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Molecular biomarkerbiomarkers — Detection of DNA in textiles derived from cotton part —

Part 2:
Overview of target sequences for use in polymerase chain reaction (PCR-)-based detection methods for cotton genetically modified (GM) events

DTS stage

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Contents

Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ~~23~~ISO/TC 34, *Food* ~~Products~~*products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis.*

This first edition, along with ISO 5354-1:—[1], cancels and replaces IWA 32:2019, which has been technically revised throughout.

A list of all parts in the ISO 5354 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

[1] Under preparation.

Introduction

Detection and identification of genetically modified (GM) cotton materials can be achieved by screening methods followed by more specific analysis of the materials. The target sequences used for screening should provide reproducible data using a variety of equipment, chemistries~~,~~ and reagents.

In this screening analysis, DNA sequences of target sequences common to many GM events are assayed in order to economically and reliably determine whether GM material is present.

The aim of this ~~standard~~document is to provide an overview of target sequences that can be found in GM cotton. Detection and identification of these target sequences can be used to determine the presence of GM events in cotton and some cotton products. ISO 5354-1 describes methods that can be used to extract DNA from cotton matrices that can subsequently be analysed with target sequences described within this ~~standard~~document.

This ~~standard~~document provides an overview of known GM cotton events.

Also included are six primer and probe sequences recommended to determine the presence of most of these GM cottons events. Only those elements for which a detection method is available are listed.

Based on elements that are detected and not detected during screening, the presence or absence of certain ~~Cotton~~cotton GM events can be evaluated and confirmed by event-specific methods. A database is available to aid in this process at the European GMO database.[7] ~~[1].~~[1]

Event-specific detection methods ~~may~~can be found via the CropLife International database[8] ~~[2]~~ and the EURL GMFF GMOMETHODS database[9] ~~JRC GMO Method Database [3].~~[1].

Molecular ~~Biomarkers—~~biomarkers — Detection of DNA in textiles derived from cotton ~~part~~ —

Part 2: Overview of target sequences for use in polymerase chain reaction (PCR-)-based detection methods for cotton genetically modified (GM) events

1 Scope

This document ~~provides~~establishes a list of target sequences that can be used to screen for the presence of genetically modified (GM) material in cotton and cotton products. ~~Its use~~

This document is ~~limited~~applicable to cottonseed, cotton leaf, cotton fibre and cotton fibre-derived materials from which sufficiently high-quality DNA can be extracted.

Methods describing the extraction of DNA from different cotton samples can be found in ISO 5354-1.

NOTE 1~~: This~~ The list of target sequences provides guidance for the screening of all currently known GM cotton events and GM cotton events that contain the same DNA sequences. Further guidance on screening of foodstuffs is provided in CEN/TS 16707 ~~[4]~~.

NOTE 2 ~~:~~ Sampling is outside of the scope of this document. Information on sampling cotton products ~~may~~can be found in ISO 1130:1975 ~~[5]~~ and in ASTM D1441-12 ~~[6]~~.

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.

ISO 16577, *Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production*

~~ISO/TS 16393:2019, *Molecular biomarker analysis — Determination of the performance characteristics of qualitative measurement methods and validation of methods*~~

~~ISO 24276, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*~~

ISO 21569 (all parts), *Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products*

~~ISO 21571, Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Nucleic acid extraction~~

ISO 21570, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*

~~ISO 21569-1, Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products — Part 1: Qualitative PCR-based DNA analysis~~

~~ISO/TS 21569-3:2020, Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products — Part 3: Construct-specific real-time PCR method for detection of P35S-pat-sequence for screening for genetically modified organisms~~

~~ISO/TS 21569-4:2016, Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products — Part 4: Real-time PCR based screening methods for the detection of the P-nos and P-nos-nptII DNA sequences~~

~~ISO/TS 21569-5:2016, Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products — Part 5: Real-time PCR based screening method for the detection of the FMV promoter (P-FMV) DNA sequence~~

~~ISO/TS 21569-6:2016, Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products — Part 6: Real-time PCR based screening methods for the detection of cry1Ab/Ac and pubi-cry DNA sequences~~

~~8~~3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 and the following apply.

ISO and IEC maintain ~~terminological~~terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
cottonseed
seed from cotton plants

3.2
cotton lint
raw fibre that has gone through the ginning process ~~of the definition~~

~~9~~4 GM element screening

~~9.1~~4.1 Principle

A screening strategy is used whereby a minimum number of tests are conducted to identify two or more of all possible GM events that are currently known to occur in cotton. Transgene target sequences for analysis can be chosen from Table 1 by the user based on the expected events or as a strategy to provide the most information with the least use of resources. Common genetic sequences, or elements, present across multiple GM constructs are initially targeted. The combined presence or absence of individual elements can be used to infer the presence of one or more GM events.

~~9.2~~4.2 Procedure

A procedure using a limited number of target sequences is chosen and may be followed up by adding further target sequences to the analysis. If detected, no further testing is required. If not-detected, further testing is necessary for up to all six elements. A minimum of two detection methods (targeting two of T-nos, P-35S, cry1Ab/Ac, pat otp/mepsps or P-FMV) are applied based on expectations of which GM event can be present, as a first screen. Screening with the six target sequences T-nos, P-35S, cry1Ab/Ac, pat, otp/mepsps, and P-FMV is likely to detect most events if they are present (see Table 1). Internationally recognized methods should be applied, if possible.

~~9.3~~4.3 Primers and ~~Probes~~probes

~~9.3.1~~4.3.1 T-nos

PCR screening for the biomarker T-nos using the following primers and probes is described in ISO/TS 21569-4. (See also ISO 21569:2005/Amd 1:2013).

