COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Honors Capstone Research (HCR) Summer Program 2020

The Regulatory Role of *tnaC* in the Universal Expression Regulation of RF2-Dependent Regulons

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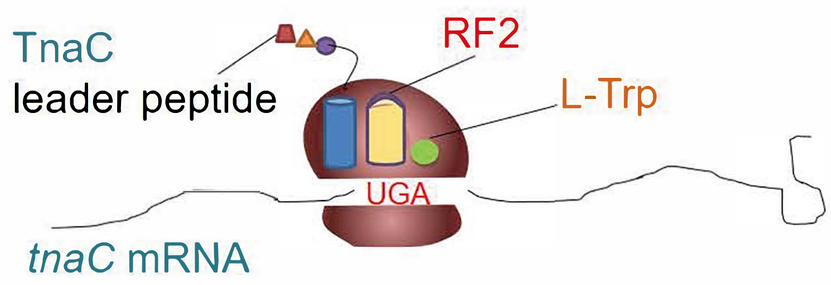
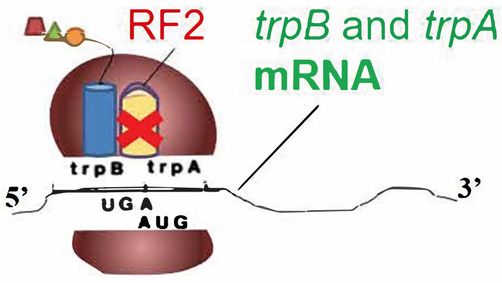
Project Overview

The concentration of the amino acid L-Tryptophan (L-Trp) is regulated by the *tna* operon and the *trp* operon in *Escherichia coli (E. coli)*. In high L-Trp concentration, the translation of the *tna* operon's *tnaC* gene is attenuated as free L-Trp molecules bind the ribosome translating this gene, stalling it at the stop codon UGA of *tnaC*. Simultaneously, release factor 2 (RF2) is thought to be sequestered within the TnaC-ribosome complex. The project aims to investigate how the different expressions of the *tnaC* gene affects the availability of RF2 as well as the expression of a specific RF2-dependent gene system in *E. coli.* It is expected that a universal depletion of RF2 as well as a lower expression of the *trp* operon's *trpB* and *trpA* gene system would occur in high L-Trp concentration.



L-Trp **stalls** the translating ribosome at the stop codon UGA, hypothetically **sequestering RF2** within the complex



