Information pest: *Pseudomonas corrugata* and *Pseudomonas mediterranea*

Pseudomonas corrugata (PC) is the causal agent of tomato pith necrosis (TPN) that generates severe losses on tomato-growing area world-wide. It is very similar to *Pseudomonas mediterranea (PM)* and has long been considered as the same bacterium. The growth of infected plants is blocked. These two bacteria are present in North America, Canada, France, UK, Netherlands, Italy, Spain, Portugal and Turkey.

Characteristic symptoms of the disease are the necrosis and/or hollowing of the parenchymatic tissue of the stem. Frequently, the first visible symptom is chlorosis of the youngest leaves. This often happens on plants where the fruit of the first truss is fully grown. With increasing extension of pith necrosis, the plant loses turgor and collapses.

Introduction

The PCR Pcorr set has been developed by Qualiplante.

The assay is based on the amplification of the PC1/1-PC1/2 and PC5/1-PC5/2 primers.
This product should be used only for research purposes.

Intended use

The PCR Pcorr is validated for the detection and the discrimination of *Pseudomonas corrugata* and *Pseudomonas mediterranea by* Duplex End-Point PCR.

Suitable samples are tomato tissues and bacterial isolates.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7PcorrP2	PCR *P. corrugata and P. mediterranea (Pcorr)* set 24
7PcorrP9	PCR *P. corrugata and P. mediterranea (P.Corr)* set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7PcorrP2-DM-	2x48 tests 7PcorrP9-DM-
Positive Control	3 tests 7PcorrP2-PC-	8 tests 7PcorrP9-PC-
Negative Control	3 tests 7PcorrP2-NC-	8 tests 7PcorrP9-NC-

Storage conditions

This set can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the set is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

Materials and equipment (not provided)

- DNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- DNA ladder and loading-dye buffer
- PCR thermal cycler
- Agarose gel reagents and apparatus

Nucleic acids extraction

Extract DNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

Reaction set-up

a) Slowly thaw Direct Master Mix by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and spin down the liquid.
c) Add 18 µl of Direct Master Mix (without DNA template) to each PCR tubes or wells of an optical-grade PCR plate.
d) Add 2 µl of DNA template to the Direct Master Mix. Do not forget to prepare a PCR tube or well of a PCR plate for the Positive Control and the Negative Control.

Components	Volume/PCR tube or well
DNA template or Positive control or Negative control	2 µl
Direct Master Mix	18 µl
Total Volume / PCR tube or well	20 µl

In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.

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Run and thermal cycling

a) Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
b) Load the PCR tubes or plate into the PCR thermal cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Initial denaturation	94°C	15 min	1
Denaturation	94°C	30 sec	35
Annealing	62°C	1 min	
Elongation	72°C	1 min 30	
Final elongation	72°C	5 min	1
Storage	4°C	∞	-

Agarose gel electrophoresis

Prepare an agarose gel **at 1,3% w/v in 1X-TBE buffer.**

Gel loading:
- load the DNA ladder (for example 100-2.000 bp DNA step ladder).
- load 10 µl of PCR products from the previous step adding the loading dye buffer (*not provided in the set)*.

Run: run the gel electrophoresis for 40-45 minutes at 80V.

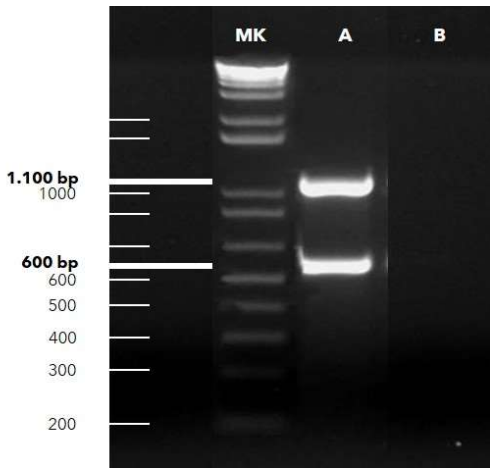
Results analysis

ANALYSIS VALIDATION

Pseudomonas corrugata is detected when a 1.100 bp DNA fragment is observed and *Pseudomonas mediterranea* is detected when a 600 bp DNA fragment is observed.

The analysis is validated when:
- ✓ 2 DNA fragments of 1.100 bp and 600 bp are visible in the positive control lane
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 1X-TBE 1,3% agarose gel showing the DNA amplification in a sample infected by *Pseudomonas corrugata* and *Pseudomonas mediterranea:*



MK: DNA ladder - **A**: Sample infected by *Pseudomonas corrugata* (1.100 bp) and *Pseudomonas mediterranea* (600 bp) *or* Positive Control of the set - **B**: Healthy sample or Negative Control of the set

RESULTS INTERPRETATION

The specific product of *Pseudomonas corrugata* is a 1.100 bp fragment and the specific product of *Pseudomonas mediterranea* is a 600 bp fragment.

- ✓ A sample is **positive** when a 1.100 bp DNA specific fragment is present in the PCR reaction; in this case, the sample is infected by *Pseudomonas corrugata.*
- ✓ A sample is **positive** when a 600 bp DNA specific fragment is present in the PCR reaction; in this case, the sample is infected by *Pseudomonas mediterranea.*
- ✓ A sample is **positive** when 2 DNA fragments (1.100 bp and 600 bp) are present in the PCR reaction; in this case, the sample is infected by both *Pseudomonas corrugata* and *Pseudomonas mediterranea.*
- ✓ A sample is negative when no fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

Fragment size		Interpretation
600 bp	**1.100 bp**	
-	-	NEGATIVE
✓	-	**POSITIVE** *Pseudomonas mediterranea*
-	✓	**POSITIVE** *Pseudomonas corrugata*
✓	✓	**POSITIVE** *Pseudomonas mediterranea* and *Pseudomonas corrugata*

POSITIVE: infected sample – NEGATIVE: healthy sample

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Special handling instructions

This set was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA/RNA and the components of the set. Do not touch any set components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the set components. Do not use reagents after their expiration date.

Troubleshooting

Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate	Repeat the test using the appropriate tools to correctly seal the plate
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the thermal cycler supplier
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In any doubt, please, contact us
Abnormal amplification	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles

No amplification reaction is observed in the positive control well, while other samples are positive:

Possible causes	Corrective actions
The positive control provided with the set was not added into the reaction well	Repeat the test. If the problem persists, please, contact us

An amplification plot is observed in the negative control well:

Possible causes	Corrective actions
Contamination of the negative control or the Master Mix with target-positive nucleic acid	Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly

Warranty and Responsibilities

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