iGEM 2012 Workshop

Teachers Workshops

2012
Schedule

9:30 AM  Registration / Breakfast
10:00 AM  Welcome
10:15 AM  Introductions
11:30 AM  Synthetic Biology / Parts
12:30 PM  Lunch
1:30 PM  Operations / iGEM Foundation
2:10 PM  Judging
2:30 PM  Assembly
3:00 PM  Break
3:30 PM  Divisions
4:00 PM  Discussion
5:00 PM  Reception
7:00 PM  End
<table>
<thead>
<tr>
<th>Name</th>
<th>Team Name</th>
<th>Role</th>
<th>iGEM Year (how did you hear about iGEM?)</th>
<th>Composition</th>
<th>Credits</th>
<th>Funding</th>
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</table>
Synthetic Biology
Based on Standard Parts

Teachers Workshops

2012

Randy Rettberg
randy@igem.org
igem.org
Synthetic biology could be the defining technology of the 21st century.
NEW DIRECTIONS  The Ethics of Synthetic Biology and Emerging Technologies

The 21st century is widely heralded as the century of biology. Building on the fundamental understanding achieved in the second half of the last century, revolutionary advances are expected to improve many aspects of our lives, from clean energy and targeted, safer medicines to new industries. Prominent among emerging technologies is “synthetic biology,” which aims to apply standardized engineering techniques to biology and thereby create organisms or biological systems with novel or specialized functions to address countless needs.

The idea of managing or manipulating biology to identify or develop specific characteristics is not new. Scientists have used DNA to create genetically
190 Collegiate
40 High School
15 Entrepreneurship
5 Regions
AlumniGEM: 10,500 Alumni

210 Labs
8,500 Physical Parts
18,000 Total Parts
1,400,000 Parts Distributed

We Moved to the iGEM Foundation!
### iGEM Growth and Scale

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Note: Extrapolation for 2013 and beyond is particularly difficult because of poster activities.
Regional Structure 2012

World Championship Jamboree
Boston

Asia Jamboree
Hong Kong

Latin America Jamboree
Bogota

European Jamboree
Amsterdam

Americas West Jamboree
Stanford

Americas East Jamboree
Pittsburgh
SN74LS14

Schmitt Triggers Dual Gate/Hex Inverter

The SN74LS14 contains logic gates/inverters which accept standard TTL input signals and provide standard TTL output levels. They are capable of transforming slowly changing input signals into sharply defined, noise-free output signals. Additionally, these have greater noise margins than conventional inverters.

Each input contains a Schottky trigger followed by a Emitter followers input driver that is TTL compatible. The Schottky trigger uses negative feedback to effectively speed-up slow input transitions and provide different input threshold voltages for positive and negative-going transitions. The hysteresis between the positive-going and negative-going input thresholds (typically 50 mV) is determined internally by resistor ratios and is essentially insensitive to temperature and supply voltage variations.

LOGIC AND CONNECTION DIAGRAMS

GUARANTEED OPERATING RANGES

<table>
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<th>Parameter</th>
<th>Min</th>
<th>Max</th>
<th>Unit</th>
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<td>+2.7</td>
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<td>V</td>
</tr>
<tr>
<td>TA (°C)</td>
<td>0</td>
<td>70</td>
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</tr>
<tr>
<td>Icc (mA)</td>
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<td>0.8</td>
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<td>Iol (mA)</td>
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ORDERING INFORMATION

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<td>14-Pin DIP</td>
<td>2000 UNITS</td>
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<tr>
<td>SN74LS14D</td>
<td>14-Pin</td>
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</table>

© Texas Instruments 1969

Publication Order Number: SN74LS14D
Minicomputer Based on TTL
Mainframes and Minicomputers

The Innovator’s Dilemma, Clayton Christensen
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?
iGEM Schedule: Assemble Your Team

