بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 3

Overview & Basic Techniques (Pt.3)

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وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: الذي له الحكمة العليا في قدره وشرعه وجزائه يوم القيامة، الذي أحسن كل شيء خلَقه، فلا يخلق شيئًا عبثًا، ولا يشرع ولا يقضي إلا بما فيه حكمة.

الورود: ورد في القرآن (٩١) مرة.

الشاهد: ﴿وَهُوَ ٱلْعَزِيزُ ٱلْحَكِيمُ﴾ [الجمعة:٣].

اضغط هنا لشرحٍ أكثر تفصيلًا

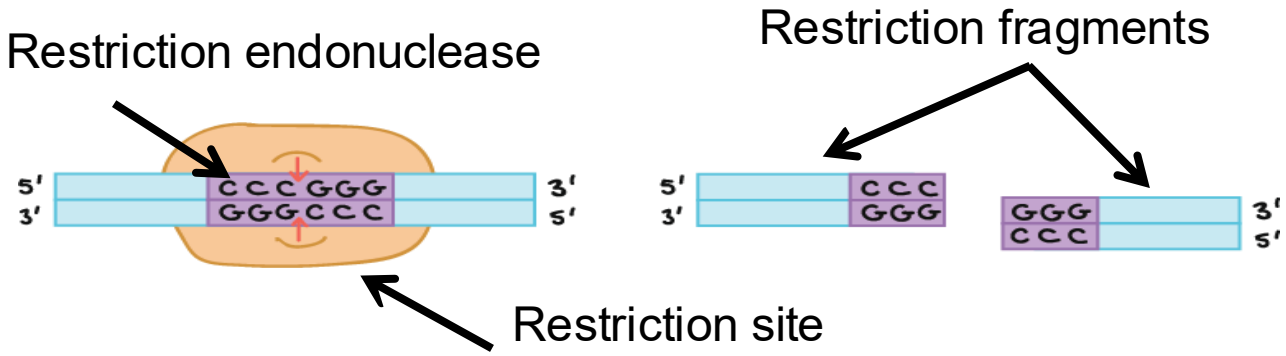
Molecular biology lec2 quiz

Click above...

❑ Restriction endonucleases (enzyme that degrade nucleic acid from within)

Endo = within
Exo = From the ends

- Endonucleases are ezymes 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:

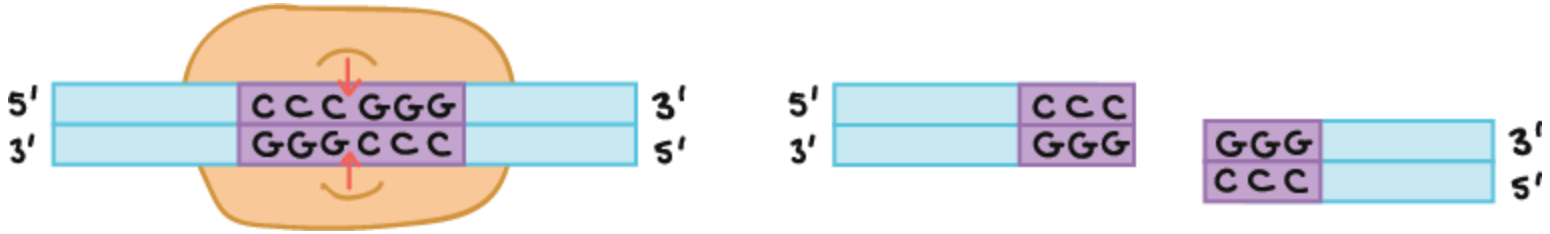
✓ Inserting their DNA into bacterial cells.
✓ Taking over the bacterial machinery to produce phage proteins instead of bacterial proteins.
✓ Causing the bacteria to burst (lysis), releasing new phages to infect other bacteria.

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.

❑ Restriction endonucleases

- They call them restriction endonucleases because they can't cleave anywhere in the DNA .. They are restricted by certain sequences
  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

