SPECTRAL CHARACTERIZATION OF DNA

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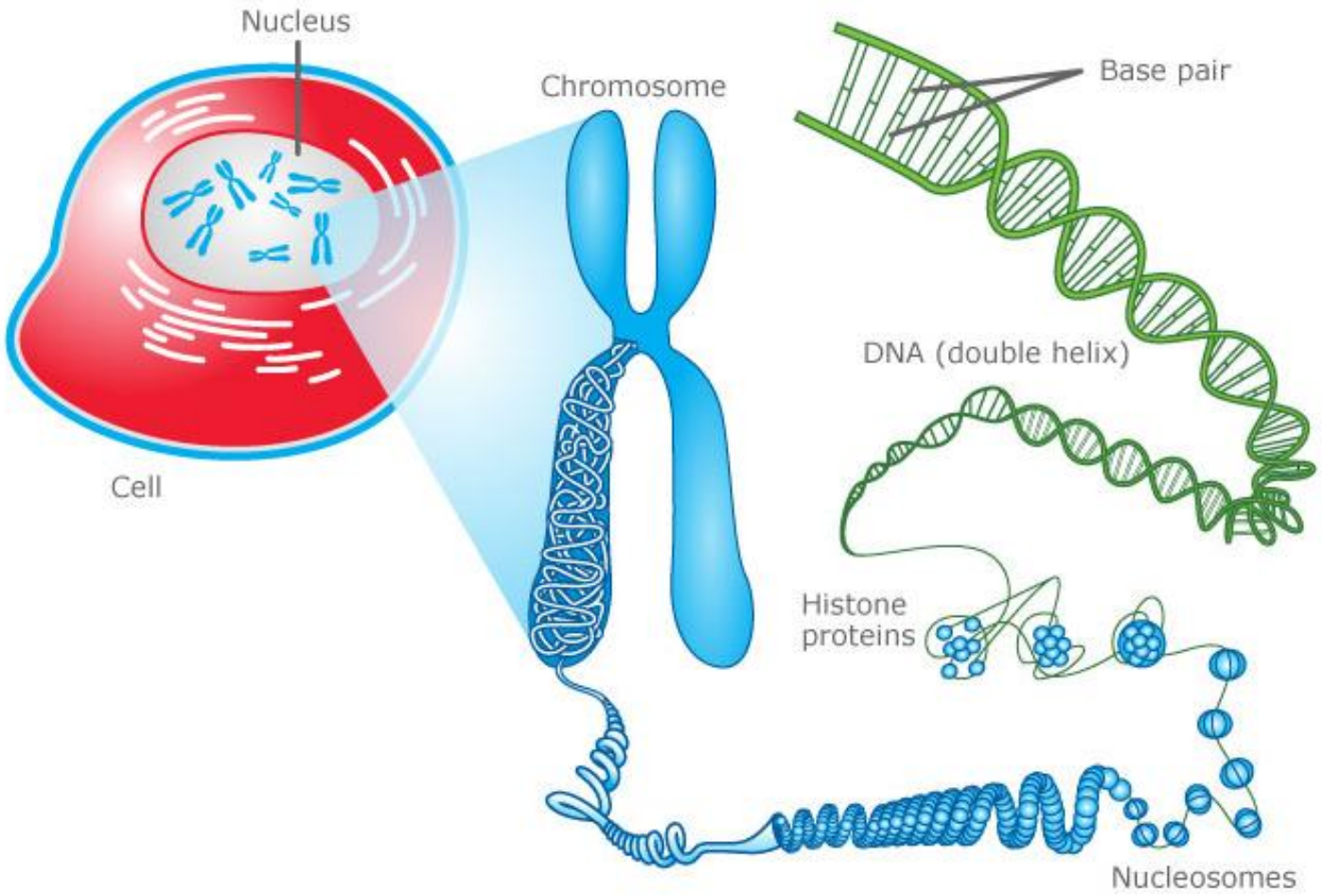
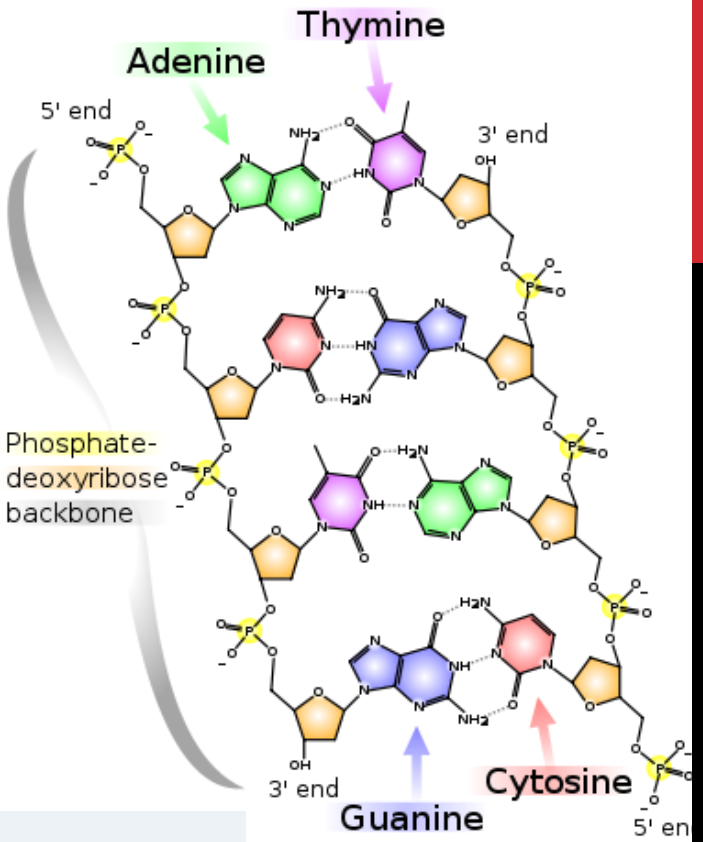
DNA

