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THE **BIOTECHNOLOGY**
EDUCATION COMPANY®

NEW

Edvo-Kit #

S-47

Edvo-Kit #S-47

Linking Food Science to Biotechnology: Unlock the Color of Candies

Experiment Objective:
In this experiment, students will investigate how gel electrophoresis unlocks the color code by investigating food dyes used to make colorful candies.

See page 3 for storage instructions.

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Experiment Components

READY-TO-LOAD™ SAMPLES FOR ELECTROPHORESIS

Store all components at room temperature

Components		Check (✓)
A	Dye Extraction Buffer	❑
B	Standard Dye Marker	❑

Experiment #S-47 is designed for 10 gels.

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ ❑
- Electrophoresis buffer (50x) ❑
- Practice Gel Loading Solution ❑
- Transfer pipet ❑
- Calibrated transfer pipets ❑
- Plastic Cups ❑
- 0.5 ml Microcentrifuge Tubes ❑
- 1.5 ml Microcentrifuge Tubes ❑

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips (optional)
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Visualization system (white light box)
- Distilled or deionized water
- Candies (suggestions: M&Ms®, Skittles®, Jelly Beans, Gum Balls)

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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Background Information: Candy Electrophoresis

FOOD COLORING

The color of food has always been an integral aspect of our culture. The early Romans believed that people not only eat with their palate, but also "eat with their eyes." For centuries, humans have used dyes from natural ingredients to add color to food, drink, clothing and other products. For example, saffron, paprika and other spices were used to provide food yellow appearance.

Companies have long been introducing color additives to a variety of products, including candies, shampoos, perfumes, drinks, etc. Color additives are used in food for several reasons. Manufacturers add colors to food to offset color loss due to product exposure to various environmental conditions, such as light, air, and moisture. Additionally, companies often add dyes to food products like beverages, jellies, pudding and condiments to make them look more attractive to consumers. Table 1 illustrates the seven commonly used food dyes in the United States. Of these dyes, Blue 1 and Red 40 are the most common blue and red dyes while Green 3 and Blue 2 are rarely used. Food containing these approved dyes are called "For Coloring Food" and have the abbreviation "FCF" preceding their names. Companies are required to list food dyes in their list of ingredients (Figure 1).

INGREDIENTS: CHOCOLATE COATING (MALTITOL, UNSWEETENED CHOCOLATE, COCOA BUTTER, SODIUM CASEINATE, MILK FAT, SOY LECITHIN, VANILLA, NATURAL AND ARTIFICIAL FLAVORS, SALT, SUCRALOSE), ROASTED PEANUTS (PEANUTS, CORN OIL), MALTITOL, LESS THAN 1% OF PURE VANILLA EXTRACT, CONFECTIONER'S GLAZE, CARNAUBA WAX, BEESWAX, TITANIUM DIOXIDE COLOR, YELLOW 5 LAKE, RED 40 LAKE, BLUE 1 LAKE, BLUE 2 LAKE, YELLOW 6 LAKE, YELLOW 6, GUM ARABIC. FR01
CONTAINS MILK, PEANUTS AND SOY.
THIS PRODUCT IS MANUFACTURED IN A FACILITY THAT USES OTHER TREE NUTS AND WHEAT.

Figure 1 – Example of Candy Ingredient Label

As the use continues to grow, concerns regarding the addition of food colors to food products also emerge. Commonly used food dyes such as yellow 5 and red 40 are believe to pose several health concerns in children, including hyperactivity and allergic reactions. The Food and Drug Administration (FDA) is responsible for regulating color additives used in a variety of products in the United States. Color additives allowed for use in foods are classified as "certifiable" or "exempt from certification". Certifiable color additives are man made which are tested by the manufacturers and the FDA to assures the quality and safety of the color additives. On the other hand, color additives that are "exempt from certification" are those that derived from natural sources such as vegetables, fruits, and minerals.

AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis is widely used to separate molecules based upon charge, size and shape. It is particularly useful in separating charged biomolecules such as DNA, RNA and proteins.

Agarose gel electrophoresis possesses great resolving power, yet is relatively simple and straightforward to perform. The gel is made by dissolving agarose powder in the electrophoresis buffer. The solution is boiled to dissolve the agarose and then cooled to approximately 60° C and poured into a gel tray where it solidifies. The tray is submerged in a buffer-filled electrophoresis apparatus, which contains electrodes.

Samples are prepared for electrophoresis by mixing them with glycerol or sucrose to give the mixture higher density. This makes the samples denser than the electrophoresis buffer. These samples can then be loaded with a micropipet or transfer pipet into wells that were created in the gel by a template during casting. The dense samples sink through the buffer and remain in the wells.

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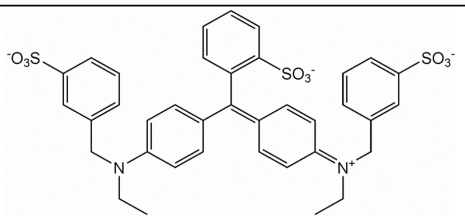
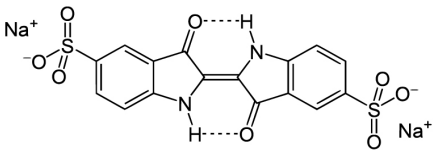
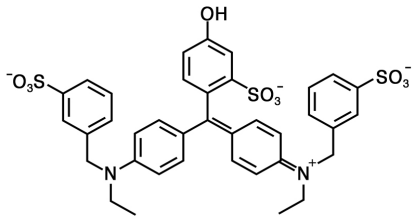
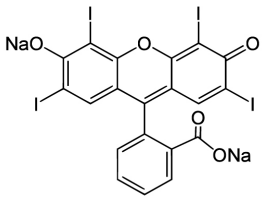
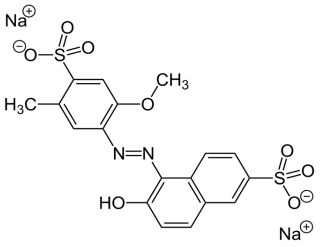
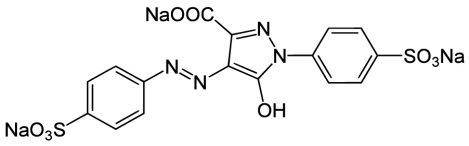
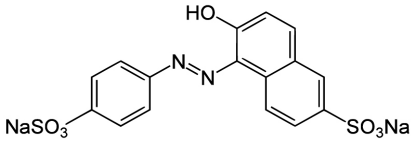
Abbreviation	Name	Shade	Structure
Blue 1	Brilliant Blue	Blue	
Blue 2	Indigotine	Indigo	
Green 3	Fast Green	Turquoise	
Red 3	Erythrosine	Pink	
Red 40	Allura Red	Red	
Yellow 5	Tartazine	Yellow	
Yellow 6	Sunset Yellow	Orange	

