

BIOL 1101

Lab 6 DNA Profiling Lab

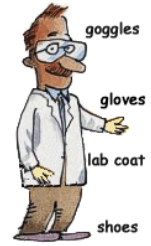
Name _____

Date _____

Lab safety: Full PPE required for this lab - lab coat, long pants, closed-toe shoes, gloves and safety goggles

Learning Objectives:

1. Describe the basic structure of a nucleotide and DNA
2. Know the function of DNA and how it is organized in cells
3. Understand the general process of extracting genomic DNA from fruit
4. Explain the function of each ingredient for DNA extraction.
5. Explain the process of DNA profiling.
6. Know the purpose of DNA fingerprinting; it's relevance in both human health and forensics.
7. Become familiar with the method of Gel electrophoresis.
8. Be able to Identify and measure the DNA bands in BP from a gel



Pre-Lab Activity:

Read the introduction below and then answer the following questions.

1. What is the function of DNA?
2. What are the three components of a nucleotide?
3. What is the basic structure of the double stranded DNA helix?
4. What is the overall charge of a DNA molecule?

Introduction:

DNA (Deoxyribonucleic acid) is a nucleic acid that contains all of the information necessary to build and maintain an organism. Encoded within this DNA are the directions for traits as diverse as the color of a person's eyes or the scent of a flower. It is found in nearly all living cells. However, the exact location of the DNA depends on the type of cell. In eukaryotes the DNA is located in a membrane-bound organelle called a **nucleus**. In prokaryotes the DNA is located within the cytoplasm of the cell.

DNA is composed of monomers called nucleotides. Each nucleotide has three primary components (Figure 1):

1. **nitrogenous base**
2. carbon-based **sugar** molecule called deoxyribose
3. **phosphate group**

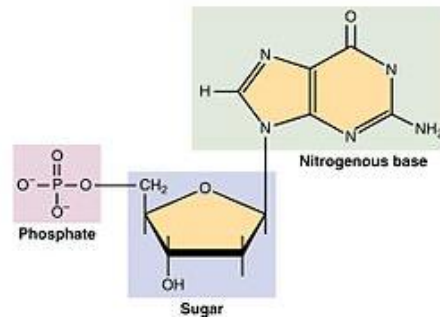


Figure 1. Basic structure of a nucleotide.

Picture Source: wikiwand (<https://images.app.goo.gl/ucavDERzq9h1Zydm7>)

There are four different DNA nucleotides, each defined by a specific nitrogenous base: **adenine** (A), **thymine** (T), **guanine** (G), and **cytosine** (C).

The most stable form of DNA is the double-stranded helix which looks like a twisted ladder (Figure 2). The two strands of DNA are connected via hydrogen bonds between the nitrogenous bases. **Base pairing rule**, states that hydrogen bonding between the nitrogenous bases are as follows: adenine pairs with thymine (A-T) and cytosine pairs with guanine (C-G). The sugar and phosphate group of the nucleotides form the backbone of the structure connected via covalent bonds. Since the phosphate groups are negatively charged and sit along the backbone of the molecule, DNA is considered to be a **negatively charged** molecule.

