DNA FINGERPRINTING

- DNA fingerprinting is a method utilized to identify individuals based on their unique DNA characteristics.
- Sir Alec Jeffery pioneered this technique in 1984.
- In India, the introduction of DNA fingerprinting was initiated by Dr. V.K. Kashyap and Dr. Lal Ji Singh.
- While the majority of human DNA is consistent among individuals, forensic scientists analyze the small variations that exist between people to establish identity.
- These variations, known as polymorphisms, play a crucial role in DNA typing. Polymorphisms are characterized by differences in the length of DNA segments at specific loci, referred to as restricted fragments. These segments are primarily composed of short repetitive nucleotide sequences known as variable number of tandem repeats (VNTRs) or minisatellites.
- Alec Jeffery discovered VNTRs, which constitute the hypervariable repeat regions within restricted fragments. These regions typically consist of a basic repeat sequence spanning 11-60 base pairs and are flanked by restriction sites.
- The number and positioning of VNTRs within a restricted fragment vary between individuals, directly influencing the fragment's length.
- Consequently, when the genomes of two individuals are cleaved using the same restriction enzyme, the resulting fragment lengths differ.
- These variations in fragment length are termed Restriction Fragment Length Polymorphisms (RFLPs), which are distributed throughout the human genome and serve as valuable markers for DNA fingerprinting.
- Remarkably, DNA fingerprints can be generated from exceedingly small samples of blood, semen, hair bulbs, or any other cell type in the body. As little as 1 microgram of DNA content is sufficient for analysis

Technique of DNA Finger Printing Involves the Following Major Steps.

(a) Extraction

DNA is obtained from cells through a process known as cell lysis. In cases where the DNA content is limited, Polymerase Chain Reaction (PCR) can be utilized to amplify the DNA, a process termed amplification.

(b) Restriction Enzyme Digestion

DNA is cut at specific sequences of 4 or 6 base pairs known as restriction sites by restriction enzymes. Hae III, derived from Haemophilus Aegyptus, is commonly used. This enzyme cleaves DNA at the sequence GGCC, generating restricted fragments which are then transferred to an agarose polymer gel.

(c) Gel Electrophoresis

- Gel electrophoresis is a technique that separates macromolecules based on their size and electric charge.
- In this process, molecules are forced through a gel matrix by an electric current, with electrodes at either end of the gel providing the driving force.
- Agarose is commonly used as the matrix due to its ability to separate DNA fragments by size via sieving effects.
- Charged particles migrate towards the cathode or anode depending on their net charge, with DNA fragments moving towards the positive electrode (anode) due to their negative charge.
- Smaller DNA fragments migrate more rapidly towards the positive pole, resulting in the arrangement of DNA fragments according to molecular weight.
- These separated fragments can be visualized by staining them with a fluorescent dye.

(d) Southern Transfer / Southern Blotting

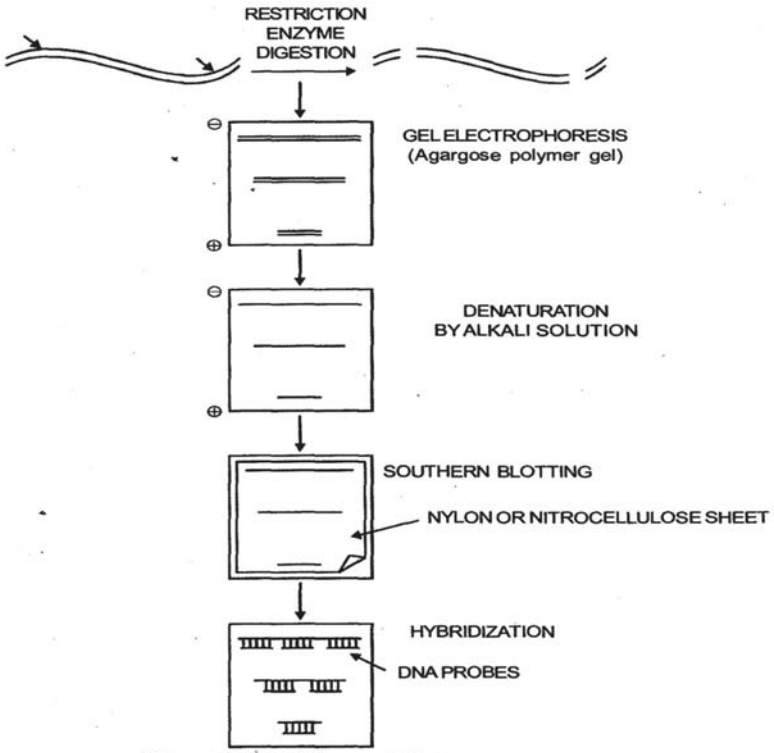
- DNA is fragile in the gel and needs to be transferred to a solid support permanently. This is achieved through Southern blotting.
- The DNA in the gel is denatured into single-stranded form and transferred to a nylon sheet through blotting.
- The nylon membrane irreversibly attaches the separated DNA fragments, a process termed Southern blotting after its inventor, Edward Southern (1970).

(e) Hybridization

- To detect Variable Number Tandem Repeats (VNTR) loci on restricted fragments, single-stranded radioactive (P32) DNA probes are used.
- These probes have sequences complementary to the DNA sequences at the VNTR loci. Multiple DNA probes are commonly used.
- The labeled probes hybridize with the VNTR loci of restricted DNA fragments.

