NAME PERIOD DATE

SICKLE CELL GENE DETECTION

Background

Sickle cell anemia, a common form of sickle cell disease, is a genetic disorder that results from a mutation in the hemoglobin B gene (*HBB*) that provides instructions for making hemoglobin B (Hb A), also known as beta-globin. This is one of two types of molecules comprising the hemoglobin protein. Hemoglobin is a globular protein that contains two alpha-globin chains and two beta-globin chains. The four-polypeptide complex contains *heme* groups that bind oxygen molecules. Thus, hemoglobin plays a critical role in delivering oxygen to cells throughout the body.

While sickle cell anemia, an autosomal recessive disorder, is relatively rare, it is estimated that 8–12% of African Americans are carriers of the disease. Diagnosis of sickle cell anemia, or identification of carrier status, can be accomplished using molecular biology techniques.

The polymerase chain reaction (PCR) produces many copies of the hemoglobin B gene, and restriction enzymes are used to differentially cut the normal and mutant alleles. Separation of DNA fragments on a gel using electrophoresis allows a technician to detect the alleles present in each DNA sample.

In this investigation, a mother, father, and their child will be analyzed for their genetic status with regard to sickle cell anemia. The mother is a 37 year-old African American woman who is pregnant with her first child. Her doctor recommended amniocentesis due to her advanced maternal age. In addition to the typical screening for Down syndrome and other chromosomal disorders more common in children born to older women, the doctor suggested a sickle cell genetic test. DNA samples from the mother, her husband, and cells obtained from the fetus have been prepared for your analysis.

Driving Questions

Does the child have sickle cell anemia? What is the genetic status of the child's parents?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

For Each Student Station

- Horizontal gel electrophoresis apparatus
- DC power supply
- Automatic micropipet, 5 to 50 µL, with tips
- Tray with 0.8% agarose gel
- QuickStrip™ DNA samples
- InstaStain® Blue card
- Plastic tray for gel staining
- Plastic wrap
- Graduated cylinder, 100-mL
- Waste receptacles (for used tips)
- Disposable gloves
- Distilled water or buffer, 75–100 mL, for staining

  OPTIONAL (for preserving a record of the result)
- Camera (USB or other)
- Permanent marker
- Transparency film (for tracing the results)

One per Class

- DNA visualization system (white light) [1]
- Spatula (for handling the gel)

[1]A visualization system is not required, but if it is available, it will allow you to optimize the view of the gel.

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- Make sure that all liquid reagents are safely stored and that areas are dry before plugging in and turning on electrophoresis equipment.
- Wear gloves when working with stain.

Investigation

Record all observations, data, explanations, and answers in your lab notebook.

1. Compare the DNA sequence for normal hemoglobin B (*Hb A*) to the mutant DNA sequence for abnormal hemoglobin B (*Hb S*), described in Table 1.

   a. Identify the type of mutation that resulted in the Hb S variant.

   b. Describe the effect the mutation has on the primary structure of the hemoglobin B polypeptide.

Table 1: Comparing the normal and mutant hemoglobin B alleles

Hemoglobin B	DNA Nucleotide Sequence (template strand for transcription) of the gene for Hemoglobin B (*HBB*)
Normal (*Hb A*)	C T G A C T C C T G A G G A G A A G T C T
Abnormal (*Hb S*)	C T G A C T C C T G T G G A G A A G T C T

2. Analyze each of the DNA sequences in Table 1 for the presence of one or more of the restriction site sequences listed in Table 2.

Table 2: Recognition sequences for various restriction enzymes

Enzyme	Restriction Site Sequence[1]
*Bam*HI	GGATCC
*Mst*II	CCTNAGG
*Sac*I	GAGCTC
*Hinf*I	GANTC

[1]"N" can be any of the four nitrogen bases.

   a. Which enzyme or enzymes would cut within the normal *HBB* sequence? How does this compare to the effect the enzyme or enzymes would have on the abnormal *HBB* sequence?

   b. In performing a diagnostic test for sickle cell disease, which enzyme would you choose to use in the digestion stage? Explain your reasoning.

3. A diagnostic test for sickle cell disease will be performed using samples from a mother, father, and their child. To accurately determine the genotype of each person for the *HBB* locus, what control DNA samples are needed?

4. Put on your safety goggles.

5. Obtain the QuickStrip DNA samples A–F and a micropipet. Set the micropipet volume to 30 µL.

Load the Gel

6. Obtain a tray with the 0.8% agarose gel and take it to an electrophoresis chamber. Identify the *positive* (red cord) and *negative* (black cord) sides of the chamber. Place the gel, with the tray, in the chamber, positioning the wells on the negative side of the chamber (the side with the black cord).

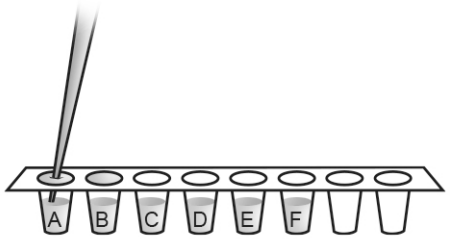
   NOTE: During electrophoresis, the DNA samples migrate through the gel towards the positive electrode. It is critical that you have the wells closest to the negative side of the chamber!

