4. Incubate at –80°C for 30 min or place in dry ice for 5 min.
5. Centrifuge at 16,000*g* for 10 min at 4° C.
6. Remove supernatant, rinse the precipitate with 1 mL 70% ethanol.
7. Dry under vacuum.
8. Make up the following master mix for one sample:
   a. 5 μL 10X RTase buffer.
   b. 5 μL Actinomycin D.
   c. 20 μL 2.5 m*M* dNTP mix.
   d. 1 μL 3' Antisense-strand primer (100 pmol/1 μL).
   e. 19 μL Sterile H_2O.
   f. 0.3 μL AMV reverse transcriptase XIL (30 U/μL).
9. Add the master mix to the dried denatured genomic RNAs, vortex, and spin briefly.
10. Incubate at 45°C for 1 h and terminate the reaction by adding 1 μL 0.5*M* EDTA, pH 8.0.
11. Add 25 μL each of phenol and chloroform:isoamyl alcohol (24:1).
12. Vortex for 3 min and centrifuge for 5 min.
13. Transfer the aqueous phase to a new microcentrifuge tube and add 50 μL chloroform.
14. Vortex for 3 min and centrifuge for 5 min.
15. Transfer the aqueous phase to a new microcentrifuge tube and add 0.1 vol of 3*M* sodium acetate and 2.5 vol of ethanol.
16. Place on ice for 30 min and centrifuge at 16,000*g* for 10 min at 4°C.
17. Rinse the precipitate with 70% ethanol and dry under vacuums.
18. Suspend in 25 μL sterile H_2O and 5 μL was subjected to a PCR reaction.
19. In a 0.5-mL microcentrifuge tube, mix in the following in order:
   a. 5 μL 10X amplification buffer.
   b. 8 μL 1.25 m*M* dNTPs.
   c. 1 μL 5' Sense-strand primer (100 pmol/1 μL).
   d. 1 μL 3' Antisense-strand primer (100 pmol/1 μL).
   e. 30 μL Sterile H_2O.
   f. 1 μL Tth DNA polymerase (4U/μL).
   g. 5 μL First-strand cDNA made from up to 1 μg dsRNA.
20. Overlay the reaction mixture with one drop (about 30 μL) of light mineral oil and briefly spin.
21. Carry out the amplification of cDNAs with a thermal cycler. Typical conditions for the synthesis of cDNA, denaturation, annealing, and polymerization are as follows:

24 PCR cycles	94°C for 1 min
	55°C for 2 min
	72°C for 3 min
Final extension	72°C for 10 min

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