



Technical Specification

ISO/TS 5354-2

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)*

**First edition
2024-04**

STANDARDSISO.COM : Click to view the full PDF of ISO/TS 5354-2:2024



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Abbreviated terms	2
5 GM element screening	2
5.1 Principle	2
5.2 Procedure	3
5.3 Primers and probes	3
5.4 Reference materials	6
5.5 GM cotton events	6
5.6 Validation	9
5.7 Interpretation and expression of results	9
5.8 Results	9
5.9 Reporting	9
6 Test report	9
Bibliography	11

STANDARDSISO.COM : Click to view the full PDF of ISO/TS 5354-2:2024

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 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 5354-1¹⁾[\[1\]](#) 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.

STANDARDSISO.COM : Click to view the full PDF of ISO/TS 5354-2:2024

1) Under preparation. Stage at the time of publication: ISO/DIS 5354-1:2023

STANDARDSISO.COM : Click to view the full PDF of ISO/TS 5354-2:2024

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	<i>Agrobacterium tumefaciens</i> nopaline synthase terminator
P-35S	cauliflower mosaic virus 35S promoter sequence
cry1Ab/Ac	synthetic fusion gene derived from <i>Bacillus thuringiensis</i> produces Cry1Ab-Ac delta endotoxin (fusion protein)
pat	synthetic form of the phosphinothricin N-acetyltransferase (PAT) enzyme derived from <i>Streptomyces viridochromogenes</i> 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 <i>Bacillus thuringiensis</i> (Bt) during sporulation.
nptII	<i>Escherichia coli</i> Tn5 transposon gene that encodes an enzyme for neomycin phosphotransferase II
cry-1Ab	crystal delta endotoxin insecticide produced by <i>Bacillus thuringiensis</i> (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 <i>Agrobacterium sp.</i> strain CP4
cry-2AB2	crystal delta endotoxin insecticide produced by <i>Bacillus thuringiensis</i> (Bt) that is expressed in cotton
cry1C	crystal delta endotoxin insecticide produced by <i>Bacillus thuringiensis</i> (Bt)
tE9	terminator derived from pea ribulose diphosphate carboxylase gene
Cry1F	crystal delta endotoxin insecticide produced by <i>Bacillus thuringiensis</i> (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'-TTCTGGACTGCCAACAATGG - 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'-TCTTTTGTGGTCGTCCTACTGC - 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.

Forward primer 5'-GTTACCCTGATTGATAGGC-3'
Reverse primer 5'-ACAGAAGACCTTTCAATATC-3'

5.3.11 T-35S

PCR screening for biomarker T-35S using the following primers is described in Reference [18].

t35S pCAMBIA c-F 5'-CGGGGGATCTGGATTTTAGTA - 3'
t35S pCAMBIA a-R 5'-AGGGTTCCTATAGGGTTTCGCTC - 3'

5.3.12 Pubi-cry

PCR screening for biomarker Pubi-cry using the following primers and probe is described in Reference [19].

Ppubi-F2 5'-ATTTGCTTGGTACTGTTTCTTTTGTC - 3'
pubi-RCry-rr-R 5'-TTGTTGTCCATGGATCCTCTAGAGT - 3'
Ppubi-T2 probe 5'-ACCCTGTTGTTGGTGTACTTCTGCA - 3'

5.3.13 cp4-epsps

PCR screening for biomarker cp4-epsps using the following primers is described in Reference [20].

CP4 Synthetic F 5'-GCATGCTTCACGGTGCAA - 3'
CP4 Synthetic R 5'-TGAAGGACCGGTGGGAGAT - 3'

5.3.14 cry- 2Ab2

PCR screening for biomarker cry-2Ab2 using the following primers and probe is described in Reference [21].

cry2Ab2 - F 5'-AATTCTAACTACTTCCCCGACTACTTC - 3'
cry2Ab2 - R 5'-ACGGAGAGGCGATGTTCTTG - 3'
cry2Ab2 - Probe 5'-TCTCTGGTGTTCCTCTCGTCGTCGCA - 3'

5.3.15 cry1C

PCR screening for biomarker cry1C using the following primers is described in Reference [22].

