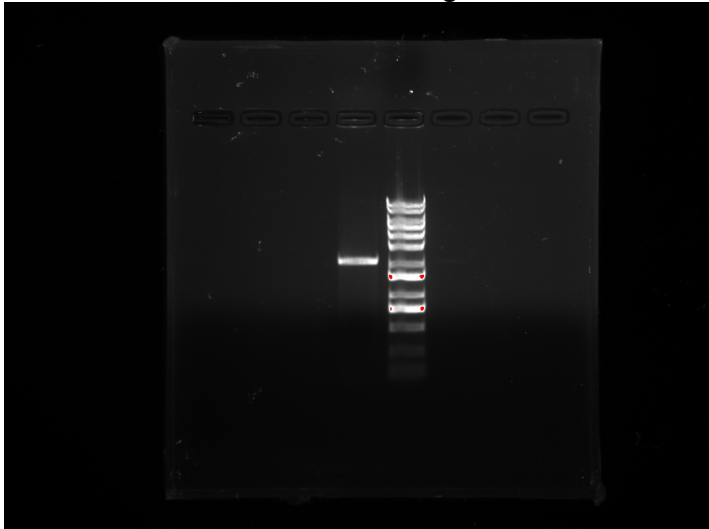


1. Inducible Promoter Assembly (promoter\_K081005-TetR\_C0040 + terminator\_B0015)

1.13 Enzyme Digestion of K081005\_C0040

K081005_C0040	1
0ul	
Spe1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	
2ul	
H2O	
7ul	

1.14 Gel Identification of the Digestion Product K081005\_C0040



Lane1: digestion products of K081005\_C0040, indicate **negative result (we have to reconstruct the plasmid)**

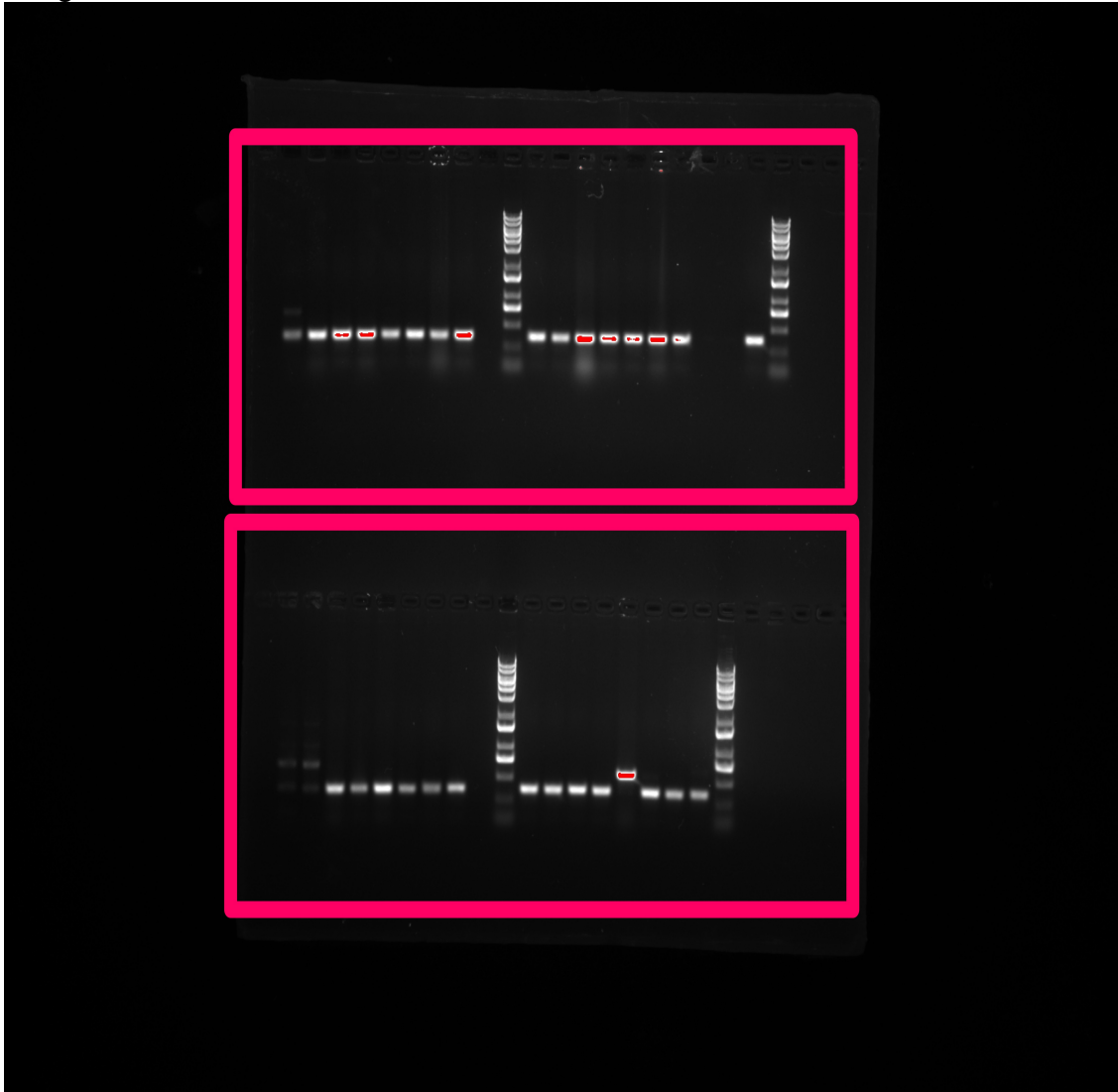
Lane2: 1KB Plus marker

1.15 Colony PCR searching for right colony

Reaction system 350ul (32samples)

