Background

Biological systems must be continuously improved to function consistently and reliably. Our team sought to develop and experimentally verify a light induced gene expression system that we hope will find use in iGEM and beyond. In 2012, we developed an app that allows anyone with access to an Android device with an LED screen the ability to illuminate cells in a controlled manner. This year, we thoroughly tested our app with a previously reported light induced expression system, and submitted biobricked versions of these genes to the Registry. Together, our tablet app and light induced expression system represent a complete toolkit for a number of potential synthetic biology applications.

The light induced expression systems we used both require multiple protein components. In general, a small molecule chromophore is synthesized in the cell, and acts as a substrate for a transmembrane light sensing protein. When the chromophore-bound sensor protein is exposed to light of the correct wavelength, a conformational change in the chromophore activates the sensor protein, which in turn phosphorylates a transcription factor. The active form of the transcription factor then mediates the expression of any gene downstream of its cognate promoter. We worked with both red and green light inducible gene expression systems this year. Both systems utilize the same chromophore, phycocyanobilin, but differ in the transmembrane light sensing proteins that bind the chromophore, and the downstream regulator proteins. Regardless of the system in question, the minimal components required are: 1) genes utilized in the synthesis of the chromophore, 2) a transmembrane light sensing protein, and 3) response regulators which are activated by the light sensitive proteins, and ultimately function as transcription factors.

Our App

E.Calight is a tablet application designed by members of our team in 2012. This year, our team wanted to continue our 2012 project by expanding the functionality of our app, calibrate it using existing light-inducible promoter systems, and determine the maximum number of experiments we can perform on a single tablet. In addition to developing routine protocols for the use of the app, we also developed new features that can accommodate a broader range of growth vessels, a virilization feature, and utilize the heat output of the processor as an incubator. By designing experiments with high reproducibility that explore the strengths and limitations of the app, we hope to create the go to system for light induced gene expression.

Results

(Right) The length of exposure to full intensity green light was examined. As exposure time decreased, a linear decrease in GFP expression levels was observed.

(Above) We conducted our experiments on the Samsung Galaxy Tab 7.7, which has a Super AMOLED display. Each pixel on a Super AMOLED display is made up of three LEDs corresponding to each of the colors in the RGB color format. This format is advantageous for the light inducible gene expression systems because it can display all of the colors in the RGB color space. Furthermore, as shown above, the RGB LEDs output spectra are highly monochromatic, with only slight overlaps between the spectra, suggesting they should be compatible with published red, green, and blue light systems.

(Above) We tested the light bleached through between adjacent wells in a 36 well plate by illuminating three wells sequentially and comparing the GFP output of cultures growing in the lit wells to the adjacent growing wells. Other wells completely surrounded by dark (D-E13) or light wells (D2, E2) were used as controls. The GFP expression level of a “dark” well next to a “light” well was up to 60% of the fully illuminated value, as a result, we recommend skipping wells when setting up experiments using this app.

(Above) In order to confirm that our Biobrick versions of the previously reported light system functioned similarly to the original constructs, the two were examined side by side using our tablet app and the light induced expression protocol we developed. While green light was observed to induce expression of GFP relative to the dark control in our Biobrick variants, the growth rate was relatively slower than that of the control plasmids. We hypothesize that this difference is due to the use of different antibiotic resistance genes between plasmids, and are currently attempting to optimize our Biobrick plasmids for growth and expression.

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