بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | FINAL 1

Overview & Gelelectrophoresis (Pt.1)

Written by : DST, NST

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وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

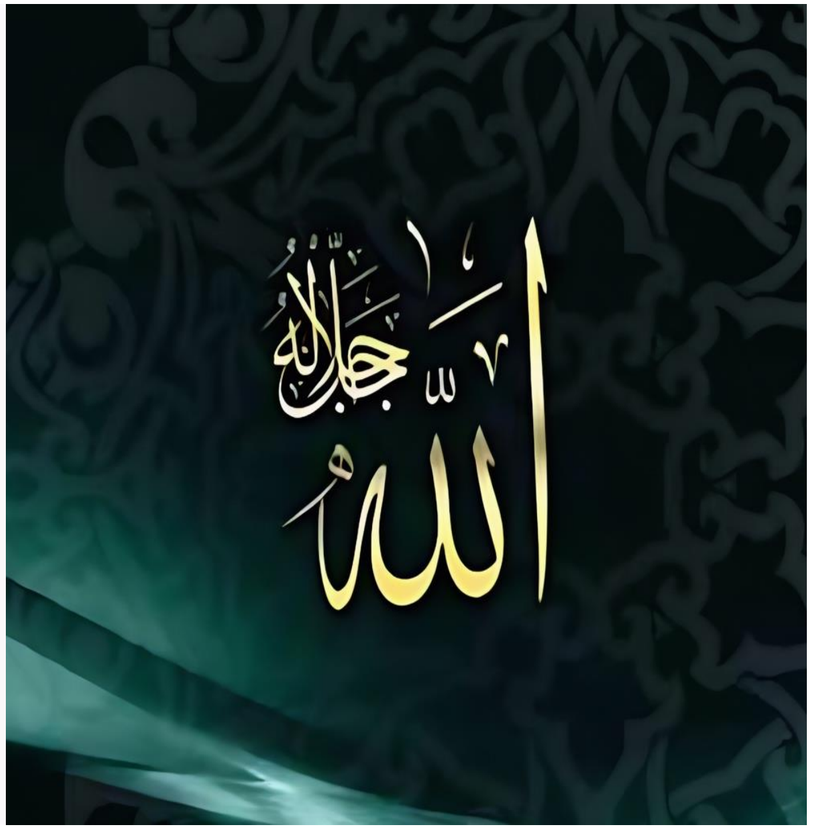
العلم بأسماء الله الحسنى أجل العلوم وأشرفها، لأن به يعرف الناس ربهم وخالقهم، خالق السماوات والأرض، ويتبع ذلك عبادته، ومحبته، وخشيته، وتعظيمه سبحانه وتعالى، ثم إنّ شرف العلم يكون من شرف المَعلوم، ولمّا كان المَعلوم من تعلّم أسماء الله الحسنى هو الله سبحانه وتعالى؛ كان شرف تعلّم أسماء الله تعالى عظيمًا.

فتظهر أهمية معرفة أسماء الله وصفاته في جوانب عدّة لا يجب أن تخفى على كل مسلمٍ، ومنها:

1. أن العلم بالله تعالى وأسمائه وصفاته أصل العلوم وأساس الإيمان، فإذا عرف العبدُ ربَّه عَبَدَهُ حقَّ العبادة. يقول الأصبهاني: "قال بعض العلماء: أول فرض فرضه الله تعالى على خلقه معرفته، فإذا عرفه الناس عبدوه قال الله تعالى: {فاعلم أنه لا إله إلا الله}". وقال: "ولو أراد إنسان أن يتزوج أو يزوِّج أو يعامل إنسانا؛ طلب أن يعرف اسمه وكنيته، واسم أبيه وجده، وسأل عن صغير أمره وكبيره، فالله الذي خلقنا ورزقنا ونحن نرجوا رحمته ونخاف من سخطه أولى أن نعرف أسماءه ونعرف تفسيرها ".
2. التلازم الوثيق بين صفات الله تعالى وما تقتضيه من العبادات الظاهرة والباطنة، فلكل صفة عبادة خاصة هي من مقتضاها، وفي ذلك يقول ابن القيم: "فلكل صفة عبودية خاصة هي من موجباتها ومقتضياتها، أعني: من موجبات العلم بها والتحقق بمعرفتها وهذا مطرد في جميع أنواع العبودية التي على القلب والجوارح: فعلم العبد بتفرد الرب تعالى بالضر والنفع، والعطاء والمنع، والخلق والرزق، والإحياء والإماتة؛ يثمر له عبودية التوكل عليه باطنا، ولوازم التوكل وثمراته ظاهرًا. وعلمه بسمعه تعالى وبصره وعلمه، وأنه لا يخفى عليه مثقال ذرة في السموات ولا في الأرض، وأنه يعلم السر وأخفى...، فرجعت العبودية كلها إلى مقتضى الأسماء والصفات".
3. . للعلم بأسماء الله الحسنى وصفاته ثمرات طيبة في التعامل مع الفتن والمصائب والمكروهات التي تصيب العبد، فالعبد إذا علم أن الله تعالى حكيم لا يفعل شيئًا عبثًا، وأنه عدل لا يظلم أحدًا، وأنه رحيم بعباده يبتليهم ليغفر لهم؛ إذا علم العبد ذلك كله رضي وصبر على المكروه والمصاب الذي ينزل به، واطمأن قلبه وفوض أمره لربه سبحانه.

وغيرها الكثير من الثمرات التي تنعكس على عبادة المسلم وجميع جوانب حياته الدنيوية...

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا



المعنى: اسم «الله» دالٌّ على كونه مألوهًا معبودًا، تألهه الخلائق محبة، وتعظيمًا، وخضوعًا، وفزعًا إليه في الحوائج والنوائب، وهو الاسم الجامع لمعاني أسماء الله الحسنى.

الورود: ورد في القرآن (٢٧٢٤) مرة.

الشاهد: ﴿إِنَّنِيٓ أَنَا ٱللَّهُ لَآ إِلَٰهَ إِلَّآ أَنَا۠ فَٱعْبُدْنِى وَأَقِمِ ٱلصَّلَوٰةَ لِذِكْرِىٓ﴾ [طه:١٤].

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اضغط هنا لشرحٍ أكثر تفصيلًا

Molecular Biology (1)

