SUBJECT

DATE 2020.05.20 陳慈晴

Place: Ming Dao high school

Temperature: 26°C

Pressure: 1 atm

Experiment: Transformation of ① T16: T7 terminator (2016 distribution kit)

② T17: T7 terminator (2017 distribution kit)

③ T18: T7 terminator (2018 distribution kit)

<preparation>

1. Sterilize styrofoam box with alcohol.
2. Sterilize pipette and clean bench with alcohol.
3. Turn on the UV light of the clean bench, sterilizing for 3~5 min.
4. Prepare ice (with the sterilized box)
5. Set ~~at~~ the dry bath incubator, 42°C.

<Protocol>

on ice:
1. add 20 μL of E-coli DH5α
2. add 2 μL of target
3. vortex
4. ice 3-5 min

5. heat shock 1 min (42°C dry bath incubator)
6. ice 3-5 min
7. add 100 μL of LB media (antibiotic free)
8. 37°C, shaking for 1 hour
9. Spread on Cm agar plate

<Result>

T16 failed

T17. T18 success

SUBJECT

DATE
2020.05.20 陳宥勝

Place: Ming Dao high school
Temperature: 26°C
Pressure: 1atm

Experiment: Banana sensor sfGFP positive control

<Preparation>

1. Put solution A, solution B, RNase inhibitor, RNase/DNase free water, banana sensor sfGFP DNA, banana trigger DNA on ice.
2. Sterilize the bench, and put on a labmat
3. Set the microplate reader machine:

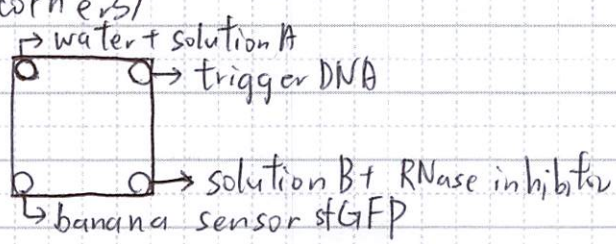
<~~Protocol~~>

+. 1) ~~38~~ Plate Type: 384 WELL PLATE, Select wells: Per step
2) ~~Temperatu~~ Description:
Temperature: Setpoint 37°C
Shake: Orbital for 1:00
Read: (F) 485,520
Start Kinetic [Run 2:00:00, Interval ~~0:00:30~~ 0:01:00]
Read: (F) 485,520
End Kinetic

<Protocol>

on ice

1. Add the reagents (seperate the droplets in different corners)



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on ice

Order, reagent \ well	A4	A5	A6	A7	A8	A9
DNAase/RNase free water	1.875					
Solution A	12.5					
Solution B	5					
RNase inhibitor	0.625					
~~banana~~ DNA template	2.5					
DNA trigger	2.5					
~~total~~	25					

	Order, reagent \ well	A1	A2
1	DNAase/RNase free H2O	2	4.5
2	Solution A	12.5	12.5
3	Solution B	5	5
4	RNase inhibitor	0.5	0.5
5	Banana sensor sfGFP	2.5	2.5
6	trigger DNA	2.5	0
	total	25	25

2. Seal the wells with microseal
3. Place the plate in the plate reader, and start the measurement

<Result> see "20200520_1.xlsx"

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<Discussion>

1. Certain components in Solution A, B and DNA may precipitate during storage. Be sure to mix well before use.
2. Add more RNase inhibitor (see the instruction on the bottle)
3. Should centrifuge before ~~usi~~ reading the plate.

SUBJECT

DATE
20.20.05.21 陳彥晴

Place : Ming Dao high school
Temperature : 25°C
Pressure : 1atm

Experiment : Agar plate preparation

<Protocol>

1. Add 200 mL dd H_2O into a ~~drun~~ duran bottle.
2. Add 3 g agar
3. Add 5 g LB broth
5. Shake
6. Seal the lid with tin foil
7. Sterilize with ~~ster~~ autoclave.

