3. Method

3.1. Virus Purification

1. Dust approx 20 *Nicotiana tabacum* (cv. White Burley) plants (at approximately the 4–5 leaf stage), with carborundum, and mechanically inoculate with virus inoculum (*see* **Notes 1** and **2**).
2. Maintain the plants at approx 23°C for 7–10 d (*see* **Note 3**).
3. Collect the inoculated leaves (ca. 200–250 g), and homogenize thoroughly in homogenization buffer (1:2, w/v) using an Omnimixer (*see* **Notes 4–6**).
4. Filter the homogenate through two layers of muslin, make 8.5% (v/v) with *n*-butanol, and stir gently for 30 min (*see* **Note 7**).
5. Centrifuge at 10,000*g* for 20 min and discard the pellet.
6. Make the supernatant solution 10% (w/v) in PEG 6000 and 0.17*M* sodium chloride.
7. Stir gently for 1 h.
8. Centrifuge at 10,000*g* for 20 min. Discard the supernatant solution.
9. Resuspend the pellet in resuspension buffer A (10% original volume of the homogenate; **step 3**; *see* **Note 8**).
10. Allow the mixture to stand overnight.
11. Centrifuge at 10,000*g* for 10 min and discard the pellet.
12. Centrifuge the supernatant solution at 150,000*g* for 2 h; discard the supernatant solution.
13. Resuspend the pellet in a small volume (ca. 2–3 mL) of resuspension buffer A.
14. Repeat **steps 11** and **12**.
15. If the virus is to be used for RNA extraction, resuspend the pellet in 2–3 mL resuspension buffer B; alternatively, use buffer A.
16. Dilute a small sample 100X in the same buffer used for final resuspension and assess the virus concentration spectrophotometrically, assuming that a suspension of 1 mg/mL has an absorption of 10 (260 nm, 1 cm path length) (*see* **Note 9**).

3.2. RNA Purification

1. Make the virus suspension (ca. 5 mg/mL in buffer B) 1% (w/v) in SDS, and vortex briefly (*see* **Note 10**).
2. Add an equal volume of phenol mix and shake vigorously for 5 min at room temperature on a wrist-action shaker (*see* **Note 11**).
3. Add chloroform mix (same volume as phenol mix) and shake again for 5 min at room temperature.
4. Add ca. 50 µL silicone grease and centrifuge at 2500*g* for 5 min (*see* **Note 12**).
5. Collect the (upper) aqueous phase, and make 0.2*M* in sodium acetate.
6. Add 2 vol of ethanol and allow to stand for at least 2 h at –20°C to precipitate the RNA.
7. Centrifuge at 12,000*g* for 20 min.
8. Add 70% (v/v) ethanol (approximately twice the volume of the original virus suspension) and vortex for a few seconds.