GSA Method

customized by

A. Malcolm Campbell and Todd Eckdahl

Eco RI

GAATTC

CTTAAG

palindrome

type II

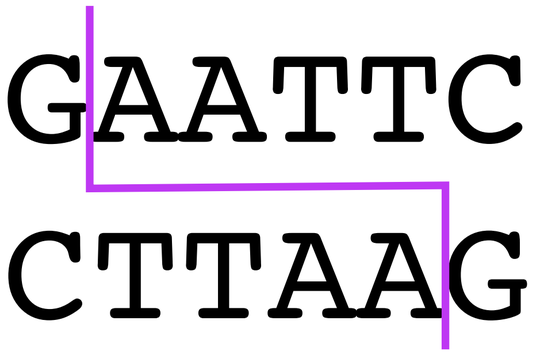
Eco RI

GAATTC
CTTAAG

palindrome

type II

Eco RI



type II

Eco RI

```
G                AATTC
CTTAA            G
```

type II

Bsa I

GAGACC

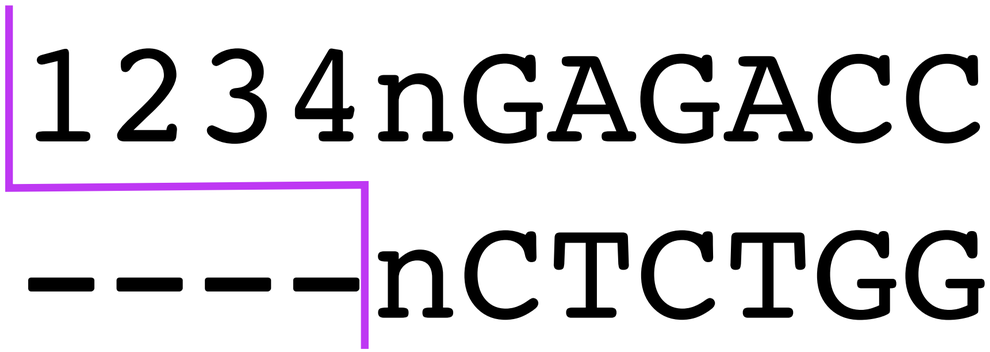
CTCTGG

G

not a palindrome

type IIs

Bsa I



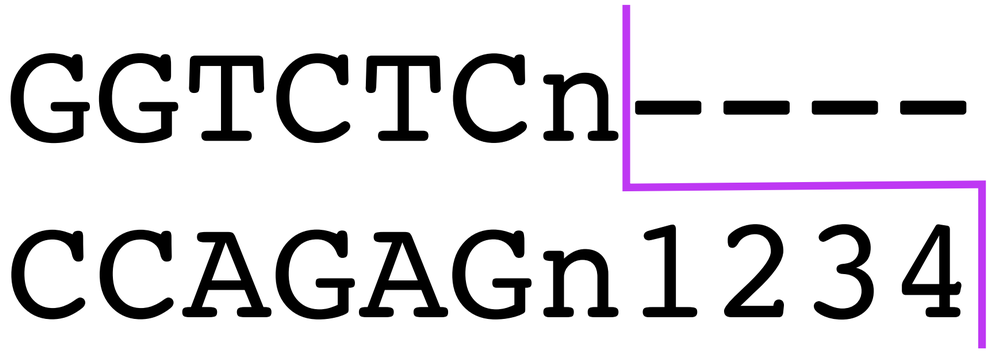
type IIs

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

Bsa I

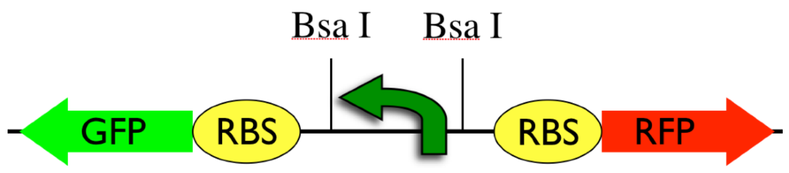
CGACtGAGACC(Pr)GGTCTCaGCGG

GCTGaCTCTGG(Pr)CCAGAGtCGCC

ligase

Bsa I

ligase



CGACtGAGACC(Pr)GGTCTCa

aCTCTGG(Pr)CCAGAGtCGCC

[redacted]GCGG[redacted]

[redacted]GCTG

[redacted]

ligase

ligase

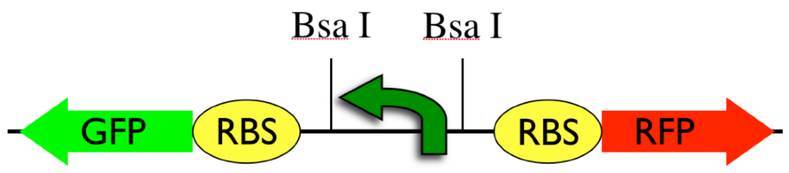
Bsa I

[redacted] **CGACtGAGACC(Pr)GGTCTCaGCGG** [redacted]

ligase

[redacted] **GCTGaCTCTGG(Pr)CCAGAGtCGCC** [redacted]

