NAME: _____ LAB DAY: _____

LIVING ENVIRONMENT TEACHER: _____ CLASS PERIOD: _____

DNA Recombination

LAB 23

Introduction: In order to remove a gene from one cell, and insert it into another cell's DNA, the gene must FIRST be **cut** from the original strand of DNA, and THEN be **implanted** into the new cell's DNA. This is accomplished by using special enzymes called **restriction enzymes.** These enzymes recognize a **specific sequence** of nucleotides, and will **cut** at this specific location. The cut out strand of DNA is said to have **"sticky ends".** When the same restriction enzyme is used to cut the other cell's DNA, it also leaves sticky ends. This allows the original DNA strand to be accepted by the other cell's DNA (think of two puzzle pieces fitting together). The original DNA strand is "glued" to the other cell's DNA by an enzyme called **ligase.**

This technique is called **DNA recombination** because you are "recombining" DNA strands after they have been cut. This process is used to insert human DNA (the original strand) into circle-shaped bacterial DNA known as a **plasmid.** Once the human gene is inserted, the bacterial cell will then begin to produce the human protein coded by the gene! If this bacterial cell divides, its offspring will also produce the human protein, providing enough for use. This technique is widely used to produce large amounts of human insulin which is needed by persons with diabetes.

Objective: In this laboratory, you will simulate how real geneticists remove a human gene and insert it into a bacterial cell.

Materials:

- **Colored pencil of your choice**
- **Scissors**
- **Tape or Glue**

Pre-lab questions:

1. What is a restriction enzyme and what does it do? _____

_____

_____

2. What are sticky-ends? _____

_____

3. The enzyme that glues sticky-ends together is called _____.

4. A circular piece of bacterial DNA is a called a _____.

5. What human protein is produced in large quantities by DNA recombinant technology? _____

Procedure:

1. Cut out the chromosome segment (s-shaped molecule) and the plasmid (ring shaped molecule). Be sure to cut out the "center" of the plasmid so you have a ring.

2. For this lab, we will simulate the use of the restriction enzyme known as **EcoRI. EcoRI** recognizes the following sequence of DNA. The "zigzag" represents the actual cut made by EcoRI:

5'	...GAATTC... 3'	CUTS	...G	AND AATTC...
3'	...CTTAAG... 5'		...CTTAA	G...

- Find the correct base sequence on the chromosome and cut it accordingly.
- Follow the example below as you cut the base sequence.

5' ...G|A A T T C... 3'
3' ...C T T A A|G... 5'

3. **Find** the same base sequence further along the chromosome, and **cut** another zigzag according to the diagram. This will give you two sticky-ends once the chromosome segment is cut. Remember, the cut out chromosome segment contains the gene for the protein we want produced by the bacterial cell.

4. **Color your cut out chromosome segment with your color pencil.**

5. **Find the same base sequence in the bacterial plasmid (Note: There is only one restriction site on the plasmid). Cut** the same zigzag that you cut in the chromosome segment. You should now be able to open the plasmid! Notice that one cut gives you two sticky ends!

6. **Insert** the gene from the chromosome segment into the plasmid so that the sticky ends match. Make sure your base pairs match (A w/ T and C w/ G).

7. **Glue or tape** the chromosome segment in place.

8. **Glue or tape** your **completed** recombinant DNA plasmid on the back of this lab for your teacher to see.

Questions to answer following laboratory

1. What did the scissors represent in this process? _____

2. What did the glue or tape represent in your model? _____

3. Why do you think you had to color your chromosome segment? _____

_____

4. What is the purpose of putting a human gene in a bacterial cell? _____

_____

What happens to the donor DNA (chromosome segment) when it gets into the bacterial cell? _____

6. What are some advantages of this recombinant DNA technology? What are some disadvantages? _____

REGENTS QUESTIONS

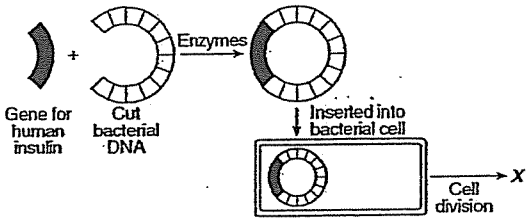
___ 1) Which statement *best* describes the result of some of the processes involved in genetic engineering?
A) They alter the arrangement of hereditary material.
B) They provide energy for mitosis and meiosis.
C) They are necessary for normal gamete formation.
D) They reduce variation in organisms that reproduce asexually.

