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EDUCATION COMPANY®

Edvo-Kit #

**S-46**

Edvo-Kit #S-46

## Linking STEM to Agarose Gel Electrophoresis

### Experiment Objective:

In this experiment, brightly colored dyes will be separated using agarose gel electrophoresis. The unique banding patterns will be analyzed using one of four different scenarios.

See page 3 for storage instructions.

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The dye samples in this experiment can be used to teach different concepts, including human genetics, disease, DNA Fingerprinting, and other basic molecular biology ideas.

**LESSON PLAN A (page 6)**

Using Dyes to Simulate a Crime Scene/DNA Fingerprinting Activity

**LESSON PLAN B (page 7)**

Using Dyes to Simulate a DNA Paternity Simulation

**LESSON PLAN C (page 8)**

Using Dyes to Simulate a GMO Detection Activity

**LESSON PLAN D (page 9)**

Using Dyes to Simulate a Cancer Gene Detection Activity

Safety Data Sheets can be found on our website: [www.edvotek.com](http://www.edvotek.com)

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## Experiment Components

### READY-TO-LOAD™ DYE SAMPLES FOR ELECTROPHORESIS

Store QuickStrip™ samples in the refrigerator immediately upon receipt.  
All other components can be stored at room temperature.

#### Components (in QuickStrip™ format)

- A Standard Dye Marker
- B Dye Sample B
- C Dye Sample C
- D Dye Sample D
- E Dye Sample E
- F Dye Sample F

#### Check (✓)

- 
- 
- 
- 
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- 

#### REAGENTS & SUPPLIES

- UltraSpec-Agarose™ Powder
- Electrophoresis Buffer (50X)
- Practice Gel Loading Solution
- 1 ml pipet
- Microtipped Transfer Pipets

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**Experiment #S-46 is designed for 10 gels.**

Store QuickStrip™ samples in the refrigerator immediately upon receipt. All other components can be stored at room temperature.

## Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet Pump
- Flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Visualization system (white light box)
- Distilled or deionized water

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

### PRINCIPLES OF GEL ELECTROPHORESIS

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. This technique possesses great resolving power, yet is relatively simple and straightforward to perform.

Agarose, a polysaccharide derived from seaweed, is commonly used to form the separation matrix used for gel electrophoresis. To make a gel, solid agarose powder is added to buffer and melted by boiling. The buffer controls the pH of the solution throughout the electrophoresis process, which is important to the charge and stability of biological molecules. Once the solution has cooled to approximately 60° C, it is poured into a gel tray to solidify. A special comb is used to form depressions in the gel called loading wells.

Once solidified, the gel is placed in a horizontal electrophoresis chamber and covered with a pH-balanced buffer. Electrodes placed at each end of the electrophoresis chamber generate current when connected to a direct current power supply. The buffer contains ions necessary to conduct the electrical current.

Samples are prepared for electrophoresis by mixing them with glycerol or sucrose, which makes them denser than the electrophoresis buffer. When the samples are loaded into the wells, the dense samples sink through the buffer and remain in the wells. An electrical current is passed through the gel to drive molecules through the gel. Generally, the higher the applied voltage, the faster the samples are separated by electrophoresis. Once the current is applied, the biomolecules in the sample are pulled into the gel matrix. At first glance, an agarose gel appears to be a solid at room temperature. On the molecular level, the gel contains small channels through which the molecules can pass. These pores act as a molecular sieve that affects the rate at which a molecule can migrate through the gel.

Factors such as the molecular charge, size and shape, together with buffer conditions, gel concentrations and voltage, can affect the mobility of molecules in a gel. For example, small DNA fragments move through these holes easily, but large DNA fragments have a more difficult time squeezing through the tunnels. Because molecules with dissimilar sizes travel at different speeds, they become separated and form discrete “bands” within the gel. Given two molecules of the same molecular weight and shape, like dyes, the one with the greater amount of charge will migrate faster. Molecules with a net negative charge migrate towards the positive electrode (anode) while net positively charged molecules migrate towards the negative electrode (cathode). Furthermore, molecules may have the same molecular weight and charge but different shapes. Molecules having a more compact shape, like a sphere, would move through the pores more quickly than molecules with a looser conformation.

