Document 3: Polymorphic genes in population

- **Define polymorphic genes:**

A gene is said to be polymorphic when it has more than 2 alleles of frequency (>1%) in a population.

- **Define a wild type allele.**

A wild type allele is the allele that codes for the most common phenotype.

- **Examples of polymorphic genes**

1. Blood group
2. Major Histocompatibility Complex
3. Beta-globin gene

Major Histocompatibility complex MHC

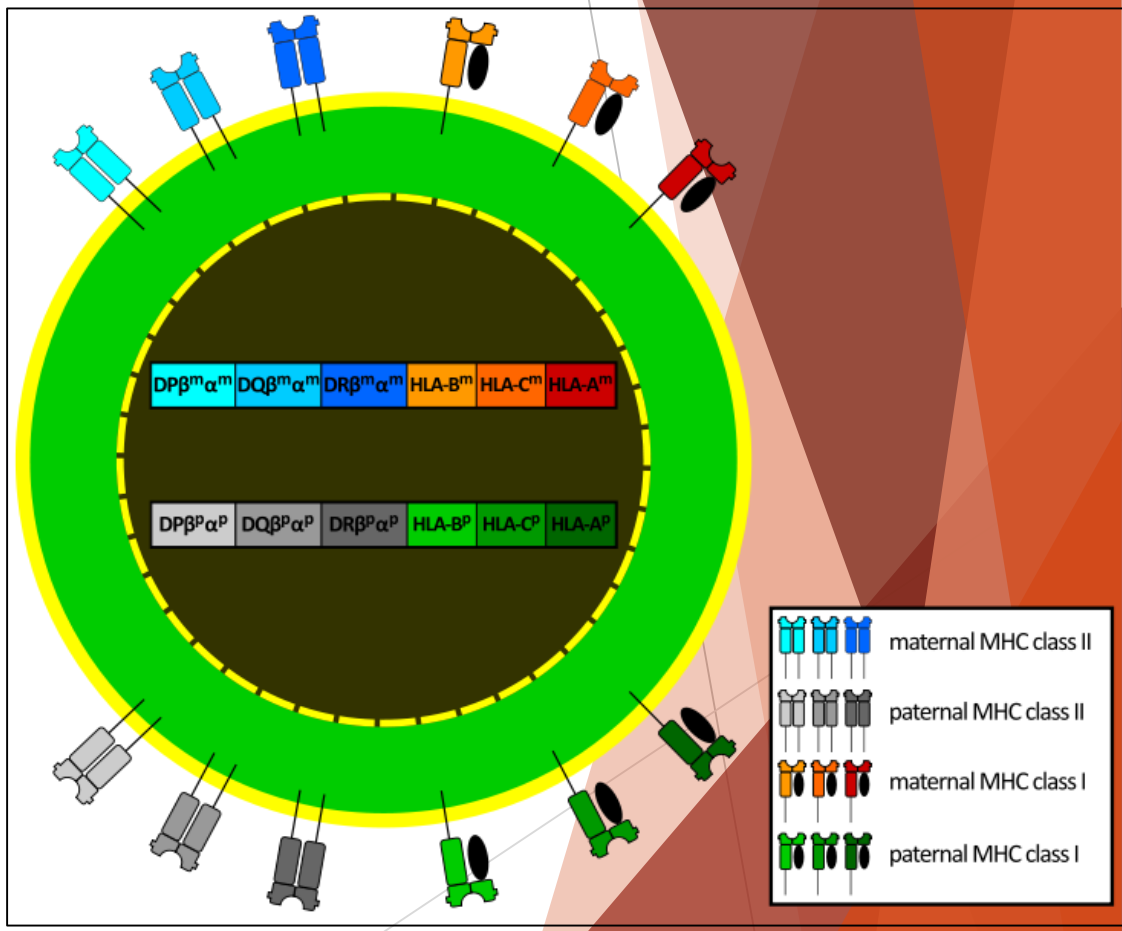
❑ Structure and Function:

- MHC is also called HLA (Human Leukocyte Antigen) because these proteins were initially described **on surface of leukocytes**

- **Structure:** large glycoprotein carried on the surface of all nucleated cells in the body

- **Function:**

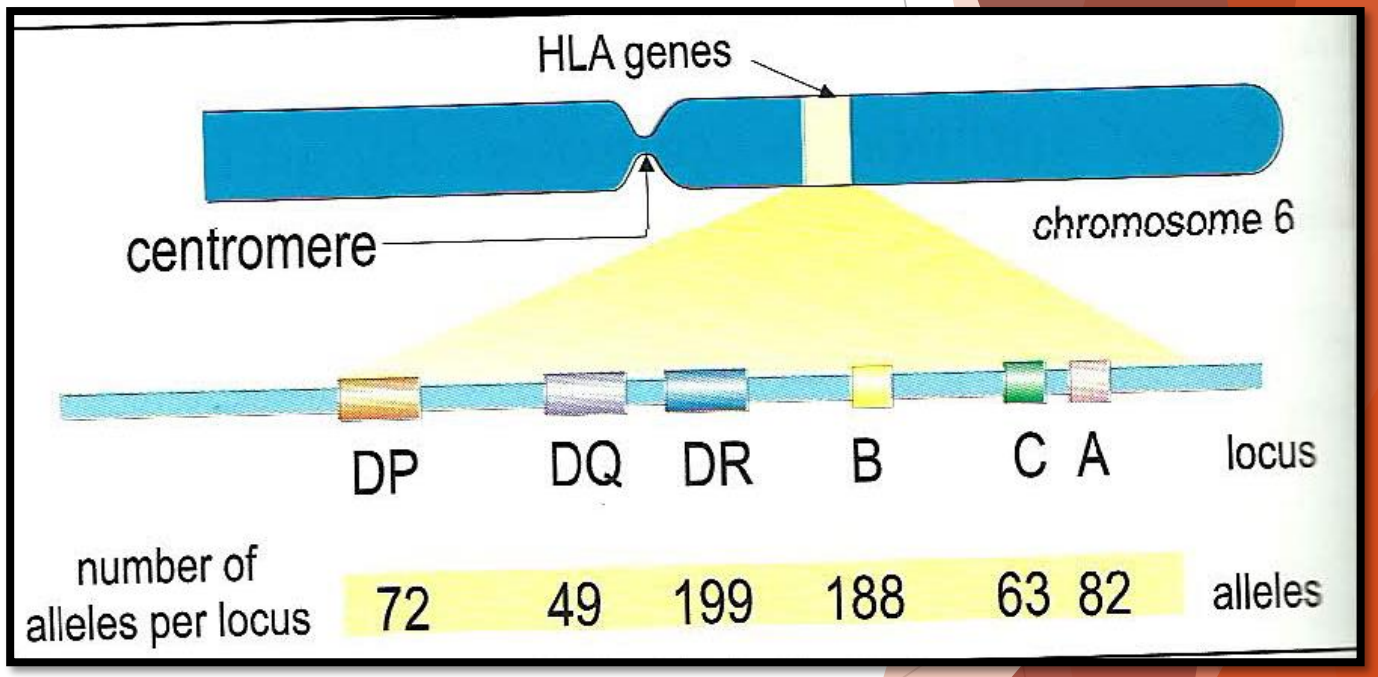
1. These proteins determine the compatibility of donors for organ transplant and are, thus, involved in graft rejection.
2. Other function will be discussed in Immune System.



Major Histocompatibility complex MHC

❑ Transmission:

- MHC is coded by **6 genes** (haplotype) carried by chromosome 6.
- Each gene has **many alleles** and alleles of each gene are **codominant.**
- **Haplotype:** set of genes very close to each other that are inherited as one block

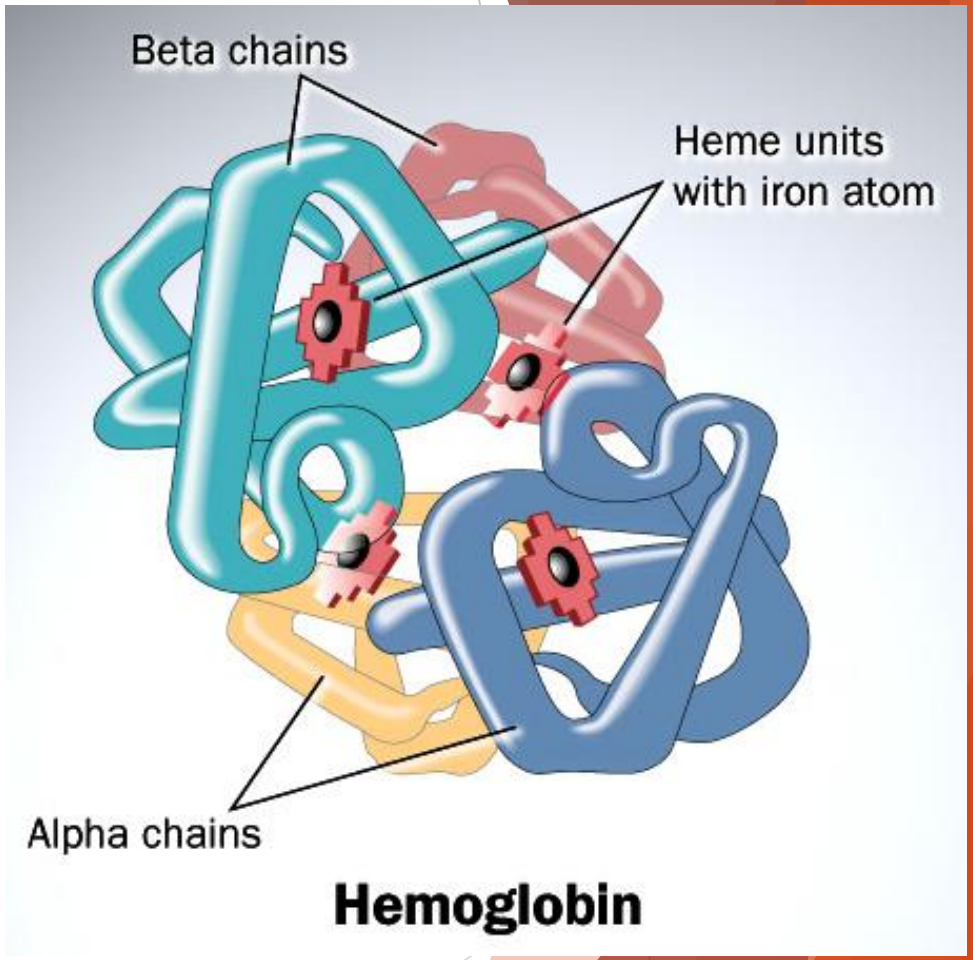


Note

Every individual has 6 genes that are involved in coding for MHC (A B C DP DQ DR) existing in 2 copies (one of paternal origin and one of maternal origin). Each gene has many different alleles and all are codominant. This makes it practically impossible for 2 individuals to have exact combination of the all 6 HLA loci. On the other hand, identical twins have same genetic information so, they have the same alleles at each of the loci in the MHC complex.