5 High School Students
5 Undergraduate Students
3 Graduate Students
3 Faculty

Utah State - iGEM 2009
iGEM Schedule: Raise Money
iGEM Schedule: Attend A Workshop

igem.org
iGEM Schedule: Get the BioBrick Parts
iGEM Schedule: Attend the Jamboree
iGEM Schedule: Attend the Jamboree
iGEM Schedule: Win Awards
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?
Projects: Bacterial Photography

c/o Jeff Tabor & colleagues
BACTOBLOOD

Researchers
Arthur Yu • Austin Day • David Tulga • Hannah Cole • Kristin Doan • Kristin Fuller • Nhu Nguyen • Samantha Liang • Vaibhavi Umesh • Vincent Parker

Teaching Assistants
Amin Hajimorad • Farnaz Nowroozi • Rickey Bonds

Advisors
John Dueber • Christopher Anderson • Adam Arkin • Jay Keasling

UC Berkeley iGEM 2007
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
A Rainbow of Colorful Cells

<table>
<thead>
<tr>
<th>Biobrick</th>
<th>Colour</th>
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<tbody>
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<td>BBa_K274100</td>
<td>Red</td>
</tr>
<tr>
<td>BBa_K274200</td>
<td>Orange</td>
</tr>
<tr>
<td>BBa_K274001</td>
<td>Brown</td>
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<tr>
<td>BBa_K274002</td>
<td>Violet</td>
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<td>BBa_K274003</td>
<td>Dark Green</td>
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<tr>
<td>BBa_K274004</td>
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</table>
Save the World: Soil Erosion

Auxin

Human Practices
Implementation
Specifications
Design
Assembly
Modelling
Testing
Can simple biological systems be built from standard, interchangeable parts and operated in living cells?

Sometimes!

Or, is biology so complex that each case is unique?

Sometimes!
SN74LS14

Schmitt Triggers
Dual Gate/Hex Inverter

The SN74LS14 contains logic gates/inverters which accept standard TTL input signals and provide standard TTL output levels. They are capable of transforming slowly changing input signals into sharply defined, clean-low output signals. Additionally, they have greater noise margins than conventional inverters. Each circuit contains a Schottky trigger followed by a Schottky level shifter and a Schottky inverter driving a TTL level output stage. The Schottky trigger uses positive feedbacks to effectively speed-up slow input transition, and provides different input threshold voltages for positive and negative-going transitions. This minimizes the extrinsic rise and negative-going transition times (typically 600 ms) is determined internally by resistor ratios and is essentially insensitive to temperature and supply voltage variations.

LOGIC AND CONNECTION DIAGRAMS

GURANTEED OPERATING RANGES

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<th>Min</th>
<th>Typ</th>
<th>Max</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
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<td>Vcc</td>
<td>Supply voltage</td>
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<td>5.5</td>
<td>5.5</td>
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<tr>
<td>Ta</td>
<td>Operating/absolute temperature range</td>
<td>0</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>I0P</td>
<td>Output Current - High</td>
<td>±0.4</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>I0N</td>
<td>Output Current - Low</td>
<td>±0.4</td>
<td>8</td>
<td>8</td>
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ORDERING INFORMATION

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<thead>
<tr>
<th>Device</th>
<th>Package</th>
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<td>SN74LS14D</td>
<td>14-Pin DIP</td>
<td>2000/2000B &amp; Mark</td>
</tr>
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</table>
High copy number assembly plasmidic backbones

The most common set of plasmidic backbones that people use to assemble BioBrick® standard biological parts together are high copy BioBrick plasmidic backbones. High copy plasmidic DNA is easily purified in high yield from cultures, so it makes obtaining enough DNA for assembly easy.

The high copy plasmidic backbones listed below have a common set of features:

1. A complete BioBrick® cloning site for easy cloning and assembly of BioBrick parts.
2. Terminators flanking the BioBrick® cloning site to insulate the vector from read-through transcription originating in the cloned BioBrick® part, device, or system.
3. Primer binding sites for the standard BioBrick® verification primers Vf2 (Bba_G00100) and Vr (Bba_G00101). These primers are located for convenient sequencing and screening by colony PCR of cloned BioBrick® parts, devices, and systems.

