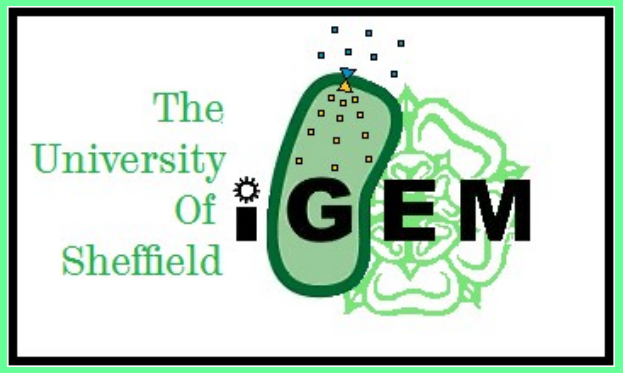
International Genetically Engineered Machines Competition

M.I.T, Nov 7th-9th 2008



An introduction to the University of Sheffield 2008 iGEM Team...

Who are we?

Gosia Poczopko

1st year Molecular and Cellular Biochemist

Dmitry Malyshev

1st year Biomedical Engineer

Eva Barkauskaite

1st year Biochemist

Hammad Karim

2nd year Engineer

Rosie Bavage

1st year Molecular Biologist

Sam Awotunde

2nd year Engineer

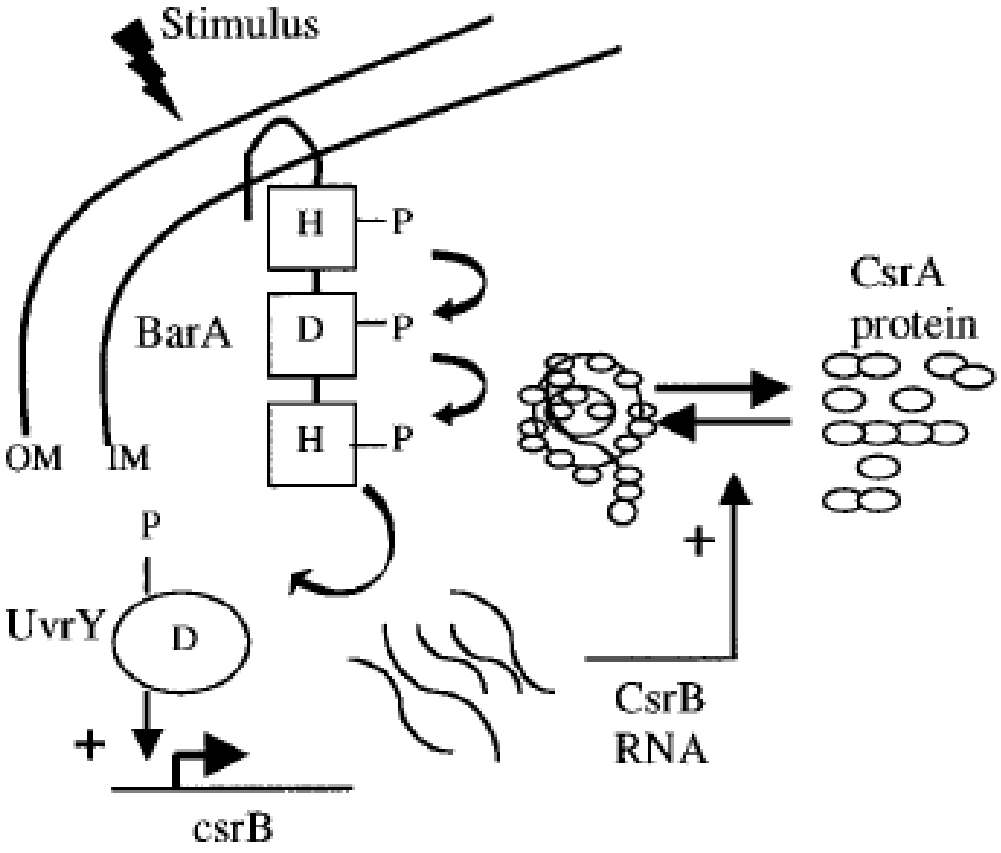
What is iGEM?

