



Forensic Evidence in Civil & Criminal Trials: DNA PROFILING

Presented By:
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HOMAGE TO PADMASHREE DR. LALJI SINGH

(Father of DNA Fingerprinting in India)

for his untiring life-time contribution in the field of DNA Technology

He left for his heavenly abode on **10th Dec 2017**

ROLE OF EVIDENCE

Evidence plays a crucial role in Civil & Criminal Cases

1. Evidence is divided into

1. Direct - Eyewitness statements / Confessions
2. Circumstantial - Requires indirect judgment or inference about what happened. Eg. Presence of Hair, Fingerprint at the scene of crime


2. Circumstantial evidence - Important in civil and criminal cases which lacks Direct Evidence.

1. Physical Evidence
2. Biological Evidence

3. Forensic science plays a **vital role in the criminal justice system by providing scientifically based information through the analysis of CIRCUMSTANTIAL EVIDENCE.**



FORENSIC SCIENCE

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- **FORENSIC SCIENCE - USE OF SCIENCE & TECHNOLOGY FOR LEGAL PURPOSE**
 - ‘Forensic Science’ - Application of various basic sciences to provide scientific evidences to court of law
 - **Forensic evidence supplied by an expert witness is usually treated as CIRCUMSTANTIAL EVIDENCE**

Locard's Principle of Exchange

1. “Whenever two objects come into contact, they always leave a trace on the other.”
2. Every criminal can be connected to his crime by contact traces carried from the scene of crime or left by him at the scene of crime

FORENSIC EVIDENCE - Physical

- Physical forensics deals with non-biological types of evidence. These may take the forms of fibers, paint chips, explosives, Questioned Document (Handwriting/ Signature)
- Forensic geology finds evidence in soils and minerals
- Forensic meteorology delves into the historical weather patterns
- Forensic Chemistry deals with chemicals, drugs etc.
- Forensic entomology studies insects
- Psychological Forensics applies the knowledge of psychology to criminal law.
- Forensic ballistics is the study of firearms
- Digital or computer forensics is the identification and examination of all forms of digital evidence.

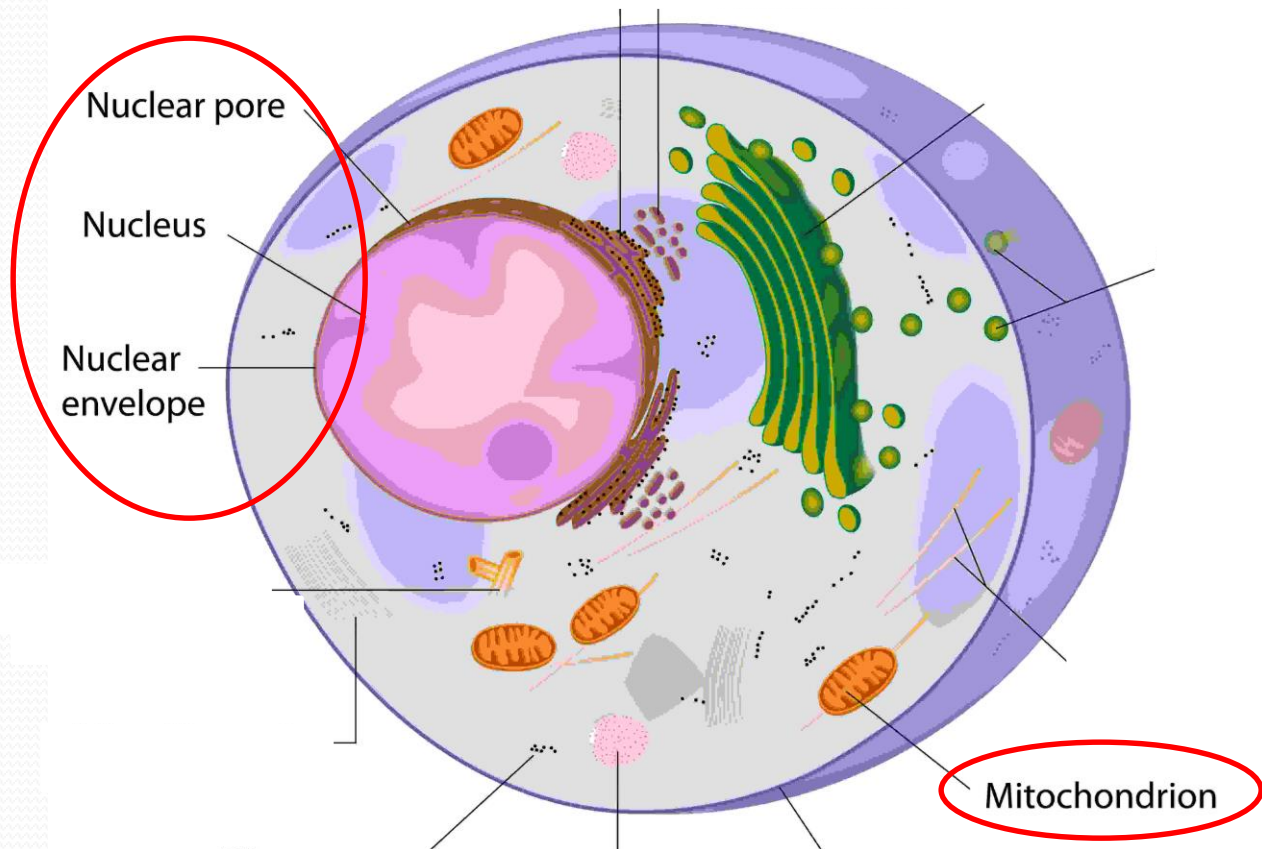


FORENSIC EVIDENCE - Biological



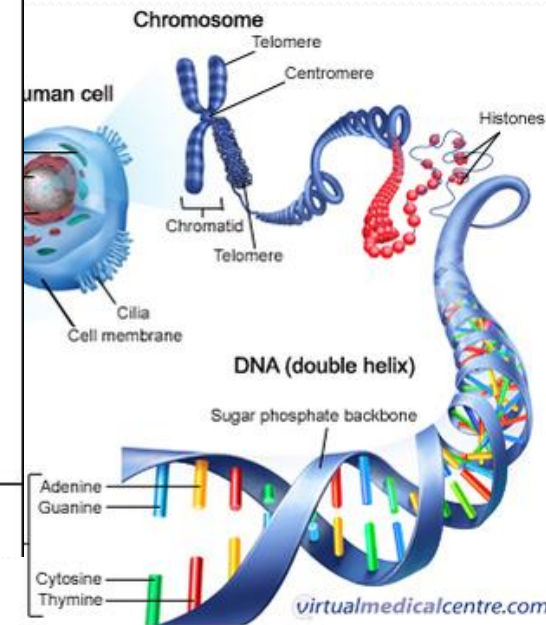
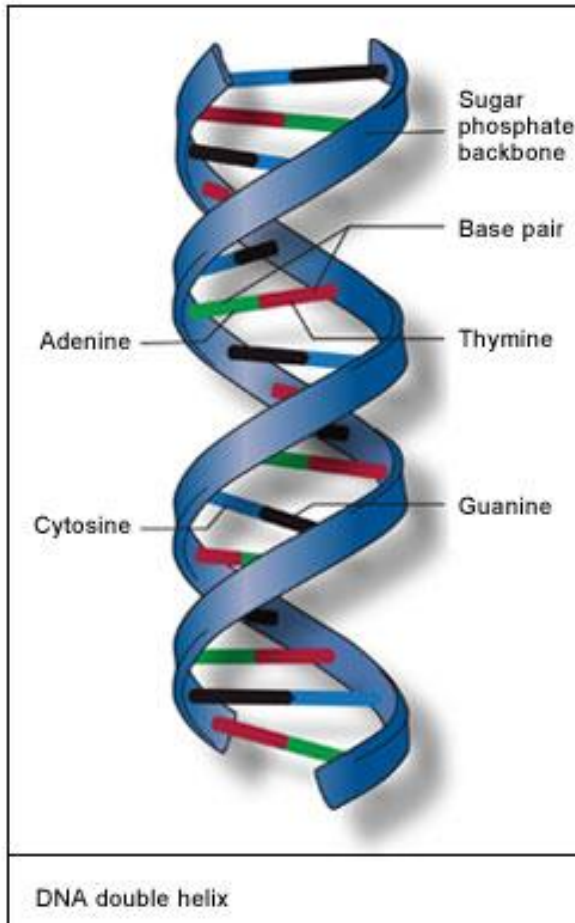
- Biological forensics techniques apply knowledge about the biological sciences to find clues and make determinations about evidence.
- The types of biological evidence that may be found at a crime scene include blood, semen, saliva, faecal material and urine, hair and bone.
- Fingerprint analysis is also a biological forensic technique.
- Forensic toxicology is examination of drug or poison
- Forensic anthropology helps identify skeletal remains
- Forensic odontology deals with the study of the teeth
- Forensic pathology is concerned with the cause of death of a victim.
- Forensic DNA

