BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,

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Programme: M.Sc., Biomedical Science

Course Code: 18BMS59C17

Course Title: Immune & Molecular Diagnostics

Unit-V

Molecular Diagnostics

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Unit V:

Molecular Diagnostics- Diagnosis of Mycobacterim tuberculosis, HCV & HIV; Conventional vs Molecular Diagnostics- Merits & Demerits. Biological warfare: Bacillus anthracis, H5N1, SARS- CoV, Chikunguniya- pathogenesis- diagnostic methods- Molecular diagnosis of single gene disorder- sickle cell anemia- Molecular HLA typing- Sequence Specific PCR (SSP), Sequence Specific oligonucleotide probe (SSOP), Sequence Based Typing (SBT)- Advantages of molecular HLA typing over serological methods- HLA typing and clinical significance- comment on Sensitivity and Specificity of clinical laboratory techniques- Quality assessment Programs (external & internal assessment Programs)

PRESENTATION: 2

Molecular Diagnosis of Single Gene Disorder

- Single Gene Disorder is a genetic condition caused by mutations in a single gene.
- These disorders follow Mendelian inheritance patterns and can be classified based on how they are inherited: autosomal dominant, autosomal recessive, X- linked dominant, or X- linked recessive.

Examples:

1. **Cystic fibrosis**
2. **Sickle cell anaemia**
3. **Huntington’s disease**
4. **Duchenne muscular dystrophy**
5. **Phenylketonuria**

Diagnosis:

1. Genetic testing:

Identifying mutations in the specific gene associated with the disorder

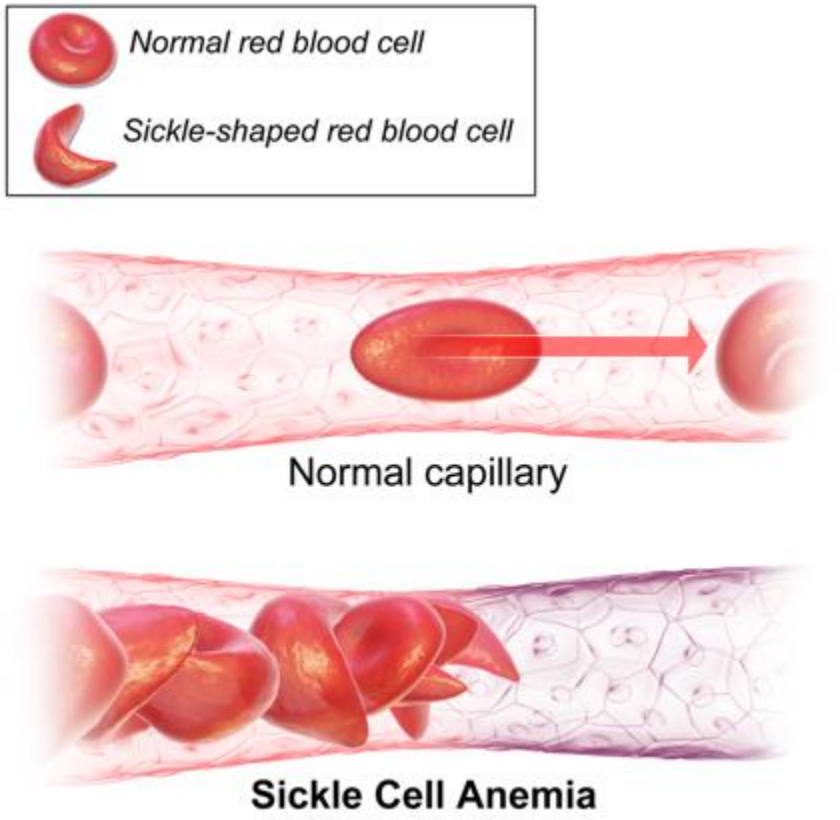
2. Biochemical test: measuring enzyme activity or metabolite levels in some cases

