no. 2

Molecular Biology

Doctor 2019 | Medicine | JU

◉ Sheet ○ Slides

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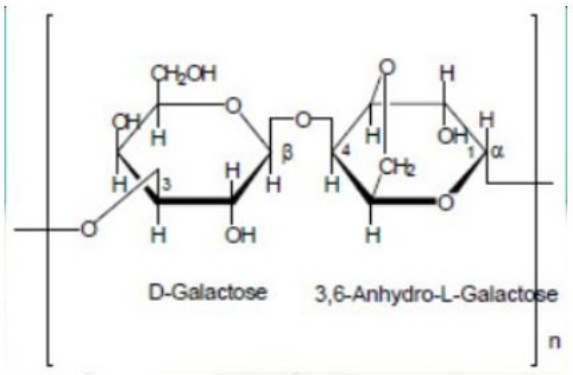
Gel Electrophoresis

Phoresis : movement through a medium

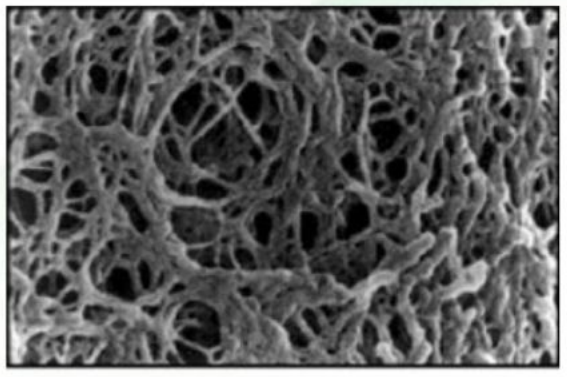
Electrophoresis : movement of DNA molecules through a medium by the influence of electrical force

The gel used in this technique is made of sugar polymers called agarose

Polymers are molecules made of repetitive monomers



Note: you don't need to memorize monomers names written in this photo



Note: agarose under EM

Pores in this gel contribute primarily in the movement of molecules and fluids through it

Remember that DNA molecules are negatively charged and their movement through the gel from the cathode (negative pole) to the anode (positive pole) is influenced by an electrical current

Gel electrophoresis in more details

The length and purity of DNA molecules can be accurately determined by the gel electrophoresis

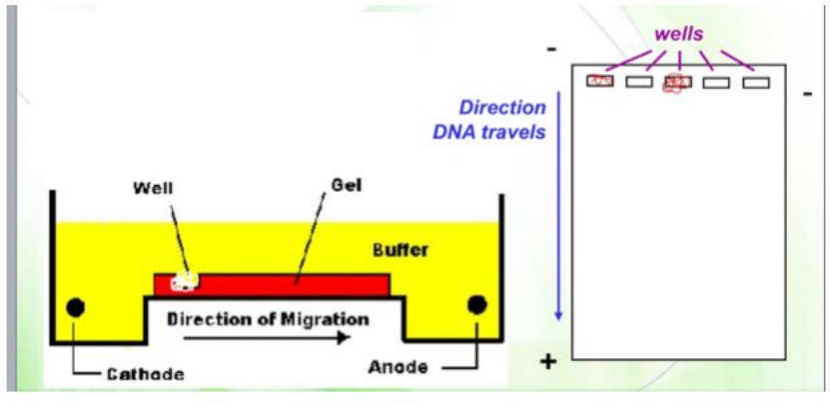
Agarose contains wells in which DNA molecules are filled

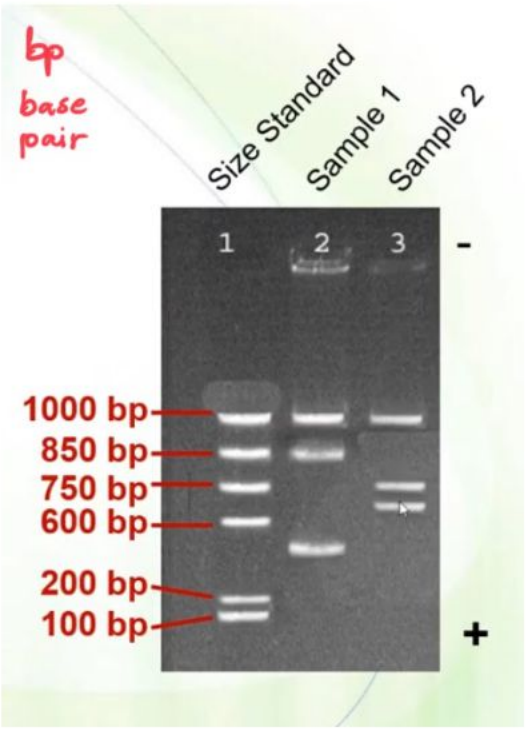
After filling wells with DNA molecules and turning on the electrical current DNA molecules start to move towards the positive pole with variations in the

