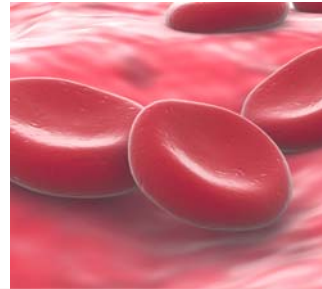


Mystery of the Crooked Cell[©]



A Sickle Cell Anemia Investigation

Student Guide

BioBus Educational Programs

Version 1

**Adapted From: © CityLab and Don DeRosa, Director of CityLab
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Version 1

STUDENT CASE BRIEF

In the Mystery of the Crooked Cell, you will become a diagnostic laboratory technician, helping the BioBus scientists determine whether patients have **sickle cell anemia**, are normal, or whether they are **carriers** of the disease. Before we can perform the diagnostic testing however, it is important that everyone involved is familiar with some of the science behind it. The information below will provide you with some of the facts necessary for you to make a diagnosis.

SICKLE CELL ANEMIA

Sickle cell anemia is an **inherited** blood disease characterized by **anemia**, and severe pain in the joints and muscle. The problem is a **mutation**, or defect, in the **hemoglobin** protein found in red blood cells. Red blood cells carry **oxygen** throughout the body, to the muscles, and organs. The part of the red blood cell that attaches to the oxygen molecules is hemoglobin protein. When the body is low on oxygen, such as after running up the stairs, normal red blood cells continue to function, keeping their plump, round shape (picture a donut, but without a hole in it). However, when people with sickle cell anemia are exposed to low oxygen levels, their red blood cells become hard and pointed, shaped like a half-moon. The cells are not able to carry oxygen to the body, and thus the person experiences a great deal of pain.

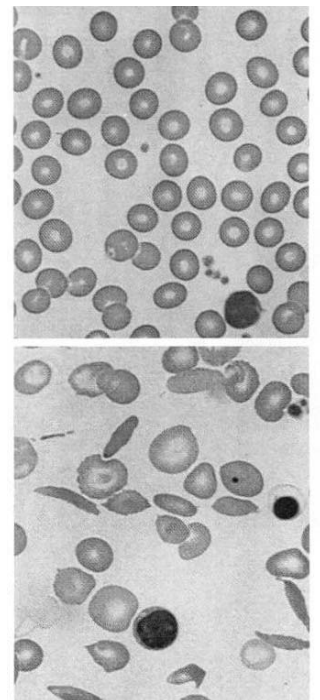
As we said earlier, sickle cell anemia is an inherited disease, which means a child receives this disease from his or her parents. For every **gene** in your body, you have one copy donated from your mother and one copy donated from your father. Therefore, you have two copies of every gene. A person needs *two* copies of the sickle hemoglobin gene in order to have sickle cell anemia; this is called being **homozygous** for sickle cell anemia. A person who does not have sickle cell anemia can be either homozygous normal (two normal copies of the hemoglobin gene) or they could be a **carrier** of the sickle cell trait referred to as **heterozygous** (they have one normal hemoglobin gene and one sickle.) Someone who carries the sickle trait does not have the disease but may pass on his or her DNA with another carrier and produce a child with the disease. It is estimated that 70,000 people have the disease in the United States and 1 in 1,000 babies are born with it each year.

Despite understanding what causes Sickle Cell Anemia there is no cure. The life expectancy of someone with the disease is their mid 40's. Patients are treated with antibiotics, pain killers, bed rest, and sometimes blood transfusions. A smaller number of patients have now been cured with bone marrow transplantation.

Fact Files

Sickle Cell Anemia and Malaria

While being homozygous for sickle cell results in a deadly disease, being heterozygous, or a carrier, can be beneficial, as it renders carriers resistant to the effects of **malaria**. This is invaluable in regions of the world where malaria kills thousands every year.



□ **Normal and Sickle Red Blood Cells.** Above is a staining of normal red blood cells. Notice they are round, uniform, and concave in the middle. Below is a staining of sickle red blood cells. Notice many are flattened, and are not uniform in shape.