CELL



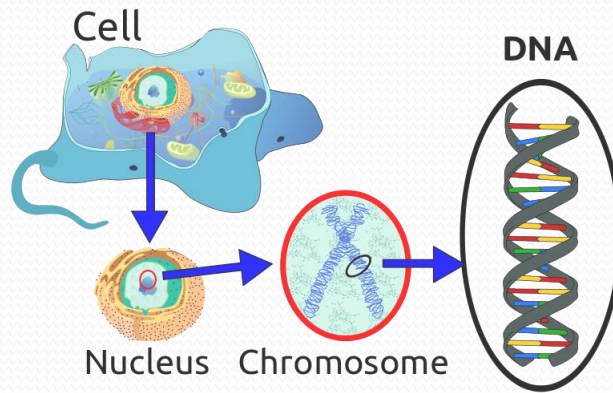
DNA

- DNA (Deoxyribonucleic acid) main constituent of the chromosome found in the form of a **double helix** and gives an individual a **PERSONAL G**
- These chromosomes such as eye, like blood group, give an individual characteristics including invisible characteristics
- In an individual, DNA is the same
- The chances of having exactly the same profile is 30, (except for identical twins)
- DNA is highly scientific in part of admissible expert evidence

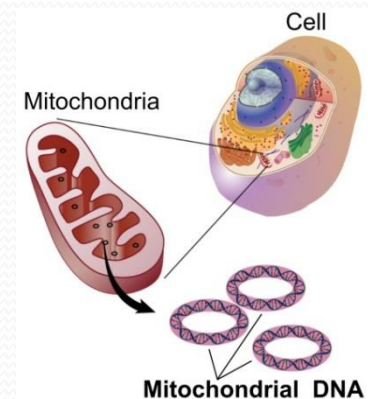


CHARACTERISTICS OF DNA

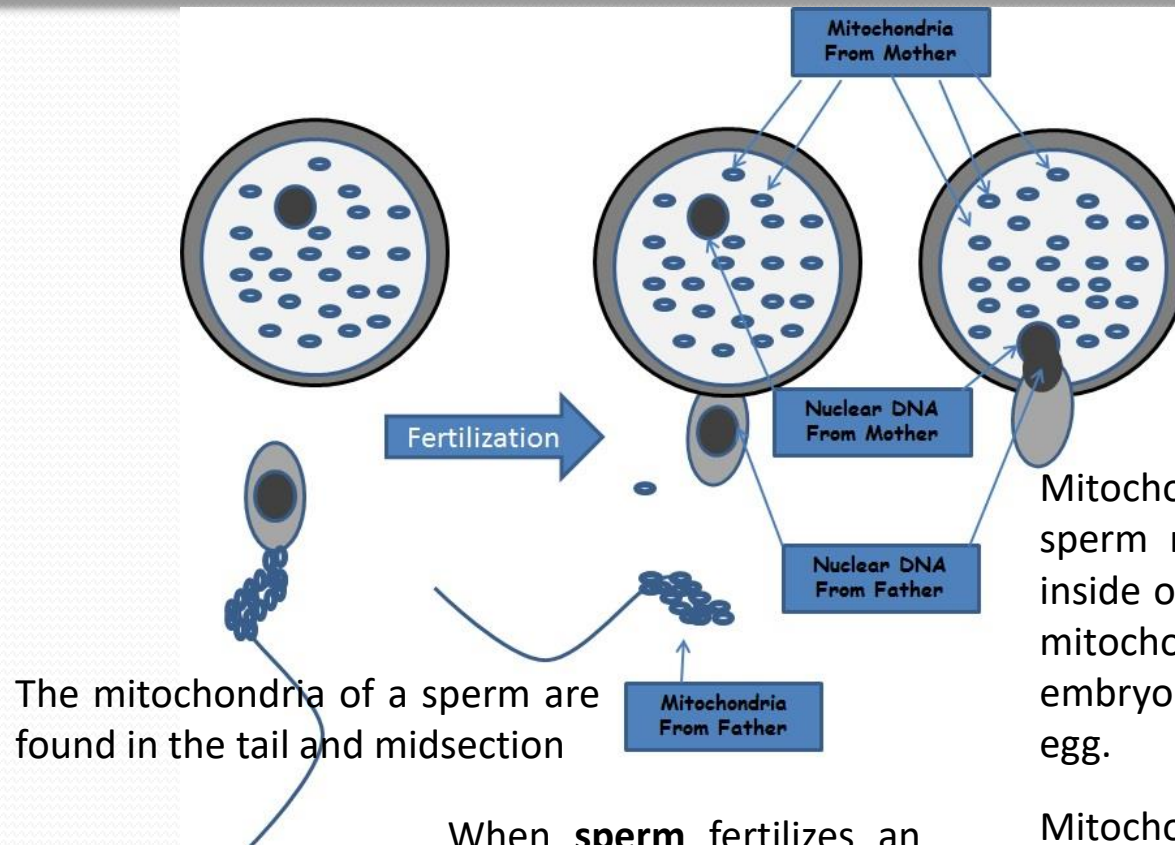
- DNA is mainly of two Types: Nuclear DNA & Mitochondrial DNA



Nuclear DNA is inherited from parents (half from mother, half from father)



Mitochondrial DNA is inherited from the mother



The mitochondria of a sperm are found in the tail and midsection

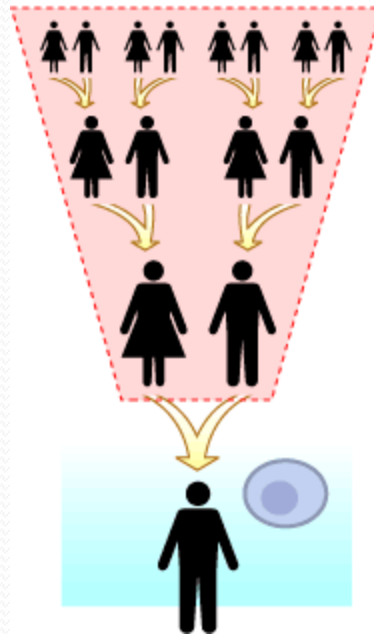
When **sperm** fertilizes an egg, the DNA-containing head of the sperm fuses with the egg, but the tail and midsection are left on the outside of the egg.

