

UNIT SIX: BIOTECHNOLOGY

Chapter 9

Objectives:

1. Identify the advantages and disadvantages of selective breeding of various organisms.
2. Explain how recombinant DNA and restriction enzymes are related to genetic engineering.
3. Analyze and explain the process of gel electrophoresis and results obtained from gel electrophoresis.
4. Relate biotechnology and genetic engineering to agriculture, medicine, and you!
5. Contrast the benefits and concerns associated with genetic engineering.

Key Vocabulary:

genetic engineering

clone

palindrome

plasmid

gel electrophoresis

sticky ends

vector

biotechnology

DNA fingerprint

restriction enzyme

gene

recombinant DNA

Human Genome Project

ethics

transgenic organism

gene therapy

Tools of the Genetic Engineer

INTRODUCTION: Scientists have learned to control the genetic technology to produce substances such as insulin, human growth hormone, interferon, and hepatitis B vaccine. The process used to produce those substances is called **recombinant DNA technology**. The steps involved in creating recombinant DNA include:

1. Scientists identify the gene that codes for the production of the protein they want to manufacture (for example: Human growth hormone).
2. After the gene has been identified, it must be isolated by cutting it out with restriction enzymes.
3. Once the gene has been isolated, it must be inserted into a plasmid. Then the plasmid is placed into a bacterium cell.
4. The bacteria replicate the plasmid containing the target gene, multiple copies of the gene become active, and the bacteria begin to produce the human protein.

BACKGROUND

INFORMATION: Growth hormone

Karen had just finished her driver's education course and had gone with her parents to take her road test. After passing her test, she met with a young woman to fill out some forms for her new license. When the woman left her chair to get the forms from the filing cabinet, Karen noticed that she was short, about 4 feet tall, she guessed. After filling out the necessary forms and having her picture taken for her license, Karen left with her parents. On the way home, Karen asked her parents if the young woman had the same thing her brother Chris had.

"I don't know. How much do you know about what Chris has?" her father asked. "I'm not sure," Karen said. "I know he has a growth hormone deficiency, because I have always heard you and mom say that when people asked why Chris was so small. And he goes to the doctor every week to get hormone shots. But, I am not sure I really understand what the problem is. I know that when Chris was younger, he was much shorter than other kids his age. The difference doesn't seem to be that large, now that he is older."

Chris was now 11 years old, and although they had their disagreements, Karen liked Chris a lot. He was a forward on the Hoover Middle School soccer team, was a rock hound, and always seemed to be in an upbeat mood. Her dad suggested that Karen accompany Chris and him on Chris' next visit to the doctor. "You can speak to Dr. Thompson about what growth hormone is," said Karen's father.

The following Monday, while Chris was being weighed and measured, Karen and her dad sat down with Dr. Thompson. Dr. Thompson was a pediatric endocrinologist, a specialist who cares for children with hormone problems. Many of the children under her care had diabetes, but some of her patients also had growth problems. "Well, Karen," Dr. Thompson said, "if you look around at your classmates at school, it is easy to see that people vary in many ways. Height, weight, skin color, eye color, intelligence, and athletic ability are good examples of individual differences. Things that vary in this way are often the result of a combination of many factors. An individual's height, for example, depends on the height of his or her parents and grandparents, on nutritional factors, and on the function of proteins or hormones from different endocrine glands, such as the pituitary, thyroid or adrenal glands."

“One of the most important of these hormones, which are proteins, is a growth hormone, which is made by the pituitary gland,” revealed Dr. Thompson. Karen remembered from her biology class that the pituitary gland is in the brain. “Abnormalities of growth hormone can occur in several ways,” Dr. Thompson explained. “Excess production of the growth hormone can result in an individual being taller than expected and having serious bone and joint problems as he or she ages. Many of the famous ‘giants’ of the past probably had that condition. Individuals who are shorter than expected may also have growth hormone problems.”

“So, Chris has a problem with the pituitary gland,” Karen deduced. “Yes, his pituitary gland is not making enough growth hormone,” said Dr. Thompson. “When Chris was about one and a half years old, your pediatrician, Dr. Kelfer, noticed that Chris wasn’t growing as fast as expected. Dr. Kelfer watches the growth of all of his patients, from birth, on a growth curve. Chris’ growth was plotted on the standard growth curve and was found to be lower than normal. We did some special tests on Chris and found he wasn’t making enough growth hormone. Fortunately, we were able to give Chris the growth hormone he needed to have close-to-normal growth.”

