

11. 1M DTT: Dissolve dithiothreitol in 10 mM Na-acetate, pH 5.5. Sterilize by filtration, dispense into suitable aliquots and store at  $-20^{\circ}\text{C}$ .
12. 0.5M EDTA, pH 8.0: Using a magnetic stirrer, suspend  $\text{Na}_2$  EDTA salt in  $\text{ddH}_2\text{O}$ . It will be necessary to adjust the pH to 8.0 by adding NaOH pellets (~20 g) to allow the salt to go into solution. Sterilize by autoclaving and store at room temperature.
13. Ethanol, 70%: Dilute 95% ethanol with sterile  $\text{dH}_2\text{O}$ , and store at  $-20^{\circ}\text{C}$ .
14. Ethanol: Store at room temperature.
15. Ethidium bromide stock (10 mg/mL): **Caution**—Wearing gloves, dissolve one 100-mg ethidium bromide tablet (Sigma E2515) in 10 mL sterile  $\text{ddH}_2\text{O}$ . Store in the dark at room temperature.
16. Guanidinium extraction buffer (**Caution**: Toxic, wear gloves): 4M guanidinium thiocyanate, 20 mM 2-(*N*-morpholino)-ethanesulfonic acid, pH 7.0, 20 mM EDTA, 50 mM  $\beta$ -mercaptoethanol (add just before use), prepared in sterile  $\text{ddH}_2\text{O}$ . **Caution**: Carry out all manipulations involving  $\beta$ -mercaptoethanol in a fume hood, because it is highly toxic and a possible mutagen.
17. High-vacuum silicon grease (Dow Corning, München, Germany): Dispense grease into autoclavable polypropylene syringe, wrap in foil, and autoclave. Store at room temperature.
18. Loading buffer: 6X orange G loading dye: Mix 0.9 g Ficoll 400, 0.015 g orange G, 400  $\mu\text{L}$  0.5M EDTA, pH 8.0, and 4.5 mL sterile  $\text{dH}_2\text{O}$ . Store at room temperature.
19. 25 mM  $\text{MgCl}_2$ : This is usually supplied with the DNA polymerase obtained commercially. Dissolve  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  in  $\text{ddH}_2\text{O}$ , and autoclave. If anhydrous  $\text{MgCl}_2$  is used, care should be taken when adding  $\text{ddH}_2\text{O}$ , because it is extremely hygroscopic. Store at room temperature.
20. Mineral oil, light white (e.g., Sigma M3516).
21. NA extraction buffer: 2% (w/v) SDS, 0.1M Tris-HCl, pH 8.0, 2 mM EDTA, pH 8.0, made from 10% (w/v) SDS, 1M Tris-HCl, pH 8.0, and 0.5M EDTA, pH 8.0, stocks. Autoclave and store at room temperature.
22. Oligo(dT)<sub>25</sub> Dynabeads (Dynal, Oslo, Norway).
23. PCR buffer (10X): An optimum buffer is generally supplied by commercial suppliers for their DNA polymerase.
24. PCR primers: 18–30 bases long, 10–100 pmol/ $\mu\text{L}$  in sterile  $\text{ddH}_2\text{O}$ .
25. Phenol: **Caution**—All phenol and chloroform-containing solutions are highly toxic; handle in fume hood wearing protective clothing, gloves, and face-shield. Do not use phenol solutions that have oxidized and have changed from colorless to light pink. It is recommended to add 0.1% (w/v) 8-hydroxyquinoline to phenol as an antioxidant, a partial inhibitor of RNase, and to chelate metal ions involved in binding RNA to proteins. The addition results in the solution turning yellow, and oxidation being visible by a color change to brown. Dispose of phenol and chloroform waste according to laboratory regulations.
26. Phenol, acid (for RNA viruses): saturated with 0.1M citrate buffer ~pH 4.5 (e.g., Sigma P4682). Store in a dark bottle at  $4^{\circ}\text{C}$ .
27. Phenol, neutral (for DNA viruses): saturated with TE (e.g., Sigma P4557). Store in a dark bottle at  $4^{\circ}\text{C}$ .