MOLECULAR BIOLOGY

بسم الله الرحمن الرحيم

FINAL – Lecture 1

Overview And Basic Techniques (pt.1)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

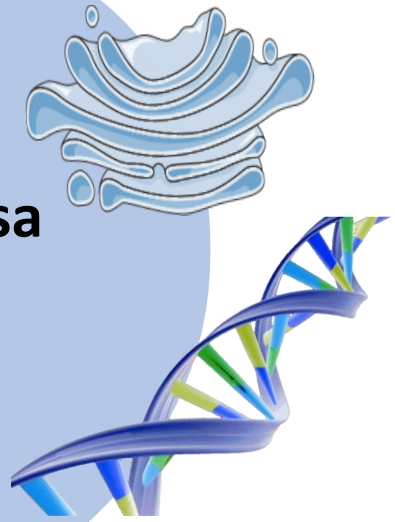
اللهم استعملنا ولا تستبدلنا

Written by :

- Mahmoud Aljunaidi
- Abd AL Rahman Musa

Reviewed by :

- Bashar Khraisat



This is a quiz of some basic concepts of Molecular Biology that we previously before, which are essential throughout this course: click on the picture

Molecular Biology (1)

Structure of nucleic acids

Prof. Mamoun Ahram

School of Medicine

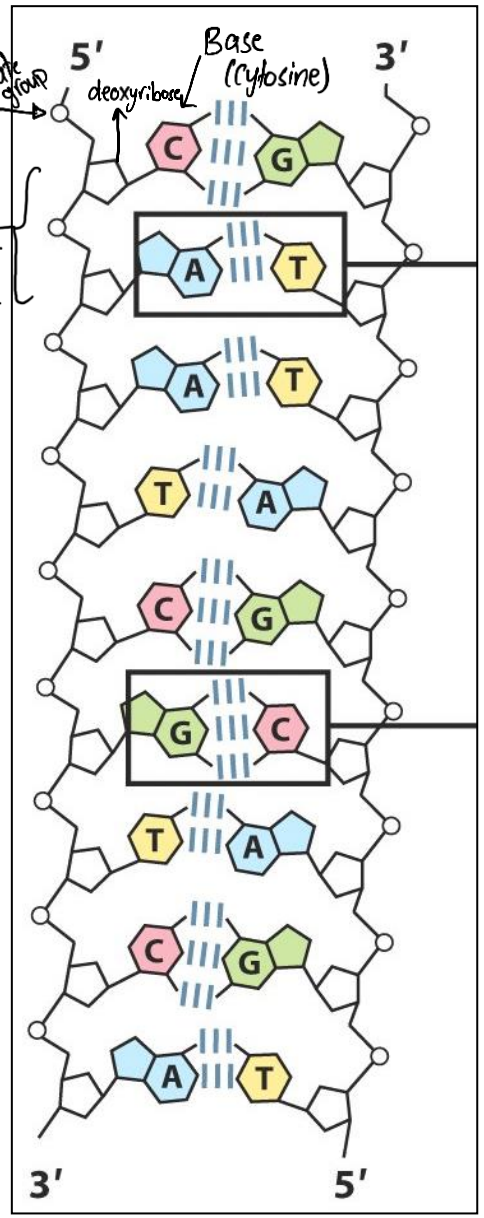
Second year, Second semester, 2024-2025

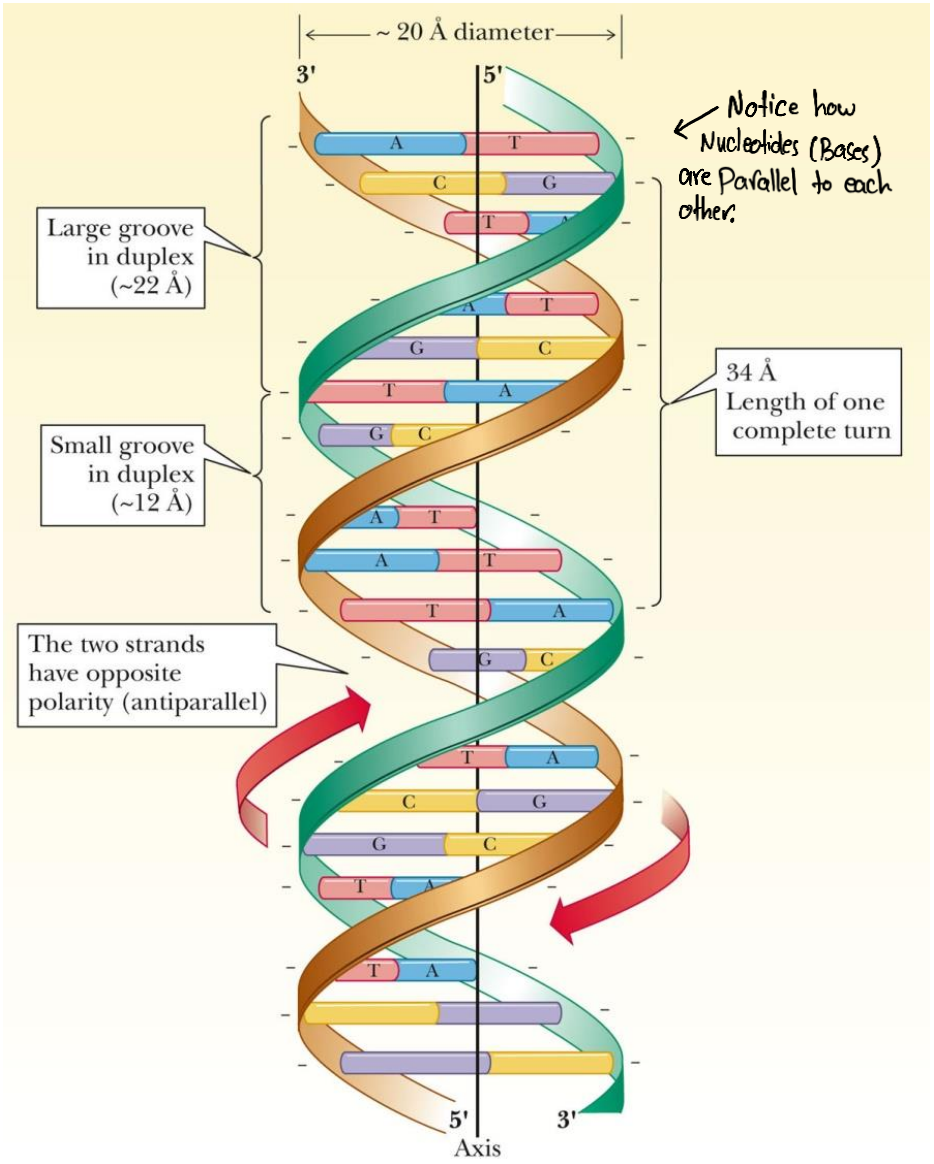
وَتَوَكَّلْ عَلَى الْحَيِّ الَّذِي لَا يَمُوتُ وَسَبِّحْ بِحَمْدِهِ ۚ وَكَفَىٰ بِهِ بِذُنُوبِ عِبَادِهِ خَبِيرًا

General overview

- **This course will cover key aspects of molecular biology:**
- Beginning with the organization of chromosomes and the genome.
- Then shifting to DNA replication.
- Followed by transcription, covering the various types of RNA molecules and their functions.
- Transitioning next to translation.
- Later addressing mutations and repair mechanisms.
- Major techniques utilized in molecular biology will be introduced as they become relevant to the topics discussed.
- Finally, the DNA repair section will include a discussion on the groundbreaking technology of CRISPR-Cas9 and its vast potential in both medicine and science.

