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Review Article | Journal of Nutritional Health & Food Science | Open Access

Special Issue: Food Safety & Hygiene

Forensics: Food Authentication Using MtDNA

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Received: July 13, 2015; Accepted: October 03, 2015; Published: October 20, 2015

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Abstract

There is a clear trend in the food international market towards labelling products with information about their composition and quality. Due to the increase in international trade in seafood and seafood products, authentication has become a necessity. There is a need for suitable methods of identification to ensure compliance with the labelling regulations and thereby to prevent the substitution of fish species.

Keywords: Food Science; mtDNA; Forensic.

Introduction

Consumers require clear and accurate information to make informed choices about their diet and the foods they buy. Their choice might also reflect lifestyle or religious concerns (preference for organic products for vegetarian, absence of pork for Jews and Muslims), or health concerns (e.g. absence of peanuts, lactose or gluten for individuals with particular allergies). Therefore, the description and/or labeling of food must be honest and accurate, particularly if the food has been processed and unable to distinguish one ingredient from another. The information that must be given is enshrined in the laws of most developed countries so that the food supplied must be exactly as in its label. In other words, the food must be authentic and not misdescribed.

According to the Ministry of Agriculture Food and Fisheries (MAFF), UK, [1] food can be misdescribed by several ways, include:

(i) Abstraction or omission of valuable constituents;

(ii) Extending or adulteration of food with a base ingredient;

(iii) The non-declaration of processes and

(iv) Over-declaring a quantitative ingredient or substitution by undeclared components.

In 1962, the Codex Alimentarius Commission was established for implementation of the joint Food and Agriculture Organization of the United Nations and the World Health Organisation (FAO/WHO) standards programme. The aims of Codex Alimentarius include protecting the health of the consumer, ensuring fair practices in the food trade, coordination of all food standard work, publishing regional and world standards, recommending international standards for individual foods and making provision with respect to food hygiene, contaminants, additives, labelling and so on. The Codex recommendations are often used by bodies like the European Union (EU) to formulate their standards [2].

According to the United States Department of Agriculture (USDA), food is considered adulterated when the food article: consists of any filthy, putrid, decomposed or diseased animal or vegetable material; is insect infested or unfit to human consumption; is prepared, packed or stored under insanitary conditions, contains any poisonous ingredients; has been substituted by any inferior or cheaper substance; has had any constituent abstracted; is packed in a container of any poisonous or deleterious substance; has any unpermitted additive present in an amount exceeding the prescribed limit; consist of a quality falling below the prescribed standard; or is not as purported or claimed [3].

It has become a challenging task to identify the species origin of meat and fish, especially in processed meat products. Furthermore, the identification of animal species is one of the areas of major concern for food hygiene laboratories. It is also of considerable importance in forensic medicine and in the quality control of animal products. According to the Minister of International Trade and Industry, the Malaysian Government is committed to make Malaysia the Hub of Halal Food. Food quality and safety has been strongly improved by the EU legislation (178/2002) on food traceability, which came into force in January 2005.

Methods of food analysis have taken advantage of the rapid development of DNA fingerprinting techniques. DNA based techniques have the advantage that one does not need a standard for each tissue because all the cells in an individual have the same DNA. DNA based techniques like FINS (Forensically Informative Nucleotide Sequencing), RFLP (Restriction Fragment Length Polymorphism), SSCP (Single-Strand Conformational

Polymorphism), Random Amplified Polymorphic DNA (RAPD) and LP-RAPD (Long-Primer Random Amplified Polymorphic DNA) and AFLP (Amplified Fragment Length Polymorphism) all can contribute to the establishment of methods for authentication [4] (Table 1). Single Nucleotide Polymorphisms (SNPs) and Restriction Fragment Length Polymorphism (RFLP) are two approaches using Polymerase Chain Reaction (PCR) (Table 1), which have proven to be very useful. Meyer et al. 1994 [5] described the use of the RFLP technique for the detection of pork in cooked meat products. In this instance the RFLP detected was in the gene encoding cyt *b*.

Today, the use of the cyt *b* gene is nearly universal for determining the species of animals, birds and fish in raw and processed food products. The cyt *b* gene is located on mitochondrial DNA (mtDNA) and thus has two advantages [6]. The mtDNA is present in multiple copies compared to nucleus DNA (nDNA) in every cell thus making its detection easier and the mitochondria are likely to remain intact during processing, thereby, minimizing DNA degradation.

Species Identification of Raw and Processed Fish and Meat

Consumers have become more demanding in the choice of foodstuff to avoid commercial frauds or for health issues as for example allergies towards specific components or ingredients [7]. Problems of authentication call for the availability of reliable and rapid methods to assess the hygienic quality of food and to identify food components in meat or fish-based foods. Species identification is important for the implementation of the labeling regulations as set by many countries [8,9]. Food labeling regulations require that the species of meat in meat products to be accurately declared to the consumer [10]. It is vital for preventing possible commercial frauds and guaranteeing the quality and the safety of meat [11]. It is very important to assess that species of high commercial value are not sold, partially or entirely substituted with other species of lower commercial value [7].

Identification of species in food is becoming a very important issue concerning the assessment of food composition, which is necessary to provide consumers accurate information about the products they purchase [12]. There is a need for a new analytical technique, which is sensitive and inexpensive to discriminate the origin of species in minced pork and beef [13]. Beef has been always adulterated with low-cost meat such as pork.

Identification of processed food is necessary as the customer has the right to be informed about products being bought and consumed [14]. Law requires that products should be labeled with official names, thus creating a foundation for discouraging fraud. Regulation by the EC legislation (178/2002) on food traceability [15] requires all stakeholders within the food supply chain must be able to identify the source of all raw materials. There is, therefore, a need for rapid methods for determining the species origin of a biological sample.

Determination of genetic relationships among closely related species is important in animal breeding program [16]. Cattle (Bos indicus), buffalo *(Bubalus bubalis),* goat *(Capra hircus)* and sheep *(Ovis aries)* belong to a single family Bovidae, order Artiodactyla. They are thought to have originated from a single ancestral species and are closely related. However, information on the extent of genetic relationships and diversities at the molecular level in these species is not yet available.

The quantitative detection of meat and fish species in mixed samples has been approached using High-Performance Liquid Chromatography (HPLC) [17]. The HPLC method has proven to be useful for the identification of many different animal species, but the detection limits are restrictive [17]. The detection of Nuclear DNA (nDNA) sequences has also been useful in this regard, but it is limited as a result of their generally low copy number [5].

