

**Course**

*Pathophysiology*

**Unit I**

*History, Trends  
and the Future*

**Essential  
Question**

*What can be  
accomplished by  
changing the  
DNA sequence?*

**TEKS**

*§130.208(c)  
3A, 4A*

**Prior Student  
Learning**

*Understanding  
of DNA*

**Estimated time**

*3-4 hours*

**Rationale**

Bioengineers make news using recombinant DNA techniques in hopes of curing genetic diseases, better understanding cancer, and improving agricultural yields. But while promising much, such techniques have presented and will continue to present society with some very difficult moral and ethical problems.

**Objectives**

Upon completion of this lesson, the student will be able to:

- Understand the role of the biotechnology industry and its impact on society
- Know the general structures and functions of DNA, RNA, and protein
- Understand the techniques used in recombinant DNA technology
- Complete a paper lab to illustrate some of the steps of recombinant DNA experiments

**Engage**

Cloning is making an identical genetic copy of something. Many helpful things can be accomplished from cloning but there are many ethical issues which can arise. Brainstorm about the positive things which might come about from this technology. What are some of the issues which might also arise?

**Key Points**

- I. Biotechnology
  - A. Made up of 3 technologies
    1. Bioprocess technology: when microorganisms are provided with nutrients and advantageous conditions, they perform processes
      - a. In existence for 10,000 years
      - b. Three of its products
        - i. bread
        - ii. cheese
        - iii. beer
      - c. Breakdown of waste at treatment sites
    2. Monoclonal antibodies: scientists fuse cancer cells, which multiply with cells that produce an antibody
      - a. Fused cells-hybridomas
      - b. Hybridomas cloned
      - c. Under bioprocess conditions produce large quantities of antibody

3. Recombinant DNA: produce substances in large amounts
  - a. Like monoclonal antibody technology
    - i. Produces substances in large amounts
    - ii. Used to study biological processes
  - b. Process of  
Gene coding for a particular protein is transferred into the host organism
    - i. Host organism multiplies and produces desired protein in volume
  - c. Example
    - i. Gene that codes for production of insulin
    - ii. Inserted into bacterium E Coli
    - iii. Bacteria is grown in large vats using bioprocess technology
    - iv. Large amounts of insulin is collected
  - d. Already developed sources
    - i. Interferon
    - ii. Human Growth Hormone
    - iii. Hepatitis B
- B. Uses living organisms
  1. To carry out chemical processes
  2. To produce substances
- C. Combines
  1. Biology with chemistry
  2. Science with industry

## II. DNA, RNA & Protein

- A. Nucleic Acids: Nucleic acids are commonly known as DNA (deoxyribonucleic acid) and RNA (ribonucleic acid).
  1. All nucleic acids contain the atoms C, H, O, N, and P.
  2. DNA stores the genetic code within structures called chromosomes. They are found within the nucleus of the cell.
  3. DNA and RNA assist with protein synthesis.
  4. RNA is responsible for transporting the genetic code from the nucleus to the ribosomes where the needed proteins are made.
  5. Structure of DNA
    - a. DNA is formed by a double helix of nucleotides.
    - b. Nitrogen bases are paired together (A-T) and (C-G). The sequence of the nitrogen bases determine the amino acids to be used in the forming of a new protein. The nitrogen bases form the rungs of the DNA molecule.
    - c. The four nitrogen bases are adenine, thymine, cytosine, and guanine
  6. Structure of RNA
    - a. RNA is formed by a single helix of nucleotides. A nucleotide is composed of a sugar, a phosphate, and a nitrogen base.

- b. Nitrogen bases are paired together (A-U) and (C-G). The sequence of the nitrogen bases determine the amino acids to be used in the protein. The nitrogen bases form the rungs of the RNA molecule.
- c. The four nitrogen bases are adenine, uracil, cytosine, and guanine
- d. The sugar is ribose. The nitrogen base is attached to the ribose and helps to form the side of the helix.
- e. The side of the helix contains a phosphate group which alternates with the ribose forming the backbone.

