Abstract

Our project aims to introduce a triplet of NOT gates in *E. coli* to create a system which is finely controlled by exposure to light. This would be demonstrated using three pigments - cyan, magenta and yellow. Projecting an image onto a lawn of our modified bacteria would allow exact replication of the image in full colour, showcasing the versatility of utilizing optical controls in a synthetic pathway. Our project is highly adaptable as it allows future iGEM teams to insert a gene of their choice into the system in place of the pigments, using the restriction enzymes BglII and BamHI.

Light, pigments and our logic gates

Exposing the *E. coli* to red, blue or green light at sufficient levels will halt the synthesis of cyan, yellow or magenta pigments respectively.

The Blue Light Module works in a very similar way, however the light sensing protein is an internal protein, rather than transmembrane. The Module was designed and ordered through DNA2.0. The Green Light Module required an inverter system, as exposure to green light allowed the phosphorylation of the intermediate protein, so the cyan pigment gene is not transcribed. We built this Module using standard 3A Assembly

The primary component of our model is a rule-based stochastic simulation of the biological pathways above, written in Kappa and executed with KaSiM. It takes the calculated activation rate and simulates how the pathways respond:

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\text{Data from the stochastic simulation is combined with the absorption spectrum of the background cell, the light sensor and pigment proteins to produce a surface that describes how the absorption spectrum of the cell changes with time.}
\]

\[
\text{where:}
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- \(S(t)\) is the index of the sum and is the i'th species of molecules
- \(I(t)\) is the absorption spectrum over time
- \(N\) is the number of molecules of species over time
- \(S\) is the absorption spectrum of sensor

There was insufficient experimental data to test the model, however preliminary trials using ballpark figures for reaction rates suggest great instability and uneven pigment mixing. Increasing the number of light regulated DNA stabilised the system and can be used to correct the mix of colours.

Results

Green Light Module

Assembled using IDT gBlocks, the Green Light Module has been shown to function as expected on exposure to white light; the production of magenta pigment is inhibited.

**BglII and BamHI output brick**

Also assembled using IDT gBlocks, this output brick responds to red light by producing GFP, clearly seen in the image to the right. Running the digested plasmid on a gel also verifies the presence of the BglII and BamHI restriction sites. Replacing GFP with a gene of your choice would require use of simple standard primer based mutagenesis protocol.

Cyan pigment with specific promoter

ompC was successfully ligated to the cyan pigment, allowing synthesis of the pigment to be solely controlled by red light alone.

Future Applications

- **High resolution images**
- **Pigments don’t necessarily have to be the output – BglII and BamHI restriction sites allow insertion of any gene**
- **Applications in a vast array of fields – medical, industrial, experimental, even artistic**
- **Possibility of use in light targeted therapies**
- **Potential use of lasers for high spatial control**
- **Theoretically, light would be the only input (simple, cheap, effective)**

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