

14. Add 0.1 vol of 3.0M sodium acetate, pH 5.2, and 2.5 vol of ethanol, mix, and then incubate for 60 min on ice.
15. Precipitate dsRNA by centrifugation at 15,000g for 15 min at 4°C in the microcentrifuge.
16. Remove supernatant and then wash the pellet with 1 mL of 70% ethanol.
17. Recover the pellet by centrifugation at 15,000g for 10 min at 4°C in the microcentrifuge.
18. Stand the open tube on the bench at room temperature until the last traces of fluid have evaporated.
19. Dissolve the dsRNA pellet (which is often invisible) in the desired volume of TE or sterile H<sub>2</sub>O. Rinse the walls of the tube well with the buffer or sterile H<sub>2</sub>O.

### **3.3.2. Direct Extraction of Viral mRNAs and Genomic dsRNAs from Plants**

1. Infected fresh rice leaves are homogenized with the RNA extraction solution (500 µL/100 mg) in a mortar and pestle.
2. Transfer the homogenate to a 1.5-mL microcentrifuge tube and centrifuge for 1 min at high speed.
3. Transfer 500 µL supernatant to a new microcentrifuge tube.
4. Add 50 µL 2M sodium acetate and 500 µL phenol, 100 µL chloroform:isoamyl alcohol. Mix thoroughly by inverting the tube after the addition of each reagent.
5. Vortex for 10 s.
6. Stand the tube for 3 min at room temperature.
7. Centrifuge for 15 min at 10,000g at 4°C.
8. Transfer the aqueous phase to a new microcentrifuge tube.
9. Add 500 µL isopropanol.
10. Stand for 5–10 min at room temperature.
11. Centrifuge for 10 min at 10,000g at 4°C.
12. The RNA pellet is washed with 1 mL 70% ethanol
13. Dry the pellet for 5 min under vacuums.
14. Suspend in 250 µL sterile H<sub>2</sub>O.
15. Add 250 µL 4M LiCl and vortex.
16. Place on ice for more than 8 h.
17. Centrifuge for 10 min at 10,000g at 4°C.
18. Supernatant contains dsRNAs and tRNAs. Collect them by ethanol precipitation. dsRNAs are further purified by CC41 treatment as in **Subheading 3.3.1., step 7**. The pellet contains viral mRNAs suitable for Northern blotting analyses.

## **3.4. RT-PCR Amplification of Genomic dsRNAs**

### **3.4.1. Basic RT-PCR**

1. Add 100 µL of DMSO to 5 µg of genomic dsRNAs in 5 µL sterile H<sub>2</sub>O.
2. Incubate at 50°C for 30 min.
3. Add 10 µL 3M sodium acetate and 300 µL ice-cold ethanol.