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Vitreoscilla Globin Promoter Cloning and Testing in *Escherichia coli*

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VITREOSCILLA GLOBIN PROMOTER CLONING AND TESTING IN *ESCHERICHIA COLI*

Abstract

In industrial fermenters, mixing limitations can cause variations in dissolved oxygen levels. This can lead to oxygen limitations for bacterial cells, such as *E. coli*, producing organic acid byproducts and reducing desired product yield.

My project goal was to develop an oxygen-sensitive promoter system capable of sensing these oxygen-limited zones and, in turn, reducing the expression of enzymes that cause byproduct formation.

I assembled a plasmid containing the *Vitreoscilla* globin (vgb) promoter, green fluorescent protein (GFP), and a backbone. I then transformed competent *E. coli* cells with the plasmid and performed GFP expression testing in oxygen-sufficient and -limited zones.

Objectives

- **Develop an oxygen-sensitive promoter system.**
- **Monitor GFP expression levels under aerobic and micro-aerobic conditions.**

Plasmid Structure and Function

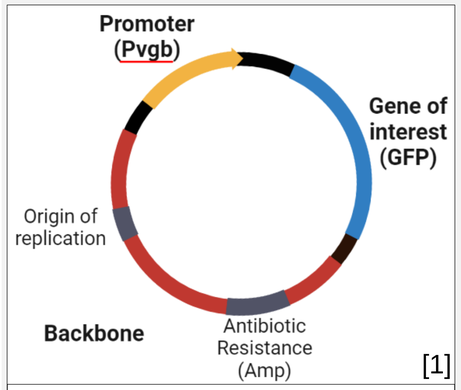

Figure 1: Experimental Plasmid Map

A plasmid is a circular DNA strand in the cytoplasm of a bacterial cell, in this case, *E. coli*.

As displayed in Figure 1, the experimental plasmid is made up of three distinct DNA segments: the vgb promoter, GFP, and backbone. The black areas represent the overlap between two of these segments.

A promoter is a DNA sequence where proteins can bind to initiate the expression of a specific gene, in this case, GFP. In this experiment, the vgb promoter is activated under oxygen-limited environments, which causes the *E. coli* cells to express GFP. The protein then accumulates and causes the cells to fluoresce green under UV light. A higher GFP expression level results in a higher fluorescence intensity.

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Method of Cloning and Assembly

Step 1: DNA templates used to create fragment copies.

Table 1. Primers and Replication Tools used for PCR

Segment	Forward Primer	Reverse Primer	Replication Tool
P_{vgb} Promoter	RK2	RK3	Q5 Polymerase
GFP	RK6	JA9	repliQa HiFi ToughMix
Backbone	JA10	JA11	

Step 2: DNA fragments assembled into plasmid.

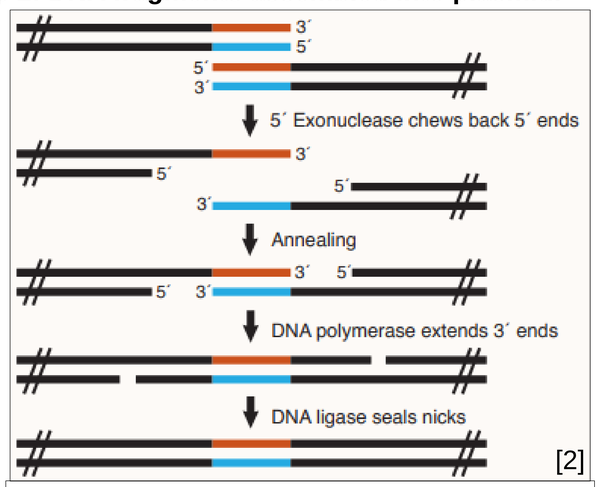

Figure 2. NEBuilder HiFi DNA Assembly Method

Results

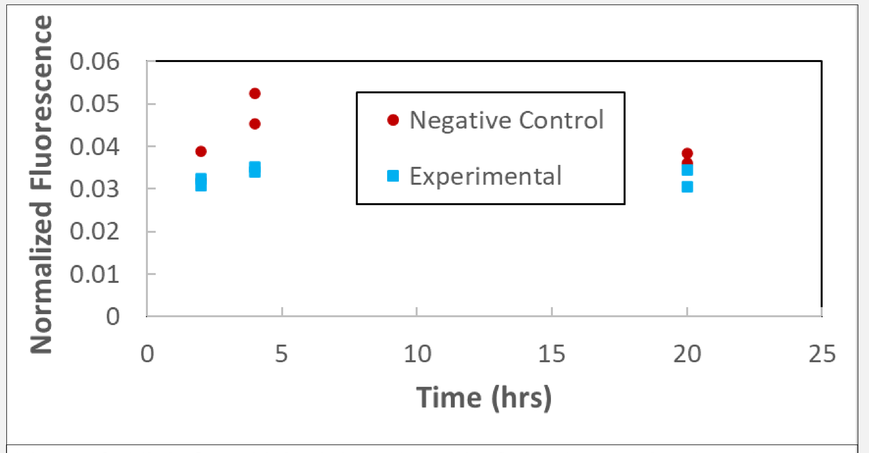

Figure 3: High Sensitivity Micro-Aerobic GFP Expression Levels

At a high sensitivity setting on the fluorescence plate reader, the experimental sample did not behave as desired. If the results were successful, one should have witnessed increasing GFP expression levels over the sample times for the experimental sample.

Conclusions and Future Work

The experimental plasmid was successfully assembled, but GFP expression testing results did not display sufficient GFP accumulation in a micro-aerobic environment.

In the future, the vgb promoter could be doubled to enhance its expression level. Additionally, various mutations of the vgb sequence could be created using a mixed PCR product. [3]

References

[1] https://app.biorender.com/illustrations

[2] https://www.neb.com/protocols/2014/11/26/nebuilder-hifi-dna-assembly-reaction-protocol

[3] http://parts.igem.org/Part:BBa_K561001

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