distances they achieve according to the sizes (the smaller the DNA molecule the greater distance it moves)

Important :

Single DNA molecule can't be seen without microscope and we don't use microscopes here which means that each sample contains thousands to millions DNA molecules migrating through movement according to their sizes (DNA molecules of the same size migrate and move the same distance regardless of their type or bases sequence)





*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 (not one DNA molecule)

* DNA is stained (that is, colored) with a dye (ethidium bromide(not used any more due to its toxicity as it can cause cancer) or radioactively labeled (32P)

*It is common that a DNA standard is used to determine the length of the examined DNA molecule.(known sizes)

Notes:

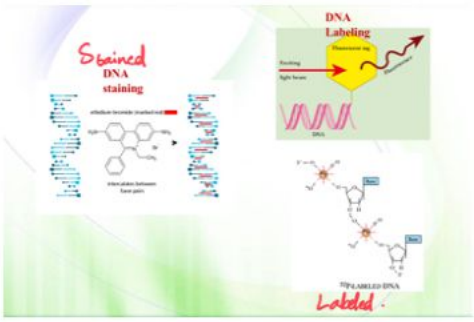
[1] 1000 bp = a total of 2000 bases each DNA molecule (1000 bases each strand)

[2] In order to be seen , DNA molecules are stained or labeled;

Stained with a dye in which the dye molecules reside in or between baese

Labeled either with a radioactive element (32P) or a fluorescent tag

And viewed after this under UV (ultraviolet) and not under normal visible light



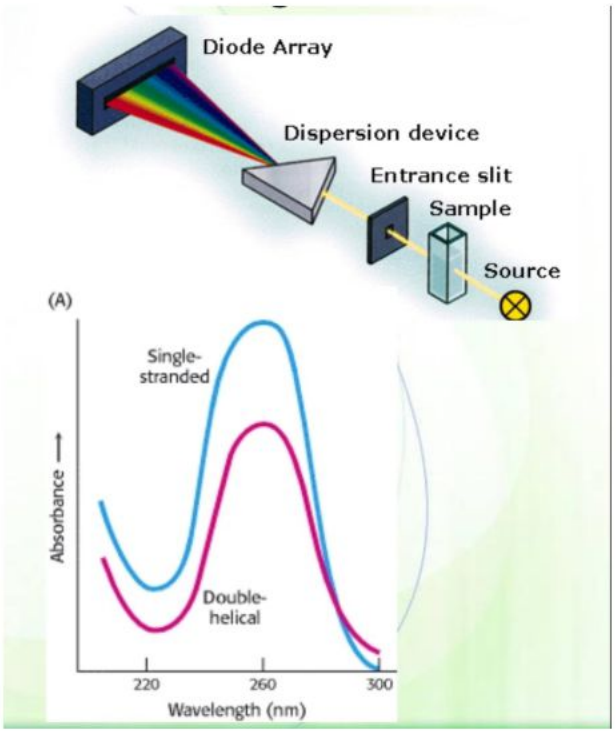
**DON'T FORGET TO WATCH THE ANIMATION

Light absorbance of nucleic acids

*Aromatic pyrimidines(T/C/U) and purines (A/G) can absorb UV light

* The peak absorbance is at 260 nm wavelength(Nucleic acids absorb light with a wavelength of 260 nm)

* Single stranded DNA molecules absorb more light than double stranded DNA molecules of same concentration (unstacked base vs. stacked bases)



* The absorbance of nucleic acids at 260 nm (A260) is constant

- dsDNA: A260 of 1.0 = 50 ug/ml

ug=microgram / 1.0 = 1 unit of light

Q: What is the concentration of a double stranded DNA sample diluted at 1:10 and the A260 is 0.1?

