Bacterial Relay Race

Penn State iGEM 2006

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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

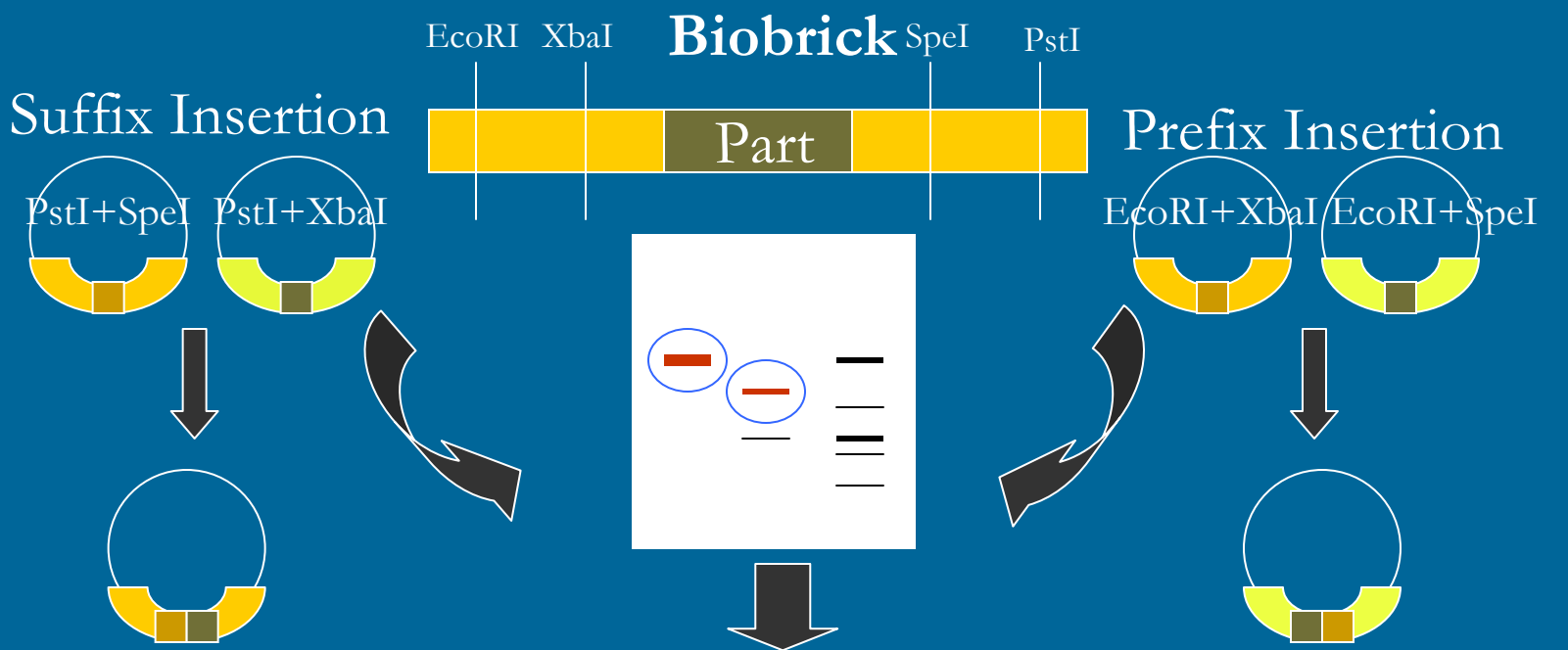
Comparing Synthetic Biology to Electrical Engineering

A Cell or System of Cells ←→ Electrical circuit
DNA, RNA, Proteins ←→ Electrical components, signals
Registry of Standard Biological Parts ("Biobricks") ←→ TTL data book
BIOSPICE ←→ SPICE

iGEM competition instructions:
Use these concepts to build something cool

Effect: Creates shared standards, worldwide!

Building With Biobricks



Ligation

Transformation

Mini-prep, restrict, sequence,

iterate

Spe I

5′...A C T A G T...3′
3′...T G A T C A...5′

Xba I

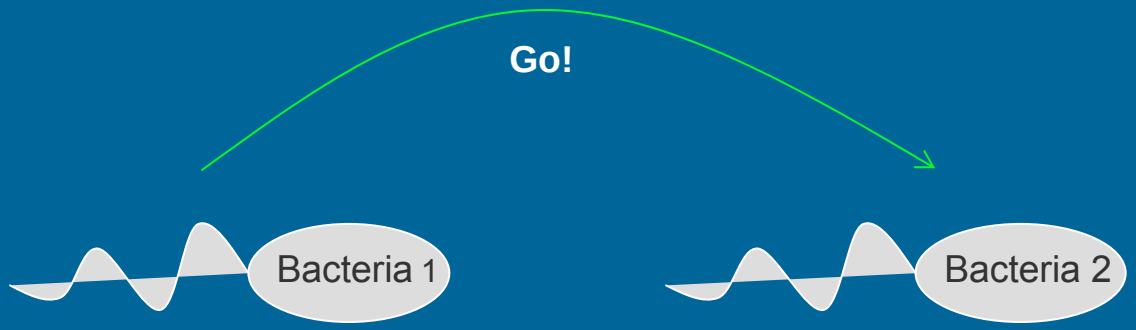
5′...T C T A G A...3′
3′...A G A T C T...5′

Complementary sticky ends

The Penn State Concept

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

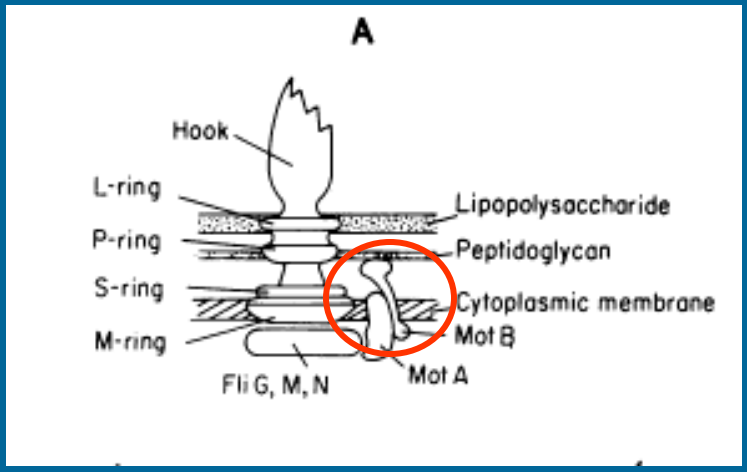


Why?

