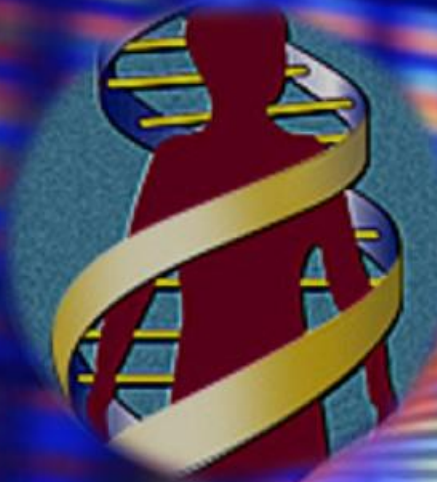


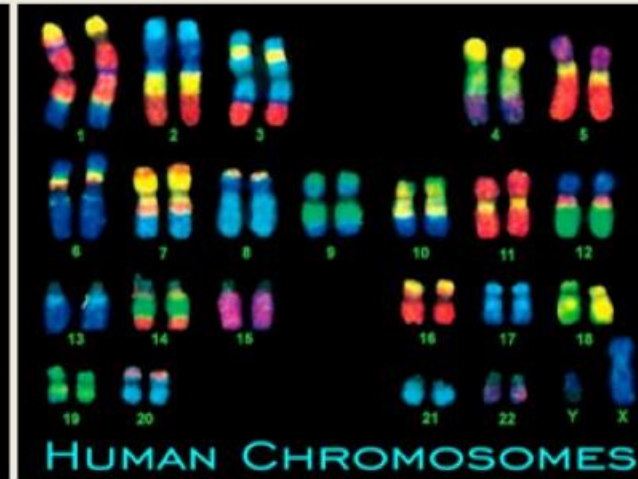
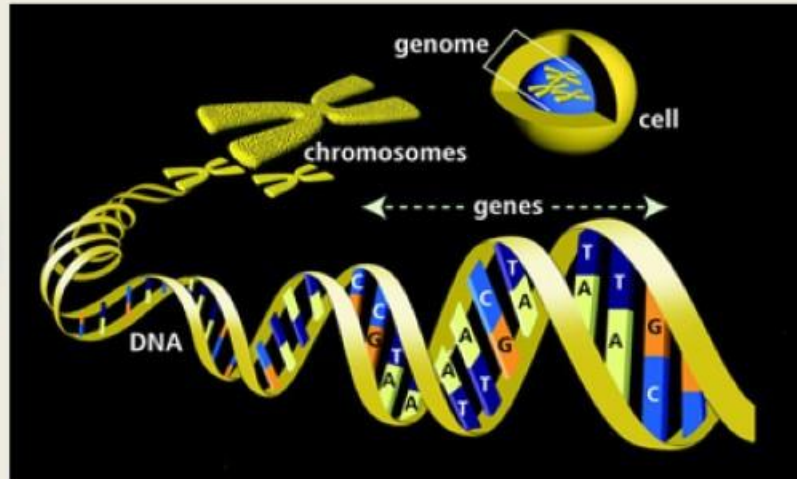


HUMAN GENOME PROJECT (HGP)





HUMAN GENOME PROJECT (HGP)

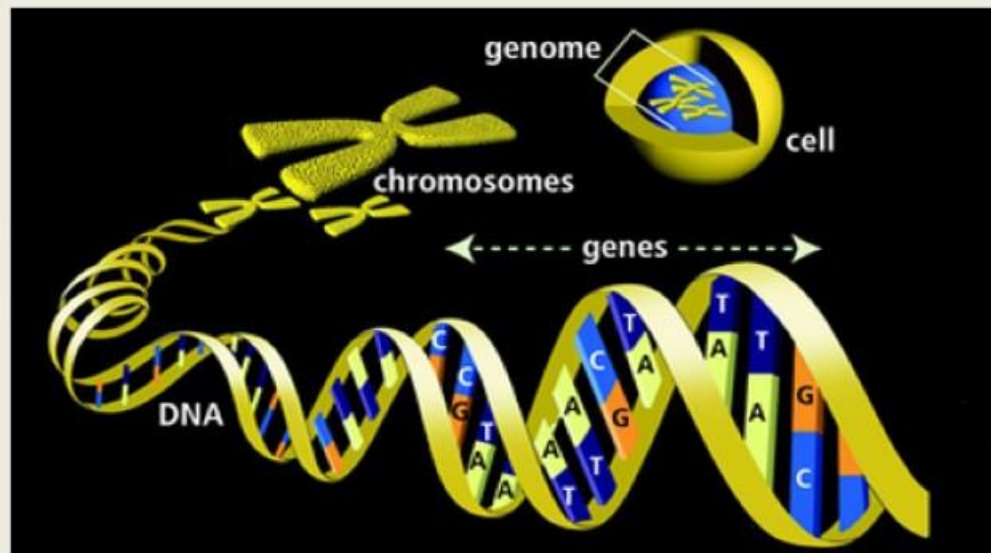


- The entire **DNA in the haploid set of chromosomes** of an organism is called a **Genome**.
- In Human genome, DNA is packed in 23 chromosomes.
- Human genome contains about **3×10^9 bp**.
- **Human Genome Project (1990-2003)** was the first mega project for the sequencing of nucleotides and mapping of all the genes in human genome.
- HGP was coordinated by **U.S. Department of Energy** and the **National Institute of Health**.

HUMAN GENOME PROJECT (HGP)

GOALS OF HGP

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✓ Identify all the estimated **genes** in human DNA.

✓ **Sequencing** of 3 billion chemical **base pairs** of human DNA.

✓ Store this information in **databases**.

✓ Improve tools for **data analysis**.

✓ **Transfer** related technologies to other sectors.

✓ Address the **ethical, legal and social issues (ELSI)** that may arise from the project.

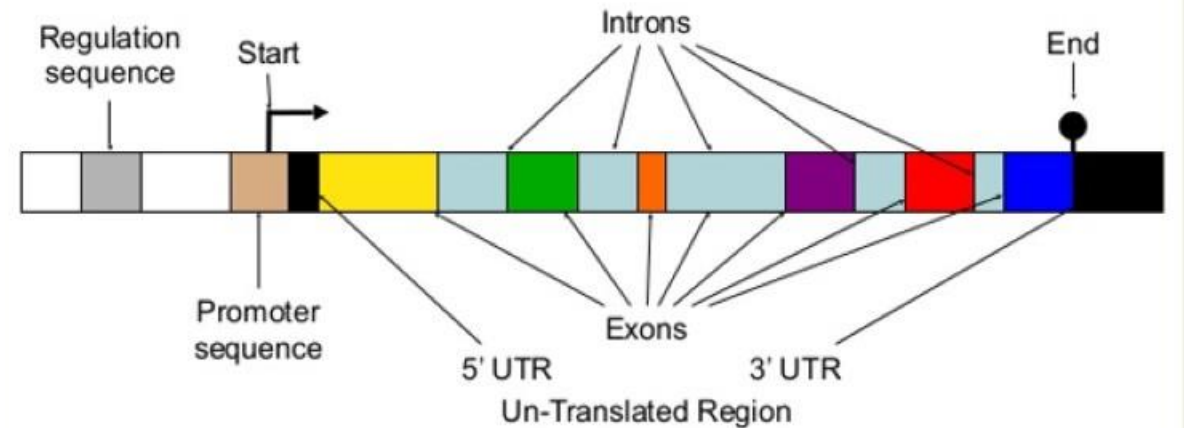
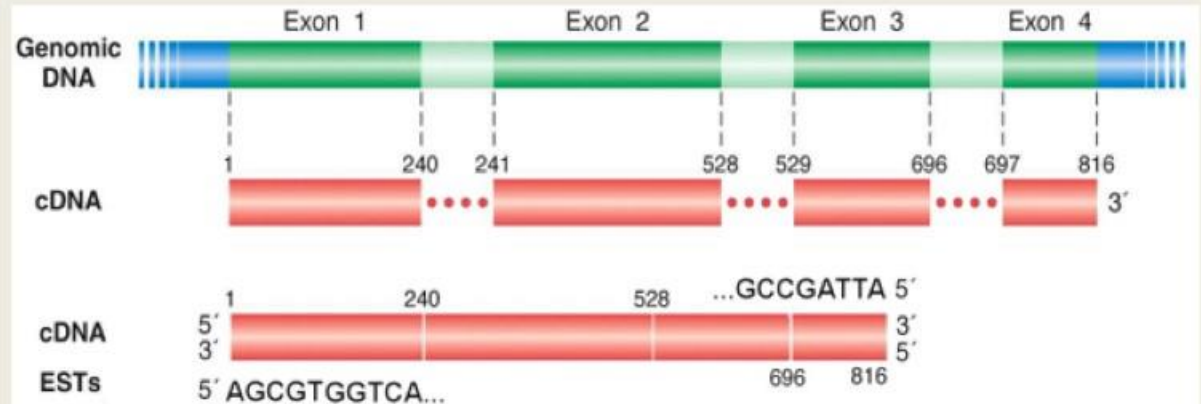
It involves 2 major approaches.

1. Expressed Sequence Tags (ESTs)

Focused on identifying all the genes that are expressed as RNA.

2. Sequence annotation

Sequencing whole set of genome containing all the coding & non-coding sequence and later assigning different regions in the sequence with functions.



HUMAN GENOME PROJECT (HGP)

PROCEDURE

Isolate DNA from a cell

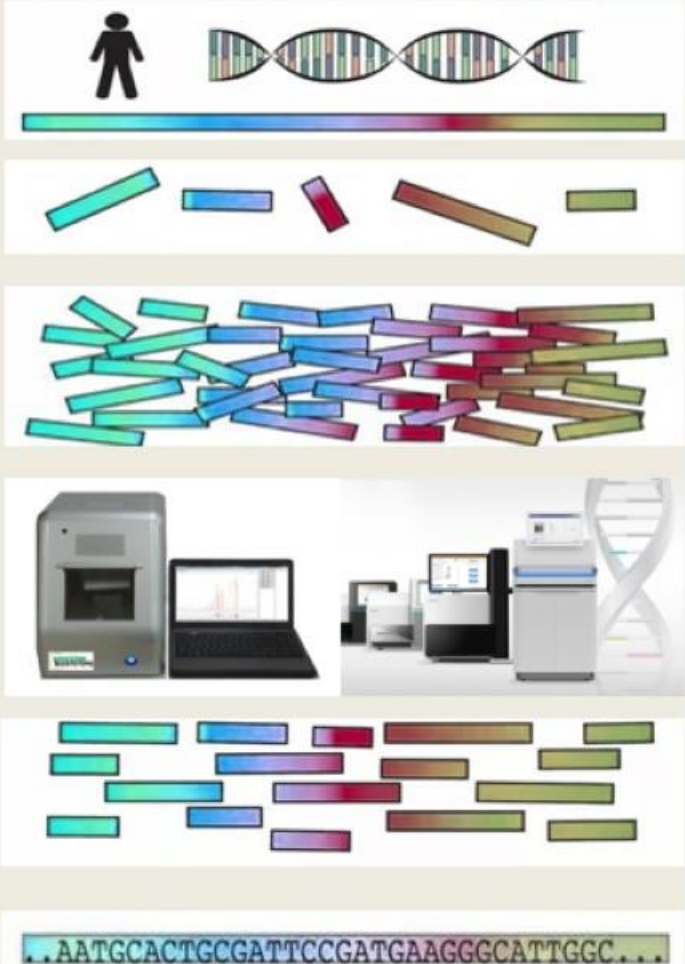
Convert into random fragments

Clone in a host (bacteria & yeast) using vectors (e.g. BAC & YAC) for amplification

Sequencing of fragments using Automated DNA sequencers (using Frederick Sanger method)

Arrange the sequences based on overlapping regions

Alignment of sequences using computer programs.



