

***Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit**

Product#TM66250 **Product Insert**

Intended Use

Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit is designed for the detection of *Candidatus* Liberibacter solanacearum specific DNA in a real-time PCR based on the use of TaqMan® technology. This kit is designed for research use only and not for use in diagnostic procedures. The lyophilized format is designed to ship the kit at ambient temperature. Upon receiving, it is important to store the kit at -20°C until the expiry date.

Background Information

Candidatus Liberibacter solanacearum was first identified in 2008 and was shown to be associated with zebra chip disease of potato, which has been observed since the 1990s with increasing economic impacts. *Candidatus* Liberibacter solanacearum is also known to primarily infect tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), tomatillo (*Physalis peruviana*), tamarillo (*Solanum betaceum*), tobacco (*Nicotiana tabacum*), and several weeds in the family Solanaceae.

The symptoms of infection include stunting, erectness of new foliage, chlorosis and purpling of foliage with basal cupping of leaves, upward rolling of leaves throughout the plant, shortened and thickened terminal internodes resulting in plant rosetting, enlarged nodes, axillary branches or aerial tubers, leaf scorching, disruption of fruit set, and production of numerous, small, misshapen, and poor-quality fruits. PCR is the method of choice for the detection of *Candidatus* Liberibacter solanacearum (Li *et al.*, 2006). Conventional PCR can be used, but real-time PCR is recommended because of its increased sensitivity (Li *et al.*, 2009).

Product Description

Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit is designed for the detection of *Candidatus* Liberibacter solanacearum specific DNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures. The detection of *Candidatus* Liberibacter solanacearum specific DNA is based on TaqMan PCR providing a simple, reliable and rapid result for the detection of *Candidatus* Liberibacter solanacearum infection. Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit includes a PCR control to monitor for PCR inhibition, and to validate the quality of the sample and the detection result. The *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit comprises Master Mix for the target and PCR control detection, Primer & Probe Mix, as well as a positive control and a negative control (nuclease-free water) to confirm the integrity of the kit reagents.

The MDx TaqMan 2X PCR Master Mix (Lyo), *Candidatus* Liberibacter solanacearum Primer & Probe Mix (Lyo) and *Candidatus* Liberibacter solanacearum Positive Control (Lyo) are provided in the lyophilized format. It is important to store the kit at -20°C upon receiving to maintain the shelf life of the kit. Once the lyophilized products are reconstituted, store them at -20°C until the expiry of the kit. For further information, please refer to instructions for storage and reconstitution.

Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit was developed and validated to be used with the following PCR instruments:

- Qiagen Rotor-Gene Q
- BioRad CFX96 Touch™ Real-Time PCR Detection System
- QuantStudio™ 7 Pro Real-Time PCR System

Kit Components

Component	Product # TM66250L (100 preps)	Volume upon Reconstitution
MDx TaqMan 2X PCR Master Mix (Lyo)	4 tubes	4 X 350 µL
<i>Candidatus</i> Liberibacter solanacearum Primer & Probe Mix (Lyo)	1 tube	1 X 280 µL
<i>Candidatus</i> Liberibacter solanacearum Positive Control (Lyo)	1 tube	1 X 150 µL
Nuclease-Free Water (Negative control)	3 X 1.25 mL	N/A
Product Insert	1	N/A

Storage Conditions and Product Stability

- All kit components should be stored at -20°C upon arrival.
- Once reconstituted, repeated thawing and freezing (>2 times) of the Master Mix and Positive Control should be avoided, as this may affect the performance of the assay. If the reagents are to be used only intermittently, they should be frozen in aliquots.
- Each kit is provided with 4 tubes of 2X PCR Master Mix and each tube is enough to run 25 reactions. It is not necessary to reconstitute all Master Mix tubes at once. The Master Mix tubes can be reconstituted as and when needed.
- All kit components can be stored for 2 years after the date of production without showing any reduction in performance.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument with FAM and HEX filter channel
- DNA Purification Kit
 - The kit is compatible with all DNA purification kits that yield high quality, inhibitor-free DNA
 - **Recommended Purification Kit:** Norgen's Plant/Fungi DNA Isolation Kit (Cat. 26200, 26900)
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Vortex mixer
- PCR reaction preparation station (Optional)

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit is tested against predetermined specifications to ensure consistent product quality.

Warnings and Precautions

- Do not store the kit at room temperature. Store the kit at -20°C upon arrival. Please refer to **Storage Conditions and Product Stability** for further information.
- Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit is intended for research purposes only. It is not intended for diagnostic use.

- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Dispose of unused kit reagents and specimens according to local, provincial or federal regulations.
- Do not substitute or mix reagents from different kit lots or from other manufacturers. Do not use components of the kit that have been stored for more than 2 years.
- The presence of PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the *Candidatus Liberibacter solanacearum* genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.
- Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

Instructions for Use

A. Reconstitution.

Each *Candidatus Liberibacter solanacearum* TaqMan PCR Lyophilized Kit is provided with 4 tubes of lyophilized MDx TaqMan 2X PCR Master Mix (Lyo), 1 lyophilized *Candidatus Liberibacter solanacearum* Primer & Probe Mix (Lyo), 1 lyophilized *Candidatus Liberibacter solanacearum* Positive Control (Lyo) and 3 tubes of Nuclease-Free Water. Please follow the steps below to reconstitute each component.

- Spin down all tubes to make sure all the lyophilized products are at the bottom of the tube.
- **Reconstitution of MDx TaqMan 2X PCR Master Mix (Lyo)**
 - Note: **Failure to dissolve the 2X PCR Master Mix (Lyo) completely might affect the efficiency of the PCR assay.**
 - 1. Add **325 µL** Nuclease Free Water (provided in the kit).
 - 2. Let the Master Mix rehydrate at room temperature for 10 minutes.

3. Invert and mix the Master Mix 10 – 20 times to completely dissolve the lyophilized cake. Check the walls of the tube for any residue and make sure to mix it by inversion.

Note: Do not vortex the Master Mix as it may affect the efficiency of the PCR assay.

4. Once completely dissolved, spin down the Master Mix tube briefly.
 5. The 2X PCR Master Mix is now ready to setup the PCR. Store the reconstituted 2X PCR Master Mix at -20°C if not used for PCR setup immediately.
 - o Note: It is not required to reconstitute all the 2X PCR Master Mix tubes at the same time. They can be reconstituted as and when needed.
- **Reconstitution of *Candidatus Liberibacter solanacearum* Primer & Probe Mix (Lyo)**
 - o **Note: Failure to dissolve the Primer & Probe Mix completely might affect the efficiency of the PCR assay**
 - 1. Add **275 µL** Nuclease Free Water (provided in the kit) and let it rehydrate at room temperature for 5 minutes.
 - 2. Briefly vortex to dissolve the lyophilized Primer & Probe Mix.
 - 3. Once completely dissolved, briefly spin down the Primer & Probe Mix tube
 - 4. The Primer & Probe Mix is now ready to setup the PCR. Store the reconstituted Primer & Probe Mix at -20°C if not used for PCR setup immediately.
 - **Reconstitution of *Candidatus Liberibacter solanacearum* Positive Control (Lyo)**
 - o **Note: Failure to dissolve the Positive Control completely might affect the efficiency of the PCR assay**
 - 1. Add **120 µL** Nuclease Free Water (provided in the kit) and let it rehydrate at room temperature for 5 minutes.
 - 2. Use a pipette with a sterile tip to dissolve the lyophilized Positive Control.
 - 3. Once completely dissolved, briefly vortex and spin down the Positive Control tube
 - 4. The Positive Control is now ready to setup the PCR. Store the reconstituted Positive Control at -20°C if not used for PCR setup immediately.

B. Sample Preparation

Purified DNA is the starting material for Norgen's *Candidatus Liberibacter solanacearum* TaqMan PCR Lyophilized Kit. The quality of the DNA template will have a major impact on the performance of the *Candidatus Liberibacter solanacearum* detection test. The user must ensure that the method used for DNA purification is compatible with TaqMan PCR. We recommend the use of **Norgen's Plant/Fungi DNA Isolation Kit (Cat. 26200, 26900)**. Norgen's Plant/Fungi DNA Isolation Kit has been fully validated with Norgen's *Candidatus Liberibacter solanacearum* TaqMan PCR Lyophilized Kit.

