BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,

Tamil Nadu, India

Programme: M.Sc., Biomedical Science

Course Code: 18BMS59C17

Course Title: Immune & Molecular Diagnostics

Unit-IV

Basic Molecular Methods

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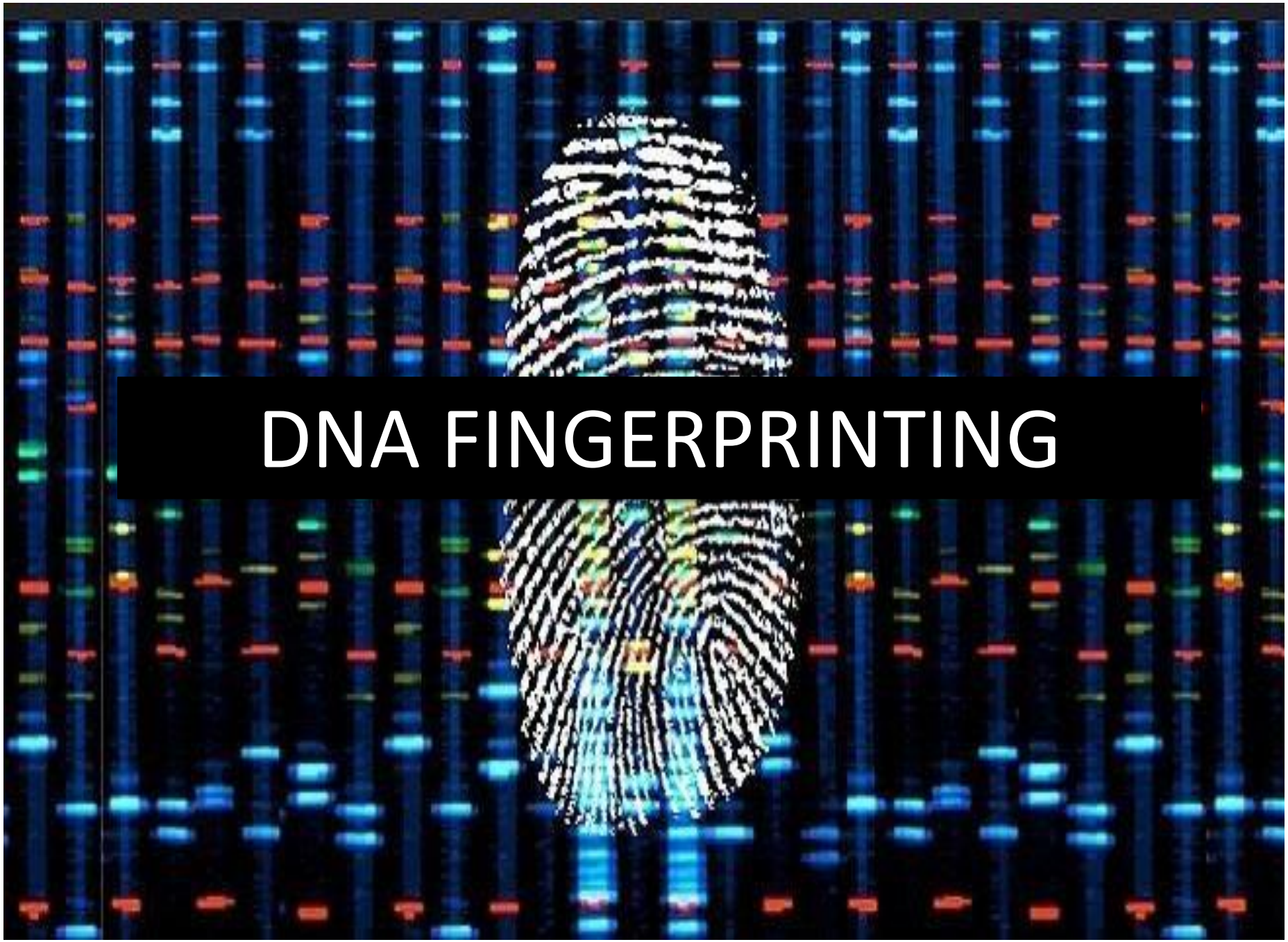
Guest Faculty

Department of Biomedical Science

Unit IV:

Basic Molecular Methods: Types of mutations- PCR based mutation detection methods- for known & unknown mutations- ASP, ASOP, TTGE, DGGE, Heteroduplexing method, SSCP & sequencing- Discuss: Primer designing for PCR- Collection, processing and storage of sample- RNA extraction- cDNA preparation, RT-PCR- Principle, methods & Applications- Real time PCR- Principle, methods & Applications. Types of dyes (SYBR Green) and probes (Taq-Man). DNA finger printing- micro (STRs) and minisatellites (VNTRs)- principle and applications.

PRESENTATION: 3


DNA FINGERPRINTING

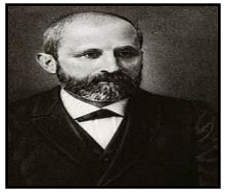
DNA FINGERPRINTING

- It is a technique used to identify individuals based on the unique patterns in their DNA.
- It exploits the variations in the genetic sequences of the individuals, making it a powerful tool in forensic science, paternity testing and genetic research.

TIMELINE

1869

Friedrich Miescher

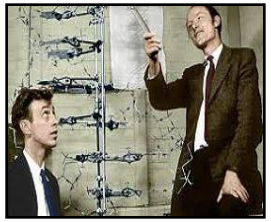


1935

Andrei Nikolaevitch

1953

Watson & Crick



1966

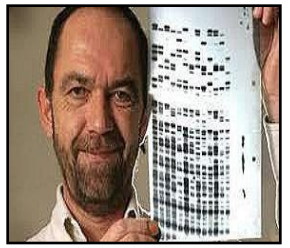
Nirenberg & Mathaei

1980

American researchers

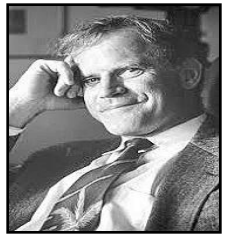
1984

Dr.Alec Jeffreys



1985

Dr.Kary Mullis



Steps in DNA FINGERPRINTING

1. Sample collection:

DNA can be extracted from various sources such as blood, saliva, hair, or skin cells.

2. DNA extraction:

The DNA is isolated from the cells in the sample.

3. DNA fragmentation:

The DNA is cut into smaller fragments using restriction enzymes, which recognize specific sequences in the DNA.

4. Gel electrophoresis:

The DNA fragments are separated by size using gel electrophoresis. Smaller fragments move faster through the gel than the larger ones.