DNA structure





- The monomer
- A double helix
- Specific base-pairing
  - A = T; G = C; Pur = pyr
    (Chargaff's rule)
- Complementary
- Backbone vs. side chains
- Antiparallel
- Stability vs. flexibility
- Groovings

Explanation of the previous slide (pt.1):

- DNA is a **polymer** made up of **nucleotides** (monomers).
- Each nucleotide contains one of four nitrogenous bases:
  - **Adenine (A)**
  - **Guanine (G)**

  } Purines, each with a two rings structure.
  - **Cytosine (C)**
  - **Thymine (T)**

  } Pyrimidines, each with a one ring structure.
- DNA strands are held together by:
  - **phosphodiester bonds** in the backbone, between nucleotides in same standard.
  - **hydrogen bonds** between complementary bases: adenine with thymine (two bonds) and guanine with cytosine (three bonds).

Explanation of the previous slide (pt.2):

- DNA: is a double(1) helical(2) (stranded) polymer(3).

(1) Because it is composed of two complementary strands.

(2) Because the two strands are going around each other in a helical pattern.(but it's not really a perfect helix, see next slide for illustration)

(3) Because each strand of DNA is composed of a sequence of nucleotides.

Explanation of the previous slide (pt.3):

❖ The DNA helical structure is not a perfect helix, but why?

- The double helix of DNA contains grooves—Major and Minor—that run along its length.
- Major grooves and Minor grooves **alternate** along the helix, with each Major groove positioned opposite a Minor groove. These grooves serve as essential **binding sites for DNA-binding proteins.**

Explanation of the previous slide (pt.4):

➢ Base pairing(1) is complementary(2).

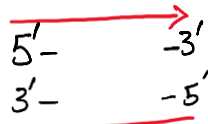
(1) Nitrogenous bases are bound to each others via hydrogen bonds, and the pairing is always between **purines and pyrimidines;** to have an equal diameter of the helix of 20Å (Angstrom).

(2) When two bases are bound to each other by hydrogen bonds they're complementary to each other.

- **A** always pair **T** (by 2 H-Bonds), and **C** always pair **G** (by 3 H-Bonds).
- Knowing that H-bonds provide strength; the DNA strands that have more C and G content are connected in a stronger manner than those strands with more A and T content.

Explanation of the previous slide (pt.5):

- In each strand of DNA there's a **backbone** composed of:
  - **phosphate groups and deoxyribose sugars.**
- And the side chains are: **the bases themselves, perpendicular to the backbone.**
- A single DNA strand has 2 ends:
  - one that starts with a phosphate group (5' end).
  - while the other starts with a sugar with a free carbon no.3 (3' end).
  - DNA is **Antiparallel** (running in the opposite direction); so each strand is in the opposite direction in relation to the complementary strand (5' end of strand 1, faces the 3' end of strand 2).
- DNA is stable yet flexible; the molecule can be bent but not easily broken bond. (Breakage of Phosphodiester bonds).



Writing the sequence of nucleic acids

DNA

5’ ...A T G G C C T G G A C T T C A... 3’
3’ ...T A C C G G A C C T G A A G T... 5’

Shows complementarity and how DNA is Antiparallel.

Here you can see that A pairs with T, and G pairs with C. (Base pairing)

OR

A T G G C C T G G A C T T C A.

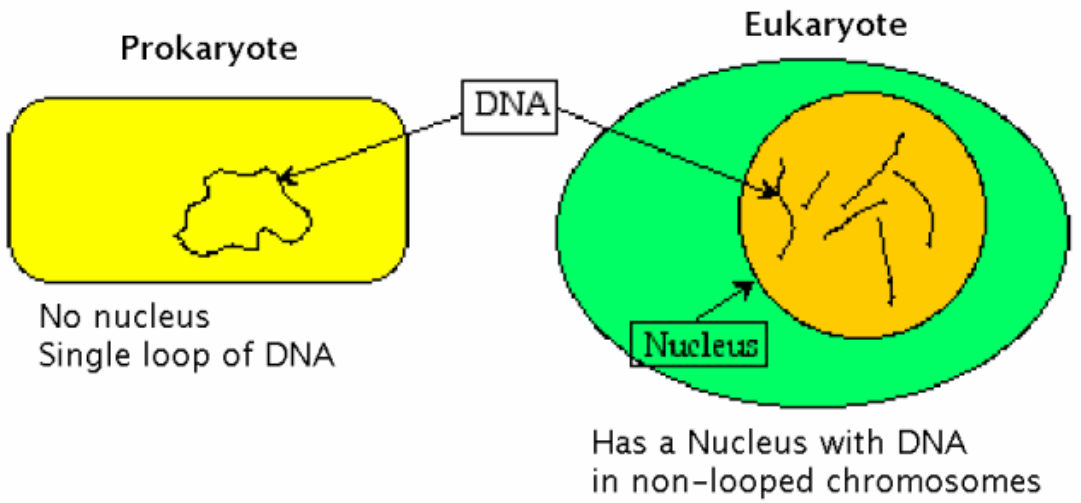
DNA sequence can be written without pointing the ends, but the left end indicated the 5’ end. And knowing that it’s DNA (double stranded) the complementary strand’s sequence can be interpreted from the given sequence.

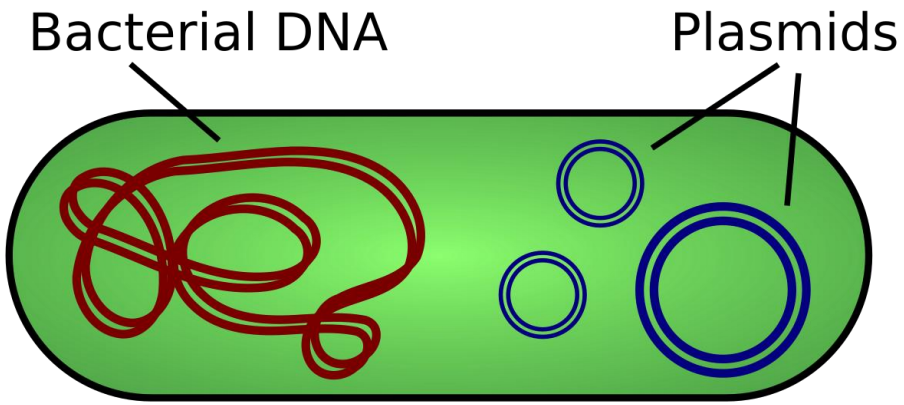
RNA

5’ ...A U G G C C U G G A C U U C A... 3’

RNA is single stranded (with some viruses as an exception), with 4 different monomers (nucleotides)→ (Adenine (A), Guanine (G), Cytosine (C) or Uracil (U)). RNA has U instead of T.

