$$\frac{50\,ng \times \frac{1\,g}{10^9\,ng}}{13826.1\,g/mol} \times \frac{1\,L}{20\times10^{-6}\,mol} \times \frac{10^6\,mL}{1\,L} = \frac{5}{13826.1\times 2}\times 10^3\,mL = 0.18\,mL$$

SUBJECT

DATE 2020.09.04 Ivy 張栢睿 - 潘建安

Place: Mingdao High School

Temperature: 24°C

Zr31 inv1 (322.01) — ZZ31 inv1 (334.27) — 0r31 inv1 (341.55)

	A1 Zr31 (+)	A2 Zr31 (−)	A3 ZZ31 (+)	A4 ZZ31 (−)	A5 0r31 (+)	A6 0r31 (−)
DNase-free water	0.96	1.14	0.97	1.15	0.97	1.15
A	2	2	2	2	2	2
B	1.5	1.5	1.5	1.5	1.5	1.5
RNase-Inh	0.2	0.2	0.2	0.2	0.2	0.2
toehold	0.16	0.16	0.15	0.15	0.15	0.15
trigger	0.18	0	0.18	0	0.18	0
total	5	5	5	5	5	5
sucrose (0.5M)	5	5	5	5	5	5

0Z31 inv1 (338.63)

	A7 0Z31 (+)	A8 0Z31 (−)	A9 blank	blank only 糖
DNase-free water	0.97	1.15	5	5
A	2	2	0	0
B	1.5	1.5	0	0
RNase-Inh	0.2	0.2	0	0
toehold	0.15	0.15	0	0
trigger	0.18	0	0	0
total	5	5	5	0
sucrose (0.5M)	5	5	5	0
ddH2O				5

	Zr31 (+)	Zr31 (−)	ZZ31 (+)	ZZ31 (−)	0r31 (+)	0r31 (−)	0Z31 (+)	0Z31 (−)	Blank	Blank only 糖
0 min	71 mg/dL	71	63	63	74	68	63	58	Er4.	Er4.

SUBJECT

DATE
2020.09.04 Ivy James

Place: Ming Dao High School
Temp: 25°C
Pressure: 1 atm

Experiment: Resuspension of treated switch

	Conc.	260/230	260/280	plasmid : H2O
pp21_B	29.89	4.4	2.75	1:5 (9/5)
zp21_A	19.41	2.96	2	2:6
zp21_B	18.91	2.88	1.84	2:6
zz21_A	22.60	3.11	2.08	2:6
zz21_B	19.92	2.84	1.83	2:6
op21_A	26.09	3.26	1.93	2:8
op21_B	46.48	4.21	2	1:8
oz21_A	48.60	2.46	0.83	1:9
oo21_B	28.59	3.31	2	1:5 (9/5)
zr146_A	61.06	5.84	3.50	1:11
zr146_B	34.71	1.56	3.51	1:6 (9/5)
zz146_A	40.75	1.90	4.13	1:7
zz146_B	24.89	3.11	1.84	2:8
or146_A	19.41	2.67	1.78	2:6
rr146_B	80.35	6.54	3.75	1:15
oz146_A	18.91	2.69	1.56	2:6
oz146_B	15.64	2.25	1.70	3:6

SUBJECT

DATE
2020.9.4. Jones

Place: Ming Dao High School.
Temp: 25°C
Pressure: 1atm.

Experiment: Transformation of toehold switch

Result: FAILED! Bacteria grow too much!

Discussion: use 5uL instead of 100uL LB+ E.coli DH5α (transformed)
to spread on the plate.
→ Redo the experiment on 2020.9.5.

SUBJECT

DATE
2020. 9.5. James.

Place: Ming Dao High School
Temp: 25°C
Pressure: 1atm.

Experiment: Transformation of toehold switch.
- pp21_B
- zp21_A, B zr146_A, B
- zz21_A, B zz146_A, B
- op21_A, B or146_A, B
- oz21_A, B oz146_A, B.

Preparation: ① Resuspension of pp21_B, oz21_B, zr146_B to 5 ng/uL and label as 0905. Others take 0904 for the amount is still enough.
② LB plate with Kan. for 40 portions (see 2020. 7. 21 Procedure).

Procedure:

ON ICE!
1. Add 33uL of E.coli DH5α competent cells into an eppendorf
2. Add 3.5uL of targeted plasmid (5 ng/uL) into the eppendorf
(3. vortex, spin down) → I forgot....
4. Ice 3-5 mins.

5. heat shock 1 min (42°C dry incubator)
6. Ice 3-5 mins.
7. Add 500uL of LB media (antibiotic free!)
8. 37°C, shaking for 1 hour
9. Centrifuge 2mins at 15000 rpm.
10. Take 435 uL of the supernant out.
11. Vortex.
12. Take 5uL of the transformed bacteria and spread on LB+Kan50 plate.
13. Incubate for 14 hours.

SUBJECT

DATE
xxx.9.6. James

Place: Ming Dao High School
Temp: x5°C
Pressure: 1atm.
Experiment: Culture Bacteria of pp21_B1,2.
zp21_A1,2
zp21_B1,2
zz21_A1,2
zz21_B1,2
op21_A1,2
op21_B1,2
oz21_A1,2
oz21_B1,2
Preparation: ① # 2 portions of LB+Kan50 (Kan100: x5uL + LB: 50mL)

SUBJECT

DATE
xxx0.9.7. James.

