iGEM 2017 Lab Notebook

Table of Contents

Exp. iGb1

I. Title: pUC57-mini-6his-aph(3′)-VIa and
Ptac-N6-specR-IMPP2.4-pUC57-mini Assembly

Performed by: Cyrillus Tan
Recorded by: Cyrillus Tan
First Entry: 6/7/2017 1:32 PM

II. Purpose: to subclone kanamycin resistant aph(3') into pUC57 backbone; to subclone spectinomycin resistance specR into Ptac-N6 backbone.

Roadmap: for lab workshop preparation
Status: ongoing

III. Procedure

1a. Plasmid Preparation

6 mL LB-amp
Donor: 1776. pUC57-simple-6his-aph(3′)-VIa
(kan resistance)
Recipient: 573. 6his-gusA-pUC57-mini
(containing ribosome binding site but no promoter)
37 deg rotator 6/7/2017 3:16 PM

6 mL LB-amp
Donor: 1212. pCDF-specR-IMBB/pSL1180
(spec resistance)
Recipient: 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini
(containing weak constitutive promoter)

37 deg rotator 6/7/2017 3:16 PM

QIAprep miniprep on 6/8/2017
elute 40 uL

Take 3 concentration measurements
573. 6his-gusA-pUC57-mini 53.92 ng/uL
1212. pCDF-specR-IMBB/pSL1180 75.645 ng/uL
1776. pUC57-simple-6his-aph(3′)-VIa 16.587 ng/uL
2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini 62.593 ng/uL

1b. Restriction Digestion

573. 6his-gusA-pUC57-mini: desire 1882 bp, not 2688 bp
30 uL sample
38 uL ddH2O
8 uL 10x 3.1 NEB buffer
2 uL NcoI
2 uL PstI
---
80 uL

1212. pCDF-specR-IMBB/pSL1180: desire 1242 bp, not 3112 bp
30 uL sample
38 uL ddH2O
8 uL 10x 3.1 NEB buffer
2 uL PstI
2 uL NheI
---
80 uL

Gel Lane:
1/ 2-log DNA ladder
2/
3/ digested 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini
4/ digested 1212. pCDF-specR-IMBB/pSL1180
5/ digested 573. 6his-gusA-pUC57-mini
6/ digested 1776. pUC57-simple-6his-aph(3')-VIa
pic: iGb1b1

Band Isolation, concentration test
573. 6his-gusA-pUC57-mini: 17.257 ng/uL
2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini: 12.763 ng/uL

1212. pCDF-specR-IMBB/pSL1180: 12.464 ng/uL
1776. pUC57-simple-6his-aph(3')-VIa: no visible band under UV, abort

pUC57-mini-6his-aph(3')-VIa assembly end hereto

1c. Ligation

20 fmole 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini = 13 ng/kb x 1.94 kb = .22 ng = 1.935 uL
20 fmole 1212. pCDF-specR-IMBB/pSL1180 = 13 ng/kb x 1.24 kb = 16.12 ng = 1.295 uL

Enz = 1 uL 1/6 dil NEB T4 DNA ligase

#	vec	ins	enz	H2O
1	2	0	1	14
2	2	0	1	13
3	2	1.5	1	11.5
4	0	1.5	1	13.5

+ 4 uL 5x T4 DNA ligase buffer
---
20 uL

17 deg water bath 6/9/2017 7:00 PM

Gel lane
1/ 2-log DNA ladder
2/ 2116: Ptac-N6-tagRFP-IMPP2.4-pUC57-mini
3/ 1212: pCDF-specR-IMBB/pSL1180
4/ #1 ligation VOE
5/ #2 ligation VOE
6/ #3 ligation VIE
7/ #4 ligation OIE
pic: iGb1c1

1d. Bacterial Transformation

Heat shock transformation with Mach 1 E. coli
spread on LB-amp/spec plate, each 100 ug/mL
37 deg room 6/10/2017 4:30 PM

