

THE BIOTECHNOLOGY
EDUCATION COMPANY®

Edvo-Kit #

116

Edvo-Kit #116

Sickle Cell Gene Detection (DNA-Based)

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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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)

- A Sick cell gene sample
- B Sick cell trait (carrier) sample
- C Normal gene sample
- D Mother's DNA sample
- E Child's DNA sample
- F Father's DNA sample

Check (✓)

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REAGENTS & SUPPLIES

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- 10x Gel Loading Solution
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- 1 ml pipet
- Microtipped Transfer Pipets

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Experiment #116 is designed for 8 gels if stained with FlashBlue™ or InstaStain® Blue (both included) or 16 gels if stained with SYBR® Safe or InstaStain® Ethidium Bromide (not included).

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

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.

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled or deionized water

Background Information

IN SEARCH OF THE SICKLE CELL GENE

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 the changes in a single nucleotide. 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.

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 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.

	T	t	
T	TT	Tt	Genotype: 1/4 TT 1/2 Tt 1/4 tt
t	Tt	tt	
			Phenotype: 3/4 dominant 1/4 recessive

Figure 1

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. Therefore, pregnancies at risk of an offspring suffering from sickle cell anemia is $8\% \times 8\%$, which equals 0.64 or 3.75%. 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.

Hemoglobin is made up of two α 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 structural change 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 polar (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.

With the advent of biotechnology, parental or fetal DNA from cells obtained from amniocentesis can now be analyzed with a high degree of accuracy. DNA from a few cells can provide sufficient DNA to amplify using Polymerase Chain Reaction (PCR). Alternative methods can include growing cells in culture to yield sufficient DNA for analysis. The basis of the test is the recognition by restriction enzymes of specific palindromic sequences in DNA. 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. The point mutation in codon 6 converting the A to T changing the sequence CCT-GTG-GAG. The palindrome recognition site of the restriction enzyme *Mst* II is CCTNAGG, where N can be any of the four nucleotides. Close examination of the sequence shows that *Mst* II will recognize the normal β globin CCT-GAG-G where N is a G, but not the mutated form.

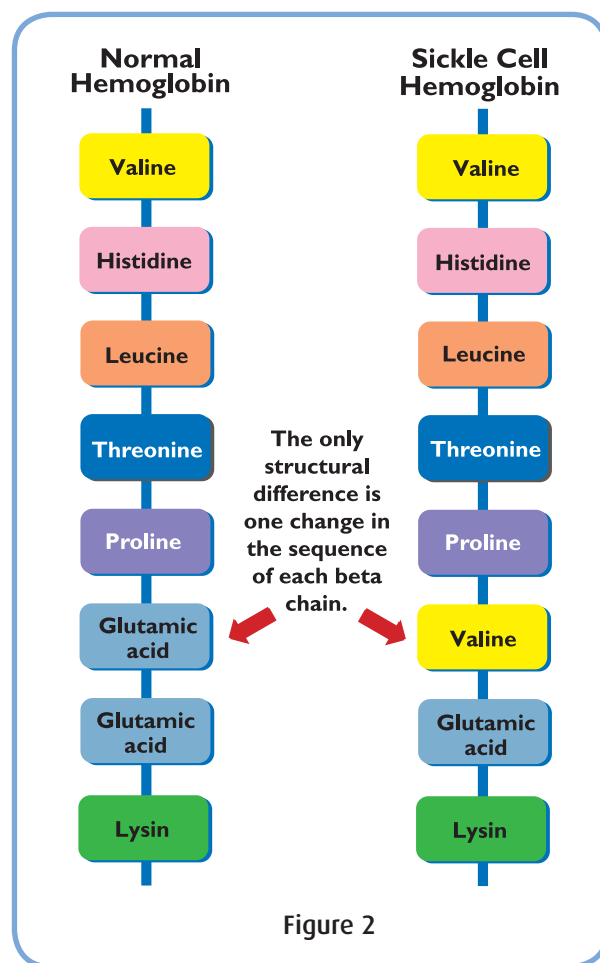


Figure 2

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.



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.