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

5.3.16 T-E9

PCR screening for biomarker T-E9 using the following primers and probe is described in Reference [23].

T-E9-R 5'-TTTTTATTCGGTTTTTCGCTATCG - 3'
T-E9-F 5'-TGAGAATGAACAAAAGGACCATATCA - 3'
T-E9-Probe 5'-FAM- TCATTAACCTCTTCCATCCATTTCACAGT-TAMRA - 3'

5.3.17 cry1F

PCR screening for biomarker Cry1F using the following primers and probe is described in Reference [24].

Cry1F-F2	5'-GACGTGGATCTTCATCTGCAATC - 3'
Cry1Fr-n2	5'-GCAACACGGCTGGCAATCG - 3'
Cry1F-P2 Probe	5'-CGCCCCCGGGATTGAAGACCCCGTAAC - 3'

5.4 Reference materials

Certified cotton certified reference materials (CRMs) from the JRC-Institute for Reference Materials (IRMM) [25] and the American Oil Chemist's Society (AOCS) [26] are available for use as positive controls in the PCR (see ISO 24276 [26]) of the screening elements, for example:

- AOCS 0804D MON15985: P-35S, T-nos, cry1Ab/Ac;
- ERM-BF422 3006-210-23x281-24-236: pat;
- AOCS 1108-A GHB614: otp/mepsps;
- AOCS 0804-B MON1445: P-FMV;
- AOCS 0906-D2 MON 88913: P-35S, T-nos, cry1Ab/Ac, P-FMV.

Results from the use of these positive controls should be interpreted with caution as they are based on DNA or seed material, as opposed to cotton lint or fabric, and so can behave differently in a PCR reaction.

5.5 GM cotton events

A list of known GM cotton events at the time of development of this document and their identifying PCR targets is provided in Table 1. For information on new GM cotton events and their corresponding genetic elements refer to the following sources:

- the European GMO database [5];
- The Convention on Biological Diversity, Biosafety Clearing-House (BCH) [28];
- ISAAA GMO database [29];
- USDA-APHIS Biotechnology Permits [30];
- CropLife database [31].

Some of the GM events described in Table 1 have never been commercialized or have only been identified in publications/reports from academic or government institutions. Their presence in a sample is highly unlikely and every effort should be made to identify them with assays that provide a high degree of confidence.

For a comprehensive list of currently commercial cotton products that contain these events, refer to the CropLife database [31].

Table 1 — Overview of target sequences in known genetically modified organism (GMO) cotton events

Genetically modified organism event name	OECD unique identifier ^[32]	Event-detection method	Detected with screening	Transgene target sequence														cry1F
				T-nos	P-35S	cry1Ac	cry-1Ab/Ac	nptII	P-FMV	cry-1Ab	pat	T-35S	P-ubi1	otp/mepsps	cp4-epsps	cry-2Ab2	cry1C	
LLCotton25	ACS-GH001-3	yes	yes	X	X													
GHB614	BCS-GH002-5	yes	yes											X				
T303-3	BCS-GH003-6	no	yes	X	X													
T304-40	BCS-GH004-7	yes	yes	X	X													
GHB119	BCS-GH005-8	yes	yes	X	X								X					
GHB811	BCS-GH811-4	yes	yes															
BXN/ BXN10211-4, BXN-10222-2, BXN10224	BXN-10211-9, BXN-10215-4, BXN-10222-2, BXN-10224-4	no	yes		X													
	3006-210-23	yes	yes			X							X					
	281-24-236	yes	yes								X	X						X
	DAS81910	no	yes								X							
MON531, MON757, MON89924	MON-00531-6, MON-00757-7, MON-89924-2	yes	yes	X	X		X	X										
MON1445, MON89383	MON-01445-2, MON-89383-1	yes	yes	X	X				X									
MON15985:	MON-15985-7	yes	yes	X	X	X	X	X							X			
MON88701	MON-88701-3	yes	yes	X	X													
MON88702	MON-88702-4	no	yes						X				X					
MON88913	MON-88913-8	yes	yes		X				X						X		X	
COT102	SYN-IR102-7	no	yes	X														
COT67B	SYN-IR67B-1	no	yes	X							X							
TAM66274	TAM-66274-5	no	yes	X														