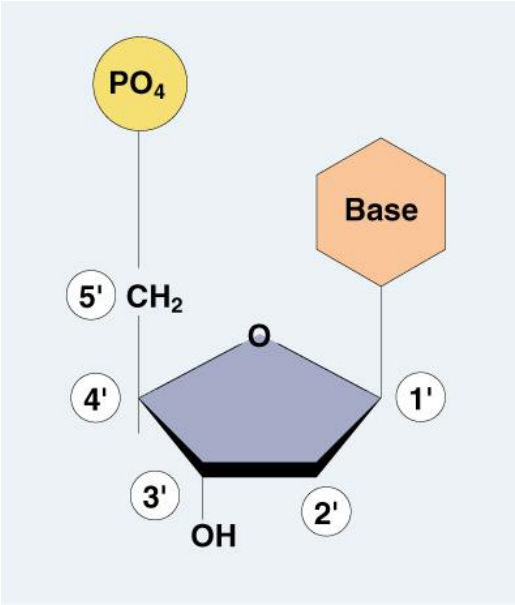
‘DEOXY RIBONUCLEIC ACID’



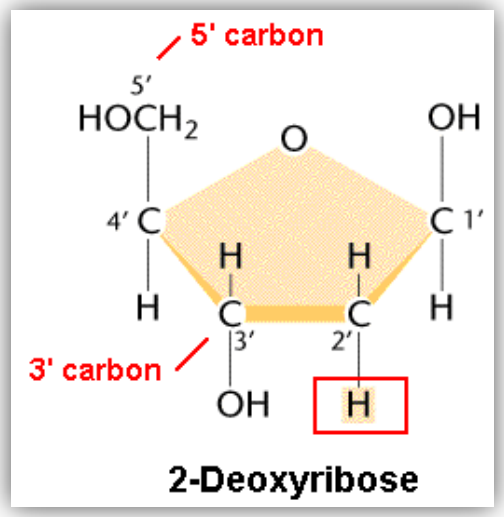
DEOXY RIBONUCLEIC ACID (DNA)

- DNA is made of 2 polynucleotide chains which run in opposite direction.
- DNA has a double helical structure.
- Each polynucleotide chain of DNA consists of monomer units called Nucleotide.
- A Nucleotide consists of 3 main components that are:
  1. sugar,
  2. phosphate,
  3. anitrogenous base.





