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# Mystery of the Crooked Cell

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An Investigation into the Molecular Basis of Sickle Cell Anemia

## Maryland Loaner Lab Teacher Packet



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# Mystery of the Crooked Cell

## Maryland Loaner Lab Overview

*The Mystery of the Crooked Cell* has two parts:

- A Pre-Laboratory classroom activity that explores the connection of hemoglobin to symptoms exhibited in sickle cell anemia.

- A laboratory activity that simulates a clinical test for sickle cell anemia using gel electrophoresis.

Teachers and students who will be performing *The Mystery of the Crooked Cell* laboratory activity using the Maryland Loaner Lab must first complete the Pre-Laboratory classroom activity. The conceptual aspects of the curriculum will be reinforced with the laboratory activity.

### Supplied by Maryland Loaner Lab Program:

Description	Quantity	Comments	Must Be Returned
Teacher packet (binder)	1	Contains all information necessary to conduct lab	Yes
Volumetric flasks	6	For Pre-Lab Activity B	Yes
Doughnut-shaped clay pieces	20	For Pre-Lab Activity B	Yes
Sickle-shaped clay pieces	20	For Pre-Lab Activity B	Yes
Practice Gels	10 Petri dishes	1 per group	Empty and Return
Practice Loading Dye	10 tubes	1 per group	Yes
Gel Electrophoresis box	1	With lid	Yes
Gel trays	6	5 trays + 1 extra	Yes
Gel combs	6	Makes 8 wells each	Yes
Gel tray dams	6 pairs	2 dams per tray	Yes
Power supply	1	For use with gel electrophoresis box	Yes
Spatula	1	For use when moving gels	Yes
Agarose (0.84g)	1 baggie	To make 6 gels	Return empty baggie
Orange-capped bottle	1	To make up agarose gels	Rinse and Return
Micropipettes	10	1 per group, either 20 $\mu$ l or 50 $\mu$ l	Yes
Micropipette Tips	5 boxes	1 box per two groups	Yes
10X TAE Buffer	3 tubes	Follow Teacher Prep for dilution	Rinse and Return
2-liter bottle	1	Used for mixing TAE buffer	Rinse and Return
Normal control samples	10 tubes ("A")	1 per group	No
Sickle control samples	10 tubes ("S")	1 per group	No
Patient (Unknown) samples	10 tubes ("P")	1 per group	No
Foam Microtube racks	10	1 per group	Yes
Microcentrifuge	1	Used to spin down sample tubes	Yes
Disinfectant Wipes	1	Used to disinfect returned equipment	Yes

### Supplied by the Teacher:

Description	Quantity	Comments
Distilled water	1350 ml	For making gels and running buffer
1-liter container	1	Used to make electrophoresis buffer
Graduated cylinder (100 ml)	1	Used for pouring gels
Disposable Cups	10	1 per group—to be a waste container at each workstation
*Goggles	varies	1 per student
*Gloves	varies	1 pair per student

**\*SAFETY: The classroom teacher must instruct students with basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.**

# Mystery of the Crooked Cell

## Maryland Science Core Learning Goals

These classroom and laboratory activities meet several of the MSDE Science Core Learning Goals:

### Goal 1.0 Skills and Processes

1.2.7 <i>The student will use relationships discovered in the lab to explain phenomena observed outside the laboratory. *</i>
1.2.6 The student will identify appropriate methods for conducting and investigation (independent and dependent variables, proper controls, repeat trials, appropriate sample size, etc.).
1.3.1 <sup>NTB</sup> The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques.
1.3.2 The student will recognize safe laboratory procedures.
1.3.3 <sup>NTB</sup> The student will demonstrate safe handling of the chemicals and materials of science.
1.3.4 <sup>NTB</sup> The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction.
1.4.2 The student will analyze data to make predictions, decisions, or draw conclusions.
1.5.1 The student will demonstrate the ability to summarize data (measurements/observations).
1.5.4 The student will use tables, graphs, and displays to support arguments and claims in both written and oral communication.
1.5.5 The student will create and/or interpret graphics. (scale drawings, photographs, digital images, field of view, etc.)
1.7.5 Students will investigate career possibilities in the various areas of science. **

### Goal 3.0 Concepts of Biology

3.3.2 <i>The student will illustrate and explain how expressed traits are passed from parent to offspring. *</i>
3.1.1 The student will be able to describe the unique characteristics of chemical substances and macromolecules utilized by living systems. (specifically proteins).
3.2.1 The student will explain processes and function of related structures found in unicellular and multicellular organisms. (transportation of materials – role of circulatory system)
3.3.3 The student will explain how a genetic trait is determined by the code in a DNA molecule.
3.3.4 The student will interpret how the effects of DNA alteration can be beneficial or harmful to the individual, society, and/or the environment.

\* Italicized CLG's are the primary focus of the laboratory activity. The other indicators are addressed, but can also be used as a guide to determine the focus of the lesson plans for the pre-lab and post-lab extension activities.

\*\* The provided DVD, MdBio Tech Council of Maryland, *Careers in Biotechnology* addresses 1.7.5.

***The Mystery of the Crooked Cell Middle School VSC***

1.A.1: Design, analyze, or carry out simple investigations and formulate appropriate conclusion based on data obtained or provided.

1.B.1.d: Describe the reasoning that lead to the interpretation of data and conclusions drawn.

3.B.2: Recognize and provide examples that human beings, like other organisms have complex body systems of cells, tissues and organs that interact to support an organism's growth and survival.

3.C.1: Explain the ways that genetic information is passed from parent to offspring in different organisms.

3.D.1: Recognize and describe that evolutionary change in species over time occurs as a result of natural variations in organisms and environmental changes.

3.D.1.b: Recognize that adaptations may include variations in structures, behaviors or physiological.

B.1.b: Indentify and describe that different individual people or groups of people are affected by an issue in different ways.

Grade 8 Health: Describe risk factors and behaviors that contribute to the development of non-communicable diseases.

# Mystery of the Crooked Cell

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## Introduction

Sickle cell anemia is a genetic disease that affects the hemoglobin molecule in red blood cells. Hemoglobin carries the oxygen that the red blood cells deliver to all the tissues and organs of the body. Normal red blood cells (having normal hemoglobin) are round like doughnuts and they are very flexible and able to move through small blood vessels in the body to deliver oxygen. Diseased red blood cells with sickle hemoglobin become hard and shaped like sickles used to cut wheat; they carry less oxygen to the body's tissues. When these hard and pointed cells go through the small blood vessels, they can cause clots and clog the blood flow. This can cause pain and tissue damage. Sickled red blood cells also do not live as long as healthy red blood cells and cause a low red blood cell count, or anemia.

Each person has two copies of the gene for 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 when considering hemoglobin A and S:

- AA** Individual is homozygous for the hemoglobin A gene. So both copies of the gene code for normal hemoglobin and the person does not have the disease.
- AS** Individual is heterozygous. One copy of the gene codes for normal hemoglobin and the other copy of the gene codes for sickle cell hemoglobin. This person does not have the disease and will not develop it later in life. However, this person is considered a **carrier** of the sickle cell hemoglobin gene. They are often referred to as having **sickle cell trait**, because they may exhibit a few of the symptoms occasionally.
- SS** Individual is homozygous for the sickle cell hemoglobin S gene. Having both copies of the gene code for diseased hemoglobin, this person suffers from sickle cell anemia.

The irregularly-shaped blood cells lead to a cascade of symptoms. The sickle-shaped red blood cells die prematurely resulting in anemia and the production of excess bilirubin (a yellow pigment resulting from the breakdown of hemoglobin protein). Jaundice, which is the yellowing of the skin and the whites of an individual's eyes, often results when the liver cannot metabolize bilirubin fast enough.

