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Screening of genetically modified organisms (GMOs) in cotton and textiles

Criblage pour la détection des organismes génétiquement modifiés (OGM) dans le coton et les textiles

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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 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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.

International Workshop Agreement IWA 32 was approved at a workshop hosted by the Netherlands Standardization Institute (NEN), in association with the Organic Cotton Accelerator, held in New Delhi, India, in January 2019.

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

0.1 General

This purpose of this document is to provide guidance to laboratories worldwide to assess, in a standardized way, whether cotton, cotton fibre and/or cotton-derived materials are produced from, or contain materials from, genetically modified (GM) cotton plants. This document is intended for non-GM cotton and textiles production lines, but it can be applied to any production line that wants to check the presence of GM cotton.

0.2 Protocol

The GM screening protocol described in this document is based on Polymerase Chain Reaction (PCR)-based methods, as these methods are the minimal set of DNA-based methods to cover all known GM-cotton events. The protocol is written for and tested to work on all four of the major commercial cotton species: *Gossypium hirsutum*, *G. barbadense*, *G. arboreum G. herbaceum*.

Cotton (*Gossypium* spp.) has been cultivated for lint for over 8 000 years. There are over 50 species in the *Gossypium* genus (Wendel et al., 2009). The *Gossypium* genome is complex, containing 2,25 to 2,43 gigabase (Arumuganathan and Earle, 1991). While GM-cotton cultivation covers a large part of global cotton production today, there are countries where the cultivation of GM-cotton is not allowed by law as well as voluntary private and/or public standards that do not allow the intentional use of genetically modified organisms (GMOs) in the cotton and textile production process. This creates a need for an adequate and harmonized protocol on the screening of cotton and cotton-derived materials for the potential presence of GM-cotton related sequences.

This document describes a procedure to screen seed, leaf and (processed) fibre samples in the cotton production chain for the potential presence of GM-related DNA elements. The protocol describes three major steps:

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

b) a method to confirm that the isolated DNA consists of amplifiable cotton DNA, i.e. suitable for PCR, preferably a low copy nuclear target;

c) A screening method consisting of a minimum set of detection methods covering all the currently known GM cotton events, to be performed on the cotton DNA isolate.

If the results of the screening methods described in this protocol are 'not detected', the likelihood that the cotton sample is (at least partly) derived from GM cotton is minimal, based on the ability of the screening methods to detect elements and constructs of the GM cotton events. GM cotton levels below the detection limit of the method or unknown GM cotton events that do not contain any of the elements or the construct tested cannot be determined by this detection method. When one or more screening methods indicate that GM elements are present, the sample should be considered as derived from GM cotton.

Further investigation for the identification of GM-cotton events present in the sample is not part of this document as such, but some guidance is provided in Annex A as to how further identification of the related cotton events can be achieved.

0.3 Structure

The structure of this document is illustrated in Figure 1. Clause 4 describes the principle of the screenings protocol. Clause 5 describes sample preparation for different types of material. Clause 6 describes the DNA isolation method that allows for successful DNA isolation from the respective cotton-related products. Clause 7 describes the DNA quality control for the different cotton species. Clause 8 describes the screening of GM-related DNA sequences in a cotton sample. Clause 9 describes recommendations on the test report (outcome). Annex A gives an overview of known GMO cotton events. Annex B gives an overview of detection methods applied by RIKILT[1)]. Annex C provides

1) https://www.wur.nl/en/Research-Results/Research-Institutes/rikilt.htm

more information on the inhouse validation as carried out by RIKILT. Annex D provides a list of the contributos to the International Workshop.

