

FINAL – Lecture # 3

# Overview and Basic techniques (pt.3)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ ﴾

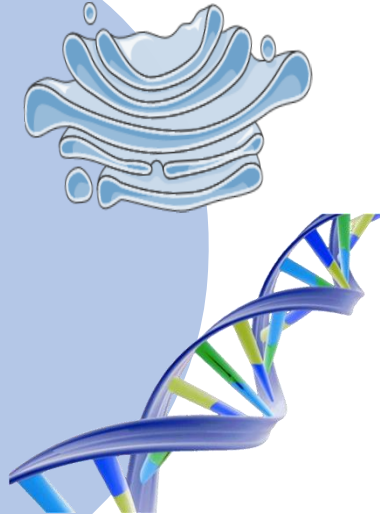
اللهم استعملنا ولا تستبدلنا

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**the doctor started the lecture with a small revision about the previous lecture make sure you studied it and here is a quiz**

**[Molecular biology lec2 quiz](#)**

# Disease detection by ASO (Cystic fibrosis)

## ASO: Allele-specific oligonucleotide

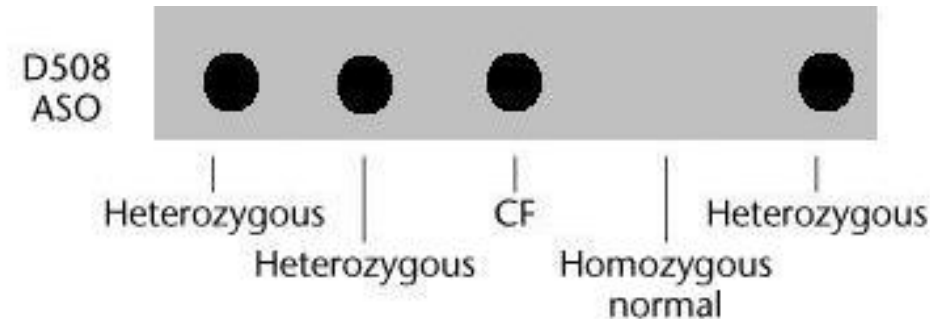
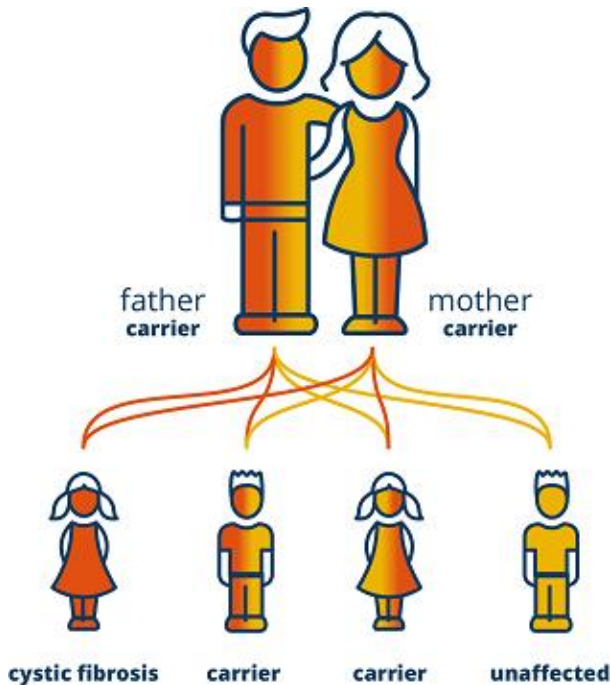
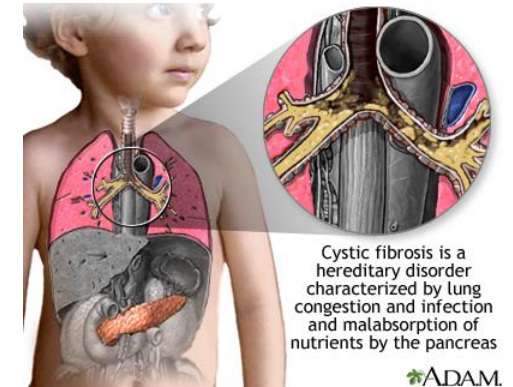
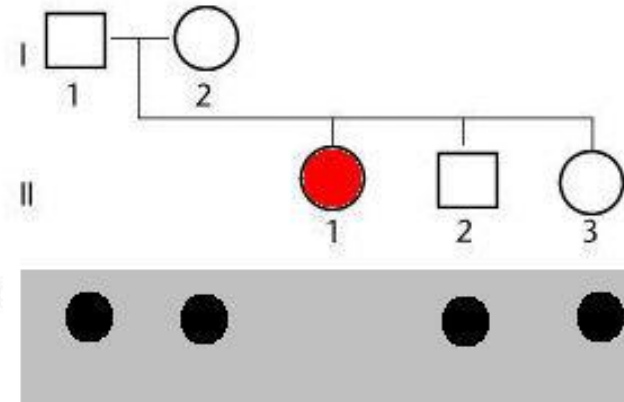
this slide from the previous lecture 😊

Cystic Fibrosis allele  $\Delta 508$  has 3bp deletion [AGA]

ASO for normal DNA 5' CACCAAAGATGATATTTTC-3'

ASO for DNA sequence of  $\Delta 508$  mutation 5' CACCAATGATATTTTC-3'

The whole genomic DNA is spotted on a solid support (a membrane) and hybridized with two ASO's, one at a time.

