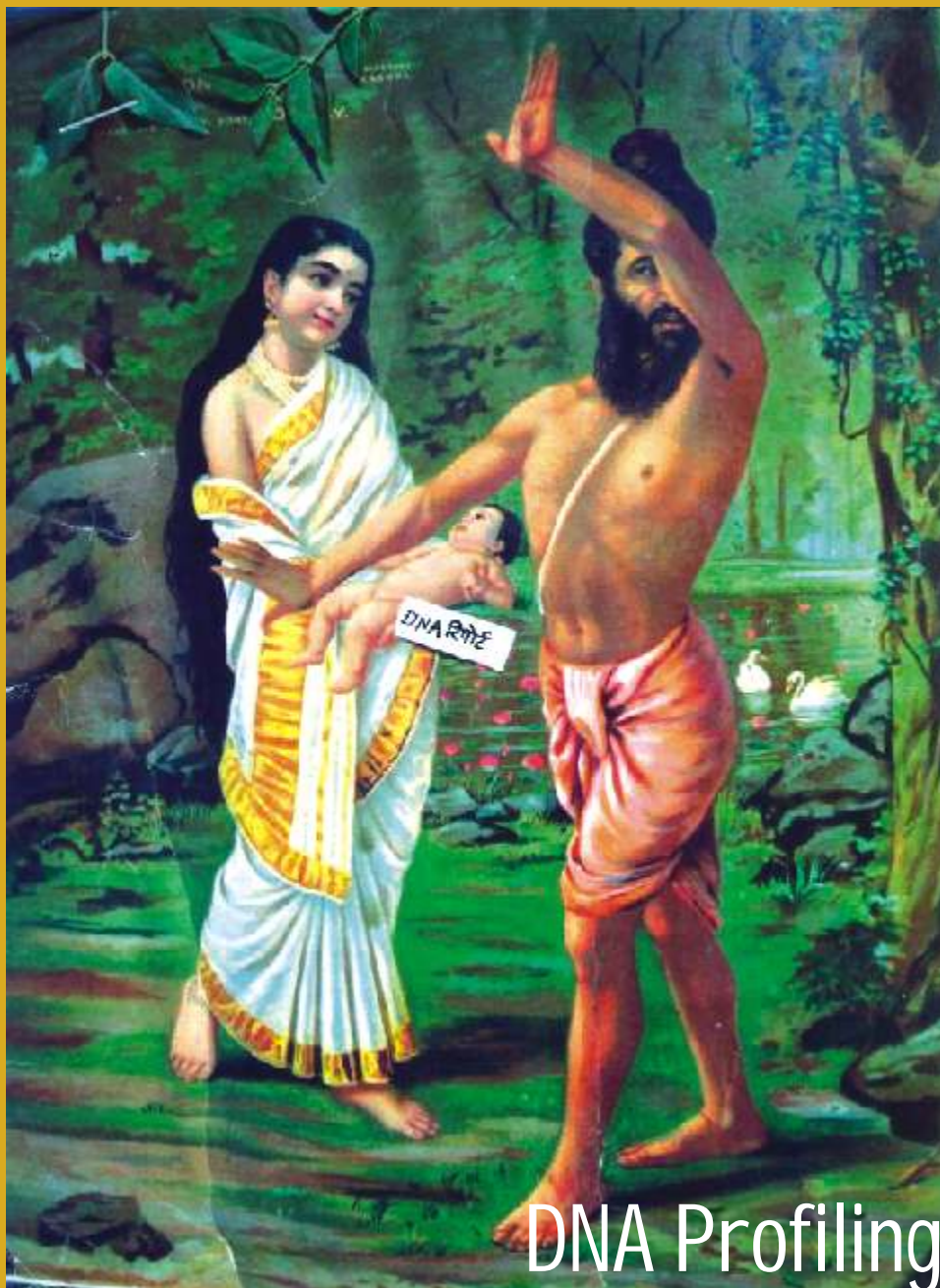


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N E W S



DNA Profiling Clinching Evidence



Department of Biotechnology
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DNA Profiling

Clinching Evidence



J Nagaraju

The discovery of the structure of DNA by Watson and Crick in the early 1960s heralded the DNA revolution with profound applications in all spheres of human life. Today DNA technologies have touched every aspect of the living world. One such important application is the serendipitous discovery by Sir Alec Jeffreys of a short hypervariable stretch of DNA with tandem repeats called minisatellites which laid the foundation of the first DNA “fingerprint”.

