WORKSHOP

Sweet Science: Exploring Complex Mixtures with Biotechnology

EDVOTEK®

Designed for the Classroom

SINCE 1987

Introduction

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

Background Information

Color is an integral aspect of our culture. Companies have long been using color additives in a variety of products, including candies, shampoos, perfumes, drinks, etc. All the colors that we see are a mixture of the three primary colors: red, green, and blue. That these three simple colors can be combined to form the myriad of colors that we see everyday makes them a great example of how complex things, such as colors, can be broken down into their individual components.

In the classroom, a great way to break down colors is by examining color additives in food. The color of food has always been important for us. The early Romans believed that people not only eat with their palate, but also "eat with their eyes". For centuring, humans have used dyes from natural ingredients to add color to food, drink, clothing, and other products. These days, manufacturers add colors to food to offset color loss due to product exposure to various environmental conditions and to make them look more attractive to consumers. As the use of color additives to food continues to grow, concerns regarding the addition of food colors to products also emerge. The seven more commonly used food dyes in the United States are shown in Table 1 on page 4. However, the FDA and independent scientists are looking into whether or not certain dyes, such as yellow 5 and red 40, are linked to hyperactivity or allergic reactions.

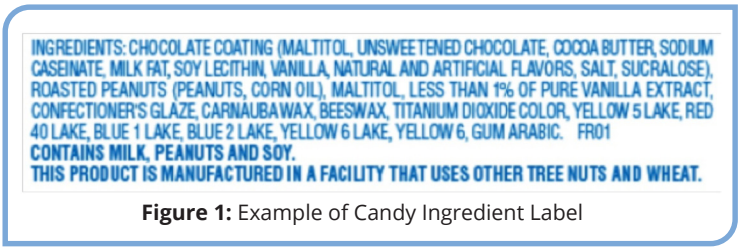

INGREDIENTS: CHOCOLATE COATING (MALTITOL, UNSWEETENED CHOCOLATE, COCOA BUTTER, SODIUM CASEINATE, MILK FAT, SOY LECITHIN, VANILLA, NATURAL AND ARTIFICIAL FLAVORS, SALT, SUCRALOSE), ROASTED PEANUTS (PEANUTS, CORN OIL), MALTITOL, LESS THAN 1% OF PURE VANILLA EXTRACT, CONFECTIONER'S GLAZE, CARNAUBA WAX, 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

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.

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.

