

Recombinant Paper Plasmids Cut-and-Paste Biotechnology

OBJECTIVE / 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. The student will illustrate some of the steps of recombinant DNA experiments.

TEKS 121.15 (c) 5A, 7C

TAKS ELA 1, 4
Science 1, 2, 3

KEY POINTS

Recombinant DNA technology is one of three technologies grouped together as biotechnology. Biotechnology uses living organisms to carry out chemical processes or to produce substances, combining biology with chemistry and science with industry. The areas comprising the current biotechnology are bioprocess, monoclonal antibodies, and recombinant DNA technology.

Not everything about biotechnology is new. Bioprocess technology has been around for 10,000 years. Bread, cheese, and beer are three of its products. When microorganisms are provided with nutrients and advantageous conditions in which to grow, they can perform a process, such as the breakdown of waste at treatment sites, or produce a product, such as alcohol in the manufacture of beer or wine.

Monoclonal antibody technology is much more recent. In the manufacture of monoclonal antibodies, scientists fuse cancer cells, which multiply continuously, with cells that produce a particular antibody. The fused cells are called hybridomas. The hybridomas are cloned, and under bioprocess conditions they produce the antibody in quantity.

Recombinant DNA technology, like monoclonal antibody technology, produces a substance in large amounts and is used to study basic biological processes. A gene coding for a particular protein is transferred into a host organism. The host organism multiplies, and the population produces the desired protein in volume. For example, the gene that codes for the production of human insulin has been inserted into the common bacterium *E. coli*. Then the bacteria can be grown in huge vats using bioprocess technology and large amounts of insulin collected. Researchers have already developed sources of interferon, human growth hormone, and hepatitis B vaccine using recombinant DNA techniques.

Steps to Recombination

To begin the recombinant DNA process, scientists must first identify the gene that codes for the production of the protein they want to manufacture. One way is to work backwards from the amino acid sequence of the desired protein to the nucleotide sequence of the gene. There are various other methods of finding genes.

After scientists have identified the gene, they must isolate it. Restriction enzymes, or endonucleases, from bacterial cells are key in this step. They are isolated from bacteria that use them to destroy, by cleaving or cutting out, any foreign DNA that gets into the bacterial cell.

What makes restriction enzymes so useful is that each recognizes and cleaves only a very specific sequence of DNA. Some restriction enzymes make a staggered cut of the DNA yielding “sticky ends,” single strands of nucleotide bases capable of binding with complementary sticky ends. By using enzymes that will cut the DNA on either side of the gene, the gene can be clipped out of the DNA strand.

Once scientists obtain the gene they are looking for, they must somehow get it into the host cell. The workhorse of this transfer step is the plasmid, a small ring-shaped piece of DNA found naturally in most bacteria. Plasmids have a region, called the replication origin that enables them to be replicated. Plasmids perform one or two other functions, such as antibiotic resistance. Multiple copies of the plasmids used in recombinant DNA technology exist normally within a bacterial cell.

After removing a plasmid from a bacterial cell, scientists cleave the plasmid using the same enzyme they used to clip out the gene. This way the sticky ends of the plasmid will match those of the gene. Sometimes, the cuts are made using one enzyme at one location and another at a second location. Regardless, it is important to use restriction enzymes that do not cut within the gene itself.

Then, the cleaved plasmid and the cleaved gene are mixed together. If all goes well, the sticky ends of the gene and the sticky ends of the plasmid come together, their complementary base pairs joined by hydrogen bonding. A DNA ligase enzyme is added, which creates a phosphodiester linkage and completes the bond.

Finally, bioengineers mix the plasmids with host bacteria. To check that the plasmid has been taken up, they test the bacteria for an innate characteristic of the plasmid. For example, if a plasmid confers resistance to an antibiotic, scientists could spread the bacteria they hope contain the plasmids on a Petri plate of agar mixed with the antibiotic. Only the bacteria containing plasmids with antibiotic resistance and the replication origin will survive. At some point, the plasmid would be tested to determine that it has not been closed without incorporating the gene.

Inside the bacteria, the plasmids are replicated so that they exist in multiple copies. The gene become active and the bacteria begin producing the desired protein.

ACTIVITIES

- I. Complete the Recombinant Paper Plasmids Laboratory Investigation.

MATERIALS / RESOURCES

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

ASSESSMENT

Successful completion of the Recombinant Paper Plasmids activity.

ACCOMMODATIONS

For reinforcement, the student will

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 and now noticeable.

REFLECTIONS

Recombinant Paper Plasmids

Follow all instructions carefully.

Materials Needed

Plasmid Pattern Sheet
Enzyme Pattern Sheet
Cell DNA Pattern Sheet
Scissors
Scotch tape
Colored map 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.
7. You should now have a plasmid with a gene spliced into it. This is your **RECOMBINANT DNA**.

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

GC	AT	TA	CG	TA	TA
CG	GC	AT	GC	GC	AT
CG	AT	GC	AT	GC	AT
CG	AT	GC	GC	TA	GC
AT	AT	CG	TA	GC	CG
GC	AT	CG	TA	GC	CG
AT	TA	CG	AT	GC	GC
GC	GC	CG	AT	GC	TA
TA	TA	CG	CG	GC	AT
TA	GC	TA	CG	CG	GC
TA	TA	TA	TA	AT	GC
CG	GC	TA	AT	AT	TA
TA	TA	TA	GC	GC	TA
TA	CG	TA	GC	GC	CG
AT	CG	AT	AT	TA	GC
AT	AT	GC	GC	TA	AT
GC	GC	GC	GC	AT	AT
GC	TA	GC	GC	TA	CG
TA	AT	AT	CG	AT	TA
CG	GC	CG	CG	CG	CG
TA	GC	TA	CG	TA	CG



= ampicillin resistance



= tetracycline resistance




= kanamycin resistance



= plasmid replication

Cell DNA

TA	GC	TA	TA	GC	TA
GC	AT	TA	TA	TA	TA
GC	GC	CG	CG	AT	CG
GC	AT	GC	GC	AT	GC
CG	TA	AT	TA	TA	AT
CG	TA	AT	CG	AT	AT
TA	CG	GC	AT	TA	CG
AT	TA	GC	TA	TA	GC
GC	TA	TA	GC	CG	GC
GC	AT	AT	TA	CG	GC
CG	AT	CG	GC	TA	GC
AT	GC	AT	CG	CG	CG
CG	TA	TA	CG	CG	CG
AT	CG	AT	TA	TA	CG
GC	AT	AT	TA	TA	TA
GC	AT	CG	TA	AT	AT
GC	GC	GC	TA	AT	GC
CG	CG	TA	AT	GC	GC
CG	AT	CG	AT	AT	AT
CG	GC	TA	AT	AT	CG
GC	GC	CG	TA	TA	CG
1	2	3	4	5	6

 = protein gene

Enzymes

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

Questions for Review

1. Give a definition of 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.