**THE TOLUENE TERMINATOR**
**UNIVERSITY OF MICHIGAN**

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**Project Description**

In-Situ Toluene Remediation

Toluene is a toxic substance used in petrol, paint, paint thinners and adhesives. Through spills and improper disposal, toluene can contaminate soil and ground water environments. Using microorganisms to clean up toluene-contaminated sites can be an effective and economical way of degrading the pollution before it can spread throughout the environment. There is concern, however, that these non-native microorganisms may upset the balance of the ecosystem through unnatural competition or horizontal synthetic gene transfer. We are engineering the Toluene Terminator as a way to neutralize toluene pollution while addressing these concerns. It will have the capabilities of sensing and mineralizing the toluene into carbon dioxide and water, but this terminator will not be back. The Toluene Terminator will have a suicide mechanism which kills the bacteria in the absence of toluene.

**Background**

Environmental contamination due to fossil fuels is a growing problem in many economies, harming agricultural interests and endangering public health. Bioremediation is an increasingly attractive means of cleaning up contamination. We aim to demonstrate the feasibility and efficacy of bioremediation through construction of a specific example—a Pseudomonas putida strain capable of eliminating toluene contamination. This device will require four basic modules: chemoreognition, chemotaxis, toluene uptake and metabolism, and a synthetic suicide circuit.

**Chemoreognition and Chemotaxis.** The construction of a final device will require all three modules, as well as additional regulatory modules so that the system can be tuned to optimize its performance. Though initial Pseudomonas mobility experiments showed encouraging results, we focused this year on the toluene metabolism and the suicide circuit. The toluene degradation mechanism is in place on the PWW0-TOL plasmid of the P. putida mt-2 strain; we seek to isolate the relevant genetic elements and characterize their function in E. coli before building a device in Pseudomonas. Likewise, we will test the efficacy of the suicide module in both E. coli and P. putida. The construction of a final device will require all three modules, as well as additional redundant kill switches.

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**Degradation Module**

**Regulation of Natural Degradation**

The goal of this project is to work with the toluene degrading abilities that already exist in P. putida mt-2 (ATCC 33015) on the PWW0-TOL plasmid. The pathway on this plasmid is composed of the upper pathway, where toluene is metabolized into catechol, and the lower meta-pathway, where catechol is converted into acetaldehyde and pyruvate. The regulation of toluene degradation on the plasmid is presented below.

**Toluene Sensor**

Our project is aimed at characterizing the Pu promoter to sense toluene. In order to characterize this promoter we created Bba_K270003, a device that contains the Pu promoter expressing GFP. To have this device function in strains other than P. putida mt-2 which already contains the XylR regulating protein, the part Bba_K270001 was created from the Pu promoter portion of the PWW0 plasmid to regulate the Pu promoter. By combining the functions of these two parts, the GFP fluorescence can be used to measure the promoter activity when induced with non-lethal levels of toluene.

**Kill Switch Module**

This suicide module utilizes the Pu promoter, which senses toluene, inputting to an inverter system containing a repressor gene and the promoter it represses. This outputs to the suicide operon, resulting in cell death in the absence of toluene. This Switch requires the culture to be grown in the presence of toluene, but allows the tunability of suicide operon expression based on the concentration of toluene to Pu promoter.

**Kill Switch with Tunable Expression**

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**Nitratre-Dependent Kill Switch**

This suicide module exploits a nitrate-dependent promoter driving expression of the holin and lysozyme proteins, followed by the Pu promoter driving the antiholin gene. When the device is released into the contaminated area, naturally-occurring nitrate stimulates expression of lysoze genes that are posttranslationally repressed by antiholin—until the toluene runs out. This design requires no special growth conditions, and ensures that the device cannot leave the contaminated area.

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**Meet Our Team!**

**Students:**
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- Nasir Fakhri
- Ian Faulkner
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**Advisors:**
- Xiaoxia (Nina) Lin
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**Nitrate-Dependent Kill Switch Modeling**

The following mathematical model examines the dynamics of the Suicide Module with Tunable Expression. See the Kill Switch Module section for further background information.

**Cell Growth and Death**

Assumption: Rate of Cell Death = Rate of (number of cells).

**Rate of Cell-Growth:**

Using the translation rate for , and taking into consideration the binding of with : 

\[
\frac{dL}{dt} = \gamma_L \cdot \frac{H_L}{k_{dH}} - \frac{dL}{dt} \cdot \frac{k}{k} - \frac{dL}{dt} \cdot \frac{k}{k}
\]

**Rate of Cell Death:**

Because lysozyme and holin are downstream of the repressor, we can set if all operating sites are free.

\[
\frac{dH}{dt} = \gamma_H \cdot \frac{H_H}{k_{dH}} - \frac{dH}{dt} \cdot \frac{k}{k} - \frac{dH}{dt} \cdot \frac{k}{k}
\]

**Production of Proteins**

Antiholin is produced constitutively at a constant rate. Since holin and antiholin form a dimeric complex:

\[
+ \cdot \frac{dH}{dt} = \gamma_H \cdot \frac{H_H}{k_{dH}} - \frac{dH}{dt} \cdot \frac{k}{k} - \frac{dH}{dt} \cdot \frac{k}{k}
\]

**Repression of Lysozyme and Holin Transcription**

Assuming transcription rate is proportional to the number of free promoter sites:

\[
\frac{dH}{dt} = \frac{dH}{dt} \cdot \frac{H_H}{k_{dH}} - \frac{dH}{dt} \cdot \frac{k}{k} - \frac{dH}{dt} \cdot \frac{k}{k}
\]

Because lysosome and holin are dimerizations of the same promoter, hence transcription rate is equal to two.

\[
\frac{dL}{dt} = \frac{dL}{dt} \cdot \frac{H_L}{k_{dH}} - \frac{dL}{dt} \cdot \frac{k}{k} - \frac{dL}{dt} \cdot \frac{k}{k}
\]

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**Future versions of this model will include coupling effects of toluene metabolism and kill switch dynamics—that is, kill switch behavior in slowly diminishing levels of toluene. For a list of variables and parameters involved in this model, please refer to our wiki page. For modeling of holin-antiholin function, refer to Berkeley's iGEM 2008 project.**