9. Centrifuge at 2500*g* for 1 min and remove the ethanol.
10. Repeat this ethanol washing at least twice more.
11. Either store the RNA pellet in 70% (v/v) ethanol at 4°C or resuspend the RNA in distilled water and store at –80°C until required.

4. Notes

1. Tobacco is a suitable source of TobRSV-infected tissue for purification purposes. However, for this and other nepoviruses, several other hosts are appropriate; *Nicotiana clevelandii* is suitable for many, but for TomRV, for example, cucumber or squash may be more appropriate.
2. The yield of virus to be expected will vary, depending on the virus to be used. For TobRSV, a conservative estimate would be 50 mg/kg; 5–20 mg/kg is typical. A convenient amount of tissue to process is 250–400 g, but this can clearly be adjusted if large amounts of virus or viral RNA are required.
3. The time at which tissue is harvested is not absolutely critical, but, in our hands, 7–10 d after inoculation has provided highest yields from inoculated tissue, when plants are maintained under the conditions described.
4. Harvested tissue may be processed immediately, or stored at –80°C for several months, until required. If stored frozen, the tissue must be brought close to 4°C before homogenization.
5. TobRSV is stable for several days at room temperature in sap. However, unless otherwise stated, it is preferable to perform all steps at 4°C to maintain maximum infectivity and integrity of the viral RNA.
6. An Omnimixer top-drive macerator is ideal for tissue homogenization; all liquid is effectively contained. Bottom-drive blenders of the Waring type are also satisfactory.
7. Clarification using *n*-butanol is appropriate for many of the nepoviruses. A useful alternative, which also gives good results, is to use chloroform (e.g., **refs. *17*** and ***18***). In this method, tissue is homogenized in homogenization buffer and chloroform (1:1:1 [w/v/v]), and the mixture allowed to stand for 30 min. The mixture is then centrifuged at 10,000*g* for 20 min, and the lower layer discarded. The supernatant solution is then processed as indicated (**steps 6–16**).
8. A critical aspect of this purification process, that is common to all procedures which include pelleting of the virus by high-speed centrifugation, is efficient resuspension of the virus pellet. Since some viruses are susceptible to aggregation, this can be difficult, although nepoviruses do not pose as severe a problem as do some other groups of viruses, (e.g., potyviruses). I use glass rods and rubber policemen (crude but effective). An additional modification is to sediment virus paricles onto a cushion of Maxidens (Nycomed), a high-density perfluorinated liquid that is immiscible with water.
9. The method described here provides a mixture of the two genomic RNAs. If it is intended to use RT-PCR to clone the sections of the genome required, then this is all that is required. However, if purified preparations of RNA-1 and RNA-2 individually are required, additional steps will need to be included