Background information

Scenario overview

In this lab, students are presented with a fictional family's medical history and must work to make a genetic diagnosis. The family depicted in this lab, the Robinson family, has two children, one of whom has had an initial test result indicating possible sickle cell disease.

Background information

Sickle cell disease is an inherited genetic disease that can lead to significant health problems including low numbers of red blood cells (anemia), repeated infections, and episodes of pain due to blockages of blood vessels. The cause of these and other symptoms is a structural change in hemoglobin, the protein responsible for carrying oxygen in red blood cells and the source of your blood's red color. A single amino acid change in one of hemoglobin's polypeptide chains can be life changing for many people.

Proteins are complex folded polymers made of amino acids. A normal protein consists of a chain of amino acid monomers anywhere from a few dozen to several thousand amino acids long. This long chain then folds into a very specific and complex three-dimensional structure. This three-dimensional structure is held together by several different types of interactions between both amino acids and other amino acids, and amino acids and the surrounding molecules in which the protein is found. An important type of these interactions is dependent on the hydrophobicity (relative attraction to water) of the different amino acids. Proteins dissolved in an aqueous (water based) solution, like the cytoplasm, typically have hydrophilic (attracted to water) amino acid side chains on the outer surface of the protein. These proteins have hydrophobic (water repelling) amino acid side chains on the inside of the protein where they are shielded from the surrounding solution.

Hemoglobin is this type of protein. Hemoglobin is a tetramer, meaning it is made of four smaller protein subunits, or polypeptide chains, that come together to make the final protein. The four smaller polypeptide chains that make up hemoglobin are two subunits of alpha-globin and two subunits of beta-globin. Each subunit can bind to one oxygen molecule and, in this way, hemoglobin is responsible for distributing oxygen throughout your body.

Sickle cell disease results from a simple substitution of just one amino acid in the beta-globin subunits of the hemoglobin protein. One hydrophilic amino acid (glutamic acid) is replaced by a hydrophobic amino acid (valine) in the sixth position of the beta-globin polypeptide chain. This change of valine for glutamic acid does not change the overall structure of the molecule, but it does mean that a single hydrophobic amino acid is now facing out in direct contact with water on the outside of the hemoglobin protein.

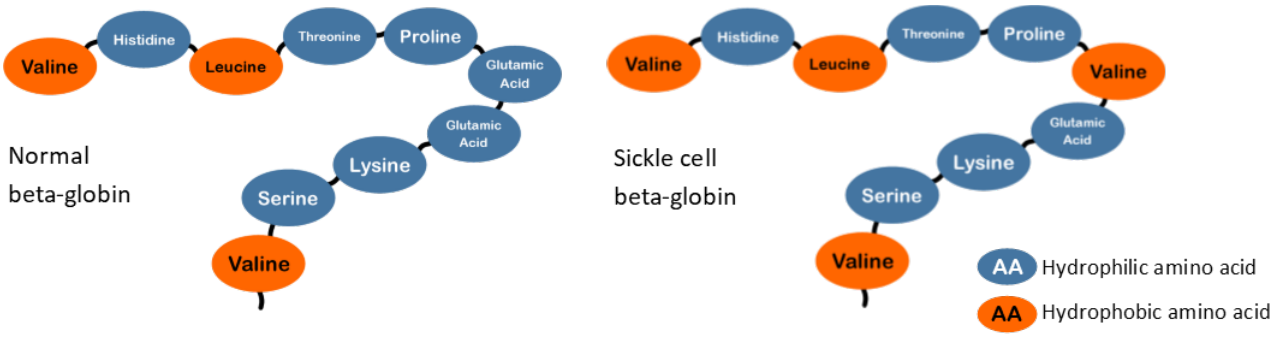


Figure 1: The first ten amino acids of normal beta-globin (left) and sickle cell beta-globin

Hemoglobin changes conformation slightly when it is bound to oxygen. In a sickle cell hemoglobin protein, when oxygen is present, the hemoglobin takes a shape that causes the hydrophobic valine to be shielded from the watery surrounding. When oxygen is absent, however, the protein changes conformation, exposing the valine to the cytoplasm. In a single hemoglobin molecule, this single hydrophobic amino acid being in contact with water would not be a significant issue. The problem occurs when many hemoglobin molecules in the same cell with the same hydrophobic valine begin interacting. The hydrophobicity of the valine means that when two valines from different hemoglobin proteins come in contact they will adhere (stick) to each other. In this way, both valines are now shielded from the surrounding cytoplasm. Because hemoglobin is a tetramer that contains two beta-globin proteins, individuals with sickle cell disease will have two hydrophobic valines on each hemoglobin protein, located on opposite sides of the protein. This means that hemoglobin proteins will start to form long chains inside the red blood cell, joined together by hydrophobic valine amino acids. When these chains become long enough, they can distort the shape of the red blood cell, giving them their namesake sickle shape.

This distorted shape is what can cause so many health problems for the individual. A major job of the spleen is to remove old or damaged red blood cells from the blood; sickled cells passing through the spleen may be recognized as abnormal and removed from the body. This lowers the average lifespan of red blood cells and the overall red blood cell count, making the person anemic. Sickled cells also tend to be much less elastic than regular blood cells, and this, combined with their abnormal shape, can cause the cells to become stuck in capillaries, the body's smallest blood vessels. Such blockages are referred to as a sickle cell crisis. Sickle cell crises tend to be incredibly painful and can lead to permanent tissue damage in the area of the blockage. When this occurs in the capillaries of the lungs, it is called acute chest syndrome and can be extremely dangerous. Because the spleen not only removes the damaged red blood cells but is also often itself damaged from their sickling events, individuals with sickle cell disease also often have reduced spleen function. As the spleen is central to the immune system, this can lead to increased rates of serious infection. To compound matters, infections can lead to conditions in the body that favor sickling. These symptoms typically first appear in babies a few months after birth because *in utero* the fetus produces a different form of hemoglobin, fetal hemoglobin, that is not affected by the sickle cell mutation.

