

Cloning Paper Plasmid DNA Using Restriction Enzyme

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:

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Clemens Wendtner



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