As we all know now, the DNA Fingerprinting technique has revolutionized the way the crime investigation is done in the world (Nature 301, 732-734, 1983; Nature 314, 67-74, 1985). This discovery also signified the power of curiosity-driven exploration of the unknown facets of science. In Jeffrey's own words “If someone had told me in 1980, 'Alec, go away and figure out a way of identifying people with DNA', I would have sat there looking very stupid and got nowhere at all”, he says. “ So, if I could tell you what I would be doing 5 years from now, I'd be very depressed, because that means I sort of know the answers” (Proceedings of National Academy of Sciences, 103, 8918-20,2006).

In India, the application of this technique as an important aid in forensics was initiated by Dr. Lalji Singh at the Center for Cellular and

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The process of DNA fingerprinting was discovered serendipitously by Alec Jeffreys, in the course of his research on human myoglobin gene at the University of Leicester in 1985. This method was used for the first time to solve two rape/murder cases in the UK in 1987.

► Molecular Biology (CCMB), Hyderabad. Singh used a multilocus probe which is different from the one used by Jeffreys. This was isolated from the banded krait (*Bkm* Probe). DBT established the Center for DNA Finger Printing and Diagnostics in 1996. Since its inception the CDFD has not only developed several new DNA signatures but also provided invaluable services to the criminal justice system of the country in more than 3500 cases (see box below).

As with any scientific advances, the methodologies involved in DNA fingerprinting have

undergone a sea change within 25 years of its first application in criminal justice delivery system thanks to the breathtaking developments such as transition from multilocus, minisatellite-based approach to single locus, microsatellite-based DNA fingerprinting. Other advances include Polymerase Chain Reaction (PCR), automated genotyping technologies which can assign allele size differences to one base resolution, identification and validation of genetically neutral microsatellite markers, multiplexing of multiple microsatellite markers, deployment of Y chromosome-

linked markers that can pick up the male DNA from a complex mixture of DNA to assign paternity and patrilineal relationship, utilization of mitochondrial genomic regions for assigning maternity and matrilineal relationship, technical improvements in DNA isolation protocol from embedded tissues, excavated and archaic bone and hair samples, development of miniature microsatellite alleles which can amplify microsatellite alleles from degraded samples, development of DNA databases of offenders, convicted individuals, of different caste populations of different religions, and so on. ►►

