Sheet no. 2

Molecular biology

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Doctor: Mamoun Ahram

Instructions:

Blue → What doctor mentioned in their slides

Black → What doctor said in the lecture

Italic → *Sheet writers' notes that doctor did **NOT** mention in the lecture*

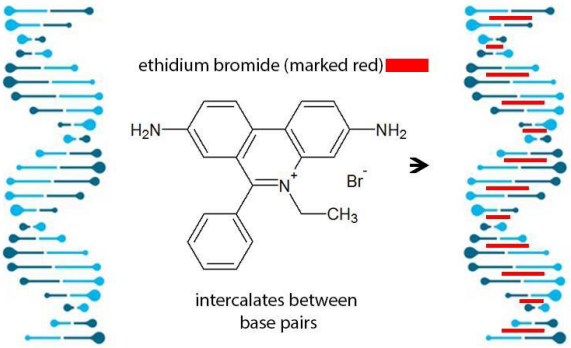
Note: All pictures are taken from doctor's slides

In this lecture, we will talk about number of basic techniques and applications. We will talk about: Electrophoresis, denaturation and hybridization (last two concepts will allow us to move on and talk about more techniques).

Note: doctor said that the main source to study this course is his lectures, Cooper book doesn't contain all the details.

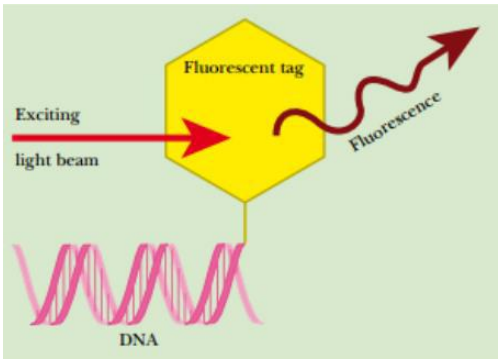
❖ DNA labeling VS DNA staining:

We can't see DNA with our own eyes because it absorbs light in the (UV) range and we can't see UV, but we can see DNA by staining (coloring) it, or by labeling it.



➢ Staining is coloring DNA so we add a stain to DNA (e.g., ethidium bromide).

➢ DNA labeling is more sensitive; it is used to see minute *(very small)* amounts of DNA. Basically, we stick something to DNA that emits a signal. We can label DNA either with **radioactive phosphorus** (it emits signal that can be detected), or we can attach a **fluorescent tag** to the DNA, so that it's not really the DNA that emits the signal, it's rather the fluorescent tag itself.



Ethidium bromide has a flat structure, and it can intercalate *(insert)*, it gets between the base pairs and gives a color that we can see.

Note: we use labeling to detect small amounts of DNA and staining for large amounts

1) Gel Electrophoresis: (الفصل الكهربائي الهلامي)

Gel: like the jelly

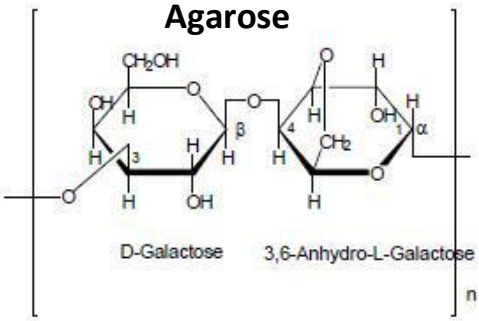
Electro: electrical field

Phoresis: movement of molecules (like DNA) through a medium.

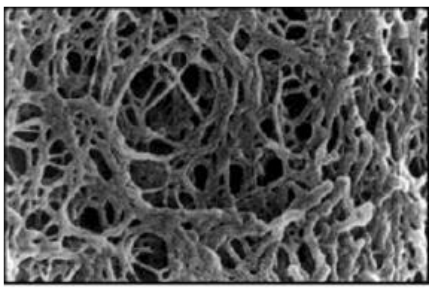
So, it means movement of DNA through an electrical field.

➢ If you put a drop of water on the top of a jelly, this water would disappear, it would go through the pores of the jelly, same thing with this gel that we use to analyze DNA.

➢ The gel is prepared from a sugar molecule known as **Agarose**. This Agarose is exactly like a jelly, we dissolve it in water and then we heat it, so that when it cools down it polymerizes, forming something like this.

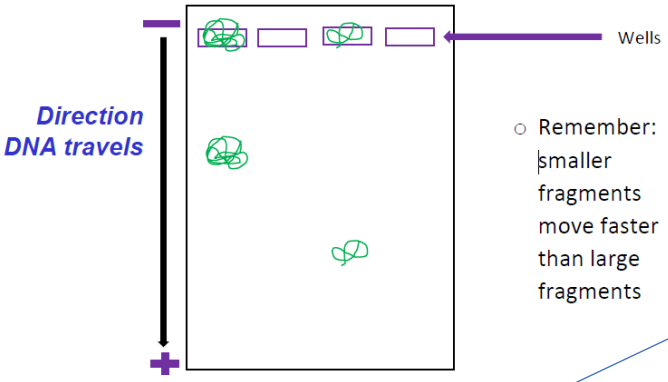


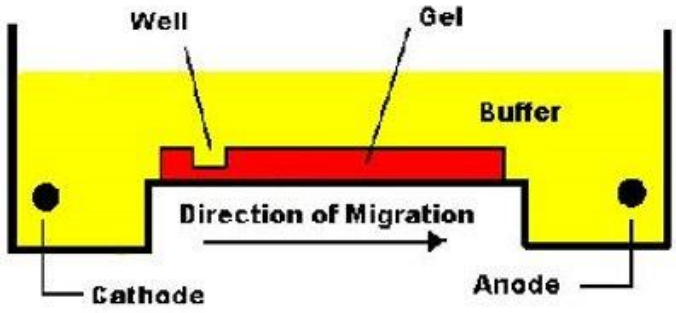
➢ This is a scanning electron image of an Agarose gel, so you can see the pores (holes) within the gel itself, so the DNA would move through these pores.



➢ Smaller DNA fragments (have few nucleotides) would move faster through the gel compared to large DNA fragments; the DNA fragments as they move will keep on hitting the solid structure of the gel so the large fragments would move slower than the smaller fragments.

➢ When we prepare a gel we create openings inside it, these openings are called **wells**. We add our samples inside the wells.



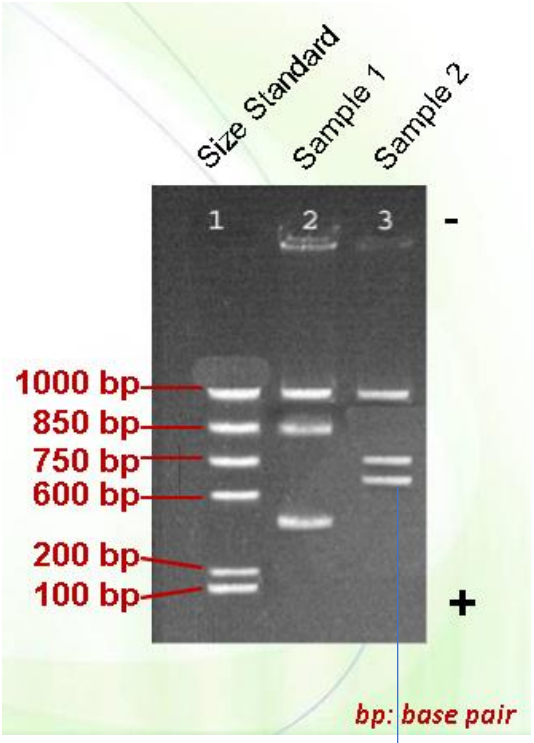


➢ We place the gel in a tank that contains a solution, and we apply an electrical field on the tank, so we have a cathode (negative pole) and the Anode (positive pole), after applying the electrical field DNA will move from the cathode toward the anode, because it's negatively charged (because of the phosphate groups).

Detection:

➢ DNA is stained with a dye (ethidium bromide) or labeled (radioactive 32P).

➢ DNA molecules of different lengths will run as "bands", the shape of the band looks like the shape of the wells, so the narrower the well, the narrower the DNA band.



We can estimate its length because it's between 750 bp and 600 bp, travels maybe closer to 750 bp so that we can estimate its size to around 700 bp.

➢ Each band contains thousands to millions of copies of DNA fragments of the same length (these copies have the same size because they move at the same speed, so they move as one band) but can be of same or different type (meaning that these DNA fragments can have the same exact sequence (identical), or they can be different but have the same size so that they move together as one band).

➢ How can we determine the size of the DNA fragments that we have in our sample?
It is common that a DNA standard is used to determine the length of the examined DNA molecule. (Known sizes)

➢ **Size standard:** it's a sample that contains DNA fragments of known sizes (length), can be purchased or prepared in labs.

➢ When we say 1000 bp it means it's a DNA that contains 2000 bases and they are paired together, so you have 1000 thousands on one strand and 1000 on the other strand.

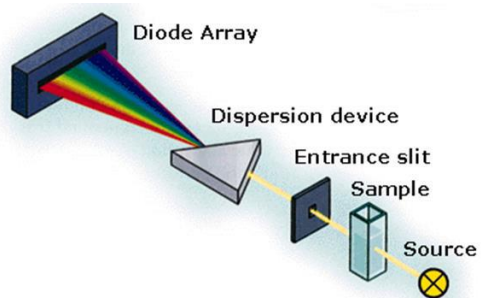
Note: *doctor advises us to watch videos on YouTube, it'd help but don't waste your time with long videos, you can visit this website.*
http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html

❖ Light absorbance of nucleic acids:

➢ DNA doesn't absorb light in the range we see; it absorbs light at 260 nanometers which is in the UV range.

➢ Why does DNA absorb light in the UV range?
Because of the aromatic rings of pyrimidines (T, C and U) and purines (A and G) that can absorb UV light. (Ring structures usually absorb light at different wavelength, in this case purine and pyrimidines absorb UV light).

➢ Using spectrophotometry, the peak absorbance can be measured at 260 nm wavelength.



➢ dsDNA (doubled stranded DNA) can absorb certain amount of light (according to the amount of DNA).

➢ dsDNA: A260 of 1.0 = 50 ug/ml (if a DNA sample absorbs one unit of light, it means that the amount of DNA in this sample is around 50 µg/ml, in other words, if I have a DNA sample that has a concentration of DNA of around 50 µg/ml it would be able to absorb one unit of light).

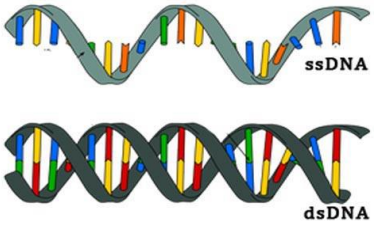
➢ What if you have a DNA sample that contains a concentration of 5 µg/ml, how much light would be absorbed?
It should absorb 0.1 of light. *(نسبة وتناسب)*

➢ What if you have a DNA sample that can absorb 0.5 unit of light, what is the concentration of DNA molecules in this sample?
25 µg/ml.

➢ What is the concentration of a double stranded DNA sample **diluted** *(مُخفّف)* at 1:10 and A260 (absorbance) is 0.1?
the question is: what is the concentration of the diluted sample, well if the amount of light is 0.1 unit it means that the concentration is 5 µg/ml, what is the concentration of the original undiluted DNA sample? well you have to consider this dilution is 1:10 so you need to **multiply the concentration that you got by 10 (dilution factor)**, so now the concentration of the original DNA sample is 50 µg/ml (0.1×10×50 = 50 µg/ml)

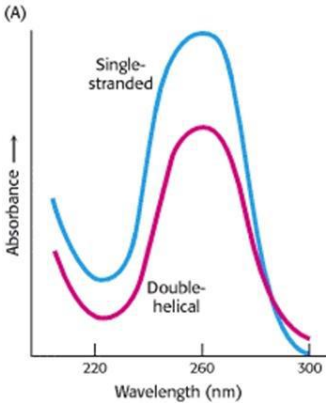
Instead of crisscross: ***concentration of DNA = 50 * absorbed light * dilution factor***
↓
In unit of light

➢ ssDNA (single stranded DNA) can absorb more light than double stranded DNA, because in the double stranded DNA, bases are stacked and they're embedded, they're hidden inside the dsDNA, on the other hand, the bases in the ssDNA are more exposed so they can absorb more light.



