Research exercise: Amplifying Signals via Riboswitch Biosensors

Follow this and additional works at: https://ecommons.udayton.edu/stander_posters

Part of the Arts and Humanities Commons, Business Commons, Education Commons, Engineering Commons, Life Sciences Commons, Medicine and Health Sciences Commons, Physical Sciences and Mathematics Commons, and the Social and Behavioral Sciences Commons

Recommended Citation
https://ecommons.udayton.edu/stander_posters/750

This Book is brought to you for free and open access by the Stander Symposium at eCommons. It has been accepted for inclusion in Stander Symposium Posters by an authorized administrator of eCommons. For more information, please contact frice1@udayton.edu, mschlangen1@udayton.edu.
Amplifying Signals from Riboswitch Biosensors

Annastacia Bennett1,2, Michael Goodson2, Nancy Kelly-Loughnane2
1. The University of Dayton, Department of Biology, 300 College Park, Dayton, Ohio
2. 711th Human Performance Wing, RHXBC, Wright-Patterson AFB, Dayton, Ohio

Introduction
- Biosensors are self-sufficient, natural systems that can report a signal based on the presence of a specific molecule — but are limited by a low signal output
- Biological amplification circuits, based on electrical circuitry concepts, will be used to produce and amplify a signal once the circuit is triggered by riboswitch-initiated protein translation that synthesizes a quorum signaling molecule (QSM)
- Cells within the circuit will be “wired” together via QSM production and promoters that respond to specific QSMs

What is a Riboswitch?

Riboswitch-Induced Amplification Circuit

Methods
- All plasmids were created via Polymerase Chain Reaction (PCR), restriction digest, and ligation. Plasmids were confirmed by sequencing and transformed into JM109 E.coli cells.

Plasmid Schematics

Reporter Cell Signal Progression Experiment Set-Up
- Each experiment set-up begins with a signaling molecule to kick-start signal progression.
- Signal progression from plated synthetic signal to cells will result in fluorescence from left to right.
- DMSO should not induce fluorescence

Amplification Circuit Signal Progression Experiment Set-Up

Results

Initial Tests of Individual Cell-Types with Signaling Molecule
- Only reporter cell-types (N & O) with synthetic signal fluoresced

‘Tuning the Circuit’ by Removing Degradation Tag
- Signal was not passing from N cells to O cells
- N cells contain LasI gene which produces 3OC12 to activate O cells
- LasI gene has a degradation tag (LVA) which lowers LasI production
- PCR and Gibson Assembly were used to remove LVA from N plasmid and new N cell-type was transformed into JM109 E.coli cells

‘Amplifying Signals from Riboswitch Biosensors’

What is a Riboswitch?

Amplification Circuit Signal Progression:
- Synthetic 3OC12 activates adjacent O
- Activated N does not activate any of the downstream bacterial colonies

Signal Progression Testing after Removal of LVA
- Signal progression from synthetic signal to O cells to N cells is occurring as proven by visual fluorescence (Plate 1)
- N cells are still not activating neighboring O cells (Plate 2)
- O cells appear to be producing C4 when ‘off’ and are activating neighboring N cells (Plate 3)
- Individual cell types are not fluorescing on their own (Plate 4)

Conclusions and Future Work
- In liquid culture, individual cell types were producing green fluorescent protein in the presence of their signal molecule
- Signal progression plate tests revealed that N cells were not producing enough 3OC12 to activate neighboring O cells
- The degradation tag was removed to increase 3OC12 production in N cells
- Testing after removal of degradation tag did not improve induction of fluorescence in O cells
- To increase fluorescence in cells and ensure the circuit will not be activated without synthetic 2AP, the circuit will be ‘tuned’ by replacing ribosome binding sites (RBS) in each of the reporter cell types, N and O.
- Experiments with the newly synthesized reporter cell-types will be performed to determine riboswitch-induced signal progression

References

Acknowledgement
We thank Prof. Chris Voigt for generously supplying plasmids and Prof. Jason Micklefield for providing sequence data. This research was funded by the Air Force Office of Scientific Research (AFOSR).