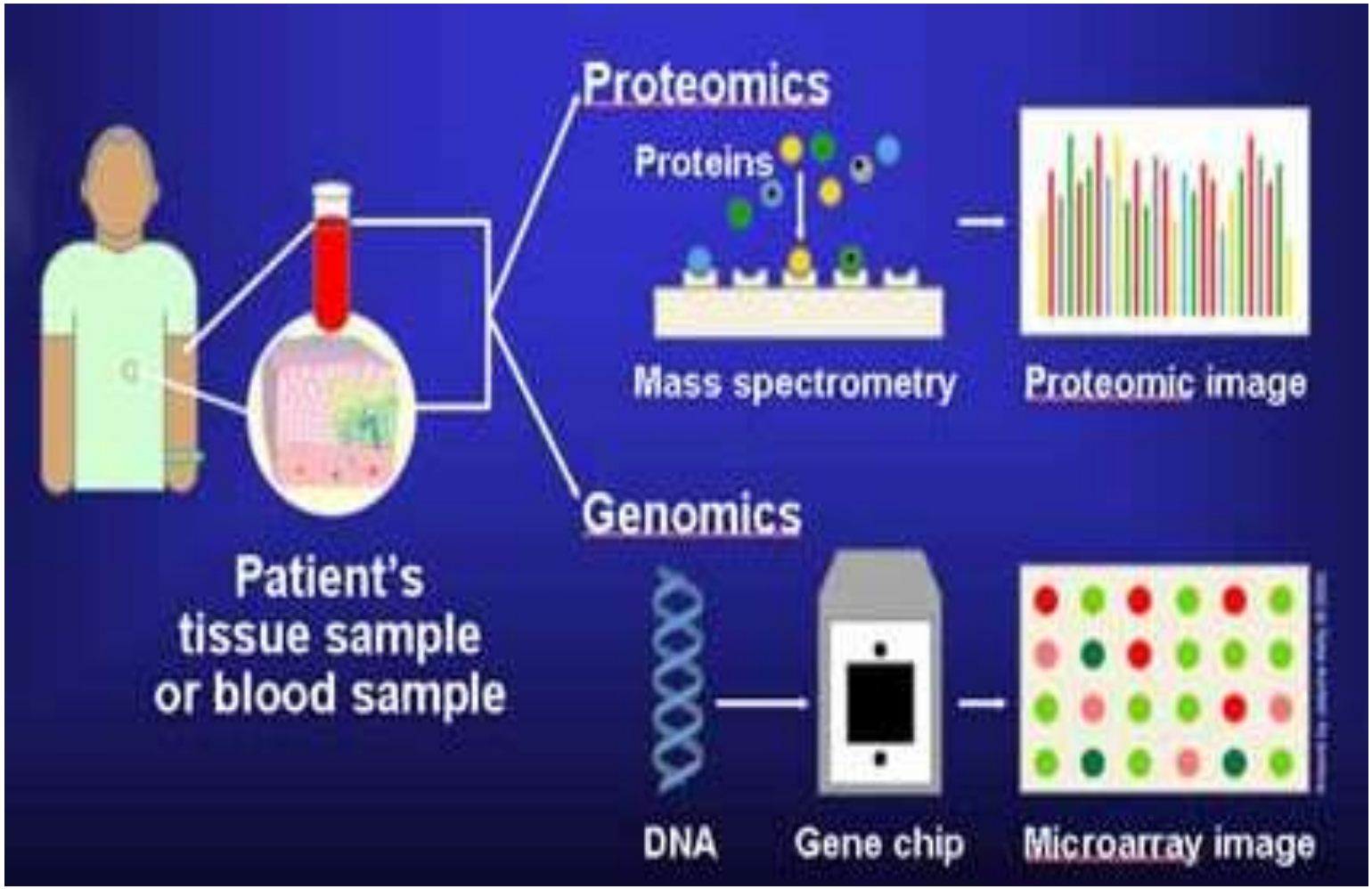
PCR IN MOLECULAR DIAGNOSIS

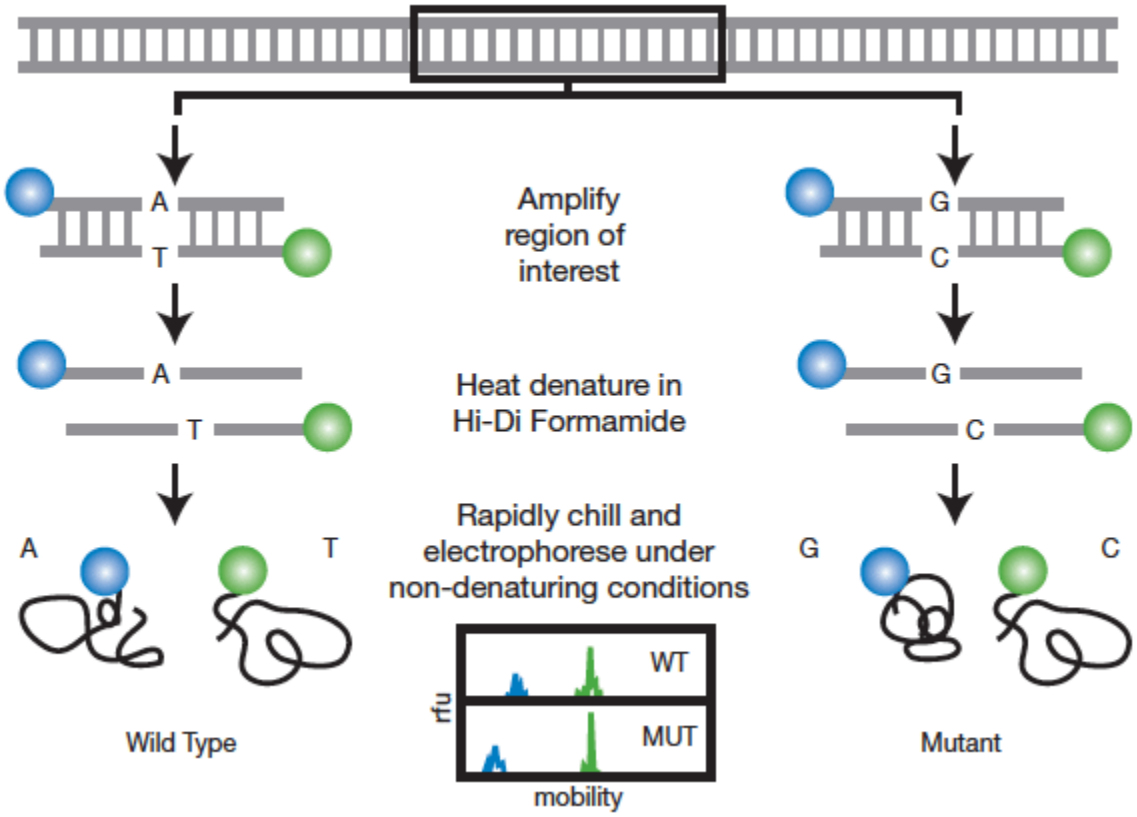
MOLECULAR DIAGNOSIS



Types of Mutation Detection Methods

- Hybridization-based
  - SSCP, ASO, melt curves, array technology
- Sequencing (polymerization)-based
  - Sequence-specific PCR, allelic discrimination
- Cleavage-based
  - RFLP, nuclease cleavage, invader