DNA concentration = 0.1 x 10 x 50 µg/ml

= 50 µg /ml

* Explanation *

The amount of light absorbed by the diluted solution is 0.1

1 > 50

0.1 > ?

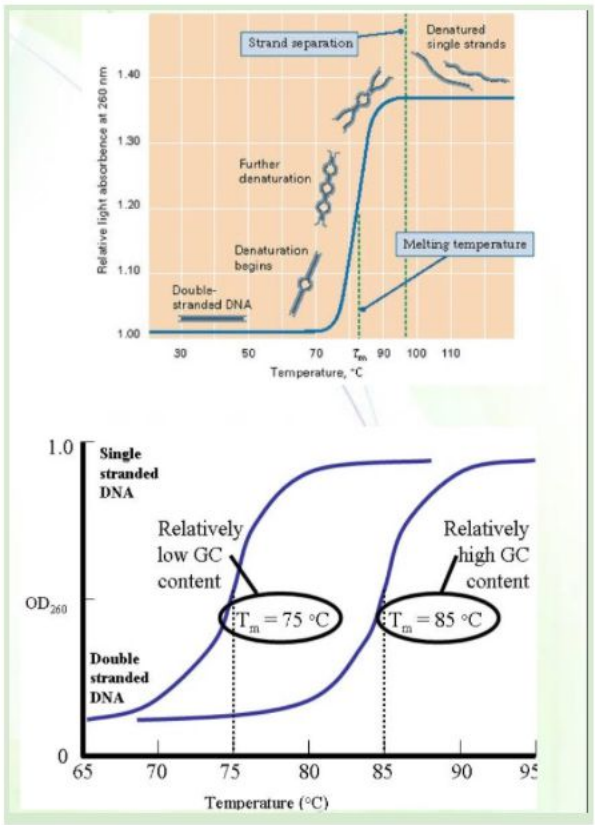
Then and according to the fact that the resulting number is the concentration of DNA molecules in the diluted solution we multiply the number by 10 (dilution factor)

Denaturation , renaturation & hybridization

*** Denaturation :the separation of DNA strands(double stranded DNA = 2 single strands) in which DNA double helix is ruined and no longer observed**

***DNA denaturation is achieved by increasing temperature**

*** The transition temperature(melting temperature) : The temperature at which 50% of DNA molecule are double stranded and 50% are single stranded (denatured)**



****Factors influencing Tm**

[1] length (greater length > higher Tm)

[2] G-C pairs (greater content of G-C pairs >greater stability > higher Tm) according to hydrogen bonds and base stalking

[3] pH (extreme pH values (very acidic / very basic)> lower stability > lower Tm)

[4] Salts and ions (according to the fact that DNA is negatively charged (repulson) , the addition of positively charged substances stabilizes it > higher Tm)

[5] Destabilizing agents (lower stability > lower Tm) ; e.g : (alkaline solutions, formamide, urea)

*****Renaturation the opposite of denaturation

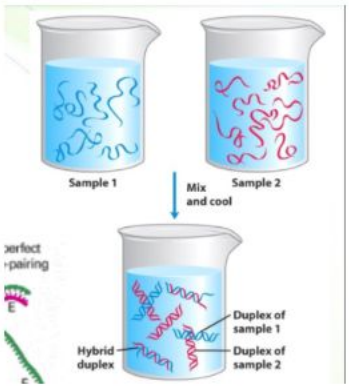
After the removal of the denaturation factor , the two single strands reform the double helix in a process known as renaturation

