

2. Collect the second instars of *N. cincticeps* in a small bottle and inject CO<sub>2</sub> gases into the bottle for 30–60 s to anesthetize. Arrange the insects on a Petri dish using a toothpick and cover them with Parafilm™ (American National Can) to immobilize.
3. Cut the point of the glass needle, and suck the inoculum into a glass needle with a syringe.
4. Inject a small amount of the inoculum (>1 µL) into the abdomen of each insect under a stereoscopic microscope.
5. Rear the insects on rice seedlings in a conical flask covered with a fine mesh screen for 10–12 d at 25°C under continuous fluorescent lights (latent periods).
6. Transfer individual insect to a rice seedling cv. Norin No. 8 grown for 5–7 d after germination in a test tube. Inoculate for 1–4 d by feeding.
7. Remove the insect, and grow the plant under fluorescent lights for 18 h at 25–27°C. About 1–2 wk after inoculation feeding, viral symptoms (white specks along veins) appear on new leaves.

### 3.2. Purification of Viruses

#### 3.2.1. Rice Dwarf Virus

1. Harvest 10–50 g fresh rice leaves.
2. Homogenize the leaves with ELISA juice press (Erich Pollahne, Germany) in three to five times (v/w) of 0.1M phosphate buffer.
3. Add 1% (w/v) Driselase while stirring at 6°C with a magnetic stirrer for 1 h.
4. Add one-third vol of chloroform, mix the extract thoroughly for 3 min with Polytron homogenizer on ice.
5. Centrifuge at 3000g for 15 min.
6. Transfer the aqueous phase to a centrifuge tube, leaving interface. Centrifuge at 62,000g in a Hitachi RP 30-2 rotor for 60 min at 4°C.
7. Decant the supernatant. Add 4 mL of 0.1M phosphate buffer containing 1% Triton X-100, store the tube overnight at 4°C.
8. Dissolve the pellet thoroughly with Teflon homogenizer on ice. Add an equal volume of carbon tetrachloride; vortex for 3 min.
9. Centrifuge at 3000g for 15 min.
10. Transfer the aqueous phase to a centrifuge tube, leaving interface. Centrifuge at 80,000g in a Hitachi RP 65 rotor for 60 min at 4°C.
11. Decant the supernatant quickly, and dissolve the pellet in 1 mL of 0.1M phosphate buffer with Teflon homogenizer on ice.
12. Overlay the suspension on a 10–40% (w/v) linear sucrose density gradient, and centrifuge at 80,000g in a Hitachi RPS 27 rotor for 60 min at 4°C.
13. By inserting a L-shaped needle into the tube from the meniscus, collect the virus zone. Transfer into a centrifuge tube.
14. Centrifuge at 80,000g in a Hitachi RP 65 rotor for 60 min at 4°C.
15. Decant the supernatant quickly, and dissolve the pellet in a small amount of 0.1 M phosphate buffer.
16. Add an equal volume of glycerol, store at –80°C.