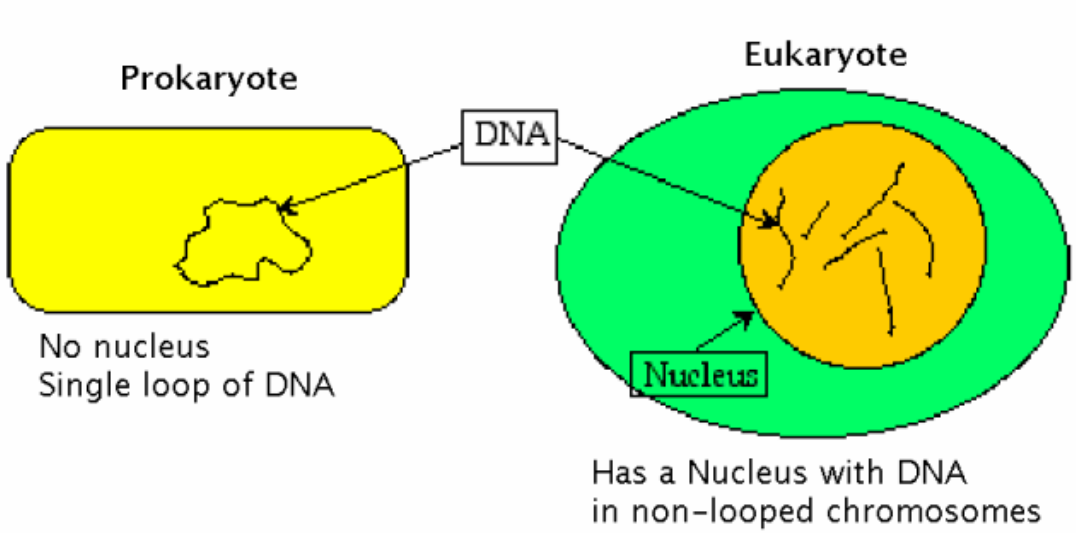
The genome of prokaryotes versus eukaryotes 1

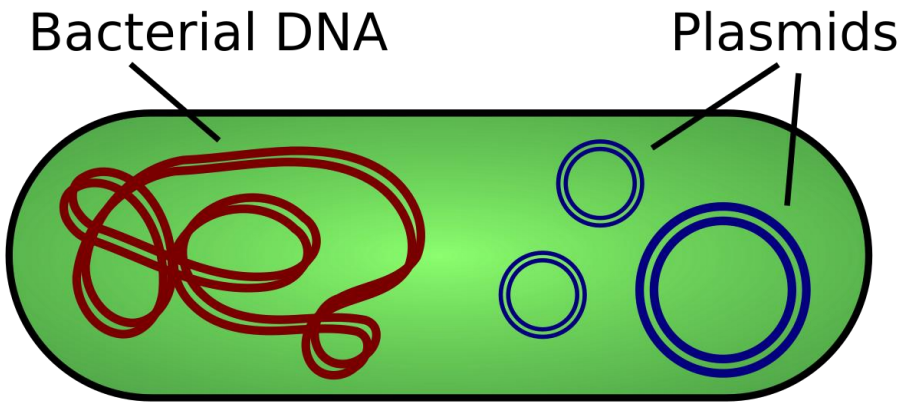




- Genome: the total genetic material of a living being (bacteria vs. human), a species (monkey vs. human) , an individual (me vs. you), or a cell (brain vs. liver), etc.
- **if they are from the same person, they should be identical**.
- Prokaryote: circular genome + plasmid
- Eukaryote: a linear, nuclear genome (chromosomes) + mitochondrial genome

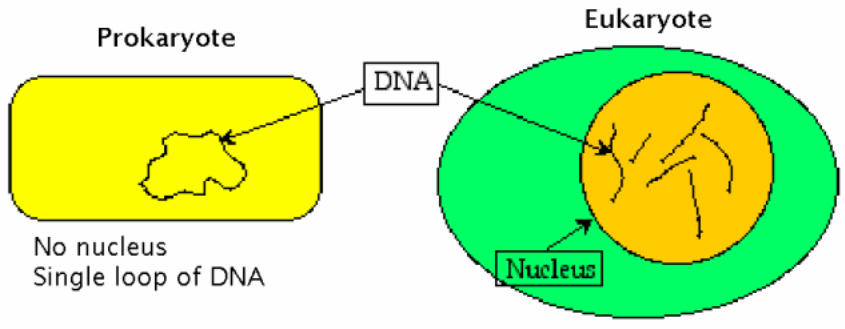
The genome of prokaryotes versus eukaryotes 2





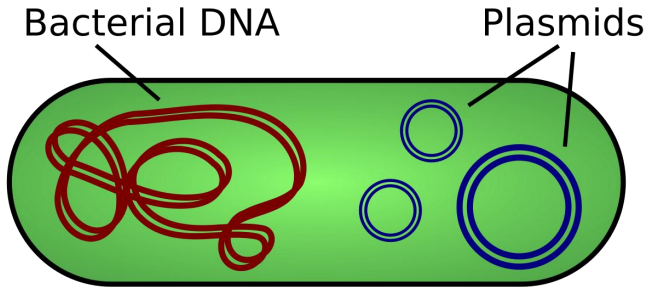
- Remember that each chromosome is made of a long dsDNA packed by Histone proteins.
- Mitochondria have their own genome (mtDNA), which is circular and less stable than the nuclear genome. In eukaryotes there are multiple copies of mitochondria in the cytosol, each of which has multiple (identical) genomes.

The genome of prokaryotes versus eukaryotes **(extra slide)**



Single circular molecule of dsDNA.

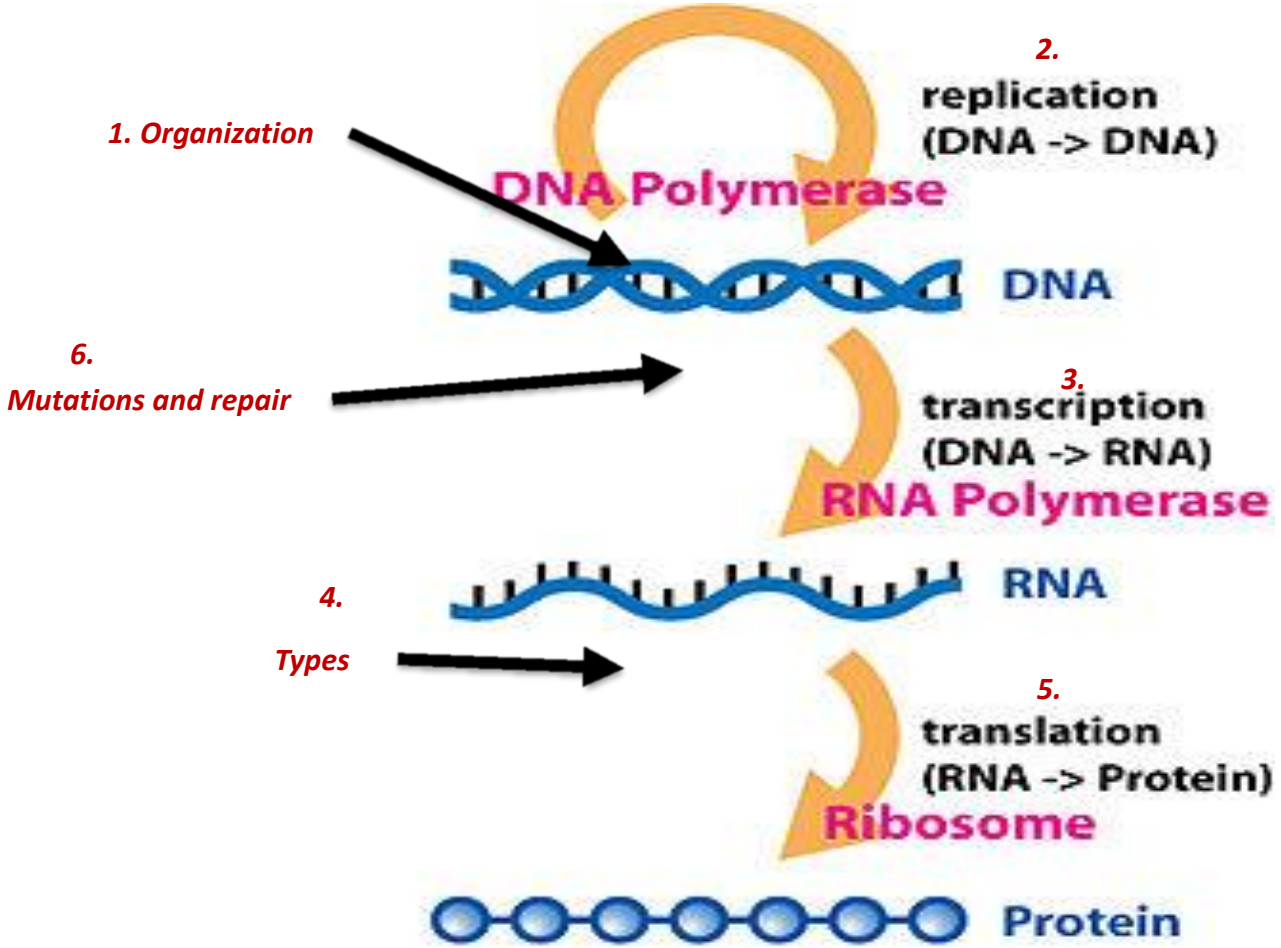
Organized as linear chromosomes enclosed by a nuclear membrane



- In addition to the single ring chromosome, bacteria have a smaller genetic material known as **Plasmid** (circular dsDNA)
- Prokaryotic cells (bacteria) have 1 copy of the ring chromosome but multiple copies of the plasmid.

