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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 5334.1-2020

**Digital PCR detection method for genetically modified plant
products - Part 1: General requirements and definitions**

转基因植物产品的数字PCR检测方法 第1部分：通用要求与定义

(English Translation)

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Foreword

SN/T 5334-2020 "Digital PCR Detection Method for Genetically Modified Plant Products" is divided into 8 parts.

--Part 1: General requirements and definitions

--Part 2: Genetically modified soybeans;

--Part 3: Genetically modified corn;

--Part 4: Genetically modified rape;

--Part 5: Genetically modified cotton;

--Part 6: Genetically Modified Potatoes;

--Part 7: Genetically modified alfalfa;

--Part 8: Genetically modified sugar beet.

This document is part 1 of SN/T 5334-2020.

This document was drafted in accordance with the rules given in GB/T 1.1-2020.

1 Scope

This document specifies the scope of digital PCR detection methods for genetically modified ingredients of genetically modified plant products, normative references, and abbreviations.

Languages, terms and definitions, principles, method establishment steps, result determination and sample storage content.

This document is applicable to the digital PCR quantitative detection of genetically modified components in genetically modified plant products.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analytical laboratory water specifications and experimental methods GB/T 19495.1 General requirements and definitions for the testing of genetically modified products GB/T 19495.2 Technical requirements for genetically modified products testing laboratories GB/T 19495.3 Nucleic acid extraction and purification method for detection of genetically modified products GB/T 19495.7 Sampling and sample preparation methods for genetically modified products

3 Terms and definitions

The following terms and definitions apply to this document.

Transgene

Introduce functional gene sequences from other species that do not exist in the parent species into the parent species through various means in the genome, it is expressed in the parent species to obtain the corresponding traits.

Genetically modified plant events Through transgenic technology, functional gene fragments are introduced into the parent plant genome, so that the parent plant can obtain heritable target traits.

The parent plants that have acquired the desired traits become transgenic plant lines.

Event-specific GMO content detection in transgenic plant lines, the plant endogenous gene sequence next to the foreign gene is called the flanking sequence. For different genetically modified plants Line, the flanking sequence is unique. Therefore, the detection method that

amplifies the target fragment located at the junction of the foreign gene and the flanking sequence is called transgenic Specific detection methods for transformation events.

Digital PCR digital PCR an absolute quantitative PCR technology. Divide the PCR system into a large number of micro-reaction systems for PCR amplification, and then react to the micro-reactions after amplification the system reads the fluorescent signal, and calculates the copy number concentration of the target sequence in the sample by calculating the positive rate and Poisson distribution.

Conversion factor multiplication factor the coefficient used when converting the ratio of the transformant-specific sequence and the copy number concentration of the internal standard gene into the content of the transgenic component (W/W).

Limit of quantitation the lowest copy number concentration within the linear dynamic range that meets the precision conditions.

Limit of detection

Within the linear dynamic range, at least 2 microreaction systems can be stably detected as the lowest copy number concentration of the positive signal.

Repeatability

By two different operators, in two different time periods, samples with the same mass fraction (including target concentration samples, detection limit Samples, quantitation limit samples and negative samples).

4 Principle

Digital PCR is a quantitative detection technology of gene copy number developed on the basis of fluorescent PCR, which is used for the absolute copy of nucleic acid template.

Determination of the number. By filling the fluorescent PCR reaction system containing template, primer/fluorescent probe, heat-resistant DNA replicase and its buffer Divide and mix, and divide the same amount into a large number (more than 10 000) of micro-reaction systems isolated from each other, so that each template is allocated independently and randomly Into the micro-reaction system; all the micro-reaction systems simultaneously perform PCR amplification reactions under the same specified conditions, according to the set fluorescence threshold Value to judge the amplification results of each micro-reaction system; the digital PCR reaction is calculated according to the positive rate of the micro-reaction system and the Poisson distribution formula should be the template concentration in the system.

5.1 Instrument and equipment

5.1.1

Analytical balance. Sensitivity 0.1 mg.

5.1.2

Biological safety cabinet (ultra-clean workbench).

5.1.3

Digital PCR system. micro-reaction system generator or other instruments with the same function, micro-reaction system fluorescence detector or its it has the same function instrument.

5.1.4

PCR thermal cycler.

5.1.5

Constant temperature incubator. temperature control accuracy ± 1.0 °C.

5.1.6

Sample crusher. It can grind samples such as cottonseed and cottonseed meal into powder of about 60 mesh.

5.1.7

Micropipette. 100 μL ~1 000 μL , 20 μL ~200 μL , 10 μL ~100 μL , 0.5 μL ~10 μL , 0.1 μL ~2.5 μL .

5.1.8

Double distilled water generator or pure water meter.

5.1.9

Vortex oscillator.

5.1.10

Nucleic acid detector. nucleic acid/protein content determination spectrophotometer, micro-spectrophotometer or other nucleic acid detector.

5.1.11

Centrifuge. centrifugal force $\geq 12\ 000$ g.

5.2 Reagents and consumables

Unless otherwise specified, the quality of reagents and materials should meet the requirements of GB/T 19495.1. Double distilled water or meet the requirements of GB/T 6682 the first-level water requirements.