Centre for DNA Fingerprinting & Diagnostics, Hyderabad

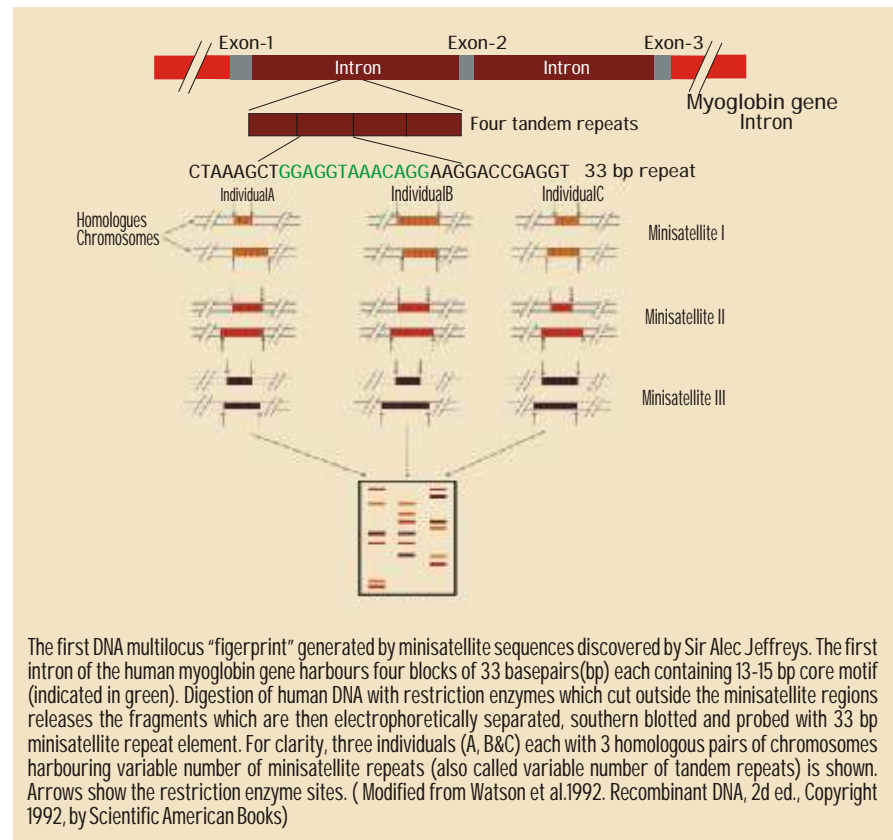
The Centre for DNA Fingerprinting & Diagnostics (CDFD), Hyderabad – one of the autonomous institutes of DBT – has analyzed and provided expert opinion in more than 3500 cases involving paternity, body identification, crime scene samples, baby exchange, rape cases, mass disaster victim identification and others. Some of the important cases handled by CDFD include: Naina Sahni Tandoor murder, Rajiv Gandhi assassination, Priyadarshini Mattoo murder, Mudhumita Shukla murder, Godhra mass burial, Nithari murders, Mangalore air crash, Samjhauta Train bombing, Nayapul hospital baby exchange etc.

Based on its own research findings, CDFD has established jointly with Agricultural and Processed Food Products Export Development Authority-Centre for Basmati DNA Analysis. The Centre has authenticated the purity of more than 2500 Basmati export samples since its establishment.

► DNA evidence, unlike latent fingerprints evidence, offers law enforcing agencies and judiciary important tools for the identification and apprehension of perpetrators of crimes such as sexual assault resulting in a very high conviction rate. In the West, DNA evidence has also provided relief to convictions long after the appeal process has been concluded, resulting in the acquittal of a large number of persons wrongly convicted based on certain conventional evidence (e.g. eye witness). This has raised troubling issues about the statutes of limitations and the principles of finality in criminal law. We all have been brought up on the notion that a “Mother is a Mother is a fact” and a “Father is a father is a belief”. Even this notion, which our society has religiously accepted has been challenged with the development of cloning, *in vitro* fertilization and surrogate motherhood. So, DNA revolution is all pervasive and hardly any segment of the society is untouched.

Genetic Basis of DNA Fingerprinting

DNA is a double helix; two strands of which spiraled around each other contain a sequence of nitrogenous bases, i.e. Adenine (A), Guanine (G), Cytosine (C), and Thymine (T). The two strands of DNA are



connected at each base: (A) will only bond with (T) and (G) will only bond with (C). Each person has about 3.3 billion base pairs (bp).

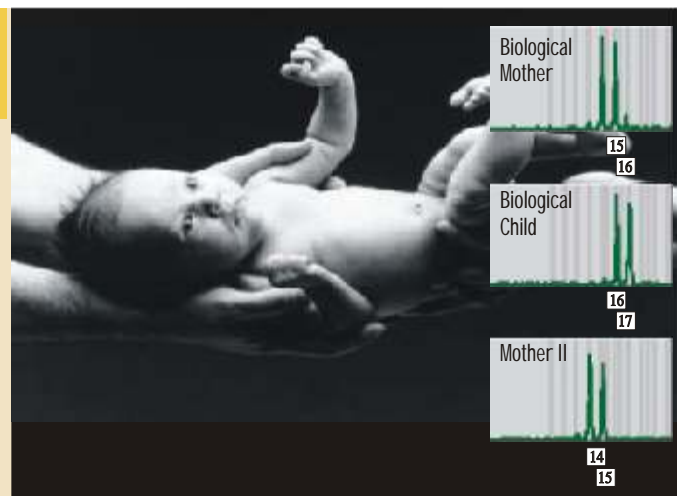
Cells in the human body contain 23 pairs of Chromosomes called homologous Chromosomes; one member of each homologous

pair is inherited from the mother through ovum and one from the father from sperm.

