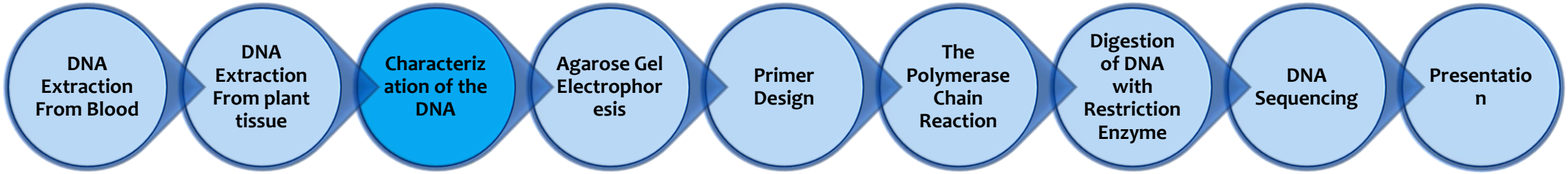
CHARACTERIZATION OF THE DNA BY: (1) THE SPECTROPHOTOMETRIC ASSAY; (2) THE MELTING TEMPERATURE (TM)

Outline


DNA Extraction From Blood
DNA Extraction From plant tissue
Characterization of the DNA
Agarose Gel Electrophoresis
Primer Design
The Polymerase Chain Reaction
Digestion of DNA with Restriction Enzyme
DNA Sequencing
Presentation

After DNA Extraction…

- **What is the next step?**

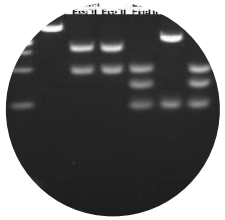
- 1-The concentration of your Extracted DNA
- 2-The purity…
- 3-the integrity (is your extracted DNA is degraded or not)
- 4-(determining the GC content)

DNA Quality and Quantity

- After DNA extraction, DNA integrity and purity must be checked.

DNA intigrity

Spectroscopic analysis



agarose gel electrophoresis

→ **GC content by measuring Tm**

UV for quantification of nucleic acid concentration

- Is determined by measuring absorbance at 260 nm,
- For a 1-cm pathlength, the optical density at 260 nm (OD_{260}) equals 1.0 is equivalent to approximately.

- **50 µg/mL double-stranded DNA (dsDNA)**
- **33 µg/mL single-stranded DNA (ssDNA)**

- **Concentration** = 50 µg/mL × A260 × dilution factor.

Purity

- Calculating the ratio between absorbance at 260 nm and 280 nm.

