10. The purity and concentration of the RNA can be measured according to the OD readings at 260 and 280 nm. The RNA preparation can also be analyzed by electrophoresis on agarose gel. A ratio of $OD_{260}/OD_{280} \geq 1.9$ indicates that the RNA is essentially free from protein.

An extinction coefficient $E^{260\ nm}_{0.1\%,\ 1\ cm} = 25$ is used to quantify the amount of RNA. To obtain high-quality, full-length, infectious gRNA, the RNA extracts should be further centrifuged in 5–20% (w/v) RNase-free sucrose gradient in 10 m*M* Tris-HCl, pH 7.5, 100 m*M* NaCl, 1 m*M* EDTA, as follows:

1. Load the RNA solution onto a continuous 5–20% sucrose gradient and centrifuge at 87,800*g* for 13 h in a swing-bucket rotor at 4°C.
2. Pass the gradient through a UV scanner-fractionator.
3. Collect fractions corresponding to the full-length gRNA, precipitate RNA with 2 vol of 95% ethanol at –20°C overnight and recover RNA by centrifugation for 10 min at 12,500*g*. Wash RNA pellets with a large volume of 70% ethanol.

3.2.2. Extraction of Viral RNA from Infected Plant Tissue

This technique can be used for extracting viral genomic and subgenomic RNAs from infected tissues or from transgenic plants expressing viral sequences. Total plant RNA is isolated by grinding frozen plant tissue in extraction buffer, extracting with phenol, and precipitating with ethanol. RNA quantity can be determined by UV absorption or by electrophoresis on agarose gels. Viral RNA can be identified from plant RNA by Northern blot analysis (*see* **Note 9**).

A variety of procedures can be used to isolate viral RNA from infected plant tissues. These include extraction in a phenol–cresol solution ***(26)*** and extraction with hot phenol–SDS ***(27)***. The highest yield of PVX RNA was achieved in the following manner:

1. Place leaves (0.4 g) in a prechilled mortar and pestle and grind to a powder in liquid nitrogen. Add 6 mL of extraction buffer A and allow to freeze.
2. Add 6 mL Tris-saturated phenol, pH 8.0, and DEPC (to a final concentration of 1%), and thaw the mixture at room temperature for 10 min. Pour the slurry into a tube and maintain on ice (*see* **Note 10**).
3. Remove large debris by centrifugation at 12,000*g* for 10 min at 4°C. Extract supernatant with phenol, then twice with 1 vol phenol:chloroform (1:1), and twice with chloroform. Transfer the aqueous phase to a new tube and precipitate RNA by adding 2 vol 95% ethanol and storing at –20°C for 1 h. Pellet RNA by centrifugation at 5800*g* for 20 min. Wash RNA in 70% ethanol, vacuum-dry for 15 min, and resuspend in 1 mL TE buffer.
4. Viral RNA can be identified from cellular RNAs by electrophoresis on a 1.5% agarose gel containing 10% formaldehyde, and by Northern blot analysis.