Generation of plasmid vectors for fluorescence reporter assay

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Abstract

Plasmids are double stranded DNA that are distinct from the typically chromosomal DNA found in cells. Plasmids are able to provide any living organism with genetic advantages which can include antibiotic resistance, virulence, and metal tolerance which makes it important to investigate since the evolutionary process of plasmids are not completely understood.

pcDNA3 RLUC POLIRES FLUC is a bicistronic reporter plasmid that expresses renilla luciferase and firefly luciferase which are both bioluminescence reporter genes. These reporter genes when excited by light at a certain wavelength will emit light back.

However when inserting a foreign DNA sequence into *pcDNA3 RLUC POLIRES FLUC* the emission from the reporter genes can be increased or decreased. In this project the utilization of PCR on plasmid *pcDNA3 RLUC POLIRES FLUC* allows for the observation on the effect of sequence *OCleader1-25* has on the light emission from the reporter genes.

Objective

- The objective of this project is to insert the new gene code into plasmid *pcDNA3 RLUC POLIRES FLUC* in between the T7 promoter region and renilla luciferase reporter gene to observe whether the inserted gene enhances or limits the reporter genes light emission.

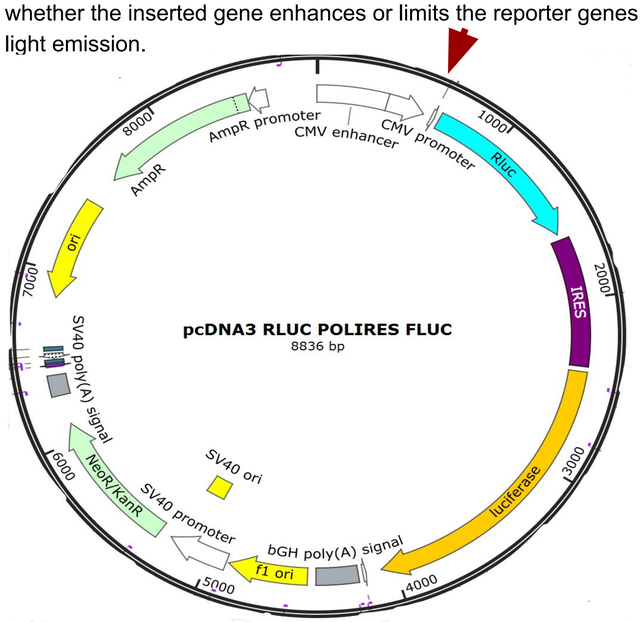
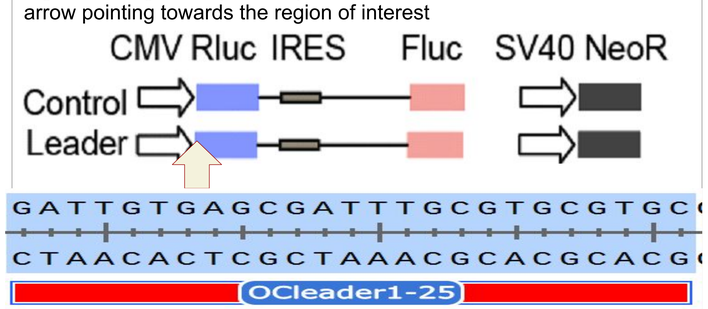


Figure 1: Diagram of plasmid *pcDNA3 RLUC POLIRES FLUC* with an arrow pointing towards the region of interest



PCR Cloning Method

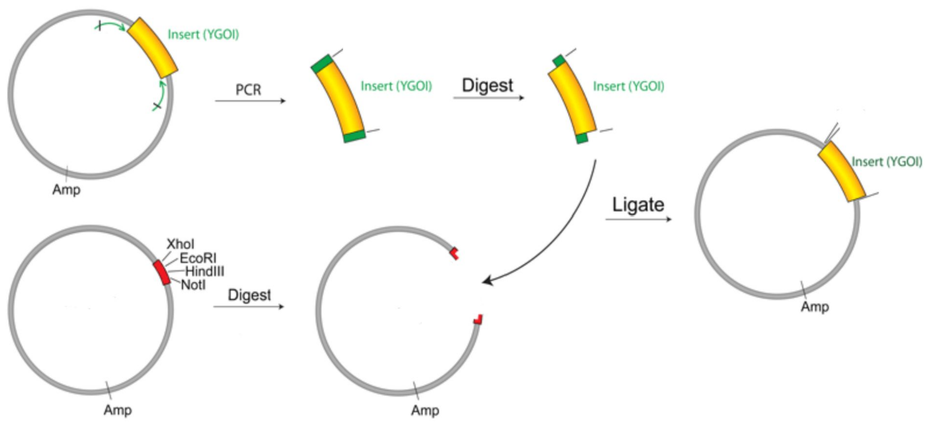


Image 2: This is the whole process of the PCR method. Start by digesting the gene of interest with enzymes and as well as using enzymes to digest the plasmid that you want to insert the gene within.Then ligate the two together and you now have the gene in the plasmid

