LSM1301 General Biology

Laboratory 2: DNA Extraction, PCR, and Gel Electrophoresis

Semester 1, AY2025/2026

STARTING TASKS

- Use alcohol and Kimwipe to clean your goggles
- Goggles and gloves needed for today's lab work

LEARNING OBJECTIVES

1. Isolate DNA from banana and spinach
2. Explain how the cell is lysed and DNA is released from cells
3. Conduct PCR and explain the principle of PCR
4. Conduct agarose gel electrophoresis and explain its principle
5. Interpret experimental data and to report data effectively

ORDER OF TASKS

1. Gel Electrophoresis of PCR samples (*45 mins to 1 hour*)
2. DNA Extraction from banana and spinach (*20 mins*)
3. View and analyse gel electrophoresis results (*5 to 10 mins*)

Previously…

Each pair of students prepared a PCR sample containing the following:

- 10 µl PCR master mix
- 2 µl Forward primer (*note down set number)*
- 2 µl Reverse primer (*note down set number*)
- 2 µl DNA template (plasmid)
- 4 µl Distilled water

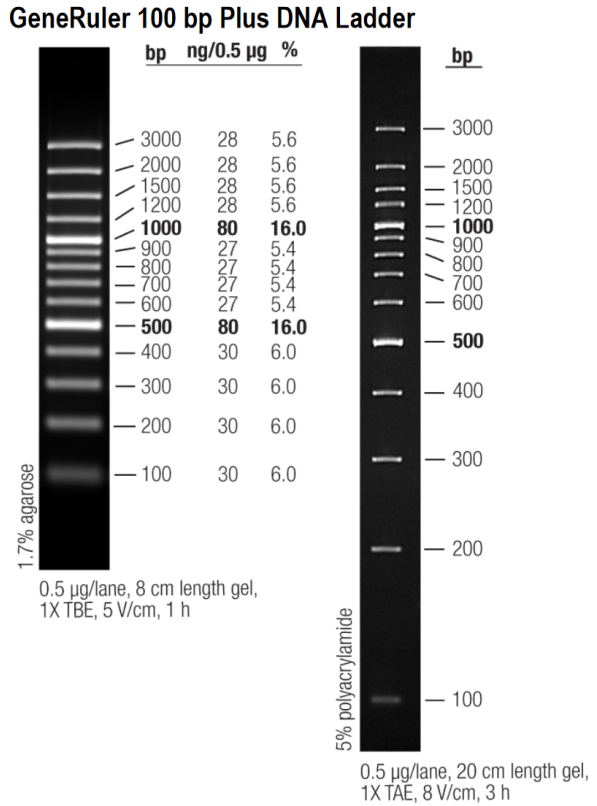
Subjected to the following PCR conditions:

STEP	TEMP.	TIME
Initial Denaturation	95°C	3 min
Denaturation, Annealing, and Extension (35 cycles)	95°C	30 secs
	55°C	30 secs
	72°C	30 secs
Final Extension	72°C	5 min
Hold	15°C	5 min

TODAY: Gel Electrophoresis

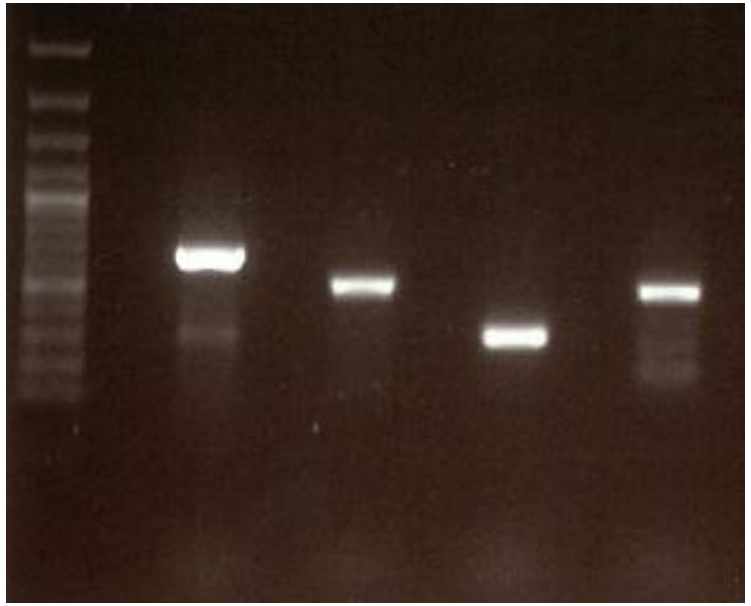
Purpose: To find out if the PCR was successful