Place: Ming Dao High School
Temp: x5°C
Pressure: 1atm.
Experiment: Plasmid Extraction, Concentration Measurement.

	x60/230	x60/280	Con (ng/uL)	
pp21_B1	x4.31	1.59	196.37	mix together
pp21_B2	4.42	1.58	170.50	
zp21_A1	3.83	1.38	192.89	
zp21_A2	4.51	1.62	193.58	
zp21_B1	3.93	1.43	x28.15	
zp21_B2	4.07	1.43	x64.x0	
zz21_A1	4.11	1.50	196.37	
zz21_A2	3.81	1.38	x15.07	
zz21_B1	3.72	1.32	x56.79	
zz21_B2	3.77	1.39	x07.41	
op21_A1	4.38	1.58	188.01	
op21_A2	4.42	1.60	192.19	
op21_B1	4.48	1.59	x06.78	
op21_B2	4.51	1.61	166.28	
oz21_A1	4.43	1.60	178.22	
oz21_A2	4.41	1.56	162.76	
oz21_B1	3.96	1.64	x31.58	
oz21_B2	4.47	1.59	169.80	

SUBJECT

DATE 2020.09.07 Inj.

Exp: Toehold test of { ① zr31 : zr31 inv1 (322.01) ② zz31 : zz31 inv1 (334.29) ③ or31 : or31 inv1 (341.55) ④ oz31 : oz31 inv1 (338.63) }

	DNase-free water	A	B	inh	toe	trig	total	sucrose (0.5M)	dd H2O
zr31 (+)	0.78	2	1.5	0.2	0.16	0.36	5	5	0
zr31 (-)	1.14	2	1.5	0.2	0.16	0	5	5	0
zz31 (+)	0.79	2	1.5	0.2	0.15	0.36	5	5	0
zz31 (-)	1.15	2	1.5	0.2	0.15	0	5	5	0
or31 (+)	0.79	2	1.5	0.2	0.15	0.36	5	5	0
or31 (-)	1.15	2	1.5	0.2	0.15	0	5	5	0
oz31 (+)	0.79	2	1.5	0.2	0.15	0.36	5	5	0
oz31 (-)	1.15	2	1.5	0.2	0.15	0	5	5	0
blank	5	0	0	0	0	0	5	5	0
blank only H2O	5	0	0	0	0	0	5	0	5

SUBJECT

DATE ~~30.09.of~~ Ivy

	0	10 min	20 min	30 min	40 min	50 min	60 min
Zr 31 (+)	73	229	443	Hi	Hi.	Hi.	Hi
Zr 31 (-)	68	142.	245.	360.	482	536.	Hi.
Zz 31 (+)	61	119.	184	264	338	442	514.
Zz 31 (-)	64	87.	117.	144	179	219.	249
Or 31 (+)	76	262	447	Hi.	Hi.	Hi.	Hi
Or 31 (-)	73	211.	374	553	Hi.	Hi	Hi.
Oz 31 (+)	68	185	269.	409	509.	Hi.	Hi
Oz 31 (-)	61	124	184.	278	351	407.	505
blank	Er4.	Er4	Er4	Er4.			
blank only H_2O	Er4.	Er4	Er4	Er4			

SUBJECT

DATE
xxx.9.7. Ivy.

Place: Ming Dao High School
Temp: x5°C
Pressure: 1atm.
Experiment: Restriction Enzyme O/N for 5V: several miR21 toeholds.
I: ① invertase
② GFP

V	DNA	10x buffer	XbaI	PstI	dd H2O	total
pp21_B	10	10	1	1	78	100
zp21_A	10	10	1	1	78	100
zp21_B	10	10	1	1	78	100
zz21_A	10	10	1	1	78	100
zz21_B	10	10	1	1	78	100
op21_A	10	10	1	1	78	100
op21_B	10	10	1	1	78	100
oz21_A	10	10	1	1	78	100
oz21_B	10	10	1	1	78	100
I						
inv 1~67	50	10	1	1	38	100
GFP 1~67	50	10	1	1	38	100

SUBJECT

DATE
2020. 9.7. James.

Place: Ming Dao High School.
Temp: 25°C
Pressure: 1atm.
Experiment: Culture bacteria of zr146_A1, 2
zr146_B1, 2
zz146_A1, 2
zz146_B1, 2
or146_A1, 2
or146_B1, 2
oz146_A1, 2
oz146_B1, 2
Preparation: 1 portion of LB+Kan50 (Kan100: 25mL + LB: 50mL)
Experiment: Put into 37°C incubator: 9/7
Ideal time for takeout: 9/8.

SUBJECT

DATE
2020.09.08

Place: Ming Dao High School
Temp: 25°C
Pressure: 1 atm
Experiment: Plasmid Extraction, Concentration Measurement

		260/230	260/280	Con (ng/μL)
OR146	A1	4.65	1.61	166.28
OR146	A2	4.30	1.52	234.31
OR146	B1	4.63	1.64	193.58
OR146	B2	4.60	1.63	187.31
OZ146	A1	4.42	1.52	236.37
OZ146	A2	4.79	1.63	194.01
OZ146	B1	4.64	1.61	194.28
OZ146	B2	4.79	1.65	197.76
ZR146	A1	4.74	1.63	206.08
ZR146	A2	4.44	1.53	216.45
ZR146	B1	4.69	1.58	188.71
ZR146	B2	4.73	1.61	208.16
ZZ146	A1	4.68	1.58	215.76
ZZ146	A2	4.93	1.61	190.10
ZZ146	B1	4.65	1.61	214.38
ZZ146	B2	4.79	1.60	208.16

SUBJECT

DATE

SUBJECT

DATE 09.08. 2020. ~~08.29~~

Experiment: Clean up of V { ① pp21B ② zp21A, ③ zp21B ④ zz21A ④ zz21B ⑤ op21A ⑥ op21B ⑦ oz21A ⑧ oz21B

I { ① GFP 1~7 ② Invertase 1~7

Mix (1) GFP 1~3, (2) Inv 1~3 together.