B. Proteins

- 1. All proteins contain the atoms C, H, O, and N.
- 2. Proteins are responsible for much of the structure of body tissues including cell membranes, collagen, keratin, and elastin.
- 3. Proteins form enzymes which act as catalysts in chemical reactions to speed up the reaction.
- 4. Proteins function as antibodies to help the body fight infection.

III. Steps to recombination

A. Identify the gene that codes for the production of the protein that is to be manufactured

- 1. Work backwards from the amino acid sequence of the desired protein to the nucleotide sequence of the gene
- 2. There are various other methods

B. Isolate the gene

- 1. Isolated from bacteria (by cleaving or cutting any foreign DNA that gets into the bacterial cell)
- 2. Restriction enzymes or endonucleases from bacterial cells are key
  - a. Recognize and cleave only very specific sequence of DNA
  - b. Some make a staggered cut of the DNA yielding sticky ends (single stranded nucleotides bases capable of binding with complimentary sticky ends)
- 3. By using enzymes that will cut the DNA on either side of the gene, the gene can be clipped out of the DNA strand

C. Transfer of the gene to the host cell

- 1. Plasmid
  - a. Small ring shaped piece of DNA found naturally in most bacteria
  - b. Contain replication origin that enables them to be replicated
  - c. Other functions include antibiotic resistance
  - d. Multiple copies exist normally within the cell
- 2. Scientists cleave plasmid using the same enzyme they used to clip out the gene
  - a. Sticky ends of plasmid will match those of the gene

- b. Cuts can be made with one enzyme at one location and another at a second location
- D. Cleaved plasmid and cleaved gene are mixed together
  - 1. Sticky ends of the gene and the sticky end of the plasmid come together
  - 2. Complementary base pairs are joined by hydrogen bonding
  - 3. DNA ligase enzyme is added
    - a. Creates phosphodiester linkage
    - b. Completes the bond
- E. Mix plasmids with host bacteria
  - 1. Test bacteria for innate characteristics of the plasmid
    - a. Example
      - i. If plasmid confers resistance to an antibiotic, scientists spread the bacteria on a Petri plate of agar mixed with antibiotic
      - ii. Only bacteria containing plasmids with antibiotic resistance and replication origin will survive
  - 2. Tested to determine that plasmid has not been closed without incorporating the gene
- F. Plasmids are replicated inside the bacteria
  - 1. Replicated so they exist in multiple copies
  - 2. Gene becomes active
  - 3. Bacteria produce the desired protein

### **Activity**

- I. Complete the Recombinant Paper Plasmids Laboratory Investigation

### **Assessment**

Successful completion of the Recombinant Paper Plasmids activity.

### **Materials**

Plasmid Pattern Sheet  
Enzyme Pattern Sheet  
Cell DNA Pattern Sheet  
Scissors  
Scotch tape  
Colored pencils

### **Accommodations for Learning Differences**

For reinforcement, the student will define the key terms.

For enrichment, the student will take a new plasmid sheet and throw away 2 of the segments. Be sure to keep the section of the plasmid that contains the replication origin. What major changes are now noticeable?

## **National and State Education Standards**

### **National Healthcare Foundation Standards**

Standard 6: Ethics

6:1 Ethical boundaries

6:11 Differentiate between ethical issues impacting healthcare

6:12 Recognize ethical issues and their implications related to healthcare

### **TEKS**

§130.208 (c) (3) (A) in all fields of science, analyze, evaluate, and critique scientific explanations by using empirical evidence, logical reasoning, and experimental and observational testing, including examining all sides of scientific evidence of those scientific explanations, so as to encourage critical thinking by the student; and

§130.208 (c) (4)(A) identify biological and chemical processes at the cellular level.

