“Scientists discover the world that exists; Engineers create the world that never was.”

Theodore von Karman
Synthetic Biology
- Foundational Principles
- Fundamental Research Review
Synthetic Biology
- Foundational Principles
- Fundamental Research Review

The Registry of Biological Parts
- Overview
- BioBricks & Standard Assembly
- Rational Design
Synthetic Biology
- Foundational Principles
- Fundamental Research Review

The Registry of Biological Parts
- Overview
- BioBricks & Standard Assembly
- Rational Design

The iGEM Competition
- History of Competition
- Project examples
Synthetic Biology
- Foundational Principles
- Fundamental Research Review
1953
Physical Sciences

1873
1897
1882
1947
1958

Biological Sciences

1953
1972
1975
1983

Electrical/Electronic Engineering

1953
1972
1975
1983

Biological Engineering

?
Existing Technologies

Biological research focuses on:
- study & analysis of naturally evolved systems
- ad-hoc construction of ‘genetically-engineered’ solutions

eg. insulin-production in bacteria
pesticide-resistance in crops
Biological research focuses on:
- study & analysis of naturally evolved systems
- ad-hoc construction of ‘genetically-engineered’ solutions
  eg. insulin-production in bacteria
  pesticide-resistance in crops

1st Generation Biotech.

Enabling Technologies:
- Polymerase Chain Reaction
- Recombinant DNA
Emerging Technologies

2nd Generation Biotech.

Enabling Technologies:

*Polymerase Chain Reaction*

*Recombinant DNA*
Emerging Technologies

2nd Generation Biotech.

Enabling Technologies:
Polymerase Chain Reaction
Recombinant DNA
DNA Sequencing
Emerging Technologies

2nd Generation Biotech.

Enabling Technologies:

* Polymerase Chain Reaction
* Recombinant DNA
* DNA Sequencing
* DNA Synthesis
Emerging Technologies

2nd Generation Biotech.

Enabling Technologies:

- Polymerase Chain Reaction
- Recombinant DNA
- DNA Sequencing
- DNA Synthesis
- Computational Modelling & Design
Emerging Technologies

2nd Generation Biotech.

Enabling Technologies:

Polymerase Chain Reaction
Recombinant DNA
DNA Sequencing
DNA Synthesis
Computational Modelling & Design

‘Synthetic Biology - Life 2.0’
The Economist, August 31st 2006
Existing Biotechnology
Application of Engineering Principles to Biological Systems

Essential to apply engineering principles:

- Modularity
- Standardisation
- Abstraction
- Decoupling
Application of Engineering Principles to Biological Systems

Essential to apply engineering principles:

- Modularity
- Standardisation
- Abstraction
- Decoupling

Consider rational engineering of novel synthetic devices and systems
Engineering Principles for Biology

Decoupling

- Rules insulating design process from details of fabrication
- Enable parts, device, and system designers to work together
- VLSI electronics, 1970’s
Engineering Principles for Biology

Decoupling
- Rules insulating design process from details of fabrication
- Enable parts, device, and system designers to work together
- VLSI electronics, 1970's

Standardisation
- Predictable performance of parts, devices & systems
- Off-the-shelf standardised components
- Mech. Eng; 1800’s
Engineering Principles for Biology

**Decoupling**
- Rules insulating design process from details of fabrication
- Enable parts, device, and system designers to work together
- VLSI electronics, 1970’s

**Standardisation**
- Predictable performance of parts, devices & systems
- Off-the-shelf standardised components
- Mech. Eng; 1800’s

**Abstraction**
- Insulate relevant characteristics from overwhelming detail
- Simple artifacts that can be used in combination
- From Physics to Elec. Eng; 1800’s
Abstraction

Insulate relevant characteristics from excessive details
Standardisation

Construction from “off the shelf” parts with known characteristics

```
gcaactagccgcatggttagtaaaggagaagaactttcactggagtgctcccaatlltagtgaactagatggc
gacgtgaagctcataagttcagtgtctccggcgaa
gtgaggggtatgcaactgtagtttaactttgaaagttaatagttaacccggaagctggtctgtcccctg
gctacctgggtggcaacattattgtagttggtgtgc
gtttgctagataccagatcatacatgaaagcgatgactcttttaatgctgaatggtacagtttttaaagatgacggtaattataaaactagggctgaagttaaattcgaaggtgacacacttgtaaatcgaatagagttaaaggggattgatttcaaagaggatggtaatattctaggccataaacttgaatataactataattcaccaactttttgatactccgcgacaagcagaagaatggaatcaaagccaccgca
gattcgtagctgtgacgcacaactagccgcatggttagtaaaggagaagaactttcactggagtgctcccaatlltagtgaactagatggc
gacgtgaagctcataagttcagtgtctccggcgaa
gtgaggggtatgcaactgtagtttaactttgaaagttaatagttaacccggaagctggtctgtcccctg
gctacctgggtggcaacattattgtagttggtgtgc
gtttgctagataccagatcatacatgaaagcgatgactcttttaatgctgaatggtacagtttttaaagatgacggtaattataaaactagggctgaagttaaattcgaaggtgacacacttgtaaatcgaatagagttaaaggggattgatttcaaagaggatggtaatattctaggccataaacttgaatataactataattcaccaactttttgatactccgcgacaagcagaagaatggaatcaaagccaccgca
```
Decoupling

