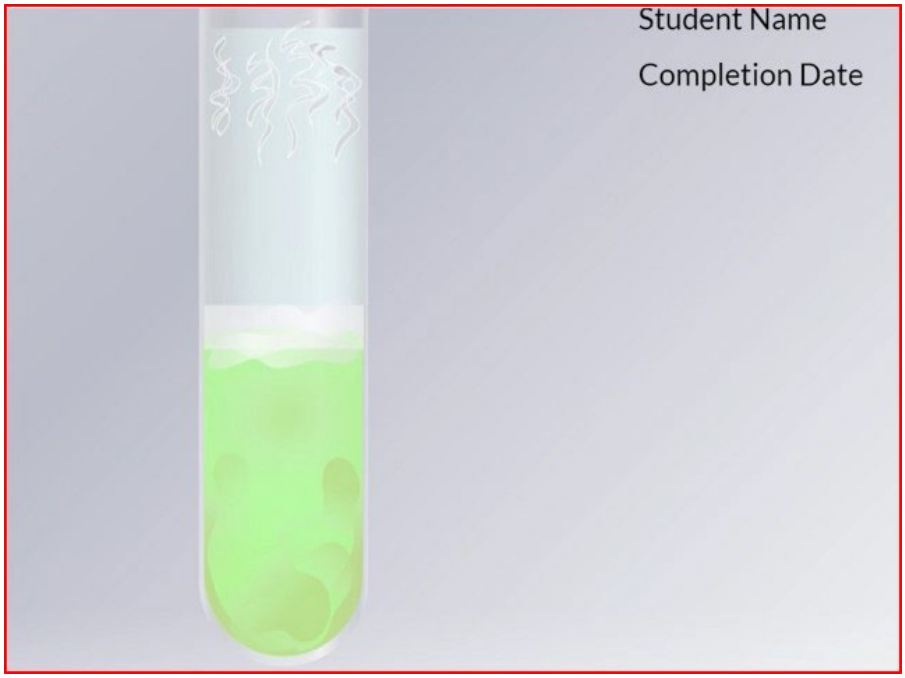
Exercise 1: DNA Extraction

Photo 1 : DNA Extraction



Question 1

Describe the functions of the detergent, salt, papain, and alcohol for the extraction of DNA.

The detergent and salt removed the cell membranes. Papain was used to separate DNA from surrounding proteins. The cold alcohol was used to precipitate the DNA out of the solution.

Question 2

How could DNA extracted in these procedures be amplified for further analysis in a biomolecular laboratory. Explain the polymerase chain reaction in your answer.

The polymerase chain reaction (PCR) could be used to amplify the DNA extracted in these procedures. Before performing PCR, a region of DNA would be identified for copying, then a set of primers would be created. The primers, template DNA, nucleotides, and Taq polymerase would be assembled in a tube, along with cofactors needed by the enzyme.

The DNA would then be heated to 96°C to separate the two DNA strands from one another. The DNA would then be cooled to 55°C which allows the primers to bind (anneal to) the target DNA.

The new DNA would then be synthesized with the help of DNA polymerase and deoxynucleotide triphosphates (dNTPs) at a temperature of 72°C. Amplification of the DNA would then occur in cycles, with each cycle exponentially increasing the number of newly synthesized DNA strands. In 2-4 hours, over 1 billion copies of a DNA fragment could be produced using this biomolecular technique.

Exercise 2: Recombinant DNA Synthesis and Translocation

Table 1: DNA Fragment Length

DNA Type	Length (bp)
Foreign	720
Plasmid	2803

Question 1

Describe the enzyme required to complete the recombinant DNA plasmid initiated by this exercise and the resulting size of molecule that would be produced. Reference Data Table 1 in your answer.

The enzyme DNA ligase would be required to join the cut DNA fragment to the cut plasmid to create recombinant DNA. The completed plasmid would have 3523 base pairs which is the sum of the fragments recorded in Data Table 1.

Question 2

Explain how the proteins coded by the recombinant DNA plasmid initiated in this exercise could be synthesized using bacteria cells.

The proteins coded by the recombinant DNA in this exercise could be produced using bacteria cells by bacterial transformation. During transformation, bacteria absorb recombinant DNA and clone the original fragment during cell division. The proteins coded by the fragment are produced by the bacteria cells in large volumes as the cells increase in number.