### THE POLYMERASE CHAIN REACTION (PCR)

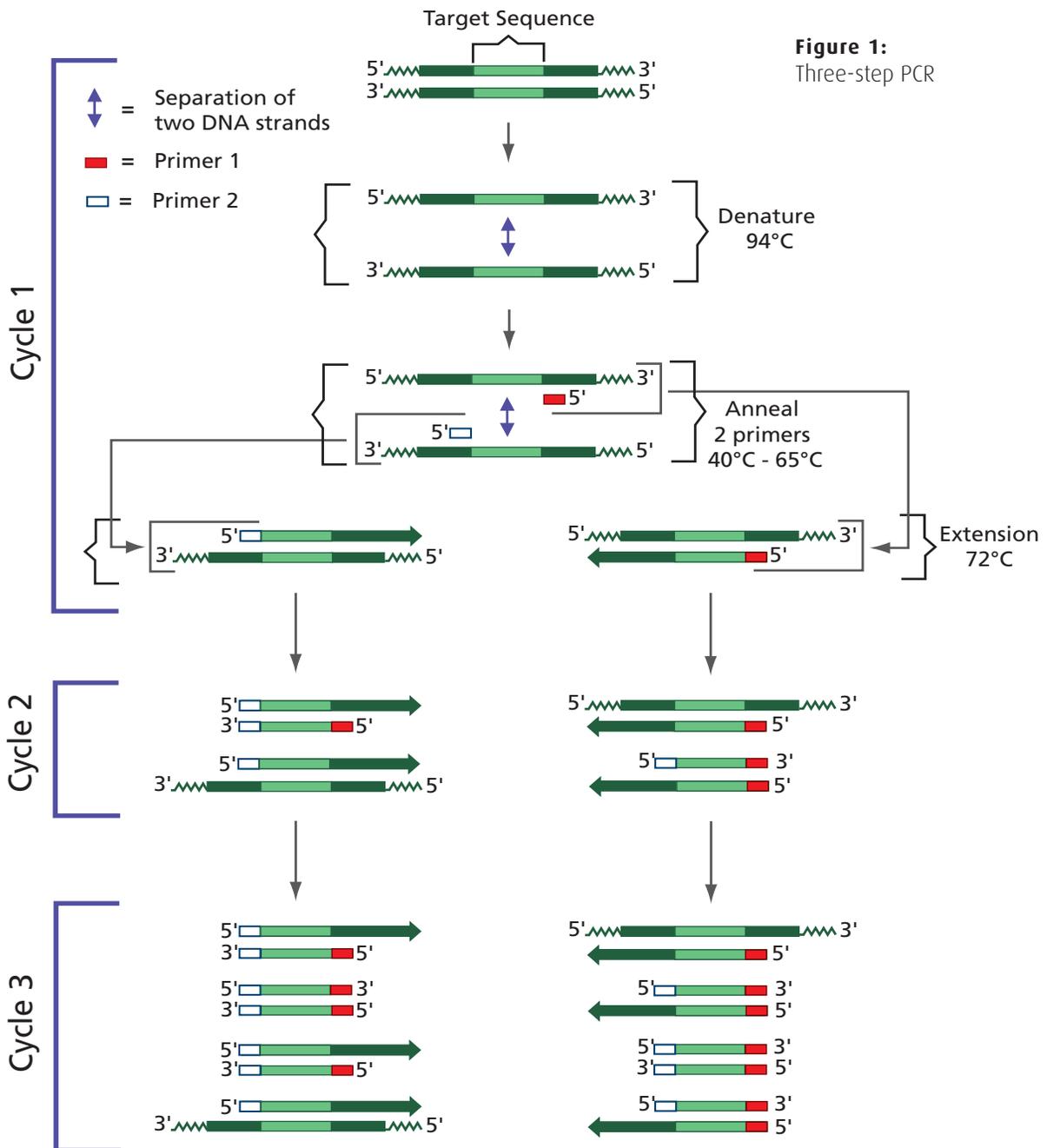
In 1984, Dr. Kary Mullis revolutionized the field of molecular biology when he devised a simple and elegant method to copy specific pieces of DNA. Recognizing that an initial step in DNA replication in a cell’s nucleus is the binding of RNA primers, Mullis discovered that he could replicate DNA in vitro using short, synthetic DNA primers and DNA polymerase I. Furthermore, because researchers can specify a primer’s sequence to target a specific gene, this method allowed for the rapid amplification of a selected DNA sequence. For the development of this technique, known today as the Polymerase Chain Reaction (or PCR), Mullis was awarded the Nobel Prize in Chemistry in 1993.

In order to amplify DNA, purified double-stranded DNA is mixed with the short DNA primers, a thermostable DNA polymerase (*Taq*) and nucleotides. The mixture is heated to 94°C to “denature” (i.e., unzip into single strands) the DNA duplex. Next, the sample is cooled to 45°C-60°C, allowing the primers to base pair with their target DNA sequences (a step known as “annealing”). Lastly, the temperature is raised again, to 72°C, the optimal temperature at which *Taq* polymerase will extend the primer to synthesize a new strand of DNA. Each cycle (denaturation, annealing, extension) doubles the amount of target DNA. Today, a specialized machine, called a “thermal cycler” or “PCR machine”, is used to rapidly heat and cool the samples. As a result, a PCR cycle can be completed in less than 5 minutes; 20-40 cycles produce sufficient DNA for analysis.



## Background Information

Because of its ease of use and its ability to rapidly amplify DNA, PCR has become indispensable in medical and life sciences labs, replacing the time-intensive Southern blot as the method of choice. For example, today's research laboratories can quickly create copies of a specific region of DNA for cloning applications. Medical diagnostics use PCR to identify genetic mutations and infectious agents. In addition, because amplification by PCR requires very little starting material, it is ideal for forensic analysis of biological samples or determination of paternity.



## Background Information

### LESSON PLAN OPTION A: USING DYES TO SIMULATE A CRIME SCENE/DNA FINGERPRINTING ACTIVITY

In this scenario, students will analyze PCR reactions obtained from different suspects and compare them to a crime scene sample.

- A Standard Dye Marker
- B Crime scene PCR reaction
- C PCR control reaction
- D Suspect 1 PCR reaction
- E Suspect 2 PCR reaction
- F Suspect 3 PCR reaction

In humans, DNA is packaged into 23 pairs of chromosomes that are inherited from an individual's biological parents. Although most of this genetic material is identical in every person, small differences, or "polymorphisms", in the DNA sequence occur throughout the genome. For example, the simplest difference is a Single Nucleotide Polymorphism (or SNP). Changes in the number and location of restriction enzyme sites result in Restriction Fragment Length Polymorphisms (or RFLPs). Short repetitive stretches of DNA at specific locations in the genome can vary in number to produce STRs (Short Tandem Repeats) and VNTRs (Variable Number of Tandem Repeats). Although most polymorphisms occur in non-coding regions of DNA, those that disrupt a gene can result in disease. Medical diagnostic tests can identify specific polymorphisms associated with disease.

Analyzing several different polymorphisms within a person's genome generates a unique DNA "fingerprint". DNA fingerprints can allow us to distinguish one individual from another. Because polymorphisms are inherited, DNA fingerprints can also be used to determine paternity/maternity (and other familial relationships). The best-known application of DNA fingerprinting is in forensic science. DNA fingerprinting techniques are utilized to interpret blood, tissue, or fluid evidence collected at accidents and crime scenes. After DNA is extracted from these samples, forensic scientists can develop a DNA fingerprint. The DNA fingerprint from a crime scene can then be compared to the DNA fingerprints of different suspects. A match provides strong evidence that the suspect was present at the crime scene.



## Background Information

### LESSON PLAN OPTION B: USING DYES TO SIMULATE A DNA PATERNITY SIMULATION

In this scenario, students will compare a child's DNA sample with his parents using the DNA fingerprinting technique.