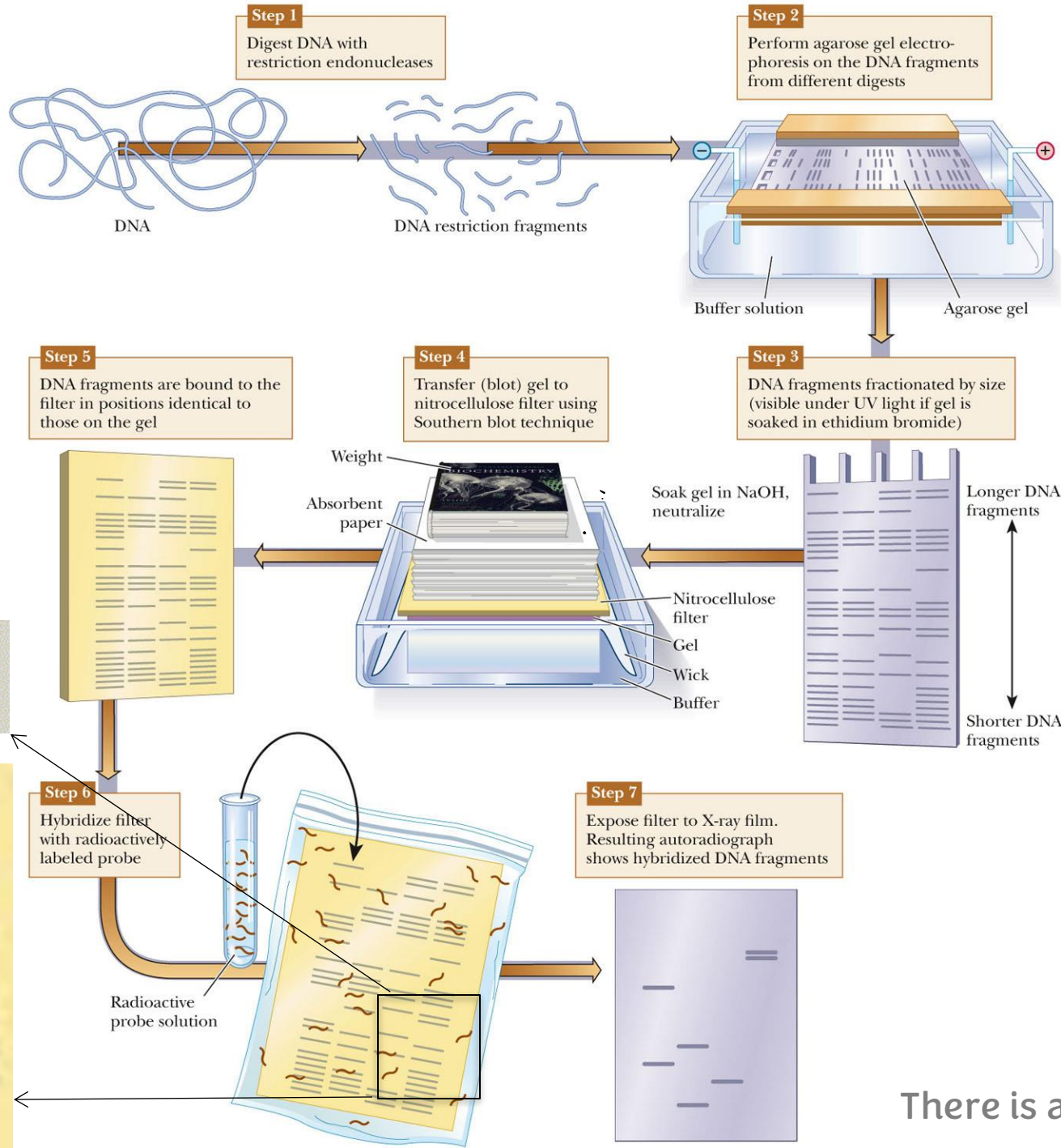


# Resources

- <http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html>
- Watch this....very important

# Southern blotting

- This technique is a combination of DNA gel electrophoresis and dot blotting
- Used to detect:
  - the presence of a DNA segment complementary to the probe
  - the size of the DNA fragment



1- the idea from this technique is that :

a) the DNA is taken from an individual .. Organism .. Cells

B) Fragment the DNA in to smaller pieces

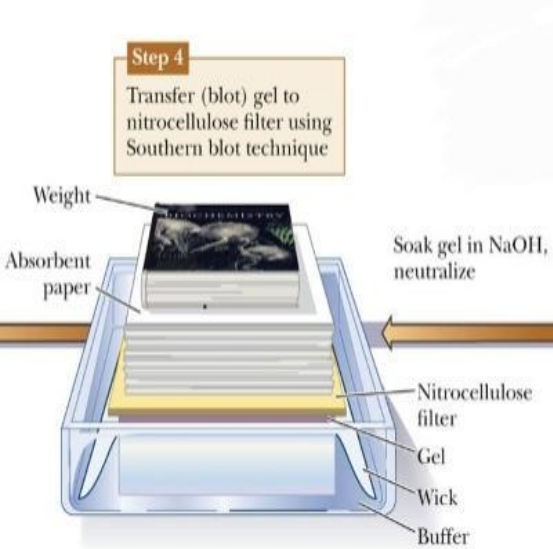
2- Then separated in the gel electrophoresis

3- At the end of the electrophoresis you have the gel with large fragments on top and the smaller ones at the bottom

.. It is gradient .. larger → smaller → smaller

There is a complement in the next slide 😊

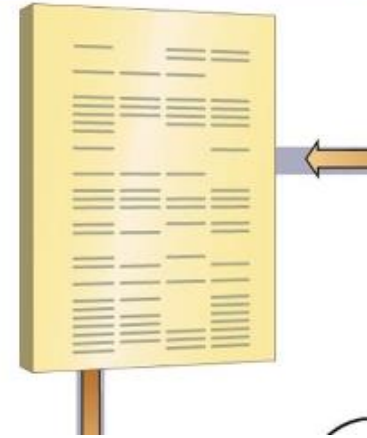




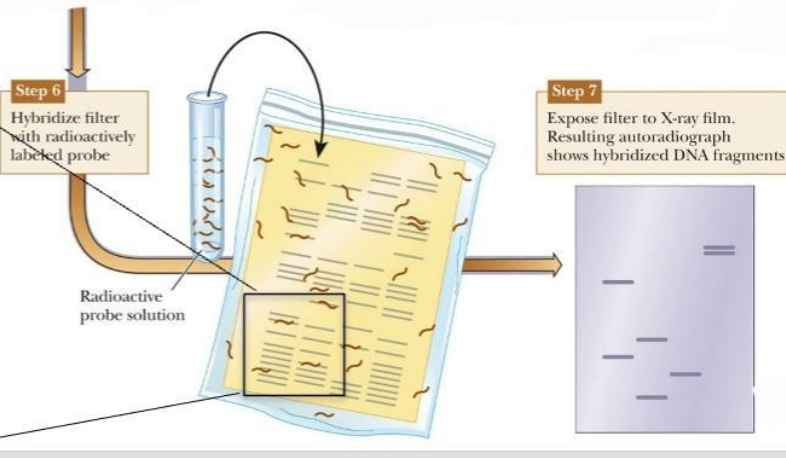
4- transfer the DNA fragments from the gel to the membrane (we put them together 🤝 like this so they transfer ) so if we look at the membrane **it looks like a replica as in the gel**