Attempts to identify beef from meat of other species of animals using various serological and immunological methods have been made by several research workers [18,19]. The myoglobin band has also been used to verify the raw meat [20]. Enzyme-Linked Immunosorbent Assay (ELISA) has been successfully applied in identifying fish species [21,22]. Several electrophoretic techniques are also now available for species identification, including Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE) [23] native or urea-Isoelectric Focusing Electrophoresis (IEF) [23-25] and Two-Dimensional Electrophoresis (2-DE) [26]. Protein electrophoretic techniques however, not reliable for resolving mixtures of meat species in highly processed meat products because the protein profiles of a single species produces a complex banding pattern, which often overlap the species-specific bands [10]. Species differentiation of meat products of closely related species is a difficult task even when raw meat is considered [27]. Recently, DNA rather than protein has been exploited for species identification due to its stability at high temperatures and its structure being conserved within all tissues of an individual. This has resulted in the development of species-specific DNA probes [28], polymerase chain reaction (PCR) assays [5], Random Amplified Polymorphic DNA (RAPD) and polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) [29].

In livestock animals, DNA markers have been used for pedigree registration, individual identification, parentage test and removal of carrier individuals with genetic diseases [11]. Molecular DNA markers have become essential tools for the

Table 1: Methods of food analysis.

Food analysis technique	
FINS	forensically informative nucleotide sequencing
SSCP	single-strand conformational polymorphism
RAPD	Random Amplified Polymorphic DNA
LP-RAPD	long-primer random amplified polymorphic DNA
AFLP	amplified fragment length polymorphism
SNPs	Single nucleotide polymorphisms
RFLP	Restriction Fragment Length Polymorphism

identification of marine species [30,31]. DNA is a relatively stable molecule, which is much better able to withstand heat processing even though it will be in fragmented form. After autoclaving, the average size of DNA fragments has been shown to be 300–400 base pairs (bp) [5,27]. DNA techniques, based on PCR, coupled to Single Strand Conformational Polymorphism Analysis(SSCP) [32] and RFLP [33] or oligonucleotide probe hybridization assays [34] have been shown to be successful with cooked meats but are cumbersome, technically difficult and time consuming. Hybridization of DNA extracted from meat to probes recognizing the species-specific satellite repeats can be used to discriminate related species, e.g. sheep versus cattle; chicken versus turkey [34].

Reports have been published which have focused on the use of species-specific primers [35,36]. However, these reports have not simplified or increased the speed of analysis for the differentiation of chicken and turkey. Recently, a multiplex PCR technique was developed for the identification of meat of domesticated animals such as cow, sheep and goat [37].

Unseld et al. 1995 [38] have amplified the mitochondrial cyt *b* gene and then the PCR products were studied by RFLP analysis. PCR-RFLP analysis of mtDNA [14] is used to identify the species origin of meat samples. Zhang et al. (2006) [39] have developed a PCR-RFLP method to distinguish species of red snappers among commercial salted fish products by amplifying mitochondrial 12S rRNA gene. They successfully discriminated the red snappers *Lutjanus sanguineus*, *Lutjanus erythopterus* from *Lutjanus argentimaculatus*, *Lutjanus malabaricus* and other morphologically similar fishes such as *Lethrinus lutjanus* and *Pinjalo pinjalo.*

An accurately labeled food product is always authentic. A number of papers describing the exploitation to authenticate food components [4] have been published. The adulteration of meats and meat products by the addition of low cost meats from different species has been reported in USA, England, Australia and Italy. Mortara goose salami, a typical product of the Lomellina zone (Italy), has to be protected because it is often substituted with lesser quality ones; typical frauds are duck (*Cairina moschata*), chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), goose (*Anser anser*), pork (*Sus scrofa*), duck (*Anas plathyrhincos*) meat or skin for the content or for the envelope [40]. Obviously this practice serves economic purposes [41]. Therefore, suitable methods are needed in order to detect this practice.

In the early 1990s, inexpensive beef was imported to Japan. As a consequence, the cheap meat competed with Holstein beef in the Japan market. In order to compete, Japan-based beef producers worked to create a first filial hybrid (F1) which is a cross of Japanese Black bulls with Holstein cows. FI beef was misbranded as Japanese Black beef, since the two breeds cannot be easily distinguished by appearance [11]. In China, there are many laws to protect the Chinese water deer (*Hydropotes inermis* Swinhoe), the Chinese muntjac (*Muntiacus reevesi reevesi* Ogilby), the black muntjac (*Muntiacus crinifrons* Sclater) and the muping tufted deer *(Elaphodus cephalophus michianus)* Swinhoe deer from illegal trade and poachers [42].

The price of Chinese alligator meat is very high, currently in excess of US 120 Dollars/kg [43]. Legal commercial products from farmed Chinese alligator's meat have appeared in the Chinese market. It is important to develop an effective method to identify the Chinese alligator meat to prevent the development of illegal trade of Chinese alligators and the hunting [43].

Implication of Adulteration

Adulteration may expose sensitive individuals who may have specific food allergies to allergens [44]. The necessity to identify different species in foodstuff is an important aspect to consider when allergic problems towards specific species are taken into account [7].

The risk associated with infectious transmissible spongiform encephalopathy in humans has discouraged many individuals around the globe from consuming beef [17]. The practice of utilizing ruminant carcasses in animal feed for livestock is responsible for the spread of Bovine Spongiform Encephalopathy (BSE), commonly known as mad cow disease, to epidemic proportions [45]. As a result, the need for sensitive detection of ruminant species remains in animal feed is a paramount agricultural issue [17].

Serious food poisoning incidents due to ingestion of toxic puffer fish or toxic dried dressed fish fillets have occasionally occurred in Taiwan [46,47]. *Lagocephalus gloveri* is the nontoxic puffer fish whose muscle contains no tetrodotoxin (TTX). The muscles of *Lagocephalus lunaris* and *Takifugu oblongus* accumulate lethal levels of TTX. Therefore *Lagocephalus gloveri* is widely used than *Lagocephalus lunaris* or *Takifugu oblongus* for the preparation of dried dressed fish fillets. It is because of morphological similarities between *Lagocephalus lunaris* and *Lagocephalus gloveri* that both the manufacturers and consumers would identify the species ambiguously. Additionally, *Takifugu oblongus*, one of the toxic puffer fish in Taiwan, is often abused as the material for the preparation of the dried dressed fish fillet [48].

Mitochondria

The mitochondria, plural for mitochondrion, (Greek: mitos, thread + chondros, granule) are small membrane-bound organelles that produce most of the energy in cells of oxygen respiring organisms. Mitochondria are currently used for the identification of meat species in food [29,36,37,49-51] and animal feedstuffs [52,53]. Cattle and bison differentiation with mtDNA have been described for the purpose of wildlife conservation [54,55].

