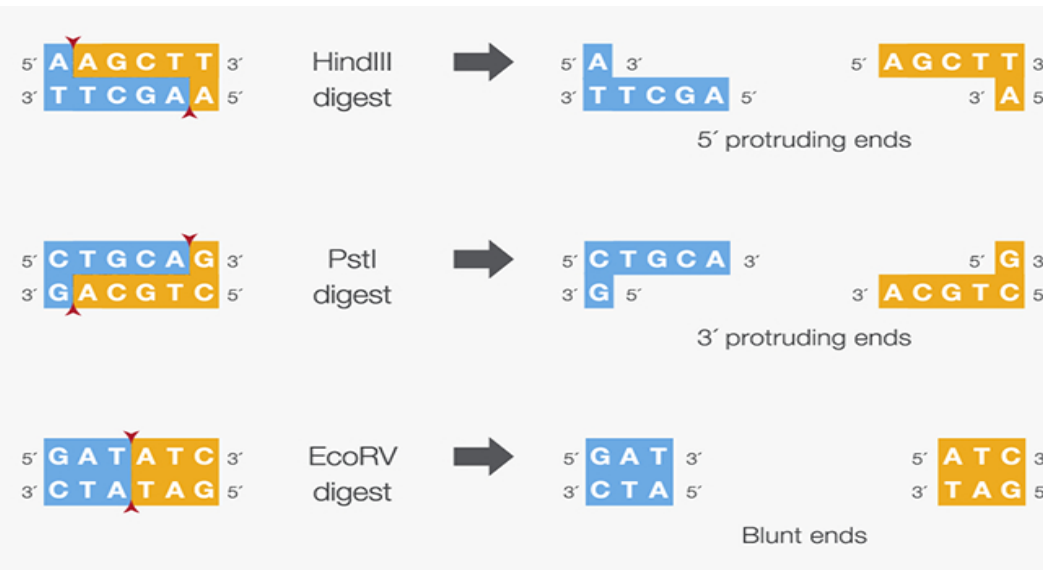
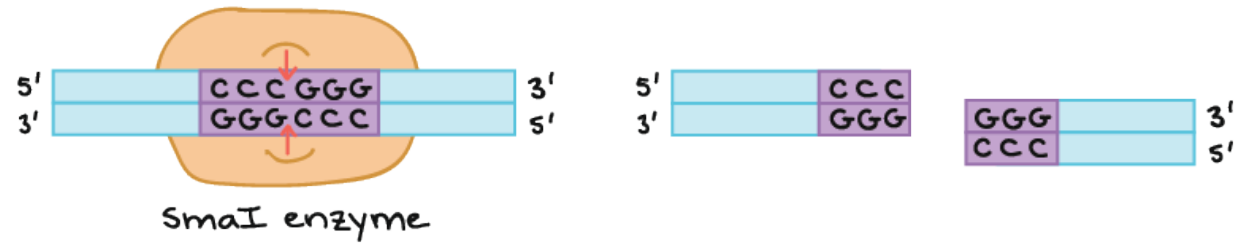

Restriction Fragment Length Polymorphism (RFLP)

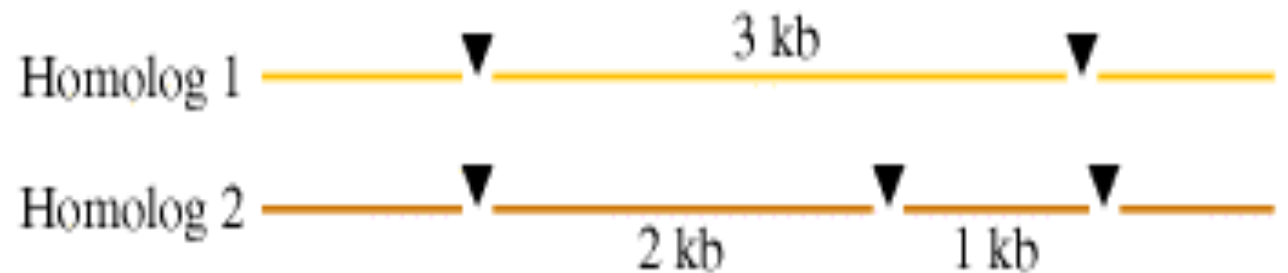
Restriction Endonucleases (RE):

- What are they?
- Types of cleavage.
- Specificity.
- Role and application.