Beta (β) globin gene

- Hemoglobin contains 2 kinds of polypeptides
  - 2 alpha
  - 2 beta
- Beta globin gene is coded by gene that has 150 alleles
  1. Many code for the normal functioning β-globin polypeptide.
  2. Few of them codes for abnormal beta globin leading to disease such as β-thalassemia.



Note: A mutation in the genes may severely alter β-globin protein leading to dysfunctional hemoglobin molecule that cannot bind iron properly

- The phenotypic consequence of a mutation depends on its location on the gene.
- The severity of the mutation in β-thalassemia depends on whether one or both alleles are affected.
- Out of the 150 mutations identified worldwide, at least 17 mutations have been reported in Lebanese population.

codon	mutation	new codon or new sequence	phenotypic consequence and clinical signs
2	subs.	C A T	functional hemoglobin
6	subs.	G T G	rod-shaped hemoglobin or fibers ; deformed RBC: sickle cell anemia
6	subs.	A A G	hemoglobin C: mild anemia
6	del.	G - G	Thalassemia severe anemia
16	del.	G G -	
17	subs.	T A G	
39	subs.	T A G	
41 - 42	del. 4 nucl.	- - - - T T	
71 - 72	ins.	T T T T A G T	
29	subs.	G G T	3 types of β- thalassemia detected in Lebanon*
30	subs.	A A G	
44	del.	T C -	

Polymorphic gene of B- globin gene is due to the presence of diverse alleles in the human population. These alleles are the results of different types of mutation insertion, deletion, or substitution of DNA nucleotide.

The severity of B- thalassemia depends on the site, type and extent of mutation of beta globin gene. In general, substitution mutations are less severe than deletion or insertion, especially if the substitution leads to an amino acid characteristically similar to that of the original amino acid (example lysine and arginine). However deletion of a long stretch of beta globin gene leads to severe thalassemia.
Moreover, one must note that the severity of disease depends whether the individual carries one or 2 mutant alleles.

Document 4: Detection of genetic polymorphism

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Genetic polymorphism

Mutation → Leads to → Difference in nucleotide sequence of the gene

↓

Different forms of the same gene

↓

Multiple alleles

↓

polymorphism

So how can we detect the existence of change in nucleotide sequence?

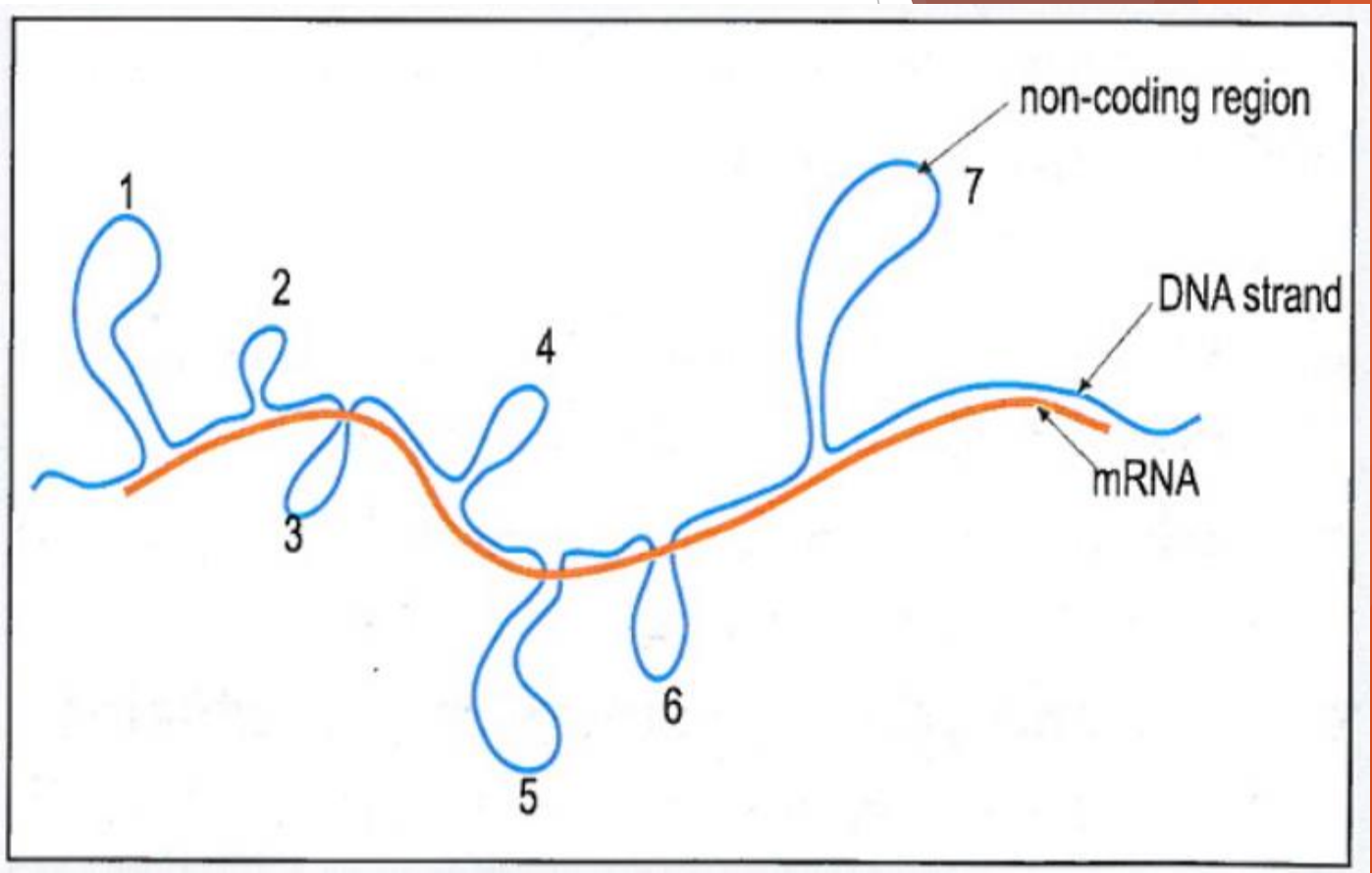
- How can we detect genetic variation?

By observing the variation of the phenotype of organisms.

- Does all the DNA we have code for proteins and thus is expressed at the level of the phenotype??

The next document is obtained by using a synthetic radioactive mRNA and the corresponding DNA strand, in the case of certain DNA strands.

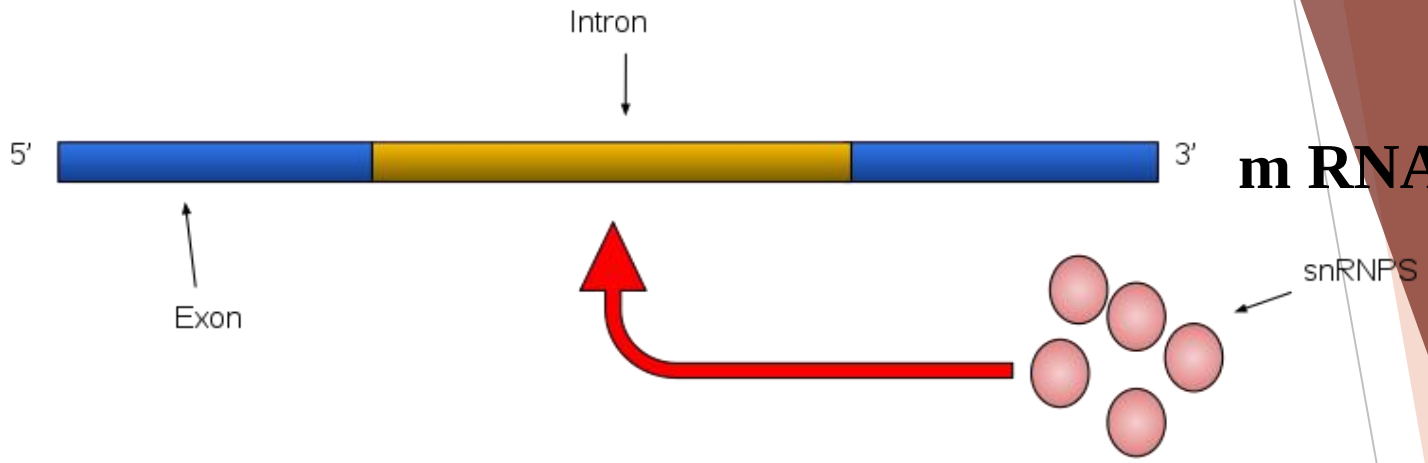
Explain how certain gene mutations might have no effect on the phenotype expression.



Explanation:

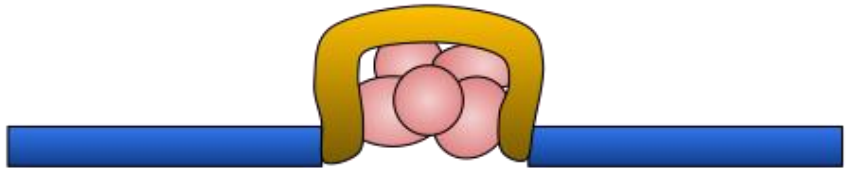
Many eukaryotic genes have interrupted coding sequences where exons (coding sequences) are separated by introns (non-coding sequences)
Between its transcription in the nucleus and its passage into the cytoplasm, where it will be translated into a polypeptide, the mRNA undergoes a maturation process.
Therefore, a synthetic mRNA which is usually established from the amino acid sequence of a specific protein, is only formed by the transcript of the coding sequences. When such a mRNA and a DNA strand are put together, they will bind by complementary base pairing, only at the level of the exons; the DNA buckles visible in the document are thus the non coding regions of the gene.
Mutations occurring in these introns and whose transcript will be eliminated during mRNA maturation, could not affect the phenotype of the individual: These are neutral mutations.

Step 1.
A group of five snRNPS's, or ribonucleoproteins, are needed to bind to the intron of pre-mRNA and remove it to leave only the exons.

