Nelson and Tremaine ***(8)*** developed an alternative protocol in which the tissue was homogenized in an acidic sodium acetate buffer to achieve clarification of the extract in the absence of organic solvents. A later improvement of this method included a polyethylene glycol precipitation step ***(9)*** and has been widely used for carmovirus purification (*see* **Note 6**). Here, we describe a different modification of the acetate buffer method that renders high yields of high-quality virus in a significantly shorter time. The method described here is based on a high-speed centrifugation to pellet the virions through a sucrose cushion. Our experience with several carmoviruses shows that the virions obtained in this way are pure enough for different virological and molecular analyses (*see* **Notes 4** and **5**). Viral RNA extraction from purified virions is carried out essentially as described previously ***(10)***.

2. Materials

All the solutions should be prepared with MilliQ water.

2.1. Virus Purification

1. Liquid nitrogen.
2. 0.2*M* Sodium acetate, pH 5.0. Autoclave and store at room temperature.
3. 10 m*M* Tris-HCl, pH 7.3. Autoclave and store at room temperature.
4. 20% (w/v) Sucrose in 10 m*M* Tris-HCl, pH 7.3. Freshly prepared in each experiment. Alternatively, the solution can be autoclaved at 121°C for no more than 15 min to avoid caramelization of sucrose.

2.2. Viral RNA Extraction

1. 4 mg/mL Proteinase K dissolved in sterile water. Store aliquoted at –20°C.
2. 10% (w/v) Sodium dodecyl sulfate (SDS) in 50 m*M* Tris-HCl, pH 8.0. Autoclave and store at room temperature.
3. 1*M* Tris-HCl, pH 7.6. Autoclave and store at room temperature.
4. 5*M* NaCl. Autoclave and store at room temperature.
5. Phenol:chloroform:isoamyl alcohol (25:24:1). Prepared and stored as described by Sambrook et al. ***(11)***. **Caution:** Phenol is highly toxic and gloves must be worn when handling it.
6. Diethyl ether or chloroform. **Caution:** Diethyl ether is highly volatile and should be stored and used in a fume hood.
7. 3*M* Sodium acetate, pH 5.5. Prepared and stored as described ***(11)***.
8. Absolute ethanol and 70% (v/v) ethanol in water. Store at –20°C.

3. Methods

When possible, keep the extract on ice or at 4°C. Also, once the virus purification (and RNA extraction) has been started, it should be carried out as quickly as possible. **Caution:** Caution must be taken with RNase contamination; gloves