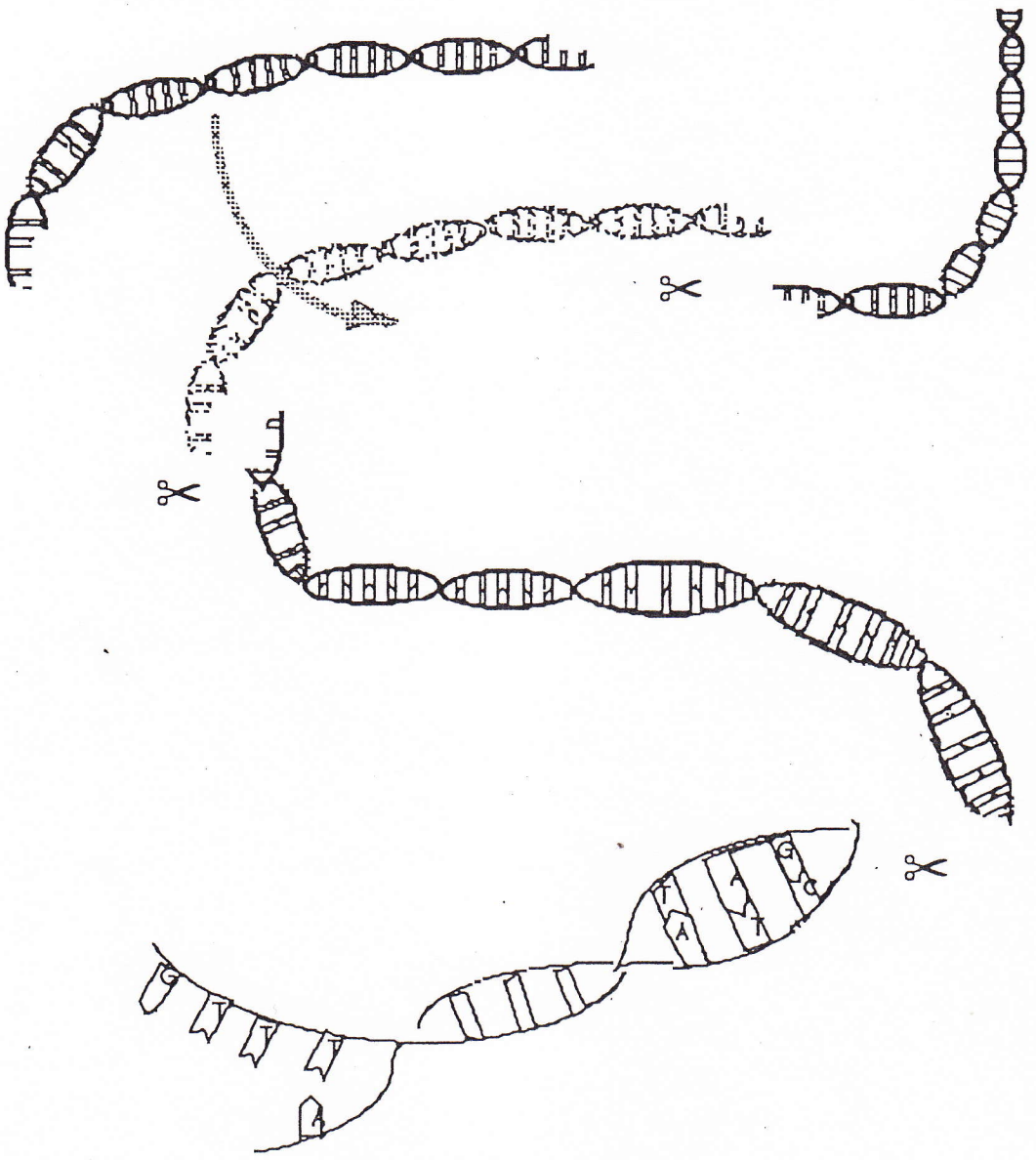
CHAPTER 38

GENETIC ENGINEERING



Chapter 38

Genetic Engineering

The only limit to our realization of tomorrow will be our doubts of today.
Franklin D. Roosevelt

38-1 Laboratory Investigation

Gene Splicing

Objective

You will be expected to be able to describe how genetic engineers splice one gene from one organism into another organism's genetic material. Also be able to give examples of the benefits of such gene splicing.

