

3. Phenol (nucleic acid grade): Equilibrate phenol with TE.
4. Chloroform:isoamyl alcohol (24:1).
5. 3M Sodium acetate, pH 5.2.

2.3.2. Direct Extraction of Genomic dsRNAs from Plants

1. 10X STE buffer: 1M NaCl, 100 mM Tris-HCl, pH 6.8, 10 mM EDTA.
2. 1X STE buffer: Dilute the 10X STE buffer with sterile H₂O.
3. CC41 cellulose powder (Whatman).

2.3.3. Direct Extraction of Viral mRNAs and Genomic dsRNAs from Plants

1. Milli-Q grade H₂O treated with 0.1% DEPC and autoclaved.
2. RNA extraction solution: 4M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, 0.1M 2-mercaptoethanol. This solution is made by mixing the following:
 - a. 250 g Guanidinium thiocyanate (Fluke) is dissolved in 293 mL Milli-Q grade H₂O.
 - b. 17.6 mL 0.75M Sodium citrate, pH 7.0.
 - c. 26.4 mL 10% Sarcosyl. The stock solution (a + b + c) can be stored for at least 3 mo at room temperature.
 - d. 0.36 mL 2-Mercaptoethanol/50 mL. RNA extraction solution (a + b + c + d) can be stored for 1 mo at room temperature.
3. Phenol (nucleic acid grade): Equilibrate phenol with Milli-Q grade H₂O.
4. 2M Sodium acetate, pH 4.0.
5. 4M LiCl.

2.3.4. RT-PCR of Genomic dsRNAs

1. Dimethyl sulfoxide (DMSO) (Spectrum grade): Flushed with N₂ gas.
2. 2.5 mM dNTPs mix, pH 8.0, in 1 mM Tris.
3. Actinomycin D (500 µg/mL).
4. AMV reverse transcriptase XL (Life Sciences).
5. 10X RTase buffer for AMV reverse transcriptase: 500 mM Tris-HCl, pH 8.3., 100 mM KCl, 40 mM DTT, 100 mM MgCl₂.
6. Tth DNA polymerase and 10X reaction buffer supplied by the manufacturer.
7. 0.5 MEDTA, pH 8.0.
8. Mineral oil (Sigma M-3516 or equivalent).

3. Methods

3.1. Isolation and Propagation of Rice Dwarf Virus

1. Preparation of inoculum: Homogenize 10–50 mg of infected rice leaves in a small mortar and pestle with 19 times (v/w) of 0.1M phosphate buffer, pH 7.3. Transfer the homogenates to a 1.5 mL microtube, and centrifuge at 1800g for 5 min at 0°C. Dilute the supernatant in 5–25 times of 0.1M phosphate buffer, pH 7.8, and use this extract as an inoculum.