Project: “Bacterial Dosimeter”

Goal: Detecting Radiation through DNA damage

Genetic Circuit:
Project: “Bacterial Dosimeter”

- Activator: RecA
- Sensor
- Reporter
- Additional Work
  - Dosimeter Device Design
  - NYC_wetware Collaboration
  - Human Practices
- Conclusions
Activator: RecA

- RecA is a protein found naturally in E. coli

Functions:
- Repairs DNA damage through recombination
- Cleaves Lambda phage cl repressor

Goal: To use RecA to activate our genetic system through its proteolytic activity.
Activator: RecA

- Challenges:
  - Common laboratory strains (DH10β) contain inactivated RecA mutation: “RecA1”
  - Not compatible with standard Biobrick assembly methods
Activator: RecA

Our question:

How to prevent recombination and loss of cloned genes, while maintaining proteolytic activity of RecA?
Activator: RecA

- **Solutions**

W: ATG...CTGCAG...CAT...CGC...GAATTTC...AAA
M: ATG...CTGCAT...CGT...CAC...GAATTTC...AAC

W = Wild type RecA
M = Mutated RecA
Red = BioBrick restriction sites

Cyan = RecA activation
Green = Arg 243
Magenta = Lys 286
Activator: RecA

- Test circuit was developed to determine if the mutations were successful.
- Mutations successful: RecA cleaves the cl repressor.
- Mutations fail: homologous double terminators will recombine.
Sensor: $\lambda$ Phage Switch

- Design based on the lambda phage lysogenic vs. lytic switch
  - Based on design by Penn State 2007 iGEM team
  - Switch is activated in presence of DNA damage

Circuit under normal conditions:

```
cl + RBS  P_{RM} O_{R3} O_{R2} O_{R1} P_{R}  RBS + Cro  RBS + TEV  B0015
```
Sensor: λ Phage Switch

DNA Damage Due to Radiation

RecA with ssDNA

cl + RBS

$P_{RM}$ $O_{R3}$ $O_{R2}$ $O_{R1}$ $P_R$

RBS + Cro

RBS + TEV

B0015

DNA Damage Due to Radiation
Sensor: λ Phage Switch

- Attached three different strength ribosome binding sites to the cl repressor gene to vary the translation rate
  - Higher RBS strength on cl repressor → higher threshold of radiation required to activate the switch
Reporter Project

- Original Part: BBa_K316007
- Part utilizes reporter enzyme immobilized by fusion to GFP
- Cloned and created a catalog of the individual fusion parts included in the original form of the part created by the Imperial College London 2010 iGEM team
Reporter

Reporter System Characteristics:
1. Fast-acting
2. Color Pigment Output
Fusion Protein Variations
Fusion Protein Variations
Reporter Project

XylE
- Codes for Catechol (2,3) dioxygenase (C230)
- C230 catalyzes the conversion of colorless Catechol into 2-hydroxymuconic semialdehyde.

GusA
- Codes for β-glucuronidase (GUS)
- X-Gluc is a colorless substrate which is converted to the same bright blue product as LacZα.
Xyle Mutation

- Original Xyle gene sequence contains two NgoMIV sites and one AgeI site
- Not compatible with Assembly 25 methods
Xyle Mutation

- Synonymous mutations were created at each site

Green = Xyle; Yellow = Assembly 25 Scar; Blue = Linker; Red = Suffix restriction sites
Modeling

- Initial repression model of the operating region promoters.
- Model was too simple, and more work needed to be done.
• The new model takes into account binding of Cro and RNAP to the Operating Region.
• This graph shows the concentration of Cro and CI proteins over time after the system is activated.
Or Data

We measured the Pr promoter on the operating region of the sensor part by using RFP.
Dosimeter Design

- Design goal: portable, durable, and reliable
- Reusability: camera and film pairing.
  - Hollow case made out of a hard plastic \( \rightarrow \) camera
  - Tray will be for one-time use \( \rightarrow \) film
- After use the tray will be developed in catechol, etc. to show the different levels of radiation present.
Collaboration with NYC-Wetware

![TS TR E. Coli Irradiation Graph](image)

- Control
- TS TR
Human Practices

○ (Re) Designing Life is a video designed to explain synthetic biology in an understandable way for a general audience.
Video: Audience

- Demographic: the Elderly
  - Untapped audience
  - Active voters

- Presentation and Survey
Video: Survey Results

I Have a Better Understanding of Synthetic Biology as a Result of Watching this Video

- Agree = 8 (50%)
- Neutral = 6 (38%)
- Strongly Disagree = 2 (12%)
Improvements to the Video

- More mature tone
- Less abstract analogies explaining synthetic biology techniques
# Parts Submitted

## Parts Submitted to Registry

<table>
<thead>
<tr>
<th>Part Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K648000</td>
<td>mCherry with terminator</td>
</tr>
<tr>
<td>BBa_K648005</td>
<td>Short Fusion Protein Linker</td>
</tr>
<tr>
<td>BBa_K648006</td>
<td>Long 10AA Fusion Protein Linker with Standard 25 Prefix/Suffix</td>
</tr>
<tr>
<td>BBa_K648008</td>
<td>TEV protease cleavage site with Standard 25 Prefix/Suffix</td>
</tr>
<tr>
<td>BBa_K648008</td>
<td>RecA Cleavage Site with Standard 25 Prefix/Suffix</td>
</tr>
<tr>
<td>BBa_K648011</td>
<td>Standard 25-Ready Xyle Reporter</td>
</tr>
<tr>
<td>BBa_K648013</td>
<td>GFP with Standard 25 Prefix/Suffix</td>
</tr>
<tr>
<td>BBa_K648028</td>
<td>Cro, Lamda Repressor which activates the lytic cycle</td>
</tr>
<tr>
<td>BBa_K648029</td>
<td>OR, operating region for Lambda switch</td>
</tr>
<tr>
<td>BBa_K648102</td>
<td>RecA (mutation in the amino acid Lys 286)</td>
</tr>
<tr>
<td>BBa_K648101</td>
<td>RecA</td>
</tr>
</tbody>
</table>
Where we are now:

RecA:
- First addition of RecA part to registry
- Introduced all five mutations into one plasmid
- Assembled complete testing construct
- Unable to test mutated RecA plasmid with test construct due to time constraints
Where we are now:

- **Sensor:**
  - Characterized Pr promoter of the operating region
Where we are now:

- Reporter:
  - Cloned each individual subunit.
  - Assembled intermediates.
  - Unable to characterize all expected reporter systems due to time constraints.
Labwork Timeline:

First Assembly | Mutated Xyle | Successfully Cloned RecA | Characterized Or Part | Completed RecA mutations | Jamboree | Sensor Testing: Gamma Facility | Characterize Complete Reporter Variations

Cloned final reporter subunit: GusA | RecA Test Circuit | Test RecA Mutant Combinations
We would like to thank our advisors Dr. Tom Richard, Dr. Howard Salis, and our mentor Mike Speer.

Penn State 2011

Additionally we would like to extend our gratitude to all of our sponsors!