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Molecular Biology

Doctor 2019 | Medicine | JU

Sheet Slides

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Recall:

DNA fragments can be separated by A gel (made of sugar material known as agarose) according to size this is a technique called **Gel electrophoresis**

Hybridization: A technique reactions are used to detect and characterize specific nucleotide sequence using Probes (Oligonucleotides). These reactions are based on the concept of complementarity

Hybridization can be imperfect (under specific conditions).

Imperfect hybridization can be controlled by changing the temperature, ionic strength of solutions, modifying GC content by using a probe with a high G≡C content.

Dot blot A technique that informs us if a specific sequence that is complementary to a probe of a known sequence exists in a larger DNA (This probe is labeled either with a florescent tag or a radioactive phosphorus).

Southern blot A technique that is a combination of DNA gel electrophoresis and hybridization by which we detect the presence of a DNA segment complementary to the probe and the size of the DNA fragment.

Restriction endonucleases

First, we should ask ourselves a question How can we fragment our DNA?

The answer is that we use specific enzymes called **Restriction endonucleases**.

Note: we need to fragment our DNA because our genome is so big... A whole chromosome can't pass through the pores in the agarose gel.

**We don't want random fragmentation we need to fragment them in certain way

Endo: inside/internal (the enzyme cut the DNA within DNA fragments).

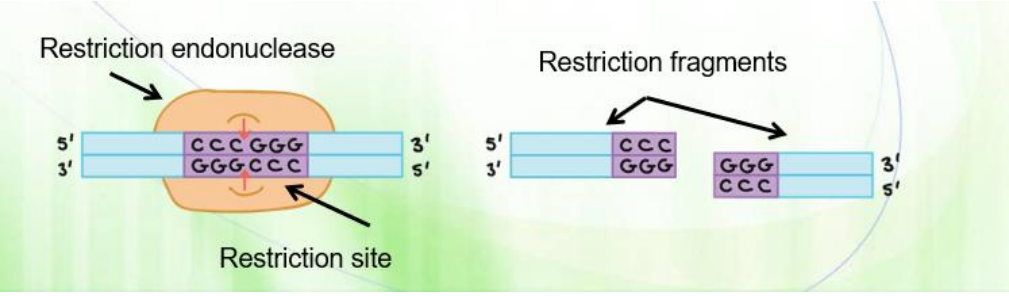
Nuclease: enzyme that degrade(cleave) nucleic acids (in this case DNA).

Restriction: because of the way these enzymes restrict the growth of bacteria (while they were discovering it) and they are restricted where to cleave the DNA when they recognize specific sequence.

****Exonucleases:** nucleases that cleave nucleotides one at a time from the end of a polynucleotide chain breaking phosphodiester bonds at either the 3' or the 5' end.

So, **Restriction endonucleases** are enzymes that degrade DNA within the molecule also they are Bacterial enzymes that recognize and cut (break) the phosphodiester bond

between nucleotides at specific sequences (4- to 8-bp restriction sites) generating restriction fragments.



In the figure shown:

The **restriction site** is the CCCGGG sequence that is recognized by **Restriction endonuclease** and this nuclease make a cut between C and G in the first strand and between C and G in the other strand generating two DNA fragments called **Restriction fragments**.

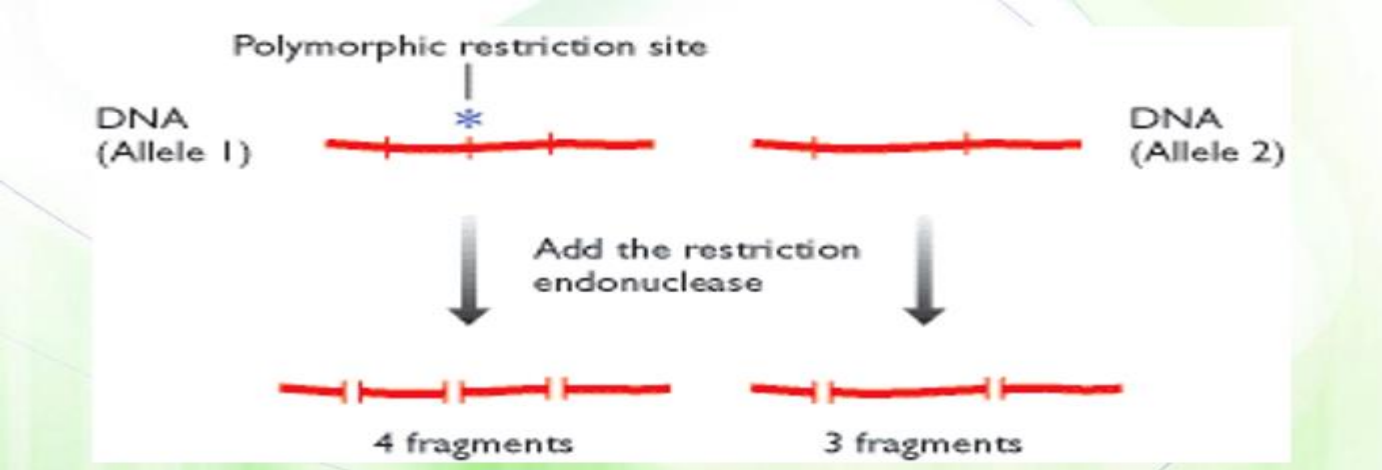
E.G: **Eco RI**

Eco RI is a restriction endonuclease enzyme isolated from species E. coli, **Eco RI** recognizes and cuts within the sequence (GAATTC).

**E. coli is A bacteria that infect meat and can be fetal... Foods that have been linked to E. coli include meat (shawarma, sprouts, spinach, lettuce, ready-to-eat salads, fruit, raw milk, and raw flour and cookie dough.

In variant 1: The sequence is GCATTC NOT GAATTC so the **ECOR1** doesn't recognize the sequence so doesn't make A cut and the DNA remain intact.

In variant 2: **ECOR1** recognize the restriction site and make A cut between A and G generating two Restriction fragments.



If we have 2 DNA fragments that have multiple restriction sites on different parts of the DNA.

In the figure shown:

Allele 1: has 3 e restriction sites that are recognized and cut by restriction endonuclease, forming four fragments.

Allele 2: has two restriction sites that are recognized and cut by restriction endonuclease, forming three fragments.

DNA polymorphisms

Morph: from morphology (structure/shape).

Polymorphism: have many different shapes.

Polymorphism: that is individual varieties in DNA sequence (genetic variants) which 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.
- We are similar in our DNA sequence by about 99.9%, and different by 0.1%, and this variation in our DNA sequences makes us unique individuals, not like each other.

Note: Multiple lengths of DNA fragments that are generated by restriction endonucleases, polymorphic in length of restriction fragments due to DNA polymorphism.

RFLP

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.

molecular fingerprinting:

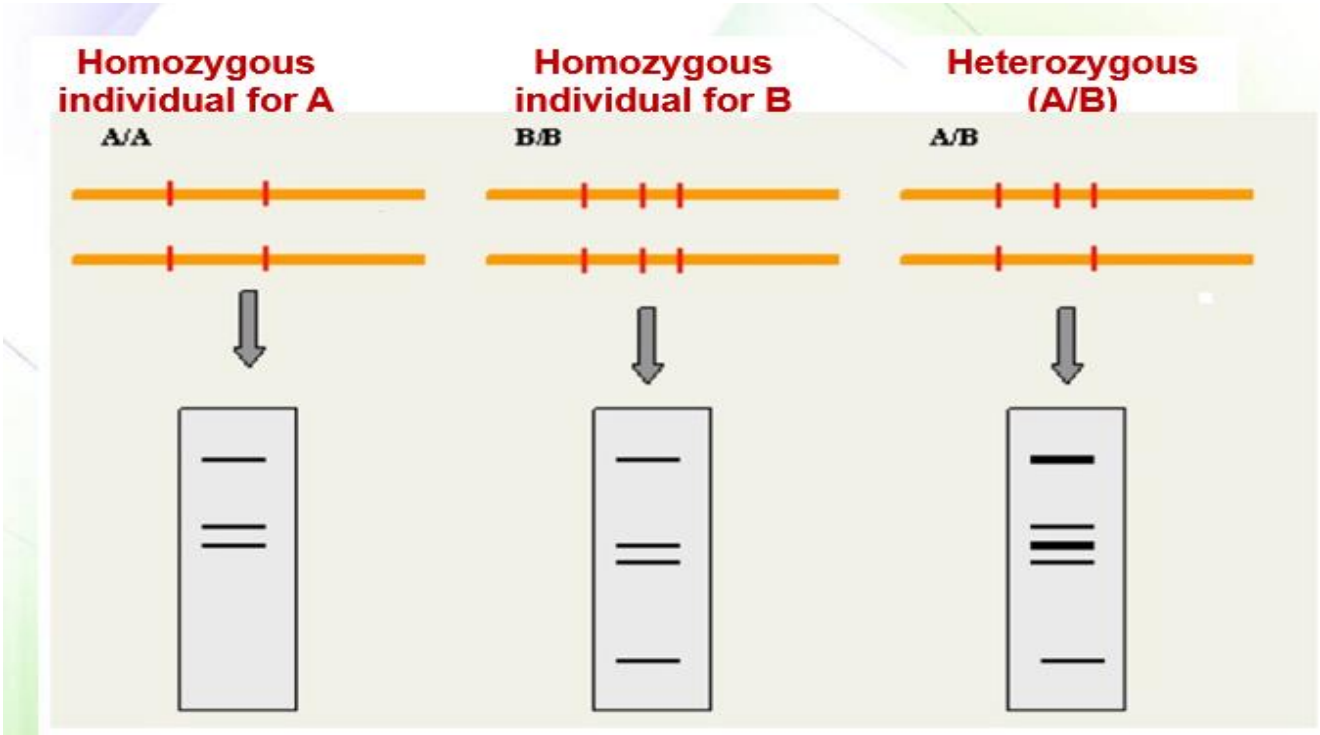
1-We have different patterns of fragments generate

