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Molecular biomarker analysis — Methods of analysis for the detection and identification of animal species in food and feed products (nucleotide sequencing-based methods) —

Part 1: General requirements

Analyse moléculaire de biomarqueurs — Méthodes d'analyse pour la détection et l'identification d'espèces animales dans les aliments et les aliments pour animaux (méthodes basées sur le séquençage des nucléotides) —

Partie 1: Exigences générales

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Foreword

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 16, *Horizontal methods for molecular biomarker analysis*.

A list of all parts in the ISO 22949 series can be found on the ISO website.

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Introduction

This document provides general guidance for deoxyribonucleic acid (DNA) sequencing for animal species detection or identification, or both, in food and feed products. DNA sequencing is the process of determining the order of the four nucleotide bases (adenine, guanine, cytosine and thymine) in a nucleic acid polymer. Nucleic acid polymers can range in length from a few nucleotides to hundreds of millions of bases.

Rapid DNA sequencing methods have been successfully validated and verified for detection and identification of animal species in food products[1]. Within the food industry, rapid, economical and high throughput access to whole genome sequences in foods has improved the control of both food quality and safety[2]. Two types of DNA sequencing methods are most widely used for food products: chain termination and high throughput sequencing[3][4][5][6][7][8].

Chain termination developed in 1977 by Frederick Sanger still bears his name. Sanger sequencing reactions can be prepared manually and electropherograms can be read directly by the user. Automated base calling capillary electrophoresis systems have mostly replaced manually read gels and rapid Sanger applications are being developed.

High throughput or next generation sequencing (NGS), including next generation short-read and third generation long-read methods, has reduced the cost of DNA sequencing, improved sequence readability and automated most of the steps from preparatory to bioinformatics. High throughput automated DNA sequencing applies base/wavelength specific fluorescence or ionic detection to determine the real-time enzymatic addition of nucleotides to a DNA template.

Sanger sequencing (dideoxy chain termination) generates high quality data for determining a single DNA sequence of an individual target. NGS, by contrast, can be used to assess millions of individual DNA fragments of mixed markers and targets at the same time.

Sanger sequencing and NGS can both be used to verify animal species composition in a food sample or compare DNA sequence results to previously defined databases to identify its animal origin, or both[9].