Int. J. Food Ferment. Technol., **10**(2): 59-65, December 2020

DOI: 10.30954/2277-9396.02.2020.3

REVIEW PAPER

Nucleic Acids Probes in Seafood Authentication

Wilwet Lidiya[1] and Karunanithi Masilan[2]*

[1]Department of Harvest and Post-Harvest Technology, Central Institute of Fisheries Education, Mumbai, India
[2]Department of Fish Processing Technology, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India

*Corresponding author: masilanakmsara@gmail.com

Paper No.: 245 **Received:** 19-10-2020 **Revised:** 29-11-2020 **Accepted:** 10-12-2020

ABSTRACT

The adulteration of fish and seafood products, in general, is a global challenge that is constantly increasing. Seafood fraud usually involves adulteration or substitution of a type of species with the meat of lower nutritional value and quality, and therefore of the lower price, aiming for economic profit. Besides the consumer financial protection issues, another important matter is the health risks posed by the occurrence of allergic reactions. For these reasons, regular controls of meat products are in great demand, to protect consumers and producers from fraud, and ensure food safety and public health. Therefore, sensitive, fast, simple, inexpensive and effective analytical methods are required to confirm the species origin of seafood-based products and detect seafood fraud. Development of nucleic acid probes paves a way to onsite detection of species within an hour and which is a growing need for food inspecting agencies. All these aspects have been revised.

Keywords: Nucleic acid probes, Seafood, Adulteration, Seafood fraud

A nucleic acid probe is a nucleic acid molecule (single-stranded DNA or RNA) with a strong affinity with a specific target (DNA or RNA sequence). Probe and target base sequences must be complementary to each other, but depending on the conditions, they do not necessarily have to be exactly complementary. The hybrid (probe–target combination) can be revealed when appropriate labeling and detection systems are used. Gene probes are used in various blotting and *in situ* techniques for the detection of nucleic acid sequences. In medicine, they can help in the identification of microorganisms and the diagnosis of infectious, inherited, and other diseases (Fig. 1).

Probe Size

Probes can range in size from as short as 10 nucleotide bases (molecular weight of 3,300) to as long as 10,000 bases or more (molecular weight of 3,300,000). The most common size range for most probes is between 14 and 40 bases. For statistical uniqueness, a minimum of 20 nucleotide bases are usually needed for a probe. Probes can be short or long. Short probes tend to hybridize nucleic acids at very high rates (in minutes) whereas longer probes may require reaction times of hours to achieve a stable hybridization, short probes do have some disadvantages. They are subject to more nonspecific hybridizations, are limited in specificity, and are more difficult to label whereas long probes hybridize more stably than short probes at high temperatures and low salt concentrations (low stringency).

Types of Probes

Mainly Probes can be classified as 3 based on their origin. They are in Fig. 2.

How to cite this article: Lidiya, W. and Masilan, K. (2020). Nucleic Acids Probes in Seafood Authentication. *Int. J. Food Ferment. Technol.*, **10**(2): 59-65.

Source of Support: None; **Conflict of Interest:** None

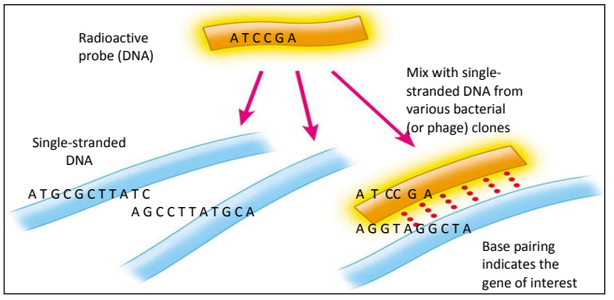

Fig. 1: Nucleic acid probe

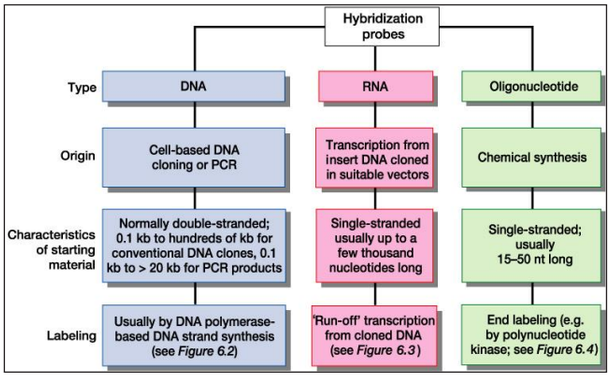

Fig. 2: Classification of probes

Labeling of probes

The detection of homologous sequences after hybridization with the probe is like finding a needle in the hay-stock. Therefore, for the success of DNA probe assay it is necessary to develop simple safe and sensitive techniques for their use. As probes transmit no signal of their own and they have to be either labeled with radioactive isotopes or coupling of non-radioactive signal molecules to the probes without impairing the hybridization ability of these probes. These signal molecules may include fluorescent antibodies, enzymes that produce color changes in dyes and chemiluminescent catalysts. Whatever label is used, it has to be attached to, or incorporated into, the nucleic acid probe. Here are 3 methods of labelling probes:

- ❑ Nick translation
- ❑ Primer extension
- ❑ End labelling

Nick Translation

It is one of the commonly used techniques for producing a radioactive probe. A purified phage or plasmid vector containing a cloned genomic or cDNA sequence is treated with a small amount of pancreatic DNase which hydrolyzes the phosphodiester bonds between nucleotides. At very low concentration the DNase produces only scattered "nicks" in one or other strand of the duplex DNA. DNA polymerase and radioactively labeled deoxynucleotides are also added to the DNA sample. Using the unharmed strand as template, the DNA polymerase synthesizes a new second strand using exposed 3′ end at a nick site as primer, which then displaces the existing DNA from the 5′ end of the nick. Radioactive nucleotides are incorporated into the new strand, so, a single standard probe is created when the duplex DNA is denatured (Fig. 3(a) and 3(b)).

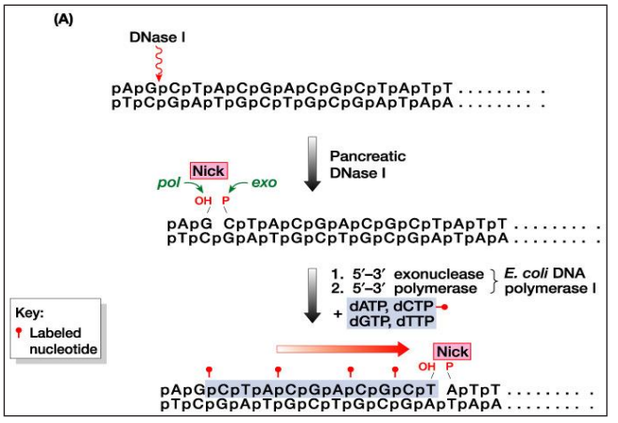

Fig. 3 (a): Nick translation

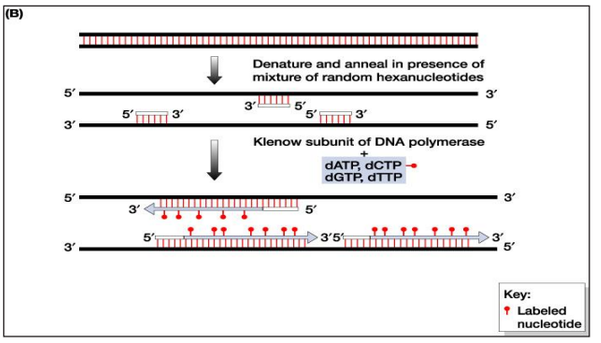

Fig. 3(b): Nick translation

Random-Primed Labeling (or Primer Extension)

Gene probes, cloned or PCR-amplified, and oligonucleotide probes can be random-primed labeled with radioactive isotopes and nonradioactive labels (e.g., DIG). Random-primed labeling of DNA fragments (double- or single-stranded DNA) was developed by Feinberg and Volgestein as an alternative to nick translation to produce uniformly labeled probes. Double-stranded DNA is denatured and annealed with random oligonucleotide primers. The oligonucleotides serve as primers for the 5′ to 3′ polymerase (the Klenow fragment of *E. coli* DNA polymerase I), which synthesizes labeled probes in the presence of a labeled nucleotide precursor (Fig. 4).

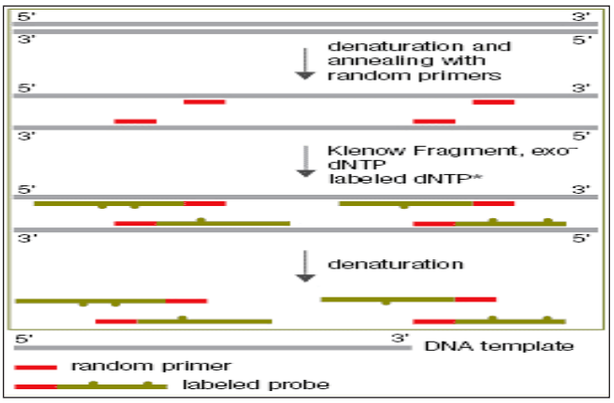

Fig. 4: Random-Primed Labelling (or Primer Extension)

End Labeling

Single-stranded oligonucleotides are usually end-labeled using polynucleotide kinase (kinase end-labeling). The label is provided in the form of a ^{32}P at the γ-phosphate position of ATP and the polynucleotide kinase catalyses an exchange reaction with the 5′-terminal phosphates. The same procedure can also be used for labeling double-stranded DNA, here fragments carrying label at one end only can then be generated by cleavage at an internal restriction site, generating two differently sized fragments which can be separated by gel electrophoresis and purified (Fig. 5).

