



**NSAI**  
Standards

Irish Standard Recommendation  
S.R. CEN/TS 17303:2019

Foodstuffs - DNA barcoding of fish and fish products using defined mitochondrial cytochrome b and cytochrome c oxidase I gene segments

**S.R. CEN/TS 17303:2019**

*Incorporating amendments/corrigenda/National Annexes issued since publication:*

The National Standards Authority of Ireland (NSAI) produces the following categories of formal documents:

I.S. xxx: Irish Standard — national specification based on the consensus of an expert panel and subject to public consultation.

S.R. xxx: Standard Recommendation — recommendation based on the consensus of an expert panel and subject to public consultation.

SWiFT xxx: A rapidly developed recommendatory document based on the consensus of the participants of an NSAI workshop.

*This document replaces/revises/consolidates the NSAI adoption of the document(s) indicated on the CEN/CENELEC cover/Foreword and the following National document(s):*

*NOTE: The date of any NSAI previous adoption may not match the date of its original CEN/CENELEC document.*

*This document is based on:*

CEN/TS 17303:2019

*Published:*

2019-03-27

*This document was published  
under the authority of the NSAI  
and comes into effect on:*

2019-04-14

ICS number:

67.120.30

NOTE: If blank see CEN/CENELEC cover page

NSAI  
1 Swift Square,  
Northwood, Santry  
Dublin 9

T +353 1 807 3800  
F +353 1 807 3838  
E standards@nsai.ie  
W NSAI.ie

Sales:  
T +353 1 857 6730  
F +353 1 857 6729  
W standards.ie

Údarás um Chaighdeáin Náisiúnta na hÉireann

## National Foreword

S.R. CEN/TS 17303:2019 is the adopted Irish version of the European Document CEN/TS 17303:2019, Foodstuffs - DNA barcoding of fish and fish products using defined mitochondrial cytochrome b and cytochrome c oxidase I gene segments

This document does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

For relationships with other publications refer to the NSAI web store.

**Compliance with this document does not of itself confer immunity from legal obligations.**

*In line with international standards practice the decimal point is shown as a comma (,) throughout this document.*

This page is intentionally left blank

**TECHNICAL SPECIFICATION**

**CEN/TS 17303**

**SPÉCIFICATION TECHNIQUE**

**TECHNISCHE SPEZIFIKATION**

March 2019

ICS 67.120.30

English Version

**Foodstuffs - DNA barcoding of fish and fish products using  
defined mitochondrial cytochrome b and cytochrome c  
oxidase I gene segments**

Produits alimentaires - Codes-barres d'ADN de  
poissons et de produits à base de poissons à l'aide de  
segments de gènes mitochondriaux du cytochrome b et  
cytochrome c oxydase I

Lebensmittel - DNA-Barcoding von Fisch und  
Fischprodukten anhand definierter mitochondrialer  
Cytochrom-b- und Cytochrom-c-Oxidase-I-  
Genabschnitte

This Technical Specification (CEN/TS) was approved by CEN on 14 January 2019 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

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



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

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

<b>Contents</b>	<b>Page</b>
European foreword.....	3
Introduction .....	4
1 Scope.....	5
2 Normative references.....	5
3 Terms and definitions .....	5
4 Principle .....	7
5 Reagents and materials.....	7
5.1 General.....	7
5.2 PCR reagents.....	8
6 Apparatus.....	9
7 Procedure.....	9
7.1 Sample preparation.....	9
7.2 DNA extraction .....	9
7.3 PCR.....	9
7.3.1 General.....	9
7.3.2 PCR setup .....	10
7.3.3 Temperature-time program.....	11
7.3.4 PCR controls .....	11
8 Evaluation .....	12
8.1 Evaluation of PCR products .....	12
8.2 Evaluation of the PCR results.....	12
8.3 Sequencing of PCR products.....	12
8.4 Evaluation of sequence data.....	13
8.5 Comparison of the sequence with public databases.....	13
8.5.1 General.....	13
8.5.2 Sequence comparison of <i>cytb</i> and/or <i>cox1</i> DNA sequences with GenBank.....	13
8.5.3 Sequence comparison of <i>cox1</i> DNA sequences with BOLD .....	14
9 Interpretation of database query results.....	15
10 Validation status and performance criteria.....	15
10.1 Collaborative study for the identification of fish species based on <i>cytb</i> sequence analysis .....	15
10.2 Collaborative study for the identification of fish species based on <i>cox1</i> sequence analysis .....	16
11 Test report.....	18
Annex A (informative) Practical laboratory experiences with the amplificability of <i>cytb</i> or <i>cox1</i> segments from tested fish species .....	19
Bibliography.....	24

## **European foreword**

This document (CEN/TS 17303:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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.

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

## **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.



## 1 Scope

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

The identification of fish 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 (*cox1*, *syn COI*) [2], [3] or both, followed by sequencing of the PCR products and subsequent sequence comparison with entries in databases [4], [5]. The methodology allows the identification of a large number of commercially important fish species.

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.

This method has been successfully validated on raw fish fillets, 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 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.

## 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.

EN ISO 24276, *Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — General requirements and definitions (ISO 24276)*

ISO 16577, *Molecular biomarker analysis — Terms 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 <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://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]

This is a free preview. Purchase the entire publication at the link below:

[Product Page](#)

- 
- Looking for additional Standards? Visit Intertek Inform Infostore
  - Learn about LexConnect, All Jurisdictions, Standards referenced in Australian legislation
-