Sickle cell disease is a recessive trait. That is, to have sickle disease, a person must have two copies of the sickle cell allele (referred to in shorthand as HbS). People who have either one or two copies of a normal beta-globin (HbA) allele will not be sick with sickle cell disease. Even though heterozygous individuals, those with one HbS and one HbA allele, do not usually show signs of sickle cell anemia, they are said to have the sickle cell trait, because they still produce some abnormal beta-globin. In these individuals' red blood cells there are abnormal hemoglobin molecules, normal hemoglobin molecules, and hemoglobin molecules that have both a normal and sickle cell version of the beta-globin subunit. In this case, when the hydrophobic valine in the abnormal beta-hemoglobin becomes exposed, the hemoglobin molecules will begin to clump together as in individuals with sickle cell disease, but because there is also normal beta-globin present, the long chains that form in sickle cell disease patients will generally not form. Without the long chains of hemoglobin, these individuals usually do not show any symptoms, though in extreme cases of prolonged low oxygen they may experience some symptoms of sickle cell disease.

The sickle cell and normal beta-globin proteins differ by a single amino acid. Likewise, in the beta globin gene, the nucleotide sequences of the normal and sickle cell alleles also differ by a single nucleotide – a change from adenine to thymine in position 20 of the beta-globin coding sequence (described further in Extension: Analysis of the Beta-Globin Coding Sequence.)

Prevalence of Sickle Cell

Sickle cell anemia is most often found in people from sub-Saharan Africa, or people whose ancestors are from sub-Saharan Africa, though it is also found at lower frequencies in peoples from some areas of the Middle East and regions of India. This distribution of sickle cell alleles reflects the historical distribution of the infectious disease malaria, which is transmitted by mosquito bites. This co-occurrence is due to the fact that carrying a single copy of the sickle cell allele confers some malarial protection (described in Extension: Sickle Cell and Malaria.) With human migration over the last few hundred years, people who are descended from those regions have brought the sickle cell allele with them all over the world. In the United States, approximately 1 in 100,000 babies will be born with sickle cell disease, but in African Americans the number of sickle cell births is much, much higher, approximately 1 in every 365 births. It is estimated that 1 in every 13 African Americans has the sickle cell trait[1].

Testing for sickle cell disease

In all states, newborn babies are routinely tested for sickle cell anemia as part of normal newborn screening procedures. Soon after birth, a doctor or nurse pricks the baby's heel to collect a drop of blood. From this drop, a medical lab tests for many different conditions. One test is to measure the amount of normal hemoglobin in the blood. If this level is too low, it does not necessarily indicate sickle cell anemia, but the child is then referred for further testing. A second confirmatory blood test is then performed. If the second test is positive, or still inconclusive, the patient is then usually referred to a hematologist who will perform genetic testing in consultation with a genetic counselor.

[1] https://www.cdc.gov/ncbddd/sicklecell/data.html.

The simplest genetic test for sickle cell is done using polymerase chain reaction (PCR) and restriction digest. PCR is a method for making many copies of a specific DNA sequence, in this case the beginning sequence of the sickle cell gene coding region. Restriction digest is a method of cutting DNA based on a defined DNA sequence.

To do a restriction digest, an enzyme is used that locates a specific DNA sequence, usually 4-8 base pairs long. The enzyme then cuts the DNA into two pieces. The mutation that causes the change from normal beta-globin to the sickle cell variant happens to be in the middle of one of these restriction enzyme recognition sites. The normal beta-globin allele has the sequence CTGAG from nucleotides 17-21 of its coding region. CTGAG happens to be the recognition sequence for the enzyme DdeI. When DNA containing this sequence is incubated with the DdeI enzyme at 37˚C, the enzyme cuts the DNA in two. In the sickle cell allele, the adenine (A) in the sequence is changed to thymine (T), meaning the sequence is now CTGTG. This means that the enzyme cannot cut the DNA into two fragments in the sickle cell allele. The difference in whether the DNA was cut or not can be observed on an electrophoresis gel.

Living with sickle cell

Most people with sickle cell trait (heterozygous for the sickle cell allele) will live normal lives, never experiencing any symptoms. Occasionally, athletes or other people undergoing extreme aerobic exercise or exercise in extreme environments will experience symptoms. People with the sickle cell trait should be careful in these circumstances. For example, there have been several high-profile cases of sickle cell trait individuals choosing to sit out of NFL games played at high altitude, such as in Denver's Mile High Stadium.

Sickle cell disease patients, on the other hand, will struggle with symptoms their entire lives and can expect a reduced life span as a result. In the United States, where sickle cell is routinely tested for and identified early in life, most sickle cell disease patients can expect to live to adulthood, with a typical life span of between 40 and 60 years. In less developed countries, childhood mortality rates due to complications with sickle cell are significantly higher.

The frequency and intensity of pain symptoms in sickle cell disease patients will vary greatly between individuals but will typically be managed through pain medication. Children with sickle cell disease are often kept on antibiotics for several years to combat the frequent infections that can often lead to childhood mortality. Children are also vaccinated against the pneumococcal pneumonia, one of the most common killers of children with sickle cell anemia.

Through fairly simple monitoring, most people with sickle cell disease can live relatively normal lives. Sickle cell crises are often started by known triggers, such as dehydration, high altitude, heavy exercise, among others. Avoiding these triggers can significantly reduce the number of sickle cell crises.

