Time Contr

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TUESDAY, 6/6/2017

Set 1 Transformation

DNA taken from igem stock:

Table1

	A	B	C	D	Name of the parts
1		Box	Row	Column	
2	pSB1A2-BBa_R0051	2	7	D	pcI
3	pSB1C3-BBa_B0032	3	5	B	0.3 RBS
4	pSB1C3-BBa_B0015	1	1	H	TT
5	pSB1C3-BBa_I13500	3	1	C	1.0 RBS - GFP
6	pSB1C3-BBa_B0034	3	9	B	1.0 RBS

pSB1A2-BBa_R0051 (iGEM DNA Kit Box 2 7D)
pSB1C3-BBa_B0032 (iGEM DNA Kit Box 3 5B)
pSB1C3-BBa_B0015 (iGEM DNA Kit Box 1 1H)
pSB1C3-BBa_I13500 (iGEM DNA Kit Box 3 1C)
pSB1C3-BBa_B0034 (iGEM DNA Kit Box 3 9B)

DNA from Kit:

Table2

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	A	B	C	D	Name of the parts
1	pSB1A2-BBa_R0062	5P	2014 Kit plate 4	pSB1A2	pluxR
2	pSB1A2-BBa_C0051	3F	2014 Kit plate 4	pSB1A2	cI
3	pSB1C3-BBa_K081007	16G	2014 Kit plate 3	pSB1C3	0.6 RBS - cI
4	pSB1C3-BBa_S0109	8H	2014 Kit plate 2	pSB1C3	0.01 RBS - cI
5	pSB1C3-BBa_P0451	15N	2014 Kit plate 3	pSB1C3	1.0 RBS - cI - TT
6	pSB1A2-BBa_P0151	9D	2014 Kit plate 4	pSB1A2	0.07 RBS - cI - TT
7	pSB1C3-BBa_E0422	6P	2014 Kit plate 2	pSB1C3	1.0 RBS - CFP (+LVA) - TT
8	pSB1K3-BBa_Q04510	18B	2013 Kit plate 5	pSB2K3	1.0 RBS - cI - TT - pcI

1µl of DNA samples were placed into 50µL of competent cell (E.Coli)

* Note that there was only one Kanamycin agar plate left and the agar was relatively thin.

WEDNESDAY, 6/7/2017

1. **Set 1 Inoculation** 11:30 a.m.

Table3

	A	B	C
1	Show colonies	Show small colonies (put back into the incubator)	Do not shows colonies (re-transform)
2	pSB1A2-BBa_R0062	pSB1C3-BBa_I13500	pSB1C3-BBa_B0034
3	pSB1A2-BBa_C0051	pSB1C3-BBa_P0451	pSB1K3-BBa_Q04510
4	pSB1A2-BBa_P0151	pSB1C3-BBa_B0032	
5	pSB1A2-BBa_R0051	pSB1C3-BBa_E0422	
6	pSB1C3-BBa_K081007		
7	pSB1C3-BBa_S0109		
8	pSB1C3-BBa_B0015		

The small number of colonies is predicted to be due to its low concentration. However, the absence of bacterial colony, especially pSB1K3, is likely due to the thin layer of Kanamycin agar plate. Other reason is the gel were destroyed during plating.

Satellite colonies were found in each Ampicillin dish.

2. **Set 2 Transformation**

Table4

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	A	B	C
1	pSB1C3-BBa_B0034	Time delay module	Box 3 9B
2	pSB1K3-BBa_Q04510	Time delay module	Box 3 8D
3	pSB1C3-BBa_E0422	Time delay module	Box 3 7D
4	pSB1A2-BBa_C0261	Biosensor module	Well 13 D Kit Plate 4
5	pSB1C3-BBa_F2620	Biosensor module	Well 4O Kit Plate 3
6	pSB1A2-BBa_E0240 (stock)	Biosensor module	Box 1 2A

THURSDAY, 6/8/2017

1. **Set 3** transformation (Our today's failure ;-( )

- pSB1C3-BBa_B0034 (Box 2 5F)
- pSB1K3-BBa_Q04510: Probably the quantity of the plasmid is low
- pSB1C3 - BBa_F2620

2. Miniprep

- [x] pSB1A2-BBa_R0062
- [ ] pSB1A2-BBa_C0051 (Redo!)
- [x] pSB1A2-BBa_P0151
- [x] pSB1A2-BBa_R0051
- [x] pSB1C3-BBa_K081007
- [x] pSB1C3-BBa_S0109
- [x] pSB1C3-BBa_B0015
- [x] pSB1C3-BBa_I13500

- [x] pSB1C3-BBa_P0451
- [x] pSB1C3-BBa_B0032
- [x] pSB1C3-BBa_E0422

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3. Check DNA Concentration (Biodrop)

Table5

	A	Concentration (ng/uL)	Salt contamination	Protein contamination
1	pSB1A2-BBa_R0062	81.49	2.508	1.874
2	pSB1A2-BBa_P0151	98.67	1.626	1.805
3	pSB1A2-BBa_R0051	168.7	0.977	1.762
4	pSB1C3-BBa_K081007	160.6	2.411	1.854
5	pSB1C3-BBa_S0109	129.5	2.787	1.891
6	pSB1C3-BBa_B0015	64.78	2.975	1.918
7	pSB1C3-BBa_I13500 (set 1)	53.74	6.146	1.937
8	pSB1C3-BBa_P0451	156.4	2.507	1.853
9	pSB1C3-BBa_B0032	79.89	1.863	1.780
10	pSB1C3-BBa_E0422 (set 1)	193.7	2.26	1.832

* pSB1C3- BBa_I13500 resul

4. **Set 2 Inoculation**
   - [x] pSB1C3-BBa_I13500
   - [x] pSB1C3-BBa_E0422
   - [x] pSB1A2-BBa_C0261
   - [x] pSB1A2-BBa_E0240 (stock)

FRIDAY, 6/9/2017

1. **Set 3** Inoculation
   - [x] pSB1C3- BBa_B0034
   - [ ] pSB1K3- BBa_Q04510 --> Continue to inoculate. The plate shows several small col
   - [x] pSB1A2- BBa_C0051 (**Set 3**)
   - [x] pSB1C3 - BBa_F2620 (Biosensor)
   - [x] pSB1A2-BBa_E0240 (Biosensor)

2. **Set 2** Miniprep
   - [x] pSB1C3-BBa_I13500
   - [x] pSB1C3-BBa_E0422
   - [x] pSB1A2-BBa_C0261 (Biosensor)

Table9

	A	Concentration	Salt contamination	Protein contamination
1	pSB1C3-BBa_I13500	271.9	2.177	1.838
2	pSB1C3-BBa_E0422 (set 2)	254.6	2.202	1.850
3	pSB1A2-BBa_C0261	144.9	2.166	1.836
4	pSB1A2-BBa_E0240	111.7	1.903	1.840
5	pSB1C3-BBa_B0034	93.10	1.787	1.822
6	pSB1A2-BBa_C0051 (set 3)	66.68	1.980	1.869
7	pSB1C3 - BBa_F2620	12.66	1.086	1.653

* F2620 concentration is very low

3. Transformation of pSB1A2-BBa_C0051 **(set 4)**

MONDAY, 6/12/2017

1. Inoculation of pSB2K3-BBa_Q04510 & pSB1A2-BBa_C0051 **(set 4)**
2. Digestion

Control:
Total volume = 20 uL
DNA amount = 1000 ng
Enzyme 1 & 2 = 0.3 uL

1X CutSmart buffer = 2 uL

DNA that will be used for the **First Ligation**

- [ ] pSB1A2-BBa_R0062 (x5)
- [ ] pSB1A2-BBa_P0151
- [ ] pSB1C3-BBa_K081007
- [ ] pSB1C3-BBa_S0109
- [ ] pSB1C3-BBa_E0422
- [ ] pSB2K3- BBa_Q04510
- [ ] pSB1A2- BBa_C0051
- [ ] pSB1C3-BBa_P0451

3. Positive Sample: Calculate the volume of DNA sample (V) and ddH2O used based on each plasmid concentration

Table6

	A	Volume of DNA sample (uL)	ddH2O (uL)	Backbone or insert?	Enzyme cutting sites
1	pSB1A2-BBa_R0062 (S&P)	12.27	5.13	Backbone	S & P
2	pSB1A2-BBa_P0151 (X&P)	10.13	7.27	Insert	X & P
3	pSB1A2-BBa_R0051 (S&P)	5.93	11.47	Backbone	S & P
4	pSB1C3-BBa_K081007 (X&P)	6.23	11.17	Insert	X & P
5	pSB1C3-BBa_S0109 (X&P)	7.72	9.68	Insert	X & P
6	pSB1C3-BBa_E0422 (X&P)	5.16	12.24	Insert	X & P
7	pSB1C3-BBa_P0451 (X&P)	6.39	11.01	Insert	X & P

4. Negative Sample (No enzymes)

Table7

	A	DNA sample volume (uL)	ddH2O (uL)
1	pSB1A2-BBa_R0062	12.27	5.73
2	pSB1A2-BBa_P0151	10.13	7.87
3	pSB1A2-BBa_R0051	5.93	12.07
4	pSB1C3-BBa_K081007	6.23	11.77
5	pSB1C3-BBa_S0109	7.72	10.28
6	pSB1C3-BBa_E0422	5.16	12.84
7	pSB1C3-BBa_P0451	6.39	11.61

5. Gel electrophoresis
- Prepare gel 30 minutes before
- Use 1% gel
1 ladder + 14 samples : 7 for positive 7 for negative = 15-16 total
For negative sample: Only show one band
- Use 18 teeths. 40 ml gel.

Result:
Successful: BBa_K081007 (X&P), BBa_S0109 (X&P), BBa_P0451(X&P), BBa_E0422 (X&P), pSB1A2-BBa_R0062 (S&P), BBa_P0151 (X&P)
Failure: E0422, R0051

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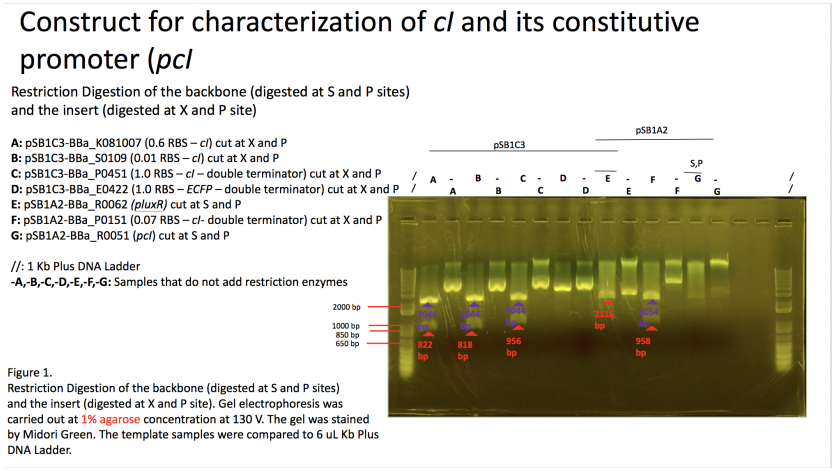

Construct for characterization of *cI* and its constitutive promoter (*pcI*

Restriction Digestion of the backbone (digested at S and P sites) and the insert (digested at X and P site)

A: pSB1C3-BBa_K081007 (0.6 RBS – *cI*) cut at X and P
B: pSB1C3-BBa_S0109 (0.01 RBS – *cI*) cut at X and P
C: pSB1C3-BBa_P0451 (1.0 RBS – *cI* – double terminator) cut at X and P
D: pSB1C3-BBa_E0422 (1.0 RBS – *ECFP* – double terminator) cut at X and P
E: pSB1A2-BBa_R0062 *(pluxR)* cut at S and P
F: pSB1A2-BBa_P0151 (0.07 RBS – *cI*- double terminator) cut at X and P
G: pSB1A2-BBa_R0051 (*pcI*) cut at S and P

//: 1 Kb Plus DNA Ladder
-A,-B,-C,-D,-E,-F,-G: Samples that do not add restriction enzymes

Figure 1.
Restriction Digestion of the backbone (digested at S and P sites) and the insert (digested at X and P site). Gel electrophoresis was carried out at 1% agarose concentration at 130 V. The gel was stained by Midori Green. The template samples were compared to 6 uL Kb Plus DNA Ladder.

*Note the 'w' shaped band might be due to non-uniform solidification of agarose gel or the old TAE buffer or unequal temperature across the gel
*Smeared DNA bands: Too much dna/ protein contamination is too high? Trobleshooting: http://bio.classes.ucsc.edu/bio20L/info/content/molbio2/molbio1/troub.htm

6. Restriction test of the two suspected pSB1A2-BBa_C0051 with concentration of 66.68 ng/uL and 146.... ng/uL respectively

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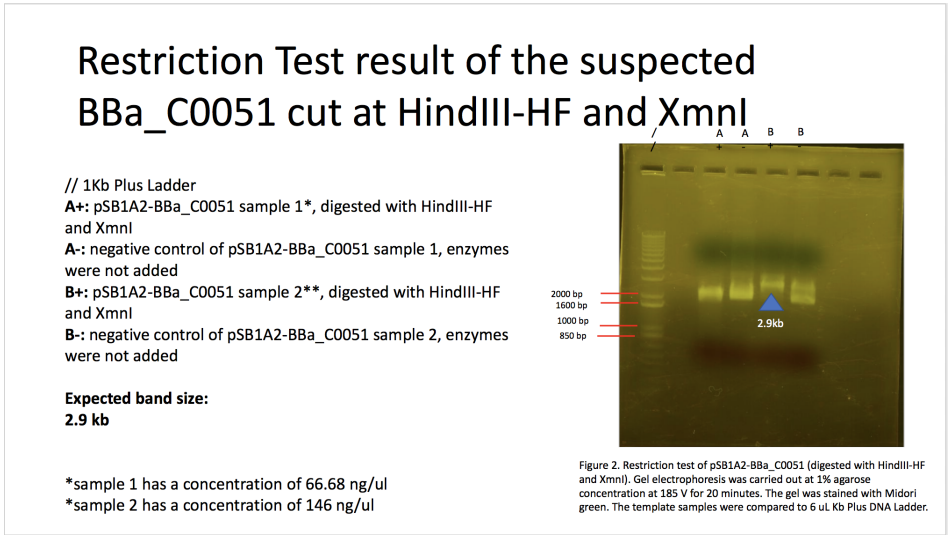

TUESDAY, 6/13/2017

1. Digestion

- Total volume = 18 uL
- Enzyme 1 & 2 = 0.2 uL each (500 ng DNA)
- CutSmart buffer = 1.8 uL (The CutSmart is expired)
- 2 samples (tubes) each

Table13

	Positive	Volume of DNA	ddH20
1	pSB1C3-BBa_E0422 (X&P)	1.96	13.84
2	pSB1A2-BBa_R0062 (S&P)	6.14	9.66
3	pSB1A2-BBa_R0051 (S&P)	2.96	12.84
4	pSB1A2-BBa_C0051 (X&P)		

Table14

	Negative	Volume of DNA	ddH2O
1	pSB1C3-BBa_E0422 (X&P)	1.96	14.24
2	pSB1A2-BBa_R0062 (S&P)	6.14	10.06
3	pSB1A2-BBa_R0051 (S&P)	2.96	13.24
4	pSB1A2-BBa_C0051		

2. Gel electrophoresis

- 1% gel
- Successful: R0062, R0051. E0422 fails due to the same reason (Undigested), so we decide to miniprep again from our new innoculated batch

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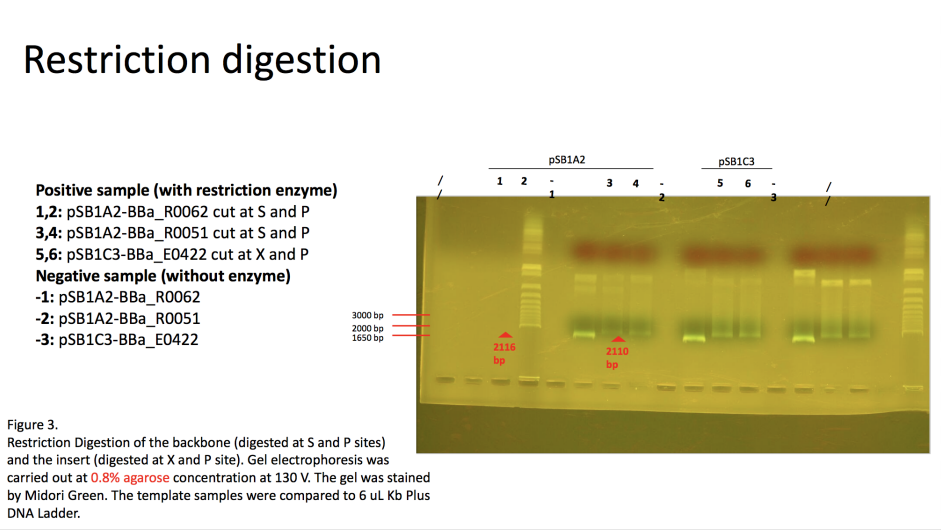

3. Miniprep pSB1K3-BBa_Q04510

WEDNESDAY, 6/14/2017

1. E0422 Miniprep (set 3)

Table17

	A	Plasmid concentration	Salt contamination	Protein contamination
1	pSB1C3-BBa_E0422 (set 3)	197.1	2.238	1.841

THURSDAY, 6/15/2017

1. Digestion - two replicons

- [ ] Total volume = 18 uL
- [ ] 500 ng DNA
- [ ] Enzyme 0.2 uL
- [ ] 10X CutSmart 1.8 uL

Table15

	Positive	Volume DNA sample	ddH2O
1	pSB1C3-BBa_B0015 (E&X)	7.72	8.08
2	pSB1C3-BBa_B0015 (S&P)	7.72	8.08
3	pSB1C3-BBa_B0032 (S&P)	6.26	9.54
4	pSB1A2-BBa_C0051 (X&P)	7.50	8.30

Table16

	Negative	Volume of DNA sample	ddH2O
1	pSB1C3-BBa_B0015 (E&X)	7.72	8.48
2	pSB1C3-BBa_B0015 (S&P)	7.72	8.48
3	pSB1C3-BBa_B0032 (S&P)	6.26	9.94
4	pSB1A2-BBa_C0051 (X&P)	7.50	8.70

3.Gel electrophoresis

- [ ] 0.8% agarose gel
- [ ] 40 ml TAE buffer
- [ ] 0.4 uL midori green
- [ ] 40mA, 130V, mins

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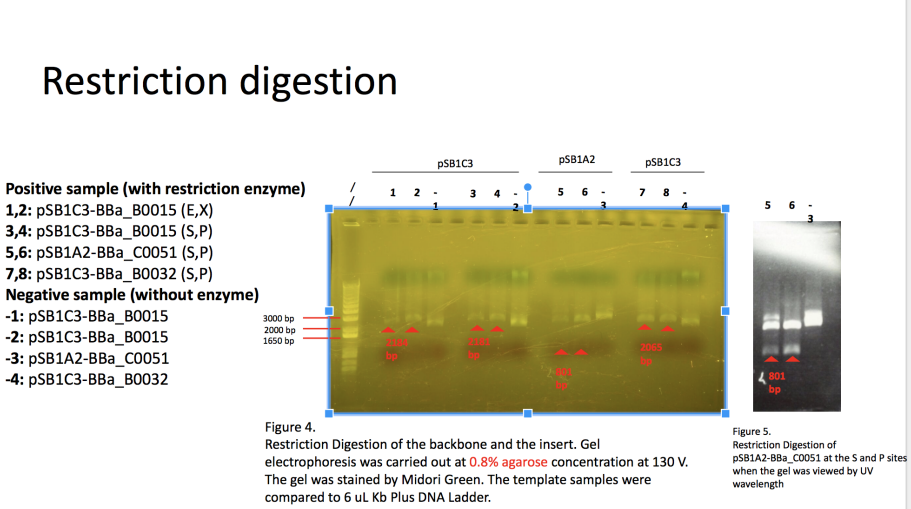

Expected results:

pSB1C3-BBa_B0015 (E&X): 15bp, 2184bp

pSB1C3-BBa_B0015 (S&P): [illegible]
pSB1C3-BBa_B0032 (S&P): [illegible]
pSB1A2-BBa_C0051 (X&P): 801bp, 2053bp

2. Digestion (Restriction Test) of E0422 (set 1 & set 2) - 2 replicons
** Note: no restriction enzyme specific for cutting in the middle of E0422 insert

Table18

	Positive control (2 Enzymes)	Volume of DNA sample	ddH2O
1	pSB1C3-BBa_E0422 (X&P) (set 1)	2.58	13.22
2	pSB1C3-BBa_E0422 (X&P) (set 2)	1.96	13.84
3	pSB1C3-BBa_E0422 (X&P) (set 3)	2.54	13.26

Table19

	Negative control	Volume of DNA sample	ddH2O
1	pSB1C3-BBa_E0422 (set 1)	2.58	13.62
2	pSB1C3-BBa_E0422 (set 2)	1.96	14.24
3	pSB1C3-BBa_E0422 (set 3)	2.54	13.66

* The DNA bands in BBa_E0422 is faded, which cannot be eluted during digestion

FRIDAY, 6/16/2017

1. Miniprep pSB1C3-BBa_E0422

Table23

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	A	Plasmid Concentration	Salt Contamination	Protein Contamination
1	pSB1C3-BBa_E0422	210.0	2.188	1.842
2	pSB1C3-BBa_E0422	169.1	2.282	1.836

1. Gel purification

Table22

	A	DNA concentration	Salt contamination	Protein contamination
1	pSB1A2-BBa_R0062 (S,P) (set 1)	5.581	0.059	1.218
2	pSB1A2-BBa_R0062 (S,P) (set 2)	10.85	0.247	1.854
3	pSB1C3-BBa_B0015 (E,X) (set 1)	4.155	0.242	1.928
4	pSB1C3-BBa_K081007 (X,P)	6.844	0.112	1.780
5	pSB1C3-BBa_B0015 (E,X) (set 2)	4.467	0.081	1.811
6	pSB1A2-BBa_P0151 (X,P)	6.098	0.289	1.488
7	pSB1A2-BBa_C0051 (X,P)	2.077	0.034	1.928
8	pSB1C3-BBa_B0015 (S,P)	6.454	0.029	1.869
9	pSB1A2-BBa_R0051 (S,P)	2.373	0.012	6.358
10	pSB1C3-BBa_P0451 (X,P)	7.523	0.055	2.135
11	pSB1C3-BBa_B0032 (S,P)	6.708	1.425	0.025

2. Digestion

Table24

	Positive Samples (2 Enzymes)	Volume of DNA sample	ddH2O
1	pSB1C3-BBa_E0422 (X&P) (set 1)	5.91	9.68
2	pSB1C3-BBa_E0422 (X&P) (set 2)	4.76	10.84
3	pSB1C3-BBa_S0109 (X&P)	7.72	7.88

* 0.3 ul of each enzyme was used in every samples

Table25

	Negative Controls	Volume of DNA sample	ddH2O
1	pSB1C3-BBa_E0422 (X&P) (set 1)	5.91	10.28
2	pSB1C3-BBa_E0422 (X&P) (set 2)	4.96	11.44
3	pSB1C3-BBa_S0109 (X&P)	7.72	8.48

3. Gel Electrophoresis

- [ ] 1.2 % agarose gel
- [ ] 20 ml TAE buffer
- [ ] 0.2 uL midori green
- [ ] 400mA, 110V, 45 mins

expected band sizes
- pSB1C3-BBa_E0422 (X&P) - 943 bp
- pSB1C3-BBa_S0109 (X&P) - 818 bp

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Digestion of pSB1C3-Bba_E0422 and pSB1C3-BBa_S0109

// : 1 Kb plus ladder
Positive Sample (With enzymes)
S+ :pSB1C3-S0109 digested at x,p
E1+ :pSB1C3-E0422 set 1, digested at x,p
E2+ :pSB1C3-E0422 set 2, digested at x,p
Negative Controls (without enzymes)
S- :pSB1C3-S0109 digested at x,p
E1- :pSB1C3-E0422 set 1
E2- :pSB1C3-E0422 set 2

C : pSB1C3-C0051 → to check the content of the DNA mixture

S concentration: 129.5 ng/ul
E2 concentration: 210 ng/ul
E1 concentration: 169 ng/ul

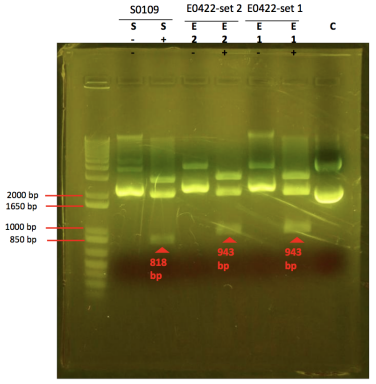


Figure 7. Digestion of pSB1C3-BBa_E0422 and pSB1C3-BBa_S0109 (digested at X and P). Gel electrophoresis was carried in a 1.2% agarose gel at 110 V for 45 minutes. The gel was stained with Midori green. The template samples were compared to 6 uL Kb Plus DNA Ladder.

*the gel electrophoresis results show 3 bands. The digestion might be incomplete because of the presence of unevaporated ethanol from miniprep process.

MONDAY, 6/19/2017

1. Gel purification of E0422 and S0109

2. Ligation
   - [ ] 1 uL 10X buffer
   - [ ] 0.5 uL Ligase (positive) 0 uL Ligase for negative
   - [ ] 0.5 uL MQ (for negative) 0 uL MQ for positive
   - [ ] Total volume = 10 uL

Table8

	(+)	backbone volume	insert volume
1	pSB1A2-BBa_R0062 (set2)-K081007	2.99	5.515
2	pSB1A2-BBa_R0062(set2)-P0151	2.49	6.012
3	pSB1A2-BBa_R0062(set2)-S0109	2.26	6.236
4	pSB1A2-BBa_R0051-E0422	4.79	3.71
5	pSB1A2-BBa_R0062(set2)-P0451	2.88	5.62
6	pSB1C3-BBa_B0032-C0051	1.35	7.15

3. Transformation
   - Remember to check the expression of CFP of pSB1A2-BBa_R0051-E0422 before transformation
   - Bacterial lawn on P0151 plate: improper spreading?

TUESDAY, 6/20/2017

4. Colony PCR
   - [ ] 5 replicons for each PCR (testing one ligated product)
   - [ ] PCR+: Use Q04510 with DNA length of 1281 bp --> Extension time = 1.281 min Annealing temperature
   - [ ] PCR-: Without DNA template
   - [ ] Sample negative:
     - [ ] From the sample negative plate (unligated)
     - [ ] Use plasmid

Table28

	A	Description	Remarks
1	pSB1A2-BBa_R0062 (set2)-K081007	1-5	Only sample 1 shows a clear band but the band size is very small (around 200-300 bp). Sample 3,4 showsimilar result but with lower intensity.
2	pSB1A2-BBa_R0062(set2)-P0151	6-10	Failed - bacterial lawn on the ligated plate --
3	pSB1A2-BBa_R0062(set2)-S0109	11-15	Failed
4	pSB1A2-BBa_R0051-E0422	16-20	Failed
5	pSB1A2-BBa_R0062(set2)-P0451	21-25	Failed
6	pSB1C3-BBa_B0032-C0051	26-30	Failed
7	pSB2K3-BBa_Q04510	PCR positive	Failed
8	pSB1A2-BBa_R0062	Sample negative (-1) from negative sample plate (without ligase)	Failed
9	pSB1A2-BBa_R0051	Sample negative (-2) from negative sample plate (without ligase)	Failed
10	pSB1C3-BBa_B0032	Sample negative (-3) from negative sample plate (without ligase)	Failed
11	MQ+Master Mix	PCR negative	

Possible problem

- The ligated products inside the colonies that survive in antibiotic agar plates may not contain inserts. It may only contain self-ligated plasmid or two backbone plasmids ligated with each other. Band should show no DNA if it's self-ligated because VR and VF2 distance is small. But -1, -2, -3 (obtained from sample negative plate; backbone that doesn't have insert) do not show any either. Cannot prove that it's the spread plates' problem.
- Cells may not be picked successfully: Too small colonies, hand shaking, too much agar picked, too much DNA stuck in the wells
- Number of cycle is 24 which should be enough
- Problems with previous ligation protocol?
- PCR does not work properly because the negative control also does not show anything

5. Inoculation (2 sets)

*another set of colonies picked (not the same as colony PCR)

Table29

	Sample	B
1	pSB1A2-BBa_R0062 (set2)-K081007	
2	pSB1A2-BBa_R0062(set2)-P0151	Failed
3	pSB1A2-BBa_R0062(set2)-S0109	
4	pSB1A2-BBa_R0051-E0422	
5	pSB1A2-BBa_R0062(set2)-P0451	
6	pSB1C3-BBa_B0032-C0051	

WEDNESDAY, 6/21/2017

6. Miniprep

Sample 1

	Sample	Concentration (ng/ul)	Protein Contamination	Salt Contamination
1	pSB1A2-BBa_R0062 (set2)-K081007	114.1	1.808	1.837
2	pSB1A2-BBa_R0062(set2)-P0151	Not done		
3	pSB1A2-BBa_R0062(set2)-S0109	98.54	1.841	1.876
4	pSB1A2-BBa_R0051-E0422	62.78	1.805	1.707
5	pSB1A2-BBa_R0062(set2)-P0451	112.3	1.831	1.802
6	pSB1C3-BBa_B0032-C0051	102.4	1.815	1.917

Sample 2

	Sample	Concentration (ng/ul)	Protein Contamination	Salt Contamination
1	pSB1A2-BBa_R0062 (set2)-K081007	89.97	1.8	1.731
2	pSB1A2-BBa_R0062(set2)-P0151	Not done		
3	pSB1A2-BBa_R0062(set2)-S0109	121.5	1.826	1.748
4	pSB1A2-BBa_R0051-E0422	16.46	1.74	1.574
5	pSB1A2-BBa_R0062(set2)-P0451	81.68	1.831	1.802
6	pSB1C3-BBa_B0032-C0051	83.18	1.801	1.801

7. Colony PCR of all constructs in the time module boxes (30 tubes in total)
- Extension: 68 Celsius, 1.45 mins. Nucleotide number = x29
- Master Mix use 5x My Taq

THURSDAY, 6/22/2017

1. PCR test on kit plate and the ligated product (with three new colonies)

image.png

We recovered unsaved changes to your entry. Click here to recover this data.

