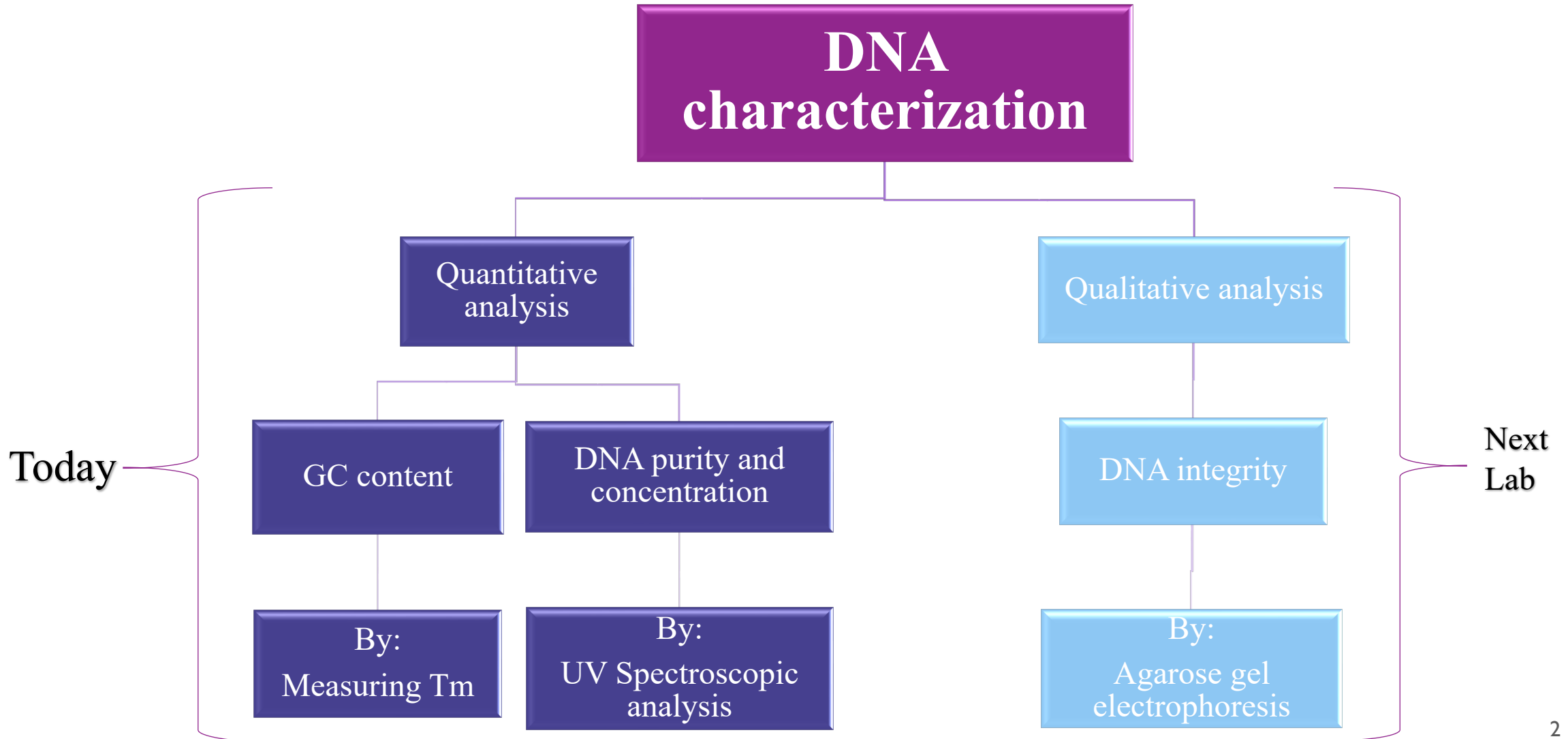




Characterization of The DNA by Spectrophotometric Assay and Melting Temperature (T_M)

After DNA Extraction... What NEXT ?



