PCR with Sucrose Prep
15 minutes

Collect the plant tissue (about 10 mg) and place on ice or freeze in liquid nitrogen

Grind in 100 µl Sucrose Solution (50 mM Tris-HCl;pH 7.5, 300 mM NaCl, 300 mM sucrose) using a fresh pipette tip, pestle, or beads.

Add 100 µl more Sucrose Solution

Immediately heat to 95-100°C for 10 min.

Centrifuge gently (6000 g) for 5-10 sec. in a tabletop centrifuge

Optional: Transfer 50-100 µl supernatant containing DNA and RNA to a new tube

Use 1-2 µl DNA/RNA per 50 µl PCR/RT-PCR reaction

Run 35-40 cycles of PCR

PCR with 'Touch-and-Go'
5 seconds

Using a fine pipette tip, puncture the leaf against a firm surface, like your finger covered with a glove

If the PCR mix is ready, touch the solution with the tip of the pipette and mix a few times to transfer most of the DNA/RNA

Run 40 cycles of PCR

For plants in the field or greenhouse, prepare PCR tubes/microtiter plates with 10 µl water in each well. Keep the tubes on ice while puncturing the leaves with a fine pipette tip against a firm surface. Mix the tip well with the solution

Add pre-prepared PCR master mix to the wells in the lab

Run 40 cycles of PCR

RT-PCR with Sucrose Prep or 'Touch-and-Go'
15 minutes to 5 seconds

RNA/DNA is prepared using either the Sucrose Solution or the 'Touch-and-Go' method

The sample is transferred to the pre-prepared "One-Step-RT-PCR" reaction-mix from Qiagen

Cycling conditions: 30 min. at 50°C for reverse transcription; 15 min at 94°C to activate the hot start*Taq* Polymerase;
40 cycles of PCR

PCR conditions

94°C for 3 min.

35-40 cycles of
94°C for 15-30 sec.
55-60°C for 30-45 sec.
72°C for 1 min. per kb amplicon

72°C for 10 min.

4°C until use