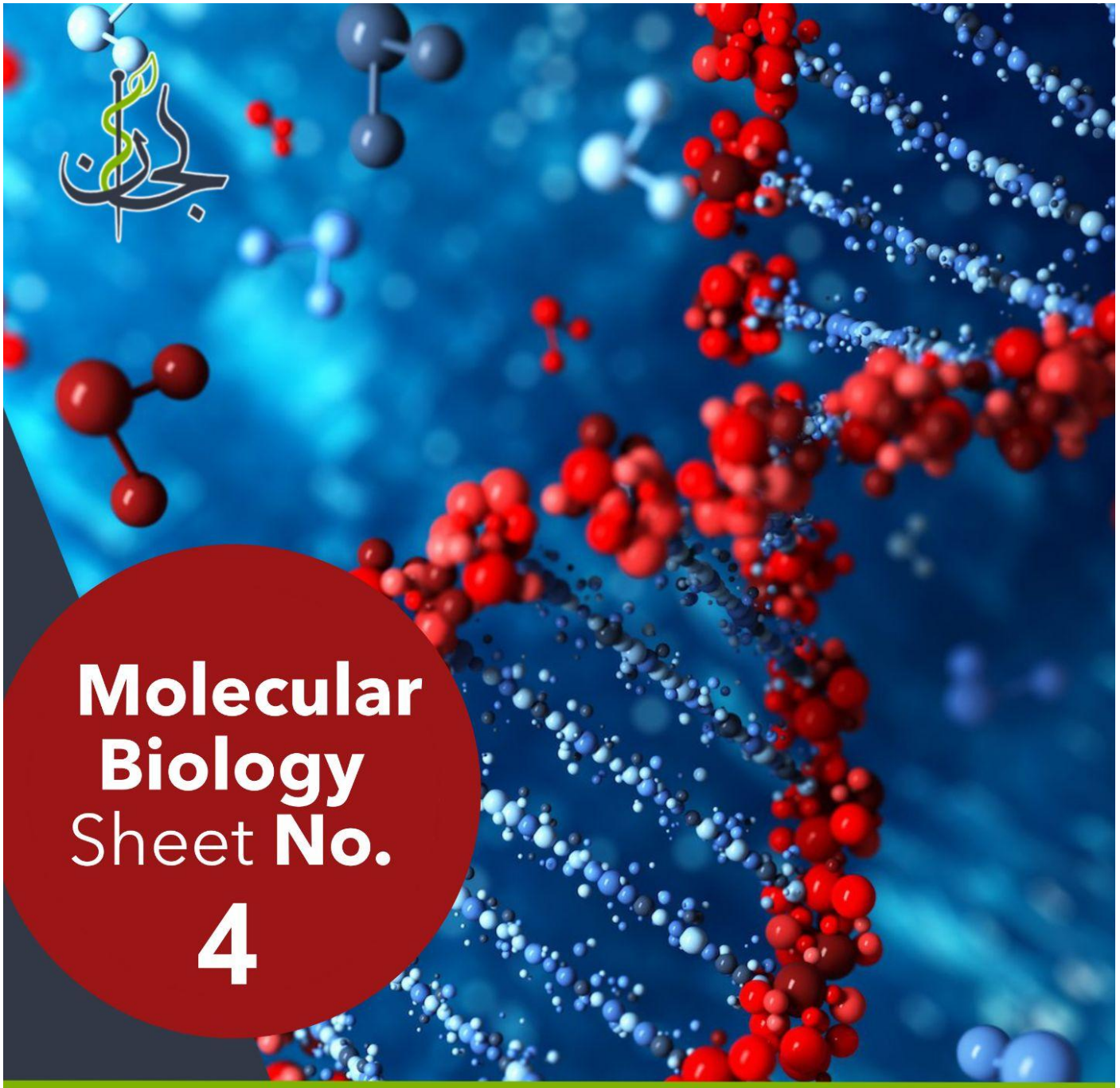

Molecular Biology Sheet No. 4

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Basic Applications (3): Restriction fragment length polymorphism (RFLP)

-In the past 2 lectures , we addressed the following subjects :

1. The characteristics of the DNA
2. Electrophoresis
3. Dot blotting and Southern Blotting

-In this lecture , we'll discuss how we can utilize these concepts in what's known as RFLP.