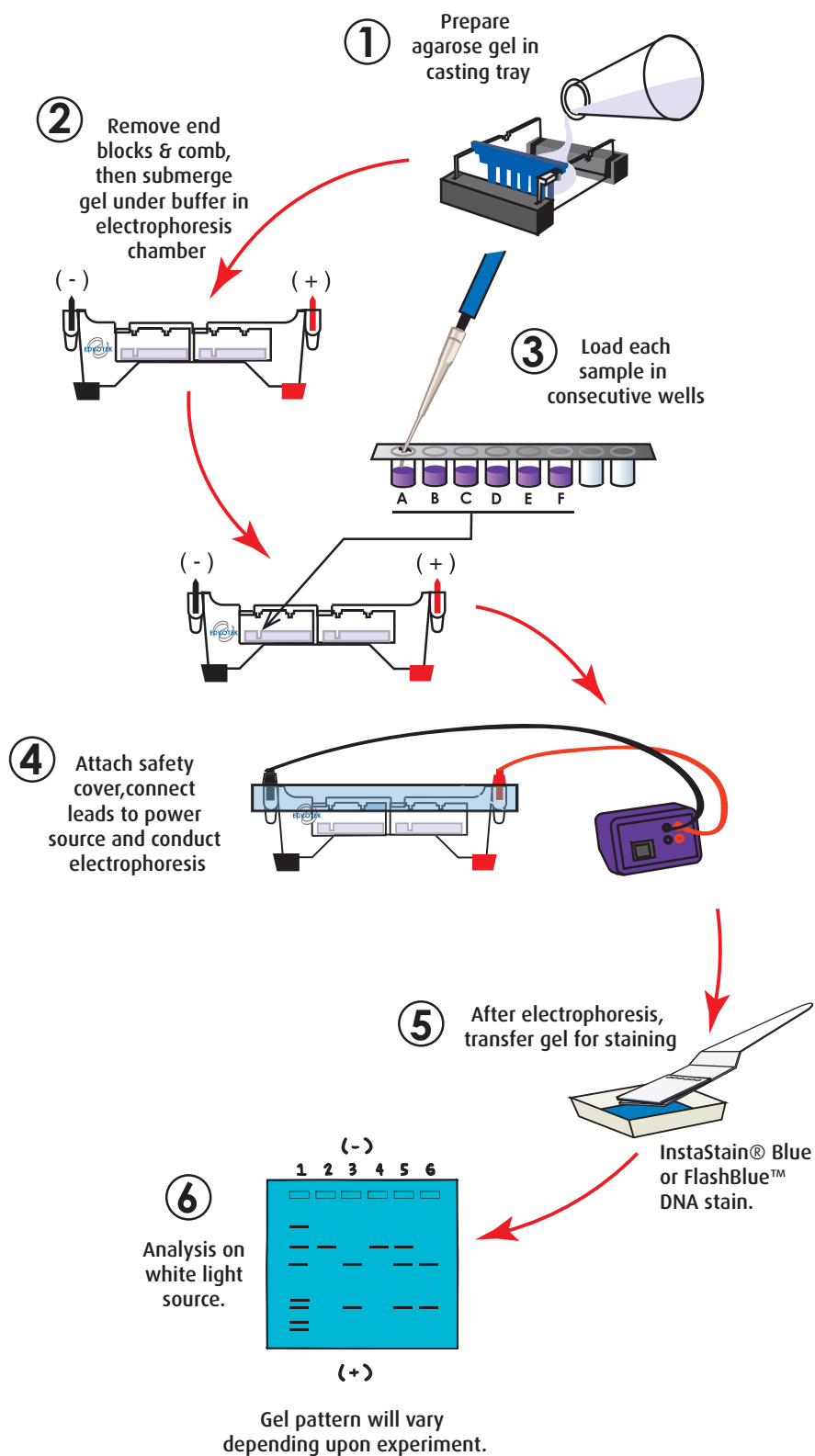
During the Experiment:

- Record your observations.