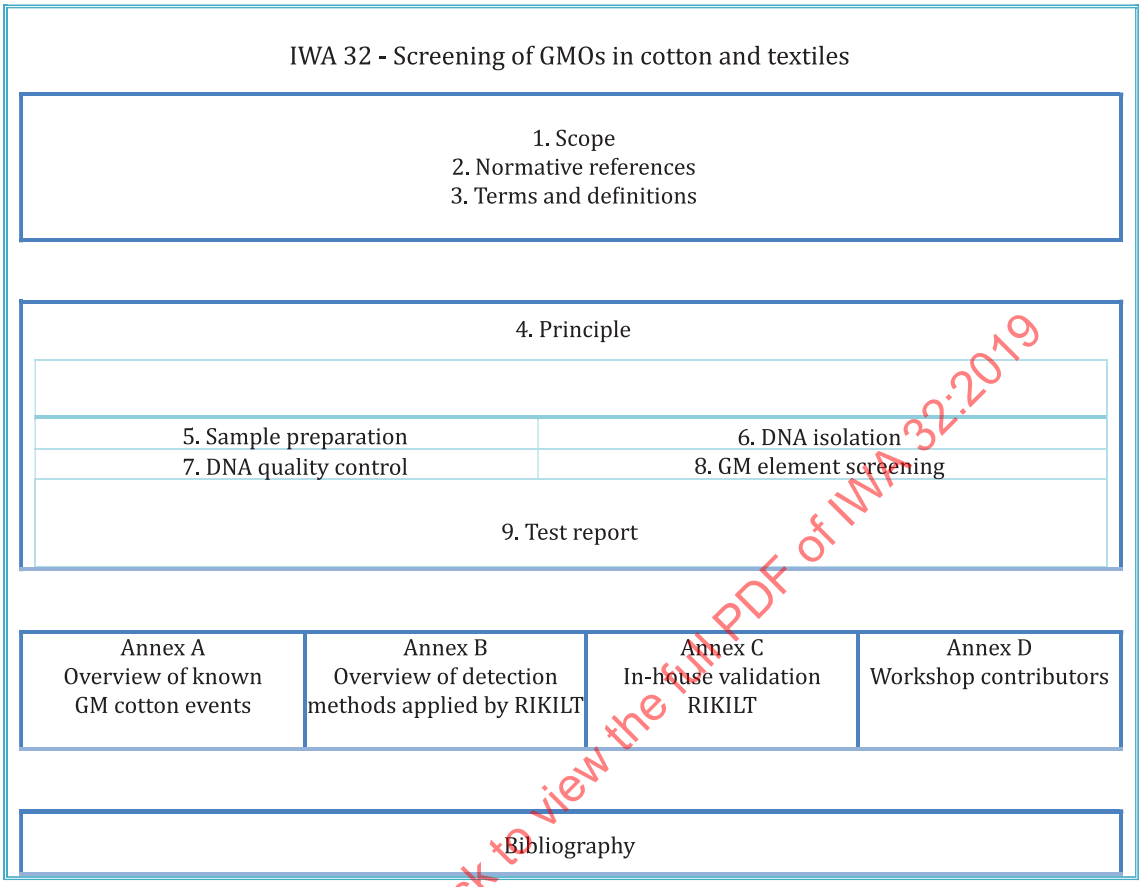

Figure 1 — Structure of this document

Screening of genetically modified organisms (GMOs) in cotton and textiles

WARNING — The method described in this document implies the use of reagents that pose a hazard to health. This document does not claim to address all associated safety problems. It is the responsibility of the user of this document to take appropriate measures for health and safety protection.

1 Scope

This document provides requirements and recommendations to laboratories that perform genetically modified organism (GMO) analyses in cottonseed, leaf, cotton fibre and cotton fibre-derived materials.

The following are within the scope of this document:

a) identifying the materials to be assessed, based on the probability of obtaining good quality, fit for purpose DNA from the materials in subsequent steps in the cotton cloth production process;

b) specifying a method for efficient DNA isolation from cotton and cotton-derived materials described under point a);

c) specifying the cotton-specific method(s) to be used as control for amplifiable DNA;

d) specifying the screening procedure that provides optimal chances to detect GMOs as a result of the performance of the lowest number of genetically modified (GM) element screening assays.

NOTE 1 The protocol allows for the screening of all currently known GM cotton events and is set up in a way that optimizes the probability of also detecting unknown GM cotton events that possibly contain similar DNA sequences. Further information is given in CEN/TS 16707.

Sampling is outside of the scope of this document.

NOTE 2 A recommended sampling method is given in ISO 6497. General guidance for the sampling of bulk materials or for cotton-based products is available in standards such as ASTM D1441-12 and CEN/TS 15568.

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 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*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

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

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

3.1
cottonseed
seed from cotton plants

3.2
cotton leaf
leaves from the cotton plant

3.3
seed cotton
raw cotton that contains both the seed and the fibre before it has been ginned

3.4
cotton lint
raw fibre that has gone through the ginning process

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

3.6
greige fabric
unprocessed textiles formed by weaving, knitting or crocheting the yarn and non-wovens

3.7
processed yarn
yarn that has undergone processing, to develop its full textile potential

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

4 Principle

This document describes a method for the screening of GMO in cotton and textiles. The screening is based on realtime PCR methods which depends on obtaining good quality amplifiable DNA. Good quality DNA samples (those fit for purpose) are defined as those where the amplification of an endogenous cotton gene (positive control) is observed. The amplification and detection of endogenous cotton is achieved through isolation methods that result in good quality DNA, applied to cotton and textiles, while the targeted amplification of six genetic elements can allow for the detection of GM-cotton in these samples.

NOTE Experimental results have shown that good quality DNA can be isolated from the production stages of cottonseed up to greige yarn and greige fabric, while it showed not to be possible to isolate amplifiable DNA in processed yarn and processed fabric. Processed yarn and processed fabric are therefore excluded from this protocol. See Clause C.3 for the assessment of isolation of good quality DNA at different cotton production stages by RIKILT.

5 Sample preparation

Homogenize the sample using suitable methods and avoiding excessive heating.

Sample preparation is dependent on sample type. Prepare samples by using either one of the following techniques: 'teasing', 'cutting', 'crushing' or 'shredding'.

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

The recommended sample preparation for different types of material is as follows.

— Cottonseed: Crush the seeds thoroughly with a suitable method. Use 100 mg in the DNA isolation procedure.

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

— Seed cotton: Seperate the seeds from the fibres, crush the seeds thoroughly with a suitable method. Use 100 mg in the DNA isolation procedure.

— Cotton lint: The fibre material can be teased thoroughly applying suitable method. Use 100 mg in the DNA isolation procedure.

— 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 isolation procedure.

— 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 isolation procedure.

6 DNA isolation

6.1 General

In order to obtain amplifiable DNA from cottonseed, cotton and textiles as per the protocol's scope, a DNA isolation method has been selected that allows for successful DNA isolation from the respective cotton-related products. This method allows for rapid purification of genomic DNA suitable for PCR with a limited number of protocol steps. The protocol works well for cotton-derived materials that can contain relatively high levels of PCR inhibitors.

NOTE 1 The DNA isolation procedure described in this document is the QIAamp® Fast DNA Stool Mini Kit. The rest of this protocol refers to the QIAamp® Fast DNA Stool Mini Kit[2)].

NOTE 2 As an alternative strategy to the DNA isolation method described below, the cotton-adjusted CTAB-protocol (e.g. CRLVL 14/05XP: JRC 2006) or any other suitable DNA isolation method can be applied, provided that this method has been proven by means of in-house validation against the QIAamp® Fast DNA Stool Mini Kit to perform equally well or better compared to the QIAamp® Fast DNA Stool Mini Kit. For seed, certified reference materials are used for validation.

6.2 Principle

The DNA isolation procedure is based on an inhibition buffer, a lysis buffer and a DNA-binding spin column. DNA binds specifically to the silica-gel membrane in the spin column, while contaminants pass through. No phenol-chloroform extraction is required. PCR inhibitors are separated from DNA by the inhibition buffer.

2) QIAamp® Fast DNA Stool Mini Kit is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

6.3 Chemicals, reagents and equipment

Use only reagents of recognized analytical grade. Appropriate facilities should be used in order to avoid contamination during the steps of preparation and measurement (e.g. uses of laminar flow benches or comparable clean facilities)[3].

Unless otherwise stated, only reagents that conform to the specifications of ISO 24276 were used.

6.3.1 Reagents

6.3.1.1 Inhibition buffer: contains lithium chloride (>=1 – 10 % w/w) and sodium dodecyl sulfate (>=1 - <10 % w/w) (e.g. Inhibitex Buffer Qiagen Cat No./ID: 51604), as provided by the manufacturer.