Bsa I **ligase**



CGACtGAGACC(Pr)GGTCTCa

aCTCTGG(Pr)CCAGAGtCGCC

[redacted]GCGG[redacted]

[redacted]GCTG

[redacted]

ligase

ligase

CGAC(promoter)

(promoter)CGCC

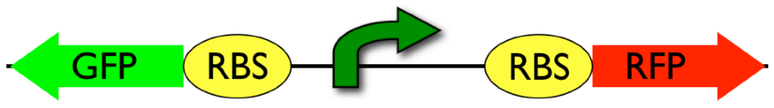
CGACtGAGACC(Pr)GGTCTCa

aCTCTGG(Pr)CCAGAGtCGCC

CGAC(promoter)GCGG

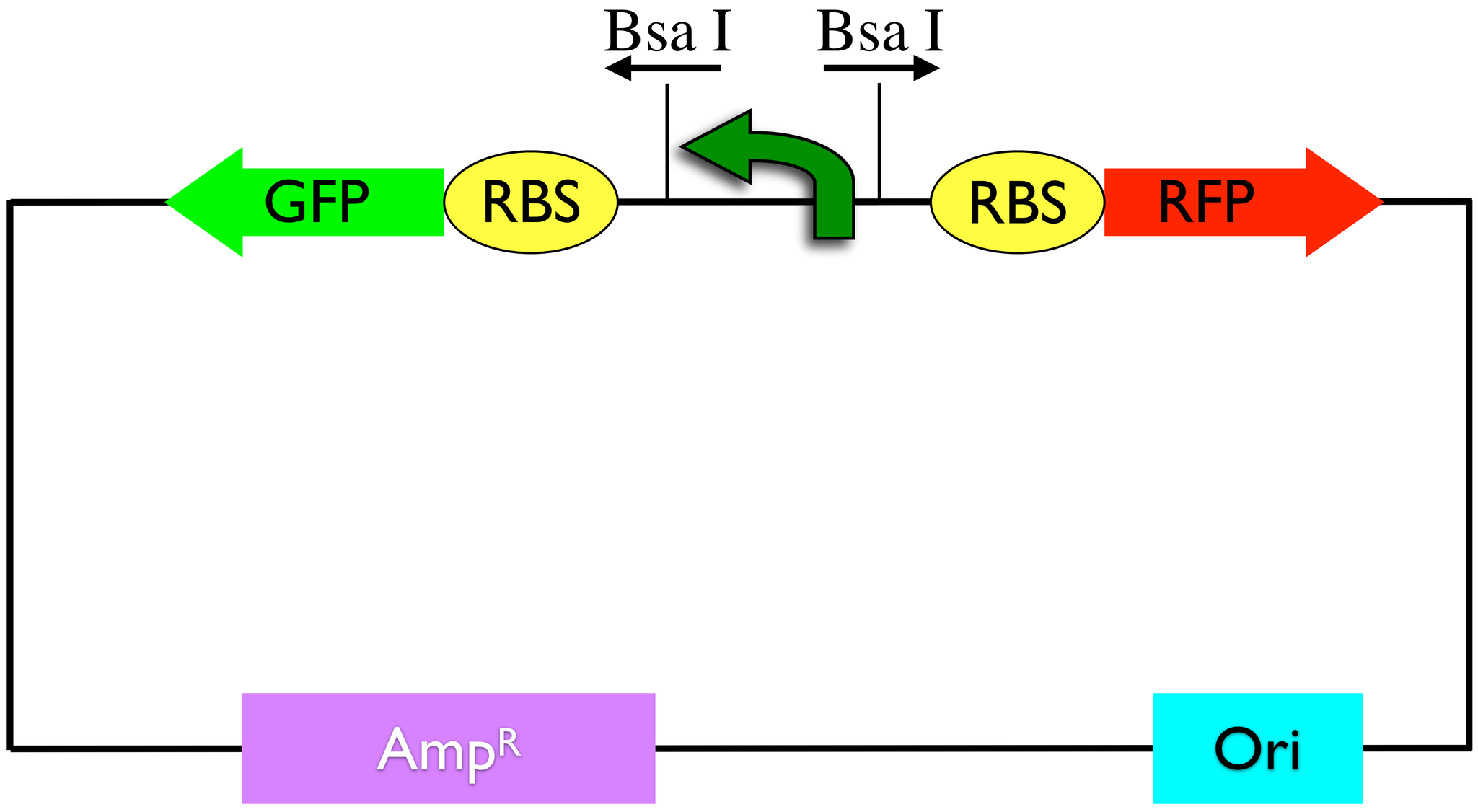
GCTG(promoter)CGCC

ligase ligase



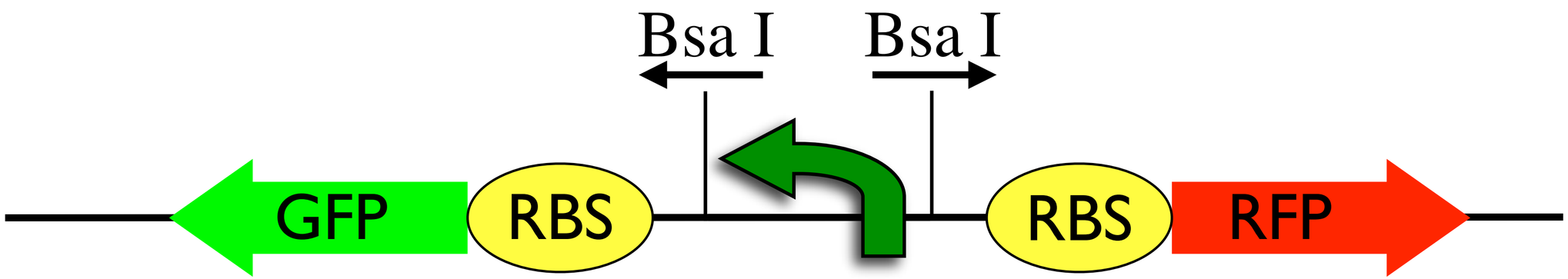
pClone Red

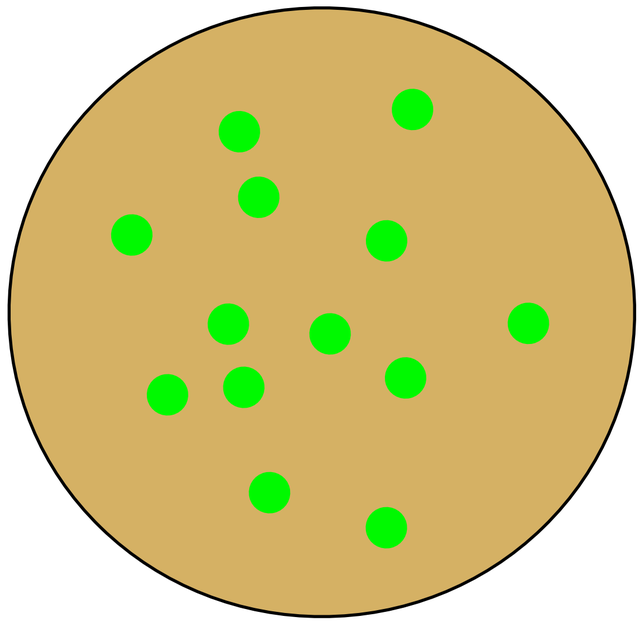
J119137



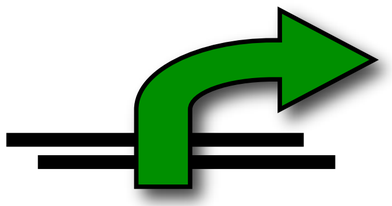
