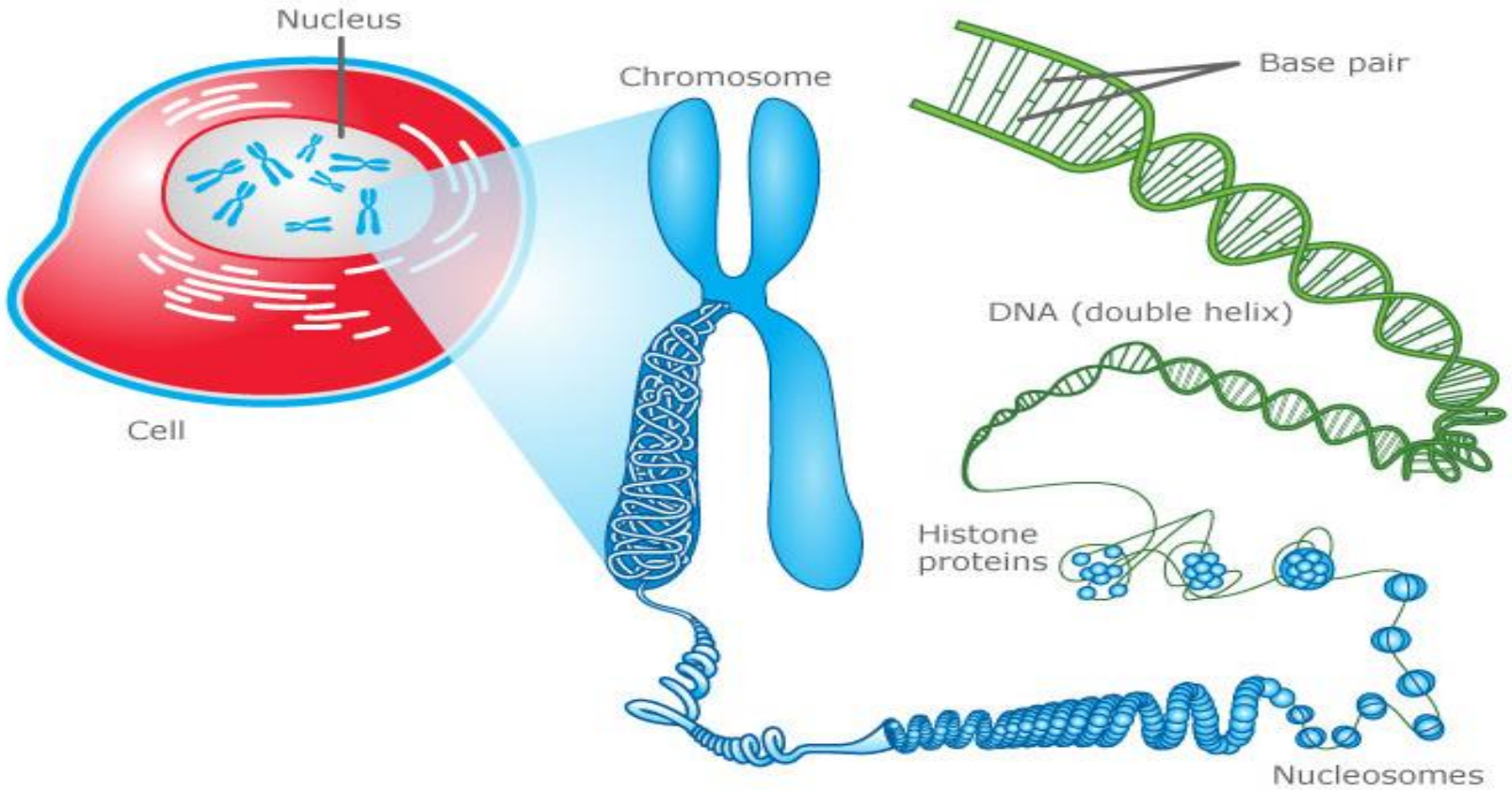
Spectral Characterization of DNA

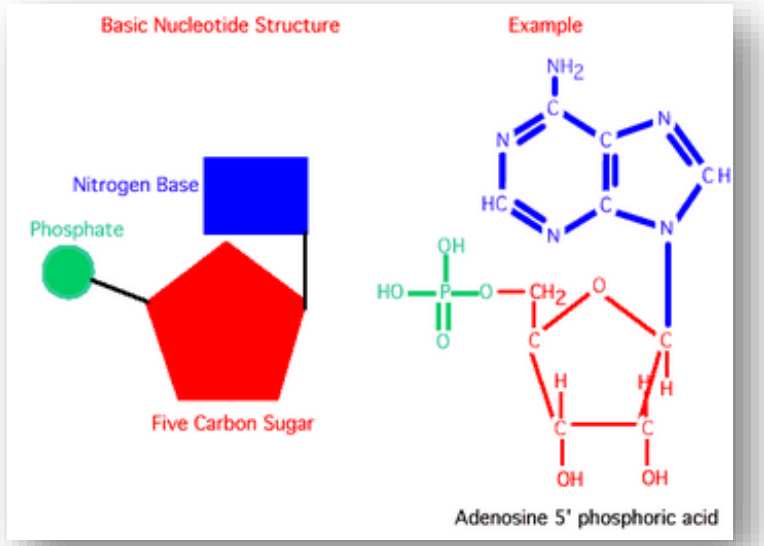
DNA = [**D**eoxyribo**n**ucleic **a**cid]