6.3.1.2 Lysis buffer: lysis buffer contains guanidine hydrochloride (>=30 - <50 % w/w) and maleic acid (>=0.1 - <1 % w/w), as provided by the manufacturer.

6.3.1.3 Wash Buffer 1; ethanol solution to denature proteins contains guanidine hydrochloride (>=50 - <70 % w/w)), as provided by the manufacturer.

6.3.1.4 Wash Buffer 2: Tris-based ethanol solution to remove salts, contains sodium azide), as provided by the manufacturer.

6.3.1.5 Ethanol 96 % to 100 %.

6.3.1.6 Elution Buffer: contains 10 mM Tris-HCl pH8.3, 0.1 mM EDTA, 0.04 % NaN_3 (sodium azide).

6.3.1.7 Proteinase K (>=1 - <10 % w/w).

6.3.1.8 Molecular biology grade water or water of equivalent purity.

6.3.1.9 DNA degrading solution (e.g. 1 % bleach) **household bleach** (hypochloric acid).

6.3.2 Apparatus and equipment

6.3.2.1 Silica-based mini spin columns, as provided by the manufacturer.

6.3.2.2 Disposable spatulas.

6.3.2.3 Sterile filter pipette tips protecting against aerosols.

6.3.2.4 Microcentrifuge tubes of 1,5 ml and 2,0 ml.

6.3.2.5 Disposable gloves (powder-free).

6.3.2.6 Analytical scale and top weigher.

6.3.2.7 Waterbath and/or thermoshaker (e.g. 24 ml × 2,0 ml).

6.3.2.8 Centrifuge for microcentrifuge tubes (at least 20 000 x g).

3) Reference to a given product is given for for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.3.2.9 Suitable prepared homogenization equipment.

6.3.2.10 Autoclave, 121 °C, 20 minutes.

6.3.2.11 Pipettes (1-10 µl, 2-20 µl, 20-200 µl, 200-1 000 µl).

6.3.2.12 Vortex.

6.3.2.13 Refrigerator.

6.3.2.14 Freezer.

6.3.2.15 Clean lab coat.

6.4 Procedure

6.4.1 General

The DNA extraction procedure comprises the following steps:

— lysis of, and separation of, impurities from samples in guanidine hydrochloride-containing buffer;

— purification of DNA on mini spin columns.

6.4.2 Protocol

All centrifugation steps should be carried out at room temperature (15 °C to 25 °C).

Perform the DNA isolation according to the protocol of the chosen isolation method or see the manufacturer's instructions.

— (1) Weigh 100 mg (+/– 10 mg) homogenized sample, as prepared in Clause 5, in a 2 ml microcentrifuge tube.

— (2) Add 1 ml inhibition buffer to each sample. Vortex continuously for 1 min or until the sample is thoroughly mixed.

— (3) Centrifuge sample at 20 000 x g for 1 min to pellet particles.

— (4) Pipette 25 µl proteinase K into a new 2 ml microcentrifuge tube.

— (5) Pipette 600 µl supernatant from step (3) into the 2 ml microcentrifuge tube containing proteinase K.

— (6) Add 600 µl lysis buffer and vortex for 15 s.

— (7) Incubate at 70 °C for 10 min.

— (8) Add 600 µl of ethanol (96 %) to the lysate, and mix by vortexing.

— (9) Carefully apply 600 µl lysate from step (8) to the silica-based spin column. Close the cap and centrifuge at 20 000 x g for 1 min. Place the silica-based spin column in a new 2 ml collection tube and discard the microcentrifuge tube containing the eluate.

— (10) Repeat step (9) until all of the lysate has been loaded on the column.

— (11) Carefully open the silica-based spin column and add 500 µl wash buffer 1. Centrifuge at 20 000 x g for 1 min. Place the silica-based spin column in a new 2 ml collection tube and discard the collection tube containing the eluate.

— (12) Carefully open the silica-based spin column and add 500 µl wash buffer 2. Centrifuge at 20 000 x g for 3 min. Discard the collection tube containing the eluate.

— (13) Place the silica-based spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at 20 000 x g for 3 min.

— (14) Transfer the silica-based spin column into a new, labelled 1,5 ml microcentrifuge tube and pipet 100 µl Elution Buffer directly onto the silica-based column. Incubate for 1 min at room temperature, then centrifuge at 20 000 x g for 1 min to elute the DNA.

6.5 Results

Resulting from the DNA extraction procedure, the extracted DNA is stored until use according to Clause 7 and Clause 8. DNA solutions may be stored at 4 °C for a maximum of 1 week or at –20 °C for long-term storage.

6.5.1 Analysis

The DNA quality is assessed according to Clause 7. The DNA isolated from the cotton samples is used both undiluted and 10 times diluted in a real time PCR and checked for amplifiable DNA for the endogenous cotton gene *SAH7* and inhibiting factors.

7 DNA quality control

7.1 General

The cotton-specific endogenous DNA marker for the Sinapis Arabidopsis Homolog 7 (*SAH7* -Baeumler et al., 2006) should be used as a positive control method for cotton. With the use of *SAH7*, one can detect all four commercial cotton species (*Gossypium hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum*). More information is provided in Annex C on the inhouse validation by RIKILT.

Amplification of the cotton-specific endogenous DNA marker for *SAH7* indicates the quality of the cotton DNA isolate. Once the *SAH7* is found to be adequately amplified, any GM-related sequence that is present above the detection limit will similarly be able to be amplified and be detected in the same sample.

7.2 Principle

A positive signal for the *SAH7* indicates there is amplifiable DNA present in the sample. To check inhibition, a 10 times dilution is also tested and is expected to give a positive signal that is theoretically 3,32 Cq values later in the PCR. If the Cq difference is less than 3,32 Cq this is an indication that there is inhibition in the sample DNA. To circumvent inhibition, a 10 times dilution is also tested to dilute the possible inhibition factors.

7.3 Chemicals, reagents and equipment, including reference materials

General requirements and recommendations related to the laboratory configuration and reagents and material used that are described in ISO 24276 apply. Reference to a given product and/or company, reagents and polymerases which lead to equal or better results may also be used.