Insulate design process from fabrication details
An Abstraction Hierarchy

DNA

ATGCTTACCGGTACGTTTACGACTACGTAGCTAGCAT
GCTTACCGGTACGTTTACGACTACGTAGCTAGCATG
CTTACCGGTACGTTTACGACTACGTAGCTAGCATGCT
TACT...
An Abstraction Hierarchy

Parts
- Promoters
- rbs
- Coding Regions
- rbs
- Terminators

DNA
- ATGCTTACCGTACGTTTACGACTACGCTAGCAT
- GCTTACCGTACGTTTACGACTACGCTAGCATG
- CTTACCGTACGTTTACGACTACGCTAGCATGCT
- TACT...
An Abstraction Hierarchy

DNA

Parts

Promoters

Logic Gates

Devices

Inputs

Outputs

Terminators

Coding Regions

rbs

rbs

rbs

rbs

ATGCTTACCGGTACGTTTACGACTACGTAGCTAGCAT
GCTTACCGGTACGTTTACGACTACGTAGCTAGCATG
CTTACCGGTACGTTTACGACTACGTAGCTAGCATGCT
TACT...
An Abstraction Hierarchy

**DNA**

ATGCTTACCGGTACGTTTACGACTACGTAGCTAGCAT
GCTTACCGGTACGTTTACGACTACGTAGCTAGCAT
CTTACCGGTACGTTTACGACTACGTAGCTAGCATGCT
TACT...

**Parts**

Promoters

**Devices**

Inputs

Logic Gates

Outputs

**Systems**

IF dark
signal-out
ELSEIF (signal-in AND light-in)
MAKE Pigment
Standard Interchangeable Parts

LEGO MINDSTORMS

Structural  Motor & Sensors  Controller

Internal Logic

Diagram showing different components and a robot.
Micro-Organisms as Genetic Machines

- Environmental Sensors
- Protein & Chemical Synthesis
- Internal Logic
- Motility
- Communication Mechanisms
Construction of a genetic toggle switch in *Escherichia coli*

**Timothy S. Gardner**†, **Charles R. Cantor*** & **James J. Collins**†

* Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA

It has been proposed† that gene-regulatory circuits with virtually any desired property can be constructed from networks of simple regulatory elements. These properties, which include multistability and oscillations, have been found in specialized gene circuits such as the bacteriophage λ switch‡ and the Cyanobacteria circadian oscillator.§ However, these behaviours have not been demonstrated in networks of non-specialized regulatory components. Here we present the construction of a genetic toggle switch—a synthetic, bistable gene-regulatory network—in *Escherichia coli* and provide a simple theory that predicts the conditions necessary for bistability. The toggle is constructed from any two repressible promoters arranged in a mutually inhibitory network. It is flipped between stable states using transient chemical or thermal induction and exhibits a nearly ideal switching threshold. As a practical device, the toggle switch is robust and more difficult to tune experimentally. In addition, the chosen toggle design does not require any specialized promoters, such as the P<sub>R</sub>/P<sub>RM</sub> promoter of bacteriophage λ. Bistability is possible with any set of promoters and repressors as long as they fulfill the minimum set of conditions described in Box 1 and Fig. 2.

The bistability of the toggle arises from the mutually inhibitory arrangement of the repressor genes. In the absence of inducers, two stable states are possible: one in which promoter 1 transcribes repressor 2, and one in which promoter 2 transcribes repressor 1. Switching is accomplished by transiently introducing an inducer of the currently active repressor. The inducer permits the opposing repressor to be maximally transcribed until it stably represses the originally active promoter.

All toggle switches are implemented on *E. coli* plasmids conferring ampicillin resistance and containing the pBR322 CoIE1 replication origin. The toggle switch genes are arranged as a type IV plasmid, as shown in Fig. 3. Although all genes and promoters are
Pattern formation is a hallmark of coordinated cell behaviour in both single and multicellular organisms. It typically involves cell–cell communication and intracellular signal processing. Here we show a synthetic multicellular system in which genetically engineered ‘receiver’ cells are programmed to form ring-like patterns of differentiation based on chemical gradients of an acyl-homoserine lactone (AHL) signal that is synthesized by ‘sender’ cells. In receiver cells, ‘band-detect’ gene networks respond to user-defined ranges of AHL concentrations. By fusing different fluorescent proteins as outputs of network variants, an initially undifferentiated ‘lawn’ of receivers is engineered to form a bullseye pattern around a sender colony. Other patterns, such as ellipses and clovers, are achieved by placing senders in different configurations. Experimental and theoretical analyses reveal which kinetic parameters most significantly affect ring development over time. Construction and study of such synthetic multicellular systems can improve our quantitative understanding of naturally occurring developmental processes and may foster applications in tissue engineering, biomaterial fabrication and biosensing.

