Technical Specification

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Molecular biomarkers — Detection of DNA in cotton used for textile production —

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

Biomarqueurs moléculaires — Détection d'ADN dans le coton utilisé pour la production textile —

Partie 2: Présentation des séquences cibles à utiliser dans les méthodes de détection reposant sur une réaction de polymérisation en chaîne (PCR) des événements de coton génétiquement modifié (GM)

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

This first edition, along with ISO 5354-1, 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.

Introduction

Detection and identification of genetically modified (GM) cotton materials are typically accomplished using polymerase chain reaction (PCR) based screening methods followed by more specific event-based analyses of the materials. Based on targets that are detected or not-detected during screening, the presence or absence of specific cotton GM events can be determined and confirmed with event-specific methods. Target sequences used with screening and event-based methods can provide reproducible data across a variety of equipment, chemistries, and reagents. In this way, DNA sequences associated with GM events can be assessed in order to economically and reliably determine whether GM material is present.

This document provides examples of screening and event-based target sequences that are found in GM cotton. PCR methods that amplify these target sequences for detection and identification can be used to determine the presence of GM events in cottonseed and some cotton products. Six primer and probe pairs are recommended for determining the presence of most GM cottons events. Only those elements for which a detection method is available are listed.

ISO 5354-1[1)][1] describes methods for extraction of PCR amplifiable DNA from cotton matrices that can subsequently be analysed for the target sequences described within this document and a taxon specific PCR reference detection method for cotton.

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Molecular biomarkers — Detection of DNA in cotton used for textile production —

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 a list of target sequences that can be used to screen for the presence of genetically modified (GM) material in cotton and cotton products.

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

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

NOTE 1 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[2].

NOTE 2 Sampling is outside of the scope of this document. Information on sampling cotton products can be found in ISO 1130:1975[3] and in ASTM D1441-12[4].

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

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

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 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

4 Abbreviated terms

T-nos	*Agrobacterium tumefaciens* nopaline synthase terminator
P-35S	cauliflower mosaic virus 35S promoter sequence
cry1Ab/Ac	synthetic fusion gene derived from *Bacillus thuringiensis* produces Cry1Ab-Ac delta endotoxin (fusion protein)
pat	synthetic form of the phosphinothricin N-acetyltransferase (PAT) enzyme derived from *Streptomyces viridochromogenes* strain Tu494
P-FMV	figwort mosaic virus promoter
otp/mepsps	optimized transport peptide/modified 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) gene
cry1Ac	crystal delta endotoxin insecticide produced by *Bacillus thuringiensis* (Bt) during sporulation.
nptII	*Escherichia coli* Tn5 transposon gene that encodes an enzyme for neomycin phosphotransferase II
cry-1Ab	crystal delta endotoxin insecticide produced by *Bacillus thuringiensis* (Bt) during sporulation.
T-35S	cauliflower mosaic virus 35S terminator (CaMV T-35S) in pCAMBIA vector
P-Ubi1	maize polyubiquitin-1 promoter
cp4-epsps	bacterial 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase, EPSPS) enzyme from *Agrobacterium sp.* strain CP4
cry-2AB2	crystal delta endotoxin insecticide produced by *Bacillus thuringiensis* (Bt) that is expressed in cotton
cry1C	crystal delta endotoxin insecticide produced by *Bacillus thuringiensis* (Bt)
tE9	terminator derived from pea ribulose diphosphate carboxylase gene
Cry1F	crystal delta endotoxin insecticide produced by *Bacillus thuringiensis* (Bt)

5 GM element screening

5.1 Principle

The PCR screening strategy described in this document is based on a method developed for detecting a minimum of two or more events known to occur in GM cotton. Transgene target sequences for analysis can be chosen from Table 1. Targets can also be selected from other references based on the anticipated 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 can be initially targeted. The combined presence or absence of individual elements can be used to determine the presence of one or more GM events.

The European GMO database is available to aid in the process of determining anticipated the targets and their respective PCR primers to be used in detecting GM cotton.[5] Event-specific detection methods are also described in the CropLife International database[6] and the EURL GMFF GMO methods database[7].

