Genetics & molecular biology

◉ Sheet ○ Slide

Number:	-3
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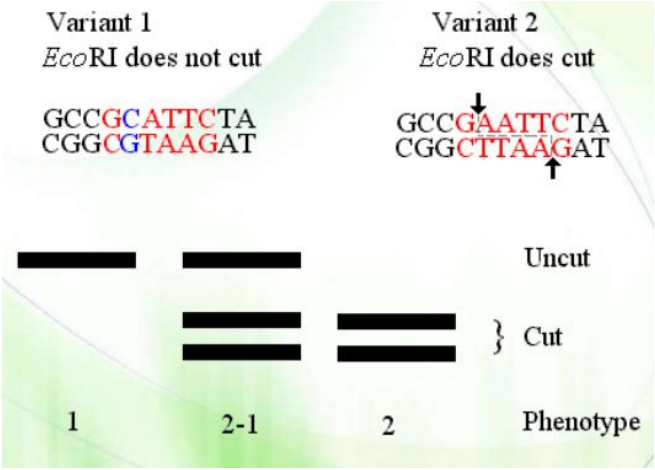
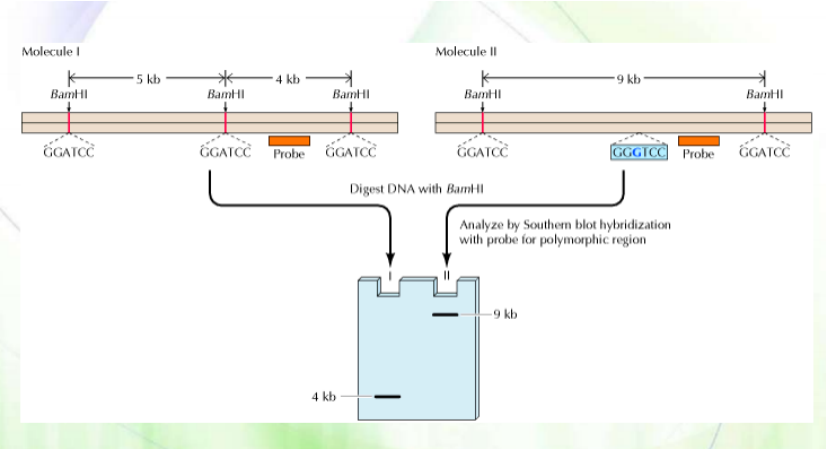
Note: The professor in this lecture started slide three for the first eight minutes or so, then someone realized that he didn't finish slide two yet. So, I typed the first 8 minutes after the lecture's main subject. Hope this doesn't complicate y'all.

We said that some people might have a certain sequence of the DNA either homozygous (same sequence) or heterozygous (different sequence). As they have two copies of DNA, one from each parent. This is the concept of allele.

So, for example, if one has this sequence (variant 2), and we added to it EcoR1. It will read GAATTC and it will make two cuts, generating two fragments. Now if we place the two fragments in gel electrophoresis, they will be separated. Assuming they are of different lengths, if a person has variant 1. So both copies of his DNA are of variant 1, EcoR1 will not recognize them and will not cut them. Now if we take them and run it through gel electrophoresis it'll appear as one single band and up (because it has a greater molecular weight or high base pair fragment.) The person with two variant one copies is homozygous. The DNA from both parents have the same length so they together. Now if a person is heterozygous, took one variant one copy and one variant two copy. The EcoR1 will cut the variant two copy but will not cut the variant one copy, leaving us with three copies on of them being larger (variant one) than the other two (variant two), when we run them through gel electrophoresis.



How can we use RFLP in the clinic?



Let's look at this example, again we have a certain DNA fragment or region in our DNA. This region has two variants as well, either we have three restriction regions or two. So if we add an endonuclease that recognizes the region it will cut variant one to four fragments, while cutting variant two to three fragments only. This is polymorphism in length of DNA fragments generated by restriction endonucleases.

If these fragments are run through gel electrophoresis we will find four fragments for variant one. We might find just three fragments as two of them are roughly of the same length.

We are not just conducting gel electrophoresis here but also southern plotting (using a probe), this means after separating the fragments by size we will add a probe. This will help us find the DNA fragment that binds to the probe ONLY.

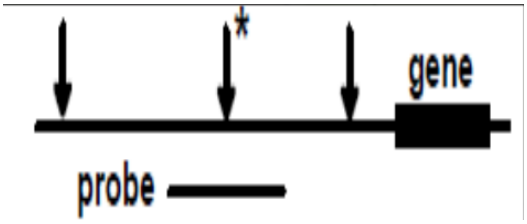
For example if we look at the figure, the probe is attached to a fragment. What is the length of the fragment? 4 kilo base pairs in variant one and 9kb in variant two.

If the person is homozygous and has only variant one, we will see just one fragment (4kb). If the person is homozygous and has variant two only, we will see one fragment as well but with the length of 9kb. If the person is heterozygous, we will see two fragments one of which will be 4kb long, while the other will be 9kb long.

The probe doesn't determine the size or the migration of the DNA fragments in the gel, it just determines where the location of the region in the gel or the membrane is.

What will be seen in the gel (right figure)?

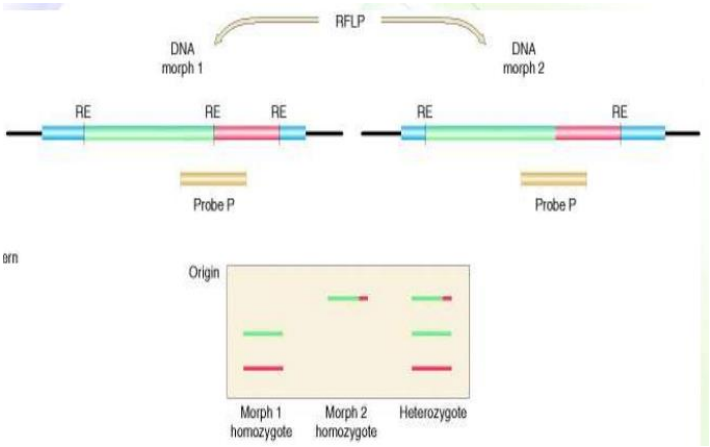
If there is a cut in the middle restriction site (indicated with the star on the figure), will the probe still attach to half of the original site? Right side of the strand or left one? Or will it just not attach?



Depends on the base pairing (hydrogen bonds). The probe can attach to just a part of the DNA sequence only if there are enough hydrogen bonds.

Looking at another example:

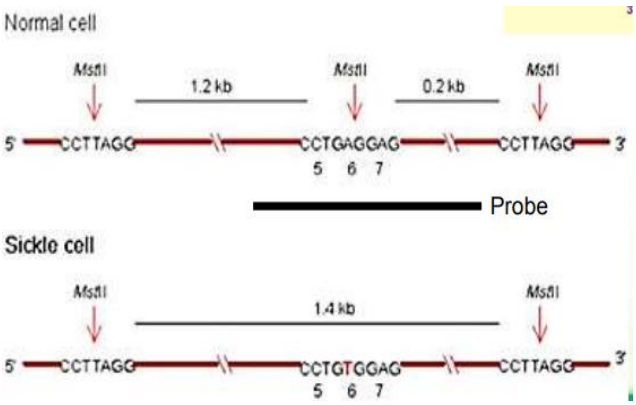
As you can see in this example the first DNA fragment has three restriction sites while the other has two and a missing one due to a different sequence, just one nucleotide is different. Now if the person has homogenous sequence with the one on the left, the probe will attach to two site (red and the green) so we will see two fragments. If he was homogenous with the one on the right then we will just see the large green+red as one fragment. If the person was heterozygous, three fragments will show up, green, red, and green+red.



The professor reexplained it in a slightly different manner, so if you didn't understand it refer to the video, minute 21.