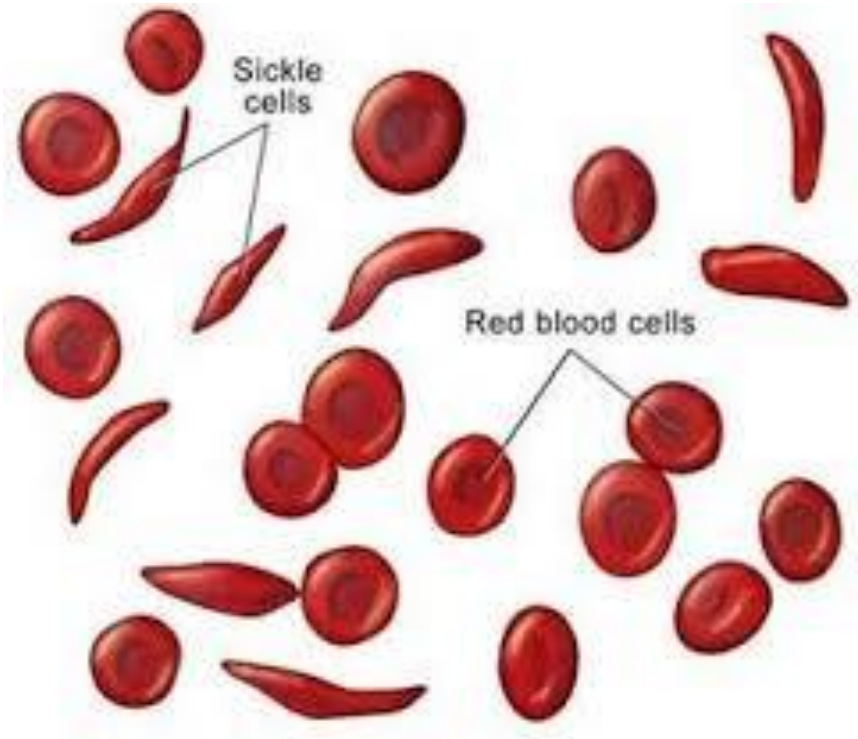
3. Family history: analysing inheritance patterns through pedigree.

Molecular Diagnosis of Single Gene Disorder: Sickle Cell Anaemia

Sickle Cell Anaemia (SCA):

- Sickle cell anaemia is a genetic disorder caused by a mutation in the **HBB** gene, leading to the production of abnormal haemoglobin (HbS) which distorts red blood cells into a sickle shape.
- The mutation is a single nucleotide substitution (GAG to GTG) at the sixth codon of the **HBB** gene, resulting in the replacement of glutamic acid with valine in the beta-globin chain.





Sickle Cell Anaemia

Molecular Diagnosis Techniques:

1.Polymerase Chain Reaction (PCR):

1. Amplifies the specific region of the **HBB** gene containing the mutation.

2.Restriction Fragment Length Polymorphism (RFLP):

1. Detects the presence of the mutation by using restriction enzymes that cut DNA at specific sequences altered by the mutation.

