MOLEBIO LAB #11: Engineering a Plasmid

Objective:

In this exercise you will use paper to simulate the cloning of a gene from one organism into a bacterial plasmid using a restriction enzyme digest. The plasmid (pUC18 plasmid) can then be used to transform bacteria so that it now expresses a new gene and produces a new protein.

1. From the white paper, cut out the pUC18 plasmid DNA in a long strip.

2. Attach the ends together to make a loop to simulate the circular DNA of a plasmid.

3. From the green paper, cut out the Jellyfish GFP gene DNA in a long strip. Leave it as a straight strip. (This is a gene from an invertebrate not a bacterium, so it is not circular.)

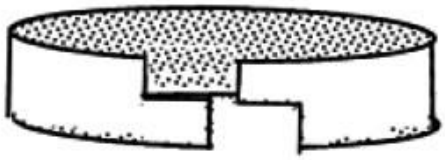
The start and stop sequences for transcribing the Jellyfish GFP gene are highlighted. These are needed to transcribe the gene properly when it is read.

In addition, the *Hin*dIII & *Eco*RI restriction enzyme cutting sites (sequences of bases) are marked in **bold** on the Jellyfish GFP gene DNA. The two restriction enzymes and their respective restriction sites are listed below. These enzymes act as "molecular scissors" to cut the DNA at these sequences in the DNA:

Restriction enzyme	Recognition site (5'→3')
*Hin*dIII	A↓AGCT T T TCGA↑A
*Eco*RI	G↓AATT C C TTAA↑G

The six letter sequence represents the nitrogen base sequence that the enzyme recognizes, and ↑ represents the place where the DNA will be cut by the enzyme. For example, *Hin*dIII cuts between A and A whenever it encounters the six base sequence AAGCTT.

4. Cut the green Jellyfish DNA as if you have used the a restriction enzyme, *Hin*dIII. Be sure to leave "sticky ends.



5. Also, cut the white pUC18 plasmid DNA as if you have preformed a restriction enzyme digest with HindIII. Be sure to leave "sticky ends."

6. Now you will incorporate the green Jellyfish GFP gene into the plasmid. Attach the sticky ends of the Jellyfish GFP gene to the sticky ends of the pUC18 plasmid and seal with "molecular glue", the enzyme ligase (scotch tape will be used in our lab).

7. You have successfully cloned a gene! You now have a single plasmid with a new gene and can use that to transform a single bacterium. The bacterium will now make green Jellyfish glow protein and will glow under black light.

Questions:

1. What is a plasmid?

_____

_____

_____

_____

2. What are restriction enzymes used for in nature?

_____

_____

_____

_____

3. What is meant by a "sticky end"?

_____

_____

_____

_____

4. Why did we cut both segments of DNA with the same restriction enzyme?

_____

_____

_____

_____

5. Why did we make sure to include the start and stop DNA sequences for the Jellyfish GFP gene in our cut segment?

_____

_____

_____

_____

6. What would have happened if we had cut both the Jellyfish GFP gene and pUC18 plasmid with the *Eco*RI restriction enzyme? Be sure to look on the paper DNA sequences to find the *Eco*RI restriction enzyme cut sites.

_____

_____

_____

_____

7. If we want to now produce a lot of this Jellyfish GFP ***protein***, what do we have to do after this first successful splicing to reach our goal?

_____

_____

_____

_____

8. Scientists have successfully transformed bacteria with human genes. Describe one current use of the technology in medicine.

_____

_____

_____

_____

_____

_____

_____

_____

Adapted from a lab developed by Kim Foglia