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Quantitative determination of genetically modified plants by digital PCR method

转基因植物品系定量检测数字 PCR 法

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1 Scope

This Standard specifies the quantitative determination of genetically modified plants by digital PCR method.

This Standard applies to the digital PCR method for quantitative determination of genetically modified maize MON810, MON89034, MIR162, genetically modified soybean GTS-40-3-2, genetically modified rice KMD event, genetically modified cotton GHB119, and genetically modified oilseed rape RT73 in seeds and physically-processed seeds.

The quantitative detection limit of this method is 0.1% (mass fraction).

2 Normative references

The following documents are indispensable for the application of this document. For dated references, only the dated version applies to this document. For undated references, the latest edition (including all amendments) applies to this document.

GB/T 6682, Water for analytical laboratory use - Specification and test methods

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

SN/T 4853, Quantitative detection of genetically modified rice - Digital PCR

3 Terms and definitions, abbreviations

3.1 Terms and definitions

The following terms and definitions are applicable to this document.

3.1.1 Event specific sequence

The neighbor-joining sequence that is recombined and produced after the exogenous DNA fragment is inserted into the recipient crop genome.

3.2 Abbreviations

The following abbreviations are applicable to this document.

Adh-1: Alcohol dehydrogenase 1

Adhc: Alcohol dehydrogenase C gene

PCR: Polymerase chain reaction

PEP: Phosphoenolpyruvate carboxylase gene

PLD: Phospholipase D gene

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 inhibitor, and can more stably, accurately, and rapidly perform a precise identification of the genetically modified organism of the trace.

At present, digital PCR includes chip-type digital PCR and droplet-type digital PCR. The chip-type digital PCR uses a microfluidic chip to realize the segmentation of the original reaction system; this segmentation method has the advantages of good stability and uniformity; but the test cost is relatively high. The droplet-type digital PCR generates tiny water-in-oil system to realize the segmentation of the reaction system; this segmentation method has fast response speed and lower segmentation cost.

In order to achieve quantitative determination of genetically modified plants by digital PCR, this Standard directly obtains the copy number content of exogenous genes (event specific sequence) and endogenous genes of genetically modified plants through digital PCR amplification reactions. The ratio (percentage) of the copy number of the exogenous gene to the endogenous gene in the sample DNA is the relative percentage content of the corresponding genetically modified plants in the sample.

7.5.2 Digital PCR reaction procedures

The digital PCR reaction procedures are shown in Tables 4 and 5.

7.5.3 Setting of the control digital PCR reaction

A positive control, a negative control and a blank control shall be set in the test. Use the genomic DNA of genetically modified plants that contains event specific sequence as the positive control; use the genomic DNA of non-genetically modified plants that contains the same endogenous genes as a negative control; use water as a blank control. In each control PCR reaction system, except for the template, the remaining components and PCR reaction conditions are the same as 7.5.1 and 7.5.2.

8 Results analysis and presentation

8.1 Quality control

Set the threshold limit according to the end-point fluorescence value of the negative control in the digital PCR results; the threshold limit shall effectively distinguish the negative reaction microcells from the positive reaction microcells in the same sample.

The negative control has endogenous gene amplification; neither the negative control nor the blank control has exogenous gene amplification.

The relative standard deviation of the amplification parallel test result (percentage of genetically modified events) shall be less than or equal to 25%.

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