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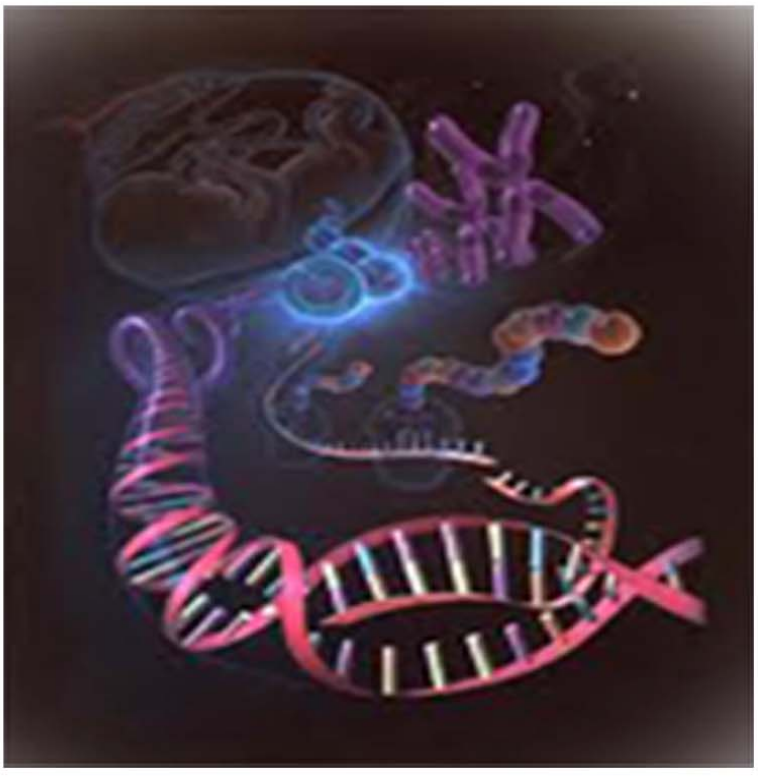

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Review Article

MOLECULAR PROBES AND THEIR APPLICATIONS

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Molecular probes are small DNA or RNA segments that recognize complementary sequences in DNA or RNA molecules that allow identification and isolation of these specific sequences from an organism. Extraordinary advances in human molecular genetics have occurred over the past decade which has revolutionized the entire scenario of biological sciences. One component of those advances is the increase in the use of molecular probes. These probes serve as the resources for a variety of applications. These molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, physiology, embryology and genetic engineering.

Keywords: DNA probe, RNA probe, cDNA probe, RFLP, DNA fingerprinting

INTRODUCTION

A stretch of DNA or RNA sequence that can detect a target sequence in the genome is called as probe. Ever since their development, they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis. The discovery of PCR (Mullis *et al*., 1986; Saiki *et al*., 1988) was a landmark in this effort and proved to be a unique process that brought about a new class of DNA profiling markers. This facilitated the development of marker-based gene tags, map-based cloning of agronomically important genes, variability studies, phylogenetic analysis, marker-assisted selection of desirable genotypes etc.

Although, initially these probes were developed and used for genetic engineering research but are now frequently used for a variety of purposes including diagnosis of infectious diseases (Katoch *et al*., 1994,1997; Kaminski *et al*., 1995; Sharma *et al*., 1996; Belak *et al*., 2009;Bexfield *et al*., 2011; Palacios *et al*., 2009), identification of food contaminants (Zhang *et al*., 2012; Goji *et al*., 2012), variety of microbiological tests (Chuba *et al*., 1998;Smorawinska *et al*., 1992) and forensic tests. Probes can also be used to identify different varieties of crop species (Cordeiro *et al*., 2001; Anderson *et al*., 1993; Nagaraju *et al*., 2002). For basic studies in molecular biology laboratories these are frequently used for

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identification and isolation of genes or related sequences.

In theory any nucleic acid can be used as a probe provided it can be labeled to permit identification and quantitation of the hybrid molecules formed between the probe and sequence to be identified. In practice, double and single standard DNAs, mRNAs, and other RNAs synthesized *in vitro* are all used as probes. DNA/ RNA probe assays are faster and sensitive so that many conventional diagnostic tests for viruses and bacteria involving culturing of the organisms are being fast replaced by molecular probe assays. While culture tests can take days or even months, molecular probe assays can be performed with in few hours or minutes.