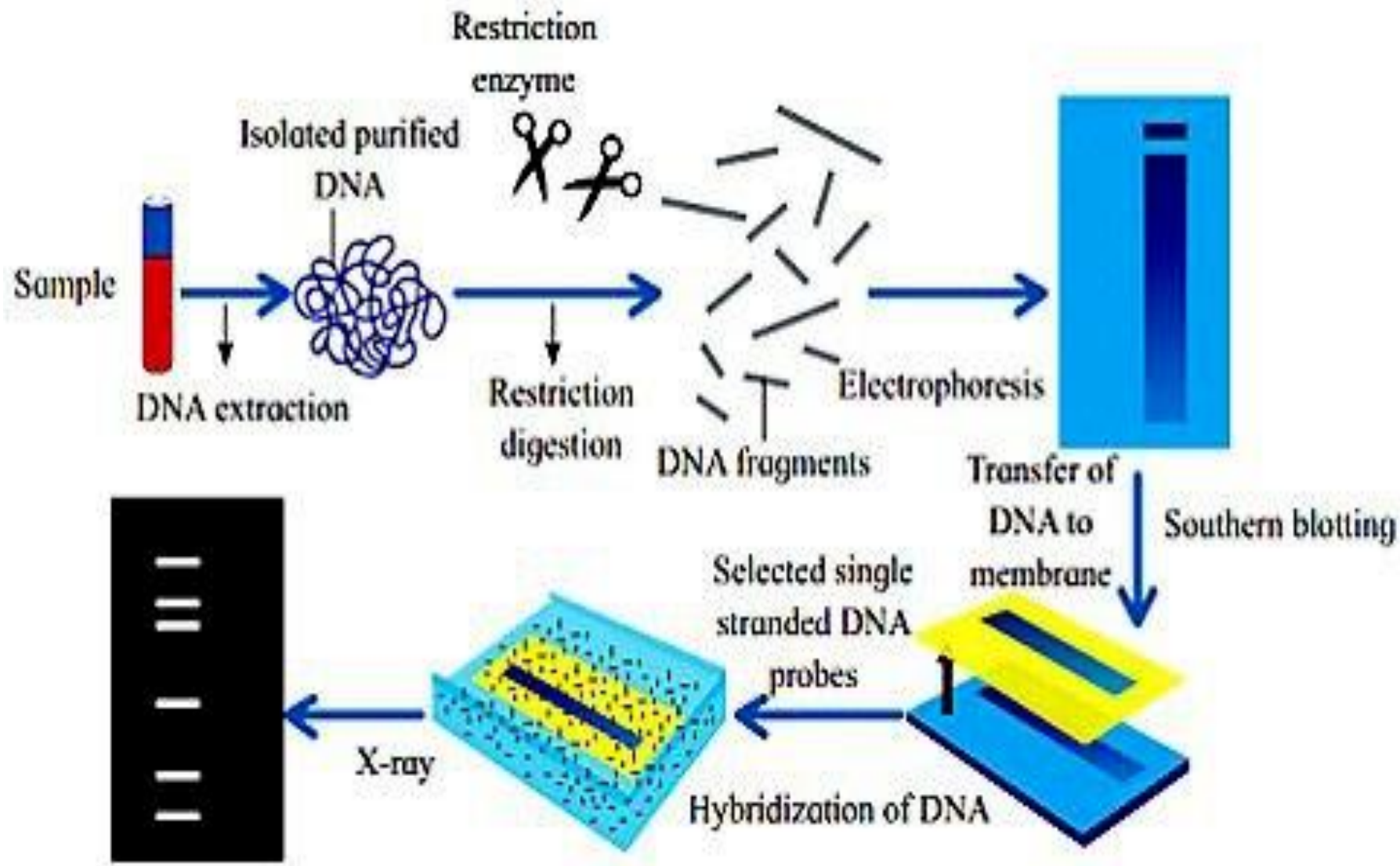
5. Southern blotting:

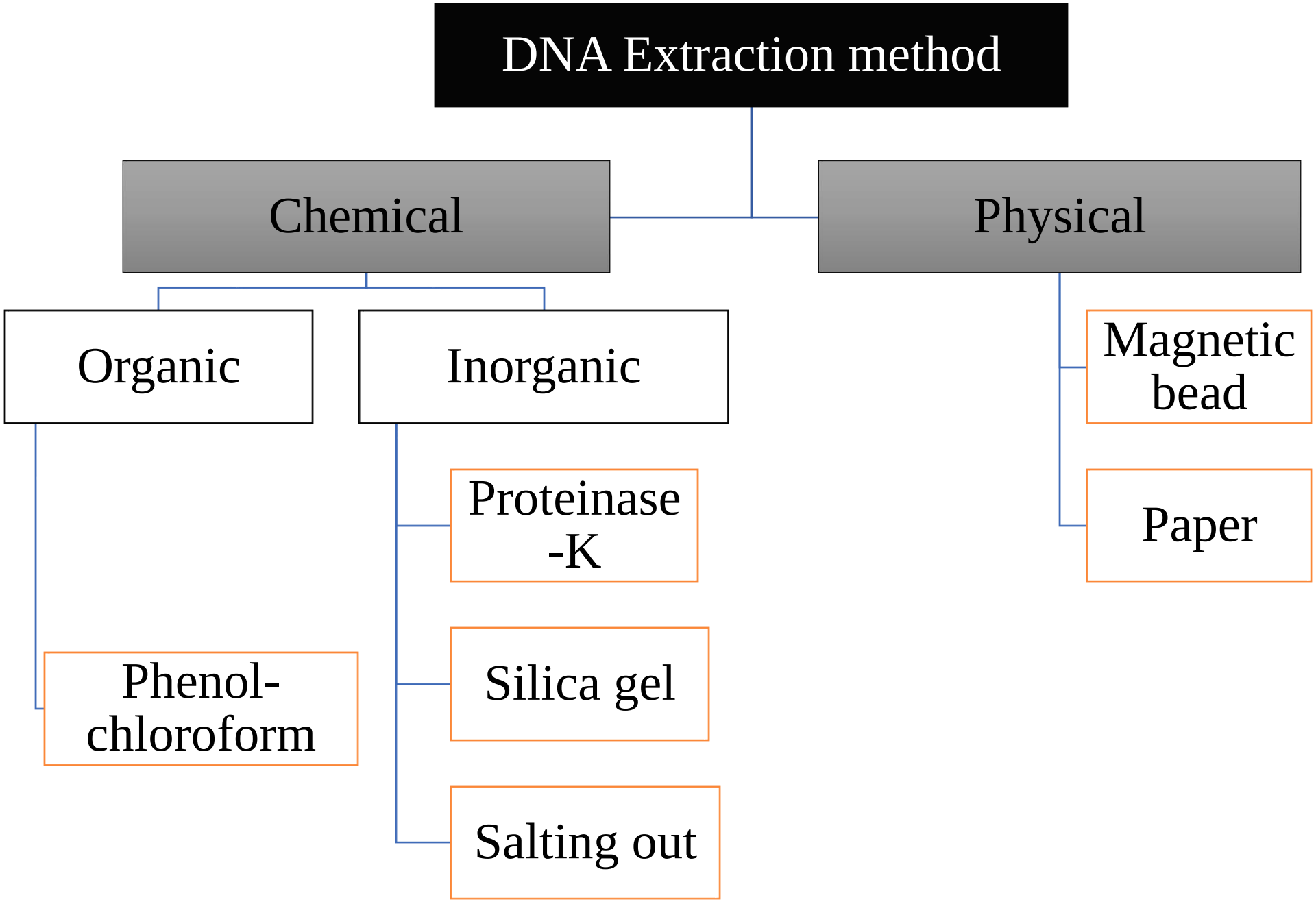
The DNA fragments are transferred to a membrane and hybridized with a labeled DNA probe that binds to specific DNA sequences.

