STEM Activity:
Synthetic Biology

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Biology Department and **GCAT**
DAVIDSON

Phillip O. Berry Academy of Technology
January 28, 2014

Outline of Presentation

Why go to college?

What is the value of an education?

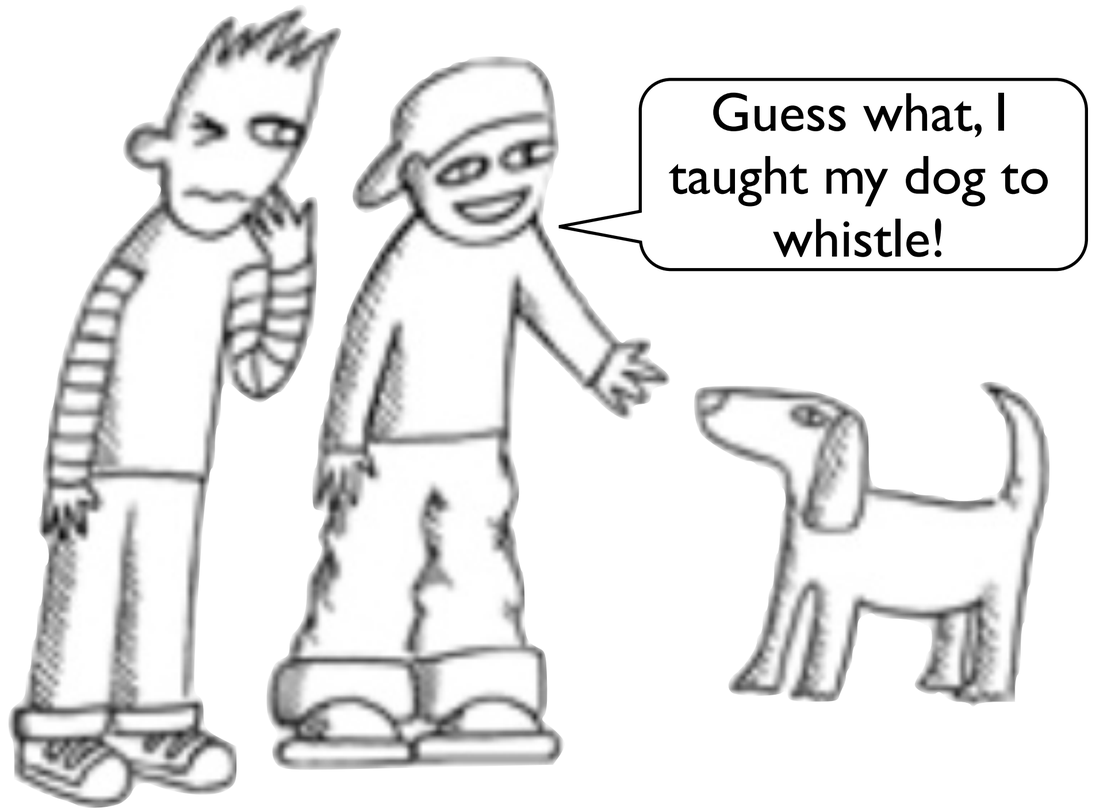
Why is research important?

What is synthetic biology?

Can you do synthetic biology research?

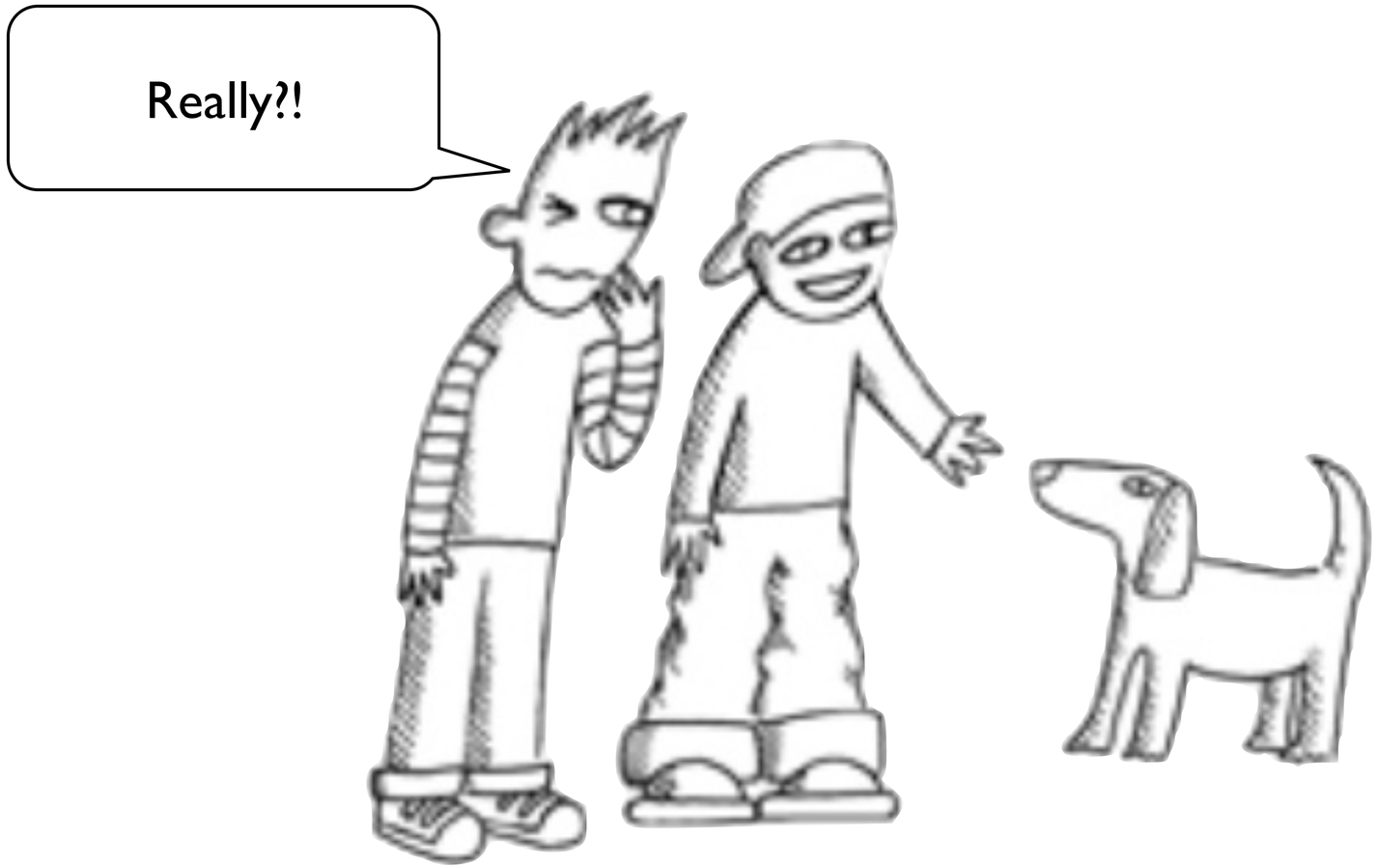
Why is research a powerful way to become educated?

Teaching *vs* Learning

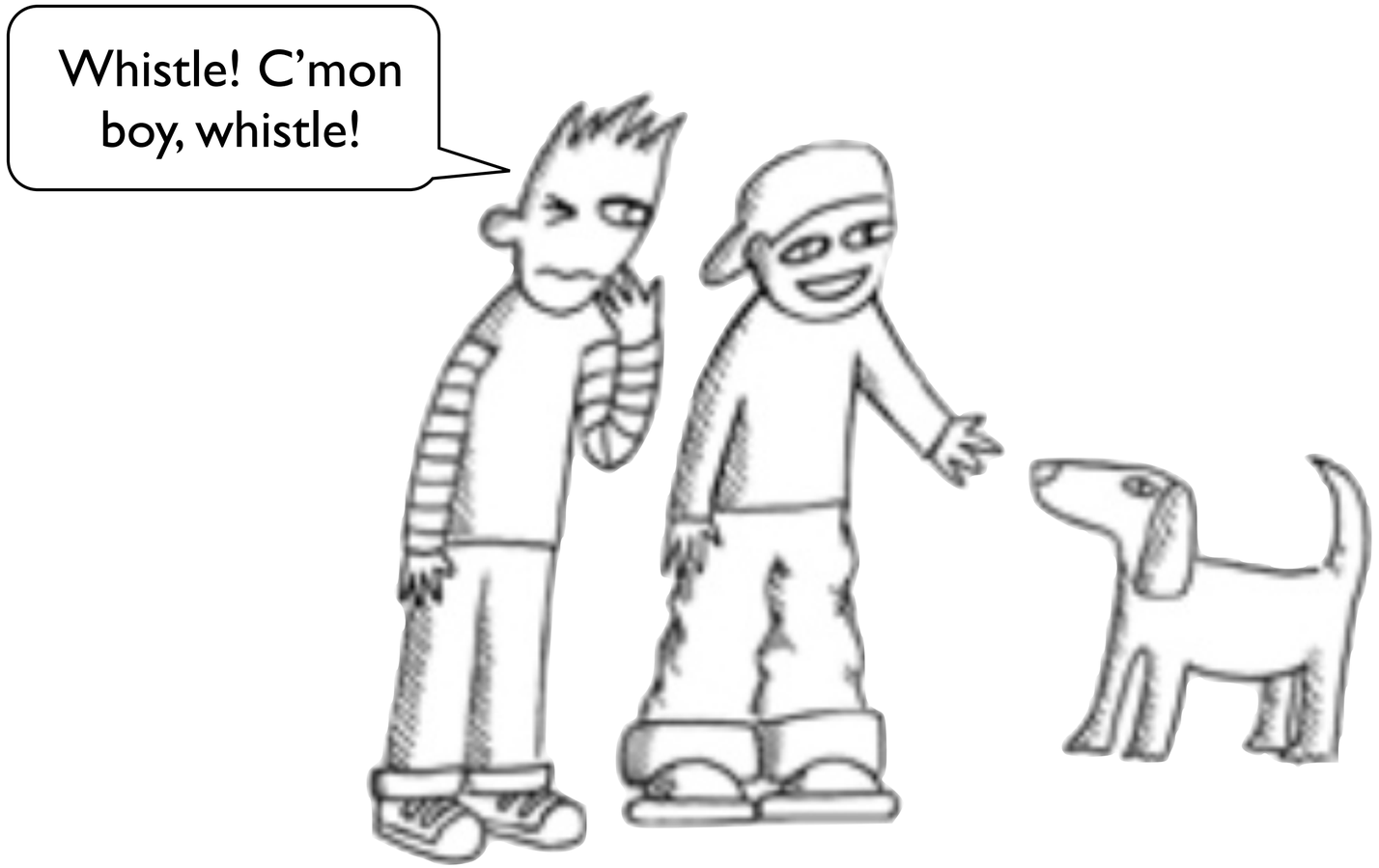


http://thegoodpeople.se/blog/?p=208

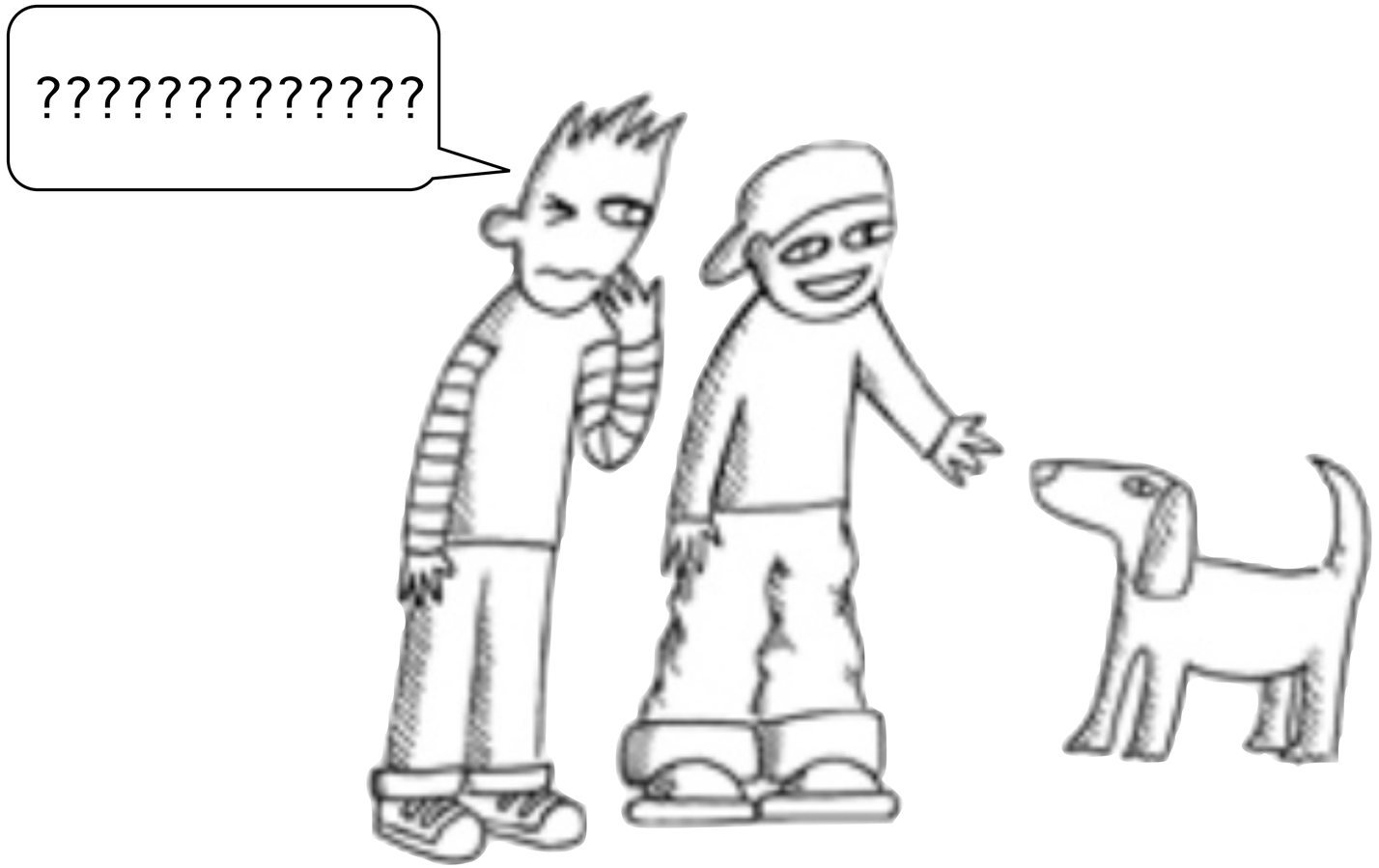
Teaching *vs* Learning



Teaching *vs* Learning



Teaching *vs* Learning



Teaching *vs* Learning

Teaching *vs* Learning

Why go to college?

http://www.creditnet.com/blog/miscellaneous/how-to-manage-student-loans

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?

Bureau of Labor Statistics, 2008

- 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

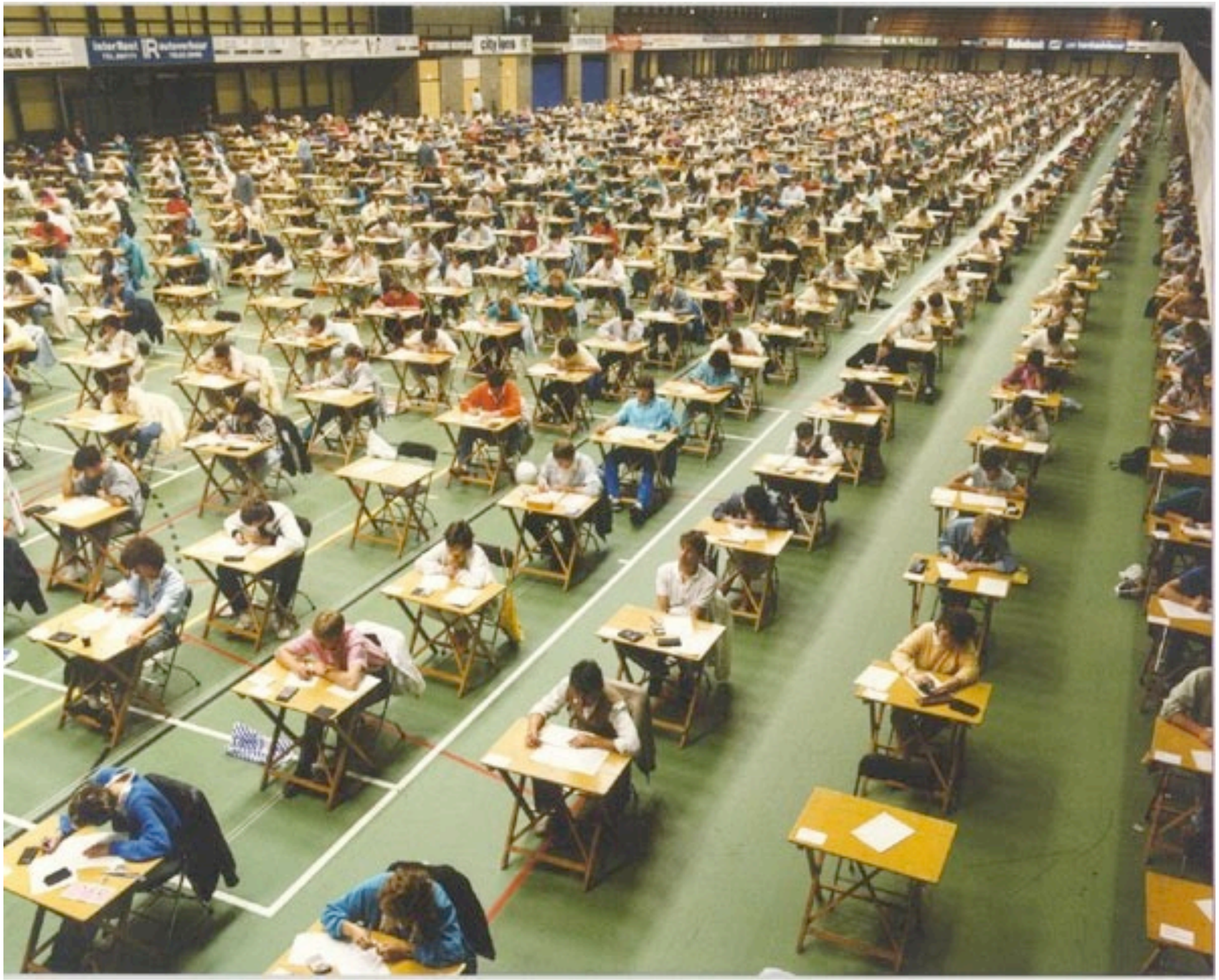
"Number of Jobs Held, Labor Market Activity, and Earnings Growth among the Youngest Baby Boomers: Results from a Longitudinal Survey"

No one *gives* you an education.

