should be worn and plasticware and glassware should be either autoclaved or treated with 15% H_2O_2 for 15 min, and then washed twice with autoclaved water.

3.1. Virus Purification

We normally extract *Chenopodium quinoa* leaves, since this is the experimental host for most of the viruses we work with. In the case of CarMV and PFBV, the tissue is collected at 6–8 d postinoculation, which is about 2–3 d after the appearance of the first viral lesions. Tissue may be kept frozen for long periods at –20°C.

1. Homogenize the infected tissue to a fine powder in liquid nitrogen with mortar and pestle. We use mortars for 5–20 g of tissue; when larger amounts are extracted, homogenization can be carried out in a food blender.
2. Wait until the homogenized leaves defrost and then add 2 vol of 0.2*M* sodium acetate, pH 5.0. Keep mixing thoroughly for up to 5 min (*see* **Note 1**). Alternatively, the fine powder can be poured into a tube containing the buffer and mixed by vortexing.
3. Filter the homogenate through cheesecloth to remove large debris.
4. Centrifuge at 7700*g* in a Beckman JA.20 rotor for 20 min. Keep the supernatant, which should be light green and free of any particulate material that would impair the next purification step.
5. Slowly, layer the supernatant (10–15 mL) on top of 5 mL 20% sucrose in 10 m*M* Tris-HCl, pH 7.3, cushion, loaded in ultracentrifuge tubes. Be careful to layer the sample on the center of the cushion surface, to avoid it sliding between the cushion and the walls of the tube. Centrifuge at 146,000*g* in a Beckman 50.2 Ti rotor for 2 h.
6. A small, lightly colored, and opalescent pellet should be visible. Discard the supernatant and drain any excess by keeping the tube upside down on filter paper for a minute or so. Resuspend the pellet in 10 m*M* Tris-HCl, pH 7.3 (ca. 0.04 of the initial volume). Make sure resuspension is complete. Resuspension will improve by pipeting in and out with an automatic pipet. If needed, pellet can be left overnight with buffer at 4°C.
7. Transfer to an Eppendorf tube. Centrifuge at top speed for 1 min in an Eppendorf centrifuge to remove any unresuspended material.
8. Keep the supernatant as purified virus fraction (*see* **Note 2**). Store aliquoted at –20°C. In general, carmoviruses are rather stable; in our experience, aliquots of purified virus stored at –20°C retained infectivity for several months.

3.2. Viral RNA Extraction

1. Incubate the purified virus at 37°C for 1 h in 400 µL of a solution containing 50 µg/mL proteinase K, 0.5% SDS, 10 m*M* Tris-HCl, pH 7.6, and 0.1*M* NaCl. We have extracted up to 2 mg of virions in a single Eppendorf tube with good yields.
2. Extract twice with 1 vol of phenol:chloroform:isoamyl alcohol.