Name _____ Date _____
AP Biology Mr. Collea

DNA Technology I: Gel Electrophoresis

Objectives: At the end of this lab students should:

- Understand the principles and practice of agarose gel electrophoresis
- Demonstrate the separation of molecules based on charge
- Examine the rate of separation of molecules based on size

Background Information:

Concepts in molecular biology are often difficult to grasp. When trying to visualize the invisible world of the molecule, students are too often confronted by abstract theory. But like everything else in the field of biotechnology, things are changing fast. In 1950, a scientist named Oliver Smithies invented gel electrophoresis. Gel electrophoresis is an advancement in biotechnology that actually allows students to separate and visualize DNA, RNA, proteins, and other polypeptides and nucleotide sequences. Genes control all life processes. Therefore, the separation of DNA and gene products provides the potential to examine all of life's processes.

What exactly is gel electrophoresis? The word electrophoresis literally means to carry with electricity. It is a **separation** technology that is the sum of its parts: **gel**, a substrate (think of Jello); **electro**, referring to electricity; and **phoresis**, from the Greek verb phoros, meaning "*to carry across*." Gel electrophoresis, then, refers to the technique in which molecules are forced across a gel by an electrical current; activated electrodes at either end of the gel provide the driving force. Arne Tiselius, a Swedish biochemist, won the Nobel Prize for chemistry in 1940 for his work with electrophoresis.

Arne Tiselius
(August 10th, 1902 – October 29th, 1971)

How does the technique work?

Separation of large (macro) molecules depends on two forces: charge and mass. When a biological sample is mixed in a buffer solution and applied to a gel, these two forces act in concert. The electrical current from one electrode repels the molecules, while the other electrode attracts them, and the frictional force of the gel material acts as a "molecular sieve," separating the molecules by size and charge. Negatively charged molecules will migrate toward the positive pole, while positively charged molecules will migrate toward the negative pole. The net negative charge of the **phosphate** backbone results in the DNA fragments having a slightly negative charge and thus will always migrate toward the positive pole. The material is roughly analogous to that of a thoroughly wetted sponge, except that in this case, the "pores" are submicroscopic. During electrophoresis, macromolecules are forced to migrate through the "pores," away from the closest electrode and toward the farther electrode when electrical current is applied. After staining, the separated macromolecules in each lane can be seen; they appear as a series of bands spread from one end of the gel to the other.

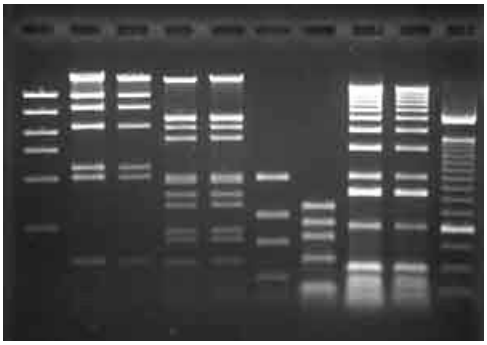


Figure 1. *Banding Pattern Produced via Gel Electrophoresis*

What's in a Gel

There are two basic types of materials used to make gels: **agarose** and **polyacrylamide**. **Polyacrylamide** is a material similar to that found in soft contact lenses and is primarily used to separate **proteins**. Agarose is a natural colloid extracted from seaweed. In the plant, it helps to resist dessication when exposed to air for extended periods of time Agarose gels have a very large "pore" size and are used primarily to separate very large molecules such as **DNA** with a molecular mass greater than 2,000 kdal. Kdal is the abbreviation for kilodalton or 1,000 daltons. A Dalton is a unit of molecular weight very nearly equivalent to the mass of a hydrogen atom, or 1.000 on the atomic mass scale. When agarose is heated to about 90°C it melts, but solidifies again when cooled below 45°C. During the solidification process, agarose forms a matrix of microscopic pores. During electrophoresis, DNA molecules wind through the pores in the gel being drawn through via the electric current.