(f) Autoradiography

- The nylon membrane containing the radioactive probes is exposed to X-rays.
- Specific bands appear on the X-ray film, indicating where the radioactive probe has bound to the VNTR.
- This results in the visualization of a specific pattern of restricted fragment lengths known as Restriction Fragment Length Polymorphism (RFLP).
- These RFLP patterns, unique to each individual, serve as a DNA signature, enabling analysts to identify individuals and assess the occurrence and frequency of specific genetic patterns.
- The probability of two unrelated individuals having the same pattern of VNTR location and repeat number is exceedingly low.
- In India, the Centre for DNA Fingerprinting and Diagnostics (CDFD) is located in Hyderabad.



Steps of DNA Fingerprinting

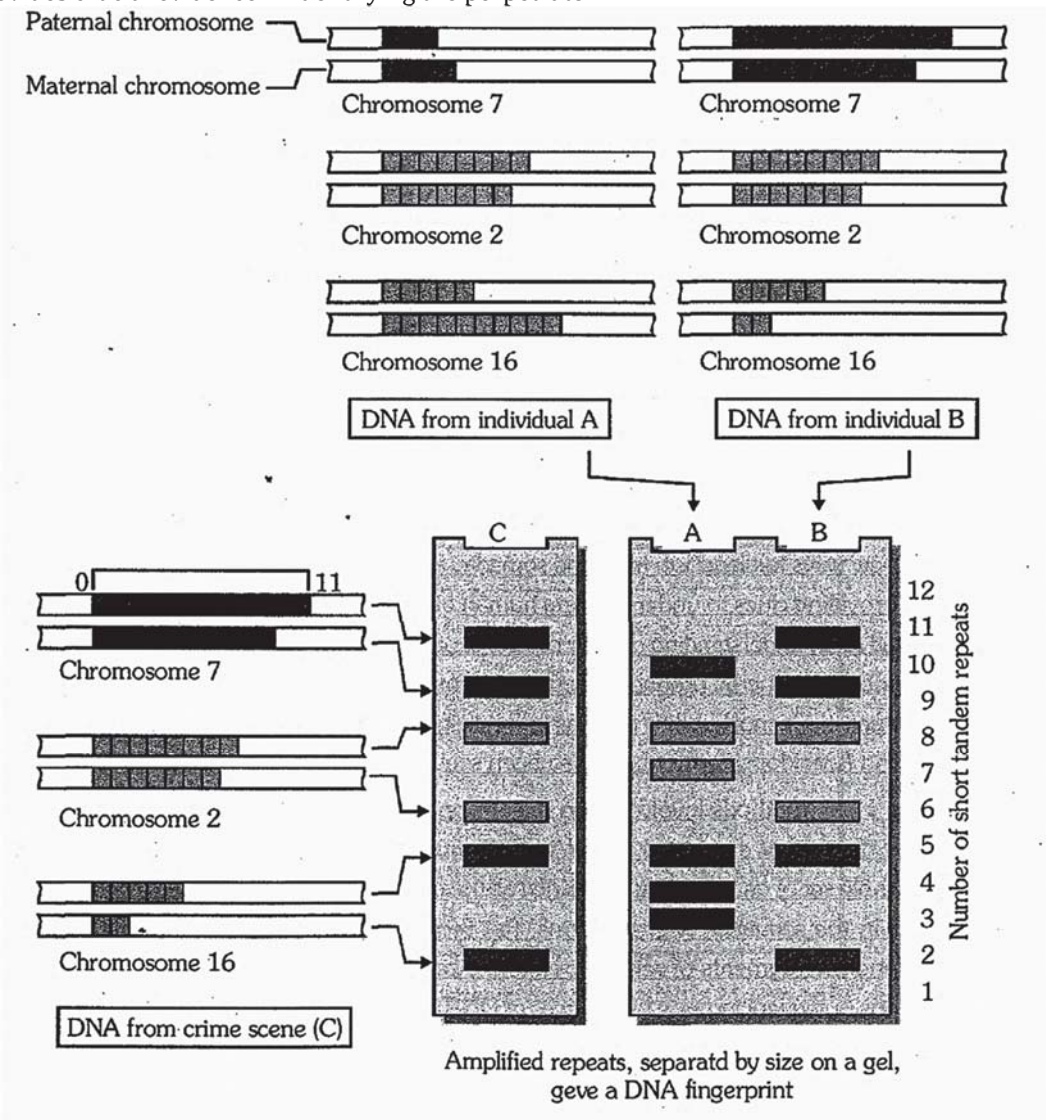
Applications of DNA Fingerprinting

1. Paternity Tests

One of the primary applications of DNA fingerprinting lies in establishing family relationships. In paternity tests, DNA samples from the child, mother, and potential fathers are collected, and their DNA fingerprints are obtained. By comparing the child's DNA fingerprints to those of the biological parents, the true father can be identified.

2. Criminal Identification

DNA fingerprinting has emerged as a valuable tool in forensic science, particularly in cases involving serious crimes such as murder and rape. To identify a criminal, DNA fingerprints of suspects are obtained from blood, hair, or semen collected from the crime scene. These fingerprints are then compared, and if a suspect's DNA fingerprint matches the one obtained from the crime scene sample, it provides crucial evidence in identifying the perpetrator.



Schematic depiction of DNA fingerprinting: A selection of chromosomes is illustrated, each exhibiting varying copy numbers of VNTR. For clarity, color codes have been employed to identify the source of each band in the gel. The two alleles (paternal and maternal) of each chromosome also exhibit distinct copy numbers of VNTR. It is evident that the DNA banding pattern from the crime scene corresponds to individual B, rather than individual A.