28. Phenol:chloroform:isoamyl alcohol (25:24:1 [v/v]): made from stocks or bought commercially. Store in a dark bottle at 4ºC.
29. Polyvinyl pyrrolidone (PVP, mol wt 40,000), 20% (w/v): Prepare fresh on day of use in sterile dH_2O.
30. Reverse transcriptase, e.g., Avian Myeloblastosis Virus (AMV) Reverse Transcriptase at 20–25 U/μL (e.g., Promega M9004).
31. RNase A: Dissolve at 10 mg/mL in sterile ddH_2O. Heat to 95ºC in a water bath for 5 min, then allow to cool to room temperature. Dispense and store at –20°C.
32. RNasin (40 U/μL, Promega), store at –20°C.
33. RT buffer (5X): This is usually supplied with the reverse transcriptase, and, for AMV-RT from Promega, the 5X buffer composition is 250 m*M* Tris-HCl, pH 8.3, 250 m*M* KCl, 50 m*M* $MgCl_2$, 2.5 m*M* spermidine, 50 m*M* DTT.
34. RT primers; random hexamers, oligo(dT), or specific downstream primer constructed or obtained commercially. Dilute stock solution to a working concentration of 100 μ*M* for random hexamers, or 50 μ*M* for oligo(dT) or specific primer. Store in aliquots at –20°C.
35. Sterile ddH_2O: Distilled deionized water sterilized by autoclaving.
36. sodium dodecyl sulfate (SDS), 10% (w/v): Weigh out in fume hood, and dissolve in warm sterile ddH_2O. Store at room temperature, and heat to ~60ºC if detergent comes out of solution subsequently.
37. Sephadex G-50 resin: Suspend 10 g of Sephadex G-50 (medium grade) in 160 mL of RNase-free sterile ddH_2O. Allow to settle, then pipet off the supernatant and wash the swollen resin three times with RNase-free sterile ddH_2O. Equilibrate the resin in 50 mL RNase-free TE and autoclave at 10 psi for 15 min. Store at room temperature.
38. 3*M* Sodium actetate, pH 5.5.
39. T7 polymerase (~10 U/μL) and reaction buffer.
40. TBE buffer (5X): 0.45*M* Tris-borate, 10m*M* Na_2 EDTA. For 1 L of 5X stock, dissolve 54 g Tris base, 27.5 g boric acid, and 20 mL Na_2EDTA pH 8.0 in dH_2O, adjust to 1 L, and store at room temperature.
41. TE: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0. Make from 1*M* Tris-HCl and 0.5*M* EDTA, pH 8.0, stock solutions and autoclave. Store at room temperature.
42. 1*M* Tris-HCl, pH 8.0: Dissolve Tris base in 900 mL dH_2O, and adjust to pH 8.0 (at room temperature) by adding concentrated HCl (~40 mL) in fume cupboard. Ensure that the pH electrode is suitable for measuring the pH of Tris-containing solutions accurately. Add ddH_2O to make 1 L of solution, and autoclave. Store at room temperature.

3. Methods

3.1. Preparation of Template

There are many methods for RNA or DNA extraction, and the method of choice will depend on virus and plant tissue. Below is one method for total nucleic acid extraction, one method for DNA extraction from recalcitrant tis-