Penn State's iGem team presents:

BACTERIAL DOSIMETER

(ONLY MY MOM CALLS ME THAT. YOU CAN CALL ME *Radicoli!*
I DETECT LEVELS OF HARMFUL RADIATION.)

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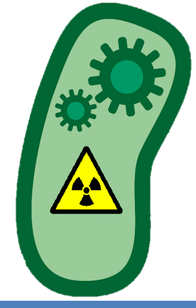
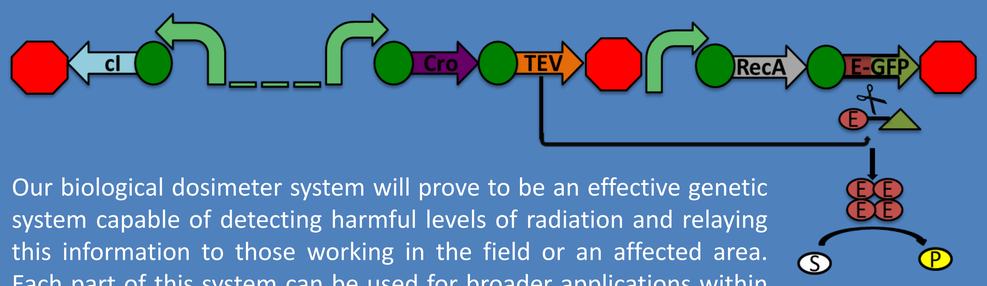


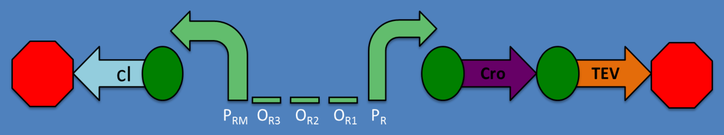
Project Abstract

Ionizing radiation and radiation pollution are significant environmental problems. Radiation pollution affects those working in nuclear power facilities as well as those who are exposed due to nuclear disasters similar to the Fukushima Daiichi nuclear reactor malfunction. The Penn State team introduced a genetic circuit into E. coli cells in order to rapidly detect and report the presence of harmful ionizing radiation. We worked to develop a robust and reliable biosensor that utilizes the lambda phage lytic-lysogenic switch coupled with a fast-acting reporter capable of producing an easily visible warning sign. We believe that the final construct may have the potential to rival current radiation detection devices, such as digital dosimeters.



Our biological dosimeter system will prove to be an effective genetic system capable of detecting harmful levels of radiation and relaying this information to those working in the field or an affected area. Each part of this system can be used for broader applications within the field of synthetic biology.

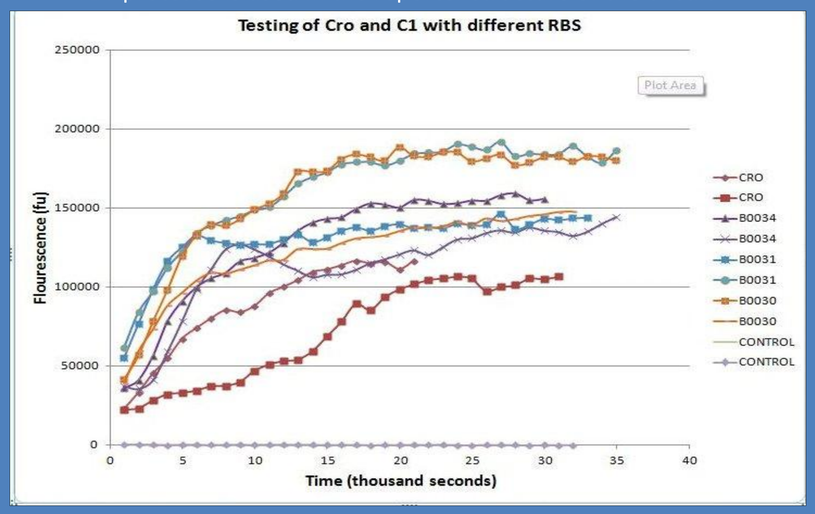
Sensor: λ Phage Switch



Design based on the lambda phage lysogenic vs. lytic switch
- Originally outlined by Penn State 2007 iGEM team
- System is activated by RecA in presence of DNA damage

Normally, P_{RM} is active and transcribes the lambda repressor (cI)
- cI binds to O_{R1} and O_{R2} and represses the P_R
- Transcription of Cro and TEV is inhibited

In the presence of DNA damage, ssDNA binds to RecA and activates the sensor
- RecA cleaves cI repressor and P_R is activated
- Cro and TEV are transcribed
- Cro binds to O_{R3} and P_{RM} is inhibited
- TEV protease is used in the reporter structure of the circuit



We measured the fluorescence of a series of test constructs we created to ensure the repression of Pr via the CI repressor. Here the CI repressor concentration was fine-tuned by 3 different RBS strengths and showed increasing levels of repression with stronger binding sites (higher conentration). This shows how our system can be used to detect various thresholds of radiation.

Activator: RecA

RecA is a protein used by E. coli to detect and repair damaged DNA. Mutations were necessary to create a RecA gene that will not recombine DNA, will be compatible with standard bio-bricking techniques, and still binds to ssDNA.