- A Standard Dye Marker
- B PCR control reaction
- C PCR reaction from Mother DNA
- D PCR reaction from Father 1 DNA
- E PCR reaction from Father 2 DNA
- F PCR reaction from Child DNA

Paternity determination based on DNA analysis (genetic DNA fingerprinting) has become an important procedure for matching children with biological fathers and mothers. Examples of recent court cases that have utilized this procedure have included rape, incest, immigration, citizenship of children to the United States and matching of children with parents who were mismatched at birth due to hospital errors. This type of testing is also used during unrest as in nations in civil war where children are often separated from parents and subsequently reunited.

An individual's DNA is identical in each and every somatic (non-reproductive) cell. Sexual reproduction randomly combines the DNA of both parents together to create a unique blend of genetic material in a new cell, thus giving rise to a distinct person. Half of one's genetic material comes from the biological mother and the other half comes from the biological father. Paternity DNA fingerprinting compares DNA samples between the child, mother, and potential father(s) and looks for a partial match among the individuals. By comparing the genetic fingerprints between alleged parents and children, we can determine paternity and maternity conclusively using DNA testing technology. DNA testing is currently the most advanced and precise technology used to determine parentage. The majority of individuals have a single and distinct set of genes, however there are rare individuals, known as chimeras that have at least two different sets of genes. There have been several cases of DNA profiling that have incorrectly shown that the actual mother was unrelated to her children.

## Background Information

### LESSON PLAN OPTION C: USING DYES TO SIMULATE A GMO DETECTION ACTIVITY

In this scenario, your students will determine if any genetically engineered genes are present in foodstuffs.

- A Standard Dye Marker
- B GMO + Control
- C GMO - Control
- D Supposed organic soy
- E Supposed organic corn
- F Supposed organic wheat

A goal of plant genetics is the development of plants that yield optimum product and have selective advantages. With the advent of biotechnology, cloning and expression of genes in GM plants have increased yields, nutritional value and enhanced quality. Plant biotechnology today offers the possibilities of modification, enhancement or suppression of gene products.

In the last half of the century, the world population more than doubled however agriculture only increased by 10%. In the same time frame, world food production per person increased by 25% due to advances in agriculture due to mechanization and biotechnology. For example, in 2002, 74% (80 million acres) of American soybeans were obtained from genetically-modified crops. The benefits of food production have not been equally distributed amongst the world population with the U.S. being both the largest producer and consumer of food.

### Approaches To Plant Biotechnology

Introduction of specific genes through biotechnology can provide advantages. As an example, a genetically modified (GM) plant can protect itself against parasites after the introduction of the endotoxin gene. Golden rice is an example of a GM crop that synthesizes a high value bioproduct. Plants can also be modified to inhibit the expression of specific genes that are involved in the ripening of fruits by maintaining and enhancing fruit flavors and extending their shelf life. The responsibility of public health and policy concerning agro-biotechnology rests on the shoulders of both the public and the biotechnology industry. It remains to be seen what long term effects altered plants will have on the ecosystem and overall biodiversity.

In order to manipulate and insert desirable genes into plants, scientists use a promoter (often 35S CMV) and a terminator (usually NOS terminator). The promoter initiates transcription and the terminator ends transcription. The 35S CMV promoter is chosen because it is already designed by nature to be highly transcribed in all plant cell types. The presence of 35S CMV and/or NOS terminator is a good indicator of a genetic modification. Since the presence of 35S CMV and/or NOS terminator are good indicators of a genetic modification, we can use PCR primers specific to these regions to provide strong evidence that a plant or food has been genetically modified.



## Background Information

### LESSON PLAN OPTION D: USING DYES TO SIMULATE A CANCER GENE DETECTION ACTIVITY

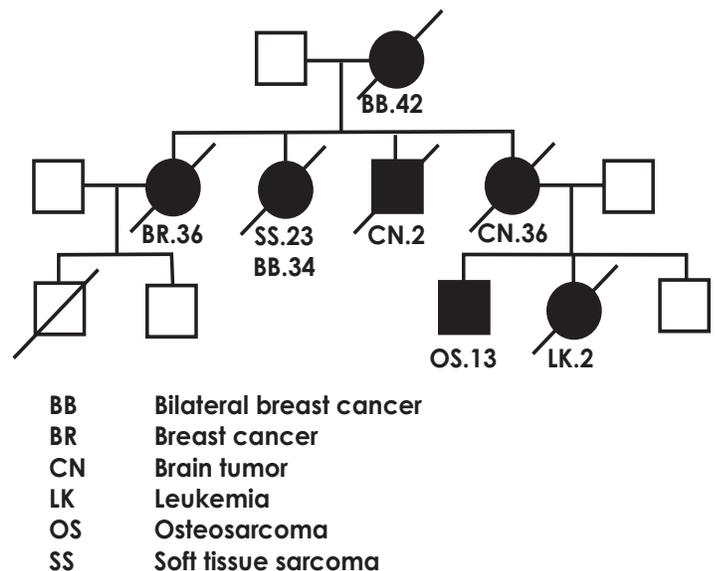
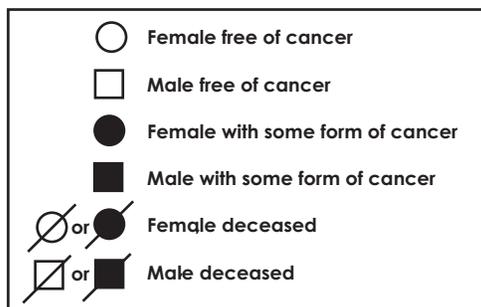
In this scenario, your students will diagnose the state of the p53 gene in individual family members.

- A Standard Dye Marker
- B "+" Cancer gene control
- C Patient 1 Tumor DNA
- D Patient 2 Tumor DNA
- E Patient 3 Tumor DNA
- F Patient 4 Tumor DNA

The study of inherited cancers has given molecular biologists the opportunity to search for genes that are critical for normal cell development and carcinogenesis. At the molecular level, cancer formation is characterized by alterations in both dominant oncogenes and tumor suppressor genes, such as p53. Suppressors are normal cellular proteins that are involved in limiting cell growth. By contrast, oncogenes are involved in promoting the growth of cells.

