
**Foodstuffs — Principles of selection
and criteria of validation for varietal
identification methods using specific
nucleic acid**

*Produits alimentaires — Principes de sélection et critères de
validation des méthodes d'identification variétale utilisant des acides
nucléiques spécifiques*

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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. www.iso.org/directives

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

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Introduction

This International Standard outlines guidelines designed to support decision-making and validation on the protocols used to produce high-quality molecular data for varietal identification.

Varietal identification testing requires high-quality markers, which are able to provide reproducible data using a variety of equipment, chemistries and reagents. Accordingly, this International Standard only addresses specific amplification methods.

The aims of this International Standard are to ensure that the methods of analysis are compatible with customer requests, to list the different steps towards method validation, and to define acceptance criteria. It also guarantees that the general principles employed in performing these analyses will be the same across all laboratories (reference material, sample size, laboratory sample, test portion, extraction, results analysis and interpretation, certificate of analysis).

Finally, this International Standard plays a role in standardizing the results obtained by different laboratories.

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Foodstuffs — Principles of selection and criteria of validation for varietal identification methods using specific nucleic acid

1 Scope

This International Standard specifies molecular tools for generating molecular profiles of varieties of plant species, enabling varietal identification, i.e. confirmation of identity in relation to one or more references.

This International Standard is applicable to various matrices, seeds, leaves, roots, industrial products composed of only one variety. Matrices presented in the form of mixtures of varieties (such as purees, compotes, flours) are excluded from the scope of this document.

This International Standard does not deal with genetic purity.

2 Normative references

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

ISO/IEC 17025:2005, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1 Terms related to variety

3.1.1

cultivar

group of cultivated plants which may be clearly defined by morphological, physical, cytological, chemical or other characteristics and which, after sexual or asexual reproduction, keeps its distinct character

[SOURCE: ISO 7563:1998, definition 1.12]

Note 1 to entry: The concept of “cultivar” is essentially different from the concept of the botanical variety “varietas”, in that

- “cultivar” is an infraspecific division resulting from controlled selection, even if empirical;
- “varietas” is an infraspecific division resulting from natural selection.

The terms “cultivar” and “variety” (in the sense of cultivated variety) are equivalent. In translations or adaptations of botanical nomenclature for particular uses, the terms “cultivar” or “variety” (or their equivalents in other languages) may be used in text.

Note 2 to entry: The names of botanical varieties and species are always in Latin form and are governed by botanical nomenclature.

3.1.2

species

group of organisms that have a high level of genetic (DNA) similarity and are capable of interbreeding; often containing subspecies, varieties or races

Note 1 to entry: A species is designated in italics by the genus name followed by the specific name, e.g. *Ananas comosus*.

3.1.3

variety

unique and uniform member of a species of plant (except for hybrid species) that retains its characteristics from generation to generation through its natural mode of reproduction

[SOURCE: ISO 5527:—, definition 2.1.7, modified — the other preferred term “cultivar” has been deleted.]

3.2 Terms related to DNA/RNA extraction and purification

3.2.1

nucleic acid extraction

sample treatment for the liberation of target nucleic acid

Note 1 to entry: The nucleic acid extraction procedure is used for isolating nucleic acids from other cellular components, such as protein, lipids, carbohydrates and other impurities in a test sample.

[SOURCE: ISO 22174:2005, definition 3.2.1, modified — Note 1 has been added.]

3.2.2

nucleic acid purification

method resulting in a more purified DNA

Note 1 to entry: A procedure or process involving sequential steps used to separate DNA and/or RNA from other components in a sample. A highly purified DNA or RNA sample contains negligible observable or measurable effects attributable to inhibitors of the polymerase chain reaction.

Note 2 to entry: In this context, purity refers to the reduction of observable and measurable effects of PCR inhibitors.

[SOURCE: ISO 22174:2005, definition 3.2.2, modified — Note 1 has been added; Note 2 has been modified.]

3.3 Terms related to PCR amplification of nucleic acids

3.3.1

amplicon

specific DNA fragment produced by a DNA-amplification technology, such as the polymerase chain reaction (PCR)

3.3.2

hybridization

specific binding of complementary nucleic acid sequences under suitable reaction conditions

[SOURCE: ISO 22174:2005, definition 3.6.3]

3.3.3

multiplex PCR

PCR that uses multiple pairs of primers in different loci combined within a single reaction mixture to produce multiple amplicons simultaneously

[SOURCE: ISO 22174:2005, definition 3.4.11, modified — the phrase following “primers” has been added.]

3.3.4

polymerase chain reaction

PCR

enzymatic procedure which allows *in vitro* amplification of DNA

[SOURCE: ISO 22174:2005, definition 3.4.1]

3.3.5**primer**

oligonucleotide of defined length and sequence complementary to a segment of an analytically relevant DNA sequence

[SOURCE: ISO 22174:2005, definition 3.4.12]

3.3.6**probe**

labelled nucleic acid molecule with a defined sequence used to detect target DNA by hybridization

[SOURCE: ISO 22174:2005, definition 3.6.1, modified — the term was originally “DNA probe”.]