SUBJECT

DATE 2020.05.21 陳靖

Place : Ming Dao high school

Template: Temperature 55°C

Pressure : ~~totem~~ 1 atm

Experiment: Culture bacteria of ~~①~~ ① 17¹T : T7 terminator (2017 distribution kit)

② 17²T : 〃

③ 18¹T : T7 (terminator (2018 distribution kit)

④ 18²T : 〃

<Protocol> see 2020.05.18 protocol of culture bacteria

SUBJECT

DATE
2020.05.21 陳彥博

Place: Ming Dao high school
Temperature: 25°C
Pressure: 1atm

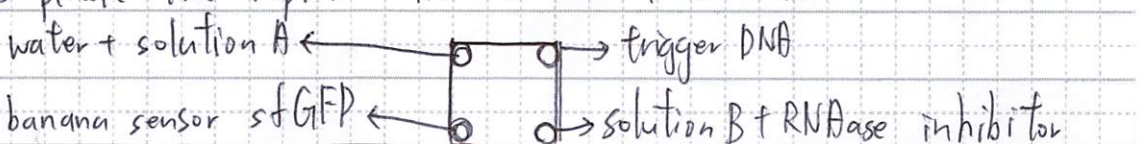
Experiment: Banana sensor sfGFP positive control

<Preparation>

1. Steilize the bench, and put on a labmat
2. Put solution A, solution B, RNase inhibitor, RNase/DNase free wate, banana sensor sfGFP DNA, control DNA template, ~~tri~~ banana trigger DNA on ice.
3. Set the microplate reader machine:
   1) Plate type: 384 well plate
   2) ~~Pl~~ select wells: Per step
   3) Description:
      Temperature: Setpoint 37°C
      Shake: Orbital for 1:00
      Read: (F) 485, 520
      Start Kinetic [Run 2:00:00, Interval 0:01:00]
        Read: (F) 485, 520
      End Kinetic

<Preparation>

on ice
1. Add the reugents
   1) Gently shake the tube before use (especially solution A and DNA)
   2) Seperate the droplets in different corners:



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on ice

3) the DNA template of A4, A5, A6, A7 = banana sensor sfGFP
the DNA template of A8 : control DNA template

Order, Reagent		A4	A5	A6	A7	A8	A9
1	DNase/RNase free water	1.875	4.375	1.875	4.375	4.875	0
2	solution A	12.5	12.5	10	10	12.5	0
3	solution B	5	5	7.5	7.5	5	0
4	RNase inhibitor	0.625	0.625	0.625	0.625	0.625	0
5	DNA template	2.5	2.5	2.5	2.5	2	0
6	banana trigger DNA	2.5	0	2.5	0	0	0
	total	25	25	25	25	25	0

2. Seal the wells with microseal
3. Centrifuge (4000 rpm, 1 min, 22°C)
4. Place the plate in the readers, check the setting, and start the measurement.

<Result>

see "20200521_1.xls"

SUBJECT

DATE
2020.05.21 Huan Jui Chang

Place : Mingdao High School
Temperature : 25°C
Pressure : 29.71 inch Hg (1006.1 mb)

Experiment : Transformation (Banana Toehold 16 DNA , Banana ~~Toehold~~ Trigger 15 Sensor sfGFP
20 Banana Toehold Invertase) (Ampicillin resistance)

<Preparation>

1. Sterilize styrofoam box
2. Turn on the UV light of BSC for 3-5 min
3. Filled ice into styrofoam box
4. Set the dry bath incubator to 42°C

<Protocol>

1. Add 20 ul of E-coli DH5α competent cell into a eppendorf.
2. Add 2 ul of targeting plasmid (100 ng/ul) into the eppendorf.
3. Vortex the previous mixture.
4. Cool the mixture on ice for 4 min.
5. Heat shock the mixture in 42°C for 1 min.
6. Add the total 22 ul mixture into 7 ml of LB+Ampicillin broth in a centrifuge tube.
7. Incubate under 37°C for 18 hours.

<Result>

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<Discussion>

In this experiment, Huan Jui Chang and James Pan forgot to check the concentration of the targeting plasmids, leading to adding too much plasmid to the competent cell. (The recommended preparation for Elite™ Competent Cells from Geneaid is ~~for for 100 ul of~~ to transform 10^{-6} ug of pUC19 plasmids into 100 ul of ~~competent~~ competent cells, which can achieve the transformation efficiency of 5.5×10^8 transformants / ug of pUC19 plasmid. In this experiment, total of 200 ng of targeting plasmid was added into 20 ul of DH5α competent cells, which is ~~*~~ 10^6 times ~~the~~ more than the recommended ratio [20 ul competent cells / 2×10^{-4} ug plasmid]) Therefore we cut off the process of incubation on plate, ~~in order to~~ and opted for incubation in centrifuge tube overnight in order to ~~reduce the growing timespan of our transformed plasmid bacteria. We decided to proceed to the colony selection on the next day to confirme the r~~ check the result of the transformation. store the transformed bactena right after checking the result of transformation through Restriction enzyme and gel electrophoresis.