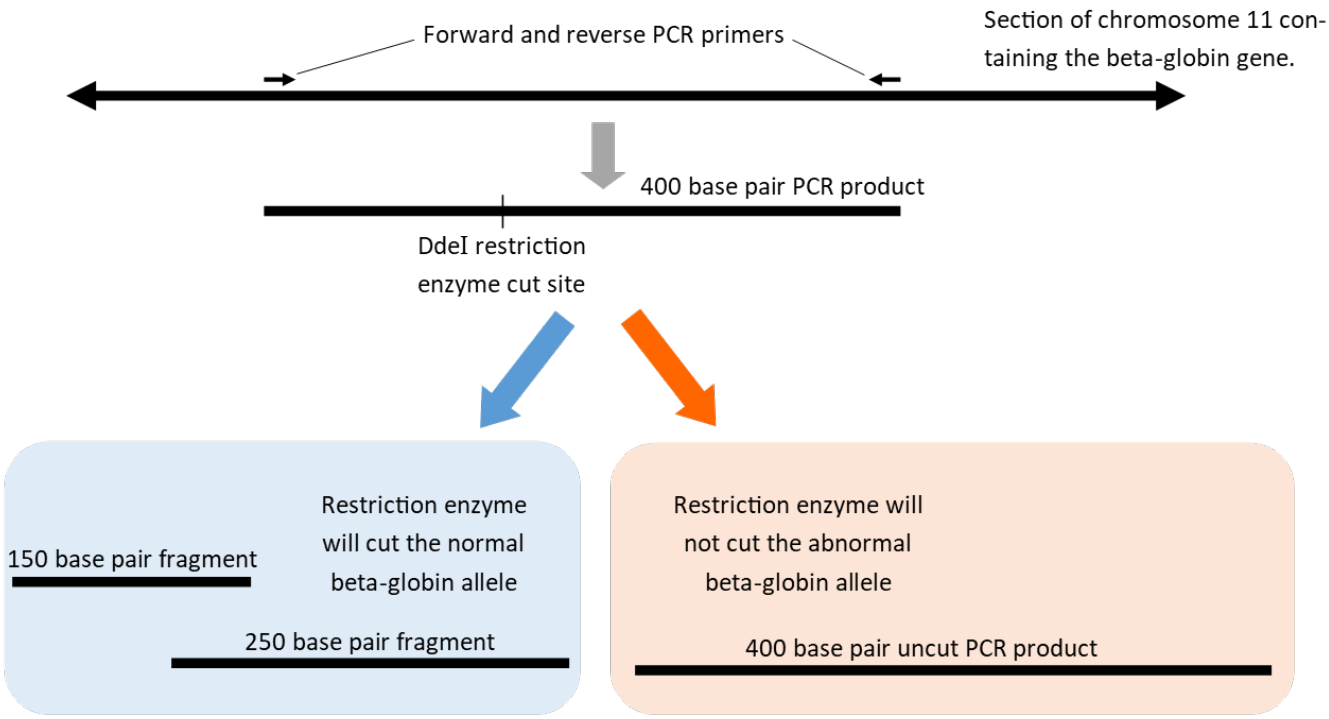
There is currently no cure for sickle cell disease, however, so patients can find themselves having to manage these symptoms for their entire lives.

Today's lab

In today's lab, you will analyze DNA samples from a family of four who were referred to genetic testing for sickle cell anemia. The DNA you have been provided represents a 400 base pair PCR product amplified from the beta-globin gene. That PCR product was incubated in the presence of the restriction enzyme DdeI at 37˚C. Your job is to run the DNA samples on an electrophoresis gel to determine for each family member whether they carry the sickle cell mutation and further assess if they are affected by sickle cell disease or sickle cell trait.

	Expected band lengths
Normal Hemoglobin	150, 250
Sickle Cell Trait	150, 250, 400
Sickle Cell Disease	400

Schematic showing how diagnostic DNA samples are produced



Important vocabulary

Polypeptide: A chain of amino acids. When a polypeptide is folded into a very specific three-dimensional form it is called a protein.

Hemoglobin: Protein found in red blood cells that binds oxygen and carries it through the body. Hemoglobin is comprised of four smaller protein subunits, two beta-globin subunits and two alpha-globin subunits.

Beta-globin: One of two globin polypeptide chains that make the protein hemoglobin. A mutation in beta-globin is responsible for sickle cell anemia.

Alpha-globin: One of two globin polypeptide chains that make the protein hemoglobin. Alpha-globin is unaffected by the sickle cell mutation.

Tetramer: A molecule made of four smaller subunits.

Hydrophobic: Lacking affinity for water. Hydrophobic molecules tend to be non-polar and lack a charge.

Hydrophilic: Attracted to water. Hydrophilic molecules tend to be polar or have a charge.

Patient medical histories

Robinson family: The Robinson family has been referred to genetic testing and counseling after their infant daughter, Marie, was identified in routine infant screening as possibly having sickle cell disease.

Jacqueline: Jacqueline is a 32-year-old female who was born in Port-au-Prince, Haiti. She immigrated to the United States with her family when she was 5 years old. She reports no abnormal medical history other than occasional migraine headaches. She has three surviving sisters, all of whom are in normal health. Jacqueline's older brother died from pneumonia as a 1-year-old while the family was still living in Haiti. Jacqueline is of primarily African descent. Jacqueline does not believe that she has ever been tested for sickle cell.

Cory: Cory is a 32-year-old male who was born and raised in the United States. Cory reports nothing abnormal in his medical history. Cory is one of three children. He has two older sisters, both surviving, who also have nothing abnormal in their medical histories. Cory is of primarily African descent. Cory has never been tested for sickle cell.

Samuel: Samuel is a 4-year-old healthy boy. Jacqueline reports that her pregnancy with Samuel was normal and that he has had no major illnesses. Samuel did not show any abnormalities in his routine infant screenings.

Marie: Marie is 2 months old. Jacqueline reports that her pregnancy with Marie was normal. A pre-natal screening program found low levels of normal hemoglobin in Marie's blood and she was referred to follow up testing. A second blood test was inconclusive.

