FRAGRANCE-PRODUCING CO SENSOR
RATIONALE AND HUMAN PRACTICES/DESIGN

• Disabled
• Natural disasters
  • Power outages
  • Reliance on gasoline generators
  • Accumulation of CO

Our project:
• Fragrance
• Energy-independent
• Transportable
• Cost-effective
EXPERIMENTATION


= CO
<table>
<thead>
<tr>
<th>Part</th>
<th>Description</th>
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<tbody>
<tr>
<td>T7</td>
<td>CO transcription activator</td>
</tr>
<tr>
<td>pCooF</td>
<td>Promoter activated by CO-CooA</td>
</tr>
<tr>
<td>pchBA</td>
<td>pchA: isochorismate synthase, pchB: isochorismate pyruvate-lyase</td>
</tr>
<tr>
<td>BSMT</td>
<td>SAM benzoic acid/salicylic acid carboxyl methyltransferase I</td>
</tr>
<tr>
<td>Chorismate</td>
<td>Endogenous molecule</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>Wintergreen smell molecule</td>
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CHEMICAL PATHWAY

Chorismate $\rightarrow$ Isochorismate $\rightarrow$ Salicylic Acid $\rightarrow$ Methyl Salicylate
DESCRIPTION

CO pathway
- CooA transcription activator
- T7 promoter

Wintergreen pathway
- pchBA and BSMT genes
- pCooF promoter
E. coli OneShot TOP10
Vectors: pCR2.1-TOPO-TA, pCDFDuet-1
T7-GFP (pCDF)
pCooF-GFP (pCDF)
T7-CooA (pCR 2.1)
CO EXPERIMENT
EXPERIMENTAL CONSTRUCTS

BL21(DE3)

Experiment
- T7-CooA, pCooF-GFP

Controls
- Negative: DE3 cells only
- Negative: pCooF-GFP
- Positive: T7-GFP
CORM-2

- Carbon monoxide releasing molecules
- CO in solution
- Safety
- Various concentrations

Tricarbonyldichloro ruthenium (II) dimer
\[ \text{[Ru(CO)\textsubscript{3}Cl\textsubscript{2}]_2} \]
PRELIMINARY METHODOLOGY

1) Anaerobic chamber
2) Cultures to 0.6 OD600 w/ glucose
3) IPTG induction
4) Add CORM-2 (100 µM)
5) Grow anaerobically
6) Fluorescence Intensity
Fluorescence vs. CO presence

<table>
<thead>
<tr>
<th></th>
<th>no CORM</th>
<th>CORM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series1</td>
<td>69675.53181</td>
<td>61376.63119</td>
</tr>
</tbody>
</table>
MODIFICATIONS

- Induced for 20 hours
- Springs to increase aeration
- Aerobic incubation
- Grow to 0.6 OD600 in flasks
- 15mL conicals
- Inducing at 37°C
- Multiple CORM concentrations
- Triplicates
- Resuspending in PBS
Fluorescence vs. CORM concentration in LB/PBS

- **Fluorescence**
- **CORM concentration (uM)**

- **LB**
- **PBS**
FINAL METHODOLOGY

1) Anaerobic chamber
2) Cultures to 0.6 OD600 with glucose
3) IPTG induction
4) Add CORM-2
5) Grow anaerobically
6) Aerobically
7) Resuspended PBS
8) Fluorescence Intensity
Normalized Fluorescence v. CORM concentration under IPTG induction
DATA WITHOUT T7-GFP

Normalized Fluorescence v. CORM concentration
under IPTG induction, no positive control
DISCUSSION

Time constraint – CO construct tested only

Possible errors
- CORM
- Anaerobic chamber
- DO content
- Mutation between transforming plasmids

Obstacles
- Sequence verification
- Creating anaerobic environment
- Designing primers
FUTURE PLANS

- Biosensor
- Anaerobic media
- Detecting other gases
- Other smells
SAFETY

- Lab training
- Chemicals
  - CORM-2
  - DMSO
  - EtBr
- Fume hood during experimentation
EDUCATION & PUBLIC ENGAGEMENT
LASA students developing bacteria to improve carbon monoxide detectors

By Carly Hess
Published: July 28, 2015, 5:55 pm | Updated: July 29, 2015, 10:04 pm

TEXAS HIGH SCHOOLERS MAY HAVE FOUND BETTER WAY TO DETECT CARBON MONOXIDE
No more worrying about the batteries in your carbon monoxide detector.

By Alan Stephenson | July 31, 2015 5:20 am

KXAN
KUT
EDUCATION & PUBLIC ENGAGEMENT

Synthetic Biology Club

2015 SXSW Create
ACKNOWLEDGEMENTS

- Dr. Ellington and all the members of the Ellington Lab
- Dr. Mishler and the UT iGEM team
- Dr. Davies
- Chris Cervini
- Joseph Oleniczak