6. Visualization:

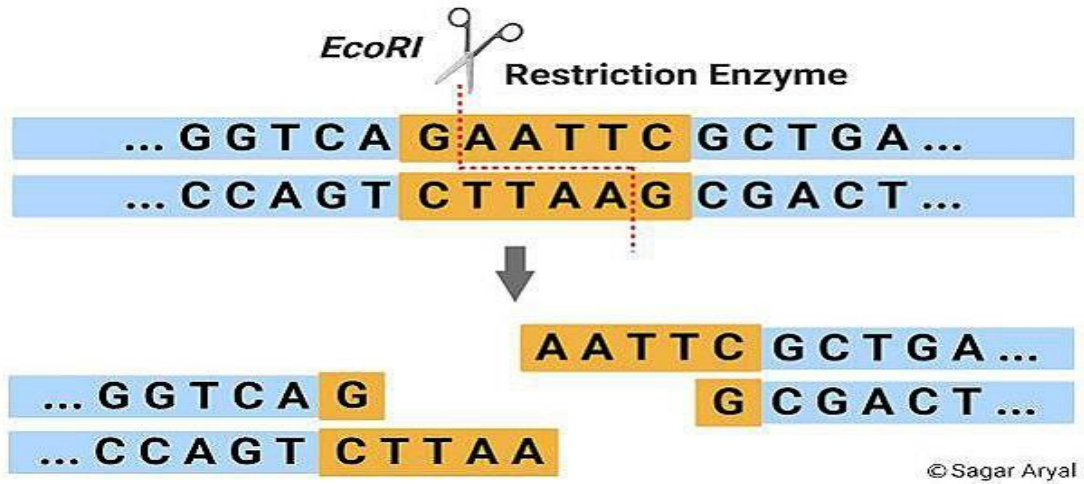
The bound probes were detected, producing a pattern of bands that represent the DNA fragments.

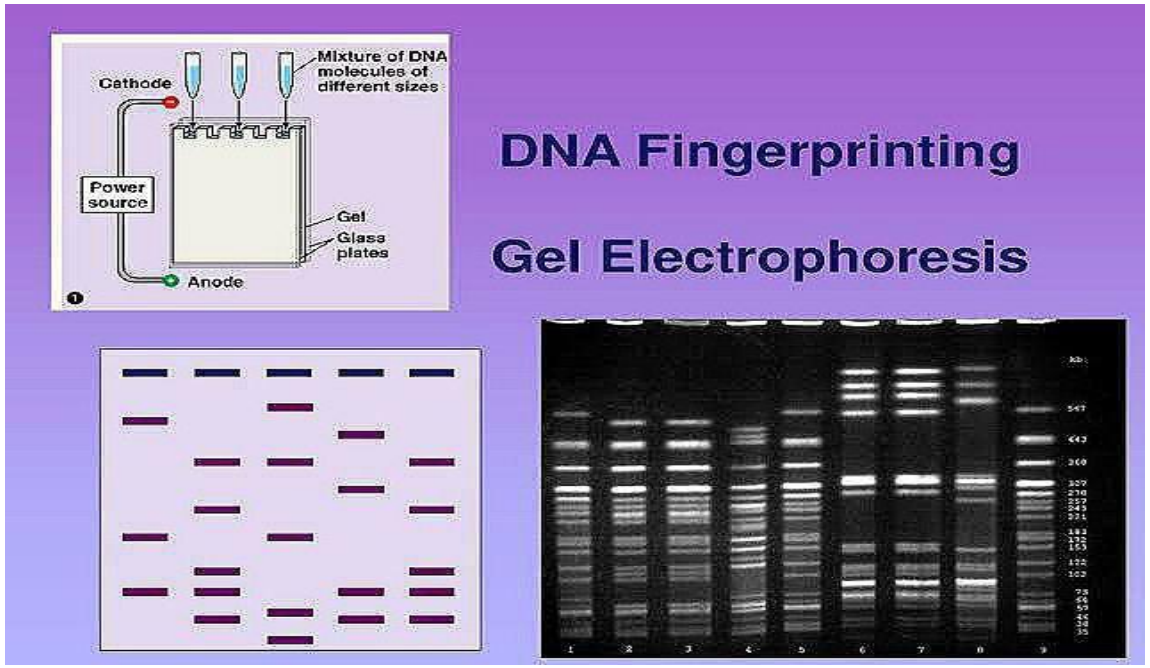
PROTOCOL


Restriction enzyme
Isolated purified DNA
Sample
DNA extraction
Restriction digestion
DNA fragments
Electrophoresis
Transfer of DNA to membrane
Southern blotting
Selected single stranded DNA probes
Hybridization of DNA
X-ray