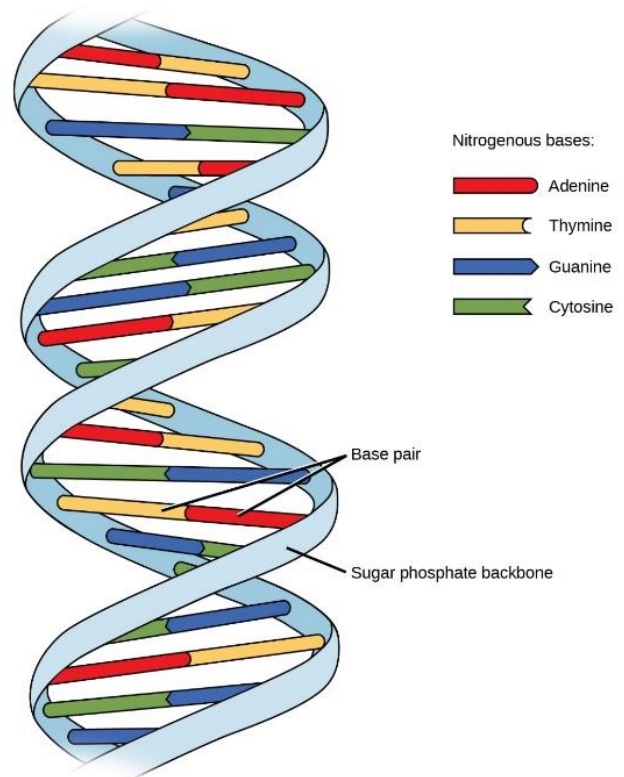


Figure 2. Structure of DNA molecule as a double stranded helix.

Picture Source: Clipart

Activity 1: DNA Isolation

Even though 99.9% of human DNA is exactly the same among individuals, there are enough variations among each of us that gives each individual a unique DNA profile, as unique as our fingerprints. **DNA**

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profiling is a forensic technique in criminal investigations, comparing criminal suspects' unique DNA profiles to DNA evidence allowing them to assess the likelihood of their involvement in the crime.

Profiling DNA involves several procedures, including **extracting** and **isolating** the DNA from the cells. In this activity you will be extracting DNA from kiwi fruit using readily available household materials.

Materials Needed

For a team of four students, you need:

- Fruit – Kiwi
- Cutting board & knife
- Extraction liquid (5% salt water)
- Detergent (shampoo, no conditioner)
- Ice cold alcohol (isopropyl alcohol) in ice bath
- Cheese cloth, scissors, and rubber band
- Glass beaker (200/250 ml), graduated cylinder (5 – 10 ml),
- Snack size Ziploc bag, plastic droppers
- Test tube (2), Test tube rack, Glass rod or paperclips

Procedure

Before starting with any step make sure the isopropanol alcohol is in the ice bath.

1. Take the skin off of kiwi and cut the fruit into small pieces. Place 6 to 8 pieces (about half a kiwi) in a Ziploc bag.
2. Measure 5 ml of extraction liquid (5% salt water) in a graduated cylinder and add to Ziploc bag.
3. Seal bag and gently smash up the fruit pieces with a beaker (roll it back and forth) or by hand.
4. Add about a quarter size amount of shampoo to the smashed components in the Ziploc bag. Seal and mix well but try to avoid making too many bubbles.
5. Allow mixture to sit at room temperature for 2-3 minutes.
6. Cut cheese cloth (double layer) into 4"/4" square and cover a 200 ml beaker with it. Place a rubber band around to hold the cheese cloth in place.
7. Filter the fruit mixture through the cheese cloth. This will remove all the solid material and almost clear liquid (filtrate) will be collected in the beaker.
8. Remove cheese cloth, careful not to spill the solids. Pour about an inch of filtrate from the beaker into a clean test tube.
9. Using a plastic dropper, take the ice-cold alcohol and very slowly, drop by drop, pour it down the side of the test tube to produce a layer of the alcohol on top of the fruit extraction liquid. Add about 15- 20 drops or until the layer is about the same height (an inch) as the amount of filtrate.
10. At the interface between the alcohol and the fruit filtrate a white shiny cloud-like substance will start forming. **Don't shake the test tube**. Do not mix the layers (alcohol and filtrate) or it will be difficult to separate the DNA. Allow the test tube to sit undisturbed for 1-2 minutes.
11. Use a glass rod or a bent paperclip to slowly spool the DNA up and out of the solution.

How it works:

- Because of their size, it is impossible to see a single DNA molecule with a naked eye. It has been suggested that it would take 300,000 DNA molecules side by side to make a bundle as thick as a single strand of human hair. Using the techniques described above, it is possible to collect a large amount of DNA to make it visible.

