

Gel Electrophoresis: How Does It Work?

Revised 5/11/96

Introduction:

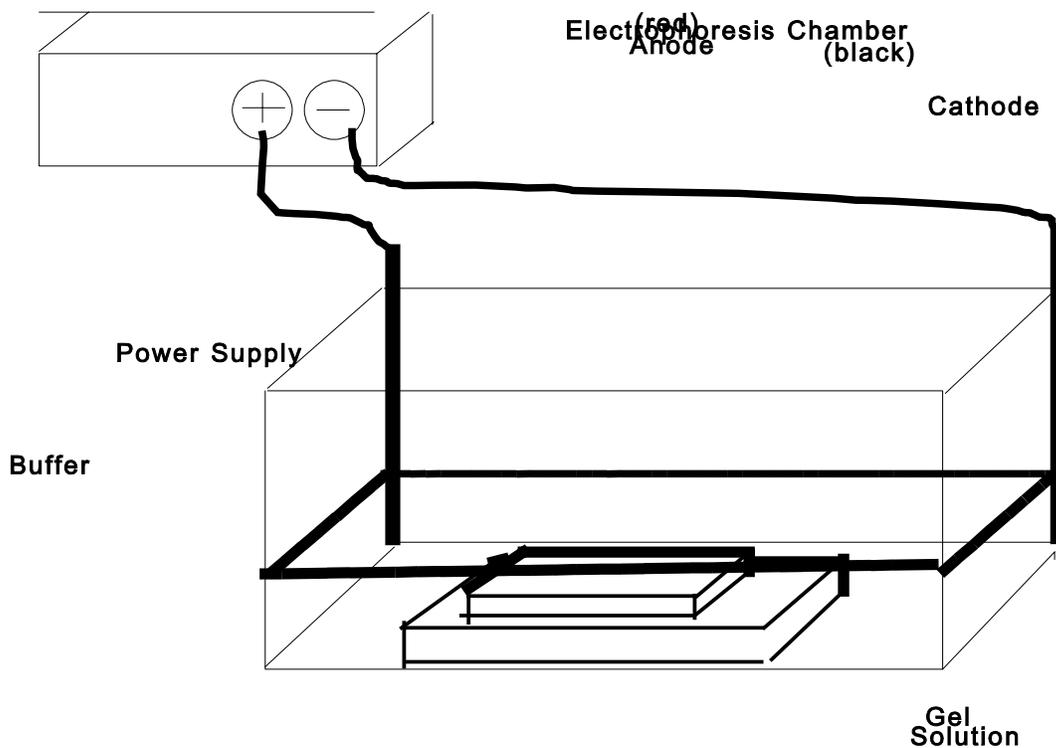
Simply put, gel electrophoresis uses positive and negative charges to separate charged particles. Particles can be positively charged, negatively charged, or neutral. Charged particles are attracted to opposite charges:

Positively charged particles are attracted to negative charges, and negatively charged particles are attracted to positive charges.

(+) ←←←← (-)

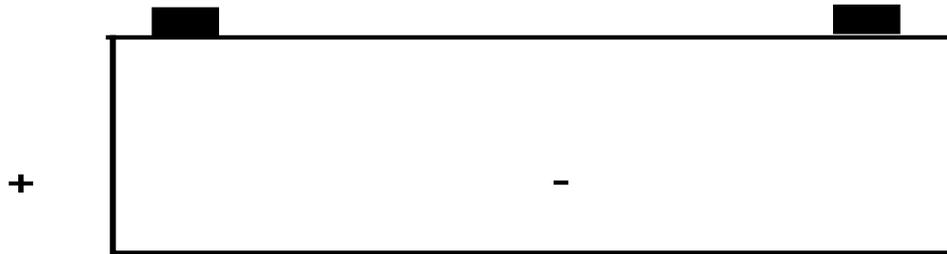
(OPPOSITES ATTRACT!)

