

Forensic DNA

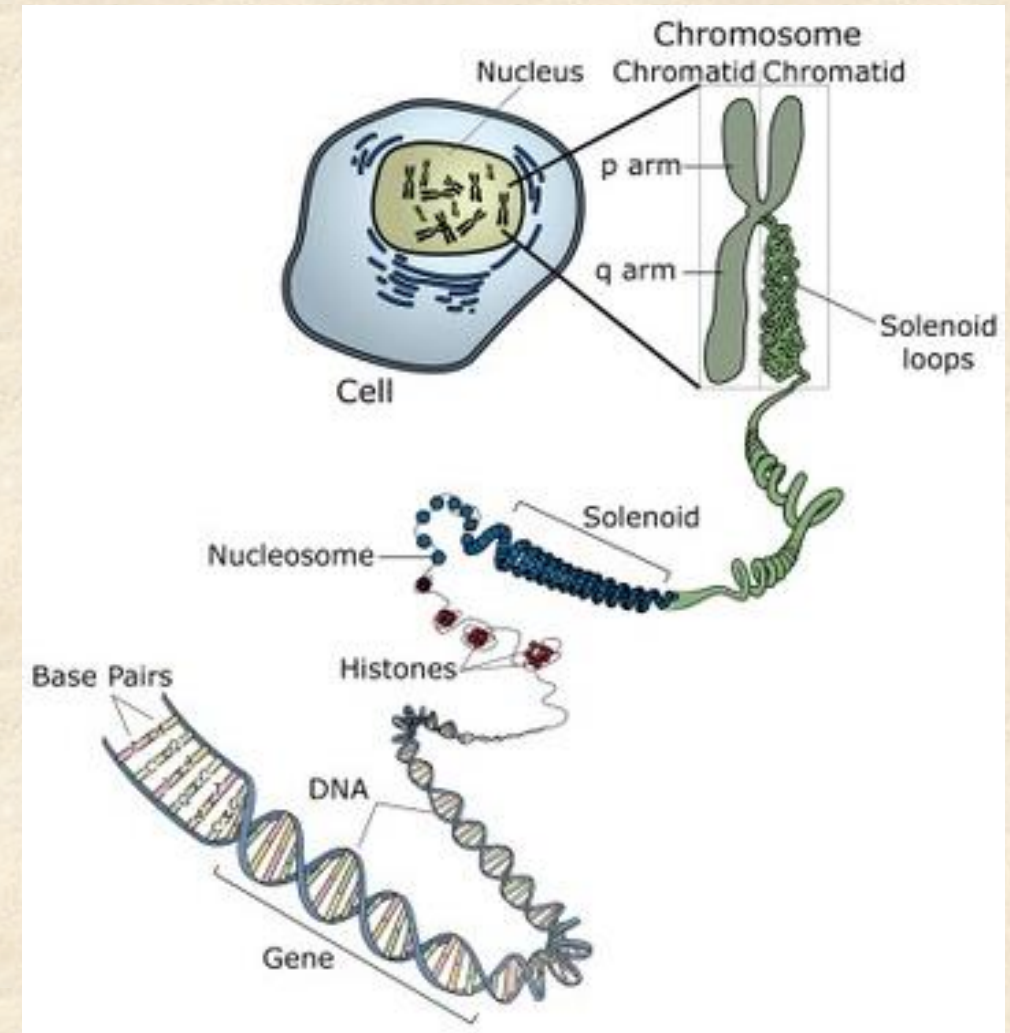
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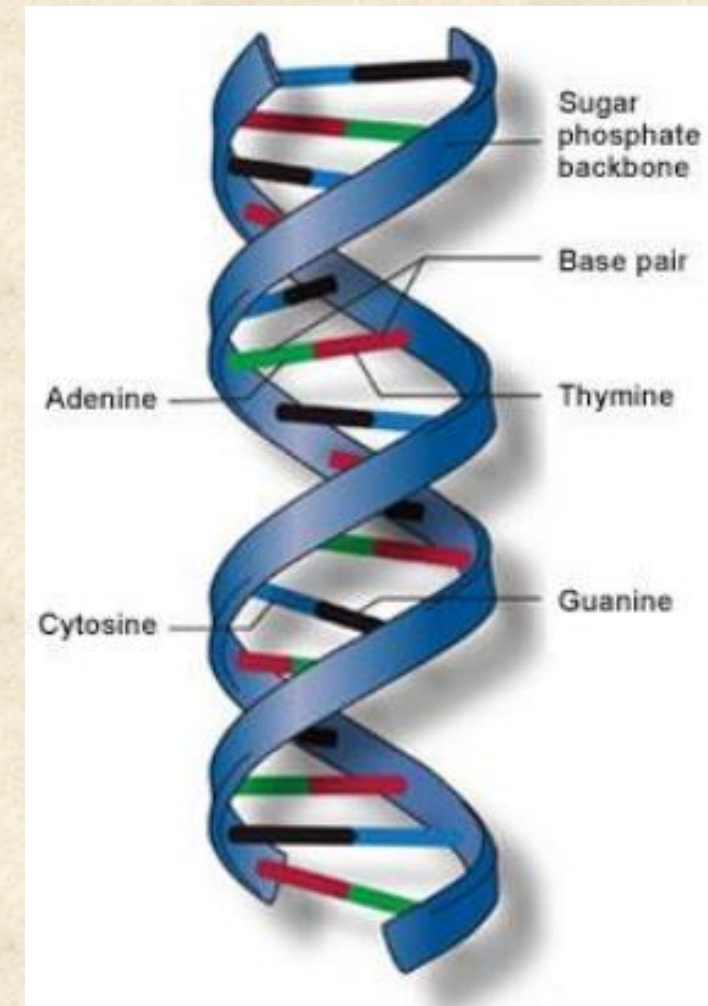
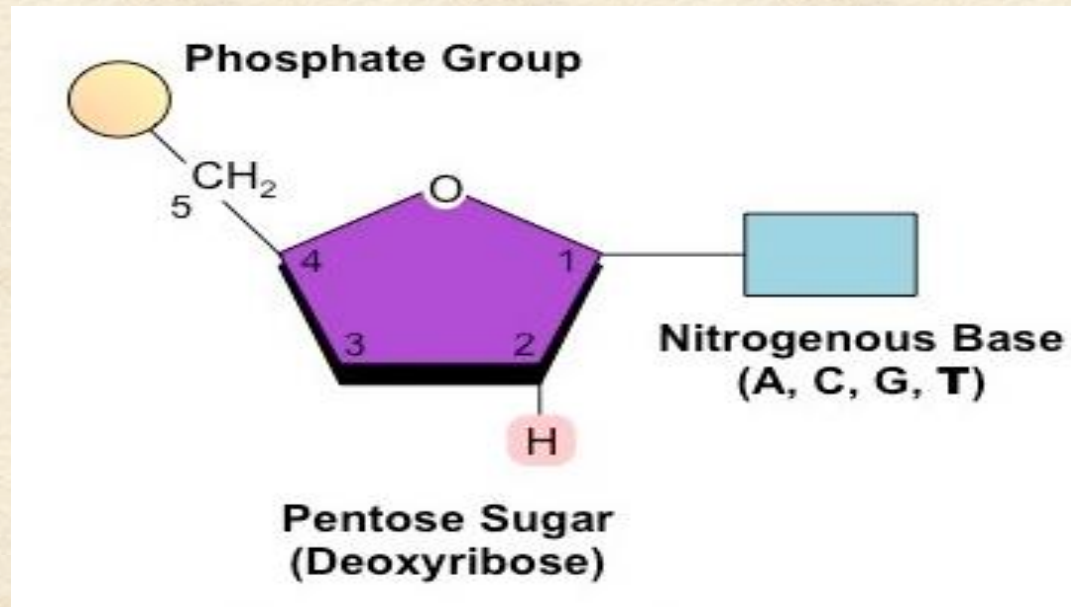
What is DNA?

- DNA = Deoxy-ribonucleic Acid
- Genetic material
- Found in all cells of the organism's body
- Human cells - 23 pairs of chromosomes
- One copy of every chromosome is from the mother and the other copy of every chromosome is from the father (Homologous chromosomes)
- 22 autosomal pairs + 1 allosomal pair (sex chromosomes)