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- When you add the shampoo, the detergent destroys cell membranes, allowing the cell contents to spill out (cell lysis). We often think that the cytoplasm of cells is quite watery. In fact, the cellular content is quite thick composed of hundreds of thousands of proteins, nucleic acid molecules and other cell components.
- Salt water is a hypertonic solution and acts as an extraction buffer, which helps to prevent the DNA from being broken down.
- Since the filtrate is thick from the cellular contents, carefully pouring the alcohol on top of the supernatant leaves two distinct layers. DNA is soluble in water, but not in alcohol; thus, at the water-alcohol interface DNA **precipitates** out of solution, allowing it to be seen. A precipitate is an insoluble solid that emerges from a liquid solution.

Questions

1. What characteristics of DNA allow it to be spooled out?
2. Is DNA spooled as a single molecule of DNA? Yes ☐ No ☐
Explain your answer.
3. Given this single strand of DNA provide the complementary strand creating a double strand of DNA.

5'--- ATCCTTGAAGCTTGACTAATGGTC ---3'

Complimentary strand →

4. What is the purpose of each of the following components in this protocol?
 - A. Shampoo
 - B. Salt water
 - C. Isopropanol (alcohol)
5. The DNA you spooled – is it pure DNA? (hint: think about the various contents of the cell)

Clean Up

Wash the test tubes in sink with soapy water, rinse with distilled water and put them upside down in the rack. Throw the fruit pulp and cheese cloth in the trash. Wash the beaker with soapy water.

Activity 2: Gel Electrophoresis Analysis

The method of **gel electrophoresis** allows use to visualize fragments of DNA. Gel electrophoresis is a basic biotechnology technique that **separates** macromolecules according to their **size** and **charge**. It is frequently used to analyze and manipulate samples of DNA, RNA, or proteins. In gel electrophoresis, samples to be separated are applied to a porous gel made of a material such as **agarose**. Agarose is a purified form of agar, a gelatinous substance extracted from red algae.

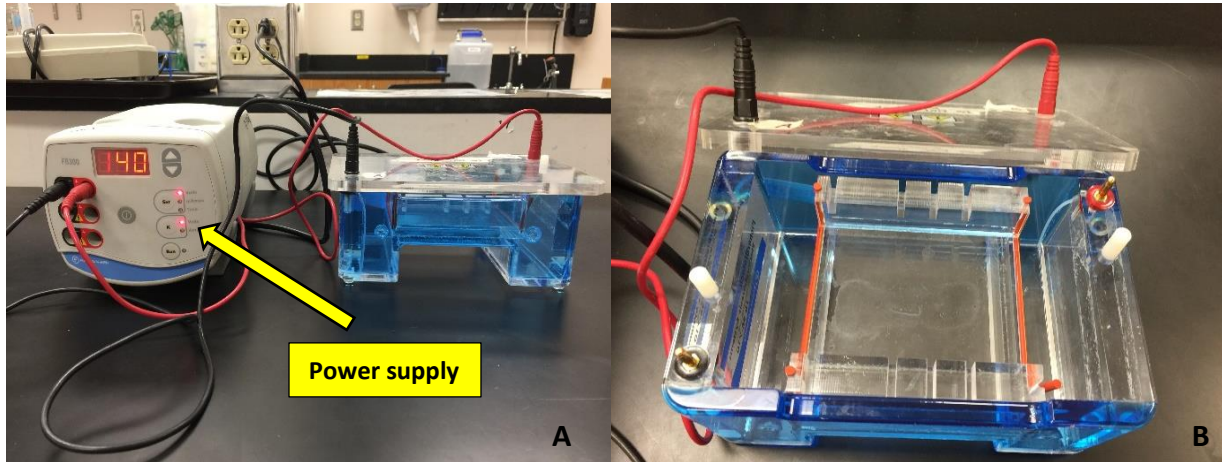


Figure 3. A. Power supply and electrophoresis unit.

