

FINAL – Lecture 2

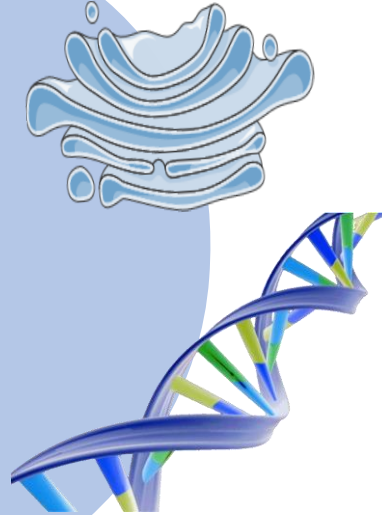
# Overview & Basic Techniques (Pt.2)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبَدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَلَكُمْ ﴾

اللهم استعملنا ولا تستبدلنا

Written by :

- Muthanna Khalil
- Ahmad Yousef



Reviewed by :

- Basil Alakhras

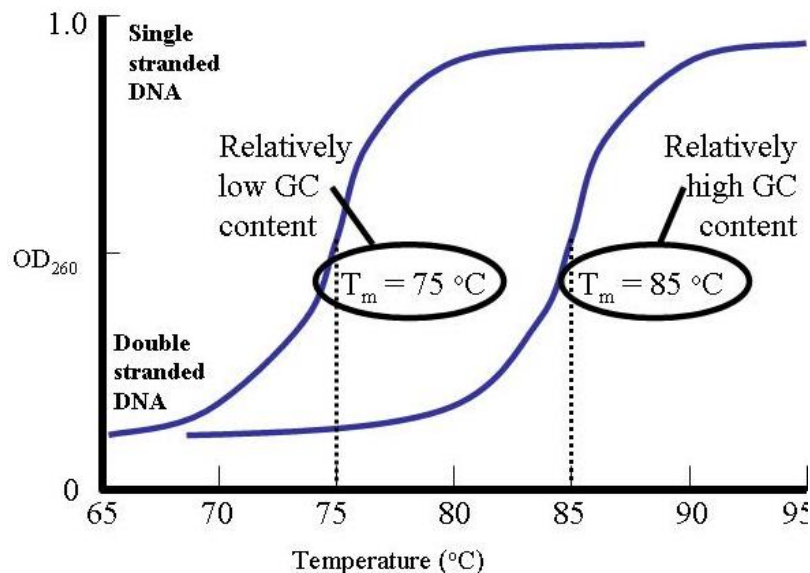
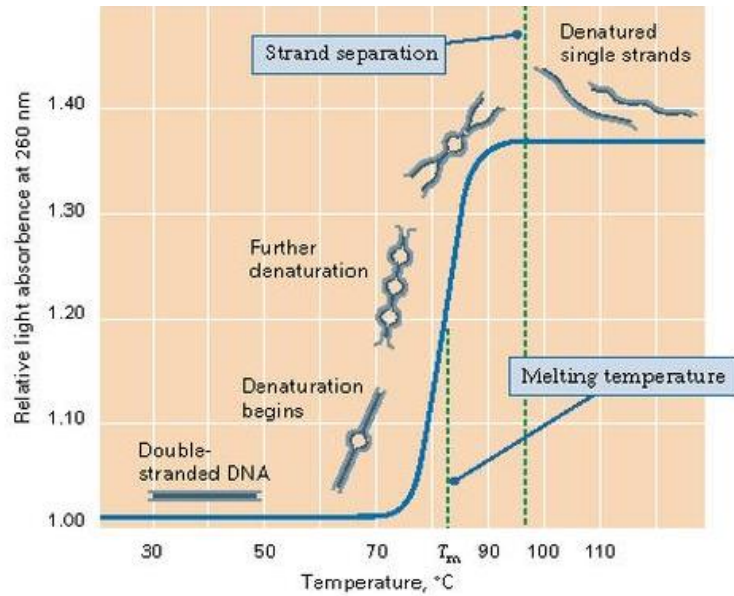


The professor started with a quick review of the last lecture, but we assume that you have already studied it, so you can safely check yourself [here](#).

صَلُّوا عَلَى الْحَبِيبِ

# Observation of denaturation

- **Denaturation:** loss of the native 3-dimensional conformation due to the breaking of noncovalent interactions within a DNA/Protein.
- In DNA, noncovalent  $\leftrightarrow$  hydrogen bonds.
- Hydrogen bonds interactions are reversible.
- The 2 strands can revert to the original state if the conditions are returned to normal (discussed later).



