STEM Activity: Synthetic Biology

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Biology Department and GCAT

Phillip O. Berry Academy of Technology
January 28, 2014
Outline of Presentation

Why go to college?

What is the value of an education?

Why is research important?

What is synthetic biology?

Can you do synthetic biology research?

Why is research a powerful way to become educated?
Guess what, I taught my dog to whistle!
Really?!

Teaching vs Learning
Teaching vs Learning

Whistle! C’mon boy, whistle!
Teaching vs Learning
Teaching vs Learning

I thought you said you taught your dog to whistle.
I did, but I didn’t say that he *learned* to whistle.
Why go to college?
Are you training for one job?

“Number of Jobs Held, Labor Market Activity, and Earnings Growth among the Youngest Baby Boomers: Results from a Longitudinal Survey”

http://www.officefurniturepics.com/tag/used-office-cubicles/
Are you training for one job?


- born in the years 1957 to 1964
- jobs from age 18 to age 42
- average of 10.8 jobs
- more jobs ages 18 - 24 than 36 - 42
- 23% held at least 15 jobs
- 14% held zero to four jobs

“Number of Jobs Held, Labor Market Activity, and Earnings Growth among the Youngest Baby Boomers: Results from a Longitudinal Survey”
No one gives you an education.

If you want one, you have to take it.

John Taylor Gotto

http://nccueagles.yuku.com/topic/6441#.T1o5-piuufqE
List jobs that pay you to memorize information that you don’t use....

http://confessionsofahighbrow.com/tag/standardized-tests/
Who thinks they can remember more factoids than a computer?
Why try to compete with computers by memorizing?

In college, you should enhance your skills that computers cannot perform.

Dr. Ben Carson
Edgar Degas
JK Rowling

Rihanna
Take Charge of Your Own Learning
Percent Americans ≥ 25 with Bachelor’s Degree

30.4% overall
14.1% Hispanics
19.9% African Americans
34.0% Caucasians

Average Annual Earnings Workers ≥ 18

- advanced degree: $74,602
- bachelors degrees: $51,206
- high school diploma: $27,915
- no high school diploma: $18,734.

http://usgovinfo.about.com/od/censusandstatistics/a/collegepays.htm
Education is the only industry where customers never complain when they get less product for their money.
Synthetic Biology Defined
What is Synthetic Biology?

Implementation 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
Real World Applications of Synthetic Biology
Land Mine Detection
Land Mine Detection
Synthetic Biology

Land Mine Detection

WARNING SIGN: The bioengineered Thales cress turns red when exposed to a mine byproduct.

COURTESY OF ARESA BIODETECTION

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

CO$_2$-neutral

1,000,000 gallons in 2008
Promoter Research
Using
Synthetic Biology
Eco RI

GAATTCC
CTTAAG

palindrome

type II
Eco RI

GAATTC
CTTAAG

palindrome

type II
Eco RI

GAATTC
CTTAAG

type II
Eco RI

G
CTTAA

AATTC
G
type II
Bsa I

GAGACC
CTCTG
not a palindrome

type IIIs
Bsa I

1234nGAGACC
----nCTCTGG

type II enzymes
Bsa I

1234nGAGACC
nCTCTGG

type IIIs
Bsa I

GGTCTCn-----
CCAGAGn1234

type IIIs
Bsa I

GGTCTCn
CCAGAGn1234

type IIIs
Bsa I

1234 nGAGACC
----- nCTCTGG

GGTCTCn

CCAGAGn 1234
GGA Ligation Method

TT + RBS + RFP

TT

RBS

RFP

BsaI + Ligase

origin

antibiotic resistance

plasmid backbone
GGA Ligation Method

TT + RBS + RFP

BsaI + Ligase

plasmid backbone

TT

RBS

RFP

origin

antibiotic resistance
CGAC\text{GAGACC(\textbf{TT}) GGTCTCa}
\text{aCTCTGG(\textbf{TT}) CCAGAG}\text{tCGCC}

\begin{align*}
\text{CGAC} & \text{(promoter)} \\
\text{CGCC} & \text{(promoter)}
\end{align*}