B. Electrophoresis unit with buffer and gel.

The gel is placed in a chamber (electrophoresis unit) that is connected to a power supply (Figure 3). All the samples are loaded into the wells of the agarose gel. To “run” the gel an electrical current is applied to the gel. The chamber is designed with a **positive electrode** (anode, red) at one end and a **negative electrode** (cathode, black) at the other end.

Electrophoresis literally means “to carry with electricity;” once the electric field is established, charged molecules migrate through the pores of the gel toward their pole of attraction. Molecules with a net negative charge (such as DNA) migrate toward the positive electrode and molecules with a net positive charge migrate toward the negative electrode. The overall charge of a molecule affects the speed at which it travels through the gel. The mobility of a molecule during gel electrophoresis depends on its **molecular size** and shape. The small pores of the gel matrix act as a sieve. Small molecules maneuver more easily through the pores than larger molecules and therefore travel relatively quickly. Large molecules encounter more resistance as they make their way through the tiny pores and therefore travel at a slower rate. (Figure 4).

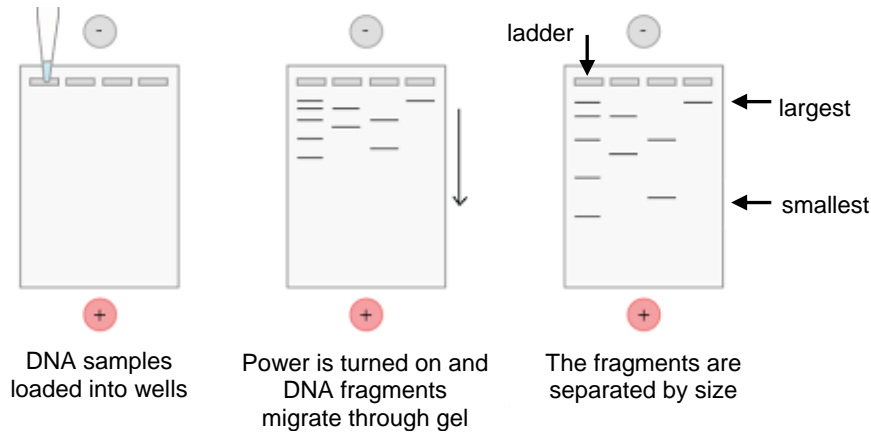
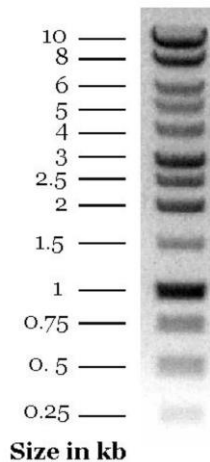


Figure 4. Negatively charged DNA fragments move through the agarose towards the positive electrode. Larger fragments move more slowly than smaller fragments. This allows fragments to be separated based on their size.

In order to see the DNA in the gel, a chemical called, **ethidium bromide (EtBr)** is added to the agarose while it was still in its molten (liquid) state. Ethidium bromide is a molecule that inserts or intercalates between the bases of double stranded DNA. Under **UV light** EtBr fluoresces, therefore DNA becomes visible under UV light in the presence of EtBr. **EtBr is a potent mutagen** (may cause genetic damage), and is moderately toxic.

Once the gel has run and a picture of the DNA bands has been taken, you can analyze the size of the DNA bands by comparing it to the lane containing the DNA ladder. A **DNA ladder** is a solution that contains known sizes of DNA. You compare your samples with the known bands to estimate the size of your DNA bands.

1 kb DNA ladder (Promega)



Exercise 1 – Running a Gel Electrophoresis

Materials Needed:

For each class section, you need:

- Gel Electrophoresis unit
- Power supply
- Running buffer (1X TAE)
- Gel with ethidium bromide
- DNA ladder
- Micropipette with tips
- UV light wand

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Protocol (Demonstrated by your instructor)

Your instructor will pipet standard DNA markers, known as a ladder, into the first well on the left side of the gel. The ladder acts like a ruler and can help you determine the size of the DNA fragments in your gel.