- Gel electrophoresis separates molecules by size and charge
- Agarose gel contains staining agent called SYBR Safe
- DNA molecules visualised under UV light after electrophoresis
- DNA standards (“ladder” or “marker”) as reference sizes



TASK 1: Gel Electrophoresis

- TA will demonstrate sample loading using 15 µl DNA ladder
- Students will load 15 µl of their own sample
- Run electrophoresis at 120V for 40 minutes
- Visualise gel under UV light
- Take a photo; use it to answer Q2 of assignment



TASK 2: DNA Extraction

Starting materials:
quarter slice of banana; one spinach leaf

↓

Place in mortar

↓

Add a few drops of salt water; grind into paste with pestle

↓

Add remaining salt water; use pestle to gently mix water + paste

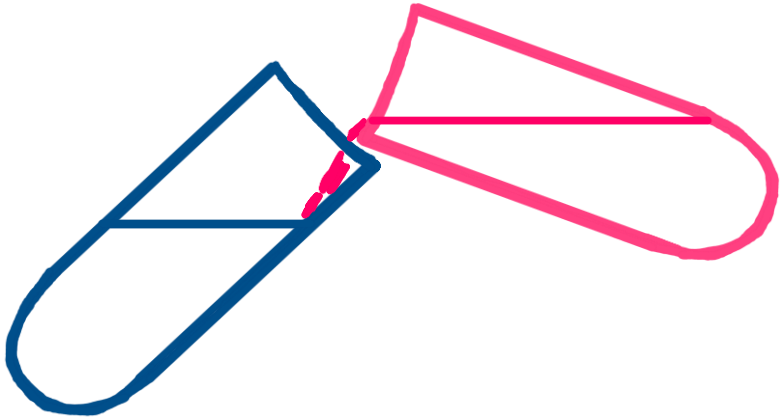
Individual Work: each student should prepare one banana extraction and one spinach extraction.

TASK 2: DNA Extraction

Transfer contents to tube containing liquid soap

↓

Cover; invert gently to mix for 2 to 3 mins



Tilt tube; gently add **ice-cold** alcohol down one side of the tube

↓

Cover; let tube rest for 5 mins (don't shake!)