SINGLE-STRAND CONFORMATION POLYMORPHISM (SSCP) ANALYSIS



SymphonyDGGE Denaturing Gradient Gel Electrophoresis

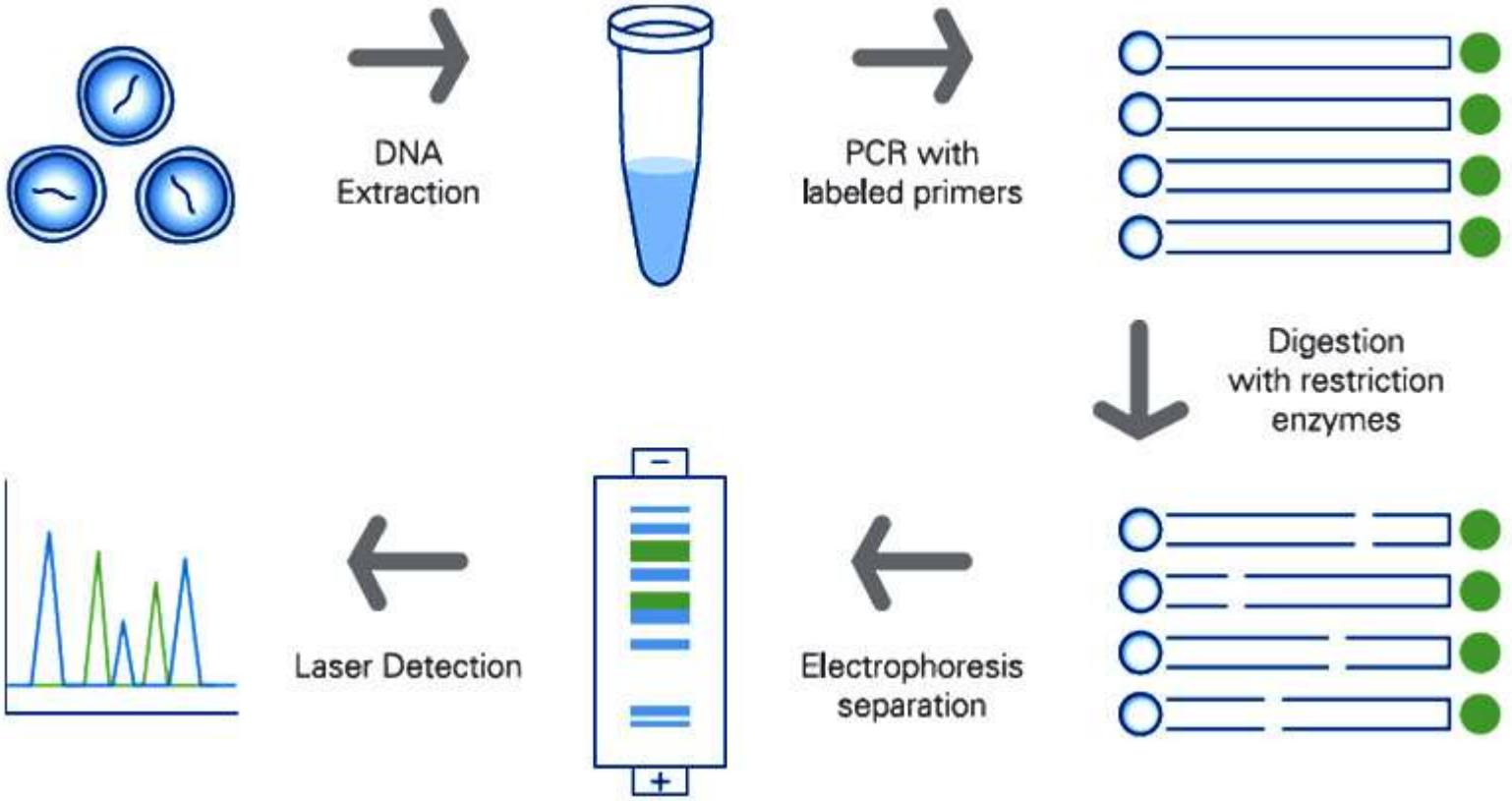
Complete system for **DNA mutation analysis**

Four-screw vertical clamp design for **rapid setup**

Maximum 96-sample throughput compatible with **microplates** and **thermal cycler blocks**

RFLP

- "Restriction Fragment Length Polymorphism"
- Basic idea:
  - If two DNA sequences have different numbers or locations of restriction sites, they will differ in their restriction enzyme digest pattern
- Uses:
  - Paternity testing
  - Criminal cases
  - Locating disease genes


DNA
Extraction
PCR with
labeled primers
Digestion
with restriction
enzymes
Electrophoresis
separation
Laser Detection

Target DNA

↓

PCR

↓

Amplified mutant gene sequence

↓

Add probe 1 and probe 2

↓

Probe 1–Nucleotide at 3'end is a mismatch. Nucleotide at this position will bulge out
Probe 2–Nucleotide at 5'end base pairs with nucleotide at position 51 of normal gene sequence

↓

Add DNA ligase

↓

No ligation of Probe 1 and probe 2
(Because of single nucleotide misalignment)

Now, we have to determine whether ligation has taken place between the two probes in both cases (normal and mutant gene sequence) or not. After hybridization and ligation,

DNA is denatured

↓

Hybridized probes are released
Reaction mixture is transferred to wells coated with streptavidin

↓

Unbound material is washed away

↓

Biotin-labeled probe DNA is present only in the wells

↓

Antidigoxigenin antibodies conjugated to alkaline phosphatase (AP) are added

↓

Then, unbound Antidigoxigenin antibodies – AP are washed away

↓

Chromogenic substrate is added

If colored product– Ligation has occurred –Normal genotype (because digoxigenin labeled probe has ligated to biotinylated probe and digoxigenin has in turn bound to antidigoxigenin antibodies)