1	pSB1C3-BBa_P0451
2	pSB1C3-BBa_I13500 (1)
3	pSB1C3-BBa_E0422
4	pSB1A2-BBa_P0151
5	pSB1C3-BBa_S0109
6	pSB1A2-BBa_R0051
7	pSB1C3-BBa_B0015
8	pSB1A2-BBa_R0062
9	pSB1C3-BBa_K081007
10	pSB1C3-BBa_B0032
11	pSB1C3-BBa_E0422 (3)
12	pSB1C3-BBa_E0422 (2)
13	pSB1C3-BBa_I13500 (2)
14	pSB1C3-BBa_B0034
15	pSB1A2-BBa_E0240
16	pSB1A2-BBa_C0051 (1)
17	pSB1C3-BBa_E0422 (4)
18	pSB2K3-BBa_Q04510
19	pSB1C3-BBa_E0422 (5)
20	pSB1A2-BBa_C0051 (2)
64	pSB1A2-BBa_R0062-S0109 (i)
65	pSB1A2-BBa_R0051-E0422 (I)
66	pSB1C3-BBa_B0032-C0051 (I)
67	pSB1A2-BBa_R0051-E0422 (i)
68	pSB1C3-BBa_B0032-C0051 (i)
73	pSB1A2-BBa_R0062-P0451 (I)
74	pSB1A2-BBa_R0062-P0451 (i)
75	pSB1A2-BBa_R0062-K081007 (I)
76	pSB1A2-BBa_R0062-K081007 (i)
77	pSB1A2-BBa_R0062-S0109 (I)

2. Gel electrophoresis

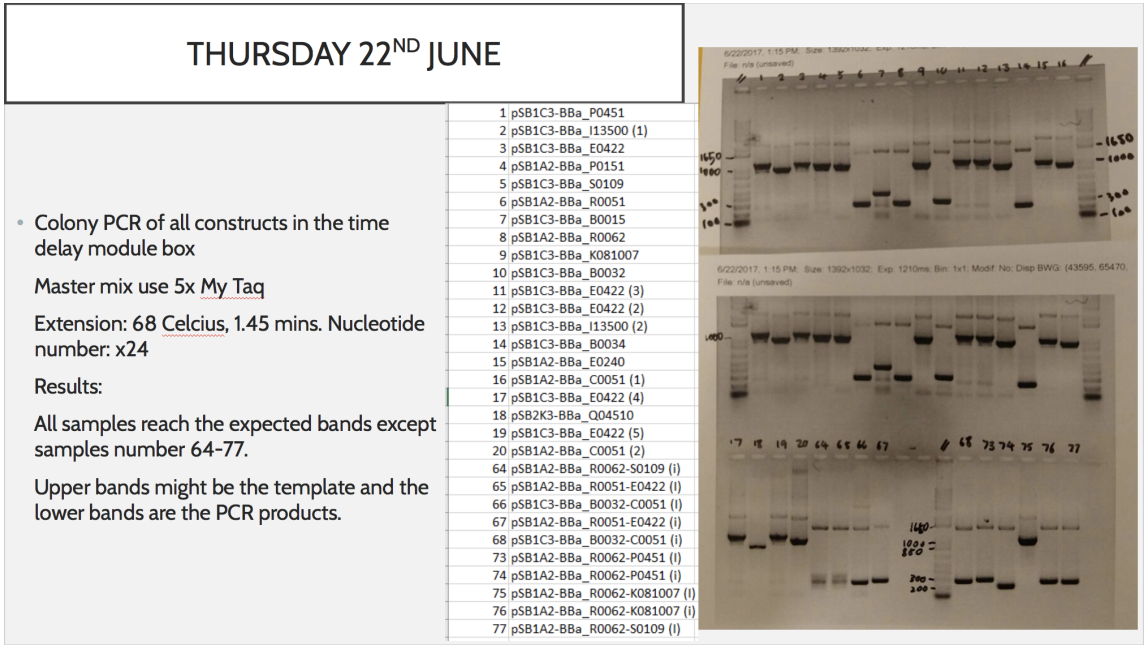
Arrangements:

Table30

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	//	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	//
2	17	18	19	20	64	65	66	67			//	68	73	74	75	76	77	

Gel photo:

Screen Shot 2017-10-31 at 2.48.52 PM.png



*result of the ligated products is not at the desired band size. Need to redo the digestion and ligation.

FRIDAY, 6/23/2017

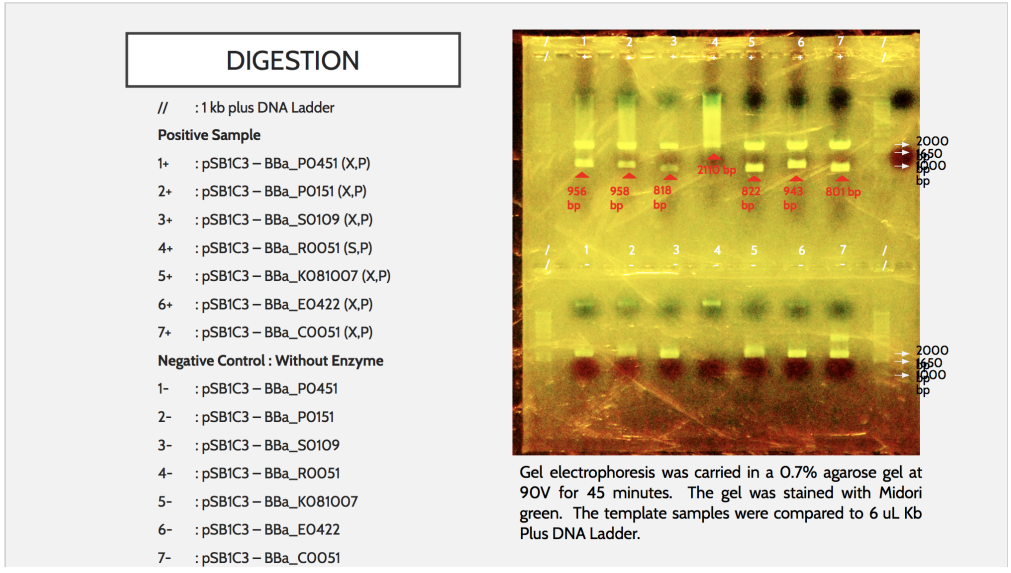
1. Digestion

Table31

We recovered unsaved changes to your entry. Click here to recover this data.

	A	Volume	ddH20	Mass (ng)
1	pSB1C3-BBa_P0451 (X,P)	12.79	2.41	2000
2	pSB1A2-BBa_P0151 (X,P)	15.2	0.2	1500
3	pSB1C3-BBa_S0109 (X,P)	3.86	11.98	500
4	pSB1A2-BBa_R0051 (S,P)	11.86	3.34	1500
5	pSB1C3-BBa_K081007 (X,P)	12.45	2.75	2000
6	pSB1C3-BBa_E0422 (X,P) Set 4	9.52	5.68	2000
7	pSB1A2-BBa_C0051 (X,P) Ken	4.86	10.34	2000

Screen Shot 2017-10-31 at 3.01.11 PM.png



* pSB1A2-BBa_R0062 (S,P) not enough for digestion
* R0051 has smeared band

2. Transformation
- pSB1C3-BBa_R0062

3. Streak plate

Table10

	A
1	pSB1C3-BBa_B0032
2	pSB1C3-BBa_F2620
3	pSB1C3-BBa_E0422 (14/6)
4	pSB1C3-BBa_E0422 (13/6)
5	pSB1C3-BBa_B0015
6	pSB1C3-BBa_S0109
7	pSB1C3-BBa_C0051
8	pSB1C3-BBa_K081007
9	pSB1C3-BBa_I13500
10	pSB1C3-BBa_P0451

MONDAY, 6/26/2017

Innoculation (2 batches)
- pSB1C3-BBa_R0062
- pSB1C3-BBa_S0109
- pSB1A2-BBa_P0151
- pSB1C3-BBa_C0051
- pSB1C3-BBa_B0015
- pSB1C3-BBa_P0451
- pSB1A2-BBa_R0051
- pSB1C3-BBa_K081007
- pSB1C3-BBa_B0032

*Check gel photo!
gel purification

Table11

	A	Concentration	Salt Contamination	Protein Contamination
1	pSB1C3-BBa_P0451 (X,P)	7.415	0.012	2.171
2	pSB1A2-BBa_P0151 (X,P)	6.946	0.082	1.760
3	pSB1C3-BBa_S0109 (X,P)	7.160	0.034	1.721
4	pSB1A2-BBa_R0051 (S,P)	8.908	0.021	1.503
5	pSB1C3-BBa_K081007 (X,P)	12.76	0.069	1.888
6	pSB1C3-BBa_E0422 (X,P) Set 4	10.55	0.113	1.901
7	pSB1A2-BBa_C0051 (X,P) Ken	14.13	0.015	2.305

Ligation

Table12

	(+)	backbone volume	insert volume
1	pSB1A2-BBa_R0051-E0422	3.99	4.51

Table20

	(-)	B	C
1	pSB1A2-BBa_R0051-E0422	3.99	4.51
2			

TUESDAY, 6/27/2017

1. Miniprep (1st batch)

Table36

	A	Concentration	Salt contamination	Protein contamination
1	pSB1C3-BBa_R0062	113.1	2.016	1.851
2	pSB1C3-BBa_S0109	79.44	2.180	1.829
3	pSB1A2-BBa_P0151	115.1	2.253	1.824
4	pSB1C3-BBa_C0051	519.0	2.296	1.840
5	pSB1C3-BBa_B0015	133.4	2.040	1.817
6	pSB1C3-BBa_P0451	116.3	2.312	1.837
7	pSB1A2-BBa_R0051	19.37	1.054	1.704
8	pSB1C3-BBa_K081007	130.7	2.226	1.848
9	pSB1C3-BBa_B0032	103.1	2.188	1.837

2. Miniprep (2nd batch)

Table38

	A	Concentration	Salt contamination	Protein contamination
1	pSB1C3-BBa_R0062	55.33	1.824	1.824
2	pSB1C3-BBa_S0109	64.86	1.861	1.861
3	pSB1A2-BBa_P0151	83.34	1.968	1.838
4	pSB1C3-BBa_C0051	362.7	2.33	1.826
5	pSB1C3-BBa_B0015	47.53	1.938	1.792
6	pSB1C3-BBa_P0451	154.2	2.261	1.831
7	pSB1A2-BBa_R0051	17.53	0.898	1.84
8	pSB1C3-BBa_K081007	125.6	2.22	1.805
9	pSB1C3-BBa_B0032	62.15	2.208	1.82

3. PCR for ligated product of pSB1A2-BBa_R0051-E0422 Expected band: 1212 bp

Table37

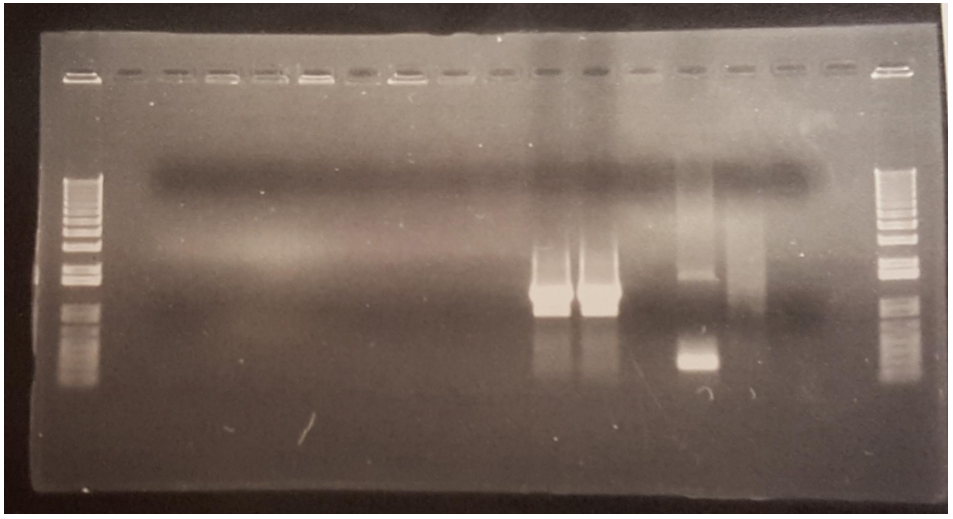
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	//	1	2	3	4	5	6	7	8	9	10	-1	-2	+	-	//		

-1 = pSB1A2-BBa_R0051 ligation negative plate
-2 = pSB1A2-BBa_R0051 from the miniprep
+ = PCR positive Q04510 (1303 bp)
- = PCR negative (MQ)

Initial denaturation should be 3 minutes!!!
Extension: 1.45 min. x29 nucleotides

1st Gel photo:

20170627_163603.jpg



Results: Only sample 9 and [illegible] remaining PCR sample left (Dilute them to 5 uL) Us

2nd Gel photo: no band is shown under blue light, so we post-stain the gel with 10X midori green. However, no band is shown after post-stain. We may switch our stain to Sybr safe in the future.

Streak plates
sample 9, 10 of colony PCR

WEDNESDAY, 6/28/2017

1. PCR check for sample 9 and 10 of pSB1A2-BBa_R0051-E0422 --> Use SYBR Safe dye instead of Midori green to prevent un-stained DNA
2. Innoculation of pSB1A2-BBa_R0051-E0422
3. Digestion:

Table39

	Sample (+)	DNA mass (ng)	DNA volume (ul)	ddH2O Volume (uL)	Cutsmart Buffer Volume (uL)	Enzyme 1 Volume (uL)	Enzyme 2 Volume (uL)
1	pSB1A2-BBa_R0051 (S & P): 17.53 ng/uL	500	28.52	16.08	5	0.2	0.2
2	pSB1C3-BBA_R0062 (S & P)	1500	13.26	2.19	1.8	0.375	0.375
3	pSB1C3-BBa_B0032 (S & P)	1500	14.55	0.9	1.8	0.375	0.375

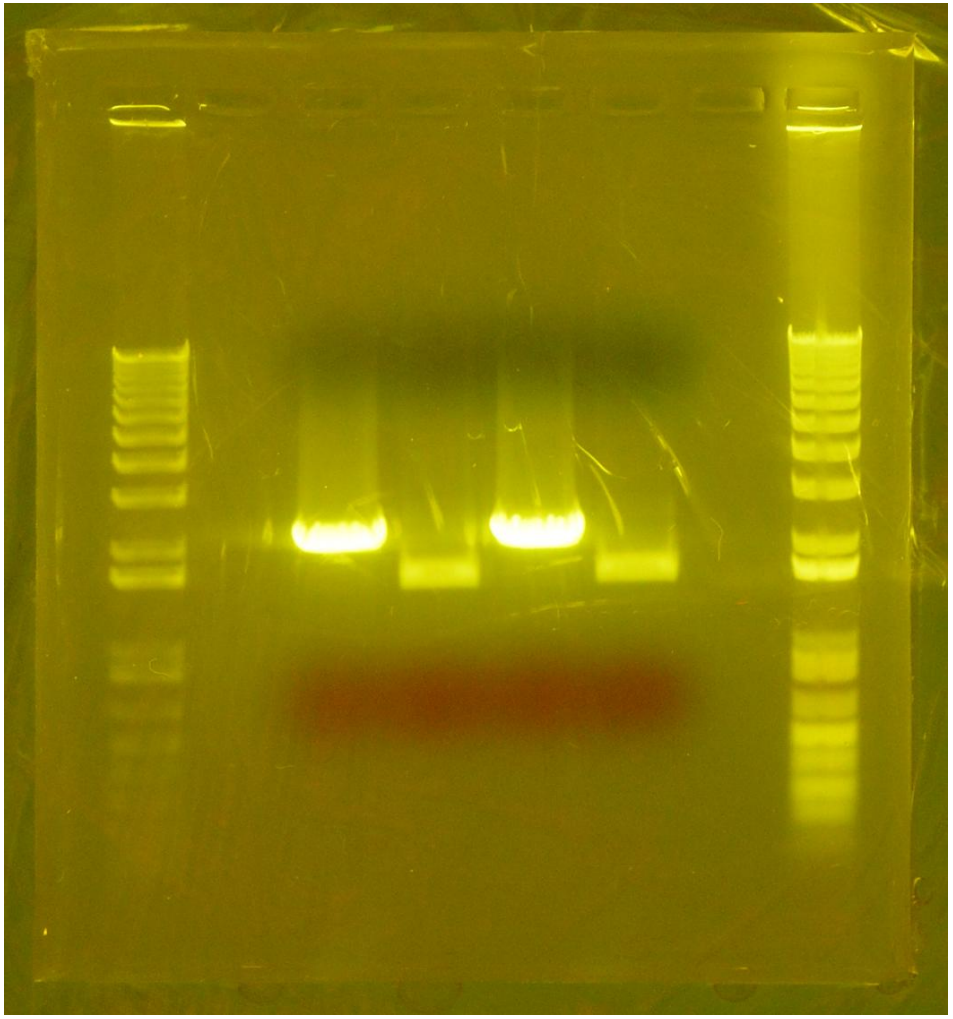
Table40

We recovered unsaved changes to your entry. Click here to recover this data.

	Sample (-)	DNA mass (ng)	DNA volume (ul)	ddH2O Volume (uL)	Cutsmart Buffer Volume (uL)	Enzyme 1 Volume (uL)	Enzyme 2 Volume (uL)
1	pSB1A2-BBa_R0051 (S & P)	100	5.70	3.3	1	0	0
2	pSB1C3-BBA_R0062 (S & P)	200	1.77	14.43	1.8	0	0
3	pSB1C3-BBa_B0032 (S & P)	200	1.94	14.26	1.8	0	0

We recovered unsaved changes to your entry. Click here to recover this data.

Gel electrophoresis c



1% Gel using Sybr safe staining(1/10 of total volume) . 130V for 30 minutes

THURSDAY, 6/29/2017

1. Restriction test of pSB1C3-BBa_C0051

Positive

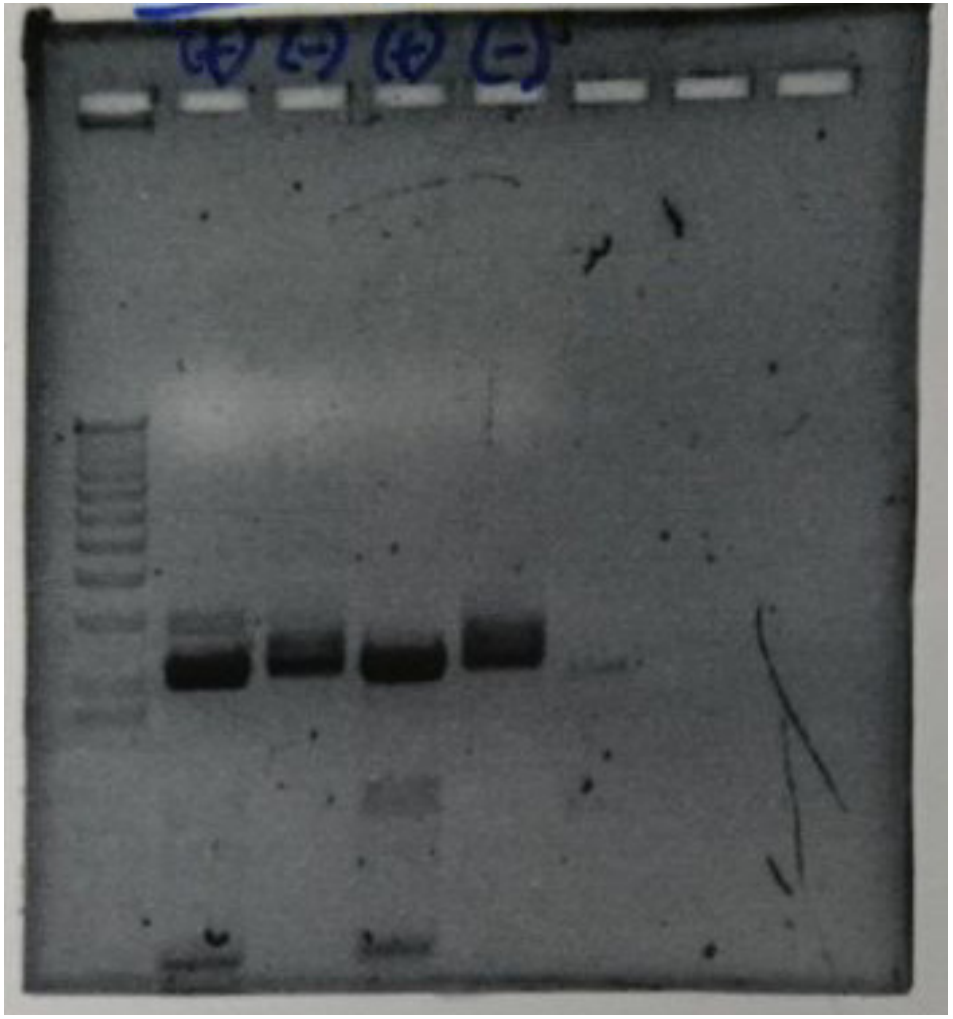
	Positive sample	DNA Mass (ng)	DNA volume (uL)	Enzyme 1 (HindIII-HF) Volume (uL)	Enzyme 2 (PvuII) Volume (uL)	Cutsmart Buffer Volume (uL)	ddH20 Volume (uL)
1	pSB1C3-BBa_C0051 (519 ng/ul)	500	0.96	0.2	0.2	1.8	14.84
2	pSB1C3-BBa_C0051 (362.7 ng/uL)	500	1.38	0.2	0.2	1.8	14.42

Table44

	Negative control	DNA Mass (ng)	DNA volume (uL)	Enzyme 1 (HindIII-HF) Volume (uL)	Enzyme 2 (PvuII) Volume (uL)	Cutsmart Buffer Volume (uL)	ddH20 Volume (uL)
1	pSB1C3-BBa_C0051 (519 ng/ul)	500	0.96	0	0	1.8	15.24
2	pSB1C3-BBa_C0051 (362.7 ng/uL)	500	1.38	0	0	1.8	14.82

We recovered unsaved changes to your entry. Click here to recover this data.

image.png



0.8 % Gel using Sybr safe staining (2ul), run in 130V for 30 mins

*Unknown small band at the positive result.

1. Miniprep of pSB1A2-BBa_R0051-E0422

Table43

	A	Concentration	Protein Contamination	Salt Contamination
1	pSB1A2-BBa_R0051-E0422 (sample 9)	76.56	1.842	1.842
2	pSB1A2-BBa_R0051-E0422 (sample 10)	93.25	1.856	1.843

3. Ligation

Table41

	Positive Sample	Backbone Volume	Insert Volume	Buffer	T4 Ligase
1	pSB1C3-BBa_R0062-K081007	2.42	6.08	1	0.5
2	pSB1C3-BBa_R0062-P0151	1.33	7.17	1	0.5
3	pSB1C3-BBa_R0062-S0109	2.64	5.86	1	0.5
4	pSB1C3-BBa_R0062-P0451	1.41	7.09	1	0.5

Table42

	Negative Control	Backbone Volume	Insert Volume	Buffer	ddH2O
1	pSB1C3-BBa_R0062-K081007	2.42	6.08	1	0.5
2	pSB1C3-BBa_R0062-P0151	1.33	7.17	1	0.5
3	pSB1C3-BBa_R0062-S0109	2.64	5.86	1	0.5
4	pSB1C3-BBa_R0062-P0451	1.41	7.09	1	0.5

FRIDAY, 6/30/2017

We recovered unsaved changes to your entry. Click here to recover this data.

1. Restriction check of p

Positive sample

	Sample	DNA Mass (ng)	DNA Volume (uL)	Enzyme (HindIII-HF) volume (uL)	Cutsmart Buffer volume (uL)	MQ Volume (uL)
1	pSB1C3-BBa_C0051 (519 ng/ul)	400	0.77	0.2	1.8	15.23
2	pSB1C3-BBa_C0051 (362.7 ng/uL)	400	1.1	0.2	1.8	14.9

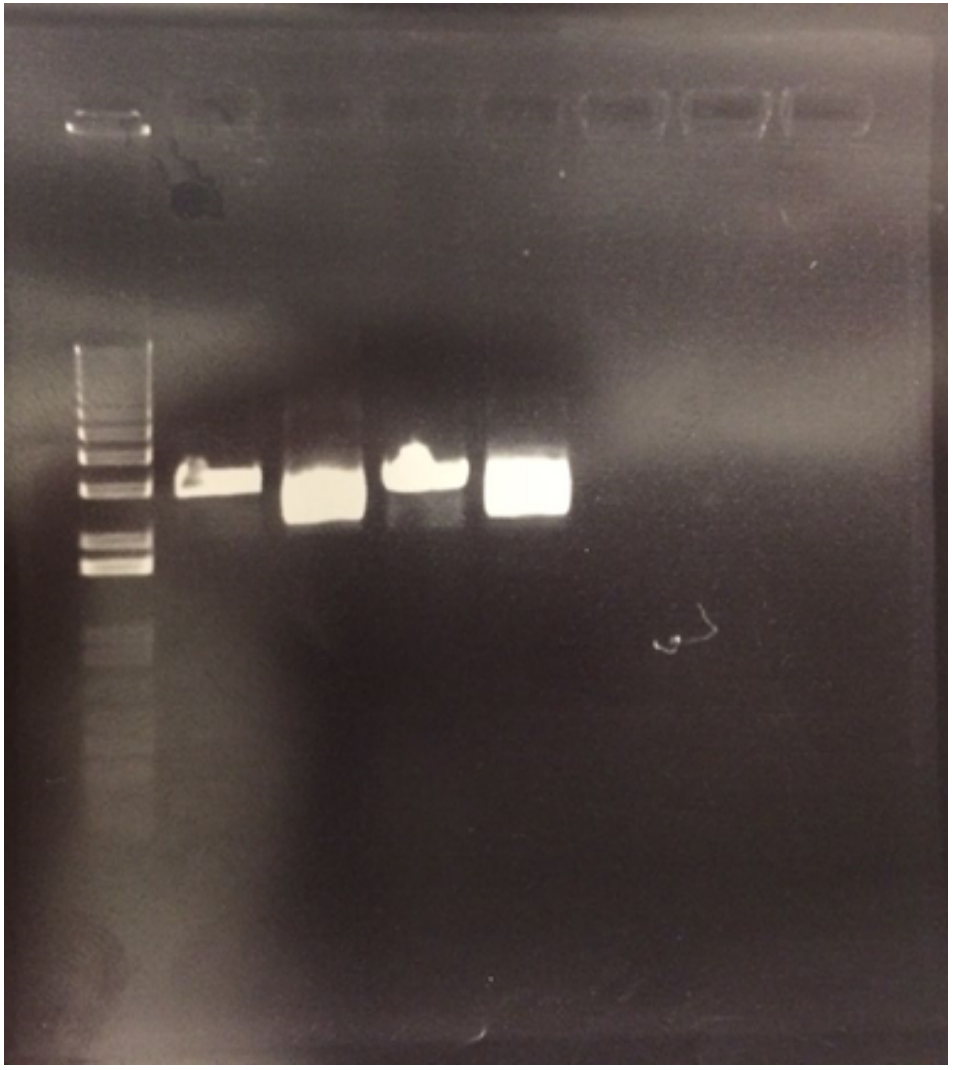
Negative control

	Negative sample	DNA Mass (ng)	DNA Volume (uL)	Enzyme (HindIII-HF) volume (uL)	Cutsmart Buffer volume (uL)	MQ Volume (uL)
1	pSB1C3-BBa_C0051 (519 ng/ul)	400	0.77	0	1.8	15.43
2	pSB1C3-BBa_C0051 (362.7 ng/uL)	400	1.1	0	1.8	15.1

Midori green: 0.2 uL
Agarose gel: 1%
Expected band: 2845

image.png

We recovered unsaved changes to your entry. Click here to recover this data.



The band sizes of positive samples are at 3000 or above. Redo the restriction check with 2 restriction enzymes.

2. Colony PCR
   - pSB1C3-BBa_R0062-P0151
   - pSB1C3-BBa_R0062-K081007
   - pSB1C3-BBa_R0062-P0451
   - pBS1C3-BBa_R0062-S0109

We recovered unsaved changes to your entry. Click here to recover this data.

Mastermix

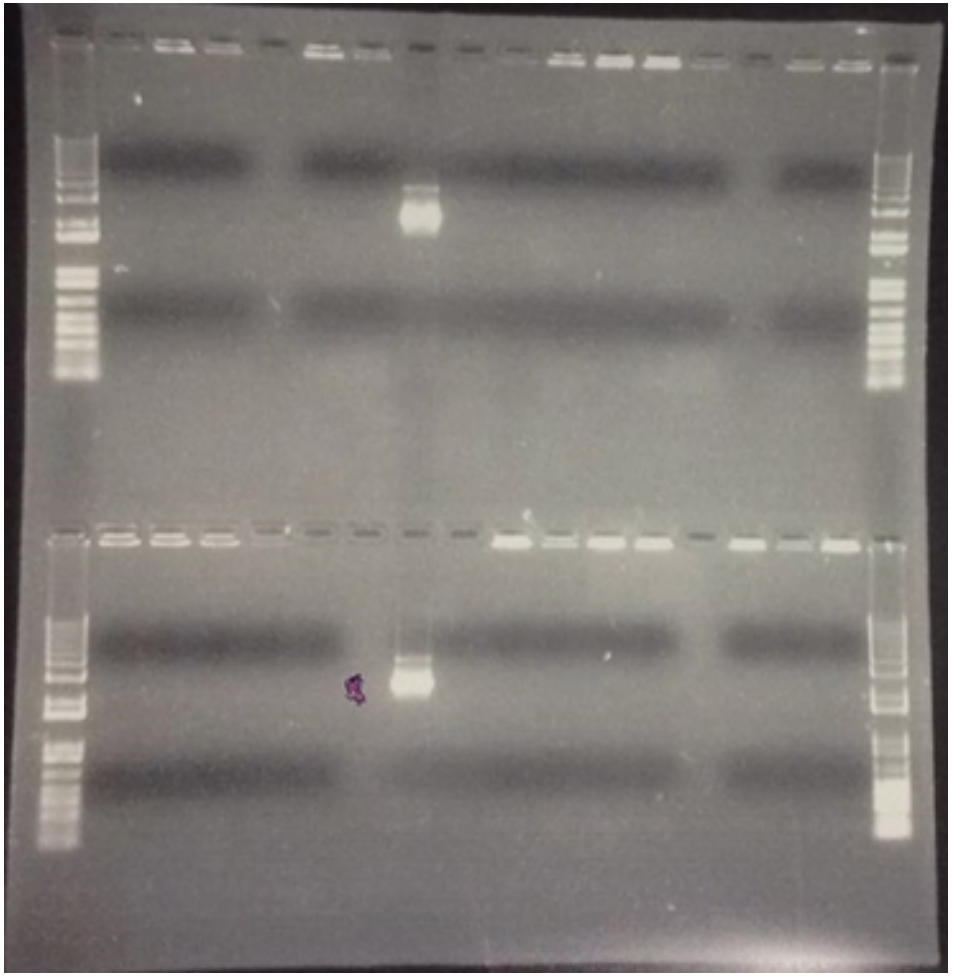
	A	B
1	MQ	386.25
2	VF2	15
3	VR	15
4	5x My Taq Buffer	120
5	Taq Polymerase	3.75

PCR Machine Settings

	A	Temperature (C)	C
1	Initial Denaturation	95	3 min
2	Denaturation	95	30 secs
3	Annealing	55	1 min
4	Extension	68	1 min 15 secs
5	Final Extension	58	5 mins
6	Holding	12	~

image.png

We recovered unsaved changes to your entry. Click here to recover this data.



The PCR+ has wrong band size and all the samples have no band. However, there are DNA stuck in the wells.