➢ ssDNA: A260 of 1.0 = 30 µg/ml (If there's a concentration of 30 µg/ml of ssDNA it can absorb 1.0 unit of light, you need more dsDNA (50 µg/ml) to absorb the same amount of light, **so this means that ssDNA absorbs more light than dsDNA.**

➢ We have the same concentration of dsDNA and ssDNA at 260 nm, ssDNA would absorb more light.

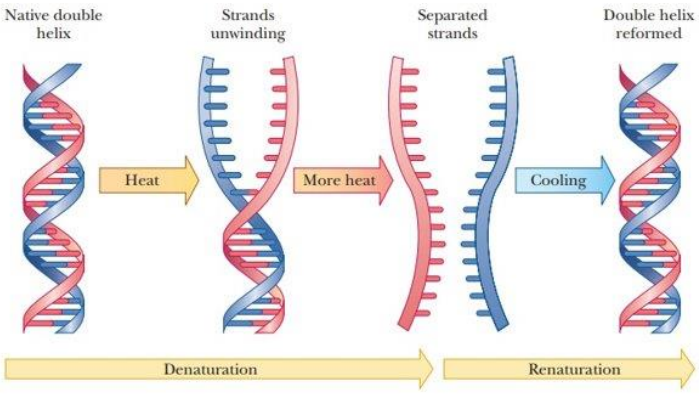


Note: *Doctor can give a problem of either ssDNA or dsDNA and we can use this method of calculation and pay attention to dilution.*

❖ Denaturation VS Renaturation

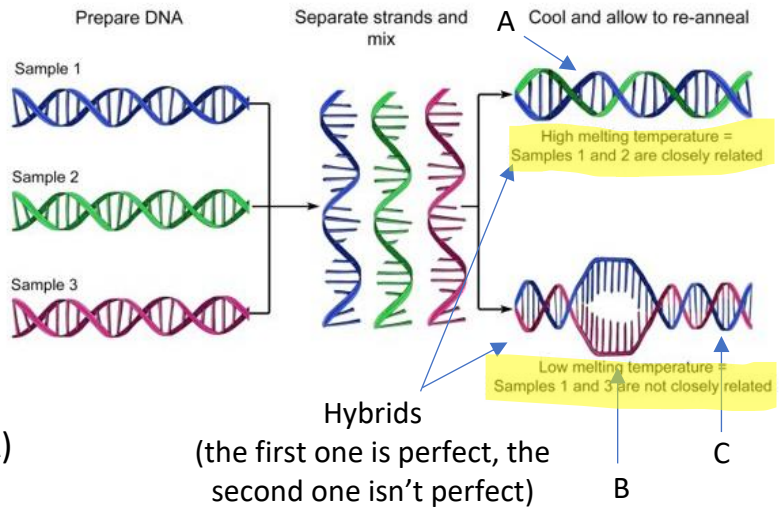
➢ **Denaturation** happens when separation of two strands by heat, when temperature increases, the hydrogen bonds between the bases will be broken.

➢ **Renaturation** happens when cooling down the DNA environment, the two strands will reform (the DNA sequencing is still complementary in both strands, so they will form double-stranded structure)



■ FIGURE 9.19 **Helix unwinding in DNA denaturation.** The double helix unwinds when DNA is denatured, with eventual separation of the strands. The double helix is re-formed on renaturation with slow cooling and annealing.

➢ **Hybridization** is reforming double-stranded DNA except that the two strands come from different sources In the following picture, we can separate the two strands in any sample by heat, and you can have a **Hybrid**



➢ The two strands bind perfectly because they're 100% complementary (A)

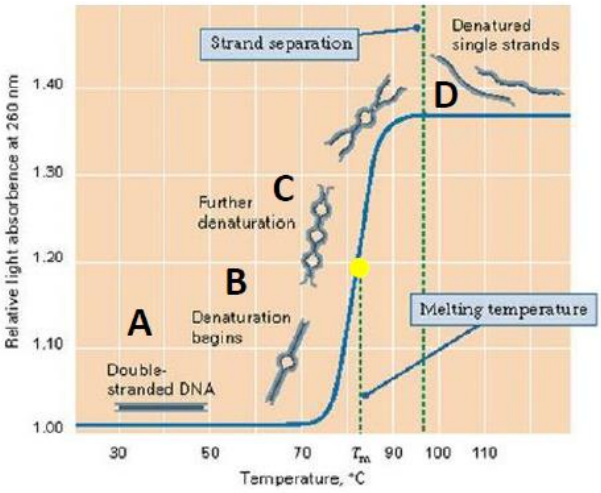
➢ The two strands don't bind perfectly because bases in (B) aren't complementary to each other thus don't form hydrogen bonds, but as long as there are enough hydrogen bonds in (C) there is formation of double-stranded DNA but not perfectly *(شوف الرسمة)*

➢ One factor that causes DNA to be denatured is temperature (the transition temperature or melting temperature (Tm)), what happens is that when we increase the temperature the hydrogen bonds between the bases are broken and the two strands get separated from each other.

