SUBJECT

DATE 楊承叡
2020.08.20 張極睿. 陳薏晴

Experiment: Improve's Transformation: Banana_amilCP, pp21_amilCP, original_amilCP
(~~Banana CP~~) (pp21CP) (OriCP)
Ban CP

Resuspend's Concentration:

	260/230	260/280	Conc. ng/ul
original_amilCP	4.16	1.62	33.29
Banana_amilCP	3.46	1.62	24.31
pp21_amilCP	1.78	1.24	70.42

Dilute to 5 ng/ul

original_amilCP: 2 ul + 10 ul ddH_2O to 5ng/ul
Banana_amilCP: 2 ul + 8 ul " " "
pp21 - amilCP: 1 ul + 13 ul " " "

Transformation:

1. Add ~~33~~ 55 ul of E.coli_DH5-α competent cells into an eppendorf.
2. Add 3.5 ul of targeted plasmid into the eppendorf.
3. Cool the eppendorf on ice for 3-5 min.
4. Heatshock the eppendorf at 42°C for 1 min.
5. Cool the eppendorf on ice for 3-5 min.
6. Add 500 ul of antibiotics-free LB media into the eppendorf.
7. Incubate the eppendorf at 37°C for 1 hour. (With shaking)
8. Centrifuge the eppendorf at 15000 rpm for 2 min.
9. Remove 436 ul of the supernatent of the eppendorf.
10. Vortex and spin down.

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11. Take 100 ul of the transformed bacteria and add it on a LB plate with Kanamycin
12. Incubate the plate for overnight. (8:32 pm → 10:32 pm)

* For step number 11, we made the following modifications with suggestions from Phil

- We add competent cells into 6 dishes:
  ① pp21_AmilCP 5 ul (less than 5 ul)
  ② pp21_AmilCP 95 ul
  ③ Banana_AmilCP 5 ul
  ④ Banana_AmilCP 95 ul
  ⑤ Original_AmilCP 5 ul
  ⑥ Original_AmilCP 95 ul
- Reason: Since this is a retransformation of IDT Product, there is are possibilities that our transformed competent cells will be too prolific after overnight incubation if we add 100 ul of competent cells on to a petri dish. So we add 2 different amount of competent cells, 5 ul and 95 ul, to insure that the bacteria has a lower chance to outgrowth the petri-dish

SUBJECT

DATE
2020.08.21 楊承叡

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

Experiment: Culture Bacteria of ~~⑧~~ ori CP ① & ②
ban CP ① & ②
pp21 CP ① & ②

Protocol:
~~1. Add 7 ml LB+Kan into 6 Centrifuge tubes~~
~~2. Add~~
1. follow 2020.05.25 culturing bacteria protocol.

SUBJECT

DATE
2020.08.22 Shen

Place: Ming Dao High School
Experiment: ① Bacteria storage ② Plasmid extraction

① 300ul of 50% glycerol
+ 600ul of O/N culture bacteria
→ -80°C Third floor → IGEM 2020 CSMU BOX
Item: oriCP 0822 ① oriCP 0822 ② banCP 0822 ① banCP 0822 ② PP21 CP 0822 ① PP21CP 0822 ②
× [b]

②

	260/280	260/230	Conc. ng/ul		260/280	260/230	Conc. ng/ul
oriCP ①	1.47 ~~2.84~~	3.89	195.67	oriCP ②	1.60	4.63	141.52
banCP ①	1.57	4.44	151.27	banCP ②	1.56	4.34	150.57
PP21 ①	1.40	3.79	214.38	PP21 ②	1.46	4.10	191.49

SUBJECT

DATE 陳薏晴
2020.08.24 張相睿-陳申霖

Place : Ming Dao High School

Experiment : Improve of AmilCP (Banana Control [BanCP①], Original [OriCP①])

Plate B ~~Plate~~

	J1	J2	J3
	Banana Control	Banana Exp	Original
DNase Free	0.97 uL ~~2 uL~~	0.6 uL	1.04 uL
Sol A	2 uL	2 uL	2 uL
Sol B	1.5 uL	1.5 uL	1.5 uL
RNase Inh.	0.2 uL	0.2 uL	0.2 uL
Plasmid	0.33 uL	0.33 uL	0.26 uL
Trigger	0	0.37 uL	0

* Trigger conc. 132.57 ng/uL , 260/230 : 4.76, 260/280 : 1.68

Plate B

	J5	J6	J7	J8	J9
	Ban_Control	Banana_Exp	Original	RFP_Control	Water
DNase Free	1.94 uL	1.19 uL	2.09 uL	0.94 uL	10 uL
Sol A	4 uL	4 uL	4 uL	2 uL *	0
Sol B	3 uL *	3 uL *	3 uL *	1.5 uL	0
RNase Inh.	0.4 uL *	0.4 uL *	0.4 uL *	0.2 uL *	0
Plasmid	0.66 uL	0.66 uL	0.51 uL	0.36 uL	0
Trigger	0 uL	0.75 uL	0 uL	0 uL	0

* RFP① conc. : 140.82 ng/uL , 260/230 : 3.76, 260/280 : 1.34

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Results: Failed, Please refer to 2020.08.24 Improve absorbance on the drive.

