FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

ENGINEERING BIOSENSORS

Comparison of Riboswitch Reporter Systems for Live Cell Imaging of Cyclic di-GMP Dynamics in *Bacillus Subtilis* Population

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INTRODUCTION

- Weiss et al. developed and tested a biosensor for the bacterial signaling molecule cyclic di-GMP in the bacteria *Bacillus subtilis* with *yfp* as the fluorescent reporter[1]
- Fluorogenic RNA aptamers could be used instead of fluorescent proteins for faster and more accurate live cell imaging
  - Aptamers are oligonucleotides that undergo structural change once bound to the target molecule
  - Fluorogenic RNA aptamers are short RNA strands that fluoresce when bound to a fluorophore

Target Sensing Platform (cyclic di-GMP responsive aptamer) + Expression Platform (fluorogenic aptamer/protein) = Biosensor (Riboswitch Reporter)

Figure 1. Definition and components of a riboswitch reporter.

Project Objective

Develop and test a cyclic di-GMP riboswitch reporter system, using fluorogenic aptamers as the expression platform instead of *yfp*; compare the two systems in vivo in *B. subtilis*

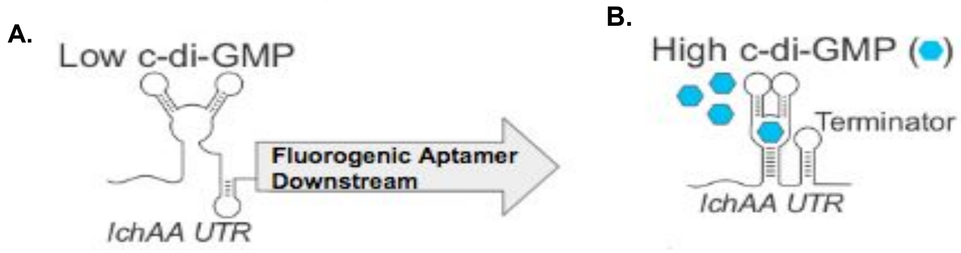


Figure 2. Mechanics of the riboswitch from *B. licheniformis* in our proposed riboswitch reporter system. In the absence of cyclic di-GMP (target molecule), downstream gene expression of fluorescent reporter occurs **(A)**. In presence of cyclic di-GMP, a transcription terminator hairpin forms and downstream gene expression is terminated **(B)**. Images modified from Weiss et al[1].

METHODS

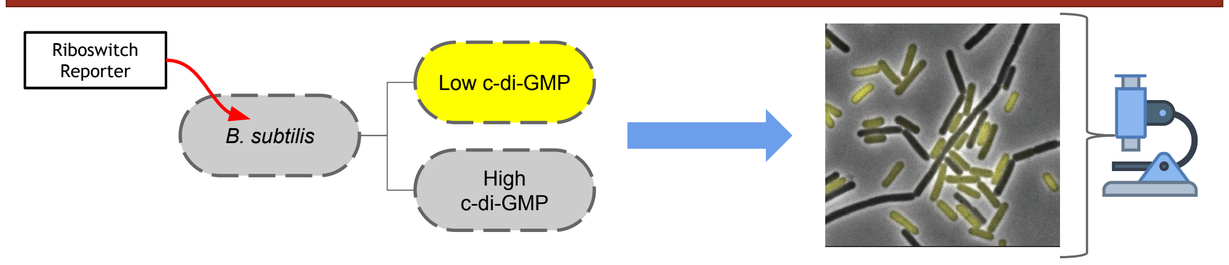


Figure 3. Research plan to use riboswitch reporter to image cyclic di-GMP dynamics in *B. subtilis* cells and expected results. Microscopy image modified from Weiss et al[1].

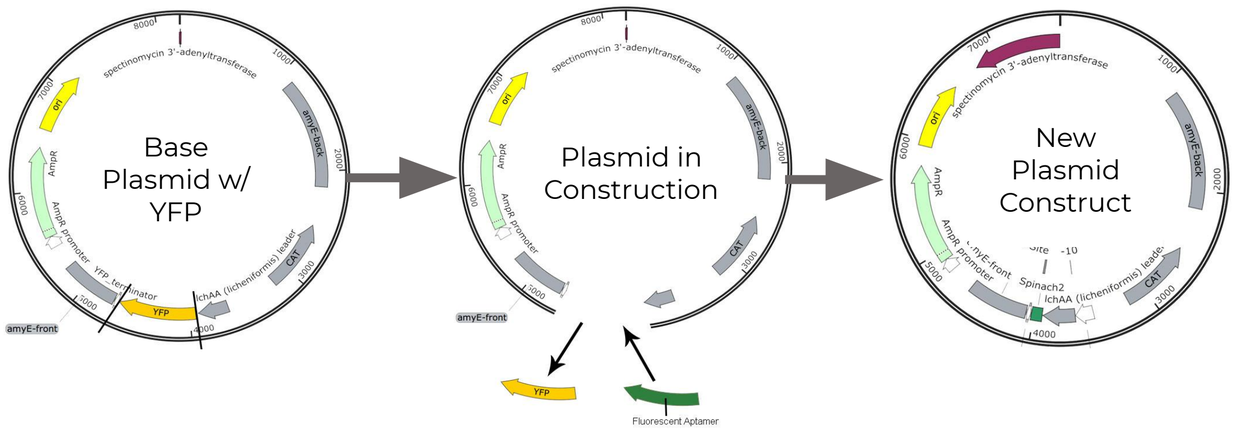


Figure 4. First plasmid is Dr. Weiss's plasmid design with yellow fluorescent protein reporter; final plasmid is plasmid with fluorogenic aptamer reporter.

