SLOVENSKI STANDARD
SIST EN 17882:2024

01-september-2024

Pristnost živil - Črtno kodiranje DNK mesa, pridobljenega iz sesalcev in ptic, z uporabo definiranih mitohondrijskih genskih segmentov citokroma b in citokroma c oksidaze I

Food authenticity - DNA barcoding of meat derived from mammals and birds using defined mitochondrial cytochrome b and cytochrome c oxidase I gene segments

Lebensmittelauthentizität - DNA-Barcoding von Fleisch und Fleischerzeugnissen von Säugetieren und Vögeln anhand definierter mitochondrialer Cytochrom b und Cytochrom c Oxidase-I-Gensegmente

Authenticité des aliments - Codage à barres de l'ADN de viande dérivée de mammifères et d'oiseaux à l'aide de segments définis du gène du cytochrome b mitochondrial et de la cytochrome c oxydase I

Ta slovenski standard je istoveten z: **EN 17882:2024**

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35.040.50	Tehnike za samodejno razpoznavanje in zajem podatkov	Automatic identification and data capture techniques
67.020	Procesi v živilski industriji	Processes in the food industry
67.120.10	Meso in mesni proizvodi	Meat and meat products

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EUROPEAN STANDARD

NORME EUROPÉENNE

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English Version

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

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European foreword

This document (EN 17882:2024) has been prepared by Technical Committee CEN/TC 460 “Food authenticity”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2025, and conflicting national standards shall be withdrawn at the latest by January 2025.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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Introduction

Fraudulent adulteration of meat in food threatens both public safety and commerce. It can affect those adhering to ethnological dietary rules, economic development and social stability. In the last three decades, globalization has taken place in the trade of food. Meat 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 meat products and fair trade. The development of reliable, harmonized and standardized protocols for the authentication of meat and meat products is necessary to ensure consumer protection and the detection of potential food fraud.

1 Scope

This document specifies a method for the identification of meat derived from mammals and birds to the level of genus or species and allows the identification of a large number of commercially important as well as exotic meat species using DNA barcoding.

This method was validated on DNA isolated from single pieces of raw meat. This method can also be used for the identification of single meat animal species in some processed products.

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

The identification of meat 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.

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*

EN 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 20813)*

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 terminology databases for use in standardization at the following addresses:

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

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

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
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.

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

```
>Sample_04_cytb
ATGGCCAGCCTCCGAAAAACTCATCCCCTTCTAAAGATTGCTAATGATGCATTAGTAGACCTTCCTGCCCCCTCTAACCTC
TCAACATTATGAAACTTCGGGTCTCTCCTAGGCCTCTGCTTAGCCGCCCAAATCTTAACAGGACTATTTCTAGCGATACAT
TATACCGCAAACGTCGAGATAGCTTTCTCATCCGTCGTACACATCTGCCGCGACGTAAATTACGGATGACTAATCCGCAAC
ATACACGCCAACGGCGCTTCTTTCTTCTTCATCTGCCTCTACCTACACATTGCACGAGGCCTATATTACGGCTCCTACTTA
TTCATAGAGACCTGAAACATTGGAGTTGTACTATTCCTTTTAGTAATAATGACCGCCTTCGTAGGCTACGTCCTCCCT
```

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.

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 – Last sentence in Note 1 to entry removed, another example is used in Note 2 to entry, 3rd bullet point in note 3 to entry deleted]

3.3
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 the sequence database of Barcode of Life (BOLD), the term similarity is used instead of identity.

3.4
query
sequence (or other type of search term) that is compared to entries in a database

3.5
query coverage
percentage of the query covered by alignment to the data base sequence

3.6
sequence similarity
identity between two or more DNA sequences measured as a percentage

[SOURCE: ISO 16577:2022, 3.7.21]

3.7
specificity
analytical specificity
diagnostic specificity
ability of a detection method to distinguish the specific organism or pathogen from other organisms, whether related or not, and the extent to which the analysis can distinguish known or unknown variants of the organism

Note 1 to entry: Specificity is a term that describes the same phenomenon as selectivity but in a different way; while selectivity is applied to analytical chemistry and physics, specificity is applied to organisms and pathogens.

[SOURCE: ISO 16577:2022, 3.3.76]

4 Symbols and abbreviations

cox1, *syn COI*	cytochrome c oxidase I gene
cytb	cytochrome b gene
bp	base pairs
dNTP	deoxyribonucleotide triphosphate
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
A	adenine
C	cytosine
G	guanine
T	thymine
Y	pyrimidine (cytosine or thymine)

5 Principle

DNA is extracted from meat and meat products derived from mammals or birds applying a suitable method. Segments of approximately 359 base pairs of *cytb* and/or approximately 540 base pairs of *cox1* are amplified by PCR. Amplification of *cox1* uses a degenerated reverse primer to increase the number of species that are able to be detected by the method. The nucleotide sequences of the PCR products are determined by a suitable DNA sequencing method, e.g. Sanger sequencing. The PCR primers used to generate the *cytb* and *cox1* amplicon are also used for sequencing. The determined sequences are evaluated by comparison to sequence entries in databases, thus allowing the assignment to a meat species or genus according to the degree of identity with the available sequences.

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

6 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized molecular biology grade and distilled, demineralized or water of equivalent purity, according to EN ISO 20813. Laboratory organization shall follow EN ISO 20813.

6.1 Thermostable DNA polymerase.[1]

[1] During the collaborative study the Maxima® Hot Start PCR Master Mix (2 x) of Fermentas GmbH (ready to use PCR buffer solution including thermostable DNA polymerase) was used for the *cytb* amplification and the BIOTAQ™ DNA polymerase of Bioline with 10 x reaction buffer and separate $MgCl_2$ solution for the *cox1* amplification. In addition to the recommended BIOTAQ™ DNA polymerase other mastermixes and polymerases were successfully used in the collaborative study. This information is given for the convenience of users of this document and does

6.2 PCR reaction buffer (including $MgCl_2$ or with separate $MgCl_2$ solution).[1]

6.3 dNTP mix (dATP, dCTP, dGTP and dTTP).[2]

6.4 Oligonucleotides, see Tables 1 and 2.

Table 1 — Oligonucleotides for amplification of the *cytb* gene region[1]

Name	DNA sequence of oligonucleotide
cytB-1	5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3'
cytB-2	5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3'

Table 2 — Oligonucleotides for amplification of the *cox1* gene region[2]

Name	DNA sequence of oligonucleotide
CO1e-H	5'-CCA GAG ATT AGA GGG AAT CAG TG-3'
CO1f-L	5'-CCT GCA GGA GGA GGA GAY CC-3'

NOTE The abbreviation of the DNA bases in Tables 1 and 2 are based on the recommendations for unambiguous, uniform, and consistent nomenclature, published by the International Union of Pure and Applied Chemistry (IUPAC) [3].

6.5 Agarose.

6.6 DNA size standard.

7 Apparatus

In addition to standard laboratory equipment, the following apparatus should be used.

7.1 UV-spectrophotometer or fluorometer, to determine the concentration of DNA.

7.2 Thermocycler.

7.3 Gel electrophoresis device.

7.4 Gel documentation system.

7.5 DNA sequencer.

8 Procedure

8.1 Sample preparation

The test portion used for DNA extraction shall be representative of the laboratory sample. In samples that consist of processed materials (e.g. convenience foods), single meat pieces shall be separated and analysed. For the analysis of samples composed of several pieces, test portions for every putative meat

not constitute an endorsement by CEN of the products named. Equivalent products may be used if they can be shown to lead to the same results.

[2] dNTPs can also be part of a commercial PCR master mix.