Examining the natural diversity of quorum sensing for orthogonal pathways

Jiaqi Wu, Computer Science
Mentor: Dr. Karmella Haynes, Assistant Professor
School of Biological and Health Systems Engineering

Background

Abstract: Homoserine lactone (HSL) quorum sensing (QS) is a bacterial cell-to-cell communication method. Bioengineers incorporate QS into genetic circuits by using the bacteria's signaling pathway as a "bio-wire". Complex genetic circuitry is inhibited by pathway overlap (crosstalk) and lack of pathways (4% have been used in synthetic systems to date). To test pathways for orthogonality (lack of crosstalk), a decoupled system was designed using a sender cell, which carries the HSL synthase, and receiver cell, which carries the QS promoter and regulator. The research will expand the synthetic biology toolbox and increase flexibility when building complex gene circuitry.

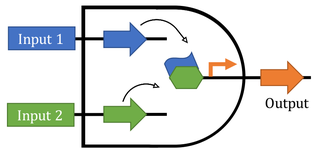


Figure 1 | An illustration of a simple genetic circuit using quorum sensing. Practical applications include smart medicines and drug discovery.

The problem

Figure 2a | In an orthogonal system, the synthase produces an HSL molecule that activates the corresponding receiver's inducible promoter.

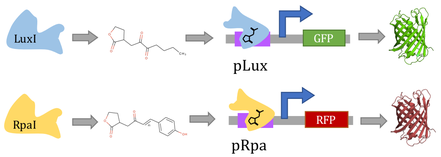
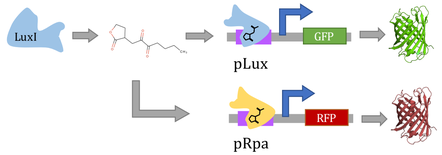


Figure 2b | When crosstalk occurs, the synthase from one system activates the inducible promoter from multiple systems, leading to unintended output when implemented in a biological circuit.



Materials & Methods

Figure 3 | System used to discover orthogonal quorum sensing components. Sender and receiver expression plasmids were constructed from BioBrick parts. Synthases, regulators, and regulator binding sequences were synthesized by IDT.

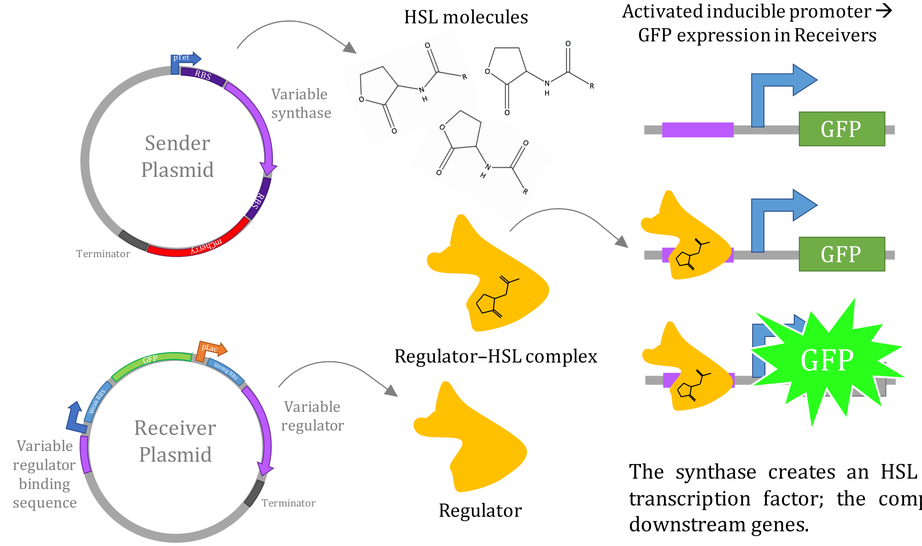
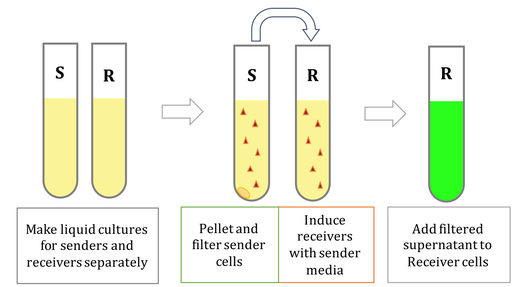


