Synthetic Biology: Insights into Natural Biology and New Technologies

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Biology and GCAT

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Mathematics and GCAT

Davidson College
November 14, 2011
Three Rules for Our Lab

1. Everyone has to learn.
2. Everyone has to have fun.
3. We try to contribute to the body of science.
What is Synthetic Biology?

Application of engineering principles and mathematical modeling to the design and construction of biological parts, devices, and systems with applications in energy, medicine, and technology.

www.bio.davidson.edu/projects/gcat/Synthetic/What_Is_SynBio.html
Synthetic Biology: Win-Win

Win #1: your design functions as expected.
Synthetic Biology: Win-Win Research

Win #1: your design functions as expected.

Win #2: your design fails but you uncover basic biology.
How is Synthetic Biology Different?

Abstraction
Modularity
Standards
Designing and modeling
Abstraction
Abstraction
Modularity

USB ports on computers
Modularity
Standardization

On a Uniform System of Screw Thread

“In this country, no organized attempt has as of yet been made to establish any system, each manufacturer having adopted whatever his judgment may have dictated as best, or as most convenient for himself.”

William Sellers April 21, 1864
Standardization

On a Uniform System of Screw Thread
Modeling of Designs
Real World Applications of Synthetic Biology
Land Mine Detection
Land Mine Detection
New weed may flag land mines

By John K. Borchardt | Contributor to The Christian Science Monitor
Production of Medicines

$1 per pill
Production of Medicines

10¢ per pill
Biofuels from Algae

$\text{CO}_2$-neutral

1,000,000 gallons in 2008
Synthetic Biology

Parts → Devices → Systems
Synthetic Biology

Parts → Devices → Systems

- Ribosome Binding Sites
- Regulatory
- RNA
- DNA
- Protein Coding
- Terminators
- Conjugation
Synthetic Biology

Synthetic Biology

Parts → Devices → Systems
How do we clone DNA?
BioBricks

BioBrick Part

BioBrick plasmid backbone + 1 part

(origin) antibiotic resistance

(http://partsregistry.org/Plasmids)
BioBricks

BioBrick plasmid backbone + 1 part

(http://partsregistry.org/Plasmids)
BioBricks

BioBrick plasmid backbone + 1 part

Eco RI

identical sticky ends

Xba I
Spe I
Pst I

BBa_B0015

origin
antibiotic resistance

(http://partsregistry.org/Plasmids)
BioBricks

put B downstream of A
BioBricks

Part A

Origin

Antibiotic resistance

Cut with Spe and Pst

Part B

Origin

Antibiotic resistance

Cut with Xba and Pst
BioBricks

Part A

origin

antibiotic resistance

Part B

gel purify

EtOH precipitate

BioBricks
BioBricks

ligate

Part A

Part B

origin

antibiotic resistance
BioBricks

mixed site = scar

transform

origin  

antibiotic resistance

Part A  Part B
BioBricks

Part A

origin
antibiotic resistance

Part B

origin
antibiotic resistance

Part A

origin
antibiotic resistance

Part B

origin
antibiotic resistance

BioBricks
Challenge:

put A upstream of B
Too Many Projects to Talk About Today

Catherine Doyle ’14

Julia Fearrington ’13
Building Bacterial Computers
Advantages of Bacterial Computation

Software → Hardware → Computation

http://www.dnamind.med.usyd.edu.au/

http://www.turbosquid.com
Advantages of Biological Computers

go anywhere - arctic, thermal vents, inside organisms

no electricity

self-replicating

no immune rejection
Self-replicating Computers

- Non-Polynomial (NP) No Efficient Algorithms

Advantages of Bacterial Computation
- Self-replicating Computers

Graphs:
- Possible Paths through the Graph
- Number of Edges in the Graph
- # of Processors
- Cell Division
One Research Project - the SAT problem
Define the SATisfiability Problem
Define the SATisfiability Problem

<table>
<thead>
<tr>
<th>Bb</th>
<th>Gg</th>
<th>Rr</th>
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<tbody>
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- Bb: Blue, Black
- Gg: Green, Grey
- Rr: Red, Brown
### Define the SATisfiability Problem

<table>
<thead>
<tr>
<th>Keys</th>
<th>Doors</th>
<th>Open Doors</th>
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<tbody>
<tr>
<td>B b G g R r</td>
<td>GorB, Gorb, Gort, gorR</td>
<td>4 open doors</td>
</tr>
<tr>
<td>G B R</td>
<td></td>
<td>3 open doors</td>
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<tr>
<td>G B r</td>
<td></td>
<td>2 open doors</td>
</tr>
<tr>
<td>g B R</td>
<td></td>
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</table>
Converting Math to Biology
Central Dogma

