CHARACTERIZATION OF THE DNA BY: (1) THE SPECTROPHOTOMETRIC ASSAY; (2) THE MELTING TEMPERATURE (TM)

The isolated and purified DNA can be characterized by different ways. In this experiment the purity and concentration of DNA obtained in the last experiment will be determined and the DNA will be characterized by measuring its melting temperature (Tm).

1-Determination of DNA purity and concentration by spectro-photometric assay:

- **UV for quantification of nucleic acid concentration:**

Analysis of UV absorption by the nucleotides provides a simple and accurate estimation of the concentration of nucleic acids in a sample. Purines and pyrmidines in nucleic acid show absorption maxima around 260nm.

For a 1-cm pathlength, the optical density at 260 nm (OD_{260}) equals 1.0 for the following solutions:

a 50 µg/mL solution of **dsDNA (double standard DNA)**

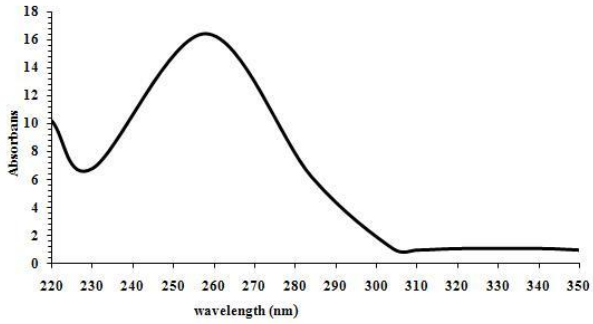
So the concentration is calculated by the equation :

Concentration = 50 µg/mL × A260 × dilution factor

2-DNA purity:

Contamination of nucleic acid solutions makes spectrophotometric quantitation inaccurate. So the OD_{260}/OD_{280} ratio for an indication of nucleic acid purity. Pure DNA has an OD_{260}/OD_{280} ratio of ~1.8. Low ratios could be caused by protein or phenol contamination. If the ratio is more than 2, then the sample is contaminated with RNA.

Absorption at 230 nm reflects contamination of the sample by substances such as carbohydrates, peptides, phenols or aromatic compounds. The ratio A260/A230 should be approximately 2.2 for pure nucleic acid samples.



3-DNA integrity using agarose gel electrophoresis:

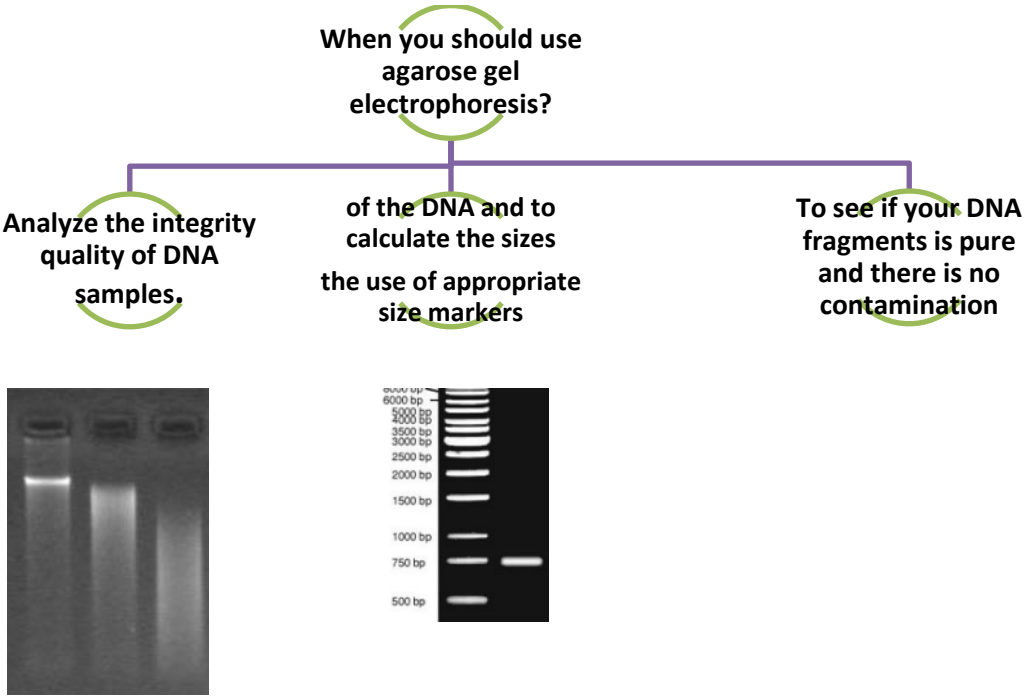
Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb

To separate DNA using agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current applied. The phosphate backbone of the DNA (and RNA) molecule is negatively charged, therefore when placed in an electric field, DNA fragments will migrate to the positively charged anode.

DNA molecules are separated by size within an agarose gel in a pattern such that the distance traveled is inversely proportional to the log of its molecular weight(3). The leading model for DNA movement through an agarose gel is "biased reptation",

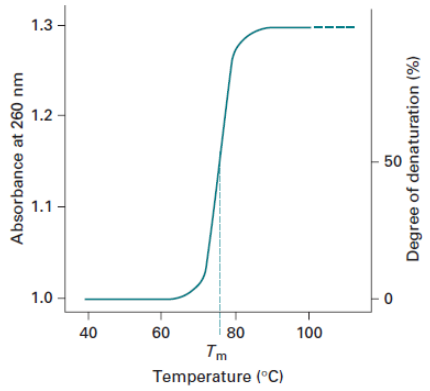
After separation, the DNA molecules can be visualized under uv light after staining with an appropriate dye.

place each sample in a quartz cuvette. Zero the spectrophotometer with a sample of solvent. For more accurate readings of the nucleic acid sample of interest, dilute the sample to give readings between 0.1 and 1.0.



3-GC content and melting temperature:

The temperature at which 50% of the DNA is melted is termed the melting temperature or T_m, and this depends on the nature of the DNA. If several different samples of DNA are melted, it is found that the T_m is highest for those DNAs which contain the highest proportion of cytosine and guanine, and T_m can actually be used to estimate the percentage (CþG) in a DNA sample. This relationship between T_m and (CþG) content arises because cytosine and guanine form three hydrogen bonds when base-paired, whereas thymine and adenine form only two.Because of the differential numbers of hydrogen bonds between A–T and C–G pairs those sequences with a predominance of C–G pairs will require greater energy to separate or denature them.



Method:

1-Meaure DNA concentration and purity from both sources (Blood and strawberry):

By using spectrophotometer:

1- For blood sample: do not dilute the sample
2- For strawberry: dilute the sample 1:10

In the spectro press:

3-Nuclic acid→1-DNA→1-10 mm→ for the unit choose microgram/ml → 2-yes (for 320 nm) → then enter dilution Factor

Write all the information that you got and explain them:

Plant source	Blood

If the absorbance of DNa at 260 nm that you got is 1.22, and the sample was diluted 1:3 Write the formula for calculating DNA concentration:……..

DNA absorption spectrum:

Measure the absorbance at the following wave length:(from 220-300 nm) using spectrophotometer:

2-Application→1-Wave length→from 220 to 300 nm→ peak on

Draw the curve that you got

2-Meaureing melting temperature:

Choose one of the DNA samples (plant or Blood), and then put it in test tube

- Melting temperature: put the DNA sample into each temperature for 5 min and then measure the absorbance
- Room temperature, 50, 60, 70, boiling
- Draw a figure between temperature and absorbance and notice the figure