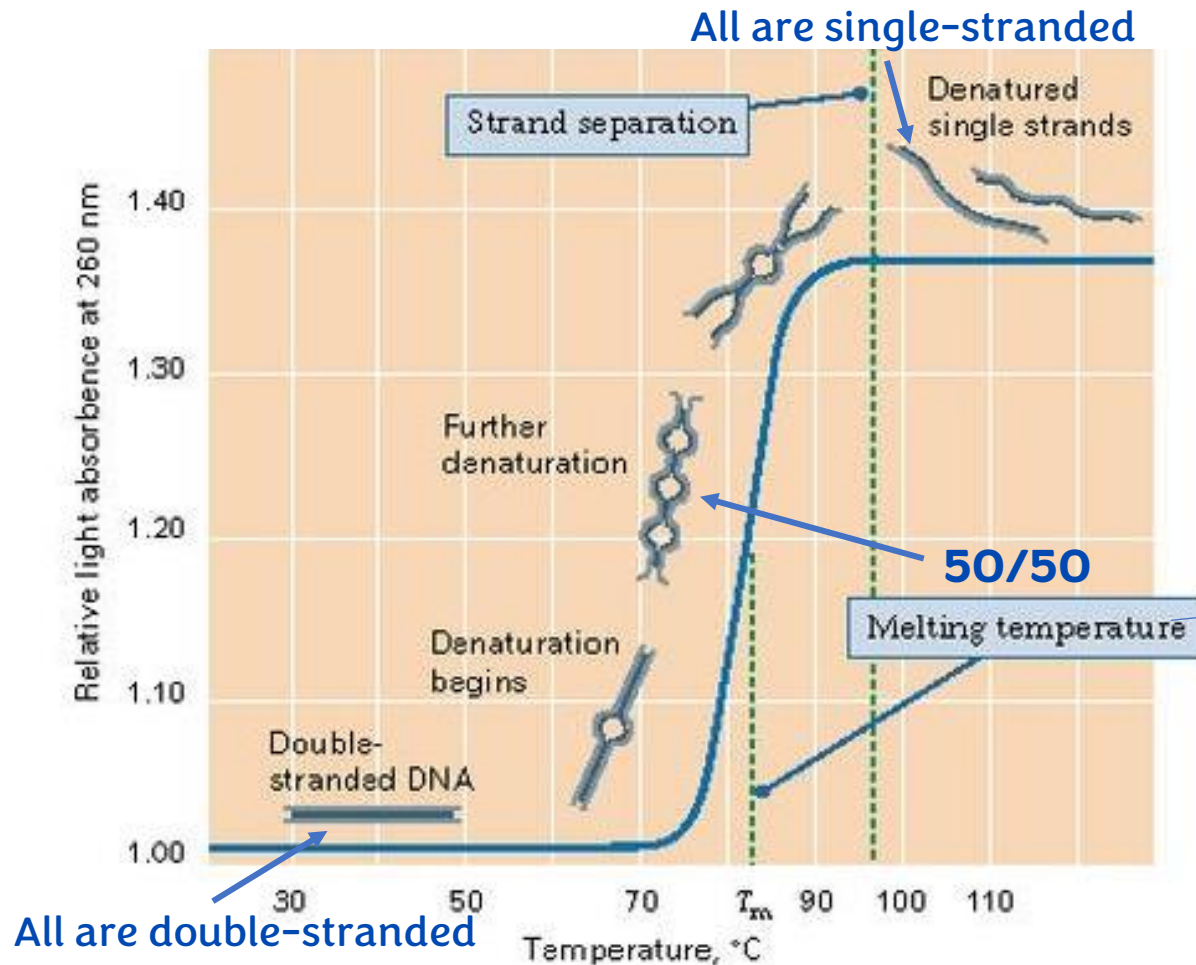
- The transition temperature or melting temperature ( $T_m$ ).

- Factors influencing  $T_m$

- Length
- G·C pairs
  - Hydrogen bonds
- pH **Cations only**
- Salts and ions
- Destabilizing agents (alkaline solutions, formamide, urea)

SEE detailed explanation of the points above on slide 5

# Just like proteins, DNA can be denatured



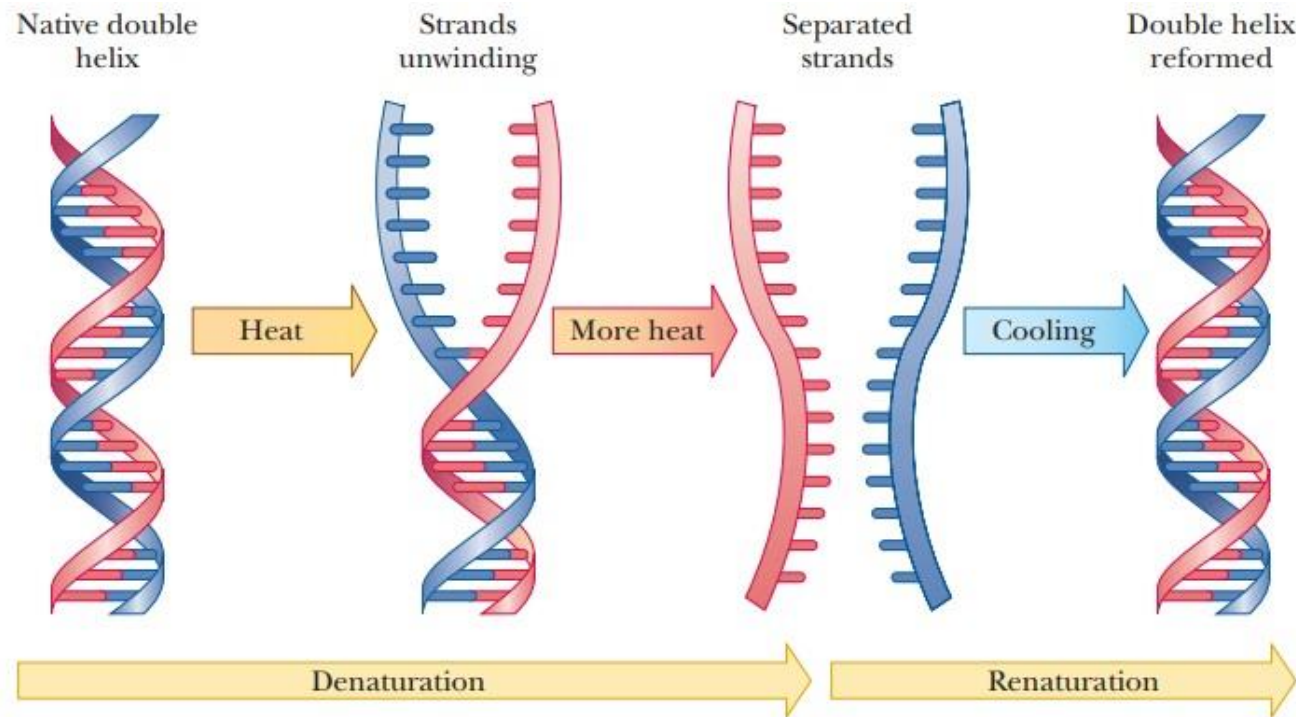
- Heat can disturb the hydrogen bonding between base pairs.
  - For 2 different DNAs, the denaturation occurs at different temperatures depending on strength of interactions of the hydrogen bonds.
  - We usually compare DNAs by their  $T_m$ , which is the temperature where 50% of the DNA molecule is double stranded and 50% has already denatured - is single stranded.

