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Science of establishing identity - Past, present and future

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INTRODUCTION

CRIME is as old as human civilisation and so is man's ingenuity to investigate crime. King Solomon's legendary dilemma in identifying who is the real mother of the child and his decision based on the psyche of the motherhood is well known. World, however, has made tremendous progress since then in the science of identification. Identification process plays a crucial role in various day-to-day activities of human life. Be it the identification of heir to the estate or be it a suspected criminal charged with an offence of murder or rape, it becomes essential that identity of the person concerned be established beyond doubt in the interest of justice. The scientific and technological advances in the process of identification of an individual, therefore, are of utmost importance specially in a forensic set up. Over the last 150 years or so, several techniques have been developed for this purpose, most of them being a reflection of scientific progress and advancement of knowledge and understanding in the field of human biology; a simple example is the fingerprints of an individual. Whatever may be the origin of a man, there can be no doubt that human beings are individualised by their fingerprints. Man realised this quite early and developed an identification system based on the fact that fingerprints of every human being are unique. Practice of making fingerprints on legal documents is also very old. To begin with, it would be worthwhile to look at the historical perspective of this well documented popular and most commonly used method for identification.

Fig. 1—Two photographs of Sir William Herschel's fingerprint of the same thumb, taken thirty years apart; *left* : taken in 1860; and *right* : taken in 1888 showing no difference except a little bit of wear and tear.

THE PAST

Fingerprints : Indicator of absolute individuality

The distinction of being the first person to document his findings about the ridges on the hands goes to the 17th Century English Botanist Dr Nehemiah Grew, Fellow of the College of Physicians and of the Royal Society. He published a paper in 1668 in Philosophical Transactions. Grew at that time had absolutely no idea that over 200 years later these ridges would be used to identify criminals at crime scenes (Geral Lambourne 1984 in the 'fingerprinting story', pg. 25 published by Harrap Limited, London). However the individuality of fingerprints was first realised in 1860 by Sir William Herschel, a British

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Administrator. He made an important observation that fingerprints of each individual were unique; hence, none of the fingerprints of two individuals matched. When he sent his observations for publication in Nature, one of the leading scientific journals, it rejected his paper with the objection that what is the guarantee that the fingerprint of a person remains the same throughout the life span of that individual. Sir William Herschel pursued his observations for 28 years and demonstrated that fingerprints taken of an individual in 1860 is the same as that taken in 1888. There was no difference in the fingerprint except a little bit of wear and tear (Fig. 1). His paper was finally published in Nature on 28th June, 1888.

This attracted the attention of a famous British biologist, Francis Galton, who did detailed study and wrote a book called "Fingerprint" in 1892. The first instance in which the person was hanged on the basis of the evidence of fingerprints took place in 1892 in Argentina. This classical approach for the identification of an individual is in vogue even today in many countries. The probability of two unrelated individuals having identical fingerprints is 1 in 10^{10} whereas the world population is about 6 x 10^9. Therefore no two unrelated individuals with identical fingerprint pattern can be found in the world. The Britishers made use of fingerprints all over the world wherever they had their empire but they never used it on themselves, until Edward Henry, another British scientist did a detailed survey of fingerprints from variety of people and classified them into various sectors and groups. This pioneering classification is used in fingerprint analysis even today. Identification through fingerprint is no doubt a powerful technique; but what if criminals do not leave any fingerprints on the scene of crime? Investigating authorities become absolutely helpless when no fingerprints of an individual suspected to be involved in the crime are available at the crime scene.

Blood as an identifier

Is it possible to identify an individual from his blood alone? It was believed earlier that the blood of every human being is the same and therefore it can be transfused from one individual to any other individual. However, such transfusion resulted in large number of deaths, the reasons for which were not immediately understood. It was Dr Karl Landsteiner who announced in 1901, one of the major medical discoveries of the century, later became known as blood group system. Dr Landsteiner demonstrated in 1901 that human blood can be classified into four distinct groups i.e., A, B, AB and O. Dr Landsteiner proposed the existence of these groups on the basis of the presence or the absence of both of the two antigens in the blood (i.e., the substances against which antibodies react) which he named A and B.

As soon as blood groups were discovered, forensic scientists adapted these characteristics for establishing identity of individuals for catching criminals. The problem with the blood group has been that if the blood group does not match, it can be said with 100% certainty that so and so cannot be the biological father of a particular child; or so and so cannot be the person whose blood was found at the scene of crime, and all this is valid even today. In fact in case of mismatch this itself could be enough and one does not have to do any more tests. But this happens very rarely. If the blood group matches, one cannot say with certainty that a particular person is a biological father of the child or he is the person whose blood stain was found at the scene of crime. This is because the probability of two people not being related and having the same blood group is very high. In a group of five, two are likely to have the same blood group. Therefore, this approach cannot be used for positive identification – one of the limitations of the blood group analysis.

In 1940, Dr Landsteiner at the age of 70 years announced the discovery of Rhesus (Rh) factor responsible for the consequent serious illness or death of 200 white babies. With the discovery of Rh factor, one has a little better probability of positive identification.

Today we know that there are more than 100 different factors in our blood, which can differ from individual to individual (Trowsdale, 1993). Most important of these factors are immunological proteins such as human lymphocyte antigens (HLAs). These antigens are found on the surface of white blood cells. By conducting about 32 different tests, including HLAs, one can have exclusion with 100% certainty; but the positive identification will again be only with a certainty of 99.7%. Can a person alleged for a crime be hanged with 99.7% probability of his being criminal? Obviously that would not be enough.