If using a different spin column-based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 3 minutes at 20,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the DNA. This will help to prevent the carry-over of any ethanol into the purified DNA, as ethanol is known to be a strong inhibitor of PCR. **Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the DNA.**

C. TaqMan PCR Assay Preparation

Notes:

- Before use, suitable amounts of all TaqMan PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly.
- Work quickly on ice.
- The amount of MDx TaqMan 2X PCR Master Mix (Lyo) provided is enough for up to 128 PCR reactions (96 sample PCR, 16 positive control PCR and 16 no template control PCR).
- For every TaqMan PCR run, one reaction containing *Candidatus Liberibacter solanacearum* Positive Control (Lyo) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of DNA samples tested per TaqMan PCR run is 6.
- To avoid any contamination while preparing the TaqMan PCR assay, follow the order outlined in Tables 1, 2 and 3 below to prepare the Negative Control, Detection Assay and Positive Control:
 1. Prepare the PCR Negative Control (Table 1)
 2. Prepare the PCR *Candidatus Liberibacter solanacearum* Assay (Table 2)
 3. Prepare the PCR Positive Control (Table 3)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) MDx TaqMan 2X PCR Master Mix (Lyo); 3) Primer & Probe Mix (Lyo); and 4) the Sample DNA or Positive Control).

1. For each TaqMan PCR set, prepare one no template control PCR as shown in Table 1 below:

Table 1. TaqMan PCR Negative Control Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix (Lyo))
Nuclease-Free Water	8 µL
MDx TaqMan 2X PCR Master Mix (Lyo)	10 µL
<i>Candidatus Liberibacter solanacearum</i> Primer & Probe Mix (Lyo)	2 µL
Total Volume	20 µL

2. Prepare the PCR reaction for sample detection as shown in Table 2 below.

Table 2. TaqMan PCR *Candidatus Liberibacter solanacearum* Assay Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix (Lyo))
Nuclease-Free Water	5 µL
MDx TaqMan 2X PCR Master Mix (Lyo)	10 µL
<i>Candidatus Liberibacter solanacearum</i> Primer & Probe Mix (Lyo)	2 µL
Sample DNA*	3 µL
Total Volume	20 µL

* The recommended amount of sample DNA to be used is 3 µL. However, a volume between 1 and 5 µL of sample DNA may be used as template. Adjust the final volume of the PCR reaction to 20 µL using the Nuclease-Free Water provided.

3. For each PCR set, prepare **one** positive control PCR as shown in Table 3 below:

Table 3. TaqMan PCR Positive Control Preparation

PCR Components	Target detection (with MDx TaqMan 2x PCR Master Mix (Lyo))
MDx TaqMan 2X PCR Master Mix (Lyo)	10 μ L
<i>Candidatus</i> Liberibacter solanacearum Primer & Probe Mix (Lyo)	2 μ L
<i>Candidatus</i> Liberibacter solanacearum Positive Control (PosC) (Lyo)	8 μ L
Total Volume	20 μ L

4. **OPTIONAL** - Experienced user protocol for PCR reaction preparation for multiple samples

Follow Table 4 and the instructions below to prepare a PCR reaction mix for multiple samples. The below example is to set up 10 PCR reactions.

Table 4. Set-Up of PCR Reaction Mix for 10 PCR Reactions

Components	Volume per 10 + 1 Reactions
MDx TaqMan 2X PCR Master Mix (Lyo)	110 μ L
<i>Candidatus</i> Liberibacter solanacearum Primer & Probe Mix (Lyo)	22 μ L
Total Volume	132 μ L

1. Aliquot 12 μ L of PCR reaction mix to PCR tubes or wells on 96 well PCR plate.
2. For the negative control, add 8 μ L of Nuclease free water and close the PCR tube cap.
3. For the detection samples, add 5 μ L of water and 3 μ L of template (DNA) to samples. Close the PCR tube cap.
4. For the positive control, add 8 μ L of *Candidatus* Liberibacter solanacearum Positive Control (PosC) (Lyo) and close the PCR tube cap.
5. Spin the tubes or 96-well plate briefly.
6. The final PCR reaction volume is 20 μ L.

D. *Candidatus* Liberibacter solanacearum TaqMan PCR Assay Programming

1. Program the thermocycler according to the program shown in Table 4 below.
2. Run TaqMan PCR assay.

Table 4. *Candidatus* Liberibacter solanacearum TaqMan PCR Program

PCR Cycle	Step	Temperature	Duration
<i>Cycle 1</i>	Step 1	95°C	3 min
<i>Cycle 2 (40x)</i>	Step 1	95°C	15 sec
	Step 2	60°C	30 sec

E. *Candidatus Liberibacter solanacearum* TaqMan PCR Assay Interpretation

Table 5. Interpretation of Assay Results

FAM (Target detection)	HEX (PCR validation)	Result
+	+	Positive
-	+	Negative
-	-	PCR inhibited

For results obtained that are not covered in Table 5, please refer to the Frequently Asked Questions.

