Synthetic Biology: Insights into Natural Biology and New Technologies

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Davidson College
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Three Rules for Our Lab

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

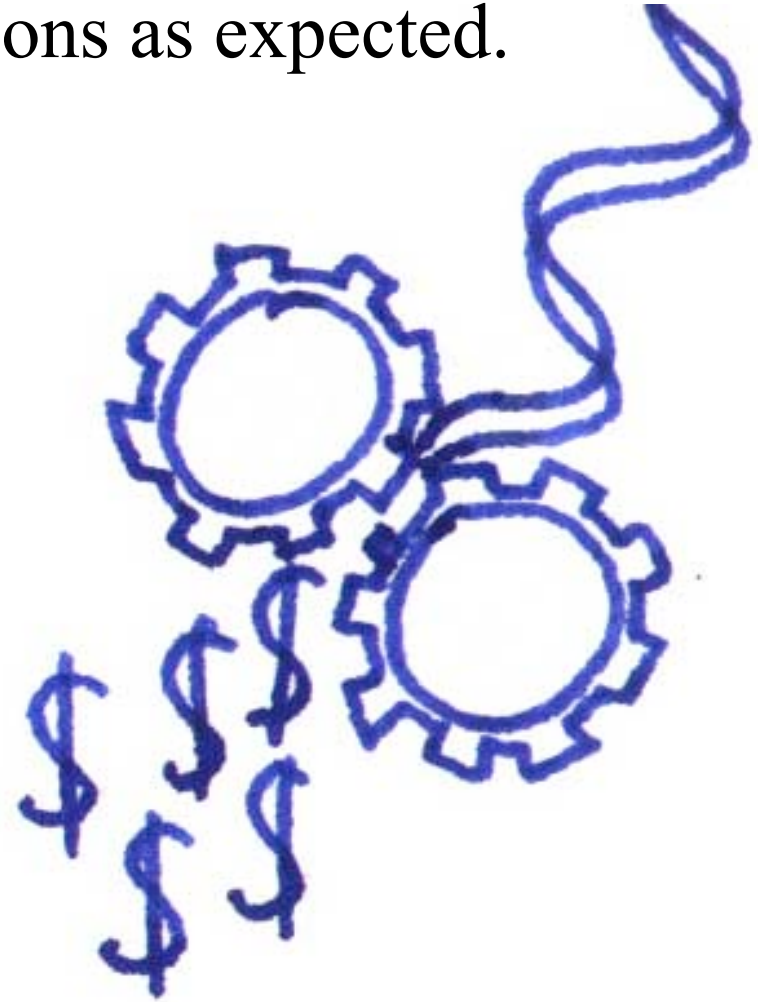
What is Synthetic Biology?

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

www.bio.davidson.edu/projects/gcat/Synthetic/What_Is_SynBio.html

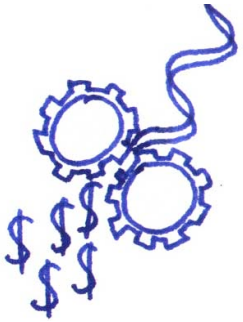
Synthetic Biology: Win-Win

Win #1: your design functions as expected.

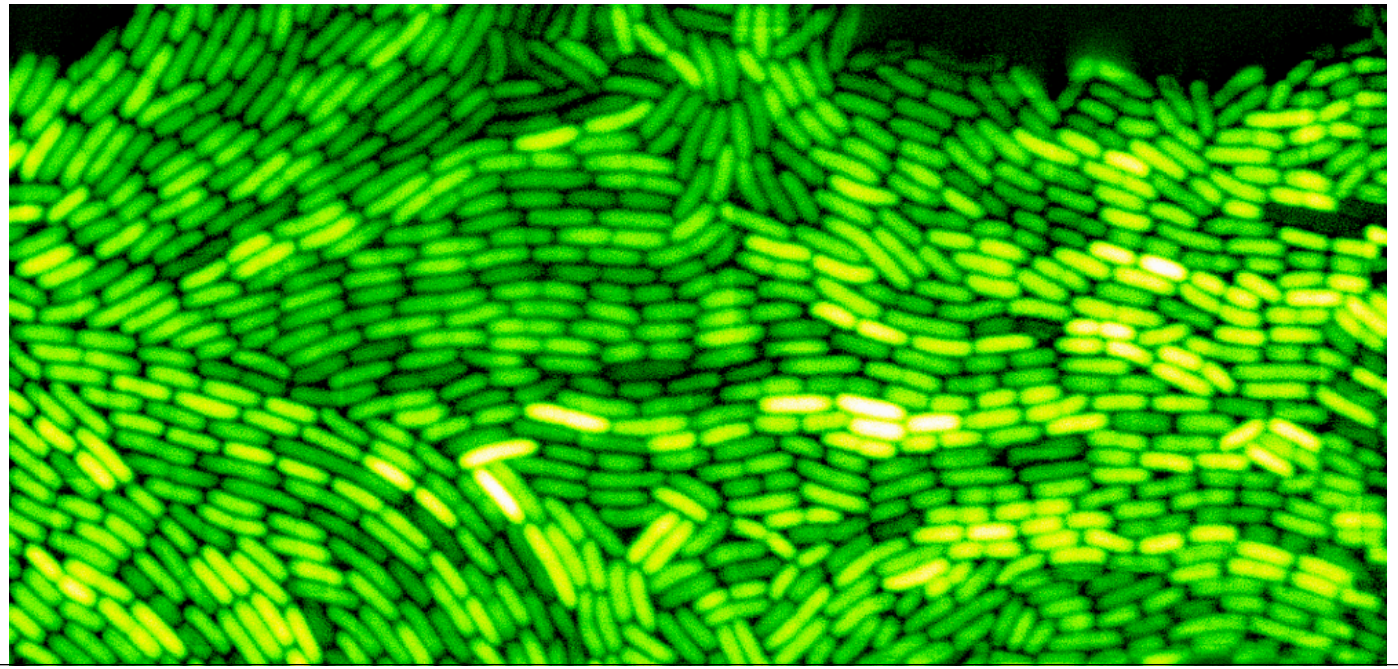


Synthetic Biology: Win-Win Research

Win #1: your design functions as expected.



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



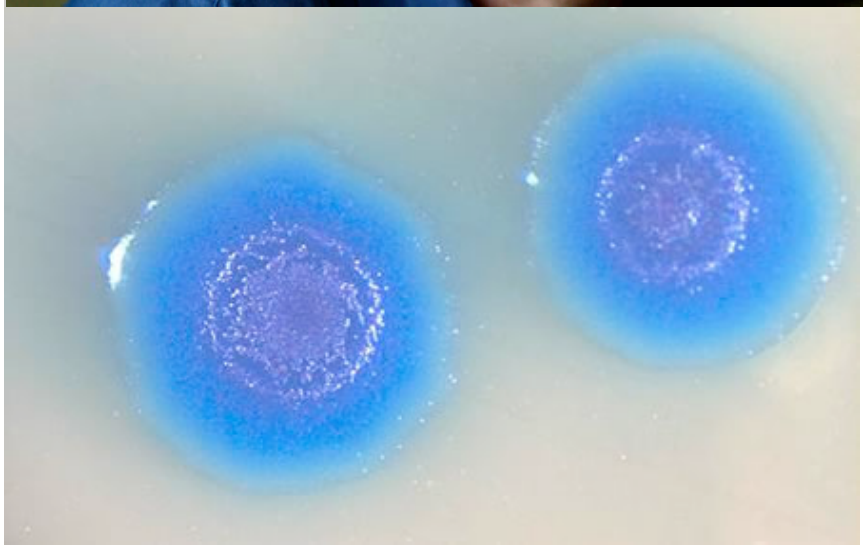
How is Synthetic Biology Different?

Abstraction

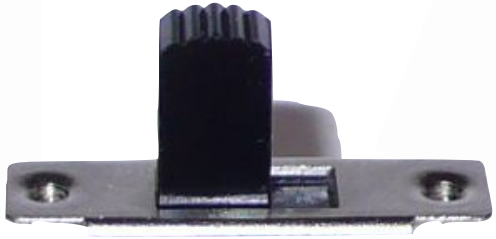
Modularity

Standards

Designing and modeling



Abstraction



Abstraction

Modularity

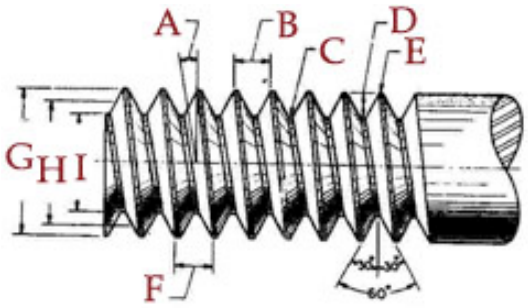
USB ports on computers

Modularity

Standardization

On a Uniform System of Screw Thread

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



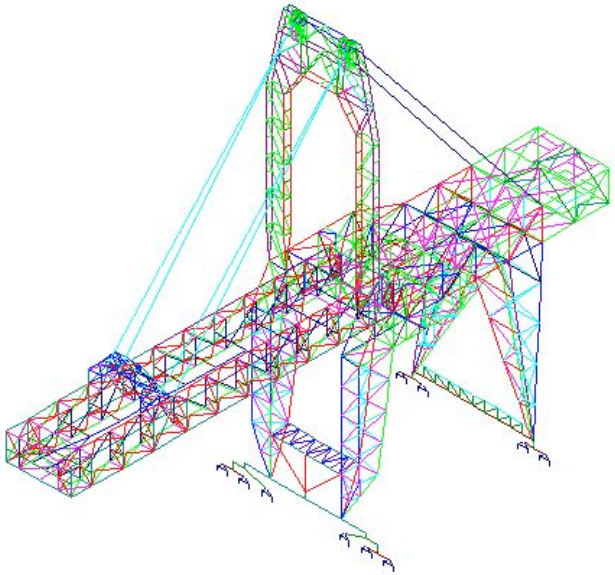
William Sellers April 21, 1864

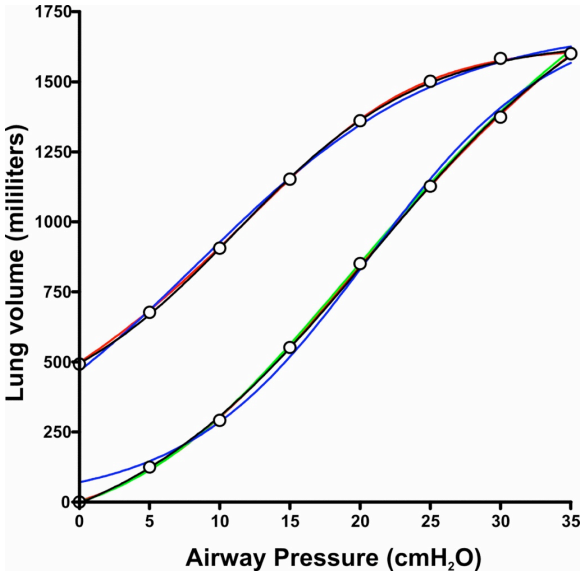
http://openwetware.org/images/b/bd/BBFRFC9.pdf

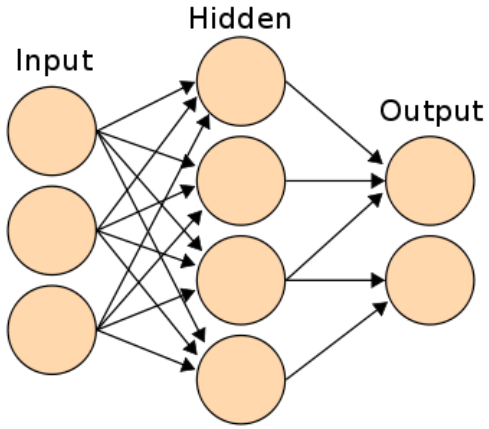
Standardization

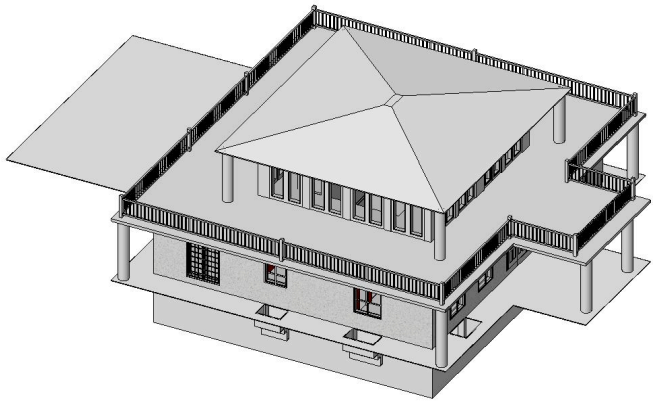
On a Uniform System of Screw Thread

Modeling of Designs



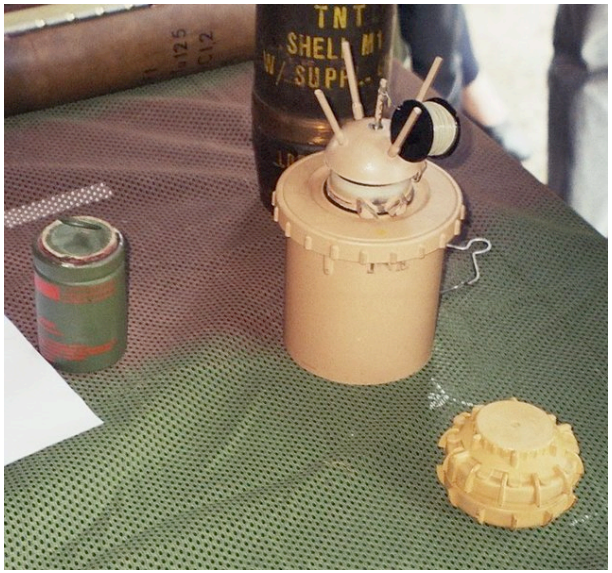






Real World Applications of Synthetic Biology

Land Mine Detection



Land Mine Detection

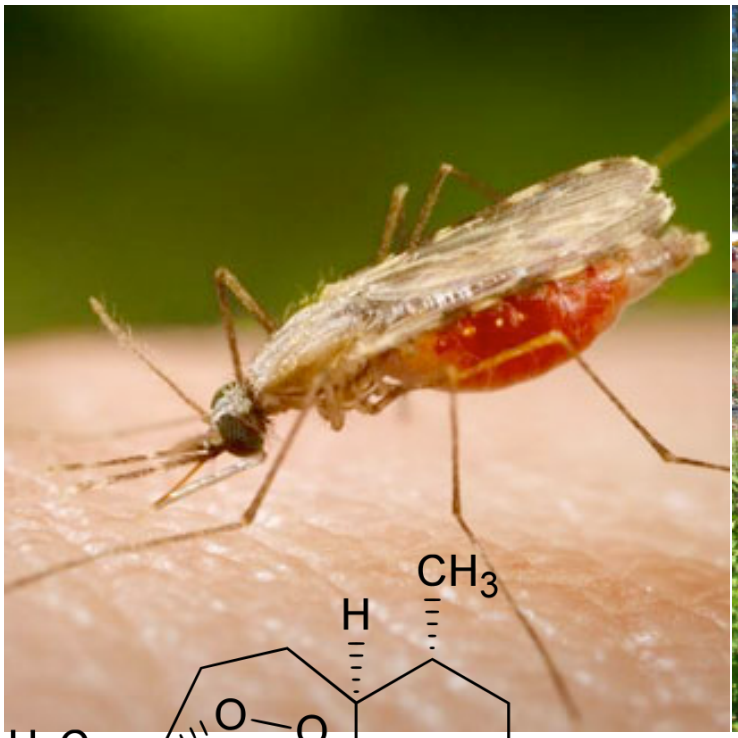
Synthetic Biology
Land Mine Detection

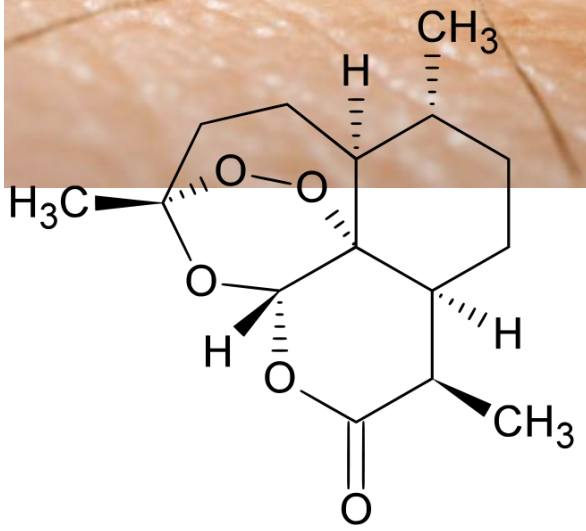
WARNING SIGN: The bioengineered Thales cress turns red when exposed to a mine byproduct.
COURTESY OF ARESA BIODETECTION