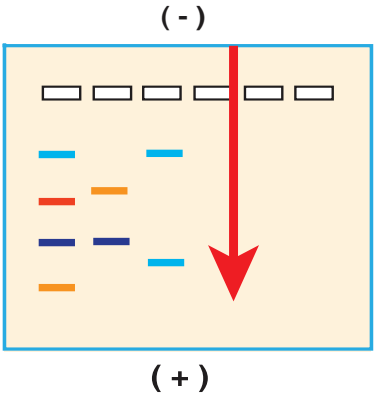
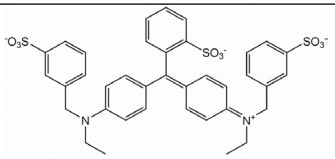
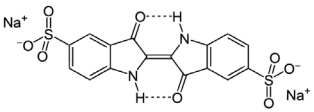
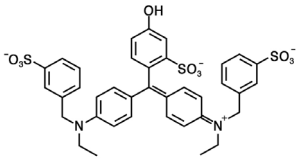
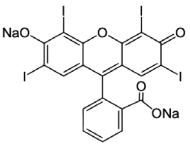
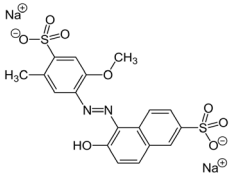
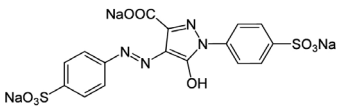
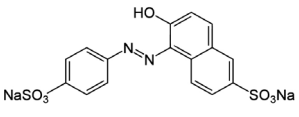
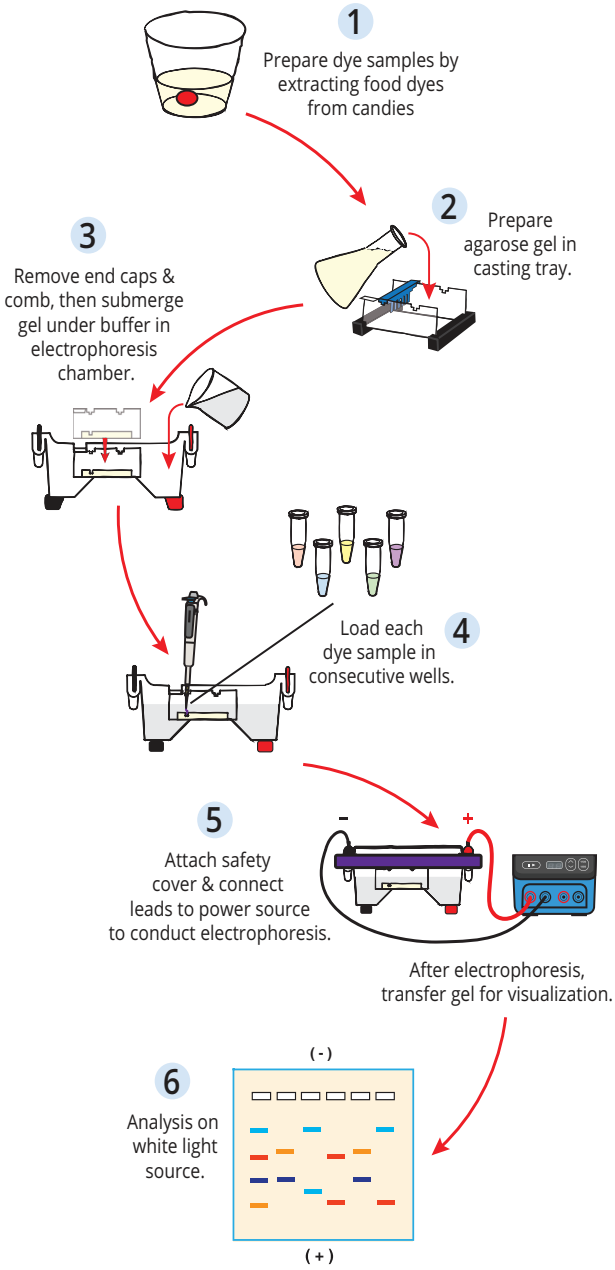


Figure 2: Overview of agarose gel electrophoresis.