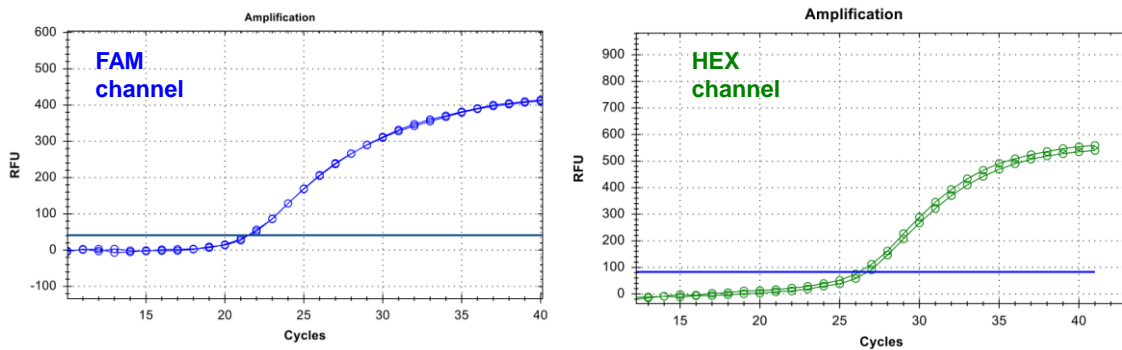


Figure 1. Example of TaqMan PCR Positive result. Both PCR signals above the baseline from FAM and HEX channel indicate the successful PCR.

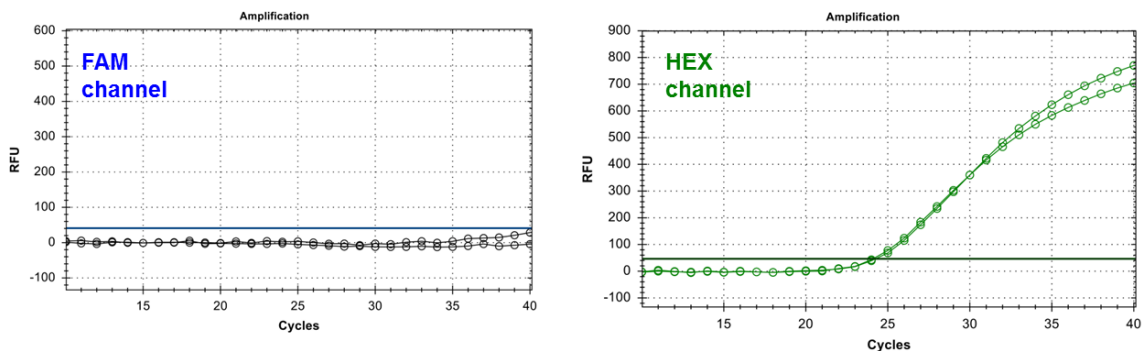


Figure 2. Example of TaqMan PCR Negative result. No target DNA was detected in FAM channel but amplification signal from HEX indicates the successful PCR.

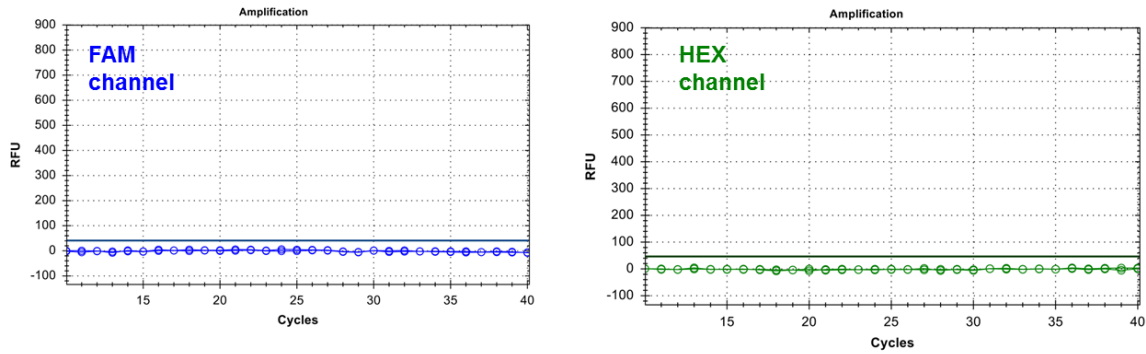


Figure 3. Example of TaqMan PCR inhibition result. No signal from both FAM and HEX channel was detected. It is suggested to repeat the sample preparation using recommended kit for DNA purification.