New weed may flag land mines

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

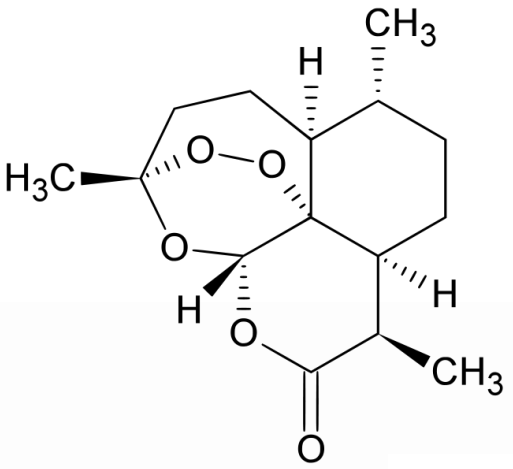
Production of Medicines



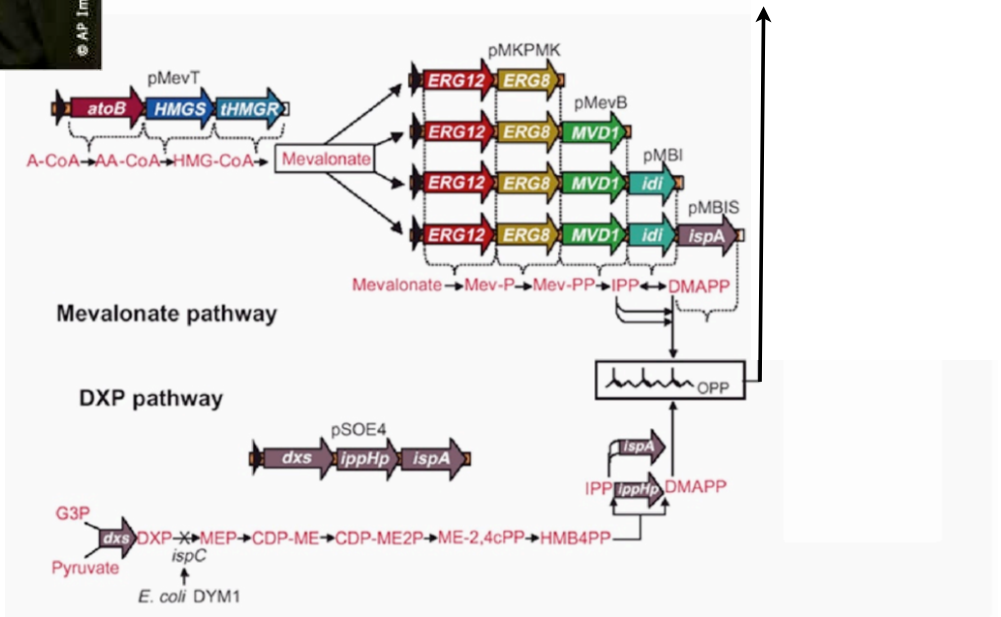


$1 per pill

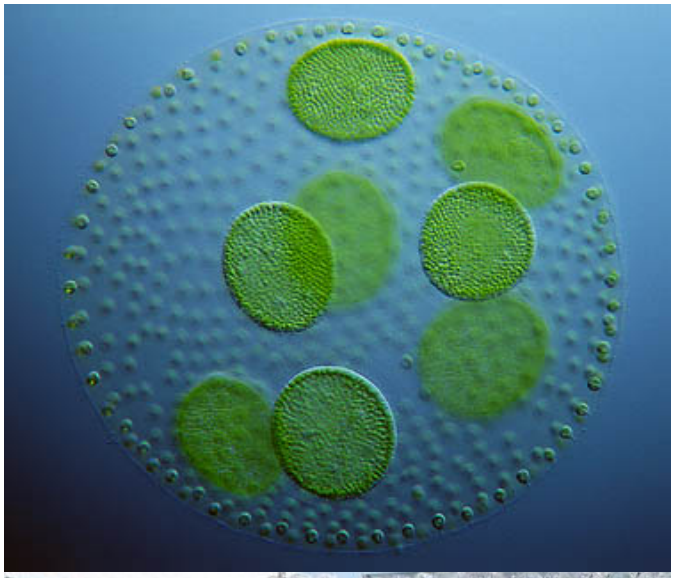
Production of Medicines



10¢ per pill



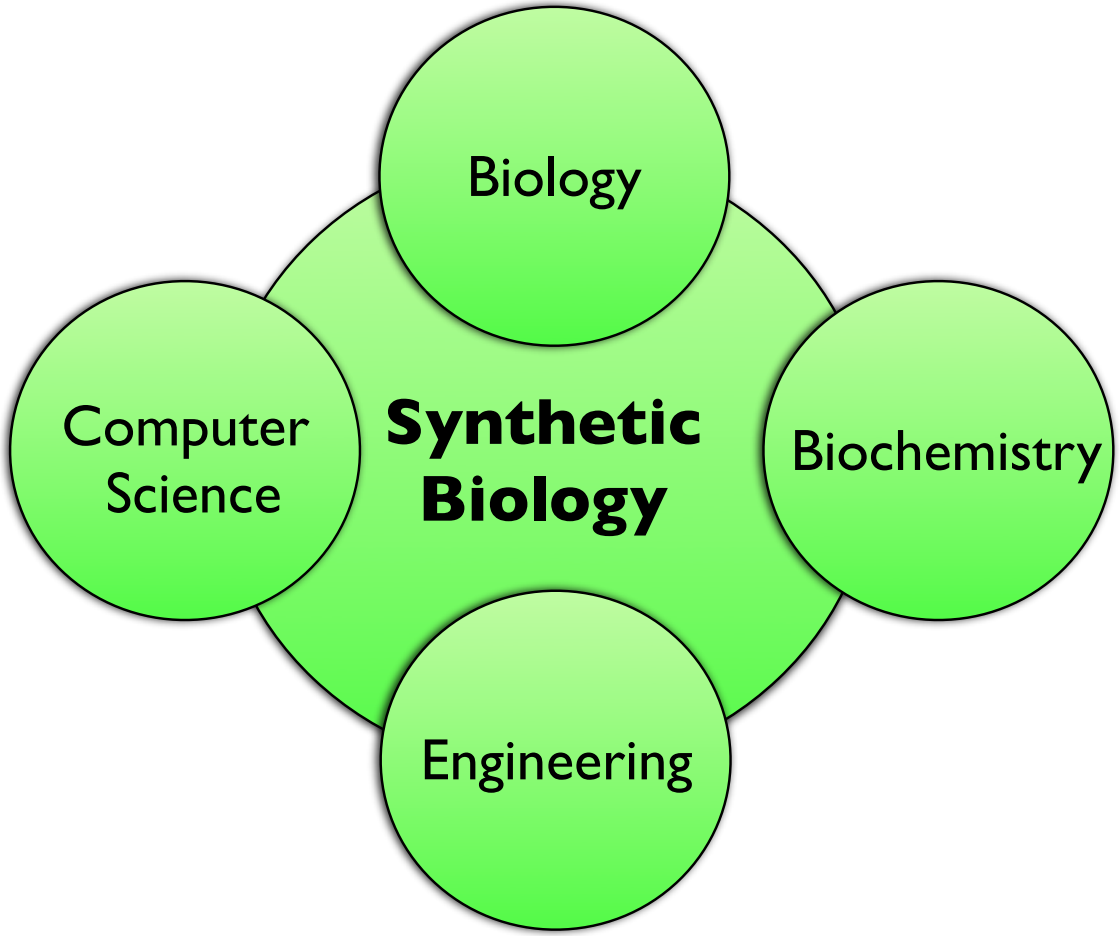
Biofuels from Algae



CO_2-neutral

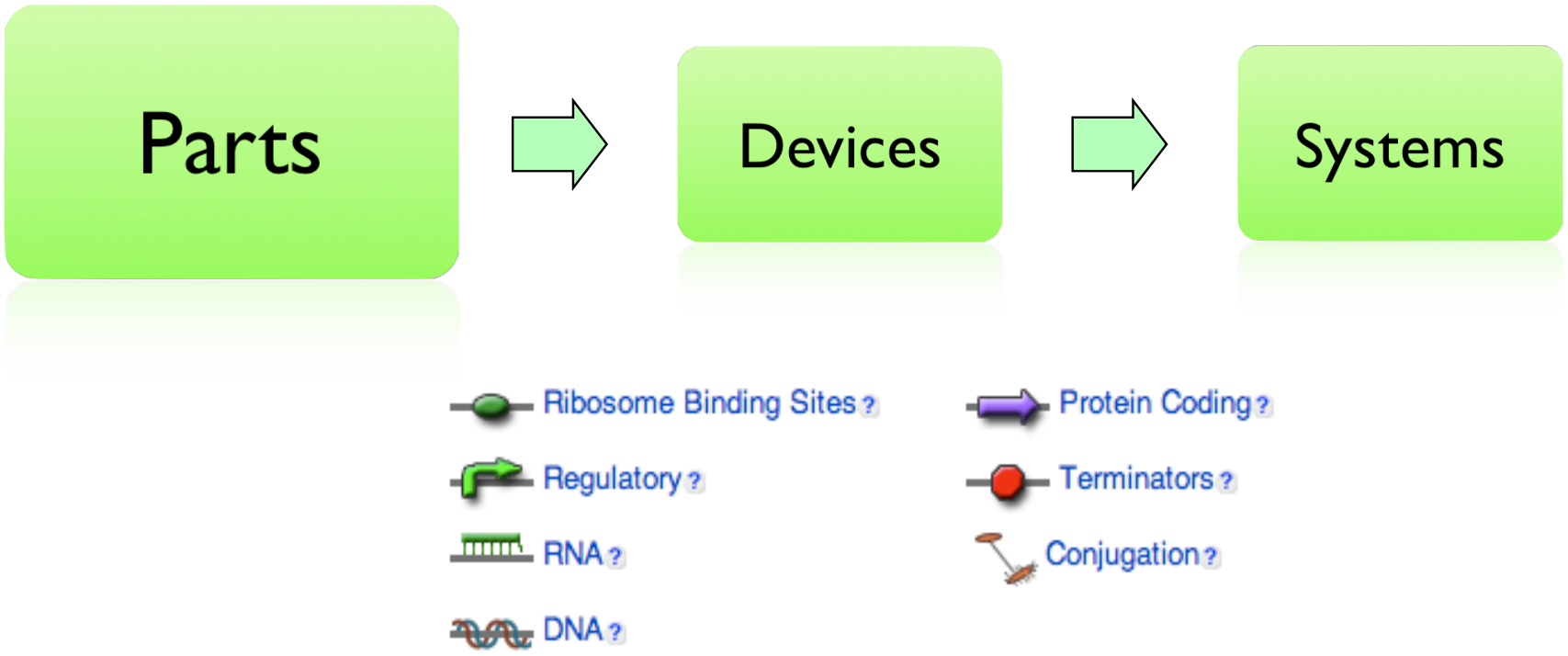
1,000,000 gallons in 2008

Synthetic Biology

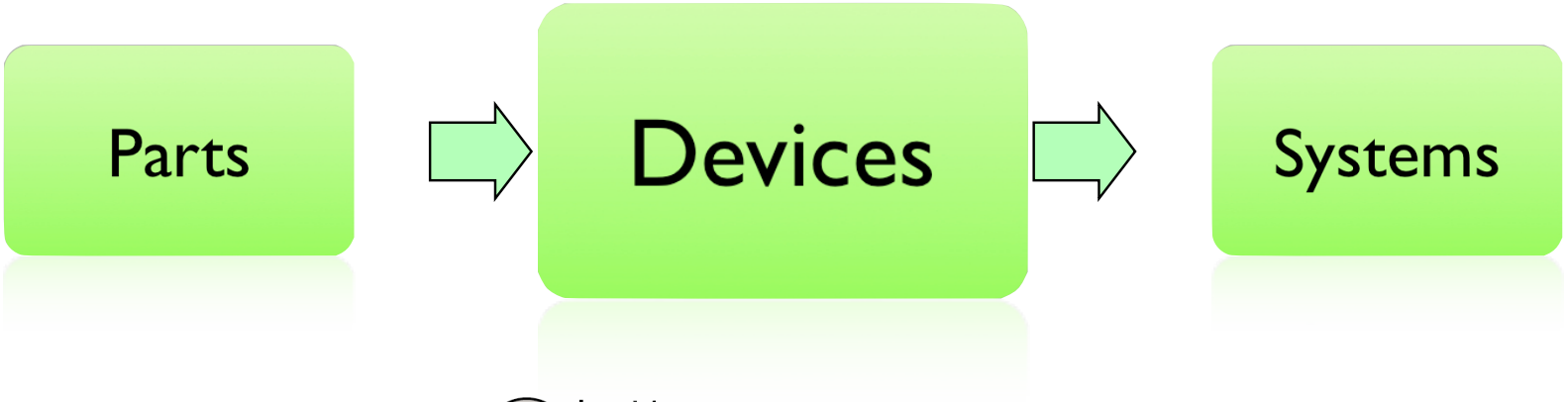


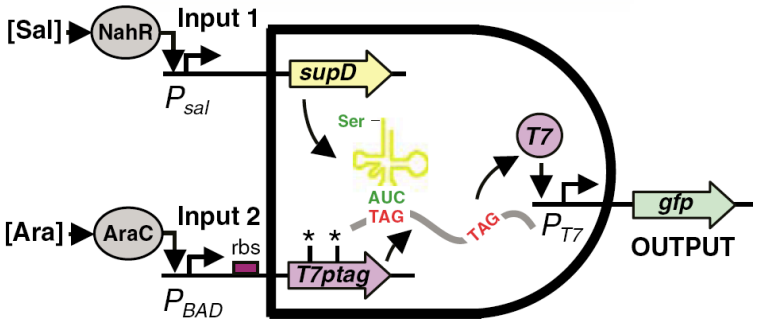
Synthetic Biology

Synthetic Biology



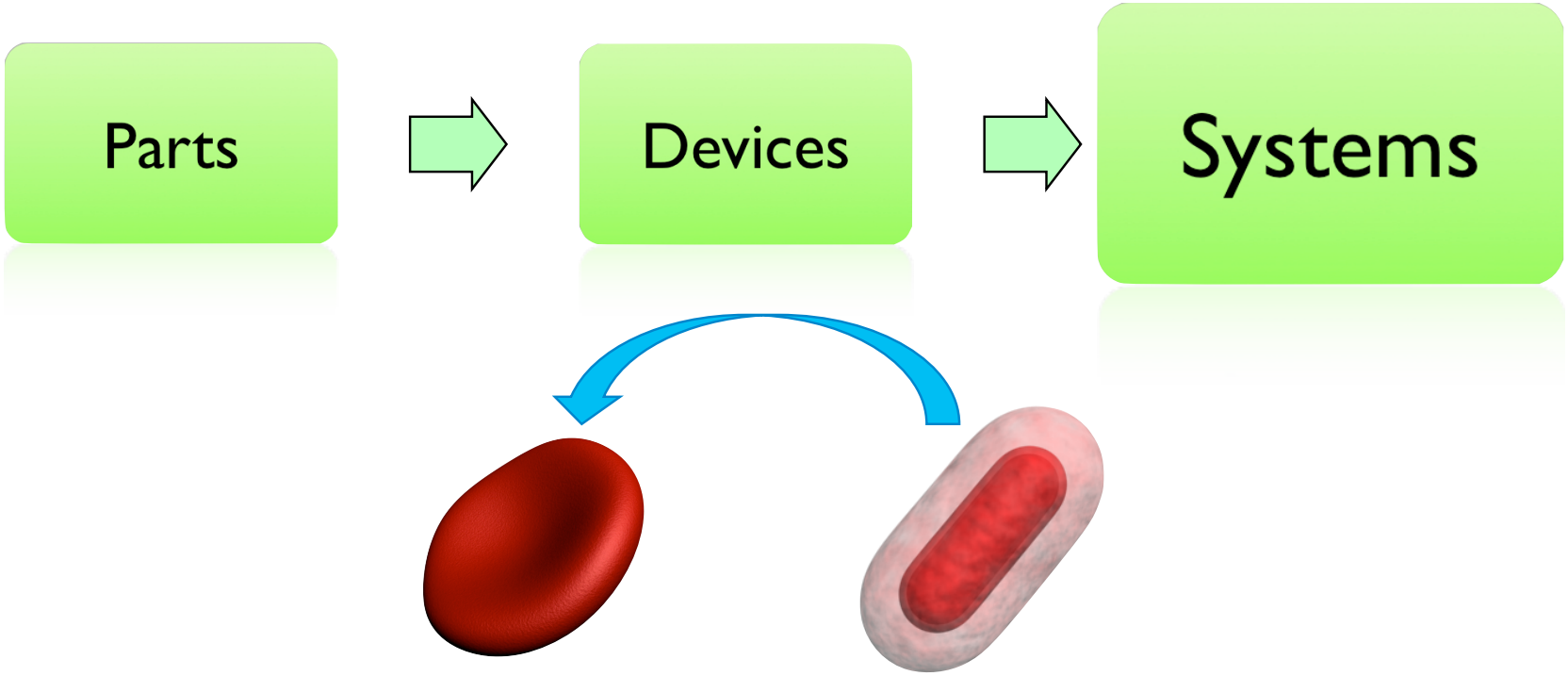
Synthetic Biology





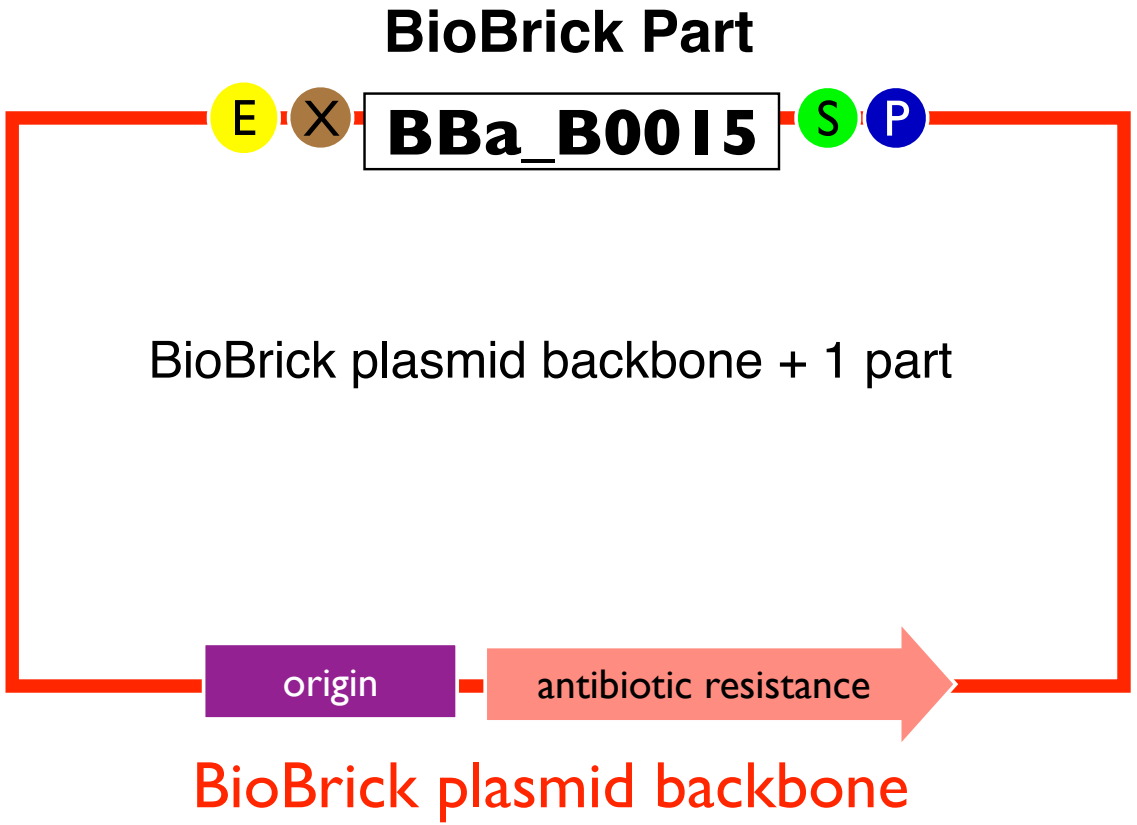
Anderson *et al. Mol Sys Bio*. 2007.

Synthetic Biology



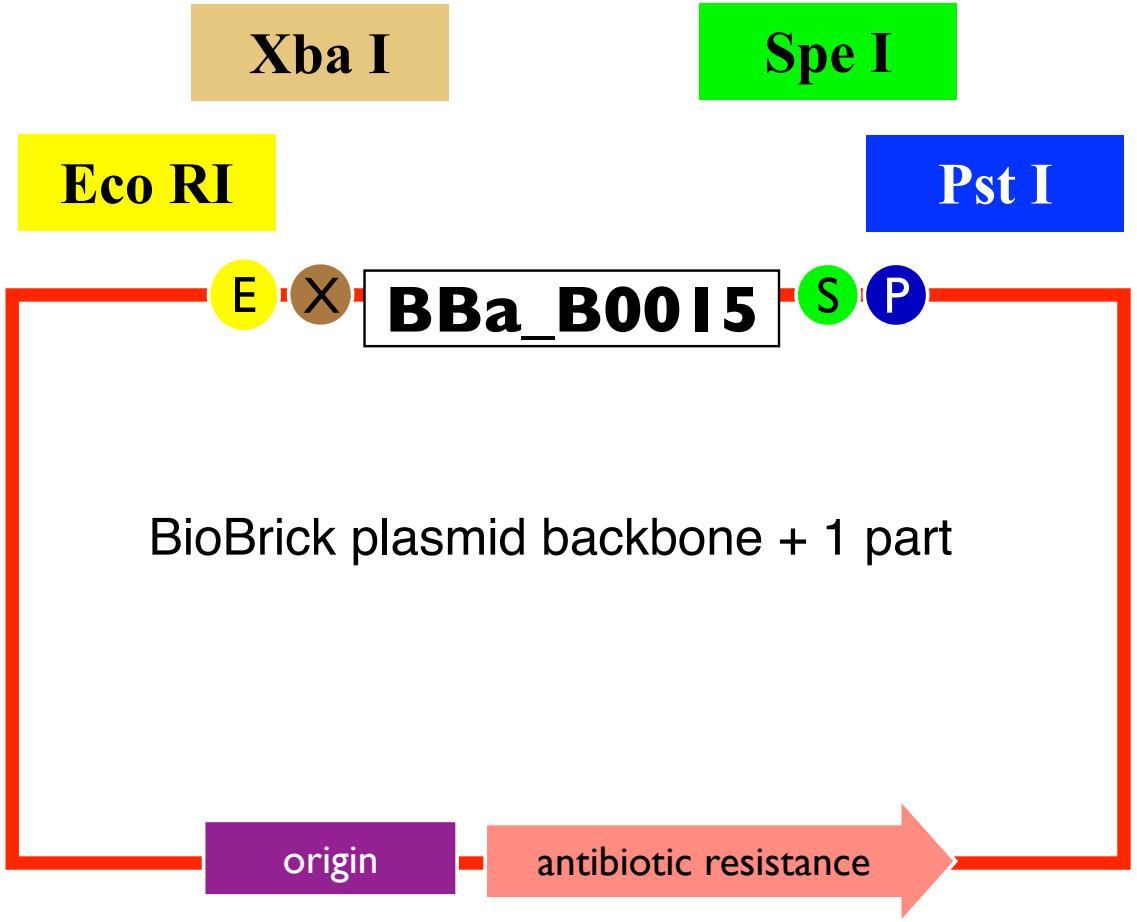
How do we clone DNA?

BioBricks



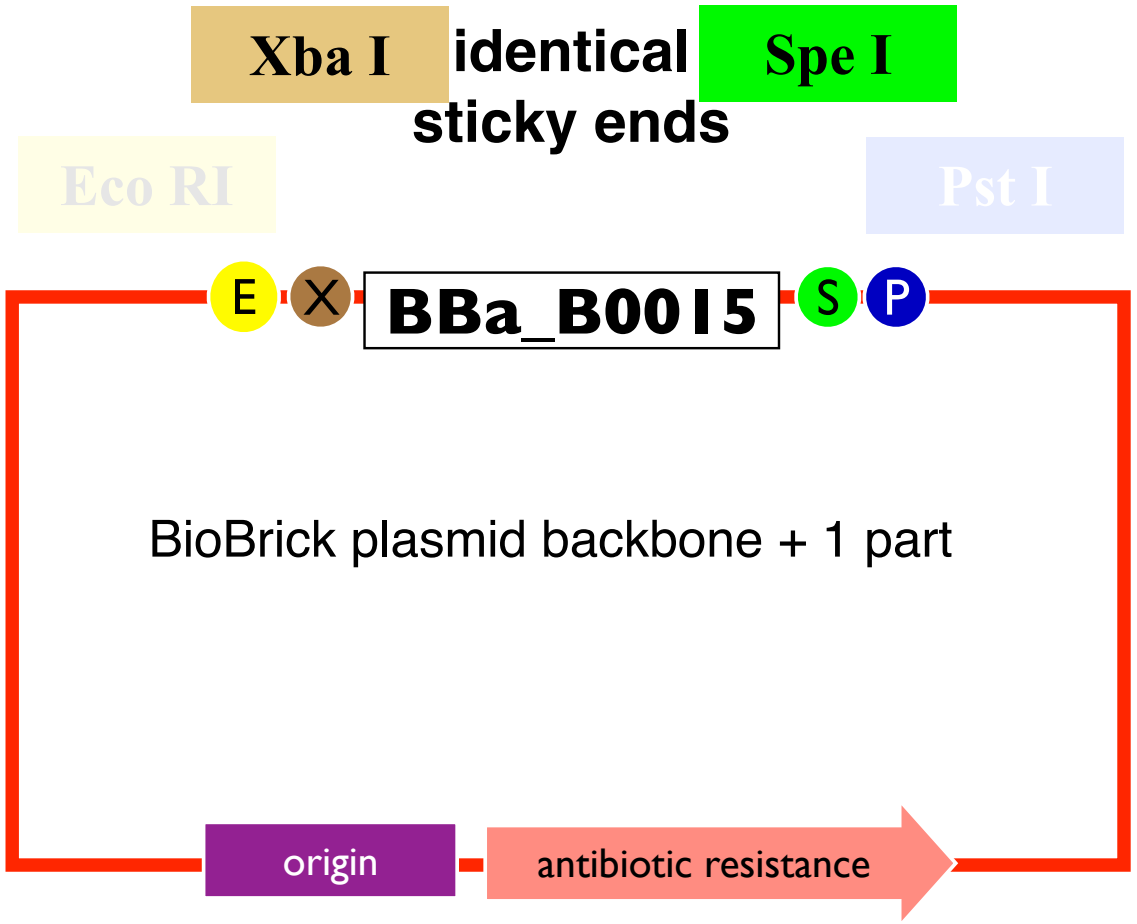
(http://partsregistry.org/Plasmids)

BioBricks



(http://partsregistry.org/Plasmids)

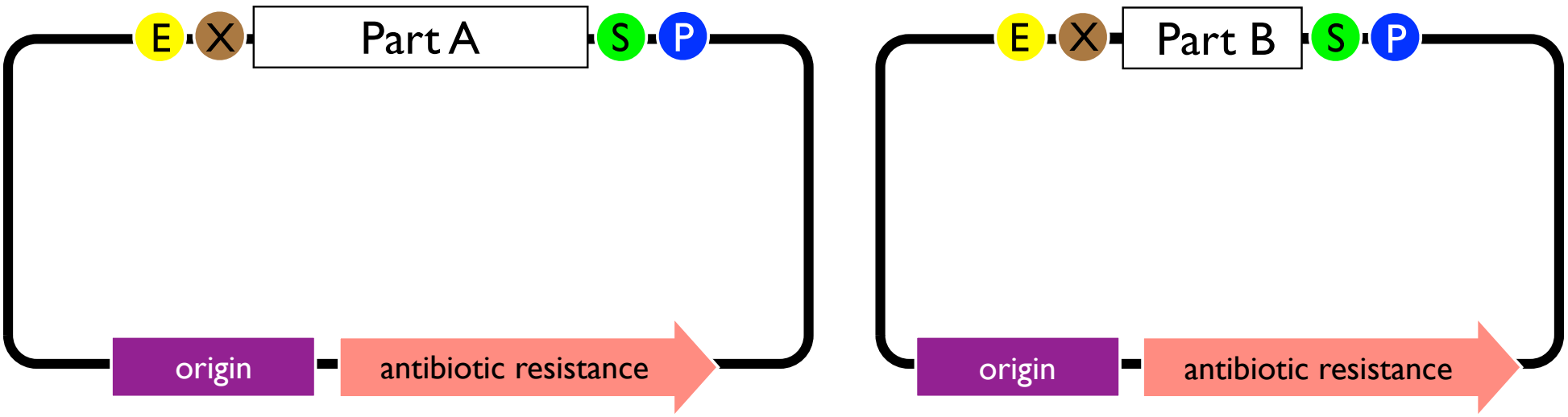
BioBricks



(http://partsregistry.org/Plasmids)

