with reverse transcription (RT-PCR) and subsequent cDNA cloning into a bacterial plasmid vector.

2. Materials

2.1. Isolation and Propagation of Rice Dwarf Virus

1. Insect culture: A colony of green leafhopper, *Nephotettix cincticeps,* is maintained on rice seedlings at 25°C under fluorescent lights for 16 h.
2. 0.1*M* Phosphate buffer, pH 7.3 and pH 7.8, containing KH_2PO_4 and Na_2HPO_4.
3. Requirements for microinjection into a vector insect are the following:
   a. Stereoscopic microscope.
   b. Fine glass needle: Stretch the 50-μL glass capillary (Drummond Scientific) with micropipet tension (Narishige, Model PB-7).
   c. 2-mL Glass syringe and silicone tubes to joint the needle and the syringe.
   d. Petri dish (9-cm diameter).
   e. Compressed CO_2 gases to anesthetize insects.
4. Rice seedlings for inoculation are prepared as follows: Soak 50–60 seeds in water for 2 d at 25°C to germinate. Transfer the individual seedling into a glass test tube (2.5-cm diameter and 13-cm length) containing 15–20 cm^3 of horticultural granular soil. Grow for 3–5 d.

2.2. Purification of Viruses

2.2.1. Rice Dwarf Virus

1. Virus source: Infected rice leaves and leaf sheaths showing clear symptoms 1–2 mo after inoculation.
2. 0.1*M* Phosphate buffer, pH 6.0, containing KH_2PO_4 and Na_2HPO_4.
3. Triton X-100.
4. Chloroform.
5. Carbon tetrachloride.
6. 40% (w/v) Sucrose in 0.1*M* phosphate buffer, pH 6.0.
7. Hitachi RP 30-2, RP 65, and RPS 27 rotors (or equivalents).
8. Glycerol.

2.2.2. Rice Black-Streaked Dwarf and Rice Ragged Stunt Viruses

1. Extraction (GMT) buffer: 0.3*M* glycine, 0.03*M* $MgCl_2$, 0.05*M* Tris-HCl, pH 7.5.
2. Carbon tetrachloride.
3. Triton X-100.
4. 40% (w/v) Sucrose in GMT buffer.

2.3. Extraction of Viral RNAs and RT-PCR Amplification of Genomic dsRNAs

2.3.1. Reagents for General Use

1. Milli-Q grade autoclaved H_2O.
2. TE: 10 m*M* Tris-HCl, 1 m*M* EDTA, pH 8.0.