Molecular probes can be broadly categorized into DNA probes and RNA probes, sometimes cDNA probes and synthetic oligonucleotide probes can also be used for various purposes.

The use of molecular probes has become todays most sophisticated and sensitive technology for a variety of uses involving biological systems both in basic and applied studies in the field of molecular biology and biotechnology including their commercial use.

Preparation of Probes

Different types of probes can be prepared in various ways.

DNA Probes

Extract the DNA from an animal or plant tissue.Digest extracted DNA with a restriction enzyme such as *Eco*RI or *Hin*d III which cuts DNA at specific sites or positions where a specific sequences recognized by the enzyme is found. Run the digested DNA on an agarose or polyacrylmide gel electrophoresis to separate fragments of different sizes. Isolate DNA of specific fragment from a particular band identified through southern blots by hybridization with specific labeled mRNA or cDNA molecules. Clone this DNA in a vector. Allow chimeric vector to infect bacteria for multiplication where it can make billions of copies. DNA probes prepared in this manner can be used for southern blotting and RFLP analysis.

RNA Probes

High specific activity RNA probes or riboprobes may also be synthesized from DNA templates cloned in expression vectors such as SP_6 (which infects *Salmonella typhimurium*) and T_7 phage (infects *E.coli*). This is achieved through RNA synthesized *in vitro* and labeled simultaneously with labeled nucleotides.

Usually SP_6 and T_7 systems can be and have been utilized to express whole RNAs, but for making a probe, only a short labeled RNA is sufficient. To enable such probes to be transcribed into uniform lengths, it is practice to linearize the plasmid by cleaving it with a restriction enzyme. The vector thus carries the input in the following order phage promoter-Enzyme 1- Enzyme 2. The template DNA is inserted at the number 1 site and composite is treated with restriction enzyme 2. So now this creates a linear DNA with a promoter, the template DNA and automatic termination site at the end cleaved with enzyme 2. The mRNAs fall of when they reach this end of the vector. Such templates reflect to run of templates and are uniform in size and are easier to isolate from the reaction mixture. RNA probes prepared in this manner can be used for northern blotting and *in situ* hybridization.

RNA probes offer several advantages over DNA probes. Since these are single stranded and

provide improved signal or hybridization blots. There is lack of competition of probe/ probe hybridization. Even though, some advantages are there wide spread presence of ribonuclease creates some problems in their preparation and use. So RNA probes are more sensitive to degradation than equivalent DNA probes, therefore extreme care must be taken in the preparation of RNA probes by keeping all glass ware free of ribonuclease.

cDNA Probes

A DNA sequence corresponding to a part of a specific gene can be obtained by reverse transcription of mRNA. cDNA thus obtained can be cloned and used as a probe.

Synthetic oligonucleotides as probes

DNA probes with known nucleotide sequence can also be synthesized chemically using automated DNA synthesizers. These synthetic probes will be efficient only when they are not more than 20-40 nucleotides in length.

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 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.

Methods for Labeling of Probes

There are two methods for labeling of probes. i.e. end labeling and nick translation.

End labeling

In this technique probe is isolated and end labeled by removing the 5'-terminal phosphate using alkaline phosphatase first and adding a ^{32}P-labeled phosphate with the help of a kinase. End labeled probes are far less labeled than the transcribed ones with labels at several nucleotides in the strand.

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.

Choice of Label

Probes can be labeled either by radioactive isotopes or can also be labeled with non-radioactive molecules such as biotin, digoxegenin etc.

Radiolabeled Probes

The most sensitive detection method employed is radioactive label. Traditionally radioactively labeled probes are used for a variety of experiments. The autoradiographic detection of labeled probes depends on the isotope used and

the specific activity which has to be high enough to permit detection after hybridization within a reasonable exposure of time or with a good signal. Several isotopes are available for radioactive labeling. The use of ^{32}P allows rapid detection of signal yet cellular localization is suboptimal because of the long path of the [^{32}P] β-rays. Autoradiographs of high quality and improved cellular localization employ ^{35}S labeled probes. ^{35}S emits γ-rays of much shorter path length. One of the major disadvantages of ^{35}S is non-specific binding of label to cell or tissues. It has been suggested that pre-hybridization in the presence of non-labeled thio alpha UTP at pH 5.5 reduces this non-specific binding. It is also possible to label nucleic acids with tritium or by iodonization with [^{125}I] iodine. Tritium labeled probes give intracellular localization, but for low abundance target molecules may require exposure of up to 100 days. This may result in high background and is unacceptably long for most purposes. Disadvantage with radiolabel is Instability of label. Different labels along with the merits and demerits were presented in Tables 1 and 2.

