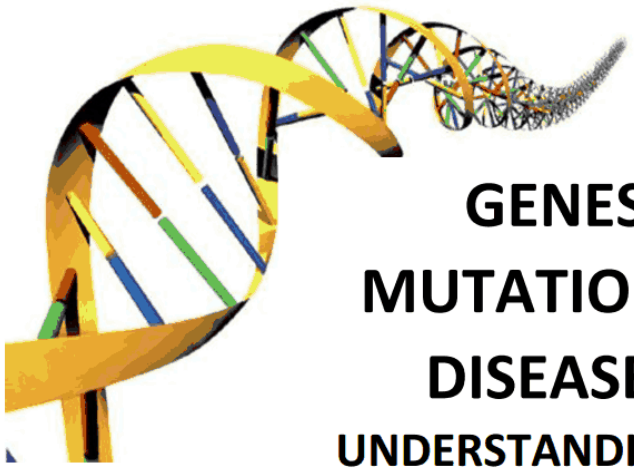
SJU SAINT JOSEPH'S UNIVERSITY

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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INSTRUCTOR GUIDE & ANSWER KEY

Please do not hesitate to contact us if you have any questions regarding the activities presented in this program.

For questions regarding the lessons, contact Dr. Edwin Li (eli@sju.edu).

For questions regarding the laboratory exercises and any troubleshooting or technical issues, contact Dr. Brian Forster (bforster@sju.edu).

The materials and recipes in this manual may need to be scaled to suit the number of students in your program.

Preparation of Nucleotide Molecule Kits

Materials Needed:
(Laboratory Preparation for 6 kits - 3 Normal sequences (N-kits) and 3 Mutant/Sickle sequences (S-kits).

- 18 strands of 4 ft pom-pom garland (LINK) (to represent backbone – sugar and phosphate)
- 306 1.5 in (38 mm) styrofoam balls (to represent nitrogenous base)
- 204 magnets (LINK)
- 408 safety Pins (to represent phosphodiester bonds)
- Green paint (to indicate phosphate group)
- Sharpie to label Styrofoam balls.

DNA Template strand

- Take a strand of pom-pom garland and lay it out on a table.
- At every third pom-pom in the garland, glue on a Styrofoam ball (need to get 17 balls onto the garland).
- Glue one magnet onto each styrofoam ball
- Paint the first pom-pom green. Then repeat at every third pom-pom.
- Once everything is dried, write in the following sequence in order:
  - For N-kit strands: T-G-A-G-G-A-C-T-C-C-T-C-T-T-C-A-G
  - For S-kit strands: T-G-A-G-G-A-C-A-C-C-T-C-T-T-C-A-G
- When you finish, you should have the following (picture is showing a portion of the template sequence).

DNA nucleotides (will need to make 17 nucleotides per kit)

- Take a strand of pom-pom garland and cut it after every third pom-pom.
- Glue a styrofoam ball onto the middle pom-pom
- Glue one magnet onto each styrofoam ball
- Paint the first pom-pom green.
- Add a safety pin to the pom-poms on each end.
- Once everything is dried, write on the styrofoam ball one of the four nitrogenous bases as needed:
  - For N-kit strands: A, C, T, C, C, T, G, A, G, G, A, G, A, A, G, T, C
  - For S-kit strands: A, C, T, C, C, T, G, T, G, G, A, G, A, A, G, T, C
- When you finish, you should have the following (picture is showing one nucleotide).

When students build their DNA model, it should look like the following (prior to folding it into a helx)

RNA nucleotides (will need to make 17 nucleotides per kit)

- Follow same procedure as you did with DNA nucleotides, except, do not add a magnet to the stytrofoam ball.
- Once everything is dried, write on the styrofoam ball one of the four nitrogenous bases as needed:
    - For N-kit strands: A, C, U, C, C, U, G, A, G, G, A, G, A, A, G, U, C
    - For S-kit strands: A, C, U, C, C, U, G, U, G, G, A, G, A, A, G, U, C

Kits:

- Place prepared DNA nucleotides in a zip-lock bag and label the bag "N" or "S" DNA nucleotides.
- Place prepared RNA nucleotides in a zip-lock bag and label the bag "N" or "S" RNA nucleotides.
- Place the two zip-lock bags and a corresponding DNA template into a container. Label the container "N" or "S"

N-kit sequences answers:

DNA template	T	G	A	G	G	A	C	T	C	C	T	C	T	T	C	A	G
Complementary DNA strand	A	C	T	C	C	T	G	A	G	G	A	G	A	A	G	T	C
DNA template	T	G	A	G	G	A	C	T	C	C	T	C	T	T	C	A	G
RNA	A	C	U	C	C	U	G	A	G	G	A	G	A	A	G	U	C
Protein	THR			PRO			GLU			GLU			LYS				

S-kit sequences answers:

DNA template	T	G	A	G	G	A	C	A	C	C	T	C	T	T	C	A	G
Complementary DNA strand	A	C	T	C	C	T	G	T	G	G	A	G	A	A	G	T	C
DNA template	T	G	A	G	G	A	C	A	C	C	T	C	T	T	C	A	G
RNA	A	C	U	C	C	U	G	U	G	G	A	G	A	A	G	U	C
Protein	THR			PRO			VAL			GLU			LYS				

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

Materials Needed: (Laboratory Preparation for 10 groups, 3 students per group)

Part I: Isolating & Visualizing DNA

- Strawberries (10)
- Ziplock bags (10)
- Cheesecloth (10 pieces that will fit over beaker)
- Scissors (10)
- 200 mL beaker (10)
- 15 mL conical tube or test tubes (10)
- Plastic bulb pipets (10)
- Glass stir rods (10)
- Water bath set to 60°C
- DNA Extraction buffer (10 mL per group)
    - To make 500 mL: Combine 50 mL shampoo, 1 gram salt, 450 mL water
- 95% Ethanol or Isopropanol (kept on ice till needed) – (3 mLs per group)
- Microcentrifuge Tubes (10)
- Deionized water (1 mL aliquots)
- 1% (w/v) agarose gel stained with SYBRSAFE® stain (LINK).
    - Alternative DNA stains include: CarolinaBLU™ stain (LINK) or Ethidium Bromide (LINK). Note that Ethidium bromide may be carcinogenic or mutagenic.
- Agarose Gel Electrophoresis Apparatus & Power Supply Box
- UV light table
- 1X TAE Running Buffer (Tris-Acetate-EDTA)
    - You can purchase 50X stock from most supply companies and dilute down to 1X (LINK).
    - Recipe: 242 g Tris Base; 57.1 mL Glacial Acetic Acid; 100 mL 0.5 M EDTA
- DNA Loading Dye (LINK)
- DNA Ladder (LINK)
- Sample of Bovine Serum Albumin in solution (protein sample for gel electrophoresis)
- Micropipettor that can pipet between 3 – 20 µL
- Pipet tips
- Tip waste container

Part II: Building a DNA molecule

- Half the groups in the class should receive the “N” Nucleotide Kit
- The other half of the groups should receive the “S” Nucleotide Kit
- Students will be working with the DNA template and DNA nucleotide bag only.

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.

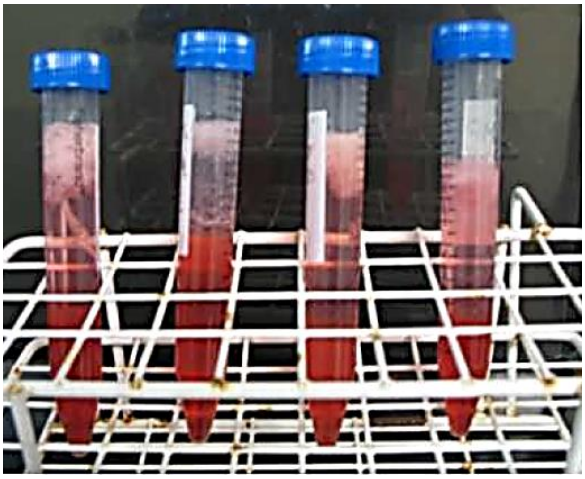
2. Add 10 mL of DNA extraction buffer (detergent, salt, water) to the bag. The detergent helps break open the ells and the salt helps remove any protein that is bound to the DNA. Press the air out and seal the bag. Mash for 1 minute.

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)?
Shown below is what the DNA should look like. The white mass seen floating is the DNA.



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.

