2.2. RNA Extraction

1. VEBA *(21a)*: 0.2*M* Tris, pH 8.5, 1*M* NaCl, 1% SDS, 2 m*M* EDTA. Autoclave and store at room temperature. VEBA must be warmed slightly before use, to resuspend the SDS.
2. Phenol:chloroform, 5:1, saturated with water (store at 4°C). Phenol should be ultrapure quality, or should be redistilled. **Caution:** Use care in handling, phenol is extremely caustic.
3. NAE: 0.3*M* sodium acetate, pH 6.0, 0.1 m*M* EDTA. Autoclave and store at room temperature.
4. 0.1 m*M* EDTA, pH 8.0, autoclave and store at room temperature.

3. Methods

3.1. Virus Purification

All centrifugation values are given as maximum relative centrifugal force (RCF). *See* **Note 5** for handling of glassware.

1. Harvest fresh plant leaf tissue, removing major ribs and stems. Use as soon as possible, or store for a short period at 4°C. Avoid any freezing of infected tissue.
2. Weigh tissue and place in a chilled blender jar (*see* **Note 6**). For each gram of tissue, add 1 mL of buffer A and 1 mL of cold (4°C) chloroform. Blend until thoroughly homogenized, about 2 min.
3. Transfer homogenate to a centrifuge bottle, and centrifuge at 15,000*g* at 4°C for 10 min.
4. Filter the aqueous phase through dampened Miracloth (*see* **Notes 7** and **8**). Transfer the filtrate to ca. 25-mL ultracentrifuge tubes, and underlay with 5 mL of cushion I. It is most convenient to use thick-walled polycarbonate tubes, because the volume can be varied, and they are available with sealable screw caps. The ultracentrifugation should be completed as soon as possible. Do not keep the virus in buffer A for more than a few hours.
5. Centrifuge at 212,000*g* for 1.5 h (*see* **Note 9**) at 4°C.
6. Pour off the supernatant, and add 4–5 mL of buffer B to each pellet. Allow the pellets to sit at 4°C overnight.
7. Vortex the pellets in buffer B briefly, pool the samples, and stir at 4°C for 2 h.
8. Centrifuge the stirred samples at 7500*g* at 4°C for 10 min. Pour off the supernatant immediately to an ultracentrifuge tube. Underlay with 5 mL of cushion II.
9. Centrifuge as in **step 5** (*see* **Note 10**). Pour off supernatant and add 2–5 mL of buffer C (depending on size) to the pellets. Allow the pellets to sit at 4°C overnight.
10. Virus can be stored in buffer C, or used for RNA extraction. To quantitate the virus yield, measure the OD_{260}. CMV has an extinction coefficient of 5 *(22)*. Virus yields vary from 100 to 1 g/kg of tissue, dependent on the strain of virus. The virus may be stored at –20°C in 50% glycerol.