Collected at 6/11/2017 1:30 PM
iGb1d1 VOE: 0 cfu
iGb1d2 VOE: 2 cfu
iGb1d3 VIE: ~700 cfu
iGb1d4 OIE: 3 cfu

1e. Restriction Mapping

Pick 3 colonies from iGb1d3 (LB-amp/spec) plate to be iGb1e1, iGb1e2, iGb1e3, culture in LB-amp 100 ug/mL
37 deg rotator 6/11/2017 3:55 PM

Miniprep
Elute 30 uL

Take 3 concentration test
1e1: 52.19 ng/uL
1e2: 83.702 ng/uL
1e3: 75.43 ng/uL

Exp. iGb2

I. Title: (TBD) Assembly

Performed by: Cyrillus Tan
Recorded by: Cyrillus Tan
First Entry: 6/11/2017 3:56 PM

II. Purpose: to subclone _ resistant _ into pUC57 backbone.

Roadmap: lab workshop
Status: ongoing

III. Procedure

2a. Plasmid preparation

Set 1
6 mL LB-amp
Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1213: pACYC-chlR-IMBB-pSL1180

Set 2
6 mL LB-amp
Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1843: ampC ADC-33-pUC57-mini

Set 3
6 mL LB-amp
Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1212: pCDF-specR-IMBB-pSC1180

Set 4
6 mL LB-amp
Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1213: pACYC-chlR-IMBB-pSL1180

Set 5
6 mL LB-amp

Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1843: ampC ADC-33-pUC57-mini

Set 6
6 mL LB-amp
Recipient: 573: 6his-gusA-pUC57-mini
Donor: 538: rpoBC v3a-c pUC57

37 deg rotator 6/11/2017 5:31 PM

Miniprep, elute amount
1v: 40 uL 1i: 40 uL
2v: 50 uL 2i: 50 uL
3v: 40 uL 3i: 40 uL
4v: 40 uL 4i: 40 uL
5v: 60 uL 5i: 60 uL
6v: 50 uL 6i: 50 uL

Take 3 concentration test

1v: 93.537 ng/uL, 1i: 84.523 ng/uL
2v: 81.859 ng/uL, 2i: 35.873 ng/uL
3v: 88.933 ng/uL, 3i: 185.915 ng/uL
4v: 67.172 ng/uL, 4i: 53.257 ng/uL
5v: 68.366 ng/uL, 5i: 76.496 ng/uL
6v: 70.134 ng/uL, 6i: 19.683 ng/uL

2b. Restriction Digestion

1v. Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
5 uL 10x buffer 2.1
1 uL SpeI-HF
1 uL PstI-HF
39 uL sample
21.6 mqH2O

67.6 uL

1i. Donor: 1213: pACYC-chlR-IMBB-pSL1180
5 uL 10x buffer 2.1
1 uL NheI
1 uL PstI-HF
39 uL sample
19 uL mqH2O
---
65 uL

2v. Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
8 uL 10x buffer 2.1
1 uL SpeI
1 uL PstI
49 uL sample
46 uL mqH2O
---
105 uL

2i. Donor: 1843: ampC ADC-33-pUC57-mini
8 uL 10x buffer 2.1
1 uL XbaI
1 uL PstI
14 uL mqH2O
49 uL sample
---
63 uL

3v. Recipient: 2113: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
5 uL 10x NEB buffer 2.1
22.5 uL sample
1 uL SpeI-HF
1 uL PstI
20.5 uL mqH2O
---
50 uL

3i. Donor: 1212: pCDF-specR-IMBB-pSC1180
5 uL 10x NEB buffer 2.1

11 uL sample
1 uL NheI
1 uL PstI
32 uL mqH2O
---
50 uL

4v. Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
Donor: 1213: pACYC-chlR-IMBB-pSL1180