Laboratory guide

Protective gloves and eyewear should be worn for the entirety of this experiment.

Gel electrophoresis - Pouring gels (before or during class period)

Important Gel Pouring Notes

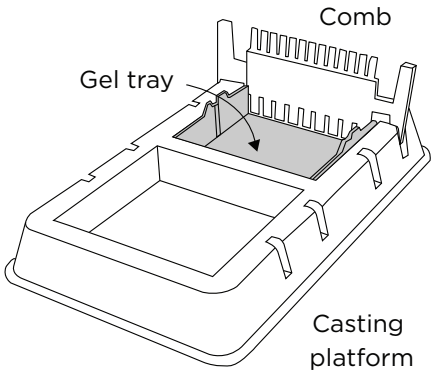
Gels can be prepared up to three days ahead of time and stored at ambient temperature, covered in air-tight plastic wrap and protected from light.

You will need four lanes plus one lane for ladder per group. It is possible for groups to share a gel by using two combs.

These instructions are designed for use with the blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.

1. **Prepare 1X TBE buffer (to be completed by teacher in advance)**
   - TBE buffer is often provided as liquid concentrate or powder.
   - Follow manufacturer's instructions to prepare 1X TBE buffer solution.

2. **Prepare a clean and dry casting platform with a gel tray and comb**
   - Place the clear gel tray in the white casting platform.
   - Place a well-forming comb at the top of the gel tray.



3. **Prepare a 2% agarose solution with a fluorescent DNA stain (e.g., SeeGreen™ or GelGreen®) using the method indicated by your instructor**

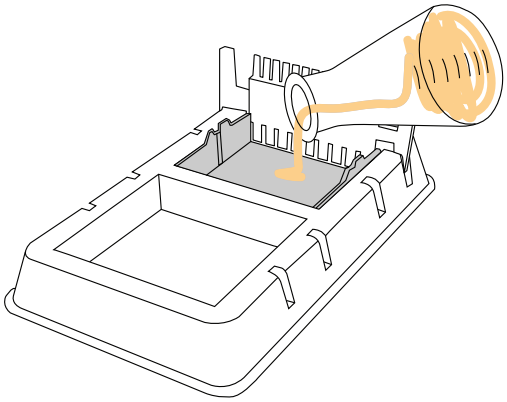
IMPORTANT NOTE: There are several ways to prepare agarose gels

- Scan the QR code for detailed instructions on how to prepare agarose gels.
- Both written and video instructions are available.

www.minipcr.com/agarose-gel/

4. **Pour the agarose solution into the prepared casting platform with a gel tray and comb**
    - The agarose solution should cover the bottom of the gel tray and the bottom 3 mm of the comb (roughly the bottom 1/3 of the comb).

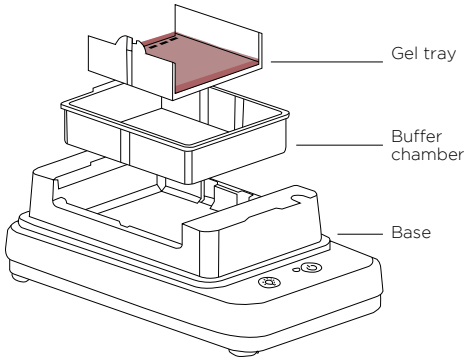
5. **Allow gel to solidify completely and remove the comb by pulling firmly upwards**
    - Gels will typically be ready in about 10 minutes.
    - Gel is ready when cool and firm to the touch.



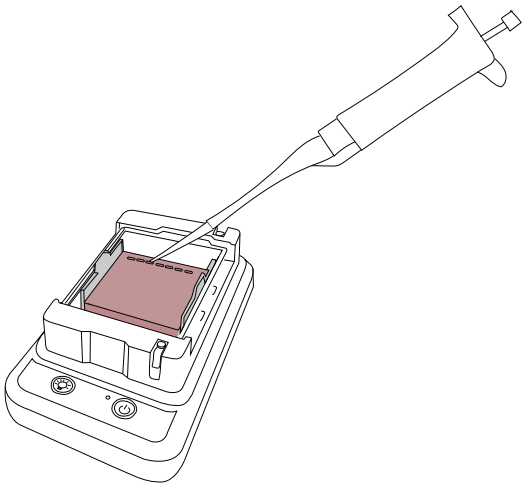
Protective gloves and eyewear should be worn for the entirety of this experiment.

Gel electrophoresis - Running the gel

These instructions are designed for use with blueGel™ electrophoresis system by miniPCR bio™. If using another electrophoresis system, these instructions may need to be adjusted according to the manufacturer's instructions.



1. **Place the gel tray containing your gel in the buffer chamber**
   - Ensure that the clear buffer chamber is inside the blueGel™ electrophoresis system.
   - The wells of the gel should be on the same side as the negative electrode, away from the power button.

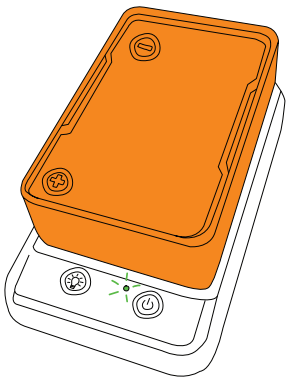


2. **Add 30 ml of 1X TBE electrophoresis buffer**
   - The buffer should just cover the gel and fill the wells.
   - Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

3. **Load samples onto the gel in the following sequence**
   - **Lane 1:** 10 µl DNA Ladder
   - **Lane 2:** 10 µl Jacqueline DNA
   - **Lane 3:** 10 µl Cory DNA
   - **Lane 4:** 10 µl Samuel DNA
   - **Lane 5:** 10 µl Marie DNA

   Note: Change pipette tips between samples to prevent contamination.

