Genetics & molecular biology

◉ Sheet ○ Slide

Number:	2
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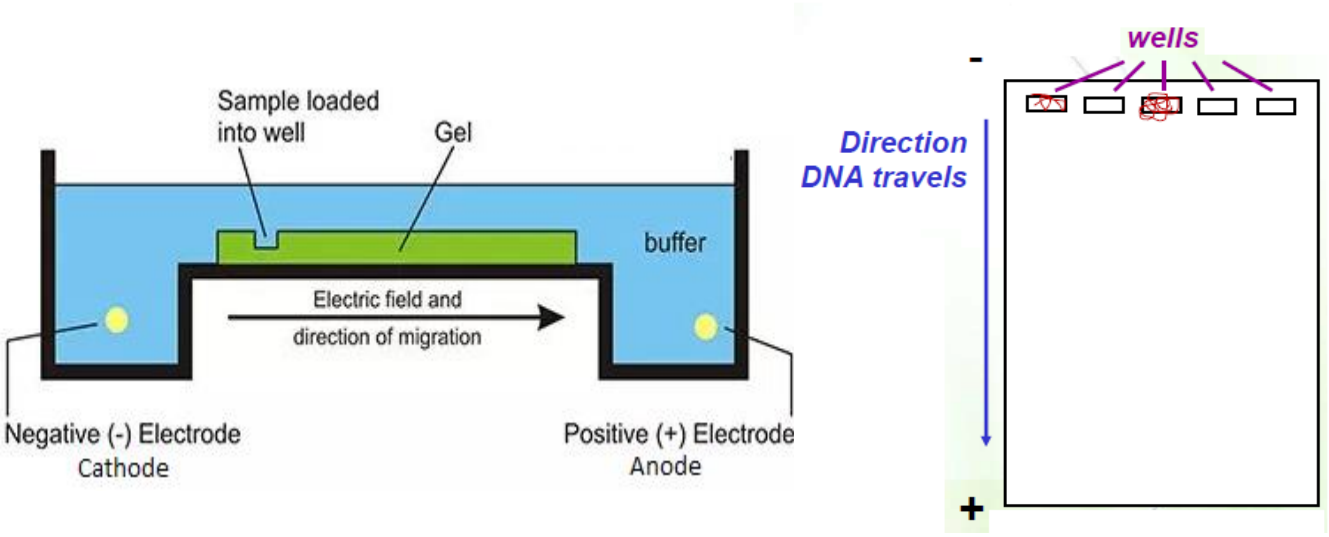
Gel electrophoresis

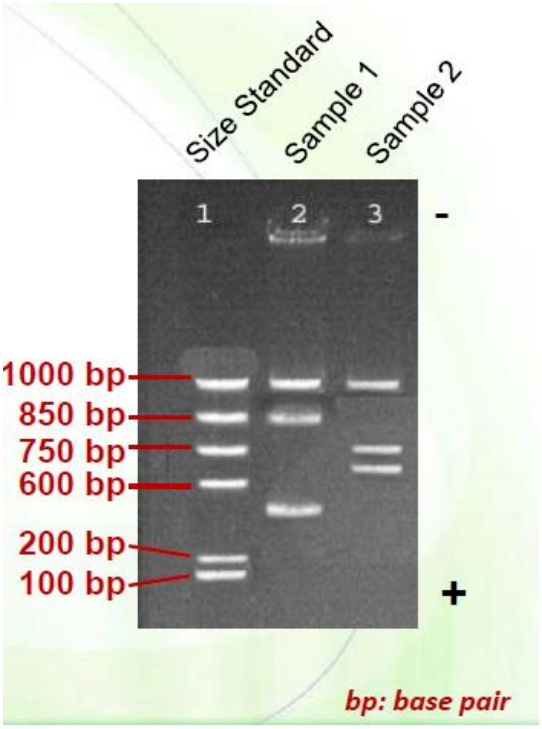
- It is a technique used to separate **DNA molecules** according to their **sizes**.
- It consists of a tank that has two poles (negative pole (cathode) and positive pole (anode)) to form an electric current. The tank also has a buffer solution (to conduct electricity).
- Inside the tank there is gel material (forms a network).
- We place our samples inside wells in the gel material.
- Naturally, DNA molecules are negatively charged because of phosphate groups, so if we applied electricity, the DNA molecules will start migrating **from the cathode toward the anode.**
- The small DNA **fragments** will migrate **faster** than the larger DNA fragments.
- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.



Gel electrophoresis can also be used to separate proteins in a similar process except a few differences:

I. Unlike DNA, not all proteins are negatively charge, so SDS page is used to denaturate and uniform the negative charge over all protein molecules.
II. Gel electrophoresis for DNA is done horizontally, however for proteins it is done vertically.

