottle to evaporate. The extraction buffer may be brought back to the bench. If you open 2-mercaptoethanol out of the fume hood, everyone will hate you, you’ll never win the lottery, and your dog will pee on your bed.

1. About 0.2 g fresh leaf tissue is placed into a 1.5 ml centrifuge tube. The tube is placed into liquid nitrogen and the frozen tissue ground using a clean plastic pestle into a fine powder. This step is crucial, must be done quickly, and the tissue not allowed to thaw.

2. 1.3 ml of extraction buffer is added.

3. Mix well by hand and leave suspension on bench for 15 minutes at RT. During this time, polyphenol compounds are extracted in to the buffer.

4. Centrifuge samples 8 minutes at max speed.
5. Remove and discard the supernatant (pellet will not be solid, nor will it adhere well to the bottom of the tube). Add 300 µl extraction buffer, and mix by hand. Shake the suspension down into the tube and add 400 µl lysis buffer. Mix well and place in pre-heated 65°C dry bath (adjust temp for various species). Incubate for at least 15 minutes. Occasional mixing throughout the incubation will increase yield. Prolonged incubation may result in higher yield of DNA.

6. Remove samples from the bath, noting the duration they were heated. Allow samples to cool to RT (about 5 minutes).

7. In the hood, add 600 µl CHCl₃:isoamyl 24:1, cap tightly, and mix very well (shaking horizontally). Centrifuge 5 minutes at 8,000 rpm.

8. Remove 600 µl of aqueous (upper) phase to a fresh 1.5 ml tube. Make certain you do not transfer any organic phase, or any solid from the interphase. Place CHCl₃-containing tubes in the hood for proper CHCl₃ disposal.

9. To the removed aqueous phase, add 400 µl isopropanol. Mix very well by multiple inversion. Place in -20°C freezer for >15 minutes.

10. Remove samples from freezer and centrifuge 10 minutes at max speed. Discard supernatant being careful not to disturb the DNA pellet (if visible).

11. Add 500 µl 70% EtOH to the pellet, mix by hand and centrifuge 5 minutes at max speed.

12. Carefully discard supernatant (pellet is even more likely to be lost in this step than in the last).

13. Place tubes with caps open on the dry bath set to 50°C.

14. When samples are dry (check frequently while drying as you don’t want to over-dry), dissolve DNA pellet in 40 µl 1X TER pH 8.0, mix well and spin samples down (use quick spin button on centrifuge). Check samples on gel, quantify, and store in the appropriate location.