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MURI Pattern Detection Circuits

Project Overview

This project seeks to engineer *E. coli* bacteria to possess pattern recognition capabilities for swarming Micro-Bio-Robots. The bacteria utilize quorum sensing to detect the size and configuration of patterns. For this project, UV radiation exposure onto lawns of bacteria serves as the pattern input. The organisms are engineered to exhibit near digital behavior for ranges of UV radiation sizes, setting forbidden band thresholds. For the circuit operation of our Null versus 1 system, the initial state of toggle set for CI production with IPTG. Later, UV induces state change for a region by cleaving CI. If the region is large enough to achieve an engineering 30C12HSL quorum, the quorum is amplified to the entire region and reported by a blue fluorescent protein. RBS's following promoters are tunable, thus controlling the strength of gene expression. Several variants of the Null versus 1 system exist, but all comprise of a Collins' toggle switch, quorum sensor, and quorum propagator. These variants may introduce additional system behaviors with additional cascades (shifting of the system's transfer curve), inhibitors (lactonase), or positive feedback. The construction of the Null versus 1 system would represent a substantial achievement in pattern detection through quorum processing, and would advance the construction of more advanced Null versus 1 versus 2 systems with both 30C12HSL and C4HSL to facilitate the detection of different pattern sizes above the null threshold.

Personal Role & Responsibilities

Currently, Gibson primers for the construction of the Null versus 1 system have been designed and vetted. An alternative, possibly more efficient construction method (recommended by Dr. Jon Babb) is being investigated. This method calls for the PCR of the full system circuit from the existing K415069. The sequencing of K415069 and the PCRing of its constituent parts will be the first task, followed by its Gibson assembly into our desired system. Once sequenced, the system circuit will undergo characterization experiments with modulating UV exposure levels and 30C6HSL concentrations.

- A more detailed workflow follows, without yet incorporating the K415069 construction method (will be detailed soon):

1. Design LuxR System Primers

-Satisfy T.anneal and other parameters.
-LuxR & LuxI Considerations
    -Design weakened RBS for Propagator LuxR
-Verify via annotations
-Check with Ron the effects of surrounding 50bp on RBS behavior

2. Order pLasORI and pRhiORI oligos.

-Check sequence with Ron and Jon

3. pLuxORI Construction

A. Anneal pLuxORI oligo & restriction digest ES

B. Restriction digest J06702 EX

C. Gel extract J06702

D. Ligate. Call this part J85012

E. Co-transform with S03119 (Ptet-RBS-LuxR)

4. pLuxORI Characterization

A. +/- AHL to detect leakiness level (no LuxR in system)

-~130 uM AHL. FACS analysis.

B. +/-AHL with S03119/J85012 (LuxR now in system)

-~130 uM AHL. FACS analysis.

C. AHL concentration modulations. May provide clues to necessary UV circle sizes.

-50, 250, 400, 600 uM AHL.

5. Lux System Construction

A. Gibson Assembly

-PCR QS: pLuxORI, LuxI, LuxR, J06702; and Prop: pLux, LuxI, LuxR, E0422 (CFP+LVA)

-Gibson reaction. QS and Prop in parallel, separate plasmids. QS in high copy # backbone.

-Biobrick assemble QS with pLPTa

-Transformations.

6. LuxR QS+Prop Characterization

A. +/- AHL to detect leakiness level (no LuxR in system)

-~130 uM AHL. FACS analysis.

B. +/-UV +/-AHL with S03119/J85012 (LuxR now in system)

-~130 uM AHL. FACS analysis.

-UV ~40 J/m^2

C. UV intensity and mask size modulations

-Mask sizes inferred from earlier AHL modulation experiments.

-Smallest possible to nearly entire plate of cells.

-UV modulation: 0, 5, 10, 20, 40, 60, 80

7. Repeat steps 5&6 for Rhi and Las systems, as well as promoter characterizatios (Steps 3-4)

<u>Goals</u>

This project holds tremendous promise as a foundational advance for various fields from synthetic biology to biomimetic materials engineering. I hope that work through this and upcoming semesters will result in a high-impact publication or patent. On a personal level, I aim to develop an analytical mindset, effective communication skills, and a strong network of collaborators.

Personal Statement

The novelty of utilizing synthetic biology as a tool to recognize patterns fascinates me. I am convinced this project lies in the forefront of an emerging branch of synthetic biology, where traditional materials are replaced by responsive, adaptable materials. I hope to develop into a better scientist, gaining vital skills in project coordination, collaboration, and time management. As a mechanical engineer, this project offers a wonderful opportunity to expand my intellectual horizons and investigate the rapidly progressing fields of biologically engineered systems.