and the RNA can be precipitated from the aqueous layer with ethanol in the presence of sodium acetate. Host DNA left in purified virus preparations will also be carried over to RNA extraction. This usually causes some problems for further cDNA synthesis and cloning experiments. Therefore, the host DNA must be removed using deoxyribonucleases (DNase) before RNA extraction. Another problem during the isolation of viral gRNA is the degradation of RNA by nucleases, mainly RNase A. To minimize RNA degradation, all tubes, tips, containers, and glasswares should be washed in DEPC-dH_2O, and the glasswares are further baked for more than 6 h at 160°C (*see* **Note 6**). To obtain full-length gRNA, which is of great importance in cloning full-length cDNAs, the viral RNA should be centrifuged in a sucrose gradient.

Host DNA contaminating the virus preparations may be removed before the isolation of RNA from the purified virions as follows:

1. To 1 mL of virus solution, add 100 μL of DNase I reaction buffer (10X) and 5 U of DNase I. Keep the mixture at 37°C for 30 min and terminate the reaction with addition of EDTA to 5 m*M*.
2. Centrifuge the DNase treated virus at 86,500*g* for 2 h and dissolve the virus pellets in DEPC-dH_2O.

Viral gRNA can be extracted according to the methods of AbouHaidar and Bancroft ***(24)***, and Maniatis et al. ***(25)***. Bamboo mosaic virus has been found to have a satellite RNA (sRNA), which is also encapsidated by CP, and the sRNA can also be extracted from purified virions (**ref. *20***; *see* **Note 7**).

1. Add SDS to the DNase I-treated purified virus solution, to a final concentration of 0.1% (w/v).
2. Add 2 vol of phenol:chloroform (1:1 v/v); keep the mixture at 40°C for 5–10 min, with occasional vortexing.
3. Centrifuge at 12,500*g* for 5 min at room temperature and transfer the aqueous phase to a new tube.
4. Add DEPC-dH_2O (0.1 vol) to the phenol:chloroform phase, vortex, and centrifuge as above. Collect the aqueous phase.
5. Combine the two aqueous phases. Extract the aqueous phase with an equal volume of phenol:chloroform and centrifuge as above.
6. Transfer the aqueous layer to a new tube and subsequently re-extract twice with 2 vol of chloroform/isoamyl alcohol (24:1, v/v).
7. Precipitate the RNA by the addition of sodium acetate to a final concentration of 0.25*M* and 2.5 vol of ice-cold 95% ethanol.
8. Keep the mixture at –70°C for 20 min (or –20°C overnight). The viral RNA is then collected by centrifugation at 12,500*g* for 10 min.
9. Rinse the RNA pellets with 70% ethanol, vacuum-dry the RNA pellet for 5–8 min, and dissolve in a desired volume of DEPC-dH_2O (or TE buffer) (*see* **Note 8**).