1.5% agarose gel. stained with midor green. 130v, Run for 40minutes.

MONDAY, 7/3/2017

1. Digestion

- [ ] pSB1C3-BBa_C0051 (
- [ ] pSB1C3-BBa_C0051 (

Mass of DNA: 500 ng
Expected band sizes (+): 801bp, 2053bp
Expected band size (-): 2854bp

Table45

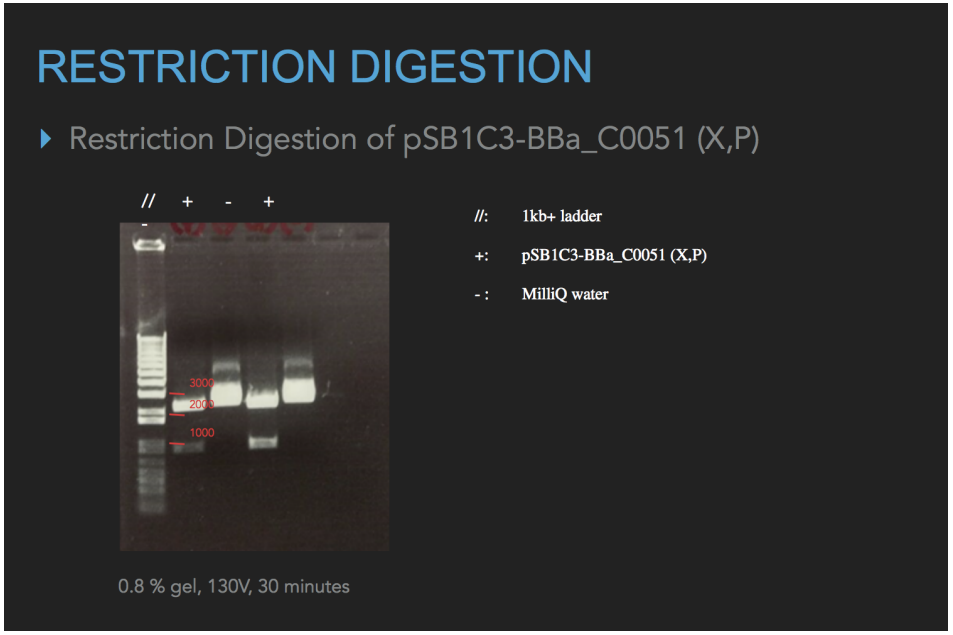
	Positive	Volume of Sample	ddH2O	Enzymes
1	pSB1C3-BBa_C0051 (519 ng/ul)	0.96	14.84	X, P
2	pSB1C3-BBa_C0051 (362.7 ng/uL)	1.38	14.42	X, P

Table46

	Negative	Volume of Sample	ddH2O	D
1	pSB1C3-BBa_C0051 (519 ng/ul)	0.96	15.24	
2	pSB1C3-BBa_C0051 (362.7 ng/uL)	1.38	14.82	

Screen Shot 2017-10-

We recovered unsaved changes to your entry. Click here to recover this data.



0.8% gel, 130V for 30 mins, stained by midori green
Gel photo:
*The length of insert (~850bp) is longer than expected length(801bp), which means our plasmid cannot be use in ligation. We need to redo the transformation.

3. Colony PCR

- pSB1C3-BBa_R0062-K081007
- pSB1C3-BBa_R0062-P0151
- positive control - pSB1C3-BBa_E0422

Table49

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B
1	MQ	206
2	VF2	8
3	VR	8
4	5x My Taq Buffer	64
5	Taq Polymerase	2

Table48

	A	Temperature (C)	C
1	Initial Denaturation	95	3 min
2	Denaturation	95	30 secs
3	Annealing	55	1 min
4	Extension	68	1 min 20 secs
5	Final Extension	58	5 mins
6	Holding	12	~

sample 1-5, -1: pSB1C3-BBa_R0062-P0151
sample 6-10, -2: pSB1C3-BBa_R0062-K081007
+: pSB1C3-BBa_E0422

Table58

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	//	1	2	3	4	5	+	-	-1	-2	6	7	8	9	10	//

Gel photo:

Screen Shot 2017-10-

We recovered unsaved changes to your entry. Click here to recover this data.

1% agarose gel. stained with midor green. 130v, Run for 30 minutes.
*The wells are bright because there are too many colonies. We need to further dilute the samples with saline.
*PCR negative control has bands. There may be contamination. We prepared a new tube of MQ today.
*PCR positive is smear and has an extra bands.
*Pick the smaller colony to increase the chance of getting the correct plasmid.

TUESDAY, 7/4/2017

1. Transformation (from kit plate)

- [ ] pSB1C3-BBa_C0051
- [ ] pSB1C3-BBa_R0040
- [ ] pSB1A2-BBa_E0040

2. Colony PCR

Mastermix composition for 20 reactions

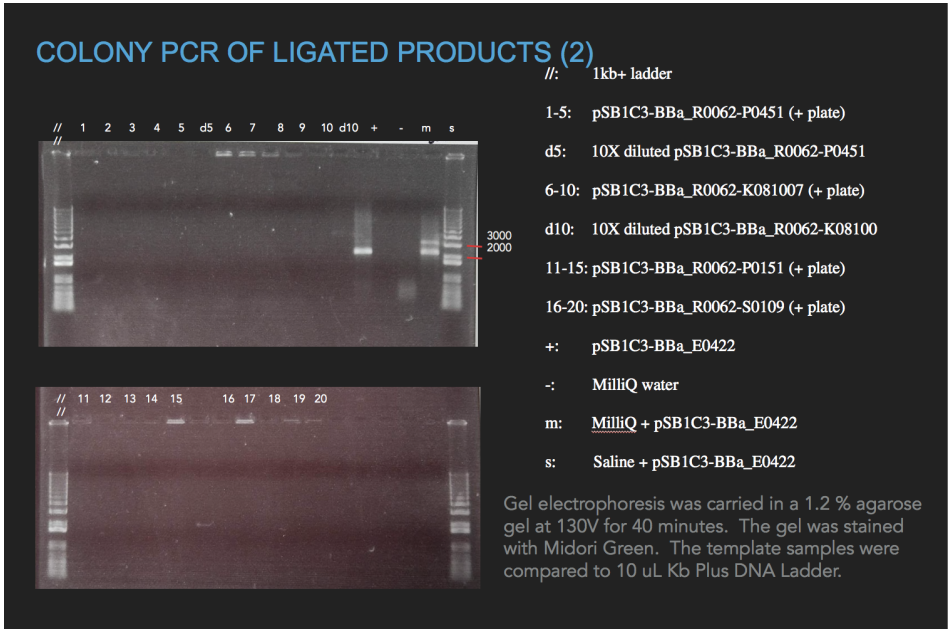
Table47

	A	B
1	MQ	382.35
2	VF2	14.85
3	VR	14.85
4	5x My Taq Buffer	118.8
5	Taq Polymerase	3.71

1-5: psB1C3-BBa_R0062-P0451
6-10: pSB1C-BBa_R0062-K081007
11-15: psB1C3-BBa_R0062-P0151
16-20: psB1C3-BBa_R0062-S0109
Gel photo:

Screen Shot 2017-10-

We recovered unsaved changes to your entry. Click here to recover this data.



3. Innoculation
- [ ] pSB1C3-BBa_R0062-K081007
- [ ] pSB1C3-BBa_R0062-S0109
- [ ] pSB1C3-BBa_R0062-P0151
- [ ] pSB1C3-BBa_R0062-P0451

WEDNESDAY, 7/5/2017

1. Minprep
- [ ] pSB1C3-BBa_R0062-K081007
- [ ] pSB1C3-BBa_R0062-S0109
- [ ] pSB1C3-BBa_R0062-P0151
- [ ] pSB1C3-BBa_R0062-P0451

Table56

	A	DNA conc,	Protein	Salt
1	pSB1C3-BBa_R0062-K081007 (1)	102.8	1.843	1.911
2	pSB1C3-BBa_R0062-K081007 (2)	153.4	1.84	2.179
3	pSB1C3-BBa_R0062-S0109 (1)	102.2	1.852	2.266
4	pSB1C3-BBa_R0062-S0109 (2)	105.1	1.84	2.056
5	pSB1C3-BBa_R0062-P0151 (1)	65.8	1.838	1.741
6	pSB1C3-BBa_R0062-P0151 (2)	112	1.836	1.964
7	pSB1C3-BBa_R0062-P0451 (1)	83.51	1.835	2.062
8	pSB1C3-BBa_R0062-P0451 (2)	54.14	1.858	2.34

2. Restriction check

Table54

	Positive	DNA Conc.	DNA volume (uL)	ddH2O	Enzymes (Vol = 0.2 uL each)	expected bands (bp)
1	pSB1C3-BBa_R0062-K081007 (1)	102.8	4.86	10.94	HindIII-HF, PvuII	2003, 926
2	pSB1C3-BBa_R0062-K081007 (2)	153.4	3.26	12.54	HindIII-HF, PvuII	2003, 926
3	pSB1C3-BBa_R0062-S0109 (1)	102.2	4.89	10.91	HindIII-HF, PvuII	2003, 922
4	pSB1C3-BBa_R0062-S0109 (2)	105.1	4.76	11.04	HindIII-HF, PvuII	2003, 922
5	pSB1C3-BBa_R0062-P0151 (1)	65.8	7.6	8.2	HindIII-HF, PvuII	2140, 925
6	pSB1C3-BBa_R0062-P0151 (2)	112	4.46	11.34	HindIII-HF, PvuII	2140, 925
7	pSB1C3-BBa_R0062-P0451 (1)	83.51	5.99	9.81	HindIII-HF, PvuII	2140, 923
8	pSB1C3-BBa_R0062-P0451 (2)	54.14	9.24	6.56	HindIII-HF, PvuII	2140, 923

Table55

	Negative	DNA conc.	DNA volume (uL)	ddH20	Enzymes volume (uL)	expected bands
1	pSB1C3-BBa_R0062-K081007 (1)	102.8	4.86	11.34	0	2929
2	pSB1C3-BBa_R0062-K081007 (2)	153.4	3.26	12.54	0	2929
3	pSB1C3-BBa_R0062-S0109 (1)	102.2	4.89	11.31	0	2925
4	pSB1C3-BBa_R0062-S0109 (2)	105.1	4.76	11.44	0	2925
5	pSB1C3-BBa_R0062-P0151 (1)	65.8	7.6	8.6	0	3065
6	pSB1C3-BBa_R0062-P0151 (2)	112	4.46	11.74	0	3065
7	pSB1C3-BBa_R0062-P0451 (1)	83.51	5.99	10.21	0	3063
8	pSB1C3-BBa_R0062-P0451 (2)	54.14	9.24	6.96	0	3063

Table57

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	//	K (1) (+)	K (1) (-)	K (2) (+)	K (2) (-)	S (1) (+)	S (1) (-)	S (2) (+)	S (2) (-)	P1 (1) (+)	P1 (1) (-)	P1 (2) (+)	P1 (2) (-)	P4 (1) (+)	P4 (1) (-)	P4 (2) (+)	P4 (2) (-)	//

Screen Shot 2017-10-

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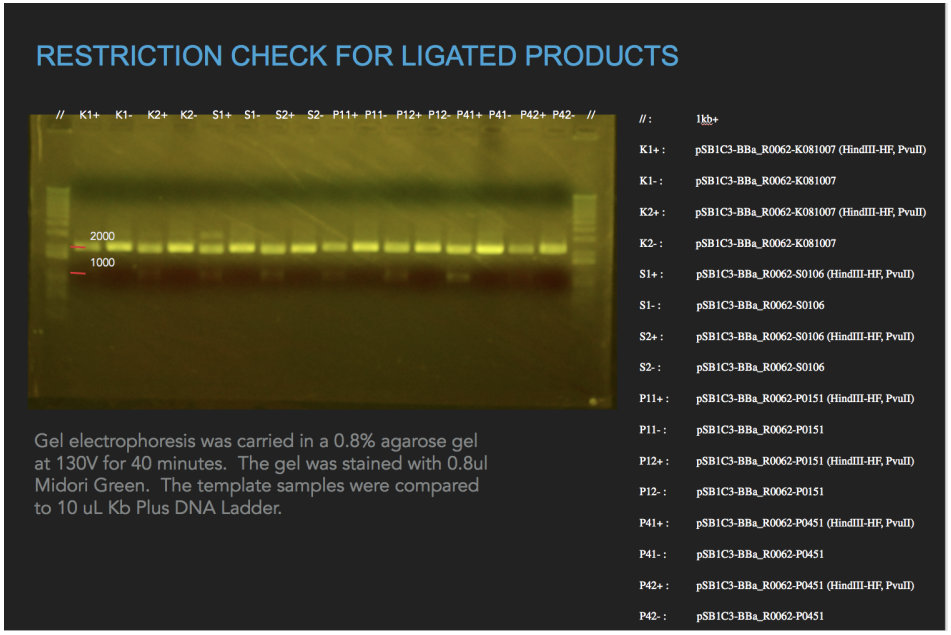
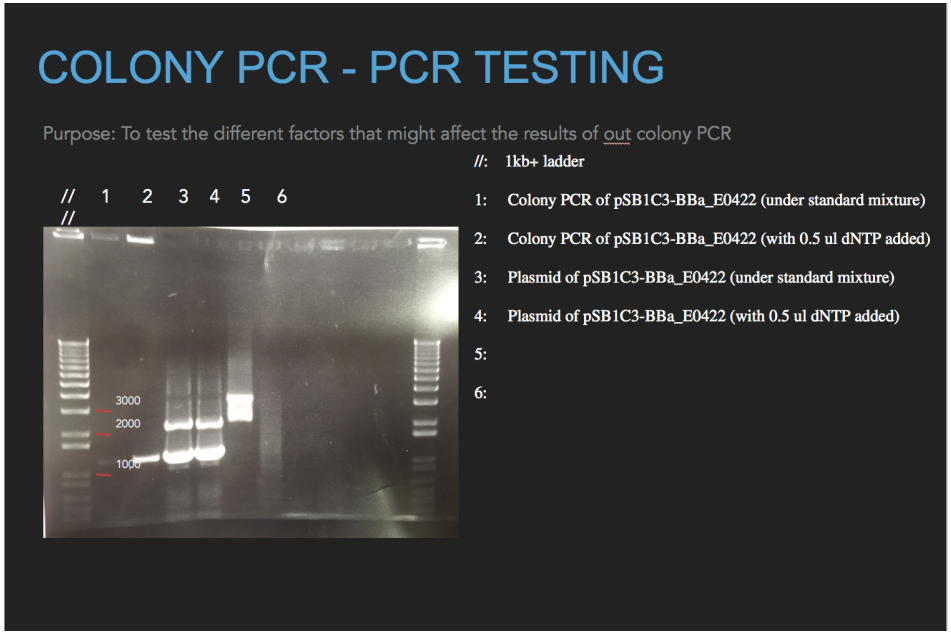


half of a 80ml 0.8% gel, stained by 0.8 ul midori green
*pSB1C3-BBa_R0062-K081007 (1) and pSB1C3-BBa_R0062-P0451 (2) do not show the band of insert
*pSB1C3-BBa_R0062-S0109 (1) shows imcomplete digestion (there is a band at 3000bp)

3. Colony PCR (Max)
positive control: pSB1C3-BBa_E0422
master mix+dNTP+template
Master mix+template
MQ+template
Saline+template
Master mix+cells+dNTP
Master mix+cells
Master mix
Master mix+dNTP

Screen Shot 2017-10-

We recovered unsaved changes to your entry. Click here to recover this data.



From left to right:

1. Colony of pSB1C3_BBa-E0422 without dNTP
2. Colony of pSB1C3_BBa-E0422 with dNTP
3. 0.5 ul of Plasmid of pSB1C3_BBa-E0422 without dNTP
4. 0.5 ul of Plasmid of pSB1C3_BBa-E0422 with dNTP
5. 0.5 ul of Plasmid of pSB1C3_BBa-E0422 in saline

THURSDAY, 7/6/2017

Second ligation

- [ ] pSB1A2-BBa_R0051-E0422 (x3)

2. Digestion

Table32

We recovered unsaved changes to your entry. Click here to recover this data.

	Positive	Volume of sample	ddH2O	D	E
1	pSB1C3-BBa_R0062-P0151 (S&P)	8.93	6.77	Backbone (left)	S&P
2	pSB1C3-BBa_R0062-P0451 (S&P)	11.97	3.73	Backbone (left)	S&P

Table33

	Negative	Volume of sample	ddH2O	D	E
1	pSB1C3-BBa_R0062-P0151 (S&P)	1.79	14.41		
2	pSB1C3-BBa_R0062-P0451 (S&P)	2.39	13.81		

Gel photo:

Screen Shot 2017-10-

1% agarose gel. stained with midor green. 130v, Run for 35 minutes.

4. Colony cracking

Protocol：

1. Grow bacterial colonies to a large size (2-3 mm) on an agar medium contining an appropriate antibiotic.

2. Using a sterile toothpick, transfer a small quantity of the colony to a master plate. Transfer the remainder of the colony to a microfuge tube containing 20 microliters of 50 mM NaOH, 0.5% SDS, 5 mM EDTA (cracking buffer).

3. Incubate the tube at 55 C for 30 min.

4. Vortex vigorously for 1 min.*

5. Add an appropriate amou[illegible]te: It may be very difficult to apply the cracking mixtu[illegible]ring electrophoresis buffer thereafter may give better result.)

6. After electrophoresis, stain the gel by soaking it for 30 minutes in a solution of ethidium bromide (0.5 microgram/ml in electrophoresis buffer).

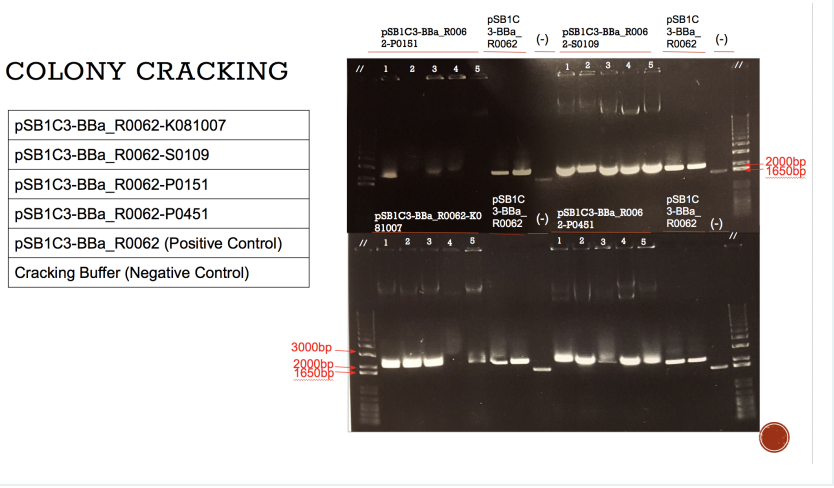
7. Under UV-illuminator, plasmid DNA should be visible between E. coli genomic DNA (20-30 kb) and low molecular weight RNAs.

* At this step, long genomic DNA is cut into smaller pieces of about 20-30 kb. Although the original protocol in "Molecular Cloning" does not contain this step, vigorous vortexing is necessary since long genomic DNA in the lysate is troublesome in loading the sample onto the agarose gel.
** Add the loading buffer just before electrophoresis, since bromophenol blue is rapidly degraded in the alkaline solution.
Plasmid as positive control

All samples are treated with lysis buffer （50 mM NaOH, 0.5% SDS, 5 mM EDTA）
Vector as comparsion with the ligated plasmid
J04450 Colony
J04450 plasmid
pSB1A2-BBa_R0051-BBa_E0422 9
pSB1A2-BBa_R0051-BBa_E0422 9 Plasmid
pSB1A2-BBa_R0051-BBa_E0422 10
pSB1A2-BBa_R0051-BBa_E0422 10 Plasmid
pSB1A2-BBa_R0051-BBa_E0422 i Plasmid
pSB1A2-BBa_R0051-BBa_E0422 I Plasmid
pSB1C3-BBa_E0422 6/6 Colony
pSB1C3-BBa_E0422 plasmid 2
pSB1C3-BBa_E0422 7/6 Colony
pSB1C3-BBa_E0422 Plasmid 3
pSB1A2-BBa_R0051 Colony
pSB1A2-BBa_R0051 plasmid miniprepped by Marcel
pSB1A2-BBa_R0051 Plasmid

Gel 0.8%
Photo:

Screen Shot 2017-10-



Conclusion: colony cracking is effective

CFP fluorescence assay
R0051 as neagtive control
9 and 10 as suspected to produce CFP

Conclusion: No CFP produced from both ligated product
P.S. No positive control so far, no useful conclusion can be drawn

5. inoculation
R0051, C0051
1 set by Kelly, and 1 set by Jenny

Transformation:
All digested parts
Positive Control: plasmid
P.S. Jenny accidentally heat shocked the cells in water bath of 52C

FRIDAY, 7/7/2017

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1. Colony PCR

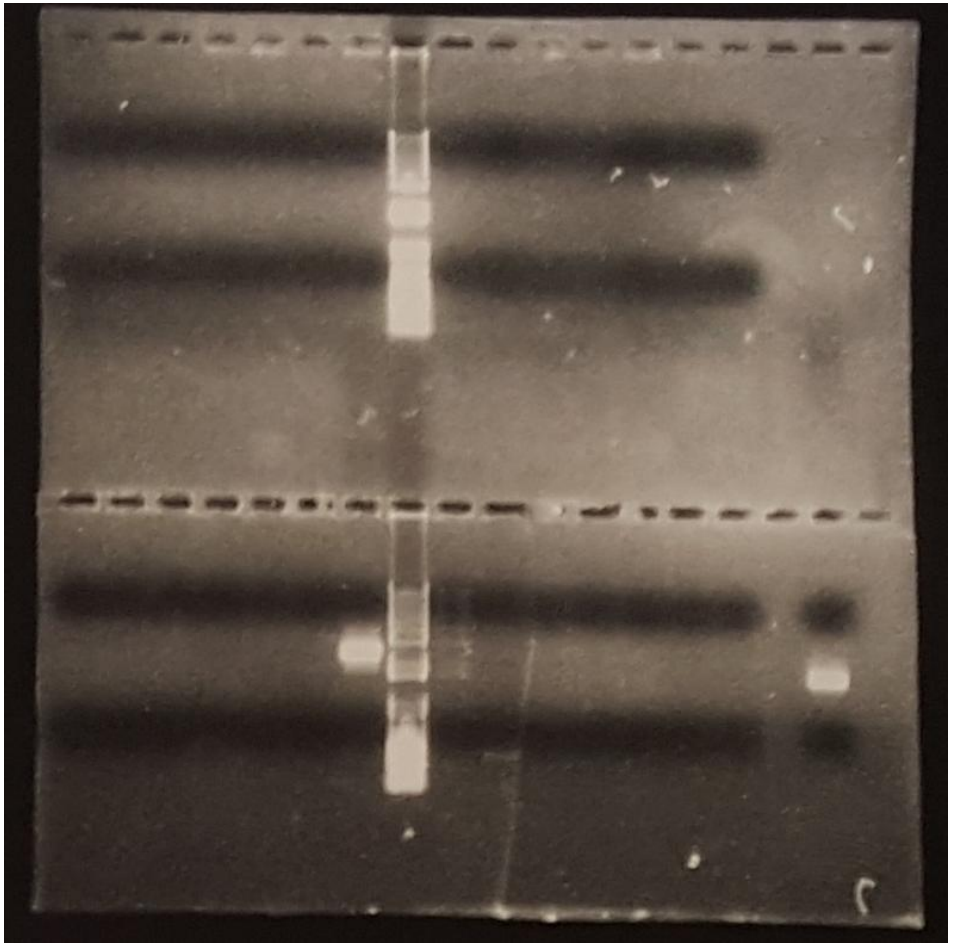
Samples used

	A
1	pSB1C3-BBa_R0062-K081007
2	pSB1C3-BBa_R0062-S0109
3	pSB1C3-BBa_R0062-P0151
4	pSB1C3-BBa_R0062-P0451
5	pSB1C3-BBa_E0422 (Streak Plate)
6	pSB1C3-BBa_E0422 (Plasmid)

Recipe for master mix

	A	Master Mix
1	MQ	416.25
2	5x My Taq Buffer	120
3	VF2	15
4	VR	15
5	10mM dNTP	15
6	Taq Polymerase	3.75

20170712_125140- Cr



1% agarose gel. stained with midor green. 130v, Run for 35 minutes.

3. Miniprep:
2 sets of R0040 inoculated by Kelly, and Jenny
2 sets of C0051 inoculated by Kelly, and Jenny
*insufficient time for Nanodrop

Table62

	A	DNA Concentration	Protein Contamination	Salt Contamination
1	pSB1C3-BBa_R0062-P0151	85.09	1.807	2.022
2	pSB1C3-BBa_R0062-P0451	113.5	1.845	2.045
3	pSB1C3-BBa_R0062-K081007	71.79	1.804	1.851
4	pSB1C3-BBa_R0062-S0109	104.9	1.784	1.881

4. master plate creation from plates transformed with ligated plasmid
9 colonies is picked for each plates

Table63

	A
1	pSB1C3-BBa_R0062-K081007
2	pSB1C3-BBa_R0062-S0109
3	pSB1C3-BBa_R0062-P0151
4	pSB1C3-BBa_R0062-P0451

MONDAY, 7/10/2017

1. Colony PCR

Table59

	A
1	pSB1C3-BBa_R0062-K081007
2	pSB1C3-BBa_R0062-S0109
3	pSB1C3-BBa_R0062-P0151
4	pSB1C3-BBa_R0062-P0451
5	pSB1C3-BBa_R0062 (Negative control)
6	pSB1C3-BBa_E0422 (Streak Plate) 0.5ul
7	pSB1C3-BBa_E0422 (Streak Plate) without dNTP 0.5ul
8	pSB1C3-BBa_E0422 (Streak Plate) 2ul
9	pSB1C3-BBa_E0422 (Streak Plate) without dNTP 2ul
10	pSB1C3-BBa_E0422 (Plasmid)
11	MX only
12	MX without dNTP

20170710-Colony PC

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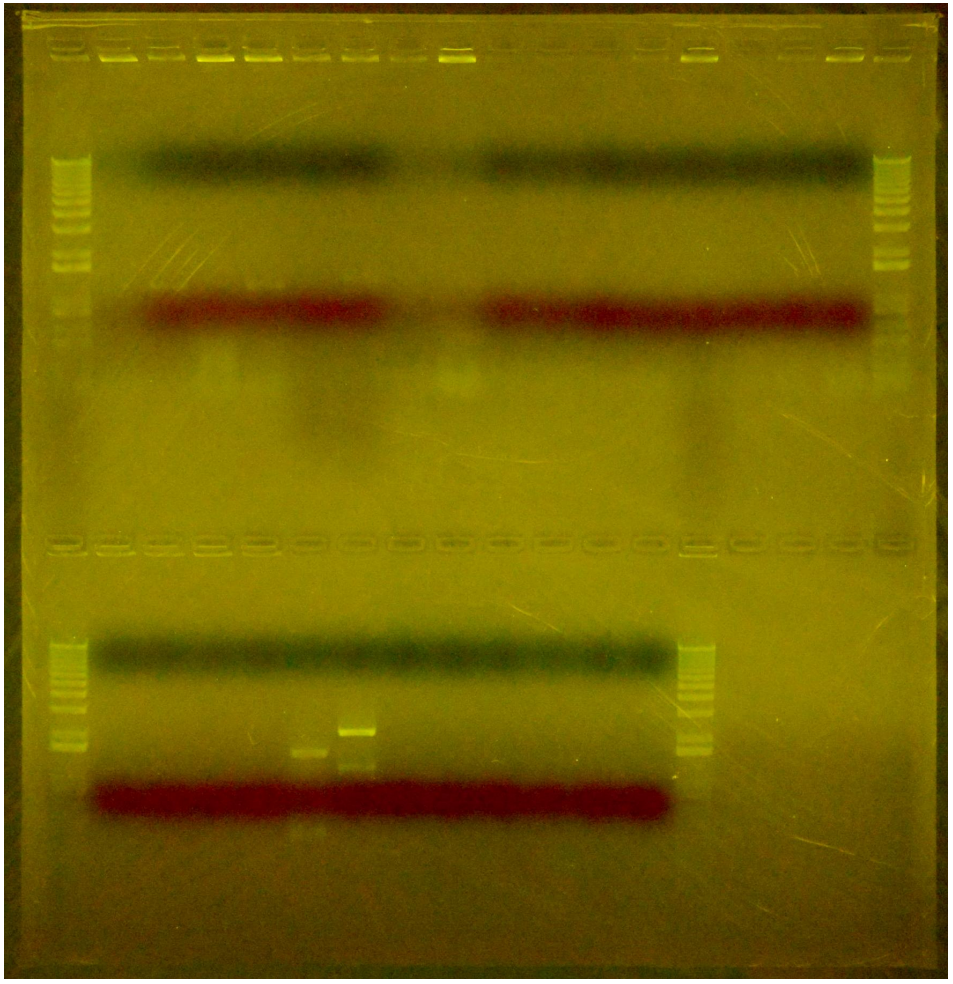

0.8% agarose gel. stained with midor green. Run for 30 minutes with 130v.