In recent years, the tumor suppressor protein, p53 has become the center of many cancer biology studies. Because it appears to be of major significance, there is great impetus to study how this gene functions in normal cells compared to cancer cells. The gene for p53 is a 53kDa nuclear phosphoprotein that functions as a transcription factor. It is located on the short arm of chromosome 17. Wild type (normal) p53 functions as a cell regulator. There is now well-documented evidence that normal p53 is a sequence specific DNA-binding protein that is a transcriptional regulator. When p53 is mutated, it loses its ability to bind to DNA. Mutations of p53 in specific hot spots promote uncontrolled cell growth and therefore function as oncogenes. For a tumor suppressor gene such as p53 to play a role in transformation in cancer, both alleles need to be altered, as shown the figure below.

**Figure 2:**  
Example of a p53 Family Pedigree



## Experiment Overview

### EXPERIMENT OBJECTIVE:

In this experiment, brightly colored dyes will be separated using agarose gel electrophoresis. The unique banding patterns will be analyzed using one of four different scenarios.

**LESSON PLAN A:** Using Dyes to Simulate a Crime Scene/DNA Fingerprinting Activity

**LESSON PLAN B:** Using Dyes to Simulate a DNA Paternity Simulation

**LESSON PLAN C:** Using Dyes to Simulate a GMO Detection Activity

**LESSON PLAN D:** Using Dyes to Simulate a Cancer Gene Detection Activity

### 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.



### 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.

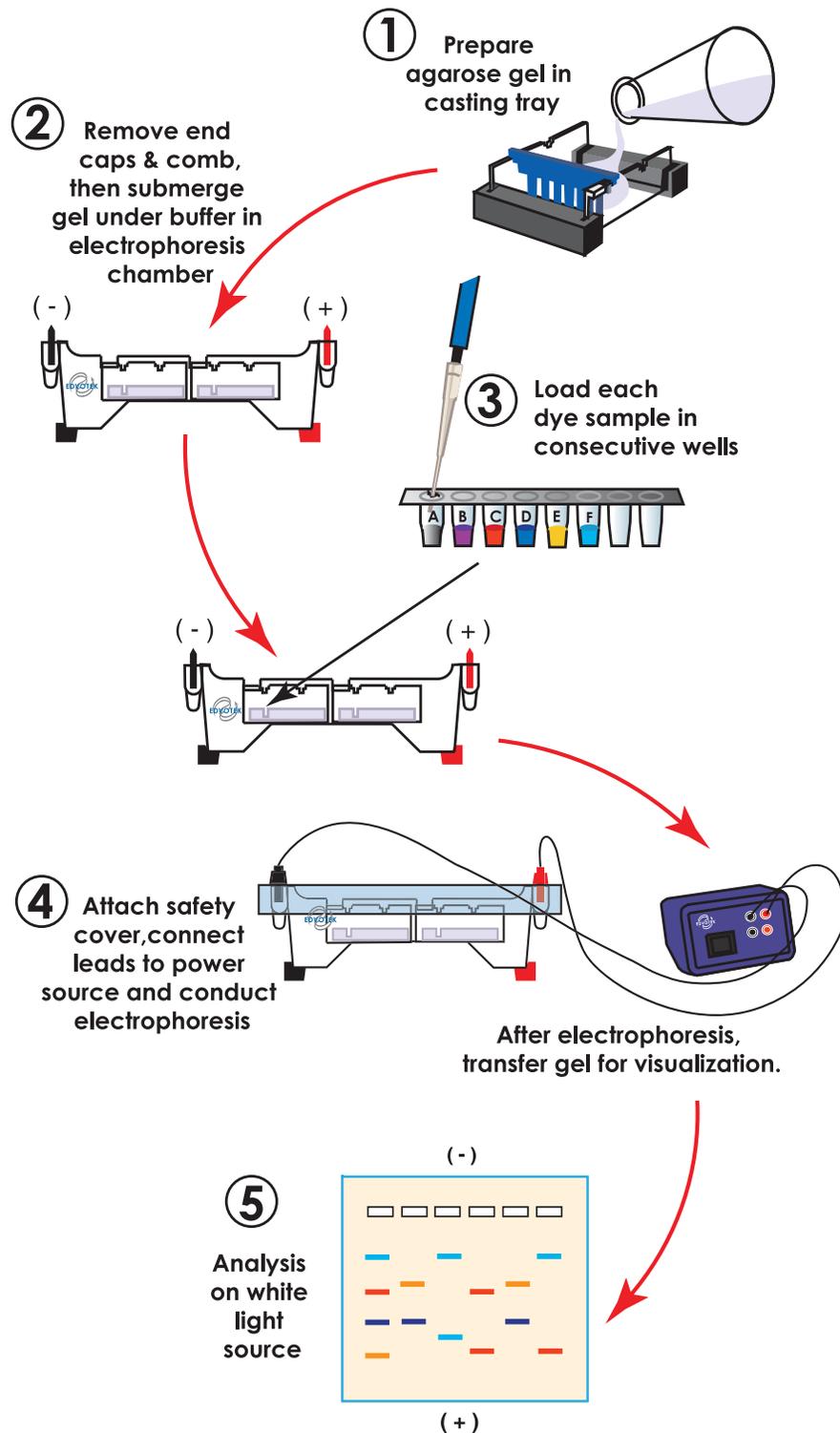
#### During the Experiment:

- Record your observations.