Karen had a lot more questions about growth hormone, and Dr. Thompson suggested she do some reading about it. Little did Karen know that she would soon become the class expert on genetic engineering because of her interest in the causes of short stature.

The school librarian helped Karen to find a book on endocrinology, the study of hormones. She read about growth hormone and found out that it is a protein that contains 191 amino acids. (How many nucleotides of DNA are there to create this protein?) This hormone is related to several other hormones involved in regulating growth and metabolism. Some children are deficient in growth hormone and do not grow as fast as expected. Doctors have developed a graph that shows how tall a boy or girl should be at different ages, such like the graph that was created to track Chris’ growth. (Would you use the same graph for both boys and girls? Why?) Some of the children who lacked the normal amount of growth hormone had inherited a recessive condition. Therefore, one child out of four was effected, on average. Fortunately, these children, like Chris, can now be treated using growth hormone manufactured by genetic engineering.

That had not always been the case, however. For many years, there was no treatment for people who lacked enough growth hormone. In the 1950’s, biologists found that they could make growth hormone from the pituitary glands of people who had died. Unfortunately, not enough people donated their pituitary glands for this purpose, and there was never enough growth hormone supply to meet the demands of the people who needed it. Even more serious was the fact that a few of the pituitaries contained a deadly virus that infected some of the children. Many of them died from this viral infection.

In the early 1980’s, other biologists found a way to get common bacteria to make growth hormone. That was an important breakthrough, because large quantities of growth hormone could not be produced, and short children were no longer limited to hormone obtained from cadavers.

Karen was thankful that genetic engineering had come up with a safe form of growth hormone for her brother to use. Because of this technology, her brother was assured a safe, reliable source of hormone that would allow him to grow at a fairly normal rate.

MATERIALS: (per team of two)

- 1 hGH mRNA sequence
- 1 vector plasmid
- 1 pair scissors

- two different color pens
- 3 pieces of scotch tape
- 1 strip of plain white paper

Part A: Identifying and Isolating the Human Growth Hormone (hGH) Gene

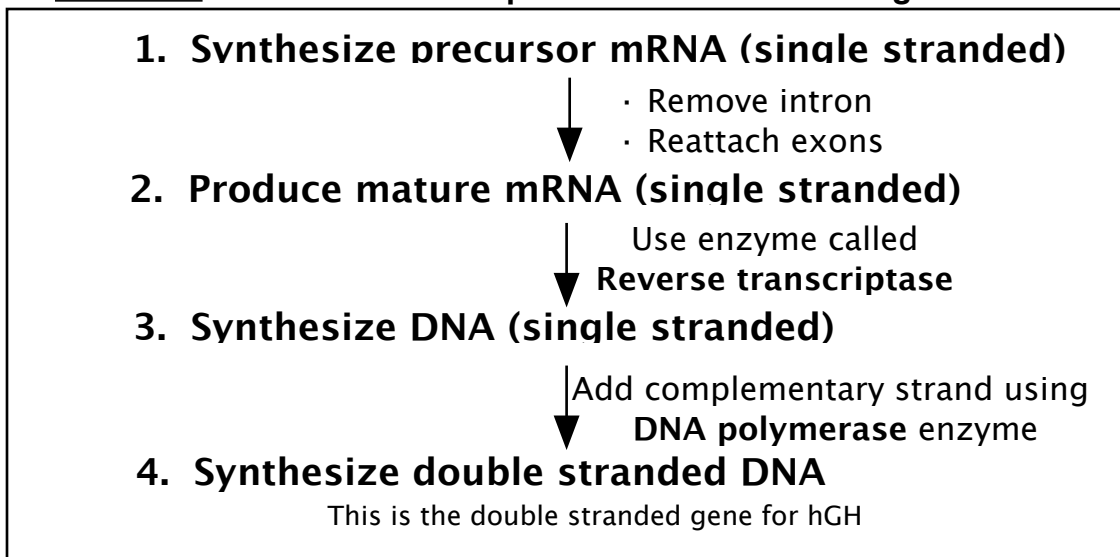
1. Answer questions "a. and b." on the questions sheet at the end of this activity.

2. Now, begin the construction of your model. The following is a portion of the mature mRNA (edited) for hGH. It has been isolated from pituitary tumor cells. Answer question "c." on your question sheet before you continue the activity.

