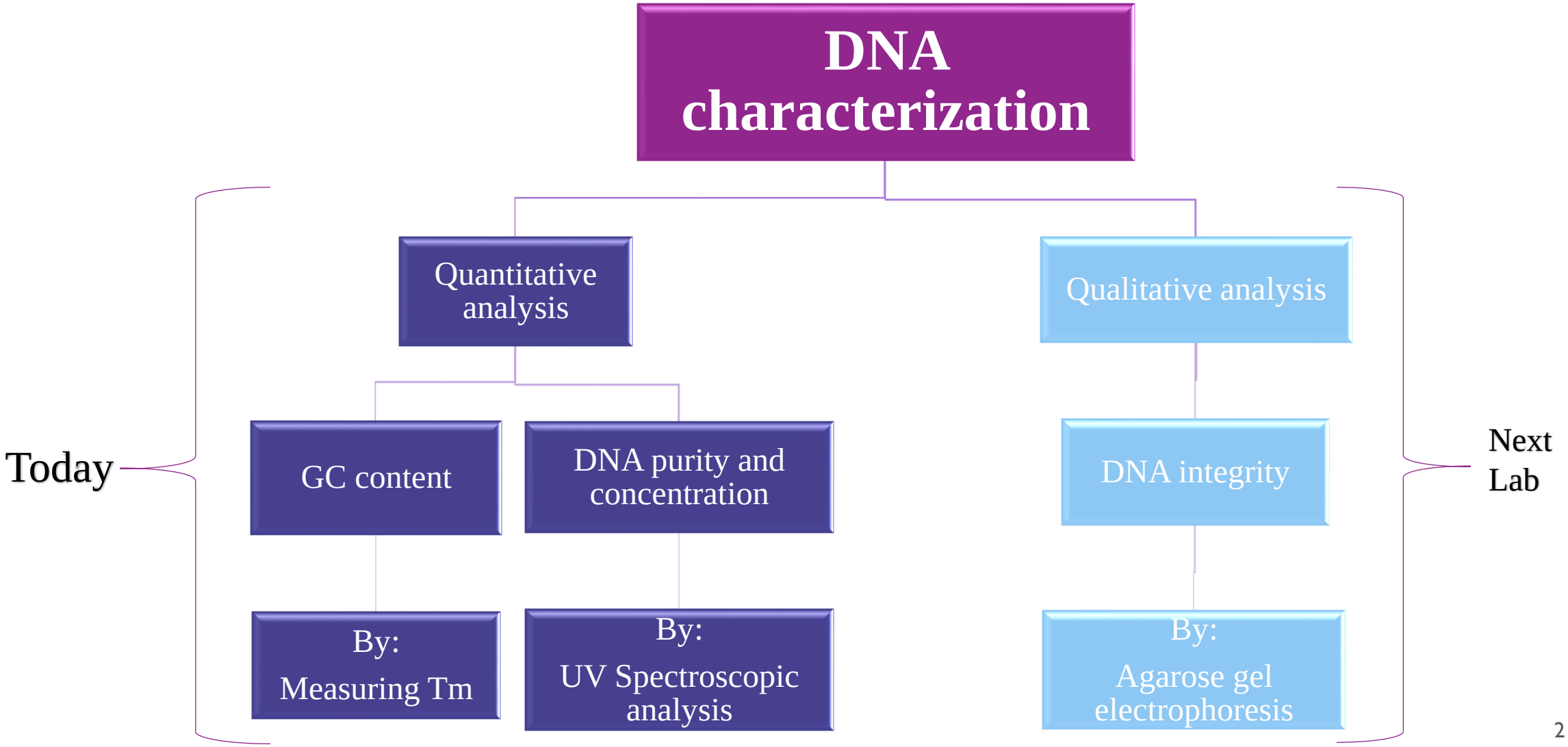
Characterization of The DNA by Spectrophotometric Assay and Melting Temperature (TM)

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 $(\mu g/ml)^{-1}cm^{-1}$.

- So:

➔ **Concentration of DNA=** (A260 / ε L) x Dilution Factor (DF).

Beer-Lambert Law:
$A = \varepsilon cl$

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**).

