# Cloning

Strong Promoter + Strong RBS + ALDH2*1 (normal) + Double Terminator (K2539100)

2018-05-28

- ALDH2 size is correct
-

PCR 0530 ALDH2 Bob + Cm Backbone (Taq)	05/30/2018

Notes:
Bob = IDT ALDH2 Wildtype
- 2600 bps
- +300 bps primer annealing
- 2900 bps total

Vector = Ligation control (ChromoProtein Cm Backbone)
- 800 bps
- +300 bps primer annealing
- 1100 bps total

Ladder (kb), BBa_K2539100, Control, ~ 2.1kb

Link to file:

-

2018-05-24
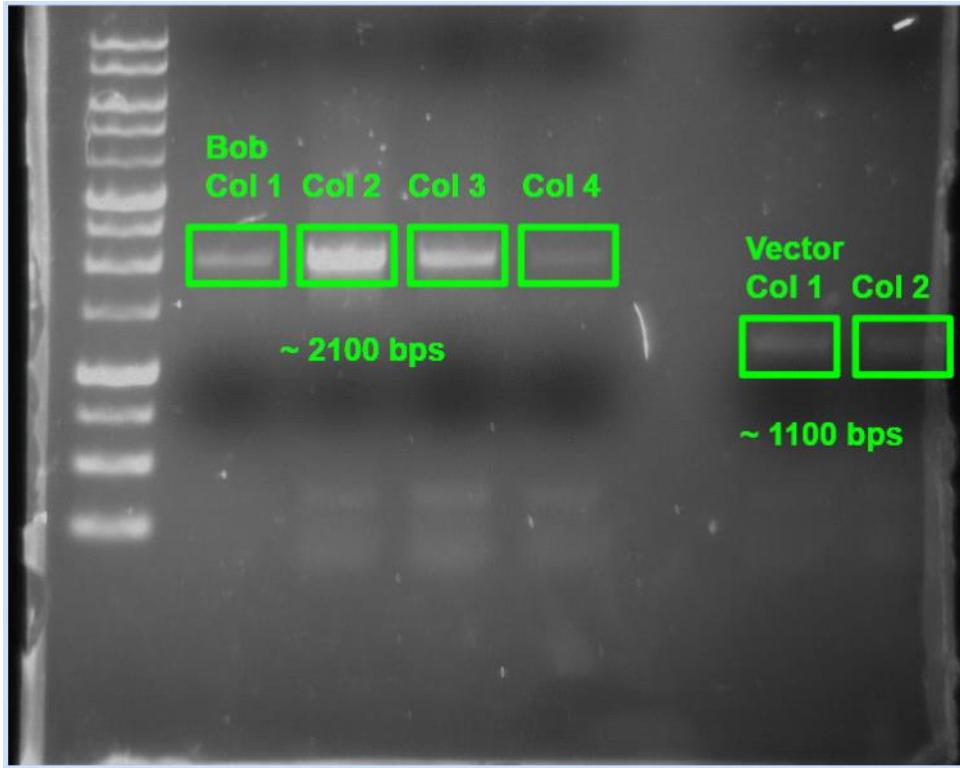
- PCR check 2 in 1 (Gel and Restreak Plates)
  - Taq polymerase
  - VF2 + VR

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min

Final Extension	72 C	7 min
Hold	4 C	

- Found colony 2 and 3 to be successful
  - Sequenced colony 2
  - COLONY 2 sequence was correct

2018-05-23

- Ligate Insert+backbone at E and P

- Transformed plasmid into Competent cell

2018-05-22

- Miniprepped BOB plasmid and Yellow Chromoprotein plasmid
- Digested BOB plasmid (at E and P) and Yellow Chromoprotein plasmid with Cm biobrick backbone

Digestion Calculator V2.0

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

ORF Insert Type	Front ▼	
Insert/ORF	202	ng/ul
Vector/Backbone	75.2	ng/ul

^^^Enter Concentration^^^

Vector/Backbone		
DNA Sample	**13.30**	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	**3.70**	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	**4.95**	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	**12.05**	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

- Ran gel to separate out DNA
- gel extracted BOB insert and Cm backbone

2018-05-21

-transform IDT ALDH2 Wildtype sequence into competent cell
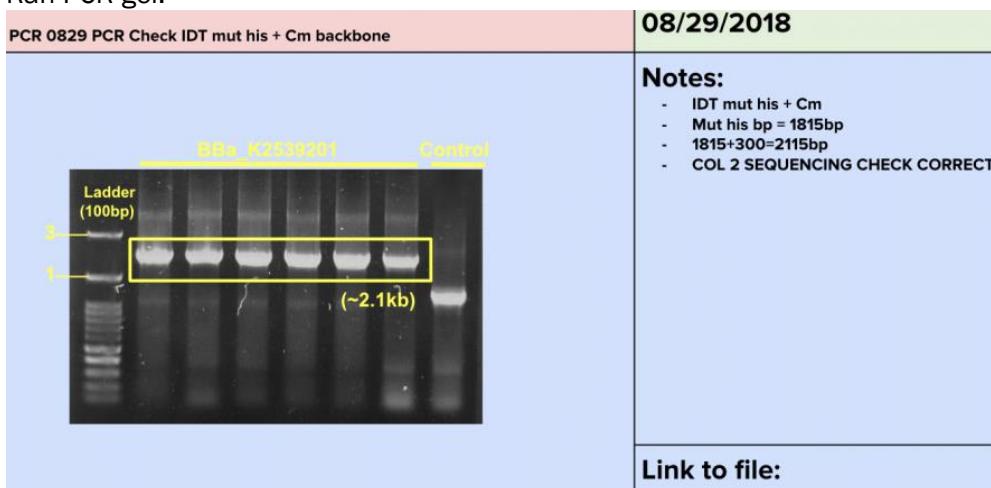
Strong Promoter + Strong RBS + 6x HIS + ALDH2*1 + Double Terminator (K2539101)