3. Allele-Specific PCR (AS-PCR):

1. Uses primers specific for either the normal or mutant sequence, allowing detection of the mutation.

4. High-Resolution Melting Analysis (HRM):

1. Differentiates between normal and mutant DNA sequences based on their melting profiles.

5. DNA Sequencing:

1. Confirms the mutation by directly sequencing the DNA.

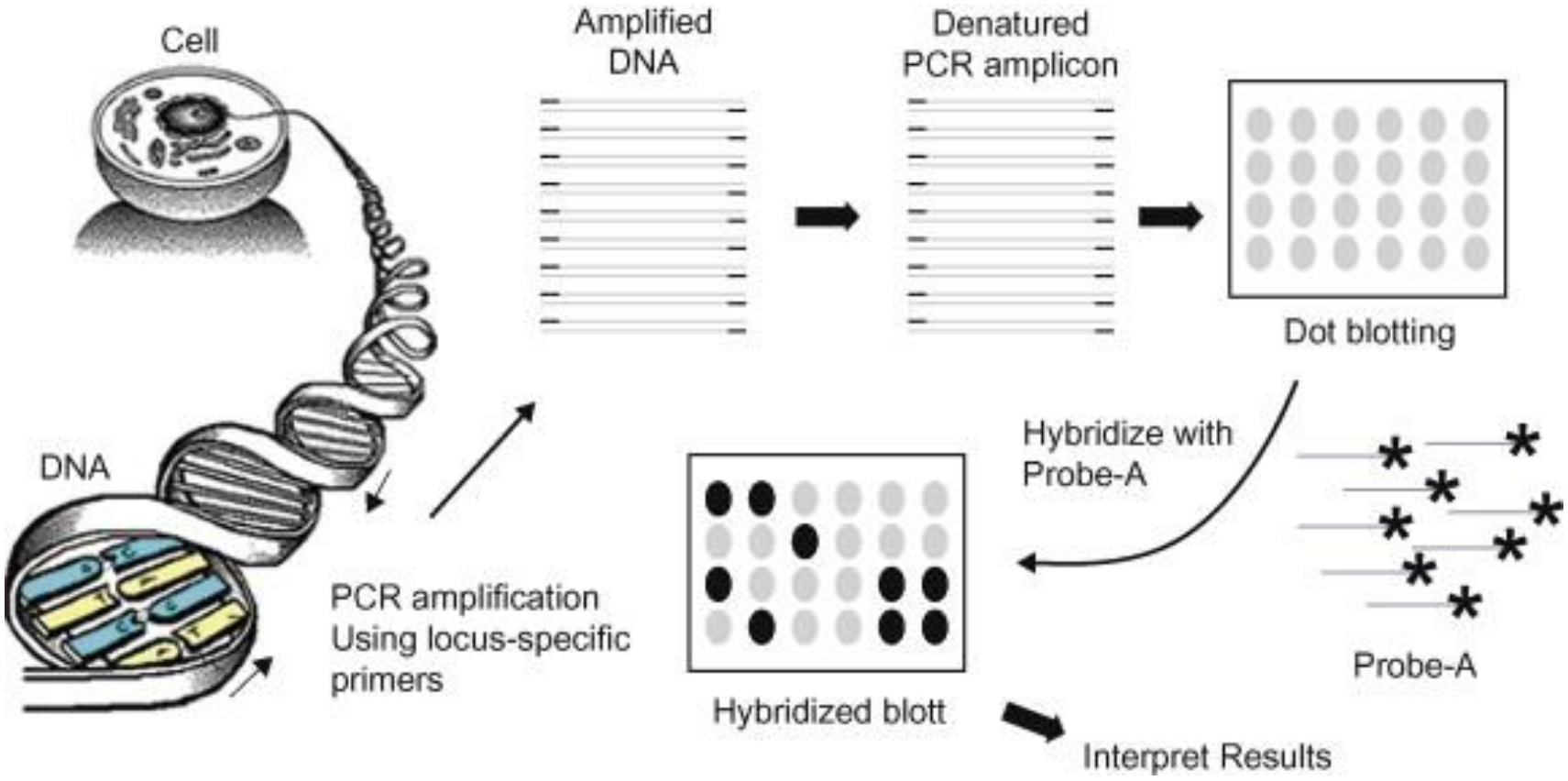
Advantages of Molecular Diagnosis:

- High sensitivity and specificity.
- Can detect carriers and diagnose the condition prenatally.
- Useful for population screening and genetic counseling.

Molecular HLA Typing

- Human Leukocyte Antigen (HLA) typing is critical for organ and bone marrow transplantation, as it ensures compatibility between donors and recipients.
- Molecular methods are now the gold standard for HLA typing due to their accuracy and resolution.