THE RECENT PAST

Besides, most of the forensic tests are based on proteins which are very labile molecules and under the environmental conditions as well as with the passage of time they get degraded. By the time forensic samples are brought to the laboratory, it takes a long time (several days) which causes degradation of proteins and therefore they become unsuitable for doing the test. Taking into account all these limitations, there was a necessity to identify biological material which on one hand is highly stable and on the other, it is so variable that is individual—specific. This dream of forensic science was fulfilled by the discovery of DNA fingerprinting in 1985 by Professor Alec Jeffreys, Leicester University, UK. Although the technique of DNA fingerprinting was pioneered by Alec Jeffreys (Jeffreys *et al.*, 1985), and is also popular amongst the developed countries such as USA, India was the third country in the world to develop its own DNA fingerprinting probe and technique in 1989 (see Singh, 1991). DNA fingerprinting would not have become so popular, had it not found its use in

Fig. 2—Schematic diagram showing cell from human body, its nucleus, chromosomes and DNA coding for protein through mRNA as an intermediary.

identification of mutilated bodies, missing children, establishing paternity or maternity, in the field of agriculture, wildlife conservation, in cases of assassinations and murders, cases of rapes, etc. It particularly became known because of its use in forensic investigations and its ability to establish the identity of an individual.

In 1985, when Alec Jeffreys demonstrated that the genetic material i.e., DNA can vary from individual to individual, it came as a surprise to the scientific community all over the world. Scientists, however, eventually confirmed that what Alec Jeffreys was saying was correct. Today, one knows that there are significant differences in the genetic material from each individual. When one does the DNA fingerprinting, which is described later, one gets the pattern something like barcodes on library books and food packets based on which the computer establishes the identity and all the details of the content. Similarly, the total number of bands mapped as per their sizes help in establishing the identity of the particular individual. By this technique it is possible to identify an individual in the entire world population, because the probability of having the same DNA fingerprint pattern for any two individuals is extremely low which could exclude the entire world population of 6×10^9 several times over, with the only exception of identical twins.

Fig. 3—Stretch of DNA sequence showing repeats of GATA.

Basis of DNA fingerprinting

All living organisms on this planet are made up of living units called cells. There are about 10 trillion cells in the human body. Each cell (Fig. 2) has a clear dense structure in the centre which is called nucleus. Nucleus contains thread-like structures known as chromosomes. There are 46 chromosomes in every cell of our body; 23 of these are inherited from the father through sperm and 23 from the mother through the egg. So the newborn child is a combination of chromosomes from both the parents. At the molecular level, chromosome is a complex entity consisting of proteins and deoxyribose nucleic acid (DNA). The DNA molecule is so small that it can be seen only when it is magnified to the extent of 200000 times under electron microscope. DNA from a single human cell measures about 2 meter in length. It is astonishing that this 2 meter long DNA is packed in a tiny nucleus, the diameter of which is barely 10 microns. This is one of the astonishing engineering feats of nature. The structure of DNA was discovered by James Watson and Francis Crick (1953) for which they were awarded the Nobel prize. They demonstrated that the DNA is made up of double helix consisting of two strands, the backbone of which is made up of sugar and phosphate groups. Thus two backbones of DNA are held together in a double helical form by four basic chemical units (bases) A, G, C & T. The structure of these bases is such that A pairs with T and G with C (Fig. 2). It is remarkable that these four bases and the sequence in which they occur in a piece of DNA essentially decide the function of this piece of DNA. Thus the piece of DNA which instructs a cell to perform a particular function is called a gene. Size of the gene is enormously variable; it can be a few hundred base pairs (RNA genes, Sry gene, etc.) or it can be several 1000 Kb in length (Muscular dystrophy gene). It is estimated that there are about 30,000-40,000 genes (International Human Genome Consortium, 2001) which are necessary and sufficient to perform each and every function of a cell of our body. However, it is interesting to note that these genes constitute only less than 2% of the total DNA found in a cell. In other words, we do not know what the role of remaining >98% of DNA present in every cell is? It has been a challenging riddle that if all the genes code for proteins and all of us carry almost same genes, then why are we so different from each other? Probably the answer to the riddle would lie in that >98% DNA present in every cell of our body; and still we do not know