SICKLE CELL ANEMIA: IT'S ALL IN YOUR DNA

DNA or **D**eoxyribo**N**ucleic **A**cid is found in the nucleus of cells. DNA is the blueprint of life, carrying all the genetic information necessary to make you who you are. DNA is made up of building blocks called nucleotides. Each nucleotide contains three parts: a sugar, a phosphate group, and a **base**. There are four bases in DNA: Adenine, Thymine, Guanine and Cytosine (we call them A, T, G, and C for short). In DNA, A always pairs with T, and G always pairs with C. These pairs of nucleotides are strung together into long chains, and take the shape of a **double helix**. Humans have about *3 billion* of these nucleotide “letters” in their DNA code. Some of this genetic code contains **genes**. Genes are pieces of DNA that carry the instructions for making **proteins**. Proteins are made up of **amino acids**. These amino acids are encoded in the order of A’s, G’s, C’s and T’s in a gene. Every three bases in a gene codes for one amino acid. For example, the base sequence CAG codes for the amino acid glutamine. The three bases that code for an amino acid are called a **codon**.

To help you visualize how DNA is organized, think of our entire genome (our entire genetic code) as one big book. Each chromosome would be a chapter in the book, and each paragraph would be a gene. Of course, if we tried to fit all 3 billion letters of our genetic code into one book, it would be over 200,000 pages long. Now that’s a lot of information to keep in a cell!

In 1949, **Linus Pauling** first proved that in sickle cell anemia, there is a problem with the hemoglobin protein. Vernon Ingram confirmed this observation when he found a genetic difference between the normal and sickle hemoglobin genes. This difference occurs at amino acid number six. In normal hemoglobin, the sixth codon is GAG (for the amino acid glutamic acid). In sickle hemoglobin, Ingram found that the sixth codon was GUG (for the amino acid valine).

Go Online!

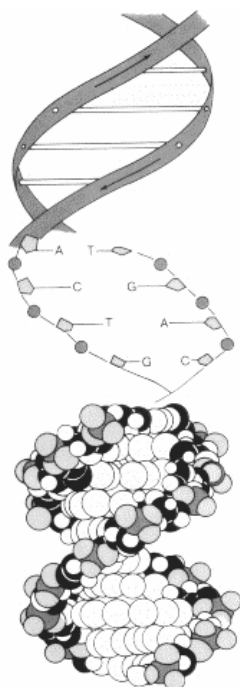
For: Double Helix Game
Visit:

http://nobelprize.org/medicine/educational/dna_double_helix/index.html



DNA Analogy

- Genome = Book
- Chromosome = Chapter
- Gene = Paragraph
- Exon = Sentence
- Intron = Blank Space
- Nucleotides = Letters



□ The structure of DNA.

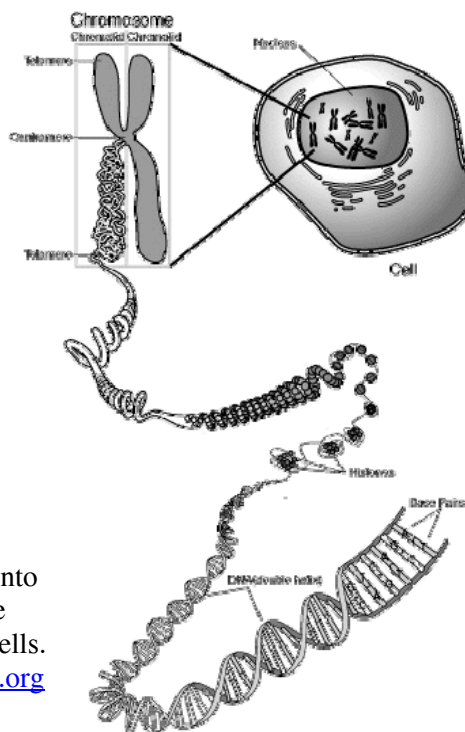
DNA is composed of a series of nucleotides which bind to each other through hydrogen bonding. In DNA, adenine always pairs with thymine and cytosine always pairs with guanine. The joining of the two DNA strands by hydrogen bonding forms the characteristic double helix structure of DNA. Photo source:

<http://academy.d20.co.edu/kadets/lundberg/images/biology/dna71.gif>

□ The organization of DNA.