### **Texas College and Career Readiness Standards**

Nature of Science: Scientific Ways of Learning and Thinking

A. Cognitive skills in science

1. Utilize skepticism, logic and professional ethics in science

3. Formulate appropriate questions to test understanding of natural phenomena

B. Scientific Inquiry

1. Design and conduct scientific investigations in which hypotheses are formulated and tested

Cross Disciplinary Standards

A. Intellectual Curiosity

1. Engage in scholarly inquiry and dialogue

D. Academic behaviors

1. Self-monitor learning needs and seek assistance when needed

3. Strive for accuracy and precision

4. Persevere to complete and master tasks

# Recombinant Paper Plasmids

**Follow all instructions carefully.**

## Materials Needed

Plasmid Pattern Sheet  
Enzyme Pattern Sheet  
Cell DNA Pattern Sheet  
Scissors  
Scotch tape  
Colored pencils

## Construction of the Plasmid

1. Locate the plasmid pattern sheet. Carefully trim off the antibiotic key on the bottom and place it to one side for later reference.
2. The plasmid pattern sheet is arranged in long strips. Starting from the left side of the page, cut the first strip off and LABEL IT #1 on the back. Cut the second strip off the paper and label it #2. Continue to cut and label each strip until you have 6 strips on your table with each one of them numbered in sequence 1 to 6.
3. With a piece of scotch tape attach strip #1 to Strip #2. Then add strip #3 to the end of strip #2. Continue attaching the strips end to end until you have ONE LONG CHAIN ending with strip #6.
4. Now take the end of strip #6 and bend it around until it is next to the beginning of strip #1. Attach the two ends to each other with a piece of scotch tape so that you now have a complete circle that will represent your plasmid.

## Location of Restriction Sites

1. Find the sheet labeled ENZYMES. Cut the enzyme sheet into 9 cards. Set the LIGASE card to one side.
2. Mark a starting point on the plasmid by drawing a line across the plasmid with a pen or pencil.
3. In the plasmid the left hand strand is always read 3' to 5' and the right hand strand is always read 5' to 3'. At the beginning spot that you have just marked on your plasmid, designate the 3' and the 5' sides of the plasmid.
4. Mark the 3' and 5' strands of the enzyme exactly as you have done the plasmid as they are read in the same directions.
5. Compare the enzyme structures with the plasmid sequences. Locate each possible restriction site by placing the enzyme card BEHIND the plasmid strand with the 5' to 3' segment showing. Match the 5' strands of the plasmid and the enzyme by sliding the

enzyme downward until you find a matching set where “cutting” may be done.

6. Take a colored pencil and mark the segment on the TOP of the plasmid exactly as the “cutting” line appears on the enzyme. Write the name of the enzyme at that location on the BACK of the plasmid.
7. Locate as many different restriction sites as you can, marking each one for later reference.

### **Construction of the Cell DNA**












1. Cut out the strips on the CELL DNA SHEET being careful to keep them in the order of 1 through 6.
2. Attach the strips to each other in the order of 1 through 6 using scotch tape to secure the strips. You should now have a long paper strip with a gene indicated in its interior.
3. The gene is in triplet code on the 3' to 5' strand and starts with methionine and ends with a stop codon.
4. Determine which restriction sites are near the gene by using the same techniques that you used on the plasmid. Be sure to mark the locations of the cutting sites on the DNA strand. Which sites you use will depend on the restriction site available in the plasmid.
5. Cut the plasmid and also the cell DNA following the cutting patterns shown on the appropriate restriction enzyme cards.
6. Match up the “sticky ends” of the gene with those of the plasmid and fasten the ends. The ligase enzyme that you placed to one side earlier will be needed to “paste” the “sticky ends” of the gene and the plasmid together.

You should now have a plasmid with a gene spliced into it. This is your

### **IDENTIFICATION OF THE NEW RECOMBINANT DNA**

1. Retrieve the antibiotic key that you saved when you cut out the plasmid strands.
2. Carefully scan the NEW plasmid and identify which of the antibiotics could successfully be used to identify the new recombinant DNA. REMEMBER THAT IF AN ANTIBIOTIC SITE ON THE PLASMID HAS BEEN CUT BY THE RESTRICTION ENZYMES IT IS NO LONGER GOING TO WORK.

