SLOVENSKI STANDARD
oSIST prEN ISO 17174:2023

01-julij-2023

Analiza molekularnih biomarkerjev - Črtno kodiranje DNK rib in ribjih proizvodov z uporabo segmentov genov, ki nosijo zapis za mitohondrijski citokrom b in citokrom c oksidaze I (ISO/DIS 17174:2023)

Molecular biomarker analysis - DNA barcoding of fish and fish products using defined mitochondrial cytochrome b and cytochrome c oxidase I gene segments (ISO/DIS 17174:2023)

Untersuchung auf molekulare Biomarker - DNA-Barcoding von Fisch und Fischprodukten anhand definierter mitochondrialer Cytochrom b- und Cytochrom c-Oxidase I-Genabschnitte (ISO/DIS 17174:2023)

Analyse de biomarqueurs moléculaires - Codes-barres d'ADN de poissons et de produits à base de poisson à l'aide de segments de gènes mitochondriaux de cytochrome b et cytochrome c oxydase I (ISO/DIS 17174:2023)

Ta slovenski standard je istoveten z: **prEN ISO 17174**

ICS:

67.120.30	Ribe in ribji proizvodi	Fish and fishery products

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Molecular biomarker analysis — DNA barcoding of fish and fish products using defined mitochondrial cytochrome b and cytochrome c oxidase I gene segments

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Contents

Page

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).

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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 34. *Food Products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

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

Food safety is a key aspect in terms of consumer protection. In the last three decades, globalization has taken place in the trade of food. Fish trade channels are becoming steadily longer and more complicated so that sophisticated traceability tools are needed to ensure food safety. Correct food labelling is a prerequisite to ensure safe fish products and fair trade as well as to minimize illegal, unreported and unregulated (IUU) fishing. In particular, the fact that fish is increasingly being processed in export countries makes the identification of species by morphological characteristics impossible. The development of harmonized and standardized protocols for the authentication of fish products is necessary to establish reliable methods for the detection of potential food fraud.

Molecular biomarker analysis — DNA barcoding of fish and fish products using defined mitochondrial cytochrome b and cytochrome c oxidase I gene segments

1 Scope

This document specifies a method for the identification of single fish and fish fillets to the level of genus or species. It allows the identification of a large number of commercially important fish species using DNA barcoding.

This method was validated on raw fish. Laboratory experiences indicate additional applicability to processed fish products, e.g. cold smoked, hot smoked, salted, frozen, cooked, fried, deep-fried samples.

The described method is usually unsuitable for the analysis of highly processed foods, e.g. tins of fish, with highly degraded DNA where the fragment lengths are not sufficient for amplification of the targets. Furthermore, it is not applicable for complex fish products containing mixtures of two or more fish species.

The identification of fish species is carried out by PCR amplification of either a segment of the mitochondrial cytochrome b gene (*cytb*) or the cytochrome c oxidase I gene (*cox1, syn COI*) or both, followed by sequencing of the PCR products and subsequent sequence comparison with entries in databases.

The decision whether the *cytb* or *cox1* gene segment or both are used for fish identification depends on the declared fish species, the applicability of the PCR method for the fish species and the availability of comparative sequences in the public databases.

Two international DNA barcoding ring trials, the first using *cytb* and the second using *cox1* gene segments, respectively identified 95 % and 87 %, of the tested samples to the fish species. All samples were correctly identified to the genus level.

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 — Vocabulary for molecular biomarker analytical methods in agriculture and food production*

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

ISO 20813, *Molecular biomarker analysis — Methods of analysis for the detection and identification of animal species in foods and food products (nucleic acid-based methods) — General requirements and definitions*

ISO 22949-1, *Molecular biomarker analysis — Methods of analysis for the detection and identification of animal species in food and feed products (nucleotide sequencing-based methods) — Part 1: General requirements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 16577 and the following 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 https://www.electropedia.org/

3.1
Alignment
Sequence alignment
arrangement of nucleic acid sequences or protein sequences according to regions of similarity

Note 1 to entry: The sequence alignment is a process or result of matching up the nucleotide residues of two or more biological sequences to achieve maximal levels of identity.