A ⟶ we have dsDNA at low temperature, so the DNA is double stranded.

B + C ⟶ as we start to increase the temperature of the DNA, the two strands get separated slowly from each other, so the hydrogen interactions between the bases are broken.

D ⟶ eventually, at very high temperatures the DNA would be all single stranded.



➢ If we decrease the temperature the DNA will renature and form double stranded DNA.

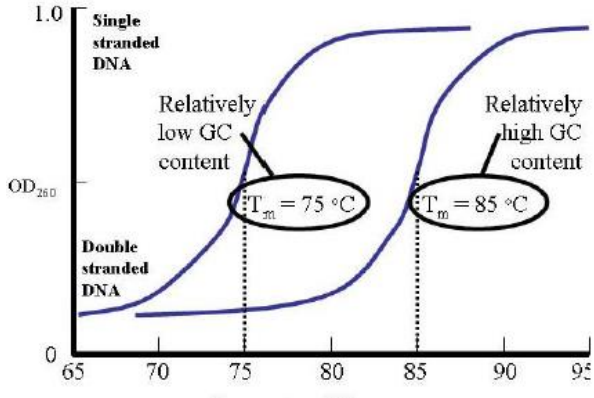
➢ Denaturation process is very slow, so it gets to a certain point (C) where 50% of the DNA molecule is single stranded and 50% is double stranded, this point is called the **melting temperature**, it's quite important because it can differ between different DNA fragments according to certain factors.

➢ **Pay attention** to the Y axis in the graph, it is the amount of light that is absorbed, notice that it gets higher, because the DNA becomes more single stranded, and ssDNA absorbs more light than dsDNA and the difference is 1 to almost 1.4 which is the difference between 30 µg/ml VS 50 µg/ml.

❖ **Factors influencing Tm**: (factors that affect the value of the melting temperature or the factors that influence how DNA is denatured):

1- Length: the longer the DNA fragment is, the higher Tm is, because you have more bases meaning that you have more hydrogen bonds, so you need more energy to denature the DNA strands from each other.

2- G≡C pairs: the higher the G.C content, the higher the Tm is, because there are 3 **hydrogen bonds** between G and C (more stable) and 2 hydrogen bonds between T and A.



Clarification: if you have the two linear fragments of the same length but you have one having more GC content than the other one the one with the higher GC content would need more energy and higher temperature to denature the DNA versus the other one.

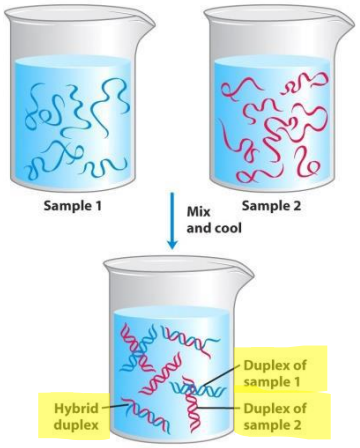
3- pH: extreme pH values (very acidic and very basic) lower stability and **lower Tm**.

4- Salts and ions: high salt concentration needs high Tm, if we're talking about possibly positive charged ions like sodium ions, they would mask the negative charges of the phosphate groups, meaning that they stabilize the DNA.