# Plasmid

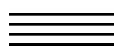
G C	A T	T A	C G	T A	T A
C G	G C	A T	G C	G C	A T
C G	A T	G C	A T	G C	A T
C G	A T	G C	G C	T A	G C
A T	A T	C G	T A	G C	C G
G C	A T	C G	T A	 G C	C G
A T	 T A	C G	A T	 G C	G C
G C	G C	C G	 A T	 G C	T A
T A	T A	C G	C G	 G C	A T
T A	G C	T A	C G	C G	G C
T A	T A	T A	T A	A T	G C
C G	G C	T A	A T	A T	T A
T A	T A	T A	G C	G C	T A
T A	C G	T A	G C	G C	C G
A T	C G	 A T	 A T	T A	G C
A T	A T	 G C	 G C	T A	A T
G C	G C	 G C	G C	A T	A T
G C	T A	G C	G C	T A	C G
T A	A T	A T	C G	A T	T A
C G	G C	C G	C G	C G	C G
T A	G C	T A	C G	T A	C G



= ampicillin resistance



= kanamycin resistance



= tetracycline resistance



= plasmid replication



# Cell DNA

T A	G C	T A	T A	G C	T A
G C	A T	T A	T A	T A	T A
G C	G C	C G	C G	A T	C G
G C	A T	G C	G C	A T	G C
C G	T A	A T	T A	T A	A T
C G	T A	A T	C G	A T	A T
T A	C G	G C	A T	T A	C G
A T	T A	G C	T A	T A	G C
G C	T A	T A	G C	C G	G C
G C	A T	A T	T A	C G	G C
C G	A T	C G	G C	T A	G C
A T	G C	A T	C G	C G	C G
C G	T A	T A	C G	C G	C G
A T	C G	A T	T A	T A	C G
G C	A T	A T	T A	T A	T A
G C	A T	C G	T A	A T	A T
G C	G C	G C	T A	A T	G C
C G	C G	T A	A T	G C	G C
C G	A T	C G	A T	A T	A T
C G	G C	T A	A T	A T	C G
G C	G C	C G	T A	T A	C G
1	2	3	4	5	6



= protein gene

# Enzymes

<div> <div>C G</div> <div>C G</div> <div>T A</div> <div>G C</div> <div>G C</div> </div> <div>Ava II</div>	<div> <div>T A</div> <div>T A</div> <div>C G</div> <div>G C</div> <div>A T</div> <div>A T</div> </div> <div>Hin dIII</div>	<div> <div>C G</div> <div>C G</div> <div>T A</div> <div>A T</div> <div>G C</div> <div>G C</div> </div> <div>Bam HI</div>
<div> <div>T A</div> <div>C G</div> <div>T A</div> <div>A T</div> <div>G C</div> <div>A T</div> </div> <div>Bgl II</div>	<div> <div>G C</div> <div>G C</div> <div>C G</div> <div>C G</div> </div> <div>Hpa II</div>	<div> <div>C G</div> <div>T A</div> <div>T A</div> <div>A T</div> <div>A T</div> <div>G C</div> </div> <div>Eco RI</div>
<div> <div>C G</div> <div>T A</div> <div>C G</div> <div>G C</div> <div>A T</div> <div>G C</div> </div> <div>Sac I</div>	<div> <div>G C</div> <div>G C</div> <div>G C</div> <div>C G</div> <div>C G</div> <div>C G</div> </div> <div>Xma I</div>	<div>LIGASE</div>

# **Recombinant Paper Plasmids**

## **Questions for Review**

1. Define recombinant DNA.
2. How many cleaving sites did you find on your plasmid?
3. List the sequences for the “stop” codons.
4. List the antibiotics that could successfully be used to test for the presence of the gene on your plasmid.
5. Explain why, if applicable, an antibiotic could not be used to test for the presence of the gene on your plasmid.
6. Which side of the DNA strand is read 3' to 5'?
7. What is the function of the Ligase card that you set to one side?
8. List at least 3 ways that recombinant DNA has been used in modern medicine to benefit mankind.