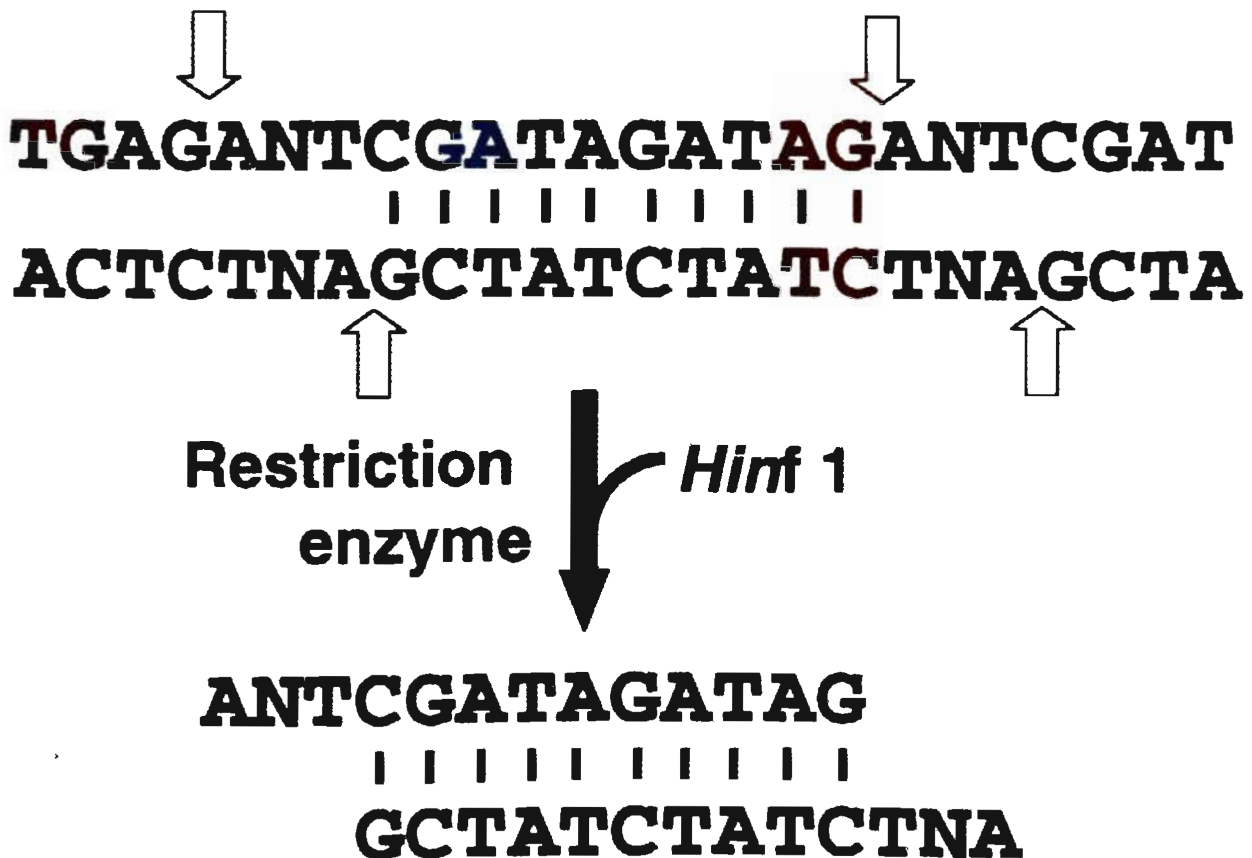

Fig. 4—Cutting of DNA at specific sites (shown by arrows) by restriction enzyme *Hinf* 1.

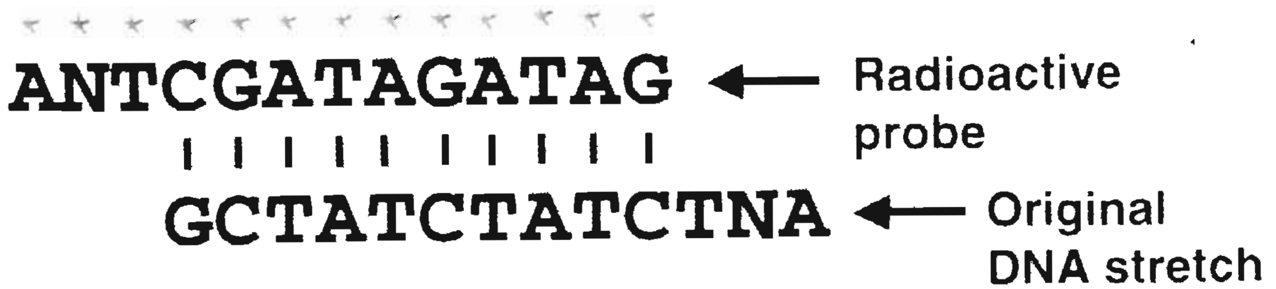

Fig. 5—Radioactively labelled DNA by the incorporation of labelled nucleotides (shown by stars).

what sense it makes for the existence of the organism. To assign function to so called Junk DNA is going to be a daunting task. It would require the help of all branches of sciences including physics, chemistry, biology, mathematics, computer science, etc. It is, however, interesting to note that a small part of this Junk DNA is repetitive in nature. Fig. 3 shows that a stretch of DNA made up of 4 nucleotides is repeated twice and there exist two copies of such repeats in the given sample. Such repeats are known as tandem repeats. These repeats could be of variable length from a few copies to several hundred copies at each location. Repeats of this kind are not known to make any biological sense. The nature of such repeats in terms of the number of copies and the frequency with which they occur in DNA varies from individual to individual. The technique by which we can detect the variation in copy number of such sequences is called DNA fingerprinting. The term DNA fingerprinting was introduced by Alec Jeffreys to emphasise that DNA from each individuals is as unique as his/her fingerprints.

HOW DNA FINGERPRINTING IS DONE

Step 1 – Isolation of DNA

Initial step of the isolation of DNA is always dictated by the source and the nature of the tissue. It depends upon whether one is isolating DNA from rocky fossils or from bones, hairs or soft tissue like kidney or liquid like blood. The crucial step in the isolation of the DNA is to break cells as well as nucleus so that contents of the cell become free; then one has to remove molecules like proteins, RNA and the cell debris from the mixture by treating with phenol and chloroform repeatedly by making use of centrifugation. DNA finally comes in the supernatant and is colourless. In a precipitated form DNA resembles cotton wool. It can then be dissolved again in appropriate buffer for further processing.

