Developing a Bacterial Model for Turing Pattern Emergence with Quorum Sensing Circuitry

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Motivation

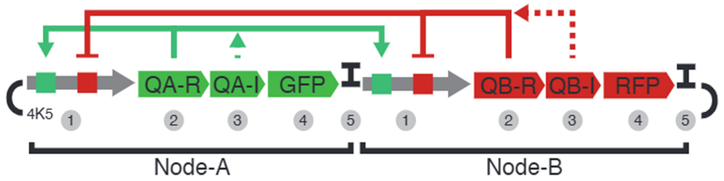
This research investigates how quorum sensing (QS) systems can drive Turing pattern formation by examining interactions between the Lux, Las, and Rpa QS systems in *E. coli*. By analyzing QS crosstalk, sensitivity, and expression dynamics, we aim to understand how two QS nodes interact to generate spatial gene expression patterns. Fluorescent reporters GFP and mCherry will quantify these patterns, informing the design of a two-operon system that mimics traditional Turing models. This work could enhance synthetic multicellular coordination, aid in engineering bacterial behaviors, and provide insights into natural biological patterning.

Objectives

- Engineer a two-node quorum sensing system in *E. coli* using Lux, Las, and Rpa systems
- Investigate crosstalk, sensitivity, and dynamic expression within and between nodes
- Quantify spatial expression using fluorescent reporters (GFP and mCherry)
- Design a synthetic gene circuit that recapitulates activator-inhibitor dynamics predicted by Turing models

Circuit Topology

** Activator A is slow, short range*
*Inhibitor B is quick, long range**

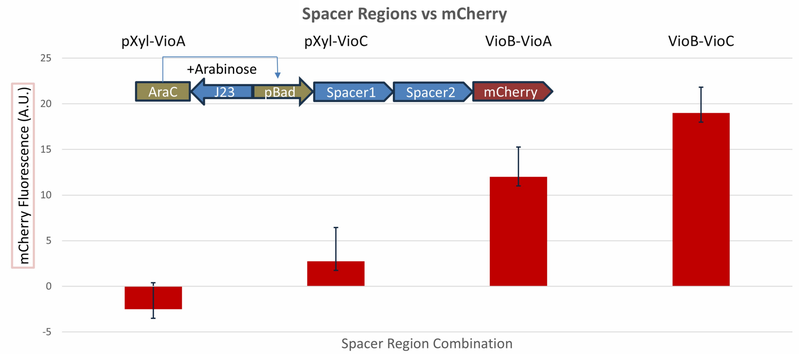


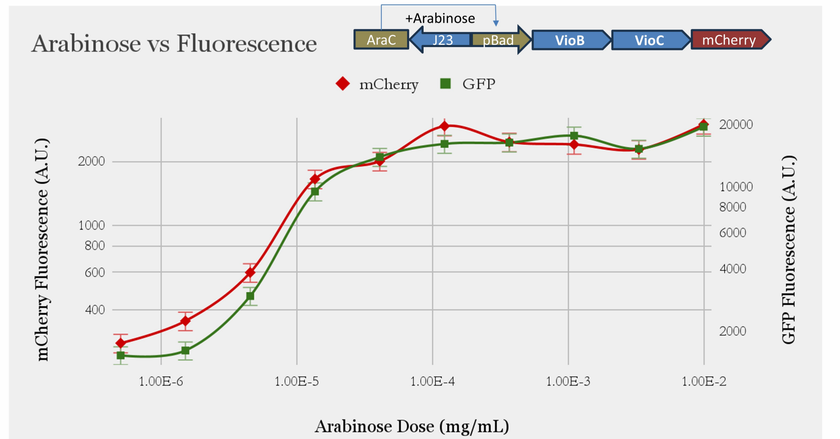
$$\frac{dA}{dt} = V_A \cdot \frac{\left(\frac{A}{k_{AA}}\right)^n}{1+\left(\frac{A}{k_{AA}}\right)^n+\left(\frac{B}{k_{BA}}\right)^n} + b_A - \mu_A A \qquad \frac{dB}{dt} = V_B \cdot \frac{\left(\frac{A}{k_{AB}}\right)^n}{1+\left(\frac{A}{k_{AB}}\right)^n+\left(\frac{B}{k_{BB}}\right)^n} + b_B - \mu_B B$$

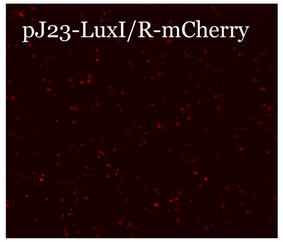
Adapted from Scholes et al., 2019

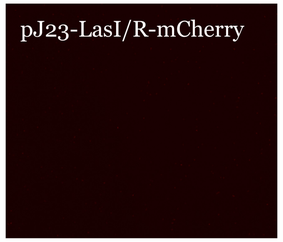
V : max regulated production rate
b : basal expression (leakage)
μ : degradation/dilution coefficient
k : Hill constants
n : cooperativity

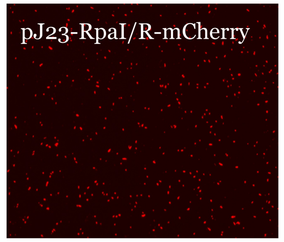
Results











Discussion and Future Work

The early findings indicate that certain spacer regions can act as premature terminators and inhibit operon expression. This consideration has been integrated into future designs, as indicated by select figures in Results.

Initial results show distinct differences in expression based on spacer sequences and QS systems. The Lux and Rpa systems display comparable interaction and toxicity profiles, with tunable sensitivity to arabinose. Using two comparable pathways will be beneficial for optimizing the circuitry. Taking this into consideration, going forward, we will:

- Grow engineered *E. coli* colonies on agar plates to observe diffusion-driven pattern formation
  - *This will allow us to engineer diffusive molecules with tunable diffusion rates/coefficients*
- Develop models to assess toxicity of QS gene parts under inducible conditions
- Mix and match QS synthases, transcription factors, and promoters to assess cross talk and feed future orthogonal designs
  - *Developing a model to assess orthogonality using error-prone PCR and library screening will help aide in developing parts that can bind with high specificity*
- *Tune RBS and plasmid copy numbers to modulate activator/inhibitor intensities*

Ultimately, these future directions aim to precisely navigate the design space where reaction-diffusion systems can emerge from living circuits. Achieving this will provide a biological foundation for synthetic pattern formation and pave the way toward more complex multicellular coordination in engineered bacterial systems, with the goal of eventually expanding toward Eukaryotic systems.

Acknowledgements

We thank Dr. Xiao Wang, Dr. Kylie Standage-Beier,
Dr. Samat Bayakhmetov, and Mahan Naseri for research guidance.
This work was financially supported by NIH grant EB031893

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