2018-09-03

- Sequencing received; sequence correct with all the cutting sites

2018-09-01

- Ran PCR gel:

PCR 0829 PCR Check IDT mut his + Cm backbone	08/29/2018

Notes:
- IDT mut his + Cm
- Mut his bp = 1815bp
- 1815+300=2115bp
- COL 2 SEQUENCING CHECK CORRECT

Link to file:

- Miniprepped colony 2, 3, 4 in water to send for sequencing

2018-08-31

- PCR Check 3-1 for IDT BOB HIS

2018-08-30

- ○ Gel extraction of the gene fragment
- ○ Ligation of IDT BOB HIS + Cm backbone (from chromoprotein)
- ○ Ligation Calculator

Ligation Calculator

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

	Conc. (ng/ul)	Length (bp)
Insert/ORF	7.0	1815
Vector/Backbone	11.7	2070

^^^Enter concentration & DNA length^^^

Ligation	
Vector/Backbone	3.71 ul
Insert/ORF	13.29 ul
Buffer	2.00 ul
Enzyme	1.00 ul
Water	0.00 ul
Total	20.00 ul

Ligation Control	
Vector/Backbone	3.71 ul
Insert/ORF	0.00 ul
Buffer	2.00 ul
Enzyme	1.00 ul
Water	13.29 ul
Total	20.00 ul

- ○ Transformation

2018-08-29

- Gene fragment of IDT ALDH2 Mut His Tag arrived
- Directly into digestion
- E and P
- 1000ng -----> diluted 20ul into 500ng/ul
- Took 5ul of 500ng/ul and digested with E and P
- Digestion Calculator
-

Insert/ORF	
DNA Sample	5.00 ul
Enzyme E	0.50 ul
Enzyme S	0.50 ul
10X NEB Buffer 2.1	2.00 ul
Water	12.00 ul
Total	20.00 ul
DNA Amount ✓	1000 ng

-
- Ran gel and saw correct gene fragment length at ~1800bp and cut the gel

ALDH2*1 basic part(K2539150)

2018-08-14

- Sequencing confirmed, all three colonies were correct
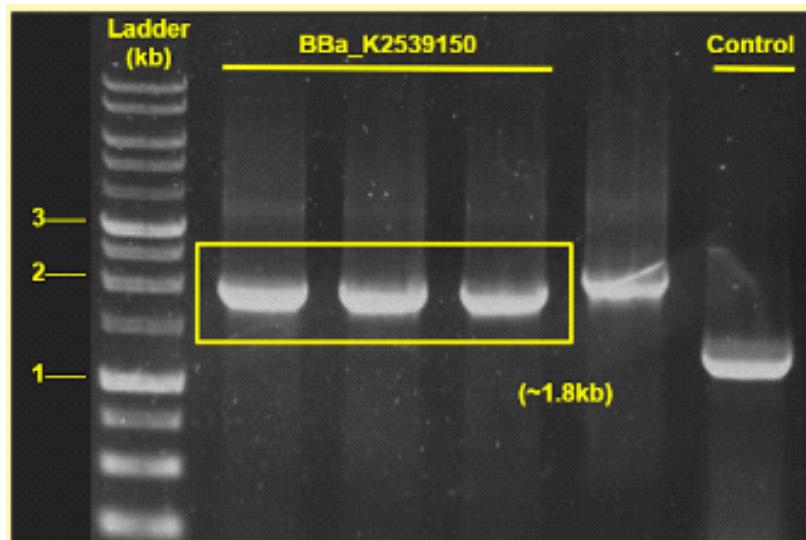
2018-08-12

- Miniprepped 2 LCs and sent for sequencing

2018-08-11

- PCR check 3-1
- PCR gel
-

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

-

-

2018-08-10

- Ligation with cm backbone, transformation

	Conc. (ng/ul)	Length (bp)
Insert/ORF	1.9	1500
Vector/Backbone	10.1	2000

^^^Enter concentration & DNA length^^^

Ligation	
Vector/Backbone	1.54 ul
Insert/ORF	15.46 ul
Buffer	2.00 ul
Enzyme	1.00 ul
Water	0.00 ul
Total	20.00 ul

Ligation Control	
Vector/Backbone	1.54 ul
Insert/ORF	0.00 ul
Buffer	2.00 ul
Enzyme	1.00 ul
Water	15.46 ul
Total	20.00 ul

2018-08-09

- Digestion, gel, gel x to cut out e and p

ORF Insert Type	Front ▼	Insert
Insert/ORF	56	ng/ul
Vector/Backbone	75.2	ng/ul

^^^Enter concentration^^^

Vector/Backbone	
DNA Sample	13.30 ul
Enzyme E	0.50 ul
Enzyme X	0.50 ul
10X NEB Buffer 2.1	2.00 ul
Water	3.70 ul
Total	20.00 ul
DNA Amount ✓	1000 ng

Insert/ORF	
DNA Sample	17.00 ul
Enzyme E	0.50 ul
Enzyme S	0.50 ul
10X NEB Buffer 2.1	2.00 ul
Water	0.00 ul
Total	20.00 ul
DNA Amount ✗	952 ng

*[DNA] too low, digest everything

↓

Recipe for 'Digesting Everything'

DNA Sample	17.9 ul
Enzyme E	0.50 ul
Enzyme S	0.50 ul
10X NEB Buffer 2.1	3.00 ul
Water	8.10 ul
Total	30.00 ul
DNA Amount	1002.4 ng