What is molecular biology?

Central dogma of molecular biology



Techniques

DNA — Makes → DNA — Makes → RNA — Used to make → Proteins

Replication

Transcription

Translation

- **This is called central dogma of molecular biology**, but there are some **exceptions** like:
  RNA makes RNA
  RNA makes DNA

DNA-protein interaction

Remember proteins can interact with DNA in a non-randomized fashion (really specific); as they recognize a specific **DNA sequence** (order of nucleotides) within a strand or within the molecule.

- Nitrogenous bases are sort of hidden inside the helical strands of DNA, except some of them are outside and more susceptible to be identified/seen by proteins, specifically A.As that make up the proteins (precisely the R groups), for example:

* Carboxylic group (-COOH) of Glutamate (Glu).

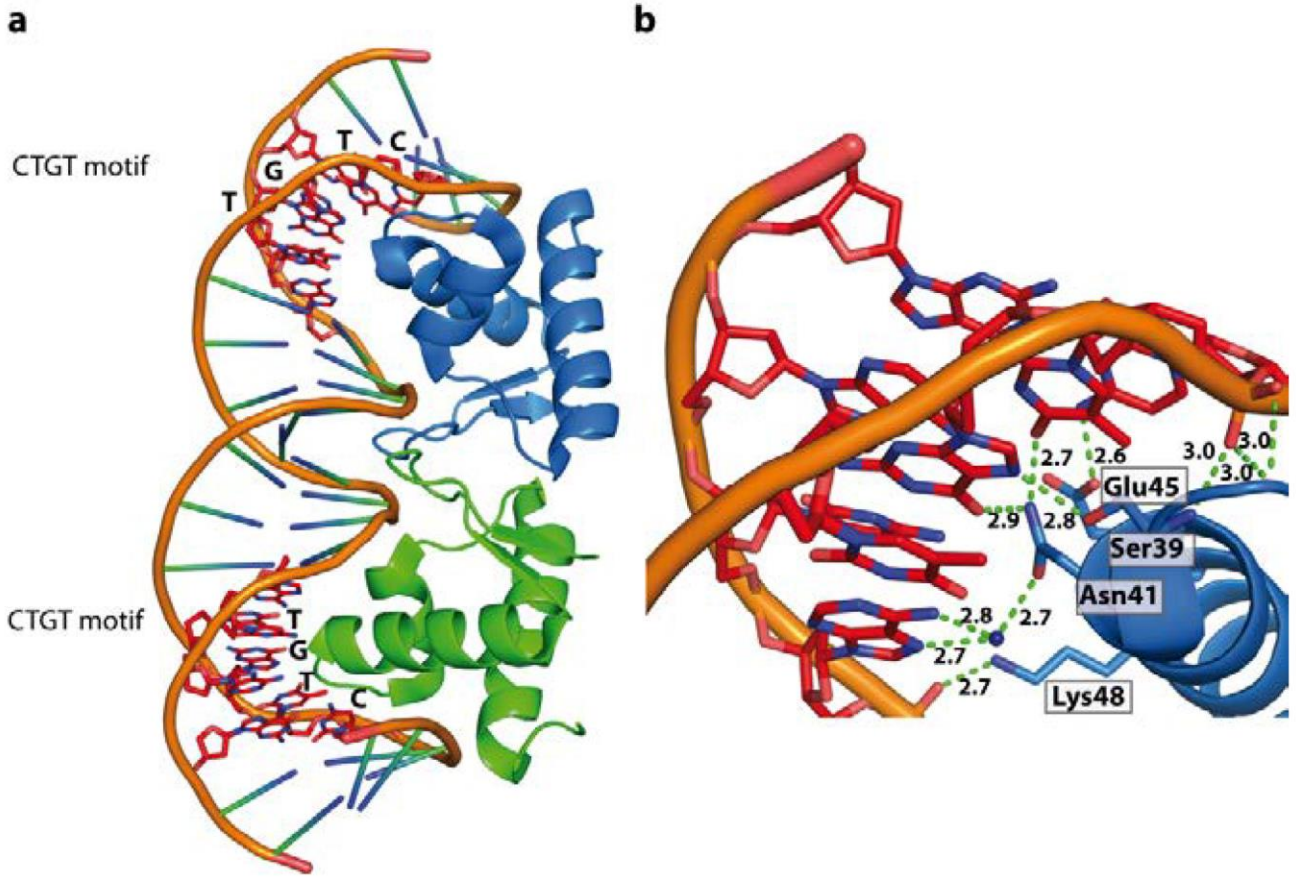
* Hydroxyl group (-OH) of Serine (ser).

* Amino group (-NH3) of Asparagine (Asn) and Lysine (lys).

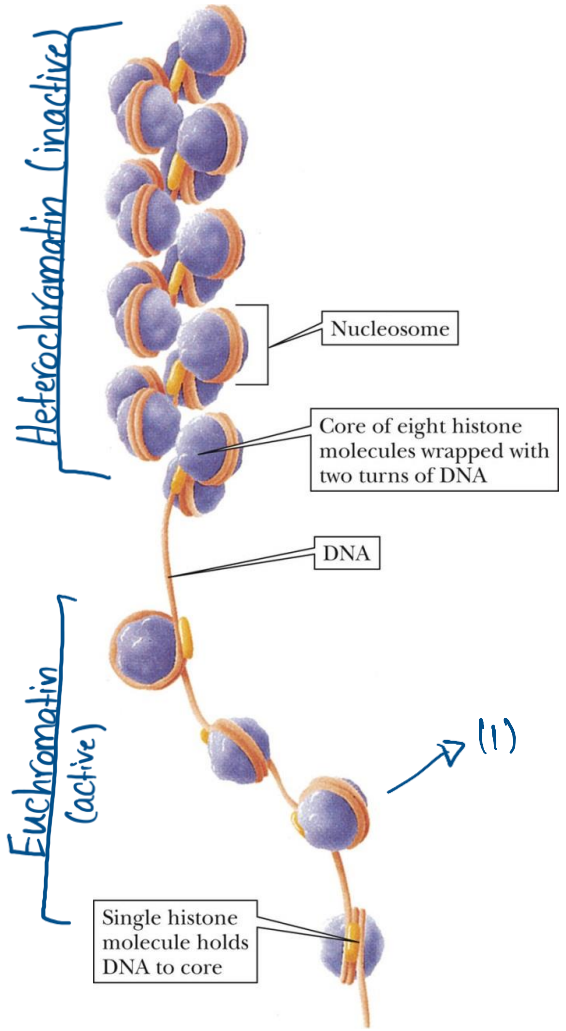
These can interact with the bases of DNA.