Separation Techniques

While there are other separation techniques, the two most prominent in biotechnology are **chromatography** and **gel electrophoresis**. Both processes can simultaneously separate many common molecular entities from molecular mixes. **Chromatography** works best for small molecules (leaf pigments, for example), or for large batch-processing requirements. **Electrophoresis** is primarily used as an analytical tool to separate large macromolecules ranging in size from 20 to 2,000 kdal.

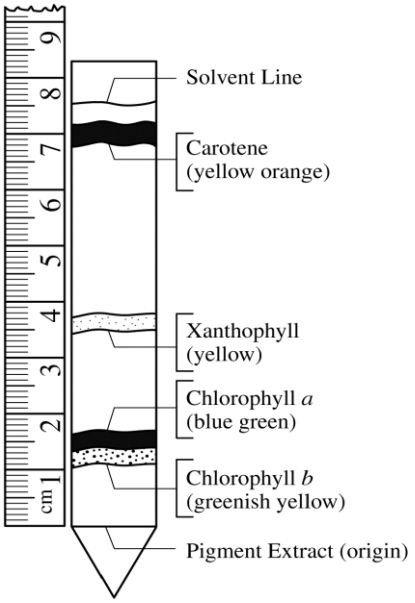


Figure 2. *Separation of Chlorophyll by Chromotography*

Pre-Lab Activity: Practice Pipetting

Materials: 1 mL vial of practice loading dye
micropipette
electrophoresis chamber
simulated gel with 24 wells

Methods:

1. Remove the lid from the electrophoresis chamber.
2. Add enough water to submerge the gel.
3. Using the micropipette, CAREFULLY place 10uL of loading dye into each well.

DO NOT puncture the bottom of the well with the tip!

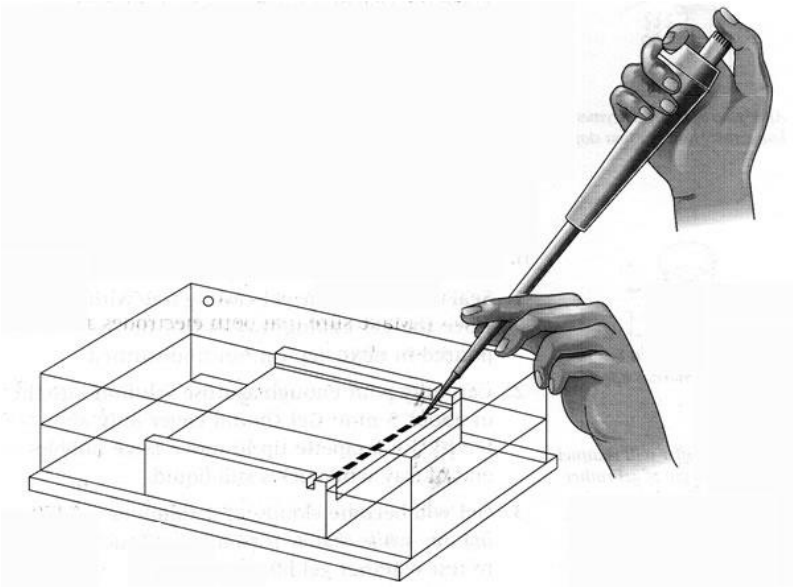


Figure 3. *Hand Positioning for Loading an Agarose Gel*

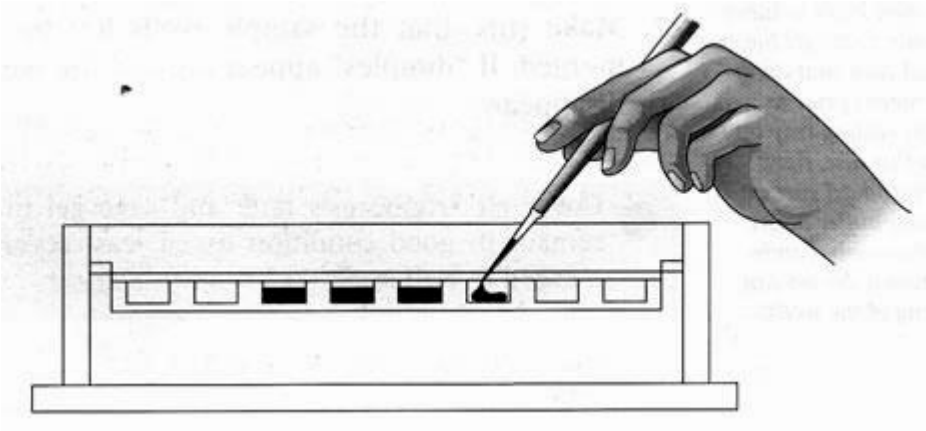


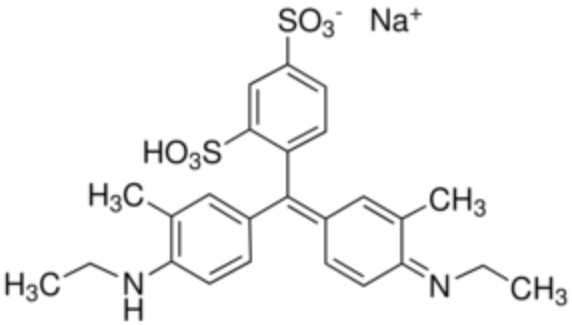
Figure 4. Loading the Gel into the Wells

Part I. A Process to Dye For

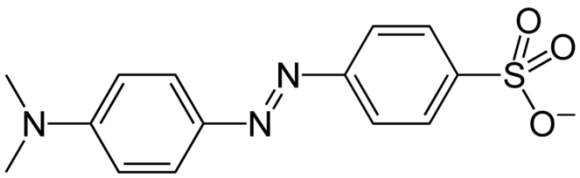
The four chemical and structural formulas of the stains/dyes we'll be using in the first part of this lab are located below. Determine their approximate molecular weights (MW) and place them in the table below. Look carefully at each structural formula to find the charge that each dye molecule possesses. Use this information to fill in the charge column below.

Table 1.

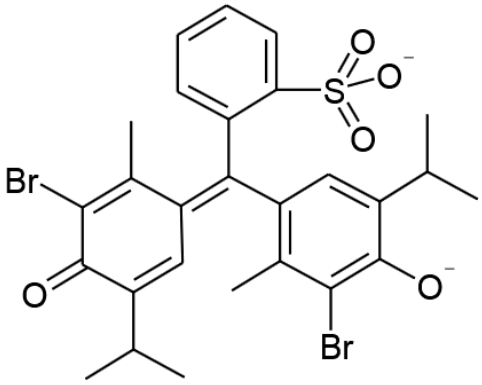
	Dye Names and Formulas	MW	Charge
1	**Xylene Cyanol** $C_{25}H_{27}N_2O_6S_2$		
2	**Methyl Orange** $C_{14}H_{14}N_3O_3S$		
3	**Bromophenol Blue** $C_{27}H_{28}Br_2O_5S$		
4	**Ponceau G** $C_{18}H_{16}N_2O_7S_2$		
5	**Unknown-I**		



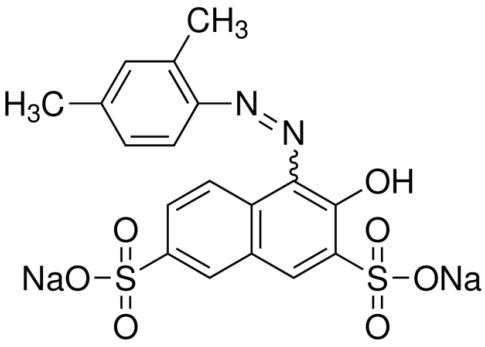
(1) Xylene Cyanol



(2) Methyl Orange



(3) Bromophenol Blue



(4) Ponceau G

Answer the following questions, based on the information in the chart on the previous page:

1. Which dye do you predict will move through the gel the fastest and why?

_____

_____

2. Which dye do you predict will move through the gel the slowest and why?

_____

_____

3. Write a brief function for each of the parts used in gel electrophoresis:

a. Agarose gel - _____

_____

b. Electrophoresis buffer - _____

_____

c. Wells in the gel - _____

_____

d. Electric current - _____

_____

4. How can we determine the approximate molecular weight and charge of the Unknown-I dye?

_____

_____

_____

_____

_____

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Gel Observations:

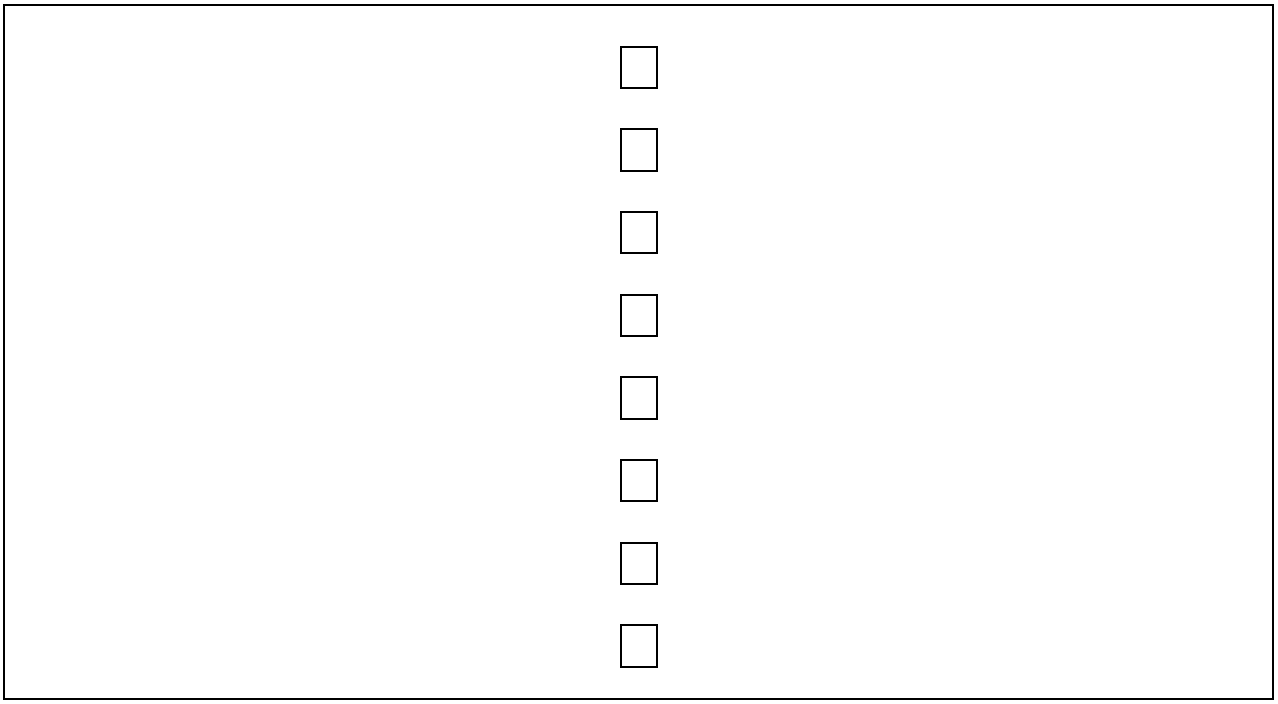

Table 2. Dye Results

Dye Name	Dye Well #	Migration Distance (mm)	Migration Direction (+/-)	Dye Molecules "Speed" Rankings
Xylene Cyanol				
Methyl Orange				
Bromothymol Blue				
Ponceau G				
Unknown - I				

According to your results, what is the approximate molecular weight and charge of the Unknown-I dye? Explain your answer.