

# CONVENTIONAL AND LATEST MOLECULAR DNA FINGERPRINTING TECHNIQUES

## Abstract

DNA fingerprint analysis is now used most frequently for paternity testing. This was the first application for DNA testing. It was most recently utilised to identify a new ancient human species. DNA fingerprinting, a revolutionary genetic technique, has emerged as an indispensable tool in various scientific disciplines, particularly in forensic investigations and genetic studies. In this study, we aimed to explore the utility of DNA fingerprinting in identifying individuals and establishing genetic relationships. The methods involved the extraction of DNA samples from diverse sources, followed by PCR amplification of specific loci and gel electrophoresis for pattern analysis. We focused on various genetic markers, such as short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs), to generate distinct profiles for each individual.

**Keywords:** DNA fingerprinting, SNP, STRs, SSCP, VNTRS, RFLP, AFLP, CAPS, RAPD.

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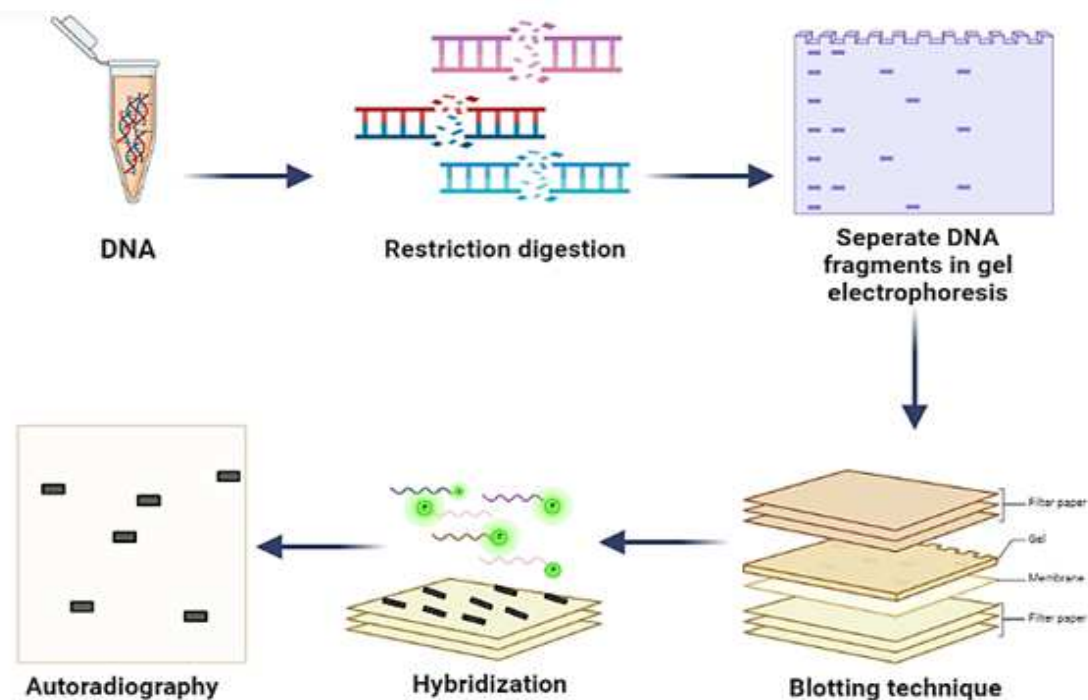
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## I. INTRODUCTION