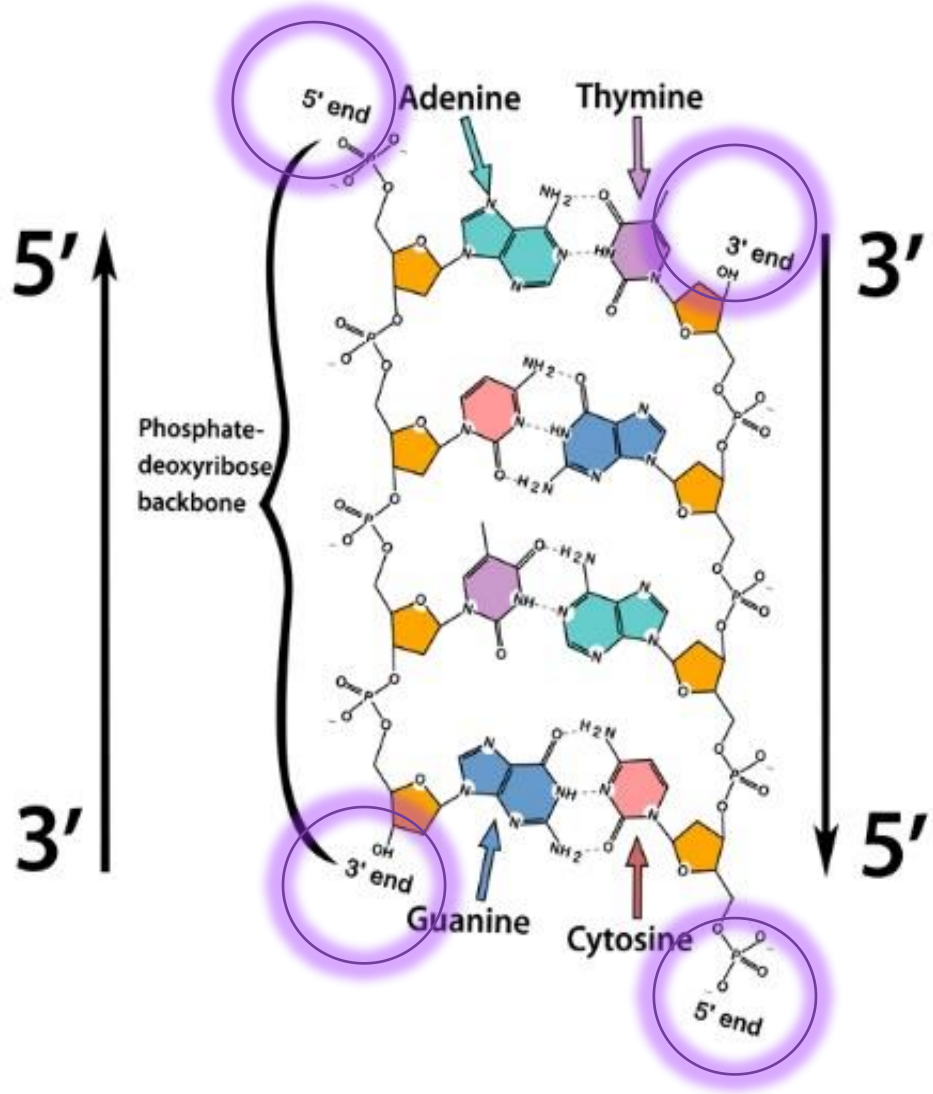
What DNA made up of :

- 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-deoxyribose).
2. Phosphate.
3. Nitrogenous base (either A,T,G or C).