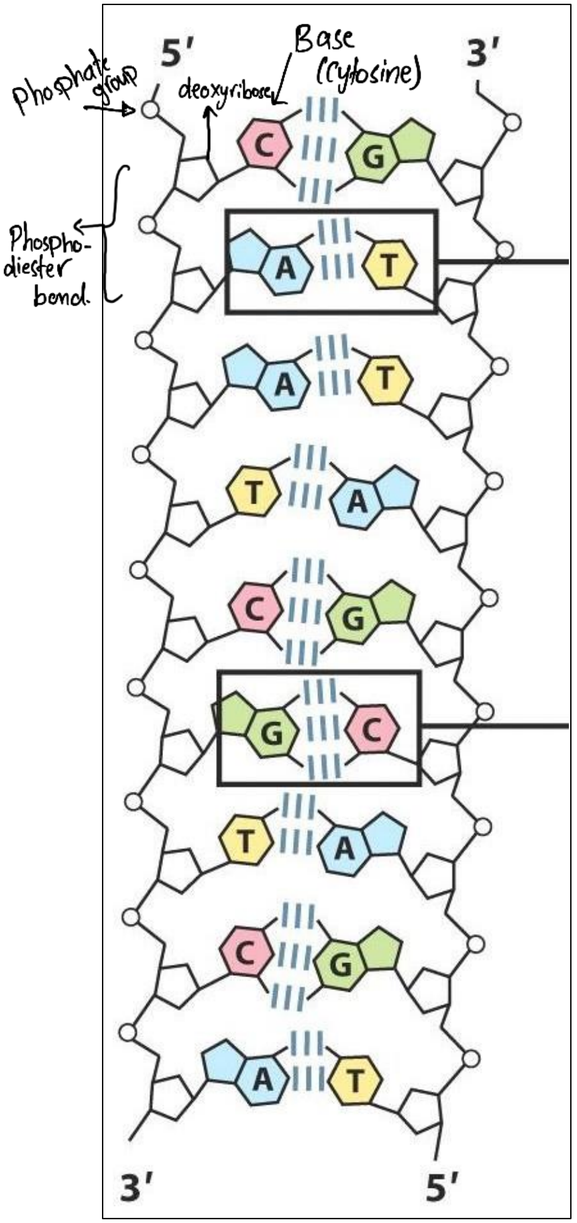
Structure of nucleic acids

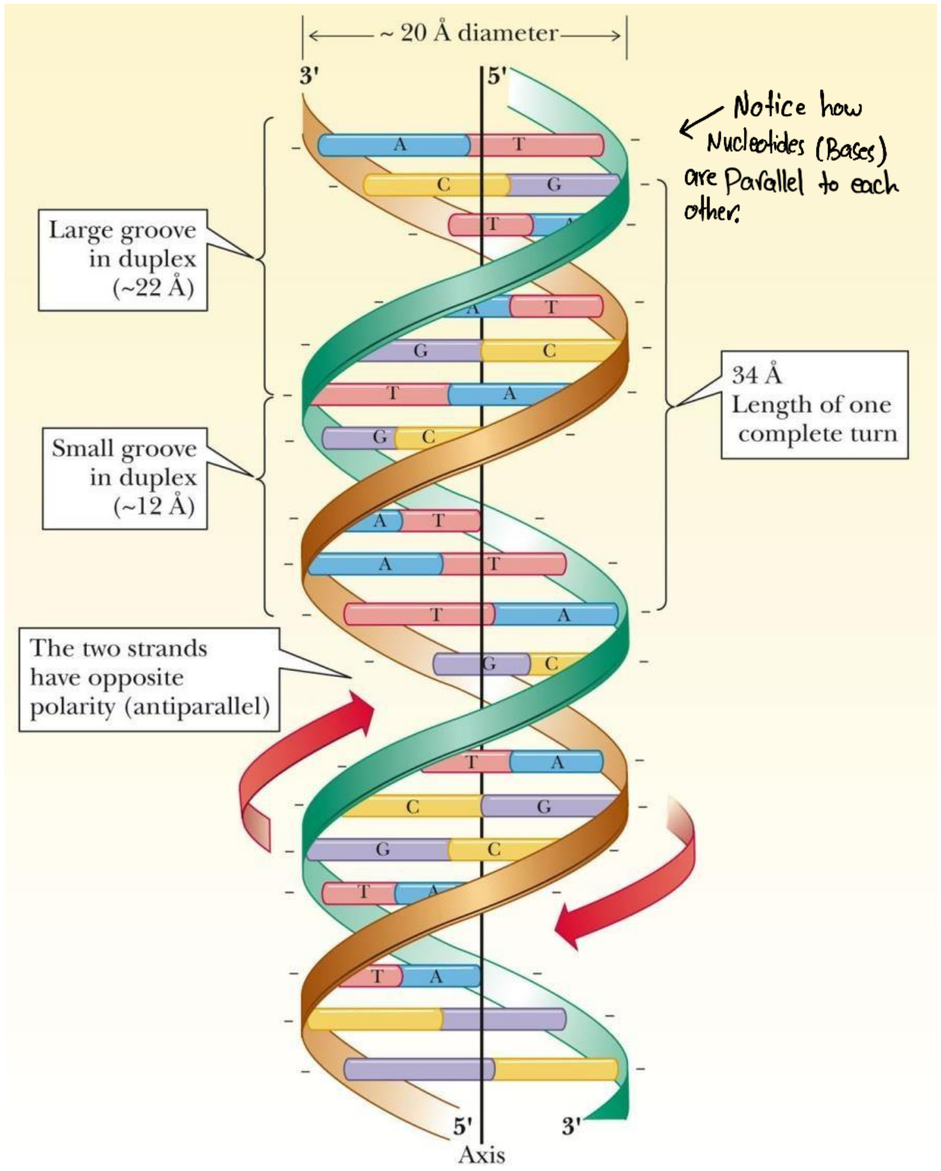
Prof. Mamoun Ahram

School of Medicine

Second year, Second semester, 2024-2025

DNA (Deoxyribonucleic acid/ Deoxyribonucleoside triphosphate) structure





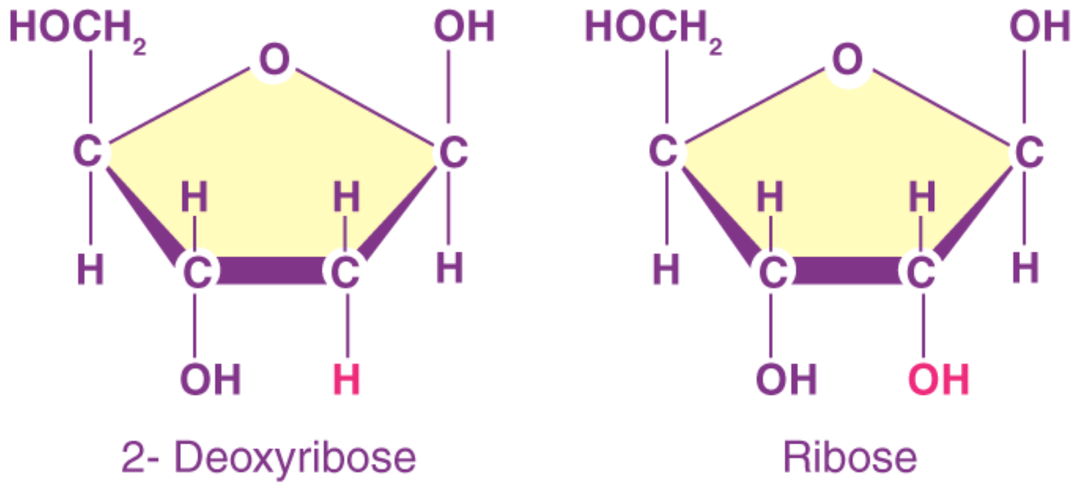
- The monomer
- A double helix
- Specific base-pairing
  - A = T; G = C; Pur = pyr (Chargaff's rule)
- Complementary
- Backbone vs. side chains
- Antiparallel
- Stability vs. flexibility
- Groovings

Explanation of the previous slide (pt.1):

- What does the name DNA (Deoxyribonucleic acid/ deoxyribonucloside triphosphate) implies ?

Deoxy : there is this a molecule that was oxygenated and it deoxygenated (there is a deoxygenated molecule) which is **deoxyribose** the deoxygenated form of ribose.

Deoxygenation is at carbon number 2



2- Deoxyribose Ribose

Acid : due to the presence of three phosphate groups, each one has a negative charge, which means 3 negative charges, so it is an acidic molecule.

Explanation of the previous slide (pt.2):

- 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.**

الاسم الصغير (Purines) للـ
Structure الكبير(2 rings)

- DNA strands are held together by:

- **phosphodiester bonds** in the backbone, between nucleotides in same standard (between carbon 3 (called 3’ carbon) and 5 (called 5’ carbon)).

✓ Prime (‘) : we write it to distinguish it from nitrogenous bases carbons.

✓ Addition of nucleotides is always at carbon number 3.

- **hydrogen bonds** between complementary bases: adenine with thymine (two bonds) and guanine with cytosine (three bonds).

Explanation of the previous slide (pt.3):

- 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.4):

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

1. **Propeller Twist:**

The individual bases within a single base pair are not perfectly flat ; they are internally rotated relative to each other, a structural deviation known as **Propeller Twist**.

2. **Stacking Optimization:**

Bases are organized at an **offset angle** along the helix axis to maximize favorable hydrophobic interactions and optimize inter-atomic distances for stabilizing **van der Waals forces** (which prevents electron cloud repulsion).

3. **Base Plane Orientation:**

The plane of the base pairs is **not oriented perpendicular** (at a 90° angle) to the central axis of the DNA helix; instead, they exhibit a slight **tilt** or **roll**.

Explanation of the previous slide (pt.5):

– 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 20**Å** (Angstrom).

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

- **A** always pair **T**(by *2H-Bonds*), and **C** always pair **G** (by *3H- 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 Tcontent.**

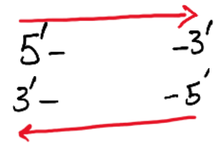
Explanation of the previous slide (pt.6):

→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.**

Antiparallel



❖ 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).

Explanation of the previous slide (pt.7):

There are two types of groves (because of the imperfection in helical shape) :

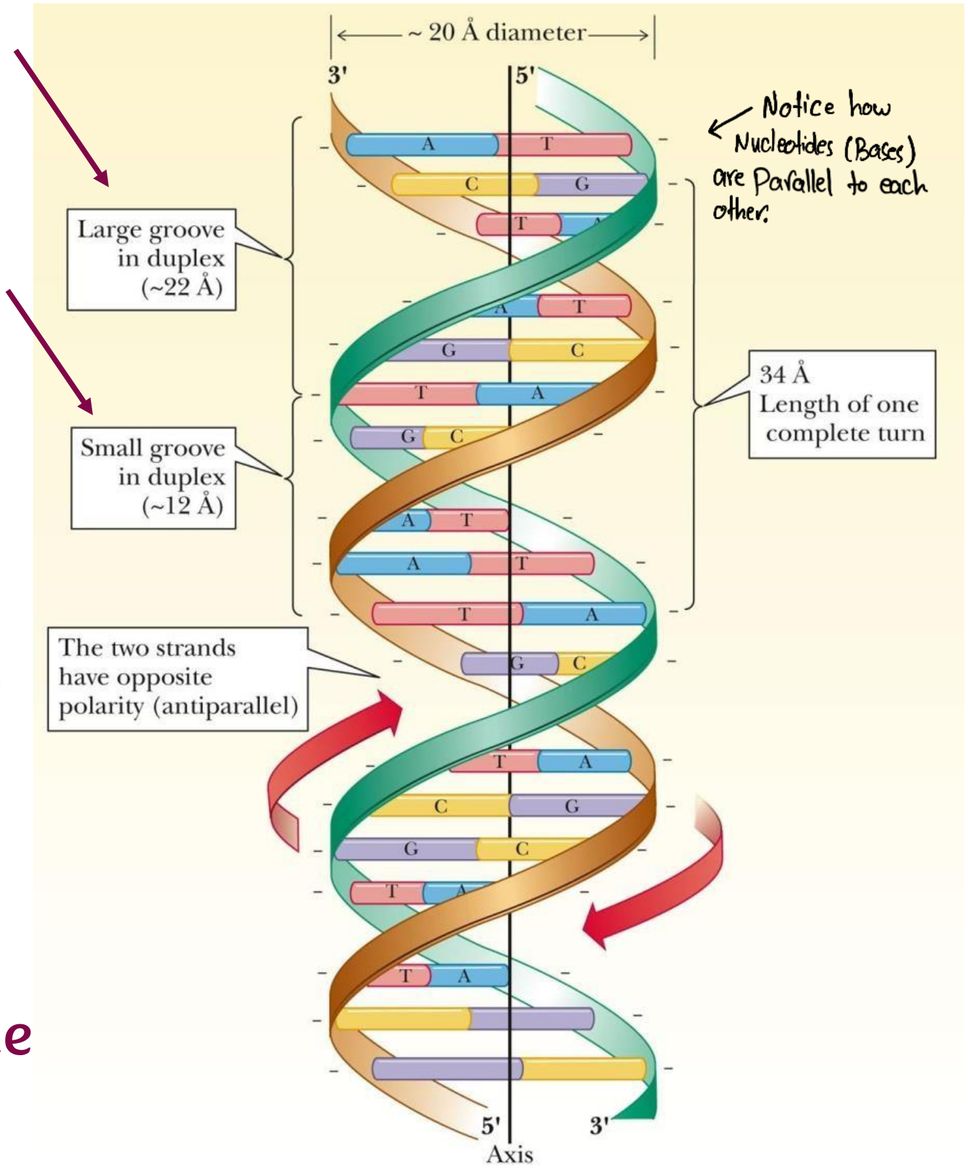
1. **Major Groves** : *wider* (Approximately **22A**).

