The Whole Script Of
SMS: Laboratory Notebook

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7. 13

Evening.

100mL LB was heated to melt and cooled down by flowing water. Till temperature had came to about 40 Celsius degree, 100 μL Kanamycin was added. The solution was quick shaken and was separated into 5 Petri dishes. Waited till the plates became hardened. αD95, which contained plasmid expressing GFP under T7 promoter, was inoculated to one of these dishes. Operation mentioned above was done in Super Clean Bench, bacteria was cultured in 37 Celsius degree for overnight.

7. 14

Noon.

Single colony harvested from bacteria cultivated overnight was inoculated to 4 mL LB Medium (contained 4 μL Kanamycin)

Solution Preparation:

1L LB medium (10g Tryptone, 5g Yeast extract, 10g NaCl and pure water added to 1L) was made and separated into equally ten conical bottles (100ml for each). 5 of which were additionally supplied with 1.5g agarose powder each. 50 mL 50% glycerol was prepared through 25mL glycerol and water to 50mL. Solution were sterilized by heating to 121 Celsius degree.

Colony PCR

Colony PCR was carried by polymerase Fast Pfu

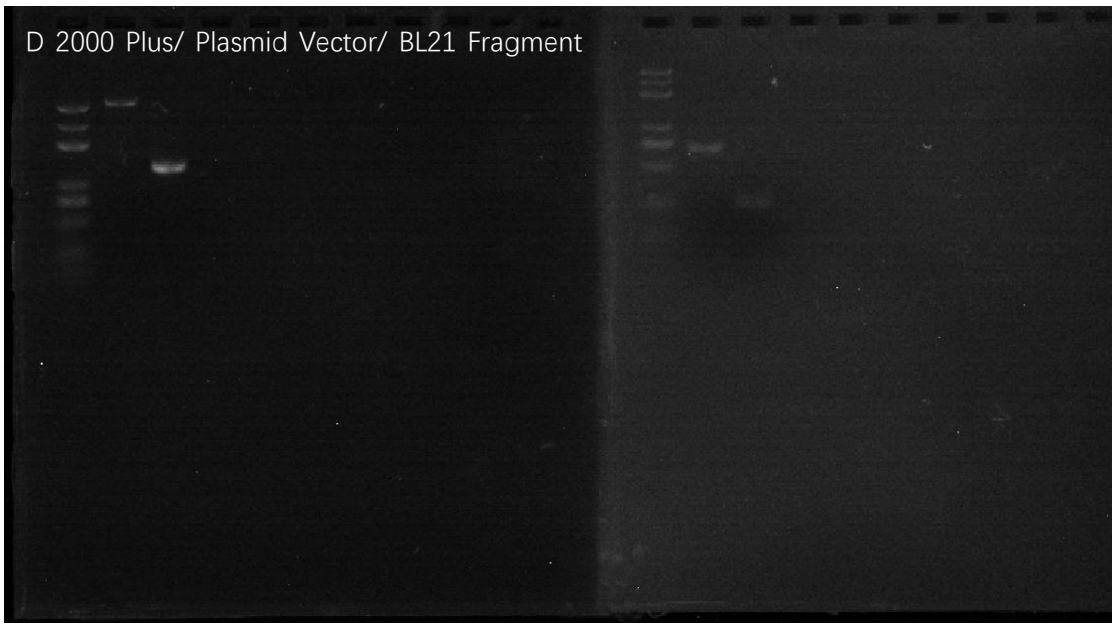
Target Sequence	Forward Primer	Reverse Primer	Template
0~210 (HpaB)	SMS-003	SMS-004	BL21
210~284 (HpaB)	SMS-005	SMS-006	BL21
284 to end (HpaBC)	SMS-007	SMS-008	BL21
Reverse PCR in plasmid vector	SMS-001	SMS-002	S059

Time for extension in each turn:

120s for S059; 25s for BL21 Fragment 3; 10s for BL21 Fragment 1 & 2

7. 15

Agarose Electrophoresis



Templates were digested by Dpa I (2μL digest enzyme + 18μL Cutsmart)

Sample Purification:

Sample Purification for PCR product was carried by Tiangen Sample Purification Kit, protocol followed.

Concentration measurement

The measurement was carried out by Nanodrop

Sample Type: dsDNA

	1		2	
A	Abs 260 -0.00075 OD / 280 -0.00045 OD	Value **0** ng/μl / **1.67** ratio	Abs 260 -0.000250002 OD / 280 0.00055 OD	Value **0** ng/μl / **NaN** ratio
B	Abs 260 0.16625 OD / 280 0.09235 OD	Value **166.25** ng/μl / **1.8** ratio	Abs 260 0.070949996 OD / 280 0.038749998 OD	Value **70.95** ng/μl / **1.83** ratio
C	Abs 260 0.03175 OD / 280 0.01785 OD	Value **31.75** ng/μl / **1.78** ratio	Abs 260 0.031949995 OD / 280 0.017349998 OD	Value **31.95** ng/μl / **1.84** ratio
D	Abs 260 OD / 280 OD	Value ng/μl / ratio	Abs 260 OD / 280 OD	Value ng/μl / ratio
E	Abs 260 OD / 280 OD	Value ng/μl / ratio	Abs 260 OD / 280 OD	Value ng/μl / ratio
F	Abs 260 OD / 280 OD	Value ng/μl / ratio	Abs 260 OD / 280 OD	Value ng/μl / ratio
G	Abs 260 OD / 280 OD	Value ng/μl / ratio	Abs 260 OD / 280 OD	Value ng/μl / ratio
H	Abs 260 OD / 280 OD	Value ng/μl / ratio	Abs 260 OD / 280 OD	Value ng/μl / ratio

BL21 Fragment

Plasmid Vector

BL21 Fragment

BL21 Fragment

Gibson Assembly:

Vector (1.8μL), BL21 Fragment 1/2/3 (0.3μL for each), 2.3μL Water, Gibson mix (2*) 5μL

7. 16

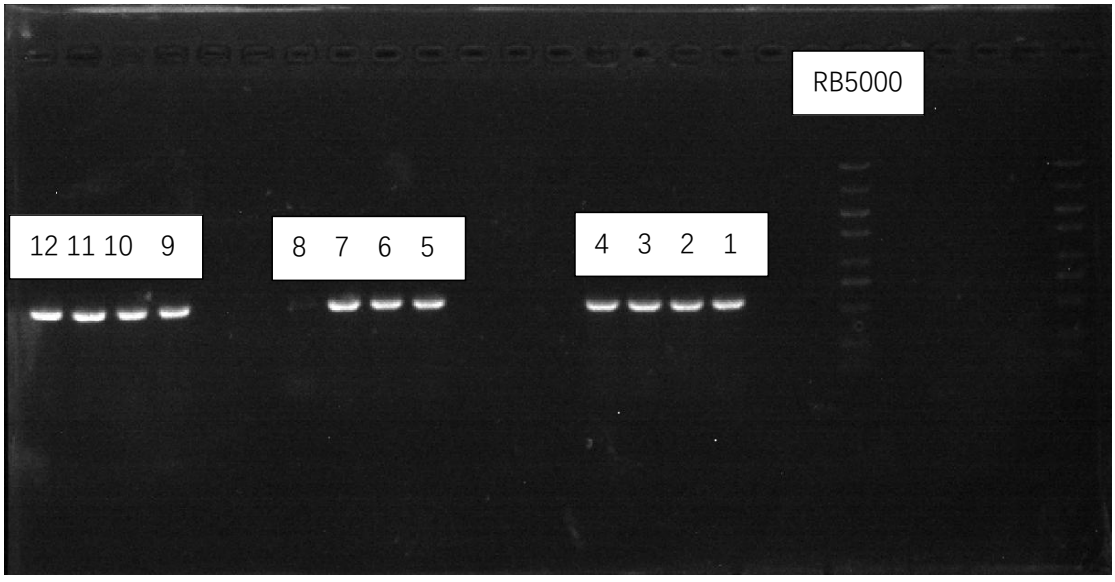
Fragments constructed by Gibson assembly were made to an inversion. 50μL competent cells were separated equally into three, each was bathed in ice for 30 minutes after products of Gibson assembly were added into the cells. 42 Celsius degree water bath for 45 seconds. 500μL LB medium was added for each and cells were cultivated for 1 hour in 37 Celsius degree shaker. 150μL of each were equally spread onto agar plates with the help of glass beads.

7. 17

Colony PCR

Colony PCR was carried by Taq mix in order to examine the product of Gibson Assembly.

Template	Forward Primer	Reverse Primer	Product
Colony	Primer Colony F	Primer Colony R	1,2,3,4,5,6,7,8,9,10,11,12
Colony	SMS_seq 001 F	SMS_seq 004 R	13,14,15,16,17,18,19,20



Another figure of gel which contained sample 13~20 was somehow lost. The results showed two stain for each sample. A probable explanation is additional sequence on E coli. Genome was targeted.