All the cells in the human body are derived by successive divisions from a single fertilized ovum. So every cell has the same chromosomal make up, except germ ►►

Child Swapping Case at Nayapul Government Hospital, Hyderabad

Ms Geetha (name changed) delivered a male child in Nayapul Government Hospital in Hyderabad. Due to premature delivery the baby was kept in an incubator for several hours. The hospital staff, mistakenly, gave the male child to another lady who had delivered a female child on the same day and offered the female child to Ms Geetha. But the latter refused to take the female child. The hospital authorities tried to prevail on Ms. Geetha to accept the female child but failed to convince her that the child was indeed hers. A police complaint was made and the media highlighted the case nationwide. At this stage, the hospital authorities approached CDFD for a DNA test. The DNA test revealed that the female child was not the biological child of Ms Geetha. After 22 days, police traced the parents who had accepted Geetha's male child and obtained DNA tests from CDFD. The DNA profiles of the male child matched with that of Ms Geetha and the child was restored to her.



Rape of a Medical Student in Delhi

The victim, a fourth-year student of the Maulana Azad Medical College, was raped at knife point on the terrace of the Mughal monument 'Khooni Darwaza' in central Delhi on November 15, 2002.

The victim filed a complaint with the police. Upon investigation, it was found that the victim had washed all her clothes after the traumatic episode. Fortunately, the police were able to recover a sanitary pad used at the time of crime which contained semen stains. The DNA profile from these semen stains matched with that of the accused. Since there was no eye witness at the scene of crime, DNA evidence nailed the accused. The trial court had awarded two suspected youths Rahul and Amit life imprisonment in January 2005.



- ▶ cells (sperm and egg cells) which have only haploid set of 23 chromosomes. The diploid set i.e. 46 is restored upon fertilization. Successive divisions result in the embryo and subsequent development of the child which inherits half of the DNA from the biological mother and half of the DNA from the biological father. This forms the basic principle to establish paternity, maternity disputes and biological relationship by DNA fingerprinting which is more appropriately called DNA profiling.

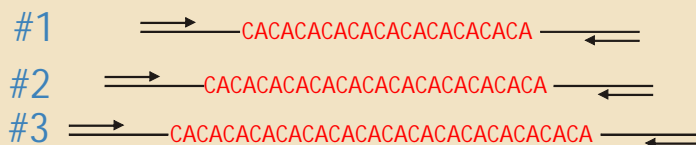
Minisatellites and Microsatellites the Magic Segments of DNA

The chemical structure of everyone's DNA is the same. The difference between individuals is the order of the base pairs in hypervariable regions of the DNA which Alec

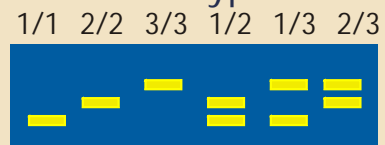
Jeffrey first discovered in the form of minisatellite repeats of 33bp with a core motif of about 10-15 base pairs long. The number of minisatellite blocks each with 33 bp varies between individuals providing length variations when electrophoretically resolved and probed with the minisatellite sequences (see graphic on pg. 158). Subsequent researches found hypervariable microsatellite repeats which are also called simple sequence repeats (SSRs) or short tandem repeats (STRs) of di-, tri-, tetra-, penta- and hexanucleotide repeats each of which is typically repeated multiple times (sometimes > 30-40 times) in the genome. These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic co-dominant markers. Alleles of these loci are differentiated by the number of

copies of the repeat sequence contained within the amplified region and are distinguished from one another (see graphic below). The individual-specific variations at microsatellite loci could be revealed by PCR amplification of SSRs using primers that flank the SSRs. The SSR loci have proved to be most useful tools in forensic genetics for the following reasons: (i) they can be amplified using PCR, as little as 1nanogram of target DNA is sufficient; (ii) they can work on DNA isolated from degraded samples since SSRs are ~ 100bp long as compared with several thousand base pairs for a typical minisatellite repeat; (iii) many SSR loci can be multiplexed in a single reaction and PCR amplified using different fluorescent dye labeled primers and run on automated genotyper with precise sizing of ▶▶

