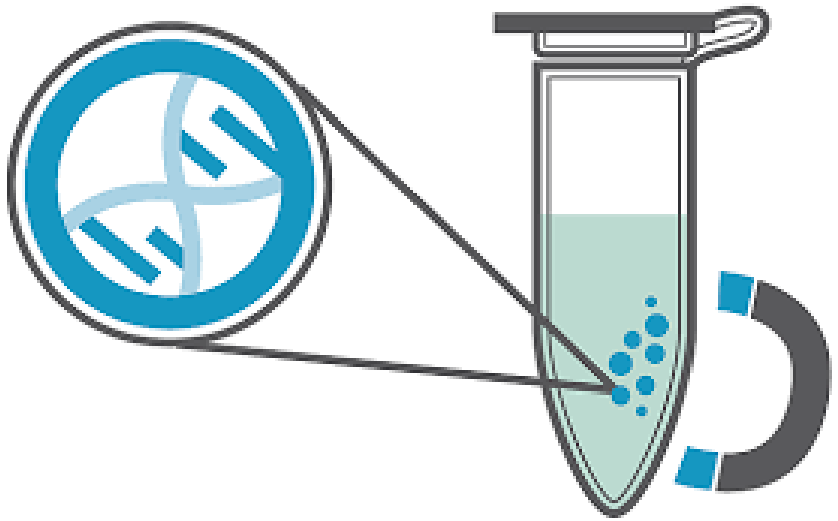
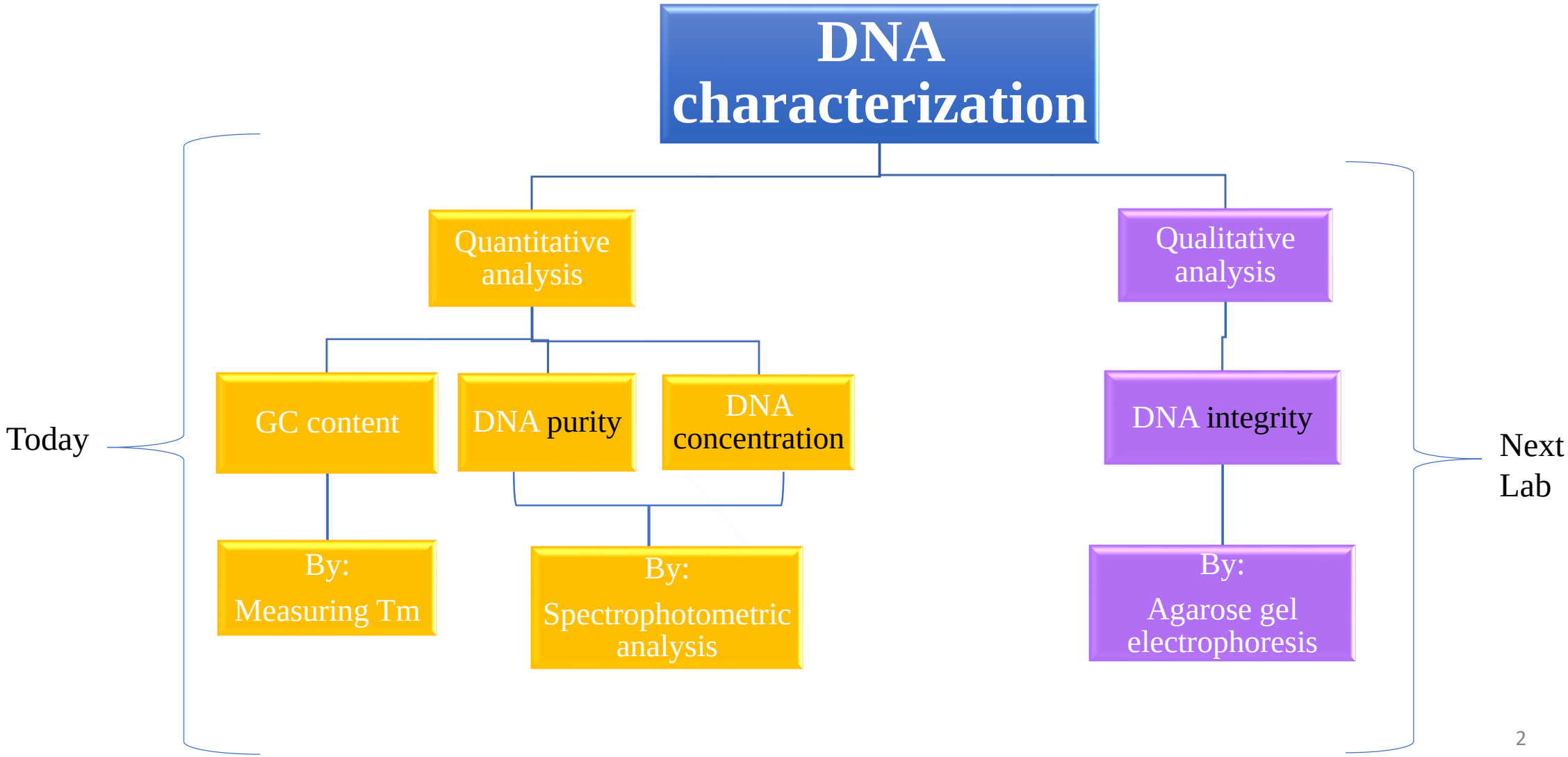
Characterization of DNA by Spectrophotometric Assay and Melting Temperature (Tm)



