Meet the team
Biofuels
Biosensors
Tuning Biosensors
Sharing
Biofuels can help solve the energy crisis.
But... analysis requires high-tech equipment.
Biofuels

How can iGEM teams detect biofuels?
What are biosensors and how can they help?

Natural sensor system + Reporter gene = Biosensor
We can sense ethanol with 2 components.

How can we make our sensors better?
Biofuels
How can iGEM teams detect biofuels?

Biosensors
How can we improve our biosensors?

Tuning Biosensors

Sharing
Directed evolution with K6340007

RFP \textit{sacB} \textit{kan}^R
Mutated sensors

![Graph showing fluorescence against concentration of ethanol (v/v)](image)

- Graph legend:
  - Green line: Uninduced
  - Red line: Induced

- X-axis: Concentration of Ethanol (v/v)
- Y-axis: Fluorescence
Mutated sensors \rightarrow Lower leak

![Graph showing fluorescence vs. concentration of ethanol](image)

- **Uninduced**
- **Induced**

**Concentration of Ethanol (v/v)**

- 0.0%
- 1.0%
- 2.0%
- 3.0%
- 4.0%
- 5.0%

**Fluorescence**

- 0
- 10
- 20
- 30
- 40
- 50
- 60
- 70
- 80
- 90
- 100
Mutated sensors → Lower leak → Higher peak

Graph:
- Y-axis: Fluorescence
- X-axis: Concentration of Ethanol (v/v)
- Two lines:
  - Green line: Uninduced
  - Red line: Induced

Fluorescence increases with increasing concentration of Ethanol.
Mutated sensors → Lower leak → Higher peak → Quantify survivors

Fluorescence vs. Concentration of Ethanol (v/v)

- Uninduced
- Induced
Sucrose sensitivity?

Graph showing the growth of bacterial cultures over time in different sucrose concentrations. The x-axis represents time in hours, ranging from 0 to 21, and the y-axis represents OD₆₀₀, ranging from 0 to 0.8. Different concentrations of sucrose (0%, 0.01%, 0.03%, 0.05%, 0.10%, 0.50%, 1.00%, 2.50%, and 5.00%) are indicated by color-coded lines. The graph compares the growth of cultures with and without sucrose, with variations in growth rates and final OD values at different concentrations.
Sucrose mutants

---

OD\textsubscript{600}

Time (h)

- Initial K634007 culture
- Post-counter selection cells
- Plasmid of post-counter selection cells in new DH10B

RFP sac\text{\textit{B}} kan\textsuperscript{R}
Evolve anything!

J23100 Responsive promoter

sensor genes

RFP sacB kan^R
Biofuels
  How can iGEM teams detect biofuels?

Biosensors
  How can we improve our biosensors?

Tuning Biosensors
  Can directed evolution help iGEM teams?

Sharing
Directed Evolution

Reporters:

A1
A2
A3
I have all these mutants...

Now what?
Introducing mutant libraries
Introducing mutant libraries

Part:BBa_K634007
Designed by Eric Walters  Group: iGEM11_Wisconsin-Madison  (2011-09-23)

Double selection cassette (tagRFP-sacB-kanR)
Introducing mutant libraries

**exaDE**
The sensor kinase ExaD responds to the presence of ethanol within the cell and phosphorylates ExaE. The phosphorylated ExaE then acts as an activating transcription factor on PexaA. The operon was studied in *Pseudomonas aeruginosa*, and found to be under transcriptional regulation by the protein AgmR (1). It was also established that by expressing exaDE under a different promoter, the system could proceed in the absence of AgmR (ibid).

This part is a promoterless monocistronic operon, ready to be put under a desired promoter. When expressed in a cell containing part K634008, genes 5' of K634008 can be expressed in the presence of ethanol.

**Mutant Library**
Hover over each offspring to see more detailed information and/or edit the library.

**Offspring A**
This mutant is from a library created through Agilent’s GeneMorph II kit, introducing single nucleotide mutations at an estimated 1/kb. The mutagenesis was performed upon the part K634008 (exaDE), and was screened using part K634007 (double selection cassette) under the exaDE-responsive promoter, PexaA. This mutant is notable due to the following observations:

- Red fluorescence: 5% increase over parent
- Sucrose negative selection: no altered growth in sucrose-containing media from parent
- Kanamycin selection: Survival observed at 8% higher kanamycin concentrations than parent

**Offspring B**
This mutant is from a library created through Agilent’s GeneMorph II kit, introducing single nucleotide mutations at an estimated 1/kb. The mutagenesis was performed upon the part K634002 (exaDE), and was screened using part K634007 (double selection cassette) under the exaDE-responsive promoter, PexaA. This mutant is notable due to the following observations:

- Red fluorescence: 2% increase over parent
Mutagenesis Tree

Offspring B
- Parent: exaDE (K634002)
- Siblings: A
- Offspring: B1, B2, B3
- Edit This Library
Better organization equals better registry
Biofuels

How can iGEM teams detect biofuels?

Biosensors

How can we improve our biosensors?

Tuning Biosensors

Powerful in theory, what comes next?

Sharing

New tools require new technologies.
Increasing awareness
Increasing awareness
Increasing awareness

@LabBadger
Tremendous thanks to:
The College of Engineering
The students of the Pfleger lab
The GLBRC, NSEC, and MRSEC
The UW-Madison REU program
iGEM HQ and the registry
John Greenler and the GLBRC
Questions?
(I know you have some)