

Recombinant Plasmid Activity Instructions

1. Cut out the Plasmid Base Sequence strips and tape them together into one long strip. The letters should all be in the same direction. Tape the two ends of the long strip together to form a circle - with the letters facing out. THIS IS YOUR PLASMID DNA.

2. Cut out the DNA Base Sequence Strips and tape them together in numerical order. This is your HUMAN DNA, which contains the gene for insulin production. The gene area is shaded.

3. Cut out the Restriction Enzyme Sequence Cards. Each card shows a sequence where a particular restriction enzyme cuts DNA.

4. Compare the sequence of base pairs on an enzyme card with the sequences of the plasmid base pairs. If you find the same sequence of pairs on both the enzyme card and the plasmid strip, mark the location on the plasmid with a pencil, and write the enzyme number in the marked area. Repeat this step for each enzyme card. Some enzyme sequences may not have a corresponding sequence on the plasmid, and that some enzyme sequences may have more than one corresponding sequence on the plasmid. In this step, you are simulating the process of choosing the correct restriction enzyme to recombine your DNA. With hundreds of restriction enzymes available, scientists must determine which one will work for the DNA they want to recombine.

5. Once you have identified all corresponding enzyme sequences on the plasmid, identify those enzymes which cut the plasmid once and only once. Discard any enzymes that cut the plasmid in the shaded plasmid replication sequence. You don't want to cut out this particular gene, because it is necessary for the bacteria to replicate itself. Which enzymes fit this criteria? _____

6. Next, compare the enzymes you chose in step 5 against the cell DNA strip. Find any enzymes that will make two cuts in the DNA, one above the shaded insulin gene sequence and one below the shaded insulin gene sequence. Mark the areas on the DNA strip that each enzyme will cut and make a note of which enzyme cuts in that spot.

7. Select one enzyme to use to make the cuts. The goal is to cut the DNA strand as closely as possible to the insulin gene sequence without cutting into the gene sequence. Make cuts on both the plasmid and the DNA strips. Make the cuts in the staggered fashion indicated by the black line on the enzyme card.

8. Tape the sticky ends (the staggered ends) of the plasmid to the sticky ends of the insulin gene to create their recombinant DNA. In the lab, DNA ligase is used to bind the strands together.

Congratulations! You have successfully created a bacterial cell that contains the human insulin gene. This bacteria will reproduce and create more bacteria with the gene. Bacteria grown in cultures can now mass produce insulin for diabetics.

Discussion Questions

1. Why was it important to find an enzyme that would cut the plasmid at only one site? What could happen if the plasmid were cut at more than one site?
2. Which restriction enzyme did you use? _____
Ask other groups what they used and compare the final transgenic plasmids. Why might there be some of different lengths?
3. Why was it important to discard any enzymes that cut the plasmid at the replication site?
4. Why is it important to cut the plasmid and the human DNA with the same restriction enzyme?
5. Do restriction enzymes exist naturally in organisms? If so, what is their purpose?
6. Why would restriction enzymes that created "blunt" ends not be as useful in recombination as those that create sticky ends?
7. In the activity, you simulated creating a recombinant bacteria organism. For each of the following materials, indicate what they represent?
Scissors _____
Tape _____

DNA BASE SEQUENCE STRIPS

1. Cut out strips along dotted lines.
 2. Tape together top to bottom in numerical order.
- Shaded region = insulin gene site

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

PLASMID BASE SEQUENCE STRIPS

1. Cut out strips along dotted lines.
 2. Tape together top to bottom in any order.
- Shaded region = plasmid replication site

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

RESTRICTION ENZYME SEQUENCE CARDS

1. Cut out cards along dotted lines.
2. Compare each enzyme sequence to the base sequences on the plasmid and DNA strips.

<p> <u>C</u> G C <u>G</u> T A G <u>C</u> G C </p> <p>Enzyme 1</p>	<p> T A <u>T</u> A C G G <u>C</u> A <u>T</u> A T </p> <p>Enzyme 2</p>	<p> C G <u>C</u> G T A A <u>T</u> G <u>C</u> G C </p> <p>Enzyme 3</p>
<p> T A <u>C</u> G T A A T G <u>C</u> A <u>T</u> </p> <p>Enzyme 4</p>	<p> G C <u>G</u> C C <u>G</u> C G </p> <p>Enzyme 5</p>	<p> C G <u>T</u> A T A A T A <u>T</u> G C </p> <p>Enzyme 6</p>
<p> C G <u>T</u> A C G G C A <u>T</u> G C </p> <p>Enzyme 7</p>	<p> G C <u>G</u> C G C C G C <u>G</u> C G </p> <p>Enzyme 8</p>	<p> A T <u>A</u> T C <u>G</u> G C </p> <p>Enzyme 9</p>