F. *Candidatus* Liberibacter solanacearum TaqMan PCR Assay Specificity

The specificity of Norgen's *Candidatus* Liberibacter solanacearum PCR Detection Lyophilized Kit is first and foremost ensured by the selection of the *Candidatus* Liberibacter solanacearum - specific primers, as well as the selection of stringent reaction conditions. The *Candidatus* Liberibacter solanacearum primers were checked for possible homologies to all microorganisms in GenBank published sequences by sequence comparison analysis.

Frequently Asked Questions

1. **How many samples should be included per PCR run?**
 - Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Detection Lyophilized Kit is designed to test 96 samples. For every 8 samples, a non-template control (Nuclease-Free Water) and a Positive Control must be included. It is preferable to collect and test 8 samples at a time.
2. **Do all Master Mix tubes need to be reconstituted upon arrival?**
 - It is not required to reconstitute all the 2X PCR Master Mix tubes at the same time. They can be reconstituted as and when needed.
3. **Would incomplete reconstitution impact the efficiency of the PCR?**
 - If there is residual lyophilized cake on the sides of the tube after reconstitution, the concentrations will not be accurate. This might affect the efficiency of the PCR assay. It is recommended to check for residue on the sides of the tube before proceeding with the PCR assay.
4. **Can the kit be stored at room temperature or 4°C upon receiving?**
 - Although the kits are stable at ambient temperatures during shipping, it is required to store the kits at -20°C upon receiving.
5. **How should it be interpreted if no PCR control signal (HEX) is detected while the target specific signal (FAM) is detected in the positive control?**
 - Tested samples(s) can be considered positive. It could happen when too much target DNA template was added due to the preferential amplification on the target.
6. **How should it be interpreted if no PCR control signal (HEX) is detected while the target specific signal (FAM) is detected in the positive control?**
 - Tested samples(s) can be considered positive. It could happen when too much target DNA template was added due to the preferential amplification on the target.

7. **How should it be interpreted if the target specific signal (FAM) and the PCR control signal (HEX) are detected in the negative control?**
- It could happen when there is carryover contamination and PCR inhibition. Repeat the assay using fresh aliquots and clean pipette tips.
8. **How should it be interpreted if no target signal (FAM) is detected in positive control?**
- It could happen when the positive control was not added. Repeat the assay.

Related Products	Product #
<i>Candidatus</i> Liberibacter solanacearum TaqMan Lyophilized Probe/Primer and Control Set	TM66210L
Plant/Fungi DNA Isolation Kit	26200, 26900

Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Product Use Restriction

Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit is designed for the detection of *Candidatus* Liberibacter solanacearum specific DNA in a real-time PCR based on the use of TaqMan technology. This kit is designed for research use only and not for use in diagnostic procedures.

Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit is intended for use by professional users such as technicians and biologists experienced and trained in molecular biological techniques including PCR.

Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Ensure that appropriate specimen collection, transport, storage and processing techniques are followed for optimal performance of this test.

The presence of PCR inhibitors may cause false negative or invalid results.

Potential mutations within the target regions of the *Candidatus* Liberibacter solanacearum genome covered by the primers in this kit may result in failure to detect the presence of the pathogen.

The respective user is liable for any and all damages resulting from application of Norgen's *Candidatus* Liberibacter solanacearum TaqMan PCR Lyophilized Kit for use deviating from the intended use as specified in the user manual.

All products sold by Norgen Biotek are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately. The kit contents are for laboratory use only, and they must be stored in the laboratory and must not be used for purposes other than intended. The kit contents are unfit for consumption. TaqMan is a registered trademark of Roche Molecular Systems, Inc

References

Li, W., Abad, J.A., French-Monar, R.D., Rascoe, J., Wen, A., Gudmestad, N.C., Secor, G.A., Lee, I.M., Duan, Y. & Levy, L. 2009. Multiplex real-time PCR for detection, identification and Diagnostic protocols for regulated pests DP 21. International Plant Protection Convention DP 21-13 quantification of 'Candidatus Liberibacter solanacearum' in potato plants with zebra chip. *Journal of Microbiological Methods*, 78: 59–65.

Li, W., Hartung, J.S. & Levy, L. 2006. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. *Journal of Microbiological Methods*, 66: 104–115.

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