DNA is tightly woven, through a complex of proteins called histones, into chromosomes. The chromosomes are housed in the nucleus of eukaryotic cells. Photo source:

www.accessexcellence.org



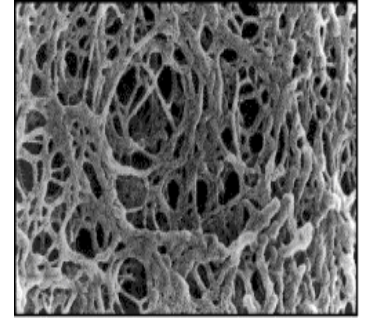
ELECTROPHORESIS AND SICKLE CELL ANEMIA

A diagnosis of sickle cell anemia is made by examining a blood smear. Carriers are identified by first exposing their red blood cells to very low oxygen; under these conditions, even blood from a carrier can be made to sickle. Then, to be certain of the diagnosis, hemoglobin agarose gel electrophoresis is used.

Agarose gel electrophoresis is used in the laboratory to separate molecules by size and charge. First, scientists make an agarose gel. **Agarose** is a sugar that comes from seaweed. When dissolved in hot water and cooled in a mold, the agarose becomes a gelatinous matrix. Think of the gel as a square piece of Jell-O with pockets in it (however you cannot eat the agarose gel). The picture to the right shows an agarose magnified under a powerful microscope. The gel is like a maze for molecules to run through. What size molecule (large or small) would be able to move through this maze faster?

To move molecules through an agarose gel, scientists use a technique known as **electrophoresis**. *Electro* refers to the use of electricity. The Greek verb *phoros* means “to carry across”. So, molecules are loaded into pockets within the agarose gel and are carried across the gel using electricity. In the gel electrophoresis chamber, there is a positive pole and a negative pole, which are used to generate the electricity. Because hemoglobin is negatively charged, it will move towards the positive pole (remember with charges, *opposites* attract).

In the case of sickle cell anemia, both the normal and sickle hemoglobin are the same size but they *do not* have the same charge. As mentioned above, sickle hemoglobin differs from normal hemoglobin by one amino acid. Sickle hemoglobin has *valine* as its sixth amino acid; it has a *neutral charge*. Normal hemoglobin has *glutamic acid* as its sixth amino acid; it has a *negative charge*. Both normal and sickle hemoglobin have a negative charge overall, but because of this one amino acid difference, normal hemoglobin is *more* negative than sickle hemoglobin. Thus, if normal and sickle hemoglobin are run through an electric field, you can easily diagnose a patient based on the different movements of the samples. Which do you think will run faster to the positive pole, normal or sickle hemoglobin? Why?



Scanning electron micrograph of an agarose gel, magnification 50,000 X. Source: www.amershambiosciences.com



Gel Electrophoresis

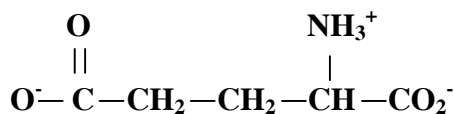
(Gel i-LEK-tro-for-rE-ss) –
The process of using electricity to separate molecules in a gel

Go Online!

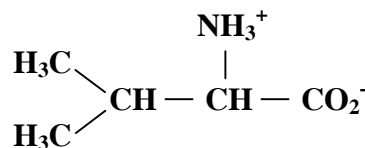
For: Gel Electrophoresis Animation

Visit:

<http://gslc.genetics.utah.edu/units/biotech/gel>



L-glutamic acid
glu



L-valine
val

□ **The amino acid structures of valine and glutamic acid.** The sixth amino acid in normal hemoglobin is glutamic acid, which has a negative charge. In sickle cell anemia, this amino acid has been changed to valine, which is neutrally charged.

