TECHNICAL SPECIFICATION

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2021-07

Molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products —

Part 2: Construct-specific real-time PCR method for detection of event FP967 in linseed and linseed products

Analyse moléculaire de biomarqueurs —

Partie 2: Méthode PCR en temps réel construit-spécifique pour la détection d'un événement FP 967 dans les graines de lin et les produits à base de graines de lin

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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).

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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 second edition cancels and replaces the first edition (ISO/TS 21569-2:2012), which has been technically revised.

The main changes compared to the previous edition are as follows:

— the single target copy integration into the genome has been updated;

— an explanation of *dfr* A/Spectinomycin resistance cassette juxtaposition has been added;

— minor typographical improvements have been made.

A list of all parts in the ISO 21569 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.

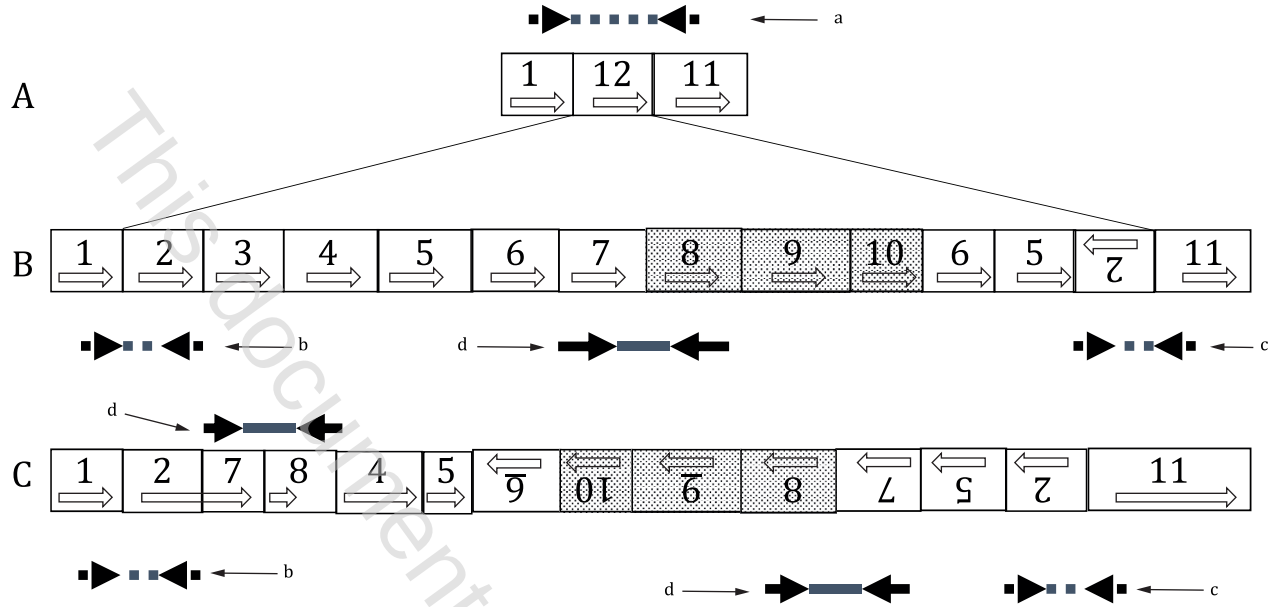
Introduction

Flaxseed (*Linum usitatissimum* L.) FP967 (CDC Triffid Flax) is the only GMO linseed flax listed in the International Service for the Acquisition of Agro-biotech Applications (ISAAA)[1]. FP967 was regenerated from a single Norlin Flax hypocotyl (regenerant number 12115) transformed with an agrobacterium/Ti plasmid system containing the NPT-11 gene encoding kanamycin resistance and a modified *Arabidopsis* acetolate synthase gene with reduced enzyme affinity for chlorosulfuron[2][3][4][5][6][7]. The *in planta* T-DNA construct includes a repeat and re-arrangement of the T-DNA forming an inverted-repeat structure of the right border, as confirmed by next generation sequencing and PCR cloning. The FP967 GM construct is stable within the recombinant plant genome and demonstrates functional resistance to the sufonylurea herbicides chlorsufuron, metsulfuron, and triasulfuron[8].