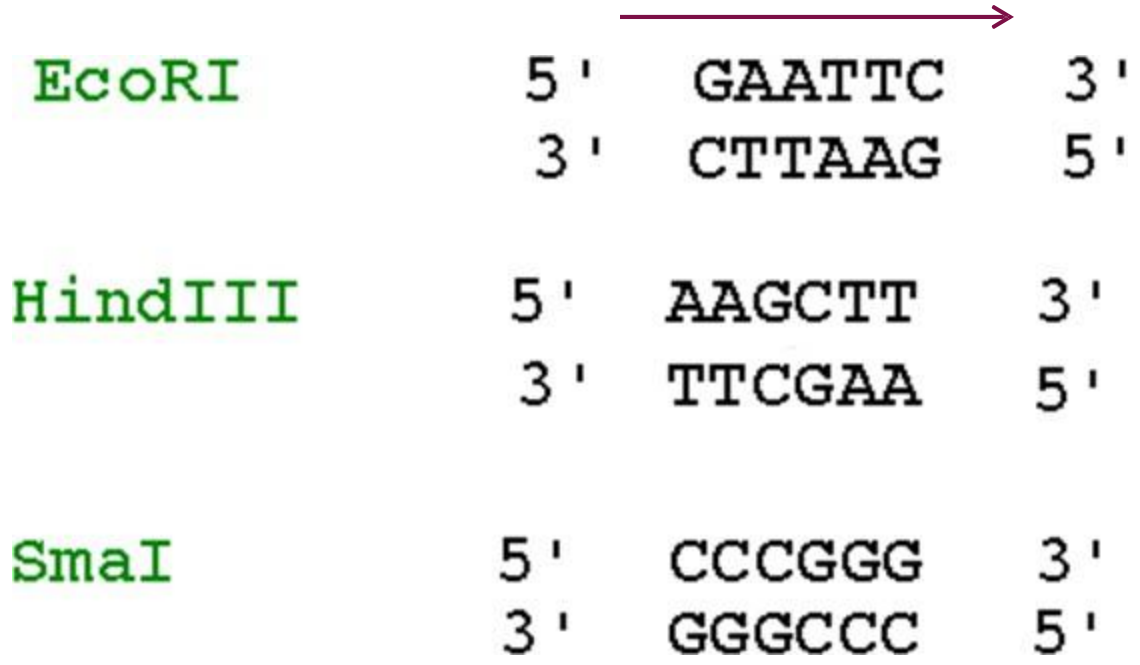


- Example : An endonuclease will recognize C C C G G G, and it cut between C and G generating these two fragments
- What do we mean by cleaving/cutting ? Cleaving/cutting the phosphodiester bond

❑ 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).

➢ These sequences are called palindromic sequences as they are read the same (5' → 3' and 3' → 5') and they are recognized by restriction endonuclease



The sequences aren't for Memorization Just memorize that we read from 5' → 3'

❑ They recognize specific sequences

- The enzyme EcoRI recognizes and cuts within the sequence (GAATTC) and cleaves the bond between G and A .
  - ➢ The endonucleases is very specific This means that if we change one nucleotide it will not cleave the bond

Variant 1

*Eco*RI does not cut

GCCGCATTCTA
CGGCGTAAGAT

The DNA stays intact

Variant 2

*Eco*RI does cut

GCCGAATTCTA
CGGCTTAAGAT

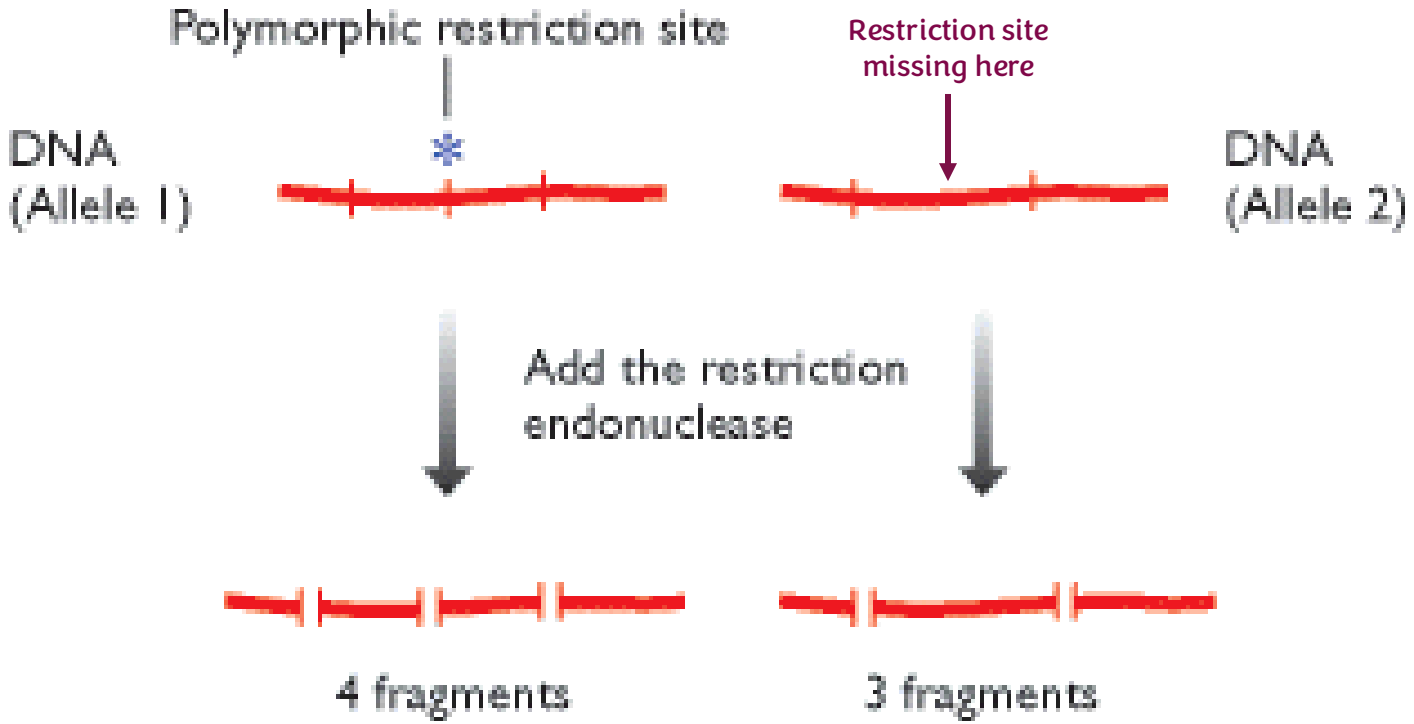
The DNA is cut into two pieces

- ➢ If the sequence differs and becomes GCATTC ECOR1 can't cleave the bond

❑ 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?