A replica is an exact copy of anything in this context it is the exact copy of the DNA

**Step 5**  
DNA fragments are bound to the filter in positions identical to those on the gel



5- now the DNA is on the membrane in the same order that was on the gel ..the largest on the top and the smaller at the bottom



6- now we add a lot of probes molecules (thousands or millions same as the DNA molecules that we add in the first step )

Someone will ask why don't we add the probe to the gel ? Why should we transfer the DNA to the membrane ?

Technically the probe will not be able to get inside the gel and bind to the DNA , but in the membrane the DNA fragments are found on the surface .

The probe has easy access to the DNA fragments , then the probe will bind to the DNA if there is a sequence that is complementary to it (hybridized to)

7- Then we can detect the signal .

There is a complement in the next slide 😊

I have 4 samples in the adjacent figure

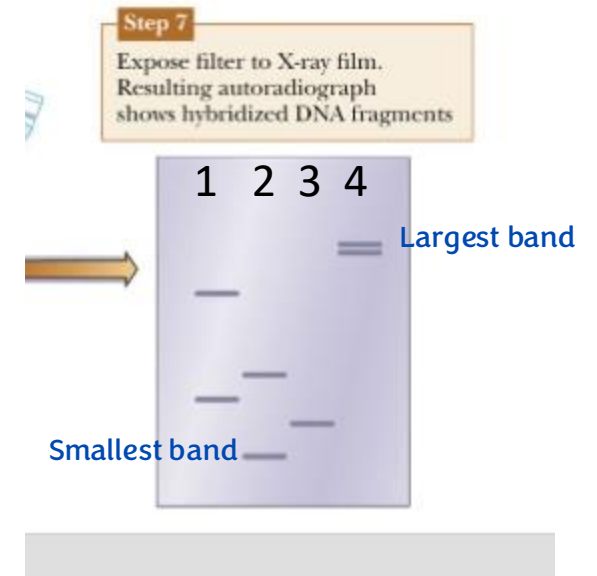
What is the information that I can raking (collecting) from it ?

The sizes of the bands .. And these bands have a sequence that is complementary to the probe

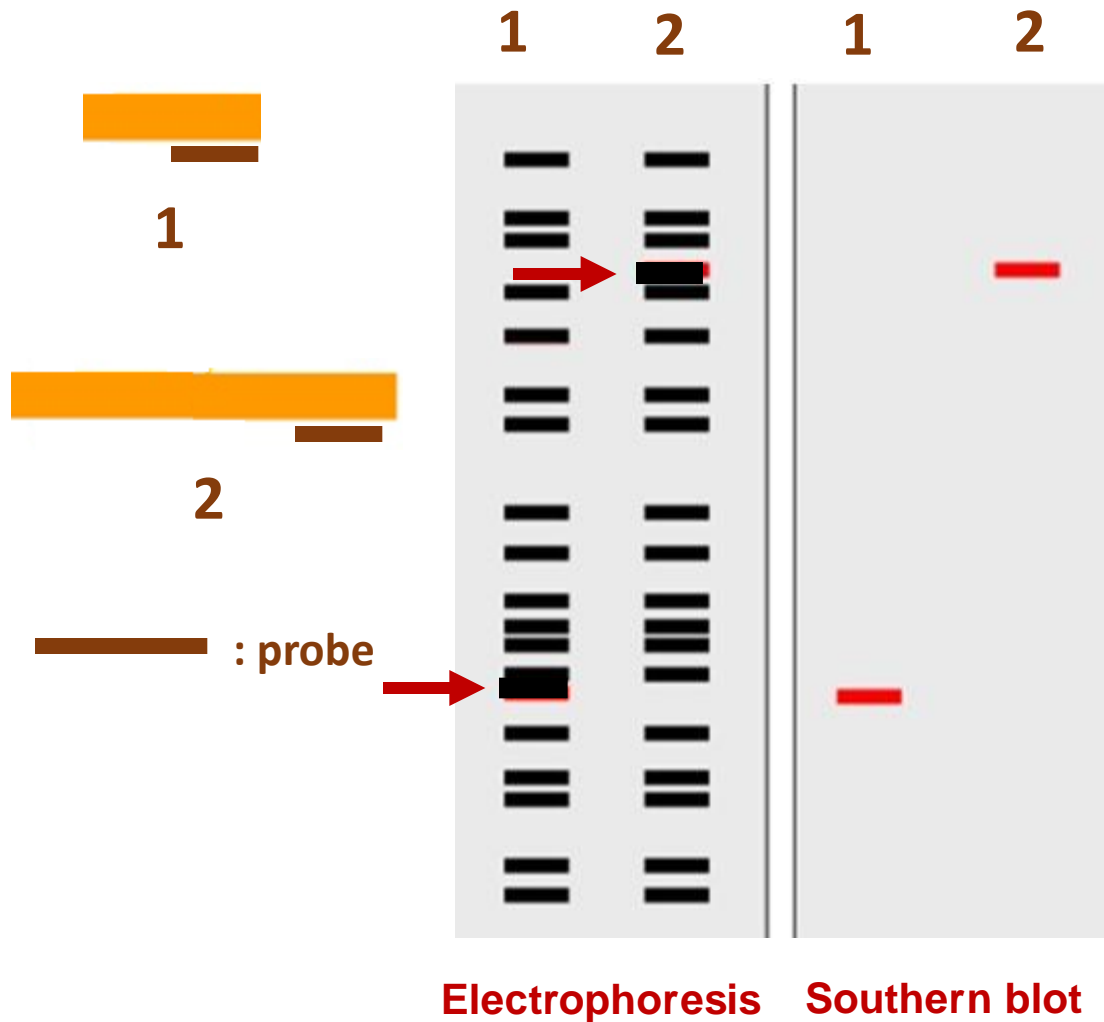
Are the 4 samples identical ? No

Note :

