

successfully by David Coates' laboratory, but the method should work with any of the column–resin combinations available for purifying plasmid DNA.

1. Freeze 0.5 g leaf material in liquid nitrogen, then grind it in liquid nitrogen in a precooled mortar and pestle.
2. Add the powder to 4 mL CTAB extraction buffer prewarmed to 65°C (for dried material, dilute 1:1 with sterile ddH₂O first), containing 1% (w/v) PVP. Add 100 µL 10 mg/mL RNase A and incubate at 65°C for 30 min.
3. Add 4 mL chloroform/isoamyl alcohol (24:1); mix gently for 5 min. Spin for 5 min (benchtop centrifuge), and collect 1 mL of the aqueous phase.
4. Extract once more with 1 mL chloroform/isoamyl alcohol, and collect 0.5 mL of the upper phase.
5. Add 1 mL of your favorite DNA-binding resin, mix, and spin for 1 min. Decant, resuspend pellet in wash buffer, and load it into the column.
6. Wash the column with 2 mL of the appropriate column wash (*see* manufacturer's instructions), spin briefly to dry the resin, then elute with 50 µL of TE preheated to 65°C.

3.1.5. Guanidinium Extraction Method for RNA

The method below is Richard Mumford's modification of the method by Logemann et al. (4) (*see* **Note 6**).

1. Place plant tissue (approx 0.2 g) in a small polythene bag and freeze in liquid nitrogen. Grind sample to a fine powder using the seam roller, and then add 2 vol (400 µL) guanidinium extraction buffer and 20 µL of fresh 20% (w/v) PVP.
2. Homogenize sample fully, then transfer supernatant to a sterile microcentrifuge tube containing ~30 µL of sterile high vacuum silicon grease, and an equal sample volume (400 µL) of acid-phenol:chloroform (5:1 [v/v]). Vortex sample to form an emulsion.
3. Just before centrifugation, add 600 µL DEPC-treated sterile ddH₂O to each sample and then centrifuge (12,000g, 15 min). This reduces the density of the aqueous phase, so that the silicon grease forms a barrier between the organic and aqueous phase.
4. Collect supernatant, transfer it to a 2-mL microcentrifuge tube, and precipitate RNA by adding 1 vol of ice-cold isopropanol and incubating at –70°C for at least 1 h.
5. Centrifuge (12,000g, 15 min), and decant supernatant, taking care not to lose the RNA pellet. Wash pellet with 500 µL cold 70% ethanol, and centrifuge for 3 min (*see* **Note 3**). Remove as much of the supernatant as possible, and dry pellet in a vacuum desiccator or laminar flow cabinet.
6. Dissolve dry pellet in 50 µL DEPC-treated sterile ddH₂O (*see* **Note 4**).

3.1.6. Guanidinium Extraction Method for RNA (II)

This is a variation adapted from that used by S. Gurr (personal communication).