Plasmid backbones are distributed by the Registry with a default insert. There are just a handful of default plasmidic inserts used in the Registry. Many of the available plasmidic backbones have the ccdB positive selection marker (Bba_P1010) as the default plasmid insert within the BioBrick® cloning site. The ccdB gene ensures that when assembling two BioBrick® parts together, the unc1 plasmid is not transformed. However, inclusion of the ccdB gene means that these vectors must be propagated in a ccdB tolerant strain, such as E. coli strain DB3.1 (Bba_V1005).

Finally, to make assembly of BioBrick® parts easier, these BioBrick® assembly plasmidic backbones are available with three different antibiotic resistance markers, so that you can use 3 antibiotic assembly methods to assemble BioBrick® parts.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Resistance</th>
<th>Replicon</th>
<th>Copy number</th>
<th>Chassis</th>
<th>Length</th>
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<tbody>
<tr>
<td>pSB1A3</td>
<td>High copy BioBrick assembly plasmid</td>
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<td>pMB1</td>
<td>100-300</td>
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<tr>
<td>pSB1A7</td>
<td>Transcriptionally insulated high copy BioBrick plasmid</td>
<td>A</td>
<td>pMB1</td>
<td>100-300</td>
<td>2431</td>
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<td>pSB1A3</td>
<td>High copy BioBrick assembly plasmid</td>
<td>AC</td>
<td>pMB1</td>
<td>100-300</td>
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<td>pSB1A3</td>
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</table>

Karmela Haynes, an instructor of the 2006 Davidson College iGEM team, designed and constructed the plasmid backbone pSB1A7. You can read more about the 2006 Davidson project in their open-access paper Engineering bacteria to solve the Burnt Pancake Problem published in the Journal of Biological Engineering.

Robbie Bryant constructed the plasmid backbone pSB1A3 in Tom Knight’s lab.
Parts, Inverting Amplifiers
Registry Contents

- Promoters
- Protein Coding
- Reporters
- RNA
- Terminators
- Signaling
- Many project parts
- Composite Parts
BioBrick Standard Assembly

Blue Part
Cut with EcoRI and SpeI

Green Part
Cut with EcoRI and XbaI

Mix and Ligate (Blue-Green Part)
The Registry of Standard Biological Parts

High copy number assembly plasmidic backbones

The most common set of plasmidic backbones that people use to assemble BioBrick® standard biological parts together are high copy BioBrick plasmidic backbones. High copy plasmidic DNA is easily purified in high yield from cultures, so it makes obtaining enough DNA for assembly easy.

The high copy plasmidic backbones listed below have a common set of features:

1. A complete BioBrick cloning site for easy cloning and assembly of BioBrick parts.
2. Terminators flanking the BioBrick cloning site to insulate the vector from read-through transcription originating in the cloned BioBrick® part, device, or system.
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The ccdB gene ensures that when assembling two BioBrick® parts together, the uncured plasmid is not transformed. However, inclusion of the ccdB gene means that these vectors must be propagated in a ccdB tolerant strain, such as E. coli strain DB3.1 (Bba_V1005).

Finally, to make assembly of BioBrick® parts easier, these BioBrick® assembly plasmidic backbones are available with three different antibiotic resistance markers, so that you can use 3 antibiotic assembly methods to assemble BioBrick® parts.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Resistance</th>
<th>Replicon</th>
<th>Copy number</th>
<th>Chassis</th>
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<tr>
<td>pSB1AC3</td>
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<td>pMB1</td>
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<td>High copy BioBrick® assembly plasmid</td>
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<td>pSB1K3</td>
<td>High copy BioBrick® assembly plasmid</td>
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</tr>
</tbody>
</table>

Karmila Haynes, an instructor of the 2006 Davidson College (GEM team, designed and constructed the plasmid backbone pSB1A7). You can read more about the 2006 Davidson project in their open-access paper Engineering bacteria to solve the Burnt Pancake Problem published in the Journal of Biological Engineering.

