3. Water-saturated phenol, containing 0.1% 8-hydroxyquinoline.
4. Chloroform.
5. Ethanol 100 and 70%, autoclaved water.

Caution: Take care in handling organic solvents (use gloves) and avoid breathing vapors.

3. Methods

3.1. Virus Purification

1. Collect infected leaves of *Nicotiana benthamiana* or *N. clevelandii* 10–14 d after inoculation, and homogenize them in cold homogenization buffer, 1 g tissue/3 mL of buffer (*see* **Notes 1** and **2**).
2. Squeeze through cheesecloth.
3. Leave the extract in ice for 30 min. Most plant proteins will precipitate because of the low pH.
4. Clarify by low speed centrifugation (12,000*g* for 10 min) (*see* **Note 3**).
5. Transfer the supernatant (where the virus particles are) into a beaker, and adjust to pH 6.0 with dilute NaOH.
6. Add 10 g PEG and 1.1 g NaCl/100 mL and dissolve with magnetic stirrer. Keep the solution on ice for 1 h.
7. Precipitate the virus particles by low-speed centrifugation (12,000*g* for 10 min) and discard the supernatant.
8. Resuspend the pellet in 0.02*M* sodium acetate, pH 5.5 (use a vortex); leave in ice for 1 h and vortex again.
9. Clarify the virus solution by low-speed centrifugation (12,000*g* for 10 min). Save the supernatant.
10. Sediment virus particles by high-speed centrifugation (90,000*g* for 1 h); discard the supernatant.
11. Let the pellet dissolve in 0.02*M* sodium acetate, pH 5.5, for several hours or overnight at 4°C. Vortex to dissolve completely the virus pellet, transfer to an Eppendorf tube, and eliminate the insoluble material by low-speed centrifugation in an Eppendorf centrifuge.

This virus preparation is now sufficently pure for RNA extraction. However, if highly purified virus is needed, further purification can be achieved through density gradient centrifugation in CsCl at equilibrium. To do so, continue as follows:

12. Dissolve 2.65 g of CsCl in 5 mL virus suspension (initial density of CsCl solution is 1.36 g/mL) and centrifuge at 90,000*g* for 16 h at 10°C.
13. Collect the sharp opalescent virus band by puncturing the tube with a syringe and removing the band. Remove the CsCl by dialysis against 50 m*M* NaCl, pH 5.5.

Virus yield ranges between 10 and 60 mg/100 g of infected tissues. The virus preparation can be stored at –70°C.