The melting temperature indicates the strength of the connection between the two strands of DNA.

# Factors Influencing Denaturation

- These 3 makes DNA require higher temperature (energy) to denature:
  1. Length
    - More base pairs → more H-bonds → stronger connection → more energy needed.
  2. G-C content
    - 3 (unlike A-T ⇔ 2) H-bonds per bp → stronger connection → more energy needed.
      - Ex: 2 DNAs with same length but one with more G-C content, that DNA will be much strongly connected.
  3. Salty conditions:
    - In DNA, repulsion occurs due to the negative charges of phosphates in the backbone; in salty conditions, cations (such as  $Mg^{2+}$ ) can stabilize the (-) charges, making it stronger, and thus more energy is needed for separation.
- Other conditions cause DNA to denature:
  - Extreme pH (especially alkaline pH); affects the hydrogen bonds.
  - Destabilizing agents (formamide, urea) they eliminate hydrogen bonds.

# Denaturation versus Renaturation



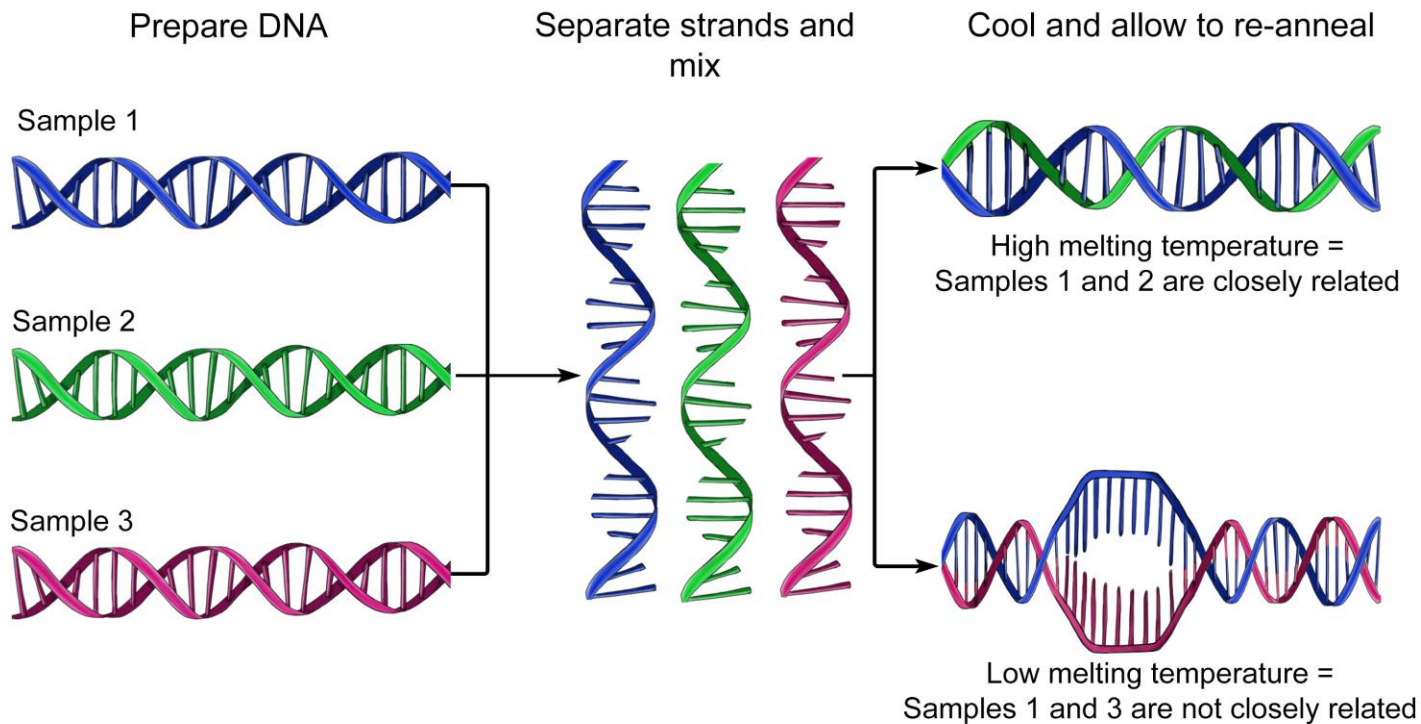
■ **FIGURE 9.19 Helix unwinding in DNA denaturation.** The double helix unwinds when DNA is denatured, with eventual separation of the strands. The double helix is re-formed on renaturation with slow cooling and annealing.

- DNA strands that were separated by denaturation can join forming a double-stranded helix again.
- This is possible because both strands are **complementary** and would favor the “renaturation”.
- For renaturation to occur, the denaturing factor should be removed.

Raise the temp. → Breaking of hydrogen bonds (denaturation)

Lower the temp. → Reformation of hydrogen bonds (renaturation)

# Denaturation versus Hybridization



- When denaturing agents are removed, any 2 DNA strands can join forming a double-stranded DNA as long as both strands are complementary, regardless of their origin.
- When 2 strands from different origins unite, it is called **“hybridization”**.

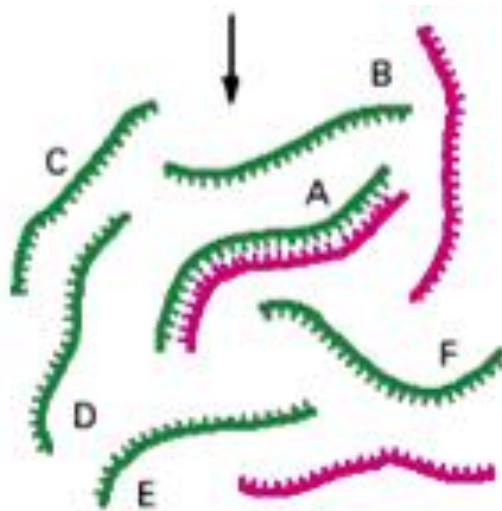
As seen in the lower image, hybridization can occur between complementary segments of DNA strands as long as there are *enough* hydrogen bonds between their complementary bases, even if not all nucleotides are complementary; this is called **“imperfect hybridization”**.

