

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*R1 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> R1	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 Answers

B Lingard



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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 **Plasmid**

Munazza Gull, 2019-06-19 This book captures in a single volume the wealth of information on the plasmid structure function and biology of all organisms that have been examined to date Plasmids exhibit wide variations in size modes of replication and transmission host ranges and the genes they carry and have provided us with a great understanding of basic life principles at the molecular level Written by experts in the field this book is a valuable source of up to date information delivering the latest impacts on studies in the areas of plasmid types genomes purification analysis and expression of recombinant proteins in bacteria Plasmid utilization in the synthesis of plasmid based vaccines plasmids as genetic tools and their applications in ecology and the evolutionary process are also covered This book is a single source of valuable information for instructors and students in advanced undergraduate and graduate courses on plasmids It will also appeal to researchers seeking to find new relationships between biological processes that are linked by plasmids to the environment

Plasmids Kimber G. Hardy, 1987

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