➢ One allele contains three restriction sites, which produces four fragments after digestion. Another allele has a sequence variation that removes one of the restriction sites, so the enzyme cannot cut at the middle site, producing only three fragments. As a result, different alleles can generate restriction fragments of different lengths (diff numbers and diff lengths of fragments).



❑ DNA polymorphisms

- Individual variations in DNA sequence (genetic variants) may create or remove restriction-enzyme recognition sites generating different restriction fragments. (it is 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 refers to variations in the lengths of the restriction fragments .
- If we add the same endonuclease to DNA from different individuals, each person will generate a unique pattern of fragment lengths. However, some fragments may be identical in both size and sequence among individuals.

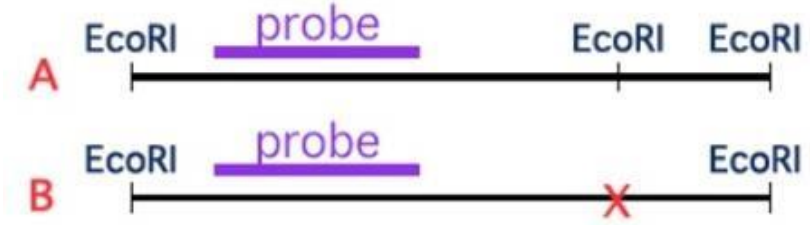
- Individuals can generate restriction fragments of variable lengths. This is known as molecular fingerprinting.
- These can be detected by gel electrophoresis by itself or along with Southern blotting.

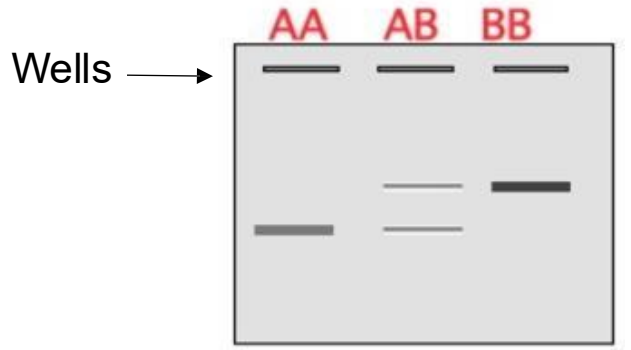
❑ Electrophoresis then blotting (Southern Blotting)

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

➢ We have two alleles in this example:

- Allele A has 3 restriction sites
- Allele B has 2 restriction sites





➢ After electrophoresis, a probe is added to the membrane.