10um F-primer		
WL14042	17.5ul	
10um R-primer		
WL14043	17.5ul	
2X		
Mix		175
ul		
Phusion DNA Polymerase		3.5ul
H2O		1
36.5ul		

the colony PCR program  
the gel identification



In the first matrix:

Lane 1-8, Lane 10-17, Lane 19: cPCR products use primer WL14042 and WL14043

Lane 9, Lane 20: 1kb plus DNA ladder

Indication: DNA fragments with the length of 0.7kb has been amplified,  
**confirming positive results**

1.15 Enzyme Digestion of TetR\_C0040

TetR_C0040
10ul
Xba1
0.5ul
EcoR1
0.5ul

cutsmart	
2ul	
H2O	
7ul	

1.16 DNA clean ( concentration: 112.4ng/ul)

1.17 Ligation of TetR\_C0040 and promoter\_K081005 (oligo6-8+oligo7-9)

pTAL-terminator_B0015	0.2u
oligo6-8	
0.3ul	
oligo7-9	
0.3ul	
Quick ligase	
Buffer	5ul
Quick	
ligase	1ul
H2O	
3.2ul	

1.18 Transformation of ligated products

1.19 DNA Purification of promoter\_K081005-TetR\_C0040 and sequence it  
**(Positive results)**

1.20 Construct promoter\_K081005-TetR\_C0040-terminator\_B0015

1.20.1 Enzyme Digestion of promoter\_K081005-TetR\_C0040 (73.6ng/ul)

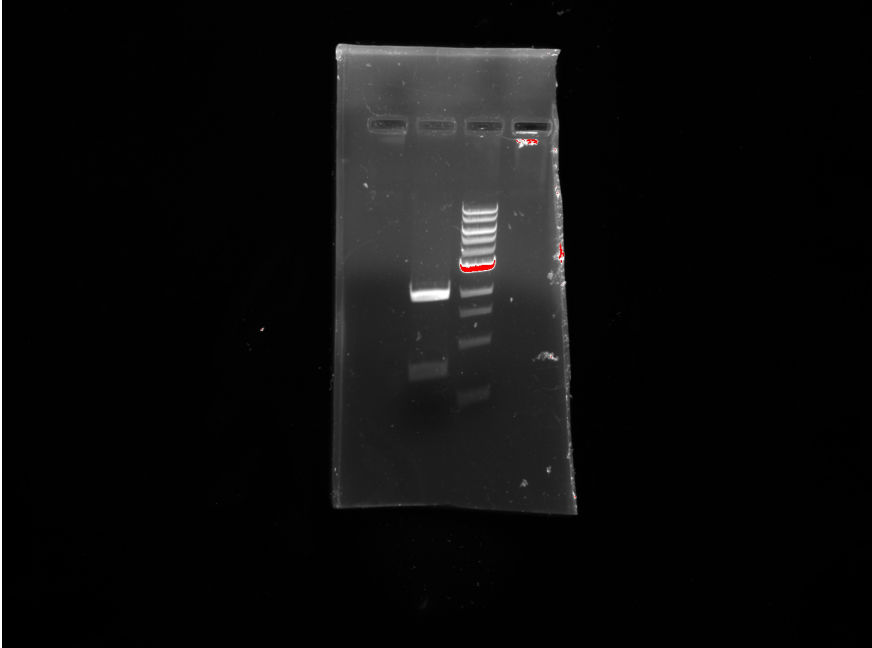
K081005_C0040	1
7ul	
Spe1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	
2ul	
H2O	
0ul	

1.20.2 Enzyme Digestion of terminator\_B0015 (114.9ng/ul)

terminator_B0015	1
5ul	
Xba1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	

2ul
H2O
2ul

1.20.3 Gel Identification and DNA Recovery



Lane 1: Products of enzyme digestion of K081005-C0040  
 Lane2: 1kb DNA ladder

1.20.4 Ligation

K081006-C0040(40.8ng/ul)	1u
termintor-B0015(23.3ng/ul)	1.5u
Quick ligase	
Buffer	5ul
Quick	
ligase	1ul
H2O	
1.5ul	