- iGEM is a rapidly increasing international competition for undergraduates in many different specialisations
  - Designed to involve undergraduates in research early in their careers
  - Over 84 teams from all around the world this year
- Premise is to expand on the principle of synthetic biology
  - Pieces of DNA are designed and standardised at each end, in the hope of building novel organisms
  - Information made publicly available
  - 'Wiki'

The Idea

- A biosensor for cholera in drinking water – machine/test/kit
- We want to hijack a pathway in E.coli and manipulate it to detect *Vibrio cholerae* quorum sensing autoinducers
- GFP marker inserted downstream
- Proof of principle in fusion kinase

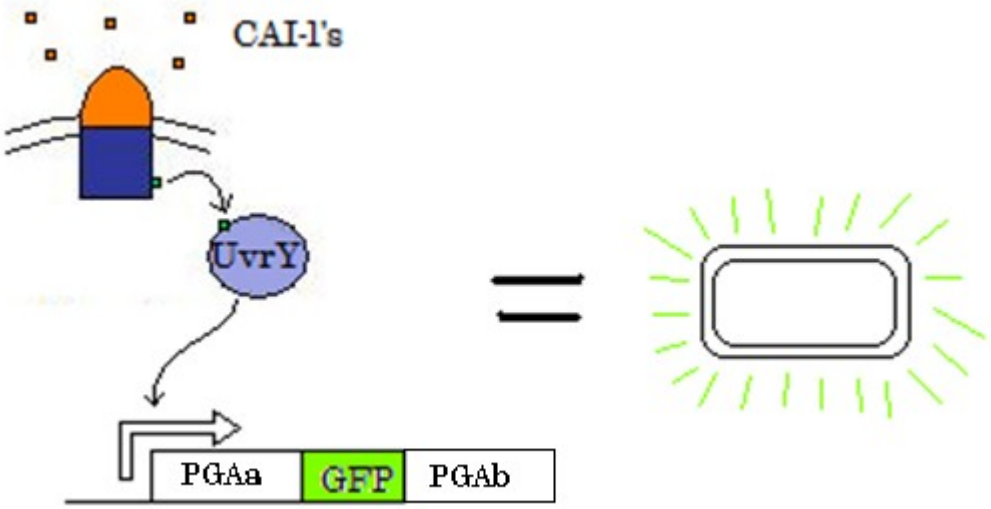
BarA Pathway



- More than 20 target genes for BarA
- Includes glycogen synthesis, glycolysis, gluconeogenesis, glycogen catabolism.
- Our target: PGA operon – role in biofilm formation

GFP into genome

- GFP will act as our reporter
- Inserted into the genome under the promoter of PGA operon between PGAa and PGAb

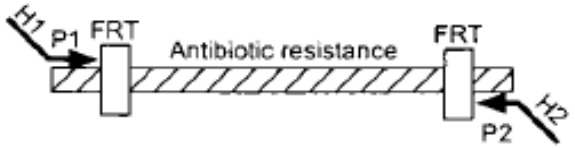


Gene Knockout

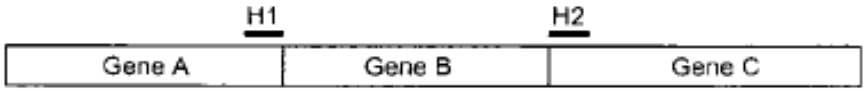
- To make sure native BarA doesn't trigger the production of GFP, we need to knock out certain genes from our strain
- Using Datsenko and Wanner's method for speeding up recombination
- PCR products provide homology, λ Red recombinase system provides faster recombination.
- Marker gene removed later

