BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biomedical Science

Course Code: 18BMS59C17

Course Title: Immune & Molecular Diagnostics

Unit-IV

Basic Molecular Methods

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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: 1

Basic Molecular Methods: Types of Mutations and PCR-based Mutation Detection Methods

- Understanding the various types of mutations and the methods used to detect them is crucial in molecular biology, genetics, and medical diagnostics.
- Mutations can be categorized based on their nature and impact on the genetic material.
- Polymerase Chain Reaction (PCR) is a widely used technique for detecting both known and unknown mutations.
- Several advanced PCR-based methods facilitate mutation detection with high specificity and sensitivity.

Types of Mutations

Types of Mutations

Mutations are changes in the DNA sequence and can be classified into several categories:

1.Point Mutations:

1. **Silent Mutation:** No change in the amino acid sequence.
2. **Missense Mutation:** Substitution of one amino acid for another.
3. **Nonsense Mutation:** Results in a premature stop codon.

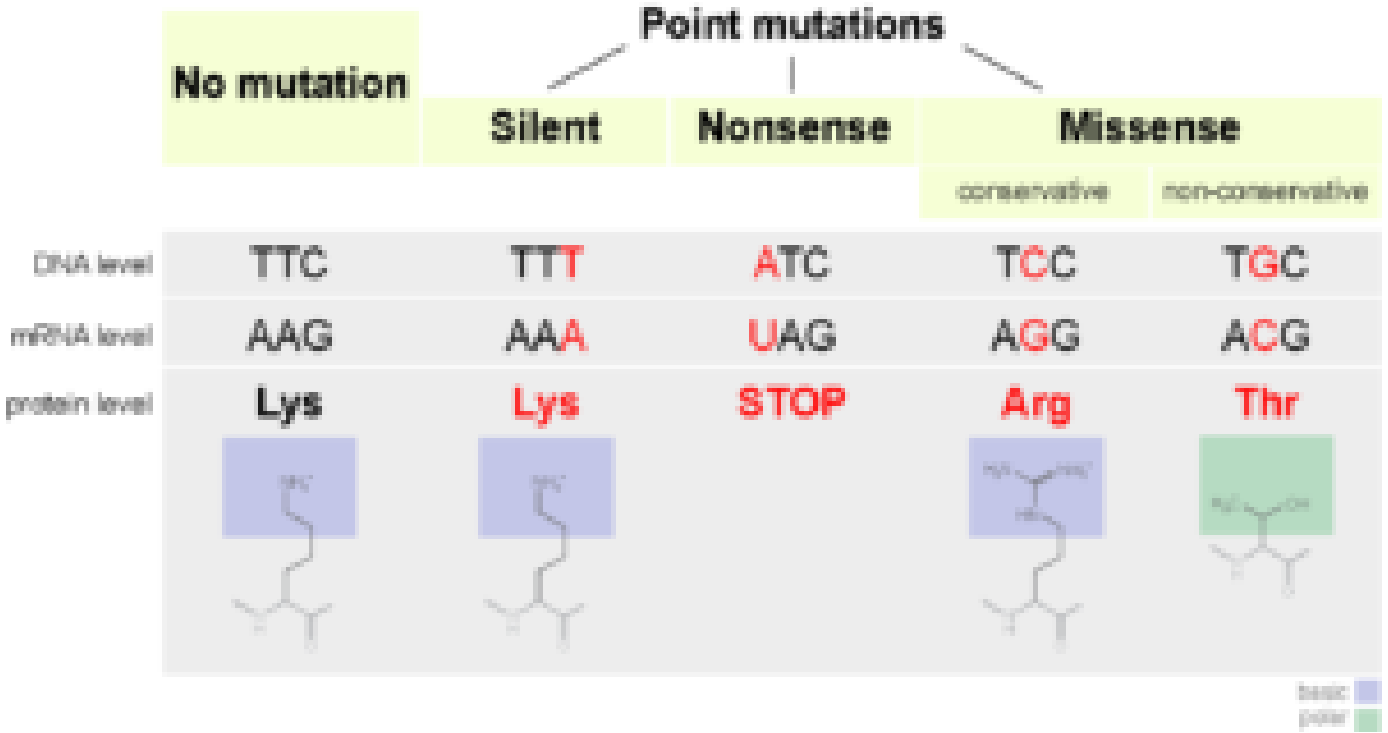


Figure: Some mutations do not change the sequence of amino acids in a protein. Some swap one amino acid for another. Others introduce an early stop codon into the sequence causing the protein to be truncated.

Insertions and Deletions (Indels):

•Small or large insertions or deletions that can cause frameshift mutations if they are not in multiples of three nucleotides.

Copy Number Variations (CNVs):

•Variations in the number of copies of a particular gene.

Structural Variants:

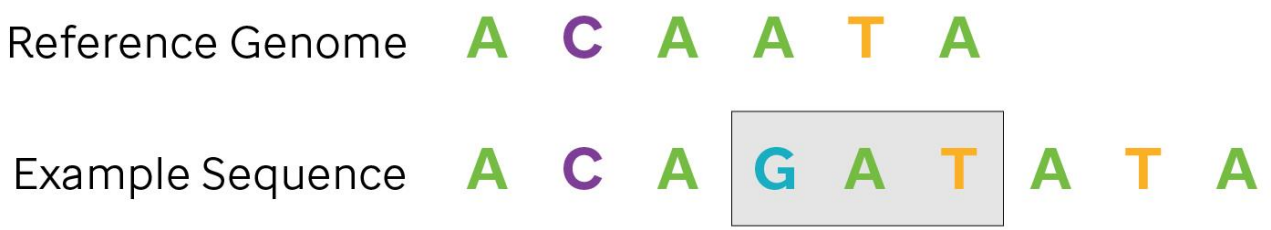
•Large-scale alterations such as inversions, translocations, and chromosomal rearrangements.