U U C G A A U A C C G A U G U C C G A G G G C C U G C U U C G A A

Figure 1 is a flow chart that summarizes the steps necessary to create the hGH gene.

FIGURE 1: Flow chart for the production of the human growth hormone gene.



3. To do this, scientists use an enzyme called **reverse transcriptase**. This enzyme, which is normally found in viruses, copies mRNA into DNA which is the opposite, or reverse, of transcription! You will choose a pen to act as *reverse transcriptase* to convert your mature mRNA sequence into DNA. You will put your sequence of DNA onto your plain piece of paper. Try to make the letters of your DNA molecule the same size as those printed on your paper. BE CAREFUL: What is the complement of uracil in DNA? the complement of adenine?

4. Make the DNA double stranded. Choose the second, different colored pen and write the complement of the DNA molecule under your template strand. CONGRATULATIONS! You have constructed an hGH gene. The technique you used to produce a gene from mRNA produces what is called **copy DNA** or **cDNA**. Now that you have created the hGH gene, answer questions "d. and e." on question sheet.

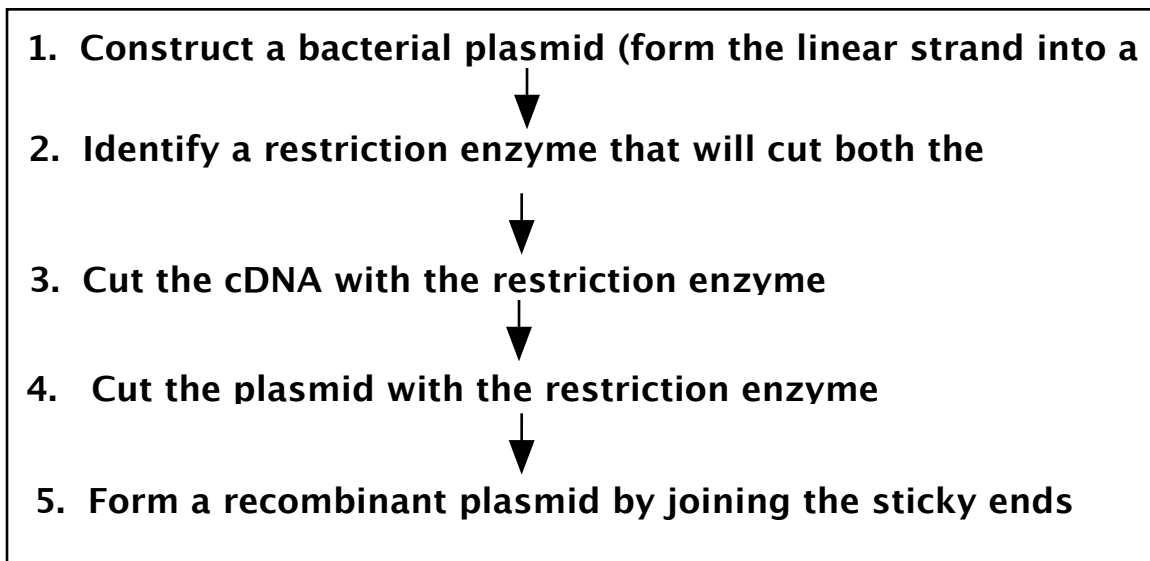
PART B: Forming Recombinant Plasmids

INTRODUCTION:

In Part A of this activity, you simulated the activities of the genetic engineer in steps 1 & 2 of the recombinant DNA process listed on page 2. You identified and constructed the hGH gene on a paper strip. In Part B, you will investigate how the hGH gene is inserted into the genome of a bacterium and how the bacterium produces hGH protein.

Figure 2 is a flow chart that summarizes the steps you will follow to create the recombinant plasmid.