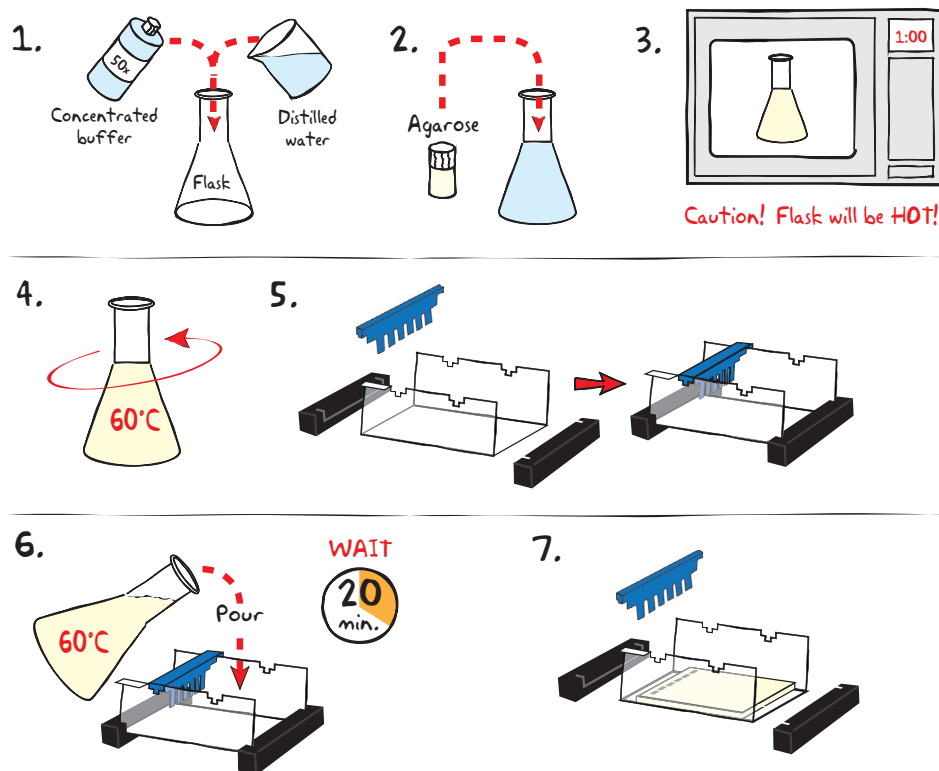
After the Experiment:

- 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



Module I: 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

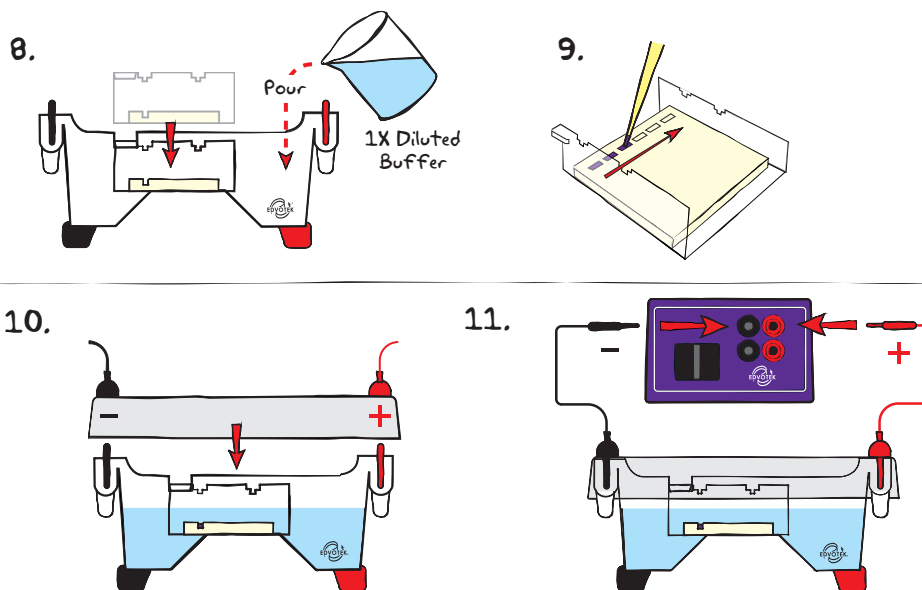
- DILUTE** concentrated 50X Electrophoresis buffer with distilled water (refer to Table A for correct volumes depending on the size of your gel casting tray).
- MIX** agarose powder with buffer solution in a 250 ml flask (refer to Table A).
- DISSOLVE** agarose powder by boiling the solution. **MICROWAVE**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

Module I: Agarose Gel Electrophoresis



Wear gloves
and safety goggles

REMINDER:

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

RUNNING THE GEL

8. **PLACE** the gel (still on the tray) into the electrophoresis chamber. **COVER** the gel with 1X Electrophoresis Buffer (See Table B for recommended volumes). The gel should be completely submerged.
9. **PUNCTURE** the foil overlay of the QuickStrip™ with a pipet tip. **LOAD** the entire sample (35 µl) into the well in the order indicated by Table 1, at right.
10. **PLACE** safety cover on the unit. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.
11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines). Allow the tracking dye to migrate at least 3.5 cm from the wells.
12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to instructions for **STAINING** the agarose gel.