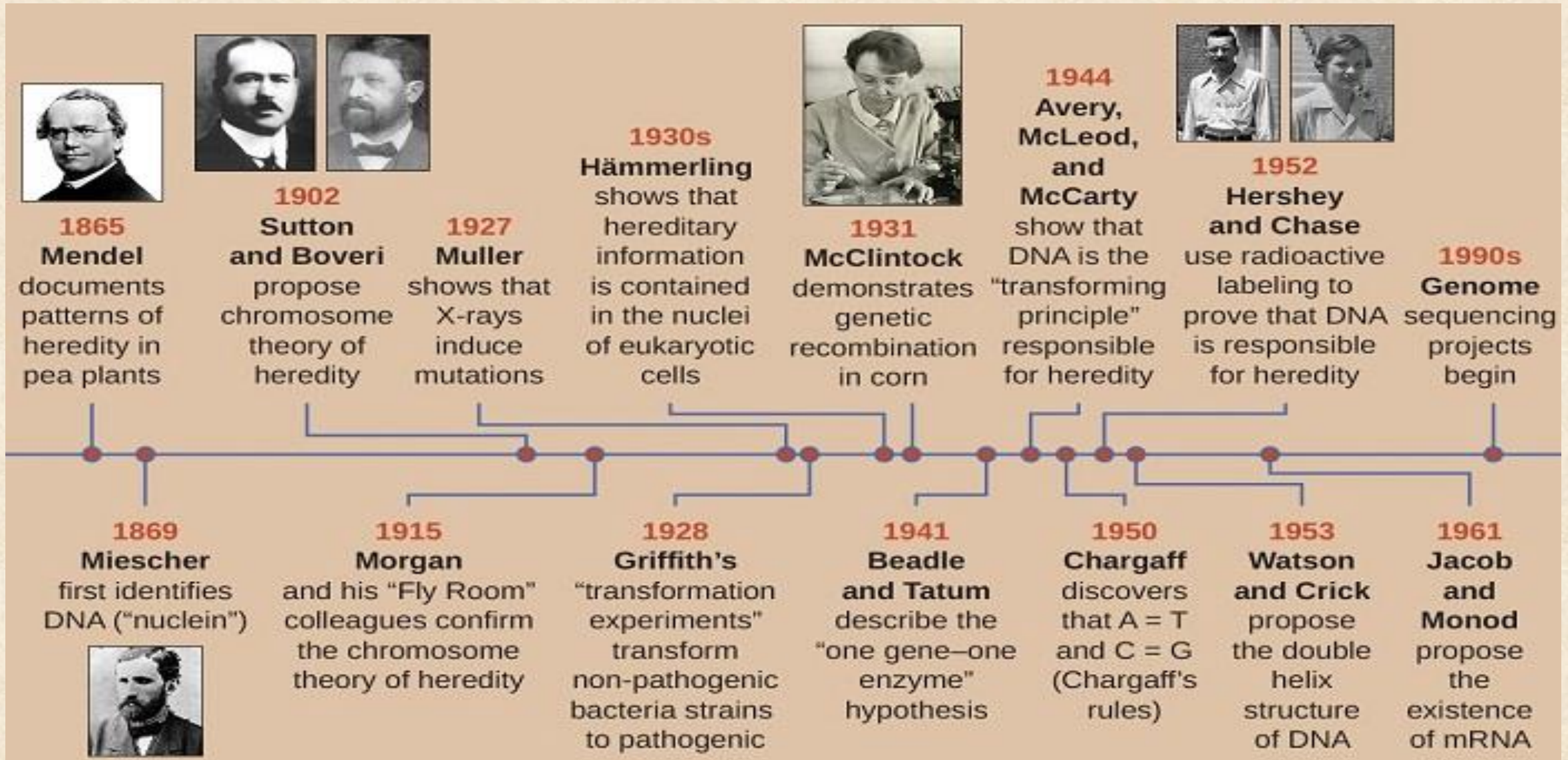


DNA structure

- Human DNA has a double-helical structure
- The building blocks of DNA are smaller units called nucleotides
- Nucleotides = sugar + phosphate group + nitrogenous base



History of DNA



PRINCIPLE OF MENDELIAN INHERITANCE



Law of Segregation

The two alleles for each gene are placed in different gametes.

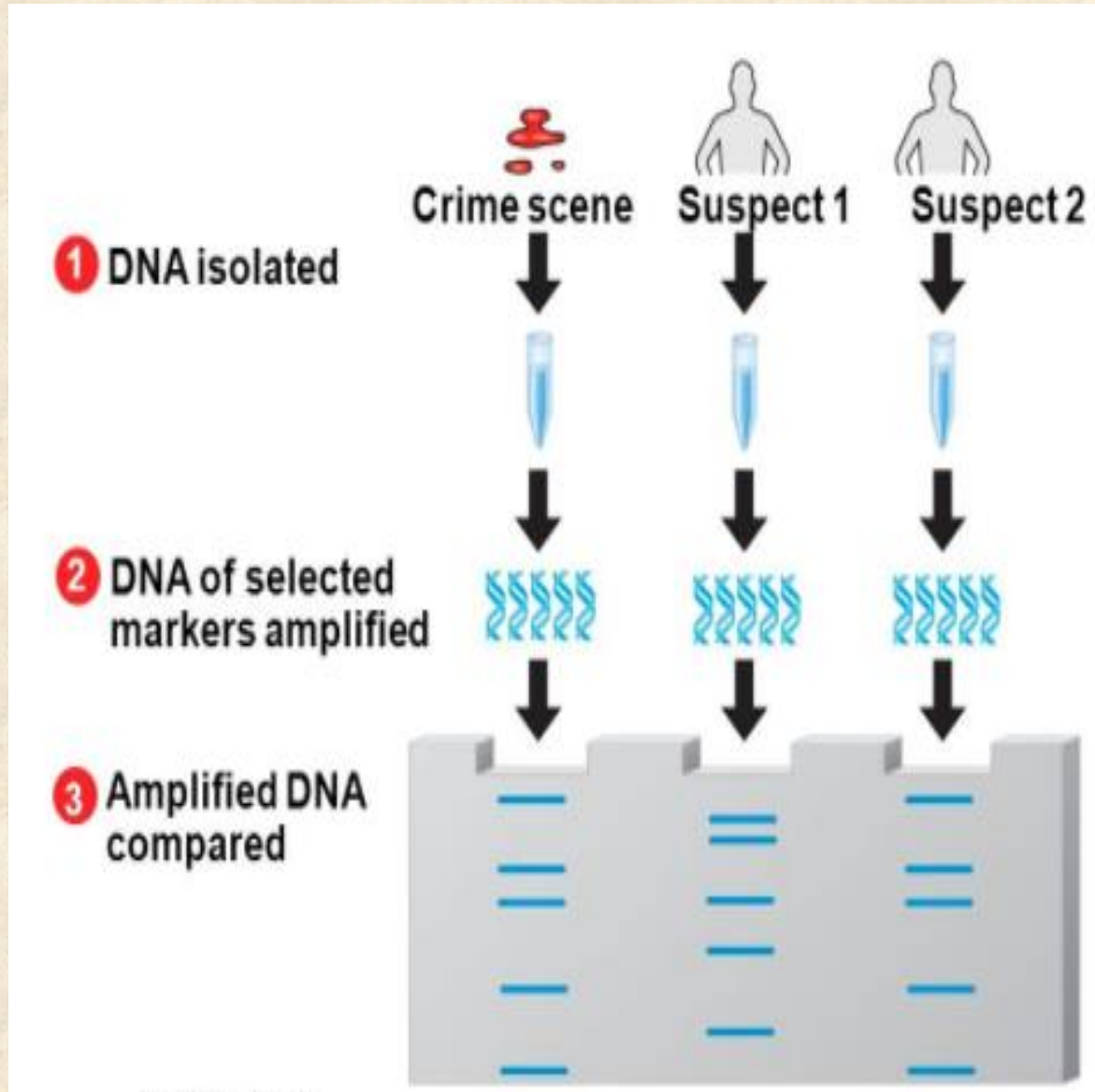
Law of Independent Assortment

The inheritance of one gene doesn't affect the inheritance of any other gene.

Law of Dominance

When two different alleles are present, only one is dominant and will be expressed.

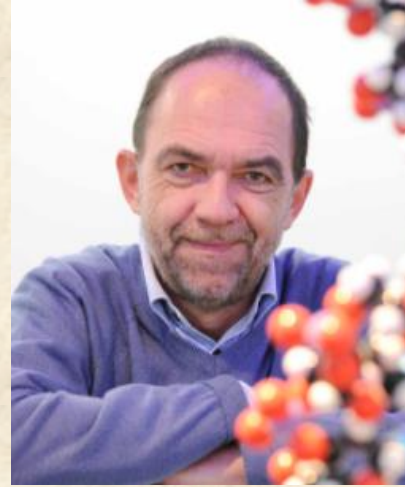
DNA Fingerprinting



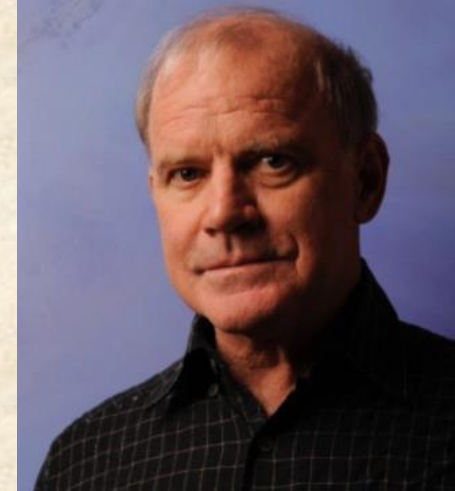
- DNA Fingerprinting is the technique used to distinguish between individuals, based on the microsatellite sequences present in each individual.
- It is also called as:
 - DNA Profiling
 - DNA Typing
 - STR Typing
 - STR Profiling
 - Genotyping

History of DNA Fingerprinting

- Professor Alec Jeffreys developed it in 1984, at University of Leicester
- He named it '**DNA Fingerprinting**'
- In 1985, Kary Mullis invented PCR technique, allowing us to magnify the quantity of DNA available to use/study
- Father of Indian DNA Fingerprinting is Late Dr Lalji Singh



Alec Jeffreys



Kary Mullis



Lalji Singh

Commonly Used Terms in DNA Fingerprinting

- VNTRs = Variable Number Tandem Repeats
- The repeating sequence is same in both individuals
- Number of repeats are different in both individuals