Infection, dehydration, overexertion, high altitude, or cold weather can bring on a sickling episode or crisis. Sometimes there is no apparent precipitating factor. People with sickle cell anemia are susceptible to fevers and infection. Patients with sickle cell anemia often will have abdominal pain (due to the spleen trying to process all the destroyed red blood cells), and joint and muscular pain (also due to the blood clots).

Currently, there is no cure for sickle cell anemia. There are treatments such as hydration, bed rest, painkillers, avoiding extreme temperatures, avoiding overexertion, and the use of antibiotics. Sometimes blood transfusions or even bone marrow transplants are required. Recent research has focused on re-expressing the fetal hemoglobin gene as a treatment for sickle cell anemia. After birth, the gene for fetal hemoglobin turns off while the gene for adult hemoglobin becomes activated. If the gene for fetal hemoglobin could be turned on again, it may compensate for the diseased adult hemoglobin and provide relief for people with sickle cell anemia.

To understand the origin of the disease, sickle cell anemia, one must understand that sickled cells serve as a protective mechanism against malaria. Malaria is a deadly disease, caused by a parasite transmitted by mosquitoes, and found in countries along the equator (in the tropics). People with sickle cell anemia are protected from malaria while those with normal hemoglobin are susceptible to it. Sickled red blood cells do not accommodate the malaria parasite. Over the years, people with sickle cell trait (those who are carriers of the sickle cell hemoglobin) migrated to other continents and that is why it is seen now in areas beyond the equator. Sickle cell disease is seen often in the African descendant populations, but is also seen in people of other ethnic groups. These ethnic groups include individuals from parts of the Middle East, Central India, and countries bordering the Mediterranean Sea, especially Italy and Greece.

This lesson is organized into two parts: a set of Pre-Laboratory classroom activities and a laboratory activity. During the Pre-Lab, students conduct learning activities and acquire clues about a mystery disease (sickle cell anemia). Each activity challenges the students to explore different aspects of sickle cell anemia. Working in groups, students manipulate models and gather data to construct an explanation of how sickle cell anemia affects the patient at the molecular level. Another Pre-Laboratory activity allows the students to practice using micropipettes and to practice loading agarose gels, as it can be a difficult skill to initially acquire. The *Practice Gel Loading Exercise* instructs students with the proper technique used to load gels and gives them the opportunity to practice before loading the samples involved in the laboratory activity. Following the Pre-Lab, students participate in the laboratory activity where they apply the concepts acquired in the Pre-Lab to test a fictional patient for the presence of sickle cell hemoglobin using gel electrophoresis.

**Note:** Additional web resources for information on sickle cell anemia are below.

<http://www.urmc.edu/medicine/genetics/sickleCell.aspx>

[http://www.sicklecelldisease.org/about\\_scd/](http://www.sicklecelldisease.org/about_scd/)

<http://www.ascaa.org/FAQ.asp>

<http://www.mayoclinic.com/health/sickle-cell-anemia/DS00324>

# Mystery of the Crooked Cell

## Pre-Laboratory Activities

**NOTE:** Groups using the Maryland Loaner Lab must first complete the Pre-Laboratory classroom activities. The conceptual aspects of the curriculum will be reinforced with the laboratory activity.

The purpose of the Pre-Laboratory Activities is to explore the connection of hemoglobin to symptoms exhibited in sickle cell anemia. It provides students with the opportunity to construct ideas and concepts about the mechanism of the disease.

### The objectives of the Pre-Laboratory Activities are:

- Observe a photograph of normal and sickled red blood cells
- Imitate the movement of red blood cells through the circulatory system to gather data and make inferences about sickle cell anemia
- Analyze an inheritance pattern using a pedigree
- Work cooperatively to explain the symptoms exhibited in sickle cell anemia
- Construct an explanation of the disease mechanism

### Pre-Laboratory Materials:

Students should work in 10 groups; each group will need to complete activities A, B, and C. Each student will need a copy of Student Pages S1-S4.

Students can work on Activities A and C at their desk or lab stations. For Activity B, set up each of two areas with the following materials:

- 3 empty volumetric flasks labeled, “Sickle Cell Patient”, “Carrier Patient” and “Normal Patient”
- Container with 10 normal red blood cells (doughnut-shaped pieces of clay)
- Container with 10 sickle-shaped red blood cells (sickle-shaped pieces of clay)

#### Activity A: Photo of red blood cells

Students will observe a picture of a blood smear showing a patient with sickle cell anemia. They are instructed to examine the image and describe what they see. They may notice the red blood cells are different shapes and that there are fewer sickled cells than round cells.

#### Activity B: Flow of red blood cells

In this activity, students will add differently shaped red blood cells to flasks that represent a patient with sickle cell anemia, a patient without sickle cell anemia, and a patient who is a carrier for the sickle cell anemia disease. Students should add a maximum of five of the appropriate type of red blood cell to each of the appropriate flasks. After students add the appropriate red blood cells to each flask, have the students try to slowly pour the red blood cells out of each flask and observe how well they flow out of the flasks. The doughnut-shaped red blood cells should flow easily, while the sickle-shaped red blood cells should mostly clog in the neck (because of their sickled shape).

#### Activity C: Pedigree with pedigree symbol key

For this activity, students will analyze the inheritance pattern of the diseased patient. They should write the genotype of each individual. For some individuals, they may be able to determine the exact genotype (for example, someone who died of sickle cell would be SS), but for others they may only be able to determine several possible genotypes. They should note that the disease appears to be hereditary, as the patient’s uncle

and grandmother were also affected and that the patient got sickle cell anemia because both parents were carriers.

### **Pre-Laboratory Engagement (10 – 15 minutes)**

Organize the students into 10 groups. Have each team read the description of the patient who came to Dr. Herrick, a Chicago physician, in 1904 (see the Patient Description sheet). Herrick (1861-1954) is credited with the discovery of sickle-shaped red blood cells. Thereafter, the disease was called sickle cell anemia based on Herrick's finding in 1904. The essential question is "What is the mechanism of the disease?" Instruct the students to make observations and gather clues about the condition described in the patient scenario. Ask the students to identify and underline any clues in the description that may help them determine the effect of the disease on the patient.

When they are finished, invite a student from each team to write one clue on the board. Discuss the clues as a class. Ask for clarification or expansion of ideas where appropriate. Encourage the students to think freely and make connections based on the evidence given in the patient description, as well as on their own experiences (students may know of someone who has, or may themselves have, sickle cell anemia or are carriers). The discussion usually leads to many good ideas about the mechanism of the disease.

### **Pre-Laboratory Exploration (15 – 30 minutes)**

Next, students will work through each of three activities. Each activity is comprised of tasks that in some way model or illustrate concepts relating to the mechanism of the disease. Give each group their set of activity guide sheets (Student Pages S2-S4), which include directions that encourage exploration. Urge the students to gather observations that may yield insights into the mechanism of the disease. Rotate the groups through all three activities, allowing about 5 - 10 minutes per activity.

