In Mystery of the Crooked Cell, you will become a diagnostic laboratory technician, helping the BioBus scientists determine whether patients have a disease called **sickle cell anemia**. Before we can perform the 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 later make your diagnosis.

**SICKLE CELL ANEMIA**

Sickle cell anemia is an **inherited** disease that affects red blood cells. The disease is caused by a **mutation**, or defect, in the **hemoglobin** protein found in red blood cells. Red blood cells, and in particular the hemoglobin protein inside red blood cells, carry oxygen throughout the body. When the body experiences low oxygen levels, such as after running up the stairs, normal red blood cells continue to function, keeping their plump, round shape (picture a doughnut without a hole in the middle). However, when people with sickle cell anemia experience low oxygen levels, their red blood cells become hard and pointed, shaped like a half-moon or sickle (hence the name of the disease). These sickled cells can stick together and form blockages in blood vessels. They are also unable to carry oxygen throughout the body. As a result people with sickle cell anemia can experience 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. Remember, we inherit our **DNA** (our genetic information) from our parents. Some of the DNA we inherit makes up our **genes**. We inherit two copies of each gene; one from our mother and one from our father. These two copies can be the same, or they can be different. A person needs to inherit **two** copies of the mutated sickle hemoglobin gene in order to have sickle cell anemia. When you have two of the **same** copies of a gene, you are **homozygous** for that trait. 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 hemoglobin gene.) Someone who carries the sickle trait does not have the disease but may pass on their 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 sickle cell anemia each year.

Despite understanding what causes sickle cell anemia there is no cure for the disease, although a few patients have been successfully cured with bone marrow transplants. Patients are treated with antibiotics, pain medicine, bed rest, and blood transfusions.

▲ **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.

**Fact Files**

**Sickle Cell Anemia and Malaria**

While being homozygous for the sickle cell trait results in a severe disease, being heterozygous, or a carrier, can be beneficial. Sickle cell carriers are resistant to **malaria** a disease carried by certain mosquitoes. This protection is valuable in regions of the world where malaria kills thousands every year.
DNA or DeoxyriboNucleic Acid is found in almost every cell. 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. The particular amino acids in a protein are determined by the sequence 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. A sequence of three bases that codes for an amino acid is 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 was the first to demonstrate that sickle cell anemia results from abnormal 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 of the protein. In normal hemoglobin, the sixth mRNA codon is GAG (which codes for the amino acid glutamic acid). In sickle hemoglobin, Ingram found that the sixth codon was GUG (for the amino acid valine).

► 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 plant and animal cells. Photo source: www.accessexcellence.org

Go Online!
For: Double Helix Game
Visit: http://nobelprize.org/medicine/educational/dna_double_helix/index.html
**ELECTROPHORESIS AND SICKLE CELL ANEMIA**

**Agarose gel electrophoresis** is a technique used 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 liquid 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 gel magnified under a powerful microscope. The gel is like a maze for molecules to move through. What size molecule would be able to move through this maze faster? A big large or a small one?

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 through the gel by electricity. In the gel electrophoresis chamber, there is a positive pole and a negative pole, which creates an electrical circuit. 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 slightly more negative than sickle hemoglobin. So, if normal and sickle hemoglobin are placed in an electric field, you can easily diagnose a patient based on the different movements of the samples through the gel. Which do you think will move faster towards the positive pole, normal or sickle hemoglobin? Why?

- L-glutamic acid (glu) with a negative charge overall.
- L-valine (val) with a neutral charge overall.

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The sixth amino acid in normal hemoglobin in glutamic acid, which has a negative charge overall. In sickle cell anemia, this amino acid has been changed to valine, which has a neutral charge overall.
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 be important clues to 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
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 using the circular codon chart (remember, 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

COMPLEMENTARY DNA:

RNA:

AMINO ACID:

The DNA sequence of bases for the first 7 amino acids in Sickle 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 A C

COMPLEMENTARY 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

<table>
<thead>
<tr>
<th>Ala</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cys</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Asp</td>
<td>Aspartic Acid</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamic Acid</td>
</tr>
<tr>
<td>Phe</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Gly</td>
<td>Glycine</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
</tr>
<tr>
<td>Ile</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>Leu</td>
<td>Leucine</td>
</tr>
<tr>
<td>Met</td>
<td>Methionine</td>
</tr>
<tr>
<td>Asn</td>
<td>Asparagine</td>
</tr>
<tr>
<td>Pro</td>
<td>Proline</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
</tr>
<tr>
<td>Ser</td>
<td>Serine</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>Trp</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Tyr</td>
<td>Tyrosine</td>
</tr>
</tbody>
</table>

The amino acid structures of glutamic acid and valine.

