

Sensing Log Book (July)

MONDAY, 7/3/2017

1. Transformation of pSB1C3-BBa_R0063 (pLux L) (2013)
2. Inoculation of pSB1C3-BBa_T9002, pSB1C3-BBa_F2620_C0261_E0240, pSB1A2-BBa_J23110_E0240, pSB1A2-BBa_J23117_E0240
3. Colony PCR

Table37

	A	B	C
1		Sample	Expected band size
2	1-5	pSB1C3-BBa_F2620_C0261_E0240	2920
3	6-10	pSB1A2-BBa_J23110_E0240 (10 mins)	1149
4	11-15	pSB1A2-BBa_J23110_E0240 (1 hour)	1149
5	16-20	pSB1A2-BBa_J23117_E0240	1149
6	+	BBa_J61002_J23110 (plasmid)	1142
7	(-)	BBa_F2620_C0261 (plasmid)	2259

- Adopted a new protocol for colony PCR:

Table38

	A	B	C
1	Reagents	Volume (μ l)	Master Mix (μ l) x25
2	MQ	12.85	321.25
3	5x My Taq (Reaction buffer)	4	100
4	10 μ M VF2	0.5	12.5
5	10 μ M VR	0.5	12.5
6	Taq polymerase	0.15	3.75
7	Template	2 /	
8	Total Volume	20	500

Table39

	A	B	C	D
1	Steps	Temperature ($^{\circ}$ C)	Time	Cycle
2	Initial denaturation	95	3 mins	1
3	Denaturation	95	30 seconds	35
4	Annealing	53	30 seconds	35
5	Extension	72	1 min 30 seconds	35
6	Final extention	72	5 mins	1

TUESDAY, 7/4/2017

Result: Transformation of pSB1C3-BBa_R0063: No colonies

1. Miniprep of psB1C3-BBa_T9002, pSB1C3-BBa_F2620_C0261_E0240, pSB1A2-BBa_J23110_E0240, pSB1A2-BBa_J23117_E0240

Results:

Table40

	A	B	C	D	E
1		pSB1C3- BBa_T9002	pSB1C3- BBa_F2620- C0261-E0240	pSB1A2- BBa_J23110- E0240	pSB1A2- BBa_J23117- E0240
2	DNA Concentration	181.3	32.02	104.2	99.82
3	Protein Contamination	1.826	1.717	1.821	1.821
4	Salt Contamination	2.007	1.231	2.305	1.821

Restriction Check:

Table41

	A	B	C	D	E	F	G
1	Reagents	pSB1C3-BBa_F2620-C0261- E0240		pSB1A2-BBa_J23110-E0240		pSB1A2-BBa_J23117-E0240	
2		(+)	(-)	(+)	(-)	(+)	(-)
3	DNA concentration (ng/ul)	32	32	104.2	104.2	99.82	99.82
4	DNA mass (ng)	300	300	300	300	300	300
5	MQ (ul)	6.425	6.825	12.92	13.32	12.79	13.19
6	DNA (ul)	9.375	9.375	2.88	2.88	3.01	3.01
7	HindIII-HF (ul)	0.2 /	/	/	/	/	/
8	NdeI (ul)	0.2 /	/	0.2 /	/	0.2 /	/
9	StyI-HF (ul)	/	/	0.2 /	/	0.2 /	/
10	Cutsmart (ul)	1.8	1.8	1.8	1.8	1.8	1.8
11	Total Volume (ul)	18	18	18	18	18	18

Restriction Check: pSB1C3- BBa_F2620-C0261-E0240 (bp: 1849, 2827)

- HinIII-HF for BBa_F2620
- NdeI for BBa_E0420

Restriction Check: pSB1A2- BBa_J23110-E0240 (bp: 202, 64, 2724)

- StyI-HF for BBa_J23110
- StyI-HF/NdeI for BBa_E0420

Restriction Check: pSB1A2-BBa_J23117-E0240 (bp: 202, 64, 2724)

- StyI-HF for BBa_J23117
- StyI-HF/NdeI for BBa_E0420

3. Transformation:

- pSB1C3-BBa_R0063 (2ul of DNA + 50ul of competent cells) (2013)
- pSB1A2-BBa_R0063 (2ul of DNA + 50ul of competent cells) (2013)

4. Colony PCR:

- Sample 1-3: pSB1C3-BBa_F2620-C0261-E0240
- Sample 4-6: pSB1A2-BBa_J23110-E0240 (10mins)
- Sample 7-9: pSB1A2-BBa_J23110-E0240 (1hr)
- Sample 10-12: pSB1A2-BBa_J23117-E0240
- (+): Ba_J61002-J2310
- (-): pSB1C3-BBa_F2620-C0261
- W: MQ

Reagents:

Table42

	A	B	C	D
1	Reagents (ul)	(+)	(-)	MasterMix (x18)
2	5X My Taq reaction buffer	4	4	72
3	10um VF2	0.5	0.5	9
4	10uM VR	0.5	0.5	9
5	Taq Polymerase	0.15	0.15	2.7
6	DNA template	2 /	/	
7	MQ	12.85	14.85	231.3
8	Total Volume	20	20	

PCR Cycle:

Table43

	A	B	C	D
1	Steps	Time	Temp. (°C)	
2	Initial denaturation	3mins	95	
3	denaturation	30sec	95	x25
4	annealing	45sec	53	
5	extension	1mins 30sec	68	
6	Final extension	5mins	68	
7	holding	∞	10	

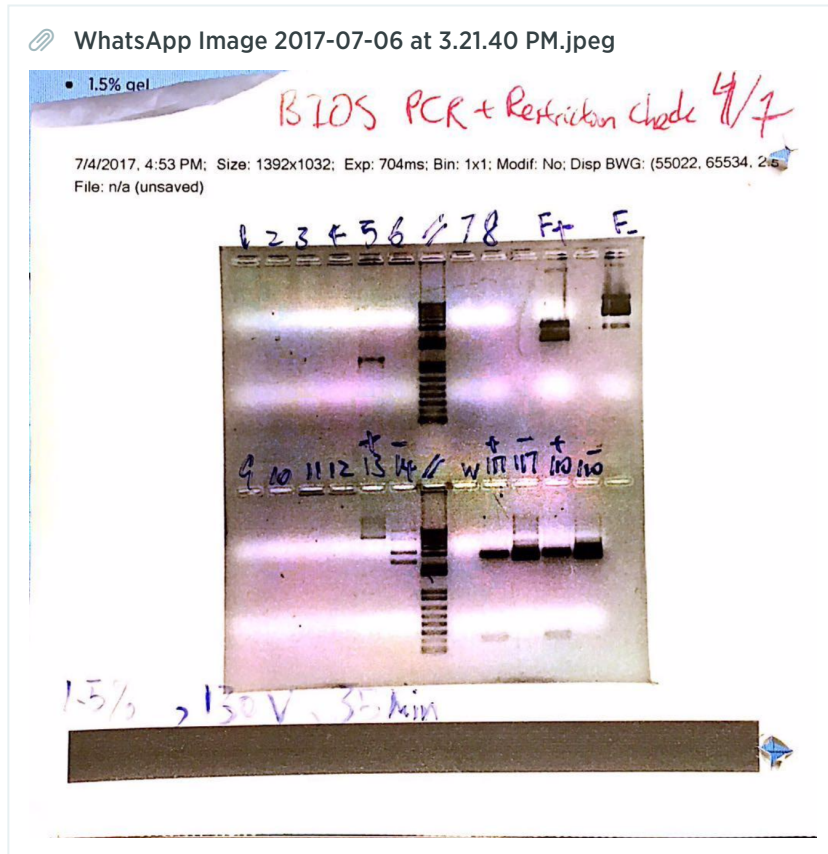
5. Gel-electrophoresis:

- 1.5% gel
- 130V, 35mins
- Colony PCR products

- Restriction check products

Results: expected bands for restriction check, not for colony PCR

Photo:



WEDNESDAY, 7/5/2017

1. Colony PCR:

- Saline solution sample 2 & 8 (7/4)

Table44

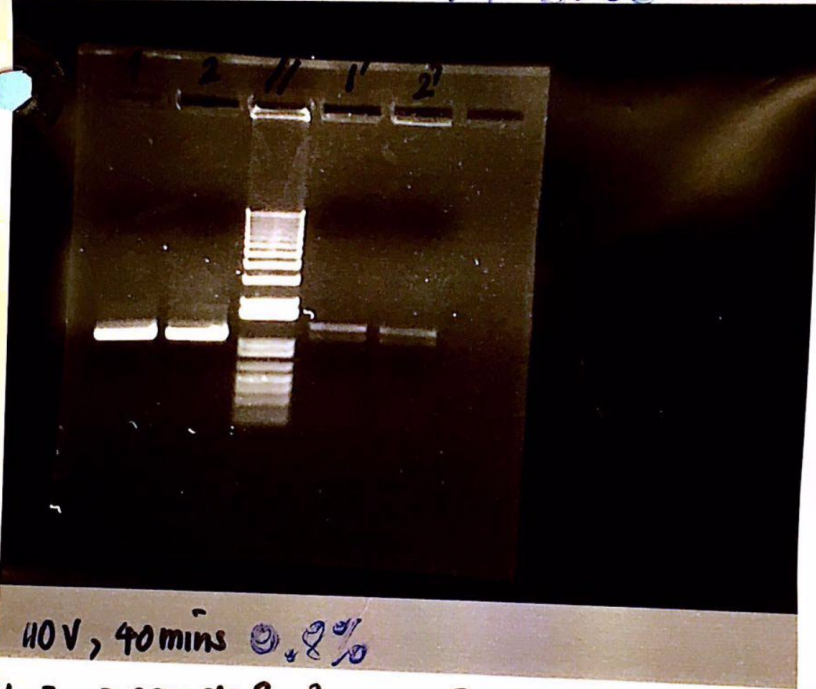
	A	B	C	D	E	F
1	Set 1:	Reagents	Volume (ul)	Set 2:	Reagents	Volume (ul)
2		MQ	14.3		MQ	12.35
3		5X MyTaq	4		5X MyTaq	4
4		10uM VF2	0.5		10uM VF2	0.5
5		10uM R	0.5		10uM R	0.5
6		Taq Polymerase	0.2		Taq Polymerase	0.2
7		DNA Template	0.5		DNA Template	0.5
8		10mM dNTP	/		10mM dNTP	0.5
9		Total Volume	20		Total Volume	20

Gel-electrophoresis photo:

WhatsApp Image 2017-07-06 at 3.21.01 PM.jpeg

7/5/2017, 5:18 PM; Size: 1392x1032; Exp: 2000ms; Bin: 1x1; Modif: No; Disp BWG: (81, 31542, 1.00)
File: n/a (unsaved)

PCR check 5/7 BIOS



110V, 40 mins @ 2%

1, 2 → sample 8 } 1 = 0.5 template
1', 2' → sample 2 } 2 = dNTPs

2. DNA extraction from kit plate:

- pSB1C3-BBa_R0063: 2014, Plate 2, 6J

3. Transformation:

- pSB1C3-BBa_R0063 (2ul of DNA + 50ul of competent cells) (2013)
- pSB1A2-BBa_R0063 (2ul of DNA + 50ul of competent cells) (2014)

THURSDAY, 7/6/2017

1. Restriction check of pSB1C3-BBa_T9002:

Table45

	A	B
1		pSB1C3- BBa_T9002
2	DNA concentration (ng/ul)	181.3
3	DNA mass (ng)	300
4	MQ (ul)	14.15
5	DNA (ul)	1.65
6	HindIII-HF (ul)	0.2
7	NdeI (ul)	0.2
8	Cutsmart (ul)	1.8
9	Total Volume (ul)	18

2.Gel-electrophoresis:

WhatsApp Image 2017-07-06 at 6.03.02 PM.jpeg

psB1C3-BBa_T9002 6/7 110V, 40 Mins
Restriction Check

7/6/2017, 4:35 PM; Size: 1392x1032; Exp: 785ms; Bin: 1x1; Modif: No; Disp BWG: (1, 23540, 1.0)
File: n/a (unsaved)



3. DNA Extraction

- psB1C3-BBa_T9002 (2015)
- pSB1A3-BBa_R0063 (2014)

4. Transformation:

- psB1C3-BBa_T9002 (2015) (2ul of DNA + 50ul of competent cells)
- psB1C3-BBa_T9002 (2014) (2ul of DNA + 50ul of competent cells)
- pSB1C3-BBa_F2620_C0261_E0240 (1ul of DNA + 50ul of competent cells)
- pSB1A2-BBa_J23110_E0240 (1ul of DNA + 50ul of competent cells)
- pSB1A2-BBa_J23117_E0240 (1ul of DNA + 50ul of competent cells)
- psB1C3-BBa_R0063 (2014) (2ul of DNA + 50ul of competent cells)
- pSB1A3-BBa_R0063 (2014) (2ul of DNA + 50ul of competent cells)

- Control: 5ul of competent cells in no antibiotic plate (see if it will form colonies?)