### **Pre-Laboratory Explanation (10 – 15 minutes)**

After all of the groups have completed the three activities, ask the groups to develop an explanation for the mechanism of the disease. Next, ask the groups to present their explanations to the entire class. Encourage students to be creative in their presentations by giving them the option to present verbally, in writing, with diagrams or concept maps, or by using role-play. Students often generate many ideas and interesting topics for discussion. Encourage the students to debate their ideas and consider them in light of the observations they made. Challenge and elaborate on students' ideas to lead them to discover the following points:

- The blood cells are irregularly shaped (seen in photo)
- The irregular shape of the red blood cells interferes with their ability to flow through the blood pathways (inferred by the activity with the flasks and red blood cells made of clay)
- The condition is inherited (as seen with the pedigrees and genotype activity)

Refer to the activities to assist the students' discovery of the above points. Activity A indicates anemia and irregularly-shaped red blood cells as seen in the photo. The blockage created in blood vessels by the sickled cells is illustrated by the normal and sickled red blood cells in the flasks in Activity B. From Activity C, the family history suggests the possibility that the condition is inherited. At this point, the students are usually curious about the name of the disease. Let them generate their own name for the condition based on their understanding of it and emphasize that their name is just as valid as the name given by Dr. Herrick. He based the name on his observations of sickle-shaped cells and the decrease in the number of red blood cells, which causes anemia and therefore, he named the disease sickle cell anemia.

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## Laboratory Explanation

The purpose of the laboratory activity is to apply the concepts developed in the Pre-Laboratory to a clinical test for sickle cell anemia using gel electrophoresis.

### **The Objectives of the Laboratory Activity are:**

- ♦ Use gel electrophoresis to distinguish normal hemoglobin from sickle cell hemoglobin
- ♦ Interpret the results of gel electrophoresis
- ♦ Demonstrate the concept and process of gel electrophoresis

Before proceeding with the laboratory investigation, it is necessary to make a logical connection to the concepts developed in the Pre-Laboratory. In doing so, the laboratory activity becomes a tool in the continuum of an ongoing problem rather than an isolated end in itself.

### **Developing the Concept for the Laboratory Activity**

With the understanding of sickle cell anemia generated by the Pre-Laboratory, ask the students to consider ways to test for the disease. A common response is to examine the blood and look for signs of anemia or sickled cells. Anemia, however, is not unique to sickle cell anemia nor are the blood cells necessarily sickled unless the patient is in crisis. Furthermore, thalassemic blood samples frequently look very similar to sickle cell blood samples (thalassemia is a hemoglobin disorder associated with the defective synthesis of hemoglobin). Since hemoglobin is the molecule affected by the disease, the conclusion is to observe the diseased or affected hemoglobin for characteristics that would distinguish it from normal hemoglobin.

### **Developing the Concept for Gel Electrophoresis**

The next goal is to help the students realize the conceptual basis of the test that will help distinguish normal hemoglobin from affected hemoglobin (sickle cell hemoglobin). Raise the question by holding up a tube containing a sample of “hemoglobin” and ask whether they can identify it as normal or abnormal (use red food coloring and water to create a light rust color which simulates the color of both normal and sickle cell hemoglobin). The students realize that they first need to see what a normal hemoglobin sample looks like in order to identify whether the unknown is normal. Place control samples of “normal hemoglobin” and “abnormal hemoglobin” next to the unknown sample. Again ask whether they can identify which sample is normal and which is affected by visually comparing the three samples of “hemoglobin”. The samples look exactly alike in the tubes. Therefore, a tool is needed to distinguish between hemoglobin samples that look identical but have different properties. The tool, gel electrophoresis, will be used in the laboratory activity. Gel electrophoresis can be used because normal hemoglobin has a net charge of  $-2$  and sickle hemoglobin has a net charge of  $-1$ , and the samples will migrate differently in a gel because of their differences in charge.

## Electrophoresis Role-Play

A role-play may be used to demonstrate the theory behind electrophoresis. Have two groups of three students come to the front of the room. Each group represents a hemoglobin protein and each person represents an amino acid. Note that both molecules have the same number of amino acids and are, therefore, the same size. Give each student a card with a number representing a charge of -1 or 0. To one group assign two -1 charges and one 0 charge. To the other group give two people 0 charges and one person a -1 charge. Consequently one group has a net charge of -2 and the other group has a net charge of -1. Point out that the difference in overall charge between the two molecules cannot actually be seen with the naked eye. However, the charge difference does make the hemoglobin react differently in an electric field. Illustrate this concept by telling the class to imagine the classroom as an electrical field with the positive pole at the back of the room and the negative pole at the front of the room. In an electrical field, the negatively charged hemoglobin molecules migrate toward the positive pole. The group with a net charge of -2 will move more quickly because it has a greater negative charge drawing it toward the positive pole. Pretend to turn on the electricity and have the two groups of students migrate as the molecules would. The groups can be distinguished by their different rates of migration with respect to their net negative charge. To check student understanding, have the students predict and demonstrate the migration if the molecules both had a charge of -2.

## The Laboratory Investigation: Protein Electrophoresis

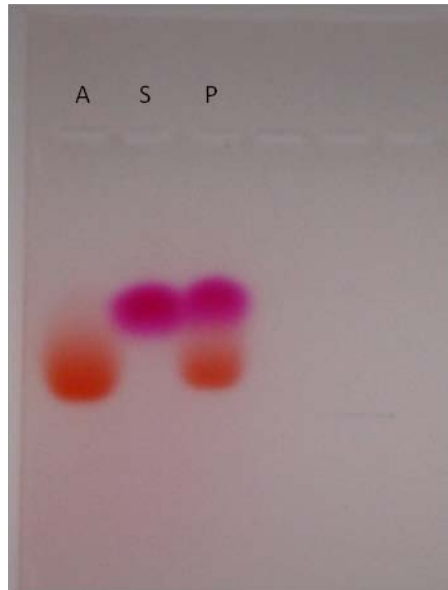
Students will work in 10 groups. Each group receives three samples of “hemoglobin”: **“A”**=Normal hemoglobin control, **“S”**=Sickle cell hemoglobin control, **“P”**=Patient hemoglobin sample. The patient samples may represent normal hemoglobin, sickle cell hemoglobin, or both in the case of a carrier. The samples of “hemoglobin” are put into an electrical field and the rates of migration compared. The negatively charged samples, either -1 or -2, will be attracted to the opposite charge and migrate towards the positive electrode in the gel box. The “hemoglobin” samples are really made up of dyes which will migrate through the gels as actual normal and sickle hemoglobin would. The agarose gels and electrophoresis buffer will be prepared in advance, but explain to the students how gels are made. Also, describe how the wells of the gels are made. It is recommended that the students load the gels dry, and then add the running buffer. The gels will run at 200 volts for 15 minutes. As the gels run, encourage the students to look through the lid or the side of the electrophoresis box to see their samples start to migrate.

## Interpretation of Results

The “Normal hemoglobin” control will have an orangish-red band which appears lower in the gel, as it runs faster with a -2 net charge. The “Sickle hemoglobin” control will have a pinkish-red band which appears higher in the gel, as it does not run as fast with only a -1 net charge. Patient results will vary. Some patient samples will display two bands (one orangish-red and one pinkish-red representative of sickle cell trait), others will be positive for sickle cell anemia (with only a pinkish-red band), while some will be negative for sickle cell anemia (with only an orangish-red band). Students may notice that the bands are different colors. This is because we use a dye, and not real hemoglobin, for this lab. You can handle this with your students in two ways: either inform them that we are using dyes and not real hemoglobin and that real hemoglobin does not differ in color between normal and sickle; or you could tell the students that the color is not a reliable indicator. Either way, make sure the students are only looking at distance migrated, rather than color, when interpreting results. Students should draw their results in the diagram on the lab protocol sheet.

## Picture of Hemoglobin Gel Electrophoresis Results:

Negative  
Electrode



Positive  
Electrode

To facilitate discussion, choose a representative gel of each outcome and put the gels on an overhead projector. Highlight the bands projected on the board with a marker. Some sample questions for discussion include:

What can be inferred from the results of the test?