Mitochondria of the sperm never reach the inside of the egg, all the mitochondria in the embryo come from the egg.

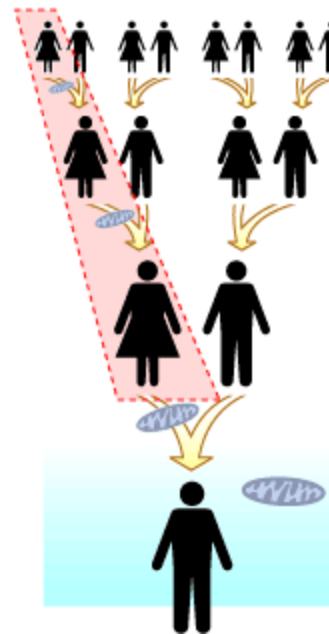
Mitochondrial DNA in a child is identical to that of the mother.

Used for proving maternal relationships in forensic investigations

Nuclear DNA is inherited from all ancestors.

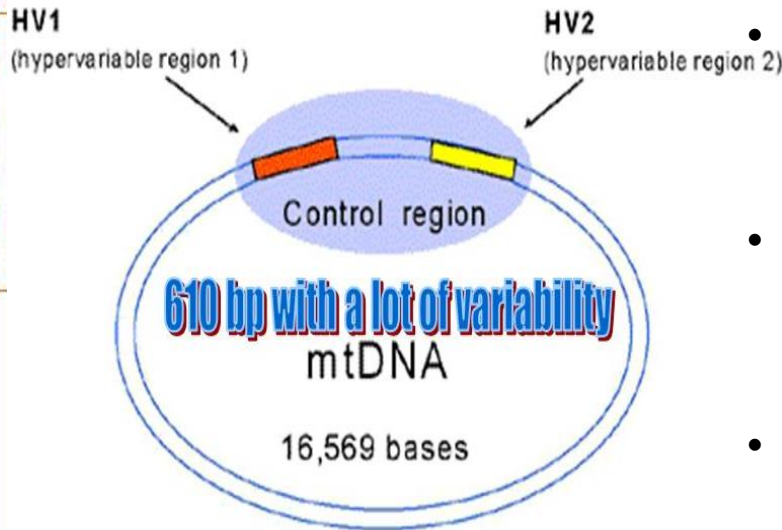


Mitochondrial DNA is inherited from a single lineage.



- Forensic analysis of mDNA is more rigorous, time consuming, and costly when compared to nuclear DNA analysis(nDNA)
- All individuals same maternal lineage will be **indistinguishable** by mDNA analysis.

- mtDNA is present in much greater quantity, more resistant and less informative than nDNA
- mtDNA will be used when "biological evidence may be degraded [i.e. charred remains] or in small quantity
- Cases in which evidence consists only of hairs, bones, teeth etc.

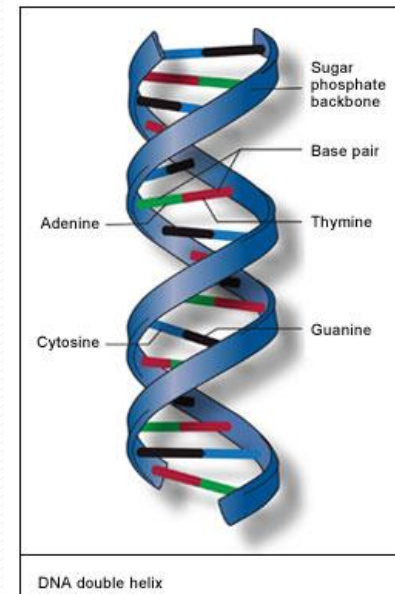


- Mitochondrial DNA is found in ring like structure has two major parts
1. Coding region 2. Control region
- The control region there are 2 regions where variations are found in humans.
- These regions are called **Hypervariable Region 1 (HV1)** and **Hypervariable Region 2 (HV2)**
- In mtDNA 3 regions namely: HVR1, HVR2 and Coding Regions are tested
- The more regions which are tested, the more stringent the comparison with another individual
- mtDNA Mismatch - not full siblings but can still have the same father.
- mtDNA match - strong possibility - related through the maternal line and they could be siblings but can have a different father.

DNA Analysis

DNA evidence has become an crucial tool in cases like

- **CRIME INVESTIGATION:** In murder or man-slaughter, sexual offences, assaults, robbery, house breaking and burglary, hoax crime and kidnapping *etc.*
- **ESTABLISHMENT OF PATERNITY AND MATERNITY:** In case of adultery, lost child and exchange of babies in the Hospitals.
- **NATURAL & MAN-MADE DISASTERS**
- **WILD LIFE CONSERVATION**
- **SEX DETERMINATION**



Types of Samples

- Seminal stains
- Blood stains
- Loose Pubic hair
- Loose scalp hair
- Saliva from bite marks
- Teeth
- Bone
- Nail scrapings
- Few plucked pubic hair & scalp hair
- Trace material from genital areas
- Anywhere nucleated cells are, we might find DNA.

An ultraviolet light may be helpful to scan the body and clothing to locate signs of semen



SOURCES FROM CRIME SCENE



Examples of sources from real cases:

- Saliva on the stamp of a stalker's threatening letter
- Skin cells shed on a ligature of a strangled victim
- Perspiration on a baseball cap discarded by a rapist was compared with the DNA in the saliva swabbed from a bite mark on a different rape victim
- DNA analysis of a single hair (without the root) found deep in a victim's throat

- The biological material should be collected for use as evidence as soon as possible due to environmental conditions
- In sexual assault cases, it is important to obtain evidence samples from the victim preferably within 12-24 hours and max within 72 hours
- With appropriate storage, DNA evidence collected properly can be analysed after the passage of any amount of time.
- The DNA profiles acquired from the samples collected from the victim's body or crime scene is compared with
 - a. Reference Sample i.e the DNA profile of the victim itself to elimination
 - b. Suspect's Sample i.e the DNA profile of the suspect



PACKAGING OF EVIDENCE

- Wearing disposable latex gloves while handling the evidence is required.
- Clothing from victim and suspect must be collected.
- The biological evidence should not be packed in plastic or airtight containers as moisture could contribute to the growth of DNA-destroying bacteria and fungi.
- Enemies of evidence: sunlight, high temperatures, bacteria, moisture
- Each article should be dried and packaged separately in a paper bag or in a well-ventilated box.
- All biological evidence (blood samples) should be refrigerated or stored in a cool location
- Standard/reference DNA specimens - such as blood or the buccal swab (swabbing the mouth and cheek).



STAGES OF DNA PROFILING



STAGE 1

Cells are broken down to release DNA.