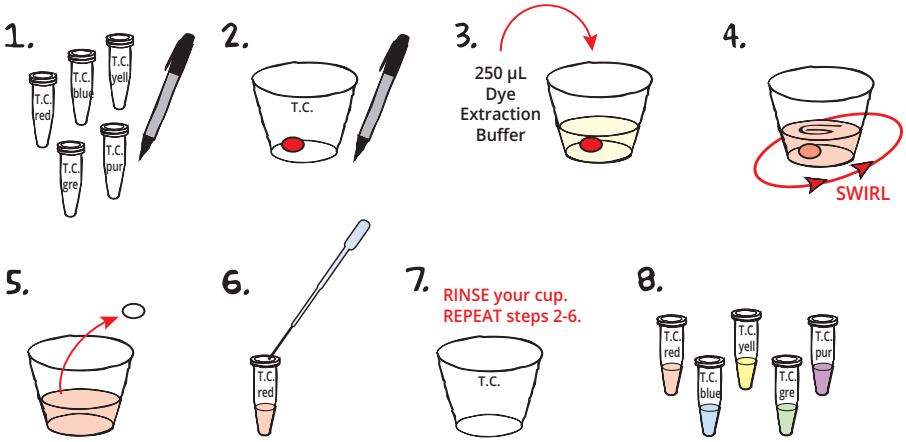
TABLE 1 – SEVEN ARTIFICIAL COLORS APPROVED BY THE FDA FOR COLORING FOOD

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	

Experiment Overview



Module I: Extraction of Food Dyes from Candy

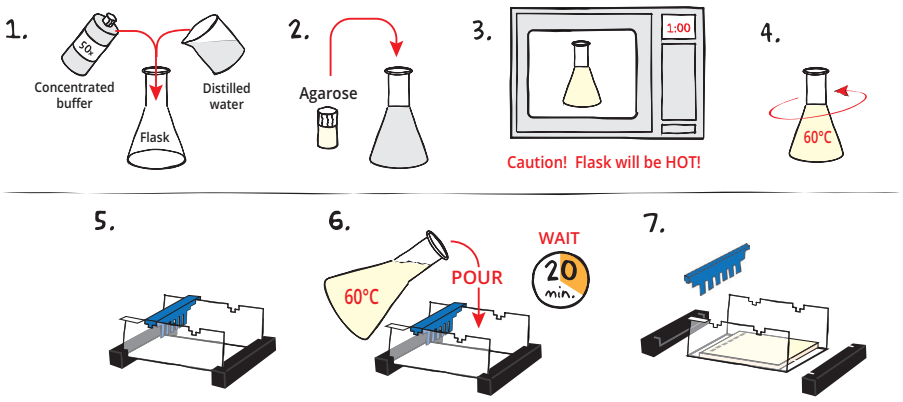


We recommend using brightly-colored candies M&M's®, Skittles®, jelly beans, & gum balls.

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** steps 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.

Module II: Agarose Gel Electrophoresis



CASTING THE AGAROSE GEL

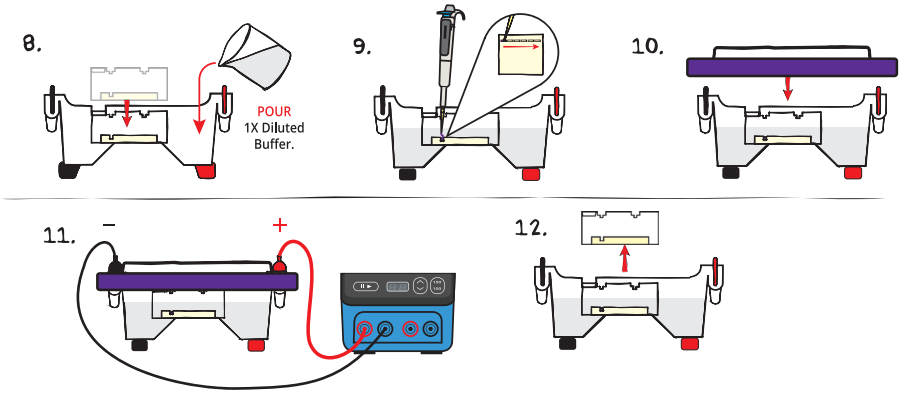
1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 mL flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **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™ Gels**

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	0.24 g	30 mL
10 x 7 cm*	0.9 mL	44.1 mL	0.36 g	45 mL
14 x 7 cm	1.2 mL	58.8 mL	0.48 g	60 mL

**Recommended gel volume for the EDGE™ Integrated Electrophoresis System (Cat. #500).*

Module II: Agarose Gel Electrophoresis, continued



RUNNING THE GEL

8. **PLACE** the gel (still on the tray) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µL) into the well in the order indicated by Table 2, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA 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). Allow the tracking dye to migrate at least 3 cm from the wells.
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
EDGE™	150 mL	3 mL	147 mL
M12	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: EDGE™	M12 & M36
Volts	Min/Max (minutes)	Min/Max (minutes)
150	10/20	20/35
125	N/A	30/45
100	15/25	40/60

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).

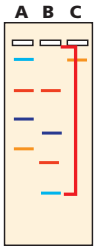


Figure 3: Measure distance migrated from the lower edge of the well to the lower edge of each band.

