Every few minutes mix the tube contents by inversion. Chlorophyll and coagulated material should collect in the upper organic phase.

4. Centrifuge the tube contents at 10,000*g* for 30 min at 12°C. Recover the lightly pigmented aqueous phase. Avoid taking any of the pelleted material or the upper, organic layer. If any of this undesired material is carried over, the aqueous phase should be centrifuged again in fresh tubes for 15 min. Filter the clarified extract through two layers of Miracloth into fresh centrifuge tubes.
5. Add 20% PEG solution to give a final concentration of 4%. Mix the tube contents by inversion and incubate on ice for 15 min. Periodically mix the tube contents. The solution should turn cloudy as the virus precipitates.
6. Pellet the virus by centrifugation at 10,000*g* for 15 min at 4°C. This should yield a whitish pellet that may be contaminated with some traces of pigmented material. Decant the supernatant and centrifuge briefly to collect residual liquid at the bottom of the tube. Pipet off the residual liquid.
7. Dissolve the pellet in 8 mL of 10 m*M* phosphate buffer (*see* **Note 6**). Centrifuge at 10,000*g* for 15 min at 4°C. Transfer the supernatant to a fresh tube.
8. Add to the supernatant 1.7 mL of 5*M* NaCl and 2.42 mL of 20% PEG. Mix tube contents and incubate on ice for fifteen min. Pellet the virus by centrifugation at 10,000*g* for 15 min at 4°C. This should yield a white viral pellet. Decant the supernatant; centrifuge briefly and pipet off the residual liquid.
9. Dissolve the pellet in 2 mL of 10 m*M* phosphate buffer. Divide the solution between two 1.5-mL microcentrifuge tubes. Centrifuge the tubes in a microcentrifuge at 13,000*g* for 30 s and pipet the supernatants to fresh microcentrifuge tubes.
10. This procedure should yield at least 20 mg of virus (*see* **Notes 7** and **8**). To determine the yields, prepare dilutions of small aliquots of the preparation and measure the absorbance at 260 and 280 nm. Estimate the yield assuming an extinction coefficient ($E^{0.1\%}_{1\,cm}$) of three. An A_{260}/A_{280} ratio of about 1:19 is expected for TMV; however, this ratio varies between different tobamoviruses.

3.2. RNA Extraction

1. Dilute an aliquot of the virion preparation to 10 mg/mL with 10 m*M* phosphate buffer. Pipet 0.8 mL of this dilution to a 2-mL microcentrifuge tube and add 0.2 mL of 5X RNA extraction buffer. Add 1 mL of phenol:chloroform; vortex briefly until an emulsion is formed, then centrifuge in a microcentrifuge tube at 13,000*g* for 5 min at room temperature.
2. Collect the upper aqueous phase without taking any of the denatured protein from the interface and transfer it to a fresh microcentrifuge tube. Repeat the extraction with phenol:chloroform twice.
3. Collect the aqueous phase from the third phenol:chloroform extraction. Add an equal volume of chloroform, and vortex briefly to form an emulsion. Separate the phases by microcentrifugation.
4. Collect the upper aqueous phase, which should contain about 0.7 mL, and divide it equally between two 2-mL microcentrifuge tubes. Add to each tube 0.1 vol of