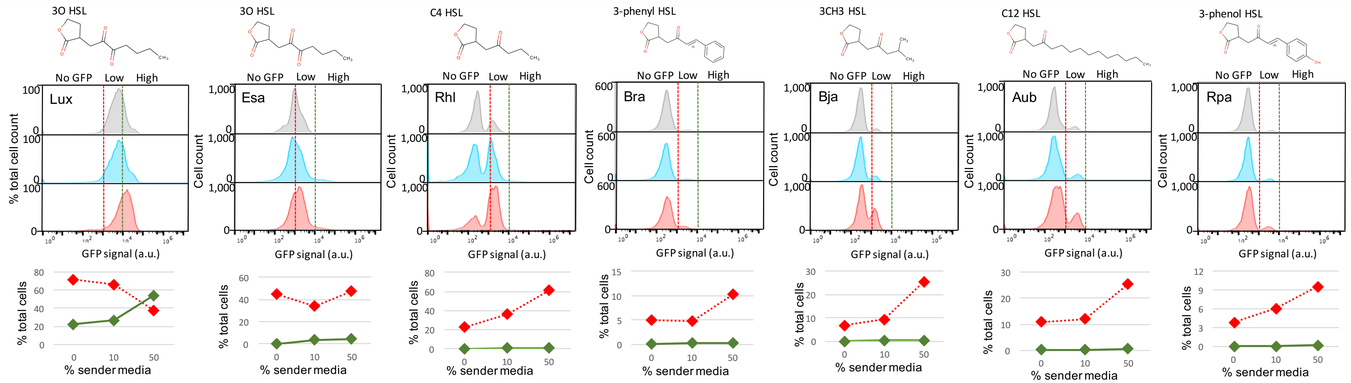
Figure 3 | Experimental workflow. BL21 *E.coli* cell lines are transformed with sender and receiver plasmids



Overnight sender supernatant is filtered and used to induce receiver cells. GFP expression in the receivers is measured using flow cytometry.

The synthase creates an HSL molecule which complexes with the regulator created by the transcription factor; the complex activates the the regulator binding sequence to express downstream genes.

Results



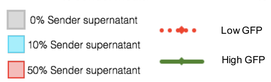


Figure 4 | Flow cytometry measurements of GFP expression. Preliminary data for receivers (BL21, *E.coli*) induced with supernatant from their endogenous sender. We ran trials with final media concentrations of 0%, 10%, and 50% sender supernatant. When induced, we see varying levels of increased GFP in all seven systems.

Conclusions

- ✓ Matching QS pairs from various species are functional in *E. coli*
- ✓ Different QS pairs produce different levels of GFP expression

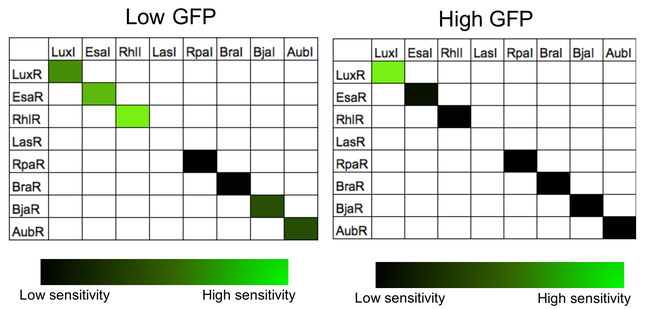


Figure 5 | Crosstalk heat maps for preliminary data.

Future Work

- ❑ Test optimal growth time for sender production.
- ❑ Complete cross-induction experiments for all sender constructs.
- ❑ Test and analyze HSL concentration.

We plan to use a cross-talk heat map to represent interactions between all senders and receivers. The Hill equation allows for a quantitative measurement of orthogonality. Each *k* value generated from the Hill equation (the HSL concentration corresponding to the half-maximal GFP expression rate) will be mapped to a corresponding saturation on a gradient scale. This map will provide a detailed profile of likely orthogonal candidates (based on squares showing low sensitivity), allowing genetic engineers to easily identify parts that fit their needs.

FURI research program

IRA A. FULTON SCHOOLS OF engineering
ARIZONA STATE UNIVERSITY