



International
Standard

ISO 17174

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

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

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Foreword

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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 document 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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This document was prepared by Technical Committee 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 460, *Food authenticity*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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 reliable, harmonized and standardized protocols for the authentication of fish products is necessary to ensure consumer protection and 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 experience indicates additional applicability to processed fish products (e.g. cold smoked, hot smoked, salted, frozen, cooked, fried and 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 does not apply to 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.

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 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 terminology 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: Alignment is a process or result of matching up the nucleotide residues of two or more biological sequences to achieve maximal levels of *identity* (3.3).

[SOURCE: ISO 16577:2022, 3.7.18, modified — “alignment” was added as the preferred term; Note 1 to entry was 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 (define) 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
ATGGCCAGCCTCCGAAAACTCATCCCCCTTCTAAAGATTGCTAATGATGCATTAGTAGACCTTCCTGCCCCCTCT
AACCTCTCAACATTATGAAACTTCGGGTCTCTCCTAGGCCCTCTGCTTAGCCGCCCAAATCTTAACAGGACTATTT
CTAGCGATACATTATACCGCAAACGTCGAGATAGCTTTCTCATCCGTCGTACACATCTGCCGCGACGTAAATTAC
GGATGACTAATCCGCAACATACACGCCAACGGCGCTTCTTTCTTCTTCATCTGCCTCTACCTACACATTGCACGA
GGCCTATATTACGGCTCCTACTTATTCATAGAGACCTGAAACATTGGAGTTGTACTATTCTTTTAGTAATAATG
ACCGCCTTCGTAGGCTACGTCTCTCCCT
```

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 — Example in Note 2 to entry was replaced; the third list item in Note 3 to entry was deleted.]

3.3

identity

extent to which two (nucleotide or amino acid) sequences have the same residues at the same positions in an *alignment* (3.1)

Note 1 to entry: Identity is often expressed as a percentage.

Note 2 to entry: In the sequence database of the Barcode of Life (BOLD), the term "similarity" is used instead of identity.

3.4

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.

3.5

query

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

3.6

query coverage

percentage of the *query* (3.5) covered by *alignment* (3.1) to the database sequence