CTAB Isolation of DNA from Fresh or Dried Tissue

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Before you start: Prepare fresh CTAB lysis buffer by adding 0.2% β-mercaptoethanol (BME) to 2X stock CTAB buffer, preparing only as much as you need for the extractions you need to do that day (i.e., **to each 1 ml of 2x CTAB, add 2 µl BME**). Heat the buffer to **60°C**.

A. Grinding and cell lysis with CTAB Buffer

1. Add 2 stainless steel ball bearings to 1.5 mL tubes (one for each sample).
2. Add a small amount of tissue into tubes (up to **20 mg** dry weight or **80 mg** wet weight).
3. Freeze the tissue by dipping the racked tubes in liquid nitrogen for about 10 seconds.
4. Transfer racks to the tissue disruptor and grind at for about **30 s** at the maximum rate.
5. Repeat steps 4 and 5 to ensure that the tissue has been turned into a fine powder.
6. Add 400 **µl** of CTAB lysis buffer prepared as above (see "before you start").
7. Vortex well to get the powdered tissue mixed with the extraction buffer, and incubate the crude extract in a **60°C** water bath for **60 min**. Mix every 15 min by inverting tubes.

B. Removal of proteins with chloroform:isoamyl alcohol (chloroform:IAA).

1. Add an equal volume **(400µl) of chloroform:IAA (24:1)** to plant extract. Use a glass dish or glass tube to handle the chloroform:IAA; it will dissolve plastic.
2. Cap tubes securely and mix by inversion **(30-50x)** to produce an emulsion.
3. Centrifuge the tubes for **20 minutes** at maximum possible rpm to separate the phases.
4. Remove aqueous (top) phase to clean tubes, **200 µl at a time** avoid the layer of gunk).

C. Precipitation, washing and drying of DNA

1. Add an **equal volume of -20°C isopropanol**. Mix gently by inversion.
2. Precipitate at **-20°C for at least 20 min** (up to several days).
3. Centrifuge for **20 min. at 4,000 rpm.** Carefully pour off supernatant.
4. Wash pellet with **500µl -20°C 70% ethanol**. Do not dislodge pellet by flicking tube, etc.
5. Centrifuge for **5 min at around 14,000 rpm** to secure pellet.
6. Aspirate off or simply decant the supernatant, being careful not to lose the pellet of DNA.
7. Dry pellet by leaving the open tubes in a cool, dry place overnight.
8. Resuspend the pellet in 50 to 100 µl TE.

Recipe for 100 mL of 2x CTAB buffer:

2g CTAB (cetyltrimethylammonium bromide)
8.2 g NaCl
10 mL 1M Tris, pH 8
4.0 ml 0.5M EDTA, pH8

Directions: warm 60 mL of deionized water on a hot plate; add the salt and the CTAB. Stir until the CTAB goes into solution (don't let it boil). Ideally, you should sterile filter this and store it in a sterile bottle. In the refrigerator, the CTAB will drop out of solution; heat to dissolve.

Notes: I've found that the single most important factor in the success of this method is the amount of tissue used. Less is definitely more, as they say. For plants that are fleshy or succulent, use less tissue. If using dry tissue, never use more than 20 mg. I have recovered as much as 40 µg of DNA from 20 mg of tissue, so you can err on the side of less tissue and still get plenty of DNA.