SLOVENSKI STANDARD
SIST-TS CEN/TS 16707:2014

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Živila - Analitske metode za odkrivanje gensko spremenjenih organizmov in njihovih produktov - Strategije presejalne analize s polimerazno verižno reakcijo (PCR)

Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Polymerase chain reaction (PCR) based screening strategies

Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Strategies für das Screening mit Polymerase-Kettenreaktion (PCR)

Produits alimentaires - Méthodes d'analyse pour la détection des organismes génétiquement modifiés et des produits dérivés - Stratégies de criblage basées sur l'utilisation de la réaction de polymérisation en chaîne (PCR)

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Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Polymerase chain reaction (PCR) based screening strategies

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (CEN/TS 16707:2014) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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Introduction

Largely, detection of materials derived from genetically modified organisms (GMOs) in a given sample employs polymerase chain reaction (PCR) analysis, specifically real-time PCR.

A general strategy for GMO detection and identification by means of PCR analysis and a stepwise approach is described.

In initial screening analysis, DNA sequences of genetic elements common to many GMOs are targeted. According to its purpose, screening is a test to rapidly and reliably sort samples into groups. Once the samples are grouped, screening facilitates and potentially reduces subsequent analytical work and results interpretation. The screening strategy should be adjusted to the scope (food, feed or seed, crop-specific etc.) of the test(s).

This document takes the general principle of GMO detection strategies as a basis and describes the underlying analytical steps for complex screening (known as the matrix-approach [2]).

The document is written primarily for screening strategies applying real-time PCR methods. Other PCR methodologies may be applicable in the same way.

The terms “screening method” and “screening strategies” are not interchangeable and have different meanings in this document.

1 Scope

This Technical Specification describes screening strategies for the detection of genetically modified (GM) DNA in food products by means of PCR methods. The strategies have been established for food matrices, but it can also be applied to other matrices (e.g. feed, seed and samples from field grown plants).

Detection of GM DNA is based on PCR methods targeting segments of transgenic DNA sequences (genetic elements, genetic constructs or insertion sites of transgenes). Various combinations of these PCR methods are involved in screening strategies. The methods are applied simultaneously or hierarchically. The general strategy is based on the matrix approach. Examples for the implementation and application of this approach are described.

In order to ensure reliable analytical results, the document also provides guidelines for the validation of the performance of qualitative PCR methods applied in screening approaches.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 21569, *Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods (ISO 21569)*

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

EN ISO 21571, *Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction (ISO 21571)*

EN ISO 24276, *Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions (ISO 24276)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 24276 and the following apply:

3.1
GMO method matrix
relational presentation (e.g. a table) of symbols or numbers

Note 1 to entry: One dimension (e.g. columns) corresponds to genetic elements and genetic constructs detected by a defined PCR method and the other dimension (e.g. rows) corresponds to GM events. The entered symbols or numbers indicate the detectability or non-detectability of the target sequence for the GM event.

Note 2 to entry: The term matrix is commonly used for a defined composition of food, but this definition is not relevant here.

3.2
GMO target matrix
relational presentation (e.g. a table) of symbols or numbers

Note 1 to entry: One dimension corresponds to genetic elements or genetic constructs present in a GMO and the other dimension (e.g. rows) corresponds to GM events. The entered symbols or numbers indicate the presence or absence of the target for the GM event and copy number, if available.

Note 2 to entry: In contrast to GMO method matrix, the GMO target matrix is independent from a detection method.

3.3
screening method
method that rapidly and reliably eliminates (screens) a large number of negative (or positive) test samples and restricts the number of test samples requiring the application of a rigorous method

Note 1 to entry: In this document, a screening method refers to PCR methods detecting the presence of several GMOs in one test.

