Background Information

A single nucleotide change in the DNA sequence of an important gene can affect health and disease. A large number of genetic diseases are identified where such changes have been correlated to changes in single nucleotides. More recently, mutations in oncogenes and tumor suppressor genes such as p53, have been associated with lung, colon and breast cancer. Other mutations in genes such as the BRCA 1 and II genes have been identified as specific markers with good potential as diagnostic tools for breast cancer.

Human genetics follows the basic findings of the Augustine monk, Gregor Mendel, who studied plant genetics in the mid-1800's. Mendelian genetics, which predicts traits inherited by offspring, is based on the inheritance of two alleles, or forms of the gene. These two alleles are inherited one from each parent. Alleles, and corresponding traits, can be either dominant or recessive. When a dominant allele is inherited, the trait coded by that allele will be apparent in the offspring. The presence of a dominant allele will, in effect, mask the trait coded by the recessive allele. To observe a recessive trait, it is required that both parental alleles be the recessive type. If both alleles are the same type, either both recessive or both dominant, the individual is said to be homozygous with respect to that trait. If an individual has one dominant and one recessive, the individual is said to be heterozygous for that trait.

	T	t
T	TT	T t
t	T t	t t

Figure 1

Genotype:

1/4	TT
1/2	Tt
1/4	tt

Phenotype:

3/4	dominant
1/4	recessive

Mendelian inheritance can be demonstrated with a 2 x 2 matrix, as shown in Figure 1. Parental alleles are placed on the sides of the matrix, and the genotype (what is genetically inherited) and phenotype (the way we look) of the offspring can be predicted. By convention, the dominant allele is denoted by an uppercase letter and the recessive allele by a lowercase letter. For example, assuming both parents each carry one dominant allele and one recessive allele, we can predict that 3/4 of their children will have the dominant phenotype and 1/4 of their children will have the recessive phenotype. Genotypically, 1/4 of the children will carry two dominant alleles; 1/2 of the children will carry one dominant and one recessive allele, and 1/4 will carry two recessive alleles. These estimates would be observed if there are a large number of offspring from two parents, as in the case of insects or plants.

Hemoglobin, which is present in red blood cells, is the carrier of oxygen to cells in the body. In capillaries carbon dioxide, which is a by product of metabolism, enters red cells and is converted to carbonic acid. The acidic pH reduces the affinity of oxygen binding to hemoglobin resulting in the release of oxygen in cells. Likewise when the bound carbon dioxide is released from red cells in the lungs there is an increase in pH which favors the binding of oxygen to hemoglobin. In individuals who suffer from certain blood diseases such as sickle cell anemia, the binding and subsequent transport of oxygen is compromised due to a single nucleotide mutation. This results in a deficiency of oxygen and carbon dioxide

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exchange in the patient. In sickle cell anemia patients, the substitution of the polar side chain (Glu) with a nonpolar hydrophobic side chain (Val) results in the polymerization of the unoxygenated form and subsequent precipitation of such polymers in red blood cells. The precipitation gives red blood cells a sickle shape due to the lack of diffusion through capillaries.

Each person has two copies of the gene of hemoglobin. Normal hemoglobin is referred to as Hemoglobin A. The letters AA are used to indicate that both hemoglobin genes are normal. The gene that causes sickle cell anemia is referred to as Hemoglobin S. There are three possible combinations of the genes for hemoglobin:

AA Individual is homozygous for the Hemoglobin A gene. So, both copies of hemoglobin code for normal hemoglobin and the person does not have the disease.

AS Individual is heterozygous. One copy of hemoglobin codes for normal hemoglobin and the other copy of the gene codes for sickled hemoglobin. This person does not have the disease and will not develop it later in life.

SS Individual is homozygous for the sickled hemoglobin S gene. So, both copies of hemoglobin code for diseased hemoglobin. This person suffers from sickled cell anemia.

