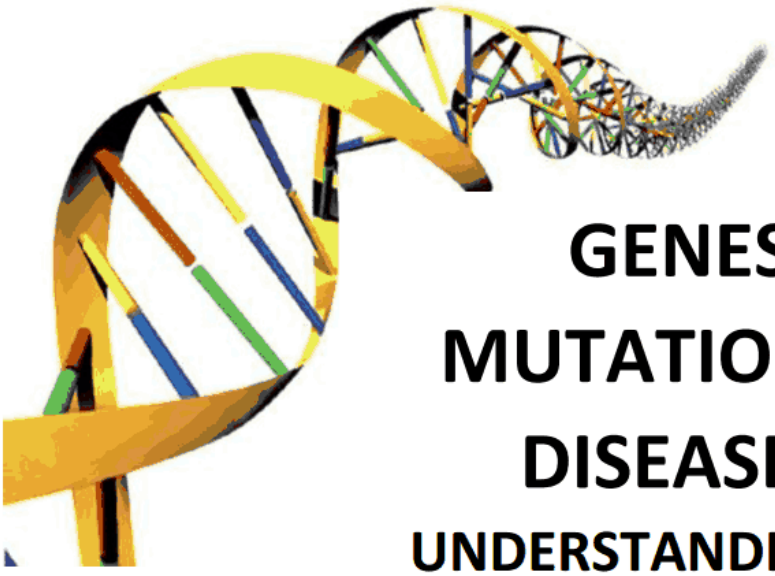
SJU SAINT JOSEPH'S UNIVERSITY

High School L.I.N.K.S.

(Learning Integrating Nature, Kids, and Science)



http://www.raogk.org/dna/

GENES, MUTATIONS & DISEASES:

UNDERSTANDING THE ORIGINS OF GENETIC DISORDERS

Laboratory exercises prepared by:

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LABORATORY SAFETY

The laboratory should be a safe and enjoyable learning environment for all. Therefore, please review the following safety rules. Your lab instructor will review these procedures during the first week of lab.

1. Follow all directions given to you by your laboratory instructor. When in doubt → ASK!!!

2. Know the locations and operations of all safety equipment, including the first aid kit, fire extinguisher, safety shower and eye wash stations. Take note of the locations of all the exits in the lab.

3. Wear clothing that will provide maximum protection. Do not wear any open toed sandals. Hats and dangling jewelry should not be worn during lab. Hair should be tied back when working near any open flame. Wear safety goggles and gloves when instructed to do so by your laboratory instructor.

4. Never eat, drink, smoke, chew gum, or apply makeup in the laboratory. Do not bring any food/drink into the lab.

5. Place items such as cell phones, purses, backpacks, sweaters, coats, and extra books under the lab bench while you are working on the day's experiment. Lab manuals should be kept with you at the bench.

6. Do only the experiments assigned and in the manner prescribed. You are not permitted to work in the laboratory unsupervised.

7. Use good judgment and care when working in the laboratory. Carry out good housekeeping practices at all times.

8. Avoid touching hot objects without proper equipment.

9. Read all reagent bottle and container labels to make certain that the vessels contain the reagents specified for the experiment, and learn of any hazards associated with the reagents.

10. IMMEDIATELY report all injuries, spills and damage to equipment, no matter how minor, to the laboratory instructor. If you spill anything on your hands, wash it off immediately.

11. When you have completed the day's laboratory exercise:
    (a) clean up your lab bench
    (b) return any equipment to their designated location
    (c) discard waste as directed by your laboratory instructor
    (d) wash your hands thoroughly (make this a routine practice)

Lab #1: Nucleotides, Nucleic Acid, DNA, genes

Introduction:
By definition, a **disease** is a deviation from the normal state of the body. Some diseases are **genetic** in nature. Genetics is the study of inheritance.

These laboratory activities are designed to show you (a) the mechanisms by which disease arises from a genetic standpoint and (b) how they can be identified experimentally. The genetic disease we will be focusing on throughout the program will be **sickle cell anemia**.

The **gene** is the fundamental unit of heredity that is passed on from one generation to the next. Scientists now know that genes are comprised of deoxyribonucleic acid (DNA). But what exactly is DNA? Our investigation begins....

Part I: Isolating & Visualizing DNA

All living organisms contain DNA within their cells. We will begin by asking the following:
Can we isolate DNA from cells?

Procedure:

1. Place a strawberry in Zip-loc bag. Press the air out and seal the bag. Mash the strawberry for 2 minutes to break open the cells.

2. Add 10 mL of DNA extraction buffer (detergent, salt, water) to the bag. The detergent helps break open the cells and the salt helps remove any protein that is bound to the DNA. Press the air out and seal the bag. Mash for 1 minute.

3. Place a piece of cheesecloth on top of a beaker and secure it with a rubber band. Cut the end of the Zip-loc bag and allow the liquid to filter through the cheesecloth into the beaker. Transfer approximately 2-3 mL of the liquid from the beaker to a clean 15 mL conical tube or test tube.

4. Slowly pour about 2-3 mL of ice-cold ethanol along the side of the conical. The ethanol should form a layer on top of the filtered extract. This will help clump DNA. We are able to see DNA because it is not soluble in ethanol.

5. Dip a glass-rod to the ethanol-extract boundary and twirl gently.

Question: What are your observations of your extracted DNA (color, texture, shape)?

_____

_____

6. Place the DNA in a clean microcentrifuge tube. A teaching fellow will add 70% ethanol to rinse the DNA. Once the DNA dries, they will add approximately 100 µL of DNA elution buffer. Incubate the DNA @ 60°C for approximately 20 minutes.

7. While you are waiting, proceed to part 2 of today's lab.

Another question arises….*How do we know that we really did isolate DNA from the strawberry?*

To answer this question, you will learn an important technique that most molecular biologists use. That technique is known as gel electrophoresis. Gel electrophoresis makes use of a gel that contains **agarose.** An **electric current** is used to separate molecules of DNA according to size.

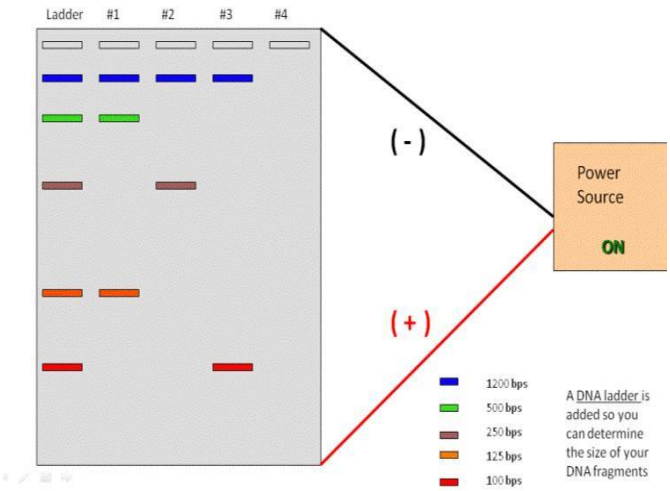


Figure 1-1. Agarose Gel Electrophoresis

A DNA nucleotide contains a phosphate group. This phosphate carries a negative charge. When DNA is loaded at the negative end of the gel and an electric charge is applied, DNA will be repelled from the negative end and migrate towards the positive end of the gel (Figure 1-1).

Agarose produces a three dimensional network for molecules to move in. As a result, larger molecules (more base pairs in the DNA molecule) stay towards the negative end of the gel since they cannot move easily while smaller molecules (less base pairs in the DNA molecule) migrate faster towards the positive end of the gel.

The gel also contains a stain known as **SYBR SAFE.** In the presence of DNA, this molecule will attach to the structure of DNA. As a result, your DNA will be visible when the gel containing the DNA is exposed to ultraviolet light.

During our fourth lab exercise, we will learn more about gel electrophoresis and use it to separate DNA molecules by size. Today, we will focus on using gel electrophoresis as a means of just observing our DNA.

Procedure:

8. After approximately 20 minutes, take out your isolated DNA. DNA is soluble in water. By placing the extract in water, the DNA will dissolve in water. Do not worry if all of the DNA dissolved.

9. Teaching fellows will help you add 20 µL of liquid from your DNA extraction to 3 µL of running dye. The dye will help sink the DNA into the gel.

10. When the entire class' DNA samples have been loaded into the gel, electrophoresis will begin. After approximately 15 minutes, the gel will be placed on the UV table. Any DNA present will appear as bands and will glow.

11. Your instructor will also load three additional lanes into the gel: one with a sample of DNA, a second lane that contains water and a third lane that contains a protein sample.

Question: Draw what you observe from the gel in the space below.

Question: According to the gel, did you isolate DNA from strawberries? Does the DNA molecule have a large # of base pairs (large molecule) or a small # of base pairs?

_____

Question: Were you able to see the protein that was loaded in the gel?