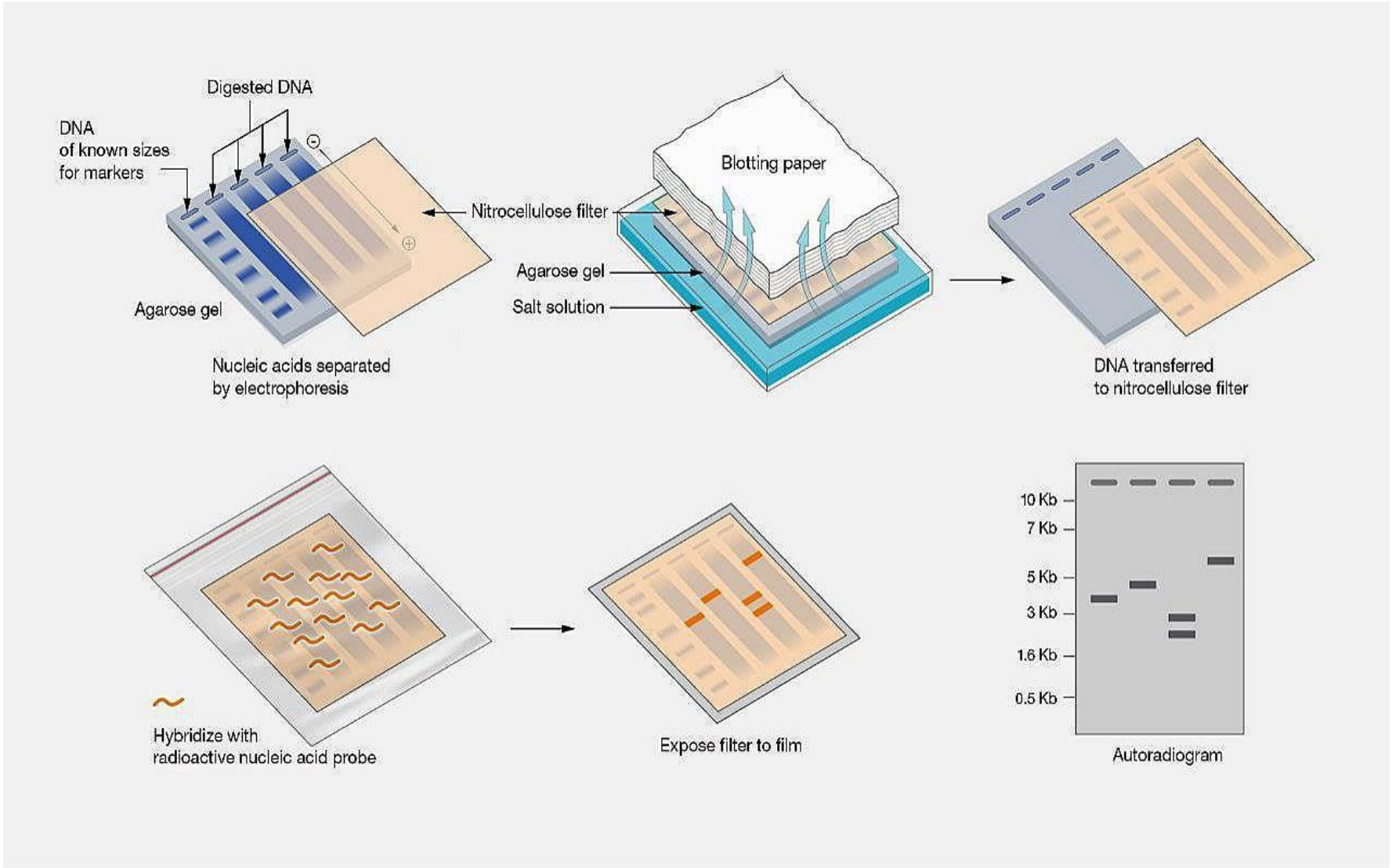

DNA Extraction method
Chemical
Physical
Organic
Inorganic
Magnetic bead
Paper
Proteinase -K
Phenol-chloroform
Silica gel
Salting out

RESTRICTION DIGESTION

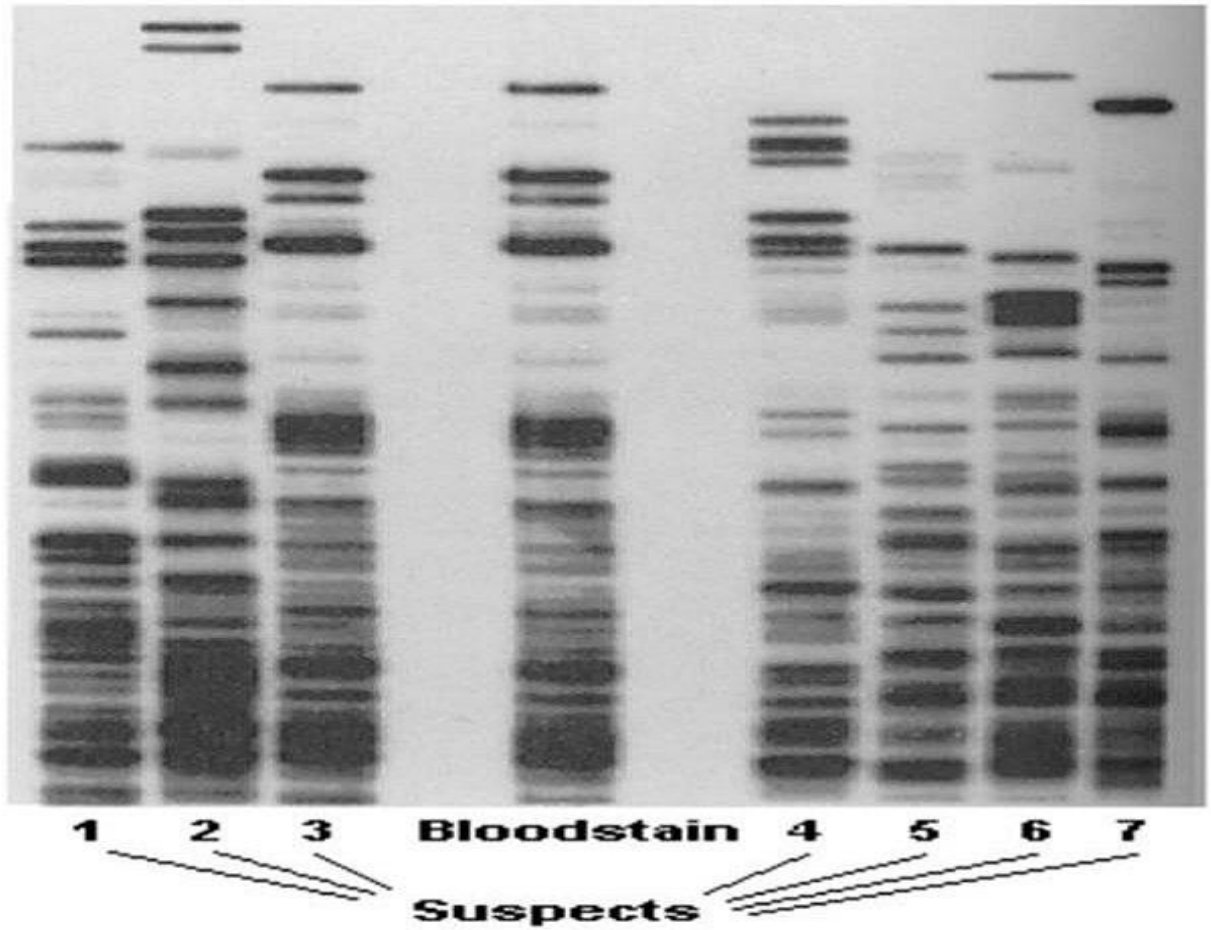




SOUTHERN BLOTTING



INTERPRETATION



TECHNIQUE

1)RFLP

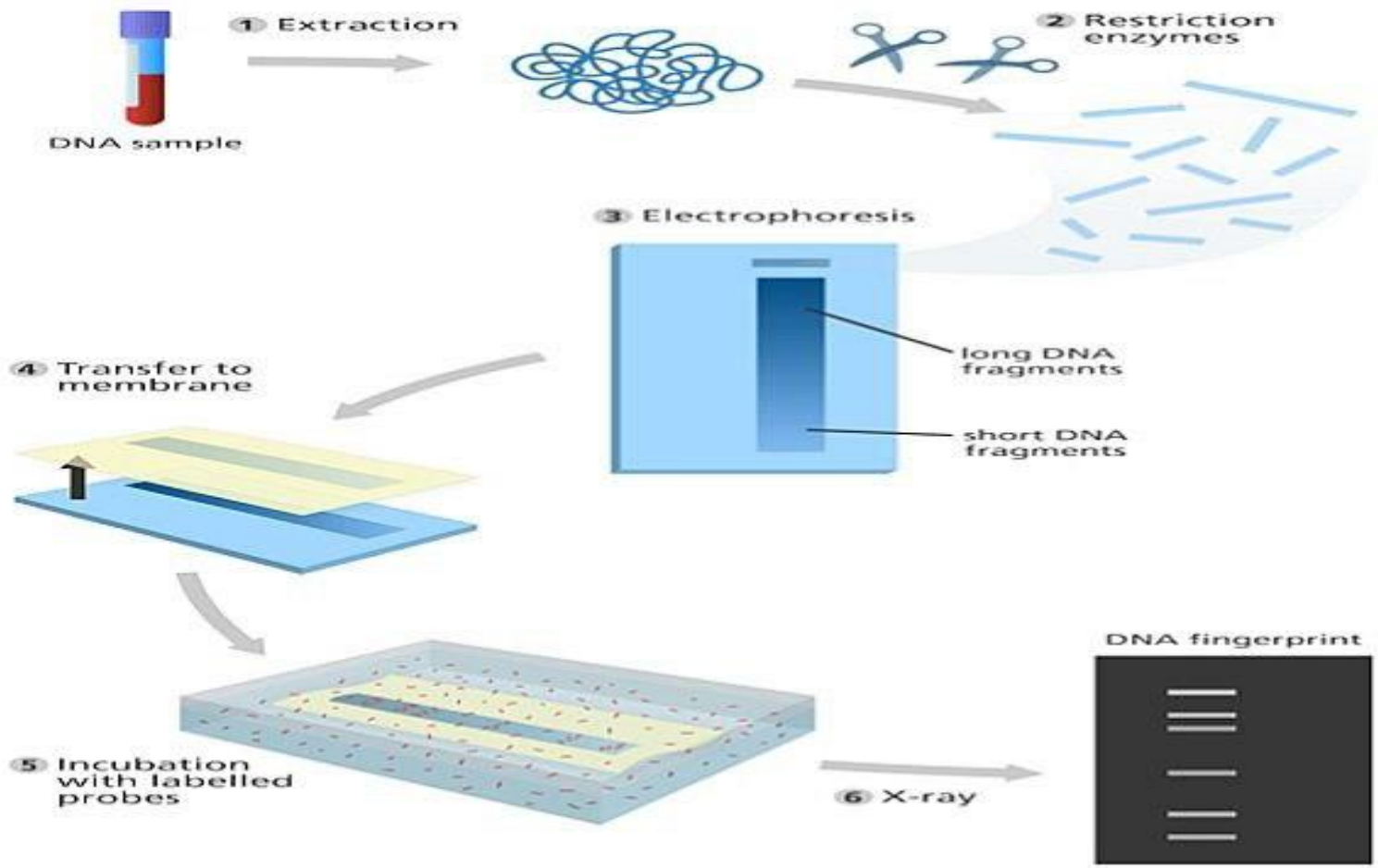


Fig 2: Showing RFLP analysis with the help of X-ray film