Background:

As prerequisites to this activity, the student needs to understand how DNA replicates and how the genetic code in DNA molecules create genetic characteristics through enzyme or protein synthesis requiring M-RNA and T-RNA.

Some people are unable to produce certain essential hormones. Diabetics are not able to produce enough of the hormone INSULIN. Insulin is produced by the pancreas and is required in order for glucose to pass from the blood stream into cells which require it for respiration. Until recently, INSULIN obtained from pigs and cattle pancreases was injected into diabetics daily. The supply had been decreasing. An unfortunate side-effect is an allergic reaction. Those found to be allergic must stop treatment, which can result in death.

Another hormone deficiency in some children involves too low a production of human growth hormone. This prevents them from reaching normal heights as adults. Some don't grow much over 3 1/2 feet tall. About 2,500 children in the United States suffer from this deficiency of hGH (human Growth Hormone) For years, the only source of hGH were sheep brains or pituitary glands from the brains of deceased humans. 500,000 sheep brains are required to obtain 5 mg of hGH. (This is about 5/1000 the weight of a paper clip.) For this reason, hGH is very scarce and extremely expensive. Also, the use of human pituitary hGH has been discontinued due to disease producing

virus contamination.

Both of the hGH and INSULIN hormones can now be produced by gene splicing. Commercial firms produce large quantities of both hormones for treatment of these deficiencies

To produce these hormones through genetic engineering, scientists had to locate the human gene that codes for insulin or hGH production. Next, the gene had to be isolated from the rest of the DNA. Then the isolated gene is inserted into a bacterial cell. The bacterial cells then divide and increase in number. All new bacteria possess the new gene which became spliced into the bacteria's own gene. As these bacteria grow in liquid cultures, they produce the desired HUMAN HORMONES. These hormones can then be removed from the culture solution. They are then purified and made ready for use in treatment of human growth hormone or insulin deficiency. Such new treatment is far less expensive and safer.

You will shortly simulate gene splicing the hGH hormone into a strand of bacterial DNA using chains of colored paper clips to represent DNA genes.

MATERIAL NEEDED:

Black, white, red and green paper clips.

PROCEDURE: (WORK IN TEAMS OF 2 OR INDIVIDUALLY)

STEP ONE: Construct a double stranded DNA molecule for the hGH (human Growth Hormone) gene. Use the following key throughout this activity:

Black clip	= Adenine	(**A**)
White clip	= Thymine	(**T**)
Red clip	= Cytosine	(**C**)
Green clip	= Guanine	(**G**)

One roll masking tape

Link the appropriate paper clips to create 2 strands of the hGH gene according to the following sequence:

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
A-A-G-C-T- T-A-T- G - G- T -C - C - C -G - G -A -C -G -A -A -G -C -T- T- C
T-T-C-G-A- A-T-A- C - C- A -G - G -G -C - C -T -G -C -T -T - C -G- A-A -G

The above sequence is a modified sequence of the actual base sequence of the first 26 nucleotide bases in the hGH gene. The entire gene contains 573 pairs of DNA bases.

STEP TWO: Place this paper clip gene model stretched out on your table with the A-A-G-C-T etc. strand above the T-T-C-G-A etc. strand. Keep the two strands matched up. [___] Tape the two strands of this gene together at the middle. [___] You will use this gene later. This hGH gene can be isolated from human cells and kept in test tubes.