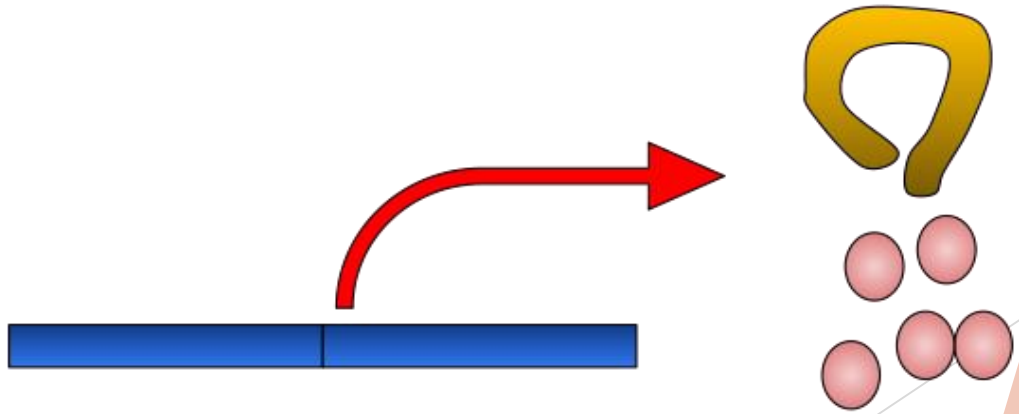


m RNA

Step 2.
The snRNP's bind to the intron and cause it to fold into bring the 5' and 3' ends of the intron closer together, making a loop. The ends of the exons also move closer together to eventually join together.



Step 3.
The intron detaches and the splice sites connect to make a mature mRNA. The introns were previously thought to be "junk" afterwards but most are used in other processes. The snRNP's detach from the intron and are used for more splicing.



Coding and non-coding regions of the genome

Only about 1 percent of DNA is made up of protein-coding genes; the other 99 percent is noncoding. Noncoding DNA does not provide instructions for making proteins. Scientists once thought noncoding DNA was “junk,” with no known purpose. However, it is becoming clear that at least some of it is integral to the function of cells, particularly the control of gene activity.

Detecting genetic polymorphism in coding regions of the genome:

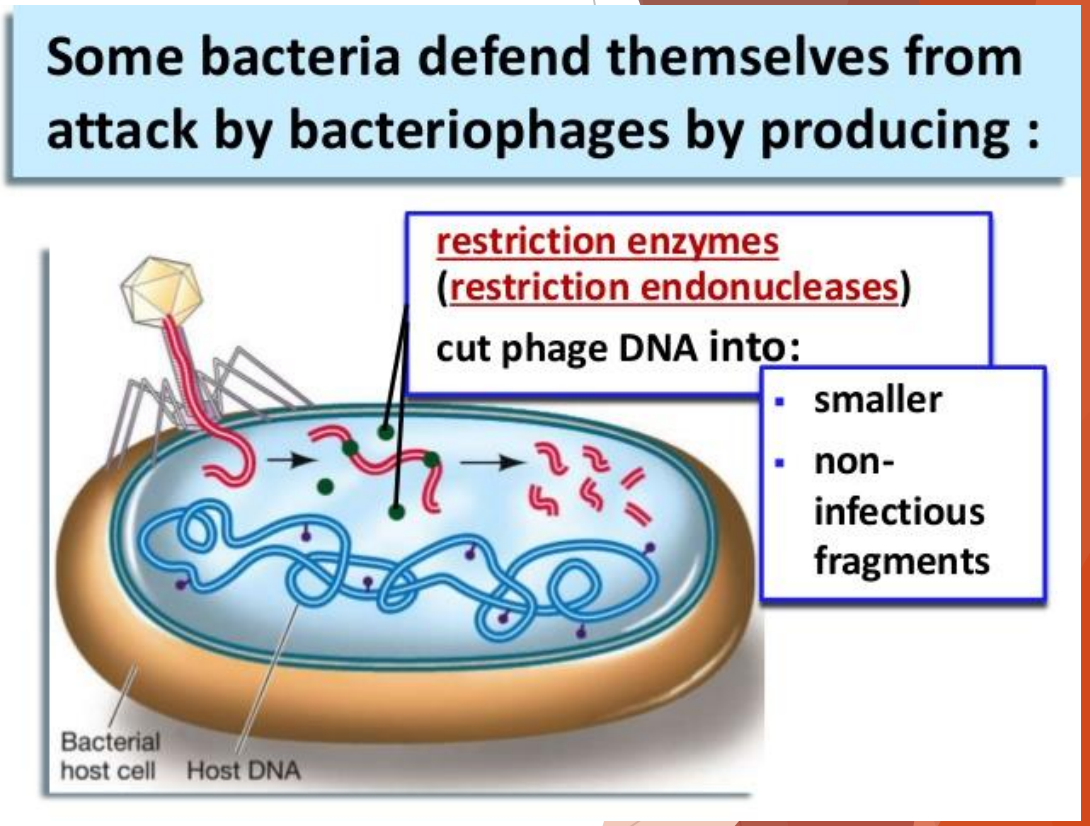
- Mutations in the coding regions of the genome can be detected by variation of the phenotype since such mutations usually affect the phenotype.
- BUT,
  Mutations taking place in the non-coding regions of the genome do not usually affect the phenotype, these mutations need certain procedures to be detected and these procedures require:
  - cutting the DNA
  - Visualizing the obtained fragments (the difference in the number and size of the observed fragments tells about the existence of genetic variation)

Cutting DNA / Restriction enzymes

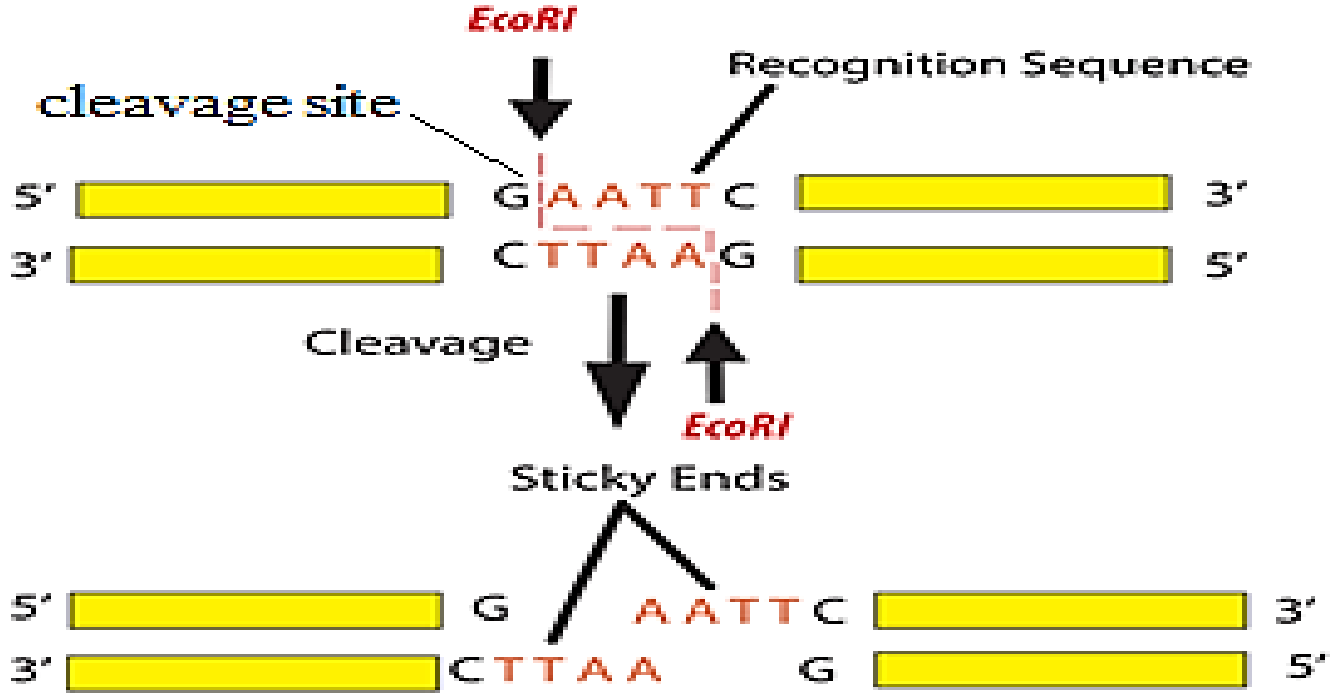
Also called **restriction endonucleases**, are biological protein scissors produced by bacteria that can cleave DNA at specific sites along the molecule.

WHY?

Bacterial cells produce these enzymes to defend themselves against bacteriophages (viruses that infect bacteria and live at their expense).



Recognition sites VS cleavage site of restriction enzymes



- Each restriction site cuts the DNA at a specific sequence (palindromic) called the recognition site. This recognition site is cleaved at a specific position of nucleotide called the cleavage site.

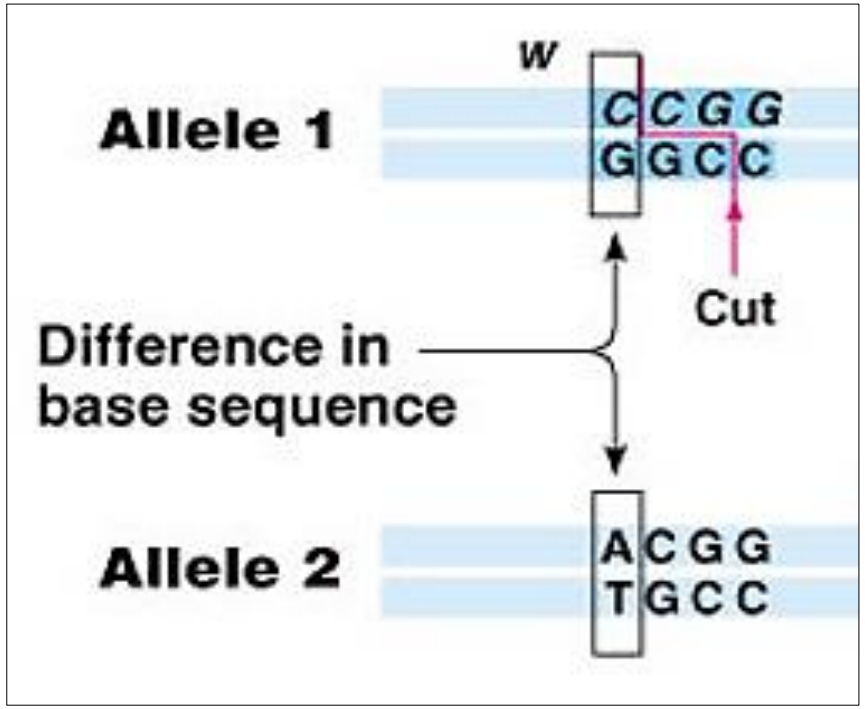
Action of Restriction Enzyme : Recognition sites VS cleavage site

Hae. III

2 restriction fragments resulting from the action of the enzyme Hae. 3 on the given DNA molecule