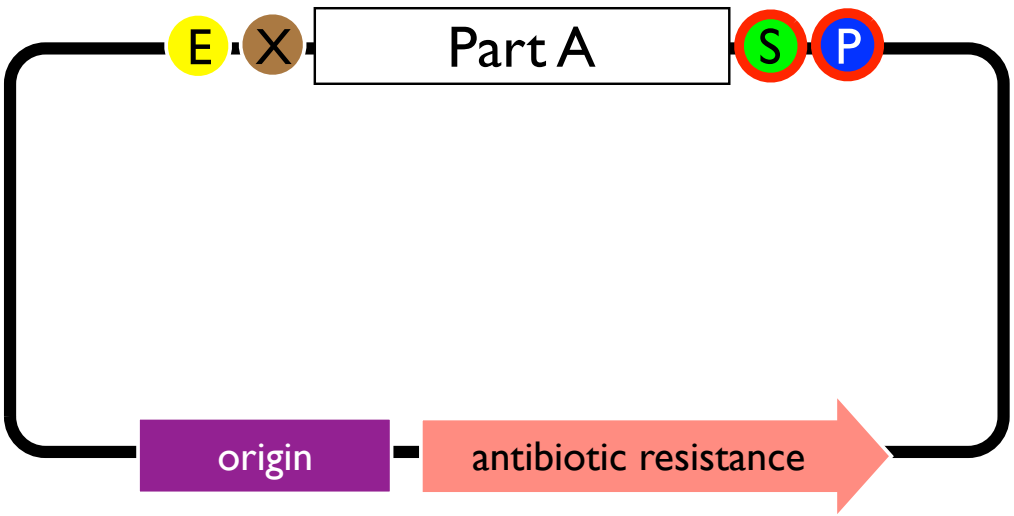
BioBricks

put B downstream of A

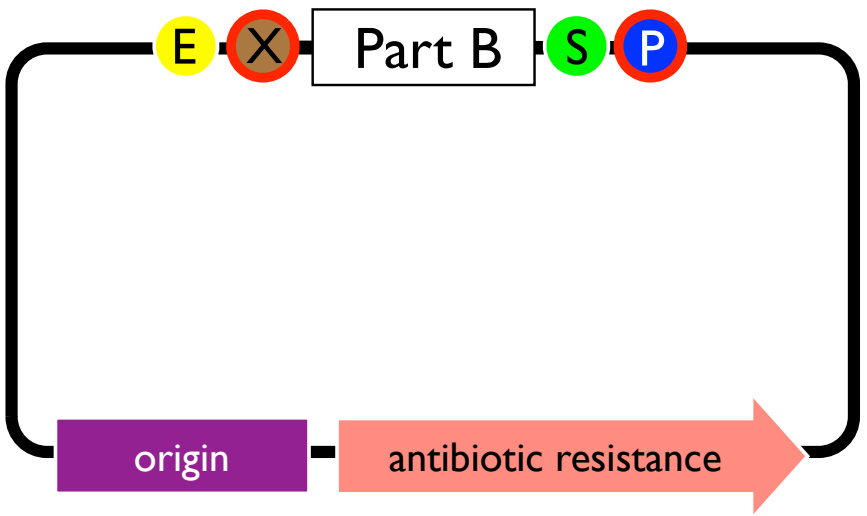


BioBricks

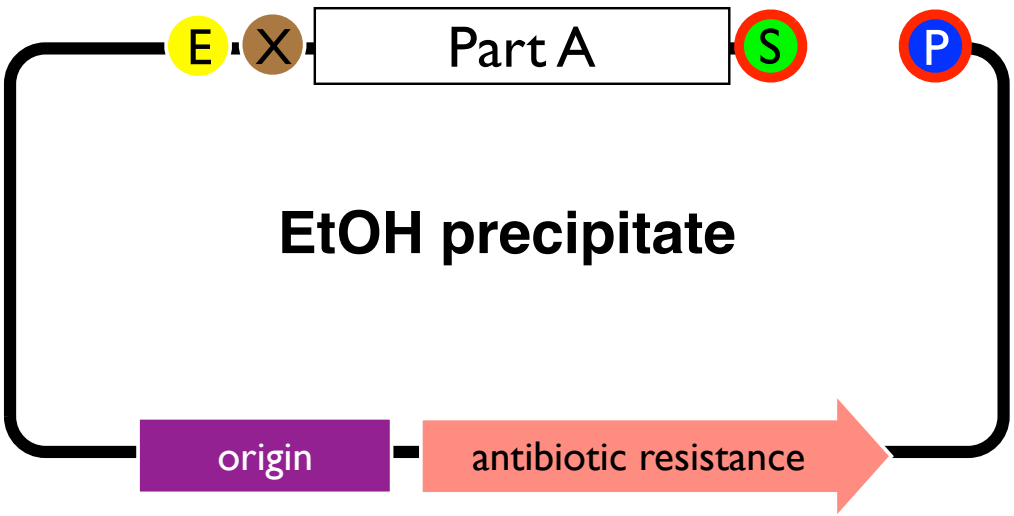
cut with Spe and Pst



cut with Xba and Pst



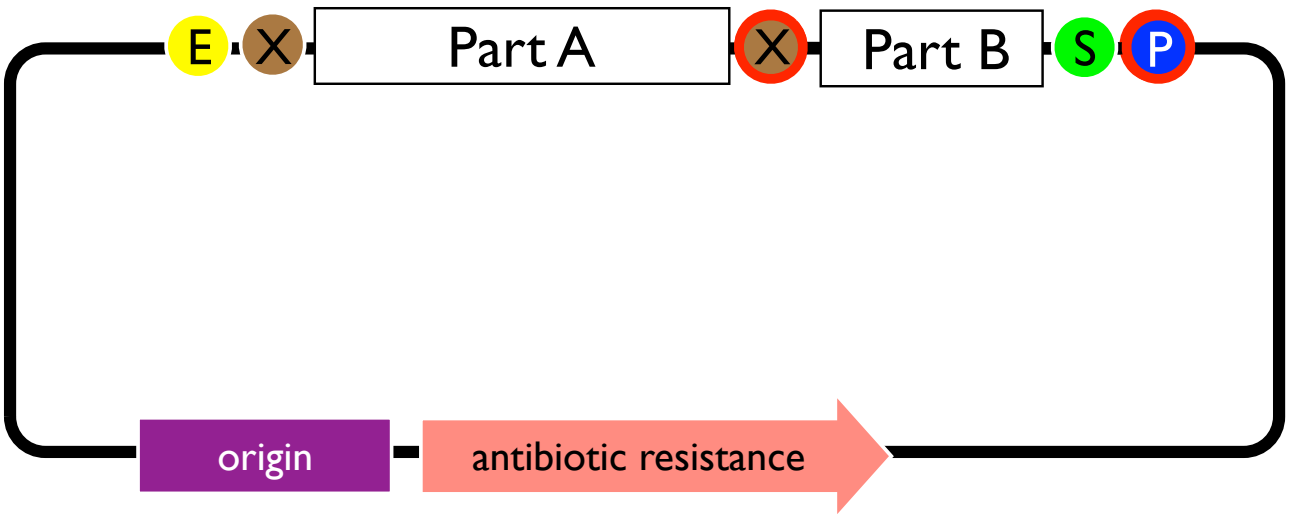
BioBricks



EtOH precipitate

gel purify

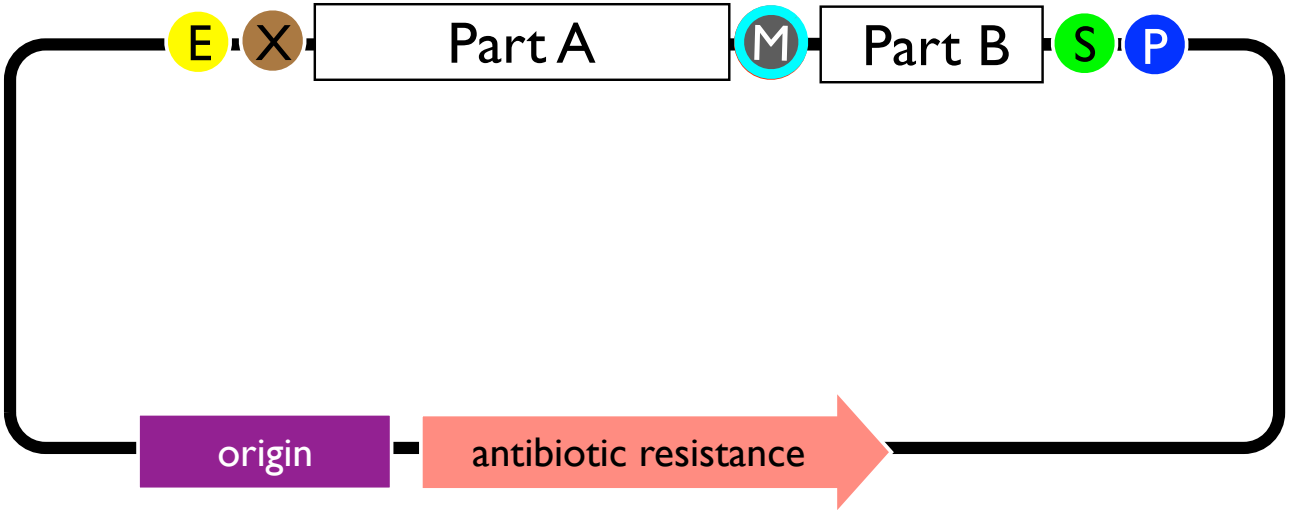
BioBricks



ligate

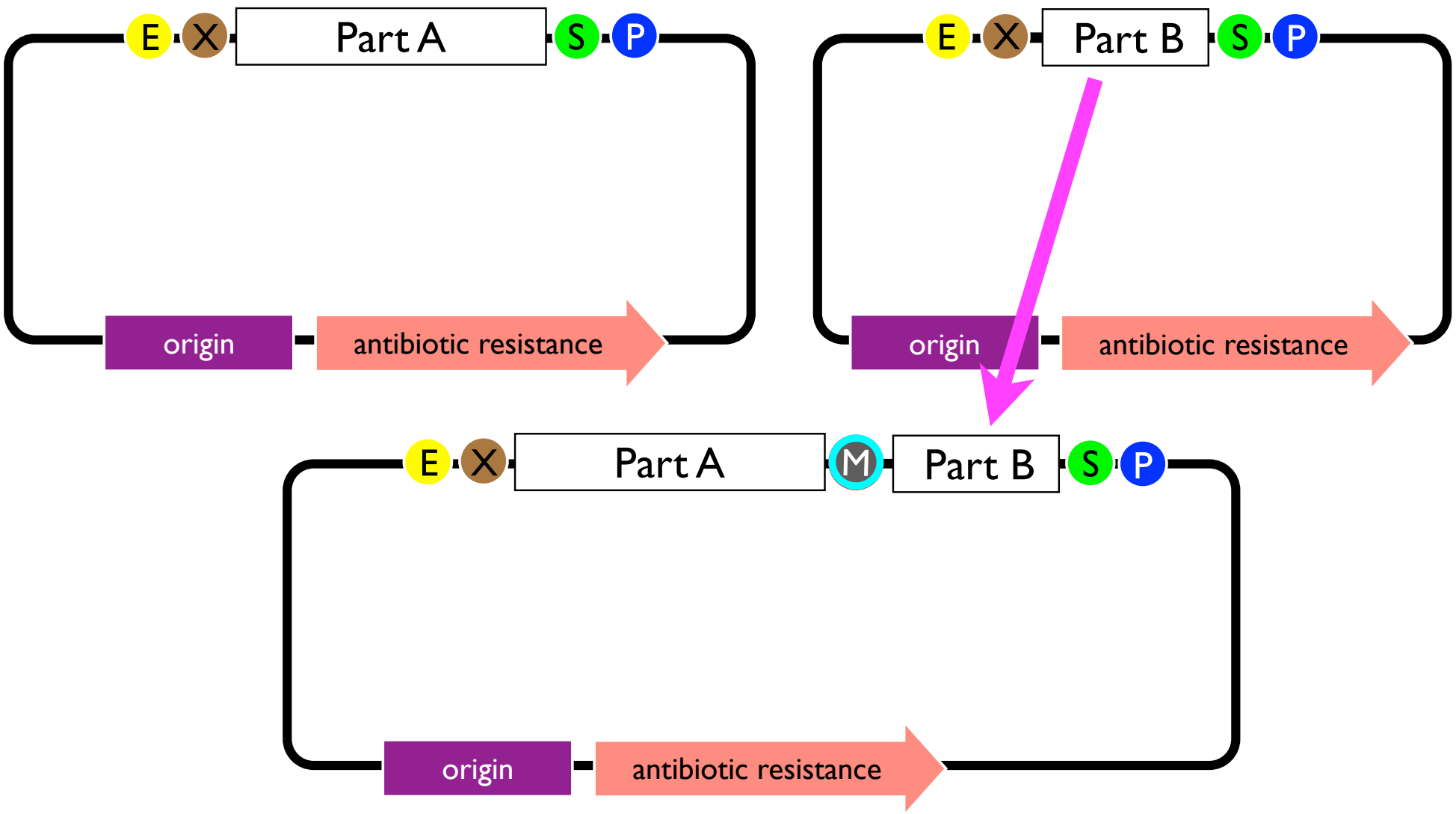
BioBricks

mixed site = scar



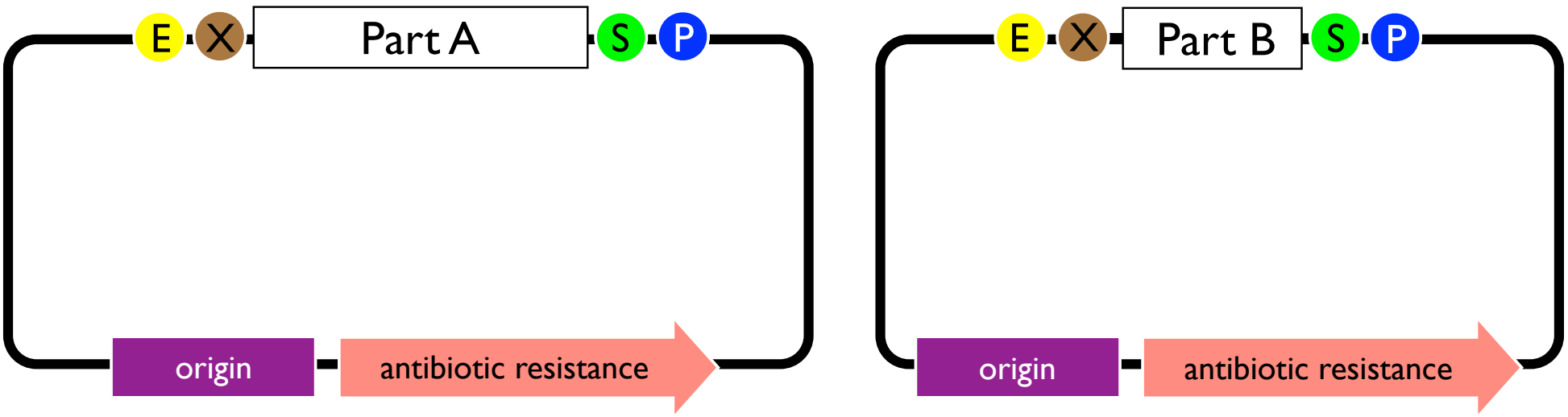
transform

BioBricks


E
X
Part A
S
P
origin
antibiotic resistance
E
X
Part B
S
P
origin
antibiotic resistance
E
X
Part A
M
Part B
S
P
origin
antibiotic resistance

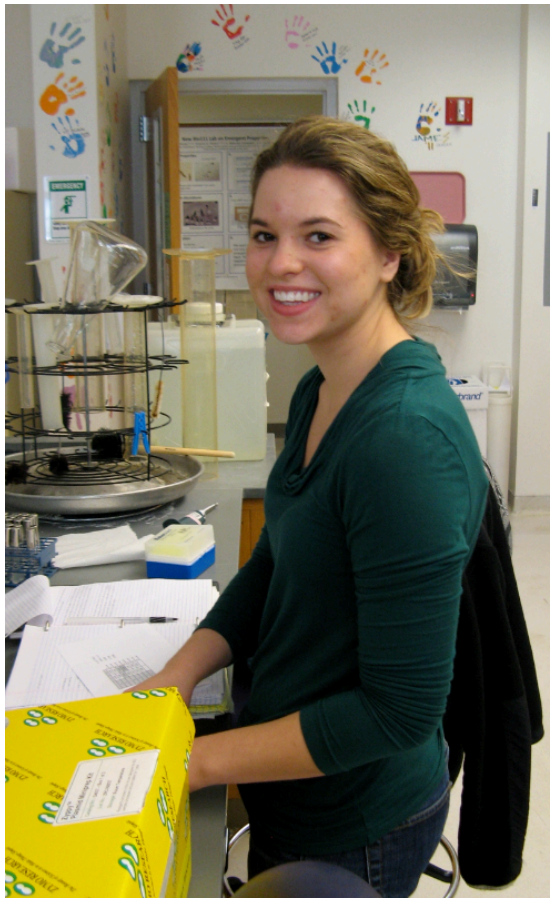
Challenge:

put A upstream of B



Too Many Projects to Talk About Today

Catherine Doyle ’14



Julia Fearrington ’13

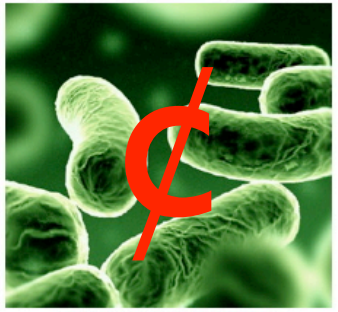
Building Bacterial Computers

Advantages of Bacterial Computation

Software ⟶ Hardware ⟶ Computation

Computation

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



http://www.turbosquid.com

Computation

Advantages of Biological Computers

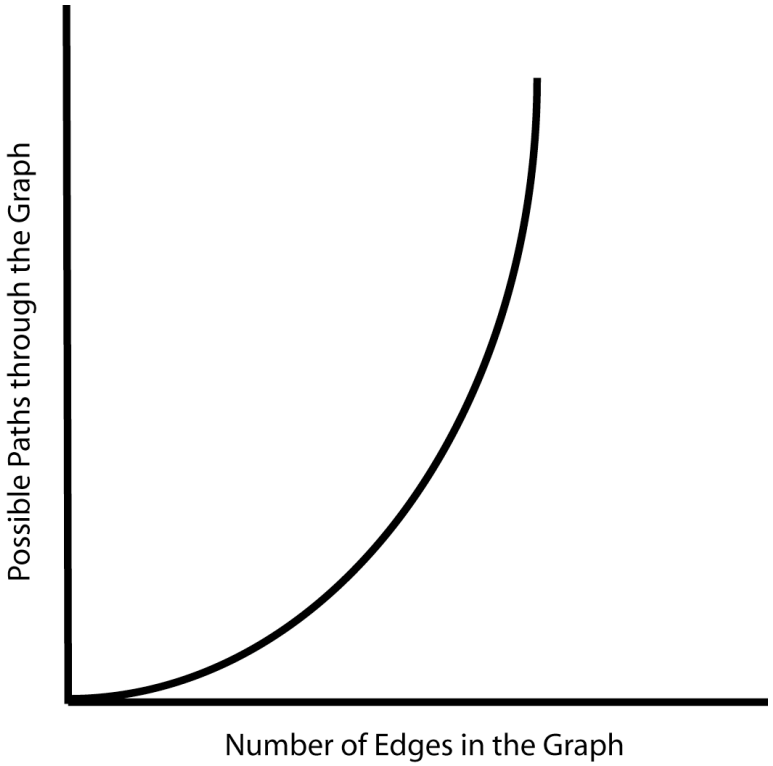
go anywhere - arctic, thermal vents, inside organisms

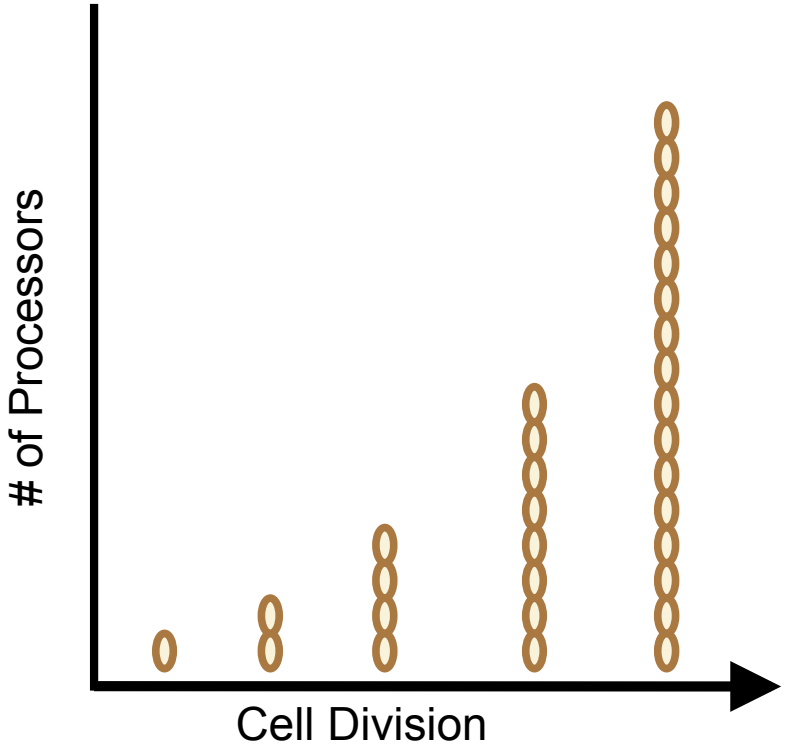
no electricity

self-replicating

no immune rejection

Self-replicating Computers

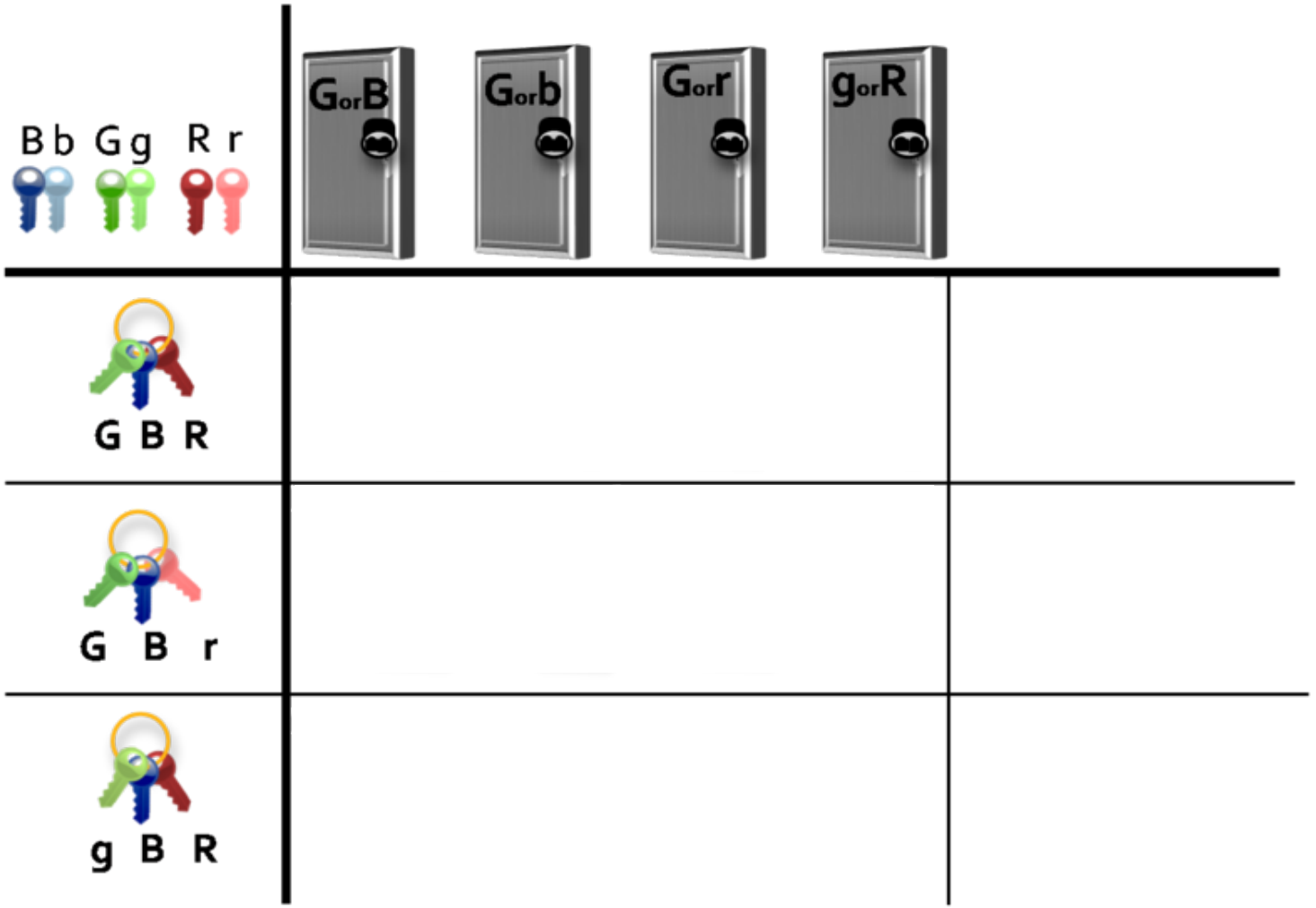




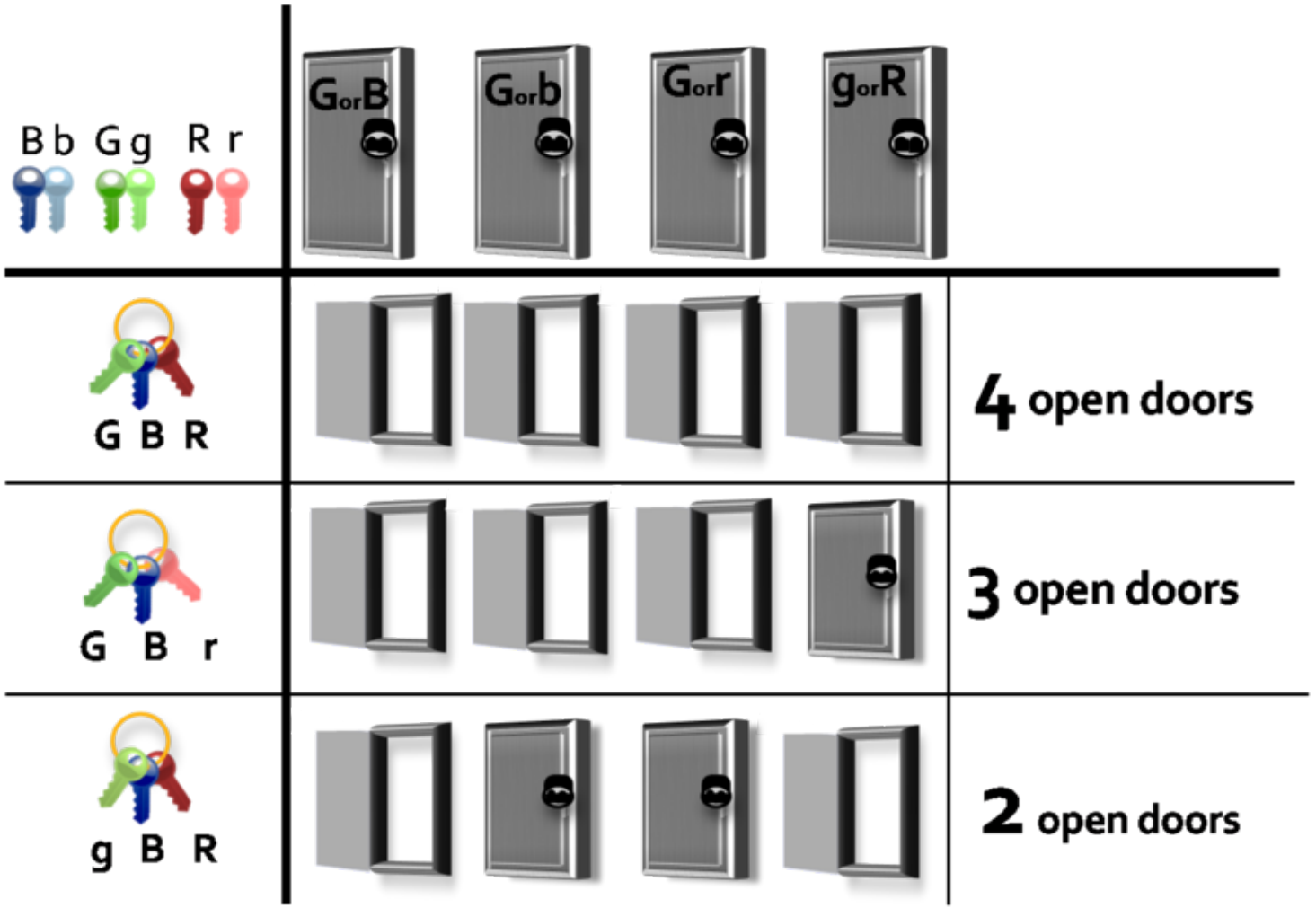
One Research Project - the SAT problem

Define the SATisfiability Problem

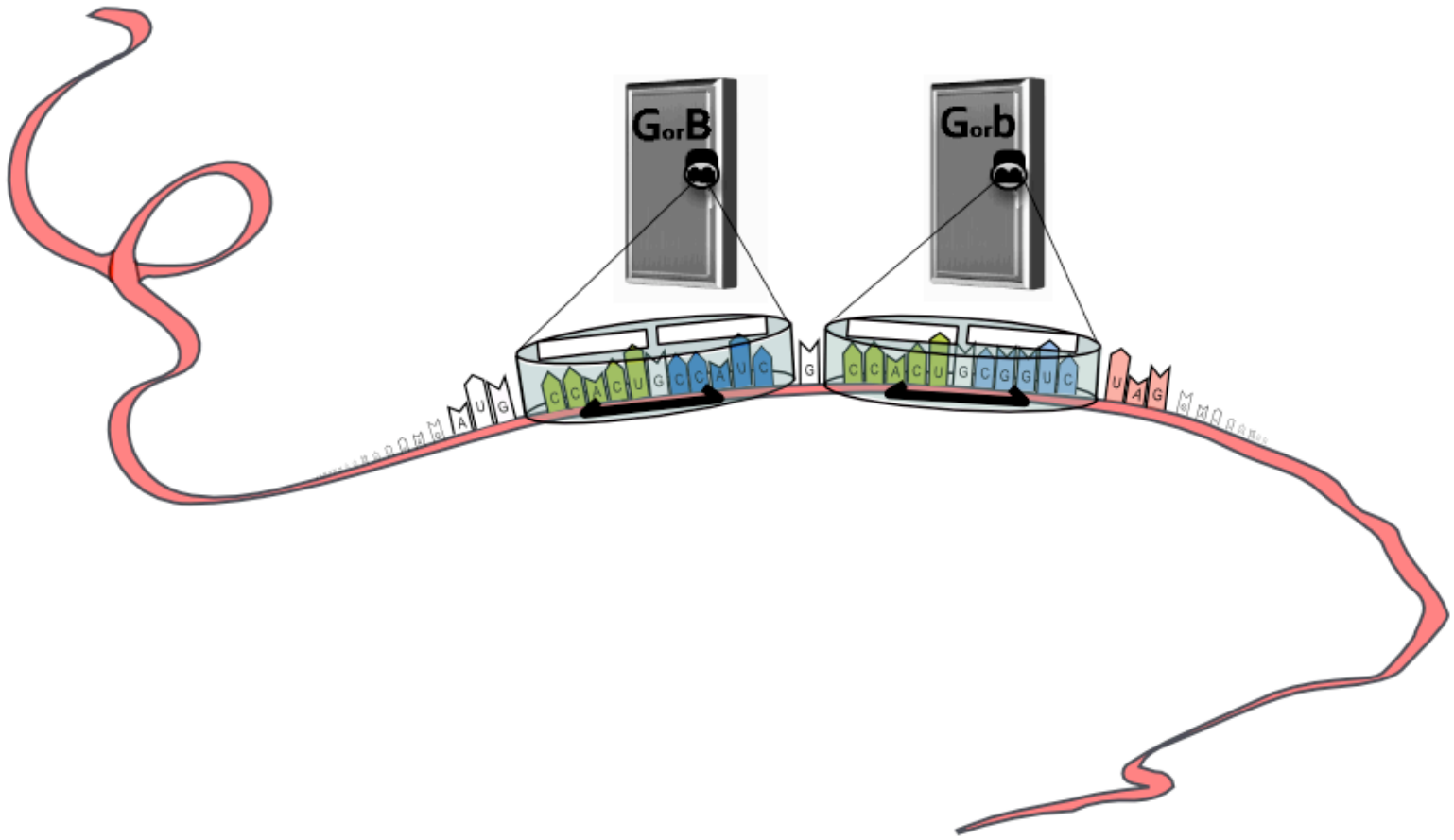
Define the SATisfiability Problem



Define the SATisfiability Problem



Converting Math to Biology



Central Dogma

DNA

atgccctactcactacctatagcgcat

transcription

mRNA

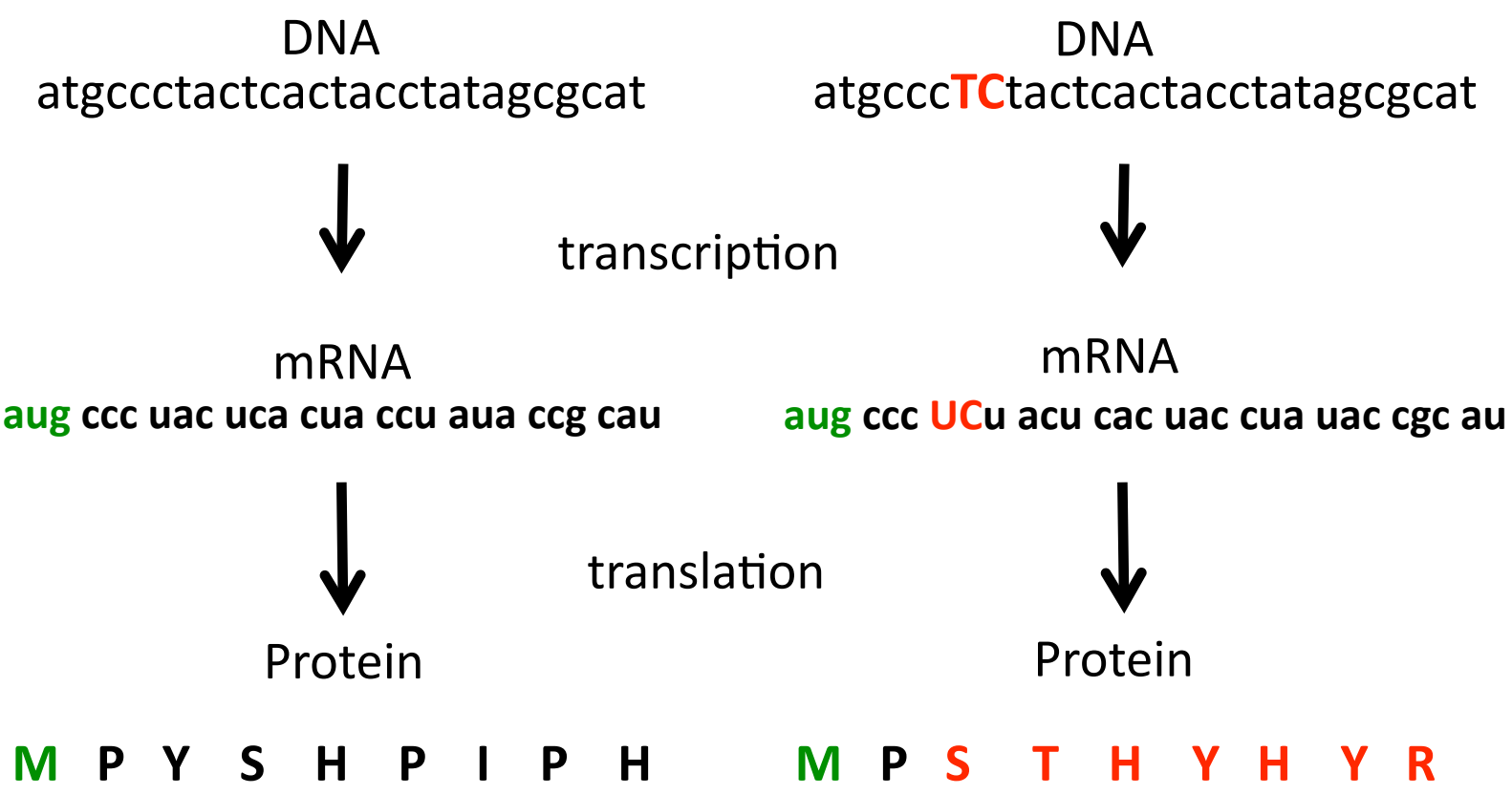
aug ccc uac uca cua ccu aua ccg cau

translation

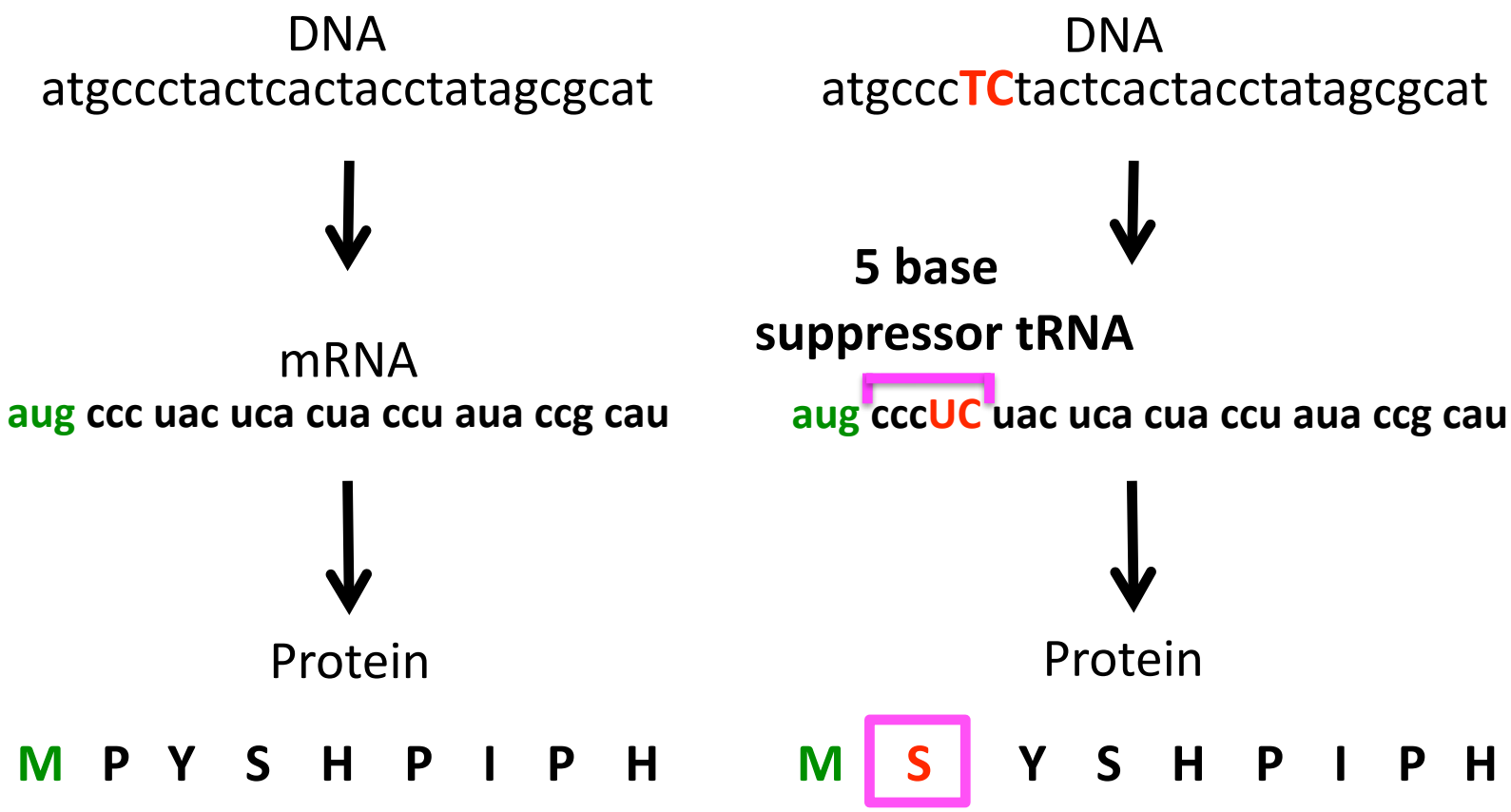
Protein

M P Y S H P I P H

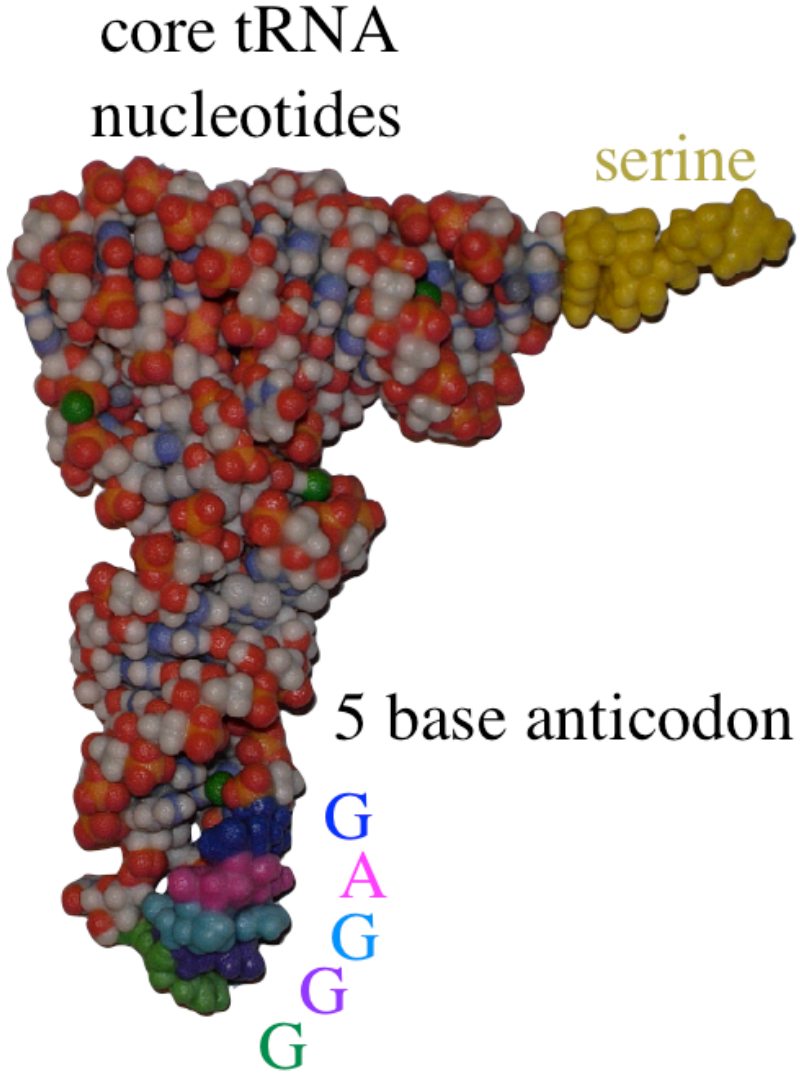
Frameshift Mutation



Frameshift Suppression



Suppressor tRNA



Coding 2-SAT Clause

G ATG NNNNN gNN NNN → satisfied

Coding 2-SAT Clause

G ATG NNNNN gNN NNN → satisfied

OR

B ATG NNN NNg NNNNN → satisfied

Coding 2-SAT Clause

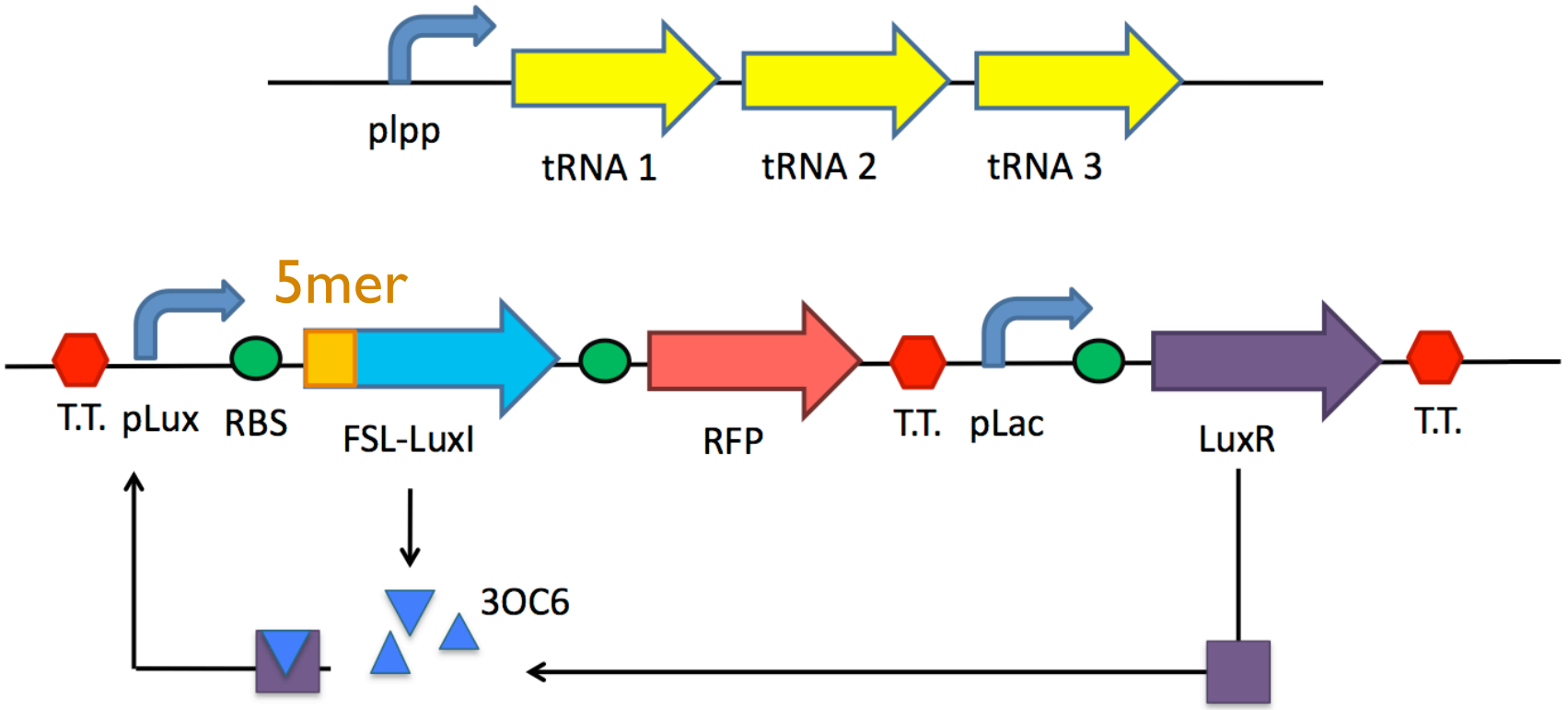
G ATG NNNNN gNN NNN → satisfied

OR

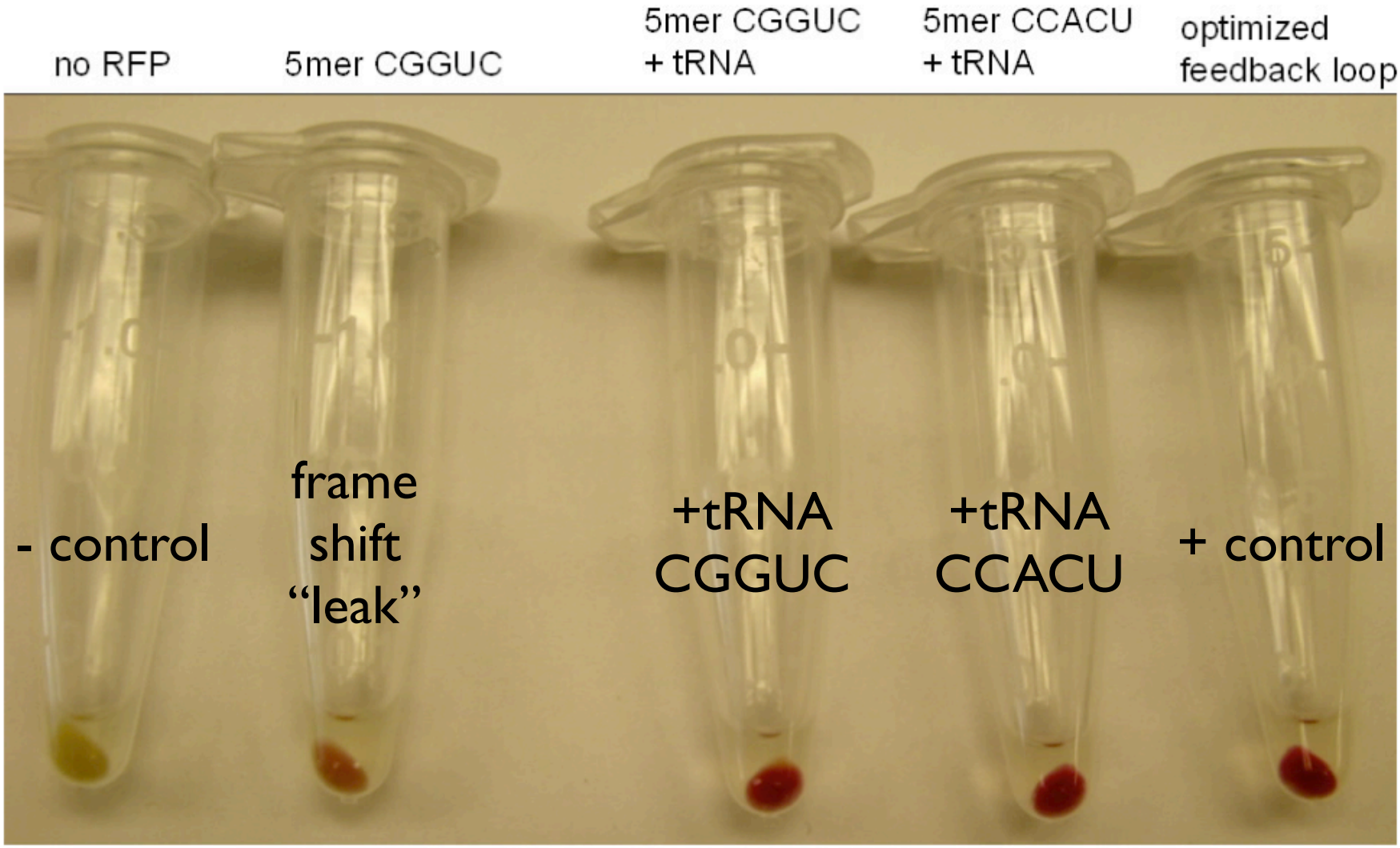
B ATG NNN NNg NNNNN → satisfied

ATG NNN NNg NNN → no satisfaction

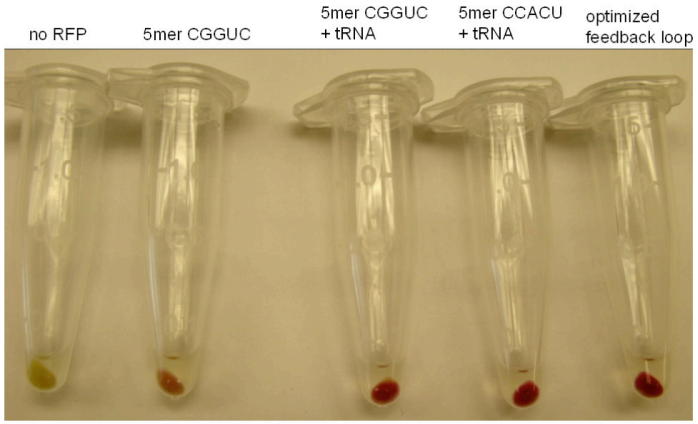
Redesign System v2.0

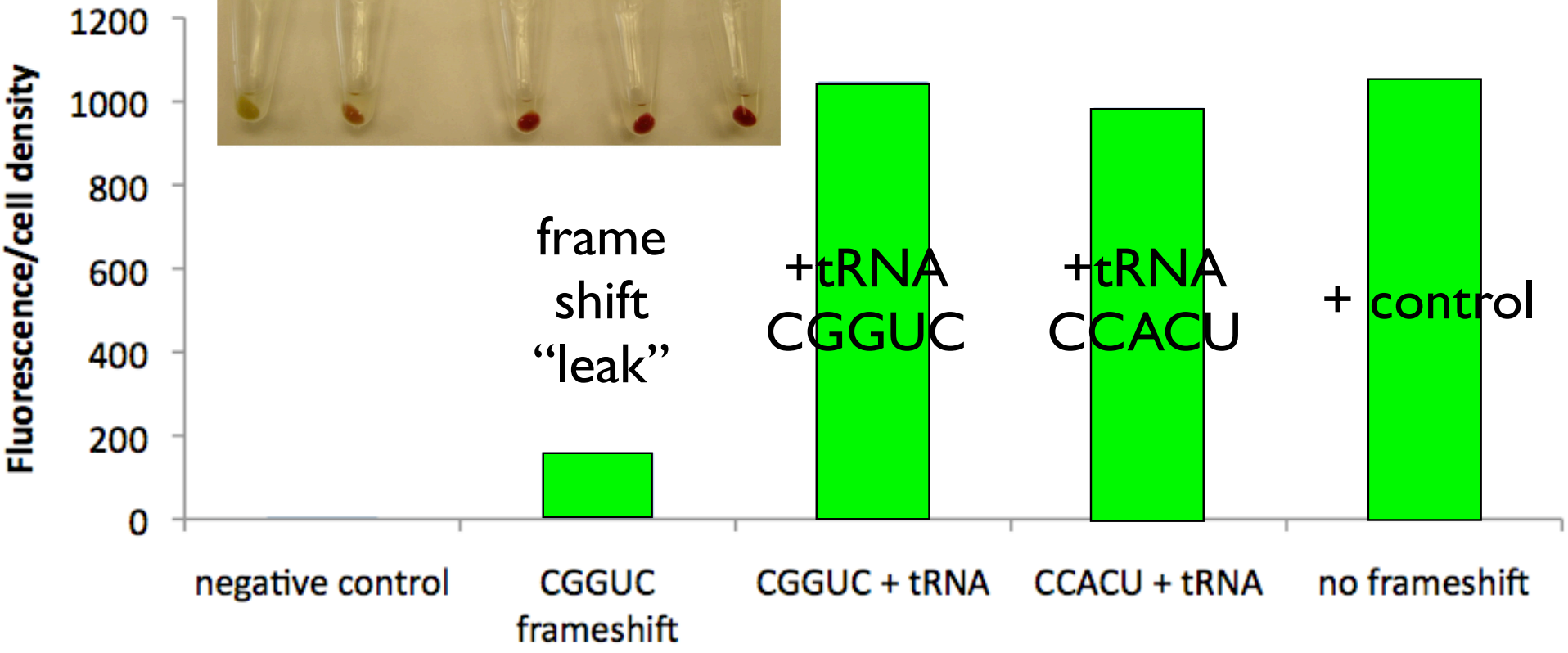


Outcomes of v 2.0

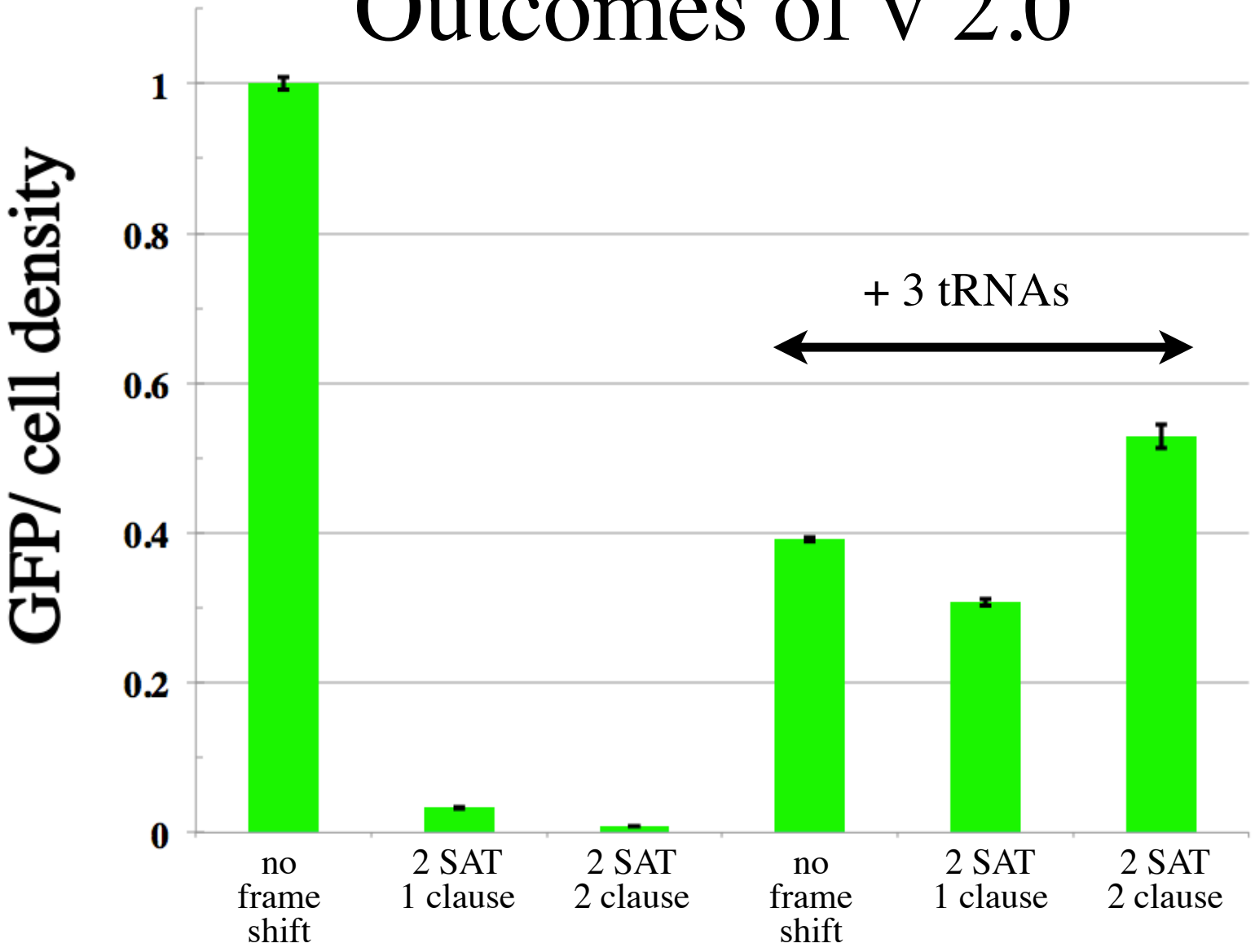


Outcomes of v 2.0

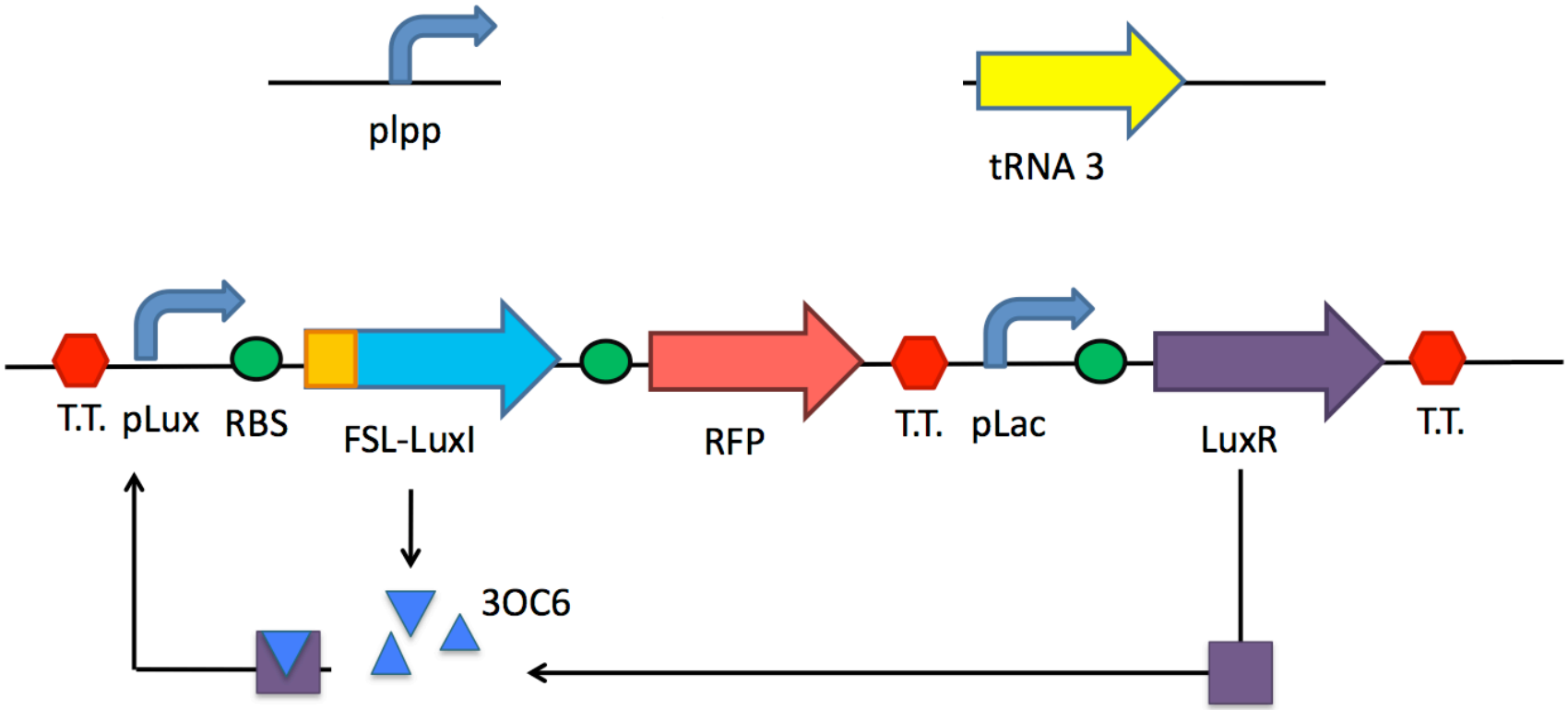




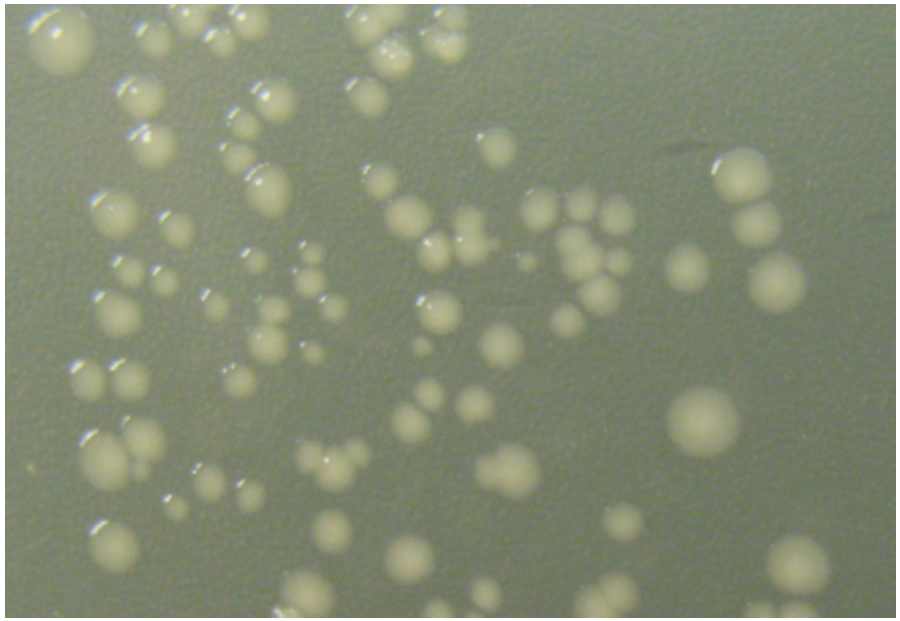
Outcomes of v 2.0

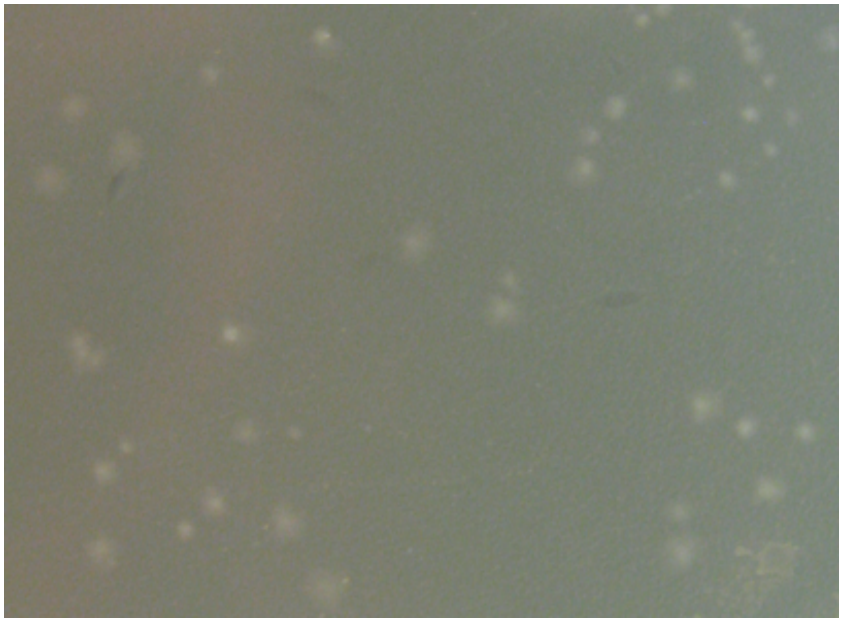


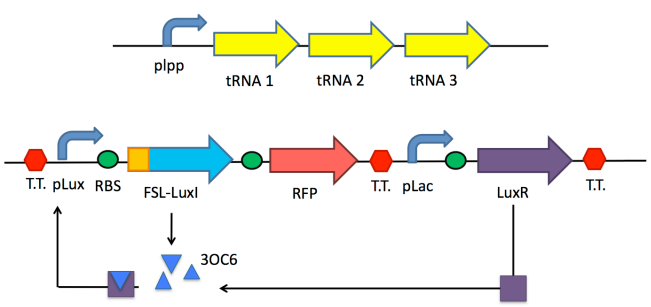
Controls….

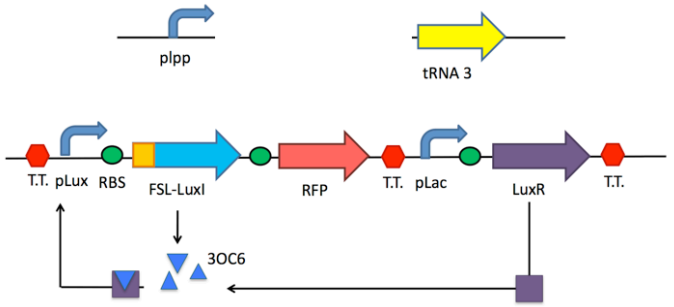


Controls....

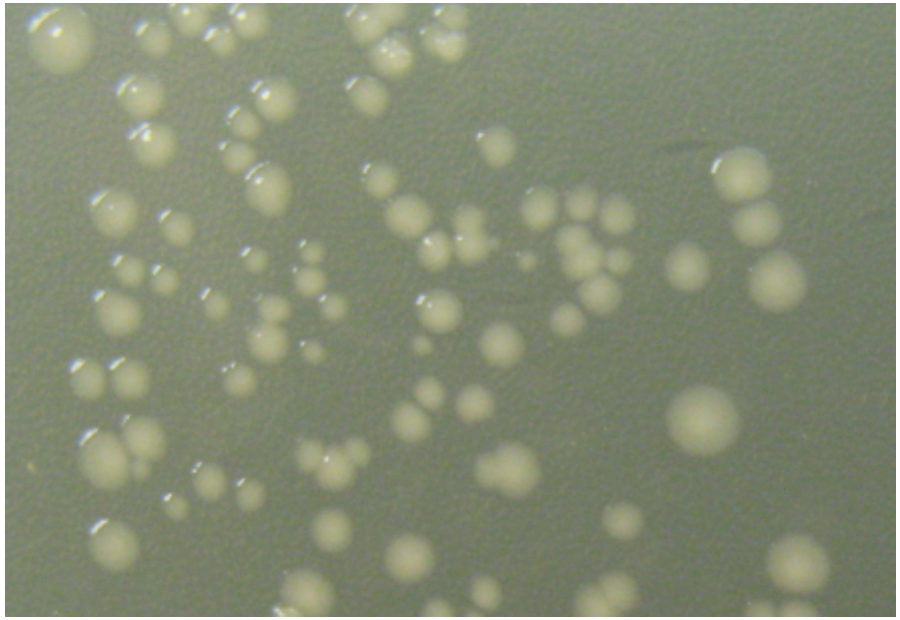


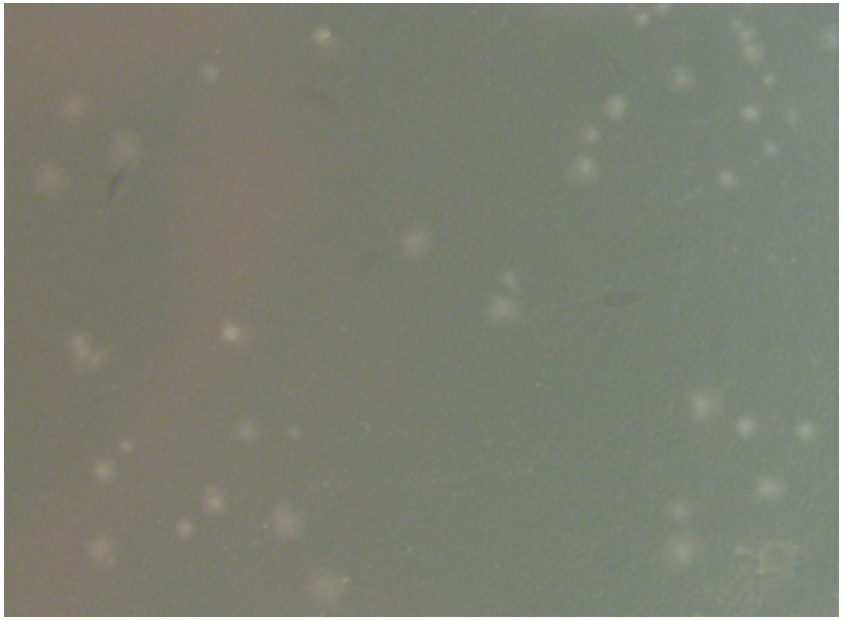


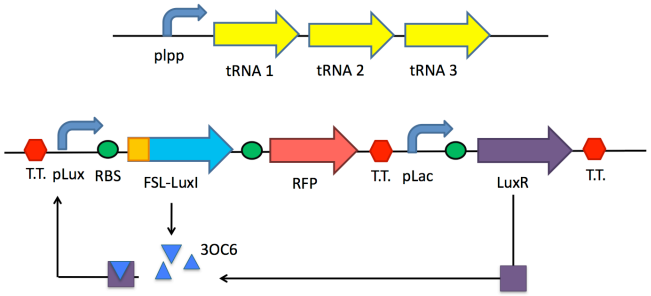

plpp
tRNA 1
tRNA 2
tRNA 3
T.T.
pLux
RBS
FSL-LuxI
RFP
T.T.
pLac
LuxR
T.T.
3OC6

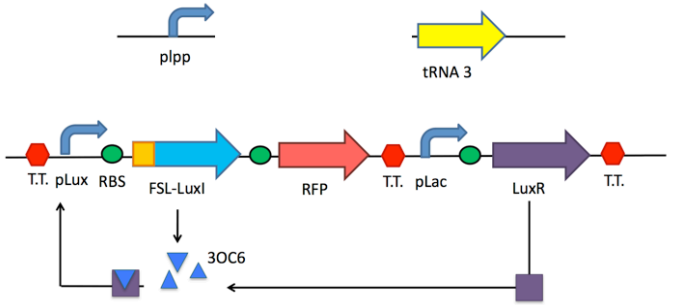

plpp
tRNA 3
T.T.
pLux
RBS
FSL-LuxI
RFP
T.T.
pLac
LuxR
T.T.
3OC6

Still working on this...



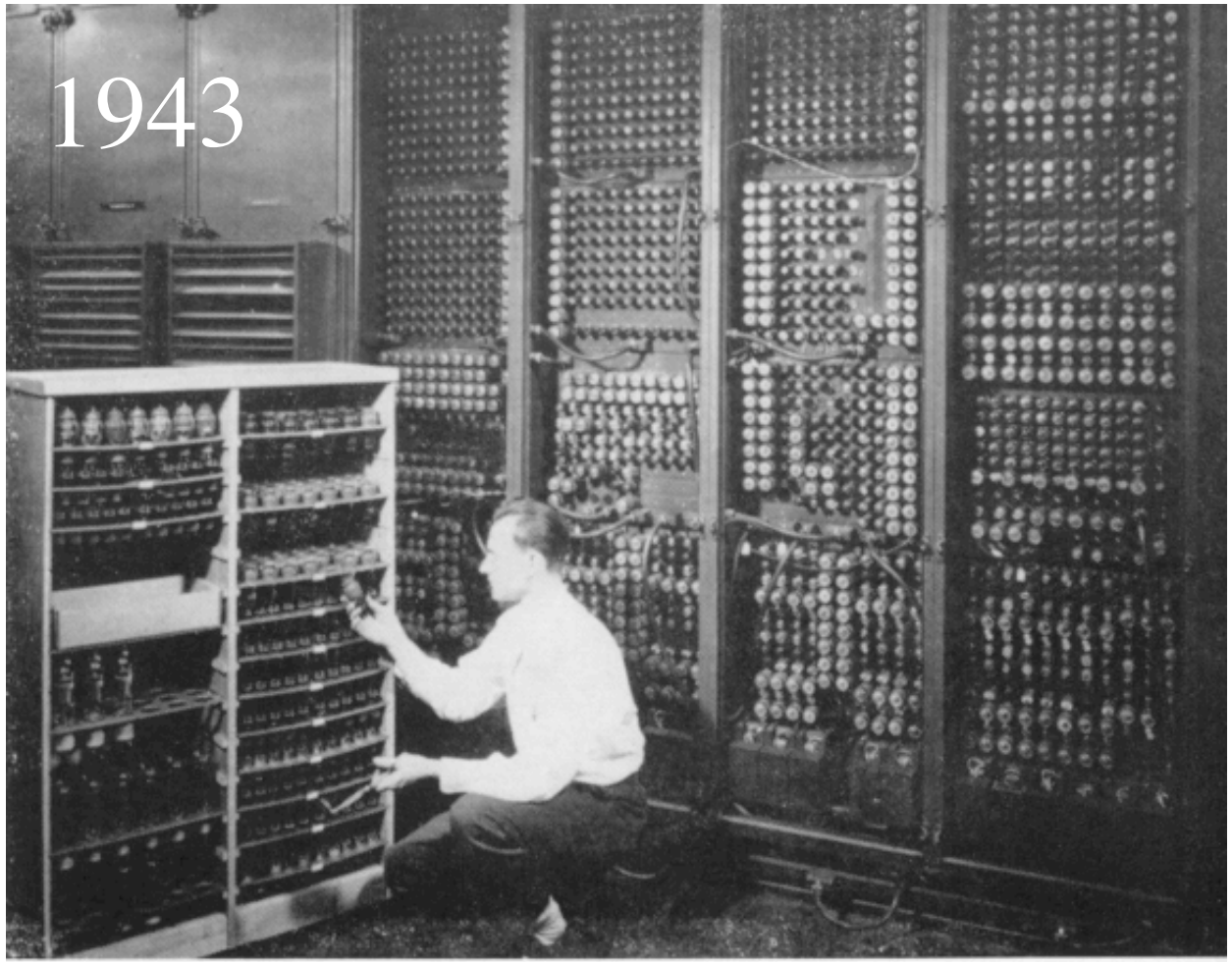






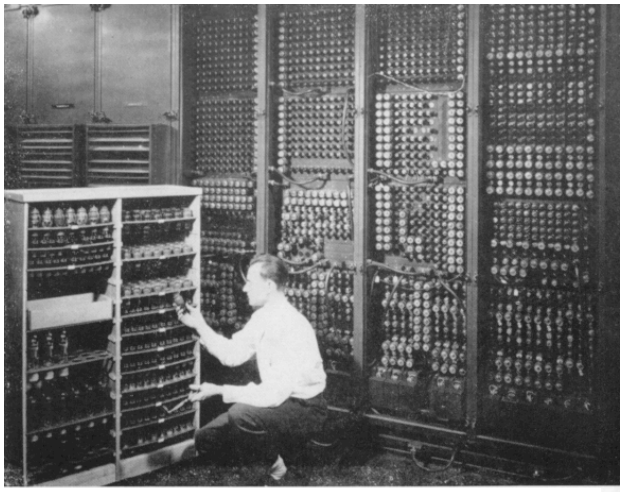
Why build bacterial computers?

Evolution of Computers



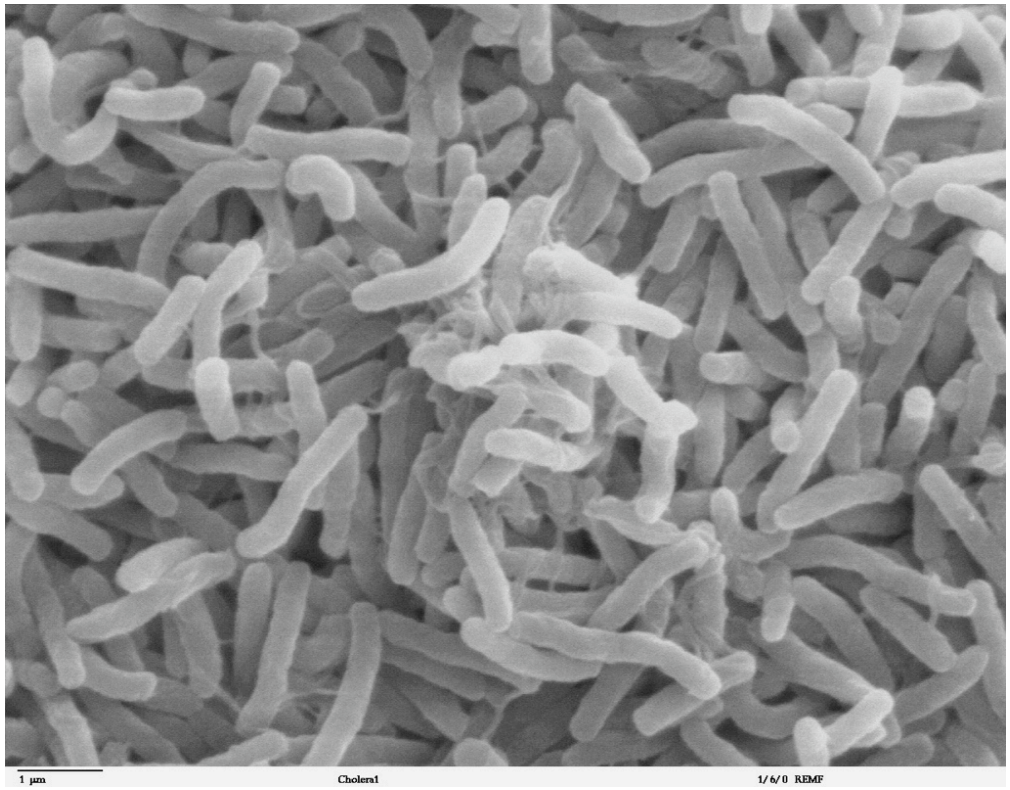
Evolution of Computers

iPhone in 2011



Evolution of Bacterial Computers

E. coli in 2011



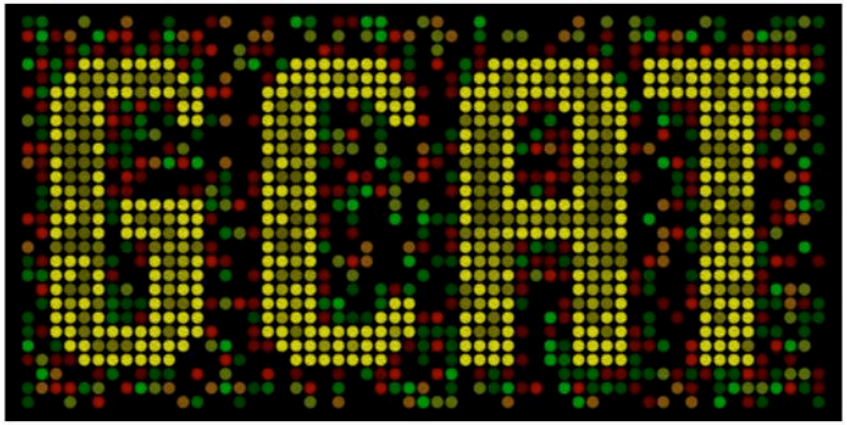
→ ?

Living Hardware
in 2021

Intro Bio Student Collaborators

DAVIDSON

Discover gold WESTERN



Intro Bio Student Collaborators

Intro Bio Student Collaborators

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

Intro students find promoter of interest.

Oligator converts into oligos.

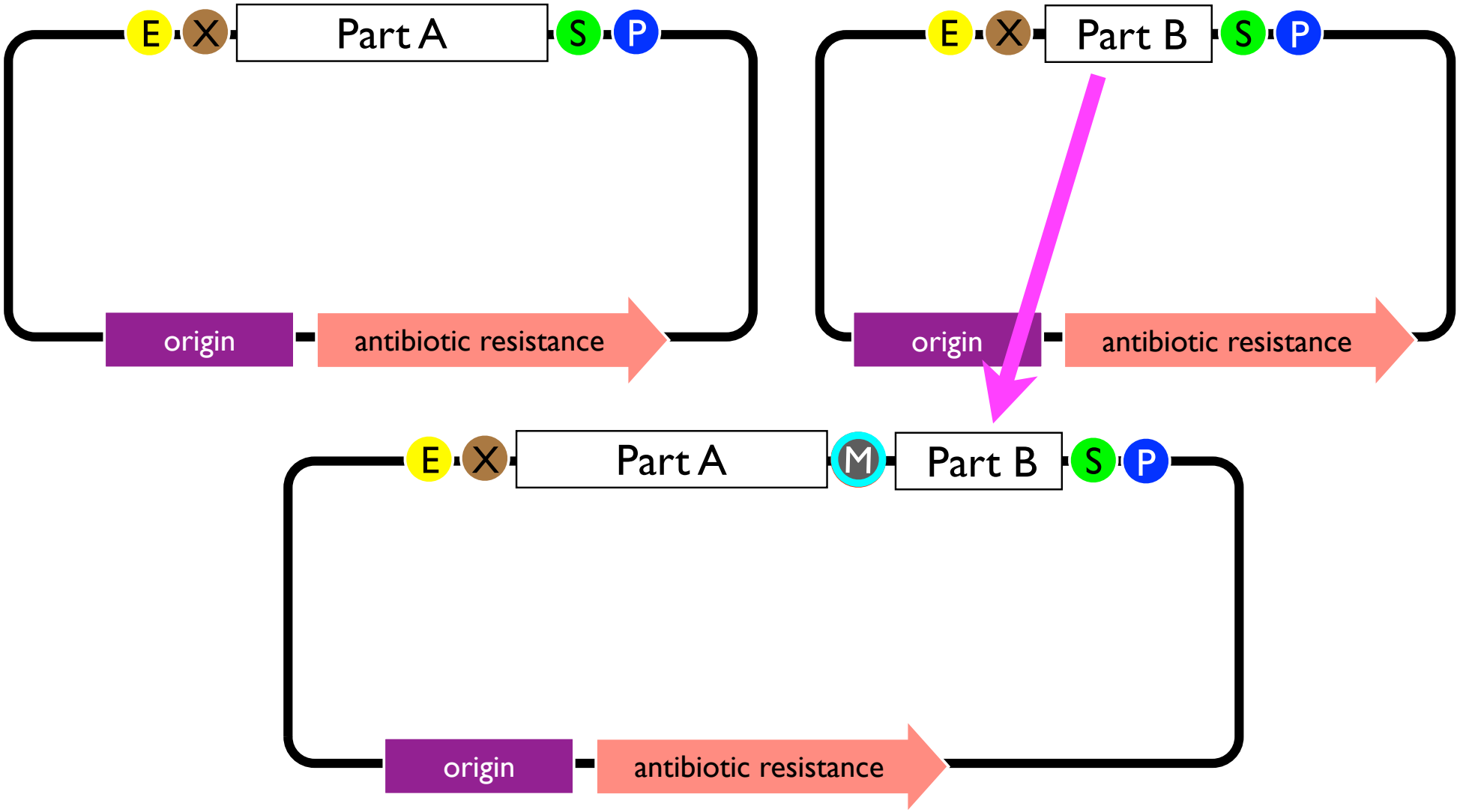
Clone new promoter.

Transform and test new promoter.

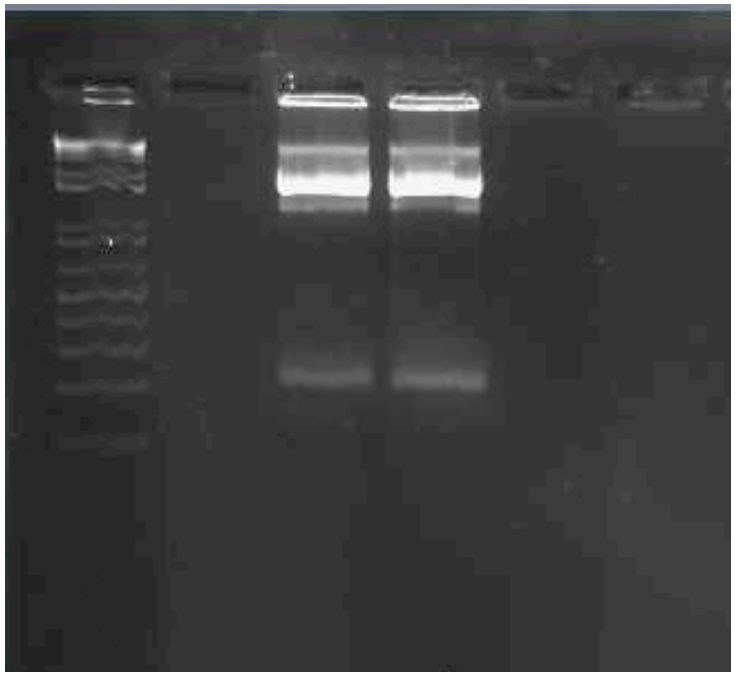
Submit to Registry of Functional Promoters (national resource).

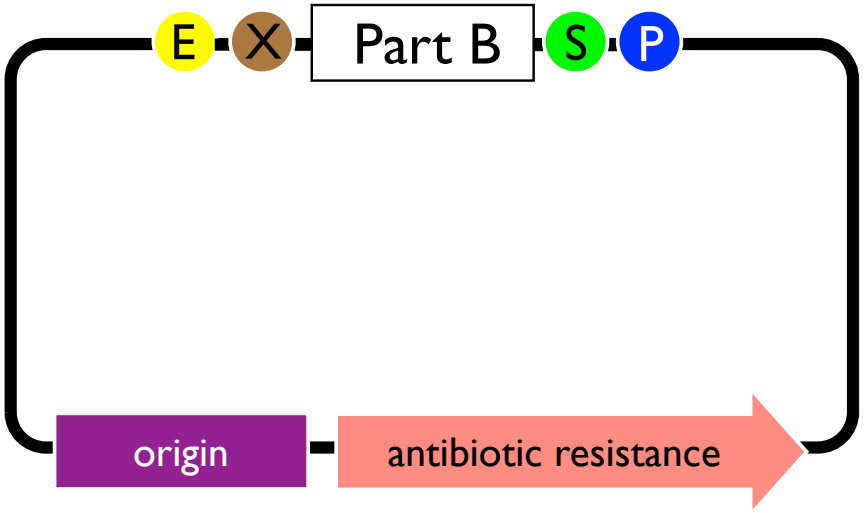
But cloning DNA is not easy….

Details of Cloning

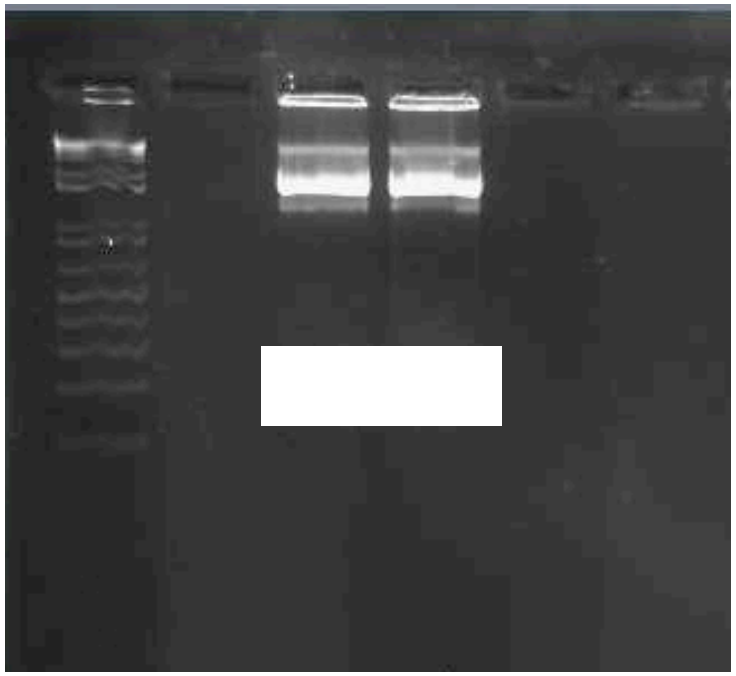


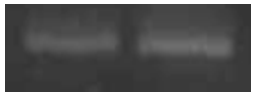
Gel Purification

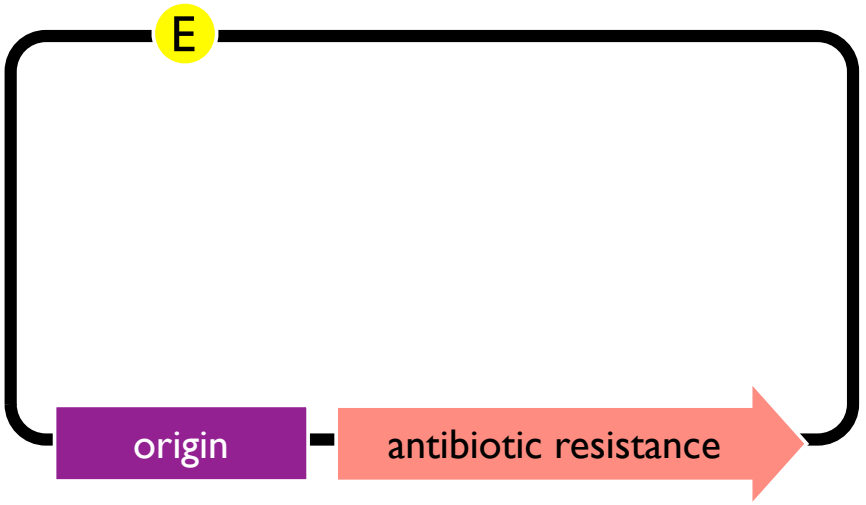




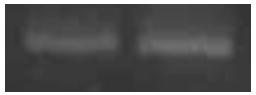
Gel Purification

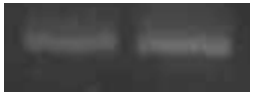






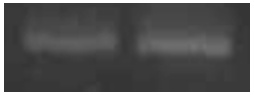
Gel Purification

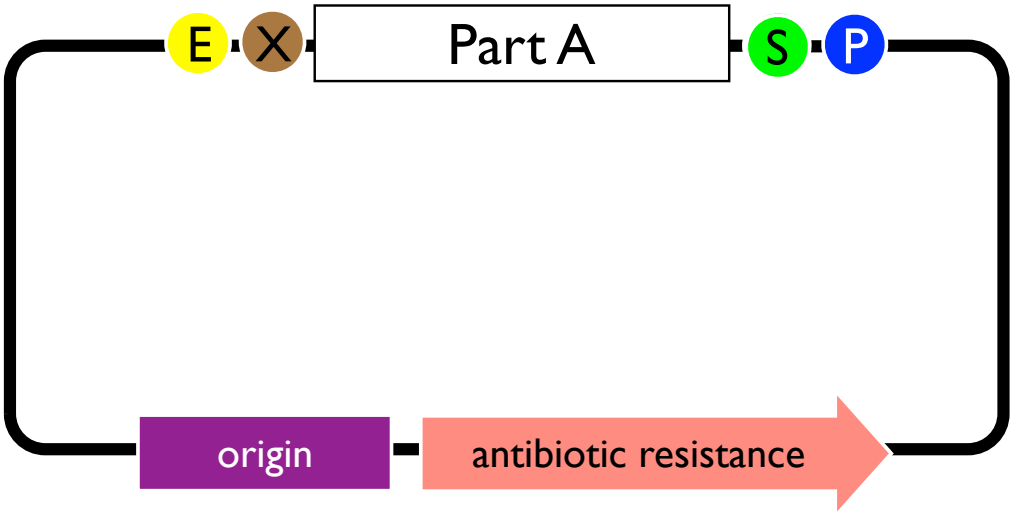


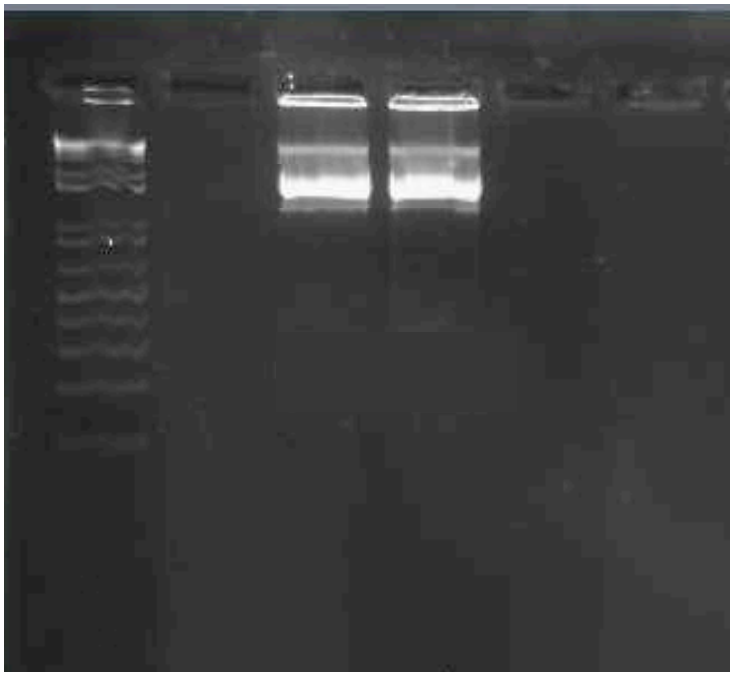


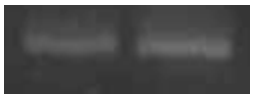
Gel Purification

Gel Purification









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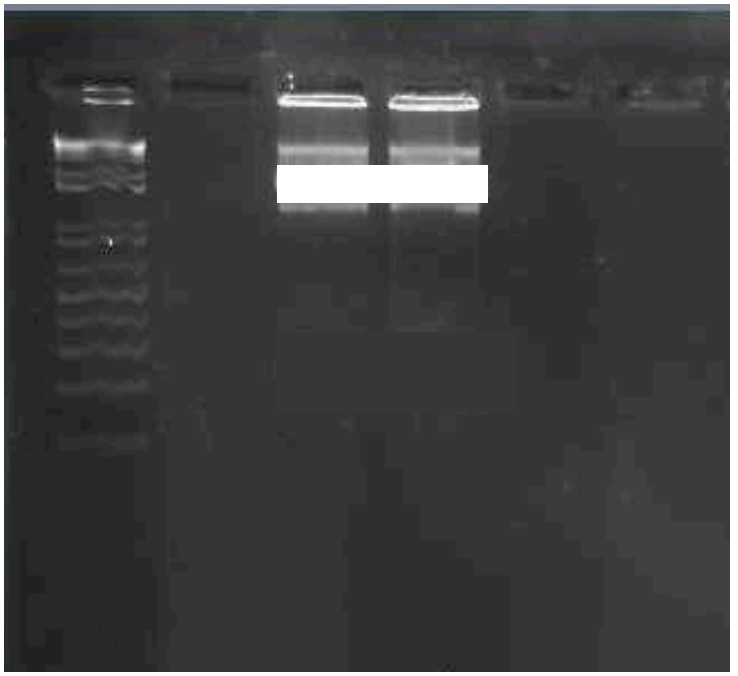

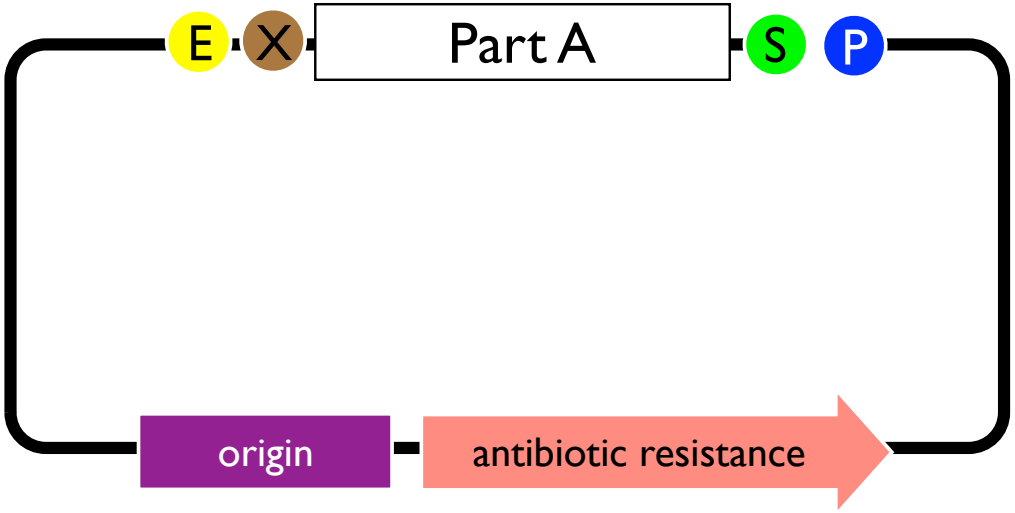

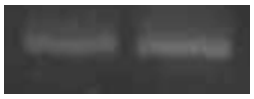

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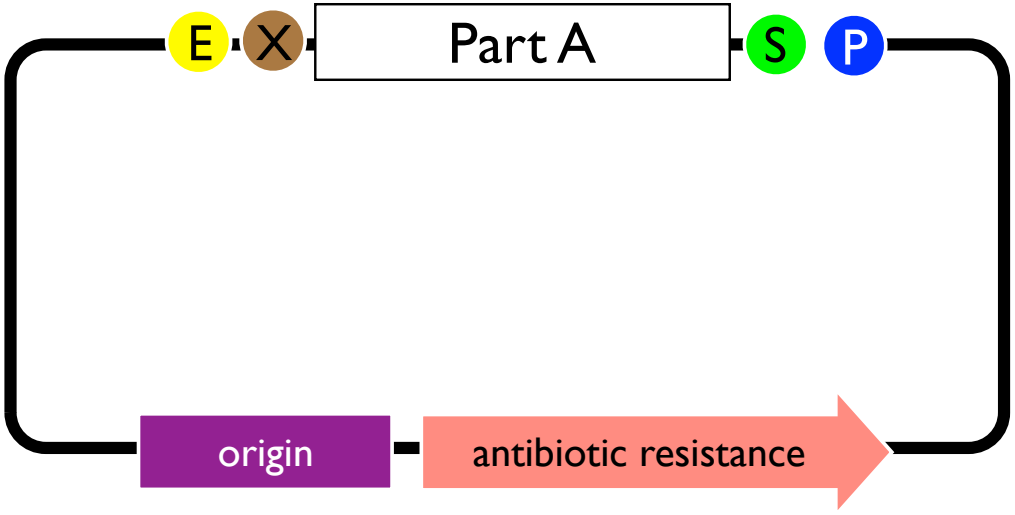

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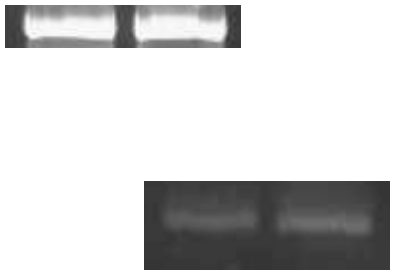
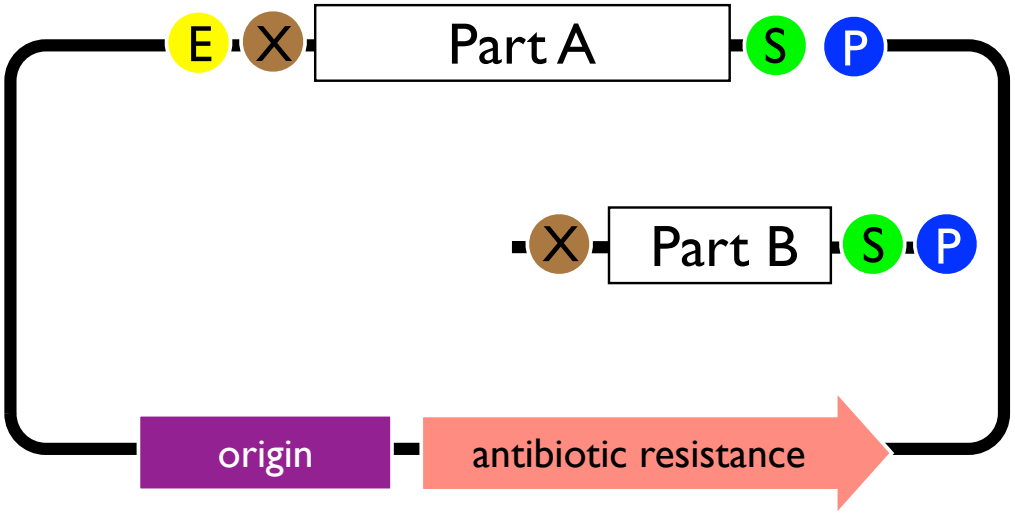
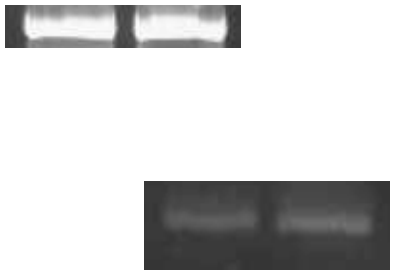
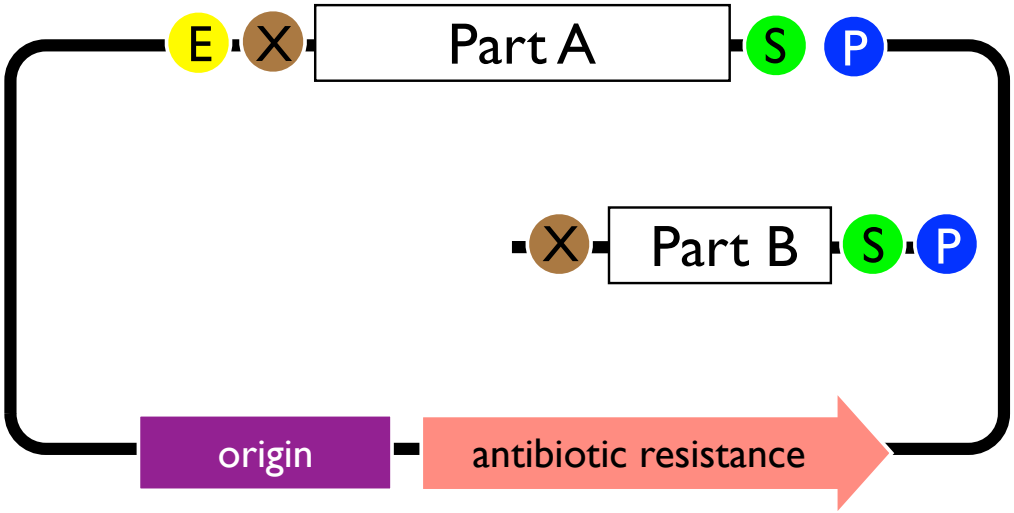


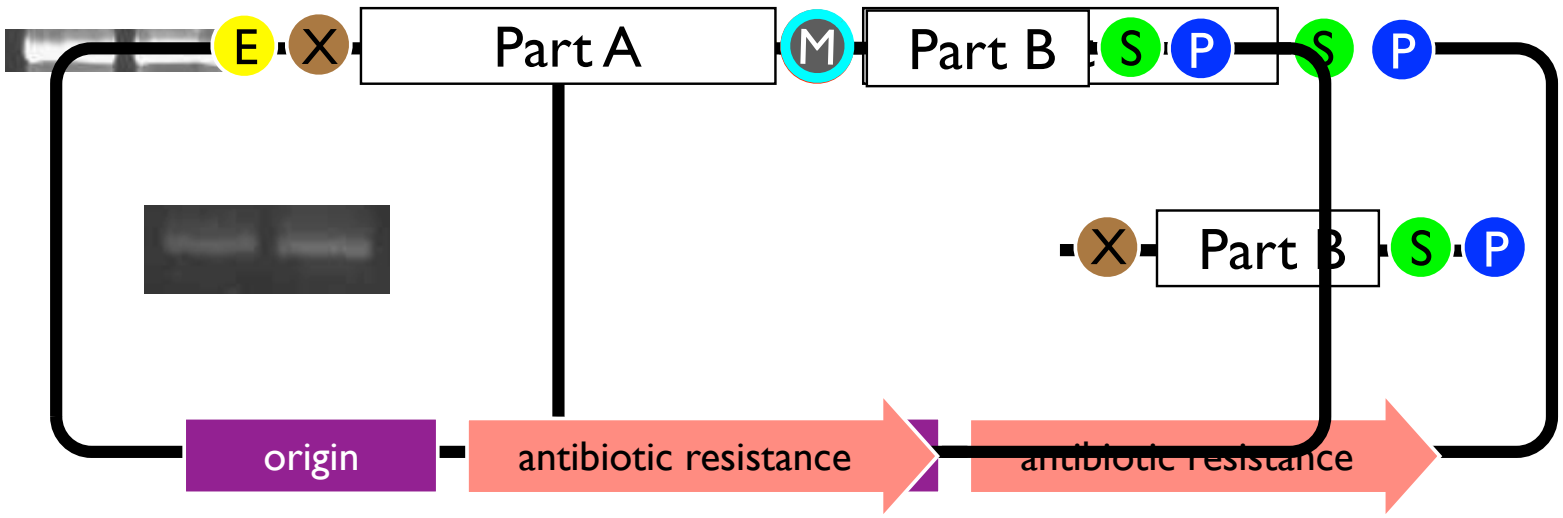


Ligation



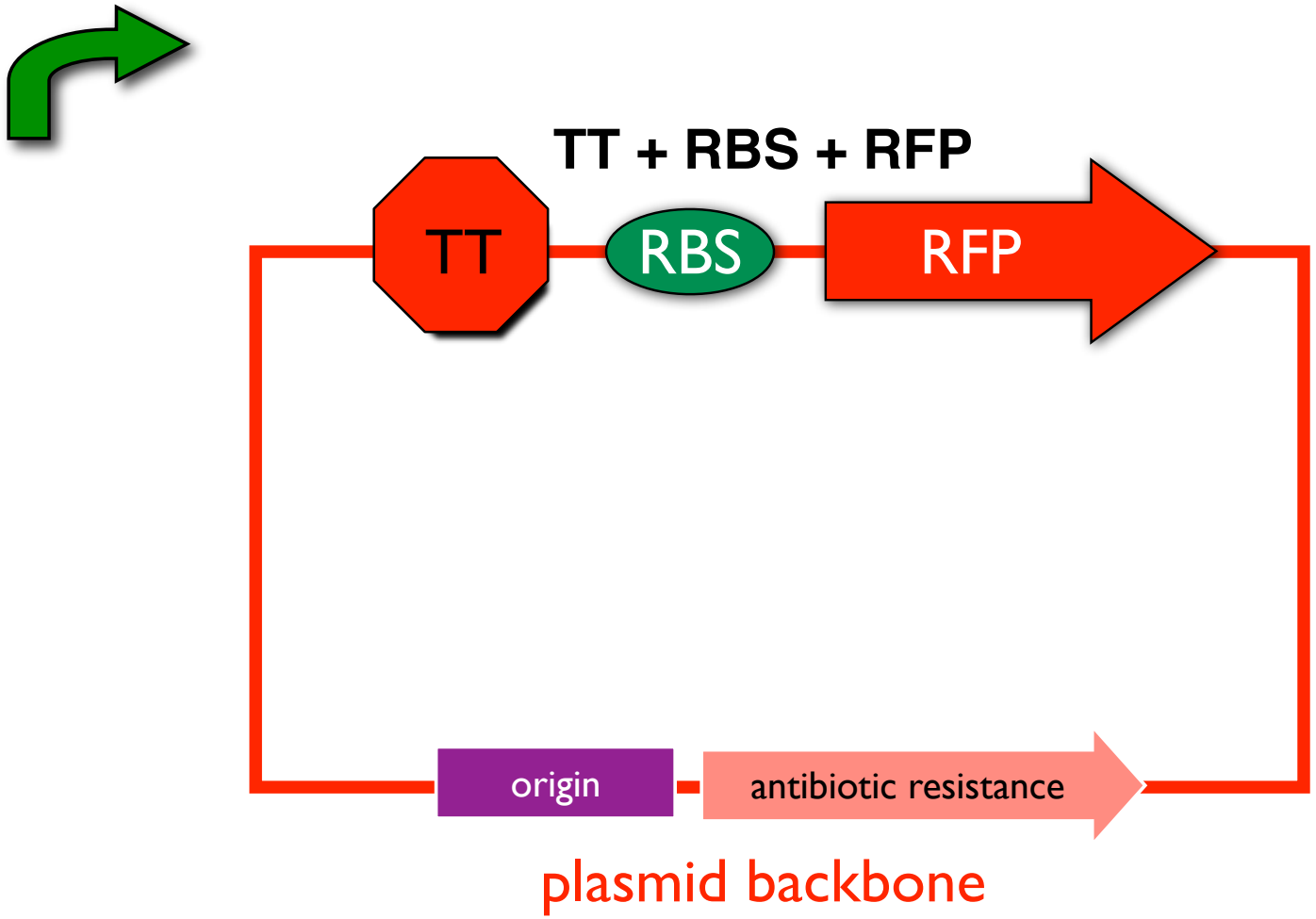


Ligation

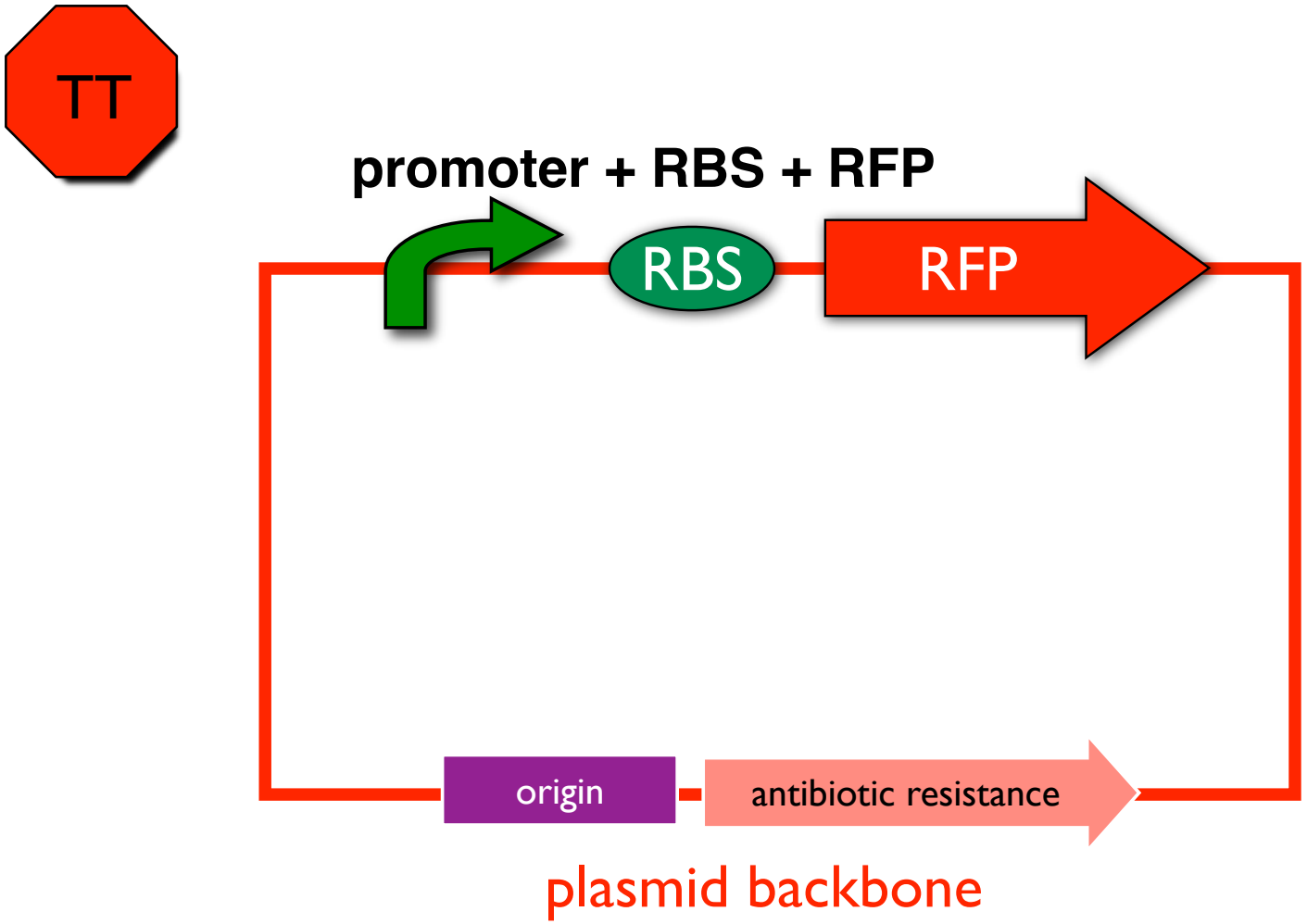

E
X
Part A
M
Part B
S
P
S
P
X
Part B
S
P
origin
antibiotic resistance
antibiotic resistance

How can we clone DNA without all the hassle?

Golden Gate Assembly Method



Golden Gate Assembly Method



Eco RI

GAATTC
CTTAAG

palindrome

type II

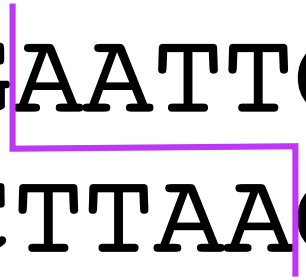
Eco RI

GAATTC
CTTAAG

palindrome

type II

Eco RI



type II

Eco RI

```
G          AATTC
CTTAA          G
```

type II

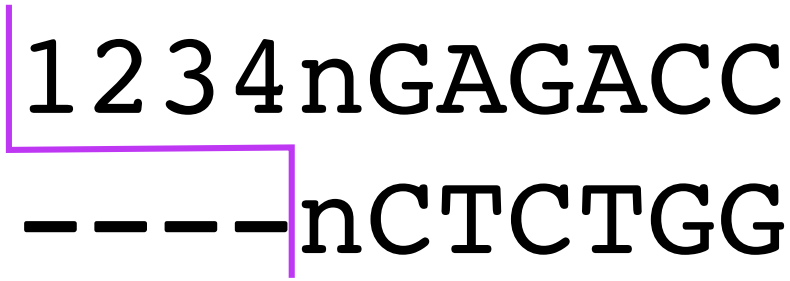
Bsa I

GAGACC
CTCTGG

not a palindrome

type IIs

Bsa I



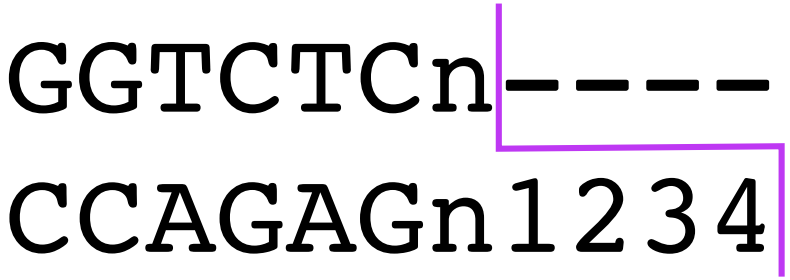
type IIs

Bsa I

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

type IIs

Bsa I



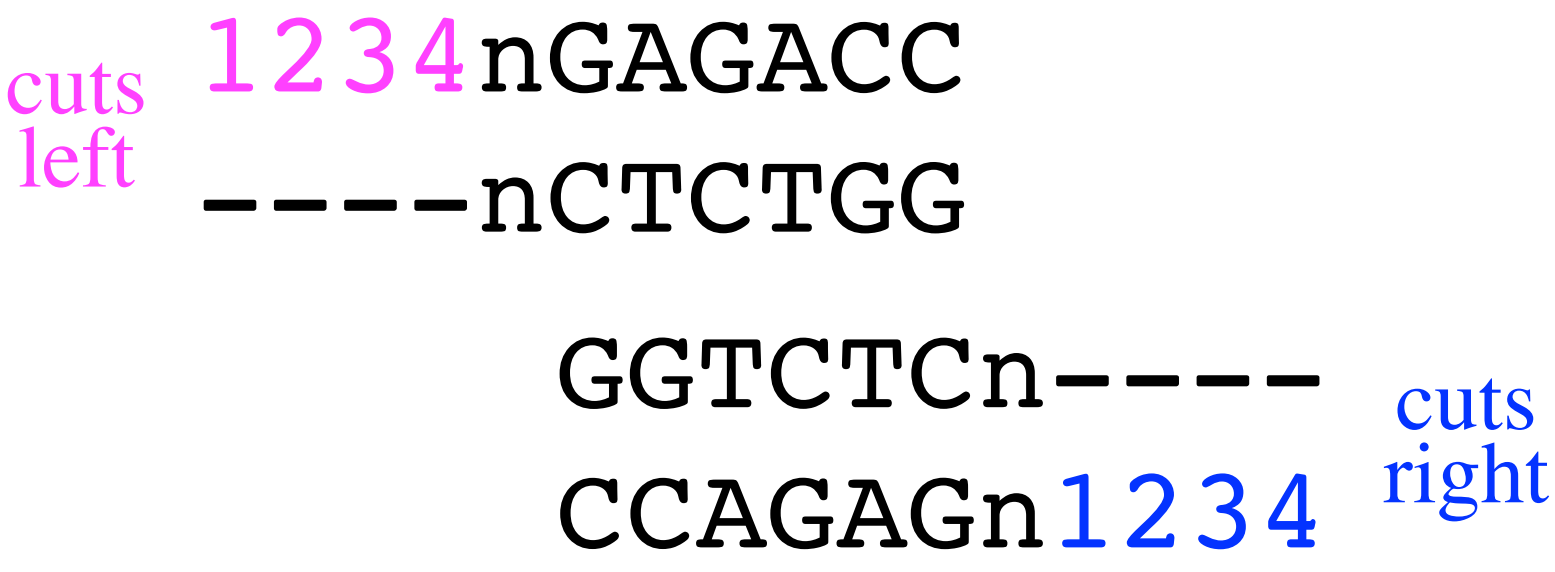
type IIs

Bsa I

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

type IIs

Bsa I

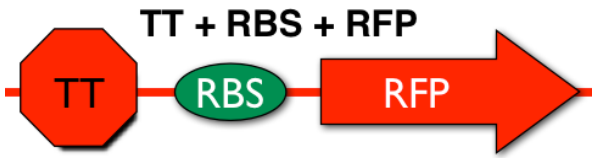

cuts
left
1234nGAGACC
----nCTCTGG
GGTCTCn----
CCAGAGn1234
cuts
right

Bsa I

CGACtGAGACC(TT)GGTCTCaGCGG

GCTGaCTCTGG(TT)CCAGAGtCGCC

Bsa I

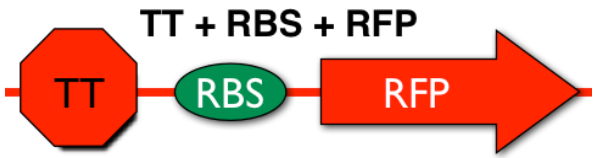


CGACtGAGACC(TT)GGTCTCa

aCTCTGG(TT)CCAGAGtCGCC

CGACtGAGACC(TT)GGTCTCaGCGG

GCTGaCTCTGG(TT)CCAGAGtCGCC



CGACtGAGACC(TT)GGTCTCa

aCTCTGG(TT)CCAGAGtCGCC

GCTG

GCGG

CGAC(promoter)

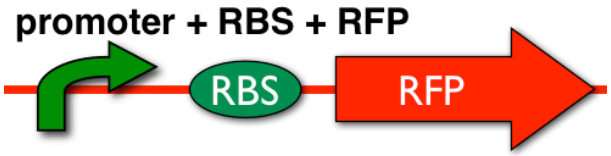
(promoter)CGCC

CGACtGAGACC(TT)GGTCTCa

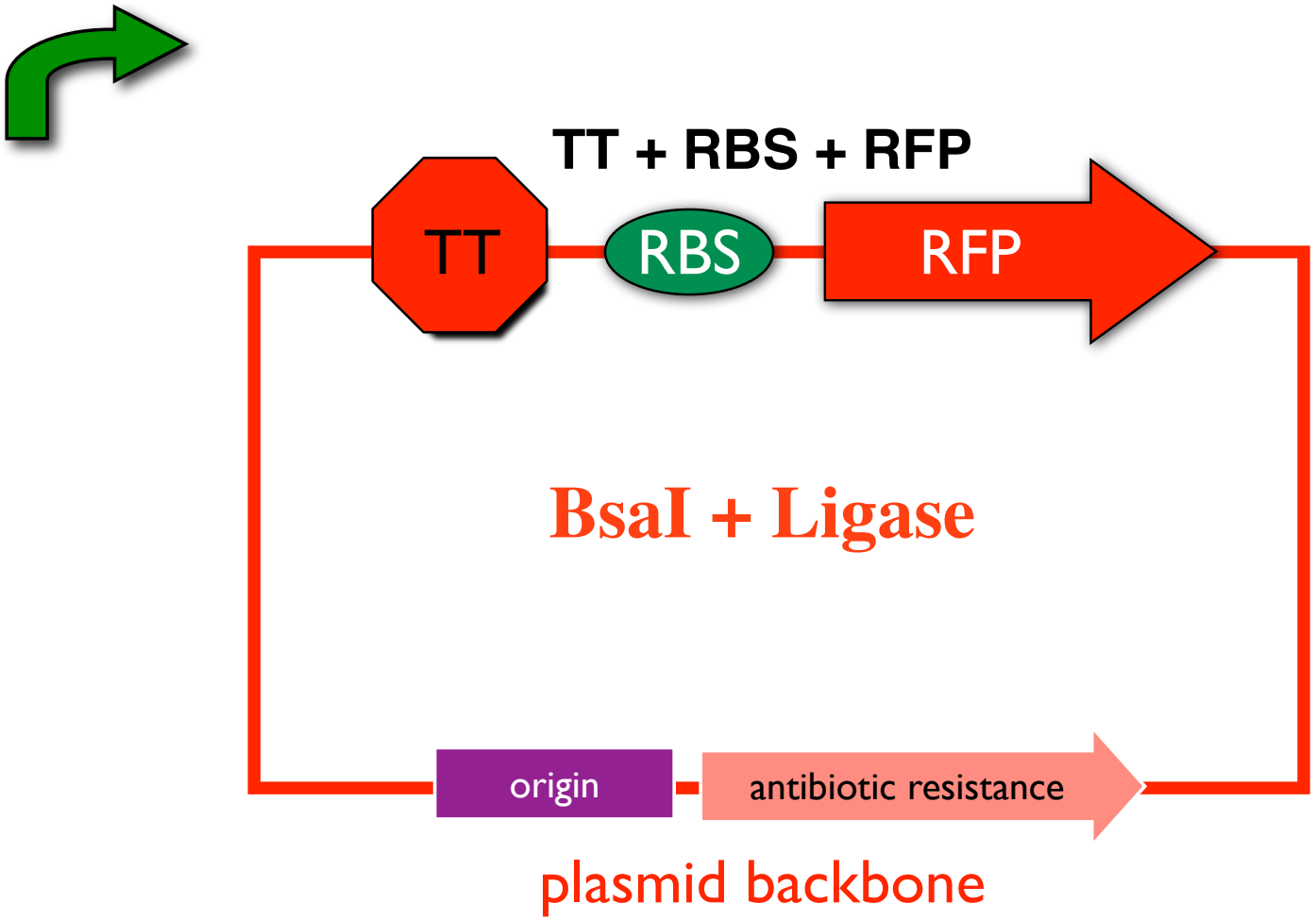
aCTCTGG(TT)CCAGAGtCGCC

CGAC(promoter)GCGG

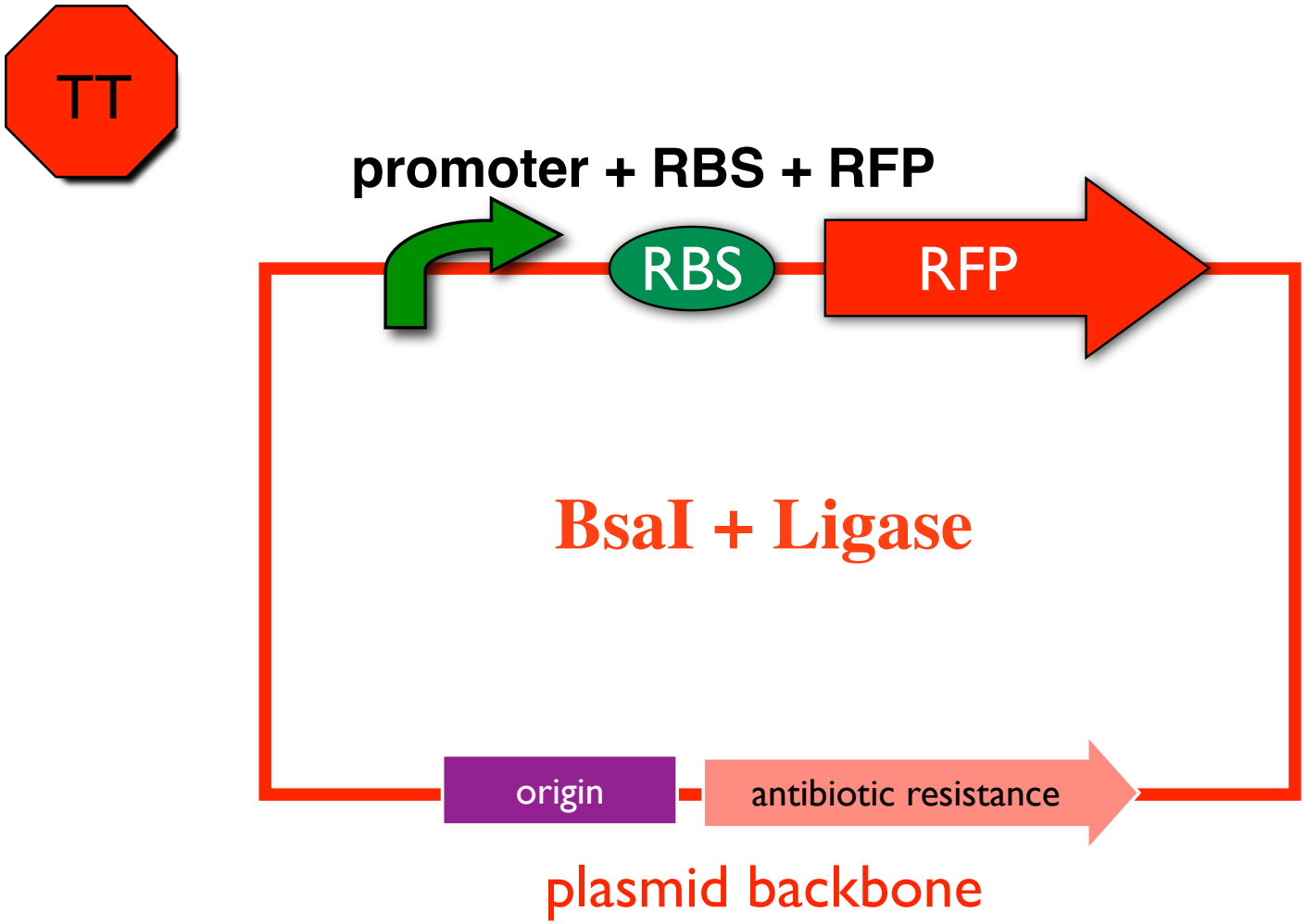
GCTG(promoter)CGCC



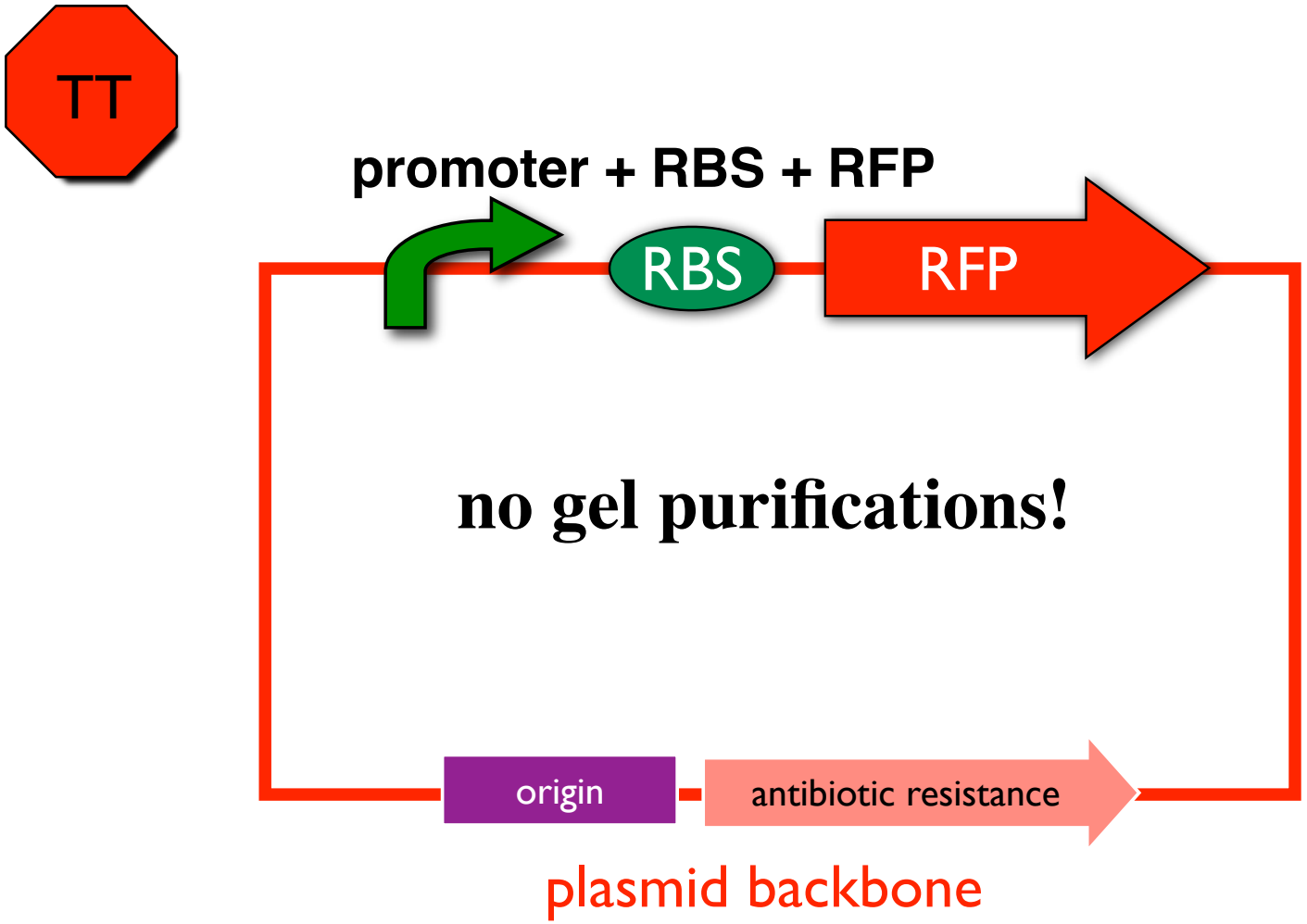
GGA Ligation Method



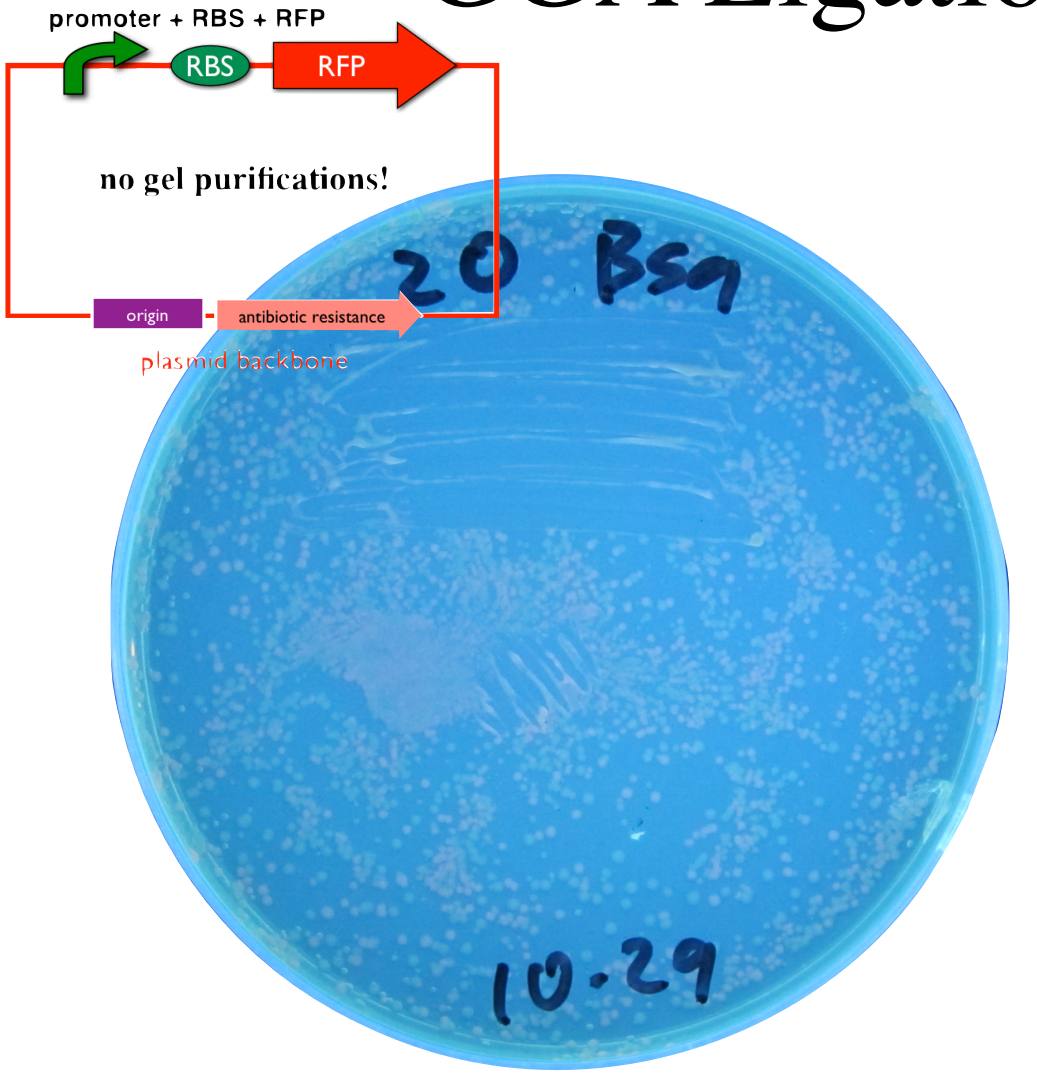
GGA Ligation Method



GGA Ligation Method



GGA Ligation Method



GGA Ligation Confirmation

All digested with R+P

1 J119022

2 J100028

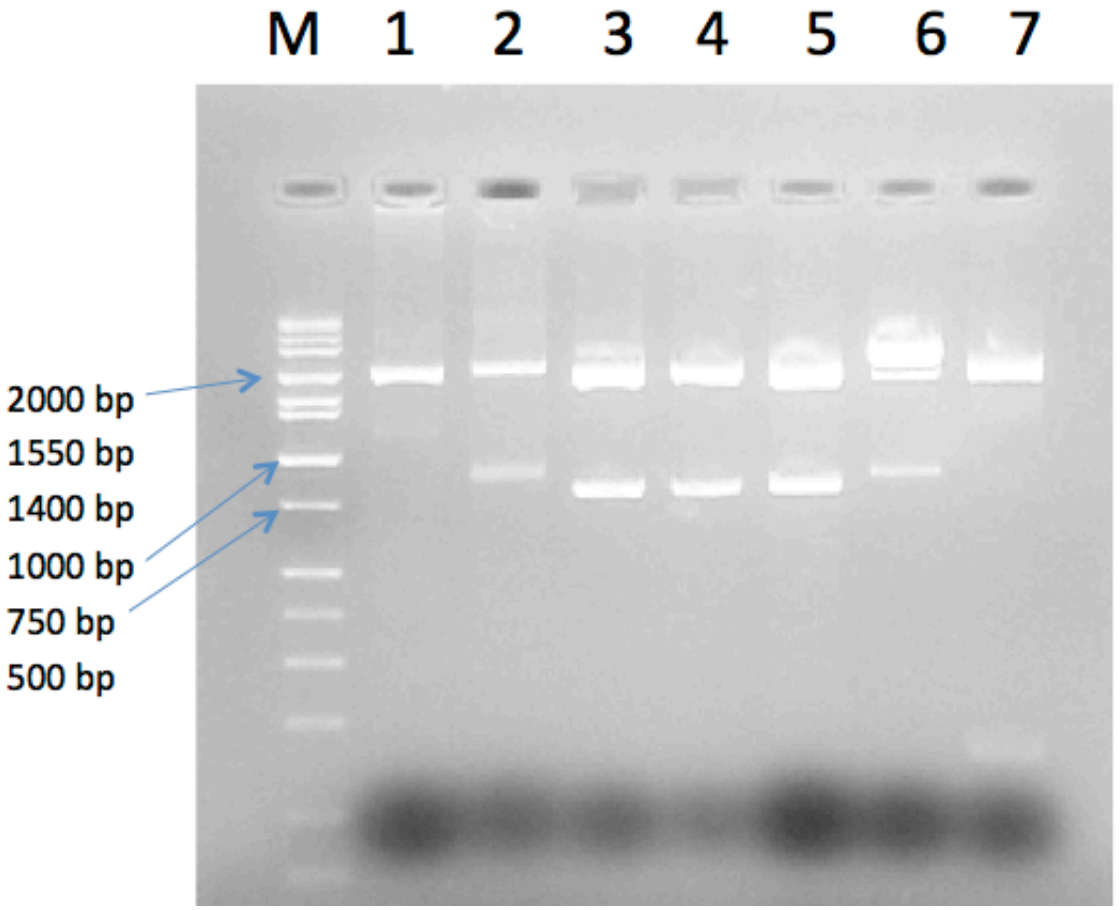
3 20 cycle red

4 20 cycle red

5 20 cycle red

6 20 cycle white

7 20 cycle white



GGA Ligation Confirmation

All digested with R+P

1 J119022

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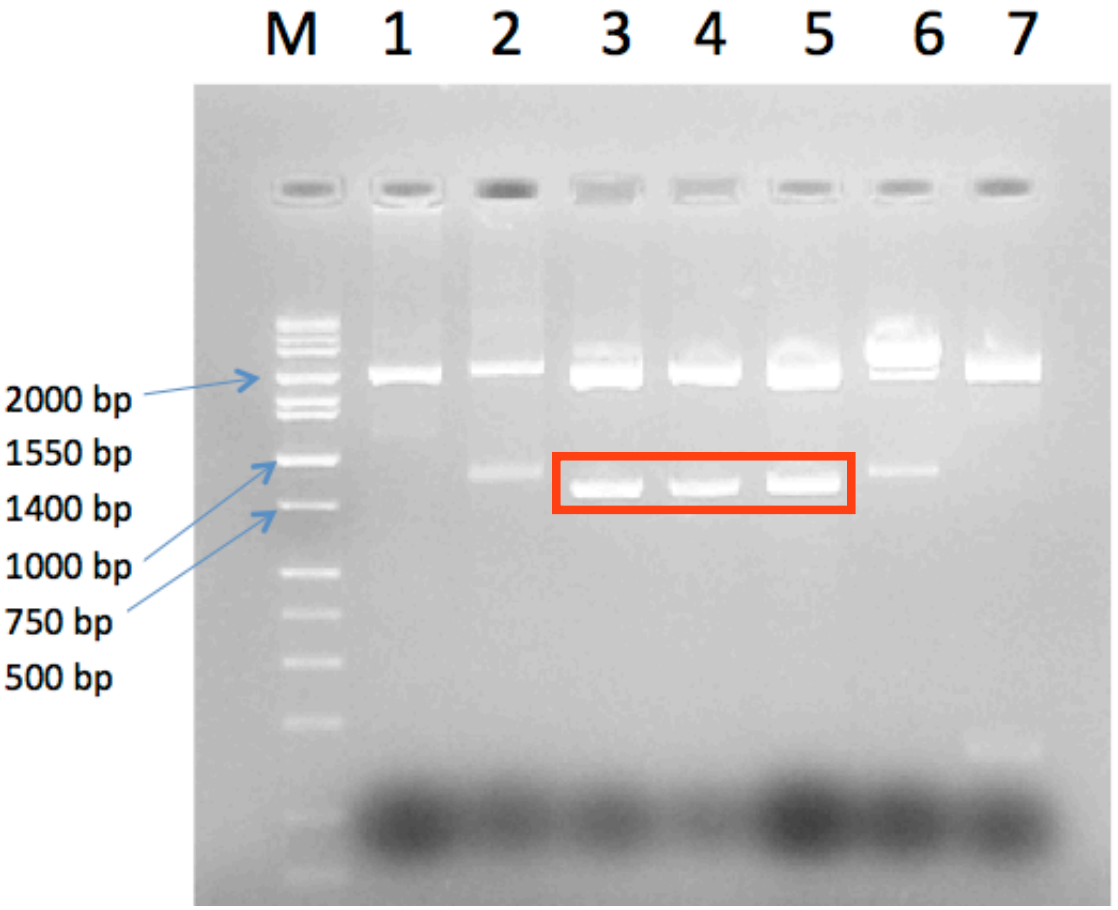
3 20 cycle red

4 20 cycle red

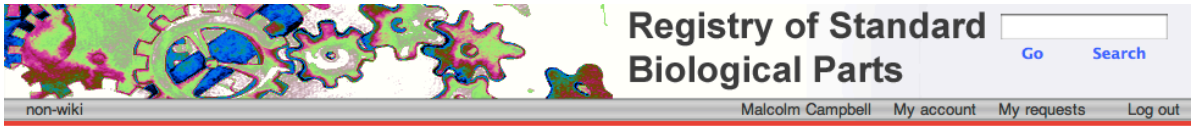
5 20 cycle red

6 20 cycle white

7 20 cycle white



Registry of Functional Promoters



Campbell M Lab Parts

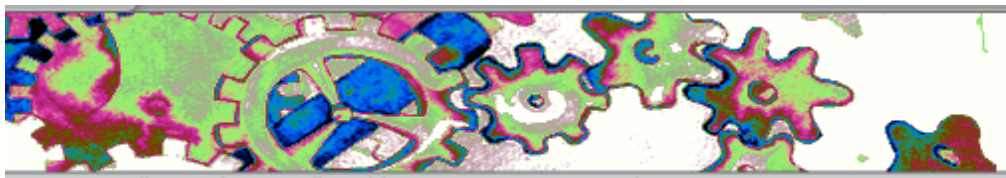
Favorite Campbell M Lab Parts *Edit*

-?-	Name	Type	Description	Designer	Length

Campbell M Lab Parts Sandbox *Edit*

-?-	Name	Type	Description	Designer	Length
	BBa_J100000	Coding	Cre with 8bp restriction sites and 1-Clause 2-SAT Problem Inserted	Eric Sawyer	1069
	BBa_J100001	Composite	pTet+RBS+Cre2SAT1Clause+pLpp+tRNA CCACU	Eric Sawyer	1357
	BBa_J100002	Composite	pTet+RBS+Cre2SAT1Clause+pLpp+tRNA CGGUC	Eric Sawyer	1357
	BBa_J100003	Generator	pTet+RBS+Cre2SAT1Clause	Eric Sawyer	1149
	BBa_J100004	Reporter	pTet+LoxP+RBS+RFP+LoxP	Eric Sawyer	870
	BBa_J100005	Other	Palindromic Stop Sequence	Eric Sawyer	221
	BBa_J100006	Intermediate	LoxP+Stop Sequence+LoxP	Eric Sawyer	305
	BBa_J100007	Intermediate	pLac+RBS+LoxP+Stop Sequence+LoxP	Eric Sawyer	533
	BBa_J100008	Composite	pLpp-tRNA CCACU-pLpp-tRNA CUAGU	Eric Sawyer	408
	BBa_J100009	Composite	pLpp-tRNA CCACU-pLpp-tRNA CGGUC	Eric Sawyer	408
	BBa_J100010	Composite	pLpp-tRNA CUAGU-pLpp-tRNA CGGUC	Eric Sawyer	408
	BBa_J100011	Composite	pLpp-tRNA CCACU-pLpp-tRNA CUAGU-pLpp-tRNA CGGUC	Eric Sawyer	616
	BBa_J100012	Intermediate	RBS-RFP-RBS	Eric Sawyer	747
	BBa_J100013	Coding	LuxI with 1 Clause 2-SAT Problem	Eric Sawyer	638
	BBa_J100014	Coding	LuxI with 2 Clause 2-SAT Problem	Eric Sawyer	652
	BBa_J100015	Composite	1 Clause 2-SAT Problem with Frameshifted LuxI and a GFP Reporter	Eric Sawyer	2757
	BBa_J100016	Composite	2 Clause 2-SAT Problem with Frameshifted LuxI and a GFP Reporter	Eric Sawyer	2771
	BBa_J100017	Composite	TT+pLux+RBS+LuxI(2-SAT 2 clause)+RBS+GFP+pLac+RBS+LuxR+tRNAs	Eric Sawyer	3395
	BBa_J100018	Protein_Domain	First Half of AspC gene	Catherine Doyle	448
	BBa_J100019	Protein_Domain	First half of ilvE gene	Julia Fearrington	457
	BBa_J100020	Protein_Domain	Second Half of AspC	Catherine Doyle	869
	BBa_J100021	Protein_Domain	First Half of PyrE	Catherine Doyle	488
	BBa_J100022	Protein_Domain	Second Half of PyrE	Catherine Doyle	280
	BBa_J100025	Protein_Domain	First half of CAT gene	James Harden	434
	BBa_J100026	Protein_Domain	second half ilvE gene	Julia Fearrington	574
	BBa_J100027	Protein_Domain	second half of TyrB	James Harden	288
	BBa_J100028	Other	placeholder insert for BsaI Golden Gate Assembly of promoter	Malcolm Campbell	877
	BBa_J100029	Regulatory	The promoter of rpoDPhs	Maggie Baay	76
	BBa_J100030	Regulatory	phoA is an inducible promoter induced by phosphate starvation.	Scott Hall	76
	BBa_J100031	Regulatory	Constitutive promoter C on Gene 1 of T7, transcribes RNA Pol.	Caroline Vrana	100
	BBa_J100032	Regulatory	proUP3 promoter	Molly Marshall	90
	BBa_J100033	Regulatory	dnakP1 promoter: Heat shock inducible	Chris Peek	101
	BBa_J100034	Regulatory	groE promoter	Margaret Stebbins	44
	BBa_J100036	Regulatory	Promoter induced by DNA damage	Erich Baker	52
	BBa_J100039	Regulatory	GalP1 Promoter-Induced By Galactose	Anaiah Toby	75
	BBa_J100040	Coding	LuxI with 3 clause 2-SAT problem	Eric Sawyer	684
	BBa_J100041	Composite	LuxI/GFP with 3 clause 2-SAT problem	Eric Sawyer	2803
	BBa_J100042	Coding	LuxI with 3 clause 3-SAT problem	Eric Sawyer	702
	BBa_J100043	Composite	LuxI/GFP with 3 clause 3-SAT problem	Eric Sawyer	2821
	BBa_J100044	Coding	LuxI with 4 clause 2-SAT problem	Eric Sawyer	704
	BBa_J100045	Composite	LuxI/GFP with 4 clause 2-SAT problem	Eric Sawyer	2823
	BBa_J100046	RNA	lpp+tRNA CCAUC (10 bp anticodon loop)	Eric Sawyer	201
	BBa_J100047	Protein_Domain	TyrB2	Julia Fearrington	
	BBa_J100048	Protein_Domain	TyrB1	Julia Fearrington	930
	BBa_K091231	Composite	LuxR producer and XOR gate	Malcolm Campbell	2772
	BBa_K091232	Composite	LuxR producer and RFP(rev) + RBS(rev) + pLux (for)	Malcolm Campbell	1916

Student Sample



Registry of Standard Biological Parts

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BBa J100033 Main Page Part Design Physical DNA Hard Information Experience Tools

Part:BBa_J100033

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

Regulatory

DNA Planning

Experience:

Get This Part

dnakP1 promoter: Heat shock inducible

dnaKP1 is naturally off, but is induced when E. coli is heat shocked, resulting in transcription downstream from this promoter.

Sequence and Features

Format: Subparts | Ruler | SS | DS Search: Length: 101 bp Context: Part only Get selected sequence

```
      1          11         21         31         41         51         61         71         81         91

1     aaatttctgc gcaaaagcac aaaaaatttt tgcatctccc ccttgatgac gtggtttacg accccattta gtagtcaacc gcagtgagtg agtctgcaaa
      tttaaagacg cgttttcgtg ttttttaaaa acgtagaggg ggaactactg caccaaatgc tggggtaaat catcagttgg cgtcactcac tcagacgttt

101   a
      t
```

Assembly Compatibility: 10 12 21 23 25

Student Sample

Registry of Standard Biological Parts

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BBa J100033 Main Page Part Design Physical DNA Hard Information Experience Tools

Part:BBa_J100033:Experience

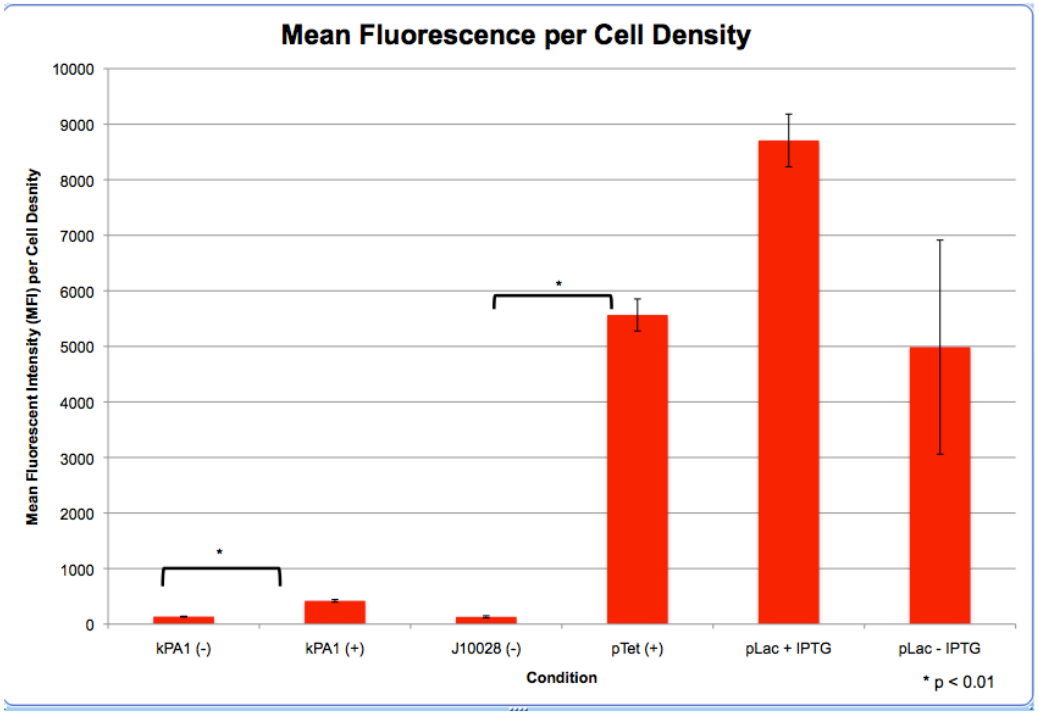
Designed by Chris Peek Group: Campbell_M_Lab (2011-09-01)

Regulatory

DNA Planning
Experience:
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_J100033 [edit]



A: Experimental:

cells containing dnaKP1 without heat shock (incubated at 37°C) B: Experimental: cells containing dnaKP1 with heat shock (incubated at 40°C) C: Negative control: part j100028 without pTet promoter D: Positive control: part j100028 with pTet promoter (always on) E: pLac promoter (part j715039) with inducer (IPTG) F: pLac

What's Next?

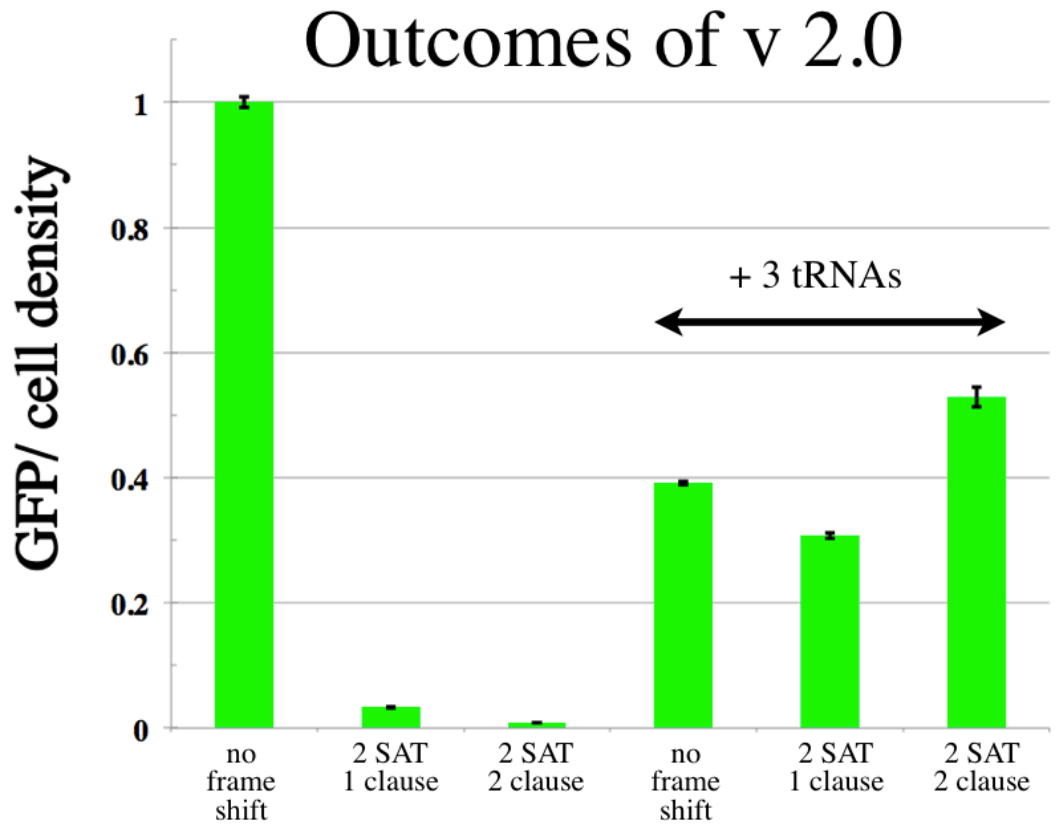
Improve the plasmid to increase phenotype.

Simplify ligation/digestion protocol.

Unleash intro bio students for real research!

Three Rules for Our Lab

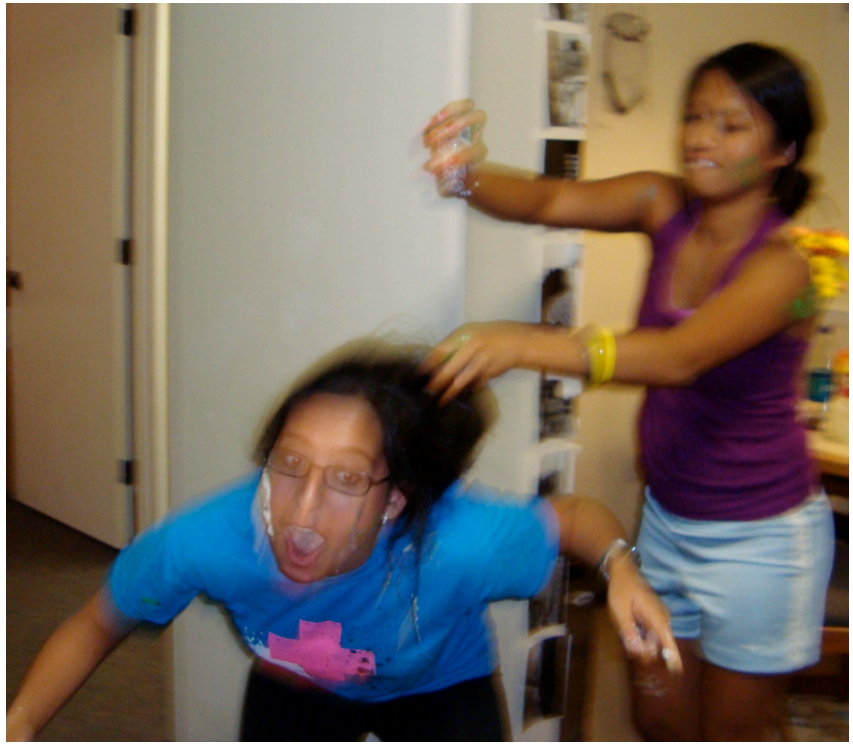
1. Everyone has to learn.



Three Rules for Our Lab

1. Everyone has to learn.

2. Everyone has to have fun.



Three Rules for Our Lab

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

Acknowledgements

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