Modeling Gene Transfer with a Plasmid

PROBLEM

How can plasmids be used to transfer new pieces of DNA into an organism?

BACKGROUND

The first step in genetic engineering is to incorporate a desired gene into a plasmid. The plasmid and gene must be prepared so that they can be joined together to form a new recombinant plasmid. One way to do this is to cut both the gene and the plasmid with restriction enzymes that leave overhanging or so-called *sticky ends*. If the sticky ends of the plasmid and the gene are complementary, they will form hydrogen bonds. The sticky ends can then be joined permanently by ligase enzymes to make a new plasmid containing recombinant DNA. In this activity you will make a model recombinant plasmid by piecing together a paper gene and paper plasmid that have been cut with restriction enzymes.

OBJECTIVES

- Explain the function of a restriction enzyme.
- Model the process of making a recombinant DNA plasmid from a desired gene and the plasmid.

Materials (per group)

scissors
2 pieces of construction paper: yellow and orange
transparent tape

Safety

Be careful when using the scissors.

Procedure

1. Cut a piece of yellow construction paper lengthwise into four equal strips. Tape the strips of paper together to form one long strip of paper.
2. Beginning on the top left edge, as shown in Figure 1, copy the following partial sequence for the pUC19 plasmid onto the strip of yellow paper. Do not leave spaces between the letters. The letters represent nucleotides in DNA. Plasmids usually contain between 5,000 and 10,000 nucleotides. The dots represent the nucleotides that are not shown.

FIGURE 1

pUC19 plasmid sequence:

```
. . . ATGACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTA
   GAGGATCCCCGGGTACCGAGCTCGAATTCACTGGCC . . .
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3. On the same strip of yellow paper, write the sequence for the complementary DNA strand directly under the sequence for the first strand.

   example: ATGACCATGATT . . .

   TACTGGTACTAA . . .

4. Trim any extra paper from the end of the yellow strip, leaving a 1-cm overhang. Tape the two ends together to make a complete circle that represents the pUC19 plasmid, as shown in Figure 2. **NOTE**: *Make sure that the DNA sequence of the plasmid is visible around the top of the circle facing outward. Label the yellow circle plasmid.*

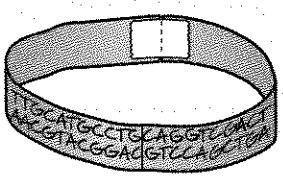

FIGURE 2

5. Using a half-sheet of orange construction paper, cut two strips of paper that are as wide as the yellow strips. Tape the two strips of orange paper together to make one long strip.
6. Copy the sequence for the *lux* gene on the orange strip of paper. The actual *lux* gene makes a chemical that causes an organism to glow in the dark. On the same strip of orange paper, write the sequence for the complementary DNA strand directly under the sequence for the first strand.

   lux gene sequence:

   CACAAGCTTAGCTTAGAGTACCTAGATAAGGCATAGATCTAGCTACGCAA
7. After you write the *lux* gene sequence, trim any excess paper from the ends so that the gene sequence fills the top edge of the paper. Label the orange strip of paper gene.
8. Identify the recognition sites for the following restriction enzymes on the pUC19 plasmid DNA. The solid lines indicate where the restriction-enzyme has made a cut within the recognition sequence. Draw lines on the yellow strip of paper labeled plasmid to show where the plasmid should be cut by the two restriction enzymes listed below. Label each side of the cut site with the name of the restriction enzyme that was used.

   HinD III

   5′— A A G C T T —3′

   3′— T T C G A A —5′

   Bam HI

   5′— G G A T C C —3′

   3′— C C T A G G —5′
9. Identify the recognition sites for the following restriction enzymes on the *lux* gene DNA. Draw lines on the orange strip of paper labeled gene to show where the gene should be cut by the two restriction enzymes listed below. Label each side of the cut site with the name of the restriction enzyme that was used.

   HinD III

   5′— A A G C T T —3′

   3′— T T C G A A —5′

Name_____ Class_____ Date _____

Bgl II

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5'—  A   G   A   T   C   T  —3'
3'—  T   C   T   A   G   A  —5'
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10. Use the scissors to cut the yellow strip of paper that represents the plasmid and the orange strip of paper that represents the gene according to your cut site markings. Be careful to leave the overhang, or sticky ends, intact.
11. Match the complementary sticky ends from the pUC19 plasmid and the *lux* gene to form a new plasmid. Tape the orange and yellow pieces together to represent the bonds that will form between the complementary strands. The result should be a circle of yellow and orange paper. In a real plasmid, the sticky ends are first held together by hydrogen bonds, and then permanent covalent bonds are formed by the ligase enzyme.
12. Tape the yellow and orange strip of paper and the extra pieces of orange and yellow paper to your laboratory recordsheet. Then, answer the questions and problems.

Name_____ Class_____ Date _____

Laboratory Recordsheet 14

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OBSERVATIONS

Fold your model recombinant DNA plasmid so that the inserted **lux** gene is easily visible. Tape your plasmid and extra pieces of DNA to the space below.

PLASMID **EXTRA DNA FRAGMENTS**

ANALYSES AND CONCLUSIONS

1. Explain what a restriction enzyme does.

2. Why are two different restriction enzymes used to cut the pUC19 plasmid and the *lux* gene DNA? What would have happened if only the HinD III enzyme was used?

3a. What does the yellow and orange circle of paper represent?

b. What do the other yellow and orange pieces of paper that you taped to this recordsheet represent?

Name_____ Class_____ Date_____

4. Describe the process you used to insert the gene into the plasmid.

5. The *lux* gene enables some organisms to make a chemical that glows in the dark. How could this gene be used to identify the bacterial colonies on a culture plate that have actually picked up the plasmid?

6. What would be the advantages of inserting into a plasmid a gene with a highly visible phenotype?

7a. From this model, how is plasmid DNA similar to the DNA found in chromosomes?

b. How is plasmid DNA different from the DNA found in chromosomes?