2. **Minor Groves** : *narrower* (Approximately **12A**).

These two types of groves are alternative* and opposite to each other**.

* : one after the other (minor then major then minor)

** : whenever there is a major grove, there is a minor one at the opposite side.



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.

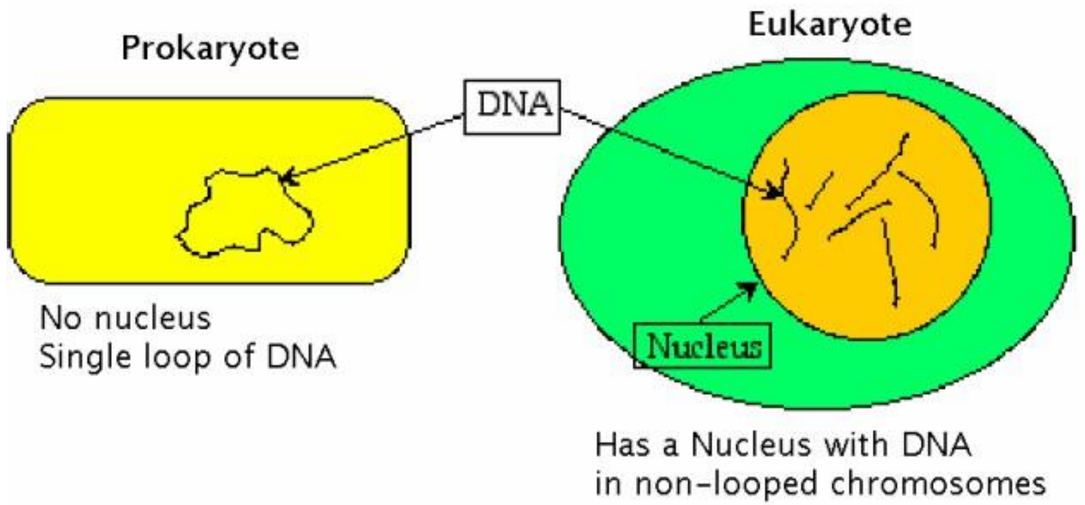
DNA sequence can be written without pointing the ends, but the left end indicated the 5’ end. And knowing that it’s DNA (double stranded) the complementary strand’s sequence can be interpreted from the given sequence.

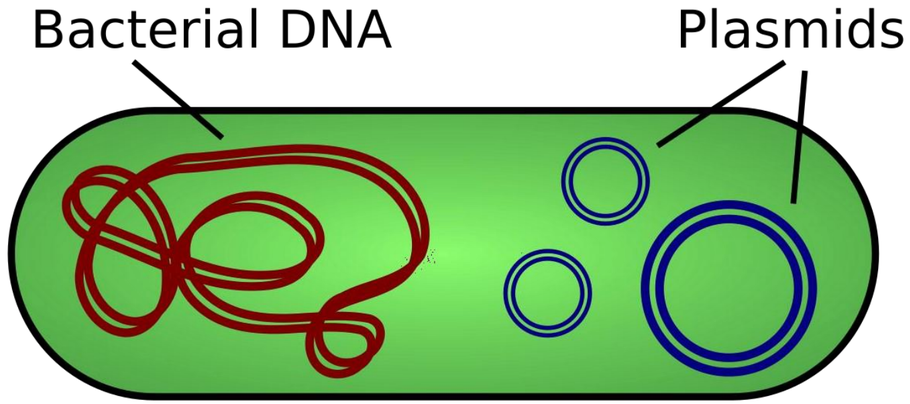
RNA

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

RNA is single stranded (some viruses are exception having it as a double helical stranded), 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

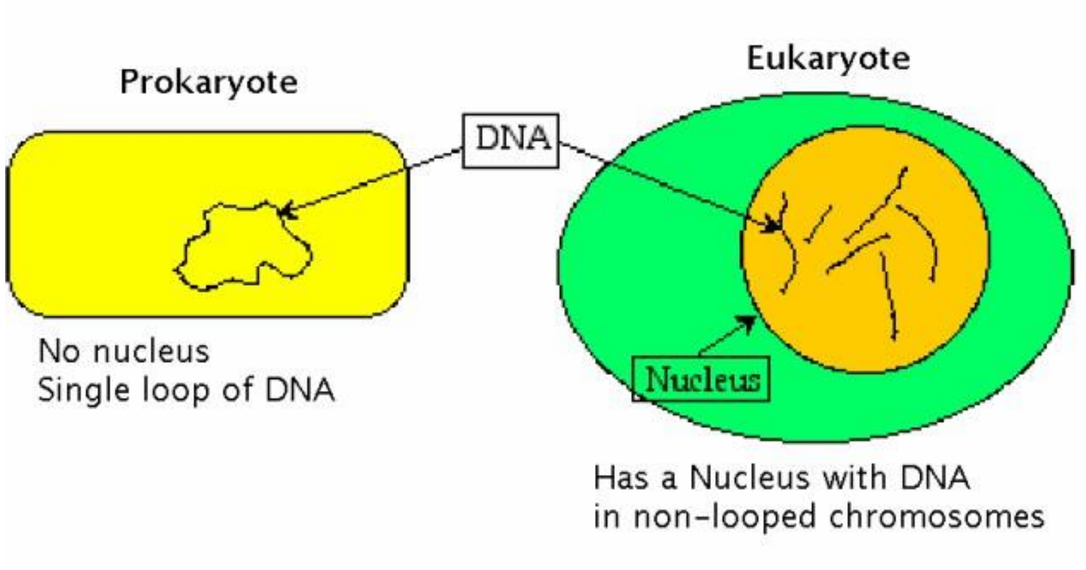


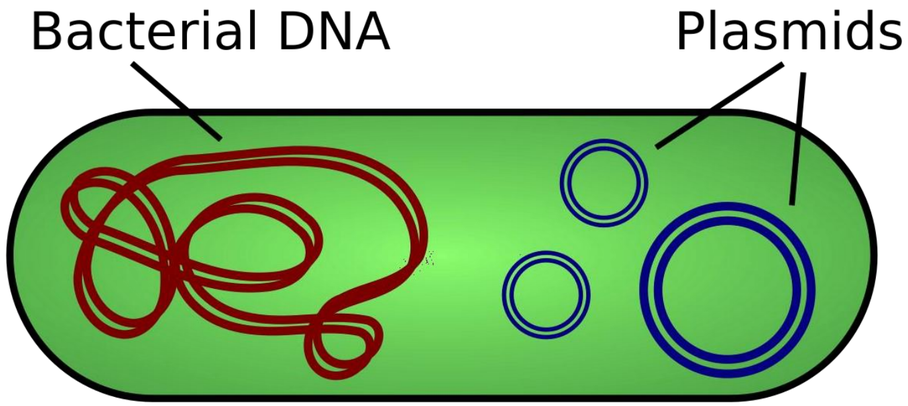


- Genome: the total genetic material of a living being (bacteria vs. human), a species (monkey vs. human) , an individual (me vs. you), or a cell (brain vs. liver), etc.
- **if they are from the same person, they should be identical.**
- Prokaryote: circular genome + plasmid **(not essential, may not br present).**
- Eukaryote: a linear, nuclear genome (chromosomes) + mitochondrial genome
- **Viruses genome can be single stranded DNA, double stranded DNA, single stranded RNA and double stranded RNA.**

I can say Eukaryotic genome or human genome, mice genome, Arabs Genome and non-Arab Genome, etc.

