Bacteria as Analog Computers to Optimize Chemical Synthesis

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Winthrop University
12 March, 2015

Outline of Presentation

What is synthetic biology?

Original research in teaching labs using SynBio.

SynBio can improve pharmaceutical production.

Are you willing to risk failure to find success?

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

Genetic engineering on a new scale.

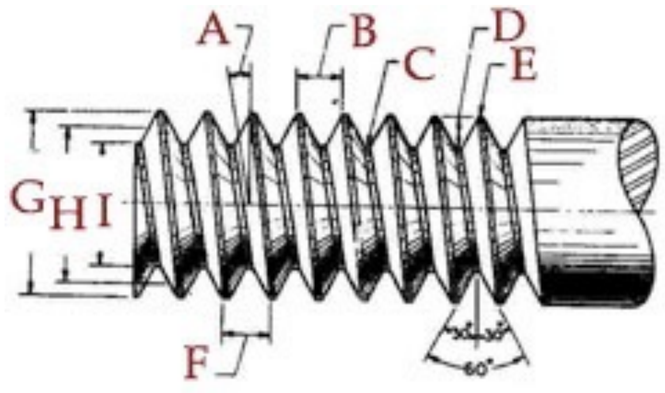
Four Characteristics:

- Standardization
- Modularity
- Abstraction
- Modeling of Designs

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."

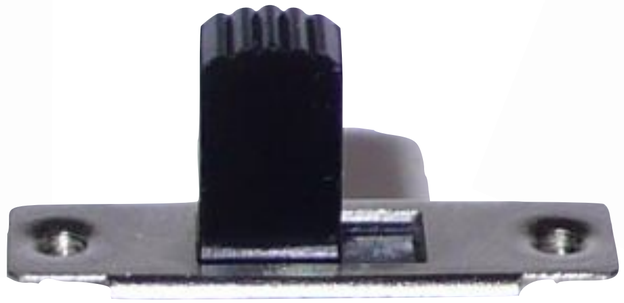


William Sellers April 21, 1864

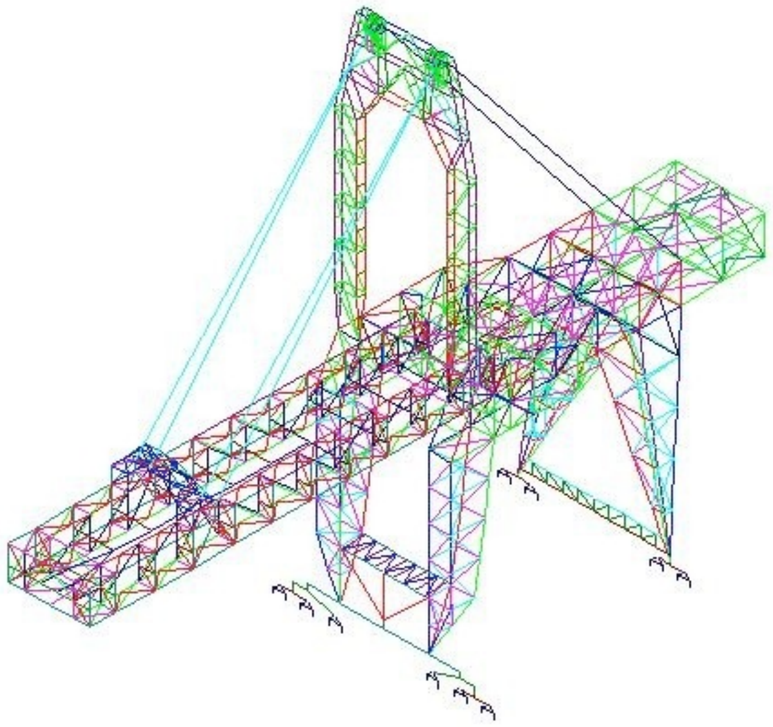
http://openwetware.org/images/b/bd/BBFRFC9.pdf

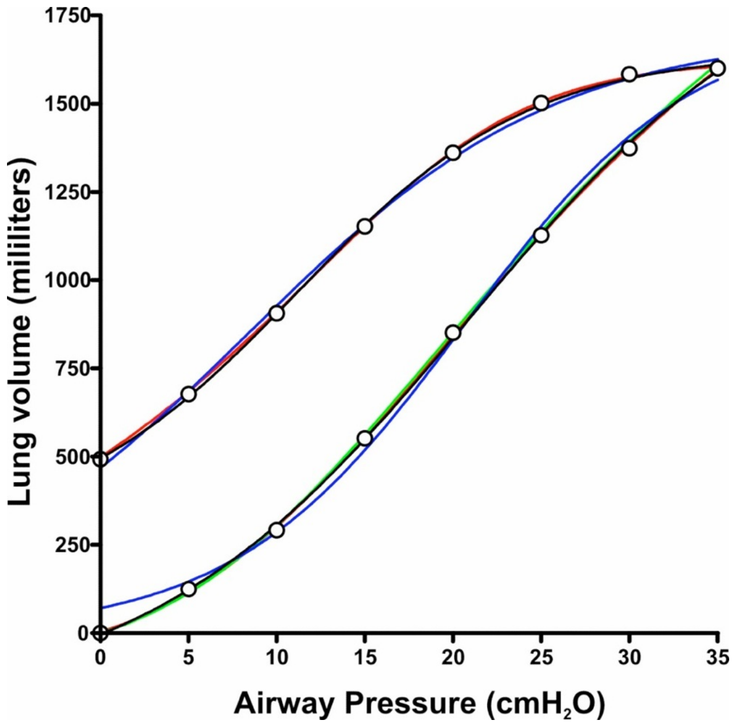
Modularity

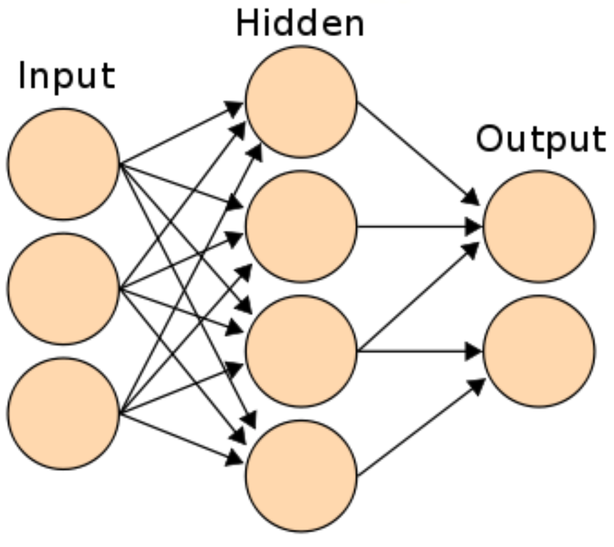
Abstraction

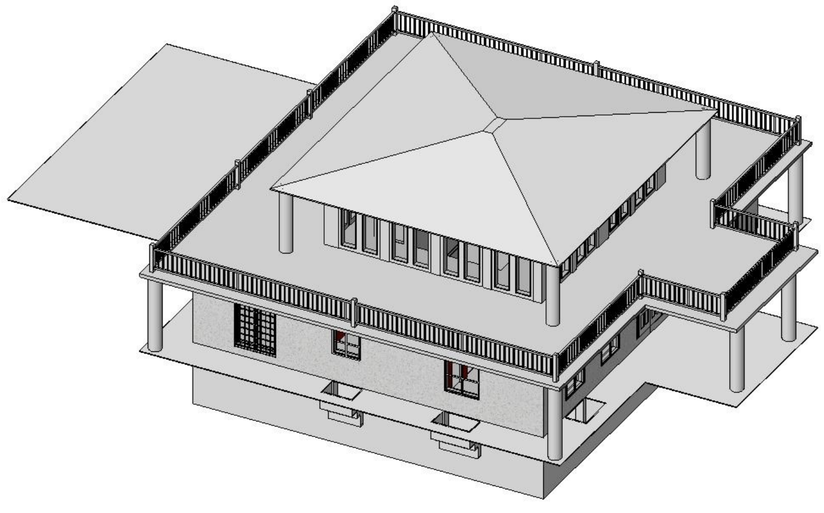


Modeling of Designs









Original SynBio Research by Undergraduates in Teaching Labs

Eco RI

GAATTC
CTTAAG

palindrome

type II

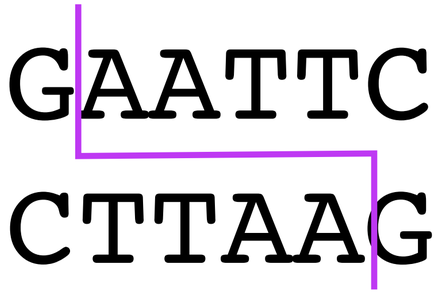
Eco RI

GAATTC
CTTAAG

palindrome

type II

Eco RI



type II

Eco RI

G AATTC

CTTAA G

type II

Bsa I

GAGACC

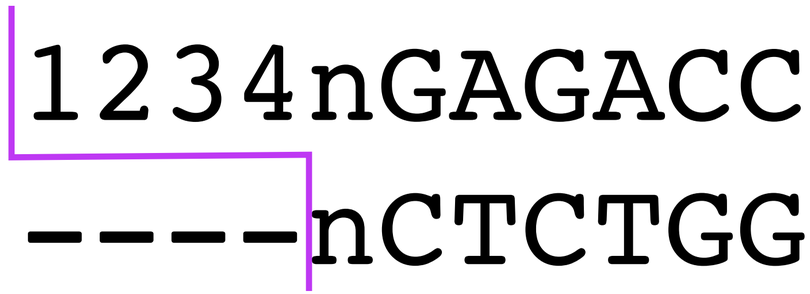
CTCTGG

G

not a palindrome

type IIs

Bsa I



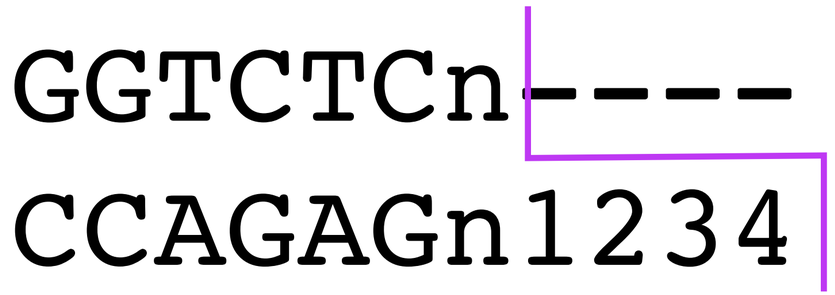
type IIs

Bsa I

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

type IIs

Bsa I



type IIs

Bsa I

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

type IIs

Bsa I

cuts left

1234nGAGACC

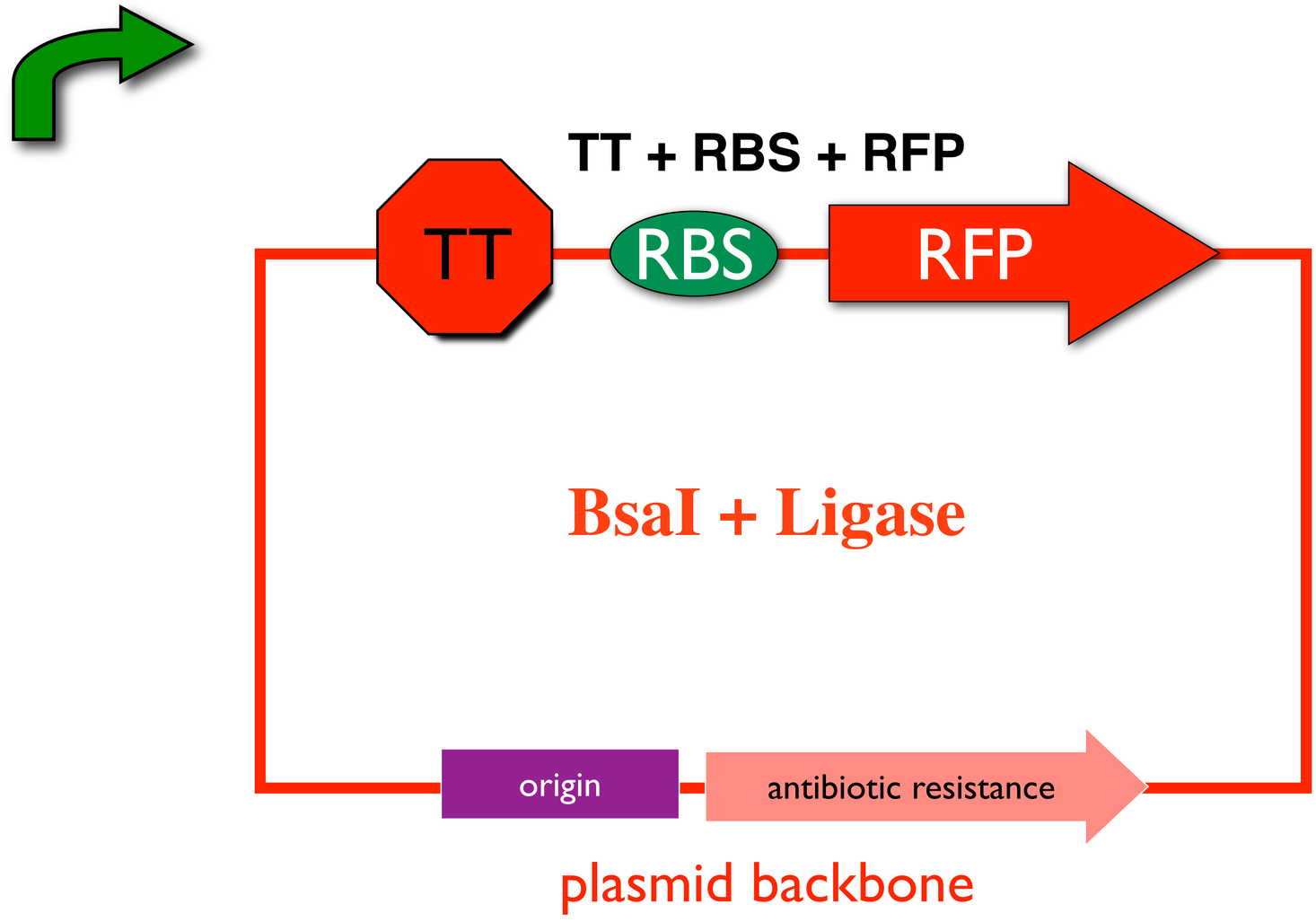
----nCTCTGG

GGTCTCn----

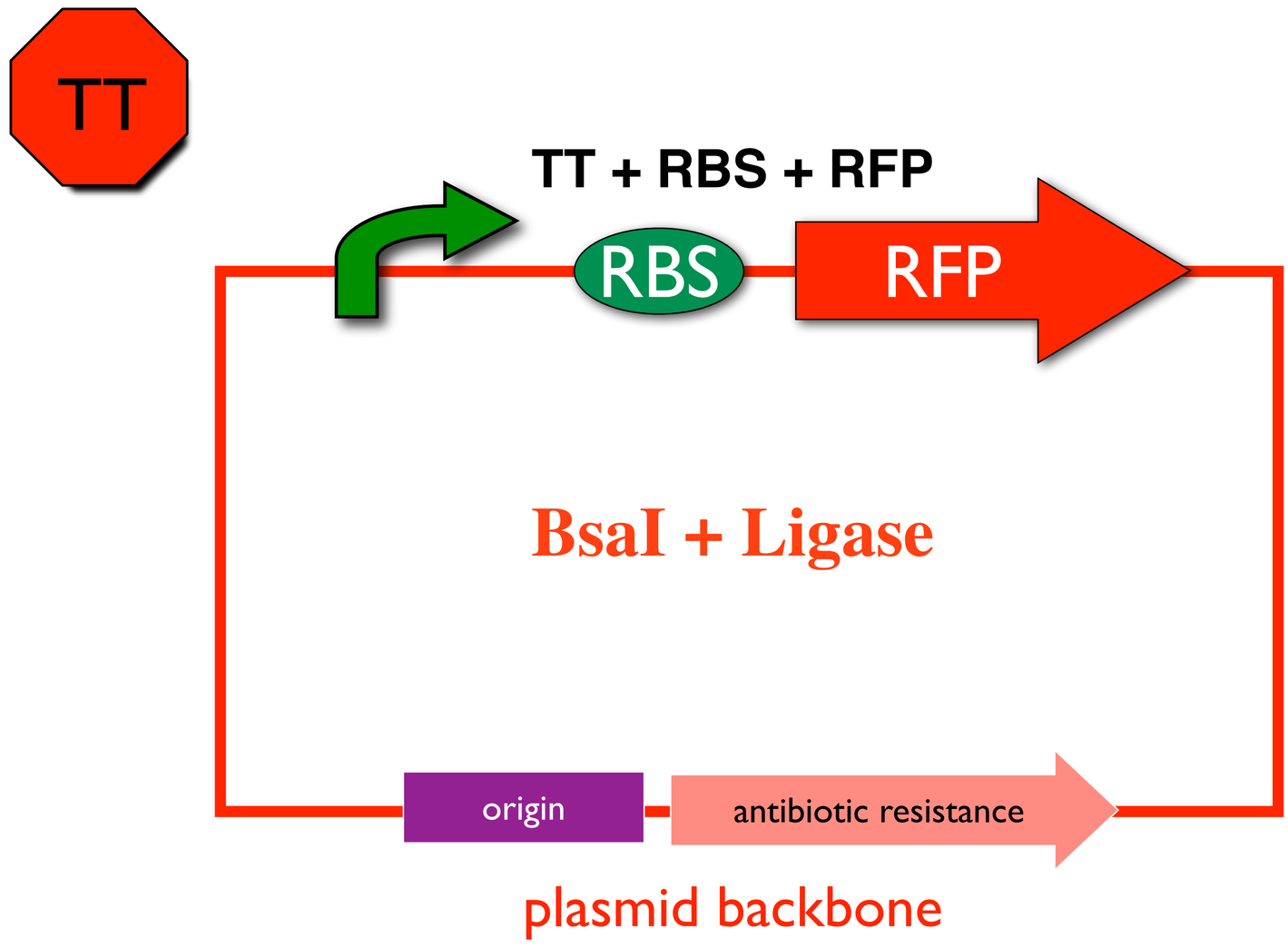
CCAGAGn1234

cuts right

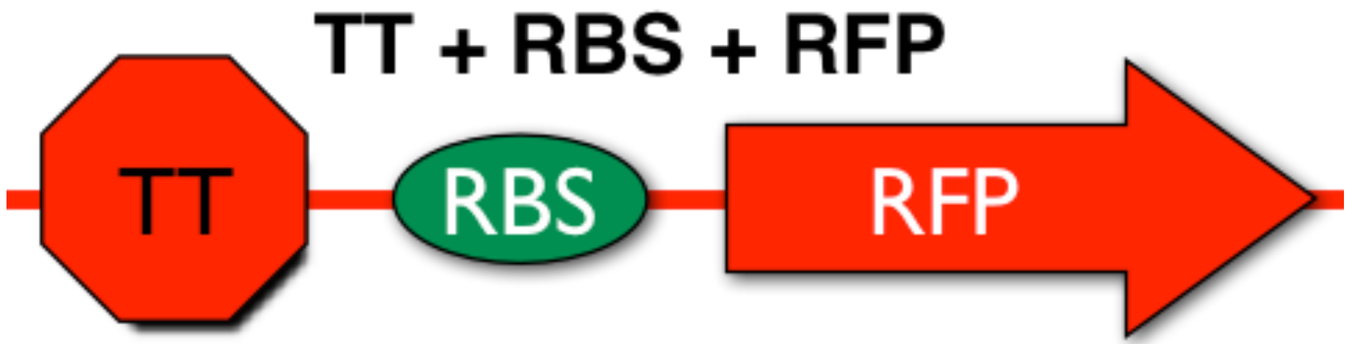
GGA Ligation Method



GGA Ligation Method



GGA Ligation Method



GGA Ligation Method

Bsa I

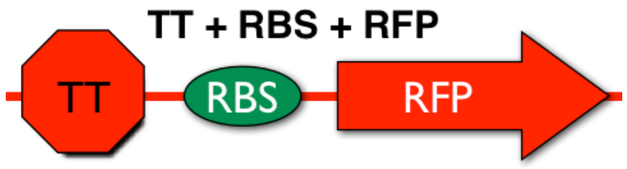
CGACtGAGACC(TT)GGTCTCa GCGG

GCTGaCTCTGG(TT)CCAGAGtCGCC

Bsa I

ligase

ligase



CGACt**GAGACC**(**TT**)**GGTCTCa**

aCTCTGG(**TT**)**CCAGAG**t**CGCC**

[redacted] **GCTG**

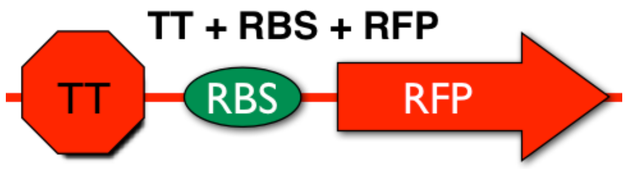
GCGG [redacted]

ligase

ligase

Bsa I

ligase Bsa I ligase



CGACtGAGACC(TT)GGTCTCa

aCTCTGG(TT)CCAGAGtCGCC

[redacted] GCTG

ligase

RBS + RFP

CGAC

GCGG [redacted]

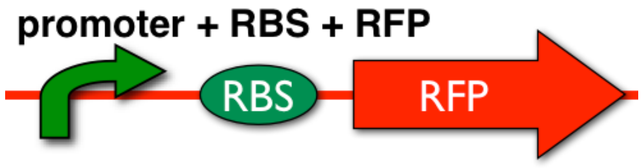
ligase

CGAC

CGAC(promoter)GCGG

GCTG(promoter)CGCC

ligase ligase



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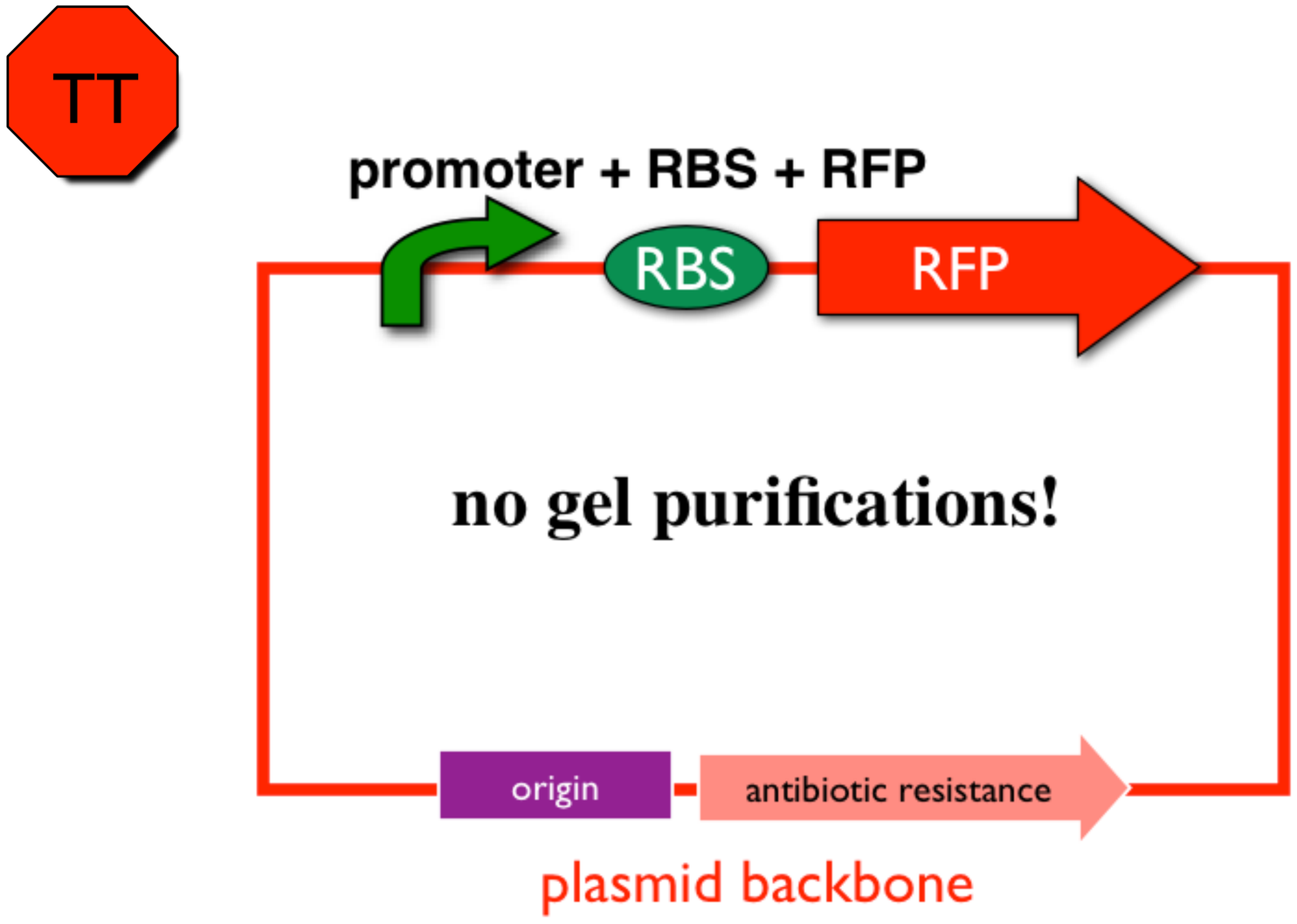
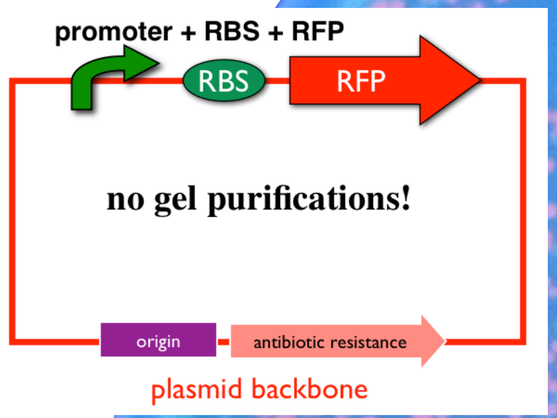
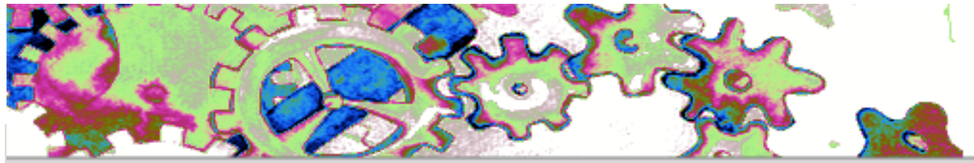



promoter + RBS + RFP
RBS
RFP
no gel purifications!
origin
antibiotic resistance
plasmid backbone

X
12 in
picked 6

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

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

Regulatory

DNA Planning

Experience: Works

Get This Part

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

Regulatory

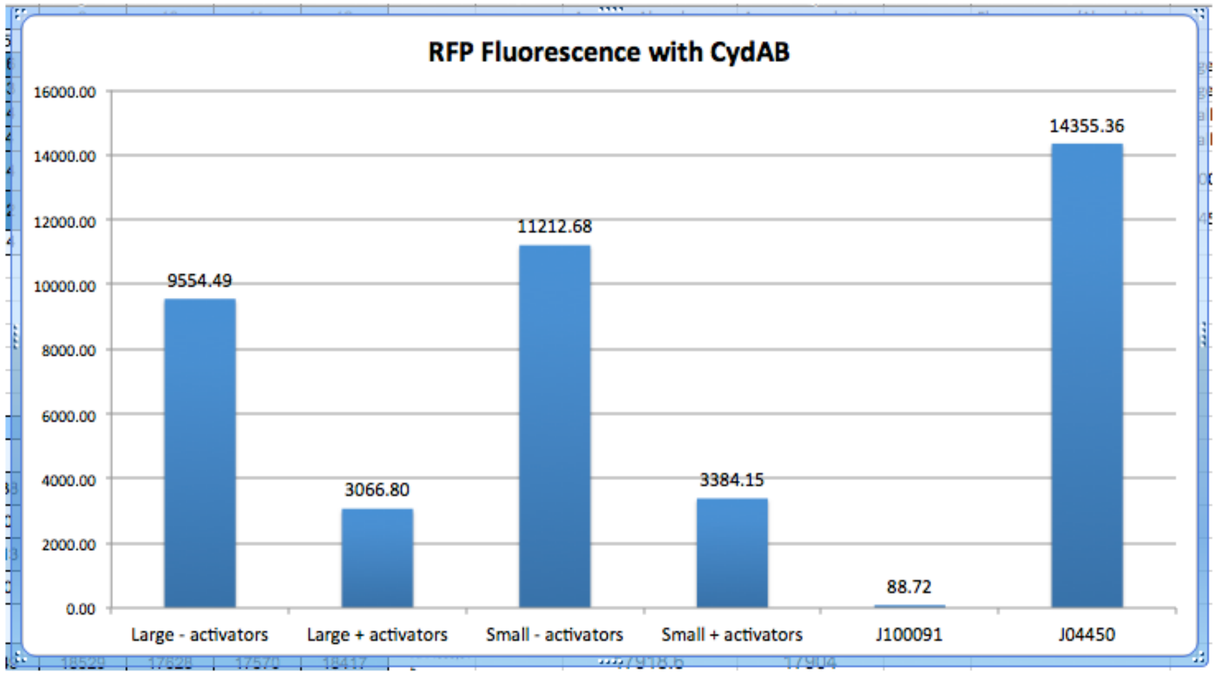
DNA Planning
Experience: Works
Get This Part

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

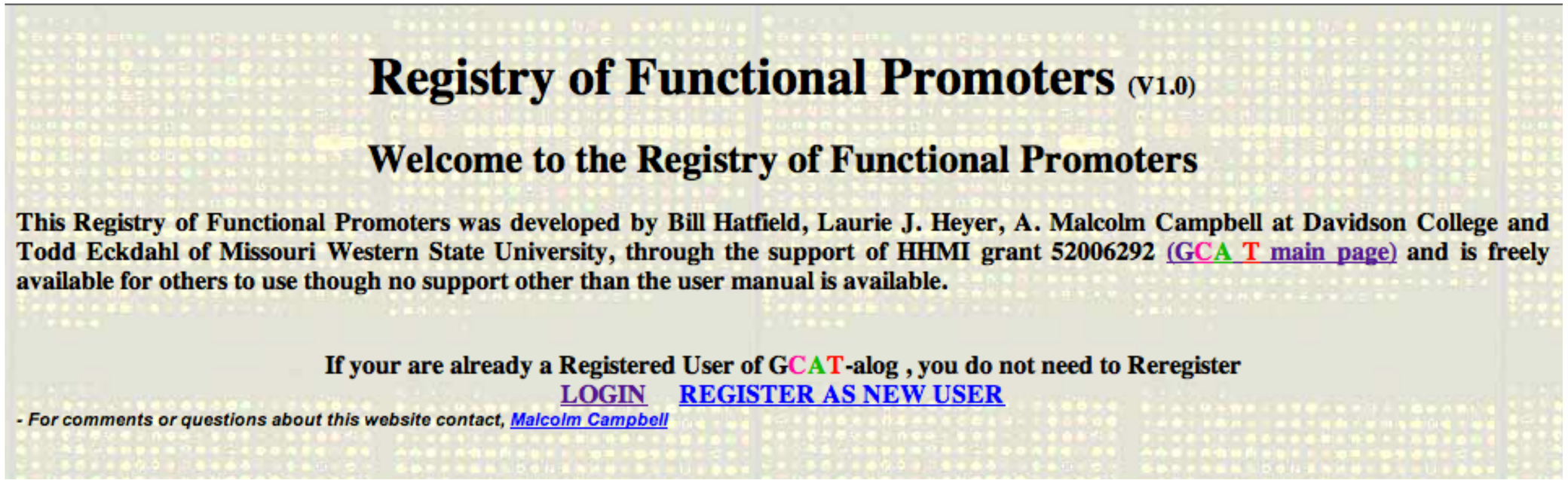
This experience page is provided so that any user may enter their experience using this part.
Please enter how you used this part and how it worked out.