STEP THREE: You now need to construct a paper clip model of **a plasmid.** A plasmid is a circular piece of DNA that makes up part of the bacteria's genetic material. Use the same color key to construct the following double-stranded DNA sequence:

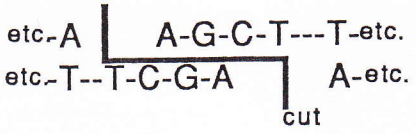
(The bacterial plasmid)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
C-G -G-A-C-C-G-T-C- A- A -G - C -T -T- C - C **PLASMID**
G-C- C-T-G-G-C-A-G -T- T- C - G -A-A- G - G

STEP FOUR: Match-up the two strands carefully and tape them together in the middle and write "Bacterial plasmid " on the tape. [___] Since bacterial plasmids are circular, attach base 1 to base 17 for both chains creating a double ring. [___]

You should now be ready to do some genetic engineering. We have one more step to perform before we can splice the hGH gene into the bacterial plasmid DNA.

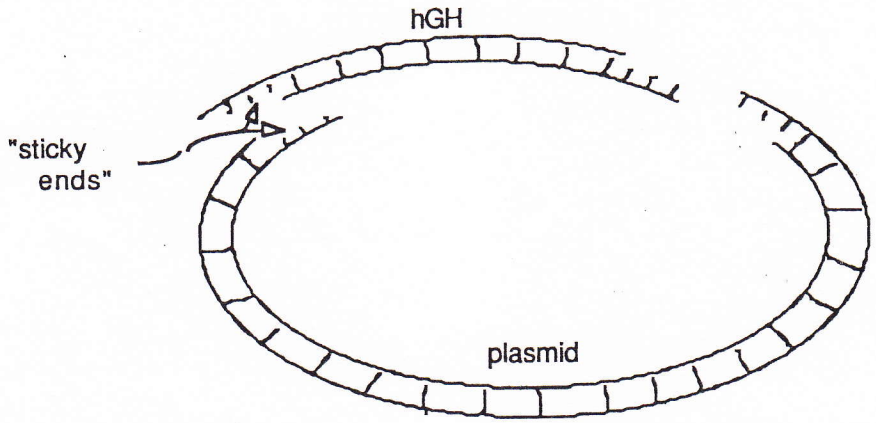
STEP FIVE: ("creating sticky ends"): Both the hGH gene and the plasmid need what genetic engineers call "sticky ends" (open complementary bases) that will attach to each other. The scientist must cut the plasmid DNA circle and the hGH gene in such a way that the cut hGH gene will splice into the plasmid according to the base-pairing rules. For this task, **RESTRICTION ENZYMES** are used. Restriction enzymes will only cut DNA in specific places. There are a variety of restriction enzymes. Some cut between adjacent G's, others between adjacent C's. We will use the <u>Hind III restriction enzyme</u> which cuts between adjacent A's in the DNA molecule. Here is how the Hind III enzyme will cut our DNA.



Find the sequence in the plasmid like the sequence above. [___] Now separate the double-strand DNA plasmid ring just as the Hind III restriction enzyme would. [___] Leave the open plasmid ring on the table and find the same sequence in the hGH DNA double strand. [___] You should be able to find 2 areas at each end of the hGH molecule. Cut these ends just as the Hind III enzyme would. [___] Discard the few clips left over and you are now ready to <u>splice!</u>

STEP SIX: SPLICING THE hGH GENE INTO THE BACTERIAL PLASMID

Move the hGH gene over to the partly open ring of the plasmid. [___] Fit the hGH gene into the plasmid ring so that A pairs with T and C with G. Don't connect them yet. [___]



Now connect the hGH gene into the ring, creating a new ring. Be sure A bonds with T and C with G. [___] Enzymes that paste the spliced DNA into a plasmid are called **LIGASES.**

You have just created a new bacterial plasmid that has the hGH gene spliced into its ring. The new DNA is called **RECOMBINANT DNA.**