Translation **termination** of *trpB* is slowed due to a **lack of RF2**

Methods

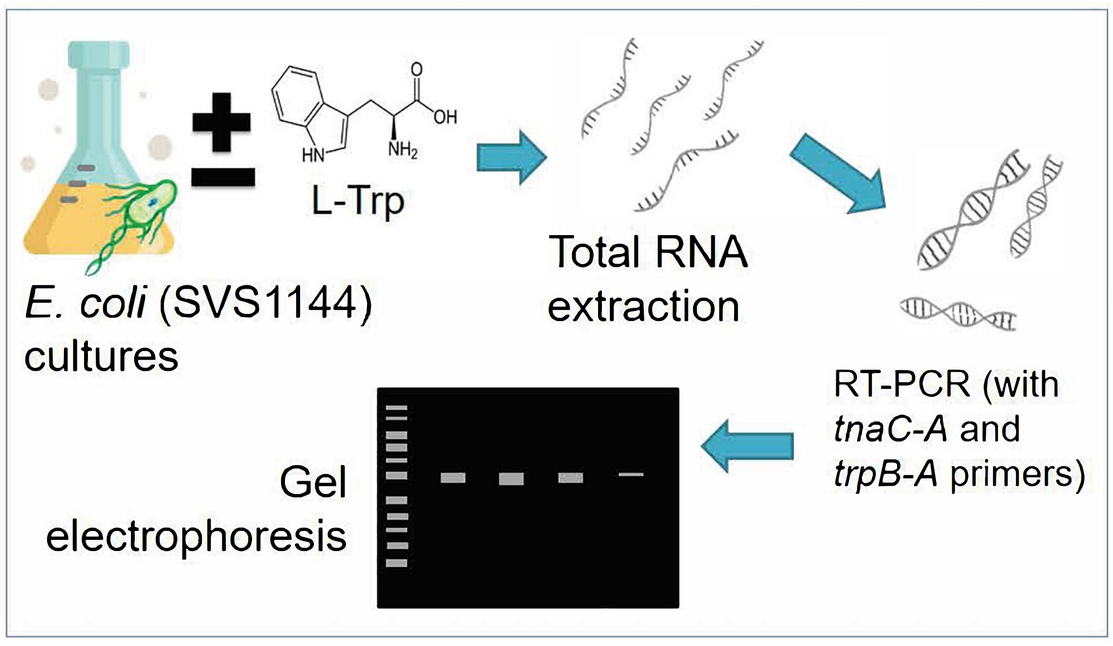


Figure 1. Experimental scheme for an investigation of the transcriptional expression regulation of *tnaC-A* and *trpB-A* gene system in conditions with and without L-Trp (100 µg/mL)

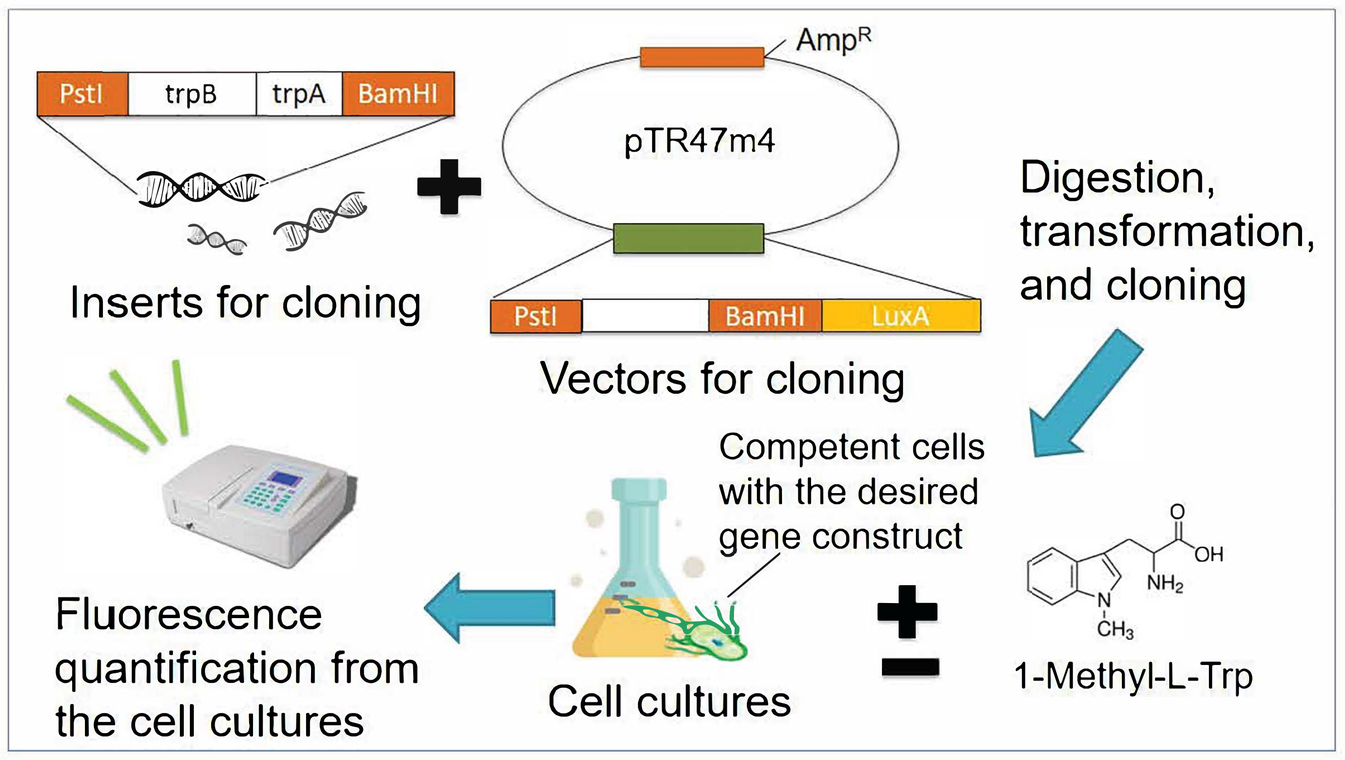
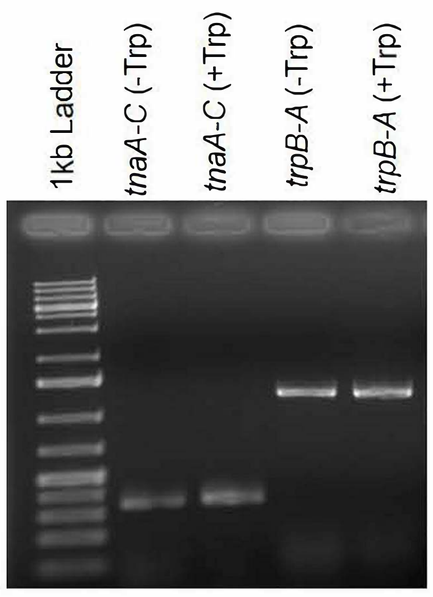