Table 1 – Seven Artificial Colors Approved by the FDA for Coloring Food

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Candy Electrophoresis

A direct current power supply is connected to the electrophoresis apparatus and current is applied. Charged molecules in the sample enter the gel matrix. Molecules having a net negative charge migrate towards the positive electrode (anode) while net positively charged molecules migrate towards the negative electrode (cathode). Within a range, the higher the applied voltage, the faster the samples migrate. The buffer serves as a conductor of electricity and to control the pH. The pH is important to the charge and stability of biological molecules.

Agarose is a polysaccharide derived from agar. In this experiment, UltraSpec-Agarose™, a mixture of agarose and hydrocolloids which renders the gel to be both clear and resilient, is used. At first glance, an agarose gel appears to be a solid at room temperature. However, on the molecular level, the gel contains microscopic pores which act as a molecular sieve, allowing the different molecules to pass through.

Food dyes are composed of ions. When these charged ions are subjected to an electric field, the molecules will migrate toward the electrode of opposite charge. Positively charged molecules will migrate toward the negative electrode, while those with a negative charge will move toward the positive electrode. Small dye fragments move through these holes easily, but large dye fragments have a more difficult time squeezing through the tunnels.

Factors such as charge, size and shape, together with buffer conditions, gel concentrations and voltage, affects the mobility of molecules in gels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete "bands" within the gel. After the current is stopped, the bands can be visualized (Figure 2).

In this experiment, students will extract several different dyes from food source. The dyes will then be analyzed using agarose gel electrophoresis and their rate of migration will be observed and measured.

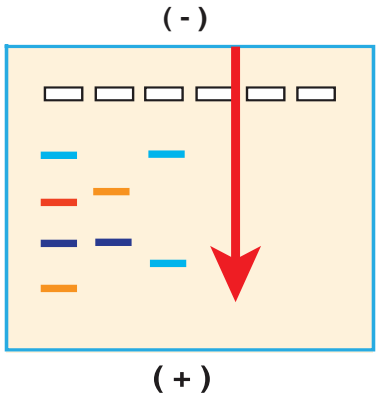

Figure 2 – Overview of agarose gel electrophoresis

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Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will investigate how gel electrophoresis unlocks the color code by investigating food dyes used to make colorful candies.

LABORATORY SAFETY

1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

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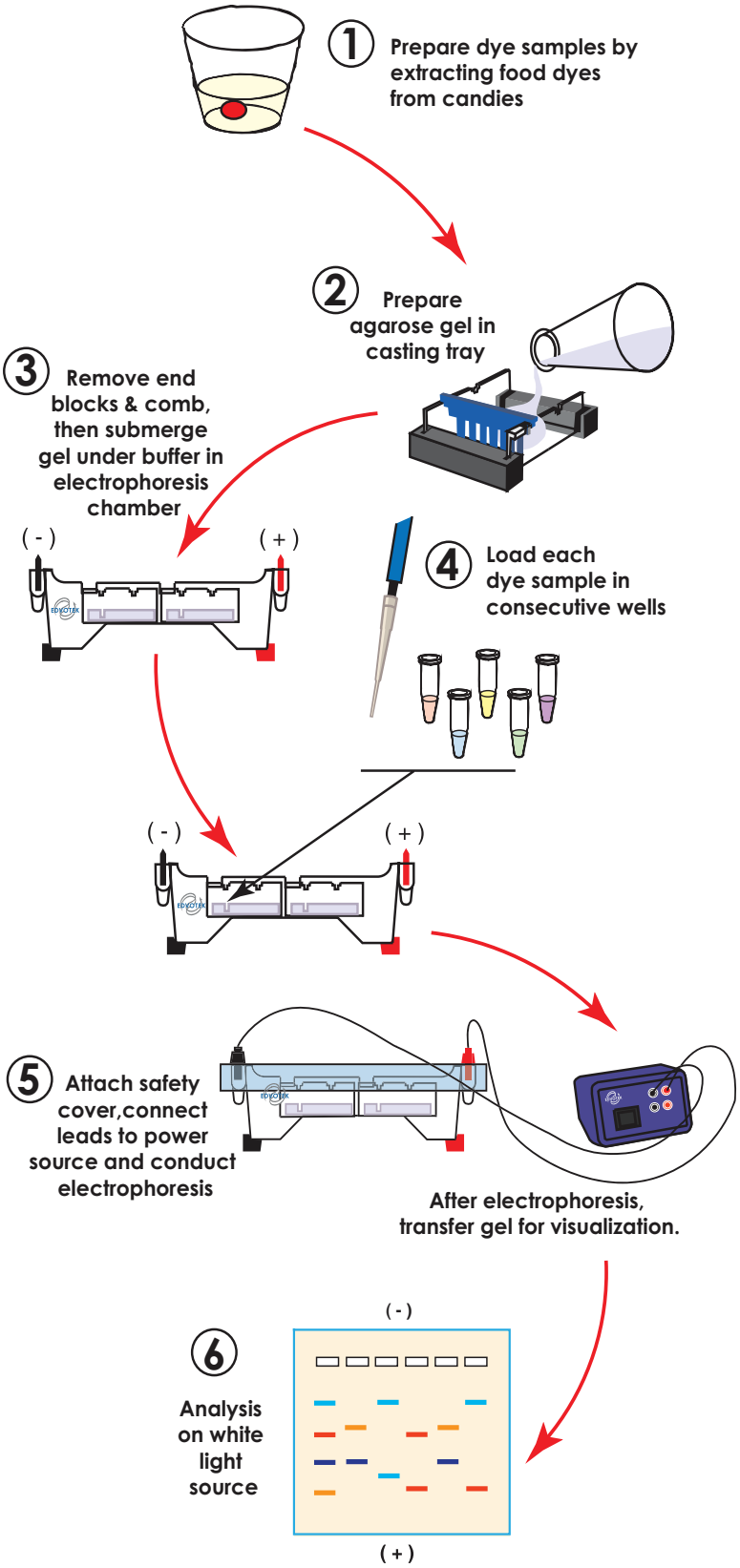
Experiment Overview



