SUBJECT

DATE 2020.06.01 陳慧晴

Place: Ming Dao high school

Temperature: 25°C

Pressure: 1 atm

~~Experiment:~~

分子數比 vector : insert = $\frac{10}{2807bp} : \frac{1}{74bp}$

1 : 1

1 : 20 = 1 : 3.79

V	3
I	1
10X Bfr	2
T4 DNA Ligase	1
H_2O	13
	20 μl

Experiment: ① Electrophoresis of 0526 I (T7 terminator)

② Gel extraction of 0526 I (T7 terminator)

~~③ Electrophoresis of V. I~~

③ Clean up V

④ Electrophoresis of V. I

~~⑤~~

<Protocol>

① see protocol of 2020.05.19 Electrophoresis (change to 25 min)

SUBJECT

DATE

② Gel extraction of I:

1. Sterilize a knife with alcohol and dd H_2O
~~2. cut the gel~~
2. Excise the gel slice contaning I(T) terminator)
3. Transfer 300 mg of the gel slice to a 1.5 ml microcentrifuge tube.
4. Add 600 ul of Gel/PCR Buffer and mix by vortex.
5. Incubate at ~~55 to~~ 60°C for 15 min, During incubation, invert the tube every 2-3 min.
6. Place a DFH Column in a 2 ml Collection Tube. Transfer 800 ul of the sample mixture to the DFH Column.
7. Centrifuge at 15,000 xg for 30 seconds.
8. Discard the flow-through and place the column back in the collection tube.
9. Repeat 6-8 step until the mixture deplet.
10. Add 400 ul of W1 Buffer to the column,
11. Centrifuge at 15,000 xg for 30 sec then discard the flow-through
12. Place the column back in the collection tube, and add 600 ul of Wash Buffer into the column.
13. Let stand for 1 min at room temperature, ~~and~~
14. Centrifuge at 15,000 xg for 30 sec then discard the flow-through
15. Put the column back in the collection tube, and ~~Centrifuge~~ centrifuge at 15,000 xg for 3 minutes.
15. Transfer the dry column to a new 1.5 ml microcentrifuge tube.
16. Add 20 ul Elution Buffer into the center of the column.
17. Incubate at 50°C for 5 min

SUBJECT

DATE

18. Centrifuge at 15,000×g for 2 min.

③ Cleanup V

1. Add 500 μl of Gel/PCR Buffer into 100 μl of V. and mix by vortex.
2. Place a DFH column in a 2 ml Collection Tube.
3. Transfer the mixture to DFH column.
4. Centrifuge at 15,000×g for 30 sec. the discard the flow-through and put the column back in the 2 ml collection tube.
5. Add 400 μl W1 to the column.
6. Centrifuge at 15,000×g for 30 sec. the discard the flow-thro
7. Add 600 μl of Wash Buffer to the column.
8. Let stand for 1 min.
9. Centifuge at 15,000×g for 30 sec the discard the flow-thro
10. Put the column back in the collection tube, and centrifuge at 15,000 ×g for 3 min.
11. Transfer the dry column to a new 1.5 ml microcentrifuge tube.
12. Add 40 μl Elution Buffer into the center of the column.
13. Incubate at 50°C for 5 min.
14. Centrifuge at 15,000×g for 2 min.

④ Electrophoresis of V. I.

See protocol of 20.20.05.19 protocol (3 μl for each sample)

SUBJECT

DATE 2020.06.01

Place: Mingdao High School

Temperature: 30°C

Atmospheric Pressure: 1 atm

Experiment: Ligation of vector and insert (Vector: T7+RBS+~~Invertase~~ RFP
Insert: terminator)

Background: After the gel electrophoresis of vector and insert, we estimated visually that the fluorescent ratio of the two is 10 : 1 (Vector : Insert). Considering the base pair differences, we roughly ~~calculating~~ calculated the amount of ~~vect~~ inserted plasmid should be 3.79 times more than ~~the~~ vector plasmid. (Amount Ratio : $\frac{10}{2804} : \frac{1}{74} \cong 1 : 3.79$) In order to meet our need for ligation reaction, which is the amount of vector and insert should be roughly same (Vector : Insert = 1 : 1), we ~~chose~~ chose not to follow the conventional ratio —— because our insert plasmid contains only 74 bp, and it would be too small to ligate properly without ~~over~~ self-ligation [Insert - Insert], we optimized the volume of vector to 3 uL and the volume of insert to 1 uL for equalling the amount of two plasmid.