Let's introduce to you an enzyme known as **restriction endonuclease:**

-**Nuclease:** is an enzyme that degrades (cleaves) nucleic acids , breaking it into different fragments.

- Examples of Nucleases :

1. Disaccharidases : An enzyme that breaks down Disaccharides
2. Lipases : a type of protein that breaks down lipids
3. Proteases : Degrades proteins
4. Esterases : Removes Ester bonds

Endo : in the middle (not exactly in the center but rather somewhere **within** the nucleic acid)

Exo : Either end of the DNA (**5**' or **3**').

after understanding these two prefixes, we can guess that :

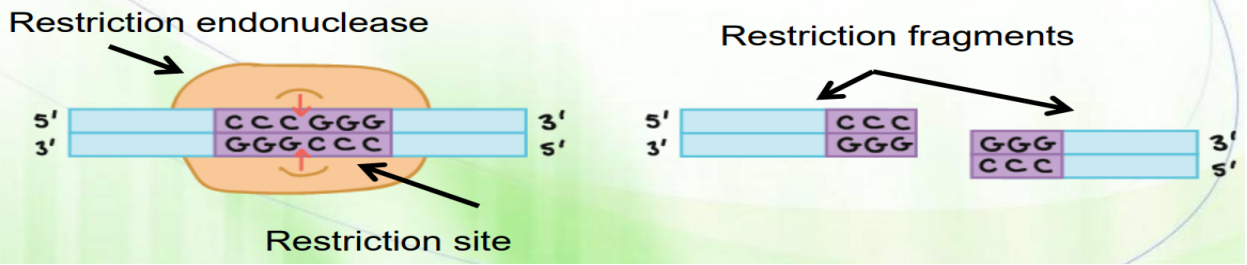
- **Endonucleases** are enzymes that degrade DNA within the molecule.

The mechanism of the enzyme(restriction endonuclease):

1-It **recognizes** a specific sequence of nucleotides within the DNA which is known as a restriction site.

2-It **cuts**(breaks) the phosphodiester bond between nucleotides there (4- to 8- bp restriction sites), **generating** restriction fragments.

Restriction endonucleases were first given the name "restriction" considering their job of restricting the growth of bacteriophages by infecting them).



Palindromic sequences:

- **Palindromic sequences** are sequences that can be read the same from left to right as they are read from right to left (on the complementary strand).
- Restriction endonuclease recognizes those palindromic sequences and cuts within their sequences.

The photo below shows some of the Restriction endonucleases (in Green) and the Palindromic sequences they act on.

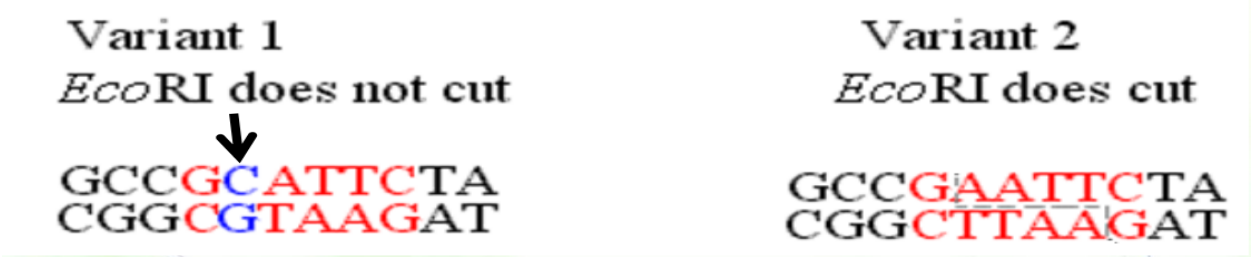
Restriction nuclease		Palindromic sequences	
EcoRI	5'	GAATTC	3'
	3'	CTTAAG	5'
HindIII	5'	AAGCTT	3'
	3'	TTCGAA	5'
SmaI	5'	CCCGGG	3'
	3'	GGGCCC	5'

Note: you don't have to memorize these enzymes and the sequences.

-EcoRI is Isolated from a bacteria called E Coli

-**Note:** E Coli is a bacteria that is found in rotten meat and is considered fatal .

-Restriction endonucleases are considered **very specific** , as If there's a **single change** in the sequence of the restriction site , it **will not** be able to cleave/break it anymore.

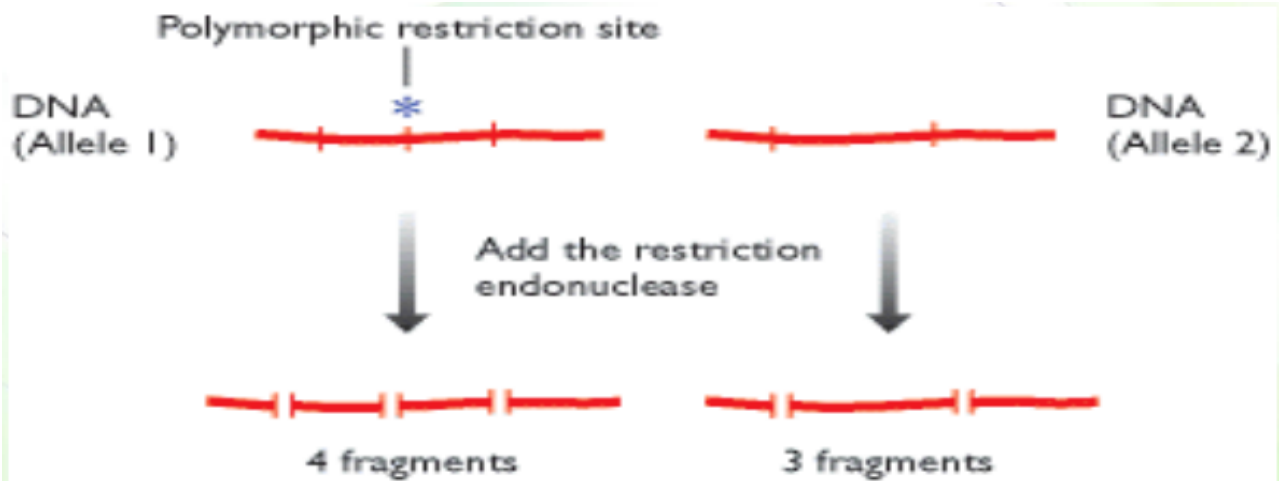


The DNA stays intact

The DNA is cut into 2 pieces

Cuts and number of fragments :

-Restriction endonucleases can cut the same DNA strand at several locations generating multiple restriction fragments of different lengths



DNA sequence can differ, as seen above , Allele 1 has a restriction site that doesn't exist on Allele 2 , So it's safe to say that this person is heterozygous for this specific polymorphic restriction site.

-In this case this person generates 7 fragments in total , that might be the same or vary in length.

-having different DNA fragments is what we call Polymorphism

(poly: many , morphism : shapes).

In simpler words, Humans have highly similar DNA sequences in their chromosomes , but there is also some differences ,and these differences create what we call Polymorphisms -So , Polymorphisms : Individual variations in DNA sequence (genetic variants) may create or remove restriction-enzyme recognition sites generating different restriction fragments.

Remember:

- Our cells are diploid.

-Alleles can be homozygous or heterozygous at any DNA location or sequence.

