EDVOTEK®

THE BIOTECHNOLOGY EDUCATION COMPANY®

Edvo-Kit #

S-52

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The Secret of the Invisible DNA: A Genetics Exploration

Experiment Objective:

In this experiment, students will explore agarose gel electrophoresis by separating fluorescent dyes. The unique patterns will be analyzed using one of two scenarios that simulate DNA testing.

See page 3 for storage instructions.

REVISED & UPDATED

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

Experiment Components

READY-TO-LOAD™ 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)		Check (√)
A	Standard Dye Fragments	❑
B	Experimental Sample 1	❑
C	Experimental Sample 2	❑
D	Experimental Sample 3	❑
E	Experimental Sample 4	❑
F	Experimental Sample 5	❑

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ ❑
- 50x Electrophoresis Buffer ❑
- Practice Gel Loading Solution ❑
- 1 ml Pipet ❑
- Microtipped Transfer Pipets ❑

Experiment #S-52 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 (optional)
- Balance
- Microwave, hot plate or burner
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Long wave UV light source (black light)
- UV Safety goggles
- 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.

Background Information

PRINCIPLES OF GEL ELECTROPHORESIS

Agarose gel electrophoresis is a common laboratory technique 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 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 influences 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 following factors affect the mobility of molecules through a gel:

1. Molecular size: On the molecular level, the gel contains small channels that act as a molecular sieve. Small molecules move through these holes easily, but larger ones have a more difficult time squeezing through the tunnels.

2. Gel concentration: The final concentration of agarose in a gel will change the size of the channels within the gel. Lower percent gels will have larger channels, making it easier to separate large molecules. Higher gel concentrations will have smaller channels, making it easier to separate small molecules.

3. Molecular charge: Given two molecules of similar size 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 those with a net positive charge migrate towards the negative electrode (cathode).

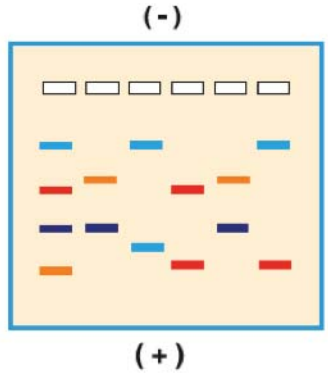


Figure 1: Separation of Dyes by Agarose Gel Elctrophoresis

4. Molecular shape: Molecules with a more compact shape, like a sphere, move through the gel more quickly than those with a looser conformation.

Because molecules with different properties travel at different speeds, they become separated and form discrete "bands" within the gel (Figure 1).

THE LAWS OF INHERITANCE

In the mid-1800's, Augustine monk Gregor Mendel established basic laws of genetics through careful experimentation using garden peas. Mendel started with several true-breeding varieties of pea plants. Each plant had a different combination of observable characteristics known as traits. He crossed different strains of plants to one another and observed the offspring over several generations. After analyzing the data, Mendel developed a model to describe the inheritance of these characteristics.

Mendel's first law, the law of segregation, stated that alternative forms of the same gene, called alleles, controlled the differences in the pea plants. Each offspring has two copies of the gene, one inherited from each parent. Next, he realized that alleles are dominant or recessive. When a dominant allele is inherited, it will mask the trait coded by the recessive allele. To impart the recessive trait, both alleles must be the recessive type. The second law of inheritance, the law of independent assortment, states that each pair of alleles will segregate separately from one another.