If you want one, you have to take it.

John Taylor Gotto

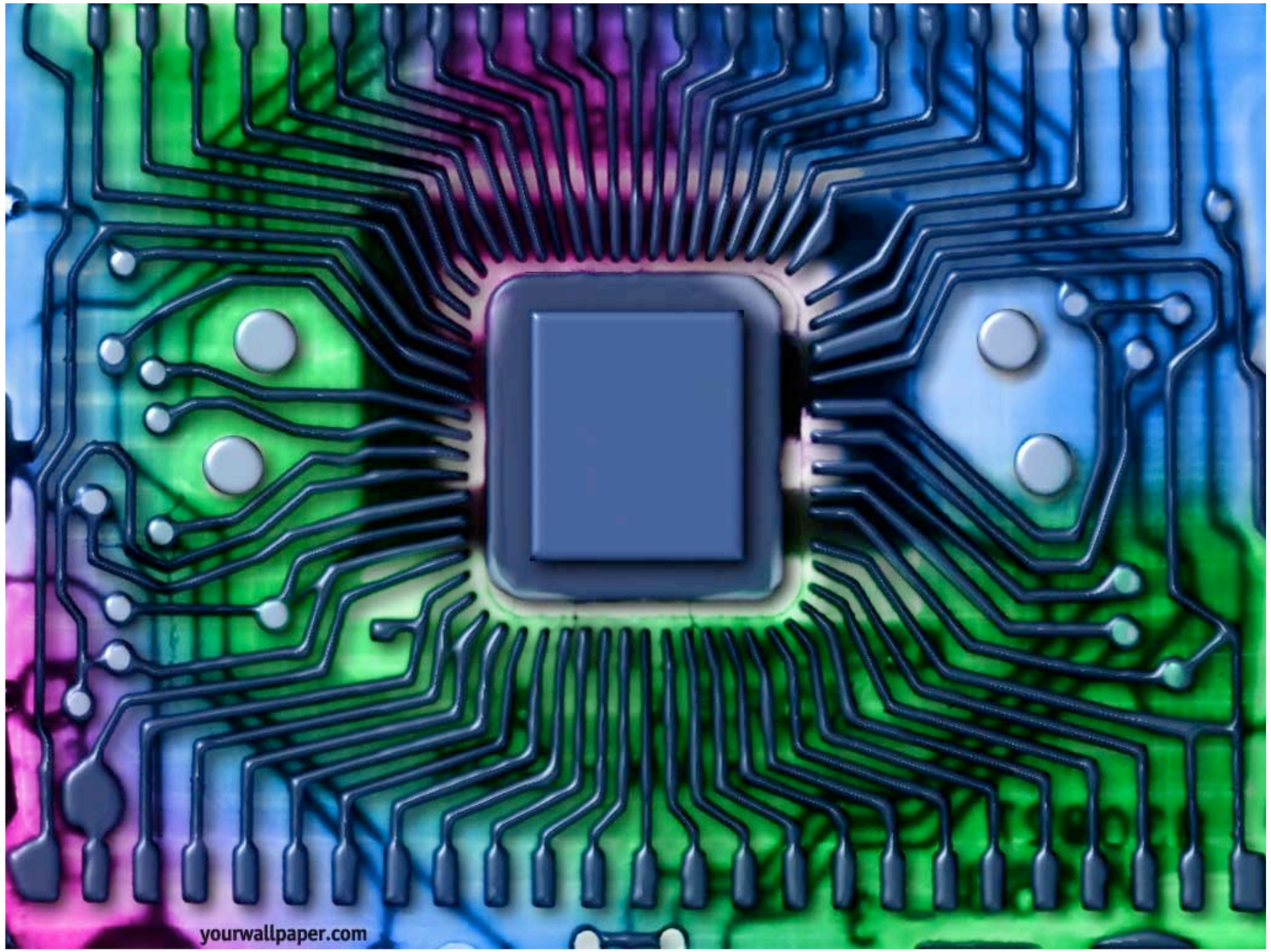
http://nccueagles.yuku.com/topic/6441#.T1o5-pjufqE



http://confessionsofahighbrow.com/tag/standardized-tests/

List jobs that pay you to memorize information that you don't use….

Who thinks they can remember more factoids than a computer?

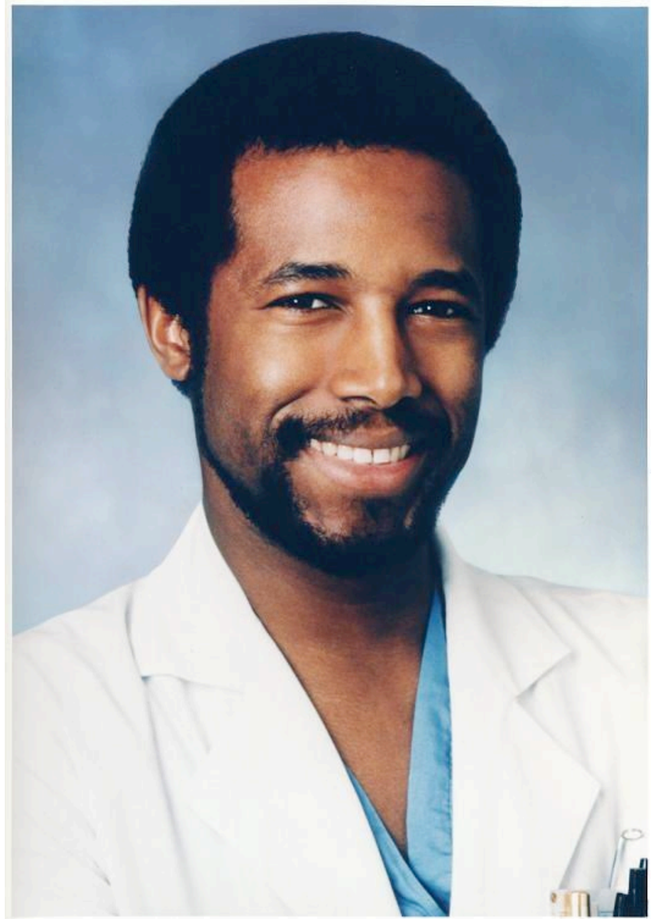


http://yourwallpaper.com/abstract/circuitboard01-1024.phtml

Why try to compete with computers by memorizing?

http://9to5mac.com/2011/12/08/apple-makes-its-2001-picks-in-the-app-store/

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



Dr. Ben Carson

Edgar Degas

Rihanna

JK Rowling

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

http://www.nytimes.com/2012/02/24/education/census-finds-bachelors-degrees-at-record-level.html

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

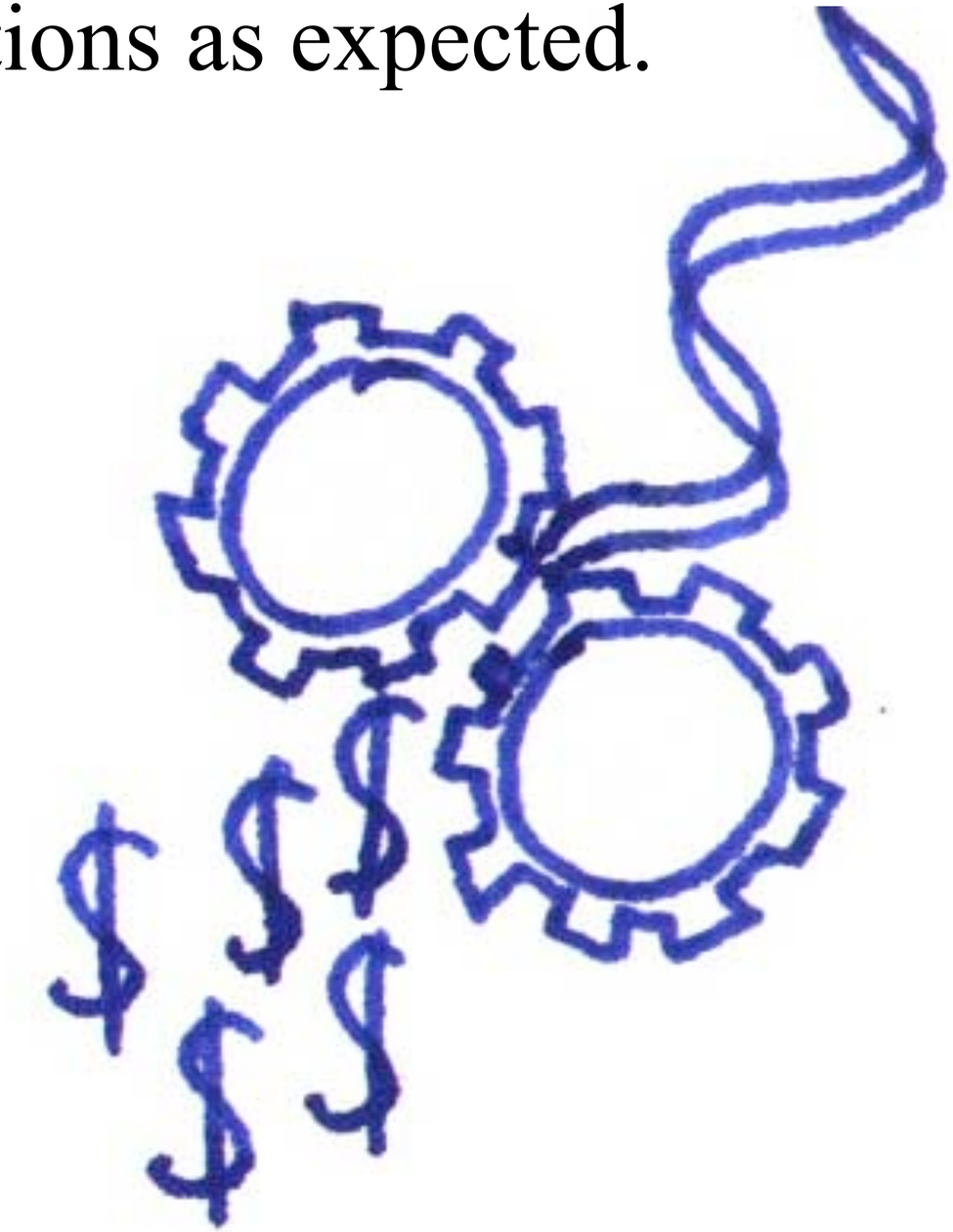
What is Synthetic Biology?

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

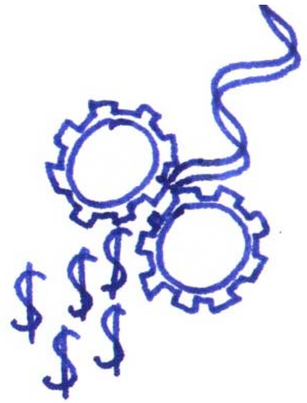
Synthetic Biology: Win-Win

Win #1: your design functions as expected.

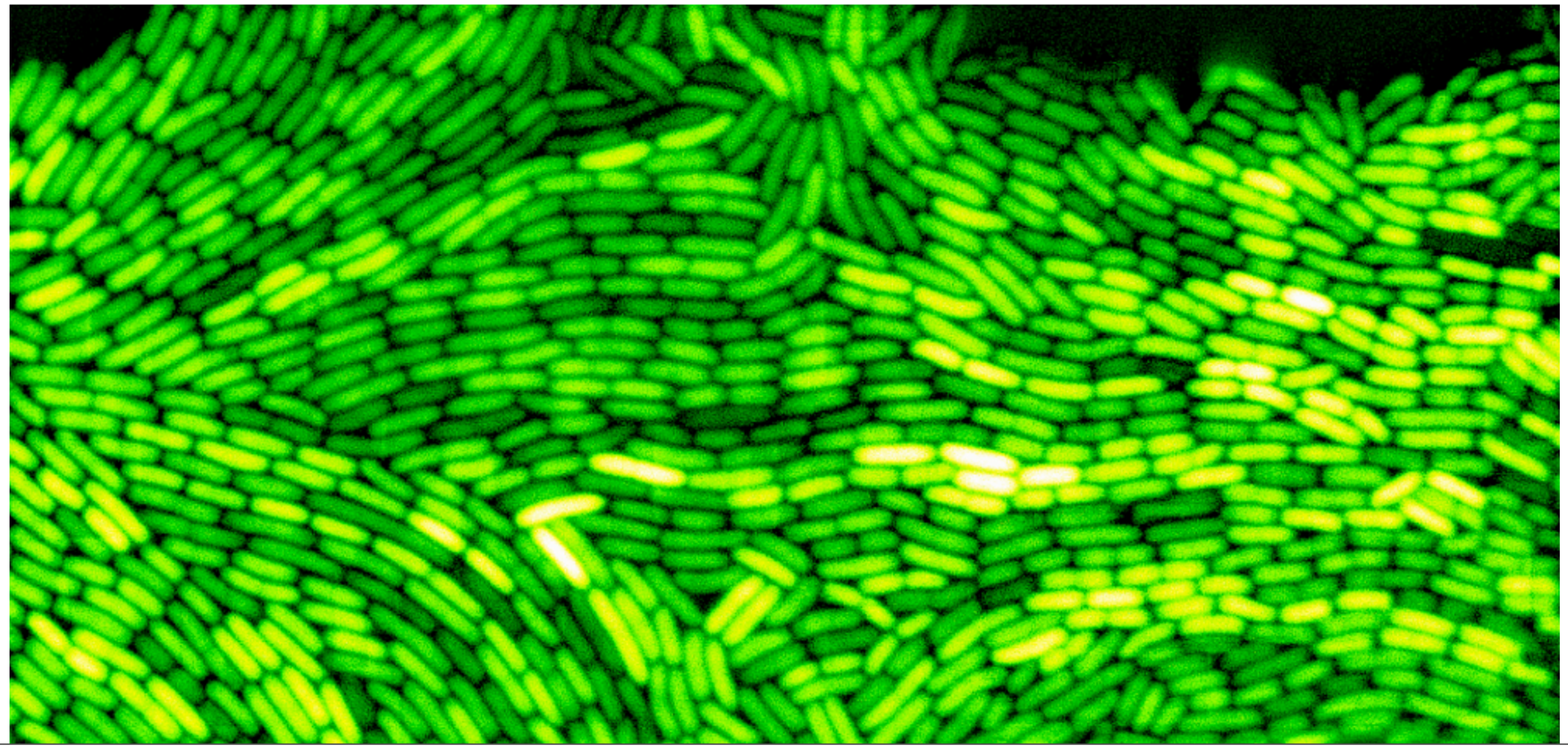


Synthetic Biology: Win-Win Research

Win #1: your design functions as expected.