_____

Part II: Building a DNA molecule

Models in science are used to describe ideas, understand biological processes and make predictions. In today's lab, we will build a model to learn about the composition and structure of DNA.

Procedure:

12. In groups, you will work to build a model of DNA. The magnets and safety pins will serve as the hydrogen and phosphodiester bonds, respectively.

Question: What are your observations of your constructed DNA molecule? Specifically....

a. Draw one of your nucleotide models. Label each component (color & identity).

b. What are the base pair rules for DNA?

_____

c. Can you form a DNA molecule using only "A" and "C" bases? Why or why not?

_____

d. Describe the shape of your model.

_____

Lab #2: Transcription/Translation/Amino Acids, Mutations

Introduction:

In the past laboratory investigation, you learned that genes are segments of DNA. In today's lab, we will continue looking at DNA and answering the following question - *how are those genes expressed?*

In gene expression, the DNA genotype is expressed as proteins, which provides the molecular basis for phenotypes we observe. The first step in protein synthesis is the synthesis of ribonucleic acid (RNA) using the gene's DNA sequence as a template. This process of DNA → RNA is known as **transcription**.

RNA contains ribose (a five-carbon sugar), a phosphate group (PO_4^-) and a nitrogenous base. There are four nitrogenous bases found in RNA: adenine (A), uracil (U), cytosine (C) and guanine (G).

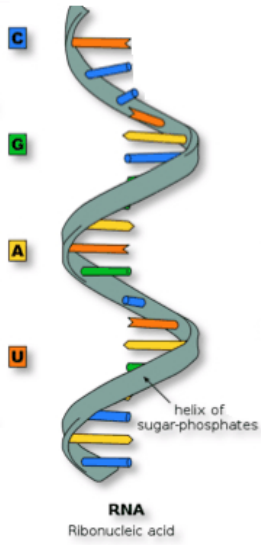


Figure 2-1. RNA
(wikipedia.com)

Part I: Transcription

Procedure

1. Look back at your observations of DNA from your last lab exercise and examine the molecule of RNA shown (Figure 2-1).

Question: Identify any similarities and/or differences between DNA and RNA.

Similarities	Differences

To synthesize RNA, a segment of a DNA molecule untwists and the hydrogen bonds between the nucleotides are broken. The nucleotides of one strand pair with complementary RNA nucleotides. When these nucleotides are joined by sugar-phosphate bonds, RNA separates from DNA.

Procedure

2. Using the DNA molecule that you designed last week, unwind your DNA molecule.

3. Looking at the DNA template strand, build the RNA that corresponds to your DNA molecule. The safety pins will serve as the phosphodiester bonds of the RNA backbone.

Transcription is the first step in protein synthesis. The type of RNA that you just made from your gene's DNA sequence is known as **messenger RNA** (mRNA). The base triplets in mRNA are known as **codons**.

Part II: Translation

Translation is the process by which a polypeptide (a subunit of a protein) is formed. The genetic code in the mRNA's codons determines the placement of amino acids (the building blocks of proteins). The codons of mRNA and the amino acids that they specify for are shown in the table on the right.

In order for translation to occur, a second type of RNA known as **transfer RNA** (tRNA) is needed. tRNA carry amino acids to the mRNA in the ribosome, the site of protein synthesis in the cell. The tRNA contains an anticodon which is complementary to an mRNA codon.

Universal Genetic Code Chart
Messenger RNA Codons and Amino Acids for Which They Code

First base	Second base U	Second base C	Second base A	Second base G	Third base
U	UUU PHE	UCU SER	UAU TYR	UGU CYS	U
	UUC PHE	UCC SER	UAC TYR	UGC CYS	C
	UUA LEU	UCA SER	UAA STOP	UGA STOP	A
	UUG LEU	UCG SER	UAG STOP	UGG TRP	G
C	CUU LEU	CCU PRO	CAU HIS	CGU ARG	U
	CUC LEU	CCC PRO	CAC HIS	CGC ARG	C
	CUA LEU	CCA PRO	CAA GLN	CGA ARG	A
	CUG LEU	CCG PRO	CAG GLN	CGG ARG	G
A	AUU ILE	ACU THR	AAU ASN	AGU SER	U
	AUC ILE	ACC THR	AAC ASN	AGC SER	C
	AUA ILE	ACA THR	AAA LYS	AGA ARG	A
	AUG MET or START	ACG THR	AAG LYS	AGG ARG	G
G	GUU VAL	GCU ALA	GAU ASP	GGU GLY	U
	GUC VAL	GCC ALA	GAC ASP	GGC GLY	C
	GUA VAL	GCA ALA	GAA GLU	GGA GLY	A
	GUG VAL	GCG ALA	GAG GLU	GGG GLY	G

After an mRNA molecule is made, it combines with a ribosome, which contains the necessary nucleic acids and proteins for protein synthesis (Figure 2-2). tRNAs carrying an amino acid joins with the mRNA to form a polypeptide chain.

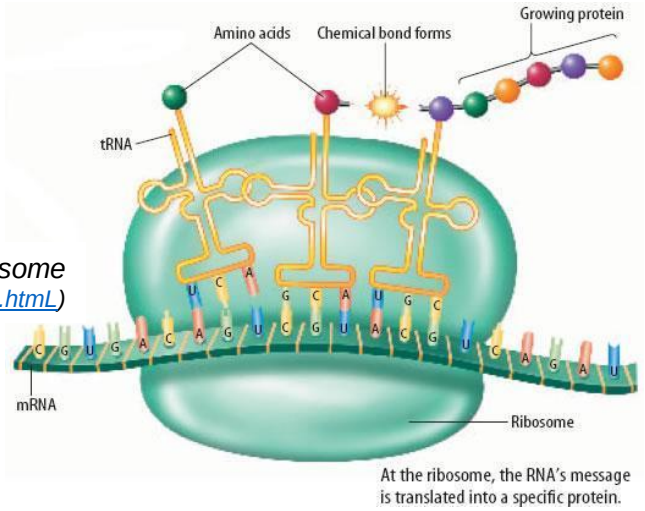


Figure 2-2. Translation at the ribosome
(biologyteksbylauryncarter.weebly.com/protein-synthesis.htmL)

Procedure:

4. Translate the mRNA that you synthesized in step of 2 of your procedure using the genetic code.

Question: Examine your polypeptide.

a. Write out the polypeptide (amino acid sequence) you constructed in the space below:

_____

b. Examine the other group's polypeptides. Does each group have the same polypeptide?

_____

c. If a group does not have the same polypeptide, write down the polypeptide they constructed.

_____

d. Examine their DNA and RNA molecule to see why they have a different polypeptide than your group.

_____

Part III: Mutations

Different groups in the last lab were assigned DNA molecules that differ in one nitrogenous base. A **mutation** is any change, no matter how minor, to a DNA sequence. These mutations can arise as the result of damage to DNA (by chemical or radiation damage) or from errors in DNA replication or repair. A mutation in a gamete (sex cell) will passed from one generation to the next.

There are several types of mutations. They can include:

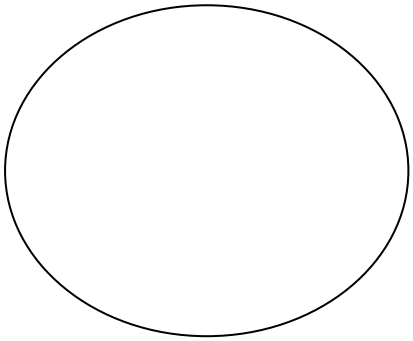
- Substitutions: are mutations that exchanges one base for another
- Insertions: are mutations in which extra base pairs are inserted into a new place in the DNA.
- Deletions: are mutations in which a section of DNA is lost, or deleted.

Since the genetic code is derived from codons, insertions and deletions can alter a gene so that its message is no longer correctly read and translated properly. These mutations are called frameshifts (they change the frame at which amino acids are added during translation).

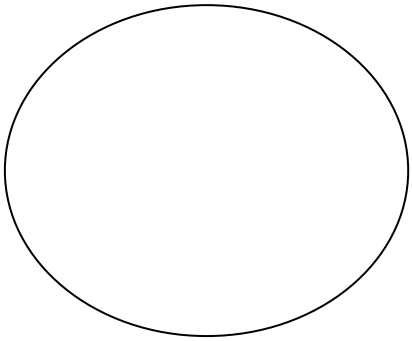
In the last laboratory exercise, you were introduced to a disease of red blood cells known as **sickle cell disease**. We will be focusing on sickle cell disease throughout this semester.

Procedure:

5. Examine the blood smears of a normal and sickle cell patient. Record your observations of the shape of normal blood cells and sickle cells.



Normal Blood Cells



Sickle Cell Blood Cells

Sickle cell is a genetic disorder that results in the abnormally shaped red blood cells. The cause of this has to do with the production of the protein **hemoglobin**. Hemoglobin, which is found in red blood cells, binds oxygen and carries it throughout the body. In sickle cell patients, a mutation is found in the DNA that results in improperly produced hemoglobin protein.

