Sep 30, 2017 Version 1

A protocol of molecular detection of phytoplasmas and Xylella spp. in post-entry quarantine for plants. V.1

PLOS One

DOI
dx.doi.org/10.17504/protocols.io.jhjcj4n

Takao Ito[1], Ryoji Nakaune

[1]Division of Grape and Persimmon Research, Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization (NARO), Higashihiroshima, Hiroshima, Japan

Takao Ito

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.jhjcj4n

External link: https://doi.org/10.1371/journal.pone.0185427

Protocol Citation: Takao Ito, Ryoji Nakaune 2017. 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.jhjcj4n**

Manuscript citation:
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. doi: 10.1371/journal.pone.0185427

Protocol status: Working

Created: August 21, 2017

Last Modified: March 28, 2018

Protocol Integer ID: 7435

Keywords: phytoplasma, xylella, real-time PCR, conventional PCR, multiplex, detection, dual priming oligonucleotides, plant quarantine

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 uploaded in the DESCRIPTION shows further details of the protocol in Japanese and English.

Attachments

PDF

Protocol-JpEn10.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 4℃

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 4℃

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: 50℃ 2 min. → 95℃ 20 sec. → 95℃ 1 sec. →	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

	60℃ 20 sec. （50 cycles） * Targets （Reporter/Quencher）：PP （FAM/NFQ-MGB）、XL （VIC/NFQ-MGB）、IC （TAMRA/None）		
	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

4 3. Data analysis

3.1. Export data

3.2. Consider positives of PP/XL at Ct<45 and IC at Ct<40.