

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 (a small plasmid) can then be used to transform bacteria so that it can express a new gene and produce a new protein.

1. From the white paper, cut out the puc18 plasmid DNA in a long strip.

AAATCGTTTCG-----



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

GCATCGAAGC-----

The atg and gag sequences for transcribing the Jellyfish GFP or Gfp gene are highlighted. These are needed to transcribe the gene properly when it is read. In addition, the HindIII & EcoRI restriction enzymes 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')
HindIII	A AGCT T T TCGAT A
EcoRI	G AATT C C TTAA G

The six-letter sequence represents the nitrogen base sequence that the enzyme recognizes, and T 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. Examine the DNA sequence for the plasmid and the jellyfish gene. Which restriction enzyme should you use to cut the plasmid? the jellyfish gene? Remember, when you cut each gene, you need to retain the start and stop sequences. Should you use the same restriction enzyme to cut both the plasmid and the jellyfish gene? Why or why not?

5. 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).

6. 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 UV light.

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