Sickle Cell Genetics Lab

Diagnosing Baby Marie™

Sicke Cell Genetics Lab:
Diagnosing Baby Marie™
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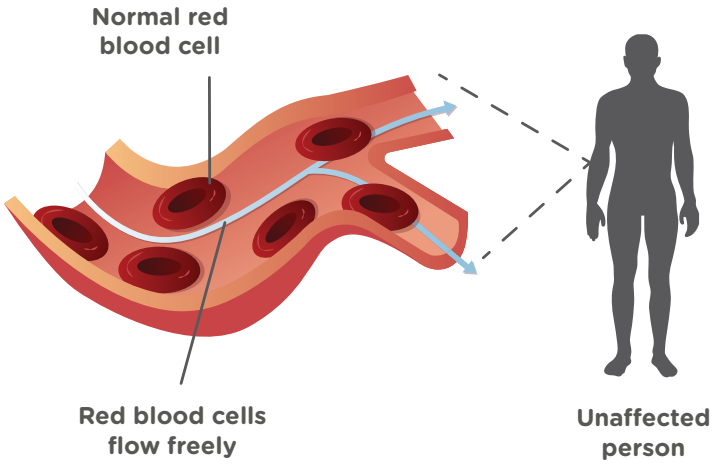
Student guide

Background information

Sickle cell disease

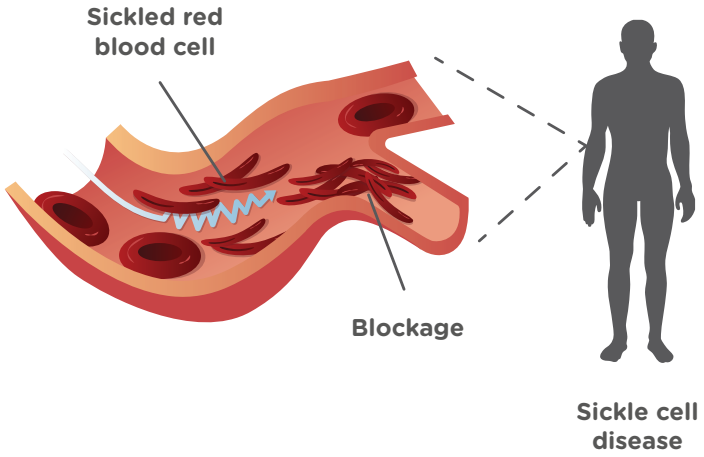
1

- **Red blood cells** carry oxygen from the lungs to cells throughout our bodies.
- Normal red blood cells are disk-shaped and very flexible, allowing them to squeeze through tiny capillaries in our circulatory system.



2

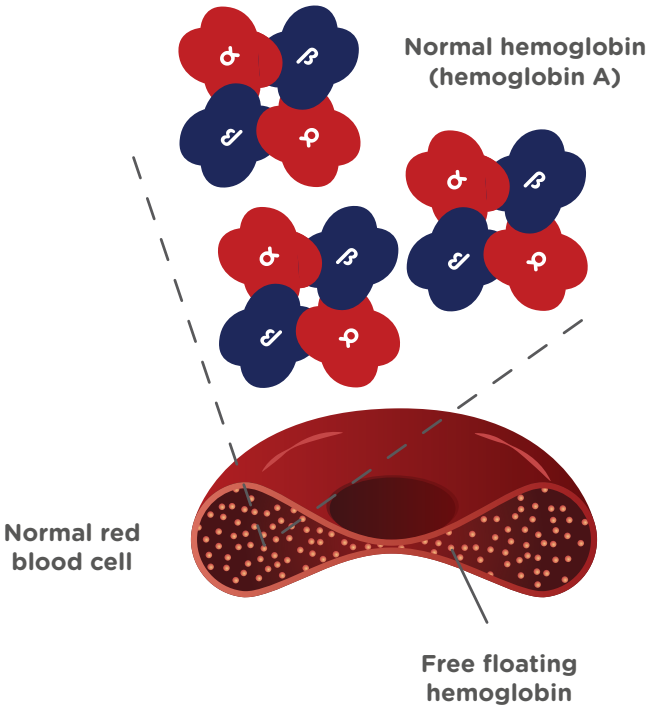
- **Sickle cell disease** is a condition that causes red blood cells to become crescent-shaped and resemble a farm tool called a sickle.
- Sickled red blood cells clump together, creating blood flow blockages that can lead to infection, pain, or even death.



The sickle cell mutation

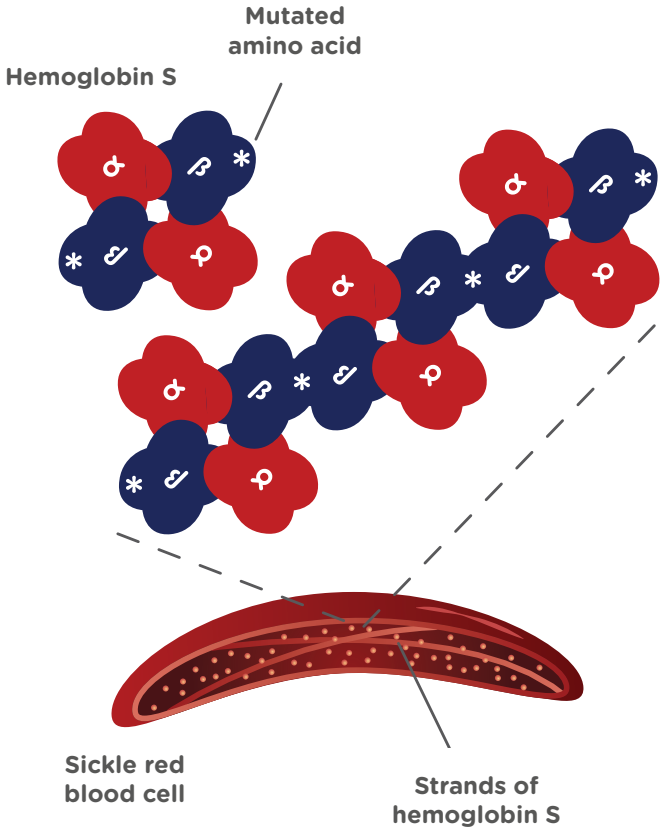
1

- **Hemoglobin** is a protein found in red blood cells that is responsible for transporting oxygen throughout the body.
- Hemoglobin is made of four protein subunits: two **alpha-globin (α-globin)** subunits and two **beta-globin (β-globin)** subunits.
- Hemoglobin proteins move freely inside the cytoplasm of red blood cells. This normal form of hemoglobin protein is called **hemoglobin A**.



2

- Sickle cell disease is caused by a **mutation** that substitutes a single **DNA nucleotide** in the *β-globin* gene.
- The mutation leads to a single amino acid substitution in the β-globin protein and causes a **hydrophobic** amino acid to face the watery cytoplasm.
- The mutated amino acids repel water and form hydrophobic links with each other, assembling long chains of hemoglobin proteins that distort the red blood cell into a sickle shape.
- This abnormal form of hemoglobin protein is called **hemoglobin S**.



Background: Stop and think

Q1. What type of mutation causes sickle cell disease?

A. Deletion of a DNA nucleotide.
B. Addition of a DNA nucleotide.
C. A single DNA nucleotide substitution.
D. A large chromosomal rearrangement.

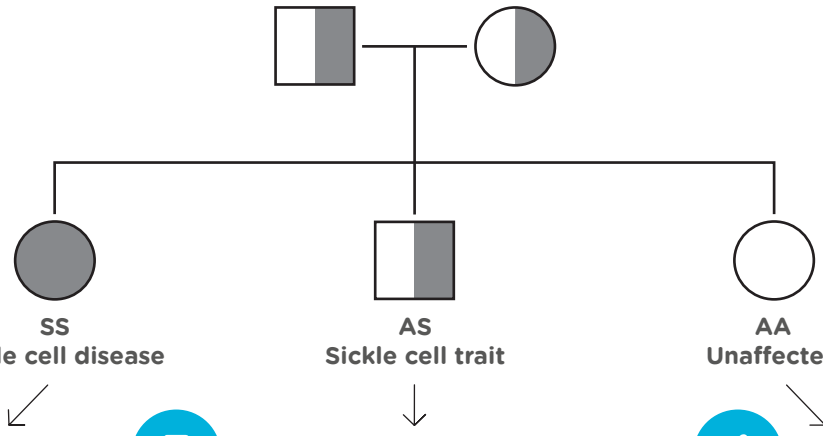
Q2. How does the sickle cell mutation affect the hemoglobin protein?

A. It prevents hemoglobin from binding oxygen.
B. It prevents hemoglobin from being produced.
C. It causes hemoglobin proteins to cluster together.
D. It increases hemoglobin's ability to bind oxygen.

Inheritance of sickle cell disease

1

- There are two **alleles** of the *β-globin* gene relevant to sickle cell disease: the normal *β-globin* allele and the mutated sickle cell allele.
- It is standard to abbreviate the normal *β-globin* allele "A" (for hemoglobin A) and the sickle cell allele "S" (for hemoglobin S).
- As you can see in the pedigree chart below, there are three possible **genotypes** relevant to sickle cell disease.



2

- Only individuals with two copies of the sickle cell allele (SS) have sickle cell disease.
- This means that sickle cell disease follows a **recessive inheritance** pattern.

3

- Individuals with one copy of the normal *β-globin* allele and one copy of the sickle cell allele (AS) are said to have **sickle cell trait**.
- People with sickle cell trait have some hemoglobin clumping, but under normal conditions, their red blood cells do not sickle, and they do not experience symptoms.

4

- Individuals with two copies of the normal *β-globin* allele (AA) are unaffected.
- AA individuals can only pass down the normal *β-globin* allele.

Background: Stop and think

Q3. True or false: A person with sickle cell disease must carry two copies of the sickle cell allele.

Today's lab

Meet the Patel family

1

- You are a doctor working with the Patels, who recently welcomed baby Marie to their family.
- Marie's routine newborn screening revealed low levels of normal hemoglobin, suggesting sickle cell disease.

2

- **Goal: you will use DNA testing to determine whether Marie has sickle cell disease!**
- Because sickle cell disease is inherited, you will also test Marie's parents, Jacqueline and Kumar, and her older brother, Lewis, to determine if they carry the sickle cell allele.

Jacqueline: Jacqueline is a 32-year-old female. She is of primarily African descent, which can be a risk factor for carrying the sickle cell allele. Jacqueline suffers from occasional migraine headaches but is otherwise healthy.

Kumar: Kumar is a 32-year-old male born in India. Indian heritage can be a risk factor for carrying the sickle cell allele. Kumar reports nothing abnormal in his medical history.

Lewis: Lewis is a healthy 4-year-old boy and has had no major illnesses. Lewis did not show any abnormalities in his routine infant screening.

Marie: Marie is 2 months old, and her newborn screening suggests sickle cell disease.

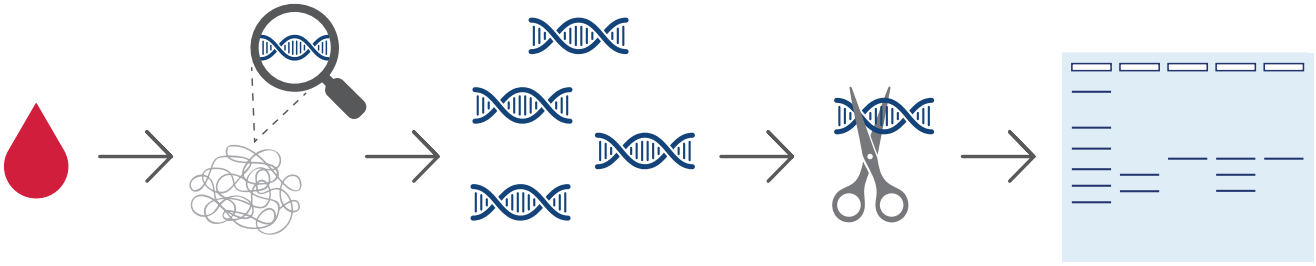
Risk factors for carrying the sickle cell allele

If sickle cell disease is so dangerous, why is the sickle cell allele relatively common in some populations? It turns out that carrying the sickle cell allele confers protection against severe **malaria**, an infectious disease spread by mosquito bites. In regions where malaria is prevalent, there is **selective pressure** for the sickle cell allele to remain in the population. The sickle cell allele is more common in people with ancestry from eastern and central sub-Saharan Africa and certain areas of the Mediterranean, Middle East, and India. All of these regions have high rates of malaria.

DNA testing

1

- A DNA test can be used to diagnose sickle cell disease.
- DNA testing involves several steps. The following procedure will determine if baby Marie or her family members carry the sickle cell allele.



Blood sample | **DNA** | **Portion of *β-globin* gene** | **Determine genotype**

DNA extraction → **Polymerase chain reaction** → **Restriction digestion** → **Gel electrophoresis**

2 A blood or tissue sample is taken from the patient. DNA is then collected from that sample.

3 Then, a method called **PCR** makes billions of copies of a section of the *β-globin* gene.

4 Next, a step to identify the sickle cell mutation is performed. This is explained on the next page.

5 You will perform the last step of the DNA test to determine if the mutated sickle cell allele is present.

Living with sickle cell disease

What if baby Marie tests positive for sickle cell disease? Advances in modern medicine allow most patients to manage sickle cell disease. Previously, standard treatments only addressed symptoms, with bone marrow transplants as the sole cure. This procedure replaces a patient's blood stem cells with healthy donor cells, producing normal red blood cells. However, finding a compatible donor is challenging. Because sickle cell disease is a genetic disease, it can be treated by modifying a patient's DNA. Recent advances have led to the first approved gene therapy treatments for patients with sickle cell disease. For more information, refer to https://links.minipcr.com/crispr_sicklecell.

Identifying the sickle cell allele using a restriction enzyme

1

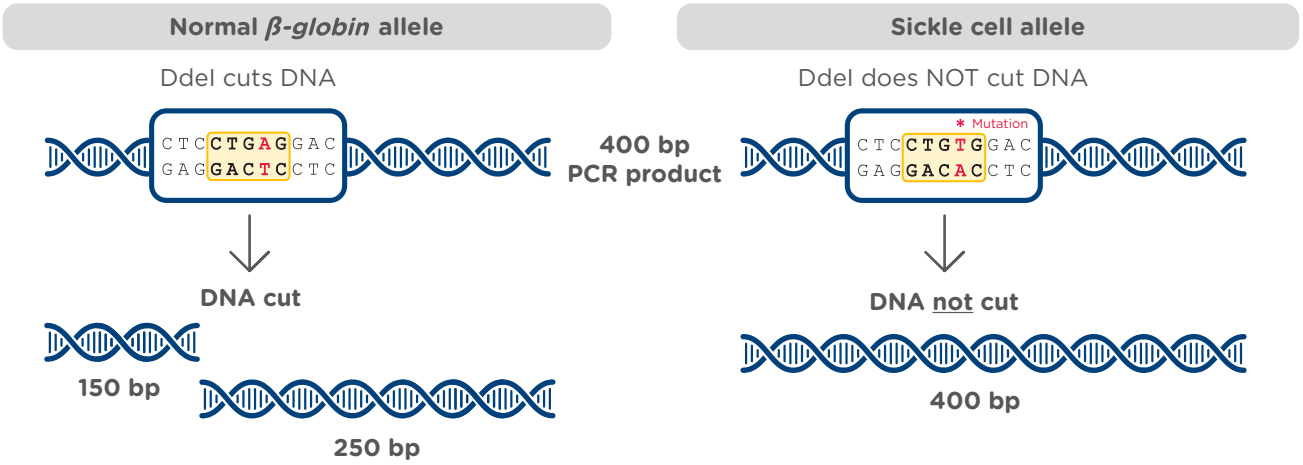
- In this lab, PCR is used to copy a 400 bp segment of the *β-globin* gene that includes the location of the sickle cell mutation.
- Then, a **restriction digestion** is used to identify if the sickle cell mutation is present.

2

- **Restriction enzymes** recognize and cut specific short **DNA sequences**. This lab uses a restriction enzyme that cuts a DNA sequence found only in the normal *β-globin* allele.
- The sequence CTGAG in the normal *β-globin* allele is cut by the restriction enzyme **DdeI**.

3

- The sickle cell mutation changes an adenine (A) to a thymine (T).
- This change means the enzyme DdeI does not cut the sickle cell allele.
- The difference in whether the DNA was cut or not can be observed using gel electrophoresis, which is explained on the next page.



Background: Stop and think

Q4. What is the role of PCR in this experiment?

A. Copy a section of the normal *β-globin* allele.
B. Copy a section of the sickle cell allele.
C. Copy a section of the normal *β-globin* allele and the sickle cell allele.
D. None of the above.

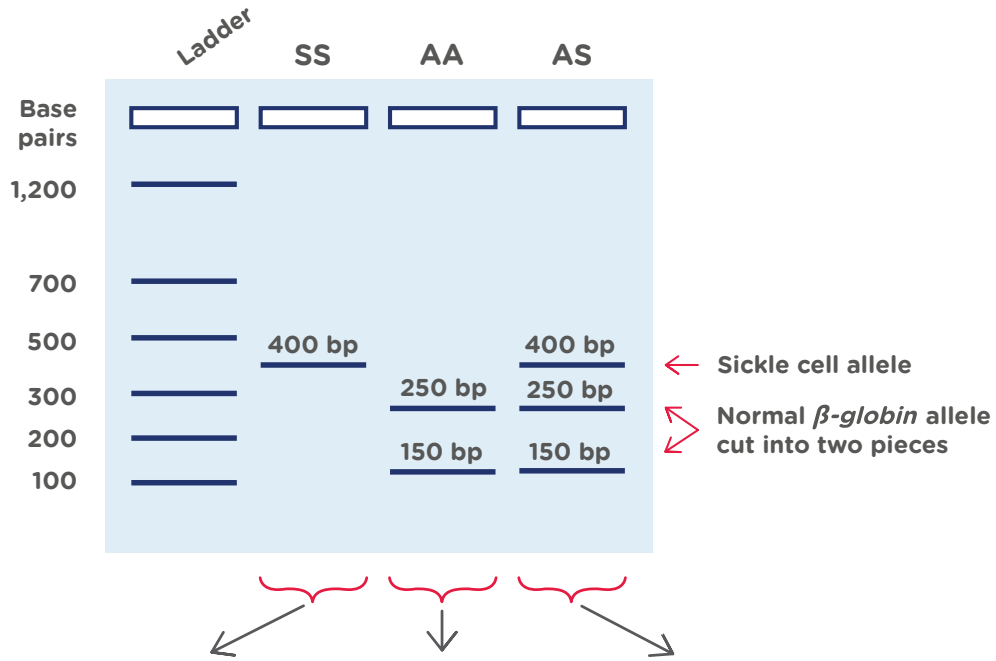
Q5. What is the role of the restriction digestion in this experiment?

A. Confirm successful DNA extraction.
B. Read the sequence of the *β-globin* gene.
C. Confirm successful PCR.
D. Differentiate between the normal *β-globin* allele and the sickle cell allele.

Interpreting gel electrophoresis results

1

- Recall that PCR was used to copy a 400 bp segment of the *β-globin* gene and then restriction digestion was performed.
- The sickle cell allele is not cut by the restriction enzyme, while the normal *β-globin* allele is cut.
- You will use gel electrophoresis to view the results and determine if the Patels carry the sickle cell allele.
- **Gel electrophoresis** separates pieces of DNA by size, with smaller pieces of DNA traveling farther.



2

Individuals who have sickle cell disease (SS) will show a single 400 bp band on the gel. This band corresponds to the sickle cell allele, which is not cut by the restriction enzyme.

3

Unaffected individuals (AA) will show two bands. These bands correspond to the normal *β-globin* allele, which is cut by the restriction enzyme into two fragments: 250 bp and 150 bp.

4

Individuals with sickle cell trait will show three bands: the 400 bp band that corresponds to the sickle cell allele, as well as the 250 bp and 150 bp bands that correspond to the normal *β-globin* allele.

Background: Stop and think

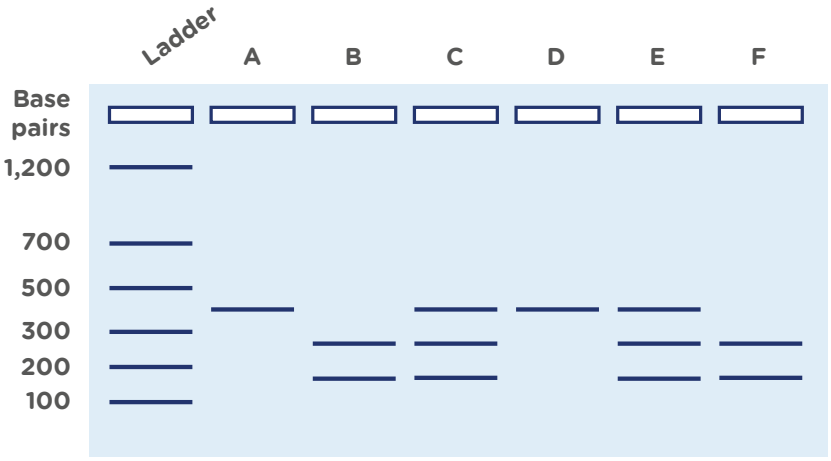
Q6. Why is gel electrophoresis a good tool for interpreting the results of a restriction digest?

A. Because it allows you to make copies of DNA.
B. Because it allows you to extract DNA from cells.
C. Because it allows you to separate DNA fragments by size.
D. Because it allows you to cut specific DNA sequences.

Imagine getting the gel electrophoresis result shown below after testing a group of patients for sickle cell disease.

Q7. Based on the gel results, which individual(s) would you diagnose with sickle cell trait?

A. Person A
B. Person B
C. Person C
D. Person D
E. Person E
F. Person F



Q8. Based on the gel results, which individual(s) would you diagnose with sickle cell disease?

A. Person A
B. Person B
C. Person C
D. Person D
E. Person E
F. Person F

Glossary

Red blood cell: A type of blood cell that delivers oxygen to cells throughout the body. Normal red blood cells are disk-shaped and quite flexible, allowing them to squeeze through small capillaries.

Sickle cell disease: A genetic disease where red blood cells take on a sickle shape. Sickled red blood cells cause blood flow blockages, leading to episodes of severe pain, organ failure, or even death.

Hemoglobin: An oxygen-binding protein found in red blood cells. Hemoglobin is composed of two α-globin subunits and two β-globin subunits.

Alpha-globin (α-globin): One of the protein subunits in the hemoglobin protein.

Beta-globin (β-globin): One of the protein subunits in the hemoglobin protein. A mutation in the *β-globin* gene causes sickle cell disease.

Hemoglobin A: Normal hemoglobin protein.

Mutation: A change or variation in the DNA sequence. Mutations can have beneficial, neutral, or harmful effects.

DNA nucleotide: A building block of DNA. Each nucleotide consists of a phosphate group, a sugar, and a nitrogenous base. There are four DNA bases: adenine (A), cytosine (C), guanine (G), and thymine (T).

Hydrophobic: Lacking an affinity for water. Hydrophobic amino acids are repelled by the watery cytoplasm of the cell.

Hemoglobin S: Abnormal hemoglobin protein in individuals with sickle cell disease. Molecules of hemoglobin S clump together and can lead to the formation of long strands that distort the shape of the red blood cell into a sickle.

Allele: One of two or more alternative versions of the same gene. Different alleles of the same gene have differences in the DNA sequence.

Genotype: An organism's genetic makeup. For a specific gene, an organism's genotype is usually a pair of alleles, one inherited from each biological parent.

Recessive inheritance: A pattern of inheritance where a trait is only present when the organism carries two copies of the same allele.

Sickle cell trait: A condition in which individuals carry one copy of the sickle cell allele. People with the sickle cell trait produce some abnormal hemoglobin protein but usually do not exhibit symptoms of sickle cell disease. However, their red blood cells can sickle under extreme environmental conditions, including low oxygen levels.

Malaria: An infectious disease caused by a parasite carried by certain species of mosquitoes. Malaria is common in certain regions where these mosquitoes are prevalent. Severe cases of malaria can lead to death. Carrying the sickle cell allele provides some protection against severe malaria infections.

Selective pressure: Environmental factors that influence an organism's ability to survive and reproduce, favoring individuals with certain traits. In areas where malaria is common, there is selective pressure for the sickle cell allele to remain in the population, even though carrying two copies of the sickle cell allele is disadvantageous to the individuals affected by sickle cell disease.

Polymerase Chain Reaction (PCR): A technique used to make multiple copies of a specific DNA segment for further study. For more detailed information on PCR, refer to https://www.minipcr.com/polymerase-chain-reaction/.

Restriction digestion: The use of a restriction enzyme to cut, or digest, a DNA sample.

Restriction enzyme: An enzyme that recognizes a specific, short DNA sequence (typically 4-8 base pairs long) and cuts the DNA at that location.

DNA sequence: The order of nucleotides, or bases, in a DNA molecule.

DdeI: A restriction enzyme that specifically recognizes and cuts a short DNA sequence present in the normal *β-globin* allele and absent in the sickle cell allele.

Gel electrophoresis: A method that separates pieces of DNA by length. For more detailed information on gel electrophoresis, refer to https://www.minipcr.com/gel-electrophoresis/.

Student lab protocol

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

1. Place the prepared gel into the electrophoresis chamber.

2. Add enough electrophoresis buffer to fill the chamber and just cover the gel.
   - You will need 30 ml of TBE buffer for a blueGel or Bandit electrophoresis system. Do not overfill the chamber.
   - If using another electrophoresis system, refer to the manufacturer's instructions for the recommended buffer type and volume.

3. Use a micropipette to load samples in the following order. To prevent contamination, use a new tip for each sample.
   - Well 1: 10 µl DNA Ladder (tube DL)
   - Well 2: 10 µl Jacqueline DNA (tube J)
   - Well 3: 10 µl Kumar DNA (tube K)
   - Well 4: 10 µl Lewis DNA (tube L)
   - Well 5: 10 µl Marie DNA (tube M)

Detailed operating instructions for miniPCR electrophoresis systems

blueGel
https://links.minipcr.com/blueGelRun

Bandit
https://links.minipcr.com/BanditViewit

4. Run the gel for 15-20 minutes.
   - The blueGel and Bandit electrophoresis systems run at a fixed voltage.
   - If using another gel electrophoresis system, set the voltage in the 70-90 V range.

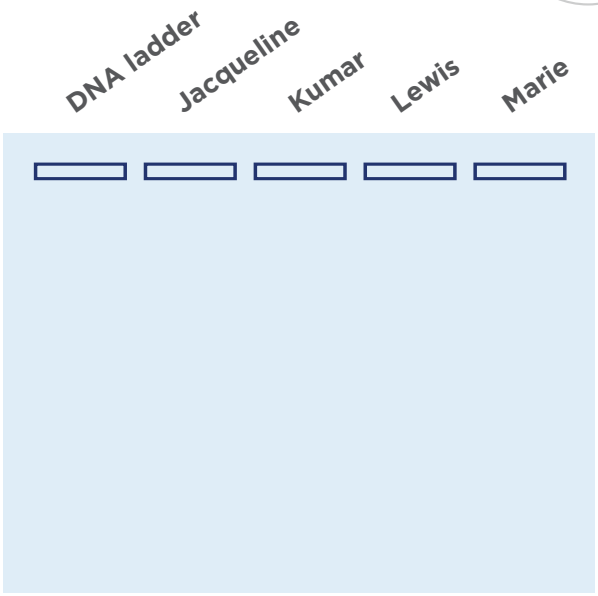
5. To visualize the DNA samples, turn on the blue light in your electrophoresis system, or move the gel to a transilluminator.

6. If needed, continue to run the gel until there is sufficient separation between the 100-500 bp bands in the ladder to interpret the results.

7. If desired, take a photo to document the results.

8. Compare the bands from the DNA samples to the DNA ladder to obtain size estimates.

Post-lab questions



Interpreting results

1. Use the schematic gel on the right to draw what your results look like. For each sample, draw the bands that you see on your actual gel.

2. Label the bands with an approximate size (in base pairs). Use the image of the ladder on the previous page to help you.

3. Use your gel electrophoresis results to complete the table below.

   A. Use checkmarks to record the gel electrophoresis results in the first two rows of the table.

   B. Record each person's genotype and diagnosis.

	Jacqueline	Kumar	Lewis	Marie
Sickle cell allele (400 bp)				
Normal *β-globin* allele (150 + 100 bp)				
Genotype (AA, AS, SS)				
Diagnosis (Unaffected, sickle cell trait, sickle cell disease)				

Critical thinking

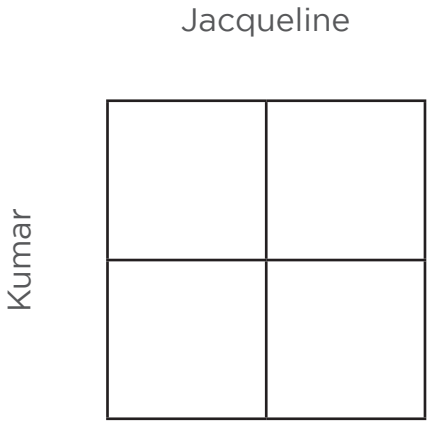
4. Imagine Jacqueline and Kumar have a third child. Fill out the Punnett square to help you answer the following questions.

   A. What is the chance this child will have two copies of the normal *β-globin* gene?

   B. What is the chance this child will have sickle cell trait?

   C. What is the chance this child will have sickle cell disease?

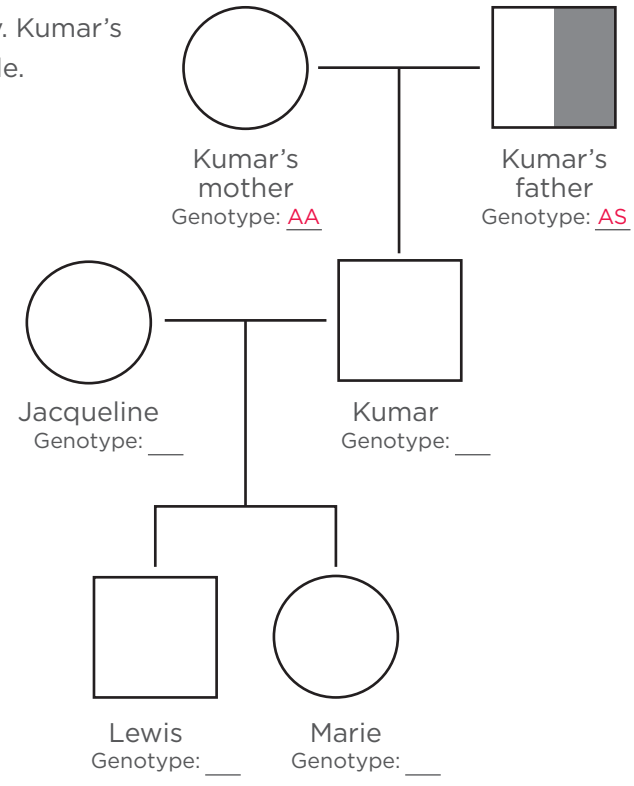
Cross: _____ x _____
Jacqueline's genotype | Kumar's genotype



5. Fill in the pedigree chart for baby Marie's family. Kumar's parents have been filled in for you as an example.

   A. Record each family member's genotype.

   B. Shade each family member's shape if appropriate to indicate the presence of the sickle cell allele.



CER table

Fill in the table based on your results from the lab. Refer to the rubric on the next page.

Question:

Does baby Marie have sickle cell disease?

Claim

Make a clear statement that answers the above question.

Evidence

Provide data from the lab that supports your claim.

Reasoning

Explain clearly why the data you presented supports your claim. Include the underlying scientific principles that link your evidence to your claim.

Score	4	3	2	1
CLAIM A statement that answers the original question/ problem.	Makes a clear, accurate, and complete claim.	Makes an accurate and complete claim.	Makes an accurate but incomplete or vague claim.	Makes a claim that is inaccurate.
EVIDENCE Data from the experiment that supports the claim. Data must be relevant and sufficient to support the claim.	All of the evidence presented is highly relevant and clearly sufficient to support the claim.	Provides evidence that is relevant and sufficient to support the claim.	Provides relevant but insufficient evidence to support the claim. May include some non-relevant evidence.	Only provides evidence that does not support claim.
REASONING Explain why your evidence supports your claim. This must include scientific principles/ knowledge that you have about the topic to show why the data counts as evidence.	Provides reasoning that clearly links the evidence to the claim. Relevant scientific principles are well integrated in the reasoning.	Provides reasoning that links the evidence to the claim. Relevant scientific principles are discussed.	Provides reasoning that links the evidence to the claim, but does not include relevant scientific principles or uses them incorrectly.	Provides reasoning that does not link the evidence to the claim. Does not include relevant scientific principles or uses them incorrectly.

We recommend that teachers use the following scale when assessing this assignment using the rubric. Teachers should feel free to adjust this scale to their expectations.

Rubric score	3	4	5	6	7	8	9	10	11	12
Equivalent	55	60	65	70	75	80	85	90	95	100

Extension: Sequence analysis of the sickle cell mutation

Restriction enzyme analysis

Recall that this activity uses the restriction enzyme DdeI to differentiate between the normal *β-globin* allele and the sickle cell allele. DdeI recognizes the following sequence:

5′...C|T N A G... 3′

3′...G A N T|C... 5′

The "N" means that the middle nucleotide of the sequence could be any of the four DNA nucleotides: A, T, C, or G. The sequences CTAAG, CTTAG, CTGAG, and CTCAG would all be cut by DdeI. The red line indicates exactly where the cut happens in the DNA strands.

The sickle cell mutation is a single nucleotide substitution near the start of the *β-globin* gene. Shown below are the first 30 base pairs of the normal *β-globin* allele and the sickle cell allele.

1. Circle the single base pair that differs between Sequence 1 and Sequence 2.

2. Find the DdeI recognition sequence and draw a box around it.

3. DdeI only cuts the normal *β-globin* allele. Based on where you identified the cut site for DdeI, is sequence 1 or sequence 2 the normal *β-globin* allele?

Mutation analysis

The sequences you analyzed on the previous page code for the first 10 amino acids of the β-globin protein. Now you will transcribe and translate the sequence to examine the effect of the sickle cell mutation on the β-globin protein sequence.

4. Transcribe each DNA sequence into mRNA.
   - To help you, the first 30 nucleotides from the template strand have been broken up into codons.
   - As an example, the first codon in Sequence 1 has been filled in for you.

5. Use the mRNA codon table on the next page to translate the mRNA into protein. As an example, the first amino acid in Sequence 1 has been filled in for you.

Seq. 1

		Coding sequence nucleotide 1 ↓			Coding sequence nucleotide 10 ↓			Coding sequence nucleotide 20 ↓			Coding sequence nucleotide 30 ↓
DNA	3′	TAC	CAC	GTA	GAC	TGA	GGA	CTC	CTC	TTC	AGA
mRNA	5′	AUG	___	___	___	___	___	___	___	___	___
Amino acid		Met	___	___	___	___	___	___	___	___	___

Seq. 2

		Coding sequence nucleotide 1 ↓			Coding sequence nucleotide 10 ↓			Coding sequence nucleotide 20 ↓			Coding sequence nucleotide 30 ↓
DNA	3′	TAC	CAC	GTA	GAC	TGA	GGA	CAC	CTC	TTC	AGA
mRNA	5′	___	___	___	___	___	___	___	___	___	___
Amino acid		___	___	___	___	___	___	___	___	___	___

6. Describe the change that occurred in the amino acid sequence.
   A. Circle the amino acid position in the diagrams above affected by the sickle cell mutation.

   B. What amino acid does the normal *β-globin* allele have in that position?

   C. What amino acid does the sickle cell allele have in that position?

7. Summarize why this single amino acid substitution leads to the mutated β-globin protein sticking together. Refer to the background information on page 13 if you need a reminder of the properties of the amino acids involved.

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: Using the Hardy-Weinberg equation

In a population that is not evolving, the prevalence of different alleles within a population are expected to remain the same over time. A population in this state is referred to as being in Hardy-Weinberg equilibrium.

The Hardy-Weinberg equation describes the genotype frequencies you would expect to see in a population that is in Hardy-Weinberg equilibrium. In its simplest form, the Hardy-Weinberg equation describes a gene with two alleles. The convention is to use the symbols p and q to represent the frequency of each allele.

For a gene with two alleles, $p + q = 1$

For examining sickle cell disease:
p = Normal *β-globin* allele
q = Sickle cell allele

The Hardy-Weinberg equation predicts the genotypic frequencies for a population that is not evolving:

$$\mathbf{p^2 + 2pq + q^2 = 1}$$

p^2 = the genotype frequency for individuals homozygous for the p allele
2pq = the genotype frequency for heterozygous individuals
q^2 = the genotype frequency for individuals homozygous for the q allele

1. The frequency of the sickle cell allele in African American populations is thought to be close to 0.04. Use this information to answer the following questions. You can use a calculator but show your work.
   A. What is the frequency of the normal *β-globin* allele?

   B. What is the predicted genotype frequency for individuals who have two copies of the normal *β-globin* allele?

   C. What is the predicted genotype frequency for individuals with sickle cell trait?

   D. What is the predicted genotype frequency for individuals with sickle cell disease?

2. In Nigeria, it is estimated that 3% of all newborn babies have sickle cell disease. Use this information to estimate the percentage of the population you expect to have sickle cell trait. You can use a calculator but show your work.

3. Remember that the Hardy-Weinberg equation assumes that a population is in Hardy-Weinberg equilibrium and makes five assumptions about the population:
   - The population size is infinite (or very large).
   - There is no net migration into or out of the population.
   - There is no mutation at the locus being tested.
   - Mating in the population is random.
   - There is no natural selection on the alleles being tested.

   Based on these criteria, would you expect the normal *β-globin* allele and the sickle cell allele to be in perfect Hardy-Weinberg equilibrium in the global population? Explain your reasoning.

Extension activity: Sickle cell disease gene therapy

Use a paper-based modeling activity to explore the first use of CRISPR/Cas genome editing to treat sickle cell disease in human patients. Visit https://links.minipcr.com/crispr_sicklecell.