8. Add 200 μL chloroform:IAA to the aqueous phase from **step 6**, vortex for 5 min, and recentrifuge.
9. Recover aqueous phase again and precipitate the RNA by adding 20 μL 3*M* NaAc and 500 μL 100% cold ethanol. Leave at –20°C overnight.

3.2. Reverse Transcription

1. Pellet the RNA from **Subheading 3.1., step 9** by centrifuging at 14,000 rpm in a microcentrifuge for 15 min at 4°C. Dry pellet briefly and resuspend in 15 μL H_2O.
2. Use a 5-μL aliquot of the resuspended RNA in the following mixture: 5 μL RNA; 5.5 μL H_2O; 1 μL RNase inhibitor; 1 μL reverse primer.
3. Incubate at 65°C for 15 min and then on ice for 5 min.
4. To each tube, add the following: 4 μL 5X superscript buffer; 1 μL 1.25 m*M* dNTPs; 2 μL 0.1*M* DTT; 0.5 μL superscript RT.
5. Incubate at 42°C for 1 h and then at 65°C for 10 min.

3.3. PCR

1. Use a 5-μL aliquot of first-strand synthesis (reaction from **Subheading 3.2., step 5**) in the following: 5 μL first-strand synthesis; 10 μL 10X *Taq* buffer; 16 μL 1.25 m*M* dNTPs; 5 μL forward primer; 5 μL reverse primer; 58.5 μL H2O; 0.5 μL *Taq* polymerase.
2. Overlay with 50–100 μL mineral oil.
3. PCR cycle (*see* **Note 4**)

4. Notes

1. Controls. It is most important to have the following negative controls to check for nonspecific binding of the superinfecting virus: extract from transgenic plant not superinfected; extract from nontransgenic plant infected with the superinfecting virus. If nonspecific binding is found, parameters such as microtiter plates and blocking agents will need to be examined (*see* **ref. *29***). Also, a positive control of superinfecting viral RNA is required for the PCR in **Subheading 3.3.**
2. Microtiter plates. Nonspecific reactions vary according to source of plate. We found rigid Falcon plates to be the most satisfactory. It is most important to fill the wells in the plate to the top in **Subheading 3.1., steps 1** and **2**, to prevent nonspecific binding in later stages.
3. This test is very sensitive and will easily detect any crossreactions between the antiserum and the CP of the superinfecting virus. Initial tests should be performed to determine if this will be a problem. If so, the antiserum can be preadsorbed with the superinfecting virus CP, preferably expressed in *Escherichia coli*, so that the superinfecting RNA does not contaminate the serum.
4. The PCR conditions will depend on factors such as the composition of the primers and size of fragment being amplified. Details on primer selection and conditions for PCR can be found in textbooks on PCR (*see also* Chapter 48).