DNA STRUCTURE



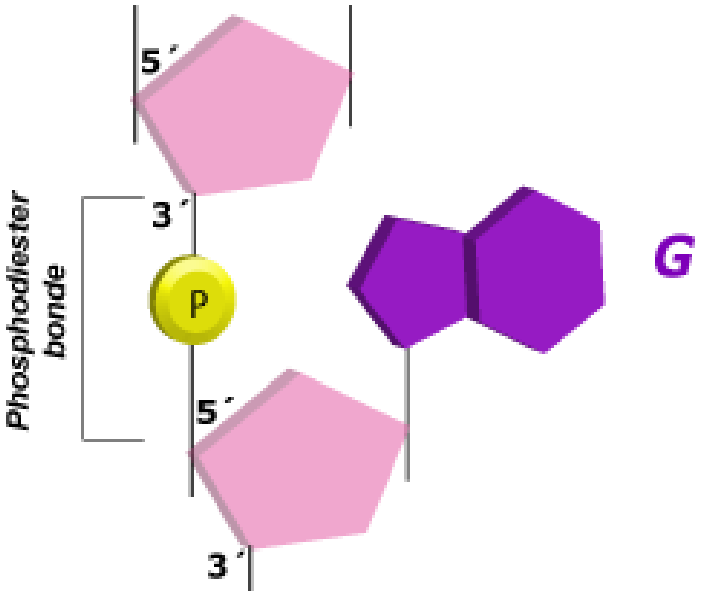
1. **Deoxyribose sugar:**

- **Is a monosaccharide 5-Carbon Sugar, Its name indicates that it is a deoxy sugar, meaning that →**

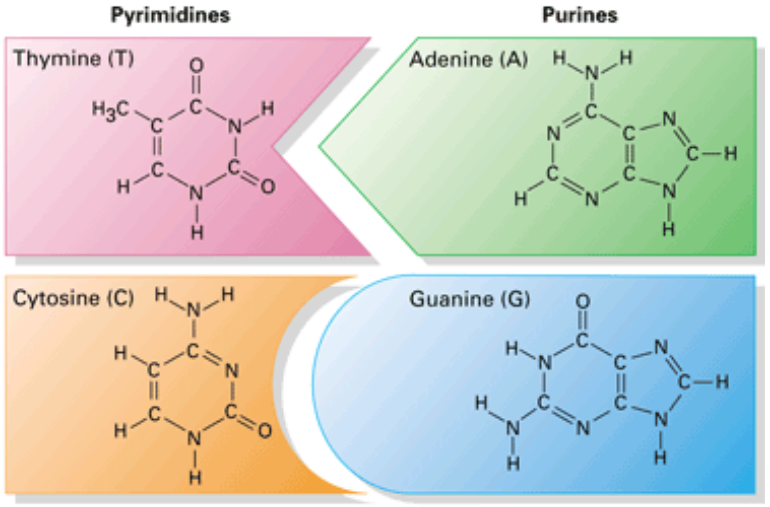
[it is derived from the sugar ribose by loss of an oxygen atom].

2. **Phosphate Group:**

- **The sugars are joined together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings.**



DNA STRUCTURE



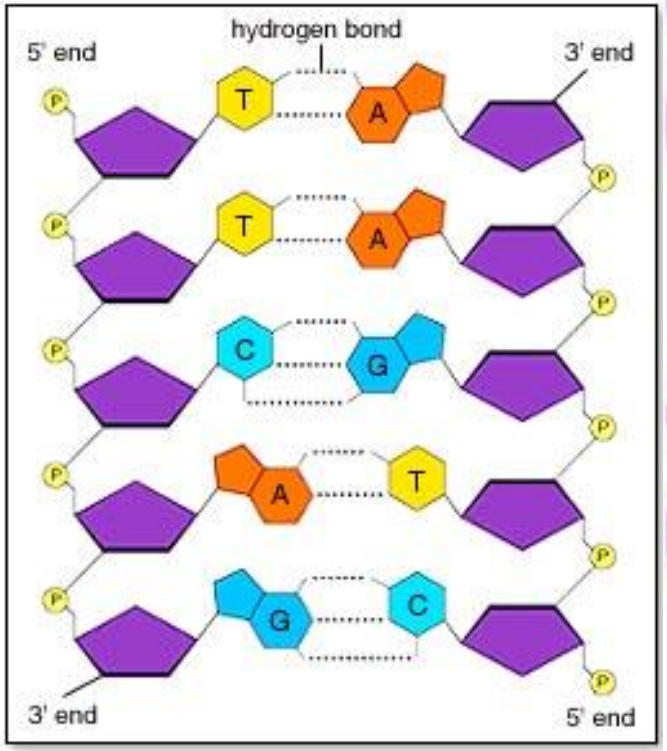
3. Nitrogenous bases:

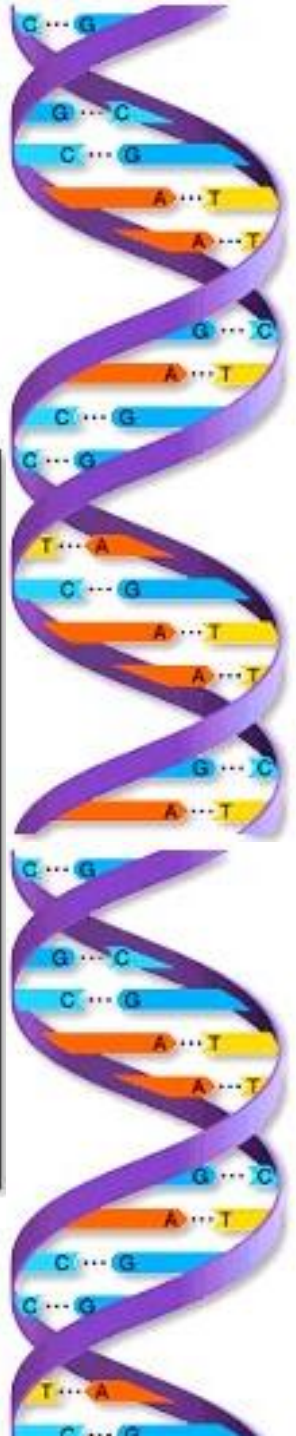
- **is a nitrogen-containing organic molecule having the chemical properties of a base**
- **They are classified as the derivatives of two parent compounds,**
  1. Purine.
     - [Adenine, Guanine]
  2. Pyrimidine.
     - [Cytosine, Thymine]

DNA STRUCTURE

4. Hydrogen bond:

- **The H-bonds form between base pairs of the antiparallel strands.**
- **The base in the first strand forms an H-bond only with a *complementary base* in the second strand.**
- **Those two bases form a *base-pair* (H-bond interaction that keeps strands together and form double helical structure).**