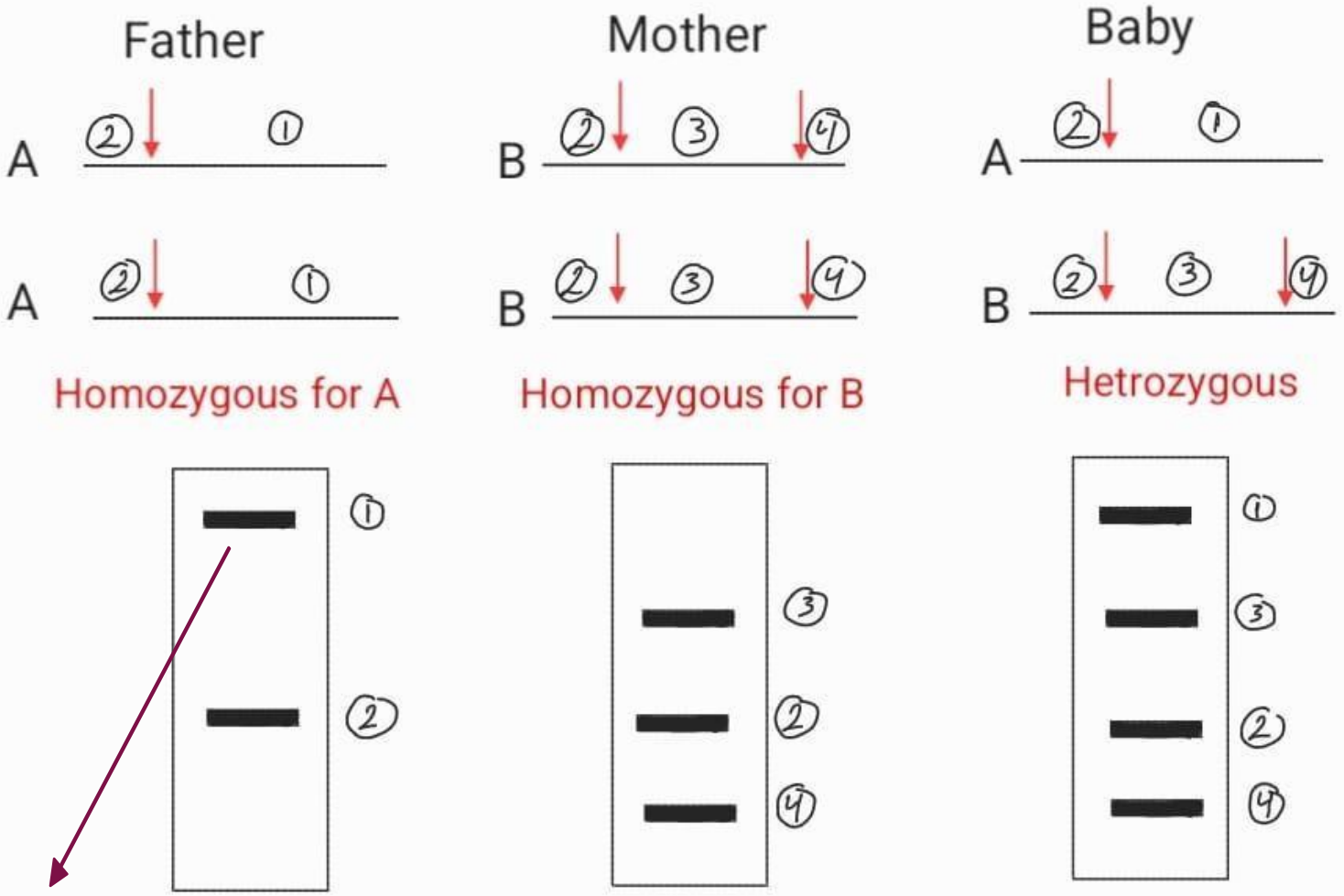
- The probe bound to Allele A detects a smaller fragment than the one bound to Allele B. An individual carrying both alleles will show two bands on the blot.

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

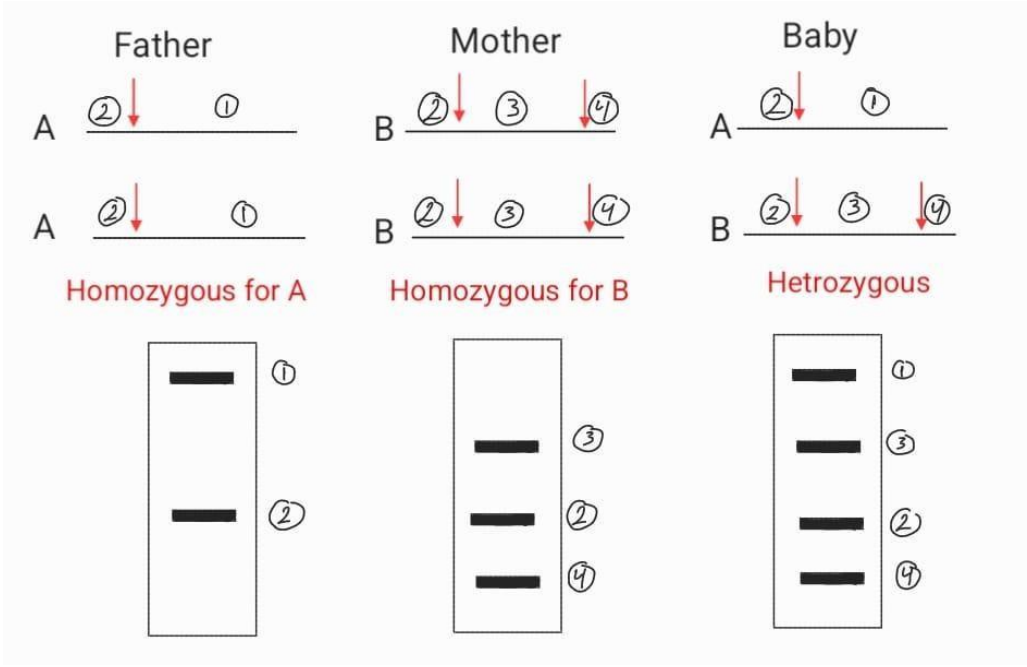
❑ Gel Electrophoresis only

- 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

❑ Gel Electrophoresis only



- In the father's DNA, the restriction digestion produces only two fragments (1 and 2). This is because he is homozygous for allele A, so both copies of the chromosome generate the same two fragments.
- The mother's DNA produces three fragments (2, 3, and 4). She is homozygous for allele B, which contains an additional restriction site, resulting in three fragments instead of two.

You can watch the prof.'s explanation for further understanding, If so, click here :) (at exactly 19:58)

- The baby is heterozygous, carrying allele A from the father and allele B from the mother. Therefore, after adding the restriction endonuclease, the baby's DNA produces all four fragments: 1, 2, 3, and 4.
- Fragment 2 is shared among all three individuals.