\text{RBS + RFP} \\
\text{RBS} \rightarrow \text{RFP}

\text{CGAC(promoter)} \\
\text{(promoter) CGCC}
promoter + RBS + RFP

- CGAC\textbf{(promoter)}\textcolor{green}{GCGG}
- GCTG\textbf{(promoter)}\textcolor{blue}{CGCC}

\textbf{ligase} \hspace{2cm} \textbf{ligase}
GGA Ligation Method

promoter + RBS + RFP

RBS

RFP

BsaI + Ligase

origin

antibiotic resistance

plasmid backbone

Tuesday, February 18, 2014
GGA Ligation Method

promoter + RBS + RFP

no gel purifications!

plasmid backbone
GGA Ligation Method

no gel purifications!

promoter + RBS + RFP

plasmid backbone
<table>
<thead>
<tr>
<th>Registry of Standard Biological Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Code</strong></td>
</tr>
<tr>
<td>Bba J100067</td>
</tr>
<tr>
<td>Bba J100068</td>
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<tr>
<td>Bba J100069</td>
</tr>
<tr>
<td>Bba J100070</td>
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<tr>
<td>Bba J100097</td>
</tr>
<tr>
<td>Bba J100098</td>
</tr>
<tr>
<td>W Bba J100099</td>
</tr>
</tbody>
</table>
A promoter (CydAB) activated by the FNR enzyme

The promoter, CydAB, was found to be activated by the FNR enzyme, which is induced by the presence of (NH4)2Fe(SO4)2 and ascorbate. The oligo includes both CydAB, the FNR binding site, and the sticky ends needed for the Golden Gate Assembly method.

Sequence and Features

<table>
<thead>
<tr>
<th>Format: Subparts</th>
<th>Search:</th>
<th>Length: 64 bp</th>
<th>Context: Part only</th>
<th>Get selected sequence</th>
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</thead>
<tbody>
<tr>
<td>1 11 21</td>
<td>31 41 51</td>
<td>61 71 81 91</td>
<td>1 ggaattgata tttatcaatg tataagctctt ggaatgggct atcaaaaaga gataaattggt ttctc</td>
<td>FNR binding -35</td>
</tr>
</tbody>
</table>

Assembly Compatibility: 10 12 21 23 25

Part: BBa_J100099: Experience

Designed by Phoebe Parrish  Group: Campbell, M. Lab  (2012-09-13)

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_J100099

We pipetted 200 microliters of one solution containing E coli cells from a small colony and the activators, one with cells from a small colony and no activators, one containing cells from a large colony and the activators, and one containing cells from a large colony and no activators. We also did a positive control with E coli cells containing a known promoter that causes red fluorescence (J04450) and a negative control with cells containing a the transcriptional terminator that does not cause red fluorescence (J100091). We tested both fluorescence of our samples using a fluorometer and the light absorbance using a spectrophotometer. We measured the fluorescence and absorbance of five samples of each solution, including a control solution that just contained the growth medium. We averaged the values for each solution and subtracted the average fluorescence/absorbance of the control. We then divided the average fluorescence by the average absorbance for each solution. These values are displayed on the accompanying graph.

![Graph of RFP Fluorescence with CydAB](image)
Registry of Functional Promoters (RFP)

Welcome to the Registry of Functional Promoters

This Registry of Functional Promoters was developed by Bill Hatfield, Laurie J. Heyer, A. Malcolm Campbell at Davidson College and Todd Eckdahl of Missouri Western State University, through the support of HHMI grant 52006292 (GCA T main page) and is freely available for others to use though no support other than the user manual is available.

If your are already a Registered User of GCAT-aIMG, you do not need to Reregister

LOGIN    REGISTER AS NEW USER

- For comments or questions about this website contact, Malcolm Campbell

gcat.davidson.edu/RFP/
### Registry of Functional Promoters (V1.0)

**SEARCH**

**Search by Entry Number**

**Entry Number**

Use ""," for multiple entries, ":" for range

**Search Criteria**

- **OR**
  - AND
    - Promoter Name
    - Part Number
    - Sequence
    - Length
    - Criterion
    - Species of Origin:
    - Constitutive
    - Regulated
  - OR
    - RBS Used for Testing:
    - ORF Used for Testing:
    - Plasmid Used for Testing:
    - *E.coli* Used for Testing:
    - Media Used for Testing:
    - Comparison Construct:
    - Comparison Plasmid:
    - *E.coli* Used for Comparison Construct:
    - Media Used for Comparison Construct:
    - Fold Difference From Comparison:
  - OR
    - Comment:
    - Direction:
      - Forward
      - Reverse
    - Status:
      - Works
      - Not Working
      - Iffy

