3.4.2. Single-Step RT-PCR

1. In a 0.5-mL microcentrifuge tube, mix in the following order:
   a. 5 μL 10X amplification buffer.
   b. 5-μL Mixture of four dNTPs, each of 5 m*M*.
   c. 1 μL 5' Sense-strand primer (100 pmol).
   d. 1 μL 3' Antisense-strand primer (100 pmol).
   e. 30 μL Sterile H_2O.
   f. 1 μL Tth DNA polymerase (4U/μL).
   g. 0.3 μL AMV reverse transcriptase XL (30 U/μL).
   h. 5 μL Template dsRNA denatured with DMSO (up to 1 μg).
2. Overlay the reaction mixture with one drop (ca. 30 μL) of light mineral oil and briefly spin.
3. Carry out the cDNA synthesis and amplification of cDNAs in the same tube with a thermal cycler. Typical conditions for the synthesis of cDNA, denaturation, annealing, and polymerization are as follows:

One cycle for cDNA synthesis	42°C for 15 min
An initial denaturation	95°C for 1 min
30 PCR cycles	95°C for 1 min
	55°C for 2 min
	72°C for 3 min
Final extension	72°C for 10 min

4. Withdraw a sample of the amplified DNA from the reaction mixture and analyze it by gel electrophoresis, Southern hybridization, or DNA sequencing.

4. Notes

1. Equipment for handling leafhoppers and planthoppers, such as cages for maintaining and tools for transferring the insects, are described elsewhere ***(6)***. Injection of viruses into the vector insects is a good alternative to a conventional acquisition feeding. Nearly 100% of the injected insects become viruliferous. The younger the larvae, the more they recover and survive after injection of the viral extract. Use larvae or male adults for inoculation feeding to prevent laying eggs into inoculated plants. Hatching eggs on the inoculated plants becomes a serious source for contamination of viral cultures. Most rice cultivars are not resistant to virus infection, but some are resistant to vector insects. The cultivar Norin no. 8 is used in our laboratory and TN 1 is used worldwide (**Subheading 3.1.**).
2. Since individual virus isolates have similar but distinct electrophoretic mobility of the genomic segments ***(5)***, viral cultures collected from fields should be examined for genomic homogeneity by PAGE of genomic dsRNAs; 50–100 ng of dsRNAs should give clear bands in 40-cm-long and 0.8-mm-thick gel after silver staining (**Subheading 3.1.**).
3. Plant materials for purification of RBSDV and RRSV are very critical for obtaining a good yield. Young and fresh leaves showing good symptoms within 1 mo