SLOVENSKI STANDARD
oSIST prEN 17882:2022

01-oktober-2022

Avtentičnost hrane - Črtno kodiranje DNK mesa in mesnih izdelkov, pridobljenih iz sesalcev in perutnine, z uporabo segmentov genov, ki nosijo zapis za mitohondrijski citokrom b in citokrom c oksidaze I

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

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

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Authenticité des aliments - Codage à barres de l'ADN de viande et de produits carnés dérivés de mammifères et volailles 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: prEN 17882

ICS:

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

oSIST prEN 17882:2022 en,fr,de

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD

NORME EUROPÉENNE

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ICS 07.080; 67.020; 67.120.10

English Version

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

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 460.

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European foreword

This document (prEN 17882:2022) has been prepared by Technical Committee CEN/TC 460 “Food authenticity”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

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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 harmonized and standardized protocols for the authentication of meat products is necessary to establish reliable methods for the detection of potential food fraud.

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1 Scope

This document describes a procedure for the identification of meat and meat products derived from mammalia and poultry to the level of genus or species.

The identification of meat species is carried out by PCR amplification of either a segment of the mitochondrial cytochrome b gene (*cytb*) [1] or the cytochrome c oxidase I gene (*COI*) [2], or both, followed by sequencing of the PCR products and subsequent sequence comparison with entries in databases [3], [4]. The methodology allows the identification of a large number of frequently used as well as exotic meat species in foodstuffs.

The decision whether the *cytb* or *COI* 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.

This method has been successfully validated on raw meat, however, laboratory experience is available that it can also be applied to processed meat products.

This document is usually 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.

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.

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ISO 16577, *Molecular biomarker analysis — Terms and definitions*

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*

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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:

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

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

3.1
alignment
process or result of matching up the nucleotide residues of two or more biological sequences to achieve maximal levels of identity

[SOURCE: BLAST Glossary]

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3.2
BLAST
Basic Local Alignment Search Tool [3]
sequence comparison algorithm optimized for speed 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 (MSP) score or high scoring signal pair (HSP) score.

3.3
BOLD
Barcode of Life Data Systems [4]
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 any researcher 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: BOLDSYSTEMS About Us]

3.4
FASTA format
text-based format for representing either nucleotide sequences or amino acid sequences, which begins with a single-line description, followed by lines of sequence data

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Note 1 to entry: The description line (defline) is distinguished from the sequence data by a greater-than (“>”) symbol at the beginning.

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EXAMPLE An example sequence in FASTA format is shown below:

> Sample_04_*cytb*
ATGGCCAGCCTCCGAAAAACTCATCCCCTTCTAAAGATTGCTAATGATGCATTAGTAGACCTTCCTGCCCCCTCTAACCTCT
CAACATTATGAAACTTCGGGTCTCTCCTAGGCCTCTGCTTAGCCGCCCAAATCTTAACAGGACTATTTCTAGCGATACATT
ATACCGCAAACGTCGAGATAGCTTTCTCATCCGTCGTACACATCTGCCGCGACGTAAATTACGGATGACTAATCCGCAACA
TACACGCCAACGGCGCTTCTTTCTTCTTCATCTGCCTCTACCTACACATTGCACGAGGCCTATATTACGGCTCCTACTTATT
CATAGAGACCTGAAACATTGGAGTTGTACTATTCCTTTTAGTAATAATGACCGCCTTCGTAGGCTACGTCCTCCCT

[SOURCE: BLAST topics, modified]

3.5
GenBank
comprehensive public database of e. g. genetic sequences [5]

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

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3.6
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 database BOLD, the term similarity is used instead of identity.

[Source: BLAST Glossary]

3.7
NCBI
National Center for Biotechnology Information
institution which houses molecular biology databases (e.g. GenBank) and provides the BLAST suite

3.8
nucleotide collection
nr/nt
non-redundant database consisting of GenBank sequences, in which identical sequences have been merged into one entry

3.9
query
sequence (or other type of search term) to which all of the entries in a data base are to be compared

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[SOURCE: BLAST Glossary]

3.10
query coverage
percentage of query covered by alignment to the data base sequence

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[SOURCE: BLAST help]

4 Principle

DNA is extracted from meat and meat products derived from mammalia or poultry applying a suitable method. Segments of approximately 359 base pairs of the *cytb* gene and/or approximately 540 base pairs of the *COI* gene are amplified by PCR. In the further course, the nucleotide sequence of the PCR product is determined by a suitable DNA sequencing method (e.g. Sanger sequencing). The sequence is 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 stored sequences.

5 Reagents and materials

5.1 General

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

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5.2 PCR reagents

5.2.1 Thermostable DNA polymerase (for hot start PCR)[1]

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

5.2.3 Deoxynucleoside triphosphate mix (dATP, dCTP, dGTP and dTTP)[2]

5.2.4 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 *COI* 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'

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5.2.5 Agarose

5.2.6 Suitable DNA length standard for assessing the amplification product length

6 Apparatus

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Apart from the usual laboratory equipment, the following equipment is required:

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

6.2 Thermocycler

6.3 Gel electrophoresis device

6.4 Gel documentation system

6.5 DNA sequencer

[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 *COI* amplification. In addition to the recommended BIOTAQ DNA polymerase other mastermixes and polymerases were successfully used in the collaborative study.

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

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7 Procedure

7.1 Sample preparation

It should be ensured that the test portion used for DNA extraction is representative for the laboratory sample. In composed samples (e.g. ready to use meals), single meat pieces have to be separated and analysed. With the analysis of samples composed of several pieces, test portions for every putative meat species are taken and analysed separately. To minimize the risk of amplifying adhering contaminants, test sample material shall not be taken from the surface of the laboratory sample. For further information regarding sample preparation, see ISO 20813.

7.2 DNA extraction

Concerning the extraction of DNA from the test sample, the general instructions and measures described in ISO 21571 should be followed, see ISO 20813. It is recommended to choose one of the DNA extraction methods described in ISO 21571:2005[3], Annex A. Alternatively, commercial kits can be used for the extraction and purification of DNA.

7.3 PCR

7.3.1 General

The primers used for the amplification of the section from the conserved areas of the vertebrate mitochondrial *cytb* gene including a variable region are universal primers. [1].

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The primers used for the amplification of the section from the mitochondrial *COI* gene were designed to amplify a segment from the 5' region of the *COI* gene [2].

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7.3.2 PCR setup

The method was validated for a total volume of 25 µl per PCR. The reagents given in Table 3 should be used for the *cytb* and *COI* PCR, respectively.

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Reagents are completely thawed at room temperature and should be centrifuged briefly before usage. A PCR reagent mixture is prepared containing all PCR components in the given concentrations except for the DNA extract. The amount of PCR mixture depends on the total volume per PCR and the total number of the reactions including a sufficient pipetting reserve.

Positive PCR results are expected when using a DNA concentration of approximately 1 ng/µl reaction solution. If it is necessary to improve the PCR result, the inserted DNA quantity may be increased (e.g. to increase the yield of PCR product) or decreased (e.g. to avoid PCR inhibition).

[3] EN ISO 21571:2005 is currently impacted by EN ISO 21571:2005/A1:2013.