PATIENT DESCRIPTION

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 the 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.

Dr. James Herrick

Name: _____

STATION A WORKSHEET

At this station you will use an overhead projector to observe the differences in both normal and sickle cell samples.

Describe (in writing or in pictures) the differences you see between the two blood samples.

STATION C WORKSHEET

At this station you will be given two sets of balloons. Each balloon represents a blood cell. One model represents a patient's blood cell and is labeled "P". The other model represents a normal blood cell and is labeled "N". You will also find a piece of flexible aluminum ducting. This ducting represents a blood vessel.

1. Keeping the blood vessel straight, investigate how the different types of blood cells travel through the blood vessel. **Record your results.**



2. However, blood vessels in the body are not straight. They have lots of turns and curves. Expand the blood vessel and create a couple of bends so the vessel looks like the picture below. Now pass the different types of blood cells through the blood vessel. Were there any differences? **Record your results.**



TRANSCRIPTION AND TRANSLATION EXERCISE

The DNA base sequences of the first seven amino acids for normal and sickle cell hemoglobin are provided below. For each, fill out the complementary DNA sequence (Hint: A binds T and C binds G). Then transcribe the *complementary* DNA into an RNA message (Hint: T is U in RNA). After transcribing the DNA, translate the RNA strand into a string of amino acids (Hint: three bases make up one amino acid.)

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

DNA: T A C C A C G T G G A C T G A G G A C T C C T C

COMPLEMENTRY DNA:

RNA:

AMINO ACID:

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

DNA:

COMPLEMENTRY DNA:

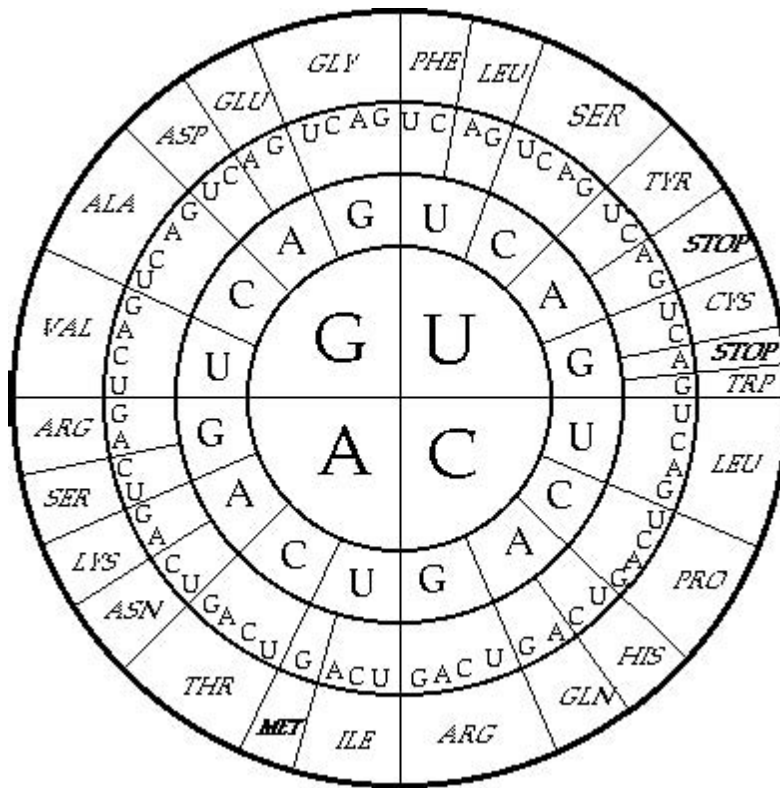
RNA:

AMINO ACID:

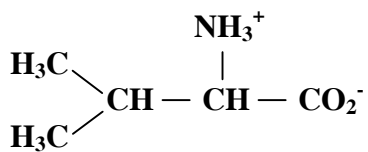
What is the difference between normal and sickle hemoglobin at the DNA, RNA and protein (amino acid) level?

How does this mutation affect the charge of sickle hemoglobin? How does that compare to the charge of normal hemoglobin?

