

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 <i>P. corrugata</i> and <i>P. mediterranea</i> (Pcorr) set 24	
7PcorrP9	PCR <i>P. corrugata</i> and <i>P. mediterranea</i> (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.

Run and thermal cycling

- 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.
- 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

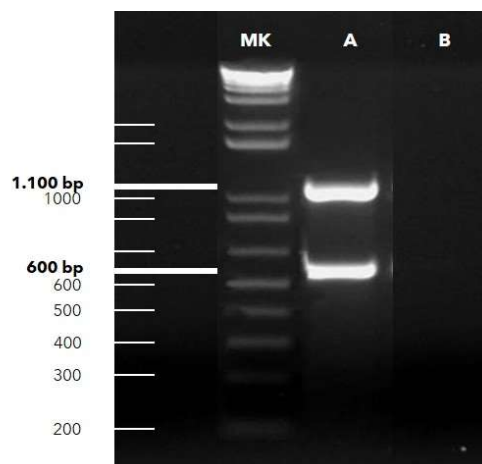
ANALYSIS VALIDATION

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 <i>Pseudomonas mediterranea</i>
-	✓	POSITIVE <i>Pseudomonas corrugata</i>
✓	✓	POSITIVE <i>Pseudomonas mediterranea</i> and <i>Pseudomonas corrugata</i>

POSITIVE: infected sample – **NEGATIVE:** healthy sample

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

Qualiplante SAS guarantees the buyer exclusively concerning the quality of reagents and of the components used to produce the Sets. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits Qualiplante SAS responsibility to the replacement of the product. No other warranties, of any kind, express or implied are provided by Qualiplante SAS.

Qualiplante SAS is not responsible and cannot anyway be considered responsible or jointly responsible for possible direct and indirect damages resulting of the use and/or the misuses of the Sets. The user consciously and under her/his own responsibilities decides for the utilization purposes of the Sets and uses it the way she/he considers most suitable in order to reach her/his goals and/or objectives. Qualiplante SAS is not responsible for the data resulting from the use of the Sets, for the utilization that the user independently decides to make of them or for the direct or indirect damages possibly resulting from the disclosure or transmission of the data themselves to third parties under any form or circumstance. This clause is automatically accepted by the user when purchasing the Sets. Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Qualiplante SAS does not encourage the unlicensed use of patented applications. The Sets may require the use of Taq Polymerase enzyme, DNA binding components and fluorochromes/quencher, often registered as trademark by companies. The product, equipment and information included in the Sets consist of assembled reagents. The Sets are designed for the services supply, quality control or any other application that is not exclusively an internal company's research and requires a specific license for PCR and Real-Time PCR use. The license and authorization for PCR and Real-Time PCR use are not included in the Sets. The user is responsible for setting prefixed goals, choosing whether or not to perform the PCR or Real-Time PCR reaction and to apply for register her/his own license.

The Sets have been internally tested by our quality control. Any responsibility is waived if the warranty of quality control does not refer to the specific Sets. The user is personally responsible for data that she/he will obtain and/or she/he will supply to third parties using these Sets. Once the sealed package is opened the user accepts all the conditions without fail; if the package is still sealed the set can be returned and the user can be refunded.

Sets components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change.