Individual 1

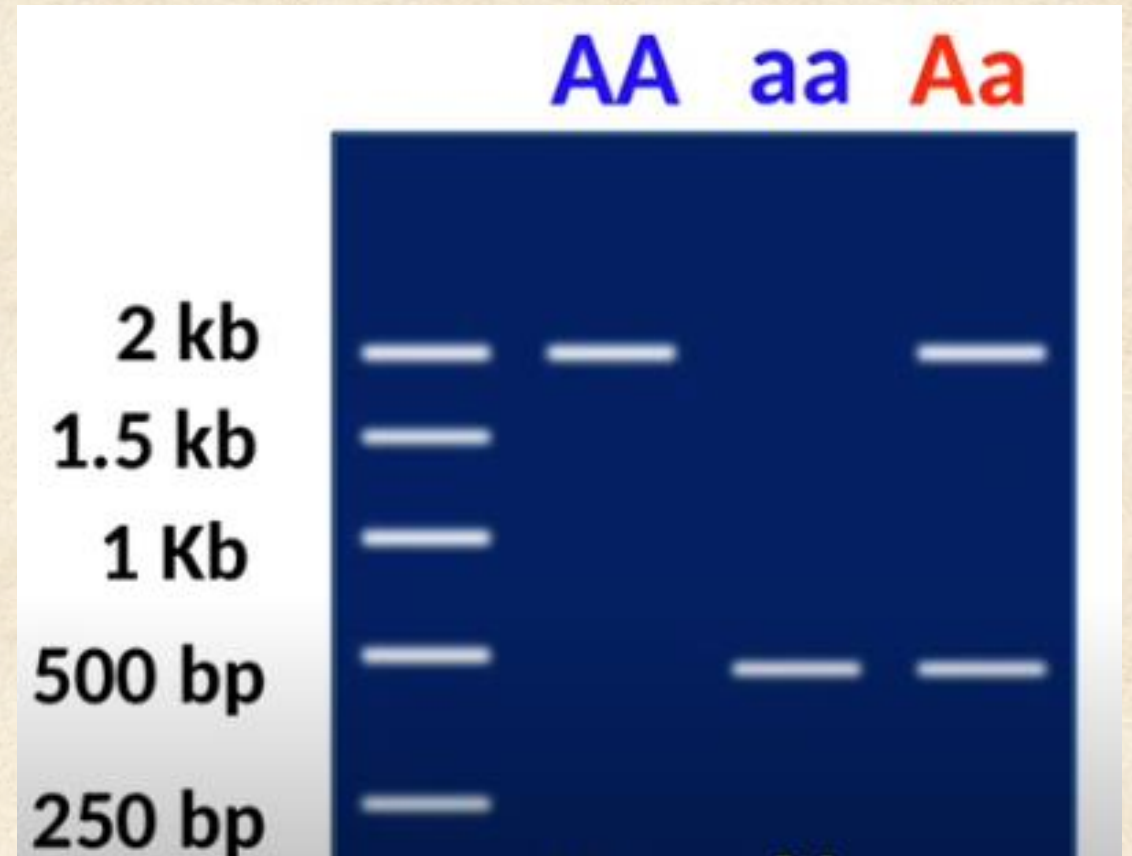
GGT AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG CGGTAAGTAGCGA

Individual 2

GGT AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG AGTCGGTAAG CGTT

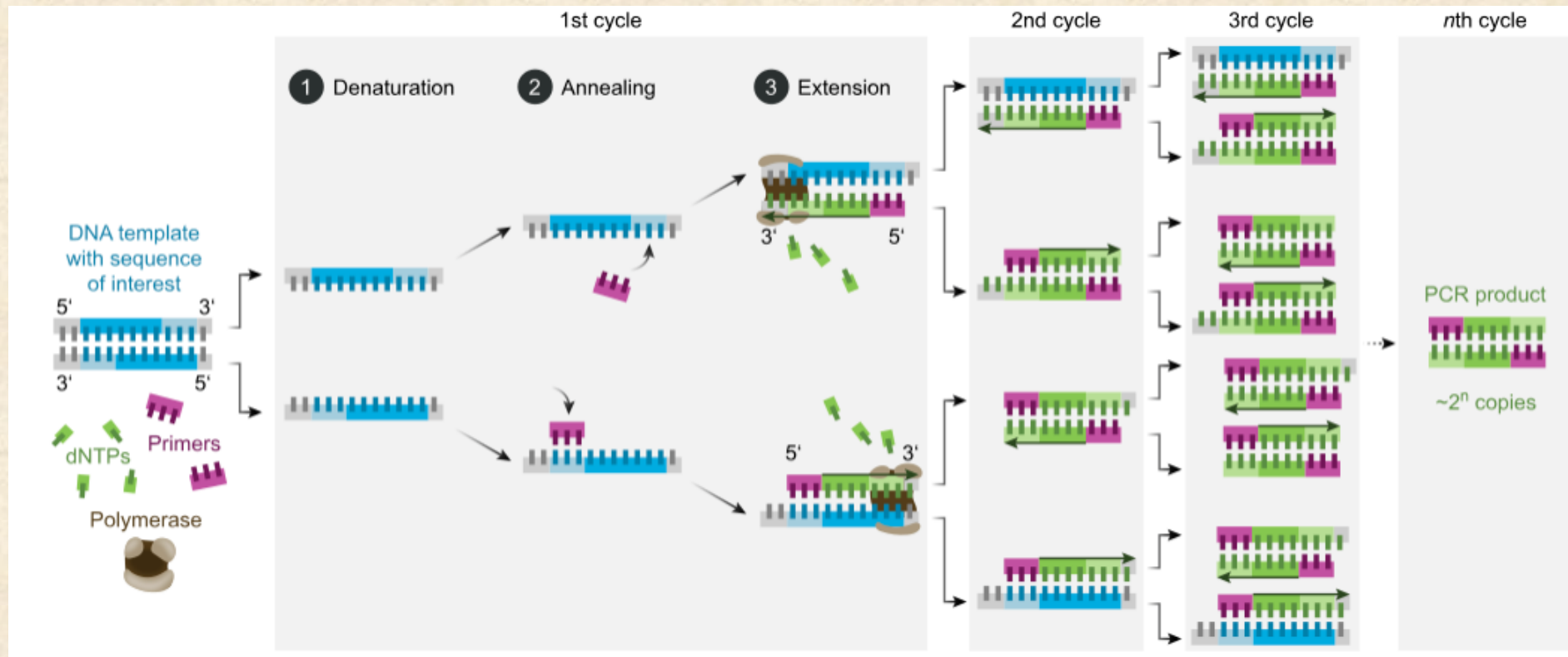
Commonly Used Terms in DNA Fingerprinting (contd.)

- RFLPs = Restriction Fragment Length Polymorphisms
- Molecular biology technique which uses Restriction Enzymes to identify variations in homologous DNA sequences
- Restriction enzymes used cut on either ends of VNTRs
- RFLPs can help differentiate individuals in a population



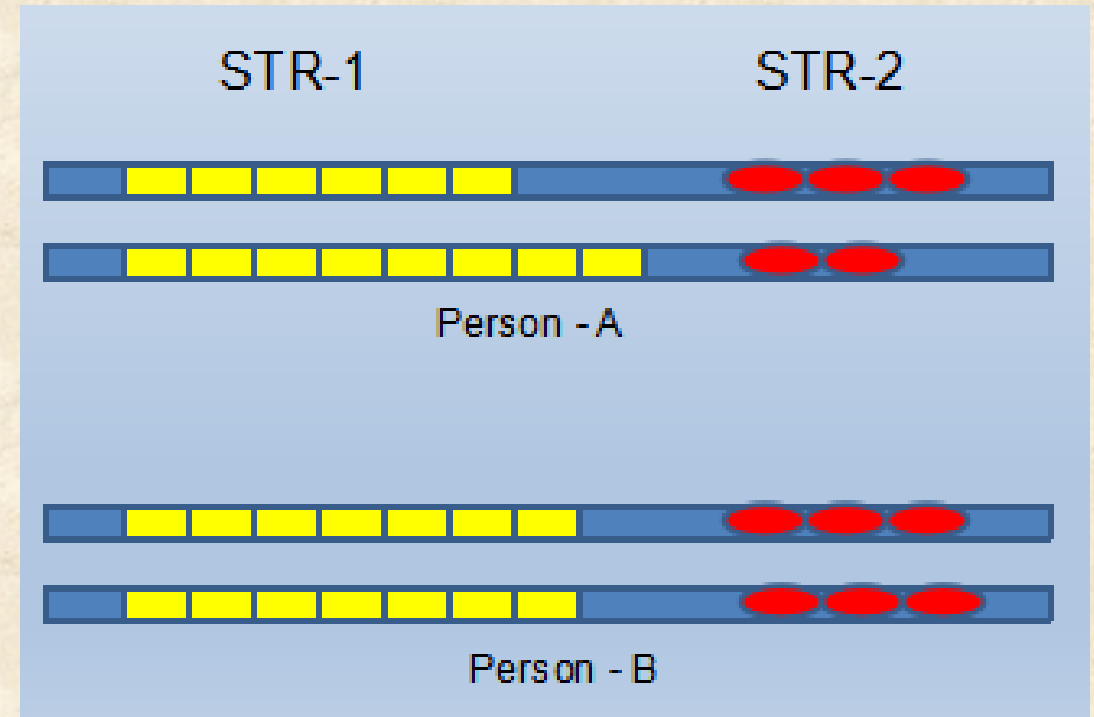
Commonly Used Terms in DNA Fingerprinting (contd.)

- PCR = Polymerase Chain Reaction
- Molecular Biology technique
- Used to make million/billions of copies of a specific DNA sample



