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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 28062-2025

Replacing GB/T 28062-2011

Code of practice for detection of Candidatus Liberibacter asiaticus by quantitative real-time PCR

柑橘黄龙病菌实时荧光定量PCR检测技术规程

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Foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for standardization work Part 1: Structure and drafting rules for standardization documents" Drafting.

This document replaces GB/T 28062-2011 "Real-time fluorescence PCR detection method for citrus Huanglongbing bacteria" and is compatible with GB/T 28062-2011 Compared with the previous version, in addition to structural adjustments and editorial changes, the main technical changes are as follows.

- Changed the sampling method for Huanglongbing samples (see 9.1, 8.2 of the 2011 edition);

- Changed the Ct value of the positive control (see 11.3.2, 10.2.2 of the 2011 edition);

- Change the fluorescent dye detection primers CQULasF04/CQULasR04 of Bacillus asiaticus to more specific primers GGTAG-3'), and added the fluorescent labeled probe HLBp (sequence. 5'-AGACGGGTGAGTAACGCG-3') (See 10.1, F.1.1 of the 2011 edition);

- Added real-time fluorescence PCR detection of the target Bacillus asiaticus ribonucleotide reductase beta gene (nrdB gene) (See 11.3.3).

Please note that some of the contents of this document may involve patents. The issuing organization of this document does not assume the responsibility for identifying patents.

This document is proposed and coordinated by the National Technical Committee on Plant Quarantine Standardization (SAC/TC271).

This document was drafted by: National Agricultural Technology Extension Service Center, South China Agricultural University, and Southwest University Citrus Research Institute.

The main drafters of this document are Feng Xiaodong, Chen Ranran, Deng Xiaoling, Zheng Zheng, Wang Xuefeng and Zhou Changyong.

The previous versions of this document and the documents it replaces are as follows.

- First published in 2011 as GB/T 28062-2011;

- This is the first revision.

Detection of Citrus Huanglongbing Disease by Real-time Fluorescence Quantitative PCR Technical regulations

1 Scope

The quantitative PCR detection procedure specifies the laboratory setting, sample collection and processing, detection operation method and result judgment, and describes the confirmation method.

This document is applicable to the real-time fluorescence quantitative PCR of Bacillus asiaticus species in host plants and vectors of Huanglongbing (Psyllid citri citri) Detection and disease identification.

2 Normative references

GB 5040

GB/T 6682

3 Terms and definitions

The following terms and definitions apply to this document.

3.1 Citrus Huanglongbing

Note. Phloemobacter is a prokaryotic organism, alpha-Proteobacteria, Rhizobiales, Rhizobiaceae, Phloemobacter Liberibacter is a Gram-negative bacterium that is difficult to culture. It is divided into three species. Liberibacter asiaticus, which is distributed in Asia, Africa and America.

3.2 Real-time fluorescence quantitative PCR quantitativereal-time PCR

In vitro amplification of trace DNA fragments. During the amplification process, fluorescent substances are released or the fluorescent substances bind to the amplified products.

The fluorescent signal is released after the fusion, and the quantitative analysis of the target DNA in the starting template is achieved by real-time monitoring of the accumulation of fluorescent signal.

3.3 Ct value cyclethresholdvalue

The number of cycles experienced when the fluorescence signal in each reaction tube reaches the set threshold in the real-time fluorescence PCR reaction.

4 Principles of the method

The real-time fluorescence quantitative PCR detection used in this document includes two detection methods. fluorescent dye method and fluorescent probe method.

The method is to design primers using the unique DNA sequence of Huanglongbing fungus, add fluorescent dyes during PCR amplification, and the fluorescent dyes react with the products formed by amplification.