Molecular HLA Typing



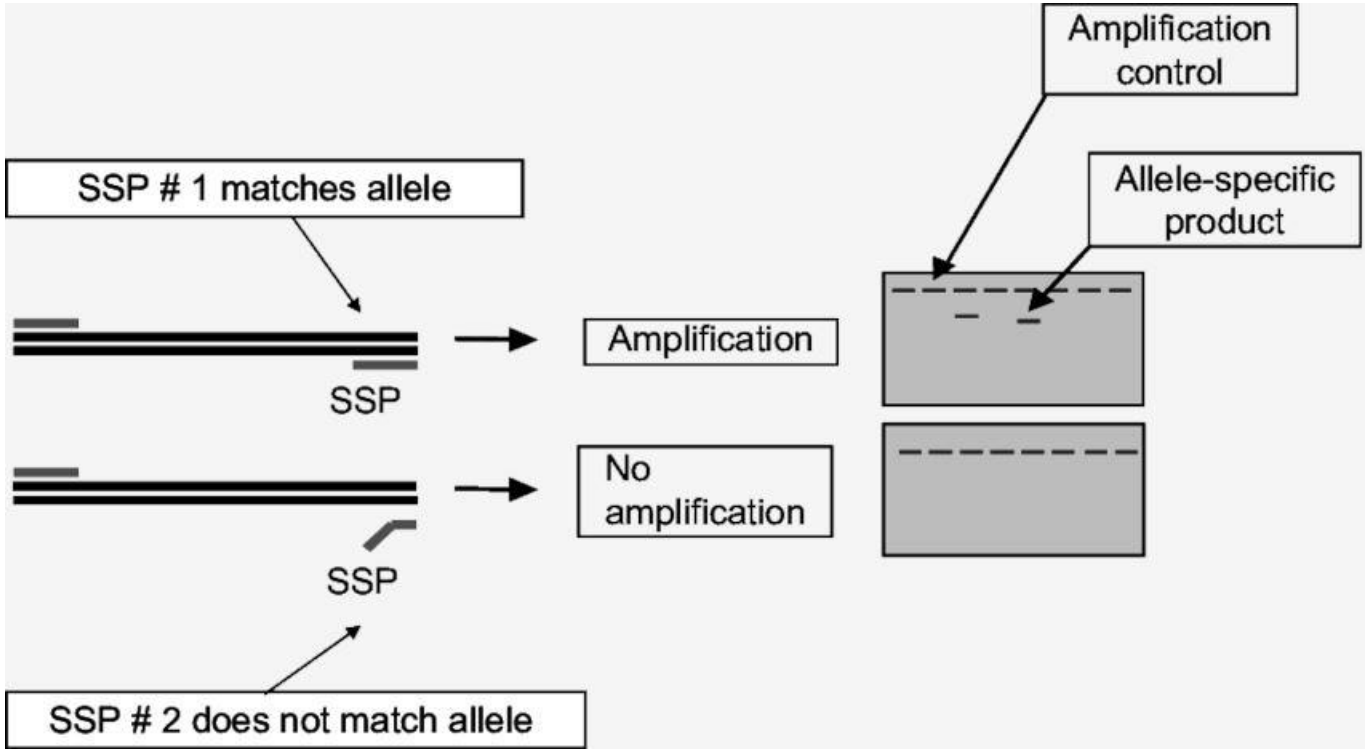
1. Sequence Specific PCR (SSP):

It is a technique that uses primers to amplify specific DNA sequences. The results are then analysed to determine the presence of alleles or other polymorphisms in DNA sample.

Methods:

- Uses primers specific to known HLA alleles.
- Each primer pair amplifies a unique HLA allele or group of alleles.
- PCR products are analyzed by gel electrophoresis.

Sequence Specific PCR (SSP)



Advantages:

•Rapid and straightforward.

•High specificity.

•Suitable for detecting specific HLA alleles.

Limitations:

•Limited resolution for detecting all possible alleles.

