Genetics & Molecular Biology

☑ Sheet ☐ Slide ☐ Handout

Number: 2

Subject: Denaturation, Hybridization and RFLP

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Observation of denaturation:

DNA is composed of two strands that are linked together by **hydrogen bonds**. These bonds are weak bonds and we can simply break these bonds by different factors (For example: Heating / pH changing). This is called **DNA denaturation.**

By heating the DNA sample gradually, you will have more and more broken hydrogen bonds and the strands are separated.

And by cooling down the sample, these strands will link together (renature) and become the same as the original sample (**Why? Because the two strands are <u>complementary</u> to each other**).

Remember from the first lecture that ssDNA can absorb light more than dsDNA. We can use this fact to observe **(1) DNA denaturation** and **(2) the melting temperature (Tm).**

The melting temperature: The temperature where the DNA is 50% double stranded and 50% single stranded. (In another way: It is the temperature where the DNA is half denatured).

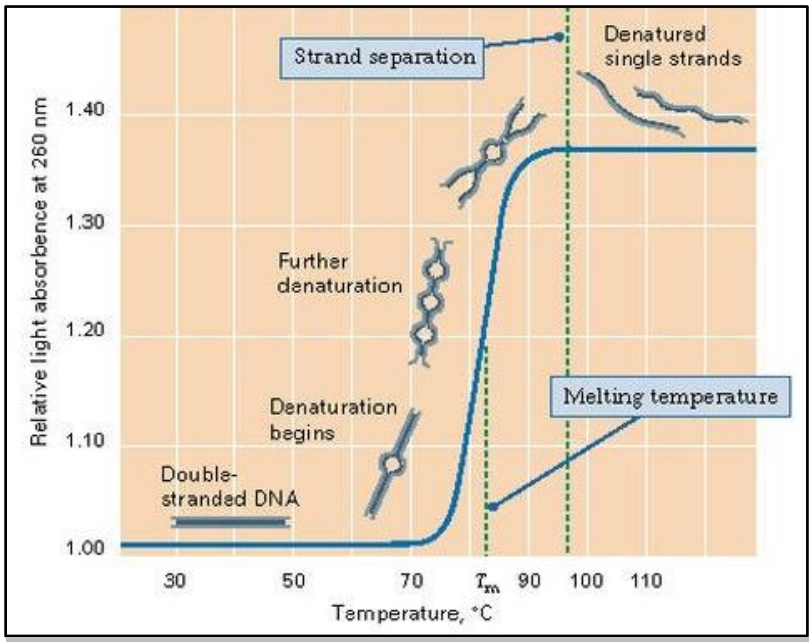

Figure 1

In **figure 1**, we have a sample of dsDNA. At room temperature, the sample absorb certain amount of light. By increasing the temperature, some DNA molecules in the sample will denature and the sample will have ssDNA along with the dsDNA. As a result, the sample will absorb more light.

Notice that somewhere in the middle of the curve the sample will contain 50% ssDNA and 50% dsDNA, and the temperature at this point is the melting temperature.

Question: If you have two samples of DNA that have the same length, is it a must for them to have the same Tm?

The answer is **No**, because there are many factors that affect Tm.

Factors influencing Tm:

1- G·C pairs.

The Higher G·C content is, the higher Tm of the sample is.

Why? Because G and C can form three hydrogen bonds (compared to two between A and T), so more energy (heat) is needed to denature the DNA.

In **figure 2**, There are two samples that have different Tm because they have different G·C content.

