Real-time Quantitative Measurement of RNA and Protein Levels Using Fluorogen-Activated Biosensors

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Materials and Methods

Model: We developed a system of differential equations to define our characterization parameters (transcriptional strength and translation efficiency). We conducted experiments based on the information needed for the model and performed non-linear regression to obtain our parameters. We then compared these values to the wild type (which we defined as the part BBa K613007) and recorded their relative values.

Experiment: Our expression strain is BL21(DE3), a strain that contains the gene for T7 RNAP, which we transformed with a high-copy plasmid (pIVEX). We filled a 96 well plate with 100µL of our transformed cells and added 200µM DFHBI into half of the wells and 10µM MG into the other half. We added IPTG and took time course measurements for 3.5 hours. We normalized our raw data using OD600 and plotted these values over time.

Mathematical Modeling of Our Synthetic Constructs

Synthetic Biologists Need “Tools” to Measure Cellular Performance

- The design and implementation of synthetic biological systems often require information on transcription and translation rates and on the impact of both RNA and protein levels on metabolic activities of host cells.
- To date, however, quantitative information about the expression strength of a promoter is difficult to obtain due to the lack of noninvasive and quick approaches to measure levels of RNA and protein in cells.

We demonstrated the use of our kit in introducing high school students to date, however, quantitative information about the expression strength of a promoter is difficult to obtain due to the lack of noninvasive and quick approaches to measure levels of RNA and protein in cells. We have also tested the kit in demonstrations to high school students. Easily shared and improved.

Here, we engineer a fluorescence-based biosensor that can provide information on both transcription strength and translation efficiency that is noninvasive, easily applied to a variety of promoters, and capable of providing results in a time frame that is short when compared to current technologies.

Human Practices: Connecting Synthetic Biology to the Society

- The kit is part of the Lending Library of Kits of DNAzone, the outreach program of the Center for Nucleic Acids Science and Technology (CNAST) at Carnegie Mellon. Kits are loaned to high school teachers in the Pittsburgh area to be used in teaching math and science.
- We demonstrated the use of our kit in introducing high school students to concepts in synthetic biology. Our kit also meets specific objectives from the Pennsylvania Academic Standards for Science, Technology and Engineering Education. and the Pennsylvania Assessment Anchors.
- We have also tested the kit in demonstrations to high school students enrolled in the Summer Academy of Math and Science (SAMS) and the AP Biology program at Carnegie Mellon.

Interactive, Relatable, Easily shared and improved

- Can remove and insert different parts, promoters, RBS, Spinach, FAP and circuit behaves accordingly.
- ‘Mini-game’ to find the best promoter
- Our kit is physical and interactive: Brings experiment/lab to students
- Everything is open source and uploaded, anyone can access and tailor it.

We used an RNA reporter, called Spinach, which binds to a dye called DFHBI.

What is Spinach?

<table>
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<tr>
<th>Promoter</th>
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<th>FAP</th>
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<tbody>
<tr>
<td>BBa_K921000</td>
<td>Spinach-DFHBI Fluorescence</td>
<td>FAP-MG Fluorescence</td>
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Normalized fluorescence intensities over a 3.5 hour time period. These wells were saturated with DFHBI and fluorescence intensities at 501nm were recorded. Solution contained 1mM MgCl₂ in M9 media. Mutant II works as expected, to operate at a lower efficiency and strength than the wild type. Mutant I performs better than the wild type.

Attributes

Yang Choo and Eric Pederson performed all biological experiments, kept lab notebooks on the biological works, and designed experiments. Peter Wei formulated the model and wrote the MARLAB program. Jesse Salazar designed the human practices circuit kit, documented it, and improved its compliance with the appropriate education requirements. All 4 members presented at the outreach presentations and worked on the wiki. Yang Choo developed the primary wiki format. We would also like to thank Dr. Robert Murphy and Dr. Eric Grotzinger for access to the lab spaces.

References

3. Shuler. PNAS 1993

What is a FAP?

Protein and RNA fluorogen-activating biosensors.
- Bind to a fluorogen (such as malachite green, dimethylindole red or thiazole orange)
- Hold the dye in the proper orientation to promote emission of photons when excited
- Are modular, can be fused to proteins or incorporated in many RNA types.

In this case, we used a FAP called Ben, which binds to malachite green (MG). Ben is an engineered version of L5, which is functional in E. coli.

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Normalized fluorescence intensities over a 3.5 hour time period. These wells were saturated with MG and fluorescence intensities at 660nm were recorded. MG was dissolved in 10% ethanol to increase saturation with MG and fluorescence intensities at 660nm were recorded. MG was dissolved in 10% ethanol to increase saturation with MG.