-

-

2018-08-08

- PCR, PCR gel

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	
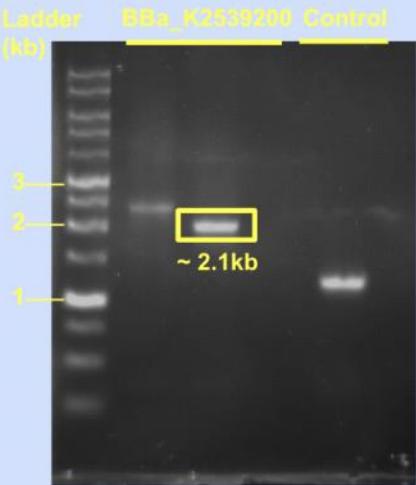
2018-08-07

- Primers ordered for cutting out BOB Basic

訂單資料

本次訂單號碼	Q1808070095
訂單登錄時間	2018-08-07 11:19 AM
訂單起算日期	2018-08-07
訂購人	Teresa Chiang
訂購人 E-mail	chiangt@tas.tw
手機號碼	0955688202
電話號碼	2873-9900 #250
郵遞區號	111
送貨地址	台北市士林區中山北路六段800號 TAIPEI AMERICAN SCHOOL

其他訂購註記或需求

Strong Promoter + Strong RBS + ALDH2*2 (deficient) + Double Terminator (K2539200)
2018-05-28

- ALDH2 mut size is correct

PCR 0528 Mutant ALDH2 Bob + Cm Backbone (Taq) — 05/28/2018

Notes:
Well #2 of mutant aldh2 bob is incorrect
Well #3 of mutant aldh2 bob is correct w/ 2100 bp
Well #5 of ligation control (glows under UV) is correct w/ 1200 bp (chromo protein 800 + 300)

Link to file:
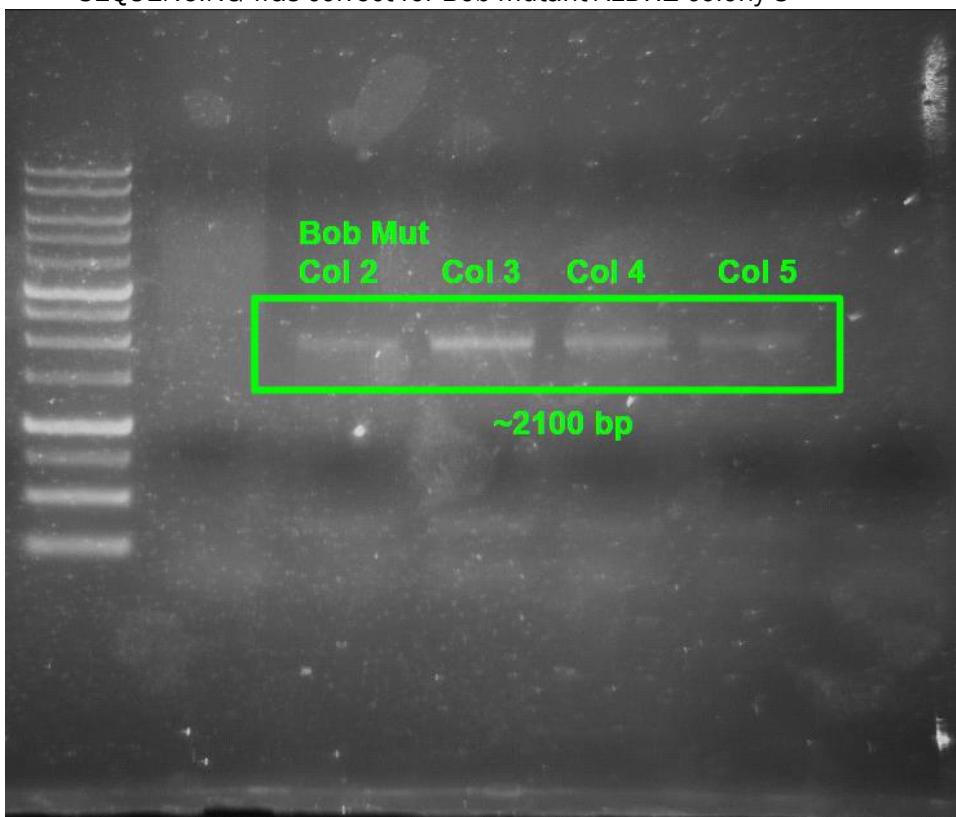
Ladder (kb) / BBa_K2539200 / Control — ~ 2.1kb

2018-05-24

- PCR check 2 in 1 (Gel and Restreak Plates)

- Taq polymerase
- VF2 + VR

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

- Found multiple colonies to be successful
    - Sequenced Colony 3 because it has the brightest bands
    - SEQUENCING was correct for Bob mutant ALDH2 colony 3

2018-05-23

- Ligate Insert+backbone at E and P

Ligation Calculator Beta

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

	Conc. (ng/ul)	Length (bp)
Insert/ORF	5.4	1800
Vector/Backbone	32.2	2000

Ligation		
Vector/Backbone	1.17	ul
Insert/ORF	15.83	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	0.00	ul
Total	20.00	ul

Ligation Control		
Vector/Backbone	1.17	ul
Insert/ORF	0.00	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	15.83	ul
Total	20.00	ul

- Transformed plasmid into Competent cell

2018-05-22

- Miniprepped BOB plasmid and Yellow Chromoprotein plasmid
- Digested BOB plasmid (at E and P) and Yellow Chromoprotein plasmid with Cm biobrick backbone
-