The advantage of mitochondrial-based DNA analyses derives from the fact that there are many mitochondria per cell and many mtDNA molecules within each mitochondrion, making mtDNA a naturally amplified source of genetic variation [37]. Recently, PCR-based methods using multicopy nDNA sequences such as satellite DNA and repetitive elements [56] have been introduced. Since the work of Kocher et al. 1989 [57], the use of mitochondrial genes and in particular the cyt *b* gene for meat species identification has been nearly universal [37,58].

It is known that individual copy number per cell of ribosomal RNA (rRNA) and mtDNA region are significantly higher than genomic DNA [59,60]. This information can be used to estimate the postmortem interval and may provide information about the place of decease itself [59]. The cyt *b* locus has been well characterized among different vertebrate groups [61,62]. Studies have revealed that the level of cyt *b* gene sequence variation is suitable for addressing general questions on inter-specific diversity [14].

Mitochondrial DNA

The mtDNA is generally 16-17 kb in size. MtDNA is a closed double-stranded (called Heavy-strand and L-strand) circle. As all cells possess only one nucleus but several hundred or thousand mitochondria, mtDNA is present in great excess over nuclear DNA in most cells [63].

MtDNA mutates occasionally and they also have a very high mutation rate, in the order of 10- to 17-fold higher than that of nDNA genes. Mutated mtDNA creates a mixed intracellular population of mutant and normal molecules which known as heteroplasmy. The dividing heteroplasmic cell randomly partitioned the mtDNA into the daughter cells. As times goes on, the percentage of mutant mtDNA in different cell lineages can drift toward pure mutant or normal (homoplasmy). This process is known as replicative segregation [64].

The mitochondrial genome is one of the best studied of all types of DNA [65]. In general, animal mtDNA is a small, circular molecule, with a high evolutionary rate and a much conserved gene order and content [66]. One of the characteristics of mtDNA is a mix of conserved regions and others showing a high substitution rate.

Mitochondrial genes have been used in studies ranging from phylogeny reconstruction and gene evolution to intraspecific phylogeography and gene flow [67,68]. MtDNA has some advantages over nDNA which has also been used to resolve evolutionary relationships among closely related species of the *Thunnus* genus [69,70]. MtDNA evolves faster than nDNA [71], probably due to inefficient replication repair. Different regions of the mitochondrial genome evolve at different rates [72] allowing suitable regions to be chosen for the question under study.

Some parts of the gene are more conserved than others due to functional restrictions [73]. Most of the variable positions seem to be located within the coding regions for transmembrane domains or for the amino- and carboxy-terminal ends [62]. MtDNA is maternally inherited in most species and does not recombine [74]. Individuals are usually homoplasmic for one mitochondrial haplotype. This means that each molecule as a whole usually has a single genealogical history through maternal lineages.

Whether the mtDNA can be considered a strictly neutral marker has been controversial [75]. Ballard and Kreitman 1995 [76] pointed out in their review that selection on any part of the mtDNA has an influence on polymorphism in the whole molecule in the population because, the lack of recombination makes mitochondrial genomes particularly susceptible to genetic hitchhiking. The heterogeneous substitution rates among lineages and the relative excess of replacement polymorphism also support the idea that selection has a role in mtDNA polymorphism.

In any case, as mtDNA exhibits a certain degree of intraspecific variability, one should always be careful when studying differences among organisms based on single base polymorphisms [77]. Terol et al. 2002 [78] reported the analysis of partial sequence of the mitochondrial cyt *b* gene to identify three tuna species. Those polymorphic sites of these sequences that did not present intraspecific variation were given a diagnostic value [78].

MtDNA analysis can be performed when the quantity and quality of DNA are insufficient for nDNA analysis or when DNA analysis through a maternal lineage is required. Analysis of mtDNA seems to have more advantages as compared with genomic DNA. MtDNA evolves much faster than nuclear and presents more sequence diversity, thus facilitating the identification of closely related species [58]. Moreover, while the required amount of tissue for mtDNA based analysis is very small, due to the high copy number of the mitochondrial genome. Techniques based on the analysis of nucleic acids such as mtDNA or nDNA present advantages over protein-based techniques as they are not dependent on tissue source, age of the individual or/ and sample damage[58] .

Cyt *b* is perhaps the best studied of all mitochondrial genes, particularly for fishes [79]. The gene has both conserved and variable regions, and has proven to be useful for investigating relationships of both closely and distantly related species. Studies of cyt *b* sequence variation have shown this region to be well adapted for studying evolutionary relationships in Actinopterygian fishes [79]. Cyt *b* of respiratory chain is found only in the mitochondria of higher animals and plants [80] and cyt *b* is the only one encoded by the mitochondrial genome [62] among the 9-10 proteins that make up complex III of the mitochondrial oxidative phosphorylation system [61]. However, nuclear pseudogenes could be potentially significant source of artifacts [29].

The cyt *b* gene is the most widely used gene for phylogenetic work for several reasons. Although it evolves slowly in terms of non-synonymous substitutions, the rate of evolution in silent positions is relatively fast [62]. The wide use of cyt *b* has created a status as a universal metric, in the sense that studies can be easily compared. Cyt *b* is thought to be variable enough for population level questions, and conserved enough for clarifying deeper phylogenetic relationships. However, the cyt *b* gene is under strong evolutionary constraints anyhow to be the best choice for resolving relatively recent evolutionary history.

Rate of mtDNA Evolution

Studies using restriction enzymes indicate that mtDNA generally evolves at elevated rates (5-10 times faster) compared to single copy nuclear gene [71]. These findings were confirmed by actual sequences, after the development of the DNA

sequencing techniques [71]. The faster mtDNA evolution is due to a higher frequency point and length mutation. The rate of silent substitution, mainly transitions, is about 4-6 times that of replacement substitution.

Several reasons for mtDNA evolution have been suggested e.g. mtDNA polymerase might not be present therefore mitochondria might lack an efficient system for the removal of pyrimidine dimers. Furthermore, greater exposure of mtDNA to oxidizing agents like radicals and superoxide might cause a higher mutation rate. A more error-prone DNA replication system, lack of editing, the high turnover of mtDNA have all been implicated as partly responsible for high rate of evolution of mtDNA [71]. They do not code for proteins involved in translation, replication or transcription [81]. Nevertheless, DNA repair enzymes (e.g. uracil-DNA glycosylase and AP endonuclease) have been identified in mammalian mitochondrial systems [82].