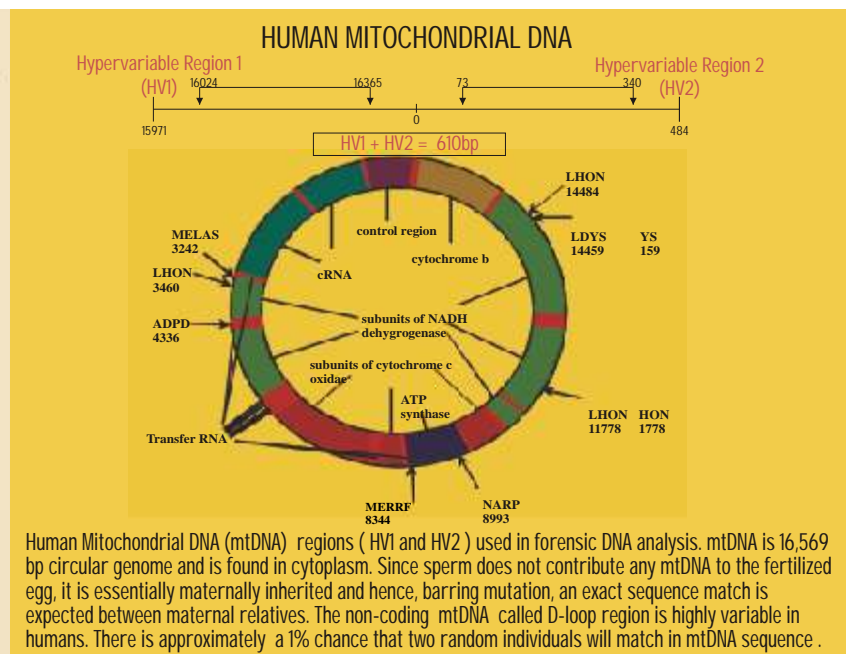
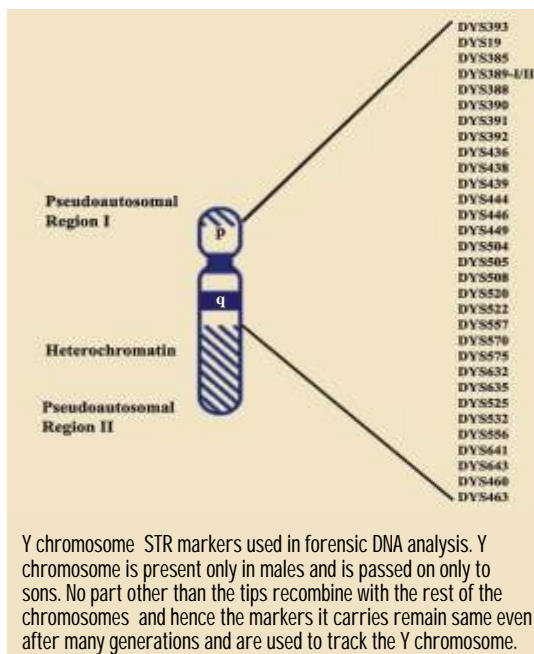
Alleles



Genotypes



Microsatellite alleles and their detection. Three alleles of different sizes at a microsatellite locus composed of a (CA) repeat are shown. The arrows indicate the locus-specific primers designed for the region flanking the (CA) repeat, used for PCR amplification. Illustrations of the various gel patterns that would be observed with different allele combinations are indicated. Homozygotes (1/1, 2/2 and 3/3) and heterozygotes (1/2, 1/3 and 2/3) could be unambiguously scored.



▶ alleles; and (iv) the entire process of SSR analysis from DNA extraction to genotyping can be completed in 2-3 hours.