1.20.5 Transformation

1.20.6 DNA Purification and Identification

DNA Identification via Enzyme Digestion

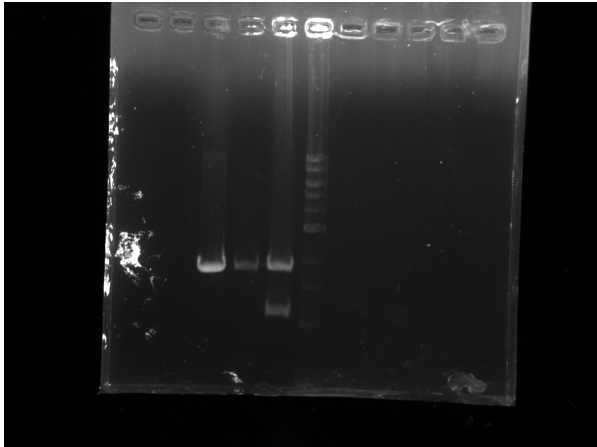
K081005\_C0040\_B0015 (1) 150ng/ul; (2) 46ng/ul; (3) 115.8ng/ul

K081005_C0040_B0015	6
ul	
Pci1	0
.25ul	
EcoR1	0



.25ul  
cutsmart  
1ul  
H2O  
2.5ul

#### Gel Identification



Notion: Bands with length less than 0.5kb have disappeared owing to long running time (over 30min)

Lane 4: 1kb DNA Ladder

Lane1-Lane3: Enzymen digestion of promoter\_K081005-TetR\_C0040-Terminator\_B0015; lane 1 and lane 2 only shows one band and the other possible 0.3kb band may run out of the gel, indicating **negative results**; lane 3 shows two bands with 1kb and 2kb bands respectively, indicating **positive results**.

1.20.7 Sequence it with WL14042

Suggest **positive results**

9.04

1.21 Construct

promoter\_K081005-TetR\_C0040-terminator\_B0015-promoter\_R0040

1.21.1 Enzyme Digestion of promoter\_K081005-TetR\_C0040-terminator\_B0015 and promoter\_R0040

Enzyme Digestion of promoter\_R0040

promoter\_R0040

17ul

Xba1

0.5ul

EcoR1

0.5ul

cutsmart

2ul

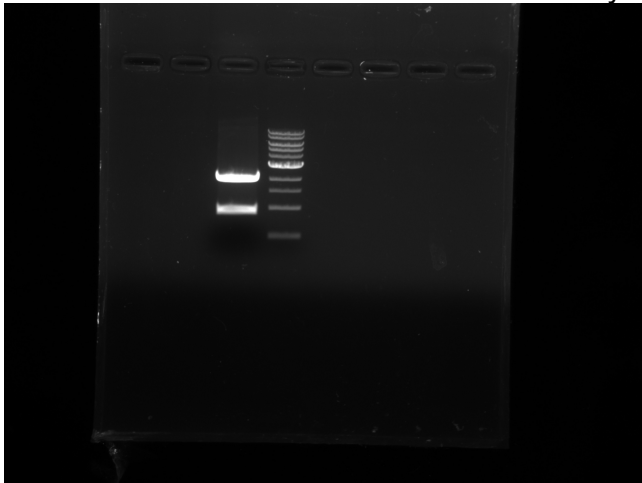
H2O

0ul

### Enzyme Digestion of promoter\_K081005-TetR\_C0040-terminator\_B0015

K-C-B	
17ul	
Spe1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	
2ul	
H2O	
0ul	

### 1.21.2 Gel Identification and DNA Recovery



Lane1: Products of enzyme digestion of promoter\_K081005-TetR\_C0040-Treminator\_B0015 and we purify the band with the length of 1kb.

Lane 2: 1kb DNA ladder

Concentration after DNA recovery:

A: R0040 107.1ng/ul;

B: promoter\_K081005-TetR\_C0040-Treminator\_B0015 30.2ng/ul

### 1.21.3 Ligation

A		
0.25ul		
B		
1.5ul		
Quick ligase		
Buffer	5ul	
Quick		
ligase		1ul
H2O		

2.25ul

A		
0.25ul		
B		
1.25ul		
Quick ligase		
Buffer	5ul	
Quick		
ligase		1ul
H2O		
2.5ul		

A		
0.25ul		
B		
1.0ul		
Quick ligase		
Buffer	5ul	
Quick		
ligase		1ul
H2O		
2.75ul		

1.21.4 Transformation and incubation

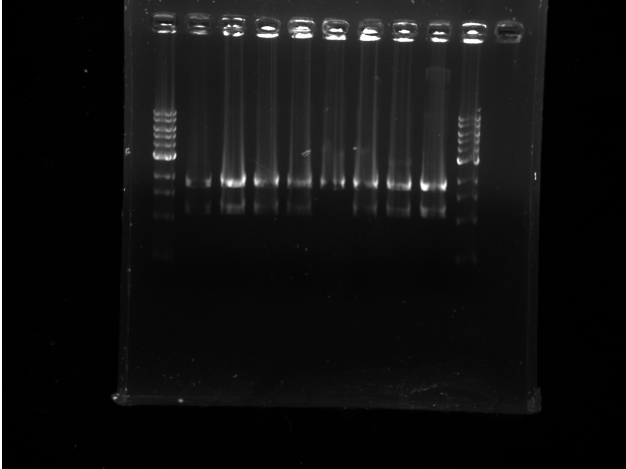
1.21.5 DNA Purification and Identification

