

Recombination LogBook (August)

WEDNESDAY, 8/16/2017

- (1) PCR of phlf
- (2) check plate
- (3) Miniprep of pSB1A2-BBa_E0240

	A	B
1		pSB1A2-BBa_E0240
2	DNA Concentration (ug/ml)	166.7
3	Protein Contamination	1.818
4	Salt Contamination	2.745

- (4) Digestion of E0240 (E,X)

	A	B	C
1		pSB1A2-BBa_E0240	Negative control
2	DNA	11.9 ul	1.79 ul
3	EcoR1	1 ul	/
4	Xba1	1 ul	/
5	Cutsmart	5 ul	2 ul
6	MQ	31.0 ul	16.2 ul
7	Total	50 ul	20 ul

- (5) Purify PCR + Digestion product
- (6) Ligation

THURSDAY, 8/17/2017

(1) Transformation of pSB1A2-pPhIF and pSB1A2-BBa_B0034-PhIF-E0240

(2) Digestion of

a. B0015-R0040 (E,S)

b. B0015-R0040 (S,P)

c. E0240 (X,P)

(3) Miniprep of (conc, protein contam, salt contam)

a. pSB1A2-CRE(BBa_J61047) x2

I. 39.50, 1.927, 3.761

II. 8.090, 1.978, -2.069

b. pSB1C3-B0034-PhIF x2

I. 74.49, 1.840, 2.922

II. 123.0, 1.826, 2.734

c. pSB1C3-B0032-PhIF x2

I. 136.3, 1.835, 0.988

II. 90.45, 1.867, 2.704

(4) Digestion of

a. pSB1C3-B0034-PhIF (X,P)

b. pSB1C3-B0032-PhIF (X,P)

Table3

	A	B	C	D	E
1	/	34 (1)	34 (2)	32 (1)	32 (2)
2	conc. (ug/ml)	123.0	74.49	136.3	136.3
3	mass used (ng)	2000	300	2000	300
4	template (ul)	16.26	4.027	14.673	2.201
5	CutSmart (ul)	2.5	2.5	2.5	2.5
6	MQ (ul)	4.24	16.47	5.827	18.30
7	Xba1 (ul)	1	1	1	1
8	Pst1 (ul)	1	1	1	1
9	total volume (ul)	25	25	25	25

(5) Gel-electrophoresis:

Expected Band sizes:

- a. pSB1C3-B0034-PhIF (X,P) (2053, 647)
- b. pSB1C3-B0032-PhIF (X,P) (2053, 648)

(6) Inoculation of

FRIDAY, 8/18/2017

(1) Miniprep of

- a. pSB-BBa_B0015-BBa_R0040 (1)
- b. pSB-BBa_B0015-BBa_R0040 (2)
- c. pSB1C3-BBa_E0240 (1)
- d. pSB1C3-BBa_E0240 (2)

Table4

	A	B	C	D	E
1		pSB-BBa_B0015-BBa_R0040 (1)	pSB-BBa_B0015-BBa_R0040 (2)	pSB1C3-BBa_E0240 (1)	pSB1C3-BBa_E0240 (2)
2	DNA concentrtration (ug/ml)	169.6	101.0	145.2	162.4
3	Protein contamination	1.831	1.803	1.833	1.796
4	Salt contamination	2.304	1.905	2.128	1.685

Result of miniprep of pSB-BBa_B0015-BBa_R0040 and pSB1C3-BBa_E0240

(2) Ligation of pSB1C3-BBa_B0040-phlf (X, P) with pSB1C3-BBa_B0015-BBa_R0040 (S, P)

(3) Colony PCR of pSB1C3-BBa_B0034-phlf -BBa_E0240

expected and size (colony pcr): 2% 150V 30 mins

psb1a2-bba_b0034-k1725040 (859)

psb1a2-bba_k1725000-pphlf (304)

(4) Streak plate of pSB1C3-BBa_B0034-phlf -BBa_E0240

(5) Transformation of pSB1C3-BBa_B0015-BBa_R0040-BBa_B0034-phlf

(6) Re-digestion of pSB1C3-BBa_B0015-BBa_R0040 (E, S), pSB1C3-BBa_B0015-BBa_R0040 (S, P) and pSB1C3-BBa_B0034-phlf (X, P)

