

ICS 65.020.30
CCS B 41

SN

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 5203-2020

**Fish species identification genetic bar code detection technical
specifications**

鱼类物种鉴定 基因条形码的检测技术规范

(English Translation)

Issued on: December 30, 2020

Implemented on: July 1, 2021

Issued by: General Administration of Customs

Table of Contents

Foreword	
1 Scope	
2 Normative references	
3 Terms and definitions	
4 Abbreviations	
5 Reagents and materials	
6 Equipment and equipment	
7 Sampling	
8 Experimental steps	
8.1 Setting up a comparison	
8.2 DNA extraction	
8.3 Determination of DNA concentration and purity	
8.4 PCR amplification	
8.5 Agarose electrophoresis	

Foreword

This document is drafted in accordance with GB/T 1.1-2020.

This document was proposed and managed by the General Administration of Customs of the People's Republic of China.

Drafting organizations of this document. Shenzhen Institute of Inspection and Quarantine, Shenzhen Customs of the People's Republic of China, Shenzhen BGI Ocean Research Institute, Shenzhen Agricultural Products Quality and Safety Inspection and Testing Center.

1 Scope

This standard document defines the sample processing, gene amplification, sequence analysis, comparison and identification and result determination in fish genetic barcode identification and other operating procedures and specifications.

This document is suitable for species identification of wild-type fishes or their remaining tissues produced by natural environment planting, and is not suitable for cross-breeding Variety.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Terms and definitions

The following terms and definitions apply to this document.

DNA barcoding

DNA fragments with sequence characteristics of biological species that can be used for species identification. This standard specifically refers to the COI in the mitochondrial genome of fish the standard fragment of gene 658 bp.

DNA barcoding identification technique A technology that uses gene fragments for biological species identification and systematic evolutionary relationship research, based on gene amplification and sequence alignment on the basis of this, it is not restricted by species development status, male and female individual and completeness of morphological characteristics, and reduces the need for taxonomy experts.

Dependency, not affected by subjective factors, high accuracy, and data sharing can be achieved with the help of the Internet.

Similarity of the sequences A value reflecting the degree of similarity between sequences, generally expressed as a percentage.

FASTA format

The term of bioinformatics, also known as Pearson format, is a format for expressing nucleotide sequences or amino acid sequences in text. nuclear the nucleotide or amino acid base is represented by a single letter, and the first line of the sequence generally starts with ">", and then the sequence name or note can be added.

Interpretation, the second line is the sequence itself, only the established nucleotide or amino acid coding symbols are allowed, such as nucleotides using ATCG.

Characters such as carriage return cannot appear in the sequence.

Example.

4 Abbreviations

The following abbreviations apply to this document.

BOLD. biological barcode data system (barcoding of life datasystem).

5 Reagents and materials

5.1

Water. Comply with the first grade water specification in GB/T 6682.

5.2

Ammonium acetate, analytically pure.

5.3

Ethanol, analytically pure.

5.4

Isopropanol, analytically pure.

5.5

Proteinase K, analytically pure.

5.6

High-fidelity DNA polymerase, dNTPs (including dATP, dTTP, dGTP, dCTP), MgSO₄, PCR buffer (without Mg

For reagents such as bromophenol blue, commercial reagents provided by professional reagent companies can be used.

5.7

Primer. Use deionized water to prepare a 100 µmol/L stock solution for each primer, and store it below -20 °C; prepare an appropriate amount before use to 10 µmol/L working solution, avoid multiple freezing and thawing. The sequence is shown in Table 1.

5.8

Standard molecular weight. 100 bp DNA marker is recommended.

5.9

Agarose, analytically pure.

6 Equipment and equipment

Benchtop centrifuge, adjustable pipettes for each range, adjustable constant temperature bath, micro-nucleic acid protein analyzer, ultra-clean workbench, PCR expansion

Increasing instrument, electrophoresis instrument, gel imaging system, refrigerator.

7 Sampling

Samples include live fish, roe, fresh frozen meat, and minced meat made from a single fish. Fresh tissues should be taken, and the muscles of adult fish should be selected first. Meat tissue (heart, brain, spleen, kidney, liver, intestine, stomach and other organs are also applicable), fish eggs to get egg membranes. If non-lethal sampling is required, cut out part. After the fin rays or part of the skin is scraped off, a small piece of muscle of 3 mm³ to 5 mm³ can be taken.

The collected tissues should be stored on ice at low temperature for later use. For long-term storage, they should be frozen at $\leq -20\text{ }^{\circ}\text{C}$ or fixed in 75% ethanol save.

8.1 Setting up a comparison

Set up blank control, negative control and positive control. Use a sample known to contain fish ingredients as a positive control, and use a sample known to contain no fish ingredients.

The divided sample is used as a negative control, and an equal volume of deionized water is used as a blank control instead of template DNA.

8.2 DNA extraction

Take 20 mg of sample tissue and cut it into a clean 1.5 mL centrifuge tube, add 600 μL of lysate (see Appendix A in A.1) and 20 μL proteinase K solution (see A.2 in Appendix A), mix well, and in a $56\text{ }^{\circ}\text{C}$ water bath for 1 h to 3 h, shake and mix gently from time to time; wait for the group. After the tissue is digested completely, cool to room temperature, add 200 μL of ammonium acetate solution (see A.3 in Appendix A), mix well, place on ice or cool at $4\text{ }^{\circ}\text{C}$ 5 min~10 min, the protein will be salted out; centrifuge at $4\text{ }^{\circ}\text{C}$, 12 000 g for 10 min, transfer the supernatant to another clean 1.5 mL centrifuge tube, centrifuge at 12,000 g for 10 min at $4\text{ }^{\circ}\text{C}$, transfer the supernatant to another clean 1.5 mL centrifuge tube, and add an equal volume Isopropanol, gently shake, place at $4\text{ }^{\circ}\text{C}$ for 15 min; $4\text{ }^{\circ}\text{C}$, 12 000 g centrifugation for 10 min to precipitate DNA; discard the supernatant, add 1 mL 75% Ethanol, mix gently, centrifuge at $4\text{ }^{\circ}\text{C}$, 12 000 r/min for 5 min, discard the supernatant, and dry; after drying, add 50 μL of deionized water to dissolve;

The DNA extraction solution should be placed on ice for later use. If long-term storage is required, it should be stored at $\leq -20\text{ }^{\circ}\text{C}$.

You can also use a proven and reliable DNA extraction and purification kit for nucleic acid extraction, and refer to the instructions for use.