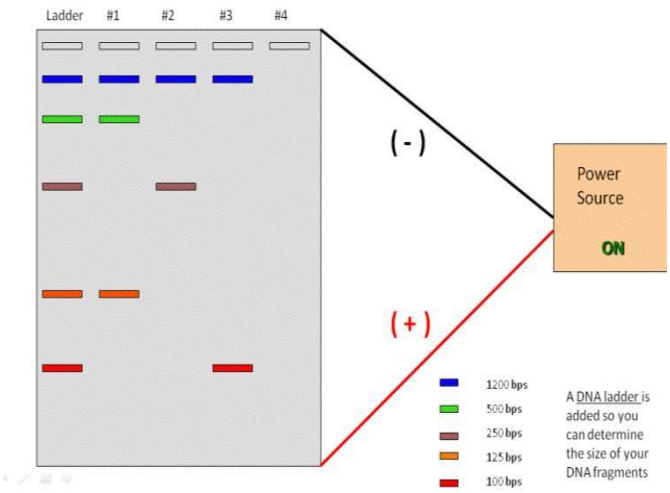


Figure 1-1. Agarose Gel Electrophoresis

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:

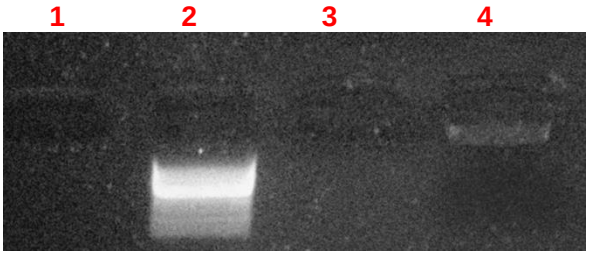
8. After approximately 20 minutes, take out your isolated DNA. DNA is soluble in water. By placing the extract in water, the DNA will dissolve in water. Do not worry if all of the DNA dissolved.

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.



Lane	*Component*
1	Water Only
2	DNA Ladder (control)
3	Protein Sample (BSA)
4	Strawberry DNA sample 1

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?
As seen in lane 4, a faint band is detected, showing the presence of DNA. Since it near the top of the gel where the sample was loaded, the DNA sample has a large # of base pairs.

Question: Were you able to see the protein that was loaded in the gel?
No. Proteins are not visible in SYBRSAFE gels that have been resolved via electrophoresis.

Part II: Building a DNA molecule

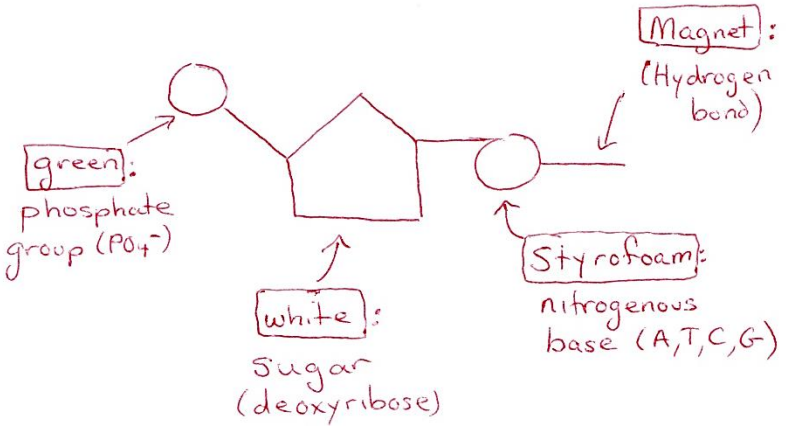
Models in science are used to describe ideas, understand biological processes and make predictions. In today's lab, we will build a model to learn about the composition and structure of DNA.

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?
A ←→ T and G ←→ C

c. Can you form a DNA molecule using only "A" and "C" bases? Why or why not?
No. The base pair rules are A ←→ T and G ←→ C.

d. Describe the shape of your model.
When DNA is unwound, it appears like a ladder. When wound up, it has a helical structure.

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

Materials Needed: (Laboratory Preparation for 10 groups, 3 students per group)

Part I: Transcription

- Half the groups in the class should receive the "N" Nucleotide Kit
- The other half of the groups should receive the "S" Nucleotide Kit
- Students will be working with the DNA template and RNA nucleotide bag only.
- Groups should continue working with the same Nucleotide kit as they did for Lab #1.

Part II: Translation

- No materials are needed for this section.

Part III: Mutations

- Microscopes
- Slides of normal blood smear (Wright stain) (LINK) (5)
- Slides of sickle cell anemia smear (Wright stain) (LINK) (5)

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).

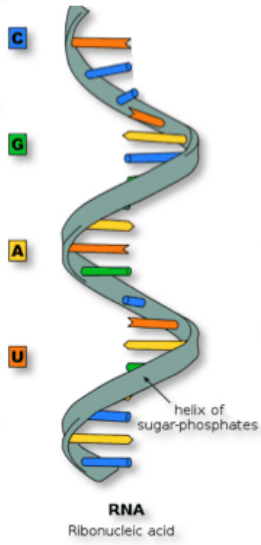


Figure 2-1. RNA
(wikipedia.com)

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
Both are nucleic acids, comprised of nucleotides (phosphate group, sugar, nitrogenous base) **Both have adenine, cytosine and guanine.**	**Different sugars (deoxyribose vs. ribose)** **Uracil in RNA, Thymine in DNA** **RNA is single stranded, DNA is double stranded**

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.

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

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.

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.

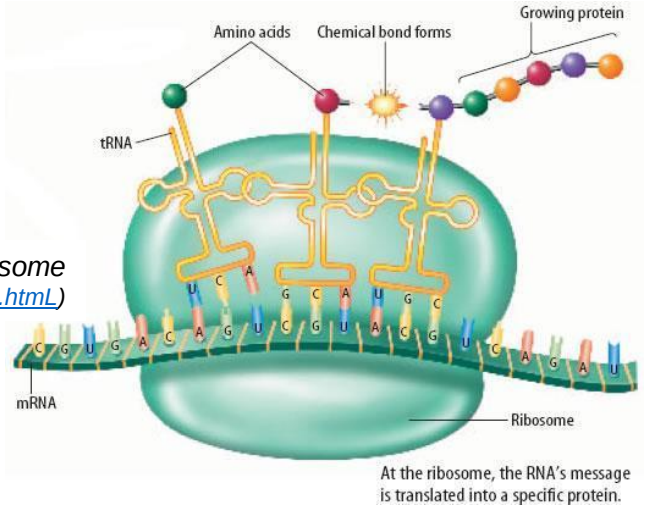


Figure 2-2. Translation at the ribosome
(biologyteksbylauryncarter.weebly.com/protein-synthesis.htmL)

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:
N kits: THR – PRO – GLU – GLU – LYS

S kits: THR – PRO – VAL – GLU – LYS

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

No. See answer to (a)

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

See answer to (a)

d. Examine their DNA and RNA molecule to see why they have a different polypeptide than your group.
The DNA template strands had one nucleotide difference (see bolded, underlined nucleotide below)
N kits: T-G-A-G-G-A-C-**T**-C-C-T-C-T-T-C-A-G
S kits: T-G-A-G-G-A-C-**A**-C-C-T-C-T-T-C-A-G

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:

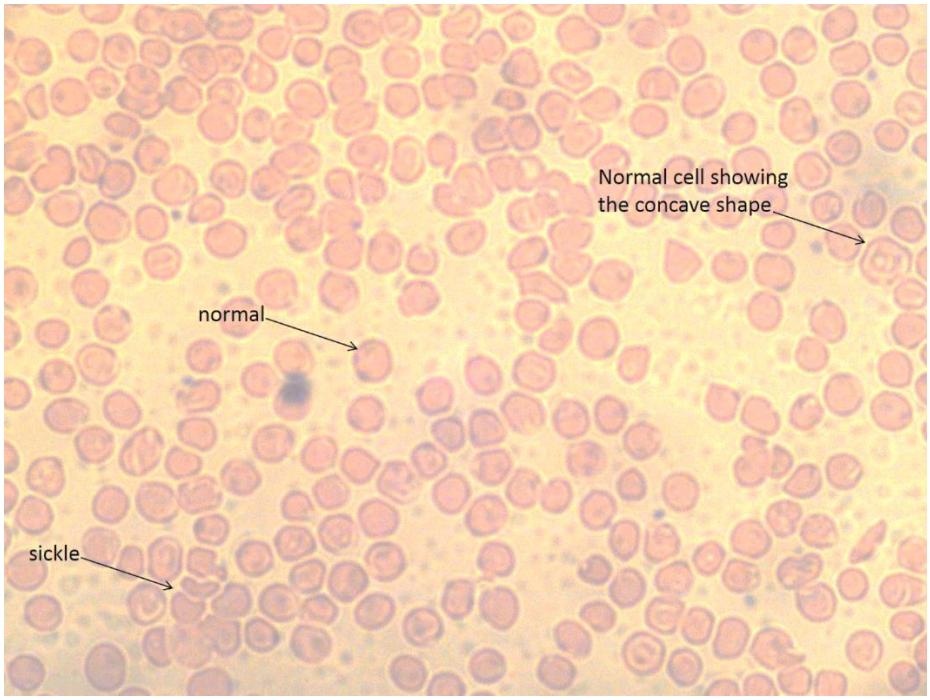
- Substitutions: are mutations that exchanges one base for another
- Insertions: are mutations in which extra base pairs are inserted into a new place in the DNA.
- Deletions: are mutations in which a section of DNA is lost, or deleted.