Digestion Calculator V2.0

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

ORF Insert Type	Front ▾	
Insert/ORF	185	ng/ul
Vector/Backbone	75.2	ng/ul

^^^Enter Concentration^^^

Vector/Backbone		
DNA Sample	13.30	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	3.70	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	5.41	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	11.59	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

-
- Ran gel to separate out DNA
- gel extracted BOB Mut insert

2018-05-21

-transform IDT ALDH2 Mutated sequence into competent cell

Strong Promoter + Strong RBS + 6x HIS + ALDH2*2 + Double Terminator (K2539201)

2018-09-01

- Received Sequencing confirmation, colony 2 sequencing correct with all the cutting sites (E,X,S,P)
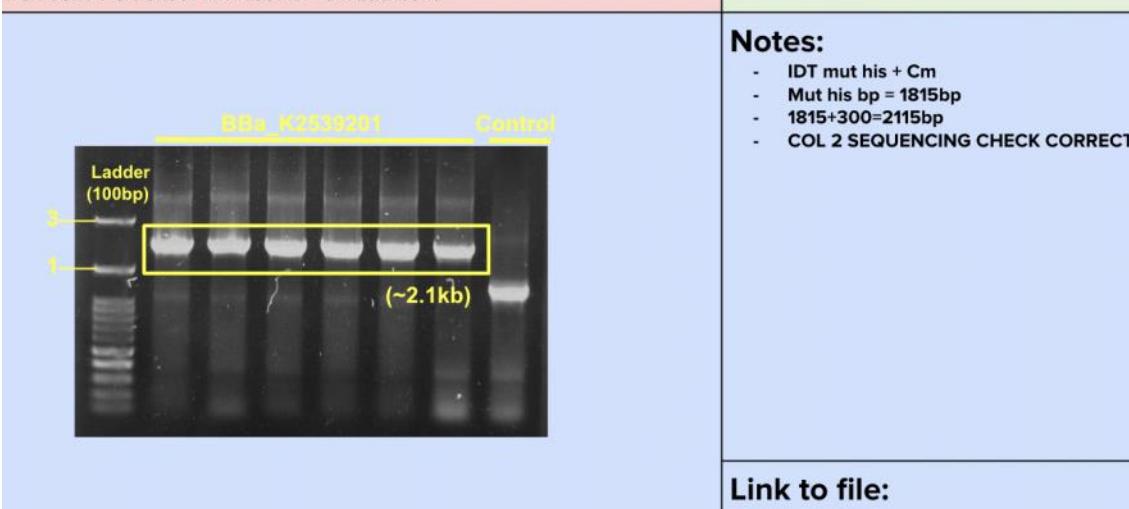
2018-08-30

- Miniprep IN WATER colonies 1 and 2 that indicated the correct bp from PCR Check for IDT Mut His

2018-08-29

- Run PCR Check 3-1 for IDT HIS Mut
  - All colonies are correct, will send for sequencing
  - PCR Gel:

PCR 0829 PCR Check IDT mut his + Cm backbone	08/29/2018

Notes:
- IDT mut his + Cm
- Mut his bp = 1815bp
- 1815+300=2115bp
- COL 2 SEQUENCING CHECK CORRECT

Link to file:

2018-08-28

- Run gel of digestion of Chromo protein + Cm backbone to obtain backbone for ligation with mut his
- Gel ex of Cm backbone
- Ligation of IDT Mut His + Cm Backbone
- Ligation Calculator:

Ligation Calculator

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

	Conc. (ng/ul)	Length (bp)
Insert/ORF	7.0	1815
Vector/Backbone	11.7	2070

^^^Enter concentration & DNA length^^^

Ligation		
Vector/Backbone	3.71	ul
Insert/ORF	13.29	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	0.00	ul
Total	20.00	ul

Ligation Control		
Vector/Backbone	3.71	ul
Insert/ORF	0.00	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	13.29	ul
Total	20.00	ul

- Transformation

2018-08-27

- Digestion of Chromo protein + Cm backbone for new cloning cycle of IDT His Mut because the sequencing sent out for colony 1 and 2 were incorrect

2018-08-24
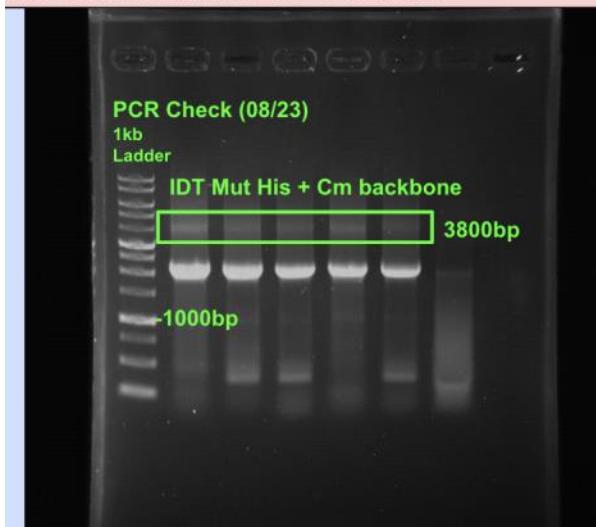
- Miniprep in WATER and send for sequencing

2018-08-23

- PCR Check 3 in 1
- Bands indicate correct length of around 3900bp
-

Notes:
- IDT mut his + Cm
- Mut his bp = 1815bp
- Cm backbone = 2070bp
- Total: 3885bp
- Sent sequencing for col 1 and 2
- SEQUENCING WERE WRONG

Link to file:

2018-08-22

- Gel Extract
- Ligation of Mut HIs with Cm backbone

- Transformation

2018-08-21

- Gene fragment of IDT ALDH2 Mut His Tag arrived
- Directly into digestion
- E and P
- 1000ng -----> diluted 20ul into 500ng/ul
- Took 6.6ul ----> 75.75ng/ul
- Digestion E and P and cut gel

Digestion Calculator V2.0

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

ORF Insert Type	Front	Insert
Insert/ORF	150	ng/ul
Vector/Backbone	182	ng/ul

^^^Enter concentration^^^

Vector/Backbone	
DNA Sample	5.49 ul
Enzyme E	0.50 ul
Enzyme X	0.50 ul
10X NEB Buffer 2.1	2.00 ul
Water	11.51 ul
Total	20.00 ul
DNA Amount ✓	1000 ng

Insert/ORF	
DNA Sample	6.67 ul
Enzyme E	0.50 ul
Enzyme S	0.50 ul
10X NEB Buffer 2.1	2.00 ul
Water	10.33 ul
Total	20.00 ul
DNA Amount ✓	1000 ng

ALDH2*2 basic part(K2539250)
2018-08-14

- Sequencing confirmed, all three colonies were correct

2018-08-12

- Miniprepped 3 LCs and sent for sequencing

2018-08-11

- PCR check 3-1
- PCR gel
-

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

-

2018-08-10

- Ligation with cm backbone, transformation
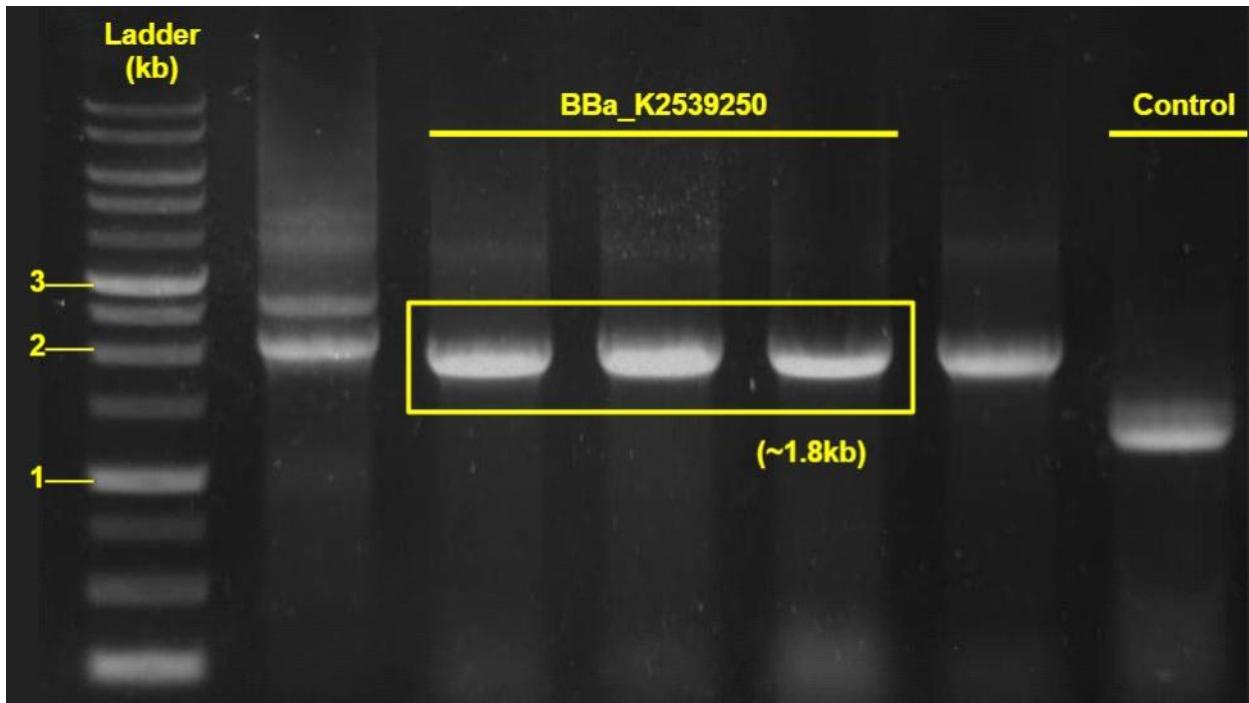
	Conc. (ng/ul)	Length (bp)
Insert/ORF	4.6	1500
Vector/Backbone	12.9	2000

^^^Enter concentration & DNA length^^^

Ligation		
Vector/Backbone	2.74	ul
Insert/ORF	14.26	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	0.00	ul
Total	20.00	ul

Ligation Control		
Vector/Backbone	2.74	ul
Insert/ORF	0.00	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	14.26	ul
Total	20.00	ul

2018-08-09

- Digestion, gel, gel x to cut out e and p

Digestion Calculator V2.0

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

ORF Insert Type	Front	
Insert/ORF	100	ng/ul
Vector/Backbone	75.2	ng/ul

^^^Enter Concentration^^^

Vector/Backbone		
DNA Sample	13.30	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	3.70	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	10.00	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	7.00	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

2018-08-08

- PCR, PCR gel

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

2018-08-07

- Primers ordered for cutting out BOB mut Basic

Oligo 合成服務訂單

訂單資料

本次訂單號碼	Q1808070095
訂單登錄時間	2018-08-07 11:19 AM
訂單起算日期	2018-08-07
訂購人	Teresa Chiang
訂購人 E-mail	chiangt@tas.tw
手機號碼	0955688202
電話號碼	2873-9900 #250
郵遞區號	111
送貨地址	台北市士林區中山北路六段800號 TAIPEI AMERICAN SCHOOL

其他訂購註記或需求

Strong Promoter + Strong RBS + alcR + Double Terminator (K2539300)

2018-06-18

- PCR gel for alcR, size is correct.

