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

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**Technical specification for DNA barcode detection of livestock
and poultry components
(English Translation)**

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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.

1 Scope

This document specifies the DNA bar code detection method for livestock and poultry components.

This document is applicable to the detection of single species samples of livestock and poultry, and not applicable to the detection of animal components in mixed samples of multiple species.

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 GB/T 27401
Laboratory Quality Control Specification Animal Quarantine GB/T 27403 Laboratory Quality
Control Specification Food Molecular Biology Testing

3.1 Terms and definitions

The following terms and definitions apply to this document.

3.1.1

Livestock

Mammals, such as pigs, cows, sheep, goats, horses, donkeys, mules, and camels, that are domesticated and can be artificially controlled to breed in captivity Camels, rabbits, cats, dogs, etc.

3.1.2

Poultry

The ornithological animals raised in captivity to obtain their meat, eggs and feathers are generally pheasant and ducks, such as chickens, ducks, geese, etc.

There are also other families of birds such as turkeys, pigeons, quail and so on.

3.2 Abbreviations

The following abbreviations apply to this document.

4 Principle

DNA barcode refers to the standard, sufficiently varied, and easy to amplify that can represent the species in the organism and relatively short DNA fragments. When encountering an unknown species or part of a species, by drawing the DNA strips of its organization Code, compare with other barcodes in the international database, if it matches with one of them, the species can be confirmed. Used in this standard PCR primers can

simultaneously amplify a segment of mitochondrion DNA from domestic animals, poultry and even more vertebrates.

Analysis and comparison for species identification.

5.1 DNA extraction reagents

5.1.1

Lysis solution, see Appendix A for the preparation method.

5.1.2

Phenol.

5.1.3

Mixture of chloroform and isoamyl alcohol (mixed in a ratio of 24.1).

5.1.4 3 mol/L sodium acetate.

5.1.5

Anhydrous ethanol.

5.1.6 70% ethanol.

5.1.7

TE buffer (Tris-EDTA buffer), see Appendix A for the preparation method.

5.2 PCR amplification reagents

5.2.1

Master Mix (2x).

6 Equipment

6.1

Electronic balance.

6.2

Micropipette.

6.3

Constant temperature water bath.

6.4

Bench-top refrigerated centrifuge.

6.5

Nucleic acid protein analyzer or ultraviolet spectrophotometer.

6.6

PCR detection system.

6.7

Electrophoresis instrument.

6.8

Gel imaging analysis system.

7.1 Introduction

7.1.1

The process of total DNA extraction, DNA concentration and purity determination, PCR amplification, and gel electrophoresis analysis of the sample shall be in accordance with GB/T 27401 and the requirements of GB/T 27403 shall be carried out.

7.1.2

For dried samples, first wash the surface with water or scrape off the animal or human DNA that may be contaminated on the surface. For fresh samples Samples should be taken from the inside of the sample.

7.1.3

Establish quality control negative control and positive sample control, and use the same method to extract DNA for negative control, positive control and test sample.

The positive control uses any kind of livestock and poultry meat samples (such as cattle, sheep, pigs, chickens, ducks, geese, etc.), and the negative control uses any non-ridged for vertebral meat samples (such as shrimp, crabs, starfish, etc.), the negative samples should be free of other animal and human DNA contamination.

7.1.4

Equivalent DNA extraction reagents can be used for DNA extraction.

7.2 Extraction of sample total DNA

7.2.1

For solid samples, such as muscles, internal organs, fur, teeth, bones, head horns, etc., they should be fully crushed, ground, and mixed.

100 mg is used as the extract; for liquid samples, shake and mix well, and take about.200 μ L as the extract.