7. 18

Strain 18 & 20 selected before were restored as bacterium 1:1 proportional to 50% glycerol solution.

Plasmid extraction

Plasmids in 18/20 were extracted by Tiangen plasmid extracting kit.

Sample Type: dsDNA

	1				2			
	Abs		Value		Abs		Value	
A	260 0.0001	OD	0.1	ng/µl	260 0.000499999	OD	0.5	ng/µl
	280 0.0001	OD	1	ratio	280 0.000499999	OD	1	ratio
B	260 0.0629	OD	62.9	ng/µl	260 0.0558	OD	55.8	ng/µl
	280 0.0325	OD	1.94	ratio	280 0.028200002	OD	1.98	ratio
C	260 0.0151	OD	15.1	ng/µl	260 0.011400001	OD	11.4	ng/µl
	280 0.008	OD	1.89	ratio	280 0.006100001	OD	1.87	ratio
D	260	OD		ng/µl	260	OD		ng/µl
	280	OD		ratio	280	OD		ratio
E	260	OD		ng/µl	260	OD		ng/µl
	280	OD		ratio	280	OD		ratio
F	260	OD		ng/µl	260	OD		ng/µl
	280	OD		ratio	280	OD		ratio
G	260	OD		ng/µl	260	OD		ng/µl
	280	OD		ratio	280	OD		ratio
H	260	OD		ng/µl	260	OD		ng/µl
	280	OD		ratio	280	OD		ratio

7. 19

18 & 20 was each inoculated into 4mL LB medium.

Taq PCR was carried for sequencing.

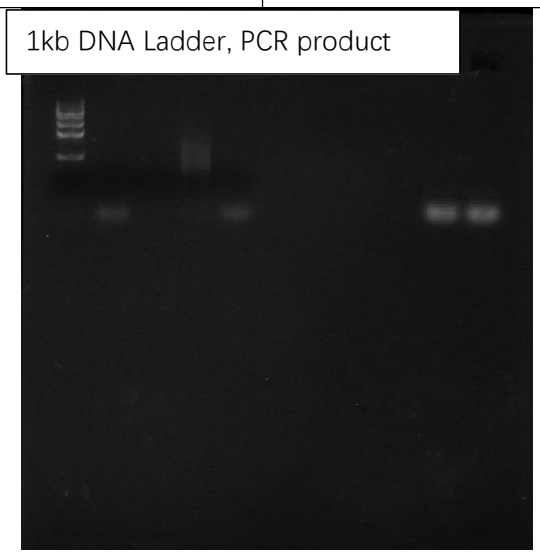
Template	Forward Primer	Reverse Primer	Product
Plasmid extracted from 18	SMS_seq 001 F	SMS_seq 004 R	Fragment of 18
Plasmid extracted from 20	SMS_seq 001 F	SMS_seq 004 R	Fragment of 20

Products were examined by agarose electrophoresis on an 1 % gel.

7. 20

Sequencing results had come out and showed plasmid 18 was what we exactly wanted. Therefore, further point mutation was carried by Fast pFu.

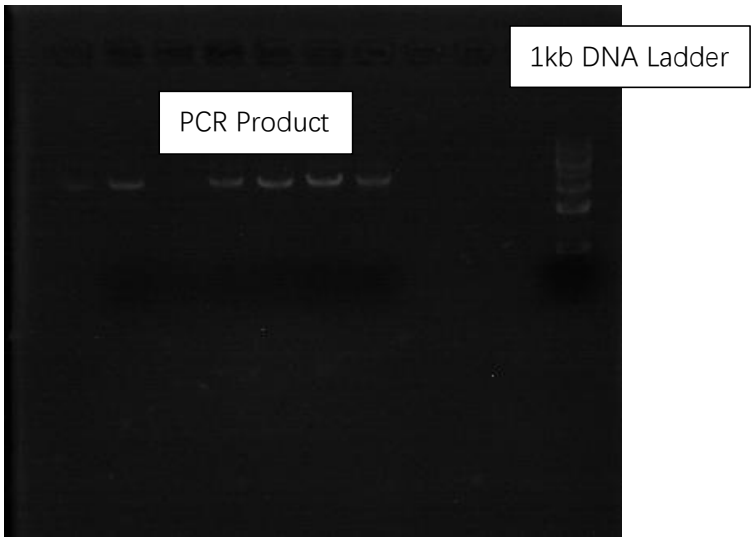
Forward Primer	Reverse Primer	Template	Extension time
SMS-009	SMS-010	Plasmid extracted from 18	20s



7. 21

Forward Primer	Reverse Primer	Template
SMS Gibson 9	SMS Gibson 10	Plasmid from 18
SMS Gibson 11	SMS Gibson 12	Plasmid form 18

Results were examined by agarose electrophoresis.



7. 22

Gibson assembly was carried with 0.6μL product of 9/10 (concentration 82.45 ng/μL), 1.7μL product of 11/12 (concentration 30.45 ng/μL) as DNA fragments. Products of Gibson assembly were inverted into αD95.

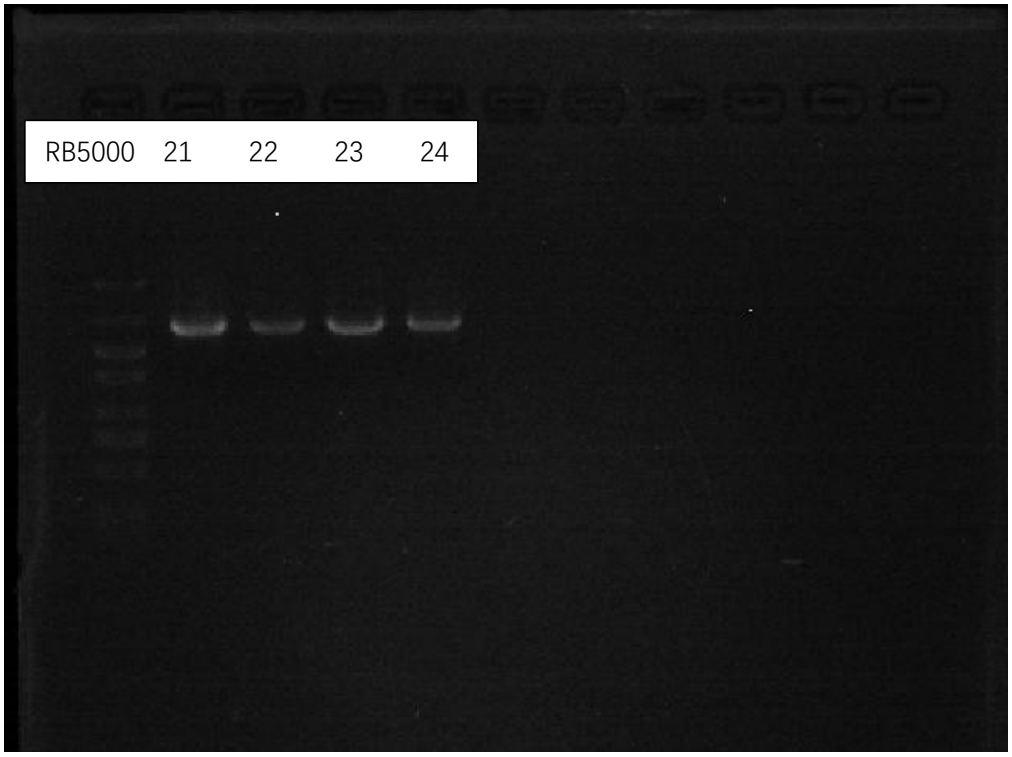
7.23

4 colony 21, 22, 23, 24 were picked and preserved on an agar plate.

PCR for sequencing was carried with Taq Mix.

Primer F	Primer R	Template
Seq 001F	Seq 004R	Colony 21, 22, 23 & 24

Agarose electrophoresis was made to examine the product.



Products were sent to sequencing as results showed good.

21, 22, 23, 24 were cultured in 5 mL LB medium in shaker overnight

7. 24

Plasmid extraction

Plasmid extraction was done by using Tiangen Mini Plasmid Kit. Plasmids were extracted from all the bacteria contained solution cultured overnight. All the products were each dissolved in 40μL water.

Solution were measured by Nanodrop.

21: 106.35ng/μL 22: 79.45ng/μL 23: 208.05ng/μL 24: 19.45ng/μL

Plasmid were transformed into BL21 competent cell by granting heat shock. After all the process, 200 μL bacteria contained medium were pipetted onto LB agar plate with kanamycin for

each. Agar plates were cultured overnight.

20200725

Based on the sequencing results, which showed plasmid 22 was a valid one.