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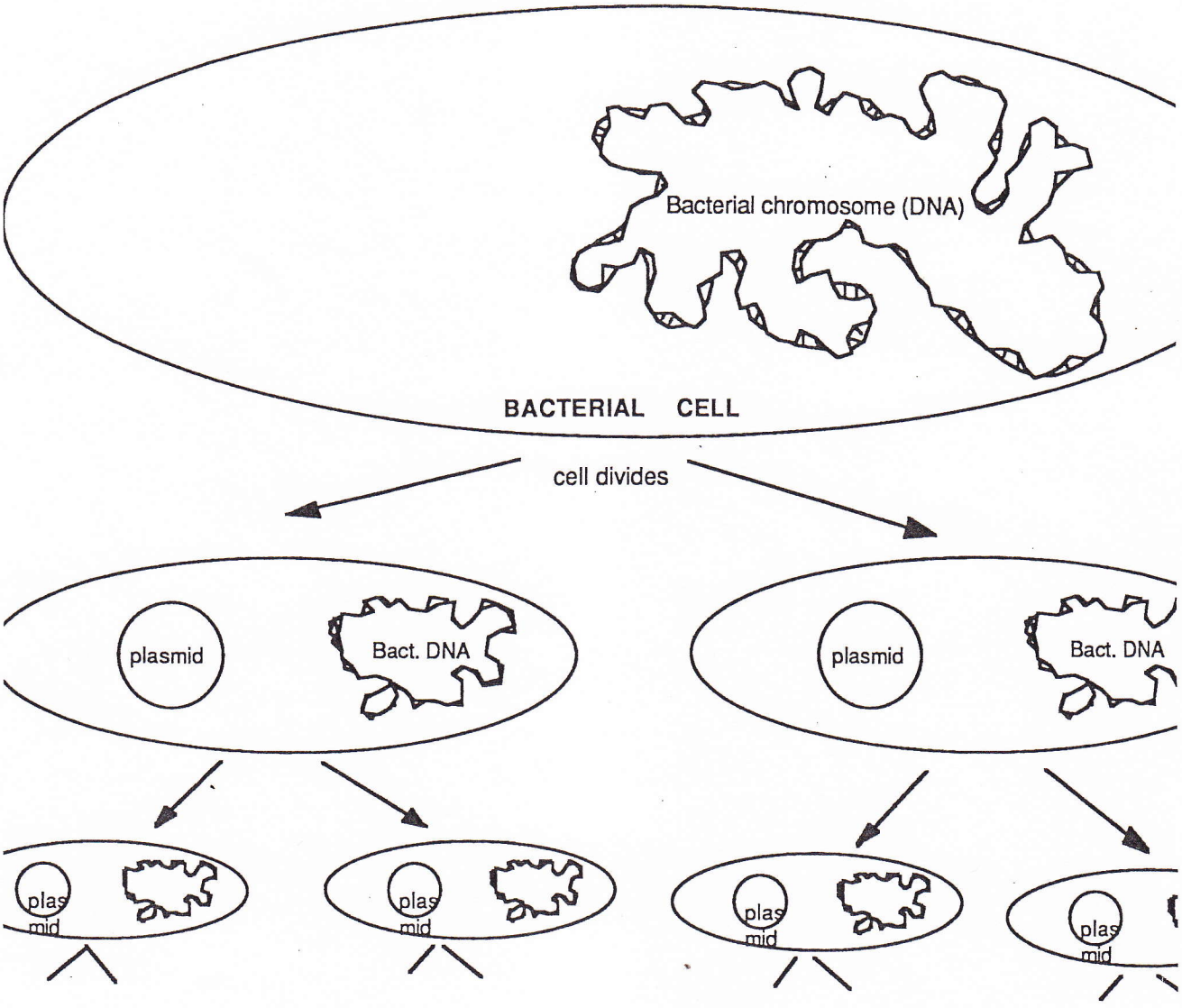
STEP SEVEN: THE BACTERIA DIVIDES:

Scientists now insert the new plasmid into bacteria. To simulate this, place a new paper clip plasmid into the bacterial cell diagram that follows. [___]



The first bacterial cell above had an hGH gene spliced into its plasmid. This cell then divides in liquid culture and the bacterial chromosome as well as the genetically altered plasmid will also divide. The cells continue to divide each 20 minutes until there are billions of bacterial cells all contianing the new human growth hormone (hGH) gene. These bacteria produce the human GROWTH HORMONE which diffuses into the liquid where it can be removed for injection into deficient humans.