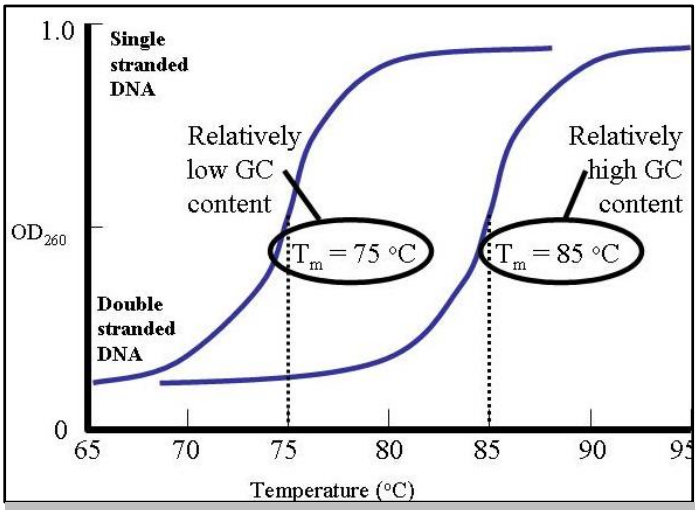

Figure 2

2- pH.

If you decrease or increase the pH to extreme values, this will affect DNA stability and break hydrogen bonds, and hence lowering the Tm.

3- Salt and ion concentration.

Increasing positively charged ion concentration will increase the Tm. (Why? Because the positive charge will neutralize the negative charges (phosphates) of the DNA so this will decrease the repulsions between DNA molecules and make DNA more stable).

4- Destabilizing agents (alkaline solutions, formamide, and urea).

These agents lower the Tm.

Urea for example, breaks hydrogen bonds so you can denature DNA easier (Decrease Tm).

5- The length of the DNA.

The longer the DNA, the higher the heat needed to denature the DNA (because it will have more hydrogen bonds) so higher Tm.

Central dogma (philosophy) of biology:

It is very simple. All what it says: DNA makes RNA, RNA makes proteins.

With time, something new is added which is RNA can make DNA (in retro viruses like HIV).

RNA:

- Consist of long, unbranched chains of nucleotides joined by phosphodiester bonds between the 3'-OH of one pentose and the 5'-OH of the next.
- The pentose unit is β-D-ribose (it is 2-deoxy-D-ribose in DNA)

- The pyrimidine bases are uracil and cytosine (they are thymine and cytosine in DNA)
- In general, RNA is single stranded (DNA is double stranded).

Types of RNA:

(We will talk about them when we discuss transcription).

The Roles of Different Kinds of RNA

RNA Type	Size	Function
Transfer RNA	Small	Transports amino acids to site of protein synthesis
Ribosomal RNA	Several kinds—variable in size	Combines with proteins to form ribosomes, the site of protein synthesis
Messenger RNA	Variable	Directs amino acid sequence of proteins
Small nuclear RNA	Small	Processes initial mRNA to its mature form in eukaryotes
Small interfering RNA	Small	Affects gene expression; used by scientists to knock out a gene being studied
Micro RNA	Small	Affects gene expression; important in growth and development

Hybridization:

Hybrid: Something that is made of two different things.

Hybridization: The process of putting two different things together.

Example: If you mixed a denatured human DNA sample with a denatured DNA from another creature (monkey, bacteria…), then you cooled the mixture. Do you expect to have a DNA that have one strand from human and one strand from the other creature?

Answer: Yes! **As long as they are complementary to each other**.

So as long as the strands are complementary to each other, they can hybridize.

Now we will talk about some techniques used in Molecular Biology.

Techniques:

1- Gel electrophoresis.
2- Hybridization techniques.

Gel electrophoresis:

(Here we talk about gel electrophoresis for DNA not proteins).

This technique consists of a gel that is made from agarose. And this gel has multiple wells (openings). In each well you insert a DNA sample. Then you apply an electrical current (At one side you put an Anode (+) and in the other side you put a Cathode (-)). The DNA will move from then negative side to the positive side through the gel pores (Why? Because the DNA is negatively charged).

Different DNA molecules will be separated **based on size** (the smaller molecules will move faster and farther than bigger molecules).

Then the DNA is stained, and the DNA molecules will appear as bands (each band has many DNA molecules that have the same size).

Note: One band can have more than one DNA type (different DNA fragments). **But they must have the same size**.

Note: The density of the bands reflects the amount of DNA fragments in it. The denser bands contain more DNA than lighter bands.

Then you can compare the results of your sample with standard DNA bands with known size.