❖ This example demonstrates how small sequence differences between individuals can produce different restriction fragment patterns, forming the basis of molecular fingerprinting.

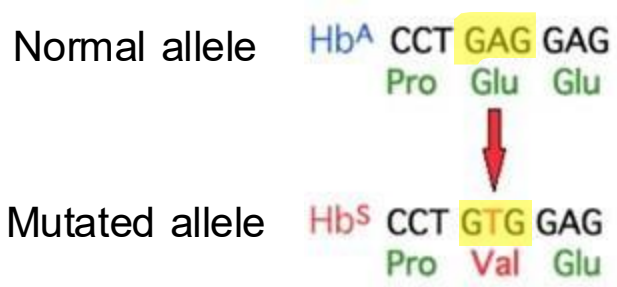
❑ RFLP in the clinic

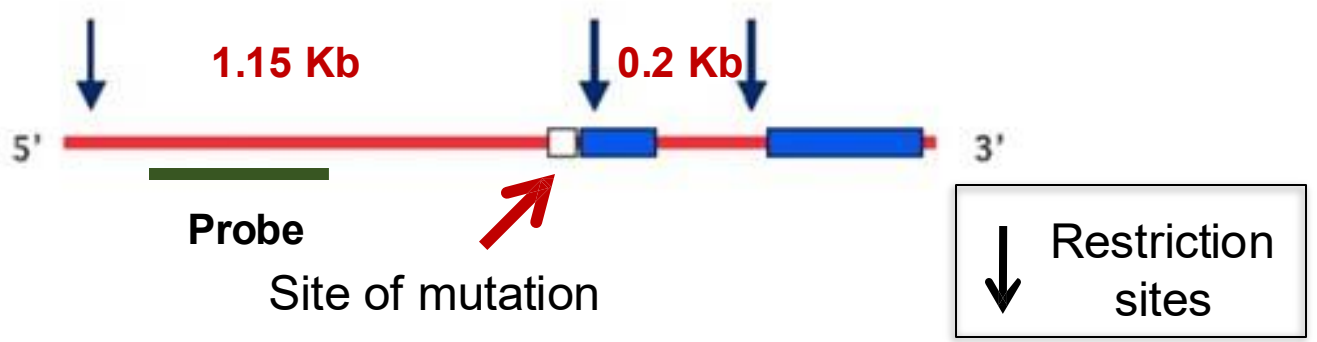
- RFLP can be used as diagnostic tools.

- 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

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

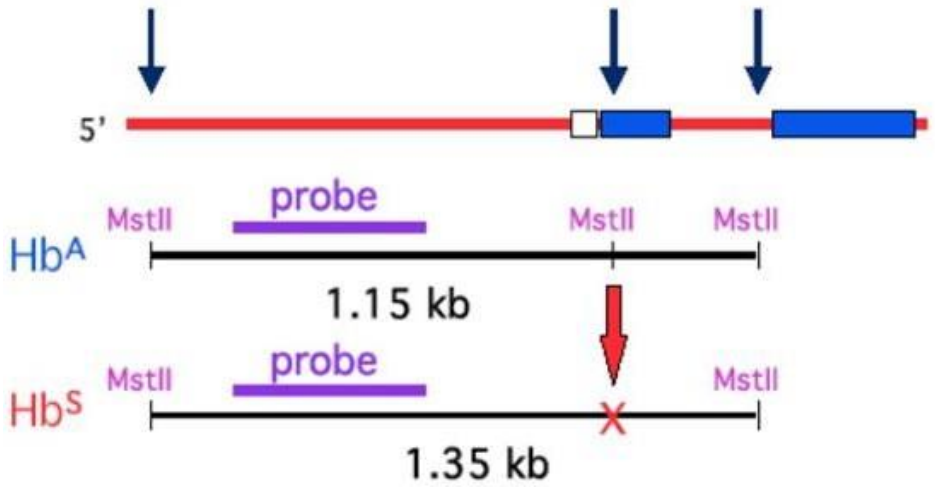
- Sickle cell anemia is caused by a mutation in one nucleotide (base) in the globin gene that is responsible for making hemoglobin.
- The position of this nucleotide happens to be within a restriction site. (the mutation is in restriction site so the endonuclease can't cut the DNA as usual)
- Individuals can be:
  - Homozygous with two normal alleles (AA)
  - Heterozygous or carriers of one normal allele and one mutated allele (AS)
  - Homozygous for the mutated alle

