EDVOTEK®

THE **BIOTECHNOLOGY EDUCATION** COMPANY®

Edvo-Kit # **S-53**

Edvo-Kit #S-53

The Mystery of the Crooked Cell

Experiment Objective:

In this experiment, students will gain an understanding of the effect of mutations in health and disease, specifically as it relates to sickle cell anemia.

See page 3 for storage instructions.

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Safety Data Sheets can be found on our website: **http://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	Normal Hemoglobin control	❑
B	Sickle Hemoglobin control	❑
C	Carrier Hemoglobin control	❑
D	Patient #1 Hemoglobin	❑
E	Patient #2 Hemoglobin	❑

Experiment #S-53 is designed for 10 gels.

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

REAGENTS & SUPPLIES

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

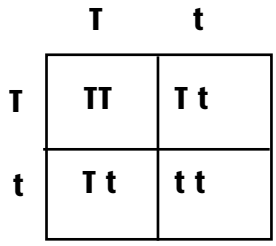
Requirements *(not included with kit)*

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Heat Source
- 500 ml Beaker or Flask
- Hot Gloves
- Distilled or deionized water
- Balance
- Automatic micropipets with tips (optional)

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

A single nucleotide change in the DNA sequence of an important gene can affect health and disease. A large number of genetic diseases are identified where such changes have been correlated to changes in single nucleotides. More recently, mutations in oncogenes and tumor suppressor genes such as p53, have been associated with lung, colon and breast cancer. Other mutations in genes such as the BRCA 1 and II genes have been identified as specific markers with good potential as diagnostic tools for breast cancer.



Genotype:

1/4 TT
1/2 Tt
1/4 tt

Phenotype:

3/4 dominant
1/4 recessive

Figure 1 - Using a 2x2 Matrix to Determine genotypes

Human genetics follows the basic findings of the Augustine monk, Gregor Mendel, who studied plant genetics in the mid-1800's. Mendelian genetics, which predicts traits inherited by offspring, is based on the inheritance of two alleles, or forms of the gene. These two alleles are inherited one from each parent. Alleles, and corresponding traits, can be either dominant or recessive. When a dominant allele is inherited, the trait coded by that allele will be apparent in the offspring. The presence of a dominant allele will, in effect, mask the trait coded by the recessive allele. To observe a recessive trait, it is required that both parental alleles be the recessive type. If both alleles are the same type, either both recessive or both dominant, the individual is said to be homozygous with respect to that trait. If an individual has one dominant and one recessive, the individual is said to be heterozygous for that trait.

Mendelian inheritance can be demonstrated with a 2 x 2 matrix, as shown in Figure 1. Parental alleles are placed on the sides of the matrix, and the genotype (what is genetically inherited) and phenotype (the way we look) of the offspring can be predicted. By convention, the dominant allele is denoted by an uppercase letter and the recessive allele by a lowercase letter. For example, assuming both parents each carry one dominant allele and one recessive allele, we can predict that 3/4 of their children will have the dominant phenotype and 1/4 of their children will have the recessive phenotype. Genotypically, 1/4 of the children will carry two dominant alleles; 1/2 of the children will carry one dominant and one recessive allele, and 1/4 will carry two recessive alleles. These estimates would be observed if there are a large number of offspring from two parents, as in the case of insects or plants.

Hemoglobin, which is present in red blood cells, is the carrier of oxygen to cells in the body. In capillaries carbon dioxide, which is a by product of metabolism, enters red cells and is converted to carbonic acid. The acidic pH reduces the affinity of oxygen binding to hemoglobin resulting in the release of oxygen in cells. Likewise when the bound carbon dioxide is released from red cells in the lungs there is an increase in pH which favors the binding of oxygen to hemoglobin. In individuals who suffer from certain blood diseases such as sickle cell anemia, the binding and subsequent transport of oxygen is compromised due to a single nucleotide mutation. This results in a deficiency of oxygen and carbon dioxide exchange in the patient. In sickle cell anemia patients, the substitution of the polar negatively charged side chain (Glu) with a nonpolar hydrophobic side chain (Val) results in the polymerization of the unoxygenated form and subsequent precipitation of such polymers in red blood cells. The precipitation gives red blood cells a sickle shape due to the lack of diffusion through capillaries.