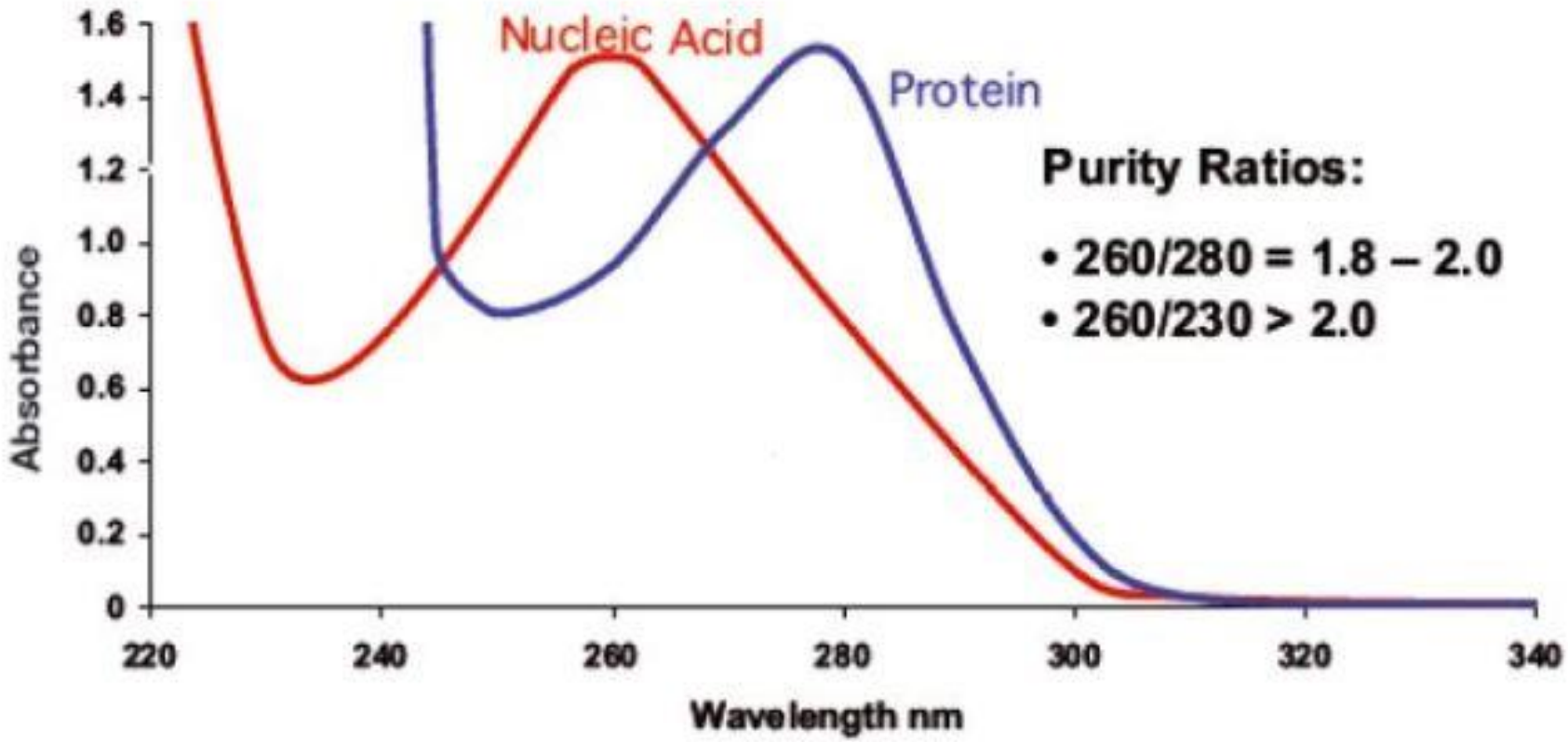
➔ What if the ration is below 1.8? What that means?

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:



Purity Ratios:

- **260/280 = 1.8 – 2.0**
- **260/230 > 2.0**

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

- GC content can be calculated by generating Tm profile (DNA melting curve).

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

DNA melting curve:

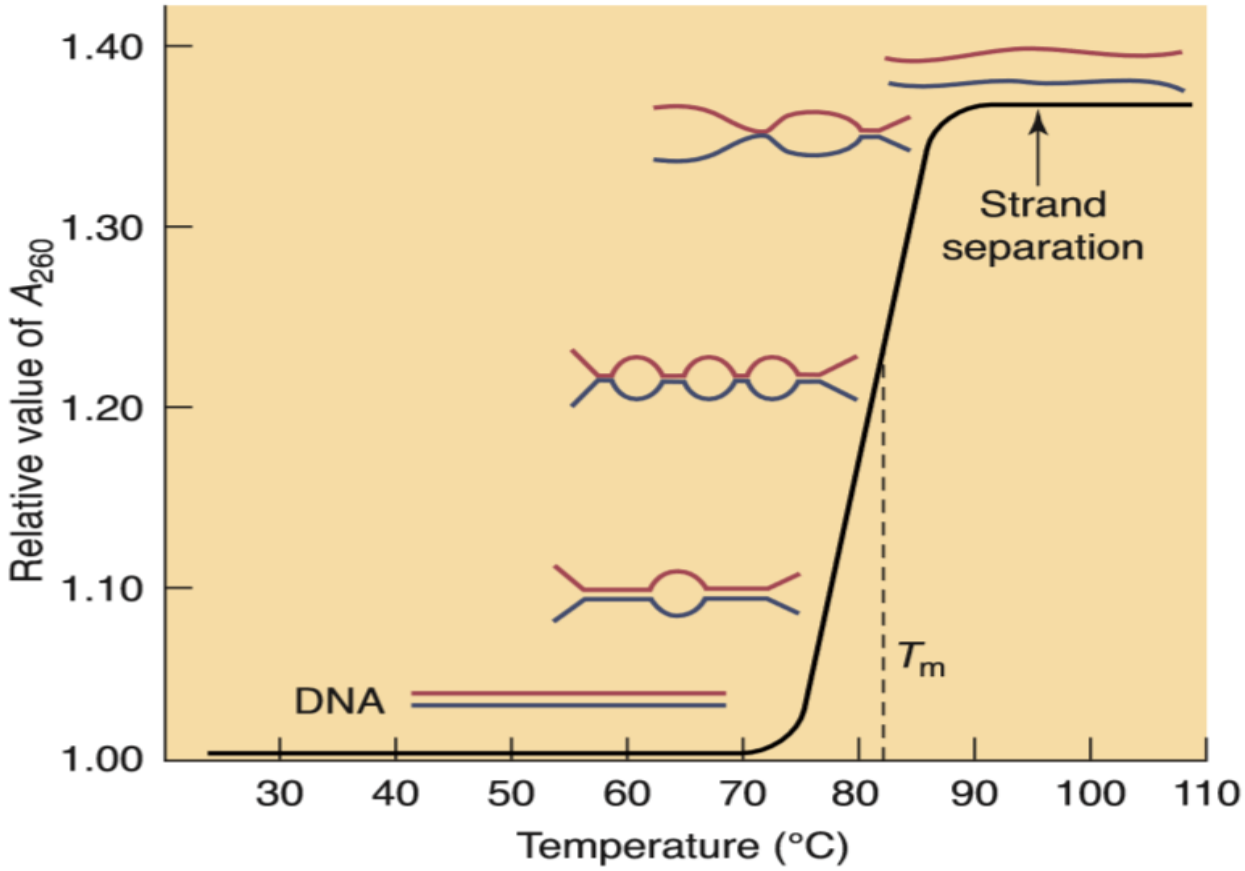


FIGURE 4.4 DNA melting curve. A melting curve of DNA showing T_m (the melting temperature) and possible molecular conformations for various degrees of melting.

Relationship between Tm and GC%:

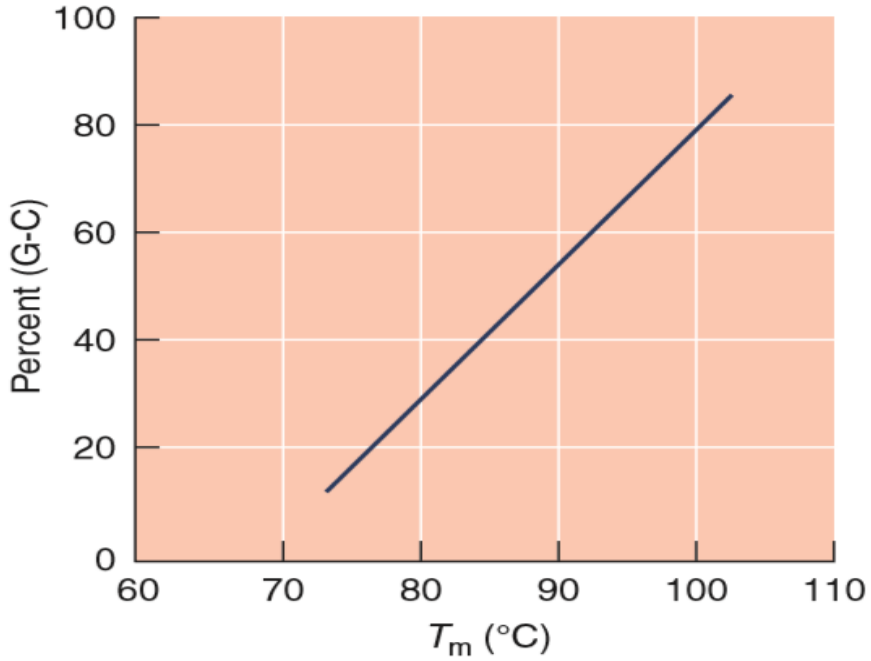


FIGURE 4.5 Effect of G-C content on DNA melting temperature. T_m increases with increasing percent of G + C.

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

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. Why it is important to know Tm of DNA?
- The DNA of each species has a specific denaturation curve.. Why?

Results:

- As in the lab sheet