7.3.1 Reagents

Use only reagents of recognized analytical grade and water conforming to grade 1 of ISO 3696.

7.3.1.1 Water.

7.3.1.2 Master mix for real-time PCR (e.g. DMML-D2-D600; Diagenode, Belgium).

7.3.1.3 Primers and Probes.

See Baeumler et al. (2006) or the JRC GMO Method Database for endogenous cotton method *SAH7* (QT-TAX-GH-021: http://gmo-crl.jrc.ec.europa.eu/gmomethods/), or see Annex B.

7.3.1.3.1 Sah7-uni-r1: GCATCTTTGAACCGCCTACTG.

7.3.1.3.2 Sah7-uni-f1: AGTTTGTAGGTTTTGATGTTACATTGAG.

7.3.1.3.3 Sah7-uni-s1: FAM-AAACATAAAATAATGGGAACAACCATGACATGT-TAMRA.

7.3.1.4 Sample DNA.

Prepared according to Clause 6.

7.3.1.5 Certified cotton reference material from IRMM or AOCS should be used as positive control in the PCR.

7.3.1.6 Filter tips.

7.3.1.7 Microcentrifuge tubes 1,5 ml and 2,0 ml.

7.3.1.8 Microcentrifuge tube racks.

7.3.1.9 PCR plates.

7.3.1.10 Aluminium foil.

7.3.1.11 Optical quality sealing tape.

7.3.1.12 Disposable gloves.

7.3.2 Apparatus and equipment

7.3.2.1 PCR thermocycler.

7.3.2.2 Freezer.

7.3.2.3 Refrigerator.

7.3.2.4 Plate centrifuge.

7.3.2.5 Centrifuge (at least 20 000 x g).

7.3.2.6 Vortex for microcentrifuge tubes and 96 well plates.

7.3.2.7 Pipettes (1-10 µl, 2-20 µl, 20-200 µl, 200-1 000 µl).

7.4 Procedure

7.4.1 General

The procedure to amplify the endogenous *SAH7* marker is a qualitative cotton endogenous screening method. For the in-house verification of this cotton endogenous screening method, the minimum performance requirements of ISO 24276 are applicable.

Perform the controls according to ISO 24276:2006, 5.2.

7.4.2 Safety precautions

No specific requirements. See ISO 24276.

7.4.3 Pre-treatment

DNA isolation according to 6.4.2.

7.4.4 Amount of sample

5 µl undiluted and 5 µl 10 times diluted DNA for each sample isolation are used in a reaction volume of 25 µl.

NOTE Two samples derive from one sample. The previous step results in two samples. Therefore, the total amount of samples is four.

7.4.5 Procedure

See the JRC GMO Method Database for the endogenous cotton method SAH7 (QT-TAX-GH-021: http://gmo-crl.jrc.ec.europa.eu/gmomethods/entry?db=gmometh&id=qt-tax-gh-021&q=sah7) as described by Mazzara et al. (2006). More information is provided in Annex B.

7.5 Results

7.5.1 Calculations

Calculate results according to ISO 21570:2005, A.1.8. No ambiguous results shall be expressed.

7.5.2 Interpretation and expression of results

According to ISO 24276:2006, Clause 6.

7.5.3 Results

The final result of the detection of *SAH7* is reported as "detected" or "not detected". When no endogenous *SAH7* is detected, there is either no DNA present, or the DNA is not of sufficient quality. Therefore, the subsequent screening should not be performed. Reporting should be carried out as specified in ISO 24276 and other applicable standards (ISO 17025).

8 GM element screening

8.1 Principle

In order to detect the potential presence of GM-related DNA sequences in a cotton sample, a minimum of two detection methods (targeting two of T-nos, P-35S, cry1Ab/Ac, pat otp/mepsps or P-FMV) shall be applied. If detected, no further testing is required. If not-detected, further testing is necessary up to all six elements.

Internationally recognized methods should be applied, if possible, e.g.:

— T-nos (e.g. QL-ELE-00-011 JRC; ISO 21569:2005/Amd 1:2013);

— P-35S (e.g. QT-ELE-00-004 JRC; ISO 21570:2005);

— cry1Ab/Ac (e.g. QL-ELE-00-016 JRC; ISO/TS 21569-6:2016);

— pat [e.g. QL-ELE-00-025 JRC; Inter-laboratory study in Food Control 73:452-461 (2016)];

— otp/mepsps (e.g. QT-CON-00-008 JRC; ISO 21570:2005);

— P-FMV (e.g. QL-ELE-00-015 JRC; ISO/TS 21569-5:2016).

NOTE 1 See the JRC GMO Method Database (http://gmo-crl.jrc.ec.europa.eu/gmomethods/).

Reagents that lead to equal or better results may also be used. Annex B provides an example of an in-house validated set of methods that could be applied as an alternative to the methods listed above.

NOTE 2 Annex A contains an overview of the tested elements and construct. The targets of these six screening methods were selected because they cover all known GM cottons events.

8.2 Chemicals, reagents and equipment, including reference materials

8.2.1 Reagents and materials

See 7.3.1.

The following is a list of primers and probes.

T-nos (ISO 21569:2005/Amd 1:2013)

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

P-35S (ISO 21570:2005)

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

cry1Ab/Ac (ISO/TS 21569-6:2016)

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

pat (Inter-laboratory study in Food Control 73:452-461 (2016))

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

otp/mepsps (ISO 21570:2005)

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

P-FMV (ISO/TS 21569-5:2016)

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

Certified cotton CRMs from IRMM or AOCS shall be used as positive control in the PCR 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

8.2.2 Apparatus and equipment

See 7.3.2.

8.3 Procedure

8.3.1 General

The in-house verification of the screening methods carried out according to ISO 24276 on detection of genetically modified organisms and derived products.

8.3.2 Safety precautions

See 7.4.2.

8.3.3 Pre-treatment

DNA used as described in Clause 6.

8.3.4 Amount of sample

See 7.4.4.

8.3.5 Procedure

See corresponding protocols mentioned in 6.1.

8.4 Interpretation and expression of results

According to ISO 24276:2006, Clause 6.

8.5 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 in the sample tested.

8.6 Reporting of data collection

The result should be recorded ensuring the reliability, reproducibility and integrity of the data according to ISO 24276:2006, Clause 7.