1. Quantifying DNA concentration

- Is determined by measuring absorbance at **260 nm**. Why?
- At 260 nm double-stranded DNA has specific absorption coefficient of $0.02 (\mu\text{g/ml})^{-1}\text{cm}^{-1}$.
- So:
→ **Concentration of DNA** = $(A_{260} / \epsilon L) \times \text{Dilution Factor (DF)}$.

Beer-Lambert Law:

$$A = \epsilon cl$$



2. DNA purity:

1. To detect nucleic acid purity from proteins contamination:

→ Calculate A_{260}/A_{280}

- Highly purified DNA samples have a A_{260}/A_{280} nm ratio of (1.8-1.9).

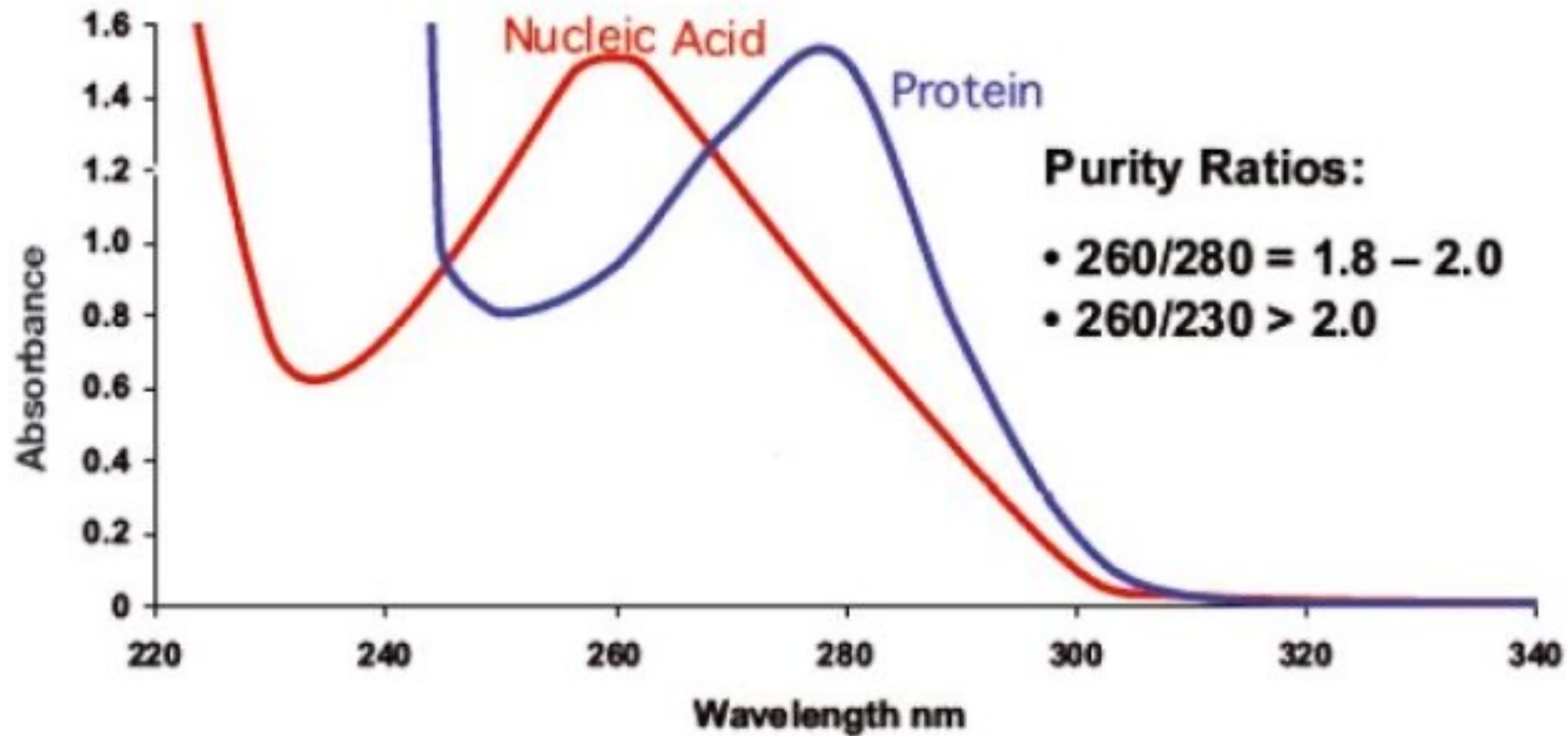
→ What if the ration is below 1.8? What that means?

