University of Jordan
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GENETICS & Molecular Biology

Number: 3

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Subject: RFLP and Intro to DNA Replication

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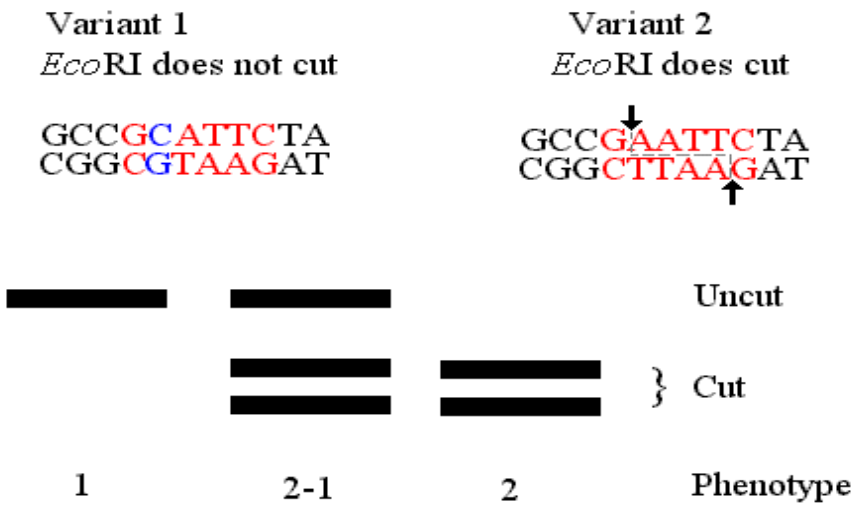
We have two alleles for every gene; one from the mother and one from the father. Sometimes, these alleles can be identical so we call them homozygous for a gene, or they could be different and we call them heterozygous for this.

Homozygous: when both alleles are identical.
Heterozygous: when the alleles are different.

Let's take an example:



Notice that "Variant 1" can't be cut by EcoR1 because there is a mutation in the fifth nucleotide (C instead of A).

Suppose that a person has two alleles of "Variant 2", then what will happen?
First of all, we should know that this person is **homozygous** for this allele.
Both alleles will be cut by EcoR1 endonuclease generating two restriction fragments that are short. These short fragments will migrate quickly in the gel (see number 2 in the picture above).

Suppose a person has two alleles of "Variant 1", and then what you expect will happen?
The DNA sequence will <u>not be cut</u> because there is a mutation in the fifth nucleotide (C instead of A).
As a results, we will end up with a relatively large DNA fragment, and it will migrate in the gel as number 1 (refer to the picture above).

Suppose a person is heterozygous for this gene, which means that he has one allele “Variant 1” and the other one “Variant 2”, then what will happen?
The “Variant 1” allele will not be cut generating one relatively large fragment; however, the other “Variant 2” allele will be cut generating two small fragments. Therefore, they will migrate in the gel as [2-1] phenotype illustrated in the picture in page 1.

Note: we have two alleles for every gene, one from mother and one from father, so when the person is homozygous **we expect to see two bands in the gel***. Each fragment refers to an allele; however, we end up just in one band, so HOW COME?!!
Remember that the fragments migrate in the gel based on their size, and since the person is homozygous the two fragments are identical, meaning that they have the same size and will migrate together in the gel, so they will end up in the same band.

*note that here we are talking about a person who is homozygous **for Variant 1.** However, if the person is homozygous **for Variant 2**, we expect to see 4 bands because each allele will be cut generating 4 fragments; however, we end up just with two bands for the same reason mentioned above.