5- Destabilizing agents (alkaline solutions, formamide, urea): formamide and urea specifically break hydrogen bonds (lower stability), so if you add urea to your sample the temperature that is needed to denature DNA should be lower, so the melting temperature would also be lower. ***(The more destabilizing agents, the lower the TM)***

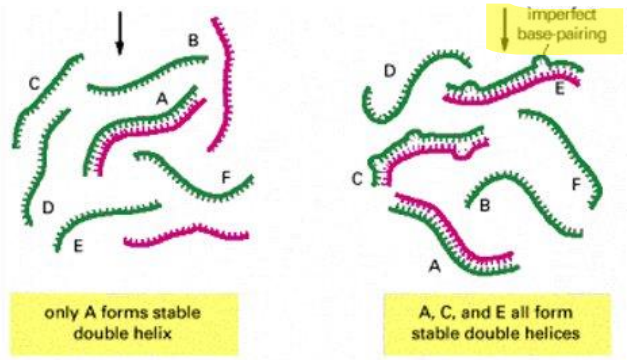
❖ Hybridization:

➢ Hybrid means formation of something from two different sources.



➢ If I say DNA hybridization, it means that I'm forming dsDNA where each strand comes from a different source.

➢ For example, here we can have two DNA fragments coming from two different sources, you have sample 1 (DNA from individual A), sample 2 (DNA from individual B), if we denature the double stranded DNA and mix the DNA from sample 1 and sample 2 there is a good chance that you would have dsDNA whereby each strand comes from a different individual.



➢ What determines whether the two strands can renature and hybridize to each other or not? **complementary base pairing**, you would have different forms of dsDNA you can have the original (the blue and red as you can see in the first picture above), or you can have a hybrid (mixed blue and red).

➢ if you take human DNA and monkey DNA, they can hybridize to each other as long as they are complementary to each other, so we can have even a hybrid between a human DNA and bacterial DNA, **it doesn't matter what the source of DNA is, what matters is if they are complementary to each other then they can hybridize to each other.**
Base pairing doesn't have to be perfect as long as you can have enough hydrogen bonding (enough base pairing), you can have hybridization between the DNA fragments.

➢ What determines whether you can have imperfect-based pairing or not? what influences hybridization?

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

If you mix the two DNA samples at **high temperature**, there is a **low chance** that you would have **imperfect** hybridization, because there is a good chance that at high temperature, the hydrogen base pairing wouldn't form. In order to allow for **imperfect** base pairing to take place, we can mix the different DNA samples at **low** temperature. Also, if we **increase** salt concentration that would **reduce** the repulsion between the phosphates and that would allow for imperfect hybridization to take place, if you lower salt concentration there is a good chance that we keep only perfect hybridization in our sample and we prevent the formation of imperfect base pairing.

➢ Hybridization can be nonspecific, so you can have the formation of double stranded DNA between a short piece of DNA and a long piece of DNA, and this hybridization can be perfect or imperfect, but if we have enough hydrogen bonds there would be formation of partially double-stranded

❖ Past paper:

1) Denaturation of DNA molecules is a necessity in southern blotting in order to allow binding between the probs and the separated DNA strands, which of the following pH values allow this to occur and specifically in southern blotting:

A) 6.5 B) 7 C) 13 D) A & C E) None

2) Calculate the concentration of double stranded DNA molecules if a **concentrated solution** of which (by a factor of 5) absorbed 2 units of light with a length of 260 nm:

A) 10 B) 20 C) 500 D) 100 E) 50

3) Melting temperature of DNA is:

A) The temperature at which the DNA strands are denatured completely.
B) The temperature at which the DNA strands are half denatured.
C) The temperature at which the DNA strands renatured.
D) None of the above.

4) All of the following regarding gel electrophoresis are true EXCEPT:

A) Agarose gel is used
B) Smaller molecules move faster than larger ones
C) Molecules move towards the positive electrode
D) The higher the density of the gel, the higher the resolution

5) When using gel electrophoresis, 1000 bp fragment size indicates:

A) A total of 1000 bases each DNA molecule.
B) A total of 1000 base pairs each DNA molecule.
C) A total of 2000 bases each DNA molecule.
D) A total of 1000 bases each DNA strand.
E) B, C&D.
F) B&C only.

Note: *if the question gives you* ***diluted solution****, then you should multiply by 5 and you get 500, but here it gives you the* ***concentrated solution*** *so you should divide the answer by 5 (A concentrated solution is one that has a relatively large amount of dissolved solute. A dilute solution is one that has a relatively small amount of dissolved solute)*

Answers:
C B B D E

وَإِذا كانَتِ النُفوسُ كِباراً
تَعِبَت في مُرادِها الأَجسامُ