TUESDAY, 7/11/2017

1. Transformation (second transformation from same batch):

- pSB3K3-BBa_E0240 2014 (From summer training box)
- pSB1C3-BBa_E0422 2015 Spring 2015 Kit Plate 2 Well 6P
- pSB1C3-BBa_E0420 2015 DNA Box 2 Column 1 Row G
- pSB1C3-BBa_I763020 Spring 2014 Kit Plate 3 (http://parts.igem.org/cgi/assembly/plates.cgi?id=3087) Well 12G (single error)
- pSB1C3-BBa_E0430 Spring 2014 Kit Plate 3 (http://parts.igem.org/cgi/assembly/plates.cgi?id=3087) Well 19K

- pSB1C3-BBa_E0432 S
- P.S.
    - E0240, GFP Generator; I763020 LVA tagged GFP Generator;
    - E0420, CFP Generator; E0422, LVA tagged CFP Generator;
    - E0430, YFP Generator; E0432, LVA tagged YFP Generator;

2. Streak plate of pSB1C3-BBa_E0422 (only 1 colony from pevious transfomation)

3. Colony Cracking
9 samples from each plate will be picked

Table61

	A
1	pSB1C3-BBa_R0062-K081007
2	pSB1C3-BBa_R0062-S0109
3	pSB1C3-BBa_R0062-P0151
4	pSB1C3-BBa_R0062-P0451
5	pSB1C3-BBa_R0062 (Positive Control)
6	Cracking Buffer (Negative Control)

Screen Shot 2017-10-

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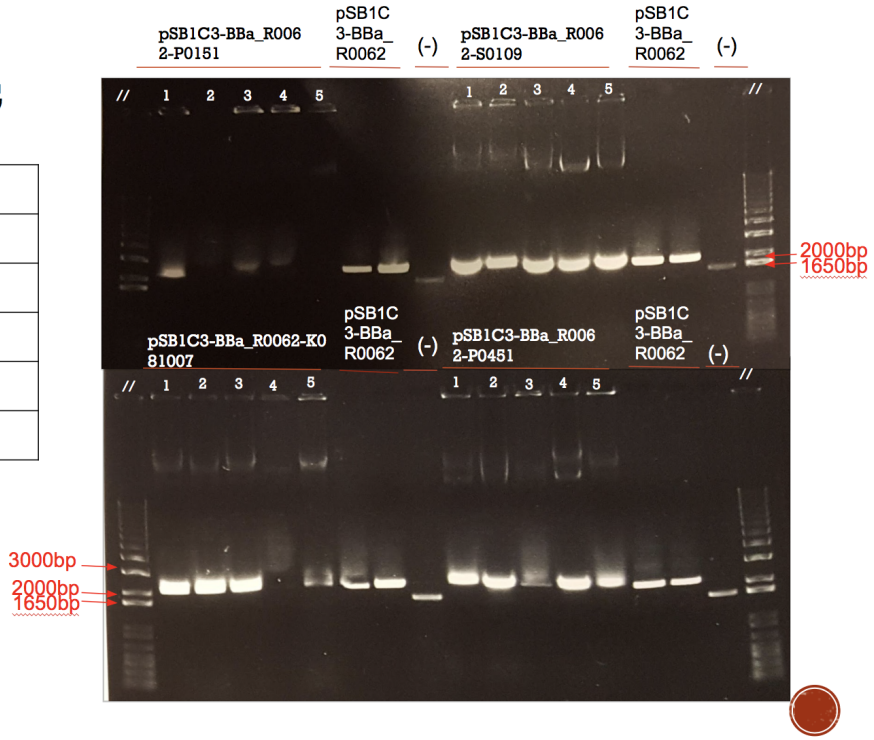
COLONY CRACKING

pSB1C3-BBa_R0062-K081007
pSB1C3-BBa_R0062-S0109
pSB1C3-BBa_R0062-P0151
pSB1C3-BBa_R0062-P0451
pSB1C3-BBa_R0062 (Positive Control)
Cracking Buffer (Negative Control)



4. Innoculation
pSB1C3-BBa_R0062-K081007
pSB1C3-BBa_R0062-S0109
pSB1C3-BBa_R0062-P0151
pSB1C3-BBa_R0062-P0451

WEDNESDAY, 7/12/2017

- Miniprep
  a. pSB1C3-BBa_R0062-P0151
  b. pSB1C3-BBa_R0062-P0451
  c. pSB1C3-BBa_R0062-K081007

d. pSB1C3-BBa_R

Table60

	Plasmid	DNA Concentration	Protein Contamination	Salt Contamination
1	pSB1C3-BBa_R0062-P0151	85.09	1.807	2.022
2	pSB1C3-BBa_R0062-P0451	113.5	1.845	2.045
3	pSB1C3-BBa_R0062-K081007	71.79	1.804	1.851
4	pSB1C3-BBa_R0062-S0109	104.9	1.784	1.881

- Restriction Check
  a. pSB1C3-BBa_R0062-P0151
  b. pSB1C3-BBa_R0062-P0451
  c. pSB1C3-BBa_R0062-K081007
  d. pSB1C3-BBa_R0062-S0109

Positive Sample of Restriction Check

	Plasmid	DNA Volume	HindIII-HF	10x Cutsmart Buffer	MQ
1	pSB1C3-BBa_R0062-P0151	3.53	0.2	1.8	12.47
2	pSB1C3-BBa_R0062-P0451	2.64	0.2	1.8	13.36
3	pSB1C3-BBa_R0062-K081007	4.18	0.2	1.8	11.82
4	pSB1C3-BBa_R0062-S0109	2.86	0.2	1.8	13.14
5	pSB1C3-BBa_E0422	1.42	0.2	1.8	14.58

Negative Controls

We recovered unsaved changes to your entry. Click here to recover this data.

	Plasmid	DNA Volume	HindIII-HF	10x Cutsmart Buffer	MQ
1	pSB1C3-BBa_R0062-P0151	1.18	0	1.8	13.85
2	pSB1C3-BBa_R0062-P0451	0.88	0	1.8	15.32
3	pSB1C3-BBa_R0062-K081007	1.39	0	1.8	14.81
4	pSB1C3-BBa_R0062-S0109	0.95	0	1.8	15.25

Screen Shot 2017-10-31 at 3.22.23 PM.png

1% agarose gel. stained with midor green. 130v, Run for 35 minutes.

- Digestion

Table64

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ
1	pSB1C3-BBa_R0062-P0151	4.87	0.2	0.2	1.8	10.93

Negative Control

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ
1	pSB1C3-BBa_R0062-P0151	0.97	0	0	1.8	15.23

201707112.jpg

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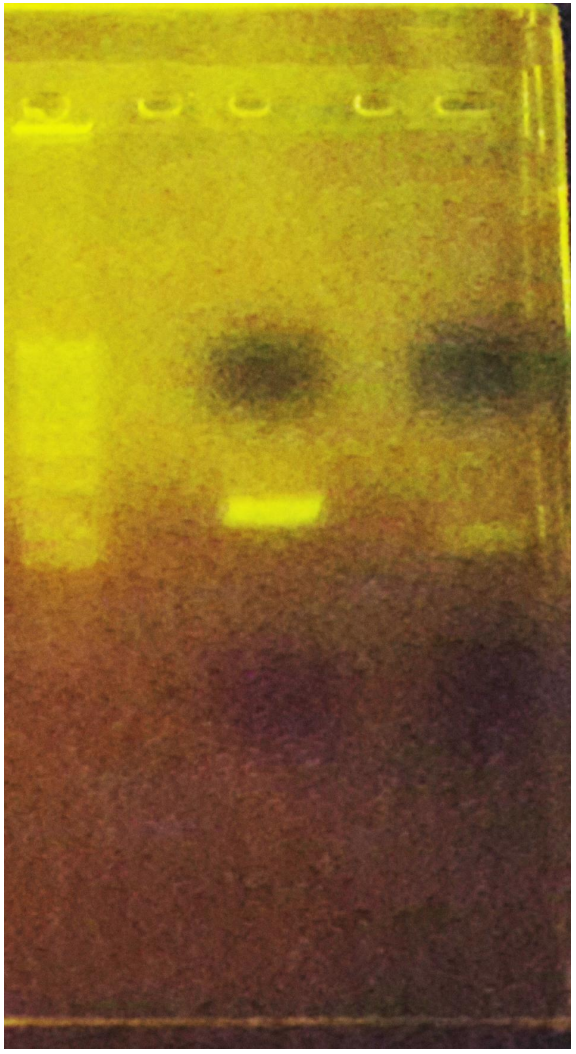

1% agarose gel. stained with midor green. 130v, Run for 35 minutes.

- Inoculation
  - [x] pSB3K3-BBa_E0240
  - [x] pSB1C3-BBa_E0422 (picked from 11/7/17 straked plate)
  - [ ] pSB1C3-BBa_E0420
  - [ ] pSB1C3-BBa_I763020
  - [x] pSB1C3-BBa_E0430

pSB1C3-BBa_E

We recovered unsaved changes to your entry. Click here to recover this data.

P.S. No colonies from most of the plate except BBa_E0240

THURSDAY, 7/13/2017

Protocol for IDT part by Yolanda
(for detailed pictures, please visit benchling...Time Module)

❀Preparation of pSB1C3-BBa_B0034-Phlf from pSB1C3-BBa_B0030-Phlf glycerol stock:

1. Inverse PCR (Use GC enhancer!!!)
    a. Primer used: FW for B0030 to B0034

[12.B0034][6.SCAR][9.part of Phlf]

    a. Primer used: RW for B0030 to B0034

*DpnI should be added to digest the template DNA after amplification

[22.part of prefix]

1. Blunt-end LIGATION (linear → circular)

End-product: pSB1C3-BBa_B0034-Phlf

❀BACKUP Plan: (full construct)

1. Amplification of IDT parts x2 (Use GC enhancer!!!)
    a. Primer used: FW for full construct part 1
    b. Primer used: RW for full construct part 1
    c. Primer used: FW for full construct part 2
    d. Primer used: RW for full construct part 2
    e. IDT: FW for B0030 to B0034

[Prefix][12.B0034][6.SCAR][781.C0062][8.SCAR][80.B0010][8.SCAR][41.B0012][8.SCAR][55.R0062][8.SCAR][14.B0031][6.SCAR][775.C0051][8.SCAR][B0010 overlapping region]

    a. IDT: RW for B0030 to B0034

[B0010 overlapping region][.SCAR][41.B0012][8.SCAR][49.R0051][8.SCAR][12.B0034][6.SCAR][603.K1725040][8.SCAR][80.B0010][8.SCAR][41.B0012][8.SCAR][48.K1725000]
[8.SCAR][12.B0034][Suffix]

1. GIBSON ASSEMBLY (Part 1 + Part 2)

(Advisors suggested using thermo fisher protocol)

1. Digestion & Ligation (STANDARD ASSEMBLY)

a. Use IDT full cor
b. pSB1C3-BBa_R

End-product: Time module full construct with .07 RBS (BBa_B0031)

☆To change RBS,

1. INVERSE PCR:
    a. Primer used: FW for B0031 to B0032Primer used: RW for B0031 to B0032Primer used: RW for B0031 to B0034Primer used: RW for B0031 to B0030Primer used: FW for B0031 to B0030 and B0034

(PS: same FW primer for B0030 and B0034)

1. Blunt-end LIGATION (linear → circular)

End-product: Time module full construct with different RBS

Amplification for IDT part:

1. Amplification of IDT parts x2 (Use GC enhancer!!!)

Primer used

	A	B	C	D
1	20	iGEM 2017 (Time Delay)	Part 1 FWD for full costruct Time delay	GAATTCGCGGCCGCTTCTAG
2	21	iGEM 2017 (Time Delay)	Part 1 REV for full construct Time delay	cgtgcgtcctcaagctgc
3	22	iGEM 2017 (Time Delay)	Part 2 FWD for full costruct Time delay	cacaagagcagcttgaggacgc
4	23	iGEM 2017 (Time Delay)	Part 2 REV for full consruct Time delay	ctgcagcggccgctactag

Time Construct 1:
[Prefix][12.B0034][6.SCAR][781.C0062][8.SCAR][80.B0010][8.SCAR][41.B0012][8.SCAR][55.R0062][8.SCAR][14.B0031][6.SCAR][775.C0051][8.SCAR][B0010 overlapping region]

Time Construct 2:
[B0010 overlapping region][.SCAR][41.B0012][8.SCAR][49.R0051][8.SCAR][12.B0034][6.SCAR][603.K1725040][8.SCAR][80.B0010][8.SCAR][41.B0012][8.SCAR][48.K1725000][8.SCAR][12.B0034][Suffix]

Protocol for Q5 PCR
https://www.neb.com/protoc

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Table66

	A	B	C
1	**COMPONENT**	**50 µl REACTION**	**FINAL CONCENTRATION**
2	5X Q5 Reaction Buffer	10 µl	1X
3	10 mM dNTPs	1 µl	200 µM
4	10 µM Forward Primer	2.5 µl	0.5 µM
5	10 µM Reverse Primer	2.5 µl	0.5 µM
6	Template DNA	1 ng (1 µl)	< 1,000 ng
7	Q5 High-Fidelity DNA Polymerase	0.5 µl	0.02 U/µl
8	5X Q5 High GC Enhancer	10 µl	(1X)
9	Nuclease-Free Water	to 50 µl	

Thermocycling Conditions for a Time Construct Part 1 PCR:

Table67

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	98°C	30 seconds
3	25 Cycles	98°C, 70°C, 72°C	10 seconds, 30 seconds, 45 seconds for 1099 bp
4	Final Extension	72°C	5 minutes*
5	Hold	4–10°C	

Thermocycling Conditions for a Time Construct Part 2 PCR:

Table68

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	98°C	30 seconds
3	25 Cycles	98°C, 72°C, 72°C	10 seconds, 30 seconds, 60 seconds for 1807 bp
4	Final Extension	72°C	5 minutes*
5	Hold	4–10°C	

*2 minutes is recommended

Gel Electrophoresis in 0.8% gel, 130V 30min

- Miniprep

Table65

	Plasmid	DNA Concentration	Protein Contamination	Salt Contamination
1	pSB1C3-BBa_E0432	146.9	1.838	2.071
2	pSB1C3-BBa_E0422	123.0	1.863	2.157
3	pSB1C3-BBa_E0240	24.88	1.932	1.672
4	pSB1C3-BBa_E0430	131.6	1.864	2.284
5	pSB1C3-BBa_E0432	155.1	1.866	2.277

- Transformations

- [ ] pSB1C3-BBa_I763020 (2016 kit4 5L)
- [ ] pSB1C3-BBa_E0420 (2014 kit3 12G)
- [ ] pSB1A2-BBa_R0051 (2015 kit3 23F)

- Digestion

Table70

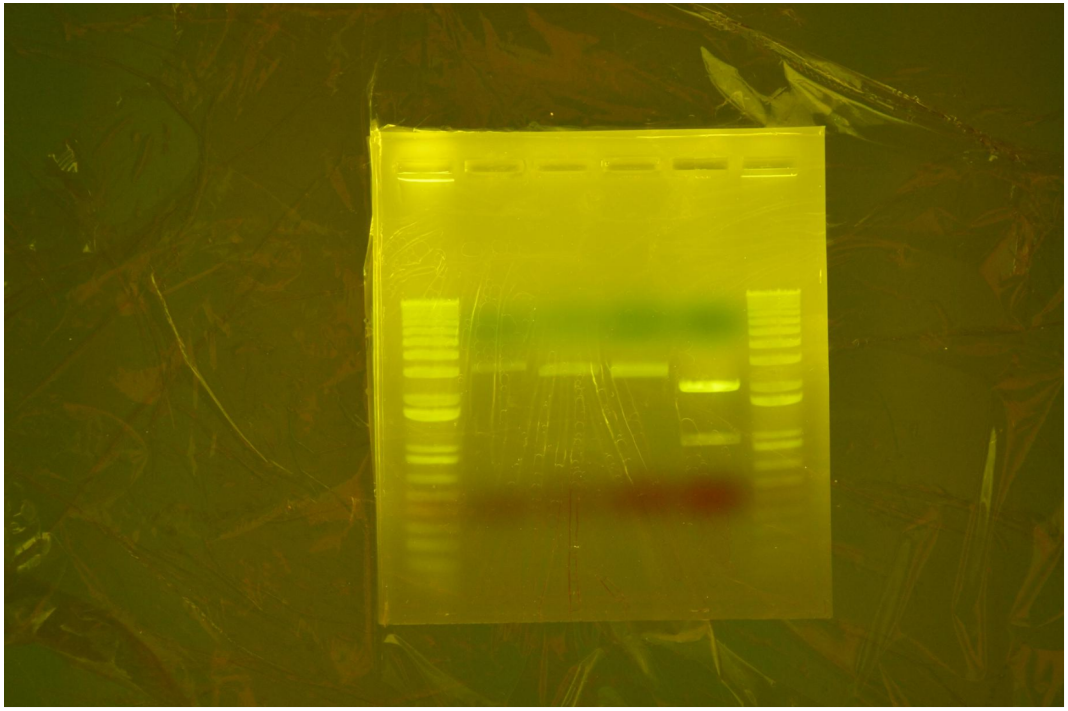
	Positive	Volume of DNA	ddH2O	Cutsmart	enzyme	Total volume (ul)
1	pSB3K3-BBa_E0240 (x,p)	40.19	4.21	5	0.3	55
2	pSB1C3-BBa_E0422 (x,p)	8.13	7.47	1.8	0.3	18

Table72

	Negative	Volume of DNA	ddH2O	Cutsmart	emzyme	Total Volume (ul)
1	pSB3K3-BBa_E0240 (x,p)	8.04	8.16	1.8	0	18
2	pSB1C3-BBa_E0422 (x,p)	1.63	14.57	1.8	0	18

20170713-Digestion

We recovered unsaved changes to your entry. Click here to recover this data.



1% agarose gel. stained with midor green. 130v, Run for 35 minutes.

*only E0422 shows correct bands

FRIDAY, 7/14/2017

- Miniprep

Table69

We recovered unsaved changes to your entry. Click here to recover this data.

	Plasmid	DNA Concentration	Protein Contamination	Salt Contamination
1	pSB1A2-BBa_R0051	19.10	1.891	0.864
2	pSB3K3-BBa_E0240	17.15	1.874	1.212
3	pSB3K3-BBa_E0240	25.80	1.870	0.928

- Digestion

Positive Sample

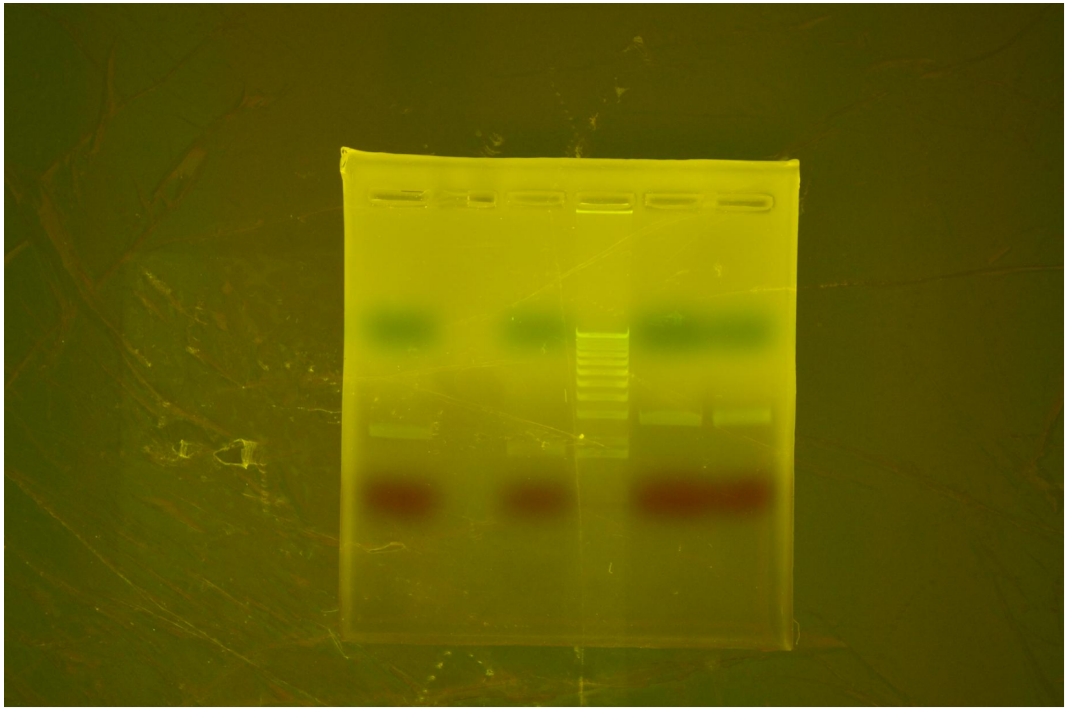
	Plasmid	DNA Volume	Enzyme 1	Enzyme 2	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1A2-BBa_R0051	26.18	0.2	0.2	5	18.42	50
2	pSB3K3-BBa_E0240	19.38	0.2	0.2	5	25.22	50

Negative controls

	Plasmid	DNA Volume	Enzyme 1	Enzyme 2	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1A2-BBa_R0051	5.24	0	0	1.8	10.96	18
2	pSB3K3-BBa_E0240	3.88	0	0	1.8	12.32	18

20170714 - Digestion

We recovered unsaved changes to your entry. Click here to recover this data.



1% agarose gel. stained with midor green. 130v, Run for 35 minutes.

- Gel Purification

Table71

	Plasmid	DNA Concentration	Protein Contamination	Salt Contamination	Cut Site
1	pSB1A2-BBa_R0051	3.131	2.768	1.469	S, P
2	BBa_E0240	0.306	-0.441	0.027	X, P
3	BBa_E0422	3.284	2.558	0.767	X, P
4	pSB1C3-BBa_R0040	8.480	2.437	1.134	S, P

- gBlock Amplification

MONDAY, 7/17/2017

- Digestion

Table74

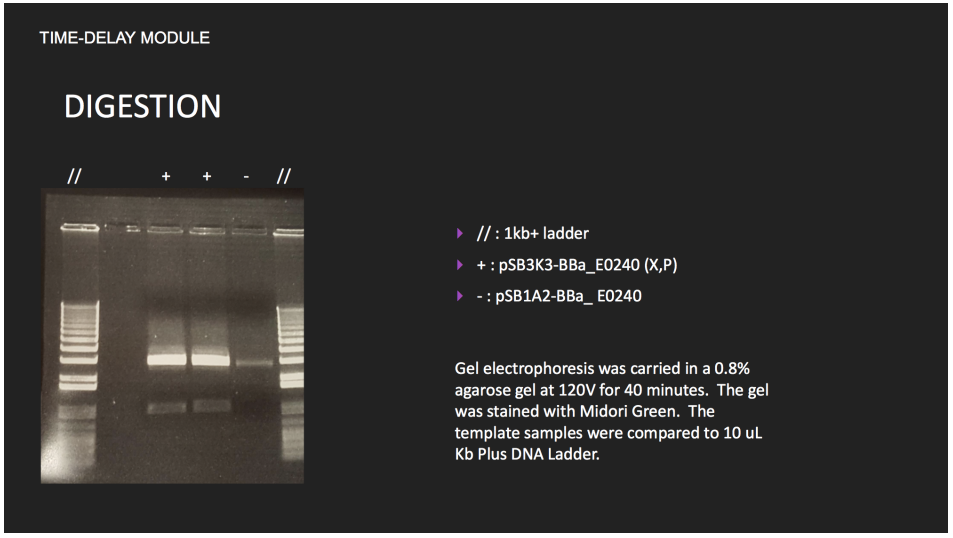
	Plasmid	DNA Volume	XbaI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB3K3-BBa_E0240	43.73	0.2	0.2	5	0.87	50

Table75

	Plasmid	DNA Volume	XbaI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB3K3-BBa_E0240	5.83	0	0	1.8	10.37	18

Screen Shot 2017-10-

We recovered unsaved changes to your entry. Click here to recover this data.



0.8% agarose gel. stained with midor green. Run for 40 minutes with 120v.

- Gel Purification

Table73

	Name	DNA Concentration	Protein Contamination	Salt Contamination	Cut Site
1	BBa_E0240	4.044	1.978	0.150	X, P

- Ligation
  - pSB1C3-BBa_R0040-E0422
  - pSB1C3-BBa_R0040-E0240

Positive Sample of Ligatic

	A	Buffer	T4 Ligase	Backbone	Insert	MQ
1	pSB1C3-BBa_R0040-E0422	1	0.5	1.90	6.60	0
2	pSB1C3-BBa_R0040-E0240	1	0.5	2.30	6.20	0

Negative Control fo Ligation 17/7/2017

	A	Buffer	T4 Ligase	Backbone	Insert	MQ
1	pSB1C3-BBa_R0040-E0422	1	0	1.90	6.60	0.5
2	pSB1C3-BBa_R0040-E0240	1	0	2.30	6.20	0.5

Ligation of R0040-E0422 shows no result while R0040- E0240 (GFP) shows

- Spread plate
  - pSB1C3-BBa_R0040-E0422
  - pSB1C3-BBa_R0040-E0422
    *Only pSB1C3-BBa_R0040-E0240 (promoter+GFP) shows fluorescence (checked under blue light & UV+microscope)
    *Possible solution: replace CFP with YFP / use another promoter to test CFP efficiency

TUESDAY, 7/18/2017

- Backbone (pSB1C3) amplification

Table76

	A	B	C
1	**COMPONENT**	**125 µl REACTION master mix**	**FINAL CONCENTRATION**
2	5X Q5 Reaction Buffer	25	1X
3	10 mM dNTPs	2.5	200 µM
4	10 µM Forward Primer	6.25	0.5 µM
5	10 µM Reverse Primer	6.25	0.5 µM
6	Template DNA	5	< 1,000 ng
7	Q5 High-Fidelity DNA Polymerase	1.25	0.02 U/µl
8	5X Q5 High GC Enhancer	25	(1X)
9	Nuclease-Free Water	53.75	

Table77

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	95°C	30 seconds
3	30 Cycles	95°C, 68-72°C, 72°C	15 seconds, 30 seconds, 1 min 10 seconds
4	Final Extension	72°C	3 minutes
5	Hold	12 °C	

Screen Shot 2017-10-

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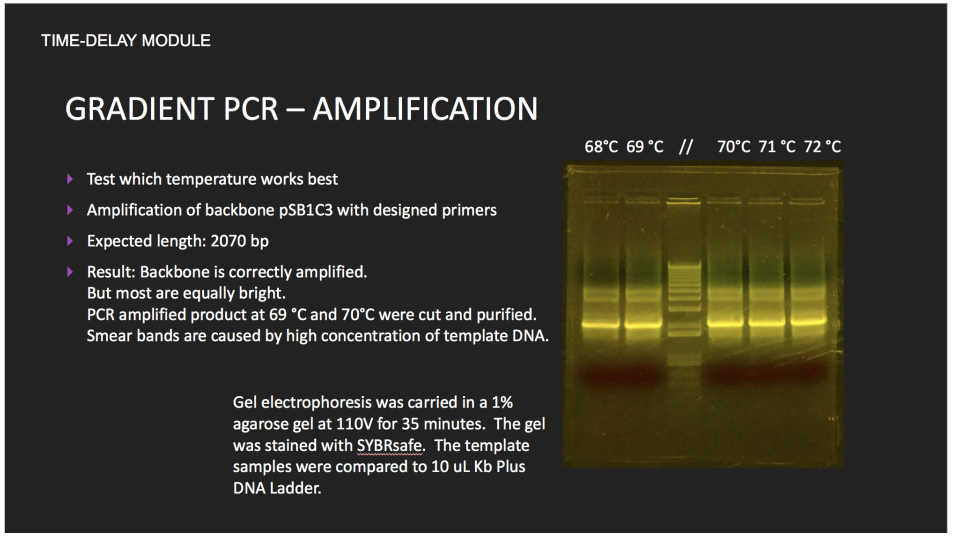


1% gel, 110 V, 35 minutes, stained with SYBRsafe
*store 69°C and 70°C samples in Time module Box2 (Sample 2 & 3 counting from left)
*redo backbone amplification tomorrow with lower concentraton [dilute 100X] of template to prevent smear bands

- Innoculation
  - pSB1C3-BBa_E0432
  - pSB1C3-BBa_E0430
- Streak plate
  - pSB1C3-BBa_E0430

WEDNESDAY, 7/19/2017

- Miniprep

Table78

We recovered unsaved changes to your entry. Click here to recover this data.

	A	DNA conc.	Protein	Salt
1	pSB1C3-BBa_E0432 (1)	141.9	1.845	2.090
2	pSB1C3-BBa_E0432 (2)	98.47	1.808	1.551
3	pSB1C3-BBa_E0430 (1)	127.8	1.831	1.780
4	pSB1C3-BBa_E0430 (2)	184.8	1.834	1.683

- pSB1C3 Backbone amplification

Table79

	A	B	C
1	**COMPONENT**	**125 µl REACTION master mix**	**FINAL CONCENTRATION**
2	5X Q5 Reaction Buffer	25	1X
3	10 mM dNTPs	2.5	200 µM
4	10 µM Forward Primer	6.25	0.5 µM
5	10 µM Reverse Primer	6.25	0.5 µM
6	Template DNA	5	< 1,000 ng
7	Q5 High-Fidelity DNA Polymerase	1.25	0.02 U/µl
8	5X Q5 High GC Enhancer	25	(1X)
9	ddH2O	53.75	

Table80

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	95°C	30 seconds
3	30 Cycles	95°C, 68-72°C, 72°C	15 seconds, 30 seconds, 1 min 10 seconds
4	Final Extension	72°C	3 minutes
5	Hold	12 °C	

- Digestion

Table81

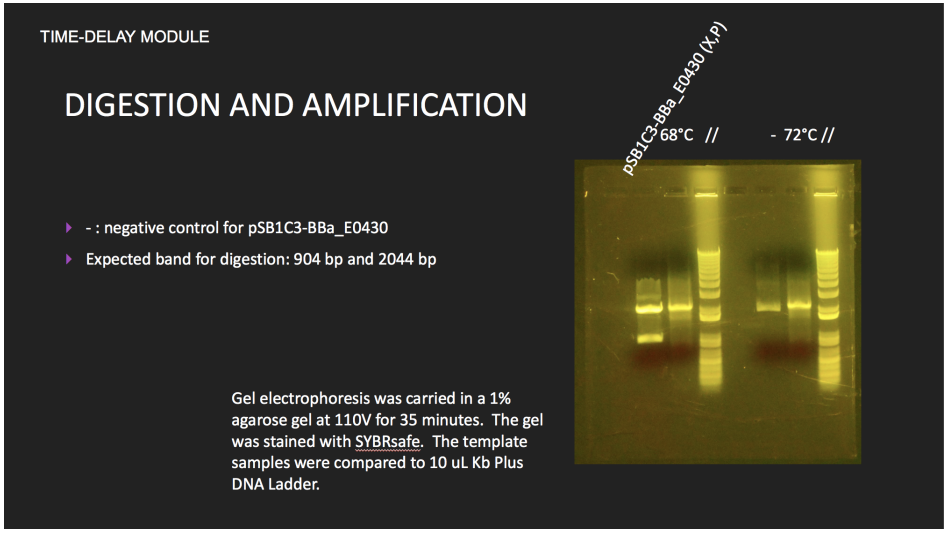
	Plasmid	DNA Volume	XbaI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_E0430	5.41	0.3	0.3	1.8	10.19	18

Table82

	Plasmid	DNA Volume	XbaI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_E0430	0.54	0	0	1.8	15.66	18

Screen Shot 2017-10-

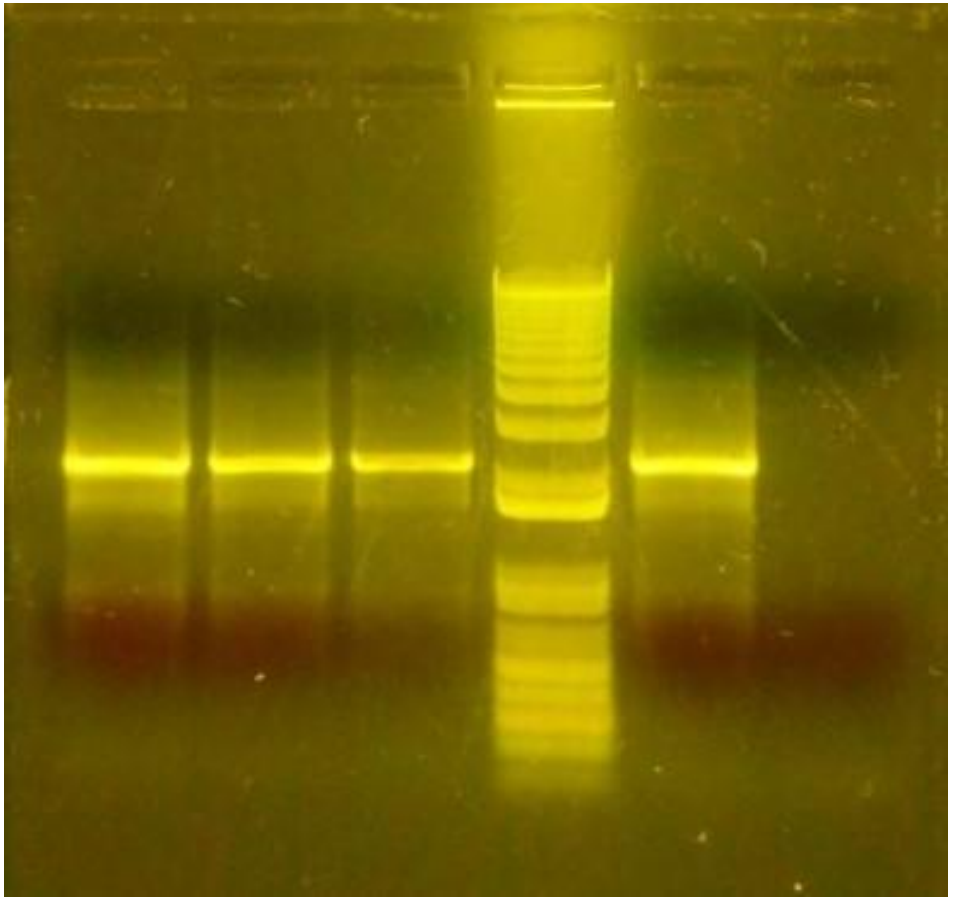
We recovered unsaved changes to your entry. Click here to recover this data.



