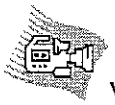
NAME :_____

Gel Electrophoresis: Dye Samples

Pre-Lab

1.  View Instructional Video :Pouring an Agarose Gel and answer the questions below.
2. Show calculations to prepare 30ml of a 0.8% agarose gel.

Instructor Initials: Calculation Correct

3. Why is it necessary to TARE the electric balance?_____
4. The purpose of the foil is _____
5. How long will you boil the solution?_____
6. The solution should be cooled below _____because _____
7. Sketch and label the parts of a gel casting tray.

8. Generate an SOP using Microsoft Word for making an agarose gel. Print SOP out and turn in with your lab activity.
9. Prepare a 0.8% agarose gel using your SOP.
10. Once your gel has solidified, your instructor will verify that the gel was made correctly.

Instructor Initials: Gel Made Correctly

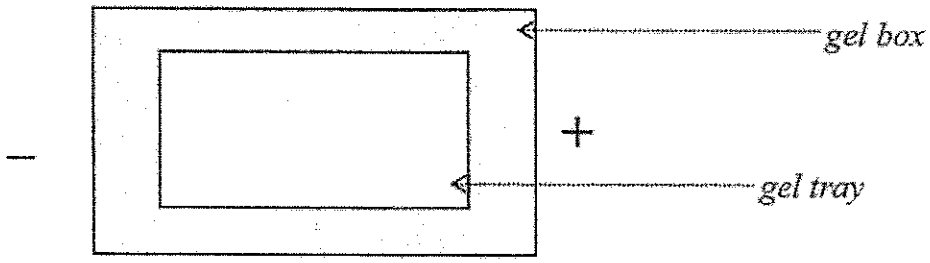
NAME :_____

Gel Electrophoresis: Dye Samples

Post-Lab

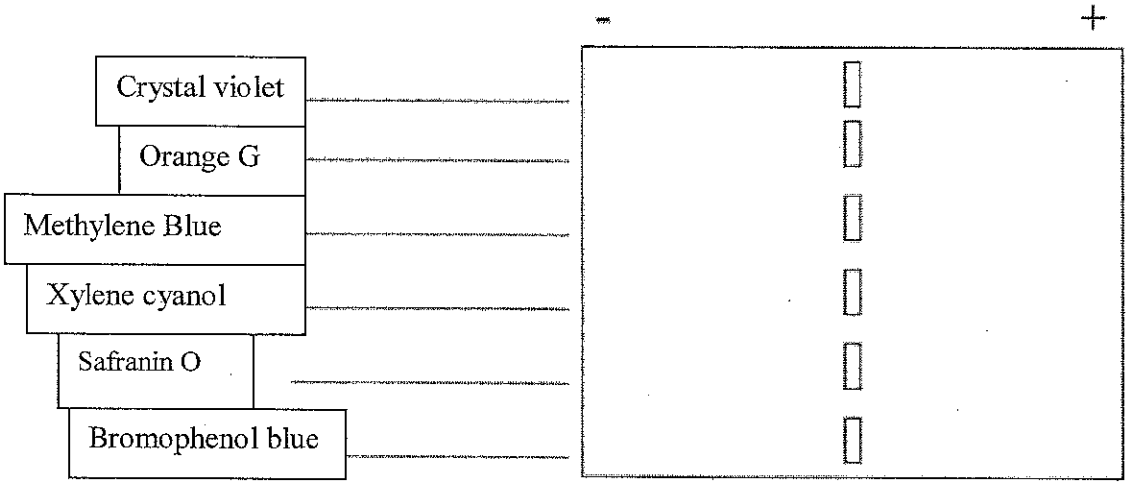
The sides of the DNA ladder are made up of alternating sugar and phosphate groups. These phosphate groups (PO_4) give DNA an overall negative charge.