Figure 1a depicts the design of the synthetic bacterial multicellular system, showing how only receivers at intermediate distances from senders express the output protein. Cell–cell communication from the senders is initiated by expression of the...
Environmnetally Controlled Invasion of Cancer Cells by Engineered Bacteria

J. Christopher Anderson\textsuperscript{1,3}, Elizabeth J. Clarke\textsuperscript{3}, Adam P. Arkin\textsuperscript{1,2*} and Christopher A. Voigt\textsuperscript{2,3}

\textsuperscript{1}Howard Hughes Medical Institute, California Institute of Quantitative Biology Department of Bioengineering University of California, 717 Potter Street, Room 257 Berkeley, CA 94720, USA

\textsuperscript{2}Physical Biosciences Division E.O. Lawrence Berkeley National Laboratory, 1 Cyclotron Road, MS 977-257 Berkeley, CA 94720, USA

\textsuperscript{3}Biophysics Program Department of Pharmaceutical Chemistry, California Institute of Quantitative Biology The University of California San Francisco, 600 16th St. San Francisco, CA 94107 USA

*Corresponding author

Bacteria can sense their environment, distinguish between cell types, and deliver proteins to eukaryotic cells. Here, we engineer the interaction between bacteria and cancer cells to depend on heterologous environmental signals. We have characterized invasin from \textit{Yersinia pseudotuberculosis} as an output module that enables \textit{Escherichia coli} to invade cancer-derived cells, including HeLa, HepG2, and U2OS lines. To environmentally restrict invasion, we placed this module under the control of heterologous sensors. With the \textit{Vibrio fischeri lux} quorum sensing circuit, the hypoxia-responsive \textit{fdhF} promoter, or the arabinose-inducible \textit{araBAD} promoter, the bacteria invade cells at densities greater than 10\textsuperscript{8} bacteria/ml, after growth in an anaerobic growth chamber or in the presence of 0.02% arabinose, respectively. In the process, we developed a technique to tune the linkage between a sensor and output gene using ribosome binding site libraries and genetic selection. This approach could be used to engineer bacteria to sense the microenvironment of a tumor and respond by invading cancerous cells and releasing a cytotoxic agent.
Production of the antimalarial drug precursor artemisinic acid in engineered yeast


Malaria is a global health problem that threatens 300–500 million people and kills more than one million people annually. Disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite Plasm odi um falciparum. Synthetic antimalarial drugs and malarial vaccines are currently being developed, but their efficacy against malaria awaits rigorous clinical testing. Artemisinin, a sesquiterpene lactone endoperoxide extracted from Artemisia annua L. (family Asterac eae; commonly known as sweet wormwood), is highly effective against multi-drug-resistant Plasmodium spp., but is in short supply and unaffordable to most malaria sufferers. Although total synthesis of artemisinin is difficult and costly, the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of.

To increase FPP production in S. cerevisiae, three genes responsible for FPP synthesis were upregulated. One gene responsible for FPP conversion to sterols was also upregulated. All of these modifications to the host were integrated into the genetic stability of the yeast. Overexpression of a truncated, soluble form of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) improved amorpha diene production approximately fivefold (Fig. 2, strain EYP208). Downregulation of ERG9, which encodes squalene synthase (the first step after FPP in the sterol biosynthetic pathway), using a methionine-repressible promoter (pMET3) increased amorpha diene production an additional twofold (Fig. 2, strain EYP225). Although sre2-1, a semi-dominant mutant allele that enhances the activity of UPC2 (a global transcription factor regulating the biosynthesis of sterols in S. cerevisiae), had only a modest effect on amorpha diene production in wild-type yeast, it increased amorpha diene production an additional twofold (Fig. 2, strain EYP225).
The ‘Repressilator’

‘A synthetic oscillatory network of transcriptional regulators’
Elowitz & Liebler
Linking *in vivo* and *in silico* experiments

Lionel Dupuy & Jonathan Mackenzie
Triggers for gene expression
Neomorphogenesis

Trigger: initiate expression of a novel gene circuit during development

Patterning: define cohort of proliferating cells via intercellular signalling

Differentiation: confer new cell fates using endogenous regulators
Synthetic Biology

In this context, Synthetic Biology might be viewed as:

- The design and construction of new biological parts, devices and systems

- The re-design of existing, natural biological systems for useful purposes
Developing an Industry

An engineering discipline based on parts must develop catalogues and suppliers of those parts.
The Registry of Standard Biological of Parts
http://parts.mit.edu
Part Types

Browse parts by projects:

- iGEM 2007
- iGEM 2006
- iGEM 2005
- Labs
- Courses
- Featured Parts

Or browse parts by part categories:

**Systems**
- Measurement
- Measurement (Under Development)
- Projects (empty)

**Devices**
- Reporters
- Inverters
- Signalling
- Protein Generator
- Composite Devices
- Measurement

**Chassis**
- E.coli Strains
- Cell-Free Systems

**Mammalian**

**Vectors**
- Plasmids

**Other**
- Yeast Parts
- Construction Intermediate
- PCR Primer
- Tags
- Other
- Deleted
- Bacteriophage T7
Cell-Cell Signalling

Cell-cell signalling devices allow communication between an individual cell and its neighbors in culture or on a plate. This capability allows synchronized behavior across a cell population or the communication of information between cells hosting different systems. A cell can send a signal and it can receive an averaged signal from all its neighbors carrying the same signalling device. The two fundamental devices to perform cell-cell signalling are therefore a Sender device and a Receiver device. The current families of sender and receiver devices are all based on the Lux system of V. Fischeri or its analogs in other organisms (see references). These two families of devices are defined below.

### Available signal senders

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Family</th>
<th>Signalling Molecule</th>
<th>Control</th>
<th>Proteins</th>
<th>Molecules</th>
<th>Cell Sec</th>
<th>Delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_F1610</td>
<td>3OC6HSL Sender Device</td>
<td></td>
<td>3OC6HSL</td>
<td></td>
<td>LuxI</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Available signal receivers

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Family</th>
<th>Signalling Molecule</th>
<th>Control</th>
<th>Proteins</th>
<th>Switch Point</th>
<th>Delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_F2620</td>
<td>3OC6HSL Receiver Device</td>
<td></td>
<td>3OC6HSL</td>
<td>R0040</td>
<td>LuxR, TetR</td>
<td>2nM</td>
<td>Seconds</td>
</tr>
<tr>
<td>BBa_F2621</td>
<td>3OC6HSL Receiver Device</td>
<td></td>
<td>3OC6HSL</td>
<td>R0063</td>
<td>LuxR</td>
<td>2nM</td>
<td>Seconds</td>
</tr>
<tr>
<td>BBa_F2622</td>
<td>3OC6HSL Receiver Device</td>
<td></td>
<td>3OC6HSL</td>
<td>R0011</td>
<td>LuxR, LacI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Available other signalling parts

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Device Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_J13261</td>
<td>Lux Receiver (J13263 with reversed part order)</td>
<td></td>
</tr>
<tr>
<td>BBa_J13263</td>
<td>Lux Receiver (HSL &amp; R0063 driven)</td>
<td></td>
</tr>
<tr>
<td>BBa_J13272</td>
<td>YFP Producer Controlled by 3OC6HSL Receiver Device</td>
<td></td>
</tr>
<tr>
<td>BBa_J13273</td>
<td>YFP Producer Controlled by 3OC6HSL Receiver Device</td>
<td></td>
</tr>
<tr>
<td>BBa_J9002</td>
<td>GFP Producer Controlled by 3OC6HSL Receiver Device</td>
<td></td>
</tr>
<tr>
<td>BBa_J0424</td>
<td>I0404,61701</td>
<td></td>
</tr>
<tr>
<td>BBa_J0426</td>
<td>I0406,61707</td>
<td></td>
</tr>
<tr>
<td>BBa_J0428</td>
<td>I0408,61606</td>
<td></td>
</tr>
<tr>
<td>BBa_J0466</td>
<td>RhlR Protein Generator</td>
<td></td>
</tr>
<tr>
<td>BBa_J3018</td>
<td>LuxR Cassette under Ppet (Other)</td>
<td></td>
</tr>
<tr>
<td>BBa_J3202</td>
<td>3OC6HSL Sender Controlled by Lac Repressible Promoter</td>
<td></td>
</tr>
<tr>
<td>BBa_J3207</td>
<td>HSL/aiiA test construct</td>
<td></td>
</tr>
<tr>
<td>BBa_J3208</td>
<td>aiIA (LVA-) protein generator driven by plac</td>
<td></td>
</tr>
<tr>
<td>BBa_J1466</td>
<td>RhlR protein generator (LVA-)</td>
<td></td>
</tr>
<tr>
<td>BBa_J13040</td>
<td>pOmpR dependent 3OC6HSL sender device</td>
<td></td>
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<table>
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<th>Control</th>
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<td></td>
</tr>
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</table>
Part: BBa_F2620

Designed by Barry Canton

3OC₆HSL Receiver Device

A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC₆HSL] is controlled by an operator [TetR]. Device input is 3OC₆HSL. Device output is PoPS produced at a LuxR-regulated operator.

Usage and Biology

Full PoPS output at high 3OC₆HSL levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see Load section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical AND function.

Sequence and Features

<table>
<thead>
<tr>
<th>Format</th>
<th>Subparts</th>
<th>Ruler</th>
<th>SS</th>
<th>DS</th>
<th>Search:</th>
<th>Length: 1061 bp</th>
<th>Context: Part only</th>
</tr>
</thead>
</table>

Device Characteristics

Get the device datasheet

Transfer Function

Latency

Specificity

Specificity preliminary data

Stability

Stability preliminary data
Part: BBa_F2620

Designed by Barry Canton

3OC₆HSL Receiver Device

A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC₆HSL] is controlled by an operator [TetR]. Device input is 3OC₆HSL. Device output is PoPS produced at a LuxR-regulated operator.