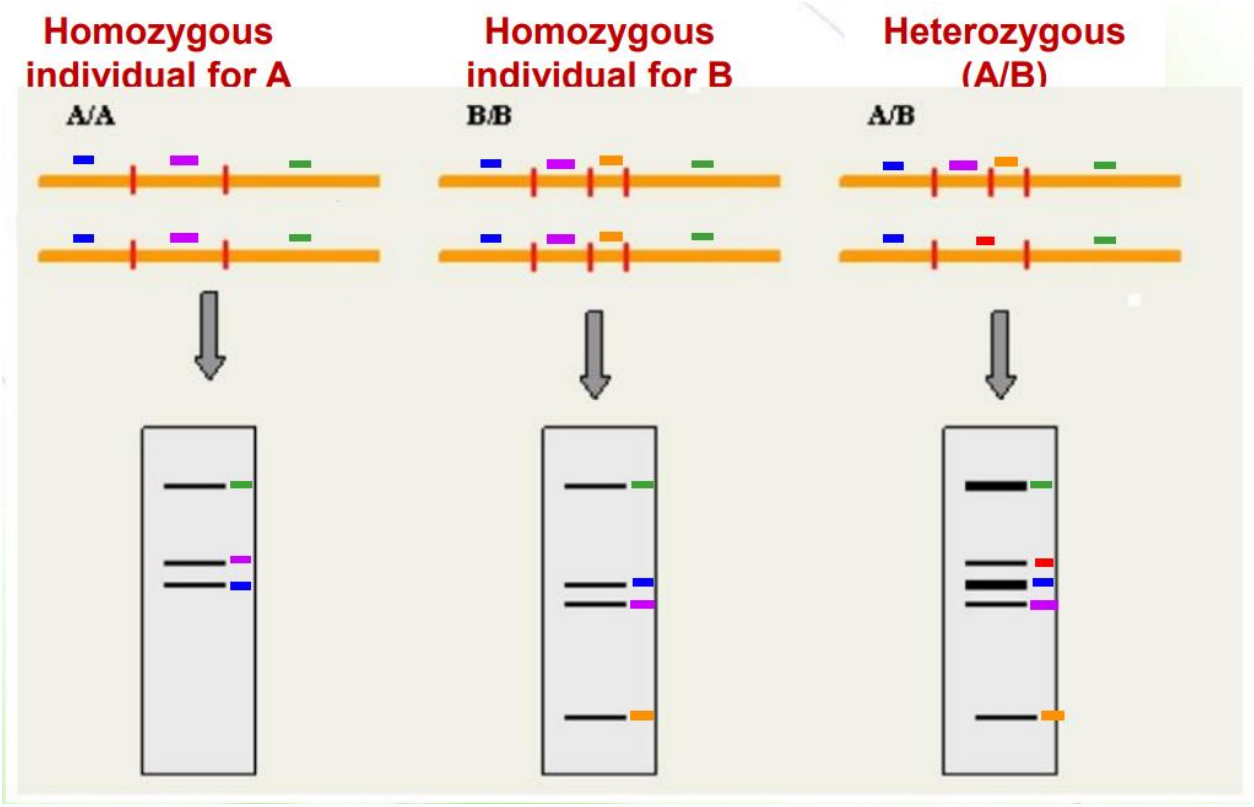
-The presence of different DNA forms in individuals generates a restriction fragment length polymorphism, or RFLP

-Individuals can generate restriction fragments of variable lengths. This is known as molecular fingerprinting. (basically, Applying the same restriction endonuclease to the DNA of 2 different individuals , will result in different DNA fragments).

-These can be detected by gel electrophoresis by itself or along with Southern blotting

Gel electrophoresis only:

The colored squares were added to help you further understand this topic



Notes :

-for a particular location on the DNA of these individuals , there are 2 possible alleles :

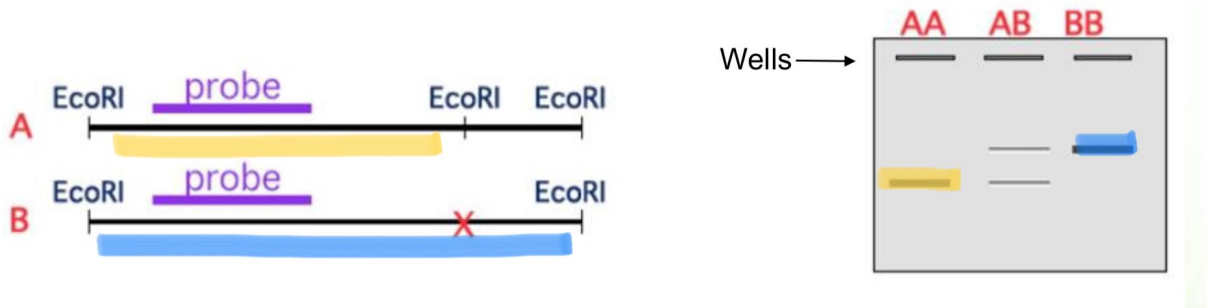
1- Allele A 2- Allele B

-DNA fragments of the same length , are seen as a one band in gel electrophoresis

To clarify this even more : looking at the result of the electrophoresis of the individual with A/A alleles, who has 6 DNA fragments , we see that there's only 3 bands in the gel , because they have the same length as seen in the picture. (the same goes for individual with B/B alleles ,except in this case , it's 8 DNA fragments seen as 4 bands in the gel)

Southern blotting:

Only DNA fragments that hybridize to the probe are detected.