How can the presence of two bands in some patient samples be explained?

# Mystery of the Crooked Cell

## Instructions for using a micropipette

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### Micropipettes

Micropipettes are precision instruments designed to measure and transfer small volumes. They are expensive and must be used with care. Their accuracy is dependent upon their proper use. Different brands of micropipettes vary in the volume range they will measure, the type of tips they fit, and the type of device used to set the volume. Be sure that everyone understands how to operate the micropipettes.

### Golden Rules of Pipetting

- 1. Don't rotate the volume adjuster beyond the upper or lower range of the pipette - this can damage it.*
- 2. Don't use a pipette without a tip on it. If this happens, liquid gets into the opening of the pipette and can damage the mechanism inside.*
- 3. Don't lay down a pipette that has a tip filled with liquid. If this happens, liquid can get inside the pipette and can damage it.*
- 4. Use new pipette tips between different samples to prevent contamination.*

### Basic Directions for Micropipette Use

#### Setting the Volume

All micropipettes have a volume control dial. Determine whether the volume window on your pipette shows tenths of microliters (0.1  $\mu\text{l}$ ) or whole microliters in the smallest place, so that you can read the scale correctly (it varies with different brand micropipettes).

#### Drawing Up and Expelling Liquid

Micropipettes have 2 stops as you depress the plunger to expel liquid. The first stop corresponds to the volume set in the window. The second stop gives a little puff of air to blow out any remaining liquid upon delivery. To draw liquid into the pipette tip, depress the plunger control only to the first stop. If you go to the second stop you will draw too much liquid into the tip. The most common pipetting error is to go past the first stop to the second stop for drawing liquid into the tip (which gives an inaccurate volume). When you are letting the liquid out of the tip, then you go to the second stop. It is worthwhile to check each student for correct technique before beginning laboratory procedures requiring use of the pipettes.

### *Using the Micropipette:*

1. Select the pipette that includes the volume range you will need.
2. Adjust the pipette to the desired volume by turning the dial. Do not turn beyond the volume range for the pipette.
3. Press a new tip onto the pipette firmly (gently tap the pipette into a tip while in the box). Get a tip without touching it with your hands; this is to prevent contamination of the samples.
4. To draw liquid into the micropipette tip:
  - a) Depress the plunger to the first stop to measure the desired volume and hold in that position.
  - b) Holding the pipette vertically, immerse the tip 1-3 mm into the liquid to be transferred.
  - c) Draw the fluid into the tip by slowly releasing the plunger. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip. If you see air bubbles, there is a problem with your volume and you will need to repeat this step to get the correct volume (either your tip wasn't immersed far enough down into the liquid or you perhaps raised your arm while releasing the plunger).
5. To dispense the liquid:
  - a) Place the tip into the container where the liquid is to be released, near the bottom.
  - b) Slowly depress the plunger to the second stop to blow out all of the liquid in the tip. Be careful not to suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just dispensed.
  - c) Eject the tip when done into a waste container by pressing the separate ejector plunger found on the top or side of the micropipette (depends on the brand of micropipette).

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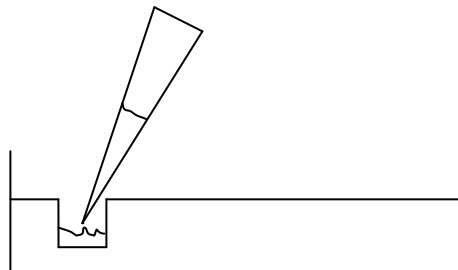
## Practice Gel Loading Exercise

“Loading gels”, or filling the wells of a gel, can be a challenging task, especially if one has never done it before. This is an opportunity to practice before you are asked to load the actual samples involved in the laboratory activity. Take your time, figure out how you feel most comfortable doing this (example: some people like to rest their elbow on the counter while loading), and practice filling a few different wells of the practice gels. Remember, this is for practice, so don’t get frustrated if you spill out of a well or if you accidentally tear the edge of the agarose gel – just try it again.

**An important thing to note about gels:** the wells appear as holes, but they really aren’t. They are more like indentations that do not go through completely to the bottom of the petri dish. This is why it is so important not to poke the micropipette tip through the bottom of the well, or else the liquid will seep into the bottom of the dish and not stay in the well.

### Loading the Practice Gels:

1. Become familiar with the feel of depressing the plunger all the way until it stops.
2. Then set the micropipette to **10  $\mu$ l**.
3. Make sure you gently tap a tip onto the end of the micropipette.
4. Remove the lid of the practice agarose gel and make sure you can clearly see the wells.
5. To suck up the practice loading dye into the tip, press down on the plunger until it stops, then place your tip into the liquid dye and slowly lift up your thumb. (Be careful not to raise your hand while lifting your thumb or you’ll get air bubbles and the volume will be incorrect)
6. Select a well to pipette the dye into.
7. Lower the tip filled with the dye into a well to be filled. Be careful not to poke through the bottom of the well or rip between the wells, or the liquid will not stay in the individual well you chose.



8. To release the dye from the tip, press down on the plunger until it stops. Next, lift up the micropipette so the tip is no longer in the well (or you may accidentally suck the liquid back into the tip).
9. Look to see if all of the dye went into the well.
10. Repeat this at least two or three times until you feel comfortable loading samples into a well. Each person in the group needs to practice loading wells in the practice gel. You do not need to change tips since you will be using the same liquid between group members.

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## Teacher Laboratory Preparation

Maryland Loaner Lab will supply reagents, equipment, and instruction for the laboratory activity for 10 groups. **Teachers must supply distilled water used for making gels and buffer.**

**Teachers must supply the students with the following handouts:**  
Pre-Laboratory Activities A, B, and C; Laboratory Protocol; and Student Worksheet.

### Prepare Student Stations (10):

- Foam microcentrifuge tube rack
- One tube each: “A”, “S”, and “P”  
(Use the microcentrifuge to spin down all samples; follow the instructions for operation found on box)
- Box of micropipette tips (1 box/2 student groups)
- One practice gel
- One practice loading dye tube
- One disposable cup (waste container for tips) (provided by teacher)

Activity:	Time needed:
Preparing Gels & Student Stations	30 minutes
Pre-Lab Activities	35-60 minutes
Practice Gel Loading Exercise	15 minutes
Laboratory Activity	30 minutes
Post-Lab Activity	10 minutes

### Shared Equipment for Multiple Groups:

- One agarose gel for every two groups (each group has 3 wells)
- One gel electrophoresis chamber (gel box) for all 10 groups
- One power supply for the gel box

## Electrophoresis: Gel Preparation and Directions for Running Gels

### Step 1 – Prepare 1X TAE Buffer (for making agarose gels and for use as a running buffer)

Buffer, and not water, must be used to make and run the gels. The buffer supplies the necessary ions to conduct electricity. The buffer received in the kit is 10X Tris-Acetate-EDTA (TAE) with 150 ml in three conical tubes, and needs to be diluted with distilled water (dH<sub>2</sub>O) to make a 1X concentrated solution.

1. Add the entire 150 ml of 10X TAE buffer to 1350 ml of distilled water in the 2-liter container provided and mix well. From this now diluted 1X TAE buffer, 120 ml will be used to make the agarose gels and 1000 ml will be used as the electrophoresis running buffer.