9 Test report

The test report shall contain at least the following information:

a) information necessary for identification of the submitted sample;

b) list of elements and/or constructs checked, detected and, if elucidated, the event(s);

c) results obtained of the screening, expressed as ‘the sample contains GM-elements’ or the ‘sample does not contain GM-elements’;

d) test method used, with reference to this document;

e) any particular points observed in the course of the test;

f) operating details not specified in this document, or regarded as optional, together with details of any incidents which might have affected the results.

Annex A
(informative)

Overview of known GM cotton events

A.1 General

This annex provides suggestions to confirm possible GM cotton events in a sample tested positive under Clause 6. Table A.1 provides an overview of known GM cotton events. The targets of the six screening methods that cover the larger part of the GM cottons events are shown in bold. Only elements for which a detection method is available are listed.

Based on elements that are detected and not detected, it should be possible to narrow down and identify the possible GM cotton source(s). Alternatively, the 'Analysis tool' (www.EUginius.eu) can be used. Here, the detected and not-detected targets aid to narrow-down the possible cotton event(s) present in the sample.

Once one or more possible candidate-events emerge, they can be confirmed by event-specific methods. For available event-specific detection methods, see the JRC GMO Method Database for fully validated methods (http://gmo-crl.jrc.ec.europa.eu/gmomethods/). For quantification, see ISO 21570.

Table A.1 — Overview of known GM cotton events

GMO	OECD Unique identifier	Event-detection method	Detected with screening	**T-nos**	**P-35S**	cry1Ac	**cry1Ab/Ac**	nptII	**P-FMV**	cry-1Ab	**pat**	T-35S	P-ubi1	**otp/mepsps**	cp4-ep-sps	cry-2Ab2	cry1C	T-E9	cry1F
TAM66274	TAM-66274-5	**no**	yes	**x**															
GHB811	BCS-GH811-4	**no**	yes											**x**					
DAS81910	DAS-81910-7	**no**	yes								**x**								
MON88701	MON-88701-3	yes	yes	**x**	**x**														
T303-3	BCS-GH003-6	**no**	yes	**x**	**x**					x									
T304-40	BCS-GH004-7	yes	yes	**x**	**x**					x									
GHB119	BCS-GH005-8	yes	yes	**x**	**x**							X							
COT67B	SYN-IR67B-1	**no**	yes	**x**						x									
GHB614	BCS-GH002-5	yes	yes											**x**					
MON88913	MON-88913-8	yes	yes		**x**				**x**						x			x	
COT102	SYN-IR102-7	**no**	yes	**x**															
3006-210-23	DAS-21023-5	yes	yes			x					**x**		x						
281-24-236	DAS-24236-5	yes	yes								**x**		x						x
LLCotton25	ACS-GH001-3	yes	yes	**x**	**x**														

NOTE For a full description of the events, see the following:

— EUginius (www.EUginius.eu)

— ISAAA (http://www.isaaa.org/gmapprovaldatabase/default.asp)

— USDA-APHIS (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status)

Table A.1 *(continued)*

GMO	OECD Unique identifier	Event-detection method	Detected with screening	**T-nos**	**P-35S**	cry1Ac	**cry1Ab/Ac**	nptII	**P-FMV**	cry-1Ab	**pat**	T-35S	P-ubi1	**otp/mepsps**	cp4-epsps	cry-2Ab2	cry1C	T-E9	cry1F
TAM66274	TAM-66274-5	**no**	yes	**x**															
MON15985: Bollgard II	MON-15985-7	yes	yes	**x**	**x**	x	**x**	x								x			
31807, 31808 etc.: BXN Plus Bollgard		**no**	yes		**x**	x	**x**	x											
1445 (MON1445), 1698 (MON89383)	MON-01445-2, MON-89383-1	yes	yes	**x**	**x**				**x**						x				
531, 757, 1076 (MON89924): Bollgard	MON-00531-6, MON-00757-7, MON-89924-2	yes	yes	**x**	**x**	x	**x**	x											
BXN/ BXN10211-BXN10224	BXN-10211-9, BXN-10215-4, BXN-10222-2, BXN-10224-4	**no**	yes		**x**			x											
GK12		**no**	yes	**x**	**x**	x	**x**	x											
GK19		**no**	yes	**x**	**x**	x	**x**	x											
SGK321		**no**	yes	**x**	**x**	x	**x**	x											
Event 1		**no**	yes	**x**	**x**	x	**x**	x											
GFM Cry1A		**no**	yes	**x**	**x**	x	**x**	x											

NOTE For a full description of the events, see the following:

— EUginius (www.EUginius.eu)

— ISAAA (http://www.isaaa.org/gmapprovaldatabase/default.asp)

— USDA-APHIS (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status)

Table A.1 *(continued)*

GMO	OECD Unique identifier	Event-detection method	Detected with screening	**T-nos**	**P-35S**	cry1Ac	**cry1Ab/Ac**	nptII	**P-FMV**	cry-1Ab	**pat**	T-35S	P-ubi1	**otp/mepsps**	cp4-ep-sps	cry-2Ab2	cry1C	T-E9	cry1F
TAM66274	TAM-66274-5	**no**	yes	**x**															
MLS 9124		**no**	yes	**x**	**x**			x				X					x		
BNLA 106 (BNBt LA-01)		**no**	yes			x	**x**												
NC 33B			yes			x	**x**												
MON88702		**no**	yes						**x**			X							
Ngwe Chi 6 Bt			yes			x	**x**												
Ngwe Chi 9 Bt		**no**	yes			x	**x**												
Sum of detectable elements per method				17	17	13	12	10	3	3	3	3	2	2	2	1	1	1	1

NOTE For a full description of the events, see the following:

— EUginius (www.EUginius.eu)

— ISAAA (http://www.isaaa.org/gmapprovaldatabase/default.asp)

— USDA-APHIS (https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/petitions/petition-status)

Annex B
(informative)

Overview of detection methods applied by RIKILT

B.1 General

The primers and probes described in this annex perform as well as those in the methods in the cited International Standards.