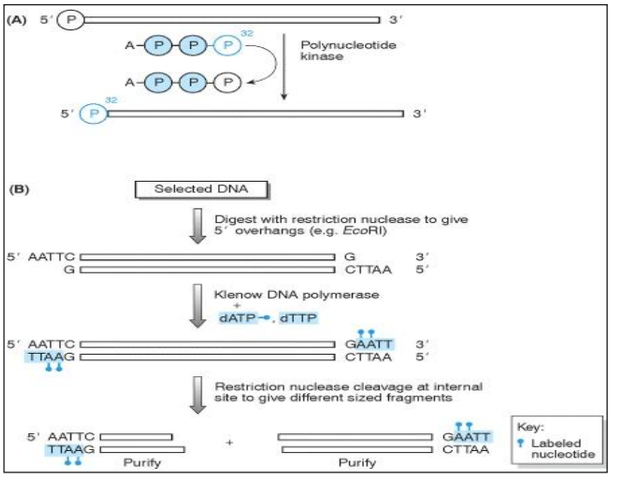

Fig. 5: End labelling

Types of Label

Radioactive Labels

Nucleic acid probes can be labeled using radioactive isotopes (e.g., 32P, 35S, 125I, 3H). Detection is by autoradiography or Geiger–Muller counters. Radiolabeled probes used to be the most common type but are less popular today because of safety considerations as well as cost and disposal of radioactive waste products. However, radiolabeled probes are the most sensitive, as they provide the highest degree of resolution currently available in hybridization assays. High sensitivity means that low concentrations of a probe–target hybrid can be detected; for example, 32P-labeled probes can detect single-copy genes in only 0.5 μg of DNA.

Non-radioactive Labels

Compared to radioactive labels, the use of nonradioactive labels have several advantages like safety, Higher stability of probe, Efficiency of the labeling reaction, Detection *in situ*, Less time taken to detect the signal. Concern over laboratory safety and the economic and environmental aspects of radioactive waste disposal have been key factors in their development and use. Some examples are as follows:

(a) Biotin

Recent advances in nucleic acid technology now offer alternatives to radio activity labeled probes. One of such procedure that is becoming popular is biotin labeling of nucleic acids. This system exploits the affinity which the glycoprotein avidin has for biotin. Avidin is commonly found in egg white. Biotinylated probes are prepared through a nick-translation reaction by replacing nucleotides with biotinylated derivatives. After hybridization and washing, detection of hybrids is done by a series of cytochemical reactions which finally give a blue color whose intensity is proportional to the amount of biotin in the hybrid. These assays employ non-toxic materials, whose half-life is longer and these probes can be prepared in advance in bulk and stored at -20°C for repeated uses. Detection of hybrids is much faster than by radioactive probes.

(b) Enzymes

The enzyme is attached to the probe and its presence usually detected by reaction with a substrate that changes color. Used in this way, the enzyme is sometimes referred to as a "reporter group," Examples of enzymes used include alkaline phosphatase and horseradish peroxidase (HRP). In the presence of peroxide and peroxidase, chloronaphtol, a chromogenic substrate for HRP, forms a purple insoluble product. HRP also catalyzes the oxidation of luminol, a chemiluminogenic substrate for HRP.

(c) Chemiluminescence

In this method, chemiluminescent chemicals attached to the probe are detected by their light emission using a luminometer. Chemiluminescent probes (including the above enzyme labels) can be easily stripped from membranes, allowing the membranes to be reprobed many times without significant loss of resolution.

(d) Fluorescence

Chemicals attached to probe fluoresce under ultraviolet (UV) light. This type of label is especially useful for the direct examination of microbiological or cytological specimens under the microscope—a technique known as fluorescent *in situ* hybridization (FISH). Hugenholts *et al.* have some useful considerations on probe design for FISH.

(e) Antibodies

An antigenic group is coupled to the probe and its presence detected using specific antibodies. Also, monoclonal antibodies have been developed that will recognize DNA–RNA hybrids.

