# Experiment (3): Characterization of DNA by Spectrophotometric Assay and Melting Temperature (Tm)

## Aim:

- Determination of the concentration and purity of extracted DNA using UV spectrophotometer.
- Determination of DNA melting temperature and GC content percentage.

## Introduction:

DNA extracts must meet downstream applications requirements. For that after each extraction approach, DNA undergo characterization process, where quantity and quality (concentration, purity and intactness) must be measured. The characterization of DNA could be performed with a number of different techniques. In this experiment, spectrophotometric and melting temperature will be used to determine DNA concentration, purity, and GC content.

***Characterization of extracted DNA by spectrophotometric assay****:* DNA concentration and purity can be determined by measuring the absorption of ultraviolet light. The DNA has a maximum and minimum absorbance at 260 nm and 234nm, respectively and the purines and pyrimidine in nucleic acid are responsible for these absorptions. At 260 nm double-stranded DNA has specific absorption coefficient of 0.02 $(\mu g/ml)^{-1}cm^{-1}$. Moreover, the $A_{260}/A_{280}$ ratio allow to detect nucleic acid purity from proteins contamination since proteins have maximum absorption at 280 nm. Highly purified DNA samples have a 260/280 nm ratio of (1.8-1.9), thus below (1.8) a significant amount of protein impurity may present within the sample. The $A_{260}/A_{230}$ ratio determined to confirm that the sample is pure from carbohydrates, peptides, ethanol or any organic compounds, and it is usually between 2 and 2.2.

***DNA melting temperature and GC content:*** The two strands of the DNA double helix separate when hydrogen bonds between the paired bases are disrupted and this can occur in vitro if the pH of the DNA solution is altered, or if the solution is heated. When DNA is heated, the double-stranded DNA (dsDNA) unwinds and separates into single-stranded (ssDNA) by breaking the hydrogen bonds between the complementary bases (A═T and G≡C). This process called DNA denaturation and it can be monitored by measuring its absorbance at 260 nm. The absorbance of DNA at 260 nm increases as the DNA becomes denatured, a phenomenon known as the hyperchromic effect. The opposite, a decrease of absorbance is called hypochromic effect. The Tm is the temperature at which 50% of the DNA is unpaired (denatured), and it is depending on both length and GC content of the DNA. The GC content of the DNA that is critical for its stability, and it can be provided by melting temperature (Tm) profile. This profile can be achieved by gradual denaturation of dsDNA into ssDNA.

PAUSE AND THINK ➔ What is the principle behind hyper/hypochromic effect?

## Principle:

DNA concentration and purity from proteins and carbohydrates, peptides, ethanol or any organic compounds can be determined by measuring the absorption of ultraviolet light. This achieved by using Beer-Lambert law and calculating the $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios. Meanwhile, when a dilute aqueous DNA solution is heated slowly, the two strands of the double helix gradually separate, leading to the formation of a single stranded DNA (denaturation). It results in an increase in absorbance at 260 nm. Temperature for midpoint of denaturation gives Tm by increasing the temperature slowly and measuring absorbance at 260 nm as melting profile can be generated. The DNA of each species has a specific denaturation curve which is dependent on the % GC content and length. In double stranded DNA, G and C base pairing is more stable and requires more heat energy to break the three hydrogen bonds to separate the strands.

## Materials:

The extracted blood and plant DNA from previous experiments, 0.1 X SSC buffer.

**Preparation of 20X SSC buffer**

Dissolve 175.3 g of NaCl, 88.2g of sodium citrate dehydrate in 8000 ml distilled water. Adjusts pH to 7.0 with diluted HCl. Make up the final volume to 1 L by distilled water.

## Protocol:

### *A. Characterization of DNA by Spectrophotometric Assay (concentration and purity):*

1. Prepare 1 ml of the sample by diluting your extracted DNA (your stock) in 0.1 X SSC buffer with 1:10 ratio.
2. Place the DNA sample in a quartz cuvette (why?) along with a second cuvette contains distal water as a blank, then set the spectrophotometer as follows:
   *Nucleic acid → DNA → 10 mm → µg/ml → yes → Enter the dilution factor.*

*OR*

2. Traditionally, measure the absorbance at 230, 260 and 280 nm.

### *B. Melting Temperature of DNA:*

1. In a microcentrifuge tube, prepare 1 ml of the sample by diluting your extracted DNA (your stock) to 10 µg/ml with 0.1 X SSC buffer.
2. In another microcentrifuge tube, pipette 1 ml of distilled water.
3. Place the tubes in a thermomixer at 25°C and allow temperature to equilibrate (5 min).
4. Immediately read the absorbance at 260 nm.
5. Raise the temperature to 50 °C, 60 °C, 70 °C, and boiling, then repeat step 4.

## Results:

### A. *Characterization of DNA by Spectrophotometric Assay (concentration and purity):*

| Wavelength (nm) | Absorbance of DNA | |
|---|---|---|
| | Rat | Plant |
| 230 | | |
| 260 | | |
| 280 | | |

- Find out the concentration of the DNA samples using the following equation:

**Concentration of DNA (µg/ml ) = ($A_{260}$ / ε L)× Dilution factor (DF)**

- Determine the purity of the DNA samples by calculating $A_{260}/A_{280}$ and $A_{260}/A_{230}$ ratios.

### B. *Melting Temperature:*

| Temperature (°C) | DNA Absorbance at 260 nm | |
|---|---|---|
| | Rat | Plant |
| 25 | | |
| 50 | | |
| 60 | | |
| 70 | | |
| Boiling | | |

- Plot the value of absorbance vs. temperature and calculate the Tm for sample DNA.
- Find out the GC content of your sample using the following formula:

**(G + C)% = (Tm - 69.3) x 2.44**

## References:

1. Surzycki S. Basic techniques in molecular biology. Springer. (2000).