Splice Site Mutations:

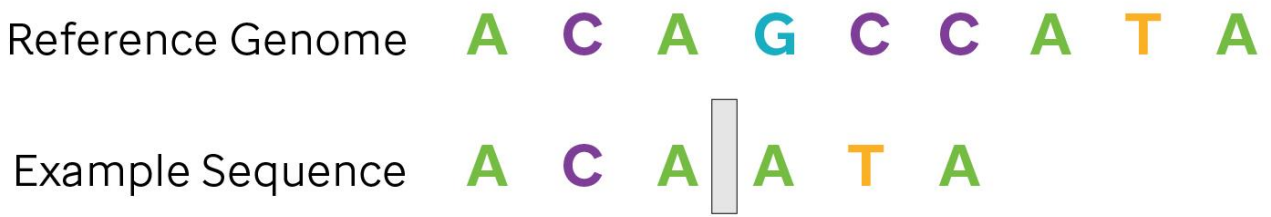
•Affect the normal splicing of mRNA, leading to abnormal protein products.

Insertion and Deletion

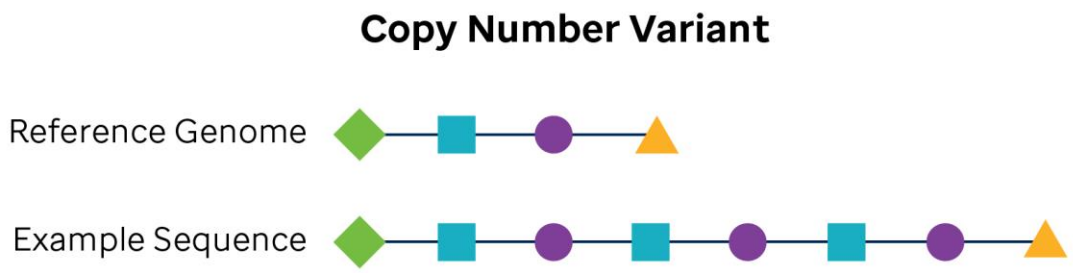
Indel: Insertion

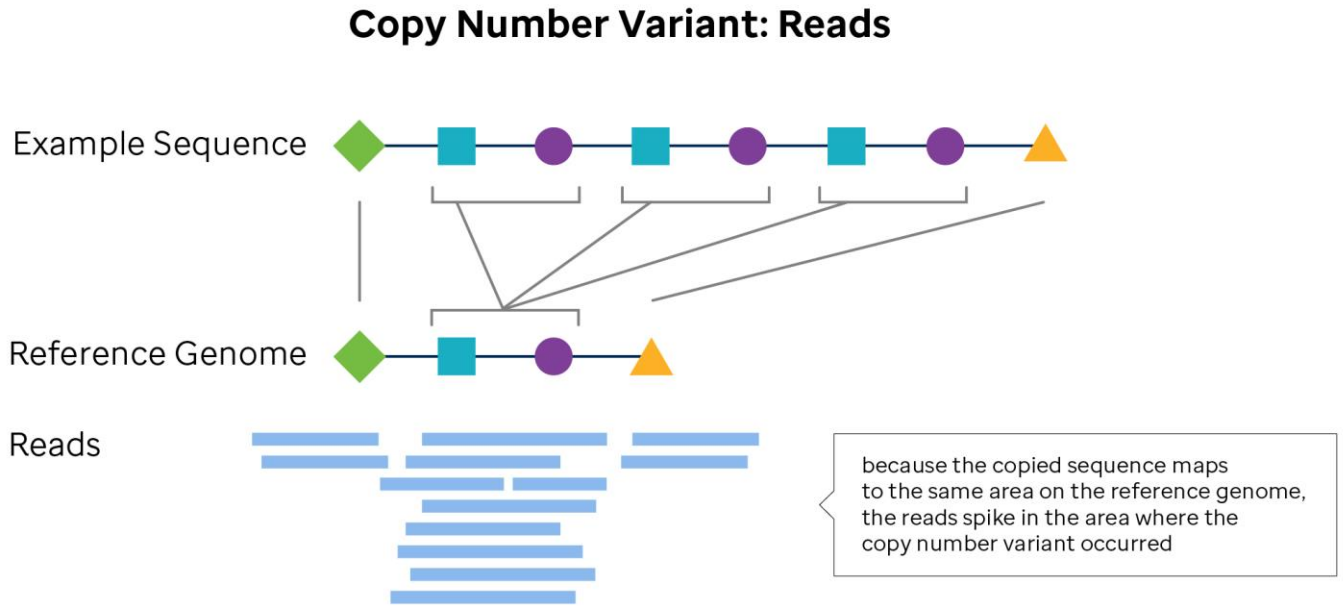


Indel: Deletion

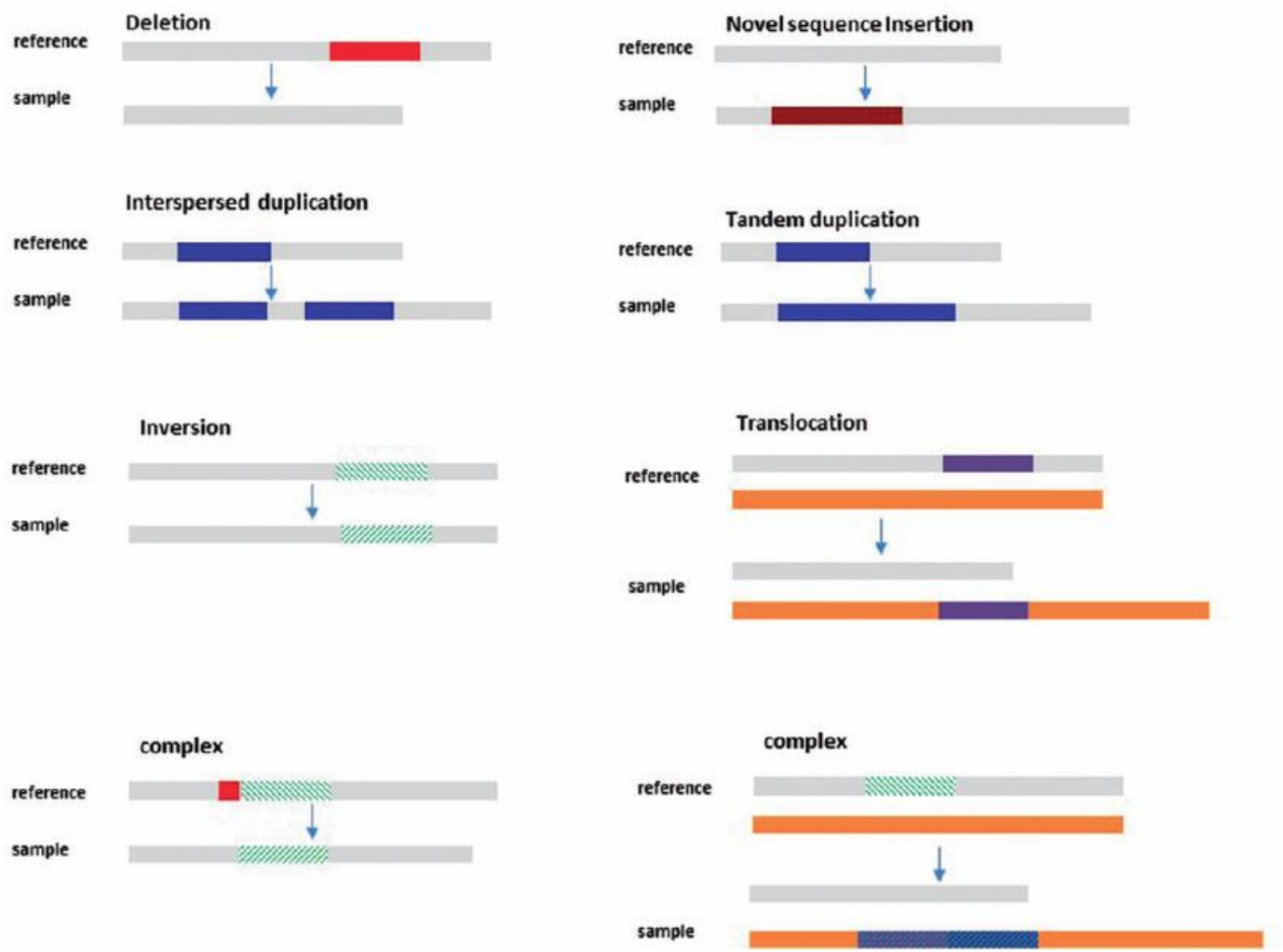


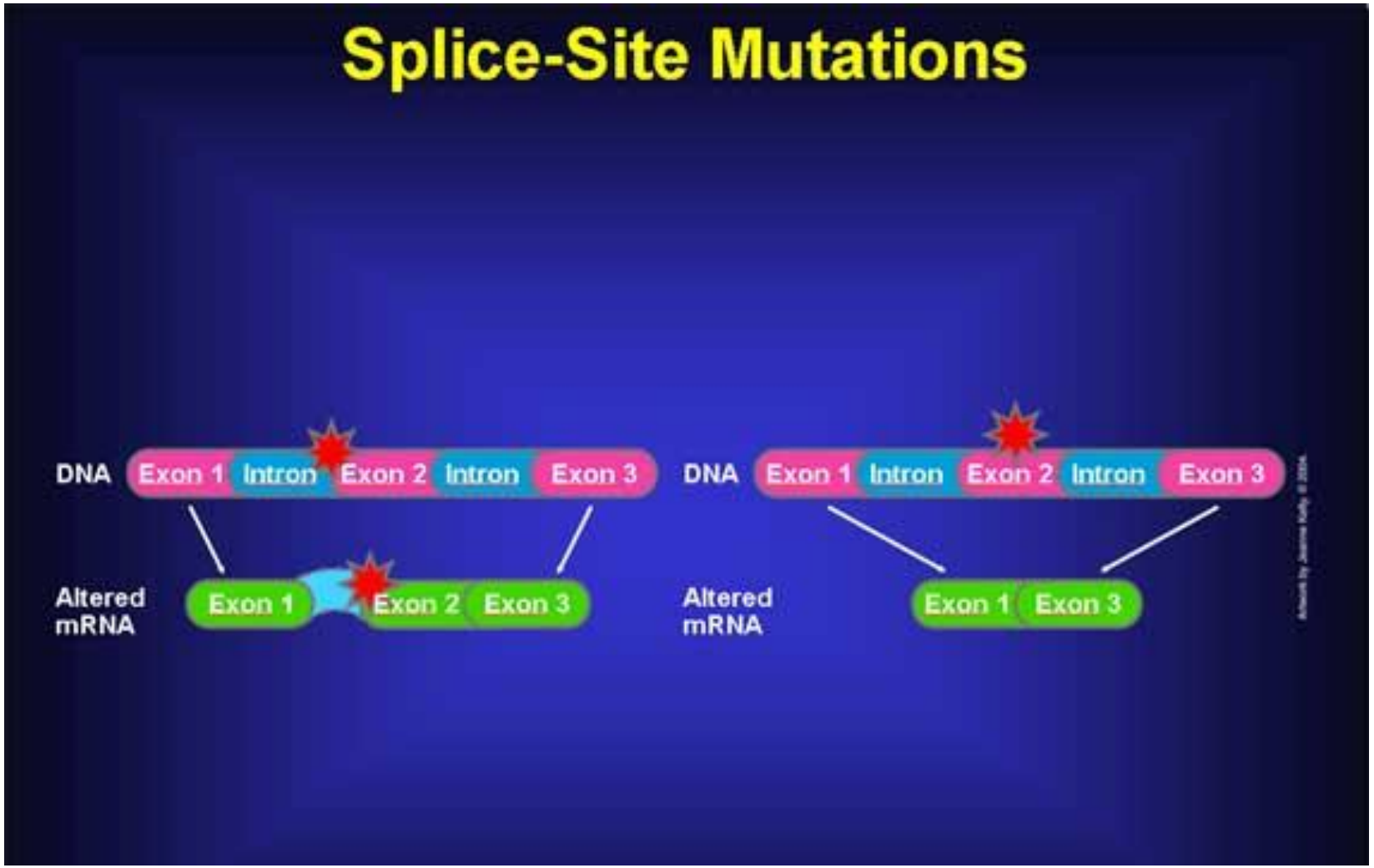
Copy Number Variations





Structural Variants




