E. COLYMPIC GAMES
Project Aims

- A functioning bacterial ‘game’ system
- A new Bio-brick
- A generic simulation applicable to bacterial communications
- Increased public and governmental awareness of Synthetic Biology
- Implementation of a synthetic biology outreach legacy
“To exploit the quorum sensing processes and engineer specific plasmids within bacterial cells to achieve the ‘Game of Life’ and ‘Rock-Paper-Scissors’ interactions.”

Playing games with bacteria!

- Ability to demonstrate complex bacterial interactions
- Fun, approachable idea
Project Idea

How the cells would defeat each other...
...Neutral Interaction using Fluorescent proteins

ROCK

How the cells would only defeat ONE opponent....
...Quorum sensing using Autoinducers

PAPER

SCISSORS
IPTG
HSL
(from RhII in paper)
Autoinducers
(activates Plas in scissors)

**‘Rock-Paper-Scissors’ Plasmid Map**

- **IPTG**
- **HSL** (from RhII in paper)
- **Autoinducers** (activates Plas in scissors)

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- **rhlR**
- **P_{J23119}**
- **P_{Pac}**
- **mCherry**
- **lasI**
- **LacI**
- **LacI**
Conway’s Game of Life

- *In silico* modelling to gain insights into the functioning of our system
- Converted plasmid maps to flow charts
- Flow charts easy to model as finite state machines
- Results visible in the form of spatio-temporal patterns
- Environment divided into layers
- Diffusion algorithm allows molecules to migrate, affecting the respective cell state-machines

- Simulator written in Tcl and uses NAP[1] for fast array processing

Figure 1: Simulation results

Game of Life

- Simple system to introduce us to concept of biological networks
- Initial drop of trigger molecule causes quorum message chain-reaction
- Red circle expands with time

Figure 2: Game of Life results
Figure 3: R-P-S beginning of simulation
Rock-Paper-Scissors

Figure 4: R-P-S mid-simulation
Figure 5: R-P-S end of simulation
Figure 6: Animated results
Simulator

- ‘What if there was a program that could simulate any system using mechanisms like ours?’

- Simulator generalised to take out custom functions and allow non-programmers to use it

- Link to latest release on wiki[2]

Wetlab

- What did we want to achieve?
- How were we going to achieve it?
- Any safety implications for team?
- Any safety implications for our schools outreach activities?

http://2009.igem.org/Team:Southampton/Project/Safety
Results - Game of life

Original digestion conditions: 2 hours at 37°C
Original ligation conditions: 2 hours at RT
Results - Rock, paper, scissors

Original digestion conditions: 2 hours at 37°C
Original ligation conditions: 2 hours at RT
Results

Original digestion conditions: 2 hours at 37°C

Original ligation conditions: 2 hours at RT

TA cloning of the insert followed by single digestions

Overnight gel purification

Dephosphorylation of the vector
**Results**

Original digestion conditions: 2 hours at 37°C
Original ligation conditions: 2 hours at RT

TA cloning of the insert followed by single digestions
Overnight gel purification
Dephosphorylation of the vector

Ligation conditions: 18 hours at 16°C
Successful cloning of P_{Tac}
Results - $P_{Tac}$

- The Tac promoter is stronger than Trp and Lac individually.

- The dual promoter is repressible by lac/ trp and can be induced by IPTG.

- The Tac promoter is the key component in the processing section from the simulations.

$P_{Tac}$ - De Boer et al., *Proc. Natl. Acad. Sci USA* 1983, 80, 21-25
Creation of construct which could test functionality

Fluorescence could then be measured upon addition of IPTG.
Achievements

- A functioning bacterial ‘game’ system

✓ A new Bio-brick

✓ A generic simulation applicable to bacterial communications
✓ Increased public and governmental awareness of Synthetic Biology
Increased public and governmental awareness of Synthetic Biology
- A functioning bacterial ‘game’ system

- A new Bio-brick

- A generic simulation applicable to bacterial communications

- Increased public and governmental awareness of Synthetic Biology

- Implementation of a synthetic biology outreach legacy
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