STAGE 2

DNA is cut into fragments using restriction enzymes

STAGE 3

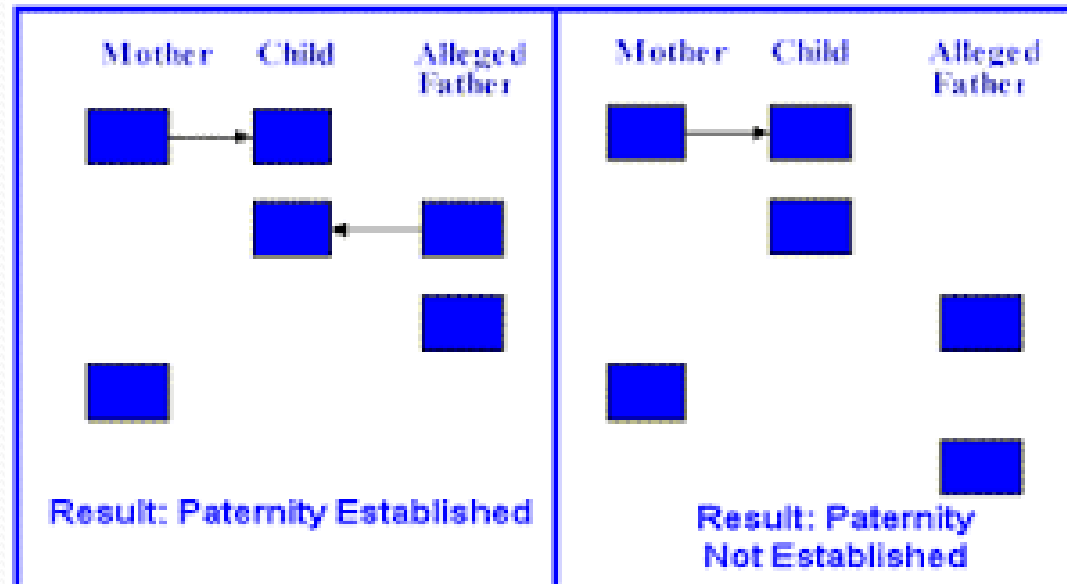
DNA Fragments are separated on the basis of size using Electric Current

STAGE 4

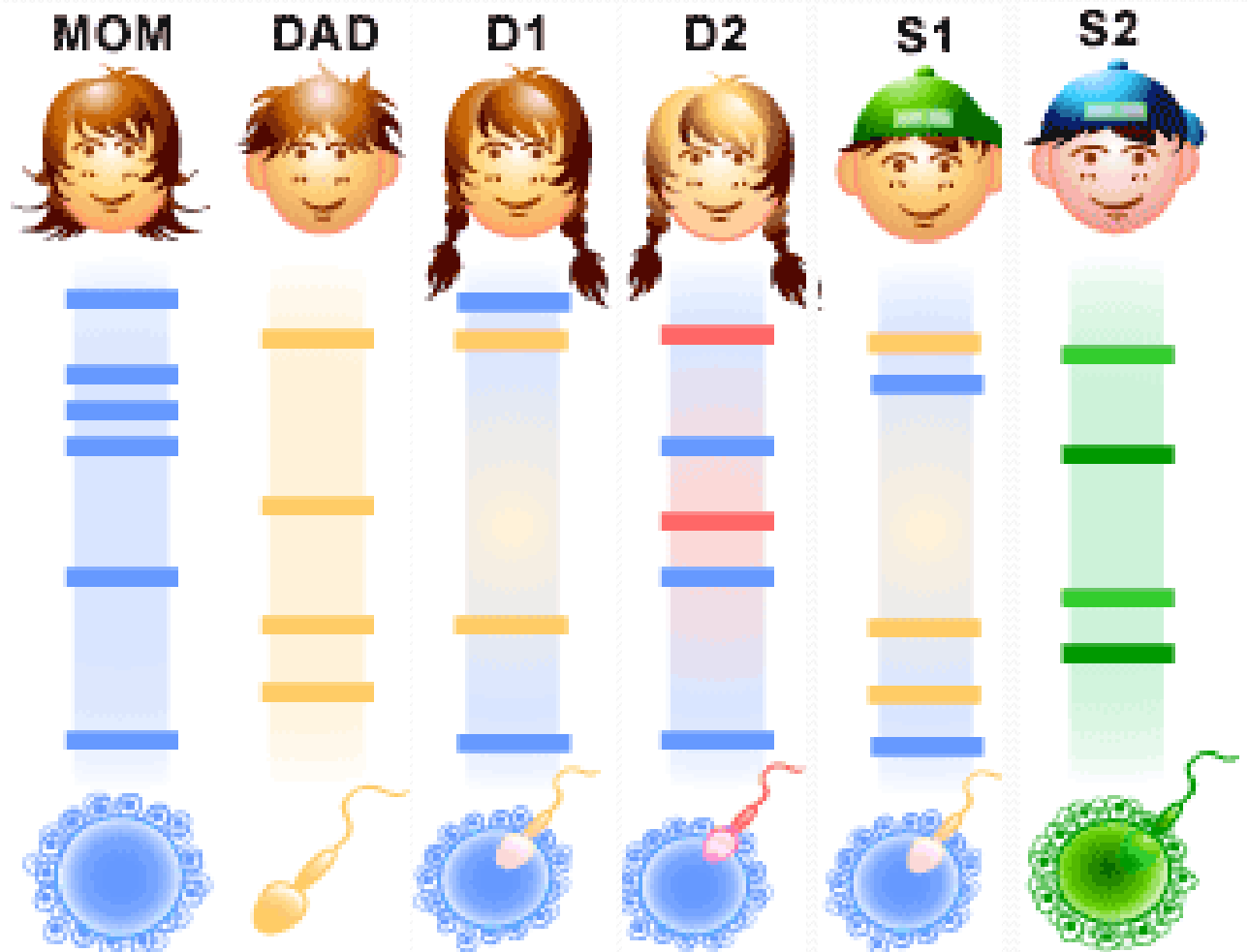
Radioactive material is added to produce a fluorescent image

A photographic copy of the DNA bands is obtained.

