

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

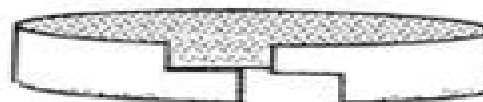
The start and stop sequences for transcribing the Jellyfish *GFP* or *Glo* gene are highlighted. These are needed to transcribe the gene properly when it is read.

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."



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