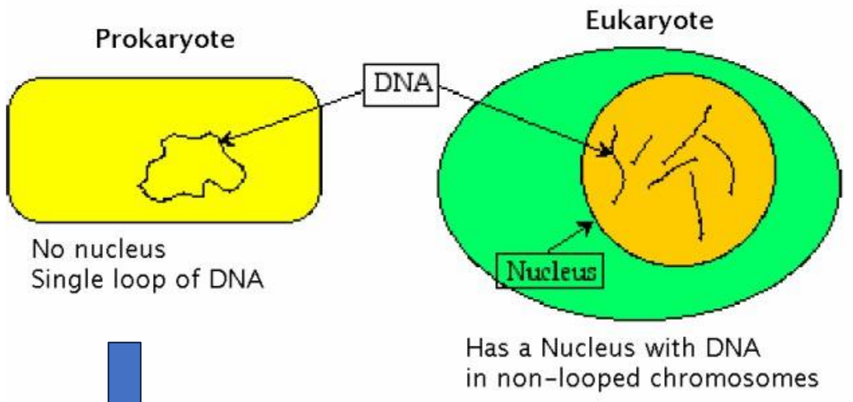
The genome of prokaryotes versus eukaryotes 2





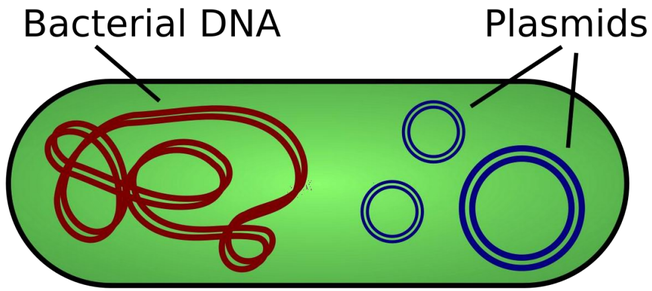
- Remember that each chromosome is made of a long dsDNA packed by Histone proteins.
- Mitochondria have their own genome (mtDNA), which is circular and less stable than the nuclear genome. In eukaryotes there are multiple copies of mitochondria in the cytosol, each of which has multiple (identical) genomes.

The genome of prokaryotes versus eukaryotes (extra slide)



Single circular molecule of dsDNA.

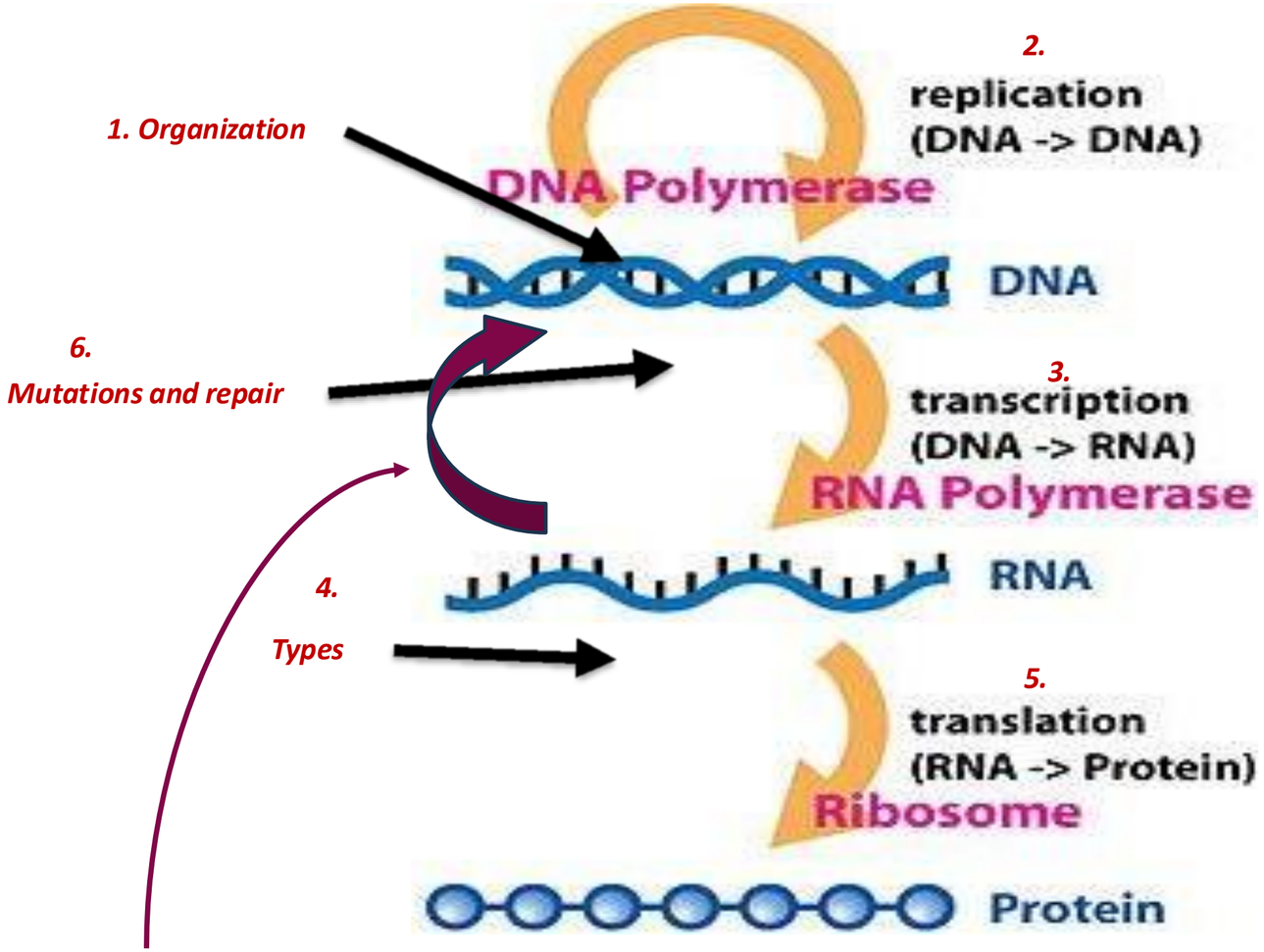
Organized as linear chromosomes enclosed by a nuclear membrane



- 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

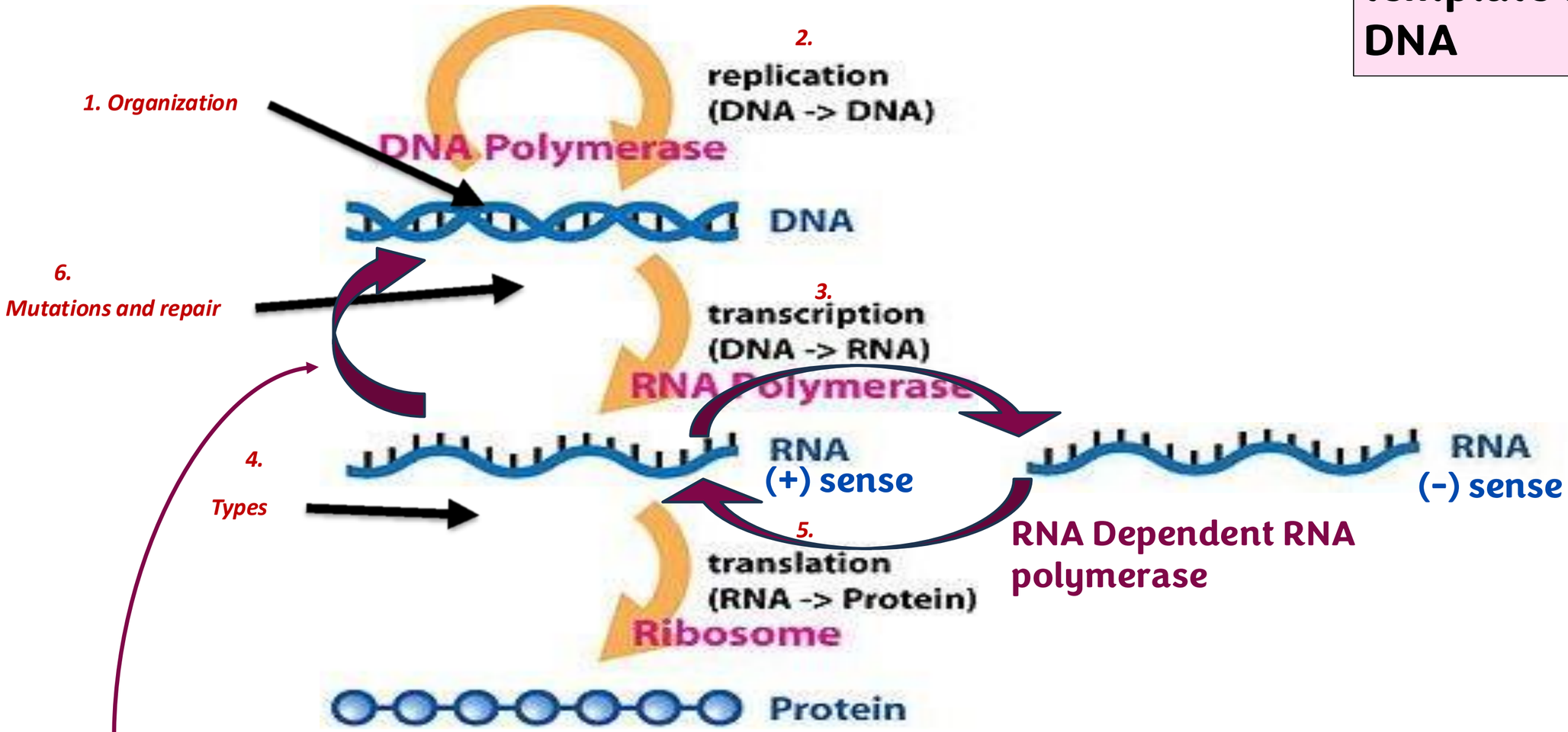
Reverse Transcription (catalysed by Rev.Transcriptase).