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.



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. GLU → VAL

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

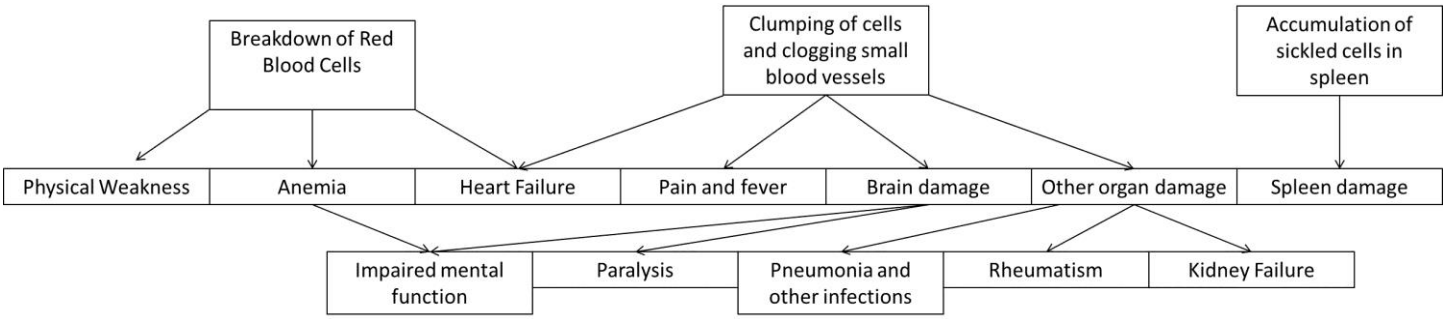


Figure 2-3. Effects of misfolded hemoglobin

Lab #3: Inheritance - PREPARATION

Materials Needed: (Laboratory Preparation for 10 groups, 3 students per group)

Part I: Mitosis

- Microscopes
- Slides of onion root tips (Hematoxylin stain) (LINK) (10)
    - You can also use whitefish blastula slides for students to see mitosis in animal cells. However students have an easier time finding the stages of mitosis and interphase using onion root tip slides.

Part II: Meiosis

- Pop-bead chromosomes (LINK) (10)
- Sordaria
    - You can either use prepared cards (LINK) for the student to use (10)
    - Alternatively, you can obtain a *Sordaria* cross plate (+ x tn). Prepare a wet-mount slide from a portion of the plate where the two strains intersect (LINK). (10 slides)

Part III: Inheritance

- Phenylthiocarbamide test paper (PTC) (LINK) (1 per person)

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.

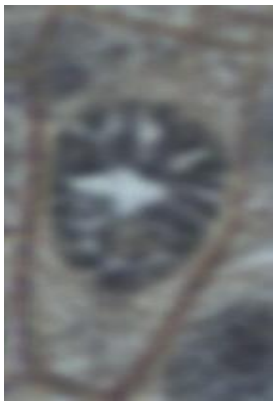
See next page for images and descriptions.

Onion Root Tip:

Interphase

Chromosomes have not condensed and can easily see the nucleus and nuclear membrane.

Prophase



Chromosomes are beginning to condense.

Metaphase

Chromosomes align in middle of cell. Can also see the mitotic spindles attached to chromosomes at kinetochore.

Anaphase

Sister chromatids are separating.

Telophase

This is late in telophase, where you can begin to see the formation of the cell plate.

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

If mitosis is not accurate, then mutations may occur in the DNA which may affect cellular functions.

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.

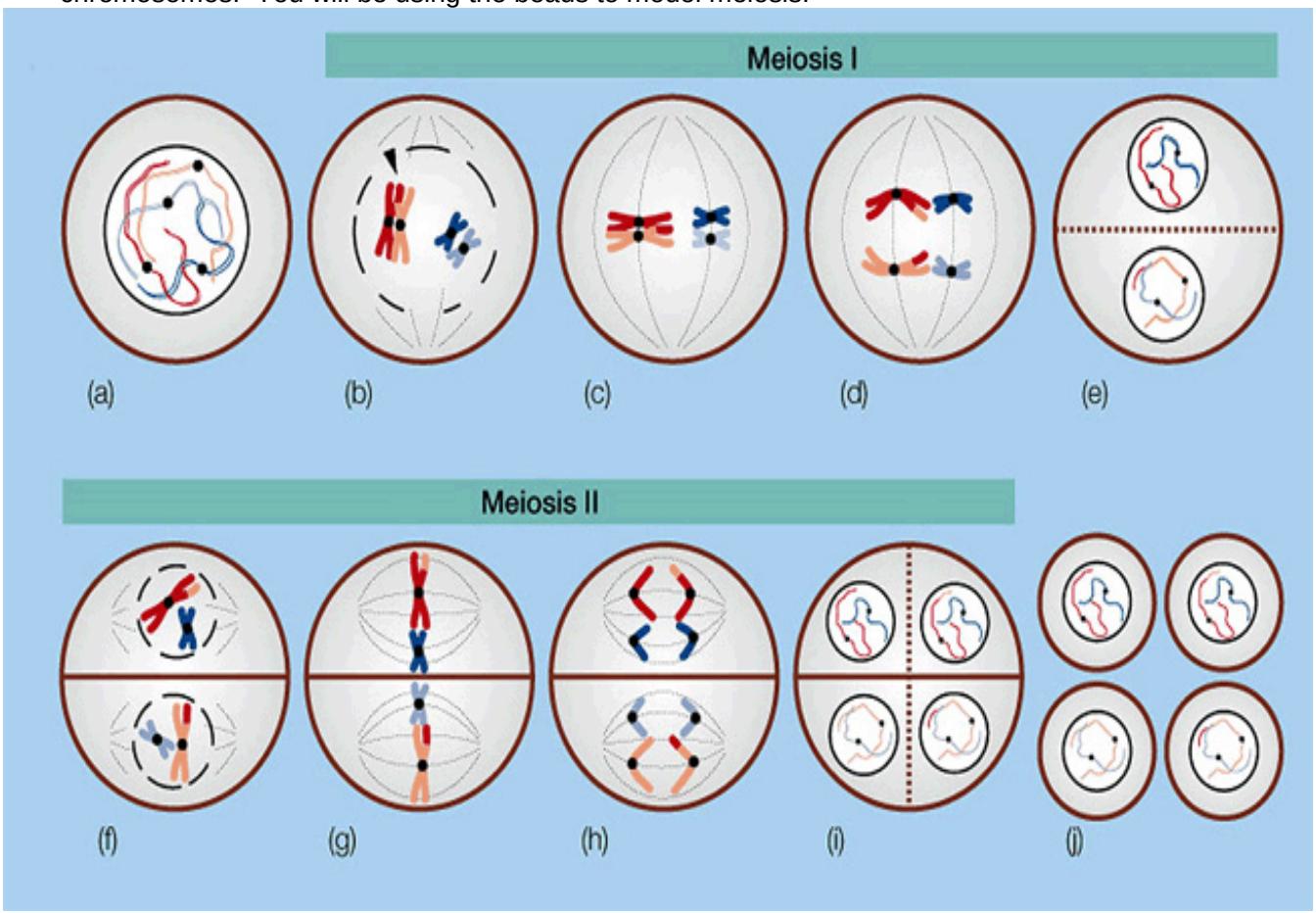


Figure 3-1. Overview of Meiosis
(http://www.tokresource.org/)

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?

Crossing-over of the non-sister chromatids of homologous chromosomes in Prophase I (cell (b) in Fig. 3-1).
Independent assortment of homologous chromosomes in Metaphase I (cell (c) in Fig. 3-1).

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.