Now about this example: Sickle cell anemia

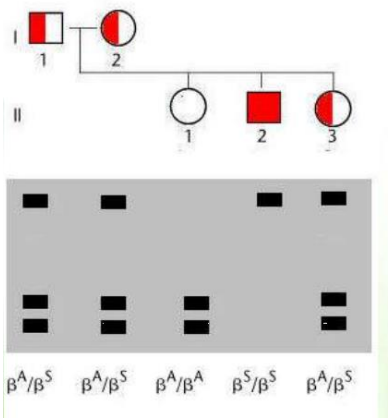
Caused by the change in one nucleotide. The place of the mutation that causes the disease is within a restriction site for one of the endonucleases. So when the gene is normal without any mutations, the endonuclease will cut the DNA. But with the mutation the endonuclease will not be able to cut the DNA.

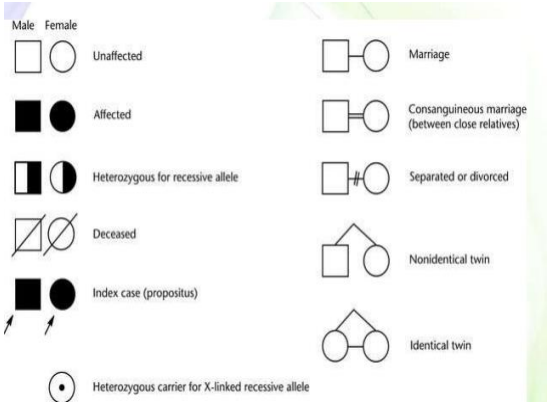


Then we used a probe that can attach to the restriction site, so if there was a cut it will attach to two fragments (the left side and the right side of the normal sequence) and if the person has the disease the probe will attach to the whole thing (larger size).

If you suspect a patient has sickle cell anemia, take his DNA and add endonuclease to it. Then run it through gel electrophoresis and at last at the probe.

- If the probe shows two bands (1.2kb and 0.2 kb) then the person is normal.
- If the probe shows one band (1.4kb) then the person has the disease.
- If the person shows three bands then the person is heterozygous (a carrier).





One and two are carriers (three bands), they got married and we investigated their children's DNA. First child just has two bands (normal), second one had just one band so the endonuclease couldn't cut therefore he is infected, and the third has three bands (carrier).

Further examples:

1. Paternity testing

The basic idea of this test is that the child's DNA can be traced to his parents. 50% each, so every band in the child's gel electrophoresis should be present in either the mother's or the father's DNA. The parents can have different bands from each other, but the child should have the same ones as their parents. So, we trace the bands of the child to the parent's DNAs and check if all of the child's bands are in the parents' DNAs. If not, then the "father" is not the true father. If there was a completely new organization of bands then the child might be adopted. (Check slide 22: D1 and D2 are the only children of the couple)

2. Forensics

DNA is collected from a crime scene and an investigation begins. The DNA is analyzed and compared to the suspects' DNA, of course if there's a match legal actions are taken.

Slide 23, there is no match, he is not the killer.

Slide 24, check the one on the left first. There is 100% match between the DNA collected and the suspect's, he was there. Check the one to the right now, we have 100% match between suspect's DNA and the DNA found in the crime scene, he was there.

If the crime scene was investigated a few days later, this gives time for bacteria to grow so bacterial DNA will be found in the evidence collected. Some DNA from the police officers might be found as well through accidently coughing, not using gloves, etc.… The DNA from crime scene might include DNA not found in the DNA of the suspect but the suspect's DNA must be present in the DNA found.

Cloning (استنساخ)

Another use of restriction endonucleases.

Making a copy of the original, the main idea is that we take a fragment of a DNA and make a lot of copies.

How can we duplicate the DNA? By using bacteria. Why? Because the doubling time of bacteria is just twenty minutes, so it is efficient. So we start with one bacterial cell that will turn to colonies (colons as they came from the same cell).

How can we use bacteria for this duplication?

As we know bacteria has plasmids, and the plasmid differs from the bacterial chromosome as one bacterial cell must have just one chromosome but can have multiple plasmids. The main idea behind it is that we take the plasmid, open it and add the insert to it. After returning it to the bacteria, we let the bacteria divide and the plasmid is passed on. So if we did this to one bacteria and left it for a day, we will have millions of bacteria and let's say each bacteria had five plasmids with the insertion (that's five million DNA fragments).

