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Genetically Modified Organism Detection Method by Digital PCR

转基因植物产品数字 PCR 检测方法

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Genetically Modified Organism Detection Method by Digital PCR

1 Scope

This Standard specifies a digital PCR method for the detection of genetically modified components.

This Standard is applicable to digital PCR detection of genetically modified plants and components, such as corn, soybean, rape, rice, potato, alfalfa, cotton and the like.

The lower limit of detection reached by this Standard is 0.1% (mass fraction).

2 Normative References

The following documents are essential to the application of this document. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) are applicable to this document.

GB/T 6682 Water for Laboratory Use – Specifications

GB/T 19495.1 Detection of Genetically Modified Organisms and Derived Products - General Requirements and Definitions

GB/T 19495.3 Detection of Genetically Modified Organisms and Derived Products - Nucleic Acid Extraction

GB/T 19495.7 Detection of Genetically Modified Organisms and Derived Products Methods for Sampling and Sample Preparation

3 Terms and Definitions

For the purpose of this document, the following terms and definitions apply.

3.1 18srRNA gene

Transcribed from 18srDNA and is a component of cellular ribosomes.

3.2 CaMV35s promoter

It is the 35s promotor from Cauliflower mosaic virus.

3.3 NOS terminator

It is the terminator from nopaline synthase gene NOS.

4 Principle

The technical principle of digital PCR is to segment the original PCR reaction system; and then to amplify and detect all small reaction systems. Through the limited segmentation of the reaction system, the entire reaction system can be more tolerant to the nucleic acid inhibitory factor; and more accurately, stably, and accurately identify trace amounts of transgenic components.

Currently, the implementation of digital PCR includes chip-type digital PCR and micro-droplet-type digital PCR. Among them, chip-type digital PCR uses a microfluidic chip to segment the original reaction system. This segmentation is featured by good stability and uniformity, but the experimental cost of this segmentation method is relatively high. The micro-droplet-type digital PCR platform segments the reaction system by generating a tiny water-in-oil system. This segmentation method has the advantages of fast response speed and low segmentation cost, but relatively speaking, the stability of this segmentation method is relatively poor, and the requirements for data analysis are relatively high. Due to the differences in the platforms of digital PCR, data collaborative analysis of different digital PCR platforms has also become the key to the development of current digital PCR detection methods.

This Standard is designed for the universal screening element CaMV35s promoter and NOS terminator and selects the specific primers and probes for digital PCR amplification; and determine the foreign screening elements of the CaMV35s promoter and NOS terminator based on the amplification results. In addition, through the collaborative comparison of chip-type digital PCR and micro-droplet-type digital PCR, the Standard method can achieve the same detection purpose in both platforms.

5 Reagents and Materials

Unless otherwise specified, only use the analytical reagents and Level-I water in accordance with GB/T 6682.

5.1 DNA extraction kit

It is recommended to select different genome extraction kits for different sample matrix

b) Pipettes ranges are 0.5μL~10μL and 0.1μL~2.5μL; and 10μL matching pipette tips.

7 Operation Procedures

7.1 Sampling

It shall be implemented in accordance with the provisions of GB/T 19495.1 and GB/T 19495.7.

7.2 Sample preparation

It shall be implemented in accordance with the provisions of GB/T 19495.1 and GB/T 19495.7.

7.3 Sample pretreatment

It shall be implemented in accordance with the provisions of GB/T 19495.1 and GB/T 19495.3.

7.4 DNA template preparation

It shall be implemented in accordance with the provisions of GB/T 19495.1 and GB/T 19495.3.

7.5 Digital PCR amplification methods

7.5.1 Sample digital PCR reaction

7.5.1.1 Internal standard gene digital PCR reaction

The internal standard gene digital PCR reactions in each sample are set up in 3 parallels. The digital PCR reaction system shall be configured according to the components and final concentrations in Table 1. After the configuration of the reaction system is completed, segment the reaction system. The chip-based digital PCR realizes this procedure through a microfluidic chip; and the micro-droplet-based digital PCR realizes the system segmentation by forming a water-in-oil structure. This procedure is performed according to the instructions of different digital PCR platforms. The effective segmentation coefficient of the digital PCR reaction must be no lower than 60% of the theoretical segmentation coefficient.

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