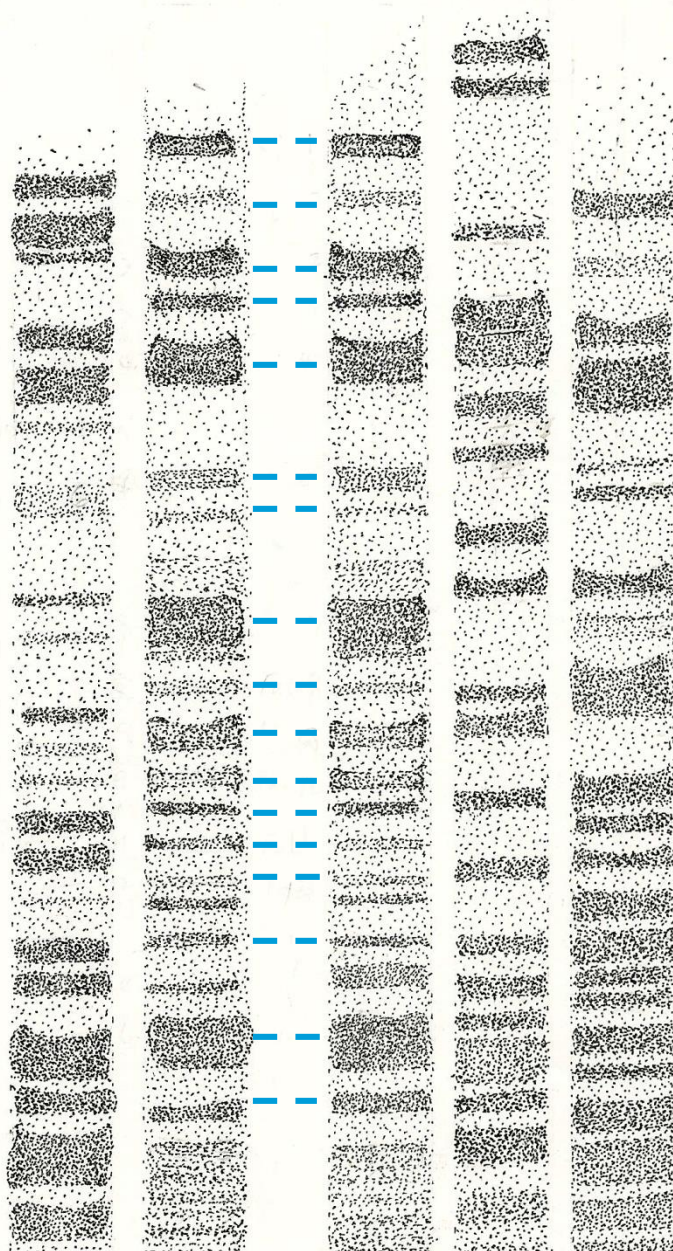
PATERNITY TEST



Example



V S S₁ S₂ S₃



V Victim

S Sample from crime scene

S₁ Suspect 1

S₂ Suspect 2

S₃ Suspect 3

Suspect 1 match those
taken from the crime scene



STR / DNA MARKERS

- DNA has 15 universally specified DNA markers with specific combination of sizes.
- These markers are highly individualised
- D8S1179, D21S11, D7S820, CSF1PO, D3S11358, THO1, D13S317, D16S539, D2S1338, D19S433, VWA, TPOX, D18S51, D5S818, FGA
- The 16th marker is Amelogenin, which determines the sex of the sample
 - XX – Female
 - XY - Male



Case: 456789 Name		MOTHER Jane		CHILD Jenny		Alleged FATHER John	
Date Collected: Test No.		1/1/2010 456789-10		1/1/2010 456789-20		1/1/2010 456789-30	
Locus	PI	Allele Sizes		Allele Sizes		Allele Sizes	
D8S1179	1.55	10	14	13	14	11	13
D21S11	2.02	27	29	29	30	29	30
D7S820	1.17	8	10	8	10	10	11
CSF1PO	1.65	11	12	11		11	12
D3S1358	1.88	14	17	15	17	14	15
TH01	2.62	6	9.3	7	9.3	7	9
D13S317	3.43	13		11	13	11	
D16S539	3.32	9	12	11	12	11	
D2S1338	4.33	19	20	20	24	23	24
D19S433	2.23	13		13		13	14
vWA	3.62	14		14	17	17	
TPOX	1.86	11		8	11	8	
D18S51	3.06	15	17	14	17	13	14
D5S818	1.35	12	13	11	12	11	12
FGA	3.55	21	22	21	24	21	24
Amelogenin		X		X		X	Y

Interpretation:

RN: 76206

Combined Paternity Index: **323,769**

Probability of Paternity: **99.9996%**

The alleged father is not excluded as the biological father of the tested child. Based on testing results obtained from analyses of the DNA loci listed, the probability of paternity is 99.9996%. This probability of paternity is calculated by comparing to an untested, unrelated, random individual of the Caucasian population (assumes prior probability equals 0.50).



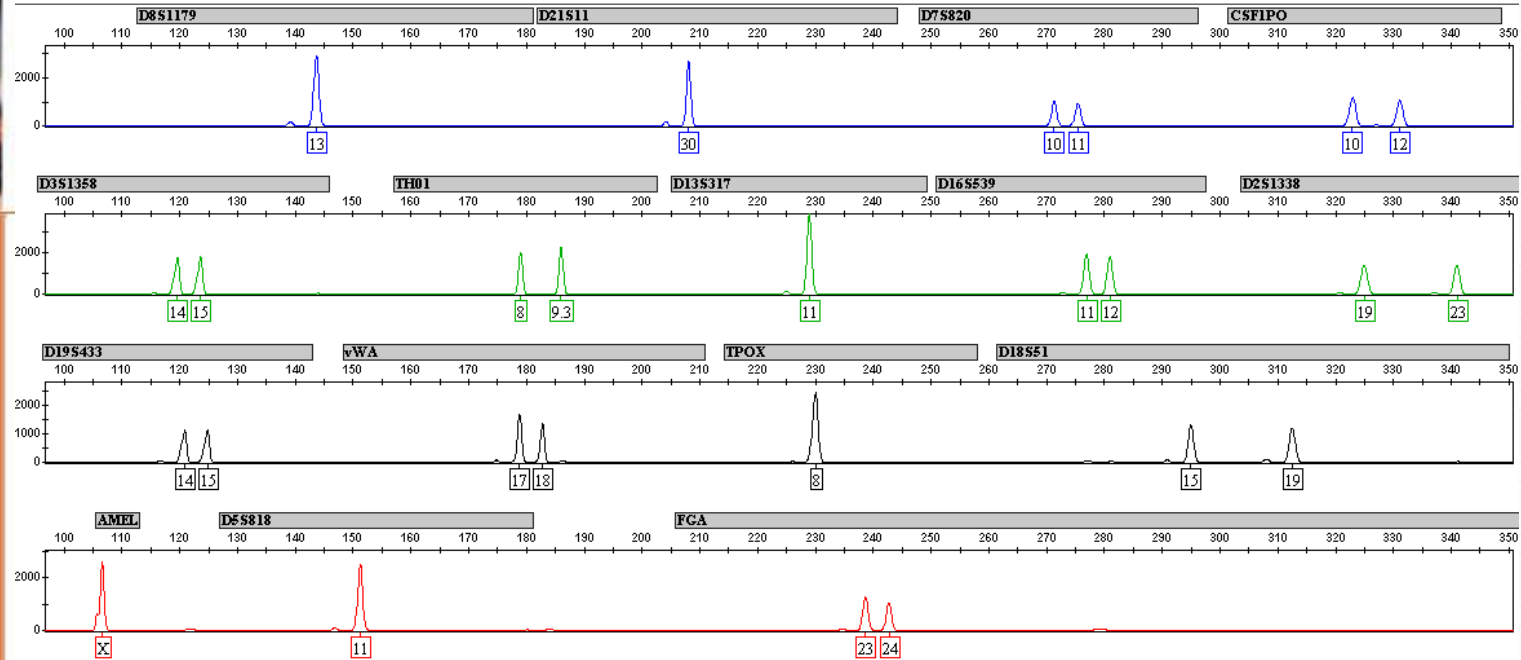
Autosomal STR Loci/ DNA Markers	<u>Santosh</u> Exh-1 Blood Sample	Blood Sample at the Crime Scene
D8S1179	13, 15	12, 11
D21S11	31, 32.2	23, 65
D7S820	10, 11	12, 8
CSF1PO	11	15
D3S11358	14, 15	12, 11
TH01	8	9
D13S317	9, 11	11
D16S539	11, 13	9, 12
D2S1338	20, 23	19, 20
D19S433	13	13
VWA	14, 16	14, 11
TPOX	9, 11	8, 10
D18S51	14, 15	12, 13
<u>Amelogenin</u>	X, Y	X, Y
D5S818	11	10
FGA	23, 25	21, 24

Table 1 Tabulated results of DNA profile comparison

Marker	DNA profile of semen found on skirt of victim	DNA profile of blood sample of accused
D8S1179	14,14	14,14
D21S11	28,32.2	28,32.2
D7S820	8,10	8,10
CSF1PO	10,10	10,10
D3S1358	15,18	15,18
TH01	6,7	6,7
D13S317	9,12	9,12
D16S539	11,13	11,13
D2S1338	22,27	22,27
D19S433	13,13	13,13
vWA	18,18	18,18
TPOX	8,11	8,11
D18S51	14,20	14,20
AMEL	X,Y	X,Y
D5S181	11,11	11,11
FGA	22,26	22,26

Full DNA Profile

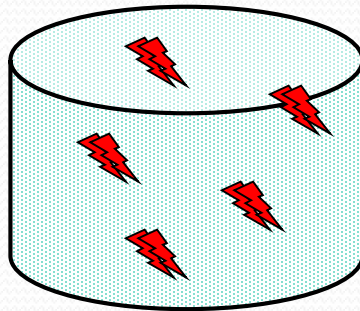
Electropherogram data generated using Life Technologies® GeneMapper® software from Thermo Fisher Scientific Inc.