Background Information

Each person has two copies of the gene of hemoglobin. Normal hemoglobin is referred to as Hemoglobin A. The letters AA are used to indicate that both hemoglobin genes are normal. The gene that causes sickle cell anemia is referred to as Hemoglobin S. There are three possible combinations of the genes for hemoglobin:

AA Individual is homozygous for the Hemoglobin A gene. So, both copies of hemoglobin code for normal hemoglobin and the person does not have the disease.

AS Individual is heterozygous. One copy of hemoglobin codes for normal hemoglobin and the other copy of the gene codes for sickled hemoglobin. This person does not have the disease and will not develop it later in life.

SS Individual is homozygous for the sickled hemoglobin S gene. So, both copies of hemoglobin code for diseased hemoglobin. This person suffers from sickled cell anemia.

The irregularly shaped blood cells lead to a cascade of symptoms. The sickle-shaped blood cells die prematurely, resulting in anemia and the production of excess bilirubin (a yellow pigment resulting from the breakdown of hemoglobin). Jaundice often results when the liver cannot metabolize bilirubin fast enough. Infection, dehydration, overexertion, high altitude, chills, or cold weather can bring on a sickling episode, or crisis. Sometimes there is no apparent precipitating factor. People with sickle cell disease are susceptible to fevers and infection.

Blood disease such as sickle cell anemia and β-thalassemias are attributed to various point mutations or other translational product aberrations. Almost 400 different hemoglobin (Hb) variants of known structure have been identified. The early recognized variants were historically assigned alphabetical initials based sequence of discovery or hematologic features.

In the United States, sickle cell anemia is of special interest since it is estimated that 8% of African Americans are carriers of the sickle trait. It is of interest to note that heterozygous individuals for Hb S have a high resistance to the malaria parasite, part of whose life cycle is spent in red blood cells. Historically, sickle cell anemia has provided a selective advantage in some regions of the world such as parts of Africa. This can also explain the reason for the high frequency of this homozygous gene amongst African Americans.