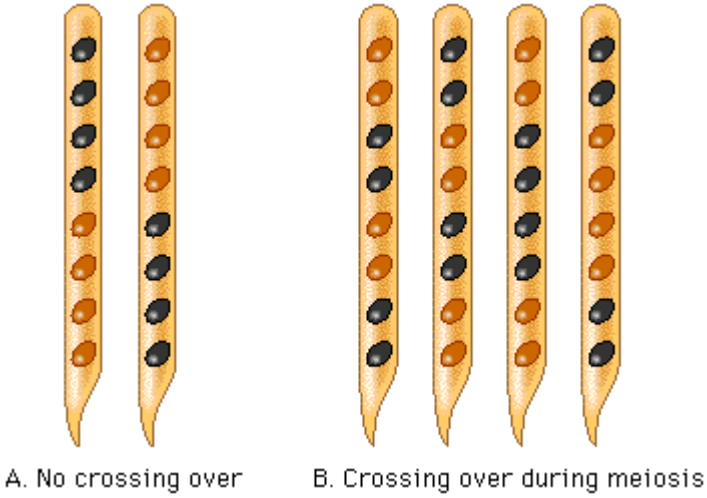


Figure 3-2. Crossing over in Sordaria
(http://www.phschool.com/science/biology_place/labbench/lab3/spores2.htmL)

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.

Answers will vary depending upon *Sordaria* preparation used.

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**.

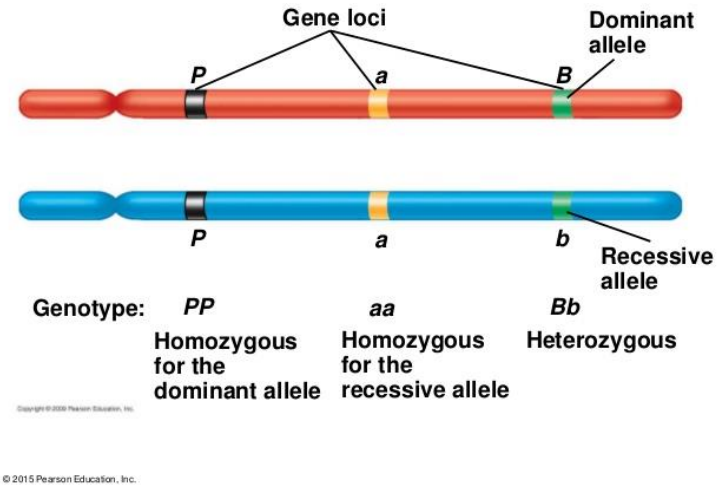


Figure 3-3. Homologous chromosomes showing homozygous and heterozygous genotypes.
(Campbell's Biology, Pearson Education)

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.

Shown below is sample data from Dr. Forster & Dr. Li's classes.

	Trait	# students	%-age of students
Earlobes	Free Earlobes	331	72.1
	Attached Earlobes	128	27.9
Hairline	Widows Peak	142	30.9
	Straight Hairline	317	69.1
Tongue Roller	Tongue Roller	358	78
	Nonroller	101	22
PTC	PTC Taster	242	52.7
	Nontaster	217	47.3
Freckles	Freckles	222	48.4
	No freckles	237	51.6
Mid-digital hair	Mid-digital hair	160	34.9
	No mid-digital hair	299	65.1
Dimples	Facial dimples	145	31.6
	No facial dimples	314	68.4
Finger Interlacing	Left/Right thumb	290	63.2
	Right/Left thumb	169	36.8
# students		459	
Red = Dominant			
Blue = Recessive			
Indicates which trait has higher frequency in the class			

Question: Does the majority of the class exhibit dominant phenotypes?
Answer will depend upon class data collected. In the sample data shown, the majority of the class do not exibit all dominant phenotypes. For example, a majority of students have straight hairlines, no freckles, no mid-digital hair and no facial dimples. These traits are all recessive 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?
Carriers still have a dominant allele which is being expressed, carrying the normal state.

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

Question: **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? **Two individuals that are closely related to one another produce offspring together.**

(b) Why would inbreeding cause a high rate of genetic disorders?
As inbreeding occurs, genetic diversity in DNA will decrease. If two closely related parents that have a genetic mutation &/or disorder have children, there is a high probability that the children will also have that mutation/disorder. If two unrelated individuals have children, then the child's DNA will have more genetic diversity and will reduce the probability of inheriting mutations/disorders.

Lab #4: Manipulation of DNA - PREPARATION

Materials Needed:

Part I: RFLP (Restriction Fragment Length Polymorphism)

- General comments on DNA samples for RFLP
    - We tell the students that the DNA samples are human samples from a hypothetical family. However, we are using prepared plasmids pCAP75, pCAP77 and pCAP88, which have been discussed previously in Arango Pinedo & Gage (2009).

    - We additionally do not use *MstII*, which would be the restriction enzyme that would be used to detect mutations in the hemoglobin gene. Rather, we use a combination of *EcoRI*, *NotI* and *SalI* such that the following results are obtained when digesting the plasmid DNA:
        - pCAP77 will give rise to 3 bands (what we define as having genotype AA) {use for Daughter}

        - pCAP88 will give rise to 4 bands (what we define as having genotype Aa) {use for Mother & Father}

        - pCAP75 will give rise to 2 bands (what we define as having genotype aa) {use for son}

    - You can alternatively purchase RFLP kits that contain both DNA samples and restriction enzymes (LINK). You can have the students go through the kit's RFLP activity so they learn how this technique is performed and analyzed. You can then show them our gel results of the hypothetical family and have them determine the genotypes and phenotypes of each member.

Materials per group (10 sets):

- RFLP Kit materials: DNA samples, restriction enzymes prealiquoted in microcentrifuge tubes, kept on ice.
- Ice bucket with ice
- Microcentrifuge Tubes
- 1% (w/v) agarose gel stained with SYBRSAFE® stain (LINK).
    - Alternatives DNA stains include: CarolinaBLU™ stain (LINK) or Ethidium Bromide (LINK). Note that Ethidium bromide may be carcinogenic or mutagenic.
- Agarose Gel Electrophoresis Apparatus & Power Supply Box
- UV light table
- 1X TAE Running Buffer (Tris-Acetate-EDTA)
    - You can purchase 50X stock from most supply companies and dilute down to 1X (LINK).
    - Recipe: 242 g Tris Base; 57.1 mL Glacial Acetic Acid; 100 mL 0.5 M EDTA
- DNA Loading Dye (LINK)
- DNA Ladder (LINK)
- Micropipettor that can pipet 10 µL
- Pipet tips
- Tip waste container

- The class will need a water bath set to 37°C

Hypothetical Family Genotype & Phenotype

(RFLP samples should be prepared as stated above to give the following results:

Family Member	Genotype	Phenotype
Father	Aa (heterozygous)	Normal hemoglobin/blood cells
Mother	Aa (heterozygous)	Normal hemoglobin/blood cells
Daughter	AA (homozygous dominant)	Normal hemoglobin/blood cells
Son	aa (homozygous recessive)	Misfolded hemoglobin/ sickled blood cells

Part II: Genetic Engineering

- Bio-Rad pGLO Transformation kit (LINK)
    - Follow the directions indicated in the kit directions to prepare the media, plasmid and *E. coli*.

Materials per group (10 sets):

- Ice bucket with ice
- 2 microcentrifuge tubes, each with 50 µL of competent *E. coli* (keep on ice)
- Microcentrifuge tube with 5 µL pGLO (keep on ice)
- Microcentrifuge tube with 500 µL Luria-Bertani broth
- One plate of Luria-Bertani (no ampicillin or arabinose added) – Label this plate: -PGLO
- One plate of Luria-Bertani with ampilcillin and arabinose added – Label this plate: +PGLO
- Microcentrifuge tube rack
- 2 sterile swabs
- Micropipettors that can pipet 5 - 250 µL
- Pipet tips
- Tip waste container

- The class will need a water bath set to 42°C
- The class will need an incubator set to 37°C

NOTE: ANY WASTE FROM PART (II) SHOULD BE TREATED AS BIOLOGICAL HAZARDOUS WASTE AND DISPOSED OF PROPERLY.

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.

If you use a different source of DNA or use a supply company's RFLP kit, you will need to edit this section to correspond to your DNA samples.

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.