___ 2) A woman has a gene that causes a visual disorder. To prevent the disorder from appearing in future generations, the defective gene would have to be repaired in the mother's
A) uterus
B) nervous system
C) reproductive cells
D) eye

___ 3) The insertion of a human DNA fragment into a bacterial cell might make it possible for
A) the cloning of the human that donated that DNA fragment
B) humans to become immune to an infection by this type of bacteria
C) the cloning of this type of bacteria
D) the bacterial cell to produce a human protein

___ 4) Many diabetics are now using insulin that was made by certain bacteria. The ability of these bacteria to produce insulin was most likely the result of
A) inserting a portion of human DNA into the ring-shaped DNA of bacteria
B) genetic mapping of bacterial DNA to activate the gene for insulin production
C) deleting many DNA segments from bacterial DNA
D) using radiation to trigger mutations

___ 5) The diagram below illustrates some key steps of a procedure in one area of biotechnology.

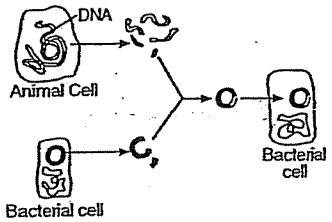


The letter *X* most likely represents
A) bacterial cells that are able to synthesize insulin
B) human cells that are able to synthesize antibodies
C) bacterial cells that are unable to synthesize insulin
D) human cells that are unable to resist antibiotics

___ 6) One way to produce large numbers of genetically identical offspring is by
A) inserting a DNA segment into a different DNA molecule
B) cloning
C) fertilization
D) changing genes by agents such as radiation or chemicals

___ 7) Which is a technique of genetic research in which genetic information is transferred from cells of one organism to cells of another organism?
A) genetic engineering
B) chromatography
C) amniocentesis
D) population genetics

Questions 8 and 9 refer to the following:



___ 8) The technique shown in the diagram represents
A) amniocentesis
B) the formation of recombinant DNA
C) the formation of a karyotype
D) animal cloning

___ 9) This process is useful in producing
A) insulin and human growth hormone
B) identical frogs
C) artificial hearts and kidneys
D) organ transplants

___ 10) In 1973, Stanley Cohen and Herbert Boyer inserted a gene from an African clawed frog into a bacterium. The bacterium then began producing a protein directed by the code found on the inserted frog gene.

Additional copies of the bacterium containing the frog gene could be produced by
A) inbreeding
B) asexual reproduction
C) grafting
D) cross-pollination

REGENTS QUESTIONS

Questions 11 through 15 refer to the following:

GENETIC ENGINEERING

Genetic engineering is a technique used by scientists to combine or splice genetic material from different organisms. Gene splicing involves changing the normal base sequences of DNA by removing a section of DNA and introducing another gene. This technique may involve the use of the bacterium *Escherichia coli*. This bacterium has one large chromosome and several small plasmids, which are ring-shaped pieces of DNA found in the cytoplasm.

Genetic engineers have been able to extract plasmids from *E. coli*. Restriction enzymes are then used to cut the DNA of the plasmid at designated places in the nucleotide sequence. These same enzymes are then used to cut a section of human DNA. This section of human DNA is then placed into the space in the cut DNA of the bacterial plasmid. The human DNA codes for the synthesis of a product such as human growth hormone. The spliced bacterial DNA, which now contains a piece of human DNA, is referred to as a hybrid. This hybridized plasmid is then transplanted into *E. coli*. When this bacterium reproduces, the hybrid DNA will be replicated. Offspring will possess the ability to synthesize the human growth hormone.