3.3.7**specificity**

property of a method to respond exclusively to the characteristic or analyte under investigation

Note 1 to entry: It describes the ability to specifically recognize the nucleic acid sequence to be detected by distinguishing it from other nucleic acid sequences, and the tendency for a primer or probe to hybridize with its intended target and not hybridize with other non-target sequences.

[SOURCE: ISO 24276:2006, definition 3.1.4, modified — Note 1 has been added.]

3.3.8**thermocycler**

automated laboratory apparatus used to repeatedly raise and lower the temperature of a sample by cycling through a series of discrete, pre-programmed steps

Note 1 to entry: This cycling of temperatures drives the PCR process.

3.4 Terms related to detection [ISO 13495:2013](https://standards.iteh.ai/catalog/standards/sist/cdc058a8-5275-433d-a061-008eda21f2f1/iso-13495-2013)

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3.4.1**electrophoresis**

method of separating electrically-charged particles by their differential migration under an electric field

Note 1 to entry: PCR products can be separated by various types of electrophoresis.

3.5 Terms related to controls**3.5.1****reference sample****reference material**

material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials

Note 1 to entry: The reference material may be either provided by the customer, internal to the laboratory, or an officially-designated reference.

[SOURCE: ISO Guide 30]

[SOURCE: ISO 24276:2006, definition 3.5.1, modified — Note 1 has been added.]

3.5.2**test control**

one or more samples that have undergone all or part of the analytical process designed for the target samples and which can reveal known alleles of the markers used, thereby signalling any process errors and providing reference alleles which can facilitate the reading of results

3.6 Terms related to markers

3.6.1

allele competition

preferential amplification of one allele over another in a heterozygote or a mixture

3.6.2

allele frequency

measure of how common an allele is in a population; the proportion or percentage of all of the occurrences of a locus that is occupied by a given allele

3.6.3

marker

genetic marker that typically applies to DNA fragments matching a given locus that gives information on the genotype of the carrier or on the genotype of neighbouring loci

3.6.4

null allele

<context of PCR> sequence variant that precludes PCR amplification of a particular target, resulting in the absence of detectable PCR product

3.6.5

repeat region

genomic region in which a particular DNA or RNA sequence occurs as multiple copies

3.6.6

simple sequence repeat

SSR

region of DNA consisting of a short (1 bp to 6 bp) sequence (repeat unit) that is tandemly repeated many (typically five to 50) times

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Note 1 to entry: SSRs are commonly known as microsatellites.

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Note 2 to entry: The number of repeat units present at a specified SSR, and thus the overall length of the SSR, often varies among individuals.

3.6.7

single nucleotide polymorphism

SNP

single nucleotide variation in a genetic sequence that occurs at appreciable frequency in the population

Note 1 to entry: SNP is often pronounced “snip”.

[SOURCE: ISO 25720:2009, definition 4.23, modified — Note 1 has been added.]

4 Quality assurance on the test results

The requirements and guidelines set out in [Table 1](#) are coded as follows:

- C: compulsory;
- R: Recommended.

Table 1 — Requirements and guidelines on the test results

STAFF		
All staff carrying out specific tasks shall be qualified based on an appropriate level of education, training, experience and/or proven competency, according to the task skills required.		
	Criteria	Requirement (C or R)
	Training in use of the equipment	C
	Handling chemicals	C
	Staff responsible for issuing opinions and interpretations shall be fully conversant with the various genetic structures and seed production systems involved, in order to provide customers with advice and/or suggest ways to interpret the results.	C
EQUIPMENT		
Maintenance operations shall be run on all apparatus and equipment following the manufacturer's instructions. Suitable apparatus calibration systems shall be made available.		
Maintenance on key equipment	The equipment used for preparing the sample	C
	Example: mixer mills	
	Pipetting equipment	C
	The autoclaves used for sterilizing the laboratory equipment and the various buffers and solutions used	C
	Storage freezer(s) designed for conserving extracts, products, mixes, etc.	C
	Refrigerator/walk-in cooler for storing extract samples, solutions and chemicals	C
	Equipment used to perform DNA amplification cycles (thermocycler, water bath, etc.)	C
	Amplicon imaging and recording system (photocopier, scanners, etc.)	C
	Electrophoresis system, genetic analyser	R
	Precision scales	C
	pH meter	R
	Robots	R
FACILITIES		
	All necessary occupational health and environmental protection measures have been taken	R
	Manual or automatically-controlled pipetting equipment specifically assigned to each workspace	C
	Two separate workspace areas	
	Pre- and post-PCR	C
REAGENTS		
	The products used shall not represent a potential source of DNA degradation or contamination. All reagents shall be conserved and used according to the manufacturer's guidelines.	C