Alec Jeffreys created DNA fingerprinting about 30 years ago. During his research, he found the minisatellite area, which was then extracted and utilised as a probe to study human DNA. A significant number of hypervariable areas in the human DNA were detected by using minisatellite probes, which were discovered to have complex patterns of bands for each individual. Each band represented one of the hypervariable DNA sections, which were interconnected (Jeffreys (2013)). Theoretically, everyone on this planet has a unique DNA fingerprint, with the exception of identical twins. Therefore, compared to traditional fingerprinting, DNA fingerprinting is distinctive, unique, and specific for identification. Using less amount than a traditional fingerprint and surviving much longer than a traditional fingerprint. It is still possible to use DNA samples that are up to 25 million years old and additionally, samples with mixed DNA can be utilised (Mishra *et al.*, 2006). The sequence of DNA, which each person gets from their biological parents, and which is the same in every cell of the body, exhibits an exceptionally high level of polymorphism and may be used to identify every person in the world at the molecular level. Except for monozygotic twins, 99.9% of the human DNA sequences are the same in every person, yet there are enough variations that allow for individual identification. DNA profiling makes advantage of highly variable repetitive sequences known as variable number tandem repeats (VNTRs), particularly short tandem repeats (STRs). Although closely related humans share a lot of the same VNTR loci, it is highly improbable that unrelated people would have the same VNTRs (Singla *et al.*, 2017). Now a days, trace information left at crime scenes for identify persons by using genetic information. DNA can be found in cells from a person's blood, semen, saliva, urine, hair, teeth, bone, or tissue, among other sources. Although DNA fingerprinting is most frequently connected with human DNA, it may also be used to identify other creatures, including plants and animals (Mercurio *et al.*, 2010). In 1990, everyone was excited about the new technique that, for the first time, used DNA markers to distinguish between individuals of people, animals, plants, and fungus (Nybom *et al.*, 2014). DNA profiling has been extremely helpful for forensic medicine. It is a reliable and well-recognized scientific technique used in the identification of disasters victims, criminal investigations, the search for missing individuals and human remains, and medical research (Garcia *et al.*, 2017). The identification of hereditary diseases in adults, infants, and unborn children is another use for DNA fingerprint technology (Bajpai (2012)). Short tandem repeats (STRs) applications for human identification as well as animal identification have coevolved. Humans and several other creatures have been the subject of population studies, kinship analyses, paternity tests, and unique identification (Cassidy and Gonzales (2005)). In order to identify high levels of polymorphism, human minisatellite probes cross-hybridize with the DNA of different species of fowl (chicken, duck, turkey, and goose). The resultant, person-specific DNA fingerprints enable differentiation even amongst closely related bird species. With a larger average proportion of large DNA fragments, chicken DNA fingerprints differ from those of humans and other animals. Low allelic pairings of variable DNA fragments found in pedigree analysis suggest that the majority of alleles are unresolved in the DNA fingerprint or are too small to be recognised (Hillel *et al.*, 1989). Since the late 1980s, DNA fingerprinting has been widely used in animal behavioural research, particularly in studies of animal reproductive behaviour (Hongyan *et al.*, 2000). The genetic development of domestic animals depends on genetic diversity, both within and across breeds. The selection of desired economic features within present commercial lines will be hampered by a loss of diversity. Therefore, preserving or expanding genetic diversity within and across commercial lines or

foreign populations is of importance to chicken breeders. DNA polymorphism is more prevalent than gene products, and it may be detected regardless of environment, age, sex, or tissue (Farrag *et al.*, 2010). In poultry, DNA fingerprinting is a potent tool for investigating genetic diversity within stocks and determining relationships among stocks, genotypically describing individuals or populations, examining the relative contribution of evolutionary forces to genetic differences between populations, using marker-assisted selection, helping with gene introgression, predicting hybrid vigour, and providing useful data for the pre-selection of populations to be used in breeding (Farrag *et al.*, 2010). Plant DNA fingerprinting is complex since it involves populations and frequently many species. evaluating the degree of hybridization in wild populations, estimating the degree of selfing in crops, determining the reproductive method, examination of the diversity of wild populations Genebank administration, Developmental and adaptive features are analysed genetically. intergeneric and interspecific crossings have been genetically analysed. Plants grown in vitro with stable genetics. locating the domestication place of Einkorn wheat Soma clonal variant identification, connections among clones, sports, and cultivars in species that are reproduced via cloning forecasting heterotic pairings in breeding, Pedigree variance is predicted, sex forecast, investigating the harm that pollution does to genetic diversity and erosion (Archak (2000)). Microsatellites are abundant in the genome, they exhibit different levels of polymorphism, alleles exhibit codominant Mendelian inheritance, tiny amounts of tissue are needed for assay, loci are conserved in related species, and they have the potential for automated assay. These characteristics make microsatellites particularly suitable as genetic markers for a variety of applications in aquaculture and fisheries research (Oreilly and Wright (1995)). DNA is extracted from samples. DNA is cleaved at a specified place using a particular restriction enzyme. The DNA fragments of varied sizes are obtained using gel electrophoresis. Transfer DNA fragments on to a nitrocellulose paper. These DNA fragments are exposed to hybridization with a radioactively tagged DNA probe which is suitable. evaluating the suspect's sample using autoradiography (Figure 1).