Table8

	A	B	C	D	E	F	G
1		pSB1C3-BBa_B0015-BBa_R0040 (E, S)	pSB1C3-BBa_B0015-BBa_R0040 (E, S) (-)	pSB1C3-BBa_B0015-BBa_R0040 (S, P)	pSB1C3-BBa_B0015-BBa_R0040 (S, P) (-)	pSB1C3-BBa_B0034-phIf (X, P)	pSB1C3-BBa_B0034-phIf (X, P) (-)
2	DNA concentration (ug/ml)	169.9	169.9	169.9	169.9	74.49	74.49
3	Mass used (ng)	800	300	800	300	800	300
4	Template (ul)	4.72	1.77	4.72	1.77	10.74	4.03
5	EcoR1 (ul)	0.2 /		0.2 /		0.2 /	
6	Spe1 (ul)	0.2 /		0.2 /		0.2 /	
7	CutSmart (ul)	2	2	2	2	2	2
8	MQ (ul)	12.88	16.23	12.88	16.23	6.86	13.97
9	Total Volume (ul)	20	20	20	20	20	20

Miniprep results

MONDAY, 8/21/2017

(1) Gel purification of

- pSB1C3-BBa_B0015-R0040
- pSB1C3-BBa_B0034-phIf

Table6

	A	B	C	D
1		pSB1C3- BBa_B0015- R0040 (1)	pSB1C3- BBa_B0015- R0040 (2)	pSB1C3- BBa_B0034- phlf
2	DNA Concentration (ug/ml)	2.137	26.67	8.039
3	Protein Contamination	1.879	1.818	0.617
4	Salt Contamination	1.000	0.930	1.595

(2) Digestion of

- pSB1A2-BBa_E0240 (X, P)
- pSB1A2-BBa_B0034-phlf-E0240 (X,P)
- pSB1C3-B0015-R0040 (S, P)

Table7

	A	B	C	D
1		pSB1A2-BBa_E0240 (X, P)	pSB1A2-BBa_B0034-phIf-E0240 (X,P)	pSB1C3-B0015-R0040 (S, P)
2	DNA concentration (ug/ml)	145	169.6	101.0
3	Mass used (ng)	2000	2000	2000
4	Template (ul)	13.793	11.792	19.801
5	Xba1 (ul)	1	1	/
6	Spe1 (ul)	/	/	1
7	Pst1 (ul)	1	1	1
8	CutSmart (ul)	2	2	2
9	MQ (ul)	2.207	4.208	0.598
10	Total Volume (ul)	20	20	20

(3) Colony PCR

(4) Transformation

TUESDAY, 8/22/2017

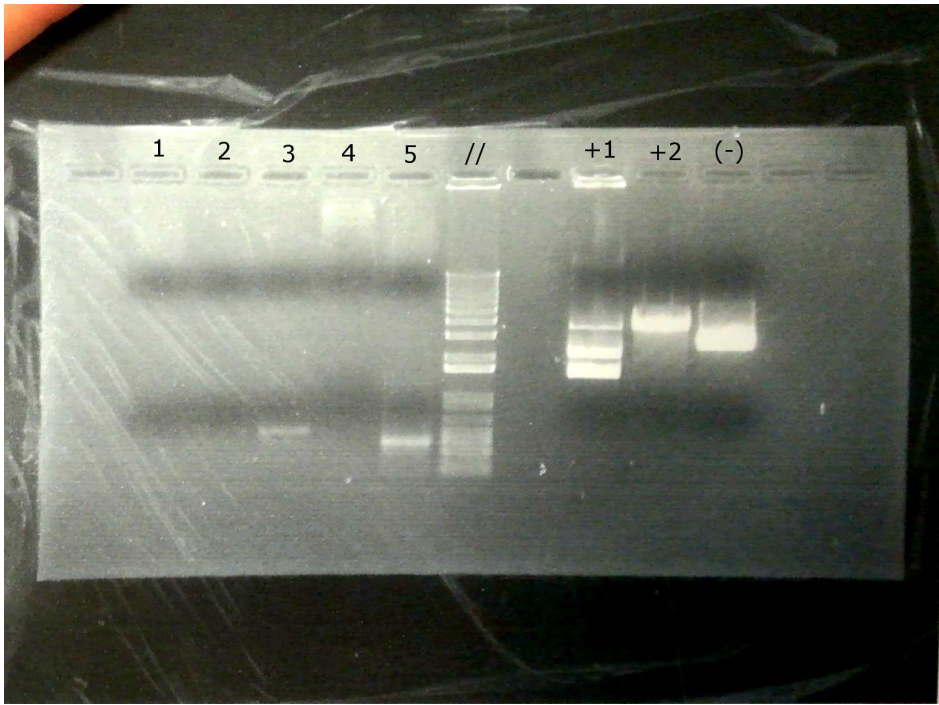
(1) Inoculation of pSB1C3-BBa_B0034-phIF

(2) Restriction check of pSB1A2-B0034-phIF-E0240

(3) Conlony PCR for pSB1C3-BBa_B0015-R0040-B0034-phIF

- 130V
- 30 mins
- 1% gel
- //: 1 kb plus ladder

WhatsApp Image 2017-08-22 at 15.45.19 (1).jpeg



colony PCR and restriction check results

1,2,3: colony PCR of pSB1C3-BBa_B0015-BBa_R0040-BBa_B0034-BBa_K1725040

4: PCR negative control

5: negative control [could be contamination]

+1: Restriction check 1st of pSB1A2-BBa_B0034-BBa_K1725040-BBa_E0240 with AseI

+2: Restriction check control of pSB1A2-BBa_B0034-BBa_K1725040-BBa_E0240 with EcoRI

[The expected band size is 3586bp which matches our result and suggests the upper band in 6th well is uncompleted digestion product]

(-): Restriction check -ve control of pSB1A2-BBa_B0034-BBa_K1725040-BBa_E0240 with no enzyme

(4) Streak plate of pSB1C3-BBa_B0015-R0040-B0034-phIF

(5) Redigestion of

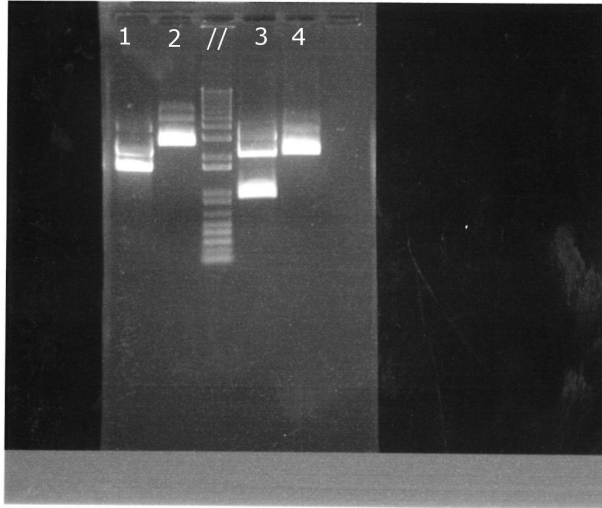
- pSB1A2-BBa_E0240 (X, P)
- pSB1A2-BBa_B0034-phIf-E0240 (X,P)
- pSB1C3-B0015-R0040 (S, P)

Table9

	A	B	C	D
1		pSB1A2- BBa_E0240 (X, P)	pSB1A2- BBa_B0034- phIf-E0240 (X,P)	pSB1C3-B0015- R0040 (S, P)
2	DNA concentration (ug/ml)	145	169.6	101.0
3	Mass used (ng)	2000	2000	2000
4	Template (ul)	13.793	11.792	19.801
5	Xba1 (ul)	1	1 /	
6	Spe1 (ul)	/	/	1
7	Pst1 (ul)	1	1	1
8	CutSmart (ul)	2	2	2
9	MQ (ul)	2.207	4.208	0.598
10	Total Volume (ul)	20	20	20

WhatsApp Image 2017-08-24 at 20.38.44 (1).jpeg

8/22/2017, 4:30 PM; Size: 1392x1032; Exp: 2000ms; Bin: 1x1; Modif: No; Disp BWG: (1, 64910, 0.45)
File: n/a (unsaved)



//: 1kb plus DNA marker

1: Digestion of pSB1A2-BBa_B0034-BBa_K1725040-BBa_E0240 (X,P)

[The expected band size is 1533bp with 2053bp, the lower band was cut]