Because opposite charges attract, we can separate particles using an electrophoresis system. Although an electrophoresis system may look very complex, it is actually quite simple. Some systems may be slightly different; but, they all have these two basic components:



1. Power Supply
2. Electrophoresis Chamber with a tray.

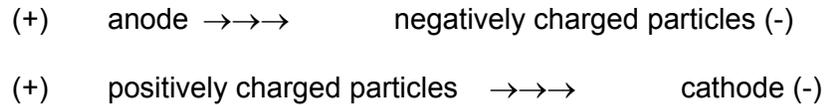
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Power Supply

The power supply supplies power (Amazing, isn't it!?!). The "power," in this case, is electricity. The electricity that comes from the power supply flows, in one direction, from one end of the electrophoresis chamber to the other.

The cathode and anode of the chamber are what attracts oppositely charged particles. (Opposites Attract!)



Inside the Electrophoresis Chamber

Inside the electrophoresis chamber, is a tray--more precisely, a **casting tray**. The casting tray consists of the following parts:

glass plate

The glass plate goes in the bottom of the casting tray. It helps the gel slide out of the casting tray when finished.

gel

The gel is held in the casting tray. It provides a place to put the small particles you wish to test. The gel contains pores that allow the particles to move very slowly toward the oppositely charged side of the chamber. At first, the gel is poured in the tray as a hot liquid. As it cools, however, the gel solidifies.

comb

The "comb" looks like its name. The comb is placed in slots on the side of the casting tray. It is put in the slots BEFORE the hot, melted gel is poured. After the gel solidifies, the comb is taken out. The "teeth" of the comb leave small holes in the gel that we call "wells."

wells

Wells are made when the hot, melted gel solidifies around the teeth of the comb. The comb is pulled out after the gel has cooled, leaving wells. The wells provide a place to put the particles you

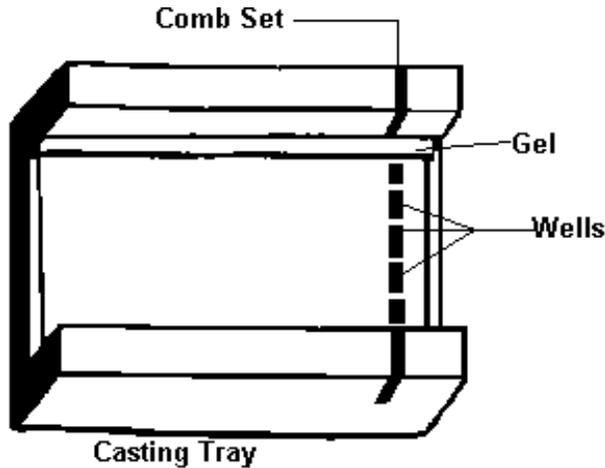
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wish to test. A person must be very careful not to disrupt the gel when loading the particles. Cracking, or breaking the gel will likely affect your results.

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particle samples

Particle samples are carefully loaded into the wells. Sometimes, dyes or other compounds are added to the particles before the particles are loaded. Many different types of particles can be loaded. The particles are usually in a solution. Each well holds around 10-25 microliters (μL). ($1 \mu\text{L}$ is 1/1000 of milliliter or 1×10^{-6} liters. A very small amount!)



buffer
 A buffer is a solution that conducts electricity. The solution is poured into the electrophoresis chamber until it *just covers* the top of the casting tray. This solution slot allows the electric current to flow from the cathode, through the buffer to the anode

CASTING TRAY

Make sure you are familiar with the terminology you have read on the previous two pages: power source, electrophoresis chamber, casting tray, gel, comb, wells, particle sample, and buffer. Do not attempt to begin the procedure until you have studied them. (Electrophoresis equipment is very expensive and must be handled very carefully, correctly.) Here is a summary of most of those concepts:

Remember, an electrophoresis system consists of two main components: 1) a Power Source, 2) an Electrophoresis Chamber with a casting tray. Here is a short review of how an electrophoresis system works.

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Summary

A gel is formed in a casting tray. The tray contains small "wells" that hold the particles you wish to test. Several microliters (μL) of the solution containing the particles you wish to test are carefully loaded into the wells. Then, a buffer, which conducts electrical current, is poured into the electrophoresis chamber. Next, the casting tray, containing the particles, is carefully placed into the chamber and immersed in the buffer. Finally, the chamber is closed and the power source is turned on. The anode and cathode, created by the electric current, attract the oppositely charged particles. The particles slowly move in the gel toward the opposite charge. The power is turned off, and the gel is taken out and inspected.