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Sequence and Features

Format: Subparts | Ruler | SS | DS

Search: Length: 1061 bp

tetR . luxr . . lux pR
R0040 B0034 C0062 B0010 B0012 R0062

Device Characteristics

Get the device datasheet

Transfer Function

Latency

Specificity

Specificity preliminary data

Stability

Stability preliminary data
Part: BBa_F2620

Designed by Barry Canton

3OC₆HSL Receiver Device

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Device Characteristics

Get the device datasheet

Transfer Function

Latency

Specificity

Specificity preliminary data

Stability

Stability preliminary data
BBa_F2620
3OC_6HSL → PoPS Receiver

Author(s): Barry Canton [bcanton@mit.edu]  Last Update: May 10, 2005

Description
A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC_6HSL] is controlled by an operator [TetR]. Device input is 3OC_6HSL. Device output is PoPS produced at a LuxR-regulated operator.

Usage
Full PoPS output at high 3OC_6HSL levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see Load section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical AND function.

Characteristics
- Input Swing: # nM 3OC_6HSL, exogenous
- Output Swing: # PoPS
- Switch Point: 2 nM 3OC_6HSL, exogenous
- LH Latency: # seconds
- HL Latency: # seconds

Key Components
- BBa_R0040: TetR-regulated operator
- BBa_C0062: luxR ORF
- BBa_R0062: LuxR-regulated operator

Transfer Function

Latency

Load
- NTP/sec/copy: # NTP per second
- AA/sec/copy: # AA per second

Compatibility
Device has been shown to work in MC4100, MG1655, and DH-5α.
Device has been shown to work on pSB3K3 and pSB1A2.
Device has been shown to work with E0430 and E0434.
Cross talk with input molecular similar to 3OC_6HSL.
Cross talk with systems containing TetR.

Registry of Standard Biological Parts
making life better, one part at a time
**BBa_F2620**

**3OC₆HSL → PoPS Receiver**

**Description**
A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC₆HSL] is controlled by an operator [TetR]. Device input is 3OC₆HSL. Device output is PoPS produced at a LuxR-regulated operator.

**Usage**
Full PoPS output at high 3OC₆HSL levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see **Load** section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical AND function.

**Characteristics**
- **Input Swing**: # nM 3OC₆HSL, exogenous
- **Output Swing**: # PoPS
- **Switch Point**: 2 nM 3OC₆HSL, exogenous
- **LH Latency**: # seconds
- **HL Latency**: # seconds

**Transfer Function**
A graph showing the transfer function with fluorescence intensity on the y-axis and 3OC₆HSL concentration on the x-axis.

**Latency**
A graph showing the latency with fluorescence intensity on the y-axis and time on the x-axis.

**Key Components**
- **Key Components**
  - **BBa_R0040**: TetR-regulated operator
  - **BBa_C0062**: LuxR ORF
  - **BBa_R0062**: LuxR-regulated operator

**Load**
- **NTF/sec/copy**: # NTP per second
- **AA/sec/copy**: # AA per second

**Comaptibility**
Device has been shown to work in MC4100, MG1655, and DH-5x.
Device has been shown to with pSB3K3 and pSB1A2.
Device has been shown to work with E0430 and E0434.
Crosstalk with input molecular similar to 3OC₆HSL.
Crosstalk with systems containing TetR.

**Registry of Standard Biological Parts**
making life better, one part at a time
F2620

Lux-receiver Device

**Description**
A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC6HSL] is controlled by an operator [TetR]. Device input is 3OC6HSL. Device output is PoPS produced at a LuxR-regulated operator.

**Usage**
Full PoPS output at high 3OC6HSL levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see Load section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical AND function.

**Characteristics**
- Input Swing: # nM 3OC6HSL, exogenous
- Output Swing: # PoPS
- Switch Point: 2 nM 3OC6HSL, exogenous
- LH Latency: # seconds
- HL Latency: # seconds

**Transfer Function**

**Key Components**
- BBa_R0040: TetR-regulated operator
- BBa_C0062: luxR ORF
- BBa_R0062: LuxR-regulated operator

**Load**
- NTP/sec/copy: # NTP per second
- AA/sec/copy: # AA per second

**Stability**
- Genetic: > # replication events
- Operational: > # replication events

**Compatibility**
Device has been shown to work in MC4100, MG1655, and DH-5x.

Device has been shown to work on pSB3K3 and pSB1A2.

Device has been shown to work with E0430 and E0434.

Crosstalk with input molecular similar to 3OC6HSL.