- 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)?

System requirements

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

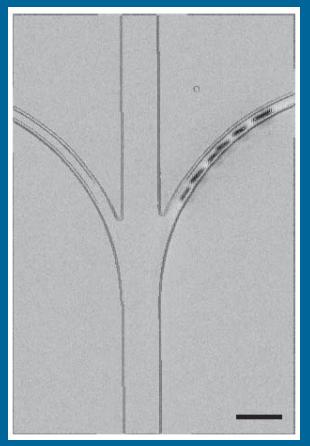
- Flagellar protein?
  - Blair and Berg[1] showed that flagellar rotation could be restored in MotB K/O cells by complementing with a functional copy on a plasmid
    - rotation restored on average in 10 min

[1]Blair, D., Berg, H.G. Restoration of Torque in Defective Flagellar Motors. *Science* 242, 1678-1681 (1988).

System requirements (cont.)

How? (cont.)

- Need:
  - Device to control movement
  - **System to direct movement**

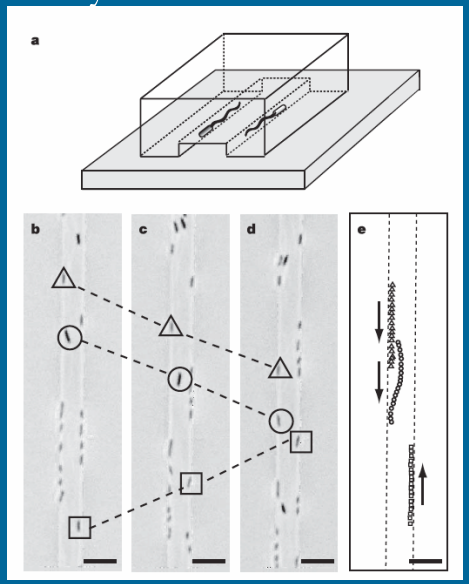


Chemotaxis-create and maintain chemical gradient

- However, adds chemical engineering challenges to already complex project

Instead:

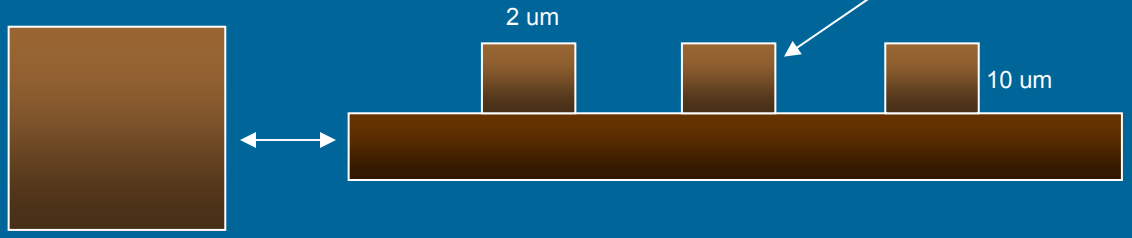
- Microchannels
  - Offer facile method for guiding bacteria
  - No gradient necessary-Whitesides & Berg[2]
  - Optimal environment constraining/directing quorum signal



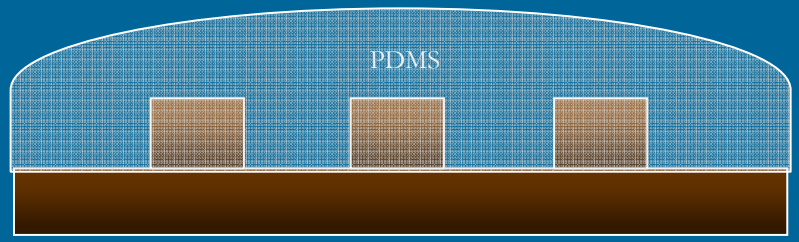
[2]Berg, Whitesides, et al. E. Coli swim on the right. *Nature*, 435, June 30, 2005.

Microchannel fabrication

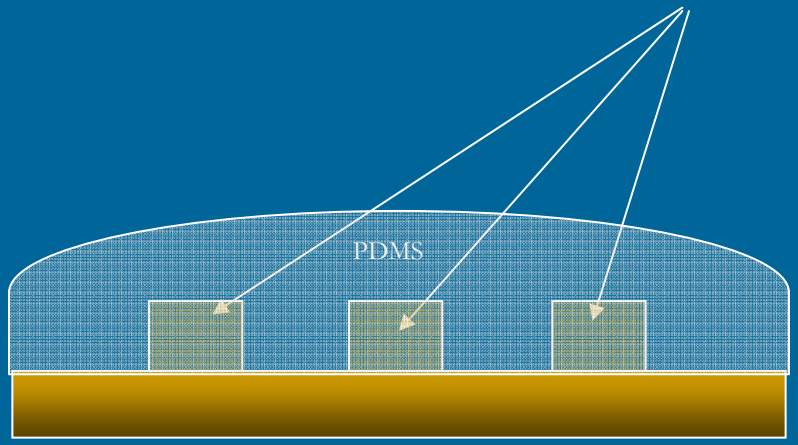
Reactive-ion etched/photolithography w/mask