#### After the Experiment:

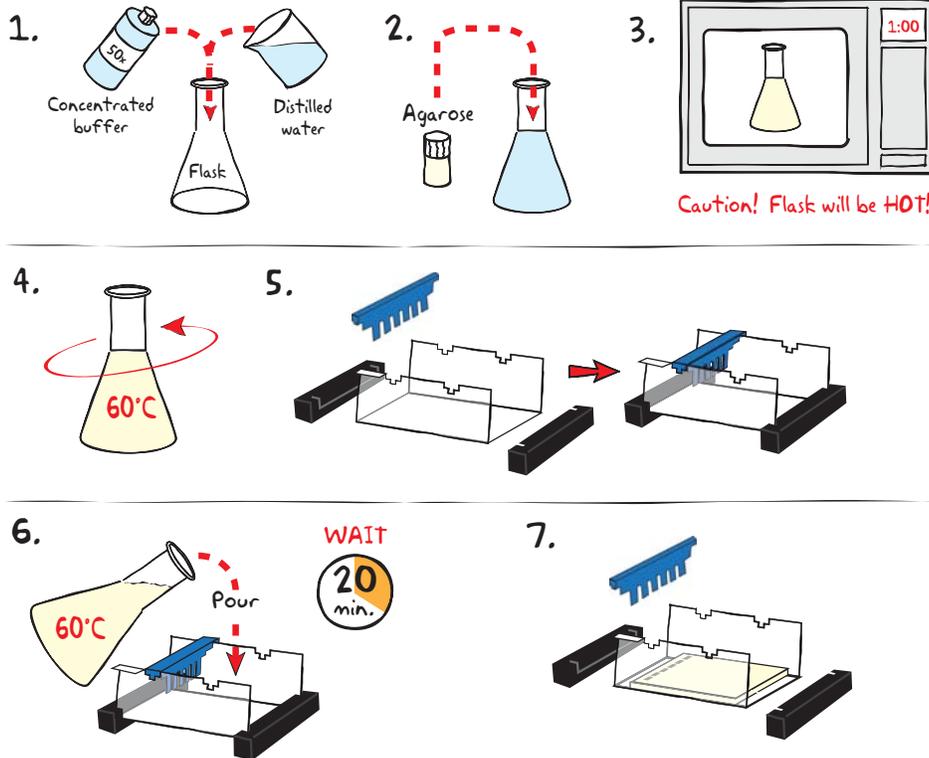
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

## Experiment Overview



Gel pattern will vary depending upon the experiment.

# 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](http://www.edvotek.com)



Wear gloves and safety goggles

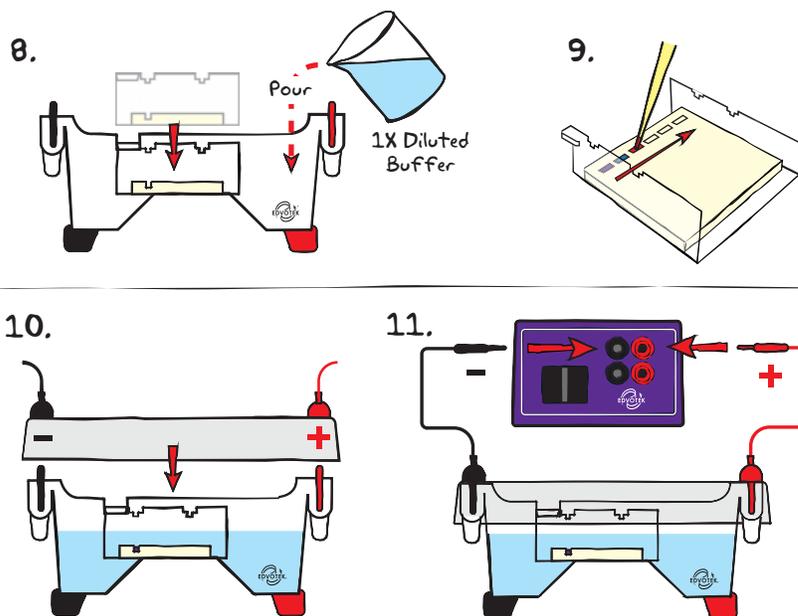
- DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
- MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
- DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from 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).
- COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
- 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.
- 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.
- 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

## Agarose Gel Electrophoresis

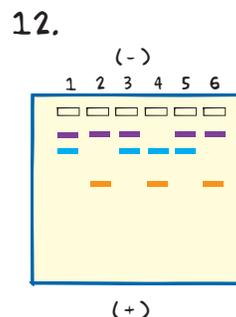


### Reminders:

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. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35-38  $\mu$ L) into the well in consecutive order. The identity of each sample is provided in Table 1.
10. **PLACE** safety cover. **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).
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and **VISUALIZE** the results. No staining is necessary.



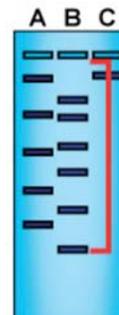
Lane	Sample	Contents
1	Tube A	Standard Dye Marker
2	Tube B	Dye Sample B
3	Tube C	Dye Sample C
4	Tube D	Dye Sample D
5	Tube E	Dye Sample E
6	Tube F	Dye Sample F

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

Volts	Electrophoresis Model		
	M6+	M12 (new)	M12 (classic) & M36
	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.

# STEM Activity

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 length. 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.

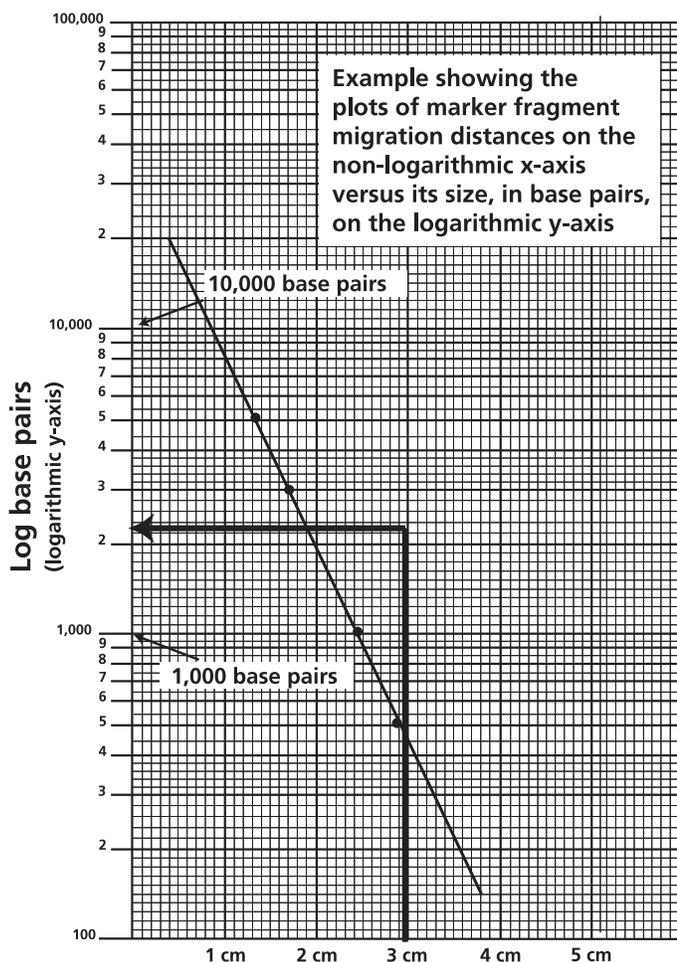
## 1. Measure and Record Migration Distances

