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Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products —

Part 5:
Real-time PCR based screening method for the detection of the FMV promoter (P-FMV) DNA sequence

Méthodes horizontales d'analyse moléculaire de biomarqueurs — Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés —

Partie 5: Méthode de dépistage PCR en temps réel pour la détection de la séquence ADN du promoteur FMV (P-FMV)

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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 on 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 the following URL: www.iso.org/iso/foreword.html.

The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

A list of all the parts in the ISO/TS 21569 series can be found on the ISO website.

Horizontal methods for molecular biomarker analysis — Methods of analysis for the detection of genetically modified organisms and derived products —

Part 5: Real-time PCR based screening method for the detection of the FMV promoter (P-FMV) DNA sequence

1 Scope

This document specifies a procedure for the detection of a DNA sequence used in genetically modified (GM) plants by means of a real-time PCR (polymerase chain reaction). The method detects a 78 base pairs long segment of the *Figwort mosaic virus* 34S promoter DNA sequence. This segment in some GM plants is indicated as FMV promoter (P-FMV) and in other GM plants as FMV enhancer (E-FMV).

The method was developed and validated for the analysis of DNA extracted from foodstuffs. It may be suitable also for analysis of other products such as feedstuffs and seeds. The procedure requires the extraction of an adequate quantity and quality of amplifiable DNA from the test sample.

The DNA sequence amplified by the P-FMV element-specific method can be detected in samples which contain DNA of the naturally occurring *Figwort mosaic virus*. For this reason, it is necessary to confirm a positive screening result. Further analyses are required using construct-specific or event specific methods.

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 21569, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods*

ISO 21570, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods*

ISO 21571:2005, *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:

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

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

4 Principle

DNA is extracted from the test portion applying a suitable method (see ISO 21571). The DNA analysis consists of two parts:

a) verification of the amount, quality and amplifiability of the extracted DNA, e.g. by a taxon-specific PCR assay (according to ISO 21569 and ISO 21570), see also Reference [1];

b) detection of the P-FMV DNA sequence in a real-time PCR, see Reference [2].

5 Reagents and materials

5.1 General

For the purpose of this document, only chemicals and water of recognized analytical grade, appropriate for molecular biology shall be used. Unless stated otherwise, solutions should be prepared by dissolving the corresponding reagents in water and be autoclaved. For all operations for which gloves are used it should be ensured that these are powder-free. The use of aerosol protected pipette tips (protection against cross contamination) is recommended.

5.2 PCR reagents

5.2.1 Thermostable DNA polymerase (for hot-start PCR).

5.2.2 PCR buffer solution (containing magnesium chloride and deoxyribonucleoside triphosphates dNTPs.

Ready-to-use reagent mixtures or mixtures of individual components can be used. Reagents and polymerases which lead to equal or better results may also be used.

5.2.3 Oligonucleotides (see Table 1)[1].

Table 1 — Oligonucleotides

Name	DNA sequence of the oligonucleotide	Final concentration in PCR
P-FMV as the target sequence (GeneBank accession number X06166[2],[3]):		
pFMV-F	5'-CAA AAT AAC GTG GAA AAG AGC T–3'	340 nmol/l
pFMV-R	5'-TCT TTT GTG GTC GTC ACT GC–3'	340 nmol/l
Probe pFMV	5'-(FAM)-CTG ACA GCC CAC TCA CTA ATG C-(BHQ1)-3'[a]	120 nmol/l

[a] FAM: 6-Carboxyfluorescein, BHQ-1: Black Hole Quencher® 1 (non-fluorescent chromophore). This information is given for convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products from other manufacturers may be used if they can be shown to give equivalent or better results.

6 Apparatus

Requirements concerning apparatus and materials shall be according to ISO 21569. In addition to the usual laboratory equipment, the following equipment is required.

6.1 Real-time PCR device, suitable for the excitation of fluorescent molecules and the detection of fluorescence signals generated during PCR.

1) In the interlaboratory trial performed for P-FMV, participants were provided with dried aliquots (per 50 reactions) of primer/probe -mixes (to be stored in dark until the start of the interlaboratory trial). Per aliquot 375 µl PCR grade water was added and allowed to settle.