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

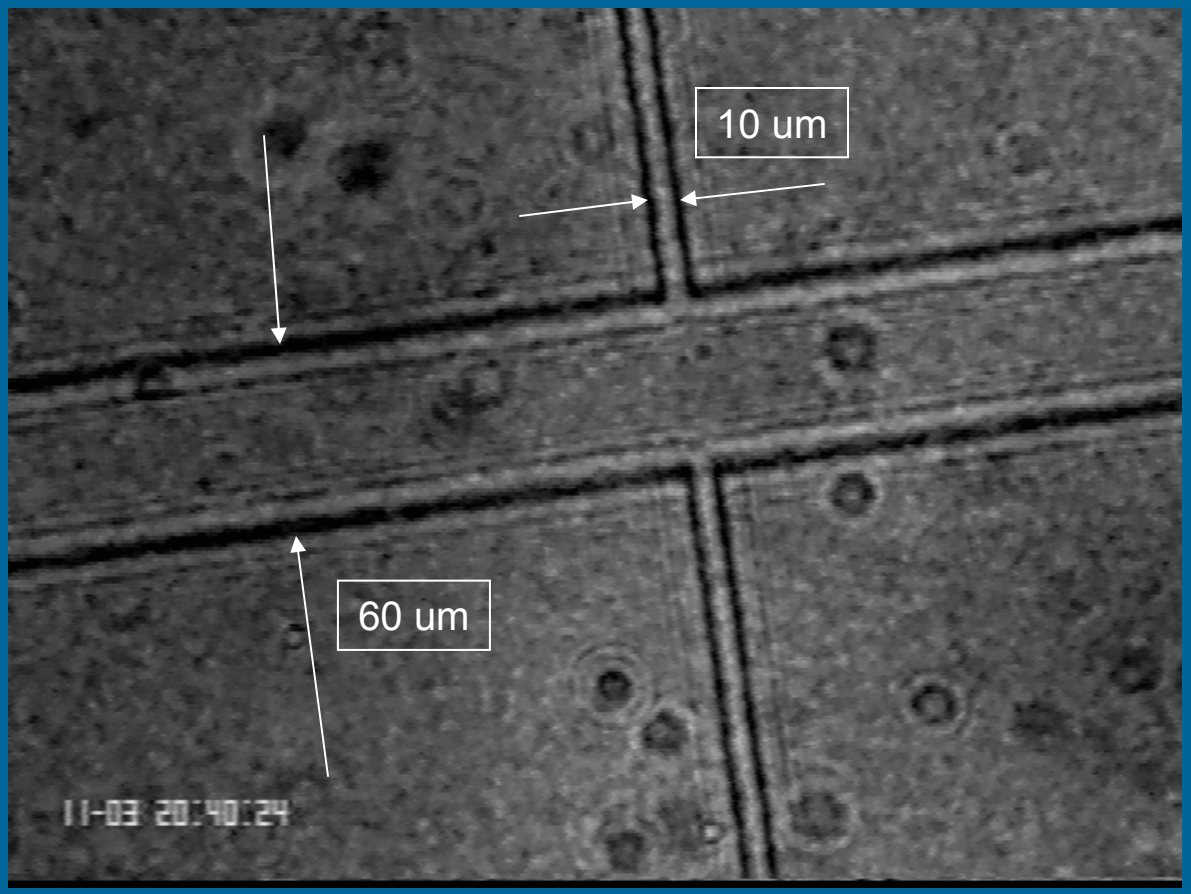


Fluid-filled microchannels

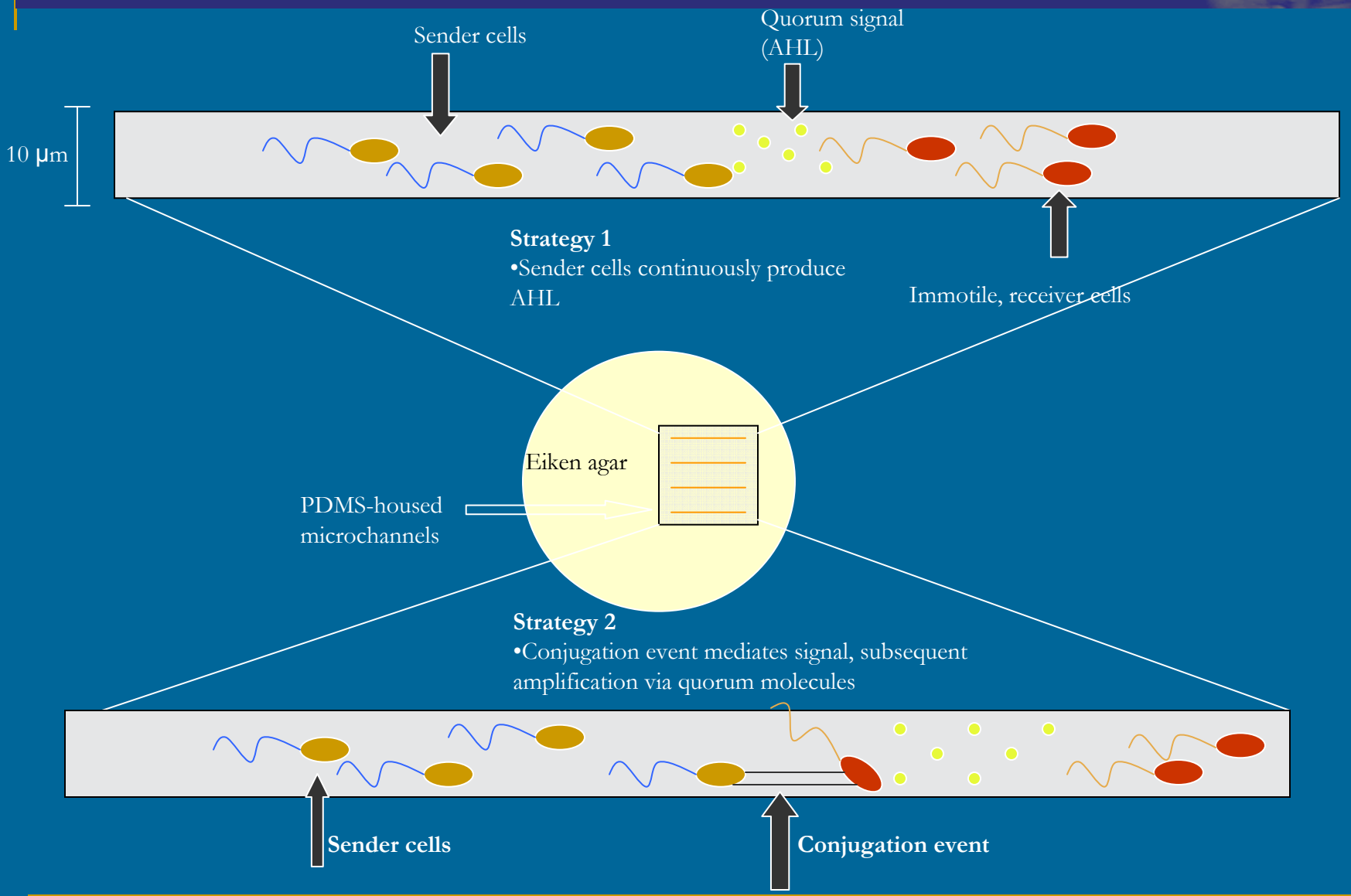


Peel off PDMS, place feature-side
down on agar (eiken) plate

Microchannel pictures



Signal representation & transfer



Strategy 1

Sender cells continuously produce AHL

<u>Advantages</u>