This bacterial cell now divides into two cells as shown. The bacterial chromosome and the new plasmid divides thousands of times producing a large population of bacteria

all containing the human growth hormone (hGH) gene. During this time the bacteria begin producing human growth hormone.

HOW TO EXTRACT THE HUMAN GROWTH HORMONE

As these bacteria continue to multiply in their culture fluid, the growth hormone molecules move out of the cells into the culture liquid. Next, scientists separate the liquid containing the hormone from the bacteria and other substances in the culture media. The purified human growth hormone extract can now be given to humans that have hGH hormone deficiency.

STEP EIGHT: Please separate all the paper clips and return them to the appropriate containers by color. [___]

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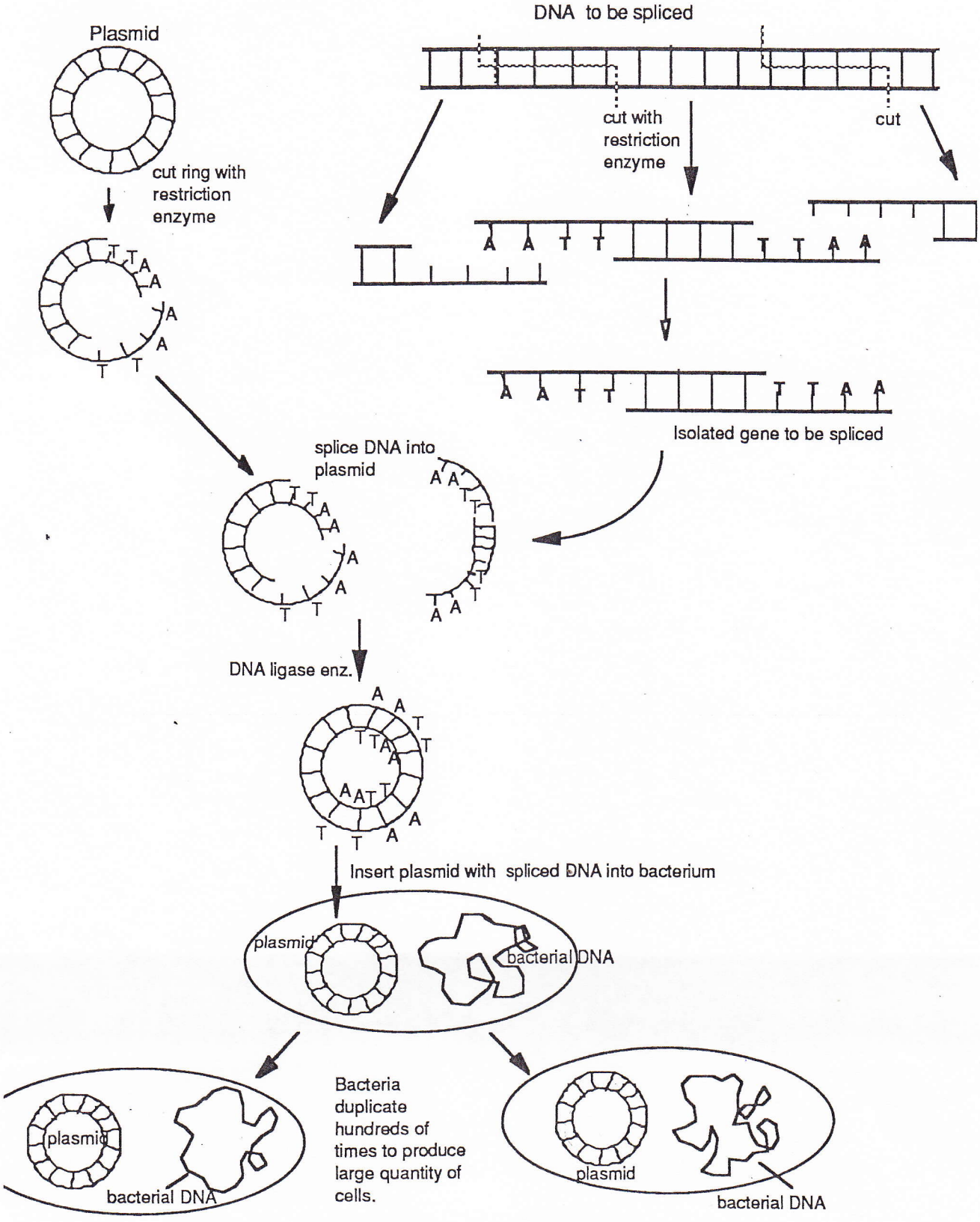
SUMMARY OF THE GENETIC ENGINEERING PROCESS