Table 1: Gel Loading

Lane	Tube	Sample
1	Tube A	Sickle cell gene sample
2	Tube B	Sickle cell trait (carrier) sample
3	Tube C	Normal gene sample
4	Tube D	Mother's DNA sample
5	Tube E	Child's DNA sample
6	Tube F	Father's DNA sample

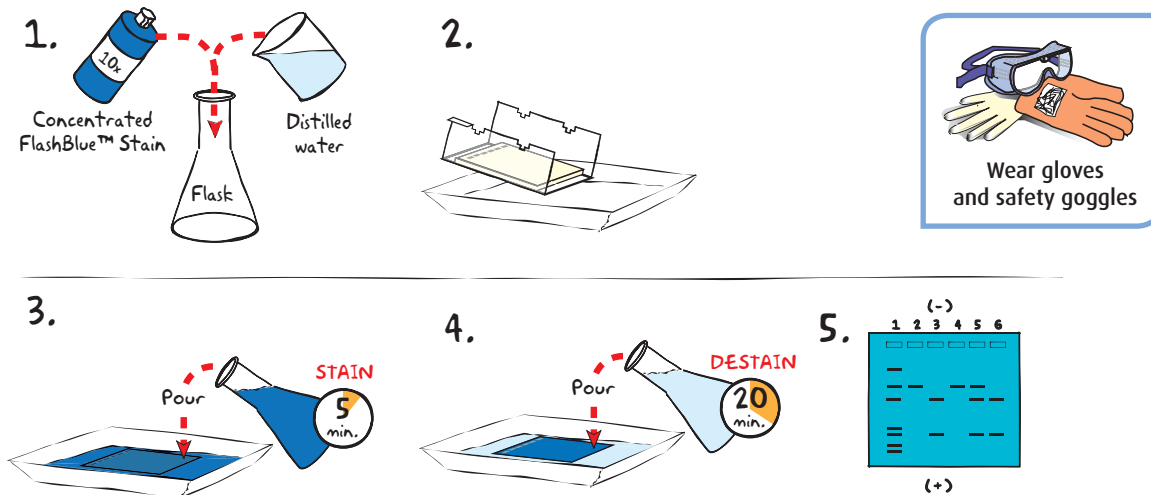
Table B
1x Electrophoresis Buffer (Chamber Buffer)

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

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

Electrophoresis Model			
Volts	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.

Module II-A: Staining Agarose Gels Using FlashBlue™

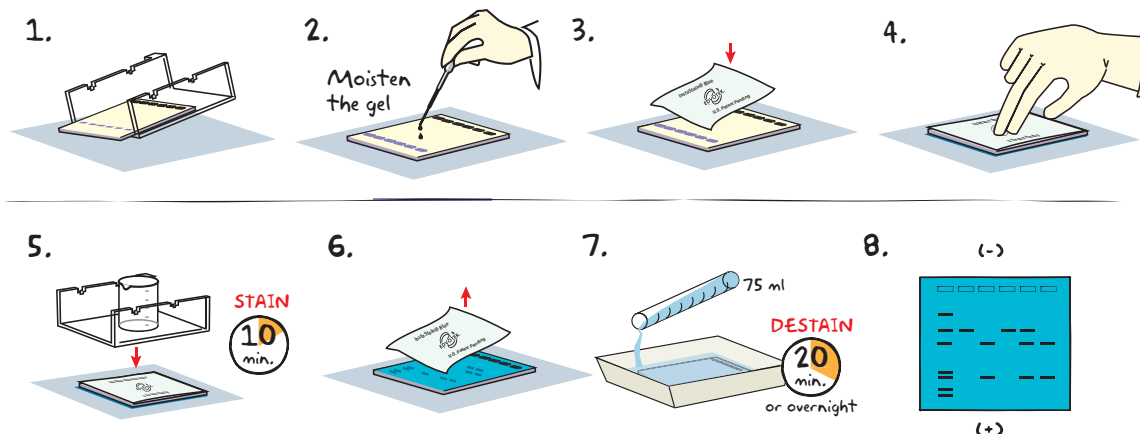


- DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
- REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
- COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
- TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
- Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

ALTERNATIVE PROTOCOL:

- DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
- COVER** the gel with diluted FlashBlue™ stain.
- SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.