1. If you were pouring a gel for DNA agarose gel electrophoresis, where would you place the comb? Draw a picture below.



2. Why is the comb placed in the center for this dye electrophoresis experiment?

3. For each known sample, add to this diagram a spot where the dye migrated.



NAME :_____

Gel Electrophoresis: Dye Samples Laboratory

Objectives

1. Understand the principles behind gel electrophoresis.
2. Become familiar with the procedure for running a gel.
3. Determine the components of an unknown dye mixture.

Materials

For Each Lab Group:

- Samples of the following dyes:
  1. Crystal violet
  2. Orange G
  3. Methylene blue
  4. Xylene cyanol
  5. Safranin O
  6. Bromophenol blue
  7. Unknown sample
- 1 microtube rack
- 1 electrophoresis gel box
- 1 0.8% agarose gel that has been cooled
- and initialed by instructor
- 250 ml beaker
- 250 ml flask of TBE buffer (1X)
- 20 µl micropipette and tips

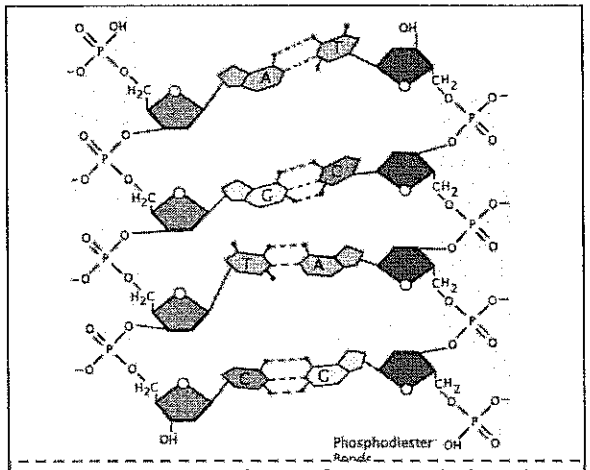


Figure 1: DNA is made up of sugar and phosphate molecules which are held together by phosphodiester bonds. The phosphate molecules on the DNA backbone are negatively charged. These phosphate molecules confer a negative charge on the entire DNA molecule. Therefore, because opposites attract, when placed in an electrical field, the negatively charged DNA will migrate toward the positive pole.

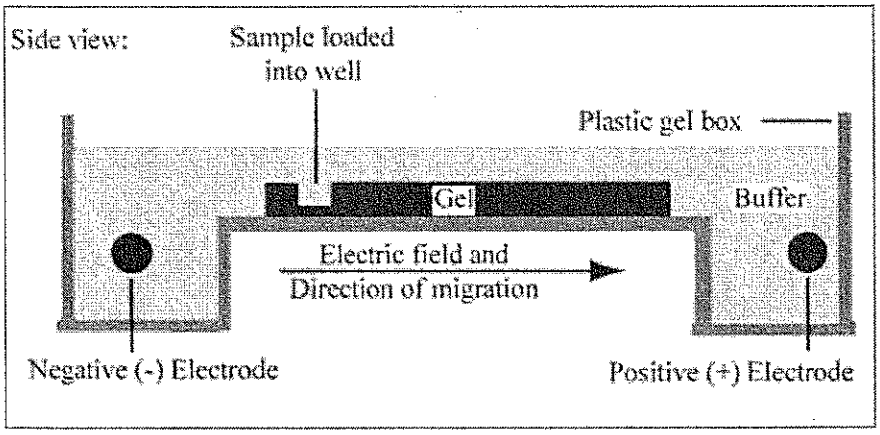


Figure 2: Because all DNA is negatively charged, regardless of the length or source, the rate of DNA migration and separation through an agarose gel depends on the size of the DNA molecule. Agarose provides a gel matrix through which the DNA molecules migrate. Smaller molecules travel faster through the gel matrix than larger molecules.

Pre-Lab: DNA Extraction

Name:_____

Go to http://learn.genetics.utah.edu/content/labs/extraction/ and complete the virtual DNA Extraction Lab. Answer the questions below.

1. Why would a scientist need to extract DNA from a cell? List 3 reasons:

2. Where is DNA found?_____

3. Where is the suspect's DNA sample taken from in the animation? _____

   a. Why here?

