Genetics
& Cell biology

◉ Sheet ○ Slides

Number

3

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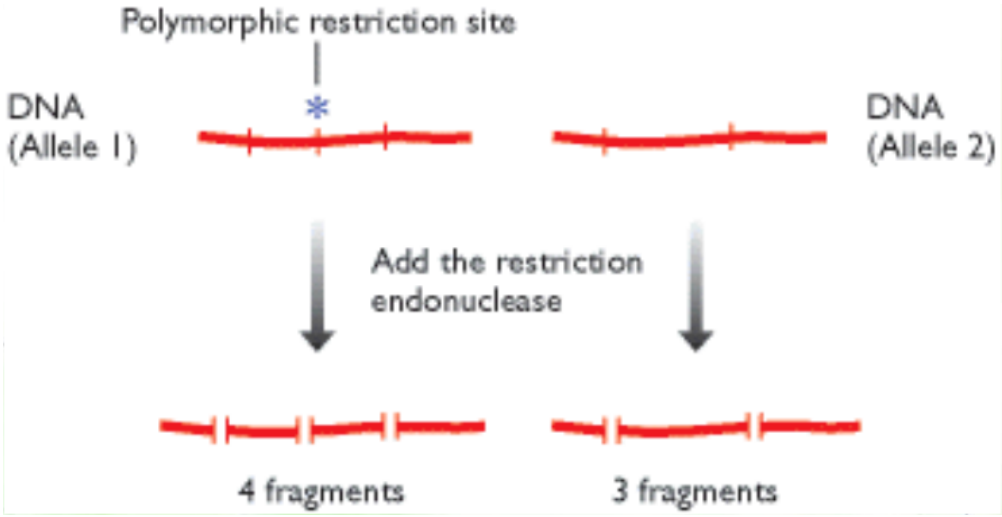
In the last lecture, we said that we can use restriction endonucleases in a technique called **Restriction fragment length polymorphism (RFLP)**; but what does that mean?

>> Assume we have two DNA fragments:-

• One has a sequence of GAATTC GAATTC GAATTC (3 restriction sites recognized by a restriction endonuclease called EcoR1 which will cut between G and A) ; If we add EcoR1 , it will make 3 cuts between G and A in each restriction site (G/AATTCG/AATTCG/AATTC) generating 4 fragments.

• The other has the same sequence except that it has a polymorphism in which one C in one restriction site is replaced by G making the sequence GAATTCGAATT**G**GAATTC; if we add EcoR1 it will make 2 cuts between A and T in the two remaining restriction sites (G/AATTCGAATTGG/AATTC) generating three fragments only having different lengths than the fragments without polymorphism.



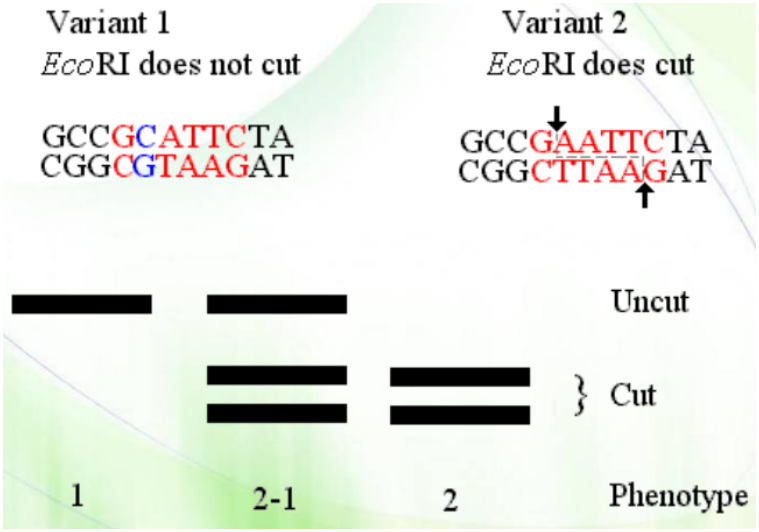
So the presence of different DNA forms in individuals results in a polymorphism in the lengths of the restriction fragments, and this is the concept of **RFLP** technique.

In the last lecture, we also talked about agarose gel electrophoresis and southern blotting techniques; these techniques are used to detect RFLP.

>>Assume we have 3 individuals and we studied a fragment in their chromosome 5, for example, because they have two chromosomes 5 (paternal and maternal):

- One of them has two *identical* fragments (paternal and maternal) without GAATTC in it; if we add EcoR1, it will not make a cut on either chromosome generating large DNA fragments.
  (we say this individual is **homozygous** for this location of the DNA)
- The second one has two *identical* fragments as well but with GAATTC in both fragments; if we add EcoR1, each fragment will be cut into two smaller fragments.
  (we say this individual is **homozygous** for this location of the DNA)
- The third has GAATTC on one fragment but not the other; if we add EcoR1; the one with GAATTC will be cut into two smaller fragments while the other will not. There will be one large fragment and two smaller ones.
  (we say this individual is **heterozygous** for this location of the DNA)

By using gel electrophoresis and southern blotting and staining, we will have these results:-



In the figure above, if we have variant 1 in the two fragments, EcoR1 will not make any cut so we will have two large uncut fragments (paternal and maternal) but they have exactly the same size so they run together and appear as one band(phenotype1). If we have variant 2 in both fragments, EcoR1 will cut both into two smaller fragments (phenotype2).

If we have variant 1 on one fragment and variant 2 in the other, the one with variant 2 will be cut into two fragments while the other will stay a large fragment generating three fragments different in length (phenotype1-2).

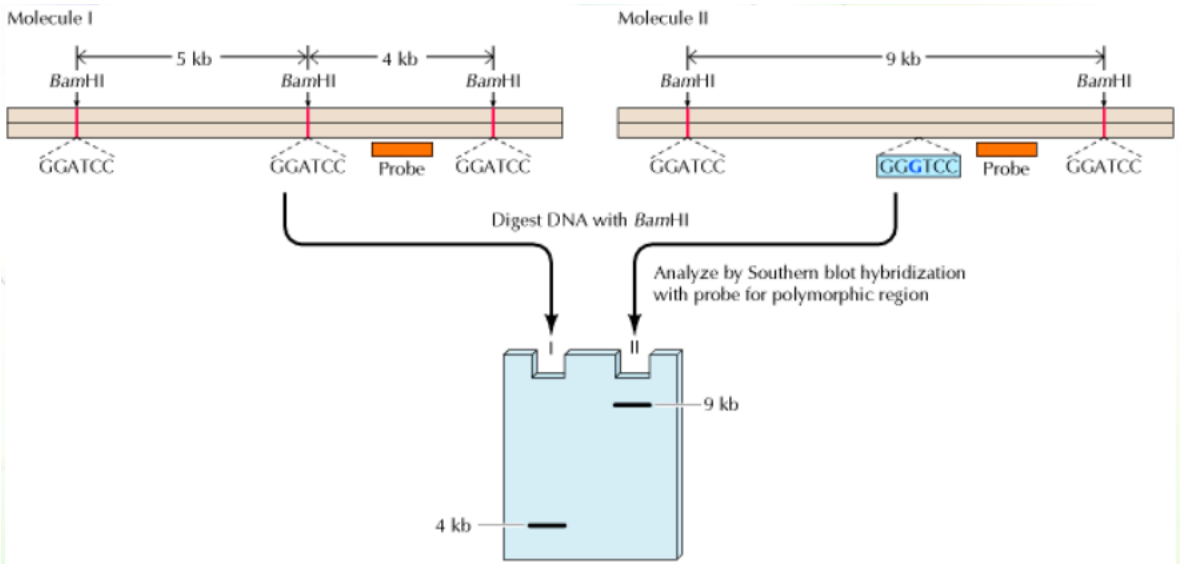
RFLP in the clinic (We will see 4 examples):-

RFLP can be used as a diagnostic tool to determine **molecularly** if the person is diseased by detection of a mutation that exists in a restriction site for an endonuclease.

So Assume that a gene contains GAATTC got mutated into GAATT**G**, and this mutation caused a specific disease (we know that this disease occur when C is replaced by G), so simply I can take this DNA fragment and add EcoR1; if it cuts, the DNA is normal, if it doesn't, the DNA is mutated at the restriction site.

In the figure below, we have two DNA fragments: One with three restriction sites for BamH1 (GGATCC) , and the other is identical but on restriction site has G instead of A (GG**G**TCC) ; We also added a Probe that recognizes a specific complimentary sequence as shown in the figure .

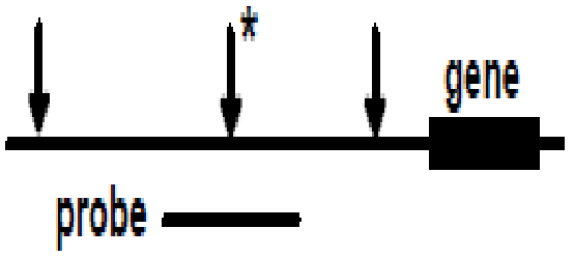
- If we added the endonuclease to the first fragment, it will make 3 cuts generating 4 small fragments. (The probe is attached to a short fragment *4 kb*)
- If we added the endonuclease to the second fragment, it will make 2 cuts generating 3 fragments. (The probe is attached to a longer fragment 9 kb)



*Notice that the fragment that has the probe when mutated is much longer than the fragment that had the probe with no mutation, so by gel electrophoresis we can determine the size of the fragment that has the probe.
(The probe is used mainly if the amount of the available DNA is **not** sufficient to be viewed on the agarose gel by itself, so the probe amplifies it).