Robbie Bryant constructed the plasmid backbone pSB1AC3 in Tom Knight’s lab.
Beginning with Digital Equipment's logic modules and experiencing a boom in the age of transistor-transistor logic (TTL), logic devices were sold as parts within families. The relevant engineering properties of each family, along with its constituent parts, were documented in Databooks. Each Databook contained an overview of the common properties of the family (e.g., operating conditions) and also dedicated a few pages to describing individual parts.

Synthetic Biology needs a similar standardization of parts, and parts interfaces and descriptions, in order to enable the systematic engineering of large scale engineered biological circuits. As Synthetic Biology is a new field, we expect that the current family (MIT BioBricks Alpha) will be replaced by still-more useful and sophisticated families, just as Coincident Current Logic was supplanted by Resistor-Transistor Logic, and that by Diode-Transistor Logic, and that by Transistor-Transistor Logic, and so on.
transfer characteristics over operating range
Fluorescent reporters generate fluorescence in response to input TIPS. The following transfer curve provides maximum, minimum, and typical values of fluorescence. Total fluorescence is a product of the transfer function, the excitation light intensity, bleaching effect, and timing effects.

<table>
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<th>Param</th>
<th>Description</th>
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<th>Unit</th>
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<td>$S_{\text{Low}}$</td>
<td>Low level output transcription (leakage)</td>
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<td>TIPS</td>
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<tr>
<td>$S_{\text{High}}$</td>
<td>High level transcription</td>
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<td></td>
<td></td>
<td>TIPS</td>
</tr>
<tr>
<td>$S_{\text{Threshold}}$</td>
<td>Threshold</td>
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<td></td>
<td></td>
<td>TIPS</td>
</tr>
<tr>
<td>$S_{\text{Slope}}$</td>
<td>Slope</td>
<td></td>
<td></td>
<td></td>
<td>TIPS</td>
</tr>
</tbody>
</table>

switching characteristics over operating range
Fluorescent proteins suffer a delay in turn on due to mRNA transcription time and dimerization time. In addition, time is required to bring the protein level into balance between generation and degradation. See figures below for turn-on and turn-off characteristics.
**insert in plasmid**

The plasmid containing the insert is:

![Diagram of BBa R0010 Plasmid with annotations](image)

**files**

Vector NTI - BBa_E0040

**container identification**

The Registry of Standard Biological Parts has moved from parts.mit.edu to partsregistry.org. References to the Registry at parts.mit.edu will be automatically redirected to the new site.

Registry News

- We are considering releasing the Registry’s DNA Repository and Library system to the Registry labs and IGEM teams. This is the system we use to keep track of parts in our freezer boxes and plates. Please check it out and let us know what you think. - June 2, 2008
- A bug that kept Internet Explorer users from seeing the Part menu on Part pages has been fixed. Now, if you go to a part, you will see menu choices for hard information and physical location. - June 2, 2008
- The sequence and features for all parts are available through DAS, the Distributed Annotation System. Learn more here - May 26, 2008
- Changes to the Registry software are underway. Check it out!
- We have a new tutorial for starting teams in the Help section
- We are starting an editorial board for promoting well-defined and useful parts to BioBrick™ part status. To join this effort check the BioBrick™ Part Program
- There is a problem with using primers VR and VF2 to PCR parts containing B0015 or B0010.
- News archive...

Report any bugs here | Request new features here | See new features here | See old bugs, requests, and features here
Part:BBa_I13600

Designed by Christopher Batten, Victoria Chou, Kenneth Nesmith

From partsregistry.org

Tet with CFP reporter (without LVA tag)

The part glows (rather weakly) with a cyan colored fluorescent protein. In the absence of the tetR protein, CFP expression is constitutive. tetR represses CFP production; this repression can be relieved by the addition of tetracycline or one of its analogs (i.e. aTc) (http://openwetware.org/wiki/aTc).

