Laboratory 2 – DNA Extraction, PCR and Agarose Gel Electrophoresis

LABORATORY SESSIONS

Date	10 September 2025 (Wednesday)			
Time	**1000 – 1200**	**1200 – 1400**	**1400 – 1600**	**1600 – 1800**
	B1	**B2**	**B3**	**B4**
Venue	**Life Sciences Laboratory 3, Block S1A Level 4**			

IMPORTANT INFORMATION

1.	You are required to put on **long bottoms (trousers, jeans, long skirts) and covered footwear**. You will not be allowed to enter the laboratory if you are wearing shorts (including knee-length), short skirts, sandals, slippers or other footwear that do not cover your feet (including Crocs).
2.	A **disposable lab coat** was issued to you during the first laboratory session. You will reuse this lab coat for subsequent sessions.
3.	Please be **punctual**. You will not be allowed to enter the laboratory if you arrive later than 5 minutes after the time the laboratory session is scheduled to begin.
4.	Please ensure that your attendance is marked. Failure to do so will result in a **50% penalty** on marks earned for the lab assignment. If you are unable to attend, please contact the teaching team as soon as possible. Please ensure that you produce acceptable official documentation, e.g. a medical certificate if you are unwell and unable to attend the lab session. Under such circumstances, an alternative lab assignment may be provided.

ASSIGNMENT

Weightage	10%
Deadline	**2359h, 16 September 2025 (Tuesday)**
Late Submission	**2359h, 17 September 2025 (Wednesday)** **50% penalty**
Missed Late Submission Deadline	**No marks**

INSTRUCTIONS

1.	Please download the relevant files for this assignment and use the answer sheet provided to submit your assignment. All answers should be TYPED for submission under **Canvas assignments.**
2.	Before submission, please rename your file according to the following format, **NUSNET UserID-Lab02**. If your NUSNET UserID is *e0765432*, the filename should be *e0765432-Lab02*. Files that are not renamed according to the stated format will be subjected to a **10% penalty**.
3.	Files are subjected to similarity checks via Turnitin upon submission. Please check the '*Similarity Report*' to ensure that you have sufficiently rephrased the materials derived from other sources, and if necessary, make the required changes and resubmit. Similarity Reports may take up to 24 hours to generate for resubmissions.
4.	Please note that marking will not be based on keywords alone but will also depend on how the explanations and descriptions are expressed.

Pre-Lab Exercise:

Please watch the video in Multimedia (Laboratory Preparation videos folder) on how to use the micropipette to load samples into an agarose gel.

INTENDED LEARNING OUTCOMES

You have observed the nucleus of your own cheek cells and that of a plant cell in the previous lab. The nucleus contains the chromosomes, which are complexes of DNA and proteins. In this laboratory session, you will conduct three experiments: isolate DNA, perform PCR (done in Laboratory 1), and analyse PCR products by agarose gel electrophoresis. After this session, you will learn how to:

a) isolate DNA from banana and spinach
b) apply your knowledge to explain how the cell is lysed and DNA is released from cells
c) conduct PCR and explain the principle of PCR
d) conduct agarose gel electrophoresis and explain its principle
e) interpret experimental data and to report data effectively

Introduction

DNA is the genetic material in many organisms. Eukaryotic cells generally have a number of chromosomes in the cell nucleus, while prokaryotic cells generally have a single, circular chromosome localized in the central region of the cell. This region is called the nucleoid. Isolating DNA from cells is a first step for understanding the nature of DNA and for many types of molecular research, such as DNA fingerprinting, medical diagnoses, and gene engineering.

Polymerase chain reaction (PCR) is a common laboratory technique used to make many copies (millions) of a particular region of DNA. PCR relies on a thermostable DNA polymerase (*Taq* polymerase) and requires DNA primers designed specifically for the DNA region of interest. In PCR, the reaction is repeatedly cycled through a series of temperature changes, which allow many copies of the target region to be produced. PCR has many research and practical applications. It is routinely used in DNA cloning, medical diagnostics, and forensic analysis of DNA.

Agarose gel electrophoresis is an effective way of separating DNA fragments based on their sizes. Agarose is isolated from the seaweed genera *Gelidium* and *Gracilaria*, and consists of repeated galactose subunits. During gelation, agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties.

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. Because DNA has a uniform mass/charge ratio, DNA molecules are separated by size within an agarose gel in a pattern such that the distance travelled is inversely proportional to the log of its molecular weight.

The agarose gel usually contains a DNA staining agent, which binds to DNA and causes it to fluoresce under UV light. Hence, after separation of DNA fragments, the gel is visualised under UV light to show the migration of the DNA fragments. Fragments of the same size are commonly termed as “bands” in the gel. The exact sizes of separated DNA fragments can be determined by plotting the base pair size for the different bands of a DNA standard (also commonly called a DNA ladder or marker)

against the distance travelled by each band. The DNA standard contains a mixture of DNA fragments of pre-determined sizes that can be compared against the unknown DNA samples.

Part 1: DNA Gel Electrophoresis

Experimental Protocol

Each pair of students would have prepared a sample for PCR during Laboratory 1. The PCR products will now be subjected to gel electrophoresis to check for the success of the procedure, and to separate the fragments by size.