So electrophoresis:

1- Tells you if the sample contains DNA or not.
2- Tells you the sizes of DNA fragments in the sample.

Hybridization techniques:

We use hybridization techniques to characterize specific nucleotide sequences.

These techniques depend on the use of **probes**.

Probe (الباحث): A short DNA fragment that is added to larger DNA fragment and binds to it (if it is complementary to it) to detect specific nucleotide sequence.

How it is done: You label the probe with a florescent tag, then you add it to the DNA. If the probe binds to the DNA then you will be able to see the DNA colored. **And so, you know that this DNA has a nucleotide sequence that is complementary to that probe** (That is the goal of the technique)**.**

Southern blotting technique:

This technique is used to detect:

A- Specific DNA segments (that are complementary to the probes).
B- The size of the DNA fragment.

How it is done:

At first, you separate the DNA fragments in your sample by **gel electrophoresis**. Then you take the gel and put it on top of a piece of paper (called membrane). The DNA bands will be **transferred from the gel to the**

membrane. The membrane will look exactly the same as the gel (the locations of the DNA fragments will remain the same).

Then you take this membrane and **add a labeled probe to it** (you know the sequence of the probe). This probe will flow over the membrane and **will hybridize to only DNA fragments that have the complementary sequence of the probe**.

So if you see the label in the membrane, you will know that the binding (hybridization) occurred.

So you can conclude that the labeled DNA fragment has this size and has a complementary sequence to the probe. (Remember we know the size from the electrophoresis).

The doctor said that you should watch the animations he provided for better understanding.

-Restriction endonucleases, RFLP, and gene cloning-

Nucleases: Enzymes that cleave nucleic acids.

They are either Exonucleases or Endonucleases.

Exonucleases: Enzymes that cleave the nucleotides from the end (there are 5' Exonucleases and 3' Exonucleases).

Endonucleases: Enzymes that cut the nucleic acid in the middle.

One type of endonucleases is called Restriction endonucleases.

Restriction endonucleases: Enzymes that cut the nucleic acid in the middle at specific sites (**They are restricted by the sequence of the DNA**).

The restriction endonucleases recognize certain sequences that are 4bp to 8bp (bp: Base pairs).

The sequence that is recognized by restriction endonuclease is called **restriction sites** and the resulting fragments (after cutting) are called **restriction fragments**.

Note: Restriction endonucleases normally found in bacteria. Bacteria use them for protection from viral/foreign DNA.

Examples of restriction endonucleases:

- EcoR1 (isolated from E. coli): recognizes **GAATTC** and makes a cut within this sequence.
- Hinf1 (isolated from H. influenzae): recognizes **ANTC** ('N' is any nucleotide). So it cuts at AATC / ATTC / AGTC / ACTC.

Restriction enzymes cut DNA in two different ways:

1- **Blunt cut:** Cut the both strands at the same site (straight cut). Results in blunt ends.

2- **Staggered (off-center):** Cut the two DNA strands at different positions generating **sticky or cohesive ends**.

Note: **EcoR1** Cuts in this way.

Note that they cut at different sites **but between the same nucleotides** (See **Figure 3**).

Figure 3

They create sticky ends because they can bind to each other again (hybridize). (Why? Because the ends are complementary to each other.

Note: The endonuclease cuts the **phosphodiester bond** between two nucleotides in each strand. **So even if the sticky ends bind again the DNA won't be stable because the phosphodiester bond is not regenerated**. There is an enzyme called **DNA ligase** (requires ATP) that regenerate these bonds and the DNA will become back as it was (DNA ligase can work in both types of cuts).

There are two types **of sticky ends** depending on the type of the endonuclease:

1- 5' overhang: the cut is closer to the 5' end (the 5' end is hanging).
2- 3' overhang: the cut is closer to the 3' end (the 3' end is hanging).

Most of the sequences that are recognized by restriction endonucleases are **Palindromic sequences**.

Palindromic sequence: Sequences that are read the same from left to right as they do from right to left on the complementary strand (**Figure 4**).