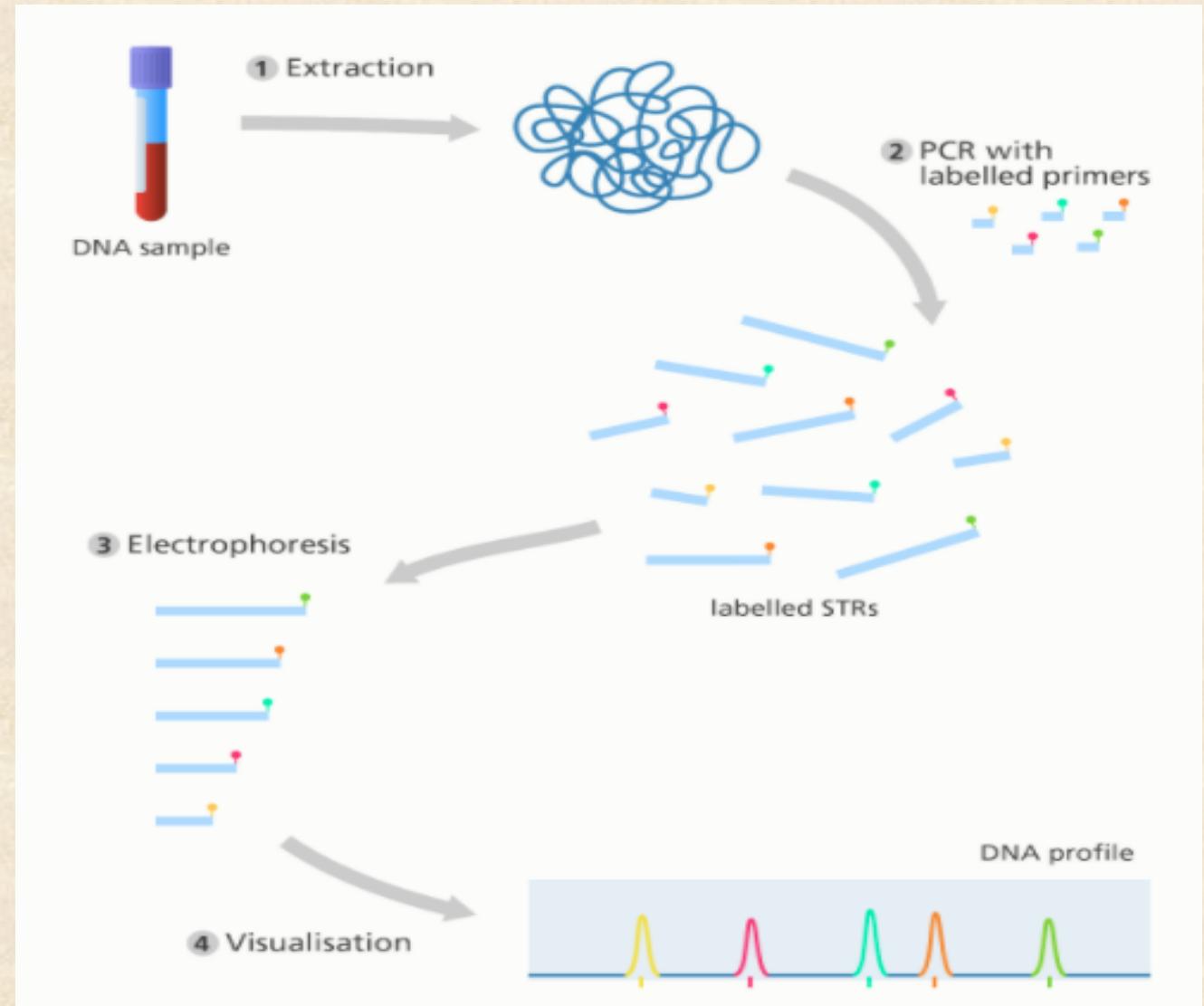
Commonly Used Terms in DNA Fingerprinting (contd.)

- STRs = Short Tandem Repeats
- DNA contains exons and introns, non-coding DNA has repetitive sequences
- Some short microsatellites (2-7bp long) or STRs (Short Tandem Repeats) repeat up to a 100 times consecutively.
- Number of repeats of these STRs are different in every individual.
- DNA Profiling targets multiple known STRs, which record unique combinations for each individual.



DNA Fingerprint Sub-steps

- Modern-day DNA Fingerprinting uses many small & large equipment to give distinct DNA profiles
- DNA Fingerprinting involves 4 sub-steps
 - DNA Isolation/Extraction
 - DNA Amplification
 - DNA Profiling
 - Visualization



Biological Samples



Saliva



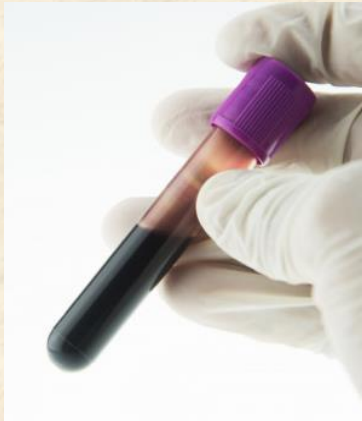
Bone



Bloodstain



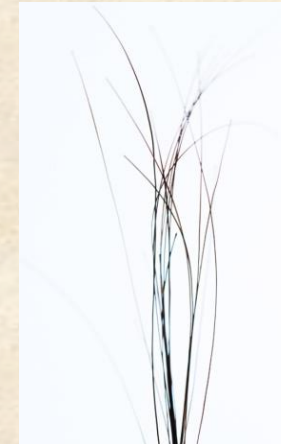
Tooth



Blood



Tissue



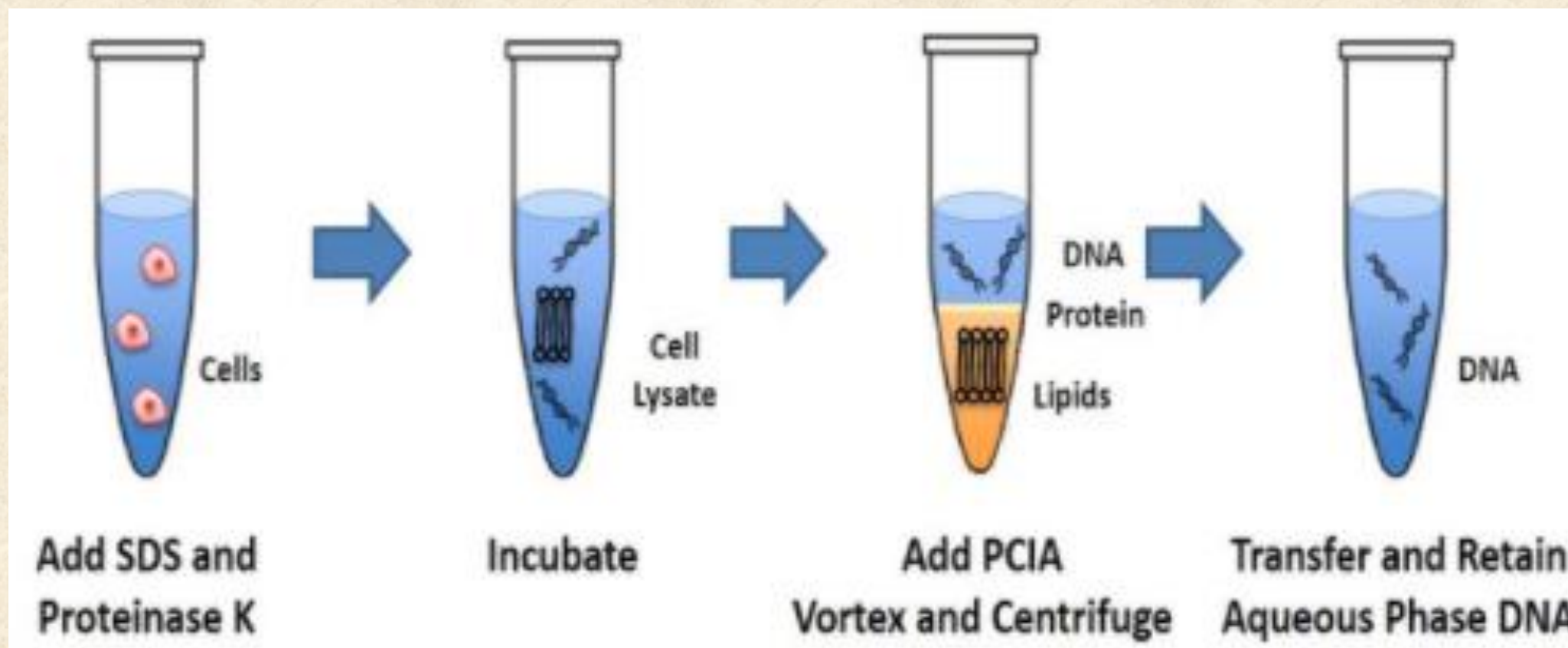
Hair



Semen

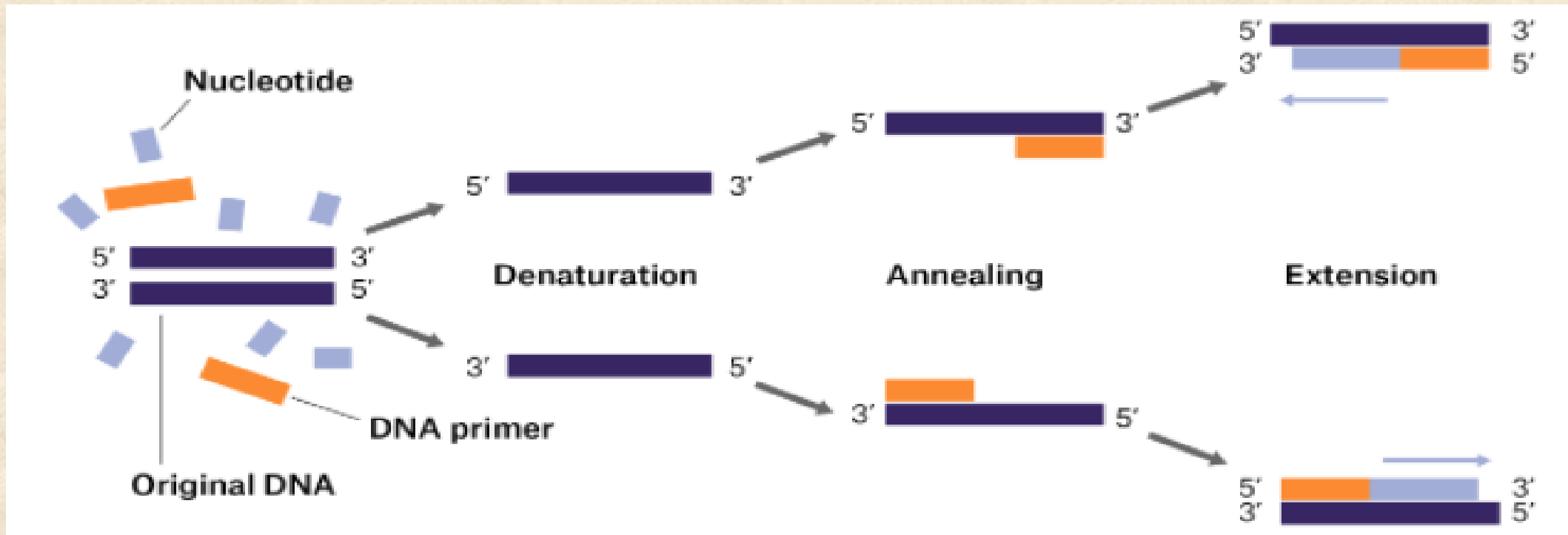
DNA Fingerprinting: DNA Extraction

- Primary step - to isolate DNA
- Pure, free DNA is separated, which is used to identify who it belongs to



