Research Quality Synthetic Biology Plasmids for Educational Uses: pClone Plasmid Family

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& **Genome** Consortium for **Active Teaching**

April 9, 2016

CURE
course-base undergrad research experience

- Reinforce core concepts
- Build core competencies
- Improve quantitative skills
- Use mathematical modeling
- Retain STEM majors
- Increase diversity of STEM
- Learn technical skills - jobs
- Work in teams
- Gain communication skills

pClone: Learning Objectives

Introductory Biology

Function of promoter
Repressor diagram
Activator diagram
Experimental design
Transformation
Type IIS restriction enzymes
GGA cloning method

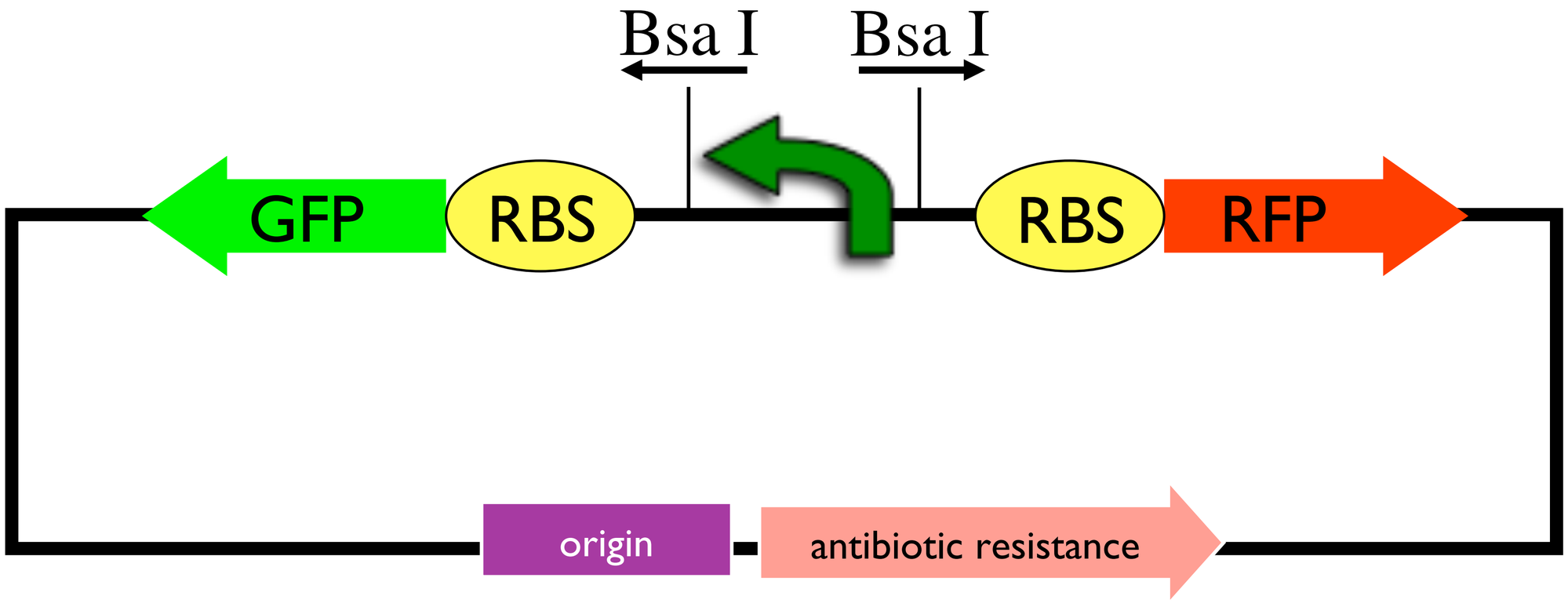
Genetics

Function of promoter
-10 & -35 sites
mutational analysis
Transformation
Verify promoter cloned
Test promoter strength
Type IIS restriction enzymes
GGA cloning method