↓

DNA will float to the top of solution

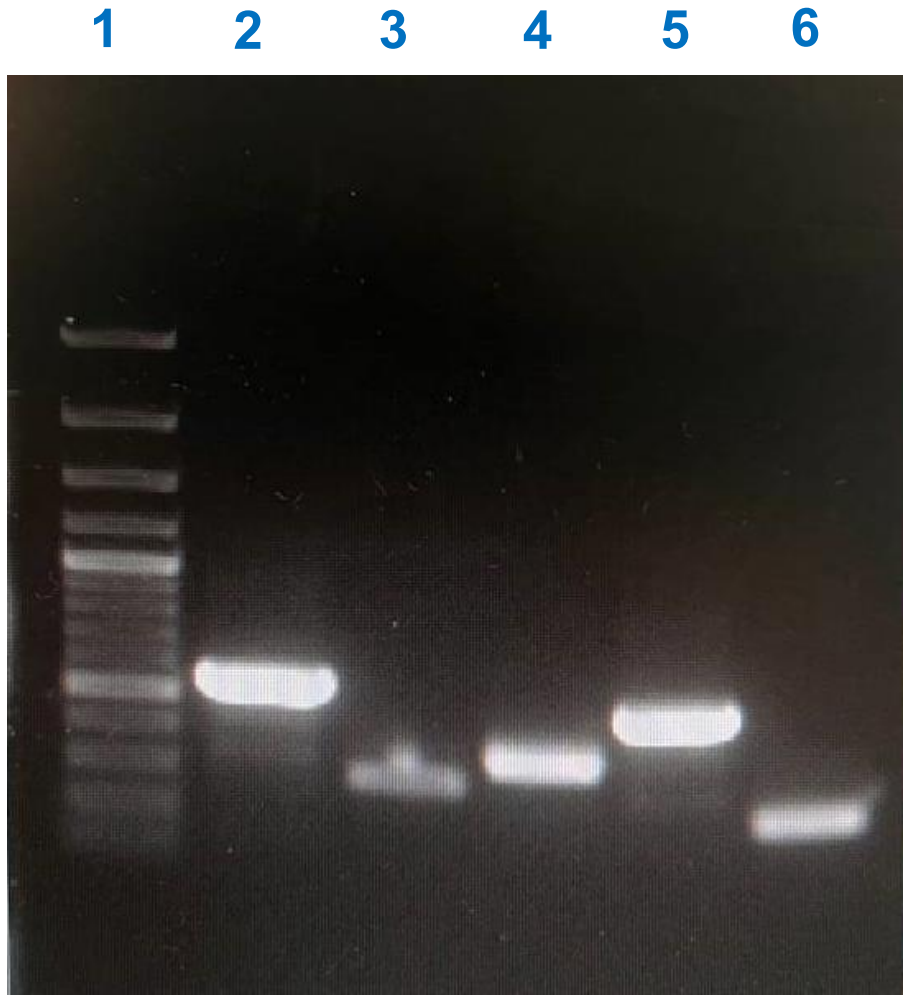
↓

To store DNA: use glass rod to twirl DNA, place in 1 mL **room temp.** alcohol

TASK 3: How to interpret/analyse gel photo

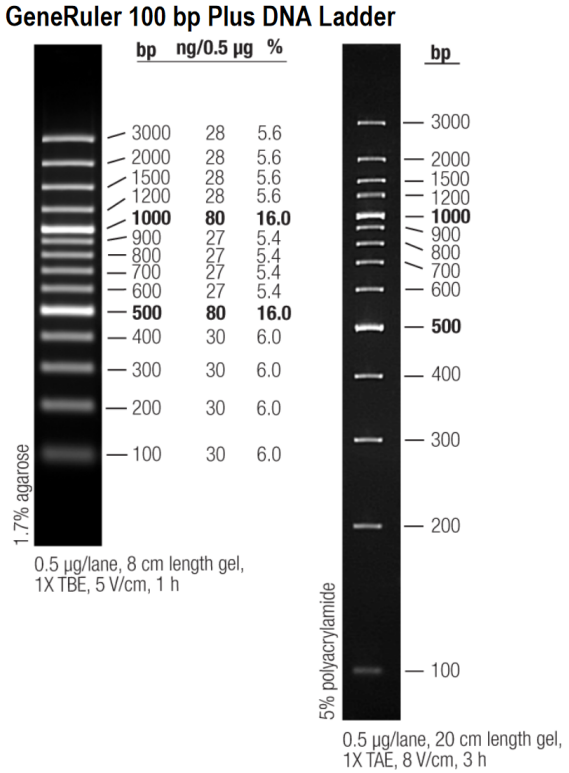
1. Label lanes for easy reference

Legend:
1: DNA marker
2: Sample 1
3: Sample 2
4: …
5: …



How to interpret/analyse gel photo

2. Identify and label DNA marker sizes



1000 bp →

500 bp →

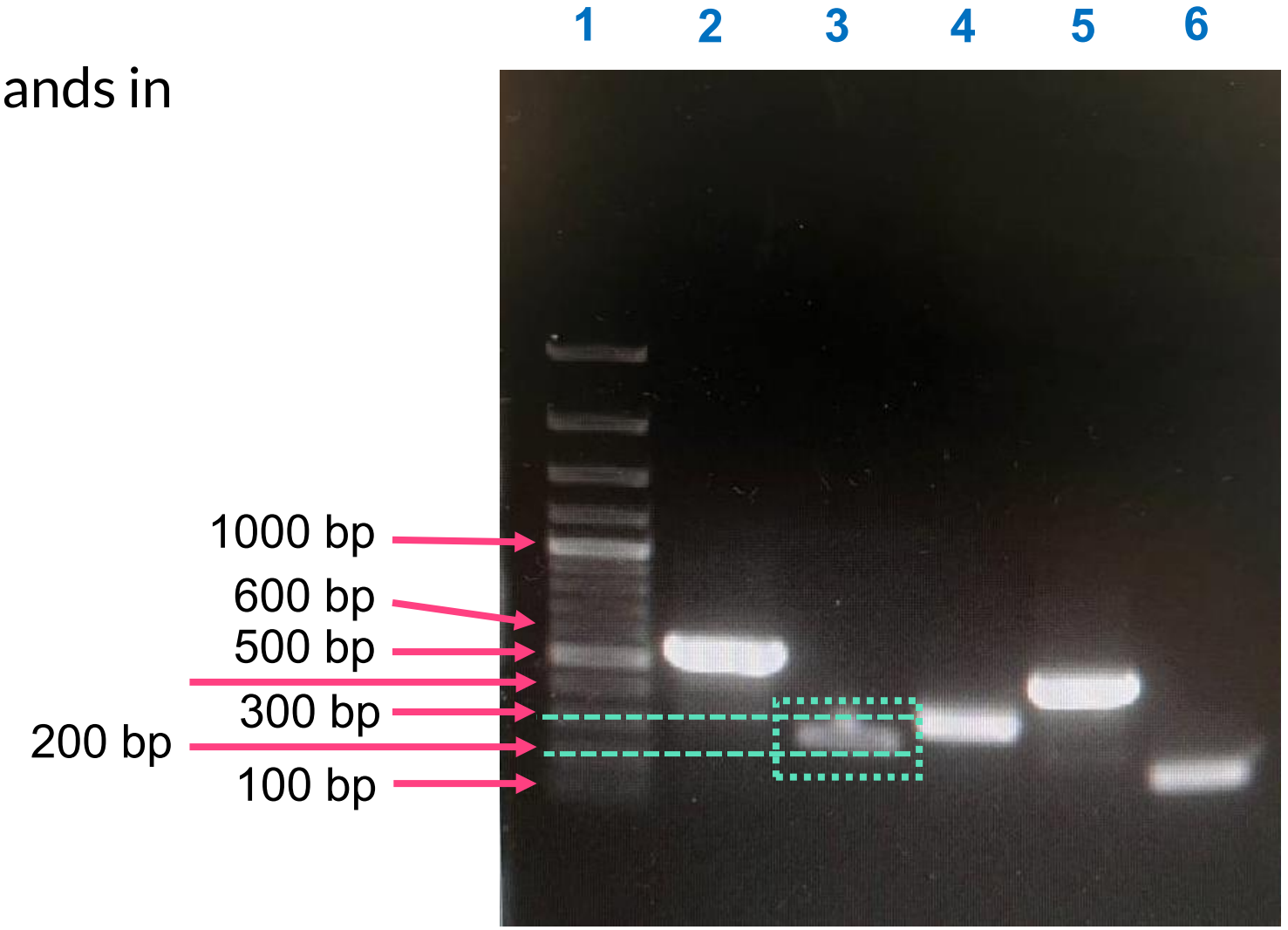
Important: Label the relevant marker sizes in the gel photo, not just the two examples shown in this photo.

How to interpret/analyse gel photo

3. Estimate size of sample bands in all lanes

e.g. The bright band in lane 3 is between 200 to 300 bp

Note: It is not necessary to draw the dotted lines around the bands, or lines aligning to the marker sizes. These lines are for demonstration only to show you how to estimate the band sizes. Avoid devising in-between sizes, such as "250 bp", which is not one of the reference sizes in the DNA ladder.



CITATIONS: HOW TO?

FYI

For certain lab assignment questions, you can cite information you obtain from scientific articles:

- You can use Google scholar (https://scholar.google.com/) or the NUS library search engines (https://nus.edu.sg/nuslibraries/databases-search) to do this
- Remember to rephrase the article's text into your own words and to cite the paper properly (below your answer).
- You may use APA citation format :
  - Author Last name, First initial. Middle initial. (Year Published). Title of article. *Title of Periodical, Volume*(Issue), page range.
- Example
  - Ross, R. P., Morgan, S., & Hill, C. (2002). Preservation and fermentation: past, present and future. *International journal of food microbiology*, *79*(1-2), 3-16.