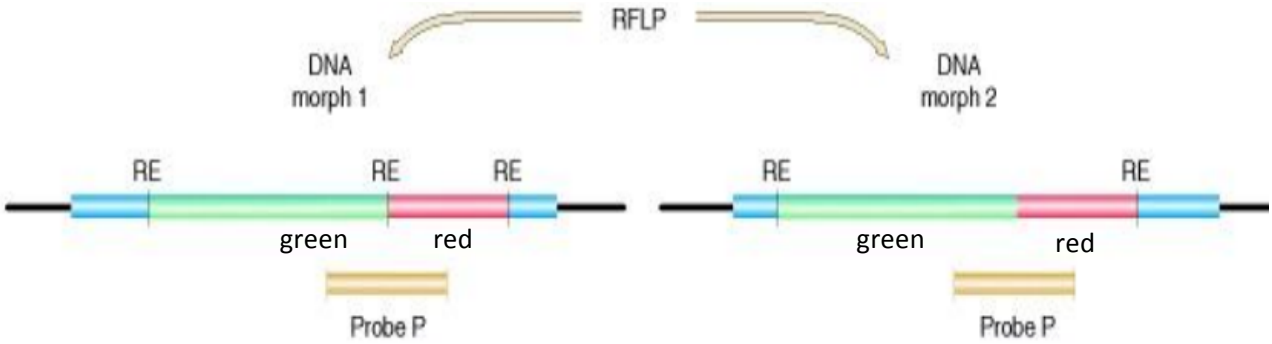
* Student's question: What if the mutated fragment can't be recognized by the probe?

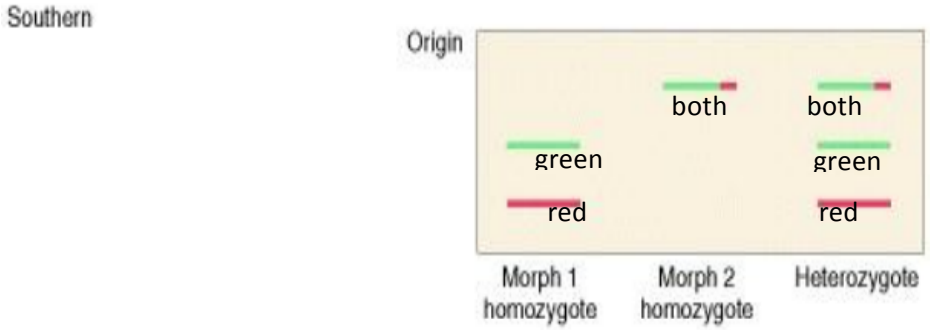
→ We use another probe.



In this figure, the place where the endonuclease usually cuts is within the sequence recognized by the probe.
So what would happen if there is a cut?

Actually, the hybridization doesn't need to be perfect as long as you have enough hydrogen bonds formed; this means that the probe can recognize both parts of the fragment if there is a cut.





In the figure above, the probe usually recognizes part of the red sequence and part of the green together. However, if there is a cut, the probe will be able to recognize the red part alone and recognize the green part alone. By using electrophoresis →

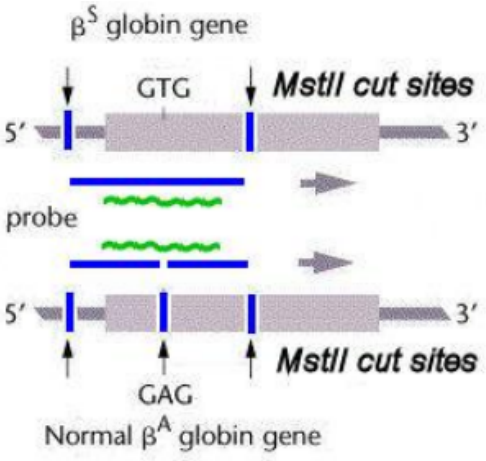
- If someone has the normal restriction site (Morph 1), we will see the red part and the green part separated.
- If someone has the mutated site (Morph 2), we will see one band of both fragments as one fragment because there was no cut within it.
- If someone was heterozygous (having both types), we will see the three bands as shown in the figure.

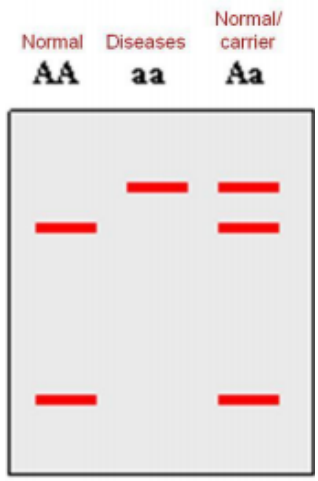
<u>Example 1:</u> Sickle cell anemia

Sickle cell anemia is disease caused by single point mutation in the globin gene; the location of the mutation has been determined and it has been found that the site of mutation is within a restriction site for an endonuclease called **MstII**, and when the gene is a normal β^A globin gene, the endonuclease makes 3 cuts and we will have 2 small fragments attached to a probe and we will see two bands by using gel electrophoresis as shown in the figure below (normal **AA**).

