Fig. 1. General gene organization of the potyvirus virus genome.

Perhaps the biggest single problem with the purification of potyviruses is aggregation of virions during the purification process. When aggregation occurs, particularly at earlier stages of purification, significant losses occur during the low-speed centrifugation (clarification) steps. Thus, many procedures utilize the nonionic detergent Triton X-100 to prevent this. Once aggregation occurs, it is difficult if not impossible to reverse. Here, we present methods that have proven successful for a number of potyviruses. Once purified virus is available, methods for purification of viral RNA are relatively straightforward.

2. Materials

2.1. General Materials

1. Organic solvents: Reagent grade chloroform ($CHCl_3$) and carbon tetrachloride (CCl_4).
2. Other reagents: Polyethylene glycol (PEG) (mol wt 6000 or 8000), Triton X-100, optical grade CsCl, optical grade sucrose, NaCl.
3. High capacity (500 mL) centrifuge capable of 10,000*g*.
4. Centrifuge tubes resistant to $CHCl_3$ and CCl_4 (polypropylene).
5. Ultracentrifuge capable of 120,000*g*.
6. Blender. We recommend the use of the 4-L Waring (New Hartford, CT) blender with a stainless steel container. This blender is rather expensive, but it has the torque to handle tough, fibrous tissues. If such a blender is unavailable, be sure to cut coarse, fibrous tissues (like grasses) to 1–2 in. or smaller lengths.
7. Stir plate and magnetic stirbars.
8. Vortexer.
9. 40°C Water bath.
10. –80°C Freezer.

2.2. Buffers for General Potyvirus Purification

1. Buffer A: 0.5*M* borate, pH 8.0 (boric acid titrated to pH with NaOH).
2. Buffer B: Buffer A with 0.15% sodium thioglycollate (grinding buffer).
3. Buffer C: 0.05*M* borate, pH 8.0, with 5 m*M* EDTA.

2.3. Buffers for Purification of Maize Dwarf Mosaic Virus

1. Buffer A: 0.1*M* ammonium citrate, dibasic, adjusted to pH 6.0 with solid KOH. A 1.0*M* stock solution can be prepared.