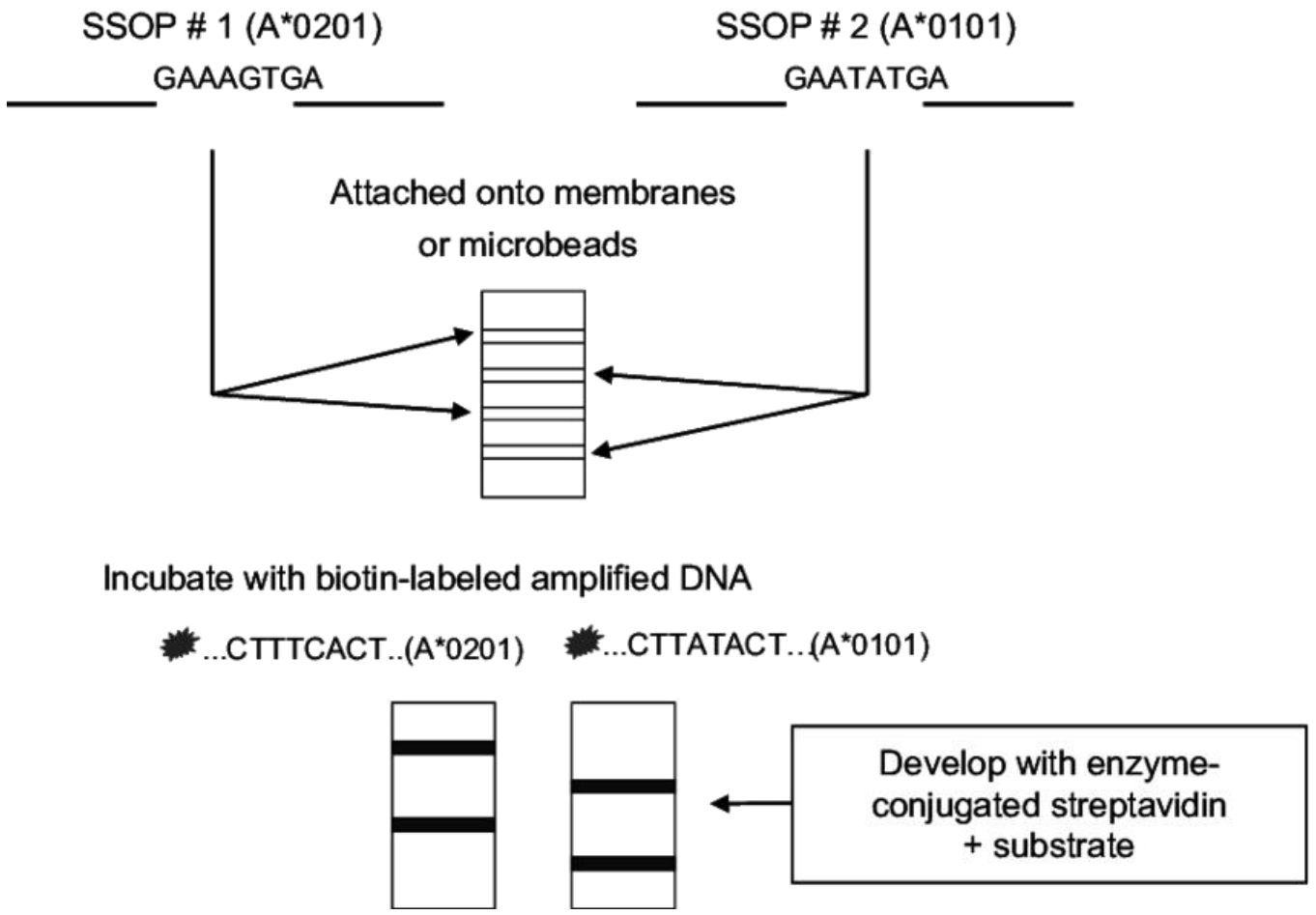
•Requires multiple reactions for broad HLA typing.

2. Sequence Specific Oligonucleotide Probe (SSOP):

Its is a synthetic DNA or RNA sequence that binds to complementary strands of DNA in cells to detect specific sequences in genetic material. These probes are usually to bases of single stranded NA that are chemically synthesized as a specified base sequence.

- **Method:**
  - PCR amplifies HLA genes, followed by hybridization with labeled probes specific to known HLA sequences.
  - The hybridization pattern is analyzed to determine the HLA type.