Splice-Site Mutations
DNA
Exon 1
Intron
Exon 2
Intron
Exon 3
Altered mRNA
Exon 1
Exon 2
Exon 3
DNA
Exon 1
Intron
Exon 2
Intron
Exon 3
Altered mRNA
Exon 1
Exon 3

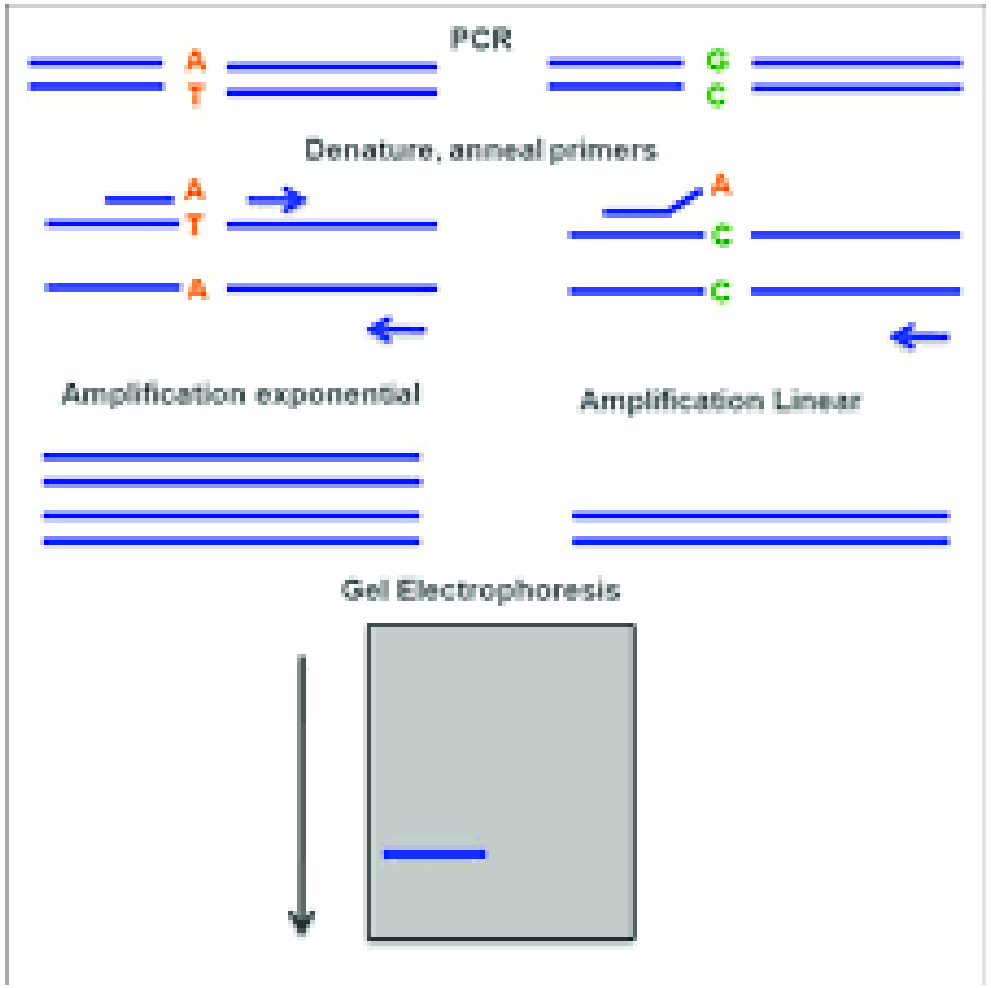
PCR-based Mutation Detection Methods

- PCR is a powerful tool in mutation detection due to its ability to amplify specific DNA sequences. Several PCR-based methods have been developed to detect both known and unknown mutations:

1.Allele-Specific PCR (ASP):

1. **Purpose:** Detects known mutations by using primers specific to the mutant and wild-type alleles.
2. **Mechanism:** ASP relies on the specificity of primer annealing to detect a single nucleotide polymorphism (SNP) or mutation. If the primer matches the target sequence perfectly, it will bind and allow PCR amplification. Any mismatch due to a mutation will prevent amplification.

