# Molecular cloning of T7 promoter +9 RBS +CI +Terminator (1-23L)

By Zhang Guosheng & Zhang Haoqian

**06.21**

Miniprep 2 CI samples,

& 9 RBS samples: 1-2I, 1-2G, 1.2M, 1-5J, 1-5N, 1-1J, 1-1H, 1-2K, 1-11N,

& 2 terminator samples: 1-23L BBa. B0015. Plasmid: PSB1AK3. Resistance: Amp

1.4H BBa B1006 Plasmid: PSB1AK3. resistance: A & K.

Save the sample spawns of 1-23L, 1-4H in -20° C

**06.22**

Double digestion for CI insert

Digestion system:

| | |
|---|---|
| 30μL | CI plasmid |
| 5μL | 10×H beffer |
| 1μL | EcoRI |
| 1μL | SpeI |
| 13μL | $ddH_2O$ |
| --- | --- |
| 50μL | Total |

Electrophoresis:

50uL system + 10uL buffer

5uL marker + 1uL buffer

Sample loading order: DL2000 marker, CI1, CI2

Extraction the nucleic acids from gel.

**06.23**

Double digestion for Terminator vector

Digestion system:

| | |
|---|---|
| 30μL | Terminator plasmid |
| 5μL | 10×M beffer |
| 1μL | EcoRI |
| 1μL | XbaI |
| 13μL | $ddH_2O$ |
| --- | --- |
| 50μL | Total |

After 90min digestion, add: 1~2μL CIAP+5μL CIAP buffer

Ligate the CI insert and Terminator vector.

Transforming the CI insert + Terminator vector ligation product.

**06.24**

Transfer the bacteria colonies with tips into tubes which filled with 5mL LB.
Each plate's got 3 colonies
CI + 1-23L terminator ligation product
CI + 1-4H terminator ligation product
6 colonies in total.

## 06.25

Miniprep 9 samples: T7P, 1-12M, 1-12O,
CI+1-4H (inert : vector = 7 : 1)1, 3, 5
CI+1-23L (inert : vector = 7 : 1)1, 2, 8
Save the spawns of these 9 samples.

## 06.26

Miniprep 12 samples:
CI
Terminators: 1-23L, 1-4H
RBS: 1-2I, 1-2G, 1.2M, 1-5J, 1-5N, 1-1J, 1-1H, 1-2K, 1-11N
The 12 plasmid samples stored in -20° C

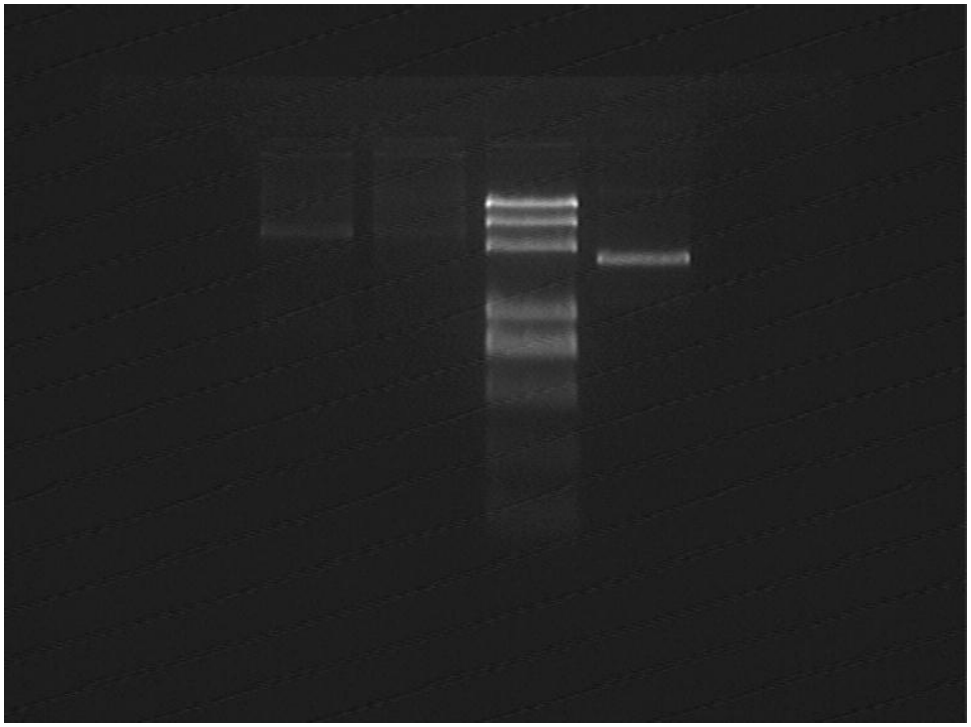
Enzymatic digestion
Samples: CI, 1-23L, 1-4H
System:
For CI insert:

| | |
|---|---|
| 5μL | plasmid |
| 5μL | 10×H buffer |
| 1μL | EcoRI |
| 1μL | SpeI |
| 38μL | $ddH_2O$ |
| --- | --- |
| 20μL | Total |

For terminator vectors 1-23L & 1-4H:

| | |
|---|---|
| 5μL | plasmid |
| 5μL | 10×H buffer |
| 1μL | EcoRI |
| 1μL | XbaI |
| 38μL | $ddH_2O$ |
| --- | --- |
| 20μL | Total |

Add CIAP into 1-4H & 1-23L vector digestion product.
Electrophoresis the digested product.
Samples loading order: CI1, CI2, M, CI plasmid (Control)



Result reveals that the double-digestion failed. The double-digested fragments can't be identified out in electrophoresis causing the concentration of plasmids in this enzymatic digestion system is too low.

Put 3 samples (CI, I-4H, 1-23L) into the shaker

**06.27**

Miniprep CI plasmid

Some improvements:

1. the EB be put in 65° C water bathing before elution;
2. 50 uL EB to elute;
3. after PW liquid, centrifuge 10 min before the next step;
4. the rpm increased to 13000 per min

A260/A280 = 1.92 A260 = 0.120 CI = 300ng.uL

Previous double digestion protocol seemed to be lack of efficiency, so we tried new protocol today.

New double digestion system:

For front insert:

| | |
|---|---|
| 10μL | plasmid |
| 2μL | 10×H beffer |
| 1.5μL | EcoRI |
| 1.5μL | SpeI |
| 5μL | dd$H_2O$ |
| --- | --- |
| 20μL | Total |

For front vector:

| | |
|---|---|
| 10μL | plasmid |
| 2μL | 10×M beffer |
| 1.5μL | EcoRI |
| 1.5μL | XbaI |
| 5μL | $ddH_2O$ |
| --- | --- |
| 50μL | Total |

Electrophoresis to test

Samples: 10μL digestion system+2μL DNA Dye

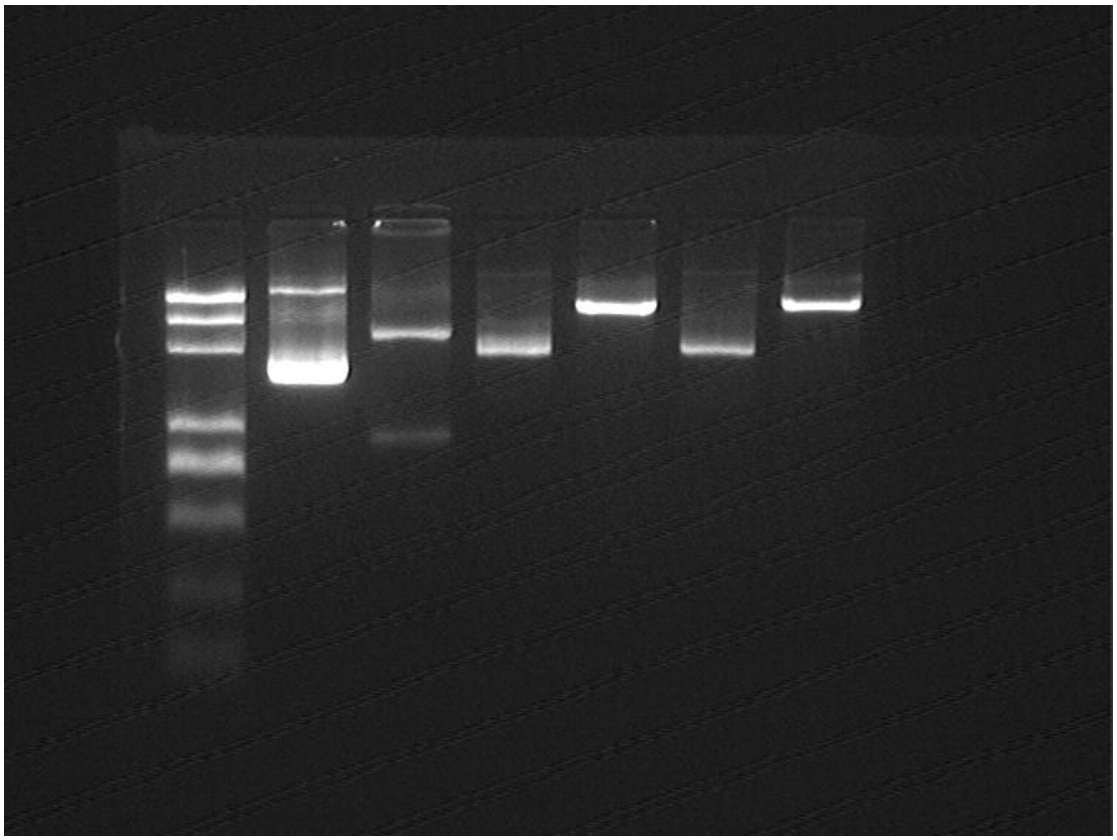
Control: 5μL plasmid+2μL DNA Dye

Marker: 10μL DL2000 plus+2μL DNA Dye

React for 10 minutes

Result (from left to right):

Marker, CI control, CI, 1-23L control, 1-23L, 1-4H control, 1-4H



Gel extraction

Extract CI insert, 1-4H vector and 1-23L vector

## 06.28

Causing the CI plasmids are not enough, we do more miniprep of CI

Miniprep 3 CI samples:

Sample 1: A260 = 0.146 Conc = 350ng/uL

Sample 2: A260 = 0.110 Conc = 275ng/uL

Sample 3: A260 = 0.111 Conc = 280ng/uL
Store them in -20 ° C

**06.29**

1. Double digestion for Terminator vector again causing the digestion product's been exhausted.
2. Transformation

(1) Got 2× 100μL DH5α competent cells from the -70℃ refrigerator and divided them equally into four EP tubes, each for 50μL.

(2) Wait for the solution to melt.

(3) Add 10μL ligation system: 1-23L 7:1, 1-23L 3:1, 1-4H 7:1, 1-4H 3:1.
Notice: 7:1, 3:1 are the ratio of insert (CI) to vector (Terminator).

(4) Keep in ice for 30min.

(5) Heat shock in 42℃ for 90s.

(6) Put the competent cells back into the ice box immediately for 2min.