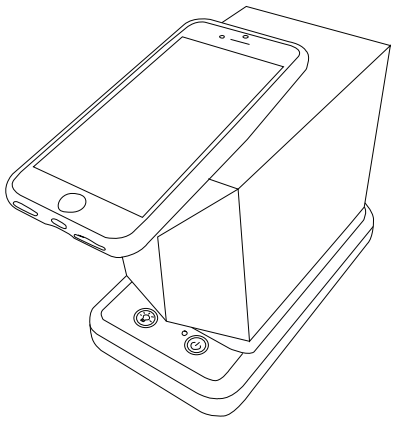
4. **Place the orange cover on the blueGel™ electrophoresis system**
   - To prevent fogging, make sure that ClearView™ spray has been evenly applied to the inside of the orange cover.
   - Match the positive and negative electrode signs on the orange lid with the corresponding positive and negative signs on the blue base.
   - The electrodes of the lid should be aligned with the metal leads on the base.
   - The orange lid should sit flush with the blue base using little force.



5. **Press the "Run" button**
   - Check that the green light beside the power button remains illuminated.

6. **Conduct electrophoresis for 20-30 minutes**
   - Note: Check the gel every 10 minutes to monitor sample migration.
   - Longer electrophoresis times will result in better size resolution. However, if run too long, small DNA fragments can run off the end of the gel or lose fluorescence.

Gel electrophoresis – Visualizing results



1. **Press the "light bulb" button to turn on the blueGel™ transilluminator**
   - For best viewing, dim lights or use Fold-a-View™ photo documentation hood with a smartphone camera.
   - Gels may be viewed at the end of the run or periodically throughout the run.
   - If the image appears hazy, wipe off the inside of the orange cover and reapply ClearView™ spray.

2. **Ensure that there is sufficient DNA band resolution**
   - Run the gel longer if needed to increase resolution.

3. **Document your results**
   - Place Fold-a-View™ photo documentation hood on the blueGel™ electrophoresis system to take a picture with a smartphone or other digital camera.
   - Compare the bands from the DNA samples to the ladder to obtain size estimates.

100 bp Ladder

Study questions

Pre-Lab: Questions after Background information.

1. Sickle cell anemia is caused by a change in the hemoglobin protein. Why then does the author spend so much time talking about beta-globin?

2. Describe why the presence of a hydrophobic amino acid on the outside of a protein can be problematic.

3. Looking at Figure 1, which amino acids do you expect to normally be found shielded from water in the final three-dimensional structure of the protein? Which amino acids do you expect to be exposed to surrounding water in the final three-dimensional structure of the protein? Justify your answer.

4. People who have sickle cell anemia produce normal amounts of hemoglobin. Why then are they anemic (have too few red blood cells)?

5. Explain why clumping hemoglobin proteins form long chains in sickle cell disease individuals.

6. Fighting infection in the body is the job of white blood cells, not red blood cells. If sickle cell disease affects red blood cells, which are responsible for carrying oxygen, why are sickle cell patients more susceptible to infection?

7. Why is sickling more likely to occur when the cell is low in oxygen?

Pre-Lab: Questions after reading family medical histories

8. After reading Jacqueline and Cory's medical histories, do you see any risk factors for sickle cell?

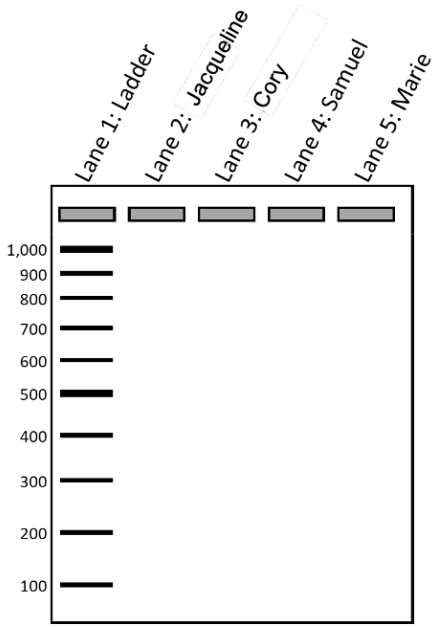
9. Is it possible for Jacqueline's brother to have died of sickle cell disease and no one else in her family to have the disease?

10. If Marie has sickle cell disease, what must we know about Cory and Jacqueline?

Lab: Questions during blueGel™ run

1. The illustration to the right shows a five-lane electrophoresis gel. Lane 1 contains a DNA ladder, showing how far bands of different size will migrate on a gel. Using the band sizes we expect from our restriction digest, predict what your gel will look like. Draw in the bands you expect to see for each individual based on the information you currently have.

2. Explain your predictions.



3. Explain the relationship between the three different sized bands we expect to see on the gel. If the difference between the HbS and HbA alleles is a single nucleotide, how do we see that difference by looking at lengths of DNA fragments?

Post-lab: Questions after DNA visualization

1. What is your genetic diagnosis of each member of the Robinson Family? State whether each family member has sickle cell disease, sickle cell trait, or is unaffected by sickle cell.

   Jacqueline:

   Cory:

   Samuel:

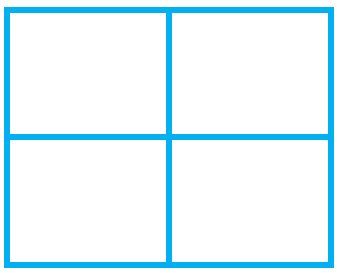
   Marie:

2. What color did the bands of DNA appear on your gel? Is DNA normally this color? Explain why it is here.

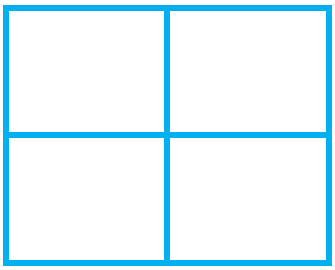
Questions using Punnett Squares and Pedigree analysis.