Applications of DNA fingerprinting in Forensic Cases

- DNA fingerprinting can be applied in the following scenarios:
- Establishment of paternity and maternity
 - Establishment of the parentage for child swapping cases
 - Establish the identity of the rapist in rape cases
 - Identification of mutilated remains

- in murder, bomb blasts, air crashes etc.
- Establishment of biological relationship for property disputes and immigrations
- Wildlife identification, and
- Seed authentication

DNA and the Law

Sir Alec Jeffreys coined the word DNA “fingerprinting” just to highlight the individual-specific exclusivity of such DNA profiles, as is the case for latent fingerprints. DNA “fingerprint” is however, not the correct term and DNA profiling

would be a more appropriate term to describe the individual-specific profile generated on the basis of an individual's DNA. Some much-publicised DNA cases across the world include the OJ Simpson case in USA identification of the remains of the Tsar of Russia and impeachment proceedings against the then President of USA for having sexual relationship with a White House Intern. In our own country, examples include Rajiv Gandhi and Beant Singh bomb blast cases, Naina Sahni *Tandoor* murder case, the Nayapul child swapping ▶▶

Human DNA Profiling Bill Salient Features

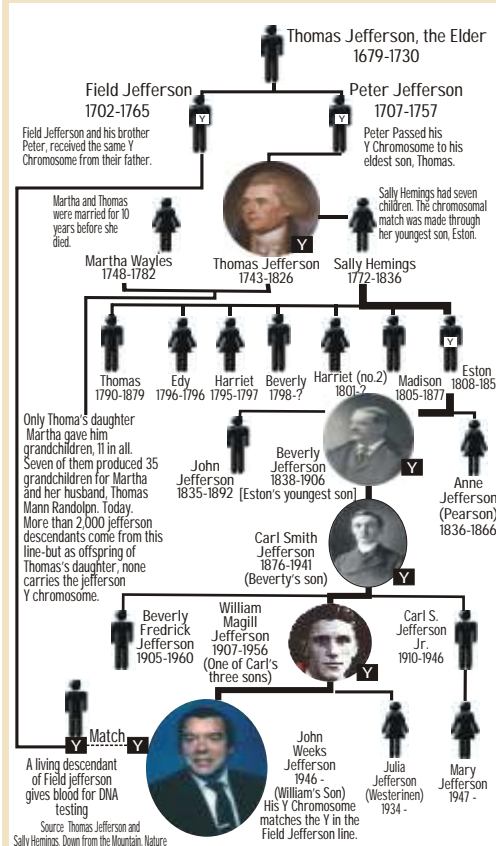
- DBT has prepared with the help of CDFD a draft Bill for Human DNA profiling for enactment in the parliament. The bill provides for:
- Collection of DNA profiles of individuals for lawful purposes
 - An Authority to create and maintain DNA databases of offenders, suspects, crime scene samples etc for detection of, and to serve as deterrents against, repeat offenders
 - Creation of a DNA Profiling Advisory Board of experts to define, establish, and modify standards and to implement quality control measures including compulsory accreditation of laboratories/agencies, undertaking DNA Profiling services (also to prevent abuse/misuse of DNA profiling to discriminate against individuals for employment, health/life insurance etc)
- The draft bill has been submitted to the Ministry of Law & Justice to examine the same from the legal and drafting angles. It will then be submitted for approval of the Cabinet before introduction in the Parliament.



► case in the city of Hyderabad (see box on pg. 158), identification of Godhra riot victims, Nithari serial murder case, rape of a medical student in Delhi (see box on pg. 159), and the recent crash of Air India flight at Mangalore. Computational advances have pushed forensic DNA revolution to the centre stage, and computational software now enables crime investigators to query the DNA profiles found at the scene of crime in large database sets which have been developed in many countries (see box on pg. 173), or even partial matches to a genetically related criminal. The crimes which involved parentage i.e. paternity/maternity can be solved even after several years of sexual assault using DNA from seminal swabs or vaginal swabs captured on a slide. This has also resulted in exchange of crime databases between different nations. We in India do not have a DNA database of criminals. The Government of India, through a Gazette Notification has created a “DNA Profiling Advisory Committee (DPAC)”. One of the mandates of D-PAC is to draft legislation for DNA. The CDFD in Hyderabad has prepared the “DNA-Profiling Bill” (see box on pg.160).

