Promega

Product Application

Automated Purification of Bacterial DNA from Phytoplasma-Infected Plant Tissue

Purify bacterial DNA from infected plant tissue using the Maxwell® RSC Instrument and Maxwell® RSC PureFood GMO and Authentication Kit.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analysis: qPCR

Sample Type: Phytoplasma-infected ash tree tissue

Input: Up to 200mg of plant tissue

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Heat block capable of 65°C, with 2.0ml tube adapters
- Plant homogenization device or punch device

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM473, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Homogenize or take punches from plant tissue using desired method.
2. Transfer up to 200mg of plant tissue per tube into 2ml tubes.
3. Create a cocktail containing 1ml of CTAB Buffer, 40µl Proteinase K, and 20µl RNase A Solution per plant tissue sample.
4. Add 1ml of this cocktail to each tube containing up to 200mg of plant tissue.
5. Vortex each plant tissue sample for 10 seconds.
6. Incubate plant tissue samples in a heat block for 30 minutes at 65°C.
7. Prepare Maxwell® cartridges as instructed in the Maxwell® RSC PureFood GMO and Authentication Kit Technical Manual (TM473), Section 5.A, Steps 1-3.
8. Following incubation, vortex each plant tissue sample for 10 seconds.
9. Centrifuge plant tissue samples for 10 minutes at ≥ 16,000 x g.
10. For each plant tissue sample, transfer 300µl of clear lysate into well #1 of a Maxwell® cartridge. Avoid transferring solid material and oil as these materials can inhibit downstream assays.
11. Trasnfer 300µl of Lysis Buffer into well #1 of each Maxwell® cartridge.
12. Run the Maxwell® RSC Instrument using the “PureFood GMO and Authentication” protocol.

Results: DNA was successfully purified from phytoplasma-infected ash tree tissue using the Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600). Phytoplasma DNA was specifically detected via qPCR and no qPCR inhibition was observed (Figure 1).

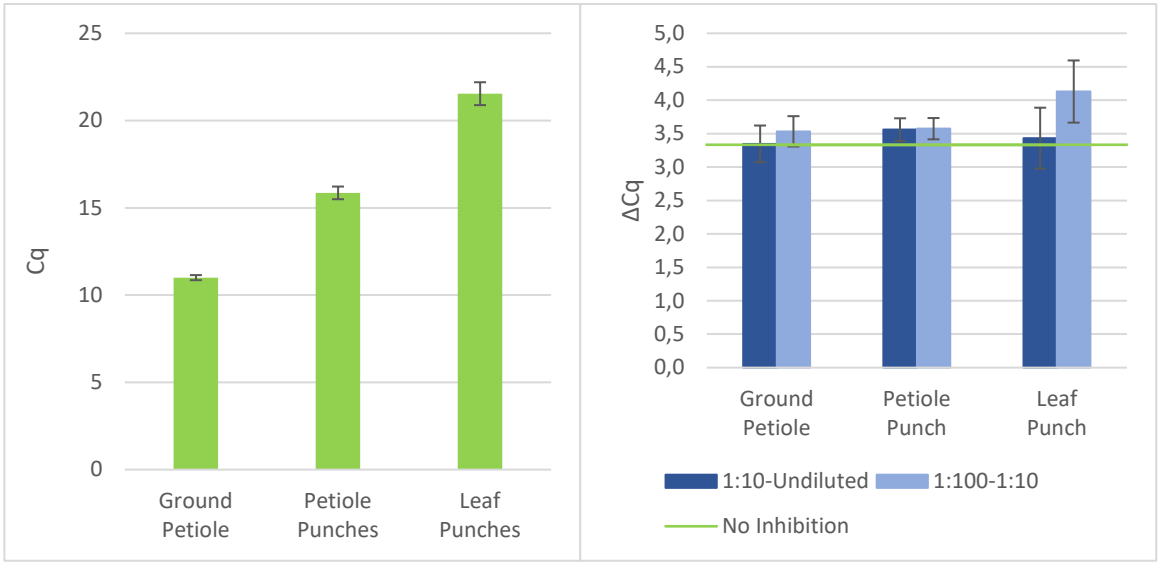

Figure 1. Cq and ΔCq results from qPCR amplification of DNA purified from phytoplasma-infected ash tree tissue. DNA was purified from 68mg of ground petiole tissue, two 4mm petiole punches, and five 4mm leaf punches from phytoplasma-infected ash tree using the Maxwell® RSC PureFood GMO and Authentication (as indicated in the method described). Undiluted, 1:10 diluted, and 1:100 diluted resulting DNA was amplified using GoTaq® qPCR Master Mix (Cat.# A6001) with primers specific to phytoplasma 16S DNA[1] (For: 5' CGT ACG CAA GTA TGA AAC TTA AAG GA 3'; rev - 5' CGA CAA CCA TGC ACC ACC TGI III ICT GAT AAC C 3'). Mean Cq values (undiluted) ± standard deviation (left) and mean ΔCq values (1:10 diluted-undiluted and 1:100 diluted-1:10 diluted) ± error (right) are displayed for triplicate purifications analyzed in duplicate reactions. A ΔCq ≥3.3 is consistent with no inhibition (green line).

References:

1. Ito T, Suzaki K (2017) Universal detection of phytoplasmas and Xylella spp. by TaqMan singleplex and multiplex real-time PCR with dual priming oligonucleotides. PLoS ONE 12(9): e0185427. https://doi.org/10.1371/journal. pone.0185427