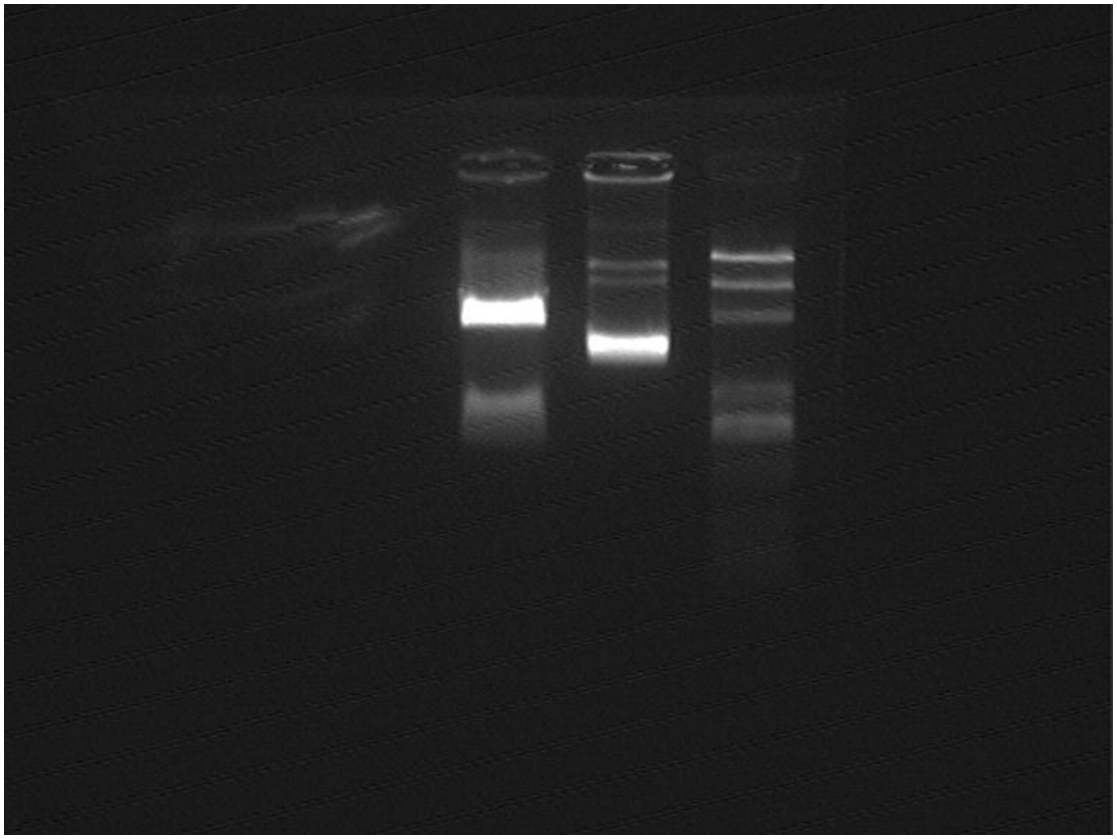
(7) Add 200uL antibiotic LB in a super clean bench and recover for 45min.

**06.30**

Double digestion of CI
System for front insert:

| | |
|---|---|
| 10μL | plasmid |
| 2μL | 10×H beffer |
| 1.5μL | EcoRI |
| 1.5μL | SpeI |
| 5μL | $ddH_2O$ |
| --- | --- |
| 20μL | Total |

4 h digested
Electrophoresis
Result (from left to right)
CI, CI plasmid control, marker



Gel: 0.072g→PN volume: 216μL
Gel extraction for CI insert

Ligation Insert:CI Vector:1-23L, 1-4H
System
(1)7:1 system

| | |
|---|---|
| 7μL | CI insert |
| 1μL | vector |
| 1μL | 10× Ligase buffer |
| 1μL | Ligase |
| --- | --- |
| 10μL | Total |

(2)3:1 system

| | |
|---|---|
| 3μL | CI insert |
| 1μL | vector |
| 1μL | 10× Ligase buffer |
| 1μL | Ligase |
| 4μL | dd$H_2O$ |
| --- | --- |
| 10μL | Total |

Control

| | |
|---|---|
| 0 | CI insert |

1μL vector
1μL 10× Ligase buffer
1μL Ligase
7μL $ddH_2O$
---
10μL Total
6 samples in total, 2 for 1-23L, 2 for 1-4H, 2 for 1-23L & 1-4H controls

**07.01**

Transformation

(1) Got 3×100μL DH5α competent cells from the -70℃ refrigerator and divided them equally into six EP tubes, each for 50μL.

(2) Wait for the solution to melt.

(3) Add 10μL ligation system: 1-23L 7:1, 1-23L 3:1, 1-4H 7:1, 1-4H 3:1, 1-23L Control and 1-4H Control.

(4) Keep in ice for 30min.

(5) Heat shock in 42℃ for 90s.

(6) Put the competent cells back into the ice box immediately for 2min.

(7) Add 200μL antibiotic LB in a super clean bench and recover for 45min.

(8) Centrifuge ,discard 100μL supernatant and smear the remaining 100μL onto the $Amp^+$ plate.

(9) Culture under 37℃.

**07.02**

Miniprep 10 samples

CI + 1-23L samples: 1-5

CI + 1-4H samples: 1-5

Measure the concentration of these 10 samples.

CI + 1-23L sample 1 400ng/uL
sample 2 385ng/uL
sample 3 900ng/uL
sample 4 875ng/uL
sample 5 515ng/uL

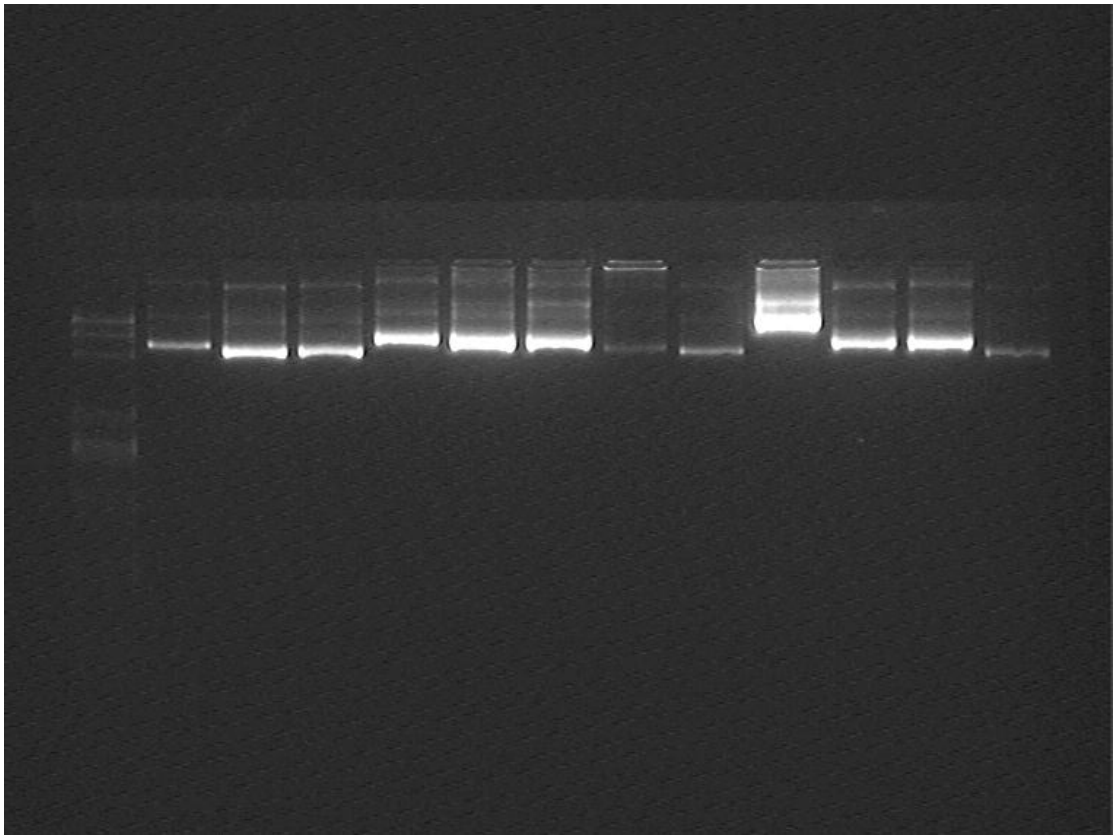
CI + 1-4H sample 1 250ng/uL
sample 2 1000ng/uL
sample 3 250ng/uL
sample 4 370ng/uL
sample 5 215ng/uL

Electrophoresis: Agarose 2%

Identify the ligation of CI+1-23L/1-4H by electrophoresis

Result (from left to right)

Marker, 1-23L vector control, CI+1-23L①~⑤ plasmids, 1-4H vector control, CI+1-4H①~⑤ plasmids



Causing the CI+1-4H samples are not very good, we do electrophoresis again: Agarose 2%.

Put the ligated 1-4H + CI plasmids into 1 EP tubes. Conc = 395ng/uL.

Miniprep for 13 samples RBS plasmid:
Nine 2009 iGEM parts samples 1-2I, 1-2G, 1-2M, 1-5J, 1-5N, 1-1J, 1-1H, 1-2K, 1-11N
Four 2008 iGEM parts samples 1004-1C, 1004-2G, 1004-3C, 1004-4C
The resistances are all $Amp^+$

Double digestion
12 RBS samples: 9 2009 iGEM parts samples 1-2I, 1-2G, 1-2M, 1-5J, 1-5N, 1-1J, 1-1H, 1-2K, 1-11N
3 2008 iGEM parts samples 1004-2G, 1004-3C, 1004-4C
System for back vector:

| | |
|---|---|
| 10μL | plasmid |
| 2μL | 10×H buffer |
| 1.5μL | PstI |
| 1.5μL | SpeI |
| 5μL | $ddH_2O$ |
| --- | --- |
| 20μL | Total |

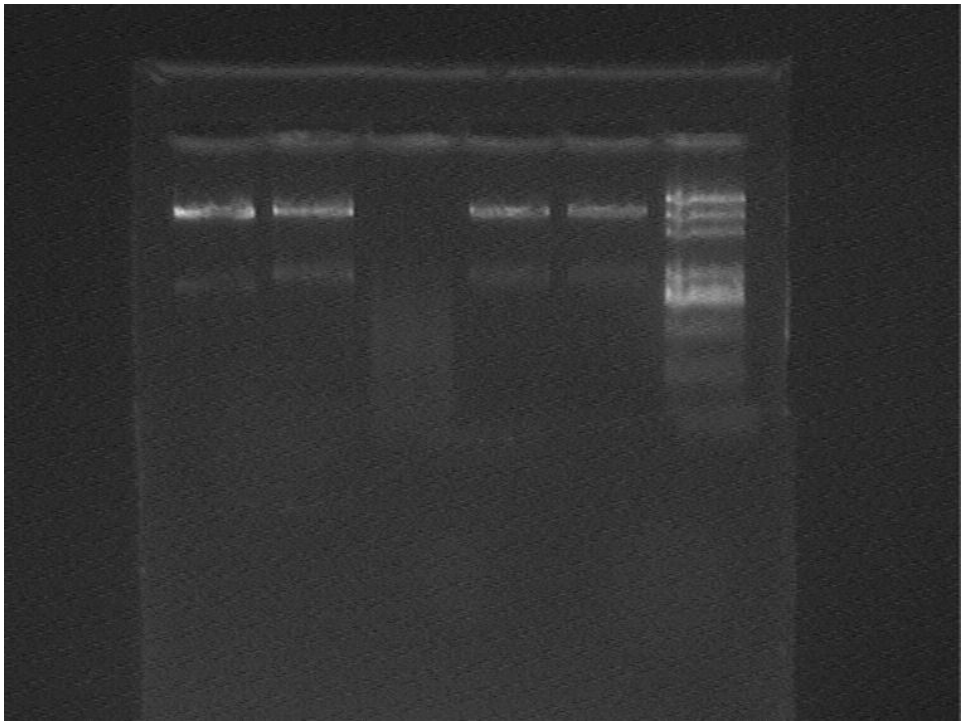
2 CI-1-23L samples & 2 CI-1-4H samples
Systems for back insert:

| | |
|---|---|
| 10μL | plasmid |
| 2μL | 10×M buffer |
| 1.5μL | XbaI |
| 1.5μL | PstI |
| 5μL | $ddH_2O$ |
| --- | --- |
| 20μL | Total |

Water bathing for 4 hours.

Electrophoresis the digestion product.

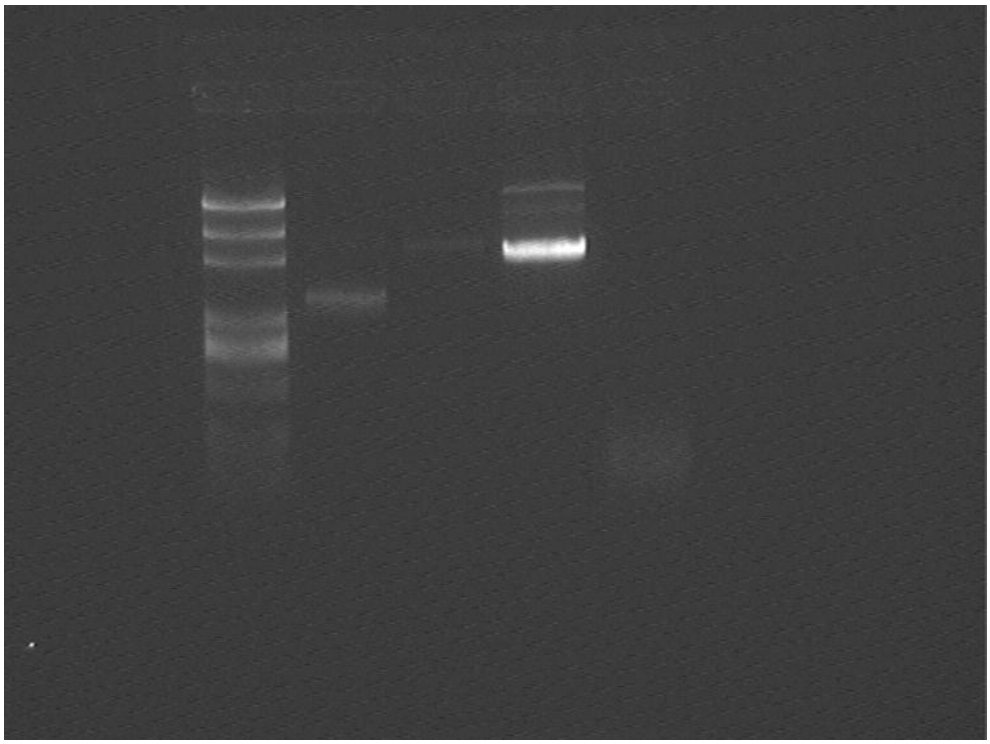
Sample loading order: CI + 1-4H sample 2, 3, 4; CI + 1-23L sample 1, 2; Marker.



The digestion of CI + 1-4H 4 failed.

Electrophoresis

Sample loading order: Marker, 1-11N control (plasmid), 1-11N digested, 1-4H4 control (plasmid), CI + 1-4H4



CI + 1-4H sample failed to be digested again.

**07.03**

Add CIAP into 12 RBS digesting vector samples.

Identify the digested ligation product of CI+1-23L/1-4H and RBS vector by electrophoresis

System:

| | |
|---|---|
| 1μL | sample |
| 1μL | DNA Dye |
| 4μL | $ddH_2O$ |

Marker:

| | |
|---|---|
| 5μL | Marker |
| 1μL | DNA Dye |

Digestions for RBS vectors failed and we digested again.

Samples: 1-11N, 1-1H, 1004-4C, 1-2M, 1-2I, 1-5N, 1-2G, 1-2K, 1-5J, 1004-3C, 1004-2G, 1-1J

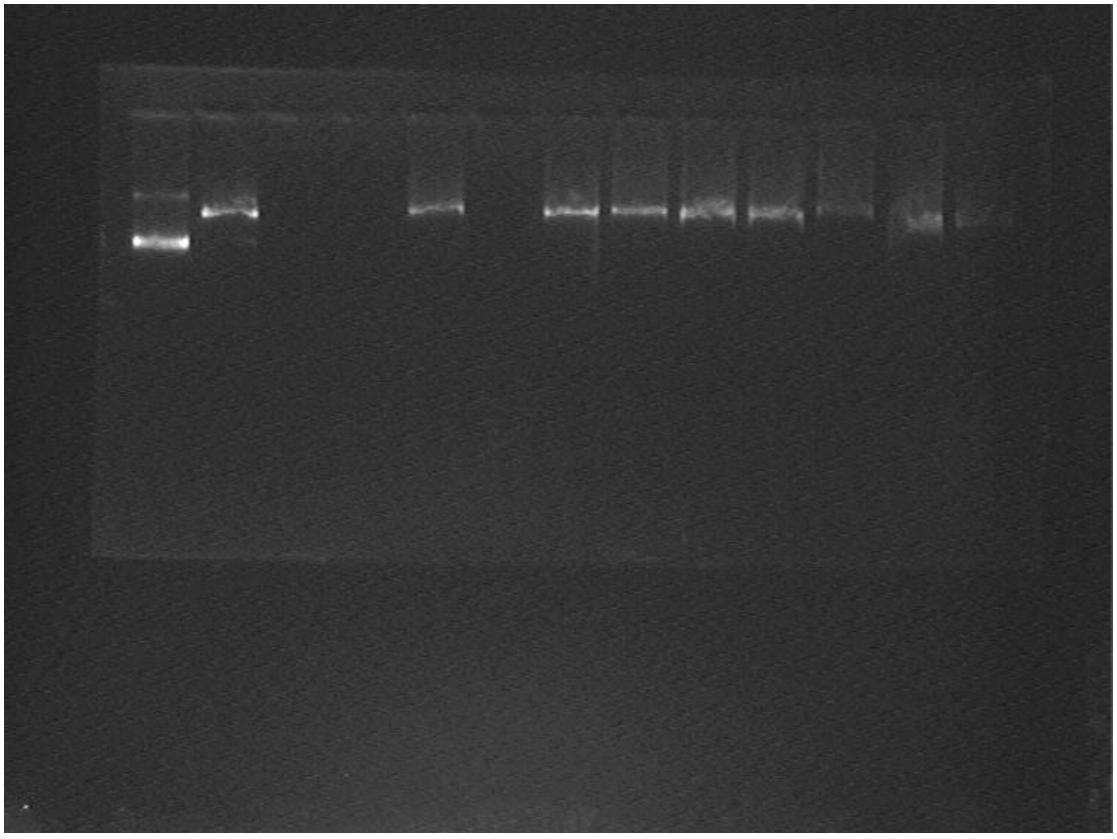
System

| | |
|---|---|
| 6μL | RBS plasmid |
| 1.5μL | SpeI |
| 1.5μL | PstI |
| 2μL | 10×H buffer |
| 9μL | $ddH_2O$ |
| --- | --- |
| 10μL | Total |

Gel Extraction for CI+1-23L/1-4H insert

Gel: CI+1-23L 0.139g→PN volume: 417μL
CI+1-4H 0.071g→PN volume:213 μL

Identify the RBS digestion products by electrophoresis
Results (from left to right)
Plasmid control, 1-2I, 1004-2G, 1004-4C, 1-5N, 1004-3C, 1-2K, 1-1H, 1-1J, 1-2G, 1-11N, 1-2M, 1-5J



There are no strips for thee 2008 iGEM parts 1004-2G, 1004-4C, 1004-3C. Perhaps the quality of the 2008 parts is bad.

DNA purification to extract RBS vector

Ligate the RBS vector and CI+Terminator insert
System

| | |
|---|---|
| 3μL | insert |
| 1μL | vector |
| 1μL | 10× Ligase buffer |
| 1μL | Ligase |
| 4μL | dd$H_2O$ |
| --- | --- |
| 10μL | Total |

**07.04**

Transforming the bacteria with the ligation product of CI + 1-4H and CI + 1-23L.

**07.05**

Transfer the transformed bacteria into the LB and incubate in shaker.

30 samples in total, all for RBS + CI + 1-23L samples, 5 samples for each RBS (1-11N. 1-2I, 1-5J, 1-1H, 1-5N, 1-2G).

Miniprep for 1-11N + CI + T×5, 1-2I + CI + T×5, 1-5J + CI + T×5, 1-1H + CI + T×5, 1-5N + CI + T×5, 1-5J + CI + T×5, 30 samples in total

Transfer the transformed bacteria into the LB and incubate in shaker.

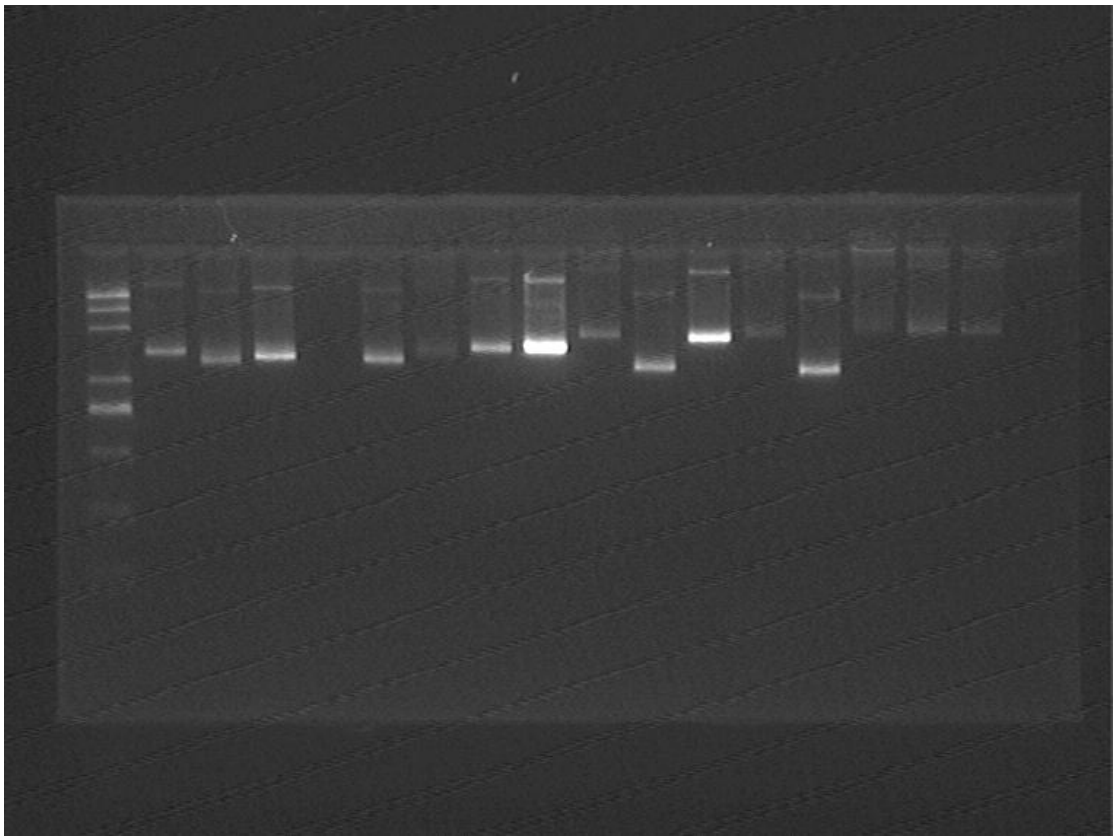
15 samples in total. 1-2M +CI +1-23L, 1-1J + CI + 1-23L, 1-2K + CI + 1-23L, 5 samples for each.

**07.06**

Identify the RBS+CI+T ligation product by electrophoresis

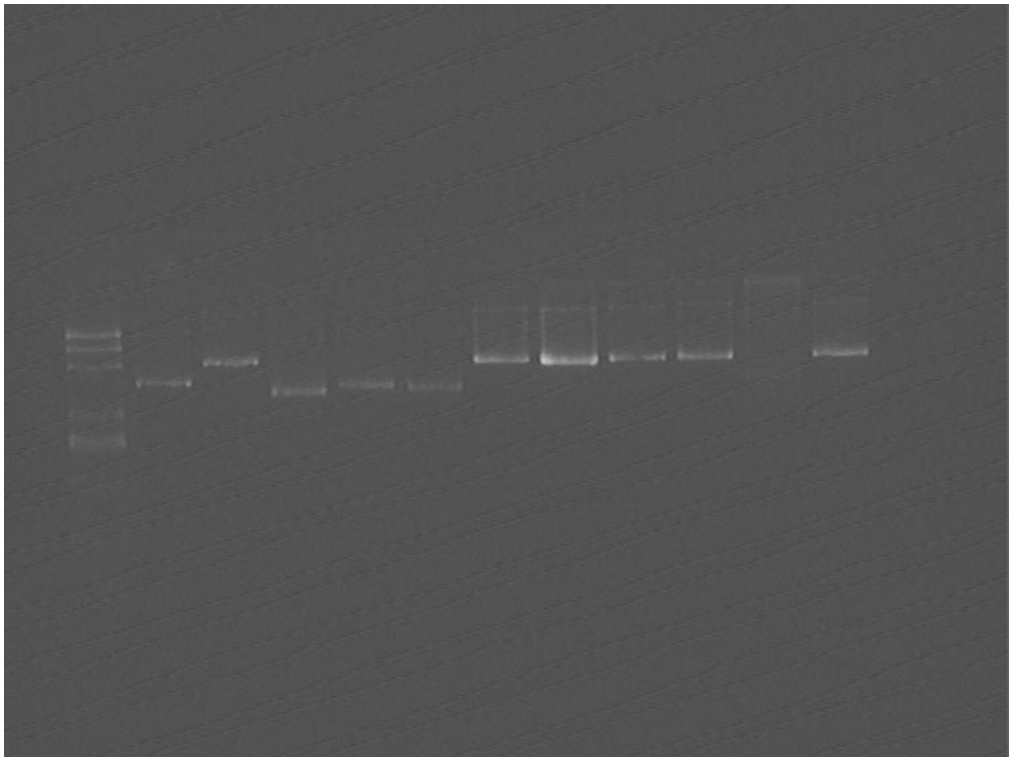
Result (from left to right)

Marker, RBS plasmid control, 1-5J+CI+T①~⑤ (plasmid), 1-2I+CI+T①~⑤ (plasmid), 1-11N+CI+T①~⑤ (plasmid)

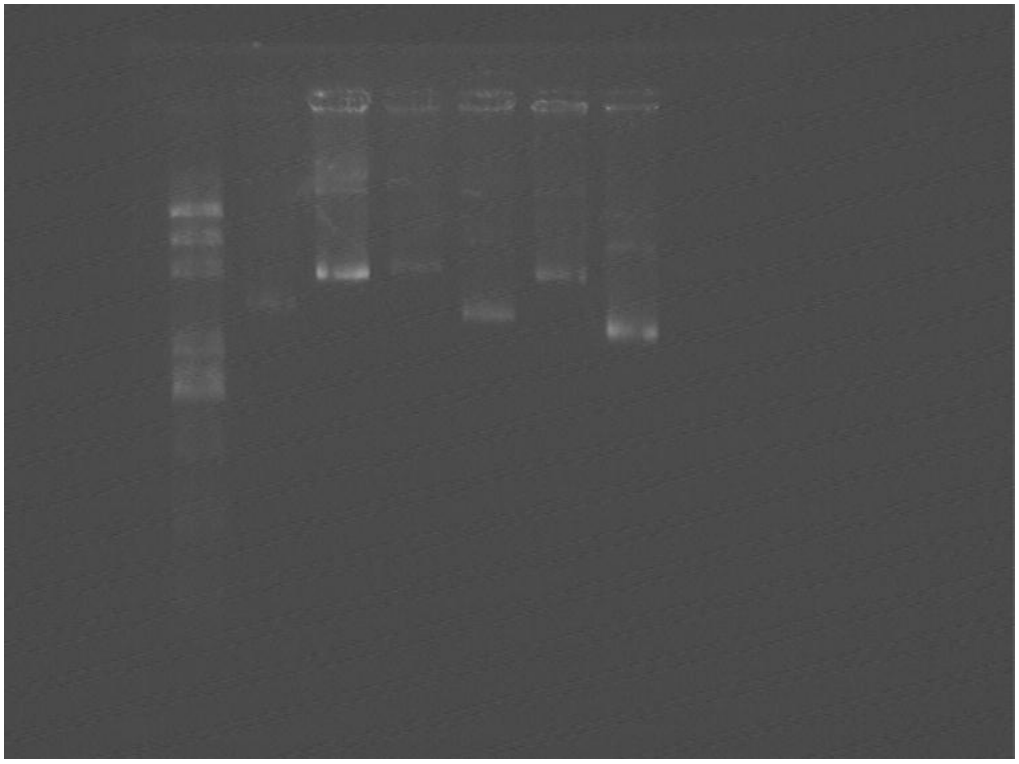


The ligation of 1-5J+CI+T failed

Marker, RBS plasmid control, 1-1H+CI+T①~⑤ (plasmid), 1-2G+CI+T①~⑤ (plasmid)



The 1-1H+CI+T1, 5 & 1-2G+CI+T1, 2, 3, 5 are the target plasmids.
Marker, RBS plasmid control, 1-5N+CI+T①~⑤ (plasmid)



The 1-5N+CI+T2, 4, 5 are the target plasmids.

Choose samples:

4μL 1H+CI+T⑤， 4μL 2G+CI+T⑤， 6μL 5N+CI+T⑤， 6μL 2I+CI+T⑤， 6μL 11N+CI+T

④

Concentration

217.5ng/μL 233ng/μL 125ng/μL 162ng/μL 123ng/μL

Double-digestion of CI+1-23L insert (causing the ligation of 1-5J+CI+T failed).

1.5μL XbaI

1.5μL PstI

2μL 10×M buffer

Plasmid

dd$H_2O$

---

20μL Total

4 hours digesting.

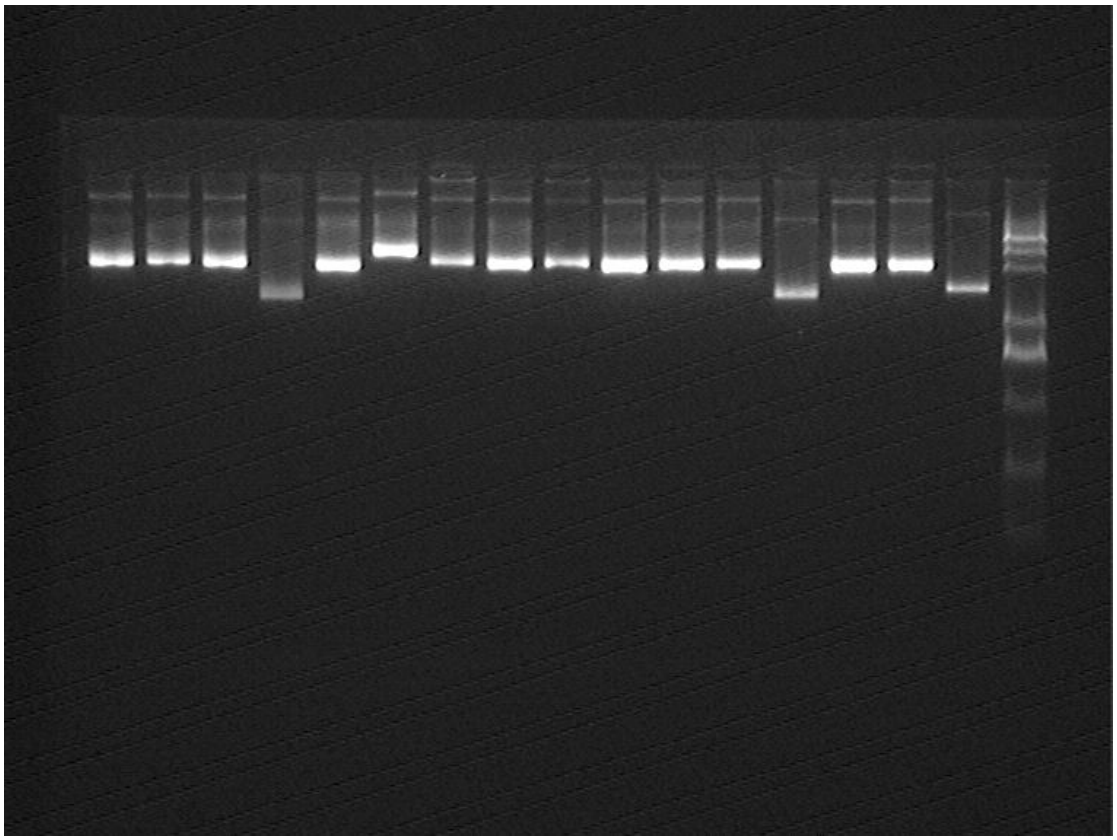
Electrophoresis to purify the CI+1-23L insert, and extraction.

**07.07**

Identify the RBS+CI+T ligation product by electrophoresis

Result (from left to right)

2K+CI+1-23L①~⑤ (plasmid), 2M+CI+1-23L①~⑤ (plasmid), 1J+CI_1-23L①~⑤ (plasmid), RBS plasmid control, Marker



The 1-2K+CI+T1, 2, 3, 5 & 1-2M+CI+T1, 2, 3, 4, 5 & 1-1J+CI+T1, 2, 4, 5 are target plasmids.

Choose samples:

[1-1H⑤,1-2G⑤,1-5N⑤, 1-2I⑤, 1-11N④, 1-2K⑤, 1-2M⑤, 1-1J④]+CI+1-23L, 8 samples in total.

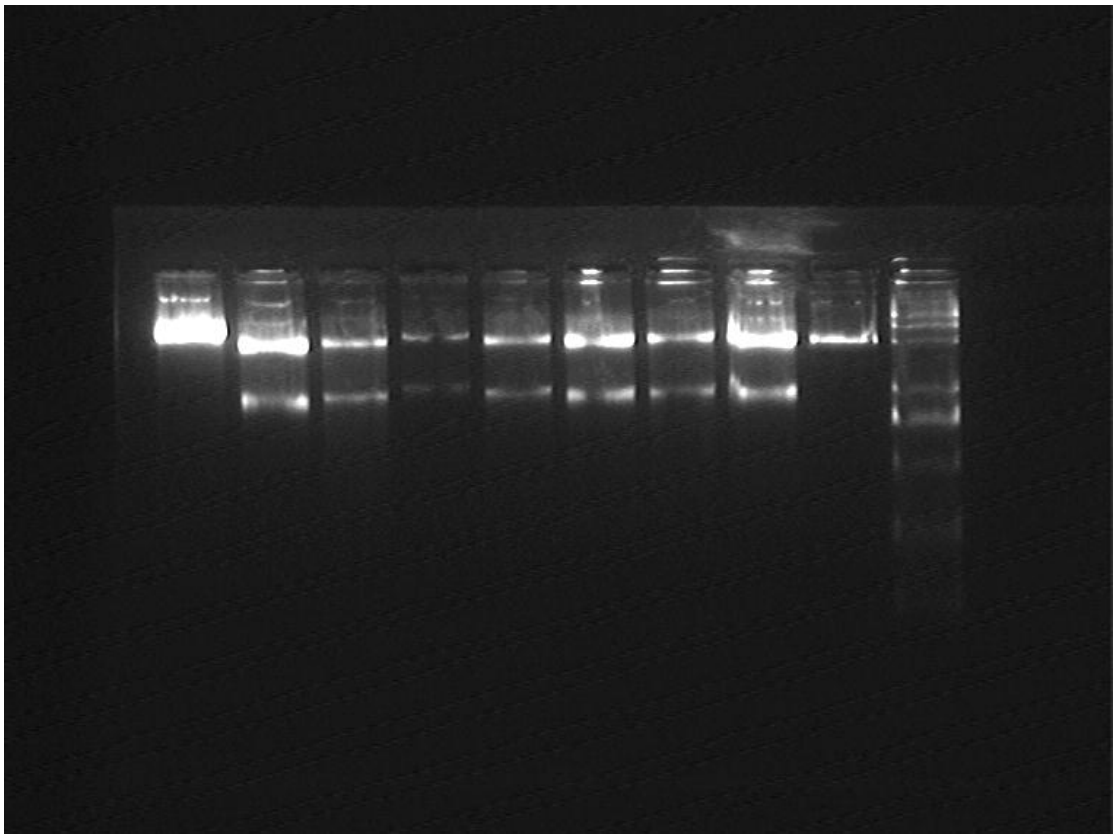
Double-digestion of RBS+CI+1-23L insert

| | |
|---|---|
| 1.5μL | XbaI |
| 1.5μL | PstI |
| 2μL | 10×M buffer |
| 10μL | Plasmid |
| 5μL | $ddH_2O$ |
| --- | --- |
| 20μL | Total |

Electrophoresis

Result (from left to right)

1-2K, 1-2M, 1-1H, 1-11N, 1-2G, 1-2I, 1-5N, 1-1J, Plasmid Control, Marker



1-2K+CI+1-23L insert digesting failed

Gel Extraction for RBS+CI+1-23L

| | | |
|---|---|---|
| Gel: 2M | 0.037g→ | PN volume: 111μL |
| 1H | 0.027g→ | 81μL |
| 11N | 0.018g→ | 54μL |
| 2G | 0.036g→ | 108μL |
| 2I | 0.028g→ | 84μL |
| 5N | 0.039g→ | 117μL |
| 1J | 0.045g→ | 135μL |

Ligate the RBS+CI+1-23L insert and T7 promoter vector

System

| | |
|---|---|
| 3μL | insert |
| 1μL | vector |
| 1μL | 10× Ligase buffer |
| 1μL | Ligase |
| 4μL | $ddH_2O$ |
| 10μL | Total |

## 07.08

Transformation for the ligation product: T7p+2M/1H/11N/2G/2I/5N/1J RBS+CI+1-23L, 7 samples in total.

Double digestion for 1-2K+CI+1-23L insert again (yesterday failed)

| | |
|---|---|
| 1.5μL | XbaI |
| 1.5μL | PstI |
| 2μL | 10×M buffer |
| 10μL | 1-2K③ plasmid |
| 5μL | $ddH_2O$ |
| 20μL | Total |

Electrophoresis and Gel Extraction for 1-2K+CI+1-23L and CI+1-23L insert (for 1-5J vector)

Gel: 2K+CI+1-23L 0.081g→PN volume: 243μL

CI+1-23L for 5J 0.100g→ 300μL

Ligate the 1-2K+CI+1-23L insert and T7 promoter vector

System

| | |
|---|---|
| 3μL | 1-2K+CI+1-23L insert |
| 1μL | T7p vector |
| 1μL | 10× Ligase buffer |
| 1μL | Ligase |
| 4μL | $ddH_2O$ |
| 10μL | Total |

Ligate the CI+1-23L insert and 1-5J RBS vector

System

| | |
|---|---|
| 3μL | CI+1-23L insert |
| 1μL | 1-5J vector |
| 1μL | 10× Ligase buffer |
| 1μL | Ligase |
| 4μL | $ddH_2O$ |
| 10μL | Total |

## 07.09

Transformation of 2 samples: T7P+1-2K+CI+T & 1-5J+CI+1-23L.

PCR identification of T7P + [1-2G, 1-11N,1-1H, 1-5N, 1-2I, 1-2M, 1-1J] + CI + T, 5 samples for each RBS samples, 35 samples in total.

System for PCR identification:

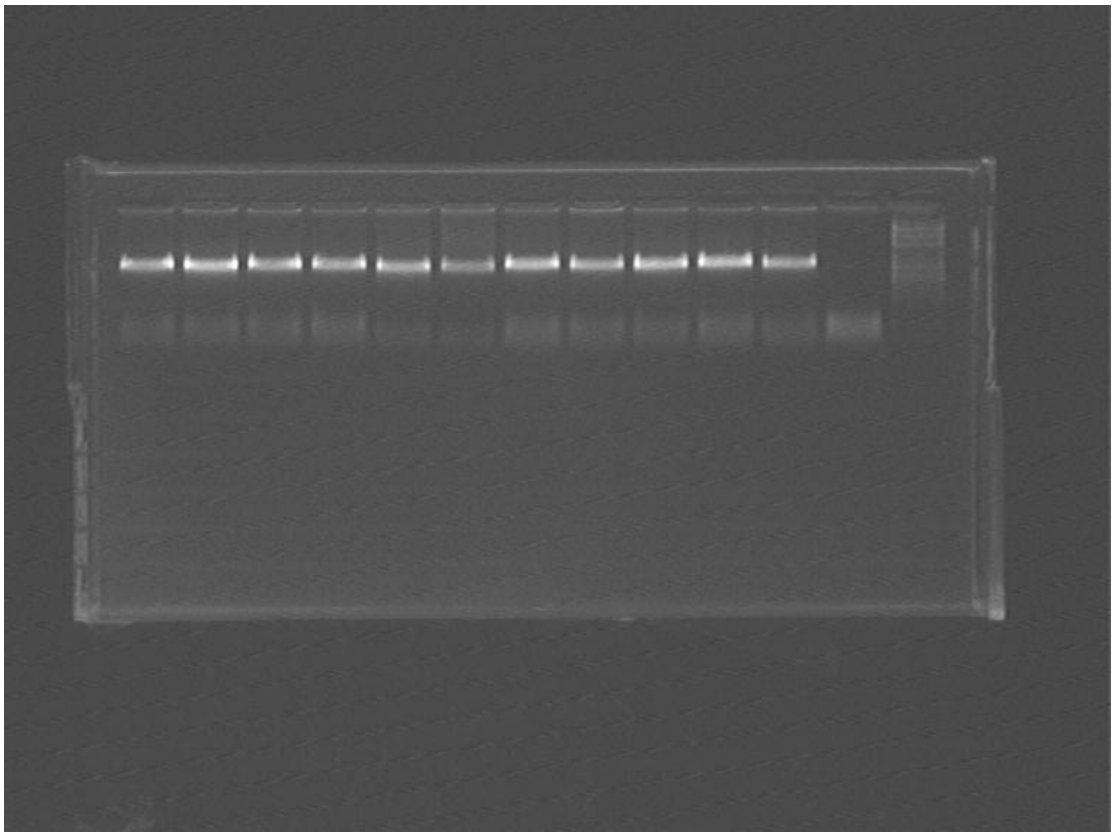
| | |
|---|---|
| 5μL | Master mix |

| | |
|---|---|
| 0.25μL | Primer For |
| 0.25μL | Reverse |
| 4.5μL | $ddH_2O$ |
| 0μL | template |
| --- | --- |
| 10μL | Total |

**07.10**

Electrophoresis 12 samples of PCR identification products.

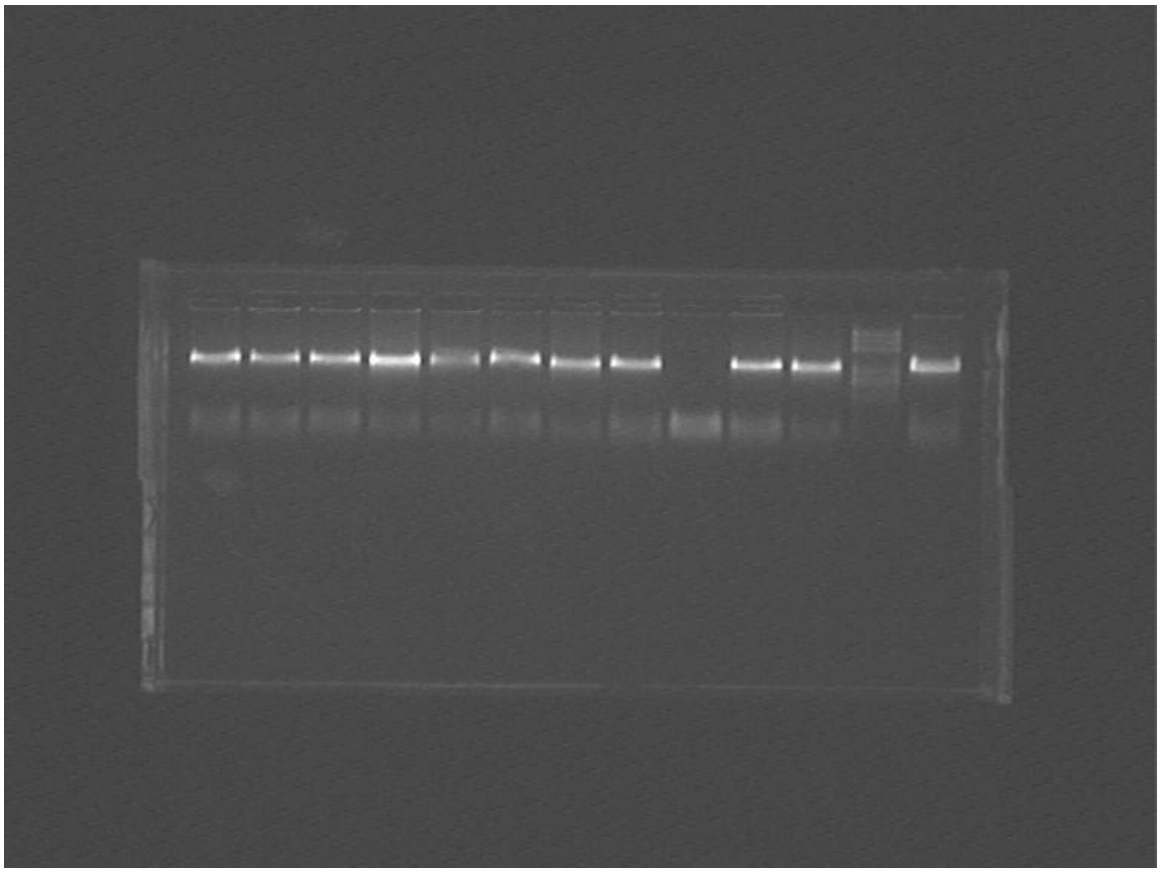
Sample loading order: T7p+5N+CI+T①~⑤, T7P+2G+CI+T①~⑤, T7P+2I+CI+TL①~ ② , Marker



The T7P+5N+CI+T①-⑤ & T7P+2G+CI+T①-⑤ & T7P+2I+CI+T①,are target spawns (PCR identified).

Electrophoresis another 12 samples of PCR identification products.

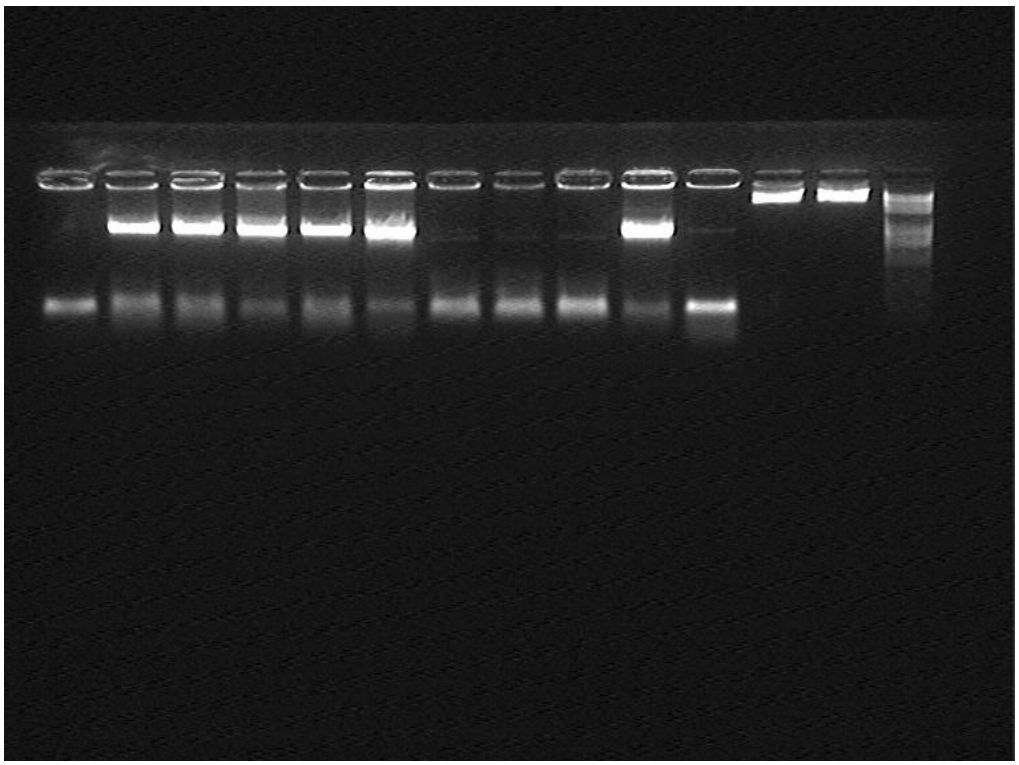
Sample loading order: T7p+2I+CI+T ③ ~⑤, T7P+2M+CI+T①~⑤, T7P+11N+CI+T①~ ④ , Marker



The T7P+2I+CI+T③-⑤ & T7P+2M+CI+T①-⑤ & T7P+11N+CI+T②-④ are target spawns (PCR identified).

Electrophoresis another 12 samples of PCR identification products.

Sample loading order: T7p+11N+CI+T⑤, T7P+1J+CI+T①~⑤, T7P+1H+CI+T①~⑤, LinMIin, Marker



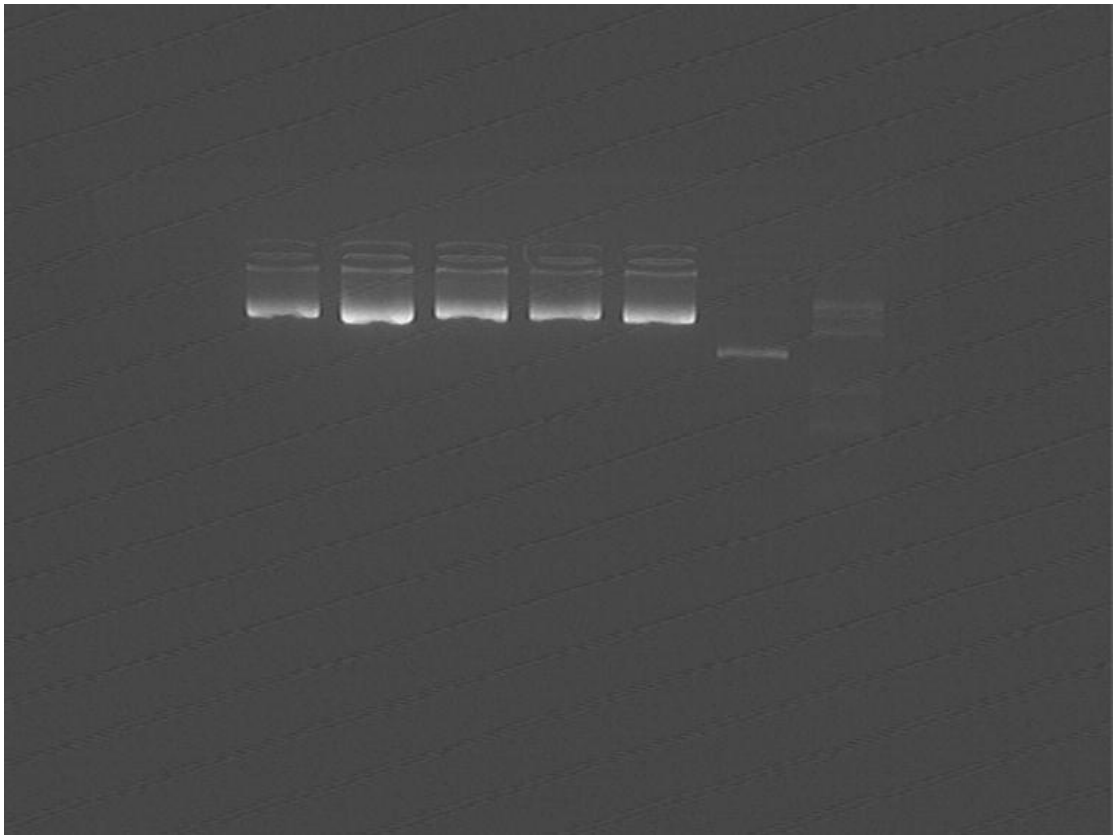
The T7P+1J+CI+T①-⑤ & T7P+1H+CI+T④ are target spawns (PCR identified).

Transfer the colonies into tubes with 5mL LB:
T7P+1J+CI+T①;
T7P+5N+CI+T①;
T7P+2G+CI+T①;
T7P+2I+CI+T①;
T7P+2M+CI+T①;
T7P+11N+CI+T②;
T7P+1H+CI+T④.

**07.11**

Store the strain of 1J①、11N②、2I①、1H④、2M①、5N①、2G① and send to sequence
7 samples in total to be sequenced:
T7P+1J+CI+T①;
T7P+5N+CI+T①;
T7P+2G+CI+T①;
T7P+2I+CI+T①;
T7P+2M+CI+T①;
T7P+11N+CI+T②;
T7P+1H+CI+T④.
The results demonstrates that T7P+1J+CI+T①;
T7P+2I+CI+T①;
T7P+1H+CI+T④;
T7P+2M+CI+T①;
T7P+2G+CI+T①;
ARE THE TARGET PRODUCTS.
While T7P+5N+CI+T①; T7P+11N+CI+T② failed.

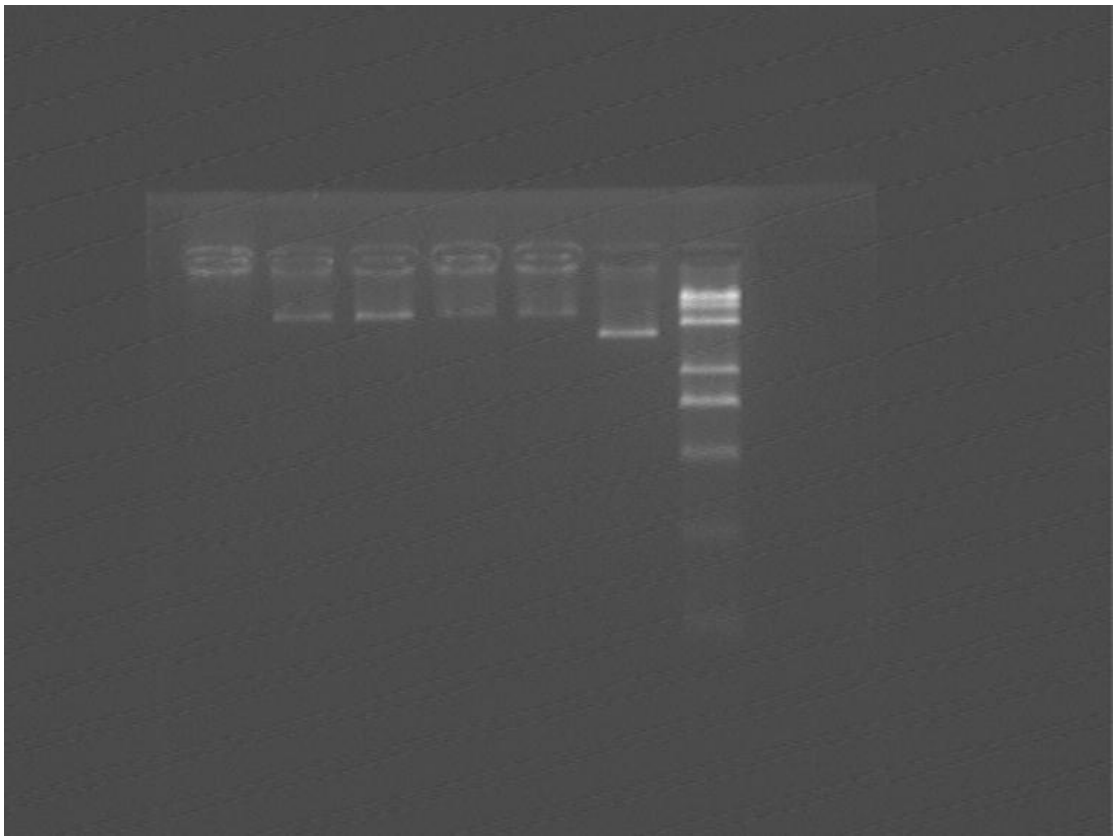
Miniprep for 5J+CI①~⑤ and T7p+2K①~⑤
Electrophoresis to identify
Result 1 (from left to right)
T7p+1-2K+CI+T①-⑤(plasmid), T7p Plasmid Control, Marker



The T7p+1-2K+CI+T①-⑤ are the target plasmids.

Result 2 (from left to right)

1-5J+CI+T①-⑤(plasmids), RBS Plasmid Control, Marker



Double digestion for 1-5J② insert:

| | | |
|---|---|---|
| 1.5μL | XbaI | |
| 1.5μL | PstI | |
| 2μL | 10×M buffer | failed |
| 6μL | 1-5J② plasmid | |
| 9μL | $ddH_2O$ | |
| 20μL | Total | |

**07.12**

Determine the concentration of 1-5J plasmid by spectrophotometer.

1-5J② 7.55ng/μL×50=377.5ng/μL

1-5J③ 2.53ng/μL×50=126.5ng/μL

Double digestion for 1-5J+CI+1-23L insert again

| | |
|---|---|
| 1.5μL | XbaI |
| 1.5μL | PstI |
| 2μL | 10×M buffer |
| 3μL 1-5J② plasmid/ 7μL 1-5J③ plasmid | |
| 12μL $ddH_2O$/ 8μL $ddH_2O$ | |
| 20μL | Total |

**07.14**

Ligate the 1-5J RBS+CI+1-23L insert and T7 promoter vector

System

| | |
|---|---|
| 3μL | insert |
| 1μL | T7p vector |
| 1μL | 10× Ligase buffer |
| 1μL | Ligase |
| 4μL | $ddH_2O$ |
| 10μL | Total |

**07.15**

Transform the ligation product of T7P+1-5J+CI+T

But the ligation failed.

**07.16**

Store the spawns of 5N＋CI②~⑤ and 11N+CI③~④.

Do the PCR identification of T7P+5N+CI+T②~⑤ and T7P+11N+CI+T③~④ using bacteria liquid; 6 samples in total.

Ligate T7P+5J+CI+T again.

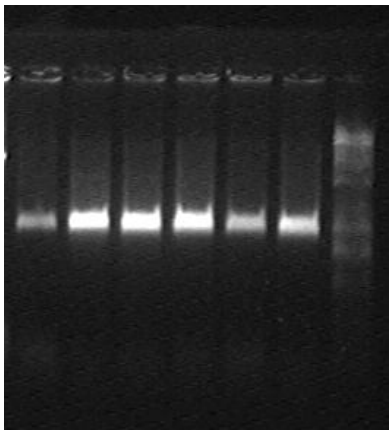
Miniprep of T7P+5N+CI+T②~⑤; T7P+11N+CI+T③~④

Concentration: 150ng/uL.

Electrophoresis to identify the 5N+CI②~⑤ and 11N+CI③~④ PCR products.

Result (from left to right)

Sample loading order:1-11N+CI③, 1-11N+CI④,1-5N+CI④, 1-5N+CI⑤, 1-5N+CI②, 1-5N+CI③, Marker



The PCR identification results show they have been successfully ligated.

**07.17**

Transformation the ligation product of T7P+5J+CI+T.

**07.19**

Store the spawns of T7p+5J+CI+1-23L ①~⑤

Bacteria Liquid PCR to identify

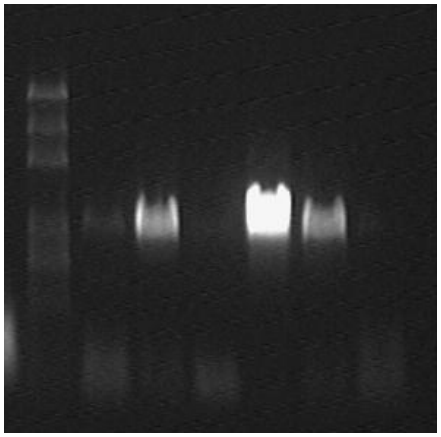
System (Master mix is exhausted)

| | |
|---|---|
| 0.5μL | Tag enzyme |
| 1μL | 10× buffer |
| 2μL | dNTP |
| 0.25μL | Forward primer |
| 0.25μL | Reverse primer |
| 6μL | $ddH_2O$ |
| | Template (Bacteria Liquid) |
| --- | |
| 10μL | Total |

Electrophorsis

Result (from left to right)

Marker, T7p+5J+CI+1-23L ①~⑤, Control



The T7p+5J+CI+1-23L ②, ④, ⑤ are target plasmids.

**07.20**

Samples below are going to be sequenced:
T7p+1-11N+CI+T ③/④; plasmids & spawns.
T7p+1-5N+CI+T ②/③/④/⑤; plasmids & spawns.
T7p+1-2K+CI+T ①-⑤; plasmid only.
T7p+1-5J+CI+T ②/④/⑤; spawns only.

**07.27**

Choose T7p+1-11N+CI+T ③/④; spawns
T7p+1-5N+CI+T ④/⑤; spawns
T7p+1-2K+CI+T ②/⑤; plasmids
T7p+1-5J+CI+T ②/④; spawns to be sequenced.
2 samples for each RBS.

**08.10**

EcoRI and PstI digest the T7p + 2K/1H/2I/2G+CI+T plasmids
Test the concentration of these 4 samples.

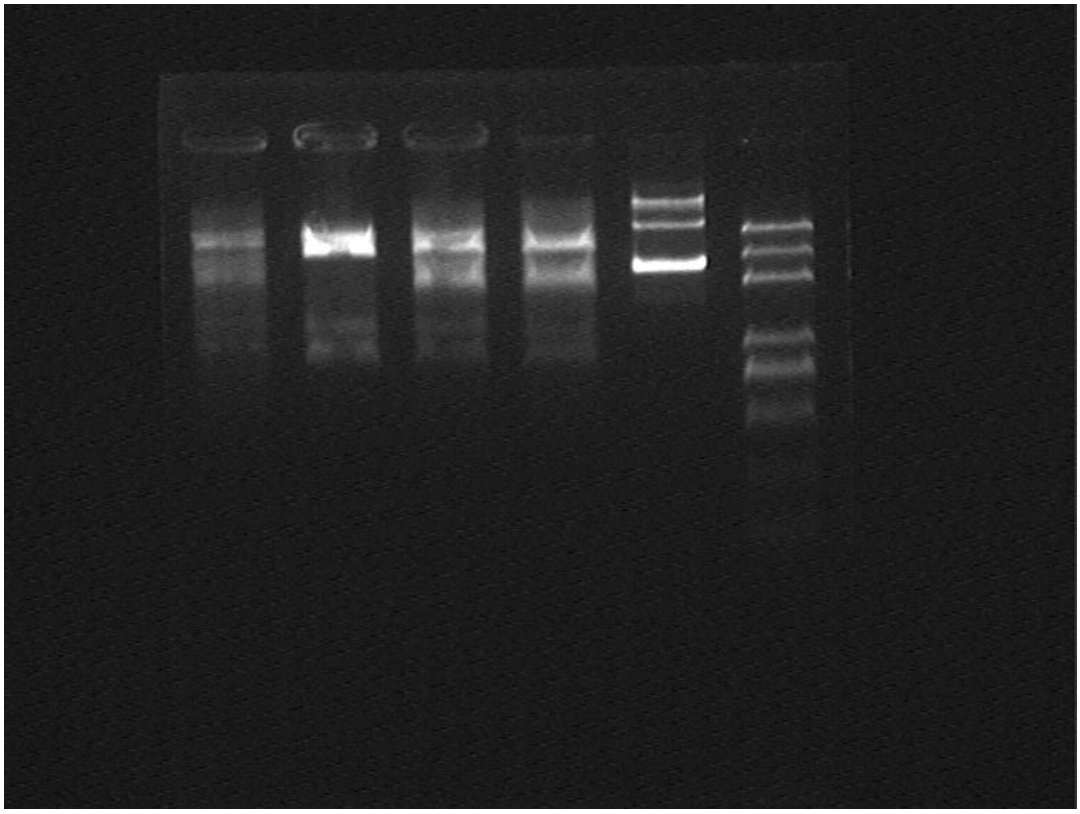
Get part 1-5C PSB3C5
1-7E PSB3K3
1-9C PSB3T5
1-1M PSB4A5
1-7G PSB4K5 for plasmid backbone
Positively transforming these 5 parts.
At the same time, transforming T7p+2K2+CI+T plasmid

Amplification of the spawns T7p+1J1/2M1/5J2+CI+T

**08.11**

Electrophoresis digested plasmids:
T7p+2K/1H/2G/2I+CI+T

4 samples in total.



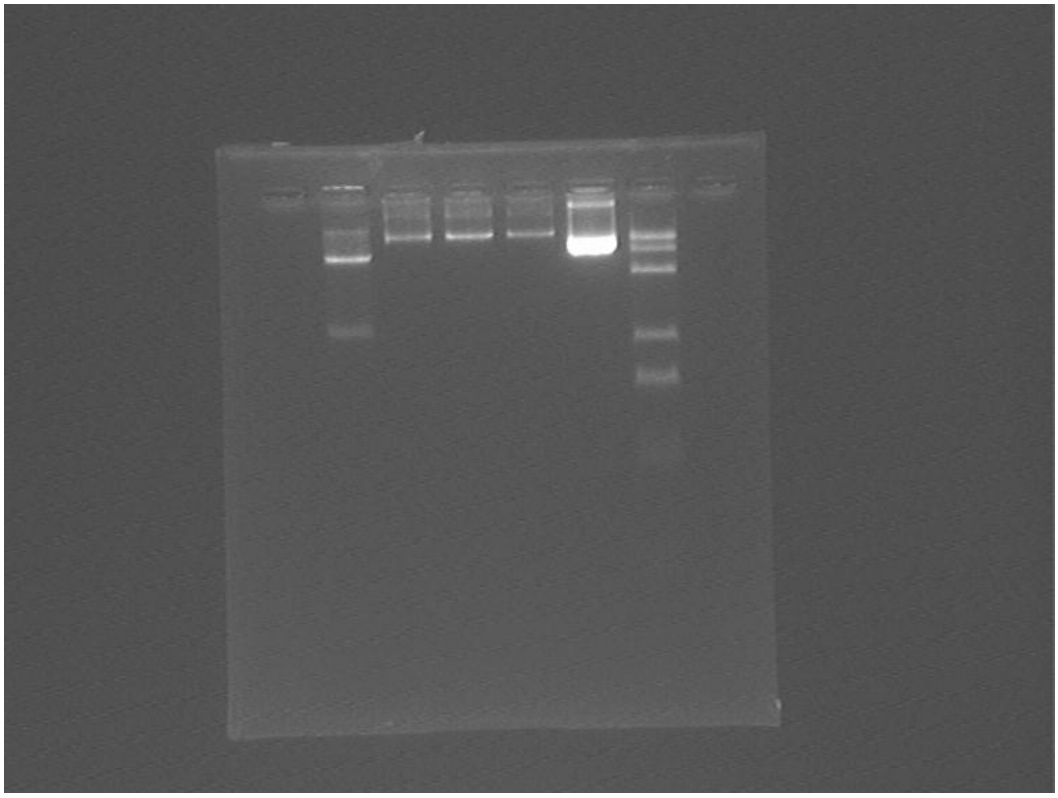
The transformation of 1-1M, 1-7E, 1-5C, 1-7G fails.
Cut gel and extract the insert.
Miniprep of T7p+5J4/2M1/1J1+CI+T
Test the concentration of these 3 samples.

EP double digest of T7p+5J4/2M1/1J1+CI+T

Transforming the 1-5C 1-7E 1-9C 1-1M 1-7G positively again.

Transfer the spawns of T7p+5N5/11N4+CI+T into tubes to amplify.

Miniprep T7p+1-2K1,2,3+CI+P
Electrophoresis to identify the plasmids.
Sample loading order: GFP, T7p+2K1, 2, 3+CI+T, control, marker



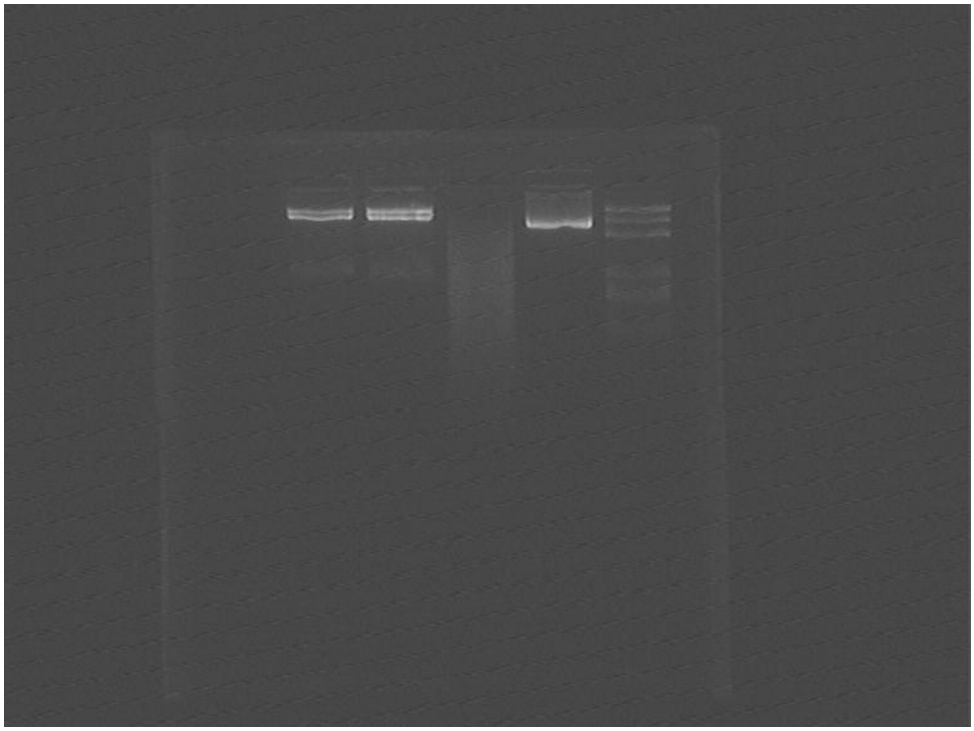
Transfer 2 colonies T7p+1-2k4,5+CI+T into tubes with 5 mL LB to amplify.

**08.12**

Electrophoresis 3 digested samples.

T7p+5J4/2M1/1J1+CI+T

Samples loading order: 1J 2M 5J control Marker

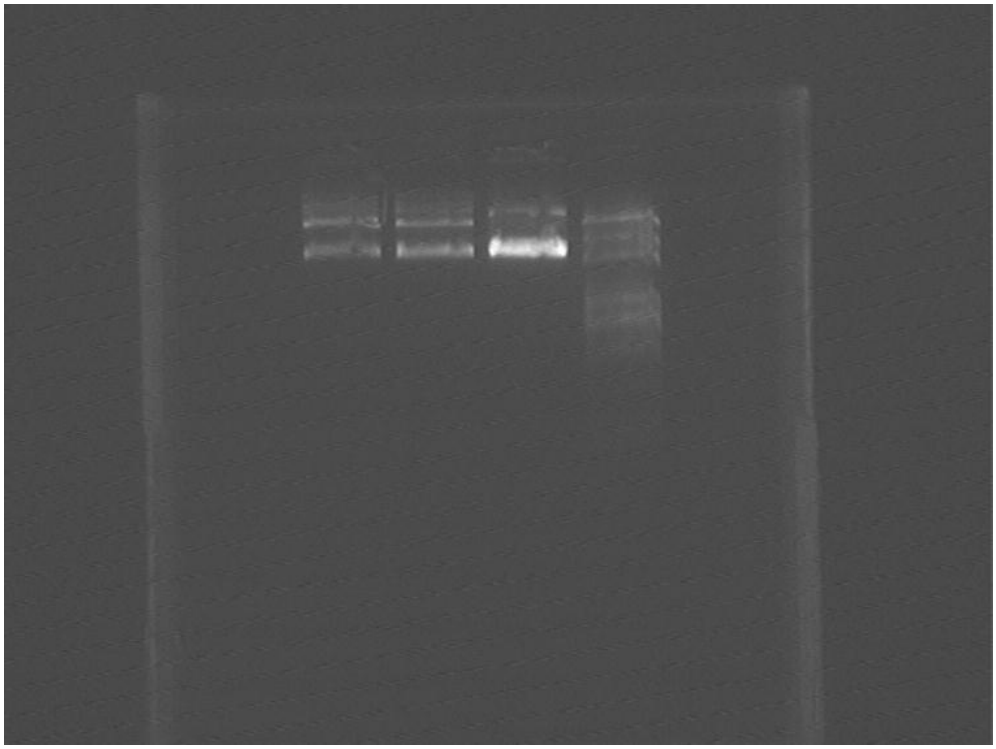


Cut gel and extract.

EP double digest T7p+5J+CI+T again.

Miniprep T7p+2K4, 5/11N/5N+CI+T, 4 samples in total.

Electrophoresis to identify the T7p+2K4, 5/11N/5N+CI+T plasmids.
Samples loading order: 1-2k4, 5, control, marker



Send bacteria liquid of T7p+11N, 5N+CI+T to be sequenced

EP double digest the T7p+5N/11N+CI+T Ligation of tetR backbone to T7p+2M+CI+T insert.

The transformation of 1-7E 1-9C 1-1M 1-7G are successful but the 1- 5C fails.
Amplify these 4 colonies.

**08.13**

Electrophoresis of digested T7p+5N/11N+CI+T, but I failed causing a stupid mistake.

EP digest the T7p+5N/11N+CI+T plasmids.

Miniprep 1-7E 1-7G 1-9C 1-1M plasmid causing the strains are low copies, we did a large miniprep.

Positive transforming 1-7E 1-7G 1-9C 1-1M.

Transfer another colonies into tubes with 10 mL LB.

Electrophoresis the digested T7p+5N/11N+CI+T plasmids again.
Sample loading order: marker control T7p+11N/5N+CI+T
But the digest failed, indicating that the ligation itself has failed before digesting.

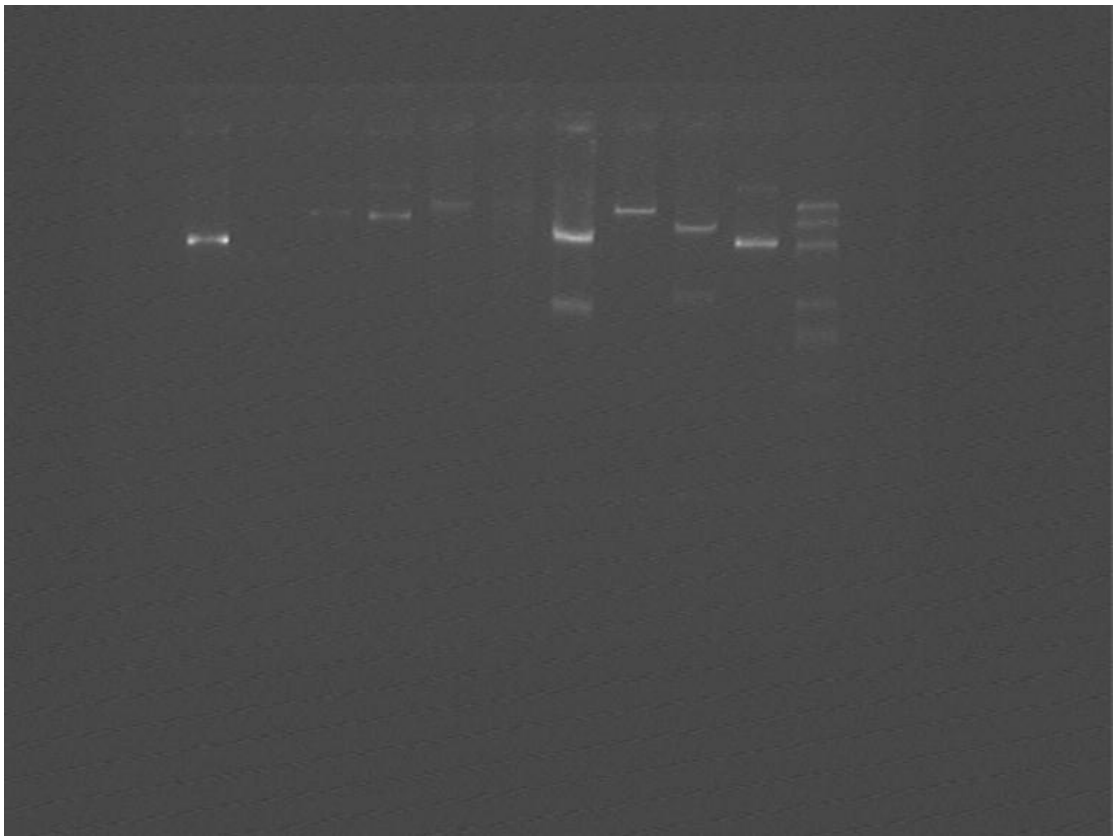
**08.14**

XP double digest of 5N5/11N5/11N1+CI+T

EP double digestion of t7p+5N/11N+CI+T

Miniprep 1-7G 1-7E 1-9C 1-1M again, causing the miniprep failed yesterday.
EP double digest of these 4 samples.

Electrophoresis the digested samples
Samples loading order: 1-1M, 1-7E, 1-7G, 1-9C, EP T7P+5N, EP+T7P+11N, XP 5N+CI5, 11N+CI1, 5 control, marker



Cut gel and extract 5N/11N+CI insert

Electrophoresis EP digested 1-9C, 7G, 7E, 1M vector
Samples loading order: M, control, 1-9C, 7G, 7E, 1M
Cut gel and extract 1M

Amplify these 4 plasmid backbone again.

**08.15**

Extract the digested and cut 5N+CI5,11N+CI5 gel.

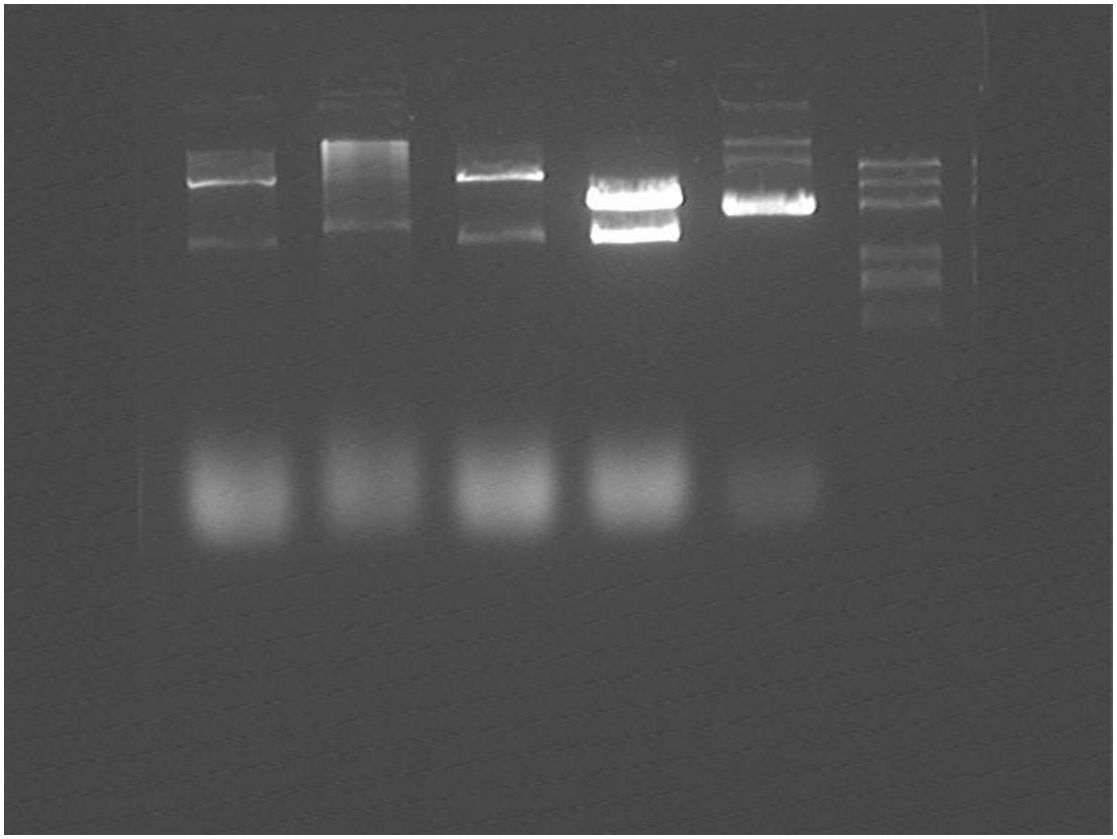
Miniprep 1-9C 7G7E 1M

Ligation T7p to 11N5/5N5+CI+T

EP double digest the minipreped 1-7E 7G 9C 1M backbones

**08.16**

Electrophoresis digested 1-7E 7G 9C 1M backbones
Samples loading order: 7G 7E 9C 1M control Marker



Cut gel and extract the vector(backbone)

Transforming the ligated T7p+11N5/5N5+CI+T 4 samples in total.

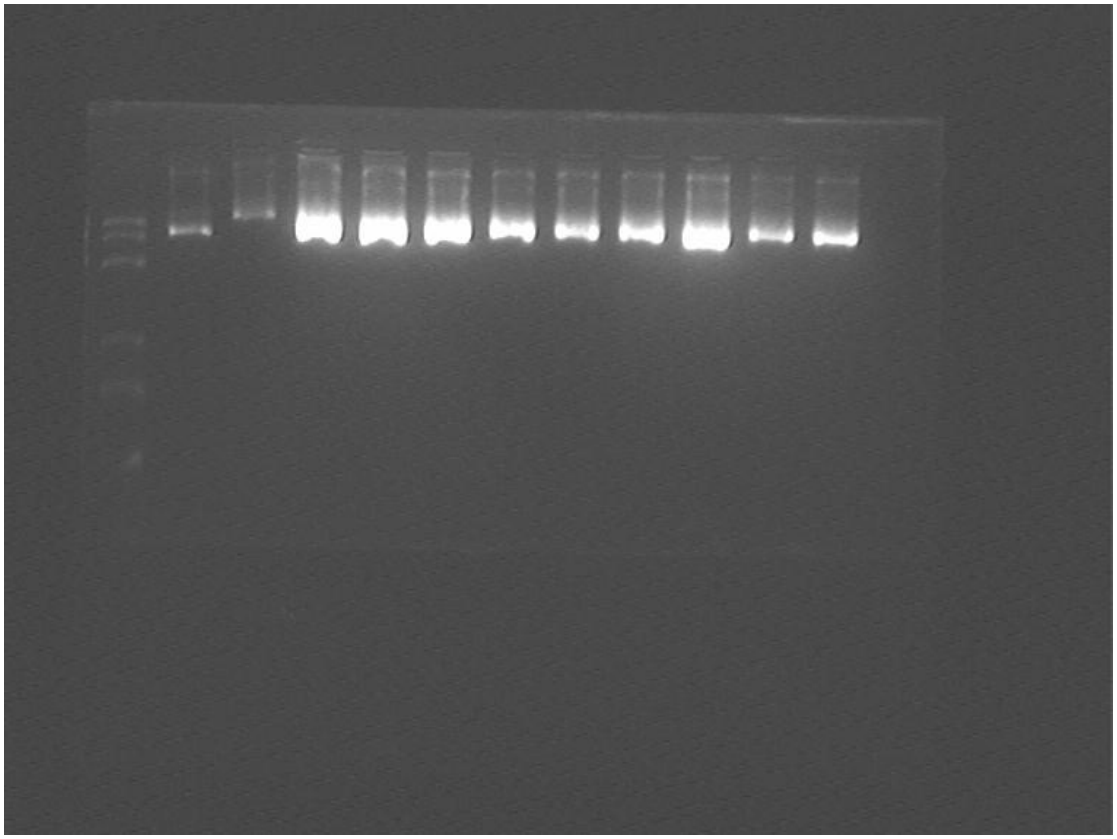
**08.17**

Transfer 20 colonies ( 5 for each sample) to amplify.

**08.18**

Miniprep the amplified T7p+11N/5N+CI+T

Electrophoresis T7p+5N+CI+T
Samples loading order: Marker Control 5NC-1-5, C+1-5



**08.19**

PCR identification of 20 samples

Electrophoresis PCR production

Samples loading order: 5N C-1-5, 11N C-1-5, marker
11N C+1-5 5N C+1-5 marker
EP double digest 5N C-4, 5; 11N C+2; 5N C+2, 3

Electrophoresis the digested 5 samples.

Samples loading order: 5N C+3, C-5, 11N C+2, 5N C+2, 5N C-4, Marker

Cut gel and ready to extract.

**08.20**

Extract the digested 5N C-5, 11N C+2, 5N C+2 insert.