5. Inoculation

- pSB1C3-BBa_T9002 (from streaked plate 2014)

FRIDAY, 7/7/2017

Results:

- No colonies in pSB1C3-BBa_T9002 (2015) & pSB1C3-BBa_T9002 (2014)
- Bacterial lawn for the control 5ul competent cells with no antibiotic
- Bacterial lawn on J23110-E0240 (bright) & J23117-E0240 (Less bright)
- No colonies on BBa_R0063

MONDAY, 7/10/2017

Results:

Transformation:

- pSB1A2-BBa_R0063: 1 colony
- pSB1C3-BBa_T9002: No colony

Inoculation & streaking:

- pSB1A2-BBa_J23117_E0240
- pSB1C3-BBa_F2620_E0240
- pSB1A2-BBa_J23110_E0240
- pSB1C3-BBa_T9002
- pSB1A2-BBa_R0063

TUESDAY, 7/11/2017

Inoculation:

- pSB1A2-BBa_R0063
- pSB1A2-BBa_J23117_E0240
- pSB1A2-BBa_J23110_E0240

Streak:

- pSB1C3-BBa_F2620_C0261_E0240
- pSB1A2-BBa_J23110_E0240
- pSB1A2-BBa_J23117_E0240

WEDNESDAY, 7/12/2017

1. PCR: 20ul Reaction

Table32

	A	B
1	1	Antisense RNA1
2	2	Antisense RNA2
3	(-)	PCR negative

Table31

	A	B
1	Reagent	
2	MQ	12.4 ul
3	5X Q5 Reaction Buffer	4 ul
4	10nM dNTP	4 ul
5	10uM VF2	1 ul
6	10uM VR	1 ul
7	Template	1 ng
8	Q5 DNA Pol	0.2 ul
9	Total	20 ul

Table46

	A	B	C	D
1		Temperature (°C)	Time	Cycle
2	Initial Denaturation	98	30 seconds	1
3	Denaturation	98	10 seconds	30
4	Annealing	53	15 seconds	30
5	Exension	72	20 seconds	30
6	Final Extension	72	5 minutes	1
7	Hold	8	infinite	/

2. Gel electrophoresis (2.5% Agarose. 130V, 40 minutes)

3. Envision Multilabel Plate reader: 96 wells plate

- pSB1C3-BBa_T9002 (T)
- pSB1A2-BBa_J23117_E0240 (J7E)
- pSB1C3-BBa_F2620_C0261_E0240 (FCE)
- pSB1A2-BBa_J23110_E0240 (JE)
- Control: pSB1C3-BBa_F2620 (F)
- Control: LB

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	T(10 [^] (-6)M)	T(10 [^] (-6)M)	T(10 [^] (-6)M)	T(10 [^] (-7)M)	T(10 [^] (-7)M)	T(10 [^] (-7)M)	T(10 [^] (-8)M)	T(10 [^] (-8)M)	T(10 [^] (-8)M)	T(10 [^] (-9)M)	T(10 [^] (-9)M)	T(10 [^] (-9)M)
B	T(10 [^] (-10)M)	T(10 [^] (-10)M)	T(10 [^] (-11)M)	T(10 [^] (-11)M)	T(10 [^] (-12)M)	T(10 [^] (-12)M)	FCE(10 [^] (-6)M)	FCE(10 [^] (-6)M)	FCE(10 [^] (-6)M)	FCE(10 [^] (-7)M)	FCE(10 [^] (-7)M)	FCE(10 [^] (-7)M)
C	FCE(10 [^] (-8)M)	FCE(10 [^] (-8)M)	FCE(10 [^] (-8)M)	FCE(10 [^] (-9) M)	FCE(10 [^] (-9) M)	FCE(10 [^] (-9) M)	FCE(10 [^] (-10)M)	FCE(10 [^] (-10)M)	FCE(10 [^] (-10)M)	FCE(10 [^] (-11)M)	FCE(10 [^] (-11)M)	FCE(10 [^] (-11)M)
D	FCE(10 [^] (-12)M)	FCE(10 [^] (-12)M)	FCE(10 [^] (-12)M)	/	/	/	/	/	/	/	/	/
E	/	/	/	/	/	/	/	/	/	/	/	/
F	J7E	J7E	J7E	/	JE	JE	JE			T(OM)	T(OM)	T(OM)
G	/	/	/	FCE(OM)	FCE(OM)	FCE(OM)	F	F	F	LB	LB	LB
H	T(10 [^] (-10)M)	/	T(10 [^] (-11)M)	/	T(10 [^] (-12)M)	/	/	/	/	/	/	/

THURSDAY, 7/13/2017

Miniprep: pSB1A2-BBa_R0063

Gel purification: Antisense RNA1 and RNA2 (Failed)

PCR of antisense RNA1 and RNA2 has the same result with 12/7

FRIDAY, 7/14/2017

Antisense RNA1 and RNA2

Run-Gel: 2% gel, 130V, 30 mins

Gel purification: Antisense RNA1 and RNA2

Results:

Table1			
	A	B	C
1		Antisense RNA1	Antisense RNA2
2	conc. (ug/ml)	2.777	7.967
3	A260/A280	3.574	4.050
4	A260/A230	0.735	0.007

Another 4 sets:

Set 1:

Template: pSB1C3-BBa_F2620 (bp: 3131)

Primers: FWD pSB1C3, REV F2620

Annealing temp. : 59.9 °C

Set 2:

Template: pSB1C3-BBa_C0261 (bp: 661+39/40=700/701)

Primers: FWD F2620-ABR1/2-C0261 + REV C0261

Annealing temp. : 56.2 °C

Set 3:

Template: pSB1A2-BBa_E0240 (bp: 876+40/41=916/917)

Primers: FWD C0261-ABR1/2-E0240 + REV E0240-pSB1C3

Annealing temp. : 56.2 °C (wrong temp used)

Set 4:

Template: antisense RNA1/2 (bp: 169/170)

Primers: VF2, VR

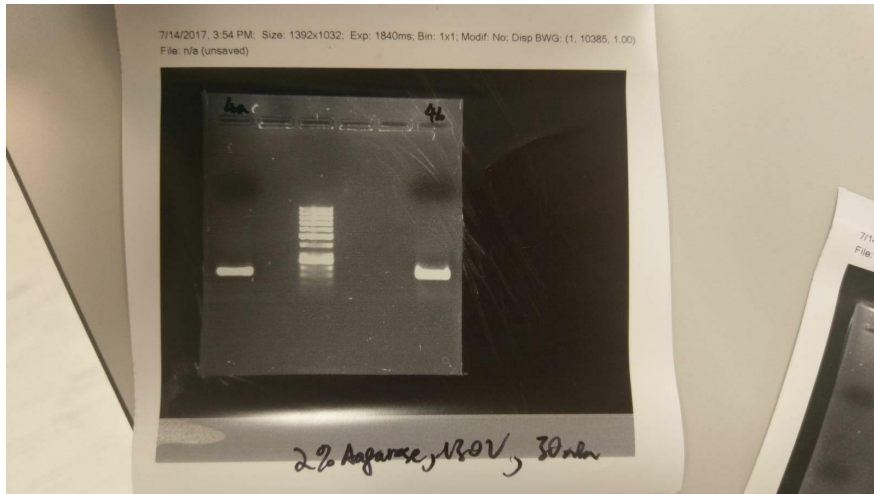
Annealing temp. : 53.0 °C

Gel-Photo:

 c3119209-3a3d-4766-9809-972e0d2ced0e.jpg



4a892eac-5f18-4b04-8c94-bd44143868b1.jpg



MONDAY, 7/17/2017

(1) PCR of set 1, 2 and 4 again

Table2

	A	B
1	MQ	12.4
2	Q5 buffer	4
3	10mM dNTP	0.4
4	Forward primer	1
5	Reverse primer	1
6	Diluted template (ng/ul)	1
7	Q5 polymerase	0.2