FIGURE 2: Flow chart for the formation of a recombinant plasmid



PROCEDURE:

1. Some bacterial DNA exists in smaller circular pieces called **plasmids** (recall that bacteria do not have their DNA bundled as chromosomes, nor do bacteria have a nucleus). Plasmids are used by genetic engineers to introduce new genes into bacteria. The plasmid acts as the vehicle or **vector** for new genes to enter that bacteria. Think of the analogy of a manufacturing plant to help you to understand the recombinant DNA process:

Manufacturing Plant

raw materials
machinery
factory
product

Recombinant DNA Process

cDNA or hGH gene
plasmid
bacteria
hGH protein (hormone)

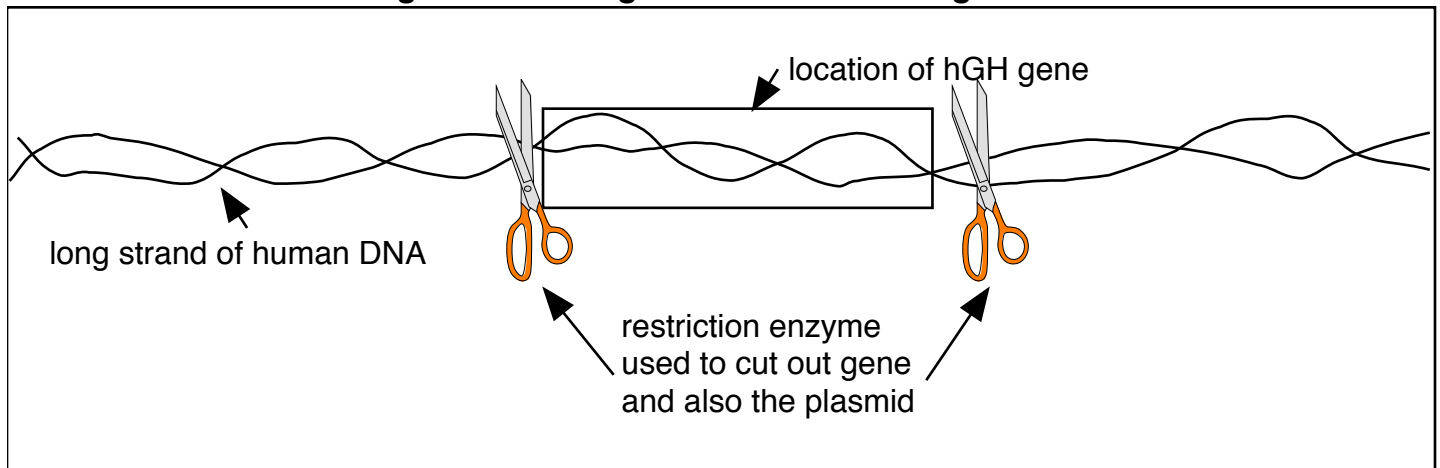
2. The first problem is to get the hGH gene (the cDNA you created in Part A) into the bacterial plasmid. To solve that problem, we must obtain a plasmid. Your teacher has created and xeroxed the bacterial plasmid for you on green paper. Because plasmids are actually circular pieces of DNA, you must bend your linear paper into a circle and use tape to permanently join the ends.

3. Answer question "f." on the question sheet before you continue.

4. Genetic engineers use enzyme scissors, called **restriction enzymes**, to cut open DNA sequences at specific locations. Each enzyme recognizes only certain DNA sequences of nucleotides and cuts within that specific sequence. When the DNA is cut, the ends become “sticky” because single-stranded fragments of DNA are left after the cut is made. These single stranded ends can attach to other **sticky ends** that have been cut with the same restriction enzyme and, therefore, have complementary nucleotide sequences. Figure 4 shows three restriction enzymes, their recognition sites, and the sticky ends the enzymes create.

Imagine that the model of the hGH gene you made in Part A is only a small part of a very long DNA molecule. Your problem is to cut out the hGH gene from this long DNA molecule and insert it into the plasmid that you produced in step 2. To do that you must use a restriction enzyme that will yield sticky ends in the hGH gene that can bind with complementary sticky ends in the plasmid.

FIGURE 3: Cutting out the hGH gene from the human genome



Examine your model of the hGH gene, which you constructed in Part A of this activity. Which of the restriction enzymes in Figure 4 can you use to remove the hGH gene from the complete DNA molecule? *Answer activity questions “g. thru j.”*

FIGURE 4: Examples of restriction enzymes

Enzyme Name	DNA Recognition Sequence and Cutting Site	Sticky Ends
BAM H1	<pre> ↓ ---G GATC C--- ↑ ---C CTAG G--- </pre>	<pre> GATCC CCTAG </pre>
HIND	<pre> ↓ ---A AGCT T--- ↑ ---T TCGA A--- </pre>	<pre> AGCTT TTCGA </pre>
Hpa	<pre> ↓ ---C CG G--- ↑ ---G GC C--- </pre>	<pre> CGG GGC </pre>