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

pClone Red

J119137

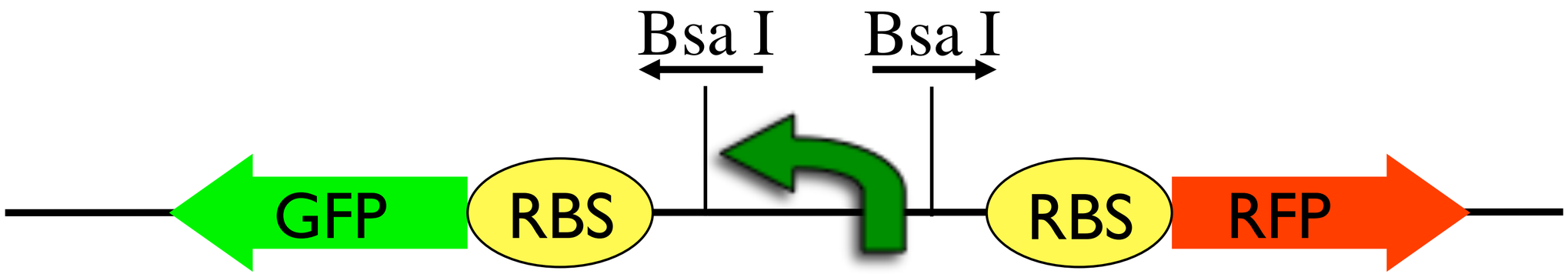


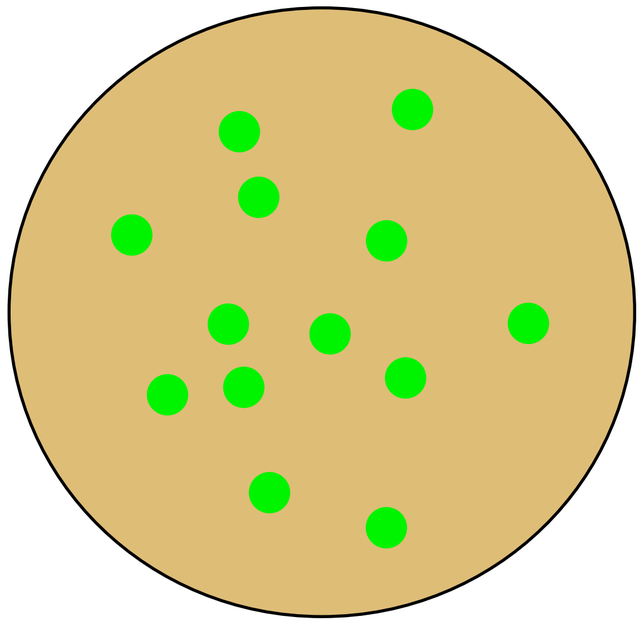
plasmid backbone

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

pClone Red

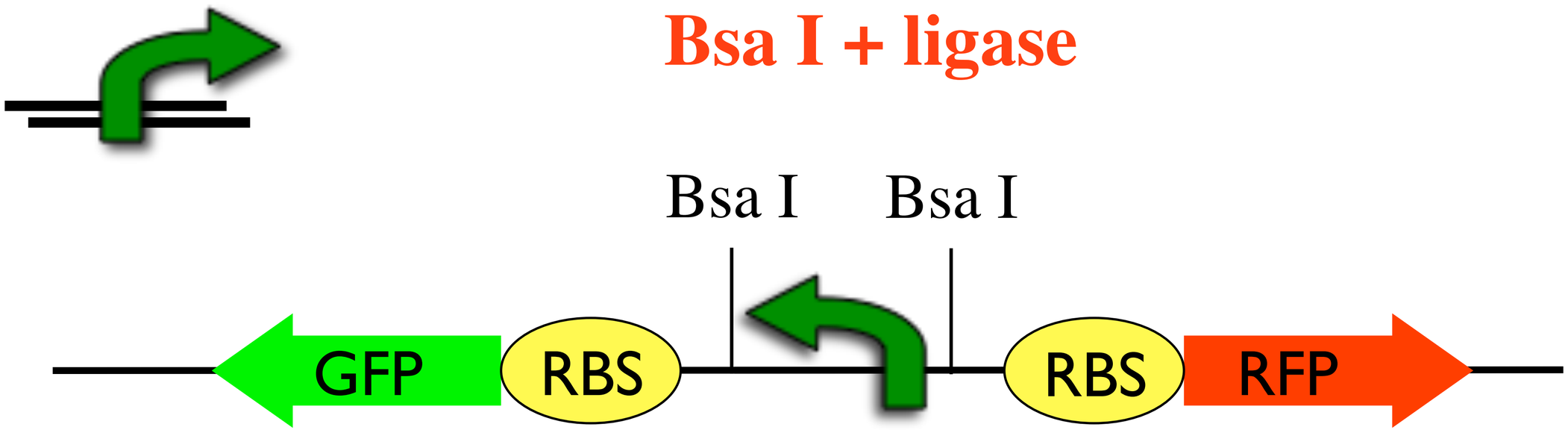
all colonies green

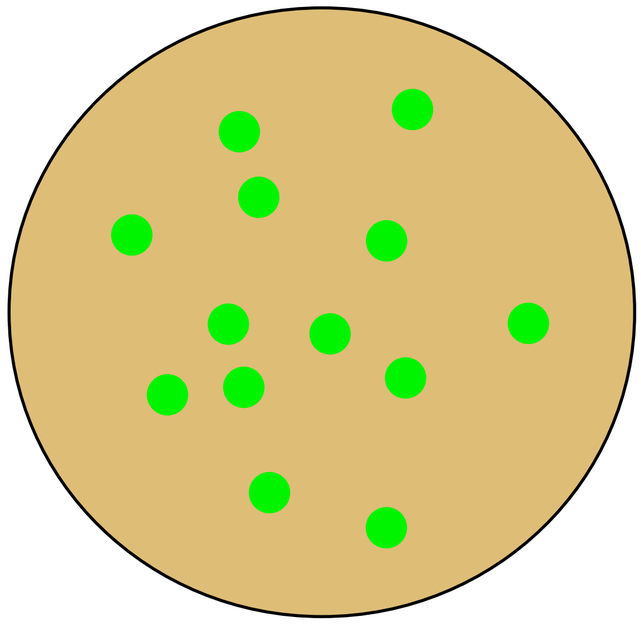




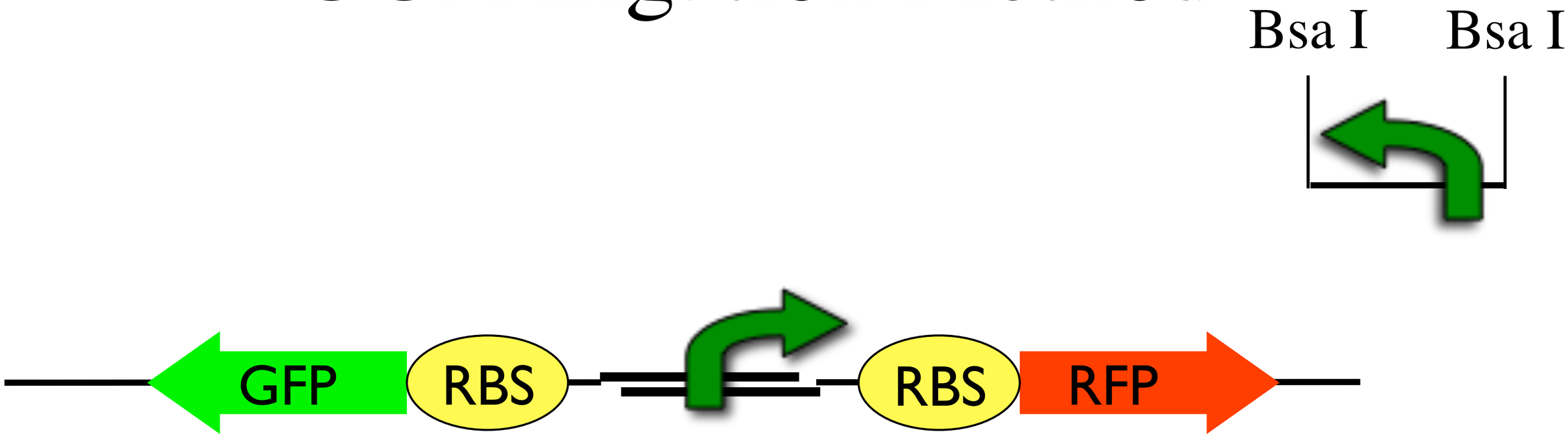
GGA Ligation Method

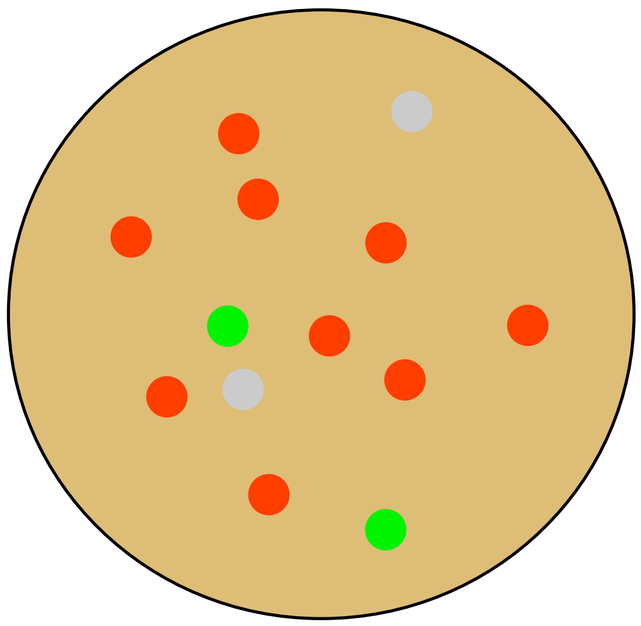
Bsa I + ligase





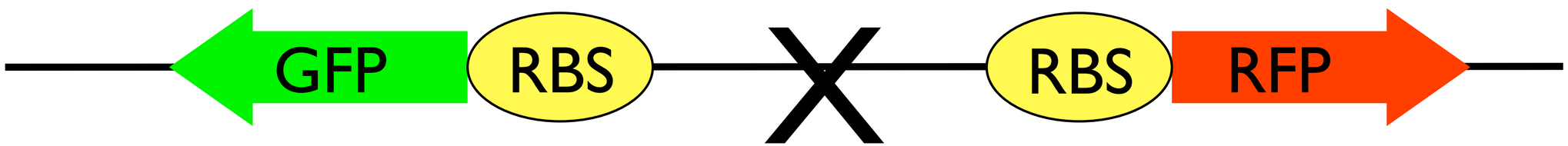
GGA Ligation Method

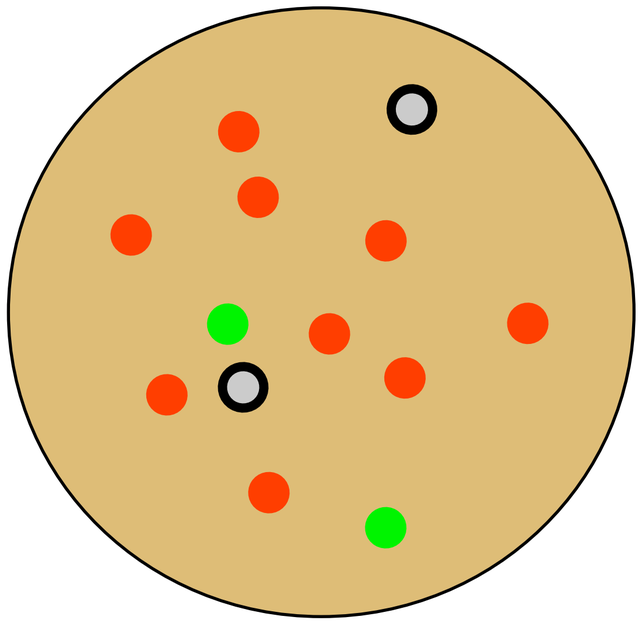




Remove Initial Promoter

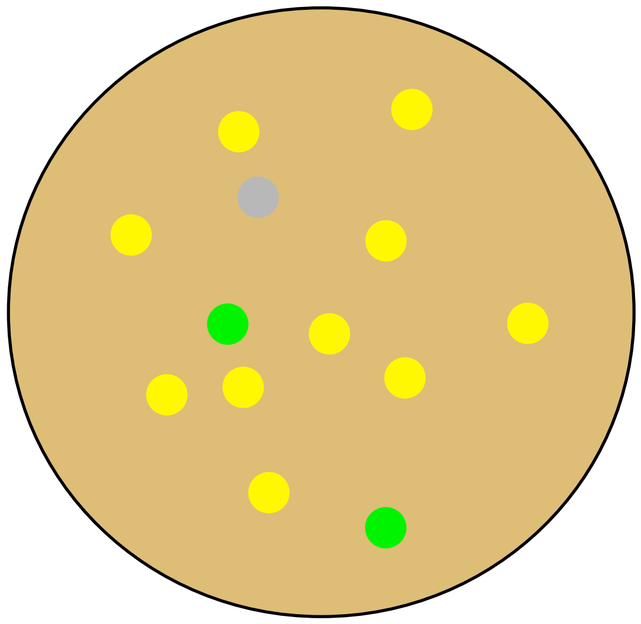
J119137





Insert Bi-directional Promoter

J119137



Insert Non-functional Promoter

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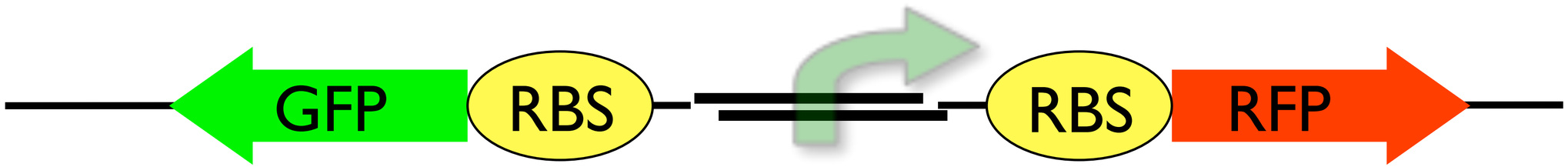
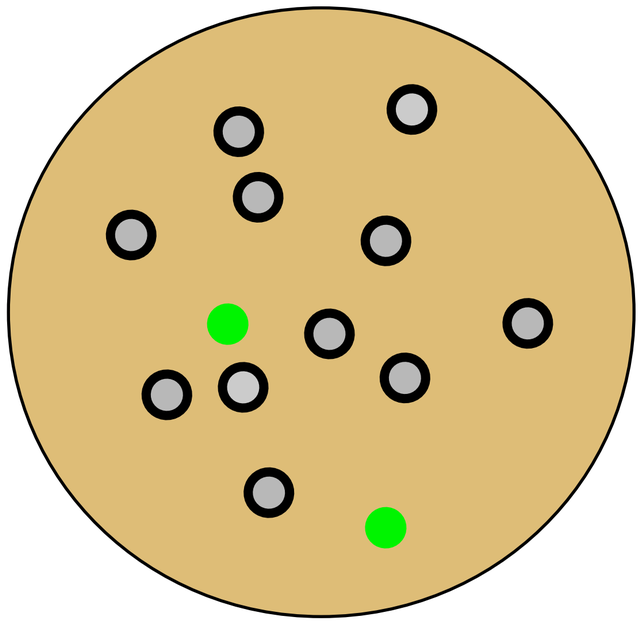
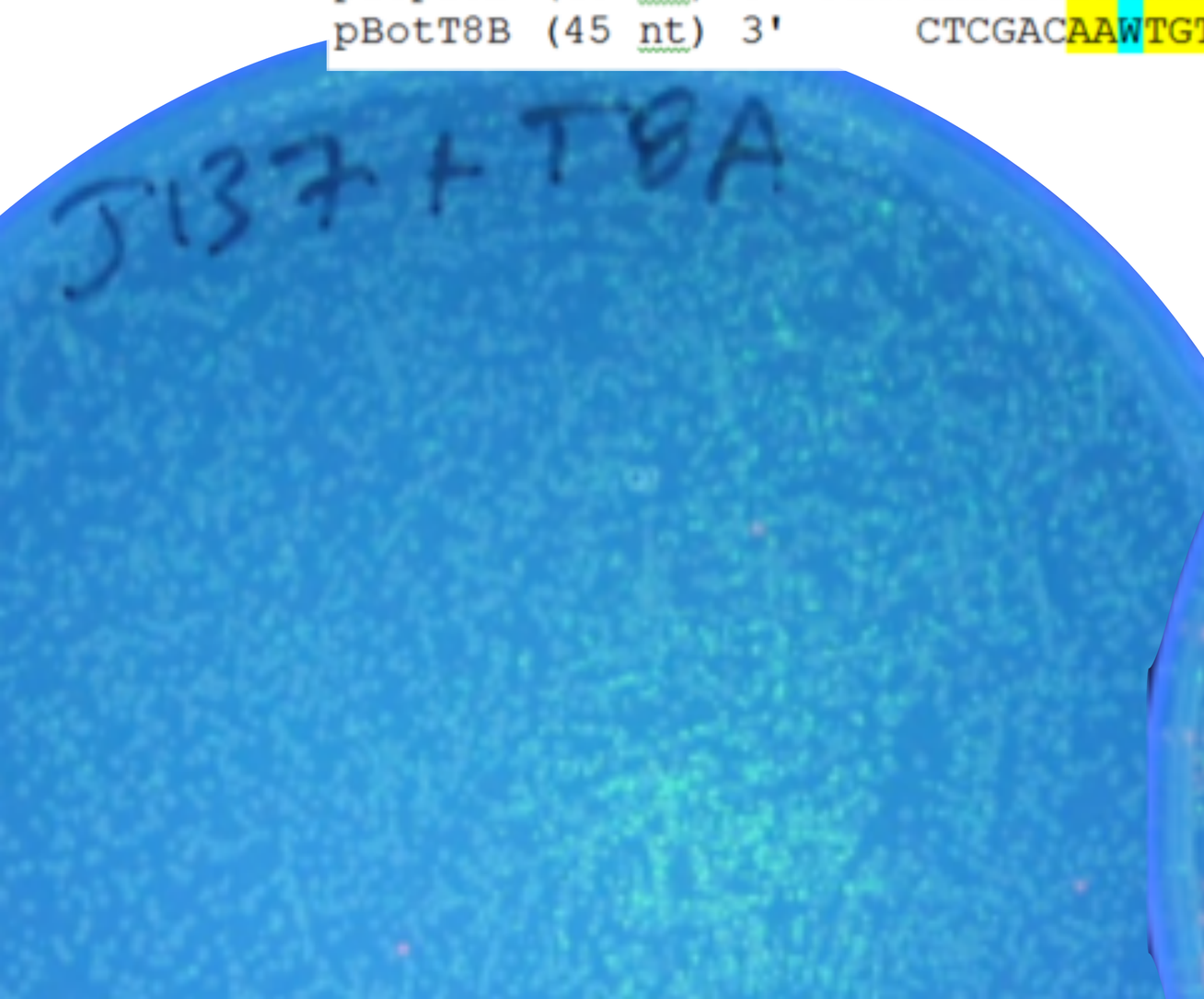


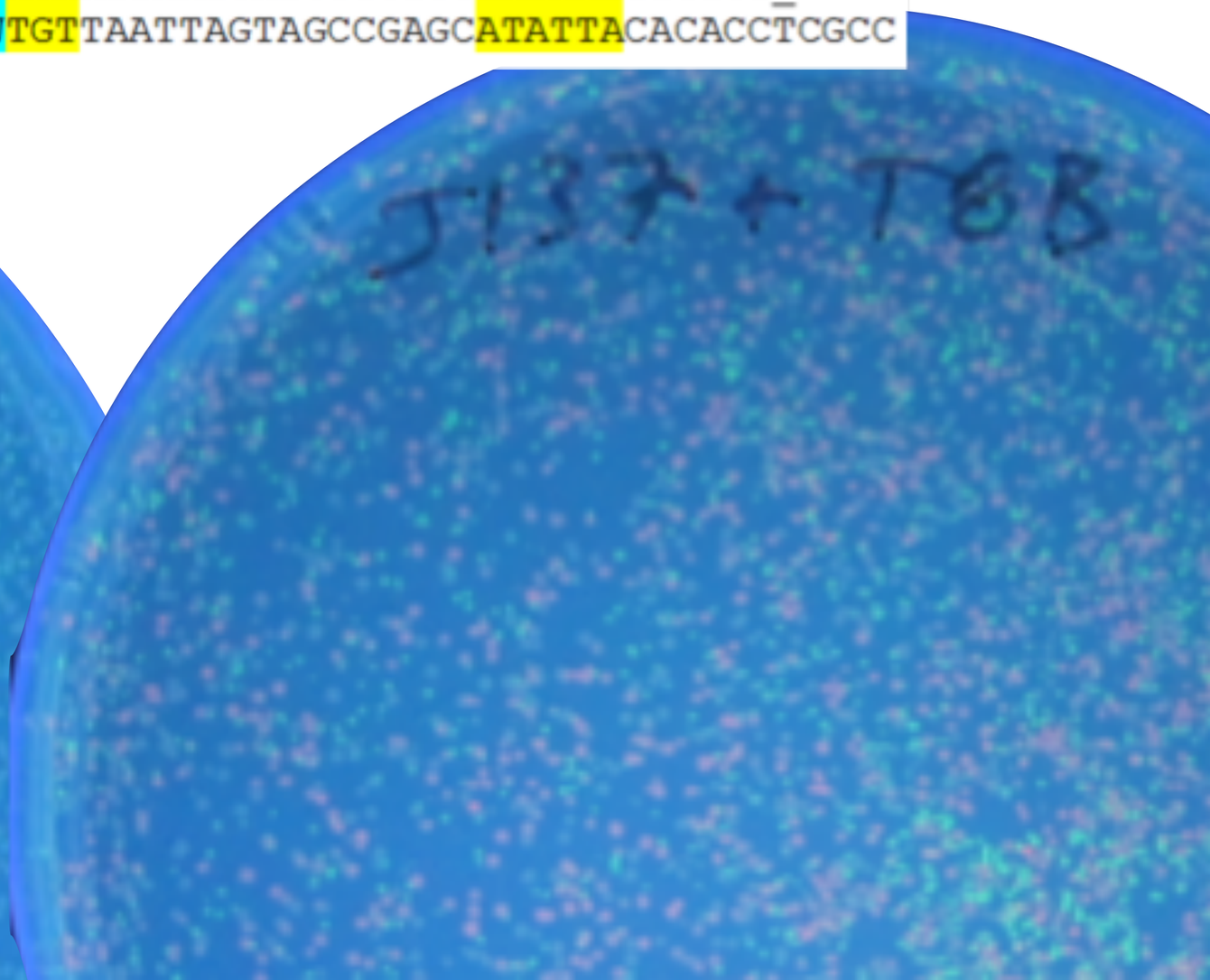


Mutating Known Promoters: *Ptac*

```
                                        ATTA DELETED
                                             V
pTopT8A (45 nt) 5' CGACGAGCTGTTGACAATCATCGGCTCGTATAATGTGTGGA
pBotT8A (45 nt) 3'      CTCGACAACTGTTAGTAGCCGAGCATATTACACACCTCGCC

pTopT8B (45 nt) 5' CGACGAGCTGTTWACAATTAATCATCGGCTCGTATAATGTGTGGA
pBotT8B (45 nt) 3'      CTCGACAAWTGTTAATTAGTAGCCGAGCATATTACACACCTCGCC
```





Phone & ImageJ to Quantify Promoter

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: Assessment Results

Introductory Biology

Function of promoter ✔
Repressor diagram ✔
Activator diagram ✔
Experimental design ✔
Transformation ✔
Type IIS restriction enzymes ✗
GGA cloning method ✔

Genetics

Function of promoter ✔
-10 & -35 sites ✔
mutational analysis ✔
Transformation ✔
Verify promoter cloned ✔
Test promoter strength ✔
Type IIS restriction enzymes ✔
GGA cloning method ✗

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pClone for CURE Laboratory Classes

1. pClone enables authentic research
2. Inexpensive & easy to prep
3. High success rate
4. Minimal training for faculty
5. Can be implemented at diverse institutions
6. Scales easily
7. Easy to disseminate research findings

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pClone: Exploring Promoters with Synthetic Biology

2 Items **Exclusive**

$215.00 - $215.00 View Details

5 / 5

Give your students the opportunity to learn and explore transcription regulation right in your classroom. This unique approach to synthetic biology was developed by college professors focused on creating a unique activity to demonstrate gene regulation. This multi-part lab will expose students to cloning, restriction enzymes, transformation, microbiology, and so much more in an effective classroom protocol.

