
**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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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

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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 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.

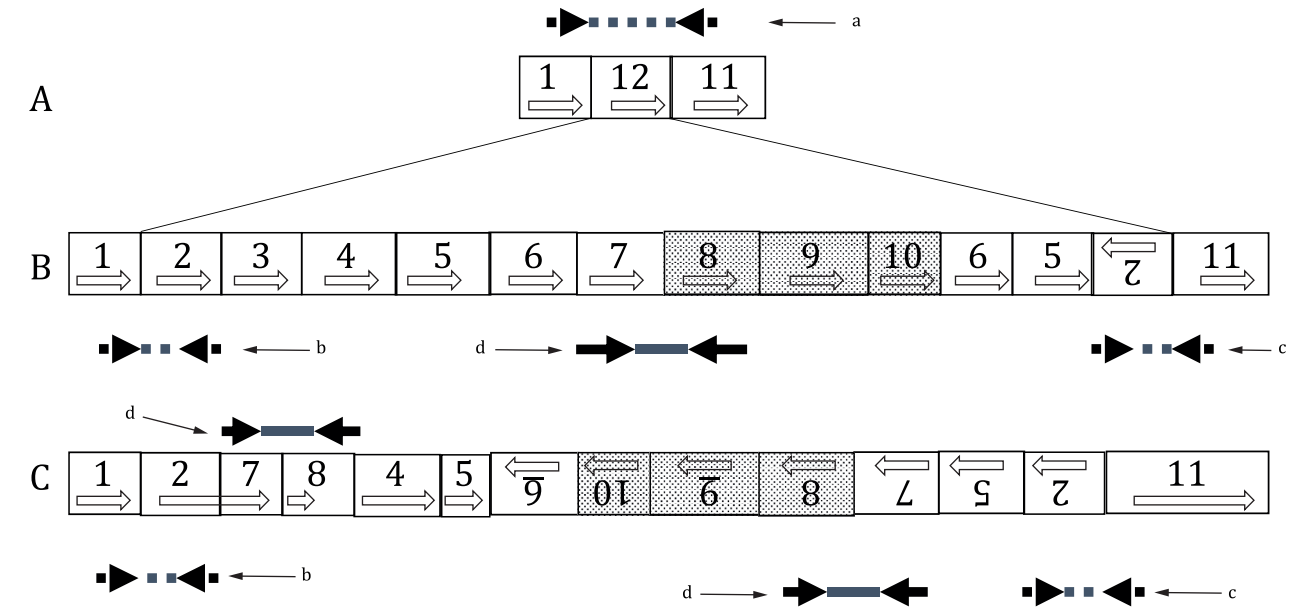
Introduction

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* acetolactate 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 sulfonyleurea herbicides chlorsulfuron, 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	<i>Arabidopsis</i> 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