5. You will use the scissors to act as the restriction enzyme you have chosen and cut the hGH gene at the proper cutting site. Mark the cutting sites you plan to use on your gene and plasmid in pencil and check these lines with your teacher BEFORE you cut anything!
6. Use the same scissors because you use the SAME restriction enzyme to cut open your plasmid. HINT: how many times do you have to cut a circle to open it?? Compare the sticky ends of the hGH gene to the sticky ends of your plasmid. *Answer question number "k." before you proceed.*
7. In nature and in the laboratory complementary sticky ends of a gene and of a plasmid are "pasted" back together by enzymes called **ligase**. Perform the function of ligase by using the pieces of scotch tape provided by your teacher to attach the sticky ends. You should be able to check your work now. *Answer question "l." to explain how you know if you correctly created recombinant DNA.*
8. *Answer the remaining questions "m. and n." and write a conclusion of the steps necessary to create recombinant DNA. Do not throw your recombinant plasmid away, it will be collected along with the completed question sheet .*

Answer the following questions as you proceed through the activity. Please write in complete sentences and be as specific as possible.

Part A: Procedure and Discussion (p.3-4)

- a. In what type of cells would you look for the human growth hormone (hGH) gene?

- b. What product, aside from hGH, would tell you that the gene for hGH is functioning in the cells you have identified above?

- c. What must be done before hGH gene is placed into the bacterium? (after you have identified mRNA)

- d. What is the name of the enzyme that produces double-stranded DNA from single stranded DNA?

- e. Now that you have identified the hGH gene, what is the next step in the production of recombinant DNA?

Part B: Procedure and Discussion (p.4-6)

- f. After the creation of the plasmid, what is the next step in the process of creating recombinant DNA?

- g. Which restriction enzymes can you use to remove the hGH gene from the complete DNA molecule? Why did you choose this enzyme over the others?

- h. Which restriction enzyme can you use to cut open the plasmid DNA? Why choose this particular enzyme?

- i. How do the enzymes used to cut out the gene and to cut open the plasmid relate?

- j. Draw the sticky ends that result when the DNA from both the human and the plasmid are cut by the restriction enzyme.

- k. What do you observe about the sticky ends of the hGH gene and the sticky ends of the plasmid?

- l. After you insert the hGH gene into the plasmid, how do you check your work?

- m. What is the next step in the production of recombinant DNA after the creation of the recombinant plasmid?

- n. What would have resulted if the hGH gene had been cut in the middle rather than at each end?

Conclusion:

Write a brief, concise paragraph, in the space below, summarizing the techniques used to create recombinant DNA. Include the following words in your paragraph and underline them: recombinant DNA, sticky ends, genetic engineering, gene, reverse transcriptase, mRNA, ligase, restriction enzymes, plasmid.

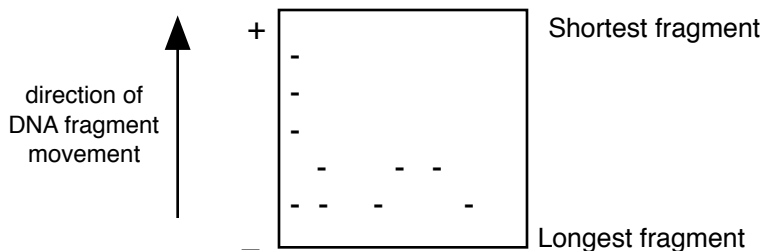
ARE YOU MY MOTHER?

DNA FINGERPRINTING (profiling) is currently being used in forensic cases to assess the probability of a suspect's involvement in certain crimes. DNA may be extracted from relatively small samples of cells such as a blood stain or a semen stain. When performed under properly controlled conditions and interpreted by an expert, forensic scientist, DNA profiling can link a suspect to a particular incident with compelling accuracy. This technique was used in the O.J... Simpson murder trial to analyze blood found at the scene and inside Mr. Simpson's car.

DNA profiling can also be used to determine the parents of a child. Assume that the Andersons and Olsons both had a child at the exact same time at Memorial Hospital. After three days, the Andersons made a complaint that they felt that the Olson's baby had been accidentally mixed up with their baby. First, the hospital said that they would check blood types to see if the babies matched the parents. Unfortunately, both sets of parents had type A blood and the babies both had type A blood so that no identifications could be made. As a result, the hospital had to use DNA profiling to check the match of the babies and their parents.

DNA profiling works in the following manner.