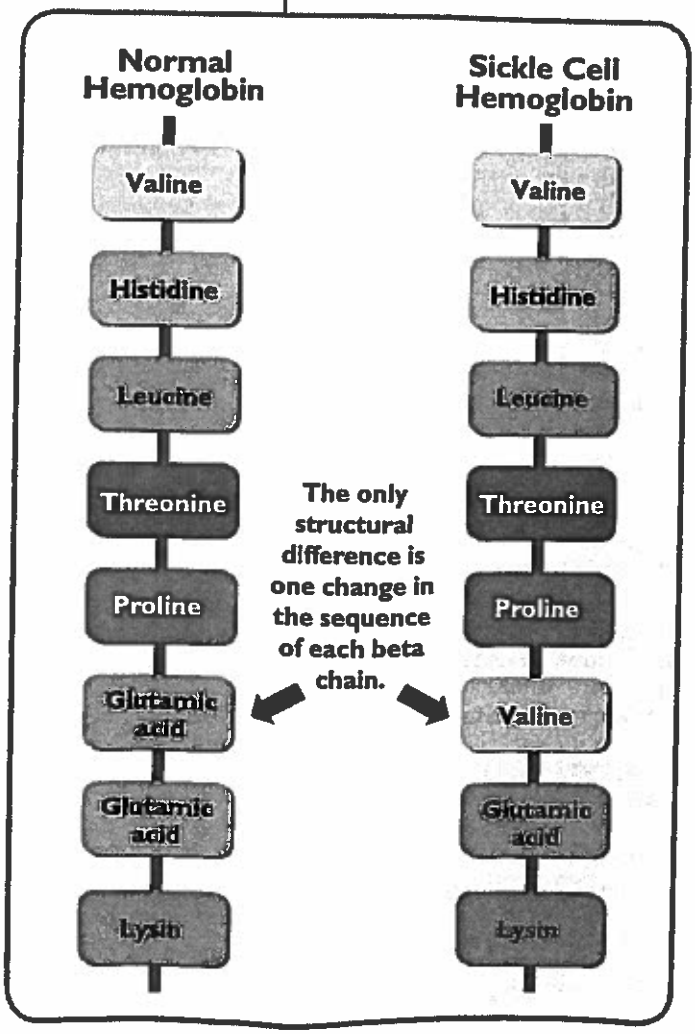
The irregularly shaped blood cells lead to a cascade of symptoms. The sickle-shaped blood cells die prematurely, resulting in anemia and the production of excess bilirubin (a yellow pigment resulting from the breakdown of hemoglobin). Jaundice often results when the liver cannot metabolize bilirubin fast enough. Infection, dehydration, overexertion, high altitude, chills, or cold weather can bring on a sickling episode, or crisis. Sometimes there is no apparent precipitating factor. People with sickle cell disease are susceptible to fevers and infection.

Blood disease such as sickle cell anemia and β-thalassemias are attributed to various point mutations or other translational product aberrations. Almost 400 different hemoglobin (Hb) variants of known structure have been identified. The early recognized variants were historically assigned alphabetical initials based sequence of discovery or hematologic features.

In the United States, sickle cell anemia is of special interest since it is estimated that 8% of African Americans are carriers of the sickle trait. It is of interest to note that heterozygous individuals for Hb S have a high resistance to the malaria parasite, part of whose life cycle is spent in red blood cells. Historically, sickle cell anemia has provided a selective advantage in some regions of the world such as parts of Africa. This can also explain the reason for the high frequency of this homozygous gene amongst African Americans.

Background Information

Hemoglobin is made up of two a chains and two β chains. The gene where the α is located is on the short arm of chromosome 16, while the β-globin gene cluster is on the short arm of chromosome 11. In addition to the adult form of Hb encoded within the β Hb cluster are the Hb forms that substitute for the adult β Hb during the various stages of development. Hemoglobin S (Hb S) is the variant form of the normal adult hemoglobin A (Hb A) in which an amino acid substitution occurs in the β polypeptide. The amino acid substitution is that of Valine (Val) in Hb S for the glutamic acid (Glu) normal Hb A hemoglobin (Figure 2). This significant finding was reported in 1957 by Vernon Ingram who was able to determine this amino acid substitution using peptide mapping analysis. These procedures are tedious and difficult. It should be noted that this predates recombinant DNA technology.



The single base mutation is an A to T in the triplet codon of the amino acid residue number 6 from the amino acid end in the beta chain. This change introduces an amino acid with a polar (neutral) side chain valine instead of the acidic (negative) residue and changes the property of the hemoglobin molecule. This substitution changes the electrophoretic mobility of Hb S compared to Hb A. At slightly basic pH, such as 8.4, Hb S will be relatively more positive than Hb A and therefore will travel slower towards the positive (anode) electrode. This change in mobility is used as a diagnostic test of the presence of Hb S.

With the advent of biotechnology, parental or fetal DNA from cells obtained from amniocentesis can now be analyzed with a high degree of accuracy. A few cells can provide sufficient DNA to be amplified using Polymerase Chain Reaction (PCR). Alternative methods can include growing cells in culture to yield sufficient DNA for analysis.

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The basis of the test is the recognition of specific palindromic sequences in DNA by restriction enzymes. In the normal β globin gene, the sequence of nucleotides that specifies amino acids 5, 6 and 7 (Pro-Glu-Glu) are CCT- GAG-GAG (see figure 2). The point mutation in codon 6 converting the A to T changes the sequence to CCT-GTG-GAG. The palindrome recognition site of the restriction enzyme *Mst* II is CCTNAGG, where N can be any of the four nucleotides. Close examination of the sequence shows that *Mst* II will recognize the normal β globin CCT-GAG-G where N is a G, but not the mutated form.

