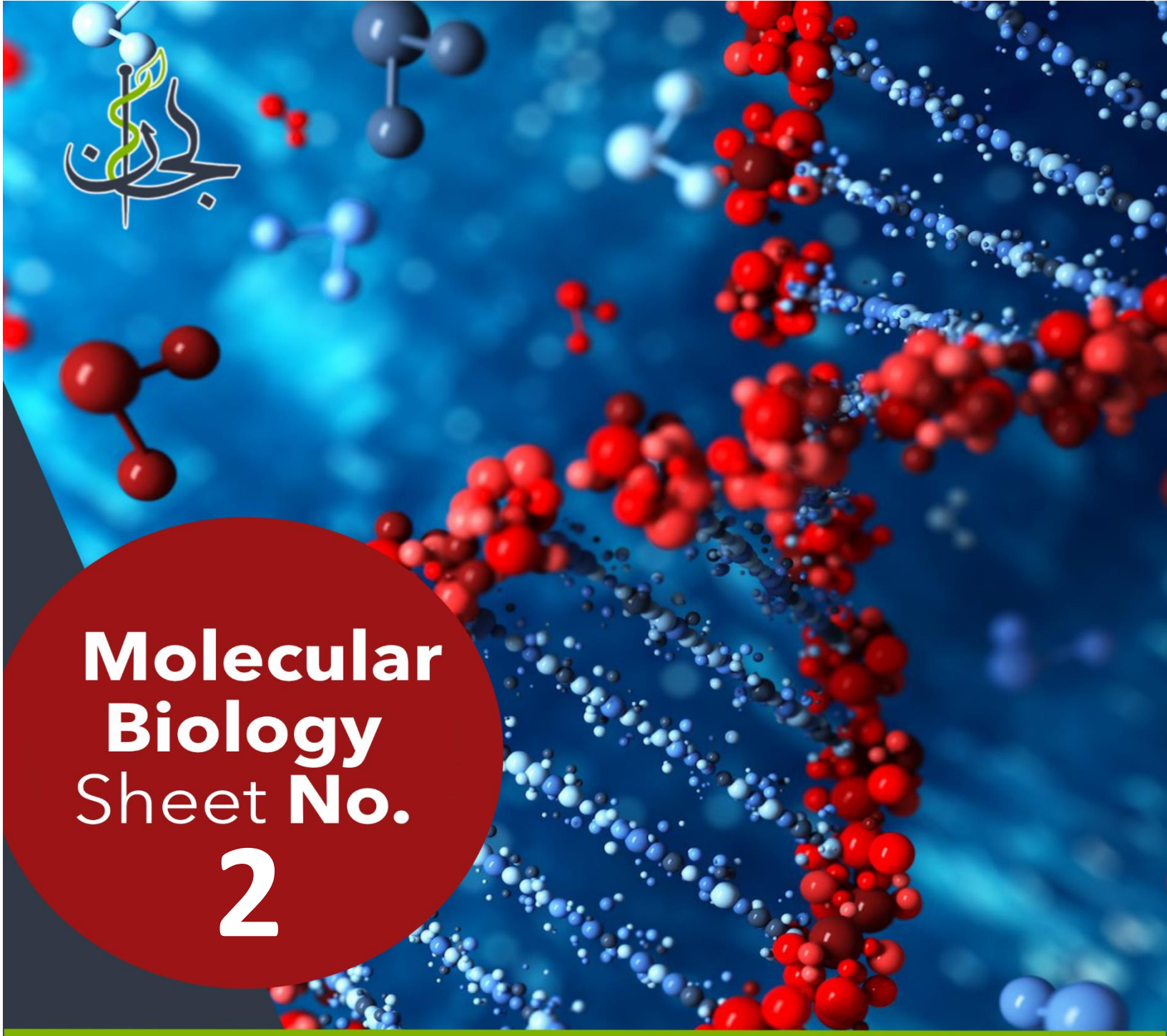

Molecular Biology Sheet No. 2

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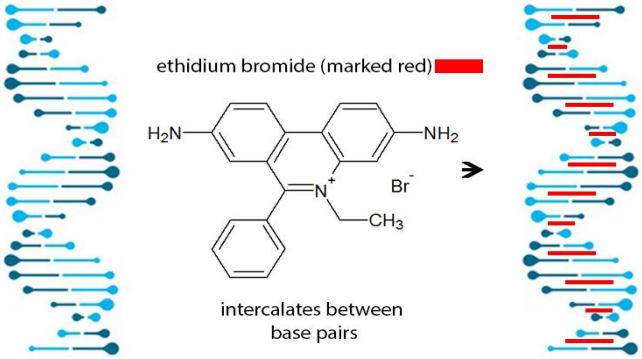
In this lecture we will talk about number of basic techniques and applications, we will talk about: 1) Electrophoresis 2) Denaturation 3) Hybridization (2+3 these two concepts will allow us to move on and talk about more techniques).

❖ DNA labeling VS DNA staining:

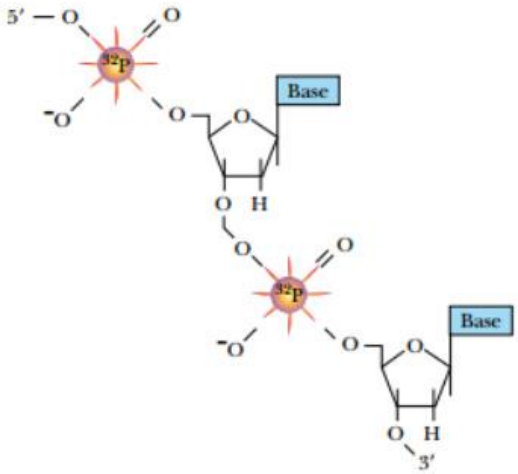
We can't see DNA with our own eyes because it absorbs light in the (UV) range and we can't see UV, but we can see DNA by staining (coloring) it, or by labeling it.

- Staining basically is coloring DNA so the idea is that we add a stain to DNA like ethidium bromide.