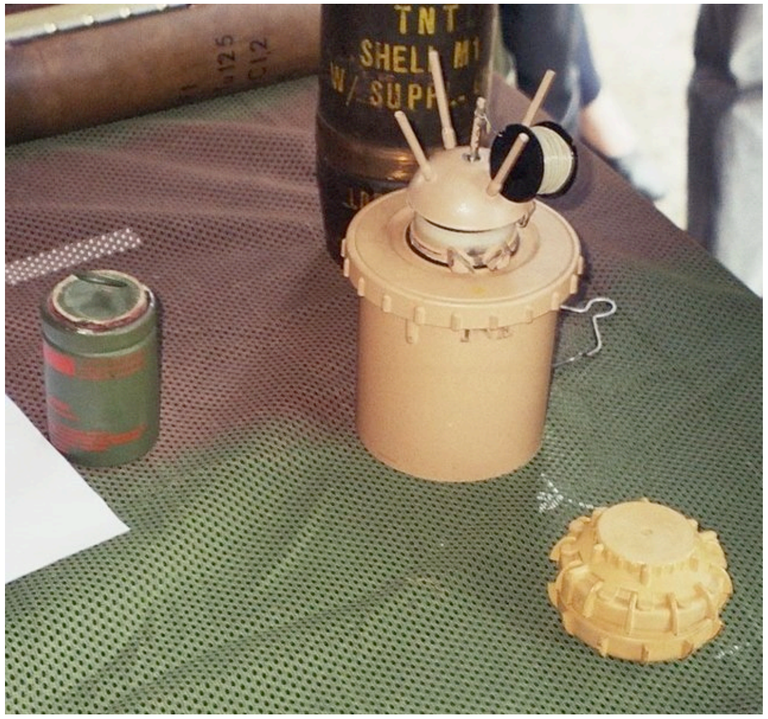


Win #2: your design fails but you uncover basic biology

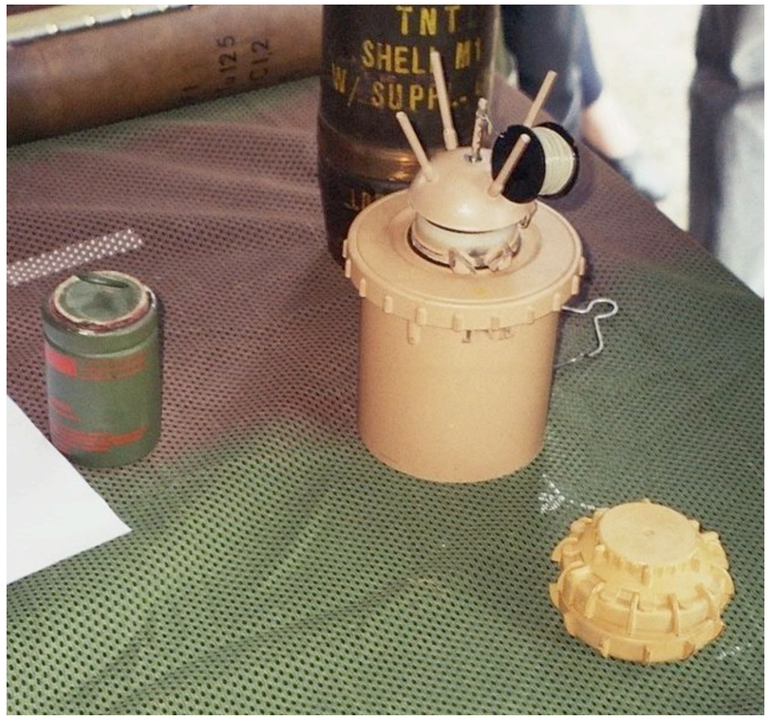


Real World Applications of Synthetic Biology

Land Mine Detection



Land Mine Detection



Synthetic Biology
Land Mine Detection

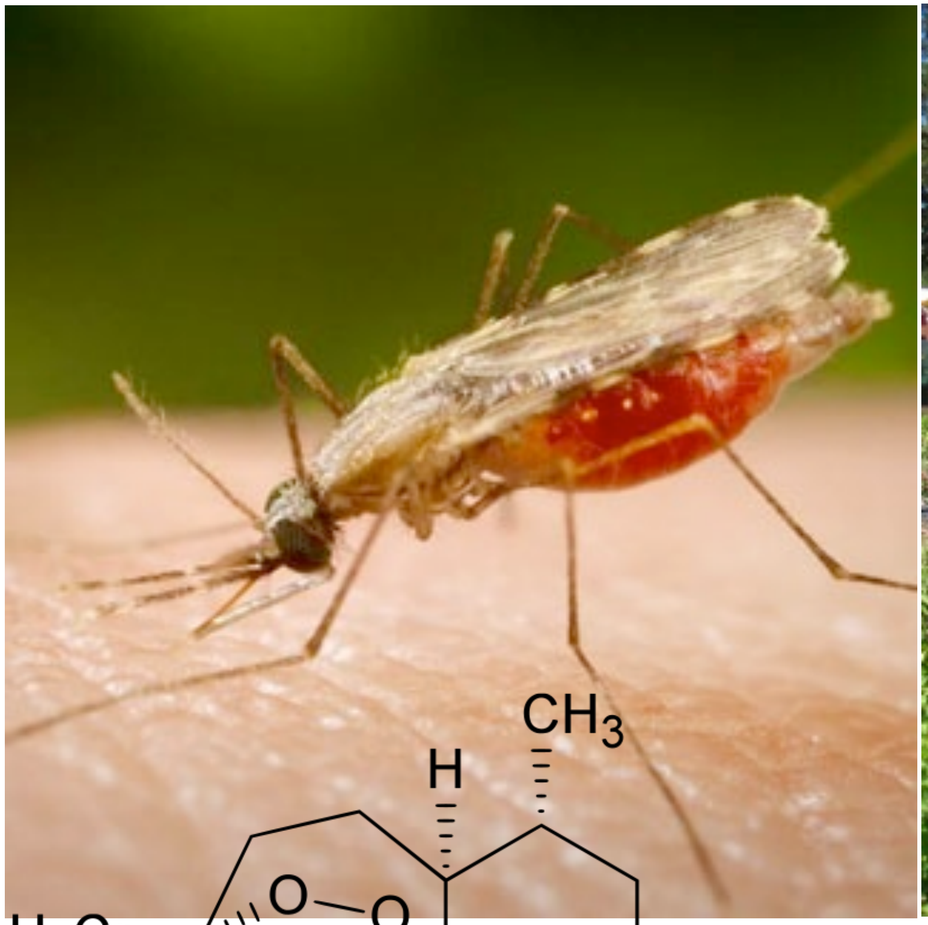
WARNING SIGN: The bioengineered Thales cress turns red when exposed to a mine byproduct.