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

What is molecular biology?

The new central dogma of molecular biology

RNA can be used as a template to synthesise DNA

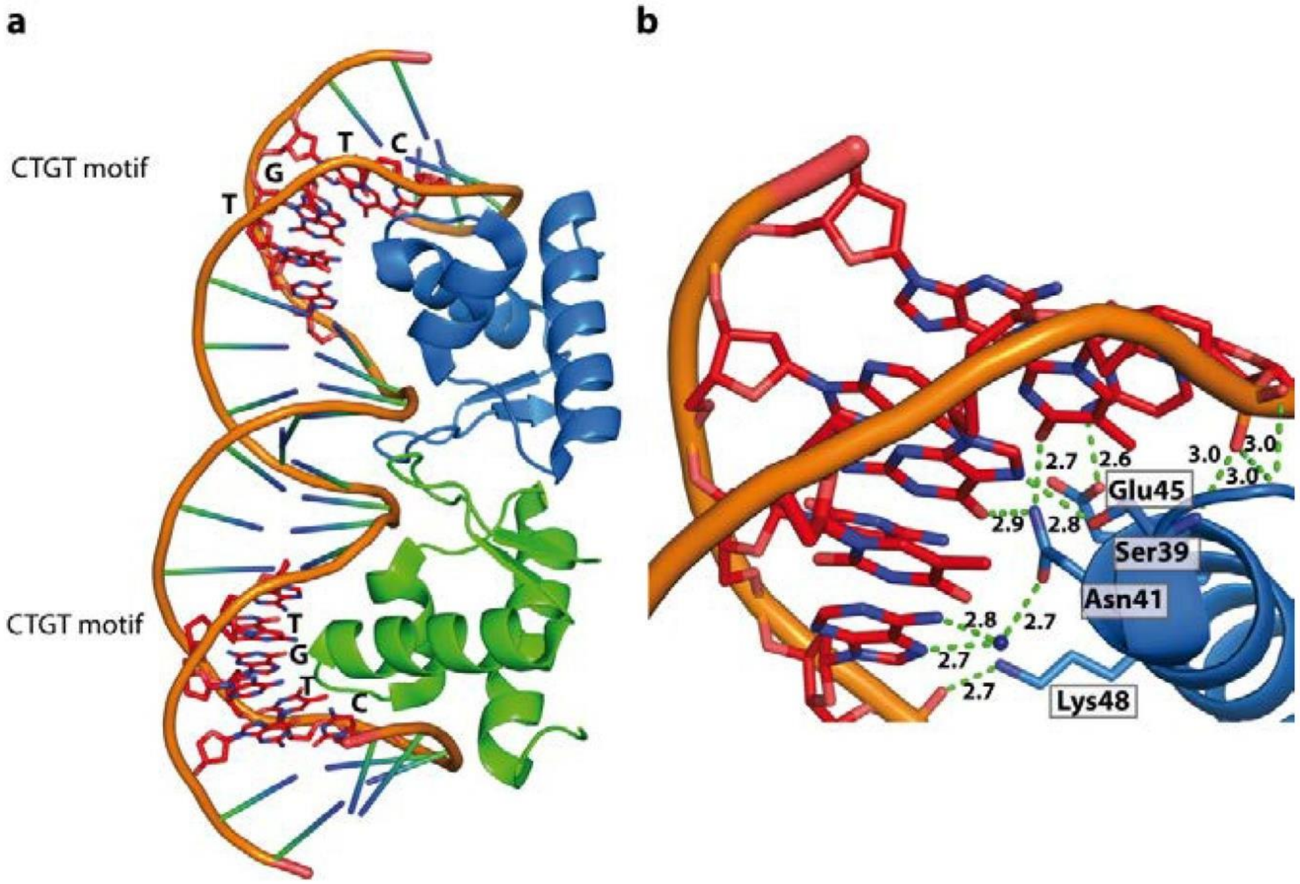


Reverse Transcription (catalysed by Rev.Transcriptase).

DNA-protein interaction

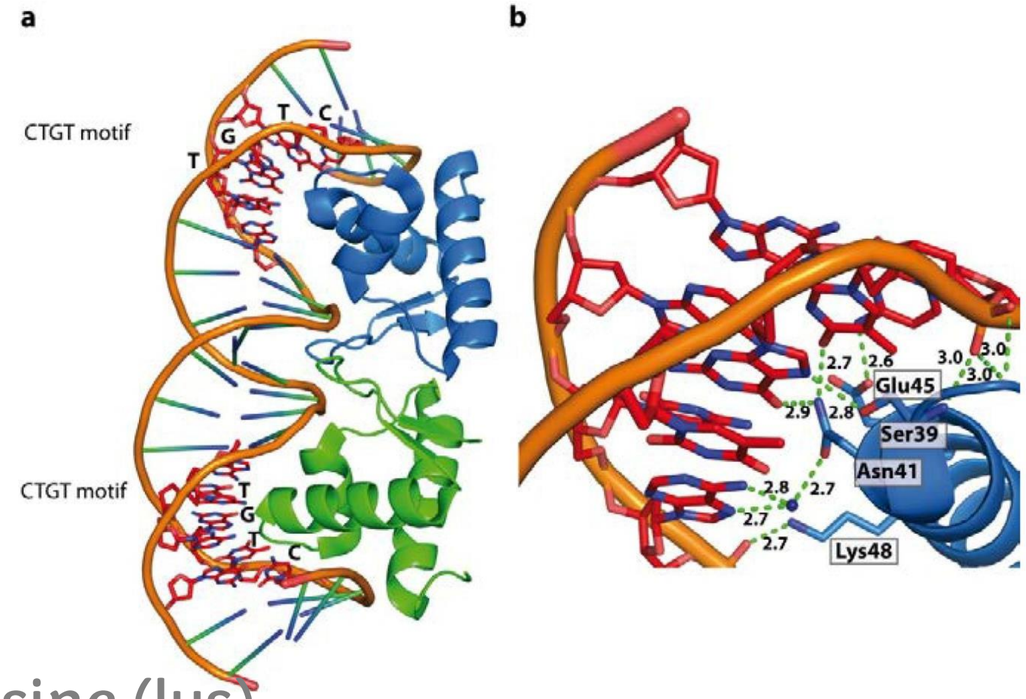
- Remember proteins can interact with DNA in a non-randomized fashion (really specific); as a specific amino acid sequence can recognize and bind to a specific **DNA sequence** (order of nucleotides) within a strand or within the molecule.

- Interactions usually take place at the major groves rather than minor ones because major groves is wider and **allow amino acids of the proteins to enter the grove and bind to a specific DNA sequence,** while minor groves are narrower and **prevent proteins from entering the grove since it is narrow.**



DNA-protein interaction

- 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:

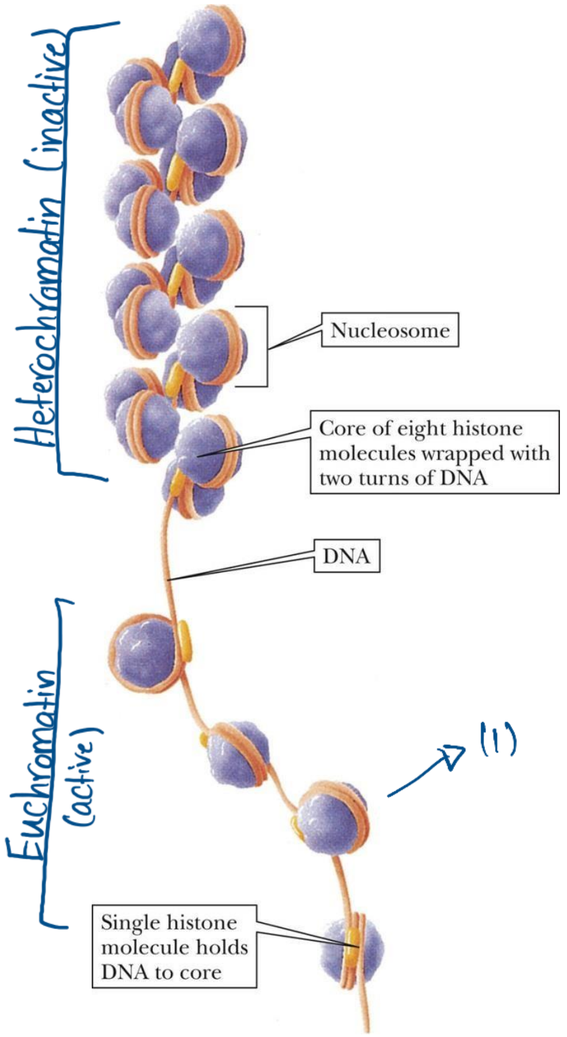


* Carboxylic group (-COOH) of Glutamate (Glu).
* Hydroxyl group (-OH) of Serine (ser).
* Amino group (-NH3) of Asparagine (Asn) and Lysine (lys).

These can interact with the bases of DNA.

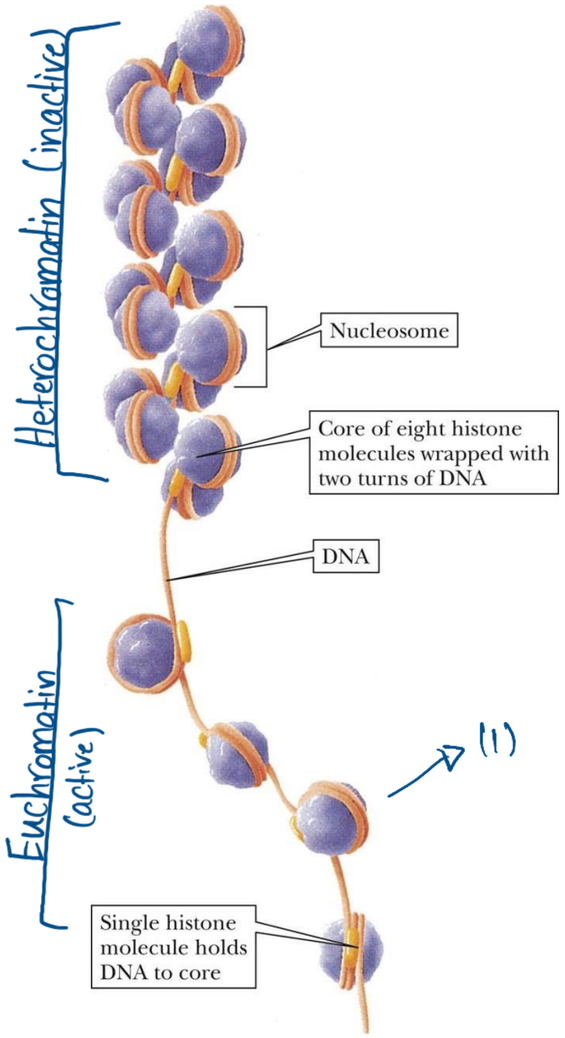
- The interactions between the bases and amino acids are **quite specific**.
- The order of the bases and the 3D overall structure of A.As (proteins) can determine the **specificity** of DNA-Protein interactions.
- Further explanation will be provided as we come across DNA-Protein interactions later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

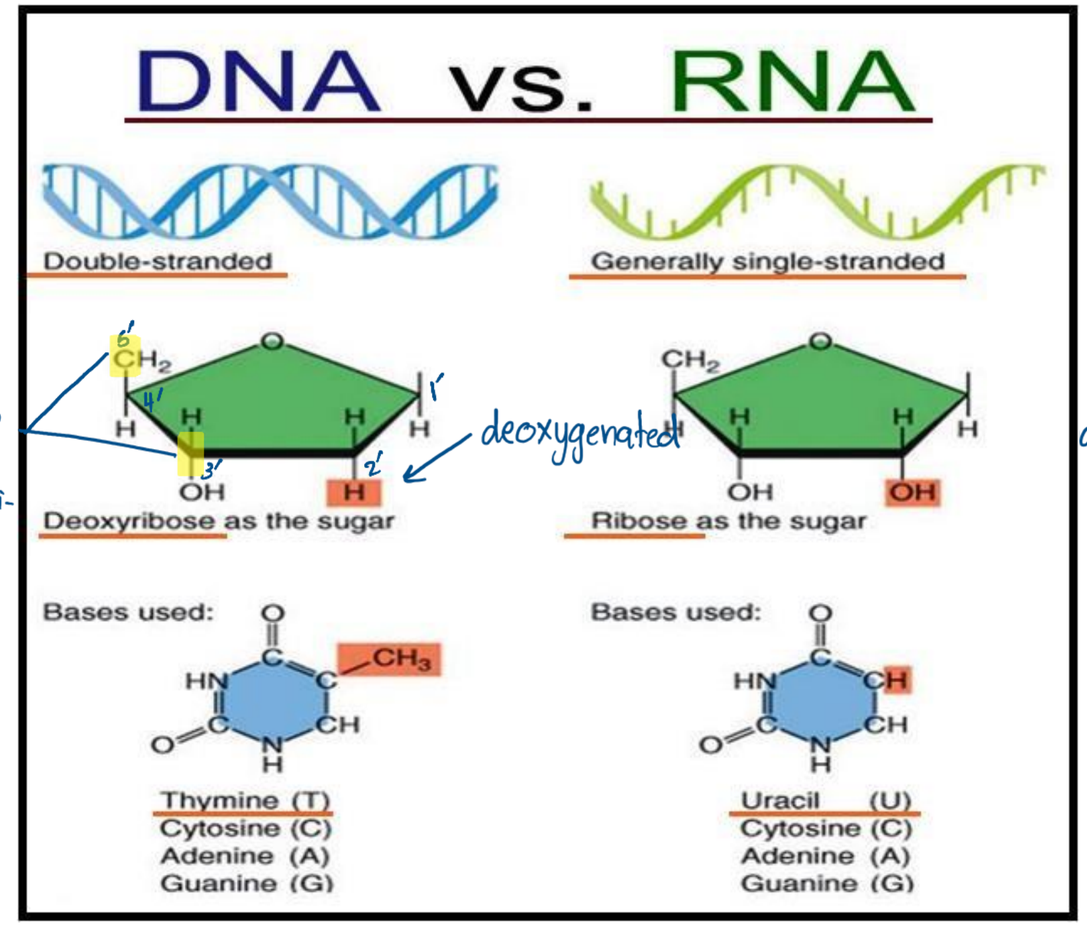


سبحان الله

Packing a DNA which is 2m long into the nucleus which is 10 micrometer in diameter is like packing a 35Km length wire into a tinnes ball.

In prokaryotes and eukaryotes (not viruses)

Take a look at the picture



These two play a role in phosphodiester bonds formation.

deoxygenated

ated

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.

Human cells have different types of RNA molecules:

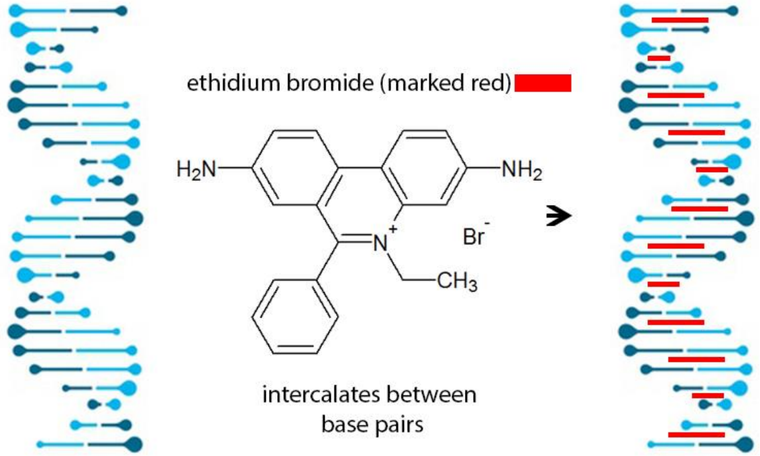
RNA species	Established function(s)	
mRNA	Messenger for protein production	Major ones
tRNA	Translation of RNA codon to amino acid	
rRNA	Enzymatic and structural part of ribosomes	
snRNA	Pre-mRNA processing	
snoRNA	Modification of rRNA	
miRNA	Repression of translation	micro RNA
piRNA	Silencing of transposons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

Techniques

In this lecture will cover one type of techniques.

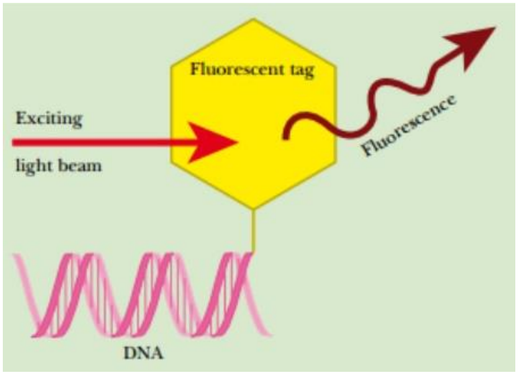
DNA labeling versus staining

DNA staining

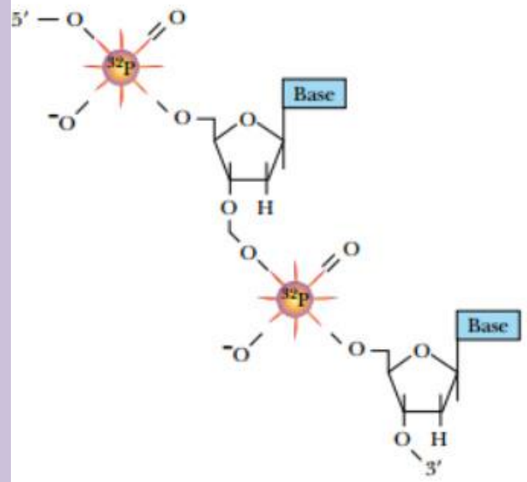


- It involves a general non-covalent interaction of a chemical dye with DNA.
- It is less specific **(can bind to any region in the DNA)** and less sensitive.
- It is usually used to detect, visualize, and/or quantify DNA.