Applications of Molecular Probes

Molecular probes are used in restriction fragment length polymorphisms (RFLPs) and related Analysis

RFLPs for Evolutionary Studies

The restriction fragment length polymorphisms (RFLPs) can be studied in a set of related species using a random or a specific DNA probe. The similarities and differences can be used to infer phylogenetic relationships. This has actually been done in a number of cases both in plants and in animals (Gill *et al*. 1991; Fukuchi *et al*. 1993; Moore *et al*. 1991; Mason-Gamer *et al*. 1998; Deshpande *et al*. 1998; Drinkwater *et al*. 1991; Mburu and Hanotte, 2005; Guimaraes, 2007).

RFLP Markers to Map the Genes in Diseased Persons and Identification of Disease

DNA polymorphisms (many forms) are differences in DNA sequence that result from point mutations, random deletions or insertions or the presence of varying number of repeated copies of a DNA fragments (tandem repeats). A polymorphism in the coding region of a gene may be detected as an alteration in the amino acid sequence of the encoded protein. It is now possible to detect polymorphisms in unexpressed regions of DNA by this analysis. Genetic disorders like sickle cell anemia, Thalassemia's, Huntington's disease and cystic fibrosis were identified through RFLP mapping analysis which demonstrates power of RFLP/Linkage analysis.

Isolation of genes using DNA or RNA Probes

Specific molecular probes can be used for isolation

of specific genes. These probes may be available either from same species or from another species can be used for isolation of genes. If probes obtained from one species used for isolation of gene from the same species they are called as homologous probes. If probes obtained from another species used for isolation of genes in other species they are defined as heterologous probes.

In situ Hybridization

In situ hybridization (ISH) is a technique which permits detection of DNA or RNA sequences in cell smears, tissue sections and metaphase chromosome spreads. The method is based on the formation of double stranded hybrid molecules which form between a DNA or RNA target sequence and the complementary single standard labeled probe. In a number of cases rye chromosomes in wheat background have been identified using this technique.

Use of Molecular Probes In DNA Finger Printing

DNA fingerprinting is a way of identifying a specific individual, rather than simply identifying a species or some particular trait. It is also known as genetic fingerprinting or DNA profiling. Inventor of this technology is Sir Alec Jeffreys in 1985. DNA fingerprinting is currently used both for identifying paternity or maternity and for identifying criminals or victims (Table 1 & 2).

Detection of changes to nucleic acid sequences

A change to the DNA sequence is a mutation, *e.g*. deletion, insertion, substitution. Changes in certain gene sequences can cause inherited diseases and their detection by probes can be diagnostic:

- Cystic fibrosis (due to a 3 bp deletion).
- Muscular dystrophies (due to various intragenic deletions).
- Phenylketonuria (due to various mutations).
- Apolipoprotein variants (some are due to a 1 bp mutation).
- Sickle cell anaemia (due to a 1 bp mutation).
- a_1-antitrypsin deficiency (due to approx. 50 different variants).

Table 1: Use of Probes in Fish species identification

Identified species	Target gene(s)	References
Four tuna species (*Thunnus obesus, Thunnus orientalis, Thunnus maccoyii, Thunnus albacares*)	cytb, 16S rRNA, D-loop region	Chuang *et al.* (2012)
Fish species	12S rRNA	Benedetto *et al.* (2011)
Atlantic salmon (*Salmo salar*)	Internal transcribed spacer (ITS)	Herrero *et al.* (2011)
Gadusmorhua	Cytochrome oxidase subunit I gene (COI)	Herrero *et al*. (2010)
European sole (*Solea solea*)	Internal transcribed spacer (ITS)	Herrero *et al.* (2011)

Table 2: Application of nucleic acid probes for detection of fish and shrimp pathogens

Pathogens	Probe used	Reference
Infectious Pancreatic Necrosis Virus (IPNV) in Atlantic Salmon	Oligonucleotide probes	Rimstard *et al.*
Penaeusmonodon type Baculovirus	Gene probe	Pizzutto *et al.*
IHHNV	Gene probe	Mari *et al.*
WSSV	Gene probe	Nunan *et al.*
HPV	Gene probe	Lightner *et al.*

Examples of the Applications of Nucleic Acid Probes in Medical Research

- Detection of tumor suppressor genes in human bladder tumors.
- Identification of *Leishmania* parasites.
- Detection of malignant plasma cells of patients with multiple myeloma.
- Diagnosis of human papillomavirus.
- Visual gene diagnosis of HBV and HCV.
- Detection and identification of pathogenic *Vibrio parahaemolyticus.*
- Detection of *Vibrio cholerae.*
- Molecular analysis of tetracycline resistance in *Salmonella enterica.*
- Identification of fimbrial adhesins in necrotoxigenic *E. coli.*
- Epidemiological analysis of *Campylobacter jejuni* infections.
- Molecular analysis of NSP4 gene from human rotavirus strains.
- Physical mapping of human parasite *Trypanosoma cruzi.*
- Detection and identification of African trypanosomes.
- Detection and identification of pathogenic *Candida* spp. and *Mycobacterium* spp.