2 colonies, named 25 and 26, were inoculated in 5 mL medium and cultured overnight in a shaker.

20200726

25 and 26 were preserved in 25% glycerol mix.

An additional BL21 strain was inoculated in medium.

20200727

L-dopa was firstly dissolved as 4 mM solution. 0μL, 22.5μL, 45μL, 67.5μL, 90μL, 112.5μL, 135μL, 157.5μL, 200μL were added into 96 well plate and supplied with water to 200μL to create a different concentration of 0mM, 0.5mM, 1mM, 1.5mM, 2mM, 2.5mM, 3mM, 3.5mM, 4mM.

Reaction was lasted for 30min, products were measured for OD400 by nanodrop.

< >	1	2	3	4	5	6	7	8	9
A	0.08869 9996	0.54269 9993	0.98170 0003	1.46140 0032	1.89370 0004	2.68729 9967	3.29169 9886	3.57879 9963	3.8455 9989

To prepare M9 medium, solutions were made.

80mL 50% Glucose (50*): 40g glucose

80mL 2.5M $MgSO_4$ (500*): 49.294g MgSO4*7H20

80mL 100mM CaCl2 (1000*): 0.88784g CaCl2

50mM 2.5% Yeast Extract (100*): 1.25g Yeast Extract

2*500mL H2O

Solution was each sterilized in 115 Celsius degree for 20 min.

20200728

0.271g tyrosine was tried to be dissolved in 30mL 50mM NaOH, but failed. It continued to fail when the concentration of NaOH was increased to 100mM. It succeeded when the concentration of NaOH had increased to 500mM.

The final concentration was 210mM (70*). 100μL solution was added into 7mL water, the pH value showed slightly above 7.

The solution was sterilized by 121 Celsius degree steam.

20200729

500mL (10*50mM) M9 medium was prepared by adding ingredients prepared before.

For each 50mM system: 1mL glucose solution, 100μL yeast extract solution, 100μL $MaSO_4$ solution and 50μL $CaCl_2$ solution.

Some LB (1000mL) was prepared as well, and equally distributed into 10 conical flasks. LB medium were sterilized by 121 Celsius degree steam for 20 min.

[Tyr]	0mM	0mM	1.5mM	3mM	6mM
Strain	BL21	pHpaBC	pHpaBC	pHpaBC	pHpaBC

20200730

1mL solution of each sample cultured overnight are centrifuged and 180μL supernatant were taken out and 20μL $NaIO_4$ was added for each. OD 400 test were taken by Nanodrop after 30 minutes reaction.

<>	1	2	3	4	5	
A	0.335799992	0.327499986	0.390399992	0.476799995	0.683600008	NaIO4
B	0.308999985	0.283199996	0.319799989	0.353100002	0.486200005	without NaIO4
C	0.335399985	0.344199985	0.4014	0.433600008	0.683200002	NaIO4
D	0.314099997	0.293900013	0.339599997	0.363099992	0.512300014	without NaIO4
Tyr concentration	BL21	0	1.5	3	6	

Since the results wasn't satisfying, we decided to try again by M9

BL21	BL21	BL21	BL21	BL21	pHpaBC	pHpaBC	pHpaBC	pHpaBC	pHpaBC	NA.	NA.
0mM TyR	1.5mM TyR	3mM TyR	4.5mM TyR	6mM TyR	0mM TyR	1.5mM TyR	3mM TyR	4.5mM TyR	6mM TyR		

This was repeated for three times in 3 lines of 96 well plate.

20200731

96 well plate was broken someone dropped it onto ground accidently.

High preservation PCR:

1, primer: T7N-F; 564N-R

2, primer: T7N-F; 179N-R

3, primer: 564C-F; T7C-R

4, primer: 179C-F; T7C-R

5, primer: vector F; vector R

Template: BL21

Extension time: 15s for 2 and 3; 90s for 1,4 and 5

Only 2 and 3 succeeded, examined by electrophoresis. However, they are discarded by accident

20200801

High preservation PCR:

1, primer: T7N-F; 564N-R

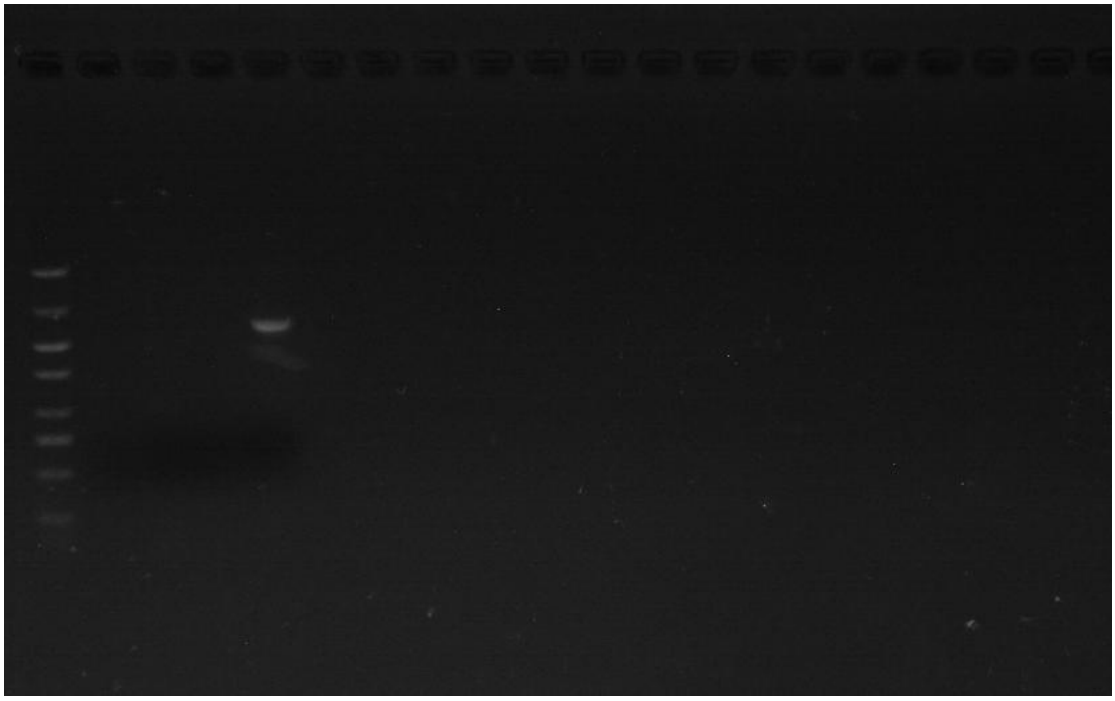
2, primer: T7N-F; 179N-R

3, primer: 564C-F; T7C-R

4, primer: 179C-F; T7C-R

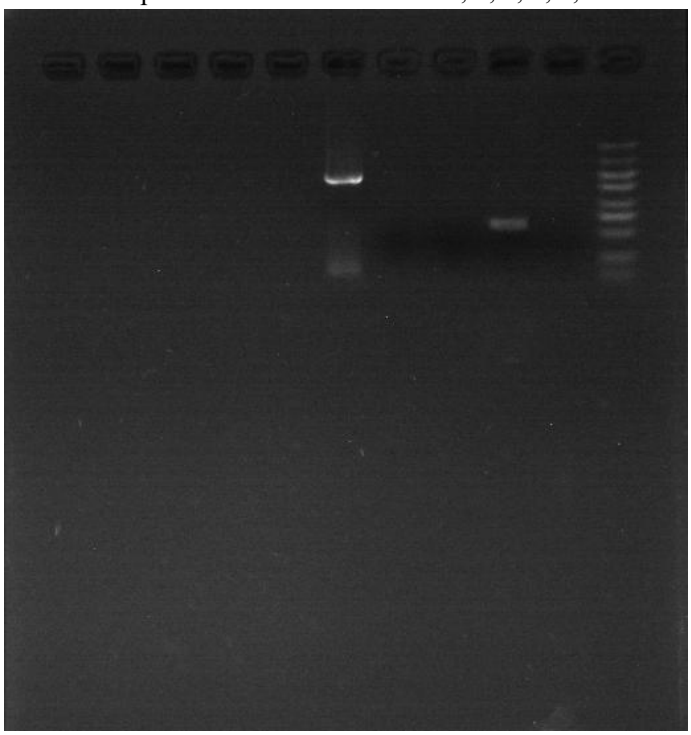
Template: BL21

Distribution: Marker; 2; 3; 5; 4



It is clear that, except lane 5 where fragment NO.4 resides, other lanes do not show positive outcomes. Thus, we conserve the successfully duplicated fragment NO.4. While debugging, we found that our devised primers for the amplification of plasmid framework have different temperatures of annealing, which could be the culprit to our constant failures. We thus re-devise primers for this sequence. For other fragments, we re-do the PCR.

Electrophoresis for the redo PCR: 1, 1, 3, 2, 1, Marker



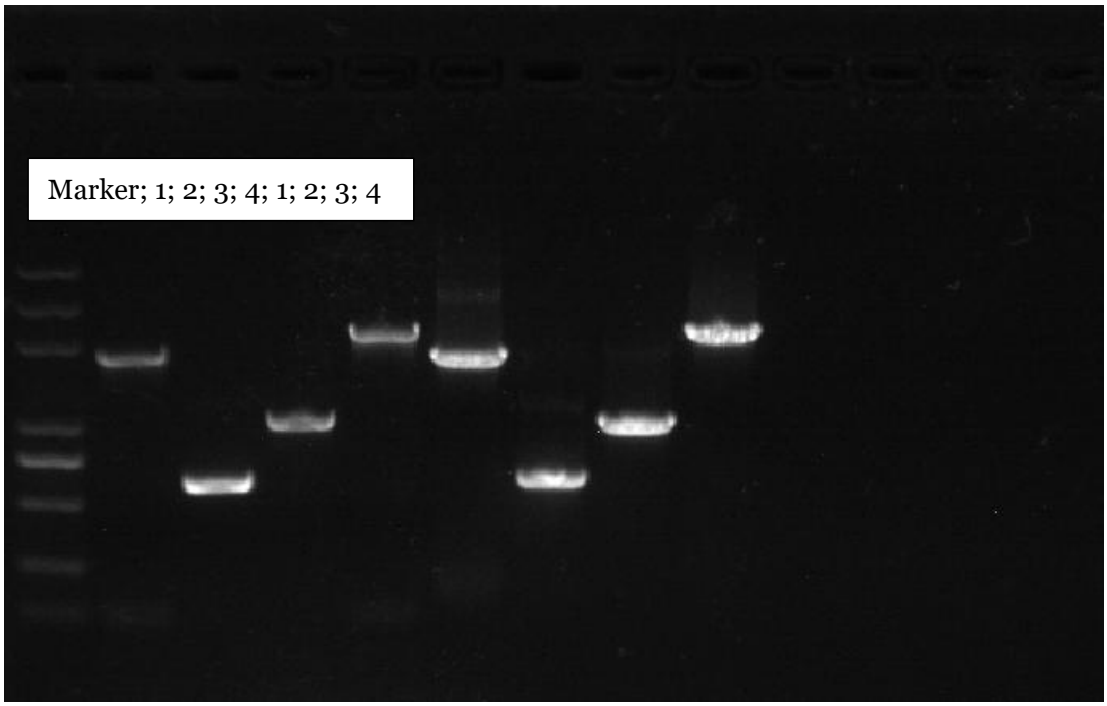
The results did not match our expectations. Failing to debug, we turned to our instructor, Kun Zhang, for help. She performed the PCR for us--when observing her operation, we recognized that, the FasPFU polymerase which we used had precipitated, and that the BL21 template which we

used contained a high concentration of glycerin. These were two reasons that were later considered as the major reasons that led to our constant failures.

The PCR was repeated by our tutor, Zhang.

20200802

Agarose Electrophoresis results:



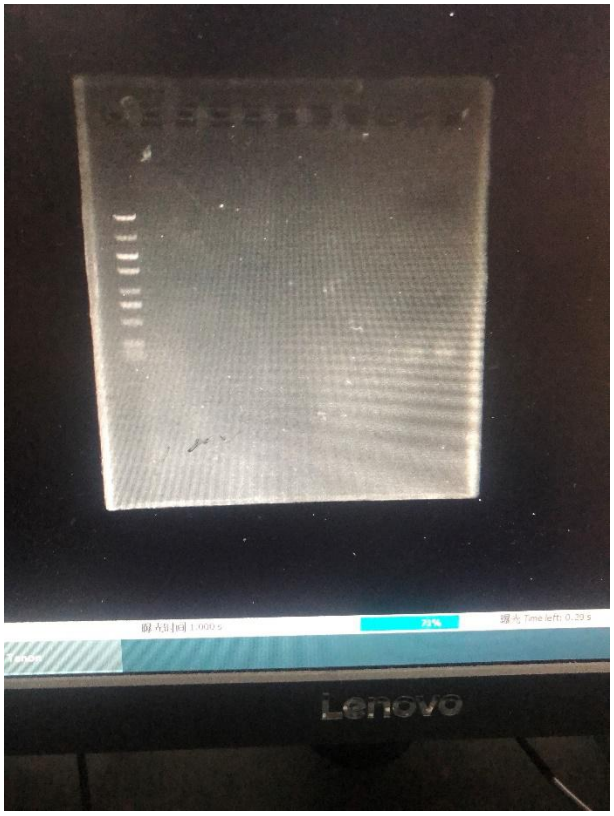
All the targeted fragments have been amplified. However, primer dimers appeared, which leads to a necessity for us to do a callback & purification of DNAs.

20200803

The re-devised primers for amplifying vector have arrived. We thus can conduct PCR experiment today.

PCR (extension time 90s) was applied on BL21 with primer Vector F-1 and Vector R to get the vector P70

The result was examined by agarose electrophoresis.



We saw no trace of PCR product--the shade below the picture could be primer dimer--given that we have never successfully amplified vector, there may be something wrong with the template.

20200804

Goldengate assembly were applied to the fragments. Products were transformed into competent cells. Final products were cultured on LB agar plates with CM.

20200805

The result showed the worst we expected. None was successful

A strain contained plasmid p70 (the vector we used) were preserved in 25% glycerol.

P70 plasmid was extracted from that by Tiangen plasmid extract kit and dissolved in 50μL water.

Levodopa standard curve was measured again with previous protocol.

<>	1	2	3	4	5
E	0.049899999	0.970899999	1.991600037	3.198299885	3.555900097
F	0.0515	1.037799954	2.029599905	3.307399988	3.64199996
G	0.0491	0.972100019	1.995300055	3.328500032	3.705499887
	0mM Tyr	1.5mM Tyr	3mM Tyr	4.5mM Tyr	6mM Tyr

The result showed a much gentle slope than reference (the one measured before as well).

20200806

Concentration of fragments yielded in previous PCRs were measured for concentration by Nanodrop.

1 (T7N (564)): 159.1ng/μL add 0.3μL in assembly

2: (T7N(179))374.6ng/μL add 0.2μL in assembly
3: (T7C(564))117.6ng/μL add 0.5μL in assembly
4: (T7C(179))167.1ng/μL add 0.3μL in assembly
5: (vector)57.4ng/μL add 1μL in assembly
6: (vector)90.3ng/μL
Fragments were mixed for Goldengate assembly.
Goldengate assembly system:
1: 2; B1; 4; 5
2: 2; B2; 4; 5
3: 2; B3; 4; 5
4: 2; B4; 4; 5
5: 1; B1; 3; 5
6: 1; B2; 3; 5
7: 1; B3; 3; 5
8: 1; B4; 3; 5
B1: VVD; B2: pMag-nMag; B3: MagFast-nMagHigh; B4: LOV

pHpaBC and BL21 were inoculated again to repeat the previous yielding test.

20200807

Goldengate products were transformed into competent cell. 200μL were inoculated onto CM LB agar plate. The bacteria were cultured overnight

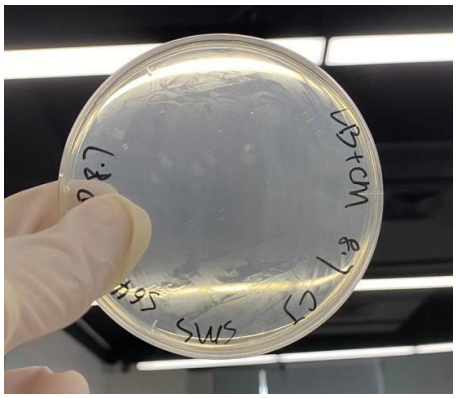
Standard curve was measured again

<>	1	2	3	4	5
A	0.041299999	0.99940002	1.952600002	3.223500013	3.7421
B	0.040199999	0.99970001	1.963799953	3.192699909	3.761899948
C	0.046500001	0.9903	2.007800102	3.247499943	3.787400007
	0mM Tyr	1.5mM Tyr	3mM Tyr	4.5mM Tyr	6mM Tyr

However, the results were abnormally low again. Though Bryan was not at lab at that time, he informed the operator, David, that the reaction time might be too short.