Each mitochondrial protein-coding gene has its own particular rate of evolution depending on factors such as functional constraints on the gene product and base compositional biases [83]. The gene coding for the subunits of Cytochrome Oxidase (CO) genes and cyt *b* genes are the more conserved genes, and the most variable ones are the NADH dehydrogenase subunit 2 (ND2) genes and Adenosine Triphosphatase (ATPase) genes [84,85]. Despite the fast rate of mtDNA evolution some genes may be highly conserved; there may be a low ceiling for the total divergence, which is partly due to nucleotide base compositional biases and strong functional constraints [83].

Restriction Enzymes

Restriction endonucleases occur ubiquitously among prokaryotic organisms [86,87]. Their principal biological function is the protection of the host genome against foreign DNA, in particular bacteriophage DNA [88]. Other functions are still being discussed, such as an involvement in recombination and transposition [89-92]. In addition, there is evidence that the genes for restriction and modification enzymes may act together as selfish elements [93].

Types of restriction enzymes

Three types of restriction modification RM (type I, II and III) have been found and were classified according to their subunit composition, cofactor requirement and mode of action [94]. The majority of all restriction endonucleases are homodimeric type II restriction endonucleases that recognize short (4–8 bp) palindromic DNA sequences [95]. Type II restriction endonucleases have been of great value to research because most of them cut within the recognition sequence, making cleavage absolutely sequence specific [96]. The type II sequence-specific endonucleases are the most widely used ones in molecular biology [97].

Restriction Enzyme Cleavage

The interaction of restriction enzyme with DNA is complex due to the large size of DNA. The restriction endonuclease binds to the macromolecular DNA, which is followed by random walking on the DNA. The nucleotide sequence is recognized primarily from the major groove, and many of these enzymes contact every single hydrogen-bonding function on the bases that are accessible from the major groove [98].

The catalysis of phosphodiester bond cleavage by restriction endonucleases can be considered as activation of the catalytic centers by the positioning of the divalent metal ion cofactors and the water molecule with the phosphodiester bond to be cleaved. After phosphodiester bond cleavage in both strands the product is released. The nicked DNA then exists only as a transient enzyme-bound intermediate, and the initial product liberated from the enzyme is the DNA cleaved in both strands [99].

Restriction endonucleases have a remarkable specificity that a single base change can reduce their activity by million fold. The degree of specificity is very important to prevent accidental cleavage of other sites of DNA sequence [95].

Principles of the PCR Method as Applied to Species Identification

PCR is an *in vitro* technique used to amplify the number of copies of a specific region of DNA which lies between two regions of known sequence. PCR makes a huge number of copies of a gene. This is done on an automated PCR thermal cycler or PCR machine. The PCR thermal cycler rapidly heats and cools the PCR reaction mixture [100].

There are three stages in PCR known as denaturation, annealing and extension. In the denaturation PCR step, the double strand melts to single stranded DNA. The DNA complimentary strands are heated to above 90°C at the denaturation step to separate the two strand of the template DNA. In the annealing step, the temperature is lowered to the optimal annealing temperature where the primers bind to the single strand created in the previous PCR step. The temperature of this stage depends on the primers and is usually 5°C below their melting temperature. In the PCR extension step, the mixture is heated up again to an optimum temperature, 72°C for the DNA polymerase enzyme. The enzyme adds bases to the primers segments to build up complementary strands of DNA identical to the original molecule. The PCR polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5' side, to make two double stranded molecules. These PCR steps are repeated for around 30 or 40 cycles [100].

Species identification in foods of animal origin is an important subject in the field of modern food control and new analytical techniques have been developed: several authors have adopted the PCR to identify species in meats [12,28,29,37,40,44,101-105]. PCR technology has also been applied to the detection of commercial fraud in food products, namely truffle species in cans [106] and "Mortara" goose salami [40].

In the field of conservation biology, the molecular genetics diagnosis for the protected species and the interrelated commercial products proved to be greatly promising [107-109]. As a molecular diagnostic tool, PCR has been applied to tissues [27,110]. Each type of sample has inherent and unique difficulties

for adequate sample preparation. Common procedures involve cell lysis to release DNA (and often host DNA as well); DNA clean-up, usually by phenol extraction and concentration by alcohol precipitation of the DNA [44] or with commercial kits [40], followed by resuspension of elution in a minimal volume.

Different PCR-based techniques, such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), PCR fingerprinting, quantitative competitive (QC) PCR, multiplex haplotype-specific PCR and nested primer PCR, have been adapted to the identification of species[16,111-113], which may be simpler and more efficient than the serological test. In particular, because of its speed, reproducibility, high sensitivity, and specificity, diagnostic PCR has been used in laboratories for the identification of animal species[112].

PCR-based methods using microsatellite [56] have been introduced recently. Microsatellites tend to be evenly distributed in the genome on all chromosomes and all regions of the chromosome. They have been found inside gene coding regions [114], introns, and in the non-gene sequences. Most microsatellite loci are relatively small, ranging from a few to a few hundred repeats. The relatively small size of microsatellite loci is important for PCR-facilitated genotyping. Generally speaking, microsatellites containing a larger number of repeats are more polymorphic, though polymorphism has been observed in microsatellites with as few as five repeats [115]. In a few fish species, Balloux and Lugon-Moulin 2002 [116] have observed alleles with very large differences in repeat numbers, predictive of an infinite allele model.

PCR analysis of species-specific mtDNA sequences is the most common method currently used for identification of meat species in food [29,36,37,49-51] and animal feedstuffs [52,53]. PCR has the potential to meet the need for better diagnostic tools. It is highly sensitive, very specific, inexpensive, and easily adapted to high volume demands [14,117]. The process is rapid, simple, and requires little manual labor [29,118]. Since PCR was first introduced in 1987 [100]researchers have made excellent progress in developing quality PCR-based tests for species identification.

Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) Markers [119] were regarded as the first shot in the genome revolution [120]. Is a technique that exploits variations in homologous DNA sequences? Traditionally, fragments were separated using Southern blot analysis, in which genomic DNA is digested, subjected to electrophoresis through an agarose gel, transferred to a membrane, and visualized by hybridization to specific probes [121].

Most recent analyses replace the tedious Southern blot method with techniques based on the PCR due to the increased amount of DNA produced by the PCR method. PCR products can be digested with restriction enzymes and visualized by simple staining with ethidium bromide [122].

RFLP method is one of the ways to provide useful markers for species identification [105,123-126]. Since specific polymorphic bands can be detected using selective restriction, RFLP is a powerful method for acquiring genome information easily. It has been widely applied for genetic relationship studies, pharmacokinetics species identification [106] and population study. Mabru et al. 2004 [106] reported the usefulness of RFLP markers as a tool to differentiate *Tuber melanosporum* (Perigord truffle) and other truffle species in cans.

