Brown iGEM Lab
Drinking water in the U.S. is among the top four public health risks posed by environmental problems.'
--Former EPA Administrator William K. Reilly

According to some estimates, arsenic in drinking-water will cause 200,000 – 270,000 deaths from cancer in Bangladesh alone. --NRC, 1998; Smith, et al, 2000

Field test kits can detect high levels of arsenic but are typically unreliable at lower concentrations of concern for human health. Reliability of field methods is yet to be fully evaluated.

Current Water Detection Methods

Bulky
Laboratory setting
Proposed Guidelines: Interface Biological and Electrical Systems

Biological

✓ Uses minimal biological machinery
✓ Direct induction of system allows for sensitive measurements
✓ Versatile construct

Engineering

✓ Compact and user-friendly system for sample analysis
✓ Economically feasible
**Objective:** To detect the presence of heavy metals in a water sample.

### Biological
- **Cell Lysis Cassette** under the control of an inducible promoter
  - Potential promoters: Arsenic, Lead, and Mercury
  - *Proof of Concept:* pBAD (Arabinose Induced) promoter

### Electrical
- Cell Lysis will cause a release of ions
  - Change in resistance/conductance of bacterial solution
Components of System

Inducible promoter (pBAD = proof of concept)

Lysis Cassette: BBa_K124003 / BBa_K124014

Holin: BBa_K124017

Endolysin: In Progress

Function Not Characterized
A Look at the Cassette
Activate Cassette With Inducer

S, R, Rz proteins are produced
Let’s take a closer look…
Outside Cell

Inside Cell
The S proteins channel through the inner membrane, then enter the membrane and initiate lysis.
The cell wall degrades & fragments
Ions leak out of cell
Figure 1: Change in optical density before and after lysis, standardized to the control. Decrease in optical density occurred several hours after induction with Arabinose.

But how long does lysis really take?

Figure 2: Visual change in optical density
Experimental Testing: Biological

- Control: (-Ara)
- Test: (+Ara)

Decrease in OD indicates lysis of cells (+Ara)
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Electrical

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  - Change in resistance/conductance of bacterial solution
Testing the Design: Resistance Apparatus Evolution

Considerations:
- Alternating Current
- Electrode Material
- Fixed Electrodes
Resistance Experimentation

Resistance of 50x Concentrated Cell Solution Before & After Lysis

**Hypothesis**
Resistance of concentrated solution will decrease after lysis.

**Conclusion**
Change seen but system noise = sensitivity
Final Apparatus

Vernier Go!Link Conductivity Probe

- Sensitivity
- Commercially Available
- Alternating Current
- Graphite Electrodes
- Fixed electrodes
- Calibration
**Hypothesis:**
Conductivity will increase during lysis.

**Conclusion:**
Conductivity increase correlates to optical density decrease as cellular lysis occurs.
Experimental Testing
Biological & Electrical

Conductance Measurements of Cell Lysis Over Time

Optical Density Measurements of Cell Lysis Over Time
Applications

1) Add H₂O to reconstitute dried E.coli
2) Add drop of sample
3) Adding sample causes S,R proteins to be expressed if the sample matches
4) Conductance is measured across
5) Circuit board determines if conductance has increased

Highly concentrated E. coli (dried)
Interface Biological and Electrical Systems

Biological

✓ Uses minimal biological machinery
✓ Direct induction of system by inducer creates a sensitive system
✓ Versatile construct

Engineering

☐ Compact and user-friendly system for sample analysis
☐ Economically feasible
Applications: Electrical Reporting System

- Potential alternative to Biological Reporters i.e. GFP

Conductance Measuring Circuit VS.

< $1.00 VS. $5,000+
Applications: Biosafety Kill Switch

“There is a need for an effective bacterial kill switch, especially for outer space applications, as we introduce organisms into new environments.”

- John Cumbers [NASA AMES]

- Auto-lysis of cells can protect against uncontrollable protein expression
- Machinery designed to be incorporated into future SynBio systems
Thank You!

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- Daniel Ludwig, Gaurab Chakrabarti
- John Cumbers, Adella Francis
- Deepa Galaiya, Jeff Hoffman, Tito Jankowski
Questions?
Appendix 1: References

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