centrifugation, the molecules move along the gradient at various rates. Larger molecules (i.e., those that have a larger sedimentation coefficient) will migrate faster down the tube than smaller ones. The density gradient medium, in this case, is usually sucrose (often in the range 10–40%). Fractions of the gradient are collected manually and the virus (or RNA) repelleted by a second round of centrifugation.

The protocols outlined below describe our preferred methods when working with CPMV (and other comoviruses). Normally, one cycle of buoyant density centrifugation is sufficient to fractionate the RNAs in a suitable form for cDNA synthesis. However, we also carry out a second round of density gradient centrifugation in those cases when it is essential to have very pure RNA (e.g., for experiments involving the inoculation of protoplasts with viral RNA).

2. Materials

2.1. Separation of Viral Nucleocomponents by Buoyant Density Centrifugation and Extraction of RNA

1. 30, 40, 50, and 60% (w/v) Nycodenz (obtained from Nycomed, Oslo, Norway) solutions buffered in 10m*M* sodium phosphate solution, pH 7.0. Autoclave. Store at 4°C (*see* **Note 1**).
2. Beckman Ultra-Clear SW40Ti and Type 40 centrifuge tubes (or similar).
3. 10 m*M* sodium phosphate buffer, pH 7.0.
4. NET buffer: 100 m*M* NaCl, 10 m*M* Tris-HCl, pH 7.4, 1 m*M* EDTA. Autoclave.
5. 10% (w/v) sodium dodecyl sulfate (SDS) solution.
6. 1:1 mixture of phenol:chloroform solution. **Caution:** Phenol is extremely toxic and caustic. Chloroform is a known carcinogen and volatile. Use only in a fume hood. Store in dark bottles at 4°C. Dispose of all waste correctly.

2.2. Fractionation of RNA by Velocity Centrifugation on Sucrose Gradients

1. 15, 20, 25, and 30% (w/v) sucrose solutions buffered in NET solution (as described in **Section 2.1.**) containing 0.1% (w/v) SDS solution. Autoclave with care (*see* **Note 4**).
2. Beckman SW40Ti tubes.
3. 3*M* Na acetate, pH 5.5.

3. Methods

3.1. Separation of Viral Nucleoproteins by Bouyant Density Centrifugation and Extraction of RNA

1. Carefully layer 2.75 mL of each Nycodenz solution in a SW40Ti tube (heaviest one first).
2. Allow to diffuse overnight at room temperature.