DNA Labeling (more sensitive)



- It usually involves a <u>covalent</u> binding of a fluorescent tag or radioactive phosphate to DNA **(we can't see the signal but we can detect it using detectors).**
- It is usually sequence-specific.
- It is more sensitive.
- It is usually used to detect and quantify DNA.



^{32}P-LABELED DNA

Further explanation :

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 the DNA a certain color.

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

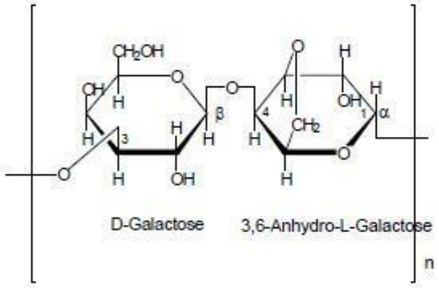
Further explanation :

DNA labeling :

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

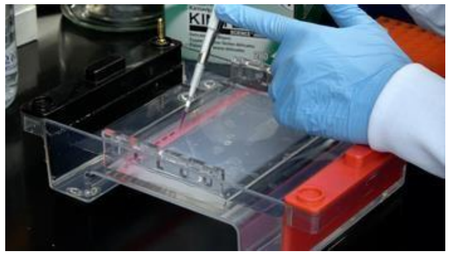
- More sensitive.
- Here the DNA itself emits the color (fluorescent signal), which can also be radioactive .
- We can use radioactive phosphate, why phosphate? Because it is present in the DNA nucleotide structure (rule : if I want to detect a specific molecule using radioactive molecule, the radioactive molecule should be a molecule or atom that present in the structure of the molecule that I want to detect and not in other molecules).
- 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.

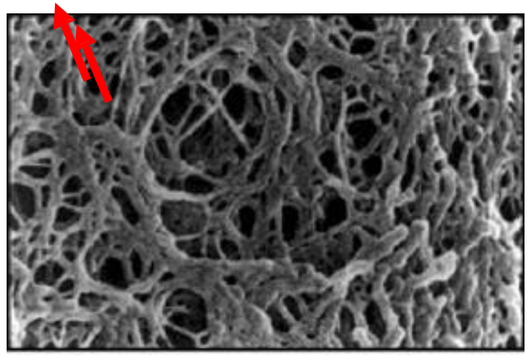
Gel electrophoresis



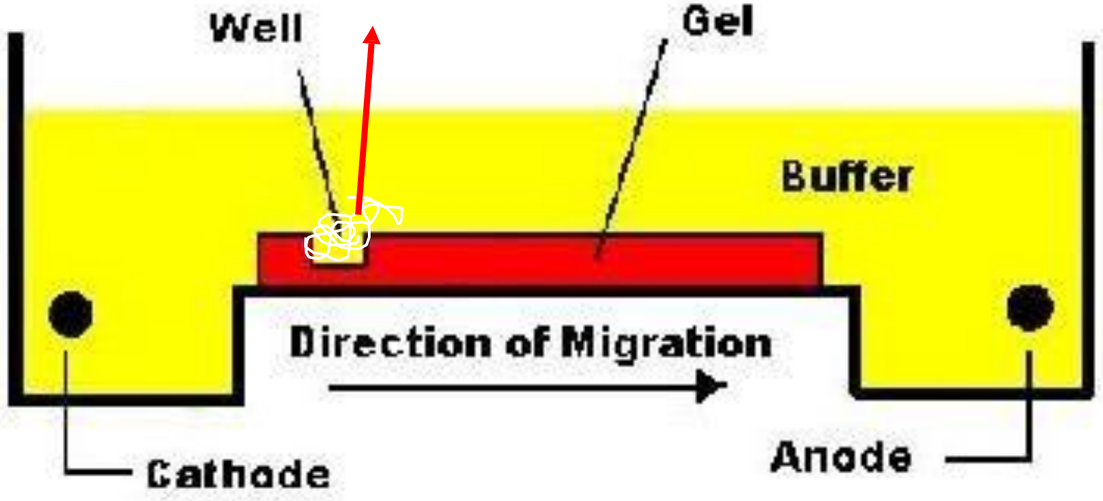
- The length and purity of DNA molecules can be accurately determined by the gel electrophoresis.

There are networks of chemical and spaces in between



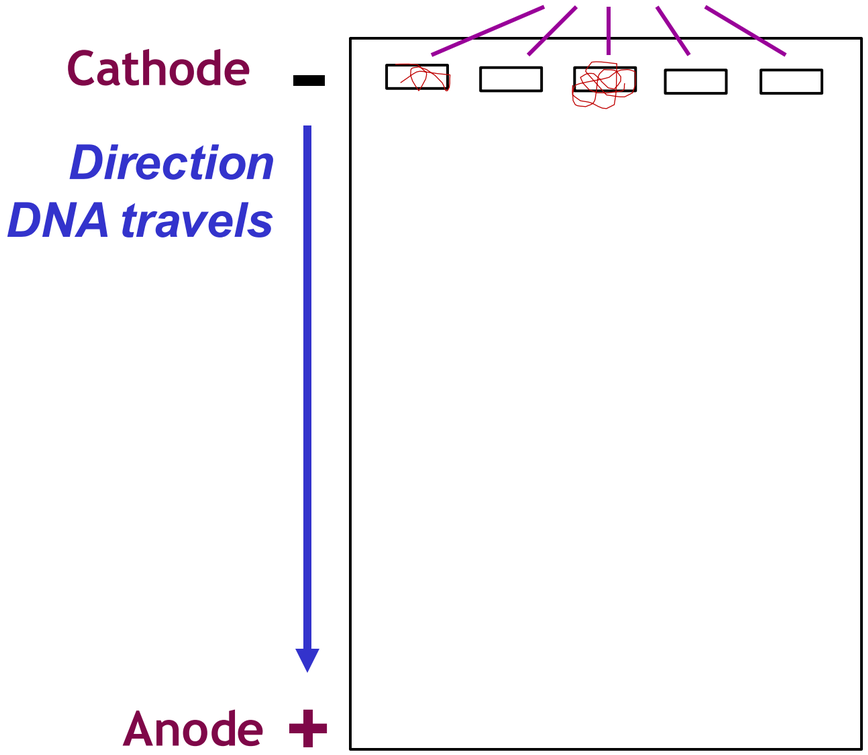


Here we add the sample



Top view of the gel

wells

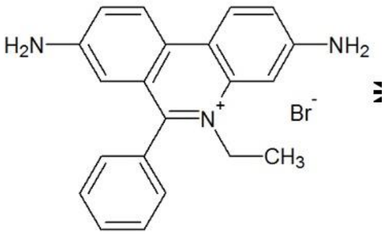


Gel Electrophoresis Overview (extra slide)

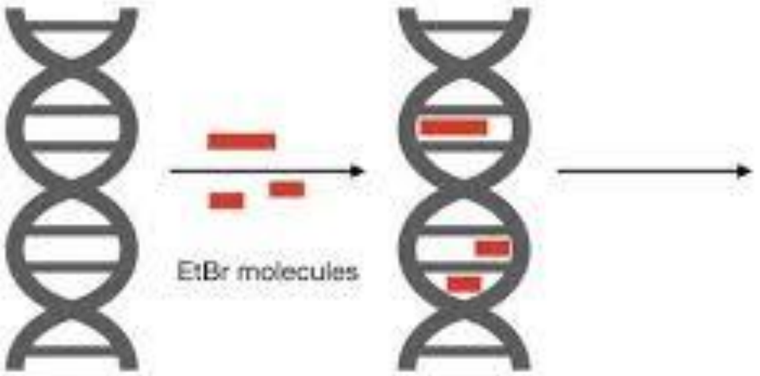
- **Purpose**: Separates DNA fragments by size.
- **Process**:

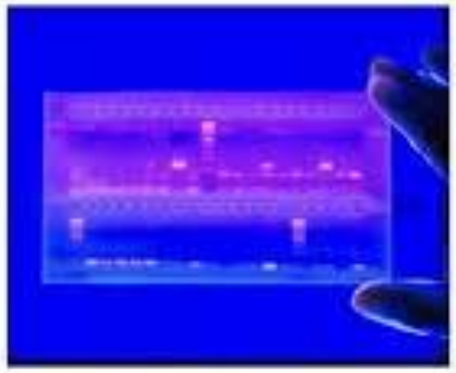
1. **Gel Preparation**: A jello-like gel is created with wells for samples.
2. **Sample Loading**: DNA samples are placed in the wells.
3. **Current Application**: An electric current is applied; DNA moves toward the positive (anode) due to its **negative charge from phosphate group.**
4. **Size-Based 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 (size).