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

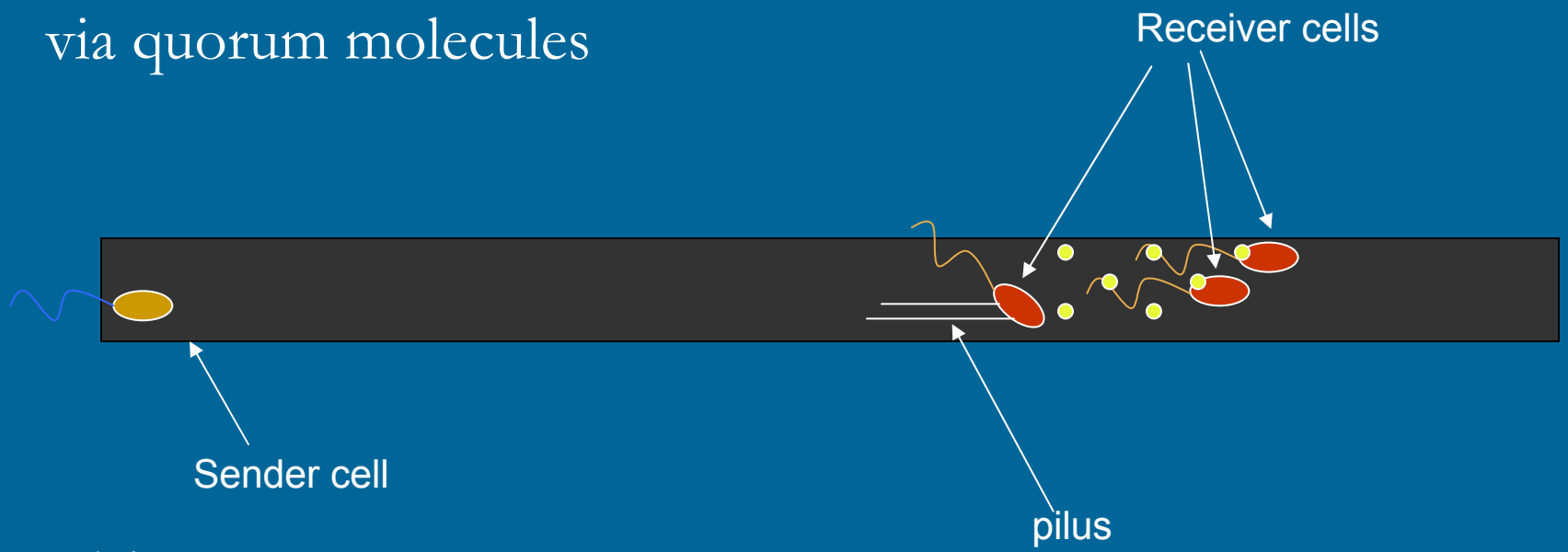
<u>Potential drawbacks</u>

Inadequate production of AHL for activation?

Leaky expression from p_{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

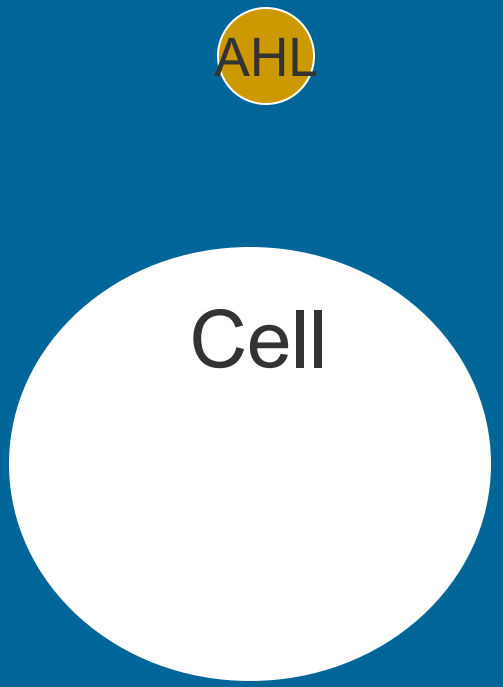
Increased time required

F^+ plasmid needed (additional DNA needed)

