SLOVENSKI STANDARD
oSIST prEN 17881:2022

01-oktober-2022

Avtentičnost hrane - Črtno kodiranje DNK školjk in proizvodov, pridobljenih iz školjk, z uporabo segmentov genov, ki nosijo zapis za mitohondrijski 16S rRNA

Food authenticity - DNA barcoding of bivalves and products derived from bivalves using a defined mitochondrial 16S rRNA gene segment

Lebensmittelauthentizität - DNA-Barcoding von Muscheln und Muschelprodukten anhand eines definierten mitochondrialen 16S rRNA□Genabschnittes

Authenticité des aliments - Codage à barres de l'ADN de bivalves et produits dérivés de bivalves à l'aide d'un segment défini du gène de l'ARNr 16S mitochondrial

Ta slovenski standard je istoveten z: **prEN 17881**

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
67.120.30	Ribe in ribji proizvodi	Fish and fishery products

oSIST prEN 17881:2022 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 17881

July 2022

ICS 07.080; 67.020; 67.120.30

English Version

Food authenticity - DNA barcoding of bivalves and products derived from bivalves using a defined mitochondrial 16S rRNA gene segment

Authenticité des aliments - Codage à barres de l'ADN de bivalves et produits dérivés de bivalves à l'aide d'un segment défini du gène de l'ARNr 16S mitochondrial

Lebensmittelauthentizität - DNA-Barcoding von Muscheln und Muschelprodukten anhand eines definierten mitochondrialen 16S rRNA-Genabschnittes

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

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

This draft European Standard was established by CEN in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and United Kingdom.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

Ref. No. prEN 17881:2022 E

Contents

Page

European foreword

This document (prEN 17881:2022) 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.

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. Seafood 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 seafood products and fair trade as well as to minimize illegal, unreported and unregulated (IUU) fishing. Seafood products are increasingly being processed in export countries. Especially bivalves are often sold without the shells. That makes the identification of species by morphological characteristics impossible.

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

1 Scope

This document describes a procedure for the identification of single bivalves to the level of genus or species.

The identification of bivalve species is carried out by PCR amplification of a segment of the mitochondrial 16S rRNA gene [1], [2] followed by sequencing of the PCR products and subsequent sequence comparison with entries in databases [5]. The methodology allows the identification of a large number of commercially important bivalve species.

This method has been successfully validated on raw mussels, however, laboratory experience is available that it can also be applied to processed, e.g. cold smoked, hot smoked, salted, frozen, cooked, fried, deep-fried samples.

This document is usually unsuitable for the analysis of highly processed foods, e.g. tins of mussels, with highly degraded DNA where the fragment lengths are not sufficient for amplification of the targets. Furthermore, it is not applicable for complex seafood products containing mixtures of two or more bivalve 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.

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*

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]

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
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

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

EXAMPLE An example sequence in FASTA format is shown below:

> Sample_04_16SrRNA gene
ATCACGTAGGATTTTAATGGGCGAACATACCAACCATTGAGACCGCCTACAGCCTCAGGATATCCGGAGCCAACATCGAGG
TCGCAAACTTTCTCATCTATAAGAACTATCAAAGAAAATAACGCTGTTATCCCCGGAGTAACTTCTTCTGTTAATCACTAA
ATAAAGTAAGTGGGTCGTCTATCAAACAAAGAAAAGAAAGAGTCTGATCTTGCTCTTTTGCTGCCCCAGCCAACAACAAAA
GTGGTAAGAATATCTCTGCCACTTAGTTAACAACTTCACGGGGTCTTCTCGTCTATCACTTATATTTAAGCATTTGCACTT
AAAATTCAATTTCATATAATTCAGCTAGAGACAGTTATAGGCTCGTCAATCCATTCACAGGGCCCCCAATTAGAGGGCCAT
AATTTAGCTACCTTAGCACGCTTTACCGCATCCGTTTAAGTCATCTCACTGGGAAGGAACGACCTACTATAAATACAGTAG
GCCATGTTTTT

[SOURCE: BLAST topics, modified]

3.4
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.

3.5

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

[Source: BLAST Glossary]

3.6
introgressed DNA
DNA sequence (allele) from one taxonomic entity (species) incorporated in the gene pool of another, divergent entity (species) [6]

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

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

[SOURCE: BLAST Glossary]

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

[SOURCE: BLAST help]

4 Principle

DNA is extracted from bivalves and bivalves products applying a suitable method. A segment of approximately 550 base pairs (bp) of the 16S rRNA gene is 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 bivalve 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.

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 Table 1)

Table 1 — Oligonucleotides for amplification of the 16S rRNA gene region [1], [2]

Name	DNA Sequence of oligonucleotide
16SAR	5'-CGC CTG TTT ATC AAA AAC AT-3'
16SBR	5'-CCG GTC TGA ACT CAG ATC ACG T-3'

5.2.5 Agarose

5.2.6 Suitable DNA length standard for assessing the amplification product length

[1] During the collaborative study the laboratories used DNA polymerases and mastermixes of different commercial providers. Amplificates were produced successfully with all used mastermixes and DNA polymerases.

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

6 Apparatus

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

7 Procedure

7.1 Sample preparation

It shall be ensured that the test portion used for DNA extraction is representative for the laboratory sample. In composed samples (e.g. seafood mixtures), single pure bivalve pieces have to be separated and analysed. With the analysis of samples composed of several pieces (e.g. bags with different scallops), test portions for every putative bivalve 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 EN ISO 21571 should be followed, see ISO 20813. It is recommended to choose one of the DNA extraction methods described in EN 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 mitochondrial 16S rRNA gene are universal primers. The primer pair 16SAR / 16SBR has been tested against a broad taxonomic range of bivalve species, and has only failed in a small minority of cases (< 5 % of species tested) [1].

7.3.2 PCR setup

The method was validated for a total volume of 25 µl per PCR. The reagents given in Table 2 should be used for the 16S rRNA PCR.

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.