Sequence and Features

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<tr>
<td>R0040</td>
<td>B0054</td>
<td>E0020</td>
<td>B0010</td>
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</tr>
</tbody>
</table>

Pictures

BBa_I13600 visualized under non-UV lightbox

BBa_I13600 visualized under 254nm wavelength UV

Retrieved from "http://partsregistry.org/Part:BBa_I13600"
Plasmids are circular, double-stranded DNA molecules typically containing a few thousand base pairs that replicate within the cell independently of the chromosomal DNA. Plasmid DNA is easily purified from cells, manipulated using common lab techniques and incorporated into cells. Most BioBrick parts in the Registry are maintained and propagated on plasmids. Thus, construction of BioBrick parts, devices and systems usually requires working with plasmids.

**Note:** In the Registry, plasmids are made up of two distinct components:

1. the BioBrick part, device or system that is located in the BioBrick cloning site, between (and excluding) the BioBrick prefix and suffix.
2. the plasmid backbone which propagates the BioBrick part. The plasmid backbone is defined as the sequence beginning with the BioBrick suffix, including the replication origin and antibiotic resistance marker, and ending with the BioBrick prefix. [Note that the plasmid backbone itself can be composed of BioBrick parts.]

Many BioBrick parts in the Registry are maintained on more than one plasmid backbone!

One of the most common tasks that biological engineers do is to assemble two parts together using BioBrick® standard assembly. To make the process of assembling two BioBrick® parts together easier, there are several kinds of assembly plasmid backbones available via the Registry.
High copy number assembly plasmid backbones

The most common set of plasmid backbones that people use to assemble BioBrick® standard biological parts together are high copy BioBrick plasmid backbones. High copy plasmid DNA is easily purified in high yield from cultures, so it makes obtaining enough DNA for assembly easy.

The high copy plasmid backbones listed below have a common set of features.

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<th>Copy number</th>
<th>Chassis</th>
<th>Length</th>
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</tbody>
</table>

Karmella Haynes, an instructor of the 2006 Davidson College iGEM team, designed and constructed the plasmid backbone pSB1A7. You can read more about the 2006 Davidson project in their open-access paper Engineering bacteria to solve the Burnt Pancake Problem published in the Journal of Biological Engineering.

Robbie Bryant constructed the plasmid backbone pSB1AC3 in Tom Knight's lab.
Part: BBa_E0840


GFP generator

BBa_E0840 takes as input a transcriptional signal (PoPS) and produce as output the fluorescent protein GFP.

Usage and Biology

- See BBa_E0040 for additional details.
- BBa_E0840 is often used to quantify the behavior of transcriptional control devices such as promoters.
- BBa_E0840 has a strong ribosome binding site.

Sequence and Features

Format: Subparts | Ruler | SS | DS
Search: Length: 878 bp  Context: Part only

Assembly Compatibility: 10 21 23 25

Parameters

| emission | Green | excitation | tag | None |

Twins

- BBa_I741026 Deleted
- BBa_I751310 Building
- BBa_S04013 Planning

Reviews

- 1 Registry Star
  Group Favorite
  Experience: Works

Categories

- //classic/reporter/et
- //GEM2006/MIT/favorites
**Part Libraries**

**Chris Anderson’s Constitutive Promoters**

![DNA sequence and RFP values]

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Registry Contents

- Promoters
- Protein Coding
- Reporters
- RNA
- Terminators
- Signaling
- Many project parts
- Composite Parts
BioBrick Standard Assembly

- **Blue Part**
  - Cut with EcoRI and SpeI

- **Green Part**
  - Cut with EcoRI and XbaI

- Mix and Ligate (Blue-Green Part)
iGEM Philosophy: Get and Give

Teams are expected to use the parts, ideas, and experience of teams in previous years.

Teams are expected to contribute their parts, ideas, and experiences.
Community Parts Collection
Registry DNA Distribution
Quality Control
## Sequence Verification

### Registry of Standard Biological Parts

- Non-wiki
- Log in / create account

### Sequence Analysis

**From partsregistry.org**

This tool is used to organize and analyze a set of DNA sequencing runs by comparing DNA sequences against parts in the Registry.

Use BLAST at NCBI to compare sequences with a large number of genomes.

