**Project Justification**

In the United States alone, it is estimated that three million people are affected by type one diabetes. Complications that arise due to the disease include heart disease, stroke, high blood pressure, blindness, kidney disease, and neuropathy. The American Diabetes Association has estimated that diabetes costs America over $200 billion per year in diagnosis and treatment[1]. Many of the complications that arise from diabetes can be prevented by testing blood glucose levels and taking insulin shots at appropriate times. Future applications of this project have the potential to make more affordable blood glucose test strips for people without access to good health care all over the world. Further research in this area could also lead to practical insulin pump technology. This could save lives and improve the quality of life for many people afflicted with diabetes.

**Project Goal**

Our goal is to create a system that can measure and report different concentrations of glucose. With further research, this could eventually be applied as indicated in Project Justification.

**Making Our Parts**

We started with three parts from the iGEM registry:
- **Bba K62102**: RBS-3, a medium-strength ribosome binding site
- **Bba E0030**: eYFP gene, codes for yellow fluorescence
- **Bba R0082**: omp-c operon, contains three binding sites for phosphorylated OmpR

To build our part our team performed restriction digests and ligations as indicated by Figure 1.

**Our System Overview**

![Figure 2 - Two component regulatory system pathway](image)

![Figure 3 - Activated system](image)

![Figure 4 - Inhibited system](image)

This means that higher osmolalities have a greater chance of down-regulating the system. In summary, as osmolality increases from very low levels, the fluorescence produced by the system also increases until the system reaches a threshold osmolality. Once the system reaches the threshold, the fluorescence will decrease with increasing osmolality due to the inherent down-regulation of the system. The activity of the system can be quantified using the fluorescence of the cells because the two-component regulatory system of EnvZ and OmpR regulates transcription of the eYFP gene, dictating the level of fluorescence.

**Testing**

To measure fluorescence our team used a 96-well plate reader. Overnight cultures of bacteria were grown in 0.5X LB media and then aliquoted into 1.5ml tubes. Serial dilutions of the desired glucose concentrations were made. Glucose was diluted using 0.5X LB media so that the concentration of LB remained constant. The glucose was administered to the cells in a fashion that did not dilute the cell densities in the cultures. Samples of each treatment were added to a 96-well plate and were imaged using the plate reader.

**Data**

**Fluorescence Wavelengths**

<table>
<thead>
<tr>
<th>Excitation Max</th>
<th>Emission Max</th>
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</thead>
<tbody>
<tr>
<td>485nm</td>
<td>520nm</td>
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</table>

**Dose Response in 0.5X LB**

If cells are grown in a high osmolality media, such as LB broth, the background fluorescence will be higher than an induced glucose response. This is because the system is already primed from the high osmolality of the media. Above is a dose response in the presence of LB media. Note how glucose, in these conditions, has an inhibitory effect on the system.

![Figure 5 - Dose Response in 1X LB](image)

**Dose Response in 0.5X LB**

To control for compounding variables, the cells assayed were grown in 0.5X LB media. This reduced the background osmolality and allowed our team a measure a valid response. In the graph you can see that the system fluoresces over background at 1% glucose and then drops back to background at 5% glucose. The increase at 1% is due to the positive regulation by the EnvZ-OmpR system. 5% glucose is a high enough concentration to induce down-regulation of the system.

![Figure 6 - Dose Response in 0.5X LB](image)

**Time Trial**

The graph below shows time trials of the system. These time trials were run in the presence 0%, 1%, and 8% glucose. We chose to do this so we could gather more comprehensive data. The first conclusion we can draw from this graph is that the response is fairly consistent over time (there is no peak in fluorescence associated with a specific time point). Also, for each time point between 0.5-4.5 hrs, the curve of the dose response remains consistent. The assay is not accurate after 4.5 hours (the response fades and the data becomes random and inconsistent). So, data was not included in the graph.

![Figure 7 - Time Trial in 0.5X LB](image)

**References**


**Dose Response in 0.5X LB**

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**Parts Created**

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<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
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<tr>
<td>Bba K621001</td>
<td>Reporter</td>
<td>OMP-R-9RBS-eYFP</td>
<td>858 bp</td>
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<tr>
<td>Bba K621000</td>
<td>Intermediate</td>
<td>RBS-eYFP</td>
<td>742 bp</td>
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</table>

**Sequence Verification**

This part has been sequence verified and works as expected.

**Growth Conditions**

- **Growth Temperature and Rate** - E. coli strain DH5-alpha used as chassis, standard growth conditions apply
- **Growth Media** - Nutrient broth or diluted LB media, since OmpR is an osmosensor, the cells must be grown in a low osmolality media to control for compounding factors. To express fluorescence it is recommended that cultures are grown in liquid media. Colonies growing on plates cannot detect osmolality and will not activate EnvZ.

**Dose Response in 0.5X LB**

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