2. To detect nucleic acid purity from carbohydrates, peptides, ethanol or any organic compounds:

→ Calculate A_{260}/A_{230}

- Purified DNA samples have a A_{260}/A_{280} nm ratio of (2-2.2).

DNA and protein absorption spectrum:





3. GC content:

- **Denaturation:** is when the double-stranded DNA (dsDNA) unwinds {dissociated "melted"} and separates into single-stranded (ssDNA) by heat or altered pH, which breaks the hydrogen bonds between complementary bases (A = T and G ≡ C).
- Hyperchromic and hypochromic effect.
- The **melting temperature (T_m)** is the temperature at which **50% of the DNA is unpaired** (denatured).
- GC content can be calculated by generating T_m profile (DNA melting curve).

$$\%(G+C) = 2.44 (T_m - 69.3)$$

DNA melting curve:

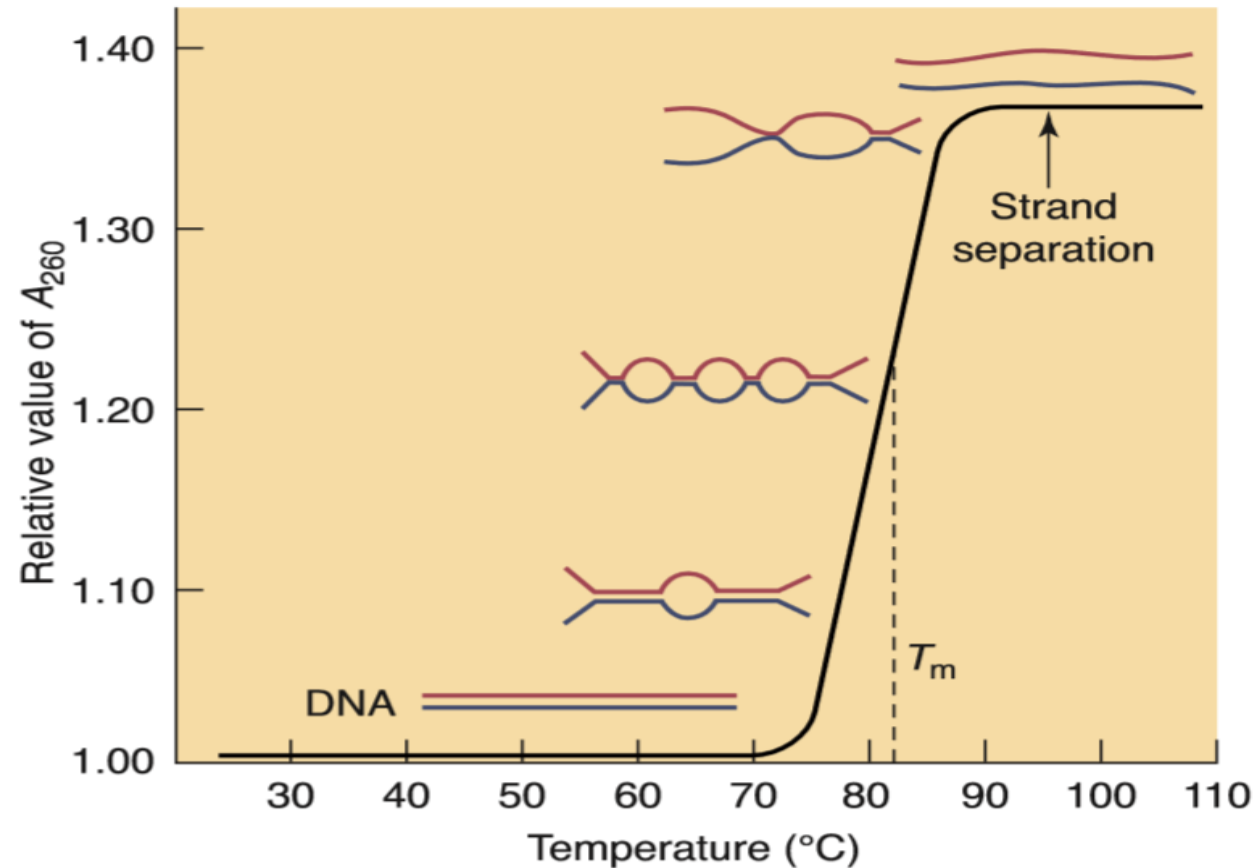