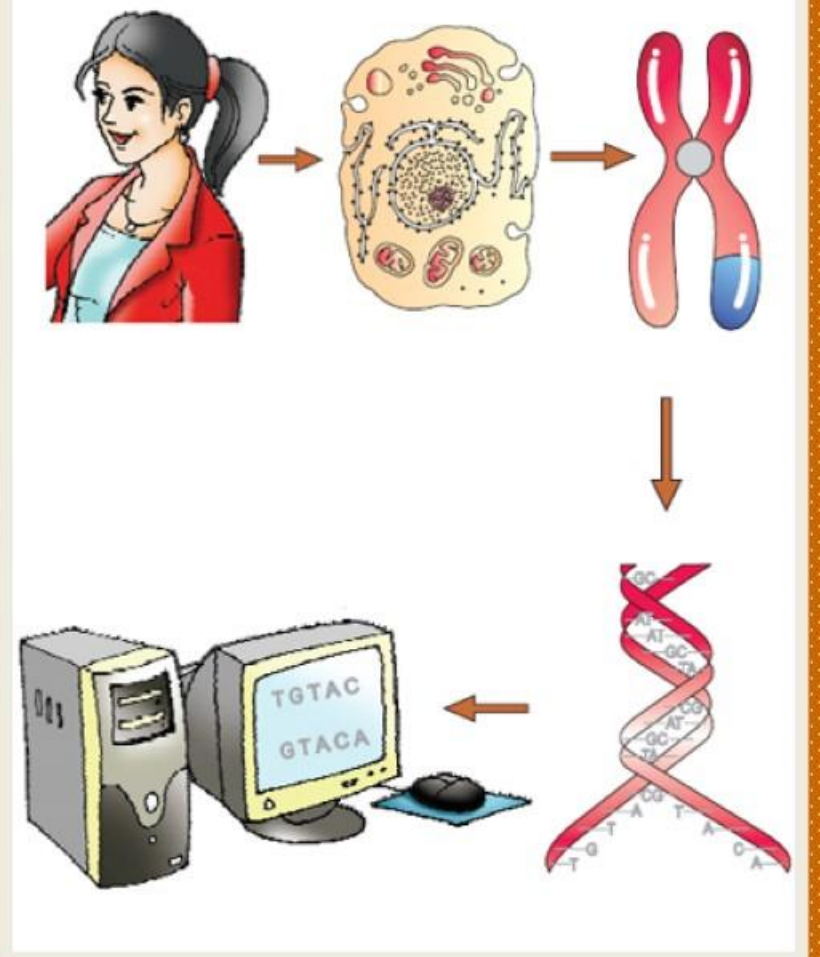
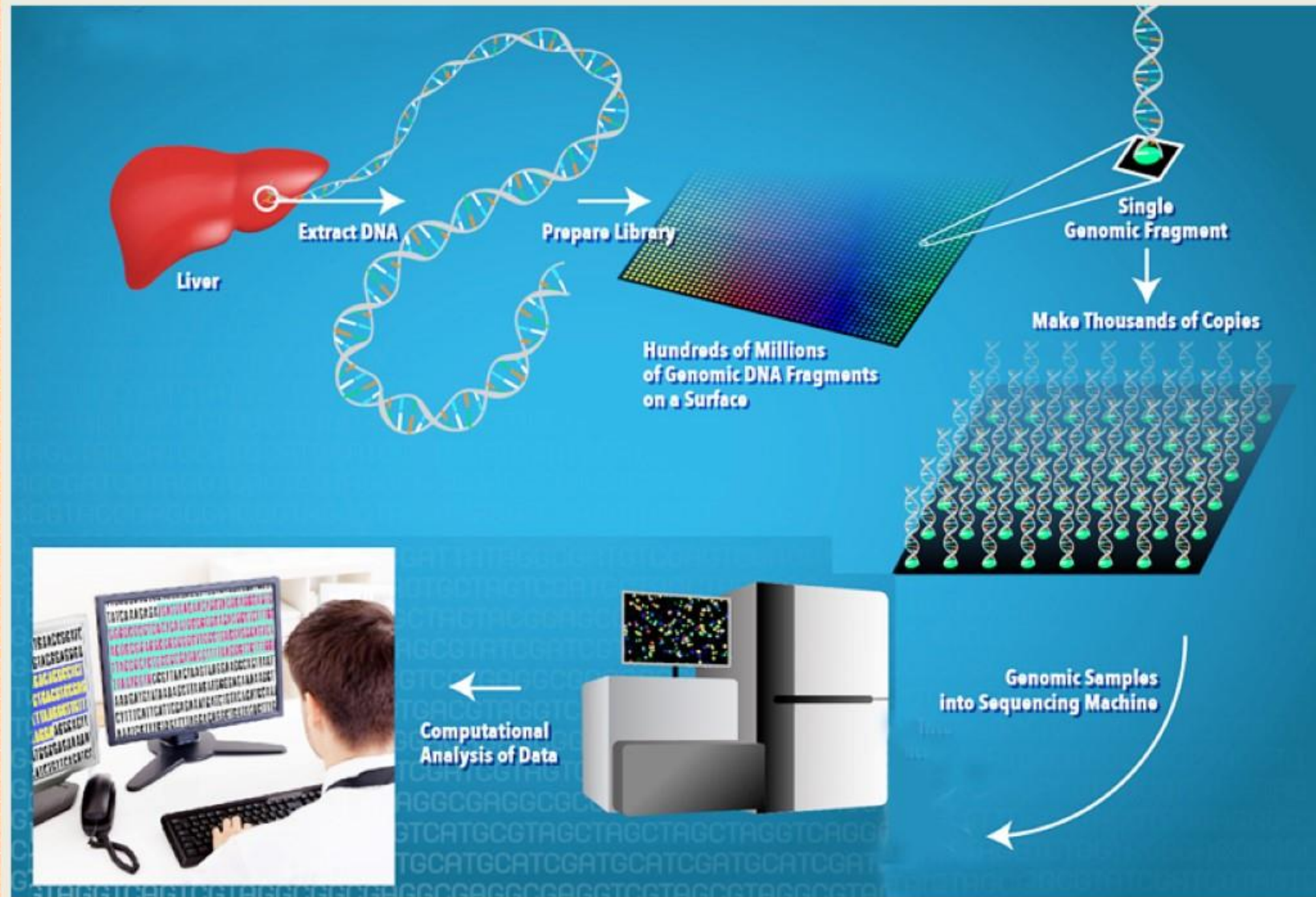
BAC= Bacterial Artificial Chromosomes

YAC= Yeast Artificial Chromosomes

HUMAN GENOME PROJECT (HGP)

PROCEDURE

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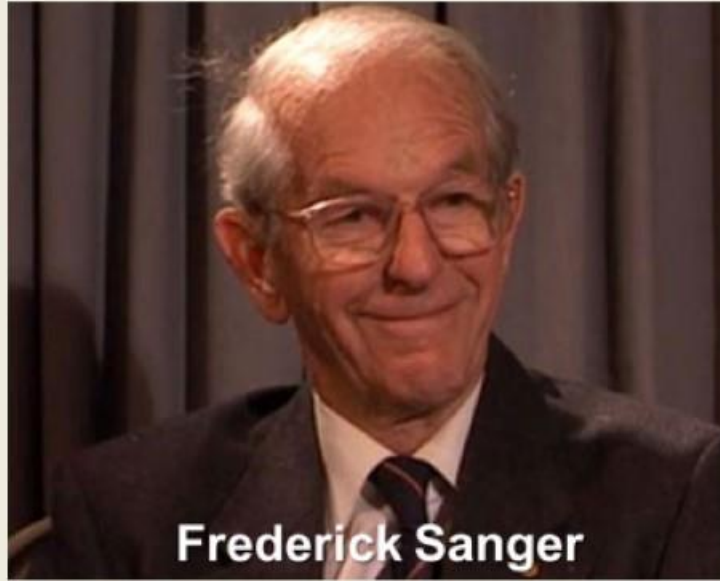


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HUMAN GENOME PROJECT (HGP)



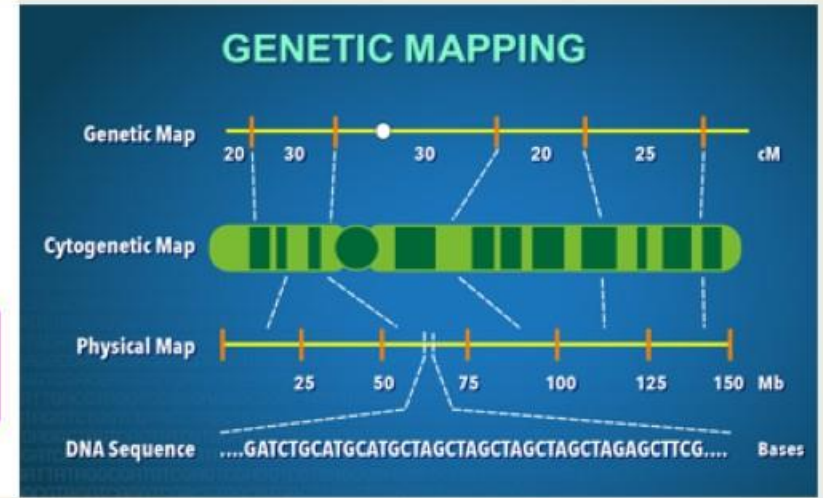
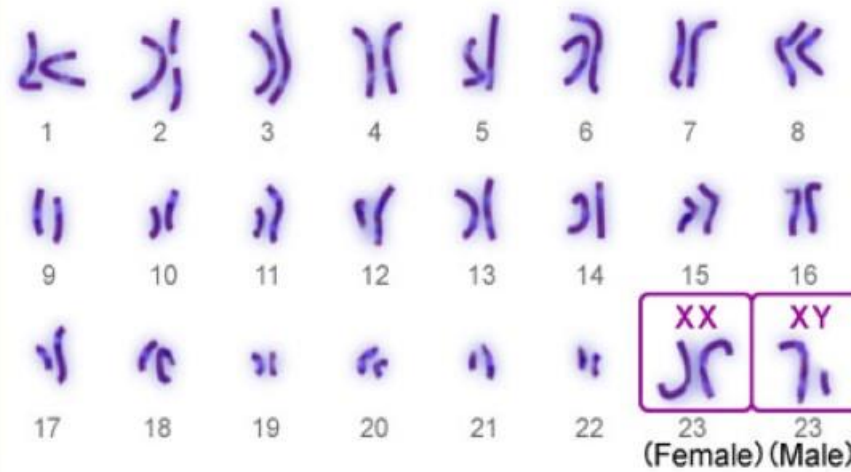
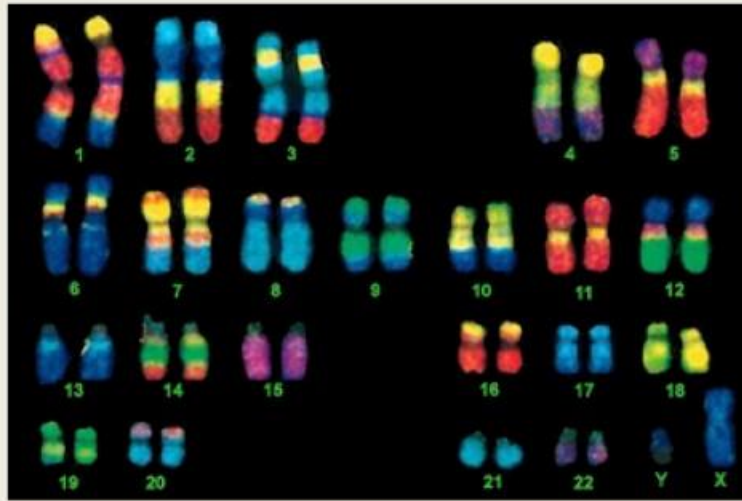
Frederick Sanger



- Sanger has also developed method for **sequencing of amino acids** in proteins.
- DNA is converted to random fragments as there are technical limitations in sequencing very long pieces of DNA.
- HGP was closely associated with **Bioinformatics**.
- **Bioinformatics**: Application of computer science and information technology to the field of biology & medicine.