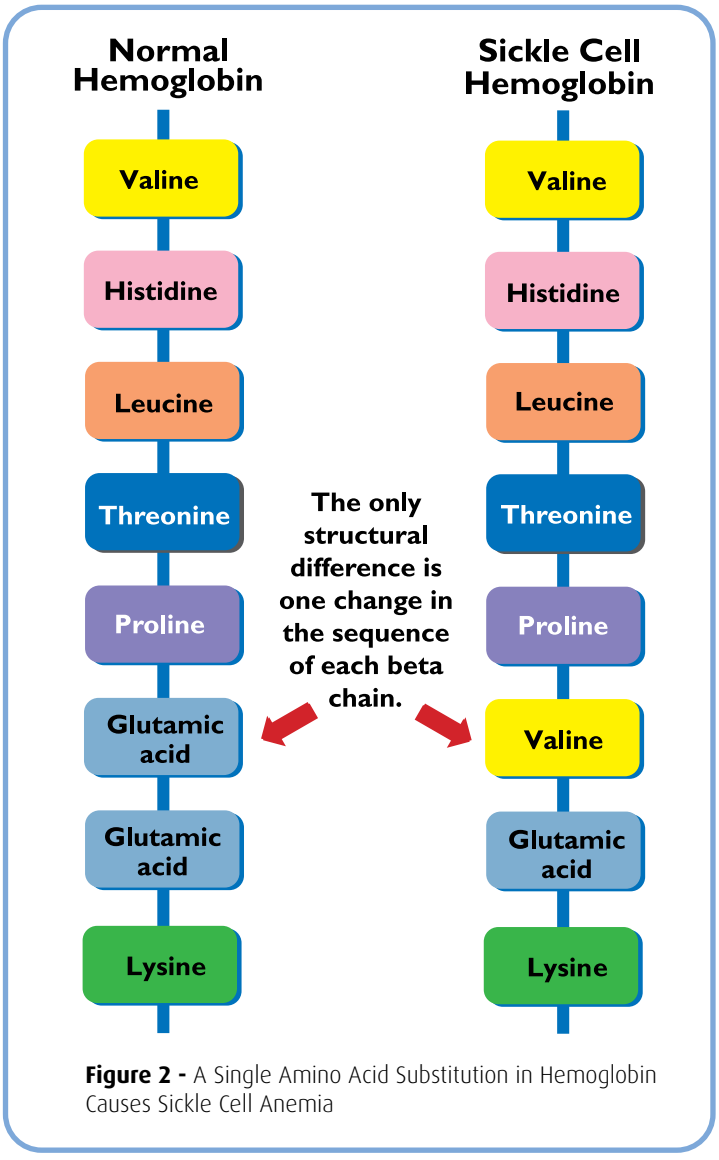


Figure 2 - A Single Amino Acid Substitution in Hemoglobin Causes Sickle Cell Anemia

Background Information

Hemoglobin is made up of two a chains and two β chains. The gene where the α is located is on the short arm of chromosome 16, while the β-globin gene cluster is on the short arm of chromosome 11. In addition to the adult form of Hb encoded within the β Hb cluster are the Hb forms that substitute for the adult β Hb during the various stages of development. Hemoglobin S (Hb S) is the variant form of the normal adult hemoglobin A (Hb A) in which an amino acid substitution occurs in the β polypeptide. The amino acid substitution is that of Valine (Val) in Hb S for the glutamic acid (Glu) normal Hb A hemoglobin (Figure 2). This significant finding was reported in 1957 by Vernon Ingram who was able to determine this amino acid substitution using peptide mapping analysis. These procedures are tedious and difficult. It should be noted that this predates recombinant DNA technology.

The single base mutation is an A to T in the triplet codon of the amino acid residue number 6 from the amino acid end in the beta chain. This change introduces an amino acid with a nonpolar (neutral) side chain valine instead of the acidic (negative) residue and changes the property of the hemoglobin molecule. This substitution changes the electrophoretic mobility of Hb S compared to Hb A. At slightly basic pH, such as 8.4, Hb S will be relatively more positive than Hb A and therefore will travel slower towards the positive (anode) electrode. This change in mobility is used as a diagnostic test of the presence of Hb S. After the DNA is isolated, it is analyzed for the Sickle Cell mutation.

With the advent of biotechnology, parental or fetal DNA from cells obtained from amniocentesis can now be analyzed with a high degree of accuracy. A few cells can provide sufficient DNA to be amplified using Polymerase Chain Reaction (PCR). Alternative methods can include growing cells in culture to yield sufficient DNA for analysis.

The patient DNA is treated with a special enzyme called a restriction endonuclease, which acts like a molecular scissor to cut DNA at specific palindromic sites. In the normal β globin gene, the sequence of nucleotides that specifies amino acids 5, 6 and 7 (Pro-Glu-Glu) are CCT-GAG-GAG (see Figure 2). Sickle Cell patients have a single point mutation in codon 6 that changes the A to T, resulting sequence to CCT-GTG-GAG. The two alleles can be distinguished between using the restriction enzyme Mst II, which recognizes the palindrome CCTNAGG (where N can be any of the four nucleotides). Close examination of the sequence shows that Mst II will recognize the palindrome and cut the normal β globin gene into smaller pieces. However, the enzyme cannot digest the mutated form of the gene because of the change of A-T base substitution, and the DNA fragment remains whole.