Selected items **Compare** **Add to Cart** **Add to List** Sort by We Recommend

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☐		pClone: Exploring Promoters with Synthetic Biology Kit (with prepaid coupon) Item #211150	$215.00	Qty 1 Add to Cart Available 5/4/16
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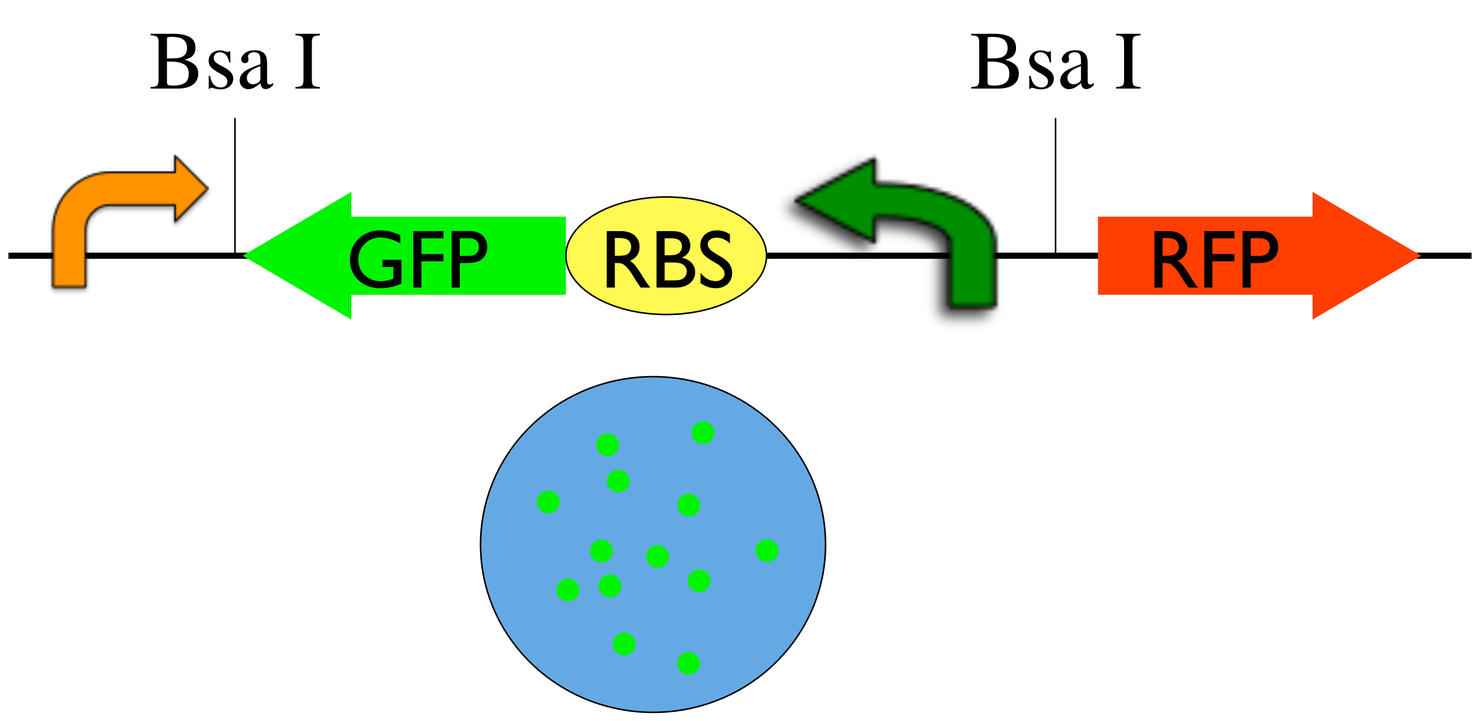
rClone: Learning Objectives

- Initiation of Translation
- RBS efficiency
- Interaction of RBS and 16S rRNA
- Alternative base pairings in RNA
- Abstraction: parts, devices, systems
- Standardization of parts
- Standardization of assembly
- Golden Gate Assembly
- Type IIs restriction enzymes
- Designing oligonucleotides
- Annealing oligonucleotides
- rClone: green versus not green
- Reporter genes
- RFP intensity quantification
- Mutagenesis for RBS function
- Consensus sequences
- RBS efficiencies in Synthetic Biology
- RBSs efficiencies in bacterial genomes
- RBS contribution to phenotype
- RBS efficiency & natural selection

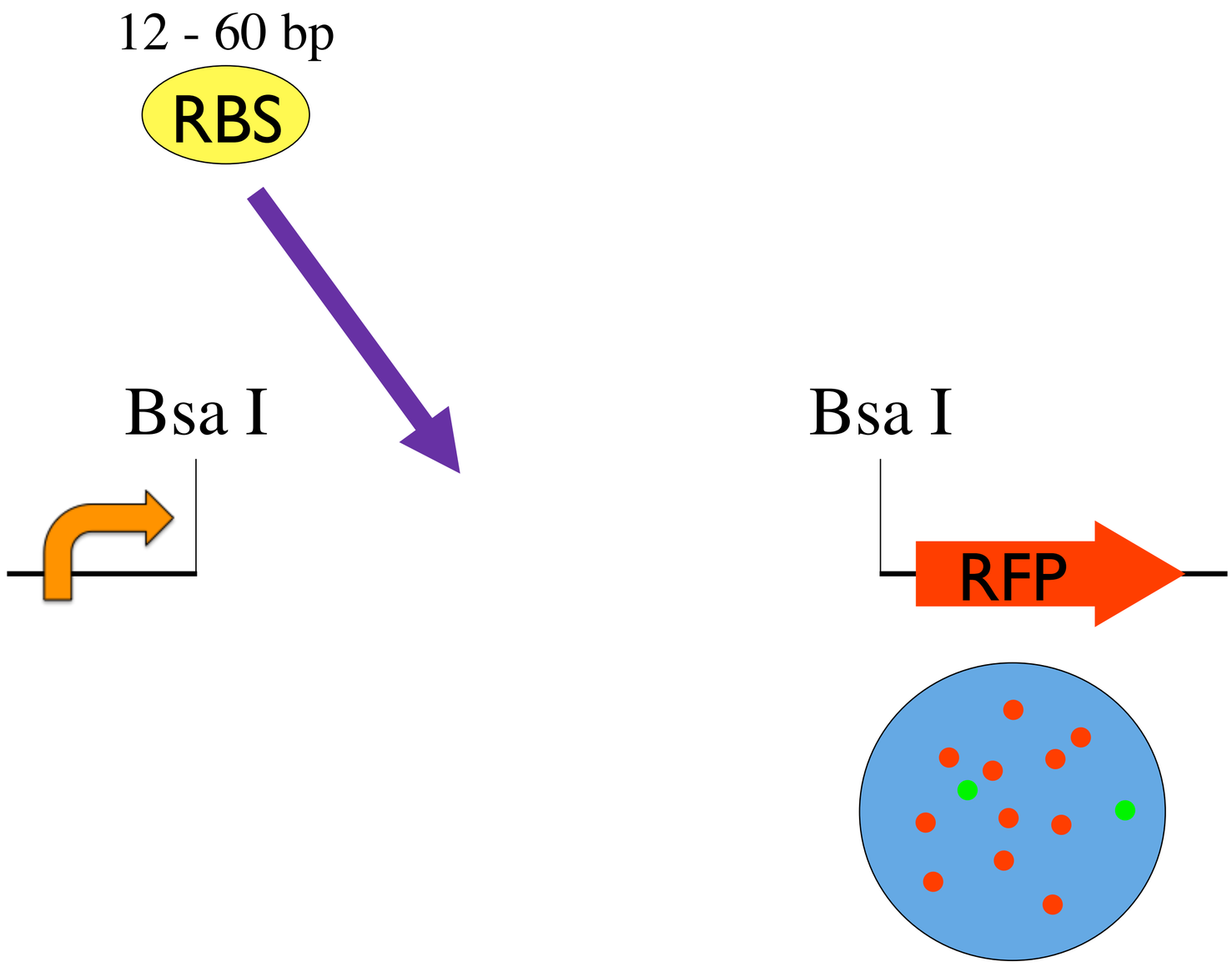
rClone Red (ribosome research)