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 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.

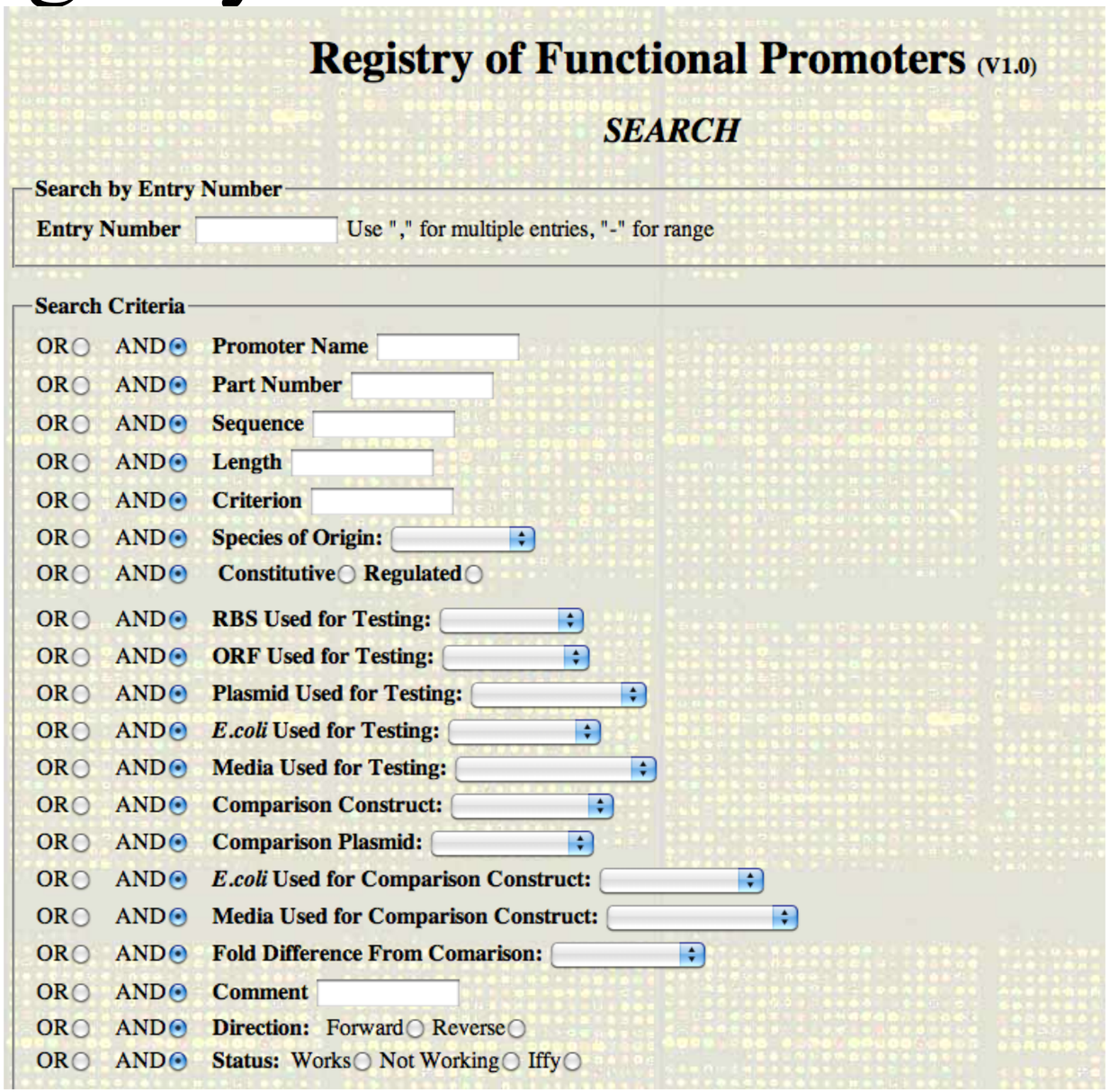


Registry of Functional Promoters (RFP)



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			pSE
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					

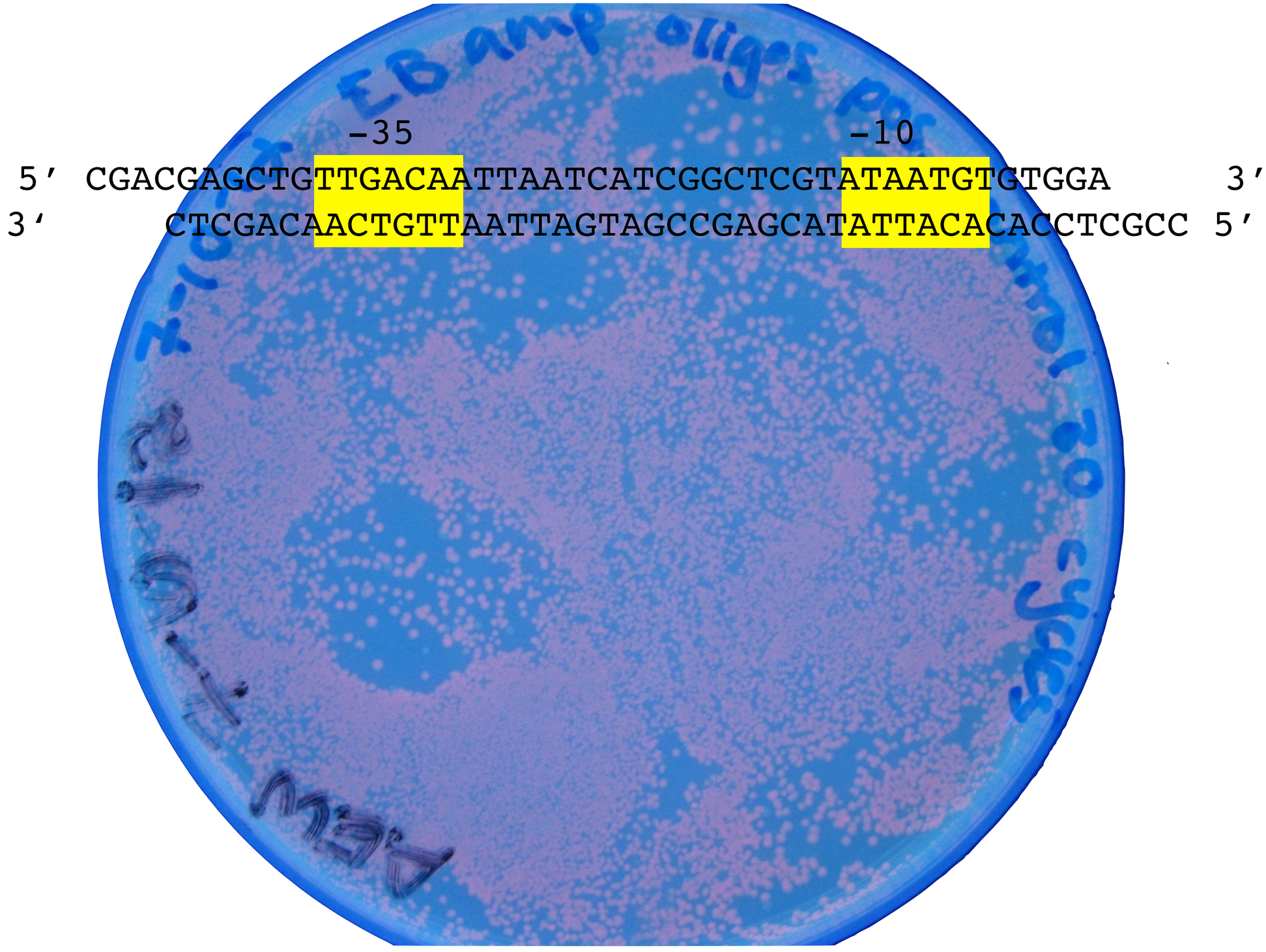
To Edit an Entry, Enter the Entry # and press "Edit Entry" [ ] Edit Entry

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

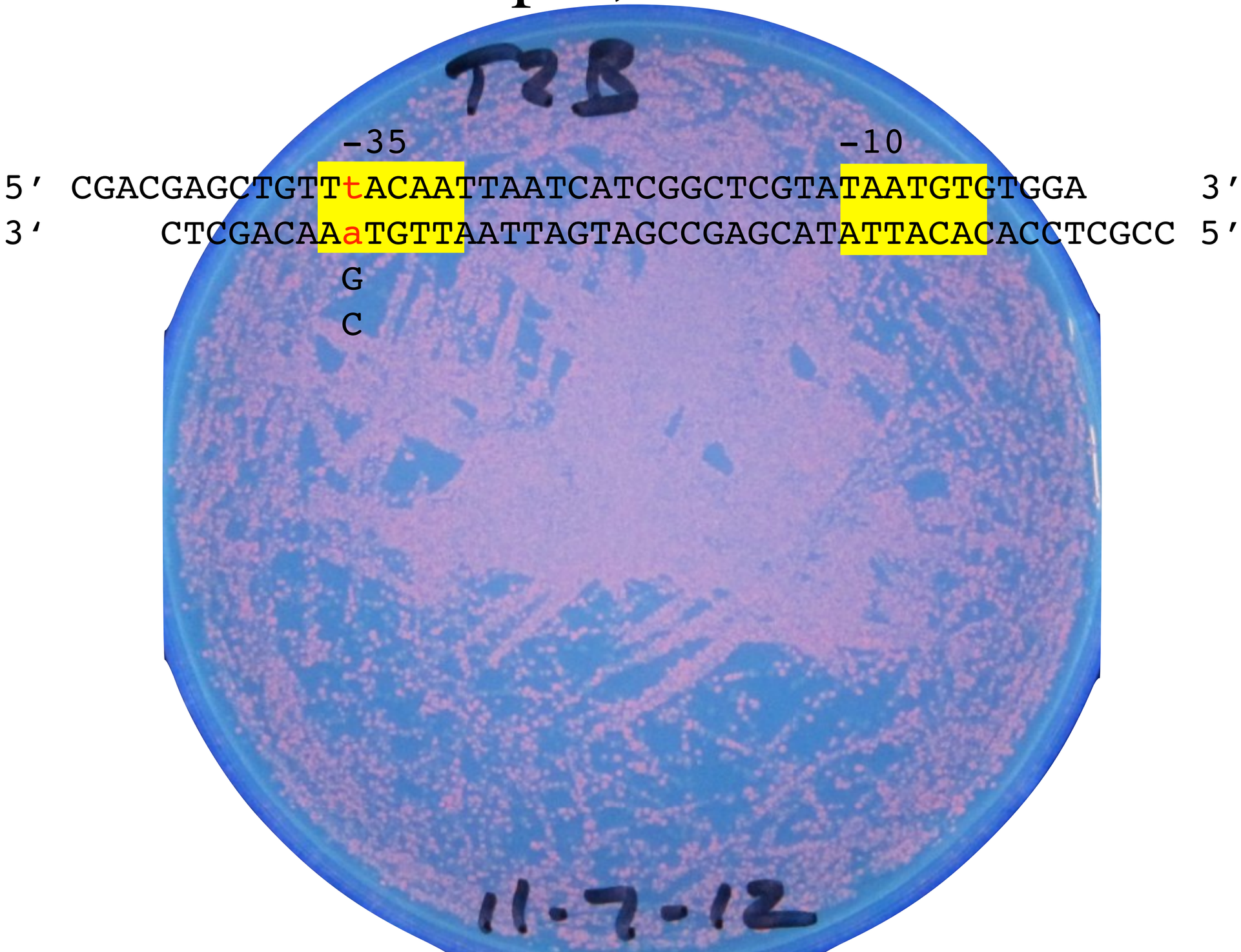
Search Again

gcat.davidson.edu/RFP/

Testing Known Promoters: Ptac

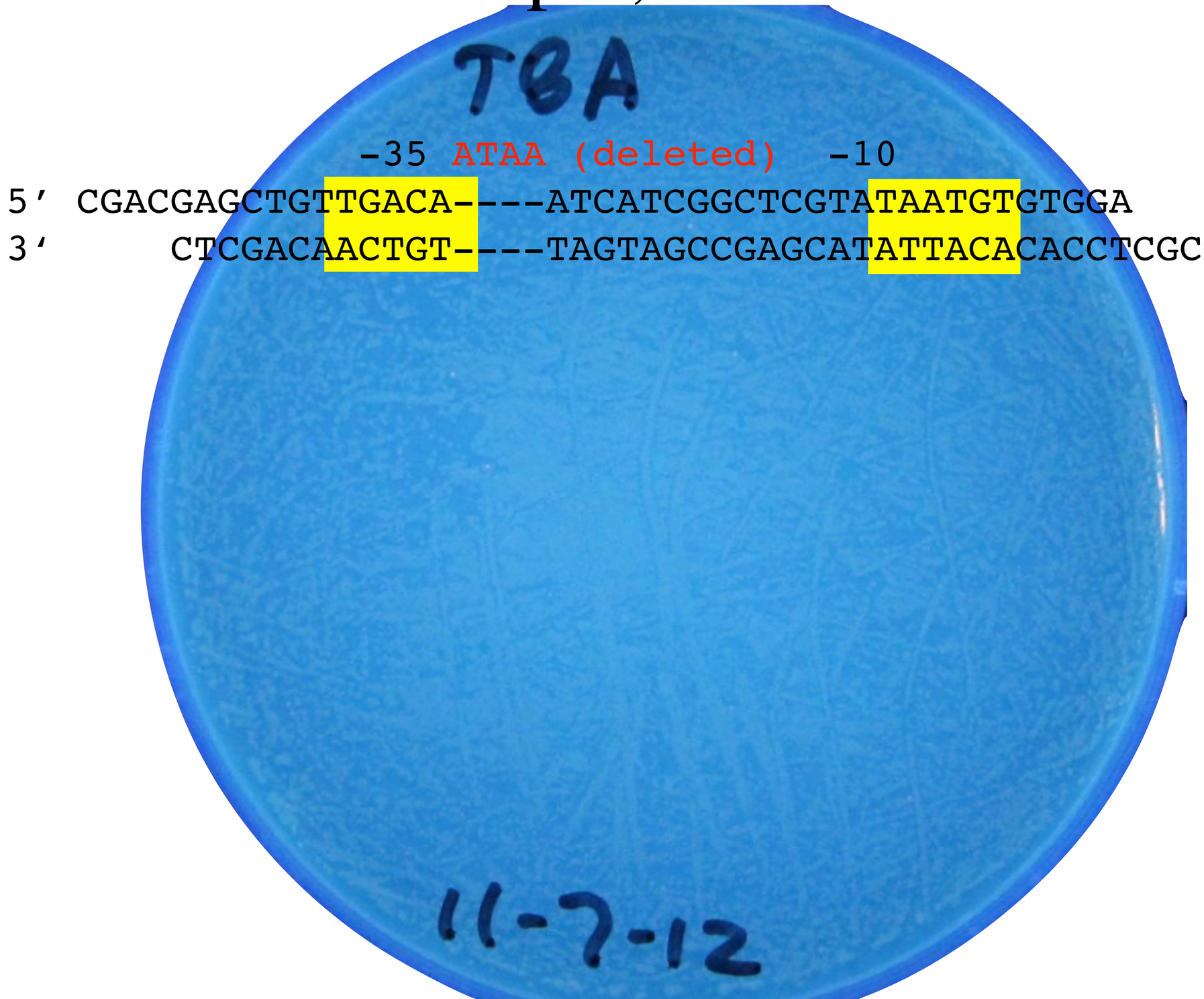


Student Sample, November 2012




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

Student Sample, November 2012



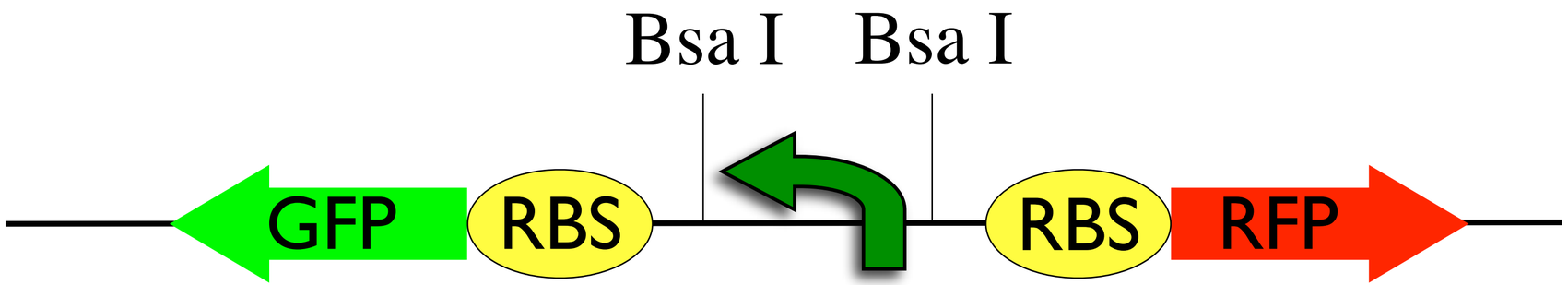
-35 ATAA (deleted) -10

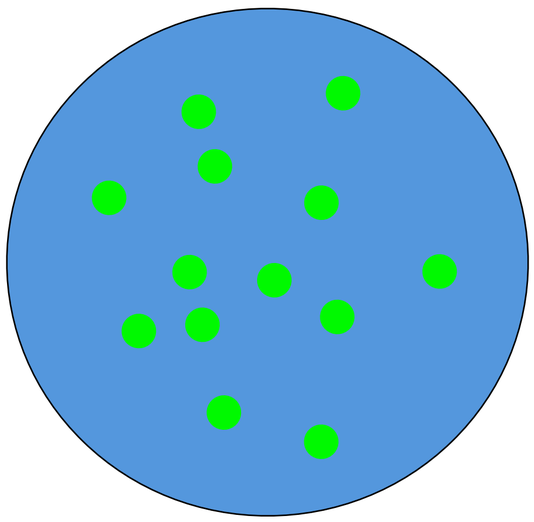
5′ CGACGAGCTGTTGACA----ATCATCGGCTCGTATAATGTGTGGA 3′

3′ CTCGACAACTGT----TAGTAGCCGAGCATATTACACACCTCGCC 5′

pClone Red

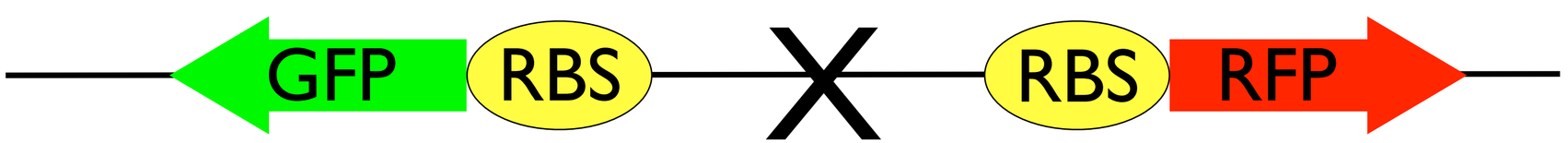
J119137

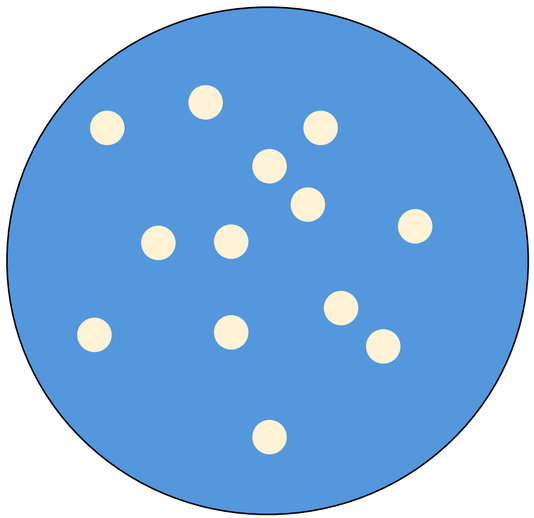




Remove Initial Promoter

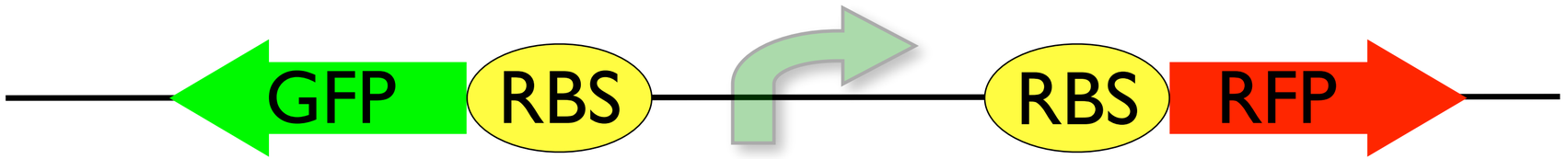
J119137

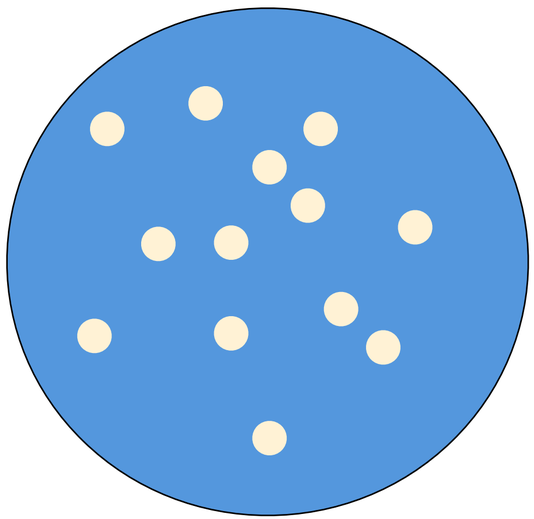




Insert Non-functional Promoter

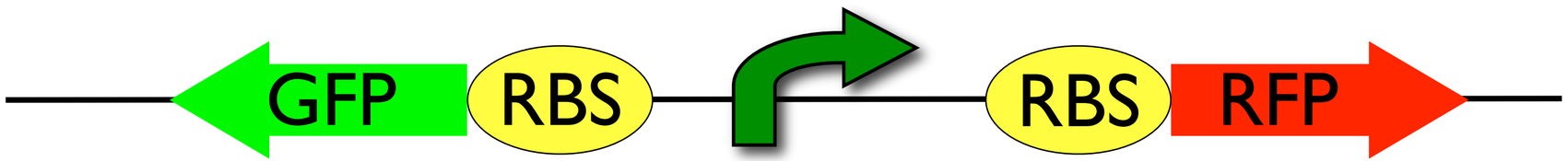
J119137

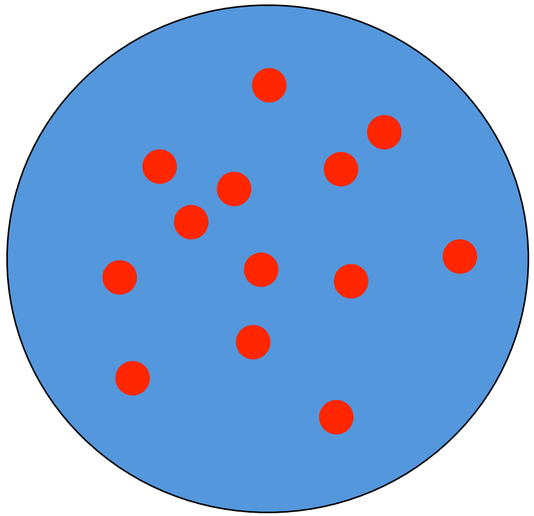




Insert Forward Promoter

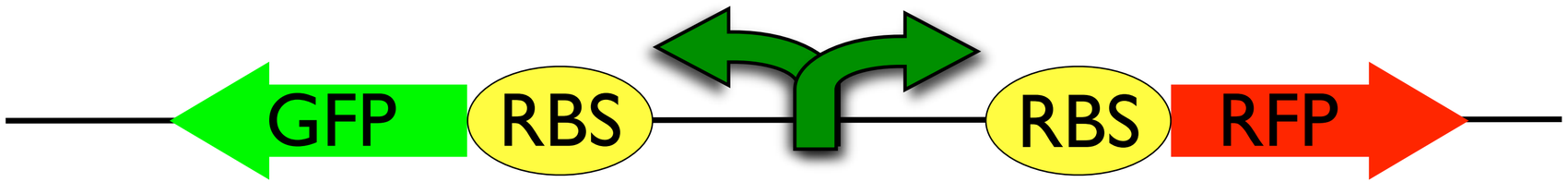
J119137

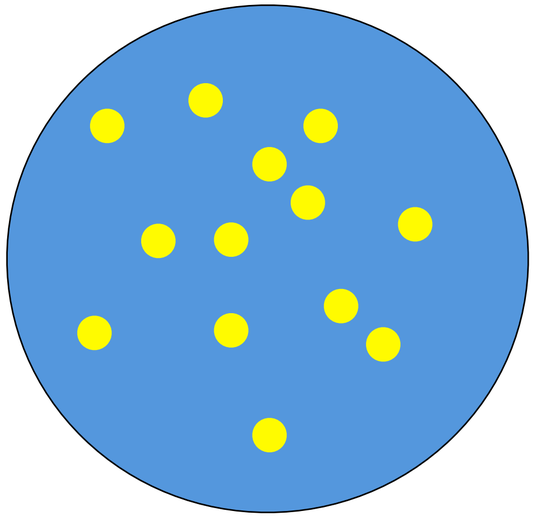




Insert Bi-directional Promoter

J119137

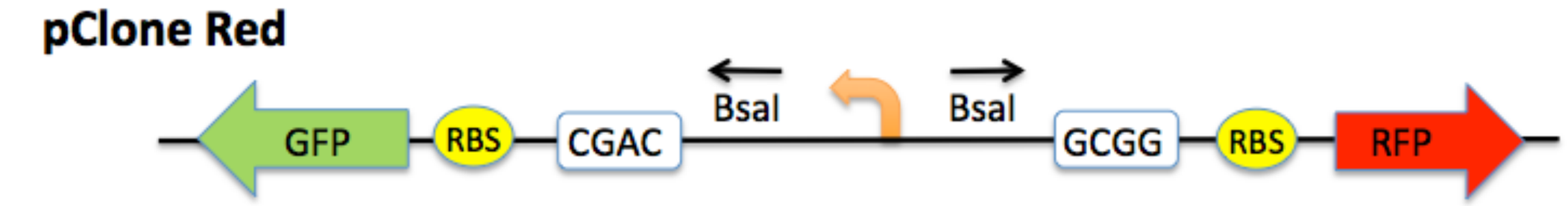




pClone Red

J119137

A



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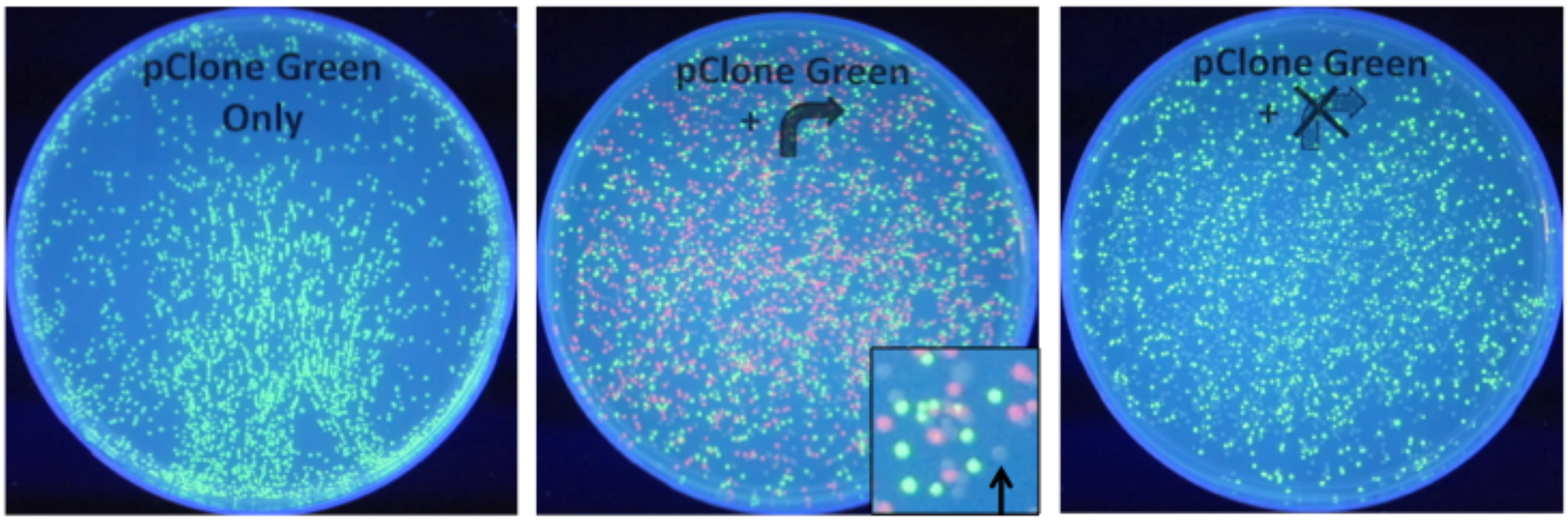
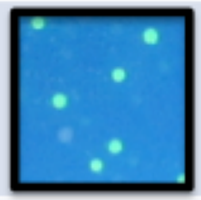
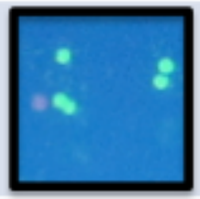
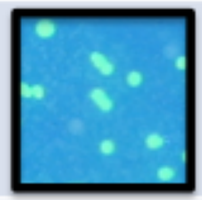
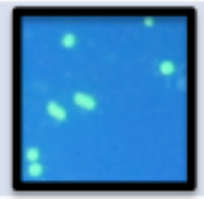
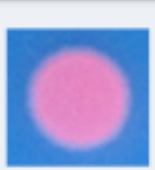
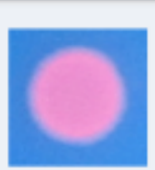
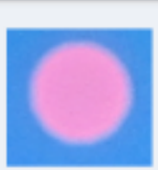
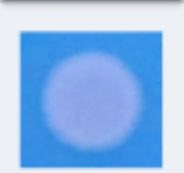
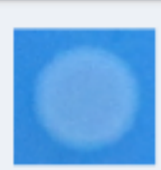
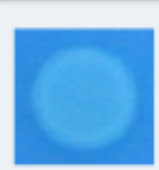


Campbell, *et al.* 2014. *CBE Life Sciences Education*. Vol. 13(2): 285 - 296.

