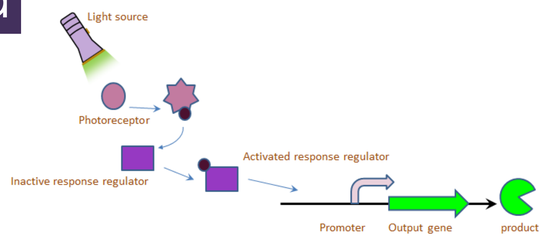
RED LIGHT, GREEN LIGHT

UNIVERSITY OF WASHINGTON iGEM 2013

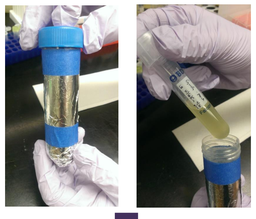
Background

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 affords any researcher 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.



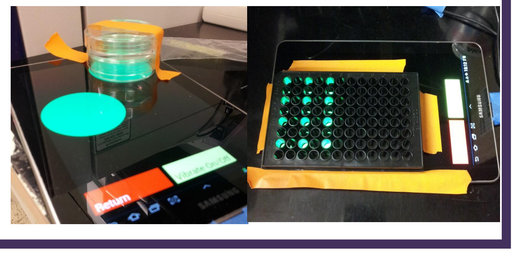
The light induced expression systems we used both require multiple protein components. In general, a small molecule chromophore is synthesized in the cell, and acts as a substrate for a transmembrane light sensing protein. When the chromophore-bound sensor protein is exposed to light of the correct wavelength, 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 modulates 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.**

The Setup



This year our team optimized a protocol for testing our specific system.

1. Overnight cultures were grown of E. coli containing plasmids with genes for all of the parts of the light system.
2. The next morning, these cultures were used to inoculate either M9 media or M9 agar.
3. The app was set up for the appropriate container (96 well plate, 60 or 100 mm petri dishes) and light conditions (including wavelength, blinking frequency and intensity)
4. A "dark" control plate was covered in aluminum foil. experimental plates were placed on the tablet and grown in a 37°C incubator with or without shaking as dictated by the experiment.
5. Cell density (absorbance) and GFP output (fluorescence) were then measured using a plate reader. **(See Results)**

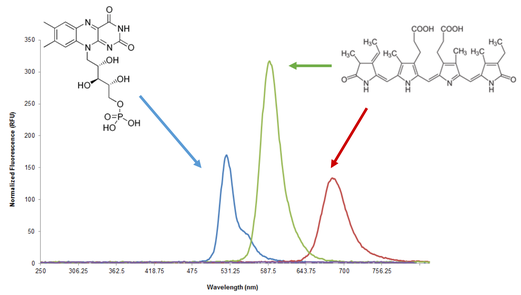


Our App

E.Colight 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 **vibration 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.

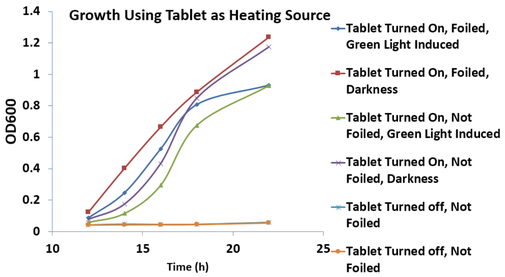
GET IT ON Google play

E. colight
Max Horowitz-Gelb
★★★★★ FREE

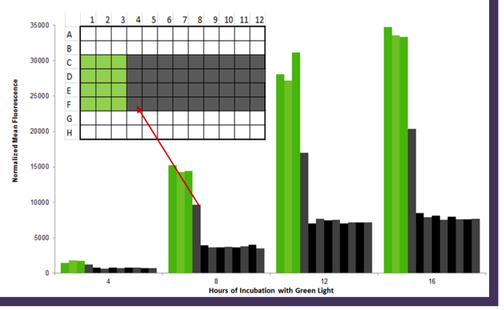


(Above) We conducted our experiments on the Samsung Galaxy Tab 7.7, which has a Super AMOLED display. Each pixel on a Super AMOLED display is made up of three LEDs corresponding to each of the colors in the RGB color format. This format is advantageous for the light inducible gene expression systems because to date, red, blue, and green light sensing systems have been reported. 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.

(Right) We tested the light bleed through between adjacent wells in a 96 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 (D5-E11) 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.

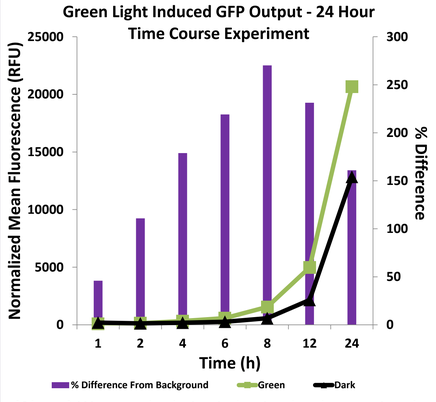


(Above) Heat generated by the tablet's processor while running the app can be used to incubate bacteria cultures. Growth was observed using the tablet as the sole heating element.

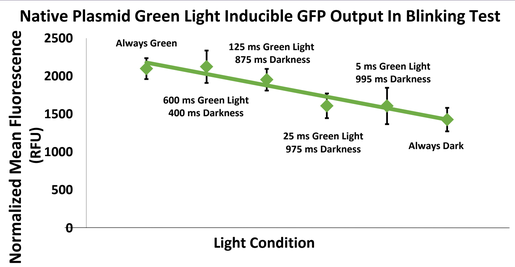


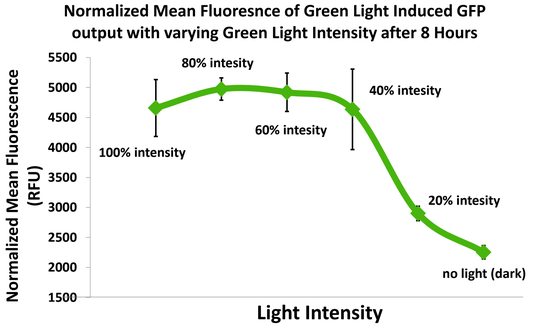
Results

(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.

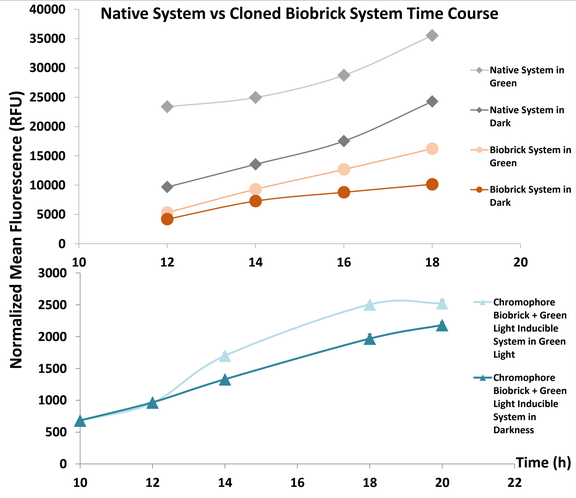


(Above) We tested relative induction levels over time by measuring the change of fluorescence over multiple time periods. **When exposed to green light continuously on a shaker in liquid culture over 24 hours, GFP levels in the system increased relative to the dark control. The largest difference between signal and background was observed after 8 hours.**





(Above) The effect of varying intensities of light on GFP induction levels was examined. Above 40% intensity, GFP expression levels appeared consistent. Below 40%, an exponential decrease in GFP expression was observed suggesting finer control can be obtained through flashing.



(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.

Team Members:

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