In addition, you can electrophorese 5 μL RNA (mixed with 3 μL of an RNase free loading dye) on a 2% agarose gel in TAE buffer (Tris-acetate-EDTA) to obtain quality and yield information. Potyviral RNA should migrate as a clear single band with little or no degradation. Store RNA preparations at –80°C (*see* **Notes 29** and **30**).

4. Notes

4.1. Potyvirus Purification

1. This procedure is designed for 200 g of infected tissue, but can be scaled-up by concomitant increase in added reagents. When working with virus hosts that result in viscous extracts (e.g., hosts in the Malvaceae or *Allium* spp.), try including 2% (w/v) Polyclar AT (Sigma, St. Louis, MO) and 0.02*M* sodium sulfite to the extraction buffer. Many procedures also will use EDTA (0.05*M*) in the extraction buffer. The need for these or other amendments can only be determined empirically.
2. (**Subheading 3.1., step 1**) Excessive mixing may cause foaming. A foam suppresser, available for the 4 L Waring blender, will reduce this. **Caution:** Protective clothing and gloves should be used when handling organic reagents. Chloroform and carbon tetrachloride cause burning pain and redness if contact with skin occurs. Inhalation or ingestion can cause central nervous system depression, with dizziness, drowsiness, and vomiting. These reagents are suspected carcinogens based on animal studies.
3. (**Subheading 3.1., step 3**) Use a separatory funnel, if necessary, to remove all carbon tetrachloride and chloroform.
4. (**Subheading 3.1., step 4**) A common source of virus loss is failure to release virus from the PEG. In many procedures, NaCl is included to aid in virus release and, although not used in some procedures, it may be important. Therefore, it may be useful to consider inclusion of 1.5–1.75% (w/v) NaCl when purifying an uncharacterized virus, if yields are unsatisfactory.
5. (**Subheading 3.1., steps 6** and **9**) Do not resuspend using vortex mixer. You can resuspend overnight at 4°C, if you wish.
6. (**Subheading 3.1., step 8**) This is about 30,000 rpm in a Beckman Ti 70 rotor.
7. (**Subheading 3.1., step 11**) It is important not to add more than 2 mL (derived from the equivalent of 50–75 g tissue) to every 35 mL sucrose gradient. Overloading by using more than 2 mg virus per gradient will likely cause virion aggregation and precipitation. Prevention of this can be enhanced by the addition of Triton X-100 to the sucrose solutions (to 0.1% final concentration) before gradient preparation. However, Triton X-100 strongly absorbs light at 254 nm and can make fractionation using a spectrophotometer (i.e., ISCO system) difficult.
8. (**Subheading 3.1., step 11**) Assumption here is that a Beckman SW 28 rotor or equivalent is used. Centrifugation times will have to be adjusted if a different rotor is used. Vertical or fixed-angle rotors can be used for sucrose density gradi-