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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

GB/T 33526-2017

Genetically modified organism detection method by digital PCR

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1 Scope

Recommended national standard giving a digital PCR method for detecting genetically modified components. It applies to the digital PCR detection of genetically modified plants and their derived material - maize, soybean, rapeseed, rice, potato, alfalfa, cotton and the like - and reaches a lower limit of detection of 0.1 % by mass fraction. The method partitions the PCR reaction into many small systems that are amplified and read individually, which makes it more tolerant of nucleic acid inhibitors and better able to identify traces of transgenic material; it is written for both of the platforms in use, chip-based digital PCR partitioned through a microfluidic chip and droplet digital PCR partitioned by a water-in-oil system, and the results of the two are made comparable. Detection targets the universal screening elements, the CaMV35S promoter and the NOS terminator, with their specific primers and probes, against the 18S rRNA internal reference gene; the internal reference reaction is run in three replicates per sample and the effective partitioning coefficient must be at least 60 % of the theoretical one. Sampling, sample preparation and nucleic acid extraction follow the GB/T 19495 series. Issued on 28 February 2017 and in force since 1 September 2017, it is a first edition.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document, including any amendments, applies.

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 - Sampling and sample preparation

3 Terms and definitions

18S rRNA gene: transcribed from the 18S rDNA and a component of the cellular ribosome.

CaMV35S promoter: from the cauliflower mosaic virus.

NOS terminator: from the nopaline synthase gene.

4 Principle

Digital PCR partitions the original reaction system into many micro-systems, each amplified and read individually. The partitioning makes the reaction more tolerant of nucleic acid inhibitors and allows traces of transgenic components to be identified more accurately and more stably.

Two platforms are covered. Chip-based digital PCR partitions through a microfluidic chip: stable and uniform, but expensive. Droplet digital PCR partitions by generating a water-in-oil system: fast and inexpensive, but less stable and more demanding on the data analysis.

The method is built on the universal screening elements - the CaMV35S promoter and the NOS terminator - with their specific primers and probes, and the results of the two platforms are made comparable.

7 Operation procedures

Sampling, sample preparation, pretreatment and DNA extraction follow GB/T 19495.1, GB/T 19495.3 and GB/T 19495.7.

The internal reference gene reaction is run in three replicates per sample.

The effective partitioning coefficient shall be not less than 60 % of the theoretical value.