Use a Punnett square to answer the following questions. Use A to represent the normal, HbA allele. Use S to represent the sickle cell, HbS allele.

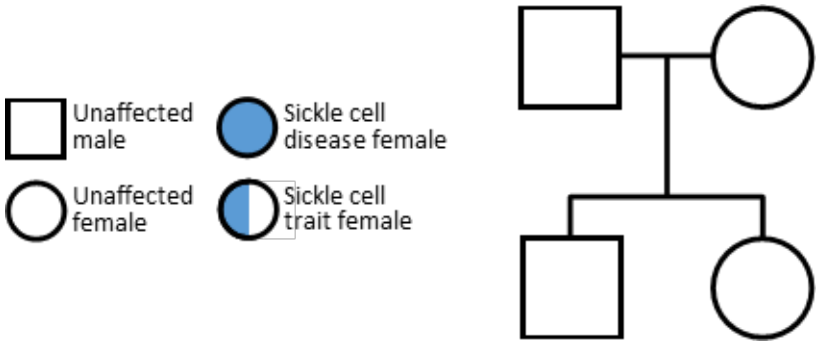
3. If two parents have the sickle cell trait, what is the chance that their child will have sickle cell disease?



4. If a person with sickle cell disease has children with a person who does not carry the HbS allele, can they have a child with sickle cell disease?



5. The following is a pedigree of the Robinson family that has not been filled in. Fill in the pedigree based on your data from the lab.

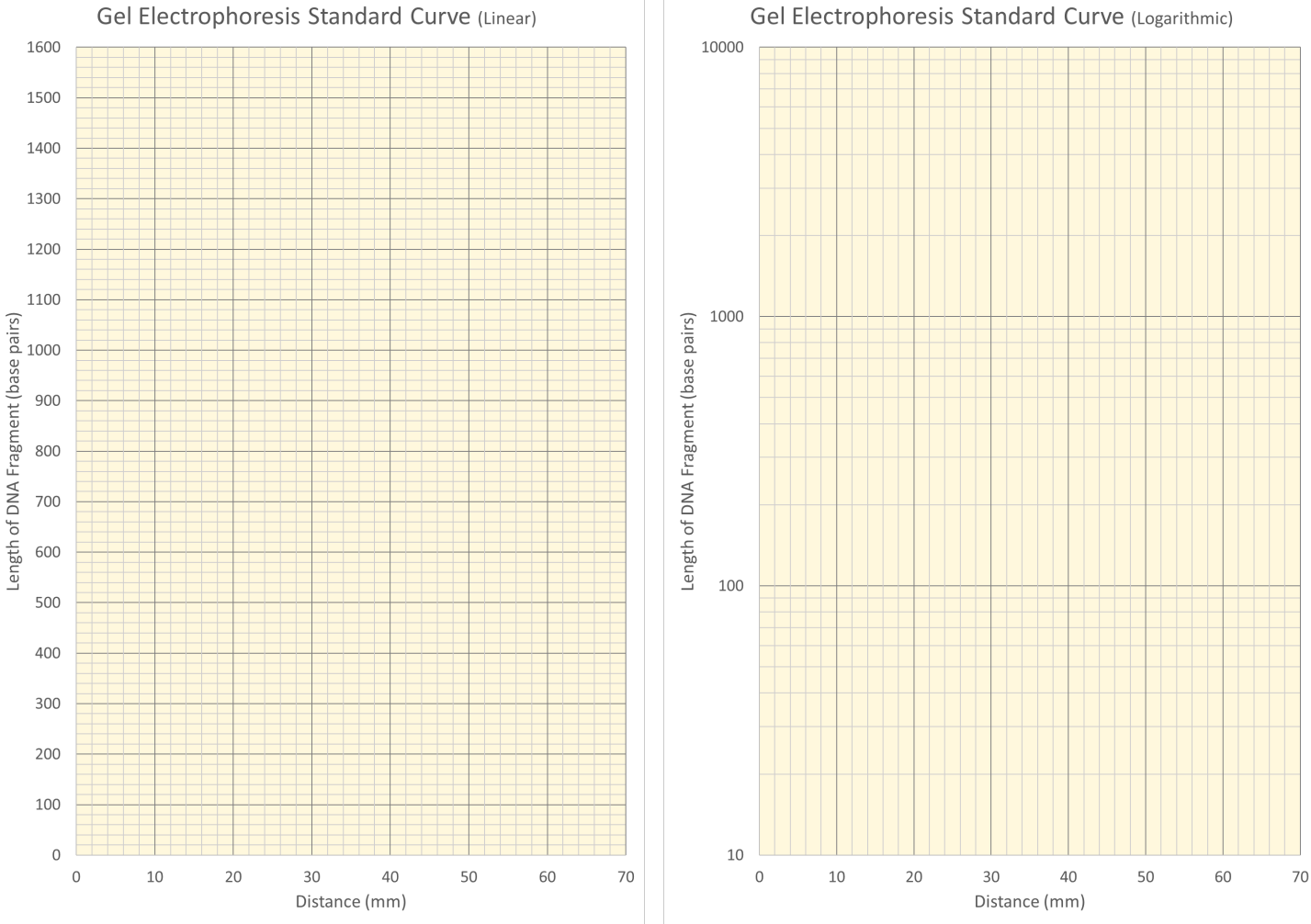


6. Give evidence from the pedigree that sickle cell disease is a recessive trait.

7. Add the rest of Jacqueline's family onto the pedigree. Assume that Jacqueline's brother was positive for sickle cell. Include every family member mentioned, and fill in as much information as possible.