The DNA molecules you built are actually the DNA sequences for the gene encoding hemoglobin!

- Normal individuals have the following DNA sequence: T-G-A-G-G-A-C-**T**-C-C-T-C-T-T-C-A-G
- Sickle cell individuals have the following DNA sequence: T-G-A-G-G-A-C-**A**-C-C-T-C-T-T-C-A-G

Question: Answer the following questions about Sickle Cell.

a. What type of mutation causes sickle cell? Circle your answer: Substitution Insertion Deletion

b. If it the mutation is a substitution, indicate below what the amino acid originally was and what it was changed to. _____ → _____

Figure 2-3 shows the results of having improperly folded hemoglobin.

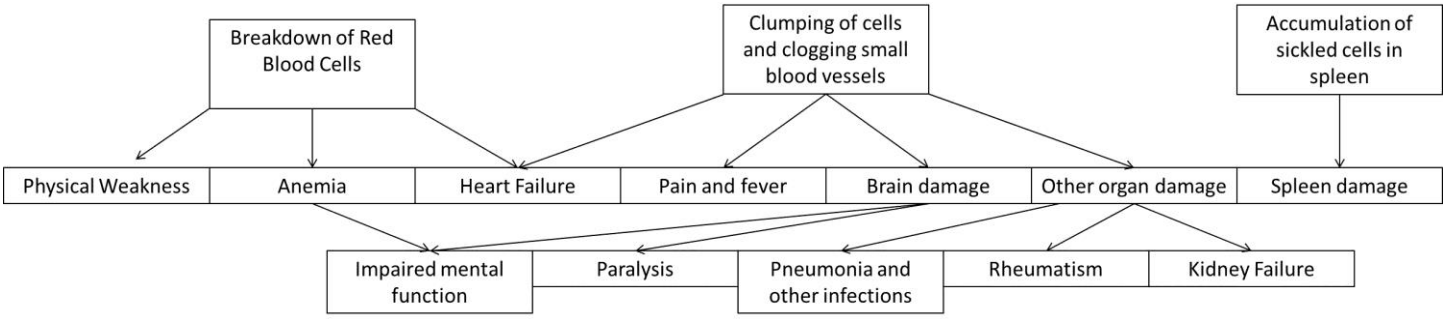


Figure 2-3. Effects of misfolded hemoglobin

Lab #3: Inheritance

Introduction:
Recall from our first laboratory exercise that one of the steps during our isolation procedure was the need to remove protein. DNA in the cell is organized into **chromosomes**, which consist of DNA and protein wrapped together. Our genes are located on these chromosomes. Genes (as you remember from our second lab) encode the information on how to make a protein

Human cells (with the exception of sex cells) each have 23 pairs of chromosomes (a total of 46 chromosomes). Since we have two of each chromosome, humans are said to be **diploid.** Half of our chromosomes came from our mother and half came from our father. A pair of chromosomes are referred to as being **homologous**. As we will see today, any deviation from the correct number of chromosomes can lead to drastic genetic disorders.

In today's exercise, we will see:
(a) how the cell maintains the proper chromosome number while it replicates
(b) how parents are able to donate half of their chromosomes to their offspring
(c) the relationship between chromosomes and genes

Part I. Mitosis

During cell replication, the cell must divide its nucleus, which houses the chromosomes. The division of a single nucleus into two genetically identical daughter nuclei is known as mitosis.

Procedure:

1. After your teaching fellows review the steps of mitosis, you and your laboratory partners will now look for the stages of mitosis in an onion root tip. Examine the slide and try to identify each stage of mitosis. As you make your sketches, remember to label the parts of the cell and describe what is happening.

Interphase	Prophase	Metaphase
_____ _____ _____	_____ _____ _____	_____ _____ _____
Anaphase	**Telophase**	
_____ _____ _____	_____ _____ _____	

Question: The human body produces 300 million cells/minute. Why is it important that mitosis must be precise and accurate?

Part II. Meiosis

With the exception of sex cells, each cell has 22 pairs of autosomal chromosomes and 1 pair of sex chromosomes. Females have 2 "X" chromosomes (XX) and males have an "X" and "Y" chromosome (XY).

During reproduction, both parents normally contribute one gamete or sex cell to the process. In females, this gamete is known as an egg and in males, this gamete is known as sperm. Each of these gametes have only 1 of each pair of chromosome and are therefore referred to as haploid.

In order for sex cells to be made, a process for reducing the number of chromosomes found in the cell is needed. This process is known as meiosis.

Procedure:

2. As your teaching fellows review the steps of meiosis (Figure 3-10, follow along using pop-bead chromosomes. You will be using the beads to model meiosis.

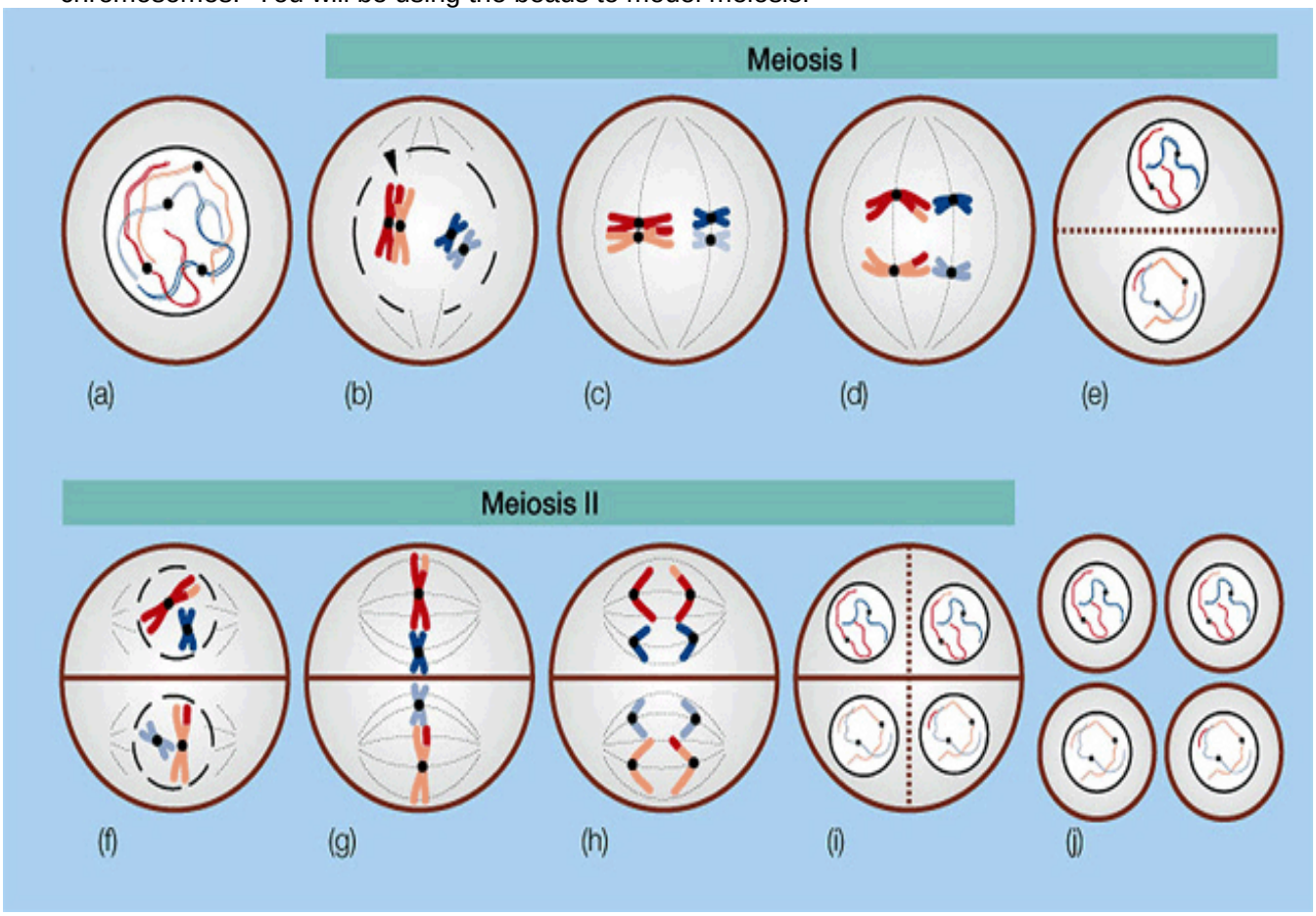


Figure 3-1. Overview of Meiosis
(http://www.tokresource.org/)

Question: If our parents give us our chromosomes and these chromosomes contain genes, why do we not look exactly like them? One reason is due to steps of "genetic diversity" that occur in meiosis. As you review the stages of meiosis, what steps allow for genetic diversity?

_____

_____

You will now look at meiosis and crossing-over in *Sordaria,* a fungus.