5v. Recipient: 2116: Ptac-N6-tagRFP-IMBB2.4-pUC57-mini
59 uL sample
5 uL 10x NEB buffer 2.1
1 uL SpeI
1 uL PstI
13 uL mqH2O
---
74 uL

5i. Donor: 1843: ampC ADC-33-pUC57-mini
59 uL sample
5 uL 10x NEB buffer 2.1
1 uL XbaI
1 uL PstI
17 uL mqH2O
---
83 uL

6v. Recipient: 573: 6his-gusA-pUC57-mini
29 uL sample
5 uL 10x NEB buffer 3.1
1 uL NcoI
1 uL PstI-HF
13 uL mQH2O
---
50 uL

6i. Donor: 538: rpoBC v3a-c pUC57
50 uL sample

12 uL 10x NEB buffer 3.1
1 uL NcoI
1 uL PstI-HF
56 uL mqH2O
---
120 uL

37 deg rotator 6/12/17 2:45 PM

Gel lane
pic: iGb2b1
1/ 1 kb DNA ladder
2/ 2b1v (2113) digested vector
3/ 2b1i (1213) digested insert
4/ 2b2v (2113) digested vector
5/ 2b2i (1843) digested insert
6/ (empty)
7/ 2a5v (2116) digested vector
8/ 2a5i (1843) digested insert

pic: iGb2b2
1/ 1 kb DNA ladder
2/ 2a3v (2113) undigested vector
3/ 2b3v (2113) digested vector
4/ 2a3i (1212) undigested insert
5/ 2b3i (1212) digested insert
6/ (empty)
7/ 2b4v (2116) digested vector
8/ 2a4v (2116) undigested vector

pic: iGb2b3
1/ 1 kb DNA ladder
2/ 2a4i (1213) undigested vector
3/ 2b4i (1213) digested vector
4/ (empty)
5/ 2a6v (573) undigested vector
6/ 2b6v (573) digested vector
7/ 2a6i (538) undigested vector
8/ 2b6i (538) undigested vector

2c. Gel extraction

gel band weight:
1v (2113), 1942 bp
1i (1213), 1428 bp
2v (2113), 1942 bp
2i (1842), 1217 bp
3v (2113), 1942 bp
3i (1212), 1242 bp
4v (2116), 1942 bp: 428 mg
4i (1213), 1213 bp
5v (2116), 1942 bp
5i (1843), 1217 bp
6v (573), 1882 bp
6i (538), 8532 bp

Elute amount: all 30 uL

Take 3 concentration test
1i: 19.48 ng/uL
2v: 29. 05 ng/uL
2i: 3.81 ng/uL
3v: 21.53 ng/uL
3i: 6.82 ng/uL
4v: -12.38 ng/uL
4i: -2.87 ng/uL
5v: -10.31 ng/uL
5i: -9.15 ng/uL
6v: 9.44 ng/uL
6i: -8.63 ng/uL

2d. Ligation for Ptac-N6-chlR-IMBB2.4-pUC57-mini assembly

2v + 1i.

Enz = 1/6 dil NEB T4 DNA ligase

Rxn	vec	ins	enz	H2O
1	1	0	0	15
2	1	0	1	14
3	1	1	1	13
4	0	1	1	14

+ 4 uL homemade 5x T4 ligase buffer

---

20 uL

3v + 1i.

Enz = 1/6 dil NEB T4 DNA ligase

Rxn	vec	ins	enz	H2O
1	1.2	0	0	14.8
2	1.2	0	1	13.8
3	1.2	1	1	12.8
4	0	1	1	14

+ 4 uL homemade 5x T4 ligase buffer

---

20 uL

17 deg water bath 6/14/17 1:13 PM

Gel lane:

pic: iGb2d1

1/ 11 (3v+1i) VOO
2/ 12 (3v+1i) VOE
3/ 13 (3v+1i) VIE
4/ 14 (3v+1i) OIE
5/ 21 (2v+1i) VOO
6/ 22 (2v+1i) VOE
7/ 23 (2v+1i) VIE
8/ 24 (2v+1i) OIE

