Disease

- Natural disaster
- Feces-contaminated water
- Food

Cause

Detection

Prevention

- Safe drinking-water
- Sanitation
- Personal and food hygiene
The Challenge

Diarrhea-causing pathogens

**EPA Standard—One Bacterium per 100 mL**

**Bacteria:**
- E. Coli
- Shigellae
- Salmonellae

**Viruses:**
- Rotavirus

**Protozoa:**
- Cryptosporidium
<table>
<thead>
<tr>
<th>Type</th>
<th>Quick</th>
<th>Cheap</th>
<th>Portable</th>
<th>Robust</th>
<th>Easily Customizable</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>✗</td>
<td>✗</td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>FRET</td>
<td>✓</td>
<td>✗</td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Electro-chemical</td>
<td>✓</td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
<td>✗</td>
</tr>
<tr>
<td>ELISA</td>
<td>✗</td>
<td>✗</td>
<td>✓</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>
Design modules

Express sensors in E. coli

Purify sensors

Add to heat-treated water sample

Cheap & Easily customizable
How to Detect?

- Membrane Antigen
- DNA Binding
Sensor Design

 SENSOR | LINKER | ENZYME

No Pathogen

No COLOR
Sensor Design

SENSOR  LINKER  ENZYME

Contaminated

BLUE OUTPUT
Split β-gal Functionality

- Omega-only
  - Negative control

- Alpha + Omega

Quick & Robust
Split βgal Functionality

Omega-only
Negative control

Alpha + Omega + Adapter
DNA Biosensor

Chimeric Probe

Targeted Genomic DNA

Functional β-gal

BLUE OUTPUT
Topoisomerase provided by: University of Pennsylvania (Hwang et al, 2006)
Mass spectrometry confirms expected protein expression.

<table>
<thead>
<tr>
<th>Mass (m/z)</th>
<th>% Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>9.0</td>
</tr>
<tr>
<td>14</td>
<td>41.8</td>
</tr>
<tr>
<td>20</td>
<td>84.6</td>
</tr>
<tr>
<td>27</td>
<td>27.4</td>
</tr>
<tr>
<td>33</td>
<td>70.2</td>
</tr>
<tr>
<td>40</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Negative control

His-TOPO

His-TOPO D168A
Topoisomerase

Test

Topo D168A
BBa_K891234

DNA BBa_K891000
DNA BBa_K891999

Results

<table>
<thead>
<tr>
<th></th>
<th>DNA</th>
<th>DNA+ protein</th>
<th>DNA+ TOPO</th>
<th>DNA+ D168A</th>
<th>No DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>relaxed circle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uncut</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>fragments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DNA BBa_K891000
DNA BBa_K891999

![DNA gel electrophoresis result]

3000bp 2500bp 2000bp 1500bp 1000bp 800bp 600bp 400bp 200bp
Streptavidin DNA adapter

- 4 Binding Sites
- Higher Sensitivity
Magainin Membrane Antigen Sensor

— Higher Sensitivity
— User Friendly
Human Practices

Background

DNA Biosensor

Further Engineering

Human Practices
thinking beyond the lab...
thinking beyond the lab...
Delivery Package

- User Manual
- Additional Buffer
Production & Distribution

Purified biosensor

X-gal

Portable
Field Implementation

What is the time course of an outbreak?

Finding the source of the outbreak?

OUTBREAK
Bayes’ Rule: how confident can we be about a positive result?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Condition Positive</th>
<th>Condition Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensor Outcome</td>
<td>True Positive</td>
<td>False Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>True Positive</td>
<td>False Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>True Negative</td>
<td>False Negative</td>
</tr>
</tbody>
</table>

Sensitivity (A): proportion of true positives accurately measured
Specificity (B): proportion of true negatives accurately measured
Prior probability (C): what is the “natural frequency” of contamination?

\[
\text{probability of true positive given positive result} = \frac{A \times C}{A \times C + (1-A)(1-C)}
\]
\[ E_1 + E_2 \overset{k_c}{\underset{k_{c-1}}{\rightleftharpoons}} E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_{cat}}{\rightarrow} E + P \]

\[
\dot{X} = \begin{bmatrix}
0 & 0 & k_{c-1} & 0 & 0 & 0 \\
0 & 0 & k_{c-1} & 0 & 0 & 0 \\
0 & 0 & -k_{c-1} & 0 & k_{cat} + k_{-1} & 0 \\
0 & 0 & 0 & 0 & k_{-1} & 0 \\
0 & 0 & 0 & 0 & -k_{cat} - k_{-1} & 0 \\
0 & 0 & 0 & 0 & k_{cat} & 0 \\
\end{bmatrix}
\begin{bmatrix}
E_1 \\
E_2 \\
E \\
S \\
ES \\
P
\end{bmatrix}
+ \begin{bmatrix}
-k_c * E_1 * E_2 \\
-k_c * E_1 * E_2 \\
-k_1 * E * S + k_c * E_1 * E_2 \\
-k_1 * E * S \\
k_1 * E * S \\
0
\end{bmatrix}
Local & Community Outreach

Teaching at ASU101

Mentoring Bioscience High School’s iGEM Team
Achievements

- Built, characterized, and submitted 3 Biobricks that worked as expected
- Confirmed feasibility and robustness of split beta-galactosidase as a reporter system
- Determined a mathematical model for testing sensitivity and specificity of biosensor technologies
- Developed and implemented an international hygiene program
- Researched field implications and considered the ethics of knowledge sharing
- Developed a distribution strategy for real-world implementation
Attributions

Research Materials
• Case Western Reserve University
• University of Pennsylvania

Sponsors
• Ira A. Fulton Schools of Engineering at ASU
• College of Liberal Arts and Sciences at ASU
• School of Biological Health Systems Engineering at ASU
• School of Life Sciences at ASU
• School of Politics and Global Studies at ASU
• Barrett, the Honors College at ASU
• Department of Chemistry and Biochemistry at ASU
• Integrated DNA Technologies

Individuals
• Dr. Karmella Haynes
• Dr. Vincent Pizziconi
• Dr. Xiao Wang
• Dr. Miles Orchinik
• Kylie Standage-Beier
• Alizee Jenck
• Jordan Nguyen
• James Alling
• Rene Davis
• Behzad Damadzadeh
• Eric Trang
• Dr. Thomas E. Grys

Non-Governmental Organizations
• Carroll Behrhorst Clinic
• SIAS Guatemala

The iGEM Foundation
Questions?
Extra Slides
DNA Biosensor

Bba_K891000
Bba_K891999
Bba_K891234
DNA Biosensor

Chimeric Probe

Targeted Genomic DNA

Functional β-gal

BLUE OUTPUT
Mass spectrometry confirms expected protein expression.
Bradford Assay confirms expected protein expression and his-purification.
DNA  DNA+ protein  DNA+ TOPO  DNA+ D168A  No DNA

relaxed circle  uncut  fragments