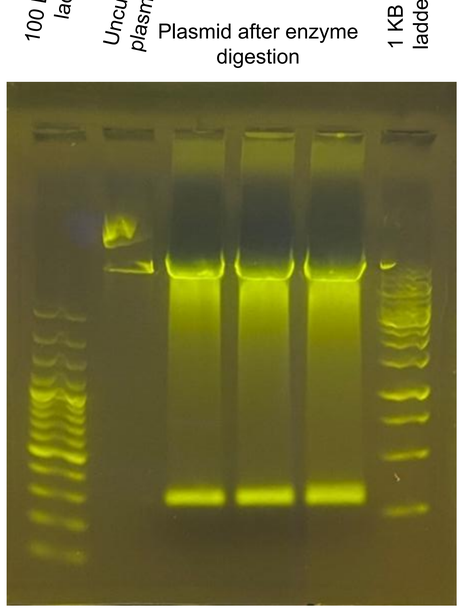


Image 4:Shows the Verification process of the plasmid after being digested by enzymes in preparation of ligation

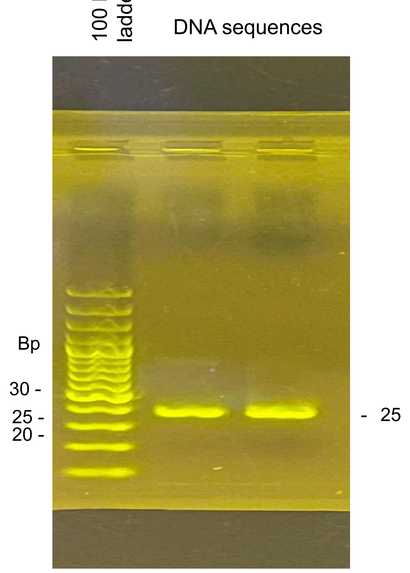
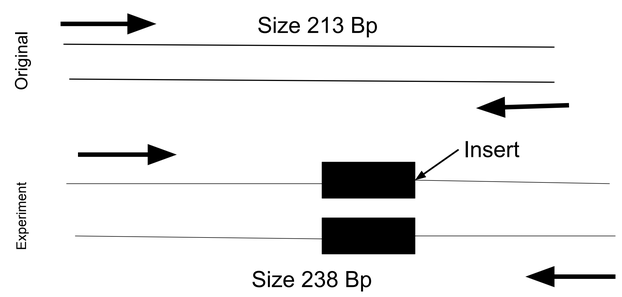
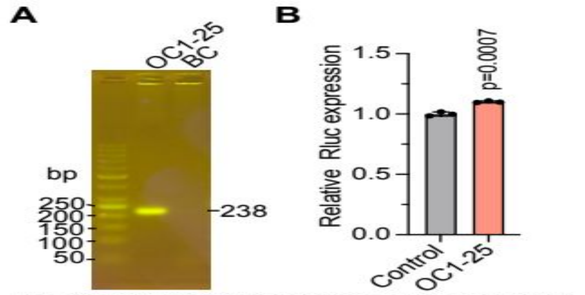


Image 5:Shows the size verification of the sequence that we will be inserting. The DNA ladder helps us see how much base pairs the sequences are.

Results





(A) Colony PCR detected an expected band with size of 238 bp.OC1-25: OC43 leader sequence 1-25 bp. BC: blank control, using water as PCR template.
(B) Fluorescence reporter assay detected a slight increase in relative Rluc expression, indicating potential positive effect of OC1-25 on translation. Control: original plasmid without insert sequence. OC1-25: generated plasmid inserted with OC43 leader sequence 1-25 bp.

Summary

- We have successfully generated a fluorescence reporter plasmid with the insertion of the interested OC43 leader sequence 1-25 bp prior to the translation site of Renilla luciferase.The fluorescence reporter assay showed that the insertion of OC43 leader sequence 1-25 bp slightly increased the expression level of Renilla luciferase.

Acknowledgements

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- Thank you to Kongpan Li and Jennifer Galvez to assisting me in my work within the lab. Your help was greatly appreciated.

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