- No exact percentage; it depends on the DNA fragments (G-C content and other factors).
- G-C are stronger than A-T.

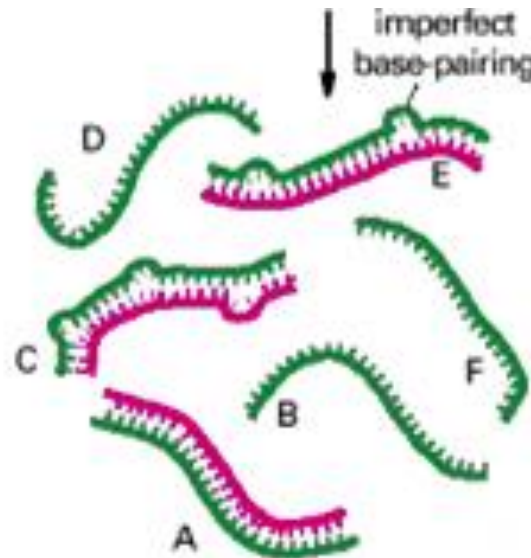
# Hybridization

➤ Regarding the second point below:  
Imperfect hybridization is less stable, but we can promote it by lowering the temperature and making the solution more ionic, stabilizing the DNA.

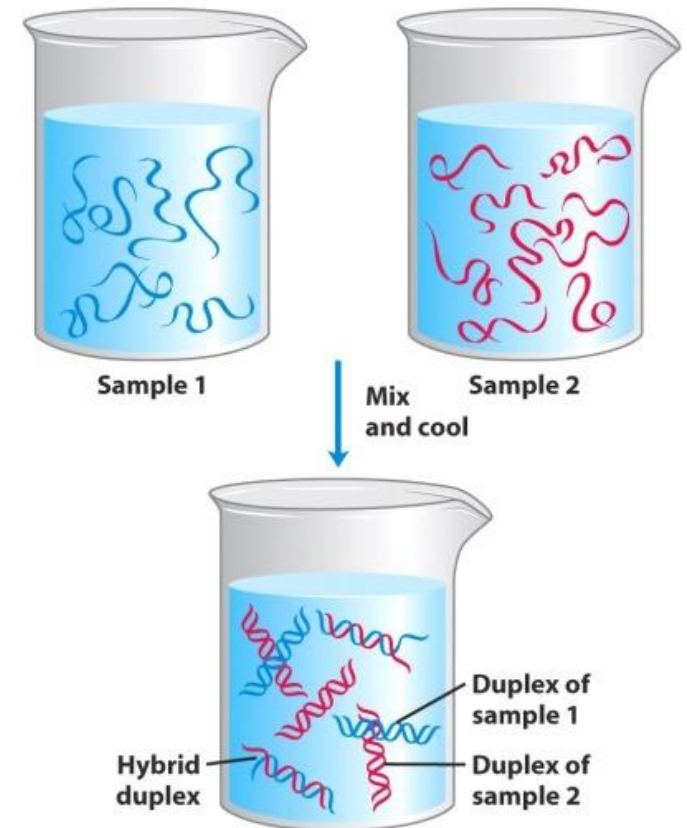
- DNA from different sources can form double helix as long as their sequences are compatible (hybrid DNA).
- Hybridization can be imperfect (when temperature is low, salt concentration is high, etc).



only A forms stable double helix



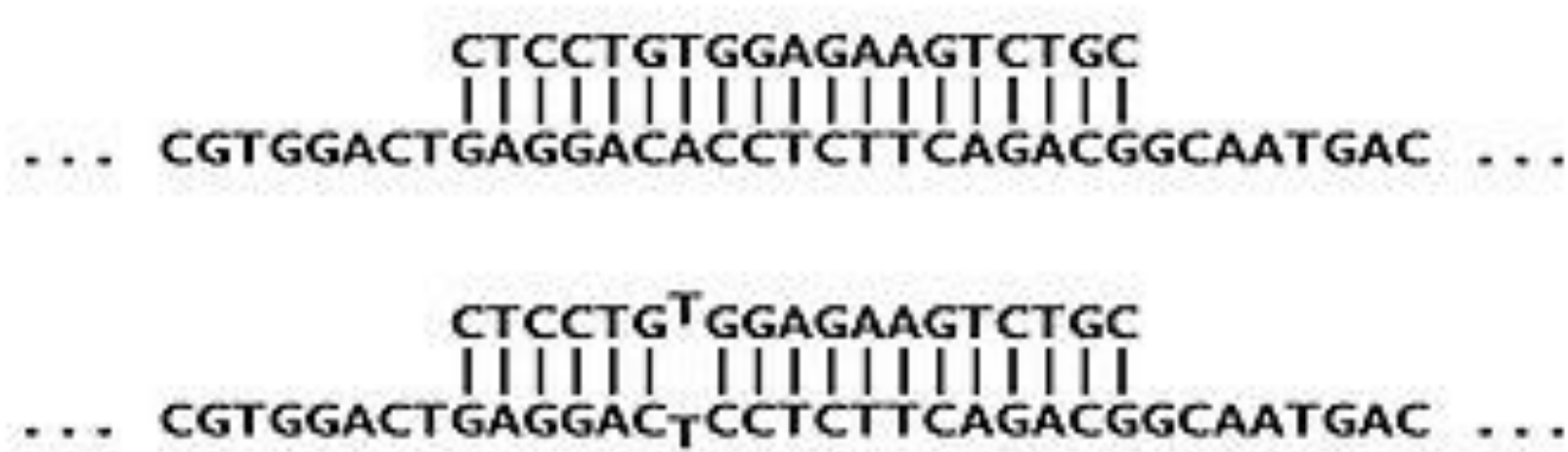
A, C, and E all form stable double helices