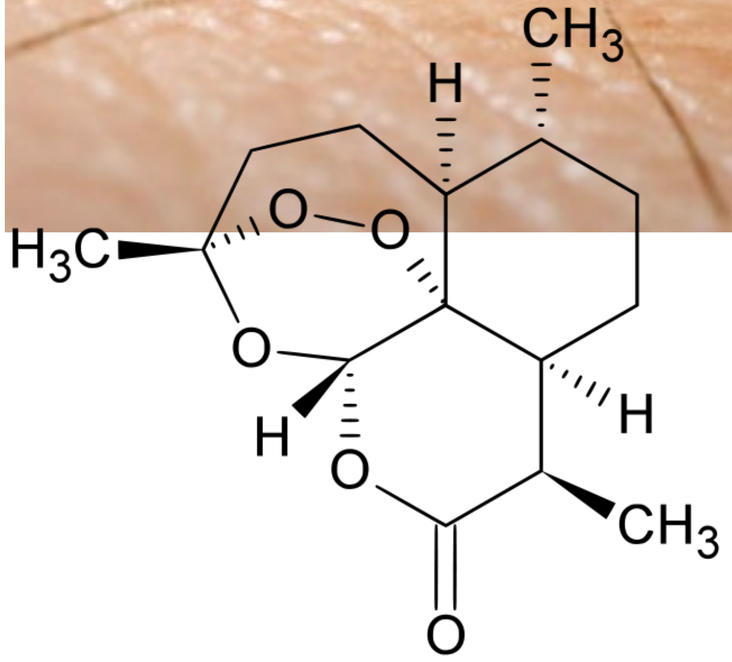
COURTESY OF ARESA BIODETECTION

New weed may flag land mines

By John K. Borchardt | *Contributor to The Christian Science Monitor*

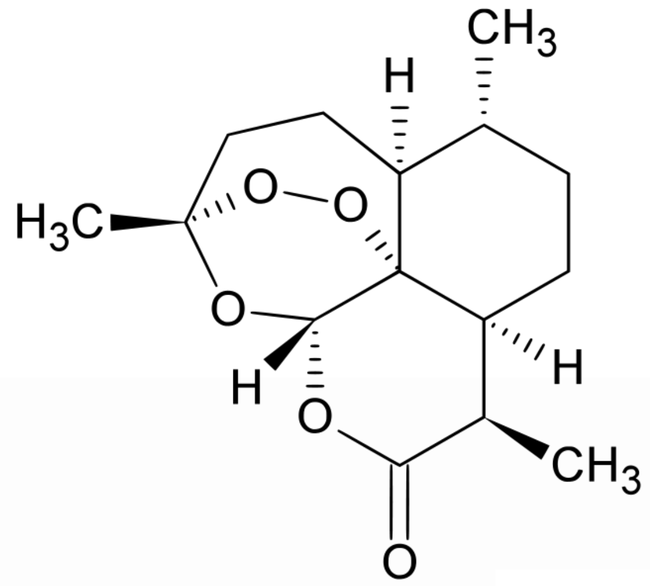
Production of Medicines



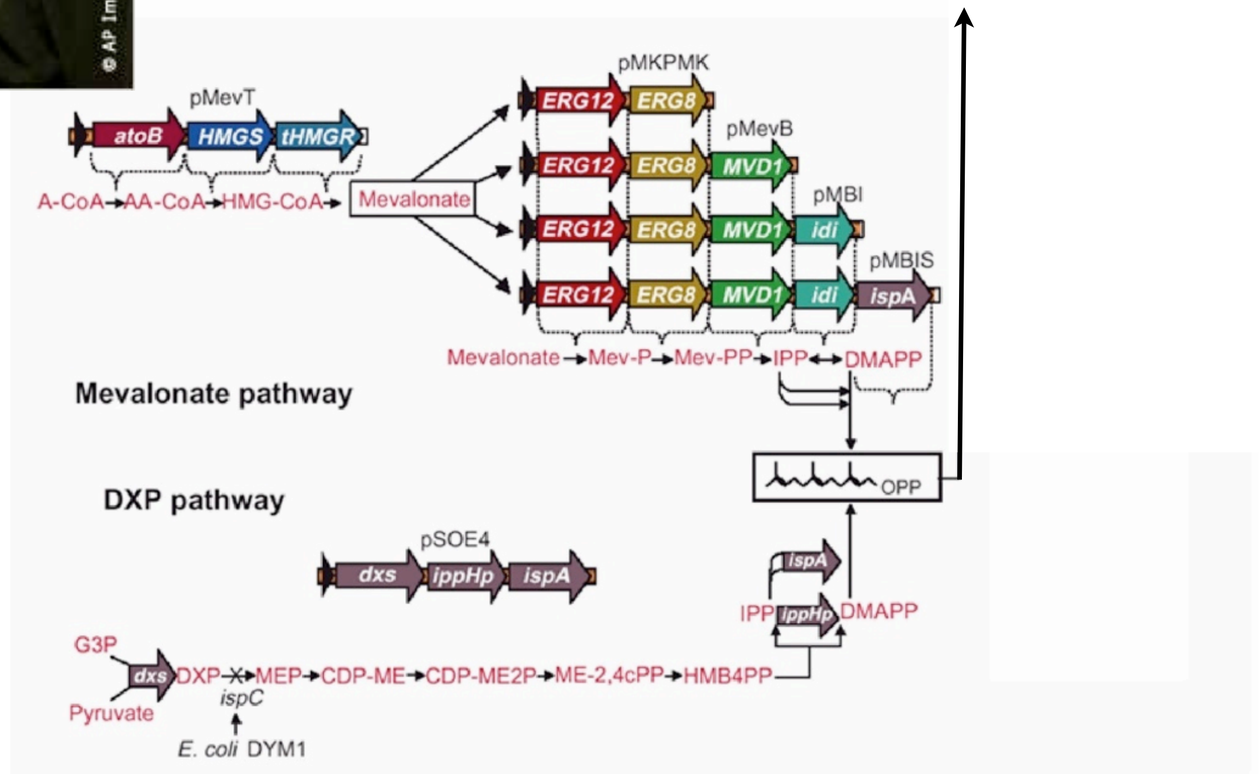


$1 per pill

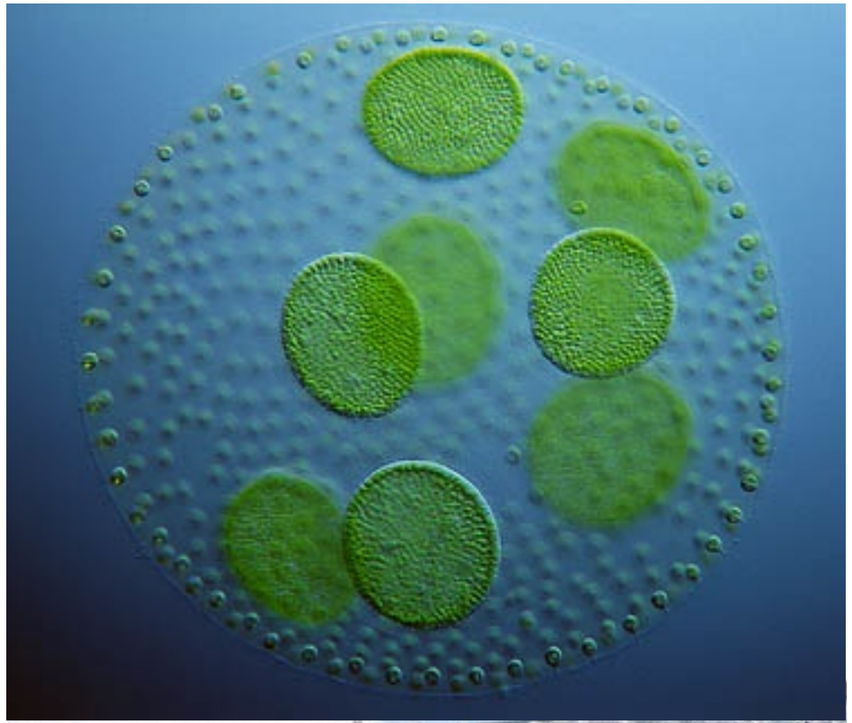
Production of Medicines



10¢ per pill



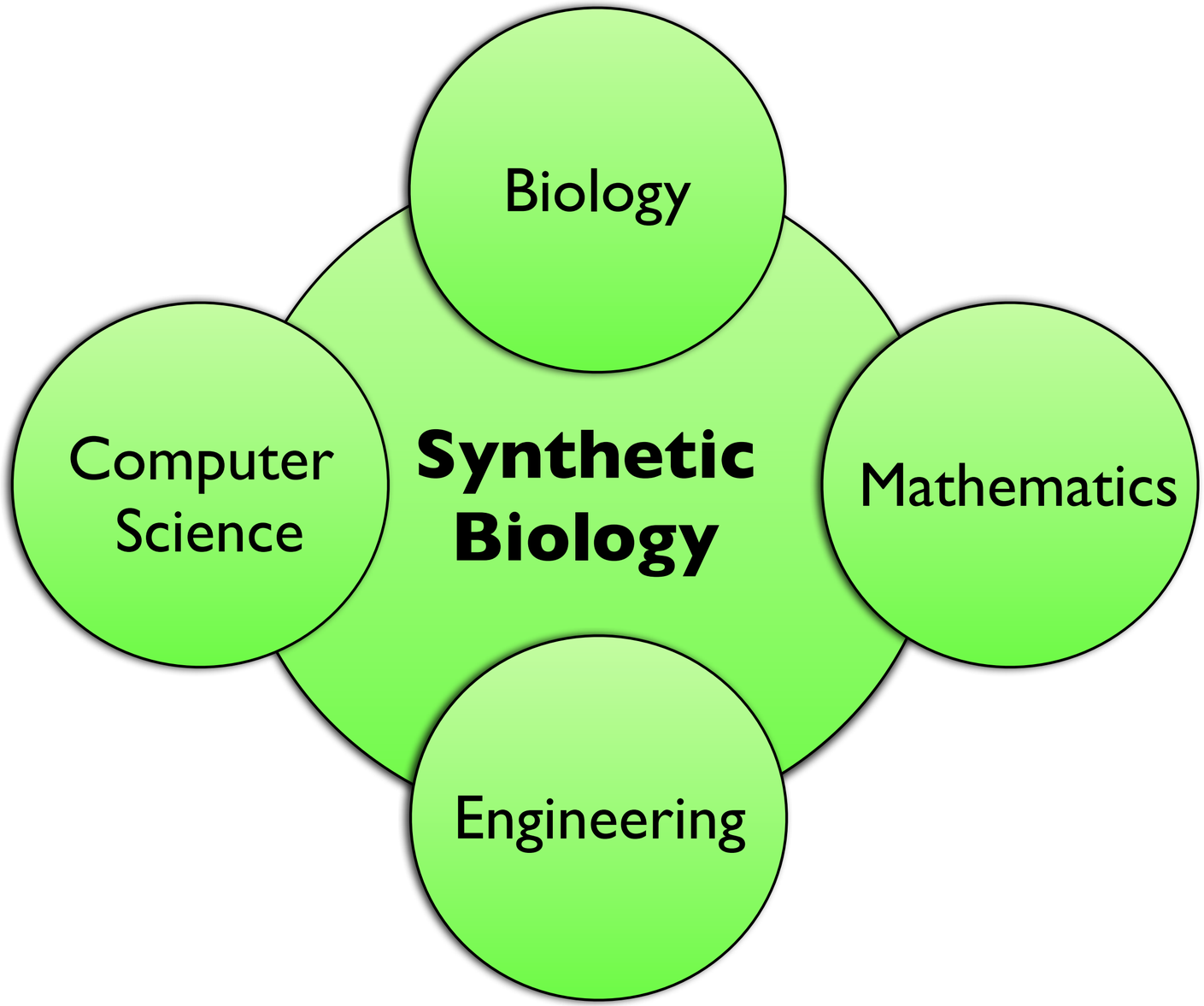
Biofuels from Algae



CO_2-neutral

1,000,000 gallons in 2008

Synthetic Biology



Promoter Research Using Synthetic Biology

Eco RI

GAATTC
CTTAAG

palindrome

type II

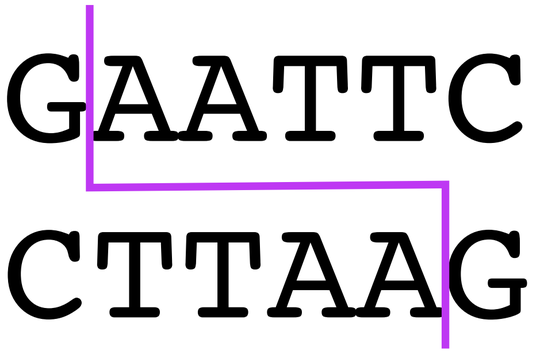
Eco RI

GAATTC
CTTAAG

palindrome

type II

Eco RI



type II

Eco RI

```
G          AATTC
CTTAA          G
```

type II

Bsa I

GAGACC

CTCTGG

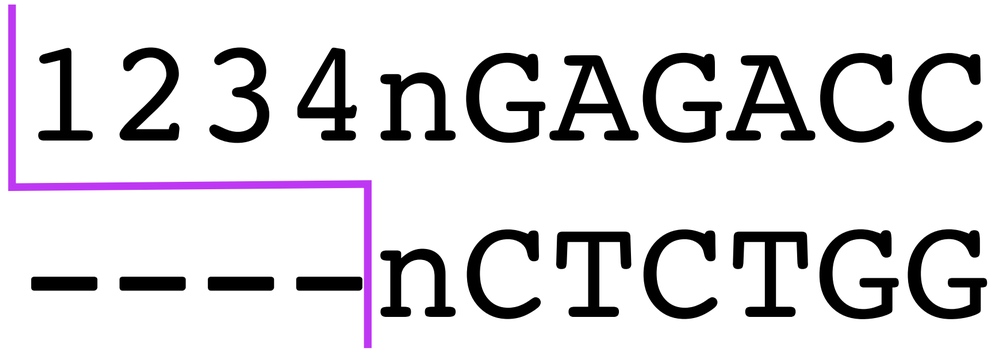
G

not a palindrome

palindrome

type IIs

Bsa I



type IIs

Bsa I

```
1234nGAGACC
    nCTCTGG
----
```

type IIs

Bsa I

```
GGTCTCn----
CCAGAGn1234
```

type IIs

Bsa I

```
GGTCTCn                ----
CCAGAGn1234
```

type IIs

Bsa I

cuts left

1234nGAGACC

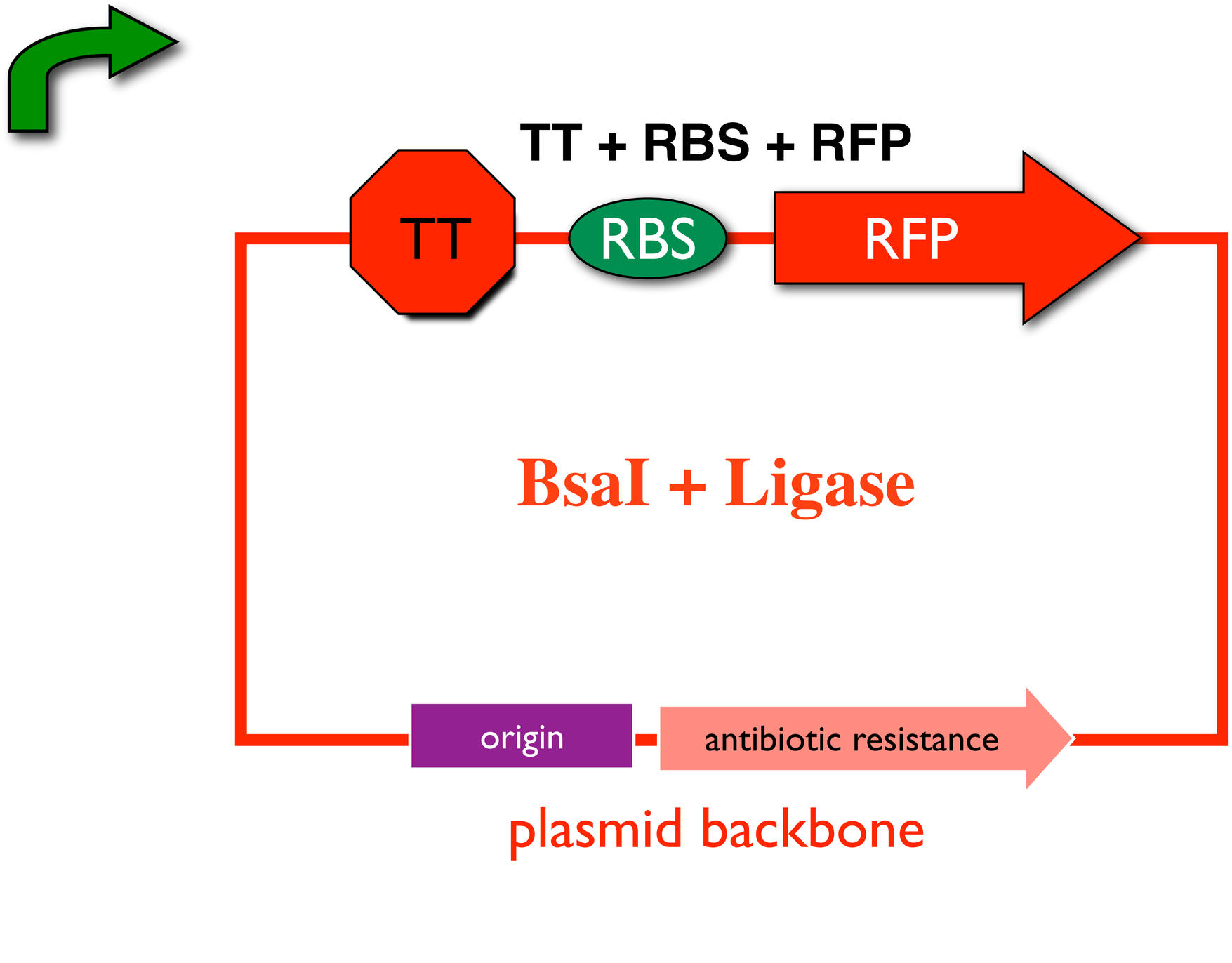
----nCTCTGG

GGTCTCn----

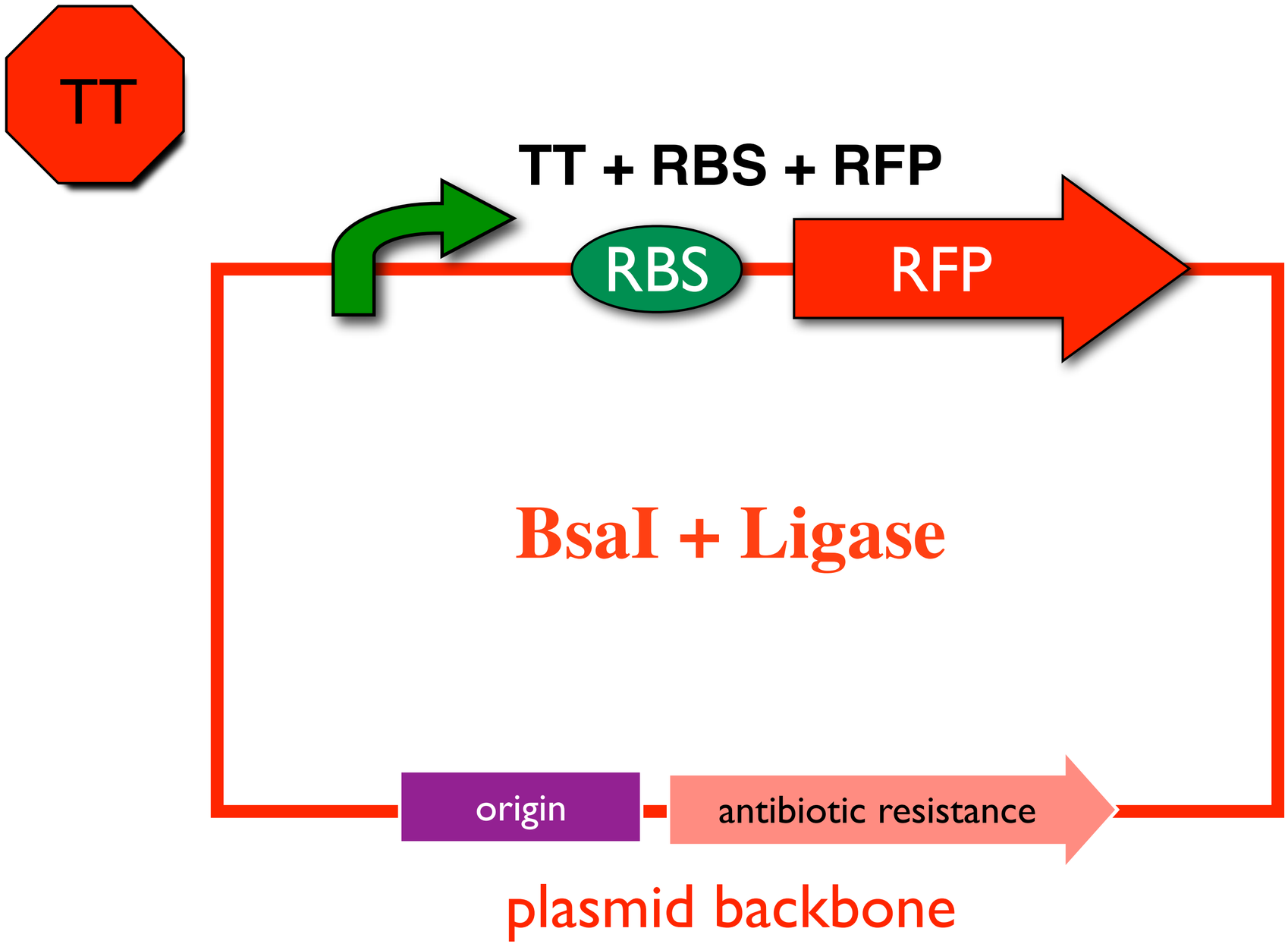
CCAGAGn1234

cuts right

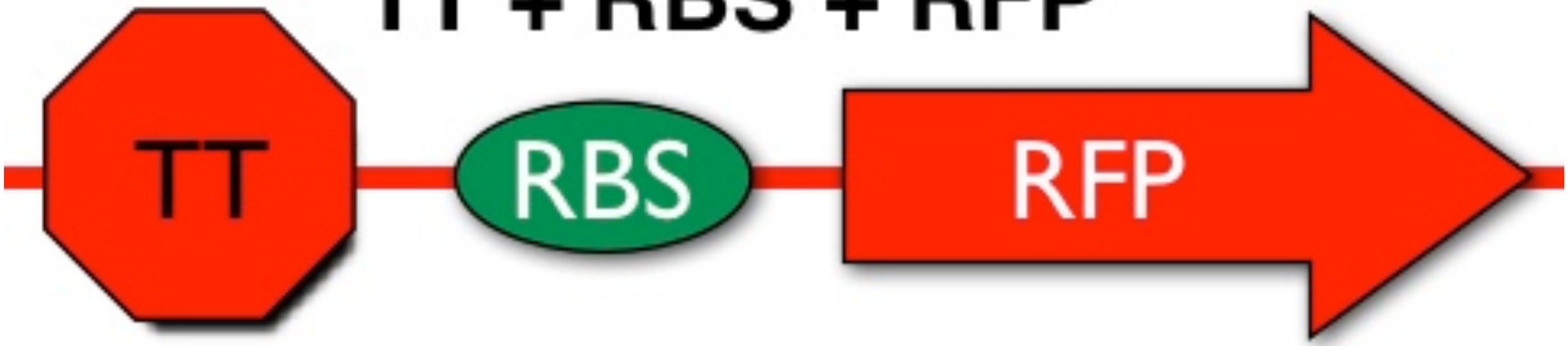
GGA Ligation Method



GGA Ligation Method



TT + RBS + RFP



Bsa I

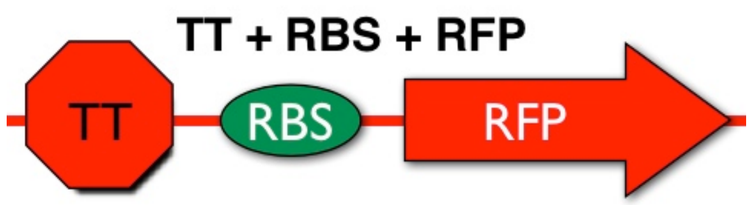
CGACtGAGACC(TT)GGTCTCaGCGG

GCTGaCTCTGG(TT)CCAGAGtCGCC

ligase

Bsa I

ligase



CGACtGAGACC(TT)GGTCTCa

aCTCTGG(TT)CCAGAGtCGCC

[redacted]

[redacted]GCTG

[redacted]GCGG[redacted]

ligase

ligase

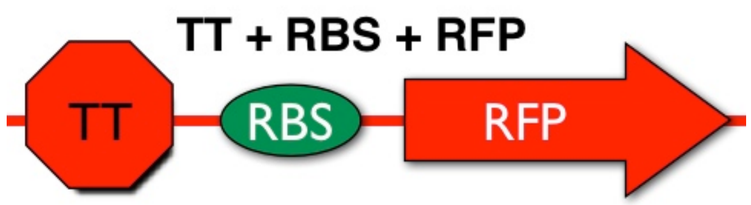
Bsa I

CGACtGAGACC(TT)GGTCTCaGCGG

GCTGaCTCTGG(TT)CCAGAGtCGCC

ligase

Bsa I ligase



CGACtGAGACC(TT)GGTCTCa

aCTCTGG(TT)CCAGAGtCGCC

[redacted]GCGG[redacted]

[redacted]GCTG

[redacted]

ligase

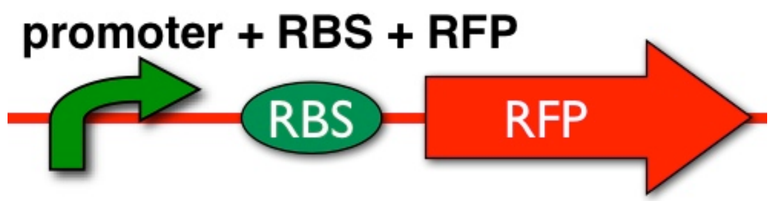
ligase

CGAC(promoter)

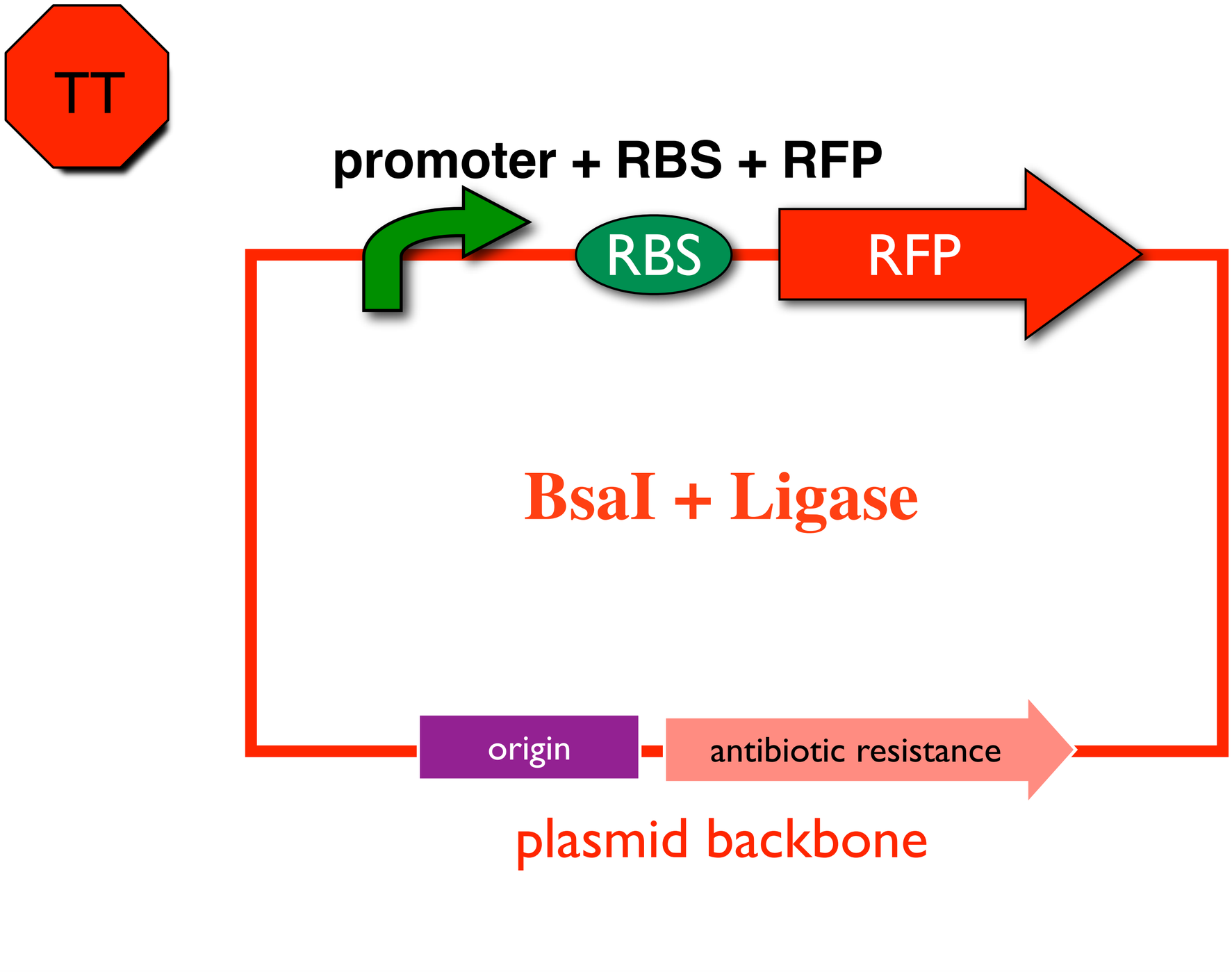
(promoter)CGCC

CGACtGAGACC(TT)GGTCTCa

aCTCTGG(TT)CCAGAGtCGCC



GGA Ligation Method



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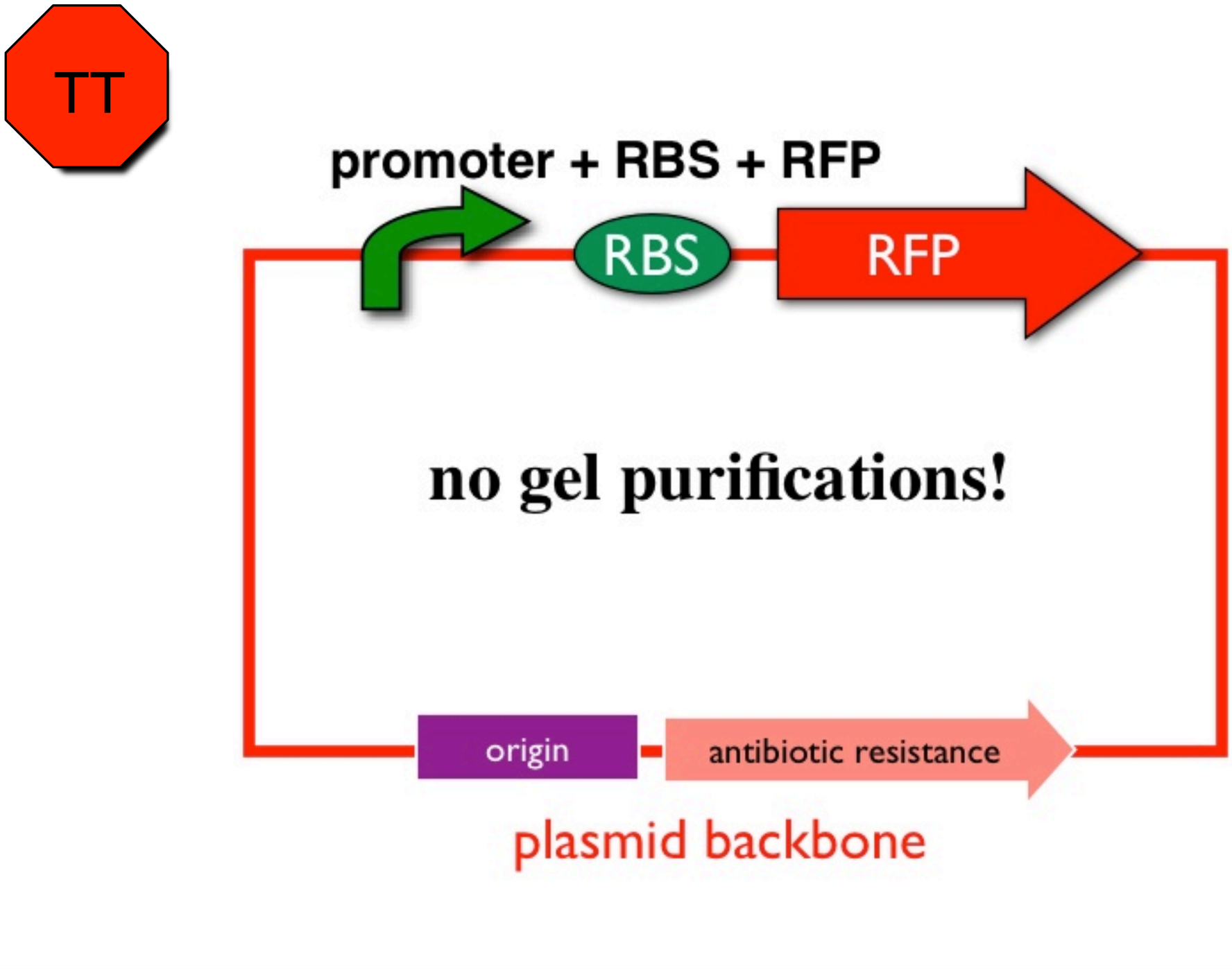
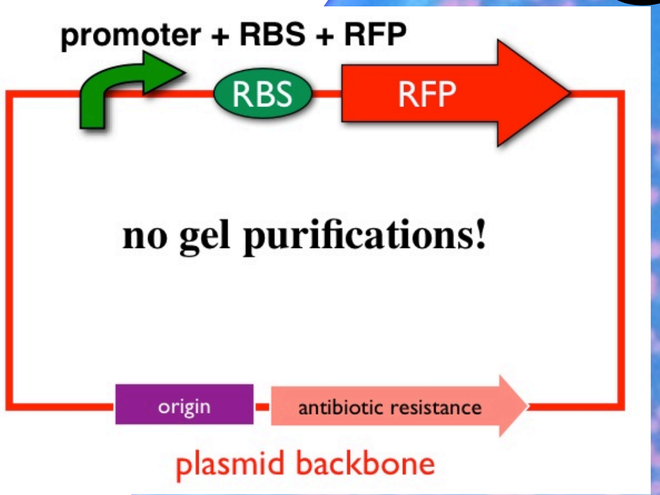
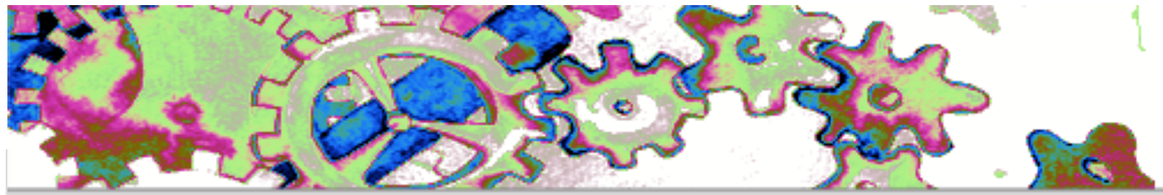