Table 1: Different Types of Labels for Labeling of Probes

Radiolabeled Probes	Non-radioactive Labelled Probes
3H, 32_P 35_S 14_C, $^{125}1$	Digoxigenin
4-(Phenoxymethyl)piperidine	Biotine
	5-Bromo deoxyuridine
	Dinitro phenyl
	Alkaline Phosphate
	Ethidium
	Sulphonation
	Luciferase
	Sodium metabisulphate
	Mercury Meta Acetate
	TetramethylRhodamine

Table 2: Merits of Radiolabeled and Non-radiolabeled Probes

Label	Resolution	Sensitivity	Exposure	Stability
32 p	+	++	7 days	0.5 weeks
35 S	++	+++	10 days	6 weeks
3H	+++	+++	14 days	>30 weeks
Biotin	+++	++	0.16 days	>52 weeks
Digoxigenin	+++	++	0.16 days	>52 weeks

Non-Radiolabeled Probes

a) Biotin Labeled Probes

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.

Advantages

These assays employ non-toxic materials, whose half-life is longer.

- 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.

LIMITATIONS

- A limitation of this technology is that very small probe contains only a small number of

biotinylated sites limiting the intensity of signal obtained. It has been solved by adding long 'tails' of biotinylated nucleotides to the probes through enzymatic methods. Sometimes the probe does not need to be labeled with biotin but only coupled with a tail.

- Another disadvantage of biotin labeled probes is that cytochemical visualization reactions lead to precipitation of insoluble material which cannot be removed and therefore the filter cannot be reused, whereas with radio labeled probes, the filters can be used repeatedly for hybridization with a number of probes one at a time.

b) Digoxigenin Labeled Probes

Digoxigenin is another chemical derived from plants and used for non-radioactive labeling of probes. An antibody associated with an enzyme (antidigoxigenin - alkaline phosphatase conjugate) is used for the detection of the presence of digoxigenin. The probes may be labeled with digoxigenin - II - dUTP supplied with a digoxigenin kit (these kits are available from any commercial firm, eg. BoehringerMannheem). The labeled and denatured probe may be used for hybridization. After hybridization the membrane or the slide may be transferred into detection buffer containing 20 ug/ ml of antidigoxigenin fluorescein and 5% (W/V) BSA (bonine serum albumin). This is incubated for 1 h at 37°C and then the membrane or slide is washed in detection buffer three times (8 min each at 37°C) and alkaline phosphatase activity was detected using 0.17 mg/ml BCIP (5-bromo 4-chloro 3-indoyl phosphate) and 0.33 mg/ml NBL (nitro blue tetrazolium) as dye substrate.

c) Alternatives to Biotin and Digoxigenin Labeling

The techniques of non-radio isotopic labeling have been further expanded and new methods have been devised for attaching other ligands (eg: hapten determinants, 2, 4 dinitrophenol, arsenative derivatives etc) to nucleotides without hampering their ability to be incorporated into DNA. These alternatives require binding of attached ligands to specific proteins that can be tagged with enzymes or fluorescent molecules. It is possible to monitor many probes simultaneously by using several different ligands since each ligand would yield a different signal. A chemiluminscent probe system has also been developed in which two different probes complementary to a continuous segment of DNA hybridize to adjacent segments of a gene. The first label is a chemiluminescent complex that emits light at a specific wavelength this emission excites the label molecule on the second probe to emit light at a different wave length which can be detected using a photomultiplier device: This process called non-radioactive energy transfer can occur only if the two probes hybridize correctly and the two labels are close to each other. This system has great fidelity and provides basic technology for a homogeneous assay. In addition DNA does not need to be immobilized and no washing steps are necessary which may be an additional advantage for large scale testing.

Applications of Molecular Probes

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

a) 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)

b) RFLP Maps and Linkage of RFLPs with Specific Genes

RFLPs have been used to prepare chromosome maps in humans, mice, fruit fly and in plants including maize, tomato, lettuce, and rice. The use of Mendelian markers for genetic mapping is sometimes limited due to non-availability of mutants. The list of markers can be increased or extended by using molecular markers which are examined in the form of RFLPs. Once a large number of RFLPs are available in a species the parents, F_1, F_2 generations can be used to study their inheritance and linkage relationship and genetic linkage maps can be prepared. In this way they can be used for plant and animal breeding (Mburu and Hanotte *et al*., 2005; Guimaraes *et al*., 2007; Parasnis *et al*., 1999; Sen *et al*., 1997; Pujar *et al*., 1999; Sant *et al*., 1999)

c) 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.

