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

Part 1: Extraction of DNA from cotton, 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 coton, de graines de coton et de matières premières issues de celles-ci

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Contents

Page

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/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 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 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).

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 (WFSR), the Netherlands.

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

1 Scope

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