The United States FBI has developed a database of convicted offenders by a statute of congress through the “DNA Identification Act” in 1994 and it named Combined DNA Index System (CODIS), which contains 13 microsatellite loci and an amelogenin locus (from sex chromosomes), which can distinguish gender. Now new multiplex systems that amplify 16 loci in a single reaction have been introduced (see graphic on pg. 173). Besides, depending on the complexity of the

The Thomas Jefferson Affair Y Chromosome Shows The Way



Y-chromosomal DNA analysis and the Jefferson-Hemings controversy: Tracking the Jefferson Y chromosome. A simplified version of the family tree, showing the transmission of the matching Y chromosomes

Thomas Jefferson (1743-1826), the third President, and author of the Declaration of Independence of the United States, was accused of having a sexual relationship with his slave Sally Hemings and fathering her children. Jefferson made no comments on this issue during his life time, but many traditionalists believed that such a liaison was not possible. However, some of Sally's descendants maintained otherwise and claimed to be the progeny of the ex President. This 200-year-old historical controversy has been recently resolved (E Lander & JJ Ellis and Foster et al. 1998. Nature, 396: 14, 26-27). The study was based on the fact that most of the male (Y) chromosome is passed intact from father to son. Females do not carry the Y chromosome. Jefferson did not have male heirs; however, he had a paternal uncle who had sons. The lineage was traced from this uncle through eight generations to the present day. The results were compared with those on the male lineage of Sally Hemings through five generations. Using Y chromosome haplotypes, in combination with biallelic markers and a minisatellite marker, geneticists were able to confirm that there is a high degree of probability that at least one of Sally's children, Eston, was fathered by Jefferson.

cases, a number of Y chromosome-STRs as well as mitochondrial sequences which are paternally and maternally (see graphics on pg160) inherited respectively are also used for forensic analysis to identify the paternity and maternity. Perhaps, the most famous case where Y chromosome-specific markers were successfully employed was that of Thomas Jefferson (1743-1826), the third U.S. President, and author of the Declaration of Independence of the United States (see graphic above).

Single Nucleotide Polymorphisms (SNPs) as Future Forensic DNA Markers

The sequencing of the human genome has revealed several important features of our genome. All of us are almost 99.9% identical. The 0.1% difference of more than 3 billion nucleotides, is however, significant in absolute numbers. Between any two individuals single nucleotide-based differences occur and this is referred to as Single Nucleotide Polymorphism also ►►

(Contd. on page 173)

Clinching Evidence

(Contd. from page 161)

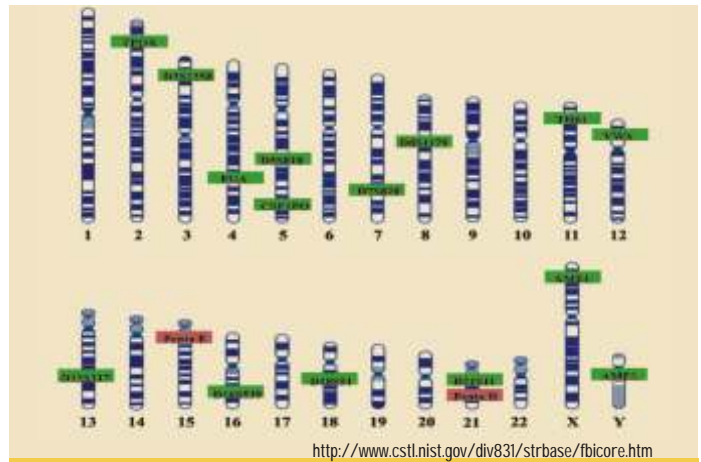
referred to as SNPs or SNIPs. Recently, SNPs are finding use in forensic genetic analysis. The practical advantage of SNP typing is that DNA template size can in principle be used on targets as small as the size of a pair of specific primer i. e., 50 bp. Hence, SNPs are quite useful in typing severely degraded material. The technical challenges of the World Trade Centre Disaster in New York have led to the application of forensic SNP typing. The European Network of Forensic Science Institutes and the US FBI Scientific Working Group on DNA Analysis Methods are assessing potentially useful multiplexes and are in the process of making global recommendation.