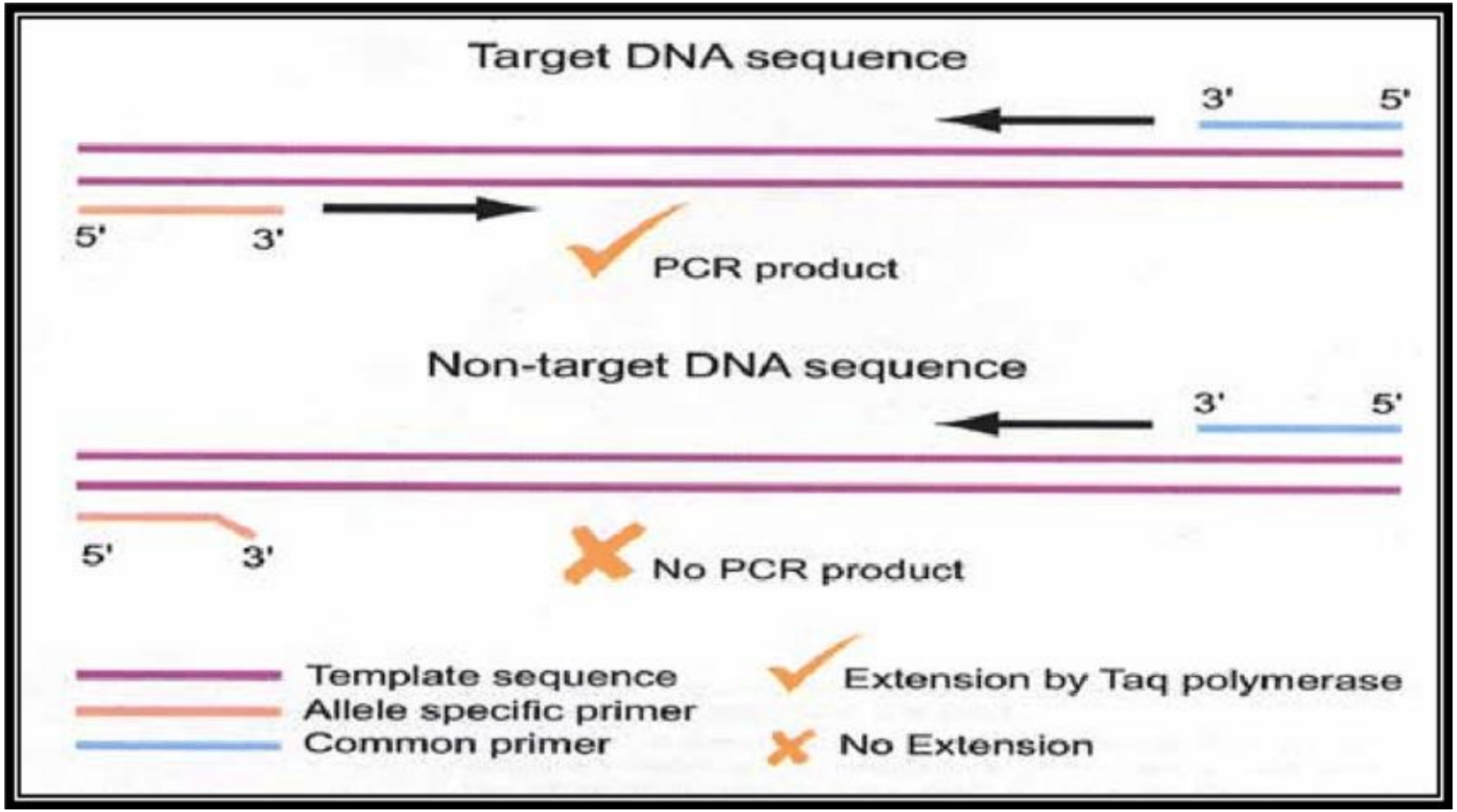
PCR


PCR
A
T
G
C
Denature, anneal primers
A
T
A
A
C
C
Amplification exponential
Amplification Linear
Gel Electrophoresis

2. Amplification Refractory Mutation System (ARMS) or Allele-Specific Oligonucleotide PCR (ASOP):

- **Purpose:** Differentiates between wild-type and mutant alleles based on primer specificity.
- **Mechanism:** This method uses two sets of primers, one for the wild-type allele and another for the mutant allele. The presence or absence of amplification in separate reactions indicates the genotype of the sample.