Gene Knockout

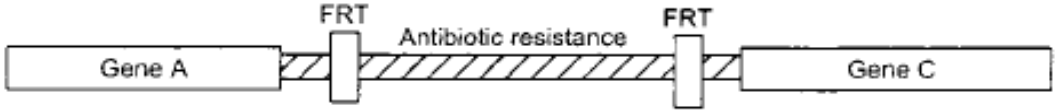
Step 1. PCR amplify FRT-flanked resistance gene



Step 2. Transform strain expressing λ Red recombinase



Step 3. Select antibiotic-resistant transformants



Step 4. Eliminate resistance cassette using a FLP expression plasmid

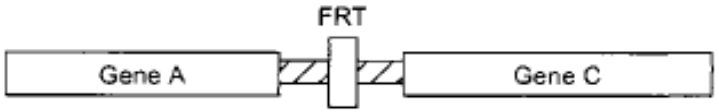


Fig. 1. A simple gene disruption strategy. H1 and H2 refer to the homology extensions or regions. P1 and P2 refer to priming sites.

Problems

- We couldn't get a knockout
- Various setbacks and little time

Fusion Histidine-Kinase

CAI-1 Synthesis

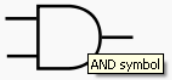
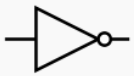
- CqsA is the synthesis machine for CAI-1's in cholera
- Bonnie Basslers lab designed plasmid and protocol for transferring CqsA into E.coli and purify the CAI-1 product – it works
- Received and used
- Mass-spec to confirm

Engineering

- Synthetic biology is the application of engineering principles and approach to molecular biology
- Mathematical modelling of the system is part of the iGEM project
- The BioBricks
- Current theories in use:
  - Boolean modelling
  - Modelling in Simbiology

Engineering – The Boolean Model

- Biological processes treated as discrete
  - i.e either ON or OFF
  - assumption that the transition between states is synchronous
- Analogy of biological systems as electrical circuit components
  - eg NOT-AND gate

Type	Distinctive shape	Boolean algebra between A & B	Truth table
AND		$A \cdot B$	INPUT A B / OUTPUT A AND B: 0 0 0; 0 1 0; 1 0 0; 1 1 1
NOT		$\overline{A}$	INPUT A / OUTPUT NOT A: 0 1; 1 0

Engineering - Simbiology Model

- Using Simbiology reaction modelling, we can model the pathway. For simplicity we split it into 3 parts
  - 1.) this equation models the sensor kinase and the response regulator
  - 2.) the phosporylation cascade
  - 3.) transcription and translation of the GFP
- Model not yet implemented for the latest project version (barA).

Comparing the 2 models

- Ideally both models should predict similar results, and the prediction should be correct.
  - However, there could be differences in the modelling results due to different model types.
  - There could be overlooked factors.

Further ideas

- Re-usuable sensor
  - Cleavable GFP/ housekeeping gene regulation – LVA tag.
  - Provided by past iGEM project = criteria for an award
- Threshold experiments
  - Modelled

Acheive: Bronze Award

- Register
- Complete and submit a Project Summary form.
- Create an iGEM wiki
- Present a Poster and Talk at the iGEM Jamboree
- Enter information detailing at least one new standard BioBrick Part or Device in the Registry of Parts
  - including nucleic acid sequence, description of function, authorship, safety notes, and sources/references.
- Submit DNA for at least one new BioBrick Part or Device to the Registry of Parts
- We've done all of these
- Silver: BioBrick characterisation...

BioBrick Characterisation

- Criteria for ‘Silver Award’
- Serious transformation issues

Sponsors

- idtDNA – £1000 gene, and 10 free primers
- iChemE - £1000 reimbursement for travel
- £2500 from Prof Poole MBB (covered all flights and hotels)
- Printing and other minor costs from MBB Funds

Our many thanks go to…

- Prof Philip Wright
- Dr Catherine Biggs
- Esther Karunakaran
- other ChELSI members
- Dave Wengraff
- Prof David Hornby
- Prof Robert Poole
- Prof David Rice
- Prof Jeff Green
- Prof Visakan Kadirkhamanatan
- The Bassler, Stafford and Karolinska Institute labs for plasmid provision.

References

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