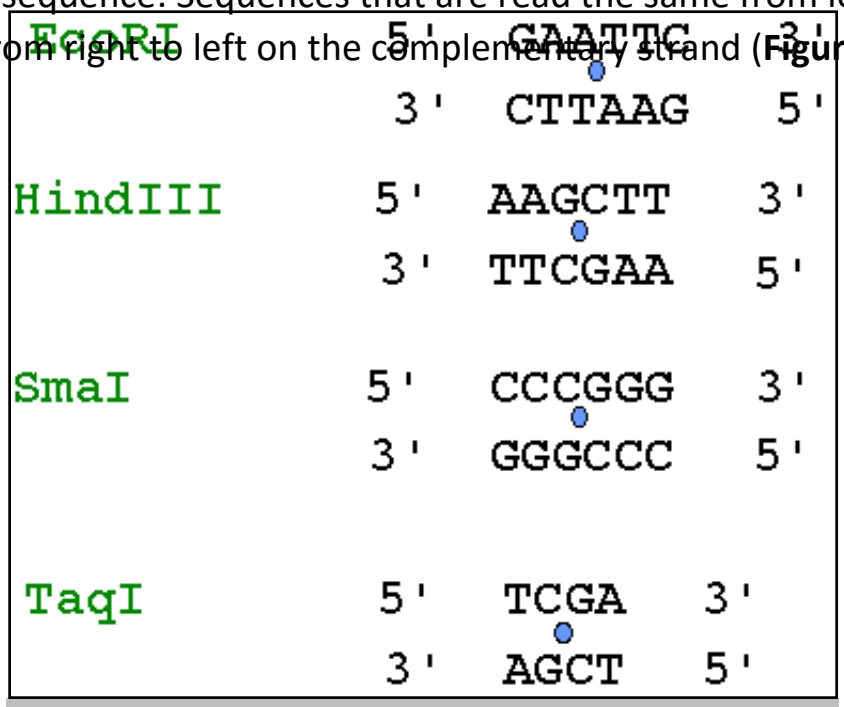

Figure 4: Palindromic sequences

Advantage of restriction endonucleases:

They are used in:

- Restriction fragment length polymorphism (RFLP).
- Cloning.

Before talking about RFLP, we will talk briefly about **DNA polymorphisms:**

DNA polymorphisms means different shapes of DNA.

As you know, in our body we have two copies of chromosomes. One copy comes from the father and the other one comes from the mother. They are almost exactly the same but there are some little differences (so they are not identical).

So for every gene (ex. Eye color gene) you have two copies, these copies are called alleles.

Alleles can be Homozygous if they are exactly the same or Heterozygous if they are different.