Step 2 – Fragmentation of DNA

DNA molecules are very large and they need to be cut further into smaller fragments. This molecular cutting is achieved by special chemical scissors known as restriction enzymes. The beauty of the restriction enzymes is that they have an ability to recognise specific sites consisting of their sequences and an ability to cut only those sites. For example, the restriction enzyme *Hinf* 1 recognises the sequence such

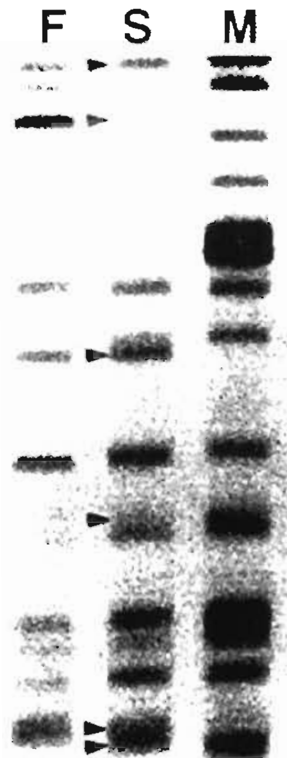

Fig. 6—Typical DNA-fingerprint patterns obtained from different related individuals using multilocus probe. Arrows indicate the paternally inherited bands in the son which are not present in the mother (F: father; S: son; M: mother)

as -GANTC- on DNA and cuts between G and A at this site as shown in Fig. 4. Several hundreds of such restriction enzymes have been isolated and are commercially available. About half a dozen of them are routinely used for DNA fingerprinting analysis.

Step 3 – Separation of DNA fragments

DNA which has been cut into smaller pieces in the previous step is separated for further examination by a process called gel electrophoresis. The matrix of the gel is prepared from agarose derived from a seaweed. The agarose is liquified by melting it and then poured in a thin glassware tray. Gel is set when it cools. DNA samples are loaded in previously made slots and voltage is applied across the gel. As DNA is negatively charged because of its phosphate groups, it moves towards the positive node when electric current is passed through the gel. Smaller size fragments move relatively faster and reach towards the positive pole earlier; the larger fragments remain behind. In between these two extremes, fragments of intermediary sizes align themselves according to their lengths.

Step 4 – Denaturation of fragments

Separated fragments are now denatured with the help of alkali (sodium hydroxide solution). These fragments are then transferred on to a membrane which is positively charged. Hence we now have essentially a single stranded fractionated DNA stuck on a positively charged membrane.

Step 5 – Detection of specific fragments

As DNA is colourless, we need some method by which we can detect all the fragments of DNA on the membrane. Therefore new complementary strands labelled with radioactivity are used. Formation of complementary base pairs with the existing single strand DNA fragments makes its detection possible with the help of radioactivity (Fig. 5). For example, if the fragment composed of ATGCG is on the membrane then we should provide radioactively labelled complementary strand such as TACGC which will form a duplex DNA by complementary base pairing. These radioactively

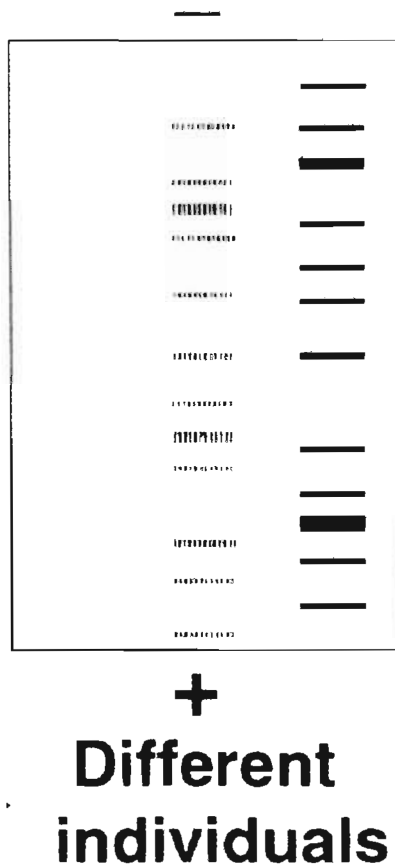

Fig. 7—DNA-fingerprints of three different unrelated individuals showing individual-specific patterns

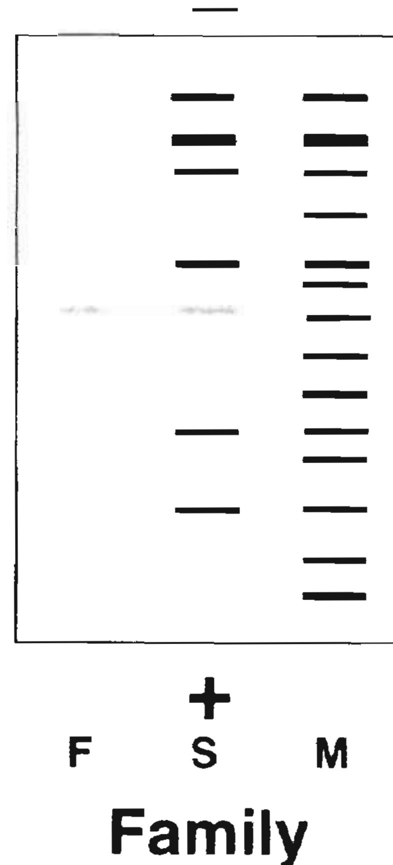

Fig. 8—DNA fingerprints of members from one family (F : father, S : son, M : mother). Every band present in the son is accounted for either being present in the father (grey bands) or in the mother (black bands). There is not a single band which is unaccounted for.