### Step 2 - Prepare a set of six 0.7 % agarose gels (5 gels for the activity with 1 gel extra.)

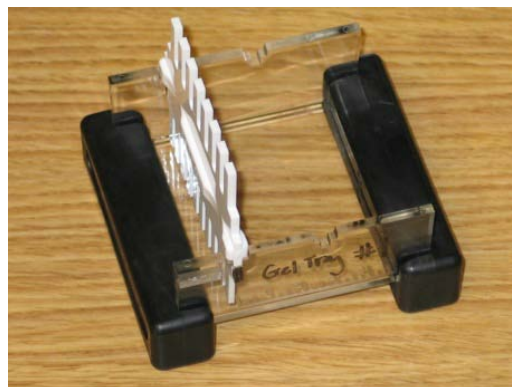
Agarose gels and running buffer may be made the night before use. This prep will make 6 small gels one of which is extra. Each gel will have 8 wells and will accommodate two groups of students with 3 samples each. Before making the agarose solution, have casting trays prepared and ready to be used (see Step 3). The kit includes one extra casting tray, which you will not need for this activity.

The glass bottle with 0.84 g agarose needs 120 ml of the diluted 1X TAE buffer from Step 1 (use the graduated marks on the bottle to fill to 120 ml). Add the buffer to the bottle and mix well with the agarose.

1. Dissolve the agarose in a microwave or on a hot plate. **The orange bottle cap must be removed before heating.** The power of the microwave may vary, but to prepare 120 ml of agarose it generally takes 1.5-2 minutes on high power. For best results place the bottle in the microwave for one minute, stir and heat for 30 more seconds, stir and heat another 30 seconds only if needed. **Do not over heat** as the liquid will boil out of the bottle and spill. It is best to microwave in small time intervals and to mix, then continue heating. The agarose must be completely in solution and well mixed. No particulate matter should be visible.
2. Cool the agarose solution to about 60°C by placing the melted agarose in a 60°C water bath or by allowing it to stand at room temperature for several minutes. **Swirl occasionally** while it is cooling to avoid rapid cooling of the agarose in the bottom of the bottle, in which it starts to solidify (or reheating will be necessary). The bottle of melted agarose solution is ready to be used when it is warm to the hand, but not too hot to handle (if it's too hot it can warp the comb and gel tray).

### Step 3- Casting Agarose Gels

1. Place the rubber dams onto the ends of each gel tray (it is easiest to lay the rubber dam on a table and, holding the gel tray, carefully press it into one corner, then use your weight to “roll” the gel tray into the second corner and repeat with the other rubber dam). **Use caution to prevent breaking the gel tray.**
2. Place the gel trays with rubber dams onto a flat surface.
3. Position the comb teeth down over the black mark (**Use the large teeth only, as it will create 8 wells of the needed size**).
4. Swirl the mixture and slowly (to avoid air bubbles) pour 20 ml cooled agarose solution into the 5 casting trays using a graduated cylinder (use a pipette tip to pop any air bubbles).
5. After the gel has hardened (about 30 minutes), gently remove the comb. It is important that the gels have completely solidified before the comb is removed.
6. Being very careful so that the gel does not slide off the gel tray, remove the two rubber dams from each end of the gel tray.
7. The gels may be stored by placing them in a zip-lock bag or other plastic container. Refrigeration is best, but not required.



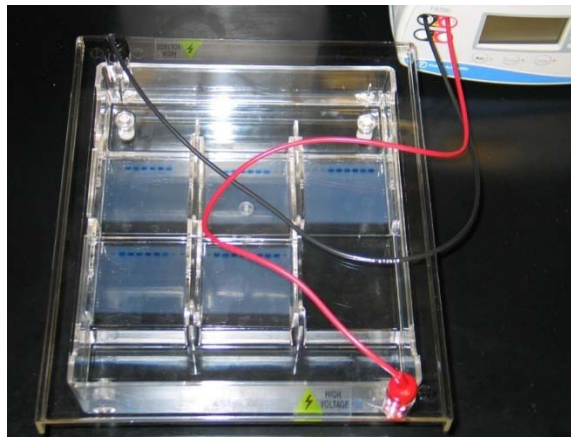
### Step 4 –Prepare Electrophoresis Running Buffer

1. Measure out 1000 ml of 1X TAE buffer from Step 1. This now is the electrophoresis running buffer that will be used to run the gels. The gel box requires approximately 1000 ml of running buffer. The buffer may be stored at room temperature or in a refrigerator.

### Step 5 – Electrophoresis of the Samples (following student Laboratory Protocol)

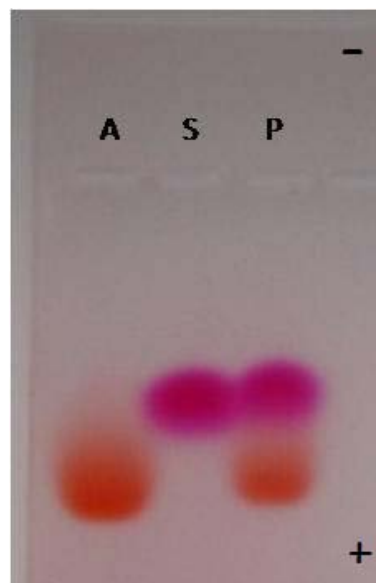
1. The electrophoresis gel box holds all six gel trays. The gel trays are labeled “1-6” with one being extra. Assign up to two student groups on one gel tray and assign three wells per group. Each gel has 8 wells, so assign wells #2-4 and wells #6-8 to the two student groups using each gel.
2. Next, the gels will be loaded dry at the students’ tables. Students will load 20  $\mu\text{l}$  of the hemoglobin samples to their assigned wells. Finally, the teacher will very carefully pick up the gel trays and add them to the gel box (notice there is a notch at the top of the gel tray that fits or “locks” into place in the gel box).

Be sure to **place the gel trays in the gel box so the ends containing the wells are closest to the black electrode**, or the samples will run backwards. This gel box holds two rows of gel trays so both rows must be oriented the same way in the gel box. (See picture).
3. Next, slowly pour 1000 ml of the **1X TAE running buffer** into the bottom chamber of the gel box (nearest the red electrode). **Do not pour the buffer directly onto the gel** or the samples may come out of the wells. The gels in the trays need to be completely submerged to run, but the top of the trays (sides) will be exposed out of the buffer while running.
4. Once gels have been placed in the gel box and the running buffer added, be careful not to disturb the electrophoresis apparatus.
5. Place the cover on the gel box matching black and red electrodes.
6. Connect the gel box with lid to the power supply, again matching black and red electrodes to the colors marked on the ports of the power supply.
7. Follow the printed directions found on the top of the power supply to start the run. The voltage selector on the power supply should be set to 200V and the timer should be set for 15 minutes.
8. To confirm proper operation of the power supply, look for bubbles rising from the electrodes and that the samples are moving in the proper direction (“running towards red”)
9. When the gels are done turn off the power supply and disconnect the lid of the gel box from the power supply.
10. Remove the gel trays from the box.
11. The hemoglobin bands are best visualized when viewed against a white background or even better on a light box.
12. When done with the running buffer it may be poured down a sink drain. Used gels can be disposed of in the trash.
13. After use, the gel box and trays should be rinsed with tap water and allowed to air dry.



## Step 6 – Interpretation of Results

The “Normal hemoglobin” control will have an orangish-red band, which appears lower in the gel as it runs faster with a  $-2$  net charge. The “Sickle hemoglobin” control will have a pinkish-red band, which appears higher in the gel as it does not run as fast with only a  $-1$  net charge. Patient results will vary. Some patient samples will display two bands (one orangish-red and one pinkish-red representative of sickle cell trait), others will be positive for sickle cell anemia (with only a pinkish-red band), while some will be negative for sickle cell anemia (with only an orangish-red band). Students may notice that the bands are different colors. This is because we use a dye, and not real hemoglobin, for this lab. You can handle this with your students in two ways: either inform them that we are using dyes and not real hemoglobin and that real hemoglobin does not differ in color between normal and sickle; or you could tell the students that the color is not a reliable indicator. Either way, make sure the students are only looking at distance migrated, rather than color, when interpreting results. Students should draw their results in the diagram on the lab protocol sheet.