1% gel, 110V, 35 minutes, stained with SYBRsafe

- Gel purification
    - pSB1C3

WhatsApp Image 20



1% gel, 110 V, 35 minutes, stained with SYBRsafe

- Ligation pSB1A2-BBa_R0051-E0240 to test pcI based on the GFP
  - R0051 expected band 2110 bp
  - E0240 is 902 bp
  - Result: the negative plate shows 4 cells (50%) with GFP. Suspected that the insert and vector ligate by chance (rare) But the streak plate of pSB3k3-BBa_E0240 alone (plate of 12/7) that was used for this ligation with digestion conc of 4.044 (17/7) does not show GFP.

- Gel purification
  - pSB1C3-BBa_E0430

- pSB1C3(72°C) *

THURSDAY, 7/20/2017

- Transformation

Table84

	A	DNA volume	igem kit plate	Well
1	pSB1C3-BBa_E0422		2016 distribution iGEM kit plate 2	6P
2				

- Gel purification
  - pSB1C3(70°C)-12ul elution buffer to increase concentration

- Ligation

Table83

	A	Buffer	T4 ligase	Backbone	Insert	MQ
1	pSB1C3-BBa_R0040-E0430	1	0.5	4.05	4.45	0.5

Result: E0430 successfully shows YFP
*use a constant promoter to check YFP (E0430)

- Transformation
  - pSB1C3-BBa_E0422

Table89

	A	B	C
1	6P	2016 Kit Plate 2	pSB1C3

Result: Transformation fails.

- Transformation of K5
  - Incubate at room temperature
  - Result: K592009 shows colonies, J04450 does not

FRIDAY, 7/21/2017

- Digest BBa_R0051 (S,P)

Table51

	Plasmid	DNA Volume	XbaI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1A2-BBa_R0051 (200 ng) (old) 14/7	10.47	0.1	0.1	1.8	5.53	20

Expected band: 2110 bp

- Redo Restriction Test for pSB1C3-BBa_R0062-P0151

Table53

	Plasmid	DNA Volume	HindIII-HF	10x Cutsmart Buffer	MQ
1	pSB1C3-BBa_R0062-P0151	3.53	0.2	1.8	12.47

Expected band: 3074 bp

- Digestion of pSB1A2-BBa_C0051 (Box 2) (547.5 ug/ml)

Table52

We recovered unsaved changes to your entry. Click here to recover this data.

	Plasmid	DNA Volume	XbaI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1A2-BBa_C0051 (547.5 ug/ml)	0.91	0.2	0.2	1.8	14.89	20

Expected band: 801 bp

Result:

Screen Shot 2017-10-31 at 3.57.32 PM.png

- Gibson assembly (for full construct)

Table26

	Positive	Volume(ul)
1	Backbone	0.759
2	construct 1	1.38
3	construct 2	2.27
4	ddH2O	0.59

Table34

	Negative	Volume(ul)
1	Backbone	0.2
2	ddH2O	4.8

Table35

	A	B	C
1	GA MX	15	*add the template mix directly to the tube filled with MX

1. Flick the tube several times, and centrifuge to collect the sample at the bottom of the tube.
2. Add template to GA MX**
3. Incubate at 50°C for one hour. *
4. Transform using 10uL reaction products.

**pipette up and down for 30times to mix thoroughly, the MX is viscous
*Pre-heat the machine for extra 5min to start polymerisation reaction before the exonuclease activativity digested the linear fragment.

- PCR (fragments amplification)

Table87

We recovered unsaved changes to your entry. Click here to recover this data.

	Box	column	row	D	E	F	G	H	I	J
1	1	9	C	BBa_K592009	amilCP, blue chromoprotein	pSB1C3	2013	1	19	E
2	3	1	F	BBa_J04450		pSB1C3	2014	2	24	O

Primers

Table88

	A	Box	Position	D
1	Trevor2013	2	8I	GA-amilCPF
2	Trevor2013	2	9I	GA-amilCPR
3	Trevor2013	3	1A	GA-BBF
4	Trevor2013	3	2A	GA-BBR

BBa_K592009

Table85

	A	B	C
1	**COMPONENT**	**75 µl REACTION master mix**	**FINAL CONCENTRATION**
2	5X Q5 Reaction Buffer	15	1X
3	10 mM dNTPs	1.5	200 µM
4	10 µM Forward Primer (GA-amilCPF)	3.75	0.5 µM
5	10 µM Reverse Primer (GA-amilCPR)	3.75	0.5 µM
6	Q5 High-Fidelity DNA Polymerase	0.75	0.02 U/µl
7	ddH2O	44.25	

Table86

	A	volume of saline with colony per tube(ul)
1	BBa_K592009	2
2	(-)	0

pSB1C3-BBa_J04450

Table91

	A	B
1	COMPONENT	25 µl REACTION
2	5X Q5 Reaction Buffer	5 µl
3	10 mM dNTPs	0.5 µl
4	10 µM Forward Primer (GA-BBF)	1.25 µl
5	10 µM Reverse Primer (GA-BBR)	1.25 µl
6	Q5 High-Fidelity DNA Polymerase	0.25 µl
7	Nuclease-Free Water	to 25 µl

Table90

	A	template volume
1	pSB1C3-BBa_J04450(without RFP coding gene)	?

- pcr clean-up

Table21

We recovered unsaved changes to your entry. Click here to recover this data.

	A	DNAconc.	Protein	Salt	length
1	BBa_K592009				669bp
2	pSB1C3-BBa_J04450(without RFP coding gene)				2433bp

MONDAY, 7/24/2017

- Colony PCR
- 10 colonies from plate*
- 2 +ve control
    - E0422 plasmid
    - E0422 colony
- 1 -ve control

*Colony of different size will be choosen, small colony is expected to contain insert

Table96

	Master mix	volume
1	MQ	198.4
2	5x My Taq buffer	57.2
3	10mM dNTP	7.15
4	10uM VF2	7.15
5	10uM VR	7.15
6	Taq Pol	1.8
7	Each tube	19.5

Table97

	A	Temperature	Time
1	Initial		
2	Denaturation		
3	Annealing		
4	Extension		
5	Final extenaion		
6	holding		

Table98

	A	Expected band	Plasmid length
1	Time module full construct	3214bp	4970

- Ligation
  - pSB1A2-BBa_R0051(S,P): 10.56
  - BBa_ E0430 (X,P): 9.953

Result:

- Gibson assembly
  - Testing assembly
    incubate at 50 degrees for 1 hr, infinite hold at 4 degree

Table27

	Positive	Volume(ul)
1	pSB1C3-BBa_J04450(without RFP coding gene)	0.314
2	BBa_K592009	0.22
3	ddH2O	4.464

- Digestion of C0051 (463.3 ug/ml)

500 ng

Table94

	Negative	DNA volume	XbaI	PstI	CutSmart	ddH2O	Total volume
1	pSB1C3-BBa_C0051 (x,p) (463.3 ug/ml)	1.08	0.2	0.2	1.8	14.72 (+) 15.12 (-)	18

Expected band size: 801 bp

- Digestion of C0051 (362.7 ug/ml)

500 ng

Table95

	Plasmid	DNA Volume	XbaI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1A2-BBa_C0051 (362.7 ug/ml)	1.38	0.2	0.2	1.8	14.42 (+) 14.82 (-)	18

Expected band size: 801 bp

- Digestion of pSB1C3-BBa_B0032 (62.15 ug/ml)

500 ng

Table50

We recovered unsaved changes to your entry. Click here to recover this data.

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_B0032 (62.15 ug/ml)	8.05	0.2	0.2	1.8	7.75 (+)	18

Expected band size: 2065 bp

100 ng

Table92

	Plasmid (-)	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_B0032 (62.15 ug/ml)	1.61	0	0	1.8	14.59	18

1%, 130 V, 40 min, SYBRsafe

Result: C0051 is not digested because of the buffer use. PstI is HF so it does not work best in Cutsmart but 3.1 (if I remember correct) but the negative sample is still weird

image.png

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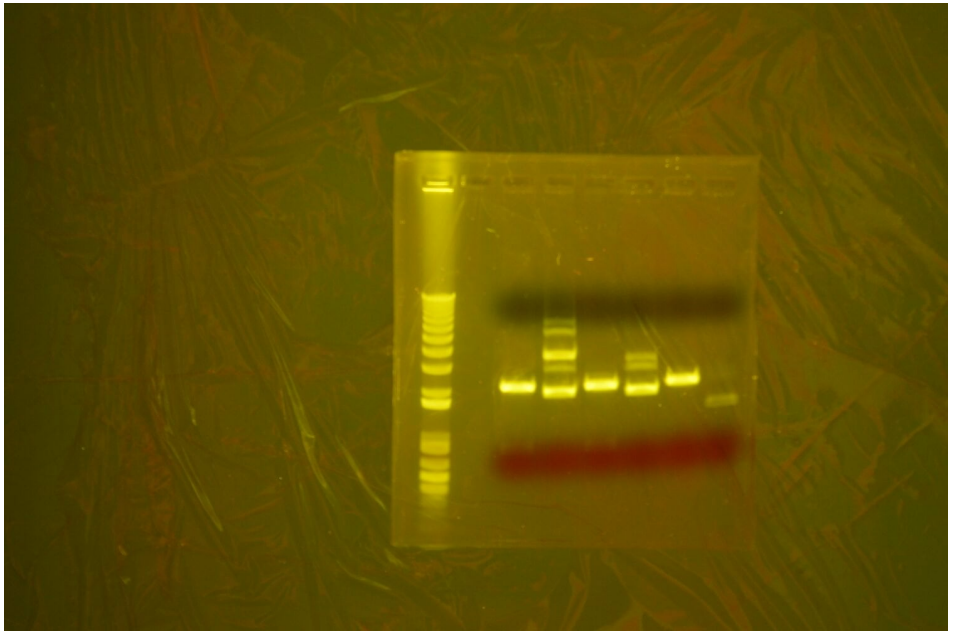

From left to right: C0051 (463.3 ug/ml) +, C0051 (463.3) -, C0051 (362.7 ug/ml) +, C0051 (362.7 ug/ml) - ,
B0032 +, B0032 -

- Innoculation and miniprep pSB1C3-BBa_C0051

TUESDAY, 7/25/2017

- Miniprep

Table105

	A	DNA conc.
1	pSB1C3-BBa_C0051 (1)	489.4
2	pSB1C3-BBa_C0051 (2)	417

- gel electrophoresis for colony PCR products
    - gel photo:

Table106

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	//	1	2	3	4	5	6	7		8	9	10	-1	-2	(-)	//

-1: pSB1C3-BBa_E0422 (colony); -2: pSB1C3-BBa_E0422 (plasmid)

image.png

- Clean up time module stock (the digested ones of June can be discarded)
- Digestion of
    - C0051 (489.4 ug/ml)

Table99

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_C0051(489.4 ug/ml)	1.02	0.2	0.2	1.8	14.78	18

500 ng +

Table100

We recovered unsaved changes to your entry. Click here to recover this data.

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_C0051(489.4 ug/ml)	0.2	0	0	1.8	1.8	18

100 ng -

- E0432 (146.9 ug/ml)

Table101

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_E0432 (146.9)	3.4	0.2	0.2	1.8	12.40	18

Table102

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_E0432 (146.9)	0.68	0	0	1.8	15.52	18

- R0051 (19.37 ug/ml)

Table103

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_R0051 (19.37)	15.49	0.2	0.2	1.8	0.51	18

Table104

We recovered unsaved changes to your entry. Click here to recover this data.

	Plasmid	DNA Volume	SpeI	PstI	10x Cutsmart Buffer	MQ	Total Volume
1	pSB1C3-BBa_R0051 (19.37)	5.16	0	0	1.8	11.04	18

image.png

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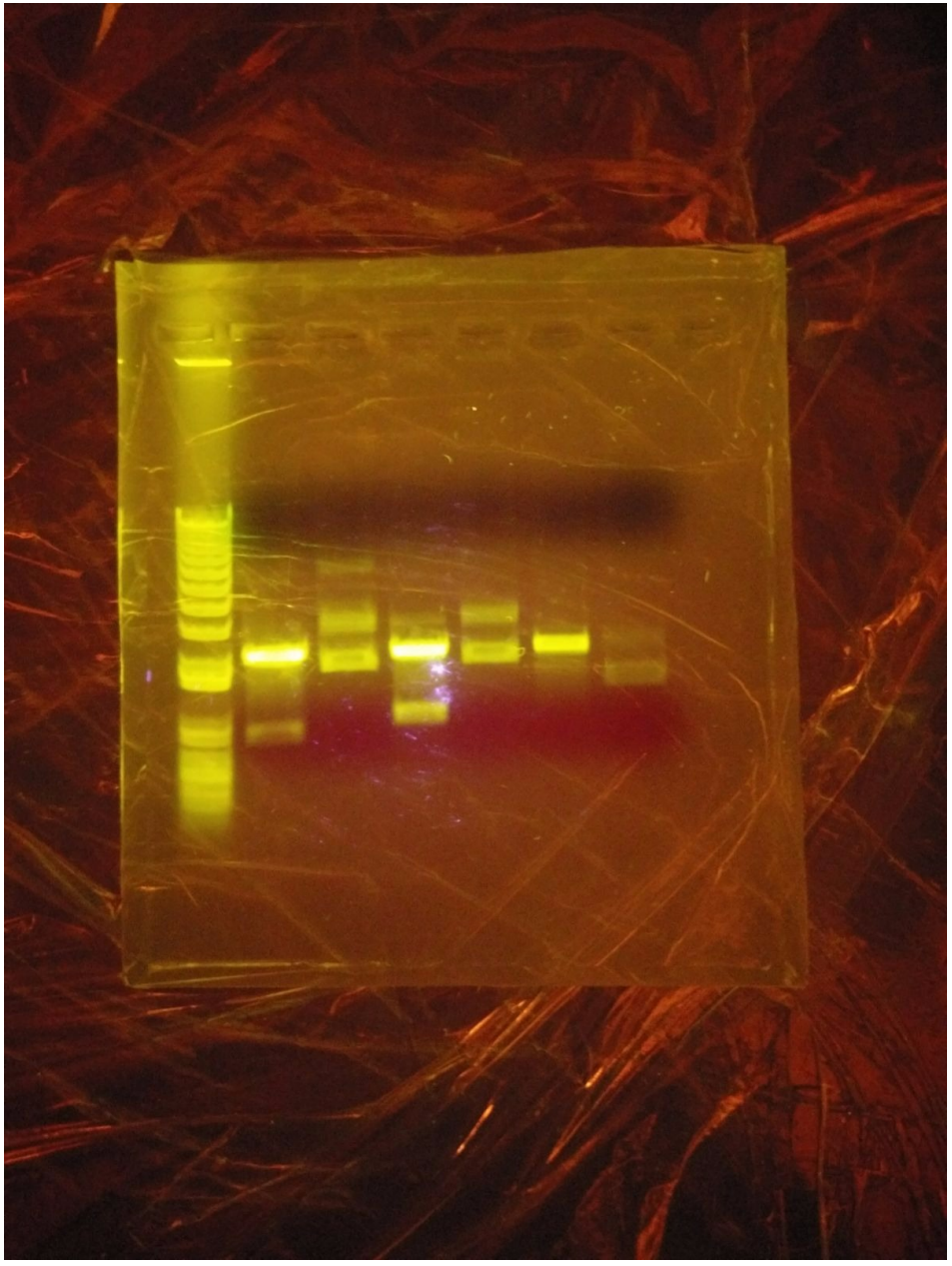

C0051+-, E0432+-,psb1c3-r0051+-

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image.png

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pSB1C3-BBa_T9002 (2kb,

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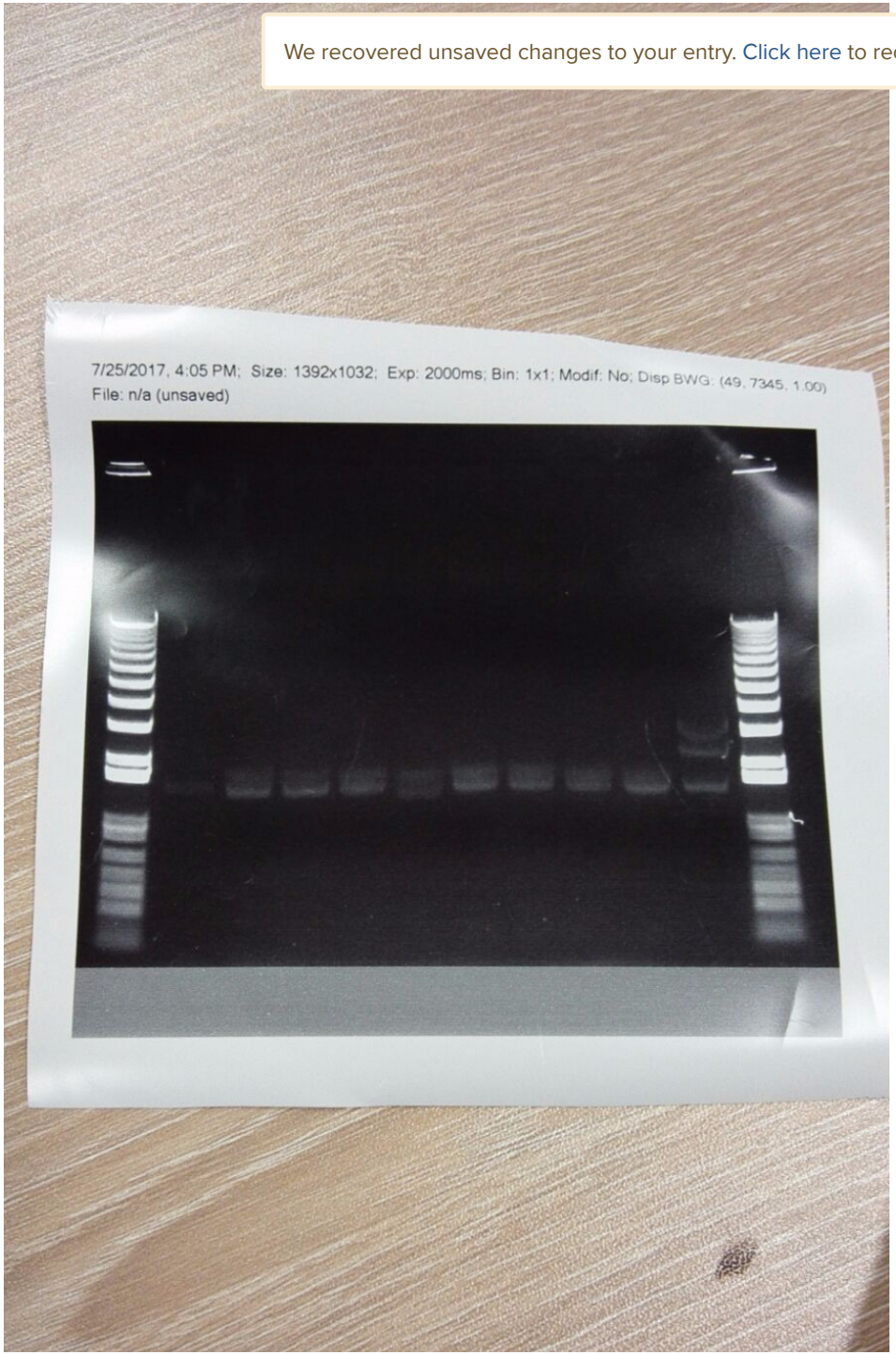
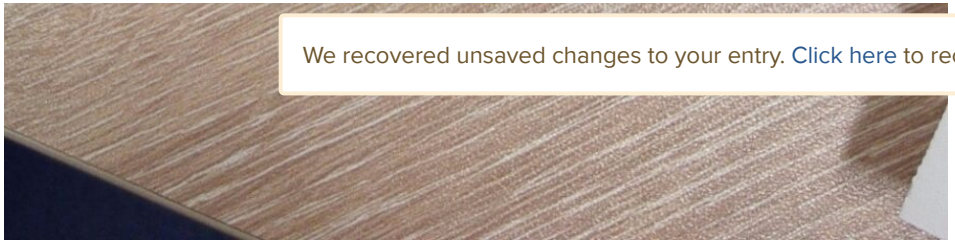




WEDNESDAY, 7/26/2017

- Gel purification

Table107

	A	DNA conc.	Protein	Salt
1	pSB1C3-BBa_C0051(x,p)	15.27	4.671	0.006
2	pSB1C3-BBa_E0432(x,p)	10.2	1.961	0.422
3	pSB1C3-BBa_R0051(s,p)	10.3	1.944	0.407

- Ligation of pSB1C3-BBa_R0051-E0432 and pSB1C3-BBa_B0032-C0051
- Digestion

Table111

	Plasmid	DNA Volume	SpeI HF	EcoI HF	10x Cutsmart Buffer	MQ	Total Volume	Expected band
1	pSB1C3-BBa_R0062-K081007 (E,S) (71.79 ug/ml)	6.96	0.2	0.2	1.8	8.84	18	882

500 ng

Final conc: 5.107

Table112

	Plasmid	DNA Volume	SpeI HF	EcoI HF	10x Cutsmart Buffer	MQ	Total Volume	Expected band
1	pSB1C3-BBa_R0062-K081007 (71.79 ug/ml)	1.39	0	0	1.8	14.81	18	882

100 ng

Table113

	Plasmid	DNA Volume	SpeI HF	EcoI HF	10x Cutsmart Buffer	MQ	Total Volume	Expected band
1	pSB1C3-BBa_R0062-S0109 (E,S) (47.53 ug/ml)	4.78	0.2	0.2	1.8	11.02	18	878

500 ng

Final conc: 7.427

Table114

	Plasmid	DNA Volume	SpeI HF	EcoI HF	10x Cutsmart Buffer	MQ	Total Volume	Expected band
1	pSB1C3-BBa_R0062-S0109 (E,S) (47.53 ug/ml)	0.96	0	0	1.8	15.24	18	878

100 ng

Table115

	Plasmid	DNA Volume	XbaI	EcoI HF	10x Cutsmart Buffer	MQ	Total Volume	Expected band
1	pSB1C3-BBa_B0015 (E,X) (47.53 ug/ml)	10.52	0.2	0.2	1.8	5.28	18	2184

500 ng

Final conc: 15.56

Table116

	Plasmid	DNA Volume	SpeI HF	EcoI HF	10x Cutsmart Buffer	MQ	Total Volume	Expected band
1	pSB1C3-BBa_B0015 (E,X) (47.53 ug/ml)	2.10	0	0	1.8	14.10	18	2184

100 ng

- Miniprep the Gibson Assembly colony (with full construct)

Table120

	A	DnA conc.	Protein	Salt
1	colony (S size)	51.85		
2	colony (M size)	47.15		
3	colony (L size)	58.31		

- Restriction test for Gibson Assembly colony (with full construct)
  - HindIII-HF: 0.2ul
  - expected bands: 1436bp, 3534bp

Table121

	positive	DNA volume	CutSmart	ddH2O
1	colony (S size)	9.64	1.8	6.16
2	colony (M size)	10.6	1.8	5.2
3	colony (L size)	8.57	1.8	7.23

500ng

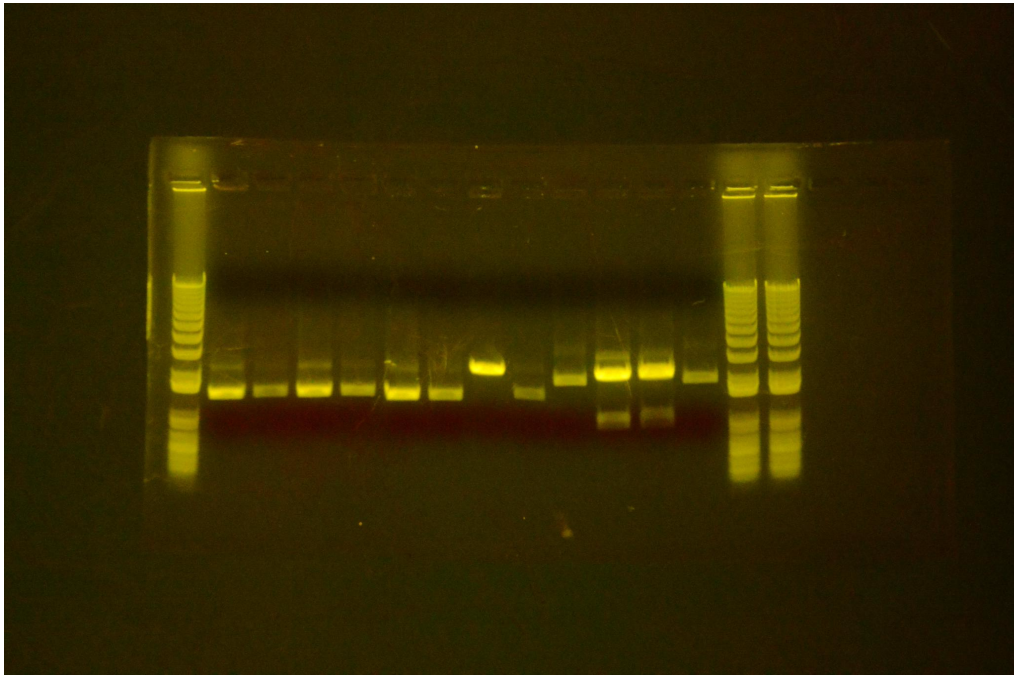
Table122

We recovered unsaved changes to your entry. Click here to recover this data.

	negative	DNA volume	CutSmart	ddH2O
1	colony (S size)	3.86	1.8	12.34
2	colony (M size)	4.24	1.8	11.96
3	colony (L size)	3.43	1.8	12.77

200ng

DSC_0248.jpg



colony(S size)+-, colony(M size)+-, colony (L size)+-, B0015+-, R0062-S0109-+, R0062-K081007+-

0.8%, 40ml gel, SYBR safe stain

- Gibson Assembly: T1, T2, pSB1C3 if no expected plasmid

THURSDAY, 7/27/2017

We recovered unsaved changes to your entry. Click here to recover this data.

- Take your tubes from
- Re-streak the ligated plate (7/7)

Table110

	A
1	pSB1C3-BBa_R0062-P0451
2	pSB1C3-BBa_R0062-K081007
3	pSB1C3-BBa_R0062-P0151
4	pSB1C3-BBa_R0062-S0109

- Ligation
  - pSB1C3-BBa_R0062-K081007-B0015
    - R0062-K081007: 1.81 ug/ml
    - B0015: 6.69 ug/ml
  - pSB1C3-BBa_R0062-S0109-B0015
    - R0062-S0109: 6.09 ug/ml
    - B0015: 2.4 ug/ml

- 2nd Gibson assembly (for full construct)
  - backbone : insert = 1:5

Table117

	Positive	Volume(ul)
1	Backbone	0.35
2	construct 1	1.592
3	construct 2	2.618
4	ddH2O	0.437

Around 6 ng backbone mass

Table118

We recovered unsaved changes to your entry. Click here to recover this data.

	Negative	Volume(ul)
1	Backbone	0.35
2	ddH2O	4.65

Around 6 ng backbone mass

Table119

	A	B	C
1	GA MX	15	*add the template mix directly to the tube filled with MX

2 Master mix
2 competent cell tubes

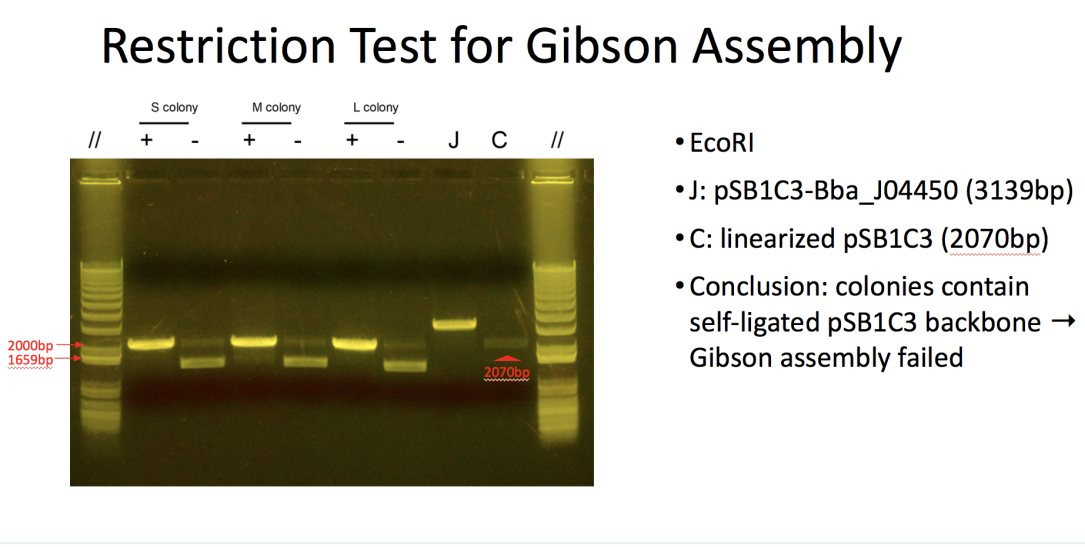
1. Add template to GA MX
2. pipette up and down for around 30 times**
3. Incubate at 50°C for one hour. *
4. Transform using 10uL reaction products.

**pipette up and down for 30times to mix thoroughly, because the MX is viscous
*Pre-heat the machine for extra 5min to start polymerisation reaction before the exonuclease activativity digested the linear fragment.

- 2nd Restriction test of GA (1st with 1:2 ratio)

Screen Shot 2017-10-

We recovered unsaved changes to your entry. Click here to recover this data.



