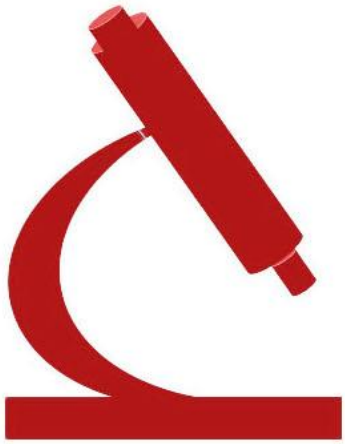

YTOLOGY

Premed 2018 - JU

◉Sheet ○Slides

Number

5

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➢ **What is the difference between Dot blot and Southern Blot?**

Southern Blot

1. The whole genome is cut into **DNA fragments**
2. These DNA fragments are then separated according to size by **gel electrophoresis**
3. If you stain the DNA, a distinct band isn't seen, but a smear is seen
4. The DNA is transferred to a membrane
5. The membrane looks exactly the same as the gel with the same smears of DNA as seen on the gel
6. A probe is then added, and binds to a fragment that is complementary to it
7. The unbound probes are then washed away
8. If one of the DNA bands is complementary to the probe, then a signal is given on an X-ray

- Using Southern Blotting, if the band gives a signal we then know:
    1. The band is complementary to the probe
    2. The size of the fragment bound to the probe

Dot Blot

1. We take the Whole Genome/ DNA molecule and add it onto a piece of paper (Membrane)
2. A probe is added (This probe is complementary to a specific sequence of a region of DNA)
3. **IF** this complementary sequence is found in a region of the DNA molecule**, the probe can bind to it.**
4. The unbound probes are then washed away
5. Because the probe is labelled, once it binds to the DNA it gives a **Signal**. If there is no signal, then that means there is no complementary region in the genome.

- Using **Dot Blot**, we are ONLY able to find out or know whether the sequence complementary to the probe is found within the DNA sample or not.

➢ **Applications of Southern Blotting**

Nuclease: An enzyme that cuts/cleaves nucleic acids (RNA or DNA). ex: DNase- cleaves DNA, RNase- cleaves RNA

Two types of Nucleases:

1. Endonuclease: (found in bacteria only) Cuts/cleaves the DNA or RNA at a site within the molecule itself.
2. Exonuclease: Cuts/cleaves the DNA or RNA from either end (starting from the 5' or 3' end), removing nucleotides one after the other (one by one).

Protease: Cleaves proteins
Lipase: Cleaves lipids
Esterases: Removes ester bonds/groups

➢ **Restriction endonucleases**:

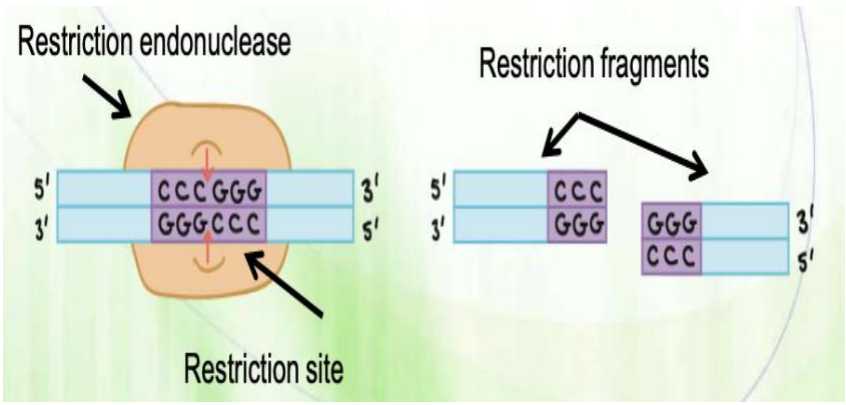
Bacterial enzymes that recognize and cut (break) the phosphodiester bond between nucleotides at specific sequences – called **restriction sites**- (the sequence's size ranges between 4 to 8 base pairs), generating separate fragments called **restriction fragments**. The enzyme cleaves these sites within the molecule.

➢ **Important terms to keep in mind:**

Restriction sites: Specific sequences found within the DNA molecule that are detected by restriction endonucleases, which cut/cleave the DNA at this specific site. These sites can be read on both DNA strands as they are exactly the same.

Restriction fragments: Separate fragments formed or generated by the cleavage of restriction sites by **restriction endonuclease**.