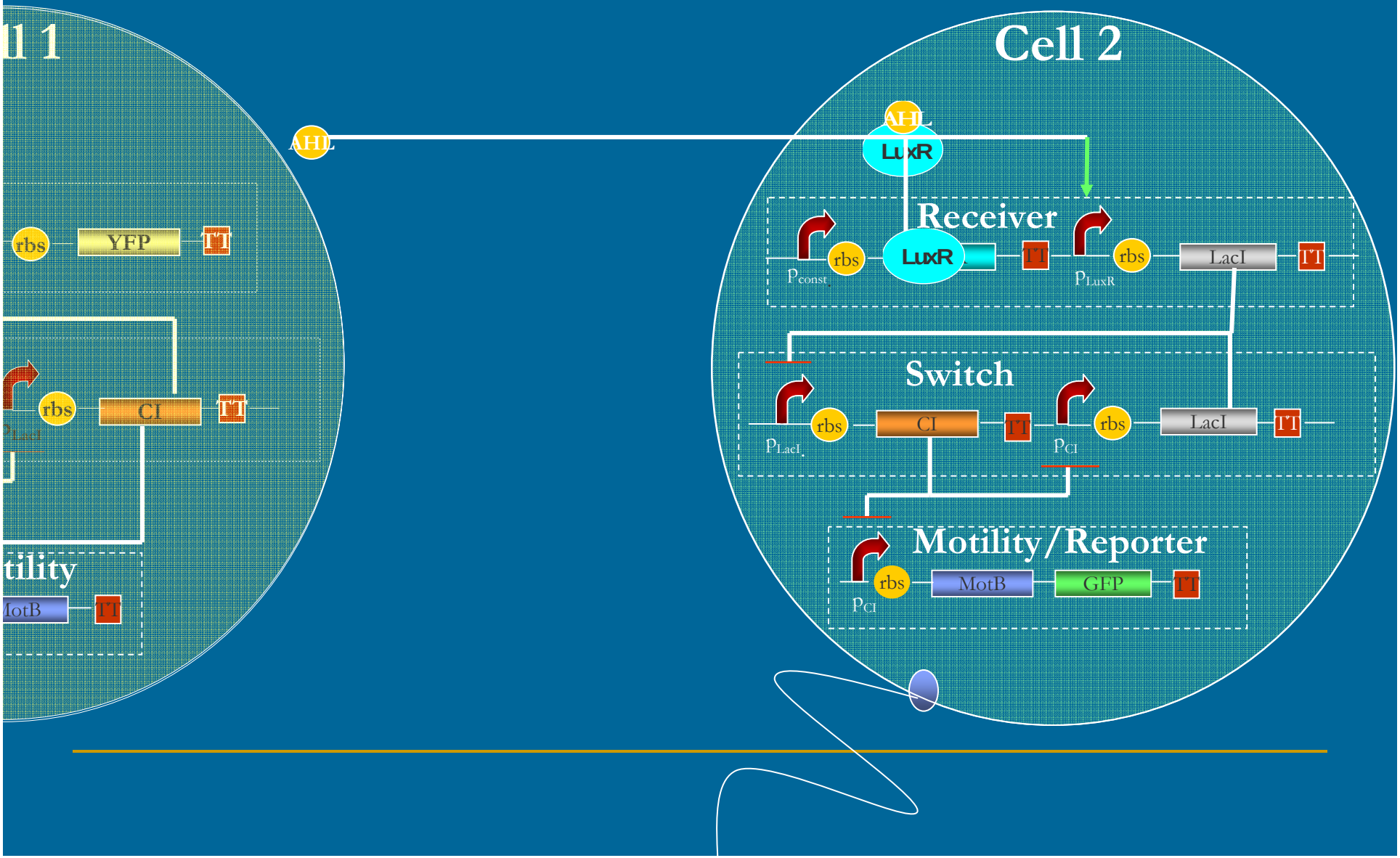
Quorum Sensing

acyl homoserine lactone (AHL)

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



Genetic control mechanism



What About the Cell?

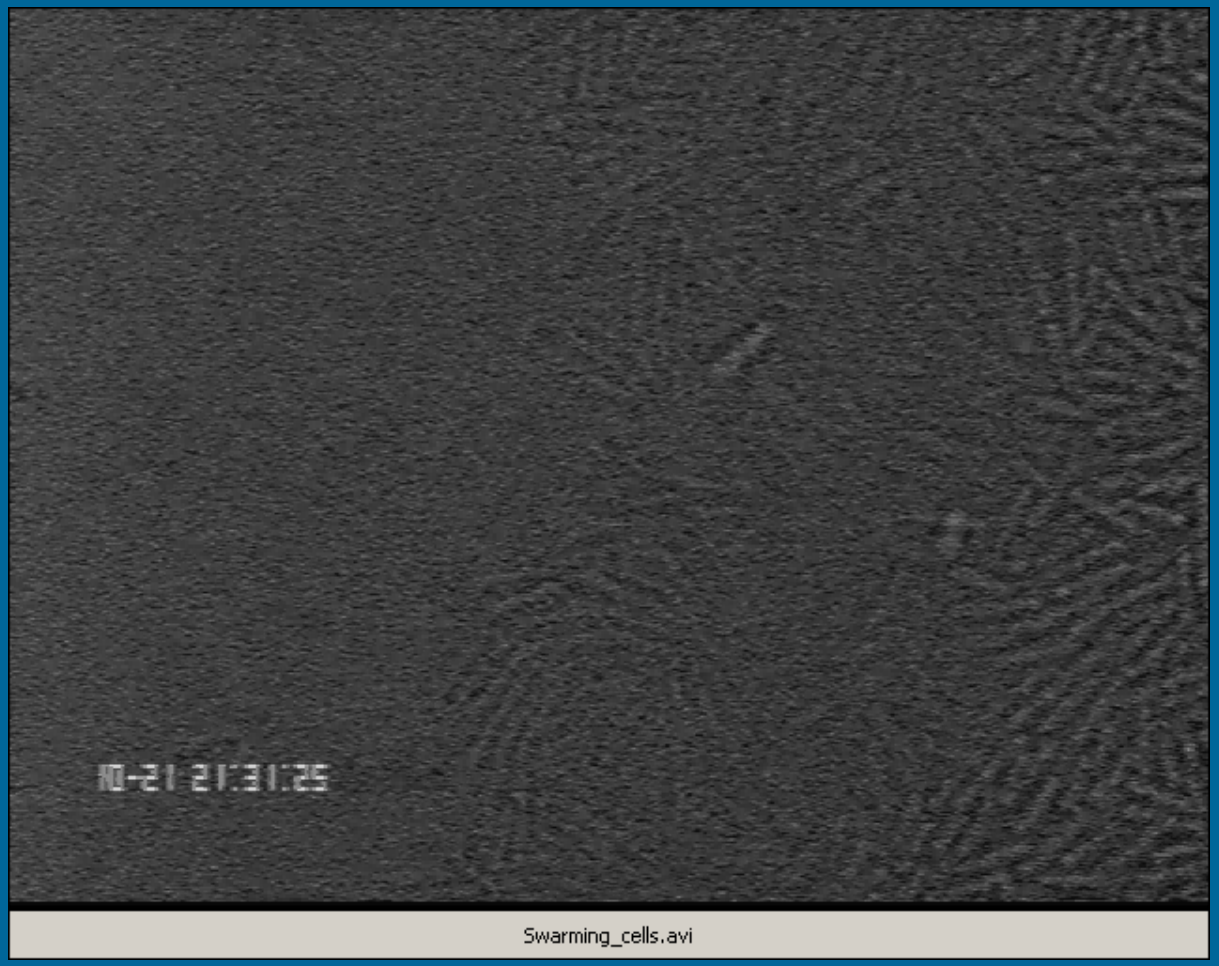
- Knock out MotB (ASKA Library)[1,2]
  - Put MotB on our plasmid, under CI control
- Knock out RecA
  - RecA will delete multiple copies of genes
- Knock out LacI
  - Preventing interference with our process

