followed by rRNA, and the final peak corresponds to viral RNA. Phenol-extract and ethanol-precipitate each fraction. Pellet RNA and resuspend in TE buffer.

7. Load one-half of each fraction onto a 1.5% agarose gel containing 10% formaldehyde and identify viral RNA by Northern blot analysis.

4. Notes

1. It is usually easy to purify those definitive members of potexviruses; most of the possible members, especially those potexviruses that only infect woody hosts, have not been successfully purified, and some purification attempts have failed ***(29)***. In those cases, virus replicative nucleic acids (dsRNAs) can be extracted from virus infected tissues and used as templates for gene cloning ***(30,31)*** and RNA analysis.
2. If the infected tissues are used as inoculum, the tissues are ground in 0.1*M* phosphate buffer, pH 7.2 (2 mL buffer/1 g tissue), and centrifuged briefly (10,000*g* for 4 min) before the extracts are used for inoculation. Virus-infected plants will be ready for virus purification 10–14 d postinoculation. Infected leaves can also be stored at –20°C. Both fresh and frozen leaf tissue can be used for virus purification.
3. For most potexviruses, pretreatment of the infected tissues is usually not necessary. In the case of potato aucuba mosaic virus (PAMV), however, infiltrating the tissues with extraction buffer under vacuum before homogenizing them is believed to be helpful ***(32)***.
4. It is recommended that centrifuge tubes be filled first with the virus solution and then that the sucrose cushion be deposited at the bottom of each tube with a Pasteur pipet.
5. The virions of potexviruses have a tendency to aggregate side-to-side or end-to-end and to break. This will result in a lower yield of purified virus and difficulties in the isolation of full-length gRNA. To avoid or minimize these problems, it is recommended that during purification, after each centrifugation step, the pellets be covered with buffer at 4°C for a prolonged period (e.g., overnight) before complete resuspension. It is also recommended to dissolve the pellets by gentle repeated pipeting and not by vortexing. Many potexviruses (e.g., papaya mosaic virus) may have a high yield of virus. It is recommended to dilute the virus preparations prior to ultracentrifugation. High concentrations of the virus result in increased viscosity and prolonged ultracentrifugation time and/or loss of yield. Pellets of diluted virus preparation are generally cleaner.
6. It is essential in the steps involving the handling of RNA that all glasswares and solutions are RNase-free. This can be accomplished by baking glasswares at 160°C for at least 6 h and making all solutions with DEPC-dH_2O.
7. Bamboo mosaic virus is the only potexvirus to contain a packaged satellite RNA (sRNA). Both gRNA and sRNAs can be extracted from purified virions and separated by electrophoresis in nondenaturing 1% low-melting agarose gel. After electrophoresis, both RNAs are then isolated from gel slices, followed by phenol extraction and ethanol precipitation ***(33)***.