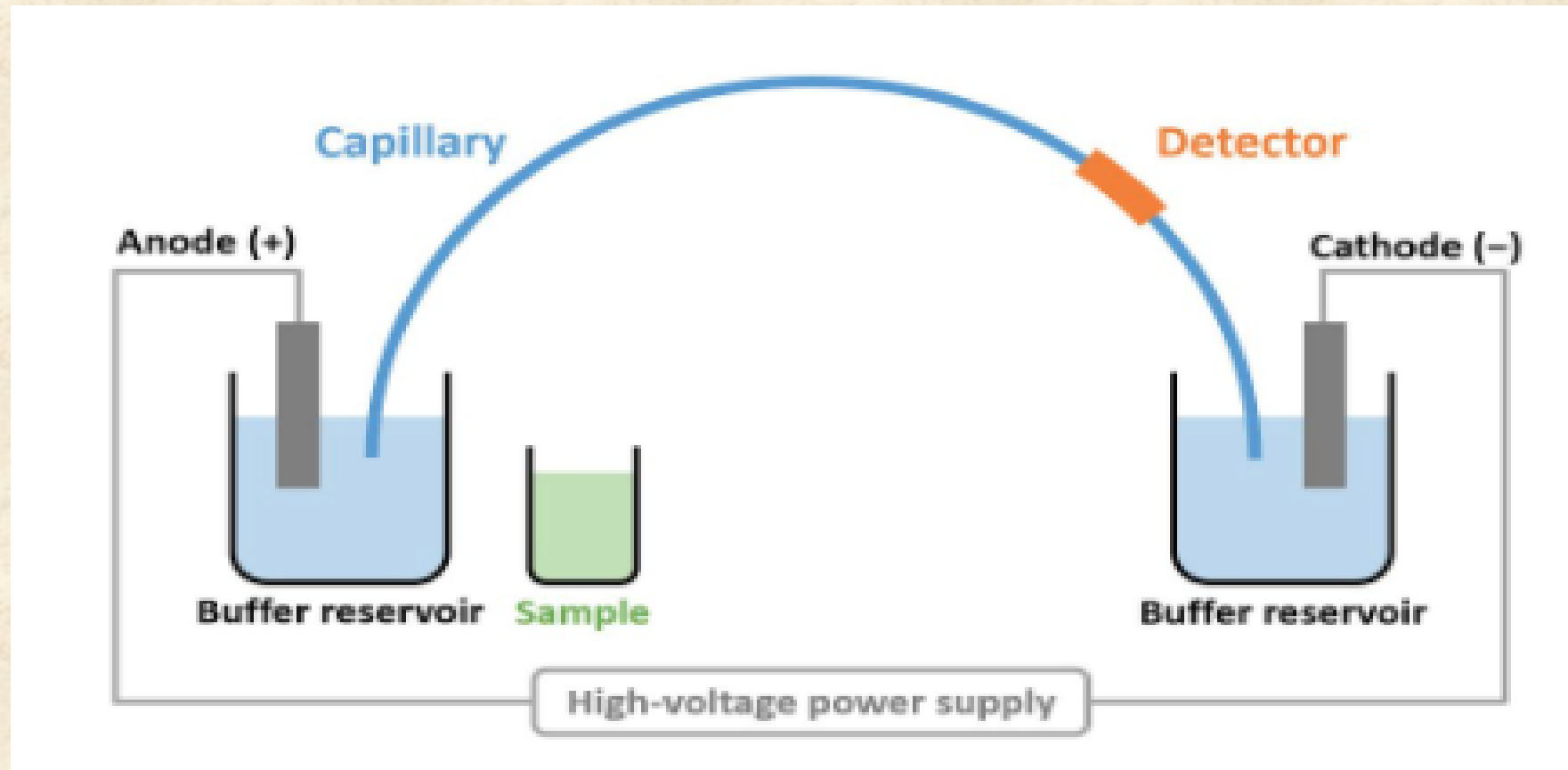
DNA Fingerprinting: DNA Amplification

- Secondary step - to magnify the quantity of DNA
- Pure, free DNA sample is increased
- Every PCR reaction needs DNA polymerase, dNTPs, primers