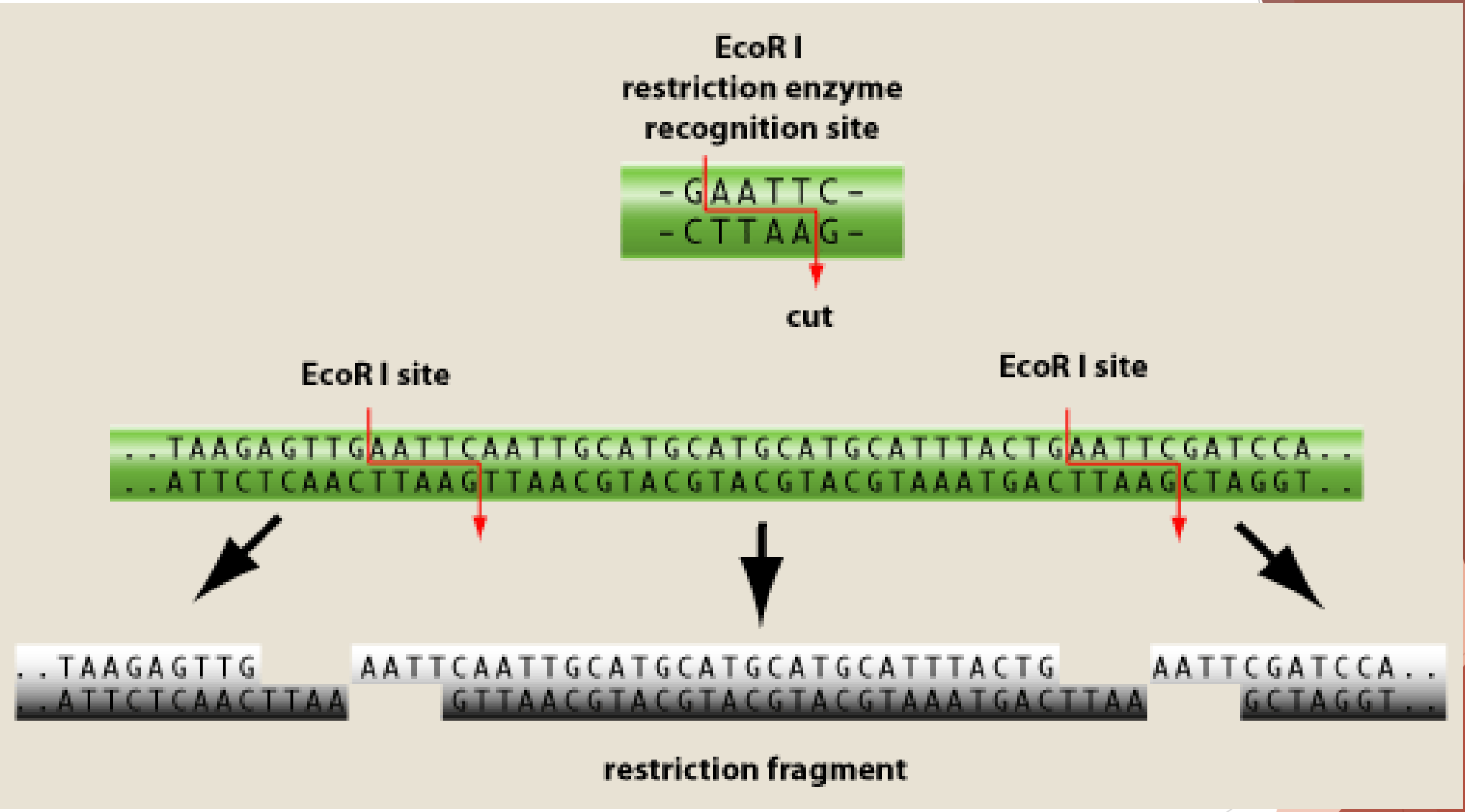
In case the recognition site of a restriction enzyme is submitted to a mutation:

- What happens?



If mutation occurs within the recognition site, the restriction enzyme will not recognize the sequence and will cut 2 different alleles of the same gene at different sites.

This yields DNA fragments that differ by their **number** and **size**.



The number of restriction fragments= number of recognition sites +1

1. How can DNA cutting by restriction enzymes help detect the genetic polymorphism??

Restriction enzymes cannot cut the 2 different alleles in same manner if they have different sequence of nucleotides **at the level of the recognition sequence of the restriction enzyme.** In this case, it will give fragments of different number and size for 2 different alleles. So the difference in the number and size of fragments indicate the occurrence of mutation and thus genetic polymorphism

How can we visualize the obtained fragments and thus have evidence of genetic polymorphism??

Gel Electrophoresis

- **How can we detect these fragments?**

  separating them by gel Electrophoresis

- **Principle:**

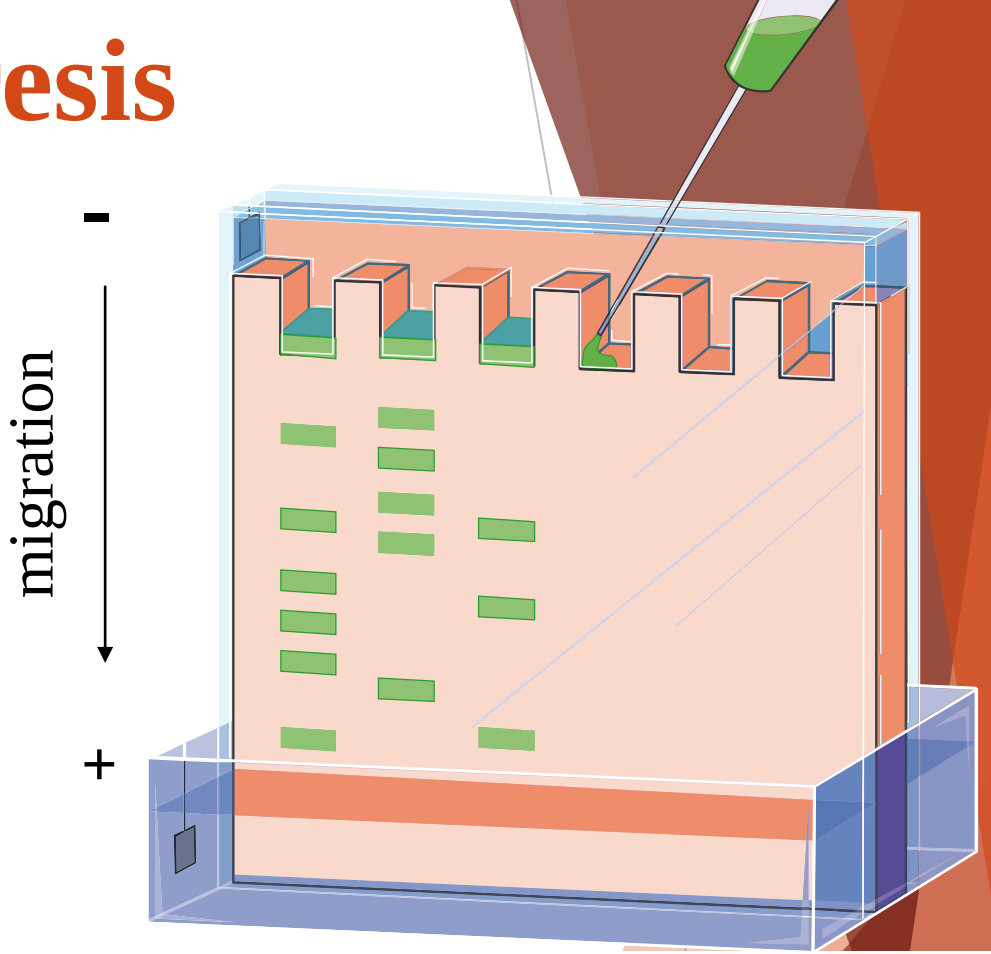
  1. DNA is negatively charged due to phosphate group.
  2. During electrophoresis bands migrate from negative pole to positive pole.
  3. Migration depends on size: smaller fragments migrates further (Larger fragments migrate slower than smaller ones)

- **Role of Ethidium Bromide (Et-Br)**

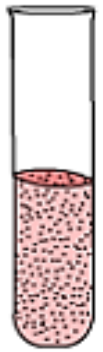
  Dye that binds to DNA and fluoresces under UV light.

- **Restriction map:**

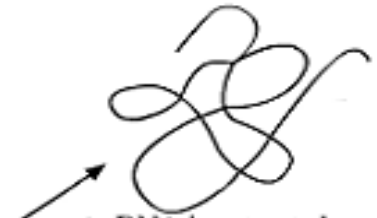
  It is the generated pattern of bands



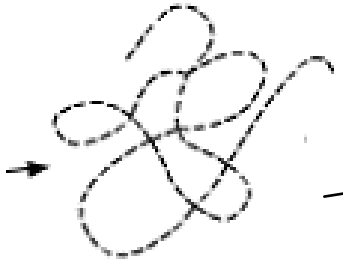
A technique that is used for the analytical separation of charged fragments of DNA by running these fragments in an electrified gel. It is used to assess genetic polymorphism i.e. to detect mutations especially in the non-coding regions of the genome which don't affect the phenotype.