In this experiment, we will perform the genetic test that identifies the Sickle Cell gene. Restriction digests from control and patient samples are analyzed by agarose gel electrophoresis, a technique that separates DNA fragments by size. In this experiment, the DNA fragments produced by restriction digest are represented by orange dyes of different sizes. The smaller band represents the normal β globin gene, whereas the larger band represents the mutant gene. By using brightly colored dyes to simulate DNA fragments, we have eliminated post-electrophoresis staining, saving you valuable classroom time.

Experiment Overview

EXPERIMENT OBJECTIVE:

In this experiment, students will gain an understanding of the effect of mutations in health and disease, specifically as it relates to sickle cell anemia.

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.

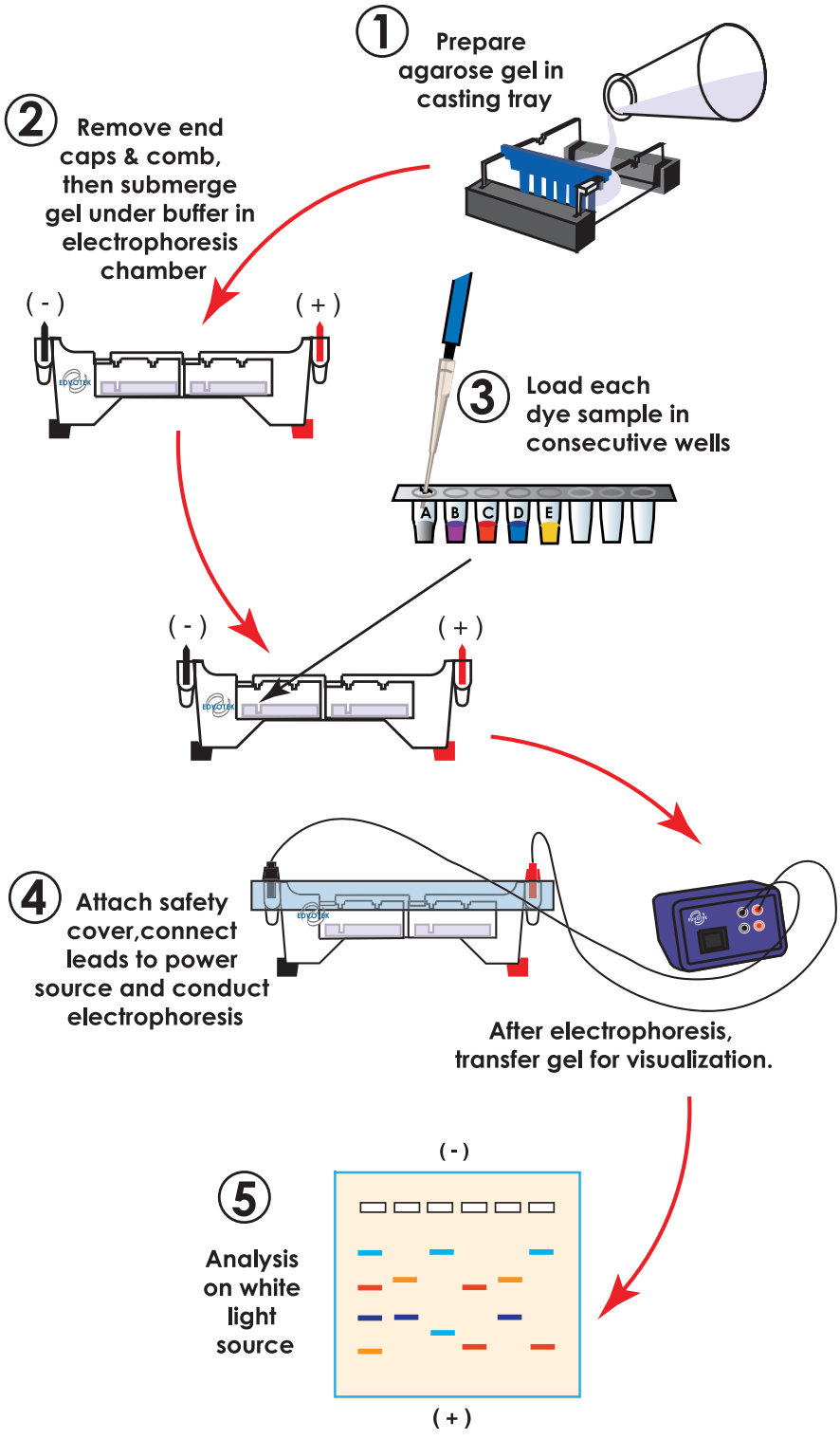
During the Experiment:

- Record your observations.