(2) Digestion of R0063 (backbone) and antisense RNA 1&2 (4a&4b) (169&10bp)

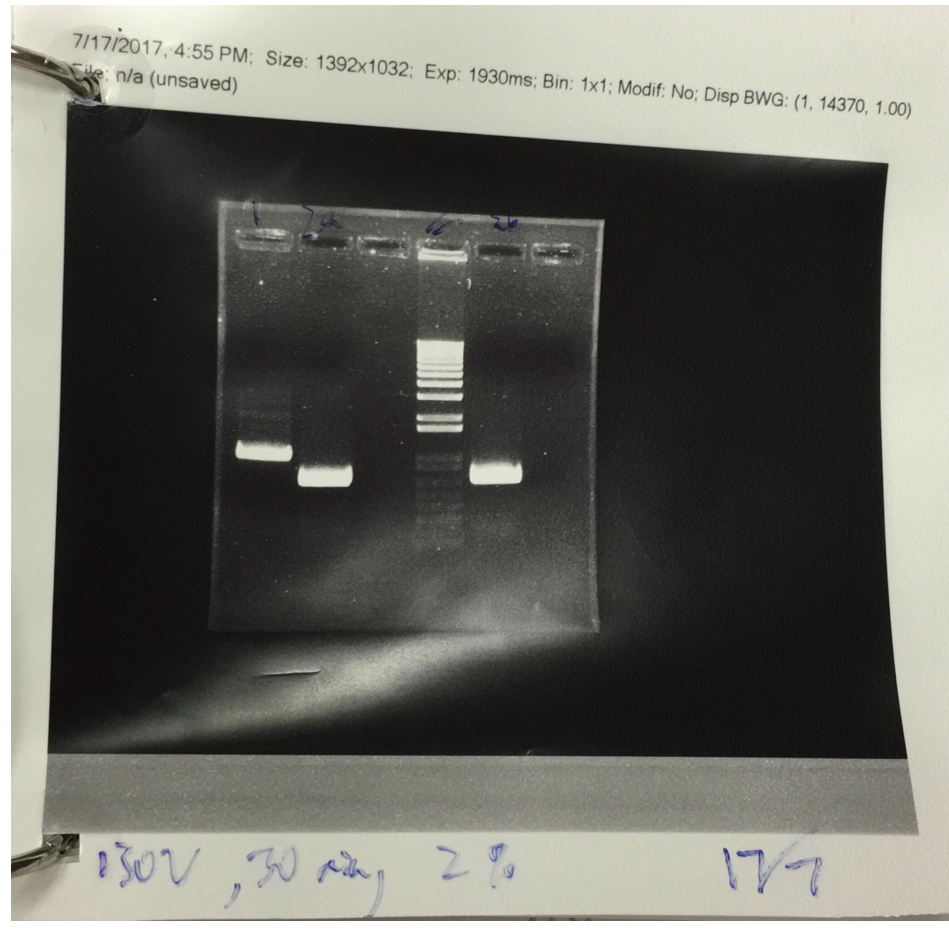
Table3

	A	B	C	D
1		pSB1A2- BBa_R0063	Antisense RNA 1	Antisense RNA 2
2	Concentration (ug/ml)	67.97	/	/
3	Mass (ng)	800	/	/
4	Water (ul)	4.03	32.1	32.1
5	DNA (ul)	11.76	12.5	12.5
6	XbaI (ul)	/	0.2	0.2
7	SpeI (ul)	0.2	/	/
8	PstI (ul)	0.2	0.2	0.2
9	Cutsmart (ul)	1.8	5	5
10	Total (ul)	18	50	50

(3) Gel electrophoresis of set 1, 2a and 2b (130V, 30 mins, 1% gel)

Gel-photo:

image.png



(4) Gel cutting fo set 1, 2a and 2b

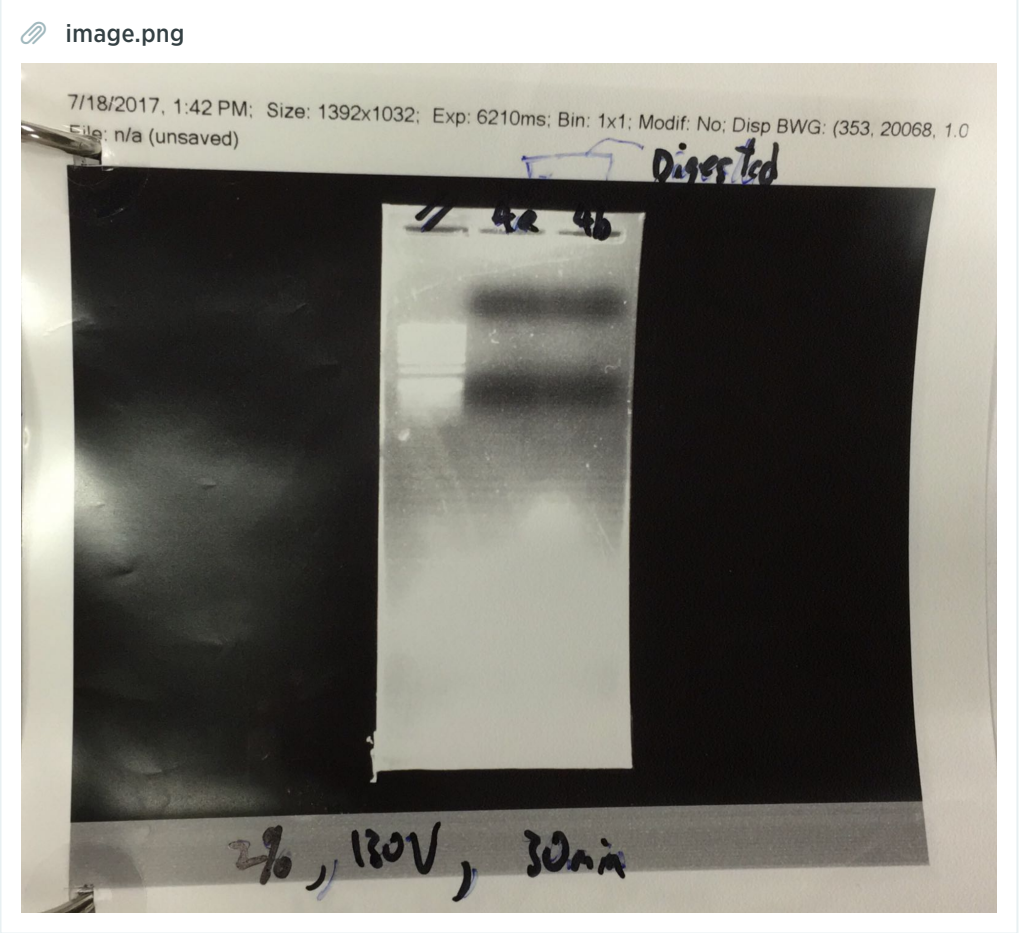
TUESDAY, 7/18/2017

(1) Gel electrophoresis

- a. Digested 4a and 4b (2% agarose, 130V, 30 mins) (112, 36, 21)
- b. Digested pSB1A2-BBa_R0063 (1% agarose, 130V, 30 mins) (113, 36, 21)
- c. PCR set 1 58°C and 59°C (1% agarose, 110V, 40 mins) (3131)
- d. Digested BBa_J23110-E0240 (Expected band size: 2038, 952), BBa_J23117-E0240 (Expected band size: 2038, 952), pSb1C3-J04450 (Expected band size: 2028, 1110)

Gel-photo:

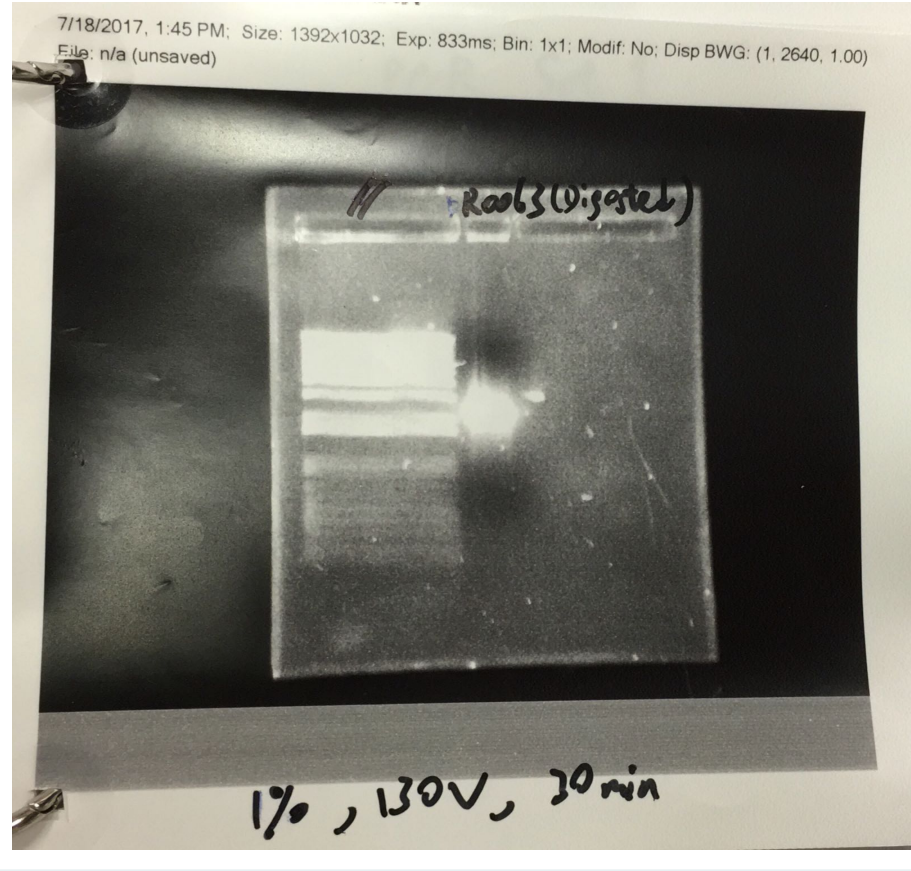
(a):



(from L-->R: //, 4, 4b)

(b):

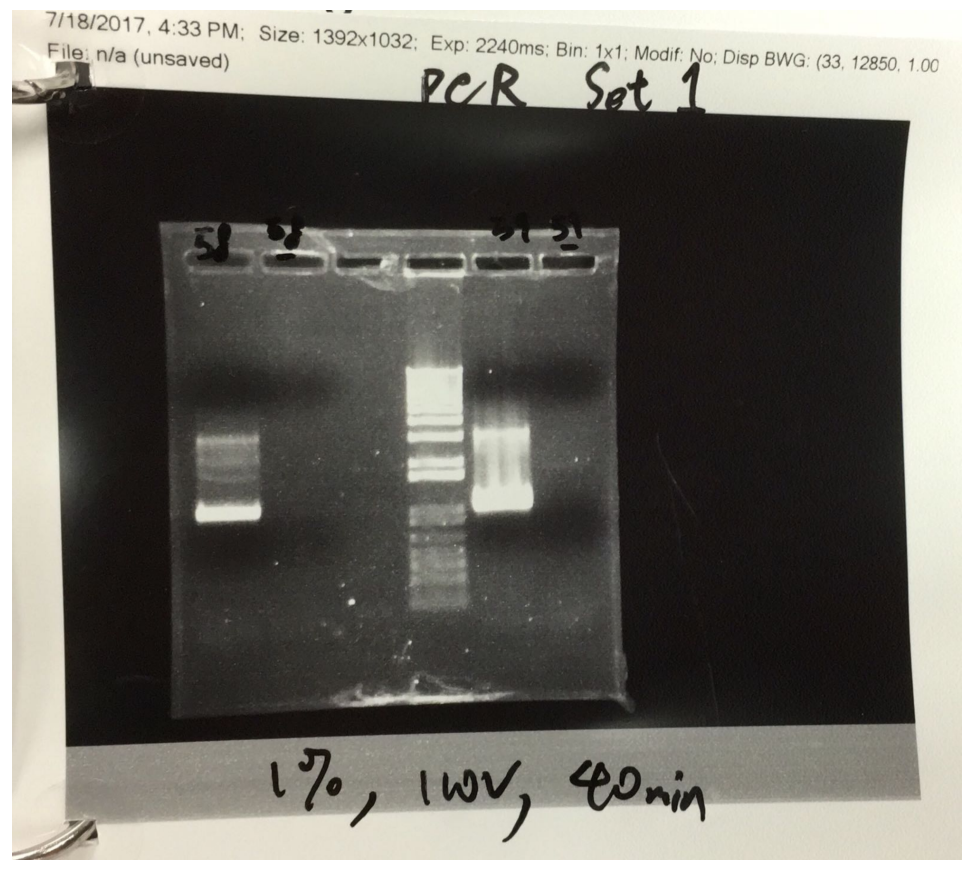
image.png



(from L--> R: //, R0063)