1. Blood cells are taken from an individual.
2. The cells are broken open and the DNA is extracted.
3. The DNA is chemically split into individual strands by restriction enzymes.
4. Another DNA restriction enzyme cuts the DNA into smaller pieces. These enzymes only cut the DNA at specific places based upon specific sequences of nucleotides.
5. The fragmented DNA is placed into an electrophoresis gel and the different sized pieces are separated using a process called **gel electrophoresis**.



6. During gel electrophoresis the DNA strands are separated by an electrical current. The negatively charged DNA strands are pulled from the negative electrode to the positive electrode at the other end of the gel.
7. The largest strands move the slowest and a short distance from the original well, while the shortest strands move the fastest and farthest from the original well.
8. People will have different sized pieces of DNA due to the action of the restriction enzyme and therefore will have a unique banding pattern or "fingerprint" on the gel. Genetically related individuals have a greater chance of having similar bands than unrelated individuals. You will be matching the

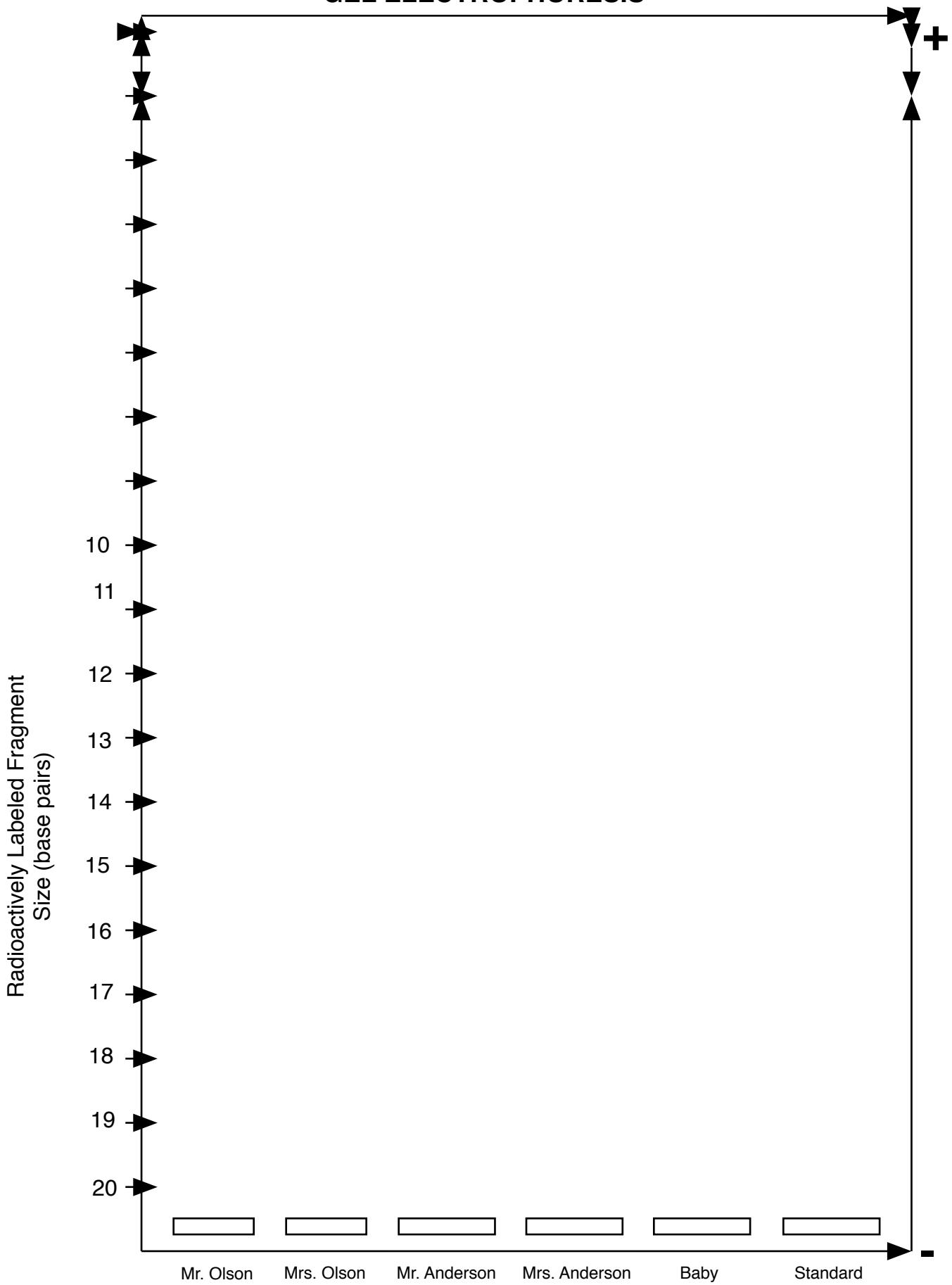
banding pattern of the baby with the banding patterns of both sets of parents. The baby belongs to the parents to whom it shares the most bands.