Note:the size of the DNA detected DNA fragment reflects **its** size, not the size of the probe.

- **RFLP in the clinic:**

-RFLP can be used as diagnostic tools.
-For example: if a mutation that **results** in the development of a disease also **causes** the generation of distinctive RFLP fragments, then we can tell:
if the person is diseased as a result of this mutation from which parent this allele is inherited

-How can we use **RFLP** in the clinic?

1. To determine if a person has disease or not.
2. To determine paternity.
3. Forensics.

Now we will explain about the use of every single example.

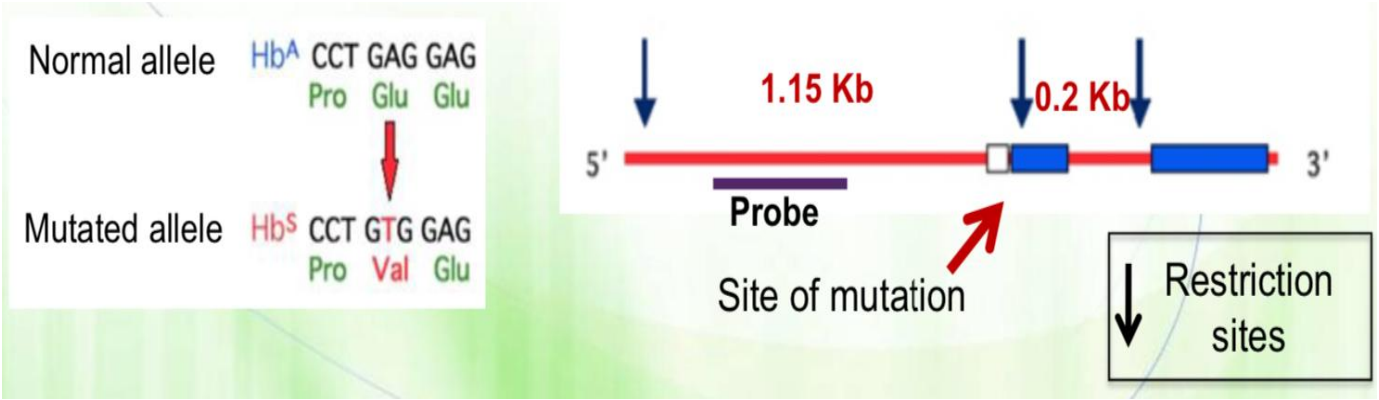
-EXAMPLE1: Disease detection by RFLP (sickle cell anemia):

- Sickle cell anemia is **caused by a mutation in one nucleotide** (base) in the globin gene that is responsible for making hemoglobin.
- The position of this nucleotide happens to be **within** a restriction site.

Individuals can be have:

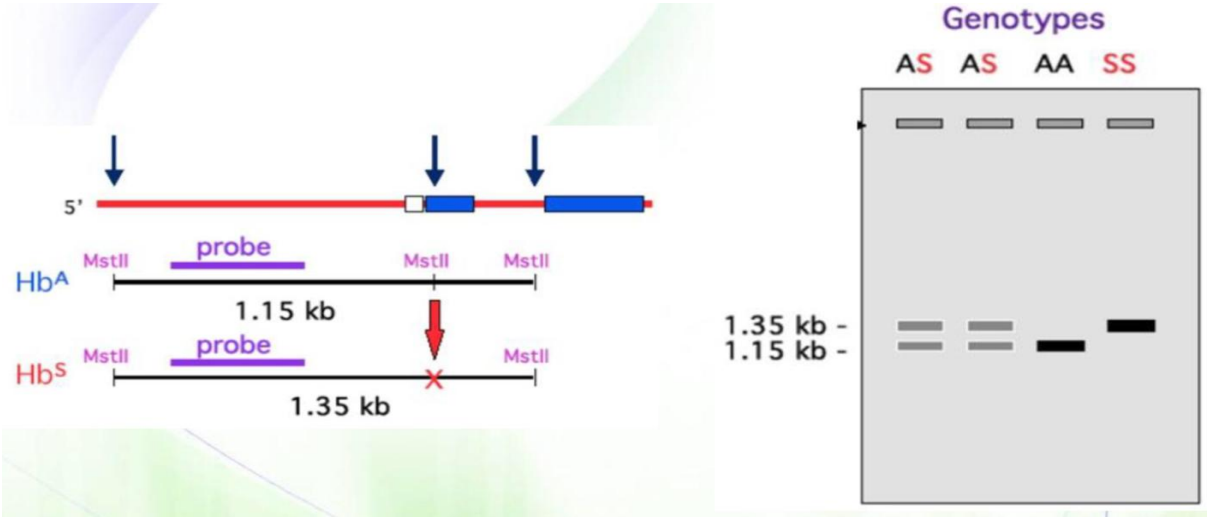
- Homozygous with **two normal alleles** (designated as **A**)
- Heterozygous or carriers of **one normal allele** and one mutated allele (designated as **AS**)
- Homozygous for the **mutated allele**, or affected (designated as **S**)

Note1: In order for a person to have the disease, this person must have both mutated alleles.
2: **S** represents sickle



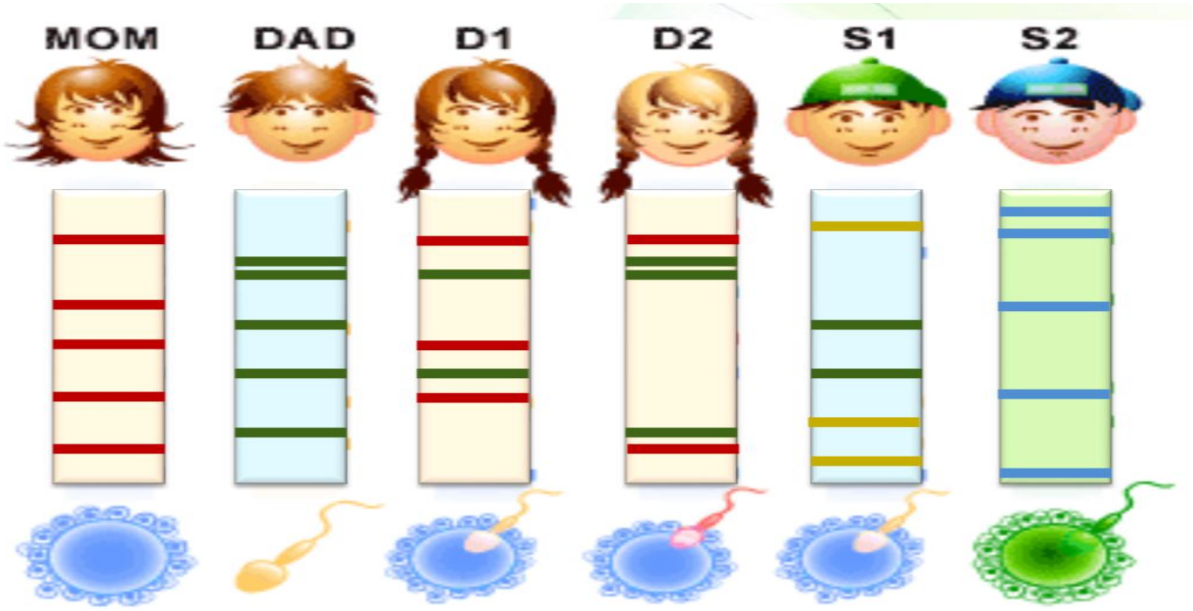
-We add the probe to recognize the sequence and the restriction nucleases would cut it.

- If the person has a **normal allele** then the length of the DNA fragments would be **1.15kb** (kilobase).
- If the person has a **mutated allele** (the restriction nucleases wouldn't be able to cut) so the length would be **1.35kb.**



EXAMPLE2: Paternity testing:

- The DNA of children must come from both parents.
- 50% of DNA comes from the father and 50% comes from the mother , but that **doesn't** mean that if the child has 6 fragments then 3 of them would come from father&3 from mother.