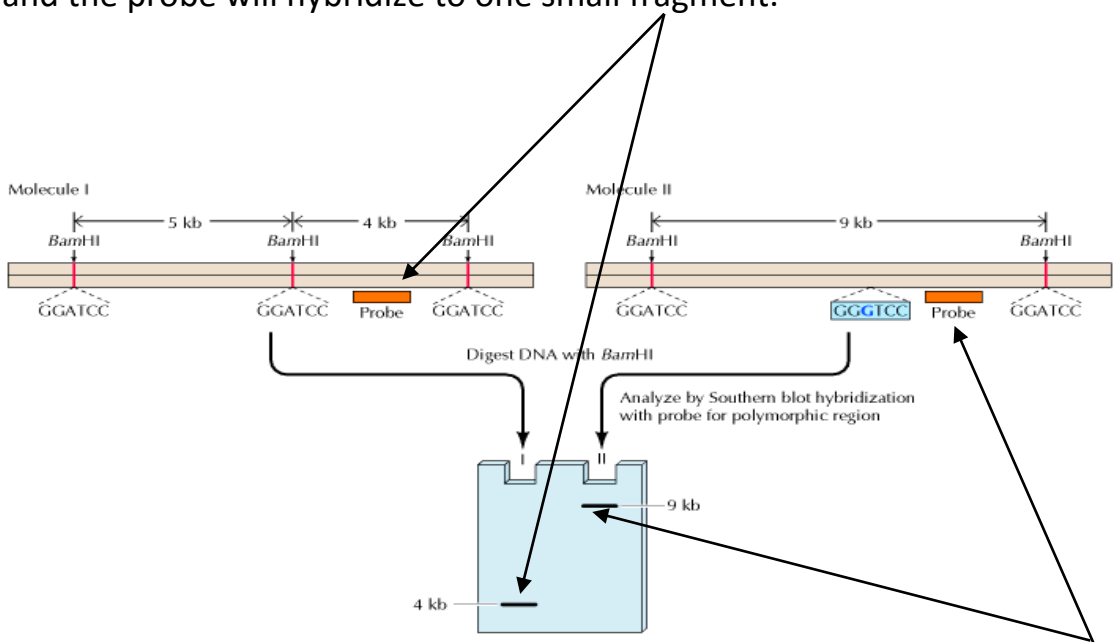
Disease Detection by RFLP (restriction fragment length polymorphism)

Let's take an example:

In this example we are using southern blot technique which means that we will use a probe which will recognize a certain sequence.

Let's say that we have this DNA fragment (as shown in the figure below) which can be recognized by Bam H1 endonuclease, so it will make 3 cuts normally, generating 4 fragments.

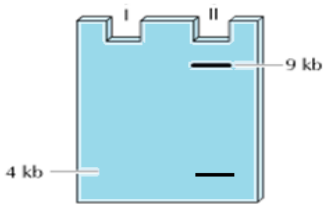
So, in normal situation the DNA sequence will be cut into 4 fragments and the probe will hybridize to one small fragment.



If we have a mutation, Bam H1 will make 2 cuts instead of 3 cuts, so we will end up with 3 fragments instead of 4 and the probe will recognize a large fragment.

Notice that the two cases mentioned previously are for a homozygous person.

Suppose a person is heterozygous, then how the gel will look like?



Now let's take a tricky example:

Assume that the probe recognizes the DNA sequence that contains **the restriction site**, meaning that if the DNA is cut, the probe will recognize part of that DNA.

Can the probe bind to the DNA while part of it is hanging?

Yes, there is a possibility. It depends on the number of hydrogen bonds.

Another example on the previous idea:

Assume that we have a probe that recognizes a DNA sequence that includes a restriction site, if it is cut we will have:

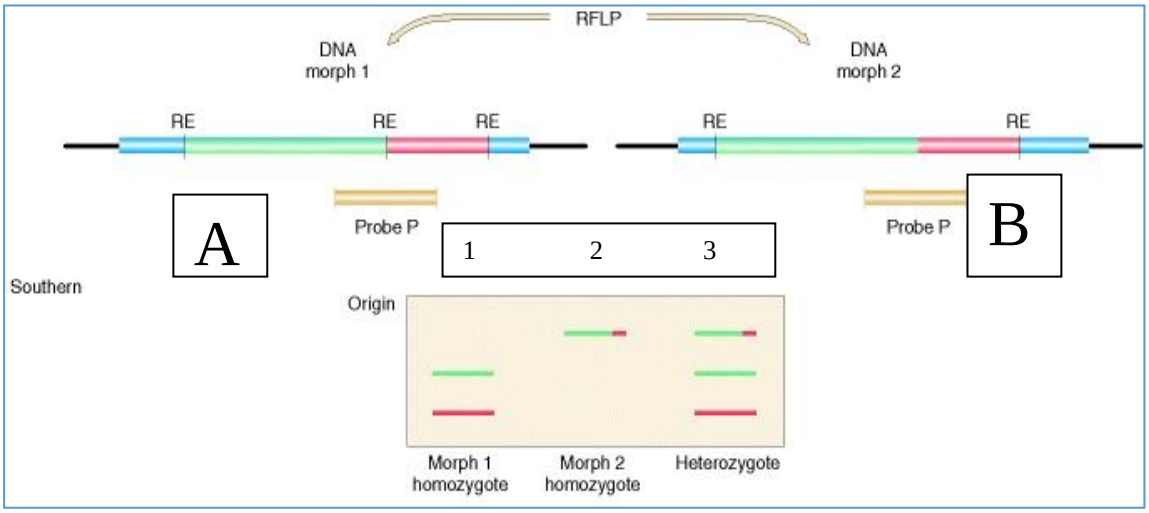
1. One large green fragment
2. One small red fragment

