Review Article

Applications of DNA Identity Testing Through DNA Fingerprinting

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ABSTRACT

The similarity of the DNA sequences between all individuals is more than 99%. However, the remaining sequence difference is unique and significant enough to distinguish between the humans. These sequences are the target of testing the identity of the organism. DNA fingerprinting is a technique used for DNA identity testing. The other two techniques are DNA profiling and DNA typing with all three often used interchangeably. The current review paper deals with the applications of DNA fingerprinting, a term which was first used in 1985 by Alec Jeffreys. Since then, the technique has found profound applications in forensics, to confirm parentage, criminal investigations, rape cases, medical diagnostics, paleontology, archaeology, plant breeding and many more. Different methods used in DNA fingerprinting include Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Short Tandem Repeats (STRs) and polymerase Chain Reaction (PCR). The paper discusses the methods briefly and highlights the salient applications of the technique.

Key words:

References

Prior to the advent of the DNA fingerprinting techniques, the identity testing in forensics was mainly carried out by ABO blood group system. Afterwards, the markers were developed which made use of the differences in the serum proteins and red blood cells enzymes and human leukocyte antigen system was used (Weedn, 1996).[1] However, it was in 1985 that Alec Jeffreys introduced the concept of identity testing based on DNA (Jeffreys et al., 1985).[2] The concept was based upon the fact that although 99% of the DNA sequences are identical between different individuals, still the difference in the DNA sequences between individuals is unique and significant to distinguish between the humans (Cooper et al., 1985).[3] Alec Jeffreys and his colleagues compared the sequence of myoglobin in seal meat to that of human and observed that some short repeating sequences were similar and when these were compared with minisatellites, they were found to be same (Wyman and White, 1980).[4] These repeating sequences were believed to be variable, genetic markers (Jeffreys et al., 1985).[2] Afterwards, radioactive probe was developed thatcould bind with the repeating sequences and revealed patterns that were unique in each human called DNA fingerprints (Jeffreys et al., 1985).[2]

DNA is the genetic component of humans. Humans inherit the DNA blueprint from the parents with each parent responsible for 50% of the DNA. No two individuals apart from identical twins share the same genetic component. Genome is defined as the entire set of DNA including all the genes in an organism. The human genome consists of about 3 billion base pairs and contains the required genetic information. This represents only about 10% of the genome. About 98% of the human genome does not code for anything and no function was associated with it traditionally (Pennisi, 2012).[5] However, recent research suggests that this part is important in regulation of transcription and translation. Figure 1 shows the double helical structure of DNA

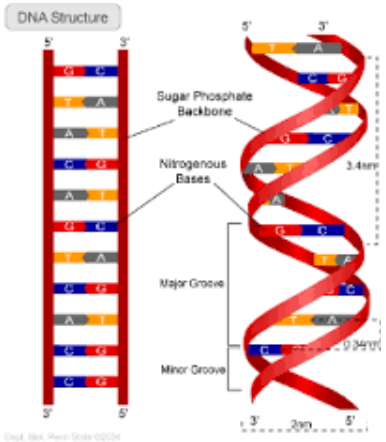

Figure 1. Double Helical Structure of DNA,

Both genomic and mitochondrial DNA can be used for forensic studies. A number of steps are

involved in DNA fingerprinting. First step includes extraction of DNA. The DNA can be extracted from any source including; blood, skin, hair, muscles or semen. The next step includes the addition of restriction enzymes which are known as the molecular scissors and cut the DNA into smaller fragments of varying size. These fragments are separated by a technique called agarose gel electrophoresis with smaller fragments moving more rapidly towards anode as compared to the larger fragments. This is followed by staining with ethidium bromide for the purpose of visualizing. The process through which the DNA is transferred onto a membrane is known as Southern Blot (Southern, 1976).[6] Afterwards, a radioactive probe is applied to the membrane, and the pattern of DNA is detected by exposing the membrane to x-ray film. The result is a pattern of DNA bands. The fingerprints of each individual are unique (Southern, 1976).[6]

Different methods are used in DNA fingerprinting. These include;

1) Restriction Fragment Length Polymorphism (RFLP)
2) Amplified Fragment Length Polymorphism (AFLP)
3) Short Tandem Repeats (STRs)
4) Polymerase Chain Reaction (PCR)

1.1 Restriction Fragment Length Polymorphism (RFLP)

RFLP's are a type of hybridization based molecular markers. The genome revolution was marked initially by introduction RFLP markers (Dodgson et al., 1997).[7] These markers have been widely used in plant and in human genetics studies (Weber and Helentjaris, 1989).[8] RFLP's have an advantage that they can detect unlimited loci and are robust markers, however they are expensive and time intensive and show relatively low level of polymorphism (Collard et al., 2005).[9] RFLP's are basically a difference in similar sequences which is detected through different fragment lengths after DNA is digested with restriction enzymes. RFLP markers are specific to a single clone. One of the advantages of RFLP markers is that they are co-dominant meaning that both alleles can be observed in the analysis and are specific to locus. The scoring process is relatively easy in RFLP. RFLP probes are DNA sequences that have been labeled and they hybridize with fragments of the digested DNA (one or more) after they have undergone gel electrophoresis. Through this they result in a blotting pattern that is unique and characteristic to a specific genotype at a specific locus.

The RFLP probes have found their applications in mapping the genome, forensic studies, paternity testing, diagnosis of genetic diseases and genotyping.

1.2 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a PCR based marker. Vos and colleagues in 1995 first made the use of this marker (Vos et al., 1995).[10] It is a multi-locus fingerprinting that overcomes the weaknesses in RFLP technique. Like RFLP, the AFLP also includes addition, deletions and base substitution between restriction sites. The technique also includes base substitutions at PCR primer binding sites. A unique characteristic of AFLP technique is the addition of adapters having known sequence to DNA fragments that have been generated by digesting the whole genome DNA. Many loci can be analyzed simultaneously. As the sequence of DNA fragments are unknown, for this reason, adapters of known sequence are joined to the ends of the fragments and are used as primer sites for PCR amplification. But as this leads to production of millions of PCR fragments, known bases are added to the 3' end to reduce the number of amplified fragments. AFLP markers are very strong in revealing genomic polymorphism. These are dominant markers.

1.3 Short Tandem Repeats (STRs)

STRs are the most recent form of DNA fingerprinting. The human genome consists of repeated sequences. The example of microsatellites within coding regions includes genetic diseases in humans, for instance the CAG repeats that are responsible for coding polyglutamine tract, which leads to mental retardation. STRs are the tandem repeats of about 2-10 bp units. Polymorphism can be detected in microsatellites from as low as five repeats (Karsi et al., 2002b).[11] The STRs are highly polymorphic due to which they have various applications in forensic science, medical genetics, plant breeding and genetics etc. The STRs have become an important DNA marker due to the fact that they can be easily

amplified by PCR and due to similar products, the analysis is much easier. The STRs are inherited as co-dominant markers. For the purpose of identifying humans, we require DNA markers with highest variation so as to differentiate between the individuals. The challenge in forensic samples is that often they are degraded or mixed, for instance, in rape cases. In STRs, due to lower mutation rate, the data is more stable and reliable. However, the use of microsatellite markers is investment and effort intensive and its development requires the identification of locus and the flanking region needs to be sequenced. For the purpose of making efficient marker, genomic DNA libraries are made which are microsatellite enriched (Kijas et al., 1994).[12]

1.4 Polymerase Chain Reaction (PCR)

PCR was developed by Kary Mullis in 1983. The invention won him the Nobel Prize in 1994. Since then, the technique has found many applications in molecular biology, forensics, medical genetics, plant and animal genomics etc. The PCR technique allows for exponential amplification of DNA sequence of interest. The process takes place in three steps namely; denaturation of DNA at 94-96^{o}C, annealing at 68^{o}C and extension at 72^{o}C. The technique makes use of heat resistant enzyme thermus aquaticus (Taq) polymerase. At every cycle the product is doubled. The real advantage with PCR is that due to its very high sensitivity, very small amounts of DNA sample can be amplified. In case of failure in results, the analysis can be repeated. However, due to this high sensitivity, contamination can become an issue.

In addition some other methods that are employed in DNA fingerprinting technique include; Y- chromosome analysis, X-chromosome STR and Single Nucleotide Polymorphism (SNP).

1.5 Applications in criminal investigations

The DNA technology is rapidly gaining recognition and attention in the criminal courts throughout the world. Prior to DNA testing, the only way to differentiate the humans was through blood types which could very easily lead towards false results. The technique has been highly successful in identifying the culprits of murder and rape cases. The forensic laboratories make the use of blood, semen, skin or hair for the purpose of investigations and match them with suspected samples. Depending on the quantity of sample and extent of degradation, several techniques can be applied to reach at a conclusion that a particular individual was responsible for a certain crime. This is a much reliable method for investigating the criminal cases. The reliability of the method can be judged from the fact that the in developed countries such as USA, the databases having profiles of DNA of criminals are used to identify them if they are believed to be involved in other crimes. On example in this regard is the CODIX (combined data index system) which contains two indexes, one is for the DNA profiles of criminals and the other one is for the evidence collected at the crimesite.

The only challenge while collecting the sample is to ensure that there is no contamination. The contaminated samples usually are not regarded as applicable for further studies. Contamination may result while collecting the sample, while transferring it to the forensic laboratory or even during the analysis. There are certain instances when the sample is not fully recovered and some of it gets lost for example, if there is wind blowing, there is possibility that the DNA may get blown away (Baldwin, 2005).[13]

2.1 Paternity testing

Apart from the criminal investigations, the major use of DNA fingerprinting has been in parentage testing (Collins, 2002).[14] Since the advent of DNA fingerprinting, the tests for paternity and maternity testing have become readily available. There are certain cases where a dispute arises regarding the actual father or mother of the child. The most common cases under this category include hospital mix-ups, adopted children cases and in-vitro fertilization cases. Prior to the DNA fingerprinting technique, the paternity testing was mainly carried out by ABO blood group and by analyzing physical traits such as attached earlobes and widow's peak. If the samples of child, mother and two disputed fathers are available, 99.99% accuracy can be guaranteed in determining the real father of the child. Just to clarify with the help of a figure, we can observe in the figure 2 where a RFLP technique is used to determine the true father from (F1 and F2) of the child (C), whereas the mother is denoted as (M). It is evident from the figure that the father (F2) seems to have more similar DNA to that of the child (C), hence it

can be concluded that F2 is the potential father of the child (Figure 2). Through this technique, many paternity cases have been resolved and the potential father has been determined. The chances of the result being false are very rare.

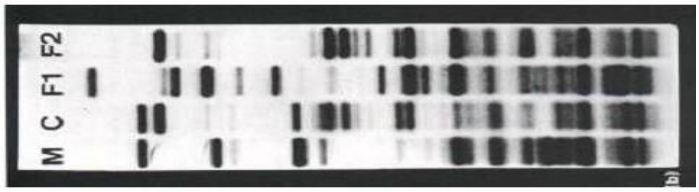

Figure 2. RFLP technique used for paternity test (Paternity Test, 2005)

2.2 Molecular archaeology

Another application of DNA fingerprinting is in molecular archaeology. The old civilizations can be studied through old DNA samples. The information regarding the cultural aspects, bloodlines, living and traveling patterns can be obtained through DNA fingerprinting. The DNA sample can be from sources including biological and skeletal remains, tissues, hair, bones, teeth, and even fossils (Christianson, 2010).[15] The chances of the sample being degraded are higher in studying archaeology. In cases, where nuclear DNA cannot be used, the mitochondrial DNA is used for studying the molecular archaeolgy.

2.3 DNA fingerprinting in plants

DNA fingerprinting through using RFLP methodology has been applied to some economically important crops including wheat and rice. Genetic maps have been developed in these crops through using RFLP technique (Marx et al., 1988)[16,26] and the technique has also has been used for identification of cultivars and genetic similarities (Hubbard et al., 1992).[17] Dallas in 1988 was able to differentiate between different rice cultivars through hybridizing restriction digested rice DNA with the human 33.6 minisatellite probe (Dallas, 1988).[18] The offspring that was studied from an individual rice plant proved to have identical fingerprints, which was the anticipated outcome due to the fact that rice is a self-pollinating crop and thus highly homozygous. In addition, Dallas ascertained the Mendelian inheritance of DNA fragments from grandparents to the second- generation offspring (F2).

Different patterns of DNA fingerprints in barley, Hordeum vulgare were observed by Ryskov and colleagues (Ryskov et al., 1988)[19] after they hybridized it with the M13 probe. Rogstad and colleagues (Rogstad et al.,1988)[20] produced similar M13 fingerprints from cottonwood trees' separate branches, Populus deltoides, and from a mother tree and its sucker plant, which went on to prove the somatic stability.