9. You will simulate the DNA profiling of the Andersons, the baby that the Andersons were given, and the Olsons. From these tests, you will determine if the Andersons were given the correct baby.

PROCEDURE:

1. Look at the standard strand of DNA. The strand takes up 2 lines and makes one strand of DNA.
2. Add the restriction enzyme. Mark in pencil where the cut sites exist. The restriction enzyme that you will use recognizes GGCC. The enzyme cuts every time this sequence occurs in the DNA and will cut between the **G and C (GG / CC)**. You will be forming fragments that end with GG and others that begin with CC.
3. Count the number of bases in each fragment. Write the number on the edge of each fragment so that you can refer back to it.
4. Add the radioactive probe to the fragments of DNA. The probe GTA recognizes complementary DNA nucleotides CAT. The fragments with the complimentary sequence of the probe are the ones that will be seen on the agar after electrophoresis has occurred. Using a highlighter, highlight the locations where the radioactive probe will attach to the complementary segments of DNA.
5. Draw the bands on the electrophoresis gel based on your counts from #4. The longest fragments move the least far and the shortest fragments moved the farthest. **ONLY plot the fragments that are radioactively labeled by the probe.**
6. Repeat steps #1-5 for all five people involved.
7. Analyze your gel. **Answer the following using complete sentences on separate paper.**
 - a. Which person(s) had the most DNA fragments radioactively labeled?
 - b. Which person(s) had the shortest piece of DNA radioactively labeled?
 - c. Which person(s) had the longest piece of DNA radioactively labeled?
 - d. Who are the parents of the baby?
 - e. Specifically explain how and why you determined your answer for question “d”.

GEL ELECTROPHORESIS



Rainbow Electrophoresis

PROBLEM: How may dyes be separated by the process of gel electrophoresis?

BACKGROUND: When researchers take cells apart to study the content of the cells, they get a mixture of different molecules. To study one particular kind of molecule, researchers must separate that kind of molecule from all others. Depending upon the physical and chemical properties of molecules, there are many ways to separate molecules from mixtures.

One method of separating molecules in mixtures is by *electrophoresis*. In the process of electrophoresis, an electrical current is applied to the ends of a gel containing a mixture of molecules. Depending upon their electrical charge and size, different molecules will move at different rates and directions in the gel. Negatively charged molecules will move toward the positive end of the gel and positively charged molecules will move toward the negative end of the gel. Generally, smaller molecules move through the gel more quickly than larger molecules. In this laboratory exercise you will have the opportunity to separate various dyes using the process of gel electrophoresis.

GOALS:

Prepare agarose gels for electrophoresis

Record data by making a sketch of your results

Infer the sizes of the dye molecules by comparing the distances they moved in the gel during electrophoresis.

MATERIALS:

power supply and cables	scoop	microwave oven
electrophoresis gel box	agarose or plain agar	various dyes
capillary pipettes	gel bed	metric ruler
gel comb	TAE or TBE buffer	1000mL beaker
colored pencils	plastic sandwich-sized bags	safety goggles

SAFETY CONCERNS:

Be sure to wear safety goggles at all times during the lab and during clean up.

CAUTION: Be aware of the electrical shock hazard when working with liquids near a power supply and when the electrophoresis equipment is operating.

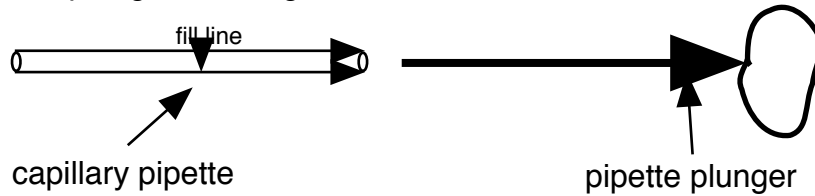
CAUTION: Be aware of the burn hazard when handling the hot flask containing melted agarose.