labelled sequences are called probes. When the membranes are incubated with probes under a proper set of conditions, radioactive probes will find their complementary partners to form duplex DNA. These membranes are subsequently put on the X-ray film in a dark room for a couple of days and bands can be seen on the X-ray film at positions where the radioactive complementary sequences have formed the duplexes. Typical DNA fingerprinting is shown in Fig. 6.

Uniqueness of DNA

There are certain important characteristics of DNA which make this material suitable for identification of an individual. Firstly, DNA remains the same all throughout the life span of human being; it does not change with age. Secondly, no matter from which tissue one isolates DNA (kidney, brain, hair, blood, semen, bone, sputum, urine, skin or from any other tissue) all give the same DNA fingerprinting pattern as long as tissue belongs to the same individual. Because of these reasons, it is immaterial which tissue is compared for DNA analysis. For example, the DNA from the semen found at the scene of crime in case of rape could be compared with the DNA isolated from the blood of the suspect. There is another remarkable property of DNA which is very essential for forensic investigations; DNA is very stable molecule. One can heat it or even boil it only to see that DNA strands are separated; this process is known as denaturation. The moment appropriate buffer conditions and temperature are provided, the strands come back and form a double helical structure. Thus DNA is comparatively more stable than other important functional biological molecules such as proteins, carbohydrates, etc. This could be one of the reasons why nature has selected DNA as a genetic material. DNA is so stable that it has been isolated from the remains of bones which were thousands of years old. It has also been isolated from some several million years old fossils. For example, DNA was isolated from insect fossils trapped in amber. Of course occasionally one finds that DNA is degraded into small pieces. However, scientists have invented ways and means to cope up with such a situation, which are described later.

Nature of DNA fingerprinting patterns

1. First generation of DNA fingerprinting probes : Multi-locus probes

A subset of minisatellites share a common 'core' sequence embedded in each repeat unit which is involved in the generation of hypervariable tandem repeated loci by serving as polymerase slippage signal. These are distributed all over the genome covering most of the chromosomes. These core-sequence probes detect variable number tandem repeats (VNTR) in the genome (Fig. 6). Some of the multi-locus probes (MLPs) that have been extensively used are Bkm (Banded krait minor satellite DNA) and Bkm-derived clone 2(8), and 33.6 and 33.15 (Tandemly repeated core-sequence downstream to α-globin gene, see Singh, 1991, 1995).

DNA fingerprinting patterns are normally reflections of existence of variable lengths of tandem repeats. As these variable regions are unique for every individual, DNA fingerprinting patterns can help in identifying a particular individual. The DNA fingerprinting patterns obtained from three different individuals are given in Fig. 7. The patterns are schematically generated on a computer for the convenience. DNA fragments in case of each individual are arranged from top to bottom in a decreasing order of length. It can be seen that these patterns are drastically different from each other and they are individual specific. None of the two individuals has all the bands exactly in the same position. However, occasionally there is a matching of one or two bands with one another but this could be purely coincidental. Another interesting example where three DNA fingerprinting patterns come from the members of the same family, the mother, the son and the father are given in Fig. 8. In these patterns, careful observation brings out certain facts such as (1) The patterns from the sample of mother and the father are entirely different and they hardly match in terms of their positions (sizes of fragments); (2) At a glance pattern from the son appears different from that of the parents, but on careful scrutiny one finds that every band present in the child is accounted for either being present in the mother or in the father. One cannot find a single band in the son which is not accounted for by his parents. This happens because 23 of the chromosomes in a child are inherited from the father and 23 from the mother which are carriers of genetic material and these bands originate from DNA. Therefore, theoretically speaking, approximately 50% of the bands present in every individual come from the father and 50% from the mother. One can also notice that every band present in the father is not inherited by the son; and every time the child is borne, 50% bands are inherited from the father in a completely random fashion. That means if one looks at the patterns from brothers and sisters, or two sons for that matter, their patterns are somewhat similar but not identical. But in each case, inheritance of the bands are accounted for by looking at the patterns of both the parents. In such cases like brother and sister, investigators make use of another parameter which is called co-efficient of band sharing. This parameter is based on the total number of bands and how many bands are common between two individuals. In such a case one should normally find a little more than 50% bands common between brother and sister; while in case of unrelated individuals, this could be 30% or less. Therefore, when one finds more than 50% bands common between two individuals, one can certainly say that these individuals are brothers and sisters. However, one cannot extend this parameter to cousins