Calculate: V : I = 1 : 20

① pp21B + GFP

V : I = $\frac{3}{2000}$: $\frac{1}{776}$ = 1 : 0.86 0.5 : 11.5

② pp21B + Inv

V : I = $\frac{5}{2000}$: $\frac{2}{1359}$ = 1 : 0.59 0.3 : 11

③ zp21A + GFP

V : I = $\frac{2}{2000}$: $\frac{1}{776}$ = 1 : 1.29 0.8 : 12

④ zp21A + Inv

V : I = $\frac{3}{2000}$: $\frac{2}{1359}$ = 1 : 0.98 0.5 : 10

⑤ zp21B + GFP

V : I = $\frac{3}{2000}$: $\frac{2}{776}$ = 1 : 1.72 1 : 11.5

⑥ zp21B + Inv

V : I = $\frac{4}{2000}$: $\frac{3}{1359}$ = 1 : 1.10 0.5 : 10

⑦ zz21A + GFP

V : I = $\frac{3}{2000}$: $\frac{2}{776}$ = 1 : 1.72 1 : 11.5

⑧ zz21A + Inv

$\frac{1}{\ }$: $\frac{1}{\ }$ = 1 : 1.47 1 : 13

SUBJECT

DATE

⑧ zz21 B + GFP
V : I = $\frac{1}{2000} : \frac{1}{776}$ = 1 : 2.58 1 : 8

⑩ zz21 B + Inv
V : I = $\frac{1}{2000} : \frac{1}{1359}$ = 1 : 1.47 1 : 13

⑪ op21 A + GFP
V : I = $\frac{3}{2000} : \frac{4}{776}$ = 1 : 3.44 1 : 6

⑫ op21 A + Inv
V : I = $\frac{1}{2000} : \frac{2}{1359}$ 1 : 2.94 1 : 6.5

⑬ op21 B + GFP
V : I = $\frac{1}{2000} : \frac{1}{776}$ = 1 : 2.58 1 : 6.5

⑭ op21 B + Inv
V : I = $\frac{1}{2000} : \frac{1}{1359}$ = 1 : 1.47 1 : 13

⑮ oz21 A + GFP
V : I = $\frac{1}{2000} : \frac{1}{776}$ = 1 : 2.58 1 : 8

⑯ oz21 A + Inv
V : I = $\frac{1}{2000} : \frac{1}{1359}$ = 1 : 1.47 1 : 13

⑰ oz21 B + GFP
V : I = $\frac{4}{2000} : \frac{3}{776}$ = 1 : 1.93 1 : 10

⑱ oz21 B + Inv
V : I $\frac{1}{2000} : \frac{1}{1359}$ = 1 : 1.47 1 : 13

SUBJECT

DATE

	dd H2O	10× buffer	V	I	ligase	t-tal
pp21 B + GFP	5	2	0.8	11.5	1	20
pp21 B + Inv	5.7	2	0.3	11	1	20
zp21 A + GFP	4.2	2	0.8	12	1	20
zp21 A + Inv	6.5	2	0.5	10	1	20
zp21 B + GFP	4.5	2	1	11.5	1	20
zp21 B + Inv	6.5	2	0.5	10	1	20
zz21 A + GFP	4.5	2	1	11.5	1	20
zz21 A + Inv	3	2	1	13	1	20
zz21 B + GFP	8	2	1	8	1	20
zz21 B + Inv	3	2	1	13	1	20
op21 A + GFP	10	2	1	6	1	20
op21 A + Inv	9.5	2	1	6.5	1	20
op21 B + GFP	9.5	2	1	6.5	1	20
op21 B + Inv	3	2	1	13	1	20
oz21 A + GFP	8	2	1	8	1	20
oz21 A + Inv	3	2	1	13	1	20
oz21 B + GFP	6	2	1	10	1	20
oz21 B + Inv	3	2	1	13	1	20

SUBJECT

DATE
2020.09.08-09.

Place: Ming Dao High School
Temp: 25°C
Pressure: 1atm.

Experiment: Transformation of ① pp21B+GFP ② pp21B+Inv.
③ zp21A+GFP ④ zp21A+Inv.
⑤ zp21B+GFP ⑥ zp21B+Inv.
⑦ zz21A+GFP ⑧ zz21A+Inv.
⑨ zz21B+GFP ⑩ zz21B+Inv.
⑪ op21A+GFP ⑫ op21A+Inv.
⑬ op21B+GFP ⑭ op21B+Inv.
⑮ oz21A+GFP ⑯ oz21A+Inv.
⑰ oz21B+GFP ⑱ oz21B+Inv.