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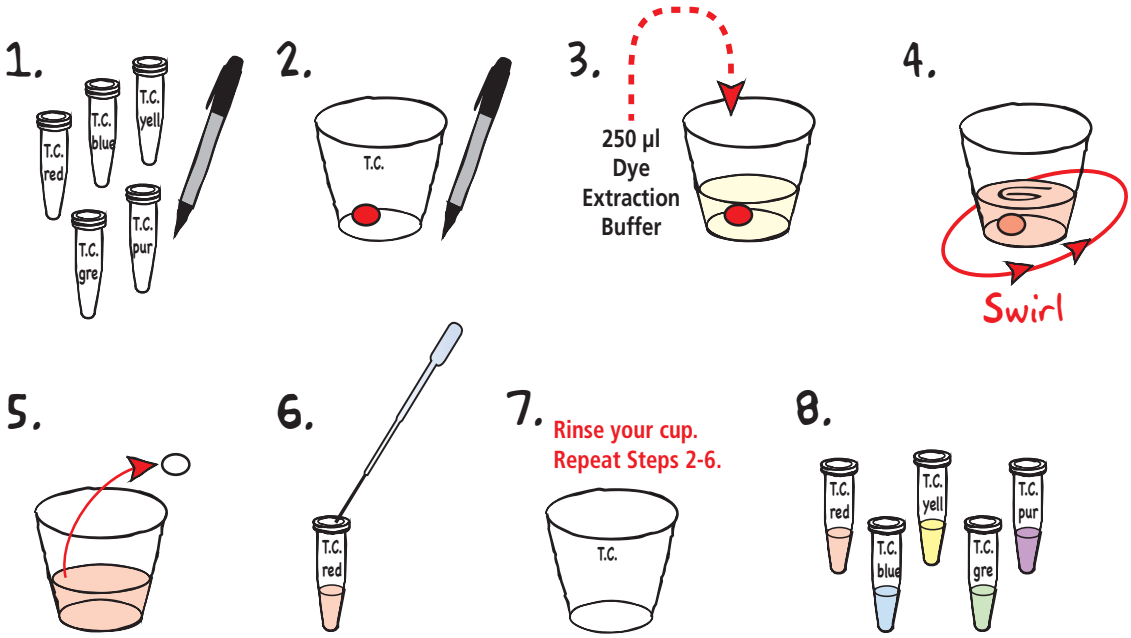
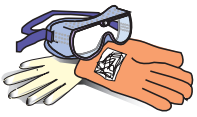
Module I: Extraction of Food Dyes from Candy



We recommend using brightly-colored candies M&M's®, Skittles®, jelly beans, and gum balls


Wear gloves and safety goggles

1. **LABEL** five microcentrifuge tubes with your initials and the colors of the candy you will be investigating.
2. **LABEL** the provided cup with your initials. **ADD** one candy to the cup
3. **ADD** 250 µl of Dye Extraction Buffer to the cup containing the candy.
4. **SWIRL** the candy gently in the Dye Extraction Buffer to dissolve the color coating until the white layer of the candy is exposed.
5. **REMOVE** the candy from the cup.
6. **TRANSFER** the dissolved color solution into the appropriately labeled microcentrifuge tube.
7. **RINSE** the cup. **REPEAT s**teps 2-6 with the remaining 4 candies.
8. **PLACE** the tubes on lab bench. **PROCEED** to Module II: Separation of Food Dyes by Agarose Gel Electrophoresis.

OPTIONAL STOPPING POINT
Dye samples may be stored in the refrigerator for up to 24 hours before performing electrophoresis.