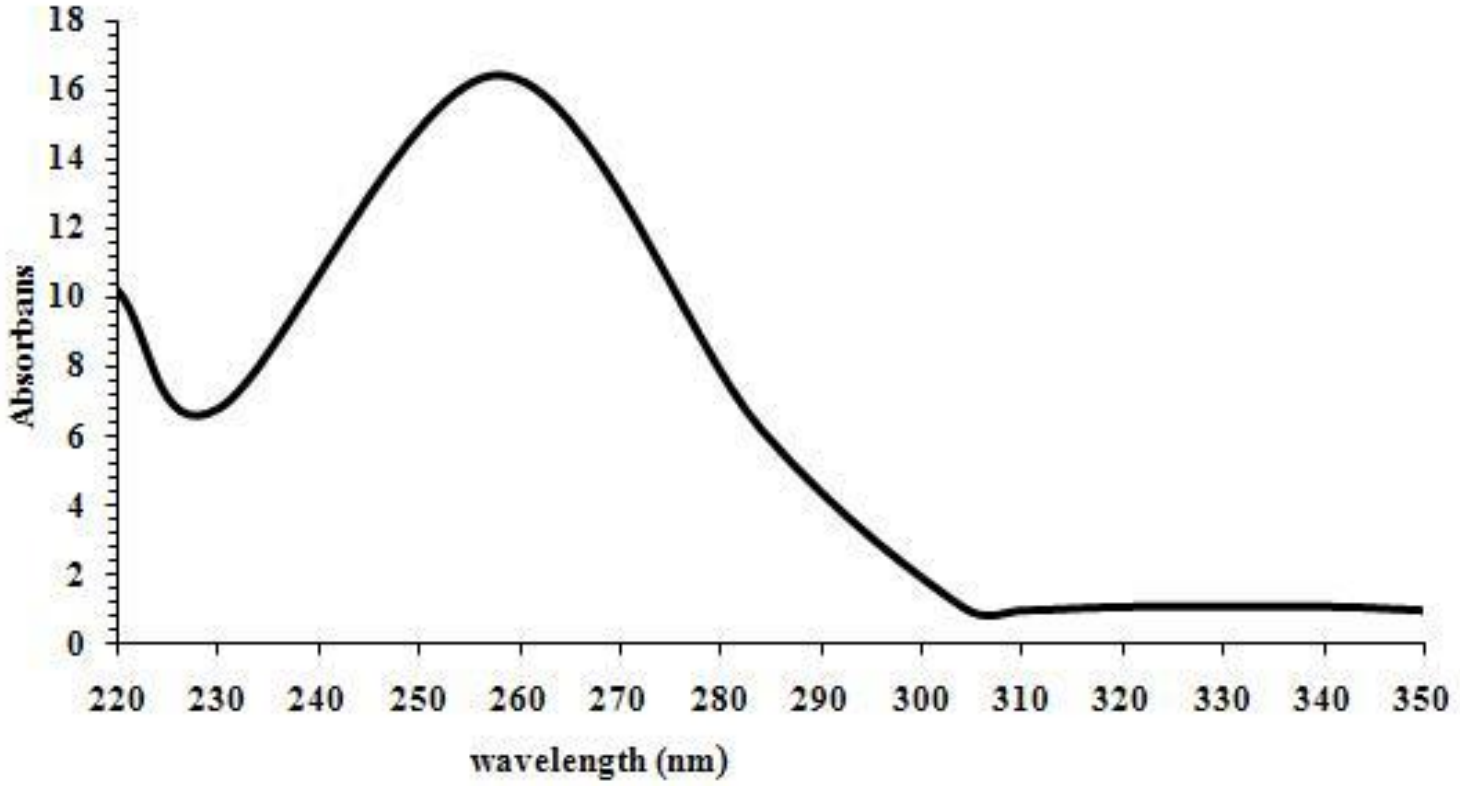
- **Contamination with protein:**
- DNA absorb light at 260 nm
- This ratio (A260/A280) is used to estimate purity because proteins absorb more strongly at 280 nm.

- **Pure DNA should have a ratio of approximately 1.8**

- **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.

- ***What is the effect of contaminated DNA on concentration?***

DNA absorption spectrum

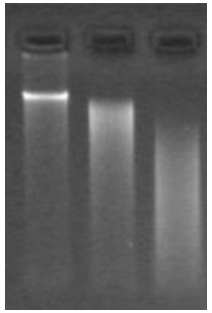


DNA integrity

- Using agarose gel electrophoresis

- Is a method of gel electrophoresis used to separate and analyze DNA or RNA molecules **by size**

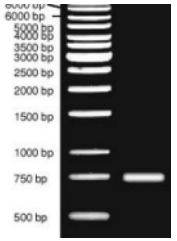
When you should use agarose gel electrophoresis?



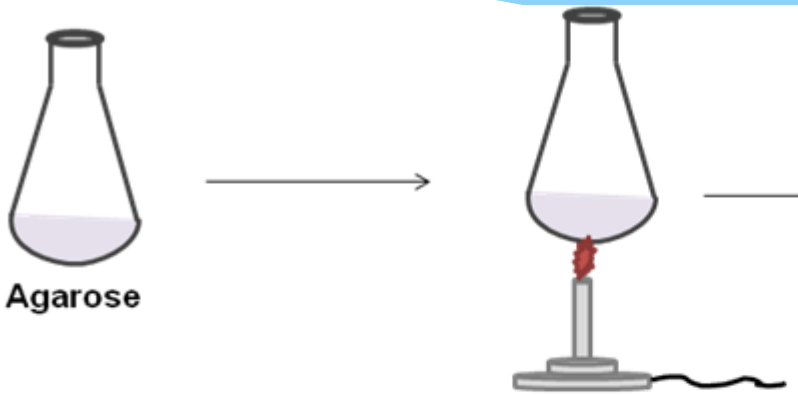
Analyze the integrity quality of DNA samples.

of the DNA and to calculate the sizes

the use of appropriate size markers

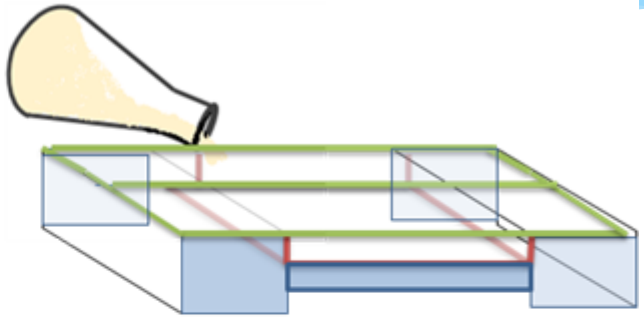


To see if your DNA fragments is pure and there is no contamination

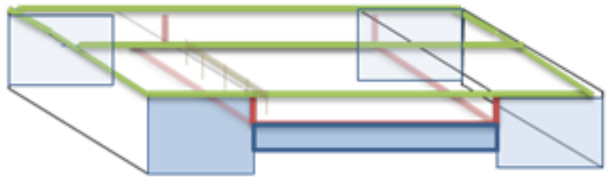


Agarose

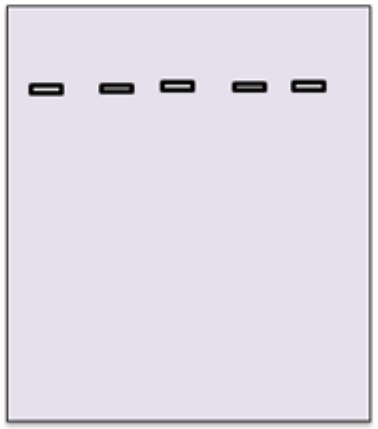
Melting Agarose



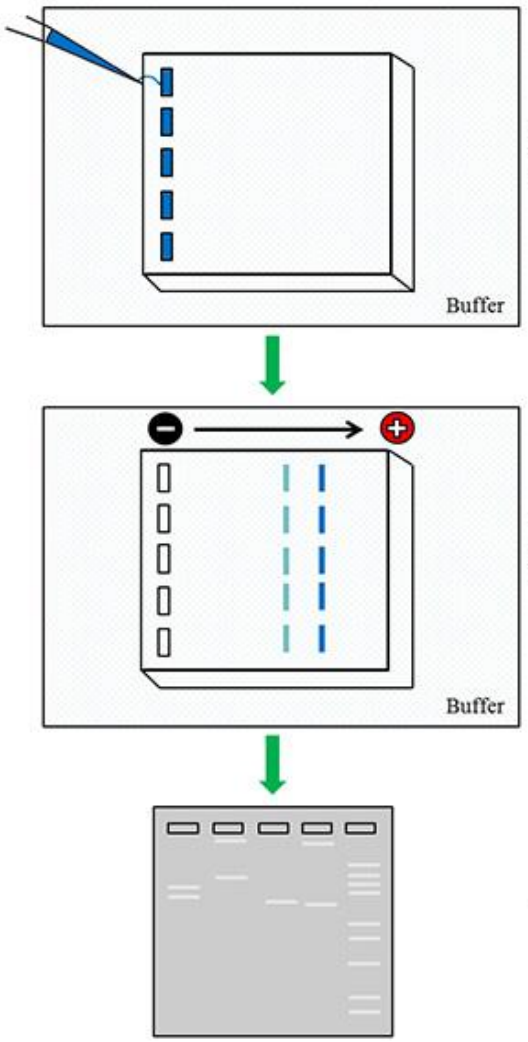
Pouring agarose in gel caster



Solidification of agarose gel



Agarose gel



DNA/RNA samples and marker loaded in the horizontal gel electrophoresis system

Direction of migration of DNA/RNA samples in horizontal gel electrophoresis system