1. Pour running buffer (1X TAE) to cover gel by 2-3 mm.
2. Load 5µl of ladder in the first lane.
3. Attach lid- red to red; black to black. Ensure tight fit and turn on the power supply (switch on back). Set to 120 V. Press run. *Note:* Be sure to periodically check the gel while running confirming the power is still on and dye is moving toward red electrode.
4. Allow 35 – 45 minutes for the gel (ladder) to run.
5. Turn power off; carefully remove the lid of the electrophoresis unit.
6. Make sure everybody put on safety glasses.
7. Plug a handheld UV lamp and make sure the UV plates are facing towards the gel, not towards you.
8. Turn off the room light and look at the gel under the UV.

Exercise 2 - Analysis of a DNA Gel

Materials needed:

For a team of four students, you will need:

- Images of DNA gel for analysis

Procedure:

Forensic scientists compare DNA banding patterns to identify individuals whom are guilty of committing crimes. A picture from such a case is given to you.

Assume that lanes 1-8 (not including the lane with the ladder) are different suspects in a criminal investigation and lane 9 contains the DNA isolated from the crime scene.

1. Estimate the size of the bands in your DNA gel and write those down in the following table.

Lanes	1	2	3	4	5	6	7	8	9
List size of DNA fragments: <i><u>largest to smallest</u></i>									

2. From the above observation, which suspect do you think committed the crime. _____
Why?

Reference:

Rolfen Peggy. Fundamentals of Biology, Cincinnati State Community and Technical College. Heiden and McNeil. 2010

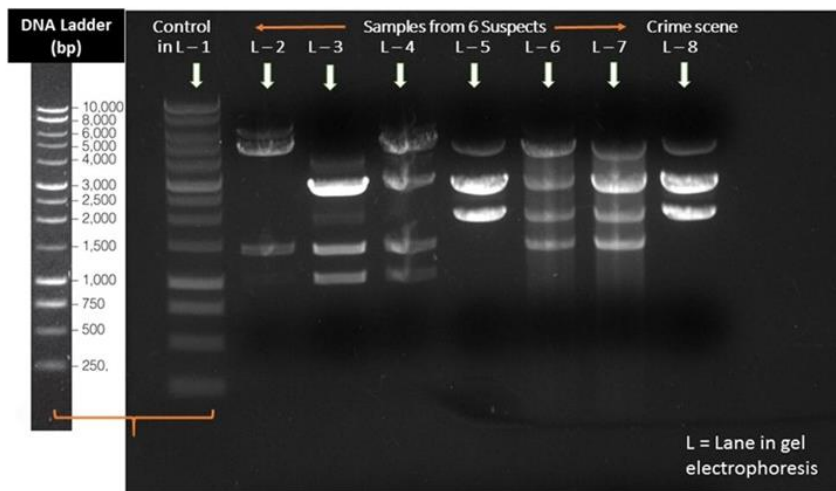
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Post-Lab Activity:

Name: _____

After completion of the lab, answer the following questions. Your instructor may require you to turn this in.

1. What is gel electrophoresis used for?
2. What makes the DNA move?
3. DNA migrates to the _____ end of the gel.
4. Long strands of DNA migrate farther than short strands of DNA. ☐ True ☐ False
5. What is ethidium bromide used for?
6. Using the image from a DNA gel electrophoresis, measure the DNA fragments (in bp) using the DNA ladder and list by size largest to smallest.



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DNA fragments (in bp)	6 Suspects (in lanes 2 – 7)						Crime scene sample (L-8)
	S-1	S-2	S-3	S-4	S-5	S-6	

7. Do any of the suspect's DNA bands exactly match those from the DNA found at the crime scene? if yes, which one(s)? _____