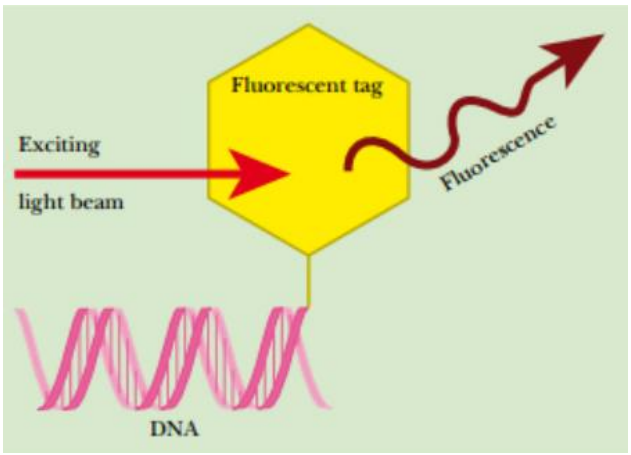
Ethidium bromide has a flat structure, and it can intercalate, it gets between the base pairs and gives a color that we can see.



- DNA labeling is <u>more sensitive</u>, it is used to see minute amount of DNA. Basically we stick something to DNA that emits(يصدر) 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, <u>so that it's not really the DNA that emits the signal it's rather the fluorescent tag itself</u>.



^{32}P-LABELED DNA



DNA labeling → detect small amounts of DNA.

DNA staining → detect large amounts of DNA.

❖ Gel Electrophoresis:

Gel → like the jelly

Electro → electrical field

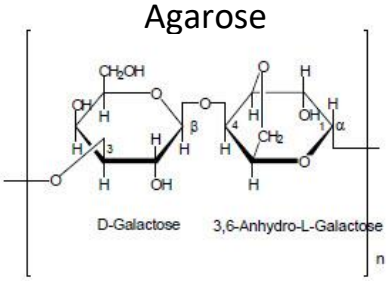
Phoresis → movement of molecules (like DNA) through a medium.