Sordaria fimicola is a haploid fungus for most of its life cycle. It becomes diploid only when two haploid cells fuse together. The diploid nucleus must then undergo meiosis to resume its haploid state. Meiosis, followed by mitosis, in *Sordaria* results in the formation of eight haploid ascospores contained within a sac called an ascus.

To observe crossing-over in *Sordaria*, one can fuse two haploid cells, one that produces black ascospores (+) and the other that produces tan ascospores (tn). When the diploid cells undergo meiosis, the asci that develop will contain four black ascospores and four tan ascospores.

The arrangement of the spores directly reflects whether or not crossing over has occurred.

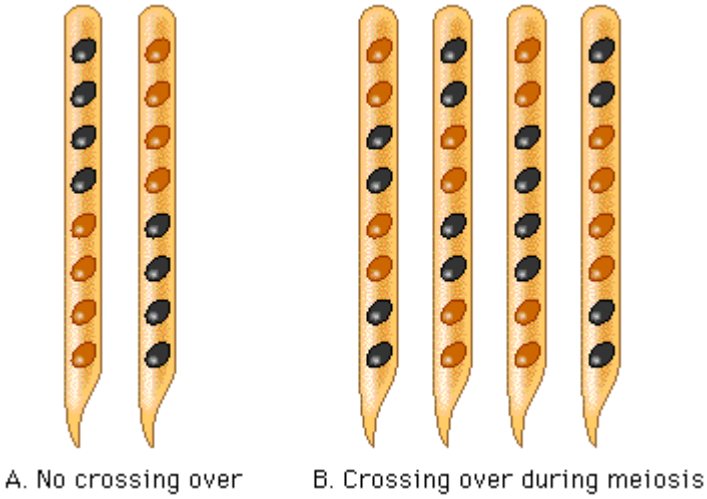


Figure 3-2. Crossing over in Sordaria
(http://www.phschool.com/science/biology_place/labbench/lab3/spores2.htmL)

Procedure:

3. Examine the cards showing a mating between (+) and (tn). Count the # of crossing-over asci and determine the percentage of asci showing crossing over. Show all work in the space below.

During reproduction, gametes fuse together to form a diploid organism. Unfortunately, errors in meiosis can occur, resulting in several genetic disorders once the gametes fuse. One such disorder is known as **Down's Syndrome**. Individuals with Down's Syndrome have three copies of chromosome #21, and present symptoms of mild to extreme mental retardation.

Part III. Inheritance

The process of meiosis and the "genetic diversity" it offers is one reason why children look like, but are not exactly identical to their parents. Another reason why we are different from our parents has to do with how the genes on the chromosomes are expressed.

Although homologous chromosomes are composed of genes for the same traits in the same order, they may have different forms of the gene. Different versions of the same gene are referred to as **alleles**.

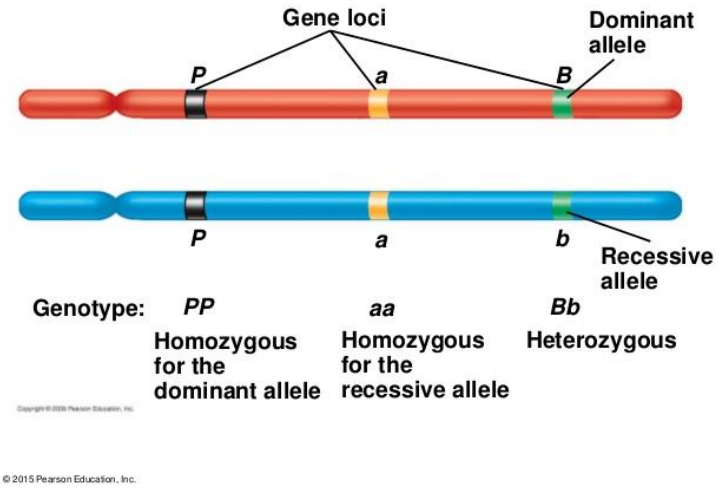


Figure 3-3. Homologous chromosomes showing homozygous and heterozygous genotypes.
(Campbell's Biology, Pearson Education)

As seen in Figure 3-3, you are said to be **homozygous** if you have two of the same allele. You are said to be **heterozygous** if you have two different alleles. The alleles you have for a particular gene is known as the **genotype**. What is actually expressed is known as the **phenotype**.

Some alleles are expressed only when the genotype is homozygous. These alleles are said to produce **recessive phenotypes**. Alleles that are expressed whether the genotype is homozygous or heterozygous produce **dominant phenotypes**.

Procedure:

4. Check off your phenotypes and the class' phenotypes in the table on the next page.

		Your phenotype	Number in Class	% of Class
Earlobes	Free Earlobes (D)			
	Attached Earlobes (r)			
Hairline	Widows Peak (D)			
	Straight Hairline (r)			
Tongue Roller	Tongue Roller (D)			
	Nonroller (r)			
PTC	PTC Taster (D)			
	Nontaster (r)			
Freckles	Freckles (D)			
	No freckles (r)			
Mid-digital hair	Mid-digital hair (D)			
	No mid-digital hair (r)			
Dimples	Facial dimples (D)			
	No facial dimples (r)			
Finger Interlacing	Left/Right thumb (D)			
	Right/Left thumb (r)			

D= dominant, r = recessive

Question: Does the majority of the class exhibit dominant phenotypes?

_____

It is important to note that although the inherited traits an individual has can be determined by their genes, usually more than one gene is involved in defining a particular trait.

Some genetic disorders are classified as dominant or recessive. In **dominant disorders**, disease state is carried on the dominant allele. Normal state is carried on the recessive allele. The disorder will occur in an individual that is either homozygous dominant or heterozygous (since in both cases, the dominant allele is present). Examples of dominant genetic disorders include:

Disorder	**Symptom**
Achondroplasia	Dwarfism
Alzheimer's Disease (one type)	Mental deterioration, usually later in life

In **recessive disorders**, disease state is carried on the recessive allele. Normal state is carried on the dominant allele. The disorder will occur in an individual that homozygous recessive. Individuals that are heterozygous are referred to as **carriers** and do not present symptoms of the disorder.

Question: Why do carriers not present symptoms of recessive disorders?

_____

_____

Sickle cell is a recessive genetic disorder. Other recessive genetic disorders can include:

Disorder	**Symptom**
Albinism	Lack of pigment in skin, hair, eyes
Cystic Fibrosis	Excess mucus in lungs, digestive tract, liver
Tay-Sachs	Mental deterioration, lipid accumulation in brain cells

<u>Question:</u> The Amish have higher rates of genetic disorders. These disorders can include dwarfism and blood diseases. It is believed that this high rate is due to the practice of inbreeding.

(a) What is inbreeding? _____

(b) Why would inbreeding cause a high rate of genetic disorders?

_____

Lab #4: Manipulation of DNA

Introduction:
Previously, we learned that DNA is transcribed into RNA and that RNA is translated to protein (The Central Dogma of Biology). We also saw that a mutation in the DNA may lead to a defect in the protein that is produced. A mutation in the hemoglobin gene of some individuals results in sickle cell.

Molecular biologists have various techniques at their disposal when working with DNA. In today's activities, you will learn about two of these techniques and how they can be applied in diagnosing and treating sickle cell disease:

(a) Restriction Fragment Length Polymorphism {RFLP}
(b) Bacterial Transformation {an example of genetic engineering}

Some bacteria produce enzymes known as **restriction enzymes**. These enzymes recognize and cut DNA molecules at specific nucleotide sequences. For example, the restriction enzyme *MstII* recognizes and cuts DNA molecules with the following sequence: C-C-T-N-**A**-G-G (where N can be either A, T, C or G).
G-G-A-N-**T**-C-C

Individuals that have sickle cell have the following sequence: C-C-T-G-**T**-G-G
G-G-A-C-**A**-C-C

Therefore, *MstII* will not recognize this DNA sequence and will not cut it.

DNA that has been digested by restriction enzymes can be visualized by gel electrophoresis (the same technology you used during the first lab to see if you extracted DNA from strawberries). Smaller DNA fragments that can easily migrate through agarose will migrate towards the bottom of the gel while larger DNA fragments will remain closer to the top of the gel.

In this exercise, you will be given DNA from a family. It is up to you to determine which individual(s) are normal, are carriers, or have sickle cell disease. You will digest this DNA with *MstII* and determine what the individual's genotype and phenotype are.

With this DNA: normal individuals will show 3 DNA bands on the gel.
carrier individuals will show 4 DNA bands on the gel.
disease individuals will show 2 DNA bands on the gel.

In order for us to do this, you will first need to learn how to use a special piece of laboratory equipment known as the micropipettor. Your teacher will review with you how to use these devices.

General Procedure for using Micropipettors:

- Make sure you have a tip on the pipette before placing it in liquid. Otherwise you could ruin the precision piston that measures the volume. Never set the pipette down with liquid in the tip, always remove the tip when finished.

