YcgF/YcgE blue reception system

Objective.

The main goal for this section of the project was to implement a system for blue light reception using the YcgF/YcgE sytem, naturally present in certain strains of *Escherichia coli.*

This reception system would be coupled both to a reporter gene (i.e. GFP) and, for the purposes of our project, a luciferase gene.

Methodology.

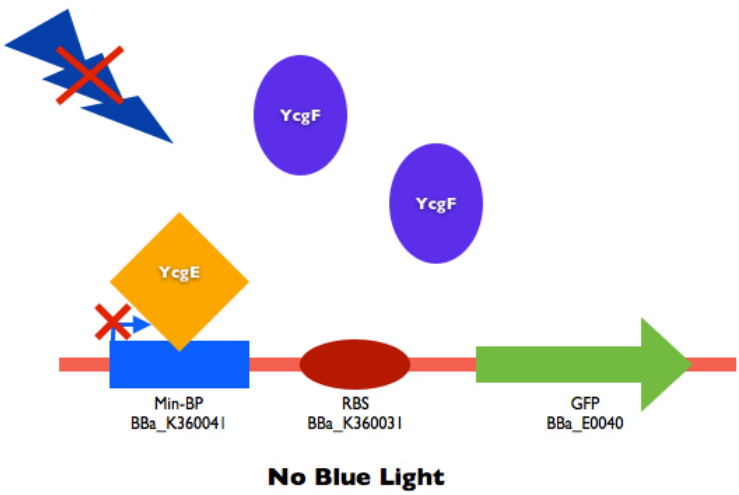
We used a modified version of the YcgF/YcgE system for blue light reception (previously reported by the K.U. Leuven 2009 Team), the modification consists in the reduction of the promoter region to 50 bp.

Originally, we tried to amplify the complete promoter region BBa_238013 (86 bp) from genomic DNA of *E. coli* K12 by PCR but we failed in several trials. So we decided to synthesize the promoter in a primer and then insert it into pSB4A5 by PCR. Because of lenght limitations in primer synthesis we reduced the promoter region to 50 bp.

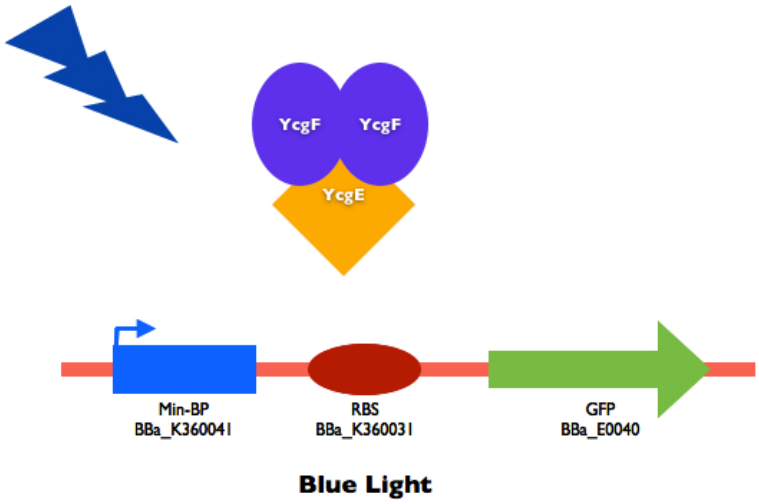
The reduced promoter retains the fundamental parts of the original one, these are the -35 and -10 box, the spacer between them, the inverted repeat 1 and 2 (inverted regions are the binding sites for the YcgE repressor) and the transcription start site. We registered this Minimum Blue Light Receptor Promoter as BBa_K360041.

In order to test the functionality of our Minimum Blue Promoter we succesfully ligated it to our Strong RBS BBa_K360031 and the GFP BBa_E0040.

The YcgF/YcgE system is based on the action of the repressor YcgE, which is bound to the promoter region when there is no blue light, thus inhibiting the transcription of any gene downstream this promoter, as shown in the next figure.



In the presence of blue light, YcgF dimerizes and now it has a great affinity for YcgE, clearing the promoter and allowing transcription to proceed.



It is reported that the response of this promoter is weak in comparison to some others standard strong promoters registered in the Registry of Standard Biological Parts. We implemented a protocol for testing the response of our Minimum Blue Light Receptor Promoter (Min-BP) in which we irradiated cells with the construction Min-BP + RBS BBa_K360031 + GFP BBa_E0040 with blue light (470 nm) leds for different times. We also irradiated with green (540 nm) and red (660 nm) light leds to discard any crosstalk of these wavelengths. GFP expression was compared to a reference: J23101 promoter + RBS BBa_K360031 + GFP BBa_E0040.

Results.

Up to now we only have qualitative results indicating that there is a weak increase in GFP expression in response to blue light exposure, as expected according to the results reported by the K.U. Leuven 2009 Team.

We are planning to perform a quantitative experiment during the next week.