L-glutamic acid (glu) and L-valine (val)
EXPERIMENTAL PROCEDURE CONTINUED:

RESULTS:

CONCLUSIONS:
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 (one of the genes that code for hemoglobin protein). 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 normal (A) and sickle (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, label each of your five cups as follows:
   Normal AA
   Carrier AS
   Sickle SS
   Non-surviving alleles
   Gene Pool

2. Place all red and white beans in the Gene Pool cup and mix them up.

3. Simulate fertilization by PICKING OUT two alleles (beans) WITHOUT LOOKING.

4. For every two beans that are chosen from the gene pool, one person will FLIP A COIN to determine whether that individual is infected with malaria.

5. Using the table on the next page, put each set of beans in the proper cup.
<table>
<thead>
<tr>
<th>Genotype (the alleles a person has)</th>
<th>Phenotype (what the alleles mean for the person)</th>
<th>Malaria (Heads)</th>
<th>Not infected (Tails)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (Red/Red)</td>
<td>No sickle cell disease, Malaria susceptibility</td>
<td>Die: place in Non-surviving cup</td>
<td>Live: place in AA</td>
</tr>
<tr>
<td>AS (Red/White)</td>
<td>No sickle cell disease, Malaria resistance</td>
<td>Live: place in AS cup</td>
<td>Live: place in AS</td>
</tr>
<tr>
<td>SS (White/White)</td>
<td>Sickle cell disease</td>
<td>Die: place in Non-surviving</td>
<td>Live for a brief time: place in SS cup</td>
</tr>
</tbody>
</table>

6. Repeat steps 3-5 until all the beans in the Gene Pool are used up.

7. At the end of the round, COUNT the number of individual red beans (A alleles) and white beans (S alleles) in the cups labeled AA and AS. These individuals survive to reproduce. RECORD these numbers in the F1 TOTAL SURVIVING ALLELES table. Put these beans back in the gene pool cup.

8. Because SS individuals do not survive to reproduce, move all beans from the SS cup into the Non-surviving alleles cup.

STOP AFTER ONE GENERATION. CHECK WITH YOUR TEACHER BEFORE GOING ON!

9. Repeat the procedure for the F2 generation, using up all the beans in your gene pool. 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 from AA and AS cups) |   |
| Number of S (WHITE) alleles surviving (Count from AS cup) |   |

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 from AA and AS cups) |   |
| Number of S (WHITE) alleles surviving (Count from AS cup) |   |

**Class Results**

On the class overhead, record your number of surviving A and S alleles from your tables above (F1 and F2). Also record the number of parent alleles you started with (this should be 75 A (red) beans and 25 S (white) beans, unless you were not given the exactly 100 beans to start with). Once all the groups have recorded their data on the class chart, record the class totals below and calculate the frequencies of A and S alleles using the formulas below:

\[
\frac{\text{Total A}}{\text{Total A+S}} \times 100 = \% \text{ Allele A} \quad \frac{\text{Total S}}{\text{Total A+S}} \times 100 = \% \text{ Allele S}
\]

<table>
<thead>
<tr>
<th>Parents</th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>S</td>
</tr>
<tr>
<td>Class Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele Frequency</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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.

**Allele 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 a normal and altered copy of a gene. They are heterozygous.

**Co-dominant** – Refers to both alleles of a genotype being expressed, resulting in both phenotypes.

**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.

**Dominant** – Refers to the allele that determines the phenotype or trait, hiding the effects of the recessive allele.

**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.

**Genotype** – The particular genes or alleles one carries.

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

**Heterozygous** – The presence of different alleles, as in having two different copies of a gene.

**Homozygous** – The presence of the same alleles, as in having two identical copies of a gene.

**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.

**Nucleus** – An organelle or part of a cell that contains genetic material.

**Phenotype** – The observable characteristic that is determined by the genotype.

**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.

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

**Recessive** – Refers to the allele where the phenotype is only seen in homozygous genotypes, or two of the same allele.

**Red blood cells** – A cellular component of blood which carries 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.