PCR-RFLP allows the amplification of a conserved region of DNA sequence using PCR, and the detection of genetic variation between species by digestion of the amplified fragment with restriction enzymes [14]. This technique has been used for speciation by exploiting DNA sequence variation within the mitochondrial D-loop region [54] and cyt *b* gene [29].

Partis et al. 2000 [14] evaluated the potential for the cyt *b* PCR-RFLP method, as developed by Meyer et al. 1995 [29] to be used as a routine analytical tool for species identification in heat-treated and fermented. All bovine species can be identified by convenient, sensitive and versatile PCR-RFLP assays [127]. The cyt *b* PCR-RFLP species identification assay was determined to be a suitable method for the identification of raw or cooked pure species [14]. Sheep and goat meats [28] were confirmed by PCR using the PCR-RFLP technique. The method is versatile as it can be used as a general screen for all vertebrate species [14]. The amplified target is also present in a high copy number in each cell, substantially increases the sensitivity of the PCR assay. The identification of species following restriction enzyme digestion was shown to be simple and straightforward by judicious choice of restriction enzymes.

References

1. Mark Woolfe, Sandy Primrose. Food forensics: using DNA technology to combat misdescription and fraud. 1993.
2. CODEX. Codex Alimentarius. Internation Food Standard. FAO/WHO Food Standards 2006.
3. USDA. United States Department of Agriculture. 2004.
4. Bossier P. Authentication of seafood products by DNA patterns. J Food Sci. 1999; 64: 189–93.
5. Meyer R, Candrian U, Luthy J. Detection of pork in heated meat products by the polymerase chain reaction. J AOAC Int. 1994; 77(3): 617-22.
6. Woolfe M, Primrose S. Food forensics: using DNA technology to combat misdescription and fraud. Trends Biotechnol. 2004; 22(5): 222-6.
7. Comi G, Iacumin L, Rantsiou K, Cantoni C, Cocolin L. Molecular methods for the differentiation of species used in production of cod-fish can detect commercial frauds. Food Control. 2005; 16: 37-42.
8. FAS. Foreign Agricultural Service 2006.
9. Mermelstein MH. A new era in food labeling. Food Technol. 1993; 47: 81–96.
10. Hird H, Goodier R, Hill M. Rapid detection of chicken and turkey in heated meat products using the polymerase chain reaction followed by amplicon visualisation with vistra green. Meat Sci. 2003; 65(3): 1117-23. doi: 10.1016/S0309-1740(02)00341-8.

11. Sasazaki S, Itoh K, Arimitsu S, Imada T, Takasuga A, Nagaishi H, et al. Development of breed identification markers derived from AFLP in beef cattle. Meat Sci. 2004; 67(2): 275-80. doi: 10.1016/j.meatsci.2003.10.016.

12. Rodriguez MA, Garcia T, Gonzalez I, Asensio L, Mayoral B, Lopez-Calleja I, et al. Development of a polymerase chain reaction assay for species identification of goose and mule duck in foie gras products. Meat Sci. 2003; 65(4): 1257-63.

13. Skrokki A, Hormi O. Composition of minced meat part A: Methods. Meat Sci. 1994; 38(3): 497-501. doi: 10.1016/0309-1740(94)90073-6.

14. Pancorbo M, Castro A, Fernández-Fernández I, Cuevas N. Cytochrome b for identification of animal species in processed food. International congress series. 2004; 1261: 592-4.

15. EC. Laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety 2002.

16. Rao KB, Bhat KV, Totey SM. Detection of species-specific genetic markers in farm animals through random amplified polymorphic DNA (RAPD). Genet Anal. 1996; 13(5): 135-8.

17. Walker JA, Hughes DA, Anders BA, Shewale J, Sinha SK, Batzer MA. Quantitative intra-short interspersed element PCR for species-specific DNA identification. Anal Biochem. 2003; 316(2): 259-69.

18. Bansal AK, Mandokhot U. Studies on differentiation of beef from meat of other species of animals. I. Comparative specificity and sensitivity of serum raised in buffalo calves against cattle antigens. J Food Sci Technol. 1988; 25: 146-55.

19. Srinivas S, Reddy PM, Reddy KS. Detection of mutton, beef and buffalo beef with antisera to species liver by double gel immuno-diffusion, immunoelectrophoresis and counter immunoelectrophoresis. J Food Sci Technol. 1991; 28: 123- 5.

20. Downey G, McElhinney J, Fearn T. Species identification in selected raw homogenised meats by reflectance spectroscopy in the mid-infrared, near infrared and visible ranges. Appl Spectrosc. 2000; 54(6): 894-9.

21. Huang TS, Marshall MR, Kao K, Otwell WS, Wei CI. Development of monoclonal antibody for red snapper (Lutjanus campechanus) identification using enzyme-linked immunosorbent assay. J Agric Food Chem. 1995; 43(8): 2301–7.

22. Kang'ethe EK, Jones SJ, Patterson RL. Identification of the species origin of fresh meat using an enzyme-linked immunosorbent assay procedure. Meat Sci. 1982; 7(3): 229-40. doi: 10.1016/0309-1740(82)90088-2.

23. Etienne M, Jerome M, Fleurence J, Rehbein H, Kundiger R, Mendes R, et al. Species identification of formed fishery products and high pressure-treated fish by electrophoresis: a collaborative study. Food Chem. 2001; 72(1): 105–12.

24. Etienne M, Jerome M, Fleurence J, Rehbein H, Kundiger R, Yman IM, et al. A standardized method of identification of raw and heat-processed fish by urea isoelectric focusing: a collaborative study. Electrophoresis. 1999; 20 (10): 1923-33.

25. Mackie I, Craig A, Etienne M, Jérôme M, Fleurence J, Jessen F, et al. Species identification of smoked and gravad fish products by sodium dodecylsulphate polyacrylamide gel electrophoresis, urea isoelectric focusing and native isoelectric focusing: a collaborative study. Food Chem. 2000; 71(1): 1-7.

26. Pineiro C, Barros-Velazquez J, Perez-Martin RI, Martinez I, Jacobsen T, Rehbein H, et al. Development of a sodium dodecyl sulfate-polyacrylamide gel electrophoresis reference method for the analysis and identification of fish species in raw and heat-processed samples: a collaborative study. Electrophoresis. 1999; 20(7): 1425-32.

27. Ebbehoj KF, Thomsen PD. Species differentiation of heated meat products by DNA hybridization. Meat Sci. 1991; 30(3): 221-34. doi: 10.1016/0309-1740(91)90068-2.