GGA Ligation Method



Student Sample, September 2012



Registry of Standard Biological Parts

		BBa_J100067	Regulatory	fadB promoter (long sequence)	Meredith Nakano	85
		BBa_J100068	Regulatory	fadB promoter (short sequence)	Meredith Nakano	61
		BBa_J100069	Reporter	Superfolder GFP	Rebecca Evans	770
		BBa_J100070	Coding	Superfolder GFP	Rebecca Evans	720
		BBa_J100071	Regulatory	cadA promoter	Ben Clarkson	334
		BBa_J100072	Regulatory	LcpxP promoter--Long cpxP promoter	Ben Clarkson	392
		BBa_J100073	Regulatory	ScpxP--Short cpxP promoter	Ben Clarkson	94
		BBa_J100074	Regulatory	Long pLux Promoter	Betsy Gammon	197
		BBa_J100075	Regulatory	CydAP1 Long Promoter	Betsy Gammon	158
		BBa_J100076	Regulatory	CydAP1 Short Promoter	Betsy Gammon	151
		BBa_J100077	Composite	J10068:K0903005	Meredith Nakano	793
		BBa_J100078	Composite	J100067:K0903005	Meredith Nakano	817
		BBa_J100079	Device	Riboswitch and GFP	Rebecca Evans	879
		BBa_J100080	Device	Riboswitch and GFP	Rebecca Evans	882
		BBa_J100081	Reporter	J100071+E0240	Ben Clarkson	334
		BBa_J100082	Reporter	J100072+E0240	Ben Clarkson	1276
		BBa_J100083	Composite	LuxI Long + RBS + GFP	Betsy Gammon	1081
		BBa_J100084	Composite	CydAP Long + RBS + GFP	Betsy Gammon	1042
		BBa_J100085	RNA	short CRISPR sequence with GFP target spacer	Caroline Vrana	240
		BBa_J100086	Composite	CydAP Short Promoter + RBS + GFP	Betsy Gammon	1035
		BBa_J100087	Reporter	J100073+E0240	Ben Clarkson	978
		BBa_J100088	Generator	J100071+J10063	Ben Clarkson	2965
		BBa_J100089	Generator	J100072+J10063 (LcpxP+LRE, Luciferase)	Ben Clarkson	3023
		BBa_J100090	Regulatory	CRISPR sequence with GFP and AmpR targets	Caroline Vrana	412
	W	BBa_J100092	Regulatory	Constitutive promoter for M1-162	Natalie Spach	50
	?	BBa_J100093	Regulatory	rrnB P1 promoter	Kayla McAvoy	60
	?	BBa_J100094	Regulatory	Lac promoter E. Coli	Cameron Bard	44
	?	BBa_J100095	Regulatory	malE1 Maltose induced promoter.	Pooja Potharaju	65
		BBa_J100096	Regulatory	PBAD Promoter from araE Gene	Elizabeth Brunner	27
	W	BBa_J100097	Regulatory	Anhydrotetracycline inducible promoter with BsaI sticky ends	Sarah Kim	55
		BBa_J100098	DNA	Promoter for the argF gene	Erin Nieusma	44
	W	BBa_J100099	Regulatory	A promoter (CydAB) activated by the FNR enzyme	Phoebe Parrish	64

Student Sample, September 2012

Registry of Standard Biological Parts

Go Search

page discussion view source history Log in / create account

BBa J100099 Main Page Part Design Physical DNA Hard Information Experience Tools

Part:BBa_J100099

Regulatory

DNA Planning
Experience: Works
Get This Part

Designed by Phoebe Parrish Group: Campbell_M_Lab (2012-09-13)

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

Format: Subparts | Ruler | **SS** | DS **Search:** **Length: 64 bp** **Context: Part only** Get selected sequence

```
     1          11         21         31         41         51         61         71         81         91
1    ggaattgata tttatcaatg tataagtctt ggaaatgggc atcaaaaaga gataaattgt tctc
```

FNR binding -35 -10

Assembly Compatibility: 10 12 21 23 25

Jeffrey Green. 1993. "Activation of FNR-dependent transcription by iron: An in vitro switch for FNR." FEMS Microbiology Letters 113 (1993) 219-222

[edit]

Student Sample, September 2012

Part:BBa_J100099:Experience

Designed by Phoebe Parrish Group: Campbell_M_Lab (2012-09-13)

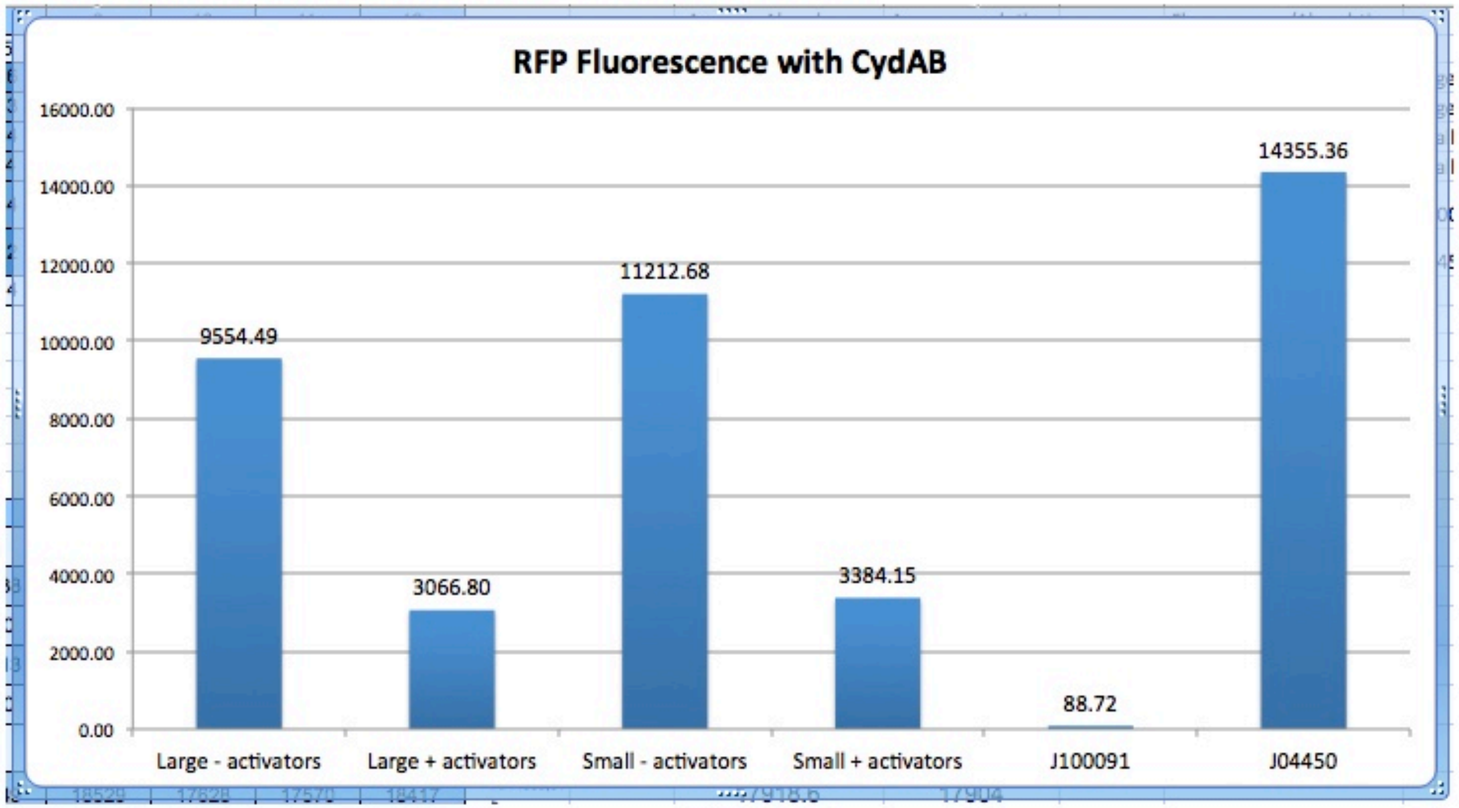
Regulatory

DNA Planning
Experience: Works
Get This Part

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 florescence (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.



Registry of Functional Promoters (RFP)

Registry of Functional Promoters (V1.0)

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.

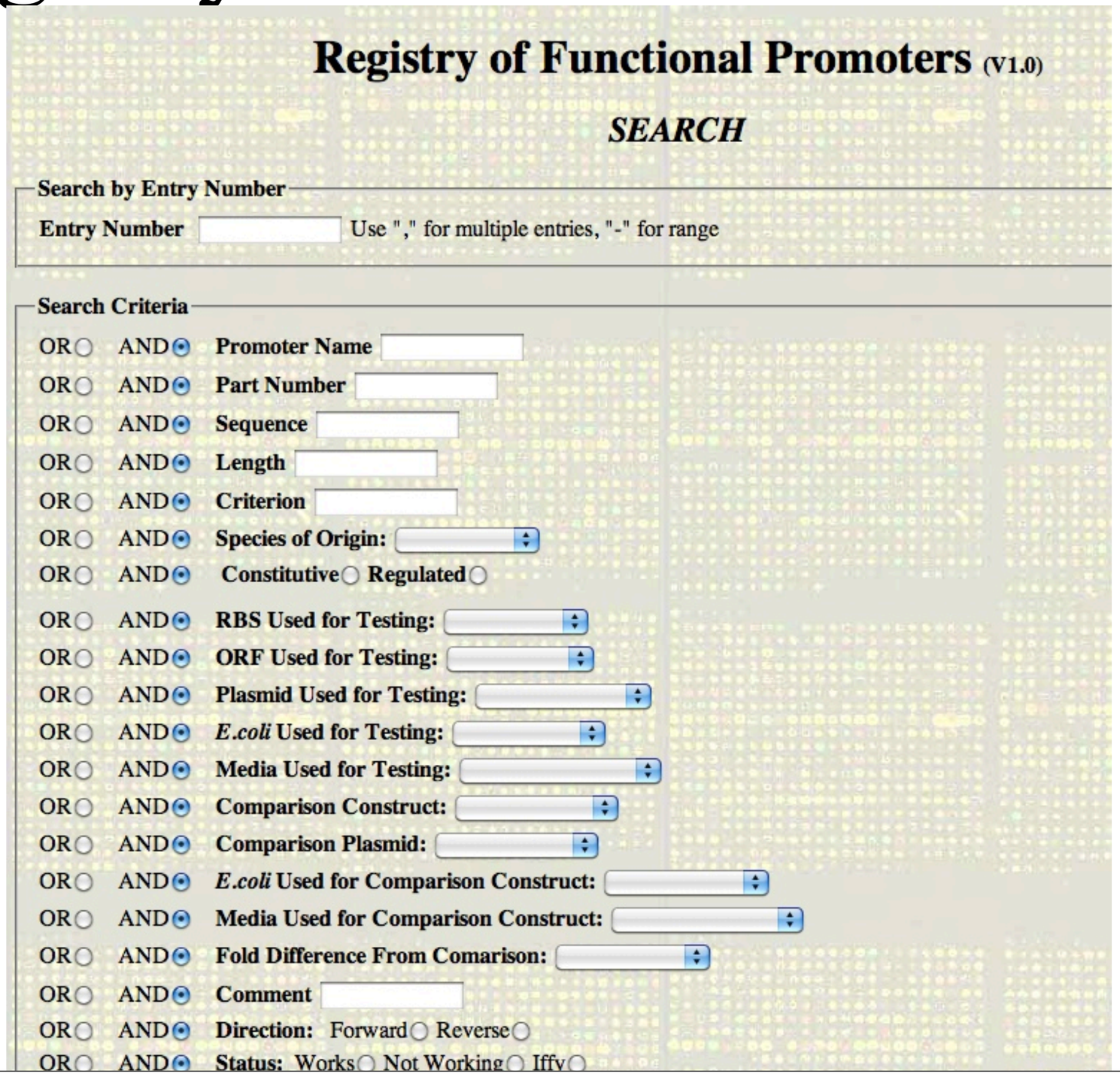
If your are already a Registered User of GCAT-alog , you do not need to Reregister

LOGIN **REGISTER AS NEW USER**

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

gcat.davidson.edu/RFP/

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

gcat.davidson.edu/RFP/

Registry of Functional Promoters (RFP)

Registry of Functional Promoters (V1.0)

SEARCH PROMOTER RESULTS

Entry No.	Promoter Name	Part Number	Sequence	Length	Citation	Species of Interest	Constitutive/ Regulated	Inducible/ Repressible	Regulator	RBS Used for Testing	ORF Used for Testing	Pla Use Te
1	TetR Repressible Promoter	R0040	tccctatcagtgatagagattgacatccctatcagtgatagagatactgagcac	54			Regulated	Repressible	TetR			pSI
2	56 bp LacI Promoter	K091110	cgttgacaccatcgaatggcgcaaaacctttcgcggtatggcatgatagcgcccgg	56			Constitutive					
3	200 bp LacI Promoter	R0010	caatacgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcac gacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaatgtgagtt agctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgt ggaattgtgagcggataacaatttcacaca	200			Constitutive					
4	LuxR & HSL Regulated Lux promoter	R0062	acctgtaggatcgtacaggtttacgcaagaaaatggtttgttatagtcgaataaa	55			Regulated	Repressible				
5	Backwards 200 LacI Promoter (right to left)	J31013	tgtgtgaaattgttatccgctcacaattccacacaacatacgagccggaagcataaa gtgtaaagcctggggtgcctaatgagtgagctaactcacattaattgcgttgcgctc actgcccgctttccagtcgggaaacctgtcgtgccagctgcattaatgaatcggcca acgcgcggggagaggcggtttgcgtattg	200			Regulated	Repressible				
6	OmpC Promoter	K199017	tttacattttgaaacatctatagcgataaatgaaacatcttaaaagttttagtatcatattc gtgttggattattctgcatttttggggagaatggact	99			Constitutive					
7	23K series very strong constitutive Promoter	J23100	ttgacggctagctcagtcctaggtacagtgctagc	35			Constitutive					