After DNA isolation:



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 $(\mu g/ml)^{-1}cm^{-1}$.
- So: ➔**Concentration of DNA=** $(A_{260} / \varepsilon L)$ x Dilution Factor (DF).

Beer-Lambert Law:

$A = \varepsilon cl$

2nd. DNA purity:

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?

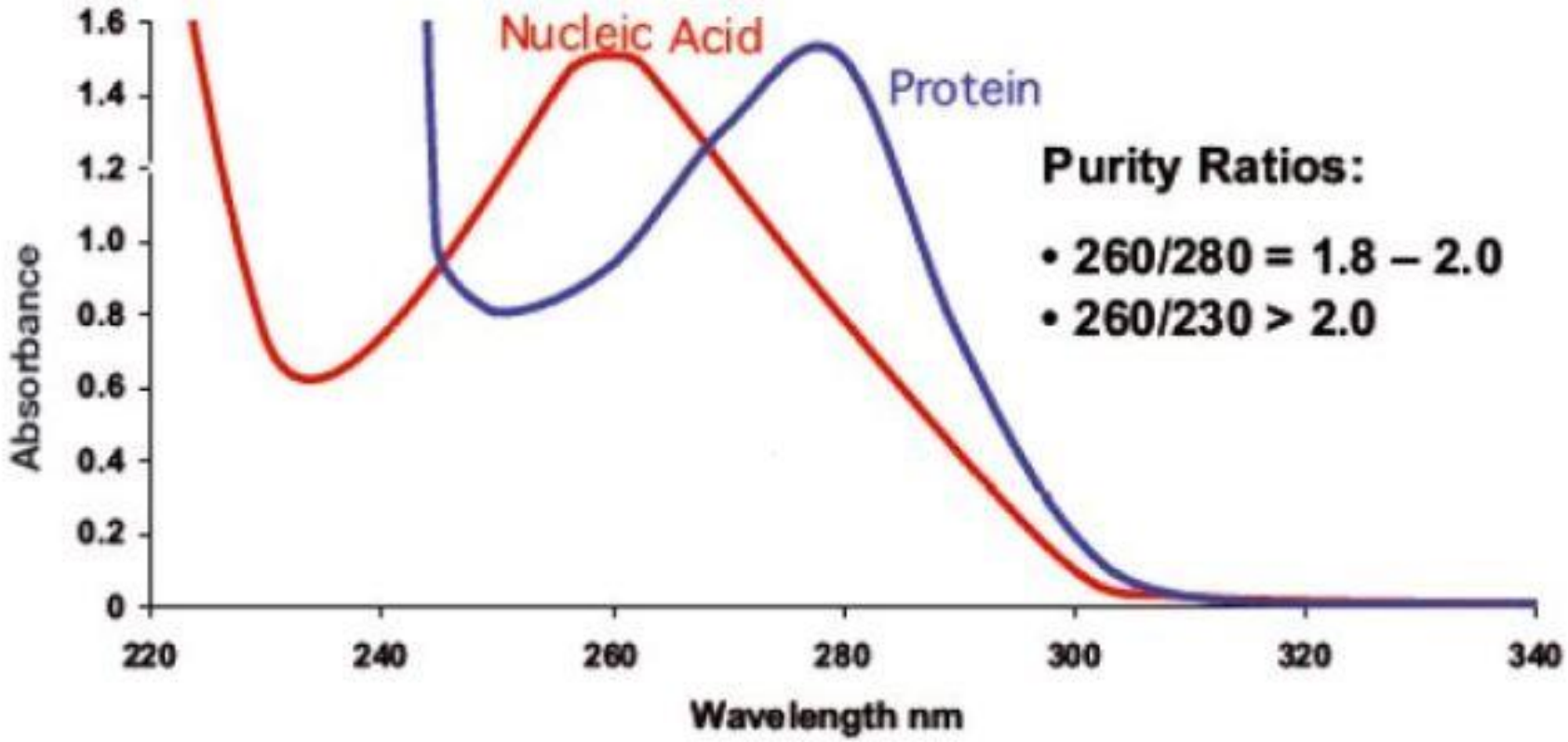
➔ What if the ratio is higher than 1.9? What that means?