**Figure 1:** DNA Fingerprinting Procedure

DNA fingerprint analysis is now used most frequently for paternity testing. This was the first application for DNA testing (Jeffreys (2013)). Using anthropology and DNA research, it is possible to identify the most likely location of origin for ancient relics like the Dead Sea Scrolls or mummies (Handt *et al.*, 1994). It was most recently utilised to identify a new ancient human species (Brown (2010)). DNA tests can potentially be useful for wildlife preservation. Using DNA evidence, it is possible to monitor endangered animals that are being slaughtered and traded illegally. The tracking of unlawful sales of whale flesh or ivory from elephants is one example of this. By comparing the DNA profile of the illegally obtained item to an animal carcass, judicial officials may take the necessary action against the poachers and eventually safeguard the endangered species (Pittera *et al.*, 2010).

## II. DNA POLYMORPHIC MARKERS

**1. Single Nucleotide Polymorphism (SNP):** Single nucleotide polymorphisms refer to variations at a solitary position within a DNA sequence that result from individual differences (<https://www.nature.com/scitable/definition/snp-295/>). A single nucleotide polymorphism (SNP) is a genetic variation that modifies a single instance of the four fundamental DNA building blocks—adenine (A), guanine (G), thymine (T), and cytosine (C)—within a segment of the molecule, and it impacts over 1% of a population (Britannica, 2022). While tri- or tetra-allelic SNPs are exceedingly rare, biallelic SNPs are the prevalent type. Within the human genome, SNPs are found on an average of one occurrence per every 1000 base pairs (bp) (Sachidanandam *et al.*, 2001; Wang *et al.*, 1998). The frequencies of SNPs can differ by several hundredfold between two locations, and the distribution of SNPs across the genome is not uniform. Non-coding regions of the genome frequently harbor a greater abundance of SNPs compared to coding regions, constituting the primary location for the majority of SNP occurrences. SNPs in noncoding areas are essential genetic or physical markers for comparative or evolutionary genomics investigations, despite the fact that they don't change the proteins they are encoding (Nickerson *et al.*, 1998). When SNPs are found in a gene's regulatory regions, they can alter the rate of transcription and therefore the amount of encoded protein produced. SNPs may modify protein function and structure in the coding areas, which can result in disease development or alter how a person reacts to a medicine or environmental contaminant. SNPs have therefore been employed as molecular markers in several pharmacogenomic and disease genetic research (Kim and Misra (2007)). In order to understand speciation and evolution, SNPs may be used to decode breeding pedigree, identify species' genomic divergence, and link genomic changes to phenotypic features, a large number of SNPs present in a group of individuals and low genotyping costs (Kumar *et al.*, 2012). The biochemical impacts of SNPs can be employed to enhance the synthesis of biomolecules. SNPs discovered through adaptive evolution benefit in strain characterization and biological pathway understanding (Liao and Lee (2010)). The availability of SNP markers across the whole genome and their suitability for high to ultra-high throughput detection technologies have led to their enormous popularity in plant molecular genetics (Mammadov *et al.*, 2012). The analysis of biodiversity in domestic and wild sheep populations, studies of resistance to intestinal parasites and foot rot, and the search for markers linked to meat and milk yield or colour inheritance traits have all benefited from the use of the new generation of molecular genetic tools known as SNP microarrays (Kawecka *et al.*, 2016).