Module II-B: Staining Agarose Gels Using InstaStain® Blue



1. Carefully **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray on to a piece of plastic wrap on a flat surface.
2. **MOISTEN** the gel with a few drops of electrophoresis buffer.
3. Wearing gloves, **PLACE** the blue side of the InstaStain® Blue card on the gel.
4. With a gloved hand, **REMOVE** air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.
5. **PLACE** the casting tray on top of the gel/card stack. **PLACE** a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Blue card is in direct contact with the gel surface. **STAIN** the gel for 10 minutes.
6. **REMOVE** the InstaStain® Blue card. If the color of the gel appears very light, reapply the InstaStain® Blue card to the gel for an additional five minutes.
7. **TRANSFER** the gel to a small, clean gel-staining tray. **COVER** the gel with about 75 ml of distilled water and **DESTAIN** for at least 20 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. To accelerate destaining, warm the distilled water to 37°C and change it frequently.
8. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.



Wear gloves
and safety goggles

NOTE:
DO NOT STAIN
GELS IN THE
ELECTROPHORESIS
APPARATUS.

ALTERNATIVE PROTOCOL:

1. Carefully **SLIDE** the agarose gel from its casting tray into a small, clean tray containing about 75 ml of distilled/deionized water or used electrophoresis buffer. The gel should be completely submerged.
2. Gently **FLOAT** the InstaStain® Blue card(s) on top of the liquid with the stain (blue side) facing toward the gel. Each InstaStain® Blue card will stain 49 cm² of gel (7 x 7 cm).
3. **COVER** the tray with plastic wrap to prevent evaporation. **SOAK** the gel in the staining liquid for at least 3 hours. The gel can remain in the liquid overnight if necessary.
4. Carefully **REMOVE** the gel from the staining tray. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

Study Questions

1. Describe the mechanism of the blood disease sickle cell anemia and how it affects its victims.
2. How many polypeptides are contained in hemoglobin?
3. What is the point mutation that causes sickle cell anemia? Where is it located?
4. Explain the methods for detecting sickle cell in patients?

Instructor's Guide

ADVANCE PREPARATION:

Preparation for:	What to do:	When?	Time Required:
Module I: Agarose Gel Electrophoresis	Prepare QuickStrips™	Up to one day before performing the experiment	45 min.
	Prepare diluted electrophoresis buffer		
	Prepare molten agarose and pour gels		
Module II: Staining Agarose Gels	Prepare staining components	The class period or overnight after the class period	10 min.

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Pre-Lab Preparations: Module II

MODULE II-A: STAINING WITH INSTASTAIN® BLUE

The easiest and most convenient DNA stain available is InstaStain® Blue. InstaStain® Blue does not require the formulation, storage and disposal of large volumes of liquid stain. Each InstaStain® Blue card contains a small amount of blue DNA stain. When the card is placed in water, the DNA stain is released. This solution simultaneously stains and destains the gel, providing uniform gel staining with minimal liquid waste and mess.

You can use a White Light Visualization System (Cat. #552) to visualize gels stained with InstaStain® Blue.

MODULE II-B: STAINING WITH FLASHBLUE™

FlashBlue™ stain is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to “equilibrate” in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

MODULE II: PHOTODOCUMENTATION OF DNA (OPTIONAL)

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.

**FOR MODULE II-A
Each Student Group
should receive:**

- 1 InstaStain® card per 7 x 7 cm gel



Wear gloves
and safety goggles

**FOR MODULE II-B
Each Student Group
should receive:**

- 10 ml 10X concentrated FlashBlue OR 100 mL 1x diluted FlashBlue
- Small plastic tray or weight boat
- Distilled or deionized water

Experiment Results and Analysis

GEL DATA EXPLANATION:

Please read about Sickle cell gene analysis using a specific restriction enzyme in the background section.

Lane 1:

Control sample for Sickle cell patient

No restriction enzyme site is available in either of the two copies of the gene. No restriction enzyme digestion of either gene. The pattern will be one band on electrophoresis.