In the figure shown:
The strands are read from 5' to 3' as CCCGGG on either strand. This specific sequence is known as the restriction site, which is cleaved to form restriction fragments by a specific restriction endonuclease enzyme.

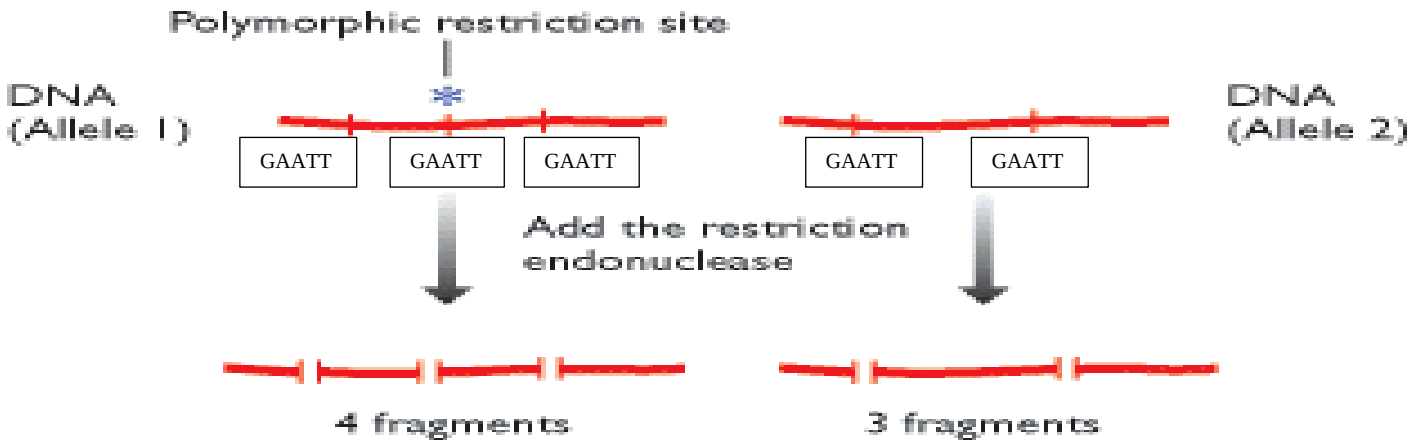


The endonuclease enzyme EcoRI recognizes and cuts within the sequence (GAATTC) and is from the bacterium E. coli.

Variant 1 *Eco*RI does not cut	Variant 2 *Eco*RI does cut
GCCGCATTCTA CGGCGTAAGAT	GCCGAATTCTA CGGCTTAAGAT

- For **Variant 1**, the sequence varies by one nucleotide, as **GCATTC** instead of **GAATTC)**. Due to this variation, the EcoRI cannot cut the DNA into fragments. The DNA remains intact.
- For **Variant 2**, DNA is cut into two pieces (restriction fragments).

➢ **What if we have two fragments with multiple restriction sites at different parts of the DNA?**



Allele 1 has **three** 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 Polymorphism**

Morph: Shape
Polymorphism: Many different shapes

DNA Polymorphism: When individual variations in DNA sequence (genetic variations) may create or remove restriction-enzyme recognition sites generating different restriction fragments.

- Remember, we are diploid
- We have identical chromosomes with genes at the same position on each.
- However, we have genetic variance, which means we have some variation in our DNA sequences that makes us unique individuals, unlike each other.

  For example: Since we are diploid, we have **two copies** of **chromosome number 5.** The first chromosome number 5 will have **GAATTC** at a certain position. And on the second chromosome number 5, at the *SAME* position/location, is **GCATTC**. So, the restriction endonuclease that **specifically** cleaves DNA within the (**GAATTC**) site would cut/cleave the First chromosome, and not the other.

➢ **At a certain position in our DNA, we can either be Homozygous or Heterozygous**

- We have alleles of the same gene (ex: Hair color)
  1. If one allele codes for the protein that gives us **black** hair, and the second allele codes for the protein that gives us **brown** hair → **Heterozygous**
  2. If one allele codes the protein that gives us **black** hair, and the second allele codes for the protein that gives us **black** hair as well → **Homozygous**

➢ What gives rise to **Polymorphism**?

Multiple lengths of DNA fragments that are generated by restriction endonucleases, (polymorphic in length of restriction fragments.)