Agarose gel after ethidium bromide staining

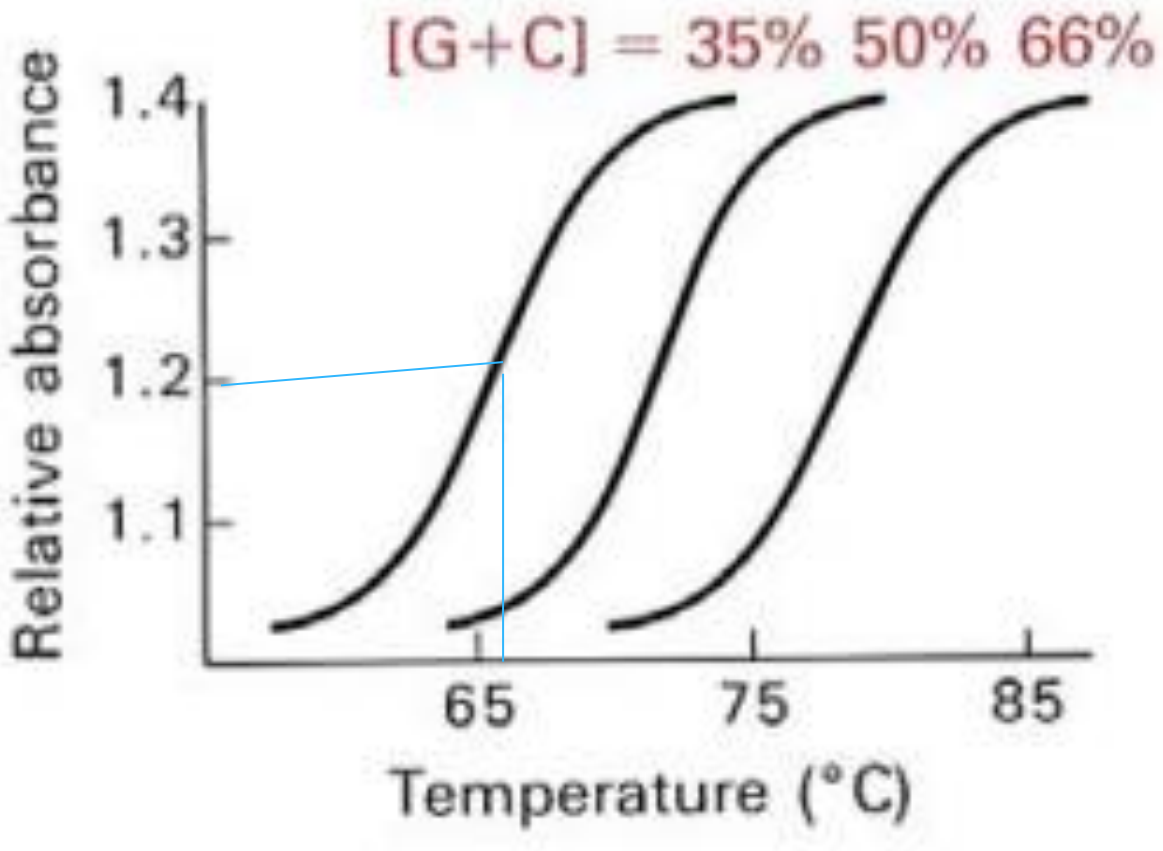
Melting temperature and GC content

- The two strands of a DNA molecule can be dissociated ("melted") into single strands by heat, which breaks the hydrogen bonds between complementary bases.

- The temperature at which a particular DNA molecule "melts" will vary. Why?
- What is the important of knowing Tm of DNA?

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

Melting Tempreture



What do you notice about the GC content in relation to Tm?

Method

- DNA concentration and purity:
- using spectrophotometer

- Melting temperature: put the DNA sample into each tempreture 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

Discussion

* Discuss the purity and DNA concentration
* Calculate the GC content

Home work

- Watch the following videos
- https://www.youtube.com/watch?v=wXiiTW3pfIM
- https://www.youtube.com/watch?v=U2-5ukpKg_Q

- And answer the questions:
- What is agarose gel electrophoresis?
- How to prepare the gel?
- How you will choose the appropriate concentration of the gel ?
- What are the things that you should consider when preparing agarose gel electrophoresis?
- What is the comb and for what is it used?
- What is loading dye? And what are the component?