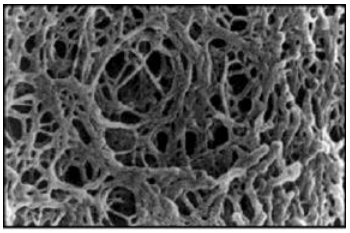
So it means movement of DNA through an electrical field.

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:

Agarose





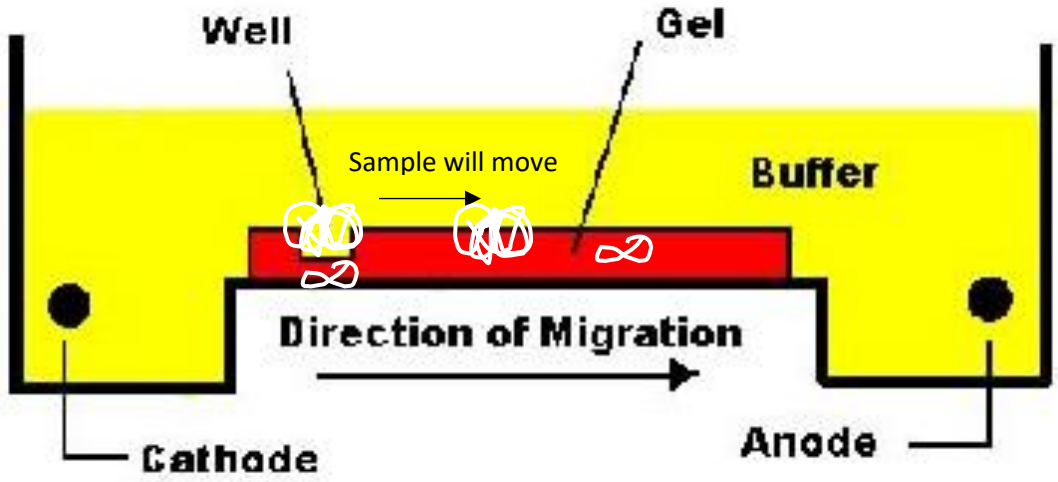
This is a scanning electron image of an Agarose gel, so you can see the pores (holes) within the gel itself, so the DNA would move through these pores.

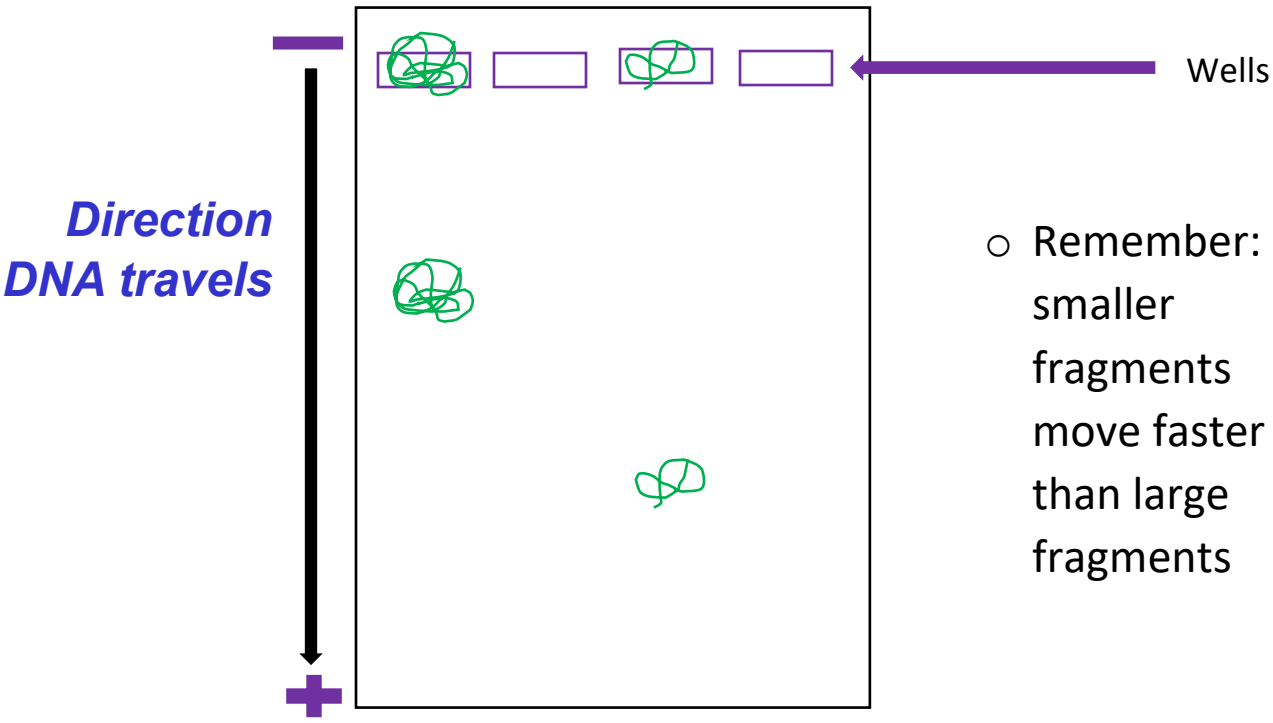
The idea here is that smaller DNA fragments (have few nucleotides) would move faster through the gel compared to large DNA fragments.

