The tobraviral CP is the 5' proximal gene on RNA-2, but it is not translated efficiently from the genomic RNA and is expressed from a 3' coterminal subgenomic RNA ***(4,5)***. The CP is thought to be an important determinant of nematode transmission ***(14)***. Additional ORFs have been identified on RNA-2 of several tobravirus isolates. Some TRV RNA-2 species have undergone recombination in the 3' region with part of the same region of RNA-1, and thus carry part or all of the RNA-1-coded 29K and 16K genes ***(3–5,15)***. TRV isolate TCM and PEBV isolate SP5, RNA-2, contain an ORF for a 29.1- and a 29.6-kDa protein, respectively ***(5,8)***. Sequence comparison of RNA-2 of nematode-transmittable and nematode-nontransmittable isolates of PEBV suggest that the 29.6-kDa protein may be involved in nematode transmission ***(16)***. There is evidence that the additional 9- and 23-kDa ORFs on PEBV (SP5) RNA-2 may also have some function in vector transmission (S. A. MacFarlane, personal communication).

2. Materials

2.1. Purification of Virus

1. Celite.
2. 30 m*M* Sodium phosphate buffer, pH 7.5.
3. Virus extraction buffer: 50 m*M* sodium phosphate buffer, 0.15% thioglycollic acid (v/v). Add 1.5 mL of thioglycollic acid to 250 mL 200 m*M* Na_2HPO_4 and 550 mL of distilled H_2O, adjust to pH 7.5 with NaOH, and make up to 1 L with distilled H_2O. Make buffer up fresh and keep at 4°C. (**Caution:** Wear gloves when handling thioglycollic acid and use in a fume hood.)
4. Commercial blender.
5. Muslin.
6. Polyethelene glycol (PEG); mol wt = 6000.
7. Sodium chloride (NaCl).
8. Chloroform:butan-1-ol 1:1 (v/v). (**Caution:** Wear gloves and use solvents in a fume hood.)
9. Sucrose cushion: 30% sucrose in 30 m*M* sodium phosphate buffer, pH 7.5.
10. 10 m*M* Tris-HCl, pH 7.5.

2.2. Extraction of Viral RNA

1. Deoxyribonuclease I (DNase I) RNase-free.
2. 1X DNase reaction buffer: 50 m*M* Tris-HCl, pH 7.9, 5 m*M* $MgCl_2$.
3. EDTA.
4. Proteinase K.
5. 1X Proteinase K buffer: 100 m*M* Tris-HCl, pH 7.4, 1 m*M* EDTA, 0.1% SDS.
6. Tris-equilibrated phenol, pH 8.0 (**Caution:** Wear gloves and use in fume hood).
7. Chloroform/isoamyl alcohol 24:1 (**Caution:** Wear gloves and use in fume hood).
8. Phenol/chloroform:isoamyl alcohol 1:1.