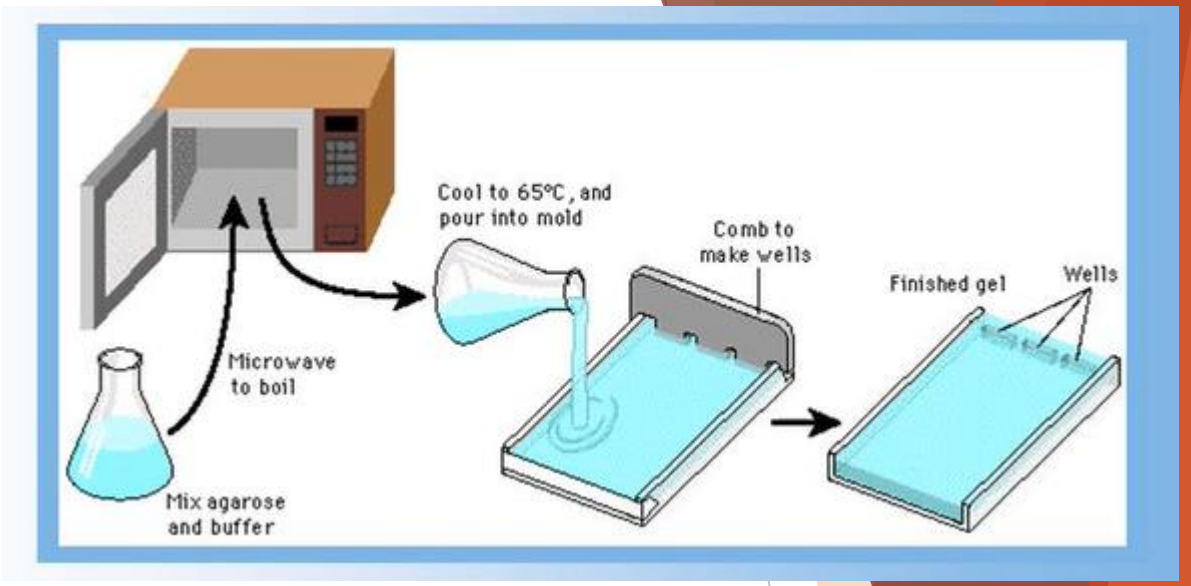
1. Blood sample

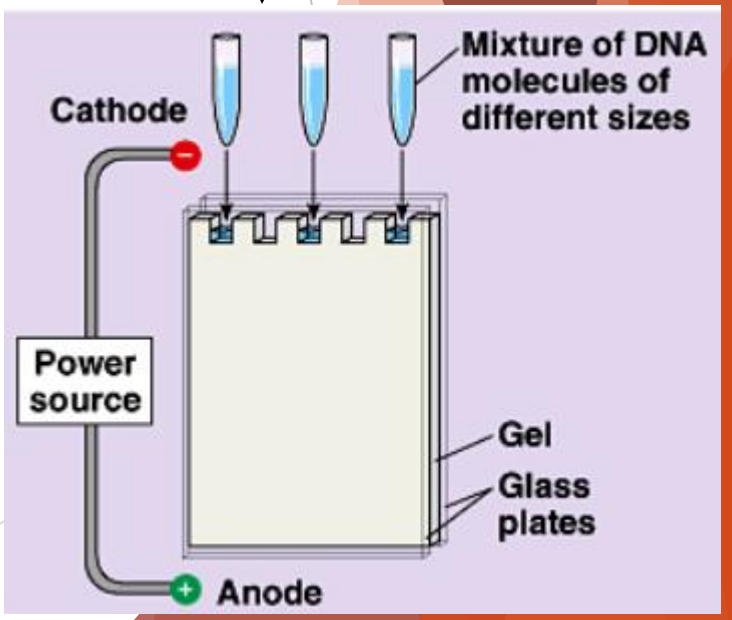


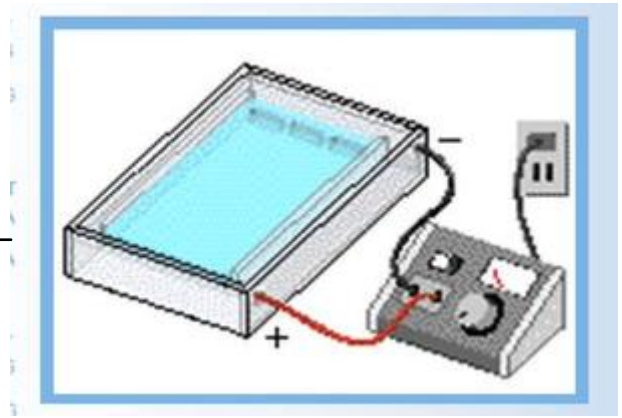
2. DNA is extracted from blood cells

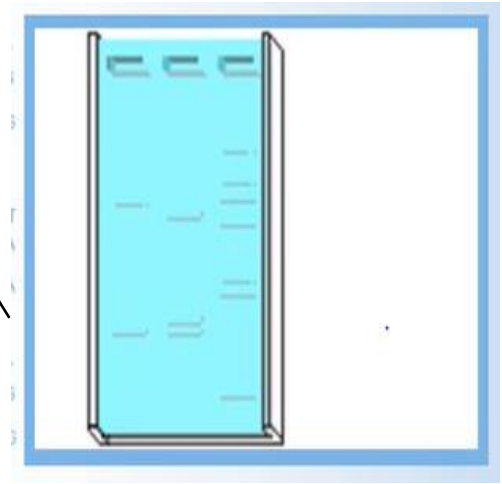


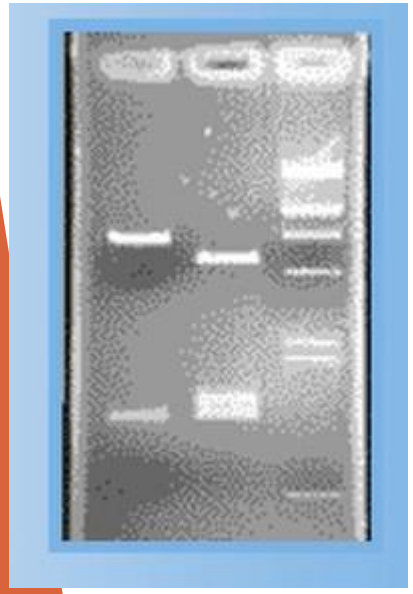
3. DNA is cut into fragments by a restriction enzyme











Procedure:

1. Extraction of DNA
2. Digestion of DNA by restriction enzymes obtaining thus a mixture of restriction fragments.
3. Preparation of agarose gel, and put it in an electrophoresis chamber.
4. Mixtures of restriction fragments to be analyzed are loaded into slots (wells) made in the agarose gel.
5. Electric current is applied so that the restriction fragments start migration through the gel from the negative pole to the positive one. The shorter fragments migrate faster i.e. further than the longer fragments.
6. Staining of the gel with ethidium bromide (E.B) in order to visualize the fragments.
7. After a certain time, the electric current is stopped and a photo of the gel can be taken.

The result is that restriction fragments are separated according to their length. Each will form a distinct band; the generated pattern of bands is referred to as restriction map.

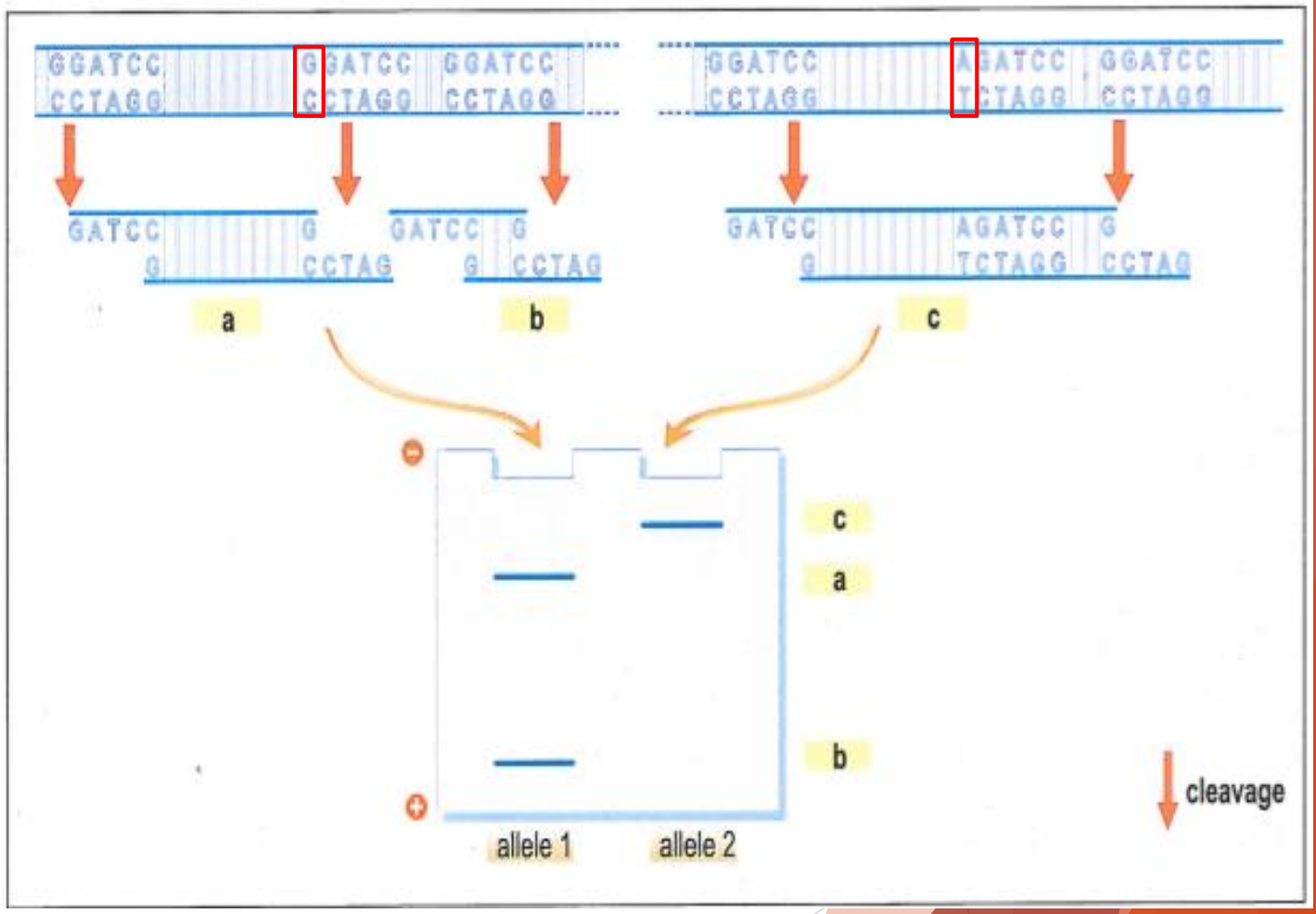
Electrophoresis of two different alleles cleaved by the same restriction enzyme

1- Indicate the number of bands obtained for each of alleles 1 and 2 after electrophoresis.

Allele 1: 2 bands, Allele 2: 1 band

2- How can you explain this difference?

The electrophoregram of allele 1shows two band which means that it is cleaved by the restriction enzyme in two fragments, that is it contains one recognition site of the used restriction enzyme, whereas the electrophoregram of allele 2 shows 1 band which means that it is not cleaved, that is it doesn't contain any recognition site of the used restriction enzyme.



