User Guide
LAMP Foc TR4 kit
Version 02 – 12/12/2024

qualiplante

Information pest: *Fusarium* Wilt

The *Fusarium* wilt in banana, also known as Tropical Race 4 (TR4) or Panama disease, is one of the most devastating diseases for global banana production, and it is caused by the fungus *Fusarium oxysporum f.* sp. *cubense* (Foc). TR4 main symptoms include wilting and yellowing of the leaves; and discoloration and browning of the pseudostem and the pseudobulb. The lack of effective treatments for this disease, together with the potential confusion of symptoms with those caused by other pathogens, underscores the need for point of care diagnostic tools for TR4. The disease is spread by contaminated soil, water, or tools.

Introduction

The LAMP Foc TR4 kit has been developed through a research project financed by France Relance where were involved CIRAD and Qualiplante, and with the partnership of Indicants project.

This product should be used only for research purposes.

Intended use

The LAMP TR4 kit is validated for the detection of Banana TR4 (VCG 01213/16) using LAMP (Loop-Mediated Isothermal Amplification) technique. LAMP technology allows for highly specific, efficient and rapid isothermal amplification, to confirm that the amplification signal and interpretation of the melting peak indicates the presence of the nucleic acids of the pathogen of interest.
This kit offers a sensitive diagnostic method to detect TR4 and permits to avoid amplicon contamination in routine diagnosis. The suitable tissue for the test consists of infected pseudo-stem samples, including infected vascular bundles, or pseudobulb tissue where lesions of the disease can be observed.

Kit format and content

Article N°	Product name
LAMP TR4 48	LAMP TR4 48 tests

Content	48 tests
TR4 primers mix	1 tube for 48 tests
Nuclease-free water	1 tube of 1,2 ml
Resuspension buffer	1 tube for 50 tests
Lyophilized enzyme	5 tubes for 10 tests
TR4 Positive Control	1 tube of 10 tests
TR4 Negative Control	1 tube for 10 tests

Storage conditions

The kit can be shipped at room temperature but upon receipt, it should be stored immediately at the recommended storage temperature: **+4°C**.

Once the TR4 Master Mix is prepared, it is recommended to proceed with the run immediately after.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the lyophilized kit is correctly stored, 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
- Thermal cycler for Isothermal amplification or Real-Time thermal cycler which allows the use of FAM channel.

Nucleic acids extraction

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

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Reagents preparation

PREPARATION OF THE TR4 PRIMERS MIX

a) Do a short spin in a centrifuge to collect the pellet at the bottom of the **TR4 primers mix** tube.

b) Add 250,00 µl (two hundred and fifty) of **nuclease free water** provided with the kit in the **TR4 primers mix** tube. Vortex vigorously and mix thoroughly by inverting 4-6 times the **TR4 primers mix** tube. Spin down the liquid by centrifuging briefly.

If not used immediately, the resuspended TR4 primers mix should be stored thereafter at -20°C.

RESUSPENSION OF THE LYOPHILIZED ENZYME

a) Do a short spin in a centrifuge to collect the pellet at the bottom of the **Lyophilized enzyme** tube.

b) Add 150 µl (one hundred and fifty) of **Resuspension buffer** provided with the kit in each **Lyophilized enzyme** tube. Vortex vigorously and mix thoroughly by inverting 4-6 times the **Lyophilized enzyme** tube. Spin down the liquid by centrifuging briefly.

c) Leave 10 minutes at room temperature**. Keep away from light exposure during this step.**

*The reconstituted **Lyophilized Enzyme** should preferably use immediately but it can also be stored at -20°C for maximum one month.*

PREPARATION OF THE MASTER MIX

a) Slowly thaw the resuspended tube of **TR4 primers mix** previously prepared (if it was frozen at -20°C) or put this tube by placing it on ice or at +4°C.

b) Briefly centrifuge the tube of **TR4 primers mix** previously resuspended to remove drops from the lid.

c) In each **Lyophilized enzyme** tube previously reconstituted, add 50 µl of **TR4 primers mix.** Vortex vigorously and mix thoroughly by inverting 4-6 times the **Lyophilized enzyme** tube. Spin down the liquid by centrifuging briefly

*One tube of prepared **Master Mix** will permit to perform 10 tests. This **Master Mix** should be used immediately.*

Reaction set up

a) Add 20 µl of the previously prepared **Master Mix** (without DNA template) to each PCR tubes or wells of an optical-grade PCR plate.

b) Add 5 µl of DNA template on each well. Do not forget to prepare one PCR tube or well of an optical-grade PCR plate for Positive Control and one for Negative Control.

