

2018 University of Iowa International Genetically Engineered Machine Team
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12 July 2018 (JH and JD)

Objective: Miniprep of plasmid Pet21

Procedure:

1. 3mL cell medium ->2mL cell lysate using Qiagen kit protocol
(Refer to Qiagen kit protocol: 1091289 02/2015 HB-1921-001)

2 August 2018 (JH and JD)



13 August 2018 (JH)

1. PCR

mmsR (Craig's: p1A102) FL
i. Mixture

(ul)	P1	P2	P3	P4 (Control)
5X Buffer	10	10	10	10
25mM dNTP	0.4	0.4	0.4	0.4

11.37uM 5'-primer	2.2	2.2	2.2	2.2
12.97uM 3'-primer	2	2	2	2
DNA Template (7.6ng/uL)	1 (7.6ng)	2 (15.2ng)	4 (30.4ng)	0
Phusion-HF Polymerase (4Unit/uL)	0.5	0.5	0.5	0.5
D.W.	33.9	32.9	30.9	34.9
Total	50	50	50	50

ii. PCR protocol: EJFGEM

	Temperature ('C)	Time
Initial melt	98	10 min
Melting	98	7sec
Annealing	72	30sec
Elongation	72	40sec
Cycle Repeat	35	
Final Elongation	72	10min
Hold	4	.

mmsR (Synthesized) Effector Domains

iii. Mixture

(uL)	e1	e2
5X Buffer	10	10
25mM dNTP	0.4	0.4
9.8uM 5'-primer	2.6	2.6
29.5uM 3'-primer	0.85	0.85
DNA Template 14.7ng/uL	1 (14.7ng)	1 (14.7ng)

Phusion-HF Polymerase (4Unit/ul)	0.5	0.5
D.W.	36.3	36.3
Total	50	50

iv. PCR protocol: EJFGEM

	Temperature ('C)	Time
Initial melt	98	10 min
Melting	98	7sec
Annealing	72	30sec
Elongation	72	40sec
Cycle Repeat	35	
Final Elongation	72	10min
Hold	4	.

2. PCR Purification

Concentration (ng/ul)

i. mmsR FL

1. P1: 10.5
2. P2: 7.3
3. P3: 6.7
4. P4(Control): 3.6

ii. mmsR Effector

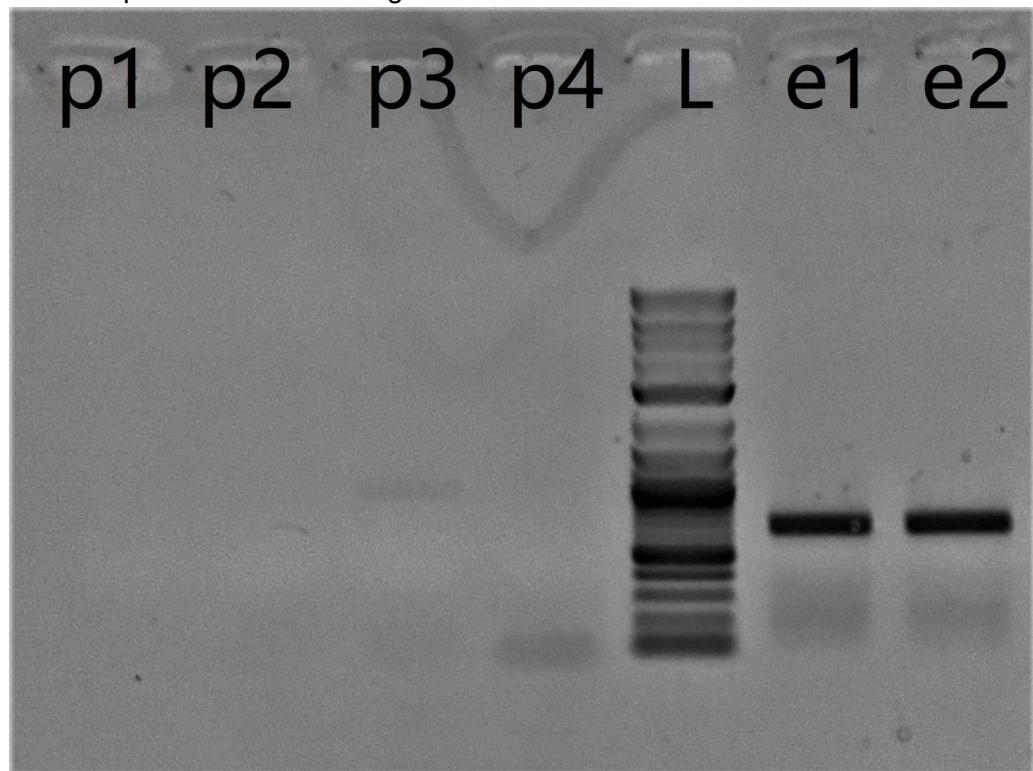
1. E1: 56.6
2. E2: 58.3

Agarose gel run

iii. P1-p4: 10ul + 2ul loading dye

iv. E1-e2: 2ul + 3ul TE buffer + 1ul loading dye

v. P1 has splashed while loading



14 August 2018 (JH)

1. PCR

a. mmsR (Craig's: p1A102) FL 2nd round

i. Mixture

P' from previous tube P3

PC used plasmid from Dr. Ellermeier's lab

(ul)	P'40	P'60	PC40	PC60
5X Buffer	10	10	10	10
25mM dNTP	0.4	0.4	0.4	0.4
11.37uM 5'- primer	2.2	2.2	2.2	2.2
12.97uM 3'- primer	2	2	2	2
P3/PC DNA Template	6 (40ng) (6.7ng/ul)	9 (60ng) (6.7ng/ul)	5 (40ng) (7.6ng/ul)	7.5 (60ng) (7.6ng/ul)
Phusion-HF Polymerase (4Unit/ul)	0.5	0.5	0.5	0.5

D.W.	28.9	25.9	29.9	27.4
Total	50	50	50	50

PCR protocol: EJFGEM

	Temperature ('C)	Time
Initial melt	98	10 min
Melting	98	7sec
Annealing	72	30sec
Elongation	72	40sec
Cycle Repeat	35	
Final Elongation	72	10min
Hold	4	.

b. Nanodrop (ng/ul):

P' 40: 33.8

P' 60: 31.2

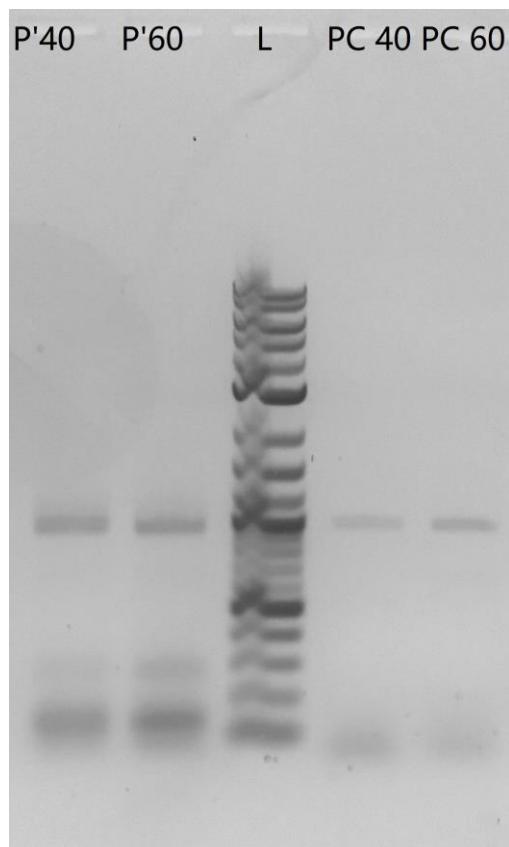
PC 40: 3.9

PC 60: 5.6

c. agarose gel run:

P': 3ul

PC: 20ul



2. PCR purification

3. Ncol / Xhol digestion

Mixture → 1hr @37°C

(ul)	mpET21 Vector (X2) 1	mmsR FL 2	mmsR Effector 3
10X Cut smart Buffer	5	5	5
DNA template (1ug)	3 (374 ng/ul)	32 (22+10) (P'40: 33.8ng/ul P'60: 31.2ng/ul)	18 (e2: 58.2ng/ul)
Ncol	1	1	1
Xhol	1	1	1
D.W.	40	11	25
Total	50	50	50

4. Gel Purification

Agarose Gel making

- i. 1% Agarose Gel X2: 100ml TAE buffer + 1g Agarose + EtBr

Gel running: 200V, 20min

Gel Extraction

5. Ligation

(ul)	Vector + effector (X2)
T4 DNA Ligase Buffer (10X)	2
Vector DNA (~5.4kB)	12
Insert DNA	4 (~0.6kB)
T4 DNA ligase	1
D.W.	1
Total	20

6. Transformation

Full length DNA lost during electrophoresis

Colonies is grown on the plate

16 August 2018 (JH)

1. PCR for full length

2.

(ul)	P'' X3 (P''1, P''2, P''3)
5X Buffer	10
25mM dNTP	0.4
11.37uM 5'-primer	2.2
12.97uM 3'-primer	2
P3/PC DNA Template	1.3 (40ng) (P' :31.2ng/ul)
Phusion-HF Polymerase (4Unit/ul)	0.5
D.W.	33.6

Total	50
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31 August 2018 (JH)

***Ligation product lost. Redo the ligation for the effector region

1.mmsR (Synthesized) Effector Domains

i. Mixture

(ul)	e3	e4	e5	e6
5X Buffer	10	10	10	10
25mM dNTP	0.4	0.4	0.4	0.4
9.8uM 5'- primer	2.6	2.6	2.6	2.6
29.5uM 3'- primer	0.85	0.85	0.85	0.85
DNA Template 14.7ng/ul	1 (14.7ng)	1 (14.7ng)	1 (14.7ng)	0
Phusion-HF Polymerase (4Unit/ul)	0.5	0.5	0.5	0.5
D.W.	36.3	36.3	36.3	37.3
Total	50	50	50	50

ii. PCR protocol: EJFGEM

	Temperature ('C)	Time
Initial melt	98	10 min
Melting	98	7sec
Annealing	72	30sec
Elongation	72	40sec
Cycle Repeat	35	
Final Elongation	72	10min
Hold	4	.

2. PCR purification

ng/ul

e3 = 60.9

E4 = 46.9

E5 = 52.0

P"1 = 36.6

P"2 = 39.4

P"3 = 40.5

3. Ncol / Xhol digestion

Mixture → 1hr @37°C

(ul)	mpET21 Vector (X2) 1	mmsR FL 2	mmsR Effector 3
10X Cut smart Buffer	5	5	5
DNA template (1ug)	3 (374 ng/ul)	24.5 (P"3: 40.5ng/ul)	16.5 (e3: 60.9ng/ul)
Ncol	1	1	1
Xhol	1	1	1
D.W.	40	18.5	26.5
Total	50	50	50

Sep.5(JH)

7. Gel Purification

Agarose Gel making

i. 1% Agarose Gel X2: 100ml TAE buffer + 1g Agarose + EtBr

Gel running: 75V, 40min

Gel Extraction

(ng/ul)

P1: 8.4

P2: 14.1

P3: 11

P4: 3.1

F: 3.9

E: 7.5

7 September 2018 (JH)

8. Ligation

(ul)	Vector + effector 1:5	Vector + effector 1:3	Vector + full length 1:5	Vector + full length 1:3	Vector
T4 DNA Ligase Buffer (10X)	2	2	2	2	2
Vector DNA (~5.4kB)	3.5 (14.1ng/ul)	3.5 (14.1ng/ul)	3.5 (14.1ng/ul)	3.5 (14.1ng/ul)	3.5 (14.1ng/ul)
Insert DNA	3.7 (~0.6kB) (7.5ng/ul)	2.2 (~0.6kB) (7.5ng/ul)	11.9(~1kB) (3.9ng/ul)	7(~1kB) (3.9ng/ul)	0
T4 DNA ligase	1	1	1	1	1
D.W.	9.8	11.3	1.6	6.5	13.5
Total	20	20	20	20	20

Room temperature for 10 min.

Transformation

Results: positive has colonies, only 1:5 full length has the colony on its plate

12 September 2018 (JH)

Objective: redo transformation with newer dh5-a cells on each ligation product and test the dh5-a direct from the company.

Results: only the plate using dh5a from the company with effector region has colonies growth present

14 September 2018 (JH)

Objective: plasmid miniprep & screening of the plasmid

Results: 6 colonies of effector region ligation and the only full-length ligation were picked and grew overnight. Went through plasmid miniprep.

E1,E2,E3,E4,E5,E6, F1

E1-F1 plasmids went through sequencing, only F1 doesn't have insertion

21 September 2018 (JD)

25uL TE Buffer into forward and effector primer

24uL TE Buffer into reverse primer

Forward Primer - 239uL of 1312 ng/uL at 11830.7g/mol

Molarity Forward Primer - 110.898 uM

Diluted- 11.0898

Reverse Primer - 239uL of 1164.5 ng/uL at 9809.3 g/mol

Molarity Reverse Primer - 118.71 uM

Diluted-11.871

Effector - 249uL of 1376.4 ng/uL at 12532.2 g/mol

Molarity Effector Primer - 109.8 uM

Diluted 10.98

PCR

HpdR from synthetic from denitrificans

(ul)	H1	H2	H3	H4(Control)
5X Buffer	10	10	10	10
25mM dNTP	0.4	0.4	0.4	0.4
11.09uM 5'- primer	2.25	2.25	2.25	2.25
11.871uM 3'- primer	2.11	2.11	2.11	2.11
DNA Template (11.9ng/uL)	.5 (5.95ng)	1 (11.9ng)	1.5 (17.85ng)	0
Phusion-HF Polymerase (4Unit/uL)	0.5	0.5	0.5	0.5
D.W.	34.3	33.8	33.3	34.8
Total	50	50	50	50

i. PCR protocol: EJFGEM

29 September 2018 (JH)

Transformation for the EF-p21a plasmid and plating.

Successful.

5 October 2018 (JH)

MINIPREP

(12:45)

Craig's lab plasmid inoculation X2

EF-p21a plasmid inoculaionX 2

Put into 5 mL LB broth with 5 uL ampicillin

37C 16~18hrs

EXPRESSION TEST

Pick 2 colonies from BL21 plate.

Put into 5 mL LB broth with 5 uL ampicillin

37C 16~18hrs

(13:25)

Autoclave 2 250 mL flasks with 100 mL LB broth

(14:30)

6 October 2018 (JH)

MINIPREP

(10:00)

Miniprep of craig's lab plasmid & EF-p21a plasmid

EXPRESSION TEST

(8:30)

100 uL ampicillin in the flask

1mL inoculation into each flask

37C 1hr then OD

(11:00)

Grow till OD -> 0.6~0.8

(13:30)

Put into 4C cooler 30 min

(14:00)

