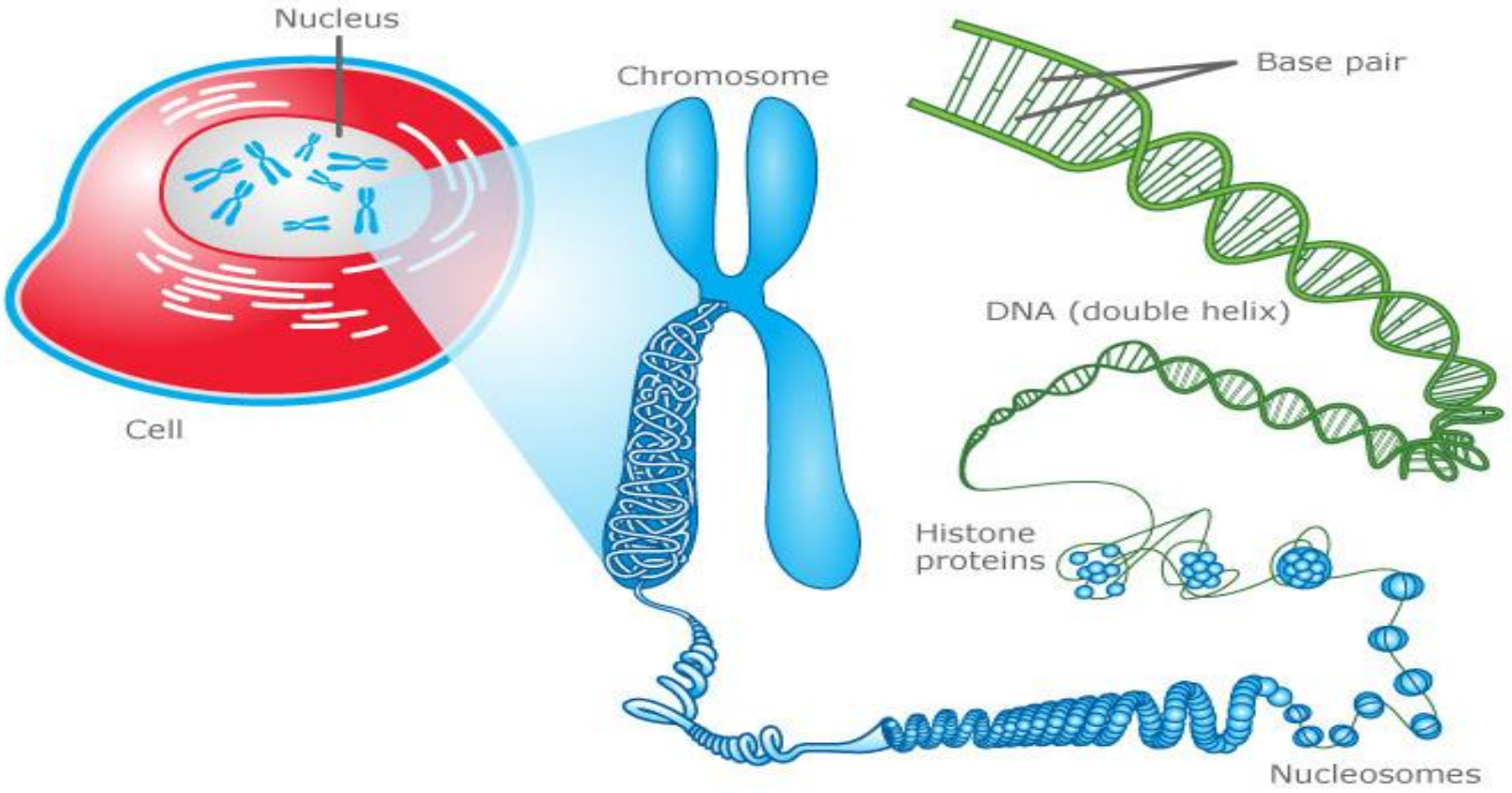
Spectral Characterization of DNA

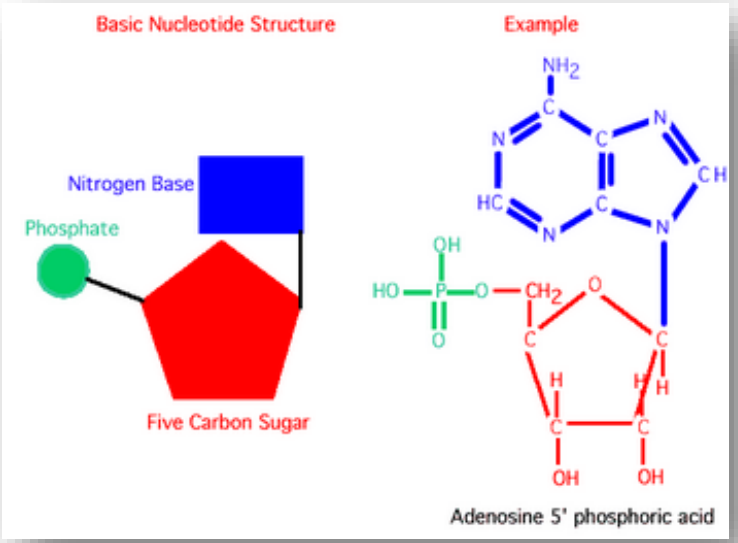