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W:  ATG...CTGCAG...CAT...CGC...GAATTC...AAA
M:  ATG...CTGCAT...CGT...CAC...GAATTT...AAC
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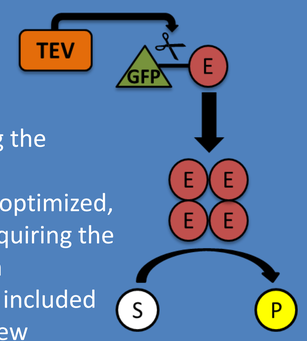
RecA Sequence

W = Wild type RecA
M = Mutated RecA
Red = BioBrick restriction sites
Cyan = RecA activation
Green = Arg 243
Magenta = Lys 286

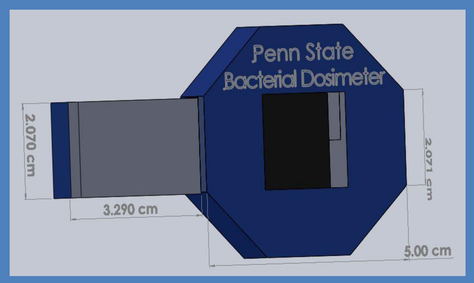
--Kawashima,H., Horii,T., Ogawa,T. and Ogawa,H. Functional domains of Escherichia coli recA protein, Mol. Gen. Genet. 193 (2), 288-292 (1984)

Reporter Project

- Cloned and created a catalog of the individual fusion parts included in the fast acting reporter BBa_K316007 from the Imperial College London 2010 iGEM team
- These parts can be quickly assembled using the Freiburg BioBricking Assembly technique
- New variations of this part can be created, optimized, and applied to many different situations requiring the use of a rapid reporter in a "in vivo" system
- Other proposed modifications by our team included the use of new fusion protein linkers and new enzyme subunits, specifically the β-glucuronidase reporter



Dosimeter Design



We designed a portable and reusable dosimeter that has a reusable shell and a one-time use tray. Our bacteria would live in gellan gum, which was designed and used by the BSC Bristol 2010 iGEM team, on the tray.

The cost analysis equation for our bacterial dosimeter is:

$$Cdosimeter = (V*Cplastic) + [n*(Cecoli + Cgellan + Cplastic) + Ccatechol$$

Based off this equation, the total cost for one time use of our device would be $10 plus cost of reporter reagents (Catechol). This price is based on using Acrylic plastic, which is UV resistant, at $30 per sheet. Additionally the cost of the Gellan Gum (Phytagel) and our bacterial are approximately $0.40 per tray. Remember, however, that upon multiple uses the marginal cost per use will decreases because the only additional costs per use will be a new tray.

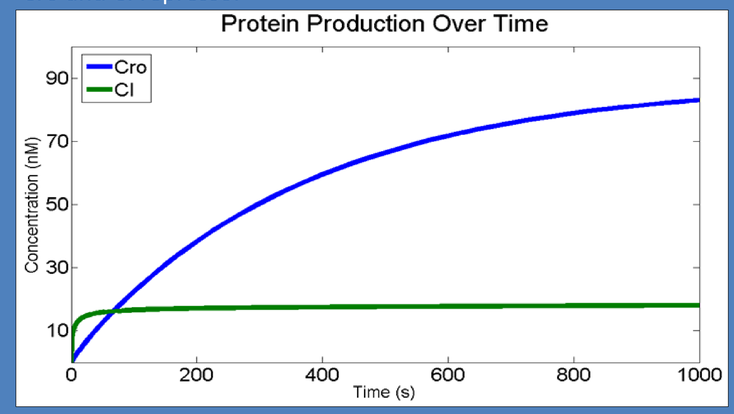
Modeling

$$\frac{dR}{dt} = \{\langle RNAP\ at\ P_{RM}\rangle_1 k_{P_{RM_1}} + \langle RNAP\ at\ P_{RM}\rangle_2 k_{P_{RM_2}}\} A_R - [R] k_{Rd}$$

The above equation corresponds to the rate of repressor production, R, that occurs at P_{RM}, while the following equation corresponds to the rate of Cro production at P_R.

$$\frac{dC_2}{dt} = \langle RNAP\ at\ P_R\rangle k_{P_R} A_{C_2}$$

By solving this simultaneous system of nonlinear differential equations, the production rate of protein at P_{RM} and P_R can be determined which in the case of the lambda phage consists of Cro and CI repressor

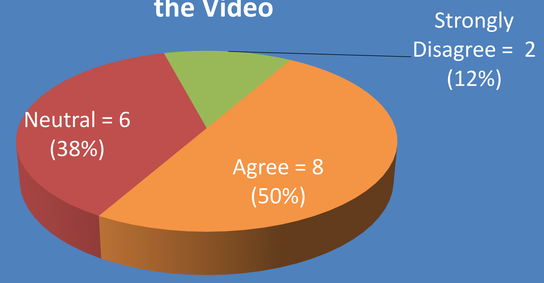


The above graph shows the concentration of Cro protein and CI repressor over time. As can be expected, both repressor and Cro are initially produced, but as CI degrades or is cleaved by RecA, its rate of production approaches zero due to the inhibitory effects of Cro on O_{R3}.

Human Practices

The Penn State team developed a film that explains synthetic biology in an understandable way for any general audience. In order to provide a unique human practices project, the video focused on reaching an elderly audience, a demographic unaddressed in previous iGEM competitions. A survey was given In order to test whether the video was effective in teaching synthetic biology.

I Better Understand Synthetic Biology from Watching the Video



50% of the audience gained a better understanding of synthetic biology from the film. But over 10% did not…