Protocol.

ddH2O : 13 uL	• Add the reagents from up to down accordingly.
10X Buffer : 2 uL	• Make sure to add DNA ligase last and keep it on ice incubator when necessary.
Vector : 3 uL	• Incubate the mixture at room temperature for 4 hr
Insert : 1 uL	
T4 DNA ligase : 1 uL	
Total : 20 uL	• Transform the plasmid into competent cells.

SUBJECT

DATE 2020.06.01 張桓睿

Place: Minghsin High School

Temperature: 36°C

Atmospheric Pressure: 1 atm

Experiment: Transformation (Plasmid: T7+RBS+~~Invertase~~ RFP +Terminator)

Competent Cells: E.coli. DH5α

Preparation: ① Sterilize the styrofoam box with alcohol

② Sterilize the pipette with alcohol

③ Turn on the UV light in the Laminar Flow for 3-5 min

④ Prepare ice in styrofoam box

⑤ Set the dry bath incubator, 42°C

Protocol:

1. Add 50 uL of E.coli DH5-α to an eppendorf.
2. Add 5 uL of plasmid to the eppendorf.
3. Vortex
4. Ice 3-5 min
5. Heatshock the mixture for 1min under 42°C
6. Ice 3-5 min.
7. ~~Add~~ Add the mixture into 500 uL of antibiotic-free LB medium.
8. Incubate it under 37°C for 1 hour.
9. Centrifuge the mixture at 15000 rpm for 2min
10. ~~Spring~~ Withdraw 450 uL of mixture and discard it.
11. Vortex the remaining mixture.
12. Spread the mixture onto Cm Agar Plate.
13. Incubate overnight (16~18 hours)

SUBJECT

DATE 2020.6.24 Shen

Place: Ming Dao High school

Temp: 25°C Pressure: 1 atm

Exp1: PCR + Plasmid DNA ↗ toehold + Invertase

10 ng/~~μ~~l DNA	1	I1 5.30
10 uM primer forward	1.5	←inv_Mfel_F
10 uM primer reverse	1.5	~~inv_Mfel_F~~
		inv_Suffix_R
10 x Buffer	5	
MgSO4	2	
H2O	33	→ 50 ul

dNTPs 5

KOD-plus DNA pol. 1

95°C 3:00 / 95°C 30' / ~~55°C~~ 60 30' / ~~72~~ 68 °C 1:00 / 72°C 5:00 / 12°C

34x

Exp2: Plasmid DNA

	260/230	260/280	Con (~~ug/mL~~ ng/uL)
RPF1 0624	4.78	1.57	94.52
			94.52
RPF2 0624	4.78	1.57	
RPF3 0624	4.04	1.49	151.27

Exp3: PCR clean up → failed

21.65 19.86 1 15.25
21.60 2 15.21
21.46 3 15.29

SUBJECT

DATE 2020.6.29 六

Place: Ming Dao High school

Temp: 26°C Pressure: 1 atm

Exp: PCR (I0) 感應的 banana sensor invertase

(※PCR w/ KODplus of banana sensor invertase (I0) in different temperature)

<Preparation> dilute I0. I1. I2. I3 (from 20.05.30) 8 小

→ 10 ng/μl

	ng/μl	water	I
I_0	100	9	1
I_1	201.92	19.192	1
I_2	185.91	17.592	1
I_3	188.71	17.871	1

< protocol >

~~PCR w/ KOD-plus of~~

DNA (10ng/μl)(I0)	1
10μM primer forward (inv_Mfe1_F)	1.5
10μM primer reverse (inv_suffix_R)	1.5
10x buffer	5
dNTPs	5
$MgSO_4$	2
※Tip=水上 KOD-plus	1
H_2O	33
	50