BCH302 [Practical]

DNA = [Deoxyribonucleic acid]

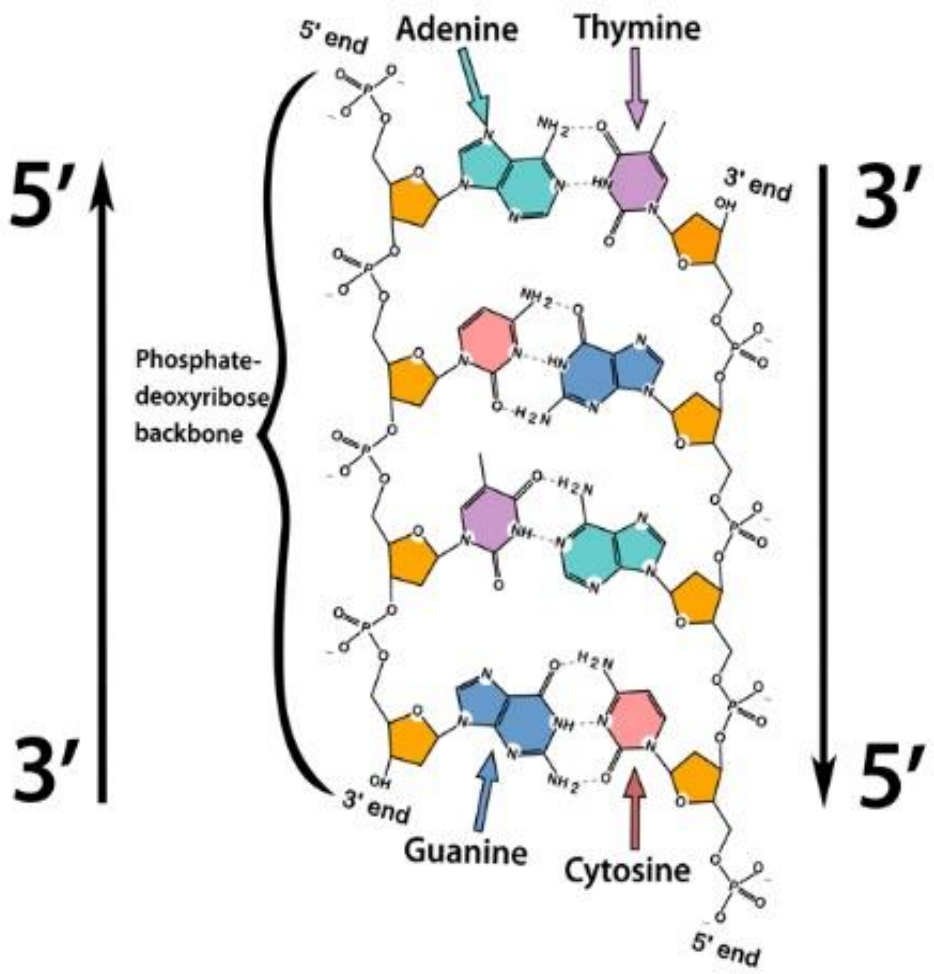


What DNA made up of :