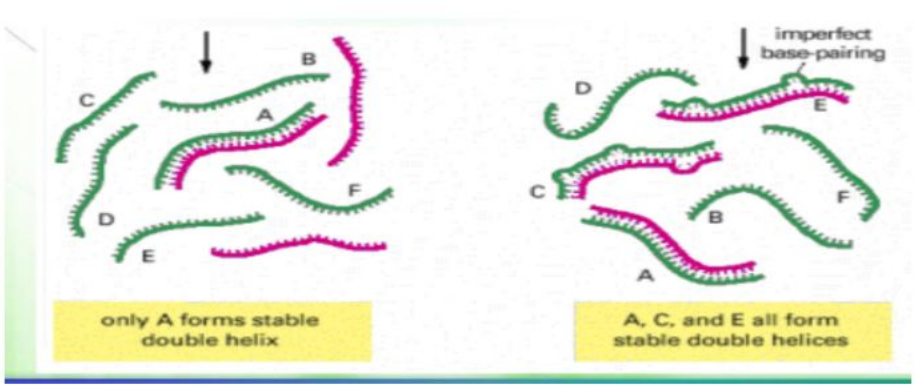
*****Hybridization

*DNA from different sources can form double helix as long as their sequences are compatible (hybrid DNA)

(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 can be :

Perfect : the two single strands are perfectly matching (complementary 100%)

Imperfect : sometimes we need to hybridize two single strands which aren't perfectly matching (1 or more bases are not complementary)

*Hybridization can be imperfect (*when temperature is low, salt concentration is high, etc*)

*Both hybridization processes require higher amounts of stabilizing factors (factors which increase Tm) but the ones required by imperfect hybridization are greater , which means it can be controlled by changing the temperature, ionic strength of solutions, GC content, etc.

Remember : As long as we have enough number of hydrogen bonds hybridization can occur
(which is something relative)

Probes

Oligonucleotides : oligo means short

Oligonucleotides = probes

* A probes is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.

*Hybridization reactions use labeled DNA probes to detect larger DNA fragments.

Mechanism

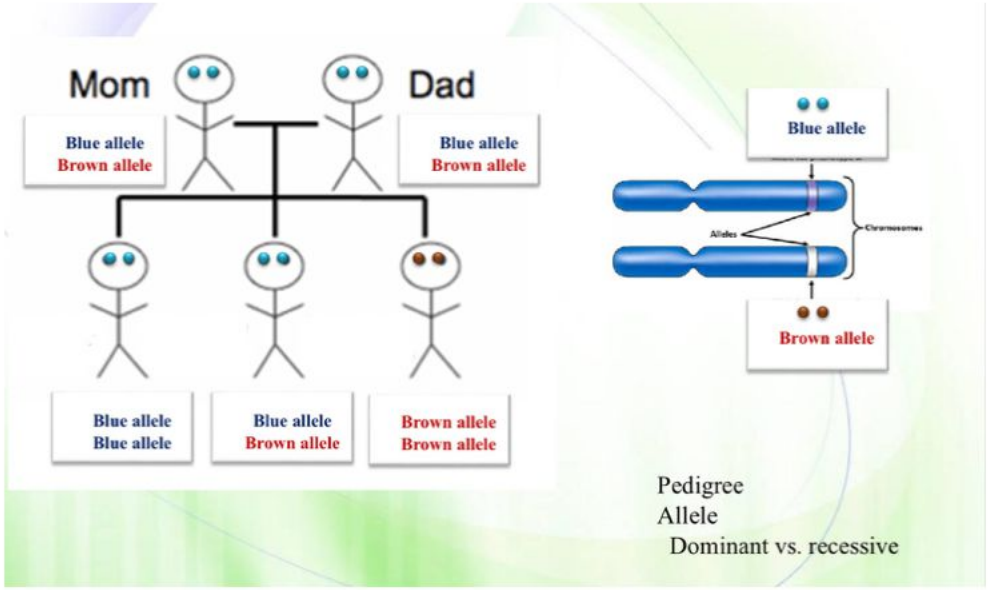
After the addition of probes and as they are highly concentrated they win the competition against the single strand bound to the complementary sequence of the the

probe on the other DNA strand (denaturation between DNA strands and hybridization between the probe and its complementary sequence occur)

Concepts to know

Allele : a specific gene on a specific chromosome responsible for a specific feature

As our cells are diploid (homologous chromosomes (maternal & paternal)) the features are maintained by two alleles and not only by one



Inheritance rules are useful in understanding alleles and how they function

Dominant سائد /Recessive متنحٍ

Pedigree (family genetic tree)

Note :