20200808

Goldengate failed again

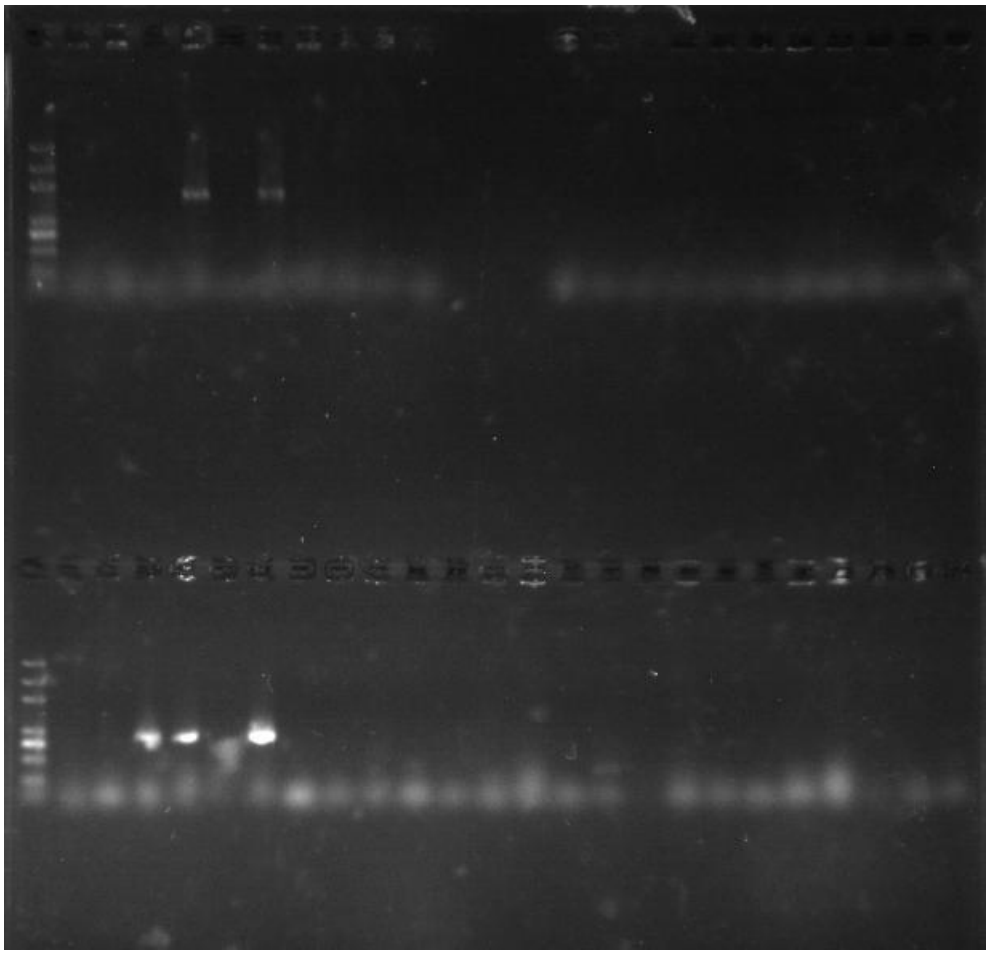


Previous PCRs were practiced again with Taq

1: 2; B1; 4; 5
2: 2; B2; 4; 5
3: 2; B3; 4; 5
4: 2; B4; 4; 5
5: 1; B1; 3; 5
6: 1; B2; 3; 5
7: 1; B3; 3; 5
8: 1; B4; 3; 5

20200809

PCR products were examined by agarose electrophoresis



The results were devastating despite .

Some more LB medium was prepared with previous protocol.

20200811

Levodopa yielding test was carried again with following distribution in a 96 well plate

0m M BL2 1	1.5m M BL21 LB	3m M BL2 1	4.5m M BL21 LB	6m M BL2 1		0mM pHpaB C LB	1.5mM pHpaB C LB	3mM pHpaB C LB	4.5mM pHpaB C LB	6mM pHpaB C LB	

LB		LB		LB							
0mM BL21 LB	1.5mM BL21 LB	3mM BL21 LB	4.5mM BL21 LB	6mM BL21 LB		0mM pHpaBC LB	1.5mM pHpaBC LB	3mM pHpaBC LB	4.5mM pHpaBC LB	6mM pHpaBC LB	
0mM BL21 LB	1.5mM BL21 LB	3mM BL21 LB	4.5mM BL21 LB	6mM BL21 LB		0mM pHpaBC LB	1.5mM pHpaBC LB	3mM pHpaBC LB	4.5mM pHpaBC LB	6mM pHpaBC LB	
0mM BL21 M9	1.5mM BL21 M9	3mM BL21 M9	4.5mM BL21 M9	6mM BL21 M9		0mM pHpaBC M9	1.5mM pHpaBC M9	3mM pHpaBC M9	4.5mM pHpaBC M9	6mM pHpaBC M9	
0mM BL21 M9	1.5mM BL21 M9	3mM BL21 M9	4.5mM BL21 M9	6mM BL21 M9		0mM pHpaBC M9	1.5mM pHpaBC M9	3mM pHpaBC M9	4.5mM pHpaBC M9	6mM pHpaBC M9	
0mM BL21 M9	1.5mM BL21 M9	3mM BL21 M9	4.5mM BL21 M9	6mM BL21 M9		0mM pHpaBC M9	1.5mM pHpaBC M9	3mM pHpaBC M9	4.5mM pHpaBC M9	6mM pHpaBC M9	

All concentration values refer to the concentrations of Tyr in each well.
After being cultured for 2h, IPTG were added 2μL for each.
The rest were cultivated overnight.

Goldengate products, pHpaBC and plasmids (B1, B2, B3, B4) were transformed into different competent cells where T7RP was expressed independently by heat shock.

To get a valid standard curve, David carried standard curve measurement again for a period. Varying concentrations were 0mM, 0.5mM, 1mM, 1.5mM, 2mM, 2.5mM, 3mM, 3.5mM, 4mM
Just after mixing:

<>	1	2	3	4	5	6	7	8	9
A	0.039999999	0.407400012	0.760200024	1.098500013	1.505900025	1.880599976	2.324899912	2.847100019	2.969899893
B	0.042199999	0.409099996	0.760900021	1.099300027	1.471600056	1.944599986	2.389899969	2.846899986	3.031899929

C	0.0421 00001	0.4081 99996	0.7674 00026	1.0977	1.5565 99975	1.9588 99975	2.3980 99899	2.9109 00116	3.0480 99995

40 minutes after mixing:

<>	1	2	3	4	5	6	7	8	9
A	0.0401 00001	0.4271 9999	0.8669 00027	1.2885 99968	1.8118 00003	2.3517 99965	3.0302 00005	3.6956 00033	3.7358 00028
B	0.0418	0.4323 99988	0.8653	1.2865 99994	1.7711 00044	2.4198 00043	3.1064 00013	3.6626 99938	3.7716 00008
C	0.0421 00001	0.4287 99987	0.8719 99979	1.2856 99964	1.8713 99999	2.4321 00058	3.1038 99956	3.7439 99958	3.7988 99889

60 minutes after mixing:

<>	1	2	3	4	5	6	7	8	9
A	0.0403 99998	0.4345 99996	0.8968 99998	1.3349 00022	1.8818 00056	2.4342 00048	3.1449 99981	3.8571 0001	3.93129 9925
B	0.0421 00001	0.4411 00001	0.8937 00004	1.3332 99994	1.8345 99972	2.5044 99912	3.2228 00016	3.8015 00082	OVER
C	0.0421 99999	0.4368 00003	0.9023	1.3314 00037	1.9378 0005	2.5174 00026	3.2321 0001	3.8847 0006	3.95370 0066

80 minutes after mixing:

<>	1	2	3	4	5	6	7	8	9
A	0.04060 0002	0.44330 0009	0.92849 9997	1.37520 0033	1.94000 0057	2.50710 0105	3.24480 0091	3.96149 9929	OV ER
B	0.04170 0002	0.44980 0014	0.91720 0029	1.37100 0051	1.89020 0019	2.58220 005	3.32319 9987	3.89910 0065	OV ER
C	0.04280 0002	0.44580 0006	0.92659 9979	1.36969 9955	1.99790 0009	2.59109 9977	3.33270 0014	OVER	OV ER