2-We have unique molecular "Fingerprints''.

3- Molecular fingerprinting are used to differentiate between individuals.

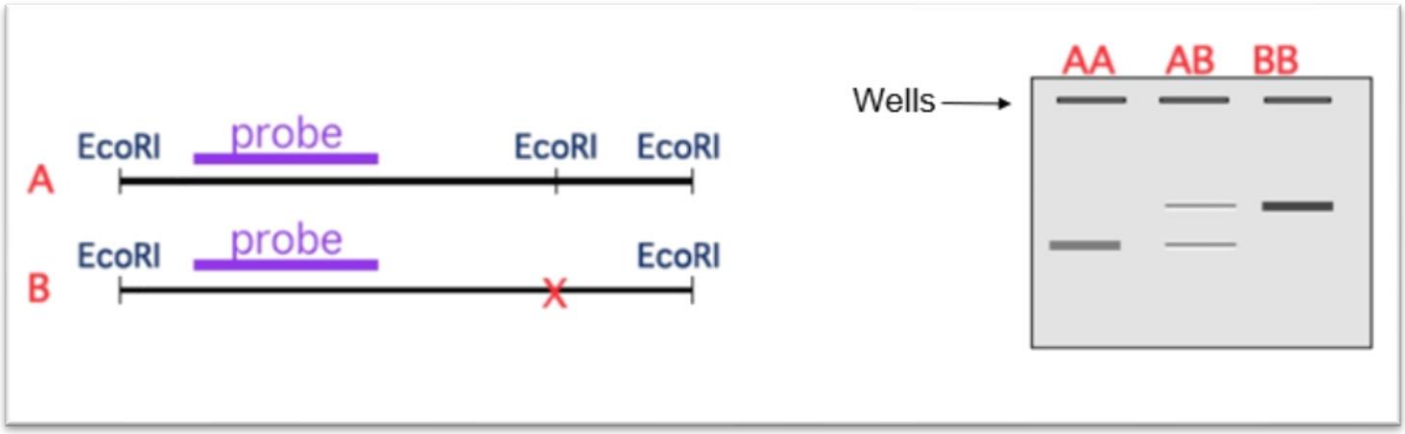
These molecular fingerprints can be detected by gel electrophoresis by itself or along with Southern blotting.

Using Gel electrophoresis only:



In the figure shown:
Homozygous for A: Three bands are formed or seen as a result of the formation of DIFFERENT LENGTHS of restriction fragments (we have six fragments, but fragments of the same length are detected as one band by gel electrophoresis)
Homozygous for B: four bands are formed as a result of the formation of 8 restriction fragments. (Again, we have 8 fragments but only 4 bands are seen since fragments of the same length appear as a single band)

Heterozygous (A/B): 5 bands are formed (We have 7 fragments, but some are identical in length and are detected as a single band)
Remember: The shorter DNA fragments the faster it travels, the longer DNA fragment the slower it travels, if two fragments traveled the same distance that mean they have the same speed and same length.
Using Gel electrophoresis then Southern blotting



In the figure shown:
DNA fragment A: has 3 restriction sites.
DNA fragment B: has only 2 restriction sites.
If someone is **Homozygous for A** one fragment out of two is detected (the outer fragment will not be detected), since only one fragment was hybridized to the probe.
If someone is **Homozygous for B** the probe will detect the long fragment (The only fragment produced).
If someone is **Heterozygous(A/B)** 2 fragments will be detected (the long fragment of allele B, and the long fragment from allele A that was hybridized).