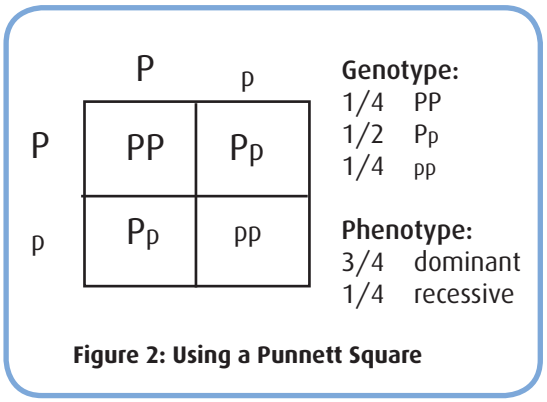


Figure 2: Using a Punnett Square

Inheritance of a single gene can be illustrated with a two-by-two grid known as a Punnett Square (Figure 2). The alleles carried by one parent are placed across the top of the grid (columns), and the alleles contributed by the other parent are placed down the side of the grid (rows). By convention, the dominant allele is denoted by an upper-case letter P and the recessive allele by a lower-case letter p. Next, the parental alleles are used to fill in the grid. Each box in the grid is assigned the allele at the head of its column and row. For example, assuming each parent carries one dominant allele and one recessive allele, the Punnett Square predicts that ¼ of the plants will receive two dominant alleles, ½ of the plants will receive one dominant and one recessive allele, and ¼ will receive two recessive alleles. This represents the genetic makeup, or genotype, of the offspring. If an individual has two of the same alleles, either recessive or dominant, they are homozygous for that trait. If an individual has one dominant and one recessive allele, the individual is heterozygous for that trait. The genotypes of the offspring determine their phenotype, or observable traits.

Mendel's laws apply to more than just plants – today, we know that these basic laws of inheritance apply to humans and other organisms as well! For example, single genes can control the appearance of the fruit fly *Drosophila melanogaster,* including wing shape and size, eye color, and body color. Different alleles of a gene can cause a wide variety of illnesses. In humans, recessive genetic disorders include cystic fibrosis, sickle cell anemia, Phenylketonuria, and many types of cancer. Dominant disorders include Huntington's Disease and Familial Hypercholesterolemia.

Table 1: Human Blood Types

Blood Type	Antigen on Red Blood Cells	Antibody in Serum
A	A	anti-B
B	B	anti-A
AB	both A & B	neither anti-A nor anti-B
O	neither A nor B	both anti-A and anti-B

Scientists have discovered some exceptions to Mendel's basic laws of inheritance. For example, certain genes may have more than two alleles. In humans, an antigen on the surface of red blood cells determines blood types. The gene that codes for this antigen has three different alleles: I^A, I^B, and i. I^A produces the A antigen, I^B produces the B antigen, and i does not produce an antigen. An individual's blood type is dependent upon the combination of these alleles (Table 1). I^A and I^B are codominant, meaning that both traits can be expressed at the same time in a heterozygous person. Both I^A and I^B are dominant to i. This means that a person who presents the A antigen (I^A I^A or I^Ai) on their red blood cells is considered to be Type A. Likewise, a person who presents the B antigen (I^B I^B or I^Bi) on their red blood cells is considered to be Type B. An AB individual (I^A I^B) has both antigens on the surface of their cells, and an O individual (ii) has no antigens on the surface of their cells.

As we learn more about the genes that control different phenotypes, we can analyze various genetic traits using DNA analysis. Specific genes are amplified using the Polymerase Chain Reaction (PCR), a biotechnology technique that allows researchers to quickly create many copies of a specific region of DNA *in vitro*. The samples are then separated using agarose gel electrophoresis.

In this electrophoresis experiment, mixtures of UV-reactive dyes represent DNA fragments of different sizes. The results of the experiment will be clearly visible upon exposure to long wave UV light. By using dyes to simulate DNA fragments, we have eliminated post-electrophoresis staining, saving you valuable classroom time. The unique banding patterns will be analyzed using one of two different scenarios simulating genetic testing (see pages 7 and 8).

SCENARIO ONE: The Genetics of Blood Type

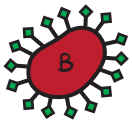
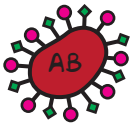
The surface antigens found on red blood cells (A and B) are carbohydrates that resemble those found on the surface of bacteria. The human body does not create antibodies when exposed to bacteria that present the same antigen as the blood cells, since the immune system will not create antibodies against its own cells. However, after exposure to bacteria with a different antigen, the human body will create antibodies to these carbohydrates. This means that a person with type A blood will produce antibodies to the B antigen. Likewise, a person with type B blood will produce antibodies to the A antigen. An AB individual will not have anti-A or anti-B antibodies, and an O will have both antibodies (See Figure 3).

The presence of these antibodies in the blood supply presents a problem for hospitals and blood banks. For example, if a person with type A blood receives a transfusion from a type B blood donor, the anti-B antibodies present in the bloodstream will recognize the B antigen on the surface of the red blood cells as foreign. The patient's immune system would mount an immune response against the foreign blood. This causes the Acute Hemolytic Transfusion Reaction (AHTR), which can result in fever, hemolysis (destruction of blood cells), kidney failure, and death.