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 solution, and we apply an electrical field on the tank, so we have a (Cathode: negative pole) and the (Anode: the positive pole), after applying the electrical field DNA will move from the cathode to the anode, because it's negatively charged (because of the phosphate groups).





- Remember: smaller fragments move faster than large fragments

Detection:

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

The 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 would be.

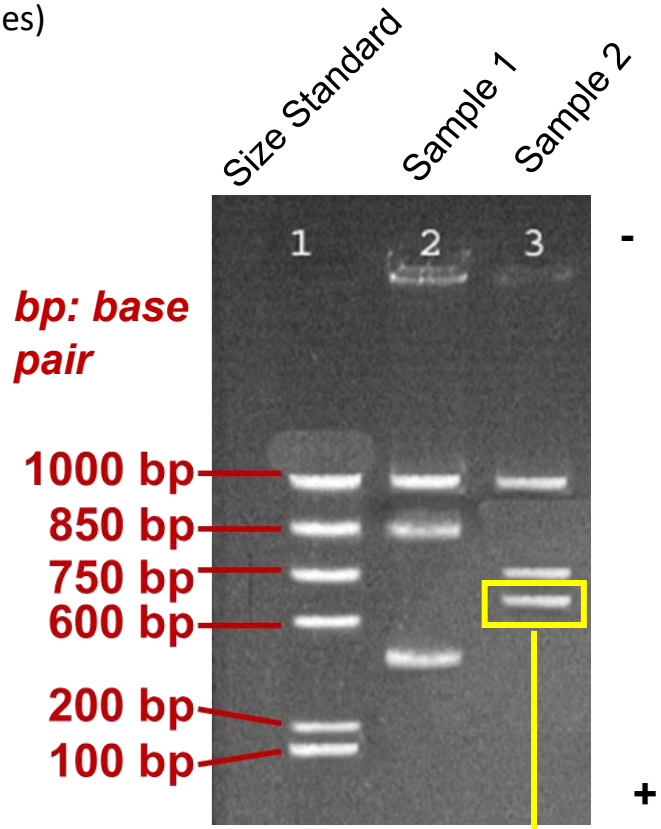
** Each band contains thousands to millions of copies of DNA fragments of the same length (these copies have the same size because they move at the same speed so they move as one band) but can be of same or different type (meaning that these DNA fragments can have the same exact sequence (identical) 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), we purchase the size standards or we can prepare it in our 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.