4. List the materials you will need to perform DNA extraction:

5. This solution is added to burst the cells open and means "to separate":_____

6. The lysis solution contains two important ingredients: _____

   a. Explain their function:

7. NaCl causes the proteins to _____.

8. Why were 2 tubes added to the microcentrifuge?

10. After the first centrifugation, the _____is found is the supernatant while the _____is found as a solid pellet at the bottom of the tube.

12. To isolate the concentrated DNA, _____is added. Because DNA is not soluble in the solution, the DNA will _____.

13. Following the second round of centrifugation, the _____is now found in the pellet at the bottom of the tube.

Lab: DNA Extraction of Wheat Germ

A complete copy of DNA is found in every cell, except red blood cells, in any organism. In order to release the DNA for analysis, scientists must break open the cells and remove structural proteins and enzymes that interfere with the DNA structure. This simplified procedure releases a great deal of DNA so that you can see it. It allows observation of DNA's physical and chemical properties. It does not, however, purify the sample enough for the strict standards of a research or forensics lab.

Materials

- 250 ml beaker
- hot plate
- Adolph's natural meat tenderizer
- non-roasted wheat germ
- ice cold 95% ethanol
- thermometer
- 15 ml test tube
- glass stirring rod
- Palmolive detergent
- distilled water
- test tube rack or 250 ml beaker
- graduated cylinders (10ml and 100ml)

Procedures

1. Add 100 ml distilled water to a beaker and heat to 50-60°C. **If your water gets to hot what will happen?**

2. Add 1.5 g wheat germ and mix until dissolved.

3. Add 5 ml detergent. Maintain 50-60°C temperature and stir for 5 minutes.

4. Add 3 g meat tenderizer. **What enzyme is in the meat tenderizer? What is its function?**

5. Maintain the 50-60°C temperature and stir for 10 minutes.

6. Remove from heat.

7. Add 6 ml of the solution to a test tube and cool to room temperature.

8. Pour 6 ml ice cold ethanol carefully down the side of the tube to form a layer.

9. Let the mixture sit undisturbed 2-3 minutes until bubbling stops.

10. The DNA will float in the alcohol. Swirl a glass stirring rod at the interface of the two layers to see the small threads of DNA.* Place DNA in 1ml of ethanol in an ependorf tube.

11. Repeat #7-10 until you have collected enough DNA (about the size of a pea).

12. Store samples in freezer.

***Draw your observations below:**

Post-Lab: DNA Extraction

1. We can't really see a DNA molecule under the microscope unless it is tightly coiled into a chromosome. Why is the DNA only visible after the addition of ethanol?

3. Why was the wheat germ raw (uncooked)?

4. What part of the cell does the detergent work on?

5. Predict what would happen if the sample was heated in boiling water.

6. What was the purpose of heating the sample?

7. Where is the first place you are able to see DNA?

Extension:
1. Bring in a fruit, vegetable or meat sample from your home.
2. Design an experiment to extract DNA from your sample.
3. Take pictures at several stages during your procedure.
4. Attach pictures in the right column and describe each step.

Pictures of fruit DNA extraction procedure

Structure of DNA and RNA-Student Resources
For Podcast 1.3 Structure of DNA and RNA

I. DNA is the genetic material in all living things. Differentiate between the following:

a. Chromosome

b. Gene

c. Nucleotide

II. Three components of a DNA nucleotide

a. Draw the deoxyribose sugar and number the carbons.

b. Show which carbon the phosphate group is on

c. Show which carbon the nitrogen base is on

i. List the four nitrogen bases. Circle the purines. Put a box around the pyrimidines.

Name: Date:

Gel electrophoresis: sort and see the DNA

Pre-class activity

Directions:

1. Go to the *DNAi* website www.dnai.org > **Manipulation > Techniques > sorting and sequencing.**
2. View the *Gel Electrophoresis* 2-D animation, and answer the following questions.