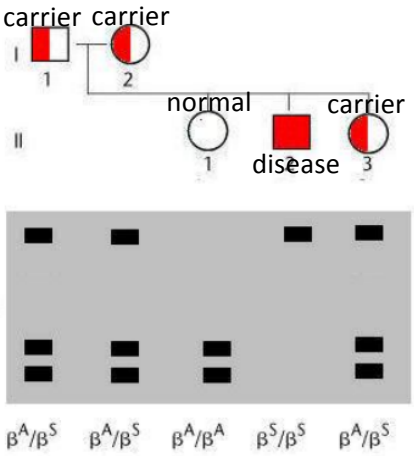
If there is a mutation (β^S globin gene), the enzyme will not make the cut in the middle and will see one large size band (disease **aa**).

If the person has the two types (one paternal and the other maternal) , he is considered a carrier **Aa** and we will see 3 bands.

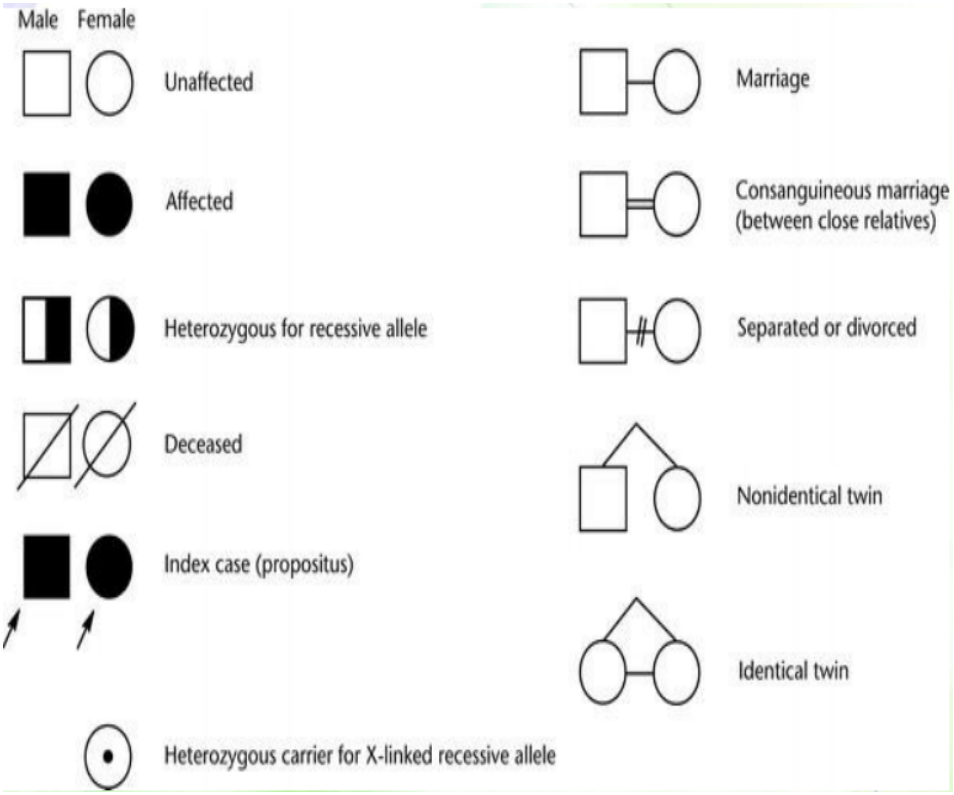




In order for an individual to have the disease, he must take the mutated copy from both parents. In the figure below both parents are carriers for the disease (one good gene and one bad gene) and will have three bands by electrophoresis; **only** the child with both defect genes <u>will have</u> the disease and we will see **only** one band of a large fragment. The <u>normal</u> child will have **only** two bands of smaller fragments, while the <u>carrier</u> will look **exactly the same** as parent.



So notice that we used RFLP to determine if the person is normal, carrier or has the disease .



These symbols will be discussed later in Genetics and they are important for the exam.

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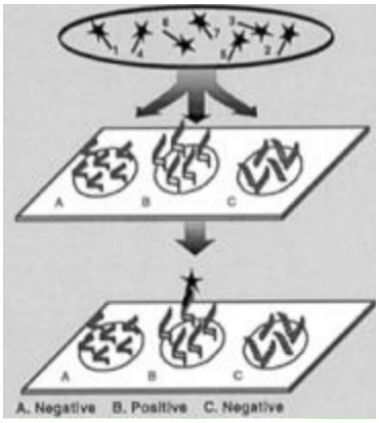
>>Notice: There is another example on this case in slide 2 page 21 similar to the previous one … Check it if you want.

