1. *Nicotiana tabacum* cv. Samsun is a good propagation host for PVX. The plants (four true-leaf-age) are lightly dusted with Carborundum (400-mesh), and inoculated with either purified PVX (1 μg/mL or higher concentration) or infected leaf extracts (*see* **Note 2**). The inoculated plants are kept under greenhouse condition (25°C, 16-h photoperiod). Two weeks postinoculation, the leaves of inoculated plants can be collected for virus purification (*see* **Note 2**).
2. Homogenize infected leaves in Tris-borate buffer containing 0.2% β-mercaptoethanol (1–2 mL/g of leaves). The subsequent steps were performed at 4°C (*see* **Note 3**).
3. Squeeze the homogenized tissue through four layers of cheesecloth. Add *n*-butanol to the sap to a final concentration of 6%.
4. Keep the mixture on ice for 45 min with constant stirring. Centrifuge for 10 min at 15,000*g* and save the supernatant.
5. Precipitate the virus from the supernatant by the addition of PEG 8000, to a final concentration of 8% in the presence of 2% NaCl, and keep the mixture at 4°C for 30–60 min.
6. Centrifuge at 15,000*g* for 10 min, resuspend the pellets in Tris-borate buffer, pH 7.5, overnight at 4°C without vortexing. The virus solution is then centrifuged three times at 7500*g* for 5 min each. Keep the supernatant.
7. Overlay the virus solution onto 4 mL of sucrose cushion in Ti 60 ultracentrifuge tubes (Beckman) (*see* **Note 4**). Pellet the virus at 86,500*g* for 3 h at 4°C. Pellets are redissolved, in the same buffer as above, overnight at 4°C, and centrifuged three times at 7500*g* for 10 min each (*see* **Note 5**).
8. The virus in the supernatant is then centrifuged in a CsCl density gradient for 17 h at 86,500*g* (15°C). The virus bands (white opalescent seen with light from beneath against a black object or in a dark room) are collected and diluted four times with Tris-borate buffer. A CsCl purification is only required for ultrapure preparations of the virus; otherwise, go to **step 10**.
9. The virus is then collected by centrifugation at 100,000*g* for 2 h. Pellets are redissolved in the same buffer overnight.
10. Optical density (OD) readings are taken at 260 and 280 nm to determine the virus purity (A_{260}/A_{280} ratio of 1.2 for PVX) and concentration using extinction coefficient 260 nm ($E^{260\ nm}_{0.1\%,\ 1\ cm}$ = 3.0 for PVX) ***(1)***. The yield is approx 0.5 to 1.0 g/kg leaf tissues. The virus particles can be negatively stained with 1% uranyl acetate and examined by transmission electron microscopy.
11. Keep the purified virus preparations at 4°C in the presence of 0.1% sodium azide. Under these conditions, the virus remains infectious for at least 3 yr.

3.2. Extraction of Viral gRNA and sgRNA

3.2.1. Extraction of Viral RNA from Purified Virions

In general, to extract viral gRNA from the purified virions, the virus is treated with SDS to strip off the capsid protein. The capsid protein is then removed by extraction with phenol. Chloroform is used to remove the phenol,