Engineering a Real-Time Living Biosensor: DNA Damage Caused by Ultra-Violet Irradiation

Craig Barcus[1] Jessamine Osborne[2] Erin Rosswurm[1] Janie Stine[1] Advisors: Dr. Jenna Rickus[1,3,4] Dr. Kari Clase[4,5] David Jaroch[3]

1: Agricultural and Biological Engineering, 2: Biological Sciences, 3: Weldon School of Biomedical Engineering, 4: Bindley Bioscience Center, 5: Industrial Technology

Purdue University, West Lafayette, IN

Motivation

- 1 million Americans are diagnosed with skin cancer every year.[1]
- Current products measure UV radiation level, *not* DNA damage.
- Our Goal: create a real-time sensor of DNA damage

Approach

- "If-Then" Construct: Promoter + reporter
  - -Promoter: *recA* of SOS system, part J22106 (activated for extreme DNA damage)
  - -Reporter: *lacZ,* part I732017 (blue/white screening on X-gal)
- *If* DNA is damaged extensively, *then* transcription of β-gal
- Essentially a reporter-gene assay

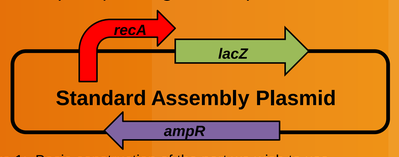
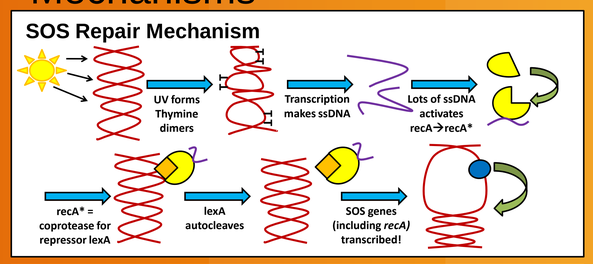
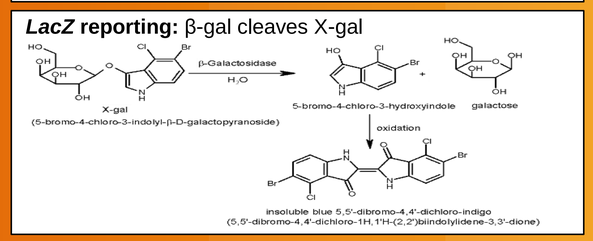


Figure 1: Basic construction of the part we wish to use.

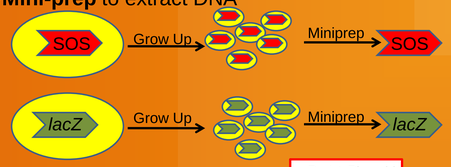
Mechanisms





Standard Assembly of the Part

- Received stabs of TOP10 cells from iGEM:
  - -*LacZ* (I732017) and *recA* promoter (J22106)
  - -Parts on standard pSB1A2 plasmids (AmpR)
- **Grow up** to make glycerol stocks
- **Mini-prep** to extract DNA



- **Digestion:**
  - -SOS: EcoRI & SpeI
  - -*LacZ*: EcoRI & XbaI
  - -Specific Enzymes
  - → SOS **in front of** *LacZ*
- **Purify** from 1% agarose gel

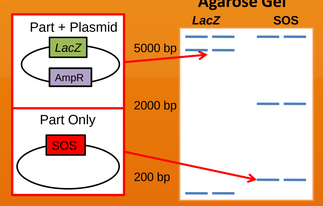
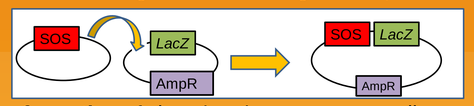


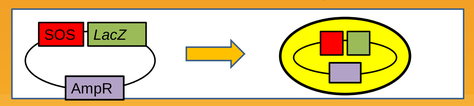
Figure 2: The construction and purification of our parts.