- Then, DNA staining (coloring) takes place using a dye (ethidium bromide) or DNA can be radioactively labeled using radioactive phosphorus (^{32}P).
- The DNA molecules of different lengths will run as "bands ".
- **Each band contains thousands to millions of copies of DNA fragments of the same length. They can be of same or different type (the sequence could be totally different).**
- It is common that a DNA standard is used to determine the length of the examined DNA molecule.
- **Size standard: It's a sample that contains DNA fragments of known lengths.**



Size standard: contains 6 bands of known lengths.

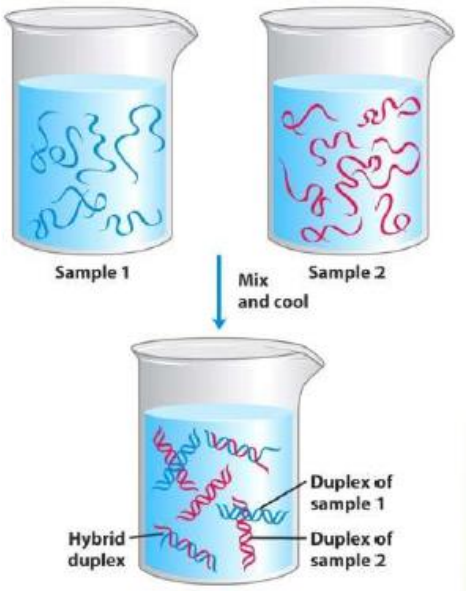
Sample 1: contains 3 bands:

Band 1: travels along with 1000 bp band, so its approximately 1000 bp.
Band 2: travels along with 850 bp band, so its approximately 850 bp.
Band 3: travels between 600 bp band and the 200 bp band, so we can estimate it (400-450bp).

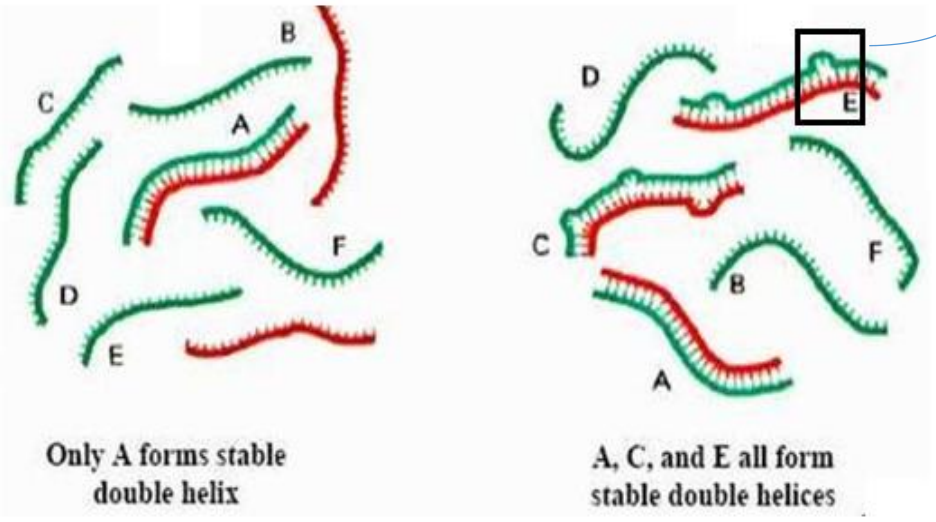
Hybridization

- It is a technique that depends on the formation of double stranded DNA, and the two strands **can be different** (from different sources).
- DNA from different sources can form double helix as long as their sequences are **compatible** (hybrid DNA).
- Hybridization can be imperfect (Mismatches may occur, not all base pairs will be compatible (eg. A with G)).

- if we heat a DNA sample the DNA strands will be separated from each other, and if we cool it again, the strands will rejoin because they are **complementary. They will still be antiparallel.**
- The same concept can be applied in hybridization, for example, if we heat DNA samples from human and monkey, then mix them and cool them again, one DNA strand from human can bind (hybridize) with another strand from the monkey as long as they are complementary**. (As long as we have enough hydrogen bonds formed between the two strands they can hybridize).**



- The human DNA is not expected to be 100% identical to the monkey DNA, and still they can hybridize because they have common sequences, but There must be enough hydrogen binding between the two strands. **it is called (imperfect hybridization).**



Imperfect base pairing because of a mismatch.

