COSMOS UC San Diego
California State Summer School for Mathematics and Science

Testing the Effect of Different Blue-Light Brightness Levels on RFP Production in pDawn Transformed E.Coli

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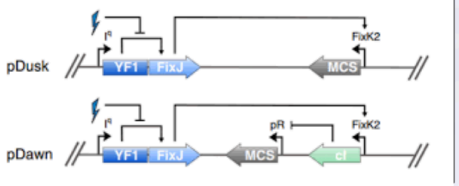
Introduction

Optogenetics is the control of organismal physiology/behavior by light. pDawn is one example of a plasmid that uses blue light to regulate light-induced gene expression. The gene expressed produces dsRed, or Red Fluorescent Protein(RFP). This protein is meant to glow either pink or red. The mechanism by which this works is an example of a histidine protein kinase, which employs a light-oxygen-voltage blue light photosensor domain. The main issue for synthetic organisms, like pDawn-transformed E.Coli, is that they rely on nonnative chromophores, which requires exogenous supplies. This specific experiment deals with finding optimal ranges of light intensity in pDawn and observing the effects.



Materials

Cell culturing

- Culture tube
- BL21 E.Coli with pDawn plasmid
- Nutrient Agar/LB/Kanamycin
- Petri dishes
- Micropipets/Sterile Loops
- Water bath shaker

Light house

- Styrofoam boards/Cardboard rings/Black tape/ Yogurt bowls
- Soldering iron
- Transistors/LED's/Wires/100K resistors
- Arduino

Procedures

Part 1 - Base pDawn Experiment

Culturing:

Refer to steps 1 and 2 of the base pDawn lab protocol.

- Ratio of Kan:LB is 31.25 microliters/mL. (468.75 microliters for 15 mL)
- Allow culture tube to incubate overnight

Preparing Agar plates:

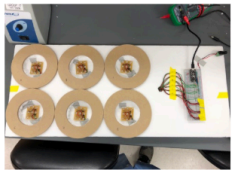
Refer to Lab 4 protocol, for technique to make the 12 Agarose gel plates

- Ratio of Kan:Agar is 1 mL/ 1L (360 microliters for 360 mL)
- Ratio of Agar:Water is 23 g/ 1L (8.28 g for 360 mL)

Growing Plates:

- Remove culture tube from incubator
- Transfer 100 microliters from culture to twelve agar plates, spread using sterile loop
- Label as "pDawn+ Slim Shady"
- Grow overnight in incubator at 37 degrees Celsius
- Testing Plates - Isolate one and shine light directly on sample for >7 hours

Part 2 - Light Housing Schematic



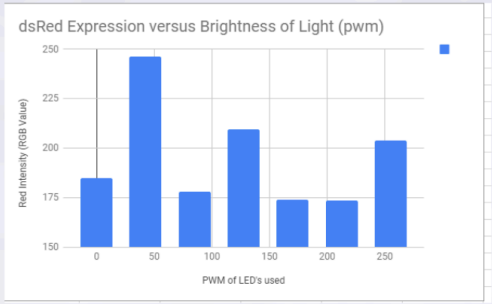
Part 3 - Testing/Analysis

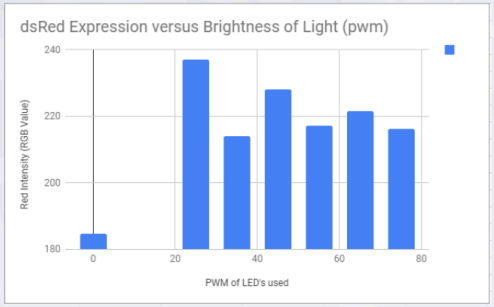
Testing:

- Turn on lights at specified intensities and pulse rates using Arduino
- Cover plates and leave overnight
- Take pictures of each plate, placing three rulers to space camera 1cm and create 1 cm square

Analysis of pictures:

- Open ImageJ and make a 1 cm by 1 cm rectangle macro of colony
- Analyze using grayscale to determine fluorescence intensity





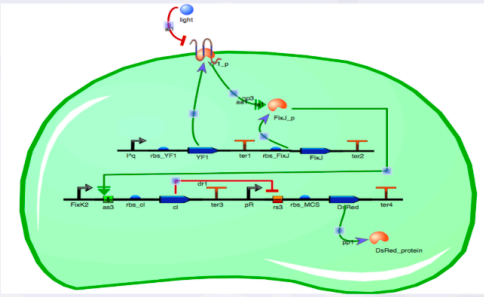
Question

Is light regulated gene expression in pDawn a binary or a spectrum? What will be the effect of different light intensities and pulsation rates on said gene expression?