*** When both alleles are the same (homozygous)**

***When they aren't (heterozygous)**

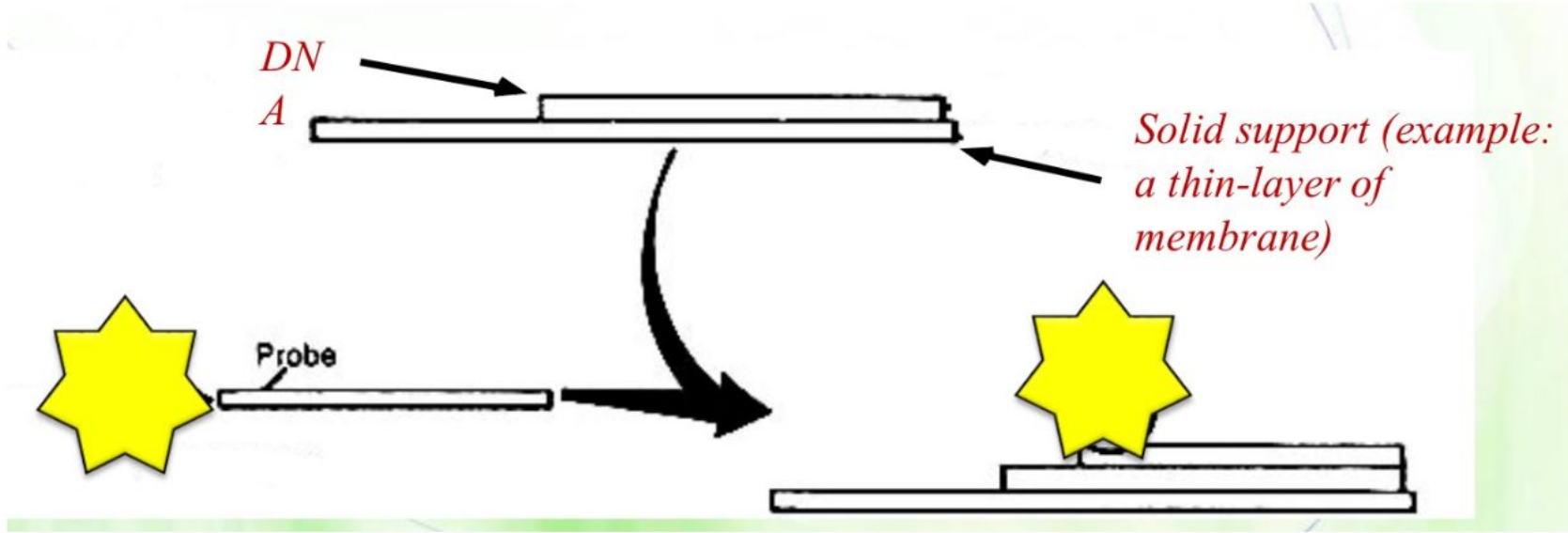
Dot plot

***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.**

Mechanism

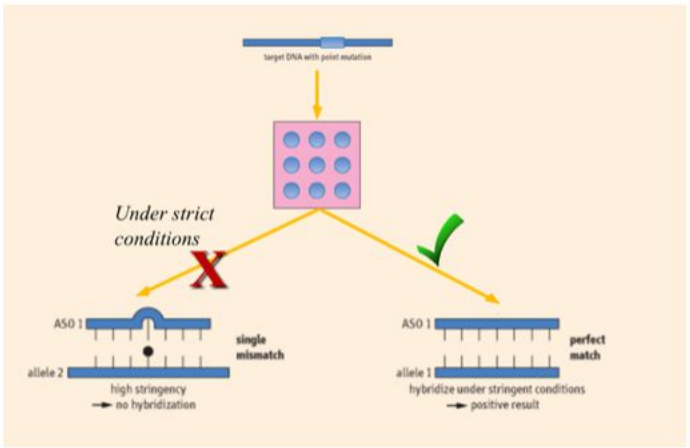
[1] DNA samples are placed on a solid support (a thin layer of membrane)

