12. The viral pellets are gently resuspended overnight in 0.5 mL of 10 m*M* extraction buffer and the optical density (OD) at 260 nm is measured on an aliquot after a 1:100 dilution. The virus concentration (C) is established according to the following formula: C (mg/mL) = OD × 100/3.2. The number in the denominator is the weight extinction coefficient ***(20)***. In general, we obtain, depending on the viral isolate, 20–80 mg/kg of leaves (*see* **Note 5**). The virus so obtained can be stored at 4°C for a few days or frozen at –20°C for several months, but its infectivity decreases abruptly after the first few days.

3.2. RNA Extraction

1. For RNA extraction, transfer the viral suspension to Eppendorf tubes and add NaCl to a final concentration of 200 m*M*. Extract with phenol for 5 min, with the tubes being placed in ice from time to time. Mix vigorously during the phenol extraction, to produce a good emulsion. Centrifuge for 5 min at 8000*g* and remove the supernatant to new sterile tubes. Repeat the extraction two times, but with phenol:chloroform rather than phenol. Add 2 vol of ethanol to the supernatant and let the RNA precipitate at –20°C. The RNA can be stored in this form for several months or years, and then collected by centrifugation at 17,000*g* for 15 min. The RNA pellet is washed once with 70% ethanol, followed by centrifugation, as above; the pellet is dried and then dissolved in a small volume of sterile water.
2. The RNA concentration can be determined by spectrophotometry at 260 nm. The concentration (mg/mL) = OD × dilution factor/25. The viral RNA constitutes approx 5% of the particle; with an 80% RNA extraction yield, we obtain 0.8–4 mg of viral RNA from a virus preparation.

4. Notes

1. Note that the host used is *C. quinoa,* which gives a local lesion response to BNYVV. The lesions differ somewhat in size and appearance, depending on the viral isolate. Isolates carrying RNA3 produce intense yellow lesions, which have a tendency to extend along the veins ***(24)***. These isolates give the best purification yield. Isolates carrying only RNA-1 and RNA-2 induce mild chlorotic local lesions and give a lower purification yield. Systemic infection hosts, such as spinach (*Spinacia oleracea*), did not give superior yields of BNYVV ***(20)***. For other furoviruses (for example, SBWMV or PMTV ***(23,25,26)***, systemic infection hosts have been used for viral propagation and purification.
2. The viral multiplication period depends on the season, being short in the summer, when the light intensity is strong, and longer in the winter. The best time to harvest infected leaves is when the lesions are well visible, but the leaves are still green. Do not wait until the leaves become senescent. For other furoviruses (SBWMV or PMTV), leaves are harvested for 3–4 wk postinoculation ***(25,26)***.
3. The clarification agent changes from one virus to another. For PCV, the organic solvent is a mixture of butanol and chloroform ***(27)***. Sap clarification with an organic solvent is omitted for viruses such as PMTV or NVMV ***(21–23)***, for