Use of Molecular Probes in Molecular Cytogenetics

a) 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.

These heterologous probes have been found to be effective in identifying gene clones during colony hybridization or plaque hybridization or on southern blots. For instance, the gene for chalcone-synthase (CHS) has been isolated from *Antirrhinum majus* and *Petunia hybrida* using heterologous cDNA probes from parsley similarly heterologous Antirrhinum cDNA probe was used for isolation of CHS gene from barley and heterologous probes from maize were used for isolation of barley genes Wx (Waxy Genes) and Al (aleurone gene). Heterologous probes should ordinarily be used with cDNA library and not with the genomic library since in the latter case unrelated genes or pseudogenes (which do not express) may be isolated and cloned. These heterologous clones if available in expression vector [Eg. $_P$GEM Blue] can also be used for getting RNA probes which have been bound to be more sensitive and efficient.

b) 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.

Satellite chromosomes with NORs (nuclear organizing regions) are recognized using probes for ribosomal DNA in wheat, barley etc. Telomeres have been identified in human and other eukaryotes using a telomeric sequence as a molecular probe showing that telomeres of all chromosomes carry the same sequence. In wheat and related species chromosomes of D genome can be identified using a D genome specific probe (PASI separated from *Aegilops squarrosa*).

This method is particularly useful if target sequences are distributed in a non-random way in tissues for the visualization of heterogenicity and the study of cell differentiation. The method of ISH for RNA-RNA hybridization is generally applicable to rapid screening of small numbers of cultured cells for expression of oncogenic mRNA. It circumvents the problem of extracting rare RNAs in sufficient amounts for detection. Similar methods have been used to visualize homeotic gene expression in developing larvae. DNA-DNA ISH is also suitable for the detection of viral genomes in sections of routinely processed archival paraffin blocks of human tissues.

USE OF MOLECULAR PROBES FOR HUMAN HEALTH CARE

As represented in Table 3 the DNA probes are being extensively utilized for diagnosis of diseases caused by parasitic protozoa and helminthes. They are also used for antenatal diagnosis of congenital diseases to allow advice on abortion of fetus, if desired. Similarly probes have been designed for the diagnosis of number of sexually transmitted diseases.

Readymade DNA probes for herpes virus and other human, animal plant viruses are available. Probes are also available for a number of human parasites from the groups protozoa and helminthes. In India, a diagnostic probe for the detection of malaria has been constructed at the Astra Research Centre India (ARCI).

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.

The vast majority of a human's DNA will match exactly that of any other human, making distinguishing between two people rather difficult. DNA fingerprinting uses a specific type of DNA sequence known as Variable Number Tandem Repeats (VNTRs) can contain anywhere from twenty to one hundred base pairs. Every human being has some VNTRs. To determine if a person has a particular VNTR, a southern Blot is performed and then the southern blot is probed through a hybridization reaction with a radioactive

Table 3: DNA Probes for Different Diagnostic Tests

Parasite	Probe Used	HybridizationTechnique Used
I Protozoa		
1. Leishmania (Kala-azar)	Kinetoplast DNA (KDNA, a repetitive DNA sequence)	Dot blots, southern blots In situ hybridization
2. Trypanosoma (Sleeping sickness)	Kinetoplast DNA (KDNA) a repetitive DNA and total parasite DNA	Southern Blot (Restriction Fragment Length Polymorphism RFLP
3. Plasmodium (Malarian Parasite; P. falciparum P. vivax and P. cynomorge)	Repetitive DNA, Synthetic oligonucleotides	Dot blots, blood-samples lysed directly on nitrocellulose filters
II Helminths		
1. Schistosomes (human blood flukes)	Ribosomal RNA gene	Dot blots, Southern blots
2. Nematodes: Wuchereria and Brugia (filaria) onchocerca (river blindness); Trichinella	Repetitive DNA sequences; Synthetic oligonucleotids; Ribosomal DNA;	Crude DNA Blood Samples dot blots
3. Taeniid Cestodes (Taenia solium)	Ribosomal DNA	Southern blots

version of the VNTR in question. The pattern which results from this process is what is often referred to as a DNA fingerprint.

