

There is also complete sequence data available for the RNA-2 species of a number of other nepoviruses, including Hungarian grapevine chrome mosaic (*11*), GFLV (*12*), RRV (*13*), and tomato ringspot (TomRV; **ref. 14**). The last of these has an unusually large RNA-2 component (7273 nucleotides), which is partially accounted for by a long 3' noncoding region. The capsid protein sequence occupies the same position in the polyprotein sequence as that of other nepoviruses. Although the genome of strawberry latent ringspot virus has much in common with the nepoviruses, the viral capsid contains two proteins,  $M_r$  29 and 44 kDa; the position of this virus within the *Comoviridae* family remains to be resolved (*15*).

Nepoviruses are usually stable and relatively easy to purify. The virus purification procedure outlined below utilizes the standard procedures of sap clarification using an organic solvent, virus precipitation with polyethylene glycol (PEG), and several cycles of differential centrifugation. It is essentially that of Mayo et al. (*16*), and works well for many of the nepoviruses. For others, however, the use of chloroform to clarify sap extracts may be preferred (*see Subheading 4.*). RNA is obtained from virions using a standard phenol:chloroform procedure.

## 2. Materials

### 2.1. Virus Purification

1. Carborundum (F 500; Carborundum, Manchester, UK).
2. Butter muslin.
3. Omnimixer (Camlab, Cambridge, UK).
4. Butan-1-ol (*n*-butanol; Merck, Luttenworth, UK).
5. PEG 6000 (Merck).
6. Homogenization buffer: 0.07M sodium phosphate buffer, pH 7.0, 0.01M in di-Na EDTA and containing 0.1% (v/v) mercaptoacetic acid.
7. Resuspension buffer A: 0.07M sodium phosphate buffer, pH 7.0.
8. Resuspension buffer B: 10 mM Tris-HCl, 50 mM NaCl, 1 mM di-Na EDTA, pH 7.5.

### 2.2. RNA Purification

1. Wrist-action shaker (Fisher Scientific, Loughborough, UK).
2. Sodium dodecyl sulfate (SDS, Merck).
3. Phenol mix: phenol, containing 0.1% 8-hydroxyquinoline, saturated with resuspension buffer B.
4. Chloroform mix: chloroform and isoamyl alcohol mixture (24:1; [v/v]), saturated with resuspension buffer B.
5. Sodium acetate.
6. 70% (v/v) Ethanol.