The BLAST database was last updated on Thu Sep 4 09:21 2008. (Update now)

#### Current Sequence Analysis

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<th>Source Plate 1000, Well 1A, Lib QC08</th>
<th>rudy</th>
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</thead>
<tbody>
<tr>
<td>Target part: BBa_B0011 (length: 40bp)</td>
<td>Linked to part info page</td>
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#### Sequence 1623 (QC08. P260: W39493_VF)

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<td>Get machine files: (Sequence)/Tracce</td>
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<td>BB Prefix found at 1258</td>
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#### Automatic Alignment

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<tr>
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<th>Confirmed Good: 40, Bad: 0, Not clear: 6, Not covered: 0</th>
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</thead>
<tbody>
<tr>
<td>Part</td>
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</tr>
<tr>
<td>1623</td>
<td></td>
</tr>
<tr>
<td>1655</td>
<td></td>
</tr>
</tbody>
</table>

**Source Plate 1000 Image Antibiotic A**
High copy number assembly plasmidic backbones

The most common set of plasmidic backbones that people use to assemble Biobrick® standard biological parts together are high copy Biobrick plasmidic backbones. High copy plasmidic DNA is easily purified in high yield from cultures, so it makes obtaining enough DNA for assembly easy.

The high copy plasmidic backbones listed below have a common set of features.

1. A complete Biobrick® cloning site for easy cloning and assembly of Biobrick parts.
2. Terminators flanking the Biobrick® cloning site to insulate the vector from read-through transcription originating in the cloned Biobrick® part, device, or system.
3. Primer binding sites for the standard Biobrick® verification primers V3 (Bba_G00010) and VR (Bba_G00010). These primers are located for convenient sequencing and screening by colony PCR of cloned Biobrick® parts, devices, and systems.

Plasmid backbones are distributed by the Registry with a default insert. There are just a handful of default plasmidic inserts used in the Registry. Many of the available plasmidic backbones have the cod8 positive selection marker (Bba_P1010) as the default plasmidic insert within the Biobrick® cloning site. The cod8 gene ensures that when assembling two Biobrick® parts together, the unc8 plasmid is not transformed. However, inclusion of the cod8 gene means that these vectors must be propagated in a cod8 tolerant strain, such as E. coli strain DB1 (Bba_V1005).

Finally, to make assembly of Biobrick® parts easier, these Biobrick® assembly plasmidic backbones are available with three different antibiotic resistance markers, so that you can use 3 antibiotic assembly methods to assemble Biobrick® parts.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Resistance</th>
<th>Replicon</th>
<th>Copy number</th>
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Karmela Haynes, an instructor of the 2006 Davidson College iGEM team, designed and constructed the plasmid backbone pSB1AT7. You can read more about the 2006 Davidson project in their open-access paper Engineering bacteria to solve the Burnt Pancake Problem published in the Journal of Biological Engineering.

Robbie Bryant constructed the plasmid backbone pSB1AC3 in Tom Knight’s lab.
<rsbpml>
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      <part_id>151</part_id>
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    <!--
    Parts from the MIT Registry of Standard Biological Parts
    -->
</rsbpml>
From: Tom Knight <tk@csail.mit.edu>
Subject: Registry parameters
Date: July 2, 2010 3:23:53 PM GMT+01:00
To: Randy Rettberg <rettberg@mit.edu>
Cc: Tom Knight <tk@csail.mit.edu>

I want to define the antibiotic resistance of the P1014 part with a parameter. I thought "resistance" would be the one, but it appears to be a switch. I need to define resistance to more than one antibiotic, as well, with units of ug/ml. Do we have a story on standard units for parameters?
gentamicin resistance cassette aph(2)

Novel gentamicin resistance gene. Derived from Genbank DQ208936, pTEX5500ts, kind gift of Barbara E. Murray. This, in turn was derived from Genbank AF016483. See pmid=16391062, pmid=9593155

Conveys resistance to genamicin at up to 12 ug/ml. It conveys low levels of resistance to kanamycin, at 1.5 ug/ml. E. coli NEB10B cells are sensitive to gentamicin at less than 0.3 ug/ml. Selection for this cassette carried out at 4 ug/ml will allow growth in Gentamicin, but inhibit other growth. With standard kanamycin concentrations of 35 ug/ml, this cassette will not convey Kanamycin resistance.

