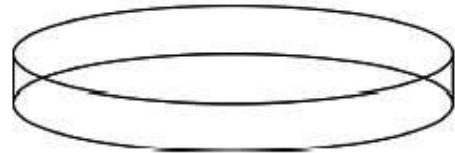


## LAB 13: Engineering a Plasmid

### **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 *Glo* 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* or *Glo* gene are highlighted. These are needed to transcribe the gene properly when it is read.

In addition, the *Hind*III & *Eco*RI restriction enzyme cutting sites (sequences of bases) are marked in **bold** on the Jellyfish *Glo* 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:

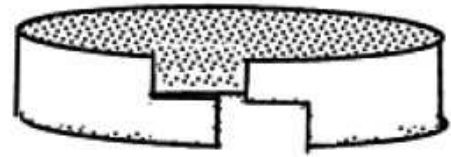
Restriction enzyme	Recognition site (5' → 3')
<i>Hind</i> III	<b>A</b> ↓ <b>AGCT</b> <b>T</b> <b>T</b> <b>TCGA</b> ↑ <b>A</b>
<i>Eco</i> RI	<b>G</b> ↓ <b>AATT</b> <b>C</b> <b>C</b> <b>TTAA</b> ↑ <b>G</b>

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, *Hind*III cuts between A and A whenever it encounters the six base sequence AAGCTT.

Name: \_\_\_\_\_

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

5. Also, cut the white puc18 plasmid DNA as if you have performed a restriction enzyme digest with HindIII. Be sure to leave "sticky ends."



6. Now you will incorporate the green Jellyfish *Glo* gene into the plasmid. Attach the sticky ends of the Jellyfish *Glo* 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?

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2. What are restriction enzymes used for in nature?

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

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Name: \_\_\_\_\_

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

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

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6. What would have happened if we had cut both the Jellyfish *Glo* gene and puc18 plasmid with the EcoR1 restriction enzyme? Be sure to look on the paper DNA sequences to find the EcoR1 restriction enzyme cut sites.

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

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Name: \_\_\_\_\_

8. Scientists have successfully produced green fluorescent mice using this Jellyfish *GFP* gene. What do we now have to do to successfully use our cloned gene to transform mice. Go to the Web site <<http://www.rpc.msoe.edu/cbm2/gfp1.htm>> to see a photo of these transformed mice.

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

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**Adapted from a lab developed by Kim Foglia**