- DNA is made of 2 polynucleotide chains which run in **opposite direction "antiparallel " .**
- DNA has a **double helical structure.**
- Each polynucleotide chain of DNA consists of monomer units of nucleotides.
- **A monomer unit (nucleotide) consists of 3 main components that are:**
  1. Pentose sugar.
  2. Phosphate.
  3. Nitrogenous base.

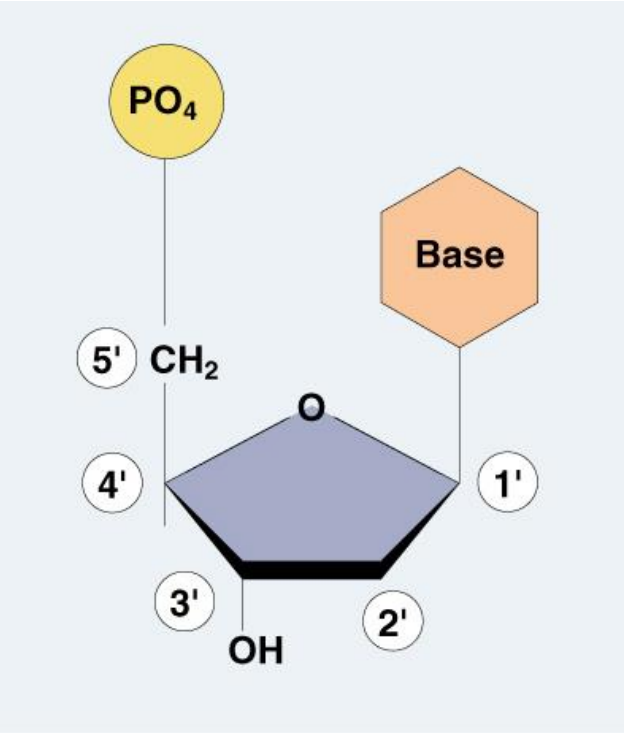


DNA double helical structure:

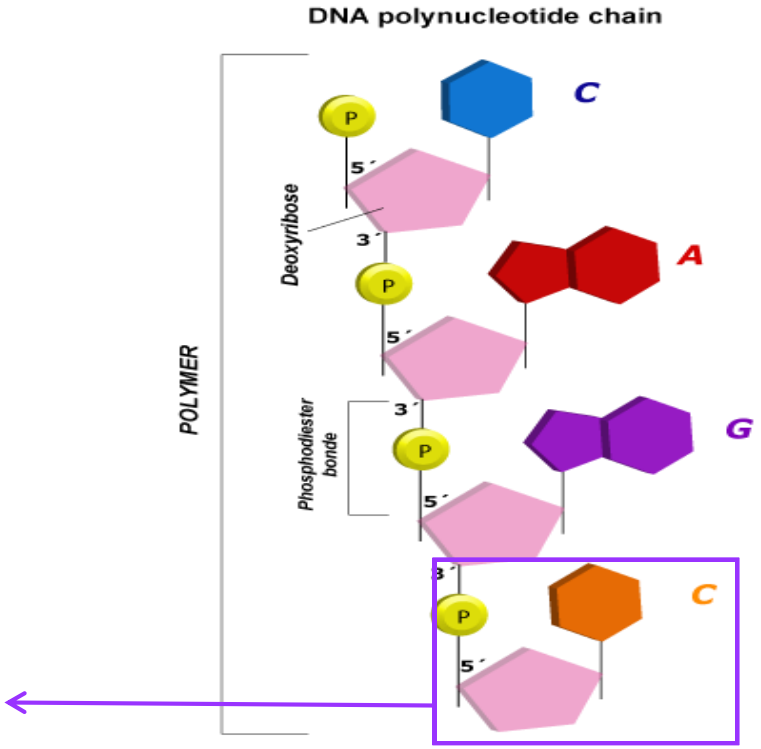


[antiparallel]