Crosstalk with systems containing TetR.
Wide range of parts & devices with modular functionality

Switches

- light
- R0051
- IPTG
- lacI
- R0011

Reporters

- J06702 mCherry Reporter
- I13504 GFP Reporter

Generators

- P0340 tetR Generator

Communication

- I13202 lux Sender Device
- T9002 lux Receiver Device
Wide range of parts & devices with modular functionality

Switches

- light → R0051
- IPTG → lacI → R0011

Generators

- P0340 tetR Generator

Communication

- iGEM 2006 DNA Registry of Standard Biological Parts
- lux Sender Device
- lux Receiver Device
BioBricks
Standardised, interchangeable parts for Biology

1. Physical Entity
   - construction and test

2. Information Modules
   - encode function allowing design & modeling
BioBricks
Standardised, interchangeable parts for Biology

EcoRI  XbaI  SpeI  PstI

B0034

P  B0034  S

Amp
1. Standard Assembly

Cut with EcoRI & SpeI

Cut with EcoRI & XbaI

Mix & Ligate

Amp

Amp

Amp

Amp
2. Device Design: Making Biology Modular
2. Device Design: Making Biology Modular

- lacI
- CI
- R0011
- RBS
- C0051
- T
2. Device Design: Making Biology Modular
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2. Device Design: Making Biology Modular
2. Device Design: Making Biology Modular
Device Design: Making Biology Modular

PoPS\textsubscript{in} \quad \text{PoPS\textsubscript{out}}

PoPS\textsubscript{in} \rightarrow \text{RBS} \quad \text{C005 I} \quad \text{T} \quad \text{R005 I} \rightarrow \text{PoPS\textsubscript{out}}

PoPS\textsubscript{out}
Device Design: Making Biology Modular

Invertor
BBa_Q04510

PoPS_{in} -> Invertor
BBa_Q04510
PoPS_{out}

PoPS_{out} vs PoPS_{in}
Device Design: Making Biology Modular

Light Sensor \( \rightarrow \text{PoPS}_{\text{in}} \) Invertor BBa_Q04510 \( \rightarrow \text{PoPS}_{\text{out}} \) Cell Death

Graph: \( \text{PoPS}_{\text{out}} \) vs \( \text{PoPS}_{\text{in}} \)
The iGEM Competition
An Engineering Question

“Can simple biological systems be built from standard, interchangeable parts and operated in living cells?

Or, is biology so complex that each case is unique?”
MIT IAP 2003/4
Independent Activities Program
1 class - 30 Students
iGEM 2005
13 Universities
BU, Caltech, Cambridge, ETH Zurich, Harvard, MIT, Oklahoma, Penn State, Princeton, Toronto, UC Berkeley, UCSF, UT Austin

Designs on life

Earlier this month, students from around the world locked horns in competition. Their challenge was to build functioning devices out of biological parts. Erika Check finds out how they got on.

Even if you’re thinking big, you usually have to start small. Especially, as a group of Swiss students found, when big means counting to infinity. The team was drawing up a blueprint for the world’s first counting machine made entirely of biological parts. Although they had their sights on a billion numbers, they opted to go no higher than two. If the plan worked, it would be a proof of principle for a much larger tallying device.

The group, from the Federal Institute of Technology (ETH) in Zurich, was one of 17 teams unveiling their projects at the first international intercollegiate Genetically Engineered Machine (iGEM) competition, held at the Massachusetts Institute of Technology (MIT) in Cambridge on 3 and 4 November. The event attracted students from all over the world to design and build machines made entirely from biological components such as genes and proteins. They drew up grand designs for bacterial E. coli-Sketches, photosensitive t-shirts, thermometers and sensors. And if none of the designs succeeded completely, that was more because of the limitations of the nascent science of synthetic biology than any lack of enthusiasm, creativity or hard work.

Synthetic biology aims to merge engineering approaches with biology. Researchers working at the most basic level are copying simple biological processes, such as the production of a protein from a gene. They break the process down into its component elements, such as a gene and the pieces of DNA and other molecules that control its activity. They then string these elements together to build a module they know will behave in a particular way — say, oscillate between producing and not producing a protein, or produce a protein that can switch another module on or off.

It is these kinds of components — oscillators and switches — that engineers order from suppliers and link together to build more complex electronic circuits and machines. Synthetic biologists are trying to develop a similar armory of biological components, dubbed BioBrick[s], that can be inserted into any genetic circuit to carry out a particular function. Scientists at MIT have established a Registry of Standard Biological Parts, a catalogue of BioBricks that theoretically from the ground up. To do so, they have commandeered a time-honoured engineering tradition: the student competition. The iGEM event began life as a project class for MIT students in 2003. Last year, it was thrown open to other US universities, and this year it went international. The organizers hope to attract 50 to 50 teams next year, including some from Asia.

Competitive culture

Much like the robot competitions that tap into students’ desire to build something cool, the iGEM competitors fire the participants’ natural curiosity — hopefully encouraging biologists to learn something from engineers, and vice versa. The selection of designs. Students from the University of Cambridge, UK, tried to make a circuit that could control the movement of Escherichia coli bacteria. They aimed to engineer the bacteria to contain a switch governing their sensitivity to the sugar maltose. With the switch off, the microbes would ignore the sugar. Tapping the switch would make the bacteria sensitive to the sugar and induce them to move towards it.

