Protocol for Fresh Samples, Silica-dried Leaves or Herbarium Samples: DNA Mini-preps

Adapted by Theresa Culley (protocol originally from Andi Wolfe's Lab)

Step 1:

SAMPLE PREPARATION & GRINDING

- Turn water bath on to 60°C at least one hour before grinding.
- Fill large basin with water; add soap
- Prepare buffer and warm to 60°C for 10 min before use:

  For ~7 samples: add approximately **15 µl** mercaptoethanol to **7.5 ml** 2X CTAB
  For ~14 samples: " " " **30 µl** " " **15.0 ml** "

- Weigh or estimate a small amount of dried tissue (e.g., 50-90 mg of leaves/stem). For fresh tissue, add about a dime-sized portion. Place the tissue in a clean mortar.
- Record sample information on data sheet
- Label a 1.5 ml eppendorf microfuge tube with sharpie
- Measure out a small volume of sterilized sand and approximately equal amount of PVP into the mortar. Remember that you can always add more sand and PVP later if needed.
- Add 500 µl CTAB buffer to mortar and GRIND tissue *thoroughly* with pestle
- Add another 500 µl CTAB buffer (for a final volume of 1000 µl) and grind again
- Carefully pour the ground material into the labeled tube
- Place mortar/pestle into soapy water

EXTRACTION AND ISOLATION OF DNA

- Incubate for 1 hr at **65-70°C** in water bath
  - Mortars/pestles can be cleaned while waiting:
    1. clean out with scrubbie in soapy water and rinse
    2. fill ~ halfway with bleach soln and soak for 15 min, pour bleach back into bleach container
    3. rinse with distilled water and fill ~ halfway with HCl soln for 15 min, pour HCl back into HCl container
    4. rinse with distilled water and prepare for autoclaving:
       - place cleansed mortars/pestles in autoclavable container, cover with foil and mark with autoclave tape

- sterilize in autoclave for 20 minutes, dry in autoclave for 10 minutes
- remove from autoclave promptly using heat-resistant gloves

- Add equal volume (~ 700 µl) chloroform: isoamyl alcohol (24:1) IN HOOD (*will nearly fill a 1.5 ml microfuge tube---holds 1.6 ml)*

- Spin for 20 min at maximum speed (13 rpm) or until you have a clear liquid above. *(Hint: if viscous region is apparent just below plant sample then you have removed from spin too early: i.e., from top to bottom you may see clear region, viscous zone; plant layer, dark zone)*

- While waiting for the samples to finish spinning, label a new set of eppendorf tubes.

- Transfer top aqueous layer phase to a new microfuge tube with SEPARATE tips. Empty old tubes into chloroform waste bottle and put tubes into large beaker in hood. Throw dry tubes out after 24 hr.

- *Optional:* repeat chloroform: isoamyl extraction if separation seems poor or if spin is inadequate. This is often necessary for *Viola* and sometimes for *Pyrus*.

DNA PRECIPITATION

- Add 540 µl COLD isopropanol (stored in freezer); invert 2-4 times and store overnight at -20 °C (or 2-3 hr @ -84 °C)

Step 2:

- Spin for 1-3 min at maximum speed (13 rpm)

- For samples with clear pellets, decant the supernatant and drain carefully with a Kimwipe. *(For samples with gelatinous material above pellet, remove all but a thin mm or so band of isopropanol with micropipette, leaving gelatinous portion and pellet intact)*

- Wash pellet with 500 µl 75% EtOH (note: some argue that DNA goes into water at 70%); invert tube several times *(gelatinous material should dissolve)*. Shake or tap the tube until the pellet becomes dislodged.

- Spin for 3 min at max speed

- Remove supernatant; dry with Kimwipe or on paper towel, leaving pellet intact (you can remove the remaining EtOH carefully with small pipette if necessary)

- Dry with speedvac or in vacuum oven (20 Hg) for 20 min, make sure ALL EtOH is gone. *(It can dry longer if needed, but the longer it dries, the harder the pellet is to get into solution)*

FINAL CLEANING & RESUSPENSION OF DNA

- Resuspend pellet in 80 µl 1X TE with 20-200 µl pipette with **filter tips;** gently pump pipette to break up pellet

- Add 8 µl of 7.5 M ammonium acetate (NH_4OAc) by putting drop on side of tube; add 180 µl of 100% (or 95%) EtOH.

- Mix and place at -20 °C <u>overnight</u> (or 2-4 hr in -80 °C ultracold)

Step 3:

- Spin 2-3 min, decant supernatant *(optional cleaning: add final wash of 75% EtOH and spin again and decant)*

- Vacuum dry and resuspend in 50-100 µl TE with separate **filter tips**. (I usually use 100 µl, but if extraction seems poor, use less TE.)

- Incubate at 37-40 °C for 15-30 min to ensure DNA is in solution

- Centrifuge a little

- Aliquot with separate tips and store at -84 °C (long-term), -20°C (temporarily), or 4 °C (active use)

- Verify presence of DNA using test gel (see below)

MAKING A GEL

- Add **50 ml** TAE buffer to **0.50 g** agarose (1% gel). Microwave until agarose is dissolved (~1 min, but watch carefully), cool flask under water until comfortable to touch.

- Add 1.20 µl ethidium bromide (TOXIC) to cooling gel soln and swirl

- Pour into gel rig with comb in place, refrigerate if solidified gel has to sit for extended period of time

- Load gel after mixing together separately on parafilm:
  5 µl ladder + 1 µl green dye (or 5 µl blue juice if used instead of green dye)
  5 µl DNA + 1 µl green dye
- Run gel at 120 V for ~20 min, look for tiny bubbles rising in buffer (runs towards red)

- Take picture under ultraviolet light, wrap gel in saran wrap and throw out

Procedure modified by T. Culley from protocol by S. Kephart via practice and discussions while in Andi Wolfe's lab; adapted in part from Doyle and Doyle (1987); K. Cullings (1992; Molecular Ecology); and D. Loockerman and R. Jansen (1996, in T. Stuessey and JS Sohmer, Sampling the Green World).

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