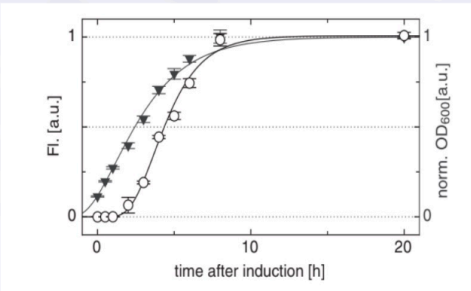
Hypothesis

Gene expression is best modeled by a spectrum for pDawn. More light exposure will increase gene expression.

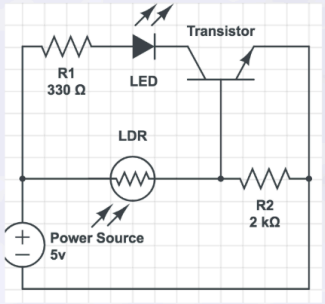
Genetics:



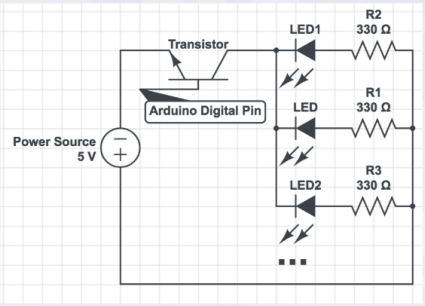
Reference results:



Electrical Model:



Testing Device (light house):



works Cited

Results and Analysis

Trial One

Using six different intensities of light (42.5 to 255 PWM) we found that the lowest PWM light (42.5) provided the most visible amount of RFP, with an ImageJ RGB value of around 246/255 on the redscale. The team concluded the other samples were bleached by excessive light. The glowing colonies were also only located on the edge of the lawn. The group thought that this could be either from extra light diffracting from the agar, or the fact that there are less layers of bacteria on the edges, which creates a more consistent red hue.

Trial Two

The group modified their experiment for Trial Two to try and solve the two issues of excessive light and edge-exclusive glowing. First, they used a smaller light range of 25-75 PWM's. Also, they moved the plates to have the LED's shine mostly on the edge of the lawn. These changes resulted in a higher mean Red value than the previous trial, but no one sample from Trial 2 produced more RFP than the one exposed to 42.5 PWM in the first trial. The second trial also lasted about 3 and a half extra hours, which the group also noted as something that could have caused the change in data trends

Discussion

Trial 1 suggested a possibility of intense light harming the samples. This is actually inconsistent with the initial hypothesis, that more light always equalled more RFP. This raised an important doubt for the team. The team then revised their hypothesis to say that there is a "Goldilocks" type range, rather than a limitless model. That is to say, optimal expression is hindered by both excessive and diminutive amounts of light. This is consistent with the literature's graph, shown to the right. Trial 2 again went against the group's initial hypothesis. This is because the data trends represented by the bar graph to the right illustrate a decline in expression when more light is added. This supports the new "Goldilocks" hypothesis, because the higher levels were still greater than 42.5 PWM's.

Conclusion

Theory

From the data collected and the Tinkercell model (Shown to the left), the group's hypothesis is not supported by the evidence. The mechanism seems to lean more towards a binary model, with a window of voltage that could be modeled better by a gradient. However, the results are best characterized as inconclusive, as the experimental design still needs revision/improvements, and needs to be done with a wider range of light intensities on a larger scale, to have a more comprehensive data set. In addition, the brightness having an inverse effect on expression also ran contrary to the group's hypothesis.

Recommendations for Future Trials

The group has many recommendations for those who attempt similar tests in the future. First, use culture tubes and a spectrophotometer as opposed to plates/software. Second, increase the distance between the LED's and the samples, as the proximity could also bleach the samples more than simple light power. Third, allow for ample time and use an abundance of resources. The experiment was severely limited by a short time frame and low budget. Finally, if all other recommendations are met, future trials should also test the effect of blinking light on the expression of the gene.

Acknowledgements: **Dr. Mauricio de Oliveira**
Dr. Carlos Vera
Aidan Zhang
Jacob Whitsel
Trudy Pachon
Kritin Karkare
Rupin Mittal