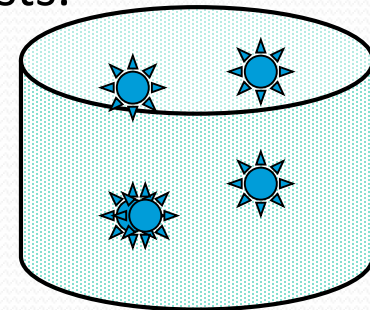
DNA PROFILE DETECTION

DNA MIXTURES

- Mixtures of victim & suspect(s)
 - How many people?
 - Previous consensual partners?
 - Contamination: scene, collection, lab?
- Mixture not always detected at all tests.



Profile A Detected



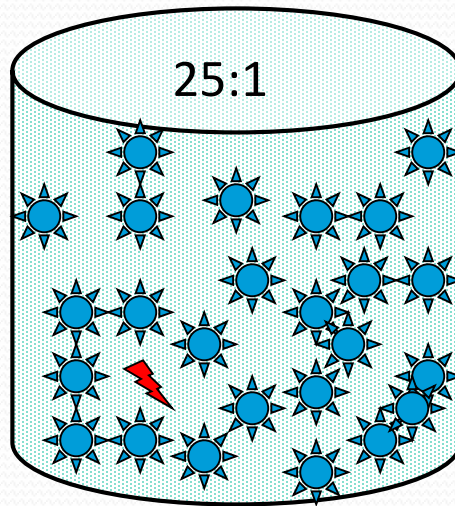
Profile B Detected

Factors:

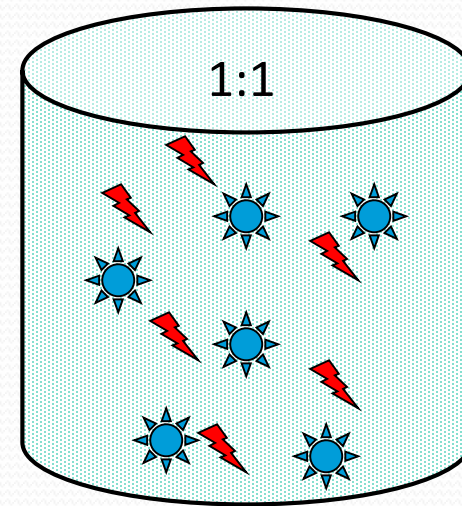
1. Quantity of DNA
2. Quality of DNA



MIXTURE DETECTION



Only Profile B Detected



Profiles A and B Detected



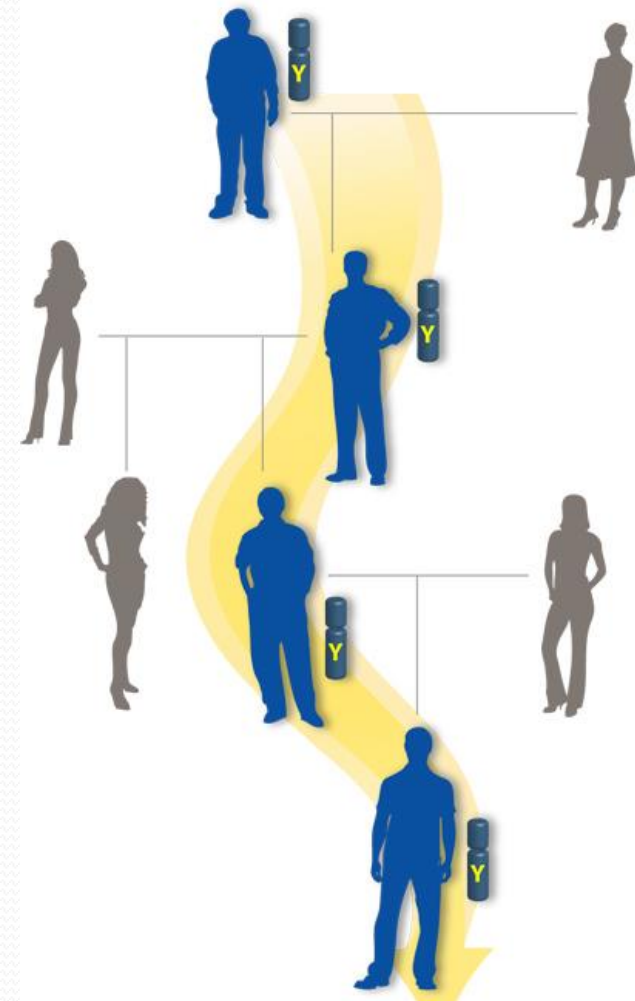
Factors:

1. Quantity
2. Quality
3. Ratio



Y-CHROMOSOME

- Y chromosome analysis is a useful technique for analysing DNA of males.
- A son inherits a Y chromosome from his biological father and he also inherits an X chromosome from his biological mother.
- Conversely, a female would inherit an X chromosome from her biological mother and an X chromosome from her biological father.
- Analysis of Y chromosomes- , which is inherited over time through males in a familial line.