Casting an agarose gel (1% agarose gel)/Set Up Electrophoresis (watch videos for details of this protocol before starting this part)

[Steps 1 – 7 have been done for you:]

1. Weigh agarose and dissolve it in TAE buffer (using beaker and microwave oven). The amount required is decided by the size of casting tray; for a small 8-well gel, between 50 to 80 mL of molten agarose is required. For 50 mL, a 1% gel of this size is equivalent to 0.5 g agarose in 50 mL of TAE buffer. Weight and volumes are adjusted according to desired final amount.
2. Allow the molten agarose solution to cool to 60°C.
3. Add the DNA staining agent SYBR Safe (stock: 10 mg/ml) to a final concentration of 0.5 µg/ml into the molten agarose, ensuring it is mixed well.
4. Assemble the gel casting tray.
5. Pour the agarose solution into the gel tray.
6. Place the comb and allow the agarose to gel at room temperature for 20 minutes.
7. Once the gel is set, place the gel tray into the electrophoresis chamber.
8. Fill the chamber with running buffer until the buffer reaches 3 mm - 5 mm over the surface of the gel. Remove the comb gently.
9. Collect your previously prepared PCR sample (from LABORATORY 1) from your TA.
10. Your TA will demonstrate how to load a sample by loading 15 µl of DNA ladder or marker (0.5µg/10µl). Students will then load 15 µl of their DNA sample into individual wells and run the electrophoresis at 120V for 40 minutes.
11. Once the run is completed, turn off the power source. With the gel still in its tray, drain the buffer into the running tank, and proceed to visualise the DNA bands by placing the gel in the GelDoc UV imaging system.

Part 2: DNA Extraction

To obtain the DNA from living cells, the cell wall (if any), plasma membrane, and nuclear envelope must be disrupted first. This is followed by the separation of the DNA from the proteins that are bound to the DNA in the chromosomes.

Two sets of the following solutions are provided for the next two experiments:

a. **10 mL** salt water (0.9% sodium chloride solution – prepared by dissolving 9 g of table salt to 1 litre of water),
b. **5 mL** liquid soap (25% liquid soap solution – prepared by adding 25 mL of liquid soap to 75 mL of water), and
c. **5 mL** ice-cold alcohol (95% ethanol – may be substituted with rubbing alcohol from any pharmacy).
d. **1 mL** room-temperature alcohol in Eppendorf tube – collect from TA when needed

(A) DNA extraction from banana

1. Take about 2-3 g of banana (quarter piece) and place it in a mortar.
2. Add a few drops of salt water and using a pestle, grind the banana into a paste.
3. Add the remaining salt water into the mortar.
4. Transfer the contents into a test tube containing 5 mL of liquid soap.
5. Cover the top of the test tube with a cap and gently mix the contents by turning the test tube upside down and right side up for 2 to 3 minutes.
6. Tilt the test tube at an angle and slowly add 5 mL of ice-cold alcohol down the side of the test tube.
7. Wait for about 5 minutes and watch as the DNA floats to the surface.
8. *Do the following only when you wish to bring the DNA home:* Use a glass rod to remove the DNA by twirling the rod in a circular motion. Place the DNA into a small test tube containing 1 mL of alcohol.

(B) DNA extraction from spinach

1. Take one spinach leaf and place it in a mortar. Ensure that the mortar is washed thoroughly before this step.
2. Add a few drops of salt water and grind the spinach leaf with a pestle.
3. Add the remaining salt water into the mortar.
4. Transfer the contents into a test tube containing 5 mL of liquid soap.
5. Cover the top of the test tube with a cap and gently mix the contents by turning the test tube upside down and right side up for 2 to 3 minutes.
6. Tilt the test tube at an angle and slowly add 5 mL of ice-cold alcohol down the side of the test tube.
7. Wait for about 5 minutes and watch as the DNA floats to the surface.
8. *Do the following only when you wish to bring the DNA home:* Use the glass rod to remove the DNA by twirling the rod in a circular motion. Place the DNA into a small test tube containing 1 mL of alcohol.

Online references:

Using a Micropipette

https://www.youtube.com/watch?v=uEy_NGDfo_8

Casting an Agarose Gel

https://www.youtube.com/watch?v=wXiiTW3pfIM

Running an Agarose Gel

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

Lab Assignment 2 (10 marks)

1. Find out the properties of the chemical structure of liquid soap. Which of the molecules of the topic 'Chemistry of Life' does the properties of the chemical structure of liquid soap remind you of? Explain the role of liquid soap in the DNA extraction procedure that you had carried out. (3 marks)
2. Include a photograph of the gel electrophoresis for your group and indicate the estimated size (number of base pairs) of each band from PCR samples on the image via comparison with the DNA marker, and explain the factors affecting the brightness of DNA bands. (4 marks)
3. If you are requested to reduce the size of the PCR fragment by 100 bp from the 3' end, what should you do? Please explain the procedures needed. (3 marks)

- The End of Laboratory 2 -

Appendix

DNA ladder/marker used in today's experiment: