Jun.26th

Construction of 7 sgRNA

Diluted primer (F1&FN) into 10µl, 3µl primer was mixed well with DNA to make template

First time PCR (20 µl)

Volume: µl

Template	**2**
DNTPs	**1.6**
High CG enhancer	**1.6**
5x Q5 buffer	**4**
Q5 DNA polymerase	**0.2**
H2O	**10.6**

Reaction time

Temperature (Celsius)	Time (second)
98	30
98	10
55	1
(Ta) 60	30
72	30
72	2 min

Second time PCR (50 μl) using rTaq system

Volume: µl

rTaq

Template (first time PCR product)	**10**
DNTPs	**4**
10 x PCR buffer	**5**
rTaq DNA polymerase	**0.5**
F1	**2**
FN (F6)	**2**
H2O	**26.5**

Reaction time

Temperature (Celsius)	Time (second)
94	5 min
94	30
60	30
72	30
Repeat 2-4	30 cycles
72	2 min
12	∞

Volume: µl

Q5 (50 μ l)

Template	10
dNTPs	4
High CG enhancer	4
5x Q5 buffer	10
Q5 DNA polymerase	0.5
F1	2
FN (F6)	2
H2O	17.5

Reaction time

Temperature (Celsius)	Time (second)
98	30
98	10
60	30
72	15
Repeat 2-4	30 cycles
72	2 min
72	∞

Jun.28th

Colony PCR

Mix together (800 µl)

pcr buffer	80
dNTPs	64
VF	32
VR	32
rTaq	8

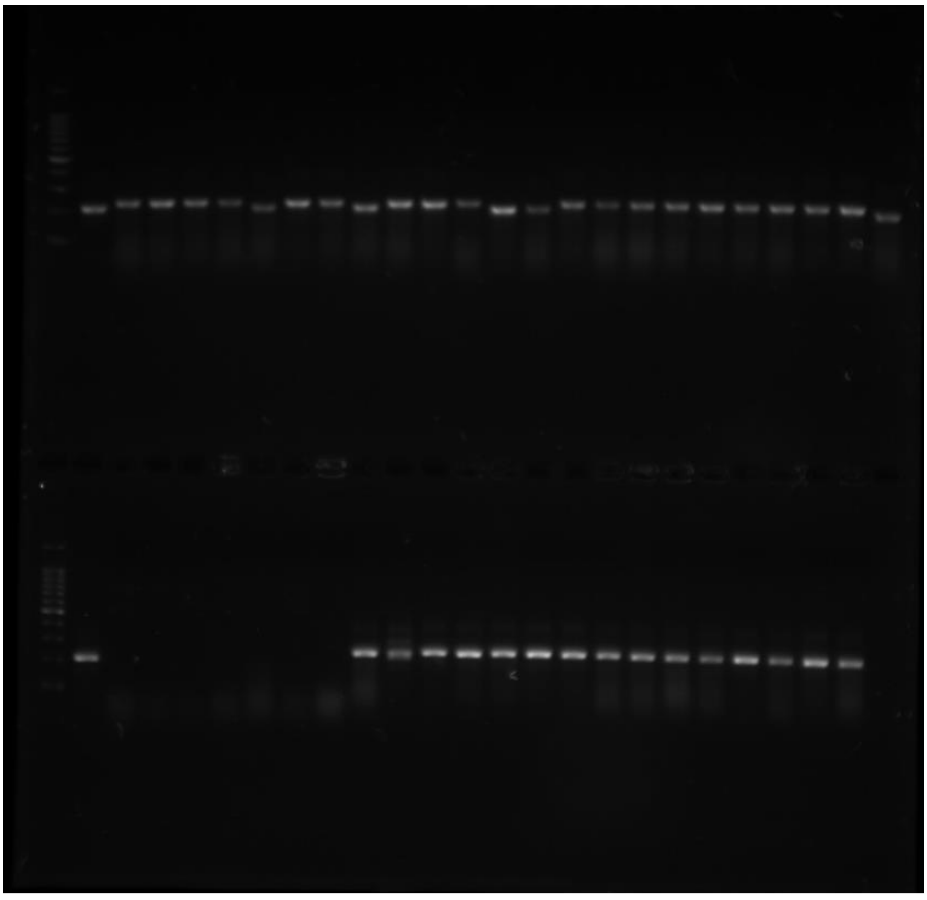
ddH20	584

- Make up mix into 1.5 ml PE tube and dispensed into eight pipes
- picked bacteria inside fume hood and put them into those pipes

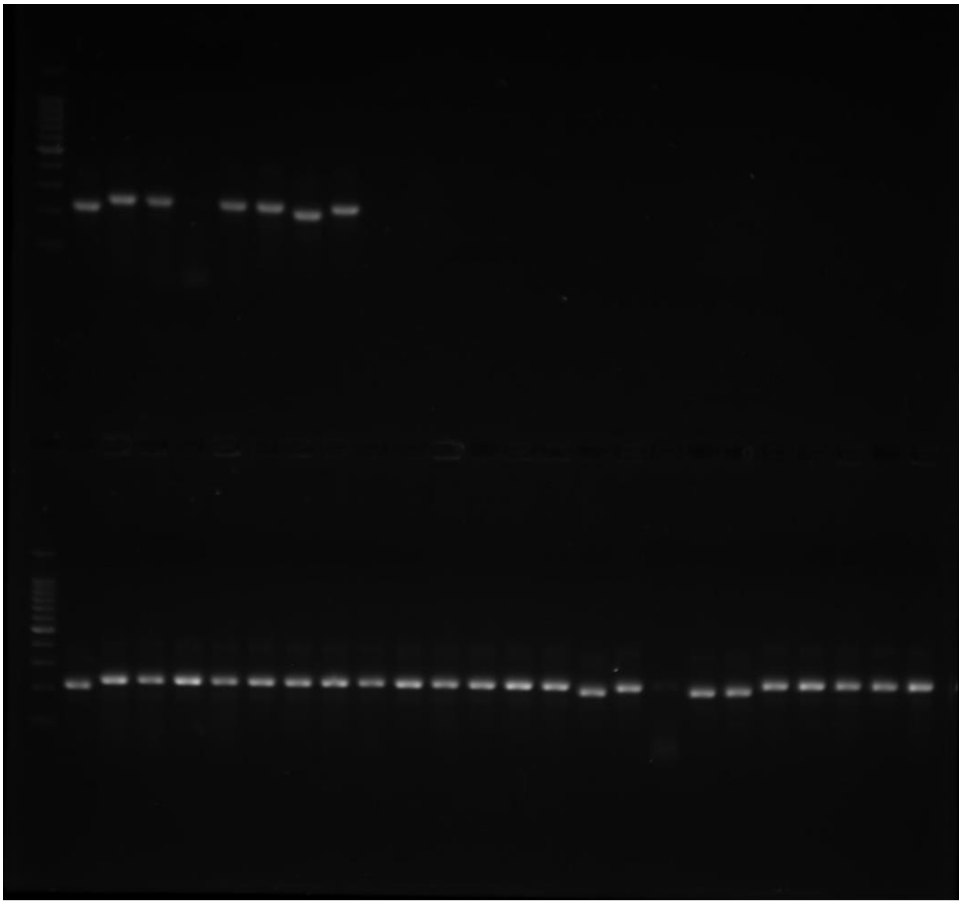
PCR

Reaction time

Temperature (Celsius)	Time (second)
94	5 min
94	30
55	30
72	30
Repeat 2-4	30 cycles
72	5 min
12	∞



2017-6-28 (1,2,3)



2017-6-28 (4,5)

Protein experiment of CT7 and NT7(without ultrasonic crashing)

Jul.3rd

Transformed plasmid into BL21 competent cells and **incubated** around 37 celcius

Jul.4th

Inoculation and pre-culture

Jul.5th

Seed-culture at 25 Celsius using 5ml tube

- Started with od 0.01 to od 0.5

Protein induction I

- Added different concentration of IPTG

Jul.6th

Protein induction II

- After 19 h, collected the bacteria (20ul bacteria plus 5ul loading buffer, 15 minutes under 98 Celsius, lowered the temperature into room temperature and stored inside -20 Celsius refrigerator)

Jul.7th

Made protein gel and gel electrophoresis to detect if we have the protein or not

- CT7
- NT7

Jul.9th

Made protein gel and gel electrophoresis to get better gel using same bacteria sample

- CT7
- NT7

7.14 分沉淀上清

Jul.10th

Enzyme digestion and ligation

- pSB1C3-va23
- pSB1C3-vb126

concentration

Plasmid name	Concentration (ng/μl)
pSB1C3	3.4
pSB1C3-va23	12.3
pSB1C3-vb126	8.5

Transformed plasmid into BL21 competent cells and **incubated** around 37 celcius (separate precipitation and supernatant)

- CFluc-dCas9-pSB1C3
- NFluc-dCas9-pSB1C3

.

Inoculation and pre-culture

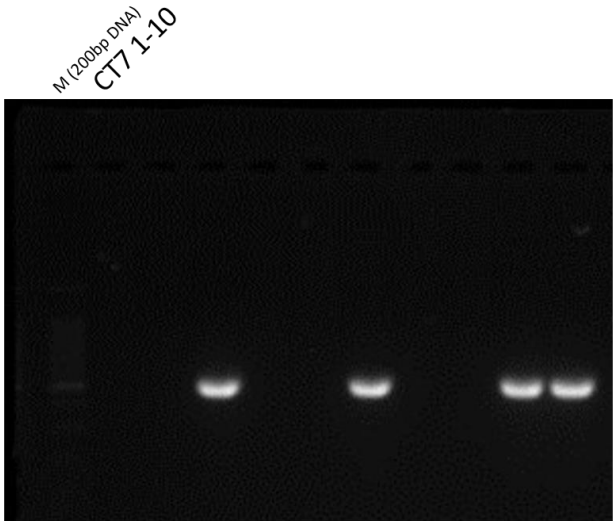
- CFluc-dCas9-pSB1C3
- NFluc-dCas9-pSB1C3

Ligation

July 11th

Restriction Digestion using BcuI and XbaI

- -PSB1C3



20170711 PCR

Small DNA Fragment Purification nanodrop

Sample	Concentration （ng/μ）
PSB1C3	3.9

- Mistake, instead of transforming and growing more pSB1C3,all of the plasmids were digested. Anyhow, we had more！

Transformation of VA83-PSB1C3, VB126-PSB1C3, PSB1C3

- The plasmid was in an empty tube with a little fluid at the bottom.

Overnight cultures

- NT7-dCas9-PSB1C3 CT7-dCas9-PSB1C3
- Miniprep the other day

Glycerol stocks

- NT7-dCas9-PSB1C3
- CT7-dCas9-PSB1C3

Overnight culture

- CFluc-dCas9-pSB1C3
- NFluc-dCas9-pSB1C3

July 12th

Made protein gel and gel electrophoresis

- CT7 1
- CT7 2
- CT73
- CT74
- NT7 1

- NT7 2
- NT7 3
- NT74

NFluc, CFluc

Seed-culture at 25 Celsius using 5ml tube

- Started with od 0.01 to od 0.5

Protein induction I

- Added different concentration of IPTG

July 13th

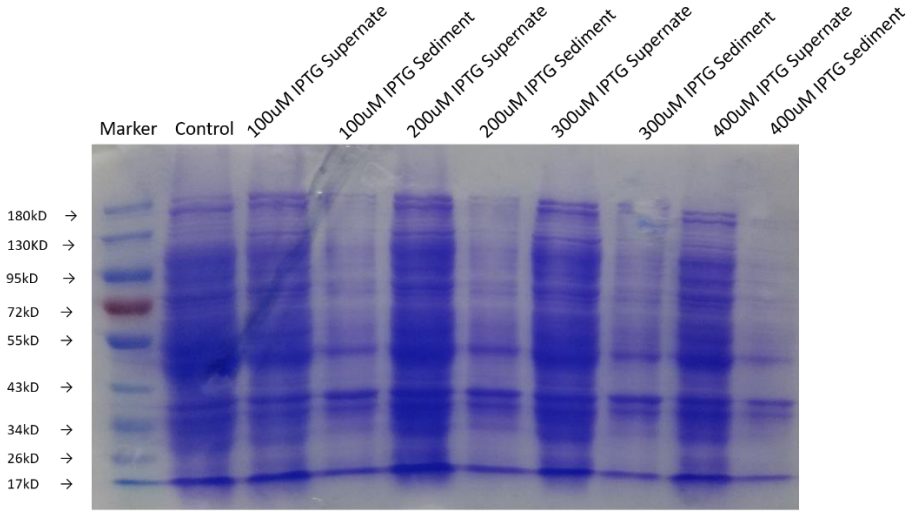
Protein induction II

- After 19 h, collected the bacteria (20ul bacteria plus 5ul loading buffer, 15 minutes under 98 Celsius, lowered the temperature into room temperature and stored inside -20 Celsius refrigerator)

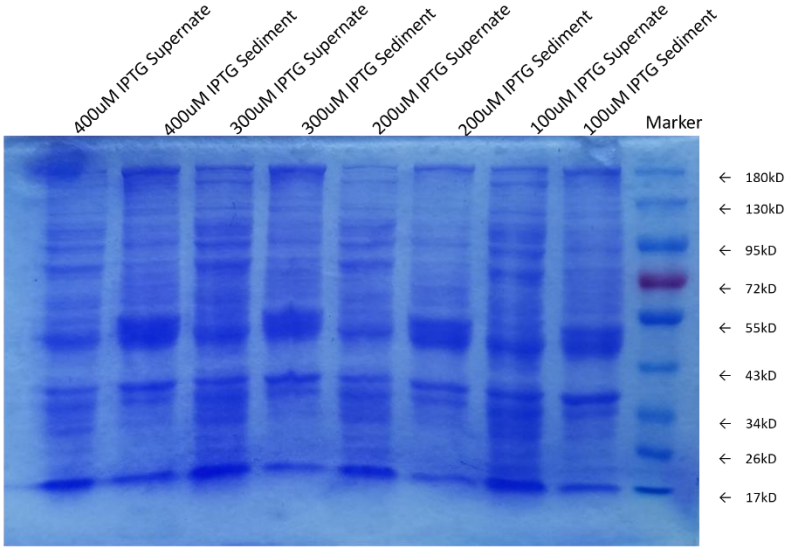
July 14th

Made protein gel and gel electrophoresis

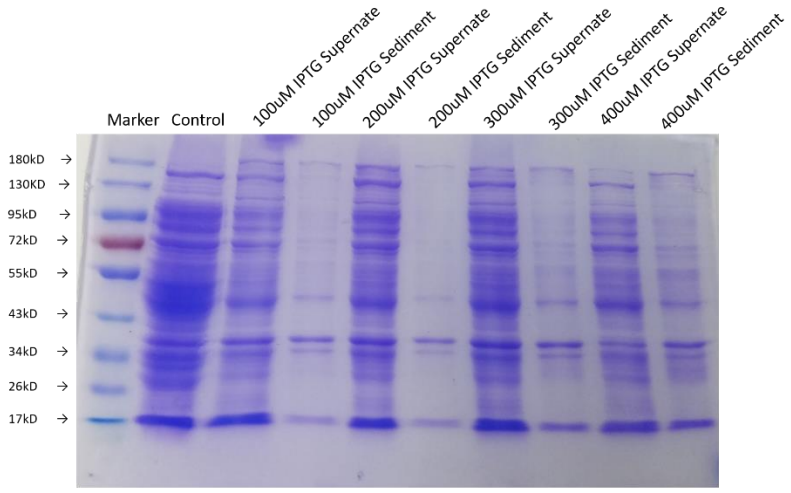
- Order: 0, marker, 0.1nM, 0.2nM, 0.3nM, 0.4nM
- NT7
- CT7
- NFluc
- CFluc



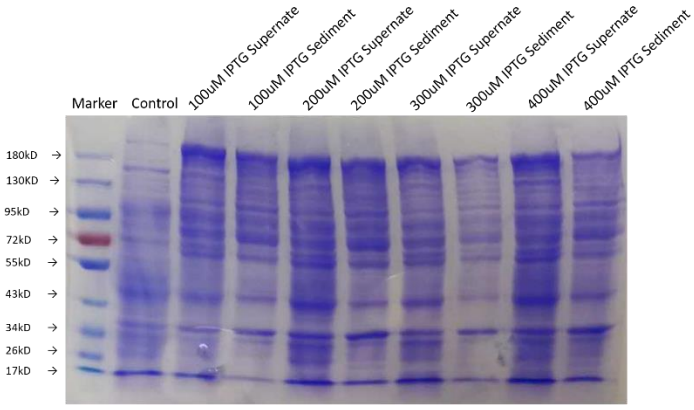
20170714 cflu-dcas9



20170714 CT7-dCas9



20170714 Nfluc-dCas9



20170714 NT7-dCas9

July 15th

Accidents

- PCR instrument power failure
- Rerun VB126-PSB1C3,VA23-PSB1C3,NT7-PSB1C3,CT7-PSB1C3 colony PCR

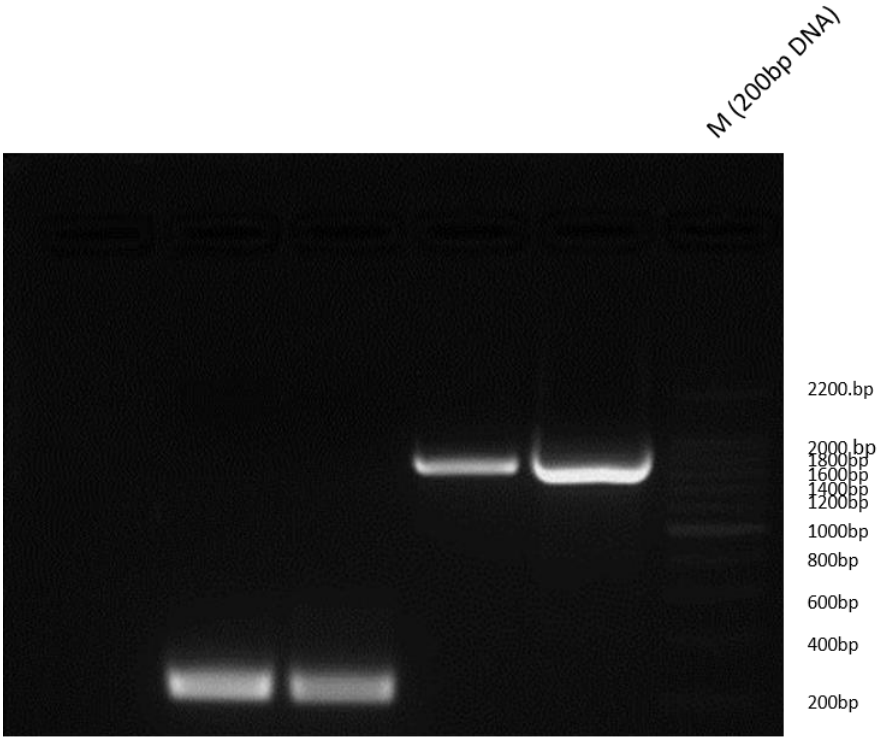
Gel electrophoresis

- Mistakes: use inappropriate primers

Possible successfully constructed plasmids

- VA23 3
- VA23 4
- VA23 5
- VA23 6
- VA23 7
- VB126 2
- VB126 7
- VB126 8
- CT7 7
- NT7 8

Re PCR plasmids or digest colony PCR products, gel electrophoresis to testify the other day.



20170715 VB126 VA23 NT7

Prepare selection plates with Chloramphenicol (Chl)

July 16th

Made protein gel and gel electrophoresis

Protein purification using kit from takara

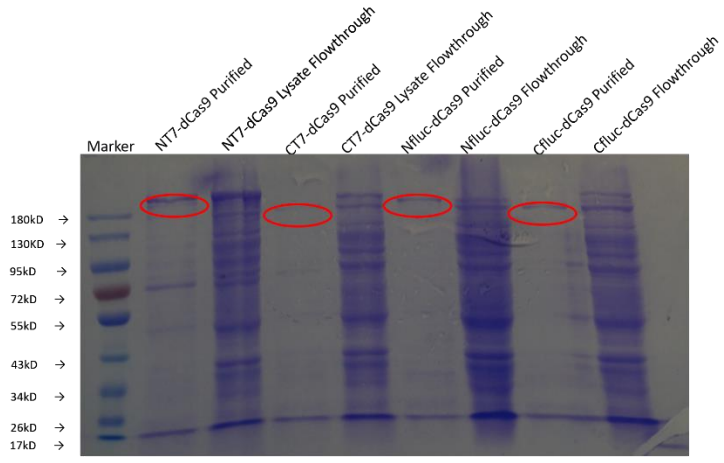
- Keep the supernatant and waste, distributed into 20 μl plus 5 μl loading buffer, 15 minutes under 98 Celsius, lowered the temperature into room temperature and stored inside -20 Celsius refrigerator

July 17th

Made protein gel and gel electrophoresis using July.16th product

Seed culture (concentration of IPTG 100Nm)

- CT7: 0, 0.05, 0.3
- NT7/NFluc/CFlc: 0,0.05,0.2



20170717 his-tag purification

July 20th

Made protein gel and gel electrophoresis using July.16th product

- NFluc 0
- NFluc 1
- NFluc 2

- CFluc 0
- CFluc 1
- CFluc 2
- NT7 0
- NT7 1
- NT7 2

RNA transcription DNase I

- 33a
- 93a
- 70b
- 147b

July 21st

Seed culture (concentration of IPTG 100Nm)

- CT7/NT7/NFluc/CFlc: 0,0.03nM,0.06nM,0.09nM.0.2nM(CT7:0.3nM)

IPTG concentration

nM	µl
0.1	5
0.03	1.5
0.06	3
0.09	4.5
0.2	10
0.3	15

OD

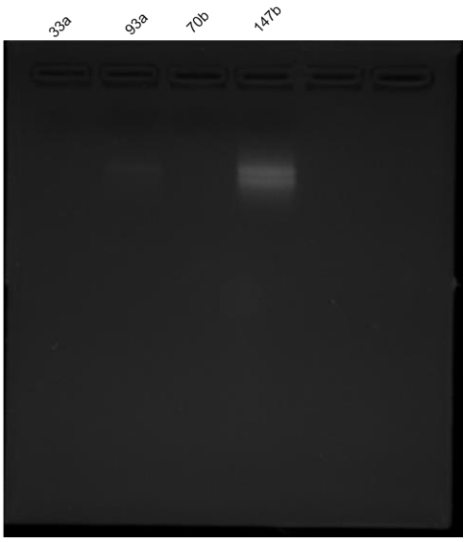
NT7	1st time	2nd time	3rd time
0	0.12	0.11	0.07
1	0.08	0.08	0.07
2	0.07	0.08	0.07
3	0.10	0.09	0.07
4	0.09	0.11	0.07
CT7			
0	0.09	0.03	0.04
1	0.11	0.09	0.05
2	0.07	0.06	0.09
3	0.09	0.09	0.08

CFluc			
0	0.07	0.07	0.15
1	0.13	0.08	0.14
2	0.09	0.07	0.13
3	0.07	0.15	0.16
NFluc			
0	0.11	0.13	0.20
1	0.06	0.17	0.19
2	0.14	0.09	0.15
3	0.11	0.07	0.19
4	0.08	0.08	0.21

RNA transcription and gel electrophoresis

- 33a
- 93a
- 70b
- 147b

20170721 第一次RNA电泳 从右到左 33A 93A 70B 147B



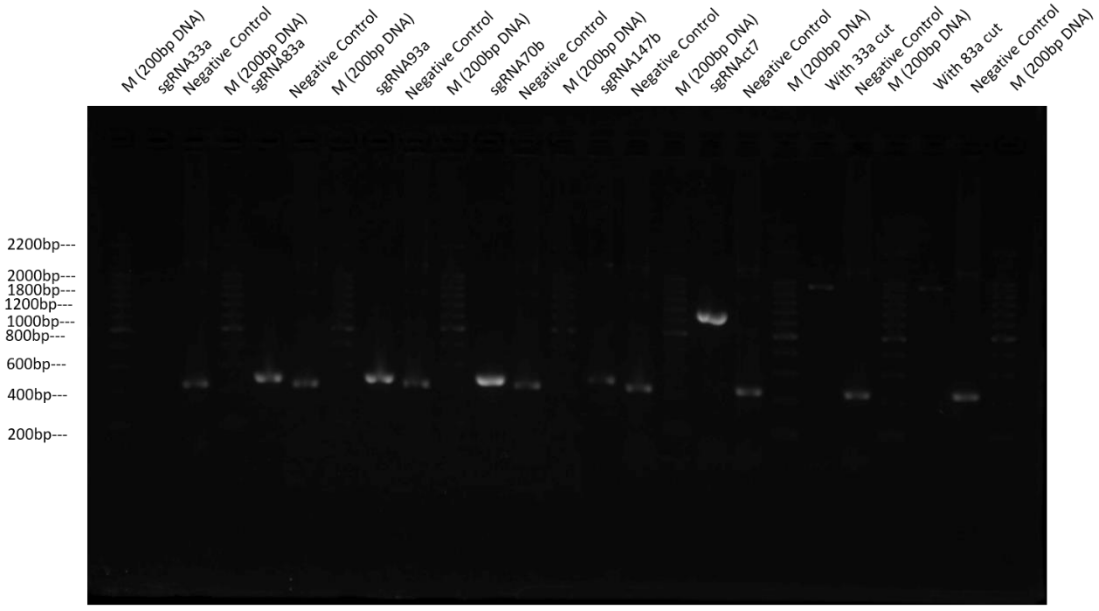
20170721 第二次RNA电泳 33a 93a 70b 147b

July 23rd

Restriction digest of plasmid using Pst1 and EcoR1

- 33a
- 83a
- 93a
- 70b
- 147b
- CT7

Gel electrophoresis of digestion product



20170723 33a 83a 93a 70b 147b ct7 pcr with 33a 83a cut

July 24th

33a

Polymerase chain reaction using rTaq

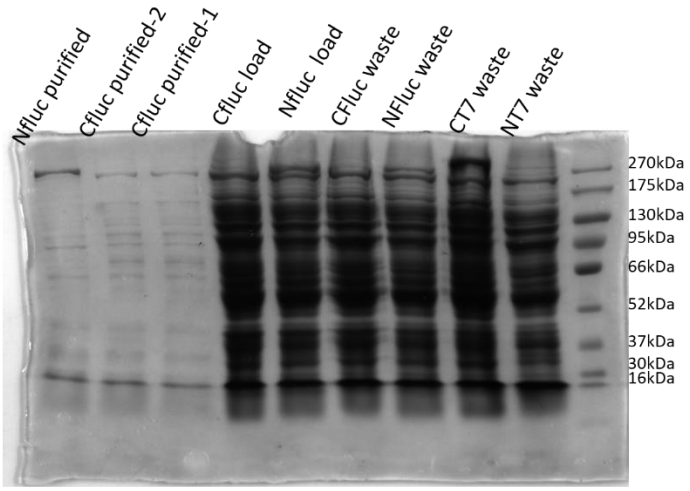
Restriction digest of plasmid using Pst1 and EcoR1

PCR using Q5

- 23a
- 126b

Protein ultrasonic crushing

- NT7
- CT7
- CFluc
- NFluc



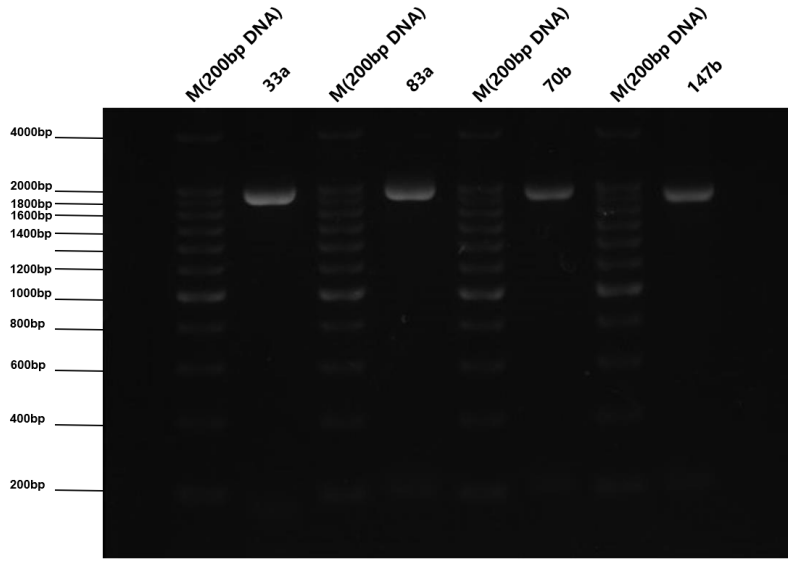
20170724 protein-2 M NT7w CT7w NFw CFw NFl CFl CFp CFp-2 NFp

July 25th

Restriction digest of plasmid using Pst1 and EcoR1

- 33a
- 83a
- 70b
- 147b

Gel electrophoresis of digestion product



20170725 33a 83a 70b 147b RD test

July 26th

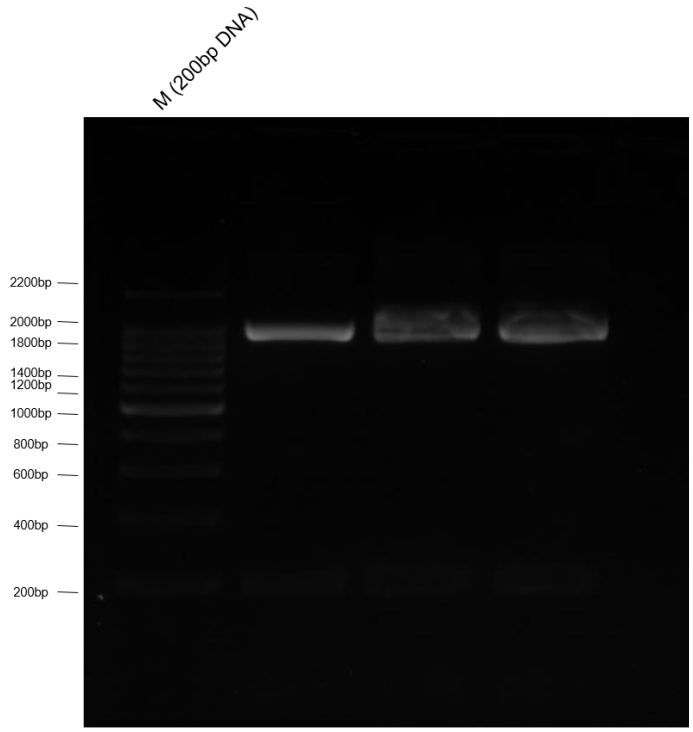
Preparation for tomorrow's experiment

Human practice

July 27th

Gel extraction dissolve in buffer EB

- 23a
- 126b
- NT7



20170727 pcr 33a-1c3,6,8,8

Restriction digest of plasmid using BamH1(0.75µ) and Bas1(0.75 µ)

- CFluc
- CT7
- NT7
- Redo 33a

July 28th

Colony PCR no control; extension length for NT7 was too short

- 23a
- 126b
- NT7

Colony PCR second time

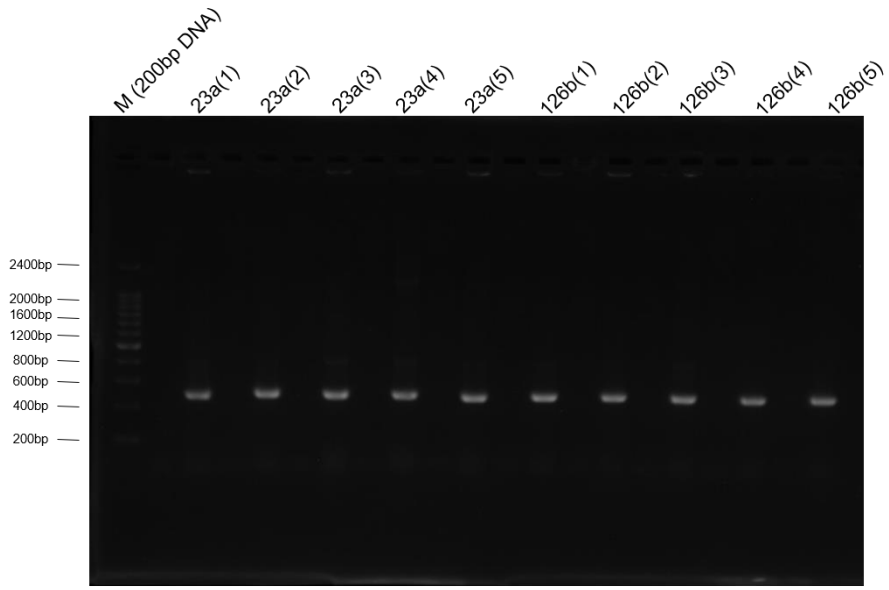
- NT7

July 30th

Colony PCR second time, control had some problem

- 23a
- 126b

Gel electrophoresis NT7 use the wrong enzyme when doing enzyme digestion



2017-7-30 23a12345 126b12345

July 31st

Plasmid extraction and nanodrop

First time

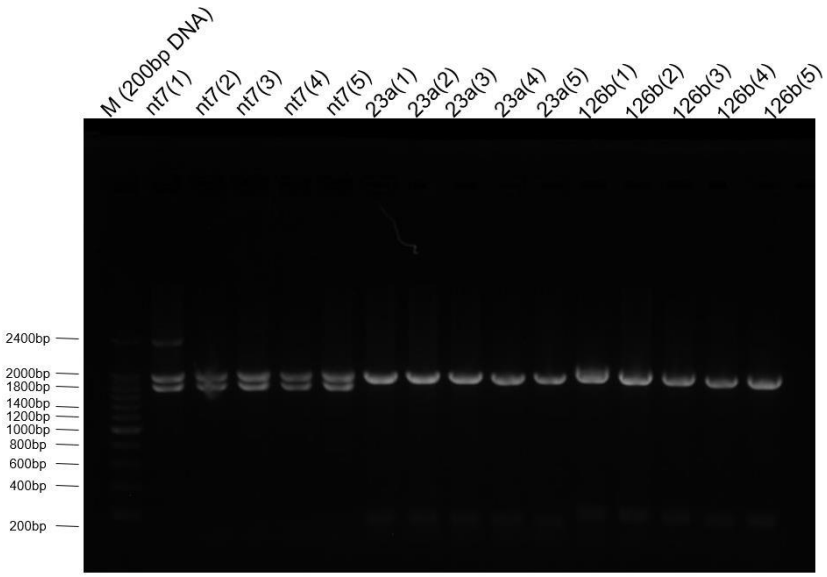
Bacteria sample	1	2	3	4	5
23a	276	255.1	266.3	302.6	273.0
126b	324.4	346.7	379.6	297.4	308.5
NT7	516.4	337.7	368.2	308.5	383.5

Second time

Bacteria sample	1	2	3	4	5
23a	273.3	246.2	267.7	302.1	271.9
126b	321.7	333.3	366.1	286.9	287.2
NT7	540.7	332.3	363.6	298.7	380.0

Restriction digest of plasmid using Pst1 and EcoR1

Gel electrophoresis of digestion product



2017-7-31 nt712345,23a12345,126b12345

Aug.1st

Sent the correct plasmid from gel electrophoresis for sequencing

Overnight culture with psb1c3 plasmid backbone

- 33a
- 83a
- 93a
- 70b
- 147b

Aug.2nd

Interlaboratory study

Transformed dna into competent cell and **incubated** around 37 celcius

- Positive control
- Negative control
- Device 1
- Device 2
- Device 3
- Device 4
- Device 5
- Device 6

Aug.3rd

Tested the sequencing result, they were all matched,

Interlaboratory study

Transferred the bacteria to the tube

Over night culture

- Positive control
- Negative control
- Device 1
- Device 2
- Device 3
- Device 4
- Device 5
- Device 6

PCR and gel electrophoresis: Q5 enzyme was not enough, so we could not see anything in the picture. We threw the gel after.

- 23a
- 33a
- 83a
- 93a
- 70b
- 126b
- 147b

Aug.4th

Visited the national gene bank and read raw fluorescence using plate reader

PCR and gel electrophoresis: the gel was too light, we threw the gel again and repeat the same experiment after

- 23a
- 33a
- 38a
- 93a
- 70b
- 126b
- 147b

Second time PCR

- 23a
- 33a
- 83a
- 93a
- 70b
- 126b
- 147b

Aug.5th

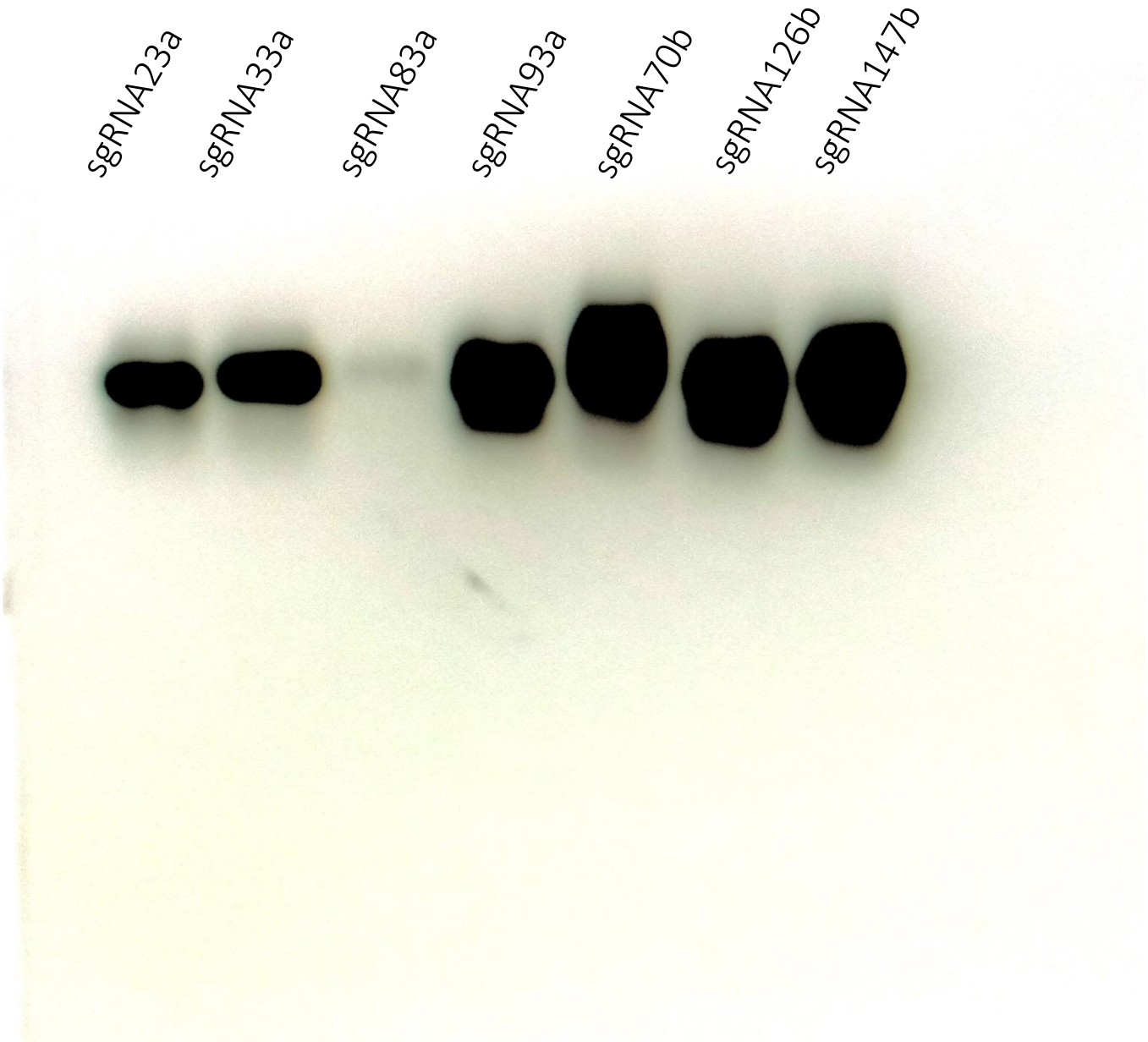
Interlaboratory study

Redo the transformation and incubation -none of them grew new bacteria

- Positive control
- Negative control
- Device 1
- Device 2
- Device 3
- Device 4
- Device 5
- Device 6

RNA transcription, purification, nanodrop and gel electrophoresis

- 23a
- 33a
- 83a
- 93a
- 70b
- 126b
- 147b



Aug.6th

Interlaboratory study

Transform of PC, NC, D1, D2,D3,D4,D5,D6

- Increased the time of culture
- Added bacteria medium without antibiotic

Aug.7th

RNA transcription

- 23a
- 33a
- 83a
- 93a
- 70b
- 126b
- 147b

Aug.8th

Found out some mistakes of our design

Design primer

August.9th

Interlaboratory study

Read raw fluorescence using plate reader (practice)

Inoculated the bacteria

August.10th

Interlaboratory study

Inoculated the bacteria

Read raw fluorescence until 2 hours

August.11th

Made protein gel and gel electrophoresis

protein purification, induction and ultrasonic crushing

- NFluc
- CT7

Interlaboratory study

Inoculated the bacteria

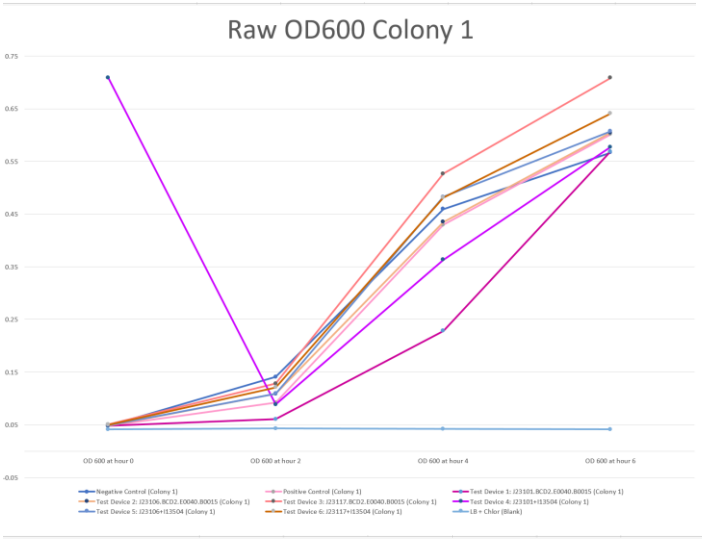
Read raw fluorescence using plate reader

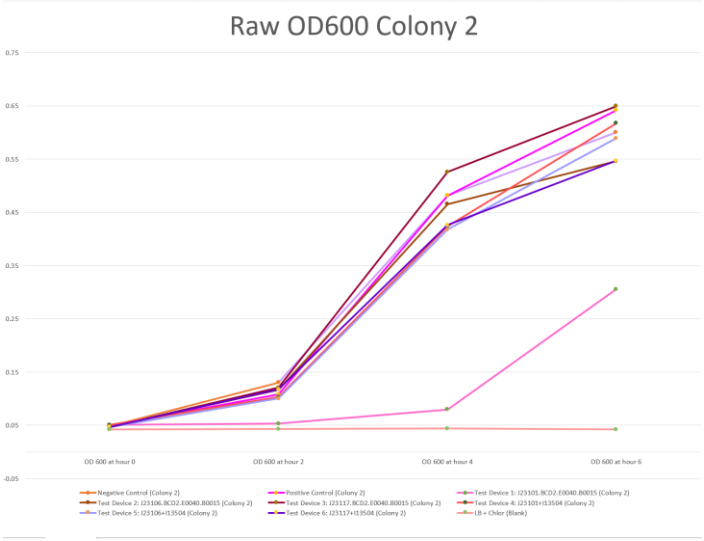
Fluorescence and OD600 in 96 holes plate

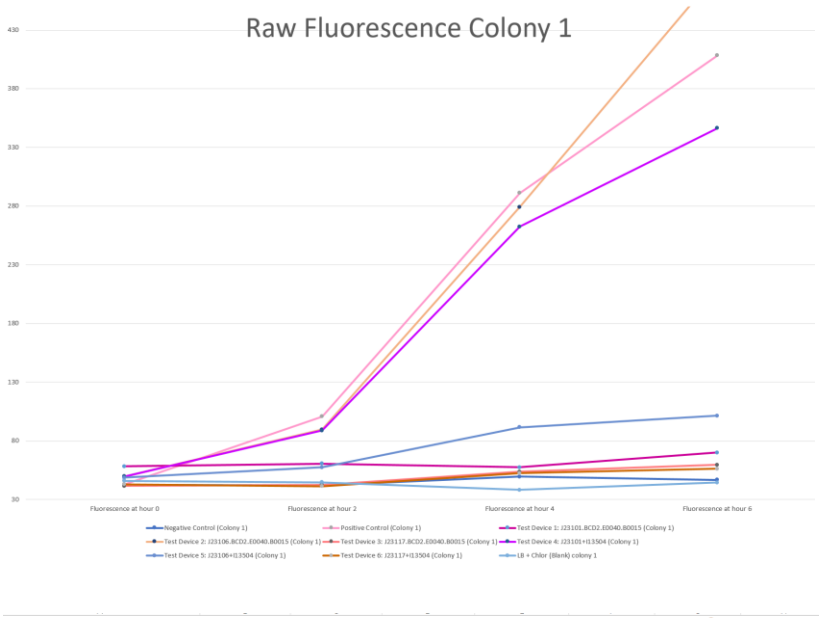
Negative control colony 1 replica 1	Positive control colony1 replica1	Device 1 colony1 Replica1	Device2 colony1 Replica1	Device3 colony1 Replica1	Device4 colony1 Replica1	Device5 colony1 Replica1	Device6 colony1 Replica1	LB +Chlor Blank colony1 Replica1
Negative control colony 1 replica 2	Positive control colony 1 replica 2	Device 1 colony 1 replica 2	Device2 colony 1 replica 2	Device3 colony 1 replica 2	Device4 colony 1 replica 2	Device5 colony 1 replica 2	Device6 colony 1 replica 2	LB +Chlor Blank colony 1 replica 2
Negative control colony 1 replica 3	Positive control colony 1 replica 3	Device 1 colony 1 replica 3	Device2 colony 1 replica 3	Device3 colony 1 replica 3	Device4 colony 1 replica 3	Device5 colony 1 replica 3	Device6 colony 1 replica 3	LB +Chlor Blank colony 1 replica 3
Negative control colony 1 replica 4	Positive control colony 1 replica 4	Device 1 colony 1 replica 4	Device2 colony 1 replica 4	Device3 colony 1 replica 4	Device4 colony 1 replica 4	Device5 colony 1 replica 4	Device6 colony 1 replica 4	LB +Chlor Blank colony 1 1 replica 4
Negative control colony 2 replica 1	Positive control colony 2 replica 1	Device 1 colony 2 replica 1	Device2 colony 2 replica 1	Device3 colony 2 replica 1	Device4 colony 2 replica 1	Device5 colony 2 replica 1	Device6 colony 2 replica 1	LB +Chlor Blank colony 2 replica 1
Negative control colony 2 replica 2	Positive control colony 2 replica 2	Device 1 colony 2 replica 2	Device2 colony 2 replica 2	Device3 colony 2 replica 2	Device4 colony 2 replica 2	Device5 colony 2 replica 2	Device6 colony 2 replica 2	LB +Chlor Blank colony 2 replica 2
Negative control colony 2 replica 3	Positive control colony 2 replica 3	Device 1 colony 2 replica 3	Device2 colony 2 replica 3	Device3 colony 2 replica 3	Device4 colony 2 replica 3	Device5 colony 2 replica 3	Device6 colony 2 replica 3	LB +Chlor Blank colony 2 replica 3

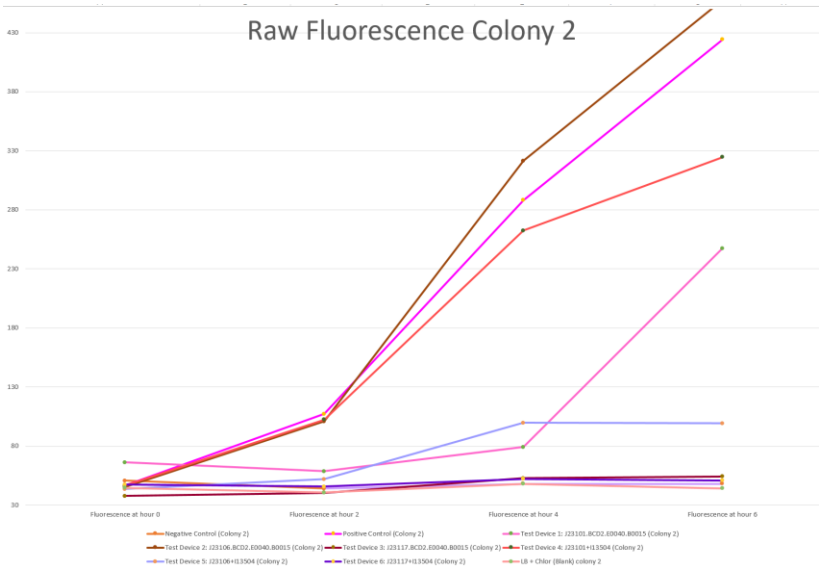
Negative control colony 2 replica 4	Positive control colony 2 replica 4	Device 1 colony 2 replica 4	Device2 colony 2 replica 4	Device3 colony 2 replica 4	Device4 colony 2 replica 4	Device5 colony 2 replica 4	Device6 colony 2 replica 4	LB +Chlor Blank colony 2 replica 4

Results









August.12th

Protein purification :protein could not be washed and pass through the filter in the tube

Made protein gel and gel electrophoresis

August.14th

Made protein gel and gel electrophoresis

Made cell culture media: the container did not go through the sterilization, so mold grew inside the medium after one month.