As a medical diagnostician, you are in the laboratory determining the blood types of five different samples that are going into the blood bank. After PCR amplification, the samples are analyzed by agarose gel electrophoresis. The largest molecular weight band (red) represents the I^A allele, the mid-sized band (blue) represents the I^B band, and the smallest band (green) represents the i allele.

Proceed to Agarose Gel Electrophoresis procedures, beginning on page 9. Then, answer the following Study Questions.

Figure 3: Blood Types

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A & Anti-B
Antibodies in Red Blood Cell	 A antigen	 B antigen	 A & B antigens	None

STUDY QUESTIONS

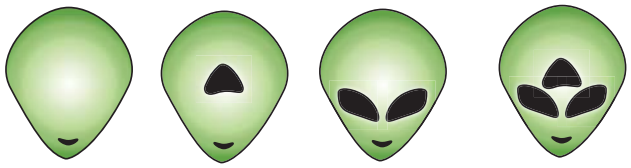
1. List and describe the factors that influence separation of molecules by agarose gel electrophoresis.
2. What are the genotypes and the phenotypes for all five patients?
3. A person with type AB blood is admitted to the hospital with a serious injury. A blood transfusion is necessary. What types of blood can be used for transfusion?

SCENARIO TWO: Martian Genetics

In the not-so-distant future, scientists discovered an alien race living on Mars. The scientists noticed that the aliens had different combinations of eyes – eyeless, one, two, or three eyes. In collaboration with the aliens, the scientists were able to decipher the genetic code and to identify the gene responsible for eye number. The trait was linked back to a gene they called "eyeless". Three alleles of eyeless were discovered -- E^1, E^2, and e. Studies show that E^1 is responsible for the one eye phenotype, E^2 for two eyes, and e is for the eyeless phenotype.

As a scientist working on Mars, you have been tasked with analyzing the eyeless gene. Three different alleles of the gene have been identified using PCR. However, you are not sure which band on the electrophoresis gel represents each individual allele. Using the phenotypes below and the results from your electrophoresis experiment to determine which band represents each of the three alleles.

Lane 1 – Control sample -- all three gene forms
Lane 2 – two eyes
Lane 3 – three eyes
Lane 4 – one eye
Lane 5 – one eye
Lane 6 – eyeless



Phenotype	Eyeless	One Eye	Two Eyes	Three Eyes
Possible genotype	ee	E^1e, E^1E^1	E^2e, E^2E^2	E^1E^2

Proceed to Agarose Gel Electrophoresis procedures, beginning on page 9. Then, answer the following Study Questions.

STUDY QUESTIONS

1. List and describe the factors that influence separation of molecules by agarose gel electrophoresis.

2. After performing the analysis, which band represents each gene form? How do you know this?

3. An E^1e alien mated with an E^1E^2 alien. Create a Punnett square to predict the genotypes and phenotypes of their offspring.

Experiment Overview

EXPERIMENT OBJECTIVE

In this experiment, students will explore agarose gel electrophoresis by separating fluorescent dyes. The unique patterns will be analyzed using one of two scenarios that simulate DNA testing.

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.