1) ASKA library Kitagawa, M., Ara, T., Arifuzzaman, M., Ioka-Nakamichi, T., Inamoto, E., Toyonaga, H. and Mori, H. Complete set of ORF clones of Escherichia coli ASKA library (A Complete Set of E. coli K-12 ORF Archive): Unique Resources for Biological Research. DNA Res 12, 291-299 (2005).

2) 2) Keio collection Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L. and Mori, H. Construction of Escherichia coli K-12 in-frame, single-gene knock-out mutants the Keio collection. Mol Systems Biol, doi:10.1038/msb4100050 (2006).

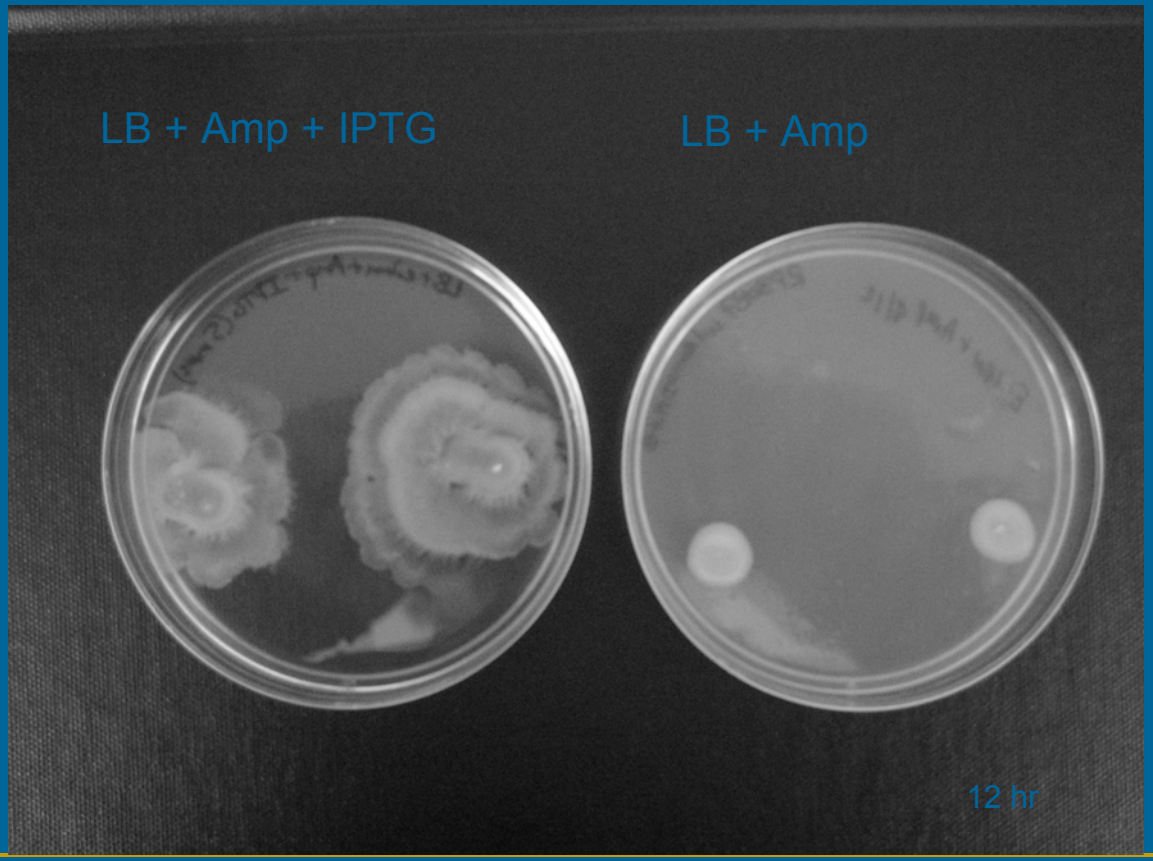