Procedure:

Result: Success (2020.9.9 jot-down)

1. Add 50 uL of E.coli DH5α
2. Add 5uL of target
3. Vortex and spin down
4. Ice 3-5 min.
5. Heat shock 1min (42°C)
6. Ice 3-5 min.
7. Add 500uL of LB media (antibiotic free)
8. 37°C shaking for 1 hour.
9. Centrifuge 15000 rpm for 2 min
10. Take 450uL of supernatant out
11. Vortex.
12. Take 100uL of the sample and drop on LB/Kan plate

SUBJECT

DATE
xxxx.09.09. James

Place: Ming Dao High School
Temp: 25°C
Pressure: 1atm
Expenment: Clean up of { ① ZZ146A ② ZZ146B ③ or 146A ④ or 146B ⑤ Zr 146A ⑥ Zr 146B ⑦ oz 146A ⑧ oz 146B

Preparation: Mix 0908. (1) GFP4~6 (2) Inv 4~6 together.

Calculation:

* Final amount of vector (V) and insert (I) ⇒ V : I = 1 : 20

* ① ZZ146A + GFP

$$V:I = \frac{3}{2000} \times \frac{2000}{3} : \frac{1}{776} \times \frac{2000}{3} = 1 : 0.86 \Rightarrow 0.5 : 8.6$$

② ZZ146A + Invertase

$$V:I = \frac{3}{2000} \times \frac{2000}{3} : \frac{1}{1359} \times \frac{2000}{3} = 1 : 0.49 \Rightarrow 1 : 9.8$$

③ ZZ146B + GFP

$$V:I = \frac{3}{2000} : \frac{1}{776} \times \frac{2000}{3} = 1 : 0.86 \Rightarrow 0.5 : 8.6$$

④ ZZ146B + invertase

$$V:I = \frac{3}{2000} : \frac{1}{1359} \times \frac{2000}{3} = 1 : 0.49 \Rightarrow 1 : 9.8$$

⑤ or 146A + GFP

$$V:I = \frac{3}{2000} : \frac{1}{776} \times \frac{2000}{3} = 1 : 0.86 \Rightarrow 0.5 : 8.6$$

⑥ or 146A + invertase

$$V:I = \frac{3}{2000} : \frac{1}{1359} \times \frac{2000}{3} = 1 : 0.49 \Rightarrow 1 : 9.8$$

SUBJECT

DATE

⑦ Or146B + GFP

$V:I = \frac{4}{2000} \overset{\times 500}{:} \frac{1}{776} \overset{\times 500}{=} 1:0.64 \Rightarrow 1:12.8$

⑧ Or146B + invertase

$V:I = \frac{4}{2000} \overset{\times 500}{:} \frac{1}{1359} \overset{\times 500}{=} 1:0.37 \Rightarrow 1:7.4$

⑨ Zr146A + GFP

$V:I = \frac{2}{2000} \overset{\times 1000}{:} \frac{1}{776} \overset{\times 1000}{=} 1:1.29 \Rightarrow 0.5:12.9$

⑩ Zr146A + invertase

$V:I = \frac{2}{2000} \overset{\times 1000}{:} \frac{1}{1359} \overset{\times 1000}{=} 1:0.74 \Rightarrow 0.5:7.4$

⑪ Zr146B + GFP

$V:I = \frac{2}{2000} \overset{\times 1000}{:} \frac{1}{776} \overset{\times 1000}{=} 1:1.29 \Rightarrow 0.5:12.9$

⑫ Zr146B + invertase

$V:I = \frac{2}{2000} \overset{\times 1000}{:} \frac{1}{1359} \overset{\times 1000}{=} 1:0.74 \Rightarrow 0.5:7.4$

⑬ OZ146A + GFP

$V:I = \frac{2}{2000} : \frac{1}{776} = 1:1.29 \Rightarrow 0.5:12.9$

⑭ OZ146A + invertase

$V:I = \frac{2}{2000} : \frac{1}{1359} = 1:0.74 \Rightarrow 0.5:7.4$

⑮ OZ146B + GFP

$V:I = \frac{2}{2000} : \frac{1}{776} = 1:1.29 \Rightarrow 0.5:12.9$

⑯ OZ146B + invertase

SUBJECT

DATE

	ddH_2O	10x buffer	V	I	ligase	Total
zz 146A + GFP	7.9	2	0.5	8.6	1	20
zz 146A + inv.	6.2	2	1	9.8	1	20
zz 146B + GFP	7.9	2	0.5	8.6	1	20
zz 146B + inv.	6.2	2	1	9.8	1	20
or 146A + GFP	7.9	2	0.5	8.6	1	20
or 146A + inv.	6.2	2	1	9.8	1	20
or 146B + GFP	3.2	2	1	12.8	1	20
or 146B + inv	8.6	2	1	7.4	1	20
zr 146A + GFP	3.1	2	0.5	12.9	1	20
zr 146A + inv.	8.6	2	0.5	7.4	1	20
zr 146B + GFP	3.1	2	0.5	12.9	1	20
zr 146B + inv.	8.6	2	0.5	7.4	1	20
oz 146A + GFP	3.1	2	0.5	12.9	1	20
oz 146A + inv.	8.6	2	0.5	7.4	1	20
oz 146B + GFP	3.1	2	0.5	12.9	1	20
oz 146B + inv.	8.6	2	0.5	7.4	1	20

? 為什麼 vector 消失了

Result: We give up this experiment for some procedures are wrong.