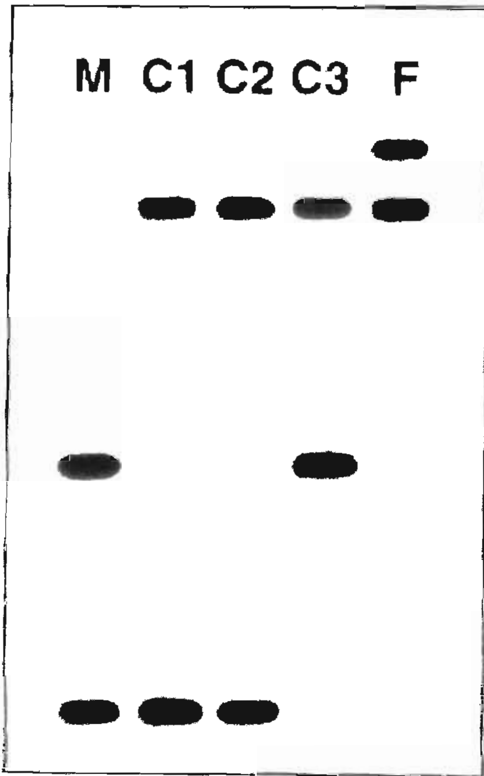

Fig. 9—DNA fingerprint patterns obtained using single locus probe. F : Father, M · Mother and C1, C2 and C3 are the children from the same family. Each child shares one band each from mother and one from father.

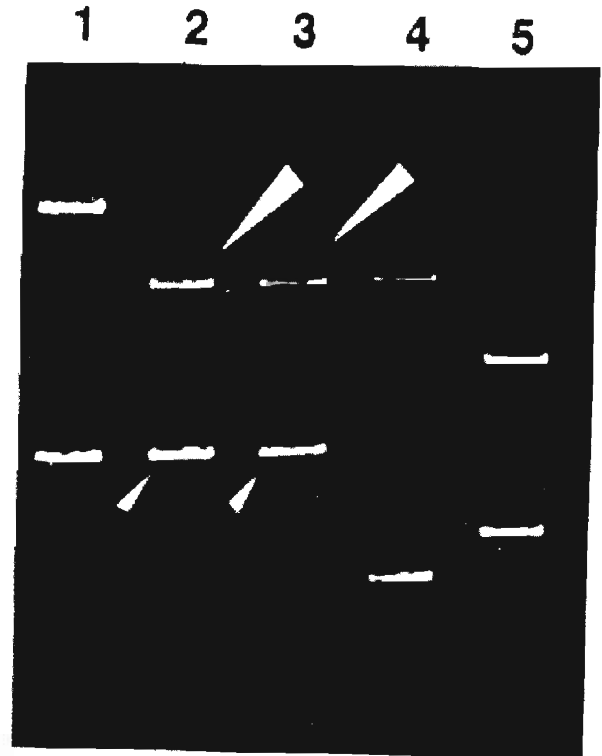

Fig. 10—PCR-based DNA fingerprinting carried out using teeth pulp and hair-roots and compared with the blood sample of the suspected parent. *Lane 1*: blood of suspected mother; *Lane 2*: teeth of the deceased; *Lane 3*: hair of the deceased; *Lane 4*: blood of suspected father; *Lane 5*: blood of unrelated individuals. Bands indicated by arrows testify that the deceased is the biological offspring of the suspected parents.

or cousin brothers because such individuals could be as good as unrelated ones.

DNA fingerprinting of identical twins

Identical twins are a special case where DNA fingerprinting patterns are absolutely identical and they match perfectly. Hence two identical twins cannot be differentiated from each other on the basis of DNA fingerprinting pattern. In a forensic set up, a challenging situation would be the case where one of the two identical twin brothers has actually committed a murder. Even if we know one of them is a suspect, it would be difficult to prove by DNA fingerprinting which one of the twin brothers has actually committed the crime – one of the serious limitations of this technology. Obviously, in such a çase, normal fingerprints, if available, may be of help in identifying which of the two twin brothers has actually committed the crime. Researchers are in search of finding such probes where the differences between two identical twins could also be mapped with certainty.

Advantages and disadvantages of MLPs

A single MLP provides sufficient number of variable bands to establish positive identity of an individual beyond reasonable doubt. An MLP cannot, however, be used reliably to type mixed samples for example in the case of multiple rape. It requires high-molecular-weight DNA in larger quantities and is technically demanding. The alleles are not well defined and their specific association with particular chromosomes is difficult to show.

2. Second generation of DNA fingerprinting probes : Locus-specific probes (single locus probes)

A probe that detects a single hypervariable locus originating from a specific region of a specific chromosome is called a locus-specific or single-locus probe (SLP). Each chromosome in an individual occurs in pair, one inherited from the biological mother and one from the biological father. If the locus is identical between mother and father, it gives only one

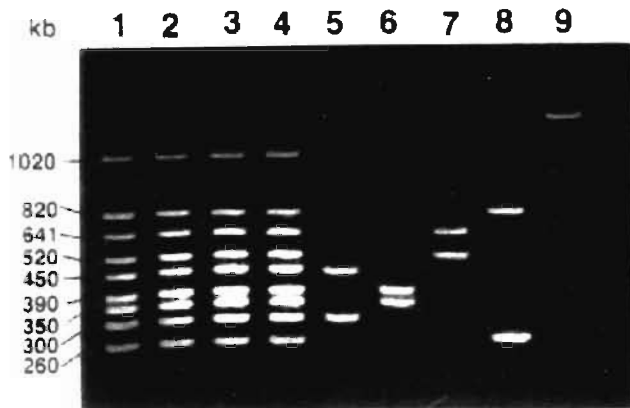

Fig. 11—Identification of various suspects from swabs of mixed blood by using PCR products from four cotton swabs (*Lanes 1-4*); four suspects (*Lanes 5-8*); and examiner as a control (*Lane 9*).

band and is designated as homozygous state and when the locus is different between the two parents, it gives two bands and is designated as heterozygous state. The hybridization patterns given by SLPs are very simple and consist of one or two fragments per individual (Singh, 1999). Loci with heterozygosities higher than 95% rarely show evidence of common alleles.