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3- Cause of difference between the two alleles

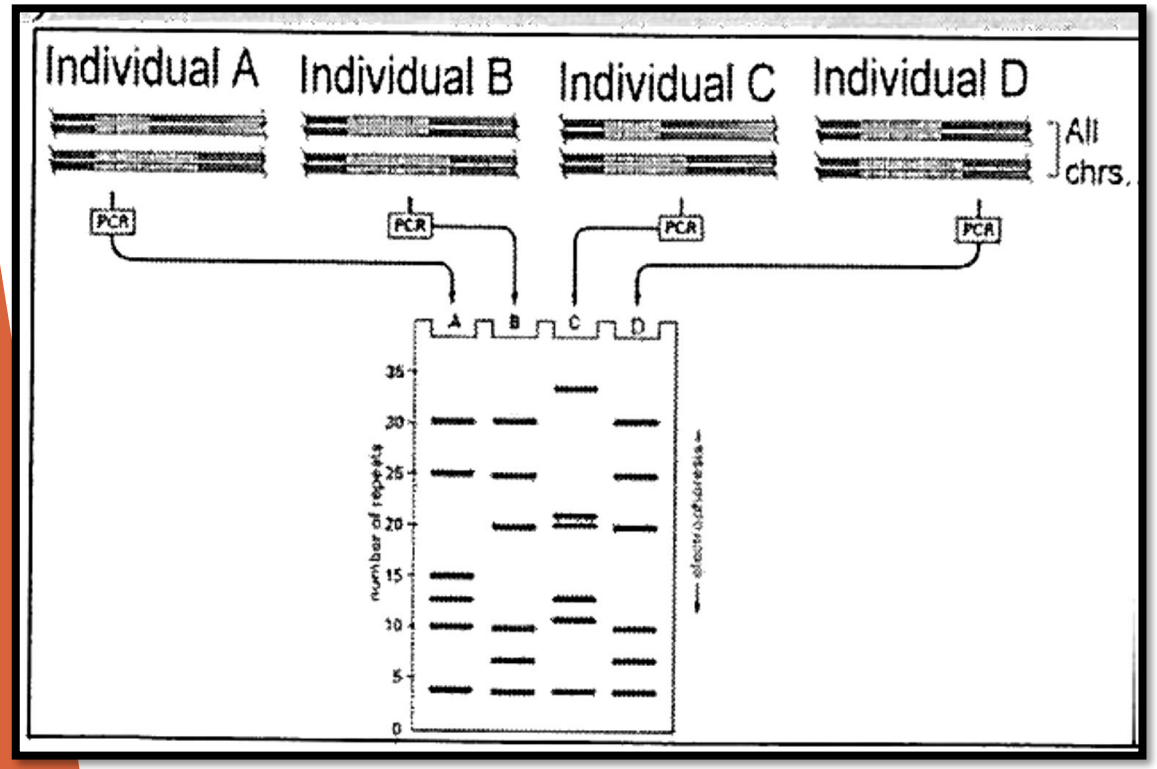
Due mutation that has occurred at nucleotide position 16 which happened to be at the level of the second recognition site and changed the its sequence and thus it can't be recognized by the corresponding R.E.

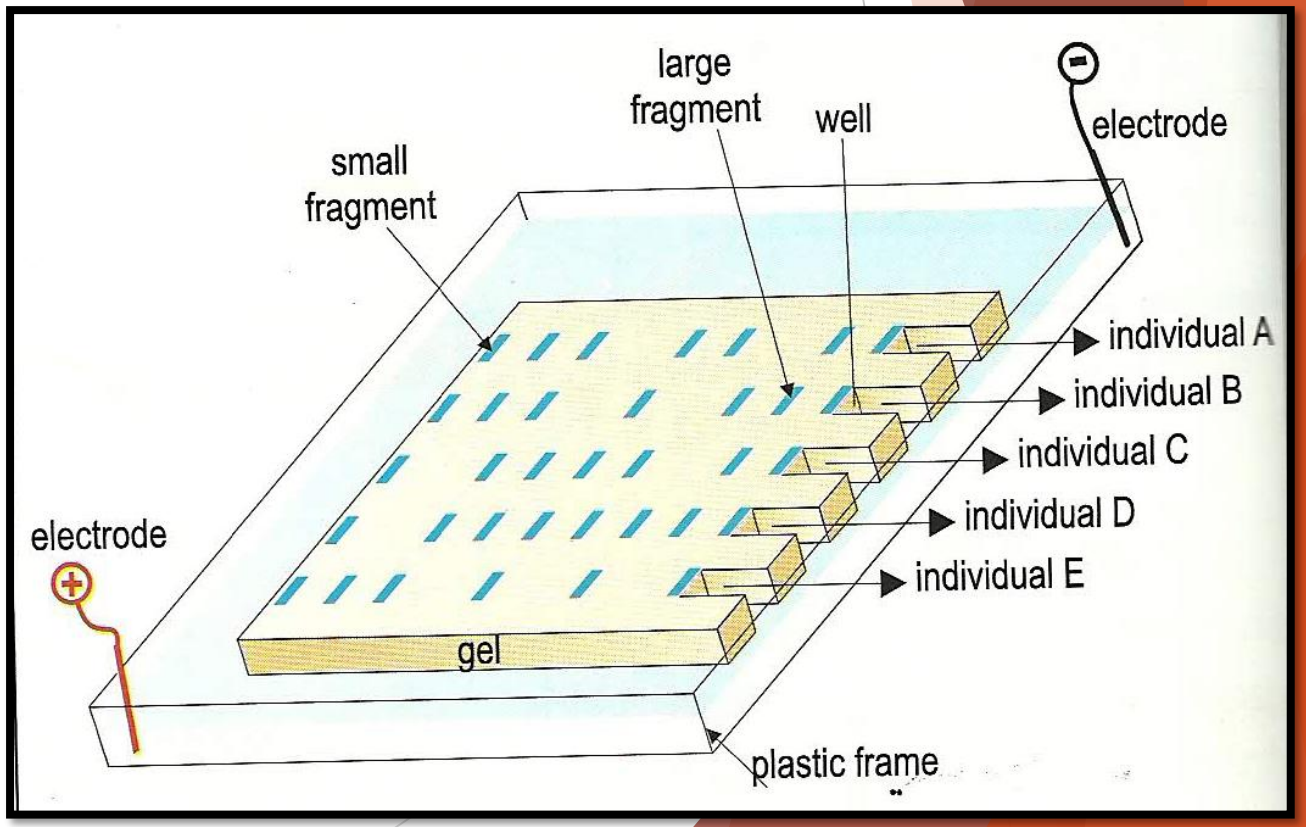
So, Can we say that genetic variation can be detected by electrophoresis??

RFLP or Restriction Fragment Length Polymorphism

- Restriction fragment length polymorphisms, or RFLPs, are differences among individuals in the lengths of DNA fragments cut by restriction enzymes (difference in the restriction maps between many individuals).
- **Aim of RFLP:**

It is used to genetically differentiate between organisms by comparing the same DNA regions in different individuals.

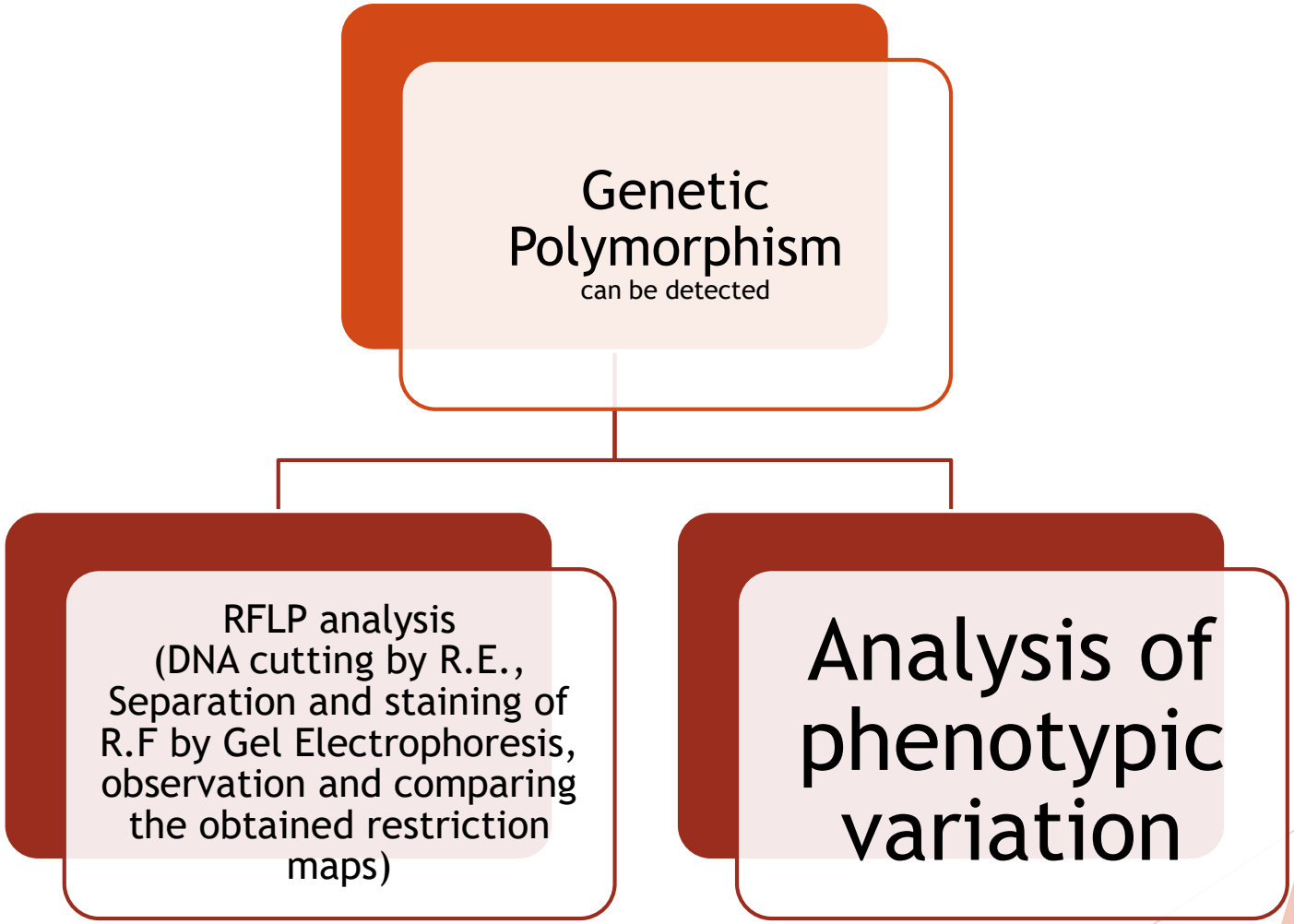




“The genetic polymorphism in a population can be assessed by analysis of phenotypic variation and by RFLP”. Explain this statement.

Genetic polymorphism can result from mutation at the level of coding regions of a gene which can be detected by phenotypic modifications. On the other hand, there are non-detectable mutations which exist in non- coding regions of DNA. These regions are not transcribed and thus don’t lead to any modification in the phenotype. These non-coding regions contain multiple restriction sites and these sites are polymorphic (restriction-site diversity). This characteristic is used to obtain different restriction maps between the individuals which called RFLP.