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

Watch this….very important

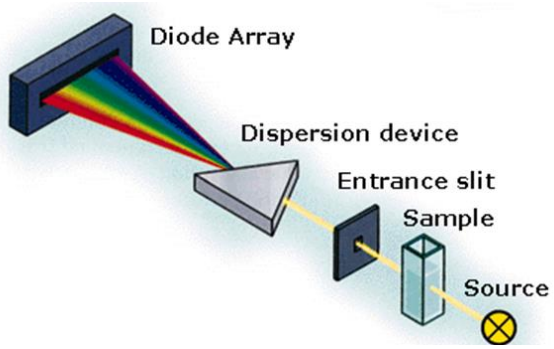
http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html

❖ Light absorbance of nucleic acids:

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

Why does DNA absorb light in the UV range? Because of the aromatic rings of pyrimidines (T,C,U) and purines(A,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 light. Certain amount (units) of light can be absorbed by dsDNA according to the amount of DNA, and we know that if a DNA sample absorbs one unit of light it means that the amount of DNA in this sample is about 50 µg/ml.
  dsDNA: A260 of 1.0 = 50 ug/ml
  In other words, if I have a DNA sample that has a concentration of DNA of about 50 µg/ml it would be able to absorb one unit of light.

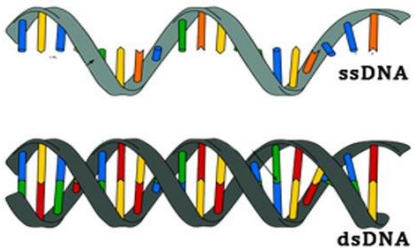
  Q: what if you have a DNA sample that contains a concentration of 5 µg/ml, how much light would be absorbed?
  It should absorb 0.1 of light.

Q: 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?

crisscross meaning that if 50 can absorb 1.0 of light then 0.5 means that the concentration is 25 µg/ml.

Q: what is the concentration of a double stranded DNA sample diluted at 1:10 and A260 (absorbance) is 0.1?

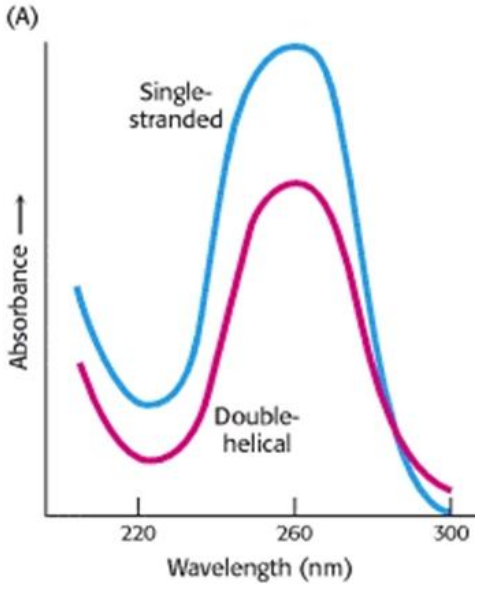
the question is what is the concentration of the diluted sample, well if the amount of light is 0.1 unit it means that the concentration is 5 µg/ml, what is the concentration of the original undiluted DNA sample? well you have to consider this dilution is 1:10 so you need to multiply the concentration that you got by 10 (dilution factor), so now the concentration of the original DNA sample is 50 µg/ml so it's 0.1×10×50 = 50 µg/ml. (Doctor can give a problem of either ssDNA or dsDNA and we can use this method of calculation and pay attention to dilution).