28. K Chikuni1, T Tabata, M Kosugiyama, M Monma, M Saito. Polymerase chain reaction assay for detection of sheep and goat meats. Meat Sci. 1994; 37(3): 337-45. doi: 10.1016/0309-1740(94)90051-5.

29. Meyer R, Hofelein C, Luthy J, Candrian U. Polymerase chain reaction-restriction fragment length polymorphism analysis: a simple method for species identification in food. J AOAC Int. 1995; 78(6): 1542-51.

30. Devey ME, Bell JC, Uren TL, Moran GF. A set of microsatellite markers for fingerprinting and breeding applications in Pinus radiata. Genome. 2002; 45(5): 984-9.

31. Paterson S, Piertney SB, Knox D, Gilbey J, Verspoor E. Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (Salmo salar L.) microsatellites. Mol Ecol Notes. 2004; 4(2): 160–2.

32. Rehbein H, Mackie IM, Pryde S, Sotelo CG, Medina I, Martin RP, et al. Fish species identification in canned tuna by PCR-SSCP: validation by a collaborative study and investigation of intra-species variability of the DNA-patterns. Food Chem. 1999; 64: 263-8.

33. Tagliavini J, Harrison IJ, Gandolfi G. Discrimination between Anguilla anguilla and A. rostrata by polymerase chain reaction-restriction fragment length polymorphism analysis. J Fish Biol. 1995; 47(4): 741-3.

34. Hunt DJ, Parkes HC, Lumley ID. Identification of the species of origin of raw and cooked meat products using oligonucleotide probes. Food Chem. 1997; 60(3): 437–42.

35. Colgan S, O'Brien L, Maher M, Shilton N, McDonnell K, Ward S. Development of a DNA-based assay for species identification in meat and bone meal. Food Res Int. 2001; 34(5): 409-14.

36. Herman BL. Determination of the animal origin of raw food by species-specific PCR. J Dairy Res. 2001; 68(3): 429-36.

37. Matsunaga T, Chikuni K, Tanabe R, Muroya S, Shibata K, Yamada J, et al. A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Sci. 1999; 51(2): 143-8.

38. Unseld M, Beyermann B, Brandt P, Hiesel R. Identification of the species origin of highly processed meat products by mitochondrial DNA sequences. PCR Methods Appl. 1995; 4(4): 241-3.

39. Zhang J, Huang H, Cai Z, Huang L. Species identification in salted products of red snappers by semi-nested PCR-RFLP based on the mitochondrial 12S rRNA gene sequence. Food Contr. 2006; 17(7): 557-63.

40. Colombo F, Viacava R, Giaretti M. Differentiation of the species ostrich (Struthio camelus) and emu (Dromaius novaehollandiae) by polymerase chain reaction using an ostrich-specific primer pair. Meat Sci. 2000; 56(1): 15-7.

41. Macedo-Silva A, Barbosa SF, Alkmin MG, Vaz AJ, Shimokomaki M, Tenuta-Filho A. Hamburger meat identification by dot-ELISA. Meat Sci. 2000; 56(2): 189-92.

42. Fang S, Wan Q. A genetic fingerprinting test for identifying carcasses of protected deer species in China. Biol Conserv. 2002; 103(4): 371–3.

43. Yan P, Wu X-B, Shi Y, Gu C-M, Wang R-P, Wang C-L. Identification of

Chinese alligators (Alligator sinensis) meat by diagnostic PCR of the mitochondrial cytochrome b gene. Biol Conserv. 2005; 121(1): 45-51.

44. Koh MC, Lim CH, Chua SB, Chew ST, Phang ST. Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. Meat Sci. 1998; 48(3-4): 275-85.

45. Brown P. Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. BMJ. 2001; 322(7290): 841-4.

46. Hwang DF, Cheng CA, Y.H. T, Jeng SS. Tetrodotoxin associated food poisoning due to unknown fish in Taiwan between 1988–1994. J Nat Toxins 1995; 4(2): 165–71.

47. Hwang DF, Hsieh YW, Shiu YC, Chen SK, Cheng CA. Identification of tetrodotoxin and fish species in a dried dressed fish fillet implicated in food poisoning. J Food Prot. 2002; 65(2): 389-92.

48. Hwang DF, Kao, C.Y., Yang, H.C., Jeng, S.S., Noguchi T. and Hashimoto, K. 1992. Toxicity of puffer in Taiwan. Nippon Suisan Gakkaishi 58: 1541–1547.

49. Lahiff S, Glennon M, O'Brien L, Lyng J, Smith T, Maher M, et al. Species-specific PCR for the identification of ovine, porcine and chicken species in meta and bone meal (MBM). Mol Cell Probes. 2001; 15(1): 27-35.

50. Montiel-Sosa JF, Ruiz-Pesini E, Montoya J, Roncales P, Lopez-Perez MJ, Perez-Martos A. Direct and highly species-specific detection of pork meat and fat in meat products by PCR amplification of mitochondrial DNA. J Agric Food Chem. 2000; 48(7): 2829-32.

51. Partis L, Croan D, Guo Z, Clark R, Coldham T, Murby J. Evaluation of a DNA fingerprinting method for determining the species origin of meats. Meat Sci. 2000; 54(4): 369-76.

52. Bellagamba F, Moretti VM, Comincini S, Valfre F. Identification of species in animal feedstuffs by polymerase chain reaction--restriction fragment length polymorphism analysis of mitochondrial DNA. J Agric Food Chem. 2001; 49(8): 3775-81.

53. Tartaglia M, Saulle E, Pestalozza S, Morelli L, Antonucci G, Battaglia PA. Detection of bovine mitochondrial DNA in ruminant feeds: a molecular approach to test for the presence of bovine-derived materials. J Food Prot. 1998; 61(5): 513-8.

54. Murray BW, McClymont RA, Strobeck C. Forensic identification of ungulate species using restriction digests of PCR-amplified mitochondrial DNA. J Forensic Sci. 1995; 40(6): 943-51.

55. Ward TJ, Bielawski JP, Davis SK, Templeton JW, Derr JN. Identifiction of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap. Anim Conserv. 1999; 2(1): 51–7.

56. Calvo JH, Rodellar C, Zaragoza P, Osta R. Beef- and bovine-derived material identification in processed and unprocessed food and feed by PCR amplification. J Agric Food Chem. 2002; 50(19): 5262-4.

57. Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, et al. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A. 1989; 86(16): 6196-200.