DNA
atgccctactcactacctatagcgcat

\[ \text{transcription} \]

mRNA
\textbf{aug ccc uac uca cua ccu aua ccg cau}

\[ \text{translation} \]

Protein
\textbf{M P Y S H P I P H}
Frameshift Mutation

DNA
atgccctactcactacctatagcgc

transcription

mRNA
aug ccc uac uca cua ccu au a ccg cau

translation

Protein
MPYSSHPIPH

DNA
atgccctactcactacctatagcgc

transcription

mRNA
aug ccc UCu acu cac uac cua uac cgc au

translation

Protein
MPSTHYHYR
Frameshift Suppression

DNA
atgcctactcactacctatatagcgc

↓

mRNA
aug ccc uac uca cua ccu aua ccg cau

↓

Protein
MPYSHPIPH

DNA
atgccccTCTactcactacctatatagcgc

↓

5 base suppressor tRNA
aug cccUC uac uca cua ccu aua ccg cau

↓

Protein
M[S]YSHPIPH
Suppressor tRNA

core tRNA nucleotides

serine

5 base anticodon

G A G G G
Coding 2-SAT Clause

G ATG NNNNN gNN NNN → satisfied
Coding 2-SAT Clause

G

ATG NNNNN

\( gNN NNN \)

\[ \rightarrow \]

satisfied

OR

B

ATG NNN NNg

\( NNNNN \)

\[ \rightarrow \]

satisfied
Coding 2-SAT Clause

\[ \text{G: ATG NNNNN } g\text{NN NNN} \rightarrow \text{satisfied} \]

\[ \text{OR} \]

\[ \text{B: ATG NNN NNG } g\text{NNNNN} \rightarrow \text{satisfied} \]

\[ \text{ATG NNN NNG } g\text{NNN} \quad \circ \rightarrow \text{no satisfaction} \]
Redesign System v2.0
## Outcomes of v 2.0

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>no RFP</td>
<td>- control</td>
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<tr>
<td>5mer CGGUC</td>
<td>frame shift</td>
</tr>
<tr>
<td>5mer CGGUC + tRNA</td>
<td>“leak”</td>
</tr>
<tr>
<td>5mer CCACU + tRNA</td>
<td>+ tRNA</td>
</tr>
<tr>
<td>optimized feedback loop</td>
<td>+ control</td>
</tr>
</tbody>
</table>

![Image of test tubes with different outcomes](image_url)

Legend:
- No RFP: Control
- Frame shift: “leak”
- 5mer CGGUC: + tRNA
- 5mer CCACU: + tRNA
- Optimized feedback loop: + control
Outcomes of v 2.0

- 5mer CGGUC + tRNA
- 5mer CCACU + tRNA
- Optimized feedback loop

Fluorescence/cell density

- Negative control
- CGGUC frameshift
- CGGUC + tRNA
- CCACU + tRNA
- No frameshift

"leak" frame shift
Outcomes of v 2.0

GFP/ cell density

+ 3 tRNAs
Controls....
Still working on this...
Why build bacterial computers?
Evolution of Computers
Evolution of Computers

iPhone in 2011
Evolution of Bacterial Computers

*E. coli* in 2011

Living Hardware in 2021
Intro Bio Student Collaborators

[Logos of Davidson and Western Universities]
Intro Bio Student Collaborators

Biologists need is a registry of functional promoters (RFP).

Intro students find promoter of interest.

Oligator converts into oligos.

Clone new promoter.

Transform and test new promoter.

Submit to Registry of Functional Promoters (national resource).

But cloning DNA is not easy….
Gel Purification
Gel Purification

- Origin
- Antibiotic resistance

Part B

Diagram of gel electrophoresis with bands indicating the separation of DNA fragments.
Gel Purification
Gel Purification

Part A
- origin
- antibiotic resistance

Part B
- S
- P
Part A

origin

antibiotic resistance

Part B

Gel Purification

E X Part A S P

S P

- X

Part B - S P
Gel Purification

Part A

origin antibiotic resistance

Part B

E • X

S • P

PSG Gel Purification
Ligation

Part A

Part B

origin

antibiotic resistance

E • X

S • P

X

X
Ligation

Part A
origin
antibiotic resistance

Part B
X
S・P

Part A
origin
antibiotic resistance

Part B
X
S・P
M
How can we clone DNA without all the hassle?
Golden Gate Assembly Method

