Dec 24, 2018 Version 6

A protocol of molecular detection of phytoplasmas and Xylella spp. in post-entry quarantine for plants. V.6

PLOS One

DOI
https://dx.doi.org/10.17504/protocols.io.vvme646

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.vvme646

Protocol Citation: Takao Ito, Ryoji Nakaune 2018. A protocol of molecular detection of phytoplasmas and Xylella spp. in post-entry quarantine for plants.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.vvme646

Manuscript citation:
Ito, T. & Suzaki, K. 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 (2017)

Protocol status: Working
We use this protocol and it's working

Created: November 25, 2018

Last Modified: December 24, 2018

Protocol Integer ID: 18061

Keywords: phytoplasma, xylella, real-time PCR, conventional PCR, multiplex, detection, dual priming oligonucleotides, plant quarantine, xylella spp, protocol of molecular detection, molecular detection, plant internal control, plant, pcr

Abstract

In the STEPS, we describe TaqMan multiplex real-time PCR to universally detect phytoplasmas (PP) and Xylella spp. (XL) with plant internal control (IC) from crude extracts. A protocol file [Protocol-JpEnXX.pdf] shows further details of the protocol in Japanese and English.

Attachments

PDF

Protocol-JpEn13.pdf
1.2MB

1 1. Extraction
1.1. Crude extraction
1.1.1. Put leaf petioles (＜50mg), a metal beads, and 1mL extraction buffer into a tube.
1.1.2. 2,500 rpm 60 sec. (the Multi-beads shocker)
1.1.3. 9,000 x g 10min 4C
1.1.4. Transfer the supernatant to a new tube. Next steps, or keep it in a freezer.

2 1.2. Isopropanol precipitation
1.2.1. Add an equal volume of cold isopropanol to the crude extract and mix.
1.2.2. 20,000 x g 5 min 4C
1.2.3. Discard supernatants and dry pellets.
1.2.4. Suspend the pellet in one-fifth volume of TE. Next steps, or keep it in a freezer.

3 2. Real-time PCR
2.1. Reagent mixture

Reagents	1 reaction	10 reactions
Sterile water	2.5	25
TaqMan FAST Advanced Master Mix	5	50
Primer mixture	1	10
Probe mixture	1	10
Total (µL)	9.5	95

2.2. Dispense 9.5 µL of the reagent mixture to PCR tubes
2.3. Add 0.5 µL of the extract (1.2.4) to the tube.
2.4. Set the tubes and run the StepOnePlus with the following parameters:
50C 2 min. → 95C 20 sec. →
95C 1 sec. → 60C 20 sec. （50 cycles）
* Targets（Reporter/Quencher）：PP（FAM/NFQ-MGB），XL（VIC/NFQ-MGB），IC（TAMRA/None）

See the next step.

4 3. Data analysis
3.1. Export data
3.2. Consider positives of PP/XL at Ct<45 and IC at Ct<40.

3.3. Refer the PDF file (a protocol in Japanese and English) in the Absract for detail.