# Hybridization can be non-specific

As long as there are *enough* H-bonds between their complementary bases



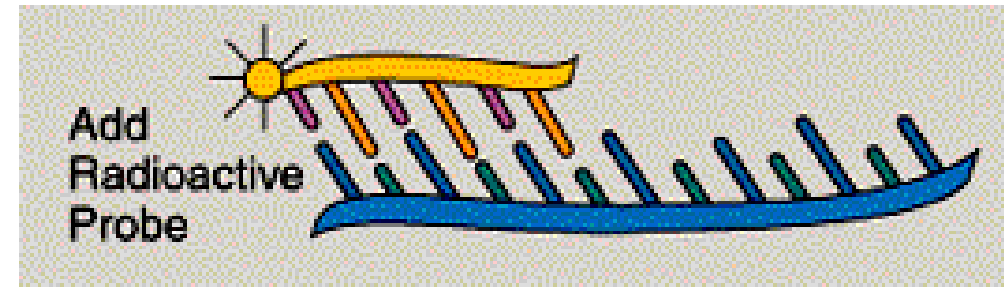
**Hybridization can be controlled by changing the temperature, ionic strength of solutions, GC content, etc.**

# Hybridization techniques

- 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

# Probes (Oligonucleotides)

Oligo → Short sequence of DNA (or RNA)  
DNA is more often because it is more stable



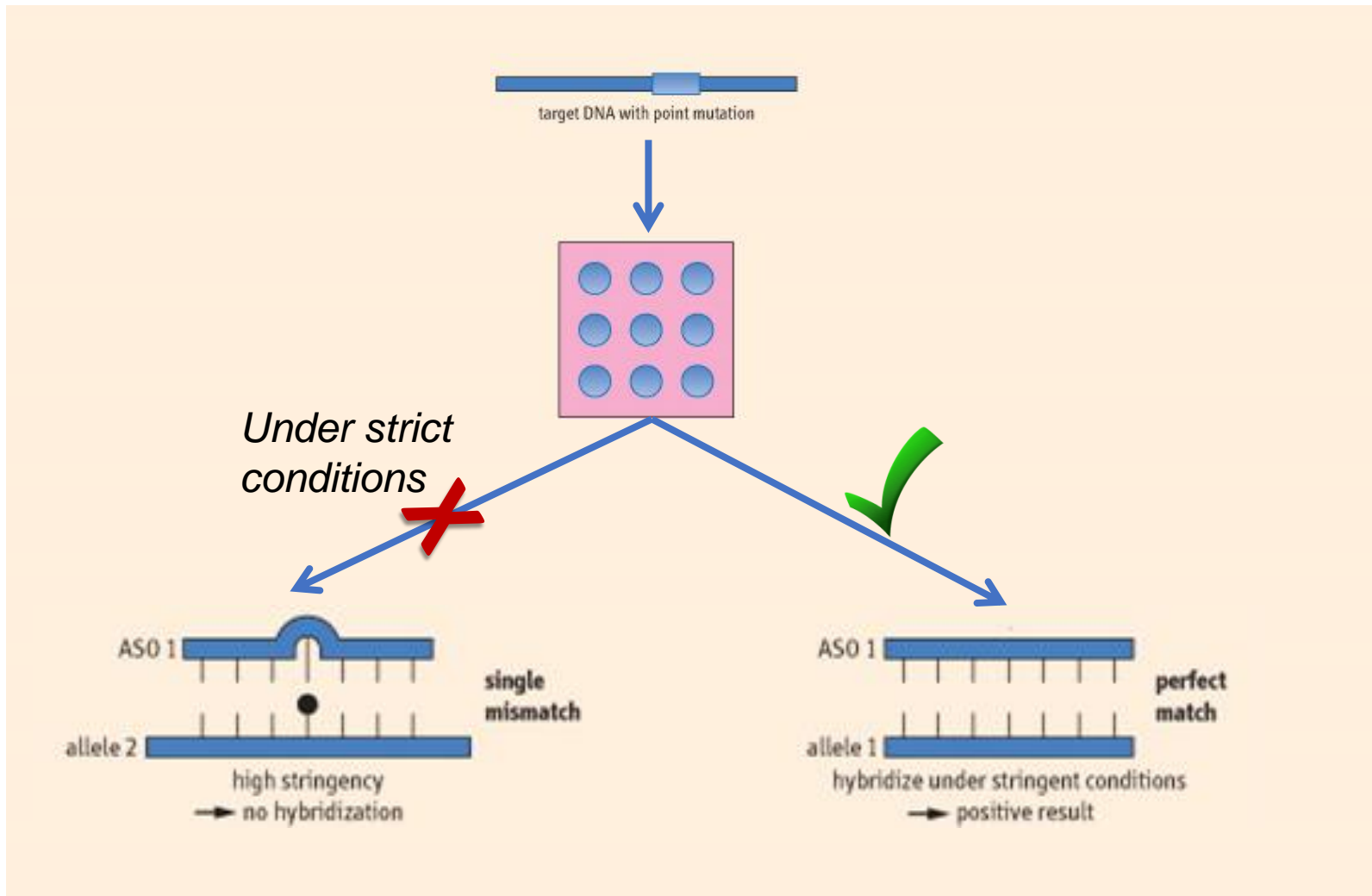
- A probe is a short sequence of single stranded DNA (an oligonucleotide) that is complementary to a small part of a larger DNA sequence.
- Hybridization reactions use labeled DNA probes to detect larger DNA fragments.

This can happen because the probe will pair with the portion of the DNA strand (by binding in an anti-parallel fashion) that is complementary to the probe (even if the probe is RNA, uracil can pair with adenine just like thymine does); a carefully designed probe must be used such that it is complementary to the segment of interest in the DNA.

Labeled meaning the probe itself will emit a signal; when it binds, this signal (radioactive or fluorescent) is detected by a by certain instruments.

# Interesting note

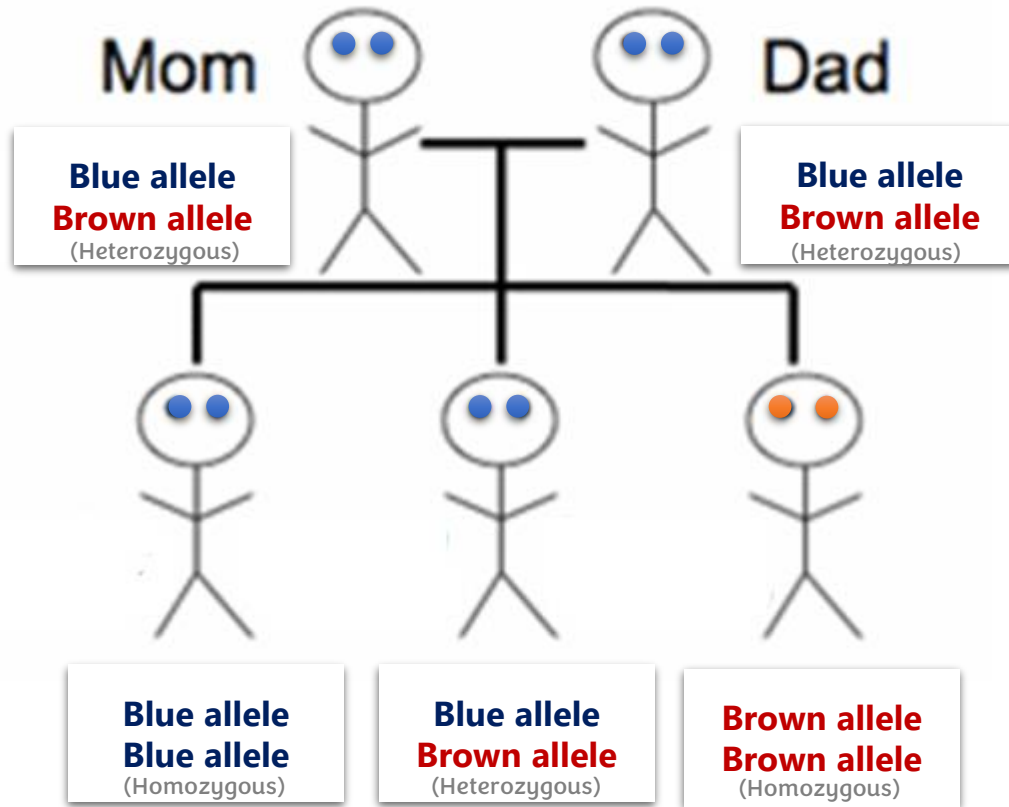
- A combination of a strand of a DNA and a strand of an RNA is possible, IF THEY ARE COMPLEMENTARY FOR EACH OTHER (OBVIOUSLY “U” RATHER THAN “T” FOR RNA)



However, also imperfect probe hybridization can occur (logically it is less common because the short nature of probes mean that they bind with less H-bonds) if enough pairing strength is present to stabilize this interaction.

# Concepts to know...

## Pedigree

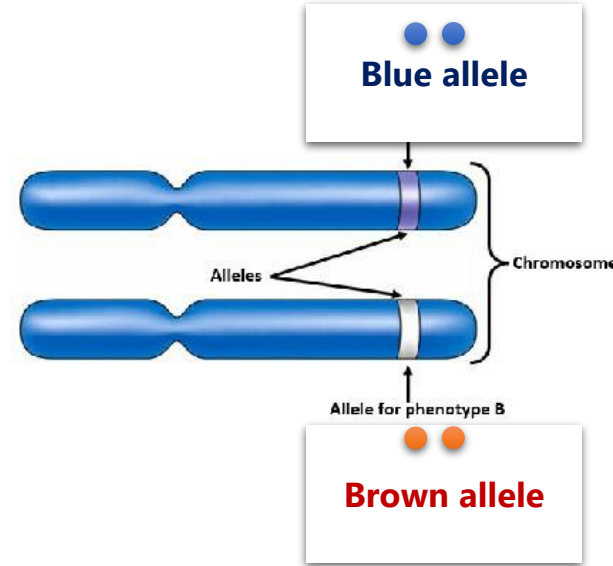


Pedigree (شجرة العائلة للمرض)

Alleles:

Dominant (سائد) vs. Recessive (متنحي)

