include pulverizing tissue in liquid N_2, either in a mortar and pestle or in a stainless steel blender container, or adding macerating enzymes prior to homogenization ***(3,5)***. However, the latter may be unhelpful or even detrimental with some isolates ***(4)***. Thorough comminution is essential. As little as 2 vol of buffer per weight of tissue will suffice for extraction. Especially with older tissue, re-extraction of the residual fibrous material can be worthwhile ***(10)***.

2. The sucrose gradients are formed in the process of freezing and thawing ***(11)***, so ensure that the gradients are not disturbed. A larger 2-mL vol of virus can be centrifuged in each tube, in which case only fill the tubes with 10 mL of the 20% sucrose solution.
3. A thorough resuspension of the pellet is essential. This can be accomplished by gentle pipeting up and down, combined with vortexing and shaking on a mechanical shaker.
4. It is essential that the sucrose gradients thaw completely to avoid crystals. They are best thawed to coincide with the timing of their use, i.e., on d 2 if a 2-d procedure is used; otherwise, on d 3. We have not noted differences in yields between the 2- and 3-d procedures.
5. The centrifugation has also been performed using a Beckman 30 rotor at 86,000*g* for 4 h.
6. These centrifugations can also be done in a microcentrifuge at 12,000*g*.
7. Each mL of virus usually represents approx 100–200 g of original starting material.
8. We employ an ISCO Model D density gradient fractionator with a Type 6 Optical Unit and a Model UA-5 Absorbance Monitor. The scale of the UV monitor is set for a range of 0.5–1.0 OD absorbtion units. The syringe speed is 2.5 mL/min and the chart speed is 60 cm/h. Virus peaks occur approx halfway along the charts, coinciding with the position of southern bean mosaic virus, if used as a marker in sister tubes ***(12)***. Published yields are usually relatively low, ranging only up to about 6 mg/kg fresh tissue ***(8)***.
9. This method has been used to purify several isolates of PLRV from the leaves and stems of *Physalis floridana* and potato plants. *P. floridana* seedlings are infected by allowing viruliferous aphids, previously fed on PLRV-infected plants, to feed overnight. Potato plants are grown from infected tubers. All plants are grown under greenhouse conditions, with supplementary light in winter. Approximate yields are 800–900 μg of virus particles per kg of plant tissue ***(7)***. Plant tissue can be stored at –20°C for several months after harvesting, but the amount of virus that can be extracted decreases with storage time; fresh material should be used whenever possible and tends to be easier to macerate in the initial stages of the extraction procedure.
10. If using 500 g or more of starting material, it is best to split the suspension into two or more batches at this stage for convenience and safety.
11. All centrifugation steps are performed at 20°C; the temperature should not fall below 15°C during centrifugation.
12. For larger quantities of partially pure virus preparation, during the first rounds of virus purification, approx 5–7 mL of 20% sucrose (w/v) in phosphate buffer