TT + RBS + RFP

TT

RBS

RFP

origin

antibiotic resistance

plasmid backbone
Golden Gate Assembly Method

TT

promoter + RBS + RFP

RBS  RFP

origin  antibiotic resistance

plasmid backbone
Eco RI

GAATTC
CTTAAG

palindrome

type II
Eco RI

GAATTCC  palindrome
CTTAAG

type II
Eco RI

<table>
<thead>
<tr>
<th>GAATTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTTAAG</td>
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</tbody>
</table>

type II
Eco RI

G
CTTAA

AATTC
G

type II
Bsa I

GAGACC  CTCTTG

not a palindrome

type II
Bsa I

1234nGAGACC

-----nCTCTCTGG

type IIIs
Bsa I

1234nGAGACC
nCTCTCTGG

type IIIs
Bsa I

GGTCTCn

CCAGAGn1234

type IIIs
Bsa I

GGTCTCn
CCAGAGn1234

type IIIs
Bsa I

cuts left

1234 nGAGACC

---- nCTCTGG

GGTCTC n---

CCAGAG A G n1234
cuts right
Bsa I

CGACTGAGACC(TT)GGTCTCaGCGG
GCTGacCTCTGG(TT)CCAGAGtCGCC

Bsa I

TT + RBS + RFP
CGACTGAGACC (TT) GGTCTCa
aCTCTGG (TT) CCAGAGtCGCC
CGAC\textsuperscript{t}GAGACC\textsuperscript{(TT)}\textsuperscript{a}GGTCTCa
\textsuperscript{a}CTCTGG\textsuperscript{(TT)}CCAGAG\textsuperscript{t}CGCC

\textbf{CGAC (promoter) (promoter) CGCC}
GGA Ligation Method

TT + RBS + RFP

TT  RBS  RFP

BsaI + Ligase

origin  antibiotic resistance

plasmid backbone
GGA Ligation Method

promoter + RBS + RFP

BsaI + Ligase

plasmid backbone
GGA Ligation Method

promoter + RBS + RFP

RBS

RFP

no gel purifications!

origin

antibiotic resistance

plasmid backbone
GGA Ligation Method

no gel purifications!
GGA Ligation Confirmation

All digested with R+P
1  J119022
2  J100028
3  20 cycle red
4  20 cycle red
5  20 cycle red
6  20 cycle white
7  20 cycle white
GGA Ligation Confirmation

All digested with R+P
1  J119022
2  J100028
3  20 cycle red
4  20 cycle red
5  20 cycle red
6  20 cycle white
7  20 cycle white
Registry of Functional Promoters

Campbell M Lab Parts

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Designer</th>
<th>Length</th>
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<td>Coding</td>
<td>Cre with 5` suppressor site and 1-copy 2-SAT promoter inserted</td>
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</tbody>
</table>

Registry of Standard Biological Parts
Student Sample

Registro de Partes Biológicas Estándares

Part: BBa_J100033

Designed by Chris Peek  Group: Campbell_M_Lab  (2011-09-01)

dnakP1 promoter: Heat shock inducible
dnakP1 is naturally off, but is induced when E. coli is heat shocked, resulting in transcription downstream from this promoter.

Sequence and Features

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<th>Subparts</th>
<th>Ruler</th>
<th>SS</th>
<th>DS</th>
<th>Search:</th>
<th>Length: 101 bp</th>
<th>Context: Part only</th>
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Assembly Compatibility: 10 12 21 23 25
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_J100033

**Mean Fluorescence per Cell Density**

- **Mean Fluorescent Intensity (MFI)** per Cell Density

Condition: kPA1 (-)  | kPA1 (+)  | J10028 (-)  | pTel (+)  | pLac + IPTG  | pLac - IPTG  | * p < 0.01

- Cells containing dnaK1 without heat shock (incubated at 37°C)
- Experimental: cells containing dnaK1 with heat shock (incubated at 42°C)
- Negative control: 
- Positive control: 

* marker indicates significant difference.
What’s Next?

Improve the plasmid to increase phenotype.
Simplify ligation/digestion protocol.
Unleash intro bio students for real research!
Three Rules for Our Lab

1. Everyone has to learn.

Outcomes of v 2.0

<table>
<thead>
<tr>
<th>Condition</th>
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+ 3 tRNAs
Three Rules for Our Lab

1. Everyone has to learn.

2. Everyone has to have fun.
Three Rules for Our Lab

1. Everyone has to learn.
2. Everyone has to have fun.
3. We try to contribute to the body of science.
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The End