DNA fingerprinting has a high success rate and a very low false-positive rate making it an extremely popular form of paternity and maternity verification. In forensics DNA fingerprinting is very attractive because it requires only a piece of a person's body like blood, hair, saliva, semen, body tissue cells.

DNA finger printing is technically demanding. There are cases on record in the US where mistakes seem to have been prompted by human error. There is a need for quality control. Every precaution should be taken to ensure preparation of high molecular weight DNA, complete digestion of the samples with appropriate enzymes and perfect transfer and hybridization of the blot to obtain distinct band with appropriate control. If necessary the analysis should be repeated. The DNA fingerprinting test is performed properly is infallible. However, there is need to interpret DNA typing with a thorough understanding of the population involved.

DNA finger printing can be used for:

1) Pedigree analysis and establishing paternity.
2) Immigration authorities.
3) In rape cases.
4) Identification of mutilated dead bodies.

5) Social security record identification.

6) In murder cases.

7) In characterization of cell cultures.

8) In animal breeding programmes.

9) In plant breeding programmes.

10) In demographic studies.

11) Twin zygosity determination.

12) Tissue culture cell line identification.

13) Mopping fine grained sequence variation in mini satellites.

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. They give new dimensions to concerted efforts of breeding and marker-aided selection that can reduce the time span of developing new and better varieties and will make the dream of super varieties come true. This ideal technology would offer absolute specificity, modularity, minimal size, deliverability (access to all cell types) and physiological neutrality (non-cytotoxic, biochemically). In this way, their use has become most versatile in the field of molecular biology and biotechnology including their commercial use.

REFERENCES

1. Anderson J A, Sorrells M E and Tanksley S D (1993), "RFLP Analysis of Genomic Regions Associated With Resistance to Preharvest Sprouting in Wheat", *Crop Sci.*, Vol. 33, pp. 453-459.

2. Belak S, Thoren P, Leblanc N and Viljoen G (2009), "Advances in Viral Disease Diagnostic And Molecular Epidemiological Techniques", *Expert Rev. Mol. Diagn*., Vol. 9, No. 4, pp. 367-381.

3. Bexfield N and Kellam P (2011), "Metagenomics and the Molecular Identification of Novel Viruses", *Vet. J.*, Vol. 190, pp. 191-198.

4. Chuba P J, Pelz K, Krekeler G, de Isele T S and Gobel U (1998), "Synthetic Oligodeoxynucleotide Probes For The Rapid Detection Of Bacteria Associated With Human Periodontitis", *J Gen Microbiol.,* Vol. 134, pp. 1931-1938.

5. Cordeiro G M, Casu R, McIntyre C L, Manners J M and Henry R J (2001), "Microsatellite Markers from Sugarcane (*Sacharum* spp.) ESTs Cross Transferable to Erianthus and Sorghum", *Plant Sci.,* Vol. 160, pp. 115-1123.

6. Deshpande A D, Ramakrishna W, Mulay G P, Gupta V S and Ranjekar P K (1998), "Evolutionary and Polymorphic Organization of the Knotted-1 homeobox in Cereals", Theor *Appl Genet.,* Vol. 97, pp. 135-140.

7. Drinkwater R D and Hetzel D J S (1991), "Application of Molecular Biology to Understanding Genotype-environment Interactions in Livestock Production", In *Proc. of an International Symposium on Nuclear Techniques in Animal Production and Health*, IAEA, FAO, Vienna, April 15-19, pp. 437-452.

8. Fukuchi A, Kikuchi F and Hirochika H (1993), "DNA fIngerprinting of Cultivated Rice With Rice Retrotransposon Probes", *Jpn. J.*

Genet., Vol. 68, pp. 195-204.

