

Genetic Engineering & Gene Splicing

Name _____
Date _____ Period ____

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:

You have already learned how DNA replicates and how the genetic code in DNA molecules creates visible characteristics through protein synthesis.

Some people are unable to produce certain essential hormones (which are a type of proteins made from DNA's code). Diabetics are not able to produce enough 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 through 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 cell then divides and increases 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 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: 4 different colored paper clips, masking tape

Procedure: (Work in teams of 2 or individually) Check off each box as you go!

Step one:

Construct a double stranded DNA molecule for the hGH gene.
For example: Adenine = blue, Thymine = white, Cytosine = red, Guanine = green

<i>Color Key for our Group</i>			
A=_____	T=_____	C=_____	G=_____

Step 1: 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.

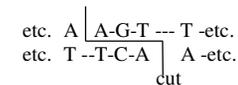
Step 2: Place this paper clip gene model stretched out on your table with the A-A-G-C-T etc. above the T-T-C-G-A etc. strand. Keep the two strands matched up. Tape the two strands of this gene together at the middle. You will use this gene later. This hGH gene can be isolated from human cells and kept in test tubes.

Step 3: 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-strand DNA sequence:

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

Step 4: 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 only one more step to perform before we can splice the hGH gene into the bacterial plasmid DNA.

Step 5 ("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 Hind III restriction enzyme which cuts between adjacent A's in the DNA molecule. Here is how the Hind III enzyme will cut our DNA.

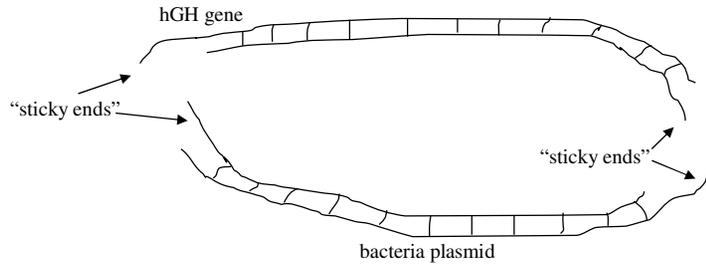


Find the sequence in the plasmid like the sequence above. Now separate the double-strand DNA plasmid ring just as the Hind III restriction enzyme would. Leave the open plasmid ring on the table and find the same sequence in the hGH DNA double strand. You should be able to find 2 areas at each end of 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 splice!

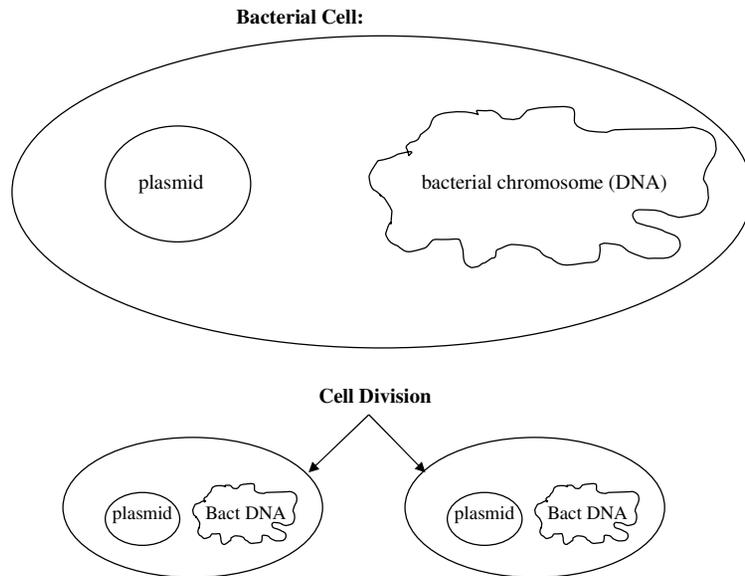
Step 6: Splicing the hGH gene into the bacterial plasmid

- 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**.

Step 7--The bacteria divides: Scientists now insert the new plasmid into bacteria. To simulate this, imagine the new paper clip plasmid put back into a bacterial cell and that this cell will divide as follows:



- The first bacterial cell above had an hGH gene spliced into its plasmid. This cell then divides in liquid culture and the bacterial chromosomes as well as the genetically altered plasmid will also divide. The cell continues to divide each 20 minutes until there are billions of bacterial cells all containing 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.

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 used by humans that have hGH deficiency.

- Step 8:** Please separate all the paper clips and return them to the appropriate containers by color.

Questions:

Please respond in your composition book! (Use part of the question in your response.)

- 1) In general, what enzymes are used to cut open the plasmid DNA ring?
- 2) What specific 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) Should the government and/or other agencies formulate rules to regulate recombinant DNA research? Why or why not?