HUMAN GENOME PROJECT (HGP)



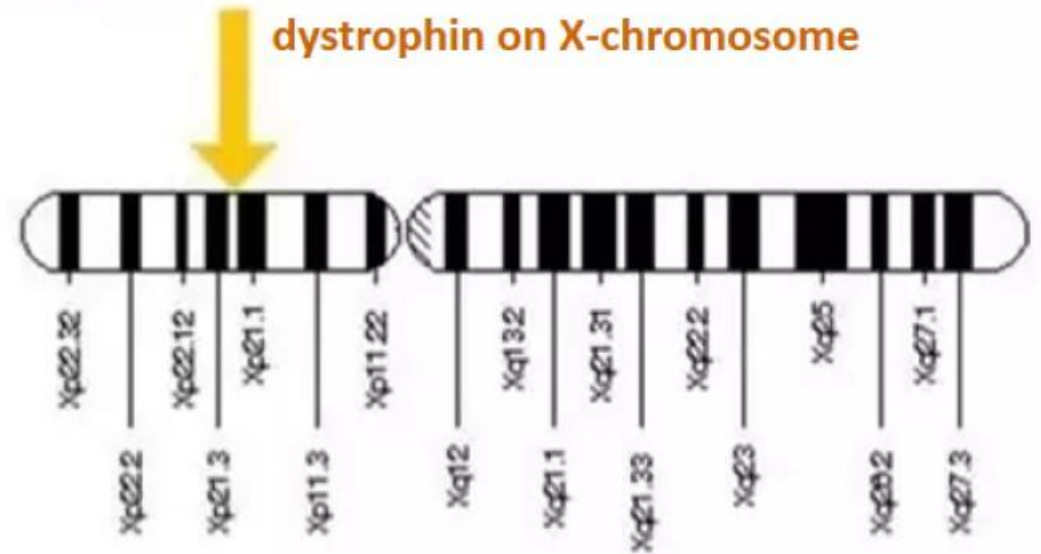
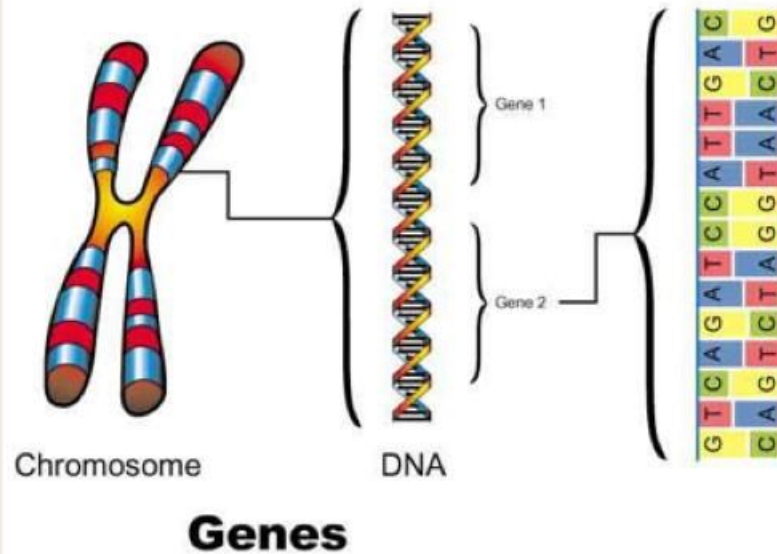
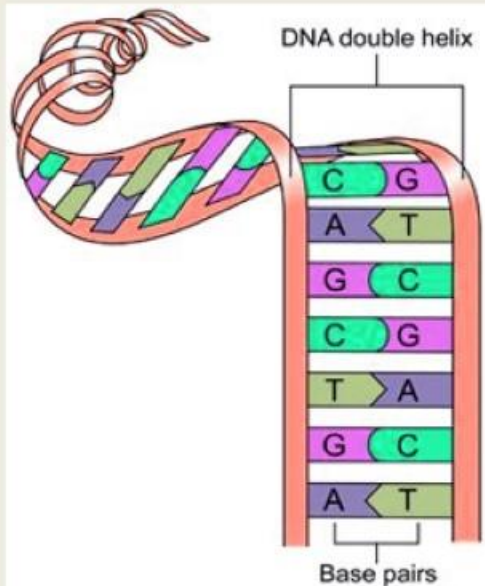
- Of the 24 chromosomes (22 autosomes and X & Y), the last sequenced one is **chromosome 1** (May 2006).
- Genetic and physical maps on the genome were generated using information on polymorphism of **restriction endonuclease** recognition sites and some **repetitive DNA sequences (microsatellites)**.
- DNA sequencing also have been done in bacteria, yeast, ***Caenorhabditis elegans*** (a free living non-pathogenic nematode), ***Drosophila***, plants (rice & Arabidopsis), etc.



HUMAN GENOME PROJECT (HGP)

SALIENT FEATURES OF HUMAN GENOME

1. Human genome contains **3164.7 million** nucleotide bases.
2. Total number of genes= about **30,000**.
3. Average gene consists of **3000 bases**, but sizes vary. Largest known human gene (**dystrophin on X-chromosome**) contains **2.4 million bases**.

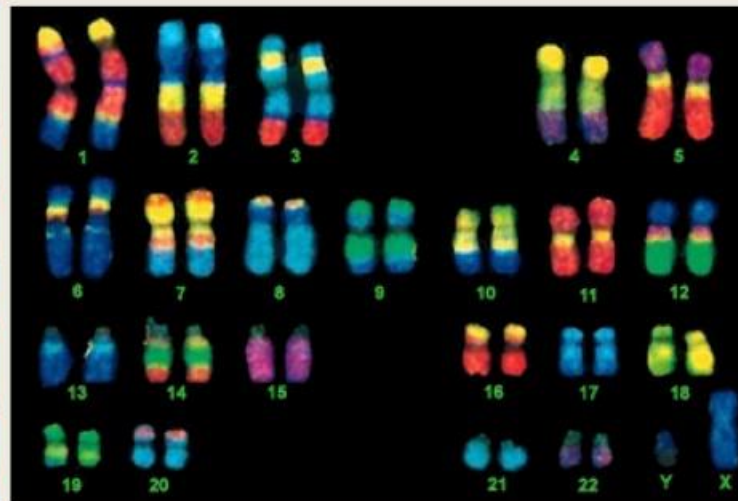




HUMAN GENOME PROJECT (HGP)

SALIENT FEATURES OF HUMAN GENOME

4. **99.9%** nucleotide bases are same in all people. Only **0.1%** (3×10^6 bp) difference makes every individual unique.
5. Functions of over 50% of discovered genes are unknown.
6. **Chromosome I** has most genes (**2968**) and **Y** has the fewest (**231**).
7. Less than **2%** of the genome codes for proteins.





HUMAN GENOME PROJECT (HGP)

SALIENT FEATURES OF HUMAN GENOME

8. Very large portion of human genome is made of **Repeated (repetitive) sequences**. These are stretches of DNA sequences that are repeated many times. They have no direct coding functions. They shed light on chromosome structure, dynamics and evolution.
9. About **1.4 million** locations have single-base DNA differences. They are called **SNPs (Single nucleotide polymorphism or 'snips')**. This helps to find chromosomal locations for disease-associated sequences and tracing human history.

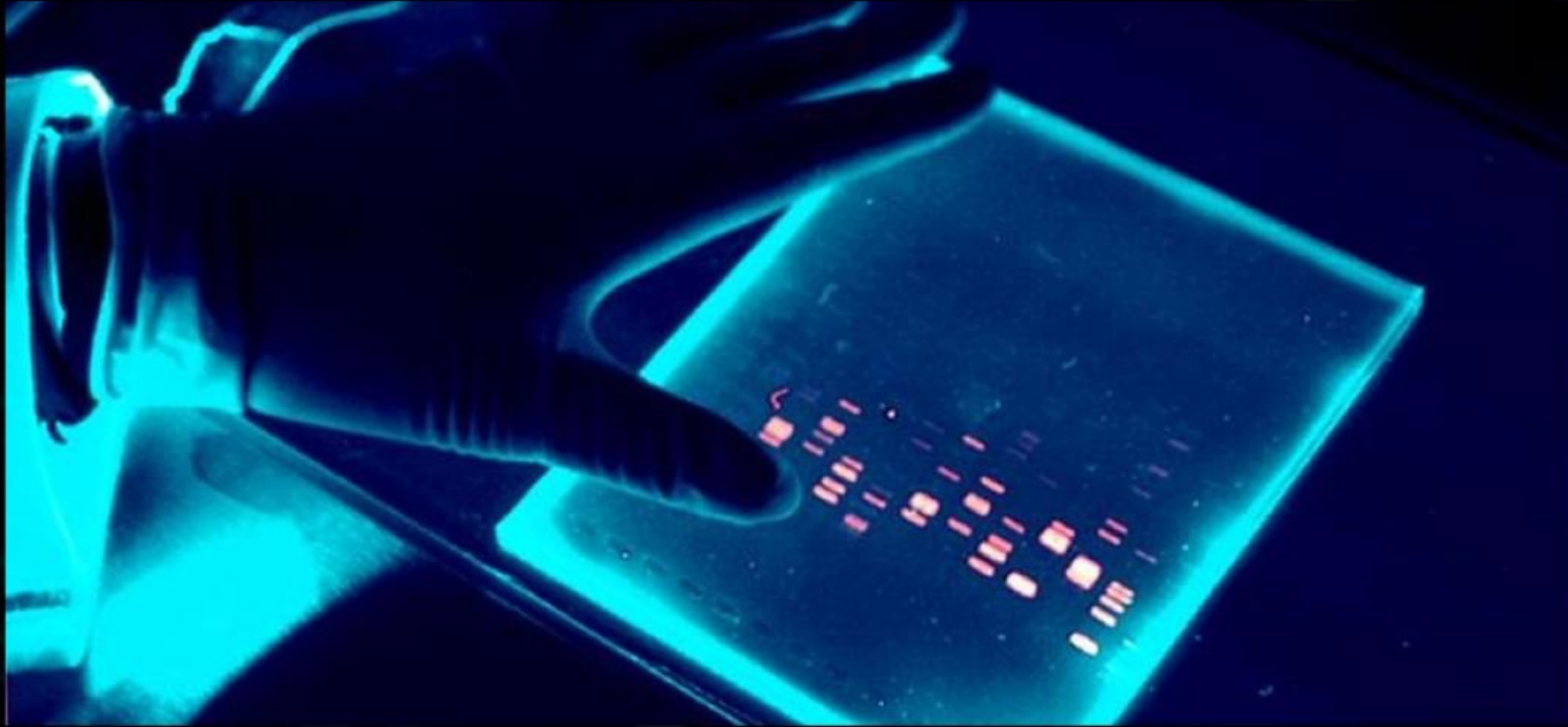
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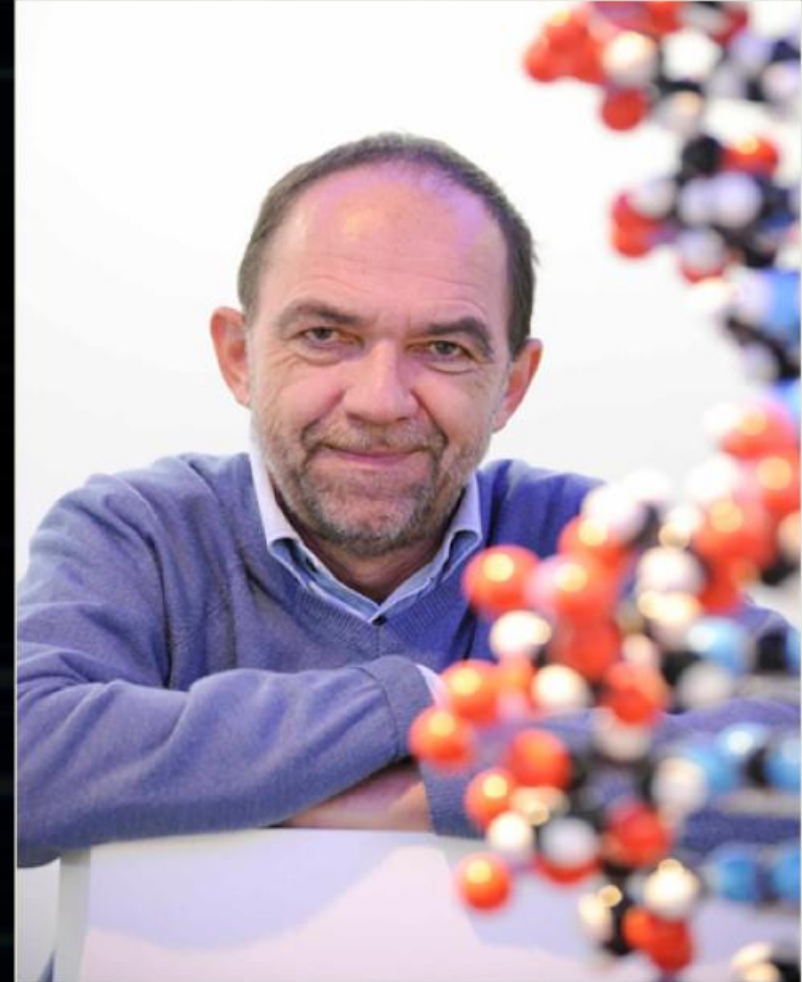