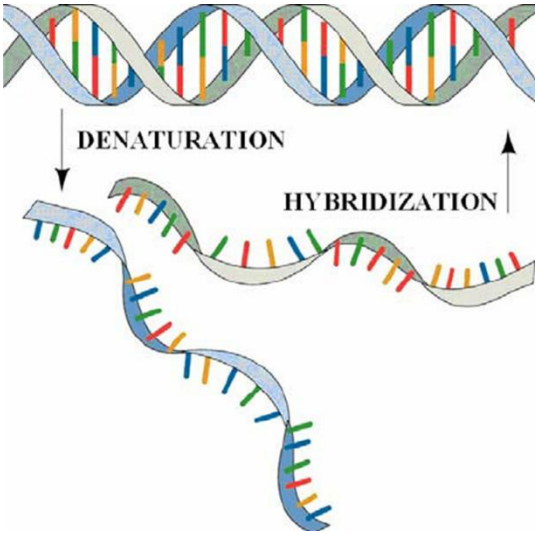
- We've also observed that ssDNA (single stranded DNA) can absorb more light than double stranded DNA, why? because in the double stranded DNA, bases are stacked and they are embedded, they are hidden inside the dsDNA, on the other hand the bases in the ssDNA are more exposed so they can absorb more 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.

In this graph:

We have the same concentration of dsDNA and ssDNA at 260 nm ssDNA would absorb more light. There is more absorbance of light by ssDNA than dsDNA.



❖ Denaturation VS Renaturation (Hybridization)



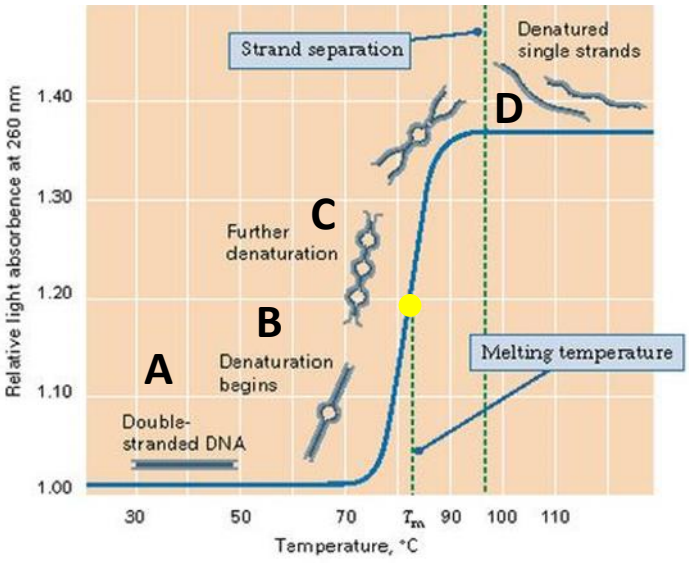
Denaturation means the separation of DNA strands (dsDNA = 2 single strands) in which DNA double helix is ruined and no longer observed.

Renaturation means that the two strands get back together forming base paring and again following the same rules of DNA structure that is complementary base pairing would be formed.

Denaturation is basically breaking up the three-dimensional structure of DNA so that you have separation of the two strands from each other, and renaturation means that the two strands get back together to their original form. Another term for renaturation is **hybridization** except that with hybridization we are talking about the formation of double stranded DNA from DNA strands of different sources that is hybrid.

<u>So what causes DNA that was stranded DNA to be denatured?</u>

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 → right here 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 would renature and form double stranded DNA again, the two strands would be complementary to each other.

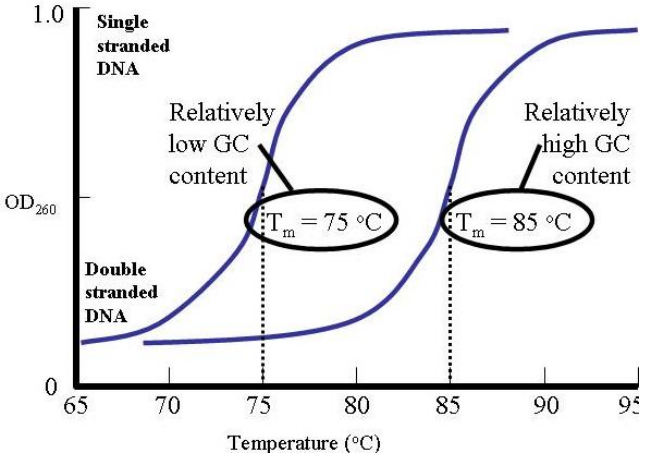
Denaturation process is very slow, so it gets to a certain point where 50% of the DNA molecule is single stranded and 50% is double stranded.