Building and Testing the System

- **Ligation** with T4 ligase to create engineered plasmid



- **Transformation** of clone into *lac*- competent cells



- **Plate** on Ampicillin/X-gal
- **Dose** with UV light to test abilities
- **Miniprep/Digest** to check successful assembly

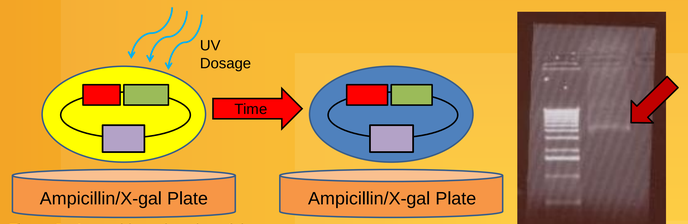


Figure 3: Assembly and testing of the part.

Modeling the SOS System

Assumptions:

- The UV irradiation is instantaneous, with a dosage of 5 J / m^2.
- The bacteria are not undergoing DNA repair at the time of irradiation.
- Thymine dimer formation by adjacent thymines is the major DNA damage occurring.
- The equilibrium point between RecA and RecA* is considered to be full induction of the SOS system.

$$\frac{d[LexA]}{dt} = \frac{a_L}{1+K_L*C_L} - b_L*C_{R^*}*C_L - e_L*C_L$$

$$\frac{d[recA]}{dt} = \frac{a_R}{1+K_R*C_L} - e_R*C_R - b_R*C_R*C_S + b_{R^*}*C_{R^*}$$

$$\frac{d[recA^*]}{dt} = b_R*C_R*C_S - b_{R^*}*C_{R^*}$$

$$[C_S] = \frac{S}{6*10^8}$$

- a_i is rate of production of i in absence of repression,
- b_i is the binding constant of component I to its activator,
- e_i is the inverse rate of breakdown of component i.
- L relates to LexA, R to RecA, R* to RecA* and S to ssDNA
- K_i is the binding constant of component i to the LexA gene.
- C_s is the concentration of single stranded DNA.

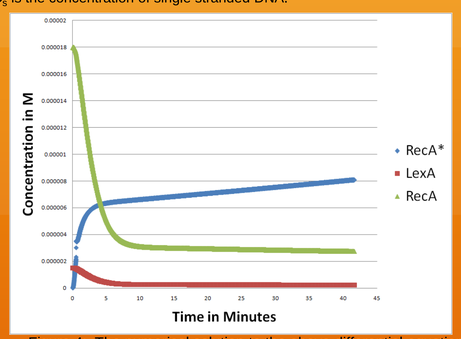


Figure 4: The numerical solution to the above differential equations. RecA and RecA* are equal at 4.2 minutes. This becomes time 0 for Xgal cleavage.

Predicted Response Time: Tens of Minutes

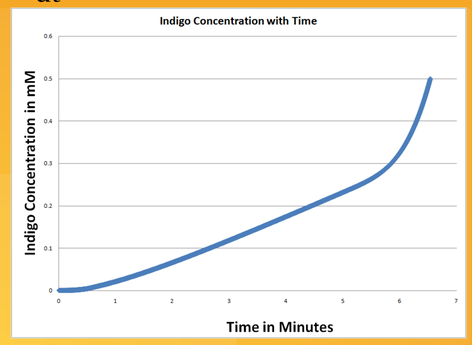
Assumptions:

- The bacteria have been on the X-gal plate sufficiently long for the X-gal to be in equilibrium with the cell.
- There is no diffusion limitation with this system.
- Half-life of beta-gal is 60 minutes. (Bachmair et al. 1986).
- The amount of X-gal within the cell exceeds Km (500mM to 0.2 mM) so that the rate of indigo formation is Vmax.
- Vmax is a function of Kcat and the concentration of the enzyme.