Nucleotide (DNA building block):



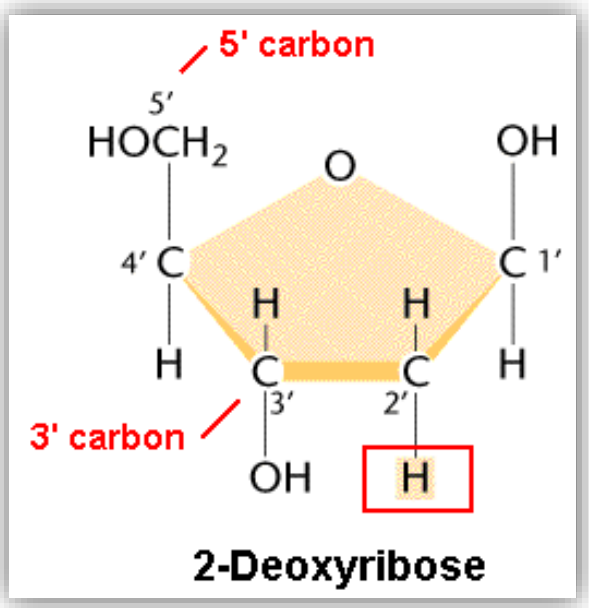
Monomer



DNA structure:

1. Deoxyribose sugar:

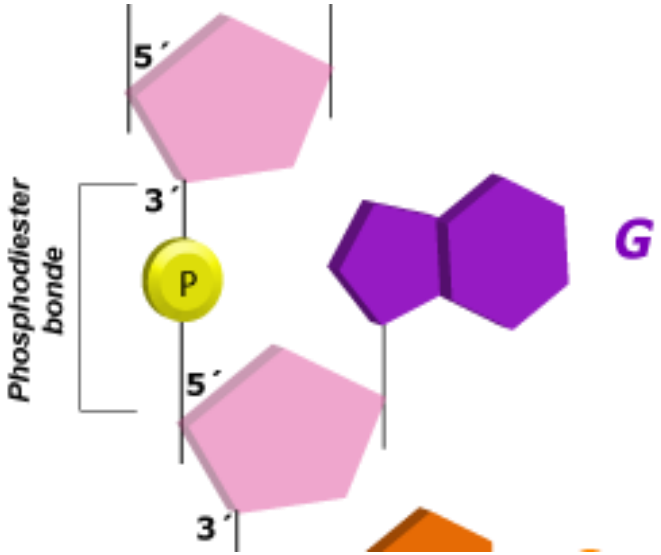
Is a monosaccharide 5-Carbon Sugar, Its name indicates that it is a **deoxy sugar**, meaning that → [it is derived from the sugar ribose by loss of an oxygen atom].



DNA structure:

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.

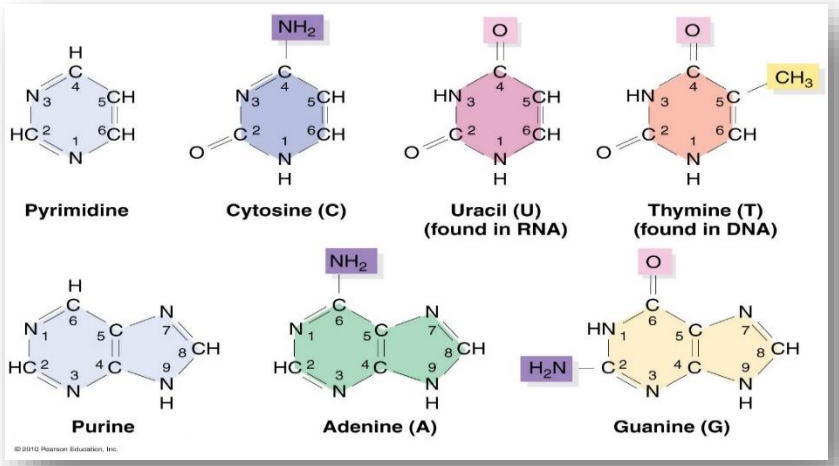


DNA structure:

3. Nitrogenous bases:

- is a nitrogen-containing organic molecule having the chemical properties of a base.

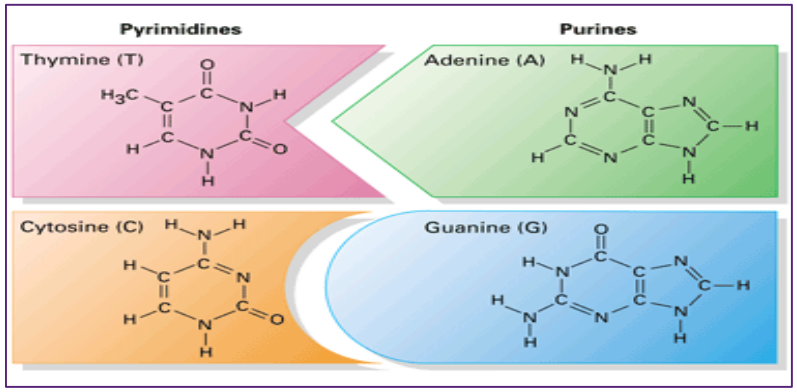
- **They are classified as the derivatives of two parent compounds:**
  1. Purine: [Adenine, Guanine]
  2. Pyrimidine : [Cytosine, Thymine]

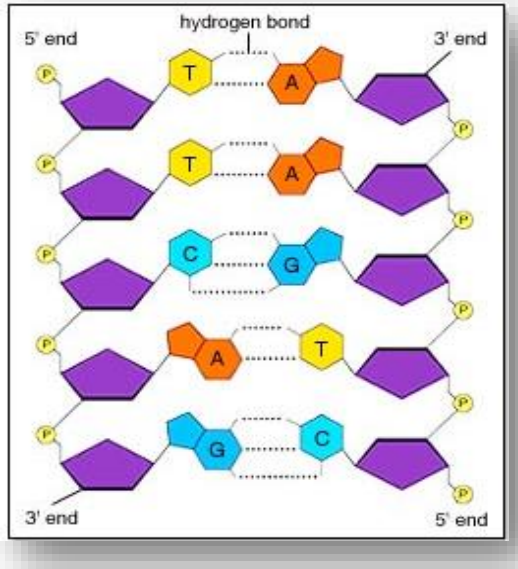


DNA structure:

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).
- Sugars and phosphates are located outside of the double helical structure.