Figure 2. Experimental scheme for an investigation of the translational expression regulation of *trpB-A* gene system in conditions with and without 1-Methyl-L-Trp (100 µg/mL). The expression is determined by the quantification of the fluorescence produced by luciferase

Results

Figure 3. RT-PCR samples on 1.5% agarose gel. The change in L-Trp concentration does not seem to induce a change in the amount of mRNA transcripts from the *trpB-A* gene system



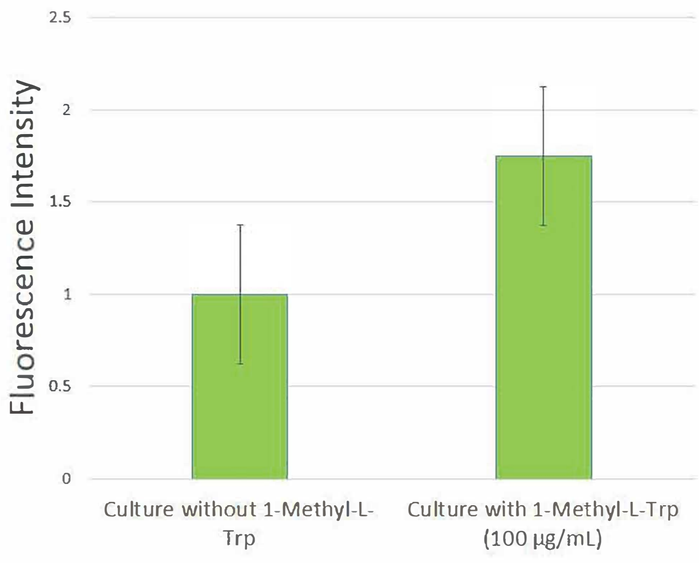


Figure 4. Predicted results for fluorescence quantification. It is expected to see more fluorescence produced in the culture with 1-Methyl-L-Trp

Future Plans

- Carry out the transformation and fluorescence quantification to confirm the hypothesis
- Extend the experiment to other similar gene systems to evaluate the effect of *tnaC* expression on the expression regulation of RF2-dependent gene systems

Acknowledgements

Sincerest thanks to Mohamed Ghazal, and other members in professor Cruz-Vera's lab for their helpful guidance on the experiments. Special thanks to Mr. Cook and UAH Honors College for funding the project.

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