SUBJECT

DATE 2020.5.22 潘建安

Place: Mingdao high ~~#~~ school

Temperature: 25°C

Pressure: 1atm.

Experiment: Plasmid extraction of ① BBa-K731721 (T7 terminator for 2018 and 2017).
② Banana trigger DNA.
③ Banana toehold sfGFP.
④ Banana toehold invertase.

Protocol: see 2020.5.19. protocol of Plasmid extraction.

Result:

	260/230	260/280	Con (ng/μL)	
T7 (2017)	4.18	1.60	179.62	do electrophoresis.
T7-2 (2017)	4.12	1.53	205.39	
T7 (2018)	4.31	1.61	196.37	
T7-2 (2018)	4.48	1.64	182.42	
trigger	4.50	1.57	127.79	
toehold sfGFP	4.62	1.56	143.60	
toehold Inv.	4.48	1.69	34.71	→ 重做 (discard).

SUBJECT

DATE
2020.5.22.

Place = Ming Dao high school.
Temperature : 25°C
Pressure : 1atm.

Experiment : Restriction enzyme + Electrophorosis of
① BBa_731721 (2018 2019)
② Banana trigger DNA
③ Bamna toehold sfGFP
~~④ Banana toehold two.~~

Preparation : Open the heating plate in advance.

Protocol : see 2020.5.19 protocol of RE + Electrophorosis.

Result:

1.

	(sample) 取sample的量	(ddH_2O) 補ddH_2O的量
T7 (2019)	3	14
T7-2 (2019)	2.5	14.5
T7 (2018)	2.5	14.5
T7-2 (2018)	3	14
trigger	4	13
toehold sfGFP	3.5	13.5.
~~toehold two.~~		

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2. We ~~found~~ find that 2017's and 2018's BBa_J31121 ~~had~~ have the same problem as 2019's. The backbone is 500bp-heavier than the normal one. As for Banana trigger DNA and Banana toehold sfGFP, their results are ideal as we think.

SUBJECT

DATE
2020.05.25 陳靖

Place: Ming Dao high school
Temperature: 25°C
Pressure: 1atm

Experiment: Banana sensor sfGFP positive control.

<Preparation>
see 2020.05.21 ~~protocol~~ preparation of positive control

<Protocol>
see 2020.05.21 protocol of positive control
*3. Centrifuge (4000 rpm, 1 min, 4°C)
run the centrifuge machine before use, make sure the temperature decrease to 4°C

<Result>
see "20200525_1.xls"

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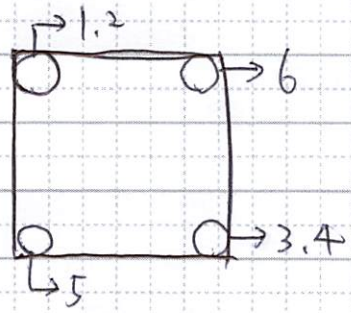
		A11	A12	A13		A14
1	DNAse/RNase free water	10.561	10.952	0.561		0
2	solution A	2	2	2		0
3	solution B	1.5	1.5	1.5		0
4	RNase inhibitor	0.2	0.2	0.2		0
5	DNA template	0.348	0.348	0.348	50ng	0
6	banana trigger DNA	0.391	0	0.391	50ng	0
	total	15	15	5		0



* concentration of DNA template (banana sensor stGFP) : 143.60 ng/μL
* concentation of banana trigger DNA : 127.79 ng/μL

calculate the amount of DNA template: 50 ÷ 143.60 = 0.348 (μL)
calculate the amount of banana trigger DNA: 50 ÷ 127.79 = 0.391 (μL)

SUBJECT

DATE
2020.05.25 馬永陽

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

Experiment: Culturing Bacteria : 18 T7 Teminator : BBa_T31721
RFP : BBa_K199118
Banana Toehold + invertase :

<protocol>

1. Add 7 mL of LB (w/ Cm) to 6 centrifuge tube.
2. Dip toothpick into frozen glycerol stock
3. Drop toothpick into the centrifuge tube.
4. Repeat step 2 & 3 two times for each sample.

