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

SN/T 5334.5-2020

**Digital PCR detection method for genetically modified plant
products - Part 5: Genetically modified cotton**

转基因植物产品的数字PCR检测方法 第5部分：转基因棉花

(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 5 of SN/T 5334-2020.

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

Certain contents of this document may involve patents. The issuing agency of this document is not responsible for identifying these patents.

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

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1 Scope

This document specifies GHB119, T304-40, GHB614, LLCotton25, MON1445, MON15985-7, MON88913, etc.

Conversion event-specific digital PCR detection method for 7 genetically modified cotton lines.

This document is applicable to the detection of the above 7 genetically modified cotton lines in cottonseed, meal, and cotton leaves. Under the quantitative test of this document the limit is 40 pg (about 20 copies) of cotton genomic DNA or 0.1% (w/w) content of genetically modified cotton line components.

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.

SN/T 5334.1 Digital PCR detection method for genetically modified plant products Part 1: General requirements and definitions

3 Terms and definitions

The terms and definitions defined in SN/T 5334.1 apply to this document.

5 Main equipment and reagents

5.2

For other equipment and reagent requirements, please refer to SN/T 5334.1.

6.1 General operation steps

The general operation steps are carried out in accordance with the provisions of SN/T 5334.1.

6.2 Digital PCR reaction system preparation

Add each component to a clean centrifuge tube according to Table 1, and mix thoroughly. Try to avoid air bubbles during PCR system preparation and transfer. Each the digital PCR of Adhc gene and transformant-specific sequence of each test sample and quality control sample should be set at least 3 parallel repeats.

Note: The total volume of the digital PCR reaction system can be adjusted according to different manufacturers and models of digital PCR machines. Try to avoid during PCR system preparation and transfer Generate bubbles.

6.3 Digital PCR reaction program

According to the requirements of the instrument, add the prepared digital PCR reaction mixture into the sample hole of the micro-reaction system generator, and press the instrument the device operation instructions generate a micro-reaction system. Place the micro-reaction system in a PCR amplification instrument, and perform PCR amplification according to the following parameters. 95 °C, 5 min (1 °C/s), 1 cycle; 94 °C, 15 s, 59 °C, 1 min (1 °C/s), 40 cycles; 98 °C 10 min (1 °C/s), 1 cycle; store the reaction product at 12 °C. Perform fluorescence detection on the microreaction system, record the number of positive and negative microreaction systems and their ratio. Calculate the Adhc gene copy number (copies/μL) and strain-specific sequence copy in the digital PCR reaction system according to the ratio.

Bay number (copies/μL).

Note: The PCR reaction parameters can be adjusted appropriately according to the different models of the gene amplification instrument.

7 Judgment and expression of results

See SN/T 5334.1 for calculation of results.

--In the test results, both the internal standard gene and the foreign gene meet the negative and blank quality control requirements, indicating that the sample has not been detected the standard Adhc gene in cotton is expressed as "No cotton components have been detected in this sample";

--In the test results, the internal standard gene result is positive, but the foreign gene meets the quality control requirements of negative and blank, said it indicates that the standard Adhc gene in cotton was detected in the sample, but the line-specific sequence was not detected, and the expression was "No genetically modified cotton was detected in this sample.

--In the test results, both the internal standard gene and the exogenous gene meet the quality control requirements of the digital PCR test, indicating that the sample was

transferred

8 Anti-pollution measures

The anti-pollution measures are specified in SN/T 5334.1.

9 Sample storage

For sample storage, see SN/T 5334.1.

Appendix A

(Informative appendix) Transgenic cotton line-specific sequence