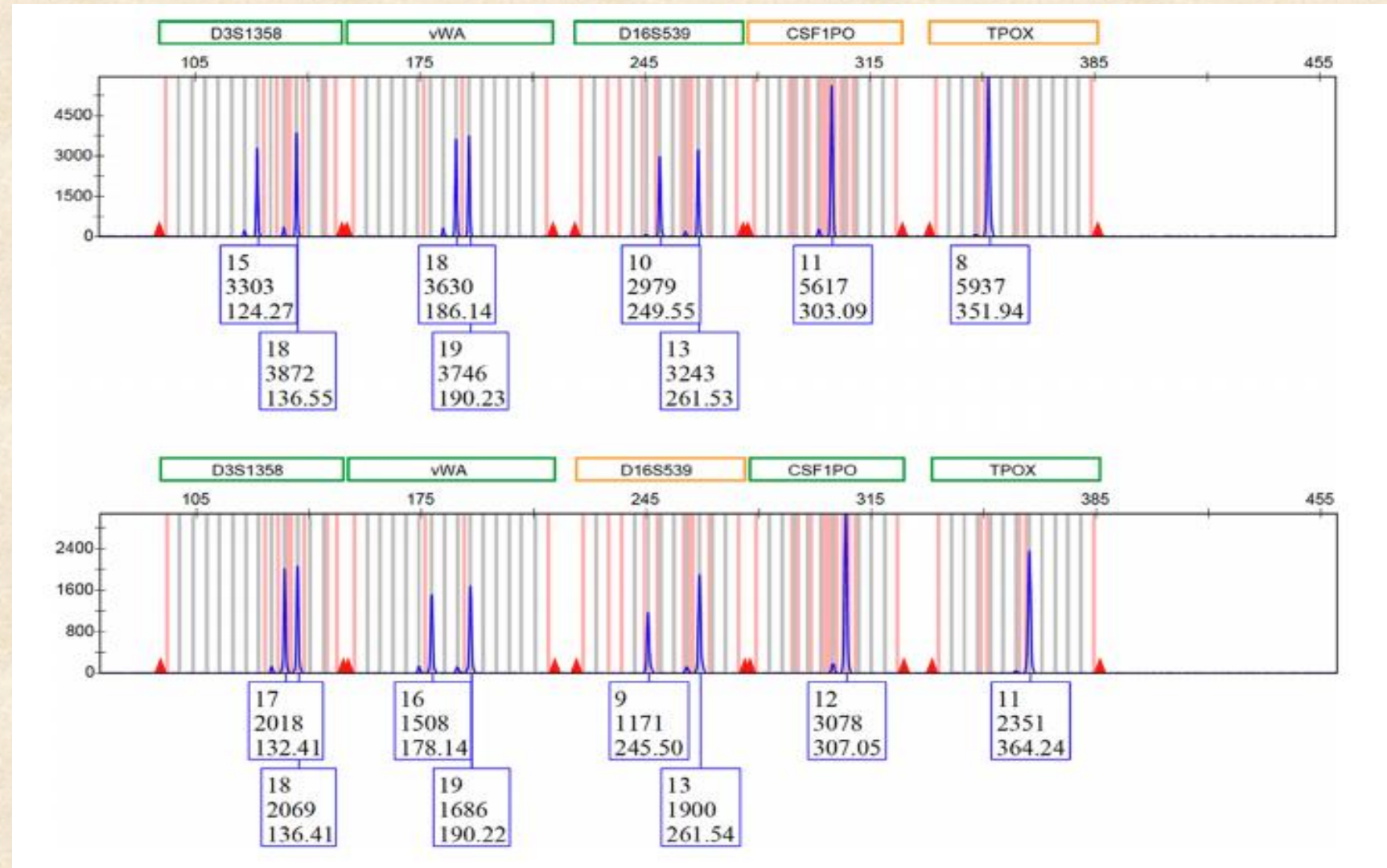
DNA Fingerprinting: DNA Profiling

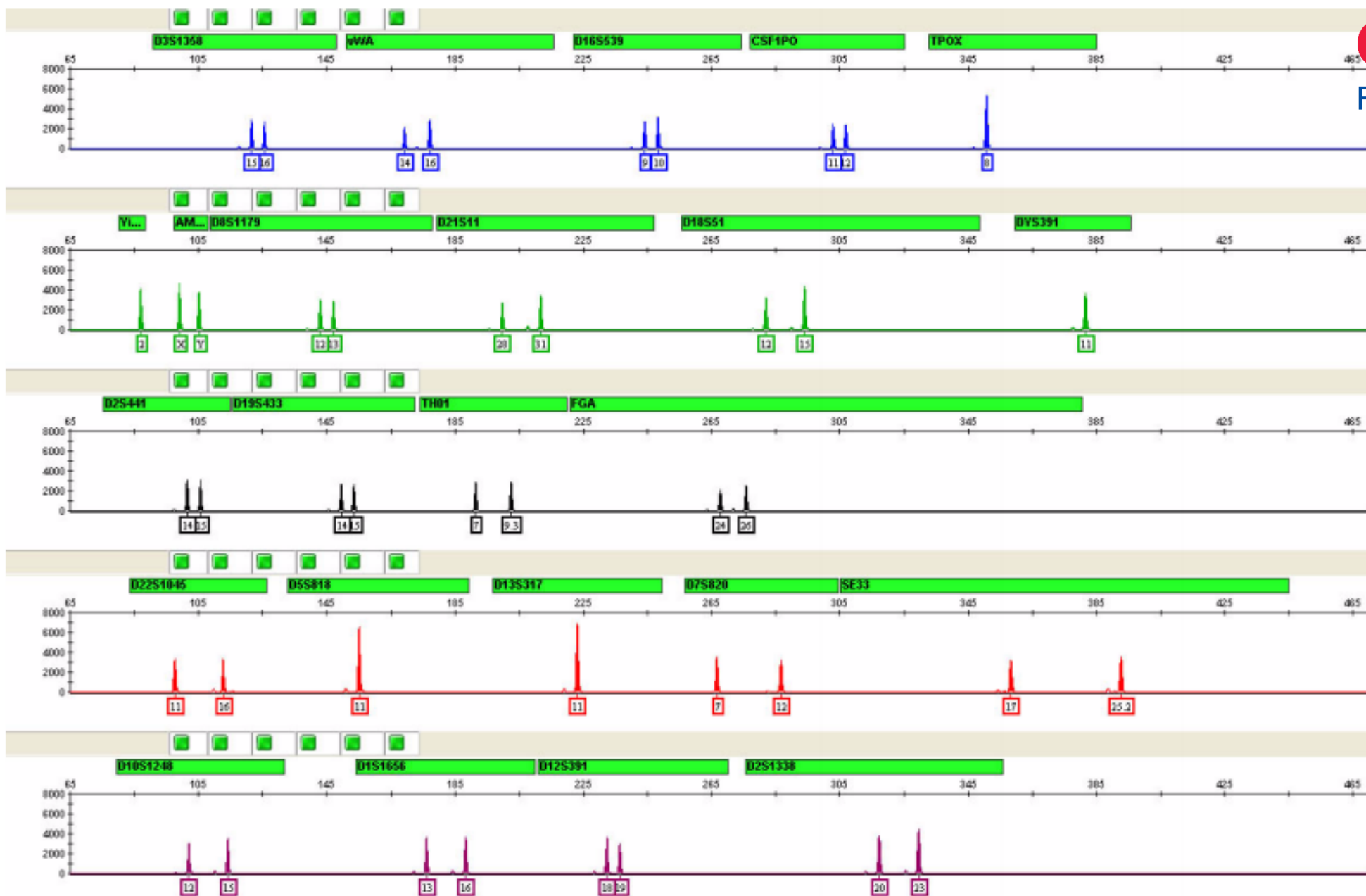
- Tertiary step - to separate and detect the STRs present in a given DNA sample
- The separation occurs on the basis of the size of the STRs



DNA Fingerprinting: Visualization

- Final step - to visualize the STRs (size & location) in the capillary for any given DNA sample
- The visualization is only possible due to the tagged primers used during PCR

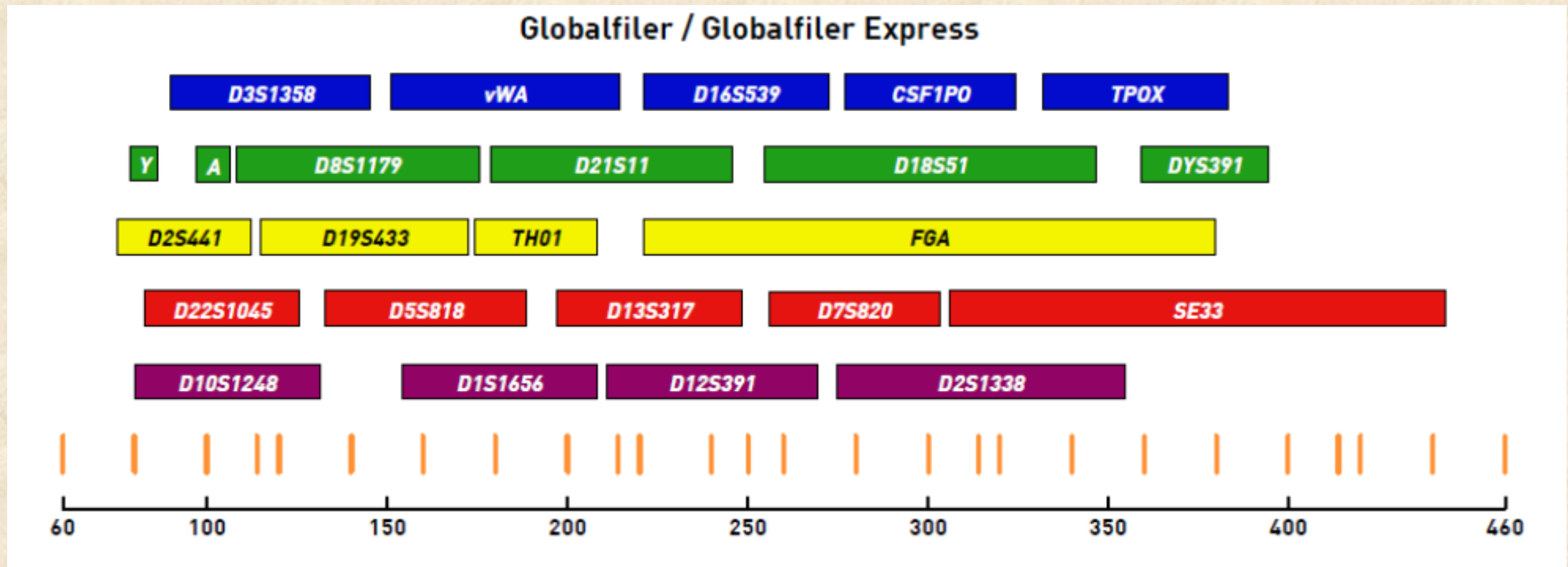






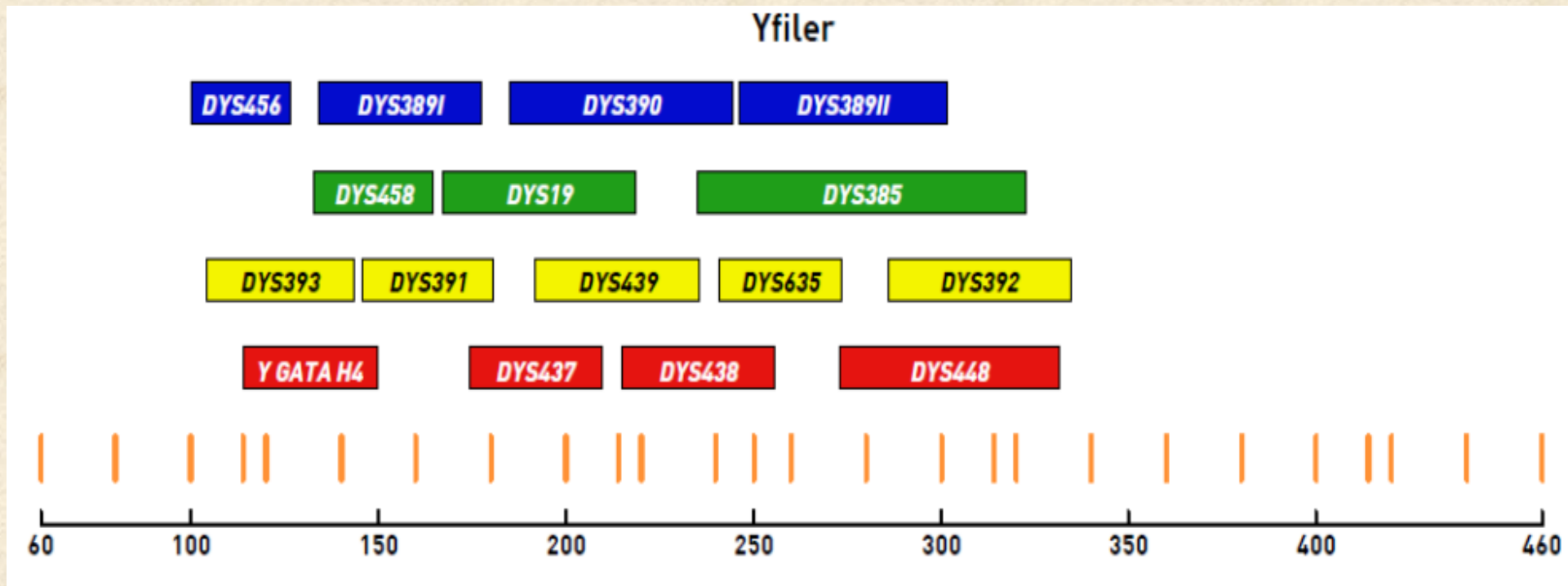
GlobalFiler Kit

- Uses 6-dye chemistry
- Provides high power of discrimination
- Rapid analysis is seen, with run time of 40 to 120 minutes