To facilitate discussion, choose a representative gel of each outcome and put the gels on an overhead projector. Highlight the bands projected on the board with a marker. Some sample questions for discussion include:

- What can be inferred from the results of the test?
- How can the presence of two bands in some patient samples be explained?

# Mystery of the Crooked Cell

## Student Worksheet Answer Key

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6. What is the function of the agarose gel?  
*The agarose gel holds the samples and serves as a matrix in which different molecules can be separated. In this lab, different hemoglobin proteins (normal and sickle) are separated by their difference in charge*
7. Why is the gel in electrophoresis buffer?  
*The gel is in electrophoresis buffer to conduct electricity through the gel.*
8. What are the charges of normal and sickle cell hemoglobin? Which electrode on the gel box will the hemoglobin protein move towards?  
*Normal hemoglobin =  $-2$  and Sickle hemoglobin =  $-1$ ; since both Normal and Sickle hemoglobin are negatively charged, they will both move towards the positive electrode (red) as opposites attract.*
9. What must you be careful of when loading the samples into the wells of the gel?  
*You must be careful not to damage the gel (accidentally poke, rip, or tear the gel) and to make sure all of your sample goes into the well and hasn't spilled out of the well.*
10. Even though the hemoglobin in the normal and sickle controls look very similar in the tubes, how do they look different in the gel (talk about migration of bands, not colors)?  
*In the gel, the Normal hemoglobin will appear lower in the gel because it has traveled further down the gel due to its  $-2$  charge. The Sickle hemoglobin will appear higher in the gel (closer to the wells) because it hasn't traveled as far down the gel as the Normal hemoglobin due to its smaller negative charge of  $-1$ . The Normal hemoglobin has a greater negative charge and will, therefore, have a greater attraction to the positive electrode and will move faster than the Sickle hemoglobin with a smaller negative charge.*
11. Does the hemoglobin in sample P look more like hemoglobin from sample A or sample S? What is your diagnosis for the patient?  
*(Results will vary among student groups) If the Patient sample has Sickle hemoglobin, then the diagnosis would be Sickle Cell Anemia based on this test result and the patient's symptoms. If the Patient sample has both Normal and Sickle hemoglobin, then the diagnosis would be that of a Carrier for Sickle Cell Anemia (or known as Sickle Cell Trait). If the Patient sample has only Normal hemoglobin, then the diagnosis is unknown as only Sickle Cell Anemia can be ruled out. Based on the patient's symptoms, they have some disease state, but cannot be accurately diagnosed by this test, so additional testing will need to be performed.*
12. Explain to the patient how you determined their test results. Assume the patient is not familiar with the test, so you'll need to describe how gel electrophoresis works.  
*Students should explain all aspects of gel electrophoresis: how the gels hold the samples, why electrophoresis buffer and electricity is used, what happens to the differently charged hemoglobin molecules, and how Normal and Sickle hemoglobin controls are used to help determine the Patient's hemoglobin results.*

# Mystery of the Crooked Cell

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## Extension Activities

### ***Discussion Topics***

The investigation can serve as the centerpiece for a variety of topics to be explored further:

#### ***Central Dogma***

Using the amino acid sequence of the affected and normal hemoglobin, the students can identify the mutation from glutamic acid (also known as glutamate) to valine that results in the change in net negative charge of the affected hemoglobin. Working back through the central dogma, they can identify the point mutation in the DNA resulting in the amino acid alteration.

#### ***Inheritance***

Genotypes can be derived from the phenotypic results expressed on the gel and the probability of inheriting sickle cell anemia can be predicted given the genotypes of the parents.

#### ***Natural Selection***

The sickle cell allele is more prevalent in races whose gene pools originate in tropical areas. People of African, Asian, and Hispanic-Caribbean descent have a higher incidence of sickle cell anemia. Selective pressure for the allele results from its ability to decrease the mortality rate of people infected with malaria. Malaria is caused by a protoctist in the genus *Plasmodium*, which is transmitted to human hosts by mosquitoes. *Plasmodia* infiltrate red blood cells where they multiply and eventually rupture the cell. Cells with sickle cell hemoglobin are less susceptible to infection by *Plasmodia*. Therefore, carriers of sickle cell trait (heterozygotes) benefit from the presence of sickle cell hemoglobin while remaining largely asymptomatic (some heterozygous individuals may show mild symptoms) with respect to sickle cell anemia.

#### ***Random Mutation/Selective Pressure***

*Plasmodium* did not infect humans 10,000 years ago; it was an avian pathogen. However, a mutation in *Plasmodium* enabled it to jump species and infect humans. Ask the students whether they think anyone could have had sickle cell anemia prior to 10,000 years ago. It often leads to a discussion concerning random mutations and the distinction between a Lamarckian and Darwinian perspective on evolution.

#### ***Treatments***

Ask the students how they would treat sickle cell anemia based on their knowledge of the disease. Bone marrow transplants, blood transfusions, and gene therapy are often mentioned. Recently, attention has been given to turning on the fetal hemoglobin gene, which is turned off shortly after birth. Hydroxyurea, which has been used to treat cancer and blood disorders, has been found to stimulate the production of fetal hemoglobin.

## *Other Activities*

- Have the students write a letter to the fictional patient explaining the results of the test and the precautions the patient may want to consider taking.
- Use the documents beginning on the next page titled, *A Closer Look at the Cause of Sickle Cell Anemia*. This activity shows students how the sickle cell trait is determined at the genetic level. Have students identify the point mutation in the DNA resulting in the amino acid alteration.
- Sickle cell anemia is an example of one genetic condition for which there is a test but no cure. Have groups of students research other inherited conditions for which there is a test but no cure - for example; cystic fibrosis, Huntington's disease, muscular dystrophy, and fragile x syndrome. Each group can make an informative display about the disease. The display could be in the form of a poster, mobile, booklet, radio broadcast, interview, role-play etc. After each group has made a presentation about the disease, create a role-play in which a genetic counselor presents a scenario, for example:

Both parents are carriers for sickle cell anemia, cystic fibrosis, or any recessive disorder. The couple decides whether or not to try to have children.

One spouse's parent has been diagnosed with Huntington's disease. The couple has two young children. The counselor asks whether they want to be tested.

The role-plays can lead to interesting discussions among the students. The teacher can facilitate by writing down ideas and issues on the board as they come up.

### *Suggested readings:*

- Pelehach, Laura. (1995). Understanding Sickle Cell Disease. *Laboratory Medicine* (26), 720-725
- Ogamdi, S. and White, G. (1993). Sickle Cell Disease. *Clinician Reviews* (February), 65-82.
- The Sickle Cell Information Center: [www.emory.edu/PED/SICKLE/news.htm](http://www.emory.edu/PED/SICKLE/news.htm)

# Mystery of the Crooked Cell

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## A Closer Look at the Cause of Sickle Cell Anemia (Post-Lab Activity)

Your research has determined that sickle cell hemoglobin differs from normal hemoglobin in the net negative charge on the proteins. This discovery is an important one; it identifies a characteristic that can be used to diagnose sickle cell anemia. However, it does not tell us what causes sickle cell anemia or why the proteins are different. Advances in molecular biology and our understanding of DNA in the past two decades have provided us with more insights into the cause of sickle cell anemia. See if you can use the following data obtained from research in molecular biology to uncover more information about sickle cell anemia.

