Information pest: *Grapevine Pinot gris virus*

Grapevine Pinot gris virus (GPGV) is a member of the genus *Trichovirus* in the family *Betaflexiviridae*. This virus was recently identified in the north of Italy, in 2012, in the variety Pinot gris. Since then, it has been reported in numerous different countries and has been confirmed in other wine and table grape varieties including Pinot noir, Traminer, Chardonnay, Merlot, Cabernet Franc, Cabernet Sauvignon, Carménère, Glera, Sauvignon Blanc and Shiraz.

Grapevines infected with GPGV can either show symptoms (delayed budburst, lead distortion and mottling, shortened shoot internodes, poor yield…) or be asymptomatic. Symptoms appear most distinct at the start of the season and are less apparent on late season growth, in both young and old vineyards.

GPGV is a graft-transmitted virus and is possibly transmitted by grapeleaf bud and blister mites.

Introduction

The SYBR *Grapevine Pinot gris virus* set has been developed by Qualiplante.

The motor *gene* (MP *gene*) was used to design the primer pair.
This product should be used only for research purposes.

Intended use

The SYBR PCR set is validated for the detection of *Grapevine Pinot gris virus* by One-Step Real-Time RT-PCR (SYBR-Green® technology). The SYBR-Green® technology allows to confirm that a sample generating an amplification signal is produced only by nucleic acids of the pathogen of interest, by interpretating the melting peak.

Suitable tissues are grapevine young leaves and bark scrapings from dormant canes.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7GPGV-S2	SYBR *Grapevine Pinot gris virus* (GPGV) - set 24
7GPGV-S9	SYBR *Grapevine Pinot gris virus* (GPGV) - set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7GPGV-S2-DM-	2x48 tests 7GPGV-S9-DM-
RT-Enzyme	24 tests 7GPGV-S2-RT-	96 tests 7GPGV-S9-RT-
Positive Control	3 tests 7GPGV-S2-PC-	8 tests 7GPGV-S9-PC-
Negative Control	3 tests 7GPGV-S2-NC-	8 tests 7GPGV-S9-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
- Thermal cycler for Real-Time PCR with filters calibrated for SYBR-Green®

Nucleic acids extraction

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

Preparation of the GPGV One-Step master mix

a) Slowly thaw Direct Master Mix and RT-Enzyme by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and RT-Enzyme and spin down the liquid.
c) In a new tube called **GPGV One-Step master mix**, mix 17,6 µl of Direct Master Mix and 0,4 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
Direct Master Mix	17,6 µl	176,0 µl
RT-enzyme	0,4 µl	4,0 µl

d) Store the **GPGV One-Step master mix** by placing it on ice or at 4°C.

Reaction set-up

a) Shake briefly **GPGV One-Step master mix** and spin down the liquid.
b) Add 18 µl of **GPGV One-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
c) Add 2 µl of RNA template to the **GPGV One-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.

Qualiplante SAS
Cap Alpha – Avenue de l'Europe
FR-34830 Clapiers

www.qualiplante.eu
info@qualiplante.eu

Components	Volume/PCR tube or well
RNA template or Positive control or Negative control	2 µl
GPGV One-Step 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

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 thermal-cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Reverse transcription	45°C	15 min	1
Enzyme activation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing	60°C	30 sec	
Elongation	72°C	30 sec	
Melt temperature	Follow the instructions of your thermal cycler		

Results analysis

The reaction for GPGV will generate a specific SYBR®-labeled amplification curve and a specific melting-curve.

Fig.1: Example of amplification curves relative to a GPGV positive sample.

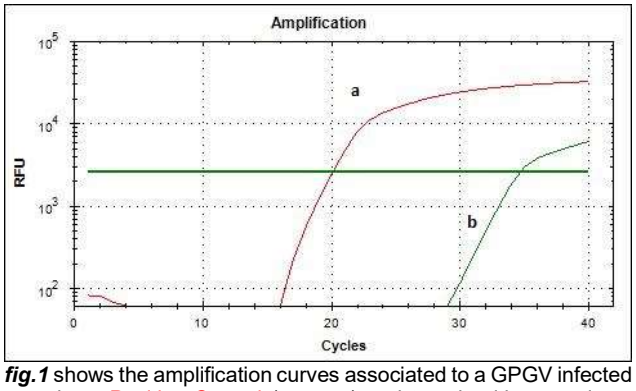

fig.1 shows the amplification curves associated to a GPGV infected sample or Positive Control (curve **a**) and to a healthy sample or Negative Control (curve **b**)

Fig.2: Example of melting curve relative to a GPGV positive sample.

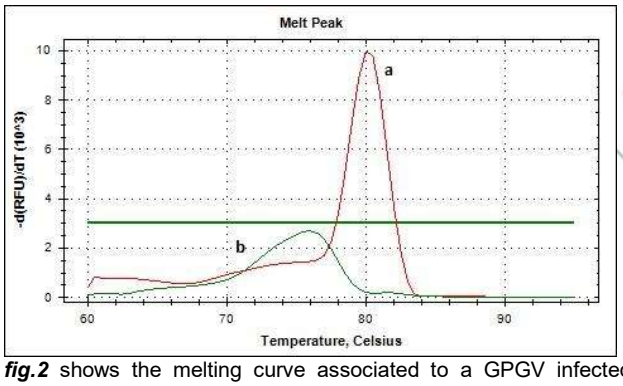

fig.2 shows the melting curve associated to a GPGV infected sample or Positive control (curve **a** - Tm=80,0°C on Biorad CFX96 machine) and to a healthy sample or Negative Control (curve **b** - None Tm)

ANALYSIS VALIDATION AND RESULTS INTERPRETATION

For a correct interpretation of results, always:

- check if results of Positive Control and Negative Control pass,
- combine amplification curves analysis with melting curves analysis,
- confirm that the samples melting temperatures match the Positive Control melting temperature.

Step 1: Check the Ct values of Positive Control and Negative Control

Well	Ct	Interpretation
Positive Control	35 or less	Go to step 2 and 3
	Above 35 or no Ct	Fail*
Negative Control	No Ct	Go to step 2 and 3
	Less than 35	Fail**

* Repeat the assay, ensuring that steps of the user guide are carefully performed
** The mix or the Negative Control was contaminated with GPGV nucleic acids. Repeat the assays after identifying and removing the potential source of contamination

Step 2: Check Ct value in the samples well

Well	Ct	Interpretation
Sample	40 or less	Go to step 3
	More than 40 or no Ct	Negative

Step 3: See melting temperature

Well	Tm	Interpretation
Sample	Tm differs no more than ± 1°C from Tm of Positive Control	Positive
	Tm differs more than ± 1°C from Tm of Positive Control	Negative

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

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