If it is not cut we will have just one large DNA fragment green and red.

Then we add a probe, the probe will bind to the green fragment and the red fragment or the whole fragment.

Now if we observe the figure below we will find 3 cases:

1- Case 1: where a person is homozygous for the "A" allele.
2- Case 2: where a person is homozygous for the "B" allele.
3- Case 3: where a person is heterozygous.



Clinical Examples

Example 1: Disease detection by RFLP (sickle cell anemia)

Sickle cell anemia is caused by a mutation in one single nucleotide.

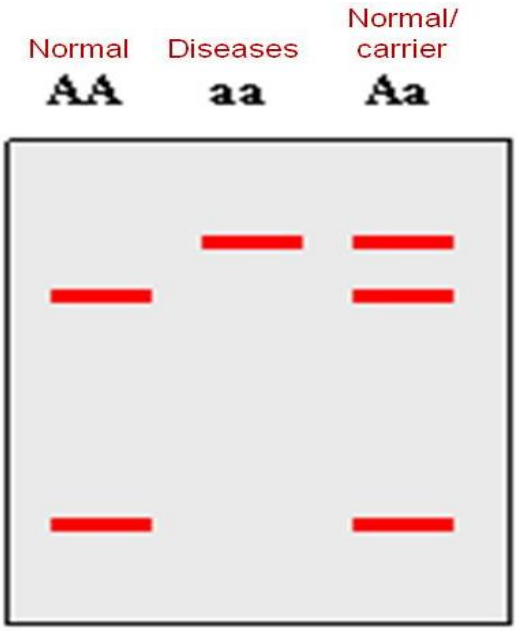
Surprisingly, the location where the mutation takes place is a restriction site for an endonuclease known as “MST1”.

If there is **no** mutation MST1 will make a cut.

If there is a mutation MST1 will not make a cut.

Observations from the figure on the right:

- The left one is a case where the both alleles are normal, meaning there is cut.
- The middle one is a case where the both alleles are mutated, meaning there is no cut.
- The right one is a case where is the person is a carrier, meaning that he has one normal allele and one mutated allele.



A normal person will have two fragments (because of the cut) which are recognized by the probe.