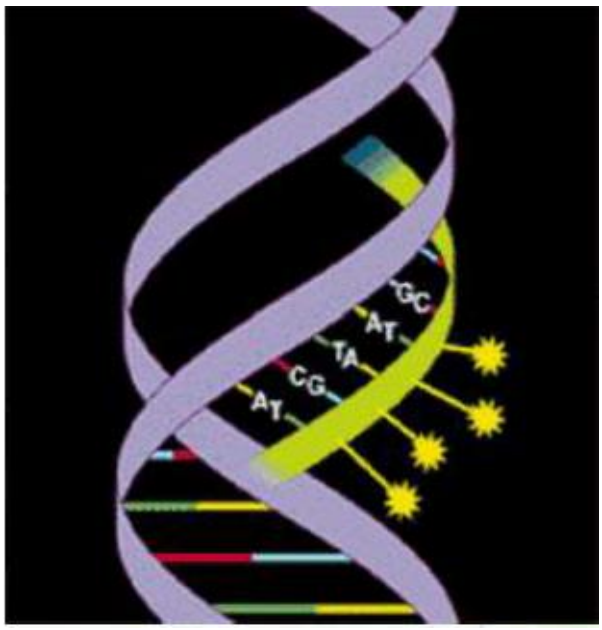
Example: A with G

For A sample: Perfect hybridization (strands are completely identical)

For sample E and C: Imperfect hybridization

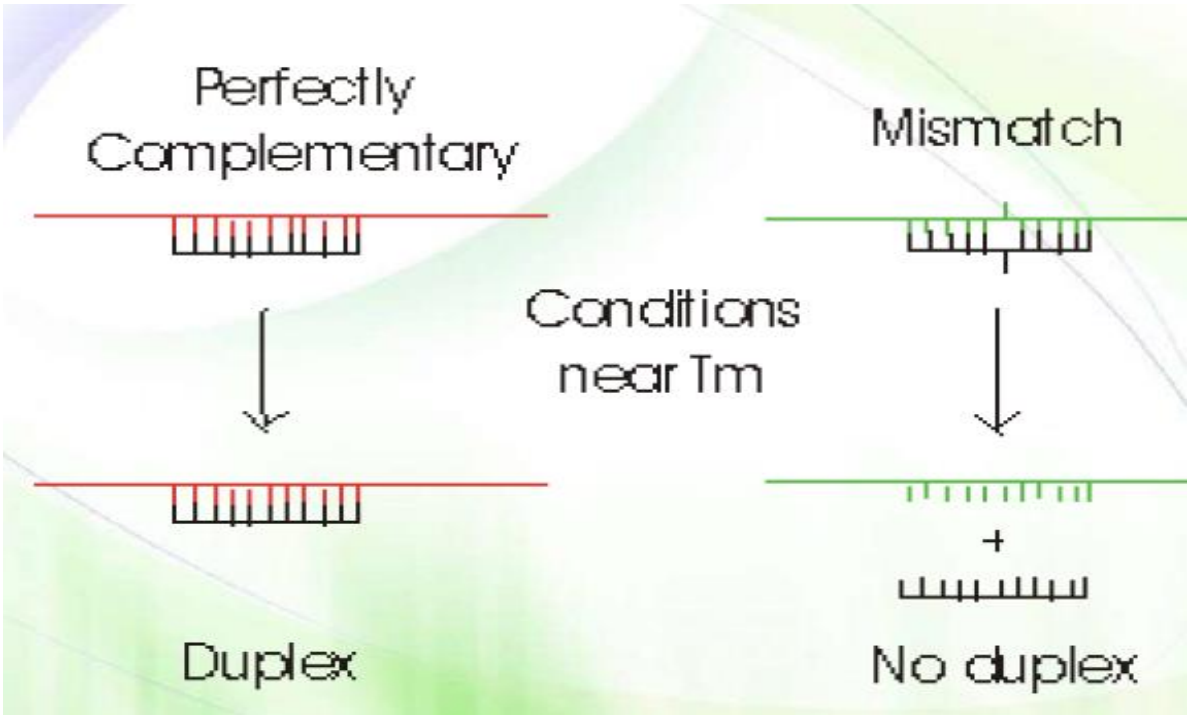
- Hybridization reactions can occur between any two single-stranded nucleic acid chains provided that they have complementary nucleotide sequences.
- Hybridization reactions are used to detect and characterize specific nucleotide sequences.

Hybridization techniques:



- Hybridization techniques depends on using ***Probes.***
- A probe is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.
- It is a known sequence of about 20 nucleotides long.
- Hybridization reactions use labeled DNA probes (labeled with radioactive phosphorus) to detect larger DNA fragments.