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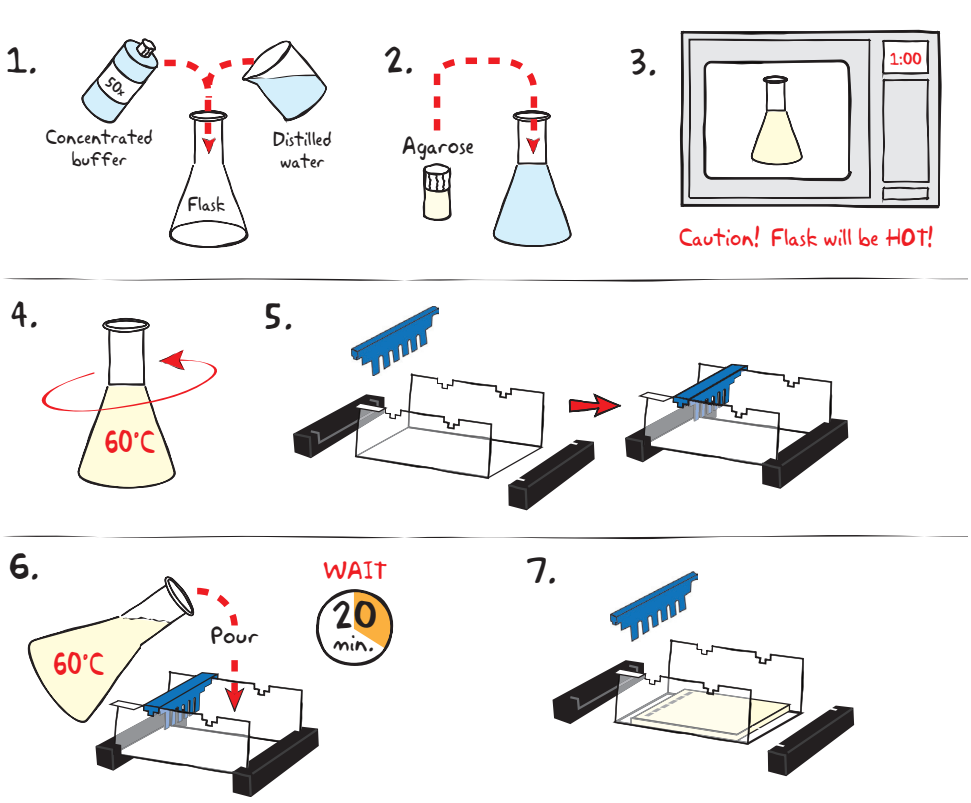
Module II: Separation of Food Dyes by Agarose Gel Electrophoresis



IMPORTANT:

If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

Wear gloves and safety goggles

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6 ml	29.4 ml	0.23 g	30 ml
7 x 10 cm	1.0 ml	49.0 ml	0.39 g	50 ml
7 x 14 cm	1.2 ml	58.8 ml	0.46 g	60 ml

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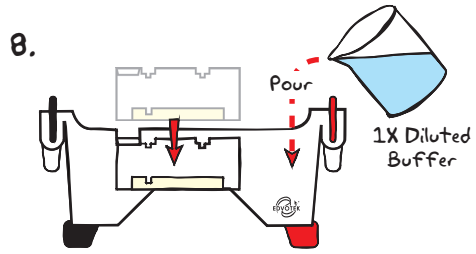
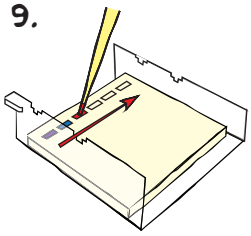
Module II: Separation of Food Dyes by Agarose Gel Electrophoresis

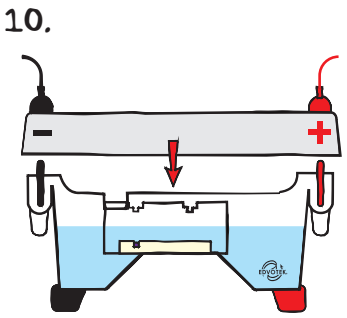
Reminders:

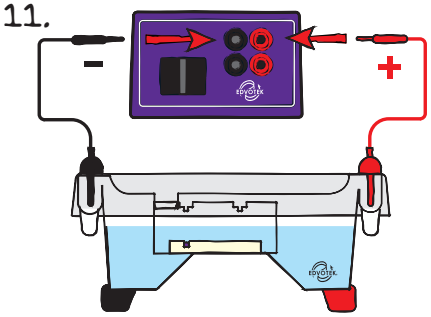
If unfamiliar with gel loading, consider performing the optional activity in Appendix C, Practice Gel Loading, prior to performing the experiment.

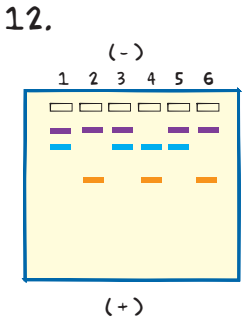
Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.











8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **LOAD** 35 µL of the Standard Dye Marker into the first well. **LOAD** the remaining dye samples into the other five lanes. Be sure to **RECORD** the color of each sample and its position in Table 1.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the negatively-charged samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **VISUALIZE** the results. No staining is necessary.

Table 2: Gel Loading

Lane	Candy Color
1	Standard Dye Marker
2	
3	
4	
5	
6	

Table B 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+ & M12 (new)	300 ml	6 ml	294 ml
M12 (classic)	400 ml	8 ml	392 ml
M36	1000 ml	20 ml	980 ml

Table C Time and Voltage Guidelines (0.8% Agarose Gel)

	Electrophoresis Model: M6+	M12 (new)	M12 (classic) & M36
Volts	Min. / Max.	Min. / Max.	Min. / Max.
150	15/20 min.	20/30 min.	25 / 35 min.
125	20/30 min.	30/35 min.	35 / 45 min.
75	35 / 45 min.	55/70 min.	60 / 90 min.

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Module III: STEM-Based Data Analysis of Food Dyes Using a Standard Curve

Agarose gel electrophoresis separates biomolecules into discrete bands, each comprising molecules of the same size. How can these results be used to determine the lengths of different fragments? Remember, as the length of a biomolecule increases, the distance to which the molecule can migrate decreases because large molecules cannot pass through the channels in the gel with ease. Therefore, the migration rate is inversely proportional to the length of the molecules—more specifically, to the $\log_{10}$ of molecule's size. To illustrate this, we ran a sample that contains bands of known lengths called a "standard". We will measure the distance that each of these bands traveled to create a graph, known as a "standard curve", which can then be used to extrapolate the size of unknown molecule(s).

1. Measure and Record Migration Distances

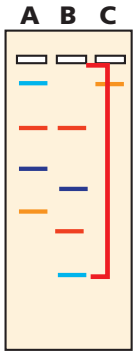
Measure the distance traveled by each Standard Dye Molecule from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters (to the nearest millimeter) in your notebook. Repeat this for each dye fragment in the standard.

