Last revised 3 August 2005 by Kevin deHaan

Miniprep protocols for isolation of plant DNA

Reference: Torres AM, Weeden NF, Martin A (1993) Linkage among isozyme, RFLP, and RAPD markers in *Vicia faba*. Theor Appl Genet 85:937-945.

DNA isolation - checklist:

tissue samples
water bath at 60 °C
fume hood
micropipettes
tips
weighing spatulas (spoon type)
1.5 ml tubes and racks
black Sharpie pen
liquid nitrogen
2% CTAB buffer
β-mercaptoethanol
24:1 chloroform:octanol
cold 95% ethanol or isopropanol
cold 70% ethanol
clean ceramic mortars and pestles
waste bottle for solvents
waste container for tips and tubes
safety goggles/glasses
gloves
RNase A
37 °C water bath
cut tips
sterile water
TE buffer

Part I: preliminary steps.

Safety: Gloves should be worn throughout protocol. Eye protection should be used when handling liquid nitrogen. Grinding steps and handling of chloroform should be performed in fume hood to minimize exposure to solvent fumes. All waste solvent solutions and used tips and tubes should be disposed of in waste containers in fume hood.

- All implements, containers and solutions should be autoclaved before use **except for** ceramic mortars and pestles, gloves and safety glasses, organic solvents such as β-mercaptoethanol, chloroform, and alcohols.
- Assemble all necessary materials (see checklist).
- Make up necessary volume of grinding buffer (1 ml/sample) by adding β-mercaptoethanol to CTAB buffer at rate of 4 µl/ml.

Part II: grinding and extraction of plant tissue. These steps to be performed in fume hood.

1) From each plant to be sampled, collect one or two young leaves (ca. 0.1 g fresh weight). If necessary, store in a labeled, standard 1.5 ml microfuge tube in refrigerator or on ice until used.

CAUTION: Liquid nitrogen exists at temperatures at and below -196 °C. It will violently boil whenever coming in contact with a substance above -196 °C. Liquid nitrogen and nitrogen gas may exit the storage container when inserting the steel receptacle. Always wear eye protection when working with liquid nitrogen.

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2) Add enough liquid nitrogen to cover one leaf sample to one mortar. When the liquid nitrogen stops violently boiling, transfer a tissue sample to a ceramic mortar (tissue should be completely covered by liquid nitrogen). Grind slowly by pressing the pestle gently on the sample until tissue is a fine powder. Heavy grinding will cause liquid nitrogen, and the plant tissues floating in it, to exit the mortar.

3) Before ground tissue thaws, add 1 ml grinding buffer. Then continue grinding until material becomes a slurry.

4) Transfer approximately 800 µl of slurry into a labeled, 1.5 ml microfuge tube.

5) Repeat steps 2-4 until all samples are processed.

6) Insert tubes in Styrofoam floats and place in 60 °C water bath. Incubate 1 hr.

Part III: solvent extractions, DNA precipitation.

1) Remove tubes from water bath and allow to cool to room temp (ca. 10 minutes on bench top).

2) Add sufficient (500-800 µl) 24:1 chloroform: octanol to nearly (but not completely) fill tube. Close tube.

3) Vortex tubes until the mixture appears uniformly green (~10 seconds).

4) Centrifuge tubes at 14,000 x g (maximum speed in microfuge) for 5 minutes to separate phases.

5) Transfer upper (aqueous) phase containing DNA into new, labeled, sterile (autoclaved) 1.5 ml microfuge tube (there should be 500 - 800 µl). Avoid transferring lower (chloroform) phase solution. If aqueous solution is cloudy, then repeat chloroform extraction steps (2-5).

6) Gently add cold 95% isopropanol (or ethanol) to nearly fill tube. Close tube and hold on ice or in freezer.
Note: it is important that the added volume of alcohol exceed the volume of the extract. Extract can be divided into multiple tubes allow more addition of alcohol.

7) After milky precipitate begins to appear at solvent interface (within 5-10 minutes), gently invert tubes several times to mix solvents. DNA may now appear as vitreous blob. Holding tubes on ice or in freezer overnight may increase DNA yield (optional)

8) Centrifuge the tube at 14,000 x g for 5 minutes. Then, pour off supernatant into sink, being careful not to dislodge white DNA pellet. Add 1 ml cold 70% ethanol. Hold on ice 10 minutes or overnight.
*Dispose of original extraction tubes as follows. Decant chloroform phase and precipitate from original tubes into labeled (chloroform waste) bottle in fume hood. Place open tubes into container in fume hood labeled for used tubes and tips.

9) Centrifuge the tube containing DNA in 70% ETOH at 14,000 x g for 5 minutes.

10) Pour off supernatant, then remove residual supernatant with pipette, being careful not to lose DNA pellet.

11) Dry DNA by placing open tubes in air flow in laminar flow hood for several hours. (Alternately, dry in speed vac).

12) Add 50 µl TE to each tube. Refrigerate overnight.

RNase treatment.

13) Make up enough RNase solution to provide 50 µl for each sample to be treated. The RNase solution is made by adding 1 µl RNase stock (10 mg/ml) to 1 ml sterile water in a microfuge tube, then vortexing for 10-15 seconds.

14) Add 50 µl RNase solution to each tube of DNA. Spin tubes for 15 seconds in microfuge to bring all solution to bottom of tubes. Mix each tube thoroughly by gently pipetting DNA/RNase solution up and down 10 times in a pipette tip that has been cut to enlarge the tip opening. Use a different tip for each sample.

15) Incubate tubes at 37 °C for 1 hour to allow digestion of RNA.

Quantification and storage.

16) Quantify DNA (methods described elsewhere) and dilute with sterile water to a final DNA concentration of 5-40 µg/ml.

17) Store DNA in refrigerator, or in freezer for long-term storage.