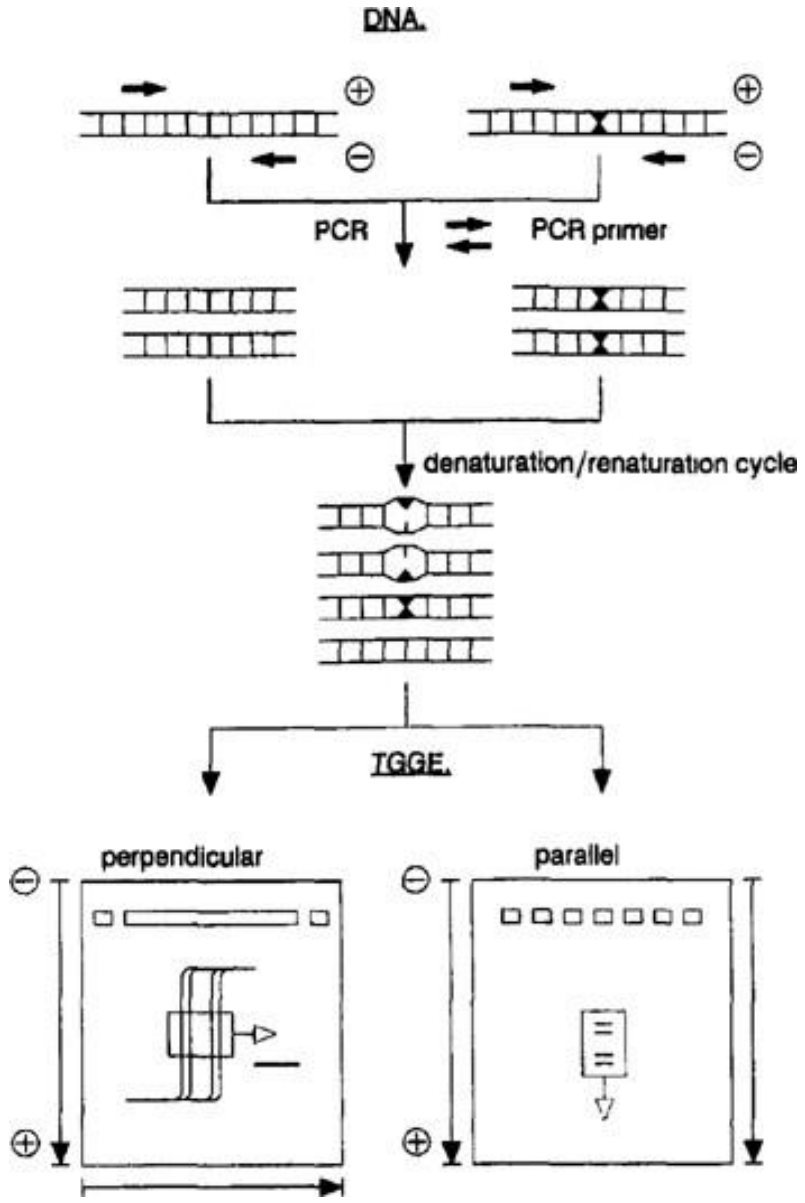
ARMS or ASOP


Target DNA sequence
3'
5'
5'
3'
PCR product
Non-target DNA sequence
3'
5'
5'
3'
No PCR product
Template sequence
Allele specific primer
Common primer
Extension by Taq polymerase
No Extension

3. Temporal Temperature Gradient Electrophoresis (TTGE):

- **Purpose:** Detects unknown mutations by separating DNA fragments based on their melting behaviors.
- **Mechanism:** DNA samples are amplified by PCR and then subjected to electrophoresis through a gel with a gradient of increasing temperature. Mutant DNA fragments have different melting properties, allowing them to be distinguished from wild-type fragments.

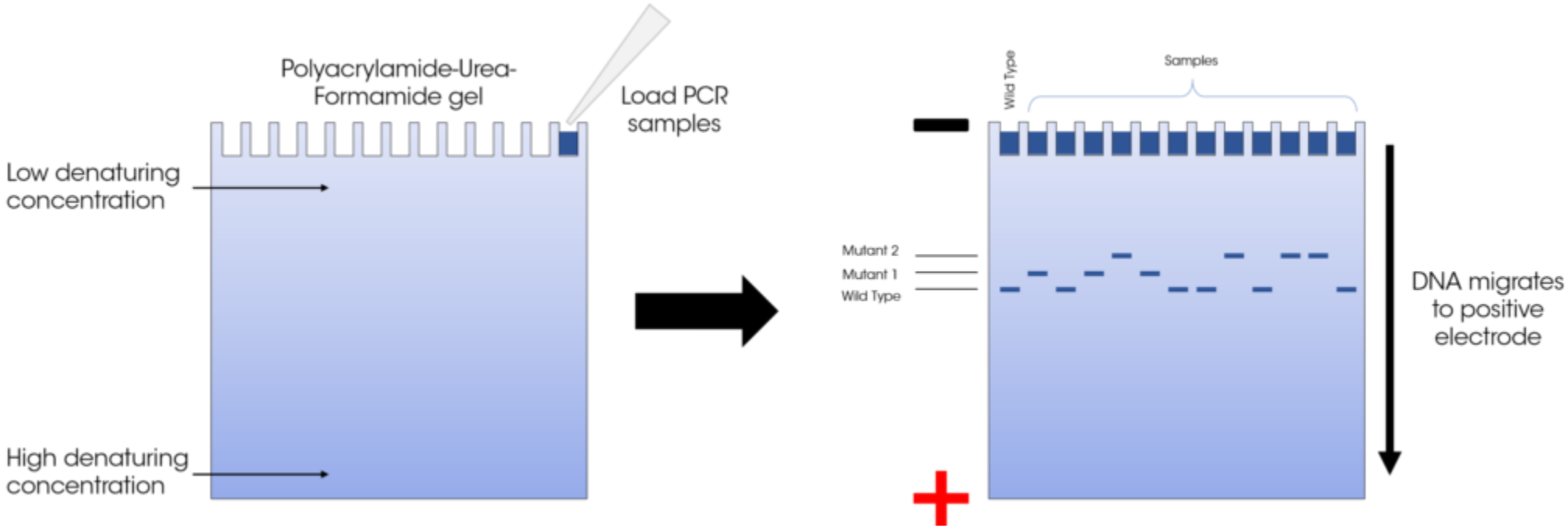
Temporal Temperature Gradient Electrophoresis



4. Denaturing Gradient Gel Electrophoresis (DGGE):

- **Purpose:** Identifies mutations based on differences in DNA melting behavior.
- **Mechanism:** PCR products are run on a gel with a chemical gradient that denatures DNA. Mutations affect the melting behavior of DNA, causing mutant and wild-type fragments to migrate differently.