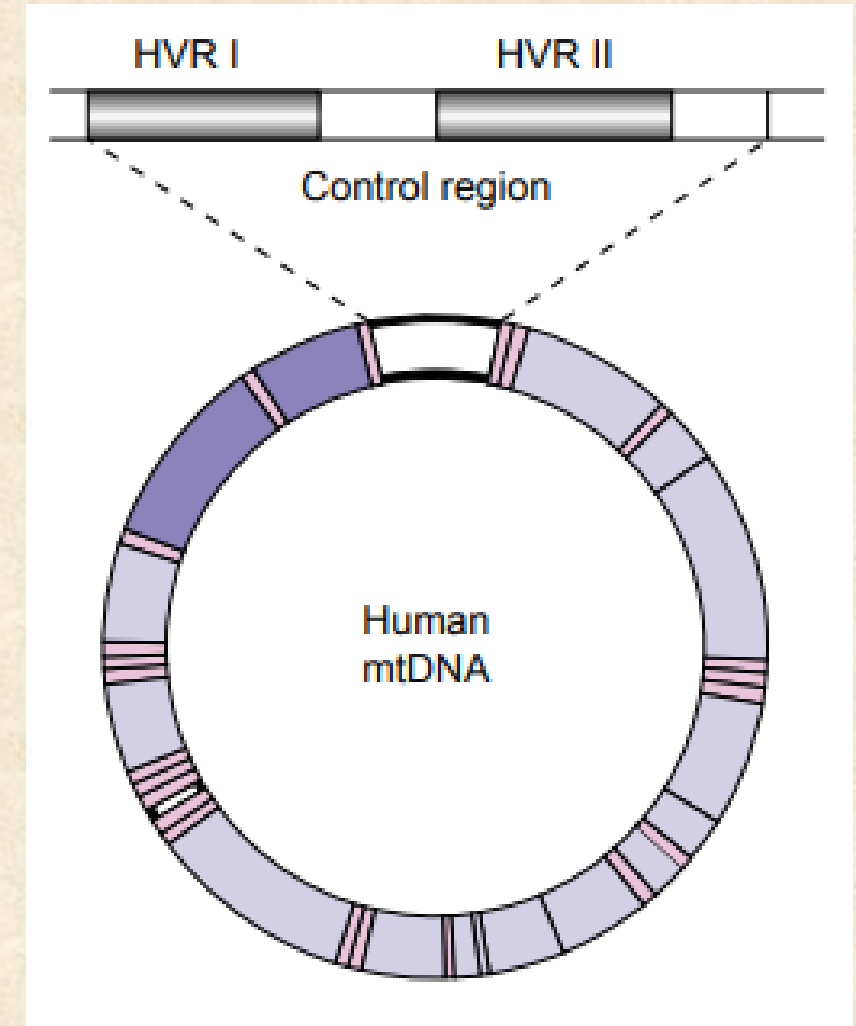
Y-Filer Kit

- Uses 5-dye chemistry
- Power of discrimination is good
- Run time of greater than 180 minutes



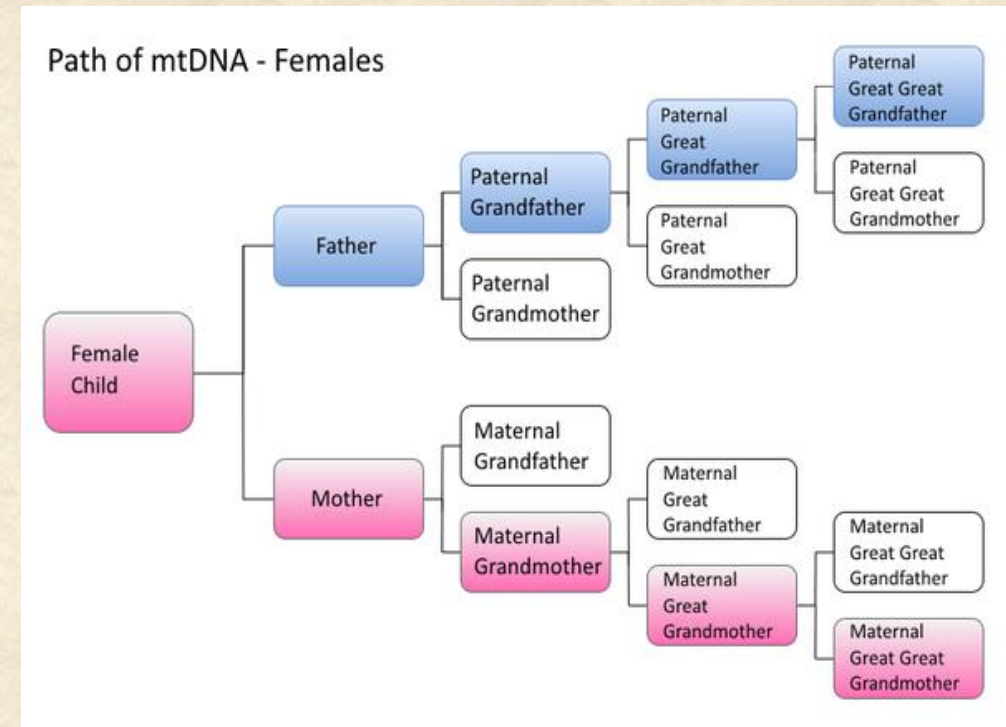
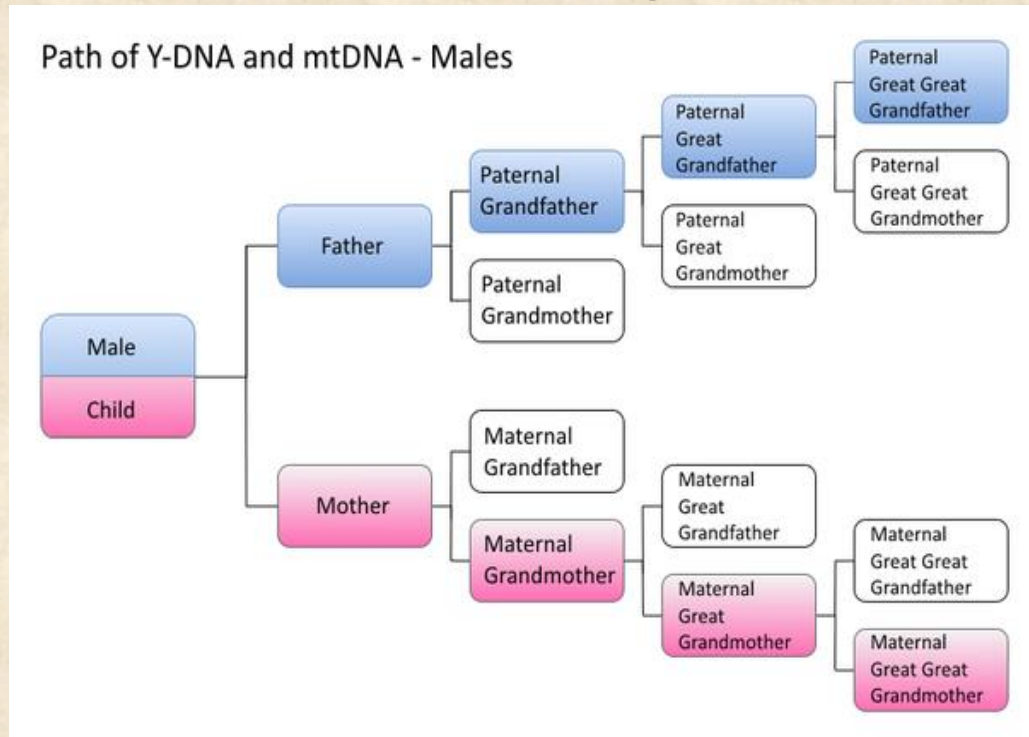
Mitochondrial DNA

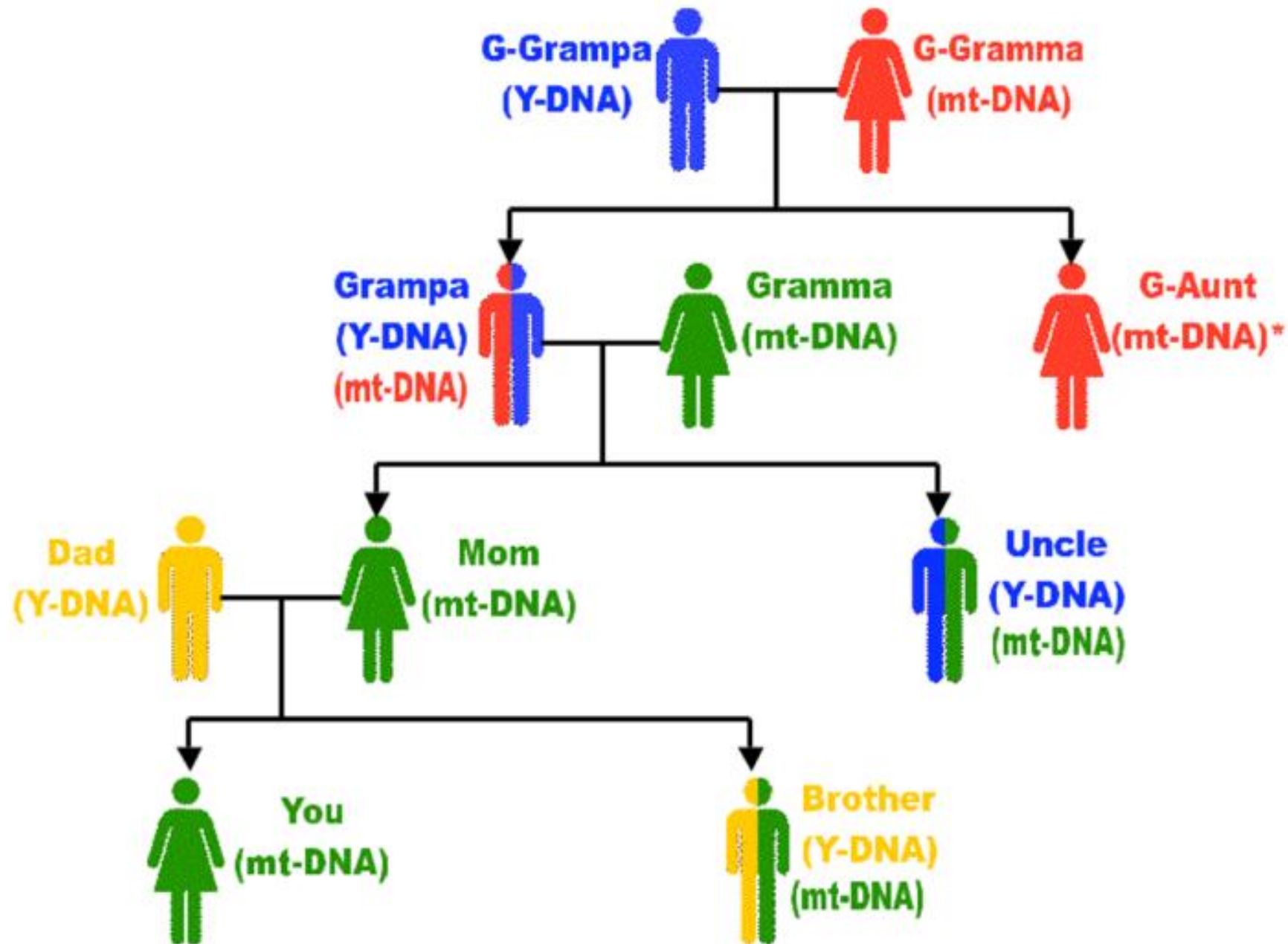
- Mitochondrial DNA (mtDNA) is present in every cell
- Multiple copies of mtDNA is present in every cell
- It is circular in structure
- Human mtDNA is 16569bp long
- The mitochondrial DNA in every child is identical to his mother's
- mtDNA is indistinguishable in the same maternal line
- mtDNA has 2 major parts:
 - Coding region
 - Control region



Mitochondrial DNA (contd.)