- It is important to press and release the plunger slowly. The tip opening is very small and it takes time to take up liquid into the tip and it also takes time to expel the liquid out. Allowing the plunger to snap back could damage the piston.

- There are three positions for the plunger: all the way up (the default position), half-way depressed (you will feel resistance) and all the way down. The purpose of the middle position is to pull up the volume set on the pipettor. The final, fully depressed position is used to push a column of air behind the liquid in order to expel it completely.

- To pipette: Place tip on micropipettors by pushing the micropipettor down onto a tip in box, depress the plunger to the first stop then lower pipette into the liquid, transfer the micropipettor with liquid to the vessel you're pipetting to, then depress the plunger again, this time pushing down all the way to the second stop.

- Properly dispose of your tips in the labeled waste container.

- A video on how to properly use micropipettors is available on the Saint Joseph's University GEP Natural Science Instructional Laboratories website: *http://www.sju.edu/int/academics/cas/resources/geplabs/Instructional%20Videos.htmL*

Part I: RFLP

Procedure:

1. On your bench are DNA samples of a family (mother, father, daughter and son) in tubes placed in an ice bucket (be sure to keep everything on ice!)

2. Add 10 µL of restriction enzyme mix to each of these tubes. *Note: when aliquoting the restriction enzyme – try not to introduce bubbles into your mix*.

3. Do NOT vortex. To mix, gently flick your tube. Keep enzyme on ice at all times.

4. Incubate the tubes for 30-45 minutes in the 37ºC water bath to allow the enzymes to function.
   ** While your DNA is incubating, begin the second part of today's lab - introducing foreign DNA**

5. After 30-45 minutes has elapsed, remove the tubes and add 3 µL of loading dye to each of your samples.

6. Carefully load DNA + loading dye into the appropriate well at the cathode end (negative) of the gel.

7. Once the gel has finished resolving, a picture of the gel will be taken. Paste your gel image in the space below.

Question: Based on your gel results, draw a pedigree chart of the family. Indicate which individual(s) are normal, carriers or have the disease.

Part II: Genetic Engineering

Another manipulation of DNA scientists perform regularly is introducing and/or removing DNA from a cell, a technique known as **genetic engineering**.

For your second lab activity, you will construct a genetically modified bacterium such that it glows when placed under ultraviolet light. To do this, you will insert a piece of DNA into *E. coli*, a bacterium. When working with bacteria, please remember:

- Before working with any bacterial culture, make sure your lab bench is clean. Wash your hands with soap and water and put on a pair of gloves.
- All open cuts and scratches must be covered with Band-Aids.
- All waste generated from work with bacteria must be placed in the BIOHAZARD WASTE container (not the regular garbage container).
- Remember to wipe down your lab bench with disinfectant when you have completed your work and wash your hands again with soap and water.

Procedure:

8. Place two tubes of *E. coli* in an ice bucket. Label one tube (+) and the other tube (-).
9. Add 5 µL of pGLO into the (+) tube. pGLO contains DNA that will allow bacteria to glow under ultraviolet light.
10. Incubate the bacterial tubes on ice for 30 minutes. During this incubation, the DNA you added will "stick" to the cell membrane of the bacteria.
11. After 30 minutes, place the bacterial tubes in a 42°C water bath for 1 minute. This "heat-shock" will make the bacterium's cell membrane more fluid, to allow the DNA that was stuck to the cell membrane to enter the cell.
12. Then return the tubes to ice for 2 minutes. This two minute incubation will allow the bacteria to recover.
13. Add 250 µL of Luria-Bertani broth. Leave the tubes out at room temperature for 10 minutes.
14. After 10 minutes, aliquot 100 µL from each tube onto the appropriate nutrient plate. Use a sterile inoculating loop to spread your bacterial culture. Your teacher will show you how to perform this step.
15. Label the plate on the bottom edge with a sharpie. Incubate the plates for 24 hours at 37°C.
16. *During the next laboratory class,* record your observations.

Question: Indicate whether any growth of *E. coli* occurred on each plate and whether or not they glow when you shine UV light on them.

(-) pGLO: _____

(+) pGLO: _____

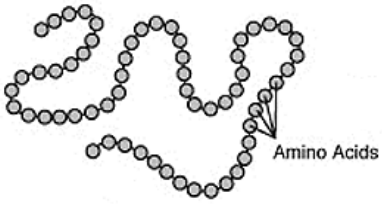
Sources. This laboratory exercise has been adapted from:

- Bio-Rad. Biotechnology Explorer: pGLO™ Bacterial Transformation Kit

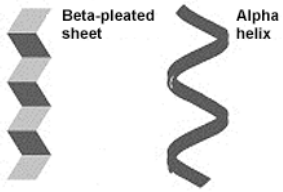
Lab #5: Protein Structure

Introduction:
Mutations in the DNA may lead to a defect in the protein that is produced. That defect could potentially have serious effects on the protein's shape and/or ability to function.

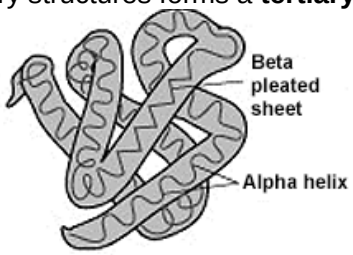
During our transcription and translation lab, you constructed a polypeptide chain of **amino acids**. You wrote this amino acid chain as a **linear or primary sequence**, as shown below:



Intermolecular forces between amino acids results in the protein chain forming **secondary structures** as shown below.



Intermolecular forces between the secondary structures forms a **tertiary structure** of a protein, as shown below.



In some proteins, tertiary structures interact with one another to form a **quaternary** structure.

** Figures for protein structure adapted from https://www.umass.edu/molvis/workshop/prot1234.htm*

Knowing protein structure, we can now examine the effect of the mutation in hemoglobin (Figure 5-1).

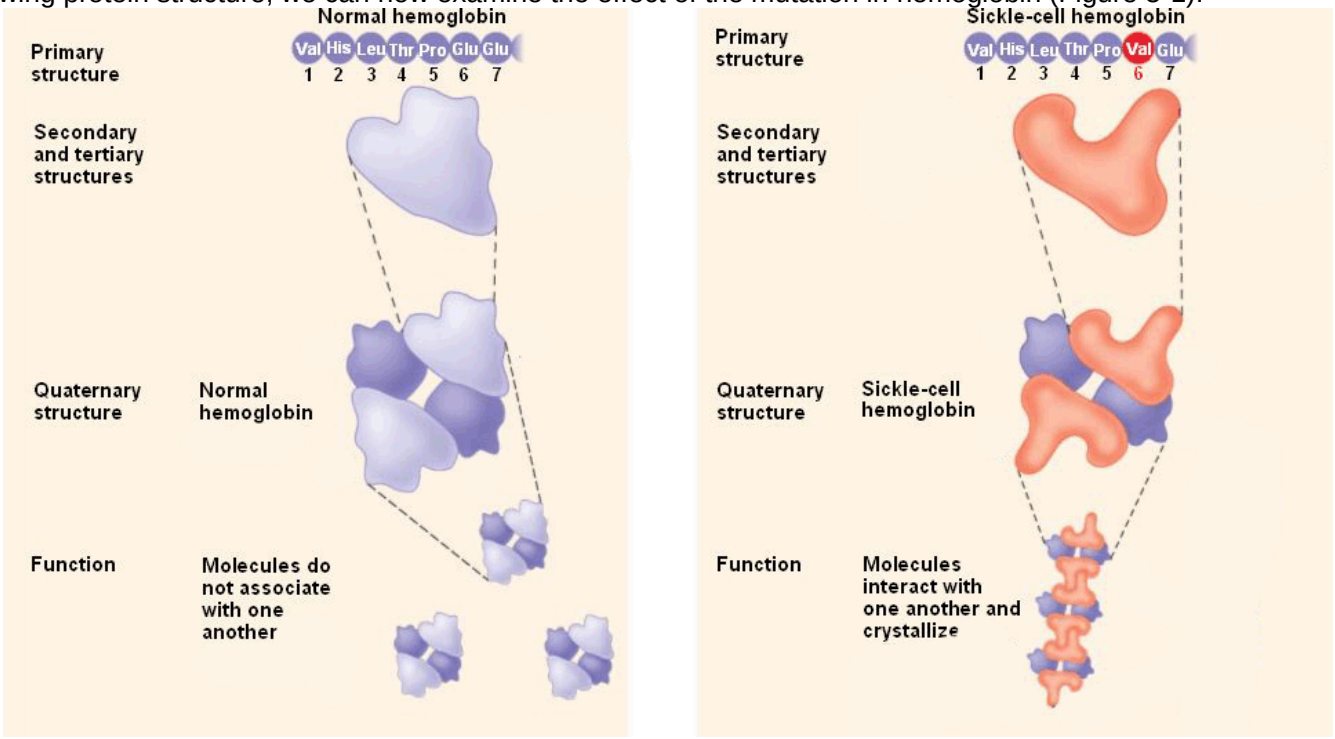


Figure 5-1. Structure of normal and sickle-cell hemoglobin
(Campbell's Biology, Pearson Education)

As you can see from the diagram, **the mutation results in a change in protein structure**. That change in protein structure results in hemoglobin interacting with one another and crystallizing. This crystallization results in the deformed shape of the red blood cell and decreases hemoglobin's ability to carry oxygen.