3.4
element-specific method
method that targets a single discrete DNA sequence of a specific genetic element

Note 1 to entry: A genetic element is a part of a gene, for example a promoter, terminator, intron or a coding sequence. Elements are often derived from naturally occurring viruses, bacteria, plants, etc.. However, elements in GMOs are commonly modified at the sequence level, relative to the original (natural) source, e.g. by altered codon-usage or specific single nucleotide polymorphisms (SNPs). This sequence modification may be based on adaptation of the nucleotide composition to the new host genome.

3.5
construct specific method
method that targets a combination of inserted DNA sequences that are only found in GMO-derived material composed of at least two elements that do not naturally co-exist in this conformation, and where the 5' and 3' end of the sequence are derived from separate genetic elements

3.6
event specific method
method that detects a specific sequence that is only present in that event

Note 1 to entry: The event may be the result of either a rearrangement or unique combination of insert and insertion locus during the transformation.

Note 2 to entry: This is commonly targeted at the integration-border region.

3.7
probability of detection (POD)
probability of a positive analytical outcome for a qualitative method for a given matrix at a given concentration

Note 1 to entry: It is estimated by the expected proportion of positive results for the given matrix at the given analyte concentration.

4 Principle

Total DNA is extracted from the sample by a suitable extraction method. The DNA quantity and quality shall be checked as specified in EN ISO 21571 to ensure the presence of sufficient analyte and to assess the presence of PCR inhibitors that could inhibit PCR amplification.

Subsequently, a set of validated PCR methods are selected and applied in a decision tree approach, for detection and identification of genetic modifications linked to the sample. This approach enables the user to choose between different courses of action. Commonly, screening starts with element-specific PCR tests prior to construct-specific and/or event-specific tests. Before, in parallel or after screening, taxon-specific tests are recommended.

Based on the combination of results at each level of the decision tree the total number of tests required for the analysis is reduced.

5 Reagents

5.1 General

The requirements and conditions for nucleic acid extraction and PCR analysis laid down in detail in EN ISO 24276, EN ISO 21569, EN ISO 21570 and EN ISO 21571 shall be followed. Only analytical grade reagents suitable for molecular biology shall be used.

5.2 PCR reagents

5.2.1 Concentrated PCR buffer solution[1)] (containing reaction buffer, dNTPs, $MgCl_2$ and Hotstart *Taq* polymerase) or equivalent.

5.2.2 Oligonucleotides at the concentrations specified in the method protocol.

6 Apparatus and equipment

See EN ISO 24276, EN ISO 21569, EN ISO 21570 and EN ISO 21571.

7 PCR analysis

7.1 General

A large number of validated PCR methods applicable for the detection of GM DNA in food samples are available in EN ISO 21569, EN ISO 21570 or are compiled in [3] and [4]. The complexity of these methods can be divided in four categories according to the targeted DNA sequence:

— taxon-specific (for the detection and identification of DNA of a species or a taxonomical group);

— element-specific (for the detection and identification of genetic elements present in more than one GMO);

— construct-specific (for the detection and identification of specific combinations of genetic elements present in a GMO as a result of genetic engineering);

— event-specific (for the detection and identification of the insertion site specific for a GM event).

Testing laboratories develop a strategy for selecting the methods according to their scope (food, feed or seed testing), and requirements of their clients (e.g. government authority or company). In addition, the incidence of products on the market containing or consisting of new GMOs may require the ad hoc implementation of new methods into an existing screening strategy in order to incorporate the detection and identification of the respective GM target DNA.

To confirm the identity of the PCR product generated by the PCR method, the method shall include a step for verification of the amplicon by an appropriate technique (e.g. probe hybridization, subsequent DNA sequence analysis or restriction enzyme digestion of the PCR product, respectively).

7.2 Screening

7.2.1 General

The first step of PCR based screening analysis is the selection of a defined set of methods targeting DNA-sequences present in several GM events. The set of methods selected depends on the availability of (validated) methods, the capability of the laboratory and the scope of the testing.

1) Ready-to-use reagent mixtures or single components may be used as PCR buffer solution. If other reagent mixtures are used than the ones stated in the method validation report, they should give comparable to or better results.