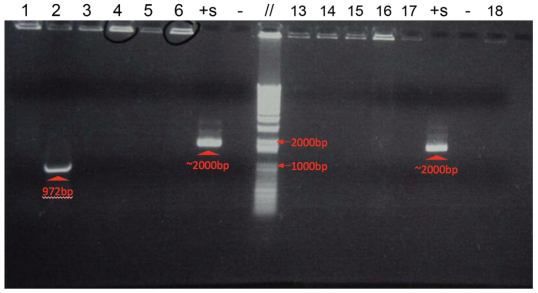
FRIDAY, 7/28/2017

- Transformation of pSB1C3-BBa_I763020 (GFP with LVA+)
  - 2015 Kit plate 3 12G
- Colony PCR of the ligated product
  - pSB1C3-BBa_B0032-C0051 (972 bp)
  - pSB1C3-BBa_R0062-K081007-B0015 (1310 bp)
    - R0062-K081007: 1173 bp
    - 1% gel
  - pSB1C3-BBa_R0062-S0109-B0015 (1306 bp)
    - R0062-S0109: 1169 bp
    - 0.8% gel

Screen Shot 2017-10-

We recovered unsaved changes to your entry. Click here to recover this data.

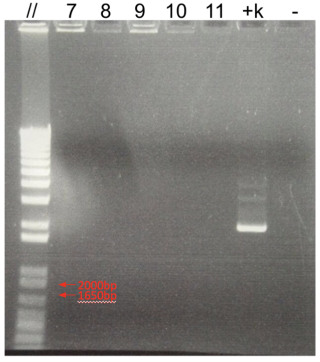
Colony PCR



0.8% gel, Midori Green, 130V, 30 mins

- 1-6: pSB1C3-BBa_B0032-C0051 (972 bp)
- 13-18: pSB1C3-BBa_R0062-S0109-B0015 (1306 bp)
- +s: pSB1C3-BBa_R0062-S0109 got from last ligation miniprep
  - Total length: 2925 bp
  - PCR's expected length: 1169 bp
- -: MQ

Screen Shot 2017-10-31 at 4.07.13 PM.png



1% gel, Midori Green, 130V, 30 mins

7-11: pSB1C3-BBa_R0062-K081007-B0015 (1310 bp)

+k: pSB1C3-BBa_R0062-K081007 got from last ligation miniprep

- +k's total length: 2929 bp
- +k's PCR expected length: 1173 bp
- -: MQ

Steps	Temperature (°C)	Time
Initial denaturation	95	3 min
Denaturation	95	30 s
Annealing	53	1 min
Extension	68	1 min 21 s (1350 bp)
Final extension	68	5 min
Holding (Storage Temperature)	4 to 12	infinity

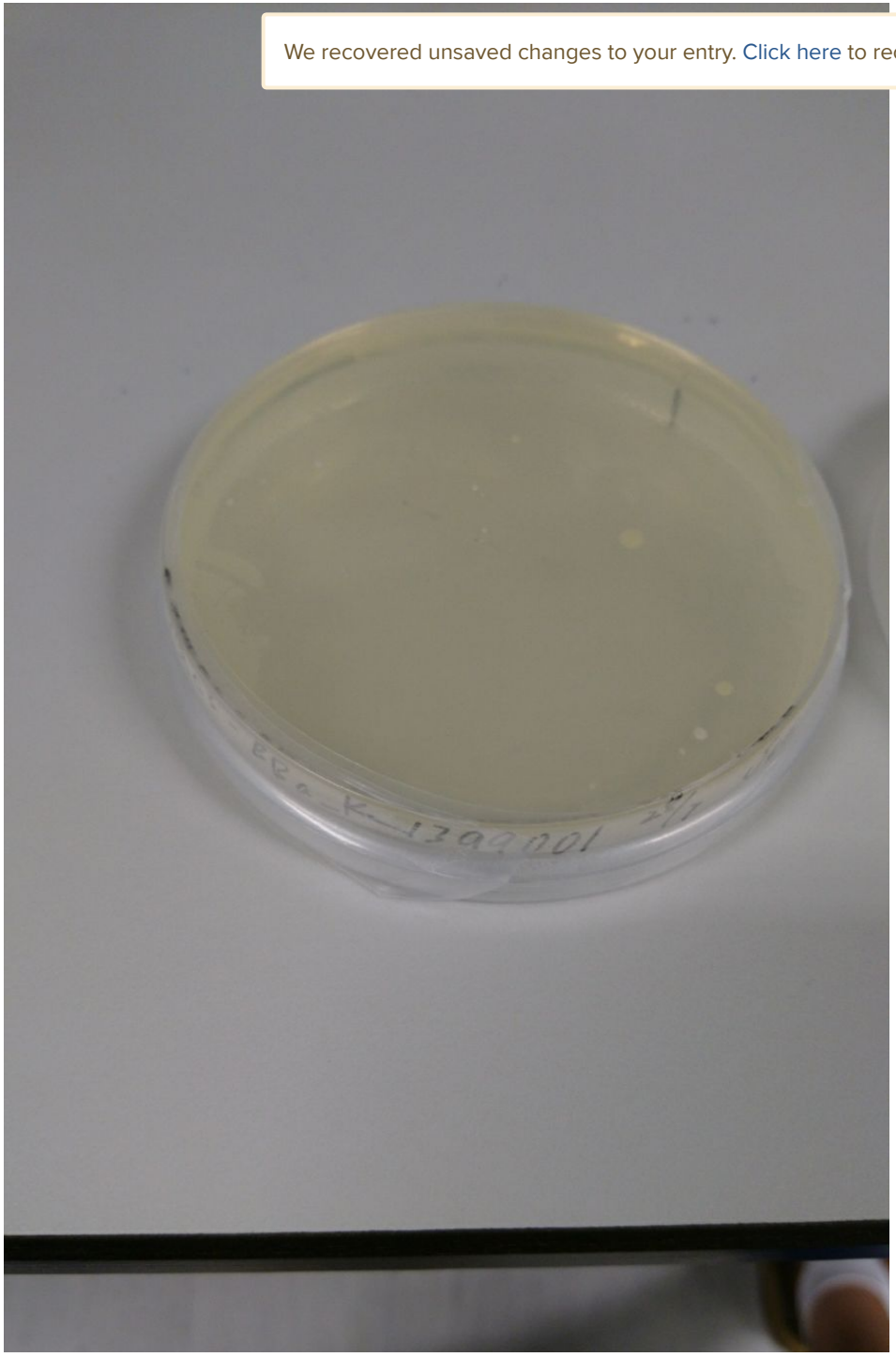
+k should have expected band of 1169 bp if pcr is successful. but we got 2000 bp while the actual template size is 2925 bp. So is it because of the circular plasmid template that makes the band looks 2000 bp? (notice the faded bands) so even the positive plasmid fails to amplify.

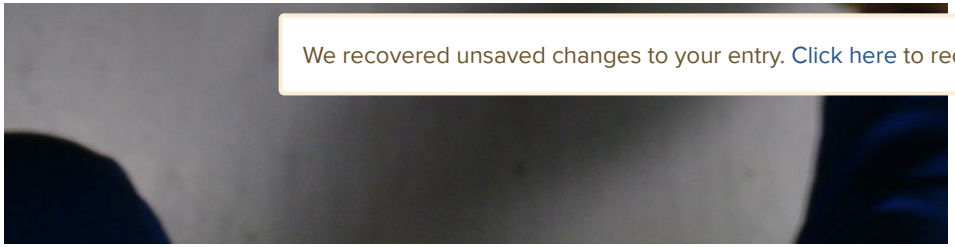
    - Gibson Assemb
- Master plate creation
- Transformation
    - pSB1C3-BBa_K1399001 RFP with LVA+ (2015 kit5 7B)

clipboard_2017-08-0

We recovered unsaved changes to your entry. Click here to recover this data.

We recovered unsaved changes to your entry. Click here to recover this data.





We recovered unsaved changes to your entry. Click here to recover this data.

Table108

	A	B	C
1	Initial denaturation	95C	3 min
2	Denaturation	95C	30s
3	Annealing	53C	1 min
4	Extension	68C	1 min 21 sec
5	Final extension	68C	5 min
6	Holding		infinity

Problem found: - Genome DNA stuck on the well. Possibility:

MONDAY, 7/31/2017

- Incubate and inoculate your pcr plates (pSB1C3-BBa_B0032-C0051 sample 2)
- pick another set of colonies (7-12) for colony pcr: Improve colony pcr
  - Flick the white tip after picking colonies for only few times. Don't scratch it around the tube's wall
  - Change annealing Temp to 55C
  - More time for extension time (at least 5 sec)
  - 10 uL master mix to save time and less genome stuck in the well?
  - Change positive control? increase initial denaturation?
  - Check if it's really vr and vf2 (NOT VR/Vf2 REV)

Table109

We recovered unsaved changes to your entry. Click here to recover this data.

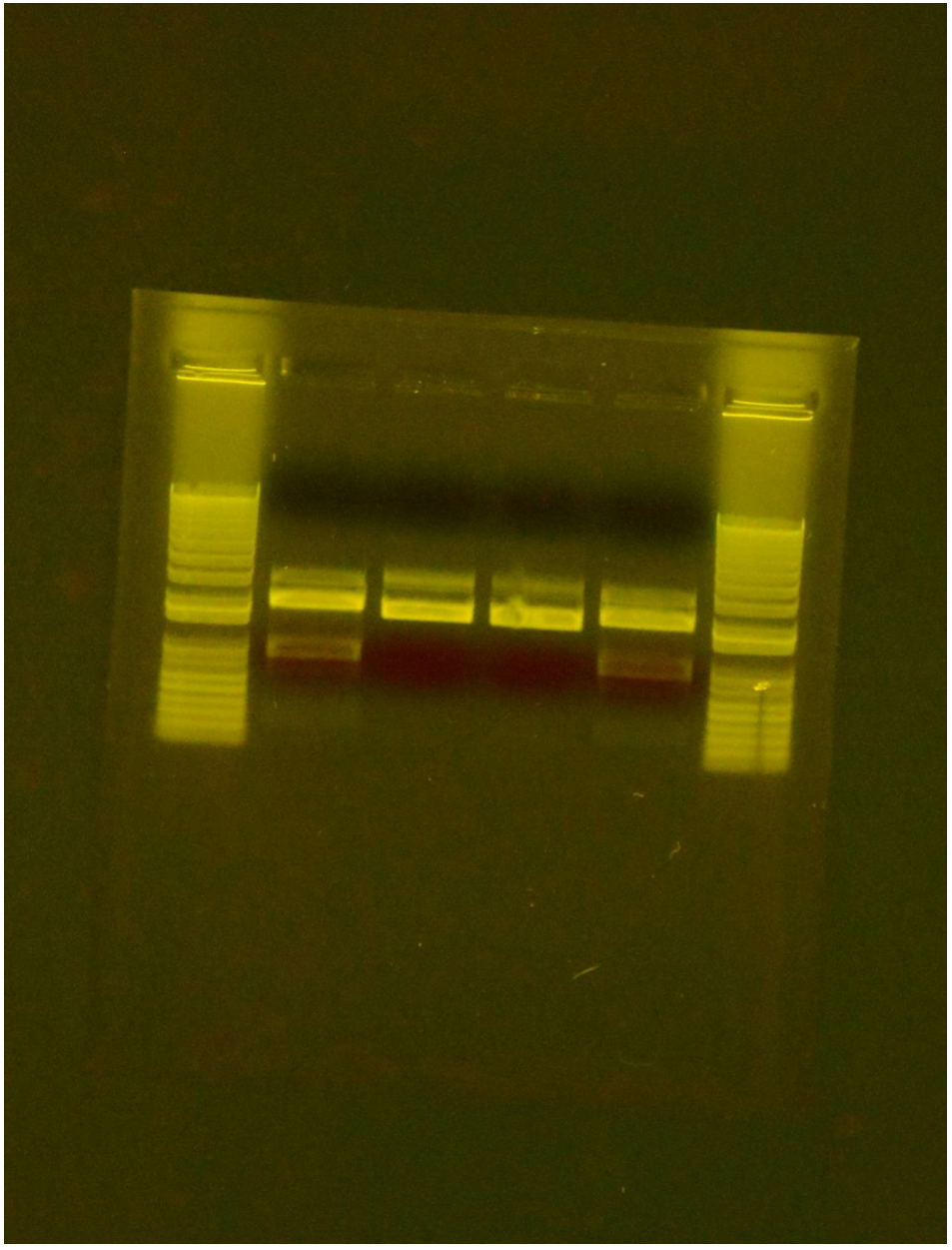
	A	B	C
1	Initial denaturation	95C	4 min
2	Denaturation	95C	30s
3	Annealing	55C	45 s
4	Extension	68C	1.23 min
5	Final extension	68C	5 min
6	Holding	8 C	infinity

1% gel Midori green 130V 30 min

- Restriction check for R0062-K081007 (926 bp) and R0062-S0109 (922 bp)
    - HindIII, PvuII

clipboard_2017-08-0

We recovered unsaved changes to your entry. Click here to recover this data.



K +, - S-, +

- colony pcr for 2nd GA

- Colony Cracking of GA
  - Colony 1-9 of 2
  - plasmid of pSB1C3-BBa_B0034, pSB1C3-BBa_P0451, pSB1C3-BBa_T9002, pSB2K3-BBa_Q04510 (2kb, 3kb, 4kb, 5.4kb) in the last lane

TUESDAY, 8/1/2017

- Digestion of pSB1C3-BBa_R0062, pSB1C3-BBa_K1399001
- Find sth that can previously amplify by PCR (B0032?)
- KC: Loading 10 uL is too much.
- pLuxR + GFP
- Restriction Test of R0062-P0451, R0062-P0151
  - HindIII, PvuIII (923 bp, bp)
  - HindIII, EcoRI (558 bp, bp)
  - 0.5%/0.8% gel
- Restriction test of B0032-C0051
  - B0032 as negative
- pSB1C3-J04450 Run electrophoresis gel
  - i use this for GA testing
- Prepare pSB1C3

Table125

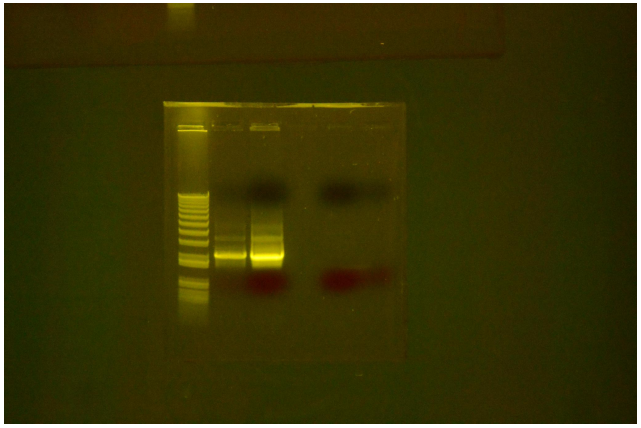
We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	**COMPONENT**	**125 µl REACTION master mix**	**FINAL CONCENTRATION**
2	5X Q5 Reaction Buffer	25	1X
3	10 mM dNTPs	2.5	200 µM
4	10 µM Forward Primer	6.25	0.5 µM
5	10 µM Reverse Primer	6.25	0.5 µM
6	Template DNA	5	< 1,000 ng
7	Q5 High-Fidelity DNA Polymerase	1.25	0.02 U/µl
8	5X Q5 High GC Enhancer	25	(1X)
9	ddH2O	53.75	

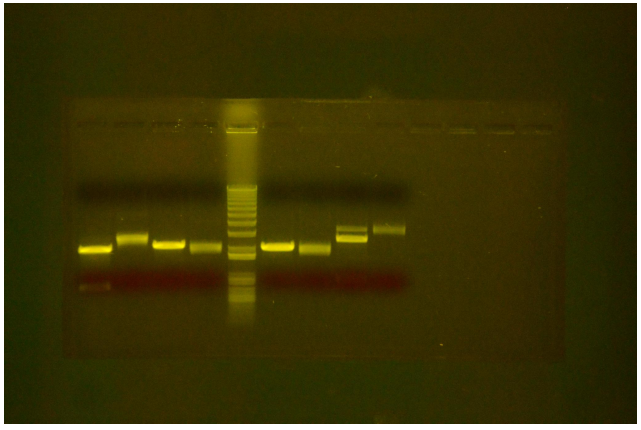
Table126

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	95°C	30 seconds
3	30 Cycles	95°C, 68-72°C, 72°C	15 seconds, 30 seconds, 1 min 10 seconds
4	Final Extension	72°C	3 minutes
5	Hold	12 °C	

DSC_0261.jpg



DSC_0262.jpg



WEDNESDAY, 8/2/2017

- Suggestion for standard assembly ligation
    - Increase chance for insert to bind with backbone? Decrease ligase.

- Gel Purification of pSB1C3

Excise the agarose gel with a clean scalpel.

• Remove the extra agarose

2. Transfer up to 300 mg of

• The maximum volume of the gel slice is 300mg.

3. Add 500 µl of FADF Buffer to the sample and mix by vortexing.

• For > 2% agarose gels, add 1000 µl of FADF Buffer.

4. Incubate at 55 °C for 5 ~10 minutes and vortex the tube every 2 ~ 3 minutes until the gel slice dissolved completely.

• During incubation, interval vortexing can accelerate the gel dissolved.

• Make sure that the gel slice has been dissolved completely before proceed the next step.

• After gel dissolved, make sure that the color of sample mixture is yellow. If the color is violet, add 10 µl of sodium acetate, 3M, pH 5.0. Mix well to make the color of sample mixture turned to yellow.

5. Cool down the sample mixture to room temperature. And place a FADF Column into a Collection Tube.

6. Transfer 800 µl of the sample mixture to the FADF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.

• If the sample mixture is more than 800 µl, repeat this step for the rest of the sample mixture.

7. Add 750 µl of Wash Buffer (ethanol added) to the FADF Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.

• Make sure that ethanol (96-100 %) has been added into Wash Buffer when first use.

8. Centrifuge again at full speed (~ 18,000 x g) for an additional 3 minutes to dry the column matrix. • Important step ! The residual liquid should be removed thoroughly on this step.

9. Place the FADF Column to a new microcentrifuge tube (not provided).

10. Add 40 µl of Elution Buffer or ddH2O to the membrane center of the FADF Column. Stand the FADF Column for 1 min.

• Important step ! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.

• Important : Do not elute the DNA using less than suggested volume (40 µl). It will lower the final yield. 11. Centrifuge at full speed (~ 18,000 x g) for 1 min to elute the DNA.

- Amplification of control insert: pCI+E0240 for Gibson assembly
  - FWD RFC10 and REV T2

Table129

	A	B	C	D	E	F	G
1	**Year**	**Box**	**Column**	**Row**	**Code**	**Name**	**Sequence (5′ to 3′)**
2	2016	5	1	H		Prefix FWD	GAATTCGCGGC CGCTTCTAGAG

  - Annealing Temp: 72 (neb), 68, 70 and 72
  - Length: 966bp

Table127

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1		25 µl REACTION	No of REACTION
2			4
3	5X Q5 Reaction Buffer	5	20
4	10 mM dNTPs	0.5	2
5	10 µM Forward Primer	1.25	5
6	10 µM Reverse Primer	1.25	5
7	Template DNA	0.5	2
8	Q5 High-Fidelity DNA Polymerase	0.25	1
9	5X Q5 High GC Enhancer (optional)	5	20
10	Nuclease-Free Water	11.25	45

Table128

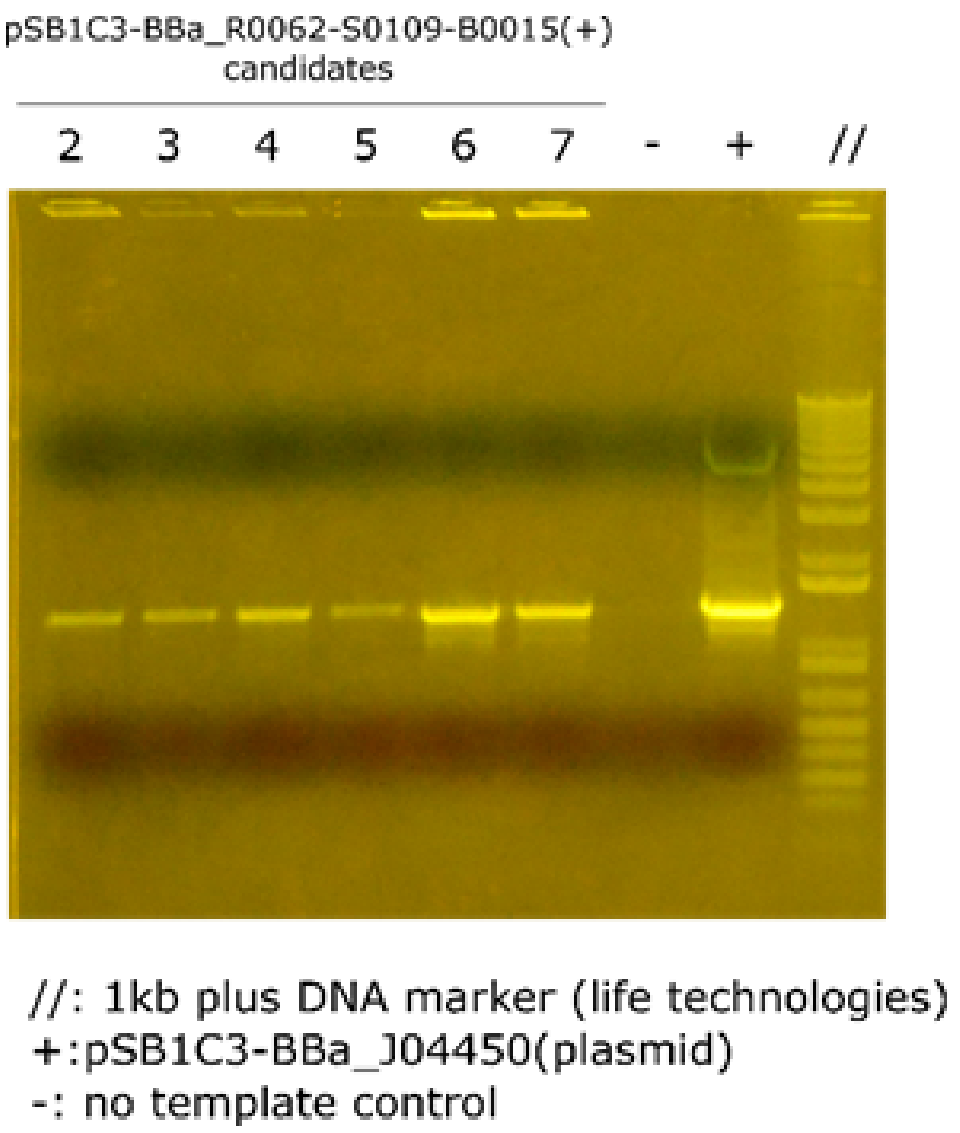
	A	B	C	D
1	STEP	TEMP	TIME	
2	Initial Denaturation	95°C	30 seconds	
3	25–35 Cycles	95°C	15 seconds	
4		68°C	30 seconds	30X
5		72°C	29s	
6	Final Extension	72°C	3 minutes	
7	Hold	8°C	inf.	

- Eric's PCR result
  - R0062-S0109-B0015: sample 5 is picked and miniprep

- R0062-K08100

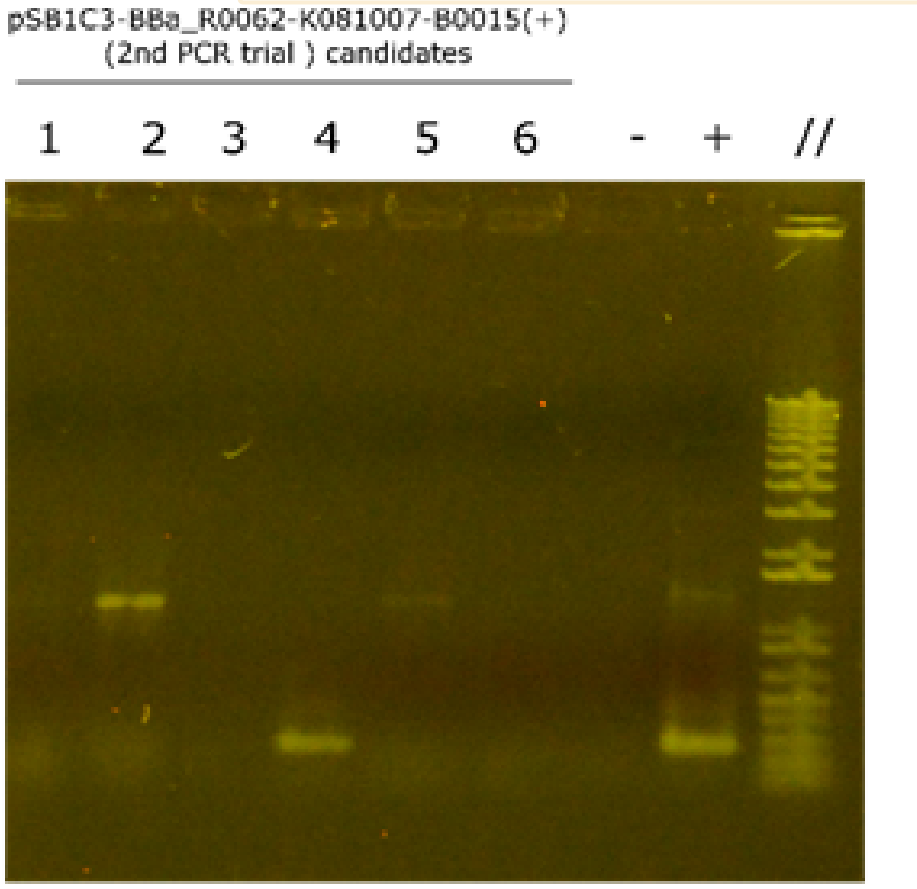
We recovered unsaved changes to your entry. Click here to recover this data.

image.png



0.8%
7 V/cm

image.png



//: 1kb plus DNA marker (life technologies)

+: pSB1C3-BBa_J04450 plasmid

-: No template control

Mastermix Recipe

Table93

We recovered unsaved changes to your entry. Click here to recover this data.

	A	x8
1	Water	98
2	Mytaq 5X buffer	32
3	VR	5
4	VF2	5
5	Taq	1.5
6	Template	Dip of colony
7	Total volume	20

Table123

	A	B	C
1	Initial denaturation	95C	3 min
2	Denaturation	95C	15 s
3	Annealing	55C	15 s
4	Extension	68C	1 min 30 sec
5	Final extension	68C	3 min
6	Holding	10 C	infinity

THURSDAY, 8/3/2017

- 3rd Gibson assembly (for full construct)
    - backbone : insert = 1:3

Table130

We recovered unsaved changes to your entry. Click here to recover this data.

	Experimental	Volume(ul)	pmol
1	Backbone	0.4375	0.007
2	construct 1	1.592	0.021
3	construct 2	2.618	0.021
4	ddH2O	0.437	

Table131

	Negative	Volume(ul)
1	Backbone	0.4375
2	ddH2O	0.56

Around 6 ng backbone mass

Table132

	A	B	C
1	GA MX	15	*add the template mix directly to the tube filled with MX

2 Master mix
2 competent cell tubes

1. Add template to GA MX
2. pipette up and down for around 30 times**
3. Incubate at 60°C for one hour. *
4. Transform using All reaction products.
5. 50ul of competent cells

**pipette up and down for 30times to mix thoroughly, because the MX is viscous
*Pre-heat the machine for extra 5min to start polymerisation reaction before the exonuclease activativity digested the linear fragment.