Questions

1. What would happen if you were to touch the gel while the electrophoresis chamber was running? What are some things that you must remember to do in order to keep that from happening?
2. What is the function of the comb, when is it used?
3. How many liters are in one microliter?
4. What is the symbol for microliter?
5. During an electrophoresis experiment, what causes the particles in a well to move?

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Gel Electrophoresis: How Does It Work?

Purpose:

To identify the basic components of an electrophoresis system and to obtain a basic understanding of their functions.

Safety Considerations:

1. Wear safety goggles and an apron.
2. NEVER PUT THE POWER SOURCE OR ELECTROPHORESIS CHAMBER NEAR RUNNING OR STANDING WATER!!!
3. ALWAYS turn off the power source when the cover is removed and the chamber is not in use.

Materials (per group):

electrophoresis chamber & power source
casting tray
masking tape
melted agarose (gel)
sample dyes on ice
buffer solution (enough to fill chamber)
toothpick micropipet (capillary tube and plunger)
100 mL distilled water

Procedure:

1. Make sure you have read the ENTIRE procedure. If you have any questions, ask now.
2. Make sure you have all of your materials (listed above).
3. Make sure you know how the comb and glass plate fit into the casting tray. And how the casting tray fits into the chamber. Practice, if you need to.
4. Preparing the Gel:
 - a. Take the casting tray out of the chamber.
 - b. Place the glass plate in the bottom of the casting tray.
 - c. Using masking tape, tape both sides of the casting tray. Wrap the extra tape around the sides and under the tray. (This tape will hold the hot melted agarose as it gels.)
 - d. Place the comb in the proper slots located on the casting tray.
 - e. In a microwave, heat the agarose until it melts. (When it is ready, you should not be able to see any particles in the agarose solution.)
 - f. Carefully, pour the melted agarose into the casting tray. (You should pour it until the agarose nearly fills the casting tray.)
 - g. If you see any air bubble ABOVE the glass plate, immediately use a toothpick and "drag" them to the side of the casting tray until they are out. (Air bubbles could affect your results if they are left in the path of the particle samples.) Note: There will probably be a flat bubble underneath your glass plate don't worry about it.
 - h. After the air bubbles are removed, DO NOT TOUCH THE TRAY OR THE GEL until it has cooled completely (you could damage the gel).

It will take 10-15 minutes for your agarose to cool enough to form a gel. As it "gels," it will turn opaque (cloudy). As it cools, get to work on Step 5.

5. Assemble your micropipet by putting the plunger and capillary tube together. Notice the lines on the side of the capillary tube. Each line represents 5 microliters. In this lab, you will need to

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measure out 15 microliters--or to the third line.

Practice measuring 15 μ L:

- a. Put the end of the micropipet into the distilled water.
- b. "Pull" up the water by pulling up the plunger.
- c. Pull the water up to the third mark (15 μ L).
- d. Leave the plunger where it is, and carefully take out the micropipet without changing the volume.
- e. Carefully wipe off the end of the capillary tube. Touch the tube to the side of the container to make sure all 15 μ L is transferred.
- f. Push out the water into another container (it doesn't matter what container you choose since it is only distilled water).
- g. There will be some water left hanging on the end of the capillary tube. Touch the tube to the side of the container to make sure all 15 μ L is transferred.
- h. Wipe off the end of the micropipet with a Kimwipe.
- i. "Rinse" the micropipet by putting it in distilled water and pulling and pushing water in and out of the capillary tube.

Note: Make sure you feel very comfortable with the micropipet before you go on to the next step.

6. Check your gel. If it has cooled enough, it should look opaque (cloudy) now. Check with your teacher if you are unsure if it is ready. If it *is* ready, move on to step 7 (Caution: follow directions very carefully).
7. Removing the comb: This step is crucial. You must remove the comb very carefully. *Gently* pull out the comb *slowly* and *evenly*, with equal pressure on both sides of the comb.
8. After the comb is removed, you are ready to load the wells.