Measure the distance traveled by each Standard Dye Fragment 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 fragment migrated on the x-axis (in mm) versus its size on the y-axis (in base pairs). Be sure to label the axes!



**Figure 4:** Semilog graph example

**Migration Distance**  
(non-logarithmic x-axis)

## STEM Activity

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.

- Locate the migration distance of the unknown fragment 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.
- 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 fragment in base pairs (refer to Figure 4 for an example). Make note of this in your lab note book.
- Repeat for each fragment in your unknown sample.

#### Quick Reference:

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

5000, 3000, 1000, 500

## STEM Highlights

### SCIENCE

- Polymerase Chain Reaction
- DNA Fingerprinting
- Paternity Determination
- GMO Identification
- Cancer Gene Detection

### TECHNOLOGY

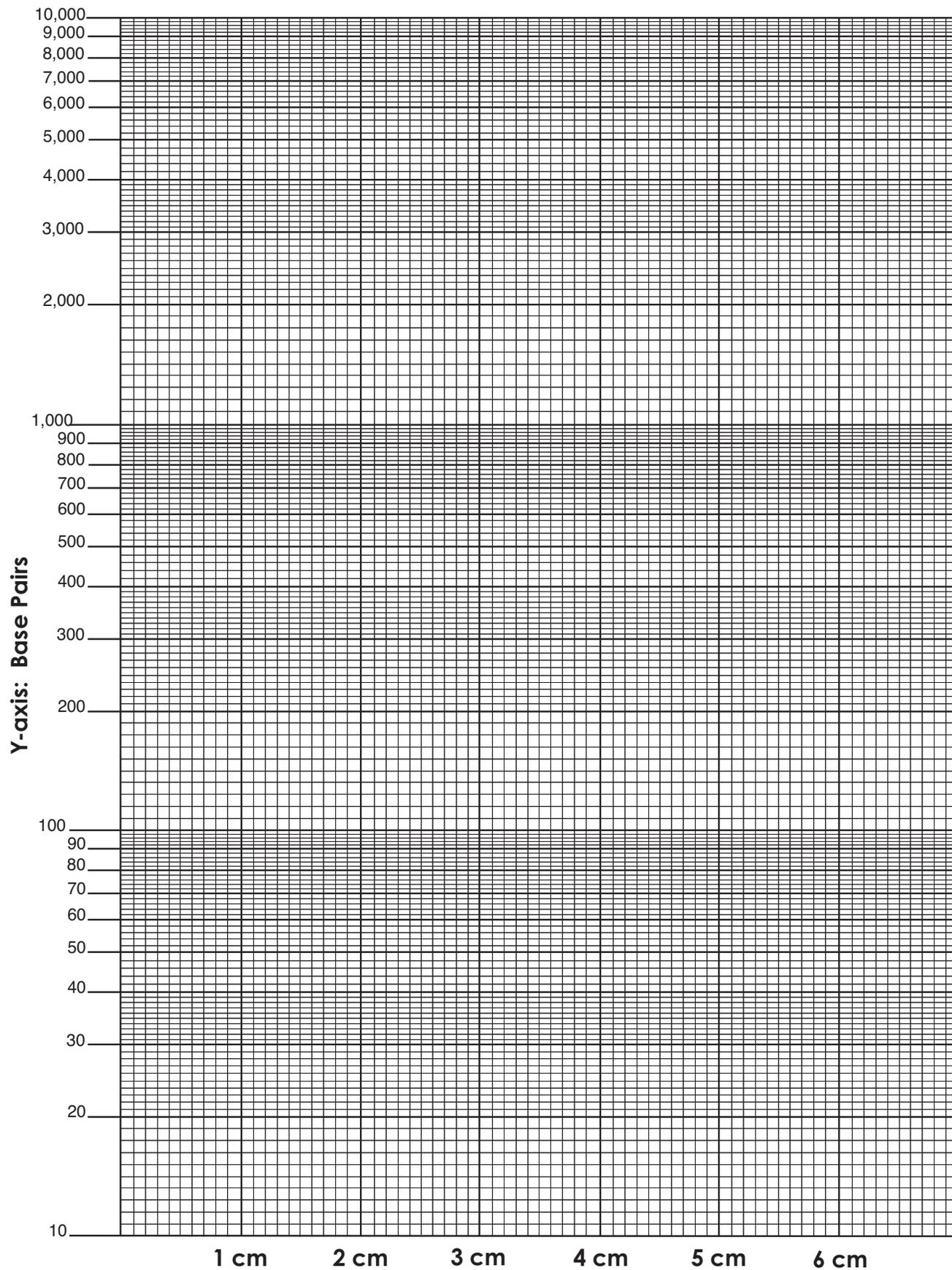
- Separation of DNA fragments by size (and dyes by charge)
- Pipetting

### ENGINEERING

- Making agarose gels
- Building electrophoresis apparatus
- Generating a current with a power supply.

### MATHEMATICS

- Making measurements.
- Plotting data using a log scale.
- Using a standard curve to extrapolate molecular weights



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## Study Questions

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1. 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?
2. What is an STR? A VNTR? How are they used in law enforcement?
3. Describe how DNA Fingerprinting can be used to determine who is the biological parent of a child.
4. What are the key benefits to creating and distributing Genetically Modified Foods? What are some of the concerns?
5. What is the difference between tumor suppressors and oncogenes? How do mutations in tumor suppressor genes like p53 cause cancer?

