Promega

Product Application

Purification of *Xylella fastidiosa* DNA from Infected Plant Tissue using the Maxwell® RSC Instrument

Purify Xylella fastidiosa DNA from infected plant tissue using the Maxwell® RSC Purefood GMO and Authentication Kit and the Maxwell® RSC Instrument.

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

Analyses: qPCR

Sample Type: Plant tissue

Input: 1g of Leaf midribs, petioles and twigs

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Purefood GMO and Authentication Kit (Cat.# AS1600)
- Bioreba extraction bag (Cat.# 430100, Bioreba)
- Hammer
- Heat block capable of 65°C
- Microcentrifuge

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. Add 1g of Leaf midribs, petioles and twigs in a Bioreba extraction bag.
2. Add 5ml of CTAB and homogenize with a hammer to grind the tissues.
3. Transfer 1ml of lysate into 1.5ml tube. **Note:** Avoid transferring solid material
4. Add 40µl Proteinase K and 20µl RNase A Solution per tube.
5. Vortex for 10 seconds.
6. Incubate in a heat block at 65°C for 30 minutes.
7. Vortex for 10 seconds.
8. Centrifuge at maximum speed for 10 minutes. Meanwhile, prepare cartridges as instructed in the technical manual (TM473).
9. Transfer 300µl of clear lysate to well #1 of a Maxwell® RSC Purefood GMO and Authentication Kit cartridge. **Note:** Avoid transferring solid material and oil as these materials can inhibit downstream assays.
10. Add 300µl of Lysis Buffer to well #1.
11. Add 100µl of Elution Buffer in each elution tube.
12. Run the Maxwell® RSC PureFood GMO and Authentication protocol on the Maxwell® RSC Instrument.

Results: DNA was successfully purified from spiked and naturally infected *Xylella fastidiosa* plant tissue samples using the Maxwell® RSC Purefood GMO and Authentication Kit on the Maxwell® RSC Instrument (Cat.# AS4500). *Xylella fastidiosa* DNA was specifically detected via probe qPCR.

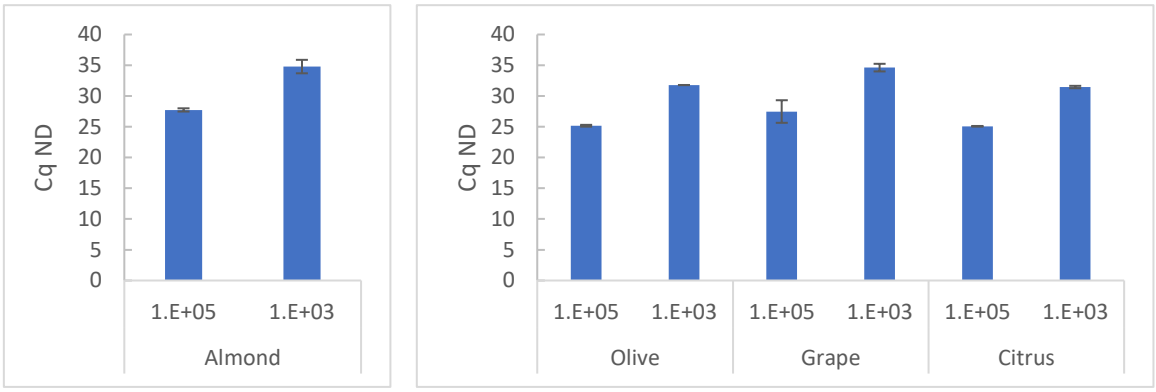

Figure 1. Cq values from probe qPCR amplification of DNA purified from *Xylella fastidiosa* spiked plant tissue using the Maxwell® RSC Purefood GMO and Authentication Kit (Cat.# AS1600). DNA was purified from 1g of almond, olive, grape and citrus leaf midribs, petioles and twigs. Lysates were spiked with 10^5 or 10^3 CFU/100µl of *Xylella fastidiosa* prior to extraction. 2µl of undiluted DNA (ND) was amplified in a 20µl reaction. **Left**. Using the Xylella Custom qPCR Kit (Custom Cat.# CS312101) that targets the 16S rRNA processing RimM protein of *X. fastidiosa*, according to the EPPO 2019 guidelines[1]. **Right**. Using the GoTaq® Probe qPCR (Cat.# A6101) and the *X. fastidiosa* primers and probe (Harper et al.)[1]. NIC (negative isolation controls) were run in parallel for each plan tissue and all of them did not amplify. Results are AVG ± STD (N=2 extractions per spiked sample, N=1 amplification per eluate).

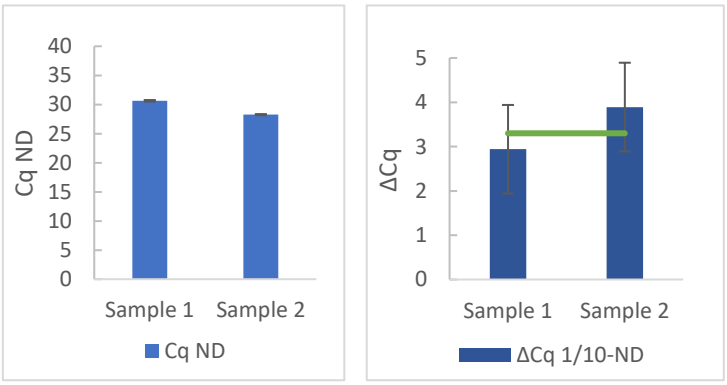

Figure 2. Cq (left) and ΔCq values (right) from probe qPCR amplification of DNA purified from *Xylella fastidiosa* naturally infected tissue using the Maxwell® RSC Purefood GMO and Authentication Kit (Cat.# AS1600). DNA was purified from 1g of *Xylella fastidiosa* naturally infected almond tree leaf midribs, petioles and twigs. 2µl of undiluted DNA (ND) or DNA diluted 1/10 was amplified in a 20µl reaction with the Xylella Custom qPCR Kit (Custom Cat.# CS312101). Results are AVG ± STD (N=2 extractions per sample, N=2 amplifications per eluate). A ΔCq ≈ 3.3 indicates no qPCR inhibition (green line).

References:

1. European and Mediterranean Plant Protection Organization. (2019). Bulletin OEPP/EPPO Bulletin: PM 7/24 (4) *Xylella fastidiosa*. 49 (2), 175–227.