SUBJECT

DATE
xxx.9.9 IrTB

Place: Ming Dao High School
Temp: 25°C
Pressure: 1atm
Expenment: Culture Bacteria of ① pp21 B + GFP ⑩ pp21 B + InV
② zp21 A + GFP ⑪ zp21 A + InV
③ zp21 B + GFP ⑫ zp21 B + InV
④ zz21 A + GFP ⑬ zz21 A + InV
⑤ zz21 B + GFP ⑭ zz21 B + InV
⑥ op21 A + GFP ⑮ op21 A + InV
⑦ op21 B + GFP ⑯ op21 B + InV
⑧ oz21 A + GFP ⑰ oz21 A + InV
⑨ oz21 B + GFP ⑱ oz21 B + InV

Preparation: 3 portion of LB + Kan50 (Kan100: ~~xx~~ uL + LB = ~~xx~~ uL)
Experiment: Put into 37°C incubator: 9/9 xx:10 xx号 45:
Ideal time for takeout: 9/10 12:10 ~ 14:10

SUBJECT

DATE
2020.09.09. James.

Place: Ming Dao High School

Temp: 25°C

Pressure: 1atm.

Experiment: Restriction Enzyme O/N for { V: several miR146 toeholds
I: invertase

Preparation: Put { 0924 I3-1 PCR
0924 I3-2 PCR
0924 I3-3 PCR } into { 0909 I8 (X.P)
0909 I9 (X.P)
0909 I10 (X.P) } invertase

Procedure:

① V

V	DNA	10x buffer	XbaI	PstI	ddH2O	Total.
zr146_A1	10	10	1	1	78	100
zr146_B1	10	10	1	1	78	100
zz146_A1	10	10	1	1	78	100
zz146_B1	10	10	1	1	78	100
or146_A1	10	10	1	1	78	100
or146_B1	10	10	1	1	78	100
oz146_A1	10	10	1	1	78	100
oz146_B1	10	10	1	1	78	100
I						
I8	50	10	1	1	38	100
I9	50	10	1	1	38	100
I10	50	10	1	1	38	100

② Dry incubator (37°) O/N.

SUBJECT

DATE

Place: Ming Dao High School
Temp: 25°C
Pressure: 1atm
Experiment: Clean up of ① Zr146 A1, Zr146 B1, ZZ146A1, ZZ146 B1, or146 A1, or146 B1, OZ146A1, OZ146 B1
② I8, I9, I10

Elution buffer: ① toehold (V): 80ml
② Inv (I): 40ml

Ligation ~~of~~

* Calculate: V : I = 1 : 20

1. Zr146 A + Inv

$V : I = \frac{3}{2000} : \frac{1}{1359} = 1 : 0.49$ 0.4 : 16

2. Zr146 B + Inv

$V : I = \frac{2}{2000} : \frac{1}{1359} = 1 : 0.74$ 0.5 : 13.5

3. ZZ146A + Inv

$V : I = \frac{3}{2000} : \frac{2}{1359} = 1 : 0.98$ 0.7 : 14

4. ZZ146B + Inv

$V : I = \frac{3}{2000} : \frac{1}{1359} = 1 : 0.49$ 0.4 : 16

5. or146 A + Inv

$V : I = \frac{3}{2000} : \frac{2}{1359} = 1 : 0.98$ 0.7 : 14

6. or146 B + Inv

$V : I = \frac{1}{2000} : \frac{1}{1359} = 1 : 1.47$ 1 : 13.6

SUBJECT

DATE

7. 0Z146A + I_{nv}

$$V:I = \frac{1}{2000} : \frac{1}{1359} = 1:1.47 \qquad 1:13.6$$

8. 0Z146B + I_{nv}

$$V:I = \frac{1}{2000} : \frac{1}{1359} = 1:1.47 \qquad 1:13.6$$

	ddH2O	10xbuffer	V	I	ligase	total
Zr146A + I_{nv}	0.6	2	0.4	16	1	20
Zr146B + I_{nv}	3	2	0.5	13.5	1	20
ZZ146A + I_{nv}	2.3	2	0.7	14	1	20
~~ZZ146B + I_{nv}~~	~~0.6~~	~~2~~	~~0.4~~	~~16~~	~~1~~	~~20~~
Or146A + I_{nv}	2.3	2	0.7	14	1	20
Or146B + I_{nv}	2.4	2	1	13.6	1	20
0Z146A + I_{nv}	2.4	2	1	13.6	1	20
0Z146B + I_{nv}	2.4	2	1	13.6	1	20
ZZ146B + I_{nv}	2.4	2	1	13.6	1	20

9. ZZ146B + I_{nv}

$$V:I = \frac{1}{2000} = \frac{1}{1359} = 1:1.47 \qquad 1:13.6$$

SUBJECT

DATE 0910.