-The interactions between the bases and amino acids are **quite specific**.

- The order of the bases and the 3D overall structure of A.As (proteins) can determine the **specificity** of DNA-Protein interactions.
- Further explanation will be provided as we come across DNA-Protein interactions later on.



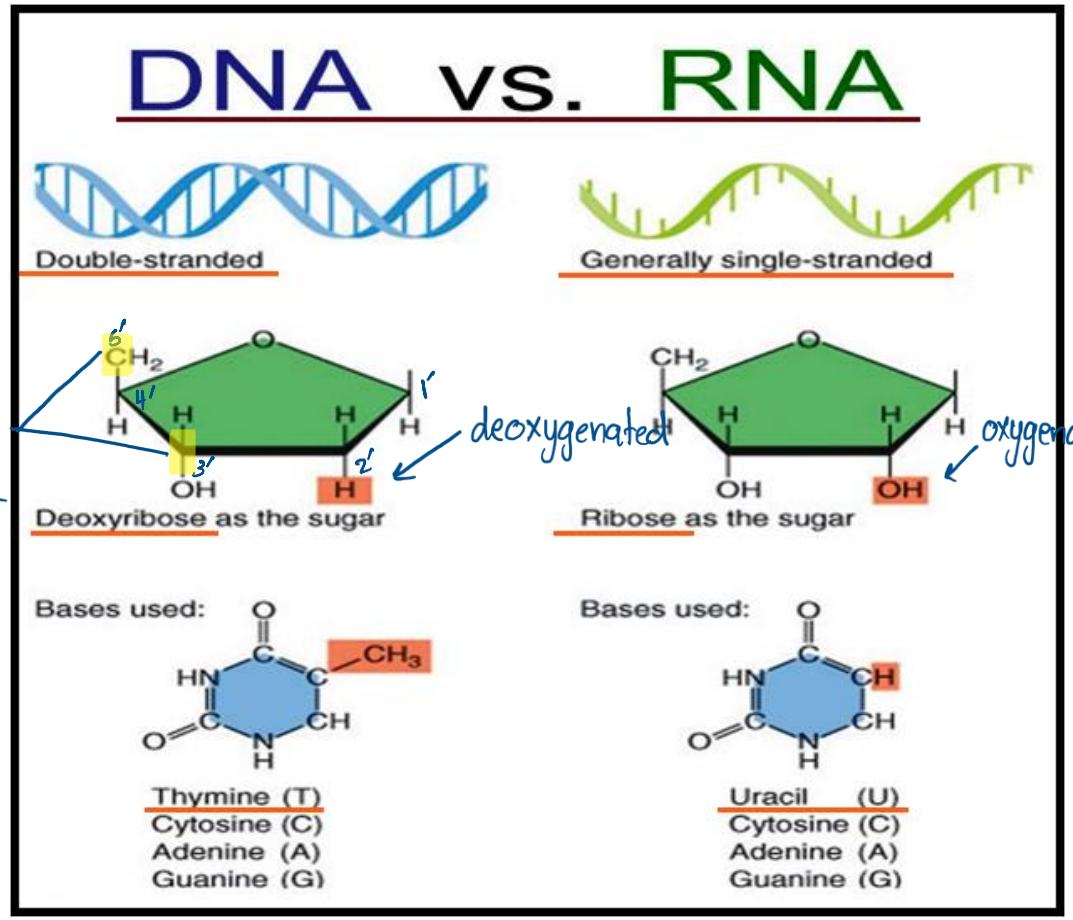
In eukaryotes…



- An exception to specific DNA-Protein interaction is the Histone-DNA interaction.
- Total DNA in eukaryotic cell is really long (~ 2m), and these 2m must fit in a really small nucleus (~10 μm). Total DNA can fit easily because DNA is wrapped around **Histone octamers** (two molecules of histones H2A, H2B, H3, and H4), looking like a string wrapped around beads[(1)], making DNA really packed.

- In eukaryotes, DNA is coiled (around a group of proteins called histones) to package the large, linear DNA.
- Eukaryotic DNA is complexed with a number of proteins, principally histones, which package DNA.
- Chromatin = DNA molecule + proteins (Histones).
- The basic structural unit of chromatin is known as a nucleosome.

In prokaryotes and eukaryotes (not viruses)



These two play a role in phosphodiester bonds formation.

deoxygenated

oxygenated

Take a look at the picture

category \ molecule	DNA	RNA
Strands	Double stranded	Single stranded (Generally)
Sugar	Deoxyribose (hence the name **D**eoxy**r**ibonucleic **A**cid)	Ribose (**R**ibo**n**ucleic **A**cid)
Bases used	Thymine (T) Adenine (A) Cytosine (C) Guanine (G)	Uracil (U) Adenine (A) Cytosine (C) Guanine (G)

Formation of the Phosphodiester bond, takes place between 3' carbon on one sugar and the 5' end of another sugar (of adjacent nucleotides)

Types of RNA

A human cell contain multiple DNA molecules, each one of which forms a distinct chromosomes, serving as templet for RNA synthesis through transcription.

mRNA	tRNA
Used to produce proteins	
large	small
	Has A.A linked to it

Human cells have different types of RNA molecules:

RNA species	Established function(s)
mRNA	Messenger for protein production
tRNA	Translation of RNA codon to amino acid
rRNA	Enzymatic and structural part of ribosomes
snRNA	Pre-mRNA processing
snoRNA	Modification of rRNA
miRNA	Repression of translation
piRNA	Silencing of transposons
lncRNA	Regulation of transcription, pre-mRNA processing, miRNA abundance and protein function

Major ones

micro RNA → miRNA: (regulating protein synthesis)

long non-coding RNA → lncRNA: Regulate … determine … Regulate and control

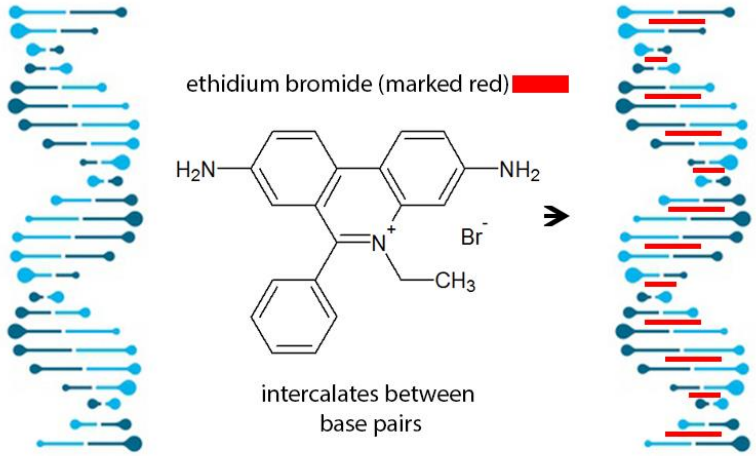
rRNA (i.e. ribosomal RNA) is responsible for forming the peptide bonds between A.As to make proteins, found in ribosomes (factories of protein synthesis).

