E. coli: A Two-Circuit System for Early Colon Cancer Detection

Joseph F. Anderson, Audrey Buckley, Yuri Patricio Cabeza, Amy Fullwood, Kellie S. Hecht, Eric W. Jones, Jordan Mackay, Justin Meek, Karl R. Nordgren, Jeffrey L. Rees, Dean James Ritchie, Brooke J. Roark, John W. Shumway, Joseph Thiriot, Hillary Walker, Joshua D. Yates and Julianne H. Grose
Brigham Young University, Provo, UT 84602

Abstract
In the initial stages of colon cancer, malignant cells give off excess heat, reactive oxygen species (ROS), and lactate. Last year, the BYU iGEM team genetically engineered E. coli to detect heat or ROS. This year we developed E. coli capable of simultaneously sensing lactate, heat and ROS, implemented a novel Cre-Lox system, and constructed a library of thermostensors. Our project uses two circuits, each with a unique reporter. The first circuit contains a periplasmic lactate sensor coupled to a reporter. The second circuit contains a RNA thermometer driven by a ROS-inducible promoter, allowing expression of Cre recombinase when both heat and ROS are present. Although heat is transient, Cre ensures continued expression of a second reporter gene. Finally, we have evolved a library of thermostensors that work in a narrow physiological range. Together, this two-circuit system may allow accurate and specific detection of early colon cancer cells.

Introduction
- “Colon cancer is...the second leading cause of cancer-related death” when both sexes are combined (American Cancer Society).
- Current detection methods are inconvenient, invasive, and expensive.
- Our idea is to engineer E. coli, a bacterium normally found in the GI tract, to detect the early signs of colon cancer (heat, ROS, and lactate).

Our Two-Circuit Design

Circuit #1: Detecting Heat and ROS
Using an AND Gate

1. Heat
2. ROS
3. GFP

Using site directed mutagenesis, we altered the binding affinity of MglB from glucose to lactate using the mutations described by Loge et al., Nature, 2003. When this mutant MglB binds lactate, it will create a cascade that leads to the transcription of a reporter gene (lac Z).

Circuit #2: Lactate Sensor

Using site directed mutagenesis, we altered the binding affinity of MglB from glucose to lactate using the mutations described by Loge et al., Nature, 2003. When this mutant MglB binds lactate, it will create a cascade that leads to the transcription of a reporter gene (lac Z).

Describing a Stable AND Gate with Cre

Cre recombinase targets two lox sequences, removing a terminator and allowing for sustained expression of GFP when the inputs are present.

Creating a Thermosensor Library

We performed mutagenic PCR on the thermosensor and screened for more sensitive and clear-cut thermoswitches from a 23°C to 37°C temperature shift in order to create a predictive model for thermosensor design.

Conclusions/Future Directions

- All components of our two circuit system were tested independently.
- The MglB lactate sensor works but need further characterization.
- Our Cre system requires an RNA thermometer that is less active at 30°C.
- We were able to model Circuit #1 with mass action enzyme kinetics.
- We created an algorithm for predicting RNA binding of thermostensors that is comparable with known algorithms such as Matlab’s modeling software.
- We submitted I1i parts to the iGEM registry (10 thermostensors, 2 lactase sensors).
- Characteristics and statistical analysis of a thermosensor library suggests that in vivo RNA behavior may be predicted by the individual G-C, A-U and G-U bond content which determine the bonding energy.
- Our results suggest that a more expensive library would enable the design of mutant thermostensors which respond to discrete temperature shifts.

For our thermosensor library, it is more useful to look at the total number of bonds than to look at the individual G-C, A-U and G-U bond content which determine the bonding energy. Our results suggest that a more expensive library would enable the design of mutant thermostensors which respond to discrete temperature shifts.

Creating an algorithm to predict RNA Structure

The path of our final algorithm [(1)] Red is the binding patterns observed in the secondary structure of our WT-thermosensor, orange is where the algorithm and these bonds agree, blue indicates differences between Matlab and our algorithm.

We adapted the Smith-Waterman method to create an algorithm that would model Thermostensor structure. In this algorithm we aligned one side of the RNA sequence with its other side, instead of aligning two DNA sequences. We then compared our algorithm to Matlab’s mfold program.

Outreach
Our team participated in Provo City’s Kids’ Science Palooza and the Central Utah Science and Engineering Fair to teach parents and children the basics of synthetic biology. To demonstrate these principles, we made BioBrick bracelets [download instructions at http://www.igem.org/BioBrickOutlet].

A BIG thank you to:
- Advisors Dr. Julienne Gross, Dr. Sean Warrick and Dr. David Kooyman.
- John Delfos and Darin Durfee for help with the math modeling.
- Dr. Hazeltrus’s lab at the University of Missouri (Trz constructs).
- BYU College of Life Sciences and MMOBIO Department for funding and support.
- New England Biolabs for providing most of the enzymes and reagents.
- Matlab: Free access to Matlab greatly enhanced our mathematical modeling.