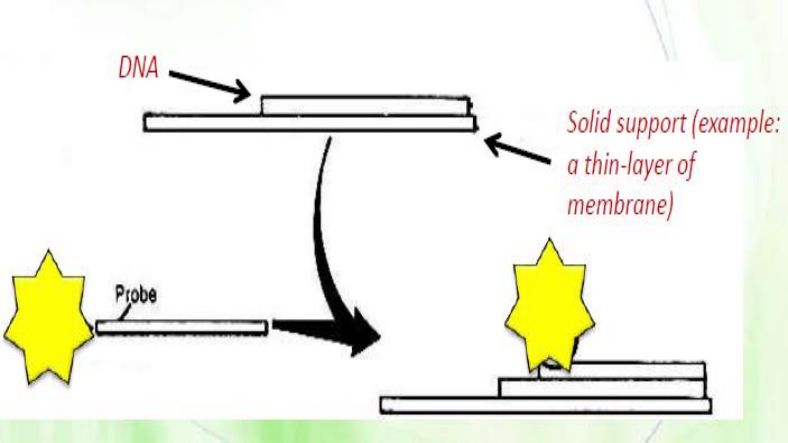
- If probes are added to a double stranded DNA that has the probe's complementary sequence, there will be competition between the probe and one of the strands to bind with the complementary strand. The probe will overcome because of its high concentration, forming hydrogen bonds with the complementary strand, so this DNA will light up or give a signal because the labeled probe is bound to it.
- If the DNA sequence is totally different from the probe's sequence (not complementary), there will be no binding (No hybridization).



Two techniques of hybridization:

1. Dot blot (blot means a spot)

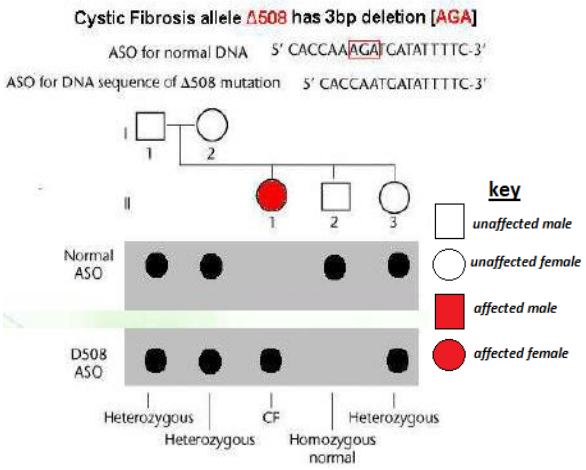
- This is a technique that informs us if a specific sequence (that is complementary to a probe of a known sequence) exists in a larger DNA.
- DNA is bound to a solid support and a labeled probe is added. If binding occurs, the sequence exists.



- It is done by taking DNA from any cell and we put it on a piece of paper (ex. Nitrocellulose paper) called **membrane** to make a spot, then we add the probe with the sequence of interest to that spot. If there is a complementary sequence, hybridization will occur and the DNA will give a signal, but if there is no complementary sequence, the DNA will not give a signal.

Application of Dot blot: Disease detection by Allele-specific oligonucleotide (ASO)

This diagram represents ***pedigree* (genetic family tree)
For the disease cystic fibrosis which affects the lungs and is Caused by deletion of 3 nucleotides in a certain gene.



In this case we use 2 probes (called allele-specific oligonucleotide), one for the normal gene and one for the affected one to detect if the normal gene or the defective gene is present, so the whole genomic DNA is spotted on a solid support (like a nylon membrane) and hybridized with two ASO's, one at a time.

DNA from all family members is taken and spotted on two pieces of the membrane, then ASO of the normal gene is added to the first spot and ASO of the defective gene is added to the other spot.

The normal gene was positive (gives a signal) in the father, the mother, the son, and the second daughter.

The defective gene was positive in the father, the mother and in both daughters.

This tells us the following:

A. The father, the mother and the third daughter are carriers and we say that they are heterozygous (have one copy of the normal allele and one copy of the defective allele).
B. The son is normal (not a carrier) and he is homozygous for the normal allele.
C. The first daughter has the disease and she is homozygous for the defective allele.

So, we made a diagnosis and confirmed the type of mutations in all family members.

> *Note: the difference between gene and allele:*
> *All humans have the same chromosome and the same genes at the same locus (for example we all have eye colour genes), what differs between people is the copy of the gene or the type of the gene which is the allele.*