Techniques

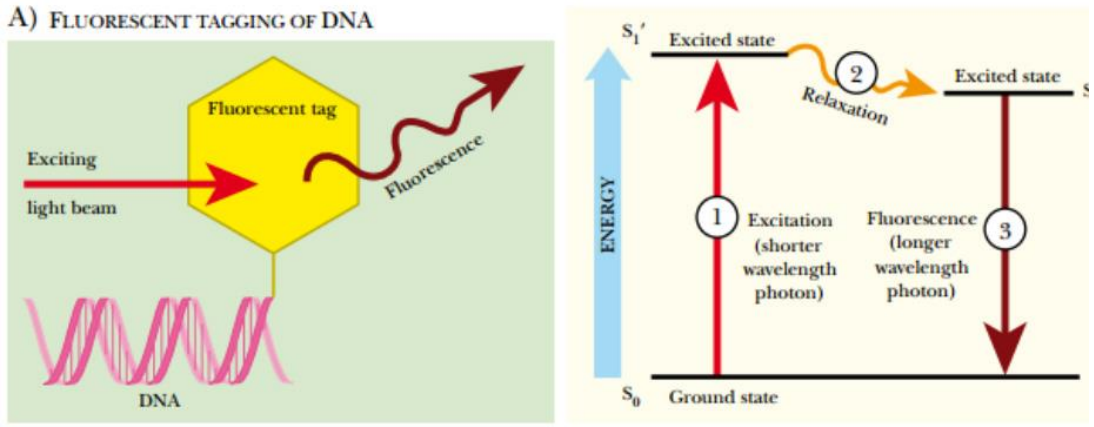
In this lecture will cover one type of techniques.

DNA labeling versus staining

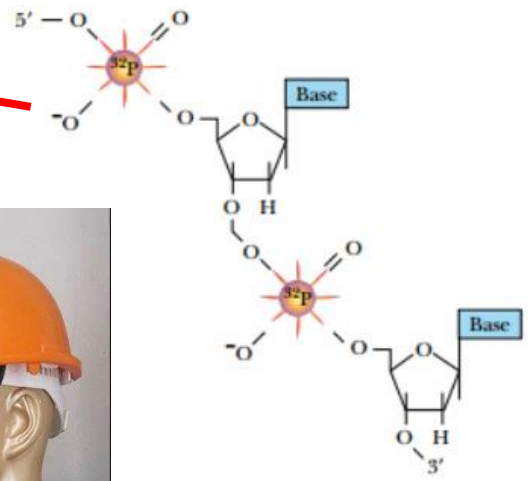
DNA staining



DNA Labeling (more sensitive)



Radioactive phosphorus



الفكرة من الصورة:

The light is emitted from an external slide **not the person itself as in DNA staining.**

Further explanation :

It's essential to understand the distinction between these two terms.

DNA staining :

Similar to staining a shirt with a paint → gives us color that we can see with our own eyes

In DNA, we add chemicals **non covalently** between DNA bases that → give us a certain color.

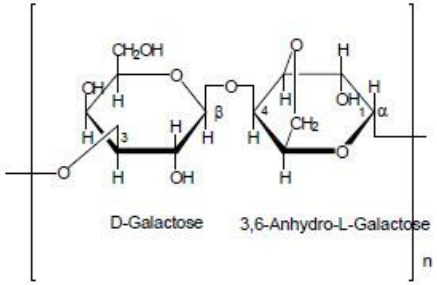
- The chemical which is attached to the DNA is responsible for the color production (not the DNA itself)

DNA labeling :

More sensitive
Here the DNA itself emits the color (fluorescent signal), which can also be radio active .

We can attach **covalently** a certain dye(molecule)→ that emits energy in the form of light with certain wavelength. when it is hit by a light with a certain wavelength (different color - different wavelength from the one emitted).
The DNA itself gives the color.

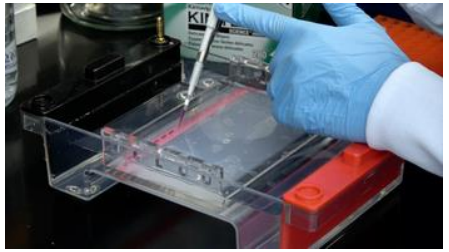
Gel electrophoresis

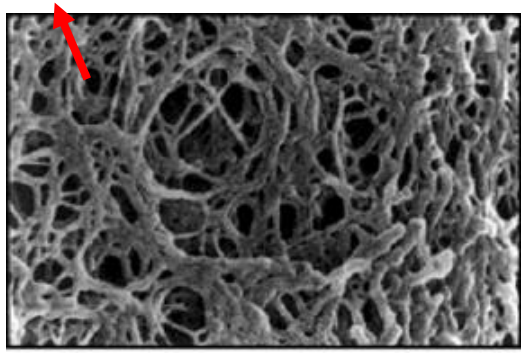


We form a gel with a certain 3d structure and gets molded

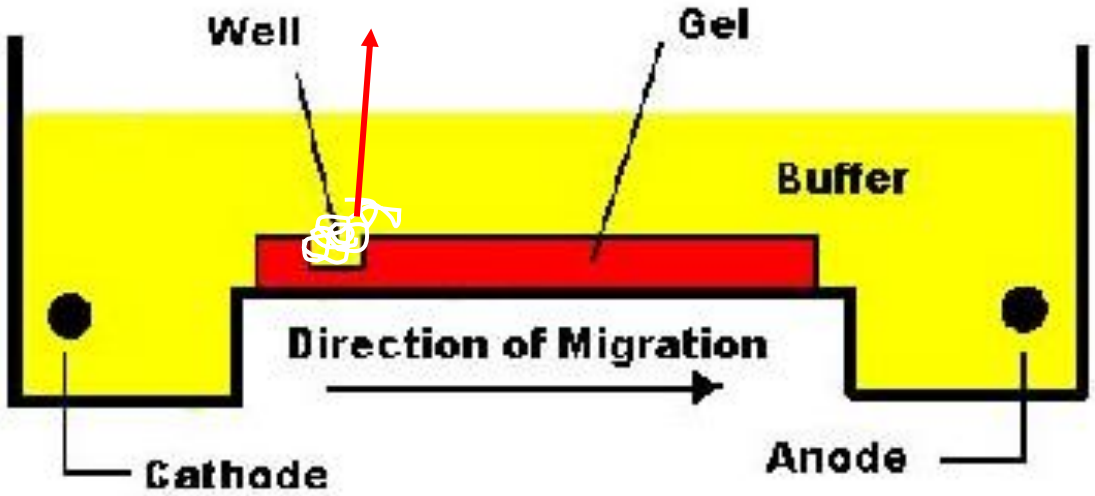
- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.

There are networks of chemical and spaces in between

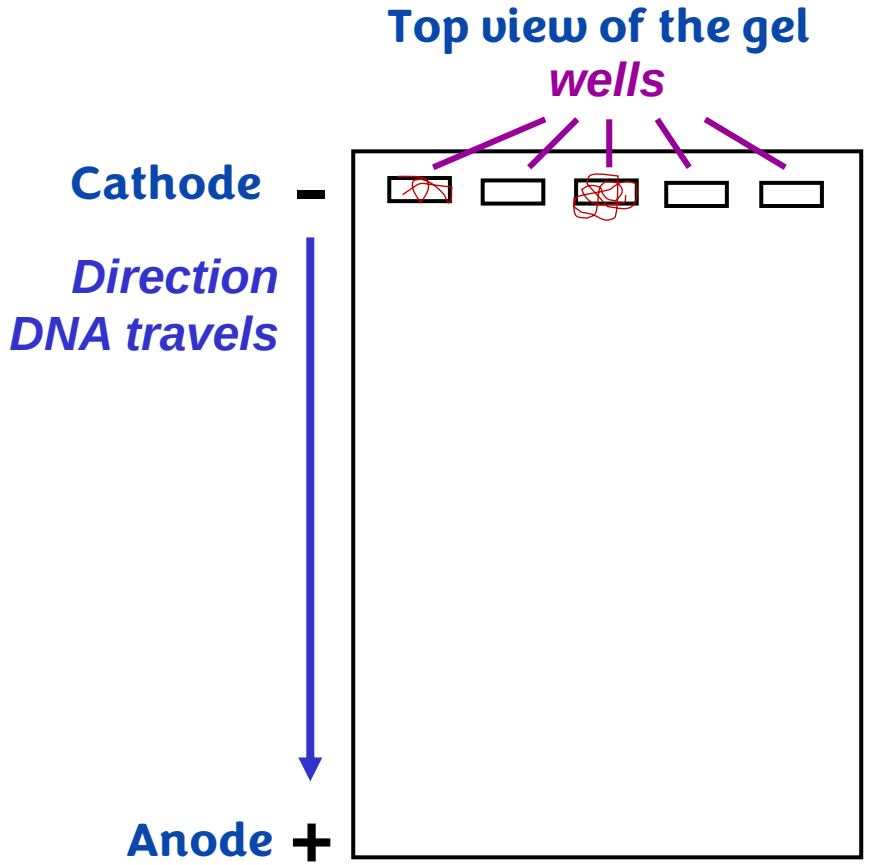




Here we add the sample



Top view of the gel



Gel Electrophoresis Overview (extra slide)