9. Gill K S, Lubbers E L, Gill B S, Raupp W J, and Cox T S (1991), "A Genetic Linkage Map of Triticumtauschii (DD) and its Relationship to the D Genome of Bread Wheat (AABBDD)", *Genome,* Vol. 34, pp. 362-374.
10. Katoch V M and Sharma V D (1997), "Advances in the Diagnosis of Mycobacterial Diseases", *Indian J Med Microbiol.,* Vol. 15, pp. 49-55.
11. Katoch V M, Kanaujia G V, Shivannavar C T *et al.* (1994), "Progress in Developing Ribosomal Rna And Rrna Gene(s) Based Probes For Diagnosis And Epidemiology of Infectious Diseases Especially Leprosy", in Sushil Kumar, Sen A K, Dutta G P Sharma R N (Eds.), *Tropical Diseases - Molecular Biology and Control Strategies*, pp. 581-87A, CSIR Publication, New Delh.
12. Kaminski D A and Hardy D S (1995), Selective Utilization of DNA Probes for Identification of *Mycobacterium* species on the Basis of Cord Formation in Primary BACTEC Cultures", *J ClinMicrobiol*, Vol. 33, pp. 1548-50.
13. Koebner R M D, Powell W and Donini (2001), Contributions of DNA Molecular Marker Technologies to the Genetics and Breeding of Wheat and Barley", *Plant breeding Review*, Vol. 21, pp. 181-220.
14. McFadden J J, Kunze Z and Seechum P (1990), "DNA probes for Detection and Identification", in McFadden J (Ed.), *Molecular Biology of the Mycobacteria,* Surrey University Press, UK, pp. 139-172.
15. Mason-Gamer R J, Weil C F and Kellogg E A (1998), "Granule-Bound Starch Synthase: Structure, Function, and Phylogenetic Utility", *Mol Biol Evol.*, Vol. 15, No. 12, pp. 1658-1673.
16. Moore S S, Sargeant L L, King T J, Mattick J S, Georges M and Hetzel D J S (1991), "The Conservation Of Dinucleotide Microsatellites Among Mammalian Genomes Allows the Use of Heterologous PCR Primer Pairs in Closely Related Species", *Genomics*, Vol. 10, pp. 654-660.
17. Mullis K, Faloona F, Scharf S *etal.* (1992), "Specific Enzymatic Amplification of DNA *In Vitro*: The Polymerase Chain Reaction", *Biotechnology,* Vol. 24, pp. 17-27.
18. Nagaraju J, Kathirvel M, Kumar R R, Siddiq EA and Hasnain S E (2002), "Genetic Analysis of Traditional And Evolved Basmati and Non-basmati Rice Varieties by Using Fluorescence Based ISSR-PCR and SSR Markers", *Proceedings of the National Academy of Sciences*, USA, Vol. 99, pp. 5836-5841.
19. Noriko Goji, Trevor MacMillan and Kingsley Kwaku Amoako (2012), "A New Generation Microarray for the Simultaneous Detection and Identification of Yersinia pestis and Bacillusanthracis in Food", *Journal of Pathogens,* Vol. 2012, pp. 1-8.
20. Parasnis A S, Ramakrishna W, Chowdari K V, Gupta V S and Ranjekar P K (1999), "Microsatellite $(GATA)_n$ Reveals Sex-specific Differences in Papaya", *TheorAppl Genet,* Vol. 99, pp. 1047-1052.
21. Palacois G, Druce J, Du L, Tran T, Birch C, Briese T, Conlan S, Quan P L, Hui J, Marshall J, Simons J F, Egholm M, Paddock C D,

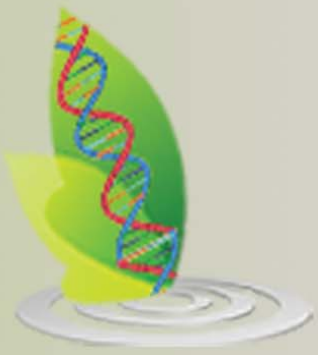
Shieh W J, Goldsmith C S, Zaki S R, Catton M. and Lipkin W I (2008), "A New Arenavirus in a Cluster of Fatal Transplant-Associated Diseases", *New Engl. J. Med.*, Vol. 358, No. 10, pp. 991-998.

22. Sharma R K, Katoch K, Shivannavar C T *et al.* (1996), "Comparisons of Sensitivity of Probes Targeting RNA vs DNA in Leprosy Cases", *Indian J Med Microbiol.,* Vol. 14, pp. 99-104.

23. Saiki R K, Gelfand D H, Stoffel S *et al*. (1988), "Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase", *Science,* Vol. 239, pp. 487-491.

24. Smorawinska M and Kuramitsu H K (1992), "DNA Probes for Detection of Cariogenic *Streptococcus Mutans*", *Oral MicrobiolImmunol,* Vol. 7, pp. 177-81.

25. Zhang X, Wu S, Li K, Shuai J, Dong Q and Fang W (2012), "Peptide Nucleic Acid Fluorescence *in situ* Hybridization for Identification of Listeria genus, Listeria monocytogenes and Listeria ivanovii", *Int J Food Microbiol.*, Vol. 157, No. 2, pp. 309-313.



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