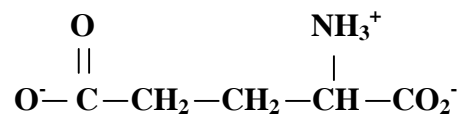
A chart of mRNA codons and their corresponding amino acids



The amino acid structures of valine and glutamic acid.



L-valine
val



L-glutamic acid
glu

Name: _____

BIOBUS LABORATORY NOTEBOOK

TITLE OF EXPERIMENT:

OBJECTIVE:

PREDICTION:

EXPERIMENTAL PROCEDURE:

BIOBUS LABORATORY NOTEBOOK CONTINUED

EXPERIMENTAL PROCEDURE CONTINUED:

RESULTS:

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CONCLUSION:

ALLELE FREQUENCIES AND SICKLE CELL ANEMIA LAB

Developed by: Jeanne Ting Chowning, BioLab, in partnership with the GENETICS Project

Objective: To observe how selective forces can change allele frequencies in a population and cause evolution to occur.

Introduction: Allele frequency refers to how often an allele occurs in a population. Allele frequencies can change in a population over time, depending on the 'selective forces' shaping that population. Predation, food availability, and disease are all examples of selective forces. Evolution occurs when allele frequencies change in a population!

In this activity, red and white beans are used to represent two alleles of β globin. The RED beans represent gametes carrying the β globin A (normal) allele and the WHITE beans represent gametes carrying the β globin S (sickle) allele. The gene pool exists in a region of Africa where malaria is highly prevalent. You are simulating the effects of a high frequency of malaria on the allele frequencies of a population.

Materials:

75 red beans, 25 white beans, 5 cups, 1 coin

Hypothesis/Prediction:

What do you think will happen to the frequencies of the A and S alleles as a result of the presence of malaria? (Will the frequency of A increase or decrease? What about S?) Formulate a hypothesis and corresponding prediction. Be sure to explain your reasoning.

Procedure:

1. Together with your lab partner, obtain five containers and label them as follows:
1) AA 2) AS 3) SS 4) Non-surviving alleles 5) Gene Pool
2. Place the 75 red and 25 white beans in the Gene Pool container and mix the beans up.
3. Simulate fertilization by PICKING OUT two 'alleles' (beans) WITHOUT LOOKING.
4. For every two beans that are chosen from the gene pool, another person will FLIP A COIN to determine whether that individual is infected with malaria.
5. Using the table below, the coin flipper tells the bean picker in which containers to put the beans.

Genotype	Phenotype	Malaria (Heads)	Not infected (Tails)
A A (Red/Red)	No sickle cell disease Malaria susceptibility	Die: place in Non-surviving	Live: place in AA
A S (Red/White)	No sickle cell disease Malaria resistance	Live: place in AS	Live: place in AS
S S (White/White)	Sickle cell disease	Die: place in Non-surviving	Live for a brief time: place in SS

- Repeat steps 3-5 until all the beans in the Gene Pool are used up.
- At the end of the round, COUNT the number of individual red beans (A alleles) and white beans (S alleles) in the containers labeled AA and AS. These individuals survive to reproduce. RECORD these numbers in the F1 TOTAL SURVIVING ALLELES table. Put them in the gene pool afterwards.
- Because SS individuals do not survive to reproduce, move all beans from the SS alleles container into the Non-surviving alleles container.

STOP AFTER ONE GENERATION.

CHECK WITH YOUR TEACHER BEFORE GOING ON!

- Repeat the procedure for the F2 generation. Record your results in the F2 TOTAL SURVIVING ALLELES table.

DATA SHEET

F1 TOTAL SURVIVING ALLELES: (very important to record)

Number of A (RED) alleles surviving (Count out all of AA and AS containers)	
Number of S (WHITE) alleles surviving (Count out of AS container)	

Put the survivors in the gene pool and create the next generation.