pClone Red

all colonies green

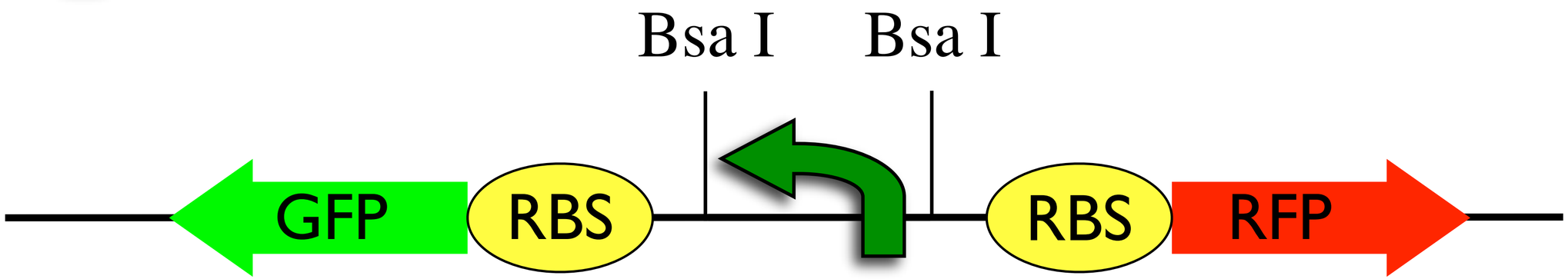


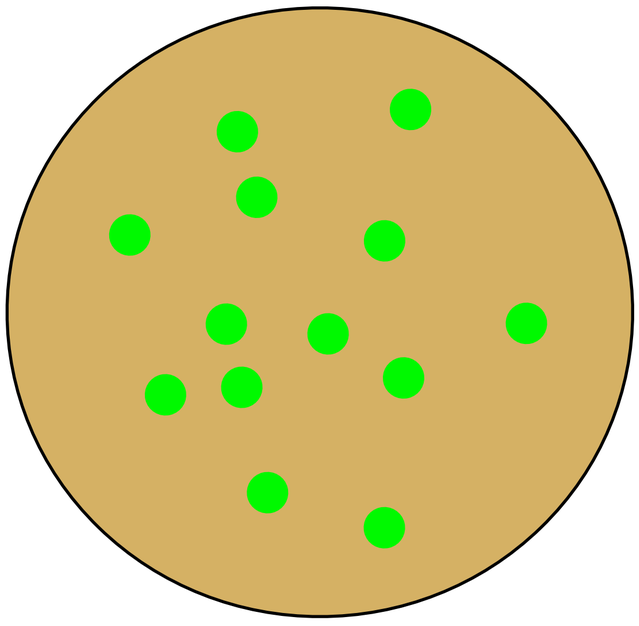


Golden Gate Assembly Method

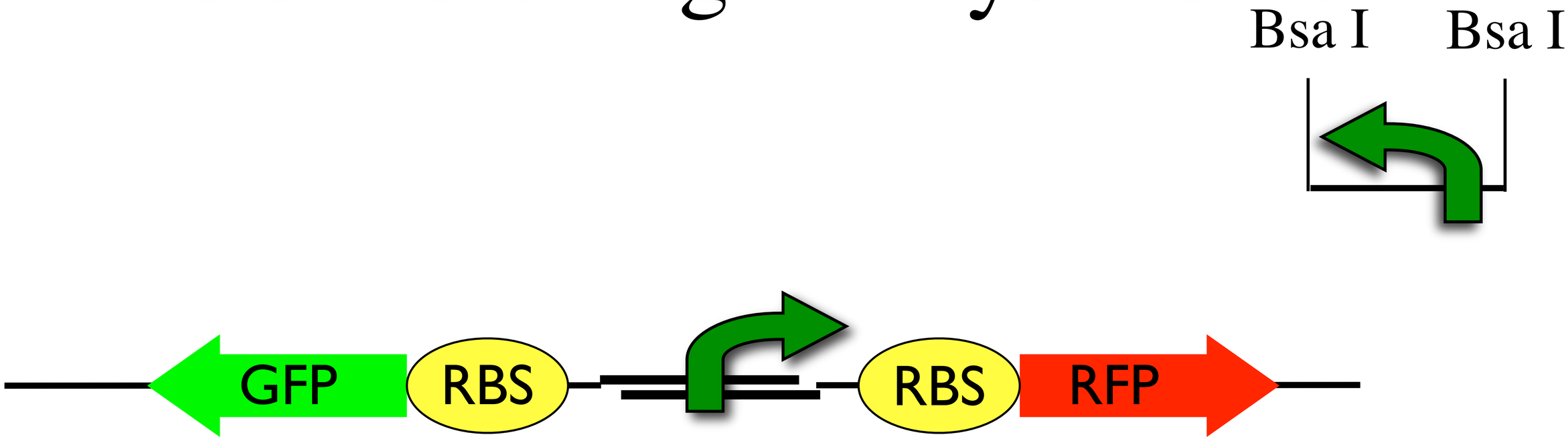


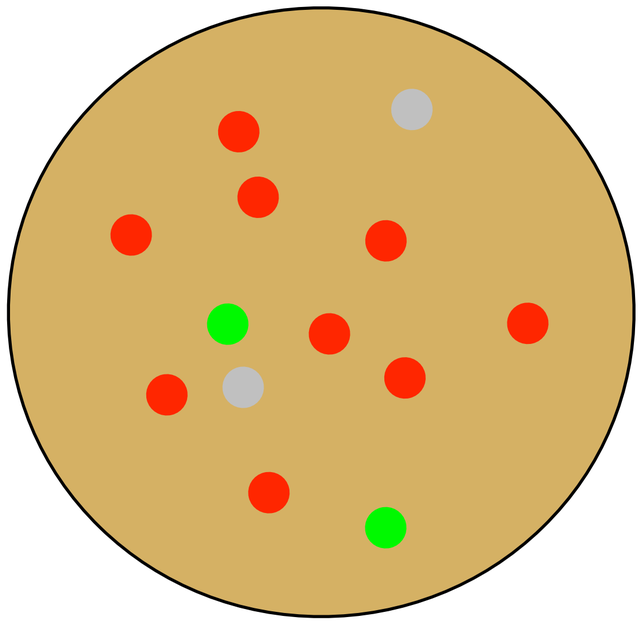
Bsa I + ligase





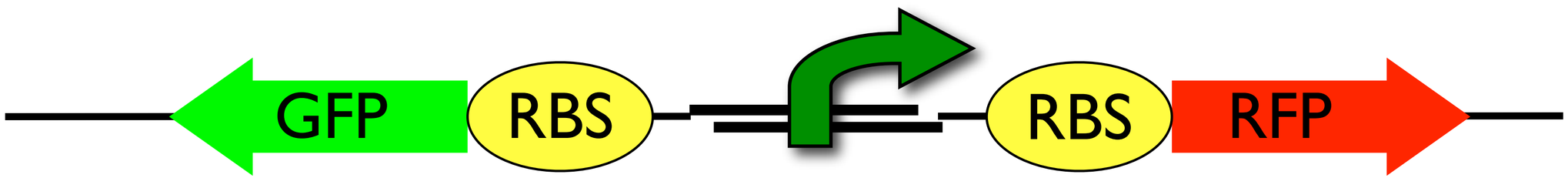
GGA Cloning Always Works

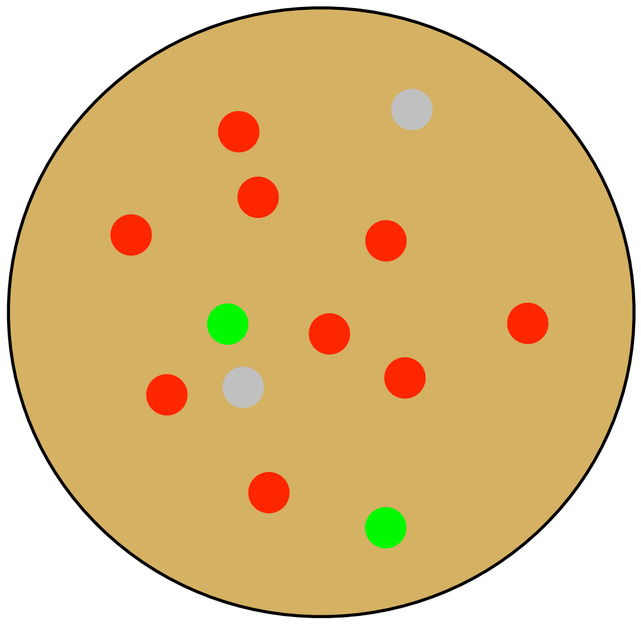




Bio113 Researchers in 3 Hour Lab

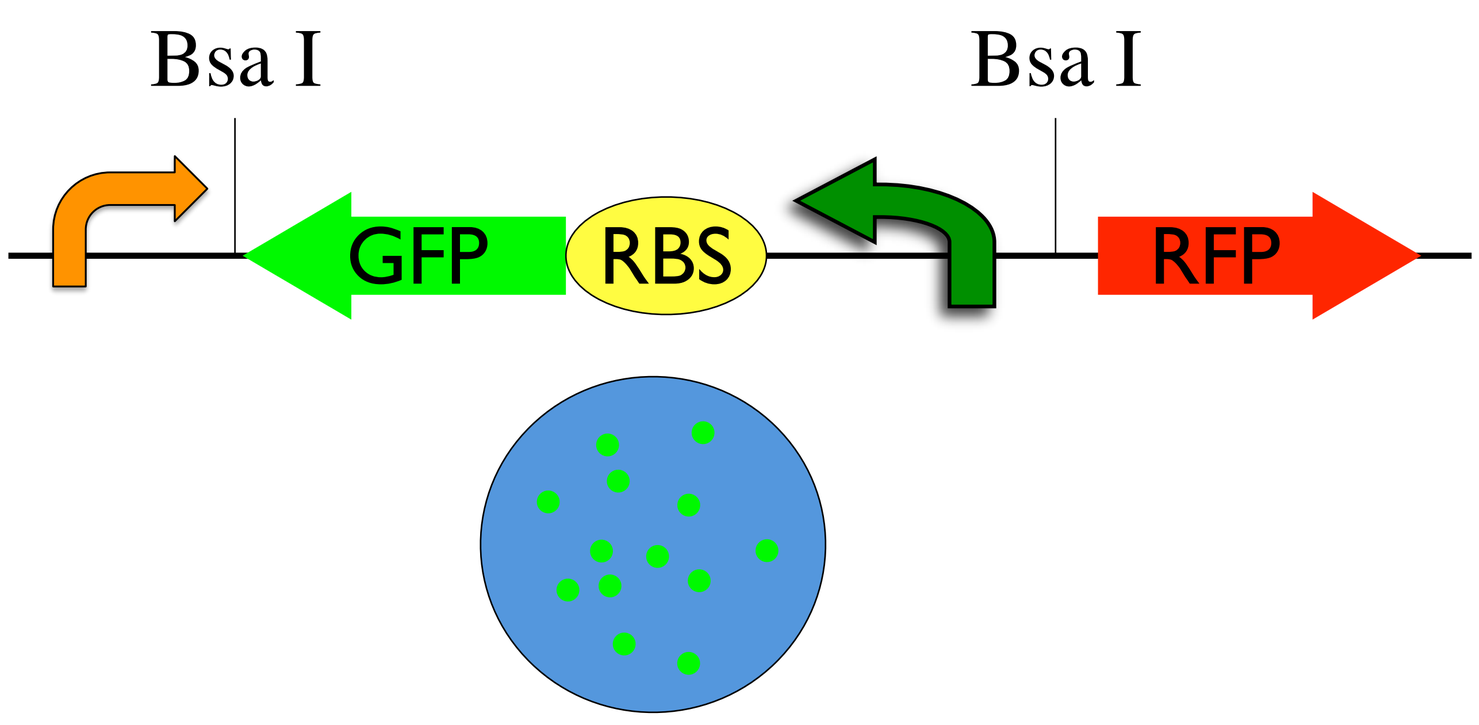
no gel purifications!





rClone Red (ribosome research)

J119384



rClone Red (ribosome research)

J119384

12 - 60 bp

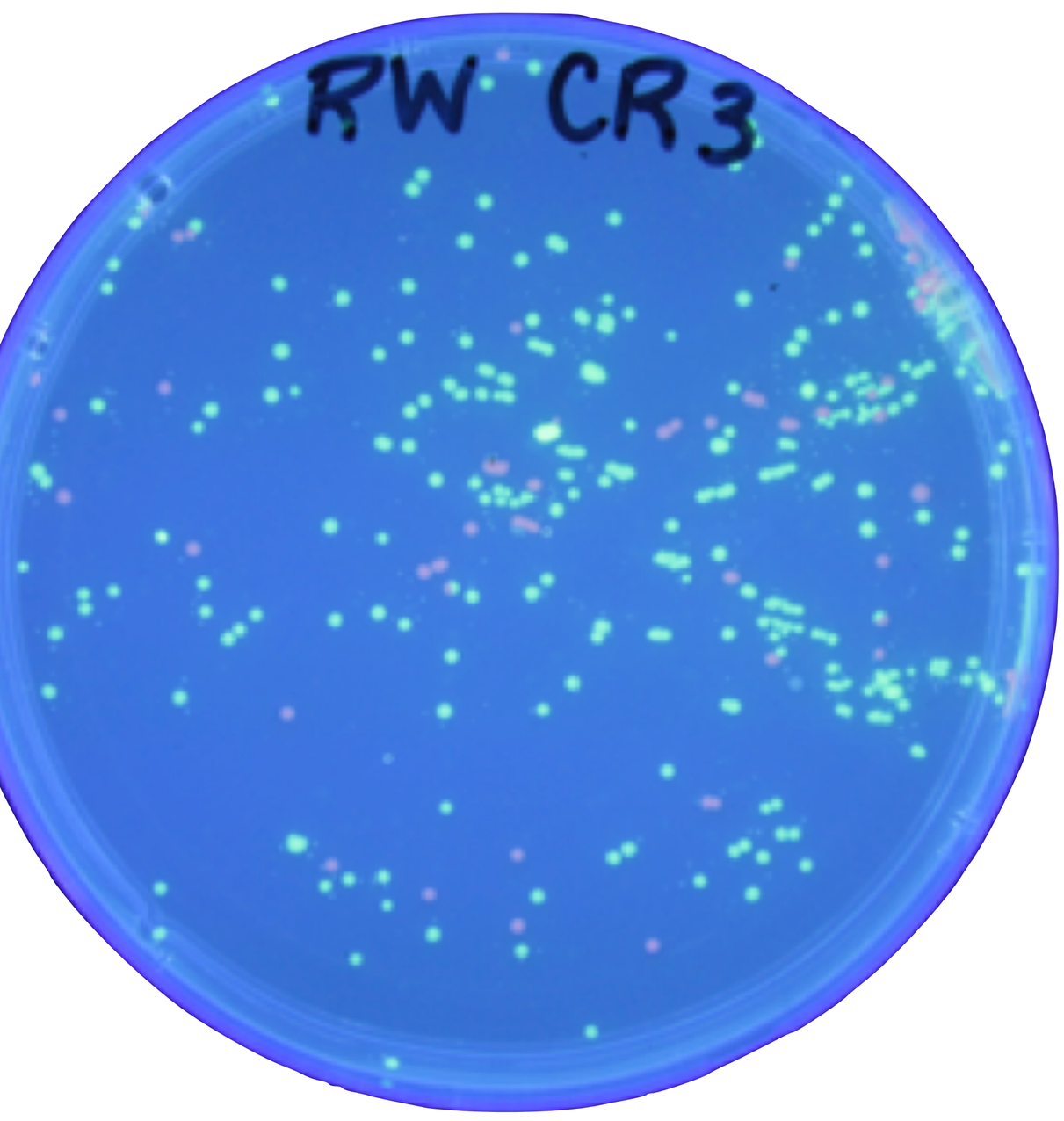
RBS

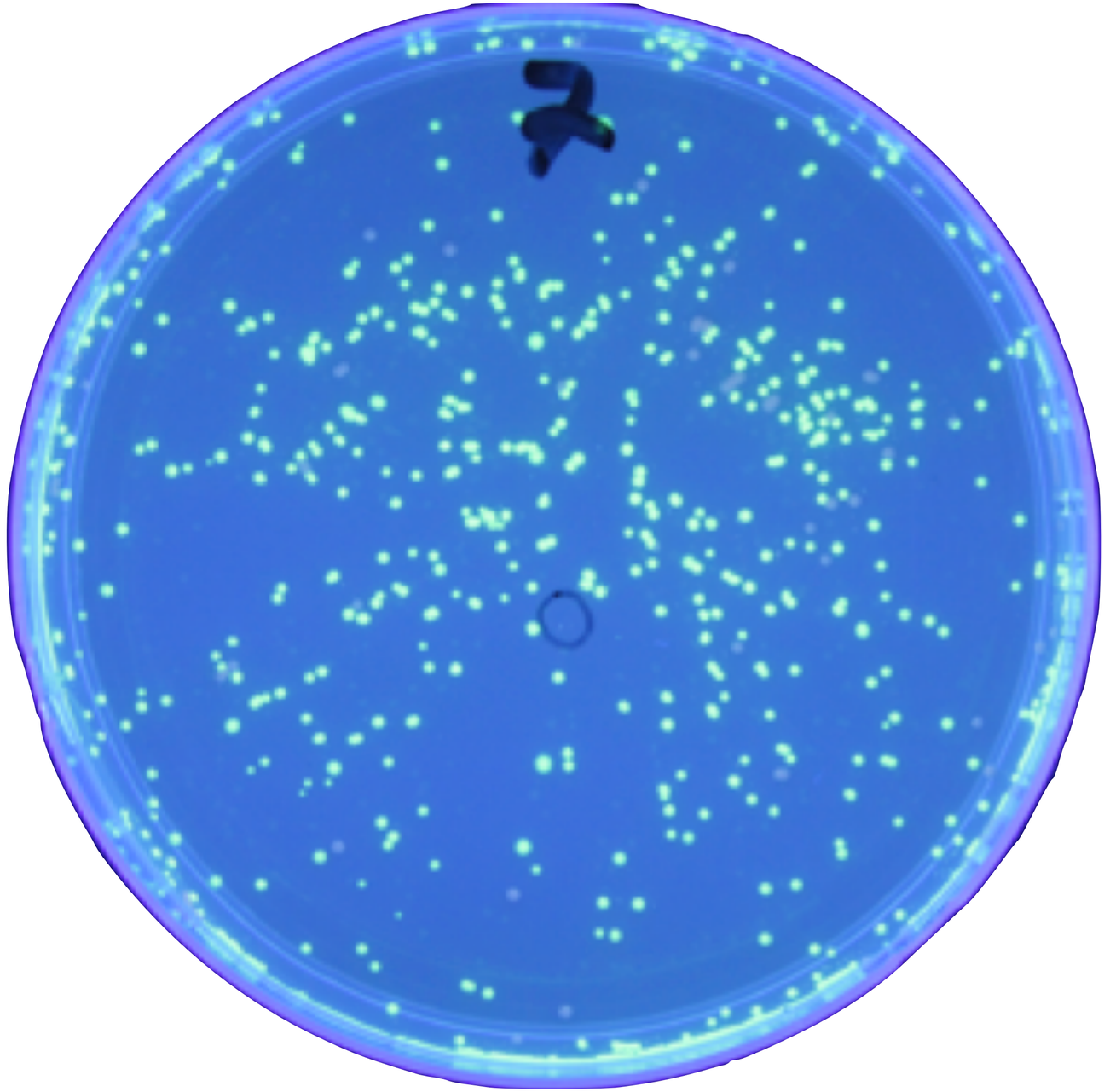
Bsa I

Bsa I

RFP

rClone Red (student-designed RBS)





Student Research, October 2016

Registry News

- Registry Release
- Registry 6.0
- Report Bugs
- Request Features
- News Archive
- Feature Box Archive

Other

- Registry API
- Safety
- Videos

Catalog

The iGEM Registry has over 20,000 documented parts. The Catalog organizes many of these parts by part type, chassis, function, and more. Browse for parts through the Registry Catalog or use the search menu.

2016 DNA Distribution

The iGEM 2016 DNA Distribution is shipping to registered teams and labs. We've added some new material this year, so be sure to read through the 2016 Distribution Handbook before using your kit.

Collections [updated!]

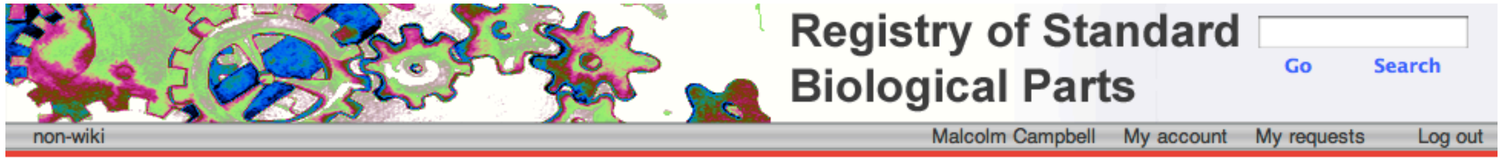
We've **updated** the Registry part collections. There are part collections for reporter proteins, plant chassis, cellulose-related parts, and more. Users can discover new parts and collections and build upon what previous iGEM teams and labs have achieved.

- Plant Chassis [UPDATED!]
- *Bacillus subtilis* [UPDATED!]

Registry Help

Protocols

Registry of Functional Promoters



Campbell M Lab Parts

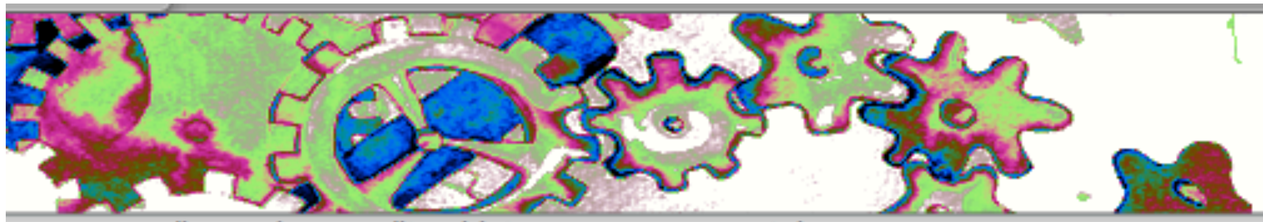
Favorite Campbell M Lab Parts *Edit*

-?-	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

Go Search

page discussion edit history move watch Macampbell My account My requests Log out

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

Go Search

page discussion edit history move watch Macampbell My account My requests Log out

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

Part:BBa_J100033:Experience

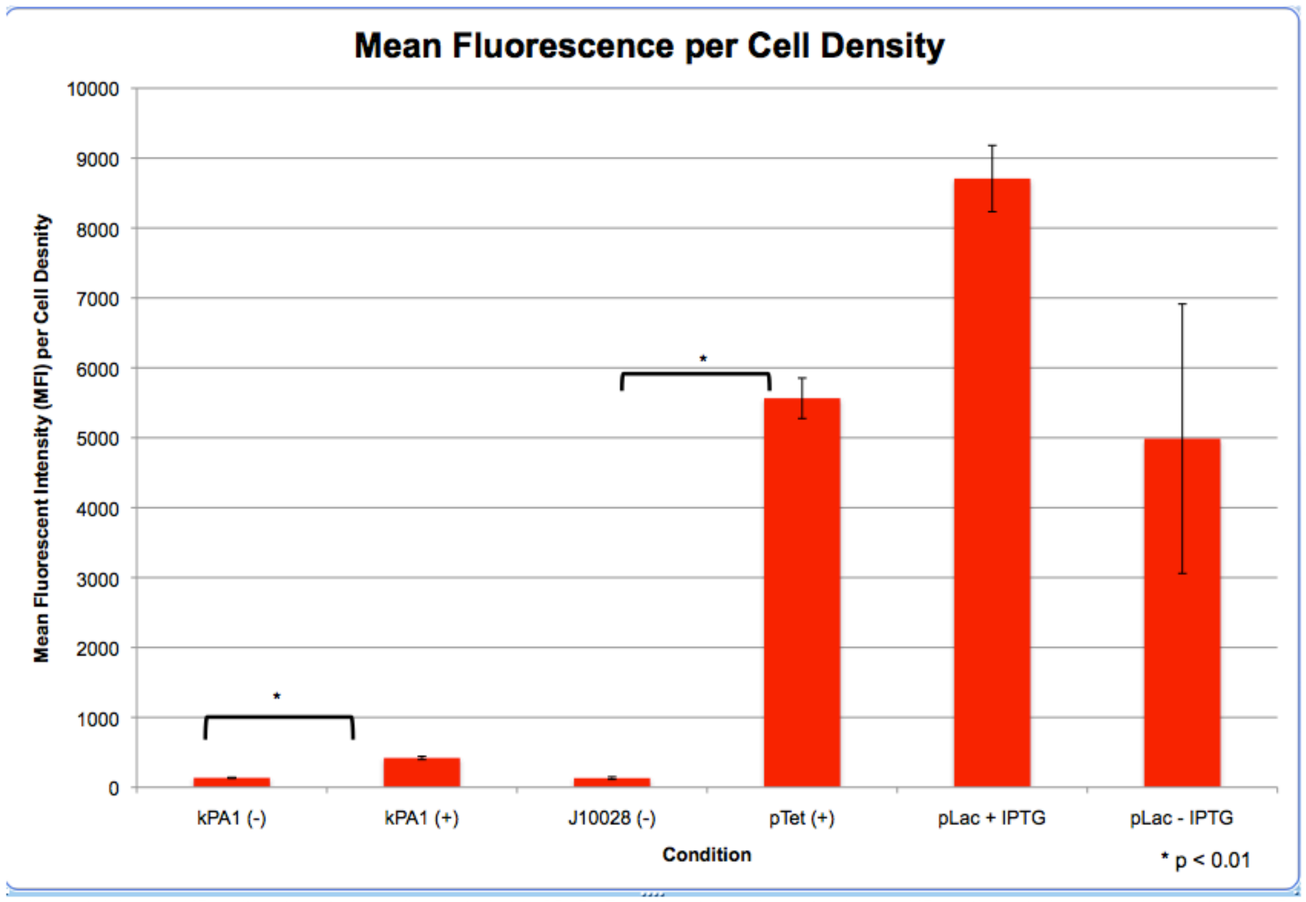
Designed by Chris Peek Group: Campbell_M_Lab (2011-09-01)

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

Bio113 Students doing Real Promoter Resarch