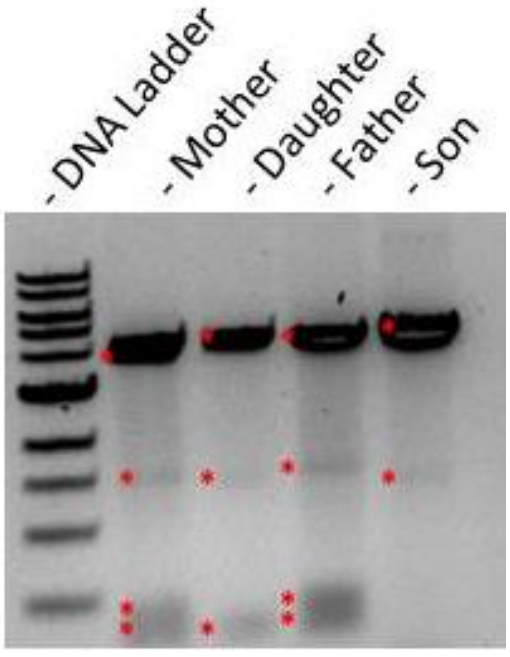
4. Incubate the tubes for 30-45 minutes in the 37ºC water bath to allow the enzymes to function.
   ** While your DNA is incubating, begin the second part of today's lab - introducing foreign DNA**

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.

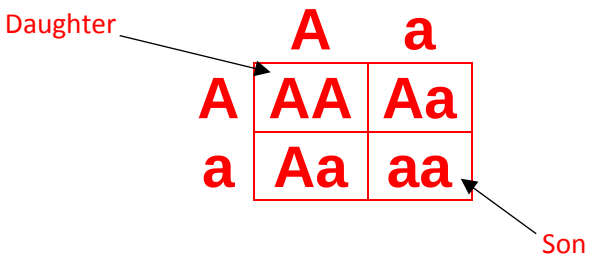
(This is the gel result we obtained using plasmids pCAP plasmids)



*Since some of the bands are hard to see, we added a * to the left of each band.*

Question: Based on your gel results, draw a pedigree chart of the family. Indicate which individual(s) are normal, carriers or have the disease.
Father & Mother are carriers (Aa), Daughter is normal (AA), son has sickle cell (aa)
See next page for more details.

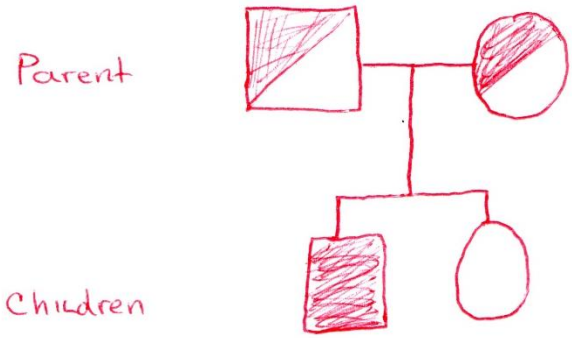
If you prepare a Punnett Square of the cross between mother and father, you can see how the daughter and the son can have those genotypes:



To prepare a pedigree, the following are used:

	**Indicates male**
	**Indicates female**
	Indicates marriage
	Connects parents to children
Fully shaded	**Indicates individual has the trait**
Half shaded	**Indicates individual is a carrier for the trait**

Answer:



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: Growth occurs, glowing does not occur

(+) pGLO: Growth occurs, glowing does occur

Sources. This laboratory exercise has been adapted from:

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

Lab #5: Protein Structure - PREPARATION

Materials Needed: (Laboratory Preparation for 10 groups, 3 students per group)

Part I: Learning about protein structure

- Amino Acid Starter Kit (LINK) (10)

Part II: Analyzing Protein Structure:

- ELISA Kit (LINK) (10 set-ups)
    - Prepare the materials as described in the Kit's Instructional manual.
    - We tell the students that the antigen is hemoglobin and that the antibodies are specific for hemoglobin. However, we simply just use the materials from the ELISA kit.
    - Each group will need each of the following (available from the kit):
        - Strip wells for ELISA.
        - "Hemoglobin" samples in individual microcentrifuge tubes and kept on ice.
            - Normal: 50 µL antigen
            - Sickle: 50 µL deionized water
            - Father: 50 µL antigen
            - Mother: 50 µL antigen
            - Daughter: 50 µL antigen
            - Son: 50 µL deionized water
        - 300 µL primary antibody kept on ice
        - 300 µL secondary antibody kept on ice
        - 300 µL enzyme substrate (keep in dark on ice till needed – substrate is light sensitive)
        - 1 mL Wash buffer
        - Paper Towels (to be used during wash steps)
        - Micropipettors that can pipet 50 - 100 µL
        - Pipet tips
        - Tip waste container

Hypothetical Family Genotype & Phenotype

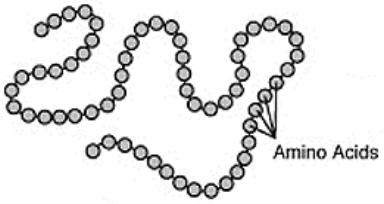
(ELISA samples should be prepared as stated above to give the following results:

Family Member	Genotype	Phenotype
Father	Aa (heterozygous)	Normal hemoglobin/blood cells
Mother	Aa (heterozygous)	Normal hemoglobin/blood cells
Daughter	AA (homozygous dominant)	Normal hemoglobin/blood cells
Son	aa (homozygous recessive)	Misfolded hemoglobin/ sickled blood cells

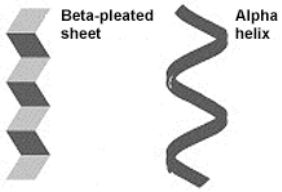
Lab #5: Protein Structure

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.

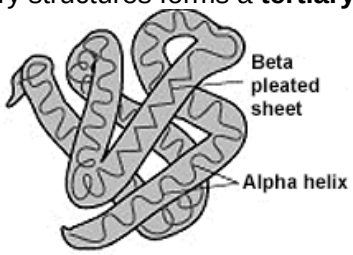
During our transcription and translation lab, you constructed a polypeptide chain of **amino acids**. You wrote this amino acid chain as a **linear or primary sequence**, as shown below:



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).

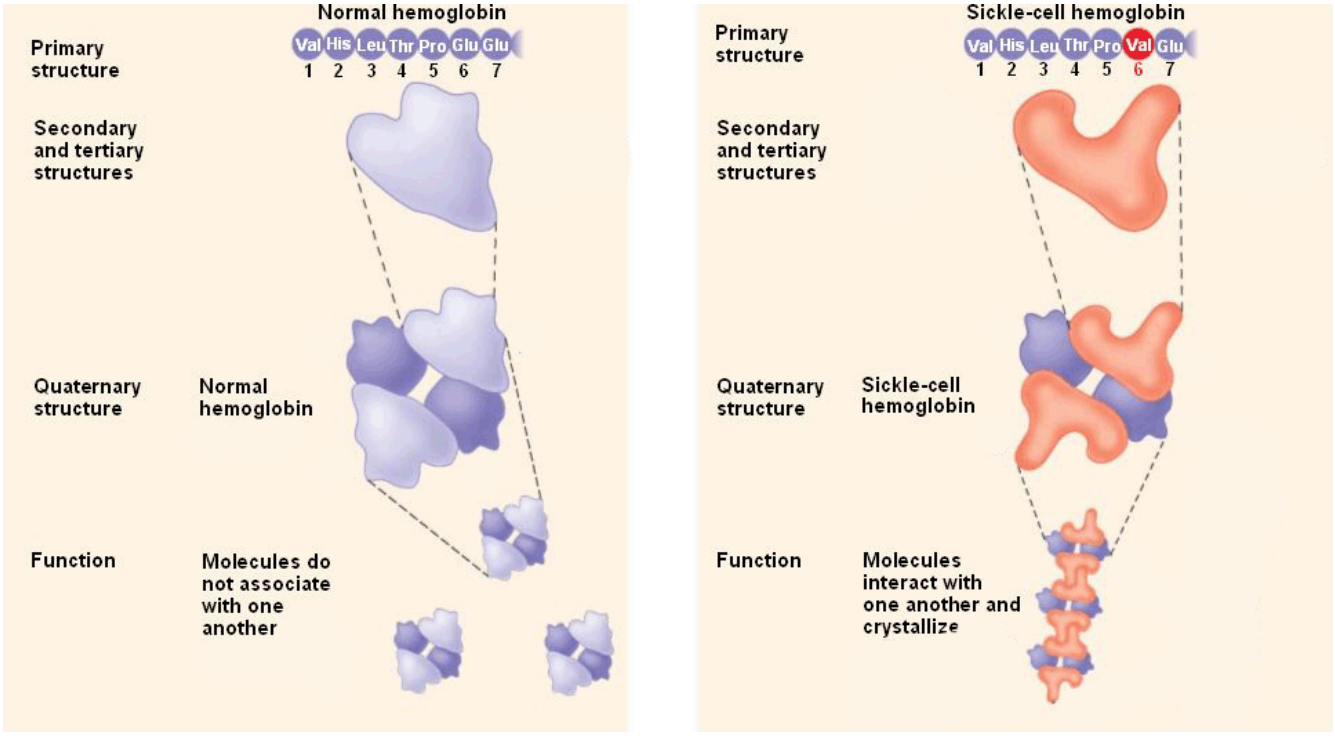


Figure 5-1. Structure of normal and sickle-cell hemoglobin
(Campbell's Biology, Pearson Education)

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 **carbon** atoms.
- This amino acid is an exception to the above observation: **glycine (it only has hydrogen as a side chain)**.
- In addition to carbon atoms, **acidic side chains** contain two **oxygen** 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 **nitrogen** 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, **oxygen, nitrogen**, and **sulfur**.