Note: only the fragment that the probe hybridizes to is detected.
**please go back and watch the video from minute 14:50 to 16:55 .

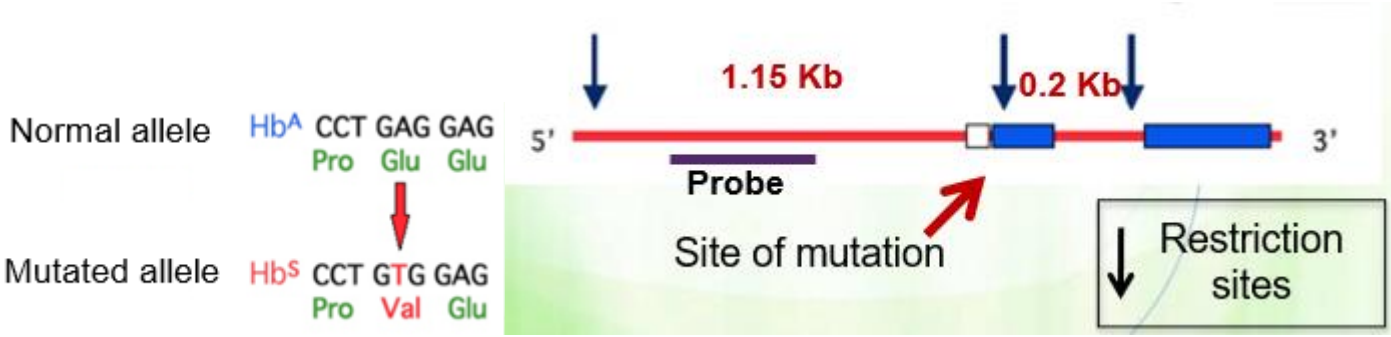
As a result, **using electrophoresis** ALL of the fragments are detected. but **using electrophoresis and southern blotting**, only fragments that are hybridized by the probe are detected (not all of them).

RFLP in the clinic

- How can we use RFLP in the clinic?
  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
    **In fact this technique was a useful technique I the past but not anymore.

Disease detection by RFLP

Example: **sickle cell anemia**



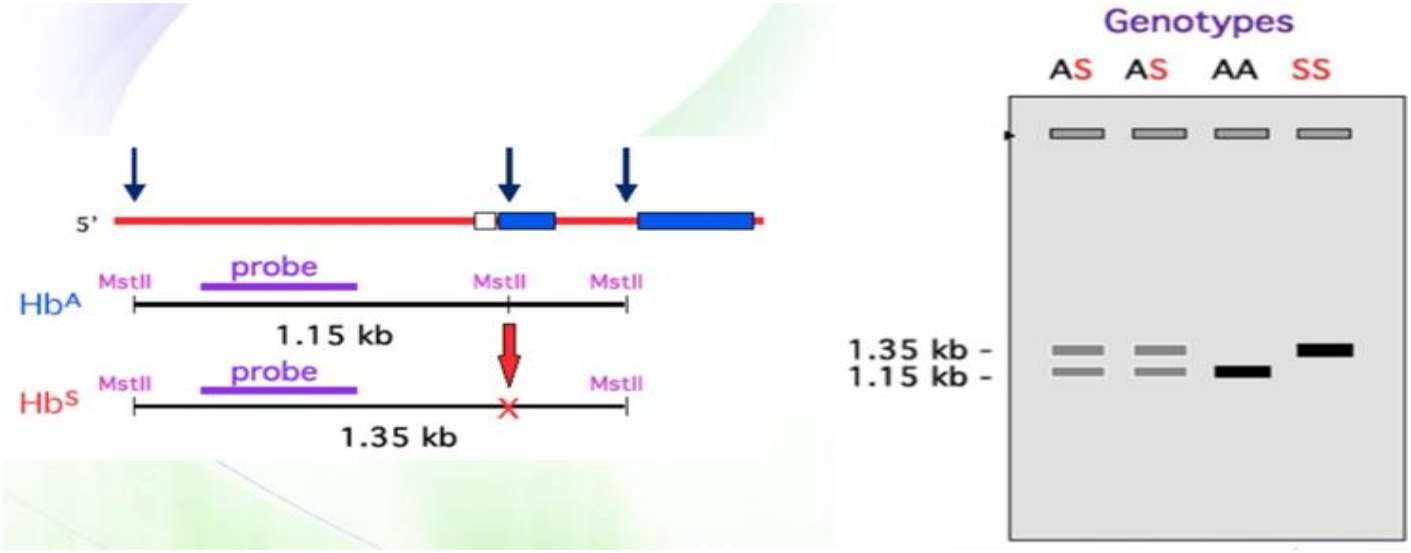
Normally a person with have CCT GAG GAG in the hemoglobin gene.