58. Wolf C, Rentsch J, Hubner P. PCR-FRLP analysis of mitochondrial DNA: a reliable method for species identification. J Agr Food Chem. 1999; 47(4):1350–5.

59. Levin BC, Cheng H, Reeder DJ. A human mitochondrial DNA standard reference material for quality control in forensic identification, medical diagnosis, and mutation detection. Genomics. 1999; 55(2): 135-46.

60. Long EO, Dawid IB. Repeated genes in eukaryotes. Annu Rev Biochem. 1980; 49: 727-64.

61. Hatefi Y. The mitochondrial electron transport and oxidative phosphorylation system. Annu Rev Biochem. 1985; 54: 1015-69.

62. Irwin DM, Kocher TD, Wilson AC. Evolution of the cytochrome b gene of mammals. J Mol Evol. 1991; 32(2): 128-44.

63. Hutchison CA, 3rd, Newbold JE, Potter SS, Edgell MH. Maternal inheritance of mammalian mitochondrial DNA. Nature. 1974; 251(5475): 536-8.

64. Cann RL, Stoneking M, Wilson AC. Mitochondrial DNA and human evolution. Nature. 1987; 325(6099): 31-6.

65. Kocher TD, Carleton KL. Base substitution in fish mitochondrial DNA: patterns and rates. Kocher TD, Steppian CA. Molecular systematics of fishes. New York: Academic Press; 1997. p. 13–24.

66. Gray MW. Origin and evolution of mitochondrial DNA. Annu Rev Cell Biol. 1989; 5: 25-50.

67. Avise JC. Phylogeography. London: Harvard Univ. Press; 2000.

68. Stepien CA, Kocher TD. Molecules and morphology in studies of fish evolution. Kocher TD, Stepien CA. San Diego, CA: Academic Press; 1997.

69. Alvarado- Bremer JR, Stequert B, Robertson NW, Ely B. Genetic evidence for inter-oceanic subdivision of bigeye tuna (Thunnus obesus) populations. Marine Biol. 1998 132(4): 547-57.

70. Bartlett SE, Davidson WS. Identification of Thunnus tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. Can J Fish Aquat Sci. 1991; 48(2): 309-17.

71. Brown GG, Simpson MV. Novel features of animal mtDNA evolution as shown by sequences of two rat cytochrome oxidase subunit II genes. Proc Natl Acad Sci U S A. 1982; 79(10): 3246-50.

72. Saccone C, De Giorgi C, Gissi C, Pesole G, Reyes A. Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. Gene. 1999; 238(1): 195-209.

73. Meyer A. Shortcomings of the cytochrome b gene as a molecular marker. Trends Ecol Evol. 1994; 9(8):278-80. doi: 10.1016/0169-5347(94)90028-0.

74. Hayashi J, Tagashira Y, Yoshida MC. Absence of extensive recombination between inter- and intraspecies mitochondrial DNA in mammalian cells. Exp Cell Res. 1985; 160(2): 387-95.

75. Rand DM, Kann LM. Excess amino acid polymorphism in mitochondrial DNA: contrasts among genes from Drosophila, mice, and humans. Mol Biol Evol. 1996; 13(6): 735-48.

76. Ballard JW, Kreitman M. Is mitochondrial DNA a strictly neutral marker? Trends Ecol Evol. 1995; 10(12): 485-8.

77. Unseld M, Marienfeld JR, Brandt P, Brennicke A. The mitochondrial genome of Arabidopsis thaliana contains 57 genes in 366,924 nucleotides. Nat Genet. 1997; 15(1): 57-61.

78. Terol J, Bargues M, Carrasco P, Perez-Alonso M, Paricio N. Molecular characterization and evolution of the protein phosphatase 2A B' regulatory subunit family in plants. Plant Physiol. 2002; 129(2): 808-22.

79. Lydeard C, Roe KJ. The phylogenetic utility of the mitochondrial cytochrome b gene for inferring relationships among actinopterygian fishes. Kocher TD, Stepien CA. New York: Academic Press; 1997. 18 p.

80. Scott T, Eagleson M. Concise Encyclopedia of Biochemistry Walt de Gruy. 1988; 2ed: 432–3.

81. Hasegawa M, Kishino H. Heterogeneity of tempo and mode of mitochondrial DNA evolution among mammalian orders. Jpn J Genet. 1989; 64(4): 243-58.

82. Tomkinson AE, Bronk RT, Linn S. Mammalian mitochondria endonucleases activities specific for ultraviolet-irradieted DNA. Nucleic Acids Res. 1990;18(4): 929-35.

83. Piercy R, Sullivan KM, Benson N, Gill P. The application of mitochondrial DNA typing to the study of white Caucasian genetic identification. Int J Legal Med. 1993; 106(2): 85-90.

84. Johansen S, Guddal PH, Johansen T. Organization of the mitochondrial genome of Atlantic cod, Gadus morhua. Nucleic Acids Res. 1990; 18(3): 411-9.

85. Roe BA, Ma DP, Wilson RK, Wong JF. The complete nucleotide sequence of the Xenopus laevis mitochondrial genome. J Biol Chem. 1985; 260(17): 9759-74.

86. Raleigh EA, Brooks JE. Restriction Modification systems: Where They Are and What They Do. de Bruijn FJ, Lupski JR, Weinstock GM. New York: Chapman and Hall; 1998.

87. Roberts RJ, Halford SE. Type II restriction endonucleases. Linn SM, Lloyd RS, Roberts RJ. New York: Cold Spring Harbor Laboratory Press; 1993.

88. Arber W. Promotion and limitation of genetic exchange. Science. 1979; 205(4404): 361-5.

89. Bilcock DT, Daniels LE, Bath AJ, Halford SE. Reactions of type II restriction endonucleases with 8-base pair recognition sites. J Biol Chem. 1999; 274(51): 36379-86.

90. Carlson K, Kosturko LD. Endonuclease II of coliphage T4: a recombinase disguised as a restriction endonuclease? Mol Microbiol. 1998; 27(4): 671-6.

91. Heitman J. On the origins, structures and functions of restriction-modification enzymes. Setlow JK, New York: Plenum Press; 1993. P.51.

92. McKane M, Milkman R. Transduction, restriction and recombination patterns in Escherichia coli. Genetics. 1995; 139(1): 35-43.

93. Naito T, Kusano K, Kobayashi I. Selfish behavior of restriction-modification systems. Science. 1995; 267(5199): 897-9.

94. Wilson GG, Murray NE. Restriction and modification systems. Annu Rev Genet. 1991; 25: 585-627.

95. Pingoud A, Jeltsch A. Recognition and cleavage of DNA by type-II restriction endonucleases. Eur J Biochem. 1997; 246(1): 1-22.