Quantify with Phone and ImageJ

J119137

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

Campbell, *et al.* 2014. *CBE Life Sciences Education*. Vol. 13(2): 285 - 296.

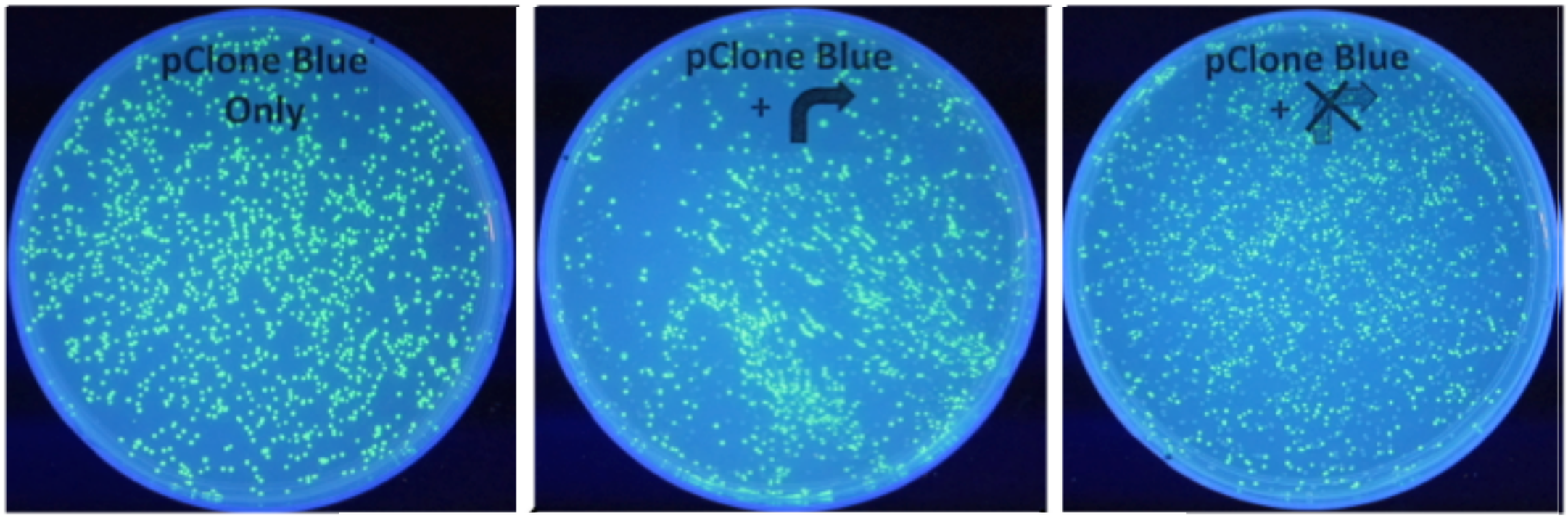
pClone Blue

J119313

A

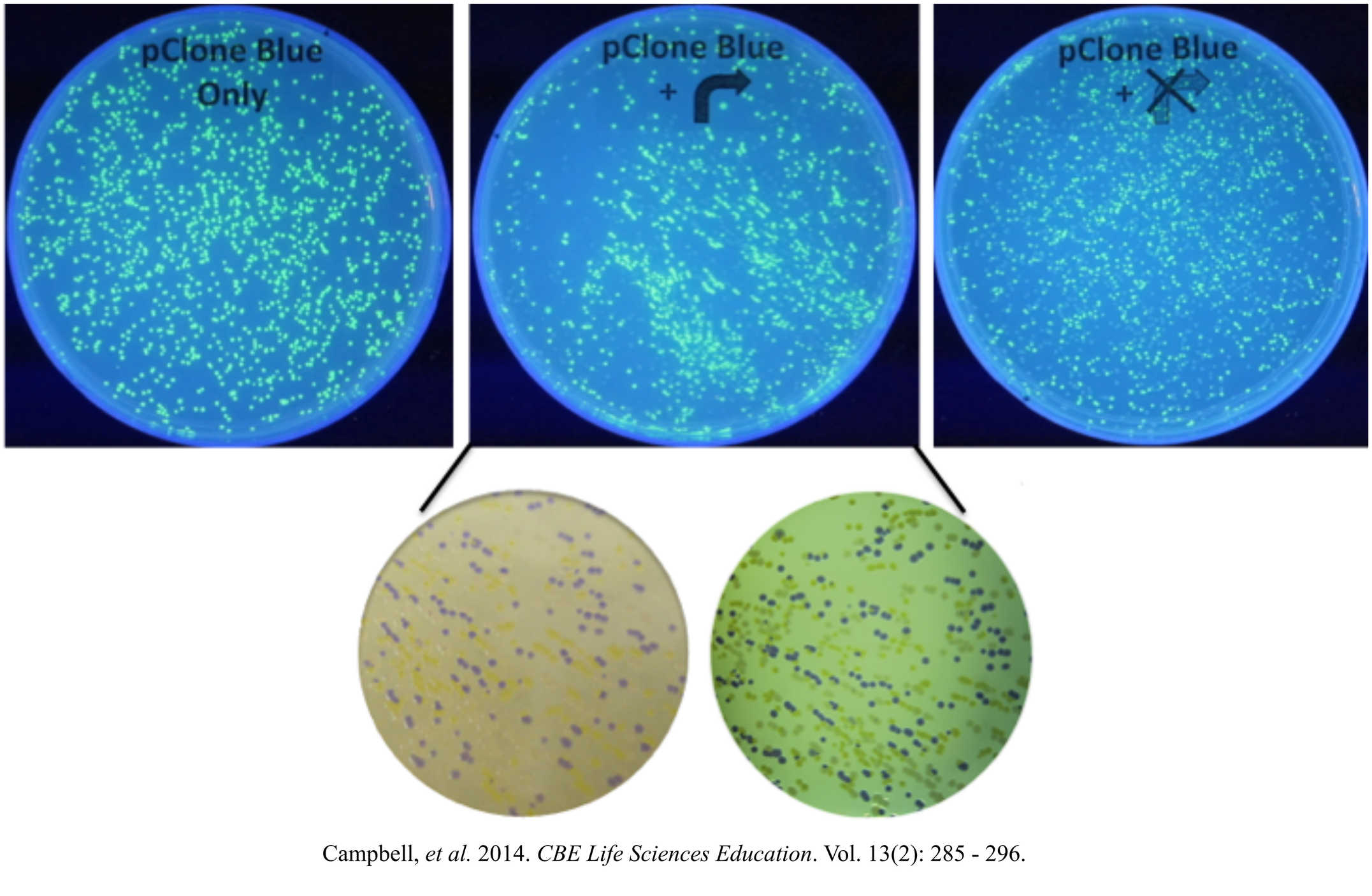
pClone Blue

B



Campbell, *et al.* 2014. *CBE Life Sciences Education*. Vol. 13(2): 285 - 296.

pClone Blue

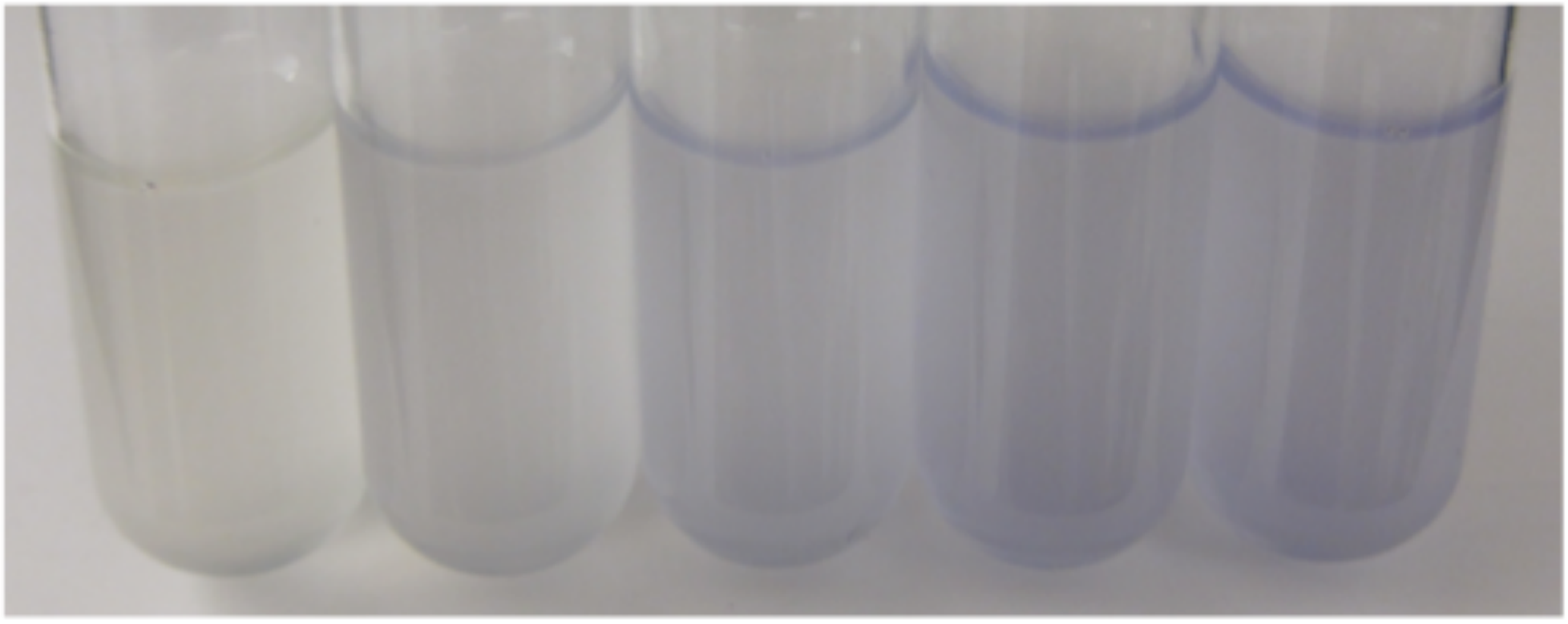


Campbell, *et al.* 2014. *CBE Life Sciences Education*. Vol. 13(2): 285 - 296.

Measure Promoter Qualitatively

J119313

A



Assessment Davidson Intro Bio

	Learning objective	Pretest experimental	Posttest experimental	Comparison course	$F(2,88)$	Effect size (η^2)	Conclusion
1	Function of promoter	43%	87%[a]	48%	8.008, $p = 0.001$	0.154	Large effect
2	Repressor diagram	23%	53%[a]	13%	7.206, $p = 0.001$	0.141	Large effect
3	Activator diagram	0%	41%[a]	0%	7.250, $p = 0.001$	0.167	Large effect
4	Experiment overview	0%	13%[a]	0%	4.538, $p = 0.013$	0.103	Moderate effect
5	Transformation method	0%	20%[a]	0%	7.374, $p = 0.001$	0.143	Large effect
6	Verify promoter cloned	50%	40%	48%	0.34, $p = 0.713$	0.008	No effect
7	Test promoter strength	43%	60%	39%	1.525, $p = 0.223$	0.034	No effect
8	Type IIs restriction enzymes	7%	50%	6%	1.873, $p = 0.16$	0.041	No effect
9	GGA method	10%	63%[a]	0%	31.929, $p < 0.001$	0.421	Large effect

[a]Significant improvement between pre- and posttest.

Assessment MWSU Genetics (soph)

	Learning objective	Pretest experimental	Posttest experimental	Control course (ecology)	$F(2252)$	Effect size (η^2)	Conclusion
1	Function of promoter	36%	59%[a]	20%	13.527, $p < 0.001$	0.097	Moderate effect
2	−10 and −35 sites	3%	70%[a]	0%	145.374, $p < 0.001$	0.536	Large effect
3	Mutational analysis	30%	75%[a]	33%	28.773, $p < 0.001$	0.186	Large effect
4	Student-designed mutation	0%	0%	0%	0, $p > 0.05$	0.000	No effect
5	Transformation method	11%	51%[a]	12%	30.731, $p < 0.001$	0.196	Large effect
6	Verify promoter cloned	19%	44%[a]	18%	10.264, $p < 0.001$	0.075	Moderate effect
7	Test promoter strength	17%	33%[a]	18%	4.421, $p = 0.013$	0.034	Moderate effect
8	Type IIs restriction enzymes	2%	29%[a]	4%	21.661, $p < 0.001$	0.147	Large effect
9	GGA method	14%	22%	14%	1.56, $p = 0.212$	0.012	No effect

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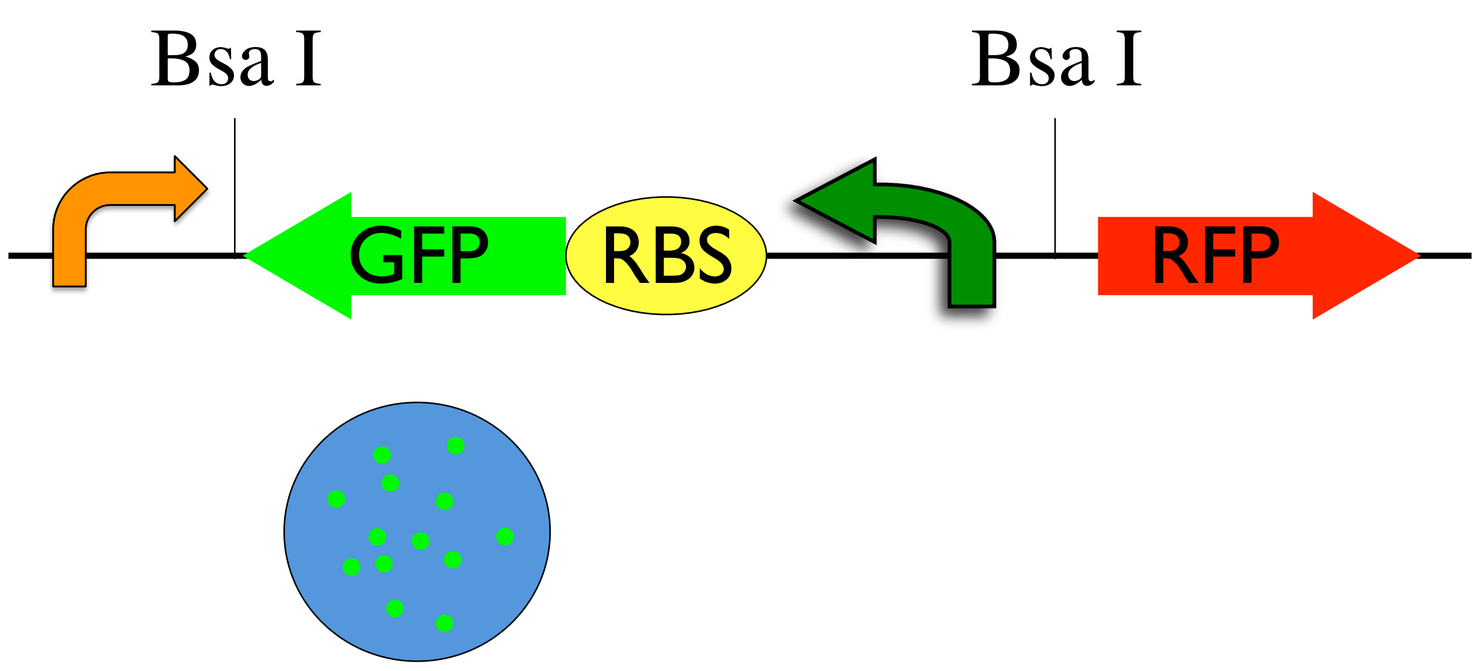
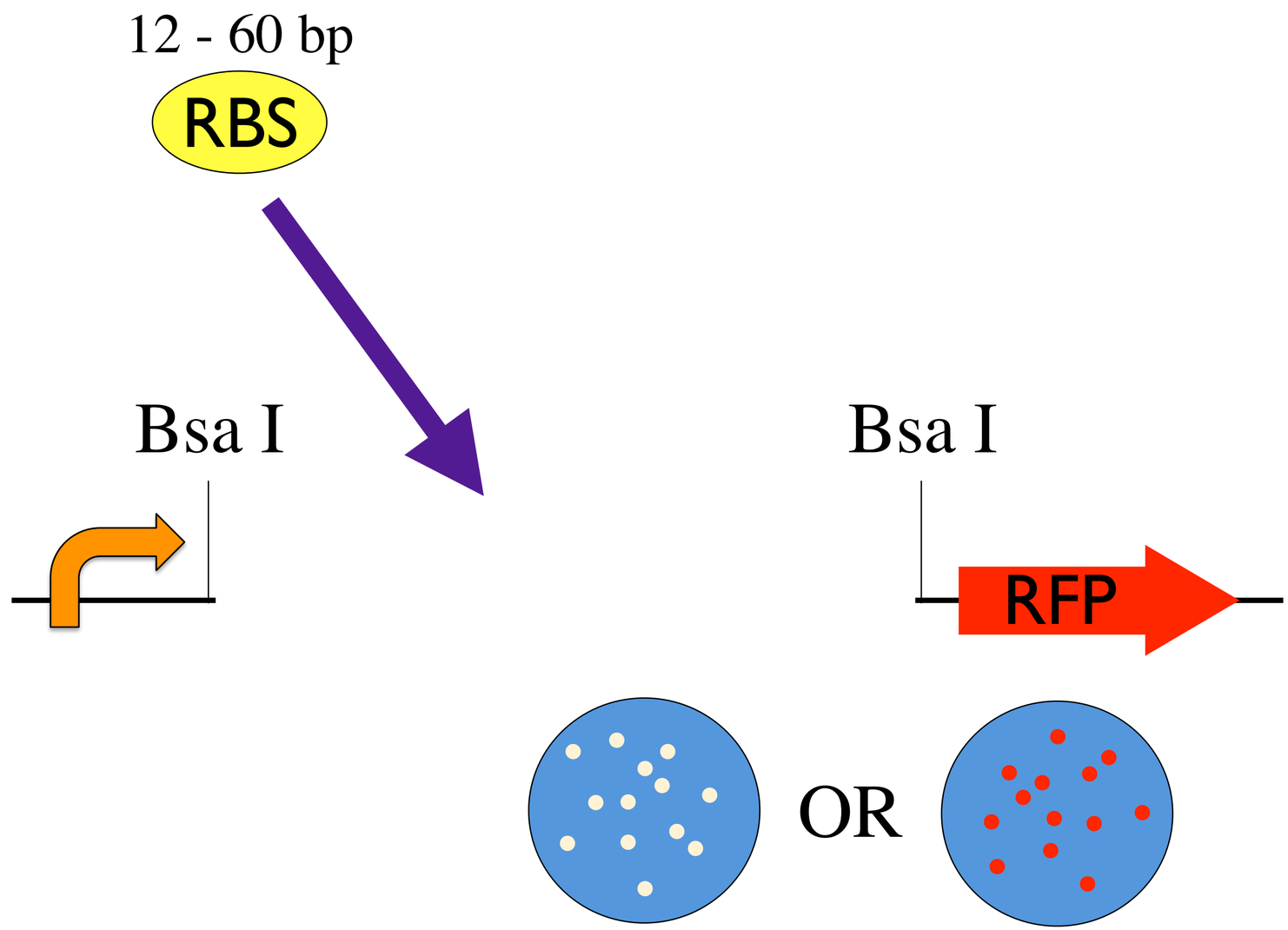
rClone Red

J119###



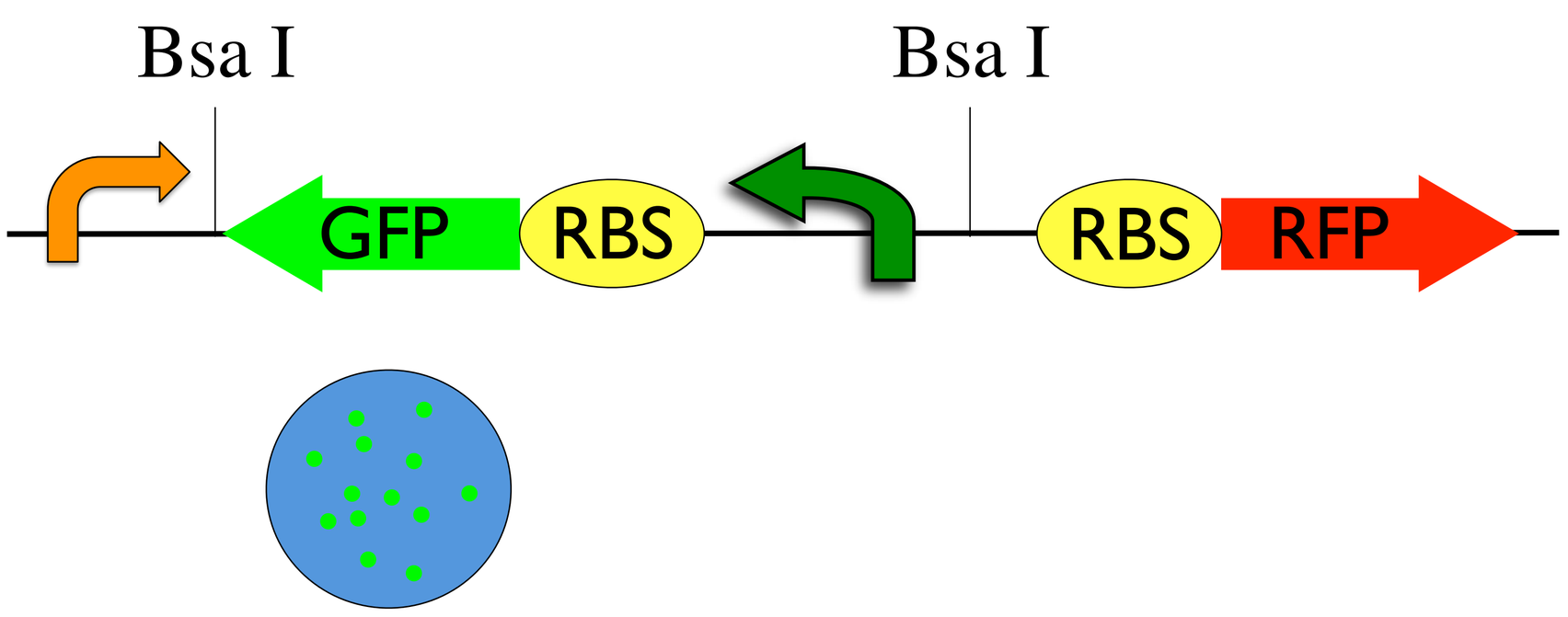
rClone Red

J119###



tClone Red

J119361



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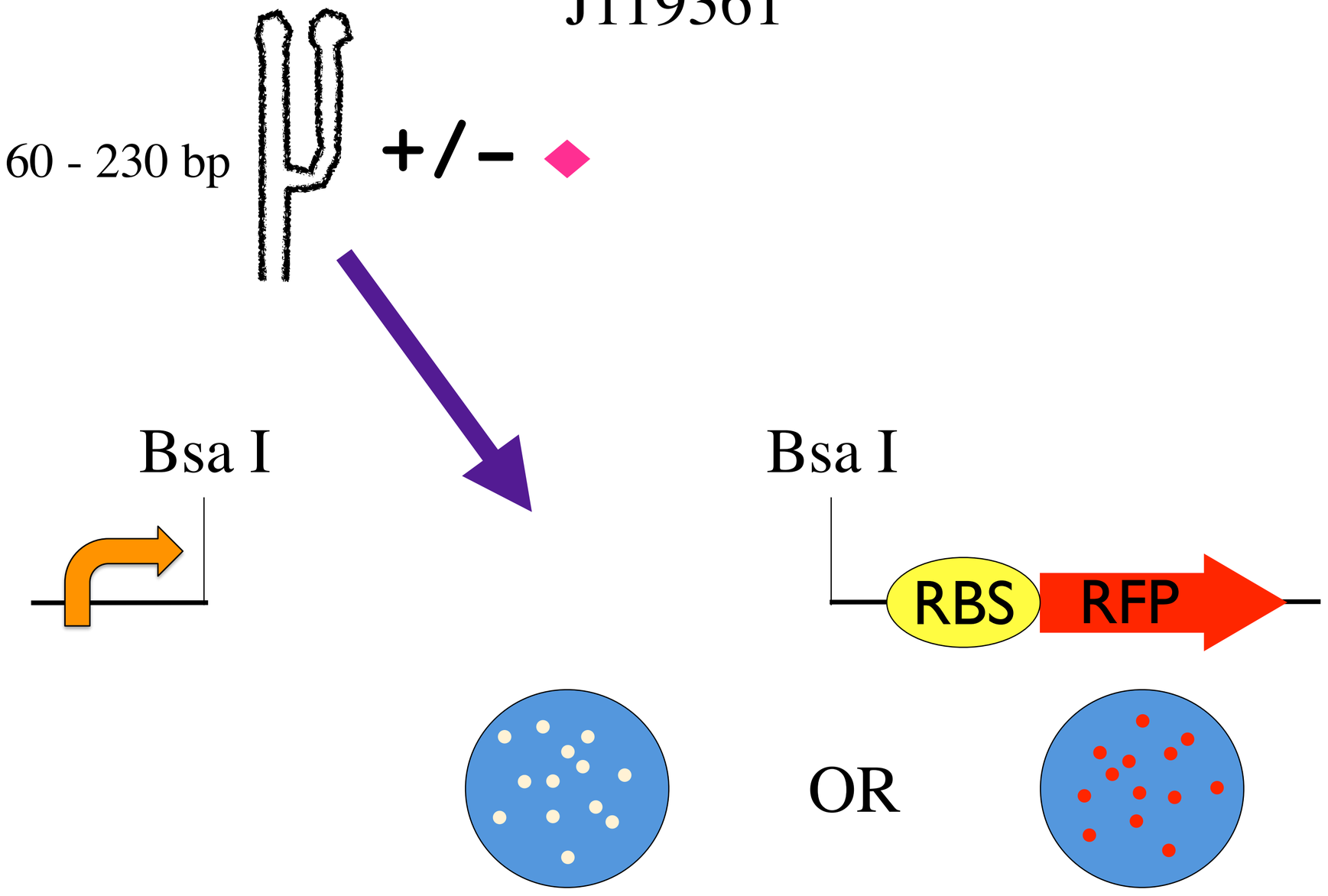
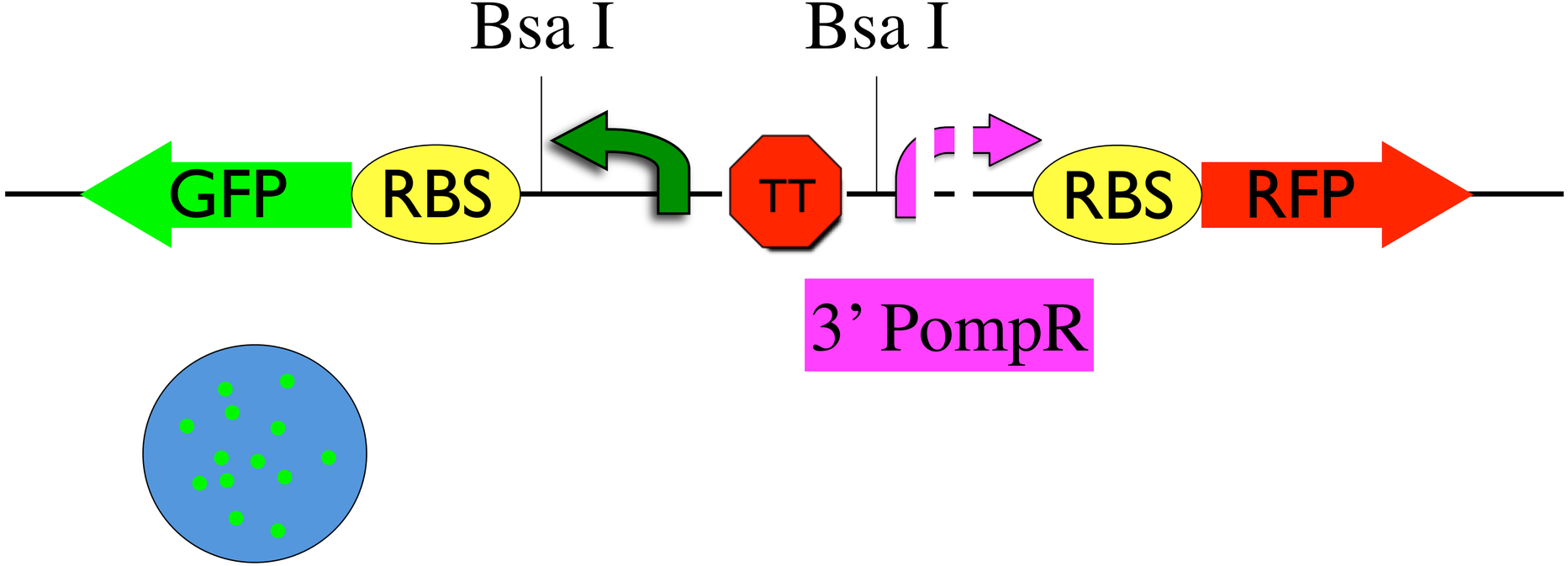