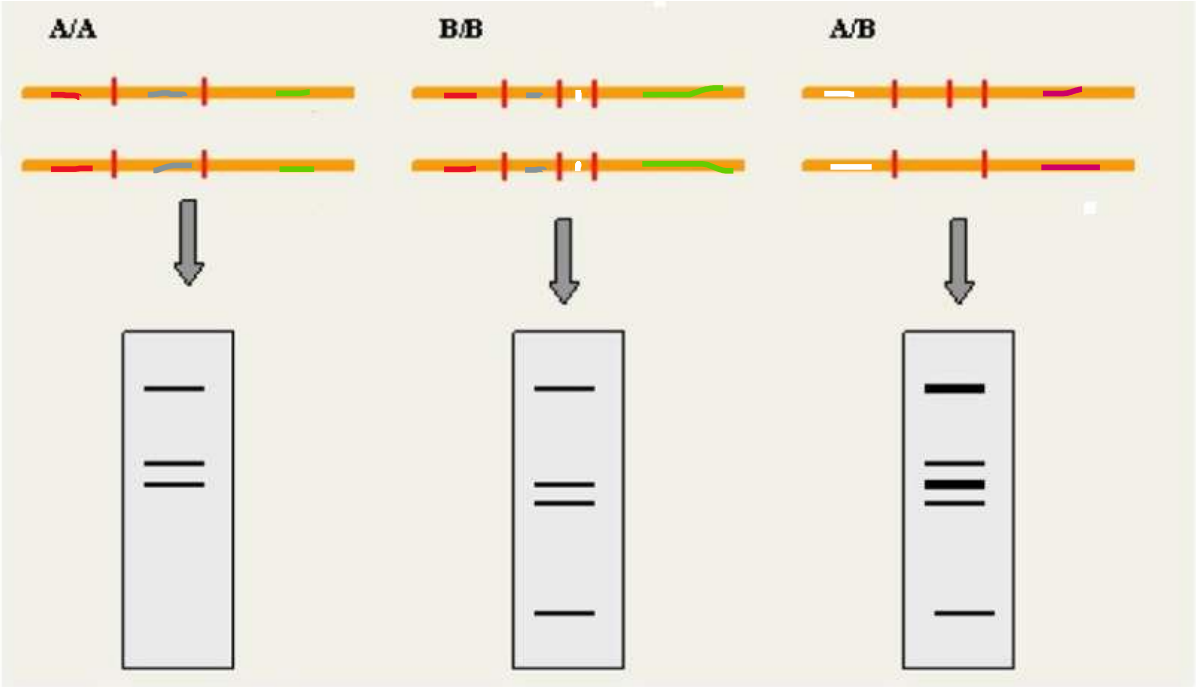
The presence of different DNA sequences in individuals generates a **restriction fragment length polymorphism, or RFLP.**

➢ **Molecular fingerprinting:**

- We have different patterns of fragments generated
- We have unique molecular "Fingerprints"
- We have variable lengths of fragments generated
- We use molecular fingerprinting to be able to differentiate between individuals
- DNA of various individuals have their own distinct restriction fragments
- These can be detected by gel electrophoresis by itself or along with Southern blotting.
- We can use molecular fingerprinting to compare between an unknown sample's DNA fragments with a known DNA sample from a known individual.

- **Using gel electrophoresis <u>only</u>**

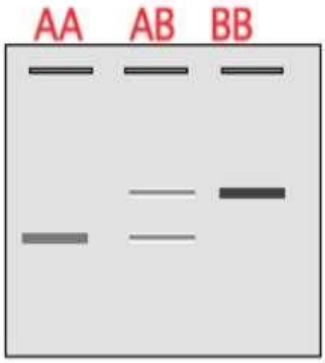
Homozygous individual for A	Homozygous individual for B	Heterozygous (A/B)



- **Same length of restriction fragment→Same speed→Same band**
- **Homozygous for A:** Three bands are formed or seen as a result of the formation of ***<u>DIFFERENT LENGTHS</u>*** 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)

- The longer the DNA fragment, the slower it is→ **Slowest/ longest is at the top**
- The shorter the DNA fragment, the faster it is→ **Fastest/shortest is at the bottom**

➢ **Using Gel Electrophoresis and then Southern Blotting**



Only DNA fragments that the probe hybridizes to is detected.