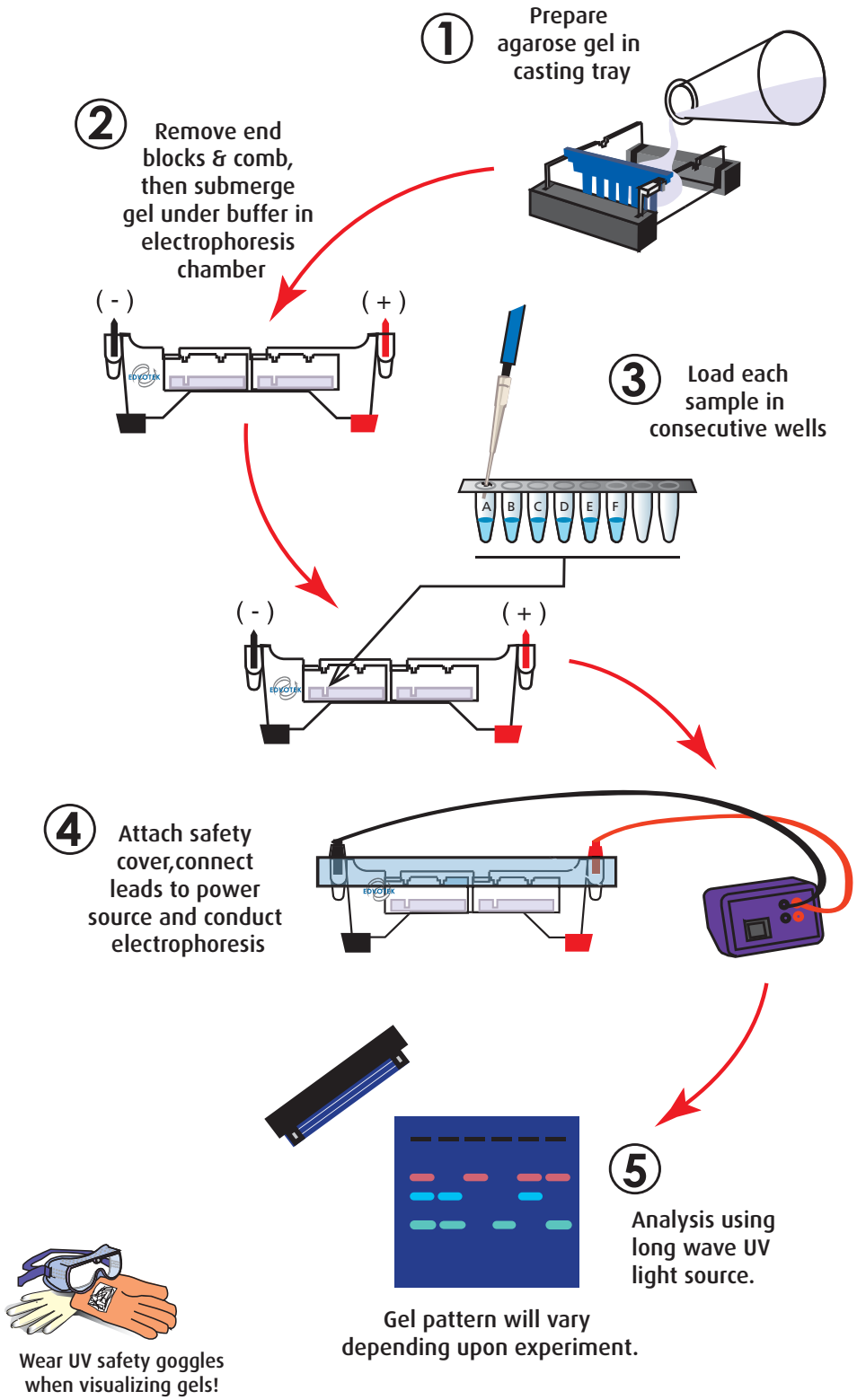
During the Experiment:

- Record your observations.

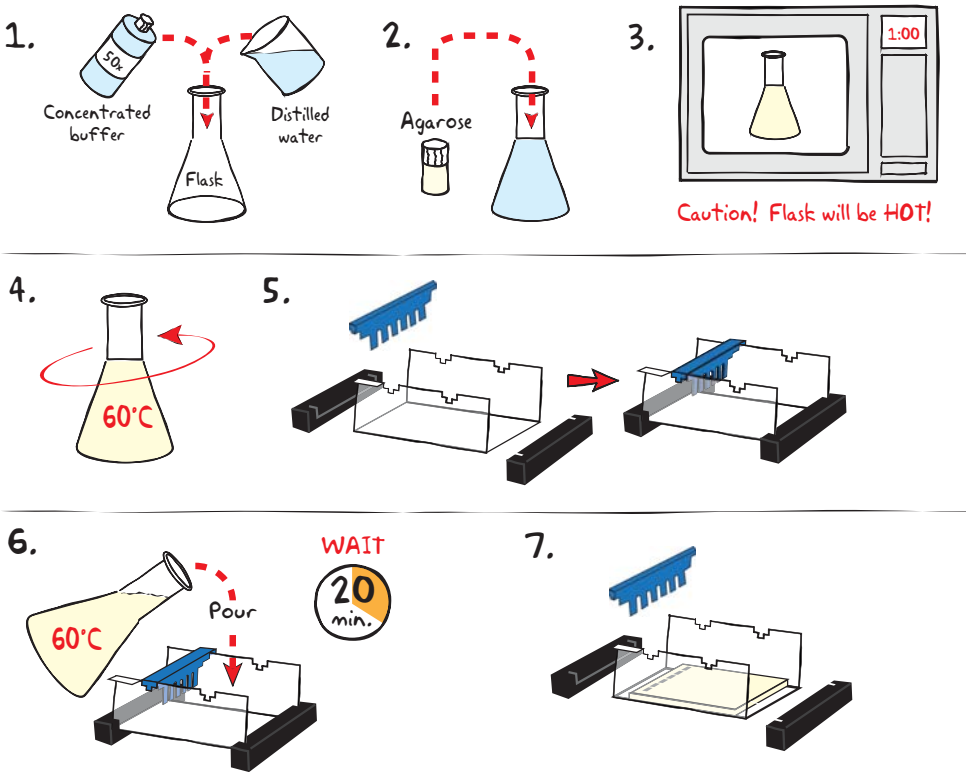
After the Experiment:

- 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.