Advantages and disadvantages of SLPs

SLPs are simpler to use compared to MLPs (Fig. 9). They require comparatively little genomic DNA. Unlike in the case of MLPs, slightly degraded DNA can also be used. SLPs are most uniquely used in identifying mixed DNA samples in cases such as multiple rape, screening of large samples by pool typing to identify a serial rapist or most potential suspects. Patterns obtained are simple and easy to interpret in courts. Chromosomal locations of these probes are well defined. However, one has to use at last a set of five or six different probes to establish identity and it is technically highly demanding.

3. Third generation of DNA fingerprinting probes : SLP detection by DNA amplification using polymerase chain reaction (SLP by PCR)

The PCR is one of the most ingenious developments in molecular biology in recent years (Mullis, 1990). It allows amplification of a single copy of a target DNA sequence, defined by oligonucleotide primers flanking the ends of the sequence, to millions of copies in a short period.

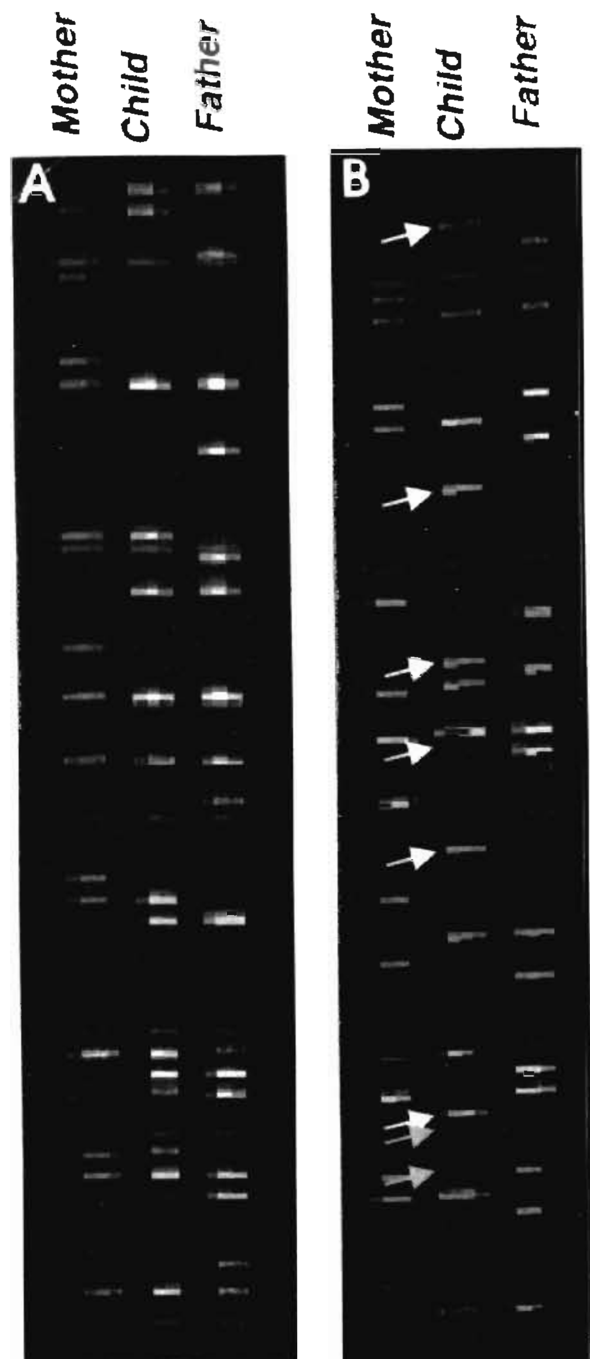

Fig. 12—STR analysis using Profiler Plus STR kit. Red color bands are the molecular weight markers, other color bands represent various STR alleles. (A) Every band present in the child is accounted for either being present in the mother or in the father, therefore they are the biological parents of this child. (B) Bands which are marked with the arrows are neither matching with mother nor with father, therefore they are not the biological parents of this child.

Advantages and disadvantages

This is ideally suited for forensic use because it allows DNA profiling of a single strand of human hair or a single drop of blood or semen or a minute blood stain or semen stain, which would otherwise be too small a quantity to be of value (Fig. 10). It also allows the typing of samples too degraded for MLP analysis. PCR-based DNA typing can be performed in a short time. It is useful in identifying mixed forensic samples and in cases of multiple rape (Fig. 11). However, one has to use at least a set of five to six pairs of different primers to establish identity; and being a very sensitive technique, contamination is a big problem, which may wrongly lead to exclusion. Therefore, utmost care is required while doing the DNA profiling. The HLA-DQ locus, with six typeable alleles was the first use of PCR in forensic case work.

Mitochondrial DNA (mtDNA) fingerprinting

Sequences of mtDNA hypervariable region are also highly polymorphic. Maternal inheritance of mtDNA makes it a unique tool in studies of populations (Thangaraj *et al.*, 1999). The use of PCR in association with restriction analysis and sequencing of any given piece of hypervariable mtDNA is ideally suited for forensic investigation, particularly in determining the maternity of any given child. mtDNA analysis was employed to determine the maternal relatives of children who were born in prison in Argentina during the military rule that lasted from 1976-1983.

