

7. Cesium chloride (CsCl) density gradient: 10 g of CsCl is mixed with 20 mL of virus solution to reach a density of 1.3667.
8. Sodium azide.

2.2. Viral gRNA Extraction

1. 10X DNase I reaction buffer (1X buffer = 20 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 100 µg/mL of acetylated bovine serum albumin [BSA]).
2. Deoxyribonuclease I (DNase I).
3. Sodium dodecyl sulfate (SDS), 10%.
4. TE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 7.5.
5. Phenol: The phenol used is pre-equilibrated with 0.1M Tris-HCl, pH 8.0, containing 0.2% β-mercaptoethanol.
6. Chloroform.
7. 70 and 95% Ethanol.
8. Diethyl pyrocarbonate-treated distilled water (DEPC-dH₂O). DEPC (0.5 mL) is dissolved in 2 mL 95% ethanol, and then mixed with 1 L dH₂O. The mixture is stored at 4°C overnight before being autoclaved.
9. RNase-free glassware.
10. Sodium acetate.
11. Triton X-100.

2.3. Extraction of Viral or Polyribosomal RNAs from Infected Plant Tissue

1. Oligo(dT)-cellulose.
2. Loading buffer A: 20 mM Tris-HCl, pH 7.5, 0.5M LiCl, 1 mM EDTA, 0.1% SDS.
3. Loading buffer B: 20 mM Tris-HCl, pH 7.5, 0.1M LiCl, 1 mM EDTA, 0.1% SDS.
4. Solution A: 0.1N NaOH, 5 mM EDTA.
5. Solution B: 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.05% SDS.
6. Extraction buffer A: 200 mM Tris-HCl, pH 9.0, 400 mM LiCl, 25 mM EDTA, 1% SDS, all in DEPC-dH₂O.
7. Extraction buffer B: 200 mM Tris-HCl, pH 8.5, 400 mM KCl, 200 mM sucrose, 35 mM MgCl₂, 25 mM EDTA, and 1% β-mercaptoethanol.
8. Sucrose cushion: 60% sucrose in 40 mM Tris-HCl, pH 8.5, 200 mM KCl, 30 mM MgCl₂, and 5 mM EDTA.
9. Buffer A: 100 mM Tris-HCl, pH 7.5, 1M KCl, 10 mM MgCl₂, and 2.5 mM puromycin.
10. Buffer B: 50 mM Tris-HCl, pH 7.5, 500 mM KCl, 5 mM MgCl₂.
11. Liquid nitrogen.

3. Methods

3.1. Purification of Virus

Many potexviruses occur in high concentration in their host plants and are relatively stable in extracted leaf sap. These properties make it possible to develop protocols for the purification of most potexviruses with high virus