The plasmid DNA now is a recombinant DNA, which means a DNA from more than one source. The plasmid itself is a vector (carrier) for the DNA insert of interest.

Know the meaning of vector, plasmid and recombinant DNA

To use any plasmid for cloning it must have at least these three features:

1) Can replicate
2) Must have one restricted site (so the endonuclease will work and open it).
3) Must have a selective marker, must have a gene that we can take advantage of where the bacterial cells that contain the plasmid can be selected. We leave in the plasmid a gene that makes the bacteria resistant to antibiotics (antibiotic resistance genes). When the plasmid is placed in the bacteria we add an antibiotic, bacteria that have the plasmid will be resistant to the antibiotic so it will live. Whereas the bacteria that doesn't have the plasmid will die as they are sensitive to the antibiotic. So when we get back to the bacteria after the replication, we will only find bacteria that took the plasmid.

Now how can we open the plasmid?

We use ***restriction endonucleases***.

- We take the plasmid, with a restriction site 'GAATTC'.
- Add EcoR1; which makes a cut and now the plasmid is open.
- Then we take the DNA insert and add EcoR1 to it as well. When the cutting occurs the ends are sticky; now when we combine the insert and the plasmid they will combine together from both ends (using DNA ligase). This is the DNA recombinant; this is how we use restriction endonucleases to make recombinant DNA.

- The plasmid is added to the bacteria. The bacteria that doesn't receive the plasmid will die, while the one that took the plasmid will be resistant.
- Then the bacteria (after the replication process) is killed; releasing the plasmid, the plasmid is collected and then use the same restriction endonuclease to remove the insert.

DNA REPLICATION SLIDE 3:

For life to continue there must be DNA replication, DNA must be relocated so that when cell division takes place one copy of the DNA goes to one cell, the other goes to the second and this is how life goes on.

General information:

- Genome: entire DNA content of a cell
  - Bacterial: usually one and circular chromosome
  - Eukaryotic: multiple, linear chromosomes complexed with histones (proteins)
- DNA is organized into chromosomes

Scientists kept on thinking about how DNA synthesize takes place, they conducted an experiment and came to the conclusion that the second model fits the best. They called it the semiconservative model as one original strand is conserved while the other one is new.

When they looked into how the DNA replicates, it turned out to be that it replicates bidirectionally (two opposite directions) from the center of the bubble. Always remember DNA replication always occurs from the 5'end to the 3' end. So any incoming nucleotide added, it has to be added at the 3' end. (END OF LECTURE, THIS IS THE FIRST 8 MINUITES OF THE LECTURE)

We will continue with DNA replication, we said that:

- DNA polymerase is the enzyme responsible for the replication.
- It works from the 5' to the 3' end all the time.
- When we start DNA synthesis, we start at one point and the replication is bidirectional.
- One of the sides is replicated continuously and is called the leading strand, while the other is called lagging strand as in this strand synthesis occurs discontinuously because the replication must go from 5' to 3' end and the leading strand should open the way for the

polymerase to start synthesizing the new fragment called Okazaki fragments that are eventually connected forming the lagging strand.

Components of DNA replication:

First of all, the enzyme responsible for DNA synthesis is DNA polymerase, and this enzyme cannot start synthesizing DNA de novo (from scratch); there has to be a starting point which the enzyme primase if responsible for. The primase makes short RNA molecules or fragments that is complementary to the starting point called primers that the DNA polymerase starts synthesizing the DNA molecule from.

Note: one primer is needed for the leading strand, but for every Okazaki fragment a primer is needed.

We start with prokaryotic cells because they are much simpler and well understood, eukaryotes will be discussed later.

Overall of the replication process:

We have a primer, DNA polymerase III is synthesizing DNA molecule and hits the primer. The primer is removed, and the DNA polymerase continues the synthesis until it hits the other Okazaki fragment, then DNA ligase connects the Okazaki fragments together. This happens with every Okazaki fragment, the reason why it is called the lagging strand.