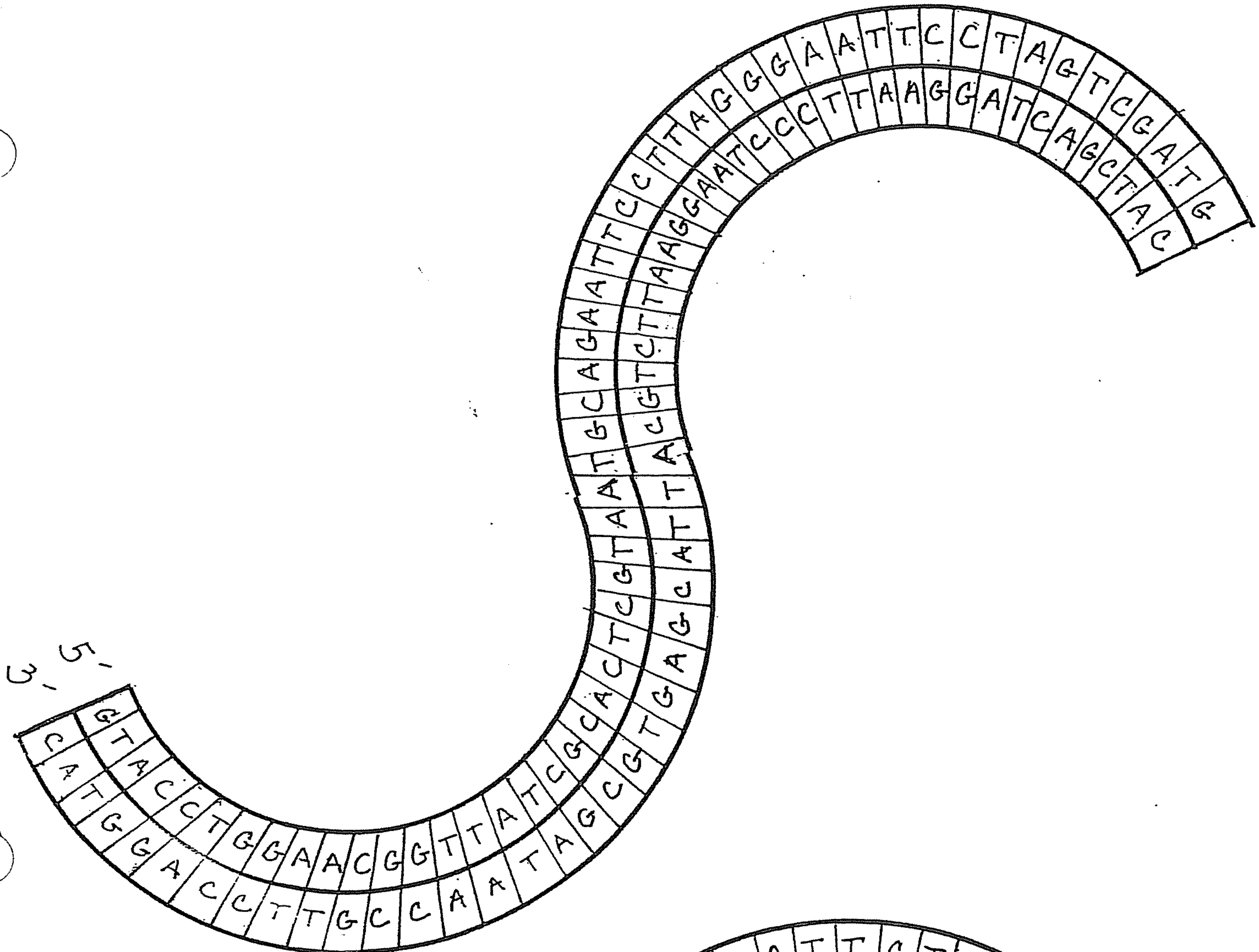
11 What is a bacterial plasmid?

12 What is a hybrid plasmid?

13 How do genetic engineers remove sections from human DNA for splicing into bacterial DNA?

14 What is *one* benefit of gene splicing?

15 Explain why it is *not* necessary to continue splicing the gene for human growth hormone into *E. coli* once cultures of the bacteria with the spliced gene are established.


5'
3'
GTACCTGGAACGGTTATCGCACTCGTAATGCAGAATTCCTTAGGGAATTCCTAGTCGATG
CATGGACCTTGCCAATAGCGTGAGCATTACGTCTTAAGGAATCCCTTAAGGATCAGCTAC