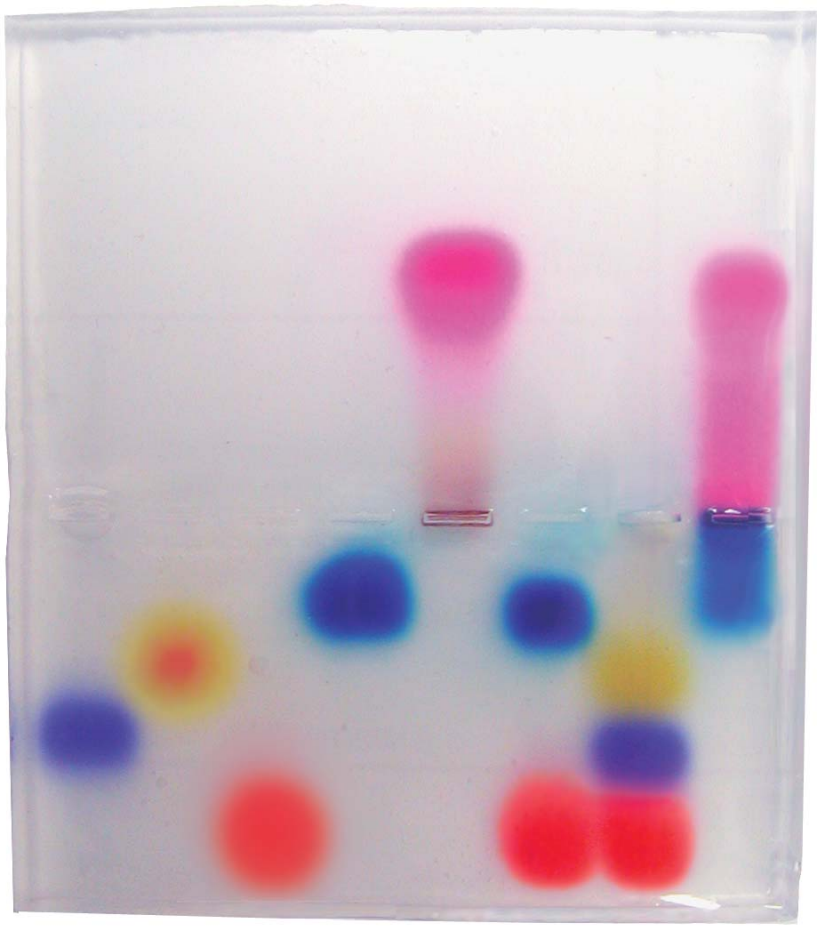
21-1147
Teacher Demonstration Kit

21-1148
8-Station Classroom Kit

Introductory Gel Electrophoresis

TEACHER'S MANUAL WITH STUDENT GUIDE



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Introductory Gel Electrophoresis

Teacher's Manual

Photocopy Masters

Introductory Gel Electrophoresis

Overview

This Teacher's Manual can be used with the Teacher Demonstration Kit (21-1147) as well as the 8-Station Classroom Kit (21-1148). Using the Introductory Gel Electrophoresis activity, students are introduced to the basic principles of macromolecule separation by gel electrophoresis. This technique is a basic tool of all modern molecular biologists. Students or teacher demonstrators will cast agarose gels and load known and unknown dyes of various sizes and charges into the wells of the gel. They will then apply an electrical current to the gel and observe how the different dyes move through the agarose gel matrix. Migration distances and electrical charges of the dyes will be evaluated and recorded. Components of the unknown dyes will then be identified on the basis of this data.

Objectives

- Students will learn the basic principles of gel electrophoresis.
- Students will be introduced to a universal technique used in biotechnology laboratories.
- Students will observe how dye molecules of different sizes and charges migrate through a gel during electrophoresis and will then draw conclusions about unknown dyes on the basis of this information.

Background Information

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 this laboratory activity, agarose gel electrophoresis will be used to separate and characterize colored dye molecules of various sizes and charges.

In gel electrophoresis, samples to be separated are applied to a porous gel medium made of a material such as agarose. Agarose is a purified form of agar, a gelatinous substance extracted from red algae. Agarose gels are made by first adding powdered agarose to liquid buffer and boiling the mixture until the agarose dissolves. This molten agarose is then cooled to about 55–60°C, poured into a gel mold called a casting tray, and allowed to solidify. Before solidification occurs, a comb is placed in the casting tray to create a row of wells into which samples are loaded once the comb is removed from the solidified gel.