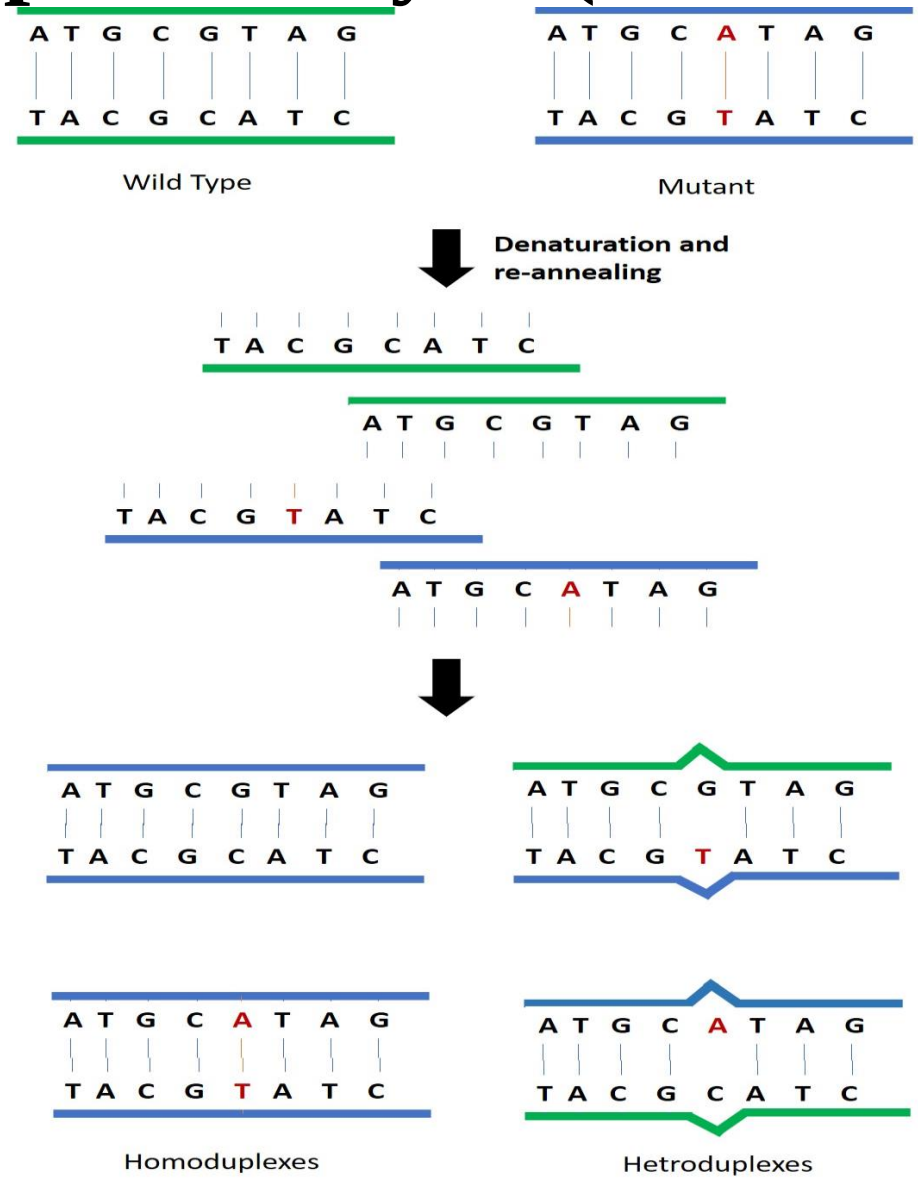
Denaturing Gradient Gel Electrophoresis (DGGE)



5. Heteroduplex Analysis (Heteroduplexing):

- **Purpose:** Detects unknown mutations by analyzing mismatches in DNA duplexes.
- **Mechanism:** PCR-amplified DNA is denatured and re-annealed. If mutations are present, mismatched base pairs (heteroduplexes) form, which migrate differently during electrophoresis compared to perfectly matched (homoduplex) DNA.

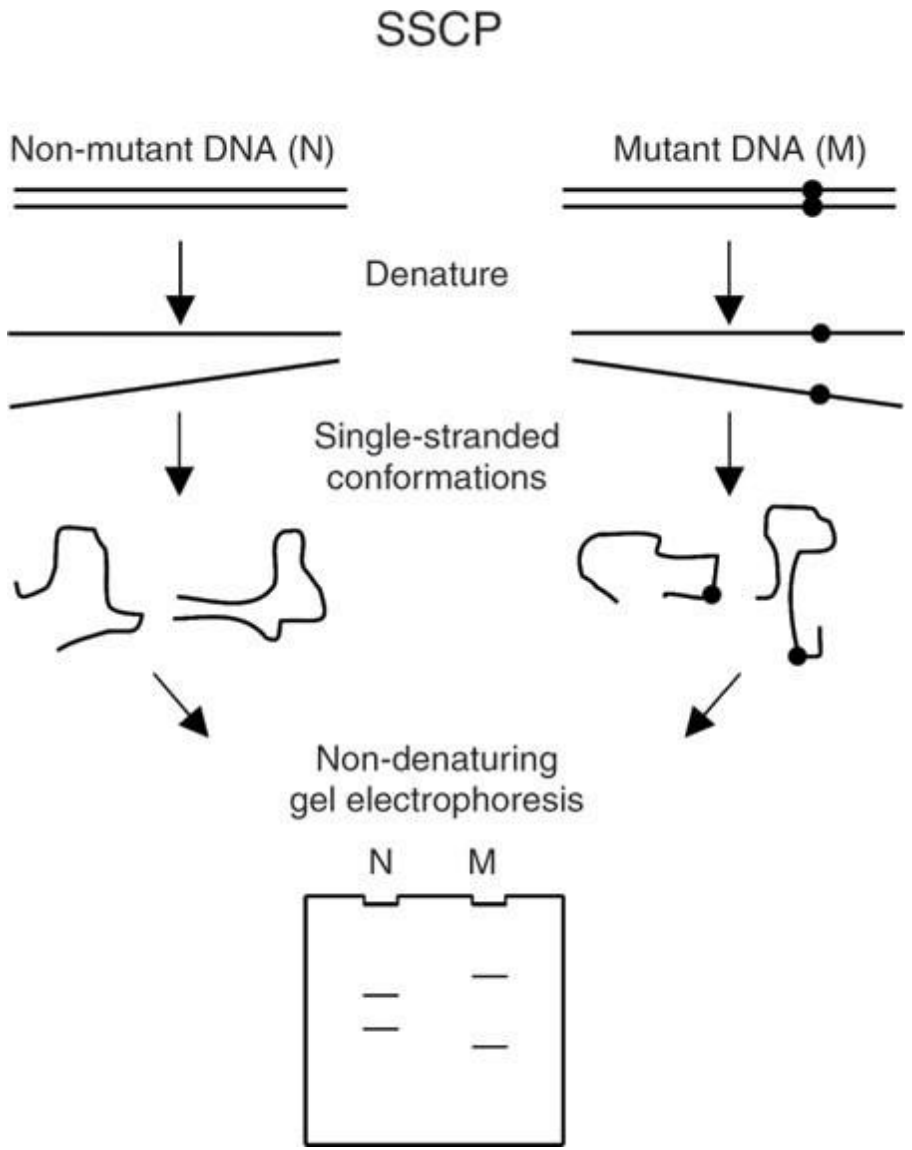
Heteroduplex Analysis (Heteroduplexing)



6. Single-Strand Conformation Polymorphism (SSCP):

- **Purpose:** Detects unknown mutations by exploiting the conformational changes in single-stranded DNA.
- **Mechanism:** PCR products are denatured into single strands and run on a non-denaturing gel. Mutations cause changes in the secondary structure of the DNA, altering its mobility during electrophoresis.

