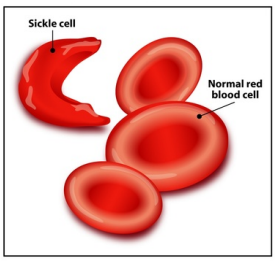
Honors Biology
Unit 5: Patterns of Inheritance

Due by Tuesday March 14!

Name: _____ Period: _____



Lab 18: Detection of Genetic Sickle Cell Disease

Introduction:

Sickle cell refers to the shape of a red blood cell, which results due to a mutation for amino acid 6 of the hemoglobin protein. This protein is part of the protein complex that carries O_2 throughout the blood. The mutation causes the red blood cell to change shape from round disc to sickle or crescent moon shape. This condition is found in all ethnicities, but is highest in people of African descent who originated from tropical areas where the disease malaria is common. This mutation provides a resistance to malaria, which helps a person fight the disease and keeps the gene in the human population. The mutation at the DNA level is a single nucleotide change. The test for the sickle cell allele relies on the differences between the wild-type or normal DNA sequence and the mutated sequence. The allelic differences can be identified using the DNA gel electrophoresis method where fragments of DNA are separated using an electrical charge.

Part A: The Genetics of Sickle Cell Disease

The first step in identification of the sickle cell mutation is to cut the DNA using a restriction enzyme, as we saw in our gel electrophoresis lab. Restriction enzymes cut DNA at particular sequences, like DNA scissors. Each restriction enzyme has its own unique cut sit. In our previous lab, we used a restriction enzyme that cuts DNA into different fragments. The number of fragments and the sizes of the fragments are unique to each person. In this situation, everyone's hemoglobin protein DNA is the same, unless it is mutated and causes sickle cell disease. In the space below, you will "cut DNA" to identify the number of fragments if a person does or does not have the sickle cell mutation.

1. Identify sickle cell mutation:

Compare the two strands below. Circle the mutated portion of the sequence.

Wild-Type (unmutated) Strand: G G T A T T T C A A T T G A A T A A C G G A A T C C A T G

Mutated Sickle Cell Strand: G G T A T T T C G A T T G A A T A A C G G A A T C C A T G

What type of mutation has occurred in the second strand? _____

2. Cutting DNA strands with restriction enzymes:

In the lab, you use the Bogus I restriction enzyme that identifies and cuts DNA at the sequence below:

AATT → AA ^ TT *where ^ represents the cut site

A wild-type strand of hemoglobin DNA will be cut at one site, resulting in two fragments. As you will see, an unmutated strand will not be cut at all. Instead, it will remain as one large fragment. On the strands below, first identify whether the strand is wild-type or mutated by comparing back to the strands in question number 1. Then use your pencil to "cut" the strands of DNA at the cut site shown above.

***Note: a person has two chromosomes or strands of DNA;*
one from a male parent and one from a female parent.

Person 1 DNA Strands:

Strand from Female Parent: G G T A T T T C A A T T G A A T A A C G G A A T C C A T G

Strand from Male Parent: G G T A T T T C G A T T G A A T A A C G G A A T C C A T G

Person 2 DNA Strands:

Strand from Female Parent: G G T A T T T C A A T T G A A T A A C G G A A T C C A T G

Strand from Male Parent: G G T A T T T C A A T T G A A T A A C G G A A T C C A T G

Due by Tuesday March 14!

Person 3 DNA Strands:

Strand from Female Parent: G G T A T T T C G A T T G A A T A A C G G A A T C C A T G

Strand from Male Parent: G G T A T T T C G A T T G A A T A A C G G A A T C C A T G

3. Identifying fragments and genotypes:

In the strands above, you cut them using the Bogus I restriction enzyme site. The number of fragments will tell you whether someone has two of the same allele of the hemoglobin gene or two different alleles. The unmutated wild-type allele is dominant to the recessive mutated sickle cell allele. Below are the possible genotypes and the symbols for sickle cell anemia detection:

	Allele Description	Possible Genotypes	Phenotype in Patient
Homozygous Dominant	Two wild-type strands	HH	No sickle cells, no disease
Homozygous Recessive	Two mutated strands	hh	Sickle cells present, anemia disease present in patient
Heterozygous	One strand wild-type, one strand mutated	Hh	Person is a carrier, no detectable sickle cells

For the three people's DNA strands previous, identify the number of fragments, whether each strand is wild-type or mutated, whether the person is homozygous dominant or recessive or heterozygous, and the person's genotype.