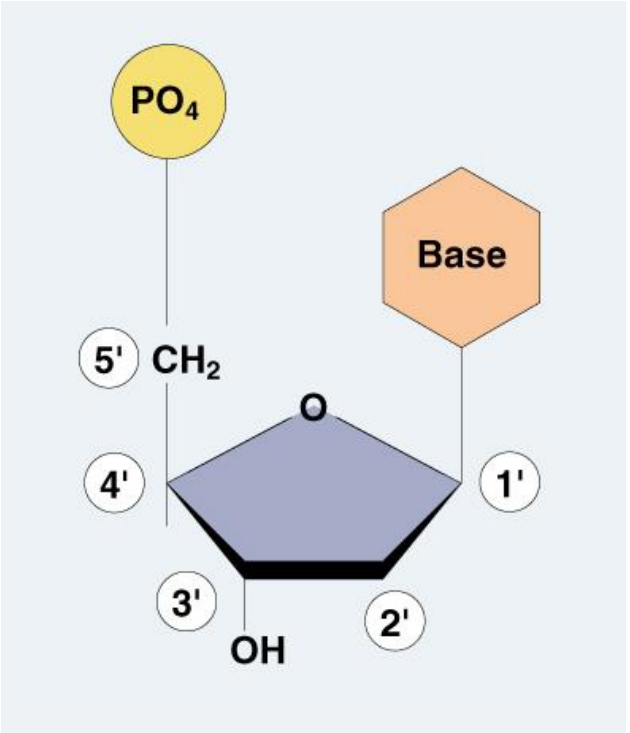


DNA double helical structure:

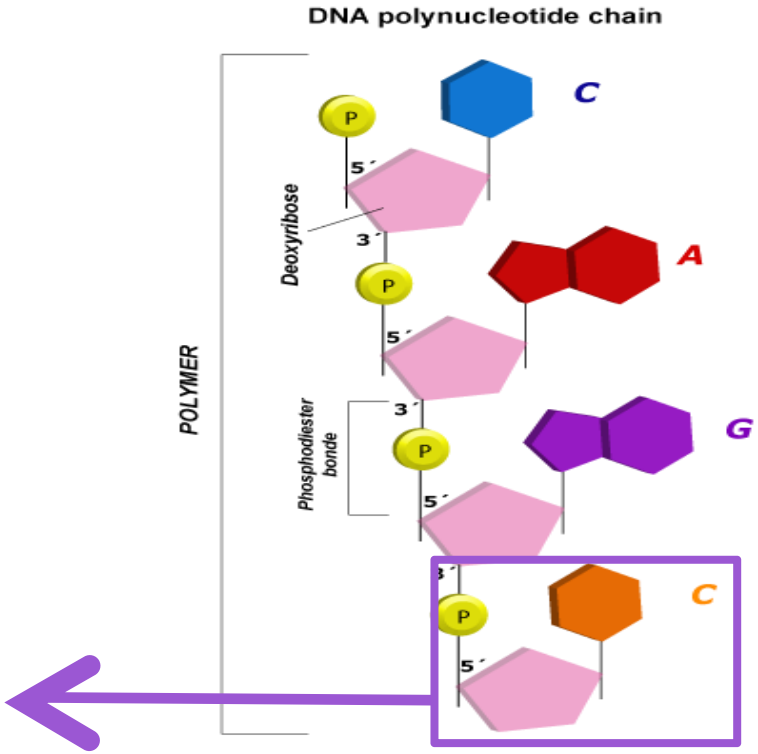


[antiparallel]

Nucleotide (DNA building block):



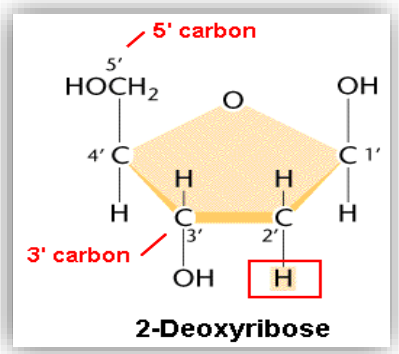
Monomer



DNA structure:

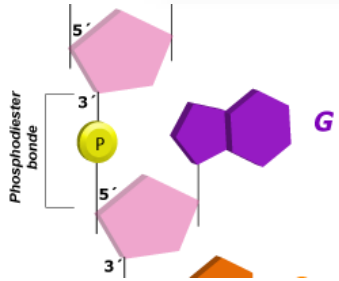
1. Deoxyribose sugar:

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].