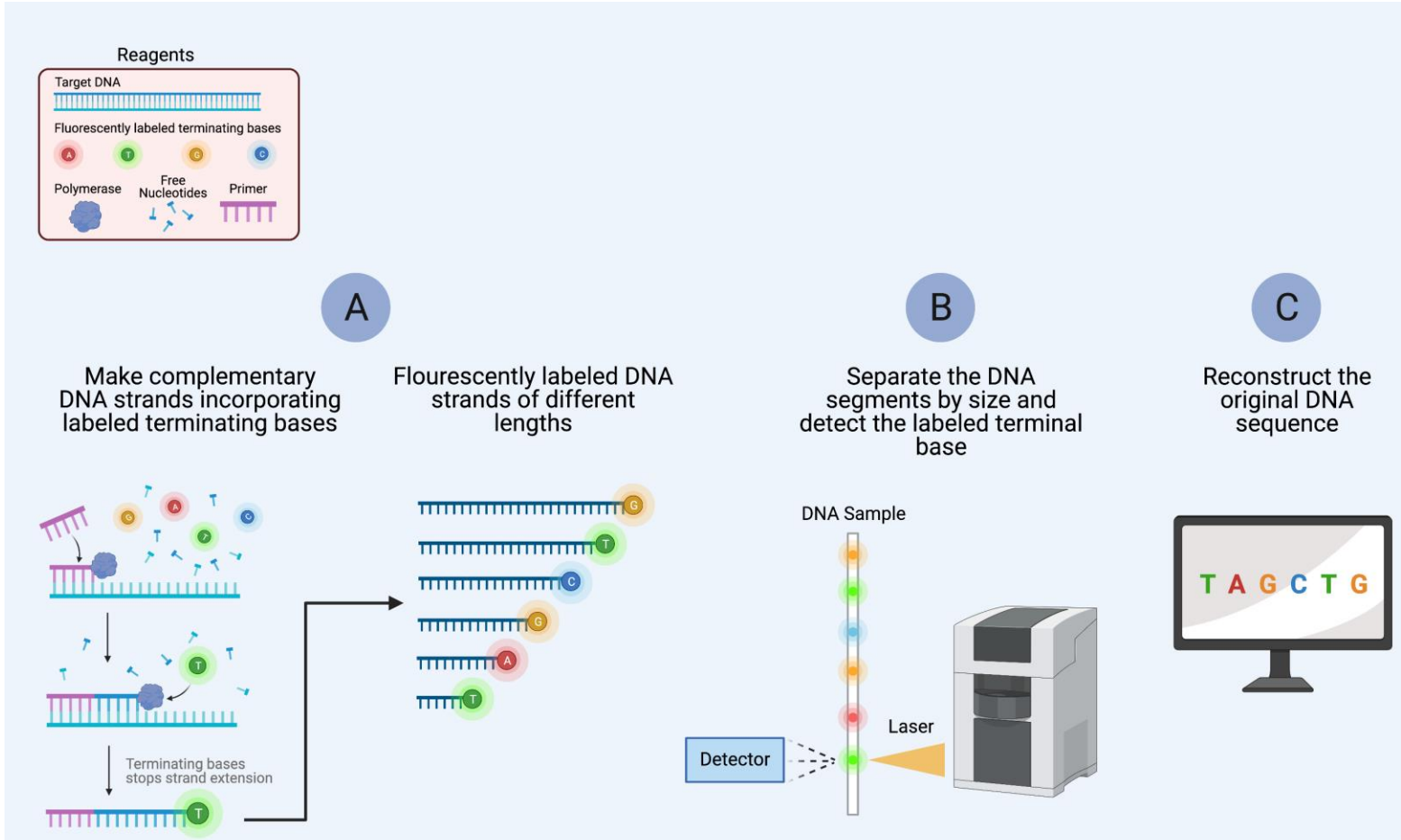
Single-Strand Conformation Polymorphism (SSCP)



7. DNA Sequencing:

- **Purpose:** Identifies both known and unknown mutations by determining the exact sequence of nucleotides.
- **Mechanism:** Sanger sequencing or next-generation sequencing (NGS) can be used. PCR amplifies the region of interest, and sequencing reveals any mutations present by comparing the sample sequence to a reference sequence.

DNA Sequencing



Applications and Significance

These PCR-based methods are critical in various fields:

- **Genetic Testing:** Detecting inherited mutations linked to genetic disorders.
- **Cancer Research:** Identifying somatic mutations in tumor DNA.
- **Pathogen Detection:** Identifying mutations in viral or bacterial genomes that confer drug resistance.
- **Pharmacogenomics:** Tailoring drug therapy based on an individual's genetic makeup

Conclusion

- PCR-based methods for mutation detection provide a robust framework for diagnosing genetic diseases, guiding treatment strategies, and advancing our understanding of genetic variability.
- Techniques like ASP, ASOP, TTGE, DGGE, heteroduplex analysis, SSCP, and sequencing offer precise and reliable means to detect both known and unknown mutations, playing a pivotal role in modern molecular diagnostics.

ACKNOWLEDGEMENT

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- Thanks are due to all the original contributors and entities whose pictures were used to create this presentation.