Bodies or remains of victims of mass disasters such as air crashes, train accidents, fire accidents, terrorist attacks, earthquakes, tsunami etc. can be effectively addressed using DNA profiling. The September 11th terrorist attack on World Trade Centre in New York in which about 3,000 people perished (www.dna-view.com/wtc.diary.htm) resulted in the largest DNA profiling ever done in the history of crime investigation. Profiling of more than 15,000 body parts recovered after the collapse of the World Trade Centre was carried out and assigned conclusively, after comparing the DNA profile of the relatives. This also enabled scientists

to develop a very high throughput software for finding DNA profile matches of thousands of individuals in a matter of seconds.

DNA profiling has proved to be infallible and unique test for identification and individualization of biological specimens for establishing their relatedness. The increased use of DNA analysis in forensic investigations, the expansion of the DNA database coupled with the automation of newer DNA analysis technologies have greatly increased the amount of DNA data being generated in crime laboratories today. The management and integration of this entire new data is becoming increasingly complex and challenging. The high level of discrimination power offered by DNA analysis technology, the faster processing of samples and the comfort level of prosecutors working with cases involving DNA evidence have greatly increased the demand for DNA analysis.

DNA profiling is not just used in criminal investigations but



<http://www.cstl.nist.gov/div831/strbase/fbicore.htm>

STR markers and amelogenin locus used in forensic DNA analysis with their chromosomal locations. The X-linked amelogenin AMELX, gives rise to a 106 bp PCR product and the Y-linked AMELY, a 112 bp amplicon. Hence, the AMELX contains a 6 bp deletion in the intron 1. Therefore, the PCR products from male sources (XY) show two bands (106 bp and 112 bp) while females (XX) show only one band, thus allowing for sex determination of unknown samples. Mutations in the AMELY may result in non-amplification of the Y allele, resulting in misidentification of the female sample.

also employed in immigration cases to prove the relationship of a visa applicant, for identification of wild life and endangered species of both plants and humans. The black buck shooting case in Rajasthan involving a famous film star was carried out at Centre for DNA Fingerprinting & Diagnostics (CDFD), Hyderabad. In a landmark case, the right of a farmer in the sale of a spurious variety of chilly seeds was established for the first time (Kumar et al. 2001, Forensic Science International 116: 63-68). In another case, the genetic identity and uniqueness of the Indian Basmati rice compared to the Pakistani Basmati was established, again by the CDFD, this case had enormous trade and export impact (Nagaraju et al. 2002, Proceedings of the National Academy of Sciences USA 99: 5836-5841).

In conclusion, while evidences can be tampered with and witnesses can turn hostile, DNA will never lie. It will indeed unravel the truth even several decades after a crime has been committed. Such are the powers of DNA Profiling! ■

Database	Website
STRbase	http://www.cstl.nist.gov/strbase/
ENFSI DNA WG STR Population Database	http://str-base.org/
Autosomal STR DNA Database	http://www.strdna-db.org/
The Allele Frequency Database (ALFRED)	http://alfred.med.yale.edu/
Y-STR haplotype reference database (YHRD)	http://www.yhrd.org/
International HapMap Project	http://hapmap.ncbi.nlm.nih.gov/index.html.en
The SNP Consortium Ltd	http://snp.cshl.org
The Combined DNA Index System (CoDIS)	http://www.fbi.gov/hq/lab/html/codis1.htm
United Kingdom National DNA Database (NDNAD)	http://www.npia.police.uk/en/8934.htm
Canada National DNA DataBank (NDDB)	http://www.nddb-bndg.org/main_e.htm
The innocents Database	http://forejustice.org/search_idb.htm