180-F CATGTAATGCATGACGTTATTTATG

180-R	TTGTTTTCTATCGCGTATTAAATGT
Tm-180	FAM-ATGGGTTTTTATGATTAGAGTCCCGCAA-TAMRA

~~9.3.2~~4.3.2 P-35S

PCR screening for the biomarker P-35S is described in ISO/TS 21569-3.

In addition, the following primers and probes are described in ISO 21570:2005.

35S-F	GCCTCTGCCGACAGTGGT
35S-R	AAGACGTGGTTGGAACGTCTTC
35S-TMP	FAM-CAAAGATGGACCCCCACCCACG-TAMRA

~~9.3.3~~4.3.3 cry1Ab/Ac

PCR screening for biomarker cry1Ac/Ab using the following primers and probes is described in ISO/TS 21569-6.

Bt-F1(mod)	GAGGAAATGCGTATTCAATTCAAC
Bt-R	TTCTGGACTGCGAACAATGG
Bt-P	FAM-ACATGAACAGCGCCTTGACCACAGC-TAMRA

~~9.3.4~~4.3.4 pat

PCR screening for biomarker pat is described in ISO/TS 21569-3 and using the following primers and probes described in Reference [10~~Debode et al. [7].~~].

pat-F	CGCGGTTTGTGATATCGTTAAC
pat-R	TCTTGCAACCTCTCTAGATCATCAA
pat-P	FAM-AGGACAGAGCCACAAACACCACAAGAGTG-TAMRA

~~9.3.5~~4.3.5 P-FMV

PCR screening for biomarker P-FMV using the following primers and probes is described in ISO 21569-5: ~~(~~2016~~).~~

pFMV-F	CAAAATAACGTGGAAAAGAGCT
pFMV-R	TCTTTTGTGGTCGTCACTGC
Probe pFMV	FAM-CTGACAGCCCACTCACTAATGC-BHQ1

~~9.3.6~~4.3.6 otp/mepsps

PCR screening for biomarker P-FMV using the following primers and probes is described in ISO 21570:2005.

GA21 3-5′	GAAGCCTCGGCAACGTCA
GA21 3-3′	ATCCGGTTGGAAAGCGACTT
GA21-2-Taq	FAM-AAGGATCCGGTGCATGGCCG-TAMRA

~~9.3.7~~4.3.7 cry1Ac

See ~~Clause 4.2.3~~4.3.3. The method is described in Reference [11~~Scholtens et al.[8].~~].

~~9.3.8~~4.3.8 nptII

The method is described in Reference [11~~Scholtens et al. [8].~~].

NPT 1-5′′	GACAGGTCGGTCTTGACAAAAAG

NPT 1-3''	GAACAAGATGGATTGCACGC
Probe	TGCCCAGTCATAGCCGAATAGCCTCTCCA

~~9.3.9~~4.3.9 cry-1Ab

See ~~Clause 4.2.3~~4.3.3. The method is described in Reference [11~~Scholtens et al.[8].~~]

~~9.3.10~~4.3.10 T-35S

The method is described in References [12] and [13~~Rischitor et al. [9][10].~~]

t35S pCAMBIA c-F	CGGGGGATCTGGATTTTAGTA
t35S pCAMBIA a-R	AGGGTTCCTATAGGGTTTCGCTC

~~9.3.11~~4.3.11 P-ubi1

The method is described in References [14] and [15~~Grohmann et al. [11] [12].~~]

pubi-F2	ATTTGCTTGGTACTGTTTCTTTTGTC
pubi-R	TTGTTGTCCATGGATCCTCTAGAGT
pubi-T2 probe	ACCCTGTTGTTTGGTGTTACTTCTGCA

~~9.3.12~~4.3.12 cp4-epsps

The method is described in Reference [16~~Barbau-Piednoir et al., 2014.~~]

CP4 Synthetic F	GCATGCTTCACGGTGCAA
CP4 Synthetic R	TGAAGGACCGGTGGGAGAT

~~9.3.13~~4.3.13 cry- 2Ab2

The method is described in Reference [17~~Dinon et al.[14].~~]

cry2Ab2 – F	AATTCTAACTACTTCCCCGACTACTTC
cry2Ab2 – R	ACGGAGAGGCGATGTTCCTG
cry2Ab2 – Probe	TCTCTGGTGTTCCTCTCGTCGTCCGCA

~~9.3.14~~4.3.14 cry1C

Cry1c – F	5'' - TTCTACTGGGGAGGACATCG – 3''
Cry1c – R	5'' -CGGTATCTTTGGGTGATTGG- 3''

The method is described in Reference [18~~X. GUO, C. HUANG, S. JIN, S. LIANG, Y. NIE and X. ZHANG [15].~~]

~~9.3.15~~4.3.15 T-E9

The method is described in References [10] and [19~~Debode et al. [7] and QL-ELE-00-024 [16].~~]

T-E9-R	TTTTTATTCGGTTTTCGCTATCG
T-E9-F	TGAGAATGAACAAAAGGACCATATCA
T-E9-Probe	FAM- TCATTAACTCTTCTCCATCCATTTCCATTTCACAGT-TAMRA

~~9.3.16~~4.3.16 cry1F

Method is described in Reference [11~~Scholtens et al. [8],~~] with minor modifications in forward primer and probe sequence.

Cry1F-F2	GACGTGGATCTTCATCTGCAATC
Cry1Fr-n2	GCAACACGGCTGGCAATCG