Questions:

1. How does the process of gel electrophoresis separate DNA fragments?

2. What is the purpose of the agarose gel?

3. What is the purpose of adding blue "tracking" dye to the DNA samples?

4. Explain why DNA has an overall negative charge.

5. Why is the fact that DNA has a negative charge so important in the gel electrophoresis process?

6. Explain how an agarose gel can separate DNA fragments of different lengths.

7. What is the purpose of ethidium bromide in gel electrophoresis?

LAB DESCRIPTION

In this experiment, samples of DNA from bacteriophage *Lambda* (48,502 base pairs in length) are cut with two different restriction enzymes, *Eco*RI and *Hin*dIII. Each enzyme recognizes and cuts at a different DNA sequence. A third DNA sample is the undigested control, while a fourth sample is cut with an unknown enzyme (either *Eco*RI or *Hin*dIII). Following incubation at 37°C the resulting restriction fragments are loaded into wells of an agarose gel and electrophoresed. An electric field applied across the gel causes the negatively charged DNA fragments to move from their origin (the sample well) through the gel matrix toward the positive electrode. The gel matrix acts as a sieve through which smaller DNA molecules migrate faster than larger ones; thus, restriction fragments of differing sizes separate into distinct bands during electrophoresis.

Following electrophoresis, the gel is removed and soaked in a chemical stain which diffuses throughout the gel and becomes highly concentrated in regions where it binds to DNA fragments. The stained gel is then exposed to medium-wavelength ultraviolet (UV) light. The stained DNA strongly absorbs UV light, loses some energy, and reemits visible light in the orange part of the spectrum. Under UV illumination, stained DNA fragments of identical size migrate to the same position in the gel and appear as a discrete orange band. By comparing the band pattern from DNA cut with the unknown enzyme to patterns obtained with *Eco*RI and *Hin*dIII, the identity of the unknown enzyme can be determined.

PROCEDURE

I. Set Up Restriction Digest

1. Use permanent marker to label four 1.5-ml tubes, in which restriction reactions will be performed: "E" *Eco*RI, "H" *Hin*dIII, "–" no enzyme, and "?" unknown enzyme.

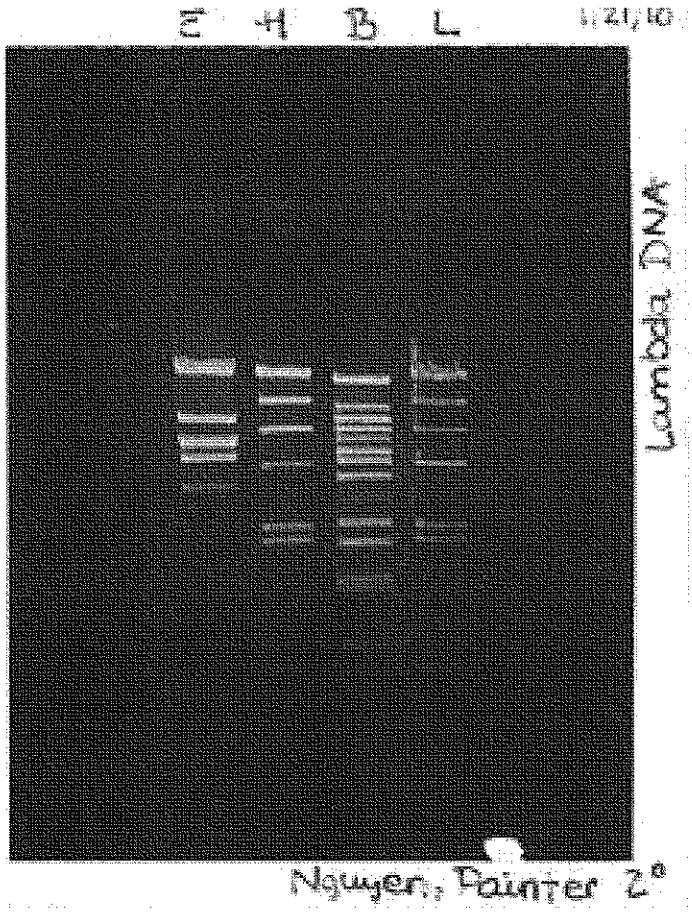
Use matrix below as a checklist while adding reagents to each reaction tube. Read down each column, adding the same reagent to all appropriate tubes; *use a fresh tip for each reagent.*