- **On A:** there are three restriction sites
- **On B:** there are two restriction sites only
- We separate these restriction fragments by gel electrophoresis and then transfer them to a membrane (piece of paper) and add a probe.
- **Only the DNA fragment that the probe hybridizes to is detected.**
- **If someone has both alleles as A,** one fragment out of two is detected, since only one fragment was hybridized to the probe. (the other fragment wasn't)
- **If someone has both alleles as B, it means the probe will detect the long fragment** (The only fragment produced)
- **If someone is heterozygous,** then the longer fragment of A (the one hybridized to the probe) and the long fragment of B are detected.

➢ Using **electrophoresis** ALL of the fragments are detected. However, using **electrophoresis AND southern blotting**, only fragments that are hybridized to the probe are detected (**not** all of them).

➢ **How can we apply southern blotting in clinics?**

- **We can detect diseases. How?**
  Say there is a mutation, a change in the DNA sequence, which resides exactly at the restriction site. It is a mutation that causes the production of a defective protein, causing a disease. The site where the mutation takes place is a **RESTRICTION SITE**.
- A change in one nucleotide at the restriction site means the restriction enzyme will NOT detect the restriction site and won't cut the DNA there.

- This generates a different fragment of a certain length that is different than what we expected.

➢ **Sickle Cell Anemia**

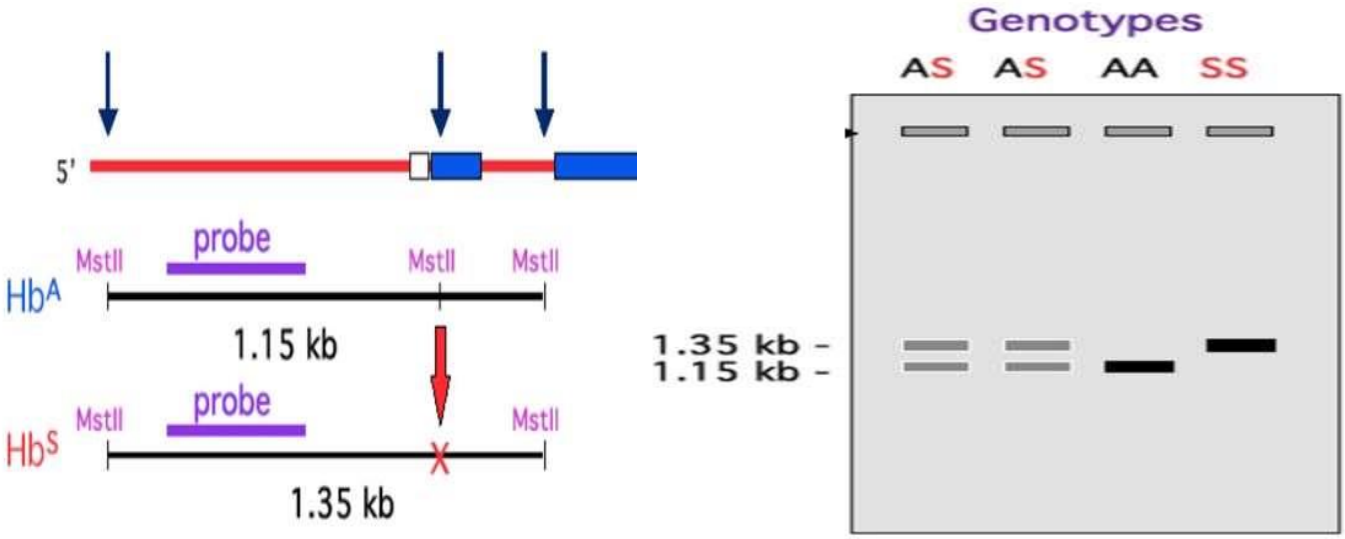
- Sickle cell anemia is caused by a mutation in one nucleotide (base) in the globin gene that is responsible for making hemoglobin.
- The position of this nucleotide happens to be within a restriction site.
- Instead of **GAG**, people with the disease have **GTG** instead.
- If restriction endonuclease is added, it won't be able to make a cut there.

How can you detect whether or not the person is a carrier? This mutation is found within a restriction site for a particular enzyme.

Individuals can be:

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

- ➢ Blue arrows indicate where the cuts are made. The probe will bind to a region of the DNA through southern blotting.
- ➢ If the mutation occurs, or arises, a cut will not be made at the site indicated as it is the site of mutation.