- The control has 2 regions where maximum variations are found in humans: Hypervariable region 1 (HV1) & Hypervariable region 2 (HV2)
- HV1, HV2 & coding regions are tested during mtDNA analysis
- **mtDNA mismatch** = not full siblings, but can have the same father
- **mtDNA match** = related through maternal line & could be siblings but have different father

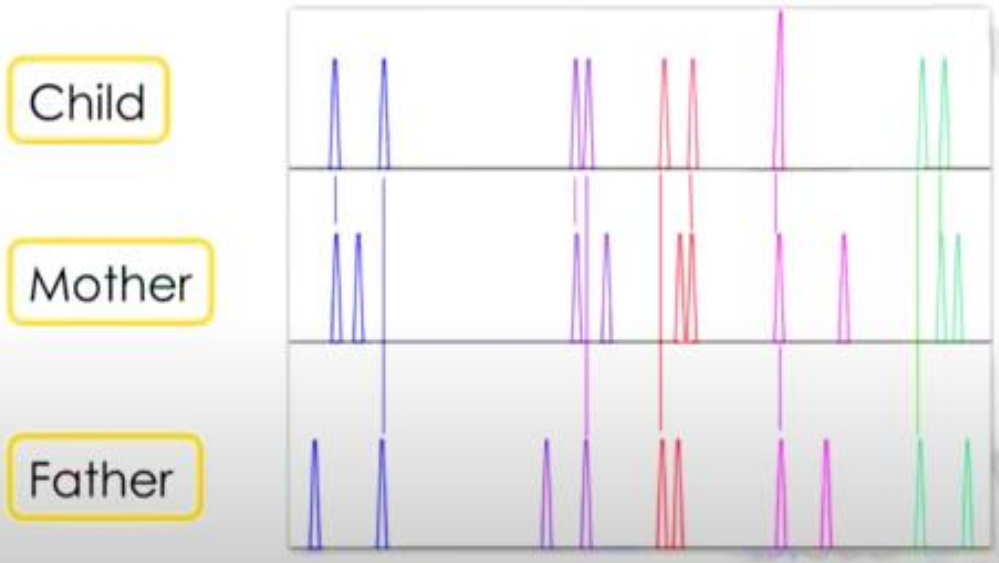




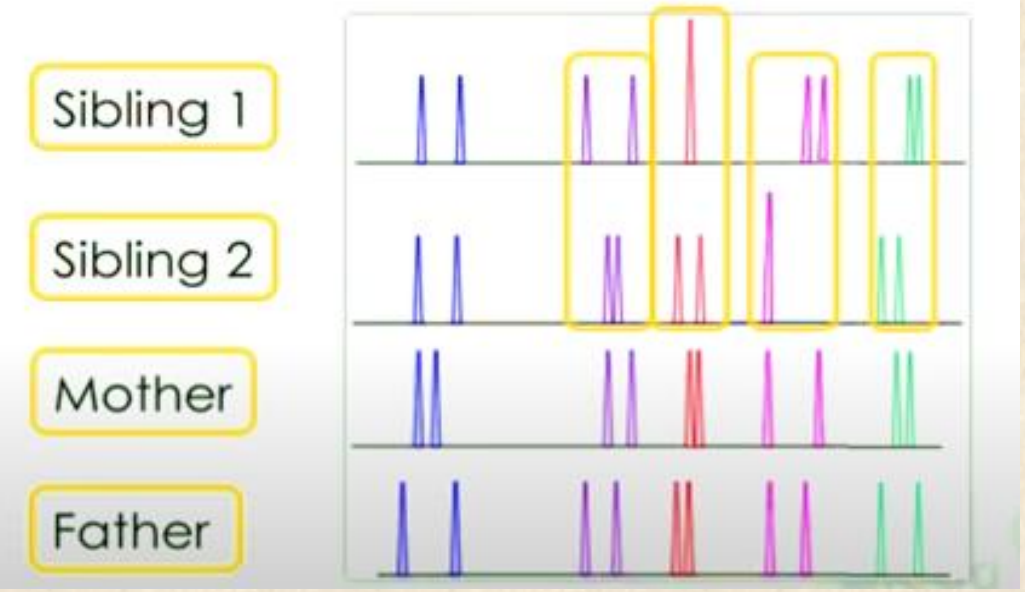
Uses of DNA Fingerprinting

- Paternity/Maternity disputes resolution
- Relationship establishment for living donor organ transplantation
- Missing persons cases
- Homicide and sexual assault cases
- Establishing identity in physical assault cases
- Identification of victims of mass disasters
- DNA tests for immigration/VISA purposes
- Ancient DNA profiling
- Child swapping cases
- Tissue or cell identification – in pathology labs
- Maternal & Paternal lineage establishment

Paternity & Maternity Testing



Sibling relationship



Identification of Victims of Mass Disasters

Example of Paternity & Maternity

- Used to identify a child's parents
- Each child inherits one set of chromosomes from each parent
- Only the repeats present in the parents can be passed on to the offsprings

DNA Marker	Father	Mother	Daughter	Son
D7S820	9, 10	10, 11	9, 11	10, 10
D3S1358	16, 17.2	15, 15.2	15, 16	15, 16
D16S539	11, 11	9, 11	11, 11	9, 11
D8S1179	13, 15	15, 16	13, 16	13, 16
CSF1PO	10, 12	10, 10	10, 12	10, 12
SE33	25, 31.2	27, 33	31.2, 33	25, 33
TPOX	8, 12	9, 13	9, 12	9, 12
Amelogenin	X, Y	X, X	X, X	X, Y

Example of Crime Scene Evidence

DNA Marker	Used Condom (from crime scene)	Suspect A	Suspect B	Suspect C	Suspect D
D7S820	10, 12	9, 9	7, 7	10, 12	8, 12
D3S1358	15, 18	13, 17	15, 15.3	15, 18	13, 18
D16S539	11, 14	11, 12	9, 11	11, 14	10, 14
D8S1179	13, 13	13, 13	15, 15	13, 13	10, 12
CSF1PO	12, 12	8, 8	10, 12	12, 12	8, 11
SE33	22, 29.2	31.2, 33	14.3, 28	22, 29.2	17, 20
TPOX	7, 9.3	9, 9	9, 12	7, 9.3	8, 9
Amelogenin	X, Y	X, Y	X, Y	X, Y	X, Y

DNA: Famous Cases

- 1st case in India - used DNA Fingerprinting for investigating - 1995
- Former Congress worker - Mrs. Naina Saini was shot by her husband, Sushil Sharma
- He tried to burn the body in the tandoor at Bagiya Restaurant, New Delhi on 2nd July, 1995 with the help of the restaurant manager, Keshar Kumar
- Charred remains of a body were recovered from tandoor
- DNA from the muscle attached to the partially charred bone were compared against that of her parents and sister
- This helped establish identity of the charred body



DNA: Famous Cases (contd.)

- DNA analysis was used during the investigation of Rajiv Gandhi's Assassination
- Carried out by a suicide bomber
- Identity established as Thenmozhi Rajaratnam (Dhanu), a member of LTTE's suicide squad, called Black Tigers



DNA: Famous Cases (contd.)

- Neeraj Grover was a television executive
- He was stabbed to death & his body was cut up & packed in bags
- The body parts with the bag were burnt in the forest
- Upon investigating, the police recovered wiped blood stains from the door latch in the suspect's house - from which they derived a DNA profile
- They also found dried bloodstains on the curtains & TV
- They recovered a few teeth, charred bones - DNA was derived and profiles were generated
- Collected DNA profiles were matched against that of his parents to establish identity

DNA: Famous Cases (contd.)

- DNA tests on bloodstains of the main accused, Ram Singh matched Nirbhaya's DNA
- A swab lifted from Nirbhaya's body showed Ram Singh's DNA
- Bloodstains recovered from the clothes of the accused, curtains and bus seats
- Bloodstains on dried leaves were the dumped Nirbhaya & her friend
- Nirbhaya's DNA profile found on the bloodstains on Vinay Sharma's undergarments and flip-flops
- Bloodstains on Vinay Sharma's jacket match with Nirbhaya's friend who was hit with an iron rod

MUKESH SINGH Age (at time of crime): 30 Occupation Part-time bus driver, cleaner Residence Ravidas Camp, South Delhi Lived in a small two-room shanty with his elder brother		VINAY SHARMA Age: 20 Occupation Gym trainer Residence Ravidas Camp Lived with his parents and sister; was doing BA from Delhi University	
PAWAN GUPTA Age: 19 Occupation Fruit seller Residence Ravidas Camp Stayed with his parents and a younger sister		AKSHAY THAKUR Age: 28 Occupation Bus cleaner Residence Ravidas Camp A school dropout, Akshay moved to Delhi a year before the incident	
RAM SINGH (Committed suicide in Tihar Jail on March 11, 2013) Age: 33 Occupation: Bus driver Residence: Ravidas Camp Lived in a two room shanty		JUVENILE Age: 17 Residence: Delhi The eldest of six children, he left his village at the age of 11 and started doing menial jobs in Delhi; was released after serving 3-yr term at a correction home.	

THANK
you