Initial Results

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



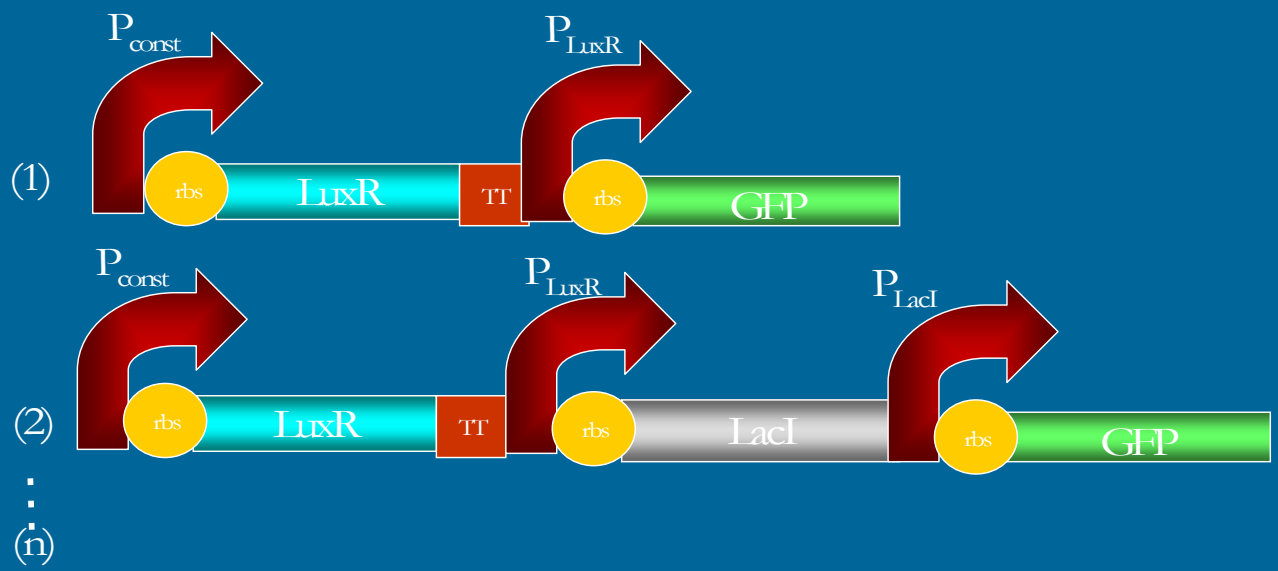
Initial Results

- Test for ability to control motility
  - Placed under control of BBa_R0010 (LacI promoter) induced with IPTG



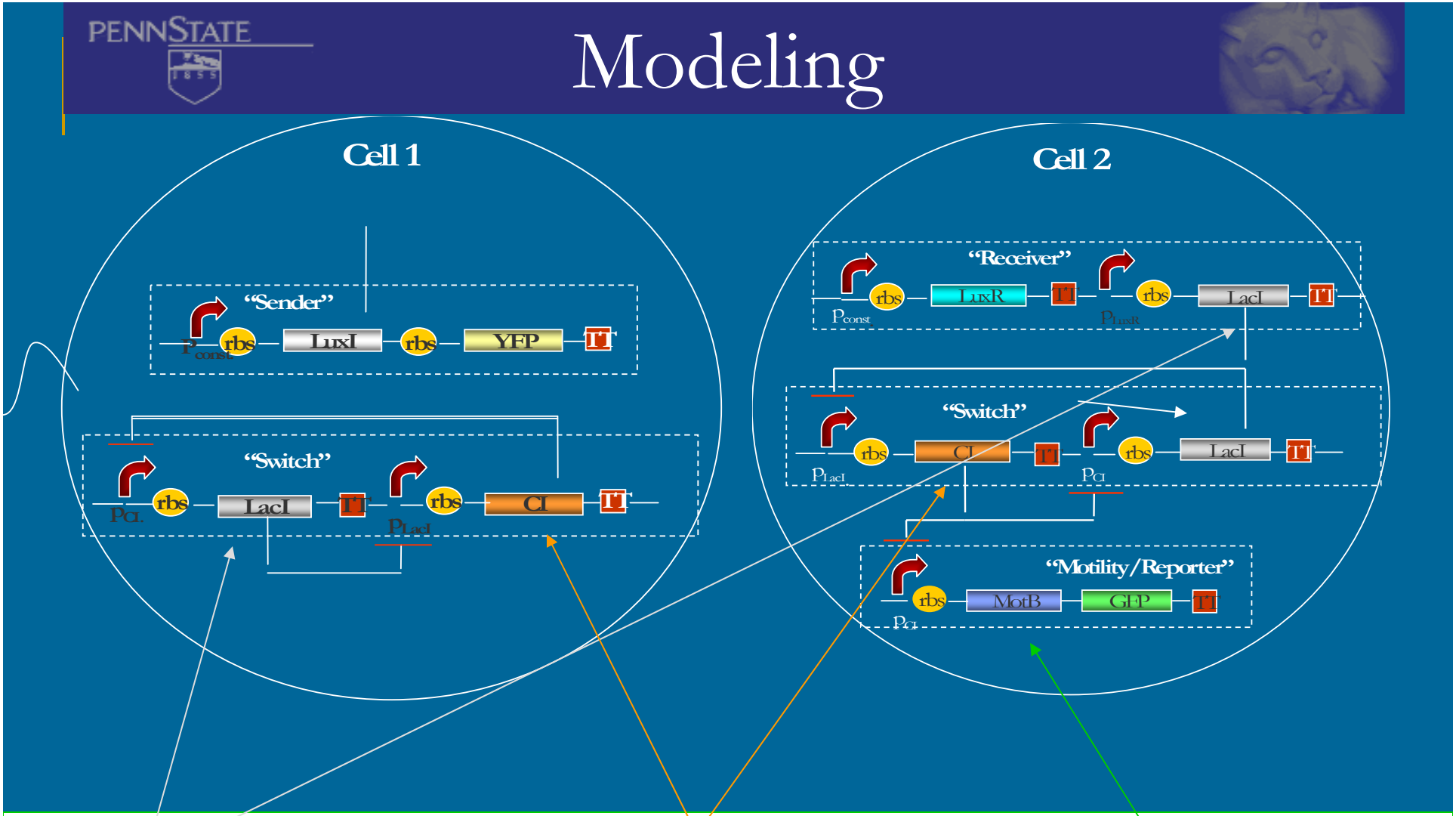
Initial Results/Progress

- 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 fluorescencing

Modeling



LacI

$$\frac{dLacI}{dt} = k_2[RlacI][r] - k_d[LacI]$$

$$\frac{dR_{lacI}}{dt} = k_3[N][L_H][P] + k_1[N][P] - k_2[R_{lacI}][r] - k_d[R_{lacI}]$$