People with sickle cell anemia have mutation in one BP as CCT GTG GAG. This will result in the formation of defected protein.

- Sickle cell anemia is caused by a mutation in one nucleotide (base) in the globin gene that is responsible for making hemoglobin (the protein responsible for the transport of oxygen).
- The position of this nucleotide happens to be within a restriction site.
- Individuals can have:
    1. Homozygous with two normal alleles (designated as A)
    2. Heterozygous or carriers of one normal allele and one mutated allele (designated as AS)
    3. Homozygous for the mutated allele, or affected (designated as S)

****So this sequence is recognized by a specific restriction endonuclease and makes a cut at blue arrows generating 2 fragment The probe will bind to a region of the DNA through southern blotting, but people with mutation does not have the restriction site so the**

endonuclease will not make a cut and the DNA remain intact.



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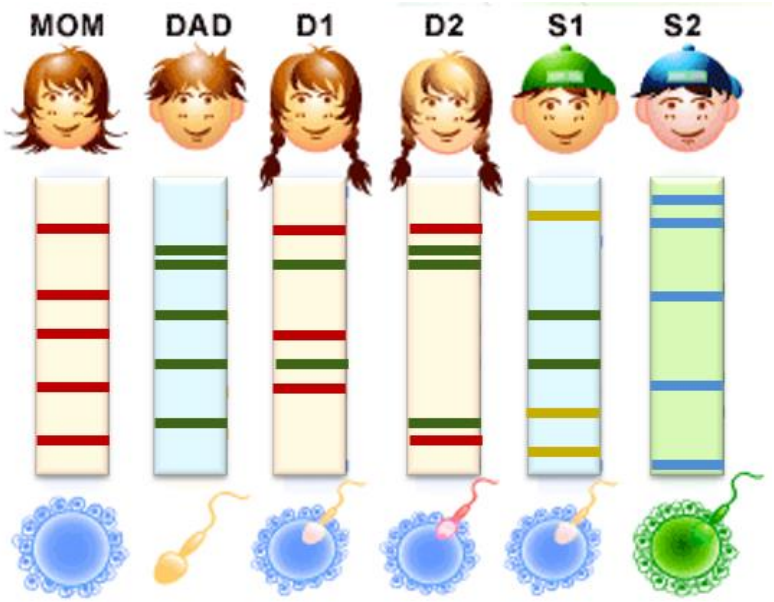
- If someone is Normal (both alleles are without mutation/homozygous) they have HbA. The enzyme makes a cut and the probe detect 1 fragment with 1.15kb.
- If someone has the mutation (both allele with mutation/ Homozygous) they have HbS. The enzyme does not make a cut and the probe detect a large fragment with 1.35kb.
- If someone is a Carrier (one allele without a mutation and one allele with a mutation / heterozygous) they have the probe will detect the shorter (1.15 kb) fragment and the longer (1.35 kb) one, having generated both of these fragments.

Paternity testing

Paternity testing is another example on using RFLP in the clinic:

- We take DNA from the parents and the child.
- We take the child DNA and add restriction endonuclease, the enzyme will make cuts generating certain fragments.
- we take the mother's DNA and add the same restriction endonuclease to generate certain fragments and the same for the father.
- We match the child's fragments to the fragments generated from the mother and father. The DNA child's restriction fragments should be 100% match to the mother's and father's restriction fragments.
- If we can match the pattern of fragments of the child's DNA to that of the mother and father's (almost 50/50) then we know that this is the child of both the individuals



In the figure shown:

D1 and D2 have restriction fragments that are the same as some from the mother and some from the father.