Enzyme Digestion

K081005\_C0040\_B0015\_R0040 (1) 62.2ng/ul; (2) 123.8ng/ul; (3) 106.7ng/ul; (4)98.9ng/ul; (5) 92.3ng/ul; (6)109.7ng/ul; (7)97.7ng/ul; (8) 110.9ng/ul

K081005_C0040_B0015_R0040	8.5ul
Pci1	
0.25ul	
Xba1	
0.25ul	
cutsmart	
1ul	
H2O	
0ul	

Gel Identification



Lane 1, Lane 10 : 1kb DNA Ladder

Lane 2-Lane 9 Products of enzyme digestion

Lane 2-Lane5, Lane 7-Lane 9 show bands with the length of 1kb, indicating **positive results**.

Lane 6 shows negative results.

#### 1.21.6 Sequence with primer WL14042

#### 1.22

Construct promoter\_K081005-TetR\_C0040-terminator\_B0015-promoter\_R0040-pTAL-terminator\_B0015

##### 1.22.1 Enzyme digestion

of promoter\_K081005-TetR\_C0040-terminator\_B0015-promoter\_R0040 and pTAL\_B0015

Enzyme Digestion of pTAL-terminator\_B0015

pTAL-B0015

17ul

Xba1

0.5ul

EcoR1

0.5ul

cutsmart

2ul

H2O

0ul

##### Enzyme Digestion

of promoter\_K081005-TetR\_C0040-terminator\_B0015-promoter\_R0040

K-C-B-R

17ul

Spe1

0.5ul

EcoR1

0.5ul
cutsmart
2ul
H2O
0ul

### 1.22.1 Gel Identification and DNA Recovery

## 2. pTAL Assembly

2.10 Pick up several colonies for further culture

2.11 DNA Purification and sequence promoter\_K081005-pTAL-terminator\_B0015 with primer WL14042

The sequence alignment shows **positive results**.

## 3. RBS Adding

### 3.1 PCR of TetR+pTet

primer F: WL14014

primer R: GCTACTAGTTTTCTCCTCTTTAATTCAGTATCTCTATCACTGAT (WL14061)

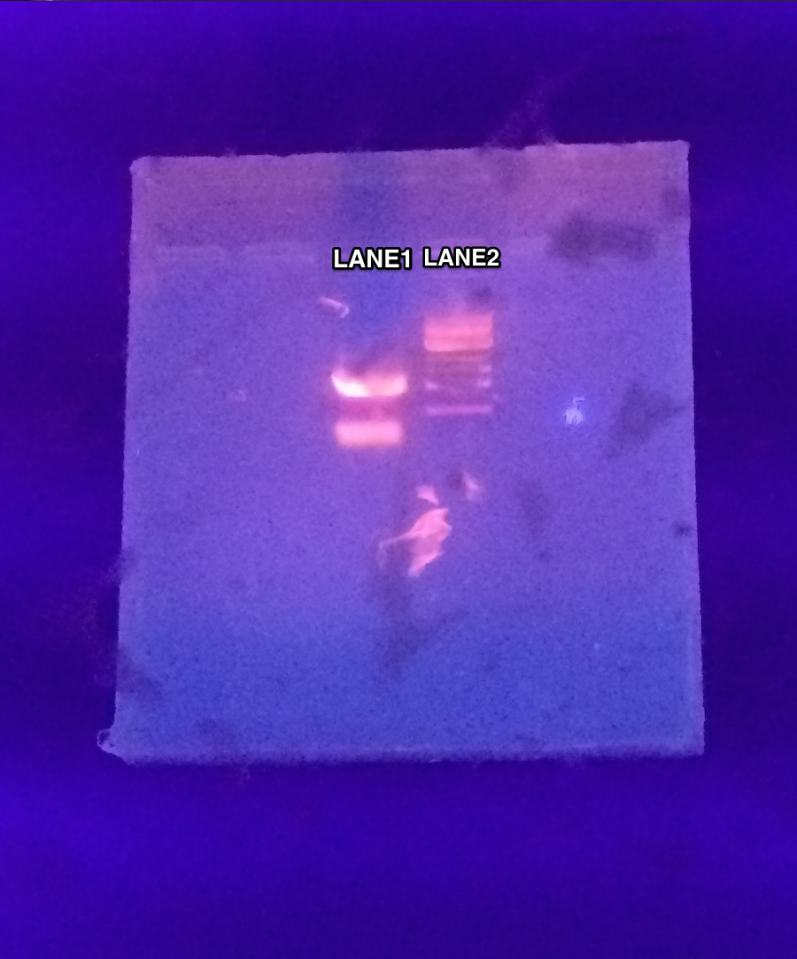
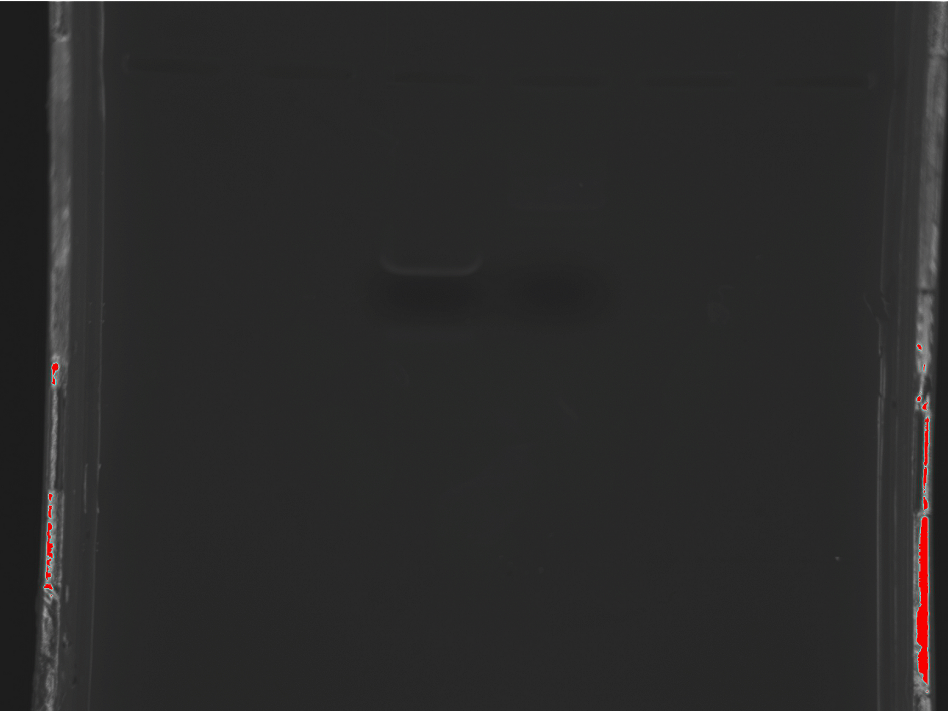
Reaction system