J119384

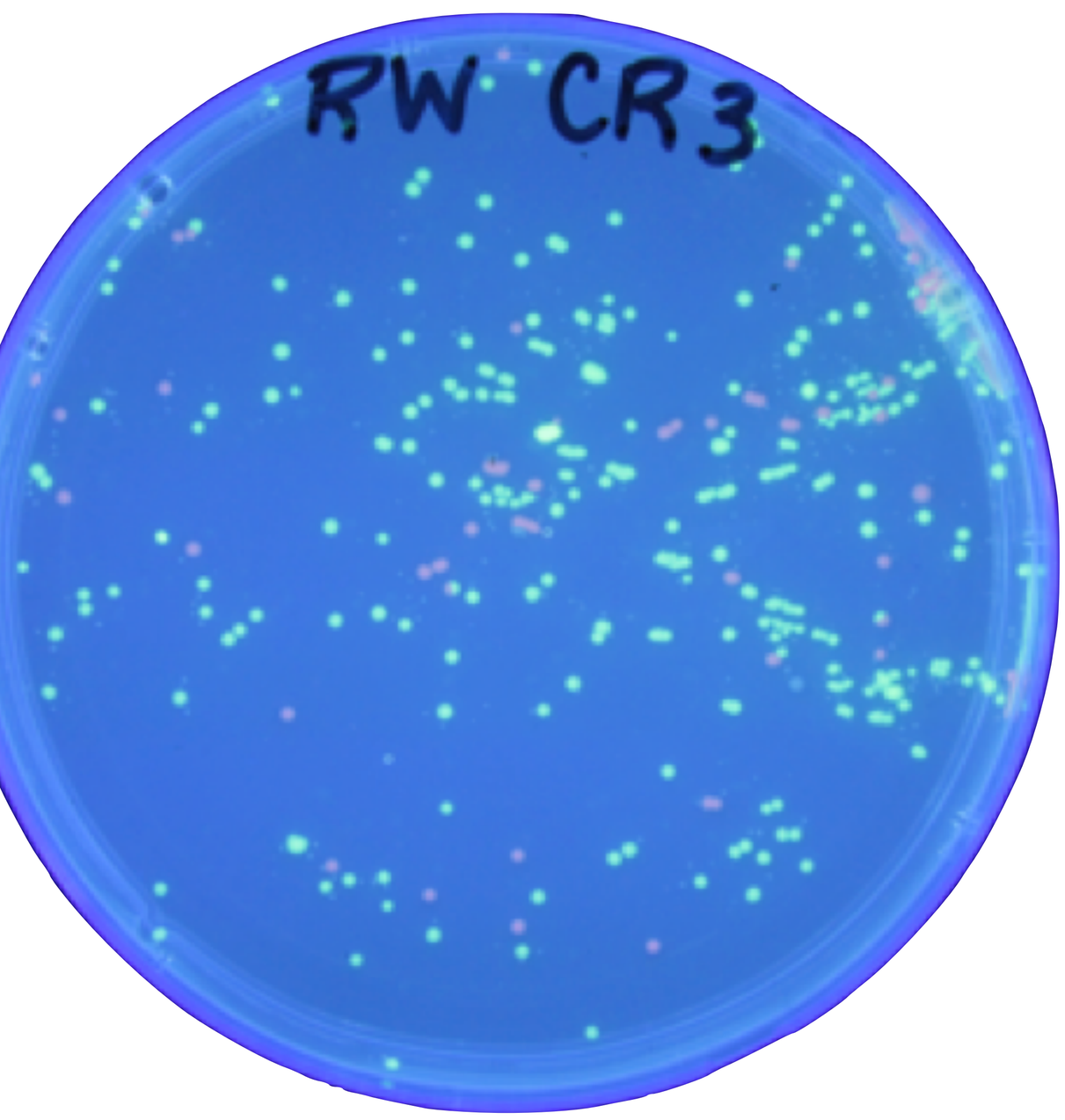


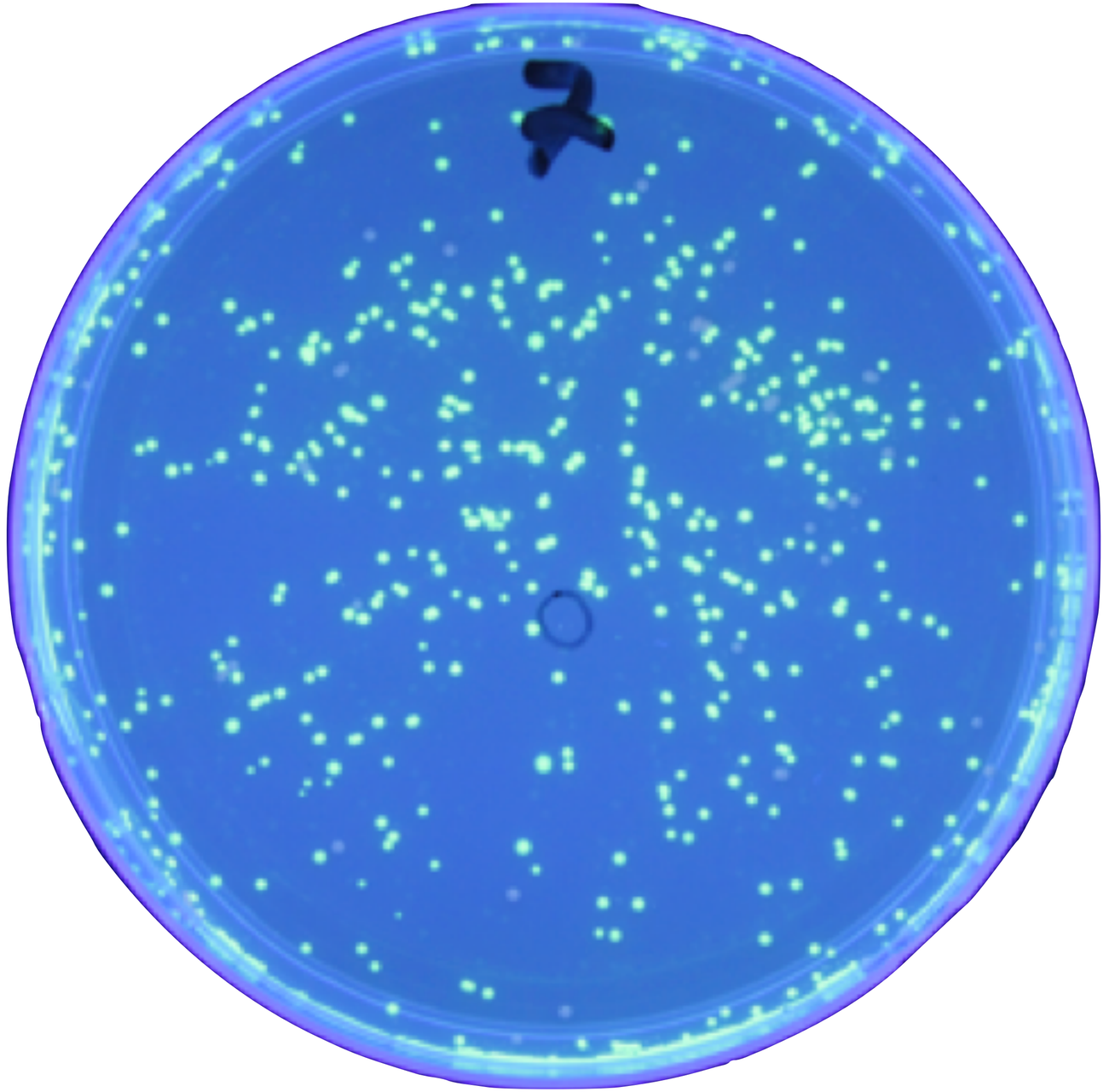
rClone Red (ribosome research)

J119384

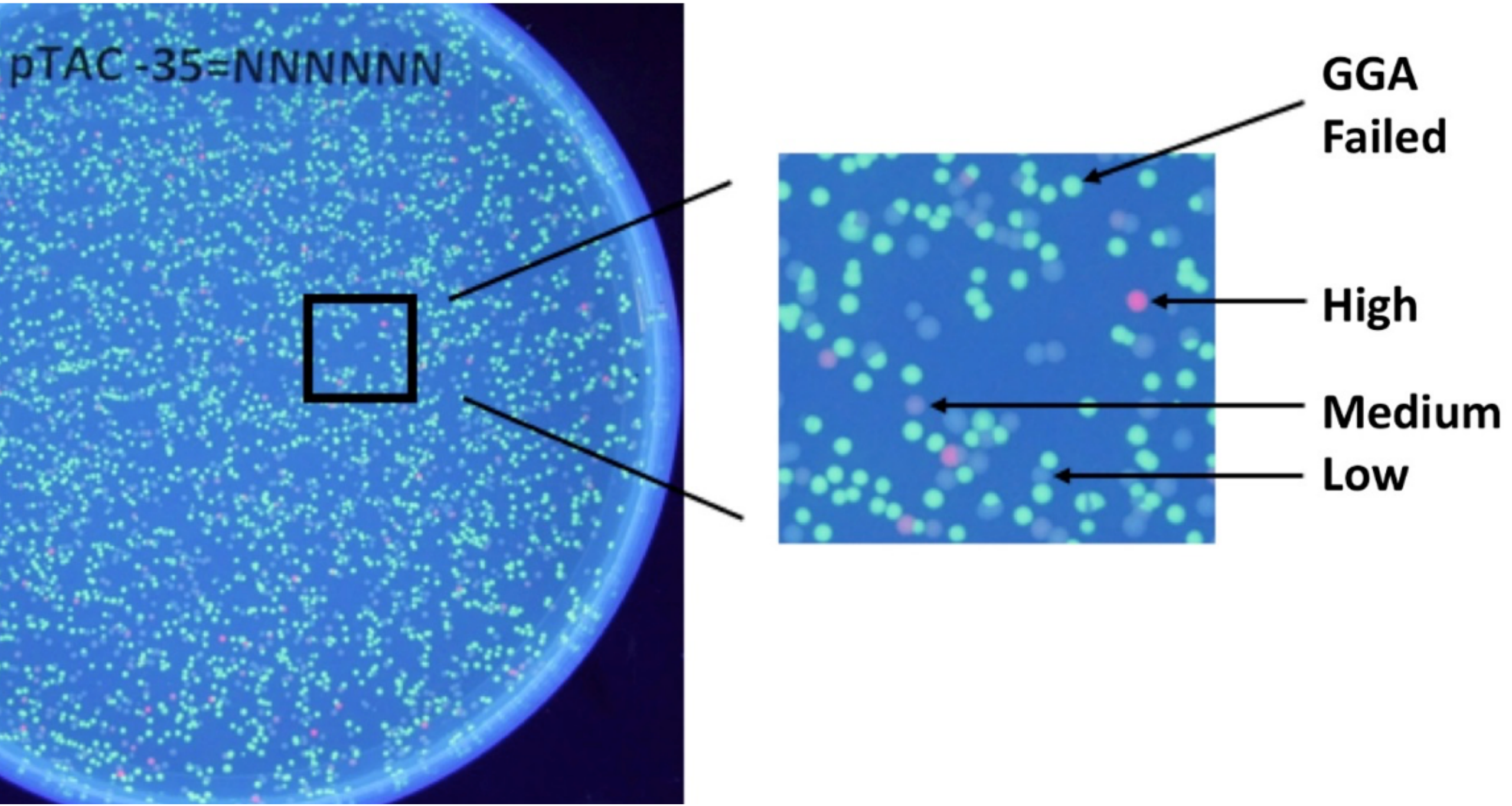


rClone Red (student-designed RBS)





rClone Red (RBS library)