Homozygous vs. Heterozygous

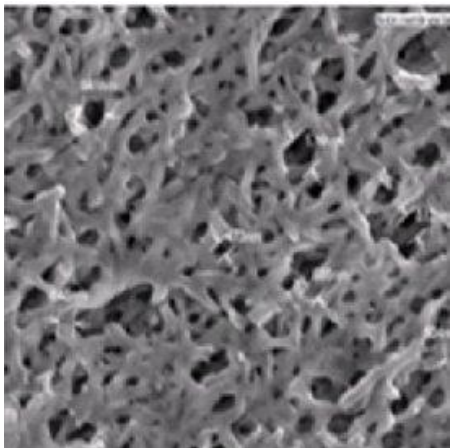
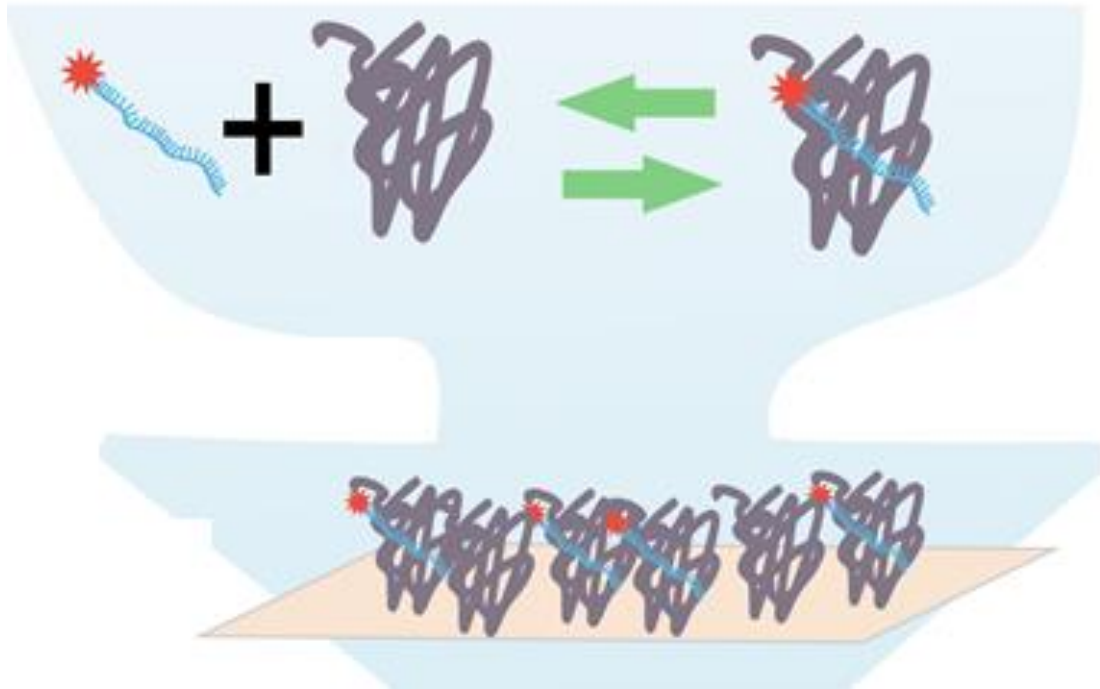


❖ An **allele** is a specific variant or form of a gene

- ❖ All humans have the same genes responsible for eye color (HYPOTHETICAL BTW), but they may carry different **alleles** for these genes (e.g., some people have an allele for blue eyes, while others have an allele for brown eyes). One of the two alleles can be dominant over the other, meaning it determines the visible characteristic, or phenotype, of eye color.
- ❖ Suppose there's a gene for eye color  
The mother has one blue allele on one chromosome and a brown allele on the other chromosome, making her heterozygous for the eye color gene.  
The child with two blue alleles is homozygous for the blue allele for the eye color gene.
- ❖ The child with two brown alleles is homozygous for the brown allele for the eye color gene.

# Dot blot

point stain



- 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.
- ❖ When I take a sample from someone, such as a blood sample, and isolate, purify, or extract the DNA (all these terms mean the same thing), I'm not just isolating a single DNA molecule, instead, I'm isolating DNA from millions of cells, meaning I'm extracting millions of DNA molecules in one sample. For simplicity, however, we represent this with just one DNA molecule.
- ❖ If I add a probe to this DNA molecule and the probe is complementary to any part of the DNA, it will bind to it, hybridize to it. This causes that specific part of the DNA to light up, not the entire molecule—only the section where the probe is bound.
- ❖ Think of it like a person wearing a hat with a light on it. In a dark room, you see the light moving, but not the person directly. Yet, you know the person is there because of the lighted hat. Similarly, when a probe binds to a DNA molecule, it illuminates that specific part, creating a detectable signal.

## More explanation on the previous slide:

- ❖ We take a membrane and add DNA to it – millions of DNA molecules. The DNA attaches to the membrane. Next, we add a probe, and anything that isn't bound is washed away. If the probe binds to the DNA (hybridizes with it), that specific part will light up, indicating the presence of the probe. This tells me that a sequence complementary to the probe exists within the DNA.
- ❖ From this, I don't know the entire DNA sequence. I only know that there is a specific nucleotide sequence in the DNA that is complementary to the probe. For my purposes, that's sufficient.



# Disease detection by ASO (Cystic fibrosis)

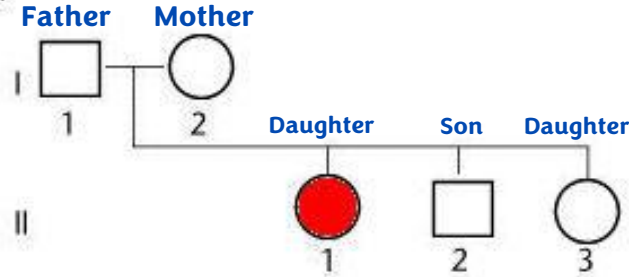
## ASO: Allele-specific oligonucleotide

- ❖ Female → Circle
- ❖ Males → squares
- ❖ لأنه الزلام راسهم مربع  
Dr. Mamoun 😊

Cystic Fibrosis allele  $\Delta 508$  has 3bp deletion [AGA]

ASO for normal DNA 5' CACCAAAGATGATATTTTC-3'

ASO for DNA sequence of  $\Delta 508$  mutation 5' CACCAATGATATTTTC-3'

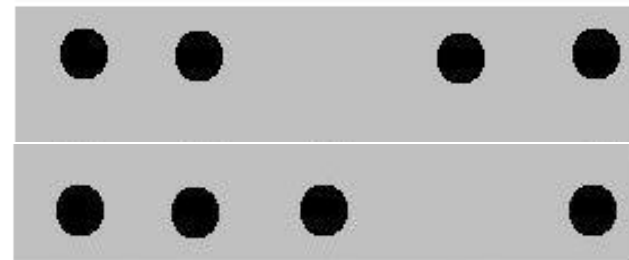


Detects normal allele

Normal ASO

Detects the mutated allele

$\Delta 508$  ASO



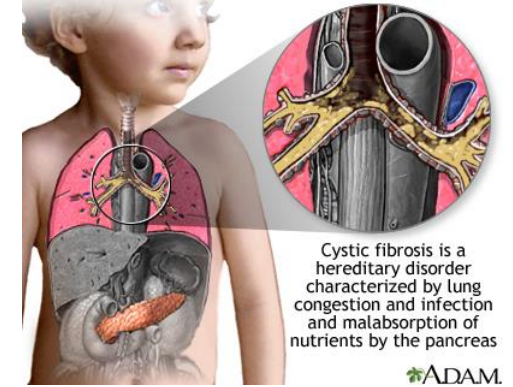
Both tests are probes

Heterozygous | Heterozygous | CF | Heterozygous  
Heterozygous | Homozygous normal