2. **Short Tandem Repeats (STRs):** Short tandem repeats (STRs), also known as microsatellites or simple sequence repeats, are brief sequences of DNA that repeat in tandem, consisting of a repetitive unit of 1-6 bp. These repetitions lead to stretches of up to 100 nucleotides in length (Tautz (1993)). STRs can be categorized into multiple groups according to their varying repeat units. On one hand, they are classified as mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats, determined by the size of the primary repeating segment. With an increase in the size of the repeating segment, the abundance of each category diminishes. Among these, dinucleotide repeats are the most commonly encountered in the human genome. STRs are further segregated into two classifications based on their repetition structures: perfect repeats (also known as simple repeats), which encompass a single repeated unit, and imperfect repeats that encompass multiple compositional repetitions (Fan and Chu, (2007)).
3. **Variable Number of Tandem Repeats (VNTRs):** Variable number of tandem repeats (VNTRs), alternatively referred to as polymorphic minisatellites, represent tandem repeat sections exhibiting diversity in the quantity of successive repeats across chromosomes within a population (Naslund *et al.*, 2005). Utilizing the count of reiterated sequences, Variable Number Tandem Repeat (VNTR) sequencing is a genotyping method offering information through a succinct and quantitative methodology (Ramazanzadeh and McNerney, (2007)). The application of recent breakthroughs has elevated the significance of multiple-locus variable-number of tandem-repeats analysis (MLVA) as a potent technique for subtyping bacteria responsible for foodborne diseases (Nadon *et al.*, 2013).

### III. APPROACHES FOR DNA FINGERPRINTING

1. **Restriction Fragment Length Polymorphism (RFLP):** This technique involves the digestion of a DNA sample by certain restriction enzymes, followed by resolve the resulting restriction fragments based on the size using gel electrophoresis (Chaudhary *et al.*, 2020). Important advantages of RFLP include genotyping, gene mapping, DNA fingerprinting, and the diagnosis of genetic diseases (Mittal *et al.*, 2011).
2. **Restriction Endonuclease:** Restriction endonucleases are enzymes which are discovered in bacteria. DNA double-strand cleavage is facilitated by restriction endonucleases. One phosphate deoxyribose bond in the backbone of each DNA strand is hydrolysed to cause cleavage, which happens once for every instance of the recognition sequence. The reaction often takes place without any energy being added (Wilson and Murray, 1991). Endonucleases were first discovered in the 1960s, and eventually made commercially available in the early 1970s. In many systems, cleavage takes place at a specific region close to the recognition sequence, either within the sequence or a few bases to one side of it. In other cases, hydrolysis occurs indefinitely far from the recognition sequence. Most restriction endonucleases require  $Mg^{2+}$  and some also need ATP or S-adenosylmethionine (Roberts and Murray, (1976)). Restrictions endonucleases may act biologically as a kind of immune mechanism that helps destroy invading genetic material. The cell's own DNA is protected from restriction endonucleases by the methylation of nucleotides found inside the recognition sequence (Arber, 1978).
3. **Amplified Fragment Length Polymorphism (AFLP):** DNA is fragmented by a pair of distinct restriction enzymes, after which the DNA fragments' extremities are linked

through oligonucleotide adapters. Selective primers are employed to amplify specific sets of DNA digestion products. The identification of polymorphism can be achieved through methods involving radioisotopes, fluorescent dyes, and silver staining (Vos *et al.*, 1995). Among the recent and highly promising methodologies, Amplified Fragment Length Polymorphism analysis (AFLP), originated by Keygene BV in Wageningen, stands out prominently. The simultaneous use of a rare cutting and a common cutting restriction enzyme (MseI and EcoRI) to cut DNA, followed by the ligation of oligonucleotide adapters with defined sequences containing the corresponding restriction enzyme sites. employing specially created primers, selective amplification of certain collections of restriction fragments. To achieve this, the 3' ends of the primer are designed to extend into the restriction fragments for a small number of randomly chosen nucleotides, while the 5' region of the primer is designed to contain both restriction enzyme sites on each side of the fragment that are complementary to the corresponding adapters and observing the amplified fragments in gel (Yadav *et al.*, 2017). A firmly established molecular marker method, amplified fragment length polymorphism (AFLP), finds diverse applications in areas such as population genetics, phylogenetics, linkage mapping, parentage investigations, and the creation of single-locus PCR markers (Meudt and Clarke (2007)).