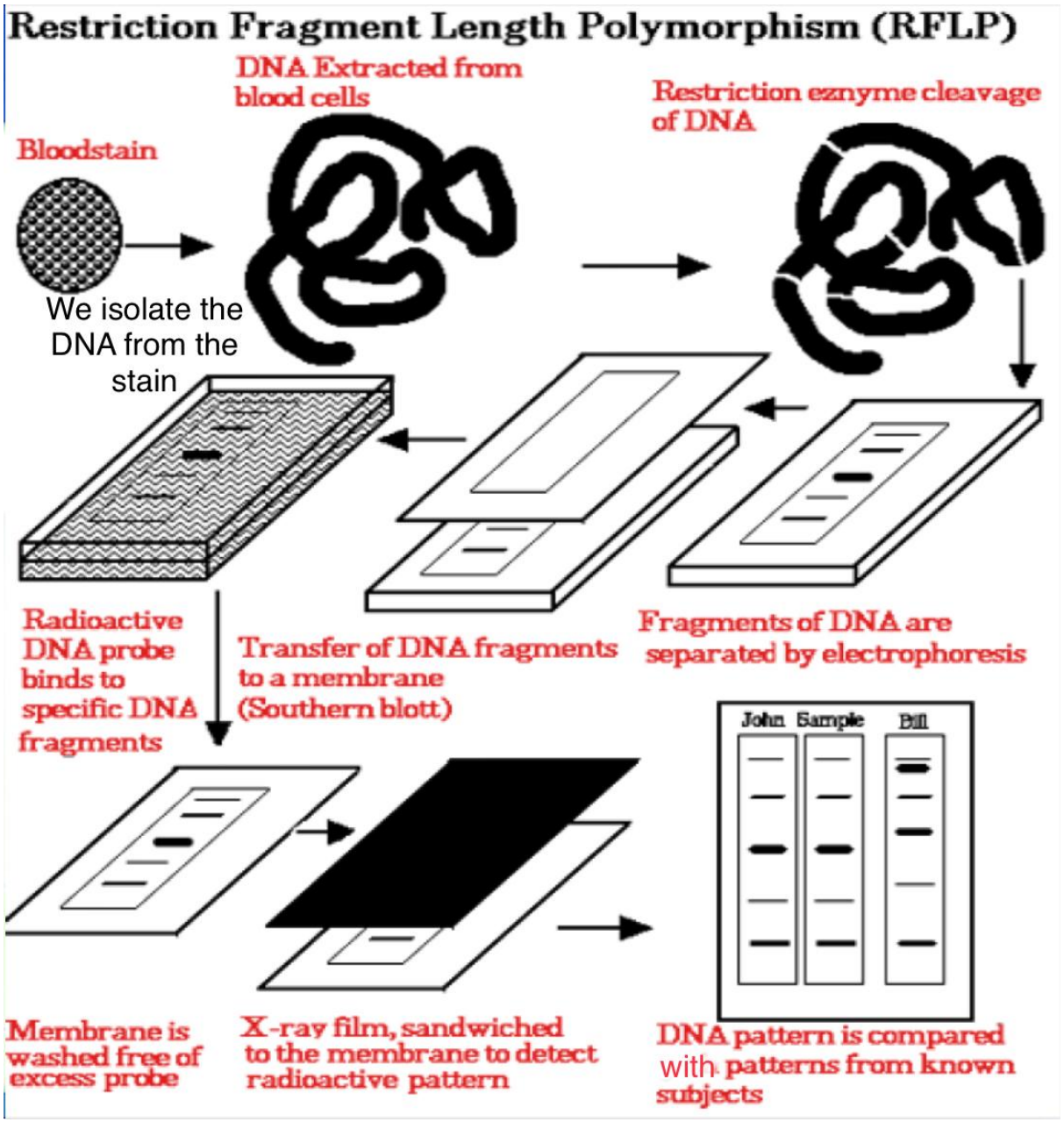
We compare the fragments.

D1&D2✅

S1: he shares DNA fragments from the dad but not from the mom , meaning he's a son of his dad's ex wife for example

S2: the fragments are totally different so we'd assume he's adopted for example.

