

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

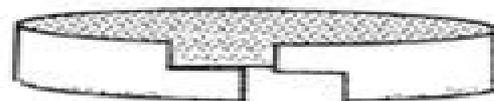
The start and stop sequences for transcribing the Jellyfish *GFP* or *Glo* gene are highlighted. These are needed to transcribe the gene properly when it is read.

In addition, the *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	A ↓AGCT T T TCGA↑ A
<i>Eco</i> 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, *Hind*III 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, *Hind*III. Be sure to leave "sticky ends."
5. Also, cut the white *puc18* plasmid DNA as if you have performed a restriction enzyme digest with *Hind*III. Be sure to leave "sticky ends."



Biology Lab Cloning Paper Plasmid Answer Deflor

Judith W. Zyskind, Sanford I. Bernstein

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E. coli Plasmid Vectors Nicola Casali, Andrew Preston, 2008-02-03 A comprehensive collection of readily reproducible techniques for the manipulation of recombinant plasmids using the bacterial host E coli The authors describe proven methods for cloning DNA into plasmid vectors transforming plasmids into E coli and analyzing recombinant clones They also include protocols for the construction and screening of libraries as well as specific techniques for specialized cloning vehicles such as cosmids bacterial artificial chromosomes l vectors and phagemids Common downstream applications such as mutagenesis of plasmids recombinant protein expression and the use of reporter genes are also described

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