PCR → replication of vector backbone and desired insert (fluorogenic aptamers: MangoIII, Broccoli, Dimeric Broccoli, SpinachII)
Plasmid Assembly → via Gibson Assembly (for Dimeric Broccoli and SpinachII) or KLD (for Broccoli and MangoIII)
Transformation into *E. coli*
Overnight Culture, Plasmid Miniprep
Sanger Sequencing
Transformation into *B. subtilis*
Live Cell Imaging → laser confocal or fluorescence microscopy

RESULTS

Sanger Sequencing to Confirm New Plasmid Constructs
Transformation into *B. subtilis* WT PY79
Transformation into *B. subtilis* ΔpdeH Mutant
Glycerol Stock of All Constructs

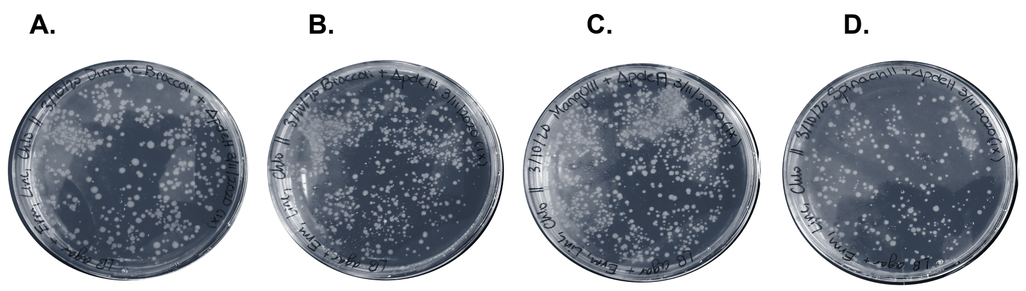


Figure 5. Agar plates of transformant *B. subtilis* ***Δ****pdeH Mutant* containing the new cyclic-di-GMP riboswitch reporter plasmid constructs **(A)** Dimeric Broccoli, **(B)** Broccoli, **(C)** MangoIII, and **(D)** SpinachII

FUTURE WORK

Next Steps

- Evaluate performance between the different fluorogenic aptamers and in comparison to *yfp* in *B. subtilis* cells, similar to the previous work by Filonov et al. in *E. coli*[2,3]
  - Applying live-cell imaging via laser confocal and fluorescence microscopy at the UMD Imaging Core Facility
  - Using flow cytometry to characterize the fluorescence of *B. subtilis* to measure levels of cyclic di-GMP with different fluorogenic aptamers
- Develop protocols for building PPGP biosensors

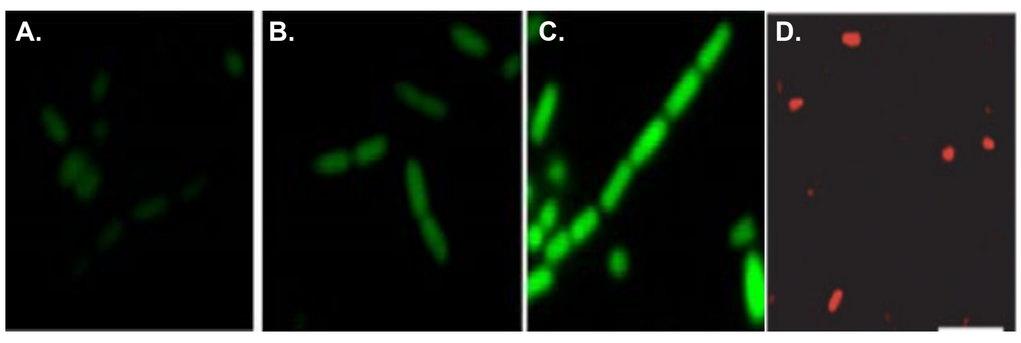
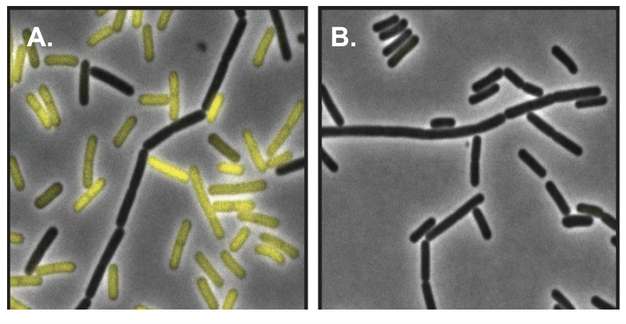


Figure 6. Microscopy images of constitutive aptamers SpinachII **(A)**, Broccoli **(B)**, Dimeric Broccoli **(C)**, and Mango **(D)** in *E. coli*.[3,4] Modified from Filonov et. al, 2014 and Neubacher et. al, 2018.

Figure 7. Microscopy images of *B. subtilis* with *yfp* riboswitch reporter construct. Wild type *B. subtilis* strain natural cyclic di-GMP variation across the community **(A)**. *B. subtilis* strain with gene for cyclic di-GMP deterioration (*pdeH*) removed **(B)**. Modified from Weiss et. al, 2019.



REFERENCES & ACKNOWLEDGEMENTS

1: Weiss et al. (2019). Single cell microscopy reveals that levels of cyclic di-GMP vary among *Bacillus subtilis* subpopulations. JB.
2: Dolgosheina et al. (2014). RNA Mango Aptamer-Fluorophore: A Bright, High-Affinity Complex for RNA Labeling and Tracking. ACS Chem. Biol. 9, 2412–2420.
3: Filonov, G.S., Jaffrey, S.R. (2016). RNA Imaging with Dimeric Broccoli in Live Bacterial and Mammalian Cells. Curr. Protoc. Chem. Biol. 8, 1–28.
4: Neubacher et al. Chem. Int. Ed. 2018, 58, 1266.

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