5X HF Phusion Buffer	10ul
10mM dNTP	1ul
Primer F	2.5ul
Primer R	2.5ul
Backbone (TetR+pTet)10ng/ul	0.5ul
Phusion Polymerase	0.5ul
H2O	33ul

### PCR Protocol

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
30Cycles	94°C	30 seconds
	55°C	30 seconds
	72°C	40 seconds per kb
Final Extension	72°C	10 minutes
Hold		4°C

Gel Identification



Lane1: PCR product of TetR+Ptet (the band with ~1kb length may be the right one)

Lane2: 1kb DNA Ladder

Gel recovery concentration: 114ng/ul

### 3.2 Enzyme Digestion:

Enzyme Digestion of pTAL-terminator\_B0015 (200ng/ul)

pTAL-B0015	
17ul	
Xba1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	
2ul	
H2O	
0ul	

Enzyme Digestion of PCR products (114ng/ul)

K-C-B-R	
17ul	
Spe1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	
2ul	
H2O	
0ul	

DNA clean

concentration

pTAL-terminator\_B0015 51.6ng/ul

PCR products 72.5 ng/ul

### 3.3 Ligation and Transformation

Ligation 1

pTAL-terminator_B0015	0.5ul
PCR products	0.3ul
Quick ligase Buffer Quick	5ul

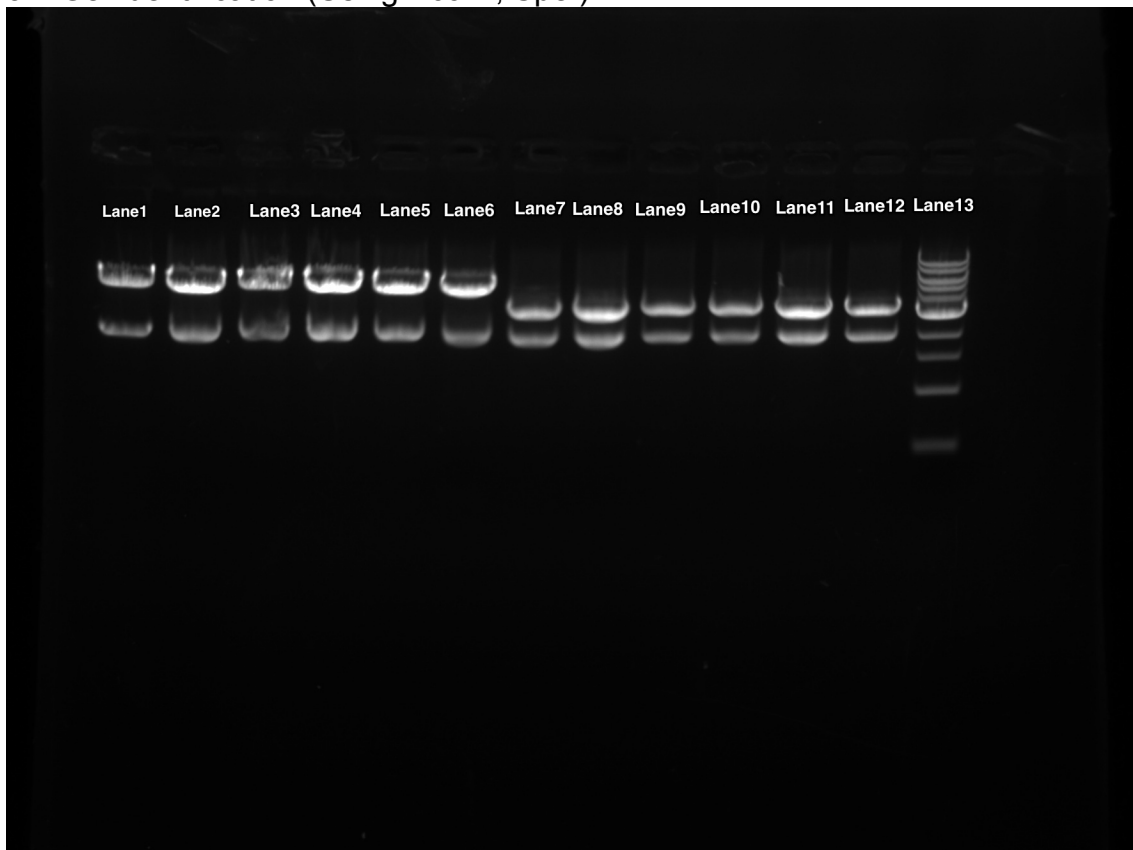
ligase	1ul
H2O	
3.2ul	

#### Ligation 2

pTAL-terminator_B0015	0.5ul
PCR products	0.25ul
Quick ligase Buffer	5ul
Quick ligase	1ul
H2O	
3.25ul	

#### Transformation

#### 3.4 Gel Identification (Using EcoRI, SpeI)



Lane1-lane6: Enzyme Digestion of constitutive pTAL and reporter system integration; two bands with length of 5kb and 2kb respectively indicate positive results;



Lane7-Lane8: Enzyme Digestion of inducible pTAL\_RBS; two bands with length of 3kb and 2kb respectively indicate positive results;  
Lane13: 1kb DNA Ladder

Sequence: indicate **positive results**.

### 3.5 Change Amp backbone

#### 3.5.1 Enzyme digestion of PSB1A3and inducible pTAL\_RBS

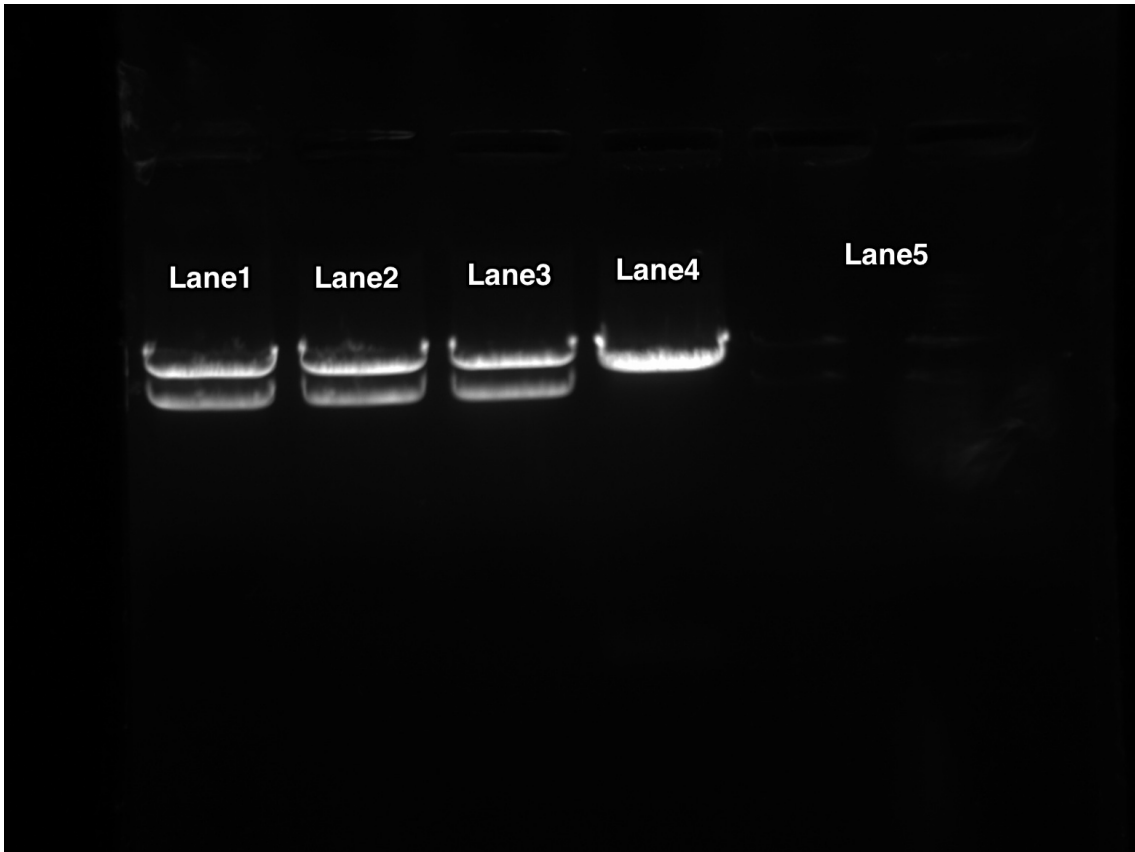
##### Enzyme digestion of PSB1A3(J23100)

