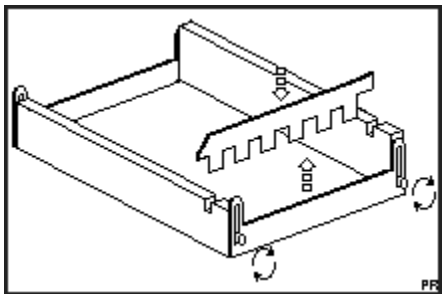
Name_____
Date_____
Period____

Agarose Gel Electrophoresis

Introduction:

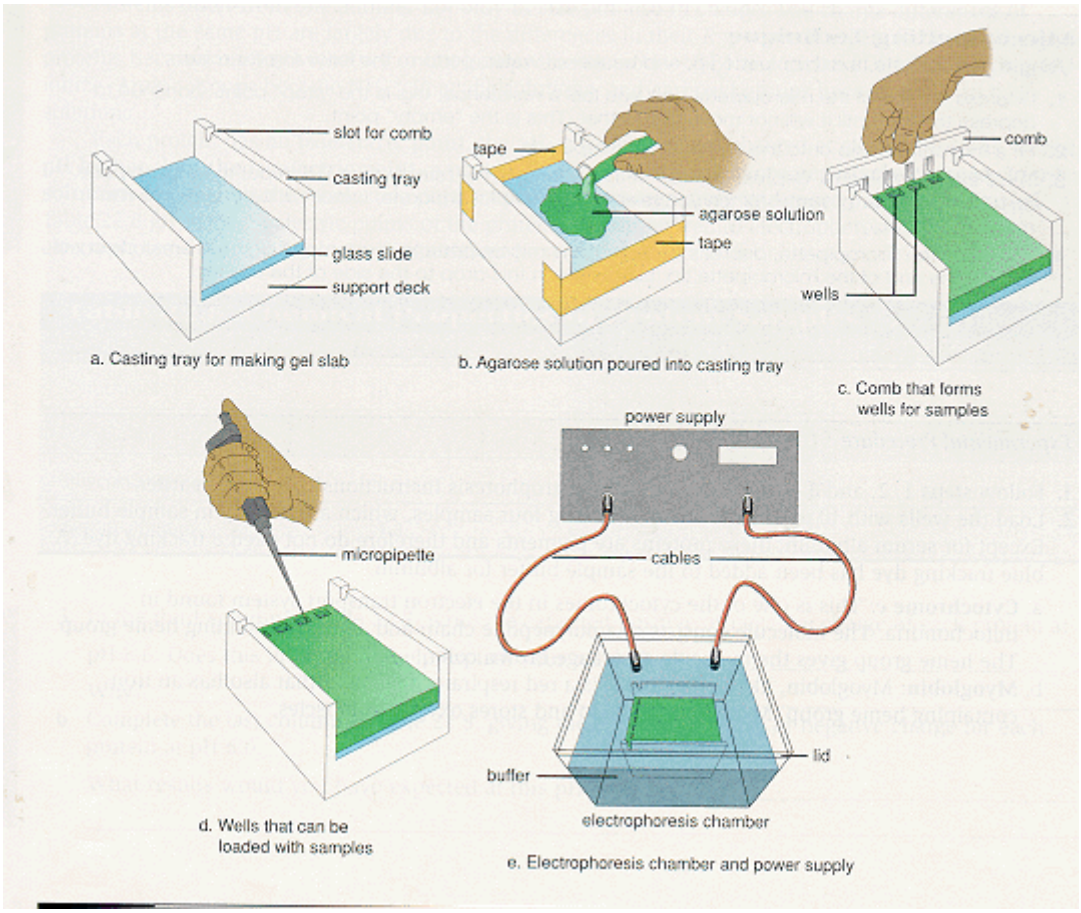
Agarose Gel Electrophoresis is a technique used very often by scientists to separate molecules. The material being separated is placed into a gel-like substance called agarose. Agarose is a substance derived from seaweed and when used in the lab has a similar consistency to jell-o.

