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

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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.

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Protocol

Step 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.

Step 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.

Step 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. → 60℃ 20 sec. 50 cycles
* TargetsReporter/QuencherPPFAM/NFQ-MGBXLVIC/NFQ-MGBIC TAMRA/None

Step 4.

3.Data analysis

3.1.Export data

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