In our last lab, you looked at the DNA to determine whether an individual had sickle cell disease. You can also look at the individual's proteins. Scientists have different ways of assessing protein structure and function. In today's lab, we will look at examples of each technique.

Part I: Learning about protein structure

In this activity, you will explore the structure of proteins and the chemical interactions that drive each protein to fold into its specific structure, as noted below.

- Each protein is made of a specific sequence of amino acids
- There are 20 amino acids found in proteins.
- Each amino acid consists of two parts — a backbone and a side chain
- The backbone is the same in all 20 amino acids and the sidechain is different in each one.
- Each side chain consists of a unique combination of atoms which determines its 3D shape and its chemical properties.
- Based on the atoms in each amino acid side chain, it could be hydrophobic, hydrophilic, acidic (negatively charged), or basic (positively charged)

When different amino acids join together to make a protein, the unique properties of each amino acid determine how the protein folds into its final 3D shape. The shape of the protein makes it possible to perform a specific function in our cells.

When examining the side chains of amino acids, we can classify them as

- Acidic
- Basic
- Hydrophobic
- Hydrophilic.

What do you think hydrophobic means? Separate the word 'hydrophobic' into its two parts — hydro and phobic. Hydro means water and phobia means fear or dislike, so hydrophobic side chains don't like water. Hydrophobic side chains are also referred to as non-polar side chains.

Now can you guess what hydrophilic means? Philic means likes or attracted to, so hydrophilic side chains like water. Hydrophilic side chains are also referred to as polar side chains.

Procedure:

1. At your bench are the 20 amino acids classified according to their chemical properties. Look at the colored spheres in each side chain. Scientists established a coloring scheme (see chart below) to make it easier to identify specific atoms in models of molecular structures.
    - Carbon is Gray
    - Oxygen is Red
    - Nitrogen is Blue
    - Hydrogen is White
    - Sulfur is Yellow

Question: Did you notice similarities of patterns in each group of side chains? Describe your observations

- **Hydrophobic** side chains primarily contain _____ atoms.
- This amino acid is an exception to the above observation: _____.
- In addition to carbon atoms, **acidic side chains** contain two _____ atoms. This is called a **carboxylic acid** functional group. Acidic side chains carry a **negative charge**.
- In addition to carbon atoms, **basic side chains** contain _____ atoms. This is called an **amino** functional group. Basic side chains carry a **positive charge**.
- Hydrophilic side chains have various combinations of these atoms: hydrogen, carbon, _____, _____ and _____.

Once you have explored the chemical properties and atomic composition of each side chain, think about how proteins spontaneously fold into their 3D shapes.

Question: Predict what causes proteins to fold into their 3D shapes.

a. Which side chains might position themselves on the interior of a protein, where they are shielded from water?

_____

b. From your knowledge of static electricity, which side chains might be attracted to each other?

_____

Procedure:

2. Unwind the 4-foot mini-toober (foam-covered wire) that is in your kit. The blue end cap represents the N-terminus (the beginning) of the protein and the red end cap represents the C-terminus (the end) of the protein.

3. Choose **15 side chains** from the chemical properties circle as indicated in the chart below. **Be sure to include <u>GLU</u>. Do not use <u>VAL</u> yet**. Mix the side chains together and place them (in any order you choose) on your mini-toober.

4. Beginning at the N-terminus of your mini-toober, add an amino acid every three inches onto the toober (3 inch marks have been already made for you on the toober).

5. Now you can begin to fold your 15-amino acid protein according to the chemical properties of its side chains. Remember all of these chemical properties affect the protein at the same time. (see photo at your lab bench associated with your kit)

Question: What are your observations about how proteins fold? Answer the following questions:

a. What happened as you continued to fold your protein and applied each new chemical property to your protein?

_____

b. Were you able to fold your protein, so that all of the chemical properties were in effect at the same time?

_____

c. If not, do you have any ideas why you weren't able to fold your protein in a way that allowed all of the chemical properties to be in effect simultaneously?

_____

d. Did your protein look like the proteins other students folded? _____
Explain: _____

Procedure:

6. Go back to your protein and introduce a mutation. Change the **GLU** for **VAL**. Then, fold your protein again according to the chemical properties of its side chains.

Question: How did your protein shape change when the mutation was introduced? Can you explain why?

_____

Part II: Analyzing Protein Structure:

Protein structure can be examined using antibodies. Antibodies are proteins produced by an organism's immune system to recognize and help destroy foreign particles.

Scientists use a procedure known as **Enzyme-Linked Immunosorbent Assay (ELISA)**. The assay takes advantage of the fact that antibodies are specific for particular amino acid sequences and hence, protein structure. Pregnancy tests are actually ELISAs! Sickle cell can also be detected via ELISA.

Figure 5-2 below is a schematic of how ELISAs are performed.

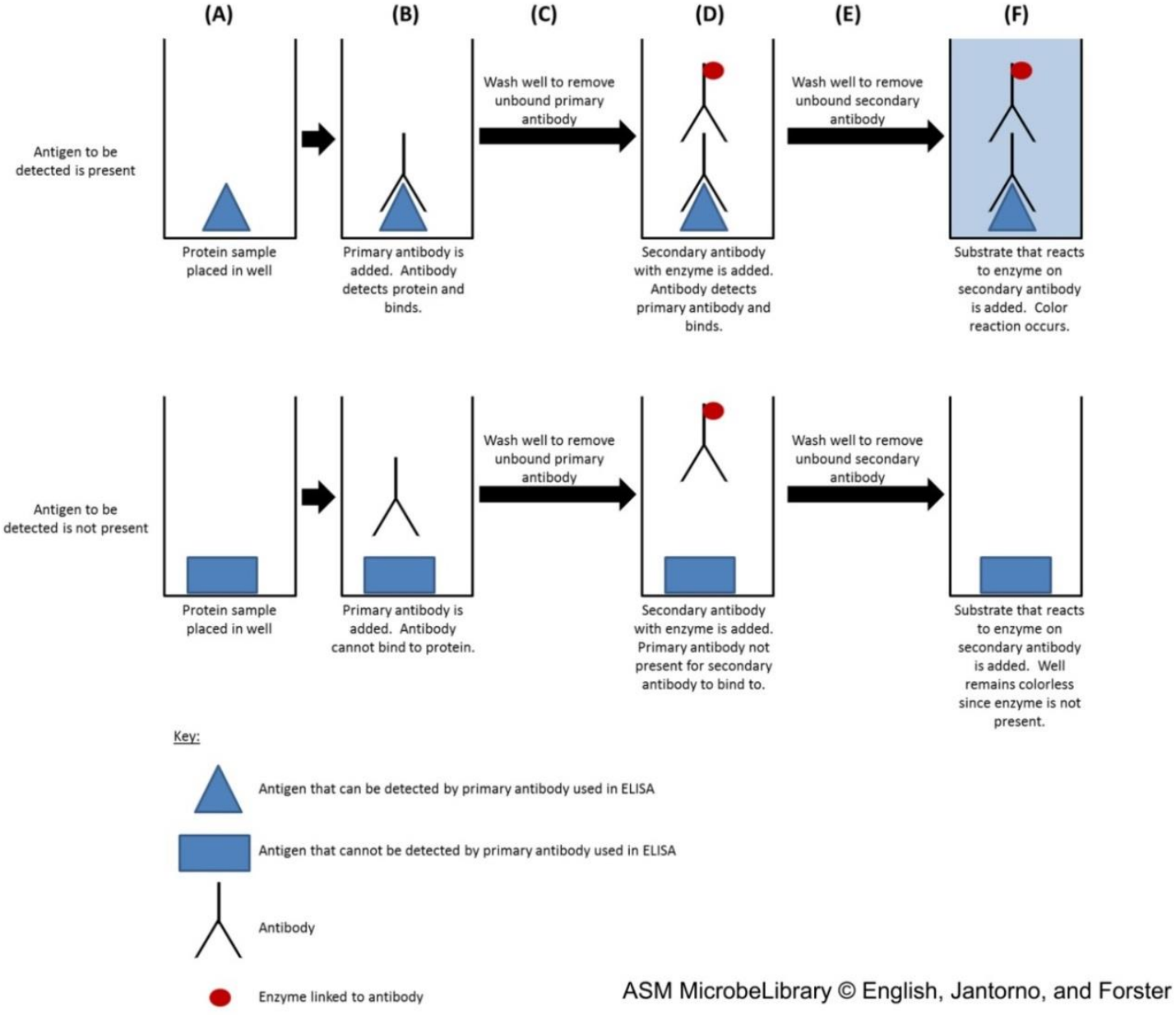


Figure 5-2. ELISA Procedure
(*American Society for Microbiology*)

In our ELISA assay, the secondary antibody is conjugated to horseradish peroxidase (HRPO). The substrate we will use is 3,3',5,5' – tetramethylbenzidine (TMB) – a colorless solution. In the presence of HRPO, TMB will turn blue.

