Characterization of DNA by Spectrophotometric Assay and Melting Temperature (Tm)
After DNA isolation:

DNA characterization

Quantitative analysis

- GC content
  - By: Measuring Tm

- DNA purity
  - By: Spectrophotometric analysis

- DNA concentration

Qualitative analysis

- DNA integrity
  - By: Agarose gel electrophoresis

Today

Next Lab
Characterization of extracted DNA by spectrophotometric assay:

1. DNA concentration.
2. DNA purity. By measuring the absorption of ultraviolet light (UV).

- DNA has maximum absorption at 260nm. WHY?
1st. UV for quantification of nucleic acid concentration:

- Is determined by measuring absorbance at **260 nm**. **WHY?**

- At 260 nm double-stranded DNA has specific absorption coefficient of 0.02 (μg/ml)^{-1}cm^{-1}.

- So:

  ➔ **Concentration of DNA** = \((A_{260} / \varepsilon \ L) \times \text{Dilution Factor (DF)}\)

Beer-Lambert Law:

\[ A = \varepsilon c l \]
1. To detect nucleic acid purity from proteins contamination:
   ➔ **Calculate** $A_{260}/A_{280}$
   **WHY?**
   • Highly purified DNA samples have a $A_{260}/A_{280}$ nm ratio of (1.8-1.9).
   ➔ What if the ratio is below 1.8? What that means?
   ➔ What if the ratio is higher than 1.9? What that means?

2. To detect nucleic acid purity from carbohydrates, peptides, ethanol or any organic compounds:
   ➔ **Calculate** $A_{260}/A_{230}$
   **WHY?**
   • Purified DNA samples have a $A_{260}/A_{230}$ nm ratio of (2-2.2).
DNA and protein absorption spectrum:

Purity Ratios:
- $\frac{260}{280} = 1.8 - 2.0$
- $\frac{260}{230} > 2.0$
*What is the effect of the contaminants on DNA concentration?

* What if the samples contaminated by proteins or organic compound?
The two strands of a DNA molecule can be dissociated ("melted") into single strands by heat or altered pH, which breaks the hydrogen bonds between complementary bases (A=T and G=C).

What that process called?

Hyperchromic and hypochromic effect.

Melting temperature profile.

The melting temperature (Tm) is the temperature at which 50% of the DNA is unpaired (denatured).

Is the Tm same for all DNA molecules? WHY?

What is the important of knowing Tm of DNA?
**DNA melting curve:**

**FIGURE 4.4 DNA melting curve.** A melting curve of DNA showing $T_m$ (the melting temperature) and possible molecular conformations for various degrees of melting.
• GC content can be calculated by generating Tm profile (DNA melting curve).

\[\% (G+C) = 2.44 \times (Tm - 69.3)\]
Relationship between Tm and GC%:

**FIGURE 4.5** Effect of G-C content on DNA melting temperature. $T_m$ increases with increasing percent of G + C.
Practical Part
**Aim:**

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

**Principle:**

- DNA and proteins have a maximum absorbance at 260 and 280 respectively.
- dsDNA will be separated to ssDNA by heat (denaturation).
- O.D at 260 nm will increase during denaturation... **Why?**
- Temperature for midpoint of denaturation **gives Tm.**
- The DNA of each species has a specific denaturation curve.. **Why?**
1. Characterization of DNA by Spectrophotometric Assay:

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Absorbance of DNA</th>
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<tbody>
<tr>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td>230</td>
<td></td>
</tr>
<tr>
<td>260</td>
<td></td>
</tr>
<tr>
<td>280</td>
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</tbody>
</table>

➔ Find out the concentration of DNA using the following equation:

\[
\text{Concentration of DNA} = \left( \frac{A_{260}}{\varepsilon L} \right) \times \text{Dilution Factor}
\]

➔ Determine the purity of the DNA.
2. Melting Temperature of DNA: 

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:

\[
\text{Percent of } G + C = (\text{Tm} - 69.3) \times 2.44
\]
Home Work:

- Watch the following videos:
  - https://www.youtube.com/watch?v=wXiiTW3pflM
  - https://www.youtube.com/watch?v=U2-5ukpKg_Q

What is agarose gel electrophoresis?