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

Part 1: Extraction of DNA from cottonseed and raw materials derived therefrom

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

Partie 1: Extraction d'ADN à partir de graines de coton et de matières premières issues de celles-ci

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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 ~~documents~~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/TS 5354-2:2024, 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

The purpose of this document is to provide guidance to assess whether cotton, cotton fibre or cotton-derived materials, or all of these, contain a specific DNA sequence or sequences. This guidance can be applied to detection of pure genetically modified (GM) cotton in textile production, detection of a specific GM cotton target sequence in other cotton and for confirming or tracing a particular species, variety or genetic marker.

While GM-cotton cultivation covers a large percentage of global cotton production today,[1] there are countries where the cultivation of GM cotton is not permitted by law, as well as voluntary, private and public standards that do not permit the intentional use of genetically modified organisms (GMOs) in the cotton and textile production process or require labelling. Due to asynchronous regulatory approvals, a GM cotton variety that is approved for growth and import and in one country can be disapproved or require labelling in another country. There has been a need for detection of a specific GM cotton event in GM (or non-GM) cotton. The detection methods submitted and approved by global regulatory agencies are available for that purpose and this method does not supplant those nor the results of those analyses.

Growers of non-GM cotton can provide traceability and certification of cottonseed to ensure that the seeds entering ~~an~~a certified cultivation scheme are not GM. If the starter seed is conventional (non-GM), which can be accurately determined depending on the availability of methods, and growers follow their certification process then the ginned fibre can be received as is without misleading consumers. This document provides evidence that DNA extraction methods are only effective and accurate for seeds and leaves. Although~~,~~ pure GM cotton can be detected at the ginned cotton stage~~.~~ and potentially at the griege yarn stage, non-GM cotton cannot be claimed if a negative result is obtained because there is a significant potential ~~of~~for a false negative result due to the lack of polymerase chain reaction (PCR) quality DNA.

The DNA sequence screening approach described in this document is based on PCR-methods. The methods described in this document are designed to work on all four of the major commercial cotton species: *Gossypium hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum*.

Cotton (*Gossypium* spp.) has been cultivated for lint for over 8 000 years. There are over 50 species in the *Gossypium* genus.[2] The *Gossypium* genome is complex, containing 2,25 to 2,43 gigabasepairs.[3]

This document describes the key factors necessary to screen cottonseed, cotton leaf and fibre samples at different stages of textile development in the cotton production chain for the potential presence of specific DNA elements. The protocol describes two major steps:

a) an effective way to isolate DNA from cotton materials;

b) a method to confirm that the isolated DNA is PCR quality DNA, i.e. suitable for PCR (preferred markers chosen for this purpose will be nuclear, and low copy number).

~~Genetically modified~~GM element screening is described in ISO/TS 5354-2[4].

The single laboratory validation studies described in this document including method development was carried out by the Wageningen University and Research Institute (RIKILT), the Netherlands.

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

Part 1:
Extraction of DNA from cottonseed and raw materials derived therefrom

1 Scope

This document specifies requirements and recommendations to laboratories that perform extraction of ~~PCR~~polymerase chain reaction (PCR) quality deoxyribonucleic acid (DNA) from cottonseed, cotton leaf and raw material derived therefrom, that is sufficient for the purpose of ~~polymerase chain reaction (~~PCR~~)~~ analysis.

This document is applicable to:

a) identifying cotton raw material from which PCR quality DNA can be extracted;

b) specifying a method for effective DNA extraction from cotton and cotton-derived raw materials;

c) specifying the cotton-specific marker(s) to be used as controls for PCR amplification of DNA.

A PCR result obtained from analysis of cottonseed, cotton leaf and to some extant raw materials derived therefrom can only indicate that it is not derived from pure genetically modified organism (GMO~~) —~~)-derived cotton. Admixtures of GMO-derived cotton cannot be detected for cotton fibre and cotton fibre-derived materials.

This document does not apply to bulk sampling of the seed, bale or processed fabric and yarn. A recommended sampling method is given in ISO 6497[5]. General guidance for the sampling of bulk materials or for cotton-based products is available in standards such as ASTM D1441-12[6] and CEN/TS 15568[7].

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 3696:1987, *Water for analytical laboratory use — Specification and test methods*~~

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

ISO 21570:2005, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*

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

ISO 24276:2006, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions*

ISO 24276:2006/Amd 1:2013, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions — Amendment 1*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 ~~Molecular biomarker analysis — Vocabulary for molecular biomarker analytical methods in agriculture and food production~~ 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
textile
woven fabric, knitted fabric, etc., formed by the interlocking of fibres and yarns having a certain cohesion and which is generally intended for clothing or furniture applications

Note 1 to entry: Textiles often include certain types of non-woven fabrics.