7. The gel should rest level and centered on the platform of the electrophoresis chamber and be submerged under the surface of dilute electrophoresis buffer. Add buffer to the chamber if the gel is not submerged.

8. Tap the QuickStrip DNA sample tubes gently on the table to ensure that the sample is at the bottom of the tubes.

9. Place a sterile tip on the end of the micropipet.

10. Pierce the protective overlay of the QuickStrip DNA sample container with the pipet tip and draw 30 µL of sample A into the tip. Make sure there are no bubbles in the tip of the pipet after you have extracted your sample.



11. Carefully place the tip of the pipet halfway into the first well of the gel. *Slowly* press the plunger of the micropipet to expel the sample into the well.

    NOTE: You should see the DNA and loading dye drop into the bottom of the well. Do not push through the "soft stop" on the pipet. Leave your thumb at the soft stop, remove the pipet tip from the well, and eject the tip.

12. Using a clean pipet tip each time, load samples B–F into the wells in consecutive order.

Run the Gel

13. Place the lid securely on the electrophoresis chamber and connect the apparatus to the DC power supply.

14. Set the power source to the required voltage. Ask your teacher what voltage is recommended for your equipment.

15. Turn on the power supply. Check that the current is flowing properly—you should see bubbles forming on the two platinum electrodes.

16. While you wait for results, copy Table 3 into your lab notebook and complete the "Expected Gel Pattern" section.

17. Conduct the electrophoresis for the length of time instructed by your teacher. When that time is up, turn off the power supply to stop the electrophoresis process.

Stain the Gel

18. Slowly pour 75 mL of water or electrophoresis buffer into the plastic tray for gel staining.

19. Put on disposable gloves. Carefully remove the gel from the electrophoresis chamber and place it into the plastic tray. (Slide the gel off the tray it was cast in.) The gel should be completely submerged in the liquid (add more liquid if necessary).

20. Place the blue dye side of the InstaStain® Blue card face down on the surface of the liquid, directly over the gel.

21. After 60 seconds, remove the card from the staining tray.

22. Cover the tray with plastic wrap and leave it undisturbed for at least 3 hours. (You can leave the gel in the tray overnight.)

23. Wearing the disposable gloves, remove the gel from the staining tray. If a light box visualization system is available, place the gel on the light box for an optimum view of the DNA bands.

24. Sketch a diagram, like the one shown below, in your lab notebook. Draw the banding patterns observed in your gel for each lane (each sample). Complete the "Actual Gel Pattern" column of Table 3.

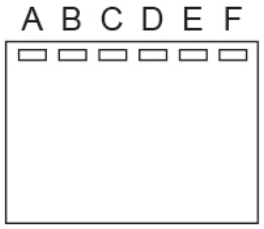


Table 3: Results of electrophoresis

DNA Sample	DNA Source	Expected Gel Pattern	Actual Gel Pattern	Genetic Status
A	Sickle cell control	RECORD THE DATA IN		
B	Carrier control	YOUR NOTEBOOK.		
C	Normal control			
D	Mother			
E	Child			
F	Father			

Data Analysis

1. What is the genetic status of a person whose hemoglobin B DNA sample produces three bands on a gel? Explain why three bands are produced.

2. What is the genetic status of a person whose hemoglobin B DNA sample produces one band on a gel? Explain why only one band is present.

3. Why do the two fragments resulting from digestion with *Mst*II travel to different locations on the gel?

4. Consider the genetic status of the mother and father tested in this investigation.

    a. If the couple decides to have another child, what is the probability that their second child will have sickle cell anemia?

    b. Is the probability of inheriting sickle cell anemia affected by the gender of the individual? Explain your reasoning.

Synthesis Questions

1. The most common cause of sickle cell anemia is a mutation that results in the amino acid valine replacing glutamic acid in the hemoglobin B gene (*HBB*). This single amino acid difference leads to significant consequences on red blood cell structure and body physiology.

    a. Valine is a neutral and non-polar amino acid. Glutamic acid is an acidic and polar amino acid. Why do different amino acids have different chemical properties?

    b. Explain why the change from glutamic acid to valine affects the structure of the hemoglobin protein.

2. Persons with sickle cell anemia are cautioned against participating in strenuous activity. The symptoms of the disease are most severe when the supply of oxygen is limited. Relate this characteristic of the disease to the location and role of hemoglobin in the body, and explain why exercise would amplify symptoms.

3. Scientists have discovered that a drug called *hydroxyurea* promotes the production of fetal hemoglobin, a form of hemoglobin present only in trace amounts after 1–2 years of age. Treatment with hydroxyurea causes many sickle cell patients to produce more fetal hemoglobin which, in turn, helps reduce polymerization of hemoglobin in red blood cells.

    a. Why would hydroxyurea be considered a treatment and not a cure for sickle cell anemia?

    b. To develop a cure for the disease, how might scientists induce the body to produce more fetal hemoglobin on its own, independent of a drug like hydroxyurea?