THE PRESENT

4. Fourth generation of DNA fingerprinting probes : Short Tandem Repeats (STRs) in human identification - present method of choice

Short Tandem Repeats (STRs) commonly termed as microsatellites are interspersed in eukaryotic genome (randomly occurring every 6-10 kb) and are known to be highly polymorphic for their length (Hancock, 1999). STRs contain two to seven repeat nucleotide sequence. The most abundant microsatellites are $(GT)_n$ and $(CT)_n$ repeats. Allele size of STRs is generally less than 350 base pairs (bp) in length. Up to 9 sets of STRs can be co-amplified using PCR in a single reaction and the fluorescence-based PCR products can be separated by polyacrylamide gel electrophoresis using ABI Prism Automated DNA sequencer (Han *et al.*, 2000).

Advantages and disadvantages

Small amount of DNA (2ng) is sufficient to co-amplify up to 10 STRs. A degraded sample can be amplified using small size STRs. STR alleles have defined size of amplification, which simplifies interpretation of the results. PCR-based STR analyses are very rapid. The ability to co-amplify and distinguish between multiple loci through the use of fluorescent multiple loci in a single reaction using fluorescent tags on markers with overlapping size ranges, and availability of automated detection technology enables STRs to be employed in high throughput situations because of which it has at present become the method of choice for genotyping (Fig. 12). More than 10 STRs can be amplified in a single tube and analysed in a single lane, provided one of the each STR primer pairs is fluorescently labelled. However, there are some disadvantages of this technology. For example, analysis of dinucleotide STRs has revealed that the Taq polymerase 'slips' during amplification resulting in the generation of artefactual stutter bands 2 bp apart; this leads to ambiguous allele designation and severely limits the reliable interpretation of such loci. Therefore, dinucleotide STRs are not generally employed in a forensic setting. Tetranucleotide repeat loci are more suitable for forensic identification.

THE FUTURE

Gene Chip Breakthrough

These world-changing biochips, formally known as DNA arrays, bear an uncanny resemblance to the chips that ushered in the information age. Instead of transisters, they are crammed with dense grids of molecular tweezers built to grip DNA. They give scientists the ability to analyse thousands of genes at once – in effect, to speed-read the book of life. Gene chips are being used in landmark studies on everything from the origin of cancer to gene mutations and genetic risks for scores of diseases. Gene chip also has potential to be used in forensic investigations for establishing identity of the suspect beyond any doubt by comparing the genetic variation using the entire genome rather than a few markers which are being presently used. For example, one of the most common forms of genomic variations is single nucleotide polymorphisms, or SNPs. The area where SNPs are receiving the most attention is their use as genetic markers for the study of complex human traits and pharmacogenomics. Genome-wide complements of SNPs are now being developed as genetic tools. Perhaps in future thousands of SNPs would be used to design oligo-microarray or a DNA chip which in turn would be used to hybridise with the fluorescence labelled DNA isolated from the forensic sample collected from the scene of crime and with the DNA of the suspect to determine the match or non-match. This will be the ultimate technology for establishing identity by comparison of thousands of variable sequences genome-wide instead of only a few marker bands used today.

Sex specific identification

Due to the lack of sensitivity and technical as well as statistical problems none of the traditional methods for male identification are being practiced for forensic applications. There are occasions when paternal or maternal lineage has to be established beyond doubt when one of the parents is absent. It has been shown that major part of the long arm of the human chromosome (Yq) is made up of polymorphic sequences which are organised into large interspersed tandemly repeated arrays. Therefore the development of Y-chromosome-linked STRs has become the system of choice for male identification (Jobling *et al.*, 1997). Y chromosome-specific STRs are more useful in rape cases to identify only the male DNA because vaginal swab of victim contains sperms as well as her own epithelial cells. Hence, DNA isolated from vaginal swab will also contain victim's DNA. In such case, Y-STR analysis is useful to detect only the culprit's DNA. Another application of Y-STRs is that if father of a male child is not available due to some reason, one can analyse his father's side male relatives to establish whether he belongs to that family or not.

To identify the maternal lineage is many a times crucial to the forensic investigation. In such a case, mitochondrial DNA (mtDNA) can be very useful because it is inherited only through maternal cytoplasm. It is known that the non-coding region of mtDNA is especially rich in polymorphism. This property of mtDNA can be used very effectively in the process of identification. However, it is also known that these regions are highly prone to frequent mutations and hence the investigator has to guard against any minor variations which he observes during the DNA fingerprinting tests.

DNA fingerprinting is now a well established technique. This is not only being used in several areas of research in modern molecular biology and genetics but also finding potential applications in our day to day life. A unique example is how the sophisticated scientific technology reaches to the common man and helps to resolve questions like establishment of paternity with accuracy which was hitherto not possible. There are several applications of DNA fingerprinting such as identification, crime investigations, wildlife conservation, authentication of plants and seeds, etc. Advances in the technology are leading to novel uses of DNA fingerprinting almost every day. It has potential to cover a range of applications such as from the identification of an individual to the conservation of biodiversity. Applications also cover various disciplines such as molecular biology, genetics, evolution, anthropology, medicine, judiciary, law, ethics, etc.

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