Genetic Transformation and Restriction Enzymes

Now that you have setup the transformation lab, let's take a look at what really occurred to make the plasmid that is being used to transform the bacteria.

Materials:

Bacterial DNA on a white sheet of paper
Jellyfish DNA on a green sheet of paper
Ligase enzyme on a small piece of yellow paper
scissors
tape or glue

Procedure:

Partner 1 – Working with Bacterial Plasmid

1. Cut out strip 1 and 2 of the bacterial plasmid DNA.
2. Place strip 2 below strip 1 and overlap and tape the ends together so you have one long strand of DNA.
3. Note that the shaded sequence represents the ampicillin-resistance gene. Ampicillin is an antibiotic.
4. Since plasmids are circular, loop the end of strip 2 back to the beginning of strip 1 and tape the ends together. You should have a loop of bacterial plasmid DNA.
5. Now find the sequence GAATTC in the **RIGHT** hand column of letters. Once you find it draw in with your pencil a line exactly as shown to the right. You should only find this sequence once.

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

6. The restriction enzyme we are using recognizes only this sequence and will cut in the same way you drew your line. The enzyme always cuts between the T & C.
7. Use your scissors to cut along the line you drew in. Make sure your lines are directly in between the T and C on both sides of the DNA strand. If you cut wrong here, you will not be able to insert the jellyfish gene in.

Partner 2 – Working with Jellyfish DNA

1. Cut out strip 1 and 2 of the jellyfish DNA.
2. Place strip 2 below strip 1 and overlap and tape the ends together so you have one long strand of DNA.
3. Note that the shaded sequence represents the glow gene. This is the gene we need to take out of the jellyfish DNA and put into the plasmid.
4. Follow steps 5-7 from Partner 1's procedure. However you need to find this sequence twice. Since we want to cut out the glow gene you should find this sequence before the shaded glow region and after this region.

Both partners

1. You will have 3 pieces of jellyfish DNA after you make the cuts. **MAKE SURE TO ATTACH THE JELLYFISH DNA THAT CONTAINS THE GLOW GENE AS SEEN BY THE SHADED REGION.** The other 2 pieces of jelly fish DNA are scraps and can be thrown away.
2. Now join the bacterial plasmid with the jellyfish DNA that contains the glow gene. Notice that the cuts you made caused there to be single-stranded pieces of DNA. These ends match up with one another according to the base pairing rule. Tape the DNA together so you have one big loop again.
3. Take a piece of ligase and cut it in half. Tape each half over the back of the joined pieces of DNA. This is an enzyme that helps join the two pieces of DNA together. It seals the cuts like a band-aid.

Questions

1. You have succeeded in making recombinant DNA. What must we do with the DNA in order for it to be expressed (get it to glow)?
2. How can we make sure that the plasmid was inserted into the bacteria making the transformation successful?
3.

Bacterial Plasmid DNA (enough for 4 groups)

1		2		1		2		1		2		1		2	
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
G	C	C	G	G	C	C	G	G	C	C	G	G	C	C	G
C	G	A	T	C	G	A	T	C	G	A	T	C	G	A	T
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
C	G	A	T	C	G	A	T	C	G	A	T	C	G	A	T
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
G	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C
G	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
C	G	C	G	C	G	C	G	C	G	C	G	C	G	C	G
C	G	T	A	C	G	T	A	C	G	T	A	C	G	T	A
C	G	C	G	C	G	C	G	C	G	C	G	C	G	C	G
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
A	T	G	C	A	T	G	C	A	T	G	C	A	T	G	C
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
A	T	G	C	A	T	G	C	A	T	G	C	A	T	G	C
T	A	C	G	T	A	C	G	T	A	C	G	T	A	C	G
T	A	C	G	T	A	C	G	T	A	C	G	T	A	C	G
C	G	T	A	C	G	T	A	C	G	T	A	C	G	T	A
T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
T	A	A	T	T	A	A	T	T	A	A	T	T	A	A	T
C	G	T	A	C	G	T	A	C	G	T	A	C	G	T	A
A	T	G	C	A	T	G	C	A	T	G	C	A	T	G	C
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
C	G	A	T	C	G	A	T	C	G	A	T	C	G	A	T
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
T	A	A	T	T	A	A	T	T	A	A	T	T	A	A	T
C	G	T	A	C	G	T	A	C	G	T	A	C	G	T	A
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
A	T	C	G	A	T	C	G	A	T	C	G	A	T	C	G
A	T	C	G	A	T	C	G	A	T	C	G	A	T	C	G
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A

Jellyfish DNA (enough for 4 groups)															
1		2		1		2		1		2		1		2	
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
C	G	G	C	C	G	G	C	C	G	G	C	C	G	G	C
C	G	G	C	C	G	G	C	C	G	G	C	C	G	G	C
C	G	T	A	C	G	T	A	C	G	T	A	C	G	T	A
A	T	G	C	A	T	G	C	A	T	G	C	A	T	G	C
G	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C
A	T	G	C	A	T	G	C	A	T	G	C	A	T	G	C
G	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C
T	A	G	C	T	A	G	C	T	A	G	C	T	A	G	C
T	A	C	G	T	A	C	G	T	A	C	G	T	A	C	G
T	A	A	T	T	A	A	T	T	A	A	T	T	A	A	T
C	G	A	T	C	G	A	T	C	G	A	T	C	G	A	T
T	A	G	C	T	A	G	C	T	A	G	C	T	A	G	C
T	A	G	C	T	A	G	C	T	A	G	C	T	A	G	C
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
T	A	A	T	T	A	A	T	T	A	A	T	T	A	A	T
C	G	C	G	C	G	C	G	C	G	C	G	C	G	C	G
T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T
A	T	G	C	A	T	G	C	A	T	G	C	A	T	G	C
A	T	C	G	A	T	C	G	A	T	C	G	A	T	C	G
A	T	C	G	A	T	C	G	A	T	C	G	A	T	C	G
T	A	G	C	T	A	G	C	T	A	G	C	T	A	G	C
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
T	A	A	T	T	A	A	T	T	A	A	T	T	A	A	T
G	C	G	C	G	C	G	C	G	C	G	C	G	C	G	C
T	A	G	C	T	A	G	C	T	A	G	C	T	A	G	C
G	C	T	A	G	C	T	A	G	C	T	A	G	C	T	A
T	A	T	A	T	A	T	A	T	A	T	A	T	A	T	A
C	G	C	G	C	G	C	G	C	G	C	G	C	G	C	G
C	G	G	C	C	G	G	C	C	G	G	C	C	G	G	C
A	T	A	T	A	T	A	T	A	T	A	T	A	T	A	T
G	C	A	T	G	C	A	T	G	C	A	T	G	C	A	T
T	A	C	G	T	A	C	G	T	A	C	G	T	A	C	G
A	T	T	A	A	T	T	A	A	T	T	A	A	T	T	A
G	C	C	G	G	C	C	G	G	C	C	G	G	C	C	G

DNA Ligase	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**
DNA Ligase	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**
DNA Ligase	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**
DNA Ligase	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**
DNA Ligase	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**	**DNA Ligase**

Each pair of students can get one piece of DNA ligase. They can then cut it in half to place over the joined sticky ends.