- ❖ the sizes of the bands don't reflect the size of the prob .. We separate the segments of the DNA before we add the prob .
- ❖ The probe is added on the membrane not the gel because its easier to access the DNA fragments; that's why we transfer DNA fragments to the membrane







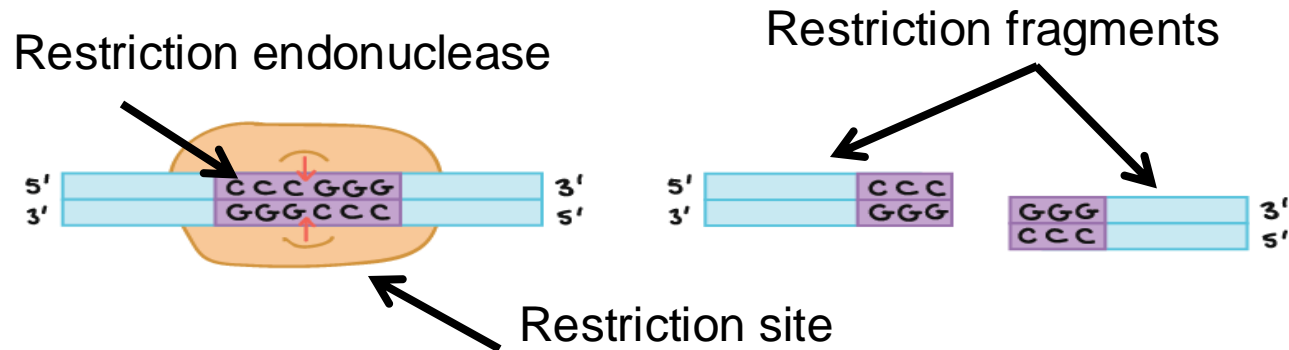
- ✓ Gel electrophoresis will be performed for two samples, resulting in multiple DNA fragments appearing as bands. The fragments will then be transferred to a membrane, and a probe will be added to bind to specific bands.
- ✓ In Sample 1, the probe will bind to a smaller DNA fragment, while in Sample 2, it will bind to a larger fragment. The probe will bind to the same target site in both samples, but the difference in fragment size will be observed.

# Restriction endonucleases

Within enzyme that degrade nucleic acid

Endo → within  
Exo → From the ends

- Endonucleases are enzymes that degrade DNA within the molecule.
- Restriction endonucleases: Bacterial enzymes that recognize and cut (break) the phosphodiester bond between nucleotides at specific sequences (4- to 8-bp restriction sites) generating restriction fragments.



Restriction endonucleases are named because they restrict the growth of bacteriophages by protecting bacteria from these viruses.

- **Bacteriophages (phages)** are viruses that infect bacteria by:

- Inserting their DNA into bacterial cells.
- Taking over the bacterial machinery to produce phage proteins instead of bacterial proteins.
- Causing the bacteria to burst (lysis), releasing new phages to infect other bacteria.

-Bacteria protect themselves by:

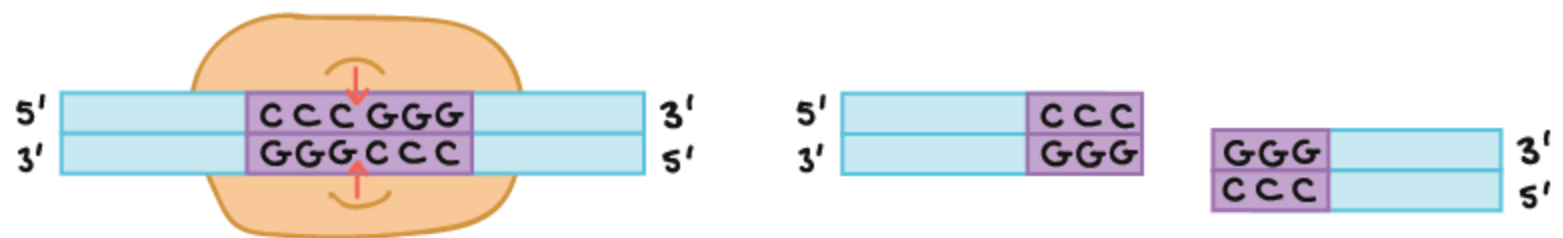
- Cleaving and degrading foreign DNA (such as phage DNA) using restriction endonucleases.
- Protecting their own DNA from cleavage through specific modifications (e.g., methylation).
- Restriction endonucleases are thus named for their role in restricting phage growth.

According to the doctor, the reason behind the naming is not critical; the focus should be on the content of the next slide.

They call them restriction endonucleases because they can't cleave anywhere in the DNA .. They are restricted by certain sequences .. That is our doctor's interpretation .

These sequences are known as restriction sites .

There are many restriction endonucleases (can be hundreds or thousands) , each can recognize a specific site and they cut within this site , generating smaller or shorter fragments, these fragments are known as restriction fragments



Example : An endonuclease will recognize C C C G G G and it cut between C and G generating these two fragments

