Our Team

• Advisor: Ms. Byford
• Seniors:
  • Steven Allen (Team Co-Leader)
  • Parth Patel (Team Co-Leader)
  • Gordon Ellison
• Juniors:
  • Will Reiber
  • Jewel Wasson
  • Paige Brown
  • India Mackinson
  • Elizabeth England
• Sophomores:
  • Claire Lamadrid
  • Heather Cheung
• Associate Members:
  • Misbah Ahmad
  • Nana Boateng
Project Choice

- BioSafety Level 1
- High School Classroom Environment
- Parts available in the registry
- Local Funding
  - Relevant to our area
    - Agriculture
    - Industry
## Original Intentions

- Two teams
- Multiple Projects
  - Alternative Energy
  - Heavy Metal Detection
- Time and resources
Original Intentions

- Biofuels
  - AceE, AceF, lpd genes
    - 1-butanol
    - *E. Coli* genes $\rightarrow$ BioBricks
  - Primers
    - Assemble one large operon
- Overexpression?
- Efficiency?
## ATSDR List

- Agency for Toxic Substances and Disease Registry
- Division of CDC Substance Priority List

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Arsenic</td>
</tr>
<tr>
<td>2.</td>
<td>Lead</td>
</tr>
<tr>
<td>3.</td>
<td>Mercury</td>
</tr>
<tr>
<td>4.</td>
<td>Vinyl Chloride</td>
</tr>
<tr>
<td>5.</td>
<td>Polychlorinated Biphenyls</td>
</tr>
<tr>
<td>6.</td>
<td>Benzene</td>
</tr>
<tr>
<td>7.</td>
<td>Cadmium</td>
</tr>
<tr>
<td>8.</td>
<td>Benzo (a) pyrene</td>
</tr>
<tr>
<td>9.</td>
<td>Polycyclic Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>10.</td>
<td>Benzo (b) Fluoranthene</td>
</tr>
</tbody>
</table>
Cadmium

• Sources
  • Industry
    • Mining / Smelting
    • Zinc, Lead, Copper
  • Water System
    • Soil / Rock Erosion
  • Nickel-Cadmium Batteries
  • Metal-Coatings
• Federal Limit = 0.005 mg/L
Cadmium

• Health Effects:
  • Affected Systems:
   • Cardiovascular
   • Respiratory
   • Gastrointestinal
  • Known Carcinogen
   • Can cause cancer in any affected system
  • Stronger effects in children
Safety

- Is our number one concern!
- High school environment
  - High traffic
- Protective Measures
- Safety Training
  - Standard Safety Procedures
  - Sterile Technique
# Cadmium Sensor

- **Mutagenic PCR**
  - U.C. Davis 2010 Protocol
  - Random Mutations through varied concentrations, buffers, and polymerase specificity
  - Possibility of increased sensitivity in promoter or reduced function
- No improvement in sensitivity
Cadmium Sensor

- Cambridge 2007 Sensitivity Tuners
  - Bacteriophage Activators and Promoters
  - Should not affect cell activity
  - 2 of 15 combinations chosen
  - Ogr and Delta Activators combined with PO Promoter
  - Hoped for 30 – 40 fold sensitivity
Construction
Sensor Model

Cadmium Factors

Transcription Factors

Ribosome

Sensor Model

Introduction
Cadmium Background
Our Project
Results & Complications
Solutions & the Future
Testing Protocol

- 4 mL LB Culture in Plastic Tubes
  - Positive Control
    - Last year’s detector
  - Negative Control
    - DH5α
  - Concentrations
    - CADMIUM CHLORIDE?? 1m???
      - 1 mM
      - 5 mM
      - 10 mM
      - 50 mM
Complication One

- Control (left) & 50 mM Cadmium Chloride (right)

- LB Fluoresces Naturally!
Results

• Luria Broth Fluoresces naturally
  • Account for growth & GFP
• Protocol
  • Spin cells down for 5 minutes
  • Resuspend in 1 mL PBS (1X)
  • Measure in Fluorescence per OD 600
Results
Our Results
Interpreting Our Results

- Initial construct is not more sensitive despite promising initial results
- Less growth at 5 mM but increased at 50 mM
- Why did this happen?
  - Possible solubility issue at 50 mM
  - Cambridge also saw reduced growth
Complications, Complications, Complications

- Detector is not functioning properly
  - Test results
- Fungus over the summer
  - Complete decontamination
  - New solutions, antibiotics, cultures, etc.
Now What?

- Completely started over
  - Transform necessary parts
  - Repeat 3X
    - Digest
      - Size check
    - Ligation
    - Transformation
      - Digest
      - PCR
Our BioBrick

- **BBa_K1042013**
  - PO Promoter + GFP with Stops

- Picture of PCR / Digest with Bands!
  - Accurate Size
Our New Parts

- Already submitted downstream part
  - K1042013
- Upstream part
  - Cadmium Promoter + P2 Ogr activator
- Entire construct
  - K1042015
  - Cadmium Responsive GFP + Ogr activator
  - Not yet tested
Bands!

- K1042015
- 1413 base pairs
Solutions & Potential Use

- Ligate Cadmium Promoter + ogr activator with Bba_K1042013
  - Full detector
  - Appropriate antibiotic resistances
- Full detector
  - Easy to interpret results
  - Self-contained kit...
  - Marketing
Future Plans

- Sensitivity Tuners?
- Cell suicide gene
- Biofuels
Sandra and Bill Hall
New England Biolabs
SignSmart
Gaston Day School