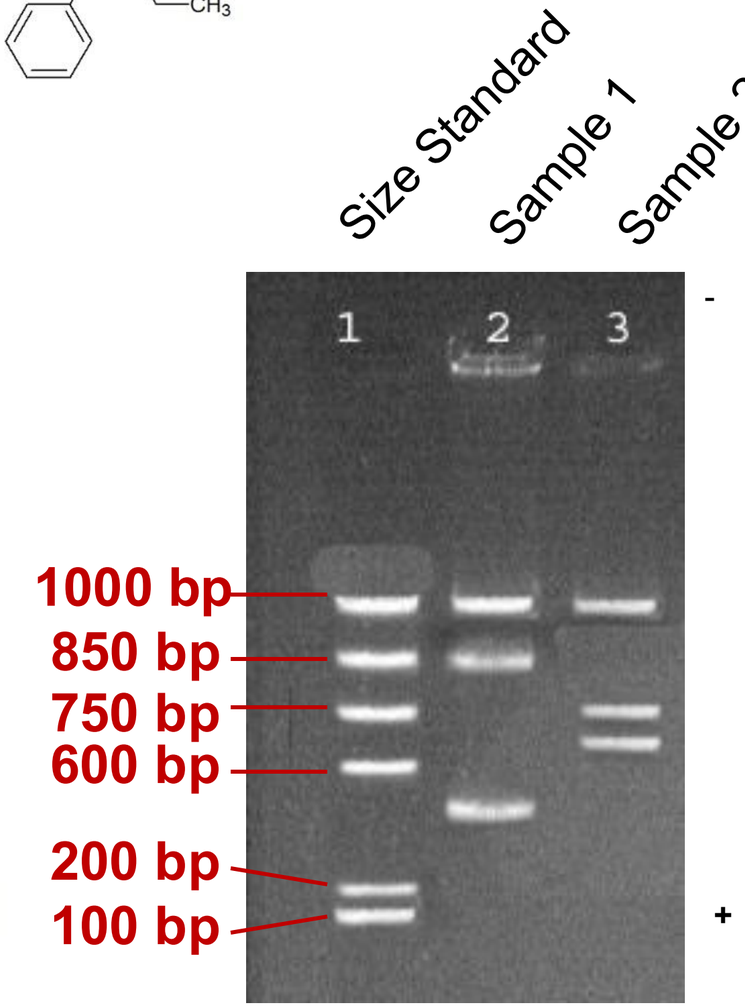
Detection



- 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)*** and observed under the uv light.
- It is common that a DNA standard is used to determine the length of the examined DNA molecule.







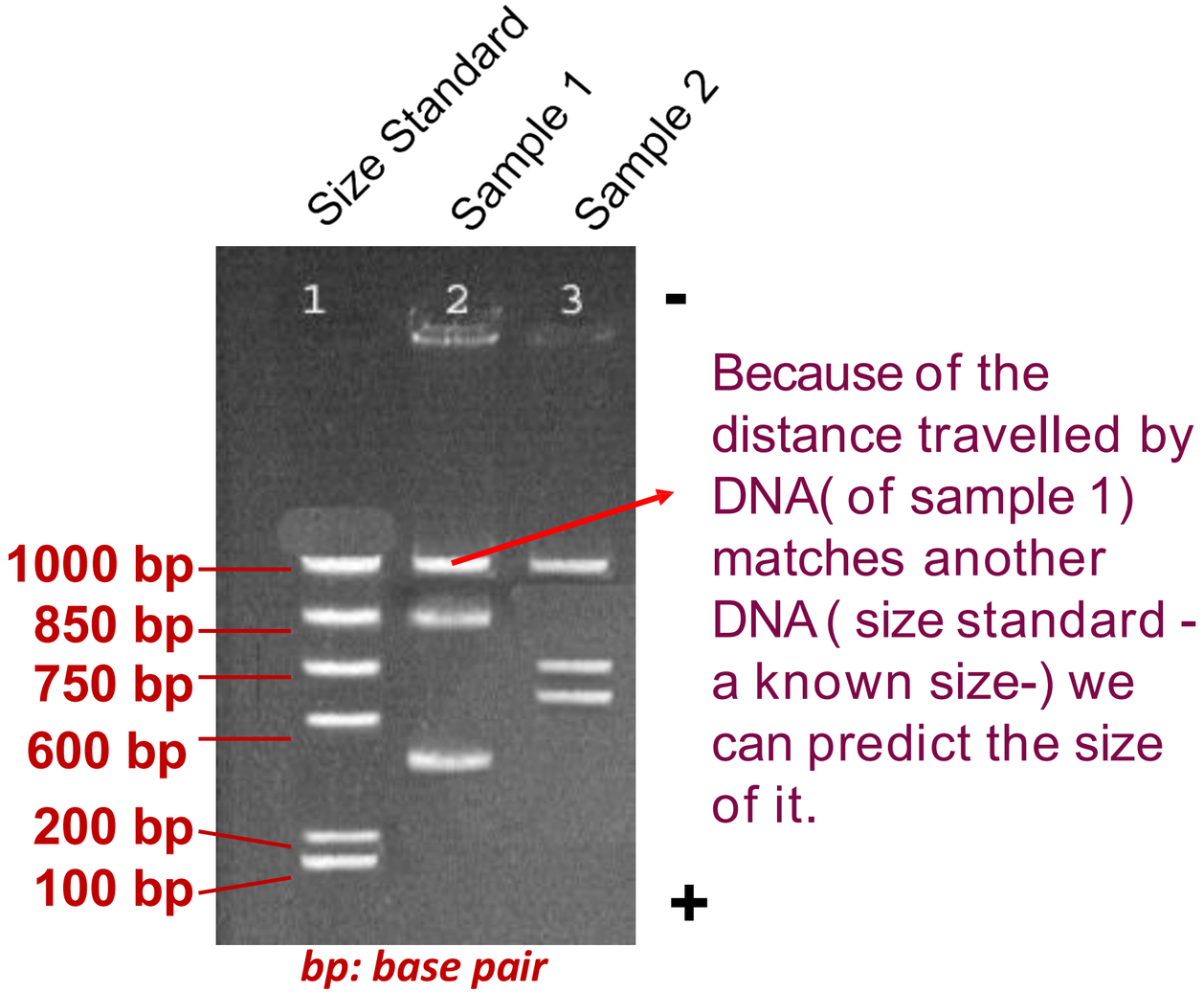
bp: base pair

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, for determining the others length in relative to the standard.**



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

Additional Resources:

رسالة من الفريق العلمي:

Post-Mid Recap

Final material before Week 10

Pathology

- [ ] Inflammation 7 (recorded)
- [ ] Inflammation 8 (recorded)
- [ ] Inflammation 9
- [ ] Inflammation 10 review (recorded)
- [ ] Neoplasia lecture 1 (online)
- [ ] Neoplasia lecture 2

Pharmacology

- [ ] Introduction to ANS in pharma Pt.1
- [ ] Introduction to ANS in pharma Pt.2
- [ ] Cholinergic drug
- [ ] Cholinergic - Blocking drug
- [ ] Adrenomimetic drug pt.1
- [ ] Adrenomimetic drug pt.2

Community

- [ ] Maternal & child health 4
- [ ] Maternal & child health 5
- [ ] Maternal & child health 6
- [ ] Growth monitoring on children (activity)
- [ ] Environmental Health
- [ ] Air pollution
- [ ] Occupational health
- [ ] Water pollution (activity)
- [ ] Injuries
- [ ] Violence against women
- [ ] Violence against children
- [ ] Bullying (activity)

Post-Mid Recap

Final material before Week 10

Metabolism

- [x] Alcohol Metabolism
- [x] Metabolism of monosaccharides and disaccharides pt.1+pt.2
- [ ] Phosphate Pentose pathway Pt.1
- [ ] Phosphate pentose pathway Pt.2+Lipid digestion and absorption Pt.1
- [ ] Lipid digestion and absorption Pt.2 (recorded)
- [ ] Degradation of fatty acids Pt.1
- [ ] Degradation of fatty acids Pt.2+ Syntheof fatty acids Pt.1
- [ ] Ketogenesis(recorded)
- [ ] Synthesis of fatty acid pt.2 + Metabolism of glycerophospholipids pt.1
- [ ] Metabolism of glycerophospholipids pt.2+ metabolism of cholesterol pt.1
- [ ] Eicosanoid metabolism (recorded)

Molecular

- [x] Structures of nucleus acids and basic techniques Pt.1
- [ ] Structures of nucleus acids and basic techniques Pt.2
- [ ] Structures of nucleus acids and basic techniques Pt.3
- [ ] Human Genome
- [ ] DNA replication Pt.1
- [ ] DNA sequencing Pt.1
- [ ] DNA sequencing (online)
- [ ] polymerase chain reaction (PCR)
- [ ] DNA replication 2
- [ ] DNA sequencing Pt.2
- [ ] Recombinant DNA technology & DNA cloning

Microbiology

- [ ] Virus Classification, Replication & Pathogenesis(online)
- [ ] Principles of Diagnosis of Virus Infections
- [ ] Principles of Treatment & Prevention of Virus Infections
- [ ] DNA viruses pt.1
- [ ] DNA viruses pt.2
- [x] Introduction to parasitology (online)
- [ ] Common protozal infection
- [ ] Common helminthic infection

قربنا نكمل المتراكم :)

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 22	Packing a DNA which is 2m long into the nucleus which is **10pm in size** is like packing a 35Km length wire into a tinnes ball.	Packing a DNA which is 2m long into the nucleus which is **10 micrometer in diameter** is like packing a 35Km length wire into a tinnes ball.
	Slide 18	**DNA** Dependent RNA polymerase	**RNA** Dependent RNA polymerase.
V1 → V2			