Experiment Overview



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

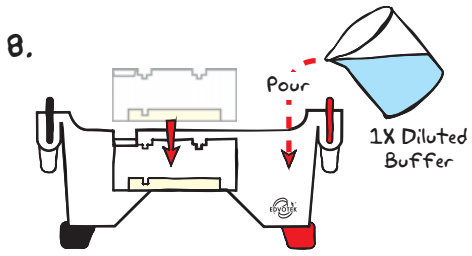
CASTING THE AGAROSE GEL

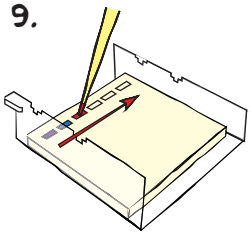
1. **DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
2. **MIX** agarose powder with buffer solution in a 250 ml flask (refer to Table A).
3. **DISSOLVE** agarose powder by boiling the solution.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

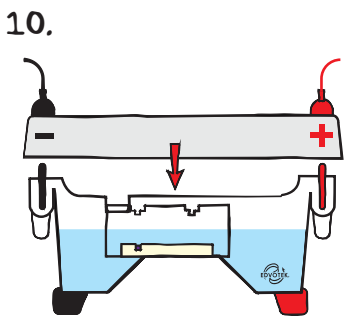


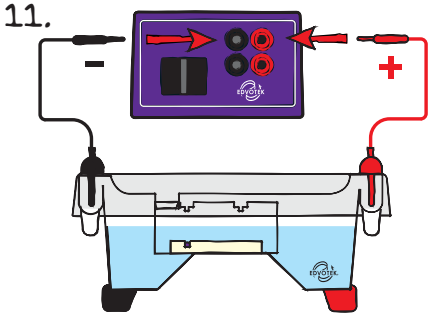


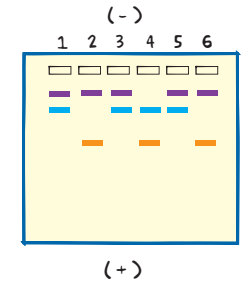
Reminders:

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 µL) into the well in consecutive order. The identity of each sample is provided in Table 2.
10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the dye 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. **Visualize the results using a long wave UV light source (black light). Be sure to wear UV safety glasses!**

Table 2: Gel Loading

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

Table B 1x Electrophoresis Buffer (Chamber Buffer)

EDVOTEK Model #	Total Volume Required	Dilution: 50x Conc. Buffer	+ Distilled Water
M6+	300 ml	6 ml	294 ml
M12	400 ml	8 ml	392 ml
M36 (blue)	500 ml	10 ml	490 ml
M36 (clear)	1000 ml	20 ml	980 ml

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

	Electrophoresis Model M6+	Electrophoresis Model M12 & M36
Volts	Min. / Max.	Min. / Max.
150	15/20 min.	25 / 35 min.
125	20/30 min.	35 / 45 min.
75	35 / 45 min.	60 / 90 min.
50	50 / 80 min.	95 / 130 min.

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 electrophoresis buffer		
Prepare molten agarose and pour gel		

Pre-Lab Preparations

PREPARING AGAROSE GELS

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.

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.

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 Electrophoresis buffer (50x), 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. Electrophoresis buffer can also be prepared in bulk. See Appendix B.

Each Student Group should receive:
- Electrophoresis buffer (50x)
- Distilled Water
- UltraSpec-Agarose™
- Ready-to-Load™ Samples

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° C as freezing will destroy the gels.

