PD ISO/TS 21569-2:2021



BSI Standards Publication

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

bsi.

National foreword

This Published Document is the UK implementation of ISO/TS 21569-2:2021. It supersedes PD ISO/TS 21569-2:2012, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its committee manager.

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient's own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

This publication is not to be regarded as a British Standard.

© The British Standards Institution 2021
Published by BSI Standards Limited 2021

ISBN 978 0 539 15975 2

ICS 67.050

Compliance with a Published Document cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 August 2021.

Amendments/corrigenda issued since publication

Date	Text affected

PD ISO/TS 21569-2:2021

TECHNICAL SPECIFICATION

ISO/TS 21569-2

Second edition
2021-07-05

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

Reference number
ISO/TS 21569-2:2021(E)

© ISO 2021



COPYRIGHT PROTECTED DOCUMENT

© ISO 2021, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

© ISO 2021 – All rights reserved

Contents

Page

Foreword ..... **iv**
Introduction ..... **v**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Principle **2**
5 Reagents and materials **2**
5.1 PCR reagents 2
5.1.4 Standard DNA for calibration 2
6 Apparatus **3**
6.1 General 3
6.2 PCR device 3
7 Sampling **3**
8 Procedure **3**
8.1 Test sample preparation 3
8.2 Preparation of the DNA extracts 3
8.3 DNA extraction 3
8.4 PCR setup 3
8.5 Temperature–time programme 4
9 Accept/reject criteria **4**
9.1 General 4
9.2 Identification 5
10 Validation status and performance criteria **5**
10.1 Robustness of the method 5
10.2 Intra-laboratory trial 5
10.3 Collaborative trial 5
10.4 Sensitivity 7
10.5 Specificity 7
11 Test report **8**
Bibliography **9**

© ISO 2021 – All rights reserved

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.

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.

© ISO 2021 – All rights reserved

Introduction

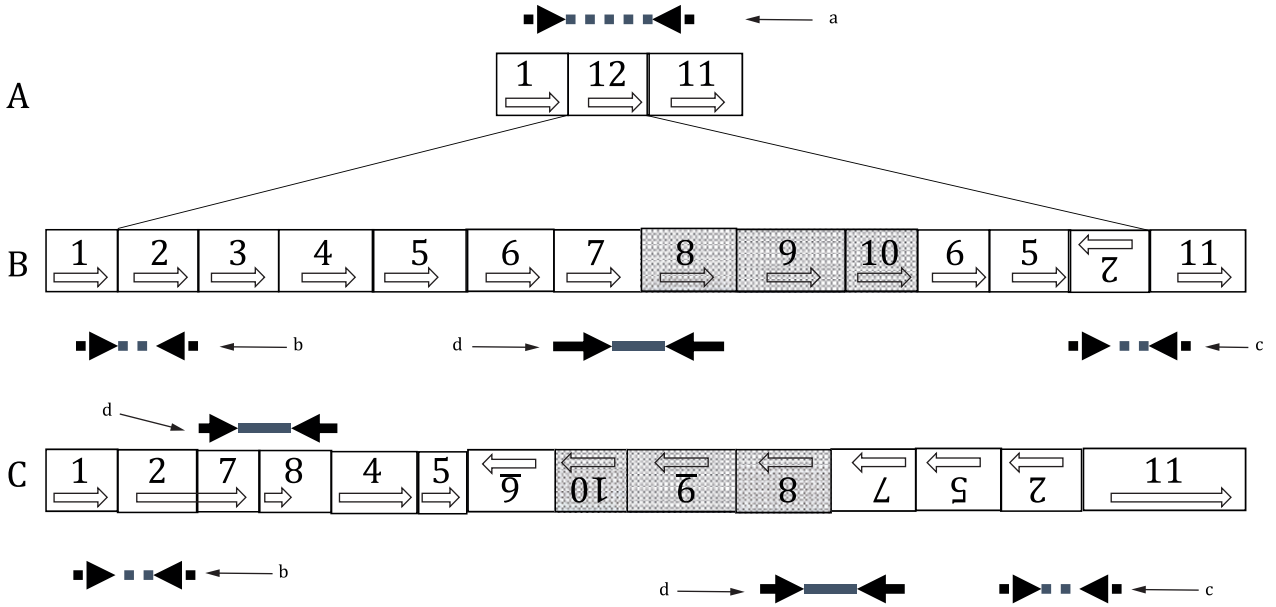
Flaxseed (*Linum usitatissimum* L.) FP967 (CDC Triffid Flax) is the only GMO linseed flax listed in the International Service for the Acquisition of Agro-biotech Applications (ISAAA)[1]. FP967 was regenerated from a single Norlin Flax hypocotyl (regenerant number 12115) transformed with an agrobacterium/Ti 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.

© ISO 2021 – All rights reserved



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

© ISO 2021 – All rights reserved

TECHNICAL SPECIFICATION **ISO/TS 21569-2:2021**

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

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/

© ISO 2021 – All rights reserved