Measure and record the migration distances of each of the fragments in the unknown samples in the same way you measured the standard bands.

2. Generate a Standard Curve.

Because migration rate is inversely proportional to the $\log_{10}$ of band length, plotting the data as a semi-log plot will produce a straight line and allow us to analyze an exponential range of fragment sizes. You will notice that the vertical axis of the semi-log plot appears atypical at first; the distance between numbers shrinks as the axis progresses from 1 to 9. This is because the axis represents a logarithmic scale. The first cycle on the y-axis corresponds to lengths from 100-1,000 base pairs, the second cycle measures 1,000-10,000 base pairs, and so on. To create a standard curve on the semi-log paper, plot the distance each Standard Dye Molecule migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!

Figure 3:
Measure distance migrated from the lower edge of the well to the lower edge of each band.



Quick Reference:

The Standard dyes have the following base pair equivalents.

Blue	5000
Red	3000
Purple	1000
Orange	500

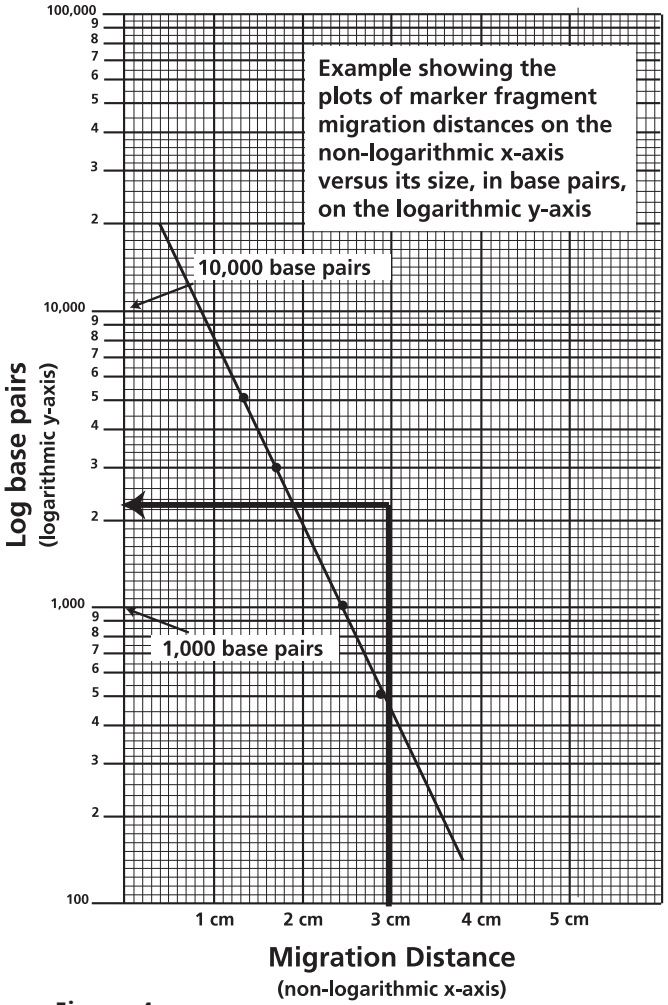


Figure 4:
Semilog graph example

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Module III: STEM-Based Data Analysis of Food Dyes Using a Standard Curve

After all the points have been plotted, use a ruler or a straight edge to draw the best straight line possible through the points. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points (see Figure 4 for an example).

3. Determine the length of each unknown fragment.

a. Locate the migration distance of the unknown dye on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.

b. From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the dye in base pairs (refer to Figure 4 for an example). Make note of this in your lab notebook.

c. Repeat for each fragment in your unknown sample.

Quick Reference:

Standard Dye marker sizes - length is expressed in base pairs.

5000, 3000, 1000, 500

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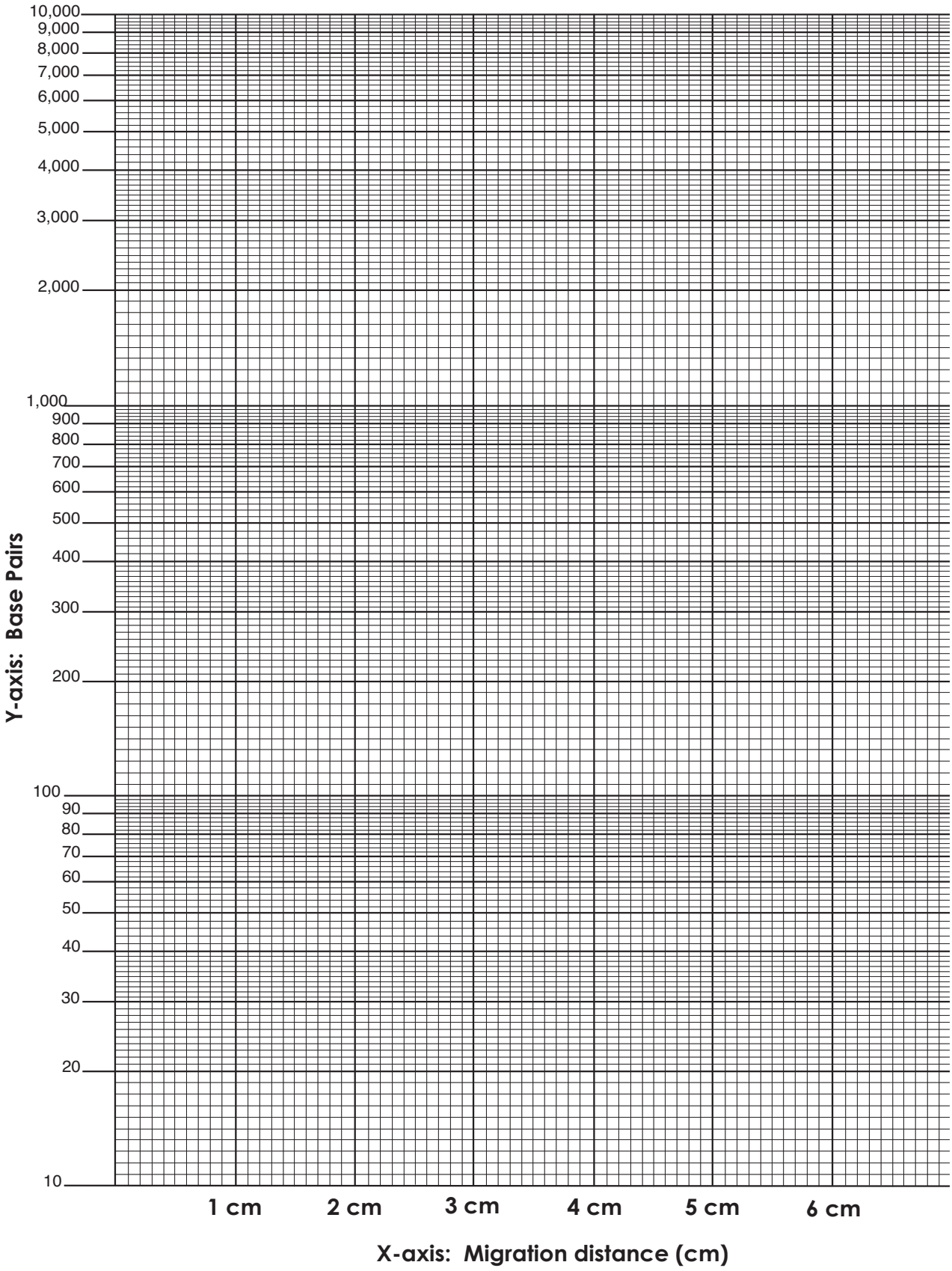