actClone Red

J100204



actClone Red

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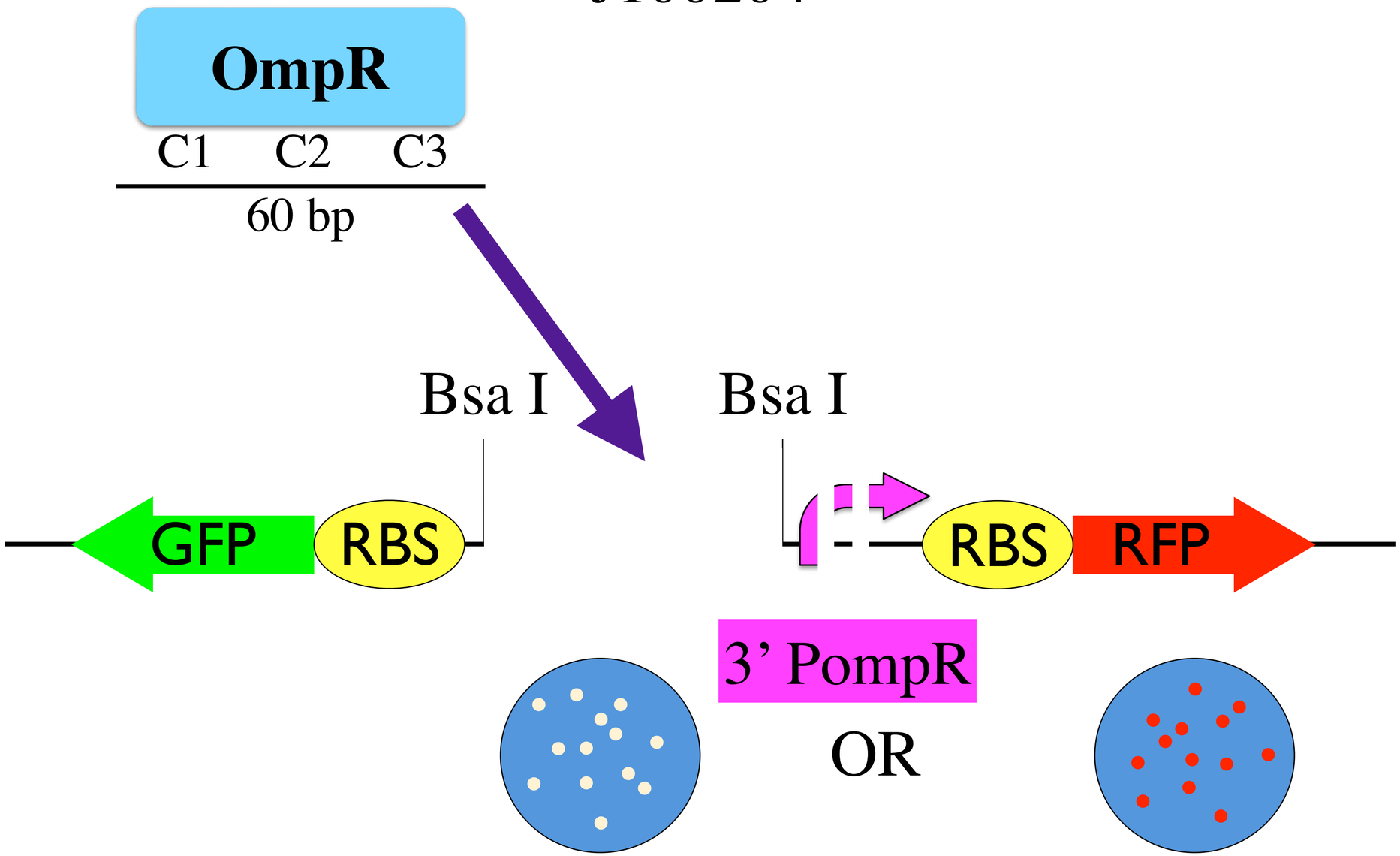
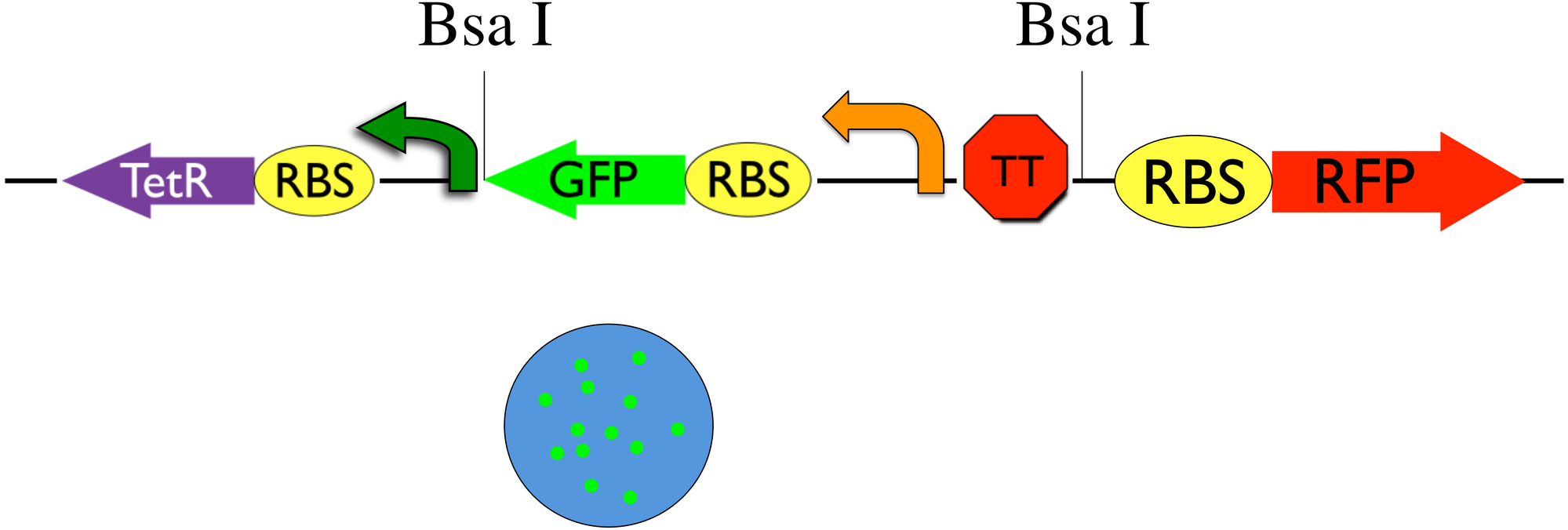


repClone Red

J100205



repClone Red

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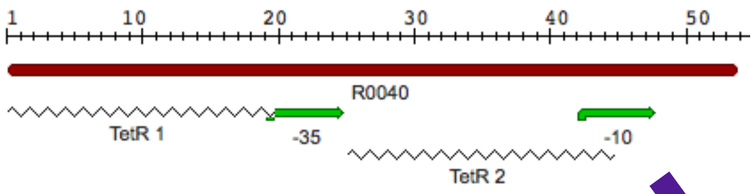
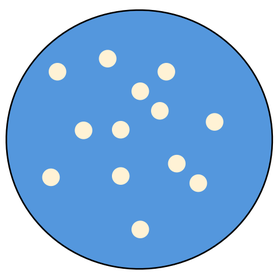


54 bp

Bsa I

Bsa I



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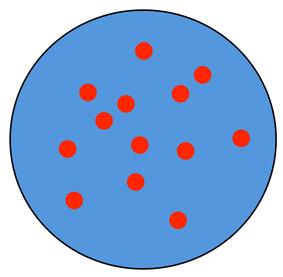


pClone: Synthetic Biology Tool Makes Promoter Research Accessible to Beginning Biology Students.

CBE Life Sciences Education. 2014. Vol. 13(2): 285 - 296.

DAVIDSON

MISSOURI WESTERN STATE UNIVERSITY

We want to design a new method to produce medications more efficiently.

What is the definition of evolution?

What is the definition of evolution?

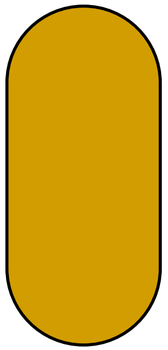
change in allele frequency in a population over time

How does natural selection work?

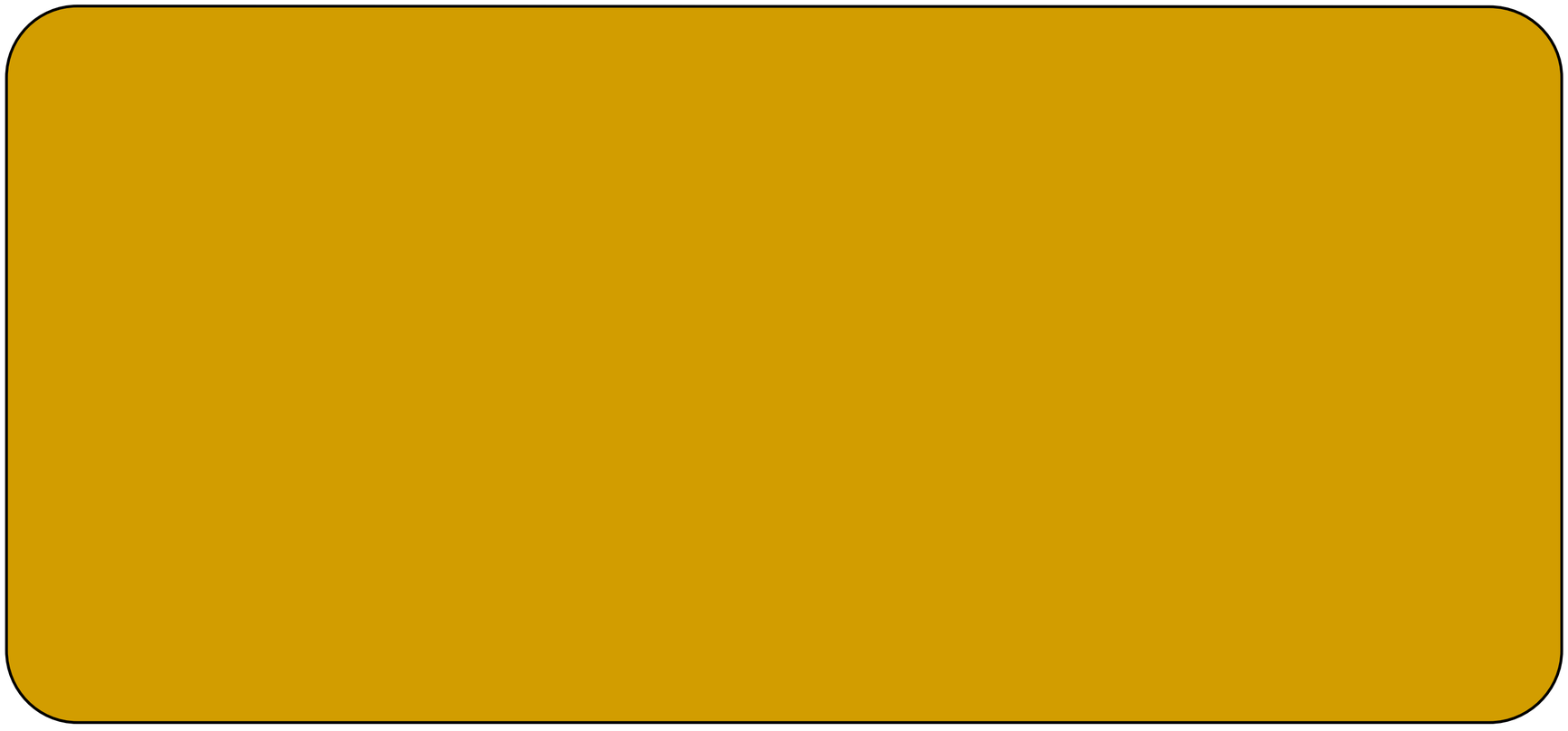
How does natural selection work?

1. over production
2. variation in the population
3. competition for limited resources
4. selective advantage
5. reproduction

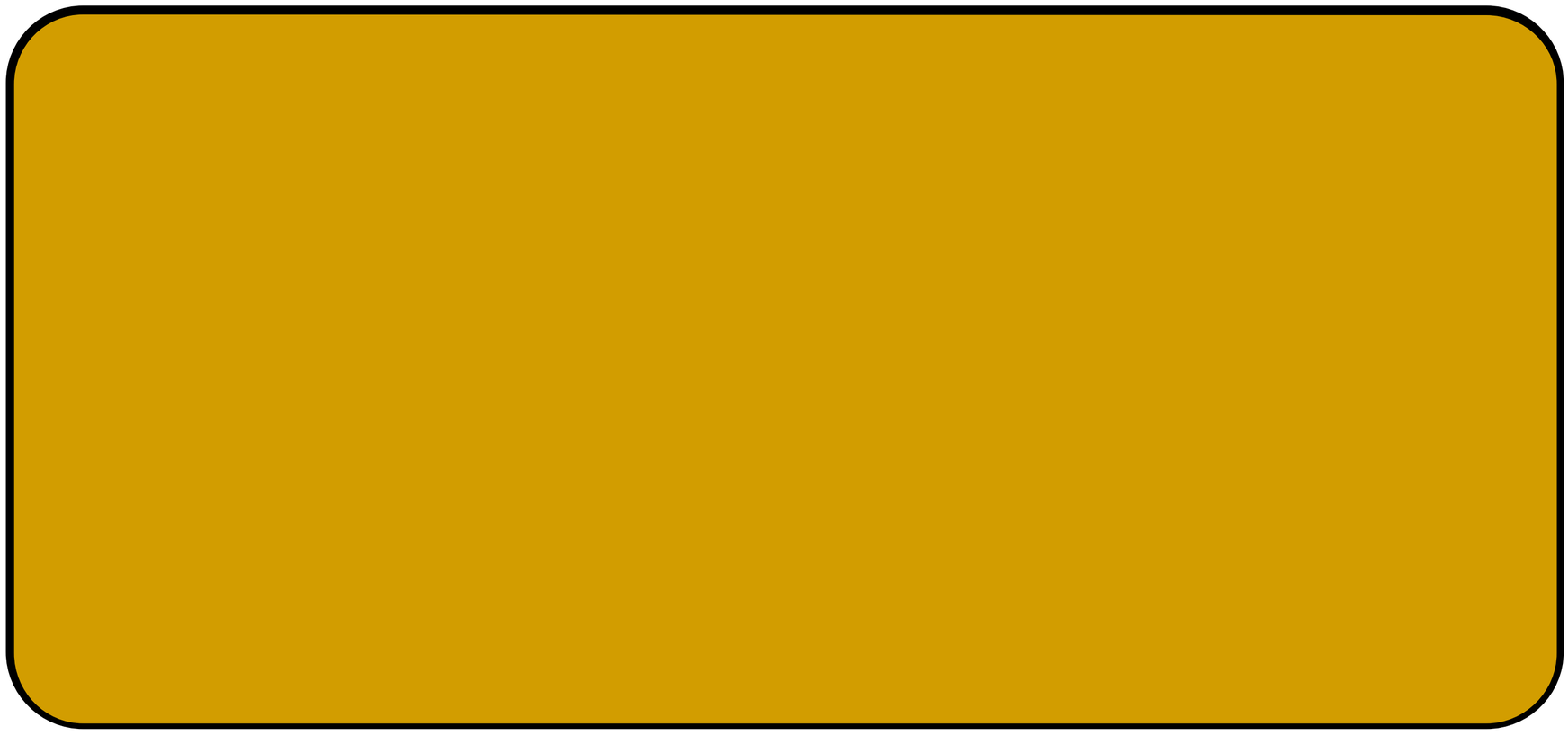
Make *E. coli* Optimize Drug Production



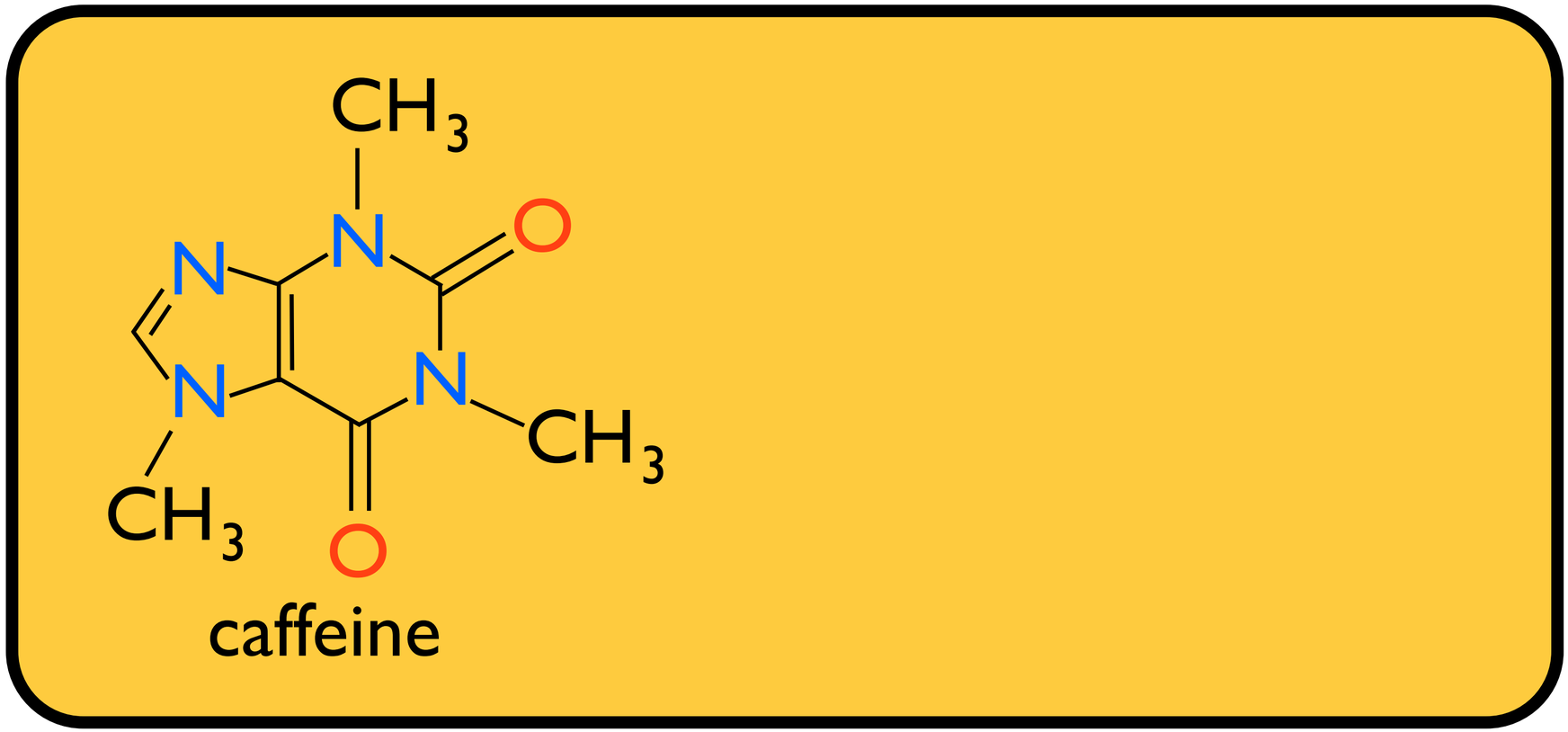
Make *E. coli* Optimize Drug Production



Make *E. coli* Optimize Drug Production

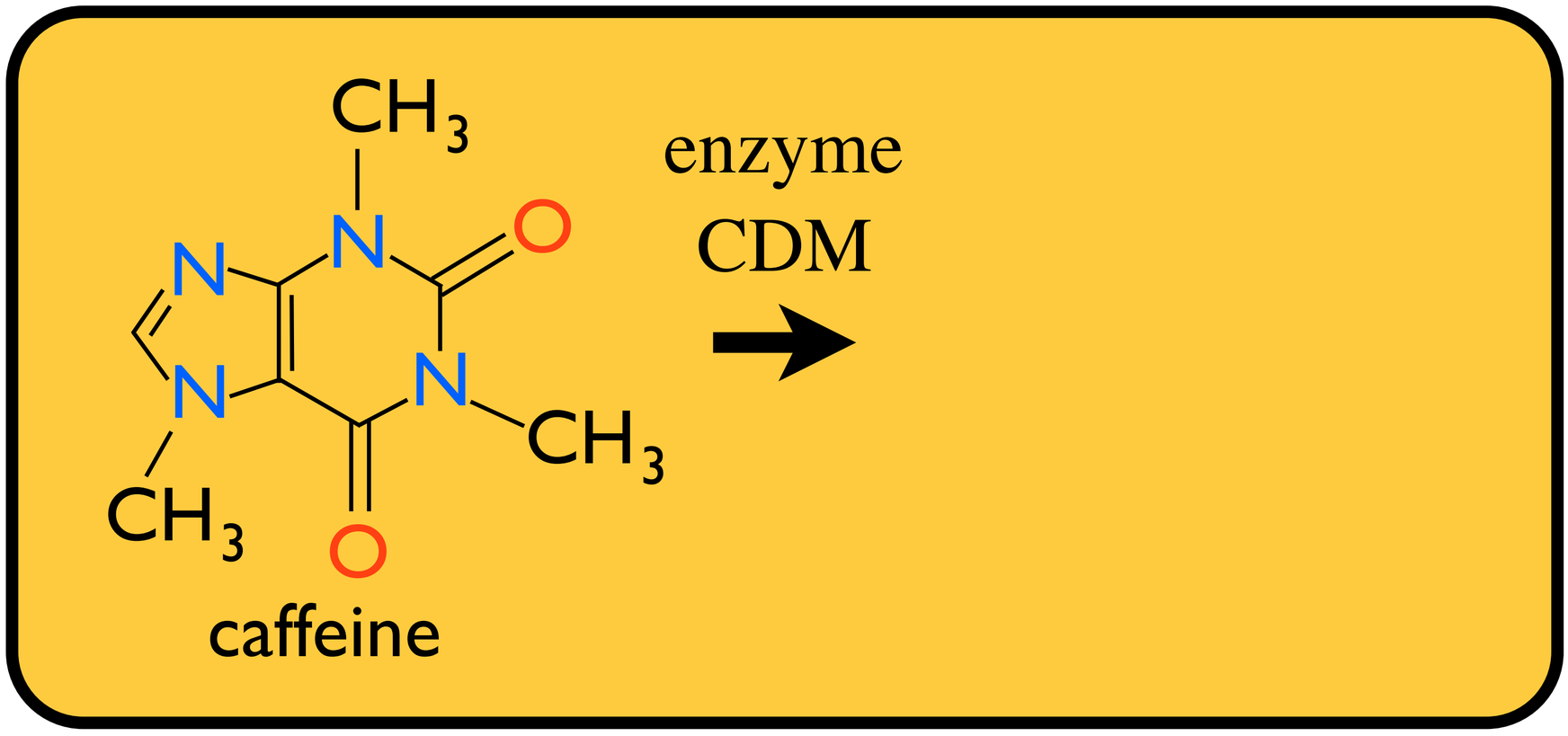


Make *E. coli* Optimize Drug Production

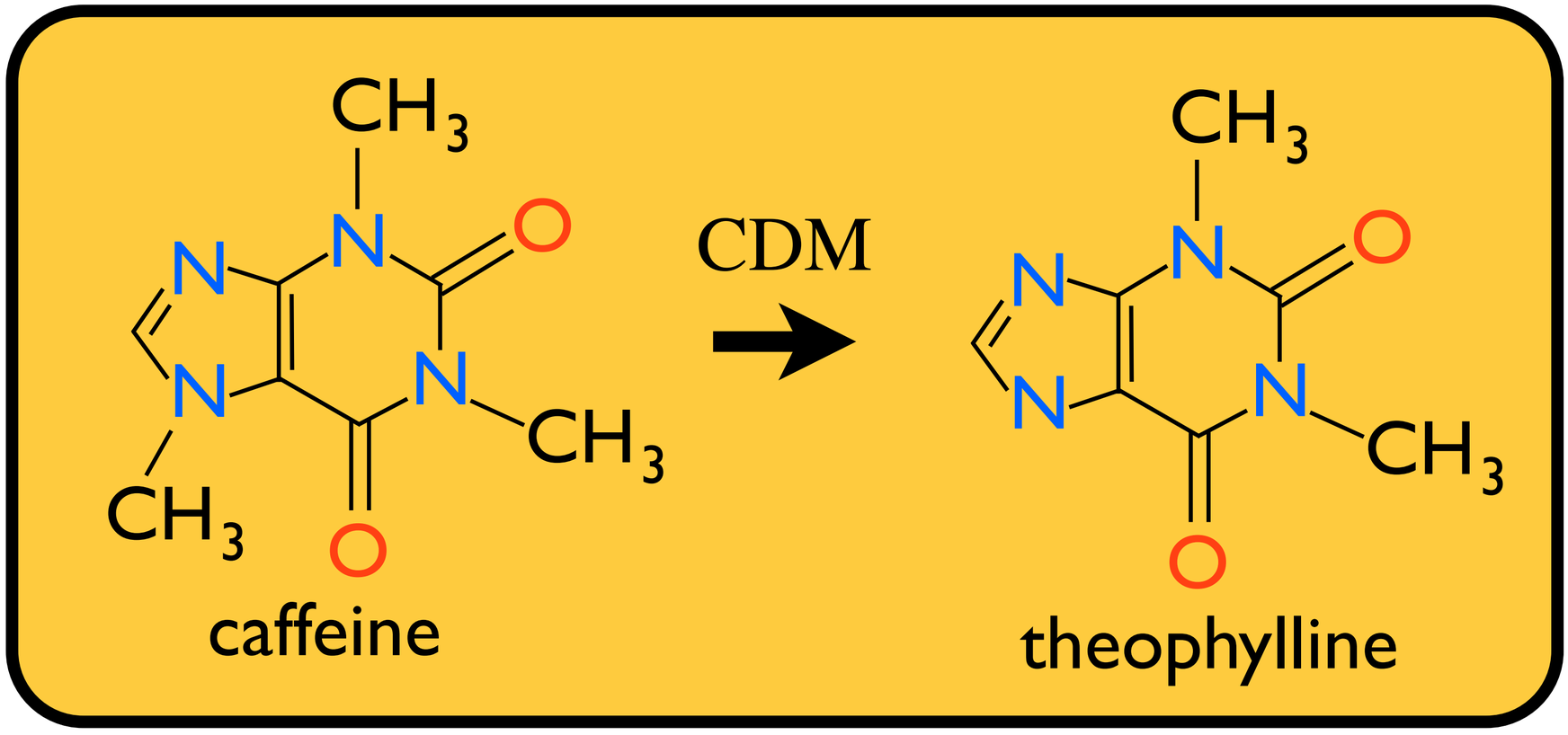


caffeine

Make *E. coli* Optimize Drug Production

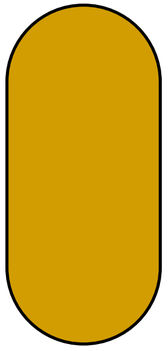


Make *E. coli* Optimize Drug Production

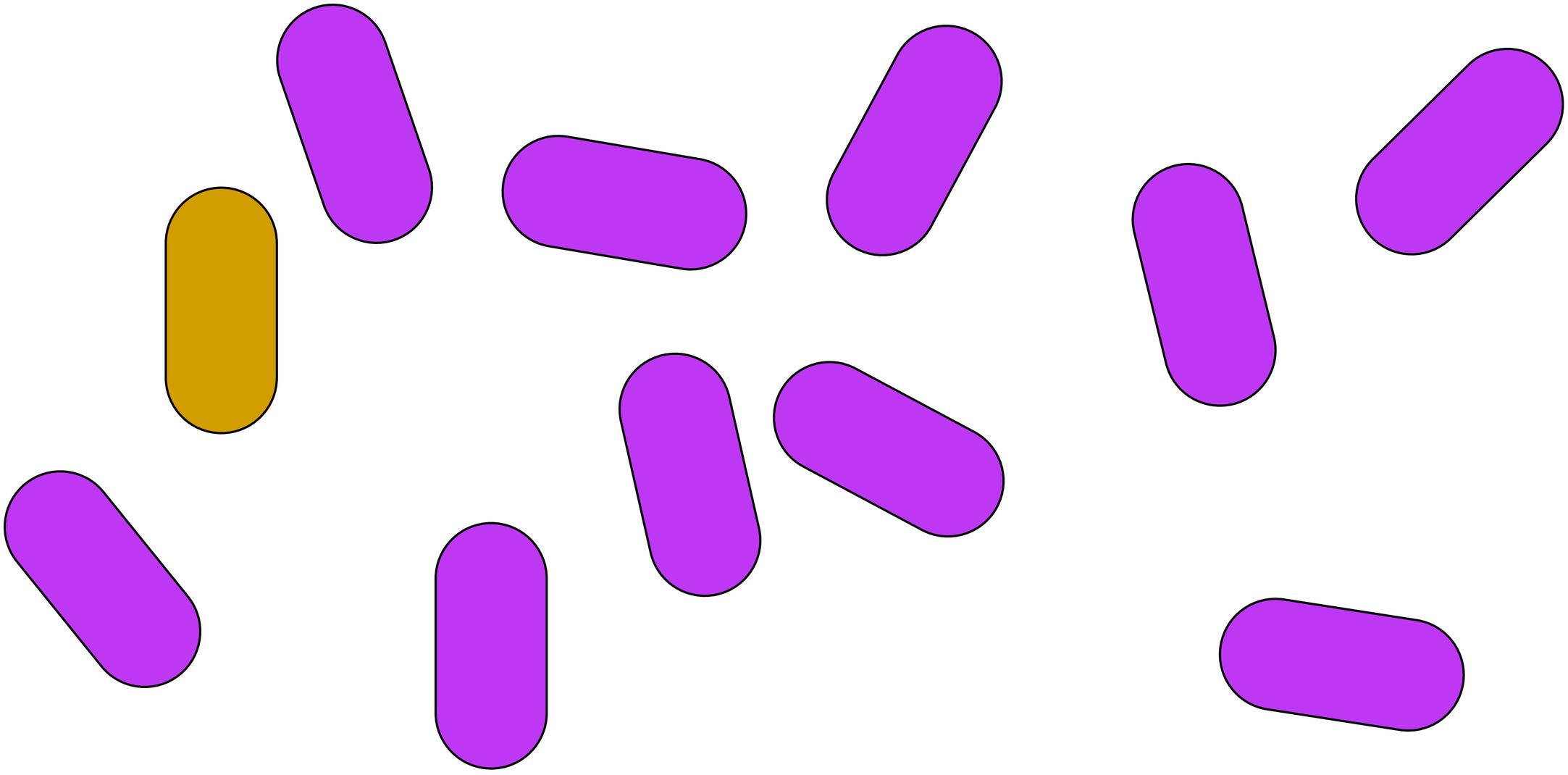


asthma medication

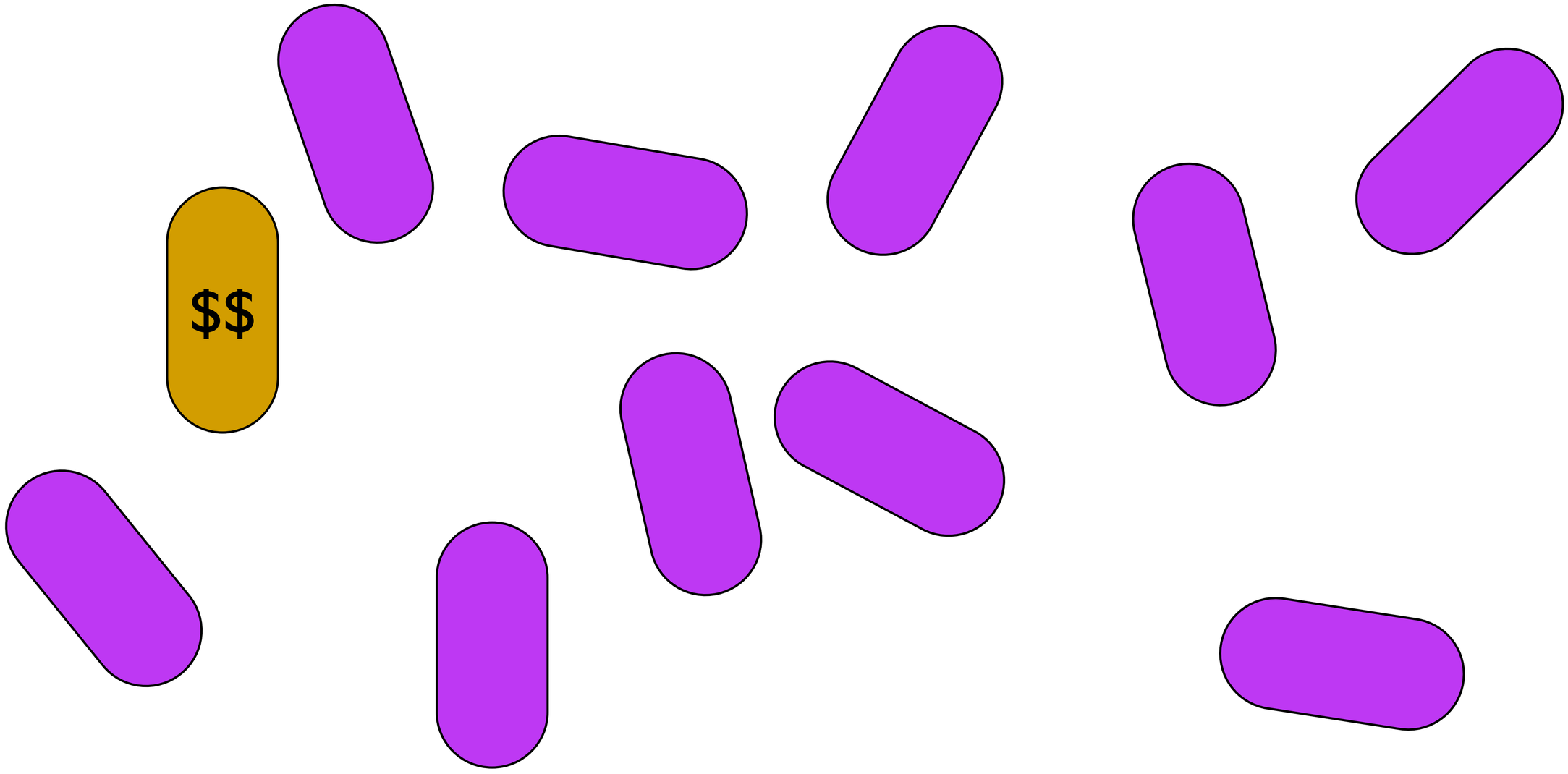
What Makes Optimization Difficult?



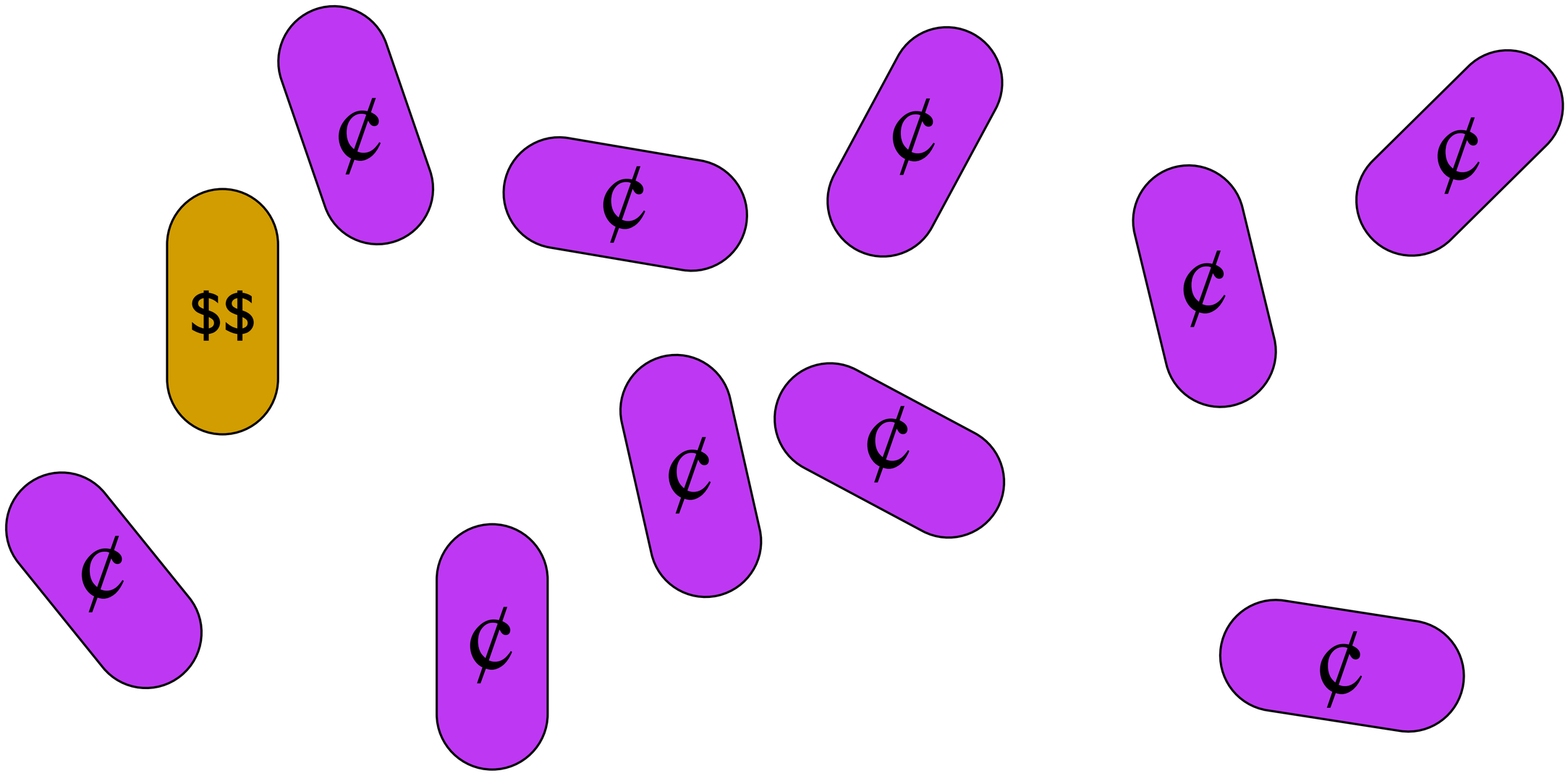
What Makes Optimization Difficult?



What Makes Optimization Difficult?



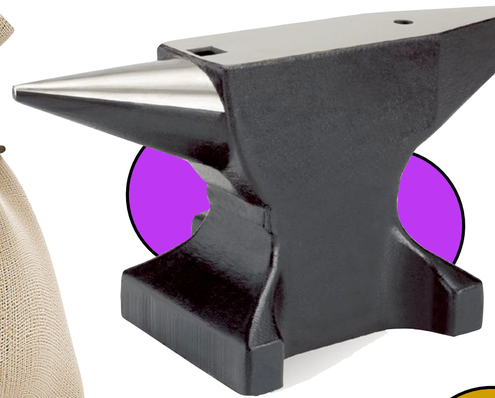
Natural Selection



Synthetic Selection

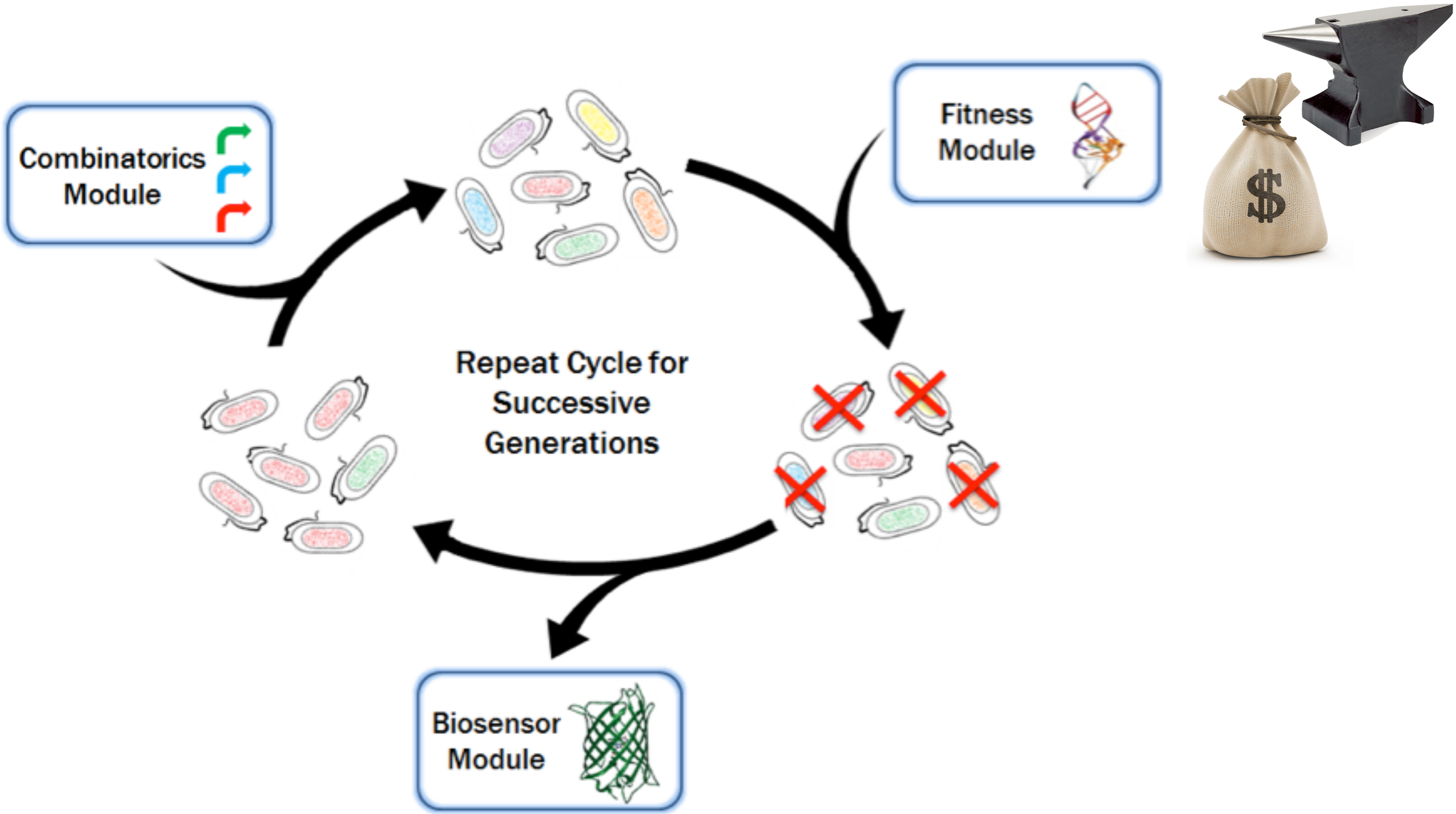
Synthetic Fitness

Synthetic Fitness

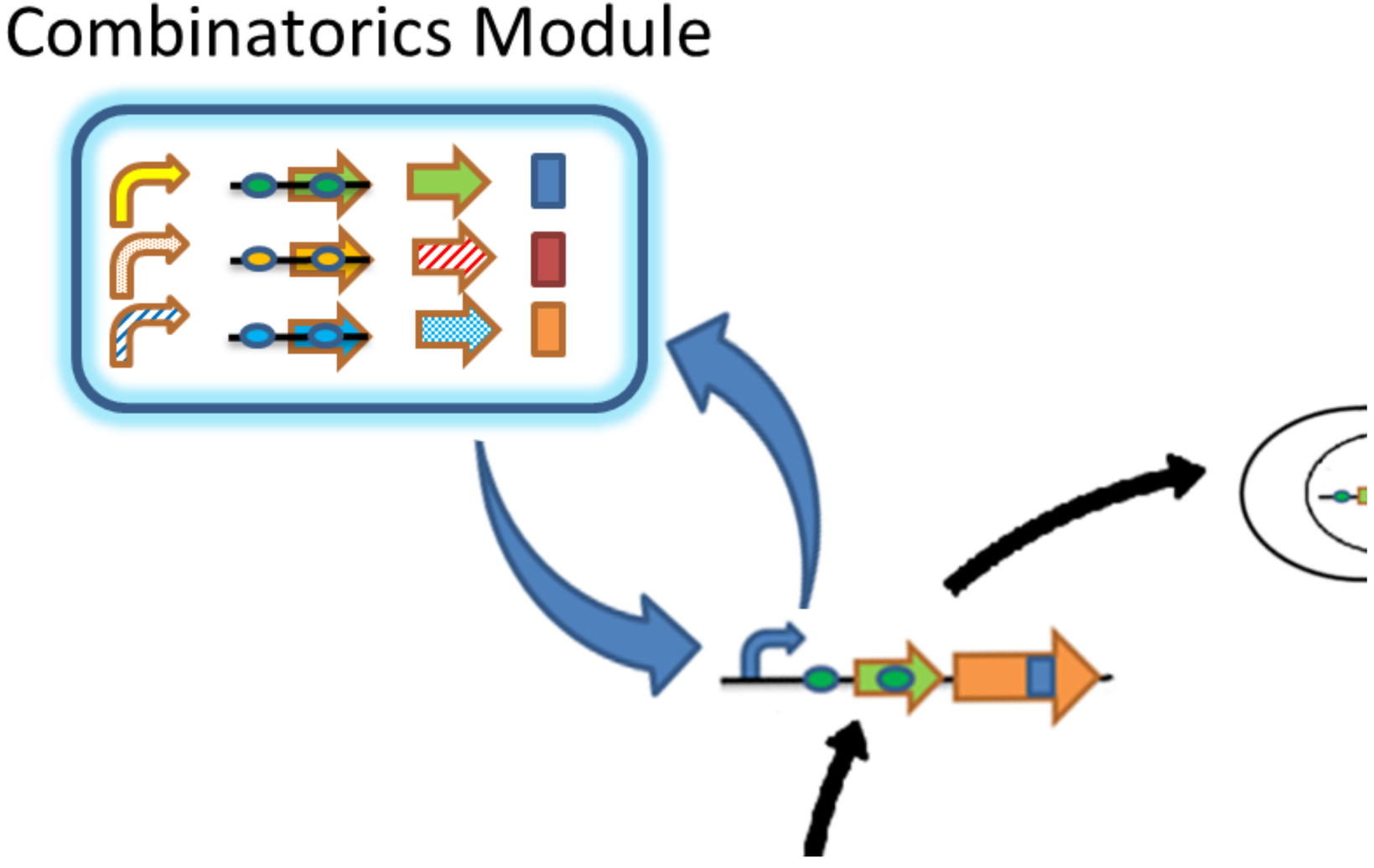


Engineering Programmed Evolution

Programmed Evolution



Programmed Evolution



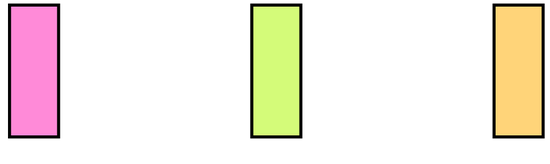
Combinatorics Cloning

Promoters (7)

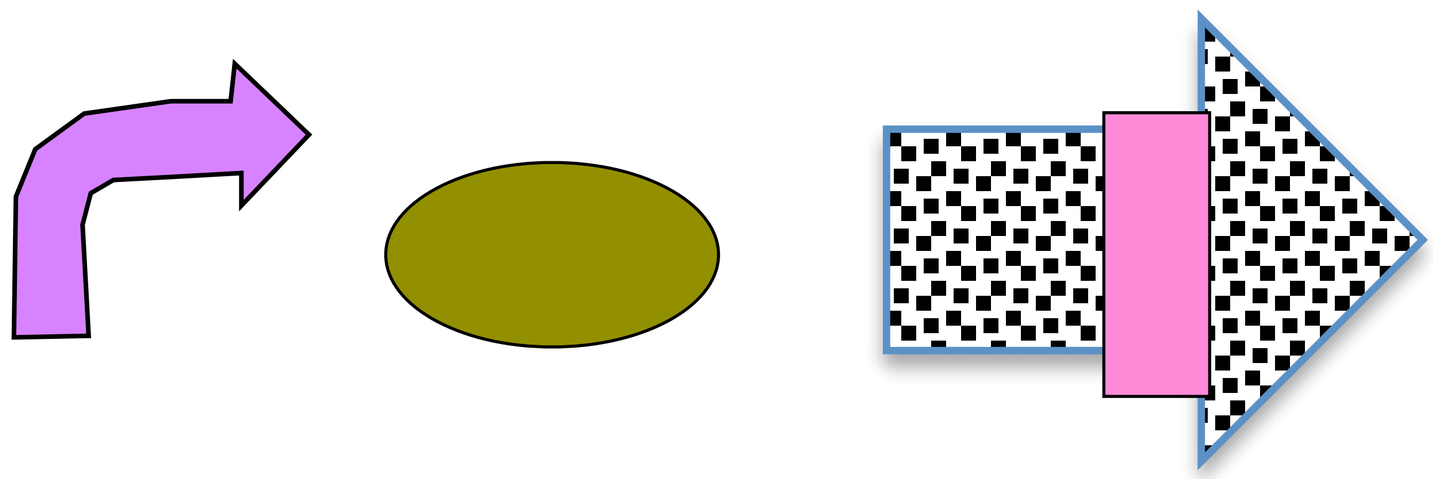
RBS (4)

Allele (6)

Degradation tag (3)

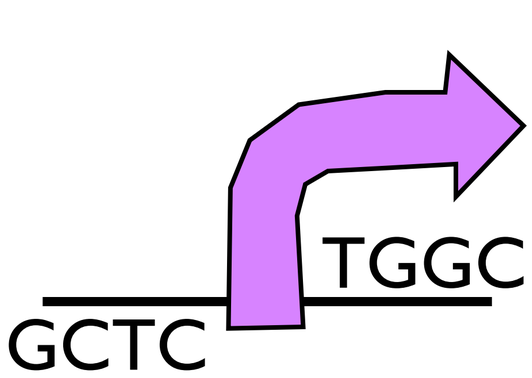


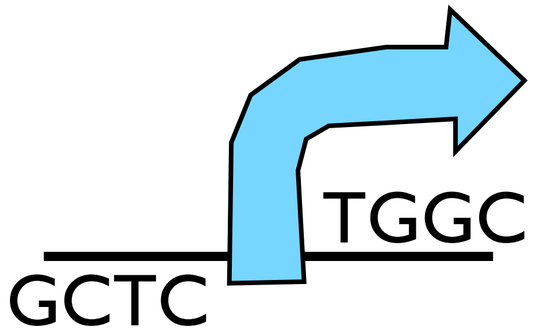
Combinatorics Cloning

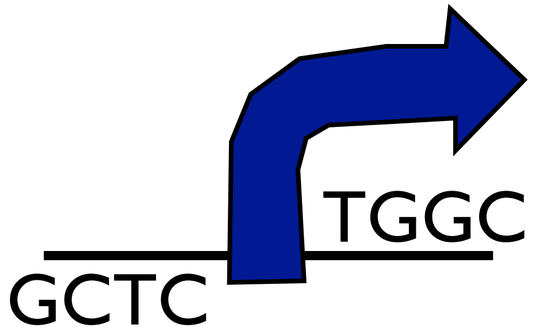


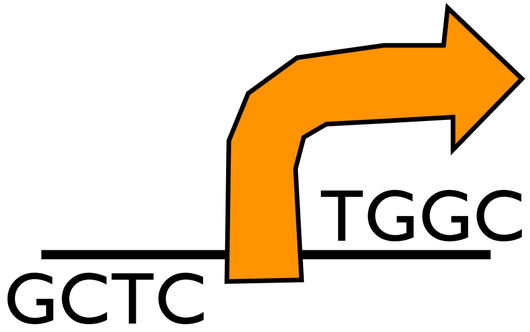
Total # constructs
= 8 x 4 x 6 x 3 = 576

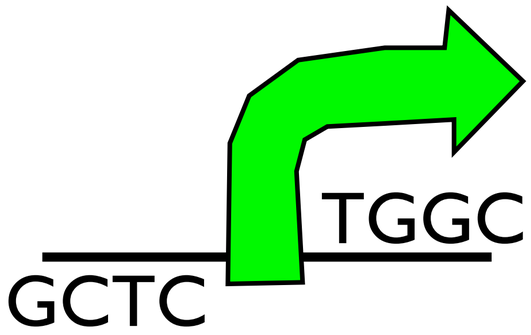
Combinatorics Cloning

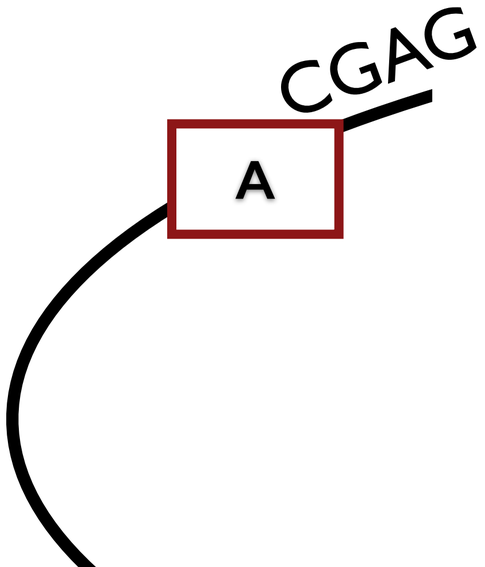


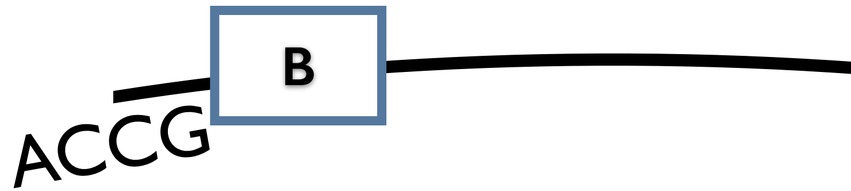




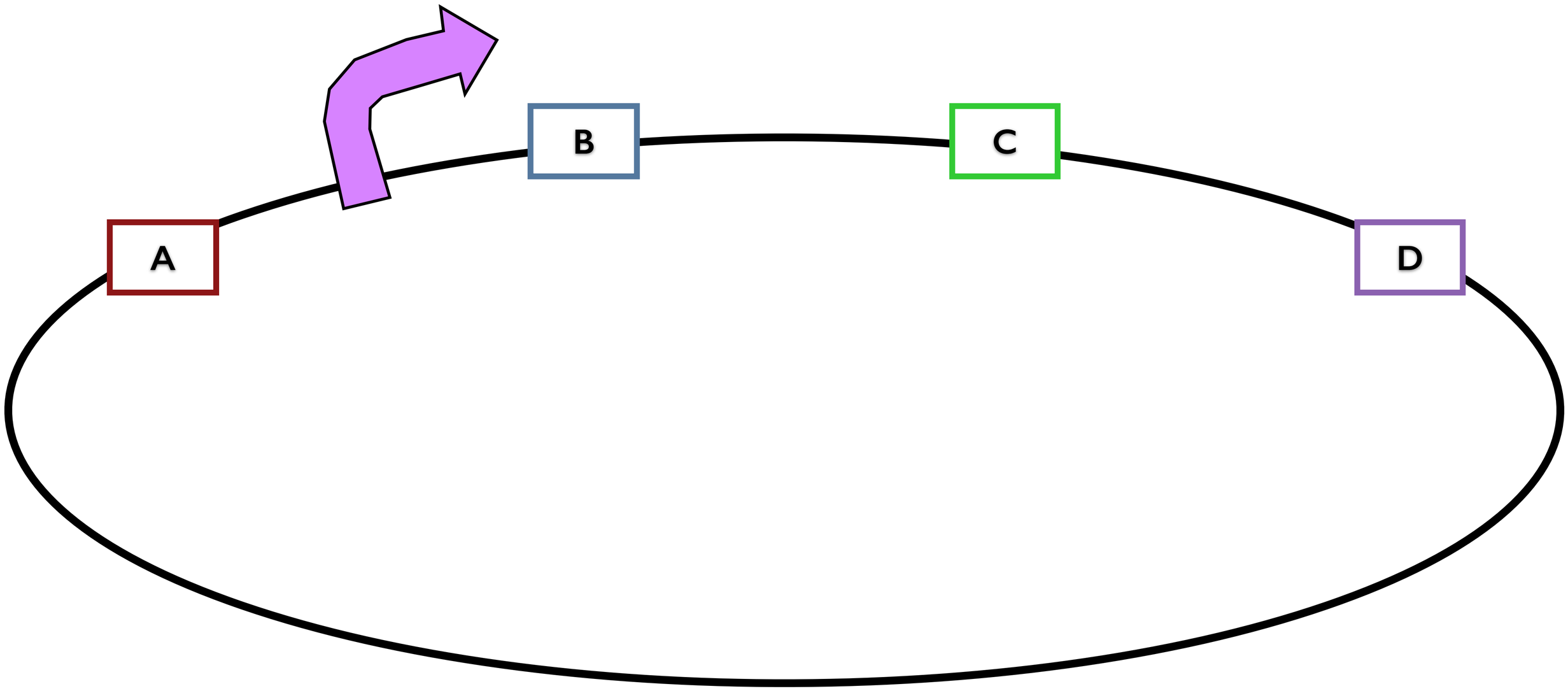




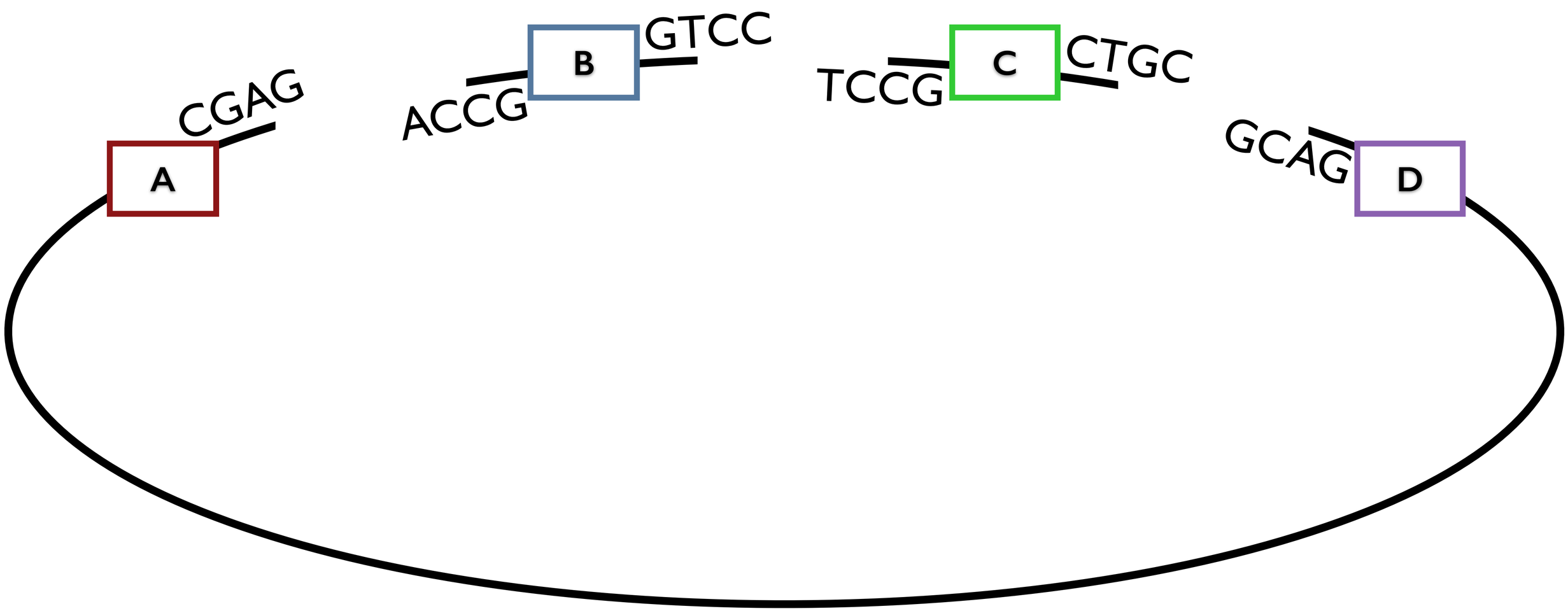




Combinatorics Cloning



Combinatorics Cloning



Finding Compatible Sticky Ends

GGAJET

Golden Gate Assembly Junction Evaluative Tool

Instructions

Sources

*Plasmid/Gene Sequences (FASTA format only):

>Sequence Name

http://gcat.davidson.edu/SynBio13/GGAJET/

* Restriction enzyme: BsaI — Currently limited to BsaI

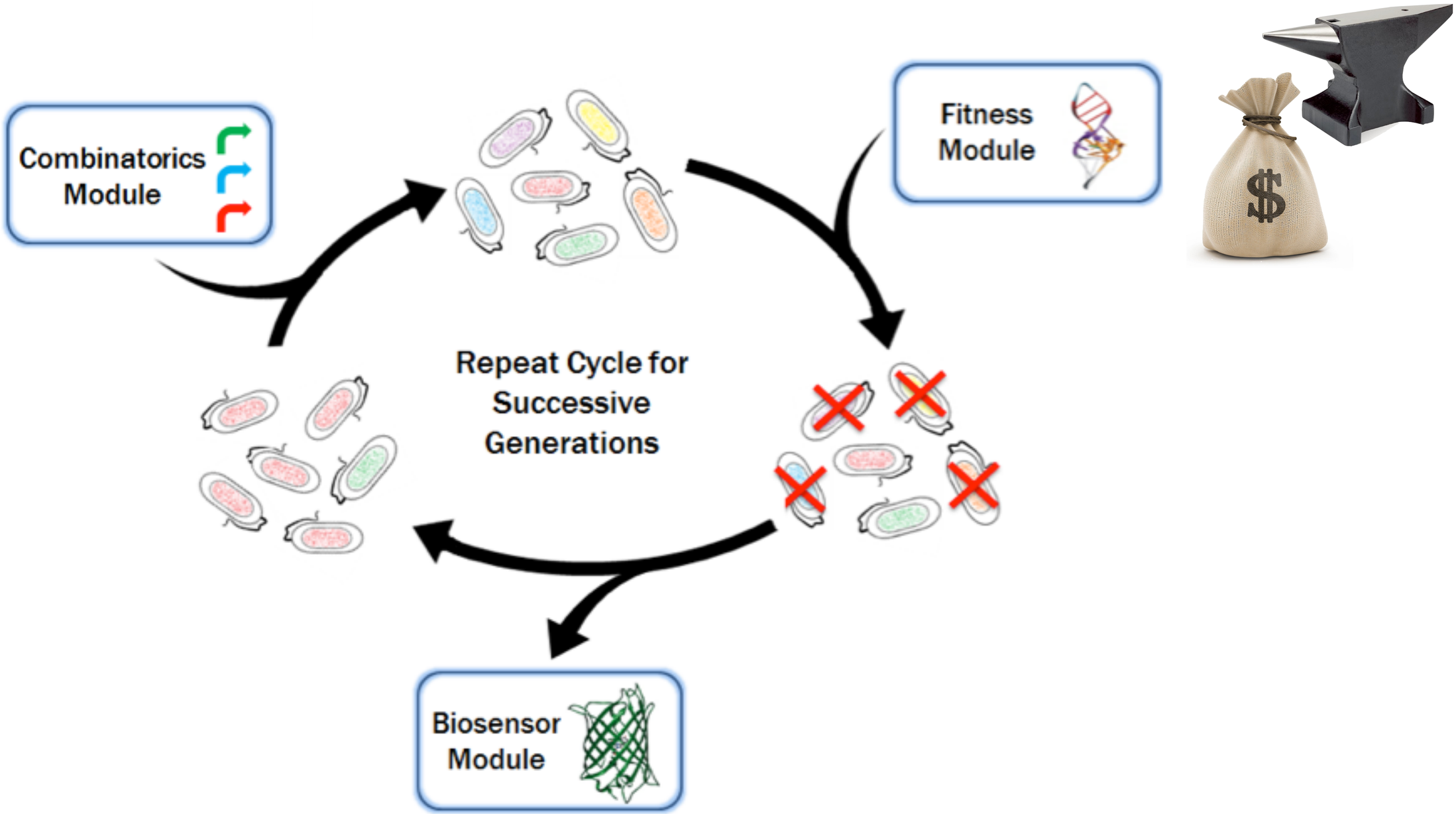
* Melting temperature (°C):

* Junction length (base pairs):

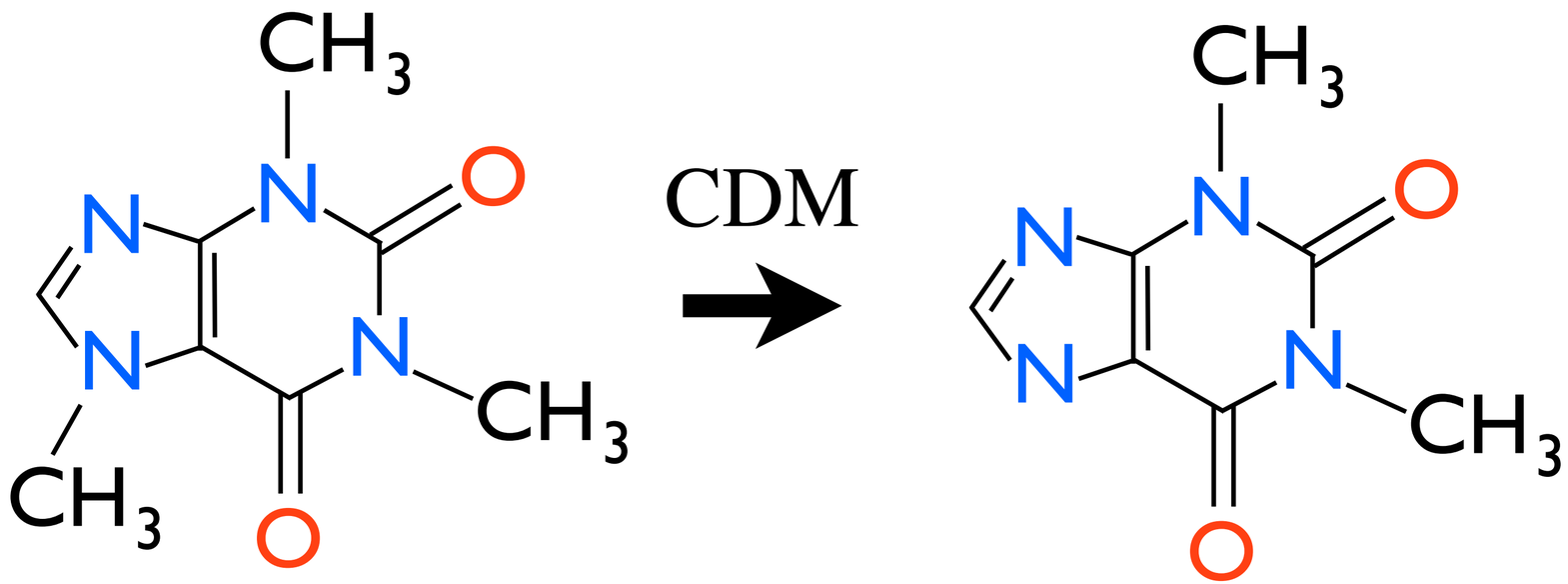
* Number of desired junctions: — Currently limited to 4 junctions

PCR annealing temperature (°C):

Programmed Evolution



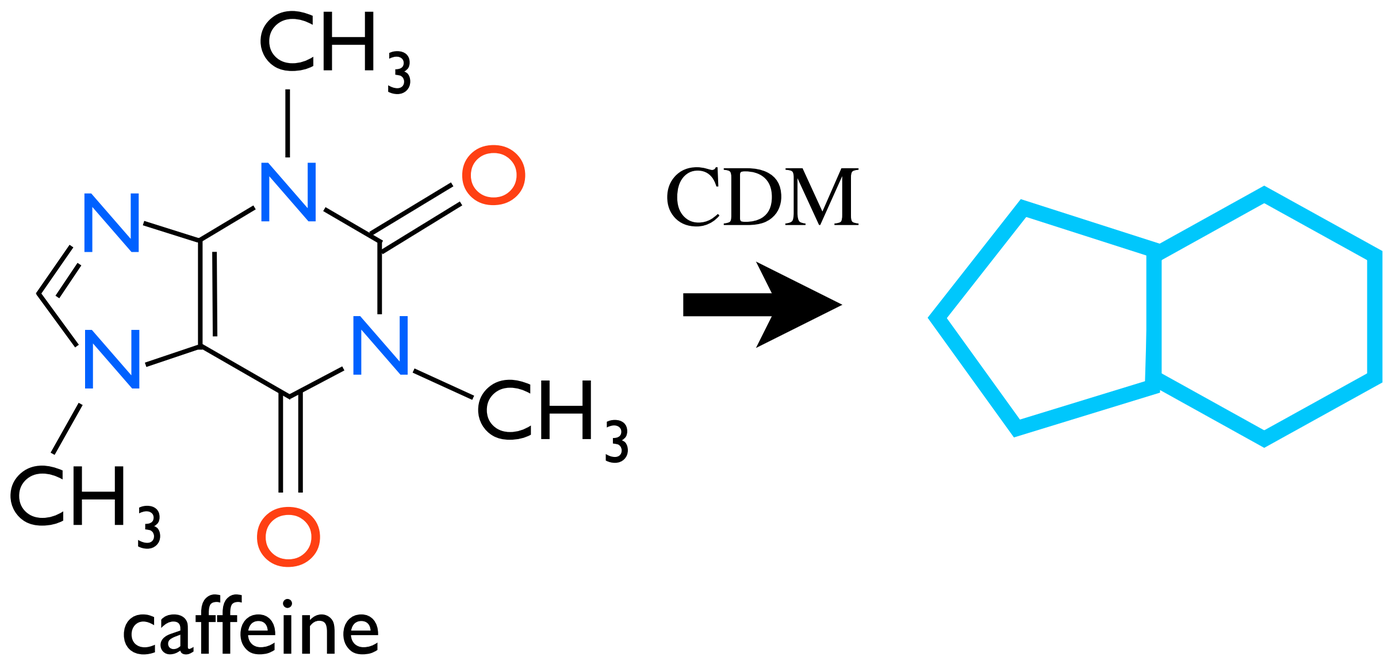
How to Build a Biosensor



caffeine

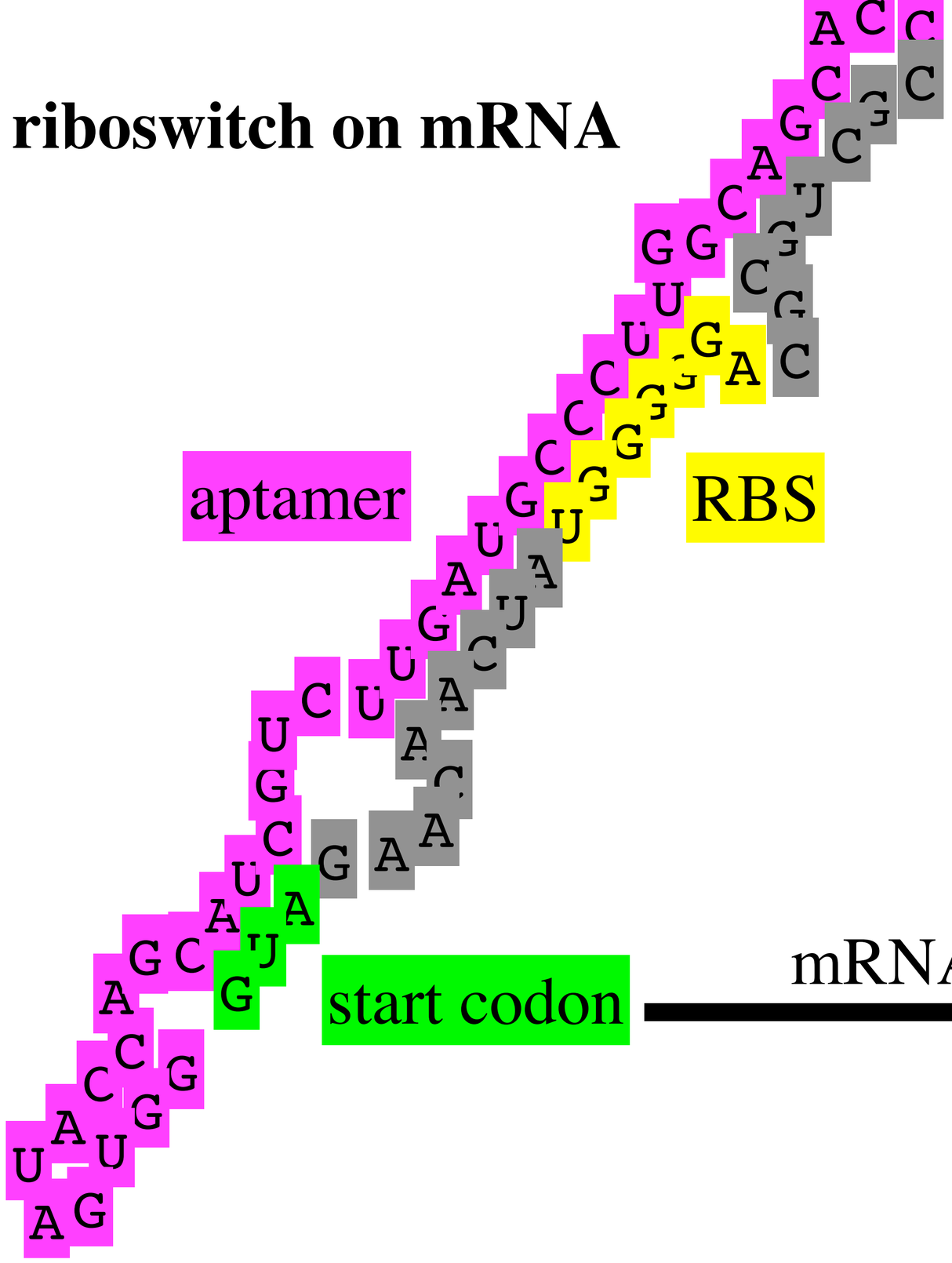
theophylline

How to Build a Biosensor



caffeine

riboswitch on mRNA

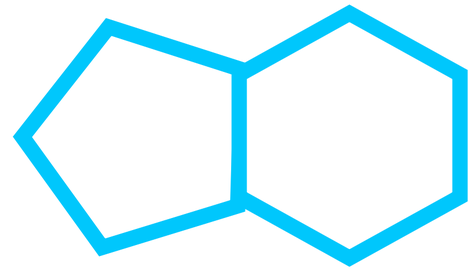


aptamer

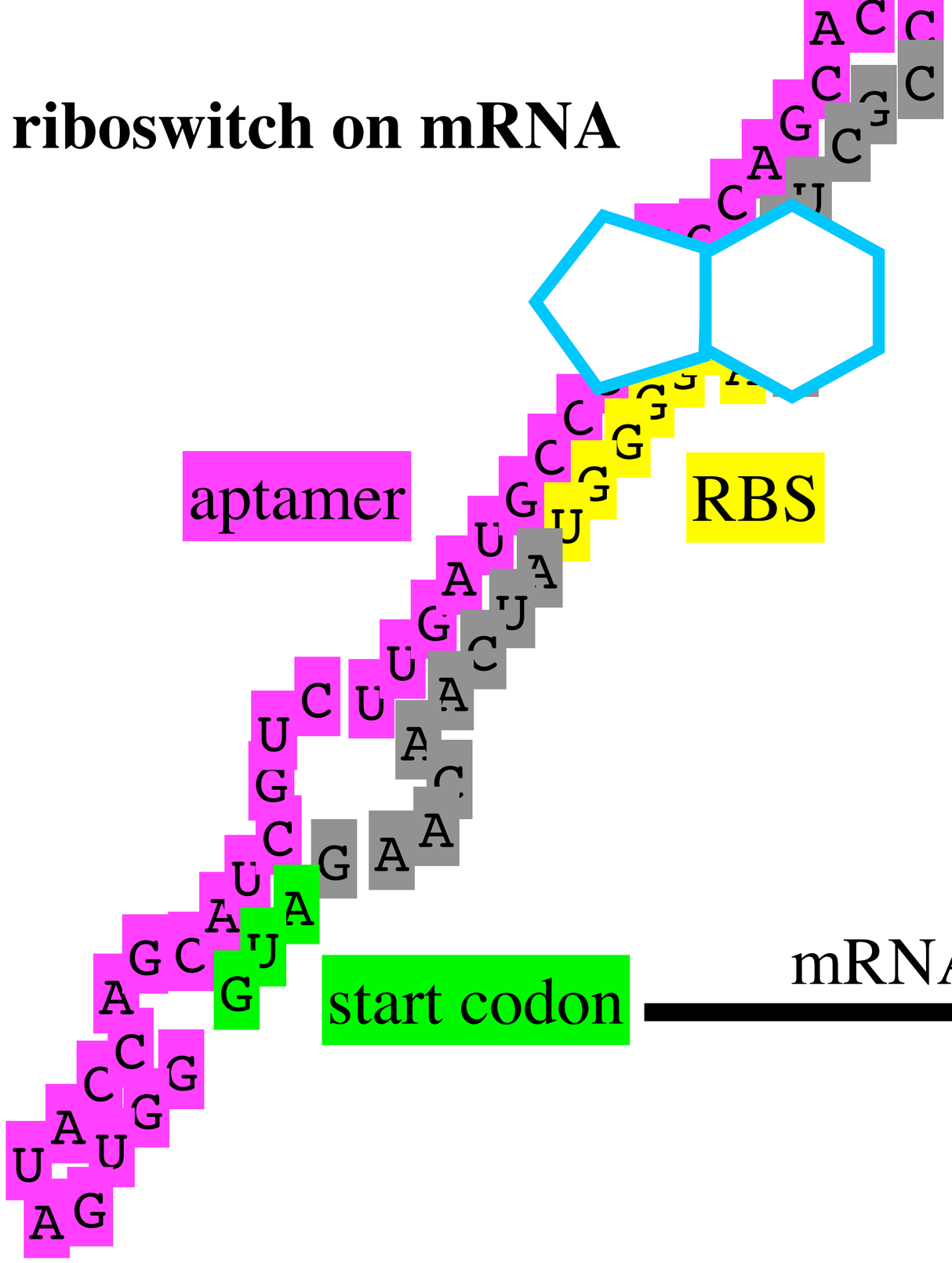
RBS

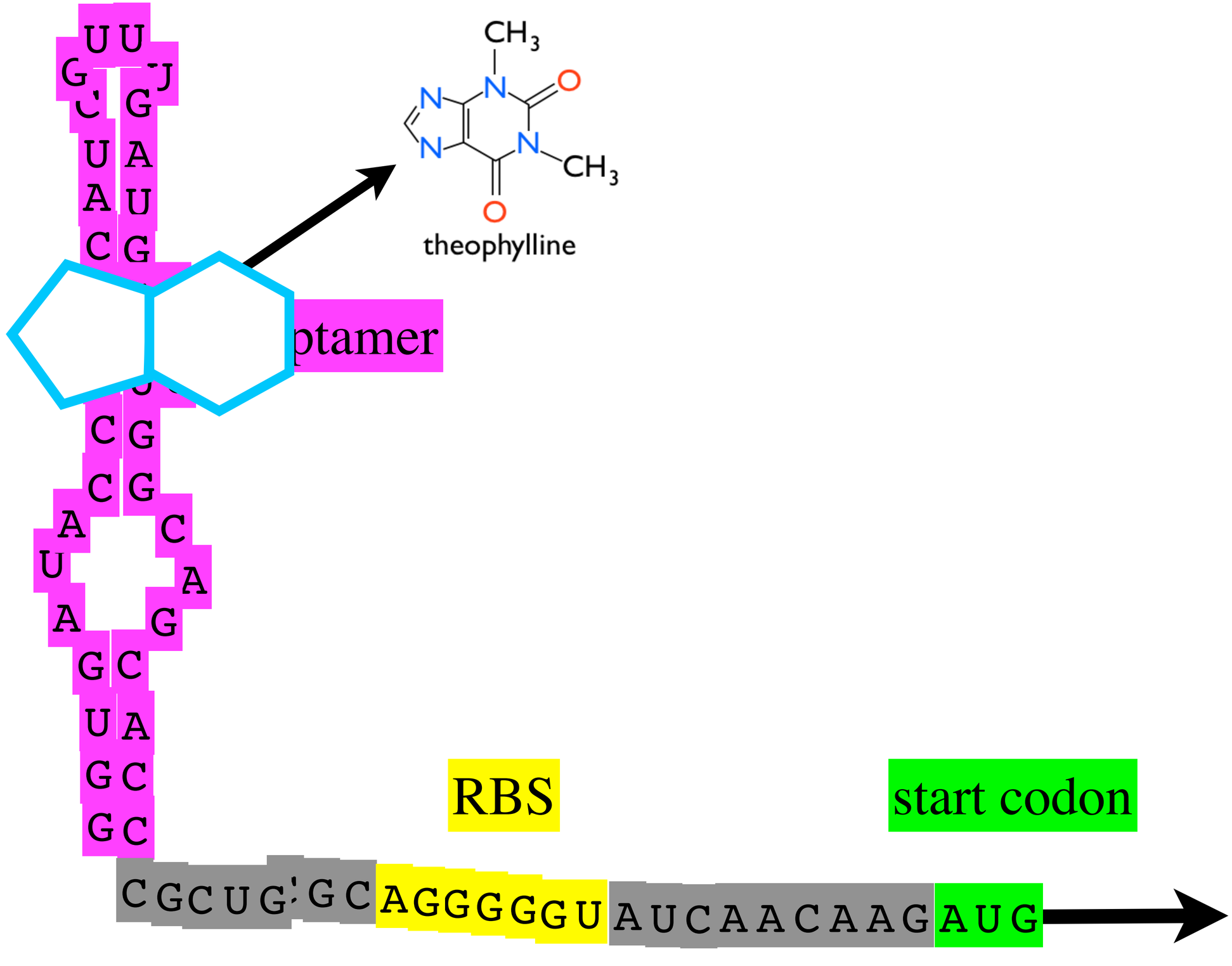
start codon

mRNA fitness gene

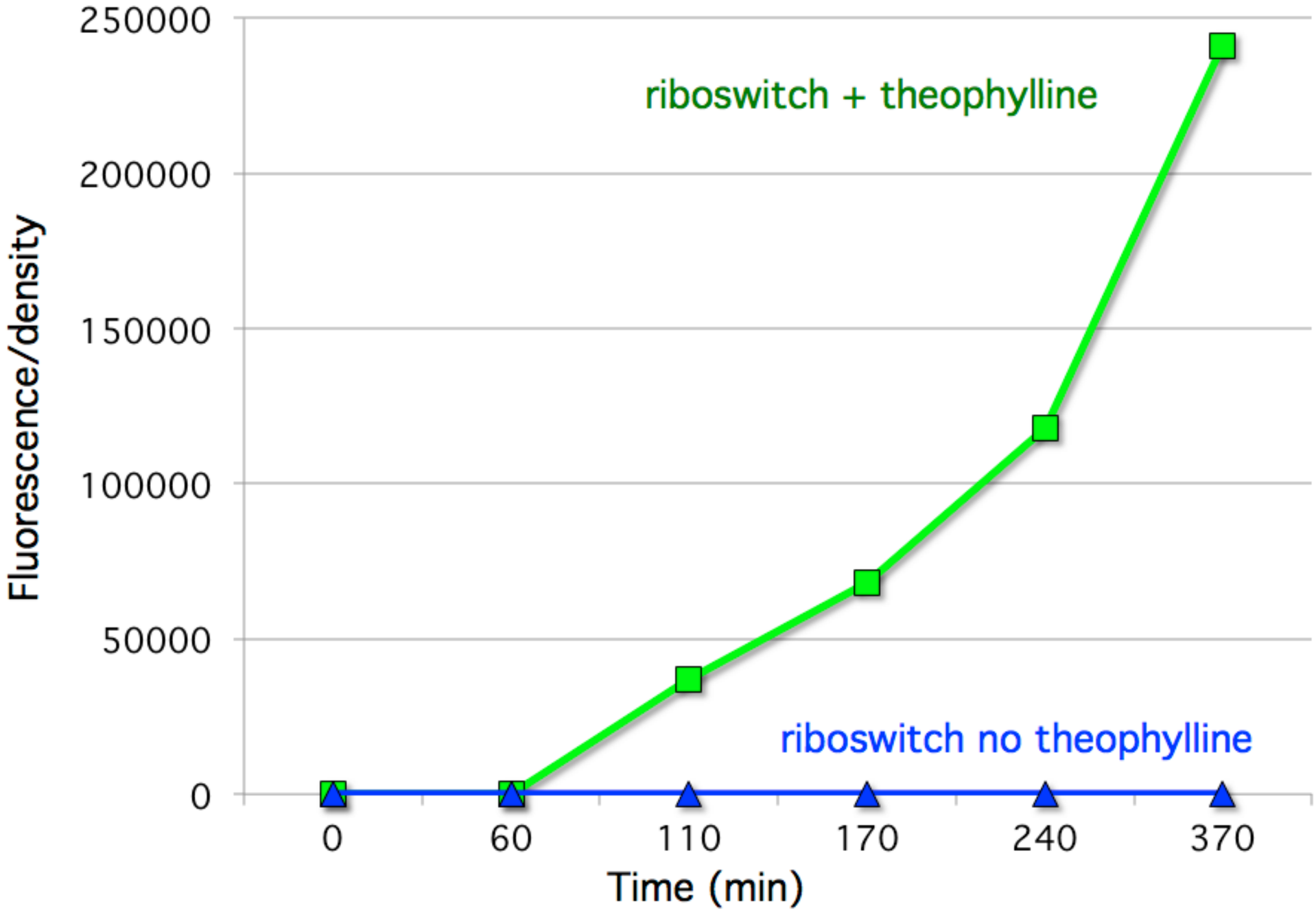


riboswitch on mRNA

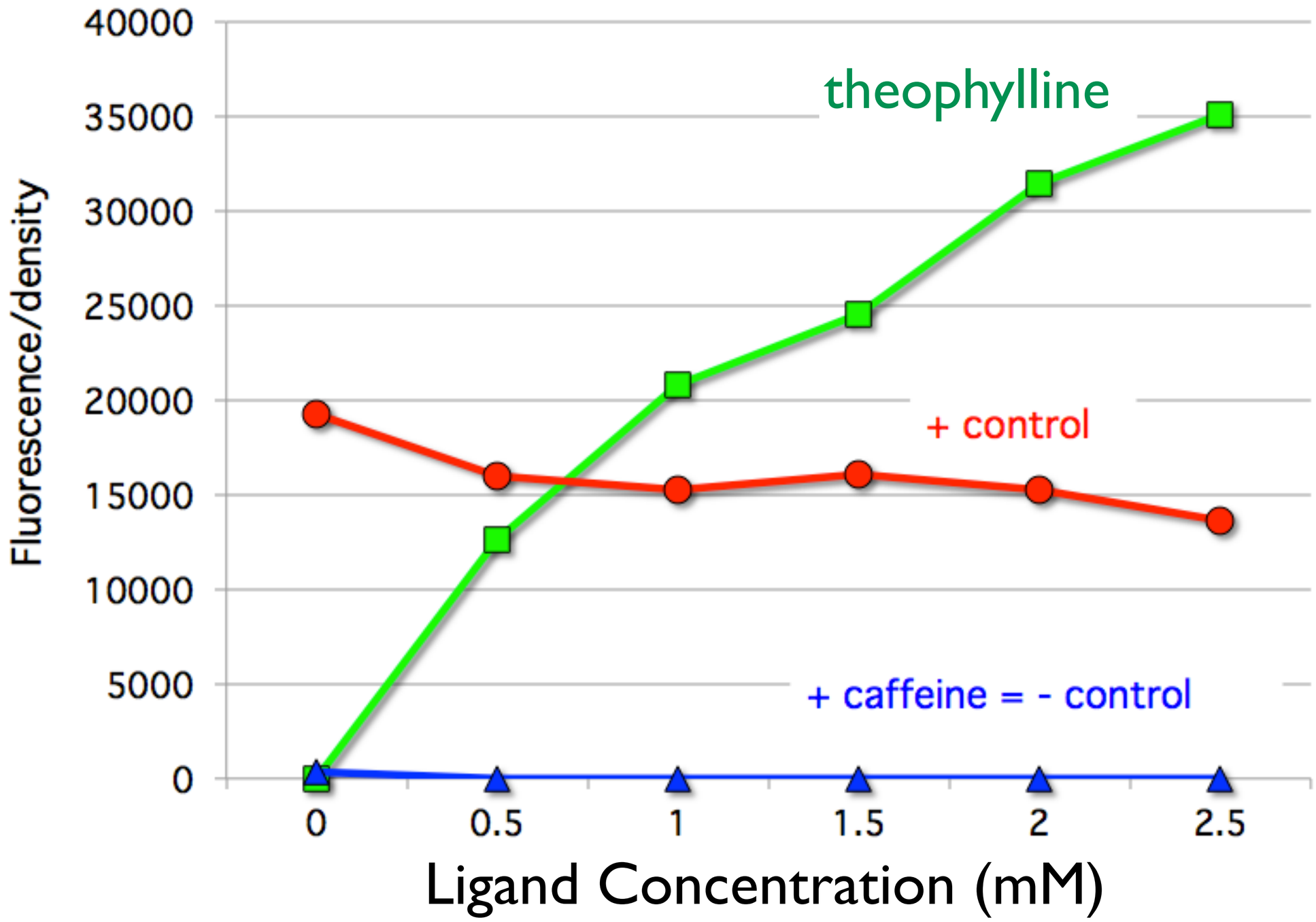



theophylline
ptamer
RBS
start codon
CGCUG GCAGGGGGUAUCAACAAGAUG

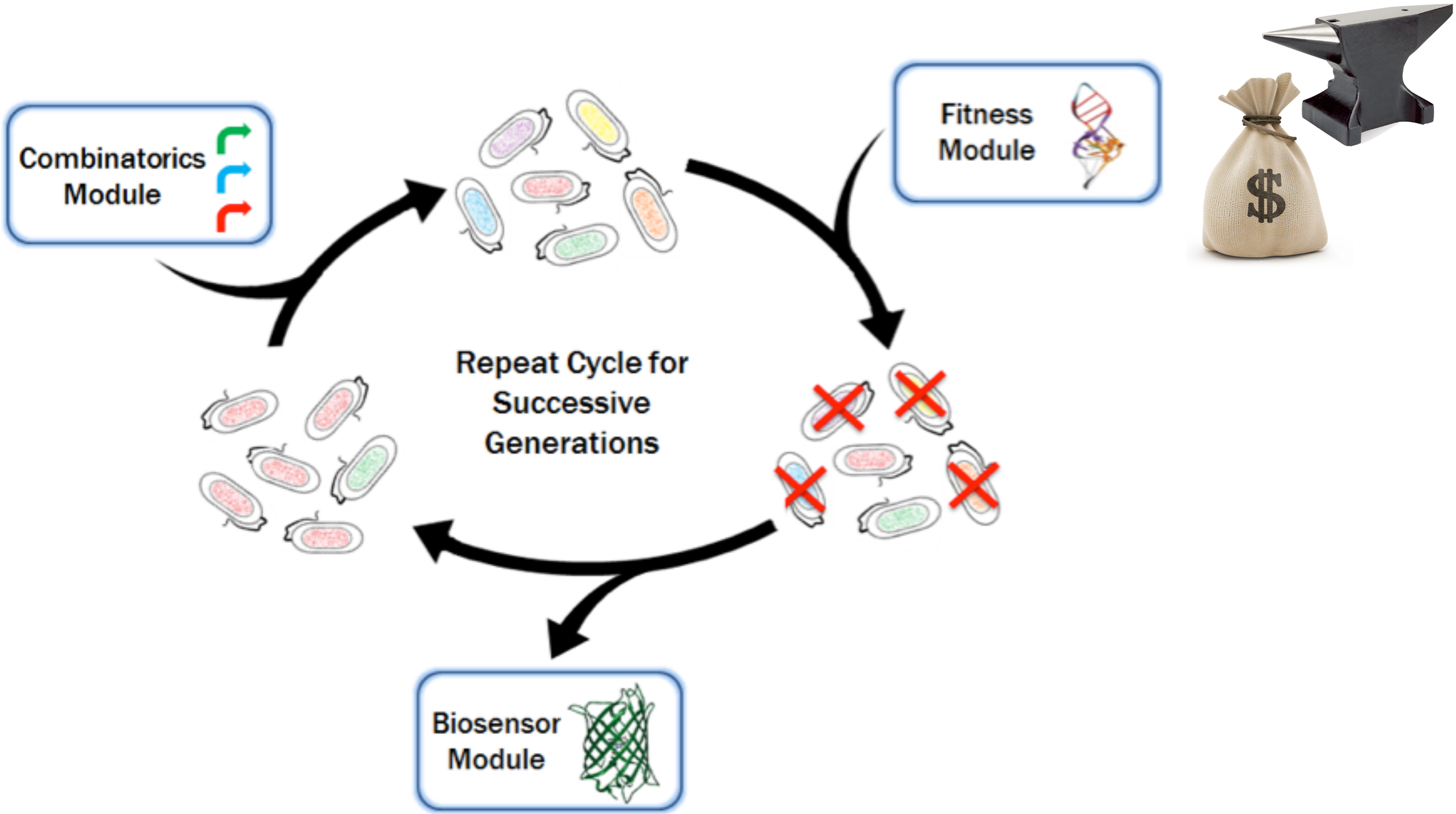
Biosensor Detects Theophylline



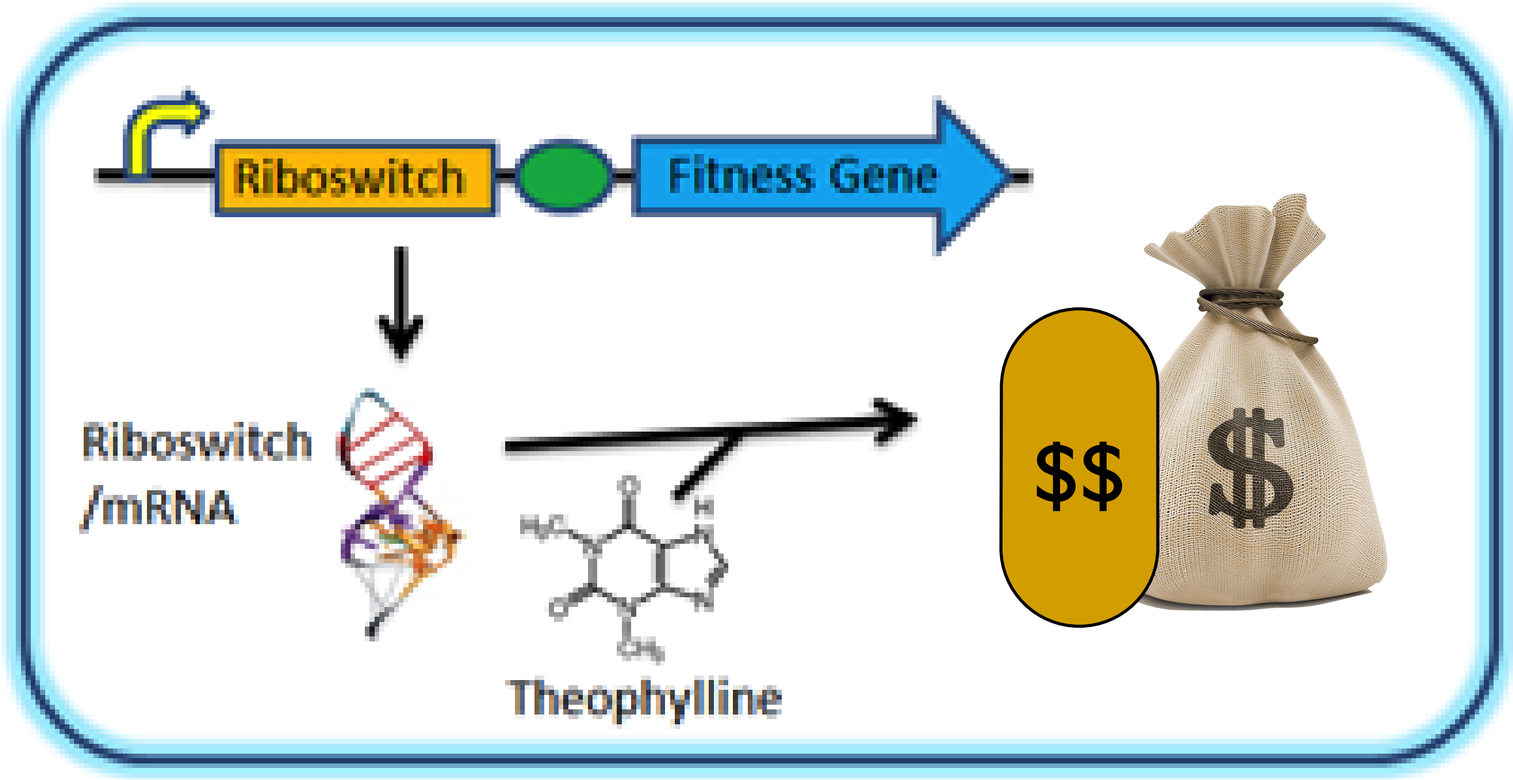
Biosensor Detects Theophylline



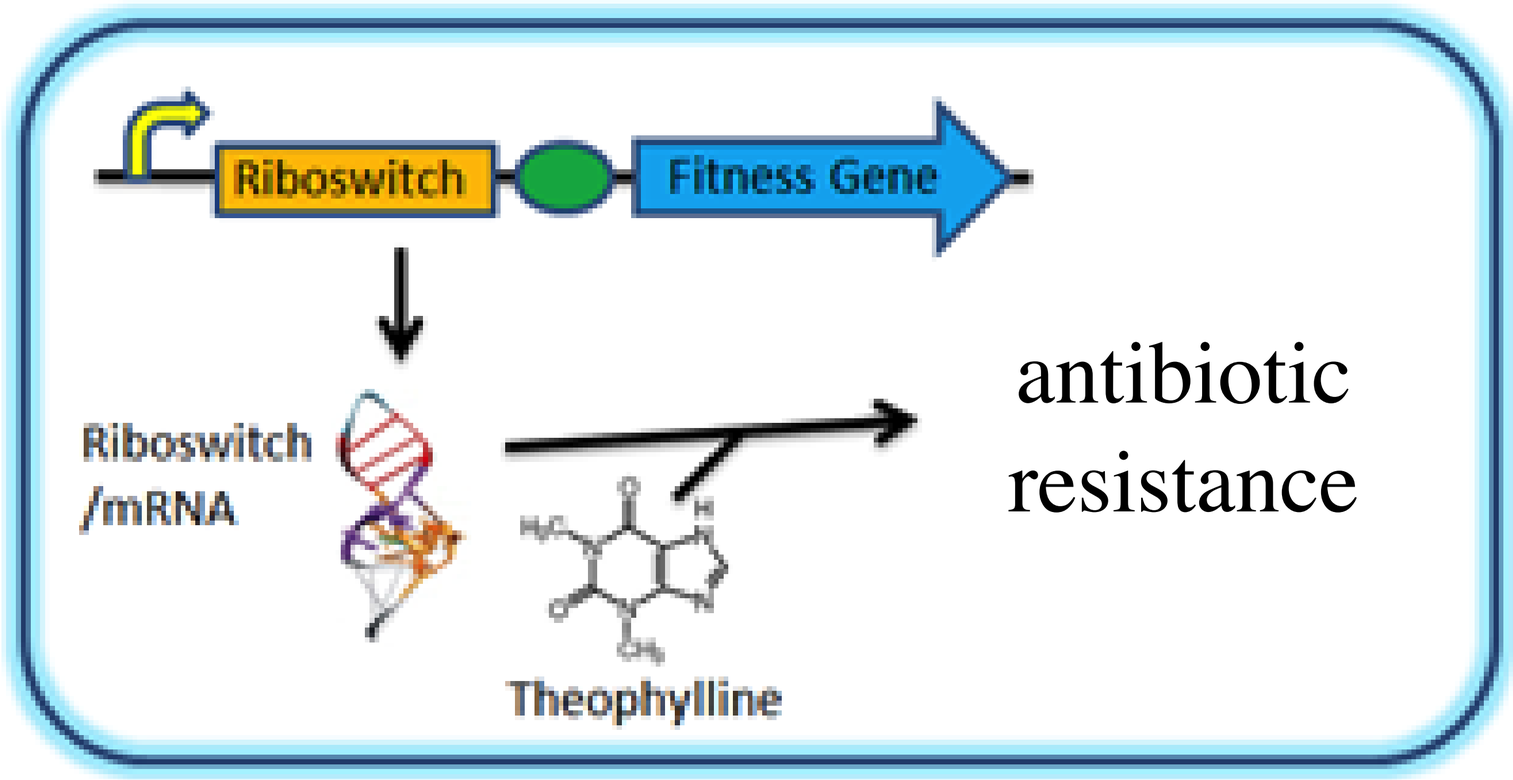
Programmed Evolution

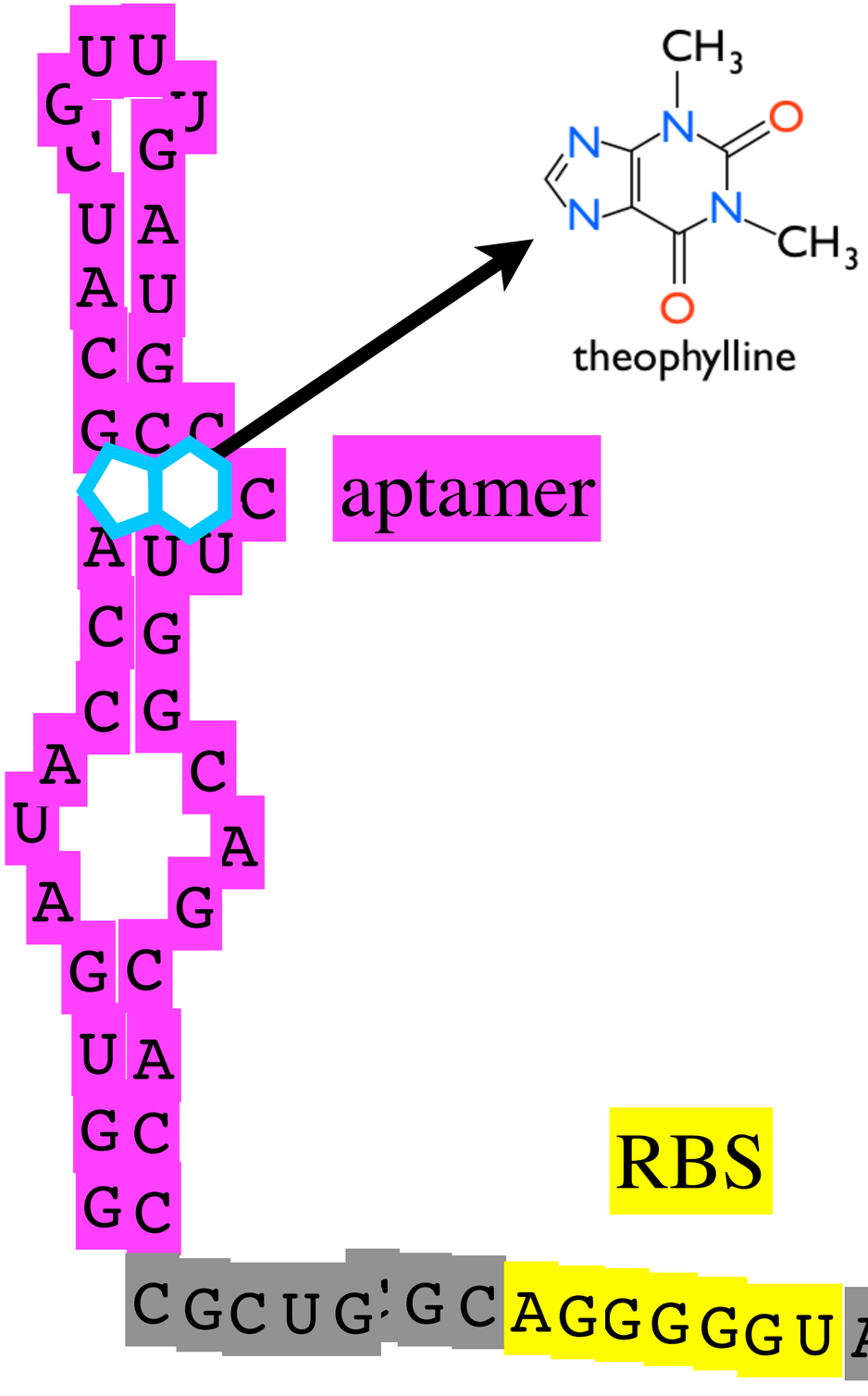


Fitness Module



Fitness Module

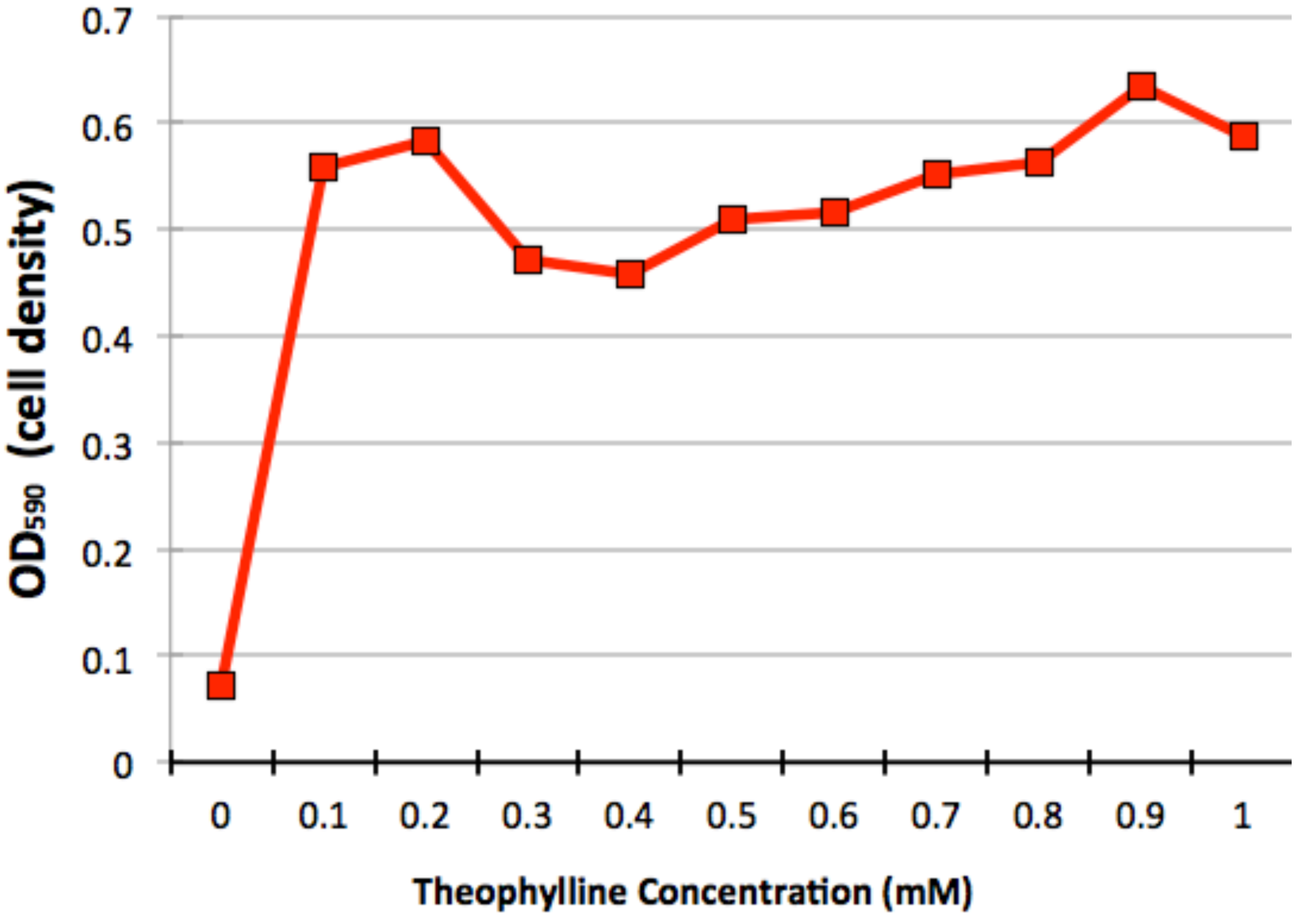



aptamer

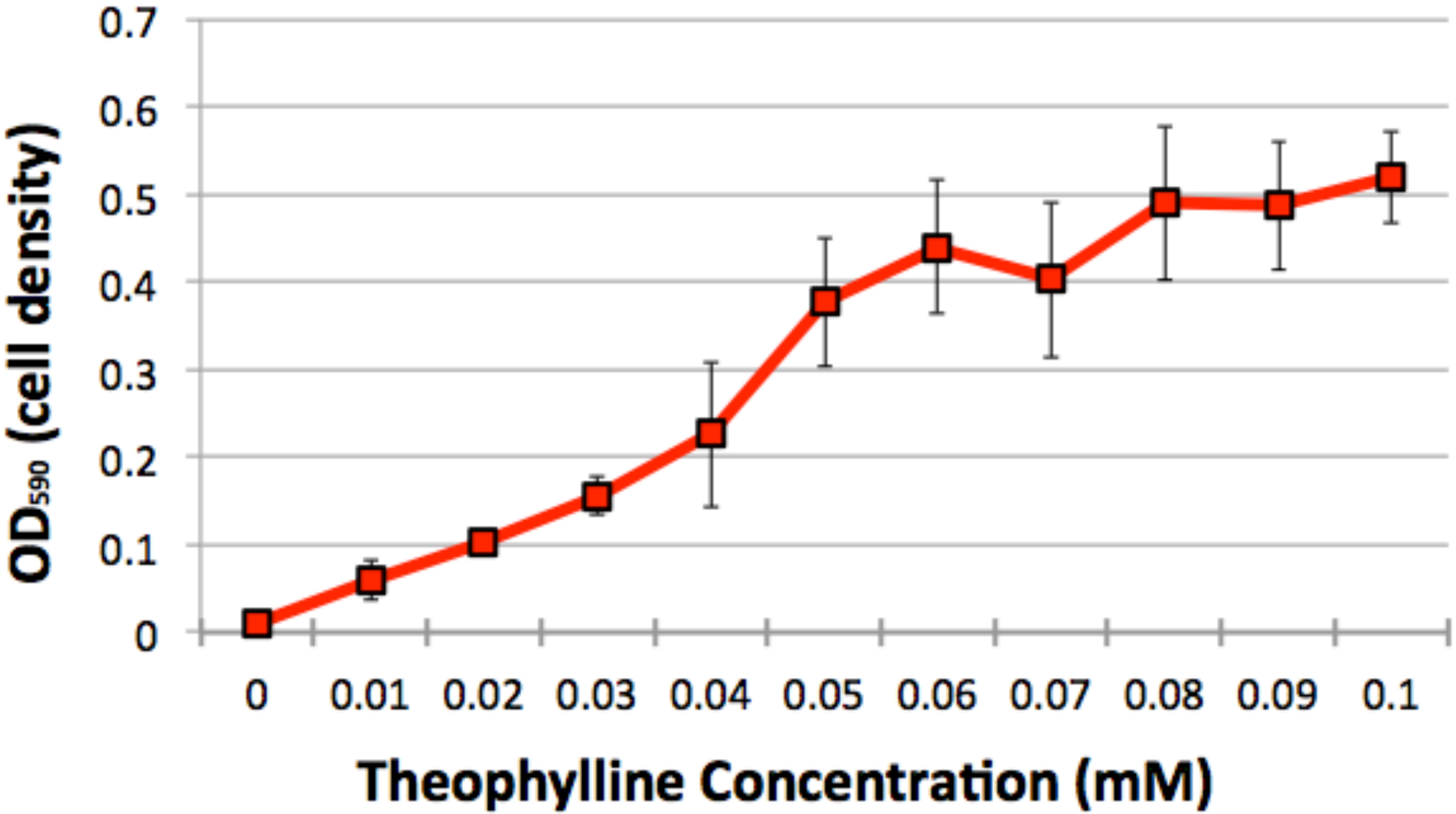
theophylline

RBS
start codon
antibiotic
resistance
CGCUG GCAGGGGGUAUCAACAAGAUG

Fitness Gene Sensitivity

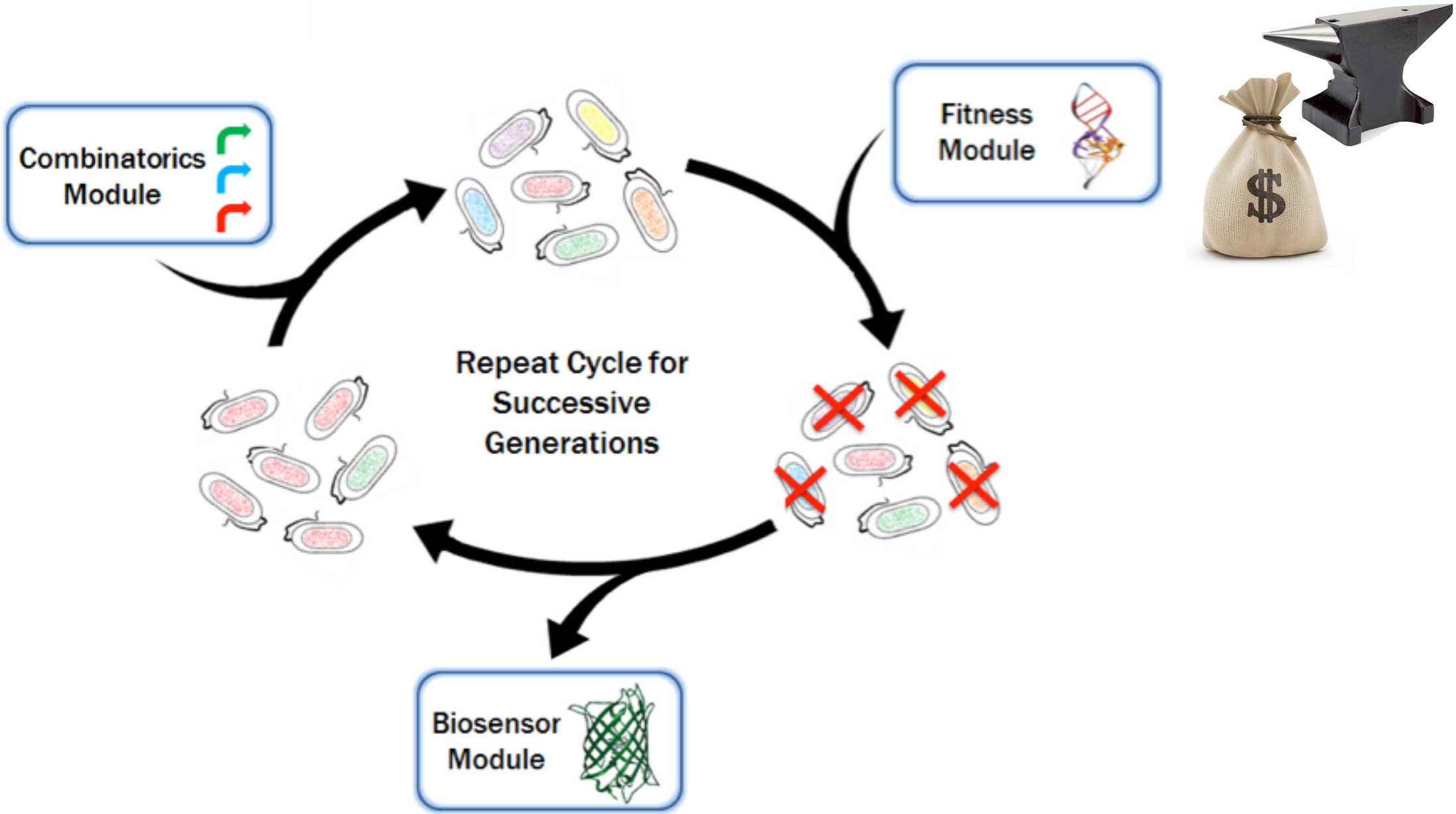


Fitness Gene Sensitivity



average +/- SEM

Programmed Evolution



How does natural selection work?

1. over production
2. variation in the population
3. competition for limited resources
4. selective advantage
5. reproduction

How does natural selection work?

1. over production
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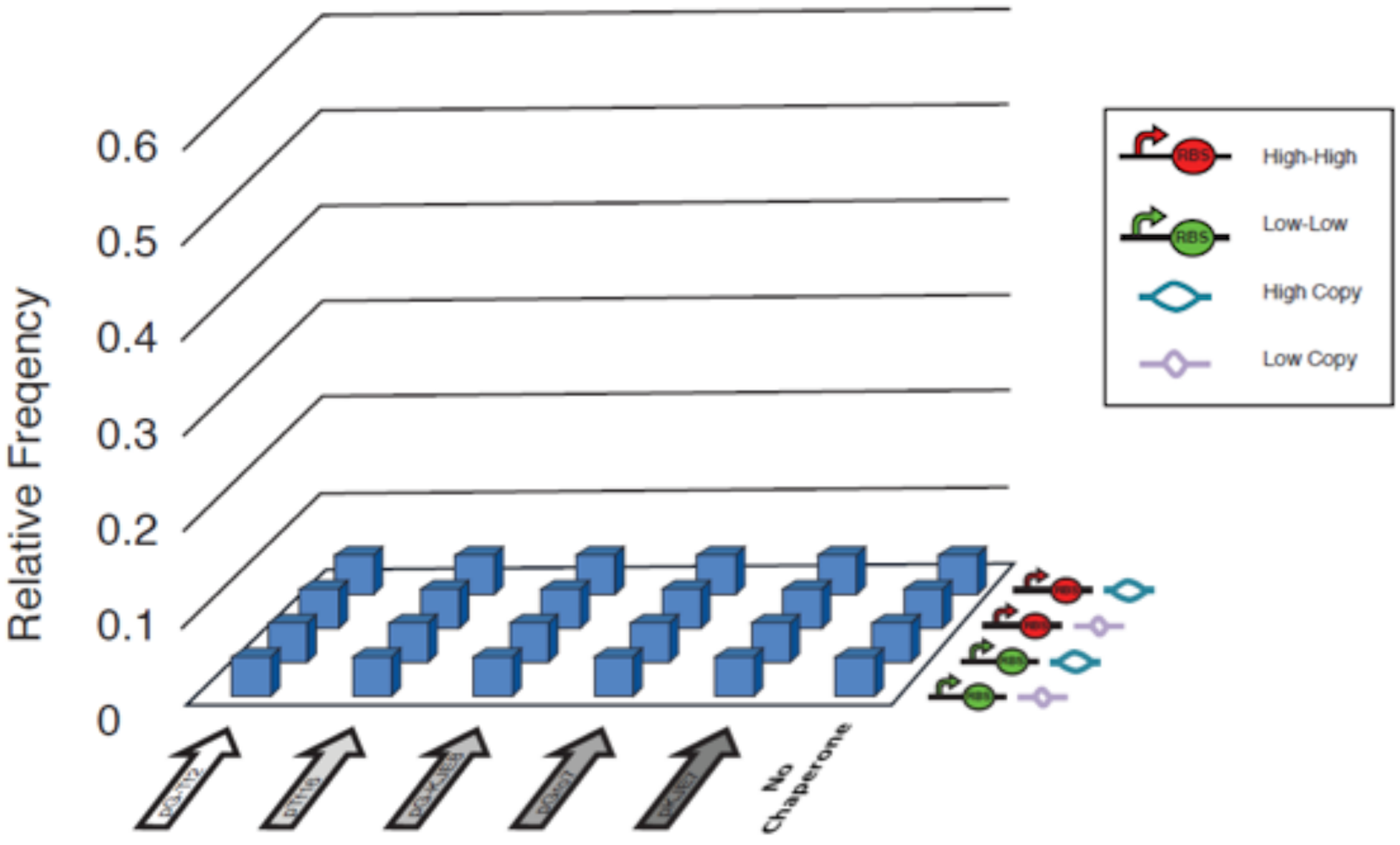
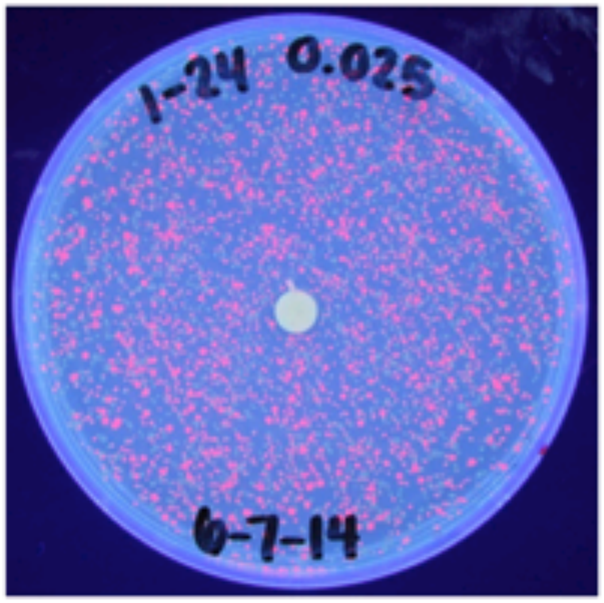
First Programmed Evolution Results

input 24 genotypes, equal proportions



First Programmed Evolution Results

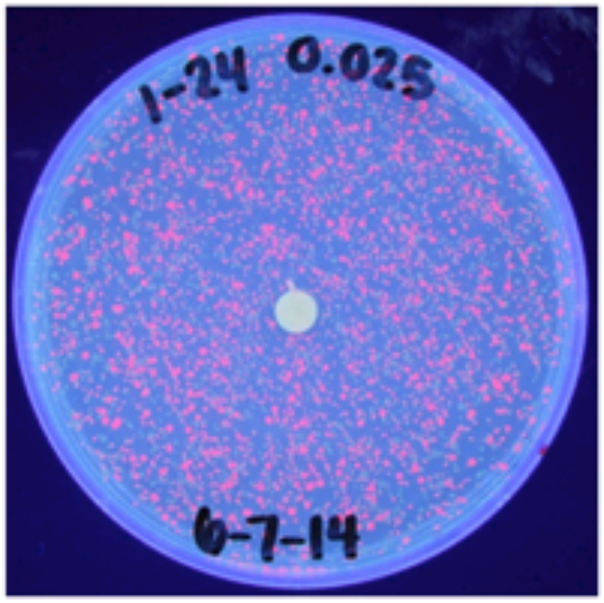


LB + Amp

H_2O

Disk

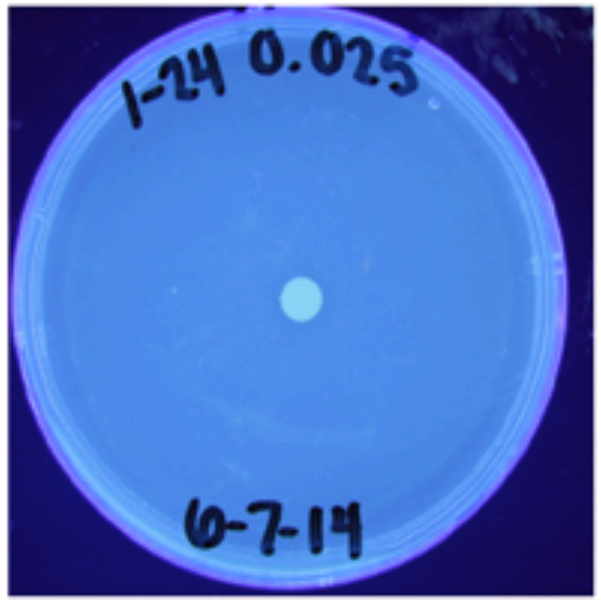
First Programmed Evolution Results



LB + Amp

H_2O

Disk

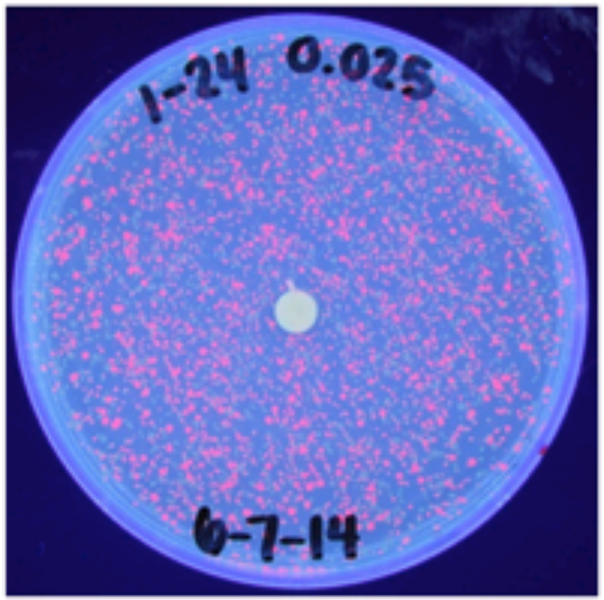


LB + Tet

H_2O

Disk

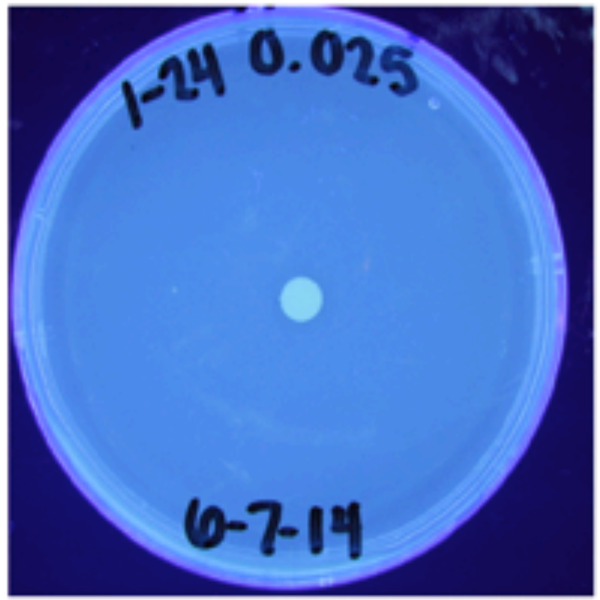
First Programmed Evolution Results



LB + Amp

H_2O

Disk



LB + Tet

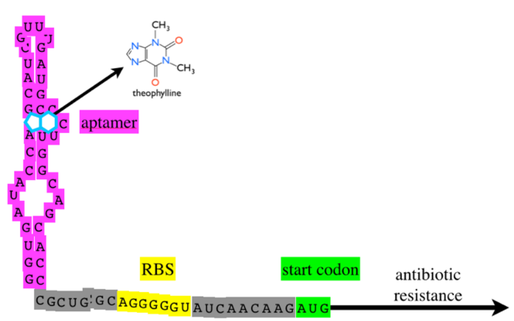
H_2O

Disk

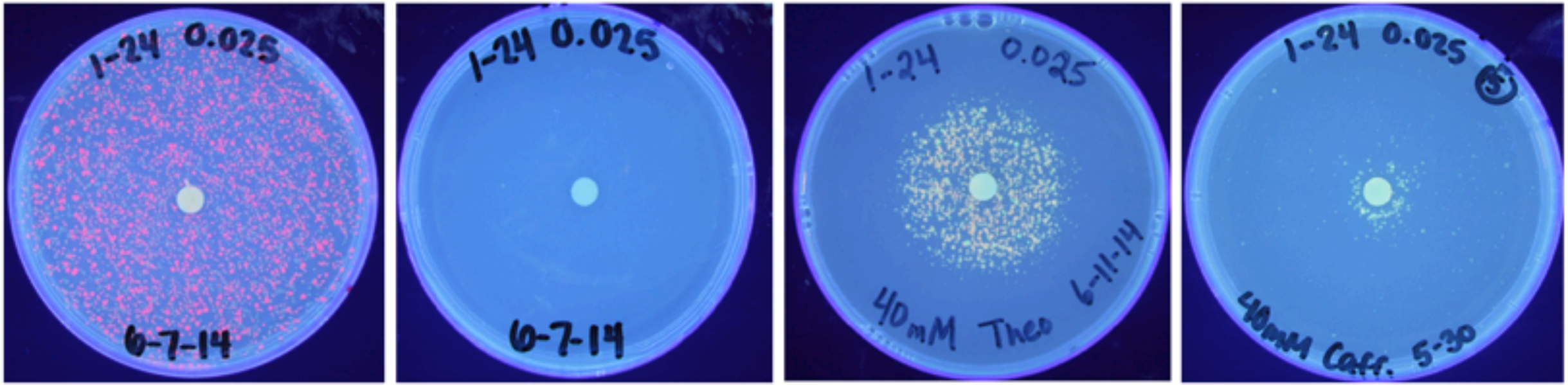
LB + Tet

40 mM Theophylline

Disk



First Programmed Evolution Results

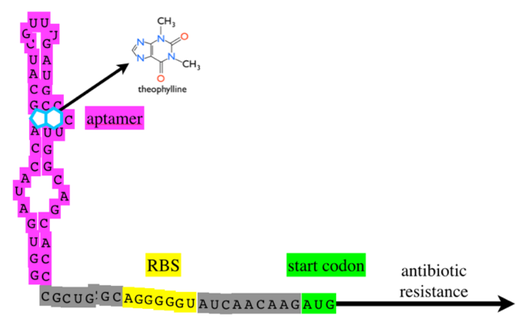


LB + Amp
H_2O
Disk

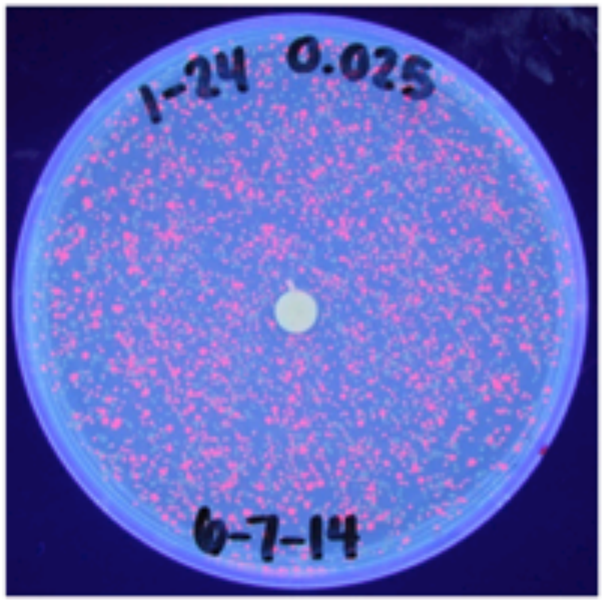
LB + Tet
H_2O
Disk

LB + Tet
40 mM Theophylline
Disk

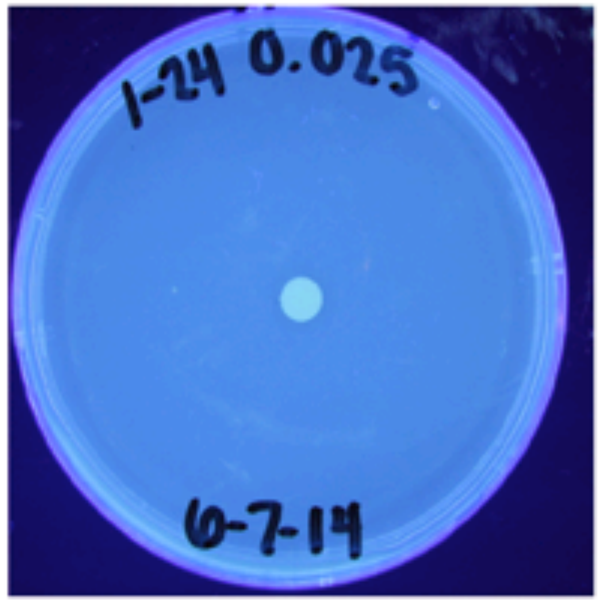
LB + Tet
40 mM Caffeine
Disk



First Programmed Evolution Results

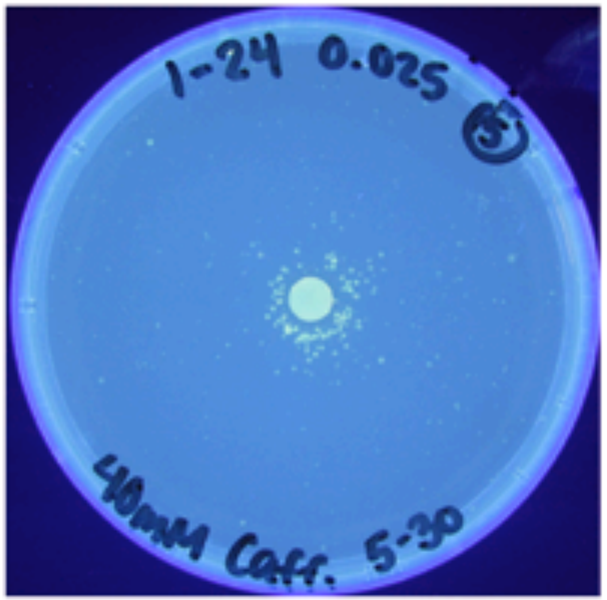


LB + Amp
H_2O
Disk

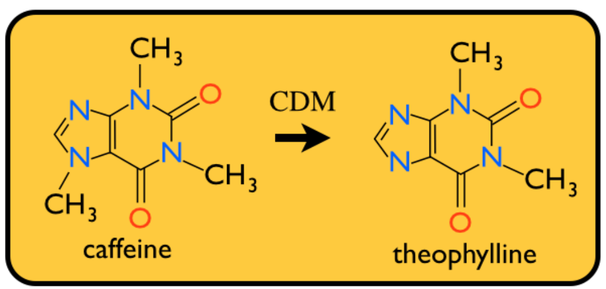


LB + Tet
H_2O
Disk

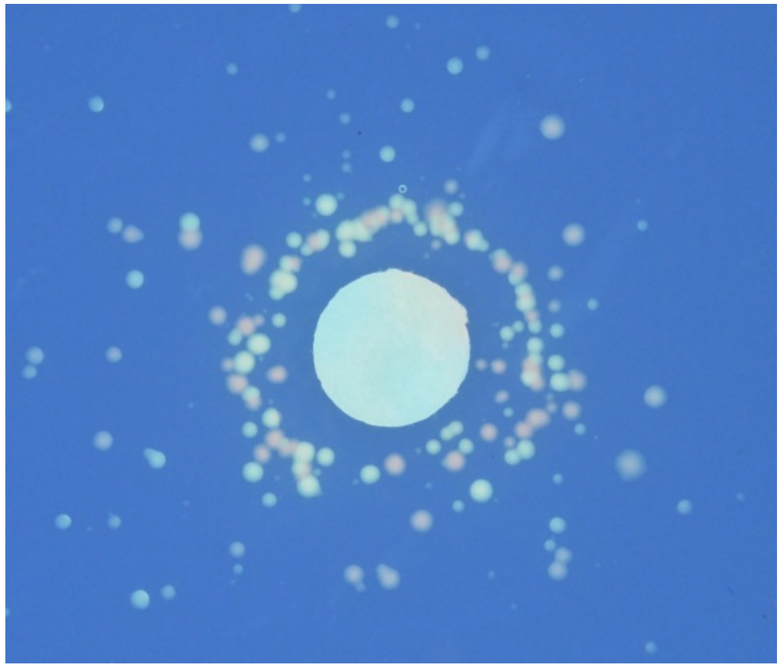
LB + Tet
40 mM Theophylline
Disk

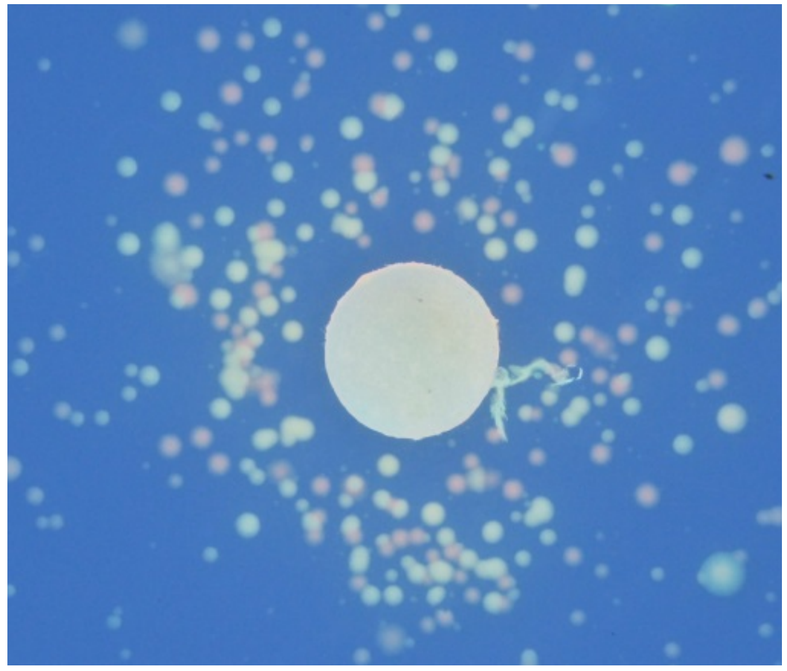


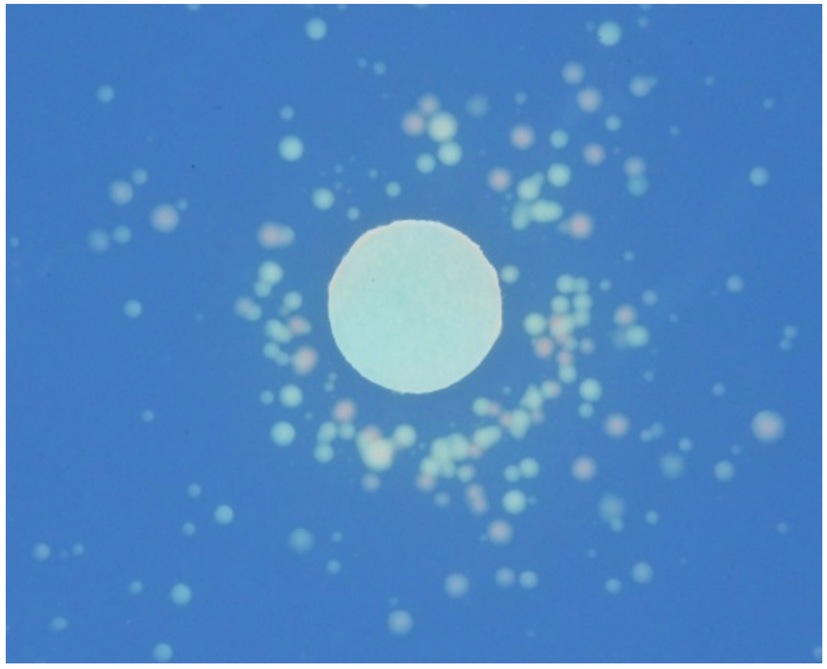
LB + Tet
40 mM Caffeine
Disk

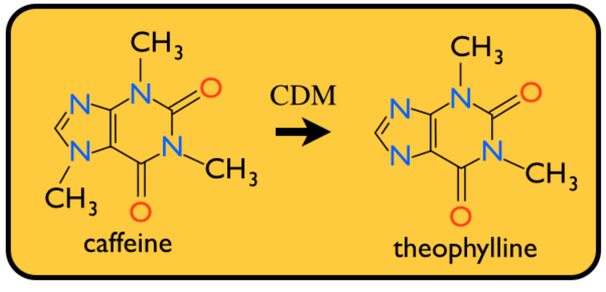


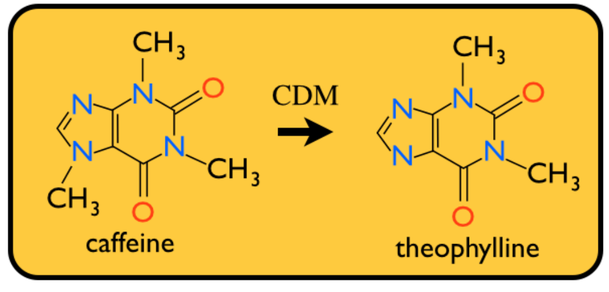
Replicated Programmed Evolution Results

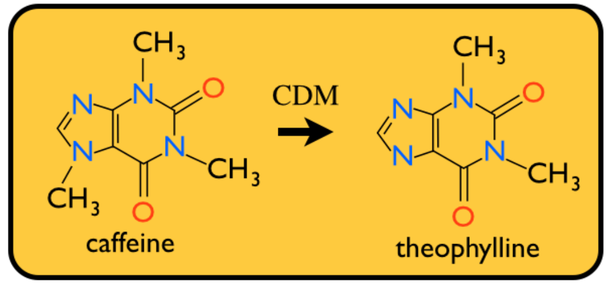






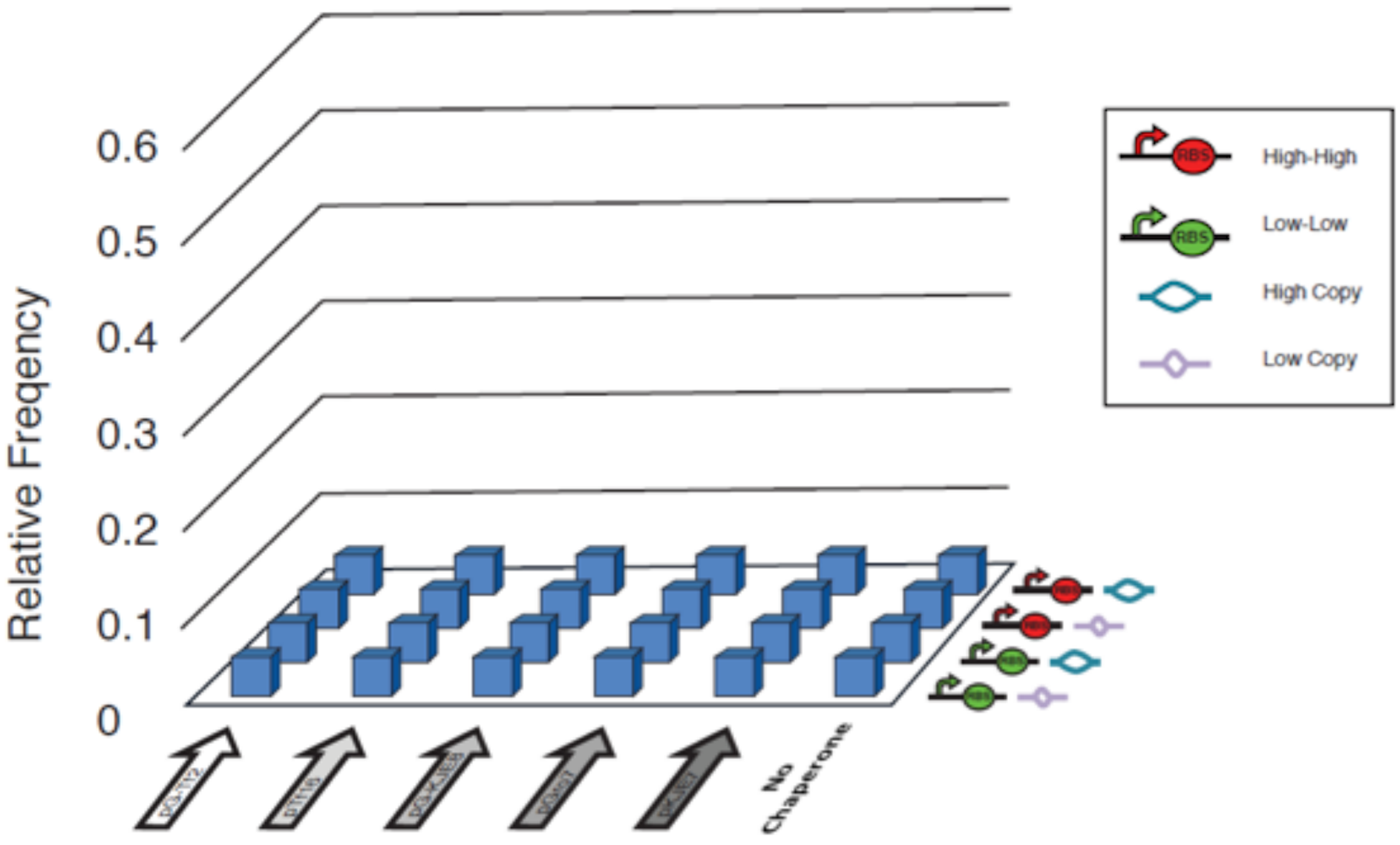






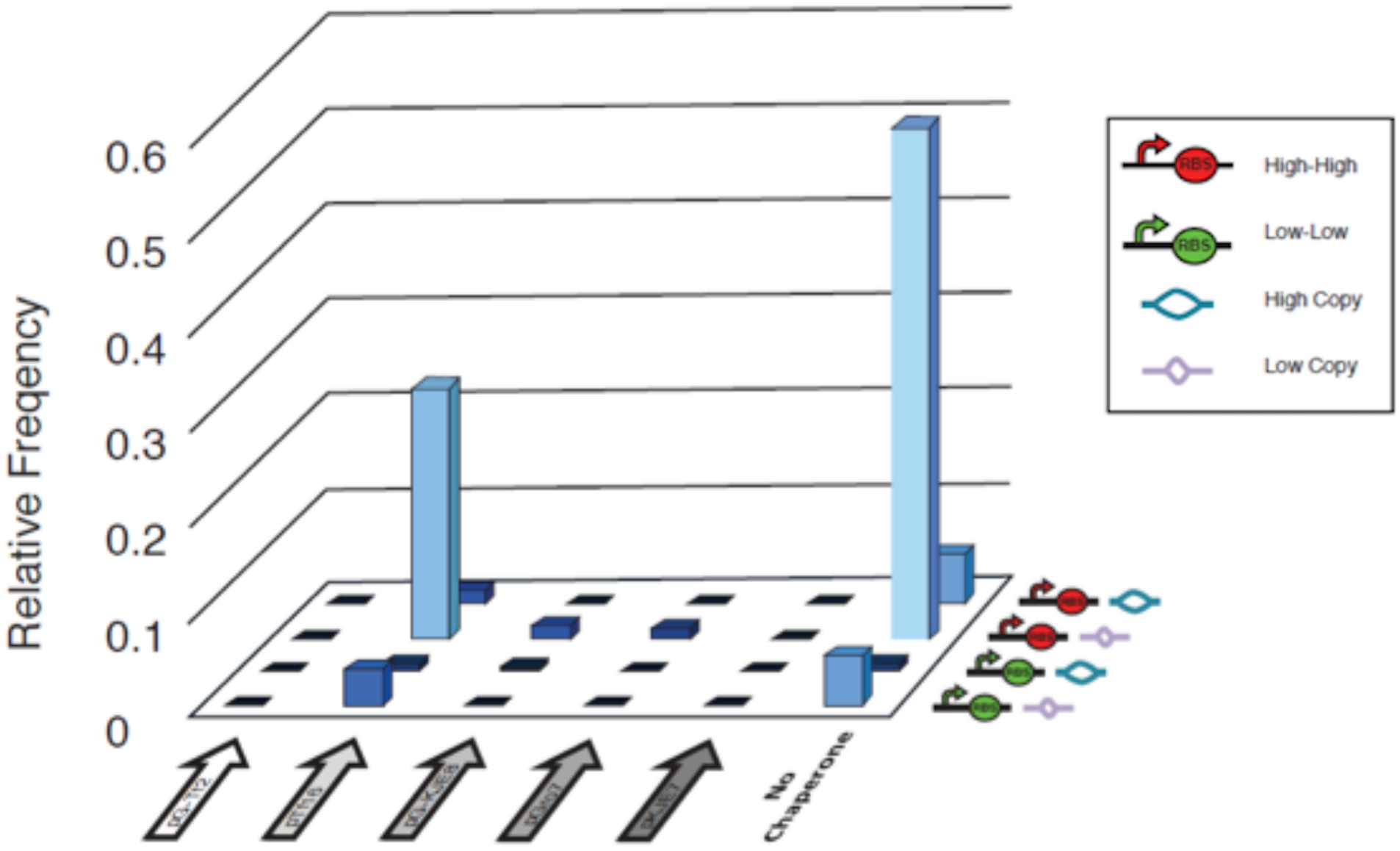
First Programmed Evolution Results

input 24 genotypes, equal proportions



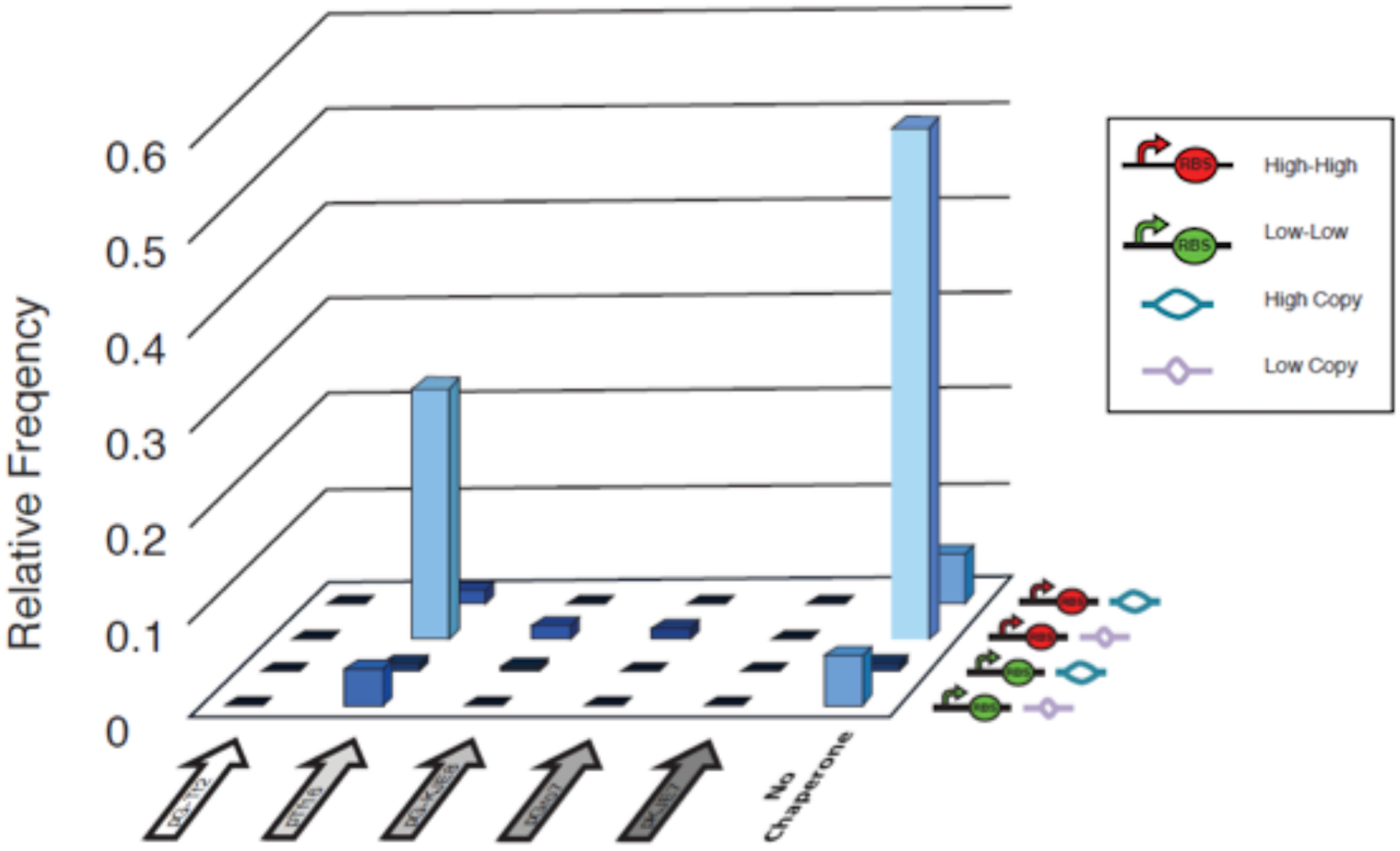
First Programmed Evolution Results

output genotypes, optimized theophylline production

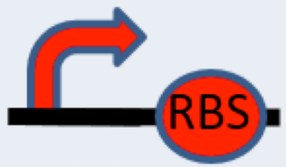
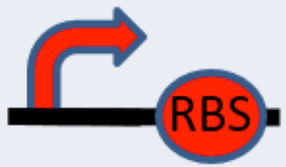
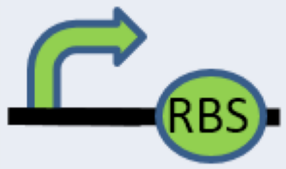
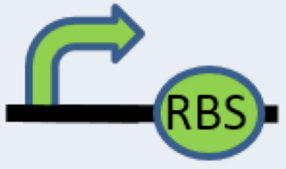
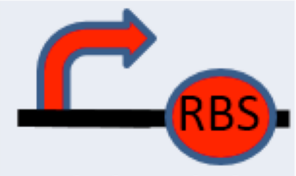


E. coli Programmed to Optimize

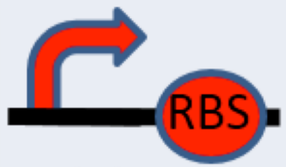
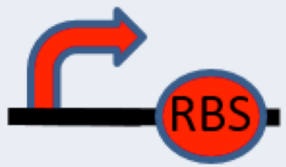
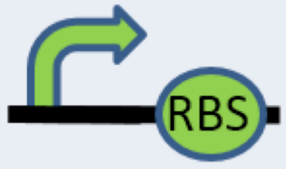
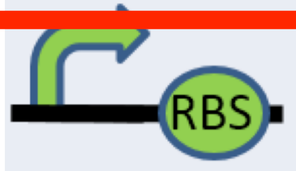
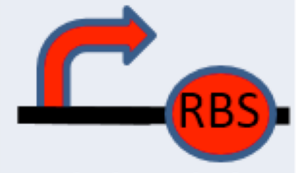
output genotypes, optimized theophylline production



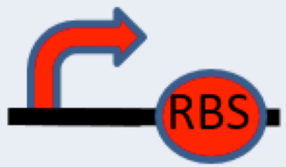
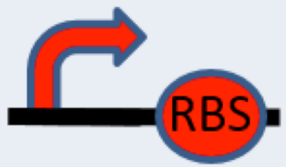
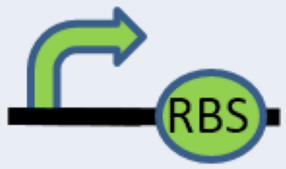
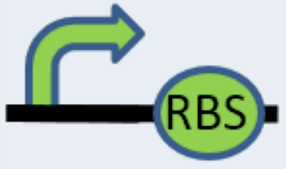
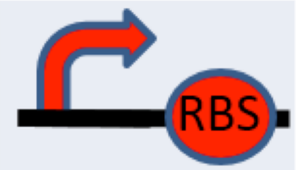
E. coli Programmed to Optimize

Promoter-RBS	Origin	Chaperone	Theophylline Production	Relative Fitness
 High-High	 Low Copy	No Chaperone	0.44	1.00
 High-High	 Low Copy		0.35	0.49
 Low-Low	 Low Copy	No Chaperone	0.43	0.10
 Low-Low	 High Copy	No Chaperone	0.14	0.01
 High-High	 Low Copy		0.19	0.00

E. coli Programmed to Optimize

Promoter-RBS	Origin	Chaperone	Theophylline Production	Relative Fitness
 High-High	 Low Copy	No Chaperone	0.44	1.00
 High-High	 Low Copy		0.35	0.49
 Low-Low	 Low Copy	No Chaperone	0.43	0.10
 Low-Low	 High Copy	No Chaperone	0.14	0.01
 High-High	 Low Copy		0.19	0.00

E. coli Programmed to Optimize

Promoter-RBS	Origin	Chaperone	Theophylline Production	Relative Fitness
 High-High	 Low Copy	No Chaperone	0.44	1.00
 High-High	 Low Copy		0.35	0.49
 Low-Low	 Low Copy	No Chaperone	0.43	0.10
 Low-Low	 High Copy	No Chaperone	0.14	0.01
 High-High	 Low Copy		0.19	0.00

Programmed Evolution of *E.coli* for Optimization of Drug Production

(*in press*, *PLOS ONE*)

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What is the secret to success?

“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
Founder of IBM

The scenery only changes for the lead dog.

The scenery only changes for the lead dog.

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