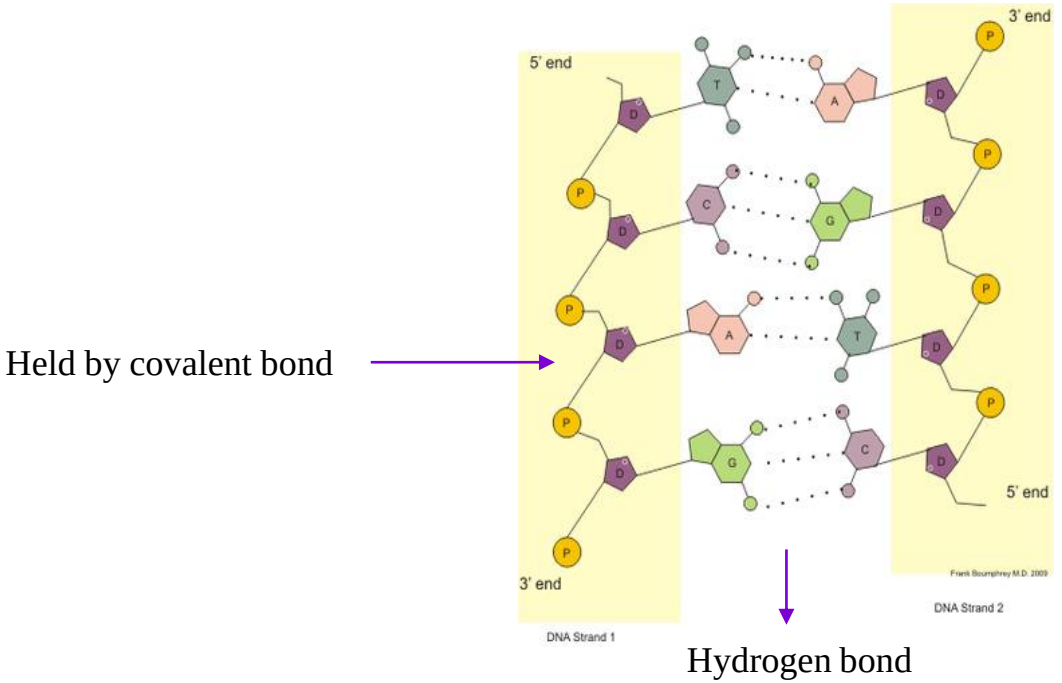




Types of bonds in DNA:

- **The backbone of the DNA** (sugars and phosphate) is held by **covalent bond** "phosphodiester bond".

- **The bases in the two strands** are linked togather by **hydrogen bond** (and hydrophobic effect between the complementary bases).

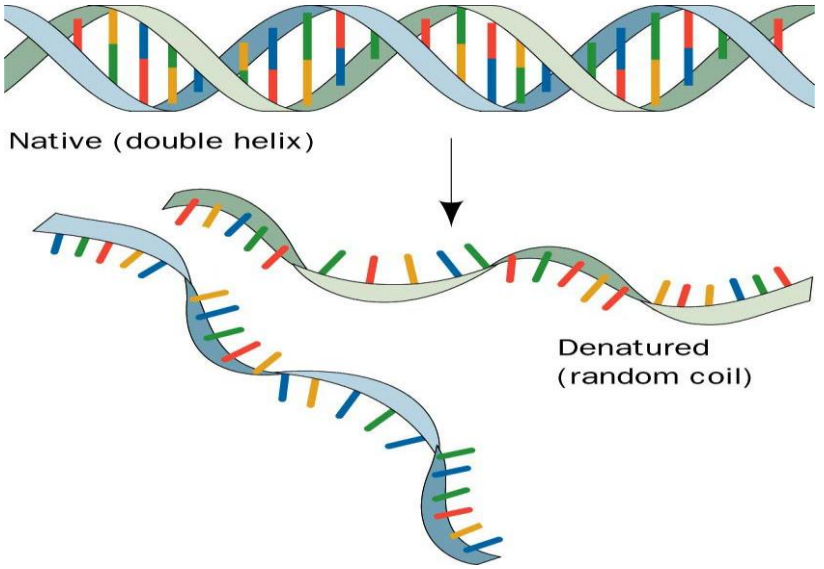


Denaturation of DNA :

- Denaturation is a process by which nucleic acids, such as DNA, lose their three-dimensional structures and consequently their primary functions.