# 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.

What to do:	When:	Time Required:
Prepare QuickStrips™	Up to one day before performing the experiment.	40 min.
Prepare diluted TAE buffer		
Prepare molten agarose and pour gel		

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## Pre-Lab Preparations:

### SEPARATION OF PCR PRODUCTS BY AGAROSE GEL ELECTROPHORESIS

This experiment requires a 0.8% agarose gel per student group. 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 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 stored under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at  $-20^{\circ}\text{C}$  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.

### SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

QuickStrip™ tubes consist of a microtiter block covered with a protective overlay. Each well contains pre-aliquoted dyes.

Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.



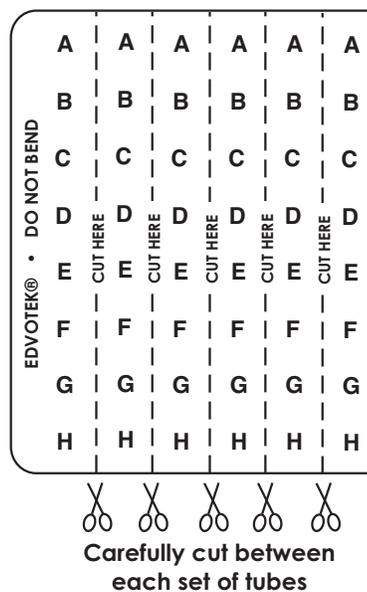
#### NOTE:

Accurate pipetting is critical for maximizing successful experiment results.

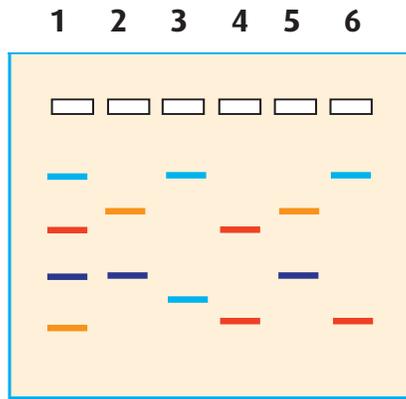
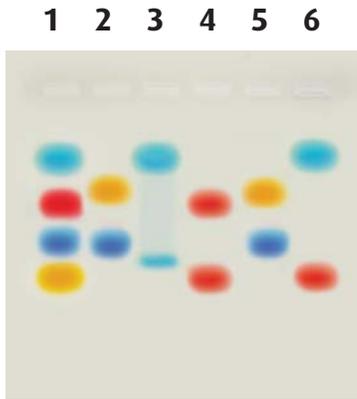
If students are unfamiliar with using micropipets, we recommend performing the optional activity found in Appendix C, Practice Gel Loading, prior to conducting the experiment.

#### Each Student Group should receive:

- 50x concentrated buffer
- Distilled Water
- UltraSpec-Agarose™
- Ready-to-Load™ Samples



## Experiment Results and Analysis



Lane	Tube	Sizes (in base pairs)
1	A	5000, 3000, 1000, 500
2	B	3250, 1000
3	C	5000, 850
4	D	3000, 550
5	E	3250, 1000
6	F	5000, 550

**NOTE:** This technique has a +/- 10-15% margin of error.

In the idealized schematic, the relative positions of dye fragments are shown but are not depicted to scale.

### Quick Reference:

Standard Dye Marker sizes - length is expressed in base pairs. 5000, 3000, 1000, 500

### LESSON PLAN OPTION A: CRIME SCENE/DNA FINGERPRINTING ACTIVITY

The results of the DNA fingerprinting experiment show an identical banding pattern between lanes 2 and 5. This is strong evidence that suspect 2 was at the crime scene.

Lane	Tube	Sample
1	A	Standard Dye Marker
2	B	Crime scene PCR reaction
3	C	PCR control reaction
4	D	Suspect 1 PCR reaction
5	E	Suspect 2 PCR reaction
6	F	Suspect 3 PCR reaction

### LESSON PLAN OPTION B: DNA PATERNITY SIMULATION

The low molecular weight band (red) in the child's DNA profile is not present in the mother's DNA profile, meaning that it must be contributed by the father. This band is only found in Father 1, suggesting he is the father.

Lane	Tube	Sample
1	A	Standard Dye Marker
2	B	PCR control reaction
3	C	PCR reaction from Mother DNA
4	D	PCR reaction from Father 1 DNA
5	E	PCR reaction from Father 2 DNA
6	F	PCR reaction from Child DNA

### LESSON PLAN OPTION C: GMO DETECTION ACTIVITY

In this experiment, the banding pattern produced by the supposed organic corn is identical to the banding pattern produced by the positive control. This suggests that the corn has been genetically modified.

Lane	Tube	Sample
1	A	Standard Dye Marker
2	B	GMO + Control
3	C	GMO - Control
4	D	Supposed organic soy
5	E	Supposed organic corn
6	F	Supposed organic wheat

### LESSON PLAN OPTION D: CANCER GENE DETECTION ACTIVITY

DNA from patients with a mutated form of p53 will match the banding pattern present in the positive control sample (lane 2). In this case, patient 3's results match the positive control, suggesting that their cancer is related to p53 mutations.

Lane	Tube	Sample
1	A	Standard Dye Marker
2	B	"+" Cancer gene control
3	C	Patient 1 Tumor DNA
4	D	Patient 2 Tumor DNA
5	E	Patient 3 Tumor DNA
6	F	Patient 4 Tumor DNA

**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](http://www.edvotek.com)

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## Appendix A

### EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
<b>Bands not visible on the gel</b>	The electrophoresis buffer was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The dyes ran off of the gel because the polarity of the leads was reversed.	Ensure that leads are attached in the correct orientation.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
<b>Very light colored band seen after electrophoresis</b>	Pipetting error.	Make sure students pipet 35 µl of dye sample per well.
<b>Poor separation of bands</b>	Gel was not prepared properly.	Make sure to prepare a 0.8% gel.
<b>Dye bands disappear when the gels are kept at 4° C.</b>	The dye molecules are small and will diffuse out of the gel.	The results must be analyzed upon the completion of electrophoresis

## 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.

- Use a 500 ml flask to prepare the diluted gel buffer
- Pour 3.0 grams of UltraSpec-Agarose™ into the prepared buffer. Swirl to disperse clumps.
- With a marking pen, indicate the level of solution volume on the outside of the flask.
- 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.
- 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.
- 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.
- 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.