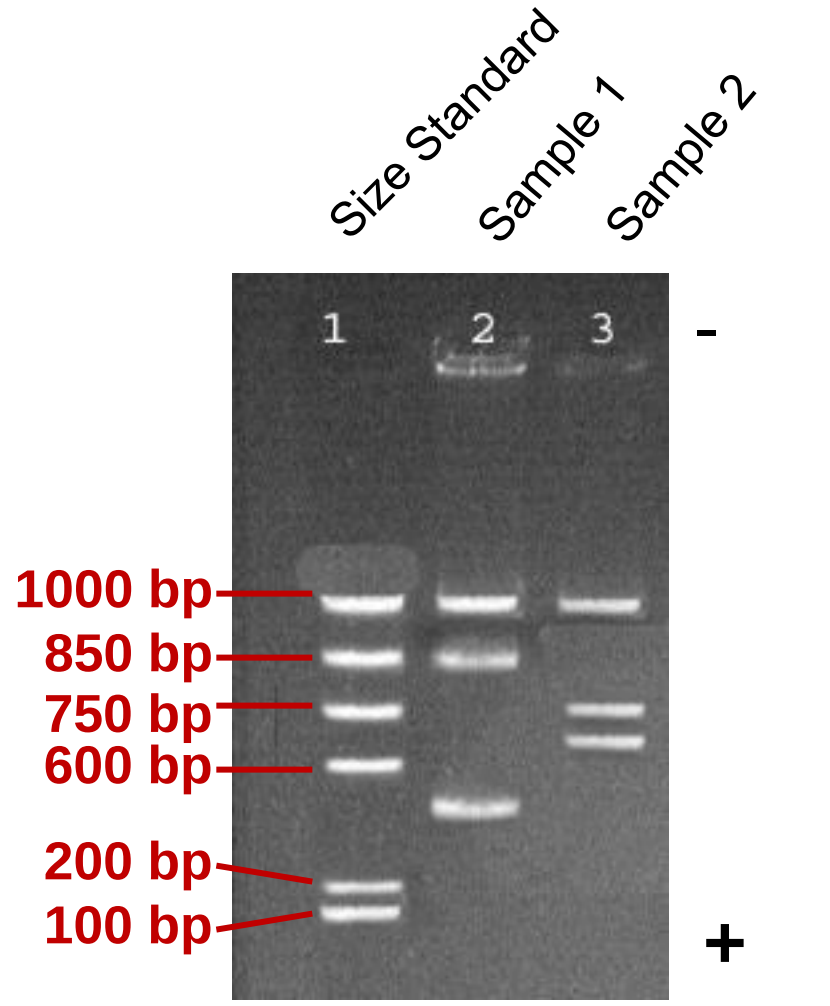
❖**Purpose**: Separates DNA fragments by size.

❖**Process**:

1. **Gel Preparation**: A jello-like gel is created with wells for samples.
2. **Sample Loading**: DNA samples are placed in the wells.
3. **Current Application**: An electric current is applied; DNA moves toward the positive (anode) due to its **negative charge from phosphate group.**
4. **Size-Based Separation**: Smaller DNA fragments travel faster through the gel's pores, while larger ones move more slowly.
5. **Result**: DNA fragments are separated by length.

Detection (pt.1)

- The DNA molecules of different lengths will run as "bands“.
- Each band contains thousands to millions of copies of DNA fragments of the same length but can be of same or different type (not one DNA molecule).
- DNA is stained (that is, colored) with a dye (ethidium bromide) or labeled (radioactive 32P).
- It is common that a DNA standard is used to determine the length of the examined DNA molecule.

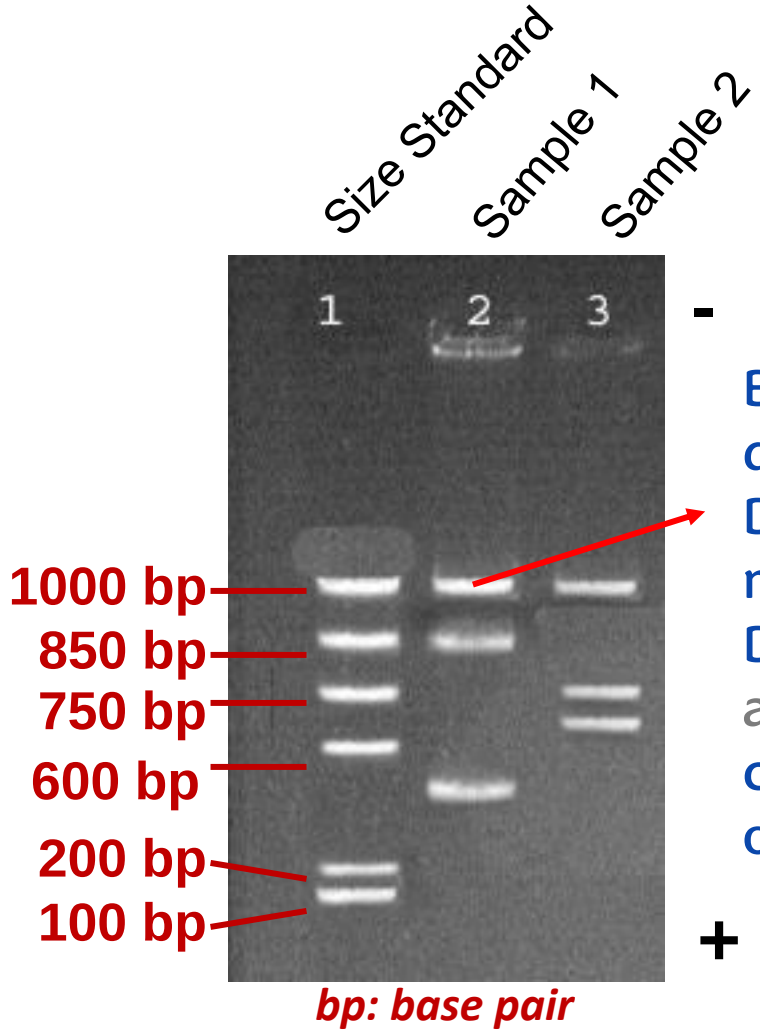


bp: base pair

Detection (pt.2)

- Each band contains millions of DNA molecules that have the same size but differs types, (different sequence of bases).
- When the amount of DNA is very low and cannot be visualized → the DNA is labeled (**using radioactive 32P**). A more powerful signal and a highly sensitive technique are then used to detect the DNA.

- Size standered: A sample of a DNA molecule that we add, that contains a DNA molecules of different sizes and **their size are known**.



Because of the distance travelled by DNA(of sample 1) matches another DNA (size standard - a known size-) we can predict the size of it

- **By applying an estimation methodology, we can determine the size of each sample band by comparing it to the size standard.**

Resources

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

- Watch this….very important

How can we measure how much DNA we have in a sample ?

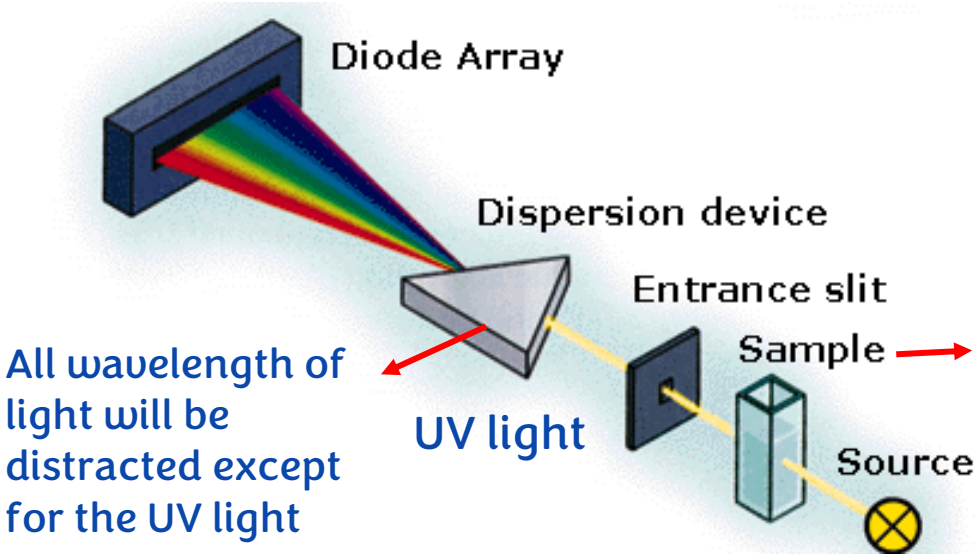
Light absorbance of nucleic acids

- Aromatic pyrimidines and purines can absorb UV light.
- Using spectrophotometry, the peak absorbance can be measured at 260 nm wavelength. **(Spectrometry (Technique) → Spectrometer (Device)).**

- The absorbance of nucleic acids at **260 nm (A260) is constant**
  - **dsDNA: A260 of 1.0 = 50 ug/ml**

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

Hint from the doctor : play with the variables in the equation and test yourself



All wavelength of light will be distracted except for the UV light

DNA then absorbs the UV light

Tells us the amount of light that is absorbed→ then we transfer this data to concentration

Further explanation :

- We can't see DNA in reality, because it absorbs the UV light and reflects everything else; that's why we have to stain it.
- **260 nm (A260)** –> The **max amount** of wavelength that DNA can absorb, A=absorption.
- **Concept:**

DNA concentration is directly proportional to its absorbance. If a DNA solution at a certain concentration absorbs a specific amount of light, then halving the concentration will result in half the absorbance.

Example:

Suppose we have a DNA solution with a concentration of 50 µg/mL, and it absorbs 1 unit of UV LIGHT. If we dilute this solution to a concentration of 25 µg/mL (half of the original concentration), it will absorb 0.5 units of light. This is because the absorbance is proportional to the concentration, so halving the concentration halves the absorbance.

Spectrophotometry technique illustration (pt.1).

❖**Sample Preparation and UV Exposure:**

- The DNA sample is placed in a vial and exposed to UV light. Only the UV wavelength reaches the DNA, as other wavelengths are filtered out.
- The DNA absorbs the UV light, and the absorption level is then detected by a sensor.

❖**Converting Absorption to Concentration:**

- The instrument provides an output indicating the amount of UV light absorbed by the sample.
- This absorption value is then used to calculate the DNA concentration in the sample.

❖**Understanding Absorption Standards:**

- The instrument is calibrated with known standards. For example:
- **Standard Concentration:** A DNA concentration of 50 µg/mL will absorb **one unit of UV light**.
- **Half Concentration:** If the DNA concentration is **25 µg/mL**, it will absorb **0.5 units of light**, as half the concentration absorbs half the light, Etc.

❖**Calculating Unknown Concentrations:** For instance, if a DNA sample absorbs **0.1 units of light** (one-tenth of the standard), the DNA concentration can be estimated as **5 µg/mL** (one-tenth of 50 µg/mL).

❖Handling High DNA Concentrations:

- When samples have very **high DNA concentrations**, such as **500 µg/mL**, the instrument may exceed its measurement limit.
- To address this, the sample is **diluted** for accurate measurement.

❖Dilution Process Example:

✓ Suppose we have a solution containing DNA at a concentration of 500 µg/mL. To dilute this solution at a ratio of 1:10, we take 1 mL of the DNA solution and mix it with 9 mL of water. This will result in a diluted solution with a concentration of 50 µg/mL (since 500 µg/mL ÷ 10 = 50 µg/mL).

- To use this diluted concentration in further calculations, we must account for this dilution factor (1:10) when applying it in formulas to ensure we obtain the correct final amount of DNA.

Additional Resources:

رسالة من الفريق العلمي:

إلى أبناء دفعة دوبامين الكرام،

لعلّ منا من يبدأ في دراسة هذه المحاضرة بعد حروب امتحانات منتصف الفصل؛ فمنّا من نجا من هذه الحرب بأقل الخسائر، ومنا من أُصيب ولم يُصِبْ جيدًا، لكن لا بدّ لنا أن نعلم أن الطريق طويل ولا مجال للاستسلام والتراخي. بالطبع، هناك مجال للتعويض (كلهم 40 علامة اللي راحوا شو يعني؟؟).

علينا أن ننسى ونتناسى ما قد حدث في فترة منتصف الفصل من الإخفاقات، وألّا ندع لها أي سبيل لإحباطنا، وأن نستفيد من هذه الإخفاقات في تطوير أنفسنا نحو الأفضل. وعلى من أحسن الصنع ألّا يغترّ بنفسه، فكما قلنا (40 علامة شو يعني) ما يعني أنه بقي أكثر من نصف العلامة.

يُقال أن هذا الفصل من أصعب الفصول، ولكن قد مررنا بنصفه وما بقي إلا القليل، فما أقوانا!

وبالتوفيق لمن بدأ في دراسة هذه المحاضرة قبل تلك الفترة.

في جميع الأحوال، وفقكم الله جميعًا وجعلكم من الأطباء ما تتمنون.

قال تعالى: «إن الله يحب المتوكلين» فالتوكل: منزلٌ من منازل الدين، ومقامٌ من مقامات الموقنين، بل هو من معالي درجات المقربين، وأعظم مقامٍ موسومٍ بمحبة الله صاحبه. فمن كان الله تعالى حسبه وكافيه ومراعيه؛ فقد فاز الفوز العظيم. فإن المحبوب لا يُعذَب ولا يُبعَد ولا يُحجَب

. واذا كنت تقرأها قبل الميد ف: **ارحم الافريج** (والله بنمزح بس , كل التوفيق)

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	Slide 19	Again, human cells contain multiple DNA molecules, each organized into distinct structures known as chromosomes.	A human cell contain multiple DNA molecules, each one of which forms a distinct chromosomes, serving as templet for RNA synthesis through transcription.
	Slide 21	The light is emitted from an external slide not the person itself as in DNA labeling .	The light is emitted from an external slide **not the person itself as in DNA staining.**
	Slide 28	Using spectrophotometry **(And the technique known as spectrophotometry)**,	Using spectrophotometry, the peak absorbance can be measured at 260 nm wavelength. **(Spectrometry (Technique) → Spectrometer (Device)).**