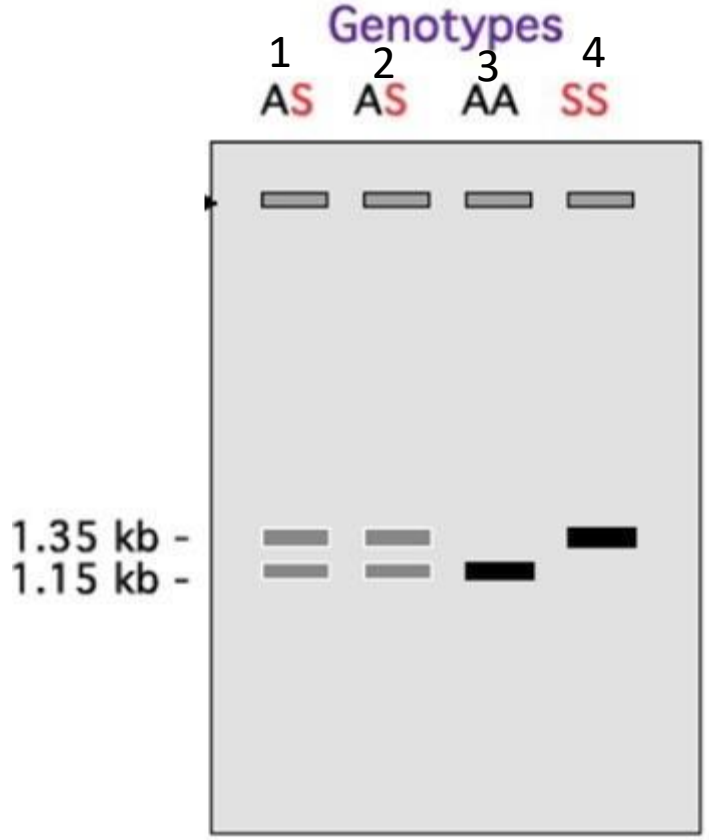




If there is a mutation the probe will detect larger fragments = 1.35kb (kilo basepair), while normally it detects fragments with 1.15kb length

- This slide shows how a mutation can be detected using the blotting technique described earlier. In a normal individual, the restriction enzyme cuts the DNA as expected, and the probe detects a single band of 1.15 kb (both alleles are normal).
- In a diseased individual, the mutation prevents the enzyme from cutting at that site, so the probe detects one larger band of 1.35 kb (both alleles are mutated).
- If the person is a carrier, one allele is normal and the other is mutated. In this case, the probe detects two bands: one at 1.15 kb and one at 1.35 kb.
- This allows us to determine whether a person is normal, a carrier, or diseased.

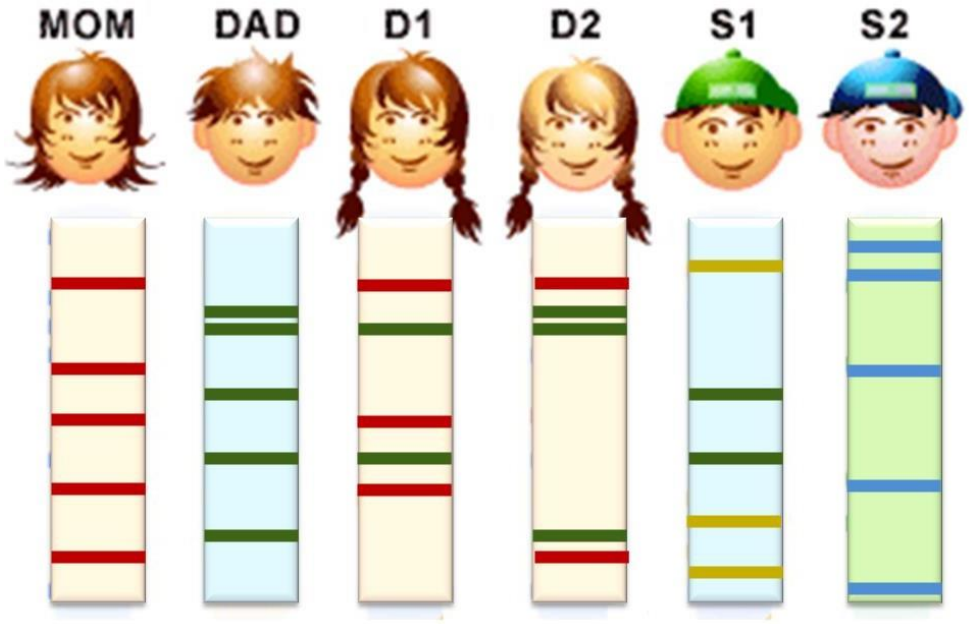




- We determine the band size using molecular weight standard.

❑ Example 2: Paternity testing

❖ How do we determine who the father and mother of someone are?



- ➢ We take DNA samples from the mother, the father, and the child.
- ➢ We apply a specific restriction endonuclease to each DNA sample and generate the fragment patterns for all three.
- ➢ The child's molecular profile should match the parents' profiles, but not necessarily 100%. Every band in the child's pattern must come either from the mother or from the father.
- ➢ We then compare each band in the child's pattern with the corresponding bands in the mother's and father's patterns.