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?
Hydrophobic side chains.

b. From your knowledge of static electricity, which side chains might be attracted to each other?
Acidic (negative) and basic (positive) side chains.

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 GLU. Do not use VAL 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?
The protein continues to fold in on itself.

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

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?
Depending on the primary sequence of amino acids, it may not be possible to fold the entire protein structure such that all chemical properties are satisfied.

d. Did your protein look like the proteins other students folded? Student answers will vary, probably they will not. Explain: Each group most likely used a different primary sequence of amino acids, which would influence how the protein folds.

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?
GLU is acidic (negative side chain) and VAL is hydrophobic. When the protein has GLU, the side chain will most likely stick out since it is hydrophilic and will interact with water. When the mutation is introduced, the protein shape will change since the VAL side chain will position itself inside the protein, away from water.

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.

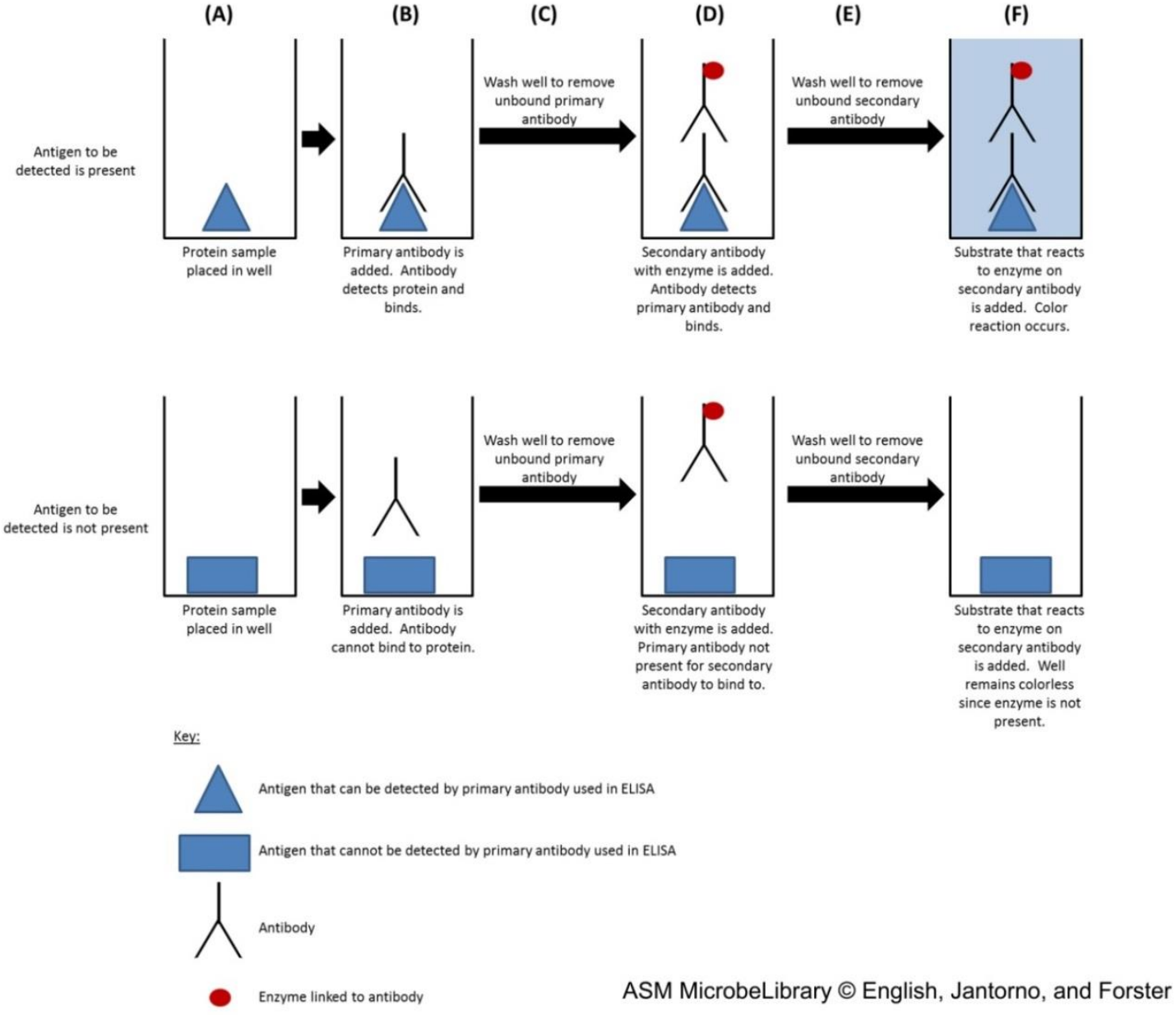


Figure 5-2. ELISA Procedure
(*American Society for Microbiology*)

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?	**Normal protein structure (blue seen)**	**Abnormal protein structure (no color seen)**	**Normal protein structure (blue seen)**	**Normal protein structure (blue seen)**	**Normal protein structure (blue seen)**	**Abnormal protein structure (no color seen)**

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

Question: Was the ELISA assay able to tell you who were "carriers?" Why or why not?
No. Both normal individuals and carriers will produce properly-folded hemoglobin. Since the ELISA looks only at protein structure, it will not be able to differentiate who carriers may be.

Sources. This laboratory exercise has been adapted from:

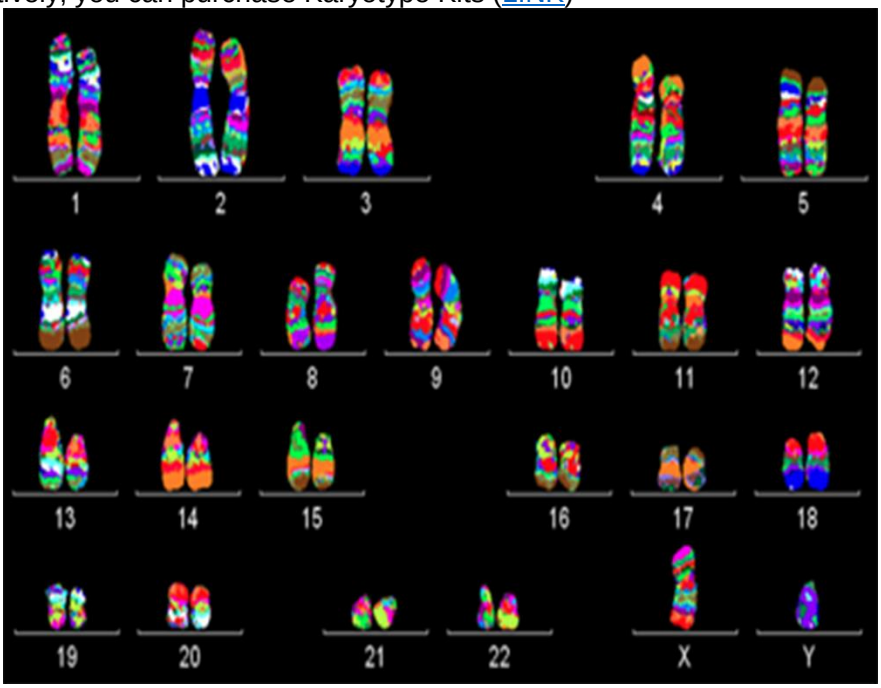
- 3D Molecular Designs: Amino Acid Starter Kit
- Bio-Rad. Biotechnology Explorer: ELISA Immuno Explorer Kit

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

Materials Needed:
(Student groups are assigned to one of 3 lab rooms. Groups then rotate through each lab. Labs are taught by teaching fellows and faculty associated with the program).