	Female Parent Strand		Male Parent Strand		Genotype	
	Wild-Type or Mutated	# of Fragments	Wild-Type or Mutated	# of Fragments	HH, Hh, or hh	Homozygous Dominant, Homozygous Recessive, or Heterozygous
Person 1						
Person 2						
Person 3						

Part B: Identification of Sickle Cell Disease

1. Observing gel electrophoresis to detect sickle cell disease:

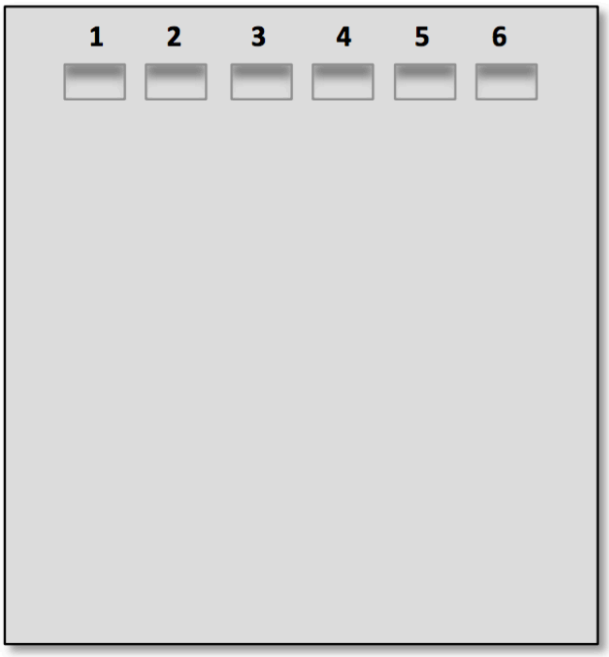
Receive a pre-prepared gel and observe it at one of the light tables. Diagram the gel on the next page as you observe it (note, there will be 13 fragments of DNA total!). Be sure to put your fragments in the correct lanes!

Clinical Scenario for Gel:

In this gel, the first three lanes (rows) are controls to compare the patients' samples in lanes 4, 5, and 6. One control shows a normal homozygous dominant person, one shows a heterozygous person carrying the sickle cell mutation, and one control shows a person who is homozygous for the sickle cell anemia gene and would present with the complications of the sickle cell anemia disease. A child was brought to his doctor suffering from chronic fatigue, headaches, joint pain, and breathlessness, all signs of many different diseases, including sickle cell anemia. The child's parents are concerned because the child's maternal great grandmother suffered from sickle cell anemia and the child's mother's uncle also suffered from sickle cell anemia. Lane 4 shows the DNA sample taken from the child's mother, lane 5 shows the child's DNA sample, and lane 6 shows the child's father.

Due by Tuesday March 14!

2. Identify sample genotypes & phenotypes:
Given the gel, identify the controls' genotypes and phenotypes. Then compare the controls to the DNA samples from the mother, child, and father and identify the genotypes and phenotypes of each.



	# of Fragments	Genotype (HH, hh, or Hh)	Homozygous Dominant, Homozygous Recessive, or Heterozygous
Lane 1: Control 1			
Lane 2: Control 2			
Lane 3: Control 3			
Lane 4: Mother's DNA			
Lane 5: Child's DNA			
Lane 6: Father's DNA			

3. Genetic testing analysis questions:

A. Given the results of observed in the gel, does the child have sickle cell anemia? Give evidence from your gel to justify.

B. Neither of the parents shows signs or symptoms of sickle cell anemia, and yet they have the genes that cause sickle cell anemia. Describe how this could be so.

C. Given the two parents' genotypes, use a Punnett square to identify the percent chance that these two people would have a child who would suffer from sickle cell anemia.

Percent chance of child with sickle cell anemia: _____

What is the percent chance these two parents would have a child who is a carrier (heterozygous) of the sickle cell mutation: _____

Due by Tuesday March 14!

4. Creating a family pedigree:

Using the clinical scenario on the second page, create a family pedigree depicting the passing of the sickle cell anemia allele throughout the family. Be sure to have the following: generations numbered (Roman numerals), people numbered (1, 2, 3, 4, etc.), people with sickle cell anemia shaded, people carrying the mutated allele half shaded. Use and **M** to identify the child's mother in this case, an **F** to identify the child's father, and a **C** to identify the child.

Lab 18 Score:			
1	2	3	4