DNA FINGERPRINTING (DNA PROFILING)

DNA FINGERPRINTING (DNA PROFILING)



- It is the technique to identify the similarities and differences of the DNA fragments of 2 individuals.
- Developed by **Alec Jeffreys (1985)**.



Alec Jeffreys

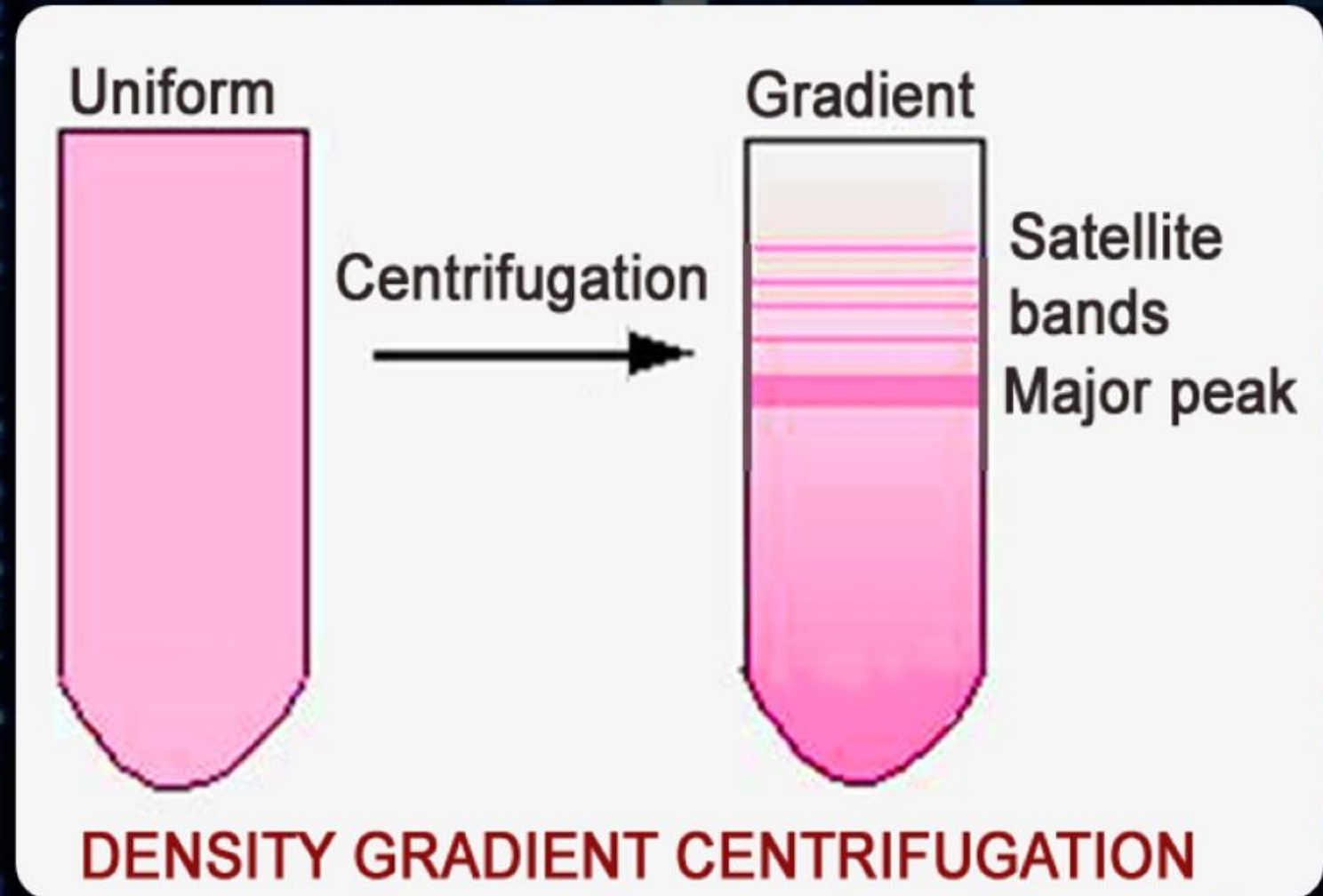
DNA FINGERPRINTING: BASIS

- DNA carries some non-coding **repetitive sequences**.
- Number of repeats is specific from person to person. E.g.



DNA FINGERPRINTING: BASIS

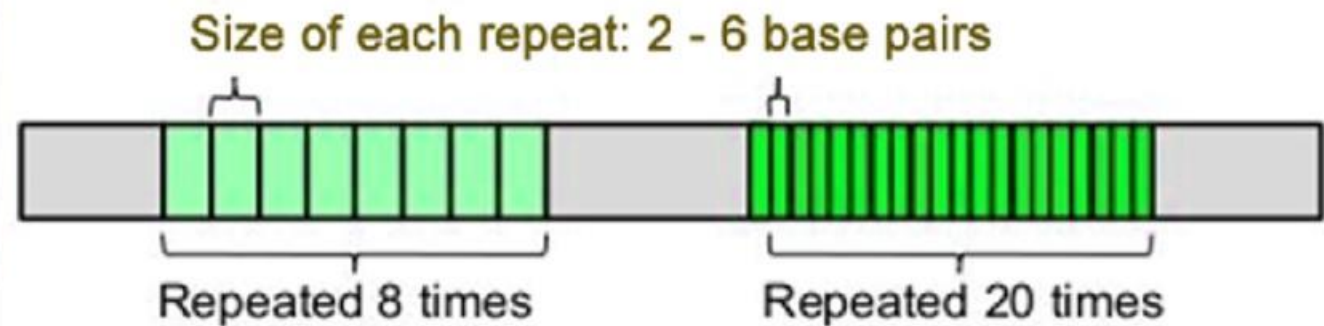
- **Repetitive DNA** can be separated from **bulk genomic DNA** as different peaks during **density gradient centrifugation**.
- The **bulk DNA** forms a **major peak** and the small peaks are called **satellite DNA**.



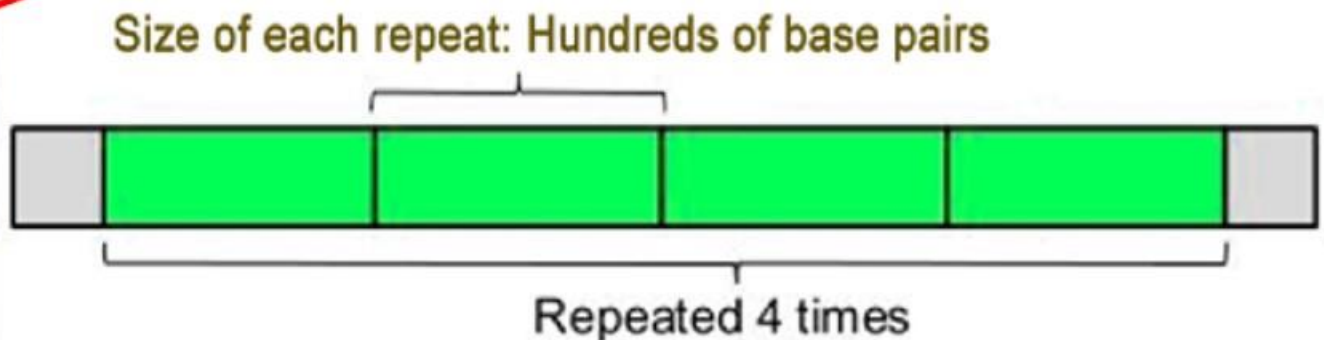
DNA FINGERPRINTING: BASIS

- Satellite DNA is classified as **micro-satellites, mini-satellites** etc. based on base composition (A:T rich or G:C rich), length of segment and number of repetitive units.
- A DNA sequence which is tandemly repeated in many copy numbers is called **variable number tandem repeats (VNTR)**.
- VNTR belongs to **mini-satellite DNA**.

Microsatellite: Short Tandem Repeats (STR)



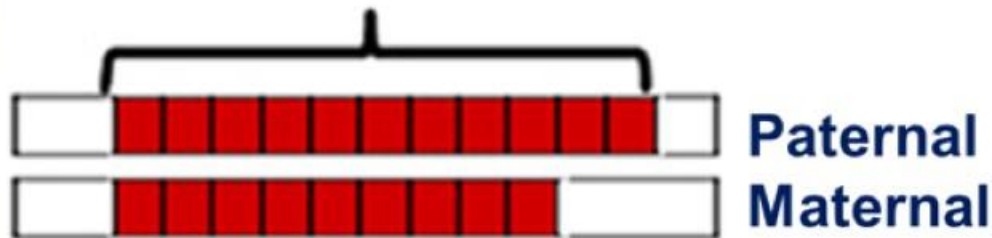
Minisatellite: VNTR



DNA FINGERPRINTING: BASIS

- In a person, copy number varies in each chromosome.
- Two alleles (paternal & maternal) of a chromosome also contain different copy numbers of VNTR.