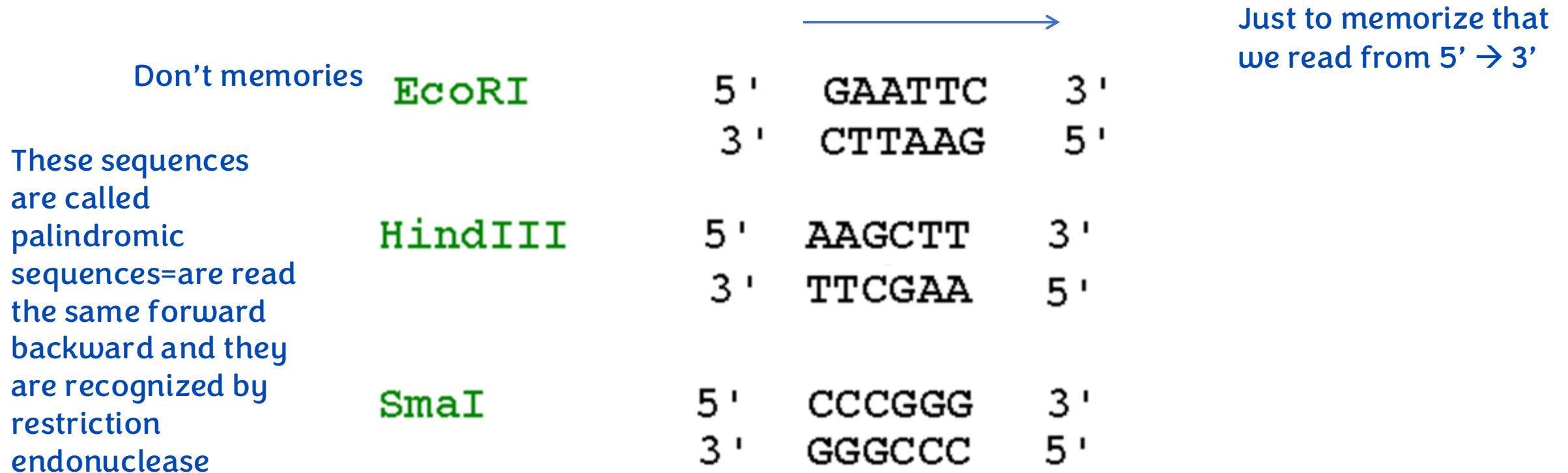
What do I mean by cut or cleave ?

Cleave or cut the phosphodiester bond

# Palindromic sequences



- The sequences recognized by restriction endonucleases—their sites of action—read the same from left to right as they do from right to left (on the complementary strand).



# They recognize specific sequences

- The enzyme EcoRI recognizes and cuts within the sequence (GAATTC).

Variant 1

*EcoRI* does not cut

GCCGCATTC TA  
CGGCGTAAGAT ↓

**The DNA stays intact**

If the sequence differs and becomes GCATTC *EcoRI* can't cleave the bond

Variant 2

*EcoRI* does cut

GCCGAATTC TA  
CGGCTTAAGAT

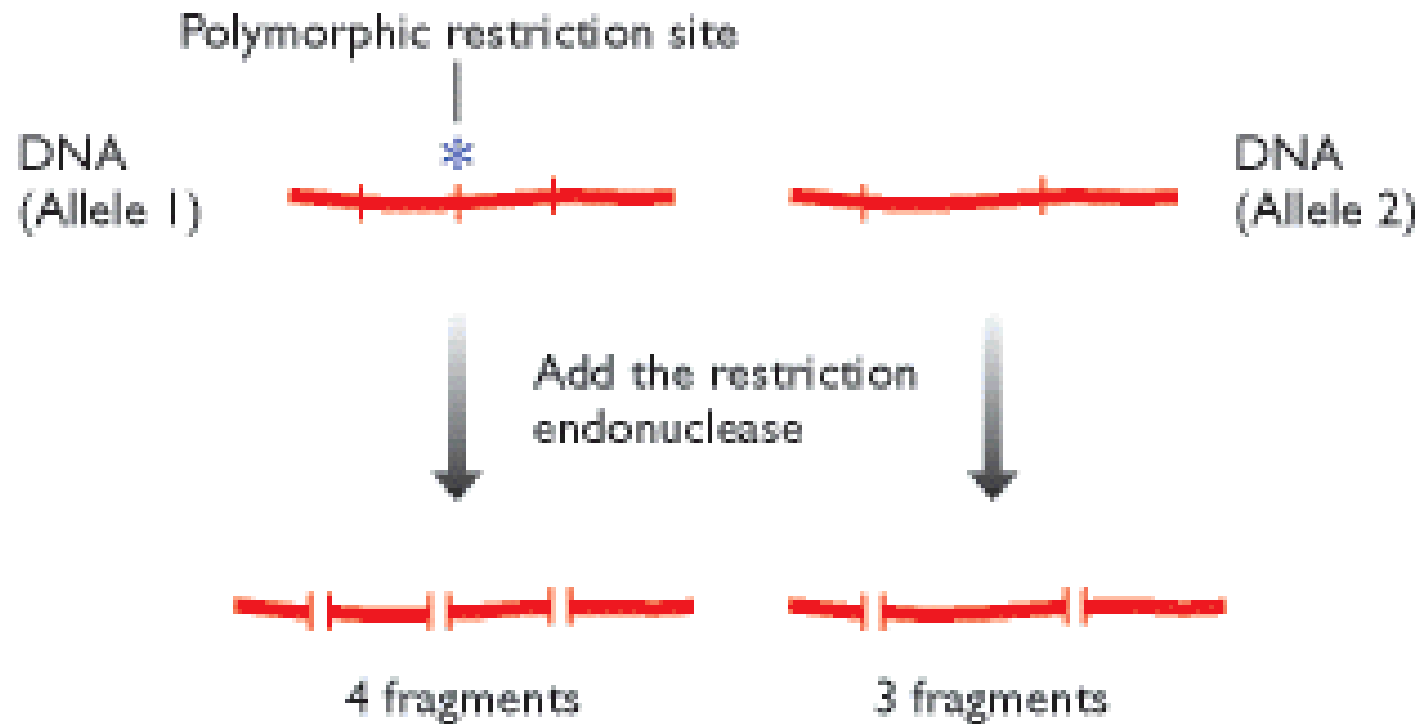
**The DNA is cut into two pieces**

The endonucleases is very specific  
This means that if we change one nucleotide it will not cleave the bond

For example: *EcoRI* recognize the sequence GAATTC and cleave the bond between G and A , it doesn't matter the top strand or the bottom one because you can flip it

# Cuts versus number of fragments

- Restriction endonucleases can cut the same DNA strand at several locations generating multiple restriction fragments of different lengths.
- What if a location on one strand is not recognized?