To Edit an Entry, Enter the Entry # and press "Edit Entry" [ ] Edit Entry

To Delete an Entry, Enter the Entry # and press "Delete Entry" [ ] Delete Entry

Search Again

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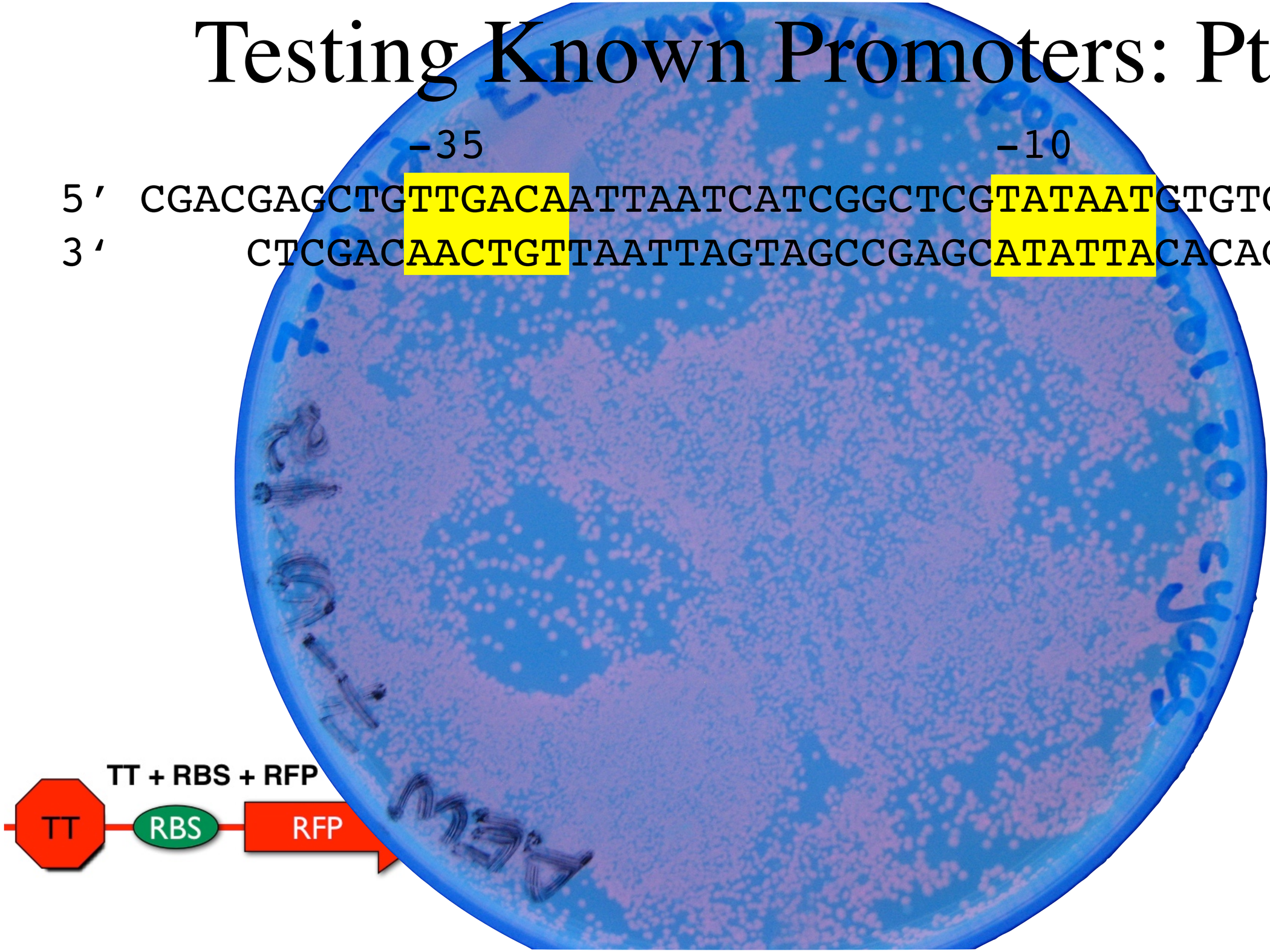
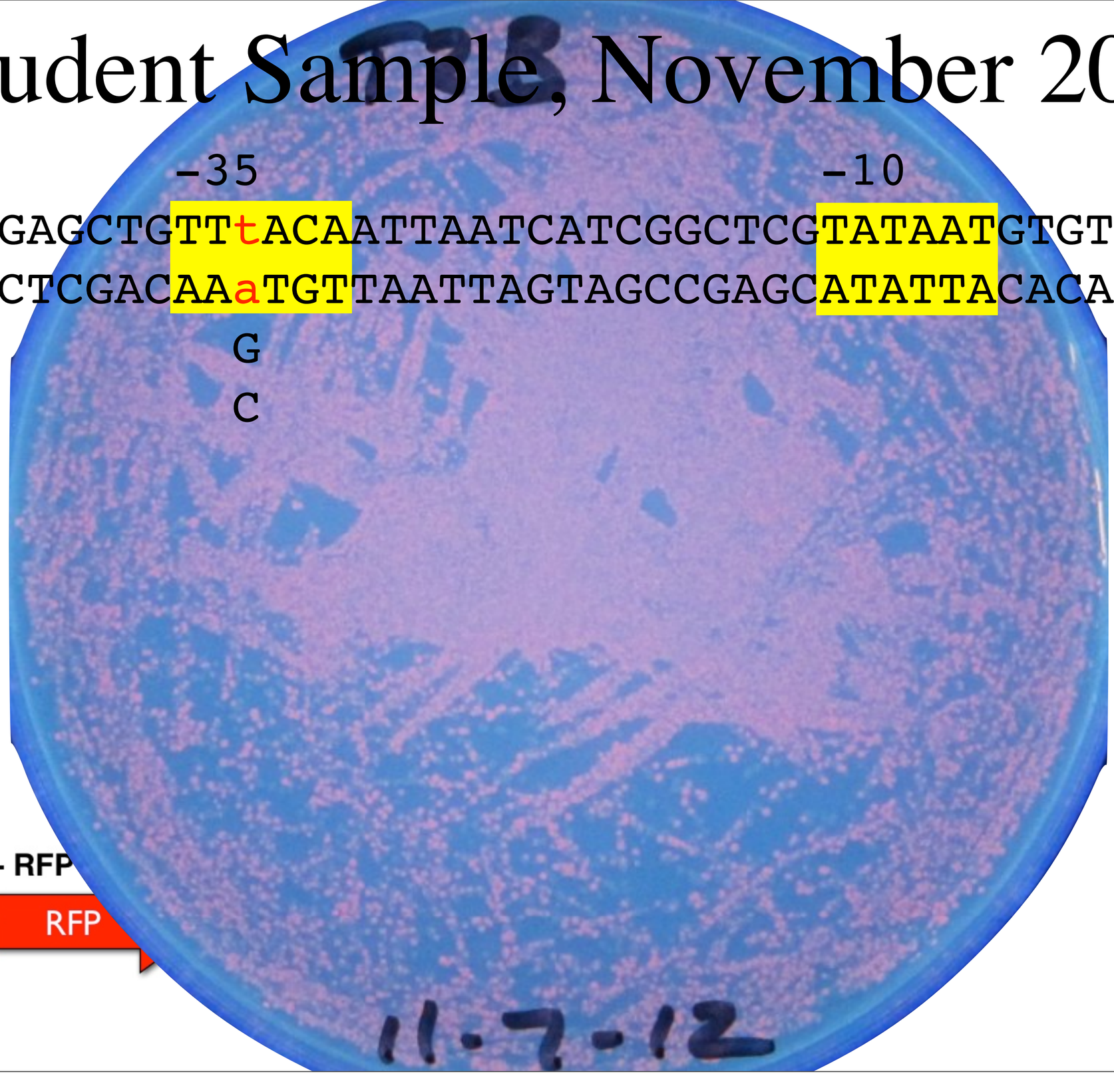
Testing Known Promoters: Ptac

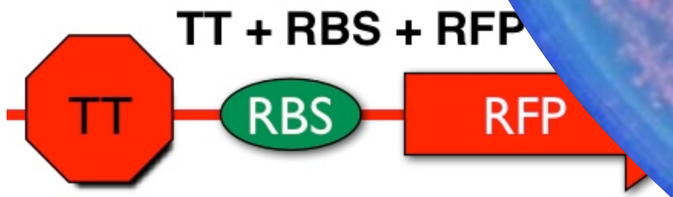
```
          -35                      -10
5′ CGACGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGA      3′
3′      CTCGACAACTGTTAATTAGTAGCCGAGCATATTACACACCTCGCC 5′
```



Student Sample, November 2012

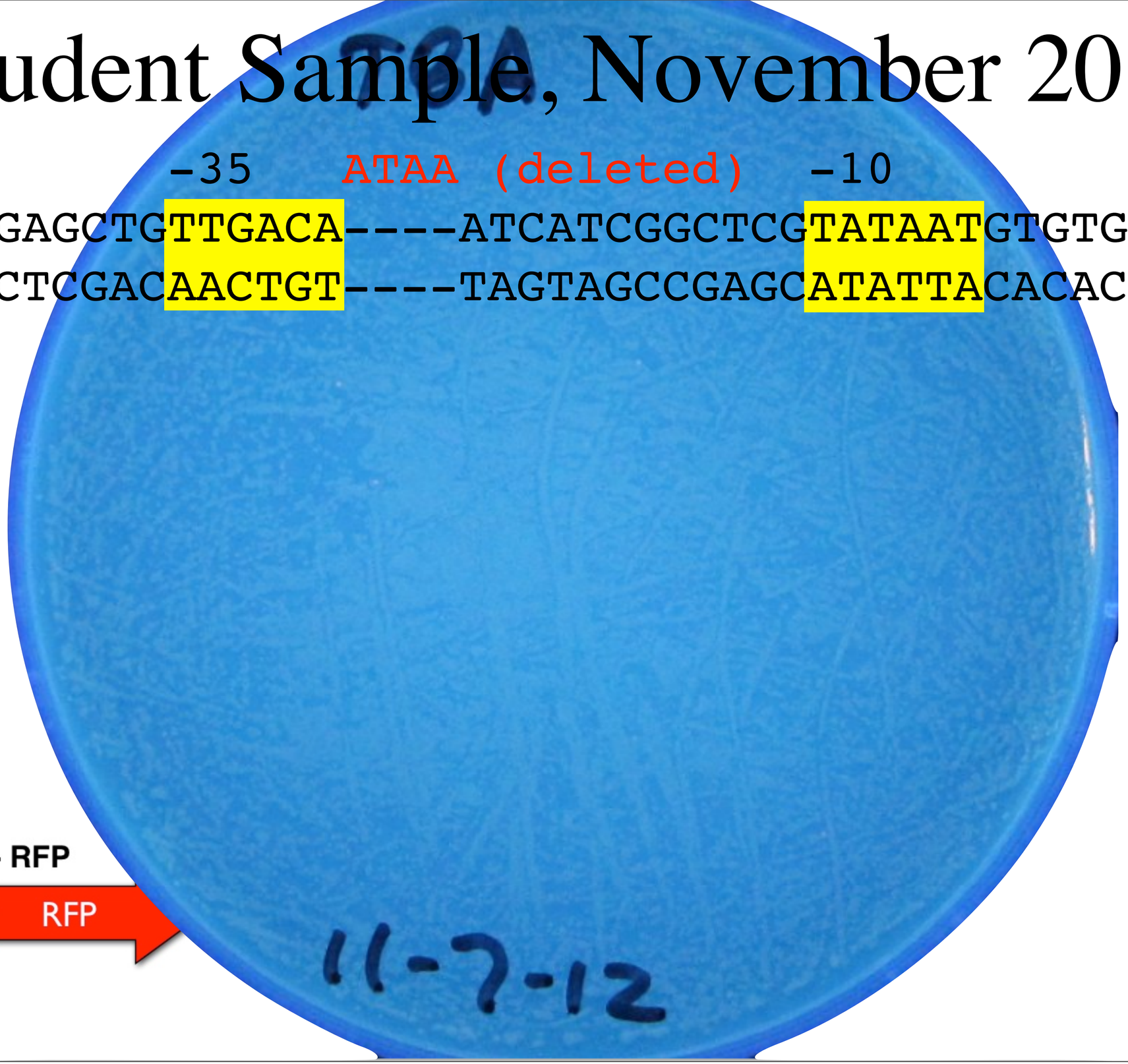
```
                -35                          -10
5′ CGACGAGCTGTTtACAATTAATCATCGGCTCGTATAATGTGTGGA      3′
3′      CTCGACAAaTGTTAATTAGTAGCCGAGCATATTACACACCTCGCC 5′
             G
             C
```

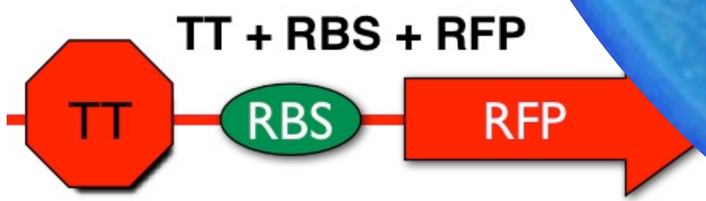




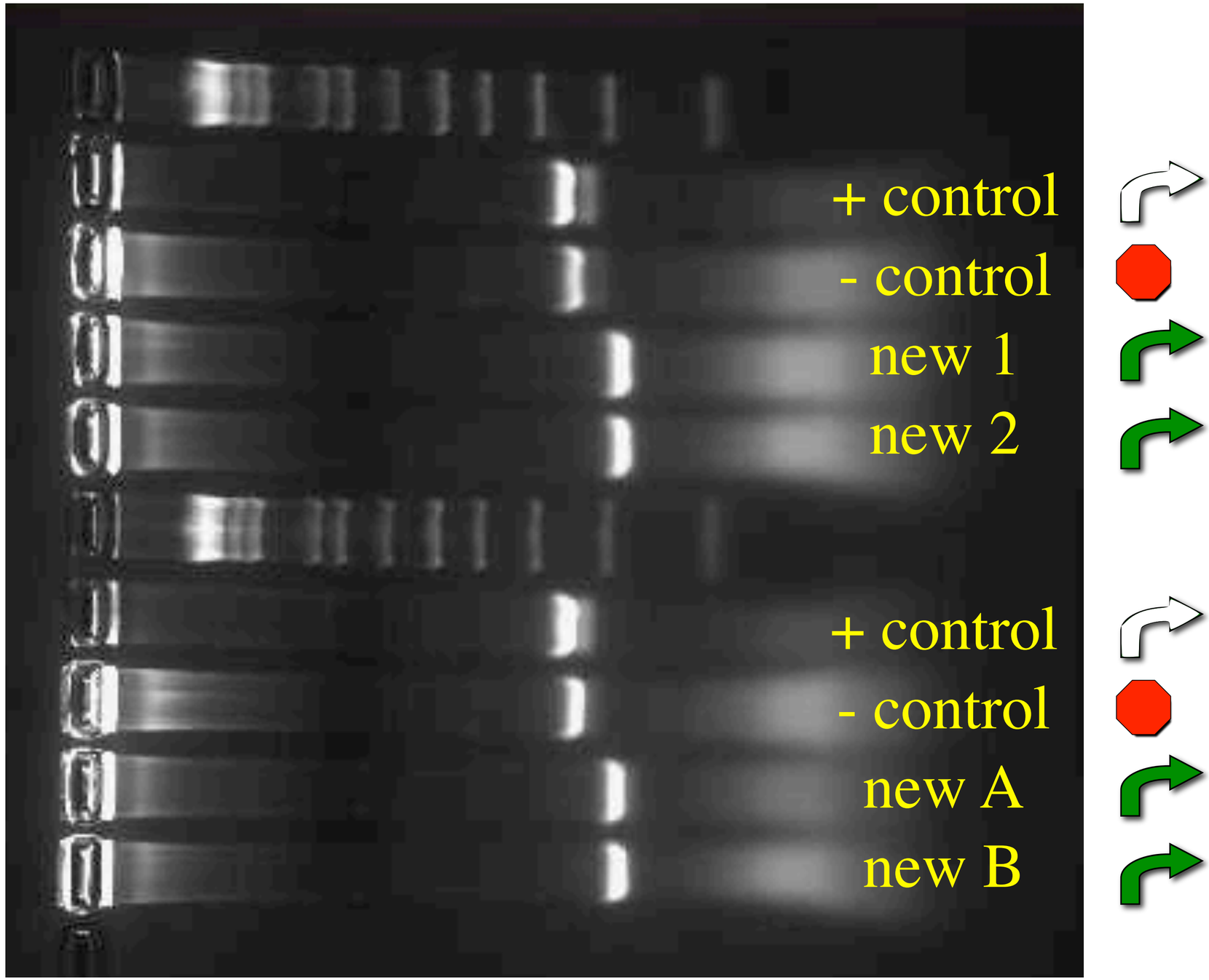
Student Sample, November 2012

```
                -35    ATAA (deleted)   -10
5′ CGACGAGCTGTTGACA----ATCATCGGCTCGTATAATGTGTGGA      3′
3′      CTCGACAACTGT----TAGTAGCCGAGCATATTACACACCTCGCC 5′
```