Y-STR ANALYSIS

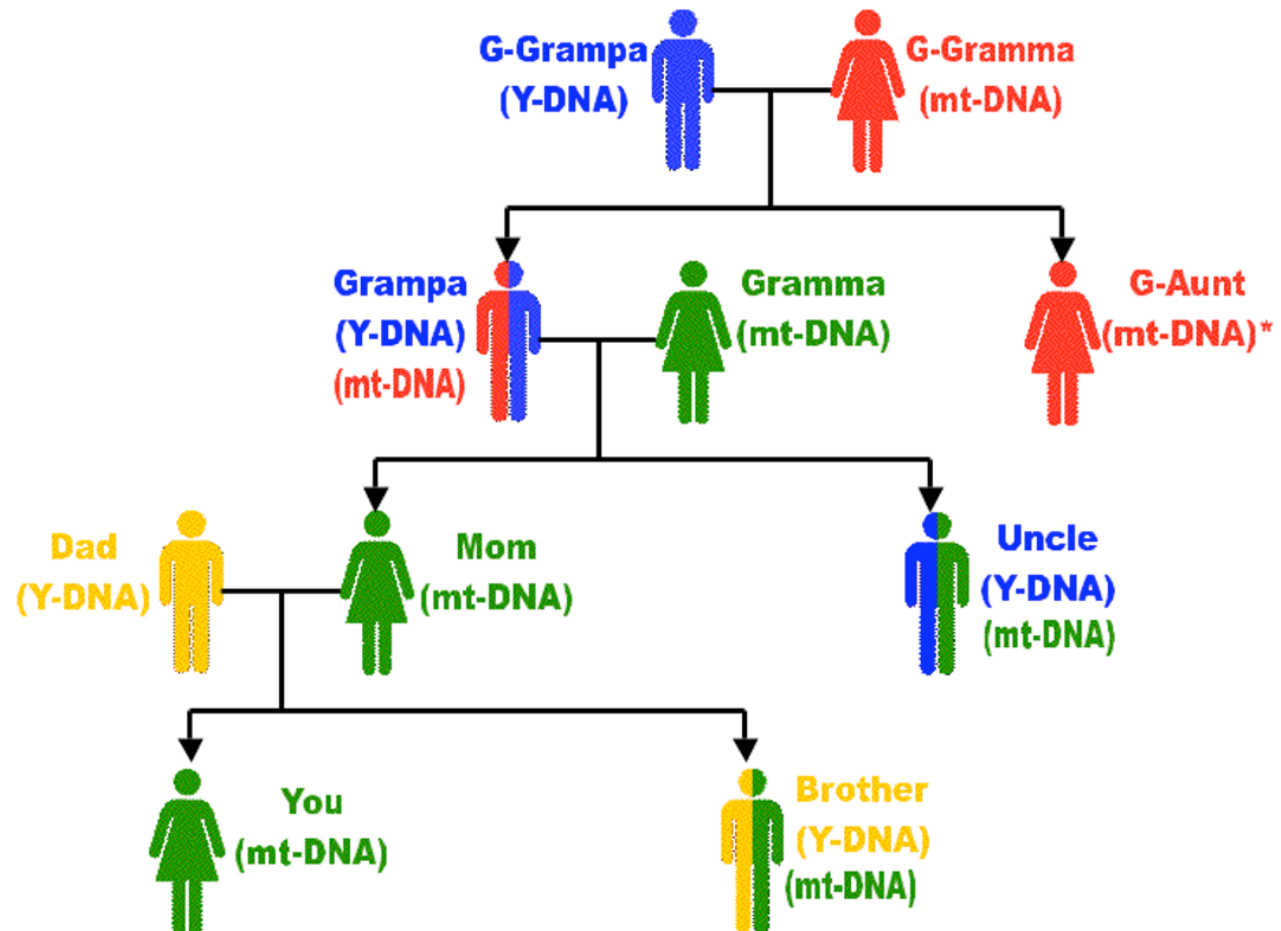
- They specially designated areas of Y-Chromosomes
- Y-STR analysis is widely used in forensic DNA analysis, in cases where standard autosomal DNA profiling is not informative.
- Y-STR can
 - (i) exclude male suspects from involvement in crime
 - (ii) identify the paternal lineage of male perpetrators
 - (iii) highlight multiple male contributors to a trace
 - (iv) provide investigative leads for finding unknown male perpetrators.
- If two or more brothers - same combination of Y-STR types - same male lineage
- If their Y-STR is different they cannot have the same father.
- All individuals same fraternal lineage will be **indistinguishable** by Y-STR analysis.



Observed Genotypes

Y STR Marker	Santosh Ex:1 Blood sample
DYS456	16
DYS389 I	12
DYS390	22
DYS389 II	28
DYS458	14
DYS19	14
DYS385 a/b	13,17
DYS393	11
DYS391	10
DYS439	13
DYS635	25
DYS392	14
Y GATA H4	11
DYS437	15
DYS438	10
DYS448	19

Inheritance of Y-DNA & mtDNA



HUMAN CHIMERA

- Mrs. McK was the first identified **HUMAN CHIMERA**. People that have two different sets of DNA are called human chimeras.
- It can happen when a woman is pregnant with fraternal twins and one embryo dies very early on. The other embryo can "absorb" its twin's cells.
- It can also happen after a bone marrow transplant
- A blood transfusion will also temporarily give a person cells from someone else, but in a bone marrow transplant, the new blood cells are permanent
- Bone marrow is the tissue inside our bones that's responsible for making white blood cells, red blood cells, and platelets.
- In bone marrow transplants, doctor uses chemotherapy or radiation to destroy all the recipient's diseased bone marrow, then a donor's healthy marrow is put in its place.





- The donor's bone marrow will keep on making blood cells that have the donor's DNA. That's how the recipient becomes a chimera.
- In "complete chimerism," 100% of the recipient's blood cells have the donor's DNA
- But when a mix of DNA from both the donor and the recipient — that's called "mixed chimerism."
- In the 1990s, scientists discovered that a pregnant woman may retain some DNA from her baby, if some fetal cells happen to migrate outside the uterus. but it's more scientifically known as "microchimerism."

CASE STUDY 1

Tandoor Murder Case



- This is the first case in India where DNA fingerprinting was used for investigation.
- Former Congress worker Mrs. Naina Sahni was shot dead by her husband Sushil Sharma.
- The body was tried to burn in the Tandoor at Bagiya Restaurant New Delhi on 2nd July 1995, with the help of restaurant manager Keshar Kumar
- Charred remains of a body in the tandoor kitchen were seized
- DNA from muscle pieces attached to her charred bone were compared with that of her parents and sister.

CASE STUDY 2

Rajiv Gandhi Assassination

- Establishing the identity of Dhanu, the suicide bomber who killed former prime minister Rajiv Gandhi
- The DNA found in the skull, charred muscle pieces on the suicide bomber's belt showed identical patterns, which proved that Dhanu was the bomber.




CASE STUDY 3 – Neeraj Grover

- In the absence of any eyewitnesses, forensic evidence played a key role in cracking the sensational Neeraj Grover murder case.
- The door latch with blood stains on it was cleaned by the accused to destroy evidence, the forensic experts managed to collect DNA samples from it.
- The blood stains also collected from the curtains television with that of the deceased
- The police recovered charred bones of the victim from Manor, which was impossible for identification.
- The forensic experts collected three teeth, femur bones and some other residue so as to extract the DNA sample from it.
- The collected DNA sample was then matched with that of his parents to establish whether the charred bones and teeth were indeed that of Grover.



CASE STUDY 4 – Sheena Bora



Four Steps for DNA testing -FIRST DAY: Part of Femur (thigh bone) of the skeletal remains was crushed. Bone cells were separated by chemically treating them to remove calcium. Calcium interferes with DNA testing (takes 24 hours)

SECOND DAY: Nuclear DNA was extracted separately from bone and blood cells of Indrani Mukerjea (takes 24 hours)

THIRD DAY: DNA profiles of bones and blood were obtained

FOURTH DAY: Fifteen DNA markers for the bone and blood samples were compared.

Fifteen universally specified DNA markers on the bone of the unknown sample and the blood sample of Indrani matched.

The sixteenth marker is Amelogenin, which determines the sex of the sample

DNA, fingerprints, analysis of bite marks sealed Nirbhaya rapists' fate: SC

DNA investigation of bloodstained clothes and body swabs linked all 5 men and a juvenile accused of the gang rape and murder of Nirbhaya

- DNA tests on bloodstains from undergarments worn by the main accused, Ram Singh, matched Nirbhaya's DNA
- A swab taken from Nirbhaya's body showed Ram Singh DNA signature,
- The DNA samples were taken from bloodstains on clothes of all five accused, curtains and seats on the bus.
- Samples were also taken from bloodstained dried leaves by the side of the highway where the pair said they were dumped.
- Nirbhaya's DNA profile showed up in bloodstains on the undergarments and flip-flops of Vinay Sharma
- Blood found on Vinay Sharma jacket matched with the woman's friend, who fought with the men before he was hit with an iron rod.