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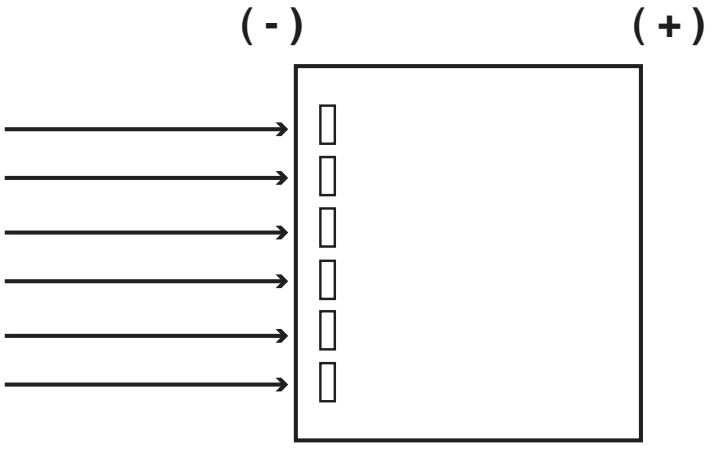
Study Questions

1. What is the purpose of gel electrophoresis?

2. What do you think are some factors that determine the distance molecules move though the gel? Give an example.

3. If you were pouring a gel to run DNA samples, where would you place the comb? Explain.

4. The diagram below represents your gel. Using colored pencils or markers draw in the results of your dye separation. Be sure to label each lane with name of the dye that was in the well.



5. Which dye was the smallest dye molecule? Explain.

6. Which dye was the largest dye molecule? Explain.

7. When determining the sizes of Dye fragments, which axis is used to plot the migration distances of the known and unknown fragments? Which axis is used to plot the sizes of the known and unknown fragments?

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Instructor's Guide

OVERVIEW OF INSTRUCTOR'S PRELAB PREPARATION:

This section outlines the recommended prelab preparations and approximate time requirement to complete each prelab activity.

Preparation For:	What to do:	When:	Time Required:
Module I: Extraction of Food Dyes from Candy	Aliquot Dye Extraction Buffer	Up to one day before performing the experiment.	10 min.
Module II: Separation of Food Dyes by Agarose Gel Electrophoresis	Aliquot Standard Dye Marker	Up to one day before performing the experiment.	40 min.
	Prepare diluted TAE buffer		
	Prepare molten agarose and pour gel		
Module III: STEM-Based Data Analysis of Food Dyes Using a Standard Curve	Photocopy/print semi-log paper	Before the class period.	10 min.

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Pre-Lab Preparations:

MODULE I: EXTRACTION OF FOOD DYES FROM CANDY

- Aliquot 1.3 ml of Extraction Buffer into 10 labeled microcentrifuge tubes. Distribute 1 tube per student group.

For Module I, each group should receive the following materials:

- 1 tube Extraction Buffer
- 1 plastic cup
- 5 small microcentrifuge tubes
- 1 small transfer pipet

MODULE II: SEPARATION OF FOOD DYES BY AGAROSE GEL ELECTROPHORESIS

Preparation of Agarose Gels

This experiment requires one 0.8% agarose gel per student group. A 7 x 7 cm gel is recommended. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

- **Individual Gel Preparation:**
  Each student group can be responsible for casting their own individual gel prior to conducting the experiment. See Module II in the Student's Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

- **Batch Gel Preparation:**
  To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix B.

- **Preparing Gels in Advance:**
  Gels may be prepared ahead and stored for later use. Solidified gels can be store under buffer in the refrigerator for up to 2 weeks. Do not freeze gels at -20° C as freezing will destroy the gels.

  Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

For Module II, each group should receive the following materials:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- Candy dye samples

NOTE:
Accurate pipetting is critical for maximizing successful experiment results.

If students are unfamiliar with using micropipets, we recommend performing the optional activity found in Appendix C, Practice Gel Loading, prior to conducting the experiment.

Additional Materials:

Each 0.8% gel should be loaded with the Standard Dye Marker and 5 samples containing dissolved colored candy coating.