4. **Cleaved Amplified Polymorphic Sequence (CAPS):** The RFLP and PCR are combined to form CAPS. The method involves DNA amplification, followed by restriction enzyme digestion (Idrees and Irshad (2014)). PCR-RFLP is another name for cleaved amplified polymorphic sequence. It was created after the PCR technique. It was initially designed as a way to identify base alterations in DNA sequence and hence serves as a diagnostic tool for hereditary diseases. It was discovered that the method would be far more useful in research on genetic diversity. One of the techniques for identification sequence variation within a DNA fragment. It serves as an alternative to direct sequencing for detecting sequence variation. It's a relatively straightforward and affordable method and requires a small quantity of genomic DNA (<https://slideplayer.com/slide/4925274/>).
5. **Random Amplified Polymorphic DNA (RAPD):** To amplify arbitrary sequences within a intricate DNA template, a lone, brief oligonucleotide primer is necessary, attaching to multiple loci. A PCR-amplified product is generated based on the primer's dimensions, length, and the genome's characteristics (Kumar and Gurusubramanian (2011)). The Random Amplified Polymorphic DNA (RAPD) technique can be harnessed for crafting specific probes, scrutinizing blends of genomic material, and achieving taxonomic classification. Noteworthy advantages of RAPD technology encompass its suitability for uncharacterized genomes, minimal DNA quantities, efficiency, and user-friendly nature (Hadrys *et al.*, 1992). Sheela *et al.* (2021) employed the randomly amplified polymorphic DNA (RAPD) method to uncover genetic variations, intraspecies divergence, and the epidemiological characteristics of *S. chromogenes* strains isolated from instances of bovine and bubaline mastitis in Karnataka. RAPD, a polymerase chain reaction-based technique for scrutinizing DNA polymorphisms, has found extensive application in genomic investigations. This approach proves potent for unveiling genetic diversity within populations and examining genetic affiliations within the realm of animal genetics and breeding. Additionally, it has the potential to explore the interplay between RAPD markers, quantitative trait loci (QTL), and marker-assisted selection (MAS) (Piyan *et al.*, 2000). Utilizing RAPD-PCR, the divergence between the Rathi and Tharparkar cow

breeds, as well as the internal variability within each breed, was effectively identified. Rathi cattle exhibited greater genetic homogeneity within the breed in comparison to Tharparkar cattle. Remarkably, a substantial level of genetic resemblance is discernible between these two breeds based on both trans-genetic similarity and the genetic distance index, suggesting a potential ancestral connection between Rathi and Tharparkar cattle (Sharma *et al.*, 2004).

According to Apostolidis *et al* (2001) the RAPDs is an effective technique for the classification testing of individual horses as well as for determining the genetic variation which was present within breeds. This is essential for an effective management strategy planned to save these species. Using a 10-base primer, the random amplified polymorphic DNA (RAPD) approach was tested for the identification of fresh meats from cattle, goats, sheep, camels, wild pigs, donkeys, cats, dogs, rabbits, and bears. These findings suggest that RAPD may be beneficial for identifying single-species meat samples (Arslan *et al.*, 2005). The efficacy of RAPD for subtype detection and pedigree analysis was evaluated by a comparison of random amplified polymorphic DNA (RAPD) among 25 isolates of *Giardia intestinalis* (SEDINOVA *et al.*, 2003). The randomly amplified polymorphic DNA (RAPD) markers were used to identify polymorphism in five breeds of chicken: White Leghorn, Rhodes Island Red, Red Cornish, White Plymouth Rock, and Kadaknath. These breeds were chosen for their early body weights, part-period egg production, and egg mass, respectively (Sharma *et al.*, 2005). Parejo *et al* (2002) used RAPD method to examine the genetic diversity and population dynamics of the critically endangered Blanca Cacerena breed of cattle. The findings make it possible to better plan mating in order to preserve genetic diversity and increase the effectiveness of conservation for the Blanca Cacerena cattle breed. In order to find polymorphic markers in dogs for possible application in linkage analyses of characteristics segregating in certain pedigrees, random amplified polymorphic DNA (RAPD) analysis was evaluated (Gu *et al.*, 1997). The RAPD markers used as a specific DNA marker for Muscovy ducks, can be used to evaluate and determine the DNA polymorphism of indigenous Muscovy ducks in Nigeria. These marker's might be useful in marker-assisted programmes for breeding Muscovy ducks, if it could be connected to a quantitative trait locus for a trait which is economically significant (Ogah and Momoh (2014)).