Agarose gels are formed just as you would make jell-o. The agarose is a fine powder that is mixed with water and a buffer solution. The mixture is heated to its boiling point. This allows the agarose to dissolve fully and disperse evenly. The solution is the poured into a casting mold. The liquid takes the shape of the tray and is allowed to set for a period of twenty to thirty minutes or until it solidifies.

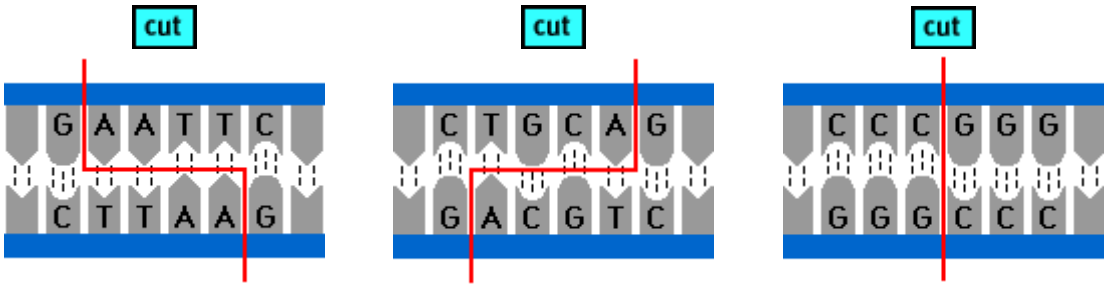


In order to separate molecules, there must be tiny slits made in the agarose gel by placing a "comb" into the mixture before it solidifies. The resulting wells can be filled with the particular substances to be separated.

The substances are separated based on their overall charge AND size. The resulting gel is a matrix of agarose with tiny holes between each agarose molecule. A direct current (DC) is passed through the gel. Positively charged molecules migrate towards the negative end of the current while the negatively charged molecules travel towards the positive end of the current. Gel itself acts as an obstacle course for the substances as they migrate to the different ends. Smaller molecules move faster through the holes between the agarose molecules. The larger the molecule the more time it takes for it to travel. At the end of the experiment the gel has various bands of separated substances.



When DNA is the substance being separated it must first be cut up into small, more manageable fragments. One of the most common ways to cut DNA is to use restriction enzymes. Restriction enzymes target specific sequences of DNA and clip the DNA in half. These “recognition sites” are symmetrical or palindromic. In other words, they are read the same on either strand! Below are 3 examples of restriction enzyme sites.



In order to see the DNA in an agarose gel it must be stained to see the bands. Another commonly used technique is to use a substance known as Ethidium bromide. Ethidium Bromide binds to DNA and when ultraviolet lights are shined on the gel the bands shine a bright color.

Materials (Per group of 6 students)

- 1 container of 6 dye samples
- Pipettes
- 1 Gel box
- 1 tray
- 30 cm of blue painter's tape
- 10 mL of buffer solution
- 0.48g of agarose powder
- 1 hot plate
- 1 100 mL Erlenmeyer flask
- 1 10 mL graduated cylinder
- 1 100 mL graduated cylinder
- 1 1000 mL graduated cylinder

Procedure:

Casting a gel:

1. Obtain 1.2mL of gel buffer and 58.8mL of water in separate graduated cylinders.
2. Weigh out 0.48g of agarose powder.
3. In a clean 100mL Erlenmeyer flask combine all three reagents (agarose powder, gel buffer and water.)
4. Place the solution over a hot plate and swirl the solution periodically.
5. Continue to swirl the contents of the flask until the solution is clear! *The solution should not boil!*
6. Allow the solution to cool until it is warm to the touch.
7. While the contents in the flask cool, use blue painter's tape to block off the edges of the gel tray. Make sure the tape is secure or you will have leaks!
8. ***Gently and slowly*** pour the content of the flask into the gel tray.
9. Place the comb into the center of the agarose gel and allow it to solidify.