Tube	DNA	Buffer	*Eco*RI	*Hin*dIII	H_2O	unknown
"E"	4 µl	5 µl	1 µl	---	---	---
"H"	4 µl	5 µl	---	1 µl	---	---
"–"	4 µl	5 µl	---	---	1 µl	---
"?"	4 µl	5 µl	---	---	---	1 µl

1. Close tube tops. *Pool* and mix reagents by pulsing in a microfuge. Make sure tubes are placed in a balanced configuration in the rotor.

2. Place reaction tubes in 37°C water bath, and incubate for a minimum of 20 minutes. Reactions can be incubated for a longer period of time.

Your gel



E: Lambda cut with EcorI

H: Lambda cut with HindIII

B: ignore this lane

L: Unknown enzyme

Lambda Base-pair Matrix

*Hin*dIII		*Eco*RI		
Distance	Actual bp	Distance	Cal. bp	Actual bp
	*27,491			*24,756
	*23,130			*21,226
	9,416			7,421
	6,557			*5,804
	4,361			*5,643
	2,322			4,878
	2,027			3,530
	**564			
	**125			

* Pair appears as single band.

** Does not appear on ideal gel, as the fragments are too small

ANALYSIS AND DISCUSSION QUESTIONS

Agarose gel electrophoresis combined with ethidium bromide staining allows the rapid analysis of DNA fragments. However, prior to the introduction of this method in 1973, analysis of DNA molecules was a laborious task. The original separation method, involving ultracentifugation of DNA in a sucrose gradient, gave only crude size approximations and took more than 24 hours to complete.

Electrophoresis using a polyacrylamide gel in a glass tube was an improvement, but it could only be used to separate small DNA molecules of up to 2000 bp. Another drawback was that the DNA had to be radioactively labeled prior to electrophoresis. Following electrophoresis, the polyacrylamide gel was cut into thin slices, and the radioactivity in each slice was determined. The amount of radioactivity detected in each slice was plotted versus distance migrated, producing a series of radioactive peaks representing each DNA fragment.

DNA restriction analysis is at the heart of recombinant DNA technology and of the laboratories in this course. The ability to cut DNA predictably and precisely enables DNA molecules to be manipulated and recombined at will. The fact that discrete bands of like-sized DNA fragments are seen in one lane of an agarose gel shows that each of the more than 1 billion l DNA molecules present in each restriction reaction was cut in precisely the same place.

By convention, DNA gels are "read" from left to right, with the sample wells oriented at the top. The area extending from the well down the gel is termed a "lane." Thus, reading down a lane identifies fragments generated by a particular restriction reaction. Scanning across lanes identifies fragments that have comigrated the same distance down the gel and are thus of like size.

1. Why is water added to tube labeled "–" in Part I, Step 7?

2. What is the function of compromise restriction buffer?

3. What are the two functions of loading dye?

4. How does ethidium bromide stain DNA? How does this relate to the need to minimize exposure to humans?

5. Troubleshooting electrophoresis: What would occur if:
   a. the gel box is filled with water instead of TBE buffer?
   b. water is used to prepare the gel instead of TBE buffer?
   c. the electrodes are reversed?

6. Examine the photograph of your stained gel (or view on a light box or overhead projector). Compare your gel with the ideal gel shown below and try to account for the fragments of DNA in each lane. How can you account for differences in separation and band intensity between your gel and the ideal gel?

7. What is the identity of the unknown restriction enzyme? Explain your answer.

8. Troubleshooting gels. What effect will be observed in the stained bands of DNA in an agarose gel:
   a. if the casting tray is moved or jarred while agarose is solidifying in Part II, Step 1?
   b. if the gel is run at very high voltage?
   c. if a large air bubble or clump is allowed to set in agarose?
   d. if too much DNA is loaded in a lane?