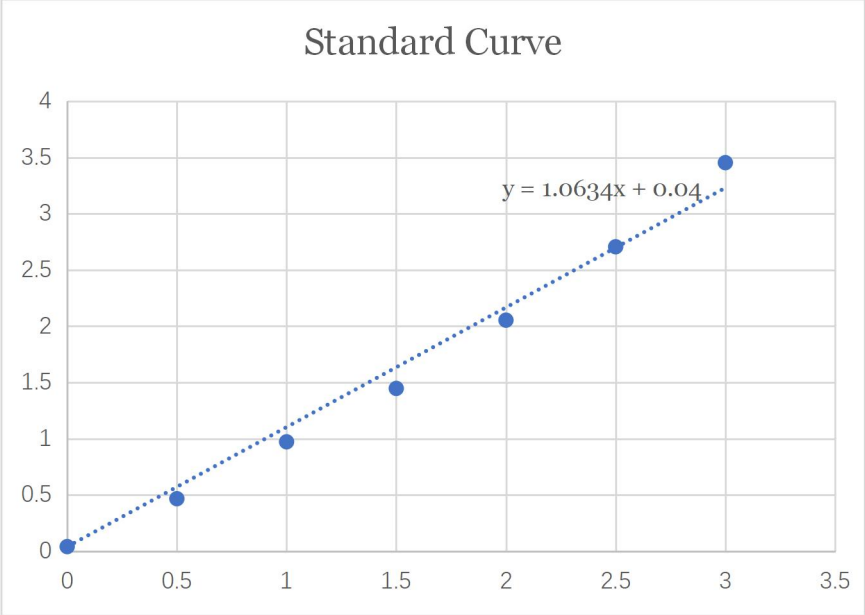
120 minutes after mixing:

<>	1	2	3	4	5	6	7	8	9
A	0.0403	0.45759 9998	0.96039 9985	1.42820 0006	2.01690 0063	2.60299 9926	3.34380 0068	3.98679 9955	OV ER
B	0.04210 0001	0.46610 0007	0.95260 0002	1.42519 9986	1.96510 005	2.68160 0094	3.42499 9952	3.96479 9881	OV ER
C	0.04170 0002	0.46000 0008	0.96179 9979	1.42279 9945	2.07809 9966	2.69039 9885	3.44289 9942	OVER	OV ER

150 minutes after mixing:

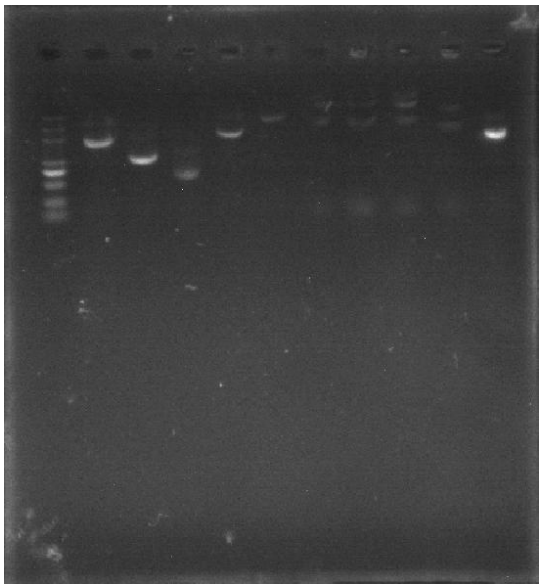
<>	1	2	3	4	5	6	7	8	9
A	0.04019 9999	0.46430 0007	0.97479 9991	1.44939 9948	2.05119 9913	2.65079 999	3.39420 0087	OVER	OV ER
B	0.04170 0002	0.47130 0006	0.96780 0021	1.44910 0018	1.99899 9953	2.73040 0085	3.48510 0031	3.98979 9976	OV ER
C	0.0418	0.46560 0014	0.97560 0004	1.44340 0025	2.11170 0058	2.73799 9916	3.48300 004	OVER	OV ER

A graph was illustrated for the last measurement.



20200812

All Goldengate failed



Previous BsaI products were examined by agarose electrophoresis.

RB5000; 1; 2; 3; 4; 6; B1; B2; B3; B4; 6

Some colonies of competent cell

OD600 measurement was carried for bacteria cultivated the day before by Nanodrop.

< >	1	2	3	4	5	6	7	8	9	10	11
A	1.038100004	1.059299946	1.039299965	1.015699983	1.045300007	0.046	1.226199985	1.250100017	1.207100034	1.196099997	1.085199952
B	1.103000045	1.072000027	0.999599993	1.013000011	1.064200044	0.0484	1.357900023	1.325799942	1.294999957	1.289899945	1.192199945

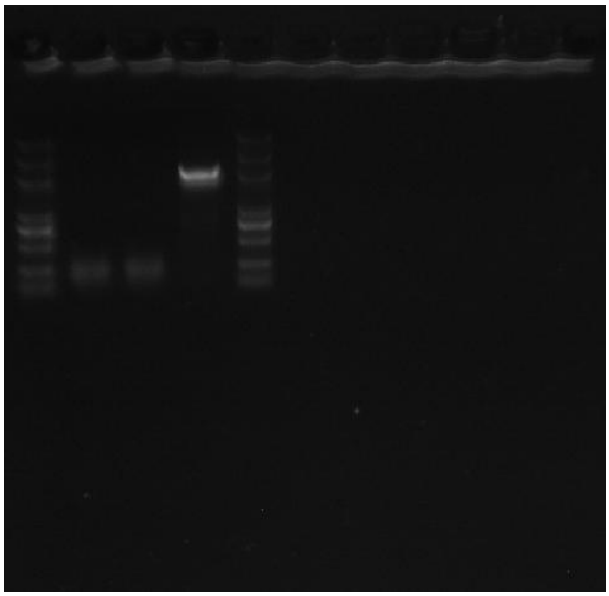
C	1.131100059	1.16229999 1	1.10699999 3	1.06009995 9	1.12039995 2	0.04699999 8	1.38320004 9	1.33449995 5	1.30820000 2	1.31690001 5	1.21500003 3
D	0.0471	0.04830000 2	0.0482	0.04760000 1	0.05079999 9	0.04699999 8	0.04720000 2	0.0471	0.04639999 9	0.04560000 1	0.04659999 9
E	0.34419998 5	0.31709998 8	0.2377	0.1426	0.04569999 9	0.04809999 8	0.23109999 3	0.21359999 5	0.06329999 9	0.04699999 8	0.04809999 8
F	0.19499999 3	0.31819999 2	0.28310000 9	0.27340000 9	0.22840000 7	0.04789999 9	0.2042	0.23520000 3	0.10469999 9	0.04859999 9	0.0546
G	0.14869999 9	0.18880000 7	0.27030000 1	0.31279999	0.28110000 5	0.04520000 1	0.07620000 1	0.22059999 4	0.04890000 1	0.04439999 9	0.0484

20200813

B1, B2, B3, B4 were extracted from bacteria cultivated.

Concentrations were measured by Nanodrop.

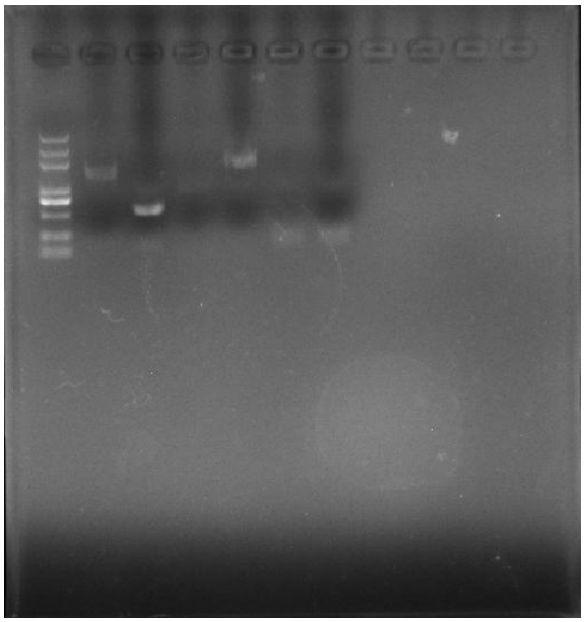
dsDNA_1		260	280	Conc ng/µl	Ratio	Sample ID
	C1(B1)	0.19085	0.0999	190.85	1.91	
	C2(B2)	0.14405	0.0747	144.05	1.93	
	D1(B3)	0.12465	0.0651	124.65	1.91	
	D2(B4)	0.15235	0.0811	152.35	1.88	



Products were examined by agarose electrophoresis (Marker, 1, 2, 3, 4, 6, B1, B2, B3, B4, 4)

20200814

PCR was carried based on templates (1; 2; 3; 4; 5; 6)



BsaI digestion (37 Celsius degree for 1 hour and 80 Celsius degree for 20 minutes) was carried on B1, B2, B3, B4

20200815

The very same cultivation was carried again.