[SOURCE: ISO 16577:2022, 3.7.18 – modified, Note 1 to entry added, alternative name added]

3.2
BLAST
basic local alignment search tool
sequence comparison algorithm optimized for speed that is used to search sequence databases for optimal local alignments to a query

Note 1 to entry: It directly approximates alignments that optimize a measure of local similarity, the maximum signal pair (MST) score or high scoring signal pair (HSP) score.

[SOURCE: ISO 20813:2019, 3.1, modified – Note 2 to entry and Note 3 to entry have been deleted]

3.3
BOLD
barcode of life data systems
informatics workbench aiding the acquisition, storage, analysis, and publication of DNA barcode records

Note 1 to entry: By assembling molecular, morphological, and distributional data, it bridges a traditional bioinformatics chasm. BOLD is freely available to anybody with interests in DNA barcoding. By providing specialized services, it aids the assembly of records that meet the standards needed to gain BARCODE designation in the global sequence databases. Because of its web-based delivery and flexible data security model, it is also well positioned to support projects that involve broad research alliances.

[SOURCE: [5]]

3.4
FASTA format
text-based format for representing either nucleotide sequences or amino acid (protein) sequences, in which nucleotides or amino acids are represented using single-letter codes

Note 1 to entry: A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line (defline) is distinguished from the sequence data by a greater-than (“>”) symbol at the beginning. It is recommended that all lines of text be shorter than 80 characters in length.

Note 2 to entry: An example sequence in FASTA format is:

> Sample_04_cytb

ATGGCCAGCCTCCGAAAAACTCATCCCCTTCTAAAGATTGCTAATGATGCATTAGTAGACCT-
TCCTGCCCCCTCTAACCTCT CAACATTATGAAACTTCGGGTCTCTCCTAGGCCTCTGCTTAGCCGCCCAAATCT-
TAACAGGACTATTTCTAGCGATACATT ATACCGCAAACGTCGAGATAGCTTTCTCATCCGTCGTACACATCTGC-
CGCGACGTAAATTACGGATGACTAATCCGCAACA TACACGCCAACGGCGCTTCTTTCTTCTTCATCTGCCTC-
TACCTACACATTGCACGAGGCCTATATTACGGCTCCTACTTATT CATAGAGACCTGAAACATTGGAGTTGTAC-
TATTCCTTTTAGTAATAATGACCGCCTTCGTAGGCTACGTCCTCCCT

Note 3 to entry: Blank lines are not allowed in the middle of FASTA input. Sequences are represented in the standard IUB/IUPAC amino acid and nucleic acid codes, with these exceptions:

— lower-case letters are accepted and are mapped into upper-case;

— a single hyphen or dash can be used to represent a gap of indeterminate length;

— in amino acid sequences, U and * are acceptable letters.

It is common to end the sequence with an "*" (asterisk) character and to leave a blank line between the description and the sequence.

[SOURCE: ISO 16577:2022, 3.1.2, modified – another example is used in Note 2 to entry]

3.5
FishBase
global biodiversity online platform on finfishes providing a wide range of information on all species currently known in the world

3.6
GenBank
genetic sequence database, an annotated collection of all publicly available DNA sequences

Note 1 to entry: GenBank at National Center for Biotechnology Information (NCBI) is part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA DataBank of Japan (DDBJ), the European Nucleotide Archive (ENA). These three organizations exchange data on a daily basis.

[SOURCE: [6]]

3.7
identity
extent to which two (nucleotide or amino acid) sequences have the same residues at the same positions in an alignment, often expressed as a percentage

Note 1 to entry: In BOLD, the term similarity is used instead of identity.

3.8
introgressed DNA
allele from one species incorporated in the gene pool of another, divergent species

Note 1 to entry: Introgression has usually happened via hybridization and backcrossing of individuals belonging to different species.

3.9
NCBI
National Center for Biotechnology Information
institution that maintains molecular biology databases and provides the BLAST suite

3.10
nucleotide collection
nr/nt
non-redundant database consisting of GenBank sequences, in which identical sequences have been merged into one entry

3.11
query
sequence (or other type of search term) that is compared to entries in a database

3.12
query coverage
percentage of query covered by alignment to the database sequence