CONCLUSION

Since, Molecular probes assays are most sensitive than conventional diagnostic methods, their use has become today's most sophisticated and sensitive technology for a variety of uses involving biological systems both in basic and applied studies. This ideal technology would offer absolute specificity, modularity, minimal size, deliverability (access to all cell types) and physiological neutrality (noncytotoxic, biochemically). In this way, their use has become most versatile in the field of molecular biology and biotechnology including their commercial use.

REFERENCES

Chuba, P.J., Pelz, K., Krekeler, G. and Gobel, U. 1998. Synthetic Oligodeoxynucleotide Probes for the Rapid Detection Of Bacteria Associated With Human Periodontitis. *J. Gen. Microbiol.*, **134**: 1931-1938.

Georgios, P., Danezis, A., Aristidis, S., Tsagkaris, Federica, Camin., Vladimir, B., Constantinos, A. and Georgiou, 2016. Food authentication: techniques, trends & emerging approaches, *Trends in Ana. Chem*., **16**: 88-93.

Herrero, B., Juan, M., Vieites, and Montserrat, E. 2011. Authentication of Atlantic salmon (*Salmo salar*) using real-time PCR. *Food Control*, **127**: 1268–1272.

Herrero, B., Madrinan, M., Vieites, J.M. and Espineira, M., 2010. Rapid identification of seaweeds in food products by PCR combined with ALF-RFLP and FINS methodologies. *J. Agric. Food Chem*., **58**: 11586–11592.

Herrero, B., Madrin, A.N., Juan, M. and Montserrat, E., 2010. Authentication of Atlantic Cod (*Gadus morhua*) Using Real Time PCR. *J. Agric. Food Chem*., **58**: 4794–4799.

Ishige, T., Itoga, S., Matsushita, K. and Nomura, F. 2016. Locked nucleic acid probe enhances Sanger sequencing sensitivity andimproves diagnostic accuracy of high-resolution melting-based KRAS mutational analysis. *Food Control*, **457**: 75–80.

Lin, W.F. and Hwang, D.F. 2007. Application of PCR-RFLP analysis on species identification of canned tuna. *Food Control*, **18**: 1050–1057.

Lockley, A.K. and Bardsley, R.G. 2000. DNA-based methods for food authentication. *Trends Food Sci. Technol*., **11**: 67–77.

Narayanan, S. 1992. Overview of Principles and Current uses of DNA Probes in Clinical and Laboratory Medicine. *Ana. of. Clini. and Labo. Sci.*, **22**: 18-24.

Rasmussen, R.S. and Morrissey, M.T. 2008. DNA-Based Methods for the Identification of Commercial Fish and Seafood Species. *Compr. Rev. Food Sci. Food. Saf.*, **7**: 280-295.

Sforza, S., Roberto, C., Tedeschi, T. and Marchelli, M. 2011. Food analysis and food authentication by peptide nucleic acid (PNA)-based technologies. *Chem. Soc. Rev.*, **40**: 221–232.

Teletchea, F. 2009. Molecular identification methods of fish species: reassessment and possible applications. *Rev. Fish Biol. Fisheries*, **19**: 265–293.

Wang, X., Sun, R., Miao, J., Li, X. and Jian- Feng, G. 2016. Hemicyanine Dyes Linked with Quaternary Ammonium Group: Near- Infrared Probes for the Detection of Nucleic Acid. *Sensors and Actuators B.*, **16**: 861-867.

Xiang, L., Wang, J., Yun Fei, B., Sun, X. and ZuHong, Lu. 2004. A novel method for screening species-specific gDNA probes for species identification. *Nucleic Acids Research*, **32**: 1086-1091.

Martineza, I., Aursanda, M., Eriksona, U., Singstadb, T.E., Veliyulinb, E. and Zwaagb, C. 2003. Destructive andnon-destructive Analytical techniques for authentication and composition analyses of foodstuffs. *Trends in Food Science & Technology*, **14**: 489–498.