Gentamicin is an important antibiotic for use in human disease. Its use should be carefully considered and alternatives sought when used in a context promoting easy horizontal transfer of the cassette to potential human pathogens, for example in mobilizable plasmids or in transposable elements (insertions sequences).
Design Notes

- PCR primers
  - gtttcttcaatccgagcccttctagag gatagttttagtcaagat, P1014-F
  - gtttcttcgccagccgcccttctagta gattccggattctaaaaaagg, P1014-R2 (after terminator) [Bad, extra A]
  - gtttctcttcacccgccgcttctagta cattattttaaatgctc, P1014-R3 (after stop codon)
  - gtttcttcgccagccgcccttctagta gattccggattctaaaaaaggatgtcgta, P1014-R4
  - gtttcttctgcacccgccgcttctagta gattccggattctaaaaaagg, P1014-R5

- XbaI site at 660 removed by mutating c->t which also improves codon usage.
  - gatgtttaacggaattttagagagaacgaatctac, P1014-XF
  - gatgttttaacggaattttagagagaacgaatctac, P1014-XF2
  - gtgatggcttctctaaatatcctctgtaaaatcatc, P1014-XR

Source

- Novel gentamicin resistance gene
- Acquired by PCR from pTEX5500ts, Genbank DQ208936, kind gift of Barbara E. Murray.
- pTEX5500ts was, in turn was derived from Genbank AF016483.

References

Part: BBa_P1014: Experience

This experience page is provided so that any user may enter their experience using this part. Please enter how you used this part and how it worked out.

Applications of BBa_P1014

The P1014 cassette conveys resistance to gentamicin at 12 ug/ml or more in high copy pSB1xx plasmids.

This is an image of a growth test plate with pSB1AC3-P1014 in rows 1 and 2, and an unrelated pSB1C3 plasmid in row 3. Cells were grown in 700 ul of LB with 20 ug/ml of chloramphenicol overnight, shaking at 37C. Row 1 has 2x serial dilutions of gentamicin, starting at 400 ug/ml on the left. Row 2 has 2x serial dilutions of kanamycin starting at 400 ug/ml on the left. Row 3 has 2x serial dilutions of Gentamicin starting at 400 ug/ml on the left. The rightmost wells have no gentamicin or kanamycin. Growth starts at 12.5 ug/ml in gentamicin, and at 1.6 ug/ml in kanamycin. Growth is inhibited in a non-resistant strain by less than 0.4 ug/ml of gentamicin. A gentamicin concentration of 4 ug/ml allows strong selection. Slightly higher concentrations might be appropriate if trying to distinguish gentamicin resistance from kanamycin resistance.
Safety

Before answering these questions on your team Safety page, be sure to read the Safety in iGEM page.

Key questions

For iGEM 2012, teams are asked to detail how they approached any issues of biological safety associated with their projects. Specifically, teams should consider the following questions:

1. Would any of your project ideas raise safety issues in terms of:
   - researcher safety,
   - public safety, or
   - environmental safety?

2. Do any of the new BioBrick parts (or devices) that you made this year raise any safety issues? If yes,
   - did you document these issues in the Registry?
   - how did you manage to handle the safety issue?
   - How could other teams learn from your experience?

3. Is there a local biosafety group, committee, or review board at your institution?
   - If yes, what does your local biosafety group think about your project?
   - If no, which specific biosafety rules or guidelines do you have to consider in your country?

4. Do you have any other ideas how to deal with safety issues that could be useful for future iGEM competitions? How could parts, devices and systems be made even safer through biosafety engineering?

Teams, please document any answers to these safety questions on your wiki safety page. Judges will be asked to evaluate your project, in part, on the basis of if and how you considered and addressed issues of biological safety. If any questions arise regarding iGEM and biological safety please send an email to safety AT igem.org.
Is iGEM Secure?