<u>Example 2:</u> Disease detection by ASO (Cystic fibrosis)

There is another technique similar to southern blotting but we don't use agarose gel to separate DNA fragments. What we do is that we take the whole DNA fragment as one spot on a paper and we add a probe; if there is a sequence complementary to the probe there will be an attachment and we can detect it to check that the sequence is present.

In the figure notice that only the spot in the middle is attached to the added probe which means it has the complementary sequence.

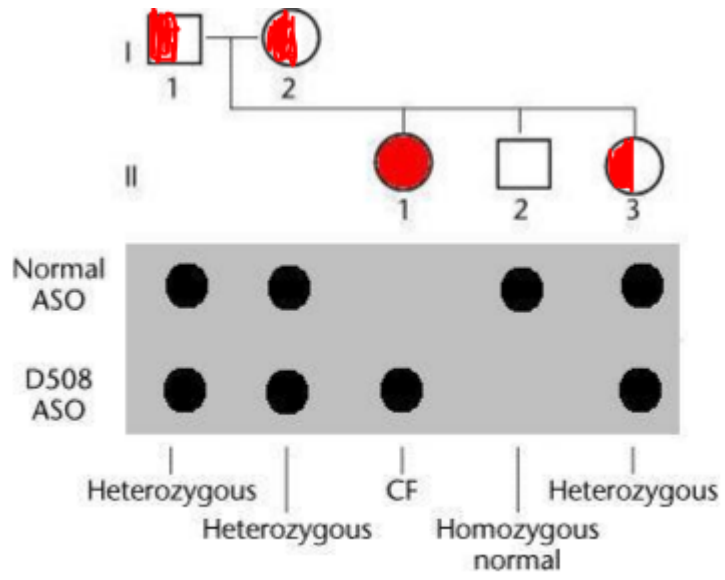


The probe we use in this technique is called **Allele specific oligonucleotide ASO**, and we use two ASOs: One for the normal sequence and one for the mutated (So it is a specific technique).

So if we take a DNA spot and added a probe specific for blue eye color allele and an attachment occurred, this means that this allele is present. And if we added a probe specific for brown eye color allele and no attachment occurred, this means this allele is not present, that's why it's called Allele <u>specific</u> oligonucleotide.

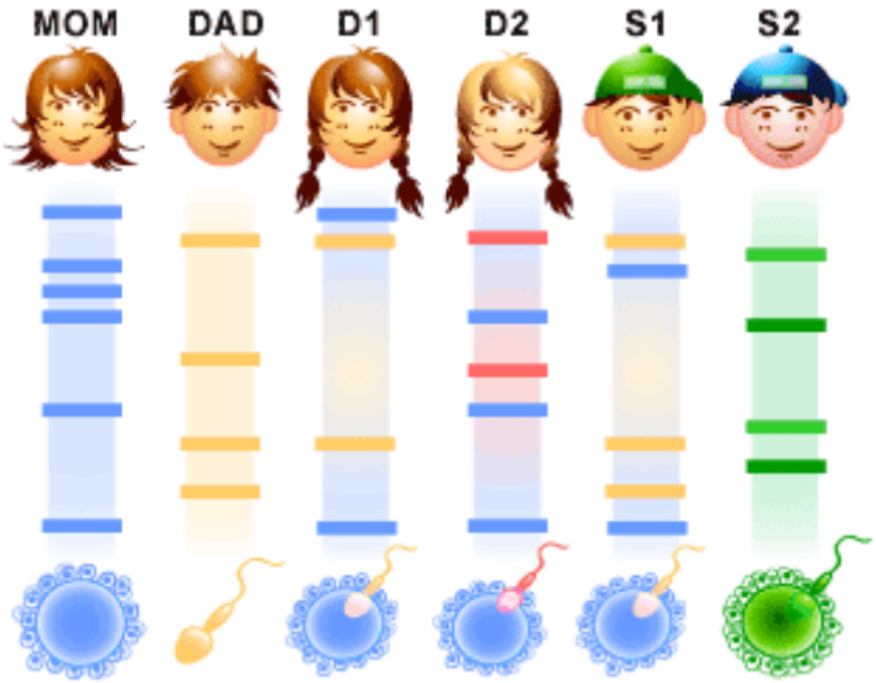
In the figure below we used this technique to detect Cystic Fibrosis allele in a family, and we use ASO for normal DNA and another ASO for mutated DNA (mutated DNA has a deletion).

- For both parents, ASOs for the normal and the mutated DNA have attached → which means they are **carriers** having good and bad genes (**heterozygous**).
- For child number one, ASO for the mutated DNA has attached → which means she has both DNA mutated (she **has the disease**).
- Child number two only ASO for the normal DNA has attached → which mean he has a **normal** DNA.
- Child 3 is the same as his parents (heterozygous).