this point is called the melting temperature, this point is 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)



if you have the two clear 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-very basic)> lower stability > lower Tm).
4- **salts and ions** → (high salt concentration needs high Tm, if we're talking about let's say possibly positive charged ions like sodium ions, they would

mask the negative charges of the phosphate groups, meaning that they stabilize the DNA)

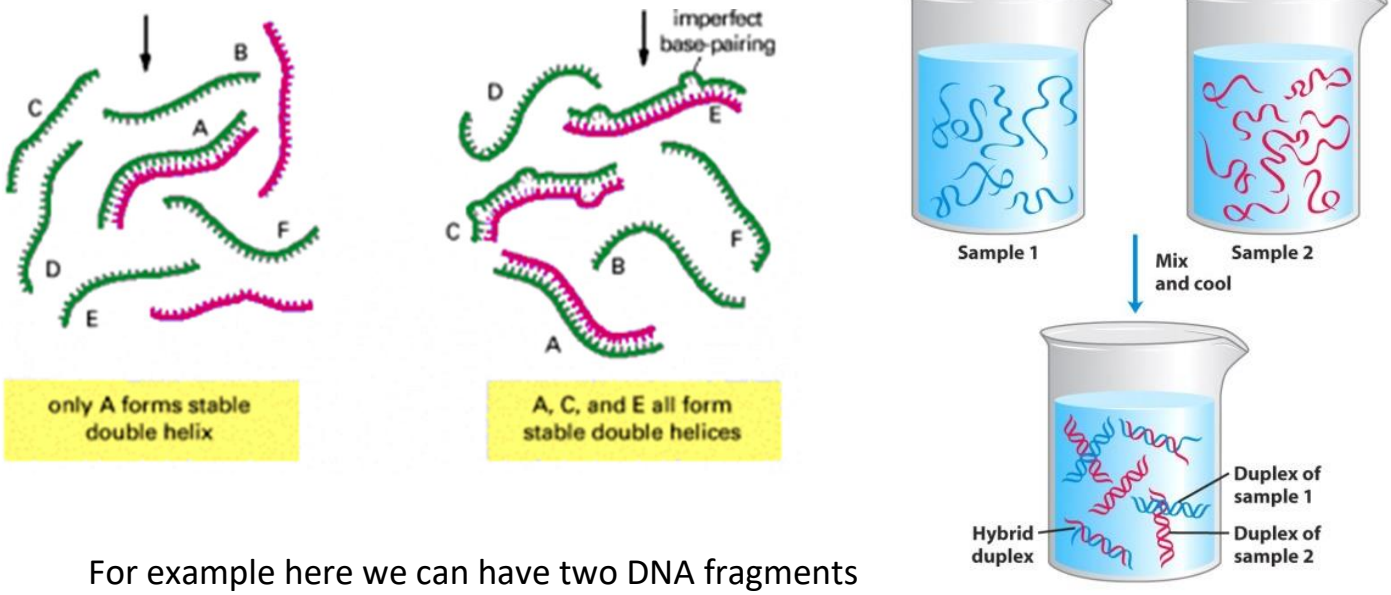
5- **destabilizing agents (alkaline solutions, Formamide, urea)** → (formamide and urea specifically break hydrogen bonds (lower stability) so if you add urea to your sample the temperature that is needed to denature DNA should be lower so the melting temperature would also be lower as well).

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

❖ 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 (let's say 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. This means that this DNA is a hybrid DNA.

what determines whether the two strands can renature and hybridize to each other or not? **complementary base pairing**, as long as the bases are complementary to each other they can form a DNA hybrid.

You would have different forms of dsDNA you can have: the original (the blue and red as you can see in the picture) or you can have a hybrid (mixed blue and red). The rule is as long as they're complementary to each other they can form a hybrid.

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?

All the other previous factors that we have just talked about: temperature, salt concentration and so on.

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 would not 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.

THE END