In the end, the group — likely almost every other entrant — had trouble assembling all of its genetic parts in time. Many of the other students also tackled problems related to bacterial communication and motion. The team from Pennsylvania ...
iGEM 2006
37 Universities

IAP 2003
30 students

SBC 2004
5 US Teams

iGEM 2005
13 Teams

http://www.ietdl.org/IET-STB
iGEM 2007
57 Universities - 750 Participants

Europe
9 Teams
Bologna
ETH Zurich
Freiburg
Ljubljana
Naples
Paris
St Petersburg
Turkey
Valencia

Asia
8 Teams
Bangalore
Chiba
Peking
Taipei
Tianjin
Tokyo
Tsinghua
USTC

North America
30 Teams
Alberta
Berkeley
Boston
Brown
Calgary
Caltech
CSHL
Davidson
Duke
Missouri
Harvard
Lethbridge
McGill
Mississippi
MIT

UK
4 Teams
Cambridge
Edinburgh
Glasgow
Imperial

Rest of World
Melbourne
Mexico
Colombia

IAP 2003
30 students

SBC 2004
5 US Teams

iGEM 2005
13 Teams

iGEM 2006
35 Teams

North America
30 Teams
Michigan
Minnesota
Penn State
Prairie View
Princeton
Rice
Stanford
Toronto
UCSF
Utah
Virginia
Virginia Tech
Waterloo
Wisconsin

North America
30 Teams
Michigan
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Minnesota
Penn State
Prairie View
Princeton
Rice
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UCSF
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Virginia
Virginia Tech
Waterloo
Wisconsin

North America
30 Teams
Mich
iGEM 2008
100 teams

Design Brief
“Design and test a simple biological system from standard, interchangeable parts and operate it in living cells.”
iGEM Synthetic Biology competition

This year the Cambridge iGEM team worked on novel systems for intercellular communication. This included new peptide signalling systems, outer membrane pores, transcription activator components and Gram positive chassis systems. For more information see the team wiki. The Cambridge team received Gold Awards and a prize for best BioBrick.
Engineering *Escherichia coli* to see light

These smart bacteria ‘photograph’ a light pattern as a high-definition chemical image.

We have designed a bacterial system that is switched between different states by red light. The system consists of a synthetic sensor kinase that allows a lawn of bacteria to function as a biological film, such that the projection of a pattern of light on to the bacteria produces a high-definition (about 100 megapixels per square inch), two-dimensional chemical image. This spatial control of bacterial gene expression could be used to ‘print’ complex biological materials, for example, and to investigate signalling pathways through precise spatial and temporal control of their phosphorylation steps.

Plants and some bacteria use a class of protein photoreceptors known as phytochromes to control phototaxis, photosynthesis and the production of protective pigments\(^1-^3\). Photoreceptors are not found in enterobacteria, such as *Escherichia coli*, so we created a light sensor that functions in *E. coli* by engineering a chimera that uses a phytochrome from a cyanobacterium.

A phytochrome is a two-component system that consists of a membrane-bound, extracellular sensor that responds to light and an intracellular response-regulator\(^3\). The response-regulators of most phytochromes do not have DNA-binding domains and do not directly regulate gene expression, so we fused a cyano-

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**Figure 1** | Light imaging by engineered *Escherichia coli*.  
**a**, The chimaeric light receptor Cph8 contains the photoreceptor from Cph1 (green) and the histidine kinase and response-regulator from EnvZ–OmpR (orange); inset, conversion of haem to phycocyanobilin (PCB), which forms part of the photoreceptor. Red light drives the sensor to a state in which autophosphorylation is inhibited (right), turning off gene expression. For details of genes, see text. **b**, Miller assay showing that Cph8 is active in the dark (black bars) in the presence of PCB and inactive in the light (white bars). There is no light-dependent activity in the absence of Cph8 (—) and there is constitutive activity when only the histidine kinase domain of EnvZ is expressed (＋), or when the PCB metabolic pathway is not included (— PCB). **c**, When an image is projected onto a bacterial lawn, the LacZ reporter is expressed only in the dark regions. **d**, Transfer function of the circuit. As the intensity of the light is increased by using a light source closer to the lawn, the expression of LacZ increases, indicating the increased detection of light. The output is measured using a UV detector.
eau d'e coli
mit igem 2006

indole deficient tnaA5^- chassis

- chorismate → SAGD → salicylic acid
  - osmY → WGD

- leucine → IAGD → 3-methylbutanal
  - NADH → isoamyl alcohol
  - osmY → BSGD

methyl salicylate

isoamyl acetate

Smell

Time

Wintergreen

Banana
The field test device

- A test tube could contain all the necessary components: Freeze dried bacteria, growth medium, indicator powder, Ampicillin salt, etc...