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:



***What is the effect of the contaminants on DNA concentration?**

*** What if the samples contaminated by proteins or organic compound?**

3ed. GC content:

- 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 ?
- The melting temperature (Tm) is the temperature at which **50% of the DNA is unpaired** (denatured).
- Melting temperature profile ?

➔Is the Tm same for all DNA molecules ? WHY?

➔What is the important of knowing Tm of DNA?

DNA melting curve:

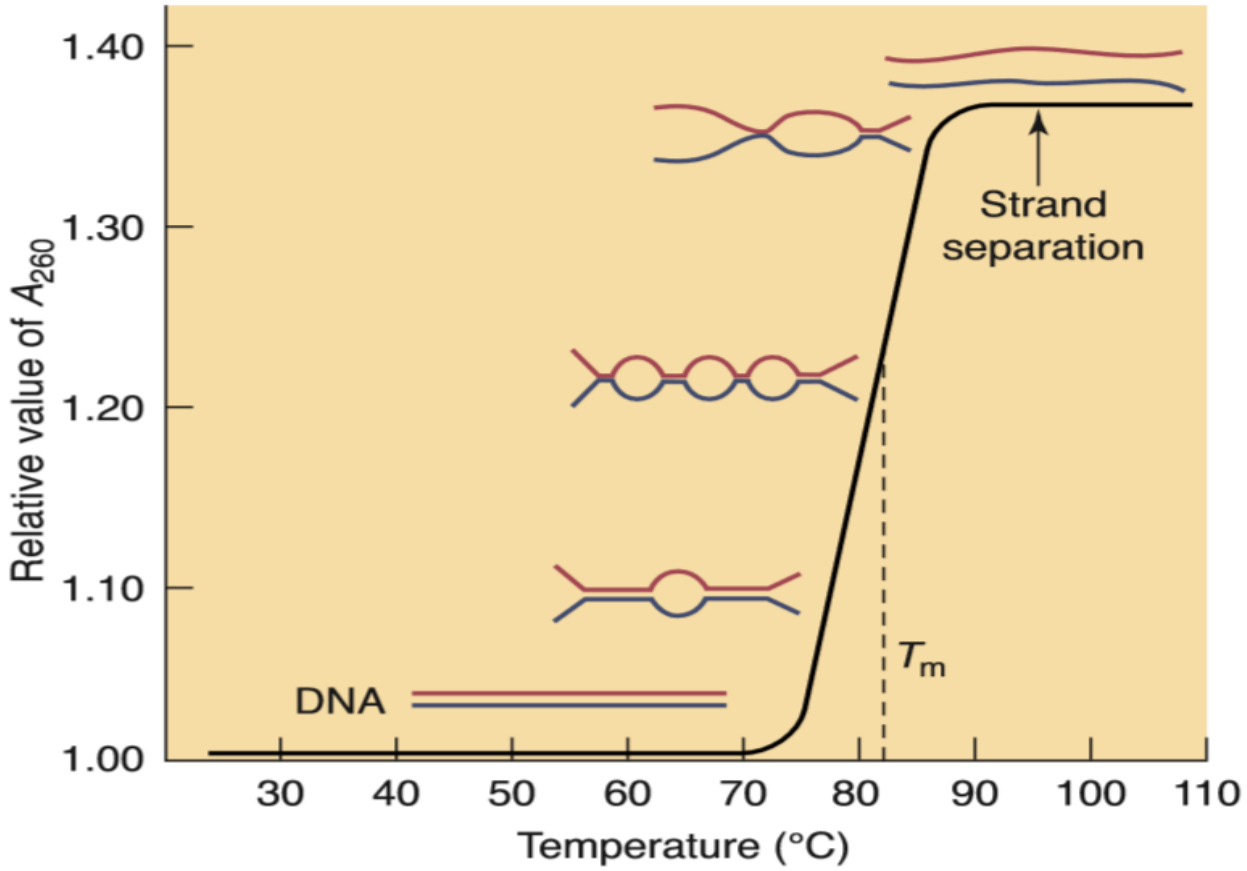


FIGURE 4.4 DNA melting curve. A melting curve of DNA showing T_m (the melting temperature) and possible molecular conformations for various degrees of melting.

