**CTAB DNA Extraction Protocol**

**Materials per sample:**

* 5 – 1.5ml microcentrifuge tubes
* Polyvinylpyrrolidone (PVP)
* 2% CTAB Lysis Buffer
  + 2% CTAB, 1.4M NaCl, 100mM Tris-HCl pH8, 100mM ꞵ-ME, 20 mM EDTA
* Phenol/chloroform/isoamyl alcohol (25:24:1)
* Chloroform
* Ice-cold 100% Ethanol
* Ice-cold 70% Ethanol
* T “low” E Buffer
  + 10 mM Tris pH 8.0, 0.1 mM EDTA

**Procedure:**

1. Use ~100 mg of plant tissue. Place sample in sterile mortar, bathe in liquid nitrogen (N2)
   * \*\*Store in -80ºC freezer for 4 hours, or -20ºC freezer overnight in a 1.5mL microcentrifuge tube if liquid N2 is not an option\*\*
2. Add 15mg of PVP, grind tissue into powder (will be a slurry if tissue is not flash frozen)
   * \*\*If grinding in 1.5mL tube skip this step\*\*
3. Using ice-cold pestle, grind frozen tissue into powder
   * \*\* If grinding in 1.5mL tube, complete step 4 first (500 µL of CTAB buffer), then grind with an ice-cold **micropestle**\*\*
4. Add 500-1000µl of 2% CTAB lysis buffer to mortar, grind again briefly
5. Pipet 500µL of slurry into a sterile 1.5ml microcentrifuge tube, vortex, use microfuge to get all liquid to bottom of tube
6. Add 4µL RNase A to microcentrifuge tube
7. Incubate tubes at 65°C for 15 minutes (water bath or heat block).

\*\*\*\*\***UNDER THE FUME HOOD\*\*\*\*\***

1. Add 500 µL of phenol/chloroform/isoamyl alcohol (25:24:1) to cell lysate.
2. Vortex thoroughly and centrifuge for 10 minutes at 14,000xg to separate the phases, making sure to balance the centrifuge.
3. Transfer upper aqueous phase to a new 1.5 mL tube

\*\*\*\*\***UNDER THE FUME HOOD\*\*\*\*\***

1. Repeat step 8, using an equal volume of phenol/chloroform/isoamyl alcohol as the newly transferred aqueous phase, vortex thoroughly and centrifuge for 10 minutes at 14,000xg.
2. Transfer upper aqueous phase to a new 1.5. mL tube.

\*\*\*\*\***UNDER THE FUME HOOD\*\*\*\*\***

1. Add 2x volume of chloroform, shake for 30 seconds, centrifuge for 5 minutes at 14,000xg
2. Transfer upper aqueous phase to a new 1.5. mL tube.
3. Precipitate the DNA by adding 2x volume of ice-cold 100% ethanol. Mix by inverting and incubate at -20°C overnight (or longer).

**Ethanol Precipitation of DNA**

1. Remove samples from previous day from the -20ºC freezer
2. Centrifuge samples at 14,000xg at 4ºC for 15 minutes. Pipet off the supernatant without disturbing the pellet.
3. Add 1 mL of ice cold 70% ethanol. Centrifuge at 14,000xg for 10 minutes at 4ºC. Pipet off the supernatant without disturbing the pellet.
4. Repeat step 14, leave a small bit of liquid (~10-20µl) at bottom of tube.
5. Quick centrifuge for 30 seconds. Carefully pipet off the remaining supernatant (usually < 20 µl, use a P200). Let samples sit open at room temperature for 10-20 minutes, examine the tubes carefully to ensure that no liquid ethanol remains. DO NOT let the pellet get over dried.
6. Suspend samples in 30-60ul of “T low E” buffer. Gently move the pellet off of the tube wall into the liquid with the pipet tip to aid suspension. Put samples in 4ºC refrigerator.