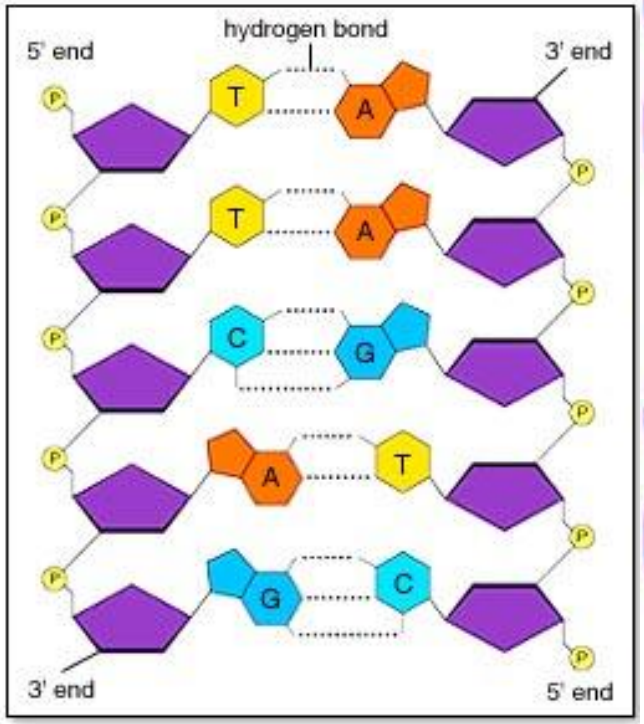


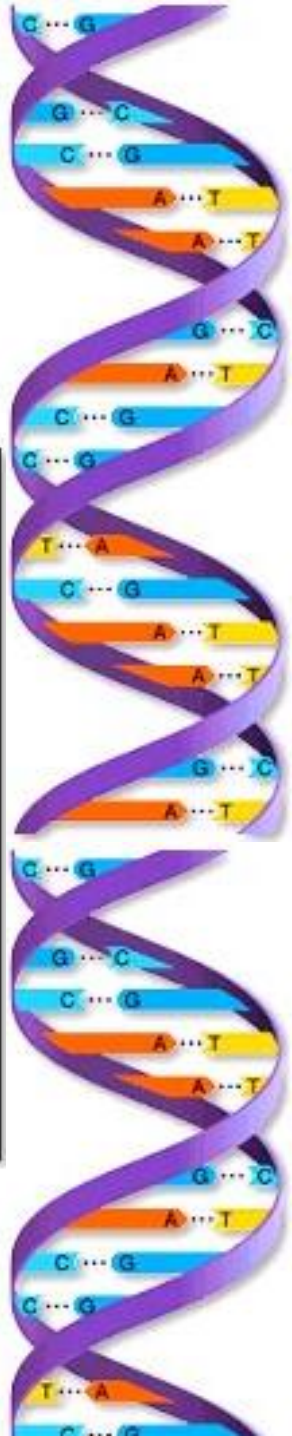
DNA STRUCTURE

- The base –pairs are:

  (A-T),(C-G).

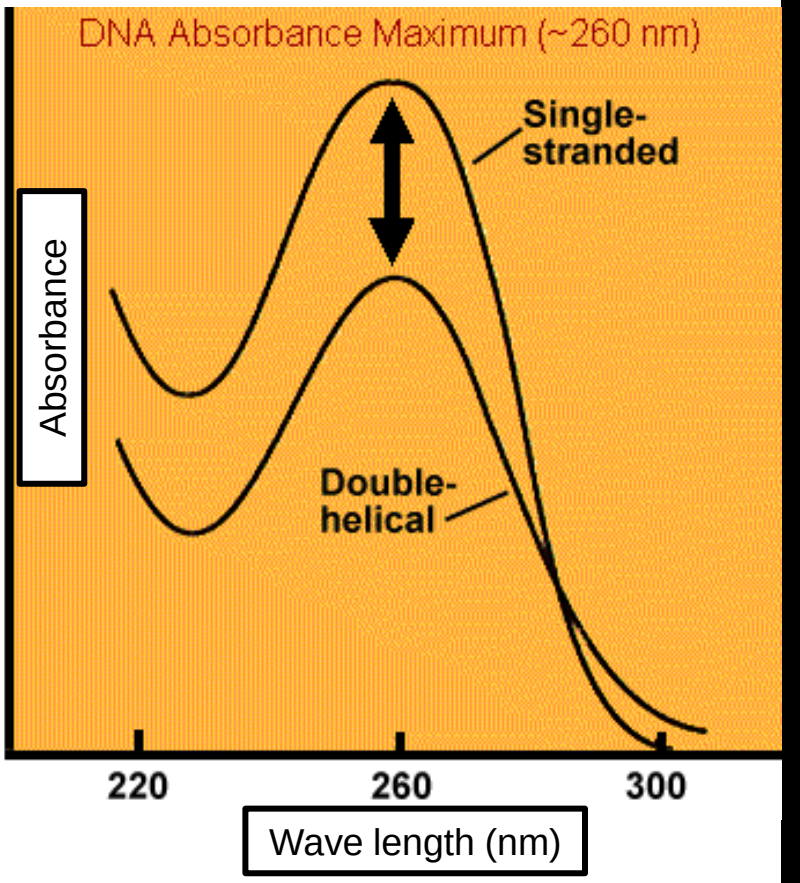
- Such interaction gives us the hint that **nitrogen-containing bases** are located **inside** of the DNA double helical structure,
- The hydrophobic bases are inside the double helix of DNA, give the **hydrophobic effect to stabilizes the double helix**.
- while **sugars and phosphates** are located **outside** of the double helical structure.





