SPECTRAL CHARACTERIZATION OF DNA
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.
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 ]
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 photodetector 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
Objective:

• To establish the wavelength 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.
**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 Cuvette.
• Spectrophotometer.
Method:

• Set and label 6 test tubes: D1, D2, D3, D4, D5, D6

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

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

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

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

• When the absorbance of solution ($A_{260} \approx 1.0$) 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.
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.
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.

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<tr>
<th>Wave length (nm)</th>
<th>Absorbance of isolated DNA</th>
<th>Absorbance of heated DNA</th>
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<tbody>
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Now

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

Thank You 😊