2: negative control of 1

3: Digestion of pSB1A2-BBa_E0240 (X,P)

[The expected band size is 902bp with 2053bp, the lower band was cut]

4: negative control of 3

(6) Overnight ligation of pSB1A2-BBa_B0015-R0040-B0034-phIF-E0240

Table10

	A	B	C	D
1		Concentration (ng/ul)	Length (bp)	Mass (ng)
2	Backbone: BR (S, P)	36.390	2243	
3	Insert: BPE (X, P)	14.410	1531	

Table11

	A	B	C
1		Positive Control	Negative Control
2	Ligase (ul)	0.5	0
3	T4 ligase buffer (ul)	1	1
4	MQ (ul)	0	0.5
5	Digested backbone (ul)	1.377	1.377
6	Digested insert (u)	7.123	7.123
7	Total	10	10

Ligation of BR-BPE

(7) Inoculation of pSB1C3-BBa_B0015-R0040-B0034-phIF

(3) Miniprep

WEDNESDAY, 8/23/2017

Typhoon, no lab work

THURSDAY, 8/24/2017

(1) Transformation of

- ligated pSB1A2-BBa_B0015-R0040-B0034-phIF-E0240

(2) Miniprep of

- pSB1C3-BBa_B0034-phIf (1)
- pSB1C3-BBa_B0034-phIf (2)

	A	B	C
1		pSB1C3- BBa_B0034- phIf (1)	pSB1C3- BBa_B0034- phIf (2)
2	DNA Concentration (ug/ml)	218.4	112.4
3	Protein Contamination	1.845	1.830
4	Salt Contamination	2.471	2.421

FRIDAY, 8/25/2017

(1) Colony PCR of pSB1C3-BBa_B0015-BBa_R0040-BBa_B0034-BBa_K1725040 with 31 cycles

Table13

	A	B	C
1	If only plasmid	Temperature (°C)	Time
2	Initial denaturation	95	3 mins
3	Denaturation	95	45s
4	Annealing	53	45s
5	Extension	68	2 min 15s
6	Final extension	68	5 min
7	Holding (Storage Temperature)	8	infinity

(2) Streak Pate

(3) Inoculation

MONDAY, 8/28/2017

(1) Digestion of

1. pSB1C3-BBa_B0015-R0040 (S,P)
2. pSB1C3-BBa_B0034-phIF-E0240 (X,P)
3. pSB1C3-BBa_B0034-phIF (X,P)

Table5

	A	B	C	D	E	F	G
1		pSB1C3-BBa_B0015-R0040 (S,P)	pSB1C3-BBa_B0015-R0040 (S,P) (-)	pSB1C3-BBa_B0034-phIF-E0240 (X,P)	pSB1C3-BBa_B0034-phIF-E0240 (X,P) (-)	pSB1C3-BBa_B0034-phIF (X,P)	pSB1C3-BBa_B0034-phIF (X,P) (-)
2	DNA concentration (ug/ml)	101.0	101.0	243.2	243.2	218.4	218.4
3	Mass used (ng)	1000	300	1000	300	1000	300
4	Template (ul)	9.90	2.97	4.11	1.23	4.58	1.37
5	Xba1 (ul)	/	/	0.2	/	0.2	/
6	Spe1 (ul)	0.2	/	/	/	/	/
7	Pst1 (ul)	0.2	/	0.2	/	0.2	/
8	CutSmart (ul)	2	2	2	2	2	2
9	MQ (ul)	7.70	15.03	13.49	16.77	13.02	16.63
10	Total Volume (ul)	20	20	20	20	20	20

Reagent used for digestion

(2) Gel purification of

1. pSB1C3-BBa_B0015-R0040 (S,P)
2. pSB1C3-BBa_B0034-phIF-E0240 (X,P)
3. pSB1C3-BBa_B0034-phIF (X,P)

(3) Ligation of

1. pSB1C3-BBa_B0015-R0040 (S,P)
2. pSB1C3-BBa_B0034-phIF-E0240 (X,P)
3. pSB1C3-BBa_B0034-phIF (X,P)

(4) Transformation of

1. pSB1C3-BBa_B0015-R0040 (S,P)
2. pSB1C3-BBa_B0034-phIF-E0240 (X,P)
3. pSB1C3-BBa_B0034-phIF (X,P)