B.2 Primers and probes

SAH7 (QT-TAX-GH-021: Baeumler et al. 2006)

Sah7-uni-r1	GCATCTTTGAACCGCCTACTG
Sah7-uni-f1	AGTTTGTAGGTTTTGATGTTACATTGAG
Sah7-uni-s1	FAM-AAACATAAAATAATGGGAACAACCATGACATGT-TAMRA

T-nos (QT-ELE-NOS ter 2-5'/NOS ter 2-3': Kuriba et al., 2002)

NOS ter 2-5'	GTCTTGCGATGATTATCATATAATTTCTG
NOS ter 2-3'	CGCTATATTTTGTTTTCTATCGCGT
NOS-Taq	FAM-AGATGGGTTTTTATGATTAGAGTCCCGCAA-TAMRA

P-35S (QT-ELE-P35S 1-5'/P35S 1-3': Kuriba et al., 2002)

P35S-1-5'	ATTGATGTGATATCTCCACTGACGT
P35S-1-3'	CCTCTCCAAATGAAATGAACTTCCT
P35S-Taq	FAM-CCCACTATCCTTCGCAAGACCCTTCCT-TAMRA

cry1Ac (QL-ELE-Cry1Ac-F(/R)-n4/Cry1AcR-n2: Scholtens et al., 2013)

Cry1Ac-F(/R)-n4	TTCAGGACCAGGATTCAC
Cry1AcR-n2	GTGAATAGGGGTCACAGAAGCATA
Cry1AcP-n3	FAM-TCTGGTAGATGTGGATGGGAAGT-TAMRA

pat (QL-ELE-Patf-n2/Patr-n2: Xu et al., 2006)

Patf-n2	GACAGAGCCACAAACACCACAA
Patr-n2	CAATCGTAAGCGTTCCTAGCCT
Patp-n2	FAM-GCCACAACACCCTCAACCTCA-TAMRA

otp/mepsps (QT-CON-00-008 JRC; ISO 21570:2005)

GA21 3-5'	GAAGCCTCGGCAACGTCA

GA21 3-3’	ATCCGGTTGGAAAGCGACTT
GA21-2-taq	FAM-AAGGATCCGGTGCATGGCCG-BHQ1

P-FMV (QL-ELE-00-015 JRC; ISO/TS 21569-5:2016)

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

B.3 Mastermix

Component	Stock	Final	µl/reaction
Diagenode DMMLD2D600	2x	1x	12,5
Primer forward	10 µM	400 nM	1
Primer reverse	10 µM	400 nM	1
Probe	10 µM	200 nM	0,5
Water			5
DNA			5
Total reaction volume			25

B.4 PCR conditions

Decontamination UNG	2 min	50 °C	1 cycle
Denaturation	10 min	95 °C	1 cycle
Amplification step 1: denaturation	15 s	95 °C	45 cycles
Amplification step 2: Annealing and extension	1 min	60 °C	

Annex C
(informative)

In-house validation RIKILT

C.1 General

This annex provides data to substantiate:

— identification of best endogenous DNA-marker;

— assessment of isolation of good quality DNA at different cotton production stages;

— assessment of DNA isolation methods for different cotton production stages.

C.2 Identification of best endogenous DNA-marker

In order to identify the best endogenous DNA marker for the detection of the four commercial cotton species (*Gossypium hirsutum, G. barbadense, G. arboreum* and *G. herbaceum*), three different DNA markers were compared.

DNA was isolated with a CTAB method in combination with a Plant DNA-isolation kit from seed (4 species) and leaf (3 species) of the cotton species in duplicate. The seed samples were obtained from different plants in the U.S.A. and India. Each isolated DNA was used in a qPCR for the endogenous controls *acp1*, *AdhC* and *SAH7*. The qPCR was performed with 50 ng and 5 ng DNA. The results of the tests are presented in Tables C.1 and C.2.

All three endogenous controls were initially developed for the detection of *G. hirsutum*. They perform equally well on *G. hirsutum*. All three endogenous controls also perform equally well on *G. barbadense*. *AdhC* does not detect *G. arboreum* and *G. herbaceum*. *Acp1* shows later Cq values compared to *SAH7* for *G. arboreum* and *G. herbaceum*. Tables C.1 and C.2 provide the outcomes for qPCR for endogenous control on cottonseed and leaf.

Conclusion: *SAH7* is the best endogenous detection method for the detection of *Gossypium hirsutum, G. barbadense, G. arboreum* and *G. herbaceum*. Therefore, the cotton-specific endogenous DNA marker *SAH7* (Baeumler et al., 2006) should be used as a positive control method for cotton. With the use of *SAH7*, all four commercial cotton species (*Gossypium hirsutum, G. barbadense, G. arboreum* and *G. herbaceum*) can be detected.

Table C.1 — qPCR for endogenous control on cottonseed

Sample (ng DNA from seed)	Cq acp1	Cq AdhC	Cq SAH7
G. barbadense 1-1 50 ng	25,04	24,72	25,06
G. barbadense 1-1 5 ng	28,41	28,21	28,32
G. barbadense 1-2 50 ng	24,74	24,74	24,61
G. barbadense 1-2 5 ng	28,09	27,99	28,09
G. hirsutum 8-1 50 ng	24,92	24,76	24,9
G. hirsutum 8-1 5 ng	28,01	28,21	28,05
G. hirsutum 8-2 50 ng	24,80	25,04	25,03
G. hirsutum 8-2 5 ng	28,03	28,62	28,50
G. arboreum 30-1 50 ng	32,07	N/A	25,68

Table C.1 *(continued)*

Sample (ng DNA from seed)	Cq acp1	Cq AdhC	Cq SAH7
G. arboreum 30-1 5 ng	34,95	N/A	28,92
G. arboreum 30-2 50 ng	32,23	N/A	25,74
G. arboreum 30-2 5 ng	35,59	N/A	29,16
G. herbaceum 35-1 50 ng	32,25	N/A	25,87
G. herbaceum 35-1 5 ng	34,9	N/A	29,14
G. herbaceum 35-2 50 ng	31,97	43,13	25,59
G. herbaceum 35-2 5 ng	35,57	N/A	28,92