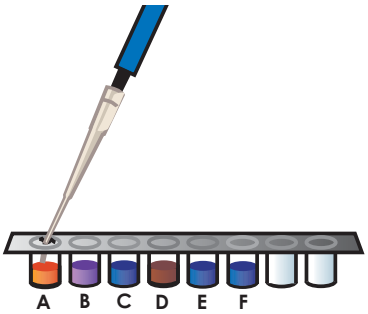
Gels that have been removed from their trays for storage should be "anchored" back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

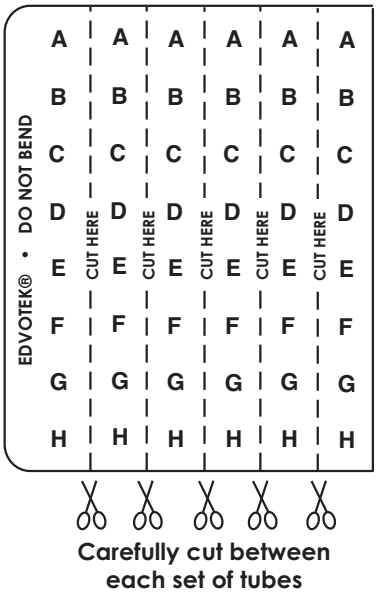
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

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.





Scenario One: Experiment Results and Analysis

Scenario One: Results Table

Lane	Tube	Sample	Result
1	A	Standard Dye Marker	--------
2	B	Patient 1	genotype: I^BI^B
3	C	Patient 2	genotype: I^AI^B
4	D	Patient 3	genotype: I^AI^A
5	E	Patient 4	genotype: I^Ai
6	F	Patient 5	genotype: ii

Scenario One: Answers to Study Questions

1. List and describe the factors that influence separation of molecules by agarose gel electrophoresis.

- *Molecular size:* Small molecules move through channels in the gel more easily than larger molecules.
- *Gel concentration:* The final concentration of agarose in a gel will change the size of the channels within the gel.
- *Molecular charge:* Molecules with more charge will migrate faster than molecules with less charge. Negatively-charged molecules migrate towards the positive electrode while positively-charged molecules migrate towards the negative electrode.
- *Molecular shape:* Molecules with a more compact shape, like a sphere, would move through the gel more quickly than those with a looser conformation.

2. What are the genotypes and the phenotypes for all five patients?

Patient 1 genotype: I^BI^B	Patient 1 phenotype: Type B blood
Patient 2 genotype: I^AI^B	Patient 2 phenotype: Type AB blood
Patient 3 genotype: I^AI^A	Patient 3 phenotype: Type A blood
Patient 4 genotype: I^Ai	Patient 4 phenotype: Type A blood
Patient 5 genotype: ii	Patient 5 phenotype: Type O blood

3. A person with type AB blood is admitted to the hospital with a serious injury. A blood transfusion is necessary. What types of blood can be used for transfusion?

Patients with type AB blood do not have antibodies against the A or B antigen. Therefore, they can receive blood from donors of all blood types.

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/Safety-Data-Sheets**

Appendix A
EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands not visible on the gel	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.
	Fluorescent dyes require a UV light for visualization.	Use a hand-held UV light (Cat. #969) or a UV transillluminator (Cat. #558) for visualization.
Very light colored band seen after electrophoresis	Pipetting error.	Make sure students pipet 35 µl of dye sample per well.
Poor separation of bands	Gel was not prepared properly.	Make sure to prepare a 0.8% gel.
Dye bands disappear when the gels are kept at 4° C.	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.

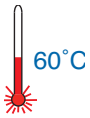
Table D — Bulk Preparation of Electrophoresis Buffer

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.

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

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:

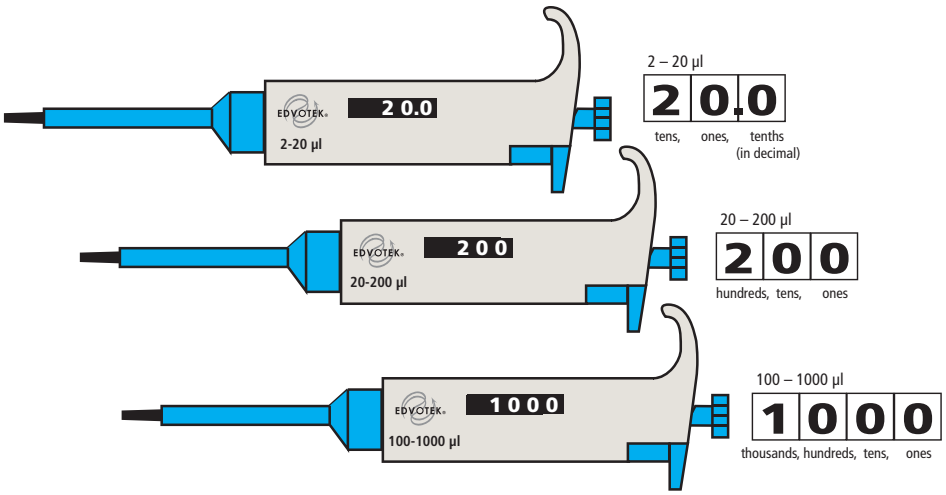
1. Cast a gel with the maximum number of wells possible.
2. After the gel solidifies, place it under buffer in an electrophoresis apparatus chamber.