Extensions

Extension: Creating an electrophoresis standard curve



1. Using a metric ruler, measure in millimeters the distance from the edge of the well to the center of each band in your DNA ladder.
2. Plot each point on the two graphs above. For each graph, the X axis is the distance traveled by each band measured in millimeters. The Y axis is the size of the band in the DNA ladder (See p. 16 for band sizes). Note that the scales of the Y axes are different for the two graphs.
3. Connect your points to make a curve/line.

Estimating unknown band size:

4. Pick one lane in your gel where there are three bands (an HbA/HbS heterozygote).
5. Measure the distance each band traveled from the edge of the well. This distance represents the X axis value for the unknown band.
6. Use the line that you drew in step 3 to estimate the size of the unknown bands.

Electrophoresis standard curve questions:

1. Describe the difference in the shape of the lines that you drew when plotting your lines on a linear scale versus a logarithmic scale.

2. Why do you think when making a graph like this people usually use a logarithmic scale?

3. The smallest band that you measured in your DNA ladder was 100 base pairs. Imagine you had a band that traveled 5 millimeters *farther* than your smallest band. On which graph would it be easier to estimate the size of that band?

4. According only to your estimates obtained from this graph, how big are the three fragments of unknown size that you measured on your gel?

5. Explain why a DNA ladder or another molecular weight marker is needed when running agarose gels.

Extension: Analysis of the beta-globin coding sequence

Shown below are the first 60 base pairs of the HbS and HbA alleles of human beta-globin coding sequence. Use these sequences to answer the following questions.

```
ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAAC
TACCACGTAGACTGAGGACTCCTCTTCAGACGGCAATGACGGGACACCCCGTTCCACTTG
          10        20        30        40        50        60
ATGGTGCATCTGACTCCTGTGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAAC
TACCACGTAGACTGAGGACACCTCTTCAGACGGCAATGACGGGACACCCCGTTCCACTTG
```

1. There are two sequences listed above. One is the normal beta-globin allele (HbA) and one is the sickle cell beta-globin allele (HbS). To distinguish between the two sequences, we use the restriction enzyme DdeI. DdeI recognizes the following sequence:

   C|TNAG
   GANT|C

   N, in this case, means that the middle nucleotide of the sequence could be any of the four DNA nucleotides. The sequences CTAAG, CTTAG, CTGAG, and CTCAG would all be cut by DdeI. The line indicates exactly where the cut happens in the DNA strand. Identify the cut site in the above sequences. Draw in the line where the restriction enzyme cuts as you see here.

2. DdeI only cuts the HbA allele (normal beta-globin). It does not cut the HbS allele (sickle cell beta-globin). Based on where you identified the cut site for DdeI, identify which strand is HbA and which is HbS.

Transcription and translation

3. The sequences above code for the first 20 amino acids of the beta-globin subunit. To make the protein, however, first the cell must transcribe the DNA sequence into mRNA. Use the bottom strand of each sequence as the template strand. What mRNA will be transcribed from each sequence?

4. Now that you have transcribed the DNA, use the mRNA Codon Table to translate the mRNA. What will the resulting amino acid sequence be for each sequence? Circle the difference between the two amino acid sequences.

5. Compare this amino acid sequence to the sequence shown in the "background information" section of the lab manual. Do you notice any differences?

mRNA Codon Table

First Position Nucleotide	Second Position Nucleotide: U		Second Position Nucleotide: C		Second Position Nucleotide: A		Second Position Nucleotide: G		Third Position Nucleotide
U	UUU	Phenylalanine (Phe, F)	UCU	Serine (Ser, S)	UAU	Tyrosine (Tyr, Y)	UGU	Cysteine (Cys, C)	U
	UUC		UCC		UAC		UGC		C
	UUA	Leucine (Leu, L)	UCA		UAA	STOP	UGA	STOP	A
	UUG		UCG		UAG		UGG	Tryptophan (Trp, W)	G
C	CUU	Leucine (Leu, L)	CCU	Proline (Pro, P)	CAU	Histidine (His, H)	CGU	Arginine (Arg R)	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Glutamine (Gln, Q)	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Isoleucine (Ile, I)	ACU	Threonine (Thr, T)	AAU	Asparagine (Asn, N)	AGU	Serine (Ser, S)	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lysine (Lys, K)	AGA	Arginine (Arg , R)	A
	AUG	Methionine (Met, M) START	ACG		AAG		AGG		G
G	GUU	Valine (Val, V)	GCU	Alanine (Ala, A)	GAU	Aspartic Acid (Asp, D)	GGU	Glycine (Gly, G)	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glutamic Acid (Glu, E)	GGA		A
	GUG		GCG		GAG		GGG		G

Extension: Sickle cell and malaria

Sickle cell disease causes lifelong illness and reduces overall lifespan. In some areas of Africa, it is estimated that 90% of children born with sickle cell disease do not live to the age of five. Alleles that reduce an individual's fitness in this way are expected to be removed from populations over time by natural selection. The question is then, why is the sickle cell allele (HbS) found at such high frequency in some areas around the world? The surprising answer: malaria.

Malaria is a blood-borne infection spread by mosquitos. The parasites that cause malaria all come from the genus *Plasmodium. Plasmodium falciparum* is the deadliest of these parasites and also responsible for most malaria infections. Humans become infected when a mosquito bites them, and *Plasmodia* then go on to reproduce in human red blood cells. When an infected person is bitten by a mosquito, the malaria parasite is taken up and can then spread to other individuals. Malaria kills over one million people every year, mostly children under five, and it infects several hundred million more, causing severe symptoms and perpetuating the transmission cycle.