- Liquid
- Solid

Protein induction: none of them reach the time of adding IPTG

- NT7
- CT7
- CFluc
- NFluc

August.15th

Protein induction NT7 and CT7 were overtime

- NT7
- CT7
- CFluc
- NFluc

Practiced streak plate method

August.15th

Added IPTG

- CFluc
- NFluc

Protein induction

- NT7
- CT7

August.18th

Added IPTG

- NT7
- CT7

Protein ultrasonic crushing

- CFluc
- NFluc

Made polyacrylamide gel, gel electrophoresis, protein purification

- CFluc
- NFluc

Sept.9th

Enzyme digestion (30µl) using Eco311 (Bsa1), 8:45 -7:30

- NT7
- CT7
- CFluc
- Pet28a 1
- Pet28a 2
- Pet28a 3
- Pet28a 4

	Concentration of pcr product	Volume of pcr product (µl)	Volume of enzyme (µl)	Volume of FD buffer (µl)	Volume of ddH20 (µl)
NT7	55.6	18	1	3	8
CT7	114.9	8.7	1	3	17
NFluc	55.2	18.11	1	3	8
CFluc	90.3	11	1	3	15
pet 28a	63.5	10	1	3	16

Concentration after enzyme digestion

	ng/ µl	A260/A280	A260/A230
HNT7	23.3	1.93	0.75
HCT7	55.7	1.71	0.64

HNfluc	**17.4**	**1.96**	**0.37**
HCfluc	**23.8**	**1.97**	**0.26**
H pet28a vector	**14.4**	**1.97**	**0.58**

Sept.10th

DNA ligation (20µl) 13:30-17:00

	Inserted sequence (µl)	pet28a vector (µl)	T4 ligase (µl)	T4 ligase buffer (µl)	ddH20 (µl)
HNT7	**2.2ul**	**3.5ul**	**1**	**2**	**11.3**
HCT7	**0.9ul**	**3.5**	**1**	**2**	**12.6**
HNfluc	**2.9ul**	**3.5**	**1**	**2**	**10.6**
HCfluc	**2.1ul**	**3.5**	**1**	**2**	**11.4**

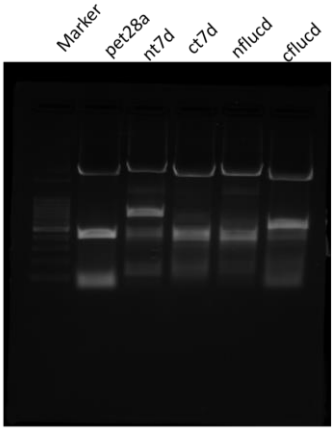
Transformed dna into competent cell and **incubated** around 37 celcius

- NT7
- CT7
- NFluc
- CFluc

Sept.14th

Transformed plasmid into competent cell(DH5α) and **incubated** around 37 celcius (Kanamycin)

- NFluc
- dCas9
- pet281-CT7-dCas9
- control



20170914 pcr pet28a nt7d ct7d nfd cfd

Sept.15th

Colony PCR (375µl) NT7 grew the least bacteria, picked up 6 each of the other plates

Volume: µl

pcr buffer	**37.5**
dNTPs	**30**
primers	**15 + 15**
rTaq	**3.75**
ddH20	**275(273.5)**

- rTaq mix: Mixed well using vortex machine and put in -20 Celsius degree refrigerator using petVF and dCas9VR

length

CFluc	**1000bp**
NT7	**2250bp**
CT7	**1500bp**
NFluc	**1800bp**

gel electrophoresis order, 7 μ l for each hole

- Marker
- CT7 1
- CT7 2
- CT7 3
- CT7 4
- CT7 5
- CT7 6
- Marker
- Nfluc 1
- Nfluc 2
- Nfluc 3
- Nfluc 4
- Nfluc 5
- Nfluc 6
- Marker
- Cfluc 1
- Cfluc 2
- Cfluc 3
- Cfluc 4
- Cfluc 5
- Cfluc 6

- Control 1
- Control 2

Sept.16th

Sway bacteria from successful pcr product from Sept.15th Kanamycin, 3 of each

- CT71
- CT72
- CT73
- NFluc1
- NFluc2
- NFluc3
- CFluc2
- CFluc4
- CFluc6

Redo colony PCR control and NT7 were

- NT71
- NT72
- NT73
- NT74
- Control 1
- Control 2
- Control 3
- Control 4

gel electrophoresis order

- Marker
- NT7 1
- NT7 2
- NT73
- NT7 4
- Marker
- Control 1
- Control 2
- Control 3
- Control 4
- CT7
- NT7

GFP/EFOR RED/LACZ synthesis using Q5 polymerase, LacZ needs to go through PCR twice

H NT7-dCas9 and H CT7-dCas9 biobrick construction

- not sure if NT7 and pet28a were connected to dCas9 or not, we tried to prove using gel electrophoresis, but without enzyme digestion, the result might be incorrect

Gel electrophoresis (second time) the gel was not put into the refrigerator, so it is too light

gel electrophoresis order; control 2 and 3 were not enough

- Marker
- CT7
- NT7
- Marker
- NT7 1
- NT7 2
- NT73
- NT7 4
- Marker
- Control 1
- Control 4

PCR and gel electrophoresis of NT7 Biobrick/CT7 Biobrick/ LacZ/GFP/ Efored

First time PCR

- GFP
- EFORED
- LacZ

gel electrophoresis order

- Marker
- NT7 Biobrick
- CT7 Biobrick
- LacZ
- GFP
- Efored

Gel extraction of CT7 biobrick

Sept.17th

Plasmid extraction plasmid were put in the box of histag

- H CFluc dCas9 1
- H CFluc dCas9 2
- H CFluc dCas9 3
- H NT7 dCas9 3
- H NT7 dCas9 4
- H NFluc dCas9 1
- H NFluc dCas9 2
- H NFluc dCas9 3
- H CT7 dCas9 1
- H CT7 dCas9 2
- H CT7 dCas9 3

Second time PCR of LacZ failed twice, we could not observe the result from the photo

Sept.30th

Transformed plasmid into competent cell(DH5α) and **incubated** around 37 celcius;

Usage:6μl plasmid each, 45μl competent cell. Also made a control.

- NFluc dCas9
- CFluc dCas9
- NT7 dCas9
- CT7 dCas9

Oct.1st

Transformed plasmid into competent cell(bl21) and **incubated** around 37 celcius;

- Pet28a-NT7-dCas9
- CT7-dCas9
- NFluc-dCas9
- CFluc-dCas9

PCR using Q5 polymerase

Enzyme digestion and gel extraction

- va23-psb1c3
- got psb1c3 plasmid backbone

Oct.2nd

Pre-culture of bl21 bacteria

- Pet28a-NT7-dCas9
- CT7-dCas9
- NFluc-dCas9
- CFluc-dCas9

Gel extraction and enzyme digestion using EcoR1 and Pst1

- Psb1c3-effored
- Psb1c3-amilgfp

Protein induction IPTG 0.2 um

- IPTG 0.2 um
- NT7-dCas9
- CT7-dCas9
- NFluc-dCas9
- CFluc-dCas9

Transformed plasmid into competent cell(bl21) and **incubated** around 37 celcius;

- Pet28a-shrpn-dCas9
- Pet28a-shrpc-dCas9

Oct.3rd

Protein gel and gel electrophoresis

- NFluc-dCas9 and CFluc-dCas9 had obvious expression

DNA ligation, transformed the plasmid into competent cell and **incubated** around 37 celcius;