❖ In our example:

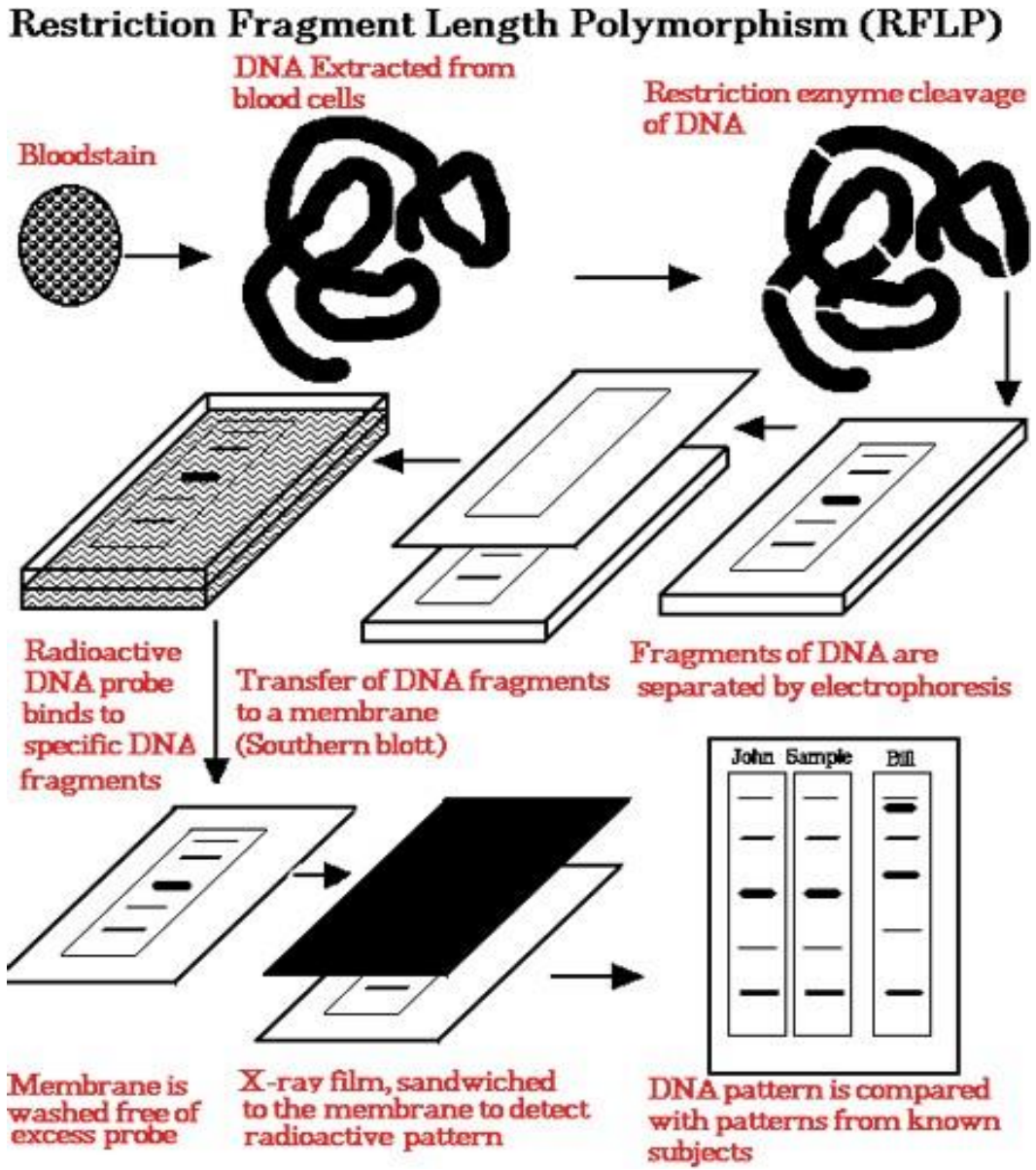
D1 and D2: All the bands in their profiles are present in either the father's or the mother's profile, so they are daughters of both parents.

S1: Some bands are not present in the mother's profile but are present in the father's profile, and some bands are not present in either parent. This indicates that the boy is the son of the father but not of the mother.

S2: None of the bands are present in either the father's or mother's profile, so he is not their son.

❑ Example 3: Forensics (طب شرعي)

How can the police detect the killer?
They have blood of the victim They have unknown blood of the criminal They take blood from the suspectes They do RFLP, the unknown DNA should match 100% the DNA of the killer If he was one of the suspects The DNA could be contaminated (the blood could be mixture of victim and killer) it's a mistake of the CSI, Bad collecting techniques.