The bacteria produce the substance dictated by the spliced DNA gene.

QUESTIONS:

1. What enzymes are used to cut open the plasmid DNA ring?

2. What type of enzyme was used to cut the hGH gene to create "sticky ends"?

3. When the hGH gene is spliced into the open plasmid, what causes it to join with the plasmid in the unique way that it does?

4. After the gene splicing procedure is completed, what kind of cell is the plasmid containing the human growth hormone gene inserted into?

SOCIAL CONCERNS ABOUT GENE SPLICING:

5. Few people have concerns about gene splicing procedures involving insulin or human growth hormone to treat people with deficiencies. An abnormally short person could definitely benefit from synthetic hGH. The human growth hormone could, however, be made available to anyone of normal height who wanted to increase their height while growing. Should the hGH be made available to anyone or should it be restricted for use for those with deficiencies only? Give reasons for your answer.

6. Since there are not many individuals who need hGH, a company that has the capability to produce hGH may not be willing to produce it because the sale of such a small amount would not pay for the company's research and development. If companies would only produce hGH if they were allowed to sell it to anyone who wants it, would you be in favor of allowing it if this is the only way we can produce it for those with serious growth deficiencies? Give reasons for your answer.

7. Some scientists and laymen have warned of the possibility of creating some new disease producing bacteria inadvertently while gene splicing for hGH and other known genes. The bacteria may cause some new human diseases for which there is no cure. Should genetic engineering be allowed to continue if this is a possibility? Explain.

8. Should the government and/or other agencies formulate rules to regulate recombinant DNA research? Why or why not?

Cooperative Learning Activity: **Science and Society**
In your cooperative learning teams, discuss your answers to questions 5 through 8. Formulate one written group response for each question that reflects the opinion of the group. If significant disagreement exists, describe these areas. How should this disagreement be handled?

38-2 Recombinant DNA

1. Based upon Lab Investigation 38-1, what is gene splicing?

Objective

You will be expected to give examples of current and planned applications of genetic engineering.

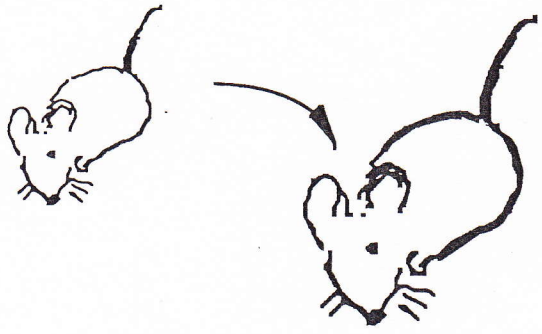
What might Mendel have thought about today's gene splicing experiments? Even forty years ago, biologists would have seriously doubted that genes from one organism could be spliced into the genetic material of a totally different species during the Twentieth Century. New breakthroughs in technique are being discovered regularly and some very interesting applications are on the horizon.