<u>Example 3:</u> Paternity testing

We take the DNA from the father, the mother and the children, and we add an endonuclease that will make many cuts in the DNA; the children should have a mixed pattern between their parents' DNA.



From the figure above:

- D1 has DNA fragments that look like the fragments of the father or the mother → she is their daughter.
- D2 has DNA fragments that like the mother's fragments but <u>not</u> the father → he is not her real father.

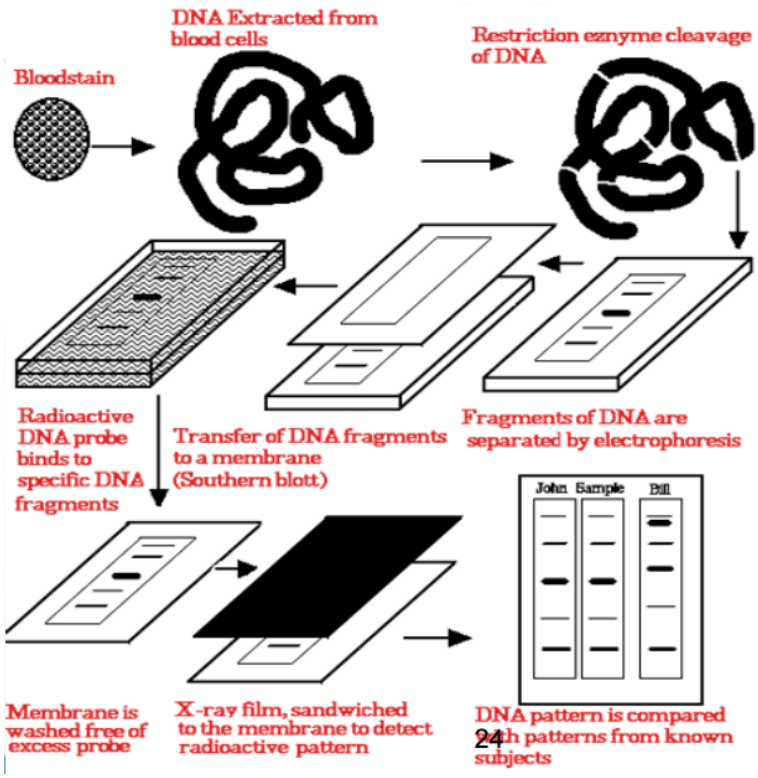
- S1 has DNA fragments that look like the fragments of the father or the mother → he is their son.
- S2 has DNA fragments that are not similar to either of the parents → he is not their son.

Example 4: Forensics

If there was a murder, and there were blood all over the place, part of this blood came from the victim and part may have come from the murderer (a part may be contaminated by bacteria, the DNA from the other part or DNA from the police officers themselves).

Now to determine the murderer out of the suspects, they take their DNA and use RFLP technique to generate a pattern for each of them (This is called **DNA fingerprinting**); We compare the pattern from the DNA extracted from the blood found in the crime scene with the pattern of each suspect.

** The pattern of the murder's blood that we took (not the one found in the crime scene) must be 100% present in the pattern of the blood we found in the crime scene. But the blood we found can contain extra fragments (bands) that resulted by contamination (So be aware!).



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Cloning الاستنساخ

Cloning means that you make several copies of one thing.

A clone is a genetically identical population, whether of organisms, cells, viruses, or DNA molecules. Every member of the population is derived from a single cell, virus, or DNA molecule.

So DNA cloning is making several copies of a DNA fragment; how is that achieved?

We take plasmids (circular bacterial DNA) as carriers (vectors) and open it up, and insert the DNA fragment we want to clone (after using an endonuclease), then close it and insert it into the bacteria and let the bacteria make several copies of the plasmids inside them, and in a short period we will have billions of copies of the plasmid carrying the DNA fragment we want to clone.

This plasmid after we inserted the DNA fragment of interest is called a **recombinant DNA** (*DNA that comes from two different sources)*.

**Bacteria have one chromosome but multiple plasmids and these plasmids replicates independently of the main bacterial genome.