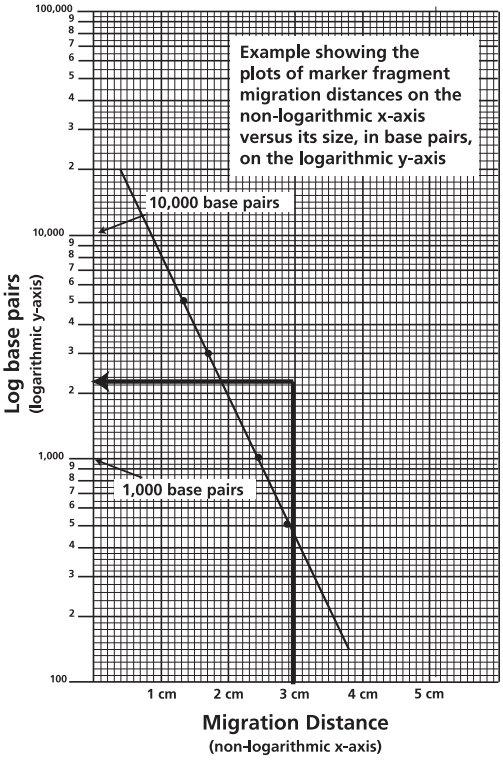
1. Measure and Record Migration Distances

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.

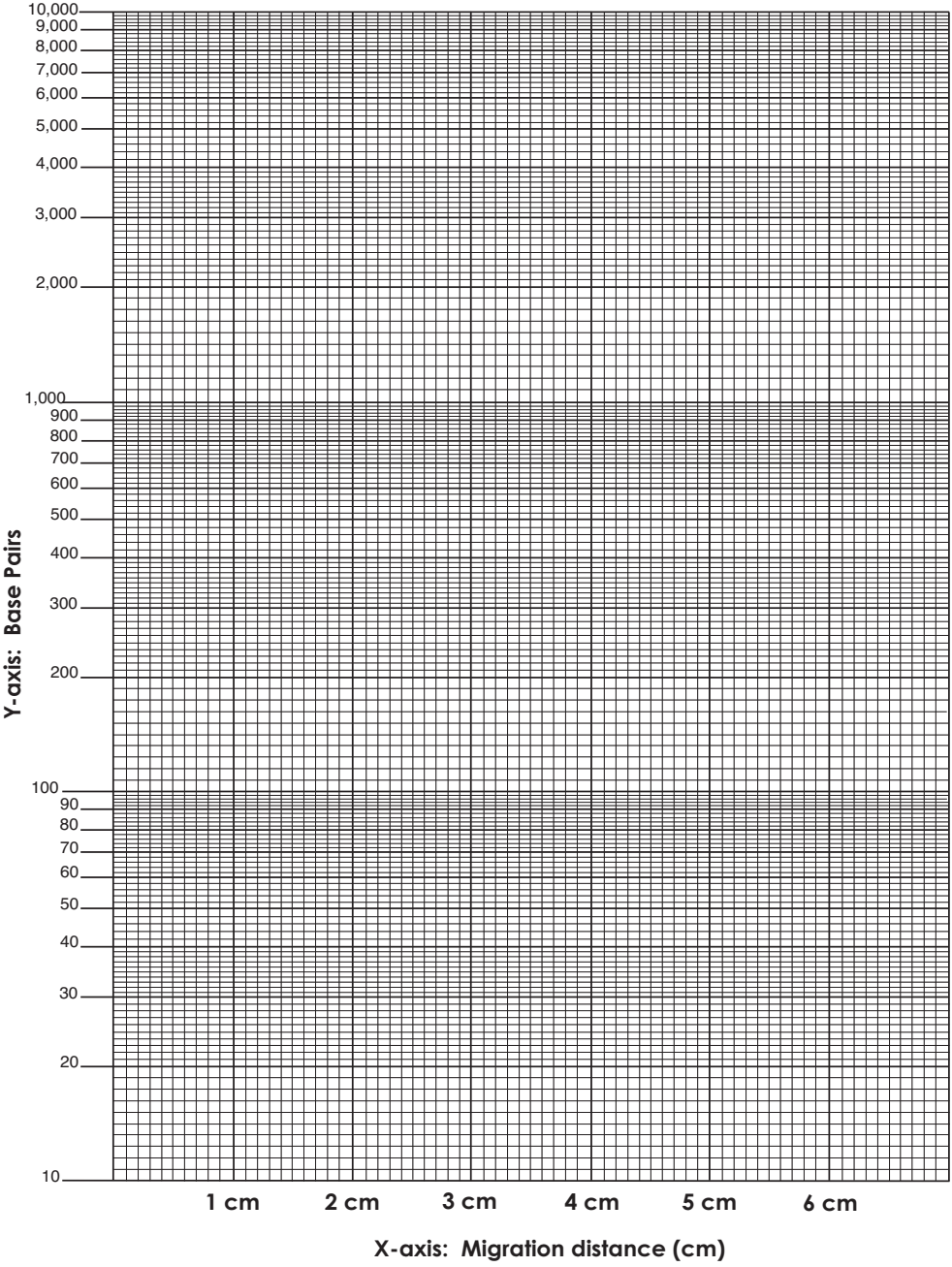
2. Generate a Standard Curve.