Therefore, a well changing color is indicative that the normal protein structure is present

Procedure:

7. On your bench are protein samples from a family (mother, father, daughter and son) as well as protein samples from patients known to have normal hemoglobin or have sickle cell disease.

8. A stack of paper towels will also be at your bench. You will need these towels for completing the ELISA.

9. Obtain a test strip and add 50 µL of each sample to separate wells. Wait 5 minutes for protein to bind.

10. Remove samples from wells by firmly tapping them on a paper towel.

11. Discard the top paper towel.

12. Add 50 µL of the primary antibody (PA) to each well.

13. Wait 5 minutes for the antibody to bind.

14. After 5 minutes, wash the wells two times (To wash - add 100 µL of wash buffer. Then, remove wash buffer by firmly tapping the wells on a paper towel. Discard the top paper towel)

15. Add 50 µL of the enzyme-linked secondary antibody (SA) to each well.

16. Wait 5 minutes for the antibody to bind.

17. After 5 minutes, wash the wells four times (same procedure as you did before in step 8).

18. Add 50 µL of the enzyme substrate to each well.

19. Wait 5 minutes. Positive samples will begin to turn blue.

20. Record your results below:

Individual	Normal	Sickle	Mother	Father	Daughter	Son
Normal or Mutant?						

Question: Look back at your RFLP analysis and pedigree chart. Are the results of the ELISA assay consistent or inconsistent with those results?

_____

_____

Question: Was the ELISA assay able to tell you who were "carriers?" Why or why not?

_____

_____

Sources. This laboratory exercise has been adapted from:

- 3D Molecular Designs: Amino Acid Starter Kit
- Bio-Rad. Biotechnology Explorer: ELISA Immuno Explorer Kit

Lab #6: Identifying and Explaining the cause of genetic disorders (FIELD TRIP)

Introduction:

Recently, three expecting couples went to the hospital for a check-up. Blood was collected from the fetus. In addition, an **amniocentesis** was performed (Figure 6-1).

Amniocentesis is a process where a sample of amniotic fluid (fluid that surrounds the fetus) is collected and analyzed. The main purpose of this analysis is to check for genetic defects in a baby.

After the amniotic fluid is collected, the fluid and fetal cells from the amniotic fluid are analyzed.

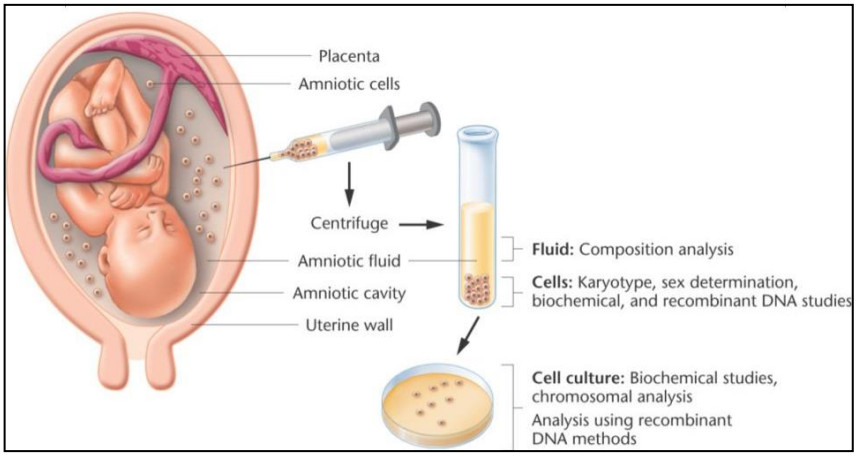


Figure 6-1. Amniocentesis
(www.healthpowerforminorities.com)

In today's activity, you will analyze these samples to develop conclusions about each fetus and their parents. Specifically, you will determine whether the fetus has a genetic disorder.

The potential genetic disorders that these fetuses may have include:

Disorder	Symptoms	Genetic cause
Sickle Cell Anemia	Sickled red blood cells	Mutation in chromosome #11 (Glu → Val)
Down's Syndrome	Varying levels of intellectual disability	Three copies of chromosome #21
Cri du chat	Abnormal larynx development	Partial deletion in chromosome #5

Using the techniques in each part of this laboratory, you will determine if each fetus has a genetic disorder. As you work through each exercise, record your results in your Data Table.

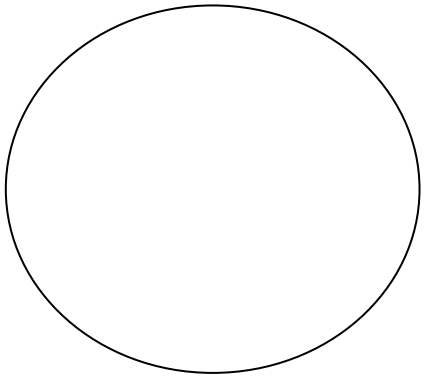
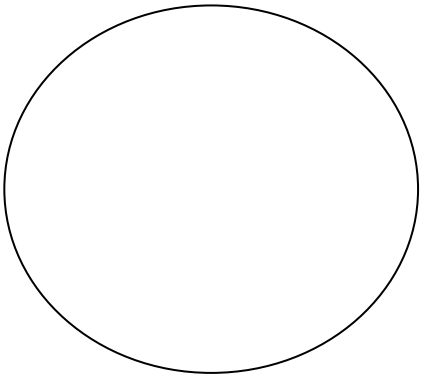
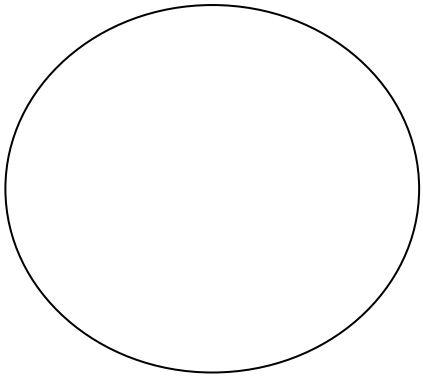
	Blood Observations	Average red blood size	Karyotype Result	Sex of fetus	Hemoglobin analysis	RFLP Results (circle result)		DNA Microarray (circle result)		Conclusion
						Ch.5	Normal / Mutation	Ch.5	Normal / Duplication / Deletion	
A						Ch.11	Normal / Mutation	Ch.11	Normal / Duplication / Deletion	
						Ch.21	Normal / Mutation	Ch.21	Normal / Duplication / Deletion	
						Ch.5	Normal / Mutation	Ch.5	Normal / Duplication / Deletion	
B						Ch.11	Normal / Mutation	Ch.11	Normal / Duplication / Deletion	
						Ch.21	Normal / Mutation	Ch.21	Normal / Duplication / Deletion	
						Ch.5	Normal / Mutation	Ch.5	Normal / Duplication / Deletion	
C						Ch.11	Normal / Mutation	Ch.11	Normal / Duplication / Deletion	
						Ch.21	Normal / Mutation	Ch.21	Normal / Duplication / Deletion	

Lab a: Phenotype Analysis – Blood & Karyotype Analysis

Part I. Blood Analysis

Procedure:
Smears of the blood collected from each fetus were prepared on microscope slides.

1. Around the room are the microscope slides with blood smears of each fetus. Examine the smears and draw your observations in the space below. Describe your observations below your sketches.

FETUS A	FETUS B	FETUS C
		

2. Using your observations, complete the Blood Observations column of your data table.

Question: Based on these observations, can you determine at this time whether any of the fetuses have a genetic disorder? If so, which one(s) and why? _____

While examining the cells, you may notice that the sizes of the red blood cells of fetuses may appear to be different. Scientists use microscopes to not only visualize cells, but to measure their size. The microscopes in the lab are connected to a camera that can be used to measure cells. A benefit of using a camera and digital measuring software is that is allows you to measure curved measurements.

Procedure:
Microscopy images of normal and abnormal red blood cells were taken. You will measure 5 normal red blood cells and 5 abnormal red blood cells.

To measure the cells, we will use Infinity Analyze, a computer program that scientists regularly use.

3. On your computer screen, you should see a Live Image of what you have focused on using the microscope.

4. Make sure the objective the microscope is set to matches the setting on the Infinity Analyze program.

5. Click “Capture”

6. Using the mouse, click Measure → Point to Point.

7. Using the mouse, click on the cell and drag a line that represents the cell’s diameter. Click again and the measurement will appear. Repeat for 5 different cells.