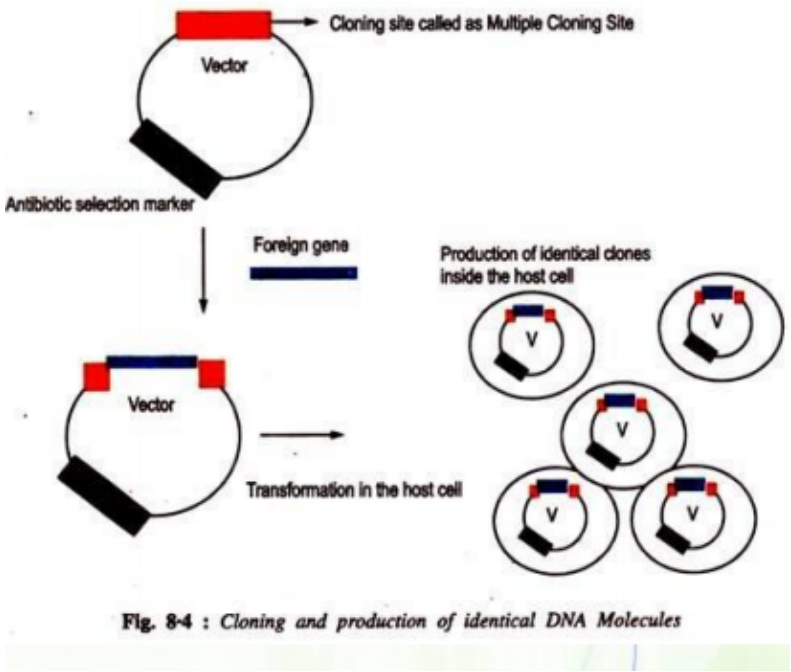
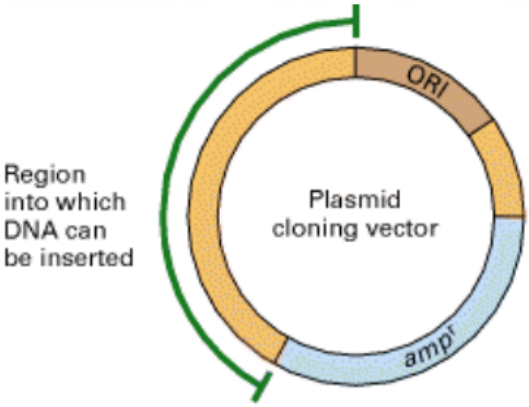


Fig. 8-4 : *Cloning and production of identical DNA Molecules*

>>Features of plasmids used for cloning:

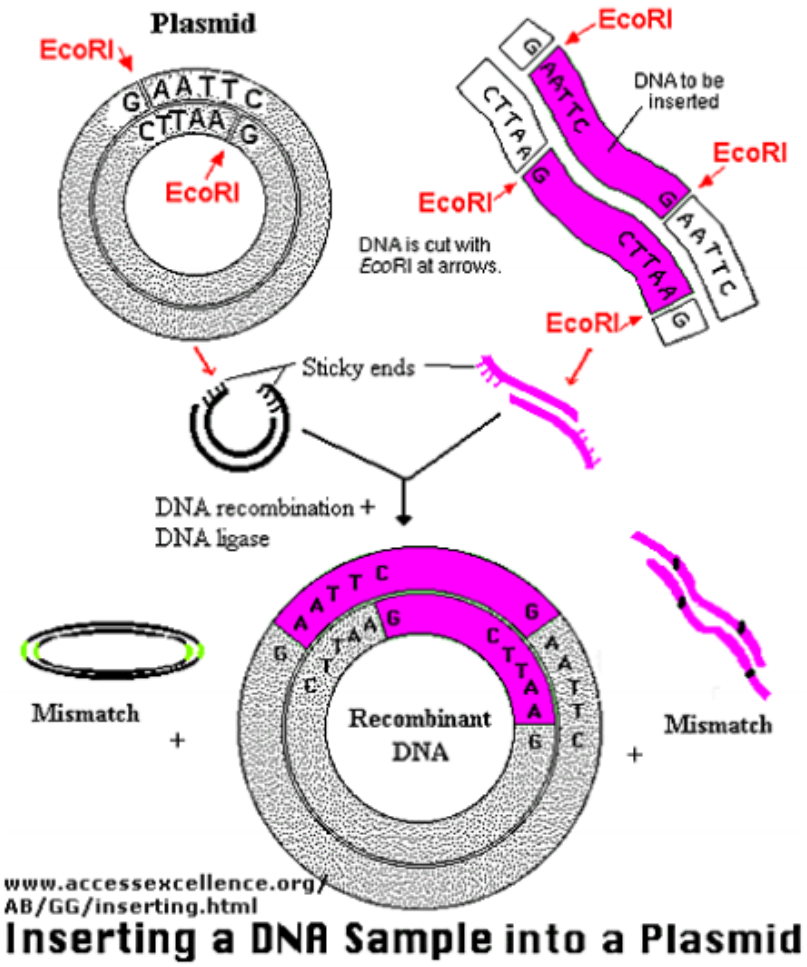
Most plasmid vectors contain at least three essential features required for DNA cloning:

1- The plasmid can replicate (make several copies).
2- The plasmid must have a selectable marker (drug resistance gene) , so that only the bacteria that have the plasmid can survive; We add an antibiotic that kills bacteria without the plasmid, while the bacteria with the plasmid are resistant to this antibiotic and survive .
3- The plasmid must have a place where I can insert the DNA fragment of interest.



How to make the recombinant DNA?