[Registry of Functional Promoters](gcat.davidson.edu/RFP/)
# Registry of Functional Promoters (RFP)

## Search Promoter Results

<table>
<thead>
<tr>
<th>Entry No.</th>
<th>Promoter Name</th>
<th>Part Number</th>
<th>Sequence</th>
<th>Length</th>
<th>Citation</th>
<th>Species of Interest</th>
<th>Constitutive/Regulated</th>
<th>Inducible/Repressible</th>
<th>Regulator</th>
<th>RBS Used for Testing</th>
<th>ORF Used for Testing</th>
<th>Plan Use Te</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TetR Repressible Promoter</td>
<td>R00000</td>
<td>cgcaatcagcttgagtgaattgacacgtactagtcagtagagtcagcac</td>
<td>54</td>
<td></td>
<td>Regulated</td>
<td>Repressible</td>
<td>TetR</td>
<td></td>
<td>pSI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>56 bp Lac Promoter</td>
<td>R00110</td>
<td>gctggaacactagonagcagcagaacactactggctggacttagtaatagtagtgac</td>
<td>56</td>
<td></td>
<td>Constitutive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>200 bp Lac Promoter</td>
<td>R00101</td>
<td>gctggaacactagcactgtgaggagctggctggacttagtaatagtagtgac</td>
<td>200</td>
<td></td>
<td>Constitutive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>LuxR &amp; HSL Regulated Lux promoter</td>
<td>R0062</td>
<td>ccacctgagctcatacagtacctcgaggagatagtagtagtagatata</td>
<td>55</td>
<td></td>
<td>Regulated</td>
<td>Repressible</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>Backwards 200 Lac Promoter (right to left)</td>
<td>J3J013</td>
<td>gctggaacactagcactgtgaggagctggctggacttagtaatagtagtgac</td>
<td>200</td>
<td></td>
<td>Regulated</td>
<td>Repressible</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>6</td>
<td>OmpC Promoter</td>
<td>K19901</td>
<td>gctggaacactagcactgtgaggagctggctggacttagtaatagtagtgac</td>
<td>99</td>
<td></td>
<td>Constitutive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>23K series very strong constitutive Promoter</td>
<td>J3J000</td>
<td>tggactatagctacgtactagctgtaggtagttaga</td>
<td>35</td>
<td></td>
<td>Constitutive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To Edit an Entry, Enter the Entry # and press "Edit Entry"  
To Delete an Entry, Enter the Entry # and press "Delete Entry"
Testing Known Promoters: Ptac

5’ CGACGAGCTGTGGACAAATTAATCATCGGCTCTGATATAATGTTGGA 3’
3’ CTCGACAACTGTAAATTAGTAGCCGAGCATATTACACACCTCGCC 5’
5′ CGACGAGCTGTGTTtACAATTAATCATCGGCTCGTATAATGTGTGGA 3′
3′ CTCGACAAaTGTTAATTAGTAGCCGAGCATATTACACACCTCGCC 5′
Student Sample, November 2012

-35 ATAA (deleted) -10

5’ CGACGAGCTGTGGACA----ATCATCGGCTCGTATAATGTGTGGA 3’
3’ CTCGACAACTGT----TAGTAGCCGAGCATATTACACCTCGCC 5’
Student Sample, September 2012

+ control
- control
new 1
new 2

+ control
- control
new A
new B
Remove Initial Promoter

J119137
Insert Non-functional Promoter

J119137
Insert Forward Promoter

J119137
Insert Bi-directional Promoter

J119137
pClone Red

A

pClone Red

GFP  RBS  CGAC  Bsal  Bsal  GCGG  RBS  RFP

B

pClone Green

Only

pClone Green

+ →

pClone Green

+ ×
pClone Blue

A

B

pClone Blue

Only

+ gfp

+ gCGG

Only + gCGG
Did you learn how to clone by GGA?