The 5 columns above each represents 1 of the 5 members of this family

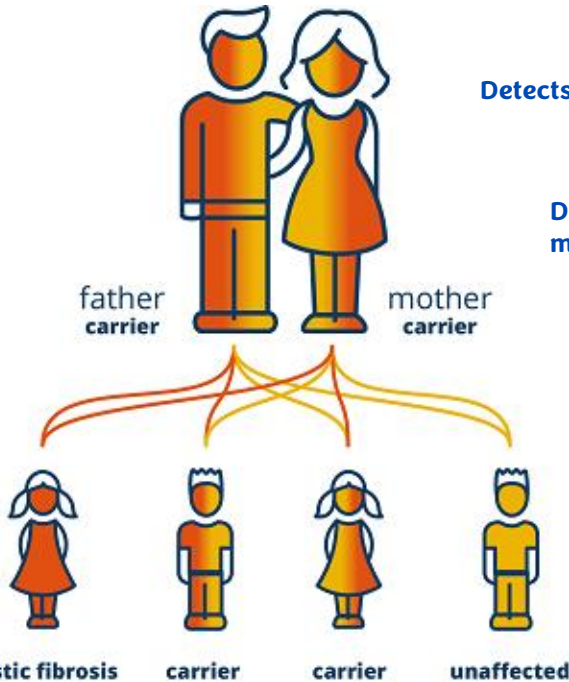
- If a member has both tests positive → they are heterozygous (carriers of cystic fibrosis)
- If a member has only one positive → they are homozygous (they either have cystic fibrosis or completely free of the mutated allele)

The whole genomic DNA is spotted on a solid support (a membrane) and hybridized with two ASO's, one at a time.



ADAM.

- ❖ Diseased child from non-carrier (and not diseased of course) parents may mean the child is adopted.



# More explanation on the previous slide:

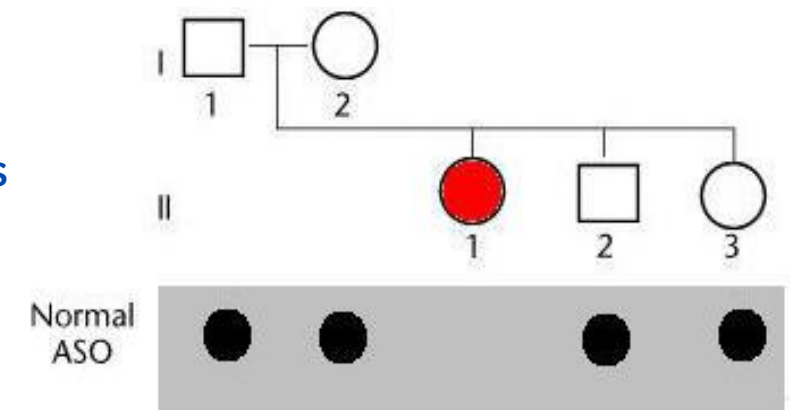
- ❖ Certain diseases can be detected, such as cystic fibrosis, using a dot blot technique. Cystic fibrosis is a genetic disorder that affects the lungs, making it difficult for affected individuals to breathe. The disease is primarily caused by a mutation, and to develop cystic fibrosis, the mutated gene must be on both chromosomes (homozygous for the mutated gene).
- ❖ In cystic fibrosis, the mutation is usually a deletion mutation. Normally, a person would have a specific DNA sequence. However, in individuals with cystic fibrosis, part of this sequence (A, G, A) is missing from both chromosomes. This deletion results in the individual being homozygous for the mutated allele. If a person has two normal alleles (no deletion), they are homozygous for the normal allele. If someone has one normal allele and one mutated allele, they are heterozygous.
- ❖ To detect this, two probes are used: one that binds to the normal allele and another that binds to the mutated allele (the allele with the deletion).
- ❖ We then take DNA samples from each family member. In the diagram, squares represent males, and circles represent females. The family members are arranged to show their relationships. We spot each individual's DNA on a membrane, labeling spots for the mother, father, and each child. Finally, we add the first probe that detects the normal allele.
- ❖ A similar approach is used for the mutated allele (using a different probe).

Cystic Fibrosis allele  $\Delta 508$  has 3bp deletion [AGA]

ASO for normal DNA 5' CACCAA[AGA]GATATTTTC-3'

ASO for DNA sequence of  $\Delta 508$  mutation 5' CACCAATGATATTTTC-3'

Some might ask whether the probe for the mutated allele could accidentally bind to the normal allele due to partial sequence similarity; To prevent this, we adjust factors like salt concentration and temperature to ensure that only perfect hybridization occurs.



# Resources

- <http://www.sumanasinc.com/webcontent/animations/content/gelelectrophoresis.html>
- Watch this....very important

# For any feedback, scan the code or click on it.



Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	2	Quiz not working	Link Fixed
V1 → V2			

# Additional Resources:

# رسالة من الفريق العلمي:

## Reference Used:

I mean come on, Dr. Mamoun MOLECULAR BIOLOGY and you are searching for something else!?

Consider watching the animations the Dr shared with us on TEAMS.

يقول النبي ﷺ: "ثلاث من كن فيه وجد بهن حلاوة الإيمان: أن يكون الله ورسوله أحب إليه مما سواهما، وأن يحب المرء لا يحبه إلا الله، وأن يكره أن يعود في الكفر بعد إذ أنقذه الله"

اللهم اهدنا بهُداك واجعل عملنا في رضاك  
أخلصوا النية في تعلمكم  
ولا تنسوننا من صالح الدعاء