Table C.2 — qPCR for endogenous control on leaf

Sample (ng DNA from leaf)	Cq acp1	Cq AdhC	Cq SAH7
G. barbadense 65-3 50 ng	N/A	N/A	N/A
G. barbadense 65-3 5 ng	30,00	30,24	30,03
G. barbadense 65-4 50 ng	N/A	N/A	N/A
G. barbadense 65-4 5 ng	30,66	30,48	30,74
G. hirsutum 69-3 50 ng	32,06	31,71	34,11
G. hirsutum 69-3 5 ng	35,52	35,22	36,30
G. hirsutum 69-4 50 ng	29,47	29,12	30,07
G. hirsutum 69-4 5 ng	32,57	32,39	33,55
G. arboreum 67-3 50 ng	N/A	N/A	36,38
G. arboreum 67-3 5 ng	38,97	N/A	32,60
G. arboreum 67-4 50 ng	N/A	N/A	37,50
G. arboreum 67-4 5 ng	N/A	N/A	34,77
AOCS 0804D cotton	24,33	24,16	24,56
water	N/A	N/A	N/A

C.3 Assessment of isolation of good quality DNA at different cotton production stages

DNA was isolated from cottonseed, cotton leaf, seed cotton, cotton lint, greige yarn and greige fabric, and processed yarn and processed fabric in duplicate using the QIAamp Fast DNA Stool Mini Kit (Qiagen). The samples were obtained from the U.S.A., Turkey and India. To confirm the presence and quality of the DNA, each isolated DNA was used in a qPCR for the endogenous control *SAH7*. The qPCR was performed with undiluted (1x) and 10x diluted (10x) DNA.

The results of the tests are presented in the Tables below. The results show that the QIAamp Fast DNA Stool Mini Kit (Qiagen) can be used to isolate good quality DNA from cottonseed, cotton leaf, cotton lint and greige yarn and greige fabric, while there is no amplification with DNA (if any) isolated from processed yarn and processed fabric.

Conclusion: The QIAamp Fast DNA Stool Mini Kit (Qiagen) is able to isolate good quality DNA (amplifiable DNA using the method for endogenous *SAH7*) from samples of cottonseed, cotton leaf, cotton lint and greige yarn and greige fabric. Processed yarn and processed fabric did not yield DNA that was successfully amplified with the endogenous target *SAH7*. Therefore, it would seem highly unlikely that from the matrices processed yarn and processed fabric sufficient DNA of good quality can be isolated that can be applied in the GMO screening.

Tables C.3 to C.7 show qPCR results of cotton samples of cottonseed, cotton leaf, cotton lint, greige yarn and greige fabric and processed yarn and/or processed fabric with *SAH7*.

Table C.3 — qPCR results of cotton samples of cottonseed with *SAH7*

Production stage: Cottonseed and seed cotton			
Sample	**Cq 1x**	**Cq 10x**	**Result**
RIK1-1	24,11	28,25	Detected
RIK1-2	23,53	28,27	
RIK8-1	24,86	28,77	Detected
RIK8-2	24,68	28,42	
RIK13-1	N/A	35,48	Detected
RIK13-2	N/A	35,83	
RIK25-1	24,43	27,57	Detected
RIK25-2	24,72	28,14	
RIK30-1	25,54	29,09	Detected
RIK30-2	25,39	29,14	
RIK35-1	25,13	28,91	Detected
RIK35-2	24,91	27,80	
RIK50-1	25,07	28,27	Detected
RIK50-2	25,27	29,07	
RIK75-1	34,63	39,38	Detected
RIK75-2	37,12	N/A	
T-1	22,19	26,12	Detected
T-2	22,63	26,14	
N/A is 'not detected'.			

Table C.4 — qPCR results of cotton samples of cottonleaf with *SAH7*

Production stage	**Sample**	**Cq 1x**	**Cq 10x**	**Result**
Cotton leaf	RIK65-1	27,18	30,79	detected
	RIK65-2	28,13	30,40	
	RIK66-1	N/A	29,57	detected
	RIK66-2	27,54	30,21	
	RIK67-1	N/A	30,46	detected
	RIK67-2	N/A	31,49	
	RIK68-1	N/A	30,72	detected
	RIK68-2	34,54	30,71	
	RIK69-1	26,06	29,29	detected
	RIK69-2	32,20	29,03	
	RIK70-1	27,35	29,09	detected
	RIK70-2	31,02	29,04	
	RIK71-1	29,80	30,05	detected
	RIK71-2	27,00	29,95	
	RIK72-1	27,55	29,34	detected
	RIK72-2	42,49	28,26	
	RIK73-1	31,05	29,01	detected
	RIK73-2	32,51	28,82	
	RIK74-1	N/A	27,72	detected
	RIK74-2	N/A	27,87	
N/A is 'not detected'.				

Table C.5 — qPCR results of cotton samples of cotton lint with *SAH7*

Production stage	Sample	Cq 1x	Cq 10x	Result
Cotton lint	RIK89-1	32,45	35,18	detected
	RIK89-2	32,74	36,04	
	RIK90-1	35,02	37,94	detected
	RIK90-2	35,16	37,82	
	RIK91-1	36,74	N/A	detected
	RIK91-2	36,25	40,48	
	RIK92-1	33,46	36,82	detected
	RIK92-2	35,11	38,11	
	RIK93-1	33,50	37,02	detected
	RIK93-2	33,15	36,36	
	RIK94-1	37,48	39,40	detected
	RIK94-2	37,01	N/A	
	RIK95-1	N/A	37,61	detected
	RIK95-2	33,15	34,71	
	RIK96-1	31,13	34,44	detected
	RIK96-2	36,51	36,14	
	RIK97-1	34,73	37,45	detected
	RIK97-2	29,95	32,95	
	RIK98-1	33,53	37,44	detected
	RIK98-2	34,35	37,16	
N/A is 'not detected'.				