5.2 Procedure

Screening with the six target sequences: T-nos, P-35S, cry1Ab/Ac, pat, otp/mepsps, and P-FMV; can detect most known cotton GM events if they are present (see Table 1). A method using all or a minimum of two or more of these target sequences can also be chosen for the analysis based on information that a GM event may be present, how the method will be performed, e.g., multiplexed, separate reactions, microarray, etc., the time and labour required and cost of materials. If the target is detected using less than six target sequences, no further testing will be required. If the target is not-detected, additional targets up to the six suggested can be added to the method. Respectively, qualitative and quantitative PCR methods should be designed and developed according to the ISO 21569 series and ISO 21570.

5.3 Primers and probes

5.3.1 General

Primers and probes that have been listed in this section have been collaboratively validated in at least one agricultural matrix. In most cases, cotton has been considered in a collaborative trial.

5.3.2 T-nos

PCR screening for the biomarker T-nos using the following primers and probes is described in ISO 21569:2005/Amd 1:2013[8], Clause B.6.

180-F	5′-CATGTAATGCATGACGTTATTTATG - 3′
180-R	5′-TTGTTTTCTATCGCGTATTAAATGT - 3′
Tm-180	5′-FAM-ATGGGTTTTTATGATTAGAGTCCCGCAA-TAMRA - 3′

5.3.3 P-35S

Quantitative PCR determination for the biomarker for P-35S using the following primers and probe is described in ISO 21570:2005[9], Clause B.1.

35S-F	5′-GCCTCTGCCGACAGTGGT - 3′
35S-R	5′-AAGACGTGGTTGGAACGTCTTC - 3′
35S-TMP	5′-FAM-CAAAGATGGACCCCCACCCACG-TAMRA - 3′

5.3.4 cry1Ab/Ac

PCR screening for biomarker cry1Ac/Ab using the following primers and probes is described in ISO/TS 21569-6[10].

Bt-F1(mod)	5′-GAGGAAATGCGTATTCAATTCAAC - 3′
Bt-R	5′-TTCTGGACTGCGAACAATGG - 3′
Bt-P	5′-FAM-ACATGAACAGCGCCTTGACCACAGC-TAMRA - 3′

5.3.5 pat

PCR screening for biomarker pat is described using the following primers and probes[11] and in ISO/TS 21569-3[12] with different primers and probes.

pat-F	5′-CGCGGTTTGTGATATCGTTAAC - 3′
pat-R	5′-TCTTGCAACCTCTCTAGATCATCAA - 3′
pat-P	5′-FAM-AGGACAGAGCCACAAACACCACAAGAGTG-TAMRA - 3′

5.3.6 P-FMV

PCR screening for biomarker P-FMV using the following primers and probes is described in ISO/TS 21569-5[13].

pFMV-F	5′-CAAAATAACGTGGAAAAGAGCT - 3′
pFMV-R	5′-TCTTTTGTGGTCGTCACTGC - 3′
Probe pFMV	5′-FAM-CTGACAGCCCACTCACTAATGC-BHQ1 - 3′

5.3.7 otp/mepsps

The junction region between the optimized transit peptide sequence (otp) and the point mutated 5-enolpyruvylshikimate-3-phosphate synthase (mEPSPS) gene from maize has served as the biomarker to detect maize event GA21.[14] The use of the following primers and probes for quantitative determination is also described in ISO 21570:2005[9], Clause C.8.

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

5.3.8 cry1Ac

PCR screening for biomarker cry-1Ac using the following primers and probes is described in Reference [15].

Cry1Ac-F(/R)-n4	5′-TTCAGGACCAGGATTCAC - 3′
Cry1AcR-n2	5′-GTGAATAGGGGTCACAGAAGCATA - 3′
Cry1AcP-n3	5′-TCTGGTAGATGTGGATGGGAAGT - 3′

5.3.9 nptII

PCR screening for biomarker npt II using the following primers and probes is described in ISO 21569:2005[16], Clause B.4.

npt II-5′	5′-GACAGGTCGGTCTTGACAAAAAG - 3′
npt II-3′	5′-GAACAAGATGGATTGCACGC - 3′
Probe	5′-TGCCCAGTCATAGCCGAATAGCCTCTCCA - 3′

5.3.10 cry-1Ab

PCR screening for biomarker cry-1Ab using the following primers is described in Reference [17] and ISO 21569:2005[16], Clause C.4.