2)PCR

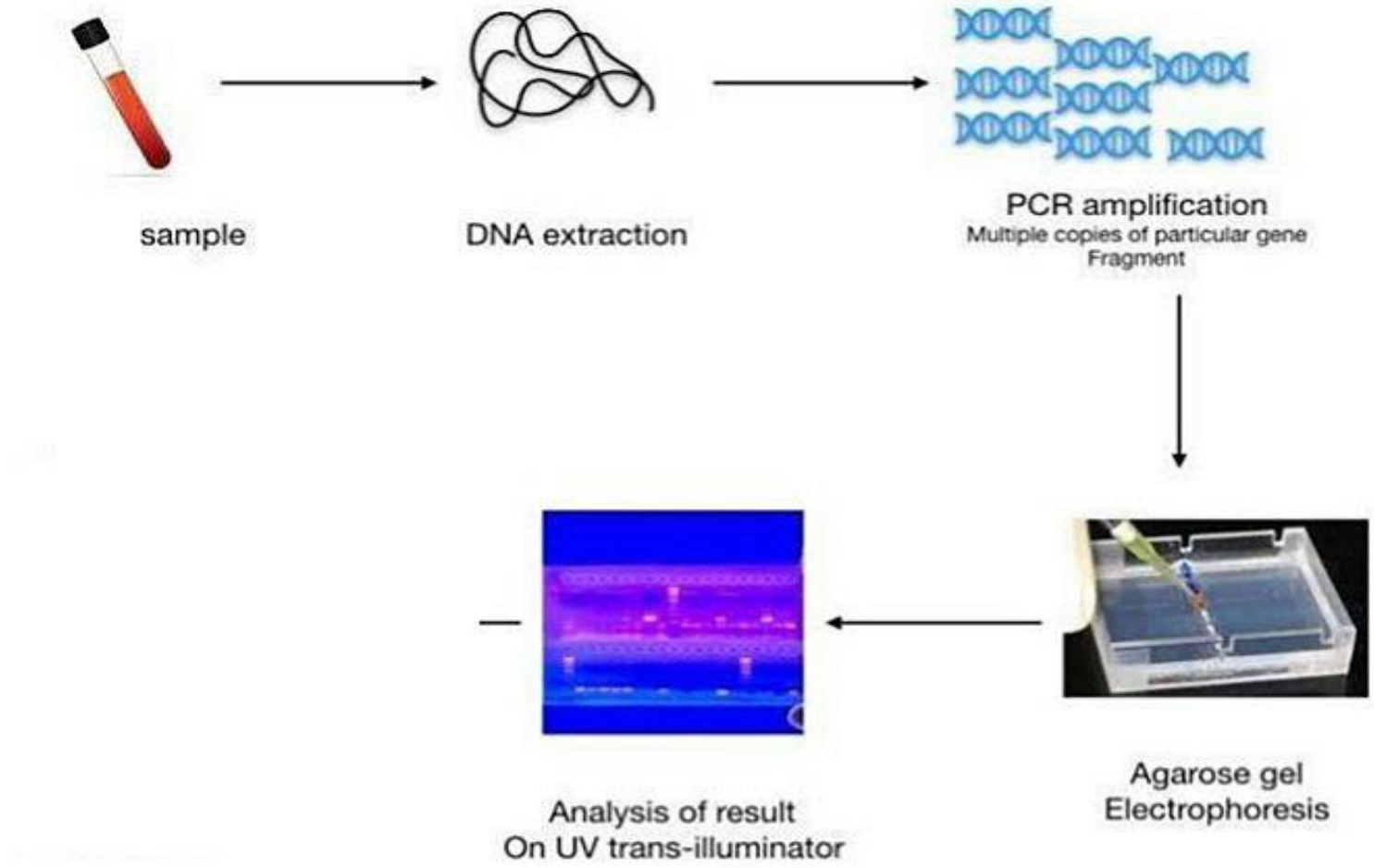


Fig 3: Showing PCR technique for DNA fingerprinting with Agarose gel electrophoresis.

3)RT-PCR

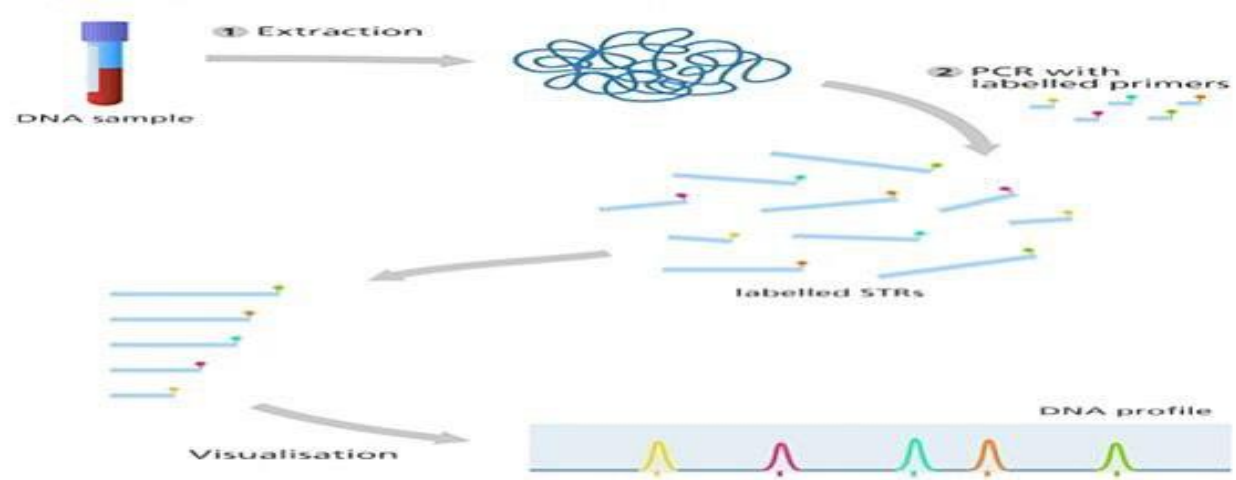
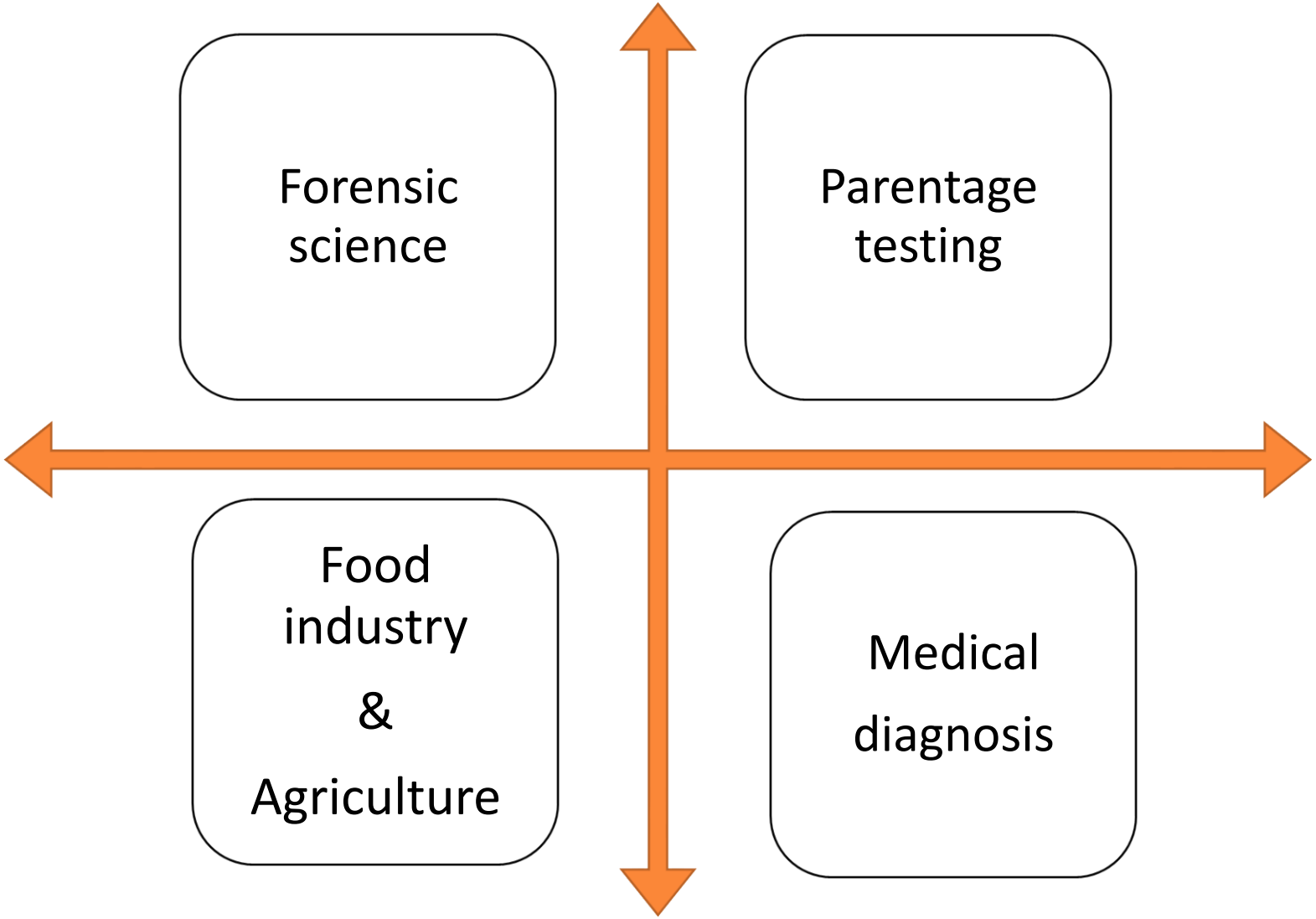