CAUTION: Be careful of the breakage hazard when working with glassware, especially the fragile capillary pipettes.

PROCEDURE:

1. Note the arrangement of lab supplies at your lab station. You will be working in groups of four for this lab. Do NOT TOUCH anything, until instructed to do so by your teacher.
2. Determine if the agarose is cool and firm by gently poking it with your finger. If it is firm, you can carefully pull out the comb from the gel by gently wiggling it back and forth and pulling it straight up.

3. Begin loading the wells of your gel with dye. The wells are the indentation compartments made from the comb. You will use the capillary pipet. Make sure you have both the pipette tube and the pipette plunger. See figure below:



4. With the plunger completely inserted into the capillary pipette, dip it into the dye container. Slowly pull up the plunger to pull the dye into the pipette. Only draw up dye to the first line on the pipette.
5. Insert the filled pipette and plunger into a gel well. Be careful you don't push down too far into the well or you will puncture the gel and your experiment won't work. To empty the dye into the gel, push the plunger down.
6. Let another group member try and repeat steps #3-5. Be sure to use all of your colors at least once. You will have extra wells that can be filled with the dye colors of your group's choice.
7. Record the position of the colored dye into the gel wells on the analysis sheet.
8. When all gel wells have been loaded, call your teacher. He or she will add buffer which acts as the medium through which the electric current will move.
9. After the buffer has been added, close the electrophoresis chamber lid, plug in the electrodes which are then connected to the power supply. Turn on the power and set power to _____.
10. After about 25 minutes, or when the dye has moved about three quarters of the gel length, turn off the power supply and unplug the electrodes. Carefully open the lid because electrophoresis generates some heat and steam might escape from the chamber.
11. Carefully remove the bed on which is your gel. Put bed over a white piece of paper and record your results on the analysis page using colored pencils.
12. Obtain a plastic sandwich bag and gently slide the gel off the bed and into the bag. This will have to be done if there is not enough time to record results on your lab day. In this case, label your bag with your group name, teacher's name, and class period number.
13. You must now create an agarose gel for the next class.
14. Pour the buffer from the chamber into the 1000mL beaker at your lab station.
15. Wash and dry out the electrophoresis chamber, the bed, and the comb. Dispose of the used capillary pipettes into the broken glass container. **DO NOT** discard the pipette plunger!
16. Let your teacher know that your group needs hot, liquid agarose. You should prepare for the agarose by placing the clean bed back into the chamber and insert the comb over the bed.

1. In the table below, record the colors of dyes used. After performing electrophoresis, observe the gel and record how the color moved or changed, its location in the gel and any other relevant information.

Well #	Dye Color	Observations
1		
2		
3		
4		
5		
6		
7		
8		

2. Draw a color picture in the space below that represents your gel's appearance. Label the charge at each end of the gel.

3. In electrophoresis, the smaller the molecules, the easier and faster they move through the gel. Which of the dyes you tested has the smallest molecules? Explain your answer.

4. Which dye in your experiment has the largest molecules? Explain your answer.

5. What charge do the pigments of dye have in this experiment? Explain your answer.

6. If we were to have used DNA instead of dye, how would we have created the different sized pieces of DNA that would be separated by electrophoresis?

7. What are three applications in which a DNA fingerprint or gel electrophoresis may be useful?

CONCLUSION: Write a paragraph summarizing this experiment and separation technique using all of the words listed and underline them in your paragraph: positive, negative, charge, size, dye, gel, movement, separation

Biotechnology Study Guide

Complete the following study guide by either defining terms or using examples to explain the meaning of the words. The more effort you use, the more successful you will be on your quest.

I. Recombinant DNA

- A. Uses

- B. Vector

- C. Plasmid

- D. Restriction Enzymes/Sticky Ends

- E. Procedure

II. Gel Electrophoresis

- ___A. Uses

- B. Restriction Enzymes

- C. How it works
 - 1. Size of DNA pieces

 - 2. Charge of pieces

- D. Are You My Mother Identification Activity

DNA fingerprint

III. **Other Important Topics**

___A. Gene Therapy

B. Genetic Engineering

C. Transgenic Organisms

D. Applications and Examples of Biotechnology Uses and Possible Abuses

IV. **Analogies**

___Relate the pictures to a biotechnology word or concept that we have studied in class:

