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

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& Genome Consortium for Active Teaching

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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

pClone Red

J119137

pClone Red

all colonies green

GFP  RBS  RBS  RFP

Bsa I  Bsa I
GGA Ligation Method

Bsa I + ligase

Bsa I          Bsa I
GGA Ligation Method

GFP → RBS → RFP

Bsa I

Diagram showing the GGA ligation method with GFP, RBS, and RFP genes connected by Bsa I restriction enzymes.
Remove Initial Promoter

J119137
Insert Bi-directional Promoter

J119137
Insert Non-functional Promoter

J119137
Mutating Known Promoters: *Ptac*

- **pTopT8A (45 nt)**
  - 5' CGACGAGCTG**TTGACA**ATCATCGGCTCGGTATAATGTGTGGA
  - 3' CTCGACA**ACTGT**TAGTAGCCGAGCATATTAACACACCTCGCC

- **pBotT8A (45 nt)**
  - 5' CGACGAGCTG**TTWACA**ATTATCATCGGCTCGGTATAATGTGTGGA
  - 3' CTCGACA**AWTGT**TAATTAGTAGCCGAGCATATTAACACACCTCGCC

- **pTopT8B (45 nt)**
  - 5' CGACGAGCTG**TTWACA**ATTATCATCGGCTCGGTATAATGTGTGGA
  - 3' CTCGACA**AWTGT**TAATTAGTAGCCGAGCATATTAACACACCTCGCC

- **pBotT8B (45 nt)**
  - 5' CGACGAGCTG**TTWACA**ATTATCATCGGCTCGGTATAATGTGTGGA
  - 3' CTCGACA**AWTGT**TAATTAGTAGCCGAGCATATTAACACACCTCGCC
Phone & ImageJ to Quantify Promoter

<table>
<thead>
<tr>
<th>Mutant</th>
<th>J119319</th>
<th>J119320</th>
<th>J119321</th>
<th>J119322</th>
<th>J119323</th>
<th>J119324</th>
<th>J119325</th>
<th>J119326</th>
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</thead>
<tbody>
<tr>
<td><strong>pClone Green plate</strong></td>
<td><img src="pClone_Green_plate.jpg" alt="Image" /></td>
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<td><strong>Isolated clones</strong></td>
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<td><strong>Expression Ratio</strong></td>
<td>4.09</td>
<td>3.94</td>
<td>3.84</td>
<td>2.04</td>
<td>1.54</td>
<td>1.34</td>
<td>3.52</td>
<td>1.00</td>
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# pClone: Assessment Results

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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

pClone: Exploring Promoters with Synthetic Biology

2 Items Exclusive $215.00 - $215.00

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.
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 II 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

12 - 60 bp

RBS

Bsa I

RFP

rClone Red (ribosome research)
rClone Red (student-designed RBS)
rClone: Assessment Results

- Initiation of Translation
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- 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 II 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

RBS  RFP

OR  (+ ♠)
tClone Red (student-designed terminators)
tClone Red (student-designed terminators)
tClone: spring 2016

- Transcription termination (TT)
- RNA folding
- Abstraction: parts, devices, systems
- Standardization of parts
- Standardization of assembly
- Golden Gate Assembly
- Type II restriction enzymes
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- 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
repClone Red

J100205
repClone Red

Ptet

J100205

54 bp

Bsa I

TetR RBS

RBS RFP

Bsa I
repClone Red

J100205
repClone Red
J100205
actClone Red

J100204

GFP → RBS → TT → RBS → RFP

Bsa I

3' PompR
actClone Red

J100204

5' PompR

\[
\begin{array}{ccc}
C1 & C2 & C3 \\
\hline
60 \text{ bp}
\end{array}
\]

Bsa I

GFP
RBS

OmpC

3' PompR

Bsa I

RBS
RFP
actClone Red

J100204

all of PompR
CURE

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