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.

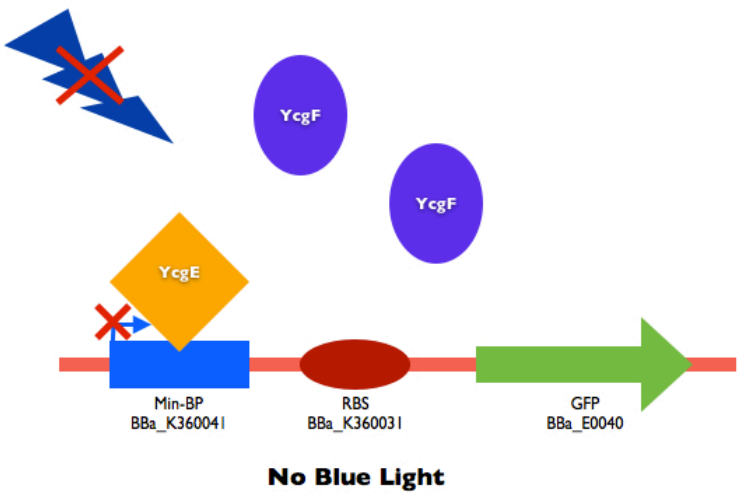
We used a modified version of the YcgF/YcgE system for blue light reception (previously reported by the K.U. Leuven 2009 Team), the modification consists in the reduction of the promoter region to 50 bp.

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.

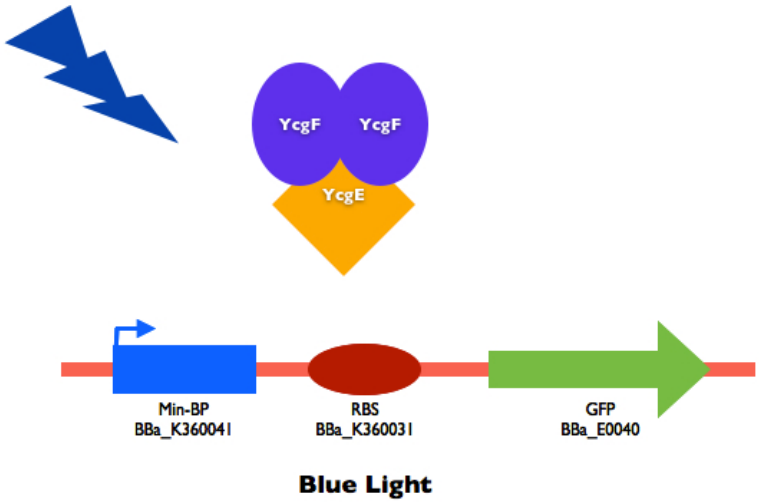
The reduced promoter retains the fundamental parts of the original one, these are the -35 and -10 box, the spacer between them, the inverted repeat 1 and 2 (inverted regions are the binding sites for the YcgE repressor) and the transcription start site. We registered this Minimum Blue Light Receptor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



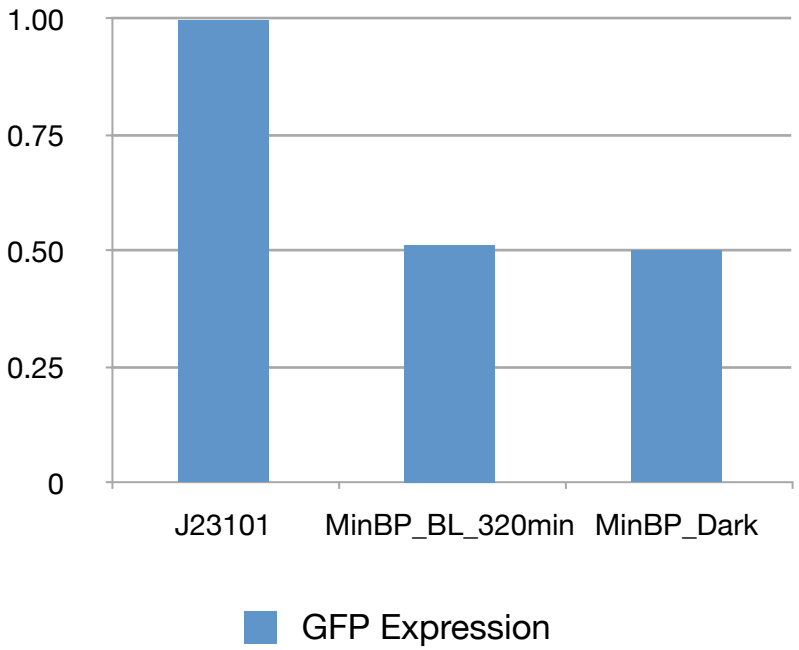
It is reported that the response of this promoter is weak in comparison to some others standard strong promoters registered in the Registry of Standard Biological Parts. We implemented a protocol for testing the response of our Minimum Blue Light Receptor Promoter (Min-BP) in which we irradiated cells 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.

We measured GFP expression of our constructions, in order to irradiate the cells with Min-BP + RBS BBa_K360031 + GFP BBa_E0040 we used blue leds and irradiated the cells for 300 minutes, we also measured GFP expression of cells that were incubated in the dark and cells with the constuction J23101 promoter + RBS BBa_K360031 + GFP BBa_E0040.

For the experiment cells were incubated overnight in M9 medium with glycerol (0.4%) as carbon source, in the morning OD600 was measured and cells diluted to 0.07 nm. After dilutions, we started irradiation with blue leds. Temperature is a very important factor in the function of the YcgF/YcgE system; it is reported that at 25 ºC the ratio between the YcgE repressor and YcgF activator allows a good repression unless there is blue ligh irradiation, so we incubated cells with this promoter at 25º C. Cells with the constitutive promoter J23101 were also incubated overnight at 37ºC.

Unless there is a increase in GFP expression in response to blue light exposure, this change is weak in comparison with cells that were not irradiated.



Conclusions and Perspectives

We have shown that our Minimum Blue Light Receptor Promoter BBa_K360041 works as expected but the response is weak compared to a standard, constitutive promoter J23101. This results are consistent with previously reported data by the K.U. Leuven 2009 Team.

In order to increase GFP expression in response to blue light, an amplifier could be used. This means to ligate a T7 polymerase downstream the Minimum Blue Promoter and the GFP downstream a T7 promoter. Additionally, we can increment irradiation time and observe response of our blue light-induced promoter.