FIGURE 4.4 DNA melting curve. A melting curve of DNA showing T_m (the melting temperature) and possible molecular conformations for various degrees of melting.

Relationship between T_m and GC%:

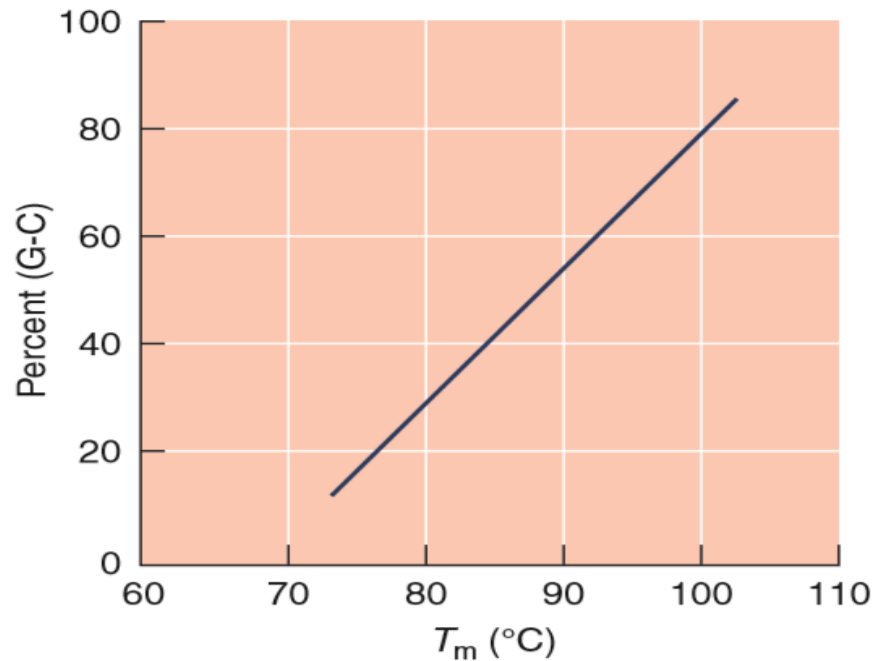
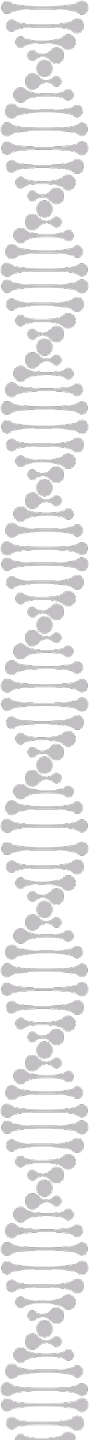


FIGURE 4.5 Effect of G-C content on DNA melting temperature. T_m increases with increasing percent of G + C.

What do you notice in the relation between GC content and T_m ?



Practical Part



Aim:

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

Principle:

- 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 T_m . Why it is important to know T_m of DNA?

The DNA of each species has a specific denaturation curve.. Why?



Results:

- As in the lab sheet



Homework:

Watch the following videos:

<https://www.youtube.com/watch?v=wXiiTW3pflM>

https://www.youtube.com/watch?v=U2-5ukpKg_Q