Exercise 3: Gel Electrophoresis

Photo 2: Electrophoresis Results

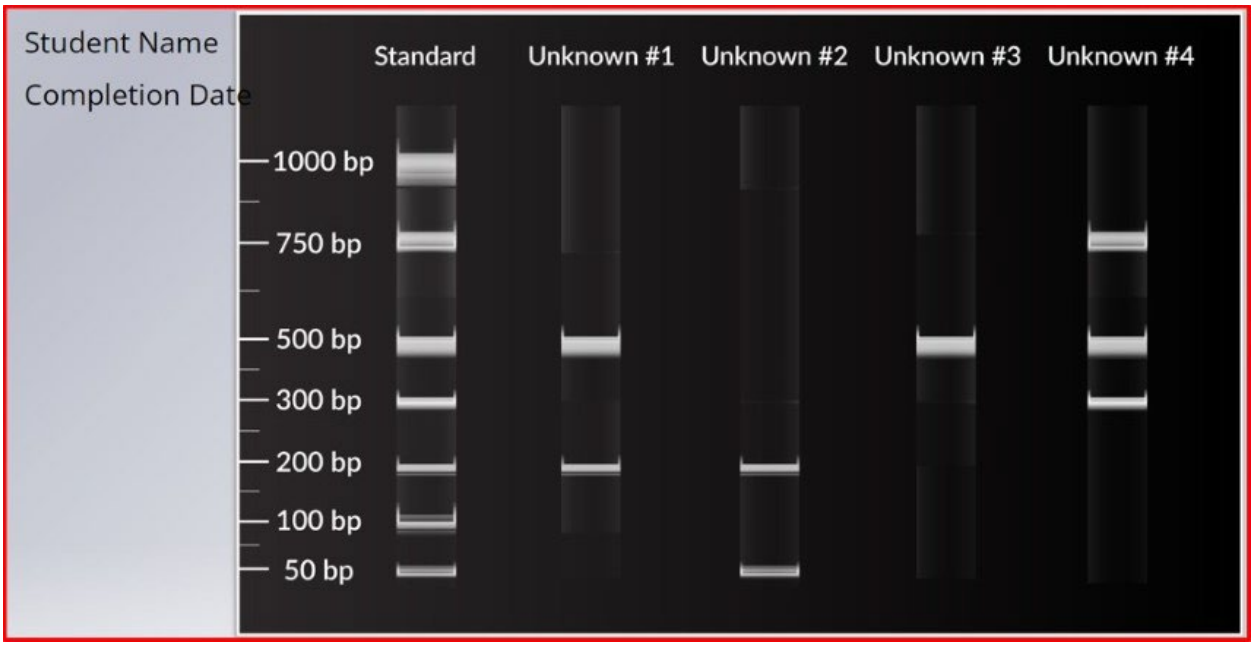


Table 2: Gel Electrophoresis Sample Identification

Sample	Fragment Length (bp)	Gene
1	500, 200	PF00210, CL0172
2	200, 50	CL0172, RPRGL2
3	500	PF00210
4	750, 500, 300	IDDMI, PF00210, PF09166

Question 1

Explain how gel electrophoresis is used to identify unknown DNA fragments.

During gel electrophoresis, molecules are separated by size and electrical charge. An agarose gel is prepared with a series of small wells in a plastic chamber. A standard mixture of identified fragments and the synthesized DNA from one or more unknown samples is added to the wells. See Figure 10. The gel is immersed by an aqueous buffer solution, and electrodes are attached to each end of the chamber. When an electric current is applied to the electrodes, the negatively charged DNA travels from the cathode (negatively charge electrode) through the agarose gel to the anode (positively charged electrode). The larger the molecule, the slower it travels through the agarose

gel. The completed gel is dyed to illuminate the separated samples which are then compared to the standards to identify the unknown fragments based on molecule size.

Question 2

Which of the unknown samples contained the DNA fragment coding for insulin? Explain your answer referencing Data Table 2 and Table 1.

Sample 4 contained the fragment with the gene IDDM1 that codes for insulin as listed in Data Table 2 and Table 1.

Extension Question

A new rainforest plant was discovered by scientists, having been used by native cultures for generations to cure fungal infections. Jane is a scientist in charge of investigating the anti-fungal properties of the new plant species. Describe the molecular techniques that Jane could use to isolate, identify, and produce the anti-fungal compound in the laboratory.

First, DNA could be isolated from plant tissue using a combination of salt, detergent, enzymes, and cold alcohol. The volume of the isolated DNA could then be increased using PCR. The amplified fragments could then be identified using gel electrophoresis. Once identified, the fragments responsible for the antifungal proteins could be inserted into plasmids and then used for cloning by bacterial transformation so than samples of the protein would be produced in a volume that could be tested on different types of fungi to determine its effectiveness as a potential drug.