Promega

Product Application

Purification of Bacterial DNA from Phytoplasma-Infected Plant Tissue Using the KingFisher™ Flex

Purify bacterial DNA from infected plant tissue using the Maxwell® HT 96 gDNA Blood Isolation System on the KingFisher™ Flex Purification System.

Kit: Maxwell® HT 96 gDNA Blood Isolation System (Cat.# A2670)

Analysis: qPCR

Sample Type: Phytoplasma-infected ash tree tissue

Input: Up to 200mg of plant tissue

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 Manuals TM368 and TM473 available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® HT 96 gDNA Blood Isolation System (Cat.# A2670)
- KingFisher™ Flex Purification System (Thermo Fisher Scientific, Cat.# 5400630)
- KingFisher™ deep-well 96 Plate (Thermo Fisher Scientific, Cat.# 95040450)
- KingFisher™ 96 Tip Comb for DW Magnets (Thermo Fisher Scientific, Cat.# 97002534)
- CTAB Buffer (Cat.# MC1411)
- 50% Ethanol
- Heat block capable of 65°C, with 2.0ml tube adapters
- Plant homogenization device or punch device

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. Vortex each plant tissue sample for 10 seconds.
5. Incubate plant tissue samples in a heat block for 30 minutes at 65°C.
6. Prepare 96-well Deep Well plates during the incubation, as indicated below:
    - Plate 1 = Tip Plate
        - Add KingFisher™ 96 Tip Comb for DW Magnets.
    - Plate 2 = Elution Plate
        - Add 110µl of Elution Buffer per well.
    - Plate 3 = 50% EtOH
        - Add 450µl 50% Ethanol per well (make fresh daily).
    - Plate 4 = Wash 3
        - Add 400µl Wash Buffer (WBA) and 50µl 50% Ethanol per well
    - Plate 5 = Wash 2

- Add 900µl Wash Buffer (WBA) and 100µl 50% Ethanol per well
- Plate 6 = Wash 1
  - Add 900µl Wash Buffer (WBA) and 100µl 50% Ethanol per well
- Plate 7 = Bind Plate
  - Add 365µl of 100% Isopropanol, 300µl Cell Lysis Buffer (CLD), and 35µl of Resin per well. **Note:** Vortex/shake resin thoroughly to resuspend before addition.

7. Following incubation, vortex each plant tissue sample for 10 seconds.
8. Centrifuge plant tissue samples for 10 minutes at ≥ 16,000 x g.
9. For each plant tissue sample, transfer 300µl of clear lysate per well into the necessary wells of the Bind Plate (Plate 7). Avoid transferring solid material and oil as these materials can inhibit downstream assays.
10. Load the plates onto the KingFisher™ Flex Purification System and run the method "PureFood_96_V1.0".

Results: DNA was successfully purified from phytoplasma-infected ash tree tissue using the Maxwell® HT 96 gDNA Blood Isolation System on the KingFisher™ Flex Purification System. Phytoplasma DNA was specifically detected via qPCR and no qPCR inhibition was observed (Figure 1).

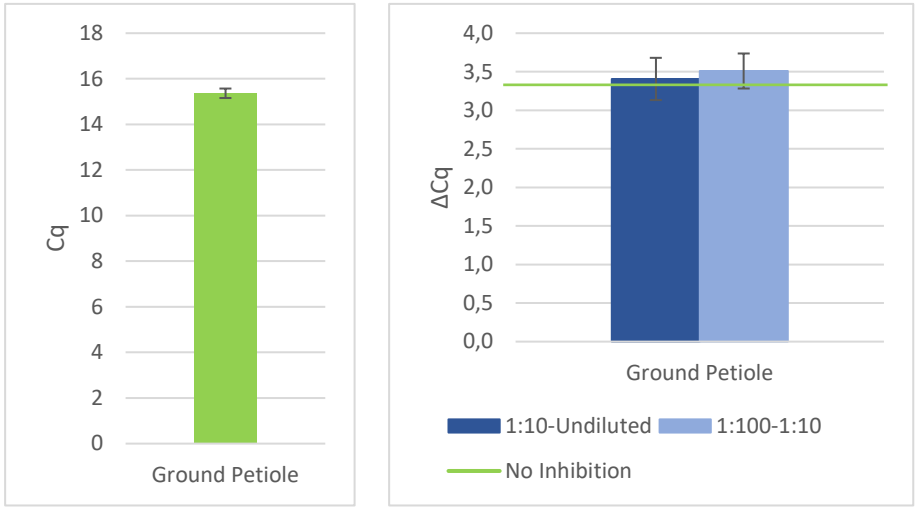

Figure 1. Cq and ΔCq results from qPCR amplification of DNA purified from phytoplasma-infected ash tree tissue. DNA was purified from 68mg of ground, phytoplasma-infected ash tree petioles using the Maxwell® HT 96 gDNA Blood Isolation System on the KingFisher™ Flex Purification System (as indicated in 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[2] (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'). The mean Cq value (undiluted) ± standard deviation (left) and mean ΔCq values (1:10 diluted-undiluted and 1:100 diluted-1:10 diluted) ± error (right) are displayed for quadruplicate purifications analyzed in duplicate reactions. A ΔCq ≥3.3 is consistent with no inhibition (green line).

Reference:

1. Applications Note: Food and Seed DNA purification on KingFisher™ Flex™. 04/2014.
2. 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