Loading the wells:

- a. After you find out what dyes you are using, draw a picture of the gel and the wells. Label which dyes you will put in each well.
 - b. When you load a gel, it is very important that you do not damage the gel in any way. You must be very careful not to "jab" the gel with the end of your pipet. Ideally, you shouldn't even *touch* the gel with the micropipet. However, you must also be careful to put the right dye *only* in the right well.
 - c. When you are ready, carefully load the wells you are using with 15 μ l of the correct dye for the particular well you are putting it into. Make sure you RINSE and WIPE your micropipet each time you measure out a new dye. If you don't, there is a really good chance that you will contaminate the samples in the other wells. (Refer to STEP 5 for pipetting technique.)
9. Peel off the tape from the sides of the casting tray. Be careful not to shake up or spill the wells.
 10. **CAREFULLY** place the loaded casting tray onto the "shelf" of the chamber with the wells placed nearest to the cathode (black). You must not tilt the gel--it will spill your samples. The slower you lower it into the buffer solution, the better chance that you will not "wash out" the wells as the buffer solution covers them.

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11. When everyone has placed their casting tray into the chamber you are using, you can place the chamber lid on top.
12. Make sure there is no standing or running water around the chamber or the power source.
13. Plug the black wires (from the cathode) into the black port of the power system. Plug the red wires (from the anode) into the red port of the power system. **DO NOT SWITCH THE WIRES!**
14. When everyone has loaded their gel, you have checked that there is no water around the system, make sure the power system is plugged in. Set the "Volts" setting to 170V and turn the power source ON. **Make no attempt to open the chamber when the power source is on--you could get electrocuted, fried, crispy.**
15. The dyes will start moving very slowly. They will probably take 10-20 minutes to separate. Ideally, you want to leave the power on, uninterrupted, until the leading dye gets very close to the end of your gel.

AS YOU ARE WAITING, YOU CAN BEGIN TO CLEAN UP.

16. After the dyes have finished their "run," **TURN OFF THE POWER SOURCE.** Take out the casting tray. Being careful not to break your gel, slide the gel out of the casting tray.
17. Below, draw a picture of your results, be sure to label everything.
18. Clean up. If you would like to save your gel, you may keep it for awhile in a plastic bag. Rinse everything with water, then with distilled water. **KEEP WATER AWAY FROM THE POWER SOURCE!**

Questions

1. Which one of your dyes moved the fastest? Which dye moved the slowest? Explain why you think that happened.
2. Did any of your samples leave more than one band? If they did, explain why you think it happened.
3. Do you think any of the dyes were identical? Similar? Why or why not?
4. Why do you think electrophoresis is an important technique for scientists to know?

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SEPARATION OF PROTEINS BY ELECTROPHORESIS**INTRODUCTION**

Proteins are made of amino acids. An amino acid has a central carbon, an amino group, a carboxylic acid group, a hydrogen, and a side chain called a R group. There are twenty different R groups and therefore twenty different amino acids. Some amino acids are neutral in charge such as valine and alanine, some are basic in charge such as lysine and arginine and some are acidic in charge such as aspartic acid and glutamic acid. Amino acids are held together by peptide bonds to form more complex structures. A dipeptide is composed of two amino acids, a tripeptide is composed of three amino acids, and a polypeptide is composed of many amino acids. A protein is a large amino acid composed of a hundred or more amino acids.

Proteins vary in the numbers and kinds of amino acids they contain. Therefore proteins vary in net charge. Electrophoresis is the movement of charged particles through a gel in an electric field. Thus proteins should be separated by electrophoresis. The following four proteins will be used in this experiment: cytochrome C, myoglobin, hemoglobin and serum albumin.

Cytochrome C is found in the mitochondria of both plants and animals. Cytochrome C is involved in cell energy production. It is an integral part of the electron transport system. Cytochrome C consists of a single lysine rich polypeptide and an iron containing heme group. This heme group is responsible for the orange red color of the protein. At a pH of 8.6 cytochrome C carries a net positive charge.

Myoglobin is found in the muscle cells where it binds and stores oxygen. It contains a heme group that is responsible for the red-brown color. At a pH of 8.6 myoglobin has a negative charge.

Hemoglobin is the major protein found in red blood cells. It transports oxygen from the lungs to the cells. It is composed of four polypeptide chains and a central heme group. The heme group is responsible for the red color of the protein. At a pH of 8.6 it is negatively charged, slightly more negatively charged than myoglobin.

Serum albumin is the a major protein found in blood plasma. It binds and transports small molecules through the blood stream. It is not colored but it binds to bromophenol blue and therefore appears blue in the presence of bromophenol blue. At a pH of 8.6 it is quite negatively charged.

PURPOSE

The object is to separate proteins according to their charge by electrophoresis.

SAFETY CONSIDERATION

Do not remove the lid from the electrophoresis chamber until the power has been turned off.

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PRE LAB QUESTIONS

Before doing this lab students should do the lab "Gel Electrophoresis: How Does it Work?" If they have not done the introductory lab or have not done it recently, the equipment and processes of electrophoresis should be explained or reviewed.

MATERIALS

electrophoresis equipment
chamber
power supply
casting tray and comb
masking tape
micropipetors
agarose
buffer
beaker
ice
distilled water

keep the following proteins on ice

cytochrome C
myoglobin
hemoglobin
serum albumin

PROCEDURE

PREPARING THE AGAROSE GEL

1. Assemble The Casting Tray

Place the clean glass slide in the tray
Place the comb in the proper position
Tape ends securely with masking tape

2. Prepare The Agarose Solution

The agarose and buffer have been combined for you by your teacher.
Check the solution, if it is not perfectly clear heat in a microwave for 2 minutes.
If the solution is perfectly clear let it cool until it can be easily handled.

3. Pour The Gel

Fill the tray two-thirds full with agarose solution. (15mL). with a toothpick carefully move any air bubbles to the side.

4. Allow The Tray To Cool

Gel should look cloudy - approximately 15 minutes

5. Remove Masking Tape

6. Remove Comb

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Place a small amount of buffer on gel by the comb
Wiggle the comb to loosen pull comb straight up and away from gel

* gel can be stored covered with buffer in a plastic container with a lid for up to a week.

APPLYING THE SAMPLE

1. Load 15 μ L of each of the proteins into the proper well.
Keep proteins on ice.
Fill the micropipet to the 15 μ L calibration.
Hold the delivery end of the pipet slightly inside the appropriate sample well.
Slowly eject the sample seal the wells with agarose.
Rinse pipet with distilled water between samples

<u>well #</u>	<u>Protein</u>
1	cytochrome C
2	myoglobin
3	hemoglobin
4	serum albumin

RUNNING THE ELECTROPHORESIS

1. Transfer the gel to the electrophoresis chamber.
2. Electrophoresis for 8 minutes.
3. Turn the power off and examine the gel.
4. Resume the electrophoresis until the bromothymol blue dye is 1 cm from the end of the gel.
5. Turn off the power.
6. Remove and examine the gel.
7. Measure the distance from the front of the well to each front edge of each protein band.
8. The gel can be stained and destained. (optional)

DATA

The students should make and label a sketch of their gel at 8 minutes and at the end of the experiment.

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CONCLUSION

QUESTIONS

1. Toward which pole would a negatively charged protein migrate?
2. Why do some proteins migrate farther in a given amount of time than other proteins?
3. Is it logical that a lysine rich protein such as cytochrome C would move the direction it does? Explain.
4. Predict how a protein rich in aspartic acid might migrate?
5. What two characteristics can be used to determine which band is which protein? Which method seems most accurate? Explain.
6. Predict how hemoglobin from a person with sickle cell anemia might migrate compared to normal hemoglobin?

ACTIVITIES

An additional activity would be to run an unknown, perhaps a combination of two of the proteins used, and have the students determine the makeup of the unknown.

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TEACHERS GUIDE

Typical Classroom Use:-First Year Class - Gel Electrophoresis:

How Does It Work.

Allows development of proper pipetting techniques, electrophoresis assembly and simple separations.

Protein Separations

Continued techniques and more complex separations with options of staining techniques.

Concept Map:

Key words for concept mapping:

Electrophoresis	Chamber
Power Source	Cathode(-)
Anode (+)	Buffer
Gel	Wells
Micropipets	Plunger
Casting trays	Comb
Gates/Dams	Sample
Electric Current	Microliter
Migration	Separation

See attached Concept Map example included in lab.

Curriculum Integration:

Biology: First Year Biology
 - Organic Compounds
 - Biotechnology
 - Genetics

Chemistry: First Year Chemistry
 - Buffers
 - Bronsted-Lowry - Acids and Bases
 - Organic Chemistry - Proteins

Background Information:

- Matter is made up of particles
- Particles maybe positively or negatively charged or neutral
- Characteristics of solutions
- Transport - Diffusion

Preparations:

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Agarose

- Food Coloring Lab - .7 g. Agarose/100mL. of buffer to be run.
- Protein Separation Lab - 1.0 to 1.2 g. Agarose/100 mL. of buffer to be run.

(100 mL. of prepared Agarose will provide enough for four (4) casting trays.)

Preparations - Microwave materials and check periodically in order to determine that Agarose particles are totally dissolved. Example - 300 mL. Agarose solution, microwave 2 - 3 minutes then check for particles, if still present continue microwaving for an additional 2 - 3 minutes then check once again. Continue until the particles are totally dissolved.

IMPORTANT Make sure cap on bottle of Agarose solution is loosened, to prevent pressure build up. May be stored at room temperature for several months, the concern would only be dehydration.

After pouring hot Agarose into casting trays, let cool. Remove any air bubbles using a toothpick, by dragging it bubble to side of casting tray.

Prepared casting trays maybe stored in a refrigerator for a few weeks. Store in a thin plastic covered container with buffer solution covering Agarose. The major concern is dehydration.

ALTERNATIVE GELS - Agar and Nutrient Agar are also excellent gel substitutes for the casting trays to be used in the Food Coloring Lab.

Proteins: Proteins are usually prepared, but need to be frozen for storage. While being used keep in ice. Overnight storage (after being taken from the freezer) should be kept in the refrigerator. NOTE - DO NOT RE-FREEZE Proteins - This will denature them.

Buffer: 10X - 100X concentrations maybe stored at room temperature for extended periods of time. 1X dilutions should be disposed when lab is completed or within a few days of preparation. If you do not wish to use the John Anderson kits, and would desire to make your own buffer, the following formula should be used to make the buffer solution:

A 8.6 pH Tris-Glycine buffer solution can be used for both the Food Coloring Lab and the Protein lab:

Tris Formula Weight 121.1

Glycine Formula Weight 75.05

Take 6.05 grams of Tris and add to 500 mL. of distilled water.

Dissolve 75.05 grams of Glycine in 600 mL. of distilled water.

Begin adding Glycine solution to Tris solution until you reach the desired pH (8.6 pH for these two labs). As you get closer to desired pH, use drops of solution to prevent raising your pH too much. After reaching the desired pH, bring final volume of Tris-Glycine buffer to one liter, by adding distilled water.

Preparation of 10X concentration of buffer:

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Take 10 mL. / 90 mL. distilled water to produce the desired 1X buffer solution. NOTE - Buffer used in making Agarose must be fresh- Leftover buffer can then be used in electrophoresis chamber.

Food Coloring: Store at room temperature.

Stain and Destaining (Optional):

Coomassie Blue R	10 mL.
Methanol	440 mL.
Water	480 mL.
Glacial Acetic Acid	80 mL.

Destaining Solution:

Methanol	100 mL.
Glacial Acetic Acid	100 mL.
Water	800 mL.

NOTE - All unused portions are stable for several months if stored in tightly capped containers at room temperature.

Source Of Materials:

Modern Biology Series: Dr. John Anderson, P.O. Box 97 Dayton, In 47941 Phone 1-800-733-6544

Time

Getting Ready: Approximate time needed for instructor 1 hour.
- Casting trays maybe made and stored for next day use.

Doing the Activity: Microwaving of Agarose, pouring casting trays, cooling casting trays, pulling combs, placing samples in wells and run trays in electrophoresis chambers, should be accomplished in one lab period (approximately 50 minutes).

Safety And Disposal:

Do's and Don'ts

- Make agarose solution with buffer you will use in electrophoresis chamber (Do Not Use distilled water)
- When preparing Agarose, microwave for a short time (2 - 3 minutes), then swirl container in the attempt to dissolve all particles, then continue to microwave and swirl until there are no visible particles in the solution.
- Fill casting tray high enough to have deep wells (up to the comb's bridge).
- If you have a chamber for one casting tray, put the tray in chamber, then load the wells with samples.
- Do not touch the sides of the well with micropipet while loading samples.

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- If you are not going to fill all the wells in your casting tray with samples, leave outside wells (lanes) empty.
- Use 1 mL. pipet to put beads of Agarose to seal loaded sample wells.
- Remember to remove all gates from casting trays before placing them in the buffer filled chamber.
- Rinse (with distilled water) and wipe (with Kimwipes) micropipet with each use when loading sample into wells.
- Always put lids on chambers to prevent electrical shock.
- Turn off power source whenever you touch chamber.

Disposal:

All materials should be disposed according to Flinn's Catalog, Methods # 24a, #26a and #26b.

Variations/Expansions:

Pre Lab Variations:

1. Fill casting trays with agar or nutrient agar instead of Agarose.
2. Micropipeting techniques. Have each student load a sample dye (food coloring) into two wells:
 - Not touching the walls of the wells with the micropipet.
 - Not putting micropipet through the bottom of the well.
 - Rinsing and wiping of micropipet after each use.
3. Demonstrate the difference in the appearance of hot clear Agarose and the cooled opaque Agarose in casting tray.
4. Discuss names, terms, functions and safety precautions of the entire electrophoresis apparatus.

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Lab Variations:

1. Use Varieties of Proteins:
 - Sickle Cell Hemoglobin
 - Animal Sera
 - Plant Auxins

Lab Expansions:

1. Determination of the Length of DNA Molecules
2. Restriction Nuclease Mapping of DNA Molecules

References:

Maniatis, Fritsch, Sambrook, Molecular Cloning, Cold Springs Harbor Laboratory, 1982.

Anderson, John N, A Laboratory Course in Molecular Biology. Department of Biological Sciences, Purdue University, West Lafayette, Indiana.

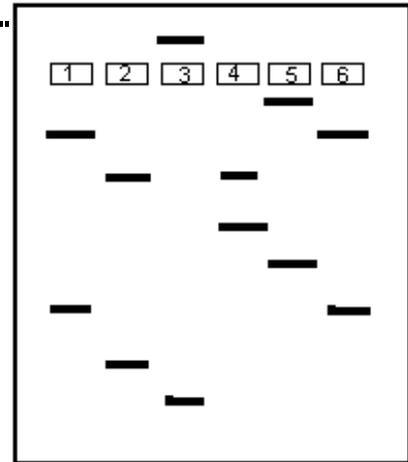
Flinn Chemical Catalog, Flinn Scientific Inc, 1993.

Assessment:

1. Pre Lab: Lab practical technique for micropipeting. Have student load two wells with two different food colors. Determine techniques of well loading and rinsing of micropipet. Wells can be reused by emptying and rinsing out food coloring.
2. Pass out a labeled diagram of electrophoresis chamber power supply, casting tray and comb, Agarose gel, buffer, micropipet, plunger and sample. Describe the function of each part of apparatus and other materials labeled.
3. Give a list of do's and don'ts in setting up, loading and running an electrophoresis apparatus.
4. You are a Negative (Positive) particle in our sample. Describe what will happen to you in each step of this lab.

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5. This gel represents six (6) different proteins run through an electrophoresis.



- A. What is the significance of those proteins having two bands?
- B. Which proteins showed the most positive and which proteins showed the most negative charges?
- C. Why did the proteins behave as you described them in B?
- D. Are any proteins similar? If so, which ones are similar?
- E. Are there any identical proteins? Explain your answer.

6. Student A ran two identical proteins in neighboring wells on the same casting tray. These two proteins migrated at two different rates. The other students ran the same two identical proteins in neighboring wells on their casting trays and found the two proteins migrated at the same rates. Explain why Student A's results may have been different.

Proficiencies Covered by the Key Elements Of The Science Proficiency Guide found on page 11:

Proficiencies covered: 3; 4; 5a, c, d, e, f; 6a, b, c, d; 7, 8, and 11.