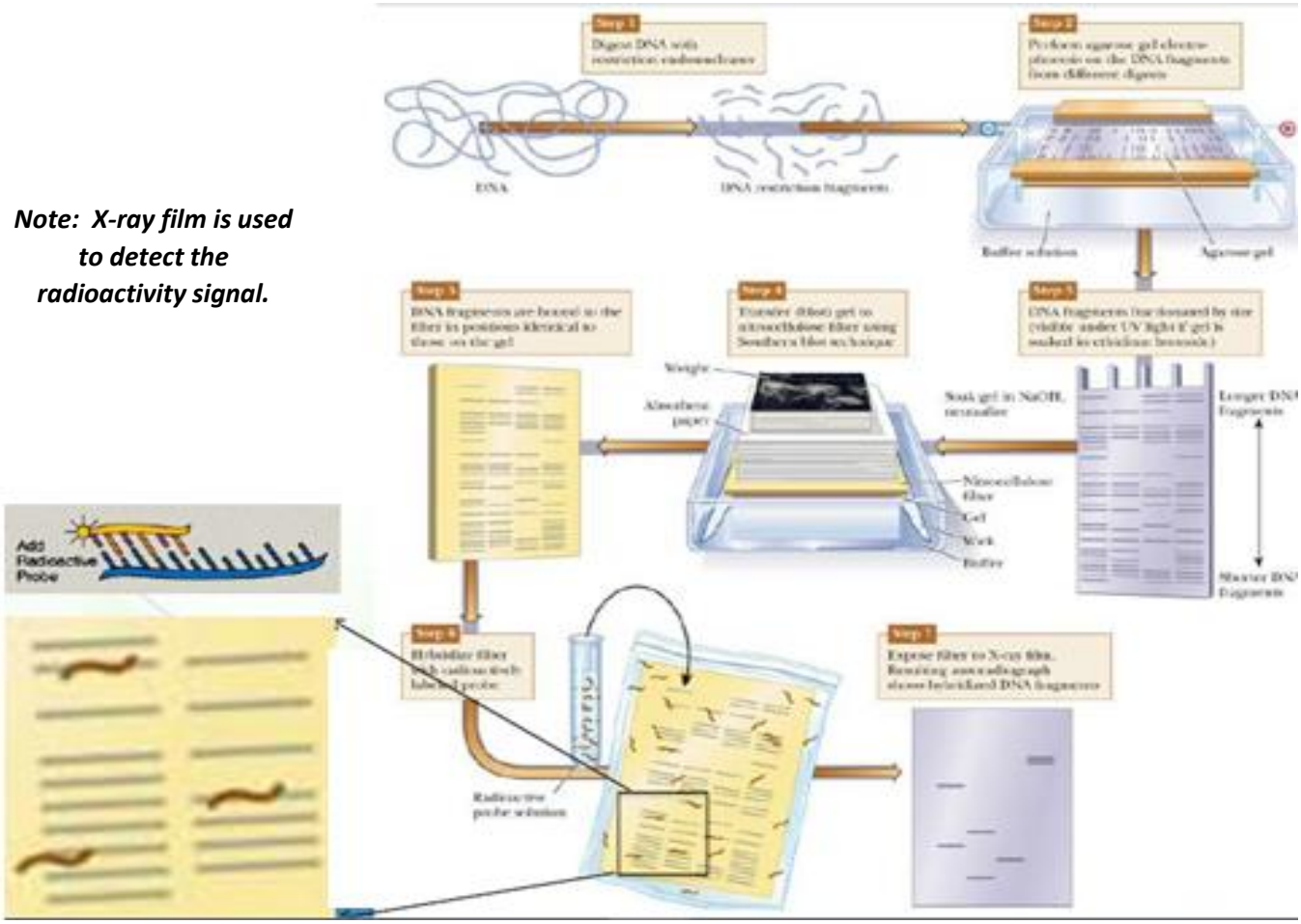
2. Southern blotting (named after the British biologist Edwin Southern)

- This technique is a combination of DNA gel electrophoresis and Dot blot

- Used to detect:
  - ✓ the presence of a DNA segment complementary to the probe.
  - ✓ the size of the DNA fragment.

- It is done by:
  1) Firstly, the DNA fragments is separated according to size by gel electrophoresis.
  2) Then, the DNA fragments are transferred and attached to the nitrocellulose paper (the membrane) so, the membrane will be an exact replica of the gel DNA bands.
  3) Finally, labeled probes are added to the membrane and the DNA bands that have a complementary sequence will give a signal, while the other DNA bands will not.

Note: X-ray film is used to detect the radioactivity signal.

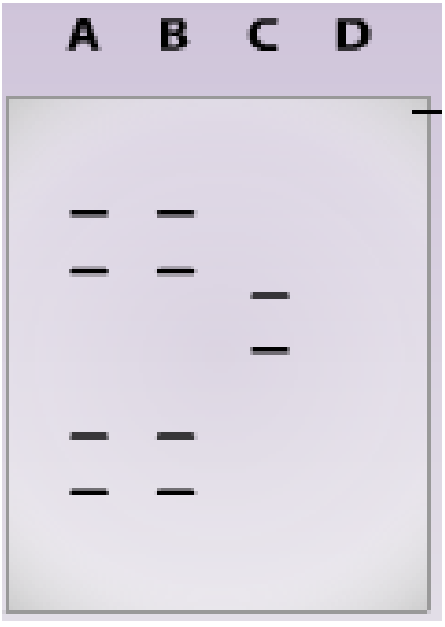


For example:

If we got this result on the right:

So what information can we get from this result?