Lab a: Phenotype Analysis – Blood & Karyotype Analysis

- Microscopes
  - You will need either a camera on the microscope so the students can measure the size of cells. If not, you can purchase stage micrometer slides and use those in measuring cells.
- 2 slides of normal blood smear (Wright stain) (LINK). Label one slide "FETUS A" and other "FETUS C"
- 1 slides of sickle cell anemia smear (Wright stain) (LINK). Label slide "FETUS B"
- Karyotype Kit
  - We make our own by printing chromosomes (see diagram below) and cutting them into sets:
    - Fetus A: Male with Down's Syndrome (trisomy 21)
    - Fetus B: Female with sickle cell anemia (shows normal karyotype).
    - Fetus C: Male with Cri Du Chat (shows normal karyotype).
  - Each group gets one set of individual chromosomes (Fetus A, B or C) and a copy of the image below so they can match each chromosome and determine if the fetuses have any chromosomal disease.
  - Alternatively, you can purchase Karyotype Kits (LINK)



Lab b: Phenotype Analysis – Protein Function (Hemoglobin)

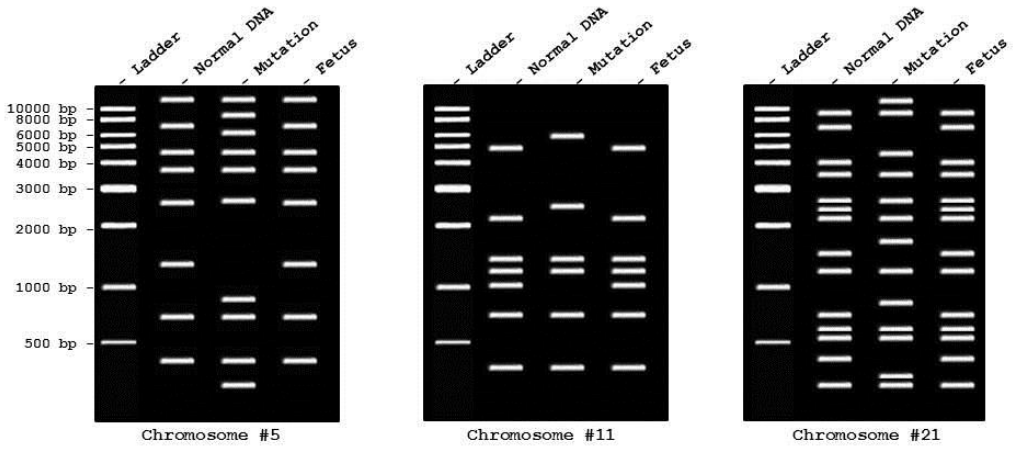
- This lab is a simulation. Students are not actually working with purified hemoglobin.
- The materials for this lab come from the Advanced Placement (AP) Biology Big Idea #4 Investigation #13 Enzyme Activity Lab (LINK)
  - Prepare the materials as described in the AP Lab Manual Link on page T218.
  - The buffers you prepare will mimic the fetal hemoglobin as described in the student's handout.
  - Fetuses A and C have normal hemoglobin, therefore use highly acidic buffer (pH 2 or 3) such that there is no color change in the presence of guaiacol indicating there is no free oxygen generated (all oxygen is captured by "hemoglobin").
  - Fetus B has abnormal hemoglobin (due to sickle cell anemia), therefore use neutral buffer (pH 7) such that there is a color change in the presence of guaiacol indicating there is free oxygen (oxygen that has not been captured by "hemoglobin").
  - Volumes needed *per group*:
    - Enzyme - Peroxidase: 2.25 mL
    - Substrate – Hydrogen Peroxide: 450 µL
    - Distilled Water – 10.5 mL
    - Fetus A "Hemoglobin" (pH 2) – 3 mL
    - Fetus B "Hemoglobin" (pH 7) – 3 mL
    - Fetus C "Hemoglobin" (pH 2) – 3 mL
    - Guaiacol - 300 µL
- Micropipettors that can pipet 100 - 750 µL
- Pipet tips
- Tip waste container
- 10 mL graduated cylinder (2)
- Ice bucket filled with ice
- Test tube rack

Lab c: DNA Analysis – RFLP and DNA Microarray

- RFLP images of each fetus

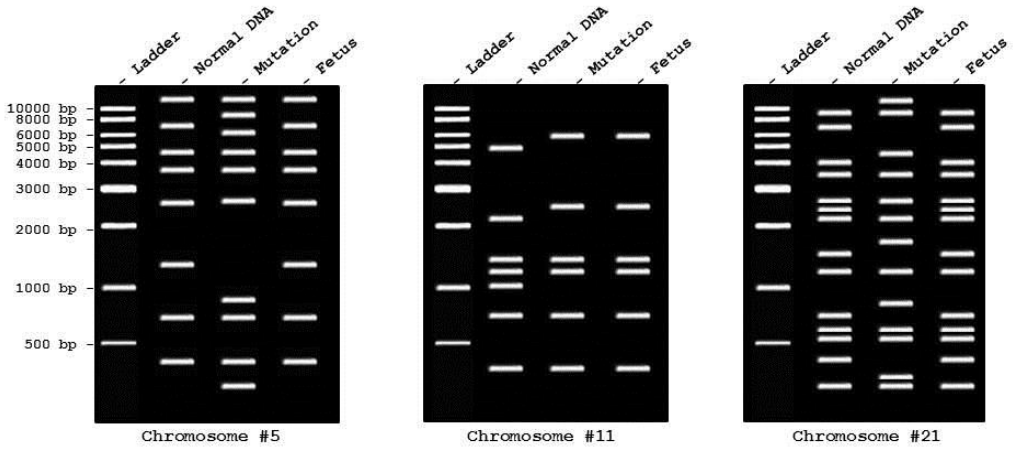
FETUS A

R.F.L.P. Results



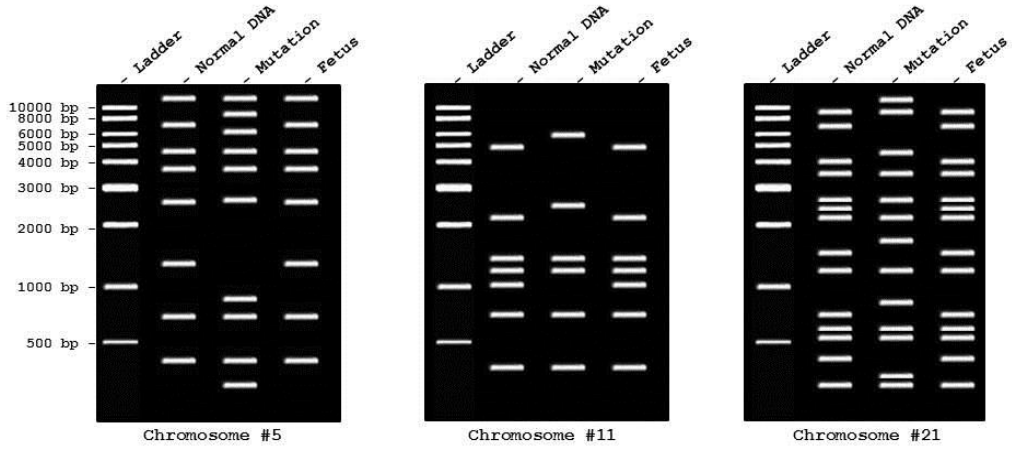
FETUS B

R.F.L.P. Results



FETUS C

R.F.L.P. Results



- Microarray Simulation Kit (LINK)
    - Using the samples provided in the kit, re-aliquot samples into microcentrifuge tubes as shown below such that it corresponds to the genes the students are investigating in this activity:

Fetus A		**Fetus B**		**Fetus C**	
Tube	**Color**	**Tube**	**Color**	**Tube**	**Color**
Fetus A, Gene A	Yellow	Fetus B, Gene A	Yellow	Fetus C, Gene A	Yellow
Fetus A, Gene B	Red	Fetus B, Gene B	Red	Fetus C, Gene B	Red
Fetus A, Gene C	Green	Fetus B, Gene C	Green	Fetus C, Gene C	Green
Fetus A, Gene D	Colorless	Fetus B, Gene D	Colorless	Fetus C, Gene D	Colorless
Fetus A, Gene E (chromosome 5)	Yellow	Fetus B, Gene E (chromosome 5)	Yellow	Fetus B, Gene E (chromosome 5)	Red
Fetus A, Gene F (chromosome 11)	Yellow	Fetus B, Gene F (chromosome 11)	Yellow	Fetus B, Gene F (chromosome 11)	Yellow
Fetus A, Gene G (chromosome 21)	Green	Fetus B, Gene G (chromosome 21)	Yellow	Fetus B, Gene G (chromosome 21)	Yellow

- Micropipettor that can pipet 2 µL
- Pipet tips
- Tip waste container
- UV lamp
- UV protection goggles

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.

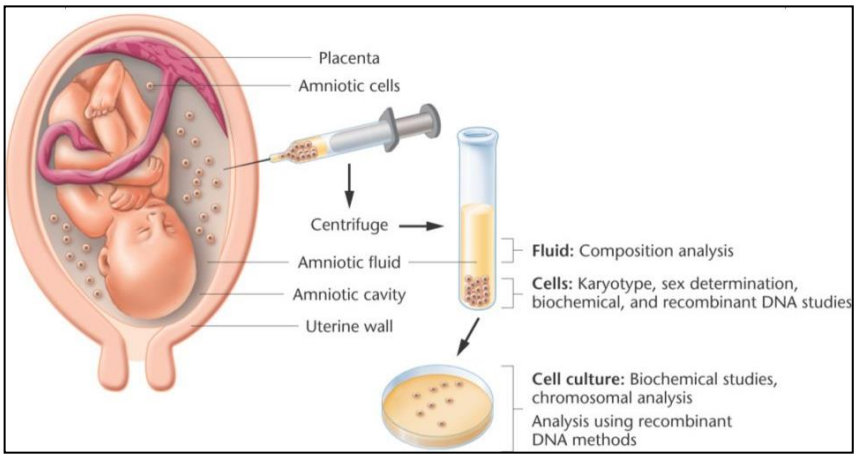


Figure 6-1. Amniocentesis
(www.healthpowerforminorities.com)

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.

ANSWERS TO IDENTIFICATIONS:

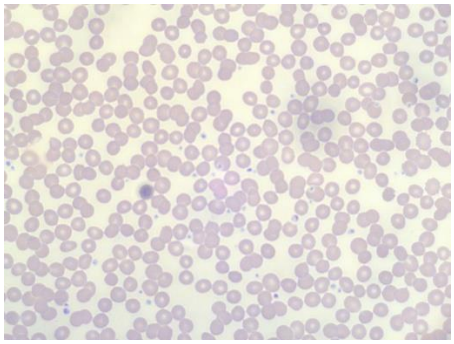
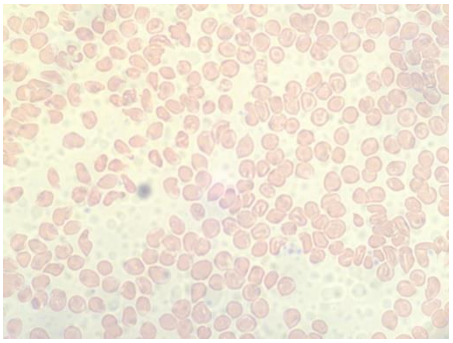
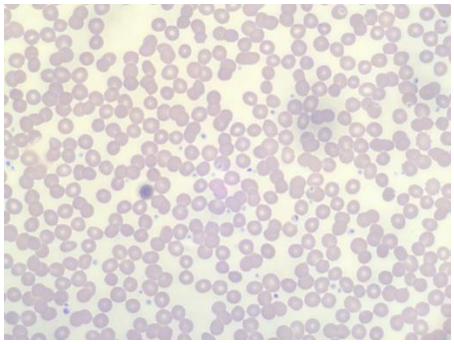
Average red blood size	Karyotype Result	Sex of fetus	Hemoglobin analysis	RFLP Results (circle result)		DNA Microarray (circle result)		Conclusion
				Ch. 5	Normal / Mutation	Ch. 5	Normal / Additional DNA / Deletion	
Student answers may vary	Trisomy 21	Male	Abnormal	Ch. 11	Normal / Mutation	Ch. 11	Normal / Additional DNA / Deletion	Downs
				Ch. 21	Normal / Mutation	Ch. 21	Normal / Additional DNA / Deletion	
				Ch. 5	Normal / Mutation	Ch. 5	Normal / Additional DNA / Deletion	
Student answers may vary	Normal	Female	Normal	Ch. 11	Normal / Mutation	Ch. 11	Normal / Additional DNA / Deletion	Sickle
				Ch. 21	Normal / Mutation	Ch. 21	Normal / Additional DNA / Deletion	
				Ch. 5	Normal / Mutation	Ch. 5	Normal / Additional DNA / Deletion	
Student answers may vary	Normal	Male	Normal	Ch. 11	Normal / Mutation	Ch. 11	Normal / Additional DNA / Deletion	Cri du chat
				Ch. 21	Normal / Mutation	Ch. 21	Normal / Additional DNA / 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
NORMAL BLOOD CELLS	**SICKLED BLOOD CELLS**	**NORMAL BLOOD CELLS**
		

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? Fetus B since abnormally shaped red blood cells (sickled shaped) are observed.

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.

Note the directions here are for the Infinity Analyze Camera Program we use on our microscopes. These directions may need to be changed according to the camera program you have on your microscopes. If you cannot measure cells with your microscope, then skip this section.

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? Student answers may vary

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).

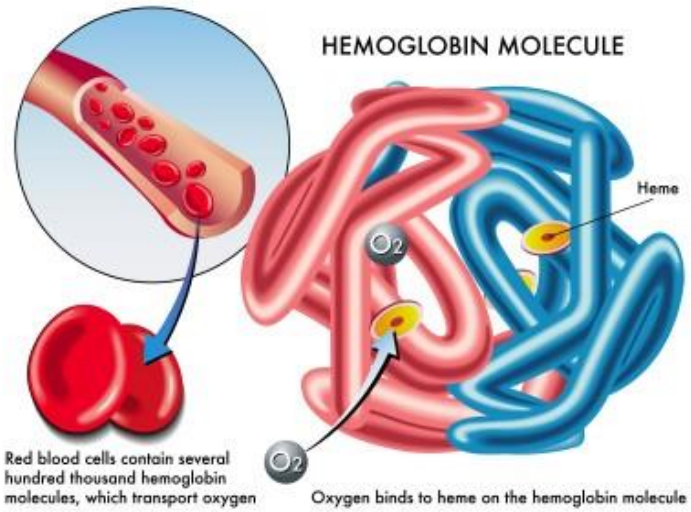


Figure 6-2. Quartnary structure of hemoglobin binding to oxygen
(*http://www.123rf.com/photo_14225607_hemoglobin.htmL*)

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,

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

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	**Student Answers will vary**	**Student Answers will vary**	**Student Answers will vary**
1 minute			
2 minute			
3 minute			
4 minute			
5 minute	**No color change should be observed indicating normal hemoglobin**	**Color change should be observed indicating abnormal hemoglobin**	**No color change should be observed indicating normal hemoglobin**

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?

No. A silent mutation would not alter the primary sequence of the protein and not affect its folding. It is additionally possible that a mutation and its subsequent change in protein shape maybe far enough away such that the active site of the protein (enzyme) keeps its proper structure.

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.
The mutant DNA had changes in the nucleotide sequence. Therefore, when the restriction enzyme was added to the DNA, different digest patterns were observed.

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.

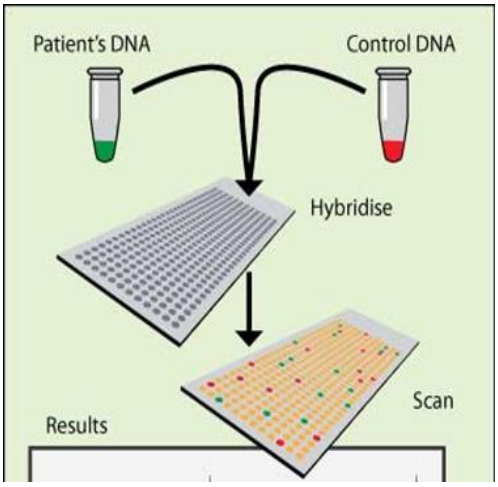


Figure 6-3. Microarray
(http://www.nchpeg.org/)

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	**Yellow**
B	**Red**
C	**Green**
D	**Colorless**

Control Wells	Fetus A Colors	Fetus B Colors	Fetus C Colors
E	**Yellow**	**Yellow**	**Red**
F	**Yellow**	**Yellow**	**Yellow**
G	**Green**	**Yellow**	**Yellow**

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? **Student Answers May Vary.**

Sources. This laboratory exercise has been adapted from:

- Edvotek – DNA/RNA Microarrays