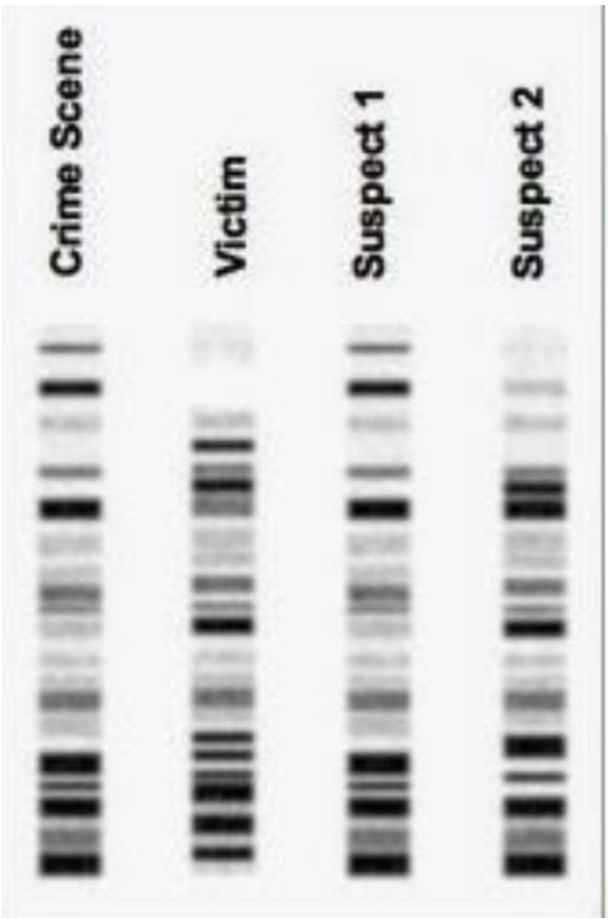
EXAMPLE3: forensics:

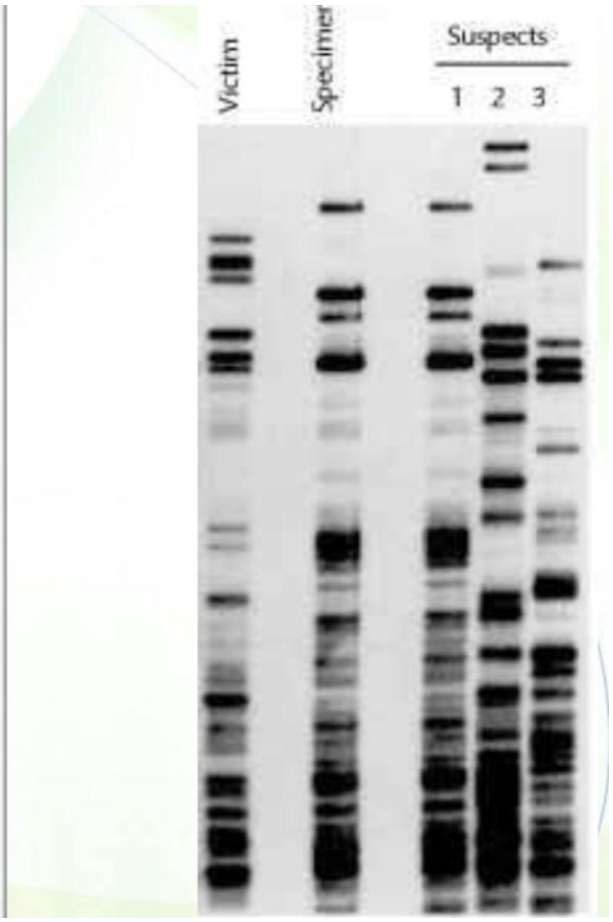


-Read the steps.

- We can clearly tell that John is the Criminal , since his DNA is identical to the sample's DNA.

Real cases:





Suspect 1✅ **Suspect1✅**

If we have a suspect and their blood in the crime scene then we should have a **total match**.

-In these cases the DNAs are clear, but in some cases DNAs may not be clear it might be: mixed with a bacterial DNA (if we found the blood after hours),or mixed with the victim's DNA (if there was a fight between the murder and the victim).

BEST WISHES