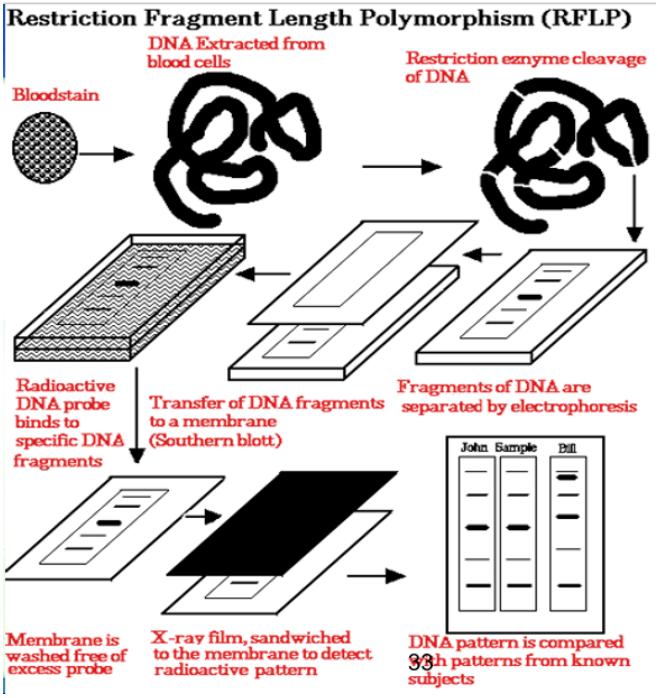
S1's fragments are similar to the fragments from the father but not the mother so we conclude he's the son of the father but not the mother.

S2's His fragments are neither similar to the mother's nor the father's. He is the son of neither of them so and maybe he is adopted.

Forensics

Forensics is another example on using RFLB. In which we match the unknown DNA from the crime scene to the DNA of the criminal.

1. We take a sample from the crime scene (victim's blood, body fluids and saliva etc..) and extract DNA from the sample.
2. They analyze the DNA and compare and match it to the DNA of the suspects.



Be attention to something that really important The unknown sample that taken from the crime scene can be contaminated because we talking about blood that is stilling a few hours in the crime scene. It can be contaminated by bacterial DNA or by police officers if they work lazy and don't do very good in collecting samples.

In the figure:

The DNA fragments from john matches the fragments from the sample from the crime scene. So probably he's the criminal.

Figure 1

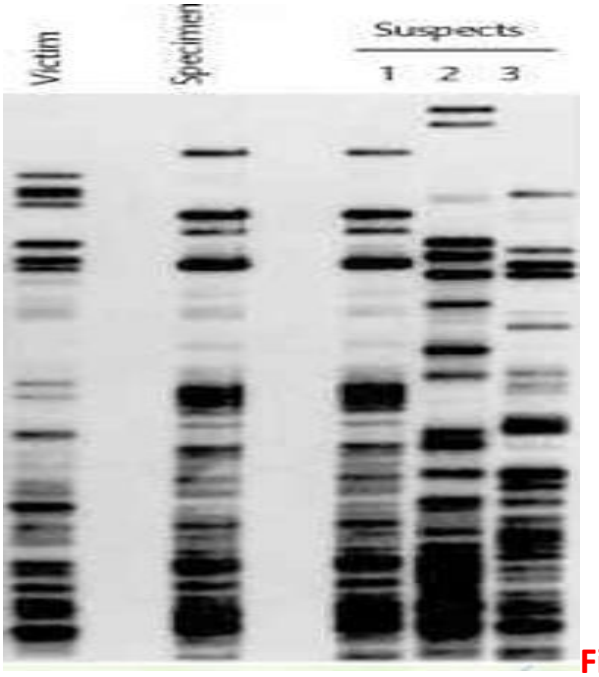


Figure 2

In the figure shown1:

The DNA fragment from suspect 1 matches the DNA fragments from the crime scene so probably he is the criminal

In the figure shown 2:

The DNA fragments from suspect 1 matches the fragments from the specimen from the crime scene.

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If you haven't watch any of the forensics crime drama television series, I would recommend **CSI: Las Vegas, Law & Order: SVU and Hawaii Five-0.**

Good Luck