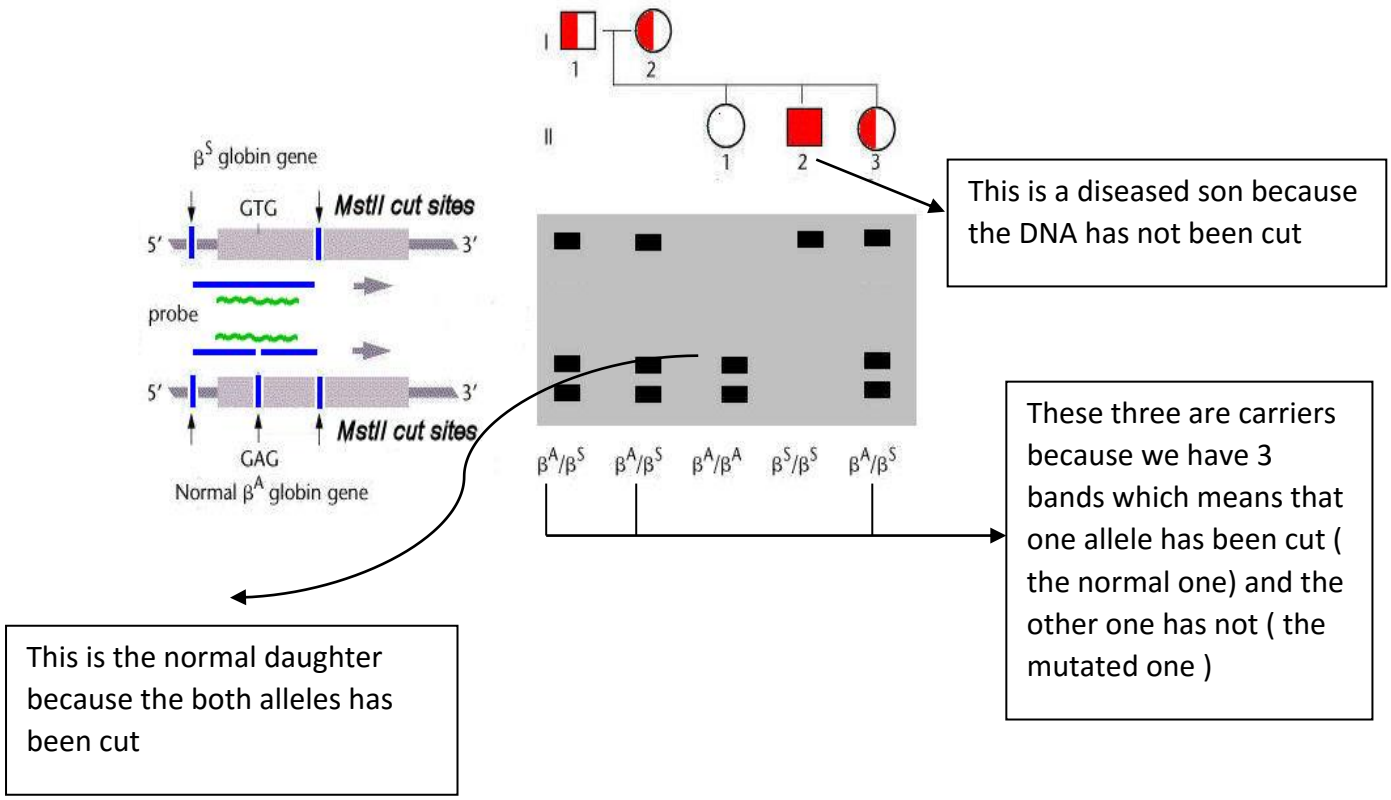
If a person has sickle cell anemia, which is when two alleles are mutated (the two alleles can’t be cut by MST1) meaning that the probe will recognize a large DNA fragment.

Assuming that the person is a carrier, he/she will have one normal allele and one mutated allele, so we will have one large fragment coming from the mutated allele and two short fragments coming from the normal allele.

Let's take another example:

In a pedigree chart (as shown below), a certain disease or phenotype is indicated by filling a square or circle. Squares indicate males and circles indicate females. If the shape is filled, it means that the person having the disease. If the shape is not filled, it means that the person is normal. If the shape is half-filled, it means that the person is a carrier.

Look at the banding pattern for the globin gene:



Example 2: Disease detection by ASO (Cystic fibrosis)

In this case there are two important differences:

The first one is that we use what we call allele specific probe, or allele specific oligonucleotide.

Cystic fibrosis can be the results of a variety of mutations. However, the most common mutation is the one shown below.

ASO for normal DNA 5′ CACCAAAGATGATATTTTC-3′

ASO for DNA sequence of Δ508 mutation 5′ CACCAATGATATTTTC-3′

By looking at the nucleotide sequence above you can notice the difference between the normal and mutated alleles which is the deletion of “AGA” sequence.