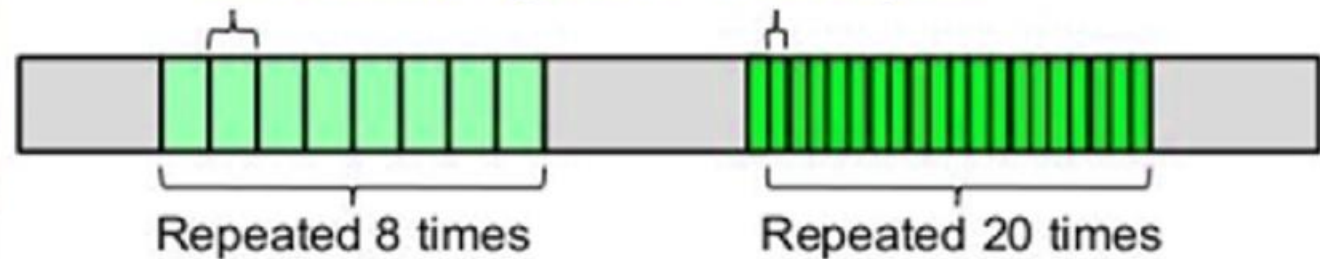
Copy number of VNTR



2 alleles of a chromosome

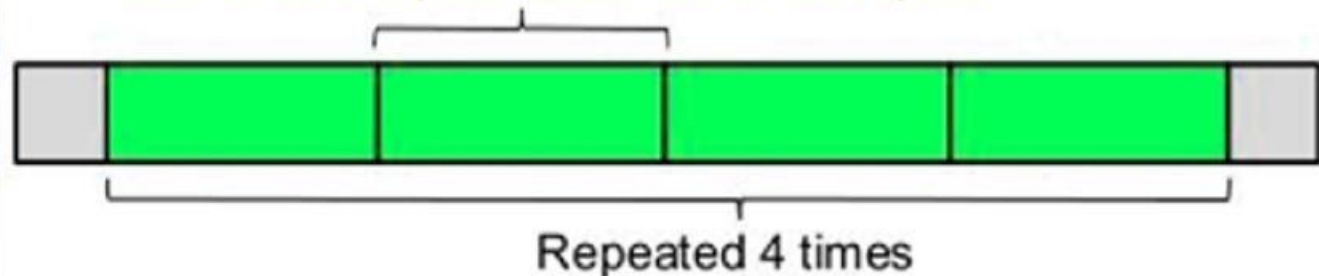
Microsatellite: Short Tandem Repeats (STR)

Size of each repeat: 2 - 6 base pairs



Minisatellite: VNTR

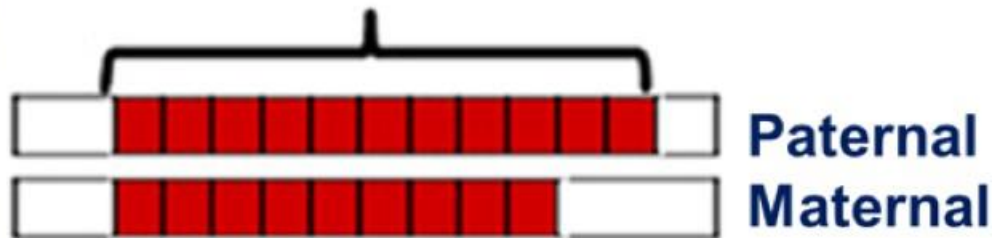
Size of each repeat: Hundreds of base pairs



DNA FINGERPRINTING: BASIS

- VNTR is specific from person to person.
- The size of VNTR varies from **0.1 to 20 kb (kilobase)**.

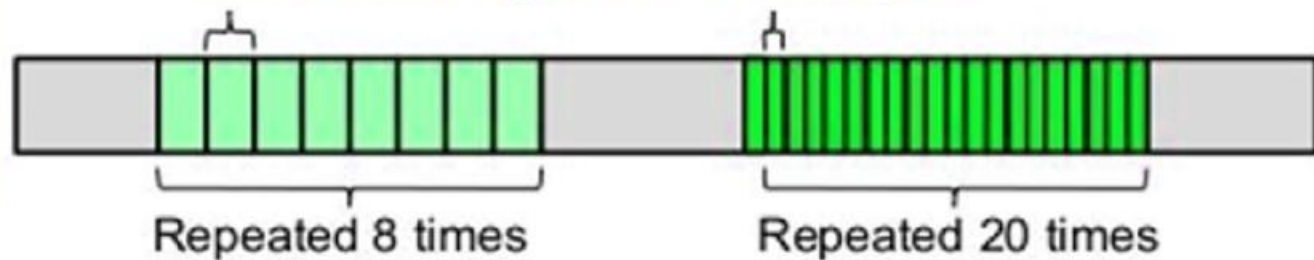
Copy number of VNTR



2 alleles of a chromosome

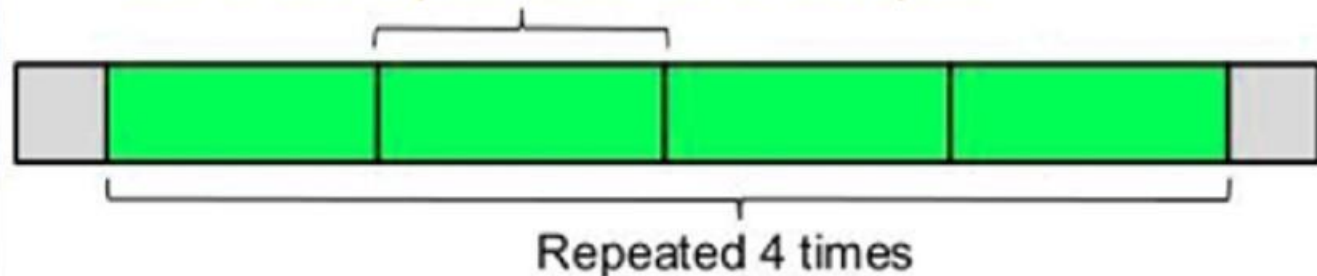
Microsatellite: Short Tandem Repeats (STR)

Size of each repeat: 2 - 6 base pairs



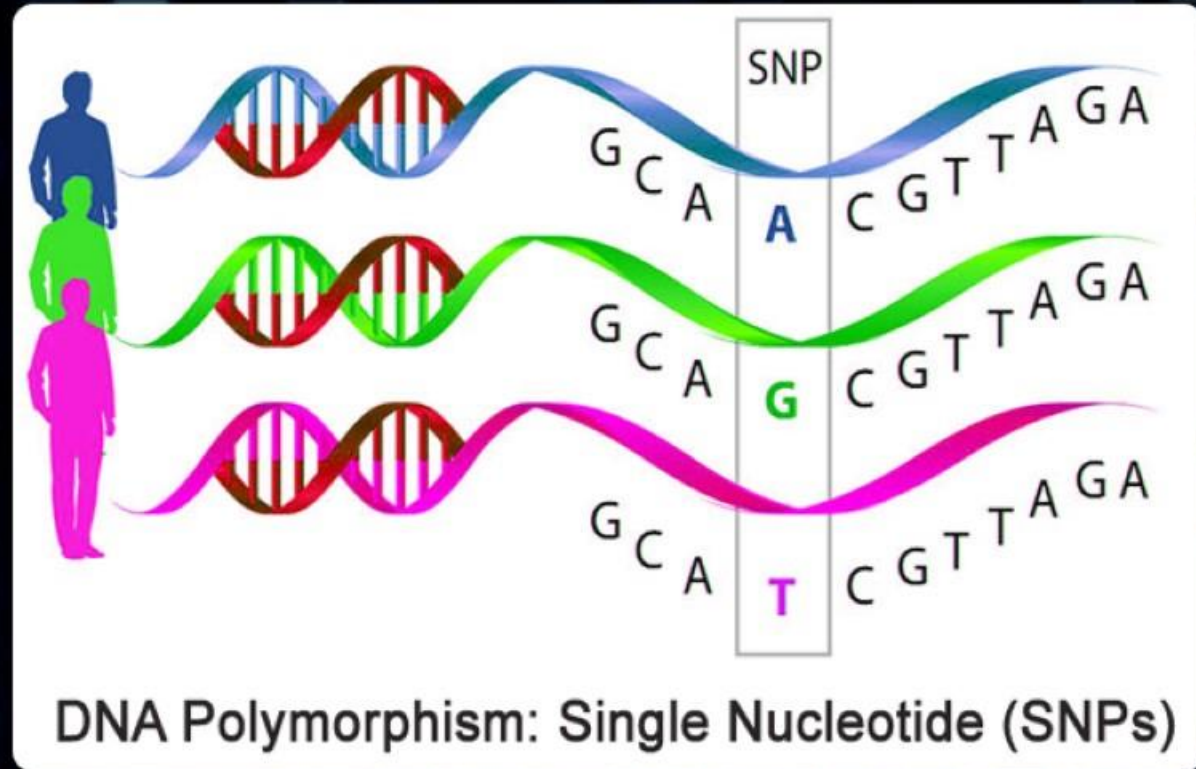
Minisatellite: VNTR

Size of each repeat: Hundreds of base pairs



DNA FINGERPRINTING: BASIS

- Any difference in the nucleotide sequence (inheritable mutation) observed in a population is called **DNA polymorphism** (variation at genetic level).
- **Polymorphism is higher in non-coding DNA** sequence because mutations in these sequences may not affect an individual's reproductive ability.
- These mutations accumulate generation to generation causing polymorphism.



Polymorphisms have great role in evolution & speciation.