0mM BL21 LB	1.5mM BL21 LB	3mM BL21 LB	4.5mM BL21 LB	6mM BL21 LB		0mM pHpaBC LB	1.5mM pHpaBC LB	3mM pHpaBC LB	4.5mM pHpaBC LB	6mM pHpaBC LB	

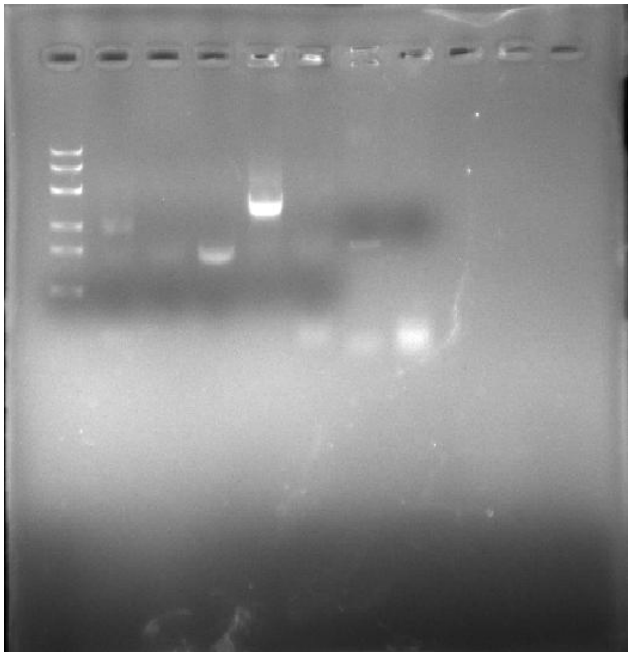
0mM BL21 LB	1.5mM BL21 LB	3mM BL21 LB	4.5mM BL21 LB	6mM BL21 LB		0mM pHpaBC LB	1.5mM pHpaBC LB	3mM pHpaBC LB	4.5mM pHpaBC LB	6mM pHpaBC LB	
0mM BL21 LB	1.5mM BL21 LB	3mM BL21 LB	4.5mM BL21 LB	6mM BL21 LB		0mM pHpaBC LB	1.5mM pHpaBC LB	3mM pHpaBC LB	4.5mM pHpaBC LB	6mM pHpaBC LB	
0mM BL21 M9	1.5mM BL21 M9	3mM BL21 M9	4.5mM BL21 M9	6mM BL21 M9		0mM pHpaBC M9	1.5mM pHpaBC M9	3mM pHpaBC M9	4.5mM pHpaBC M9	6mM pHpaBC M9	
0mM BL21 M9	1.5mM BL21 M9	3mM BL21 M9	4.5mM BL21 M9	6mM BL21 M9		0mM pHpaBC M9	1.5mM pHpaBC M9	3mM pHpaBC M9	4.5mM pHpaBC M9	6mM pHpaBC M9	
0mM BL21 M9	1.5mM BL21 M9	3mM BL21 M9	4.5mM BL21 M9	6mM BL21 M9		0mM pHpaBC M9	1.5mM pHpaBC M9	3mM pHpaBC M9	4.5mM pHpaBC M9	6mM pHpaBC M9	

High preservation PCR was carried with Down PCR program

Template	Primer F	Primer R	Extension Time
1	T7N-F	T7N-564-R	80s
2	T7N-F	T7N-179-R	80s
3	T7C-564-F	T7C-R	80s
4	T7C-179-F	T7C-R	80s

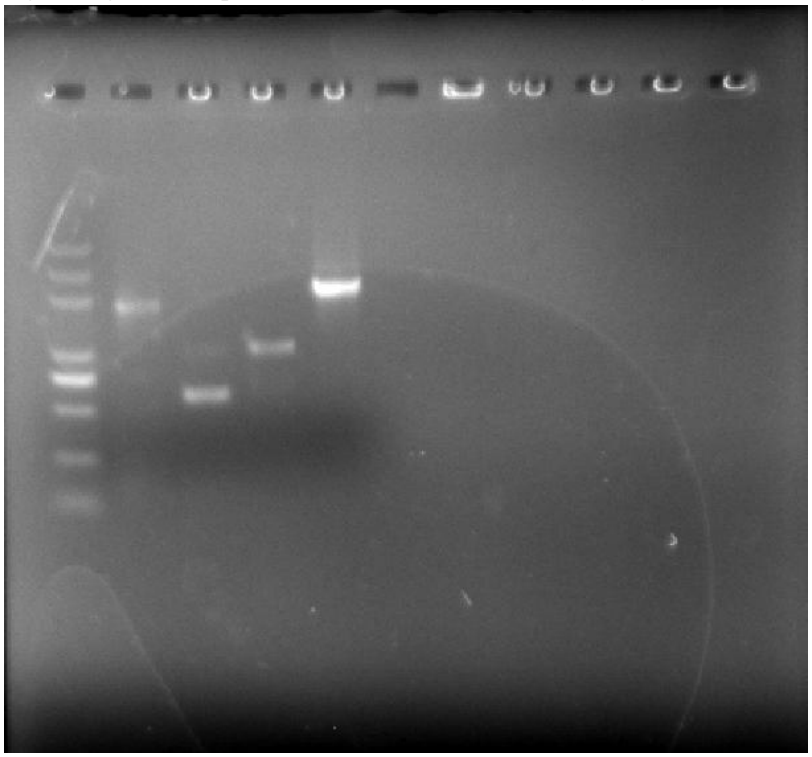
Taq PCR were carried for template 3 and template 6 (Primers: vector-F & vector-R)

The products were examined by agarose electrophoresis:



20200816

Agarose gel electrophoresis examination before recycling:



BsaI products were recycled by Tiangel Purification Kit.

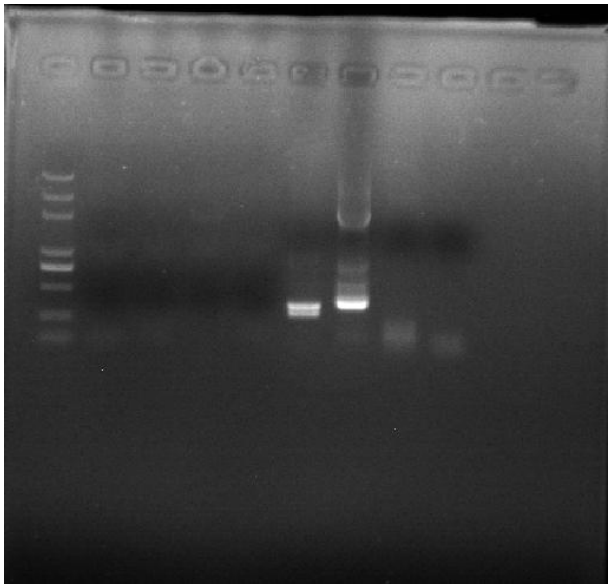
20200818

Template	Forward primer	Reverse Primer
1+3+B1	T7N-F	T7N-F
2+4+B1	T7C-R	T7C-R

5	Vector-T7-F	Vector-T7-R
P70	Vector-T7-F	Vector-T7-R

Taq PCR were carried

Agarose gel electrophoresis was carried to examine purification products and PCR products.



Distribution: DL5000; 1; 2; 3; vector; 1+3 B1; 2+4 B1; 5; P70

20200819

	1	2
C	ddH2O	ddH2O
D	P70	22
E	1	2
F	3	4

500μL P70+500μL 50%glycerol →-20%

Purification & Plasmid Extraction

Done by Tiangen plasmid extraction Kit

Products are measured by ? for DNA concentration

Electrophoresis (1% agarose

Marker

Trans5K PCR product

Fly	1μL
Fly Buffer	20μL
F	2μL
F	2μL
dNTPs	8μL

DNA	0.1μL
H2O	66μL

2×Taq Mix	15μL	390(×26)
F	2μL	25μL
R	2μL	25μL
H2O	11μL	290μL

20200821

1) Delete three base pairs

			templates
①	DEL-F/DEL-R	A 7.3kb	pHpaBC-22
②	DEL-F/DEL-R1	B 2.7kb	pHpaBC-22

2.1+2.2 Gibson ①transform directly

2) Change the source of HpaBC

③	SMS-003/SMS-008	B 2.1kb	pHpaBC from 家恒	Fragment of HpaBC
④	SMS-001/SMS-002	A 5.2kb	pHpaBC-22/S059	Vector-

3) Mutate the locus of HpaBC (from MOPU)

⑤	Locus 201	SMS-003/004	C659bp	pHpaBC from 家恒片段
⑥	Locus 201 284	SMS-006/210F-F	247bp	
⑦	Locus 284	SMS-007/008	1286bp	

5、6、7+4 Gibson

4) T7+Photoswitches

Vector-T7-R1/vector-T7-F	3.5kp	P70
T7N-F1/T7N-564-R1	1.5kp	BL21

DpnI dispose
Agarose gel extraction: Vector T7N 5
DNA purification: 2.3.4.6

Fragment1	
Cutsmart	5μL
DNA	50μL
DPnI	1μL
H2O	4μL

37 Celsius degree 1h

1. Take 5μL, and then do the purification

2. Take 3μL Gibson+2μL H2O

20200824

1. Gel extraction fragment Vector "5" T7N-564

2. Gibson assembly 3+4:

   Agarose gel: 100mL TAE buffer+1g agarose powder+ dye (brown bottle) Gold View

PCR product/ restriction enzyme cutting product
Color: sample
No Color: PCR/3μL product of restriction enzyme cutting+ 2μL loading buffer (containing glycerin dye)