[SOURCE: ISO 16373-3:2014[8], 2.1]

3.2
cottonseed
seed from cotton plants

3.3
cotton leaf
leaf from cotton plants

3.4
seed cotton
fuzzy seed
raw cotton that contains both the seed and the fibre before it has been ginned

3.5
cotton lint
raw fibre that has gone through the ginning process

3.6
greige yarn
unprocessed long continuous length of interlocked *cotton lint* (3.5) that results from the cleaning and subsequent spinning of the cotton lint

3.7
greige fabric
unprocessed *textiles* (3.1) formed by weaving, knitting or crocheting yarn and non-woven fabric

3.8
processed yarn
yarn that has undergone processing to develop its full textile potential

3.9
processed fabric
fabric that has undergone processing to develop its full textile potential

3.10
polymerase chain reaction quality deoxyribonucleic acid
PCR quality DNA
~~deoxyribonucleic acid~~DNA template of sufficient length, chemical purity~~,~~ and structural integrity to be amplified by PCR

[~~Source~~SOURCE: ISO 24276:2006~~][9],~~ 3.~~11~~2.3]

3.11
cotton matrix control
sample that can be identified as cotton derived based upon polymerase chain reaction amplification and detection of the SAH7 gene in its extracted DNA

3.12
cycle quantification
Cq
cycle in real-time polymerase chain reaction at which the fluorescence signal from the reaction crosses a threshold level at which the signal can be distinguished from background levels

[SOURCE: ~~Adapted from~~ ISO 16577:2022, modified — Other terms and note to entry deleted~~][10].~~]

4 Principle

This document describes the requirements for preparation and screening of specific DNA in cotton and textiles. It describes conditions for obtaining DNA for detecting a specific DNA element and a scheme for detecting an endogenous cotton gene (positive control). The amplification and detection of endogenous cotton DNA sequences is achieved through extraction methods that result in PCR quality DNA. In application to cotton samples PCR quality DNA allows for the detection of specific cotton DNA sequences. Admixtures of GM cotton were not analysed.

PCR quality DNA may be isolated from the production stages of cottonseed up to and including greige yarn and greige fabric. A PCR result obtained from analysis of cotton fibre and cotton fibre-derived materials can only indicate that it is not derived from pure GMO-derived cotton. Admixtures of GMO-derived cotton cannot be detected for cotton fibre and cotton fibre-derived materials.

Processed yarn and processed fabric were examined as part of the development of this document, but it was found that PCR quality DNA could not be isolated from this material.

Further screening for GM-content-based real-time PCR methods is described in ISO/TS 5354-2[4].

5 Identification of a suitable cotton endogenous DNA marker

The identification of a suitable endogenous DNA marker for the detection of the four commercial cotton species (*Gossypium hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum*) was undertaken in a study ~~of~~by RIKILT ~~(~~(The Netherlands). Three different potential DNA markers were compared: ~~1) cotton fiber-specific acyl carrier protein (ACP1) gene, 2) alcohol dehydrogenase C (AdhC) and 3) *Sinapsis arabidopsis* homolog 7 (SAH7) [11].~~

a) cotton fibre-specific acyl carrier protein (ACP1) gene;

b) alcohol dehydrogenase C (AdhC);

c) *Sinapsis arabidopsis* homolog 7 (SAH7).[9]

Formatted: Definition, Don't adjust space between Latin and Asian text, Don't adjust space between Asian text and numbers

SAH7 outperformed AdhC and ACP1 markers, showing earlier amplification and consistent amplification of the four commercial cotton species (*G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum*). Data supporting this conclusion is presented in Annex A.[10][11][12, 13]. In cases where there ~~is~~was a lack of a signal for the larger quantity of DNA, no further analysis was done to elucidate the cause for PCR inhibition.

6 Test sample preparation

The test sample should be homogenized using suitable methods and avoiding excessive heating. Sample preparation is dependent on sample type. Prepare samples by using one of the following techniques: teasing, cutting, crushing or shredding.

Prepare at least two replicates per sample. Include appropriate negative and positive controls, as specified in ISO 21571 on DNA extraction.

Suitable test sample preparation methods for different types of material are as follows:

— Cottonseed: Crush the seeds thoroughly with a suitable method. Use 100 mg in the DNA extraction procedure. Ensure that seeds are free of small fibres/lint, i.e~~.~~, delinted.

— Cotton leaf: Crush the leaves thoroughly with a suitable method. Use 100 mg in the DNA extraction procedure.

— Seed cotton: Separate the seeds from the long fibres and crush the seeds thoroughly with a suitable method. Use 100 mg in the DNA extraction procedure.

— Cotton lint: The fibre material can be separated from the seed and teased thoroughly by applying a suitable method. Use 100 mg in the DNA extraction procedure.

— Greige yarn: Cut the yarn with a suitable method into small parts of a maximum of approximately 0,5 cm length. Use 100 mg in the DNA extraction procedure.

— Greige fabric: Cut the fabric with a suitable method in small parts of a maximum of approximately 0,5 × 0,5 cm in size. Use 100 mg in the DNA extraction procedure.

7 Assessment of DNA extraction methods for different cotton production stages

7.1 General

Different extraction methods for obtaining PCR quality DNA from different cotton materials in the production process from cottonseed to textiles were tested to determine if any gave better results across specified matrices. Cottonseed matrix tested most consistently and gave best yields on multiple extract methods. In the WFSR study, cotton samples from the cotton production process were used to compare five DNA extraction methods. The samples were obtained from sources in the ~~U.S.A.,~~United States of America (USA), Turkey and India. To confirm the presence and PCR quality of the extracted DNA, each isolated DNA was used in a quantitative PCR for the endogenous control SAH7.[11][10]~~, quantitative~~ Quantitative PCR assays were performed in duplicate with undiluted DNA. The five DNA extraction methods that were examined included:

a) ~~1)~~ a cetyltrimethylammonium bromide (CTAB) method;

b) ~~2)~~ a commercial kit based on magnetic beads;

c) ~~3)~~ a CTAB method in combination with a ~~Plant~~plant DNA-extraction kit;[12] ~~[14];~~

d) ~~4)~~ a CTAB-cotton Community Reference Laboratory (CRL) VL-14/05XP method ~~and~~ ;