We take the plasmid and cut it with an endonuclease (EcoR1 as an example GAATTC) opening it up and generating a linear DNA molecule, and we do the same to the DNA fragment; as you know EcoR1 will generate sticky ended DNA fragment with an overhang, so the open plasmid can close again due to these sticky ends, but because both the plasmid and the DNA fragment are cut with same enzyme (thus have the same overhangs), the ends of the fragment can attach to the ends of the open plasmid (they are complimentary to each other).
*study the figure below for better understanding

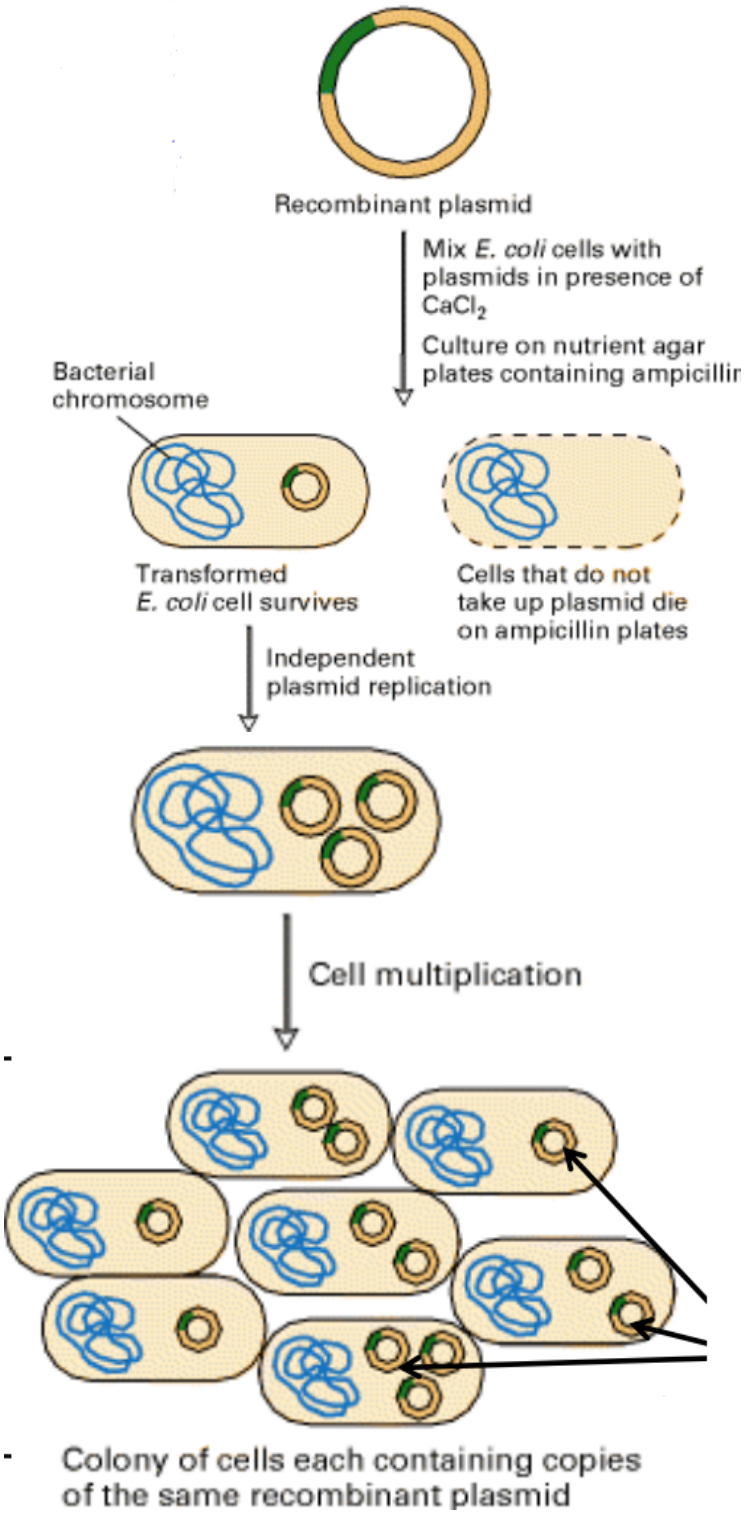


>> After that we add a DNA ligase to close the recombinant DNA via phosphodiester bond.

Then we insert the recombinant DNA in the bacterial cells, and because only this plasmid carries an antibiotic resistant gene, adding an antibiotic will kill all the bacteria without this plasmid and only bacteria with plasmid will survive.

Then they will multiply generating billions of copies in a short time with a lot of plasmids inside them, and then we extract this plasmid of the bacteria.

>> LOOK at the figure below …


Recombinant plasmid
Mix E. coli cells with plasmids in presence of $CaCl_2$
Culture on nutrient agar plates containing ampicillin
Bacterial chromosome
Transformed E. coli cell survives
Cells that do not take up plasmid die on ampicillin plates
Independent plasmid replication
Cell multiplication
Colony of cells each containing copies of the same recombinant plasmid

THE END