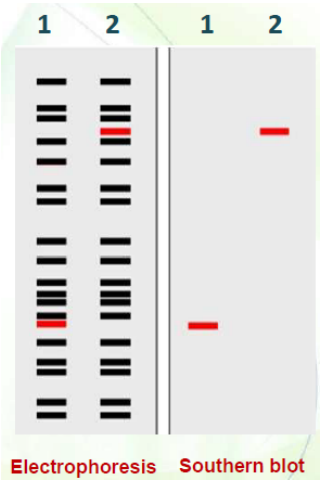
1) A and B samples are identical.
2) C sample has some complementarity with the probe, but it is different from A and B
3) D sample is totally different and does not have any complementarity with the probe.



these are the bands that gave signals.

Notice the difference between southern blotting and gel electrophoresis.

**The probe does not determine and does not affect the migration or the size of the DNA fragments because the probe is added after the separation.



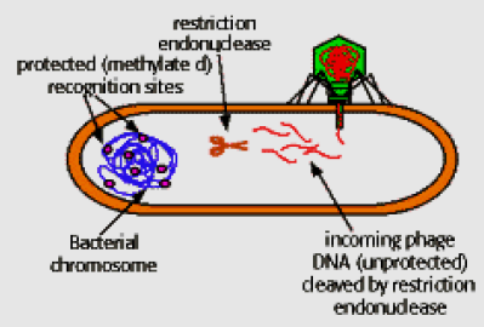
For further understanding watch this animation:
https://drive.google.com/open?id=1pAjsu0-lRjBNuq23qorvSTzAPPASeTI4

Endonucleases

- Nucleases are enzymes that degrade nucleic acids like Deoxyribonucleases (DNases) and Ribonucleases (RNases).
- Nucleases are classified into Endonucleases and Exonucleases.
- Endonucleases are enzymes that degrade nucleic acids within the molecule rather than from either end.
- exonucleases remove nucleotides from either end (one at a time).
- Restriction endonucleases: Enzymes that recognize and ***cut (break)*** the ***phosphodiester bond*** between nucleotides at specific sequences (4-to 8-bp restriction sites) generating restriction fragments.
- Type II restriction endonucleases: Always cleave at the same place generating the same set of fragments.
    - ✓ EcoRI (isolated from E. coli) cuts at 5'-GAATTC-3‘

- There are hundreds of restriction endonucleases.

- Biological purpose of restriction endonucleases: They are present in bacteria to protect them from bacteriophages that infect bacteria by transferring their DNA into them restricting their growth.

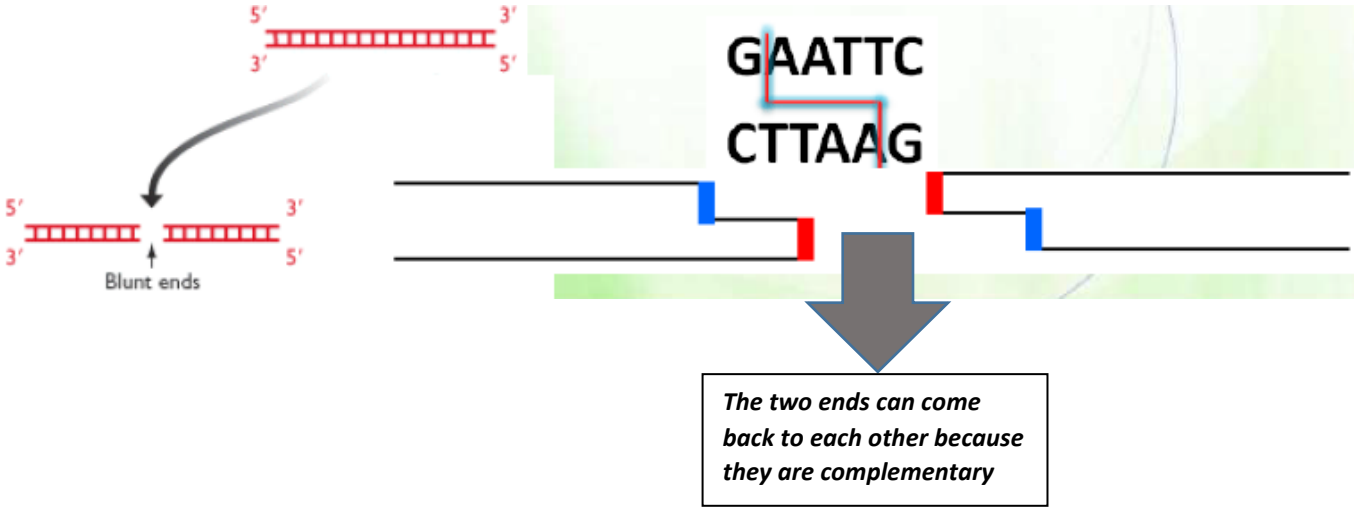


Endonucleases are called Restriction endonucleases because they restrict the growth of the phages.