Place: Ming Dao High School
Temp: 25°C
Pressure: 1atm
Experiment: Plasmid Extraction, Concentration measurement

	260/230	260/280	Con (ng/μl)
pp21B-GFP	4.52	1.56	255.44
pp21B-Inv	4.60	1.31	326.32
op21A-GFP	4.30	1.30	310.60
op21A-Inv	4.32	1.33	322.01
op21B-GFP	4.29	1.49	233.63
op21B-Inv	4.51	1.41	294.42
oz21A-GFP	4.54	1.48	292.33
oz21A-Inv	4.67	1.42	322.72
oz21B-GFP	4.70	1.21	383.02
oz21B-Inv	4.77	1.35	351.83
zp21A-GFP	4.59	1.59	245.24
zp21A-Inv	4.62	1.42	321.29
zp21B-GFP	4.70	1.62	173.31
zp21B-Inv	4.61	1.46	305.65
zz21A-GFP	4.55	1.58	247.96
zz21A-Inv	4.58	1.47	305.65
zz21B-GFP	4.56	1.50	282.63
zz21B-Inv	3.76	1.44	307.76

SUBJECT

DATE 9.10

Place: Ming Dao High School

Temp: 25° C ; pressure = 1atm

Experiment:

protocol: RE & electrophoresis of (21)

10x: 38 μl

↓ RE1.2: 9.5 μl

	ddH2O	DNA	CT	
pp21B-GFP	15.04	1.96	3	
pp21B-Inv	15.41	1.53		
op21A-GFP	15.39	1.61		
op21A-Inv	15.45	1.55	3	
op21B-GFP	14.86	2.14		
op21B-Inv	15.30	1.70		
oz21A-GFP	15.29	1.71		
oz21A-Inv	15.45	1.55		
oz21B-GFP	15.69	1.31	3	
oz21B-Inv°	15.58	1.42		
zp21A-GFP	14.96	2.04		
zp21A-Inv	15.44	1.56		
zp21B-GFP	14.11	2.89	3	
zp21B-Inv	15.36	1.64		
zz21A-GFP	14.98	2.02		
zz21A-Inv	15.36	1.64		
zz21B-GFP	15.23	1.77	3	
zz21B-Inv	15.38	1.62		

2020.09.10

↓ result

SUBJECT

DATE

Place: Ming Dao High School
Temp: 25°C
Pressure: 1 atm.
Experiment: Culture Bacteria of: ① ZR 146A + InV ⑤ OR 146A + InV
② ZR 146B + InV ⑥ OR 146B + InV
③ ZZ 146A + InV ⑦ OZ 146A + InV
④ ZZ 146B + InV ⑧ OZ 146B + InV

Preparation: 2 portion of LB + Kan 50 (Kan 100 = 22.5 mL, + LB = 45 mL)
Experiment: Put into 37°C incubator
Ideal time for takeout.

SUBJECT

DATE 2020.09.12 Iris Shen

Place: Ming Dao High School
Temp: 25°C
Pressure: 1 atm
Experiment: ① Bacteria storage ② Plasmid extraction
③ Restriction enzyme + gel electrophoresis

I. Bacteria storage
1. 300 ul of 50% steride glycerol
2. 600 ul of o/N culture bacteria
3. -80°C

① ZZ146A + Inv(I) →Backup ② ZZ146A + Inv(II) →Backup ③ ZZ146B + Inv(I) →Backup
④ ZZ146B + Inv(II) →Backup ⑤ ZR146A + Inv ⑥ ZR146B + Inv
⑦ OR146A + Inv ⑧ OR146B + Inv ⑨ OZ146A + Inv
⑩ OZ146B + Inv

II.

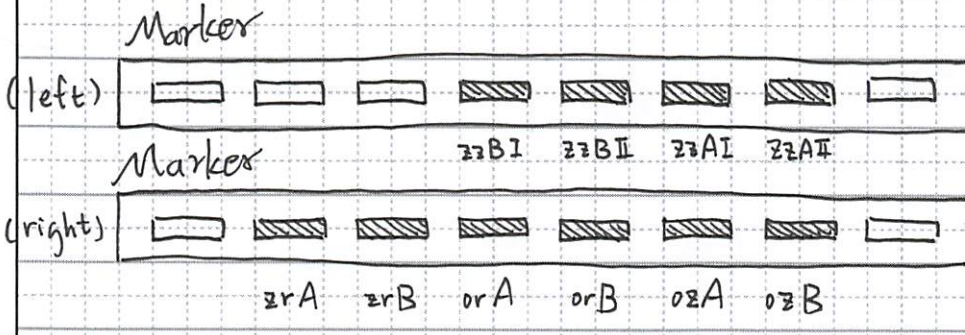
	260/230	260/280	ng/ul		260/230	260/280	ng/ul
ZZA(I)	4.54	1.44	313.44	ZZA(II)	4.44	1.48	290.24
ZZB(I)	4.53	1.49	293.72	ZZB(II)	4.52	1.51	286.77
ZRA	4.48	1.46	307.76	ZRB	4.55	1.50	284.01
ORA	4.56	1.45	304.24	ORB	4.45	1.53	268.94
OZA	3.71	1.18	323.44	OZB	4.53	1.49	289.55
ddH2O	1.62	1.20	1.62	ddH2O	1.57	1.11	1.21

SUBJECT

DATE 2020.09.12 Shen 2/11

10× : 24 μl
RE : 6 μl (XbaI, PstI)

~~protocol~~	ddH₂O	DNA	CT	
ZZ 146 A + Inv (I)	15.40	1.60	3	
ZZ 146 A + Inv (II)	15.28	1.72	3	
ZZ 146 B + Inv (I)	15.30	1.70	3	
ZZ 146 B + Inv (II)	15.26	1.74	3	
Zr 146 A + Inv	15.38	1.62	3	
Zr 146 B + Inv	15.24	1.76	3	
Or 146 A + Inv	15.36	1.64	3	
Or 146 B + Inv	15.14	1.86	3	
OZ 146 A + Inv	15.45	1.55	3	
OZ 146 B + Inv	15.27	1.73	3	