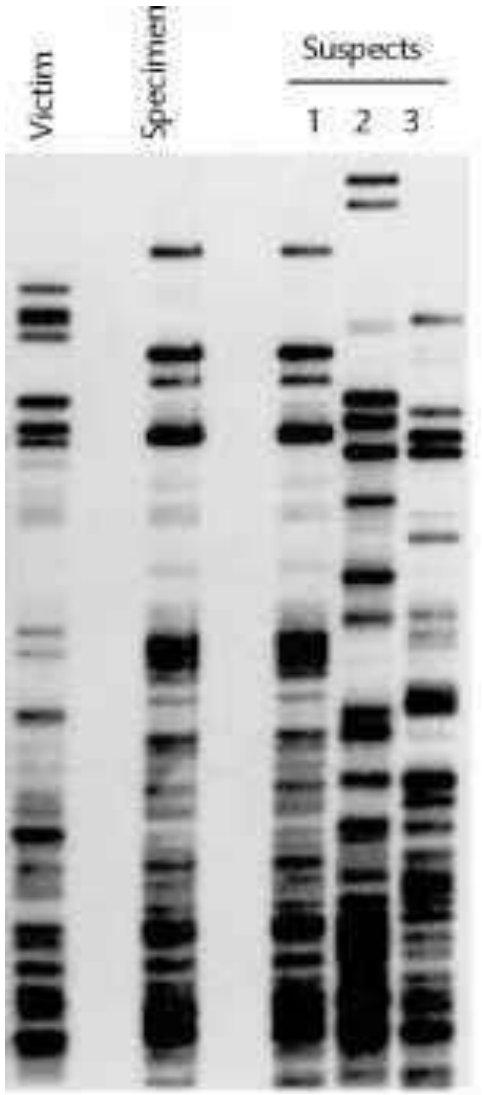


❑ Real cases

The crime scene's pattern exactly matches suspect 1

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

The specimen's pattern exactly matches the suspect1

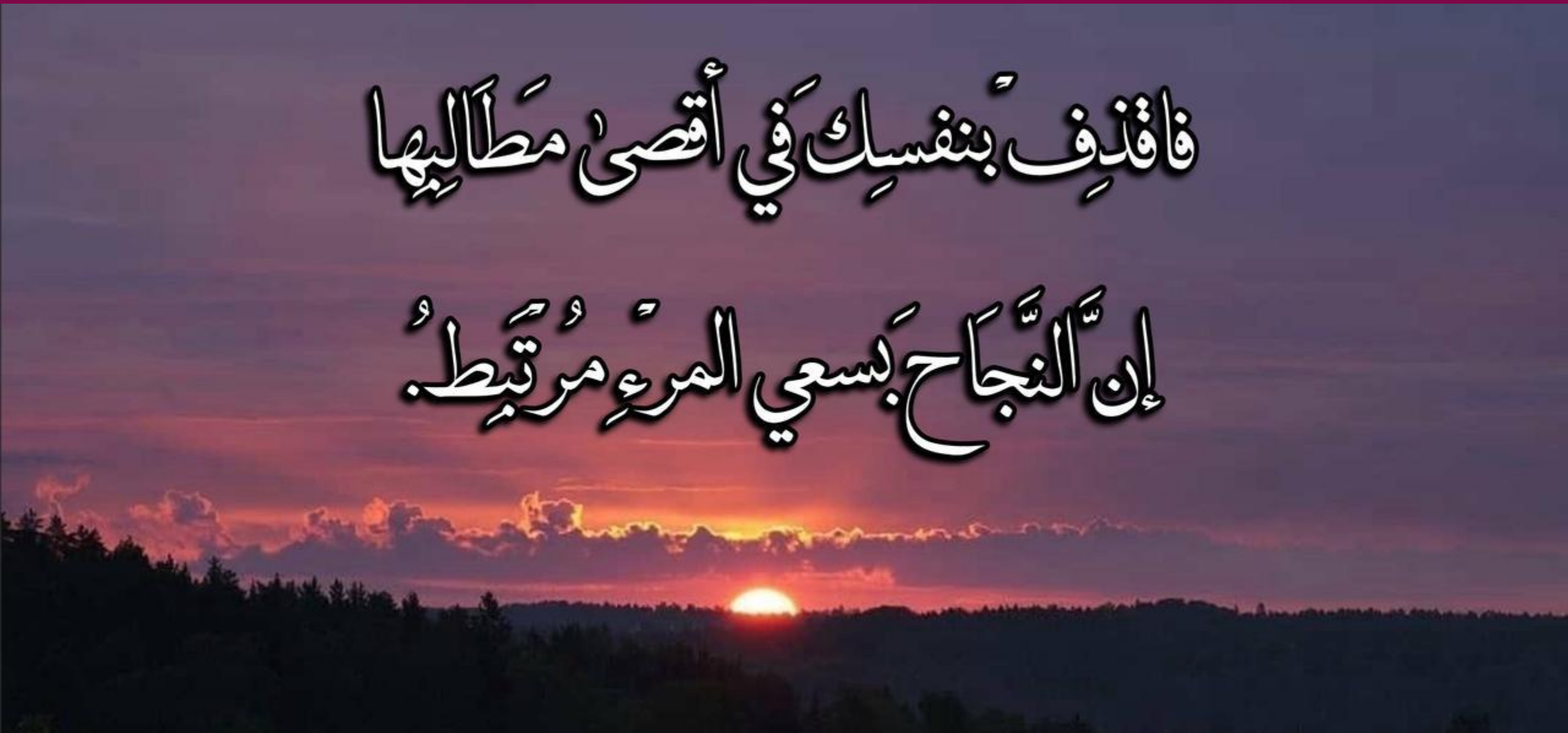


Resources

- http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html

- Watch this….very important

رسالة من الفريق العلمي:



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			
V1 → V2			