Name _____ **Period** _____

Ms Foglia • AP Biology **Date** _____

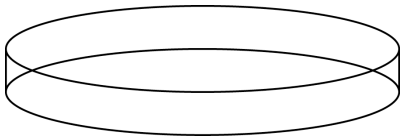
LAB ___: CLONING PAPER PLASMID

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.

AAATCGTTTGC……

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 a vertebrate not a bacterium, so it is not circular.)

GGATCGAAAGC……

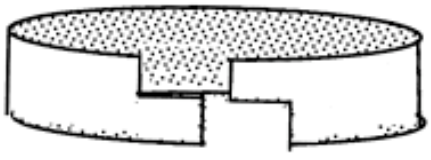
The start and stop sequences for transcribing the Jellyfish *GFP* or *Glo* gene are highlighted. These are needed to transcribe the gene properly when it is read.

In addition, the HindIII & EcoR1 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')
***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, HindIII 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, 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 *GIo* gene into the plasmid. Attach the sticky ends of the Jellyfish *GIo* 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 <u>start</u> and <u>stop</u> DNA sequences for the Jellyfish *Glo* gene in our cut segment?

_____

_____

_____

_____

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.

_____

_____

_____

_____

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?

_____

_____

_____

_____

_____

_____

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.

_____

_____

_____

_____

_____

_____

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

_____

_____

_____

_____

_____

_____

PRINT ON WHITE PAPER

Plasmid (puc18) DNA sequence

5′ 3′

GAATCCGAAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGC**AAGCTT**GGCTACCGTGTACCTG

CTTAGGCTTCGAGCCATGGGCCCCTAGGAGATCTCAGCTGGACGTCCGTACG**TTCGAA**CCGATGGCACATGGAC

Plasmid (puc18) DNA sequence

5′ 3′

GAATCCGAAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGC**AAGCTT**GGCTACCGTGTACCTG

CTTAGGCTTCGAGCCATGGGCCCCTAGGAGATCTCAGCTGGACGTCCGTACG**TTCGAA**CCGATGGCACATGGAC

Plasmid (puc18) DNA sequence

5′ 3′

GAATCCGAAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGC**AAGCTT**GGCTACCGTGTACCTG

CTTAGGCTTCGAGCCATGGGCCCCTAGGAGATCTCAGCTGGACGTCCGTACG**TTCGAA**CCGATGGCACATGGAC

PRINT ON GREEN PAPER

Chromosomal DNA (GFP gene) from Jellyfish: HindIII & EcoR1 restriction sites are marked in bold

5′ 3′

GTGCGCG**AAGCTT**CCTTACTCCAGAGC**GAATTC**TCTGGTCATTTTCTAGGCTATATACTTCTA**AAGCTT**TTCTG

CACGCGC**TTCGAA**GGAATGAGGTCTCG**CTTAAG**AGACCAGTAAAAGATCCGATATATGAAGAT**TTCGAA**AAGAC

GFP gene

Chromosomal DNA (GFP gene) from Jellyfish: HindIII & EcoR1 restriction sites are marked in bold

5′ 3′

GTGCGCG**AAGCTT**CCTTACTCCAGAGC**GAATTC**TCTGGTCATTTTCTAGGCTATATACTTCTA**AAGCTT**TTCTG

CACGCGC**TTCGAA**GGAATGAGGTCTCG**CTTAAG**AGACCAGTAAAAGATCCGATATATGAAGAT**TTCGAA**AAGAC

GFP gene

Chromosomal DNA (GFP gene) from Jellyfish: HindIII & EcoR1 restriction sites are marked in bold

5′ 3′

GTGCGCG**AAGCTT**CCTTACTCCAGAGC**GAATTC**TCTGGTCATTTTCTAGGCTATATACTTCTA**AAGCTT**TTCTG

CACGCGC**TTCGAA**GGAATGAGGTCTCG**CTTAAG**AGACCAGTAAAAGATCCGATATATGAAGAT**TTCGAA**AAGAC

GFP gene