SUBJECT

DATE 2020/09/19 Ivy

Place: Ming Dao High School

Temp: 25°C

Pressure: 1 atm

Experiment: ① Toehold test of { ① zr3l : zr3l inv | ② zz3l : zz3l inv |
③ or3l : or3l inv | ④ oz3l : oz3l inv }

② Sucrose, preparation

	DNase-free water	A	B	inh	toe	tri	total
zr3l (+)	0.78	2	1.5	0.2	0.16	0.36	5
zr3l (-)	1.14	2	1.5	0.2	0.16	0	5
zz3l (+)	0.99	2	1.5	0.2	0.15	0.36	5
zz3l (-)	1.15	2	1.5	0.2	0.15	0	5
or3l (+)	0.99	2	1.5	0.2	0.15	0.36	5
or3l (-)	1.15	2	1.5	0.2	0.15	0	5
oz3l (+)	0.99	2	1.5	0.2	0.15	0.36	5
oz3l (-)	1.15	2	1.5	0.2	0.15	0	5
blank	5	0	0	0	0	0	5
blank only Mix	5	0	0	0	0	0	5

SUBJECT

DATE

	kit total	Sucrose (0.5M) + H2O		0 min	10 min	30 min
zr31 (+) a	1	9	0	20	60	232
zr31 (+) b	1	9	0	20	67	
zr31 (+) c	1	9	0	22	57	
zr31 (-) a	1	9	0	26	48	149
zr31 (-) b	1	9	0	23	47	
zr31 (-) c	1	9	0	23	46	
zz31 (+) a	1	9	0	21	30	64
zz31 (+) b	1	9	0	23	36	
zz31 (+) c	1	9	0	22	34	
zz31 (-) a	1	9	0	20	31	58
zz31 (-) b	1	9	0	18	29	
zz31 (-) c	1	9	0	18	26	
0r31 (+) a	1	9	0	19	76	222
0r31 (+) b	1	9	0	24	85	
0r31 (+) c	1	9	0	23	79	
0r31 (-) a	1	9	0	22	63	189
0r31 (-) b	1	9	0	23	64	
0r31 (-) c	1	9	0	20	56	
0z31 (+) a	1	9	0	16	36	
0z31 (+) b	1	9	0	18	42	
0z31 (+) c	1	9	0	20	42	
0z31 (-) a	1	9	0	85	103	
0z31 (-) b	1	9	0	86	111	
0z31 (-) c	1	9	0	79	103	

SUBJECT

DATE

	kit total	(0.5M) sucrose	dd H2O	0 min	60 min	30
blank a	1	9	0	65	72	
blank b	1	9	0	66	72	
blank c	1	9	0	68	70	
blank only H2O a	1	0	9	Er4	Er4	
blank only H2O b	1	0	9	Er4	Er4	
blank only H2O c	1	0	9	Er4	Er4	

② Preparing sucrose.

$M = \frac{0.02 mol}{0.02 L}$ (water). ↱ M.W. = 342 ⇒ M = 6.84 g (sucrose)

SUBJECT

DATE

	DNase free water	A	B	inh	toe	tri	total
zz146 A (1) (313.44)		2	1.5	0.2	0.16		5
zz146 B (1) (293.72)		2	1.5	0.2	0.17		
zr146 A (307.76)		2	1.5	0.2	0.16		
zr146 B (284.01)		2	1.5	0.2	0.18		
0r146 A (304.29)		2	1.5	0.2	0.16		
0r146 B (268.99)		2	1.5	0.2	0.19		
0z146 A (323.44)		2	1.5	0.2	0.15		
0z146 B (289.55)		2	1.5	0.2	0.17		

SUBJECT

DATE

	free water	A	B	inh	toe	tri	total
Zr146 A (+)	0.18	2	1.5	0.2	0.16	0.36	5
Zr146 A (-)	1.14	2	1.5	0.2	0.16	0	5
Zr146 B (+)	0.76	2	1.5	0.2	0.18	0.36	5
Zr146 B (-)	1.12	2	1.5	0.2	0.18	0	5
ZZ146 A(I) (+)	0.78	2	1.5	0.2	0.16	0.36	5
ZZ146 A (I) (-)	1.14	2	1.5	0.2	0.16	0	5
ZZ146 B (I) (+)	0.77	2	1.5	0.2	0.17	0.36	5
ZZ146 B (I) (-)	1.13	2	1.5	0.2	0.17	0	5
Or146 A (+)	0.78	2	1.5	0.2	0.16	0.36	5
Or146 A (-)	1.14	2	1.5	0.2	0.16	0	5
Or146 B (+)	0.75	2	1.5	0.2	0.19	0.36	5
Or146 B (-)	1.11	2	1.5	0.2	0.19	0	5
Oz146 A (+)	0.79	2	1.5	0.2	0.15	0.36	5
Oz146 A (-)	1.15	2	1.5	0.2	0.15	0	5
Oz146 B (+)	0.77	2	1.5	0.2	0.17	0.36	5
Oz146 B (-)	1.13	2	1.5	0.2	0.17	0	5
Blank	5	0	0	0	0	0	5
H_2O	5	0	0	0	0	0	5