Anneal the two ssDNA molecules and ligate them into the pClone plasmid.
1. Cut out each piece surrounded by dotted lines.
2. Assemble the dsDNA promoter Ptac using the two ssDNA pieces of paper.
3. Tape together the dsDNA once you have it assembled.
4. Use scissors to perform the function of BsaI. Cut out the existing promoter in pClone.
5. Be very careful when you cut to produce the sticky ends that will remain attached to pClone. You might want to use a pencil to draw where you will cut before you start.
How strong is your promoter?
## Quantify with Phone and ImageJ

<table>
<thead>
<tr>
<th>Mutant</th>
<th>J119319</th>
<th>J119320</th>
<th>J119321</th>
<th>J119322</th>
<th>J119323</th>
<th>J119324</th>
<th>J119325</th>
<th>J119326</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pClone Green plate</strong></td>
<td><img src="pClone_1.png" alt="Image" /></td>
<td><img src="pClone_2.png" alt="Image" /></td>
<td><img src="pClone_3.png" alt="Image" /></td>
<td><img src="pClone_4.png" alt="Image" /></td>
<td><img src="pClone_5.png" alt="Image" /></td>
<td><img src="pClone_6.png" alt="Image" /></td>
<td><img src="pClone_7.png" alt="Image" /></td>
<td><img src="pClone_8.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Isolated clones</strong></td>
<td><img src="isolated_1.png" alt="Image" /></td>
<td><img src="isolated_2.png" alt="Image" /></td>
<td><img src="isolated_3.png" alt="Image" /></td>
<td><img src="isolated_4.png" alt="Image" /></td>
<td><img src="isolated_5.png" alt="Image" /></td>
<td><img src="isolated_6.png" alt="Image" /></td>
<td><img src="isolated_7.png" alt="Image" /></td>
<td><img src="isolated_8.png" alt="Image" /></td>
</tr>
<tr>
<td><strong>Expression Ratio</strong></td>
<td>4.09</td>
<td>3.94</td>
<td>3.84</td>
<td>2.04</td>
<td>1.54</td>
<td>1.34</td>
<td>3.52</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Measure Promoter Qualitatively
Three Rules for Student Research

1. Everyone must learn.
Three Rules for Student Research

1. Everyone must learn.
2. Everyone must have fun.
Three Rules for Student Research

1. Everyone must learn.
2. Everyone must have fun.
3. We try to contribute to science.

25 undergraduate co-authors

Papers of the year 2008 & 2009
Without basic research, there can be no applications....

After all, electricity and the lightbulb were not invented by incremental improvements to the candle.

former French President Nicholas Sarkozy
Skills Most Sought After by Employers

1) Communications Skills
2) Analytical/Research Skills
3) Computer Literacy
4) Flexibility
5) Interpersonal Abilities
6) Leadership Skills
7) Multicultural Sensitivity
8) Organizational Skills
9) Problem-Solving/Creativity
10) Teamwork

http://www.quintcareers.com/job_skills_values.html
Skills Improved During Research

1) Communications Skills
2) Analytical/Research Skills
3) Computer Literacy
4) Flexibility
5) Interpersonal Abilities
6) Leadership Skills
7) Multicultural Sensitivity
8) Organizational Skills
9) Problem-Solving/Creativity
10) Teamwork

http://www.quintcareers.com/job_skills_values.html
Personal Values Employers Seek in Employees

1) Honesty/Integrity
2) Adaptability
3) Dedication/Tenacity
4) Dependability
5) Loyalty
6) Positive Attitude
7) Professionalism
8) Self-Confidence
9) Self-Motivated
10) Willingness to Learn

http://www.quintcareers.com/job_skills_values.html
Personal Values Improved by Research

1) Honesty/Integrity
2) Adaptability
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7) Professionalism
8) Self-Confidence
9) Self-Motivated
10) Willingness to Learn

http://www.quintcareers.com/job_skills_values.html
Would you rather settle for a blue collar B
or try for an A and risk failure?
“Would you like me to give you a formula for success? It's quite simple, really. **Double your rate of failure.** You are thinking of failure as the enemy of success. But it isn’t at all. You can be discouraged by failure or you can learn from it, so go ahead and make mistakes. Make all you can. Because remember that’s where you will find success.”

Thomas J. Watson
The scenery only changes for the lead dog.
The scenery only changes for the lead dog.
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