Integrating T7 RNA Polymerase Mutants into Biological Pathways

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Abstract

Wildtype T7 RNA Polymerase, being such an active, specific, orthogonal, and popularly used polymerase, is an ideal tool for gene expression in synthetic biology. Thus, building off the prior work done at the University of Texas to create a panel of T7 polymerase mutants, we are currently working on integrating these newly developed RNA polymerase variants into biological pathways (Meyer, Ellefson, Ellington 2015). The primary system of focus in this research is the Chorismate to p-AS pathway. With the use of additional polymerases, the control of this pathway can be improved. By transforming cells with plasmids containing variant T7 promoter sequences and reporter genes, the effectiveness of this new panel of polymerases can be ascertained.

Introduction

The use of polymerase/promoter pairs is essential to gene expression within biological pathways. T7 is one polymerase often used due to its high level of activity, specificity, and independences from wild-type T7.. There is a interest in utilizing mutated T7 RNAP variety in the Chorismate pathway in order to create a feedback loop and different channels of control that are independent from each other.

Methodology

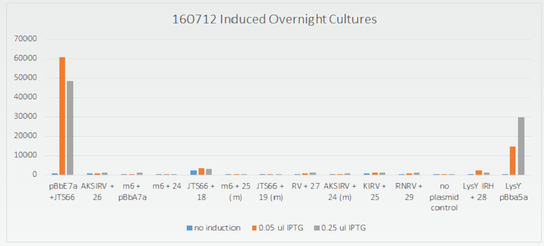
- Plasmids containing T7 promoters and RFP/GFP reporters constructed using CPEC protocol Mini-F plasmids removed from T7 express cells using mini-prep protocol (Qiagen)
- Mini-F plasmids transformed using commercial cells
- Transformed cells made competent
- Cells transformed with plasmids containing promoter and reporter sequences
- Cells inoculated and induced
- RFP/GFP signal measured with 96 well plate reader (Carothers Lab Protocol)

Results

Three cell-types have been deemed unsuccessful for these purposes;

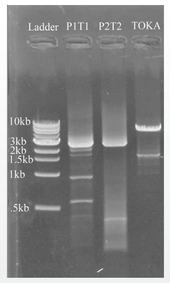
- Agilent's strain of BL21 due to its sensitivity to the T1 virus,
- New England Biolabs' BL21 without and with a lysozyme gene, due to its interference with polymerase-reporter communication.

Results



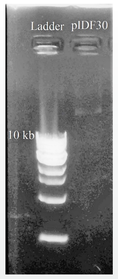
Fluorescence of Induced Overnight Cultures using BL21

Using T7 Express, 13 plasmids were transformed. Each plasmid contains a unique combination of T7 promoter and reporter. In the initial attempt to assemble a plasmid with two T7 promoters, the six fragments did not bind. In the second round, segments were assembled in parts.

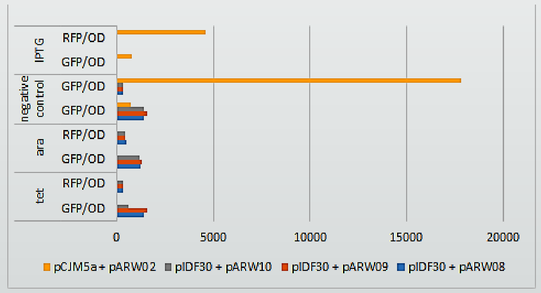


Left: Gel displaying pIDF30 fragments

Right: Gel displaying completed pIDF30



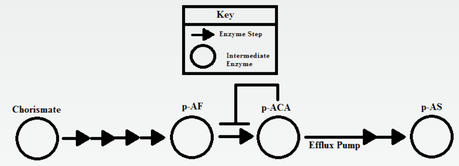
As of now, the double transformed cells are not fluorescing as expected.



Fluorescence of Induced Overnight Cultures using T7 Express Cells

Discussion

Designing a feedback loop into the Chorismate pathway is an application of this research.



Chorismate to p-AS Pathway

To preserve the health of the cell and the success of the pathway, a feedback loop must be incorporated by using an additional polymerase/promoter pair. This polymerase will express an inhibitor gene, which when triggered by the presence of p-ACA will inhibit the production of the enzyme just before the p-ACA step.

Acknowledgments

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Works Cited

Meyer, Adam J., Jared W. Ellefson, and Andrew D. Ellington. "Directed Evolution of a Panel of Orthogonal T7 RNA Polymerase Variants for *in vivo* or *in vitro* Synthetic Circuitry." *ACS Synth. Biol. ACS Synthetic Biology* 4.10 (2015): 1070-076.