2. Phosphate Group:

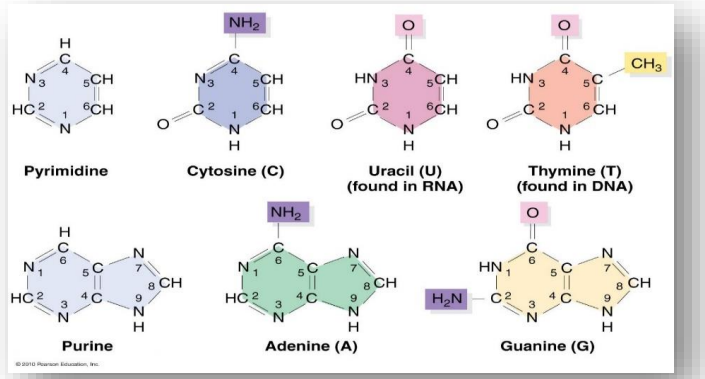
The sugars are joined together by phosphate groups that form **phosphodiester bonds** between the **third** and **fifth** carbon atoms of adjacent sugar rings.



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]

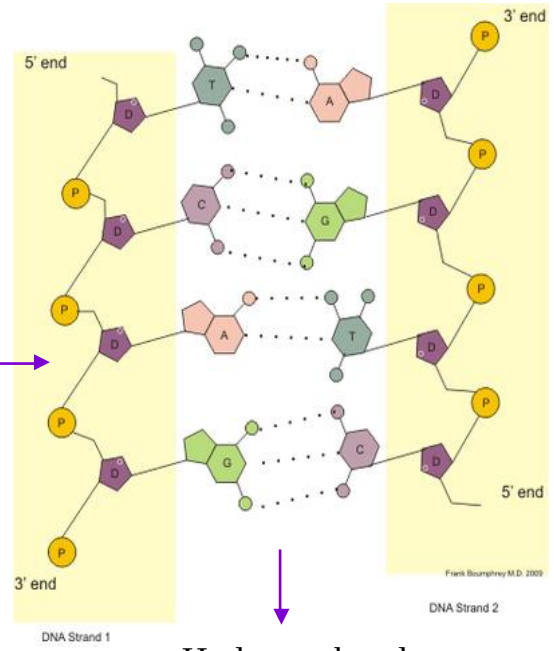


Types of bonds in DNA:

- **The backbone of the DNA** (sugars and phosphate) is held by **covalent bond** "phosphodiester bond" ➔ Sugars and phosphates are located outside of the double helical structure.

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

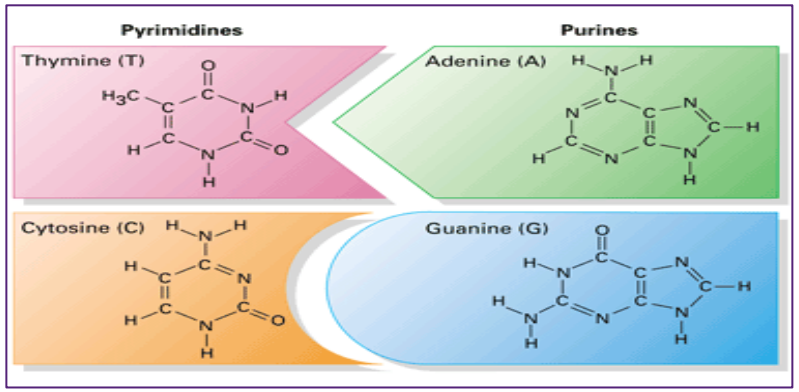
Held by covalent bond
(phosphodiester bond)



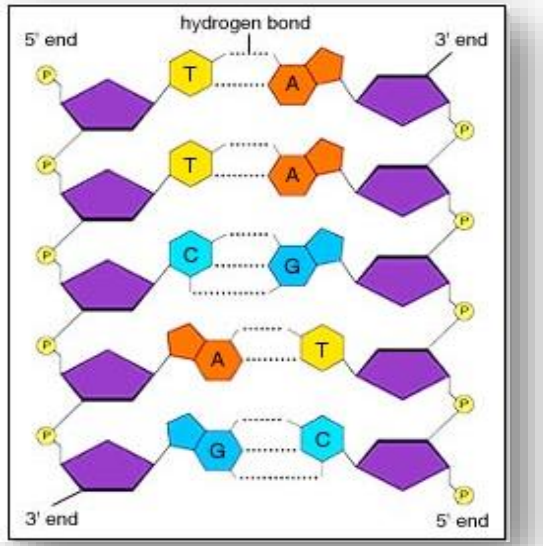
Hydrogen bond

Hydrogen bond:

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



Complementarity

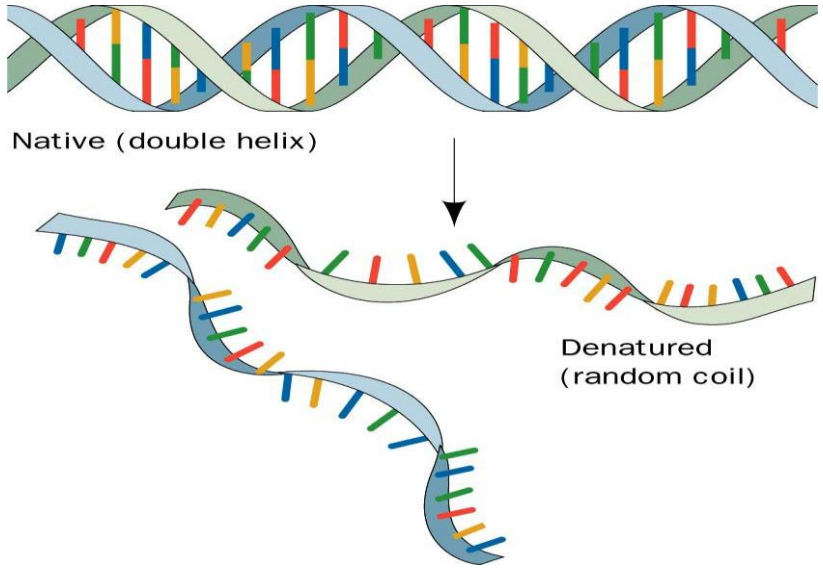


Denaturation of DNA :

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

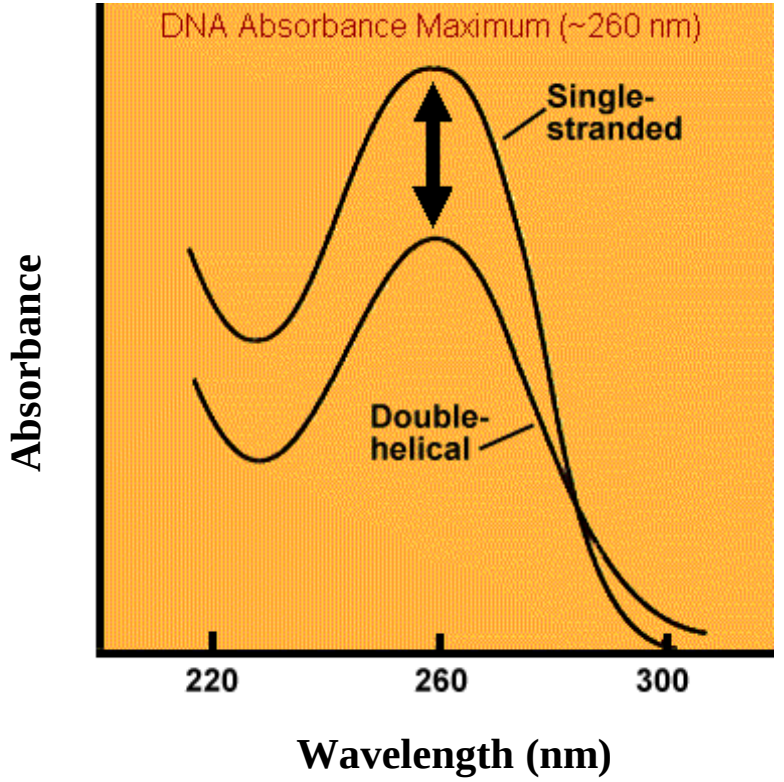
- **Many different substances or environmental conditions can denature nucleic acids, such as:**

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.