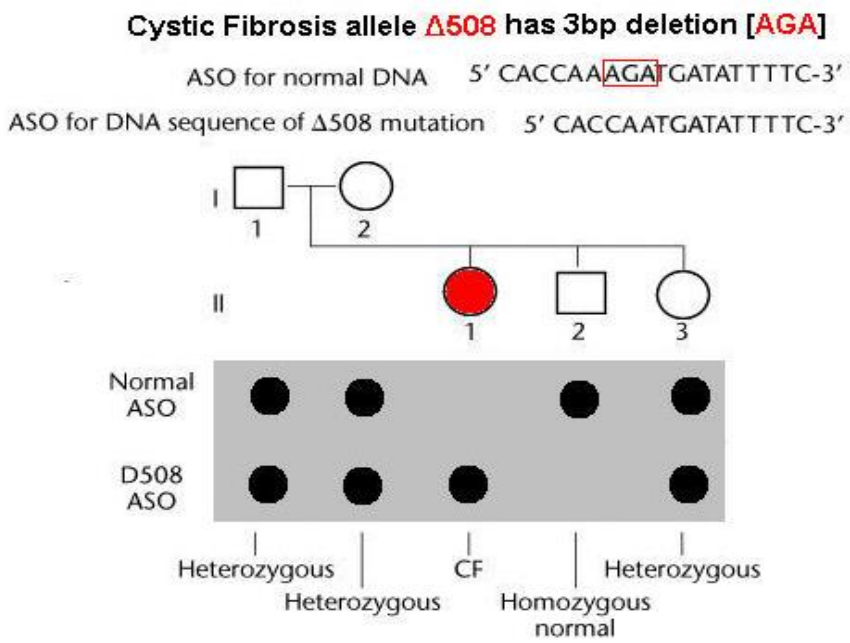
Thus, we can use Allele Specific Oligonucleotide “ASO”, which is a probe that is specific for a certain allele based on a sequence.

We have 2 probes: a probe that recognizes a normal allele and a probe that recognizes a mutated allele.

The main differences between the 1st and 2nd clinical examples are:

1. Unlike the 1st clinical example, we do not use a general probe.
2. We are not doing gel electrophoresis, southern blotting or even fractioning the DNA. What happens in the cystic fibrosis example is that we take the whole DNA and we put it as one spot and then we add the probe. If the probe is hybridized to the DNA then there is a signal and if it is not there will not be a signal.

We have two probes; one will recognize the normal allele and the other one will recognize the mutated allele.



The figure above is a pedigree chart (which looks like a family tree).

The father’s DNA can be recognized by both probes, what does that mean?

He is a carrier, because he has a normal allele and a mutated allele. Therefore, he is **heterozygous**.

What about the mother?

She is also a carrier, because she has a normal allele and a mutated allele. She is also **heterozygous**.

What about daughter number 1?

Her DNA is recognized by the mutated probe but not the normal probe. This means that both alleles are mutated and she is **homozygous** and has the disease.

What about son number 2?

Both alleles are recognized by the normal probe not by the mutated one which means that both alleles are normal. He does not have the disease and he's not a carrier. He is **homozygous normal**.

What about daughter number 3?

She is a carrier because she has a normal allele and a mutated allele.

She is **heterozygous**.

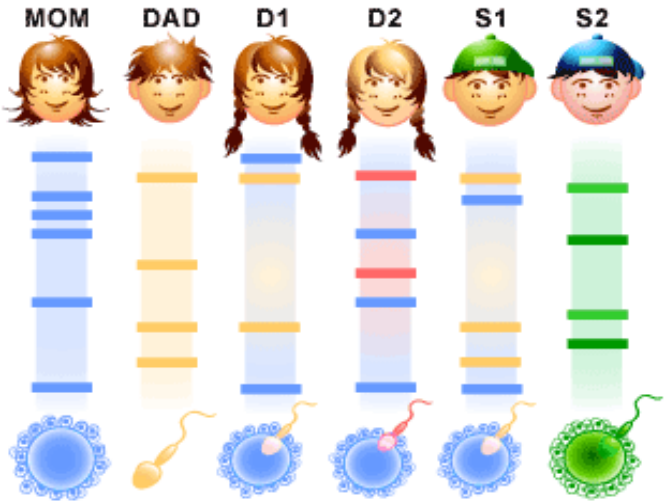
Example 3: Paternity testing

Paternity testing is performed to determine the parents of a child or multiple children. A paternity test includes DNA samples collected from a mother, a father and children. Again, the main goal of this test is to determine if these parents are the biological parents for these children or not.

*Every single DNA fragment the child has must come from both parents, but not necessarily all of them from the mother or all of them from the father. Therefore, collectively all DNA fragments must come from both parents.

Let's take an example ☺

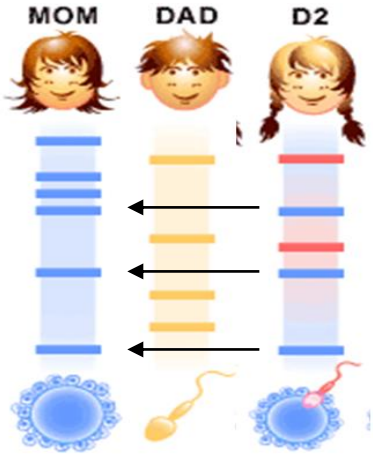
Observe the figure on the right carefully.



Let's start with D1:

The first and last DNA fragments come from the mom and the 2nd and 3rd fragments come from the dad. We can conclude that she is the daughter for the mom and the dad.

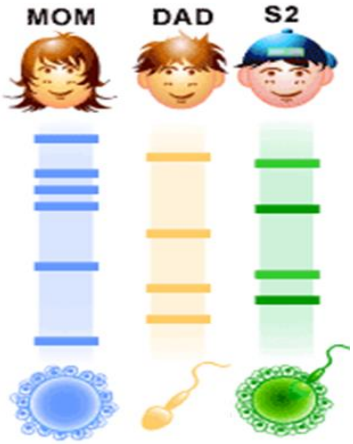
Now let's talk about D2:



She is related to the mom but not the dad. Look at the figure to the right, you can see that there are DNA fragments in the daughter that match DNA fragments in the mom, however, that's not the case with the dad.

S1 is the same as D1, so he is a son for this mom and this dad.

Let's talk about S2:



By looking at the figure, you can clearly see that none of the DNA fragments of S2 correspond or match DNA fragments of this mom or this dad. We can conclude that S2 is not a son for this mom or this dad.

Example 4: Forensics

Another use of RFLP is in Forensics & CSI (crime scene investigation). In the crime scene, blood spots help identify the murderer by doing DNA analysis (we compare possible suspects' DNA with that in the crime scene). In this case, all of the DNA fragments of that suspect must COMPLETELY match the unknown sample, except that sometimes we may have extra DNA bands that come from contamination by the detective/investigator (for many reasons like not wearing gloves, etc...), by bacteria that may grow in blood, or even by the victim.

Cloning

Cloning is another way we could take advantage of restriction endonucleases.

What is cloning?

It is basically making an exact copy of living tissue with the same genetic material.

Bacterial cloning normally happens when one bacterial cell grows into a colony and every cell is a clone of the original cell.

Clone means that every cell has the same exact copy of the chromosome as the original cell.

Plasmid: is an extra chromosomal genetic material which replicates independently of bacterial chromosome.

For DNA cloning it means that I'm making copies of a certain DNA fragment.

Suppose I have a fragment of interest and I can't do any experiments with only one DNA fragment. I have to make many copies of it to do some laboratory work. I clone this DNA fragment using a vector (vector is a carrier of a DNA fragment). Bacterial plasmids are considered excellent vectors.

What we basically do is that I open a plasmid, insert the fragment of interest inside the plasmid, close it, and then transfer the plasmid into a bacterial cell that can replicate and make copies of this plasmid. This is a

type of recombinant DNA technology where we produce a recombinant DNA molecule which is made from different sources.

In order to clone a DNA fragment, a plasmid or any other vector must have at least three of these characteristics:

1) It must replicate independently of bacterial chromosomal DNA (bacterial cell may have multiple copies of plasmid while it has only one original copy of its chromosomal DNA).
2) Capability of inserting a foreign DNA fragment into this vector or plasmid, otherwise it's useless.
3) It must contain a selective marker, meaning that the bacterial cell that contains that plasmid must survive and the one that doesn't have the plasmid must be removed or eliminated.
   How do I do this? We can have an antibiotic resistance gene in the plasmid and as the bacterial cell has the plasmid, the antibiotic won't affect it and it will survive.

Students' Questions:

Q1) Does this process (cloning) help creating pathogenic resistant bacteria?
No, because we don't use pathogenic bacteria as E. coli and these cells are susceptible to other antibiotics as well.
The real causes of developing that resistance is due to evolution, natural selection, and overuse (misuse) of antibiotics.

Q2) Since bacteria regularly have mutations, is this process efficient to make an exact copies of DNA fragments?
Yes it is, we can have mutations generated and so it must be frequently tested for any mutations in the plasmid.

Q3) If we can use PCR to replicate DNA, then why do we do this?
PCR is easier and faster than cloning but sometimes we need to do this.

Q4) How can we make recombinant DNA (insert a DNA fragment into a plasmid)?
Restriction endonucleases added to the plasmid make a staggered cut, so the ends of the plasmid are sticky or hanging. We use the same restriction endonucleases to cut the DNA fragments generating sticky

ends as well. When we mix them together there are different possibilities that can occur , one of them is getting the plasmid of interest –where the sticky ends of the fragment hybridize with those in both ends of the plasmid.

Nevertheless, there are cases where the plasmid can close itself without having that DNA fragment or multiple plasmids come together. These different possibilities are due to the same sticky ends. Here we eliminate all other DNA molecules of no use.

Q5) is it possible to insert the DNA fragment into the bacterial chromosome?
NO

Q6) What is the application for this process (cloning)?

1. For laboratory experiments
2. Production of insulin, growth hormones and vaccines.
3. Study the function of a certain promoter or certain bacterial gene.

Q7) Can we isolate the fragment and inject it into a human cell?

Yes.

We finished the 1st slide. ☺

DNA replication a general mechanism:

In order for life to continue, DNA must be replicated.

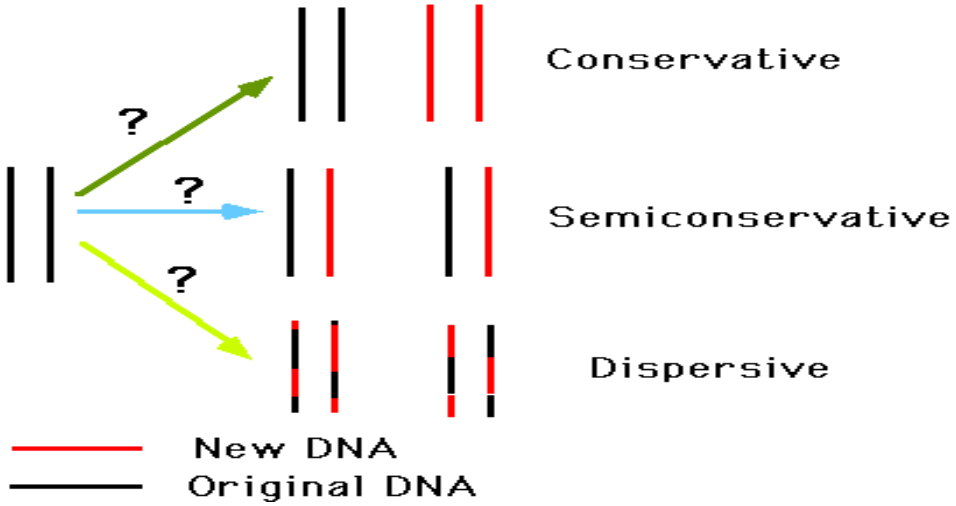
Some basic information:

- The entire DNA content of the cell is known as genome.
- DNA is organized into chromosomes.
- Bacterial genome: usually a single and circular chromosome.
- Eukaryotic genome: multiple, linear chromosomes complexed with proteins known as histones.

Long time ago they knew that DNA is replicated and the idea was **how it is replicated?**

A parent cell has dsDNA and each of its two daughter cells have an exact copy of the parent dsDNA.

3 models were depicted to explain DNA replication:



1- Conservative model:

When the parental DNA is replicated, the two DNA strands will go to one daughter cell and the new strands will go to the other daughter cell.

2- Semi conservative model:

When parental DNA is replicated, each daughter cell will end up with one old strand and one new strand.

3- Dispersive model:

A random process generating daughter cells with DNA strands that are a mixture of old fragments and new fragments.

After conducting many experiments, scientists determined that the semi-conservative model was the real mode of DNA replication.

- The sheet is over -

Sorry for any mistakes.

”فرق كبير بين من حاول الوصول فما بلغ و اخر فر من المعركه دون ادنى مقاومه“

د.خالد ابوشادي