- These tubes could then be given to local villagers to monitor their own water quality themselves

- A good alternative to the widely used Gutzeit method

www.Macteria.co.uk
The iGEM Competition

Undergraduate teams compete to design biological systems from standard parts
The iGEM Competition

Undergraduate teams compete to design biological systems from standard parts

Pulls together students from different disciplines
- engineering, life sciences, computing, maths
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Undergraduate teams compete to design biological systems from standard parts

Pulls together students from different disciplines
- engineering, life sciences, computing, maths

Challenges them to initiate a novel scientific programme and learn & share new skills
The iGEM Competition

Undergraduate teams compete to design biological systems from standard parts

Pulls together students from different disciplines - engineering, life sciences, computing, maths

Challenges them to initiate a novel scientific programme and learn & share new skills

New educational model in an exciting new field, placing students at the cutting edge of research
The iGEM Competition

Undergraduate teams compete to design biological systems from standard parts

Pulls together students from different disciplines - engineering, life sciences, computing, maths

Challenges them to initiate a novel scientific programme and learn & share new skills

New educational model in an exciting new field, placing students at the cutting edge of research

Allows students to make a valuable contribution to the research field & community, while in pursuit of their own goals
iGEM 2007 Wiki
International Genetically Engineered Machine Competition

teams jamboree participate help about registry

Peking wins the Grand Prize
the Peking iGEM 2007 team triumphantly hoists the Grand Prize BioBrick trophy

Results of the Jamboree sat & sun, nov 3-4

iGEM 2007 is now officially concluded! Congratulations to all!
- Results
- See the medal winners
- Media (including links to videos and photos)

iGEM? Hundreds of undergraduates all over the world spend their summer making Synthetic Biology a reality by participating in the annual International Genetically Engineered Machine competition.

iGEM through the years
- 2008
- 2007
- 2006

Learn More

calendar
- Jamboree roster + fees due fri 12 Oct 12
- iGEM wiki frozen + parts postmarked fri 26 Oct 07
- Jamboree! sat-sun 3-4 Nov 07
- Registry + BioBrick sun-tue
Cambridge Philosophical Society & Cambridge Synthetic Biology Society
One Day Symposium

Synthetic Biology:
Molecular Bioengineering for the 21st Century

Monday 3rd December 2007
Starts 9am, Pippard Lecture Theatre,
Cavendish Laboratory, Department of Physics.

Speakers include:
Ron Weiss, Alfonso Jaramillo, Georg Seelig,
Jim Haseloff, James Brown, Cambridge iGEM 2007 team
Abstract Submission Deadline: 9th December 2007

Registration opens early January

Speakers include:
Tom Knight, Adam Arkin & Jaroslav Stark

www.biosysbio.com
Summary

Rapid emergence & development of sequencing, synthesis and computational tools & technologies have made possible the rational engineering of biological systems.

One must apply the engineering principles that have underpinned the development of mechanical and electrical engineering fields in order to deal with biological complexity.

Synthetic Biology looks set to contribute to future improvements in the microbial, plant and animal cell engineering that are clearly needed for the renewable technologies of the 21st century.
Thank Yous

Jim Haseloff
Jim Ajioka
Gos Micklem
Duncan Rowe
Randy Rettberg
Tom Knight
Drew Endy

Cambridge iGEM 2005/6/7
www.synbio.org.uk

Resources for learning assembly techniques
- protocols
- video podcasts
  lecture material
  how-to-do-it info
- local sources of expertise
- external links
2 week crash course in Synthetic Biology

July 2008
Lecture course and brain storming
July-August-September: Practical laboratory
Composition of the 2008 Team

Student team:
10 UROP summer studentships + externally funded students

Support:
James Brown, Department of Plant Sciences
James Godman, Department of Plant Sciences
Dr. Duncan Rowe, Department of Genetics
Dr. Alex Kabla, Department of Engineering

Faculty:
Dr. Jim Ajioka, Department of Pathology
Dr. Jim Haseloff, Department of Plant Sciences
Dr. Gos Micklem, Cambridge Computational Biology Institute
Dr. Jorge Goncalves, Department of Engineering
Dr. Lorenz Wernisch, MRC Biostatistics Unit
Benefits

- engineers & biologists working together
- open-ended learning
- project management and teamwork
- national & international scientific exchange
- scientific presentations
- scientific publication
- practical training in Synthetic Biology
iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences
iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences

3rd Yr Engineer
Systems & Control + Biology Options
iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences

iGEM 2005 Cambridge Team Member

3rd Yr Engineer
Systems & Control + Biology Options
iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences

Completed MEng
Electrical Engineering - continued iGEM Project

iGEM 2005 Cambridge Team Member

3rd Yr Engineer
Systems & Control + Biology Options
iGEM, Synthetic Biology & Me

Graduate Student in Synthetic Biology
Haseloff Lab, Dept. of Plant Sciences

iGEM 2006 Ambassador
based at MIT’s Registry, supporting 10 teams

Completed MEng
Electrical Engineering - continued iGEM Project

iGEM 2005 Cambridge Team Member

3rd Yr Engineer
Systems & Control + Biology Options
Synthetic Biology