pSB1A3
17ul
Spe1
0.5ul
EcoR1
0.5ul
cutsmart
2ul
H2O
0ul

##### Enzyme digestion of pTAL\_RBS

pSB1A3
17ul
Spe1
0.5ul
EcoR1
0.5ul
cutsmart
2ul
H2O
0ul

#### 3.5.1 Gel Separation

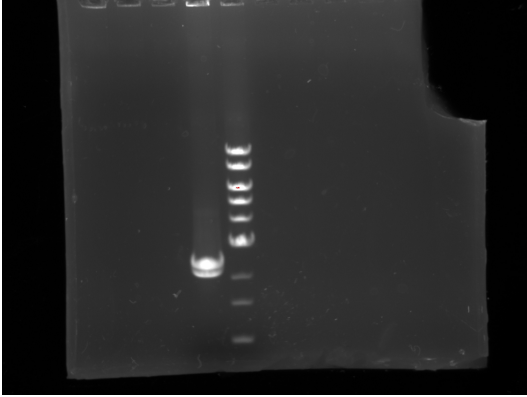


Lane 1-Lane3: Enzyme digestion of pTAL\_RBS  
 Lane 4: Enzyme digestion of PSB1A3(J23100)  
 Lane 5: 1kb DNA Ladder

4. Intergration of Constitutive pTAL and Reporter system  
 4.1 Enzyme Identification of Constitutive pTAL (300ng/ul)

Constitutive pTAL	5ul
Spe1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	
1ul	
H2O	
3ul	

Gel Identification



Two bands with length of 2.0kb and 2.5kb indicates **positive results**.

#### 4.2 Enzyme Digestion of Constitutive pTAL and Reporter system

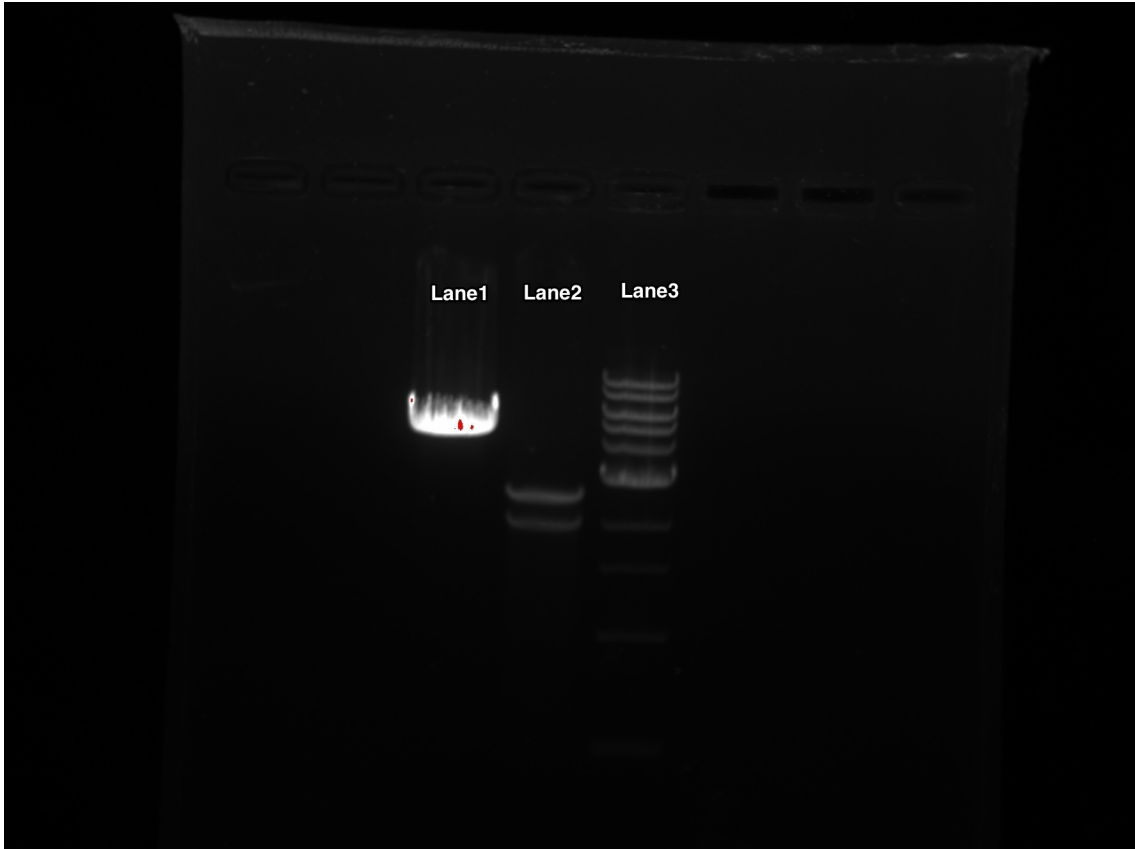
Enzyme Digestion of K081005\_pTAL-terminator\_B0015-**Amp**(300ng/ul)