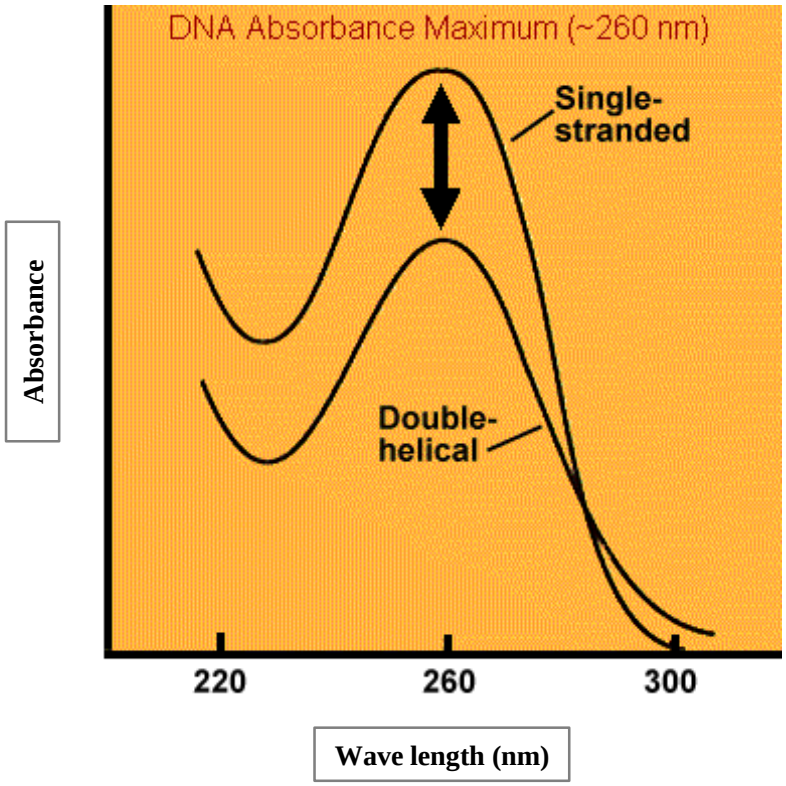
- **Many different substances or environmental conditions can denature nucleic acids, such as:**

1. Strong acids, organic solvent.
2. Heating.
3. Exposure to Radiation/ UV light.



Optical density of DNA :

- Nucleic acid have **maximum absorbance at 260 nm**, It absorbs at this wavelength because of the **nitrogenous bases** (A, G, C and T) of DNA.

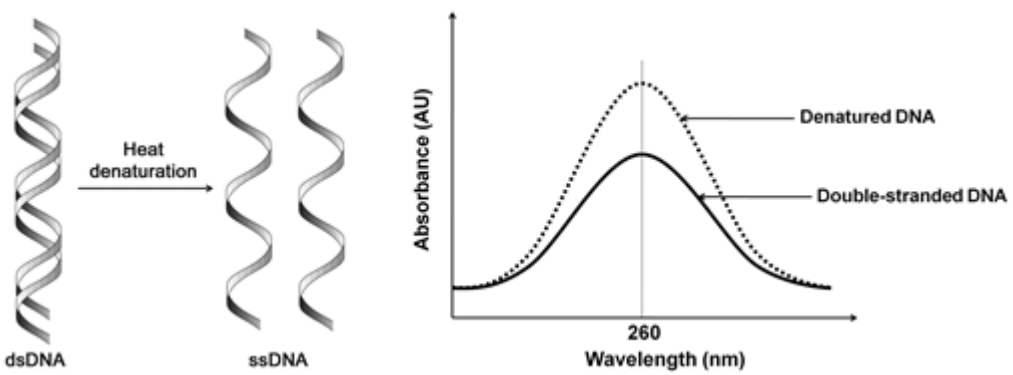


Hyperchromicity:

- **In general :** It is the increase of absorbance (optical density) of a material.
- The hyperchromicity of DNA that occurs when the **DNA duplex is denatured.**
- When DNA denatures [e.g. by heat], it's strands separate, allowing more light to be absorbed by the non-stacked bases[single DNA strands].

➔ Due to denaturation of DNA the bases become exposed to the surface and able to absorb more light at 260 nm.