-

2018-06-17

- PCR check 2 in 1 (Gel and Restreak Plates)
  - Taq polymerase
  - VF2 + VR

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

2018-06-16

- Ligation using alcR insert and chromoprotein backbone
  run PCR 3 in 1

Ligation Calculator Beta

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

	Conc. (ng/ul)	Length (bp)
Insert/ORF	4.4	2600
Vector/Backbone	32.2	2000

Ligation		
Vector/Backbone	0.68	ul
Insert/ORF	16.32	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	0.00	ul
Total	20.00	ul

Ligation Control		
Vector/Backbone	0.68	ul
Insert/ORF	0.00	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	16.32	ul
Total	20.00	ul

2018-06-15

- Received alcR from IDT
- Digested and ran gel today (using E and P)

Digestion Calculator V2.0

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

-
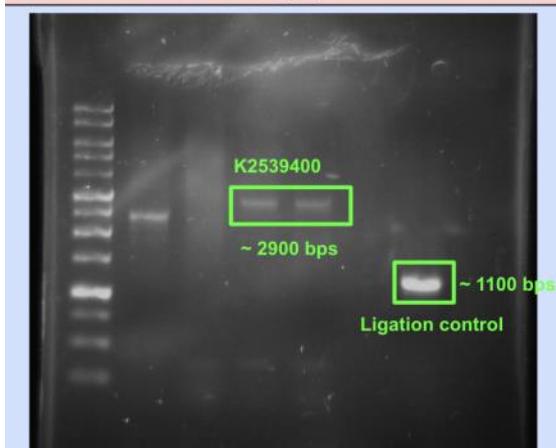
ORF Insert Type	Front ▼	
Insert/ORF	100	ng/ul
Vector/Backbone	75.2	ng/ul

^^^Enter Concentration^^^

Vector/Backbone		
DNA Sample	13.30	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	3.70	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	10.00	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	7.00	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

- Ran gel to separate out DNA

PalcA (alcR and Ethanol-Induced Promoter) + Strong RBS + ALDH2*1 + Double Terminator (K2539400)
2018-05-28

- PCR gel for PalC+ALDH2, size is correct.

PCR 0528 BOB Prom + Cm Backbone (Taq)	05/28/2018

Notes:
Bob Prom = Palc+ALDH2 Wildtype
- 2600 bps
- +300 bps primer annealing
- 2900 bps total

Vector only = Ligation control (ChromoProtein Cm Backbone)
- 800 bps
- +300 bps primer annealing
- 1100 bps total

Link to file:
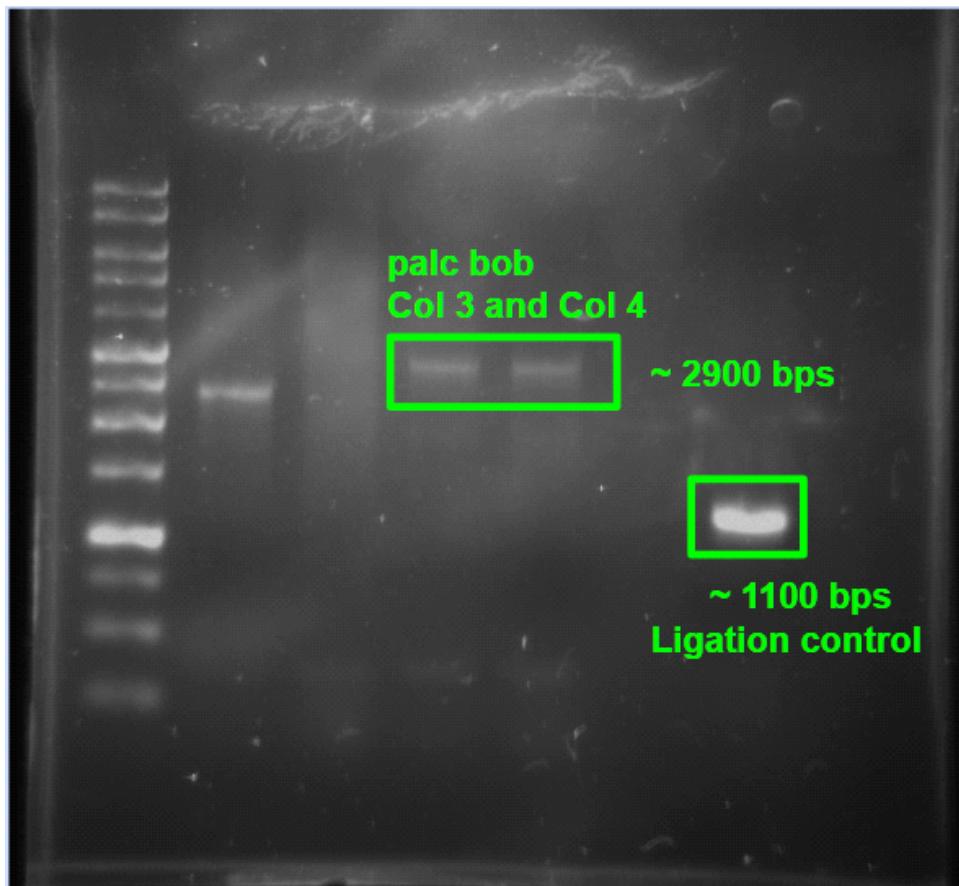
2018-05-24

- PCR check 2 in 1 (Gel and Restreak Plates)
  - Taq polymerase
  - VF2 + VR

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

- Found colony 3 and 4 to be successful
- SEQUENCE FOR COLONY 3 and 4 WERE CORRECT

2018-05-23

- Ligation using PacI insert and chromoprotein backbone
  run PCR 3 in 1
  -gel ex, nanodrop, ligation, transformation for alcohol sensing promoter

Ligation Calculator Beta

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

	Conc. (ng/ul)	Length (bp)
Insert/ORF	4.4	2600
Vector/Backbone	32.2	2000

Ligation		
Vector/Backbone	0.68	ul
Insert/ORF	16.32	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	0.00	ul
Total	20.00	ul

Ligation Control		
Vector/Backbone	0.68	ul
Insert/ORF	0.00	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	16.32	ul
Total	20.00	ul

2018-05-22

- Digested and ran gel today (using E and P)
  - Bob prom. Is a gene fragment-> have to go directly to digesting

Digestion Calculator V2.0

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

ORF Insert Type	Front ▼	
Insert/ORF	100	ng/ul
Vector/Backbone	75.2	ng/ul

^^^Enter Concentration^^^

Vector/Backbone		
DNA Sample	13.30	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	3.70	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	10.00	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	7.00	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

- Ran gel to separate out DNA
- gel extracted BOB Prom insert
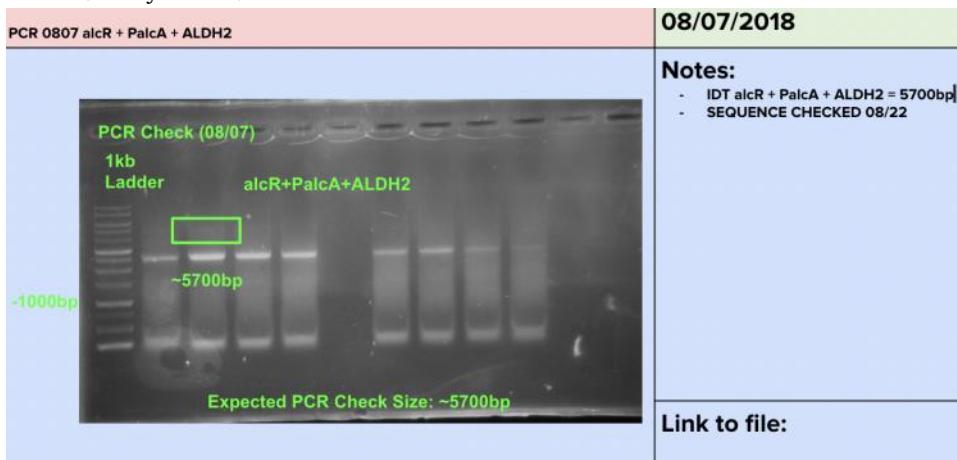
Strong Promoter + Strong RBS + alcR + Double Terminator + PalcA Promoter + Strong RBS + ALDH2*1 + Double Terminator (K2539450)

2018-08-22

- Sequence of alcR + Palc colony 2 and colony 3 are correct

2018-08-07

- cloned alcR+PalcA+ALDH2
- Colony 2 and 3 are correct

PCR 0807 alcR + PalcA + ALDH2

08/07/2018

Notes:
- IDT alcR + PalcA + ALDH2 = 5700bp
- SEQUENCE CHECKED 08/22

PCR Check (08/07)
1kb Ladder
alcR+PalcA+ALDH2
~5700bp
-1000bp
Expected PCR Check Size: ~5700bp

Link to file:

2018-08-06

- Ligation of alcR as insert and PalcA ALDH2 as vector

2018-08-03

- Digestion of alcR and PalcA
- AlcR cuts with E and S
- PalcA cut with E and X

-
Vector/Backbone		
DNA Sample	4.27	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	12.73	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	4.31	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	12.69	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

-

PalcA Promoter + Strong RBS + GFP + Double Terminator (K2539500)
2018-08-14

- Sequencing confirmed, all three colonies were correct

2018-08-12

- Miniprepped 3 LCs and sent for sequencing

2018-08-11

- PCR check 3-1
- PCR gel
-

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

-

2018-08-10

- Ligation with cm backbone, transformation

-

2018-08-09

- Digestion, gel, gel x to cut out e and p

Digestion Calculator V2.0

Made by Justin Yang (iGEM 2016-17, '18)

ONLY EDIT CELLS HIGHLIGHTED IN GREEN!

ORF Insert Type	Front ▼	
Insert/ORF	100	ng/ul
Vector/Backbone	75.2	ng/ul

^^^Enter Concentration^^^

Vector/Backbone		
DNA Sample	**13.30**	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	**3.70**	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	**10.00**	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	**7.00**	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

2018-08-08

- PCR, PCR gel

DH2O	8
Forward primer	1
Reverse primer	1
TAQ polymerase Master mix	10
1 bacterial colony	

Step	Temp	Time
Initial Denaturation	95 C	5 min
35 cycles	94 C	30 secs
	50 C	45 secs
	72 C	3 min
Final Extension	72 C	7 min
Hold	4 C	

2018-08-07

- Primers ordered for cutting out BOB mut Basic

訂單資料	
本次訂單號碼	Q1808070095
訂單登錄時間	2018-08-07 11:19 AM
訂單起算日期	2018-08-07
訂購人	Teresa Chiang
訂購人 E-mail	chiangt@tas.tw
手機號碼	0955688202
電話號碼	2873-9900 #250
郵遞區號	111
送貨地址	台北市士林區中山北路六段800號 TAIPEI AMERICAN SCHOOL
其他訂購註記或需求	

Strong Promoter + Strong RBS + alcR + Double Terminator + PalcA Promoter + Strong RBS + GFP + Double Terminator (K2539550)