Lane 2:

Control sample for heterozygous Sickle cell trait

One of the pair of genes (the normal gene) contains the restriction enzyme site. This gene is cut by the restriction to produce two smaller pieces of DNA. The second copy of the gene has a mutation within the palindrome. This change does not allow the enzyme to cut the mutated gene into two smaller pieces. The pattern will yield three DNA bands on electrophoresis.

Lane 3:

Control sample for Normal homozygous patient

Both normal genes contain the restriction enzyme palindrome and both genes are digested to give the two smaller DNA bands.

Lane 4:

Mother DNA sample analysis

The mother is heterozygous for the sickle trait. One of the pair of genes (the normal gene) contains the restriction enzyme site. This gene is cut by the restriction to produce two smaller pieces of DNA. The second gene has a mutation within the palindrome. This change does not allow the enzyme to cut the mutated gene into two smaller pieces. The pattern will yield three DNA bands on electrophoresis.

Lane 5:

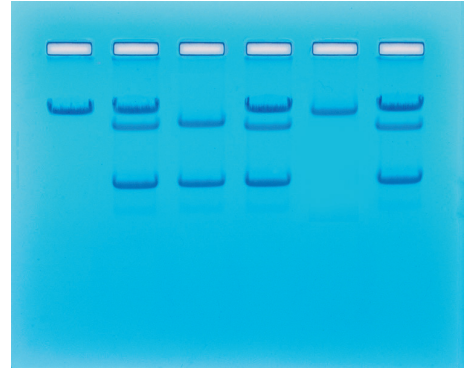
Child DNA sample analysis

The child is homozygous for the sickle trait and suffers from sickle cell anemia. Both genes have the mutation in the restriction enzyme palindrome. Neither will be cut by the restriction enzyme and the pattern will be a single DNA band on electrophoresis.

Lane 6:

Father DNA sample analysis

The father is heterozygous for the sickle trait. One of the pair of genes (the normal gene) contains the restriction enzyme site. This gene is cut by the restriction to produce two smaller pieces of DNA. The second gene has a mutation within the palindrome. This change does not allow the enzyme to cut the mutated gene into two smaller pieces. The pattern will yield three DNA bands on electrophoresis.



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

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

Appendices

- A EDVOTEK® Troubleshooting Guide
- B Bulk Preparation of Electrophoresis Buffer and Agarose Gels

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Appendix A

EDVOTEK® Troubleshooting Guides

PROBLEM:	CAUSE:	ANSWER:
Bands are not visible on the gel.	The gel was not prepared properly.	Ensure that the electrophoresis buffer was correctly diluted.
	The gel was not stained properly.	Repeat staining.
	Malfunctioning electrophoresis unit or power source.	Contact the manufacturer of the electrophoresis unit or power source.
After staining the gel, the DNA bands are faint.	The gel was not stained for a sufficient period of time.	Repeat staining protocol.
	The background of gel is too dark.	Destain the gel for 5-10 minutes in distilled water.
DNA bands were not resolved.	Tracking dye should migrate at least 3.5 cm (if using a 7x7 cm tray), and at least 6 cm (if using a 7x14 cm tray) from the wells to ensure adequate separation.	Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).
DNA bands fade when gels are kept at 4°C.	DNA stained with FlashBlue™ may fade with time	Re-stain the gel with FlashBlue™
There is no separation between DNA bands, even though the tracking dye ran the appropriate distance.	The wrong percent gel was used for electrophoretic separation.	Be sure to prepare the correct percent agarose gel. For reference, the Ready-to-Load™ DNA samples should be analyzed using a 0.8% agarose gel.
There's not enough sample in my QuickStrip™.	The QuickStrip™ has dried out.	Add 40 µL water, gently pipet up and down to mix before loading.

Appendix B

Bulk Preparation of Electrophoresis Buffer and 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. Measure 30 ml for a 7 x 7 cm tray, 50 ml for a 7 x 10 cm tray, and 60 ml for a 7 x 14 cm tray. **For this experiment, 7 x 7 cm gels are recommended.**
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.

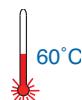


Table E Batch Prep of 0.8% UltraSpec-Agarose™					
Amt of Agarose (g)	+	Concentrated Buffer (50X) (ml)	+	Distilled Water (ml)	Total Volume (ml)
3.0		7.5		382.5	390