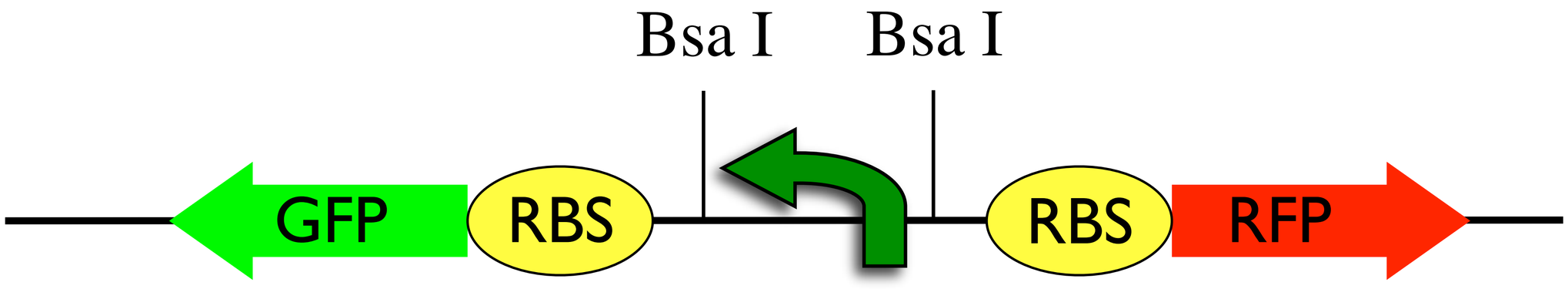


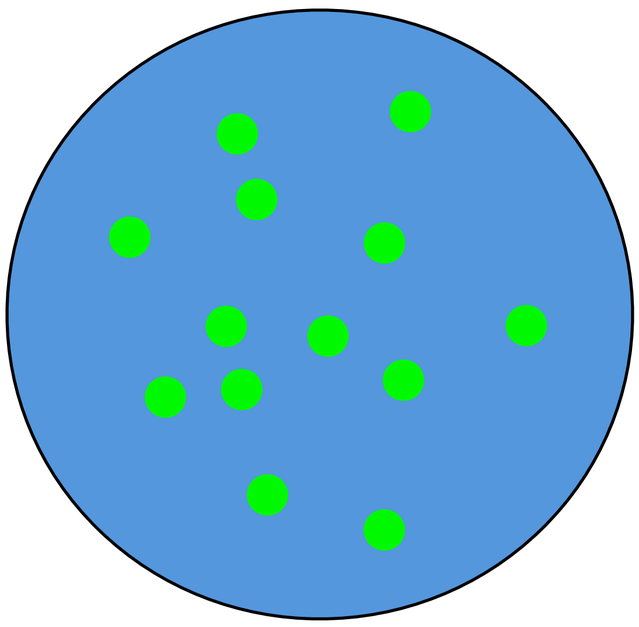
Student Sample, September 2012



pClone Red

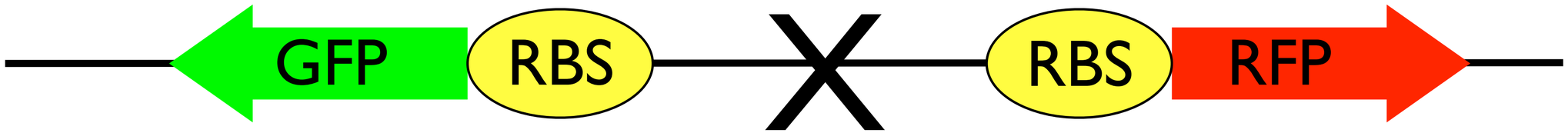
J119137

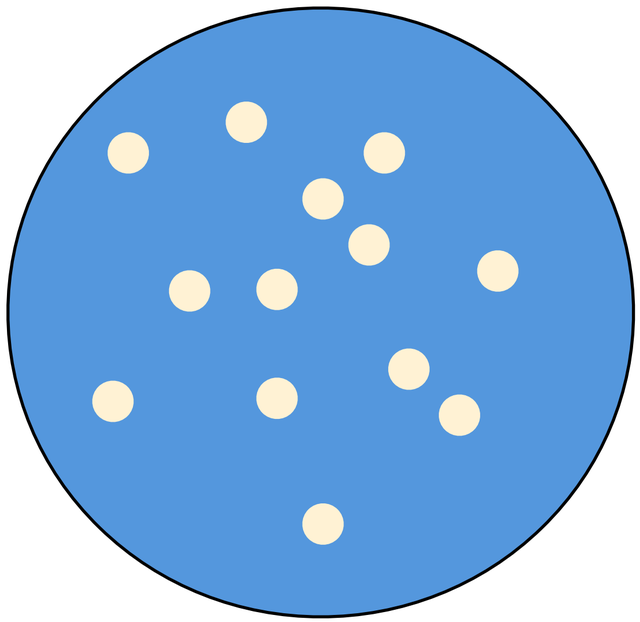




Remove Initial Promoter

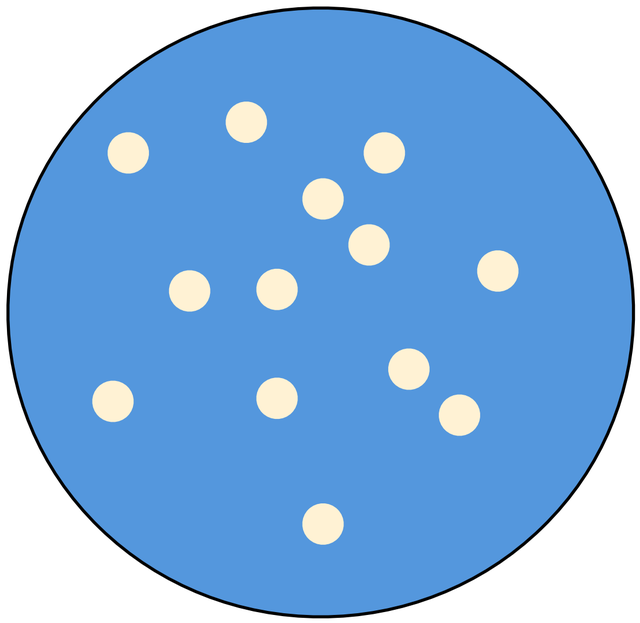
J119137





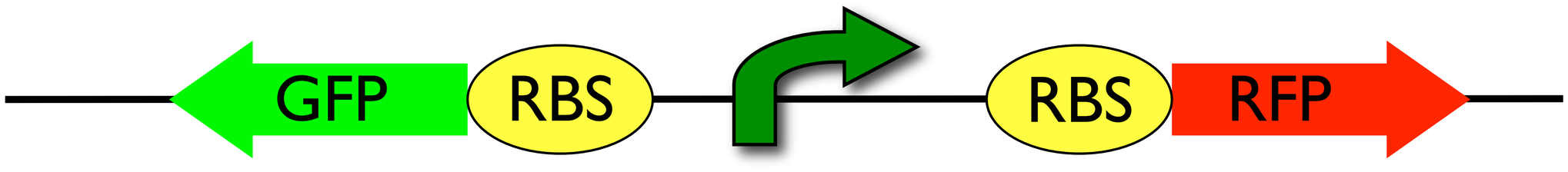
Insert Non-functional Promoter

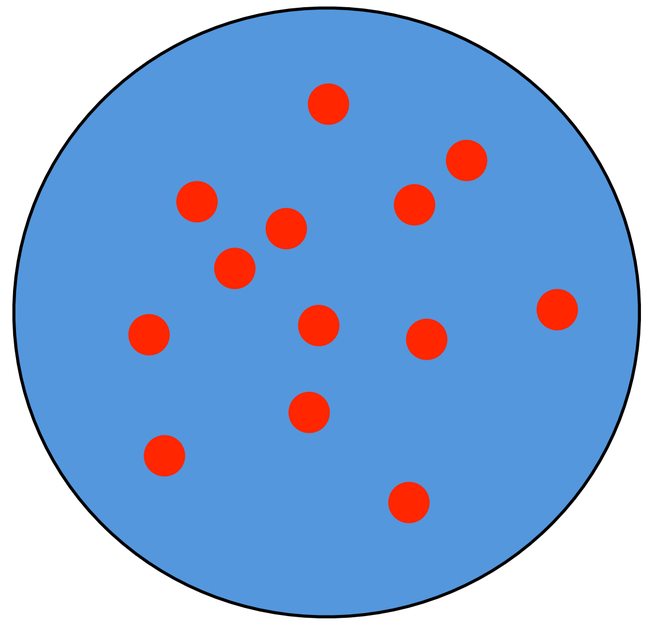
J119137



Insert Forward Promoter

J119137





Insert Bi-directional Promoter

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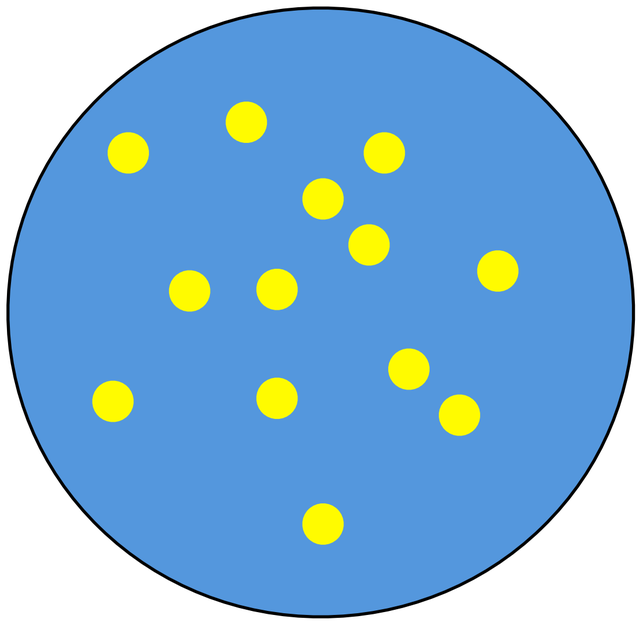
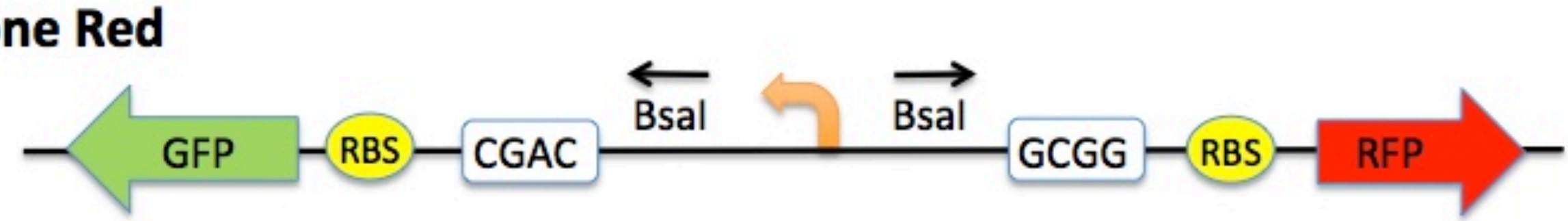
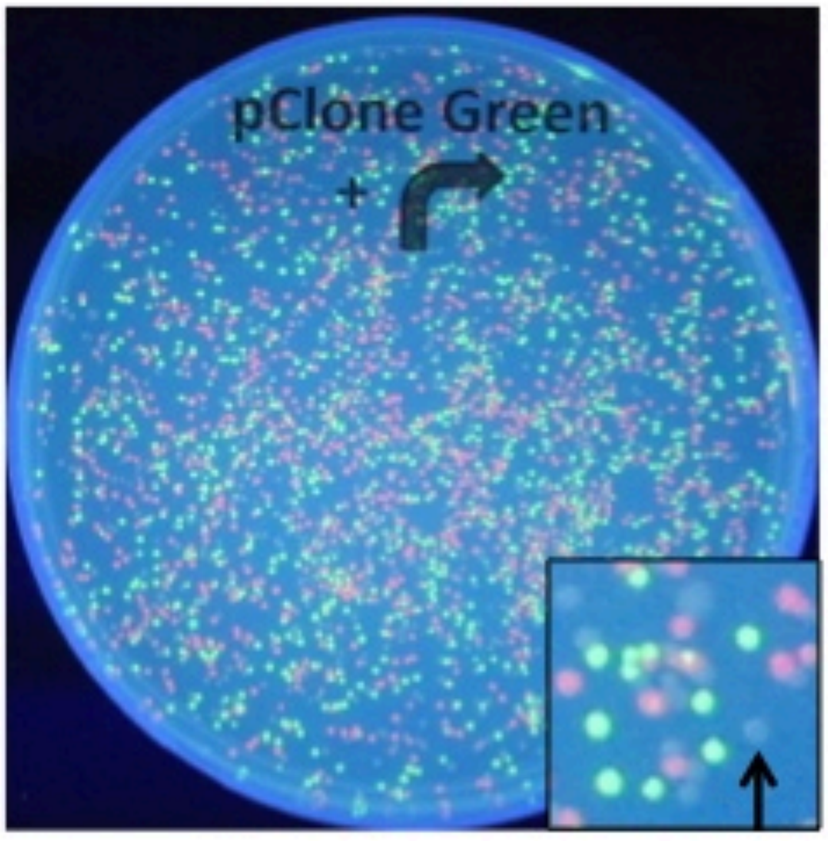


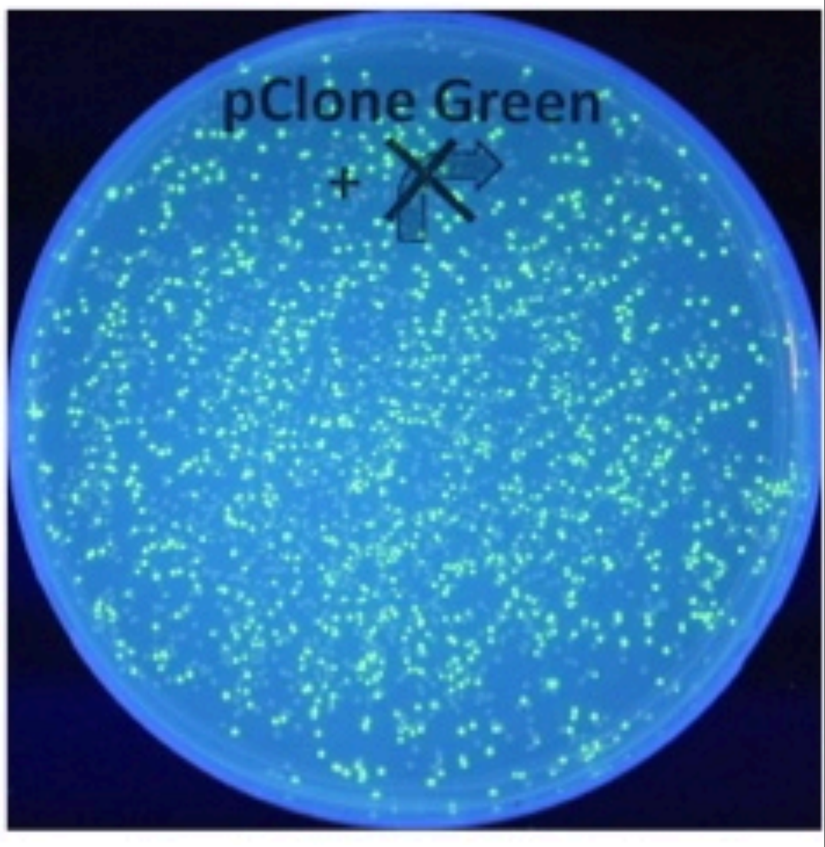
pClone Red

A



B

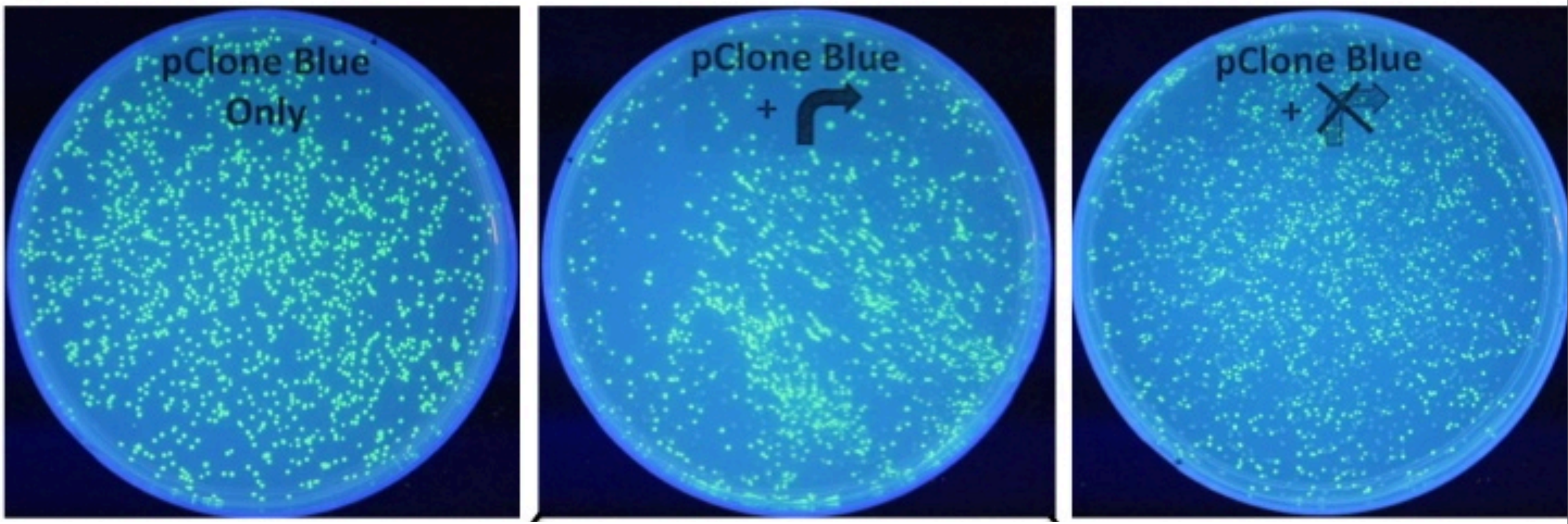




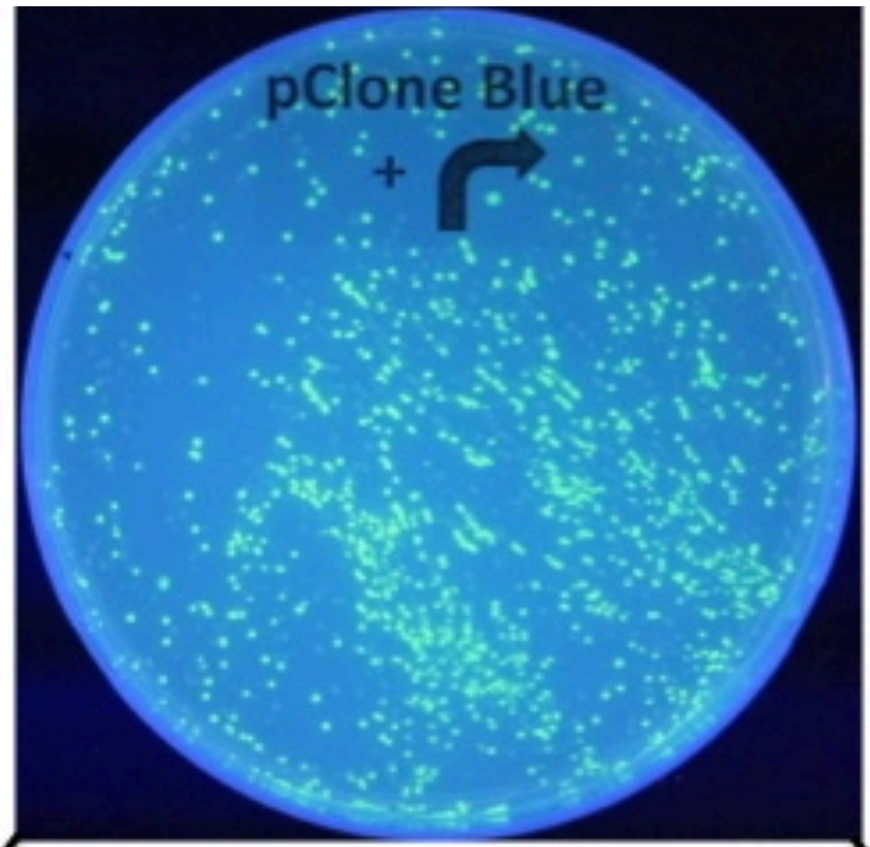
pClone Blue

A

B




pClone Blue
Only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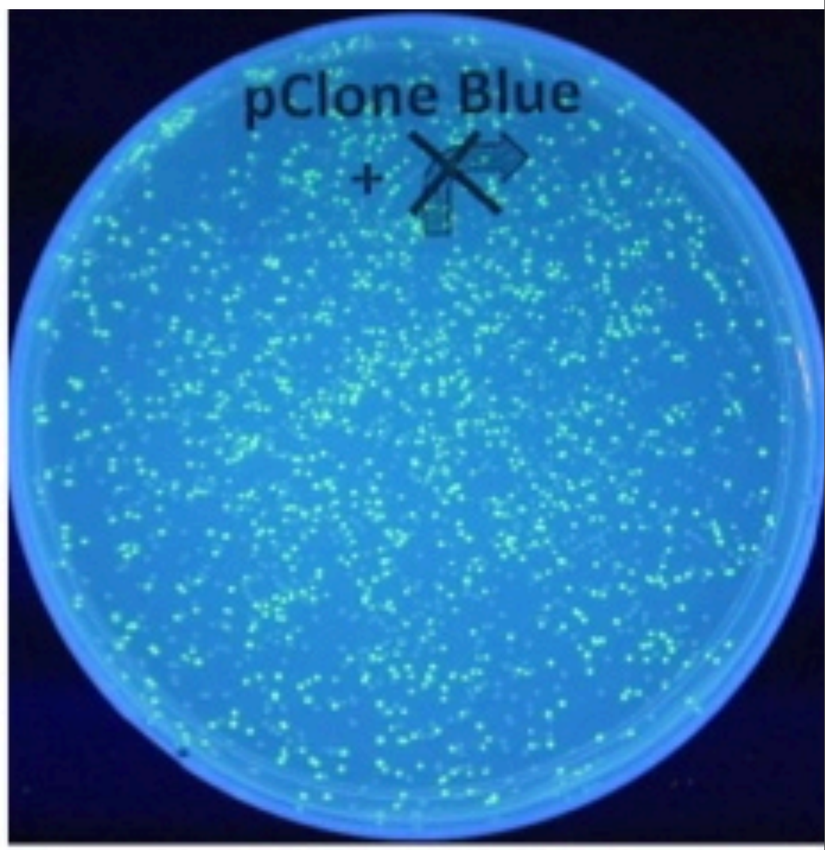
+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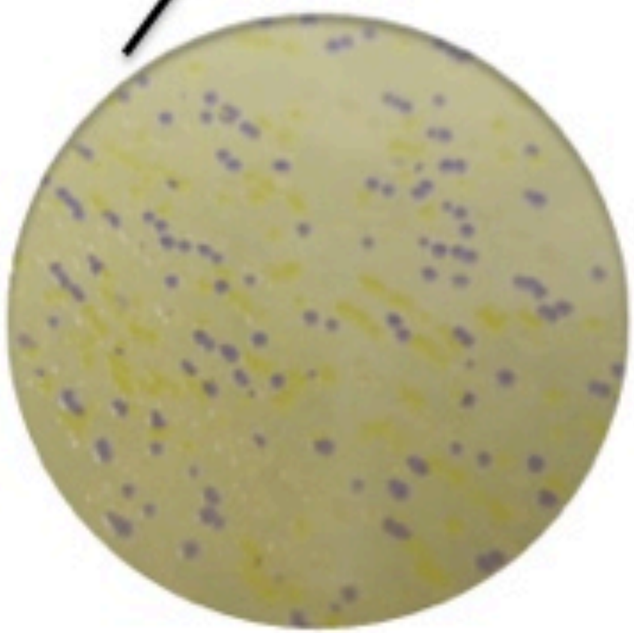
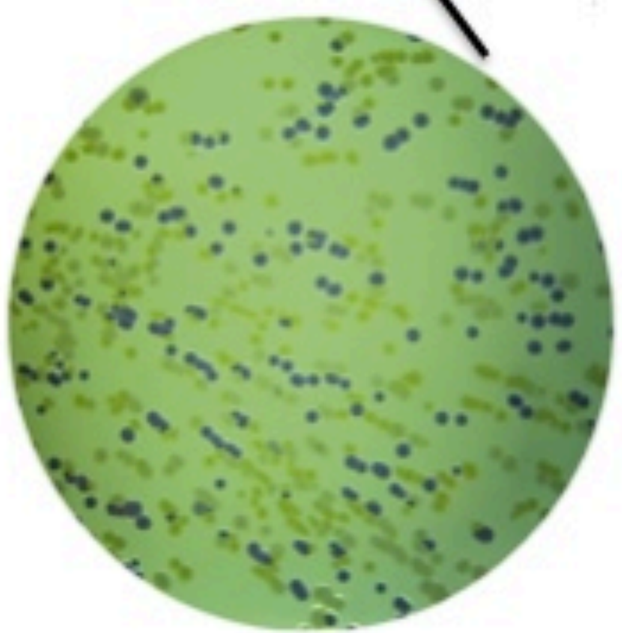