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

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.

Appendix C
Practice Gel Loading



Wear gloves and safety goggles

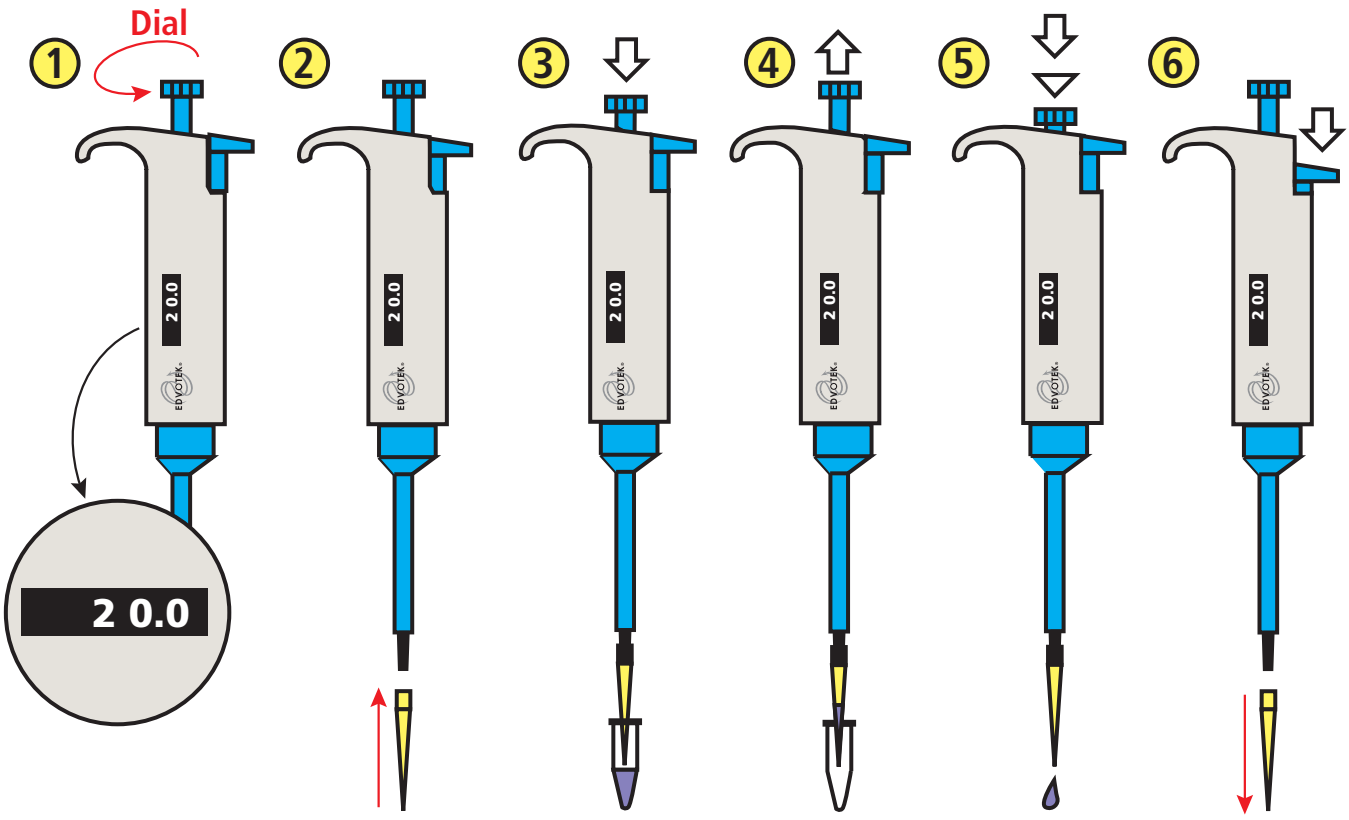
SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

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


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.