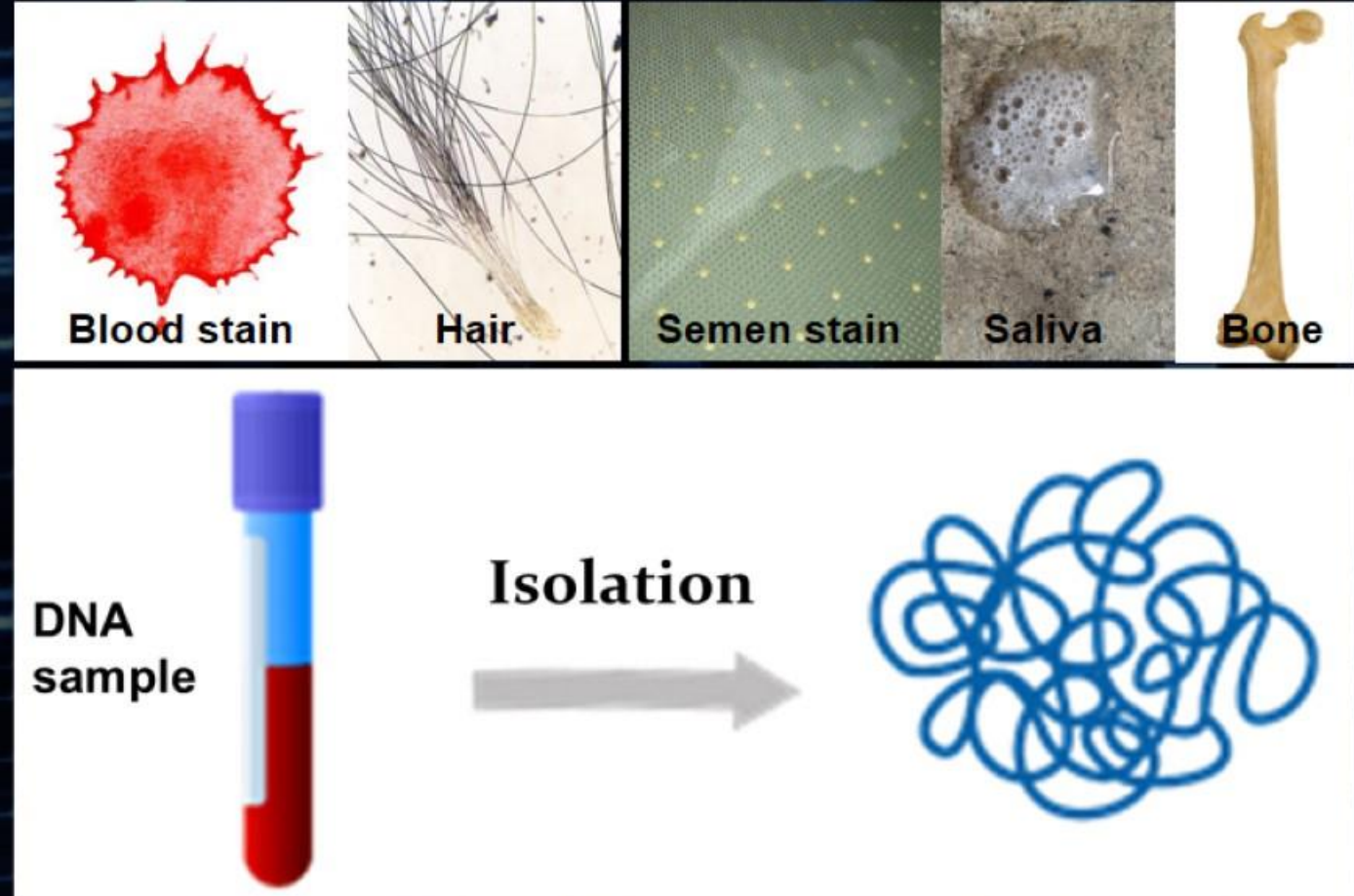
DNA FINGERPRINTING: STEPS

SOUTHERN BLOTTING TECHNIQUE

1

Isolation of DNA

(from any cells or blood stains, semen stains, saliva, hair roots, bone, skin etc.).



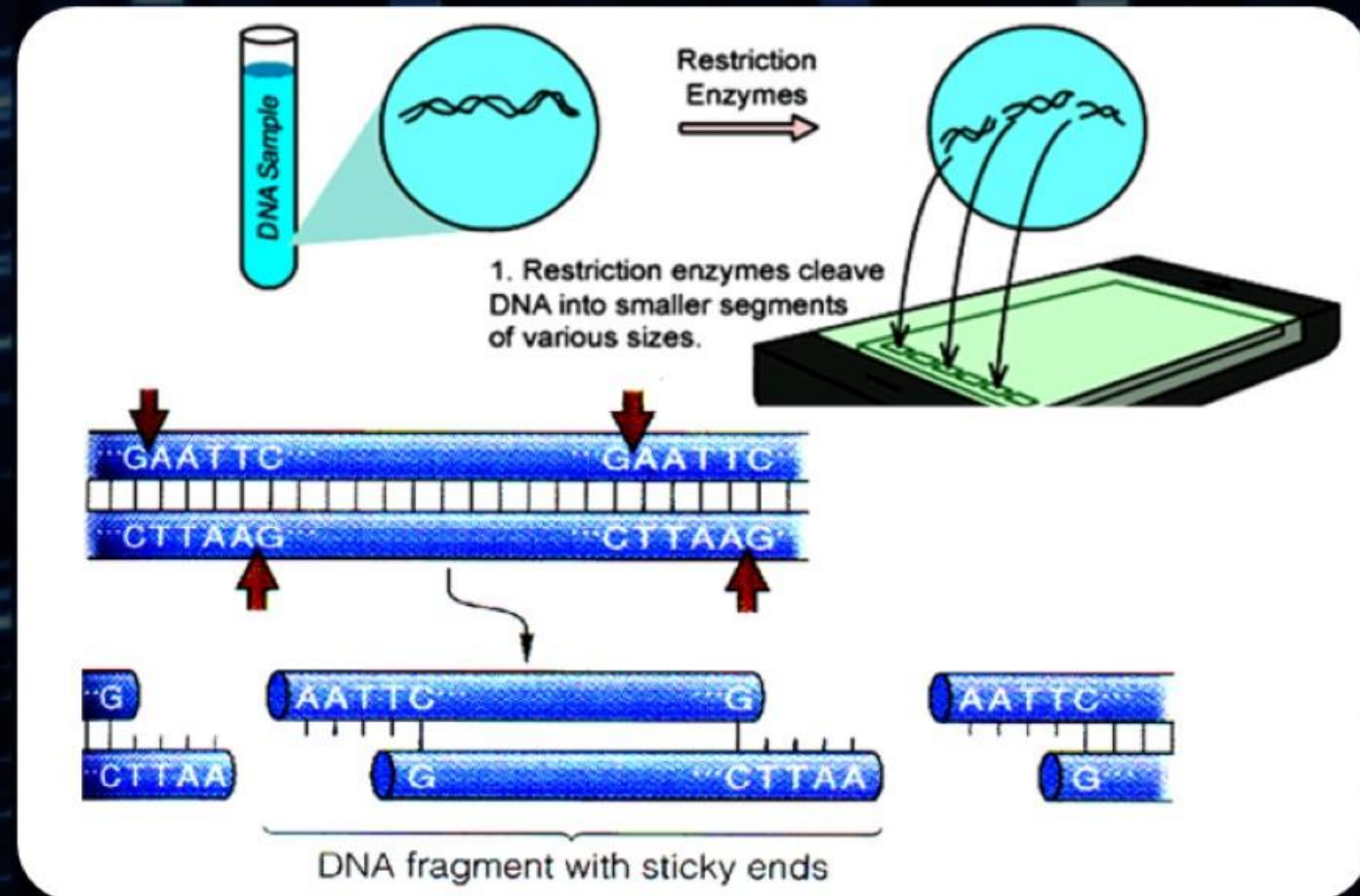
DNA FINGERPRINTING: STEPS

SOUTHERN BLOTTING TECHNIQUE

2

Digestion of DNA

By restriction endonucleases.



DNA FINGERPRINTING: STEPS

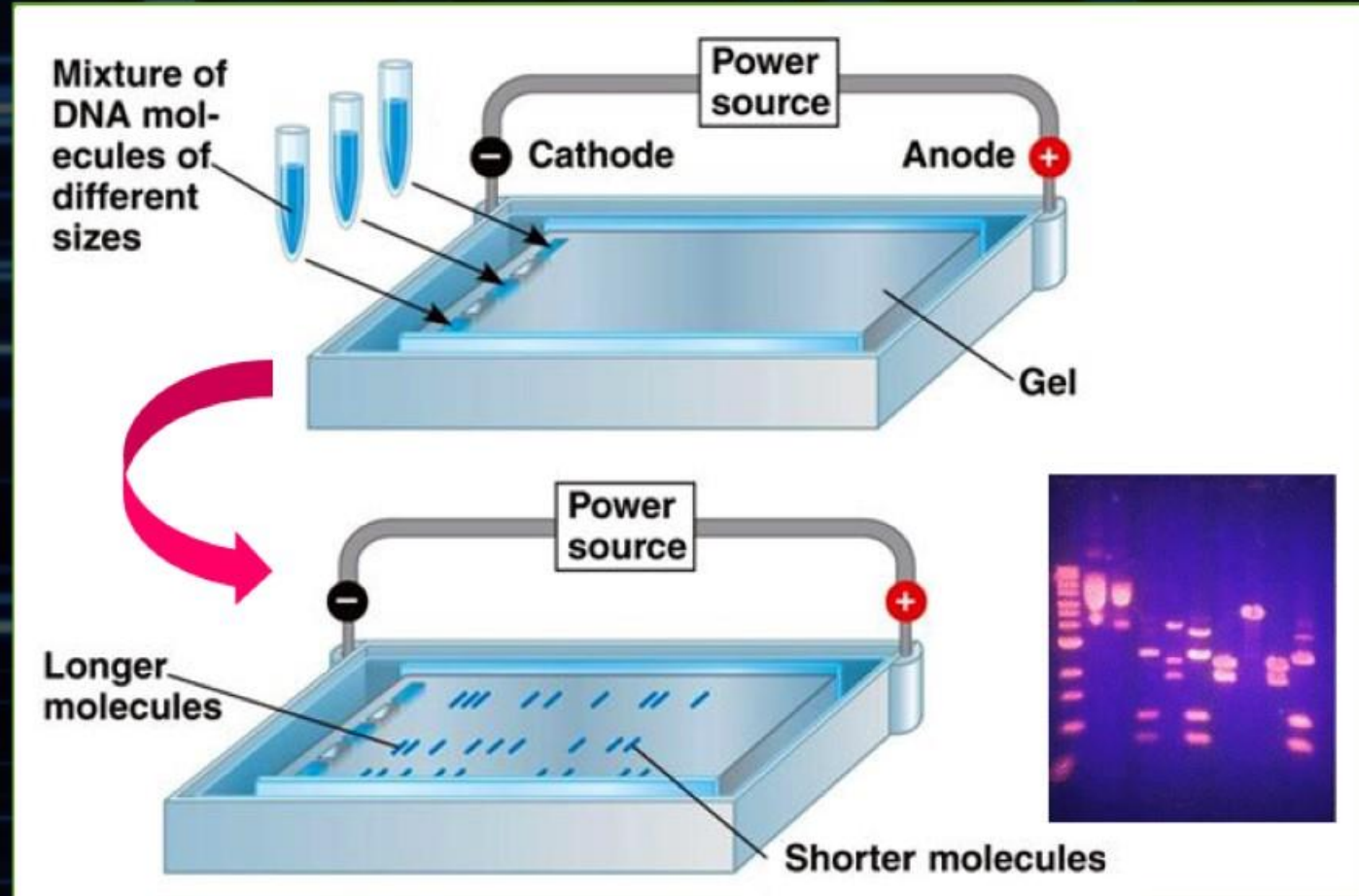
SOUTHERN BLOTTING TECHNIQUE

3

Separation of DNA fragments

By gel electrophoresis.

Then treat the DNA fragments with alkali solution (NaOH) to denature DNA bonds in the gel into single strands.



DNA FINGERPRINTING: STEPS

SOUTHERN BLOTTING TECHNIQUE

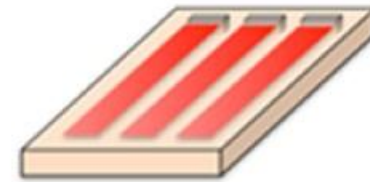
4

Transferring (blotting) of DNA fragments to synthetic membranes such as nitrocellulose or nylon.

