The Brown iGEM Lab is student-run and consists of five undergraduate students. This year, the team split up to work on two projects. We began our training in the spring semester with a lab class in Synthetic Biology. With the gracious support of the UTRA Program and various departments, we work throughout the remainder of the summer on our projects. With the guidance of graduate mentors and faculty advisors across a multitude of departments, our lab is currently working on two such projects: a novel electrical reporting system and the other a novel threshold switch. In the spirit of Synthetic Biology, our team’s project incorporates aspects of electrical engineering, biochemistry, genetics, and microbiology.

**The Problems**

**Heavy Metal Detection in Water Supplies**
- 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

Around the world, contamination of drinking water is an immense problem that is difficult and expensive to detect with current technology. As such, many 3rd World countries are unable to effectively diagnose the problem across the millions of water supply sources that exist.

**Biosafety: Bacterial Kill Switch**

There exists a need for an effective method of inducing controlled bacterial cell death in synthetic biological systems. Currently, there is a biosafety concern with respect to regulating genetically engineered organisms.

**BioBrick™ Additions**

Brown submitted 3 parts to the Registry of Standard Biological Parts:
- **Bba_K124003**: The Cell Lysis Cassette provided by John Mekalanos’ lab at Harvard Medical School. The Cassette includes the S, R, and Rz genes. It can be coupled with any promoter.
- **Bba_K124014**: S105 allele provided by Ryland Young at Texas A&M University. A variant of the S Wld Type, its 8 base pairs are deleted eliminating the expression of the S105 antiholin.
- **Bba_K124017**: Full Cell Lysis Cassette with the S105, R, and Rz genes provided by Ryland Young. The S105 gene has been deleted from the natural lambda phage cassette, thus increasing the speed of lysis.

**Applications**

**Proposed Toxin Sensor**

The sensor uses cells engineered to lyse in the presence of a certain inducer using a lysis cassette. In the proposed design, the bacterial cells will lyse in the presence of a heavy metal toxin, such as Arsenic, Lead, or Mercury.

When these cells lyse, intracellular charged particles are dispersed throughout the solution, thereby increasing its conductivity. The relative change in conductivity should indicate degree of toxicity.

**Benefits:**
- Compact Design
- Economically Feasible
- A potential circuit has been designed for this sensor—potential cost: $0.50

**Biosafety: Bacterial Kill Switch**

In synthetically engineered biological systems, there is a potential for uncontrollable and undesirable protein expression. The lysis cassette provides an antagonist mechanism as an emergency kill switch in such undesirable circumstances. The lysis cassette containing a holin and endolysin has been Bio-Blocked by Brown iGEM and is available for Synthetic Biologists to use in their constructs.

**Benefits:**
- Minimal Biological Machinery
- Direct Activation by Inducer

**Conductance Measurement of Cell Lysis as a Reporter of Toxin Presence**

Brown iGEM found that both problems could potentially be solved by one genetic construct: the lysis gene cassette.

The Lysis Gene Cassette is a genetic construct designed by Ryland Young at Texas A&M University. The cassette causes a cell to lyse upon induction of the promoter to which it is attached.

**Experimentation: The Apparatus**

**Final Resistance Apparatus**

Our final resistance measurement apparatus featured a Data Acquisition Card, circuit board amplifiers, and a LabVIEW computer program to collect the data. This apparatus and computer software permitted us to control the voltage being output into solution as well as the type of current (AC or DC). We elected to output an alternating current in order to keep the cells from electroporating.

**Significant Changes:**
- Used a six well plate (allowing for multiple tests in parallel), a PDMS elastomer mold and embedded pendant wires.
- By using an induct wire we were able to avoid Faraday chemistry and by using a cast mold, we were able to keep the distances between the electrodes fixed.

**Problems:**

After significant testing, we came to the realization that our measurement algorithm and setup would not allow for sufficiently consistent and accurate results in the form of resistance measurements.

**Conductivity Probe**

The Vernier Conductivity Probe provided:
- Fixed electrode position
- Precise calibration
- Alternating current
- Electrode body graphite electrodes

This marked a change in our strategy for measuring electrical changes in solution after induced lysis. We made a switch to measuring conductance, the inverse of resistance, because of this commercially available measuring device.

**Experimentation: Biological**

**Optical Density**

Optical Density is a well-established means of detecting cell death. By taking OD measurements over time, we tested the functionality of the Lysis Cassette after induction. Our testing was done with the Cell Lysate Cassette (Bba_K12403) under an Arabinose induced promoter.

**Benefits:**
- Simple
- Commercially available measuring device

**Conductance**

After testing the Lysis Cassette’s functionality via Optical Density measurements, we tested the change in conductance of the cellular solution as lysis proceeded.

**Benefits:**
- Direct measurement of conductivity
- Linearly proportional to concentration of lysed cells

Acknowledgements & Special Thanks

- Professors Gary Wessel, Teysha Palmore, Jerry Daniels, Carlos Azarnejad, and JIKeeling for their continuing support
- Graduate Advisors: Adrian Reich, Diana Donovan, Jamie Gagnon with whom we could never have gotten this far
- Special Thanks to Dr. Ryland Young (Texas A&M) and Mekalanos Lab (Harvard) for providing us DNA parts
- Daniel Ludwig for his perseverance neveirending help
- Brown University UTRA Program Undergraduate Teaching and Research Assistancehips for their continual support in helping Brown iGEM
- Brown University Multidisciplinary Lab, Departments of Biology & Medicine, Molecular Biology and Biochemistry, Computational Biology, and Engineering
- Corporate Sponsors Thermofisher and NanoDrop for their gracious donations

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