3. Load 0.5–0.75 mL (7.5–10 mg) of virus suspension on top surface of the gradient.
4. Centrifuge sample at 163,500*g* for 23 h at 15°C.
5. Observe separated viral components with a beam of white light shining directly through the length of the tube. Remove bands of interest using syringe fitted with a 21-gage needle.
6. Dilute virus suspension by adding at least 9 vol of 10 m*M* sodium phosphate solution. Spin in a Beckman Type 40 tube at 93,000*g* for 4 h at 4°C (*see* **Note 2**).
7. Extract RNA by resuspending the pellet in NET buffer, made 2% (w/v) with respect to SDS. Warm to 55°C. After 2–3 min, add 1 vol phenol:chloroform and vortex vigorously. Separate two phases by low speed centrifugation and remove top (aqueous) layer to a fresh tube (*see* **Note 3**).
8. To precipitate the RNA, add 2.5 vol absolute ethanol to aqueous phase and freeze overnight at –20°C (or 30 min at –70°C).
9. Pellet the RNA by centrifuging the sample at 12,000*g* for 10 min. Wash with absolute alcohol, recentrifuge for 5 min and dry briefly in a vacuum desiccator. Dissolve RNA in sterile dH_2O and store at –70°C. Examine on denaturing agarose gel (**Fig. 1**).

3.2. Fractionation of RNA by Velocity Centrifugation on Sucrose Gradients

1. Layer 2.75 mL of each sucrose solution in a SW40Ti centrifuge tube.
2. Allow to diffuse for 3–4 h at room temperature (or at 4°C overnight).
3. Load approx 50 μg RNA on to the top surface of the gradient.
4. Spin at 130,000*g* for 12 h at 15°C.
5. Puncture the bottom of the tube with a needle and collect approx 0.4 mL fractions in sterilized large microcentrifuge tubes.
6. Determine the presence of the RNA by removing a small sample (5-μL aliqout) of each of the fractions and electrophorese on a formaldehyde (denaturing) agarose gel (*see* Chapter 25).
7. Pool appropriate fractions and precipitate the RNA by adding 0.1 vol 3*M* Na acetate soution, pH 5.5, and 2.5 vol absolute ethanol.
8. Pellet RNA in a microcentrifuge and wash with absolute alcohol. Respin and dry pellet and dissolve in sterile dH_2O. Store at –70°C.

4. Notes

1. Nycodenz is a nonionic derivative of benzoic acid (systematic name 5-(*N*-2, 3-dihydroxypropylacetamido)-2, 4, 6-tri-iodo-*N*, *N′-bis*(2,3 dihydroxypropyl) isophthalamide. The original experiments characterizing the use of Nycodenz for isopycnic centrifugation of plant viruses have previously been described by Gugerli ***(1)***. Our experience has shown that this material is the most suitable density gradient medium for separation of comovirus components. Initial experiments in our laboratory showed that CPMV nucleoproteins will separate well in CsCl gadients, but when the RNA is extracted from them, it is often degraded and of very poor quality. This has never been the case when Nycodenz is used.