- Aliquot 40 µl of the Standard Dye Marker (comp. B) into labeled microcentrifuge tubes and distribute one tube of ladder per student group.

MODULE III: STEM-BASED DATA ANALYSIS OF FOOD DYES USING A STANDARD CURVE

Prepare copies of semi-log paper and distribute one copy per student group.

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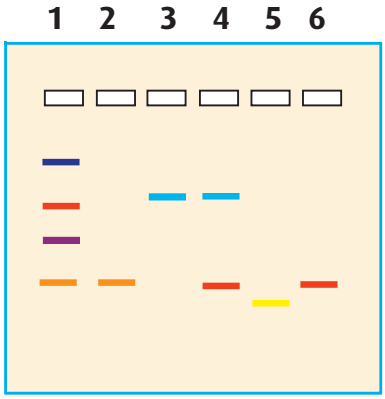
Experiment Results and Analysis



NOTE:
In the idealized schematic, the relative positions of dye fragments are shown but are not depicted to scale. No positively charged dyes are shown.

Lane	*Sample*
1	Standard Dye Marker
2	Dye extracted from Candy #1
3	Dye extracted from Candy #2
4	Dye extracted from Candy #3
5	Dye extracted from Candy #4
6	Dye extracted from Candy #5

Quick Reference:

Standard Dye marker sizes - length is expressed in base pairs.

5000, 3000, 1000, 500

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Please refer to the kit insert for the Answers to Study Questions

Appendices

A EDVOTEK® Troubleshooting Guide

B Bulk Preparation of Agarose Gels

C Practice Gel Loading

Safety Data Sheets can be found on our website: **www.edvotek.com**

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Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
The dye sample was very light after the extraction procedure.	Not enough candy used for extraction.	Try dissolving the dye off of multiple candies of the same color.
I was not able to extract color from my food samples.	Not enough food sample used for extraction.	Try dissolving the dye off of multiple candies of the same color. Also be sure that the waxy coating, if any, is dissolved.
Very light colored band seen after electrophoresis	Pipetting error	Make sure students pipet 35 µl of dissolved colored candy coating per well.
	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.

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Appendix B
Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

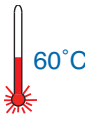
Table D — Bulk Preparation of Electrophoresis Buffer

50x Conc. Buffer	+	Distilled Water	Total Volume Required
60 ml		2,940 ml	3000 ml (3 L)

Batch Agarose Gels (0.8%)

For quantity (batch) preparation of 0.8% agarose gels, see Table E.

1. Use a 500 ml flask to prepare the diluted gel buffer
2. Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.



6. Dispense the required volume of cooled agarose solution for casting each gel. The volume required is dependent upon the size of the gel bed and DNA staining method which will be used. Refer to Appendix A or B for guidelines.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.

Note:
The UltraSpec-Agarose™ kit component is usually labeled with the amount it contains. Please read the label carefully. If the amount of agarose is not specified or if the bottle's plastic seal has been broken, weigh the agarose to ensure you are using the correct amount.

Table E — Batch Prep of 0.8% UltraSpec-Agarose™

Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390

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Appendix C
Practice Gel Loading

Accurate sample delivery technique ensures the best possible gel results. Pipetting mistakes can cause the sample to become diluted with buffer, or cause damage to the wells with the pipet tip while loading the gel.

If you are unfamiliar with loading samples in agarose gels, it is recommended that you practice sample delivery techniques before conducting the actual experiment. EDVOTEK electrophoresis experiments contain a tube of practice gel loading solution for this purpose. Casting of a separate practice gel is highly recommended. One suggested activity is outlined below:

1. Cast a gel with the maximum number of wells possible.

2. After the gel solidifies, place it under buffer in an electrophoresis apparatus chamber.

   Alternatively, your teacher may have cut the gel in sections between the rows of wells. Place a gel section with wells into a small, shallow tray and submerge it under buffer or water.

Note:
The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

3. Practice delivering the practice gel loading solution to the sample wells. Take care not to damage or puncture the wells with the pipet tip.

   - For electrophoresis of dyes, load the sample well with 35-38 microliters of sample.

   - If using transfer pipets for sample delivery, load each sample well until it is full.

4. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.

5. Replace the practice gel with a fresh gel for the actual experiment. **Note: If practicing gel loading in the electrophoresis chamber, the practice gel loading solution will become diluted in the buffer in the apparatus. It will not interfere with the experiment, so it is not necessary to prepare fresh buffer.**

Note:
If you do not wish to pour extra agarose gels, Edvotek® DuraGels™ (Cat. S-43) can be used as a substitute. Edvotek® DuraGels™ are reusable polymer gel models that allows students to gain experience with gel loading before performing agarose gel electrophoresis. The use of DuraGels™ eliminates the preparation time, expense, and waste of pouring actual agarose practice gels.

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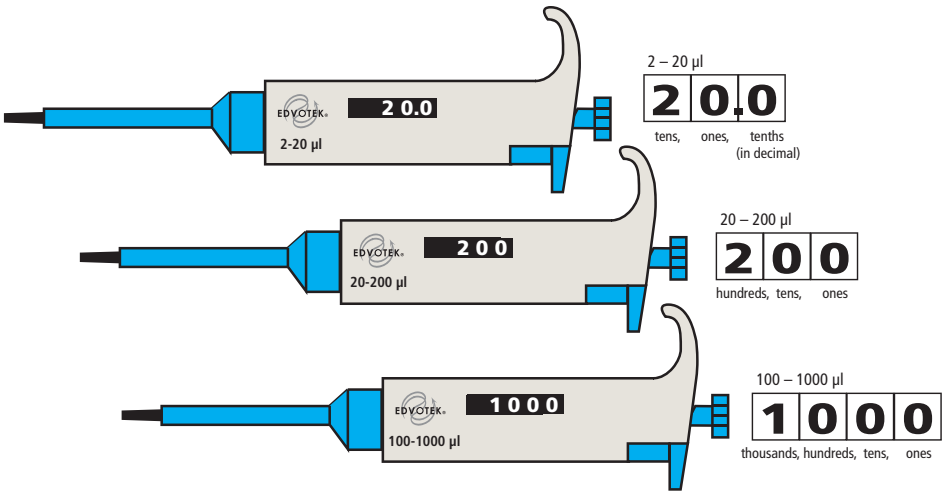
Appendix C
Practice Gel Loading



SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

1. **CHOOSE** the correct micropipet for the volume you are measuring. Make sure that the volume to be measured **DOES NOT EXCEED** the upper or lower volume setting of the micropipet.