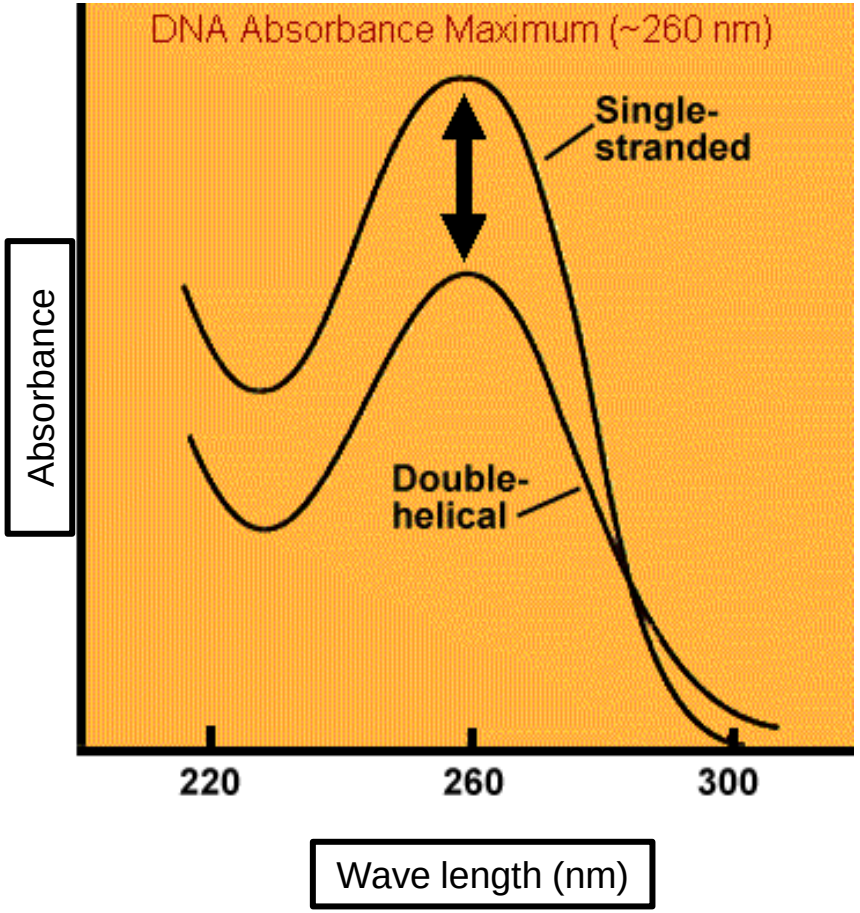
OPTICAL DENSITY OF DNA

- **Nucleic acid** would be expected to have maximum **absorbance** at 260.
- In a spectrophotometer, a sample is exposed to ultraviolet light at **260 nm**, and a photo-detector measures the light that passes through the sample.
- The more light absorbed by the sample, the **higher the nucleic acid concentration** in the sample.(**Nitrogenous bases)**



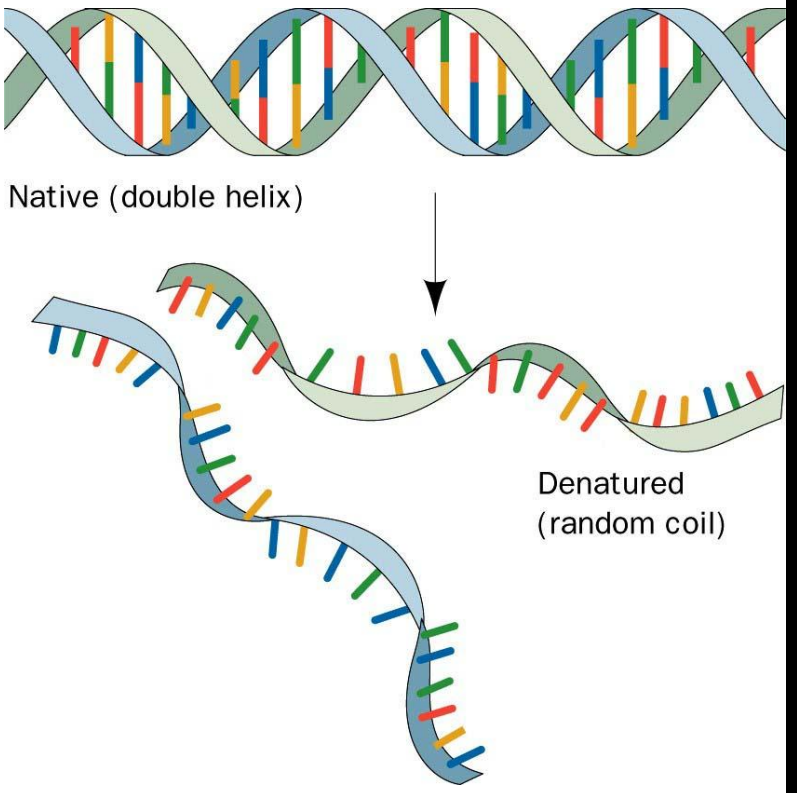
HYPERCHROMICITY

- The **increase of absorbance** (*optical density*) of a material.
- The most famous example is the **hyperchromicity of DNA** that occurs when the DNA duplex is denatured.
- The opposite, a decrease of absorbance is called **hypochromicity.**



DENATURATION OF DNA

- Many different substances or environmental conditions can denature DNA, such as:
  - strong acids, organic solvent
  - heating
  - Exposure to Radiation/ UV light



EXPERIMENT OF DAY

SPECTRAL CHARACTERIZATION OF YEAST DNA

Objective:

- To establish the wave length that represent the maximum absorbance for DNA.
- To establish the hyperchromic effect on DNA.

Principle:

- The double helix of DNA are bound together mainly by the stacking interactions, hydrogen bonds and hydrophobic_effect between the complementary bases. .
- When DNA in solution is heated above its melting temperature (usually more than 80 °C), the double-stranded DNA unwinds to form single-stranded DNA.

EXPERIMENT OF DAY

SPECTRAL CHARACTERIZATION OF YEAST DNA

Principle:

- In single stranded DNA the bases become unstacked and can thus absorb more light.
- In their native state, the bases of DNA absorb light in the 260-nm wavelength region.
- When the bases become unstacked, the wavelength of maximum absorbance does not change, but the amount absorbed increases by 30-40%.
- a double strand DNA dissociating to single strands produces a sharp cooperative transition.

EXPERIMENT OF DAY

SPECTRAL CHARACTERIZATION OF YEAST DNA

Materials:

- **DNA concentrated sample(extracted from yeast).**
- **1X saline solution (NaCl with Tri Sodium Citrate).**
- **Quartz Cuvtte.**
- **Spectrophotometer.**

EXPERIMENT OF DAY

SPECTRAL CHARACTERIZATION OF YEAST DNA

Method:

- **Set and lable 6 test tube : D1, D2, D3,D4,D5,D6**

✓1. In D1 pipette 0.5 ml of isolated DNA (extracted from Yeast) and add to it 4.5ml of 1X saline-citrate. Mix it very will.

- **Measure the absorbance of D1 at 260nm if it is > 3 :**

✓2. In D2 pipette 0.5 ml of D1 ,add to it 4.5ml of 1X saline-citrate. Mix it very will.

- **Measure the absorbance of D2 (if the absorbance is greater than 1,dilute the solution until you obtain A260 of 1 or slightly less).**

EXPERIMENT OF DAY

SPECTRAL CHARACTERIZATION OF YEAST DNA

Method:

- When the absorbance of solution (A260≈1.0) is obtained read the absorbance of the solution at the following wave lengths:

(240,245,250,255,260,265,270,275,280)

- **using 1X saline as a blank.**

EXPERIMENT OF DAY

SPECTRAL CHARACTERIZATION OF YEAST DNA

Method:

- Now take the dilution tube which give an absorbance=1 ,cover the tube and put it in boiling water bath for 15 min
- **Immediately measure the absorbance at the following wave lengths:**

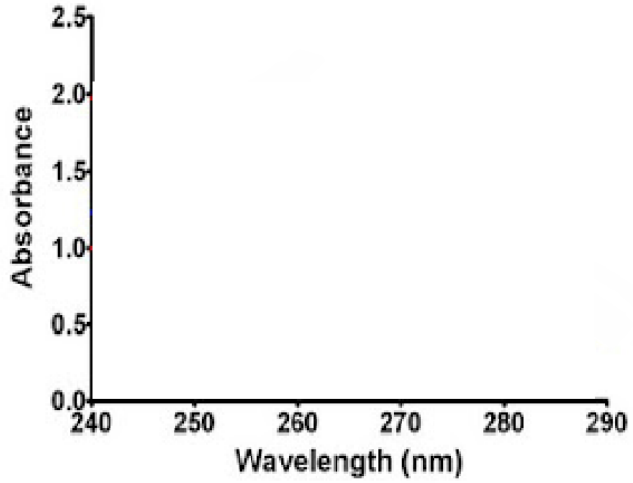
  (240,245,250,255,260,265,270,275,280)
- using 1X saline as a blank.

EXPERIMENT OF DAY

SPECTRAL CHARACTERIZATION OF YEAST DNA

Results:

- ✓ **Plot The absorption spectra of the native DNA solution and the denatured DNA against wave lengths.**
- ✓ **Record Your result and write your comment in the discussion.**



Wave length (nm)	Absorbance of isolated DNA	Absorbance of heated DNA
240		
245		
250		
255		
260		
265		
270		
275		
280		

.. Now ..

‘ Wear your gloves and lab coat
And Act Like a biochemist ’

Thank You

☺