**Document 1:** The DNA base sequences of the first seven amino acids for both normal and sickle cell hemoglobin

**Document 2:** A chart of mRNA codons and their corresponding amino acids

**Document 3:** The structural formulas for the amino acids and their corresponding charges

# Document 1

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## The DNA Base Sequences of the First Seven Amino Acids for Normal and Sickle Cell Hemoglobin

The DNA sequence of bases for the first 7 amino acids in **normal** hemoglobin is:

5' **GTGCACCTGACTCCTGAGGAG** 3'  
3' **CACGTGGACTGAGGACTCCTC** 5'

The DNA sequence of bases for the first 7 amino acids in **sickle cell** hemoglobin is:

5' **GTGCACCTGACTCCTGTGGAG** 3'  
3' **CACGTGGACTGAGGACACCTC** 5'

### REMINDER:

5'...**ATGGCCTGGACTTCA**...3' **Sense strand of DNA**  
3'...**TACCGGACCTGAAGT**...5' **Antisense strand of DNA**



Transcription of Antisense Strand

5'...**AUGGCCUGGACUUCA**...3' **mRNA**



Translation of mRNA

Met-Ala-Trp-Thr-Ser- **Peptide**

## Document 2

### A Chart of mRNA Codons and Their Corresponding Amino Acids

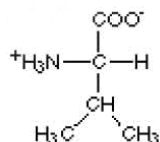
#### SECOND BASE OF CODON

		SECOND BASE OF CODON					
		U	C	A	G		
FIRST BASE OF CODON	5' End	U	C	A	G	3' End	THIRD BASE OF CODON
	U	Phe	Ser	Tyr	Cys	U	
		Phe	Ser	Tyr	Cys	C	
		Leu	Ser	Stop	Stop	A	
		Leu	Ser	Stop	Trp	G	
	C	Leu	Pro	His	Arg	U	
		Leu	Pro	His	Arg	C	
		Leu	Pro	Gln	Arg	A	
		Leu	Pro	Gln	Arg	G	
	A	Ile	Thr	Asn	Ser	U	
		Ile	Thr	Asn	Ser	C	
		Ile	Thr	Lys	Arg	A	
		Met(Start)	Thr	Lys	Arg	G	
	G	Val	Ala	Asp	Gly	U	
		Val	Ala	Asp	Gly	C	
		Val	Ala	Glu	Gly	A	
Val		Ala	Glu	Gly	G		

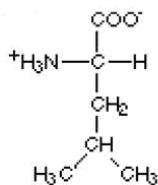
# Document 3

## The Structural Formulas for the Amino Acids

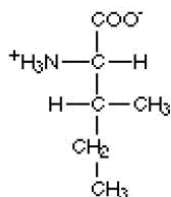
### Amino acids with hydrophobic side groups:



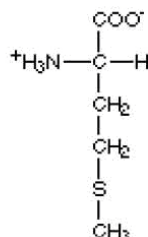
Valine  
(val) V



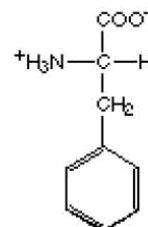
Leucine  
(leu) L



Isoleucine  
(ile) I

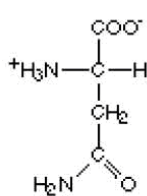


Methionine  
(met) M

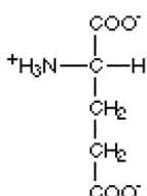


Phenylalanine  
(phe) F

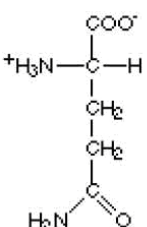
### Amino acids with hydrophilic side groups:



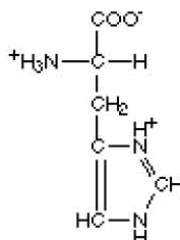
Asparagine  
(asn) R



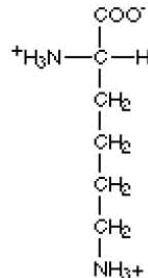
Glutamic acid  
(glu) E



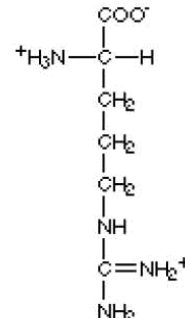
Glutamine  
(gln) Q



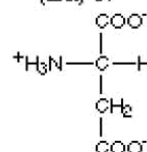
Histidine  
(his) H



Lysine  
(lys) K

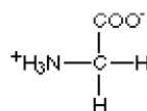


Arginine  
(arg) N

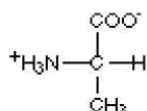


Aspartic acid  
(asp) D

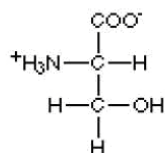
### Amino acids that are in between:



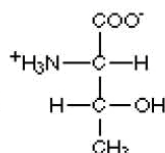
Glycine  
(gly) G



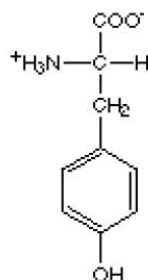
Alanine  
(ala) A



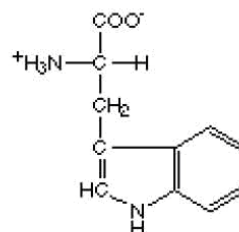
Serine  
(ser) S



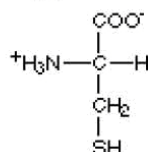
Threonine  
(thr) T



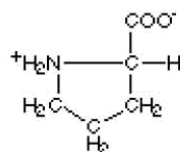
Tyrosine  
(tyr) Y



Tryptophan  
(trp) W



Cysteine  
(cys) C



Proline  
(pro) P

Name of group members: \_\_\_\_\_



## Mystery of the Crooked Cell

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### Patient Description Sheet

In 1904, a student from the West Indies came to a Chicago physician, Dr. James Herrick, with a puzzling condition. Below is a summary of some of the observations Dr. Herrick made. Your job is to learn more about this condition and to find out how the disease affects the body. Read the description below and underline the information that you think may provide important clues that will help you understand the disease.

The patient reports feeling well most of the time. But he also reports odd reoccurring events. For instance, one day after a short swim, he became so tired that he could hardly move. He became short of breath and complained of pain in his joints and muscles, especially his arms and legs. He felt unusually weak and required bed rest lasting a few weeks. These symptoms occurred repeatedly during his youth. He also had frequent fevers and infections.

The patient complained of fatigue and soreness in the joints. Upon inspection, the whites of his eyes had a yellowish tint. He complained of pain in the left abdominal area, which was tender to the touch.

A family history reveals that he has two brothers and three sisters. None of them have this condition. His uncle and his grandmother often had similar symptoms. His grandmother died a young woman. His parents do not have this condition.

*James Herrick*

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Names of group members: \_\_\_\_\_

## Mystery of the Crooked Cell

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### Activity A Guide Sheet

Observe the photograph below of human red blood cells from a patient who has the disease sickle cell anemia.

Describe in the space below what the red blood cells look like.



[www.pathology.vcu.edu/education/dental2/lab8.html](http://www.pathology.vcu.edu/education/dental2/lab8.html)

Name of group members: \_\_\_\_\_

## Mystery of the Crooked Cell

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### Activity B Guide Sheet

The flasks at this activity represent the pathways of blood in the body. As blood flows away from the heart, the arteries become narrower. Red blood cells must flow freely through the body in order for the blood to do its job of delivering oxygen and picking up wastes.

