sue, and two methods for extraction of RNA using a RNase-inactivating extraction buffer. Degradation of RNAs can also be minimized by working rapidly and performing operations at 0–4ºC whenever possible.

3.1.1. Extraction and Purification of Total Nucleic Acid **(2)**

1. Place plant tissue (approx 1 cm^2) in a small polythene bag and freeze in liquid nitrogen. Grind sample to a fine powder using the seam roller (*see* **Note 1**). As the leaf powder thaws, add 300 µL of nucleic acid extraction buffer, homogenize sample, working quickly, and transfer it to a 1.5-mL microcentrifuge tube (*see* **Note 5**).
2. Immediately add 150 µL of phenol (acid phenol for RNA viruses, neutral phenol for DNA viruses) and 150 µL of chloroform. Vortex to mix thoroughly.
3. For isolation of RNA, incubate at 70°C for 5 min.
4. Centrifuge at room temperature (12,000*g*, 10 min), and transfer 200 µL of the supernatant to a sterile microcentrifuge tube (*see* **Note 2**). For RNA viruses, add 2 µL of 1*M* DTT and 1 µL of RNasin as ribonuclease inhibitors. Quick-freeze RNA templates in liquid nitrogen before and after column purification, to minimize RNase activity, and store at –80°C.
5. Fractionate the supernatant on a 1-mL Sephadex G-50 column (*see* **Subheading 3.1.2.**), or purify polyadenylated RNAs using oligo$(dT)_{25}$ Dynabeads (Dynal, Oslow, Norway) (*see* **Subheading 3.1.3.**).

3.1.2. Sephadex G-50 Column Purification

1. Plug the bottom couple of mm of a 1-mL disposable syringe with sterile glass wool. Add 1 mL of equilibrated Sephadex G-50 resin to the plugged syringe, and then insert this into a 15-mL centrifuge tube and centrifuge (700*g*, 4 min).
2. Add more resin until the packed column volume is approx 0.9 mL. Wash the column twice by adding 100 µL TE and recentrifuging (700*g*, 4 min).
3. Apply 100 µL of thawed nucleic acid to the column; put a sterile microcentrifuge tube under the column before recentrifuging (700*g*, 4 min) to collect the purified extract.

3.1.3. Dynabeads Oligo$(dT)_{25}$ Purification of Polyadenylated RNA

Extract polyadenylated RNAs from 100 µL of the supernatant according to manufacturer's conditions, with the modification that hybridization of RNA to the magnetic beads is carried out for 10 min at room temperature.

3.1.4. DNA Purification

This method was developed to extract DNA from dried fig leaves (the usual methods produce a small brown, latex-like ball), and optimized by M. Cornell, D. MacGregor, and P. Gaunt. It is loosely based on the Rogers and Bendich CTAB nucleic acid extraction method ***(3)***, followed by purification using DNA-binding resins. Magic Minipreps™ from Promega (Madison, WI) were used