Table C.6 — qPCR results of cotton samples of greige yarn and greige fabric with *SAH7*

Production stage	Sample	Cq 1x	Cq 10x	Result
Greige yarn and/or greige fabric	RIK15-1	N/A	38,64	detected
	RIK15-2	N/A	41,09	
	RIK16-1	36,11	36,01	detected
	RIK16-2	35,06	36,42	
	RIK17-1	33,37	37,60	detected
	RIK17-2	34,28	38,36	
	RIK103-1	36,90	N/A	detected
	RIK103-2	35,48	N/A	
	RIK104-1	35,87	39,65	detected
	RIK104-2	36,43	N/A	
	RIK105-1	35,10	37,98	detected
	RIK105-2	35,84	37,74	
	RIK106-1	36,64	N/A	detected
	RIK106-2	37,90	40,08	
	RIK107-1	37,59	N/A	detected
	RIK107-2	36,14	N/A	
	RIK108-1	37,00	39,60	detected
	RIK108-2	35,17	38,47	
	RIK109-1	35,71	40,19	detected
	RIK109-2	35,25	39,91	
	RIK110-1	36,25	37,68	detected
	RIK110-2	37,18	N/A	
	RIK111-1	34,73	38,79	detected
	RIK111-2	36,13	37,60	
	RIK112-1	N/A	39,71	detected
	RIK112-2	37,56	N/A	
	RIK113-1	37,18	N/A	detected
	RIK113-2	36,38	39,78	
	RIK114-1	37,07	N/A	detected
	RIK114-2	39,90	N/A	
N/A is 'not detected'.				

Table C.7 — qPCR results of cotton samples of processed yarn and processed fabric with *SAH7*

Production stage	Sample	Cq 1x	Cq 10x	Result
Processed yarn and/or processed fabric	RIK18-1	N/A	N/A	not detected
	RIK18-2	N/A	N/A	
	RIK19-1	N/A	N/A	not detected
	RIK19-2	N/A	N/A	
	RIK20-1	N/A	N/A	not detected
	RIK20-2	N/A	N/A	
	RIK21-1	N/A	N/A	not detected
	RIK21-2	N/A	N/A	
	RIK22-1	N/A	N/A	not detected
	RIK22-2	N/A	N/A	
	RIK23-1	N/A	N/A	not detected
	RIK23-2	N/A	N/A	
	RIK24-1	N/A	N/A	not detected
	RIK24-2	N/A	N/A	
	RIK115-1	N/A	N/A	not detected
	RIK115-2	N/A	N/A	
	RIK116-1	N/A	N/A	not detected
	RIK116-2	N/A	N/A	
	RIK117-1	N/A	N/A	not detected
	RIK117-2	N/A	N/A	
	RIK118-1	N/A	N/A	not detected
	RIK118-2	N/A	N/A	
	RIK119-1	N/A	N/A	not detected
	RIK119-2	N/A	N/A	
	RIK120-1	N/A	N/A	not detected
	RIK120-2	N/A	N/A	
	RIK121-1	N/A	N/A	not detected
	RIK121-2	N/A	N/A	
Positive control (cotton)	AOCS 0306A	29,17		detected
	AOCS 0306A	29,00		
Negative control	Water	N/A		not detected
	Water	N/A		
N/A is 'not detected'.				

C.4 Assessment of DNA isolation methods for different cotton production stages

Different DNA isolation methods were used to study the applicability to isolate good quality DNA for the different cotton materials from cottonseed to textiles. The samples were obtained from the U.S.A., Turkey and India. To confirm the presence and quality of the DNA, each isolated DNA was used in a qPCR for the endogenous control *SAH7*. The qPCR was performed in duplicate with undiluted DNA.

DNA isolation methods assessed:

— a commercial kit based on magnetic beads (see Table C.8);

— CTAB method (see Table C.9);

— CTAB method in combination with a Plant DNA-isolation kit (see Tables C.1 and C.2);

— CTAB-cotton CRLVL-14/05XP (see Table C.10);

— QIAamp Fast DNA Stool Mini Kit (Qiagen) (see Tables C.3 and C.11).

The CTAB method in combination with a Plant DNA-isolation kit was only performed on cottonseed and leaf (see Table C.1) With the CTAB-cotton DNA isolation method CRLVL-14/05XP, DNA isolation from reference material derived from seed was confirmed by amplification with the endogenous gene *acp1* (see Table C.10).

For the tested methods and production stages, the results show that only the QIAamp Fast DNA Stool Mini Kit (Qiagen) is capable to isolate DNA from cottonseed, cotton lint and greige yarn and greige fabric. From the production stage 'Processed yarn and processed fabric', DNA isolation was not possible. Other DNA isolation methods yielded little or no DNA, and subsequent qPCR with the endogenous control was not successful.

Conclusion QIAamp Fast DNA Stool Mini Kit (Qiagen) is the only DNA isolation method of the set that was tested that is capable to isolate DNA from cottonseed, cotton lint and greige yarn and greige fabric.

Table C.8 shows DNA isolated with a commercial kit based on magnetic beads. Each isolated DNA was used in a qPCR for the endogenous control *SAH7*.

Table C.8 — DNA isolated with a commercial kit based on magnetic beads

Production stage	Sample	Cq1	Cq2	Result
Cottonseed	RIK79-1	30,38	28,76	detected
	RIK79-1	30,66	29,14	
	RIK14-1	29,54	30,97	detected
	RIK14-1	29,44	30,74	
Cotton lint	RIK96-1	N/A	37,29	not detected
	RIK96-1	N/A	N/A	
	RIK94-1	N/A	40,70	not detected
	RIK94-1	N/A	N/A	
Greige yarn and/or greige fabric	RIK104-1	N/A	N/A	not detected
	RIK104-1	N/A	N/A	
	RIK103-1	N/A	N/A	not detected
	RIK103-1	N/A	38,80	
	RIK109-1	N/A	40,99	not detected
	RIK109-1	N/A	42,26	
	RIK108-1	N/A	N/A	not detected
	RIK108-1	41,08	43,19	
	RIK17-1	N/A	N/A	not detected
	RIK17-1	N/A	40,31	
Processed yarn and/or processed fabric	RIK21A-1	42,26	N/A	not detected
	RIK21A-1	39,93	N/A	
	RIK118-1	N/A	N/A	not detected
	RIK118-1	N/A	N/A	
	RIK18-1	N/A	N/A	not detected
	RIK18-1	N/A	N/A	
Positive control (cotton)	AOCS 0804D	24,23		detected
Negative control	water	N/A		not detected