- **This action is calling the hyperchromic effect.**



- Note:

The opposite, a decrease of absorbance is called hypochromicity

Practical part

Experiment 1 : Spectral characterization of DNA

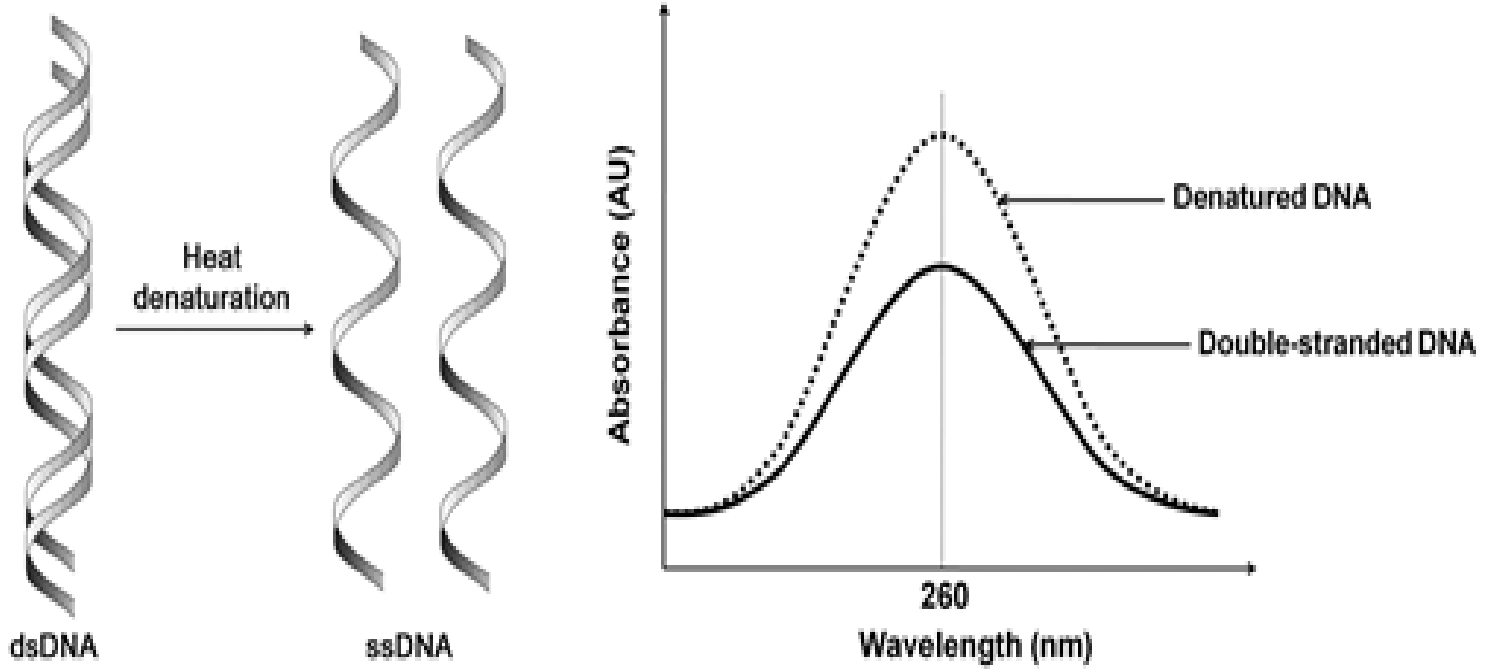
Objective:

- To determine the wave length that represent the maximum absorbance for DNA (the optimum wave length for DNA).
- To establish the effect of temperature on the absorbance of DNA or [hyperchromic effect].

Principle:

1- **The double helix of DNA** are bound together mainly by 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.

2-In single stranded DNA the bases become unstacked and can thus absorb more light.
In their native state, the bases of DNA absorb light at 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%.


Heat
denaturation
dsDNA
ssDNA
Absorbance (AU)
Denatured DNA
Double-stranded DNA
260
Wavelength (nm)

Experiment 1 : Spectral characterization of DNA

Method:

1. Measure the absorbance at the following wavelengths:(240,245,250,255,260,265,270,275 and 280 nm). Using distal water as a blank.
2. Cover the tube and put it in boiling water bath for 15 min.
3. Immediately measure the absorbance at same wave lengths.
4. Plot the absorption spectra of the native DNA solution and the denatured DNA against wavelengths.

Results:

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