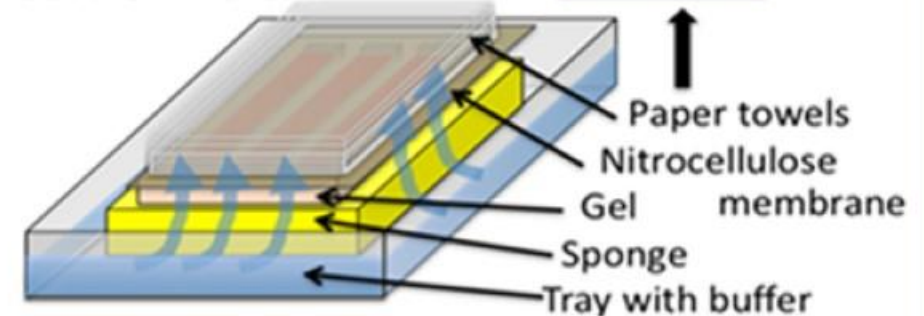


Nitrocellulose membrane

Electrophoresis



Southern Transfer



Paper towels

Nitrocellulose membrane

Gel

Sponge

Tray with buffer

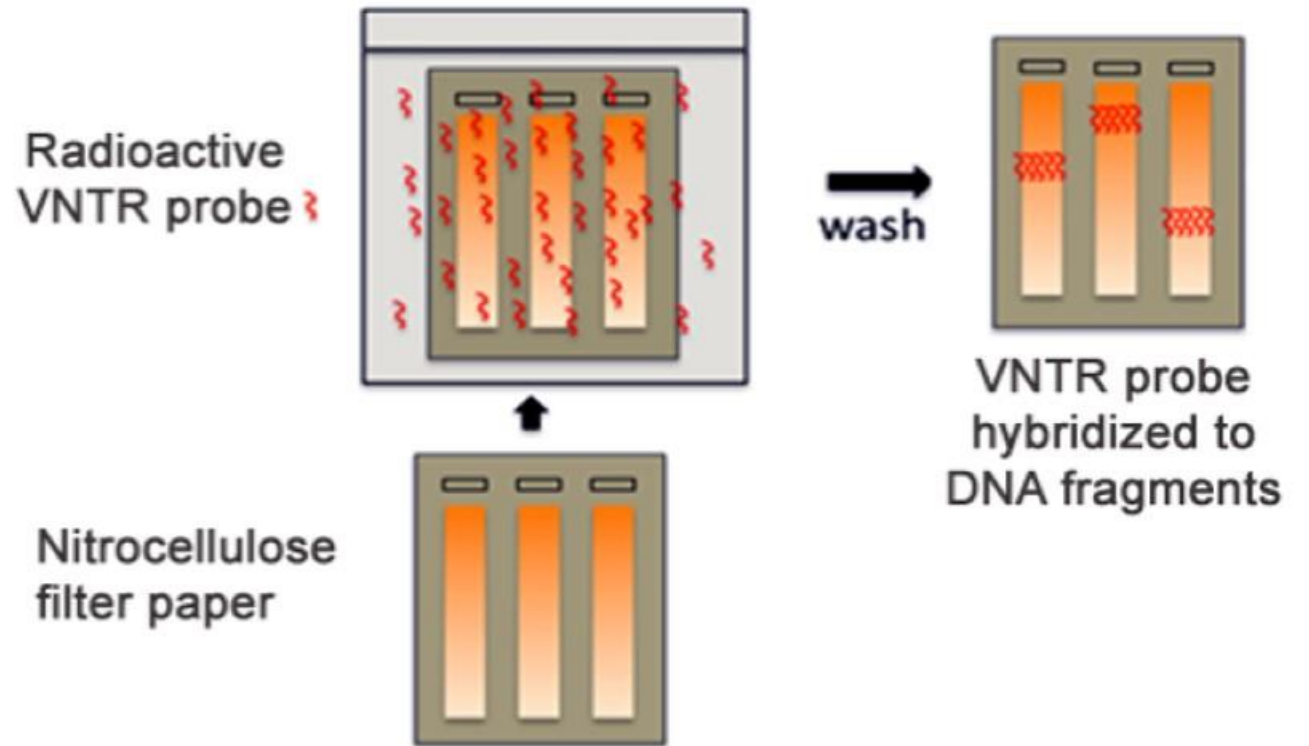
DNA FINGERPRINTING: STEPS

SOUTHERN BLOTTING TECHNIQUE

5

Hybridization using radioactive labelled VNTR probe.

*(Place the nitrocellulose filter paper in a solution of radioactive labeled single stranded DNA probe. DNA probe binds with the complimentary sequences of the DNA fragment on the membrane to form a **hybridized DNA**).*



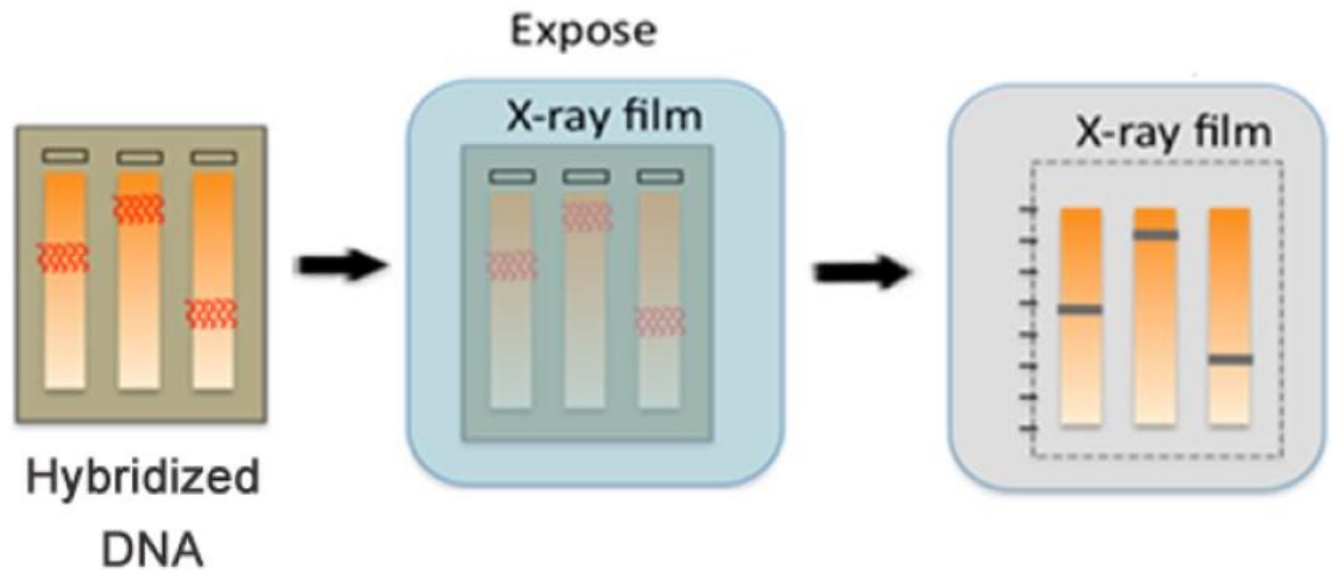
DNA FINGERPRINTING: STEPS

SOUTHERN BLOTTING TECHNIQUE

6

Detection of hybridized DNA by **Autoradiography**.

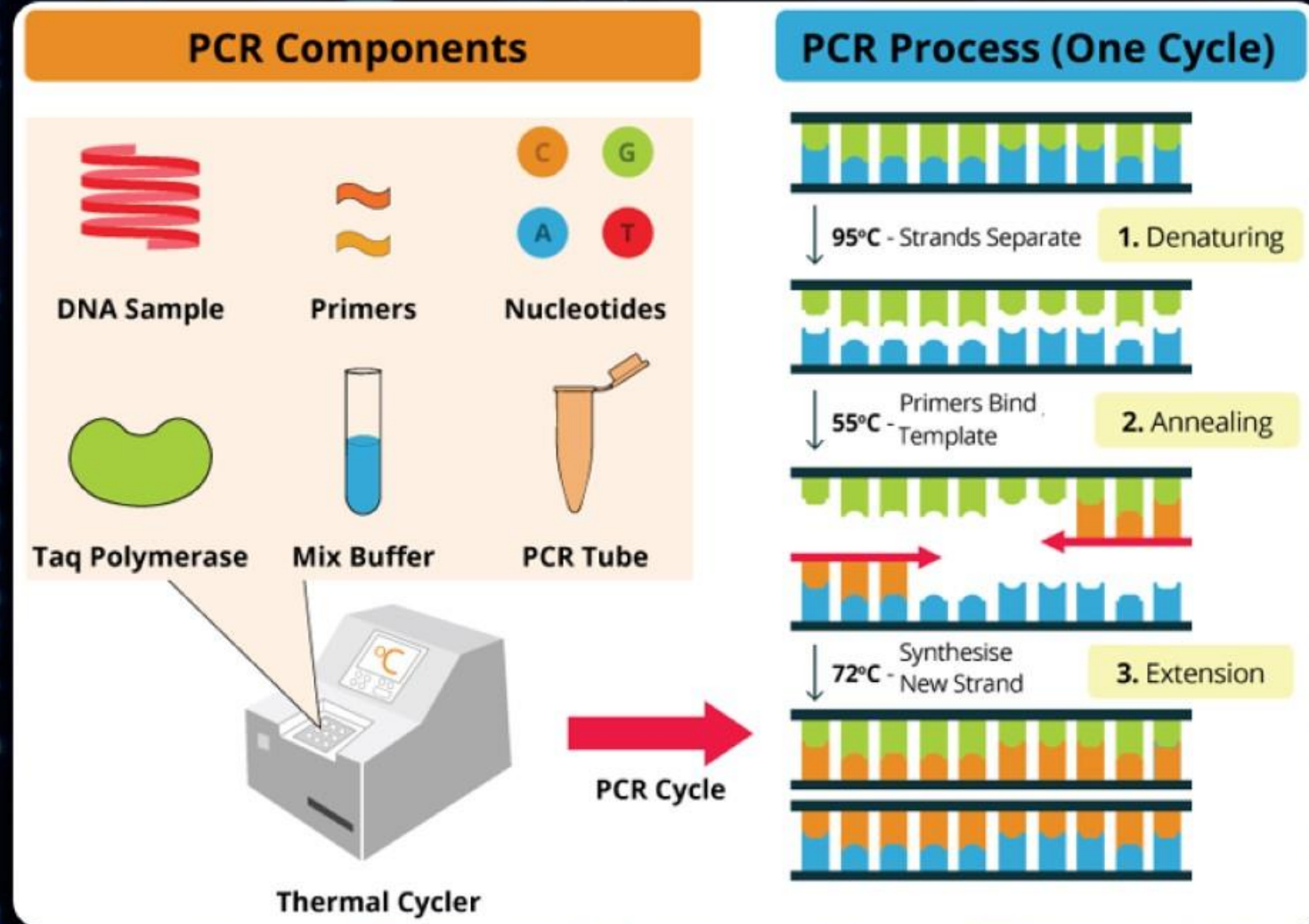
(Hybridized DNA is photographed on to an X-ray film).