2. **DETERMINE** the units measured by the micropipet by looking at the volume setting. The setting will appear in the window on the side of the micropipet. Note that the different micropipets use different scales for their measurements. Some micropipets are accurate to a tenth of a microliter, while others are accurate to one microliter.

3. **SET** the volume by twisting the top of the plunger. In general, twisting the plunger clockwise reduces the volume, and twisting the plunger counter clockwise increases the volume.

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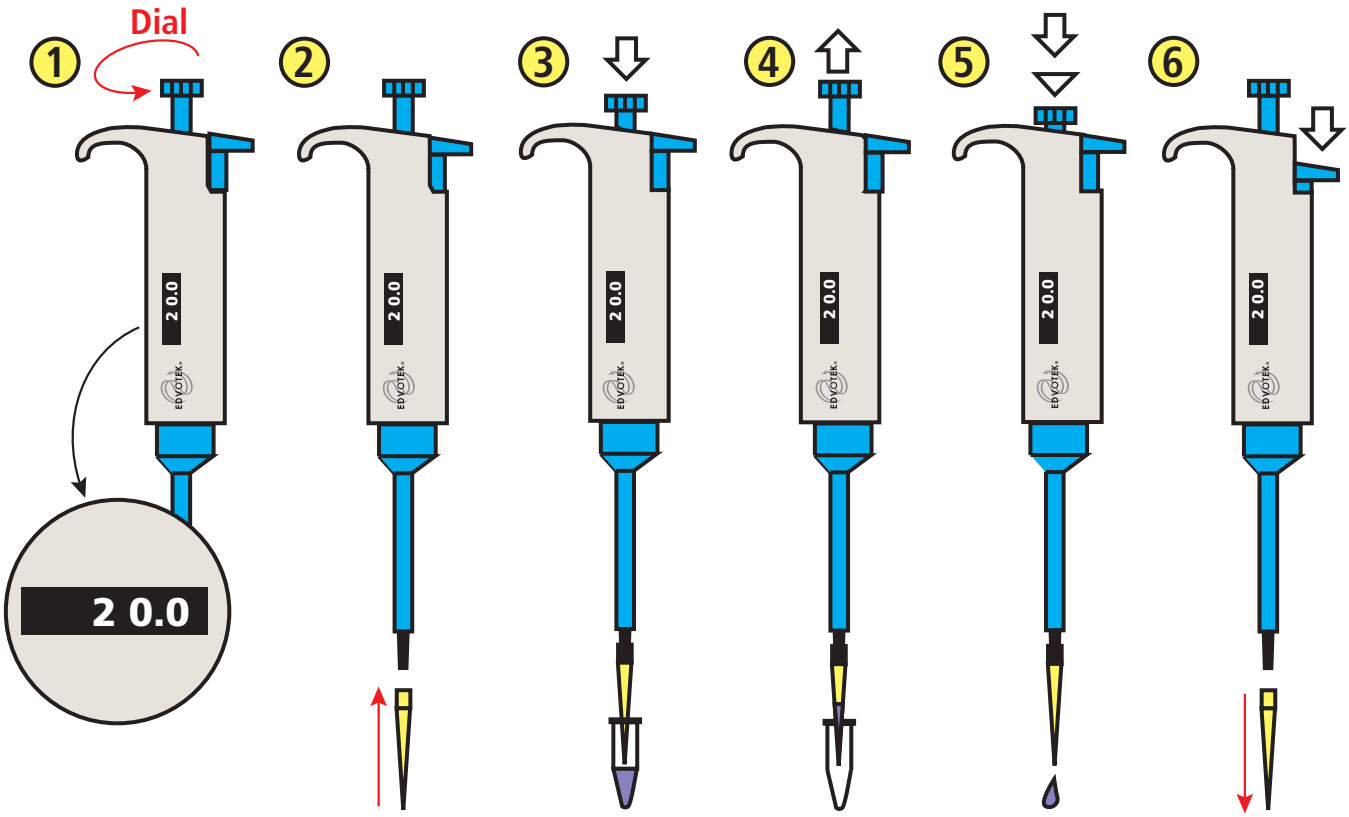
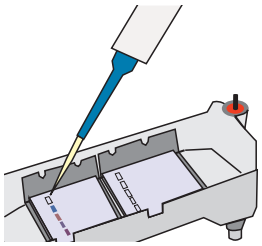
Appendix C
Practice Gel Loading



MEASURING LIQUIDS WITH A MICROPIPET

Wear gloves and safety goggles

1. **SET** the micropipet to the appropriate volume by adjusting the dial.
2. **PLACE** a clean tip on the micropipet.
3. **PRESS** the plunger down to the first stop. **HOLD** the plunger down while placing the tip beneath the surface of the liquid.
4. Slowly **RELEASE** the plunger to draw sample into the pipette tip. Position the pipet tip over the well. Be careful not to puncture or damage the well with the pipet tip.
5. **DELIVER** the sample by slowly pressing the plunger to the first stop. Depress the plunger to the second stop to expel any remaining sample. **DO NOT RELEASE** the plunger until the tip is out of the buffer.
6. **DISCARD** the tip by pressing the ejector button. Use a new clean tip for the next sample.



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