- If a person is **normal** (two good alleles -homozygous-), they have HbA (hemoglobin A). The enzyme will make a cut at three sites and the probe will detect one fragment **(1.15 kb (kilobases))**
- If a person has the **disease** (two bad alleles -homozygous-), they have HbS (Hemoglobin S <Sickle>). The enzyme will not cleave the DNA and the probe will detect the large DNA fragment **(1.35 kb)**
- If a person is a **carrier** (one good and one bad allele -heterozygous-), the probe will detect the shorter **(1.15 kb)** fragment and the longer **(1.35 kb)** one, having generated both of these fragments.

- <u>Another way we can use southern blotting in clinics:</u>

- **Paternity testing**

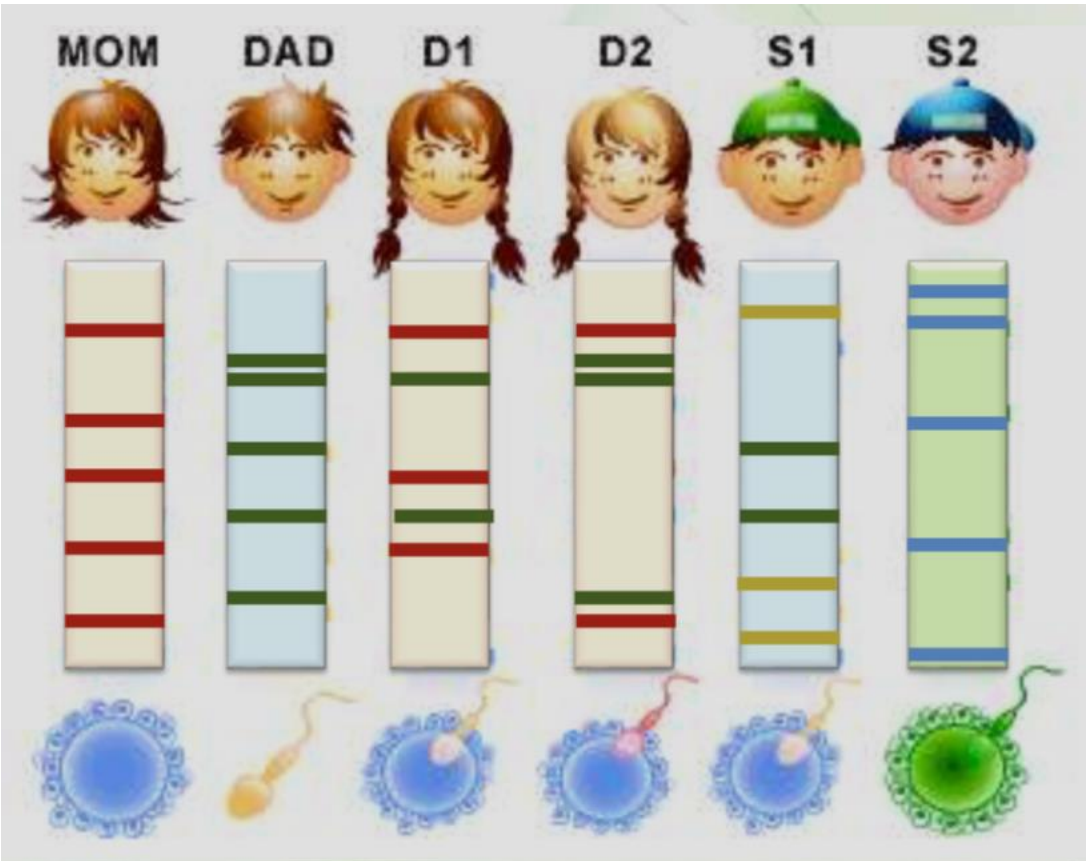
- We take all of our DNA from our parents.
- If we take the child's DNA and add restriction endonuclease, it would make cuts at certain sites, generating certain fragments.
- If we take the mother's DNA and add the same restriction endonuclease, fragments are generated.
- We do the same for the Father's DNA.
- All of the child's restriction fragments generated should almost 100% match both the mother and the father's.
- We match the child's fragments to the fragments generated from the mother and father's DNA

- 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.

D1 and D2 have restriction fragments that are the same as some of the mother's and father's generated DNA fragments.

S1's fragments come from none of the mother's but some of the father's. He is the father's son, not the mother's.