2018-06-26

- cloned alcR+PalcA+GFP
- Colony 3 is correct
- Ran PCR gel

PCR 0626 alcR + PalcA + GFP

06/26/2018

Expected 4000 bp

Ladder (kb)   BBa_K2539550

Notes:
- IDT alcR + PalcA + GFP
- alcR = 2838
- PalcA + GFP = 1763
- total : 4900
- SEQUENCE CHECKED

Link to file:

2018-08-06

- Ligation of alcR as insert and PalcA **GFP** as vector

2018-08-03

- Digestion of alcR and PalcA
- AlcR cuts with E and S
- PalcA cut with E and X

-
Vector/Backbone		
DNA Sample	**4.27**	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	**12.73**	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	**4.31**	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	**12.69**	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

NICE Lactococcus Lactis: GFP+pNZ8008 Vector & ALDH2*1+pNZ8008 Vector
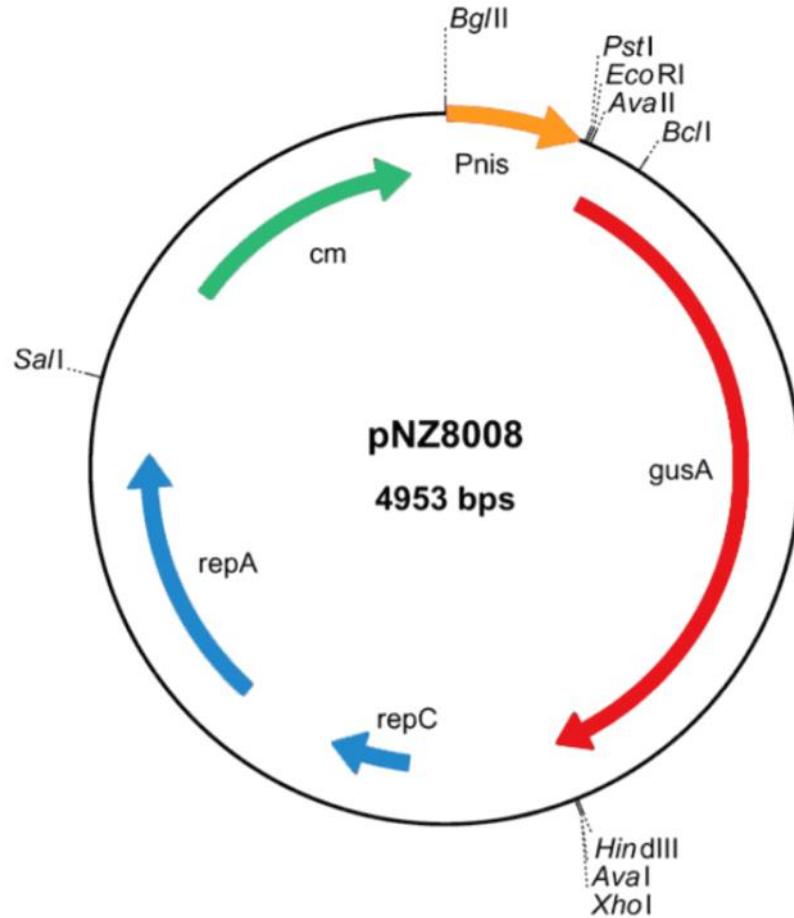
2018-08-22

- Ran PCR

2018-08-21

- Digestion of GFP and pNZ8008 at E and P

Figure: pNZ8008 plasmid map, 4953 bps, showing restriction sites BglII, PstI, EcoRI, AvaII, BclI, HindIII, AvaI, XhoI, SalI, and features Pnis, cm, gusA, repA, repC.

GFP+pNZ8008:

ORF Insert Type	Front ▾	Insert
Insert/ORF	212	ng/ul
Vector/Backbone	113	ng/ul

^^^Enter concentration^^^

Vector/Backbone		
DNA Sample	8.85	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	8.15	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	4.72	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	12.28	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

ALDH2*2+pNZ8008:

ORF Insert Type	Front ▾	Insert
Insert/ORF	240	ng/ul
Vector/Backbone	113	ng/ul

^^^Enter concentration^^^

Vector/Backbone		
DNA Sample	8.85	ul
Enzyme E	0.50	ul
Enzyme X	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	8.15	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

Insert/ORF		
DNA Sample	4.17	ul
Enzyme E	0.50	ul
Enzyme S	0.50	ul
10X NEB Buffer 2.1	2.00	ul
Water	12.83	ul
Total	20.00	ul
DNA Amount ✓	1000	ng

- Ligation of GFP and ALDH2*2 as insert and pNZ8008 as vector

GFP+pNZ8008

	Conc. (ng/ul)	Length (bp)
Insert/ORF	2.9	720
Vector/Backbone	8.8	5000

^^^Enter concentration & DNA length^^^

Ligation		
Vector/Backbone	8.65	ul
Insert/ORF	8.35	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	0.00	ul
Total	20.00	ul

Ligation Control		
Vector/Backbone	8.65	ul
Insert/ORF	0.00	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	8.35	ul
Total	20.00	ul

ALDH2*2+pNZ8008:

	Conc. (ng/ul)	Length (bp)
Insert/ORF	3.0	1500
Vector/Backbone	8.8	5000

^^^Enter concentration & DNA length^^^

Ligation		
Vector/Backbone	5.49	ul
Insert/ORF	11.51	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	0.00	ul
Total	20.00	ul

Ligation Control		
Vector/Backbone	5.49	ul
Insert/ORF	0.00	ul
Buffer	2.00	ul
Enzyme	1.00	ul
Water	11.51	ul
Total	20.00	ul

- Transformation