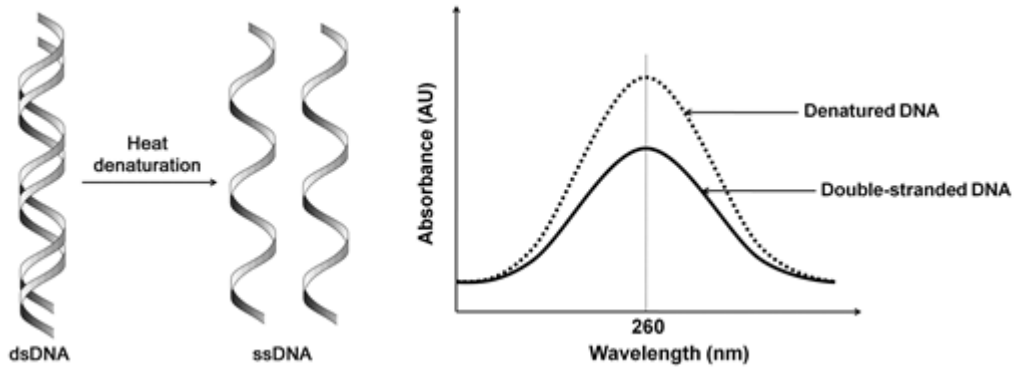


Hyperchromicity:

- **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:**

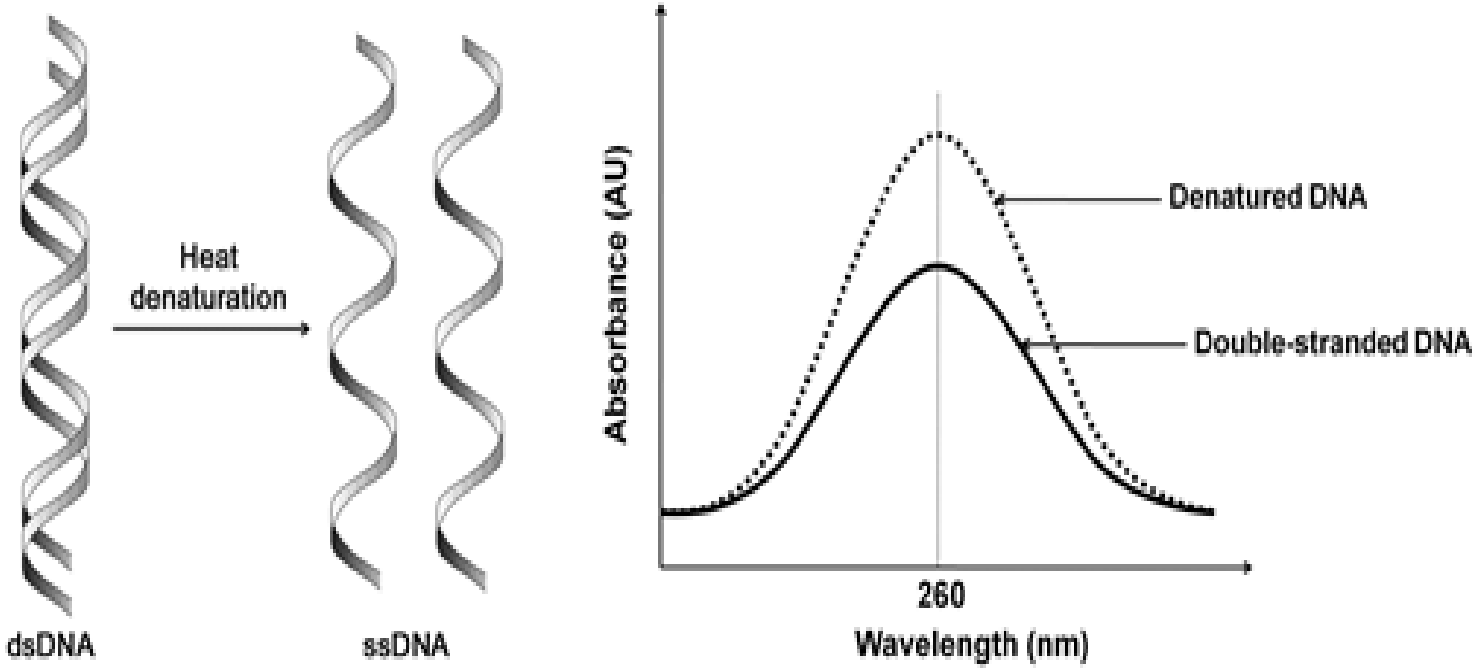
- To determine the optimum wave length for DNA.
- To establish the effect of temperature on the absorbance of DNA or [hyperchromic effect].

➢ **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. Place the sample on the thermomixer at 90 °C 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:

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