SUBJECT

DATE

		0min	30min
Zr146_A (+)	a	19	370
	b	22	379
	c	17	294
(-)	a	20	273
	b	22	346
	c	16	237
Zr146_B (+)	a	18	507
	b	21	576
	c	17	543
(-)	a	20	455
	b	19	535
	c	18	498
ZZ146_A (+)	a	18	100
	b	13	102
	c	19	111
(-)	a	15	85
	b	16	86
	c	16	74
ZZ146_B (+)	a	18	51
	b	18	58
	c	15	51
(-)	a	17	38
	b	15	44
	c	16	43

		0min	30min
OR146_A (+)	a	20	218
	b	21	246
	c	15	216
(-)	a	17	137
	b	19	153
	c	18	146
or146_B (+)	a	15	38
	b	14	40
	c	17	46
(-)	a	18	31
	b	21	37
	c	17	31
OZ146_A (+)	a	18	15
	b	17	13
	c	14	14
(-)	a	15	50
	b	17	48
	c	14	51
OZ146_B (+)	a	18	253
	b	20	328
	c	19	312
(-)	a	19	206
	b	18	231
	c	21	221

SUBJECT 12:12 a.m.

DATE

		0 min	30 min
B	a	Er 4	Er 4
	b	Er 4	Er 4
	c	Er 4	Er 4
H2O	a	Er 4	Er 4
	b	Er 4	Er 4
	c	Er 4	Er 4

SUBJECT

DATE

		free water	A	B	inh	toe	tri	total
pp21B (3>6.32)	(+)	0.79 ✓	2	1.5	0.2	0.15 ✓	0.36 ✓	5
	(−)	1.15 ✓	2	1.5	0.2	0.15 ✓	0	5
OP21A (3>2.01)	(+)	0.78 ✓	2	1.5	0.2	0.16 ✓	0.36 ✓	5
	(−)	1.14 ✓	2	1.5	0.2	0.16 ✓	0	5
OP21B (>9.42)	(+)	0.77 ✓	2	1.5	0.2 ✓	0.17 ✓	0.36 ✓	5
	(−)	1.13 ✓	2	1.5	0.2 ✓	0.17 ✓	0	5
02>1A (3>2.72)	(+)	0.79 ✓	2	1.5	0.2 ✓	0.15 2.17 ✓	0.36 ✓	5
	(−)	1.15 ✓	2	1.5	0.2 ✓	0.15 2.17 ✓	0	5
02>1B (3>1.83)	(+)	0.8 ✓	2	1.5	0.2 ✓	0.14 ✓	0.36 ✓	5
	(−)	1.16 ✓	2	1.5	0.2 ✓	0.14 ✓	0	5
zp21A (3>1.29)	(+)	0.78 ✓	2	1.5	0.2 ✓	0.16 ✓	0.36 ✓	5
	(−)	1.14 ✓	2	1.5	0.2 ✓	0.16 ✓	0	5
zp21B (305.65)	(+)	0.78 ✓	2	1.5	0.2 ✓	0.16 ✓	0.36 ✓	5
	(−)	1.14 ✓	2	1.5	0.2 ✓	0.16 ✓	0	5
zz21A (305.65)	(+)	0.78 ✓	2	1.5	0.2 ✓	0.16 ✓	0.36 ✓	5
	(−)	1.14 ✓	2	1.5	0.2 ✓	0.16 ✓	0	5
zz21B (307.76)	(+)	0.78 ✓	2	1.5	0.2 ✓	0.16 ✓	0.36	5
	(−)	1.14 ✓	2	1.5	0.2 ✓	0.16 ✓	0	5
B		5	0	0	0	0	0	5
H20		5	0	0	0	0	0	5

SUBJECT

14:52

DATE

		0min	10min	30min			0min	10min	30min
pp21 B (+)	a	18	180	525	oz21 B (−)	a	15		29
	b	21	205	660		b	14		36
	c	19	195	534		c	15		36
pp21 B (−)	a	23	212	588	zp21 A (+)	a	18		70
	b	23	208	583		b	17		62
	c	23	203	559		c	15		64
op21 A (+)	a	21	27	58	zp21 A (−)	a	18		62
	b	19	29	63		b	16		61
	c	22	26	55		c	11		35
op21 A (−)	a	20	24	40	zp21 B (+)	a	19		448
	b	19	24	41		b	18		425
	c	19	23	35		c	21		466
op21 B (+)	a	20	41	139	zp21 B (−)	a	17		444
	b	19	~~45~~	173		b	19		482
	c	18		148		c	18		428
op21 B (−)	a	20	45	137	zz21 A (+)	a	12		34
	b	18		143		b	18		48
	c	18		131		c	16		47
oz21 A (+)	a	~~14~~ 17		31	zz21 A (−)	a	15		29
	b	22		38		b	15		27
	c	16		33		c	15		31
oz21 A (−)	a	21		21	zz21 B (+)	a	13		71
	b	21		22		b	13		72
	c	21		23		c	15		76
oz21 B (+)	a	13		~~53~~ 34	zz21 B (−)	a	15		60
	b	14		~~53~~ 53		b	10		55
	c	15		47		c	14		59

SUBJECT

DATE

14:52

		0 min	10 min	30 min
Blank	a	Er4		Er4
	b	Er4		Er4
	c	Er4		Er4
H_2O	a	Er4		Er4
	b	Er4		Er4
	c	Er4		Er4