After the Experiment:

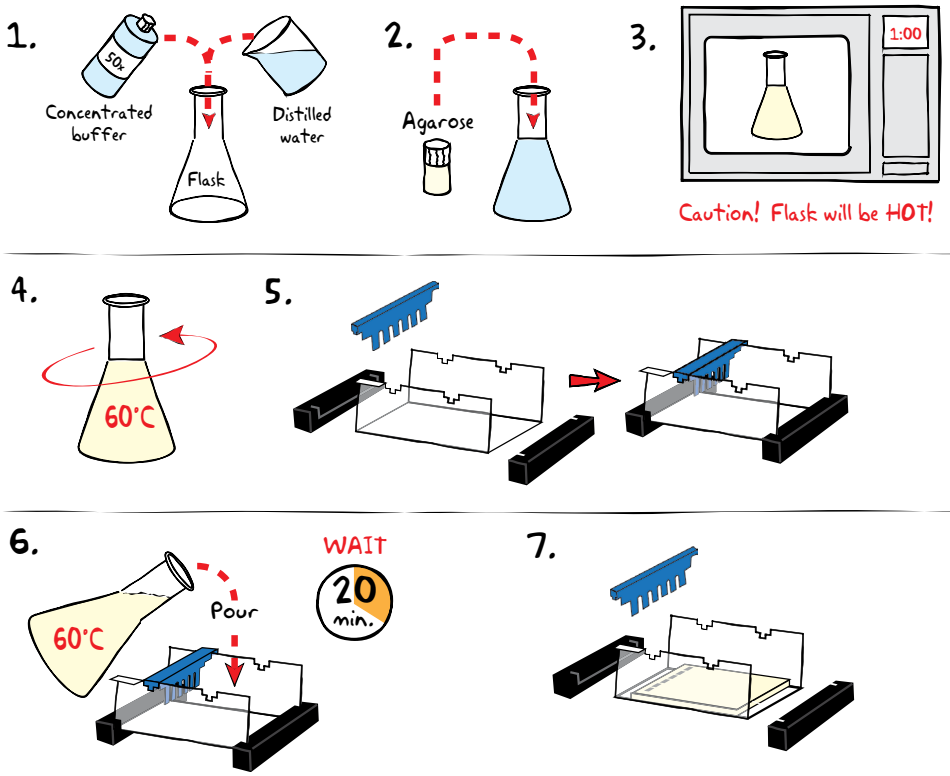
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Experiment Overview



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**

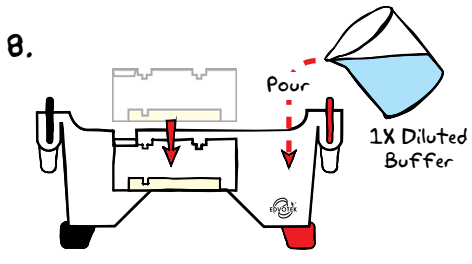
Wear gloves and safety goggles

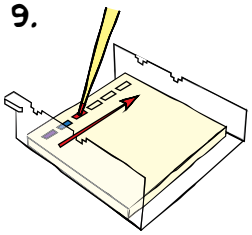
1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask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

Table A — Individual 0.8% UltraSpec-Agarose™ Gel

Size of Gel Casting tray	Concentrated Buffer (50x)	+ Distilled Water	+ Amt of Agarose	= TOTAL Volume
7 x 7 cm	0.6ml	29.4 ml	0.24 g	30 ml
7 x 15 cm	1.2 ml	58.8 ml	0.48 g	60 ml