Discussions:

① We exclude the intefere of synthesis kit by measuring the excitation and emission spectrum of mRFP, which turned out to be normal.

② We suspected that the issues may stem out from the process of maturation for camilCP after protein synthesis.

SUBJECT

DATE
2020.08.25 Ivy

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

Experiment: Improve of AmiCP { ① original (oriCP) ② improved (banCP) + trigger ③ improved (banCP)

Plate B

	oriCP		banCP+tri		banCP		H2O		
	J11	J12	J13	J14	J15	J16	J17	J18	J19
DNase free H2O	1.04	4.16	0.6	2.4	0.99	3.88	5	0	0
A	2	8	2	8	2	8	0	0	0
B	1.5	6	1.5	6	1.5	6	0	0	0
RNase inhibitor	0.2*	0.8	0.2*	0.8	0.2*	0.8	0	0	0
Plasmid	0.26	1.04	0.33	1.32	0.33	1.32	0	0	0
Trigger	0	0	0.37	1.48	0	0	0	0	0
total	5	20	5	20	5	20	5	20	0

SUBJECT

DATE 2020.08.26

楊承叡、潘建安
Ivy、張桓睿

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

Experiment: Improve of Amil CP: ① ban CP + 1x tri
② ban CP + 2x tri
③ ban CP without tri

Protocol:

1. Plate B

	J21	J22	J23	J24
DNase free H_2O	2.4	0.92	3.88	20
A	8	8	8	0
B	6	6	6	0
RNase inhibitor	0.8	0.8	0.8	0
Plasmid	1.32	1.32	1.32	0
Trigger	1.48	2.96	0	0
total	20	20	20	20

2. Plate reader
wavelength: 588nm
Temp: 37°C
Time: 4:00:00, Interval: 00:02:00
Oribital: 0:01:00

Result: Increase trigger ~~CAN~~ CAN'T increase the yield of CP.

SUBJECT

DATE
2020.08.26 楊承翰·張恒寧

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

Experiment: ~~Improve of~~ Culture bacteria of Banana Trigger 1 & 2

Protocol:

1. Add 7 ml of LB media with ampicillin into a centrifuge tube.
2. Take out frozen bacteria from -80°C and place it on ice.
3. Use a tooth pick to pick the bacteria, and mix thoroughly with the LB media.
4. Incubate overnight.

SUBJECT

DATE 2020.08.27 張桓睿.陳甲霖.陳虹婷

Place : Mingdao High School

Temperature : 24°C

Experiment : ~~Ffff~~ Plasmid Extraction of Banana Trigger ; Triple Replication of Improve

0827 BT1 0827 BT2

* Banana Trigger 1 : 166.98 ng/ul * Banana Trigger 2 : 178.22 ng/ul

Plate B

	K1	K2	K3	K4	K5
	Ban_Tri	Ban_Con	Ori	Water	BlanK
DNase_Free	2.68 ✓	3.88 ✓	4.18 ✓	20 ✓	
Sol A	8 ✓	8 ✓	8 ✓	0	
Sol B	6 ✓	6 ✓	6 ✓	0	
RNase_Inh	0.8 ✓	0.8 ✓	0.8 ✓	0	
Plasmid	1.32 ✓	1.32 ✓	1.02 ✓	0	
Trigger	1.20 ✓	0	0	0	
Total	20 ul	20 ul	20 ul	20	X

	K6	K7	K8	K9	~~Blank~~ K10
DNase Free	2.68 ✓	3.88 ✓	4.18 ✓	20 ✓	
Sol A	8 ✓	8 ✓	8 ✓	0	
Sol B	6 ✓	6 ✓	6 ✓	0	
RNase_Inh	0.8 ✓	0.8 ✓	0.8 ✓	0	
Plasmid	1.32 ✓	1.32 ✓	1.02 ✓	0	
Trigger	1.20 ✓	0	0	0	X

	K11	K12	K13	K14	K15
DNase Free	2.68 ✓	3.88 ✓	4.18 ✓	20 ✓	
Sol A	8 ✓	8 ✓	8 ✓	0	
Sol B	6 ✓	6 ✓	6 ✓	0	
RNase_Inh	0.8 ✓	0.8 ✓	0.8 ✓	0	
Plasmid	1.32 ✓	1.32 ✓	1.02 ✓	0	