K081005pTAL-B0015	10ul
Xba1	
0.5ul	
EcoR1	0
.5ul	
cutsmart	
2ul	
H2O	
7ul	

Enzyme Digestion of reporter system (138ng/ul)

pTAL-B0015	
10ul	
Spe1	
0.5ul	
EcoR1	
0.5ul	
cutsmart	
2ul	
H2O	
7ul	

Gel Identification and recovery



Lane1 : Enzyme Digestion of K081005\_pTAL-terminator\_B0015-**Amp**  
 Lane2: Enzyme Digestion of reporter system ( 2 bands, 2kb and 2.5kb)  
 Lane3: DNA 1kb Ladder

#### 4.2 Ligation and Transformation

##### Ligation A

constitutive pTAL	0.2ul
reporter system	2ul
Quick ligase Buffer	5ul
Quick ligase	1ul
H2O	1.8ul

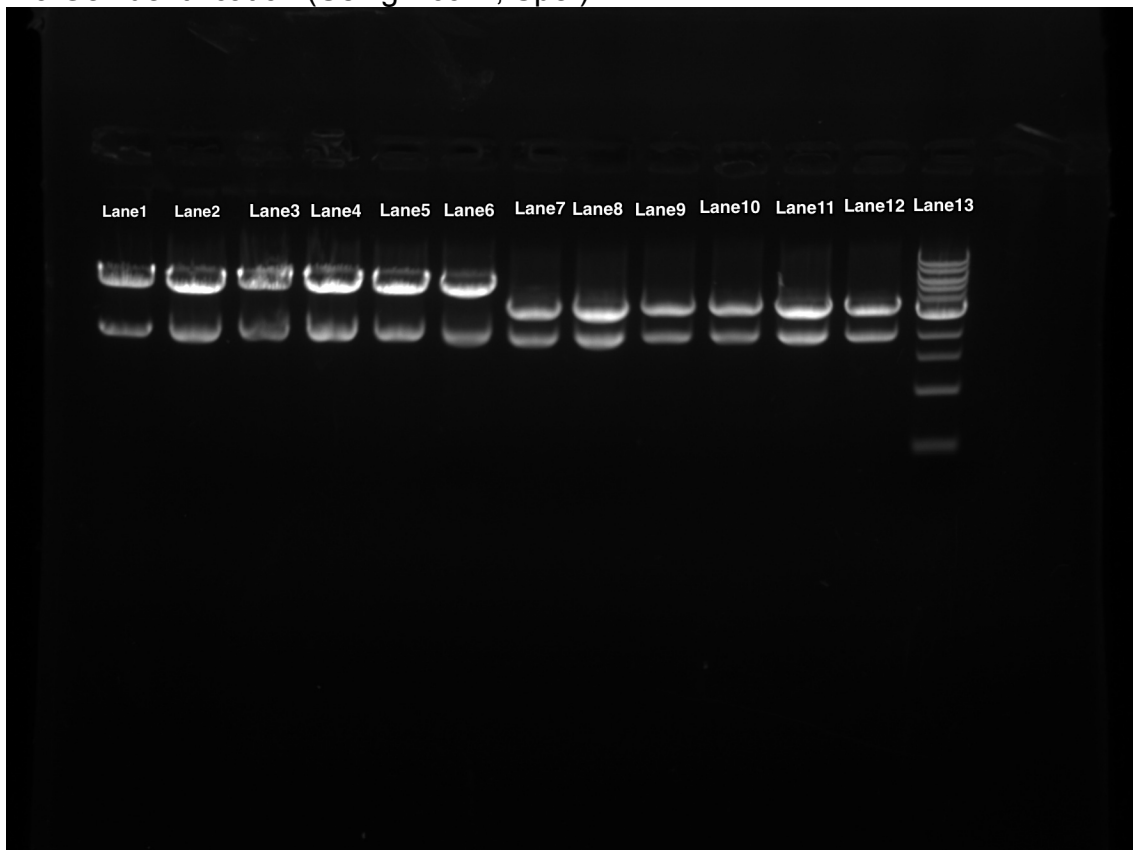
##### Ligation B

constitutive pTAL	0.3ul
-------------------	-------

reporter system		3ul
Quick ligase Buffer	5ul	
Quick ligase		1ul
H2O		
0.7ul		

Transformation.

#### 4.3 Gel Identification (Using EcoRI, SpeI)



Lane1-lane6: Enzyme Digestion of constitutive pTAL and reporter system integration; two bands with length of 5kb and 2kb respectively indicate positive results;

Lane7-Lane8: Enzyme Digestion of inducible pTAL\_RBS; two bands with length of 3kb and 2kb respectively indicate positive results;

Lane13: 1kb DNA Ladder

Sequence: indicate **positive results**.

5. TALE Assembly (constitutive)

5.1 Transformation

5.2 cPCR Identification

Primer: WL13075 WL12016

Gel