$$\frac{d[indigo]}{dt} = \frac{Vm*[Xgal]}{Km+[Xgal]} \Rightarrow \frac{d[indigo]}{dt} = V_m \quad Vm = K_{cat}*[\beta gal]$$

$$\frac{d[indigo]}{dt} = K_{cat}*[\beta gal]$$

Equation solved using Runge-Kutta 4th order in Mathcad



Next Steps

- Perform additional experiments comparing UV radiation to SOS signal
- Compare and refine theoretical model
- Perform experiments with bacteria under UV with different SPF levels
- Develop a sustainable bacterial gel with a shelf life of >3 months
- Create a bio-sensor patch

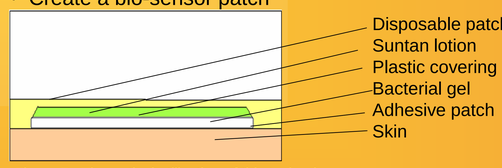


Figure 7: Theoretical utilization of this technology

Safety

- Negligible safety issues with project:
  - - Pre-engineered *E. coli* cannot sustain a population outside lab environment
  - - Biological Safety Level I only (low risk)
- Project reviewed and approved by the Institutional Biosafety Committee (IBC)
  - - IBC oversees rDNA research at Purdue
  - - No characteristics of protocol are considered hazardous

Acknowledgments

Materials and Support Provided By:

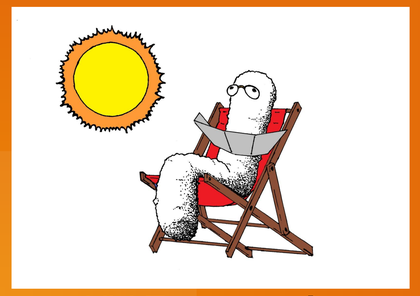
- Biology Teaching Labs
- Simran Banga
- Bob Stephenson
- Dr. Kari Clase
- Dr. Luo
- Dr. Jenna Rickus
- Dr. Larisa Avramova

Funding Provided By:

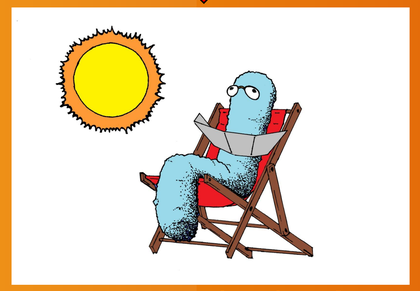
- College of Agriculture
- College of Engineering
- Agricultural and Biological Engineering Department
- Bindley Bioscience Center
- Oncological Sciences Center

References

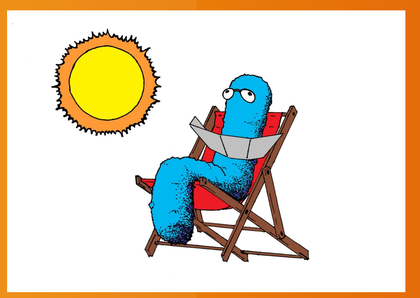
1. Aksenov, S. V., E. A. Krasavin, et al. (1997). "Mathematical model of the SOS response regulation of an excision repair deficient mutant of Escherichia coli after ultraviolet light irradiation." Journal of Theoretical Biology **186(2): 251-260.**
2. Berg, J. T., JL. Stryer, L. (2006). Biochemistry. New York, NY, W.H. Freeman and Company.
3 .bios.niu.edu/johns/recdna/blue_white.htm.
4. Sharp, A. K., G. Kay, et al. (1969). "KINETICS OF BETA-GALACTOSIDASE ATTACHED TO POROUS CELLULOSE SHEETS." Biotechnology and Bioengineering **11(3): 363-&.**
5. Warner, J. B. and J. S. Lolkema (2002). "LacZ-promoter fusions: the effect of growth." Microbiology-Sgm **148: 1241-1243.**



UV Exposure→
DNA damage



SOS Induction→
LacZ Transcribed



LacZ Reporting →
X-gal Cleavage