- Gibson Assembly troubleshoot

- Since we tried 1 [illegible] from 50C to 60C, because it might be that [illegible] very strong.
- GC content of overhangging sq of pSB1C3 is around 60%

FRIDAY, 8/4/2017

Improvement of Colony PCR

Procedures:

- Picking:
  - Barely touch the colony
- Saline preparation:
  - Stir the tips in saline for 5s
- Transfering to Mastermix:
  - Excessive: Saline with 5ul Transfered
  - Regular: Saline with 2ul transfered
  - Reduced: Saline with 0.5ul transfered
  - Direct contact with mastermix for 2s without stiring or pippetting
  - Direct contact with mastermix for 5s without stiring or pippetting
  - Plasmid DNA: 0.5ul

Colony PCR of GA

- Expected size: 1279
- Primer
  - Time Construct 1 Reverse Primer
  - VF2
- extension 77s

- +ve :pSB1A2-BBa_R0062-P0451 (pluxR-1.0 RBS-cI-TT)
  - Expected size: 271bp

- Digestion of RFP

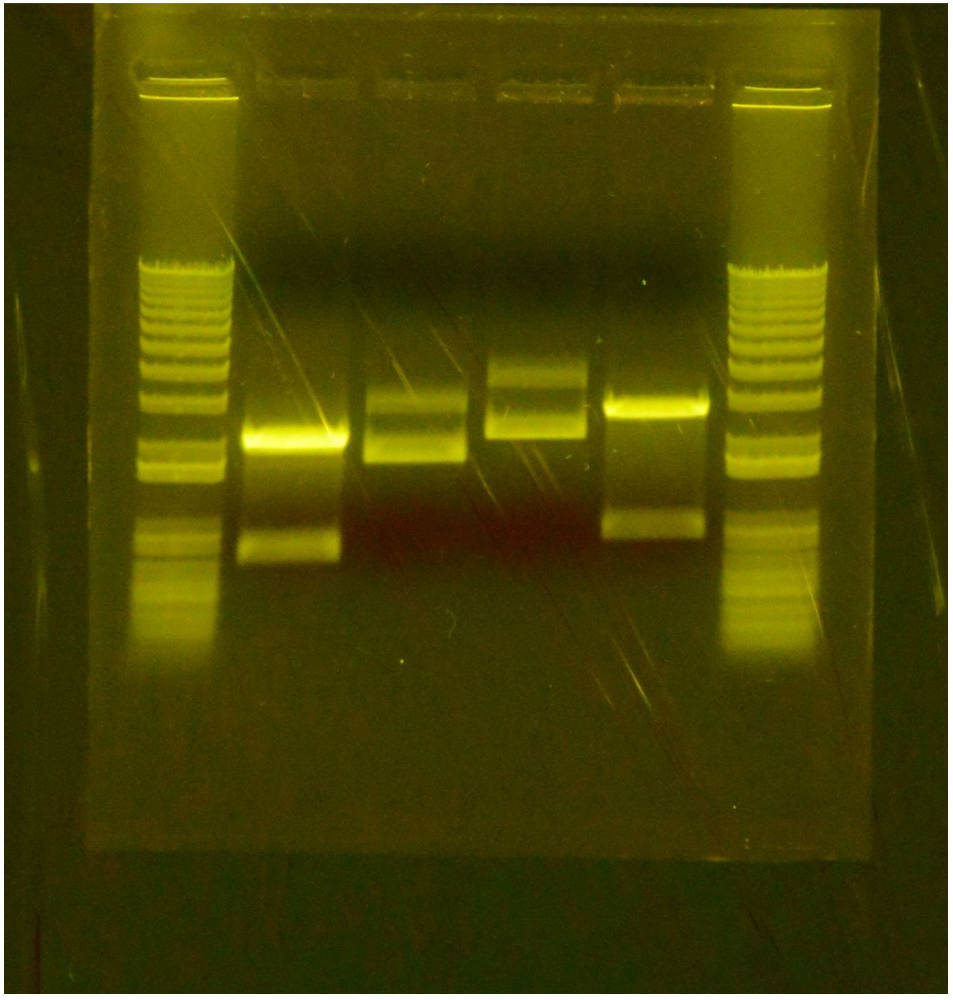
Table177

	Plasmid	DNA Volume	SpeI HF	EcoI HF	10x Cutsmart Buffer	MQ	Total Volume
1	RFP	8 .16	0 .3	0.3	1.8	1 0.11	18
2	RFP (-)	2 .63	0	0	1 .8	1 4.55	1 8

flowRoot3430.png

We recovered unsaved changes to your entry. Click here to recover this data.

	pSB1C3-BBa_K1399001		pSB1C3-BBa_E0430		
//	+	-	+	-	//



MONDAY, 8/7/2017

- Restriction Test of
  - pSB1C3-BBa_R
    - Expected band if there is an insert: 922 bp, 2140 bp (two bands)
    - If no insertion: Only pVull cuts the backbone. Expected size will be: 2199 bp linear single band
  - pSB1C3-BBa_R0062-K081007-B0015 (HindIII, PvuII)
    - Expected band if there is an insert: 926 bp, 2140 bp.
    - If no insertion: Expected size will be: 2199 bp linear single band

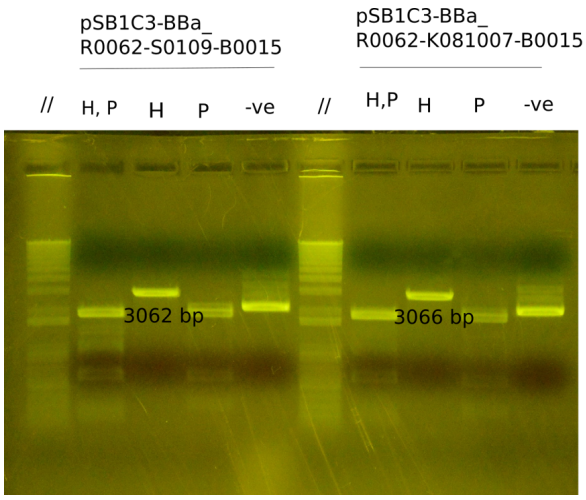
pSB1C3-BBa_R0062-S0109-B0015

	A	HandIII-HF, PvuII	HindIII-HF	PvuII	-ve
1	DNA	2.88 uL	2.88 uL	2.88 uL	2.88 uL
2	HindIII-HF	0.5 uL	0.5 uL	0 uL	0 uL
3	PvuII	0.5 uL	0 uL	0.5 uL	0 uL
4	10X Cutsmart buffer	1.8 uL	1.8 uL	1.8 uL	1.8 uL
5	MQ	12.3 uL	12.82 uL	12.82 uL	13.32 uL
6	Total	18 uL	18 uL	18 uL	18 uL

pSB1C3-BBa_R0062-K08

	A	HandIII-HF, PvuII	HindIII-HF	PvuII	-ve
1	DNA	4.3 uL	4.3 uL	4.3 uL	4.3 uL
2	HindIII-HF	0.5 uL	0.5 uL	0 uL	0 uL
3	PvuII	0.5 uL	0 uL	0.5 uL	0 uL
4	10X Cutsmart buffer	1.8 uL	1.8 uL	1.8 uL	1.8 uL
5	MQ	10.9 uL	11.4 uL	11.4 uL	13.32 uL
6	Total	18 uL	18 uL	18 uL	18 uL

flowRoot3512.png



H = HindII-HF

P = PvuII

-ve = No enzymes

Expected band:
pSB1C3-BBa_R0062-S0109-B0015: 2140 bp, 922 bp
pSB1C3-BBa_R0062-K081007-B0015: 2140 bp, 926 bp
If no insertion, there will be 2199 bp band shows
when cutted by single enzyme

Result:
The band cut with HindII-HF got expected band
PvuII causes star activity
Restriction test cannot be concluded although results shows that
the linear DNA band cut by HindIII-HF is around 3000 bp (not ~2200 bp)

- Streak and re-inoculate R0062-K081007-B0015
- Inoculate the pSB1C3-BBa_B0032-C0051 (sample 2 from fridge)

- Gel purification of RFP (K1399001)
- Ligation
    - pSB1C3-BBa_K1399001-B0015 (RFP with terminator)

We recovered unsaved changes to your entry. Click here to recover this data.

Table133

	+	B	–	D
1	Ligase	0.5	MQ	0.5
2	T4 ligase buffer	1	T4 ligase buffer	1
3	Backbone	3.86	Backbone	3.86
4	Insert	4.64	Insert	4.64
5	Total	10	Total	10

- Screening GA product
  - Colony PCR of 10-20

Table124

	A	B	C	D
1	Reagents (ul)	Volume (ul)	MasterMix (*x)	1.1
2	MQ	13.875	166.5	183.15
3	5X MyTaq Reaction Buffer	4	48	52.8
4	10 mM dNTP	0.5	6	6.6
5	10 um VF2	0.5	6	6.6
6	10 uM VR	0.5	6	6.6
7	Taq Polymerase	0.125	1.5	1.65
8	Template	0.5	6	6.6
9	Total	20	240	264

Table137

	A	B	C
1	Colony PCR		
2	Steps	Temperature (°C)	Time
3	Initial denaturation	95	3 min
4	Denaturation	95	30 s
5	Annealing	53	45 s
6	Extension	68	1 min 22 s
7	Final extension	68	5 min
8	Holding	8	infinity

    - Colony Cracking of 1-9

TUESDAY, 8/8/2017

- Miniprep the pSB1C3-BBa_B0032-C0051 (sample number 2 from last PCR) and
- Miniprep the pSB1C3-BBa_R0062-K081007-B0015

- Restriction test of
    - pSB1C3-BBa_R0062-S0109-B0015 (All enzymes use cutsmart)
        - Expected band (HindIII, PvuII-HF): 922 bp, 2140 bp (two bands)
        - If no insertion: Only pVull cuts the backbone. Expected size will be: 2199 bp linear single band
        - Expected band (EcoRI-HF, PstI-HF): 2029 bp, 1033 bp

    - pSB1C3-BBa_R0062-K081007-B0015 (All enzymes use cutsmart)
        - Expected band if there is an insert: 926 bp, 2140 bp.
        - If no insertion: Expected size will be: 2199 bp linear single band
        - Expected band (EcoRI-HF, PstI-HF): 2029 bp, 1037 bp

Table134

We recovered unsaved changes to your entry. Click here to recover this data.

	A	HandIII-HF, PvuII-HF	HindIII-HF	PvuII-HF	EcoRI-HF , PstI	-ve
1	DNA	5.43 uL	5.43 uL	5.43 uL	5.43 uL	5.43 uL
2	HindIII-HF	0.2 uL	0.2 uL	0 uL	0.2 uL	0 uL
3	PvuII-HF	0.2 uL	0 uL	0.2 uL	0.2 uL	0 uL
4	10X Cutsmart buffer	1.8 uL	1.8 uL	1.8 uL	1.8 uL	1.8 uL
5	MQ	10.37 uL	10.57 uL	10.57 uL	10.37 uL	10.77 uL
6	Total	18 uL	18 uL	18 uL	18 uL	18 uL

Use 0.8% gel

Table135

	A	HandIII-HF, PvuII-HF	HindIII-HF	PvuII-HF	EcoRI-HF , PstI	-ve
1	DNA	4.13 uL	4.13 uL	4.13 uL	4.13 uL	4.13 uL
2	HindIII-HF	0.2 uL	0.2 uL	0 uL	0.2 uL	0 uL
3	PvuII-HF	0.2 uL	0 uL	0.2 uL	0.2 uL	0 uL
4	10X Cutsmart buffer	1.8 uL	1.8 uL	1.8 uL	1.8 uL	1.8 uL
5	MQ	11.67 uL	11.87 uL	11.87 uL	11.67 uL	12.07 uL
6	Total	18 uL	18 uL	18 uL	18 uL	18 uL

Use 0.8% gel

- pSB1C3-BBa_B0032-C0051
  - Expected band if there is an insert (HindIII, PvuII-HF): 863 bp, 2003 bp
  - Expected band if there is an insert (EcoRI HF, PstI-HF): 837 bp
  - If no insertion: Only pVuII cuts the backbone. Expected size will be: 2083 bp linear single band
  - *Ask Eric if you should test it with H,P or E,P or all?

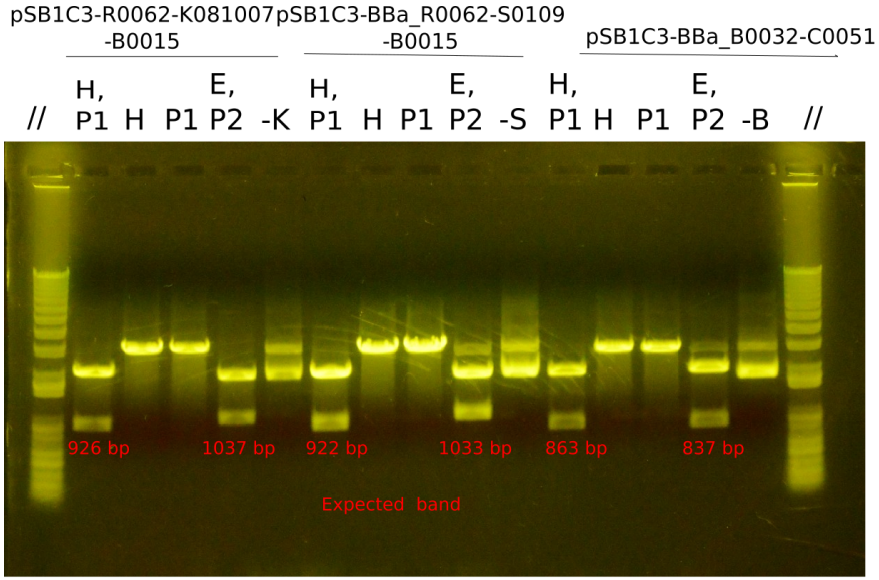
pSB1C3-BBa_B0032-C00

We recovered unsaved changes to your entry. Click here to recover this data.

	A	HandIII-HF, PvuII-HF	HindIII-HF	PvuII-HF	EcoRI-HF, PstI	-ve
1	DNA	5.15 uL	5.15 uL	5.15 uL	5.15 uL	5.15 uL
2	HindIII-HF	0.2 uL	0.2 uL	0 uL	0.2 uL	0 uL
3	PvuII	0.2 uL	0 uL	0.2 uL	0.2 uL	0 uL
4	10X Cutsmart buffer	1.8 uL	1.8 uL	1.8 uL	1.8 uL	1.8 uL
5	MQ	10.65 uL	10.85 uL	10.85 uL	10.65 uL	11.05 uL
6	Total	18 uL	18 uL	18 uL		18 uL

Use 1% gel

flowRoot3778.png



H = HindIII-HF
P1 = PvuII-HF
E = EcoRI-HF
P2 = PstI-HF

0.8% gel 130 V 35 minutes

Stained with syber safe

- Colony PCR of pSB1C3-BBa_K1399001-B0015
    - Expected band Vf2, Vr: 1165 bp
    - Template size: 2921 bp
    - Increase enzyme to 0.2 uL/reaction

*Improvement: More Jessica's Taq. Round it up!

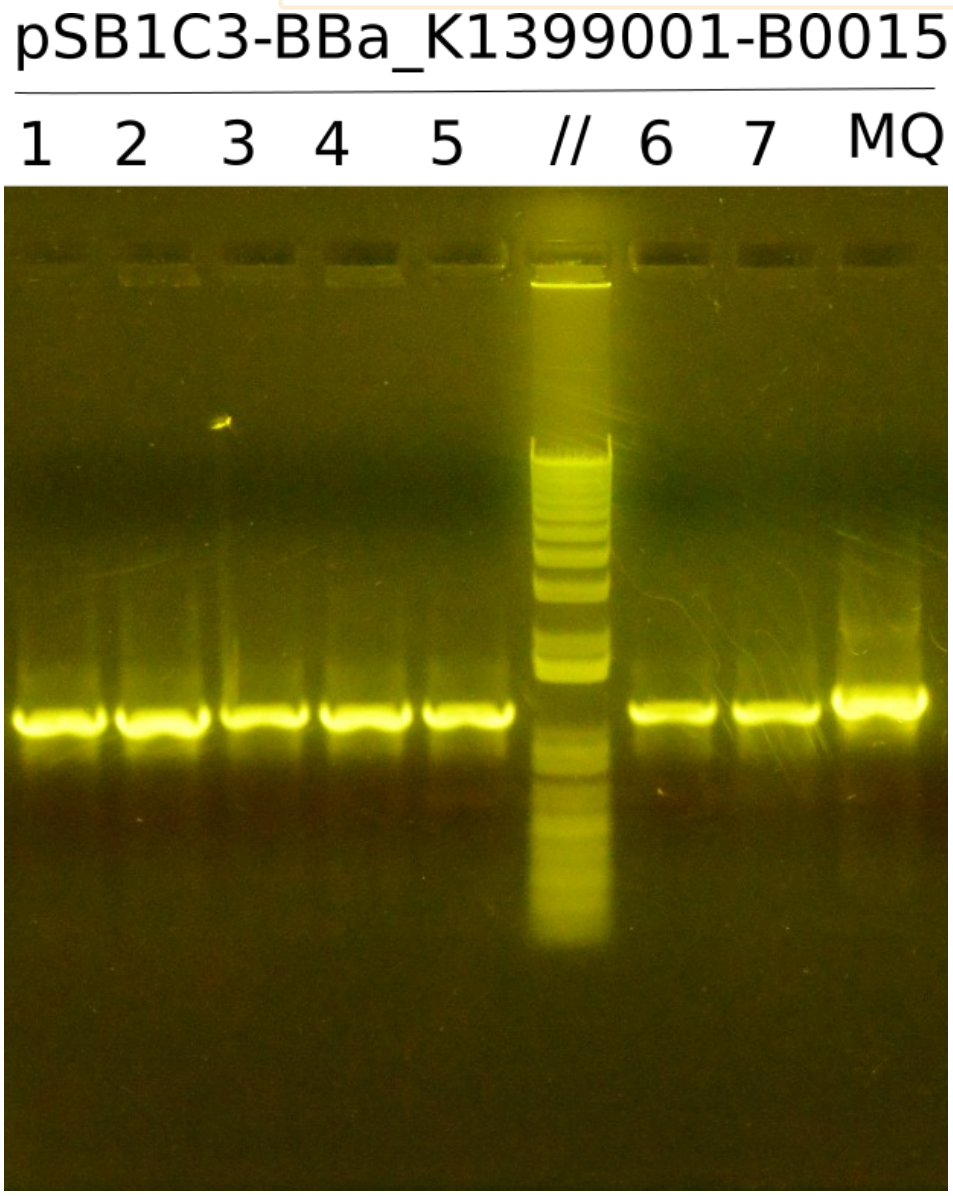
Table139

	A	B	C
1	Colony PCR		
2	Steps	Temperature (°C)	Time
3	Initial denaturation	95	3 min
4	Denaturation	95	15 s
5	Annealing	55	30 s
6	Extension	68	1 min 24 s
7	Final extension	68	5 min
8	Holding	10	infinity

Use 15 s for denaturation because Taq is susceptible to long-term high temperature condition.

path4220.png

We recovered unsaved changes to your entry. Click here to recover this data.



Stained with syber safe. Number 6 is chosen to inoculate and do restriction check (correction: MQ-->PCR+)

*Further improvement: To make the lower part of the gel clearer:

1. Run the gel until it rea
2. Load less DNA. Try 2

- Streak pSB1C3-BBa_R0062-S0109-B0015
- Innoc pSB1C3-BBa_K1399001-B0015 for restriction check tmr
- Transformation of pSB1A2-BBa_B0034 from database Box 2 5F

- Testing the MyTaq Buffer
  - Labelled MyTaq 1-5
  - pSB1C3-BBa_R0051-BBa_E0240 (Expected 1164)
  - It's suspected that the primer maybe problematic

Table136

	A	B	C	D
1	Reagents (ul)	Volume (ul)	MasterMix (*x)	1.1
2	MQ	13.875	83.25	91.575
3	5X MyTaq Reaction Buffer	4	24	26.4
4	10 mM dNTP	0.5	3	3.3
5	10 um VF2	0.5	3	3.3
6	10 uM VR	0.5	3	3.3
7	Taq Polymerase	0.125	0.75	0.825
8	Template	0.5	3	3.3
9	Total	20	120	132

Table138

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	Colony PCR		
2	Steps	Temperature (°C)	Time
3	Initial denaturation	95	3 min
4	Denaturation	95	30 s
5	Annealing	53	45 s
6	Extension	68	75 s
7	Final extension	68	5 min
8	Holding	8	infinity

WEDNESDAY, 8/9/2017

- Miniprep Max's tube (Gibson Assemblied Product; No. 3)
- Miniprep pSB1C3-BBa_K1399001-B0015 (Sample 6) for restriction check
- Inoculation of pSB1A2-BBa_B0034
- Restriction check pSB1C3-BBa_K1399001-B0015
    - Expected band if has insert (PvuII-HF): 929 bp, 1992 bp
    - If no insert (PvuII-HF): 2921 bp
- Digestion of pSB1C3-BBa_K1399001-B0015
    - Expected band: 897 bp, 2053 bp
- Digestion of pSB1C3-BBa_B0032-C0051
    - Expected band: 819 bp, 2047 bp

Try 2uL ladder
Run until it reaches 80% of the gel

pSB1C3-BBa_K1399001-B

	A	PvuII-HF for restriction check	XbaI-HF, PstI	XbaI-HF, PstI-HF	-ve
1	DNA	5.58 uL	5.58 uL	5.58 uL	5.58 uL
2	PvuII	0.2 uL			
3	XbaI-HF		0.2 uL	0.2 uL	
4	PstI		0.2 uL	0.2 uL	
5	10X Cutsmart buffer	1.8 uL	1.8 uL	1.8 uL	1.8 uL
6	MQ	10.42 uL	10.22 uL	10.22 uL	10.62 uL
7	Total	18 uL	18 uL	18 uL	18 uL

1% gel 130 V 40 mins

digestion as insert pSB1C3-BBa_B0032-C0051 (97.12 ug/uL)

	A	XbaI, PstI-HF	-ve
1	DNA	5.15 uL	5.15 uL
2	EcoRI-HF	0.2 uL	
3	SpeI-HF	0.2 uL	
4	10X Cutsmart buffer	1.8 uL	1.8 uL
5	MQ	10.65 uL	11.05 uL
6	Total	18 uL	18 uL

1% gel 130 V 40 mins SYBRsafe

- Restriction Check for GA3 with full construct (56.50 ug/ml) - 500 ng
    - expected bands: 1436bp, 3534bp

GA3

We recovered unsaved changes to your entry. Click here to recover this data.

	A	XbaI, PstI-HF	-ve
1	DNA	8.85 uL	8.85 uL
2	HindIII-HF	0.2 uL	
3	10X Cutsmart buffer	1.8 uL	1.8 uL
4	MQ	7.15 uL	7.35 uL
5	Total	18 uL	18 uL

text3534.png

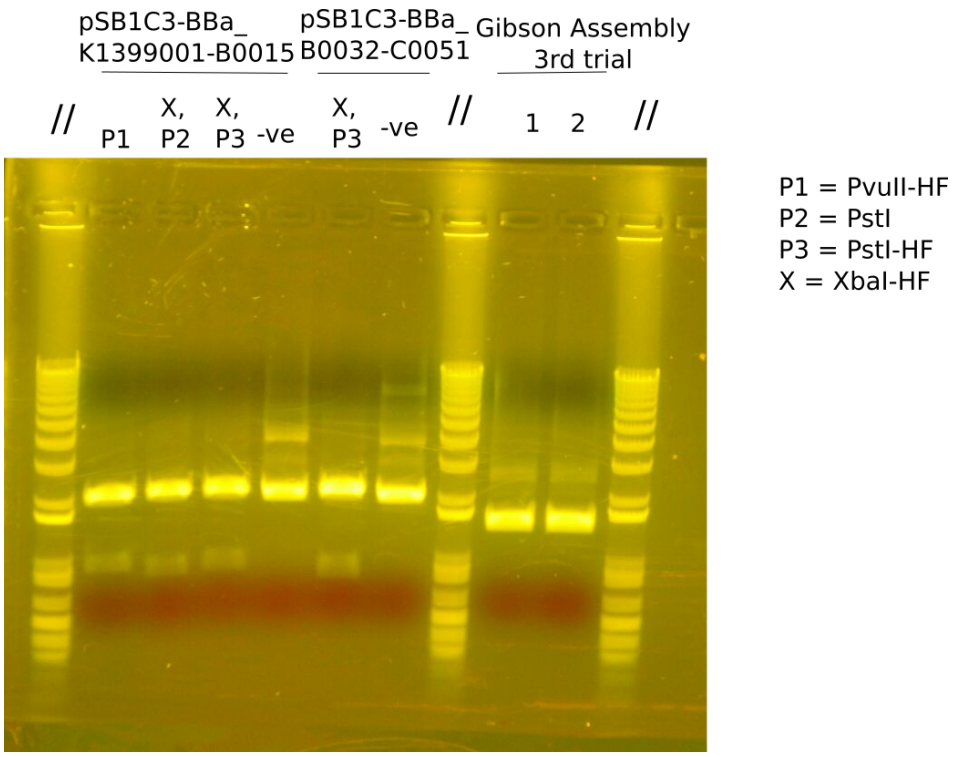


Table140

We recovered unsaved changes to your entry. Click here to recover this data.

	+	B	–	D
1	Ligase	0.5	MQ	0.5
2	T4 ligase buffer	1	T4 ligase buffer	1
3	Backbone		Backbone	
4	Insert		Insert	
5	Total	10	Total	10

- Miniprep the pSB1A2-BBa_B0034

- Inoculate pSB2K3-BBa_I13018, pSB1C3-BBa_R0062-K081007-B0015, pSB1C3-BBa_R0062-S0109-B0015
- Gibson assembly 4 (50 ng for each)

Table144

	A	B
1	pSB1C3 (conc: 27....)	
2	Insert1	
3	Insert2	
4	enzyme	15
5	Total V	~27

THURSDAY, 8/10/2017

- Colony PCR of pSB1C3-BBa_B0032-C0051-B0015
    - Total length: 3003 bp. PCR length (VF2, VR): 1247 bp
    - +: pSB1C3-BBa_R0062-K081007-B0015 (1310 bp)
    - - cloning (if no insert): pSB1C3-BBa_B0015 (443 bp)

Table142

	A	B	C
1	Reagents (ul)	Volume (ul)	MasterMix (*11.7)
2	MQ	5.4	63.2
3	5X MyTaq Reaction Buffer	2	23.4
4	10 um VF2	0.25	3
5	10 uM VR	0.25	3
6	Jessica's Taq	0.25	1.3
7	Template	0.1	Dip colony
8	Total	8 uL	
9			

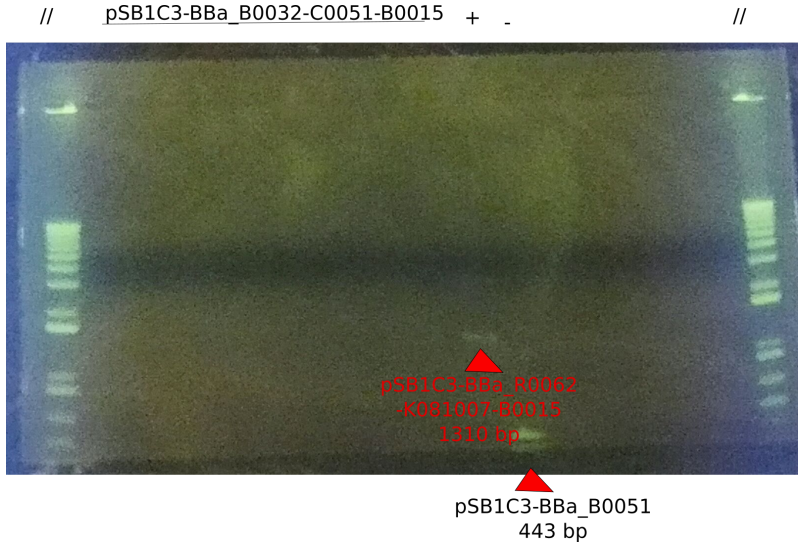
This time try it without adding dNTPs

Table141

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	Colony PCR		
2	Steps	Temperature (°C)	Time
3	Initial denaturation	95	3 min
4	Denaturation	95	15 s
5	Annealing	55	30 s
6	Extension	68	1 min 24s
7	Final extension	68	5 min
8	Holding	10	infinity

text4446.png



- Miniprep pSB1C3-BBa_R0062-K081007-B0015, pSB1C3-BBa_R0062-S0109-B0015
- Re-transform pSB2K3-BBa_I13018

- Colony PCR of Gibson
    - PCR length
    - +: pSB1C3-BBa_R0062-K081007-B0015 (274 bp)
    - - cloning (if no insert): pSB1C3
    - Colony PCR of GA
    - Expected size: 1279 bp
- Primer
    - Time Construct 1 Reverse Primer
    - VF2

Table145

	A	B	C
1	Reagents (ul)	Volume (ul)	MasterMix
2	MQ	5.4	70.2
3	5X MyTaq Reaction Buffer	2	26
4	Time Construct 1 Reverse Primer	0.25	3.25
5	10 uM VF2	0.25	3.25
6	Jessica's Taq	0.25	3.25
7	Template	dip	dip
8	Total	8 uL	

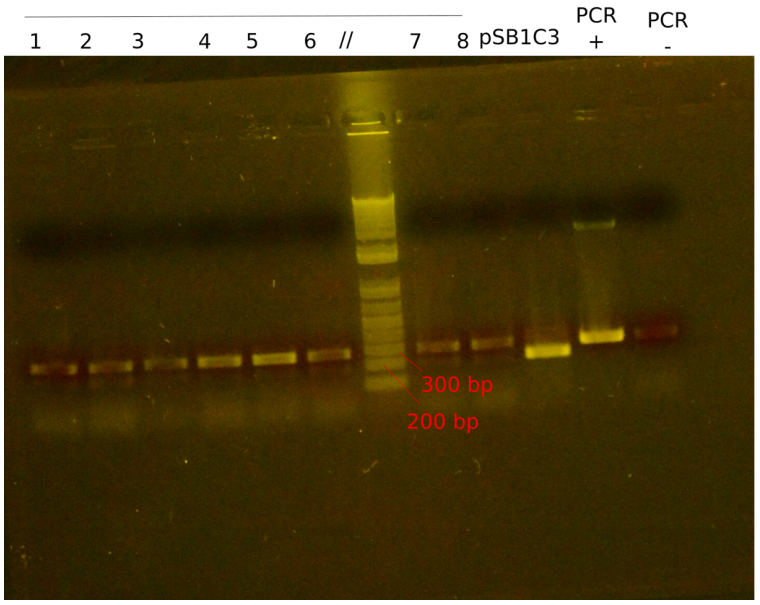
Table146

	A	B	C
1	Colony PCR		
2	Steps	Temperature (°C)	Time
3	Initial denaturation	95	3 min
4	Denaturation	95	15 s
5	Annealing	55	30 s
6	Extension	68	1 min 24 s
7	Final extension	68	5 min
8	Holding	10	infinity

We recovered unsaved changes to your entry. Click here to recover this data.

path5161.png

Gibson Assembly Test (4th Trial)



Primers used:
Vf2, Part 1 Rev for full construct time delay

PCR +: pSB1C3-BBa_R0062-K081007-B0015
(expected band: 274 bp)
(Total length: 3066 bp)

PCR -: No construct

pSB1C3: Expected band 314 bp

Notice the PCR- shows unexpected band. Hypothesis: contamination in Rev primer time delay construct.
However, the PCR+ and pSB1C3 show lighter bands than others. pSB1C3 supposes to generate band that is bigger than 300 bp but the picture shows band less than 300 bp.

