Triggering Pigment Production in *E. Coli*

Mike Davies, Shuna Gould, Siming Ma, Vivian Mullin, Megan Stanley, Alan Walbridge, Crispian Wilson

*Celebrating 800 Years of Innovation at Cambridge University*
The Cambridge 2009 iGEM team has created a Kit of Parts that will facilitate the design and construction of biosensors in the future.

We have developed a set of Sensitivity Tuners and a set of Colour Generators.
Bacterial Biosensors: the Detection of Environmental Pollutants

- Bacterial biosensors - an alternative to chemical methods
- Still selective and sensitive
- Inexpensive
- Less labour intensive
- More accessible
Bacterial Biosensors: **Bobolitriens**

**Lack of self-contained output** – Reliance on reporters in Registry – Require additional technology to read output

**Colour Generators** – Bacterial pigments – Visible, user-friendly output

**Sensitivity Tuners**

- PoPS converts promoter
- Change sensitivity of upstream promoters
- Limited by sensitivity of promoter

**Bacterial Biosensors: Solutions**

- Sensitivity Tuners
- Colour Generators

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Bacterial Biosensors: A prototype

Inducer concentration:

- 0
- low
- high

The colour readout indicates concentration of inducer
Bacterial Biosensors: How to build a bacterial biosensor with these parts

- **SENSOR**
  - Promoter sensitive to input

- **SENSITIVITY TUNER**
  - Phage activator

- **COLOUR GENERATOR**
  - Activator sensitive to input

Chemical IN → Sensor → Sensitivity Tuner → Colour Generator → Pigment OUT
Sensitivity Tuners: Introduction

- A Sensitivity Tuner allows adjustment of sensitivity to input.
- A combination of different Tuners in parallel allow measurements of a range of discrete input concentrations.
Design: *an Input to Output Device*

PoPS in

Phage activator

Activator sensitive promoter

PoPS out

Transcriptional and Translational Characteristics

Activator Concentration

Promoter Characteristics

PoPS in

PoPS out
"Amplifiers"
- GFP output controlled by phage promoter
- RFP output controlled by pBad input
- Characterized as an “amplifier” by ratio of RFP to GFP
Model gene characteristics at steady state using Law of Mass Action
- pBAD is repressed by repressor X* which binds to arabinose
- Assume transcription and translation are linear functions of PoPS
- Model protein concentrations as dynamic, since these change slowly
- Allow for protein degradation
The model contains a large number of constants

\( A \) priori modelling requires arbitrary values to be chosen

Maximum reporter production rate is sigmoidal with inducer concentration

**Modelling Results:** *Sigmoidal Behaviour*

- Reporter Degradation rates at multiple input concentrations of arabinose
- Model for maximum fluorescence rate
Curve Fitting: Hill Function

\[
\frac{d[GFP]}{dt} = \frac{a \cdot [Arabinose]^n}{k^n + [Arabinose]^n} + c
\]

A model Sensitivity Tuner

- Half-maximal induction (k)
- Concentration of Arabinose
- Peak rate
- Increase in rate (a)
- Basal rate (c)
- Rate of GFP expression

1 RPU

Hill coefficient (n)
Sensitivity Tuners: *Changing the sensitivity of an upstream promoter*

- Constructs were tested on high copy against pBAD characteristics
- Output triggered at much lower arabinose concentration when Sensitivity Tuner included

![Graphs showing maximum normalised GFP production vs. arabinose concentration for pBAD -> GFP and pBAD -> Construct 91 -> GFP.]
Sensitivity Tuners: *Characterisation*

- 15 Cambridge 2007 constructs moved down to low copy plasmid
- High throughput testing
- 3 repeats of 3 colonies over 8 concentrations
- OD and fluorescence measured
- Standard Promoter included on plate to allow for RPU measurements

<table>
<thead>
<tr>
<th></th>
<th>P2 ogr</th>
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<th>phiR73 delta</th>
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<td>I746375</td>
<td>I746385</td>
<td>I746395</td>
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</table>
Sensitivity Tuners: *Software*

- Matlab graphical interface developed to allow data to be viewed in several ways
- Standard promoter data allows for RPU characterisation
Curve Fitting: *Hill Function*

- Non-linear least squares method used to fit Hill functions to measured data
- Each fit produces the parameters of the Hill function, enabling construct to be quantitatively analysed
Sensitivity Tuners: Parameters

- A range of Sensitivity Tuners were successfully characterised on low copy
- Good range in sensitivity: 10x range in half-maximal induction
- Hill coefficients of 2 – 3 when concentration resolution is sufficient
- Wide range of rate increases, from 0.3RPU to 1.2RPU
Sensitivity Tuners: *Design*

- A standard kit was designed using well characterised candidates
- Tuners can be used with any promoter
- Any device can be placed downstream of the construct

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<th>PSP3 pag</th>
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**Colour Generators: Choosing pigments**

- **Diversity**:
  - Colour
  - Bacterial Origin

- **Design**
  - Standard Assembly
  - PCR
  - Synthesis

- **Potential for Manipulation**
  - Single gene systems
  - Multigene systems with colourful intermediates
  - Supplements to media

- **Color Generators**:
  - Violacein
  - Melanin
  - Carotenoids
Violacein: Background

- Quorum-sensing controlled pigment from Chromobacterium violaceum
Violacein: Design & Synthesis

K274002

BamHI  BglII  BclI

VioD  VioB  VioE

VioA  VioC

DNA 2.0

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Violacein: Design & Synthesis