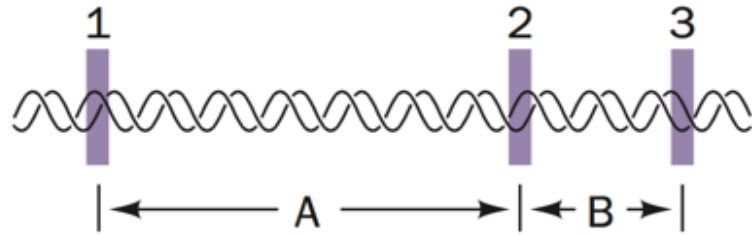
Polymorphisms and genetic variation:

- Individuality and genetic differences → create and eliminate restriction sites.
- > 1%.
- Sequence changes affect recognition sites ? → affect restriction fragment length.



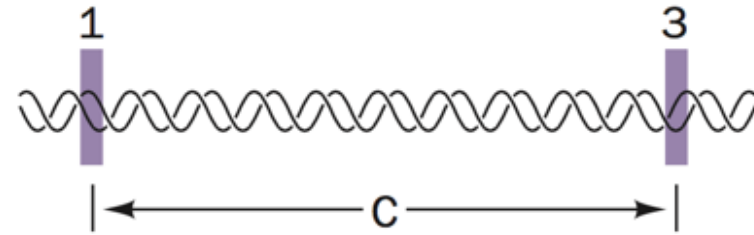
Chromosome I

DNA has
3 target sites



Chromosome II

DNA has only
2 of the target sites

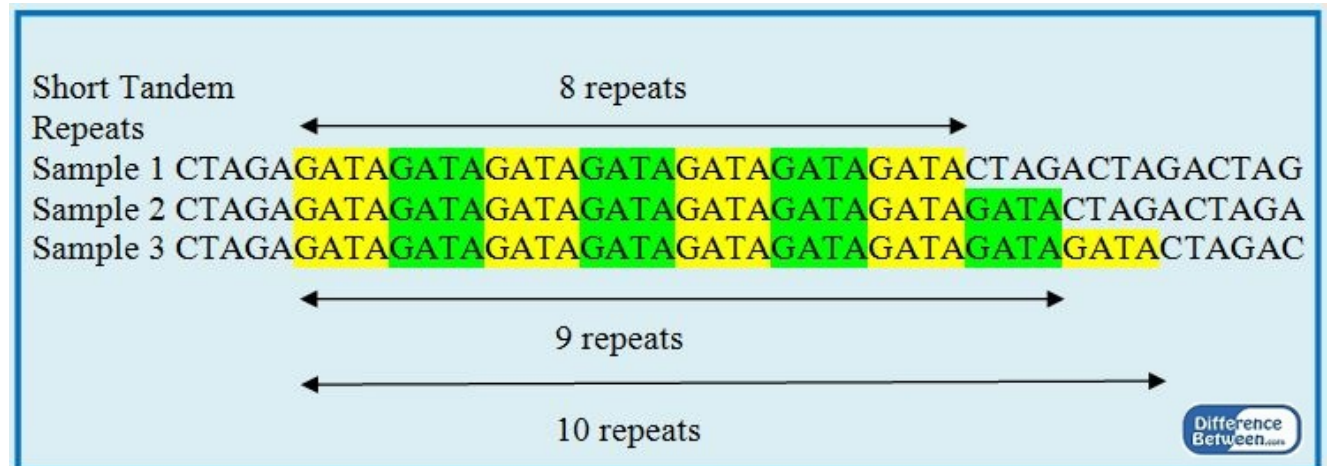


Cleave with
restriction enzyme
and electrophorese

Fragment C is
the size of
 $A + B$ combined

Restriction fragment length polymorphism (RFLP):

- Restriction fragment length polymorphism (RFLP) is an inherited difference in the **pattern of restriction**.
- It was invented in 1984 by the English scientist Alec Jeffreys.
- Analyse the DNA of genes, analysis of unique patterns in DNA fragments.
- Effect depend on the loci..



Principle:

- Restriction endonucleases cut lengthy DNA into short pieces.
- Each restriction endonuclease targets different nucleotide sequences.
- The distance between the cleavage sites of a certain restriction endonuclease differs between individuals.
- Hence, the length of the DNA fragments will differ across both individual organisms and species.