Components	Volume/PCR tube or well
DNA template or Positive control or Negative control	5 µl
Prepared Master Mix	20 µl
Total Volume / PCR tube or well	25 µl

c) Seal carefully the PCR tubes or PCR plate and centrifuge briefly to collect contents at the bottom of the PCR tubes or wells of the plate. Protect from light during set-up and before thermocycling.

Run and thermal cycling

Load the PCR tubes or plate into the LAMP machine or thermal cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time
Amplification	65°C	30 min
Annealing	98°C-70°C ramping at 0.05°C per seconds (with fluorescence measurement)	

LAMP reaction must be performed in an equipment dedicated to LAMP isothermal amplification (e.g Genie II, OptiGene) or in a real-time PCR equipment. If a real-time PCR machine is used, it is necessary to activate the detection of fluorescent emissions corresponding to the FAM channel to measure the fluorescence every minute during the 30 minutes step and to plot the annealing curve.

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Results analysis

The LAMP reaction will generate a specific fluorochrome-labelled amplification curve and a specific melting curve.

ANALYSIS VALIDATION AND RESULTS INTERPRETATION

For a correct interpretation of results, always:

- step 1: check the TTR values of the **Positive Control** and the Negative Control.
- step 2: check the TTR value in the samples well.
- step 3: check annealing peak value.

STEP 1: Check the TTR values of the **Positive Control** and the Negative Control

Well	TTR value (Time to result value)	Interpretation
Positive Control	30 or less	Go to step 2 and 3
	Above 30 or no TRR	Failed (*)
Negative Control	No TRR	Go to step 2 and 3
	Less than 30	Failed (**)

(*) Repeat the assay, ensuring that the instructions are carefully followed.
(**) The mix or the Negative Control was contaminated with target nucleic acids. Repeat the assays after identifying and removing the potential source of contamination.

STEP 2: Check the TTR value in the samples well

Well	TTR value (Time to result value)	Interpretation
Sample	30 or less	Go to step 3
	Above 30 or no TRR	Negative

STEP 3: Check annealing peak value

Well	Ta value (Annealing peak temperature)	Interpretation
Sample	Ta differs **no more** than ± 1°C from Ta of **Positive Control**	**POSITIVE**
	Ta differs **more** than ± 1°C from Ta of **Positive Control**	NEGATIVE

Fig.1: Example of amplification curves relative to Foc TR4 positive sample and negative sample:

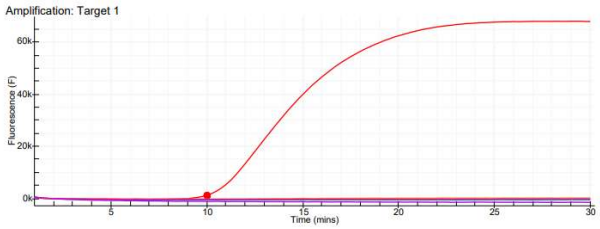

fig.1 shows the amplification curves associated to TR4-positive sample (red curve) and to negative sample or Negative control of the kit (pink curve) on Optigene Genie 2® machine.

Fig.2: Example of melting curves relative to a Foc TR4-positive sample and negative sample:

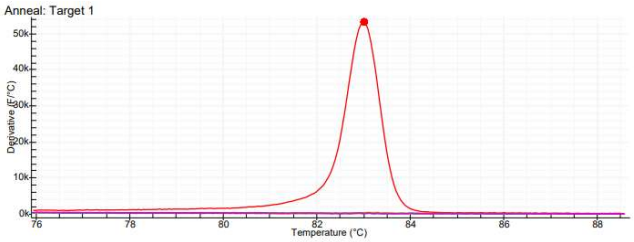

fig.2 shows the melting curve associated to TR4-positive sample (red curve - Tm = 83°C) and to negative sample or Negative control of the kit (pink curve) on Optigene Genie 2® machine.

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Special handling instructions

This kit 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 kit. Do not touch any kit 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 kits with different batch numbers in the same test procedure. Do not interchange reagents with other kits. 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 kit 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 kit 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 Kits. 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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Kits components are intended, developed, designed, and sold for Research Purpose Only. Product claims are subject to change.

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