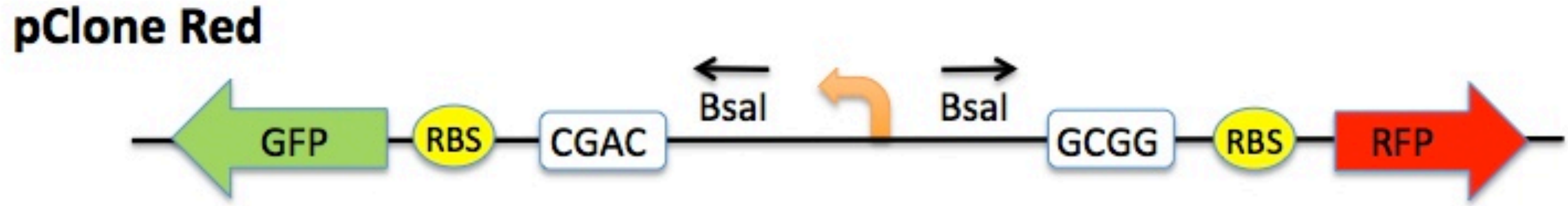
Did you learn how to clone by GGA?

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?

A



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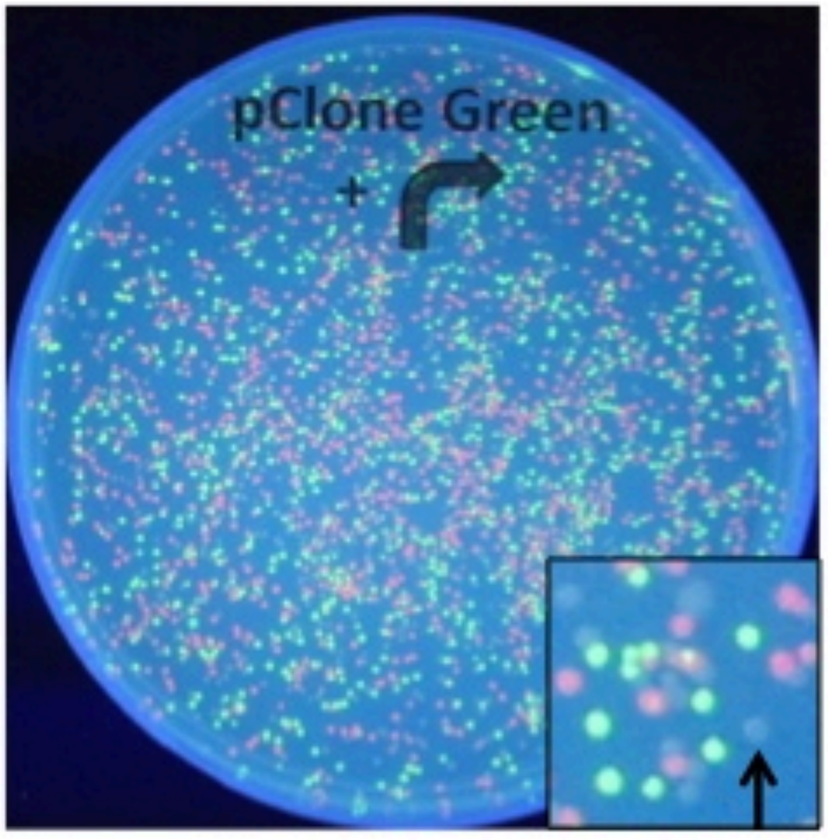
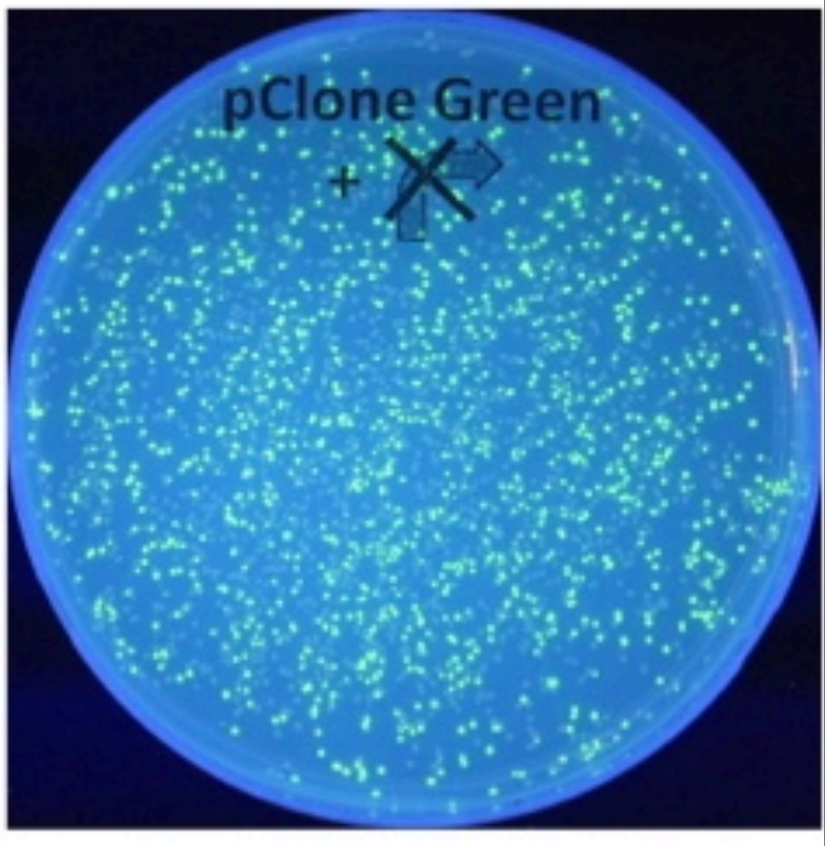
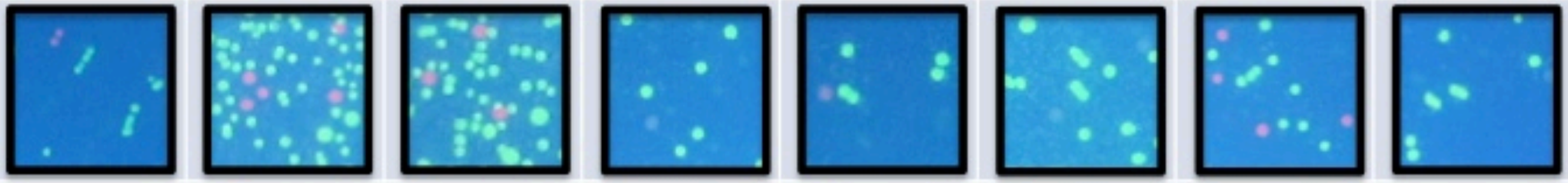
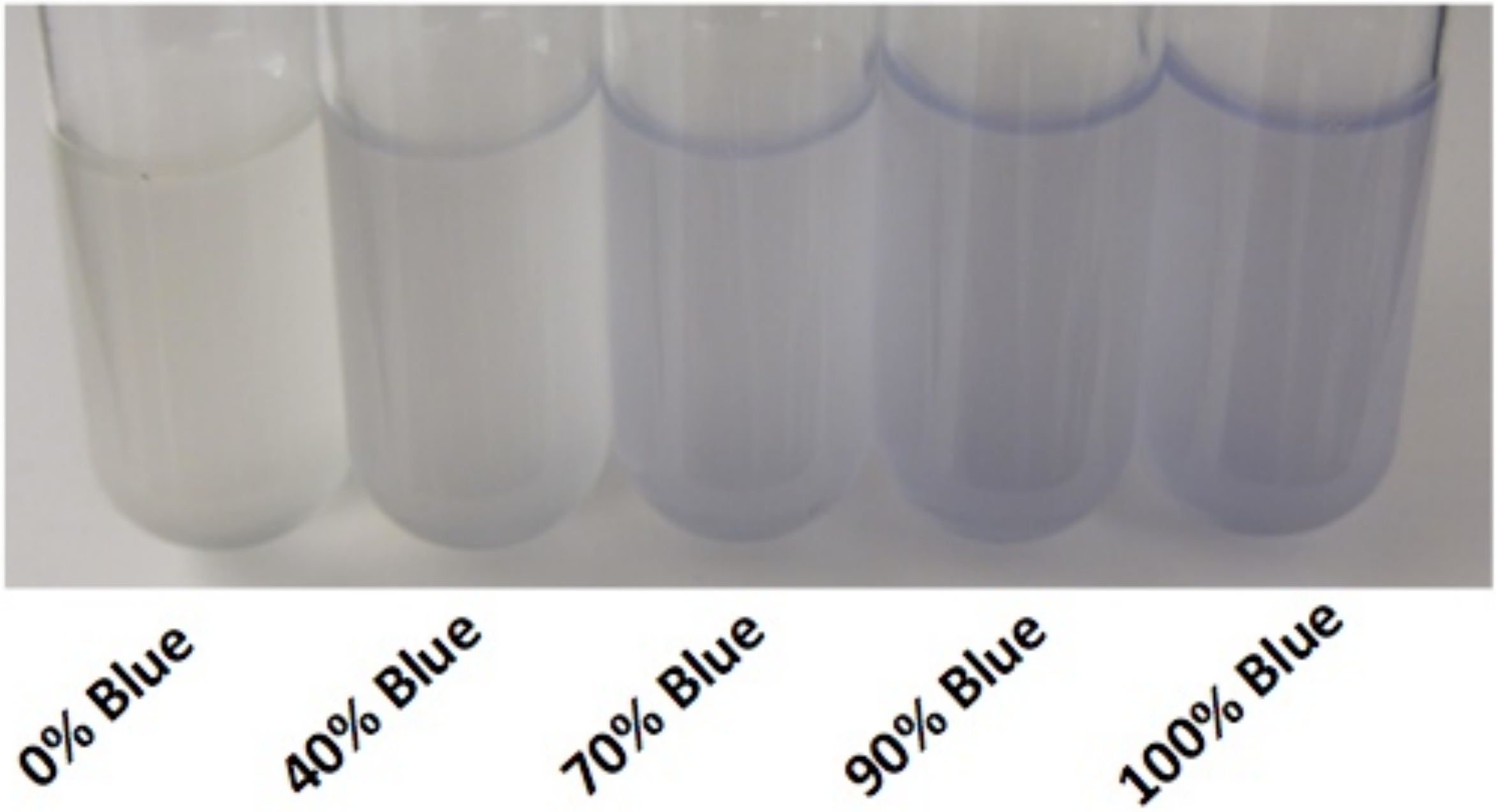




Quantify with Phone and ImageJ

Mutant	J119319	J119320	J119321	J119322	J119323	J119324	J119325	J119326
pClone Green plate								
Isolated clones								
Expression Ratio	4.09	3.94	3.84	2.04	1.54	1.34	3.52	1.00

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.

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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

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Skills Improved During Research

1) Communications Skills
2) Analytical/Research Skills
3) Computer Literacy
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10) Teamwork

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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

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Personal Values Improved by Research

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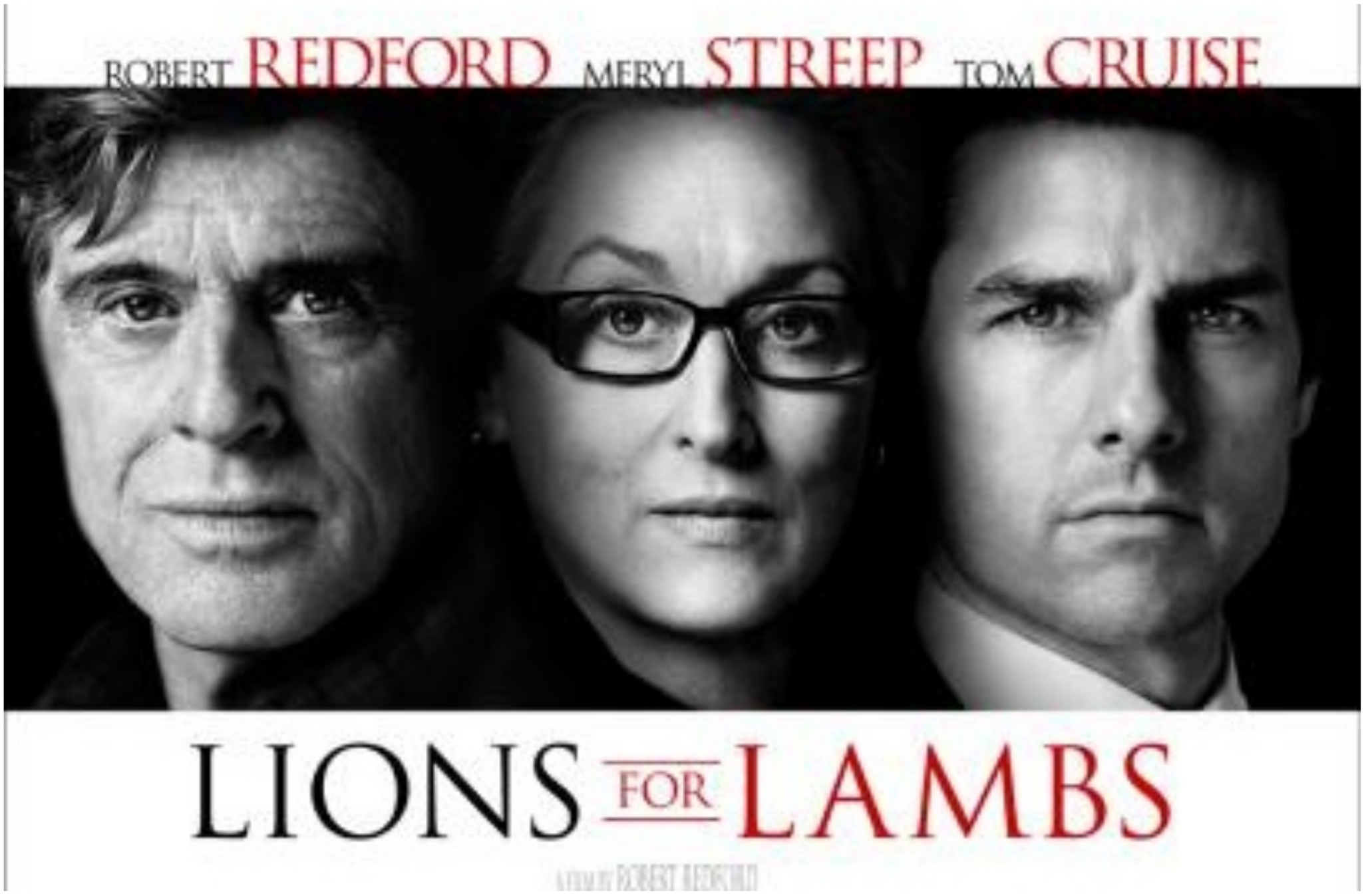
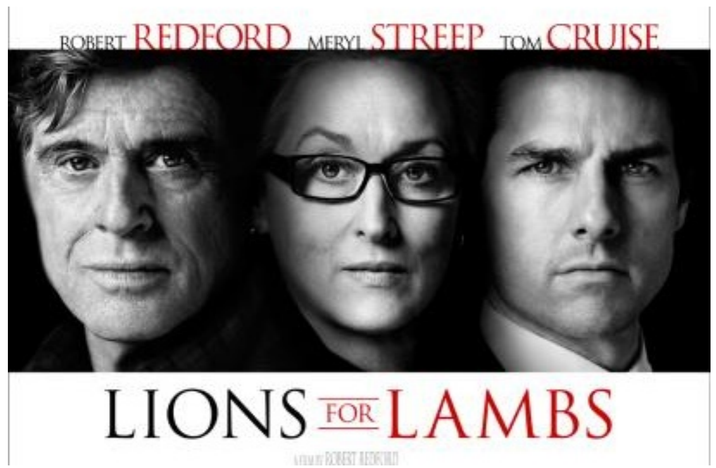
Your College Education Put Into Focus



Your College Education Put Into Focus

Would you rather settle for a blue collar B

or try for an A and risk failure?



Thomas J. Watson, founder of IBM

“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.

Tuesday, February 18, 2014

The scenery only changes for the lead dog.

Acknowledgements

Faculty: Laurie Heyer, Jeff Poet, Todd Eckdahl, Karmella Haynes, Pat Sellers, Mark Barsoum

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Genome Consortium for Active Teaching (**GCAT**)
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The End