2.4 DNA fingerprinting in anthropology

It was during late 1980's that frequency distribution of Variable Number Tandem Repeats (VNTRs) were applied as genetic markers to differentiate between ethnic populations (Balazs et al.,1989).[21] Moreover, due to the fact that VNTRs have non-coding nature, high rates of mutation and greater genetic diversity, for this reason McComb et al. used VNTR RFLP distributions for questions regarding peopling of Americans and for characterizing the genetic structure of Siberian population (McComb et al., 1995).[22] However, recently due to advancements and greater developments, more effective ways have been introduced to determine the genetic makeup of the individuals by using STRs. These markers are used for information on ancestors and for building the human origin for knowing the evolutionary history of human beings. For instance, the STRs have in the past been successfully applied to show whether the Basque populations are remnants of Europe or of North Africa. For this purpose, the sample of children, adults and surrounding Spanish community were taken (Zlojutro et al., 2006).[23] The STR loci analysis went on to reveal that the Basques are related to Spanish community and distant North African populations. It can be said that with the advancements made in technology, the genetic markers will also be more effectively used to play a role in anthropological genetics which will in turn lead to better investigation of disease associations and in studying the disease causing genetic variations. Moreover, the applications of DNA fingerprinting will give us a better understanding on the origins and evolution of humans and other organisms.

Analysis

It goes without saying that DNA fingerprinting has become a powerful technique with applications in forensics, molecular biology, gene therapy, plant breeding, child disputes, paternity testing, archaeology, anthropology and many more. The

technique is based on the fact that no two individuals apart from the identical twins possess the same genetic component. Since 1987, when DNA fingerprinting was first used for forensics, the technique has become more sensitive, easier and more cheap (Collins, 2002).[14] The major advantages of the technique over traditional methods include; it has higher sensitivity, it can generate authentic results from small amounts of the DNA

source even if it gets degraded, further, the technique has much higher potential to discriminate between individuals even at times up to 1 million times higher as compared to traditional methods (Sullivan, 1994, Bell et al. 2022, Iqbal et al, 2021, Shamala t al. 2023).[24,25,26,27]

The developments and effective use of DNA fingerprinting technique has played a significant role in solving many criminal cases, resolving child disputes, deciding the potential father and proved the innocence of many. Most courts in USA accept DNA fingerprinting results to decide the cases. The method to be used mainly depends on the nature of the sample and the expertise and facilities available. All the methods have their own advantages and their own level of reliability. If only a small amount of the DNA sample is available, PCR is employed as it is the most sensitive method to amplify DNA. The chances that a certain analysis will result in misidentification of the culprit are very rare.

There is extensive ongoing research in world related to DNA fingerprinting. Technological developments will further strengthen the technique. With the passage of time and increasing awareness, more laboratories will employ it for various purposes mentioned above. Investments made in establishing world class laboratories with high-tech equipment will be important. This will ensure that maximum benefits are achieved from the technique. Necessary skills will have to be inculcated and human resource will need to be trained in the best laboratories of the world. It will be necessary to keep the pace with the latest developments and in making the use of most recent methods. The professionals working in the area of DNA fingerprinting will need to incorporate the most advanced methods and sharing of experiences both at national and international level will be integral to overcoming the shortcomings and barriers.

4. Comments/conclusion

It is evident that the DNA fingerprinting methods which are based on differentiating organisms based on the differences in their genetic component i.e. DNA, have played a decisive role in many areas such as in forensics, crime cases, paternity testing, child disputes, rape cases, plant and animal genomics, molecular archaeology, anthropology and many more. With the passage of time and increasing expertise, the DNA fingerprinting methodologies will further improve, gain more recognition and become more reliable. DNA fingerprinting also provides an edge in its ability to review previous cases that were analyzed and decided based on traditional tests. From the experiences suggested in the paper, it can be concluded that DNA technology and the databases for forensic data will further develop as we gain more expertise and facilities around the world areimproved.

5. Future research agenda

- The need of the hour is to inculcate the necessary skills in technical manpower and improve the laboratory facilities for DNA fingerprinting technique.
- Collaboration between laboratories in developing and developed world will hold the key for improving capabilities. Joint research programmes will be a useful step forward.
- Human resource development in this area will be central for effective use of the technique.
- Necessary training will have to be provided to the technical manpower to operate and maintain the high-tech equipments. The already present equipment will have to be
- upgraded.
- Concerted effort will be required on part of all stakeholders including government, scientists, researchers and legislators for making DNA fingerprinting mandatory part of forensic laboratories, criminal courts and research laboratories.
- The role of governments will be central in ensuring effective legislations and implementation in this regard. Funds and sufficient grants will have to be provided to the researchers and scientists to undertake the necessary research related activities in the already present laboratories.
- Interaction among the scientists, researchers, academicians, clinicians and government will have to be strengthened.
- The research outcomes must be disseminated to a wider segment of society through seminars, conferences/ workshops (at local, regional and international level) and awareness programmes will have to be launched.
- The curriculum at college and university level must include topics related to forensic sciences.

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