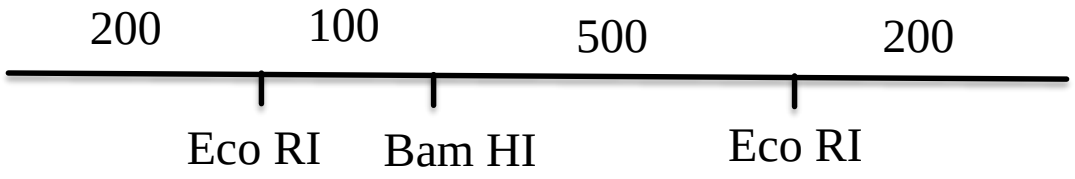
Original Problem: How can we detect genetic variation?

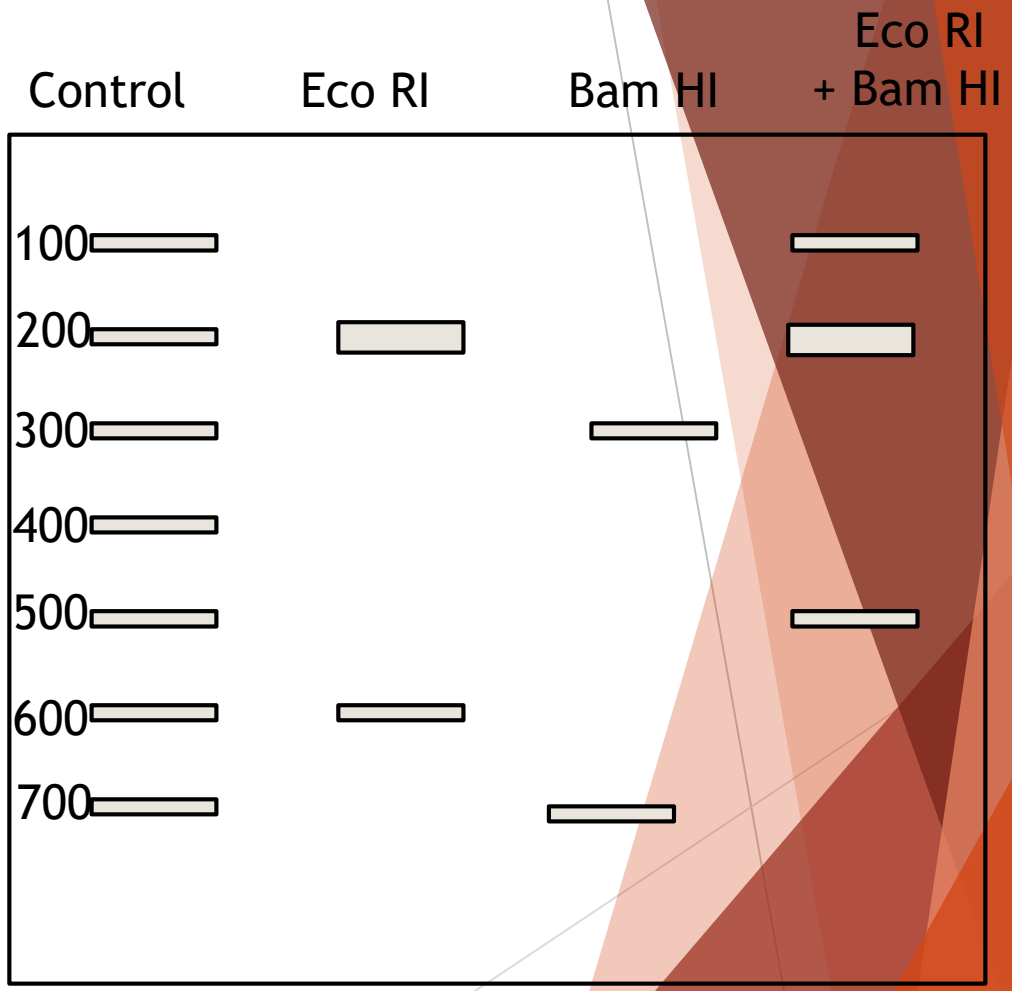


Application 1:

Two restriction enzymes (Eco RI and Bam HI) were used to cut a piece of DNA. The results are represented in the document below (the thick band corresponds to two fragments).

Locate the respective restriction sites of enzymes Eco RI and Bam HI (one possibility)



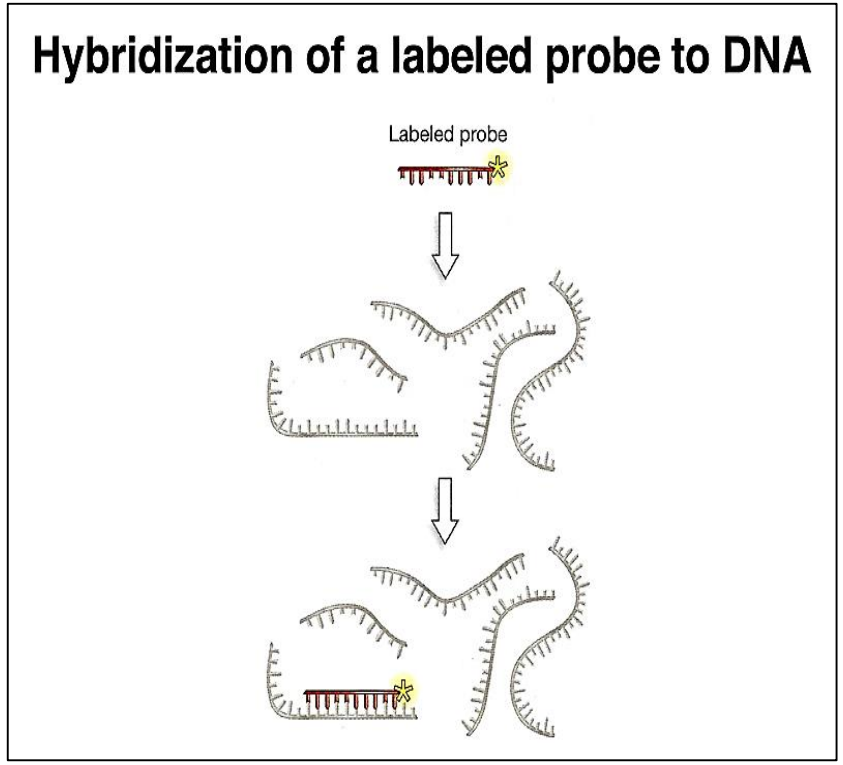


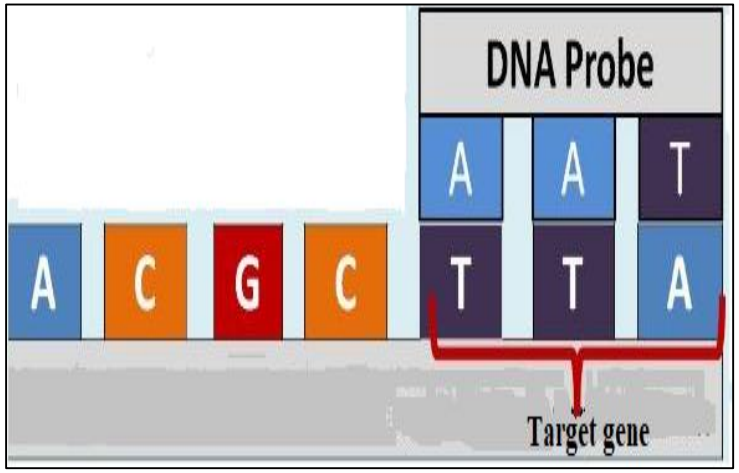
Document 5: Genetic identity of individuals

Because of the large number of polymorphisms observed in humans, it is virtually certain that each of us is genetically unique (except identical twins). **How can we determine genetic identity of individual?**

Fluorescent In Situ Hybridization (FISH):doc.a

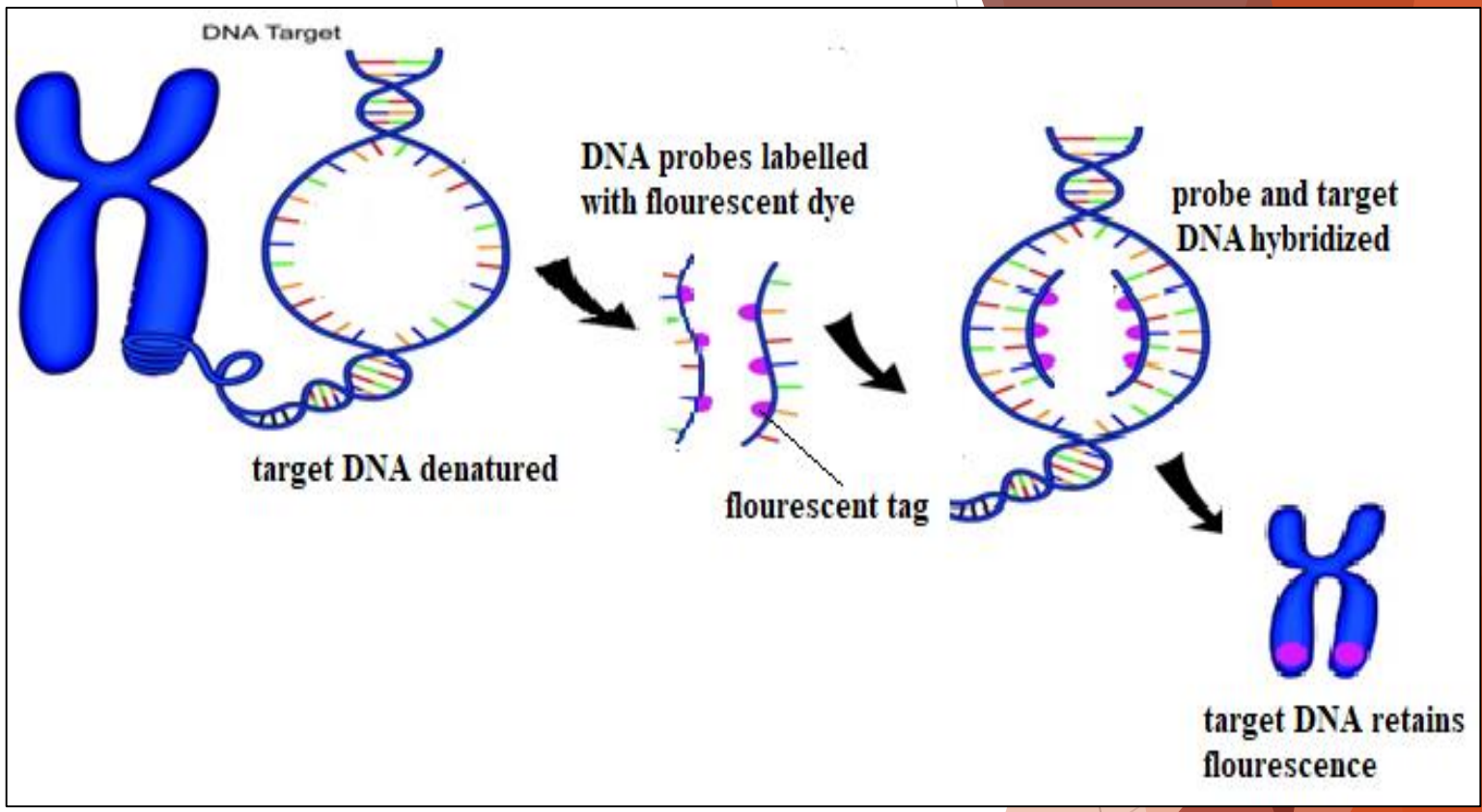
- **Definition:** it is a mapping technique (procedure) that allows to locate the positions (loci) of specific DNA sequences on chromosomes (to locate genes on a chromosome).





