

## Information pest: *Cherry leaf roll virus*

*Cherry leaf roll virus* (CLRV) is common in many wild and cultivated woody plant species. This virus is known to infect at least 36 plant families and natural hosts including olive, elm, ash, elderberry, beech, rhubarb, dogwood and lilac. It was first described in 1955 by Posnette and Cropley as causing a disease of sweet cherry (*Prunus avium* L.) in England.

CLRV belongs to the subgroup-C of the genus Nepovirus (family *Comoviridae*).

Symptoms include leaf roll, leaf yellowing, early dropping of leaves, stunted growth, and plant dieback. Plants can also be infected without exhibiting symptoms.

CLRV is readily transmitted by grafting and by seed and pollen in several host species.

## Introduction

The PCR *Cherry leaf roll virus* set has been developed by Qualiplante based on Werner et al. (1997). A verification was performed by Qualiplante (data not published) and the performance characteristics of the set are the same as the original publication. The primer pair amplifies a 416 bp product of the CLRV coat protein gene.

*This product should be used only for research purposes.*

## Intended use

The PCR set is validated for the detection of *Cherry leaf roll virus* (CLRV) in One-Step End-Point RT-PCR.

The distribution of the virus in a tree can be irregular; however, the detection from flowers, young leaves, old leaves, fruit, dormant wood, and roots is possible. The time of year when the sample is taken is also critical and can greatly affect the test results. Optimal sampling is during spring, or early summer, but can vary from season to season depending on the weather conditions. CLRV has been reported to be easily detectable throughout the year in tissue of male inflorescences, leaf buds, leaves, single seeds and cortical tissues of young twigs. The virus can also be detected in roots, meristems and within tubules in pollen, ovules and mature seeds.

## Set format and content

Two sets are available for 24 and 96 tests.

| Article N° | Product name                             |
|------------|--|
| 7CLRV-P2   | PCR <i>Cherry leaf roll virus</i> set 24 |
| 7CLRV-P9   | PCR <i>Cherry leaf roll virus</i> set 96 |

| Content           | set 24                   | set 96                     |
|-------------------|--------------------------|----------------------------|
| Direct Master Mix | 24 tests<br>7CLRV-P2-DM- | 2x48 tests<br>7CLRV-P9-DM- |
| RT-Enzyme         | 24 tests<br>7CLRV-P2-RT- | 96 tests<br>7CLRV-P9-RT-   |
| Positive Control  | 3 tests<br>7CLRV-P2-PC-  | 8 tests<br>7CLRV-P9-PC-    |
| Negative Control  | 3 tests<br>7CLRV-P2-NC-  | 8 tests<br>7CLRV-P9-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)

- RNA 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 cycling
- Agarose gel reagents and apparatus

## Nucleic acids extraction

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

### Preparation of the CLRV 1-Step master mix

- Slowly thaw **Direct Master Mix** and **RT-Enzyme** by placing it on ice or at 4°C.
- Shake briefly **Direct Master Mix** and **RT-Enzyme** and spin down the liquid.
- In a new tube called **CLRV 1-Step master mix**, mix 17,5 µl of **Direct Master Mix** and 0,5 µl of **RT-Enzyme** per reaction. Do not forget to count the **Positive Control** and the **Negative Control** in the number of reactions to prepare.

| Example:                 | 1 rxn   | 10 rxns  |
|--------------------------|---------|----------|
| <b>Direct Master Mix</b> | 17,5 µl | 175,0 µl |
| <b>RT-enzyme</b>         | 0,5 µl  | 5,0 µl   |

- Store the **CLRV 1-Step master mix** by placing it on ice or at 4°C.

### Reaction set-up

- Shake briefly **CLRV 1-Step master mix** and spin down the liquid.
- Add 18 µl of **CLRV 1-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
- Add 2 µl of RNA template to the **CLRV 1-Step master mix**. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the **Positive Control** and the **Negative Control**.

| Components   | Volume/PCR tube or well |
|--|-------------------------|
| RNA template or <b>Positive control</b> or <b>Negative control</b> | 2 µl                    |
| <b>CLRV 1-Step master mix</b>                                      | 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 thermal-cycler and follow the thermal cycling below:

| Steps                    | Temp (°C) | Time   | Cycle(s) |
|--------------------------|-----------|--------|----------|
| Reverse transcription    | 50°C      | 15 min | 1        |
| Enzyme activation        | 95°C      | 10 min | 1        |
| Denaturation             | 95°C      | 30 sec | 45       |
| Annealing and elongation | 60°C      | 60 sec |          |
| Storage                  | 4°C       | ∞      | -        |

### Agarose gel electrophoresis

Prepare an agarose gel at **1,5% w/v in 1X-TAE buffer**.

#### Gel loading:

- load the DNA ladder (for example 100-1.000 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 50-60 minutes at 80V.

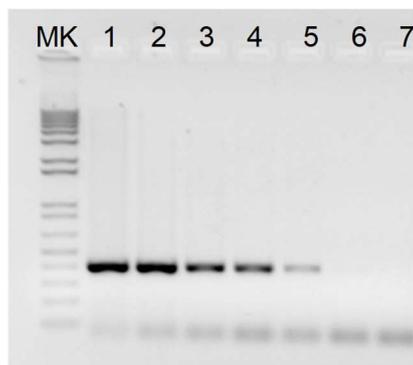
### Results analysis

#### ANALYSIS VALIDATION

*Cherry leaf roll virus* is detected when a 416 bp DNA fragment is observed. The analysis is validated when:

- ✓ 1 DNA fragment of 416 bp is visible in the positive control lane.
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 1X-TAE 1,5% agarose gel showing the DNA amplification in a sample infected by CLRV:



**MK:** DNA ladder – **1 to 5:** **CLRV positive sample at different concentration** or **Positive Control** - 1: 20 ng of total RNA - 2: 2 ng of total RNA - 3: 0,2 ng of total RNA - 4: 0,1 ng of total RNA - 5: 0,02 ng of total RNA - **6:** healthy sample or **Negative Control** - 7: no template control.

#### RESULTS INTERPRETATION

The specific product of CLRV is a 416 bp DNA fragment.

- ✓ A sample is **positive** when a 416 bp specific DNA fragment is present in the PCR reaction.
- ✓ A sample is **negative** when no fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

| Fragment size<br><b>416 bp</b> | Interpretation                                   |
|--------------------------------|--|
| -                              | Negative   |
| ✓                              | <b>POSITIVE</b><br><i>Cherry leaf roll virus</i> |

## 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 seal correctly 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 case of doubt, 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, 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.