8. Record your data in the table below.

Cell #	Normal Red Blood Cell Size (µm)	Abnormal Red Blood Cell Size (µm)
1		
2		
3		
4		
5		
Average		

9. Using your observations, complete the average red blood cell size column of your data table.

Question: Based on these observations, do you believe there is a significant difference in the sizes between the normally and abnormally shaped red blood cells? _____

Part II: Karyotype Analysis

Karyotypes are photographs or diagrams of chromosomes. To prepare a karyotype, condensed two-chromatid chromosomes are treated with **Giemsa** staining. The dye stains regions of chromosomes that are rich in the DNA base pairs adenine (A) and thymine (T). This stain produces dark bands in the chromosomes. To prepare a karyotype, the chromosomes are generally arranged in pairs and in order of size. Members of each pair are identified based on size, location of the centromere, and banding pattern

Procedure:
The fetal cells from the amniocentesis were collected and the chromosomes were extracted. You will now examine these chromosomes.

10. Using the magnetic chromosome images at your station, karyotype one of the fetuses on the board.

11. Based on your karyotype, determine the sex of the fetus and whether the fetus has any chromosomal genetic disorder. Be sure to walk around to the other groups so you can see their karyotypes.

12. Record your conclusions in the Karyotype and sex of fetus columns of your data sheet.

Lab b: Phenotype Analysis – Protein Function (Hemoglobin)

Recall from our previous laboratories that hemoglobin is a protein found in red blood cells that carries oxygen throughout the body. The shape (structure) of hemoglobin is ideal to carry oxygen molecules (Figure 6-2).

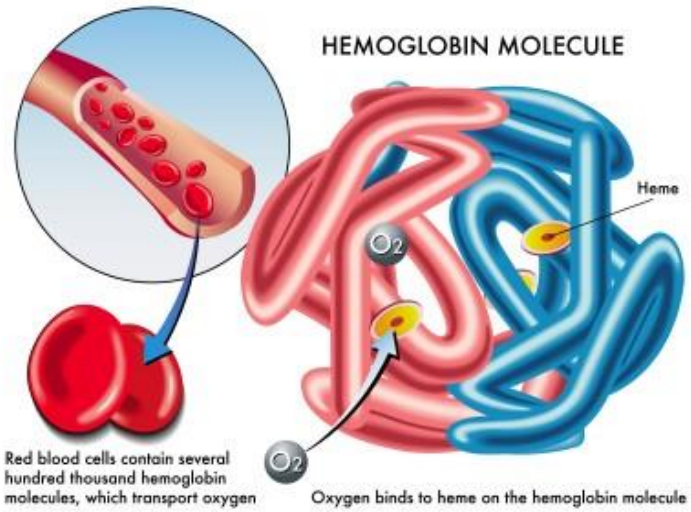


Figure 6-2. Quartnary structure of hemoglobin binding to oxygen
(*http://www.123rf.com/photo_14225607_hemoglobin.htmL*)

An alteration to a protein's structure can affect the shape of the active site and thus, its function. In sickle cell patients, the Glu→Val mutation (which we have discussed previously) makes hemoglobin stick to each other. As a result, the function of each hemoglobin is reduced. Therefore, the mutant hemoglobin can carry less oxygen than the normal hemoglobin.

In this study, we will look at hemoglobin's ability to bind oxygen.

To generate oxygen, we will use peroxidase (the same enzyme used in the ELISA assay). In the presence of peroxidase,

$$H_2O_2 \rightarrow 2H_2O + O_2 \text{ (gas)}.$$

We will add hemoglobin to each reaction and determine whether it will bind oxygen. To measure free oxygen (O_2), you will use an indicator known as **guaiacol.** In the presence of free oxygen, guaiacol will turn brown. The darker the color, the more oxygen is available.

Procedure:
Hemoglobin from the fetuses red blood cells were isolated. You will analyze the hemoglobin.

1. Each group will test each fetus' hemoglobin.

Enzyme (peroxidase)	750 µL
Substrate (peroxide)	150 µL
Distilled water	3.5 mL
Hemoglobin	3 mL
Guaiacol	100 µL

Using clean 16 x 150 mL test tubes, prepare your enzymatic reaction (one for each reaction).

2. Cover the test tube with Parafilm, gently mix, and place the tubes back in the test tube rack at the room temperature. Immediately begin timing the reactions.

3. Record the observed color for each tube at 0 minutes. Use the following scale

	Hemoglobin A	Hemoglobin B	Hemoglobin C
0 minute			
1 minute			
2 minute			
3 minute			
4 minute			
5 minute			

4. Using your observations, complete the Hemoglobin column of your data table. Indicate whether the fetus has normally or abnormally functioning hemoglobin.

Question: Based on these observations and your experience with protein folding, do you believe protein function will always be affected if a mutation has been introduced into the protein? Why or why not?

_____

_____

Sources. This laboratory exercise has been adapted from:

- The College Board. Advanced Placement Biology Lab Manual (2001) – Lab 13 Enzyme Activity

Lab c: DNA Analysis – RFLP and DNA Microarray

Part I: RFLP analysis

Procedure:

1. The fetal cells from the amniocentesis were collected and the DNA from these cells were extracted. A Restriction Fragment Length Polymorphism experiment was performed to determine whether any mutations were present in chromosomes 5, 11 and/or 21.

2. Examine the RFLPs at your station and determine whether a mutation is present in each of the chromosomes. Record your conclusions in the RFLP columns of your data sheet

Question: Explain why the normal DNA was different from the mutant DNA in the RFLP experiment.

_____

Part II: DNA Microarray

Although the karyotyping results of each fetus may indicate show a correct number of chromosomes, the amount of DNA in each of the chromosomes may be incorrect. Too much or too little could lead to a genetic disorder.

You decide to perform a DNA microarray analysis (Figure 6-3). Microarrays can detect (up or down) gene regulation and compare mRNA levels.

The microarray is a map that is covered with dots consisting of DNA from known locations on each of the 46 chromosomes. The test looks for imbalances in the amount of chromosomal material between DNA from a control and the fetus' DNA.

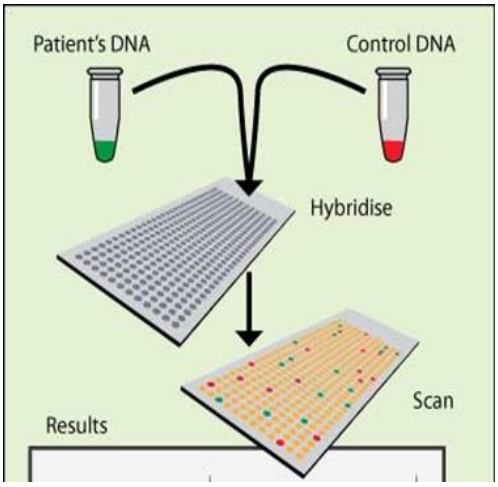


Figure 6-3. Microarray
(http://www.nchpeg.org/)

When the DNAs are mixed onto the microarray, one of three colors could develop :

Yellow:
there is an equal amount of chromosomal DNA between both the patient and control.

Red:
there is more chromosomal DNA in the control…therefore the fetal chromosome is missing DNA

Green:
there is more chromosomal DNA in the fetal DNA…therefore the fetus' chromosome had additional DNA

3. Obtain a microarray strip. Each well indicates
   - Spot A: Control reaction: equal amount of fetal and control DNA
   - Spot B: Control reaction: less fetal DNA than control
   - Spot C: Control reaction: more fetal DNA than control
   - Spot D: Negative control – no reaction
   - Spot E: Chromosome #5 detection
   - Spot F: Chromosome #11 detection
   - Spot G: Chromosome #21 detection

4. Apply 2 µL of DNA sample to spots on your microarray (the fetal DNA samples in front of you have already been mixed with control DNA). Allow the spots to dry for 5 minutes at room temperature.

5. Visualize the spots using a UV lamp. Be sure to wear eye protection.

6. Record your results below and on the board. If there is time, you can run samples E through G for the other fetuses.

Control Spots	Colors
A	
B	
C	
D	

Control Wells	Fetus A Colors	Fetus B Colors	Fetus C Colors
E			
F			
G			

7. Based on the results of the microarray, determine whether there is a normal amount of DNA, extra DNA or less DNA in the fetus' chromosomes as compared to the control. Record your conclusions in the DNA Microarray column of your data sheet.

Question: Some scientists consider microarrays the future in diagnosing genetic disorders. Some however disagree. They say that DNA microarrays are difficult to interpret because in any cell some genes are always on or always off. Others state that finding causation from just the microarray data is virtually impossible. What are your thoughts? Do you agree or disagree with these concerns? Why?

_____

_____

_____

Sources. This laboratory exercise has been adapted from:

- Edvotek – DNA/RNA Microarrays