3ed. GC content:

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

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

Relationship between Tm and GC%:

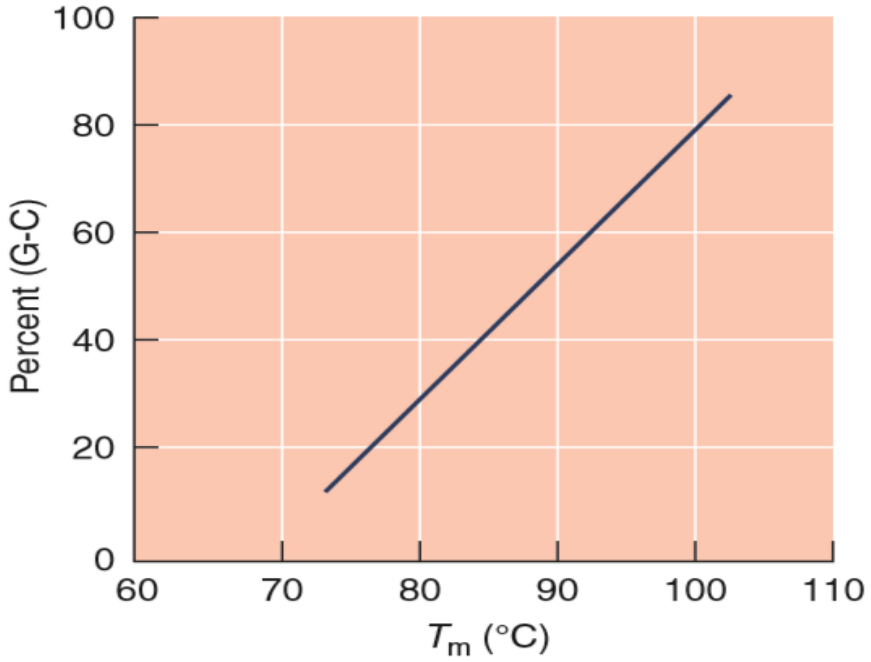
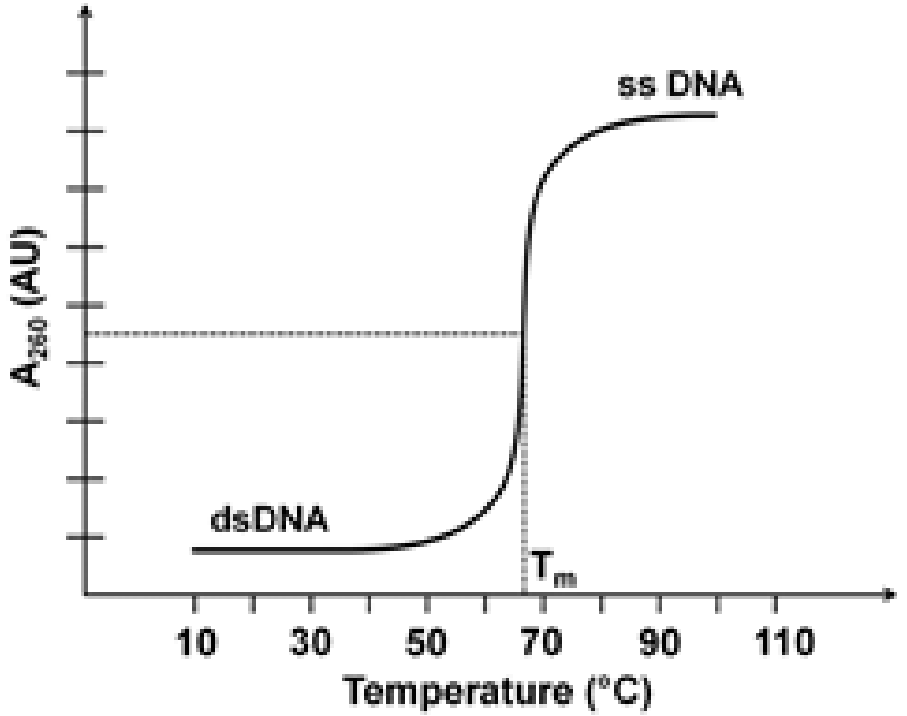


FIGURE 4.5 Effect of G-C content on DNA melting temperature. T_m increases with increasing percent of G + C.



What do you notice about the GC content in relation to 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.
- The DNA of each species has a specific melting curve.. Why?

Results:

1. Characterization of DNA by Spectrophotometric Assay:

Wavelength (nm)	Absorbance of DNA	
	Blood	Plant
230		
260		
280		

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

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

➔ Determine the purity of the DNA.

Results:

2. Melting Temperature of DNA:

Temperature (°C)	DNA Absorbance at 260 nm	
	Blood	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:

Percent of G + C = (Tm - 69.3) x 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?