(c):

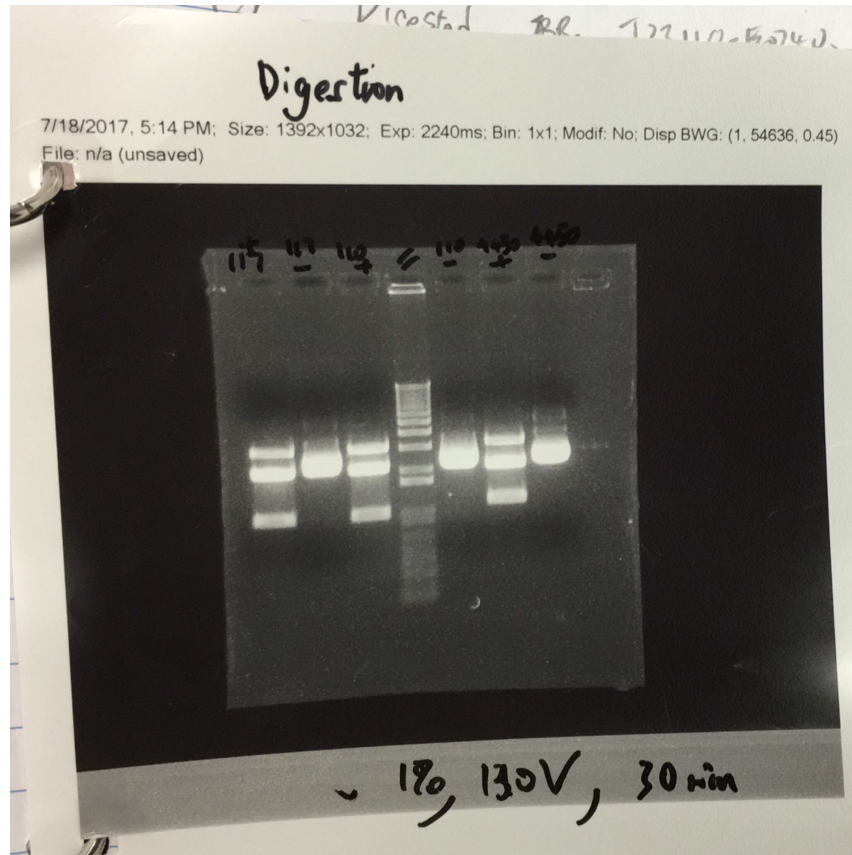
image.png



(from L-->R: 58+, 58-, //, 59+, 59-)

(d):

image.png



(from L-->R: 117+, 117-, 110+, //, 110-, 4450+, 4450-)

(2) Gel purification of set 2a ad 2b for Gibson assembly and digested pSB1A2-BBa_R0063 and digested RNA 1&2

Table4

	A	B	C	D
1		digested pSB1A2- BBa_R0063	2a	2b
2	DNA (ug/ml)	11.04	14.87	9.506
3	Protein contamination	1.828	1.676	2.110
4	Salt contamination	0.016	0.032	0.027

No result on gel for digested RNA 1&2

(3) PCR for set 1 (pSB1C3-BBa_F2620) with 58°C and 59°C annealing temperature

Table5

	A	B	C
1		Volume (ul)	MasterMix*4.5
2	MQ	12.4	55.8
3	Q5 buffer	4	1.8
4	dNTP	0.4	1.8
5	10uM Fwd	1	4.5
6	10uM Rev	1	4.5
7	Q5 polymerase	0.2	0.9
8	Template	1	
9	Total	20	

Table6

	A	B	C	D
1		Temperature (°C)	Time	Cycles
2	Initial denaturation	98	30s	
3	Denaturation	98	10s	30
4	Annealing	58/59	15s	
5	Extension	72	1min 21s	
6	Final extension	72	5mins	

1 58: set 1 with 58°C annealing

1 58 (-): negative control with MQ

1 59: set 2 with 59°C annealing

1 59 (-): negative control with MQ

(4) Digestion of pSB1A2-BBa_J23110-E0240 and pSB1A2-BBa_J23117-E0240 as inserts, pSB1C3-BBa_J04450 as backbone

(5) PCR of antisense RNA 1&2

WEDNESDAY, 7/19/2017

(1) PCR of antisense RNA 1&2

(2) Digest antisense RNA 1&2 (same as 7/17 protocol)

(3) Purify pSB1C3, BBa-J23110-E0240, BBa-J23117-E0240

Table7

	A	B	C	D
1		pSB1C3	BBa-J23110-E0240	BBa-J23117-E0240
2	DNA (ug/ml)	6.674	3.310	6.482
3	Protein contamination	2.496	2.770	4.373
4	Salt contamination	0.011	0.017	0.011

(4) PCR of set 1 Gibson assembly (gradient template)

A: 62 °C

B: 61.4 °C

C: 60.2 °C

D: 59.2 °C

(5) Gel-Electrophoresis:

- Antisense RNA 1/2 (2% gel, 130V, 45mins)

- Set 1 for Gibson Assembly (1% gel, 130V, 30mins)

Gel-photo:

THURSDAY, 7/20/2017

(1) Colony PCR of pSB1C3-BBa_J23110-E0240 and pSB1C3-BBa_J23117-E0240

Table8

	A	B	C
1	Reagents	Volume	MasterMix
2	MQ	14.35	129.15
3	dNTP	4	36
4	10mM dNTP	0.5	4.5
5	10mM VF2	0.5	4.5
6	10mM VR	0.5	4.5
7	Taq Polymerase	0.15	1.35
8	Template	0.5	/
9	Total	20	

Samples:

7.1-3: pSB1C3-BBa_J23117-E0240

0.1-3: pSB1C3-BBa_J23110-E0240

S7-: pSB1A2-BBa_J23117-E0240

S0-: pSB1A2-BBa_J23110-E0240

P-: MQ

(2) Touchdown PCR:

Table9

	A	B	C
1	Temperature	Time	Cycle
2	98	30s	
3	98	10s	x10
4	70 to 60 (-1 degree/cycle)	15s	
5	72	45s	
6	98	10s	x20
7	60	15s	
8	72	45s	
9	72	2mins	

MasterMix is the same as (18/7) Q5 protocol

(3) Digestion of Antisense RNA 1/2 from (17/7)

Table10

	A	B	C	D	E
1		Antisense RNA 1	Antisense RNA RNA 2	Negative Control 1	Negative Control 2
2	MQ (μl)	21.35	11.35	11.75	11.75
3	DNA (μl)	6.25	6.25	6.25	6.25
4	Xbal	0.2	0.2	0	0
5	PstI	0.2	0.2	0	0
6	Cutsmart	2	2	2	2
7	Total	20	20	20	20

Table10 Digestion of Antisense RNA

(4) Gel electrophoresis of

(a) colony PCR and touchdown PCR

(b) Digestion of antisense RNA 1 and 2

FRIDAY, 7/21/2017

(1) Touchdown PCR of pSB1C3-BBa_F2620 for Gibson Assembly

Table11			
	A	B	C
1		Volume (ul)	MasterMix*4.5
2	MQ	12.4	55.8
3	Q5 buffer	4	1.8
4	dNTP	0.4	1.8
5	10uM Fwd	1	4.5
6	10uM Rev	1	4.5
7	Q5 polymerase	0.2	0.9
8	Template	1	
9	Total	20	

Table11 Master

Table12				
	A	B	C	D
1	Temperture	Time	Cycle	
2	98	30s	1	Initial denaturation
3	98	10s	10	Denaturation
4	70 to 60 (-1 degree each cycle)	15s		Annealing
5	72	1.5 mins		Extensive
6	98	10s	20	Denaturation
7	60	15s		Annealing
8	72	1.5 mins		Extension
9	72	2 mins	1	Final extension

Table12 Touchdown PCR

(2) Colony PCR of pSB1C3-BBa_J23110 and pSB1C3-BBa_J23117-E0240

Table13

	A	B	C
1	Reagents	Volume	MasterMix
2	MQ	14.35	129.15
3	dNTP	4	36
4	10mM dNTP	0.5	4.5
5	10mM VF2	0.5	4.5
6	10mM VR	0.5	4.5
7	Taq Polymerase	0.15	1.35
8	Template	0.5	/
9	Total	20	

Table13 Colony PCR of pSB1C3-BBa_J23110 and pSB1C3-BBa_J23117-E0240

- (3) PCR of Antisense RNA 1 and 2
- (4) Diges Antisense 1 and 2

MONDAY, 7/24/2017

- (1) Gradient PCR pSB1C3-BBa_F2620
 - A+, A-: 61.4 °C
 - C+, C-: 61.1 °C
 - E+, E-: 60.4 °C
 - G+, G-: 39.9 °C

Table14

	A	B	C
1	Reagent	Volume (μl)	MasterMix (x9) (μl)
2	Water	12.4	111.6
3	5 X Q5 reaction buffer	4	36
4	dNTP	0.4	3.6
5	10μM Fwd	1	9
6	10μM Rev	1	9
7	Q5 polymerase	0.2	1.8
8	Template	1	
9	Total Volume	20	

Table14 MasterMix for PCR of pSB1C3-BBa_F2620

(2) Colony PCR pSB1C3-BBa_J23110-E0240 and pSB1C3-BBa_J23117-E0240

Table15

	A	B	C
1	Reagents	Volume	MasterMix
2	MQ	14.35	129.15
3	dNTP	4	36
4	10mM dNTP	0.5	4.5
5	10mM VF2	0.5	4.5
6	10mM VR	0.5	4.5
7	Taq Polymerase	0.15	1.35
8	Template	0.5 /	
9	Total	20	

Table15 MasterMix for Colony PCR pSB1C3-BBa_J23110-E0240
adn pSB1C3-BBa_J23117-E0240

(3) Digest Anti RNA 1 and 2

Table16			
	A	B	C
1		Antisense RNA 1	Antisense RNA 2
2	Concentration (ug/ml)	/	/
3	Mass (ng)	/	/
4	Water (ul)	32.1	32.1
5	DNA (ul)	12.5	12.5
6	Xbal (ul)	0.2	0.2
7	Spel (ul)	/	/
8	PstI (ul)	0.2	0.2
9	Cutsmart (ul)	5	5
10	Total (ul)	50	50

Table16 Digestion of Anti RNA 1 and 2

TUESDAY, 7/25/2017

1. Miniprep of pSB1C3-BBa_J23110-E0240 adn pSB1C3-BBa_J23117-E0240

Table17			
	A	B	C
1		pSB1C3-BBa_J23110-E0240	pSB1C3-BBa_J23117-E0240
2	DNA	98.43	54.92
3	Protein Contamination	1.808	1.776
4	Salt Contamination	1.877	1.967

Table17 Miniprep result of pSB1C3-BBa_J23110-E0240 adn pSB1C3-BBa_J23117-E0240

2. PCR of antisense RNA 1 and 2

3. Digestion of antisense RNA 1 and 2

Table18		A	B	C	D	E
1			Anti 1 (+)	Anti 2 (+)	Anti 1 (-)	Anti 2 (-)
2	DNA concentration ($\mu\text{g}/\mu\text{l}$)	/	/	/	/	/
3	DNA mass (ng)	/	/	/	/	/
4	Water (μl)		12.6	7.6	13	13
5	DNA (μl)		10	10	10	10
6	XbaI		0.2	0.2	0	0
7	PstI		0.2	0.2	0	0
8	Cutsmart (μl)		2	2	2	2
9	Total volume (μl)		25	25	25	25

Table18 Digestion of Antisense RNA 1 and 2

4. Gel purification of set 1

Table19		A	B	C
1			Column A (61.4 °C)	Column E (60.4 °C)
2	DNA		3.643	13.71
3	Protein Contamination		2.217	1.778
4	Salt Contamination		0.378	0.478

Table19 Result of Gel Purification of set 1

WEDNESDAY, 7/26/2017

1. Gel purification of digested Antisense RNA 1 and 2

Table20			
	A	B	C
1		Anti 1	Anti 2
2	DNA	5.588	6.649
3	Protein Contamination	1.557	1.822
4	Salt Contamination	0.029	0.049

Table20 Result of Gel Purification of Digested Anti 1 and 2

2. Gibson Assembly of pSB1C3-BBa_F2620, BBa_C0261, BBa_E0240
pSB1C3-BBa_F2620-C0261-E0240 (ABR 1) (+):

Table21					
	A	B	C	D	E
1		Conc. (ng/ μ l)	Length	Volume (μ l)	Mole Ratio
2	pSB1C3-BBa_F2620	13.71	3131	2.18	1
3	BBa_C0261	14.87	700	0.899	2
4	BBa_E0240	9.109	716	1.92	2
5	Total			5	

Table21 Gibson Assembly

Negative:

Table22		
	A	B
1		Volume (μ l)
2	Backbone	2.18
3	MQ	2.82

Table22

pSB1C3-BBa_F2620-C0261-E0240 (ABR 2) (+):

Table23					
	A	B	C	D	E
1		Conc. (ng/ μ l)	Length	Volume (μ l)	Mole Ratio
2	pSB1C3- BBa_F2620	13.71	3131	3.73	1
3	BBa_C0261	9.5	700	0.602	2
4	BBa_E0240	11.26	916	0.655	2
5	Total			5	

Table23

Negative:

Table24		
	A	B
1		Volume (μ l)
2	Backbone	3.73
3	MQ	1.27

3. Ligation of pSB1A2-BBa_R0063 to both of Antisense RNA 1 and 2

RNA 1:

Table25

	A	B	C	D
1		Positive	Negative	Mole Ratio
2	Ligase (μ l)	0.5	0	
3	10x Buffer (μ l)	1	1	
4	MQ (μ l)	0	0.5	
5	Backbone (μ l)	5.84	5.84	1
6	Insert (μ l)	2.66	2.66	3
7	Total	10	10	

RNA 2:

Table26

	A	B	C	D
1		Positive	Negative	Mole Ratio
2	Ligase (μ l)	0.5	0	
3	10x Buffer (μ l)	1	1	
4	MQ (μ l)	0	0.5	
5	Backbone (μ l)	6.14	6.14	1
6	Insert (μ l)	2.36	2.36	3
7	Total	10	10	

4. Transformation of pSB1A2-BBa_R0063-Antisense RNA 1 and 2 and pSB1C3-BBa_F2620_C0261_E0240 (ABR1) and pSB1C3-BBa_F2620_C0261_E0240 (ABR2)

THURSDAY, 7/27/2017

1. Gibson assembly of pSB1C3-BBa_F2620_C0261_E0240 (ABR1), psB1C3-BBa_F2620_C0261_E0240 (ABR2)

pSB1C3-BBa_F2620_C0261_E0240 (ABR1):

Table27

	A	B	C	D	E
1		Concentration (ng/ μ l)	Length	Volume (μ l)	Mole Ratio
2	pSB1C3-BBa_F2620	13.71	3131	1.184	1
3	BBa_C0261	14.87	700	1.218	5
4	BBa_E0240	9.109	916	2.601	5

pSB1C3-BBa_F2620_C0261_E0240 (ABR2):

Table28

	A	B	C	D	E
1		Concentration (ng/ μ l)	Length (bp)	Volume (μ l)	Mole Ratio
2	pSB1C3-BBa_F2620	13.71	3131	1.138	1
3	BBa_C0261	9.5	700	1.836	5
4	BBa_E0240	11.26	916	2.027	5

2. Ligation of pSB1A2-BBa_R0063-Anti1 and pSB1A2-BBa_R0063-Anti2

pSB1A2-BBa_R0063-Anti1:

Table29

	A	B	C	D	E
1		Concentration (ng/ μ l)	Length (bp)	Volume (μ l)	Mole Ratio
2	Antisense RNA 1	5.588	169	5.104	10
3	pSB1A2-BBa_R0063	11.04	2221	3.39	1

pSB1A2-BBa_R0063-Anti2:

Table30

	A	B	C	D	E
1		Concentration (ng/ μ l)	Length (bp)	Volume (μ l)	Mole Ratio
2	Antisense RNA 2	6.649	170	4.757	10
3	pSB1A2-BBa_R0063	11.04	2221	3.743	1

3. Transformation of pSB1C3-BBa_F2620_C0261_E0240 (ABR1) and (ABR2), pSB1A2-BBa_R0063-Anti1 and Anti2, pSB1C3-BBa_J23110_E0240 and pSB1C3-BBa_J23117_E0240

FRIDAY, 7/28/2017

1. PCR of set 1, 2a and 2b

Set 1:

Table33

	A	B	C
1	Reagent	Volume	MasterMix x2.5
2	Water	10.4	26
3	5x Q5 reaction buffer	4	10
4	dNTP	0.4	1
5	10 μ M Fwd	1	2.5
6	10 μ M Rev	1	2.5
7	Q5 polymerase	0.2	0.5
8	Template (μ l)	3	/
9	Total Volume	20	

*Use one more negative control with only 3 μ l of template to run gel.

Condition:

Table34

	A	B	C	D
1		Temperature (°C)	Time	Cycle
2	Initial denaturation	98	30s	1
3	Denaturation	98	10s	33
4	Annealing	60.3	15s	33
5	Extension	72	1 min 30s	33
6	Final Extension	72	2 mins	1

Set 2a & 2b:

Table35

	A	B	C
1	Reagent	Volume	MasterMix x3.5
2	Water	10.4	36.4
3	5x Q5 reaction buffer	4	14
4	dNTP	0.4	1.4
5	10µM Fwd	1	3.5
6	10µM Rev	1	3.5
7	Q5 polymerase	0.2	0.7
8	Template (µl)	3	/
9	Total Volume	20	

Use one more negative control with only 3µl of template to run gel.

Conditions:

Table36

	A	B	C	D
1		Temperature (°C)	Time	Cycle
2	Initial denaturation	98	30s	1
3	Denaturation	98	10s	33
4	Annealing	56.2	15s	33
5	Extension	72	30s	33
6	Final Extension	72	2 mins	1

2. Digestion of pSB1A2-BBa_R0063, antisense RNA 1 & 2

Table47

	A	B	C	D	E	F	G
1		pSB1A2-BBa_R0063		Antisense RNA 1		Antisense RNA 2	
2		+	(-)	+	(-)	+	(-)
3	DNA concentration (ng/μl)	67.97	67.97	/	/	/	/
4	DNA mass (ng)	800	800	/	/	/	/
5	Water (μl)	4.03	11.2	12.1	16.5	12.1	16.5
6	DNA (μl)	11.8	5	10	6	10	6
7	Xbal	/	/	0.2	/	0.2	/
8	SpeI	0.2	/	/	/	/	/
9	PstI	0.2	/	0.2	/	0.2	/
10	Cutsmart (μl)	1.8	1.8	2.5	2.5	2.5	2.5
11	Total Volume (μl)	18	18	25	25	25	25

MONDAY, 7/31/2017

1. Gel electrophoresis of set 2a, 2b and digested pSB1A2-BBa_R0063

2. Gel purification of set 2a, 2b and digested pSB1A2-BBa_R0063

Table48

	A	B	C	D
1		pSB1A2-BBa_R0063	2a	2b
2	DNA Concentration (µg/ml)	13.56	7.675	6.195
3	A260/A280	2.067	2.089	1.939
4	A260/A230	0.073	0.016	1.000