Agarose Gel Electrophoresis

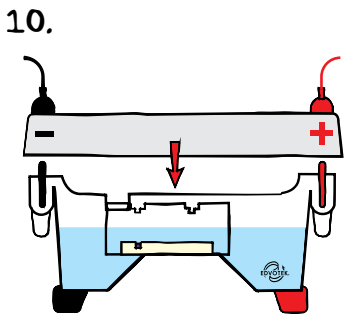


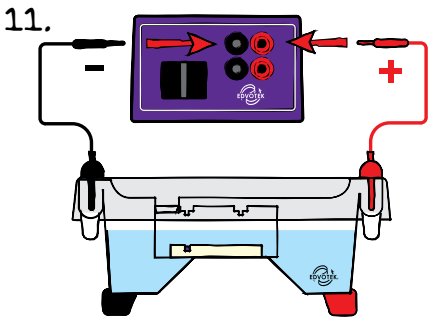


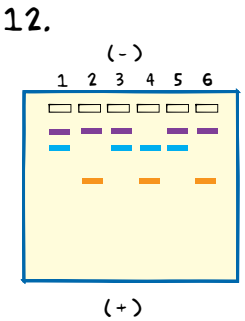
Reminders:

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 1.
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 and **VISUALIZE** the results. No staining is necessary.

Table 1: Gel Loading

Lane		
1	Tube A	Normal Hemoglobin control
2	Tube B	Sickle Hemoglobin control
3	Tube C	Carrier Hemoglobin control
4	Tube D	Patient #1 Hemoglobin
5	Tube E	Patient #2 Hemoglobin

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

Critical Thinking and Hypothesis Development

1. Based on the evidence obtained from analysis of the gel, which patient has the sickle cell trait? Explain.
2. What is the variable in this experiment?
3. What would you change in the experiment if you had to do it over again?
4. Write a hypothesis that would reflect these changes.

Study Questions

1. Why is it important to position the sample wells near the negative electrode?
2. Why is it important to use a new pipet or wash the pipet between uses?
3. How will you be able to tell which patient has the sickle cell trait?
4. Explain what happens to patients afflicted with sickle cell anemia?
5. What are the possible gene combinations for hemoglobin?

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		

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 Electrophoresis 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. Electrophoresis buffer can also be prepared in bulk. 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° 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.

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.

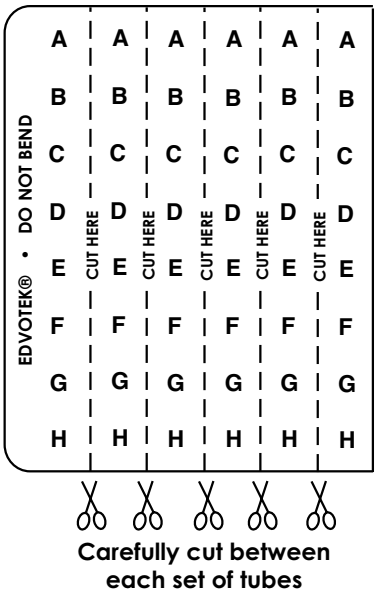
Each Student Group should receive:
- 50x Electrophoresis Buffer
- Distilled Water
- UltraSpec-Agarose™
- Ready-to-Load™ Samples

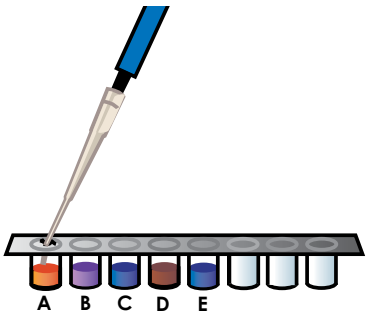
SAMPLES FORMAT: PREPARING THE QUICKSTRIPS™

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

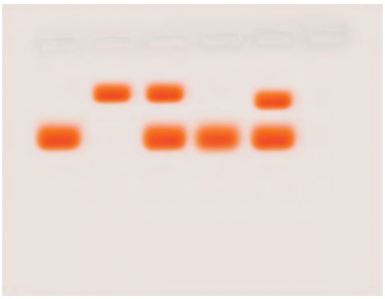
Using sharp scissors, carefully divide the block of tubes into individual strips by cutting between the rows (see diagram at right). Take care not to damage the protective overlay while separating the samples.