Procedure:

1. Denaturation of DNA
2. Adding a probe that make a hybridization with the complement DNA.
3. Washing to eliminate the probes that don’t make hybridization.
4. Analyze probe signals using a fluorescent microscope.

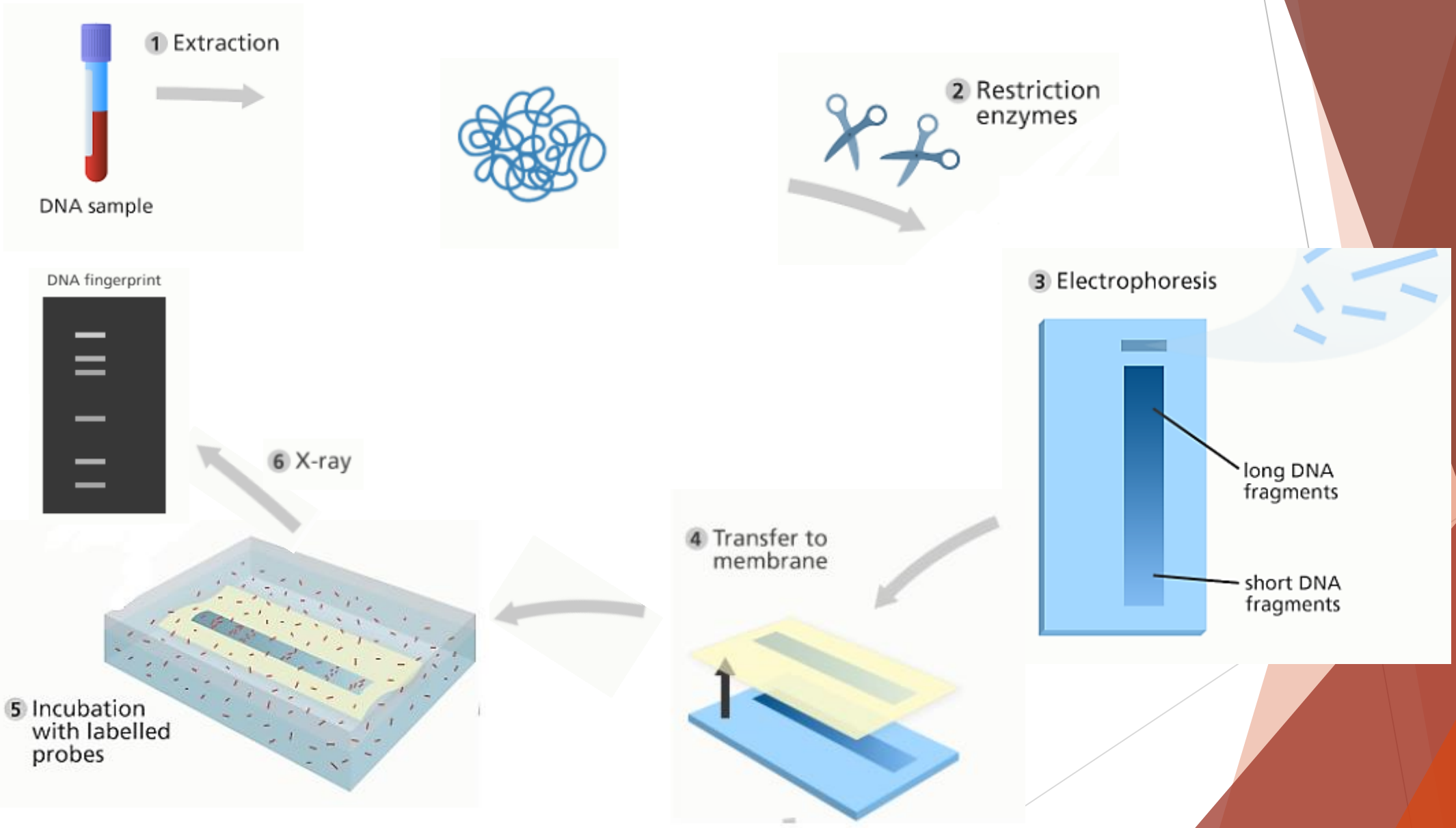


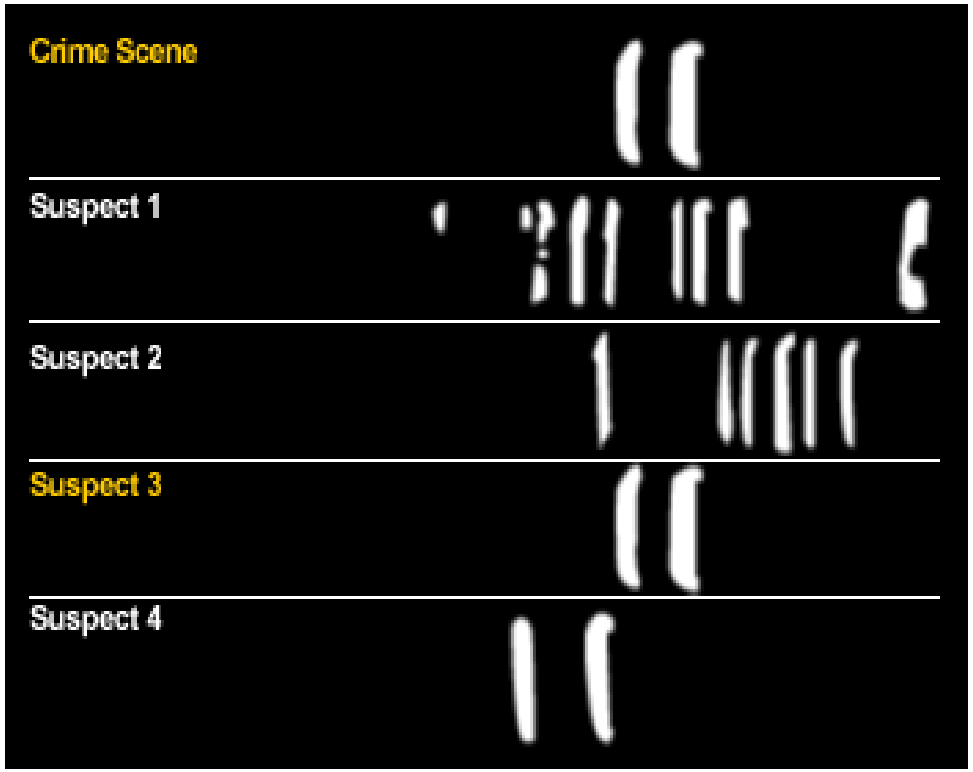
Probe: a known sequence of radioactive or fluorescent DNA used to hybridize the denatured DNA molecule by complementarity.
Denaturing: Separation of DNA strands by high temperature or using NaOH.
Hybridization: binding of probe to studied bands/ gene by complementarity.
Blotting: transferring

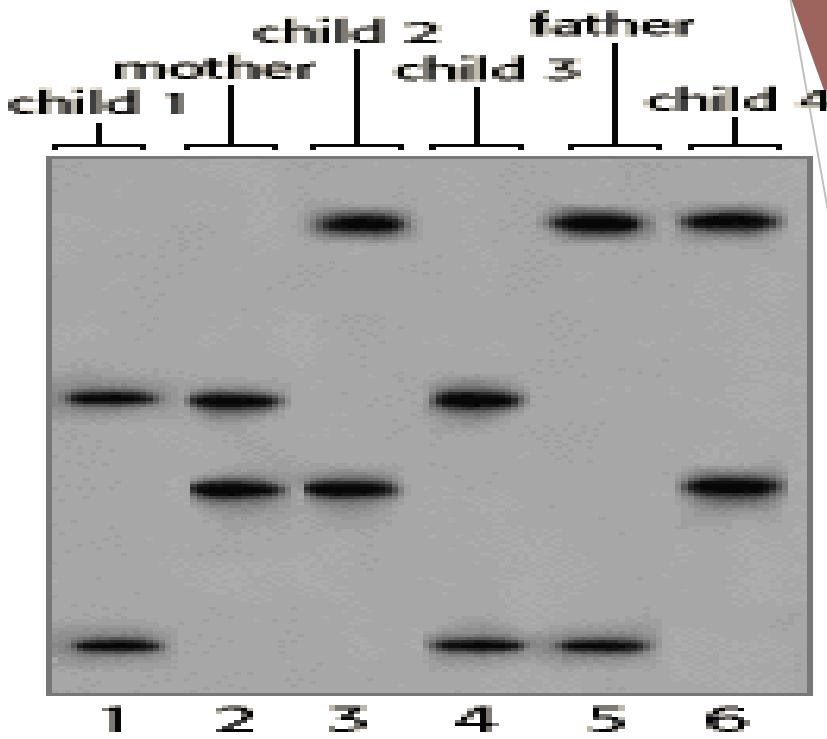
DNA fingerprint/Jeffrey's technique or southern blotting:

- **DNA fingerprinting** (DNA typing, DNA profiling, genetic fingerprinting, genotyping, or identity testing) in genetics is a molecular genetic method used to identify an individual using hair, blood, semen, or other biological samples, based on unique patterns (polymorphisms) in their DNA.
- Just like your actual fingerprint, your DNA fingerprint is something you are born with, it is **unique** to you.
- The probability of having two people with the same DNA fingerprint that are not identical twins is very small

DNA fingerprinting







DNA fingerprinting is an application of RFLP and it is used for:

- Paternity Test
- Forensic science (crimes, victims after plane crush..)

DNA fingerprints of children should be similar to their parents' fingerprints, although they are not the same. Some bands will match one parent and other bands can match the other parent. With the bands of both of those parents, they make the bands and the identity of the child.