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!

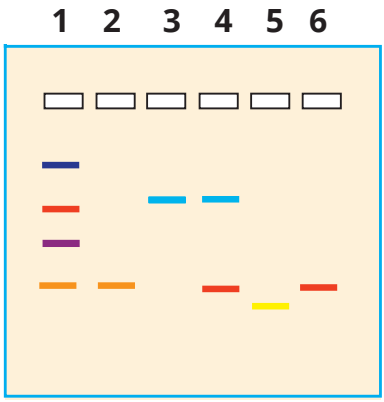


QUICK REFERENCE:
The Standard dyes have the following base pair equivalents.

Blue	5000
Red	3000
Purple	1000
Orange	500


10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)

Experimental Results and Analysis



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

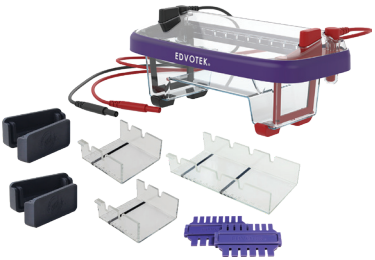
NOTE:
In the idealized schematic, the relative positions of dye fragments are shown but are not depicted to scale. No positively charged dyes are shown.

QUICK REFERENCE:

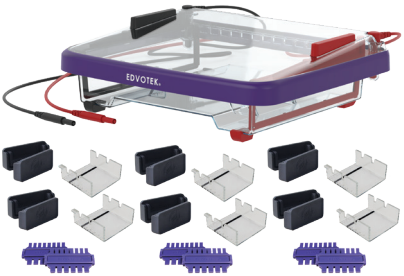
Standard Dye marker sizes - length is expressed in base pairs.

5000, 3000, 1000, 500

Related Products



M12 Complete™ Electrophoresis Package
For 1 or 2 Lab Groups
Cat# 502-504



M36 HexaGel™
For 1 to 6 Lab Groups
Cat# 515

DuoSource™
100/150 V, for 1 or 2 Units
Cat# 509

QuadraSource™
10-300 V, for 1 or 4 Units
Cat# 5010

White Light LED Transilluminator
Cat# 552

EDVOTEK® Variable Micropipette
5-50 µL Micropipette
Cat. # 590

Fixed Volume MiniPipet™
35 µL MiniPipet™
Cat. # 587-2

Linking Food Science to Biotechnology: Unlock the Color of Candies

For 10 Groups. NGSS-aligned with MS-PS1. Investigate how agarose gel electrophoresis unlocks the color code used by food scientists to make colorful candies. Students will extract color activities from common candies and separate the dyes on agarose gel electrophoresis. A fun lab extension involves the use of candy to build a DNA model.
Cat. #S-47