[2] Labeled probes are then added to hybridize with their complementary sequence

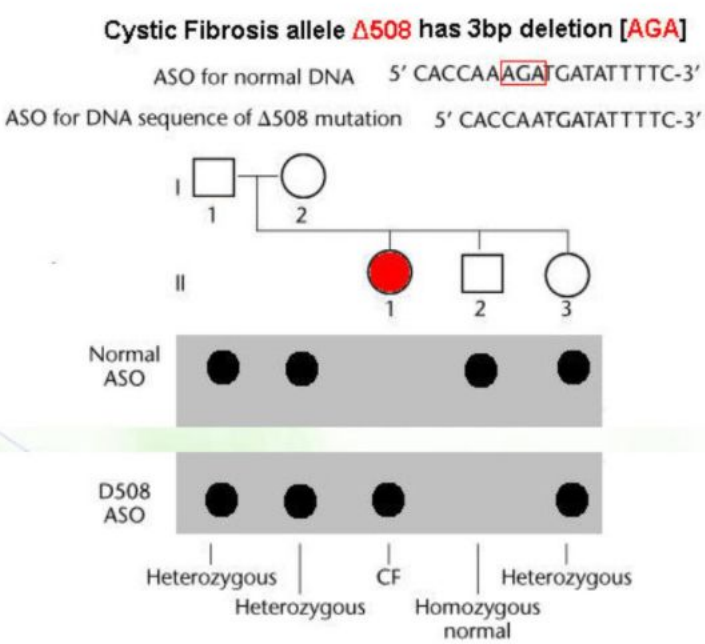


Note :

As we said before , imperfect hybridization can be induced under strict conditions and is possible as long as we have enough number of hydrogen bonds .However ; as dot plot is specific to a particular sequence of bases , imperfect hybridization is *prohibited* .



****Dot plot in the detection of cystic fibrosis**



***The whole genomic DNA is spotted on a solid support (like a nylon membrane) and hybridized with two ASO's, one at a time.**
***Cystic fibrosis results from the deletion of 3 nucleotides**

*2 types of probes are used in the detection of cystic fibrosis and are called allele specific oligonucleotides (ASO)
One for the normal sequence
One for the abnormal sequence (3 nucleotides missing)
And as we said one at a time

Heterozygous individuals have both sequences one on each allele (normal, abnormal)
Homozygous individuals have the same sequence on both alleles (normal ,normal /
normal ,abnormal)

Note:
As diseases' alleles are recessive heterozygous individuals aren't considered sick