- 6. Single-Strand Conformation Polymorphism (SSCP):** SSCP is the electrophoresis separation of single-stranded nucleic acids based on minute variations in sequence, frequently a single base pair, which produces a distinct secondary structure and a discernible change in mobility through a gel (Wakchaure *et al.*, 2015). Non-denaturing polyacrylamide gel electrophoresis can often identify conformational changes in DNA fragments of up to 300 bp caused by a single nucleotide variation. The concept behind denaturing gradient gel electrophoresis (DGGE) is that the existence of a mismatch will affect the double stranded DNA's melting point. The electrophoretic mobility will decrease when the melting point is achieved in a poly-acrylamide gel that has a gradient of denaturant. The heteroduplex and homoduplex strands are separated using a more advanced variation of this process called denaturing high performance liquid chromatography (DHPLC) (Vignal *et al.*, 2002). Although SSCP has been used extensively in medical diagnostics, there is little studies in population genetics. A quick, easy, and sensitive way to identify genetic variation is by SSCP analysis. Many DNA mutations and polymorphisms can be found by using SSCP analysis. SSCP is more

frequently employed to examine polymorphisms at single loci, particularly when utilised for mutation identification and medical diagnostics. It is a method for screening the DNA fragments in various research and diagnostic applications because of its simplicity, utility, and high rate of mutation detection (Wakchaure et al., 2015). SSCP may detect insertions, deletions, and rearrangements. Identification of Novel gene mutations, which improves our knowledge of genetic disorders. SSCP is a reliable and rapid screening method that may be used to detect nucleotide alterations (Sharma, Rishi (2021)).

#### **IV. MOLECULAR DIAGNOSTICS APPLICATION**

DNA fingerprinting is a reliable technique to determine the parentage and paternity of farm animals. Identification of alleles in the DNA profiles of the putative parents and their offspring is the objective of parentage testing (Phavaphutanon, Janjira (2011)). In recent years, these molecular markers, particularly DNA-based markers, have been widely used in a variety of fields including pathogen identification, sex determination, gene mapping, individual identity, genetic distance estimation, and evolution. They have also been used to determine twin zygosity and free martinism, identify disease carrier animals, and perform demographic studies (Athe et al., 2018). The majority of severe illnesses are caused by faulty animal genomes rather than infectious disease-causing organisms. A specific illness is resistant to or susceptible to certain allelic variants in the host genome. The identification of heterozygous carriers, or else phenotypically indistinguishable from healthy individuals, is made possible by DNA polymorphism that occurs within a gene. This is useful for understanding the molecular mechanism and genetic control of various genetic and metabolic disorders. Identification of carrier animals for the faulty recessive allele in bovine leucocyte adhesion deficiency in cattle has been done using the PCR-RFLP technique. Pre-implantation embryos sex can be determined using molecular markers. The Y-chromosome specific (male-specific) DNA sequence can be used as probes to achieve this. There has been an irreversible loss of genetic diversity among our local animal breeds as a result of the widespread crossbreeding of foreign animals with local breeds in order to capitalise on heterosis. Since it promotes a high level of heterozygosity in the population, genetic diversity conservation is crucial (Ebegbulem & Ozung (2013)). DNA amplification-based systems can be used in forensic veterinary applications such as identifying the species of meat and carcasses. There are primers for sheep, goats, and cattle that are species-specific. The majority of domestic animal species have been utilised to detect parentage using species-specific primers for the amplification of regions with a variable number of tandem repeats (Pfeffer et al., 1995).

#### **V. CONCLUSION**

DNA-fingerprinting has emerged as a significant tool of genetic analysis in medicine, veterinary medicine, and other fields. These molecular markers, especially DNA-based markers, have been extensively used in a variety of fields recently, including disease detection, sex determination, gene mapping, individual identity, genetic distance estimate, and evolution. In the field of animal genetics and breeding, genotyping, gene mapping, and the diagnosis of genetic diseases the fingerprint technique is a potent tool for identifying gene diversity in populations and analysing genetic relationships.



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