Published event-specific assays for FP967 have been described[8][9]. One generates two products from the recombinant and one product from the non-recombinant[8]. The other generates a single product but requires an internal control PCR test for linseed-specific (*Linum usitatissimum*) stearoyl-acyl carrier protein desaturase 2 gene (SAD)[9]. Event-specific assays are most useful for proprietary and breeding uses when exact identity or copy number of a transgene is required.

The FP967 PCR assay described in this document is construct-specific[10]. It generates a 105 bp product spanning the junction between the T-nos and dfrA1 elements of the transgene construct. Construct-specific assays are usually used as generic GM screening tools able to cross-detect different GM events carrying the same gene fusion. Because FP967 is the only flaxseed construct to carry a spectinomycin selectable marker and the only listed GM flax event, the described assay is conclusive for genetically modified identification among approved GMOs. It has been widely accepted and deployed and has been effective identifying and eliminating unwanted adventitious presence from unrelated breeding lines and commercial stocks. It is also more sensitive than reported for the available event-specific test because there are two copies of the target in the recombinant (see Figure 1). Adding event-specific testing options to the testing portfolio would require considerable effort (especially experimental comparison and validation to recommend one of the available event-specific assays) with no ultimate benefit to the final purpose.

Next generation sequencing and PCR cloning of the T-DNA of FP967 revealed a repeat and rearrangement of an internal T-DNA fragment forming an inverted-repeat structure of the right border of the T-DNA in the flax genome. Although, there is only a single copy of the FP967 T-DNA, the order and arrangement of the NOS gene, the *Arabidopsis* acetolactate synthase (NP_001189794.1), pBR322 (J01749.1), neomycin phosphotransferase II (AY909580.1), and the *Escherichia coli* spectinomycin resistance/dihydrofolate reductase (SpecR/DHFR) region are no longer consistent with the original plasmids used to transform FP967[8]. This rearrangement was not anticipated in the development of the construct specific assay. Figure 1 provides a graphic depicting the genomic position of the insert, the anticipated recombinant structure and the deduced recombinant structure based on DNA sequencing. It also shows the location of the event and construct-specific PCR assays on each of these.



Key

A	insertion site of flax genome	5	pBR322	12	FP 967 insertion site
B	anticipated recombinant T-DNA	6	left inside homology	a	Non-recombinant PCR.
C	deduced recombinant T-DNA	7	nopaline synthase	b	Left side event specific PCR.
1	flaxseed genomic region 1	8	spectinomycin resistance gene	c	Right side event specific PCR.
2	right border	9	chimeric neomycin phosphotransferase	d	Construct specific PCR.
3	undetermined sequence	10	*Arabidopsis* acetolactate synthase		
4	left border	11	flaxseed genomic region 2		

NOTE As a result of the rearrangement of the T-DNA in the recombinant two copies of the target amplicon were formed. This increases the sensitivity of the construct specific assay.

Figure 1 — FP967 insertion into the flax genome

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1 Scope

This document specifies a procedure for the detection of a DNA sequence present in a genetically modified linseed (*Linum usitatissimum*) line (event FP967, also named as "CDC Triffid"). For this purpose, extracted DNA is used in a real-time PCR and the genetic modification (GM) is specifically detected by amplification of a 105 bp DNA sequence representing the transition between the nopaline synthase gene terminator (*Tnos*) from *Agrobacterium tumefaciens* and the dihydrofolate reductase gene (*dfrA1*) from a Class 1 integron of *Escherichia coli*.

The method described is applicable for the analysis of DNA extracted from foodstuffs. It can also be suitable for the analysis of DNA extracted from other products such as feedstuffs and seeds. The application of this method requires the extraction of an adequate amount of amplifiable DNA from the relevant matrix for the purpose of analysis.

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 — Terms and definitions*

ISO 21569, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative 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, *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 terms and definitions given in ISO 16577 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/