CI

$$\frac{dCI}{dt} = k_4[R_{CI}][r] + k_R[CI_2] - k_d^{CI}[CI]$$

$$\frac{dR_{CI}}{dt} = kCI[P][N_{CI}] - k_1[R_{CI}][r] - k_d^{CI}[R_{CI}]$$

$$\frac{dL_{CI}}{dt} = k_2[I]^4[N_{CI}] - k_3[L_{CI}]$$

MotB

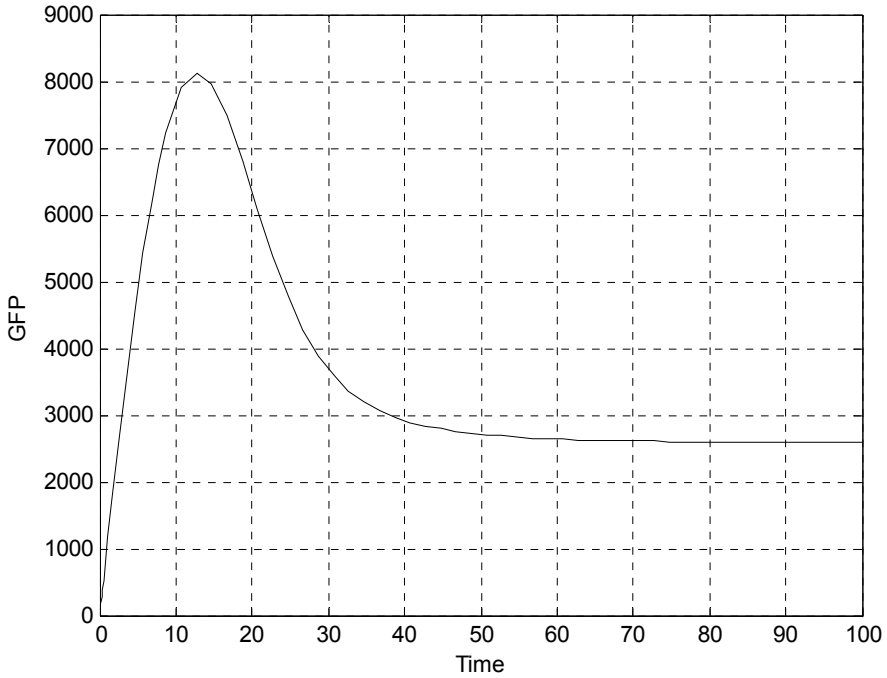
$$\frac{dM}{dt} = k_B[R][N] - k_d^M[M]$$

$$\frac{dR}{dt} = k_m[P][N] - k_B[r][R] - k_d^R[R]$$

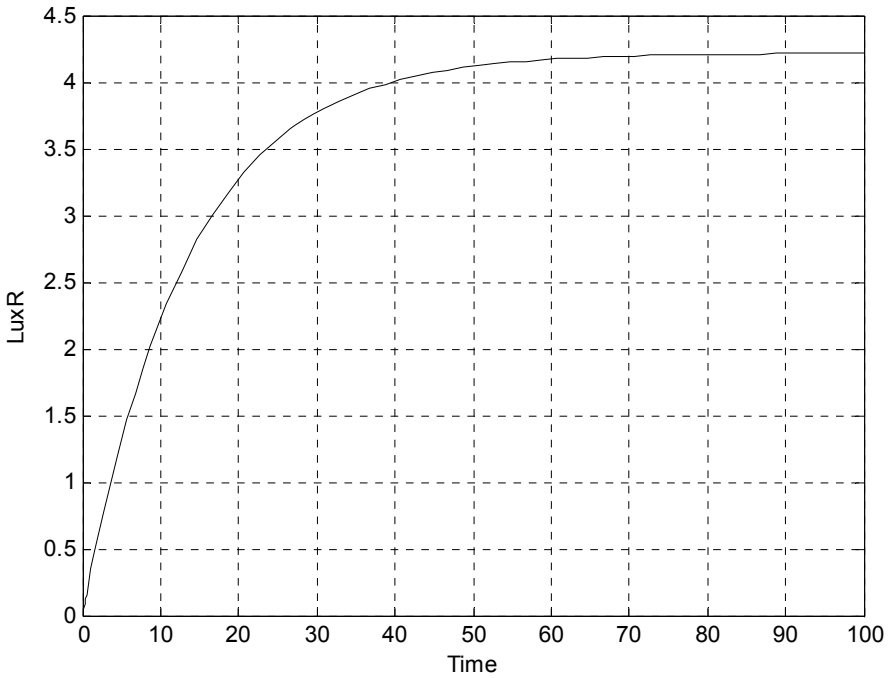
$$\frac{dN}{dt} = k^{-1}[N][CI_2] - k_m[P][N] - k^{+1}[N][CI]^2$$

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!

Acknowledgments

MIT for hosting 2005 iGEM Jamboree

We have been supported by:

- Dean of Undergraduate Research's Office
- Huck Institute of the Life Sciences
- The departments of Agricultural & Biological, Chemical, and Bio-Engineering
- Eberly College of Science
- Center for Nanoscale Science NSF MRSEC
- Materials Research Institute
- Penn State Nucleic Acid Facility for providing PCR primers

The Dorothy Foehr Huck and J. Lloyd Huck Institutes of the Life Sciences
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National Science Foundation
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Departments:
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Faculty Advisors:
S.P. Walker, Drs. R. Balasubramanian, W. Hancock, P. Cirino, M. Tien, D. Farber, P. Weiss, B. Nixon, J. Regan, V. Narayanan, and T. Richard