Security

"Biology should be more fun. It should be about exploring the world around us. We should want to get out there and do things. We should be able to do things more easily. Securing biology should be something that helps us do that. It cannot be something that gets in the way."

Scientific research continues to bring us new and unexpected knowledge, technologies and approaches. Synthetic biology, being on the very cutting edge of what is possible, promises unprecedented opportunities for health, wealth and better living. But science and technology can be used for destructive purposes as well as for constructive ones. Refining our control of biology opens up chances to intentionally cause harm to humans, animals, plants and the environment that just did not exist before. That’s why it is important now, more than ever, for us to think about how others might use what we are doing in ways we would not be happy with.

Preventing Malign Use

Securing biology is not a simple task. It is not something those outside biology could, or should, do alone. Equally, this is not something that biologists can do by themselves (our focus, as the name implies is on the biology). This is a truly interdisciplinary problem - one that means we will need to work together, in new ways, with new partners, to find an approach that provides benefits for all. Given the interdisciplinary nature of synthetic biology, there are three things you can do right now to help us secure our science.

1. Include something in your project description and presentations that demonstrates that you have thought about how others could misuse your work
2. Contribute to community discussions on policy and practice

As a participant in iGEM, there are three things you can do right now to help us secure our science.
“...So to build a virus from scratch, a terrorist would simply order consecutive lengths of DNA along the sequence and glue them together in the correct order. This is beyond the skills and equipment of the kitchen chemist, but could be achieved by a well-funded terrorist with access to a basic lab and PhD-level personnel.”

James Randerson, The Guardian, June 14, 2006
Different Chassis
Modules: A ‘Black Box’
Original Sequence

In Spring 2011, pSB1C3 was sequenced using several primers. The current sequence reflects the most recent sequencing of pSB1C3 SP 4000 Well 2A. However for posterity, we have included the old sequence that was originally specified in the Registry (PSB1C3-Te) as images of the original sequence with markers pointing out the changes made:

Subversions

Freiburg's pSB1C3 variant of 2010
Future of the Registry

SynBERC

iGEM in Europe

SB Corp

MIT

iGEM in Asia

ASM
What we want
NEW DIRECTIONS
The Ethics of Synthetic Biology and Emerging Technologies

Presidential Commission for the Study of Bioethical Issues
December 2010
**Ajax-Based Editing**

The image shows a screenshot of the Registry of Standard Biological Parts on iGEM.org. The page is labeled as `Part:BBa_K199103:Composite_Edit` and is designed by Kin Lau with the Group: iGEM09_MoWestern_Davidson (2009-11-12).

**CAUTION - This page is under construction:**

1. There will be software error notices.
2. You cannot save changes yet.

- **BBa_R0011**
  - Promoter (lacI regulated, lambda pL hybrid)

- **RFC[10]**
  - tactag

- **BBa_I0462**
  - luxR Protein Generator

- **BBa_B0034**
  - RBS (Elowitz 1999) -- defines RBS efficiency
  - tactag

- **BBa_C0062**
  - luxR repressor/activator, (no LVA?)
  - Sequence has a barcode more...

- **RFC[10]**
  - tactag

- **BBa_B0015**
  - double terminator (B0010-B0012)

**Additional Links:**
- Recent changes
- Recent part changes
- What links here
- Printable version
- Related changes
- Permanent link
- Upload file
- Privacy policy
- Special pages
- Disclaimers
Company recalls ground beef after E. coli reports

By the CNN Wire Staff
August 28, 2010 6:53 p.m. EDT

(CNN) -- Cargill Meat Solutions Corp. has recalled about 8,500 pounds of ground beef that may be contaminated with E. coli, the U.S. Department of Agriculture announced Saturday.

The move came after three people, two in Maine and one in New York, were identified as becoming ill from a strain of E. coli, the government said.
Europe Workshop Schedule

9:30 Welcome
9:40 Team Introductions
11:10 Break
11:40 Synthetic Biology / Parts
12:40 Operations/iGEM Foundation
13:25 Lunch (Rm. 2006)
14:25 Judging/Assembly
14:45 Divisions
15:15 Forum Discussion
16:15 End