1. Take 2μL loading buffer

2. Take 3μL sample, and then mix it with the loading buffer

3. Mix the solution with pipette

4. Sampling

Gibson

Vector:	50ng	inset	20ng
	2×Gibson Mix		5μL
	4 (50ng)		0.2μL
	3 (20ng)		1.5μL
	H2O		3.3μL

50°C 1h

Transformation: 3+4 pET28a-HpaBC-DDC (→put into the 4°C fridge)

1. Pipette 50μL→3+4

2. Take 2μL pE728 a-HpaBC-DDX→??

Ice bath 30min (clean bench)

42°C (metal bath) 45s

After that, put it right on the ice 2min

On the clean bench, 500μL LB/tube

37°C recovery 1h

20200827
Bacteria culture DEL-3bp ①②③ 500μL+μL glycerol 50%
Plasmid extraction
Measure the concentration, gel extraction

30μL/tube			8?
	2×Taq Mix	15μL	120
	F	1μL	8μL
	R	1μL	8μL
	H2O	13μL	104μL
Tm	55°C	72°C	1：20s

Plasmid: 0.5
5000rpm 1min
Discard 250μL supernatant
Repetitive blowing and suction
Resuspend thallus
Spread the solution all over the plate

10×T4 ligase Buffer 1μL			
T4 ligase 0.5μL			
BsaI 0.5μL			
① T7N564 1μL	② T7N179	③ T7N564-LOV	④ T7C-179-LOV
⑤ T7C564 1μL	⑥ T7C179	⑦ T7C-564-LOV	⑧ T7C-179-LOV
Vector 1μL	Vector	Vector	Vector
(9)	(9)	(12)	(12)
(37°C 5min 16°C 10min)×10			
37°C 10min	50° 5min	85°C 5min	16°C ∞

20200828

PCR: 16 tubes
Blue pen marked: 1-16
Positive control: “plasmid 22” +0.5μL
Primer: SMS-seq 001F & cexu-HpaBC-R
Chemical transformation: 564-B1 179-B1
564-B4 179-B4
Gel preparation
Cell culture

20200829
Bacteria colony PCR
3+4replace HpaBC DEL3 3+4 replacement

Taq Mix		
	7.5μL×30=225μL	
F	0.5μL×30=15μL	
R	0.5μL×30=15μL	
H2O	6.5μL×30=195μL	15μL×30

Taq Mix	13oμL	25μL×5=125	Tm 55°C
F	10μL	2μL	72°C 1‘30s
R	10μL	2μL	
H2O	110μL	21μL	
H2O	260μL	50μL	

T7 polymerase divide into two parts: (two locus) 564 179	
A: 564-VVD-VVD	E: 179-pMag-nMag
B: 564-pMAG-nMAG	F: 179-pMagFast-nMagHigh
C: 564-pMagFast2-nMagHigh	G: 564-LOV
D: 179-VVD-VVD	H:179-LOV

A: 564-VVD-VVD	(4)
B: 564-pMag-nMag	(12)
C: 564-pMagFast2-nMagHigh	(19)
D: 179-VVD-VVD	(27)(29)(31)
E: 179-pMag-nMag	(39)
F: 179-pMagFast2-nMagHigh	(41)
G: 564-LOV	(50)(55)
H: 179-LOV	(63)(64)

Design the mutated primer: HpaBC from MODu & substitutional primer 1 SMS-008-F2

	Negative	Positive	Experiment
0	×	×	×
2h	×	✓	✓

3 4h	×	✓	✓
5 6	×	✓	✓
7 8	×	✓	×
9 10	×	✓	×
11 12	×	✓	×

17:00→2groups Without light (negative)

With light (experiment)

After the sampling, make sure that the experiment group is kept in dark.

19:00→3groups Without light (negative)

Experiment

21:00 With light (positive)

23:00 take 3 examples each from 3 groups

Plasmid extraction 2

transformation BL21

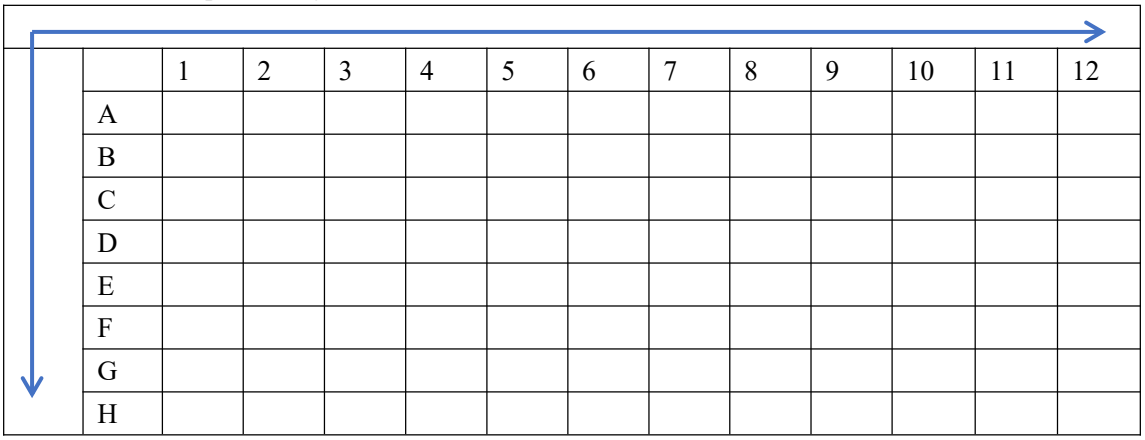
T74A1

	Negative	Positive	Experiement		200μL/hole
0	×	×	×		
2	×	✓	✓	S2	600μL
4	×	✓	✓	S4	
6	×	✓	✓	S6	
8	×	✓	×	S8	1.2mL
10	×	✓	×		
12	×	✓	×		
24	×	✓	×		

Every sample:

1. Centrifuge, and abandon the supernatants (12000rpm, 15min)
2. Add 600μL PBS, and resuspend
3. Centrifuge, and abandon the supernatants; then, add 600μL PBS, resuspend
4. Add samples to ELIASA

Direction of sample adding



	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

① S2-S10 5 packets

② S12,S24 positive 8, 10 , 12 5packets

③ Positive 24, negative6-12 5packets

④ Negative 24, blank 2packets

WT2-14	WT2-21	WT2-30	WT2-38	10mL LB+Kan+CM
MD6-14	MD6-21	MD6-30	MD6-38	

2 tubes for each sample: 10mL LB+ Kan+ 20μL bacteria solution
T74A1-WT2 T74A1-MD6
1 tube for each sample: 10mL LB (without antibiotics) +20μL bacteria solution
T74A1 10μL
1 tube for each sample: 10mL LB (without antibiotics) +20μL bacteria solution
Culture the bacteria separately with pipette

T74A1 T74A1+WT2 T74A1+MD6
Put the bacteria colony into the 1mL PBS and then resuspend it
400μL PBS+100μL bacteria solution vortex
Take 200μL/hole, add it into the ELIASA
Every sample needs to be measured twice, and then measure OD600

Supernatant: take 450μL and then add 50μL NaI2O4 solution
After the vortex, take 200μL/hole and add samples to ELIASA
Every solution needs to be measured twice, and then measure OD400

With light			WT2/4			21		
Without light			7			16		
With light	WT214	21	30	38	74	MD62	30	38

	①	②	③	④	⑤	⑥	⑦	⑧
Without light	WT214	21	30	38				

8h

5: WT2 21+

12: WT2 30-

13: WT2 74+

14: MD6 21-

15: WT2 14-

		(1)	(2)	(3)	(4)
With light	WT2	14	21	30	38
		(5)	(6)	(7)	(8)
	MD6	14	21	30	38
		(9)	(10)	(11)	(12)
Without light	WT2	14	21	30	38
		(13)	(14)	(15)	(16)
		14	21	30	38
		(17)	(18)	(19)	
		T74A1	T74A1-WT2	T74A1-MD6	

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	5	6	7	8	9	10	11	12
B	1	2	3	4	5	6	7	8	9	10	11	12
C	13	14	15	16	17	18	19	8h				
D	13	14	15	16	17	18	19					
E	1	2	3	4	5	6	7	8	9	10	11	12
F	1	2	3	4	5	6	7	8	9	10	11	12
G	13	14	15	16	17	18	19	16h				
H	13	14	15	16	17	18	19					

B plate 24h 28h

C plate 32h 44h

C plate: A-B: 44h 10/04 9:38

C-D: 28h