pic: iGb2d2

1/ 31 (2v+1i) VOO
2/ 32 (2v+1i) VOE
3/ 33 (2v+1i) VIE
4/ 34 (2v+1i) OIE

5/ 61 (3v+1i) VOO
6/ 62 (3v+1i) VOE
7/ 63 (3v+1i) VIE
8/ 64 (3v+1i) OIE

2e. Transformation Ptac-N6-chlR-IMBB2.4-pUC57-mini plasmid into E.coli

2v+1i: (21,22,23,24; 31,32,33,34; 41,42,43,44)
Rxn 1 (VOO): 1 ng ligation = 0.7 uL
Rxn 2 (VOE): 1 ng ligation = 0.7 uL
Rxn 3 (VIE): 1 ng ligation = 0.5 uL
Rxn 4 (OIE): 1 ng ligation = 1 uL

3v+1i: (11,12,13,14; 61,62,63,64)
Rxn 1 (VOO): 1 ng ligation = 0.77 uL
Rxn 2 (VOE): 1 ng ligation = 0.77 uL
Rxn 3 (VIE): 1 ng ligation = 0.5 uL
Rxn 4 (OIE): 1 ng ligation = 1.1 uL

25 uL E. coli Mach 1
85 uL spread on LB-amp plates
37 deg incubate 6/15/17 12:36PM

Results: 6/16/17 9:30 AM
11 (VOO): 0 cfu/85 uL
12 (VOE): 1 cfu/85 uL
13 (VIE): 26 cfu/85 uL
14 (OIE): 6 cfu/85 uL
21 (VOO): 3 cfu/85 uL
22 (VOE): 41 cfu/85 uL
23 (VIE): ~400 cfu/85 uL
24 (OIE): 3 cfu/85 uL
31 (VOO): 0 cfu/85 uL
32 (VOE): 0 cfu/85 uL
33 (VIE): ~300 cfu/85 uL
34 (OIE): 0 cfu/85 uL
41 (VOO): 0 cfu/85 uL

42 (VOE): 0 cfu/85 uL
43 (VIE): ~1000 cfu/85 uL
44 (OIE): 310 cfu/85 uL
61 (VOO): 0 cfu/85 uL
62 (VOE): 1 cfu/85 uL

63 (VIE): 3 cfu/85 uL
64 (OIE): 0 cfu/85 uL

2f. Re-streak colonies from plates

select 3 colonies each from plate 13, 23, 63
to be 2f11, 2f12, 2f13; 2f21, 2f22, 2f23; 2f61, 2f62, 2f63
cultured in 6 mL LB-amp,chl (amp 100 ug/mL, chl 34 ug/mL)
37 deg rotator 6/16/17 11:30 AM

2g. Restriction mapping of Ptac-N6-chlR-IMBB2.4-pUC57-mini

Plasmid preparation for group 2g11, 2g12, 2g13; 2g21, 2g22, 2g23; 2g61, 2g62, 2g63

Elute amount:
2g11, 2g12, 2g13: 30 uL
2g21, 2g22, 2g23:
2g61, 2g62, 2g63: 40 uL

Take 3 concentration test:
2g11: 53.92 ng/uL
2g12: 51.537 ng/uL
2g13: 52.567 ng/uL
2g21
2g22
2g23
2g61: 63.3 ng/uL
2g62: 47.489 ng/uL
2g63: 93.424 ng/uL

For 2g11, 2g12, 2g13, 2g21, 2g22, 2g23; 2g61, 2g62

Digested with ScaI, expect 2 fragments, 1841 bp and 1529 bp

20 uL plasmid
15 uL mqH2O
1 uL ScaI
4 uL 10x NEB buffer 3.1
---
40 uL

37 deg incubated 6/17/17 4:06 PM

Gel lane:
pic iGb2g1
pic iGb2g2

Select sample 11 and make -80 deg frozen stock