Temperature	Absorbance

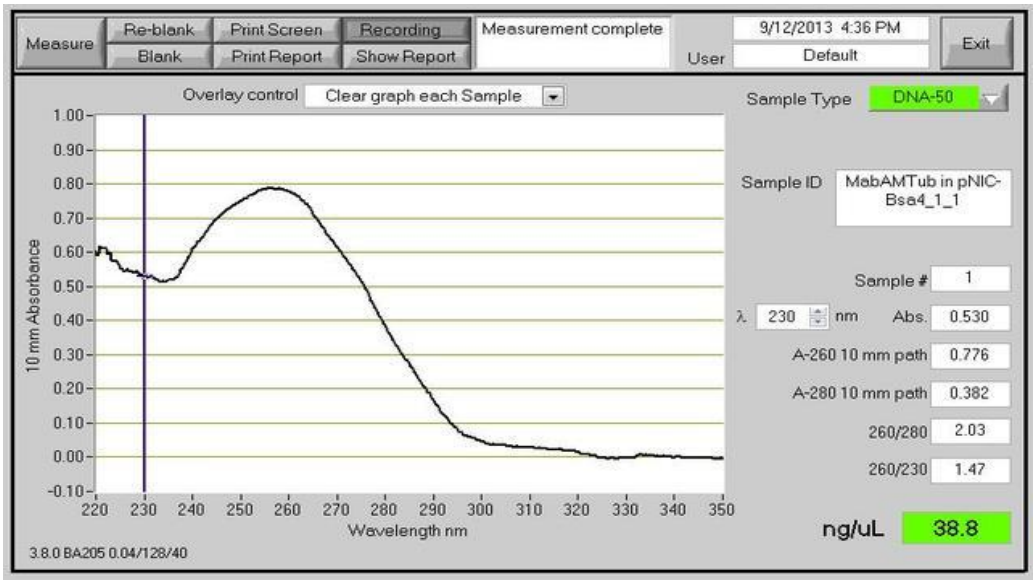
Discussion:

- How pure is your DNA samples? Reflect on the possible sources of contaminations in your DNA samples from different sources?. What your sample should have?
- Discuss the curve of your melting temperature is it the same figure that it suppose to be or not and why?
- Determine the GC content if you can
- The relationship between the temperature and GC content,
- Compare your result with other group that choose different DNA source,

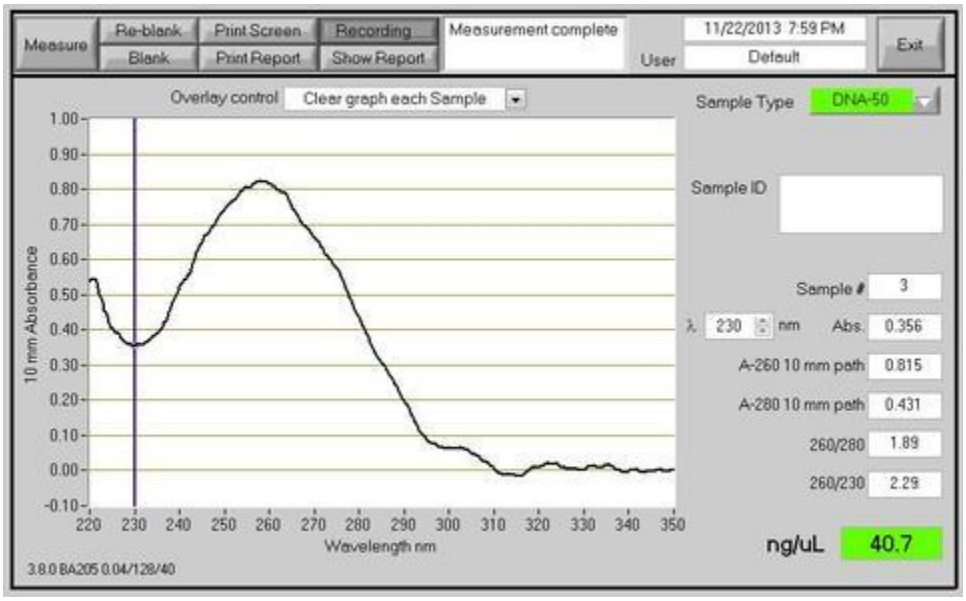
Questions:

1-Why does the melting temperature of the DNA sample depends on the GC content?

Discuss the following result of DNA, Picture-1:



Picture-2



References:https://www.ncbi.nlm. nih.gov/pubmed/22546956

Principles and Techniques ofBiochemistry and Molecular Biology