96. Roberts RJ, Macelis D. Redase- restriction enzymes and methylases. Nucleic Acids Res. 2001; 29(1): 268-9.

97. Redaschi N, Bickle TA. DNA restriction and modification systems. Neidhardt FC. Washington, DC: ASM Press; 1996.

98. von Hippel PH, Berg OG. Facilitated target location in biological systems. J Biol Chem. 1989; 264(2): 675-8.

99. Hollfelder F, Herschlag D. The nature of the transition state for enzyme-catalyzed phosphoryl transfer. Hydrolysis of O-aryl phosphorothioates by alkaline phosphatase. Biochemistry. 1995; 34(38): 12255-64.

100. Mullis KB, Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. 1987; 155: 335-50.

101. Abdulmawjood A, Buelte M. Identification of Ostrich meat by restriction fragment length polymorphism (RFLP) analysis of cytochrome b gene. J Food Sci. 2002; 67(5): 1688–91.

102. Chang KC, Beuzen ND, Hall AD. Identification of microsatellites in expressed muscle genes: assessment of a desmin (CT) dinucleotide repeat as a marker for meat quality. Vet J. 2003; 165(2): 157-63.

103. Fei S, Okayama T, Yamanoue M, Nishikawa I, H. M, Tsuji S. Species identification of meats and meat products by PCR. Animal Sci Technol. 1996; 67(10): 900–5.

104. McVeigh HP, Bartlett SE, Davidson WS. Polymerase chain reaction/direct sequence analysis of the cytochrome b gene in Salmo salar. Aquacult. 1991; 95(3-4): 225–33.

105. Sanjuan A, Comesana AS. Molecular identification of nine commercial flaffish species by polymerase chain reaction-restriction fragment length polymorphism analysis of a segment of the cytochrome b region. J Food Prot. 2002; 65(6): 1016-23.

106. Mabru D, Douet JP, Mouton A, Dupre C, Ricard JM, Medina B, et al. PCR-RFLP using a SNP on the mitochondrial Lsu-rDNA as an easy method to differentiate Tuber melanosporum (Perigord truffle) and other truffle species in cans. Int J Food Microbiol. 2004; 94(1): 33-42.

107. Baker CS, Palumbi SR. Which whales are hunted? A molecular genetic approach to monitoring whaling. Science. 1994; 265(5178): 1538–9.

108. Cronin MA, Palmisciano DA, Vyse ER, Cameron DG. Mitochondrial DNA in wildlife froensic science: species identification of tissues. Wildlife Soc Bull. 1991; 19(1): 94-105.

109. DeSalle R, Birstein VJ. PCR identification of black caviar. Nature. 1996; 381: 197–8.

110. Saez R, Sanz Y, Toldra F. PCR-based fingerprinting techniques for rapid detection of animal species in meat products. Meat Sci. 2004; 66(3): 659-65. doi: 10.1016/S0309-1740(03)00186-4.

111. Axayácatl Rocha-Olivares. Multiplex haplotype-specific PCR: a new approach for species identification of the early life stages of rockfishes of the species-rich genus Sebastes Cuvier. J Exp Marine Biol Eco. 1998; 231: 279-90.

112. Miguel AP, Begona PV. Identification of commercial canned tuna species by restriction site analysis of mitochondrial DNA products obtained by nested primer PCR. Food Chem. 2004; 86: 143-50.

113. Wolf C, Luthy J. Quantitative competitive (QC) PCR for quantification of porcine DNA. Meat Sci. 2001; 57(2): 161-8.

114. Liu ZQ, Wang YQ, Zhou KY, Han DM, Yang XG, Liu XH. Authentication of Chinese crude drug, gecko, by allele-specific diagnostic PCR. Planta Med. 2001; 67(4): 385-7.

115. Karsi A, Cao D, Li P, Patterson A, Kocabas A, Feng J, et al. Transcriptome analysis of channel catfish (Ictalurus punctatus): initial analysis of gene expression and microsatellite-containing cDNAs in the skin. Gene. 2002; 285(1-2): 157-68.

116. Balloux F, Lugon-Moulin N. The estimation of population differentiation with microsatellite markers. Mol Ecol. 2002; 11(2): 155-65.

117. Al-Talib H, Yean CY, Al-Khateeb A, Hassan H, Singh KK, Al-Jashamy K, et al. A pentaplex PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus and Panton-Valentine Leucocidin. BMC Microbiol. 2009; 9: 113. doi: 10.1186/1471-2180-9-113.

118. Al-Talib H, Yean CY, Al-Khateeb A, Hasan H, Ravichandran M. Rapid

detection of methicillin-resistant Staphylococcus aureus by a newly developed dry reagent-based polymerase chain reaction assay. J Microbiol Immunol Infect. 2014; 47(6): 484-90. doi: 10.1016/j.jmii.2013.06.004.

119. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980; 32(3): 314-31.

120. Dodgson JB, Cheng HH, Okimoto R. DNA marker technology: a revolution in animal genetics. Poult Sci. 1997; 76(8): 1108-14.

121. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol. 1975; 98(3): 503-17.

122. Liu ZJ, Cordes JF. DNA marker technologies and their applications in aquaculture genetics. Aquacult. 2004; 238(4): 1-37.

123. Cespedes A, Garcia T, Carrera E, Gonzalez I, Sanz B, Hernandez PE, et al. Polymerase chain reaction-restriction fragment length polymorphism analysis of a short fragment of the cytochrome b gene for identification of flatfish species. J Food Prot. 1998; 61(12): 1684-5.

124. Hold GL, Russell VJ, Pryde SE, Rehbein H, Quinteiro J, Vidal R, et al. Development of a DNA-based method aimed at identifying the fish species present in food products. J Agric Food Chem. 2001; 49(3): 1175-9.

125. Russell VJ, Hold GL, Pryde SE, Rehbein H, Quinteiro J, Rey-Mendez M, et al. Use of restriction fragment length polymorphism to distinguish between salmon species. J Agric Food Chem. 2000; 48(6): 2184-8.

126. Sotelo CG, Calo-Mata P, Chapela MJ, Perez-Martin RI, Rehbein H, Hold GL, et al. Identification of flatfish (Pleuronectiforme) species using DNA-based techniques. J Agric Food Chem. 2001; 49(10): 4562-9.

127. Verkaar EL, Nijman IJ, Boutaga K, Lenstra JA. Differentiation of cattle species in beef by PCR-RFLP of mitochondrial and satellite DNA. Meat Sci. 2002; 60(4): 365-9. doi: 10.1016/S0309-1740(01)00144-9.