- Psb1c3-effored
- Psb1c3-amilgfp

Protein pre-culture

- Shrpn-dCas9

Seed-culture

- After 12hrs

Cell-free system test （promega l1020）

- The kit did not work

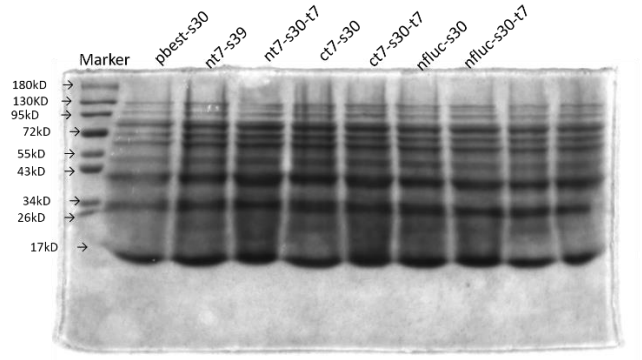
Oct.4th

Protein purification using kit from takara and **buffer exchange** using 30 K MWCO concentrator from thermo

- NT7-dCas9
- CT7-dCas9
- NFluc-dCas9
- CFluc-dCas9
- The amount of protein we got were lower than 0.5ng/μl

Cell-free system test

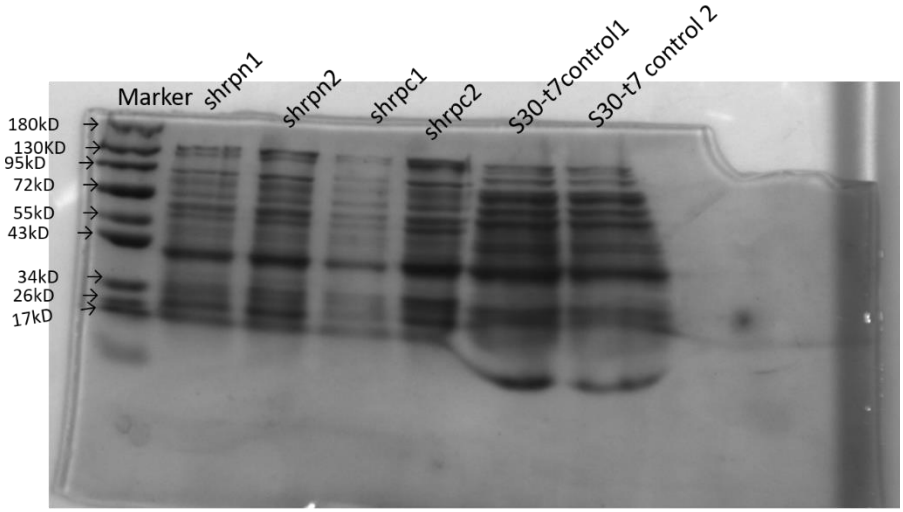
- The kit did not work again



10.4 cell free M; pbest-s30; nt7-s39; nt7-s30-t7; ct7-s30; ct7-s30-t7; nfluc-s30; nfluc-s30-t7; cfluc-s30; cfluc-s30-t7

Made protein gel and gel electrophoresis using protein induction product

- 0.2um is enough for us to induce protein



10.4 shrpn12 shrpc12 s30-t7 control12

Colony PCR

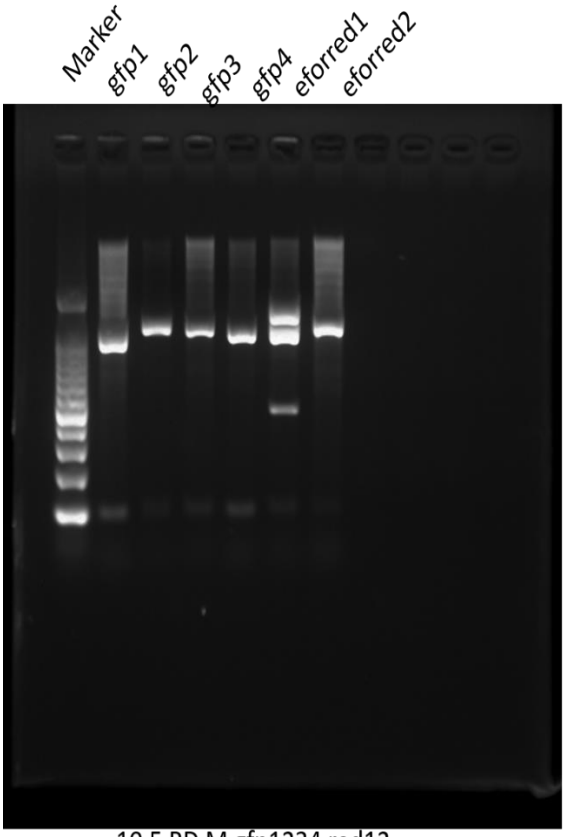
- Successfully elected correct eforred plasmid

Oct.5th

Plasmid extraction

Enzyme digestion using EcoR1 and Pst1

- One of effored was conducted successfully



10.5 RD M gfp1234 red12

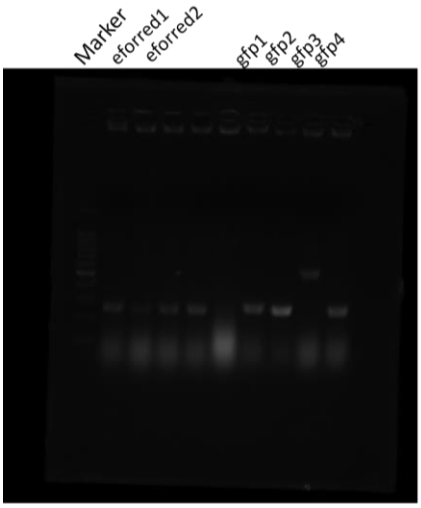
Protein purification and gel electrophoresis of hrp

- Successful

Oct.6th

Colony PCR

- Determined the correct colony

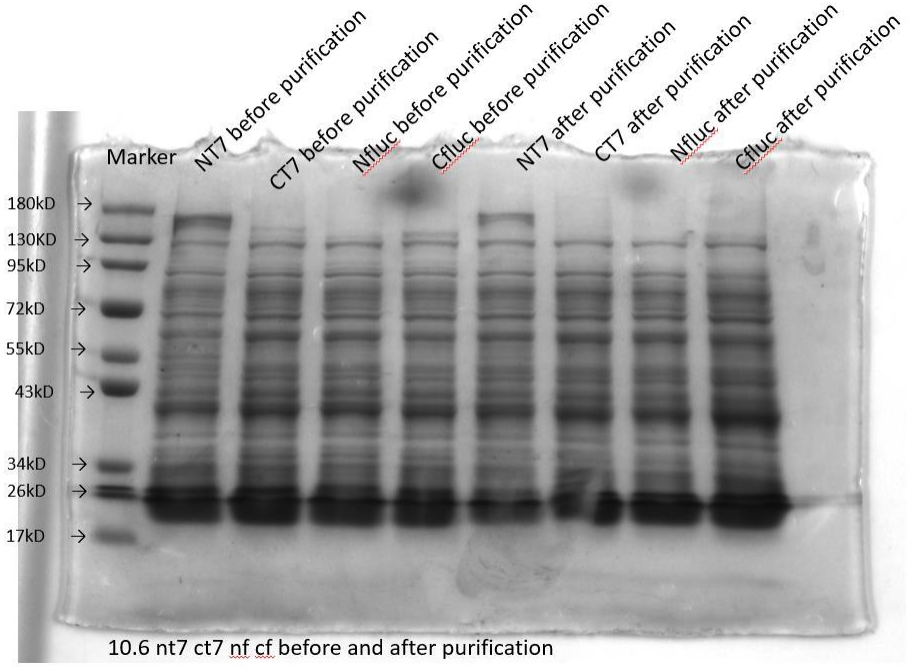


1006 eforRed 1234 GFP12345 colonypcr

Inoculation and pre-culture bacteria from colony PCR

Tried to find the reason of low concentration from protein purification using protein gel before and after protein purification from sds-page

- Ability of attaching protein on the tube was really low for the takara protein purification kit. Protein could not bind to the filter membrane easily



10.6 nt7 ct7 nf cf before and after purification

Protein induction

- Pet28a-shrpn-dCas9
- Pet28a-shrpc-dCas9
- CT7-dCas9
- NFluc-dCas9
- CFluc-dCas9
- NT7-dCas9

Tested the effect of concentrator

- Concentrator could not dilute protein with low molecular weight, also too much protein would block the filter memberane

Oct.7th

Collected the correct plasmid and enzyme digestion using EcoR1 and Pst1

- Psb1c3-amilgfp
- Psb1ce-eforred
- Successfully constructed

Extracted correct plasmid and sent to BGI for sequencing