Synthetic Biology = design and engineering of biological systems that aren’t found in nature

Why would we want to do this?
- Want to understand natural systems. One of the best ways to understand a system is to change it or make new, related ones
- To fully “understand” a system, we should be able to predict the outcome when we change the system
- For molecular biology, this means:
  - designing new gene circuits and networks
  - modeling the designed systems & predicting their properties
  - making & testing the designs
  - updating our understanding from the model/test agreement

Engineers often look at biological systems & think that the systems are equivalent to electronic circuits

e.g.,

- fluorescent proteins vs. light bulbs or LEDs
- transcription factors vs. transistors or logic gates
- repressors vs. NOT gates
- activators vs. OR/AND gates
- polymerases (transcriptional machinery) vs. batteries

and so on...

Are they right?
→ raises the possibility that biological parts (genes, proteins, etc.) could be combined using the rules established for analog/digital circuits
The Repressilator = engineered genetic circuit designed to make bacteria glow in an oscillatory fashion
= “repressor” + “oscillator”
What other kinds of circuits can be built?
First, we need some more parts!

Some of the other parts available include:
- various sensors
  - light, dark, heat, cold
- more switches, logic gates
  - more repressors, activators
- parts for intracellular communication
  - helpful if cells could tell each what condition they’re in
    → quorum sensing
- parts for signaling the output of circuits
  - fluorescent & luminescent proteins
Bioluminescence – occurs when bacteria are at high density
→ bacteria communicate in order to establish their density

Australian pinecone fish

Hawaiian bobtail squid

~10^{10} Vibrio bacteria/ml fluid
Fish uses to hunt for prey

~10^{11} Vibrio bacteria/ml fluid
in light organ in squid mantle
Squid uses for disguise (light shines downward, looks like moonlight)

Quorum sensing: chemical-based bacterial communication

Neighboring bacteria produce HSL also
if enough bacteria around, HSL builds up, activates bioluminescence

LuxI protein makes HSL (homoserine lactone)

HSL diffuses in/out of cells

LuxR protein (transcription factor) binds HSL, becomes active

Light (bioluminescence)

Promoter for LuxR
An application of quorum sensing
Programming population control into bacteria with a simple designed circuit

HSL = homoserine lactone
HSL-dependent activator makes HSL
kills cell

& the engineered circuit works ...

squares = experimental data
lines = predictions from model

The behaviour can be predicted with a simple model

\[
\frac{dN}{dt} = kN(1 - N/N_m) - dEN
\]

rate of cell growth

<table>
<thead>
<tr>
<th>Rate of Killer Protein Production</th>
<th>Rate of HSL Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\frac{dE}{dt} = k_E A - d_E E)</td>
<td>(\frac{dA}{dt} = v_A N - d_A A)</td>
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</tbody>
</table>

amount of HSL

killer protein synthesis rate

killer protein degradation rate

HSL synthesis rate

HSL degradation rate


Standardization of parts: the iGEM “BioBricks” project
Standardization of parts: the iGEM “BioBricks” project

The iGEM Registry is a growing collection of genetic parts that can be mixed and matched to build synthetic biology devices and systems.

As part of the synthetic biology community's efforts to make biology easier to engineer, it provides a

Featured on the Registry

CRISPR and Cas9 on the Registry

Want to use CRISPR? We have lots of CRISPR and Cas9 parts for different organisms! During iGEM 2013, twelve teams worked on CRISPR as part of their project. Freiburg and MIT used mammalian cells, Duke used S. cerevisiae and the others such as UBC, Paris Bettencourt and Penn State worked with E. coli.

Registry of Standard Biological Parts

Frequently Used Parts

The iGEM Registry has a vast collection of parts that have been contributed over the course of our 10 year history. We have more than 20 thousand parts that iGEMers, graduate students, postdocs, PI's and even high schoolers have contributed to the collection. Some of our parts have been used many, many times. In general, these are the older parts in our collection that have served as the foundation of many projects. The job of every iGEMer and contributor to the Registry is to make parts users will want to in coming years. With over 20,000 parts already in our collection, this is not an easy feat. You will need to create great parts for them to become among our most used.

We have many obsession binding sites in our collection. You may want a strong RBS or one for your organism of choice. However, if you're not sure, you might want one that many other people have used, I will just work it. At this time of writing, our most frequently used part is 860x_BioBrick. This obsession binding site, first described by Michael Elowitz in 1999 has been used over 8000 times in the history of the Registry. You can find the number of uses for each part in the part quick reference box at the top of each part page.

The purpose of this page is to showcase the most highly-used parts in our collection so you may see how iGEMers on different teams approach making great parts.

Top 10 Most Used Parts on the Registry

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<tr>
<th>Rank</th>
<th>Name</th>
<th>Description</th>
<th>Length</th>
<th>Created By</th>
<th>Uses</th>
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<td>RBS_Elowitz</td>
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Can simple biological systems be built from standard, interchangeable parts and operated in living cells? Or is biology simply too complicated to be engineered in this way?

iGEM’s broader goals include:
- To enable systematic engineering of biology
- To promote open & transparent development of tools for engineering biology
- To help construct a society that can productively apply biological technology

2004: MIT, UT, Princeton, Boston University, Cornell
2005: 13 teams (the above + UK, Germany, more...)
2006: 32 teams, incl. Japan/Latin America/Korea/India/more Europe
54 teams in 2007, 84 teams in 2008, 112 teams in 2009, 130 teams in 2010, 165 teams in 2011, and 245 teams in 2012 and 2013...

UT’s 2004/2005 iGEM project – build bacterial edge detector

Adapted from Zack Simpson
How does edge detection work in principle?

A computer might visit each pixel in turn, and check to see if it is bordered by both black & white pixels. If yes, highlight the pixel.

Is this pixel part of an edge?

No

No

Yes

Light-dependent gene expression

Bacterial photography


“Light cannon” developed by Aaron Chevalier, UT undergraduate

The first bacterial photograph (coliroid?)... 


Escherichia darwinia

Image: Aaron Chevalier
On to the edge detector...

Light

Dark

The edge detector circuit in more detail
It works!

Projected Mask

Photo strain

Edge detector strain

Tabor et al., Cell 137(7):1272-1281 (2009)
UT’s 2012 iGEM project – build caffeine biosensor

Decaffeination and Measurement of Caffeine Content by Addicted Escherichia coli with a Refactored N-Demethylation Operon from Pseudomonas putida CBB5

Erik M. Quackenbush, Michael J. Hammerling, Jean M. Surette, Brian B. O’Conner, Ben Stasi, Ryan N. Andel, Maxie Z. Spayd, James E. Fedorow, Mani V. Subramanian, and Jeffrey T. Barick

Basic idea

Block de novo guanine synthesis
Convert caffeine to xanthine
Addict E. coli bacteria to caffeine

ACS Synthetic Biology 2013, 2, 301−307