↓

If no colored product – No ligation – Mutant genotype

MISMATCH CHEMICAL CLEVAGE

This protocol describes the use of the chemical cleavage of mismatch (CCM) method to assess whether a region of DNA contains mutations and to localize them. Compared with other mutation-detection techniques (such as single strand-conformation polymorphism (SSCP) analysis, denaturing high-performance liquid chromatography (DHPLC) and denaturing gradient gel electrophoresis (DGGE)) that detect mutations in short DNA fragments and require highly specific melting temperatures, CCM has a higher diagnostic sensitivity suited to the detection of mutations in tumor genes, and can analyze amplicons ≤2 kb in length. To detect mutations, PCR heteroduplexes are incubated with two mismatch-specific reagents. Hydroxylamine modifies unpaired cytosine and potassium permanganate modifies unpaired thymine. The samples are then incubated with piperidine, which cleaves the DNA backbone at the site of the modified mismatched base. Cleavage products are separated by electrophoresis, revealing the identity and location of the mutation. The CCM method can efficiently detect point mutations as well as insertions and deletions.

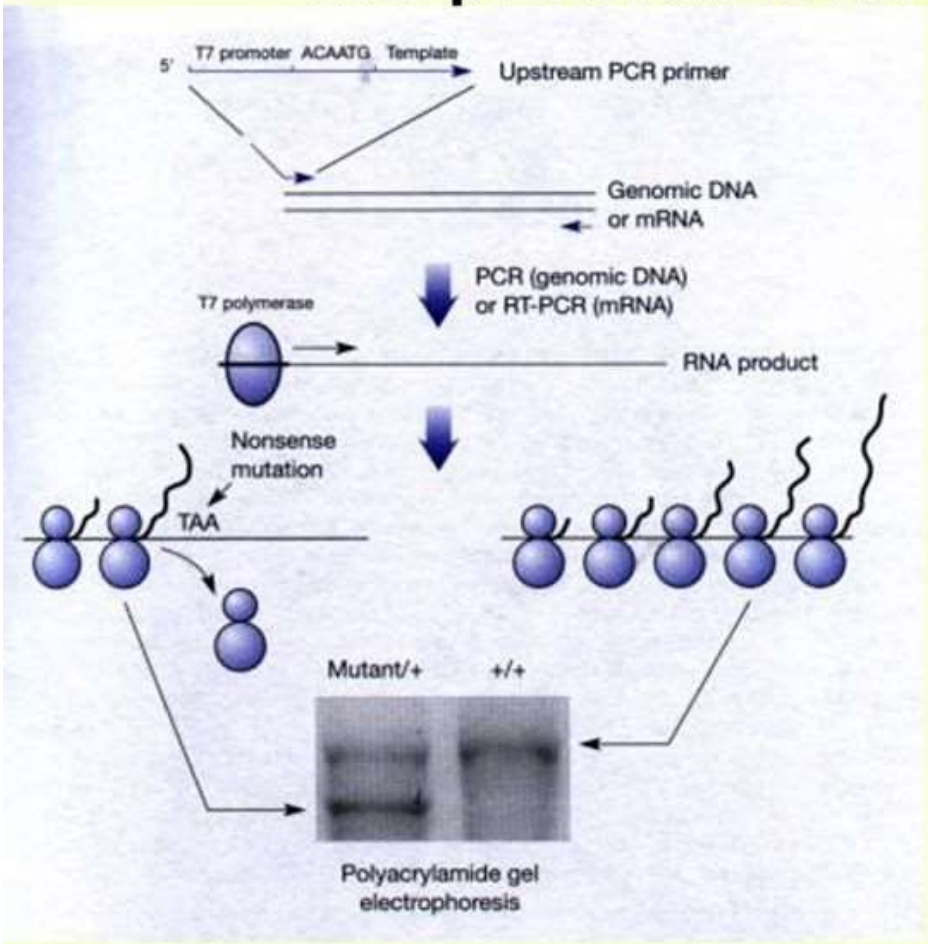
ALLELE-SPECIFIC PCR

- A diagnostic or cloning technique which is based on single-nucleotide polymorphisms (SNPs) (single-base differences in DNA).
- It requires prior knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3' ends encompass the SNP.
- PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer, so successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence.

Protein truncation test (PTT)

- This test is designed to search for a mutation in a gene by finding it directly in the protein encoded by this gene, when the consequence of the mutation is essentially the shortening of the peptide sequence (stop mutation, frameshift mutation, partial deletion, mainly in the very big genes).

Detection of a *BRCA2* mutation by the protein truncation test



The individuals gene is first amplified by PCR. The 5' PCR primer has incorporated the T7 promoter to enable RNA transcription and the resulting DNA is transcribed into RNA so that an *in vitro* translation system can be used to synthesise the protein product. Following electrophoresis on PAGE gel, the size of the protein is compared to that of the normal protein.

Thank you