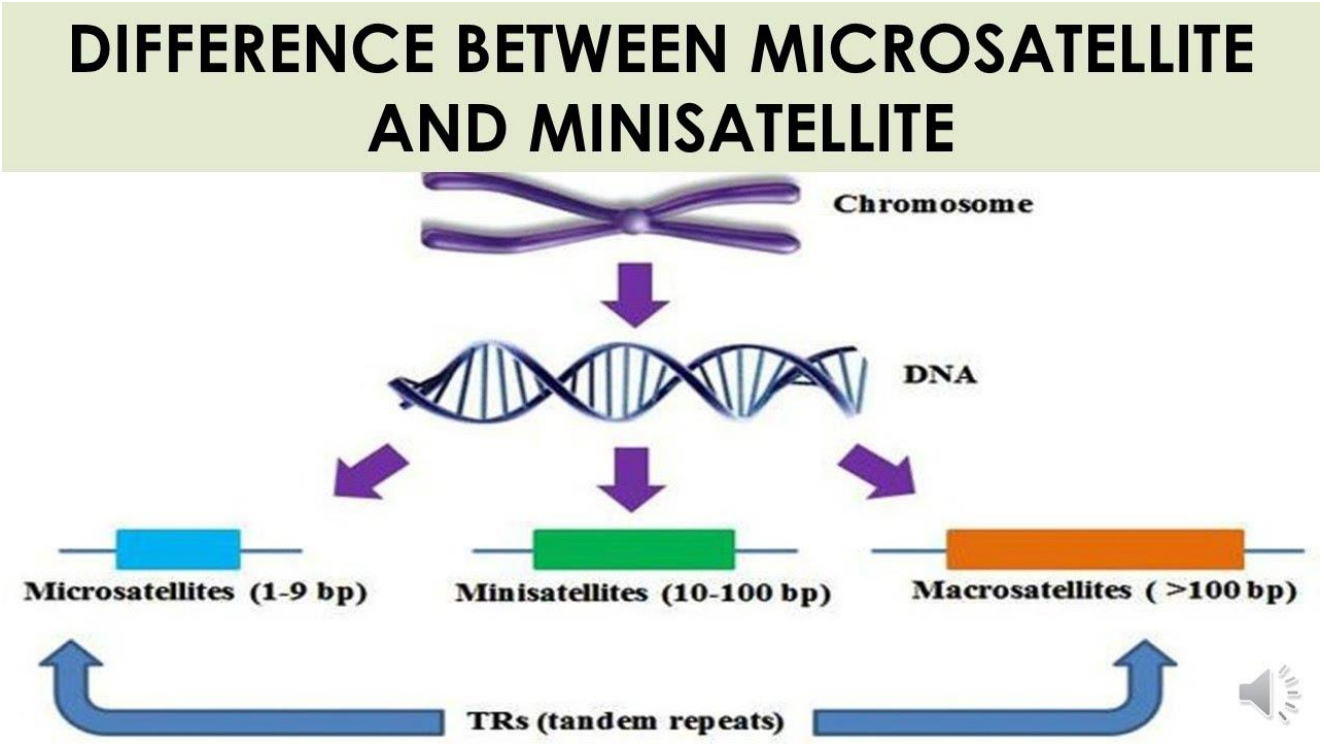
Fig 4: (a) RT- PCR with fluorescent labeled primer
(b) RT-PCR machine (No use of electrophoresis) result show on screen

APPLICATIONS



Microsatellites and Minisatellites:

- Microsatellites (STRs) and minisatellites (VNTRs) are types of repetitive DNA sequences found in the genome. These sequences are highly polymorphic, making them valuable in various genetic studies, including forensics, population genetics, and disease research.



Microsatellites (STRs)

Principle

- **Microsatellites**, also known as Short Tandem Repeats (STRs), are repeating sequences of 1-6 base pairs of DNA. The number of repeat units can vary significantly among individuals, leading to genetic diversity.

Structure:

- Consist of repeat units such as (CA)n, (GA)n, (TTA)n.
- Found throughout the genome, particularly in non-coding regions.

Mechanism of Polymorphism:

- Microsatellite polymorphism arises due to slippage during DNA replication, which leads to variations in the number of repeat units.

Applications

1.Forensic Identification:

1. STR analysis is widely used in forensic science for DNA fingerprinting. The high variability in STR loci among individuals makes it possible to distinguish between different individuals.
2. STR profiles are generated by amplifying several STR loci using PCR and analyzing the length of the repeat regions using capillary electrophoresis.

2. Paternity Testing:

•STR markers are used to compare the DNA profiles of a child and potential parents. The likelihood of paternity is determined based on the inheritance of STR alleles.

3. Population Genetics:

•STRs are used to study genetic diversity within populations, migration patterns, and evolutionary relationships.

4. Genetic Mapping:

•Due to their high density and polymorphism, STRs are useful markers in genetic linkage studies and genome mapping.

5. Disease Association Studies:

•STR polymorphisms in certain genes are linked to diseases. For example, trinucleotide repeat expansions (e.g., CAG repeats) are associated with disorders like Huntington's disease.

Minisatellites (VNTRs):

Principle:

- **Minisatellites**, also known as Variable Number Tandem Repeats (VNTRs), are longer repeating sequences of 10-100 base pairs. Like microsatellites, the number of repeat units varies among individuals.

Structure:

- Consist of repeat units such as (GGGCAGGAX)n.
- Often found in the subtelomeric regions of chromosomes.

Mechanism of Polymorphism:

- VNTR polymorphism results from recombination or unequal crossing over during meiosis, leading to variations in the number of repeat units.

Applications:

1.DNA Fingerprinting:

1. VNTR analysis was one of the first methods used in DNA fingerprinting. Though now largely replaced by STRs, VNTRs are still used in some forensic and identity testing.

2.Genealogical Studies:

1. VNTRs are used to trace ancestry and lineage due to their high variability and inheritance patterns.

3. Disease Susceptibility and Diagnosis:

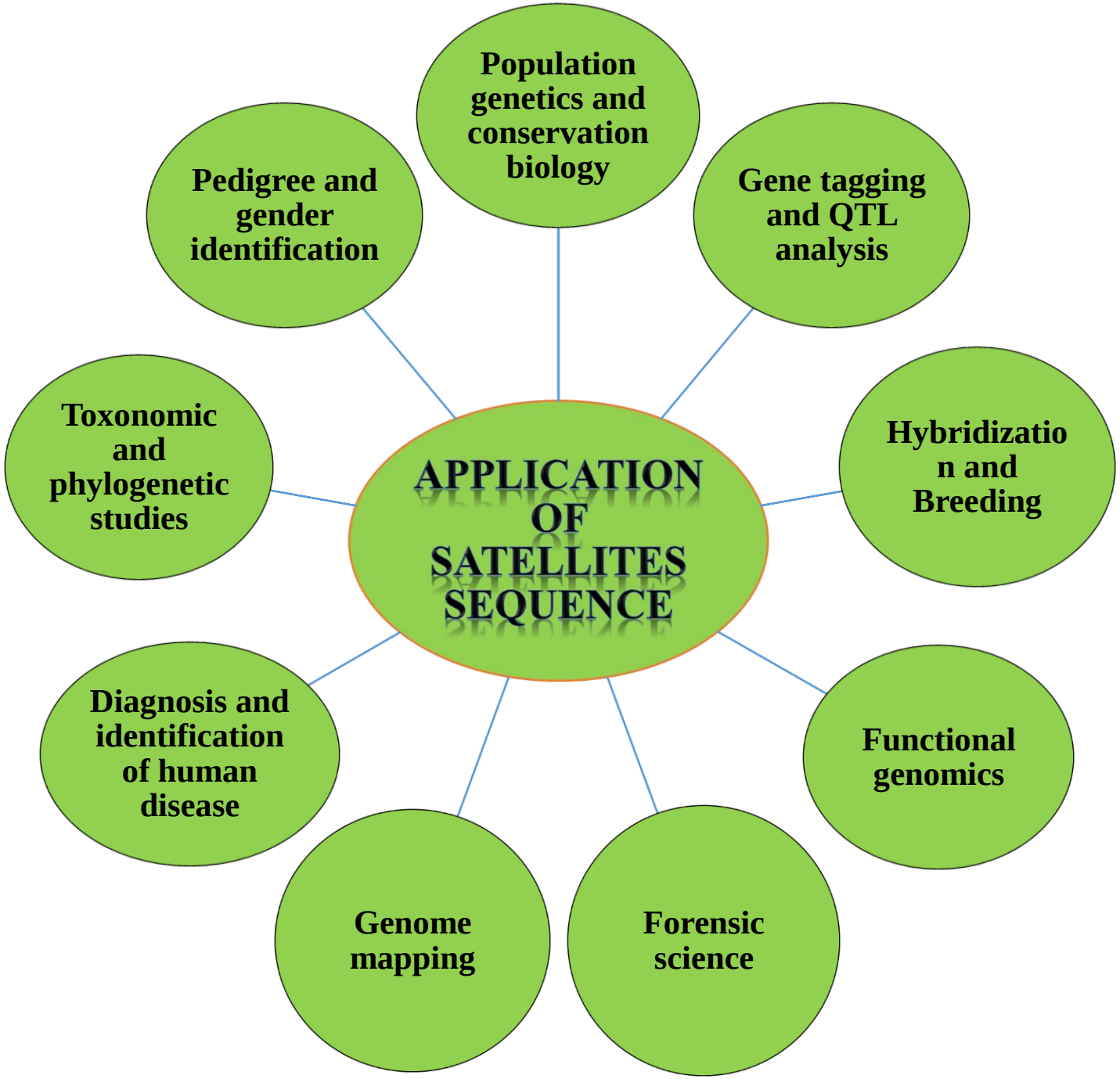
•Variations in VNTRs are associated with susceptibility to certain diseases. For instance, the insulin gene VNTR is linked to the risk of Type 1 diabetes.

4. Population Studies:

•VNTR markers help in studying genetic relationships and variations within and between populations, providing insights into human evolution and migration.

5. Clinical Genetics:

•VNTRs in specific genes are used as markers for genetic disorders. For example, the VNTR in the promoter region of the MAOA gene is associated with behavioral traits and psychiatric conditions.


APPLICATION OF SATELLITES SEQUENCE
Population genetics and conservation biology
Gene tagging and QTL analysis
Hybridizatio n and Breeding
Functional genomics
Forensic science
Genome mapping
Diagnosis and identification of human disease
Toxonomic and phylogenetic studies
Pedigree and gender identification

Comparative Overview of STRs and VNTRs

Feature	**Microsatellites (STRs)**	**Minisatellites (VNTRs)**
Repeat Unit Length	1-6 base pairs	10-100 base pairs
Typical Location	Throughout the genome	Subtelomeric regions
Polymorphism Mechanism	Replication slippage	Recombination and unequal crossing over
Analysis Technique	PCR followed by electrophoresis	Southern blot or PCR
Common Applications	Forensics, paternity testing, genetic mapping	Genealogical studies, disease diagnosis
Use in Forensics	Widely used due to ease of PCR amplification	Less common, replaced by STRs

Conclusion:

- Microsatellites (STRs) and minisatellites (VNTRs) are essential tools in genetic research and practical applications like forensic science and medical diagnostics.
- Their high polymorphism and widespread presence in the genome make them ideal for studies requiring genetic differentiation among individuals or populations.
- Advances in molecular techniques continue to enhance the utility of these markers, contributing to the growing field of genomics and personalized medicine.

ACKNOWLEDGEMENT

- The presentation is being used for educational and non-commercial purposes.
- Thanks are due to all the original contributors and entities whose pictures were used to create this presentation.