The casting tray and solidified gel are then placed in an electrophoresis chamber that has wire electrodes at each end. The gel is covered with an ion-containing buffer, such as tris-borate-EDTA (TBE), that controls the pH of the system and conducts electricity. The comb is then carefully removed from the gel and samples are loaded into the resulting wells using a pipet. Samples for gel electrophoresis are mixed with a small amount of sucrose or glycerol to increase their density. This causes the samples to sink to the bottom of the well when loaded.

Once all the samples have been loaded into the wells, the chamber is connected to a power supply and an electrical current (usually 50–150 V) is applied to the gel. The chamber is designed with a positive electrode (anode) at one end and a negative electrode (cathode) at the other end. Electrophoresis literally means "to carry with electricity;" once the electric field is established, charged molecules in the samples migrate through the pores of the gel toward their pole of attraction. Molecules with a net negative charge 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. Highly charged molecules migrate more quickly through the gel than weakly charged molecules.

The mobility of a molecule during gel electrophoresis also depends on its molecular size and shape. The small pores of the gel matrix act as a sieve that provides great resolving power. 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.

Size and net charge are factors that together determine how quickly molecules will travel through the gel, and thus what their migration distance will be. Small size and strong charge increase a molecule's migration rate through the gel. Large size and weak charge decrease the migration rate. (Note: In electrophoresis of DNA, since all the samples have the same charge, their migration rate is based solely on size).

In this activity, five known dye samples and three unknown dye mixtures will be subjected to agarose gel electrophoresis. Some of the dyes will be attracted to the negative electrode and some to the positive electrode, depending on their overall charge. Each of the known dyes will exhibit a unique gel migration distance that relates to its molecular size and net charge. Students will identify the components of the unknown dye mixtures by comparing the migration distances and direction of migration of the unknown dyes to those of the known dye samples.

Note: Electrophoresis of dyes is a simple and useful introduction to gel electrophoresis. Though it differs in some ways from most electrophoresis that your students will later encounter, the differences are just as instructive as the similarities. For example, the dye components clearly illustrate the separation process as it occurs, whereas DNA and protein gels must be stained to show the bands of separation. Also, seeing specific dye molecules run specific distances through a gel makes it easy later to understand the use and action of the loading dye in DNA separations. Creating wells across the center of a gel and watching dye components run in different directions on the basis of their electric charge makes it easy later to understand the use and action of SDS in coating protein samples to eliminate charge as a potential factor in their separation.

Materials

The materials provided are designed for use with the Teacher Demonstration Kit or the 8-Station Classroom Kit only. Carolina Biological Supply Company disclaims all responsibility for any other use of these materials.

Materials included in the Teacher Demonstration Kit (21-1147)

- 1 bottle of 0.8% melt-n-pour agarose, 60 mL (made with 0.48 g of agarose in 60 mL of 1× TBE)
- 1 bottle of 20× TBE buffer, 20 mL (enough to make 400 mL of 1× TBE solution)
- bromphenol blue, 30 µL
- methyl orange, 30 µL
- ponceau G, 30 µL
- xylene cyanol, 30 µL
- pyronin Y, 30 µL
- unknown #1, 30 µL
- unknown #2, 30 µL
- unknown #3, 30 µL
- 8 needle-point pipets
- 1 ruler
- 1 plastic tray

Needed, but not supplied for the Teacher Demonstration Kit (21-1147)

- gel electrophoresis chamber
- power supply (minimum 50-V capability)
- roll of masking tape
- rack for microcentrifuge tubes
- 400-mL (or larger) container
- boiling water bath or microwave oven
- distilled water (380 mL)
- 55–60°C water bath (optional)

Materials included in the 8-Station Classroom Kit (21-1148)

agarose powder, 4 g
(to make 500 mL of 0.8% agarose solution)
1 bottle of 20× TBE buffer, 200 mL
(to make 4 L of 1× TBE solution)
bromphenol blue, 500 µL
methyl orange, 500 µL
ponceau G, 500 µL
xylene cyanol, 500 µL
pyronin Y, 500 µL
unknown #1, 500 µL
unknown #2, 500 µL
unknown #3, 500 µL
64 needle-point pipets
8 rulers
8 plastic trays
Teacher's Manual
Student Guide
(photocopy master at the end of this Teacher's Manual)

Needed, but not supplied for the 8-Station Classroom Kit (21-1147)

8 gel electrophoresis chambers
8 power supplies (minimum 50-V capability)
roll of masking tape
rack for microcentrifuge tubes
4-L (or larger) container
1-L flask or beaker
boiling water bath or microwave oven
distilled water (3800 mL)
55–60°C water bath (optional)

Time Requirements

Teacher Preparation

Time Required	*Activity*
5 minutes	Prepare 1× TBE buffer
5 minutes	Spin down or tap down dye reagents
10–25 minutes	Prepare 0.8% agarose solution

Laboratory Procedure

Time Required	*Activity*
*20 minutes	Casting agarose gel(s)
10–15 minutes	Loading of gel(s)
15–25 minutes	Electrophoresis
15 minutes	Examination and discussion of results

*Gel(s) can be cast and stored in the electrophoresis chamber(s) covered with 1× TBE buffer prior to loading for up to 2 days.

Teacher Tips

- You may cast gels during one lab period and store them for as long as 2 days in the electrophoresis chamber covered in 1× TBE buffer before loading the gels with dye samples and performing electrophoresis.
- The dye particles in unknown #3 tend to settle out of solution over time. For this reason, mix the unknown #3 dye sample as well as possible before use by vortexing the mixture or by sharply tapping the bottom of the tube with your finger several times.
- If you are using the 8-Station Classroom Kit (21-1148), the dye samples must be shared among the laboratory workstations in the classroom.
- It is recommended that gels be electrophoresed at a voltage of 100–150 V. Electrophoresis at this voltage will take ~15–25 min. Gels may be run at lower voltages, but the total electrophoresis time will be longer. Gels should not be run below 50 V because the dye samples will become diffuse if they separate at a slow rate.
- **A gel must be analyzed promptly after electrophoresis.** Do not wait until a subsequent lab period. Over time, the dye bands diffuse and become difficult to distinguish.
- The migration distances in the unknown dye mixtures may not exactly match their counterparts in the known dye samples (they may differ by 1–2 mm). This is due to interactions between different types of dyes in the dye mixtures as they move through the gel. Such interactions can sometimes affect ultimate migration distance slightly.

Safety Tips

If using the 8-Station Classroom Kit (21-1148), remind students to use safe laboratory practice at all times. Employ close supervision when students are connecting the electrophoresis chamber to the power supply. The power supply should not be turned on until the lid to the chamber is securely in place and the electrical leads are properly inserted into the inputs. The power supply should be turned off before the lid is removed and the leads are disconnected.

Preparation

Prepare 1× TBE Buffer

Tris-borate-EDTA (TBE) is a stable buffer solution and can therefore be made several days ahead of time and stored in a carboy or other container at room temperature until ready for use. Prepare the solution in the following manner:

For the Teacher Demonstration Kit (21-1147):

To prepare the 1× concentration of TBE, add 20 mL (the entire bottle) of 20× TBE buffer to 380 mL of distilled water in a 400-mL (or larger) container. Rinse any residue from the 20× TBE buffer bottle with some of the freshly mixed 1× TBE and add it to the container of 1× TBE. Stir until the solution is completely mixed. Label the container "1× TBE."

For the 8-Station Classroom Kit (21-1148):

To prepare the 1× concentration of TBE, add 200 mL (the entire bottle) of 20× TBE buffer to 3800 mL of distilled water in a 4-L (or larger) container.

Rinse any residue from the 20× TBE buffer bottle with some of the freshly mixed 1× TBE and add to the container of 1× TBE. Stir until the solution is completely mixed. Label the container "1× TBE."

Spin Down or Tap Down Dye Reagents

Reagents often become spread around the storage-tube wall or cap during shipping. Therefore, dye samples should be collected at the bottom of their storage tubes prior to setting up workstations using one of the methods listed below. Tubes containing dye samples can be spun down or tapped down several days before the laboratory session and stored upright in a microcentrifuge tube rack.

1. Spin the dye tubes briefly (~30 sec) in a microcentrifuge designed to hold 1.5-mL tubes.
2. Tap the bottom of each tube sharply on the bench top until all of the liquid has collected in the bottom of the tube.

Prepare 0.8% Agarose Solution

Caution: Use oven mitts or heat-resistant gloves when handling the 0.8% agarose solution.

Prepare the 0.8% agarose solution at least 30 minutes before the gels are to be cast.

For the Teacher Demonstration Kit (21-1147)

Melt the agarose by heating the bottle in one of the following ways:

1. Loosen the cap on the bottle of 0.8% agarose. Heat the bottle in a microwave oven on high in 1-minute intervals until the agarose has completely melted. To prevent the agarose from boiling over, swirl the bottle between each minute of heating. Watch the bottle closely. If the agarose begins to boil too vigorously, carefully remove it from the heat source until the liquid settles. Resume heating if needed.
2. Loosen the cap on the bottle of 0.8% agarose. Heat the bottle in a boiling water bath until the agarose is completely melted (~6–12 minutes). The water level should be just above the level of the agarose in the bottle. To prevent the agarose from boiling over, swirl the bottle every few minutes during heating. Watch the bottle closely. If the agarose begins to boil too vigorously, carefully remove it from the heat source until the liquid settles. Resume heating if needed.

The 0.8% agarose solution will become clear as the agarose melts. Swirl and observe the bottom of the bottle to ensure that no solid agarose remains. Allow the bottle to cool until it can be held in a bare hand without pain. It should still feel warm and be around 55–60°C. At this point, you can use the agarose immediately or hold it at this temperature in a 55–60°C-water bath until you are ready to use it.

For the 8-Station Classroom Kit (21-1148)

Add 4 g (the entire amount) of powdered agarose to 500 mL of prepared 1× TBE buffer in a clean 1-L flask or beaker. Dissolve the agarose by heating the mixture in one of the following ways:

1. Heat the uncovered flask/beaker in a microwave oven on high in 1-minute intervals until the agarose is completely dissolved. To prevent the agarose from boiling over, swirl the bottle every few minutes during heating. Watch the flask/beaker closely and stop heating at the first sign of vigorous boiling.
2. Cover the flask/beaker and heat in a boiling water bath until the agarose is completely dissolved. The water level should be just above the level of the agarose mixture. To prevent the agarose from boiling over, swirl the flask/beaker every few minutes during heating. Watch the flask/beaker closely and stop heating at the first sign of vigorous boiling.
3. Heat the uncovered flask/beaker on a hot plate until the agarose is completely dissolved. If possible, use a hot plate with magnetic stirring capability and place a magnetic stir bar in the flask/beaker. If using a stir bar, stir at a continuous moderate rate. Otherwise, swirl the bottle every few minutes during heating to prevent the agarose from boiling over. Watch the flask/beaker closely and stop heating at the first sign of vigorous boiling.

The 0.8% agarose solution will become clear as the agarose dissolves. Swirl the flask/beaker and hold it up to the light to ensure that no undissolved agarose remains. Allow the flask/beaker to cool until it can be held in a bare hand without pain. It should still feel warm and be around 55–60°C. You can use the agarose immediately or hold it at this temperature in a water bath until you are ready to use it.

Set Up Workstation(s)

Provide each workstation with the following materials on the day of the laboratory session:

8 needle-point pipets
1 plastic tray
1 gel electrophoresis chamber
1 power supply

Instructions

These instructions are written for use with the Carolina™ Gel Electrophoresis Chamber (21-3668). If you do not have this equipment, modify these instructions to suit your particular apparatus.

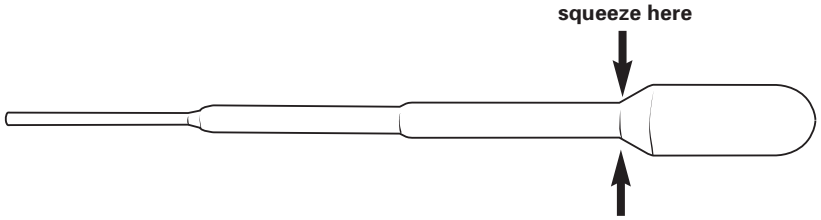
Casting the Agarose Gel(s)

1. Seal the open ends of the gel-casting tray with masking tape so that no seams or gaps appear. **Insert the well-forming comb in the middle set of grooves over the red stripe in the casting tray.** This will create a row of wells in the middle of the gel once the gel has formed.

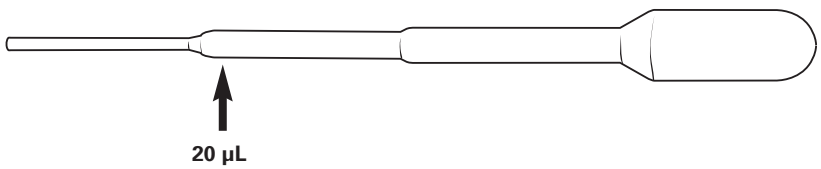
2. Carefully fill the casting tray to a depth of ~7 mm with the prepared 0.8% agarose solution; the liquid gel should cover about 1/2 the height of the comb teeth.
3. While the agarose is still liquid, move large bubbles and debris to the perimeter of the tray with the well-forming comb. Return the comb to its position in the middle set of grooves in the casting tray.
4. Allow the gel to sit undisturbed while it solidifies. Be careful not to move or jar the casting tray during this time. Once the agarose has hardened, the gel will appear cloudy. The gel will solidify in 10–15 minutes.
5. Once the agarose has solidified, remove the masking tape at the ends of the casting tray to unseal the gel. Place the casting tray and the gel into the electrophoresis chamber oriented with the red stripe toward the positive (red) end, and the black stripe toward the negative (black) end.
6. Fill the electrophoresis chamber with 1× TBE (tris-borate-EDTA) buffer to a level that just covers the surface of the gel.
7. Slowly and carefully remove the comb from the gel without tearing the wells. Make sure the sample wells left by the comb are completely submerged in 1× TBE buffer. If "dimples" appear around the wells, slowly add more 1× TBE buffer until they disappear.
8. The gel is now ready to load with dye samples. If you will be loading the gel at another time, cover the electrophoresis tank with the lid to prevent the gel from drying out.

Loading the Gel(s)

Dye samples will be loaded into the gel using plastic pipets. For better control during pipetting, squeeze the pipet where the stem meets the bulb as shown in the illustration below.



A very small volume of dye (20 µL) will be loaded into each well of the gel. A 20-µL volume is slightly more than the needle tip of the plastic pipet will hold. See the illustration below.



9. Load dye samples into the wells (also called lanes) from left to right following the order listed below. To load the first sample (bromphenol blue) into the well, draw 20 µL of dye into a plastic pipet (see illustration on page 10).

Using your dominant hand, steady the pipet over the well above the buffer layer. Rest the elbow of your dominant arm on the lab bench to stabilize your hand. Expel any air from the pipet so that the dye is at the very tip. Using your non-dominant hand, guide the pipet through the surface of the buffer and position it directly over the well (do not force the pipet tip into the well). Slowly expel the dye into the well (see Figure 1 below). The dye will sink to the bottom of the well because it has been mixed with sucrose to increase its density.

Repeat this process for each dye sample, continuing from left to right in the order shown below. Use a clean plastic pipet for each dye sample. Be sure to check the label on each dye tube before you load to ensure that it matches the intended order.

Order of Loading

lane 1	bromphenol blue
lane 2	methyl orange
lane 3	ponceau G
lane 4	xylene cyanol
lane 5	pyronin Y
lane 6	unknown #1
lane 7	unknown #2
lane 8	unknown #3

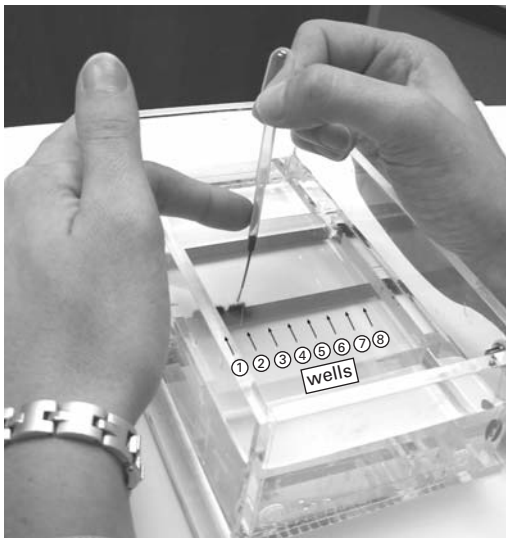


Figure 1. Loading the methyl orange dye sample into the second well of the gel.

Gel Electrophoresis

10. Once all the dye samples have been loaded, place the lid on the electrophoresis chamber. Orient the lid with the positive end of the chamber connected to the red (positive) cord and the negative end of the chamber connected to the black (negative) cord. Then connect the electrical cords to the power supply, with the positive lead in the positive input (red to red) and the negative lead in the negative input (black to black). If using a multi-channeled power supply, make sure both electrical leads are connected to the same channel.

11. Turn on the power supply and set it to the desired voltage. Watch as the dyes slowly move into the gel and separate over time. Do not allow any of the dyes to run off the gel. Run the gel until the band in lane 3 is 0.5 cm from the end of the gel.

12. Once the desired separation of dyes has been achieved, turn off the power, disconnect the leads from the inputs, and remove the top of the electrophoresis chamber.
13. Carefully remove the casting tray and slide the gel into the plastic tray. Gel results should resemble the image (Figure 2) at the right. See the cover of the manual for a color image.

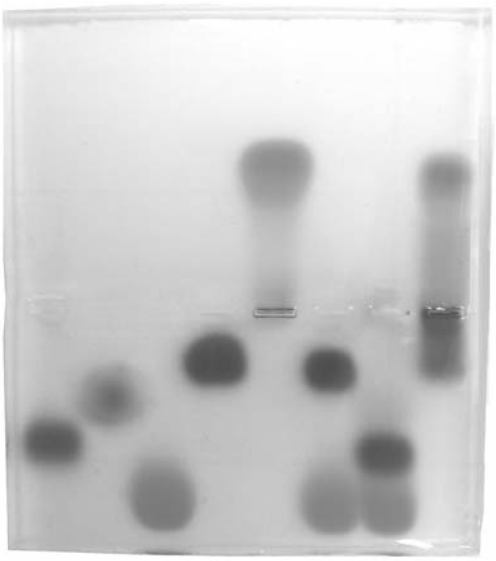


Figure 2. Gel image of expected results.

Analysis of Results

(likely results are in italics—see Student Guide for blank chart)

14. In the table below, record the number of dye bands in each lane and the direction of migration (positive or negative) for each band. Determine the migration distance of each dye in the known and unknown samples by measuring the distance from the center of the starting well to the center of the dye band with the ruler provided. If the dye moved toward the positive pole, mark the migration distance as a positive number. If the dye moved toward the negative pole, mark the migration distance as a negative number.

Lane	Sample	Number of Bands	Direction of Migration (positive or negative)	Migration Distance (cm) *Actual distances may vary slightly.*
1	bromphenol blue	*1*	*positive*	*2.4 cm*
2	methyl orange	*1*	*positive*	*1.5 cm*
3	ponceau G	*1*	*positive*	*3.4 cm*
4	xylene cyanol	*1*	*positive*	*0.9 cm*
5	pyronin Y	*1*	*negative*	*–2.4 cm*
6	unknown #1	*2*	*positive*	*0.9 cm, 3.4 cm*
7	unknown #2	*3*	*positive*	*1.5 cm, 2.4 cm, 3.4 cm*
8	unknown #3	*2*	*negative and positive*	*0.8 cm, –2.4 cm*

Questions
(answers in italics)

1. Based on the direction of migration, migration distance, and the appearance of your gel, what dye components were present in each of the unknown dye mixtures?

   unknown #1: xylene cyanol and ponceau G

   unknown #2: methyl orange, bromphenol blue, and ponceau G

   unknown #3: xylene cyanol and pyronin Y

2. Which dye molecule traveled farthest through the gel? Which traveled the shortest distance through the gel? What properties affect migration distance?

   Ponceau G migrated farthest through the gel. Xylene cyanol migrated the shortest distance through the gel. The size of a molecule and the degree of its molecular charge affect migration distance. Small or highly charged molecules migrate farther than large or weakly charged molecules.

3. What was the charge of the dye molecules that migrated toward the positive electrode and of the dye molecules that migrated toward the negative electrode? How do you know?

   Dyes that migrated toward the positive electrode had a net negative charge. Dyes that migrated toward the negative electrode had a net positive charge. This migration pattern occurred because opposite charges attract.

4. Why is electrical current necessary for separating molecules by gel electrophoresis?

   Charged molecules loaded in a gel move into the gel through an electrical attraction to the oppositely charged pole. Without the electrical current, migration would not be possible.

5. Why is the porous matrix of agarose gels an essential component of molecule separation by gel electrophoresis?

   The pores provide a passageway for samples to move through the gel; if the gel were not porous, migration would not be possible. The matrix of miniscule pores also acts like a sieve, which contributes to molecule separation. Small molecules maneuver more easily through the pores of the gel than larger molecules and thus migrate more quickly than the larger, slower molecules.

Name _____

Date _____

Introductory Gel Electrophoresis

Introduction

Gel electrophoresis is a basic biotechnology technique that separates macromolecules according to their charge and size. It is frequently used to analyze and manipulate samples of DNA, RNA, or proteins. In this laboratory activity, agarose gel electrophoresis will be used to separate and characterize colored dye molecules of various sizes and charges.

In gel electrophoresis, samples to be separated are applied to a porous gel medium made of a material such as agarose. Agarose gels are made by pouring a molten solution of agarose and buffer into a gel mold called a casting tray. Before the agarose solidifies, a comb is placed in the casting tray to create a row of wells into which samples are loaded once the comb is removed from the solidified gel. The casting tray and solidified gel are then placed in an electrophoresis chamber that has wire electrodes at each end. The gel is covered with a buffer that controls the pH of the system and conducts electricity. The comb is then carefully removed from the gel and samples are loaded into the resulting wells using a pipet.

Once all the samples have been loaded into the wells, the chamber is connected to a power supply and an electrical current is applied to the gel. The chamber is designed with a positive electrode (anode) at one end and a negative electrode (cathode) at the other end. Molecules with a net negative charge migrate toward the positive electrode and molecules with a net positive charge migrate toward the negative electrode because opposite charges attract.

The overall charge of a molecule affects the speed at which it travels through the gel. Highly charged molecules migrate more quickly through the gel than weakly charged molecules. The size and shape of the molecule also affects how quickly it travels through the gel. Agarose gels contain a matrix of minuscule pores that acts like a sieve. Small molecules maneuver more easily through the pores of the gel than larger molecules, allowing them to migrate relatively quickly.

Size and net charge are factors that together determine how quickly molecules will travel through the gel, and thus what their migration distance will be. If a molecule is small or highly charged, this will increase its migration rate through the gel. If a molecule is large or weakly charged, this will decrease its migration rate through the gel.

In this activity, five known dye samples and three unknown dye mixtures will be subjected to agarose gel electrophoresis. Some of the dyes will be attracted to the negative electrode and some to the positive electrode depending on their overall charge. Each of the known dyes will exhibit a unique gel migration distance that relates to its molecular size and net charge. You will identify the components of the unknown dye mixtures by comparing the migration distances and direction of migration of the unknown dyes to those of the known dye samples.

Instructions

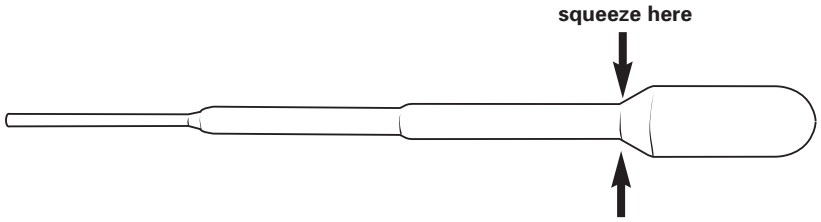
These instructions are written for use with the Carolina™ Gel Electrophoresis Chamber (21-3668). If you use other equipment, modify these instructions accordingly.

Casting the Agarose Gel(s)

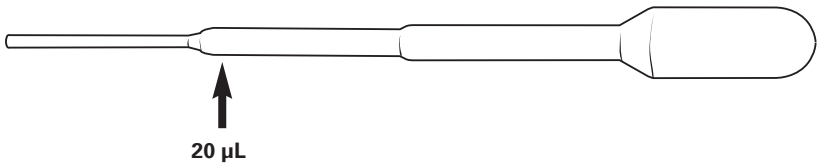
1. Seal the open ends of the gel-casting tray with masking tape so that no seams or gaps appear. **Insert the well-forming comb in the middle set of grooves over the red stripe in the casting tray.** This will create a row of wells in the middle of the gel once the gel has formed.
2. Carefully fill the casting tray to a depth of ~7 mm with the prepared 0.8% agarose solution; the liquid gel should cover about 1/2 the height of the comb teeth.
3. While the agarose is still liquid, move large bubbles and debris to the perimeter of the tray with the well-forming comb. Return the comb to its position in the middle set of grooves in the casting tray.
4. Allow the gel to sit undisturbed while it solidifies. Be careful not to move or jar the casting tray during this time. Once the agarose has hardened, the gel will appear cloudy. Gel solidification will occur in 10–15 minutes.
5. Once the agarose has solidified, remove the masking tape at the ends of the casting tray to unseal the gel. Place the casting tray and the gel into the electrophoresis chamber, orienting the red stripe toward the positive (red) end, and the black stripe toward the negative (black) end.
6. Fill the electrophoresis chamber with 1× TBE (tris-borate-EDTA) buffer to a level that just covers the surface of the gel.
7. Slowly and carefully remove the comb from the gel without tearing the wells. Make sure the sample wells left by the comb are completely submerged in 1× TBE buffer. If "dimples" appear around the wells, slowly add more 1× TBE buffer until they disappear.
8. The gel is now ready to load with dye samples. If you will be loading the gel at another time, cover the electrophoresis tank with a lid to prevent the gel from drying out.

Loading the Gel(s)

Dye samples will be loaded into the gel using plastic pipets. For better control during pipetting, squeeze the pipet where the stem meets the bulb as shown in the illustration below.



A very small volume of dye (20 mL) will be loaded into each well of the gel. A 20-µL volume is slightly more than the needle tip of the plastic pipet will hold. See the illustration below.



9. Load dye samples into the wells (also called lanes) from left to right, following the order listed below. To load the first sample (bromphenol blue) into the well, draw 20 μL of dye into a plastic pipet (see illustration above).

   Using your dominant hand, steady the pipet over the well above the buffer layer. Rest the elbow of your dominant arm on the lab bench to stabilize your hand. Expel any air from the pipet so that the dye is at the very tip. Using your non-dominant hand, guide the pipet through the surface of the buffer and position it directly over the well (do not force the pipet tip into the well). Slowly expel the dye into the well (see Figure 1 below). The dye will sink to the bottom of the well because it has been mixed with sucrose to increase its density.

   Repeat this process for each dye sample, continuing from left to right in the order shown below. Use a clean plastic pipet for each dye sample. Be sure to check the label on each dye tube before you load to ensure that it matches the intended order.

   Order of Loading

lane 1	bromphenol blue
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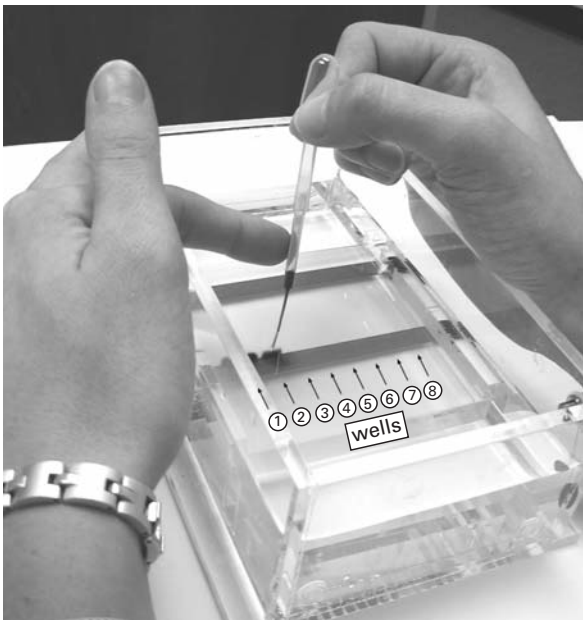

Figure 1. Loading the methyl orange dye sample into the second well of the gel.

Gel Electrophoresis

10. Once all the dye samples have been loaded, place the lid on the electrophoresis chamber. Orient the lid with the positive end of the chamber connected to the red (positive) cord and the negative end of the chamber connected to the black (negative) cord. Then connect the electrical cords to the power supply, with the positive lead in the positive input (red to red) and the negative lead in the negative input (black to black). Make sure both electrical leads are connected to the same channel if using a multi-channeled power supply.

11. Turn on the power supply and set it to the desired voltage. Watch as the dyes slowly move into the gel and separate over time. Do not allow any of the dyes to run off the gel. Run the gel until the band in lane three is 0.5 cm from the end of the gel.

12. Once the desired separation of dyes has been achieved, turn off the power, disconnect the leads from the inputs, and remove the top of the electrophoresis chamber.

13. Carefully remove the casting tray and slide the gel into the plastic tray.

Analysis of Results

14. In the table below, record the number of dye bands in each lane and the direction of migration (positive or negative) for each band. Determine the migration distance of each dye in the known and unknown samples by measuring the distance from the center of the starting well to the center of the dye band with the ruler provided. If the dye moved toward the positive pole, mark the migration distance as a positive number. If the dye moved toward the negative pole, mark the migration distance as a negative number.

Lane	Sample	Number of Bands	Direction of Migration (positive or negative)	Migration Distance (cm)
1	bromphenol blue			
2	methyl orange			
3	ponceau G			
4	xylene cyanol			
5	pyronin Y			
6	unknown #1			
7	unknown #2			
8	unknown #3			

Questions

1. Based on the direction of migration, migration distance, and the appearance of your gel, what dye components were present in each of the unknown dye mixtures?

2. Which dye molecule traveled farthest through the gel? Which traveled the shortest distance through the gel? What properties affect migration distance?

3. What was the charge of the dye molecules that migrated toward the positive electrode and of the dye molecules that migrated toward the negative electrode? How do you know?

4. Why is electrical current necessary for separating molecules by gel electrophoresis?

5. Why is the porous matrix of agarose gels an essential component of molecule separation by gel electrophoresis?

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