4. Notes

1. This protocol works well for BMV and CCMV. In the case of BBMV, especially if the virus is extracted from broad bean leaves, the ascorbic acid should be added to virus extraction buffer. The basic protocol can be scaled-down for virus extraction from mg quantities of leaf tissue. In this case, the centrifugation steps are accomplished using Eppendorf tubes and a microcentrifuge, and ultracentrifugation steps are omitted. The entire procedure should be done on ice or in cold room (*see also* **ref. *9***).
2. Pellet should appear clear and glassy.
3. 1 OD_{260} corresponds to 0.2 mg of the virus. The purity of virion RNA samples can be estimated by measuring the ratio of UV absorbency at 260/280. A ratio of 1.8–2.0 indicates that the RNA sample contains no, or only residual, amounts of proteins.
4. The virus will preserve its biological activity for years when stored in storage buffer at –70°C. To avoid thawing and refreezing of the samples, store the virus in small aliquots. Virus yield: 0.5–5 mg/g infected leaf tissue.
5. The procedure gives good yields of viral RNA for three bromoviruses. The RNA purification procedure should be done entirely on ice or in a cold room.
6. High-quality phenol saturated with 0.1*M* Tris buffer, pH 7.0 (e.g., from Gibco-BRL) should be used for RNA isolation. High-quality water (double-distilled) should be treated with DEPC overnight at 37°C, followed by autoclaving.
7. High-quality chloroform stabilized with isoamyl alcohol (mix 1 vol of isoamyl alcohol with 24 vol of chloroform) is recommended for RNA isolation.
8. Store the RNA preparations in small aliquots of DEPC-treated water at –20 to –80°C. The RNA preparation is stable for years. Avoid thawing and refreezing of the samples. Yield: 0.03–0.1 mg/g infected leaf tissue.

References

1. Speir, J. A., Munshi, S., Wang, G., Baker, T. S., and Johnson, J. E. (1995) Structures of the native and swollen forms of cowpea chlorotic mottle virus determined by X-ray crystallography and cryo-electron microscopy. *Structure* **3,** 63–78.
2. Bancroft, J. B., Hills, G. J., and Markham, R. (1967). A study of self-assembly process in a small spherical virus: formation of organized structures from protein subunits in vitro. *Virology* **31,** 354–379.
3. Jacrot, B. (1975) Studies on the assembly of a spherical virus. II. The mechanism of protein aggregation and virus swelling. *J. Mol. Biol.* **95,** 433–446.
4. Lane, L. (1979) Bromovirus Group. No 215 in CMI/ABB Descriptions of Plant Viruses, Commonw. Mycol. Inst. Assoc. Appl. Biologists, Kew, Surrey, UK.
5. Bujarski, J. J., Nagy, P. D., and Flasinski, S. (1994) Molecular studies of genetic RNA-RNA recombination in brome mosaic virus, in *Advances in Virus Research*, vol. 43 (Maramorosch, K., Murphy, F., and Shatkin, A., eds.).
6. Ahlquist, P., Bujarski. J. J., Kaesberg, P., and Hall, T. C. (1984) Localization of the replicase recognition site within brome mosaic virus RNA by hybrid-arrested RNA synthesis. *Plant Mol. Biol*. **3,** 37–44.