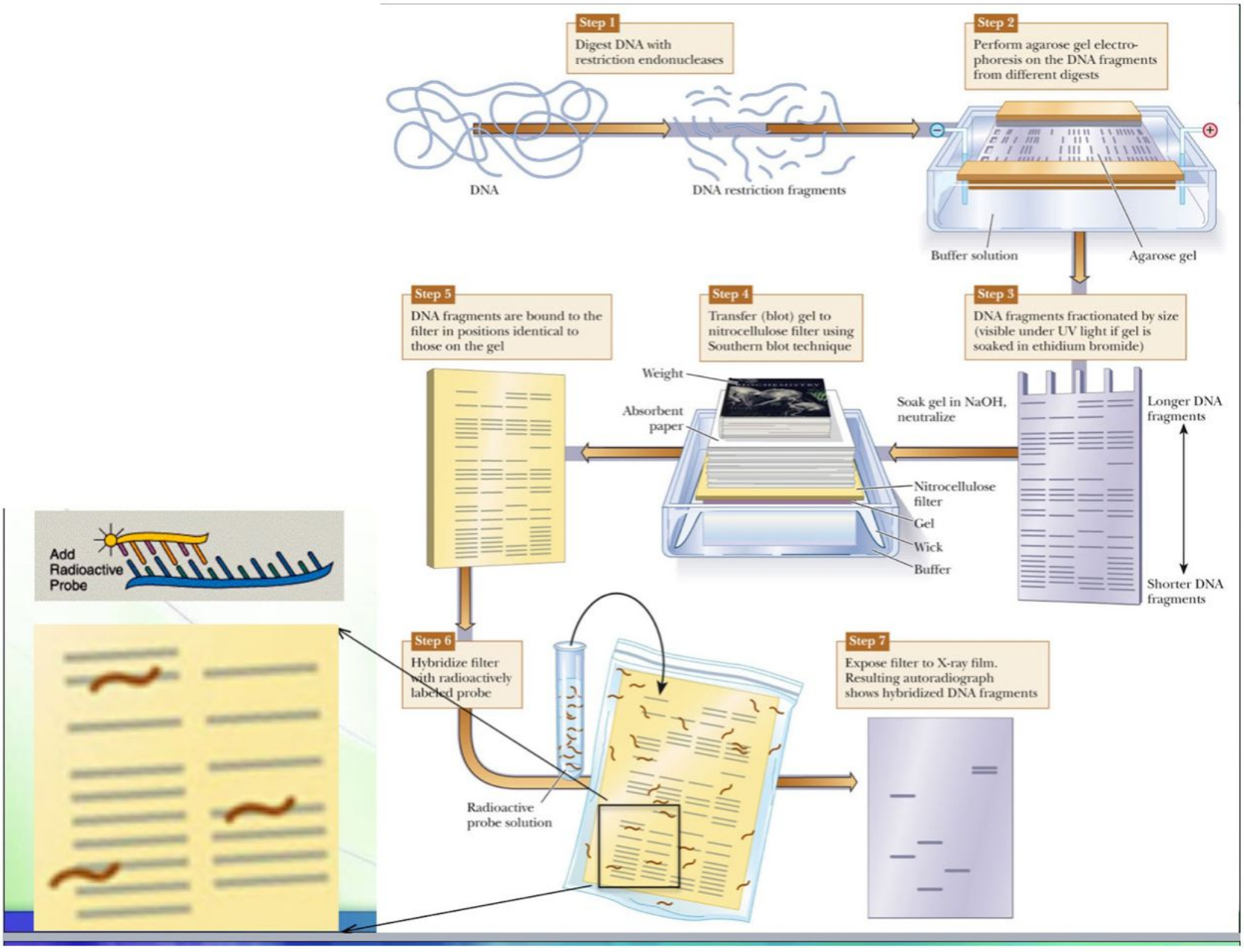
Southern blotting

*This technique is a combination of DNA gel electrophoresis and hybridization
*Used to detect:
[1] the presence of a DNA segment complementary to the probe
[2] the size of the DNA fragments

Mechanism

[1] gel electrophoresis
[2] transfer of DNA fragments to a thin membrane (nylon) , then this membrane represents a replica and is a mirror image of the DNA fragments order on the gel
[3] addition of the labeled probes which hybridize with their complementary sequence

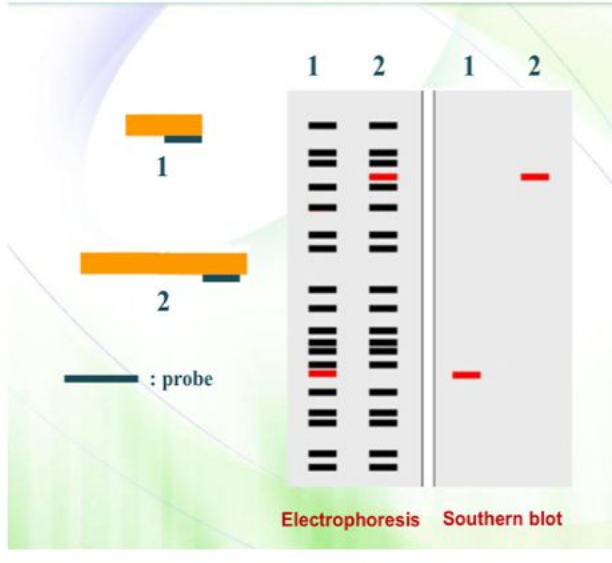
[4] in order to detect these fragments (the ones bound to the probes) an x-ray film is placed on the top of the membrane for the radioactive points to hit



Note :

Humans' genomes are similar with 99.9% of their sequences , which means the difference is 0.1%

This 0.1% can be detected by southern blotting but not by gel electrophoresis because of the great number of DNA bands



https://m.youtube.com/watch?v=tlMvUR9wBGY

Don't forget to watch this video

See your goals

Understand the obstacles

Create a positive mental picture

Clear your mind of self doubt

Embrace the challenge

Stay on track

Show the world you can do it !

The end

We wish you all the best