K274002

VioA → VioB → VioC → VioD → VioE

BamHI / BglII / BclI

GGATCC AGATCT
CCTAGG TCTAGA

DNA 2.0

Cambridge 2009
Violacein: Design & Synthesis

K274002

VioA → VioB ← VioC → VioD → VioE

BamHI / BglII / BclI

G

G A T C T

C C T A G

VioA ← VioB → VioC → VioD → VioE

K274003

DNA 2.0

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Violacein: Expression & Quantification
Violacein: Expression & Quantification

Absorbance at 584nm

- Absorbance (mM)
- Wavelength

Violacein and TOP10 absorbance comparison at 584nm.
**Violacein: Colour Logic**

- **If** $A =$ constitutive, $B = $ inducible

<table>
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<tr>
<th>$A$</th>
<th>$B$</th>
<th>Output</th>
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<tr>
<td>0</td>
<td>0</td>
<td>No colour</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td><strong>GREEN</strong></td>
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<tr>
<td>0</td>
<td>1</td>
<td>No colour</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td><strong>VIOLET</strong></td>
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</table>

**Colour Output**

- Device working:  
- Presence of $B$:  
- **VIOLET**
Melanin: Background

- Brown pigment made in many animals and bacteria via the action of a tyrosinase (MelA)
- Isolated melA from *Rhizobium etli*.
- Media supplemented with copper sulphate and tyrosine
• Used native RBS and planned to remove forbidden restriction sites using PCR

• Strong candidate for a biosensor reporter
  – Strong pigment production
  – Single gene
Carotenoids: Background

Lycopene

β-Carotene
Carotenoids: Background

Lycopene

β-Carotene
Carotenoids: Background

Lycopene

Non-mevalonate Pathway (already present in *E. coli*)

pyruvate → glyceraldehyde-3-phosphate → Farnesyl pyrophosphate (Colourless precursor)

Farnesyl pyrophosphate → CrtE → CrtB → CrtI → CrtY

β-Carotene
Carotenoids: Standard assembly

Enzymes coding sequences from *Pantoea ananatis* (Enterobacteria)

Farnesyl pyrophosphate
(Colourless precursor)

\[ \text{CrtE} \quad \rightarrow \quad \text{CrtB} \quad \rightarrow \quad \text{CrtI} \quad \rightarrow \quad \text{CrtY} \]

\[ \text{Lycope} \text{ne} \]

\[ \beta\text{-Carotene} \]
Carotenoids: *Standard assembly*

Expression in *E. coli* strain MG1655
Carotenoids: Expression and Quantification
Carotenoids: Expression and Quantification

**β-Carotene:**
1.5 µg per mL culture
Proof of Concept: Pigment Induction

**SENSOR**
- Promoter sensitive to input

**SENSITIVITY TUNER**
- Phage activator
- Activator sensitive promoter

**COLOUR GENERATOR**
- Pigment producing device

**Pbad promoter**
- IO500
- CrtE
- CrtB
- CrtI

**IRCT K274120**

**IOCT K274220**
**Proof of Concept: Pigment Induction**

**β-Carotene:**

1.3 µg per mL induced culture

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**Carotene extraction in acetone with and without arabinose**

- **No arabinose**
- **Induced by 1mM arbinose**
- **Control**
- **5 µg carotene**

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Absorbance (unit)</th>
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<td>400</td>
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<tr>
<td>520</td>
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<tr>
<td>540</td>
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**Absorbance at 456nm (β-carotene)**

- **No arabinose**
- **Induced by 1mM arbinose**
- **Control**
- **5 µg carotene**

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<th>Absorbance (unit)</th>
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<td>0.30</td>
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<td>0.33</td>
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**1mM arabinose**

**No arabinose**

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# BioBricks: Sensitivity Tuners

![Diagram](image)

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<th>K274370</th>
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BioBricks: Colour Generators

VCG K274002
BCG K274001
RCG K274100
OCG K274200
GCG K2742003

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BioBricks: Systems

R0011  CrtE  CrtB  CrtI

I0500  CrtE  CrtB  CrtI

R0011  CrtE  CrtB  CrtI  CrtY

I0500  CrtE  CrtB  CrtI  CrtY

RCT  K274110

OCT  K274210

IRCT  K274120

IOCT  K274220
Further Work: for our Project

- Show compatibility with promoters in Registry
- Expand kit of parts
  - Phage activators and phage promoters
  - Pigment-producing operons from other bacterial species
Multiplexing Information: Accessible and Informative Biosensors

- Arsenic
- Mercury
- Lead
- As + Hg
- Ag + Pb
- Hg + Pb
- As + Hg + Pb
The Cambridge 2009 iGEM team...

...would like to say a few thank yous
Thank You...

...to Jeremy Minshull and his colleagues at DNA2.0 for their generous offer to help us build and synthesize the violacein operon.
Thank You... to all our sponsors

Cambridge 2009
Thank You...

Advisors:
Dr. Jim Ajioka
Dr. Jim Haseloff
Dr. Gos Micklem
Dr. Tom Ellis
Dr. Duncan Rowe

...and especially James Brown

Summary of Achievements:
Designed 23 New Biobricks
Characterised 15 Biobricks already in the registry

Friends:
Caitlin Cockerton
Daisy Ginsberg
James King
Tuur Van Balen

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Summary of Achievements:
Designed 23 New Biobricks
Characterised 15 Biobricks already in the registry
It’s Mike’s birthday today…hopefully he’s not looking at the screen!

We’d like to sing him happy birthday, so join us!

On 3.....

1
2
3!!!