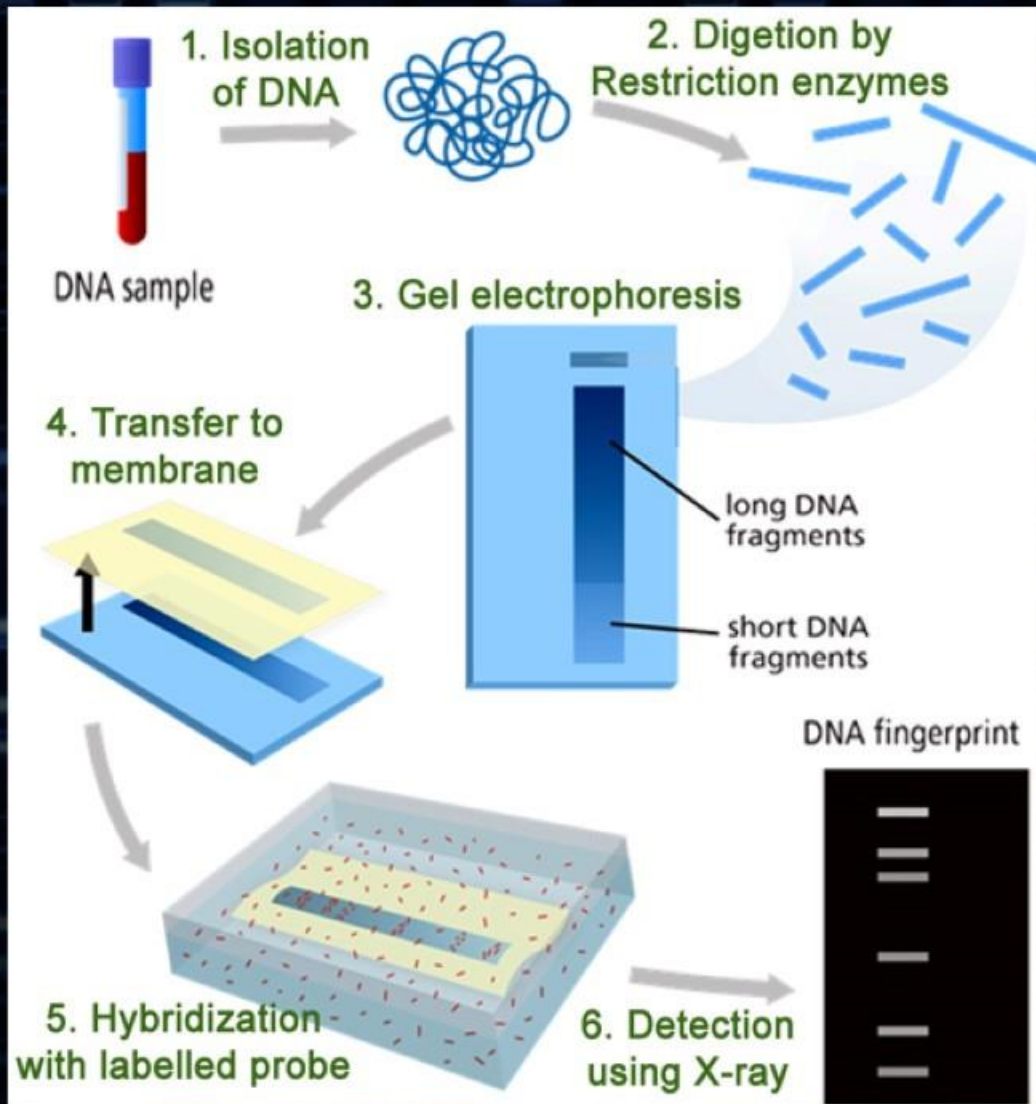
The autoradiogram gives an image in the form of dark & light bands. It is called **DNA fingerprint**.

DNA FINGERPRINTING (DNA PROFILING)

- DNA fingerprint differs in everyone except in **monozygotic (identical) twins**.
- The sensitivity of the technique can be increased by use of **polymerase chain reaction (PCR)**. Therefore, DNA from a single cell is enough for DNA fingerprinting.



DNA FINGERPRINTING: AT A GLANCE



1. Isolation of DNA.

2. Digestion of DNA by restriction endonucleases.

3. Separation of DNA fragments by gel electrophoresis.

4. Transferring DNA fragments to synthetic membranes.

5. Hybridization using radioactive labelled VNTR probe.

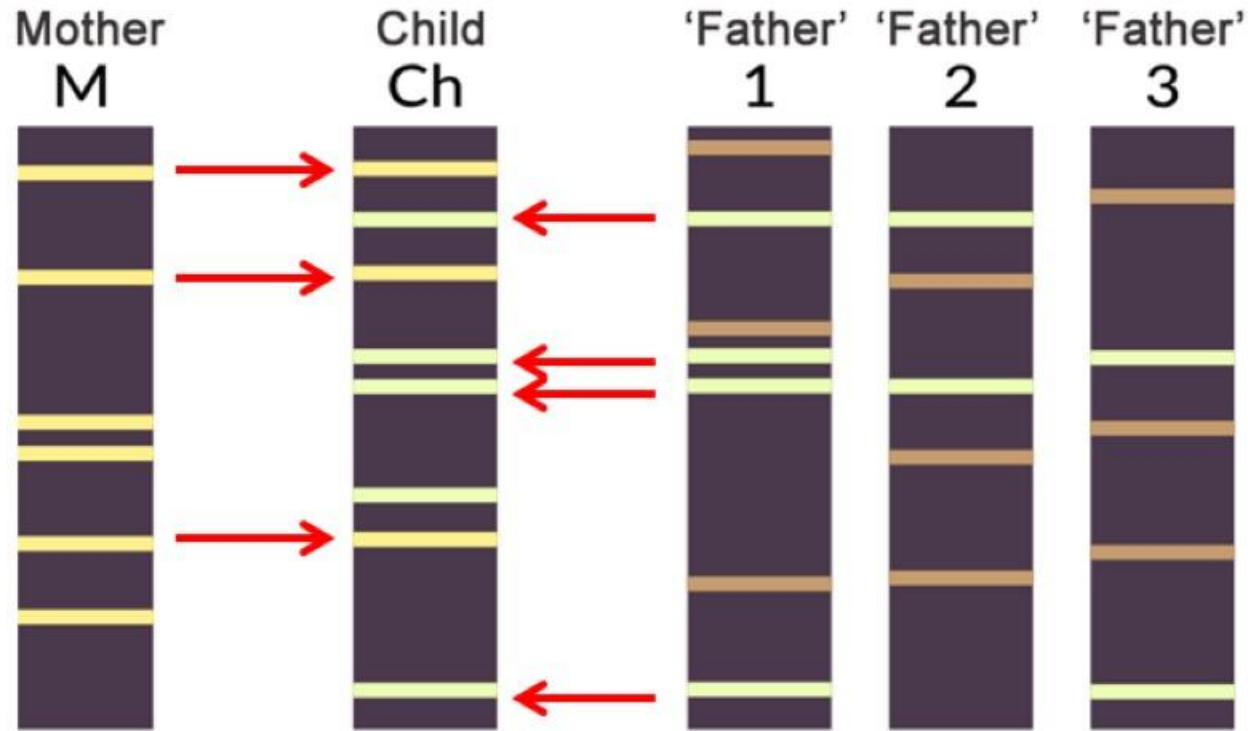
6. Detection of hybridized DNA by autoradiography.

DNA FINGERPRINTING: APPLICATIONS



- Forensic tool to solve paternity, rape, murder etc.
- For the diagnosis of genetic diseases.
- To determine phylogenetic status of animals.
- To determine population and genetic diversities.

DNA FINGERPRINTING: APPLICATIONS



Solving paternity using DNA fingerprinting

Here, person 1 is likely to be the father of child

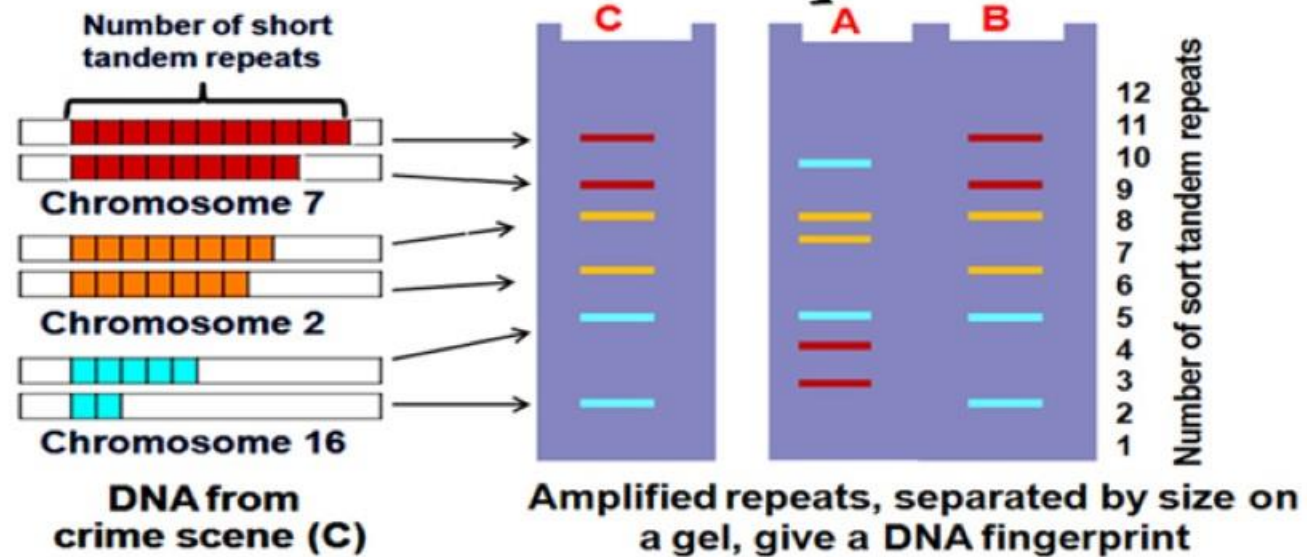
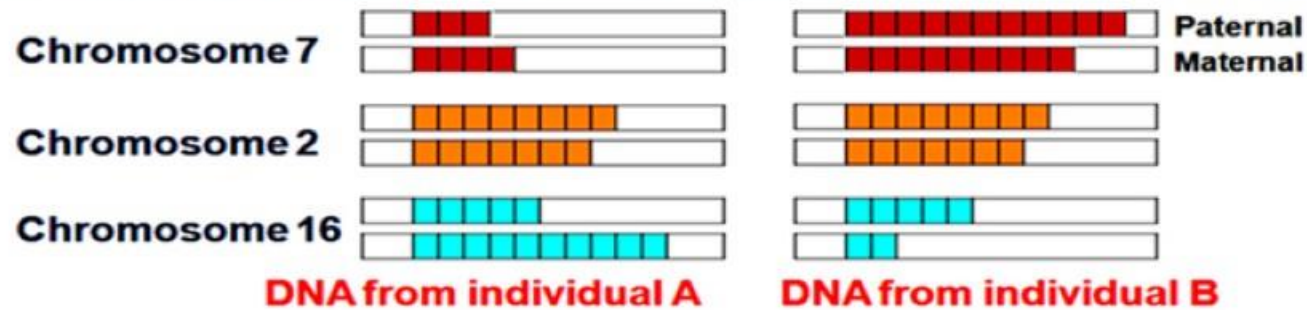
- Forensic tool to solve paternity, rape, murder etc.

- For the diagnosis of genetic diseases.

- To determine phylogenetic status of animals.

- To determine population and genetic diversities.

DNA FINGERPRINTING: APPLICATIONS



■ Forensic tool to solve paternity, rape, murder etc.

■ For the diagnosis of genetic diseases.

■ To determine phylogenetic status of animals.

■ To determine population and genetic diversities.

Solving a crime with the help of DNA fingerprinting