Allele is a type of gene

- An allele has 3 restriction sites generating 4 fragments
- Another allele has different sequence with 2 restriction sites so the endonuclease can't cut the site in the middle, generating 3 fragments
- So we can have restriction fragments with different lengths



# DNA polymorphisms

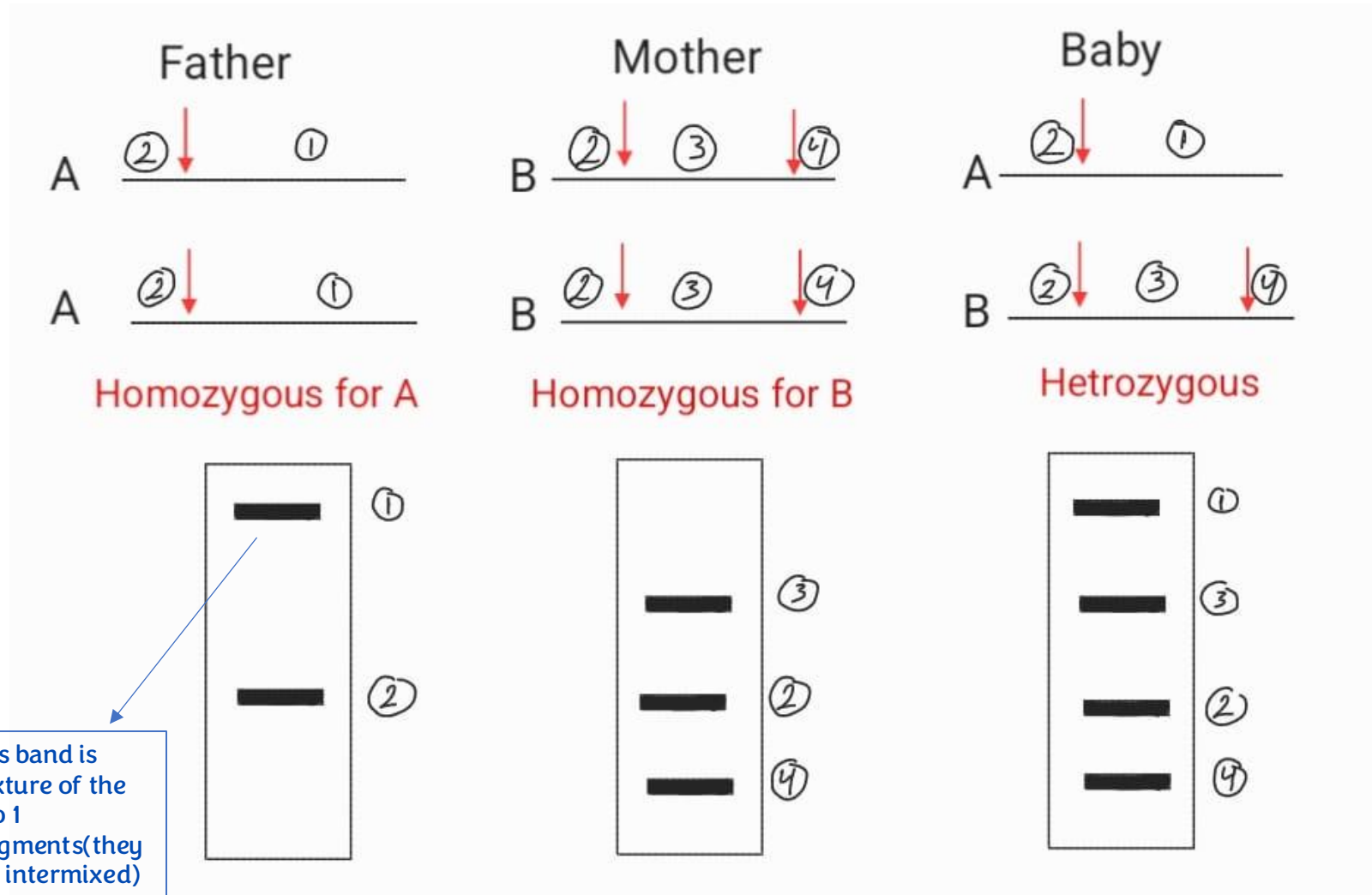
- Individual variations in DNA sequence (genetic variants) may create or remove restriction-enzyme recognition sites generating different restriction fragments. (its called polymorphism, poly=multiple, morph=shape)
- multiple shapes of DNA and we call it genetic/molecular fingerprinting; each one of us has his own DNA sequence.
- Remember:
  - Our cells are diploid.(having two types of every chromosomes one from the father the other from the mother)
  - Alleles can be homozygous or heterozygous at any DNA location or sequence.
  - We are different but we all have the same DNA sequence, the similarity in DNA sequence among people is 99.9%.

# Restriction fragment length polymorphism

- The presence of different DNA forms in individuals generates a restriction fragment length polymorphism, or RFLP.
- Which is multiple shapes in the length of restriction fragments(DNA fragment generated by restriction endonucleases)
- If we add same endonuclease to each one of us DNA, every one will generate different fragments length from the other.
- Some of the fragments will be totally identical in size and sequence.
  
- Individuals can generate restriction fragments of variable lengths. This is known as molecular fingerprinting.
- These can be detected by gel electrophoresis by itself or along with Southern blotting.

# example

- We have 3 DNA molecules from father, mother and the baby, one chromosome with two alleles A,B.
- If we add restriction endonuclease which make cuts as presented in the figure( the red arrows)
- The numbers 1,2,3,4 represent DNA fragments resulting from the restriction endonuclease
- $1 > 3 > 2 > 4$  regarding the size(we used electrophoresis)



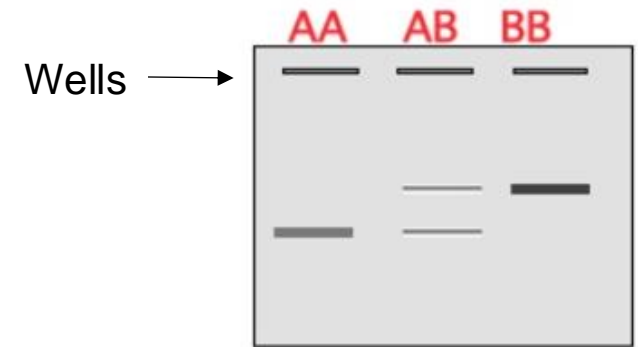
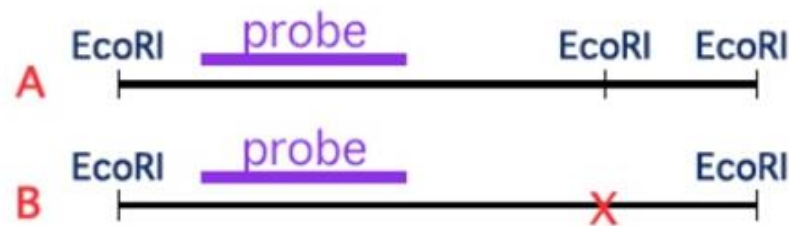
see next slide

- In fathers DNA the resulting restriction fragments are only two 1,2 (in both DNA fragments of the two alleles; since its homozygous)
- The mothers DNA generate more fragments; it has an extra restriction site, and also she has homozygous chromosome with allele B. this result in only 3 fragments 2,3,4.
- The baby has hetrozygous chromosomes (A AND B alleles) one from the father the other from the mother, so when we apply the restriction endonuclease his DNA will generate the four fragments. 1,2,3,4
- Fragment 2 is common among the three individuals.
- This example illustrate the slight differences in individuals DNA (the molecular fingerprinting)

# Electrophoresis then blotting

- Only DNA fragments that hybridize to the probe are detected.

- We have in this example two alleles
- Allele A has 3 restriction sites
- Allele B has 2 restriction sites
- After electrophoresis we will add a probe the probe bounded to A will detect smaller fragment size than the one bounded to B
- So someone who has the two alleles they will be detected as two bands



**Note: the size of the detected DNA fragment reflects its size, not the size of the probe**

Recall that we add the probe after the fragments have separated based on their size; it just detect the fragment



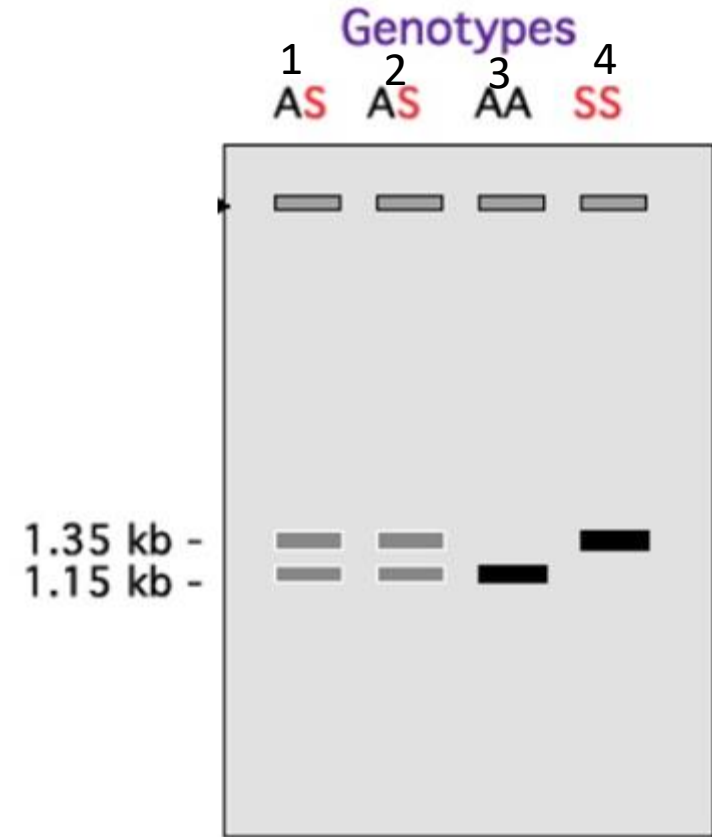
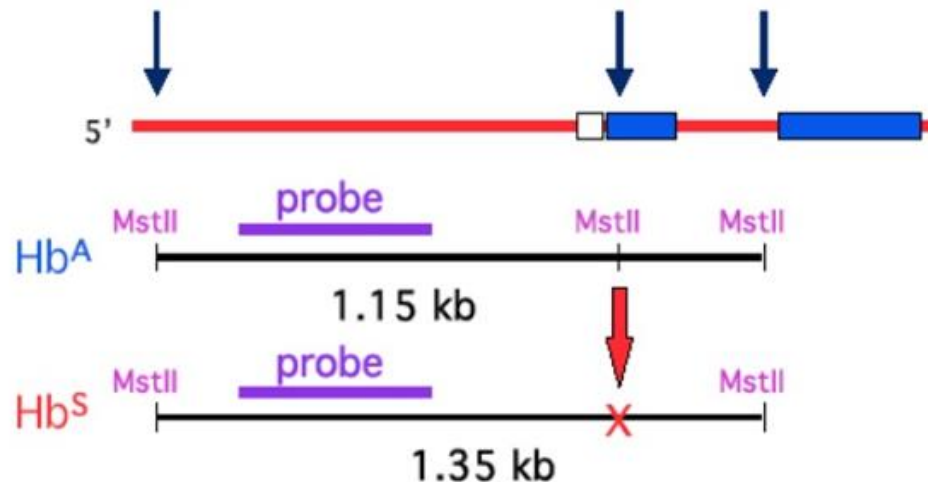
# RFLP in the clinic

- RFLP can be used as diagnostic tools.
- 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





- This slide represent the process of detecting the mutation by following over blotting technique as illustrated previously.
- In the normal case the enzyme make the cut and the probe will detect one band with 1.15kb length (the two alleles are normal).3
- In diseased person the mutation cause prevention of enzyme cut so the probe will detect one band with larger size 1.35kb (disease is caused when the two alleles are mutated).4
- If the person was carrier of the disease then one alleles is normal and the other is mutated; the probe will detect two bands 1.35kb band and 1.15kb band. 1,2
- This is how we know if the person was normal, carrier or diseased.



We determine the band size using molecular weight standard.

# Example 2: Paternity testing

## How we know who is the father and mother of someone?

-We need to take

1)DNA from the mom

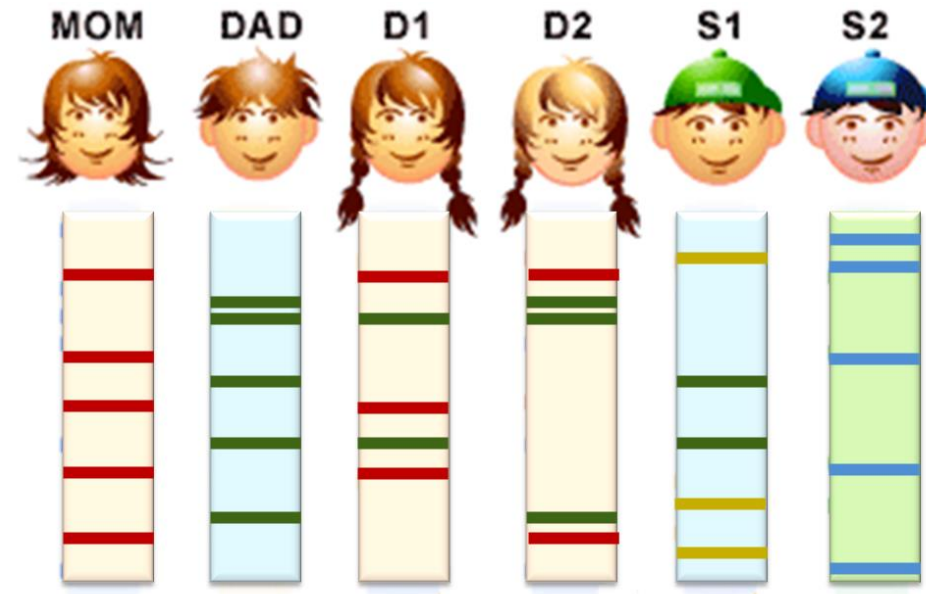
2)DNA from the dad

3) DNA from The children

-We put certain restriction endonuclease and we get the patterns of each individual.

\*Our molecular profiling should go back to mother's and father's molecular profile but not 100%(Every band in the child profiling should be either same as the mother's one or the same as father's one).

-We compare each band in child pattern with the mom and dad patterns.



## In our example

- D1,D2 all the bands are presented either in father's or mother's profile so they are daughters of the mother and father
- S1 has a bands that are not Presented in mother profile but presented in father's profile and some bands not presented in either, this means that the boy is the son of the father but not the mother.
- S2 All the bands are not presented in either father's nor mother's profile so he's not their son.



# Real cases

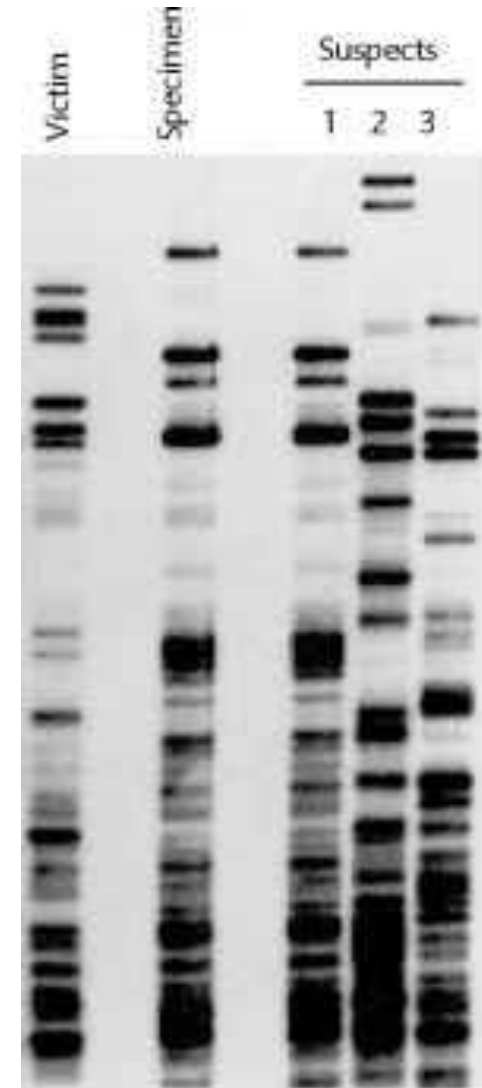
The crime scene Exact match the suspect1

Don't forget that the DNA could be contaminated and it really affect the results.

If you want to hear the story of a very famous football player and his wife about the effect of contaminating go back to 46:35 of the lecture really interesting go watch it.



The specimen Exact match the suspect1



# For any feedback, scan the code or click on it.



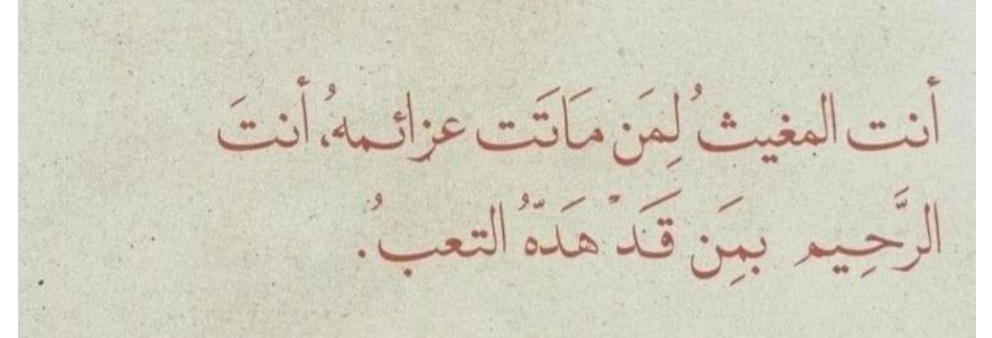
Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	7	The probe has E-Z axis (the orientation of binding the probe to the DNA )	<b>The probe has easy access to the DNA fragments</b>
V1 → V2			



Additional Resources:

رسالة من الفريق العلمي:



استعينوا بالله وما تنسوا أهلنا في غزة من دعائكم