In this activity, the flasks represent the human body (specifically the veins and arteries where blood flows). The clay pieces represent red blood cells

- Doughnut-shaped red clay pieces represent red blood cells with normal hemoglobin protein
- Sickle-shaped (or banana-shaped) red clay pieces represent red blood cells that contain mutated hemoglobin that causes the cell to change shape (sickle)

Create three flasks to represent the following patients by filling each bottle with a maximum of 5 pieces of the appropriately-shaped red blood cells:

1. Normal patient
2. Sickle Cell patient
3. Carrier

Putting your finger over the top of the flask, slowly turn the flask upside down and observe the red blood cells as they flow through the neck of the flask, which represents an artery in the body.

After you are finished, empty the flasks for the next group.

#### Answer the following questions:

1. What do the doughnut-shaped red pieces of clay represent?
2. What do the curved, or sickle-shaped, red pieces of clay represent?
3. What do the flasks represent?
4. Describe the movement of the red blood cells in each flask:
  - a. Normal Patient:
  
  
  
  
  
  
  
  
  
  
  - b. Sickle Patient:
  
  
  
  
  
  
  
  
  
  
  - c. Carrier Patient:

Name of group members: \_\_\_\_\_

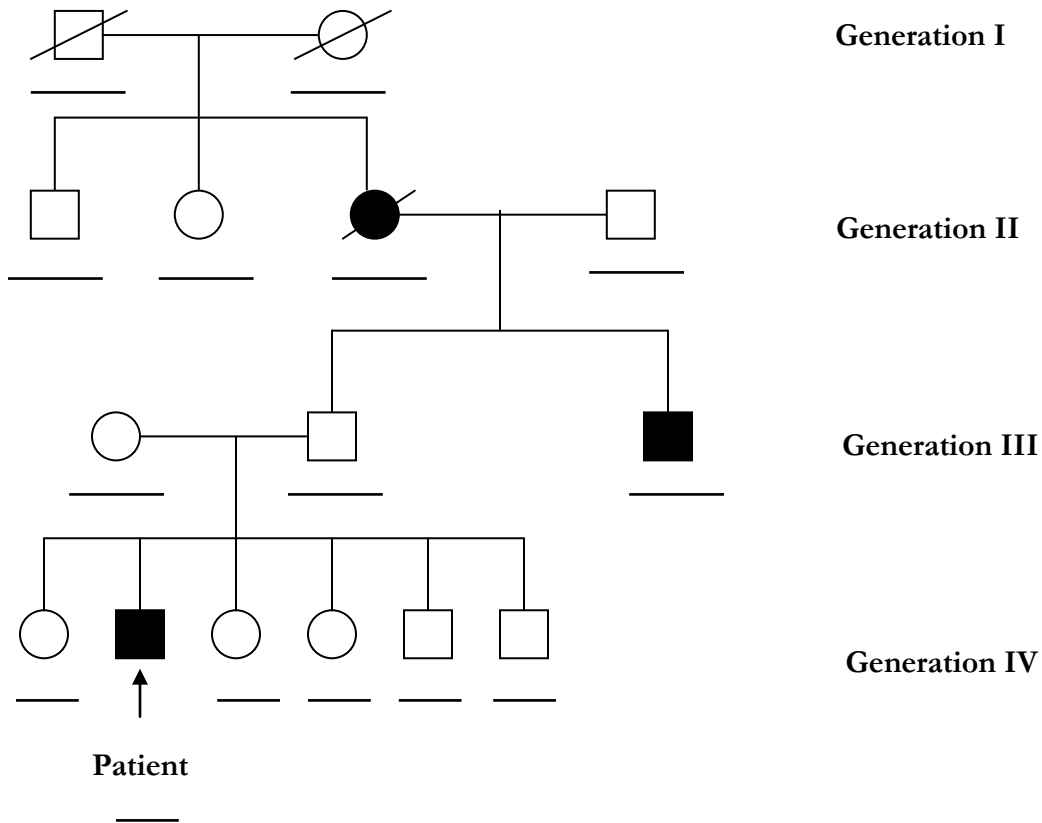
# Mystery of the Crooked Cell

## Activity C Guide Sheet

For the family history below, write the possible genotype or genotypes on the line under the symbol for each individual. Some individuals may have more than one possible genotype, while for some individuals you will be able to determine their exact genotype.

Normal Hemoglobin gene = A

Sickle Hemoglobin gene = S



**Key to symbols**

□ Male

○ Female

⧘ Deceased

┆ Offspring

■ Male affected

● Female affected

— Parents

# Mystery of the Crooked Cell

## Laboratory Protocol

### Loading Samples

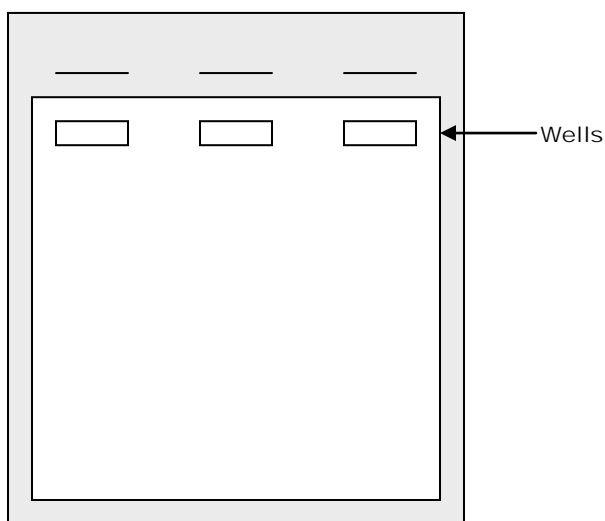
1. Your teacher will assign you a gel and a set of well numbers. Write your well numbers in the table:

Gel Tray Number:	
	Well Numbers
Normal Hemoglobin (A)	
Sickle Cell Hemoglobin (S)	
Patient's Hemoglobin (P)	

2. Load **20  $\mu$ l** of each of the normal hemoglobin control **"A"**, the sickle cell hemoglobin control **"S"**, and the patient's hemoglobin sample **"P"** into your assigned wells of the gel.  
**Use a new pipette tip between the different samples to prevent contamination.**
3. Once all samples are loaded into the gel, your teacher will place the gels in the electrophoresis box, pour electrophoresis buffer into the gel box and cover it with the lid. Your teacher will hook the box up to a power supply and start running the gel at 200 volts for 15 minutes.

### Sample Analysis

4. Observe the banding patterns on your gel. Do you see a difference between the normal and sickle cell hemoglobin controls? Compare the patient's hemoglobin to the normal and sickle cell controls; which one is it similar to?
5. Draw what you see on the gel in this picture. Be sure to label the wells with sample names, label both the positive and negative electrodes, and draw the direction of migration of the samples.



# Mystery of the Crooked Cell

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## Student Worksheet

6. What is the function of the agarose gel?
  
7. Why is the gel in electrophoresis buffer?
  
8. What are the charges of normal and sickle cell hemoglobin? Which electrode on the gel box will the hemoglobin protein move towards?
  
9. Why must you be careful of when loading the samples into the wells of the gel?
  
10. Even though the hemoglobin in the normal and sickle controls look very similar in the tubes, how do they look different in the gel (talk about migration of bands, not colors)?
  
11. Does the hemoglobin in sample P look more like hemoglobin from sample A or sample S? What is your diagnosis for the patient?
  
12. Explain to the patient how you determined their test results. Assume the patient is not familiar with the test, so you'll need to describe how gel electrophoresis works.