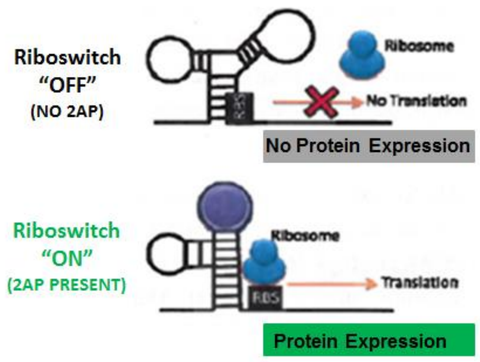
Amplifying Signals from Riboswitch Biosensors

Annastacia Bennett[1,2], Michael Goodson[2], Nancy Kelly-Loughnane[2]
1- The University of Dayton, Department of Biology, 300 College Park, Dayton, Ohio
2- 711th Human Performance Wing, RHXBC, Wright-Patterson AFB, Dayton, Ohio

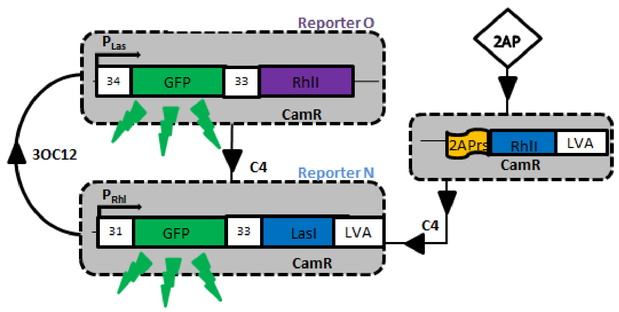
Introduction

- 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?



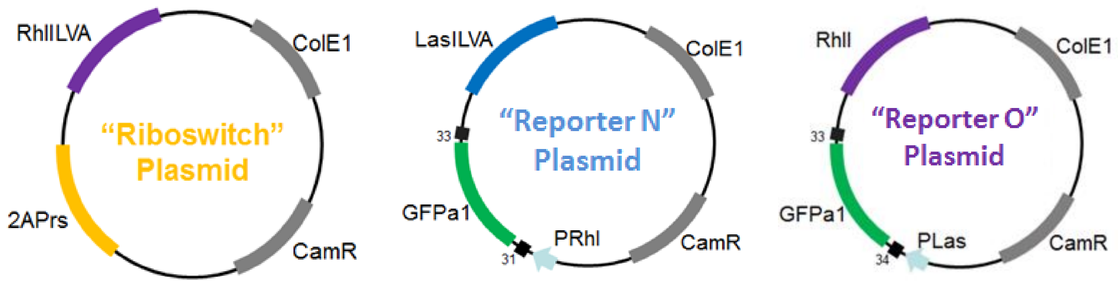
Riboswitch-Induced Amplification Circuit



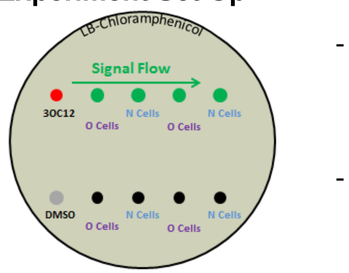
Methods

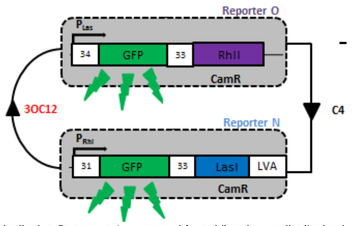
All plasmids were created via Polymerase Chain Reaction (PCR), restriction digest, and ligation. Plasmids were confirmed by sequencing and transformed into JM109 *E.coli* cells.

Plasmid Schematics



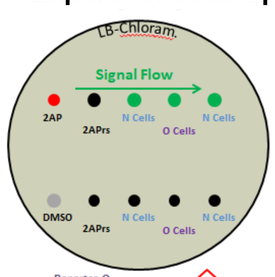
Reporter Cell Signal Progression Experiment Set-Up

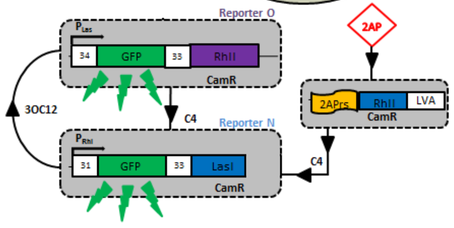




- Each experiment set-up begins with a signaling molecule to kick-start signal progression.
- Signal progression from plated synthetic signal to cells will result in fluorescence from left to right.
- DMSO should not induce fluoresce

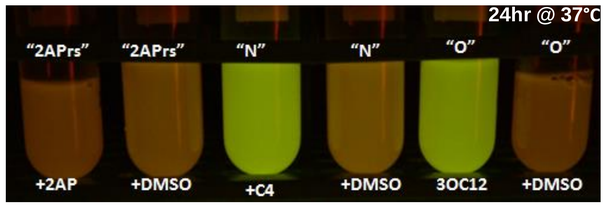
Amplification Circuit Signal Progression Experiment Set-Up





Results

Initial Tests of Individual Cell-Types with Signaling Molecule



Initial tests with individual cell-types and their respective synthetic signal molecules were successful

- ✓ 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

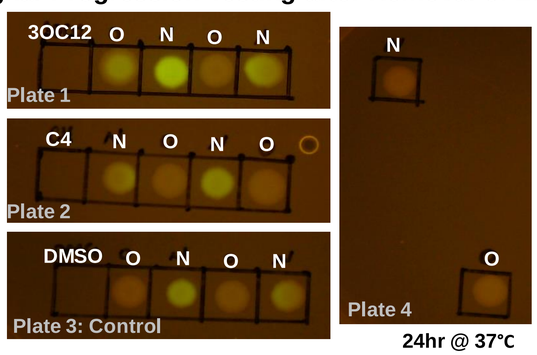
Signal Progression (without Riboswitch):

- Synthetic 3OC12 activated adjacent O
- O activated adjacent N, but O and N afterward do not fluoresce

Amplification Circuit Signal Progression:

- Synthetic 2AP activates adjacent N
- Activated N does not activate any of the downstream bacterial colonies

Signal Progression Testing after Removal of LVA



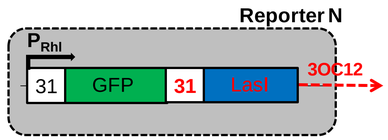
24hr @ 37°C

- 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)

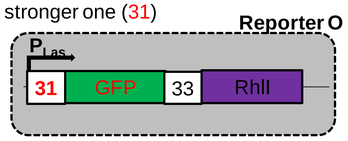
Conclusions and Future Work

- 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 fluoresce 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

Plasmids after Ribosome Removal



Increase production of 3OC12 in N cells by replacing weaker RBS (33) with a stronger one (31)



Increase GFP in O cells by replacing weaker RBS (34) with a stronger one (31)

References

[1] Park, Miso, Shen-Long Tsai, and Wilfred Chen. (2013) "Microbial Biosensors: Engineered Microorganisms as the Sensing Machinery ." *Sensors*: n. pag. Web.
[2] Dixon et al. (2012) Angew Chem Int Ed Engl. 51:3620
[3] Lynch and Gallivan (2009) Nucleic Acids Res 37:184

Acknowledgements

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) .

UNIVERSITY of DAYTON