RFLP workflow:

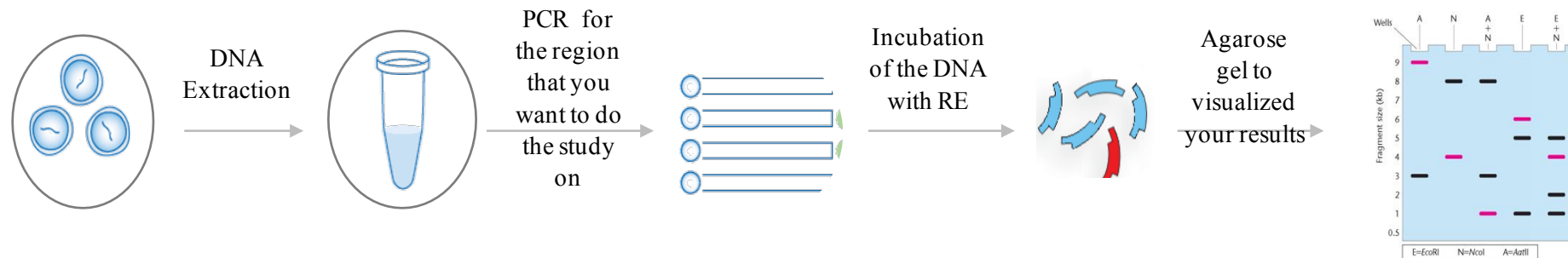
1st DNA Extraction.

2nd Perform PCR for the region of interest.

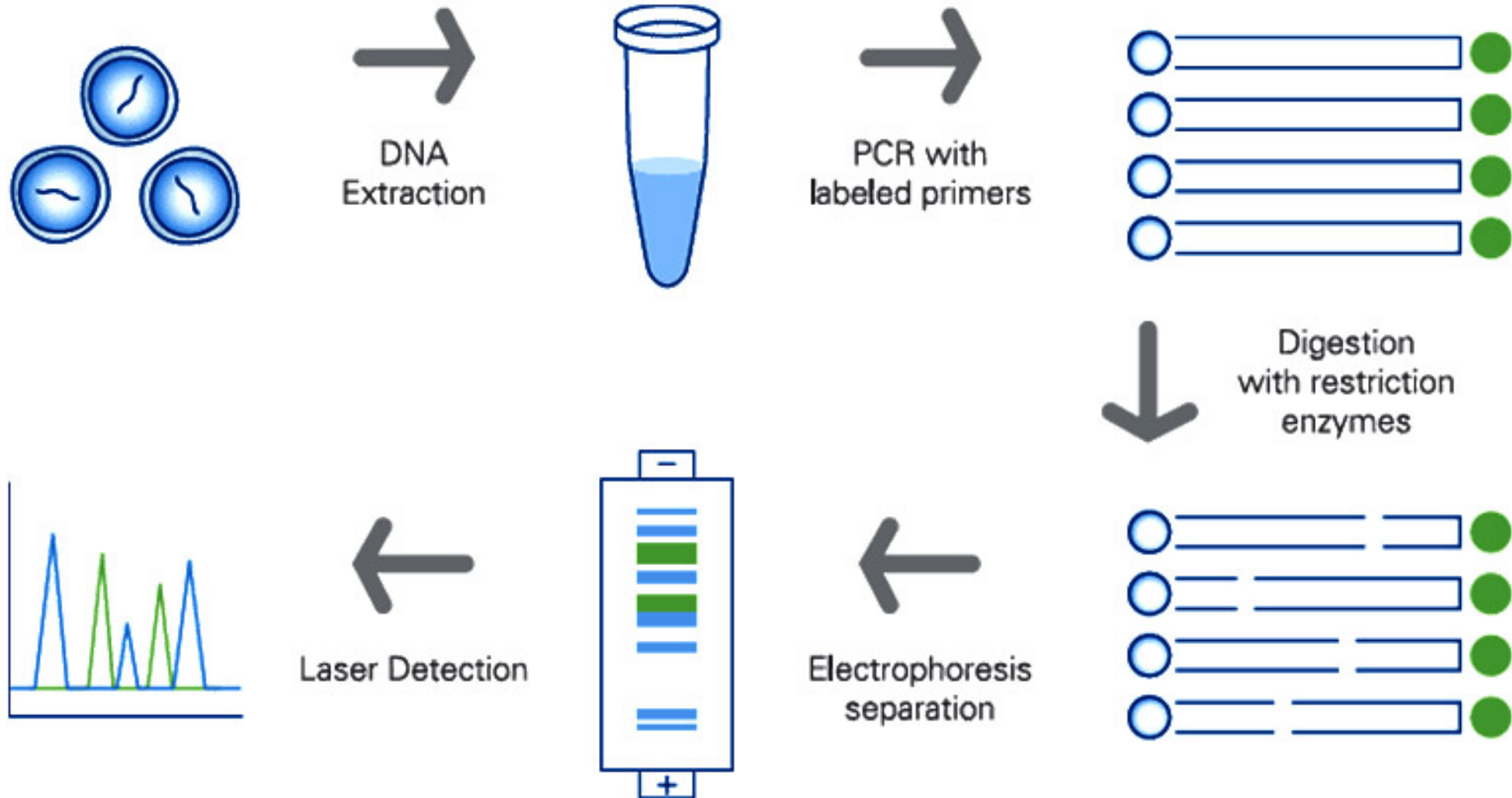
3rd DNA Fragmentation by RE.