Sequence Specific Oligonucleotide Probe (SSOP



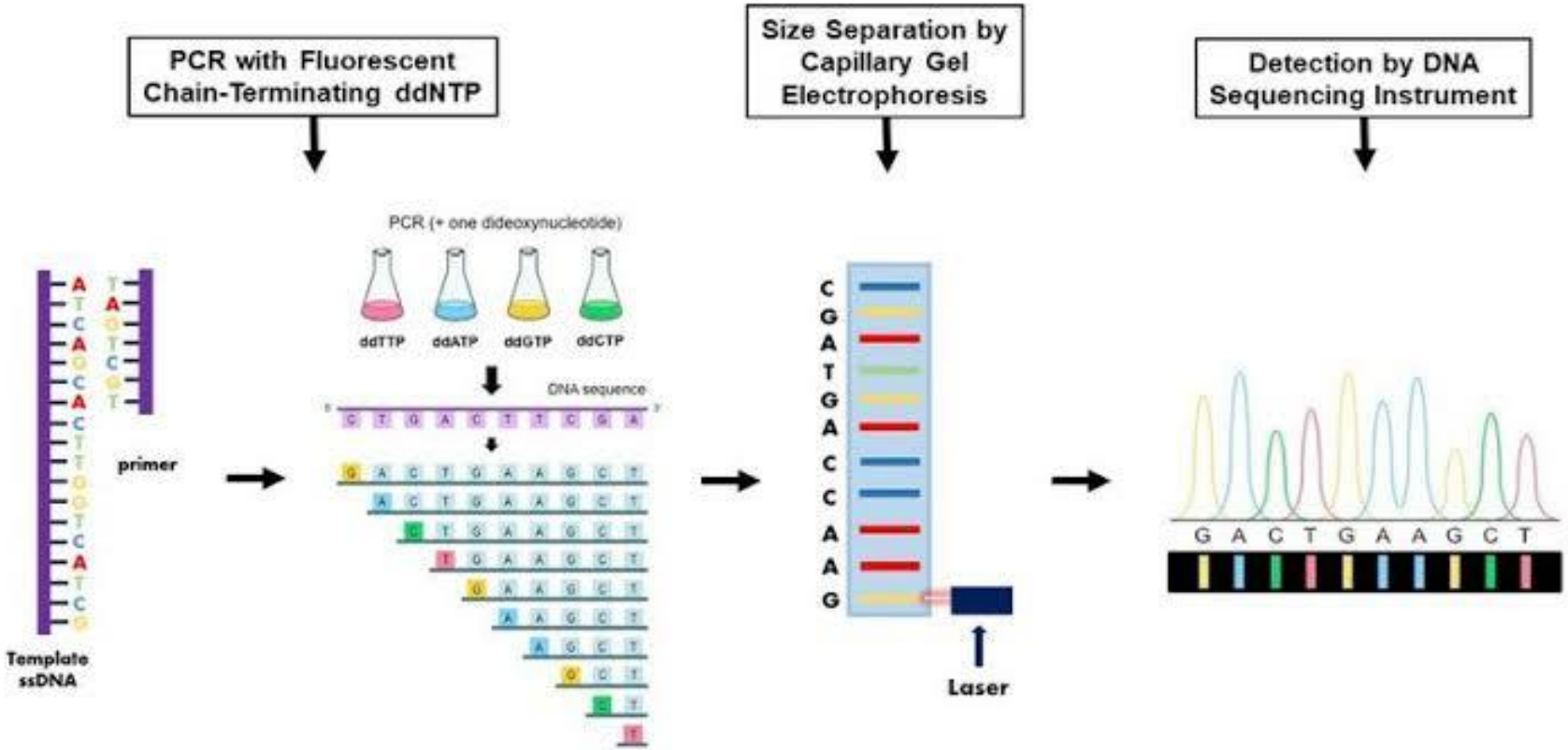
- **Advantages:**
  - Higher resolution than SSP.
  - Can handle multiple samples simultaneously.
- **Limitations:**
  - More complex and time-consuming than SSP.
  - Requires specialized equipment for probe hybridization and detection.

3. Sequence Based Typing (SBT):

It is a method that involves sequencing genes and comparing the results to published libraries.

- **Method:**
  - Involves direct sequencing of HLA genes using Sanger sequencing or next-generation sequencing (NGS).
  - Sequences are compared to reference databases to determine HLA types.

Sequence Based Typing (SBT)



- **Advantages:**
  - Highest resolution, able to distinguish between closely related alleles.
  - Provides comprehensive HLA typing data.
- **Limitations:**
  - More expensive and labor-intensive.
  - Requires high technical expertise and bioinformatics support.

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.