3*M* sodium acetate and 2.5 vol of ethanol. Mix and incubate the samples at –20°C for 15 min to precipitate the RNA.

5. Pellet the RNA by microcentrifugation at 13,000*g* for 15 min at 4°C. This should yield a visible white pellet. Decant the supernatant; centrifuge briefly to collect residual liquid at the bottom of the tubes and pipet this off without disturbing the pellet. Dry the pellet under vacuum for a few minutes. Add 0.2 mL of DEPC-treated water and dissolve the pellet by gently vortexing.
6. This procedure should yield about 200 μg of RNA (*see* **Note 9**). Prepare dilutions of the dissolved RNA and measure the absorbance at 260 and 280 nm. The preparation should have an $A_{260/280}$ ratio of 2.0. The RNA yield can be estimated by assuming that a 40 μg/mL solution of RNA has an absorbance of 1.

4. Notes

1. Anyone using this protocol should familiarize themselves with the health and safety hazards related to 2-mercaptoethanol, butan-1-ol, diethylpyrocarbonate, phenol, 8-hydroxyquinoline, isoamyl alcohol, and chloroform. All manipulations involving these chemicals should be carried out in a fume hood.
2. If Miracloth is unavailable, muslin can be substituted. However, muslin is less efficient at retaining particulates and its use is likely to lead to poorer purification.
3. Before commencing purification, it is advisable to prepare a reasonably homogeneous viral population that has the desired phenotype. This can be achieved by passaging the virus several times through a local lesion host at high viral dilutions, preparing inocula for each subsequent passage from individual lesions. The final inoculum should be tested on a range of diagnostic host species before inoculation onto the host species from which the virus is to be purified.
4. Choice of a propagation host is an important consideration in virus purification, and it is worthwhile testing a range of host plants in advance. The choice of host plant is determined not only by the level of virus accumulation, but also by aspects of the host plant's biology. Host plants that are not heavily lignified should be chosen to facilitate homogenization of host tissue, and hosts containing high levels of secondary products, which might interfere with the purification procedure, should be avoided. The levels of virus that accumulate in the host plant are dependent on the growth conditions used; extremes of temperature and light should be avoided. Hosts that the author has found useful for purification of the definitive tobamoviruses (tobacco mosaic virus, tomato mosaic virus, tobacco mild green mosaic virus, odontoglossum ringspot virus, ribgrass mosaic virus, and turnip vein clearing virus) include *Nicotiana benthamiana*, *N. clevelandii*, and *N. tabacum* cultivars.
5. The initial steps of this purification process should be carried out at room temperature, because use of lower temperatures may result in precipitation of salts.
6. Though the tobamoviruses are stable in a wide range of chemical environments and are thermotolerant, the particles are susceptible to fragmentation. Therefore, dissolution of the viral pellet should be performed by gentle stirring with a teflon-coated rod, and the use of vortexers or tissue homogenizers, which produce high