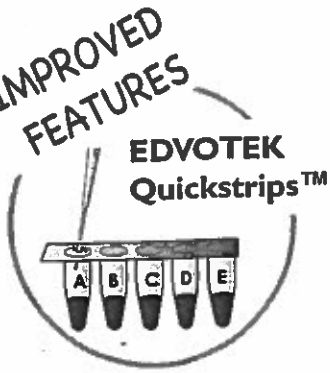
Activity Two - Conducting Agarose Gel Electrophoresis

ELECTROPHORESIS SAMPLES

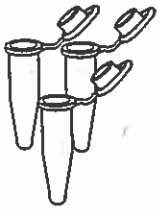
Samples in EDVOTEK Series 100 and S-series electrophoresis experiments are packaged in one of two different formats:

1. Pre-aliquoted Quickstrip™ connected tubes (new format)

   To remove samples from the Quickstrip™ tubes, simply pierce the foil top with the micropipet tip and withdraw the sample.

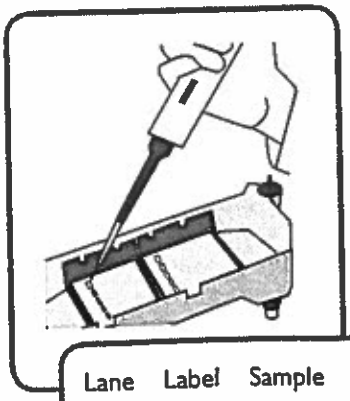


Quickstrips patent pending



2. Individual 1.5 ml or 0.5 ml microtest tubes

Your instructor may have aliquoted these into a set of sample tubes for each lab group. Alternatively, you may be required to withdraw the appropriate amount from the experiment stock tubes.



LOADING THE SAMPLES

1. Check the Sample Volumes

   Sometimes a small amount of sample will cling to the walls of the tubes. Make sure the entire volume of sample is at the bottom of the tubes before starting to load the gel.

   - If your samples are in Quickstrip™ connected tubes, tap the foil top of the strip so samples fall to the bottom of the tubes.
   - If your samples are in individual 1.5 ml or 0.5 ml microtest tubes, briefly centrifuge the sample tubes, or tap each tube on the tabletop to get all the sample to the bottom of the tube.

Lane	Label	Sample
1	A	Normal Hemoglobin control
2	B	Sickle Hemoglobin control
3	C	Carrier Hemoglobin control
4	D	Patient #1 Hemoglobin
5	E	Patient #2 Hemoglobin

2. Load Samples

   Load each of the dye samples in tubes A - E into the wells in consecutive order. The amount of sample that should be loaded is 15 µl.

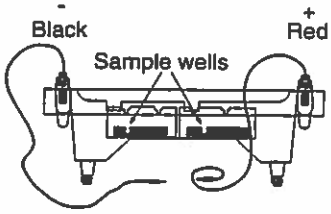
Activity Two - Conducting Agarose Gel Electrophoresis

RUNNING THE GEL

3. After the samples are loaded, carefully snap the cover down onto the electrode terminals.

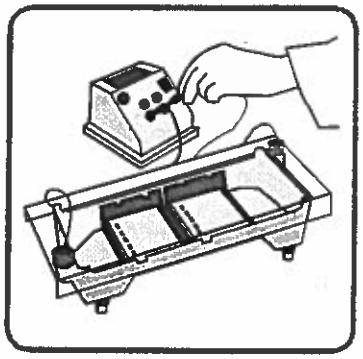
Reminders:

During electrophoresis, the samples will migrate through the agarose gel towards the positive electrode. Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



the negative and positive color-coded indicators on the cover and us chamber are properly oriented.

4. Insert the plug of the black wire into the black input of the power source (negative input). Insert the plug of the red wire into the red input of the power source (positive input).



5. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor. General guidelines are presented in Table C.

6. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

Table C Time and Voltage

Electrophoresis of Dyes

Volts	Recommended Time
125	20 min
70	45 min
50	1 hr 30 min

7. After approximately 10 minutes, you will begin to see separation of the colored dyes.

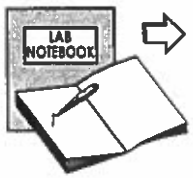
8. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.

9. Document the gel results.

   A variety of documentation methods can be used, including drawing a picture of the gel, taking a photograph, or scanning an image of the gel on a flatbed scanner.

Staining is not required for Experiment # S-53, but results must be analyzed upon completion of the electrophoretic separation. Because dye molecules are extremely small they will diffuse out of the gel. Therefore, the gel cannot be saved.

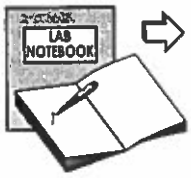
Critical Thinking and Hypothesis Development



Record the following in your Laboratory Notebook or on a separate sheet of paper:

1. Based on the evidence obtained from analysis of the gel, which patient has the sickle cell trait? Explain.
2. What is the variable in this experiment?
3. What would you change in the experiment if you had to do it over again?
4. Write a hypothesis that would reflect these changes.

Study Questions



Record the answers to the following Study Questions in your Laboratory Notebook or on a separate sheet of paper, as instructed by your teacher:

1. Why is it important to position the sample wells near the negative electrode?
2. Why is it important to use a new pipet or wash the pipet between uses?
3. How will you be able to tell which patient has the sickle cell trait?
4. Explain what happens to patients afflicted with sickle cell anemia?
5. What are the possible gene combinations for hemoglobin?