Table 1 (continued)

Genetically modified organism event name	OECD unique identifier ^[32]	Event-detection method	Detected with screening	Transgene target sequence														
				T-nos	P-35S	cry1Ac	cry-1Ab/Ac	nptII	P-FMV	cry-1Ab	pat	T-35S	P-ubi1	otp/mepsps	cp4-epsps	cry-2Ab2	cry1C	T-E9
BNLA 106 (BNBt LA-01)	not available	no	yes			X	X											
Event 1	not available	no	yes	X	X	X	X	X										
GFM Cry1A	not available	no	yes	X	X	X	X	X										
GK12	not available	no	yes	X	X	X	X	X										
GK19	not available	no	yes	X	X	X	X	X										
MLS 9124	not available	no	yes	X	X			X				X				X		
NC 33B	not available		yes			X	X											
Ngwe Chi 6 Bt	not available		yes			X	X											
Ngwe Chi 9 Bt	not available	no	yes			X	X											
SGK321	not available	no	yes	X	X	X	X	X										
Sum of detectable events per element				17	17	13	12	10	3	3	3	3	2	2	1	1	1	1

5.6 Validation

Validation of qualitative methods should conform to ISO/TS 16393^[33].

5.7 Interpretation and expression of results

Interpretation and expression of results should be carried out as described in ISO 24276:2006^[27], Clause 6.

5.8 Results

The final result of each target-specific detection method is reported as “detected” or “not detected”. When one or more targets are “detected”, the sample contains GM elements that are used in GM cotton. When the results show “not detected” for all six targets, this is considered indicative of the absence of GM at or below the limit of detection in the sample tested. Alternatively, the material may be analysed for the presence of further target sequences.

5.9 Reporting

The result should be recorded ensuring the reliability, reproducibility and integrity of the data in accordance with ISO 24276:2006^[27], Clause 7.

6 Test report

The test report shall contain at least the following information:

- a) all information needed to identify the laboratory sample;
- b) any particular information relating to the laboratory sample (e.g. insufficient size, degraded state);
- c) statement about date and type of sampling procedure(s) used;
- d) date of receipt;
- e) storage conditions, if applicable;
- f) analysis start/end date, if applicable;
- g) person responsible for the analysis;
- h) size of the laboratory sample and test sample;
- i) results according to the requirements of the specific method and the units used to report the results and the calibrators and the calculation method used;
- j) list of elements and/or constructs checked, detected and, if elucidated, the event(s);
- k) results obtained of the screening, expressed as “GM-elements were detected in the sample” or the “GM-elements were not detected in the sample”;
- l) limit of detection (LOD) of each of the methods employed for each component tested, as assessed given the DNA concentration used in the reaction;
- m) test method used, with reference to this document, i.e. ISO/TS 5354-2:2024;
- n) any particular points observed in the course of the test;
- o) significant operating details not specified in this document, or regarded as optional, together with details of any incidents that can have affected the results.
- p) any particular observations made during testing;
- q) any deviations, additions to or exclusions from the test specification;

ISO/TS 5354-2:2024(en)

- r) requirements as specified in the test report clauses of the ISO 21569 series or ISO 21570;
- s) any statements required as specified regarding quality assurance.

Information shall be given with regard to the units.

The measurement uncertainty and its level of confidence shall, on request, be made available to the user of the results.

STANDARDSISO.COM : Click to view the full PDF of ISO/TS 5354-2:2024