T_1	22.22 g	T_1 15.26g
R_2	20.46 g	T_2 15.16g
R_1	22.47 g	R_1 15.19g
T_2	20.22 g	R_2 15.27g

T1 2.14	T1 1.98	T1 2.07
T2 2.24	T2 1.98	T2 2.05
R1 2.24	R1 1.98	R1 2.07
R2 2.06	R2 1.98	R2 2.04

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2. vortex both eppendorf.
3. put both eppendorf in the heat incubator (37°C) and store overnight.

Discussion:

1. Dai Rou and James initially misunderstood what experiment we were ~~doing~~ supposed to do. We thought we're ~~doing~~ checking ~~the~~ whether the cultured bacteria are successfully. So ~~the true~~ we have actually done the protocol incorrectly.

⇒ correct protocol:

(1) culture bacteria: T and R

(2) plasmid extraction: T_1 and R_1, R_2 (T_2)

(3) mix T_1 and T_2 into an eppendorf, so that the amount of DNA is enough for RE

(4) do the same to R_1 and R_2

(5) measure the concentration of T_1+T_2 and R_1+R_2

(6) add reagents of vector plasmid (R) and insert plasmid (T)

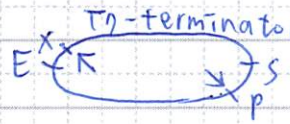
(7) vortex both eppendorf

(8) heat incubator (37°C) overnight

2. principle:

vector plasmid

insert plasmid



↓ gel extraction of ~~insert~~ plasmid after RE (cut the ~~gel of~~ plasmid fragments

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that we want off the gel after electrophoresis)

↓ ligation of V and I

E X T7-RBS-RFP T7-terminator S P

3. mind that the reagents of V and R should not contain more than 5% of glycerol (there's ~~glycer~~ about 50% of glycerol in each enzyme solution so that they won't be frozen under -20°C) or else the RE will perform "star activity"; which means RE will ~~sta~~ cut the plasmids at random ~~sights~~ sites.

4. mind the order of adding the ~~reg~~ reagents; the easier a solution gets contaminated, the latter it should be added into the eppendorf. Hence, the order should be: ddH2O → bfr → DNA → RE

★ (the order still need to be confirmed by teacher!!!)

~~5. according to discussion 1., the reason of mixin~~

SUBJECT

DATE 2020.05.27 陳彥晴

Place: Ming Dao high school
Temperature: 25°C
Pressure: 1atm

Experiment: Banana sensor sfGFP positive control

<Preparation> see 2020.05.21 preparation

<Protocol> see 2020.05.25 protocol

			5ong template/trigger 3ong ...				
			A16	A17	A18	A19	A20
左上	1	DNAase/RNase free H2O	0.561	0.952	0.850	1.091	0
	2	A solution	2	2	2	2	0
右下	3	B solution	1.5	1.5	1.5	1.5	0
	4	RNA inhibitor	0.2	0.2	0.2	0.2	0
左下	5	toehold GFP	0.348	0.348	0.209	0.209	0
右上	6	trigger	0.391	0	0.235	0	0
		total	5	5	5	5	0

*concertration: toehold GFP = 143.60 ng/μL, trigger = 129.79 ng/μL

<Result>

1. A16. A17 success
2. A18. A19 failed, due to adding the wrong amount of toehold GFP

see: 20200527_1.xls

SUBJECT

DATE 2020.05.27 蕭子道.

Place: Mingdao High School

Temperature: 25°C

Pressure: 1 atm

Experiment: Plasmid extraction of banana toehold invertase

Protocol: Modification of 19.05.2020 Protocol of Plasmid extraction

Result:

	260/230	260/280	Con(ug/nL)
ddH2O (blank)	0.36	0.82	0.30
I1	3.53	1.49	31.91
I2	2.81	0.96	23.16
I2 (test2)	2.96	0.83	21.50
ddH2O	1.77	3.35	3.72
ddH2O	0.81	0.83	1.62