F2 TOTAL SURVIVING ALLELES: (very important to record)

Number of A (RED) alleles surviving (Count out all of AA and AS containers)	
Number of S (WHITE) alleles surviving (Count out of AS container)	

Class Results

On the class overhead, record your number of A alleles surviving for the next generation and number of S alleles surviving from both the F1 TOTAL SURVIVING ALLELES and F2 TOTAL SURVIVING ALLELES tables. Then record the class totals below and calculate the frequencies using the formula below.

Using the formulas below, calculate the % allele frequency for each allele in each generation:

$$\frac{\text{Total A}}{\text{Total A+S}} \times 100 = \% \text{ Allele A}$$

$$\frac{\text{Total S}}{\text{Total A+S}} \times 100 = \% \text{ Allele S}$$

Class Results Table

	Parents		F1		F2	
	A	S	A	S	A	S
Class Total						
Allele Frequency						

ANALYSIS QUESTIONS

Answer in complete thoughts!

1. What do the red and white beans represent in this simulation? What does the coin represent?
2. What do you think “Allele frequency” means? How are allele frequencies related to evolution?
3. What are the “selective forces” in this simulation (the forces changing the allele frequencies)?
4. What was the general trend you observed for Allele A over the three generations (did it increase or decrease)? What was the general trend for Allele S over time? Was your hypothesis supported?
5. Do you anticipate that the trends in question 4 will continue for many generations? Why or why not?
6. Since few people with sickle cell anemia (SS) are likely to survive to have children of their own, why hasn't the mutant allele (S) been eliminated? (Hint: what is the benefit of keeping it in the population?).
7. Why is the frequency of the sickle cell allele so much lower in the United States than in Africa?
8. Scientists are working on a vaccine against malaria. What impact might the vaccine have in the long run on the frequency of the sickle cell allele in Africa? (Would it increase or decrease? Why?)

Challenge Question:

What differences might the advent of new technology to diagnose and treat sickle cell disease and trait make on the frequencies of the A and S alleles in the population?

GLOSSARY OF TERMS

Agarose – A sugar isolated from red algae or seaweed, commonly used as a thickening agent in food. Agarose is used to make gels in gel electrophoresis.

Allele – One member of a pair or series of genes that occupy a specific position on a specific chromosome. For example, there are many different alleles for eye color, blue, brown, green, etc.

Allelic frequency – Refers to how often a particular allele occurs in a population.

Amino Acid - A molecule that is combined with others to form proteins.

Base – One of the molecules that form DNA and RNA.

Carrier – A person who has both the normal and mutant copies of a gene. They are heterozygous.

Codon – A set of three bases along the mRNA that codes for a particular amino acid.

Crisis – The time period when a person with sickle cell anemia is suffering from the symptoms of disease.

Gel electrophoresis – A scientific technique which uses electricity to separate molecules in a gel.

Gene – A Hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic in an organism.

Genome – The total genetic content contained in a haploid set of chromosomes in eukaryotes.

Hemoglobin – A protein component of red blood cells which binds oxygen.

Heterozygous – Having different alleles at one or more corresponding chromosomal loci.

Homozygous – Having the same alleles at a particular gene locus on homologous chromosomes.

Linus Pauling – The first scientists to show that sickle cell anemia was a problem with hemoglobin.

Malaria – A tropical disease carried by mosquitoes which can be fatal and is characterized by chills and fevers.

Micropipette - A scientific piece of equipment used to measure microliters, small amounts of liquid.

Missense mutation - A mutation where a nucleotide change results in the replacement of one amino acid for another.

mRNA – A type of RNA that serves as a template for protein synthesis.

Protein – A large molecule composed of one or more chains of amino acids in a specific order; the order is determined by the base sequence of nucleotides in the gene that codes for the protein.

Punnet square - A method for determining the offspring genotypes from two parents.

Red blood cells – A cellular component of blood which carried oxygen throughout the body.

Sickle Cell Anemia – An inherited blood disease that affects the hemoglobin protein in red blood cells.

Transcription – The synthesis of an RNA copy from a sequence of DNA.

Translation - The process in which the genetic code carried by mRNA directs the synthesis of proteins from amino acids.