①	②	③	④	⑤	⑥	⑦	⑧
65	64.3	63	61.1	58.8	56.9	55.7	55

< result >

2020.06.29 broken.tif

2020.06.29_final.tif

* ① ② ~~③~~ 台 spin down

★★ enzyme 下一 spin down vortex

clean up ②③④⑤⑥⑧

40 μl left in tube

SUBJECT

DATE

cleanup ②③④⑤⑥⑧

<protocol>

refer to 2020.06.01

40 μl left in tube, add 10 μl of water

elution buffer : 20 μl

~~<result~~

<result>		con	260/230	260/280
	ddH2O	0.36	1.72	1.48
⑧	PCR I 0629	6.08	~~2.03~~	0.94
	PCR I 0629	6.08	1.77	0.94
	ddH2O	2.17	4.69	3
	ddH2O	0.98	1.95	1.04

SUBJECT

DATE 2020.06.30 Shen

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

① Toehold_invertase + Tri + Sucrose 全部
② Toehold_invertase + Sucrose 沒加Tri
③ Toehold_invertase + Tri
④ Sucrose
⑤ Blank (Water)

	①	②	③	④	⑤
H_2O	0.661	1.052	0.661	1.3	5
A	2	2	2	2	0
B	1.5	1.5	1.5	1.5	0
inhi	0.2	0.2	0.2	0.2	0
toe	0.248	0.248	0.248	0	0
tri	0.391	0	0.391	0	0
Sucrose	3.5	3.5	0	3.5	0
H_2O	0	0	3.5	0	3.5

2 hr (37°C)

1 hr (75°C)

glucose (mg/dL)

00'00"	64	29	42	42	Er4
10'00"					
20'00"		Er4	116	20	Er4
30'00"	139		Er1	±	
40'00"	105	44	168	18	Er4
50'00"	151	Er4	186	15	Er4
60'00"	Er4	66	Er3		

* ③ H_2O to成 Sucrose

< prepare sucrose >

13.69g sucrose
add water to 40 ml

SUBJECT

DATE
2020.06.30 Iris

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

Experiment: characterization → In vitro protein synthesis test.

Protocol:

1. Add the reagents (on ice) for synthesis of mRFP into well of the 384-well plate

*mRFP → put in B9

DNase / RNase free water: 10 μL
Solution A: 4 μL
SolutionB: 3 μL
RNase inhibitor: 0.4 μL
RFP + terminator: 1.1 μL
Total: 10 μL

2. Add the reagents (on ice) for blanking into one well of the 384-well plate

* Blank → put in B11

DNase/RNase free water: 5 μL
Solution A: 2 μL
Solution B: 1.5 μL
RNase inhibitor: 0.2 μL
Total: 5 μL.

	B9	B10	B11	B12	B13
*	DNaseF	1.5 μL	1.3	5	0
	Solution A	4 μL	2	0	0
	SolutionB	3 μL	1.5	0	0
	RNaseI	0.4 μL	0.2	0	0
	DNA Template	1.1 μL	0	0	0

B9	B10	B11	B12	B13
RFP	\	blank	5μL ↓ free water	\

SUBJECT

DATE

3. Seal the plate with microseal (on ice)
4. Centrifuge (4000 rpm, 0.01 (1 min), 4°C)

~~Plate Reader~~

5. Put into the plate reader
   - 37°C
   - 2 hour
6. Take the 384 well plate out of the plate reader and remove its seal.
7. Apportion 5 uL of mRFP reagent into a new well.
8. Add RIPA Buffer into the original well of mRFP till total volume of ~~20~~ uL. 25
9. Put into the Plate Reader.

<result>
Data: 550 - 600 overflow

~~<revise>~~
<revise>
- Original: Excitation peak 584nm, Emission peak 607nm
  ↓
  [600, 580]
- Diluted: 20X
- Set: 550 - 650 by 1.
- Gain: auto

SUBJECT

DATE
2020. 6. 30. James.

SUBJECT

DATE
2020.06.30 Ivy

Place : Ming Dao High School
Temperature : 35°C
Pressure : 1 atm

Experiment : PCR : invertase (T7 promoter + RBS + invertase + T7 terminator)
PCR clean up

< Protocol >

10 ng/μl DNA template	1
10 μM primer forward	1
10 μM primer reverse	1
2x Master Mix	25
dd H_2O	22
total	50

	95°C	5 min
35x	95°C	30sec
35x	55°C, 50°C	30sec
35x	72°C	2 min
	72°C	5 min
	12°C	∞

①.②.③ : 55°C
④.⑤.⑥ : 50°C

SUBJECT

DATE

<result>

260/230	260	280	Con(ng/ul)
3.48	4.07	2.52	203.31