Add IPTG 100 uL stock

1. Room temp 8 hrs

2. 18C 8hrs

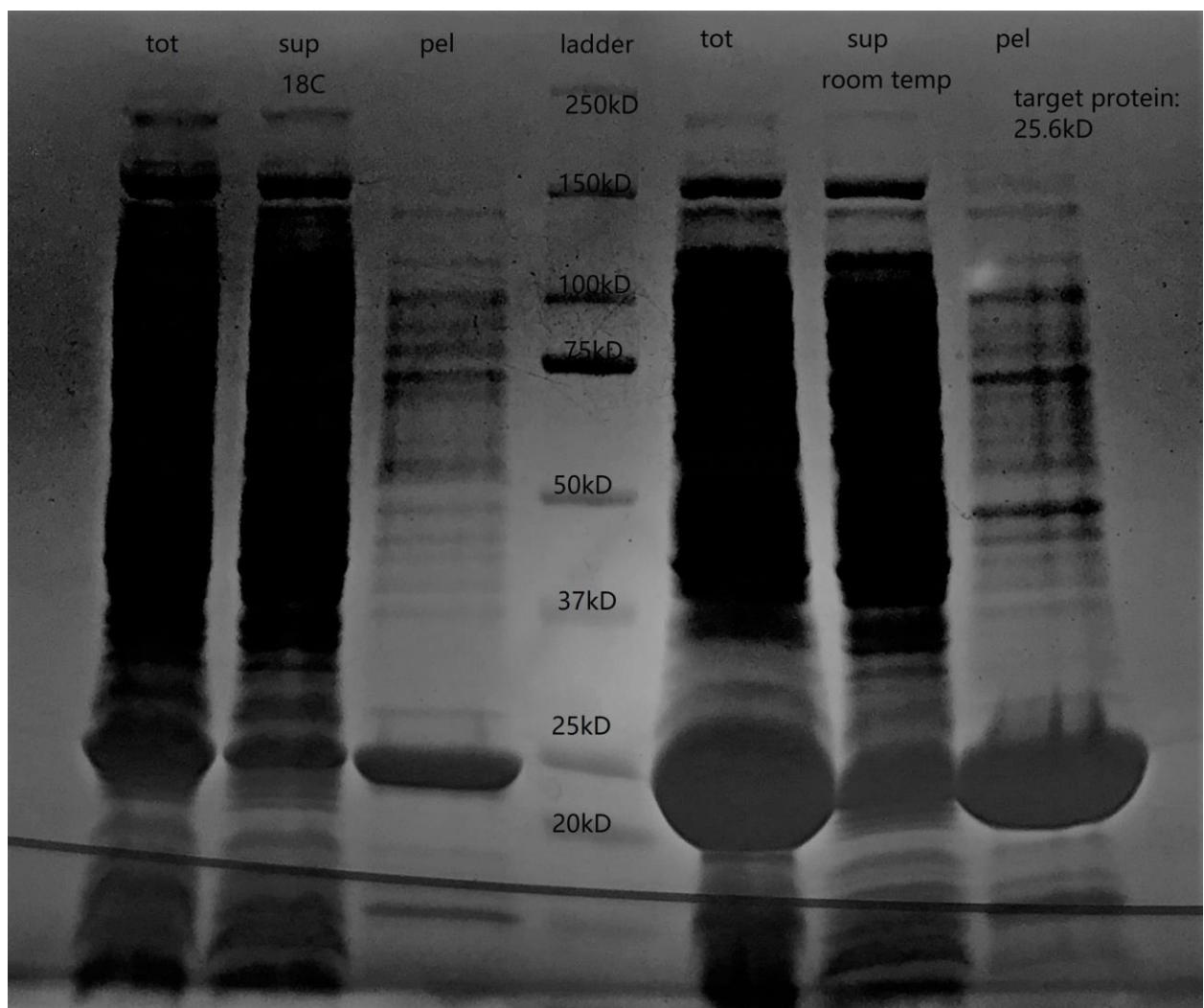
(22:00)

Into 50 mL falcon tube spin down 4400 rpm 30min

Freeze in -20C

(22:30)

12 October 2018 (JH)



Protein harvested and run on gel, results show that the BI21 expresses effector region protein