Evolving Biosensors
UW-Madison 2011  Enhance your senses!
Meet the 2011 UW-Madison iGEM team
Madison is a major center for biofuel research.
Biofuels can help solve the energy crisis.
Analysis requires high-tech equipment
Biofuels

How can iGEM teams detect Biofuels?

Biosensors

Tuning Biosensors

Tuning BioBricks
Solution: make biofuel-specific biosensors

Natural sensor system
+ Reporter gene
Biosensor
Two-Component Ethanol Sensor

Fluorescence (OD normalized) vs. Concentration of Ethanol (v/v)

- Uninduced
- Induced

Genetic elements:

- $P_{BAD}$
- exaD
- exaE
- P
- $P_{exaA}$
- RFP

Legend:

- Blue: Uninduced
- Red: Induced
One-Component Alkane Sensor

One-Component Alkane Sensor

Fluor/OD

0.0% 0.1% 1.0%

0% Arabinose

RFP

P_{alkS}

P_{BAD}

alkS

P_{alkB}

CGTCAGCTATAAGGCTACTAGCGATCGACATAAGGCTACTAGCGATCGACTA

RFP
One-Component Alkane Sensor

Fluor/OD vs. Arabinose Concentration

- 0% Arabinose
- 0.2% Arabinose
Second Generation Sensors

• Increase Dynamic Range
• Reduce Cross-Talk
• Enhancing Metabolite Sensitivity
Biofuels

How can iGEM teams detect Biofuels?

Biosensors

Show response, but not ideal

Tuning Biosensors

Tuning BioBricks
A multi-functional device for directed evolution

- **RFP**: Fluorescent Reporter
- **sacB**: Counter-Selection Marker
- **kan^R**: Selection Marker
Directed evolution: Iteratively tuning sensors
Directed evolution: Iteratively tuning sensors

- Mutated Sensor Culture
  - Less Leaky Mutants

Diagram shows the iterative process of directed evolution, starting with a mutated sensor culture and transitioning to a culture with less leaky mutants.
Directed evolution: Iteratively tuning sensors

- Mutated Sensor Culture → Less Leaky Mutants → Highly Inducible Mutants
Directed evolution: Iteratively tuning sensors

- Mutated Sensor Culture
- Less Leaky Mutants
- Highly Inducible Mutants
- Quantified Sensor Expression
Directed evolution: Iteratively tuning sensors

**Graph:****

- **Y-axis:** Fluorescence
- **X-axis:** Concentration of Ethanol (v/v)
- **Lines:**
  - Blue: Uninduced
  - Red: Induced

**Legend:**

- Mutated Sensor Culture
Directed evolution: Iteratively tuning sensors
Directed evolution: Iteratively tuning sensors
Directed evolution: Iteratively tuning sensors
RFP for quantitative expression analysis
sacB: Counter-Selection Marker

OD<sub>600</sub> vs. Time (h)

- 0% Sucrose
- 0.01%
- 0.03%
- 0.05%
- 0.10%
- 0.50%
- 1.00%
- 2.50%
- 5.00%

RFP  sacB  kan<sup>R</sup>
Kan<sup>R</sup>: Selection Marker

![Graph showing the relationship between Kan concentration (mM) and OD. The graph compares non-induced and induced states.](image)

- **Kan concentration (mM)**:
  - 0
  - 0.1
  - 0.2
  - 0.3
  - 0.4
  - 0.5
  - 0.6
  - 0.7
  - 0.8

- **OD**:
  - 0
  - 0.1
  - 0.2
  - 0.3
  - 0.4
  - 0.5
  - 0.6
  - 0.7
  - 0.8
  - 1

**Lines in the graph**:
- Blue line: non-induced
- Red line: induced
Biofuels

How can iGEM teams detect Biofuels?

Biosensors

Show response, but not ideal

Tuning Biosensors

Several selections simplifies screening

Tuning BioBricks
Other uses for BioBrick directed evolution

- Enhancing logic gates
- Increasing dynamic ranges
- Reducing cross-talk
- Enhancing metabolite specificities
A universal device to evolve sensor systems
Preparing the registry for large mutant libraries

- New parts page
- Append existing parts page
Preparing the registry for large mutant libraries

**Part: BBa_K634002_B2**

Designed by Kenneth O. Xu, Group: iGEM11_Wisconsin-Madison (2011-09-16)

**Offspring B2**

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-B (exaDE), and was screened using part K634007 (double selection cassette) under the exaDE-responsive promoter, PexA. This mutant is notable due to the following observations:

- Red fluorescence: 8% increase over parent
- Sucrose negative selection: 12% greater unduced survival in sucrose-containing media than parent
- Kanamycin selection: Survival observed at 6% higher kanamycin concentrations than parent

Based on these criteria, K634002-B2 was selected as a candidate for both decreased leaky expression as well as increased induced expression of the above ethanol sensing system. It is an improvement by all standards over its successor K634002-B.

**Mutant Library**

Hover over each offspring to see more detailed information and/or edit the library.
Biofuels
How can iGEM teams detect Biofuels?

Biosensors
Show response, but not ideal

Tuning Biosensors
Several selections simplifies screening

Tuning BioBricks
New tools require new technologies
Next steps

- Evolve alkane sensor for arabinose-insensitivity
- Evolve sensors for tighter regulation
- Increase sensor dynamic response range
- Tune RBS of *sacB* and *Kan<sup>R</sup>*
Acknowledgements

• The College of Engineering for providing lab space
• The students of the Pfleger Lab
• The UW-Madison REU in Nanotechnology, Chemical & Biological Engineering, and Renewable Energy
• iGEM HQ and the registry
• John Greenler of GLBRC

Questions?