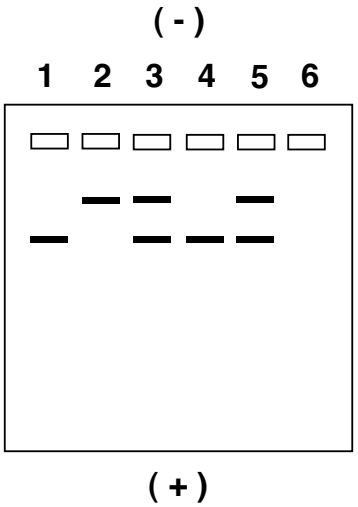
Each lab group will receive one set of tubes. Before loading the gel, remind students to tap the tubes to collect the sample at the bottom of the tube.





Experiment Results and Analysis





Idealized results are shown in the figure at left. Actual results will yield bands of varying intensity. The idealized schematic shows the relative positions of the bands, but are not depicted to scale.

Lane	Tube	Sample	Results
1	A	Normal Hemoglobin Control	One low molecular weight band -Enzyme cuts both copies of the β globin gene.
2	B	Sickle Hemoglobin Control	One high molecular weight band -enzyme does not cut either copy of the β globin gene.
3	C	Carrier Hemoglobin Control	One high and one low molecular weight band - Enzyme cuts one copy of the β globin gene but not the either.
4	D	Patient #1 Hemoglobin	One low molecular weight band – patient does not have sickle cell gene because enzyme cuts both copies of the β globin gene.
5	E	Patient #2 Hemoglobin	One high and one low molecular weight band – Patient is a carrier because enzyme cuts one copy of the β globin gene but not the either.

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: **http://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.
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.

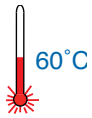
Table D — Bulk Preparation of Electrophoresis Buffer

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.

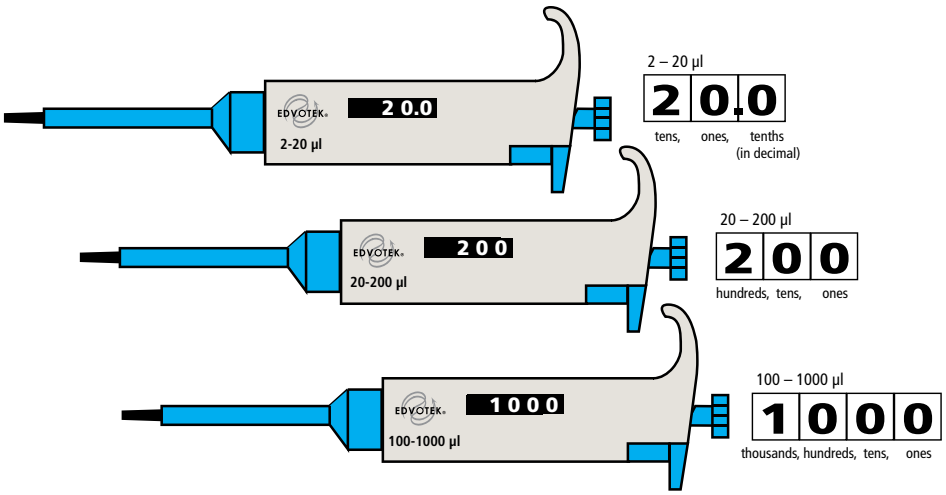
4. If you need more practice, remove the practice gel loading solution by squirting buffer into the wells with a transfer pipet.