Gene Splicing Reviewed

If you performed Lab Investigation 38-1, you will recall that a gene (a length of DNA) from one species can be cut by special enzymes from the entire DNA strand. Then a new gene segment from the same or a different organism can be substituted. If you completed the lab investigation, you simulated removing the **human growth hormone** gene and spliced it into a bacterial DNA strand. The bacteria can now make human growth hormone for our medical use. Gene splicing results in a piece of DNA that contains a new recombined section from another piece of DNA. This new form of DNA is called **recombinant DNA.**

Some Current Applications

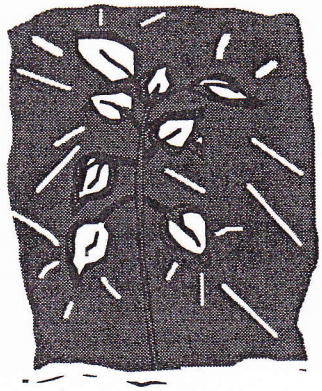
Gene splicing described so far has not involved altering an organism's genetic makeup from birth. Such experiments have been successful. To accomplish this the new gene must be introduced into an egg, sperm or zygote. Then the new individual that develops from this zygote will have an altered genetic makeup. Various genetically altered mammals have been produced this way. This is done by injecting DNA (gene) into a fertilized egg. This zygote is then placed into the uterus and allowed to develop in the normal manner. When born, the offspring clearly shows that it has the genetic trait injected. One such example involved a mouse egg that was injected with a gene for **rat** growth hormone. Rats usually grow four to six times larger than mice. When the animal was born, it grew into a giant mouse. Let's hope no one injects elephant growth hormones into rats!



The growth hormone gene from trout has been injected into carp zygotes. The carp that hatched grew faster and bigger than normal.

This technique might be used in fish farms to produce cheaper fish for market.

Scientists have worked on producing vaccines, including one for AIDS, using recombinant DNA techniques. The DNA from a disease producing virus is altered so it will not cause an infection but still cause the body of a person to produce antibodies that will kill the healthy virus. This genetically altered virus is injected as a vaccine in hopes of producing immunity to the disease.



A variety of plants have been genetically altered by gene manipulation. Biologists at the University of California have successfully isolated the gene from fireflies responsible for the insect's ability to glow in the dark. This gene was inserted into tobacco plant cells resulting in plants that glow in the dark! Potatoes, tomatoes, corn and squash have been genetically engineered to create species resistant to disease or insect pests. One goal involves creating a plant that will produce its own fertilizer.

The Human Genome Project

In 1990, molecular biologists began an ambitious project designed to identify and place in order, all 3.1 billion molecular "letters" of DNA found in every cell. This total collection of all human genes is called the human **genome** and the endeavor to "map" the gene sequence is called the **Human Genome Project.** At a White House ceremony on June 26, 2000, scientists announced that they have the human DNA sequence 99% complete, "a feat that ranks among the most important in the history of biology and a milestone expected to set the agenda for medical research for most of the 21st Century." This marks the final stage of a $2 billion effort to map the locations and sequences for every A, T, C and G in the DNA of all 46 chromosomes. President Clinton said, "Today we are learning the language in which God created life. We are gaining ever more awe for the complexity, the beauty, the wonder of God's most divine and sacred gift." Even with the entire genome mapped, scientists only know the sequences that correspond to a handful of specific genes. The next phase of the project will be to determine which genes are represented by particular known sequences. The current draft of the human genome is available on the GenBank web site. Its current web address is www.ncbi.nlm.nih.gov/GenBank/GenBankOverview.html. Examples of two known nucleotide sequences are the **insulin gene** and the **human growth hormone gene**. Biologists want to determine the DNA base sequence for every specific human gene. With this information, scientists would likely be able to alter many traits for future offspring. Most, if not all, genetic diseases might be eliminated. At present the process required to sequence one gene is a slow one, requiring many weeks of work. At the present rate for determining gene structure, all human genes could not be sequenced for many decades. Over six Seattle area labs have worked on the Genome Project. Within the next 10 years, it is predicted that people will have their DNA analyzed to tell them how likely they are to develop different forms of cancers, Parkinson's disease or other gene-based illnesses. "In 20-25 years, the first wave of new genome-based drugs should hit the drugstore shelves." One genome scientist predicts that this research will extend the average human life span to 90-95 by the year 2050.