rClone: Assessment Results

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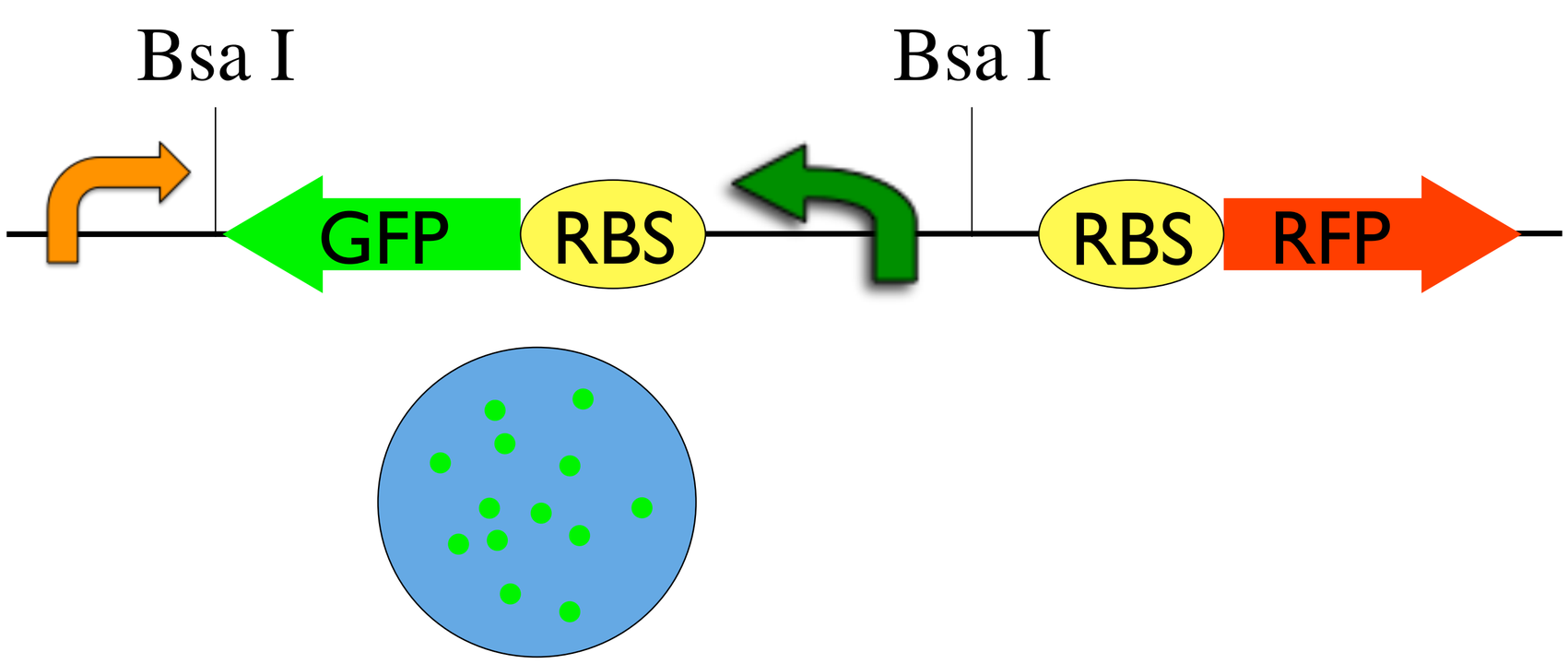
- Consensus sequences
- RBS efficiencies in Synthetic Biology
- RBSs efficiencies in bacterial genomes
- RBS contribution to phenotype
- RBS efficiency & natural selection

tClone: Learning Objectives

- Transcription termination (TT)
- RNA folding
- Abstraction: parts, devices, systems
- Standardization of parts
- Standardization of assembly
- Golden Gate Assembly
- Type IIs restriction enzymes
- Designing oligonucleotides
- Annealing oligonucleotides
- tClone: green versus not green
- Reporter genes
- RFP intensity quantification
- Mutagenesis for RBS function
- Consensus sequences
- Transcriptional readthrough
- Operon directionality
- TT efficiencies in Synthetic Biology
- TT efficiencies in bacterial genomes
- TT contribution to phenotype
- TT efficiency & natural selection

tClone Red (terminator research)

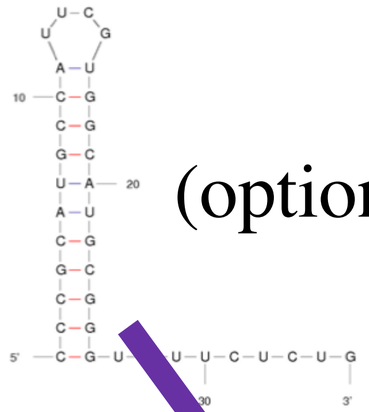
J119361



tClone Red (terminator research)

J119361

60 - 230 bp



(optional ligand)

Bsa I

Bsa I

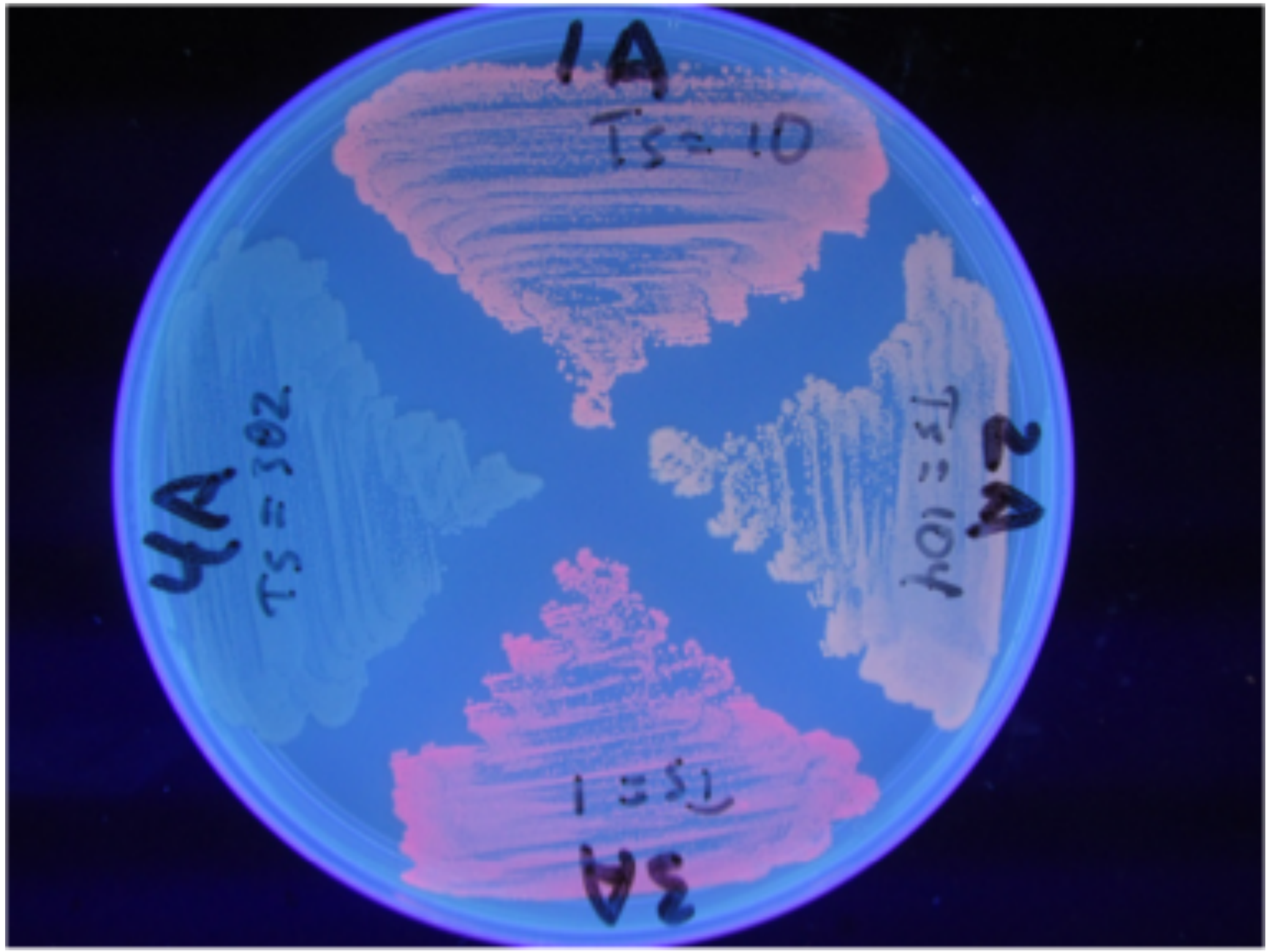
RBS

RFP

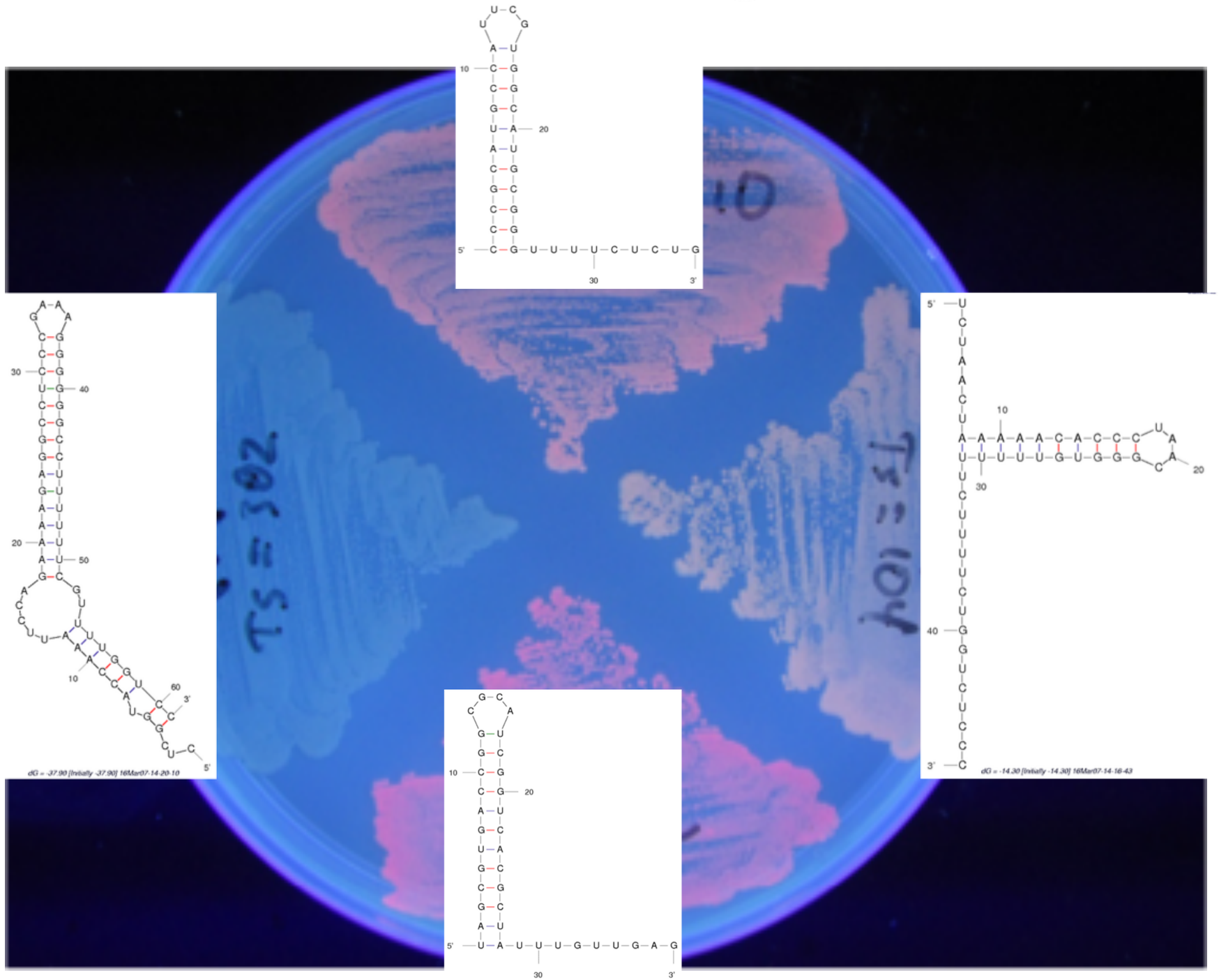
OR

(+)