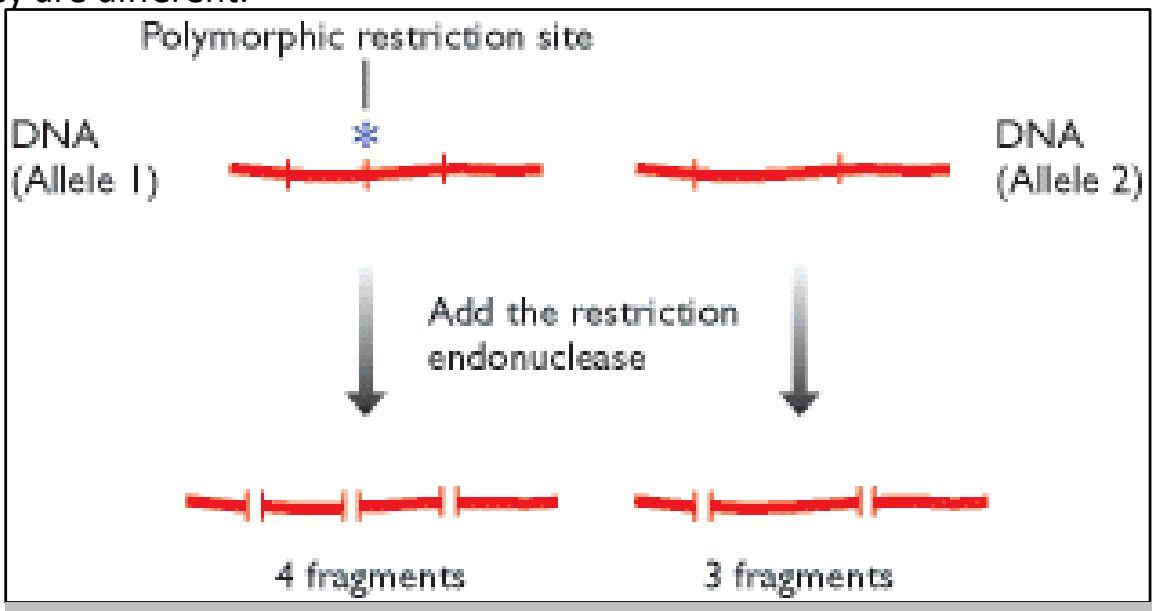

Figure 5

See the example in **Figure 5**, we have two DNA fragments that are exactly the same except in one location. They can be cut by a restriction endonuclease. The first DNA fragment has 3 restriction sites while the other one has 2 restriction sites. So after adding the endonuclease, the first fragment will result in 4 restriction fragments while the second one will result in 3.

Restriction fragment length polymorphism:

The presence of different DNA forms in individuals generates **differences in the length of the restriction fragments** (this is called RFLP).

We can use RFLP followed by Gel electrophoresis or Southern blotting.

Example:

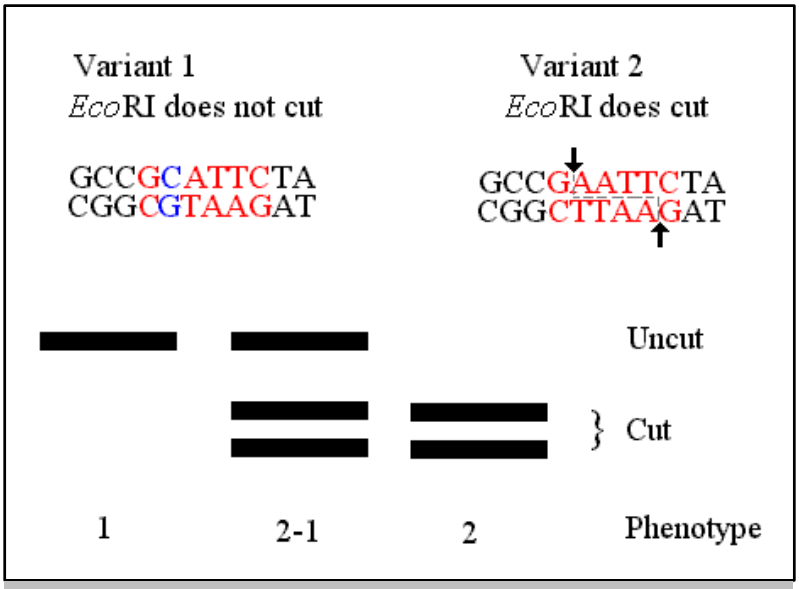

Figure 6

We have two DNA fragments (1 and 2 in the figure above).

Fragment 1 has G**C**ATTC sequence which can't be cut by EcoR1.

Fragment 2 has GAATTC sequence which can be cut by EcoR1.

Case 1: Let's say that there is a person that has two copies of allele that has fragment 1 (variant 1 in the figure above). So the fragments won't be cut and the test will result in long fragments. And if then we used gel electrophoresis they will move slowly.

Case 2: Let's say that there is a person that has two copies of allele that has fragment 2 (variant 2 in the figure above). So both fragments will be cut and the test will result in two shorter fragments (for each allele). And if then we used gel electrophoresis they will move fast.

Note that in both case 1 and case 2 the two person are homozygous.

Case 3: Let's say that there is a person that has one allele that has fragment 1 and the second allele has fragment 2. This form is heterozygous and this will generate a long fragment from the uncut allele and two shorter fragments from the second allele that has been cut.

So we can know if the persons is homozygous or heterozygous for a specific gene using RFLP.