Creating the buffer:

1. In a 1000mL graduated cylinder put 8mL of the buffer solution.
2. Add water to 400mL.
3. Mix the contents of the graduated cylinder.
4. Carefully pour the contents into the gel box.

Loading the gel:

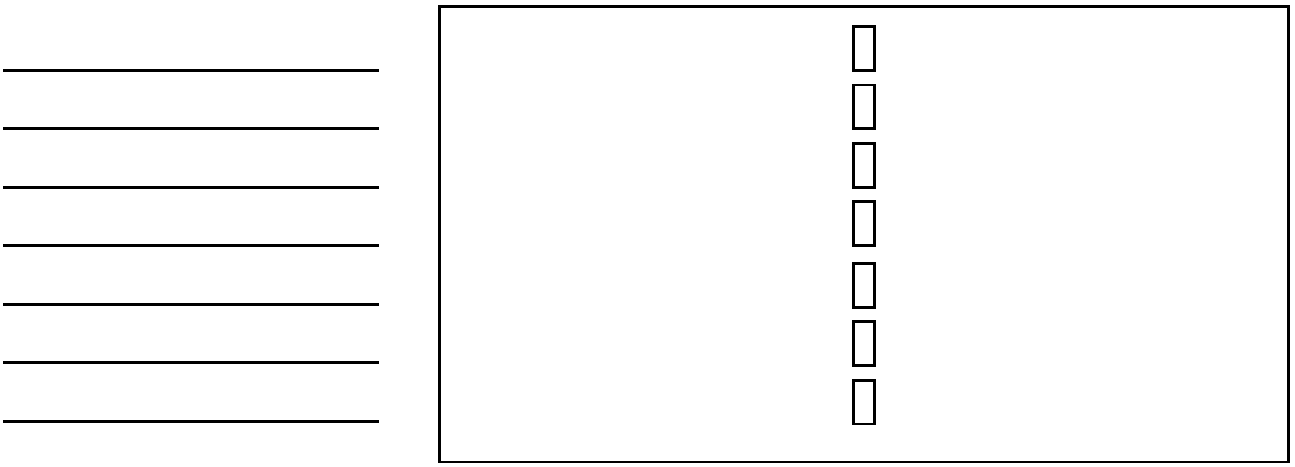
1. Once the agarose gel solidifies ***gently*** remove the comb and then remove the tape.
2. Submerge the gel (including the casting tray) in the gel box.
3. **From this point on you must NOT move the gel box at all!**
4. Using disposable pipettes load each sample into a separate well (created by the comb). Each student should load one gel. Record this information in data table one. ***Please remember to be careful***. You must make sure the pipette is IN a well or you will load the sample into the buffer solution and it will be lost. Also, be sure not to insert the pipette too far or you will puncture the gel.
5. Once all of the samples are loaded, cover the gel box and plug the leads into the electrical power source. Allow the gel to run for 30-45 minutes.
6. When the electrophoresis is complete, prepare a sketch of your gel. Use colored pencils to show the colors of the samples as originally loaded, and the bands that resulted after electrophoresis. Be sure to show each band in its proper position. **COLORED SKETCH REQUIRED.**

Results:

DATA TABLE ONE: Title: _____

LANE	COLOR	NAME OF PERSON LOADING
1		
2		
3		
4		
5		
6		
7		
8		

Sketch of completed electrophoresis



Analysis:

1. What determines the direction of DNA movement in a gel?
2. Why does DNA travel to the positive pole? (Be specific.)
3. What determines the rate of DNA movement in a gel?
4. How does electrophoresis separate the dye pigments?
5. Why do the dyes move in the direction they do once the power is turned on?
6. What charge is carried by the pigments in this separation? Support your answer.
7. Do any of the banding patterns resemble one another? If so, how might you interpret that?
8. How can a human being be identified from a blood sample using gel electrophoresis?