Types of cleavages

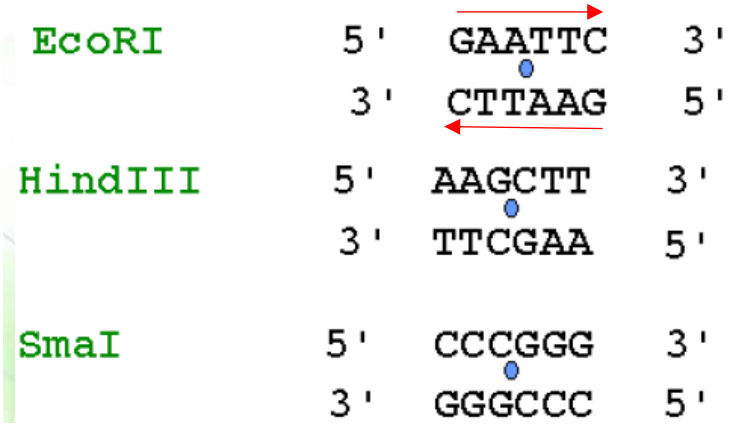
Restriction enzymes cut DNA in two different ways:

- Blunt: enzymes cut at the same position on both strands giving a blunt ended fragment.
- Staggered (off-centre): enzymes cut the two DNA strands at different positions generating sticky or cohesive ends (The DNA fragments have short single-stranded overhangs at each end), but they are not stable because there is no phosphodiester bond at the edges.



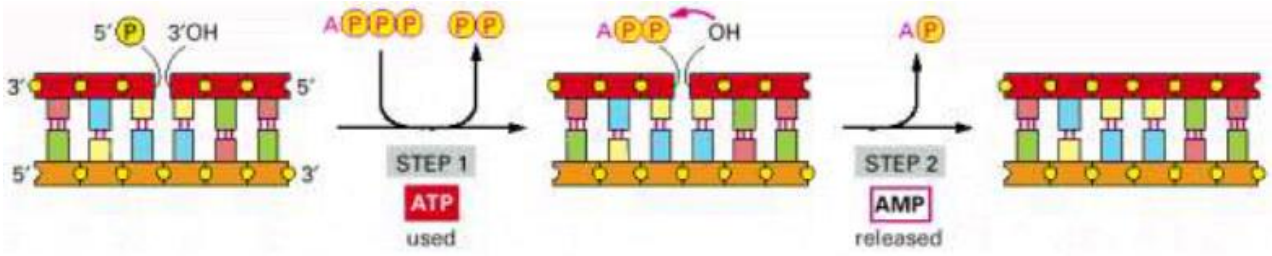
Palindromic sequences:

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



DNA ligase:

- We said that the cohesive ends can come back to each other, but we need to re form the phosphodiester bond by the enzyme DNA ligase.
- It covalently joins DNA ends (example, restriction fragments).
- It catalyzes the formation of phosphodiester bonds between the 3'-hydroxyl group of one strand and the 5'-phosphate end of another strand.



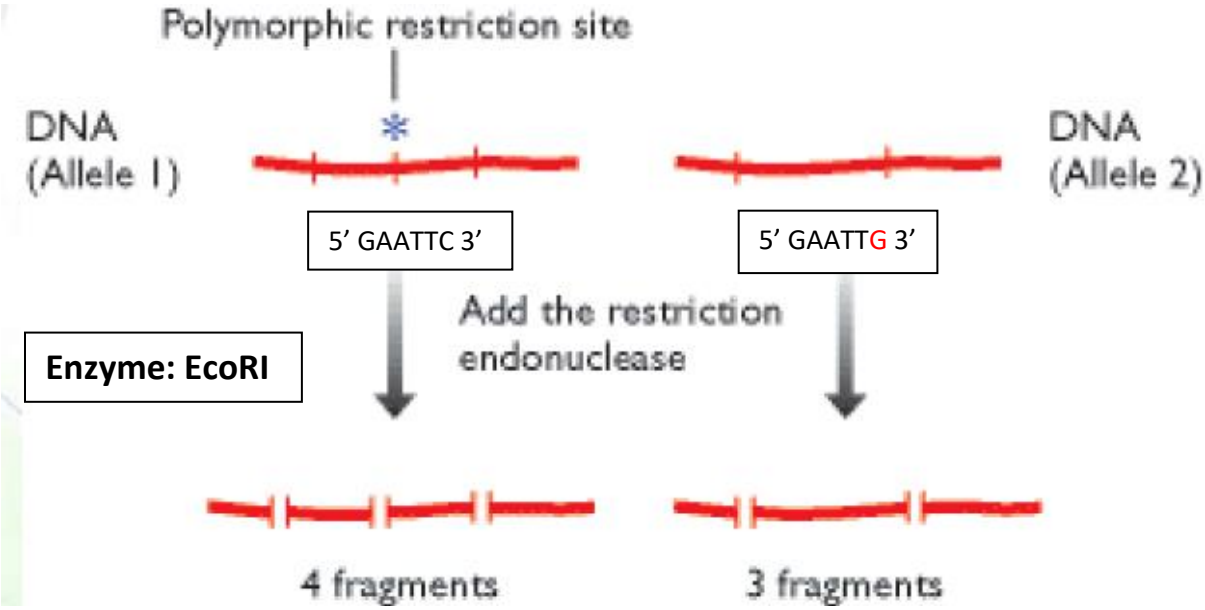
Advantage of restriction endonucleases:

1) ***Restriction fragment length polymorphism (RFLP)***
2) ***Cloning***

DNA polymorphisms:

- All people genome is 99.99% identical, so we all have the same chromosomes and the same sequences, but we have polymorphisms.
- The differences may be in a single nucleotide at a specific position.
- Our genome is formed of 3.2 billion base pairs, and it contains about 0.01% polymorphisms (millions of genetic mutations).
- Remember: our cells are diploid (have one copy from the father and one from the mother), so alleles can be homozygous or heterozygous).
- Individual variations in DNA sequence (genetic variants) may create or remove restriction-enzyme recognition sites generating different restriction fragments.

For example:



The enzyme cannot cut at the site of the polymorphism with different sequence than the normal restriction-enzyme recognition site and this results in different number of fragments.

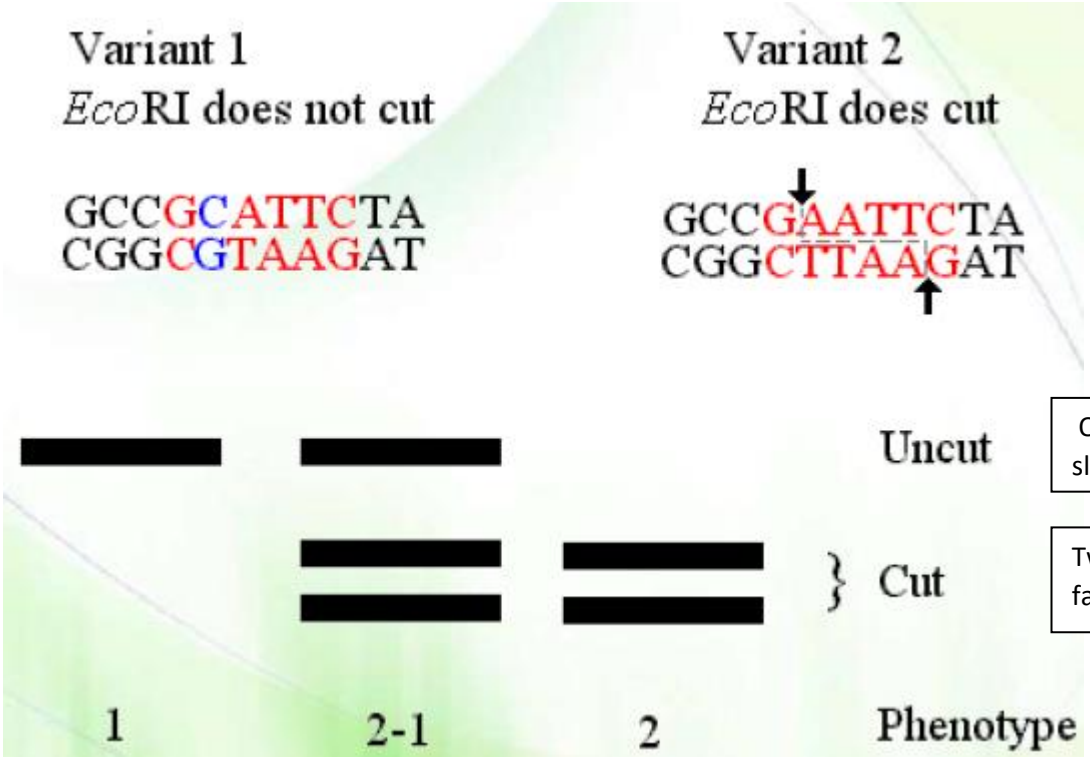
this is called Restriction fragment length polymorphism (RFLP) (we have differences in the length of fragments that are formed by the endonucleases).

Restriction fragment length polymorphism (RFLP):

- The presence of different DNA forms in individuals generates a restriction fragment length polymorphism, or RFLP.
- These can be detected by:
  - ✓ Gel electrophoresis
  - ✓ Southern blotting

For example:

Every person has two copies of the gene from his parents.



One long and slow fragment

Two shorter and faster fragments

1) If person has 2 copies of type 1 (homozygous for that sequence), he will have only one band because it doesn't matter where the source of DNA is (from dad or mom), as long as they are the same size, they will migrate together as 1 band.
2) If person has 2 copies of type 2, he will have 2 bands (4 DNA fragments each 2 of the same length).
3) If person is heterozygous, he will have 3 bands, 1 band for the uncut fragment and 2 for the cut fragment.

Good luck