5. Replace the practice gel with a fresh gel for the actual experiment.

   Note: 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.

Appendix C
Practice Gel Loading



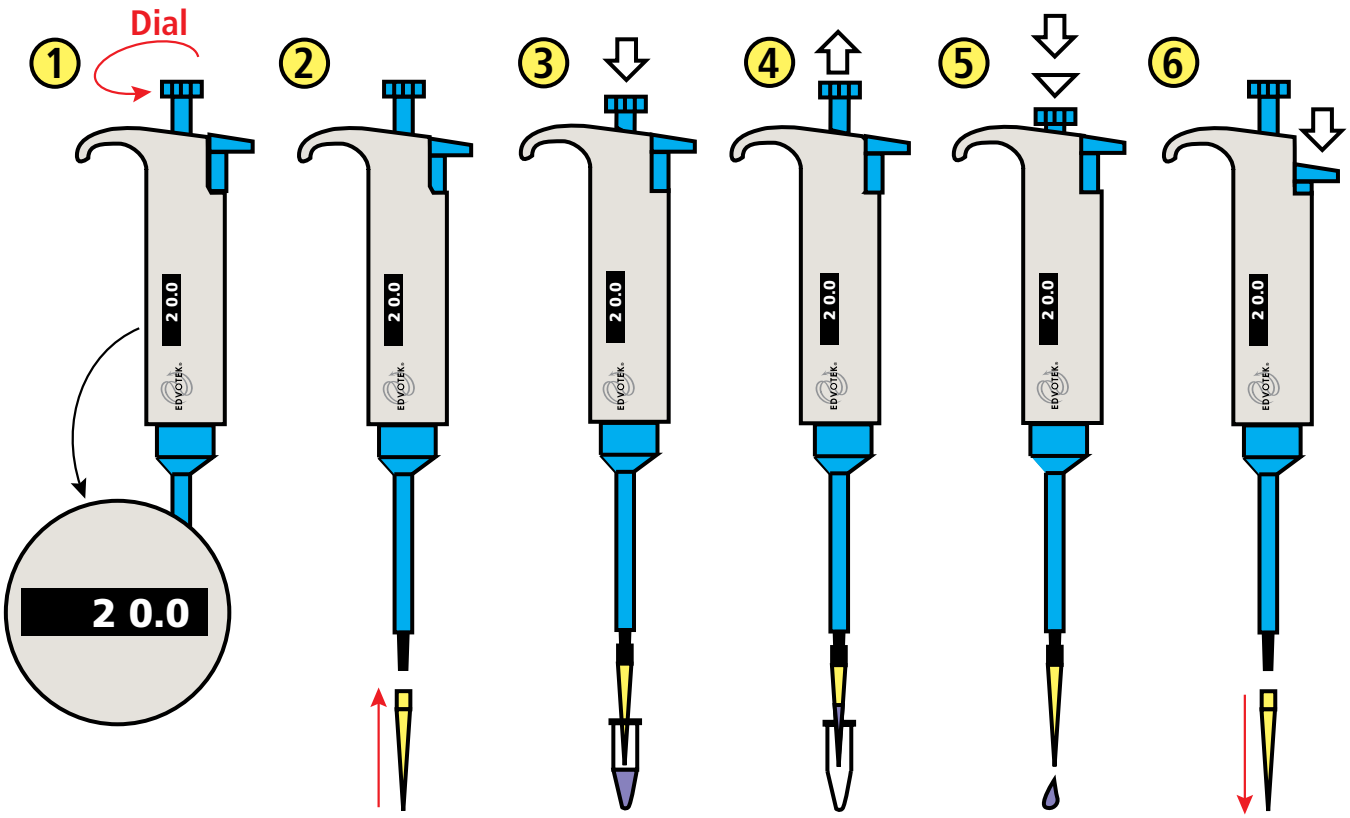
SETTING THE VOLUME OF AN ADJUSTABLE VOLUME MICROPIPET

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


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.