6. Resuspend virus pellet in approx 40 mL of 30 m*M* sodium phosphate buffer, pH 7.5, clarify as in **Subheading 3.1., step 7**, and then split into two tubes before extracting with chloroform:butanol.
7. Chloroform:butanol extractions should be repeated until no contaminants are visible at the interface.
8. The purification procedure can be stopped at **step 10** of **Subheading 3.1.**, and the viral pellet resuspended in 20–30 mL 10 m*M* Tris-HCl, pH 7.5. This should result in a virus preparation at approx 1 mg/mL, from an initial 200–300 g of infected tissue. To determine the exact virus concentration, measure the OD at 260 nm (*see* **Subheading 3.1., step 12**). If a purer virus preparation is required, then the viral pellet should be resuspended in 20–30 mL 30 m*M* sodium phosphate buffer, pH 7.5, and centrifuged through a sucrose pad to remove any remaining contaminants.
9. Carefully layer 7 mL of virus onto 3 mL of 30% sucrose in 30 m*M* sodium phosphate buffer, pH 7.5, and sedimented by centrifugation for 2–3 h at 140,000*g* and 15°C in a swing-bucket rotor.
10. The virus suspension should clear after incubation with proteinase K.
11. TRV RNA should be visible as two bands of approx 6.8 kb and 1.8–3.9 kb, and PEBV as two bands of approx 7.0 and 3.4 kb on denaturing agarose gels. Additional smaller bands may also be visible: These are subgenomic RNA species.

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