4th Gel Electrophoresis.

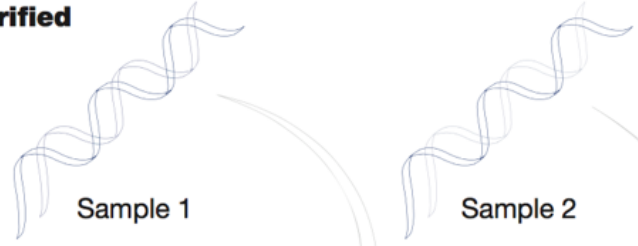
5th Visualization of Bands.



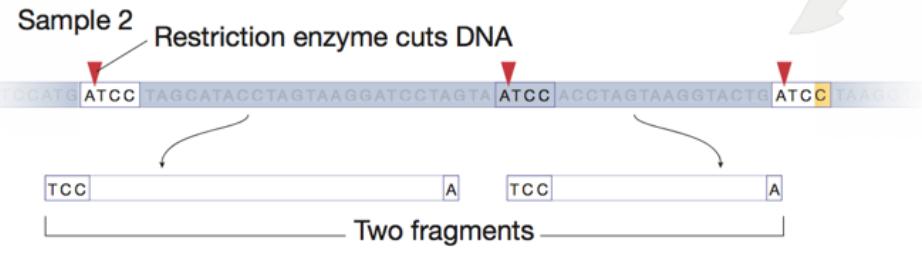
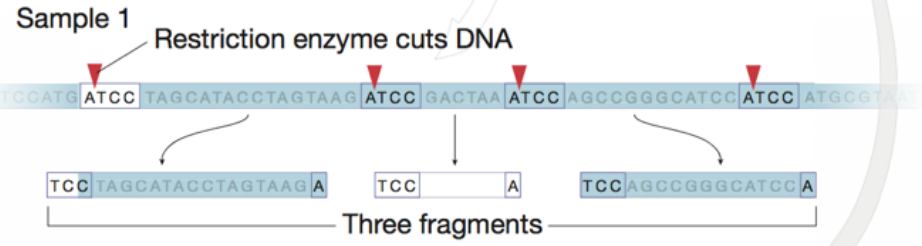
RFLP workflow:



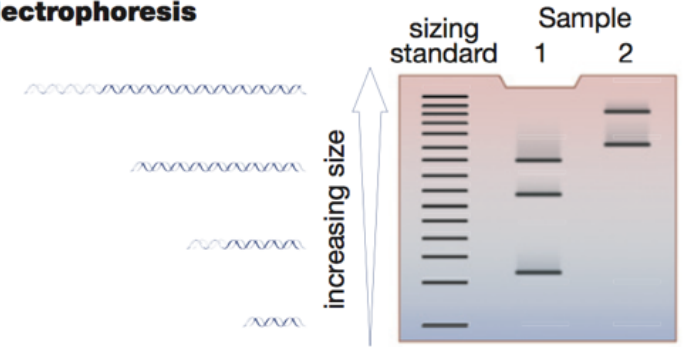
1. DNA Purified



2. DNA Fragmentation



3. Gel Electrophoresis

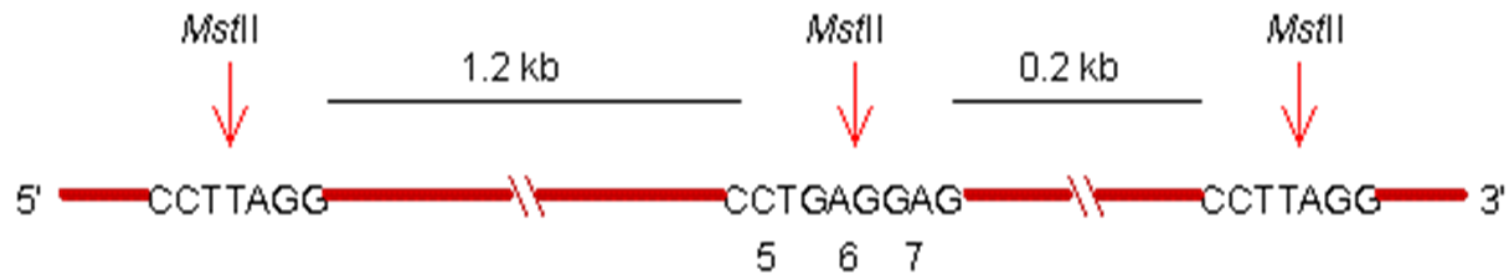


Applications of RFLP :

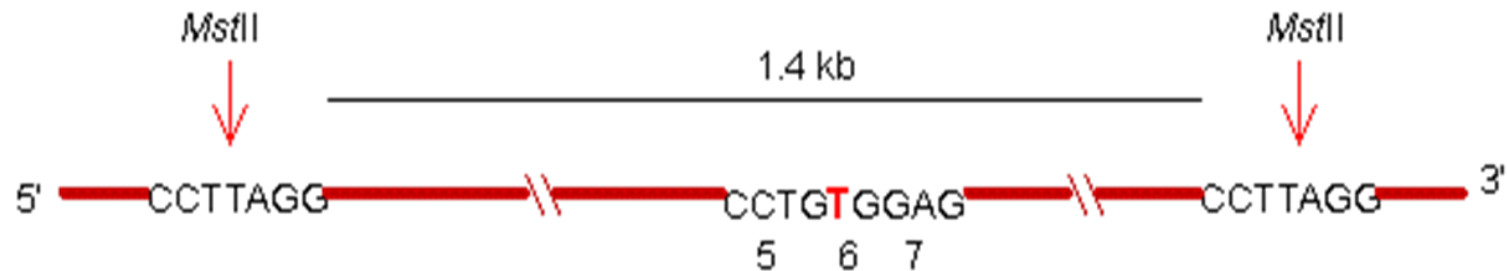
- Status of genetic diseases (Sickle cell disease, thalassemias).
- Source of a DNA sample.
- Genetic mapping.
- To identify a carrier of a disease-causing mutation in a family

Example of using RFLP in the detection of mutation in disease state:

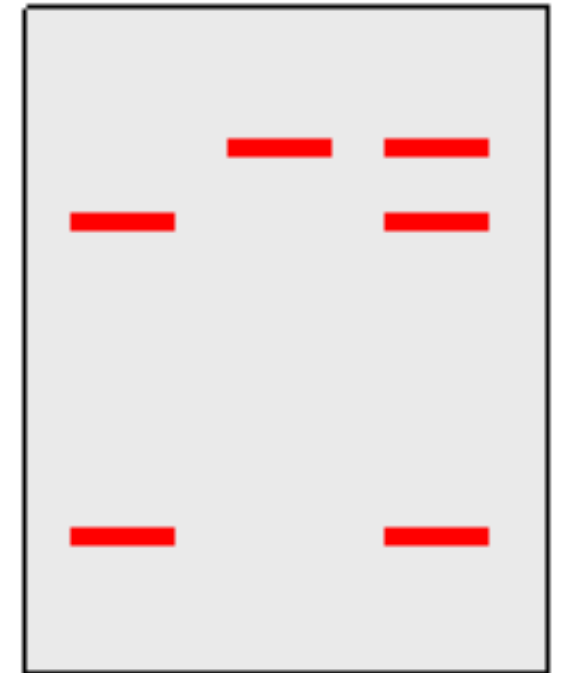
Normal cell



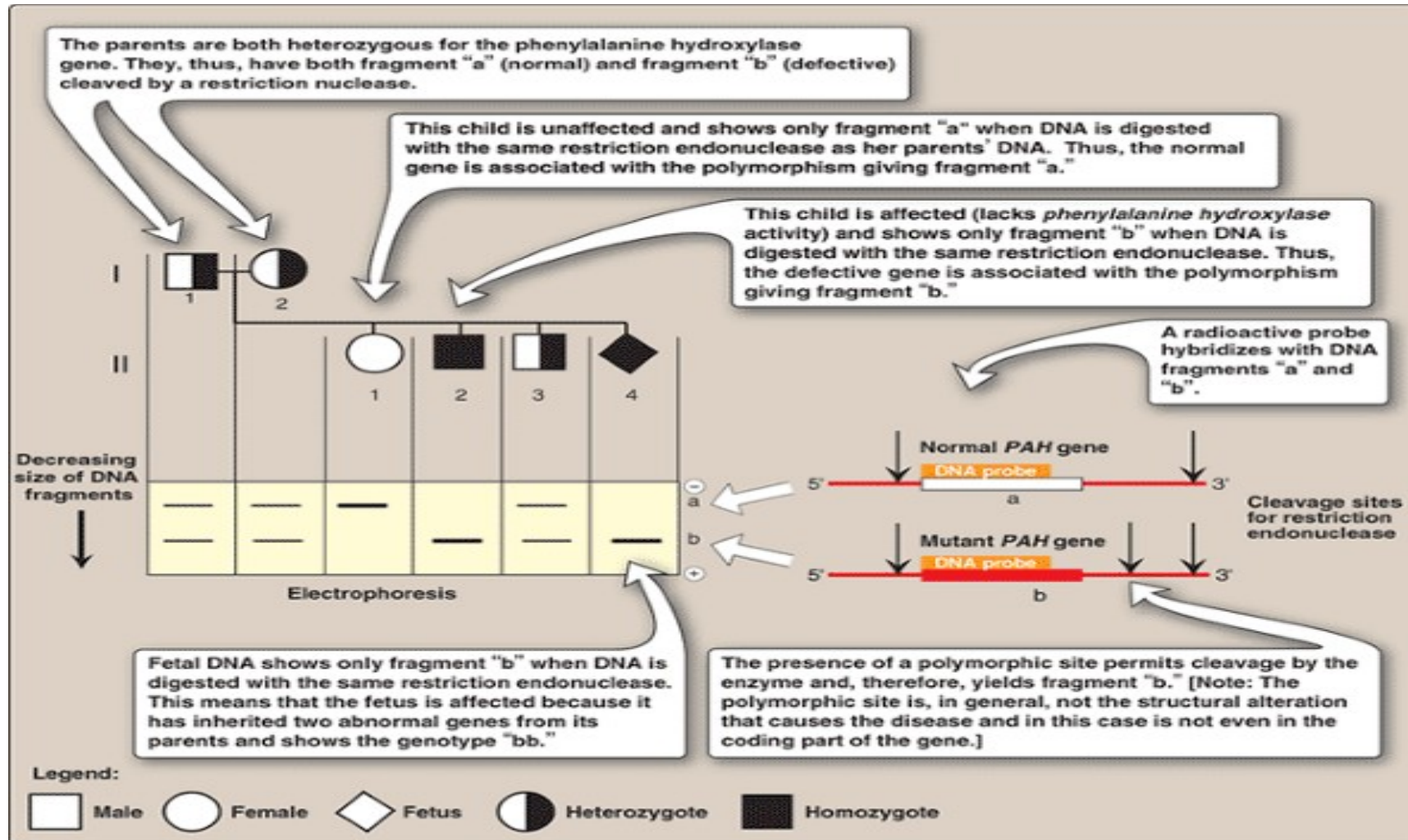
Sickle cell



*Mst*II restriction site:
'5-CCTNAGG-3'



Example of using RFLP in the detection of mutation in disease state:



Example of using RFLP in paternity test:

