miniOne®
MINILABS

Candy Color Electrophoresis MiniLab

Student's Guide

Cat# M3009

Version 021419

Table of Contents

Laboratory Safety

1. Do not eat, drink, or apply cosmetics in the lab. Do not eat food used in laboratory activities.
2. Wear lab coats, gloves, and eye protection as required by district protocol.
3. Use caution when using all electrical equipment such as electrophoresis units.
4. Heating and pouring molten agarose is a splash hazard. Use caution when handling hot liquids. Wear eye protection and gloves to prevent burns.
5. Wash your hands thoroughly after handling biological materials and chemicals.

Objectives

In this hands-on MiniLab kit, you will explore the significance of food dyes in candy, their chemical properties and visualize how color combinations separate on a gel. Dyes are extracted from the hard shell candies provided and run on an agarose gel. You can use the information about mass and size of each molecule to make some initial predictions about which molecules will migrate the furthest. An optional Inquiry Extension allows you to bring in candies to test against the ones provided.

Introduction

Browse the candy section of your local supermarket and you will see an incredible range of eye-catching colors. But read the labels and you will see that there are only a handful of different chemicals that are used to give color to the candies. This is because, in the United States, food additives must be rigorously tested to make sure they are safe for human consumption before being used in consumer products. Many dyes don't make the cut.

Five common food dyes and their chemical structures are listed in Appendix A. Some candies contain a single dye. For example, if you favor Twizzlers® and Swedish Fish®, you are consuming Red 40. The rainbow colors of Skittles®, M&Ms®, Tic Tacs®, gummy worms, and jelly beans are created the way an artist mixes paint to create new shades.

Food labels list all the ingredients in the package, but if the package contains a variety of colors and flavors, how do you know which chemicals are in which candy color? In this hands-on activity, you will extract dyes from candies and separate dye molecules on the MiniOne® Electrophoresis System. Results are analyzed by applying knowledge of dye chemical properties and understanding of electrophoresis to identify the chemicals that give each candy its color.

Enough reagents and supplies are provided to analyze nine candies per student group. Candies in six colors are provided with the lab. As an Inquiry Extension, you can bring in up to three candies per group to analyze alongside the candies provided. A variety of candies can be used but we have found that candies with a hard, solid color shell, such as Reese's Pieces®, Skittles®, and M&Ms®, provide the best results as the dye is concentrated and easier to extract, making the dyes more visible when separated.

> Since some candies can be made in plants where various foods are used, please let your teacher know if you have any allergies before you handle any candy.

Instructions specific for the Inquiry Extension are given in green.

Additional resources:

For an introduction to the history and economic significance of food dyes, see the MiniOne® Colorful Dye Electrophoresis MiniLab (Cat# M3007) Student's Guide

For a history of food dye regulation, see the US Food and Drug Administration website:
https://www.fda.gov/ForIndustry/ColorAdditives/RegulatoryProcessHistoricalPerspectives/default.htm

NSTA Learning Center provides detailed information on the chemistry of food dyes:
https://learningcenter.nsta.org/products/symposia_seminars/fall08/FDA/webseminarl.aspx

Pre-Lab Questions

1. When you buy candies at the store, all ingredients, including any dyes, are listed on the package. However, if the candies have multiple colors in the same package, like Skittles® or M&Ms®, there is no indication which dye is in which candy color. Describe how you could use electrophoresis to determine which dye goes with which candy color.

2. Small molecules (low molar mass) travel faster through an agarose gel than large molecules. Molecules with higher electrical charge travel faster than molecules with lower electrical charge. **Charge/mass** ratio is a convenient metric for estimating the relative speeds of migration of molecules through a gel. Would you expect molecules with high charge/mass ratio to travel faster or slower than molecules with low charge/mass ratio?

3. Examine the candies you have at your lab bench and **Appendix A: Dye Chemical Structures.** Make a hypothesis: which candies do you think contain a single dye and which do you think contain two or more dyes?

4. Two blue dyes are listed in **Appendix A.** Using your knowledge of electrophoresis, explain how you will distinguish the two on a gel.

5. Two yellow dyes are listed in **Appendix A.** Using your knowledge of electrophoresis, explain how you will distinguish the two on a gel.

6. Examine the MiniOne® electrophoresis chamber and the gel casting system and the charges on the color dyes using **Appendix A: Dye Chemical Structures.** To separate the dyes shown in **Appendix A,** should the wells be closer to the negative electrode or the positive electrode? Explain your reasoning.

7. Why do companies add color to food products? What flavor do you think of when you see a yellow candy? What about blue? Purple?

Inquiry Extension:

8. In the space below, write down the names and colors of the three additional candies you will be testing, and the names of any dyes listed on the package under Ingredients. Dye names usually contain a color and a number, such as "FD&C Yellow 6". If you are uncertain whether an ingredient is a dye, use the Internet to look it up.

Candy name and color	Dye names from package

9. If there is more than one color candy in the package, circle the names of the dyes that you hypothesize are in the candy color you have selected to test.

10. Compare the list of dyes in your table above to the list of dyes in Appendix A. If there are any dyes listed on your candy packages that are not in the Appendix, record them below. Use the Internet to look up the molar mass and electric charge of these dyes.

Instructions

Common workstations

- Microwave
- Gloves, eye protection, lab coats

Student group workstations

- MiniOne® Electrophoresis System - including carriage, photo hood, power supply, buffer tank, and **grey** viewing platform.
- MiniOne® Casting System
- MiniOne® 2-20 µL Micropipette
- Universal fit micropipette tips, 10 per group
- One candy of each color up to 6 colors per station (included with the MiniLab), and any additional candy you are using if doing the **Inquiry Extension**
- Aliquoted dye extraction buffer
- Dye extraction tray(s)
- 1% agarose gel cup
- Aliquoted TBE running buffer
- Fine point permanent marker
- Waste container for pipette tips
- MiniOne® 20-200 µL Micropipette (optional)

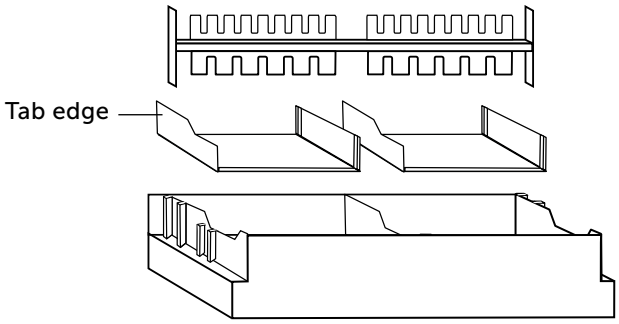
Experimental Procedures

Part I - Cast your agarose gel

Follow the instructions below to cast a 1% agarose gel. If you are analyzing only the six candies supplied with the kit, use the 6-well side of the comb.

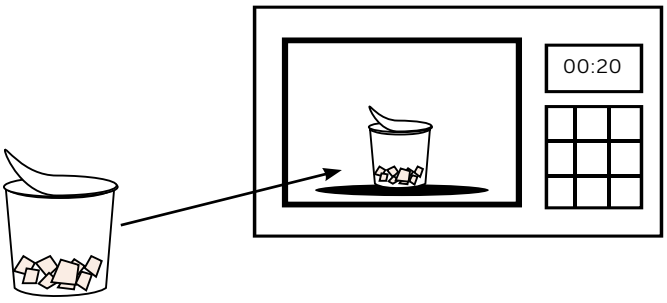
How to Cast a Gel

1. Place the MiniOne® Casting Stand on a level surface and place gel trays in the two cavities. For proper tray orientation place the tab edge of the tray on the left side. Insert the comb into the slots at the top of the casting stand with the 6-well side facing down. 💡**If you are doing the Inquiry Extension, use the 9-well side of the comb.**

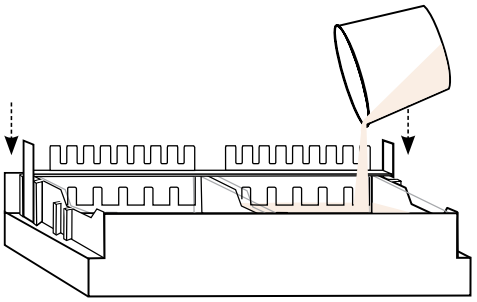


2. Partially peel the film off a gel cup and microwave for 20 seconds. Allow to cool for 15 seconds. **DO NOT microwave more than 5 gel cups at a time.**

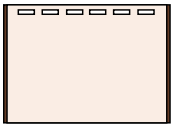
   ⚠**Safety requirement:** Adult supervision required if students are handling gel cups!

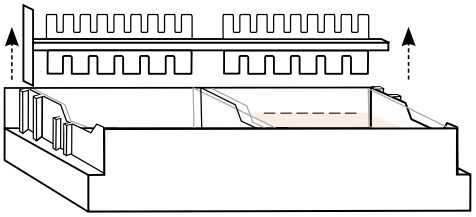


3. **One gel cup is for making one agarose gel!** Slowly pour the hot agarose solution into a gel tray. Make sure there are no air bubbles in the agarose solution. Let the agarose gel solidify for 10 minutes or until opaque. **DO NOT disturb the gel until time is up.**



4. Carefully remove comb when gel is ready. Remove gel tray with solidified gel from Casting Stand and wipe off any excess agarose from the bottom of the tray.





Part II - Extract dyes from candies

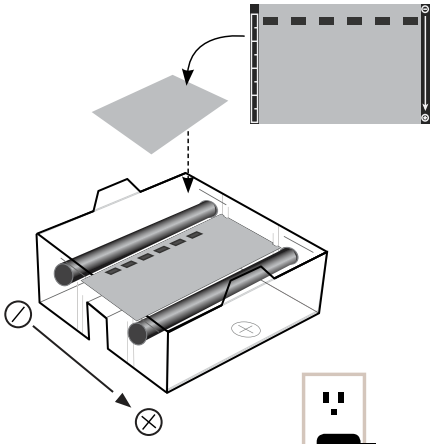
1. For each color you will be testing, add 100 µL Dye Extraction Buffer to a well of the dye extraction tray.
2. Place one candy in each well with the extraction buffer.
3. If you are analyzing candies that are too large for the wells of the tray, use a separate cup that will fit the candy and 200-500 µL Dye Extraction Buffer.
4. Allow the color to dissolve for about 2 minutes or until most of the color on the outside of the candy has dissolved. When the candy appears white on the side in contact with the solution, remove it from the tray. Dissolving too long will release molecules from the candy that will interfere with electrophoresis.

Part III - Analyze dyes with gel electrophoresis

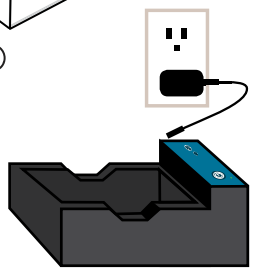
Follow the directions below to load and run the agarose gel to analyze your dye samples. Load 10 µL of each sample into the wells of the gel. Use the Data Table at the end of this section to record which sample is loaded into each well, and the initial color of the sample.

How to Load a Gel

1. Ensure the **grey** viewing platform is in the tank if it is not already installed and put the gel (still on the gel tray) into the tank.

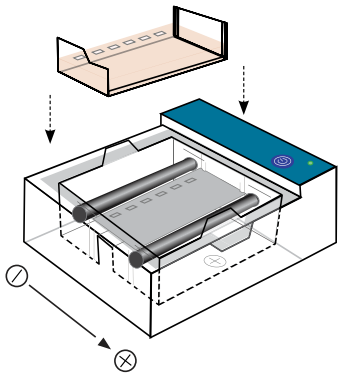


2. Plug the power supply into the wall and carefully insert the other end into the back of the MiniOne® Carriage.



3. Place the gel tank into the carriage so the carbon electrodes are touching the gold rivets and the tank sits level with the carriage.

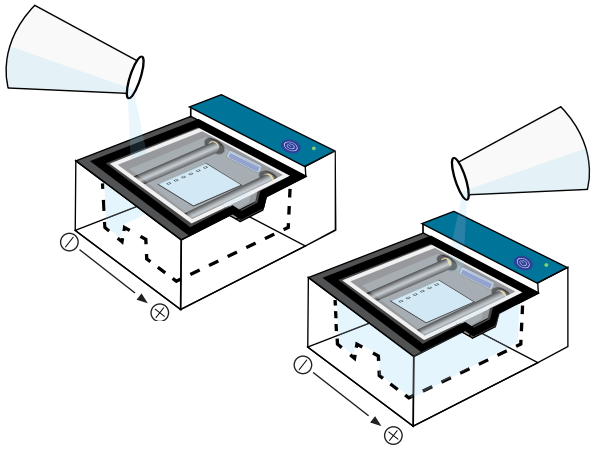
4. Place the gel tray with the gel into the gel tank. The gel tank should not have any buffer in it when putting the gel tray with gel into it.



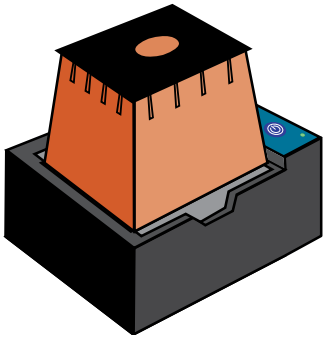
Note: Place gel tank with the gel on the gel tray into carriage **before** pouring buffer in.

5. Turn the low intensity blue LED on by pressing the button on the carriage.

6. Measure 135 mL of TBE running buffer and pour into **one side** of the gel tank. Watch the air push out between the gel tray and viewing platform. Once air has been removed from under the gel tray, pour remaining buffer into the **other side** of the gel tank.

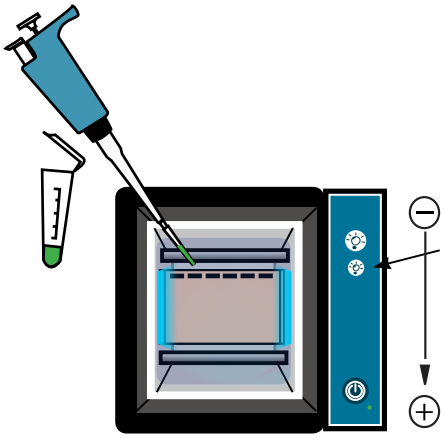


7. Place photo hood on the carriage.



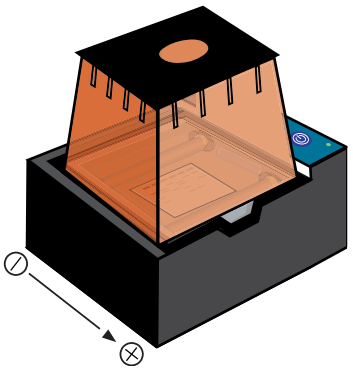
8. Press the power button which should now be a solid green light. If green light is **solid,** turn off the unit and proceed to loading gels.

9. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading. Load 10 µL per well by pipetting directly out of the extraction tray. Load one color per well. Remember to change pipette tips for each sample. **Load your samples according to their order on your Data Table. (see Page 11)**



Run, Visualize and Capture Image

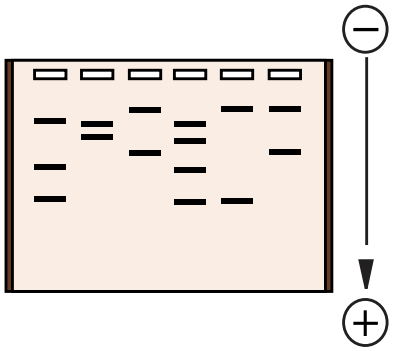
1. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.



The green power LED will not turn on if:

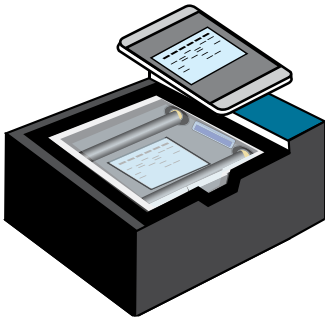
- **The tank is not properly placed inside the carriage**
- **There is no buffer in the tank**
- **The buffer is too concentrated or too diluted**
- **The photo hood is not on the carriage**
- **There is too much or too little running buffer**
- **The power supply is not plugged in. Check by turning on the blue LEDs**

2. Check the migration of the bands (~every five minutes).



3. Allow the gel to run for approximately 15-20 minutes or until the fastest moving dyes have migrated 75% of the distance through the gel. When your run in complete, turn off the power by pressing the button.

4. **Document your results.** At the end of the run, take photos following these steps: Remove the photo hood and turn off the blue LED light. Hold your cell phone or camera about three inches above the tank and take a picture of your gel.



Clean Up

1. After collecting data and documenting results, remove the photo hood and unplug the power supply from the wall and from the back of the MiniOne® Carriage. Remove the clear running tank from the carriage and remove the gel and tray from the running tank.
2. Pour the used running buffer down the drain or into a waste beaker. Throw the gel away. Rinse the clear plastic running tank, gel tray, comb, and casting system with DI or distilled water. Allow the tanks to fully air dry before storing.
3. Use a paper towel or kimwipe to gently wipe the gold rivets in the carriage (where the electrodes connect) to ensure all moisture is removed. Wipe up any buffer that may have spilled into the black carriage. Follow any additional directions the instructor gives for cleanup and storage.

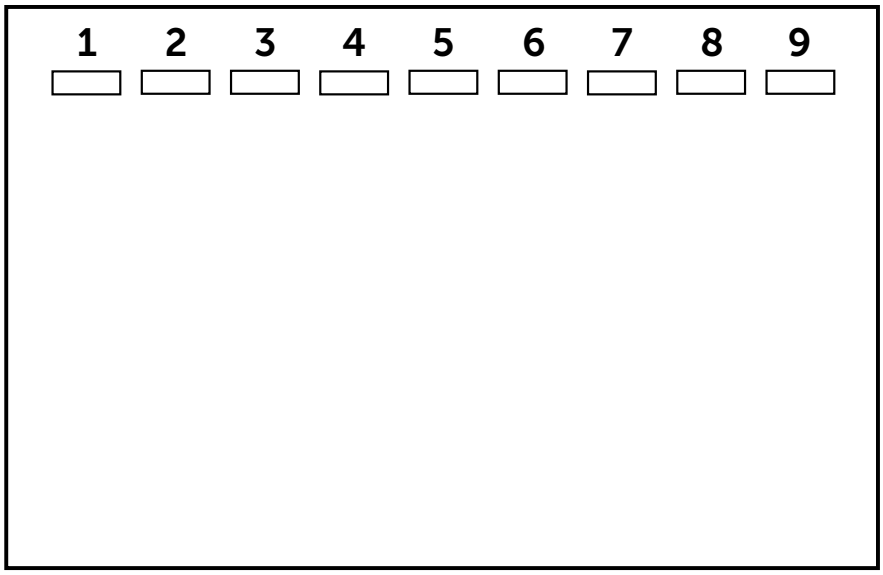
Data Table:

Use lanes 7 through 9 for Inquiry Extension

Well #	1	2	3	4	5	6	7	8	9
Original Color									
Final Colors									
Names of Dye(s)									
Molar Mass(es) (g/mol)									
# negative charge(s)									
Charge/ Mass									

Gel Analysis Worksheet

Directions: After running the DNA samples on the gel, record an image of the gel and draw the results or tape the image on the template below:



Lane 1: _____

Lane 2: _____

Lane 3: _____

Lane 4: _____

Lane 5: _____

Lane 6: _____

Lane 7: _____

Lane 8: _____

Lane 9: _____

Post-Lab Analysis Questions

1. Record the final colors of the bands in your gel on the Data Table.

2. Which candy colors contain a single dye? Explain your reasoning.

3. Which candy colors contain mixtures of two or more dyes? Explain your reasoning.

4. Calculate the change/mass ratio of each dye in **Appendix A: Dye Chemical Structures.** Using this information and your understanding of electrophoresis, match the color of the candy with the names of the dyes it contains. Record your answers in the Data Table.

5. Were there any lanes where the dyes did not separate completely? How can you tell? Justify your conclusion based on the charge/mass ratios you calculated above.

6. If you love Skittles® but are allergic to Yellow 5, which colors should you avoid?

7. Debate question: synthetic vs. natural dyes as food additives. Discuss as a class the differences and the pros and cons of each.

Inquiry Extension:

8. Compare the bands in lanes corresponding to the three candies you brought in with the bands from the six provided candies. Are there any dyes in your candies that can't be matched to one of the dyes in the Skittles®? If so, do these bands correspond to the dyes you identified in Question 10 of the Pre-lab Questions?

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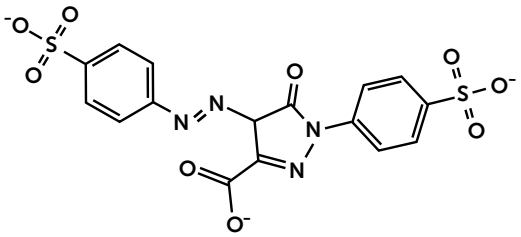
9. Are any of your candies the same color as one of the six Skittles®? If so, are the dyes used to create the color the same or different? Justify your answer by referring to your electrophoresis results.

10. Besides candies, what are some other commercial products that contain dyes that you could analyze with electrophoresis? Do you think that these products contain the same dyes that you have investigated here? Why or why not?

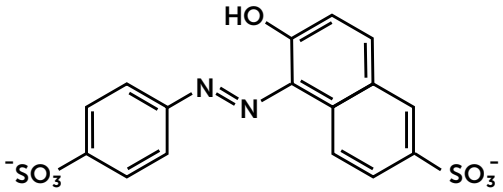
Appendix A - Chemical Structures of Dyes from Skittles® Label

FD&C Yellow 5



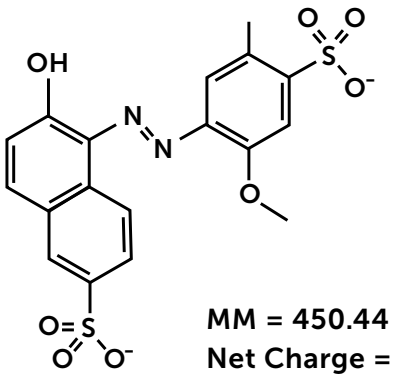
MM = 465.39 g/mol
Net Charge = -3

FD&C Yellow 6



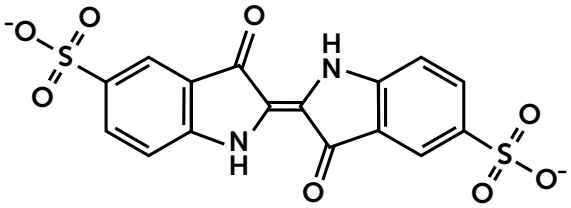
MM = 406.39 g/mol
Net Charge = -2

FD&C Red 40



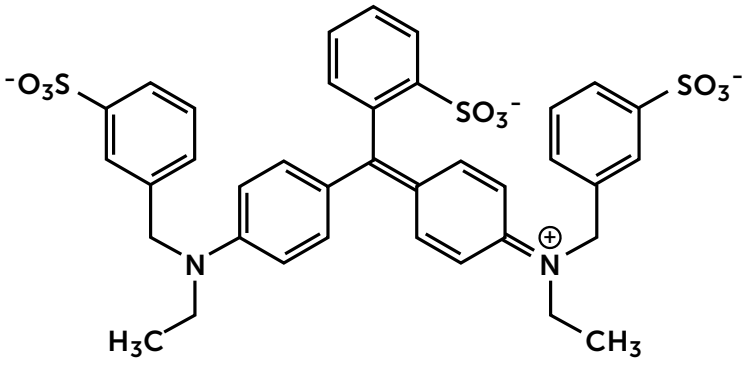
MM = 450.44 g/mol
Net Charge = -2

FD&C Blue 2



MM = 420.37 g/mol
Net Charge = -2

FD&C Blue 1



MM = 746.87 g/mol
Net Charge = -2

MM = molar mass

Appendix B - Gel Electrophoresis

Gel electrophoresis is a technique used in many areas of science to separate and analyze the components of complex chemical mixtures. Mixtures of DNA, RNA, proteins, or dyes can be separated into their individual components based on molecular size and electrical charge using a separation matrix within an electric field.

The gel used in gel electrophoresis is a tangle of polymers forming a three-dimensional matrix with water-filled pores through which molecules migrate. A higher density of polymers creates smaller pores. Like the holes in a sieve or colander, the size of the pores has to be the appropriate size for the molecules being separated. Gels can be made from different substances depending on the application. One of the most commonly used and effective materials is agarose, a polymer extracted from seaweed. Agarose gels are formed (or cast) by pouring molten (melted) agarose into a tray where it solidifies into the desired shape as it cools. A comb is placed while the agarose is molten and then removed after the agarose gel is solidified. This creates wells into which the samples are loaded.

After the gel solidifies it is placed in an electrically conductive buffer between parallel positive (⊕ anode) and negative (⊖ cathode) electrodes. A voltage is applied between the electrodes, creating a uniform electric field within the gel. Molecules in the wells begin to move under the influence of the electric field: positively charged molecules migrate toward the ⊖ cathode and negatively charged molecules migrate toward the ⊕ anode.

The speed of a molecule's movement in an electric field is determined by the strength of its electric charge relative to its size. This is quantified as the charge to mass ratio. Speed of movement within a gel is also influenced by the size of the molecule relative to the pores in the gel. The polymers in the gel are like an obstacle course: smaller molecules maneuver easily through the pores, traveling faster and farther than large, bulky molecules. However, a large molecule can move faster through a gel than a smaller molecule when the strength of its charge relative to its mass is significantly higher. Shape can also affect how a molecule moves through the gel. Long spaghetti-like molecules will move slower than compact molecules, which slip easily through the pores. Molecules of the same size, shape, and charge will move together and form a distinct band. If multiple types or sizes of molecules are present in the sample, they will separate from each other and each will form a distinct band.

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