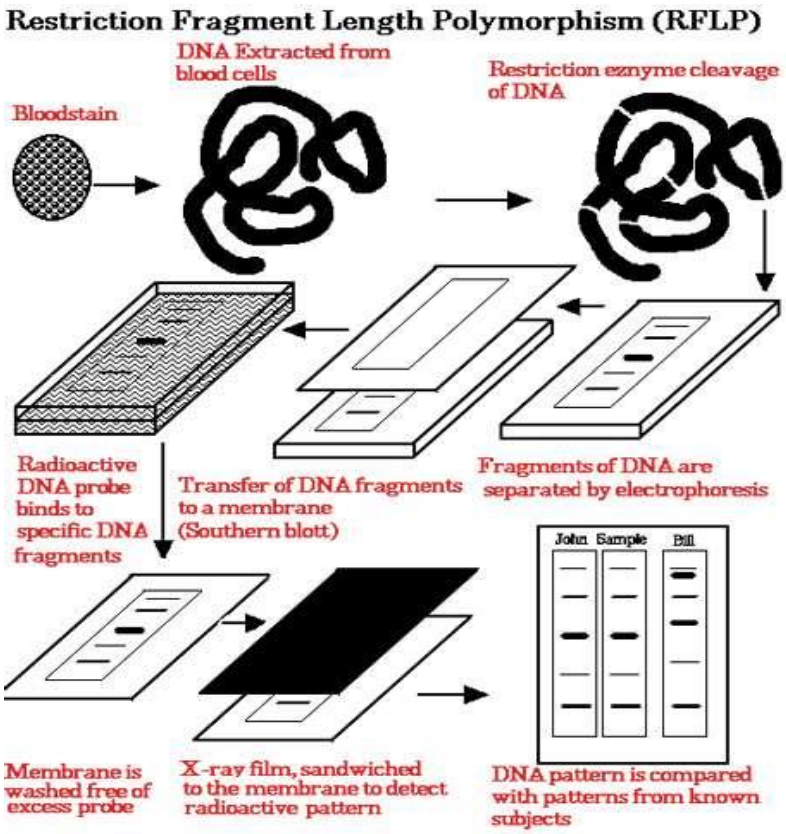
S2: His fragments are neither similar to the mother's nor the father's. He is the son of NEITHER of them.



➢ **Forensics:**

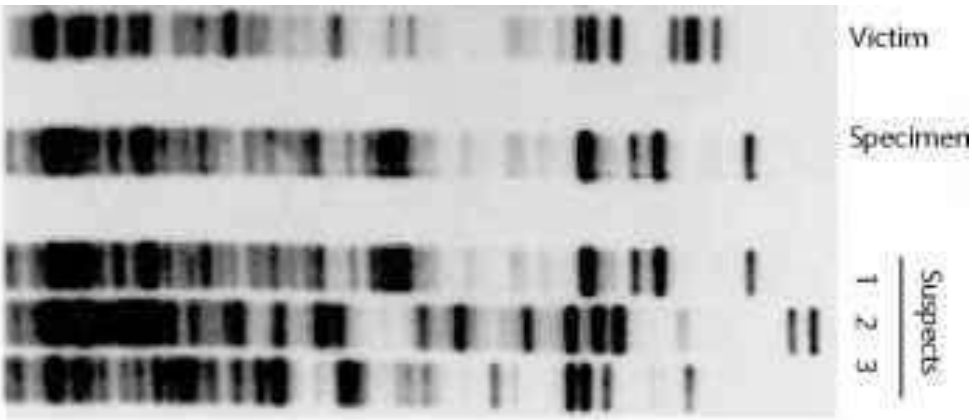
We use molecular fingerprinting to match the sample DNA analyzed to the DNA of the criminal.

1. They extract the DNA from the sample (saliva, victim's blood, body fluids)
2. They analyze the DNA
3. They compare and match it to the DNA of the suspects.



Suspect 1:

is the criminal/murderer as the DNA pattern matches the pattern of DNA analyzed from the sample taken at the crime scene.



Issues faced when using DNA analysis and molecular fingerprinting in this case (forensics):

1. Sometimes the blood is exposed for a long time before it is tested, and so the sample could be contaminated with bacteria (bacterial DNA could interfere with the human DNA).
2. If no gloves are worn.
3. When someone contaminates the sample deliberately (on purpose).

Good Luck ☺