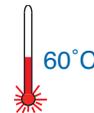


Table E Batch Prep of 0.8% UltraSpec-Agarose™					
Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390

## 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.

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:**

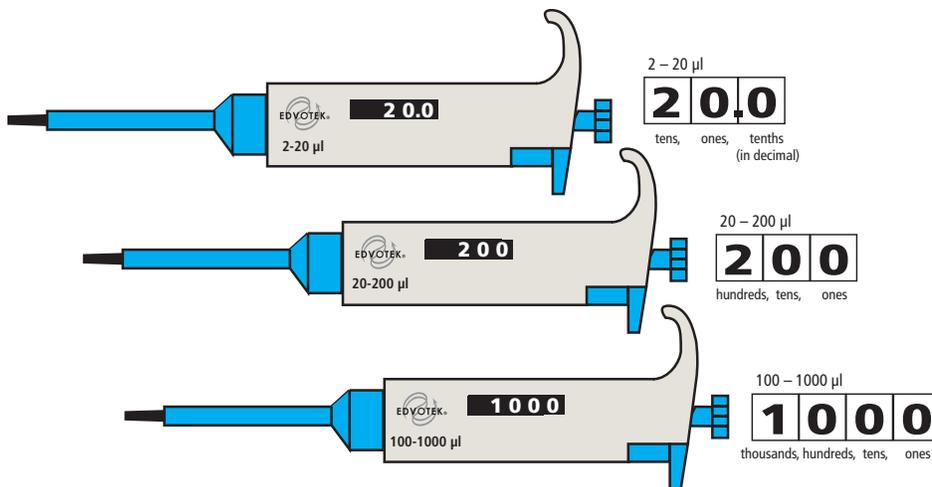
The agarose gel is sometimes called a "submarine gel" because it is submerged under buffer for sample loading and electrophoretic separation.

**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.

## Appendix C

### Practice Gel Loading

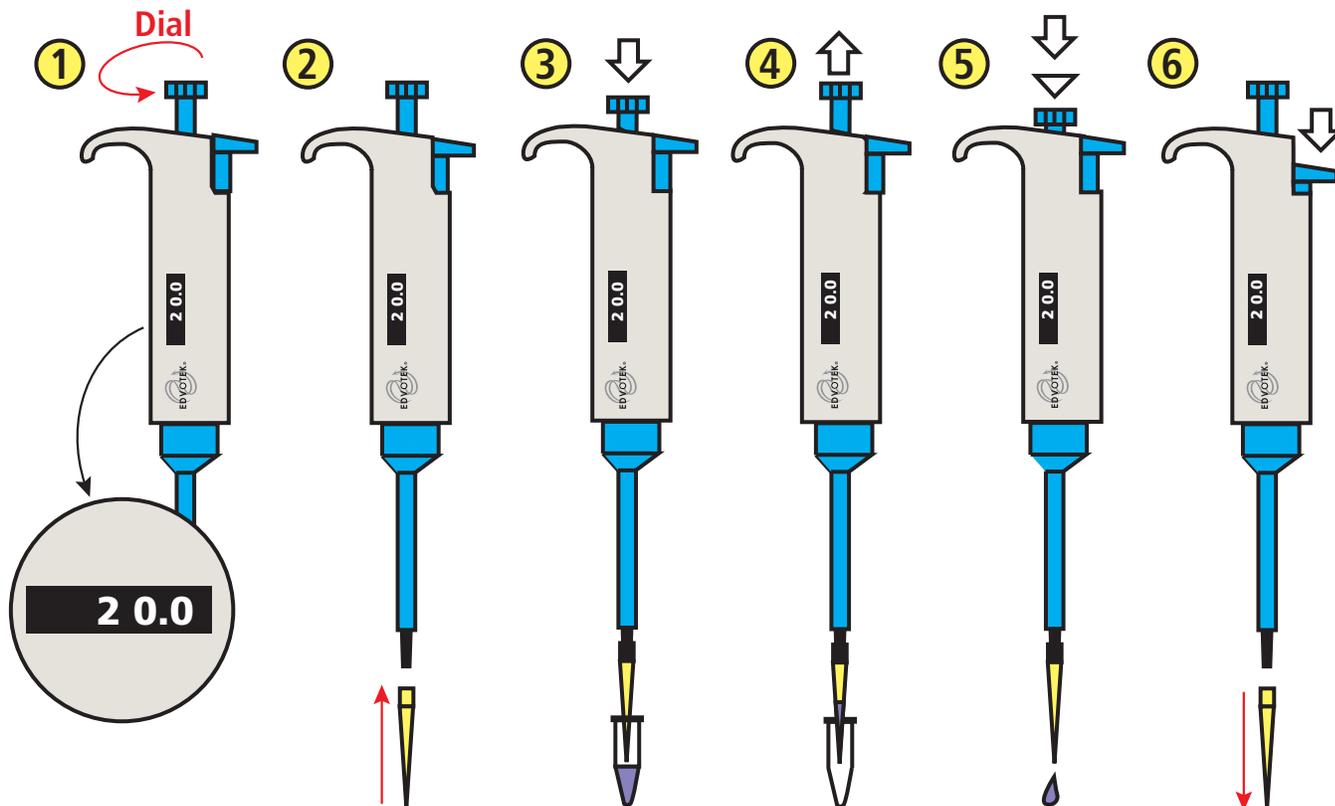


#### SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

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.

## Appendix C

### Practice Gel Loading



#### MEASURING LIQUIDS WITH A MICROPIPET

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.