DNA Fingerprinting

Criminologists can identify a person by his or her fingerprints. Just as each person has a unique set of finger prints, they also have a DNA structure different from anyone else. For this reason, scientists can identify people from DNA samples. They test blood samples, sperm samples, hair or other cell material left at the scene of the crime. The nucleotide base sequence is determined and compared with the suspect's known sequence. A local Washington woman was recently murdered in her apartment. Blood and other cell materials were left in the apartment by the murderer. The samples were DNA "fingerprinted" and compared to blood DNA prints of a suspect. The suspect has since been charged with the murder.

2. Briefly describe how gene splicing is accomplished.

3. Give four examples of how genetic engineering is currently used to alter the genetic characteristics of an organism.

4. What is Project Genome?

5. What is DNA fingerprinting and how is it used?

6. List a few applications of genetic engineering that, in your opinion, would be acceptable to society.

7. List a few applications of genetic engineering that, in your opinion, would not be acceptable to society. State why.

38-3 Genetics Review

Objective

You will be expected to be able to apply the principles of genetics to solve the problems in this set. These problems are similar to what you can expect on the unit exam.

GENETICS REVIEW PROBLEMS

1. Write the First and Second Laws of Probability.

2. A person rolls a six-sided pencil on the desk. The pencil's trade name is printed on one side only. What is the chance that the pencil will stop with the name up?

3. A person mixed up one deck of cards in one shoe box. He mixed another deck in a second box. He placed a hand in each box and drew out a card with each hand. What is the chance that he would have drawn a red card with both hands? If he continues drawing, list all the possible paired combinations he could get and the fractions of each.

4. Which Law of Probability is involved in computing the above chances?

5. A man with blue eyes marries a woman with brown eyes (heterozygous). List every possible offspring and the fractions of each.

6. In daffodils, yellow is dominant to orange flowers. Cross a homozygous yellow with a homozygous orange and give the genotype and phenotype of the F_1 offspring.

7. Now cross an F_1 offspring from 6 above with a plant just like itself. Show all possible F_2 offspring genotypes and phenotypes and fractions of each.

8. In daffodils, the short variety is dominant to the tall variety. Cross a yellow short plant (homozygous for both traits) with an orange tall plant. Show all possible offspring.

9. Cross a yellow short (heterozygous for both traits) with an orange flowered plant that is short, but carries a gene for tallness.

10. A colorblind man marries a woman with normal vision. Their first daughter is not colorblind. Their first son is colorblind. What is the genotype of each parent? What is the chance that their next son will <u>not</u> be colorblind?

11. Cross a person with type B blood (heterozygous) with a person with type A blood (heterozygous). What are the possible offspring?

12. A man with type A blood and a woman with type **A** blood have a type **A** girl and a type **O** boy. Is the man really the father? Explain your answer and show genotypes.

13. In pea plants, tall and round are dominant to short and wrinkled seeds. A gardener crossed a tall round with a short wrinkled plant and planted 80 seeds from this cross. The first plant to come up was short with wrinkled seeds. What is the genotype of the tall round parent?

14 What is DNA and how would you describe its structure? Draw a labeled representation of the DNA molecule to answer this question.

15. How does the code in DNA manage to produce a particular trait? Explain.

16. What is cloning and how is it accomplished?