tClone Red (student-designed terminators)



tClone Red (student-designed terminators)



tClone: spring 2016

- Transcription termination (TT)
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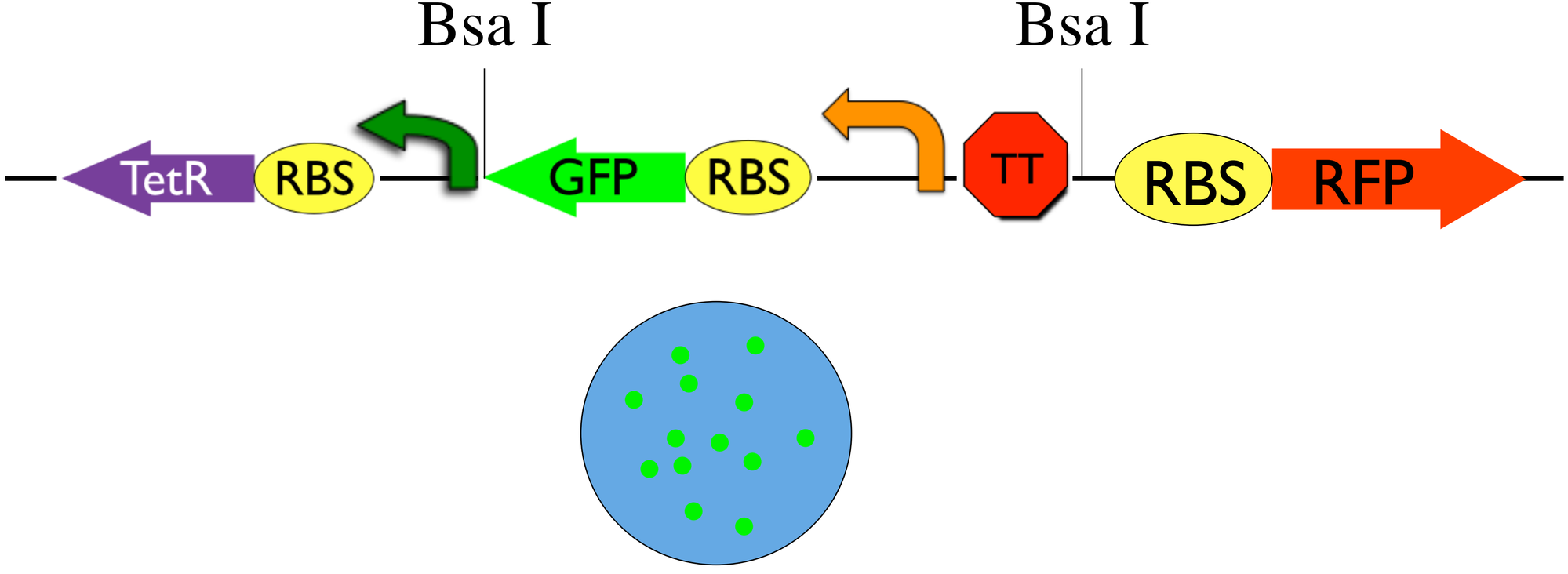
- TT efficiencies in bacterial genomes
- TT contribution to phenotype
- TT efficiency & natural selection

repClone Red

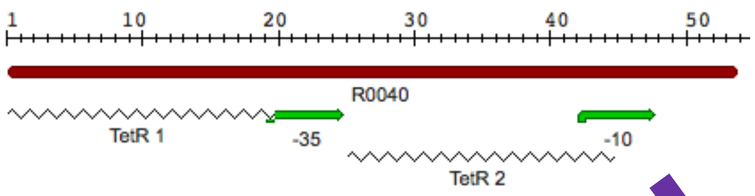
J100205



repClone Red

J100205

Ptet



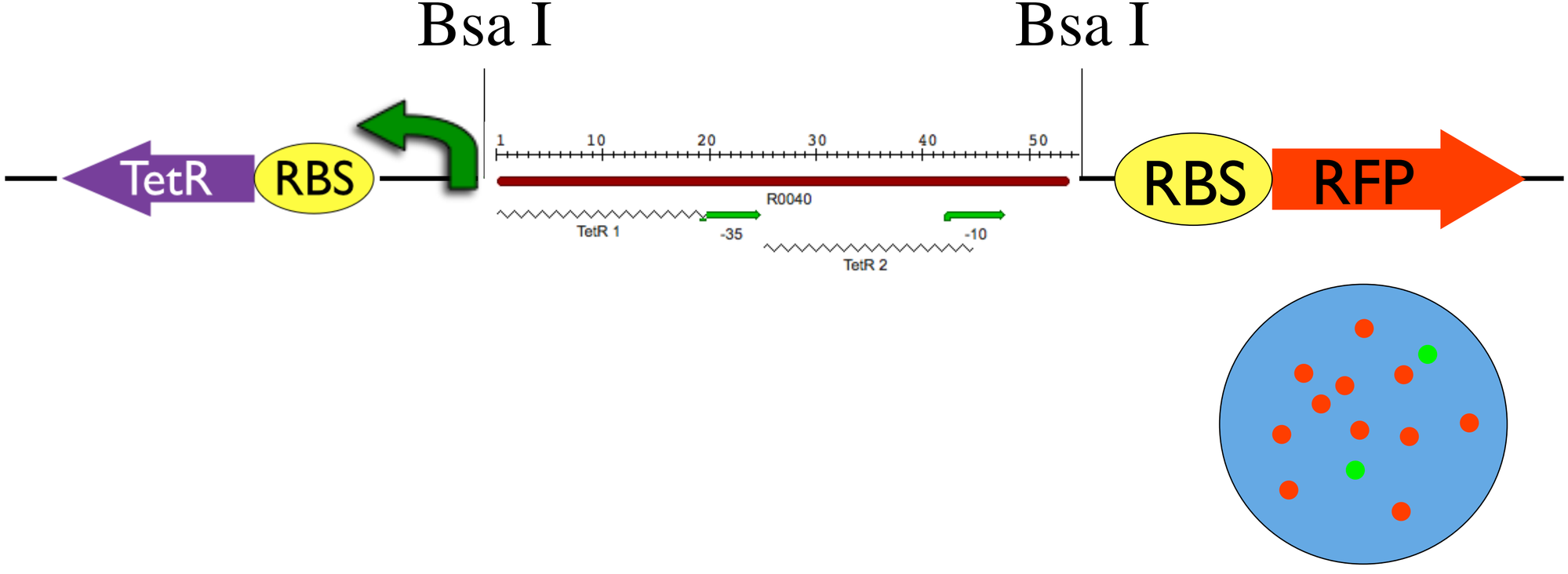
54 bp

Bsa I

Bsa I

repClone Red

J100205



repClone Red

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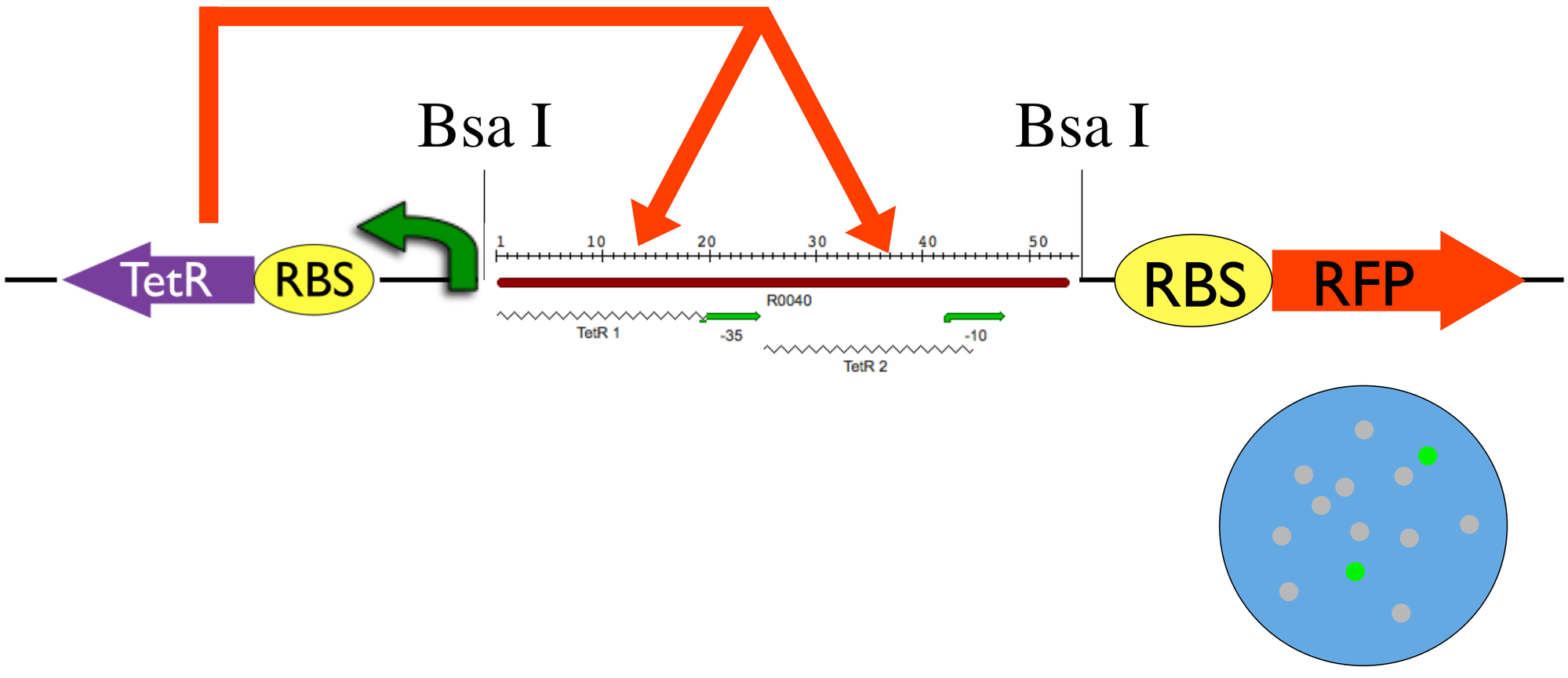
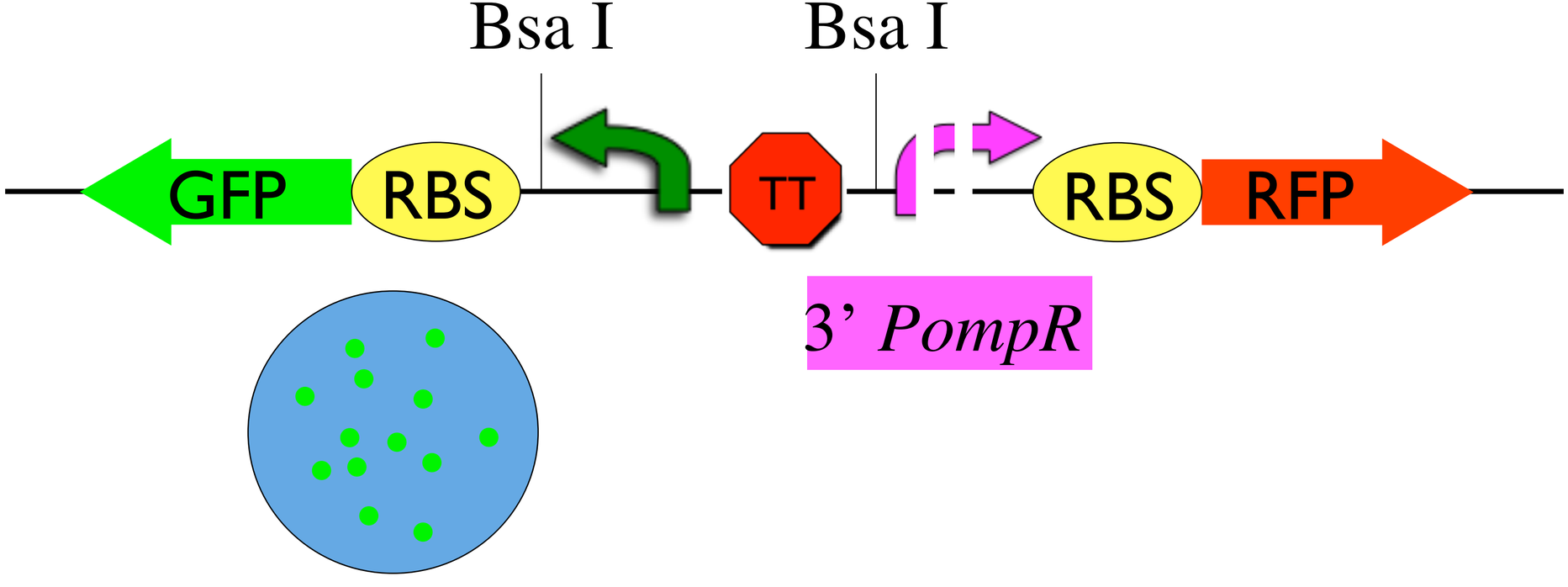


actClone Red

J100204



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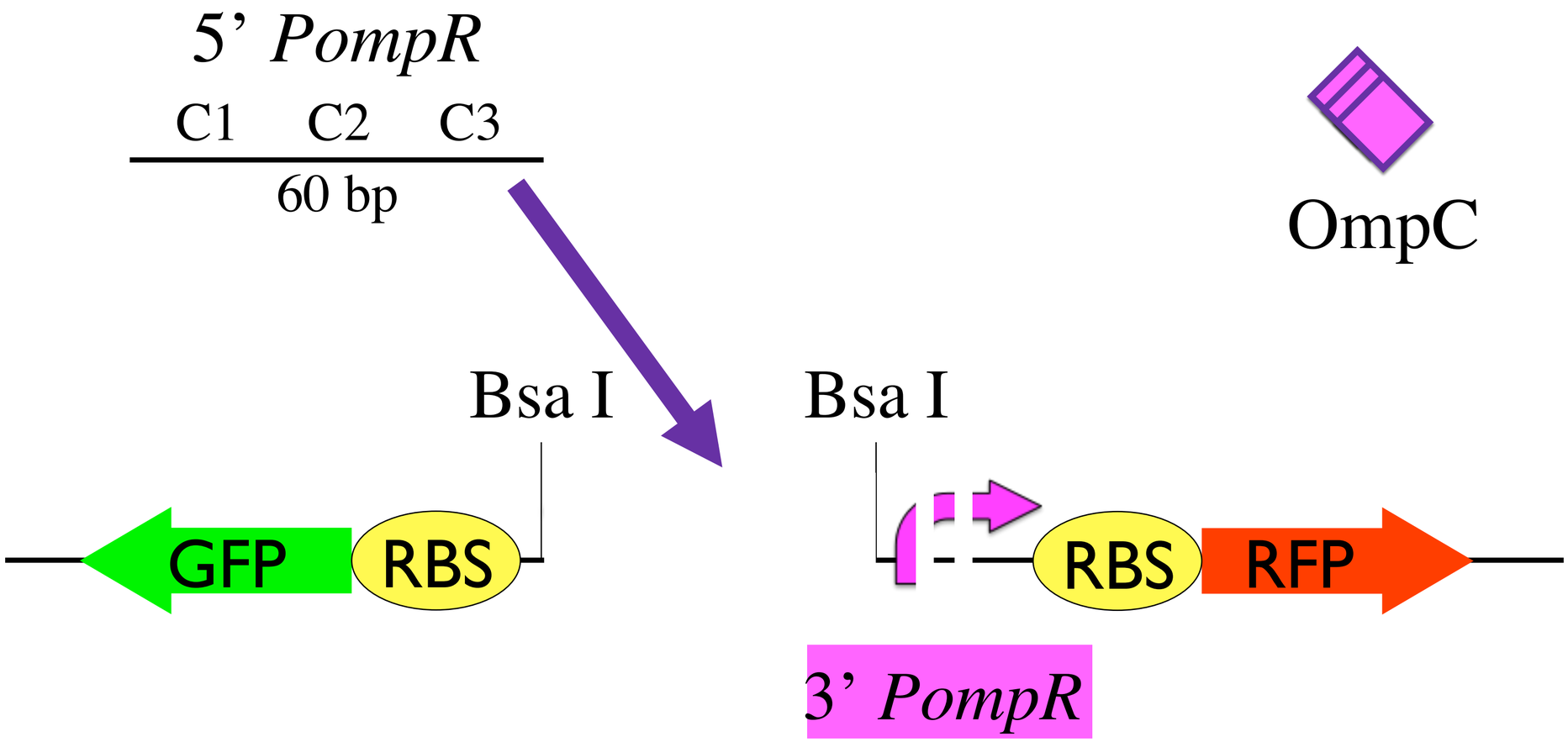

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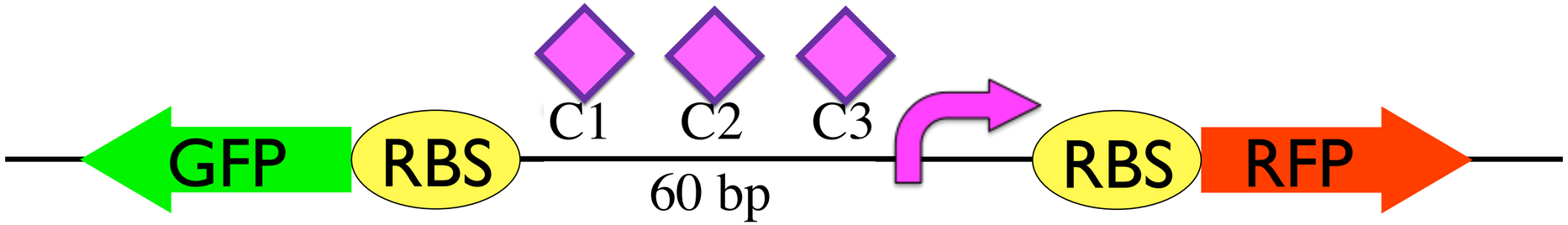
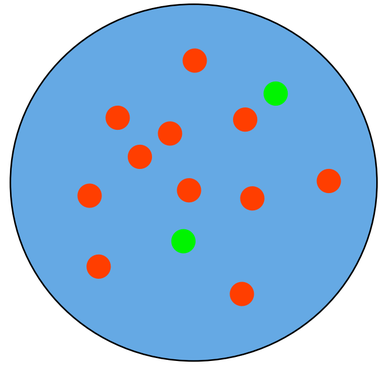


all of *PompR*



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- Laurie Heyer (Davidson)
- many undergraduate and high school students (MO & NC)