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NEW EMERGING TECHNOLOGIES IN DNA PROFILING

LCN DNA / TOUCH DNA

- Low Copy Number (LCN) DNA / Touch DNA is a profiling technique used by few countries, especially in cold cases.
- Touch DNA is the next wave of DNA testing that doesn't require blood or semen samples.
- It analyses skin cells or sweat from fingerprints left behind when assailants touch victims, weapons or anything else at a crime scene.
- This technique has dramatically increased the number of items of evidence that can be used for DNA detection.
- Usually very small amounts of DNA are deposited.
- LCN has been able to show results even 25 years after the crime



Common Sources of Touch DNA

From Hands:

Gloves
Knife handles
Weapon handles
Firearm grips
Plastic bag handles
Steering Wheels
Rope
Shoe laces
Electrical cords



TOUCH DNA EVIDENCE...

What does it mean


Shows linkage or association but....

- DNA recovered from an object may not be from the last person to touch it. Factors include:
 - Length of contact
 - Good cell shredded or not
 - Vigorous contact vs. incidental
- DNA profiles recovered from touch evidence are often mixtures – multiple individuals
 - Elimination known(s)
 - Lawful owner
 - Crime scene personnel, officers

RAPID DNA

- New rapid DNA profiling technologies are emerging to help address the challenges like time and expertise.
- This is a system which automates DNA profiling from a simple cheek swab, generating results in about 90 minutes.
- The "swab in, profile out process" takes less than five minutes of hands-on time and performs all necessary steps of DNA analysis without human intervention.
- Reagents in disposable cartridges are loaded onto the system with up to seven buccal (cheek) swab samples.
- After a sample run is started, samples are processed with no further user interaction.
- The system extracts DNA and software analysis to generate full human identification profiles.





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- The findings are then used to search the linked DNA database or compared to swabs taken from suspects.
 - This integration enables law enforcement agencies to reduce the time it takes to generate a DNA profile and make decisions while arrestees are in custody.

EVIDENTIARY VALUE OF DNA PROFILING

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- 1,37,458 rape cases pending for trial across India (2015)(NCRB)
 - In Delhi, the numbers of rape cases have tripled over the last 5 years, with increase of 277 % from 572 in 2011 to 2,155 in 2016.
 - Conviction rate has fallen from 49 % to 29 % in the last 3 years (between 2012 and 2015) in Delhi
 - In 2016, the conviction rate crime against women fell to 18.9% (NCRB)
 - More than 34,651 rapes were registered in 2015 (NCRB) and the annual report of the Centre for DNA Fingerprinting and Diagnostics (CDFD) for 2015-16 shows that they have received 99 DNA cases specifically for rape from different states.
 - DNA is the world's greatest crime fighting tool.
 - Lack of scientific methods in investigations is hampering justice delivery

- 
- When DNA is collected, it often goes into large backlogs due to India's lack of DNA testing infrastructure.
 - A study shows when DNA is available the prosecutions, 'charging rate' was 8 times higher than cases that did not have DNA casework that matched a known suspect.
 - UK completes DNA testing on over 60,000 crime scenes annually & India (13 times larger in population than the UK), collectively complete DNA testing on less than 7,500 cases annually.


Recently, in the Bhanwari Devi case, the CBI had to send the victim's bones to the FBI, USA, for identification.

This is a sad reflection of the present status of DNA technology in India.

EVIDENTIARY VALUE OF DNA

- DNA Technology potential “*genetic eyewitness*”
- Its regarded as the standard of excellence for the development of impartial, unbiased scientific evidence to ensure accuracy, transparency and fairness in criminal justice system.
- And to make this a fool-proof evidence there is requirement
 - **MAN** (Training, expertise, integrity etc.)
 - **MATERIAL** (Quality, integrity, chain of custody etc.)
 - **METHOD** (Standard Operating Procedure)






DNA Profiling is a complex scientific procedure, and its success lies in the skill and expertise of I.O., who is the first player to handle the “sample” and if he fails to do so the whole exercise of diagnostic centre goes waste.

The strange case of the 'time travel' murder

To avoid contamination in DNA samples the I.O. must use:

- Clean hands with gloves
- Clean instruments to lift the sample
- Even in the mortuary the un-cleaned instruments may adversely affect DNA profiling
- Sterile environment as much as possible in packaging etc.

- 
- Sneezing or coughing over evidence
 - Person touches their mouth, nose or other part of the face and then touches the area that may contain the DNA to be tested.
 - Scene personnel can deposit hairs, fibers, or trace material from their clothing
 - Wind can carry in contaminants


ISSUES BEFORE THE COURT

Defence challenges the “**scientific procedure**” adopted in DNA probe.

- Whether the testing procedure is reliable?
- Whether tests were performed properly?
- Whether the conclusion is acceptable?



Santosh K. Singh vs. CBI

- 
- Priyadarshini Matoo, 23, was allegedly raped and strangled to death in her house in N. Delhi in Jan 1996, by her fellow student S. K. Singh
 - DNA test was conducted and test confirmed and connect the crime with criminal.
 - But the Defence argued and challenged the **“scientific procedure”** adopted in DNA probe.

The Session Judge pronounced:

“Though I knew, he is the man (Santosh Singh), who committed the crime, I acquit him, giving him the benefit of doubt.”

SUGGESTION - The Procedure adopted should be such that NO benefit of doubt is given to the accused.

CHAIN OF CUSTODY

This concept itself is of utmost importance.

- Forensic evidence to be admissible in the court of law must be beyond any doubt.
- It also refers to the **security** and **reliability** of the police and forensic/diagnostic labs handling DNA samples.

SUGGESTION - Ensuring that there should not be any break in the “**chain of custody**” (Proper docketing, documentation etc.) to have DNA as admissible evidence.



Pantangi Balarama Venkata Ganesh vs. State of A.P. (Cr LJ, 2003, 4508):

Court relied on an article by Dr. Lalji Singh, and accepted DNA profile as conclusive evidence & observed “....**the DNA test gives the perfect identity. It is a very advance science**”

NIRBHAYA CASE

The DNA profiling, which has been done after taking due care for quality, proves to the hilt the presence of the accused persons in the bus and their involvement in the crime”

The bench referred to the statement of Dr BK Mohapatra, one of the prosecution witnesses in the case, and said he has testified that once a DNA profile was generated, its accuracy is 100 per cent.



ISSUES BEFORE THE COURT IN CIVIL MATTERS

1. Privacy and Ethical Issues

- S.C. observation in **Gautam Kundu vs State of West Bengal** regarding legitimacy: *“The court must examine carefully the consequences of ordering the blood test; whether it will have **the effect of branding a child as bastard and the mother as an unchaste woman**”*

2. Chances of misuse of DNA profile

3. Chances of genetic discrimination in marriage, education, social relations etc.

CONCLUSION

- Emphasis on Training: For I.O.
- The Procedure adopted should be such that NO benefit of doubt is given to the accused.
- No break in the **“chain of custody”**. (Proper docketing, documentation etc.) to have DNA as admissible evidence.
- Well-equipped accredited laboratories to handle DNA samples and evidence
- Joint efforts at public -private sector
- Training and information for law enforcement, policymakers to facilitate more informed decisions about effective DNA evidence collection and testing.
- Standardization of Procedure for DNA profiling
- A national DNA database should be created which will be immensely helpful





THANK YOU

FOR ANY QUERIES

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