Outline

1) “Synthetic Biology”/iGEM background
2) Penn State team project idea
3) System requirements/approach to problem
4) Strategies
5) Subtasks
   • Microfabrication
   • Circuit design
   • DNA construction
   • Strain construction
   • Modeling/Parameter estimation
   • Construct testing
6) Initial Results
7) Future
8) Conclusions
Background

Standardization is a critical part of Synthetic Biology

Comparing Synthetic Biology to Electrical Engineering

A Cell or System of Cells $\leftrightarrow$ Electrical circuit
DNA, RNA, Proteins $\leftrightarrow$ Electrical components, signals
Registry of Standard Biological Parts (“Biobricks”) $\leftrightarrow$ TTL data book
BIOSPICE $\leftrightarrow$ SPICE

iGEM competition instructions:
Use these concepts to build something cool

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Effect: Creates shared standards, worldwide!
Building With Biobricks

Suffix Insertion

PstI+SpeI
PstI+XbaI

Biobrick

EcoRI XbaI SpeI PstI

Part

Prefix Insertion

EcoRI+XbaI EcoRI+SpeI

Ligation

Transformation

Mini-prep, restrict, sequence, iterate

Spe I

5′...ACTAGT...3′
3′...TGATCA...5′

Xba I

5′...TCTAGA...3′
3′...AGATCT...5′

Complementary sticky ends
Idea: Build a bacterial relay race

- motile bacteria move along a channel carrying a signal
- encounters a second stationary bacteria
- turns on a switch controlling the latter’s motility

Why?
- Great for lab downtime (during restriction digests?)
- Fun to bet on
- Just need bacterial high jump & pole vault to start bacterial Olympics;
- Programmable control of motility;
- Future device for information transportation (a new Pony Express)?
How?

Needs

• **Device to control movement**
• System to direct movement

Options to control movement

• The Che transduction system?
  • knocking out parts of the system creates tumbling/running mutants
    – but we want stationary cells

• Flagellar protein?
  • Blair and Berg\(^1\) showed that flagellar rotation could be restored in MotB K/O cells by complementing with a functional copy on a plasmid
    • rotation restored on average in 10 min

System requirements (cont.)

How? (cont.)

• Need:
  • Device to control movement
  • System to direct movement

Chemotaxis-create and maintain chemical gradient
  • However, adds chemical engineering challenges to already complex project

Instead:

• Microchannels
  • Offer facile method for guiding bacteria
  • No gradient necessary-Whitesides & Berg\(^2\)
  • Optimal environment constraining/directing quorum signal

Microchannel fabrication

Reactive-ion etched/photolithography w/mask

Pour PDMS, spin coat
At 200 rpm for 20 sec,
cure 4 hrs
@ 70 °C

Fluid-filled microchannels

Peel off PDMS, place feature-side down on agar (eiken) plate
Microchannel pictures

60 um

10 um

60 um
Signal representation & transfer

Sender cells

Quorum signal (AHL)

Immotile, receiver cells

Strategy 1
• Sender cells continuously produce AHL.

Strategy 2
• Conjugation event mediates signal, subsequent amplification via quorum molecules.

Eiken agar

PDMS-housed microchannels

10 μm

Sender cells

Conjugation event
Strategy 1

Sender cells continuously produce AHL

Advantages
Diffusible quorum signals have been functional activators in previous synthetic networks with luxR/AHL-controlled promoter

Potential drawbacks
Inadequate production of AHL for activation?
Leaky expression from $p_{\text{luxR}}$
Strategy 2

Conjugation event mediates signal, subsequent amplification via quorum molecules

**Advantages**
AHL doesn’t outpace sender bacteria

**Potential drawbacks**
Leaky expression of LuxI in receiver cells causes premature motility
Increased time required
F⁺ plasmid needed (additional DNA needed)
Quorum Sensing

acyl homoserine lactone (AHL)

- Diffuses freely across cell membrane
- Can act in a positive feedback loop causing exponential growth
Genetic control mechanism

Cell 2

Receiver
LuxR

Switch
Cl

Motility/Reporter
MotB
GFP

Cell 1
What About the Cell?

- **Knock out MotB (ASKA Library)**
  - Put MotB on our plasmid, under CI control

- **Knock out RecA**
  - RecA will delete multiple copies of genes

- **Knock out LacI**
  - Preventing interference with our process

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Initial Results

Swarming Cells: grew swarming wild type (RP437) cells on Eiken agar plates
Initial Results

• Test for ability to control motility
  • Placed under control of BBa_R0010 (LacI promoter) induced with IPTG
Initial Results/Progress

Testing fluorescence by induction with AHL:

Using flowcell cytometry to measure fluorescence
Varied AHL concentrations from $10^{-6}$ M to $10^{-11}$ M
After one hour up to 17% of the cells were fluorescing
Modeling

Cell 1

\[ \frac{dLac}{dt} = k_2[R_{LacI}][r] - k_d[LacI] \]
\[ \frac{dR_{LacI}}{dt} = k_3[N][L_{PT}][P] + k_1[N][P] - k_2[R_{LacI}][r] - k_d[R_{LacI}] \]

Cell 2

\[ \frac{dCI}{dt} = k_4[R_{CI}][r] + k_R[CI_2] - k_d^{CI}[CI] \]
\[ \frac{dR_{CI}}{dt} = kCI[P][N_{CI}] - k_1[R_{CI}][r] - k_d^{CI}[R_{CI}] \]
\[ \frac{dL_{CI}}{dt} = k_2[I]^4[N_{CI}] - k_3[L_{CI}] \]
\[ \frac{dM}{dt} = k_M[R][N] - k_d^{M}[M] \]
\[ \frac{dR}{dt} = k_n[P][N] - k_b[r][R] - k_d^{R}[R] \]
\[ \frac{dN}{dt} = k_1[N][CI_2] - k_n[P][N] - k^{+1}[N][CI]^2 \]
Theoretical Curves

GFP vs Time

LuxR vs Time

*Simulated using Matlab’s stiff differential equation solver ode15s.
Future Modeling Considerations

• Sensitivity analysis so that experiments can be performed that target the most sensitive components of the system.

• Stochastic modeling - how noisy is the system? Some molecular species may be low in number (e.g. promoters), so this is an important question.
Future

• Model-parameter estimation, sensitivity analysis
• Continue DNA construction
• Optimize microfabrication for delivery & placement of cell 2
• Build and implement feedback circuit in cell 1
• Biobrick conjugation machinery
• Clone, clone, clone
Conclusions

Learning Experiences:
- Modeling offers important insight into function
- Laboratory organization is crucial
- As are teamwork and communication!

Scientific Progress:
- Added a new part to the registry
  - We “biobricked” motB, and now it is available for all teams to use
- Some subassemblies work, several are being tested
- Ready to parameterize model

Athletic Goal:
- Ready to Race - Beijing 2008!
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