When the malaria parasite infects red blood cells, it can cause those cells to sickle if the sickle cell version of beta-globin is present. For people who are homozygous for the HbS allele, this can be extremely dangerous. In individuals who are heterozygous, carrying one HbS allele and one HbA allele, however, just enough of their infected cells sickle that the spleen will remove those cells from the body, lowering their overall rate of infection from the *Plasmodium* parasite. Because of this, people with sickle

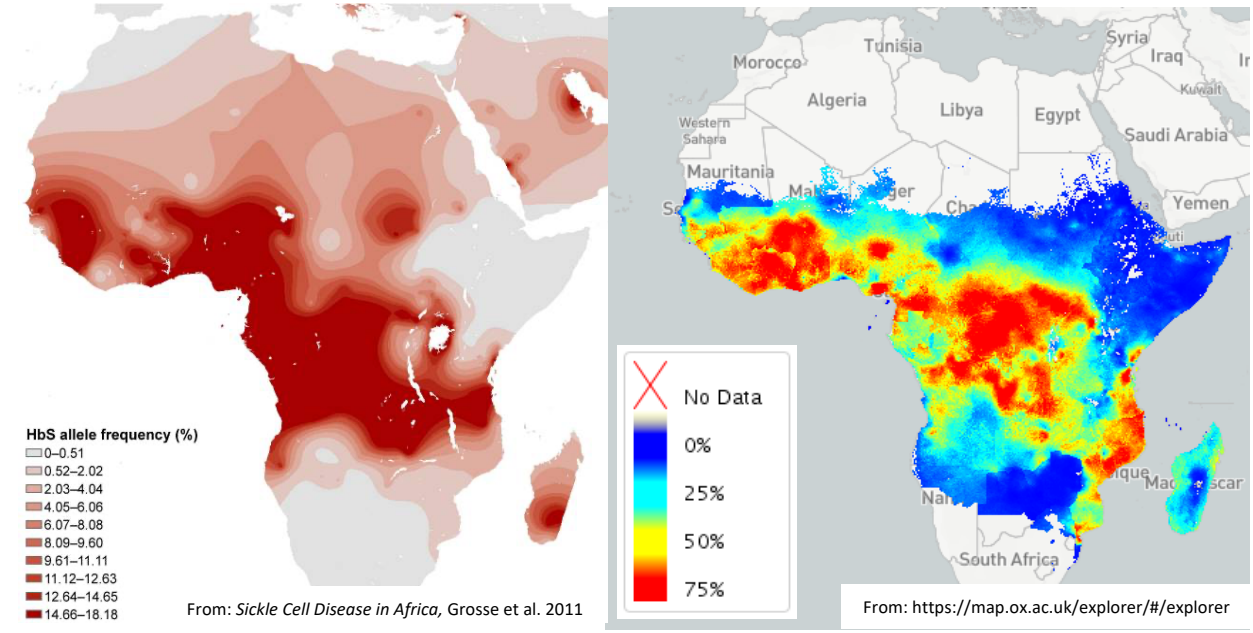


Figure 2.
Map A (Left) – Frequency of the HbS allele in Africa.
Map B (Right) – Percentage of 2-10-years-olds infected with *Plasmodium falciparum*, the parasite responsible for malaria, in the year 2000.

cell trait show some resistance to malaria. These people still get sick with malaria, but they are less likely to die from it. Looking at maps of the distribution of malaria and sickle cell anemia indicates how clearly the two maladies interact. In Africa, in areas where malaria is common, sickle cell is also extremely common; in areas where malaria is absent, generally so is sickle cell.

This is a case of what is known as *heterozygote advantage*. In heterozygote advantage, both forms of the homozygote are for some reason less fit than the heterozygote. In the case of beta-globin, an individual who is homozygous for HbA has normal fitness related to blood function, but is more likely to die from malaria. A person who is homozygous for HbS is likely to die from sickle cell anemia. People who are heterozygous, however, are less likely to die from malaria and show few if any symptoms of sickle cell.

When the sickle cell allele is rare in the population, most of the HbS alleles will be found in heterozygotes, and very few people will actually get sickle cell disease. In areas where malaria is present, these individuals will benefit from malarial protection and pass on the HbS allele, increasing its frequency in the population. As the sickle cell allele becomes more common, however, more individuals will be born as HbS homozygotes. Because sickle cell disease, at least until very recently, was nearly always fatal, homozygous individuals would not pass on their alleles, thereby lowering the sickle cell frequency. As long as malaria is present, the HbS and HbA alleles will remain balanced in this way, leading to the other name for this phenomenon, a balanced polymorphism.

Sickle cell and malaria questions

1. Looking at the rates of sickle cell anemia in Map A, describe what region of Africa has the highest rates of the HbS allele?

2. Compare Map A to Map B, and explain the relationship you see.

3. The Atlantic slave trade that brought millions of Africans to the American continent generally brought people from the western coast of central Africa. Knowing this, would you expect sickle cell disease to be a serious concern in African American populations or not?

4. As described above, in areas of high malaria prevalence, having sickle cell trait can be to your advantage. Which seems like more of a *disadvantage* in these areas, having sickle cell disease, or having normal hemoglobin? Explain your answer.

5. Based on your previous answer, which allele do you expect to be more common in populations where malaria is present, the HbA allele or the HbS allele?

Extension: Calculating with Hardy-Weinberg Equilibrium

p = frequency of the HbA allele

q = frequency of the HbS allele

$p + q = 1$

$p^2 + 2pq + q^2 = 1$

1. In African American populations in the United States the frequency of the HbS allele is thought to be close to .04. Use Hardy-Weinberg equilibrium to estimate the frequency of the African American population that will have normal hemoglobin, the sickle cell trait, and sickle cell disease.

2. In Nigeria, it is estimated that 3% of all new born babies have sickle cell disease. Use Hardy-Weinberg equilibrium to estimate p and q for this population.

3. Using your answers for p and q from the previous problem, what percentage of the population do you expect to have the sickle cell trait, but not sickle cell disease?

4. We used Hardy-Weinberg equilibrium to give us estimates of expected sickle cell frequencies in different populations. Would you expect the HbA and HbS alleles to be in perfect Hardy-Weinberg equilibrium? Why or why not?