FRIDAY, 8/11/2017

- Colony PCR of GA4 and GA5 (55)
  - +: pSB1C3-BBa_R0062-K081007-B0015 (274 bp)
  - pSB1C3
  - PCR-

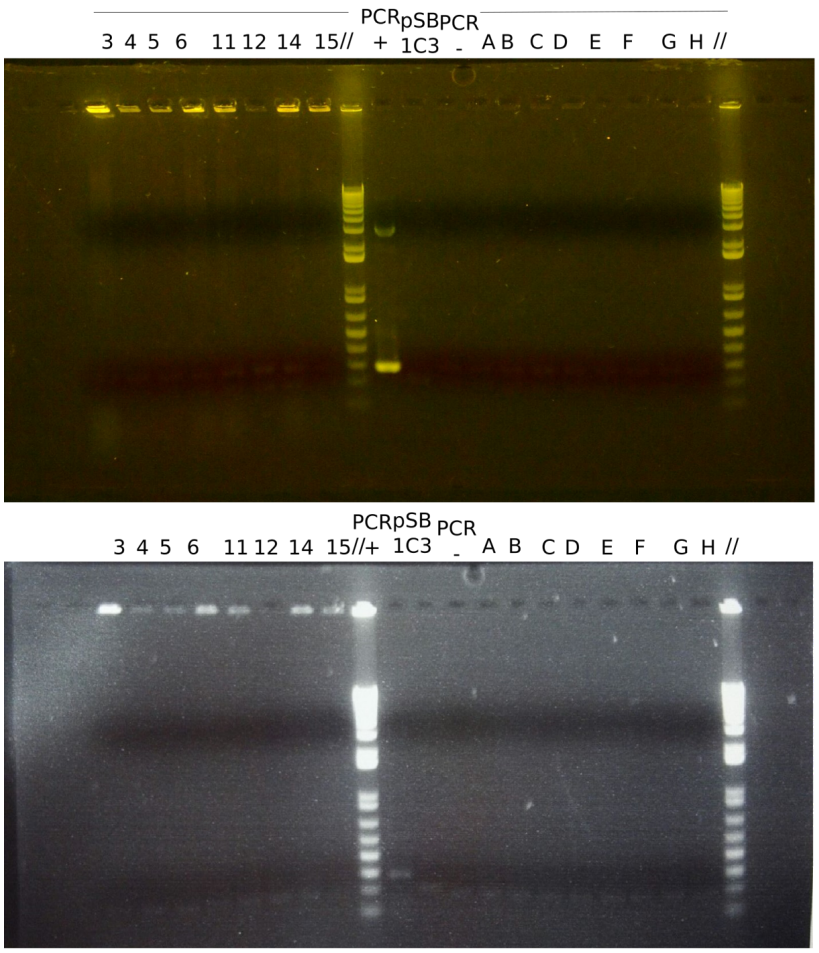
Table149

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	Reagents (ul)	Volume (ul)	Master Mix
2	MQ	5.4	129.6
3	5X MyTaq Reaction Buffer	2	48
4	10 um VF2	0.25	6
5	Time construct 1 Rev primer	0.25	6
6	Jessica's Taq	0.25	3
7	Template	Dip colony	Dip colony
8	Total	8 uL	
9			

text4630.png

Gibson Assembly (4th trial) Gibson assembly 5th trial)



150V, 30 minutes, SYBR Safe staining
Up: Gel photo taken with blue light
Down: Gel photo taken with UV light

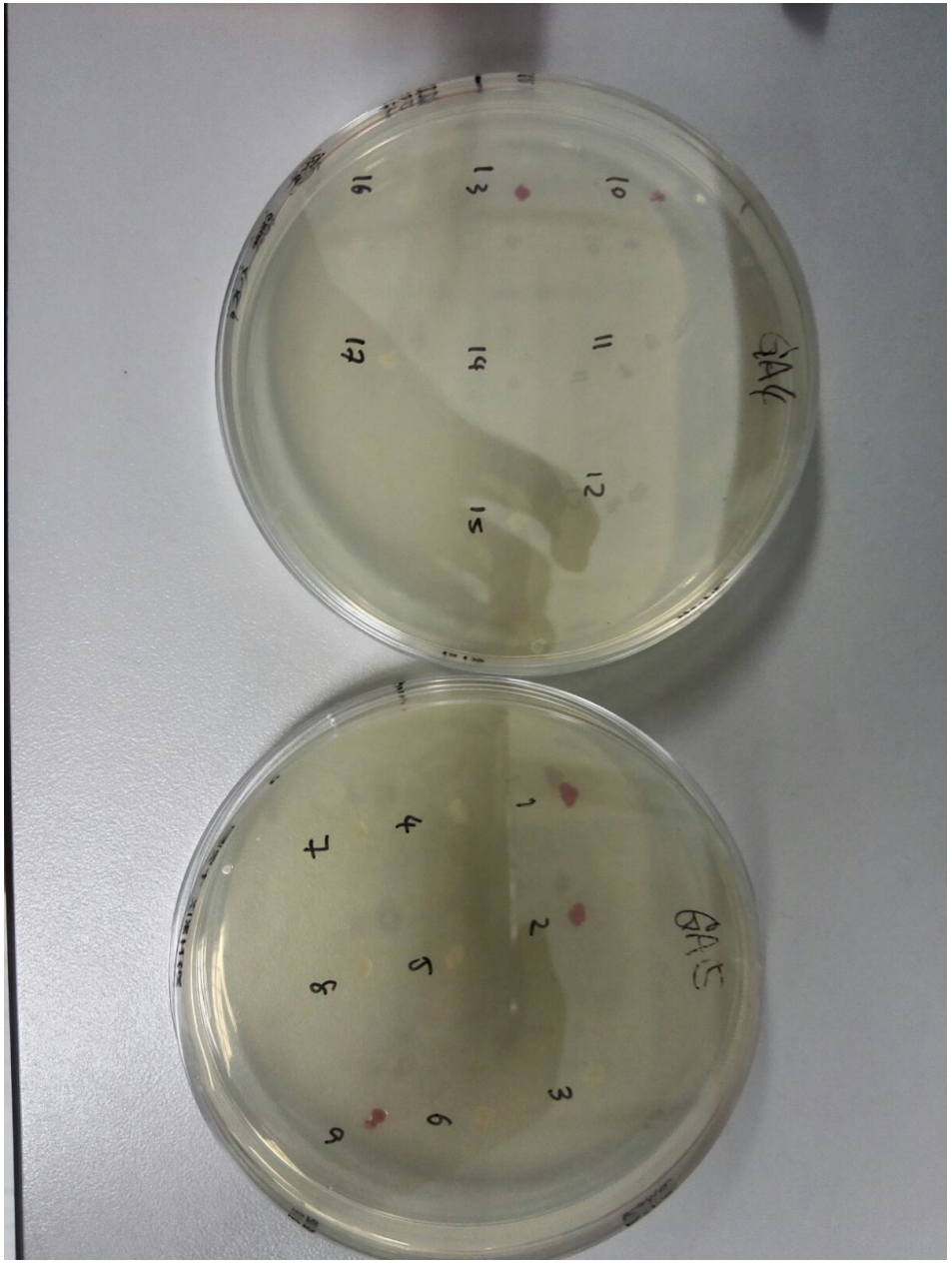
PCR+: pSB1C3-BBa_R0062-K081007-B0015

Expected band for Gibson Assembly: 1279 bp
Expected band for PCR +: 274 bp
Total length of PCR+: 3066 bp

PCR- shows band
Probably because of the contamination from our time module construct 1 primer since I did the same colony PCR except changing the reverse primer from VR to time module construct twice.

clipboard_2017-08-1

We recovered unsaved changes to your entry. Click here to recover this data.



- Redo colony PCR of E
  - Expected band
  - +: pSB1C3-BBa_R0062-K081007-B0015 (1310 bp)
  - Sample -: pSB1C3-BBa_B0015 (443 bp)

Table147

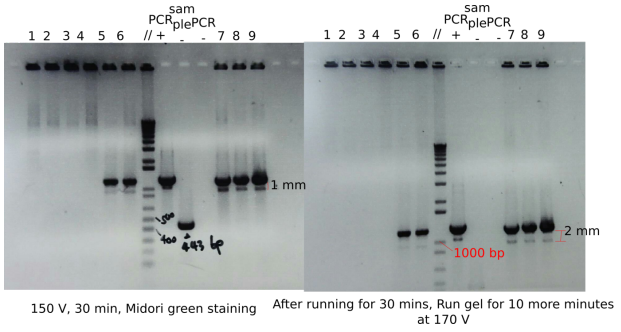
	A	B	C
1	Reagents (ul)	Volume (ul)	Master Mix
2	MQ	5.4	86.4
3	5X MyTaq Reaction Buffer	2	32
4	dNTPs		4
5	10 um VF2	0.25	4
6	10 uM VR	0.25	4
7	Jessica's Taq	0.25	2
8	Template	Dip colony	Dip colony
9	Total	8 uL	
10			

Table148

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	Colony PCR		
2	Steps	Temperature (°C)	Time
3	Initial denaturation	95	3 min
4	Denaturation	95	15 s
5	Annealing	55	30 s
6	Extension	68	1 min 24 s
7	Final extension	68	5 min
8	Holding	10	infinity

path5551.png



150 V, 30 min, Midori green staining

After running for 30 mins, Run gel for 10 more minutes at 170 V

pCR+: pSB1C3-BBa_R0062-K081007-B0015
(Expected band: 1310 bp)
Sample -: pSB1C3-BBa_B0015
(Expected band: 443 bp)

Expected band for the replicon 1 to 9: 1247 bp

There are two bands shown in each replicon so I decided to run gel with longer time to get the second photo.measure the separation between the two bands.

Hypothesis: The excess that were digested may be self-ligated so there is a small band with size of roughly 1110 bp (size of the insert with backbone after amplified by PCR) while the expecgted size of the pSB1C3-BBa_B0032-C0051-B0015 is 1247 bp.

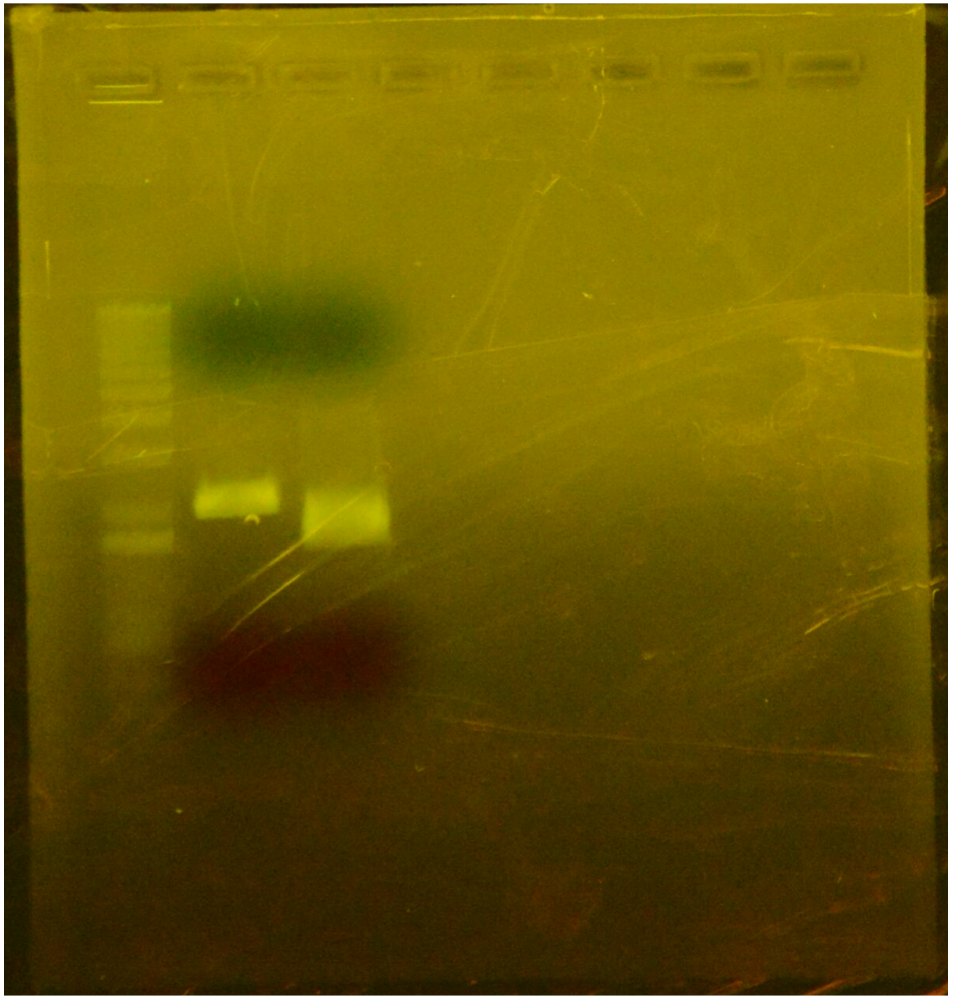
So I chose the band with less smear with no curved shape (sample 5)

- Miniprep pSB2K3-BBa_I13018
- Digest pSB1C3-BBa_B0034

text5582.png

pSB1C3-BBa_B0034

// S,P -



130V, 30 mins. Midori Green

- Streak plate pSB1C3-BBa_B0032-C0051-B0015 (sample 5), pSB2K3-BBa_I13018

We recovered unsaved changes to your entry. Click here to recover this data.

MONDAY, 8/14/2017

- Transformation of pSB1C3-BBa_S03119 (15mins for first cold shock)
- Innoculate pSB1C3-BBa_B0032-C0051-B0015
- Ligate pSB1A2-BBa_B0034 (S,P) with the pSB1C3-K1399001-B0015 (X,P) --> 0.1 RBS-RFP-TT
    - incubate overnight at room temperature

Table143

	+	B	–	D
1	Ligase	0.5	MQ	0.5
2	T4 ligase buffer	1	T4 ligase buffer	1
3	Backbone	4.51	Backbone	4.51
4	Insert	3.99	Insert	3.99
5	Total	10	Total	10

- Gibson Assembly. How to test the contamination of time module primers?
    - Testing the Primer: Suspect Primer contamination
    - Materials:
        - Make New set of Primer: VF2 and Time Reverse Primer 1 (10x dilution with MQ)
        - Old Primer (VF2 by katie made last week, TRE1,1 made by me)
        - MyTaq Buffer (1) will be used
    - Setup:
        - old-: MX
        - new-: MX
        - old+: pSB1C3-BBa_R0062-K081007-B0015 (274 bp)
        - new+: pSB1C3-BBa_R0062-K081007-B0015 (274 bp)
        - old-: pSB1C3 (linearised)
        - new-: pSB1C3 (linearised)
        - old-: pSB1C3 (J04450)
        - new-: pSB1C3 (J04450)

Table152

	A	B	C
1	Reagents (ul)	Volume (ul)	MasterMix (*x)
2	MQ	5	40
3	5X MyTaq Reaction Buffer	2	16
4	10 mM dNTP	0.25	2
5	10 um VF2	0.25	2
6	10 uM VR	0.25	2
7	Taq Polymerase	0.25	2
8	Template	0	0
9	Total	8	64

Table151

	A	B	C
1	Reagents (ul)	Volume (ul)	MasterMix
2	MQ	5.4	70.2
3	5X MyTaq Reaction Buffer	2	26
4	Time Construct 1 Reverse Primer	0.25	3.25
5	10 uM VF2	0.25	3.25
6	Jessica's Taq	0.25	3.25
7	Template	dip	dip
8	Total	8 uL	

Table150

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1	Colony PCR		
2	Steps	Temperature (°C)	Time
3	Initial denaturation	95	3 min
4	Denaturation	95	15 s
5	Annealing	55	30 s
6	Extension	68	1 min 24s
7	Final extension	68	5 min
8	Holding	10	infinity

P.S. 24X

- 2nd Colony PCR (GA4 &GA5)

Table153

	A	B	C
1	Reagents (ul)	Volume (ul)	MasterMix (19X)
2	MQ	5.4	129.6
3	5X MyTaq Reaction Buffer	2	48
4	Time Construct 1 Reverse Primer	0.25	6
5	10 uM VF2	0.25	6
6	Jessica's Taq	0.25	3
7	Template	dip	dip
8	Total	8 uL	

Table154

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C	D
1	Steps	Temperature (°C)	Time	
2	Initial denaturation	95	3 min	
3	Denaturation	95	15 s	
4	Annealing	55	30 s	2-4: 24 cycles
5	Extension	68	1 min 32s	
6	Final extension	68	5 min	
7	Holding	10	infinity	

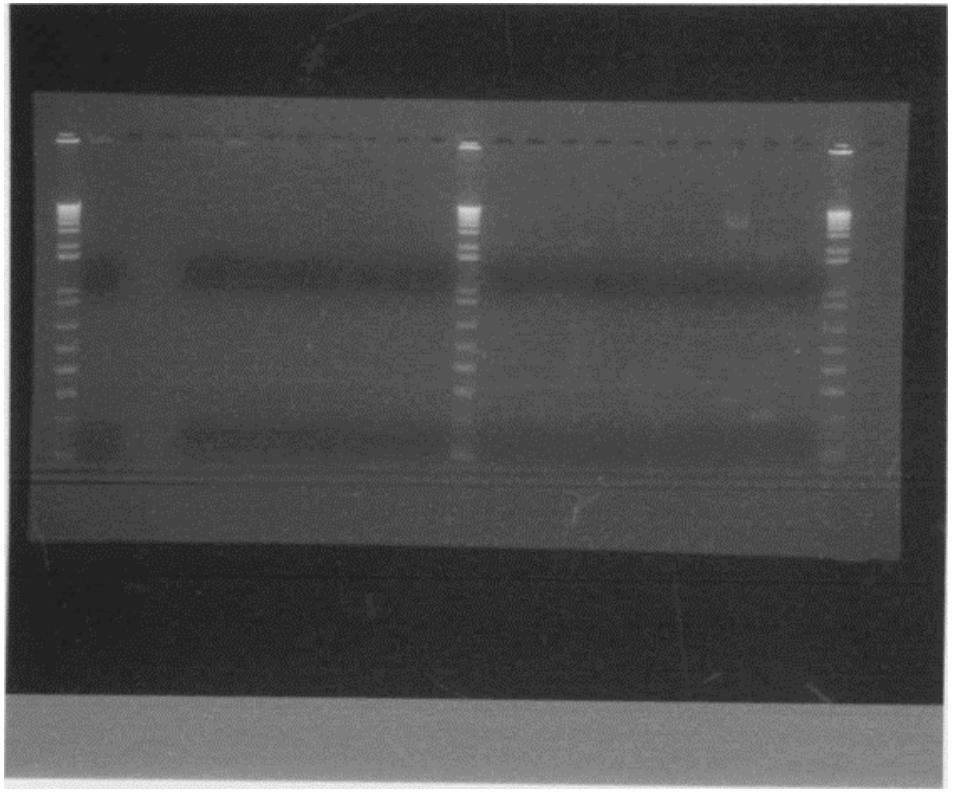
overnight

TUESDAY, 8/15/2017

- make CHL plates
- Gel electrophoresis of 2nd colony PCR (GA4 & GA 5)
    - expected band of GA: 1279bp
    - PCR+:274
    - linearized pSB1C3: 314bp (full length: 2070 bp)
    - if contamination occurs, change new set of reagents (primers, buffer, MQ)
    - if colony pcr still fails, innoculate the picked colony
    - // 1 2 3 4 5 6 7 8 A // B C D E F G H + 1c3 - // (1-8:GA4, A-H:GA5)
    - 40ml, 2 %, SYBR safe stained

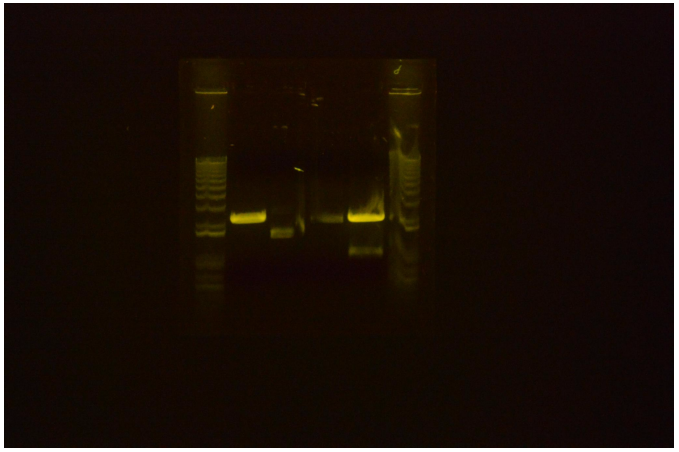
Gel_20170815_1110.p

We recovered unsaved changes to your entry. Click here to recover this data.



- Digestion of E0240(X,P) & pSB1C3-BBa_R0062(S,P)
  - pSB1A2-BBa_E0240(X,P-HF): 902bp, 2053bp
  - pSB1C3-BBa_R0062(S-HF,P-HF): 2107bp, 18bp
  - R+-, E-+

DSC_0297.jpg



- 3rd Colony PCR (GA4 & GA5)
  - expected band of GA: 1279bp
  - PCR+:274
  - linearized pSB1C3: 314bp (full length: 2070 bp)

Table157

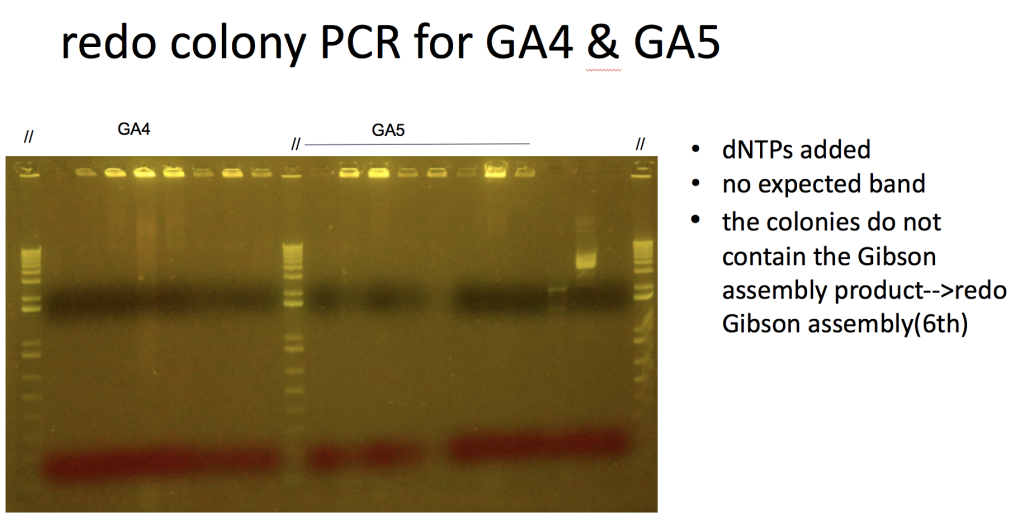
	A	B	C
1	Reagents (ul)	Volume (ul)	MasterMix (19X)
2	MQ	5.4	129.6
3	5X MyTaq Reaction Buffer	2	48
4	Time Construct 1 Reverse Primer	0.25	6
5	10 uM VF2	0.25	6
6	dNTPs	0.25	6
7	Jessica's Taq	0.25	4
8	Template	dip	dip
9	Total	8.5 uL	

Table158

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C	D
1	Steps	Temperature (°C)	Time	
2	Initial denaturation	95	3 min	
3	Denaturation	95	15 s	
4	Annealing	53	30 s	2-4: 24 cycles
5	Extension	68	1 min 22s	
6	Final extension	68	5 min	
7	Holding	10	infinity	

Screen Shot 2017-10-31 at 4.27.44 PM.png



- Innoculation of pSB1C3-BBa_B0032-C0051-B0015, pSB1A2-BBa_E0240, pSB3K3-BBa_E0240
- Transformation of ligation product (pSB1A2-BBa_B0034-K1399001-B0015), pSB1C3-BBa_S03119

We recovered unsaved changes to your entry. Click here to recover this data.

WEDNESDAY, 8/16/2017

- Miniprep pSB1C3-BBa_B0032-C0051-B0015, pSB1A2-BBa_E0240, pSB3K3-BBa_E0240

Table155

	A	DNA conc.	Protein	Salt
1	pSB1C3-BBa_B0032-C0051-B0015	277.6	1.881	2.321
2	pSB1A2-BBa_E0240	209.8	1.876	2.26
3	pSB3K3-BBa_E0240	69.25	1.91	2.147

- Digestion of pSB1C3-BBa_B0032-C0051-B0015(X,P) & pSB1C3-BBa_R0062(S,P)
  - pSB1C3-BBa_B0032-C0051-B0015(X,P): 959bp, 2044bp
  - pSB1C3-BBa_R0062(S,P): 2107bp, 18bp

Table160

	positive	DNA conc.	DNA mass	DNA vol	CutSmart	ddH2O	each enzyme	total
1	pSB1C3-BBa_B0032-C0051-B0015(X,P)	177.6	1000	3.6	1.8	12	0.3	18
2	pSB1C3-BBa_R0062(S,P)	55.33	500	9.04	1.8	6.56	0.2	18

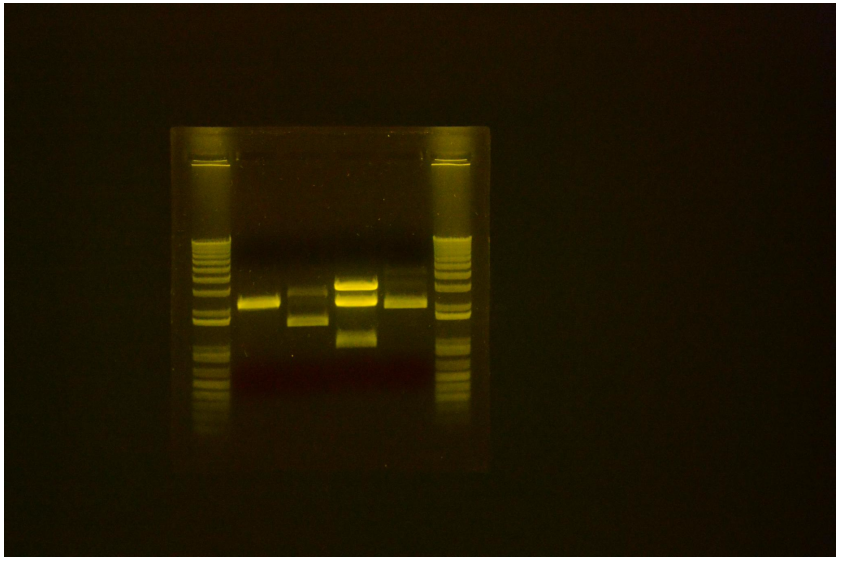
Table161

	negative	DNa conc.	DNA mass	DNa vol	CutSmart	ddH2O	enzyme	total
1	pSB1C3-BBa_B0032-C0051-B0015(X,P)	177.6	200	0.72	1.8	15.48	0	18
2	pSB1C3-BBa_R0062(S,P)	55.33	200	3.61	1.8	12.59	0	18

  - R+-, B+-

DSC_0303.jpg

We recovered unsaved changes to your entry. Click here to recover this data.



the bands shown in pSB1C3-BBa_B0032-C0051-B0015(X,P)+ aren't correct, and it shouldn't show that many bans. It may be caused by contamination/ligation problem.

- gel purification

Table156

	A	DNA conc.	length
1	pSB1A2-BBa_E0240(X,P-HF)		902
2	pSB1C3-BBa_R0062(S-HF,P-HF)_1		2107
3	pSB1C3-BBa_R0062(S-HF,P-HF)_2		2107

- Transformation
  - pSB1C3-BBa_S03119 (4ul of DNA)
  - from 2016 kit3 5A

- Innoculation & strek plate
  - pSB1A2-BBa_B0034-K1399001-B0015
  - pSB1C3-BBa_B0032-C0051-B0015
  -

THURSDAY, 8/17/2017

- Miniprep
  - pSB1A2-BBa_B0034-K1399001-B0015
  - pSB1C3-BBa_B0032-C0051-B0015

- Restriction check
  - pSB1C3-BBa_B0032-C0051-B0015
  - 2107, 896 (ScaI-HF, CS)
  - Result: same as -ve control-->B0032 fails to ligate with the insert?

- Digestion
  - pSB1A2-BBa_B0034-K1399001-B0015 (X,P-HF): 897bp, 2053bp
  - pSB1C3-BBa_B0032-C0051-B0015 (X,P-HF): 959bp, 2044bp
  - pSB1A2-BBa_E0240 (X,P-HF): 902bp, 2053bp
  - pSB1A2-BBa_R0040 (S-HF,P-HF): 2115bp, 18bp

  *XbaI has problem.

- PCR for pSB1C3
  - template: pSB1C3-BBa_J04450

Table159

	Box	Column	Row	D
1	5	4	B	fwd_RFC10suffix
2	5	5	B	rev_RFC10prefix

Table163

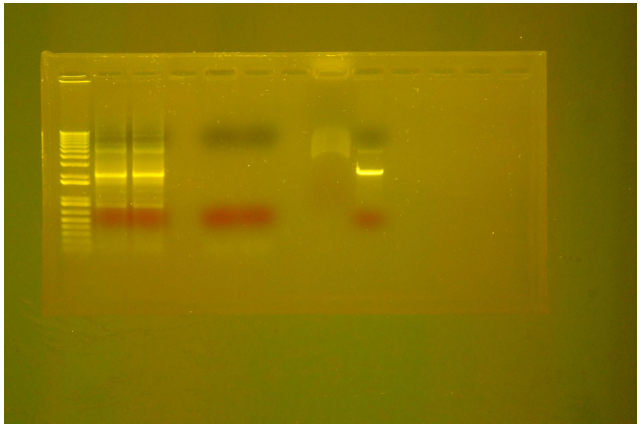
We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C	D	E	F
1		Positive	Negative		Linear 1C3 (Black Marker)	Linear 1C3 (Red Marker)
2	5X Q5 Buffer	10ul	10ul		9ul	9ul
3	10uM dNTPs	1ul	1ul			
4	10uM FWD RFC10suf	2.5ul	2.5ul			
5	10uM REV RFC10pre	2.5ul	2.5ul			
6	pSB1C3-BBa_J04450	2ul	0			
7	Q5 DNA polymerase	0.5ul	0.5ul			
8	MQ	31.5ul	32.5ul			
9	Total	50ul	50ul			

Table162

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	95°C	30 seconds
3	30 Cycles	95°C, 68°C, 72°C	15 seconds, 15 seconds, 1 min 15 seconds
4	Final Extension	72°C	5 minutes
5	Hold	10 °C	

DSC_0306 (1).jpg



We recovered unsaved changes to your entry. Click here to recover this data.

- Gibson assembly
  - [x] new master mix

- Innoculation
  - pSB1A2-BBa_B0034-K1399001-B0015
  - pSB1C3-BBa_B0032-C0051-B0015
  - pSB1A2-BBa_E0240
  - pSB1A2-BBa_R0040
  - pSB1A2-BBa_R0051
  - pSB1A2-BBa_R0062
  - pSB1C3-BBa_F2620

FRIDAY, 8/18/2017

- Miniprep

- Digestion
  - pSB1A2-BBa_R0051 (S-HF,P-HF): 2110bp, 18bp
  - pSB1C3-BBa_F2620 (S-HF,P-HF): 3113bp, 18bp
  - pSB1C3-BBa_R0062 (S-HF, P-HF): 2107bp, 18bp

  - pSB1A2-BBa_P0151 (X,P-HF): 958bp, 2053bp
  - pSB1C3-BBa_P0451 (X,P-HF): 956bp, 2044bp

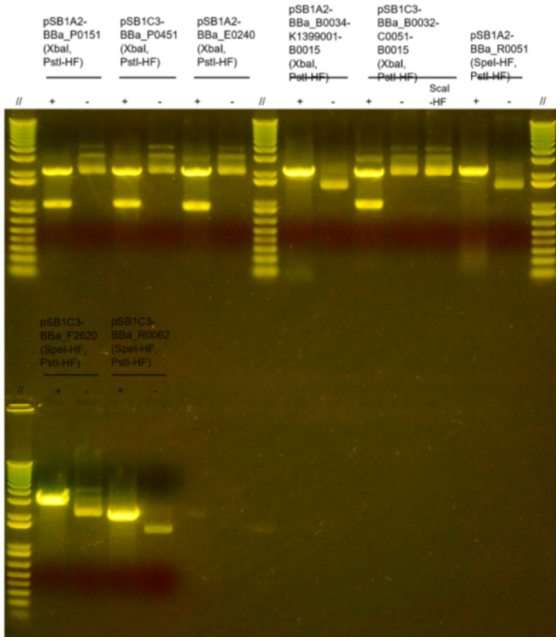
- pSB1A2-BBa_E
- pSB1A2-BBa_B
- pSB1C3-BBa_B0032-C0051-B0015 (X,P-HF): 959bp, 2044bpm

Table178

	Plasmid	DNA Volume	Enzyme 1	Enzyme 2	10x Cutsmart Buffer	MQ
1	pSB1A2-BBa_P0151 (+)	8 .69	0 .3	0.3	1.8	7 .31
2	pSB1A2-BBa_P0151 (-)	1 .74	0	0	1 .8	14.46
3	pSB1C3-BBa_P0451 (+)	6.49	0 .3	0 .3	1 .8	9 .51
4	pSB1C3-BBa_P0451 (-)	1 .3	0	0	1 .8	1 4.9
5	pSB1A2-BBa_E0240 (+)	3.72	0 .3	0.3	1.8	1 2.08
6	pSB1A2-BBa_E0240 (-)	0.74	0	0	1.8	1 5.46
7	pSB1A2-BBa_B0034-K1399001-B0015 (+)	8 .73	0 .3	0 .3	1 .8	7 .02
8	pSB1A2-BBa_B0034-K1399001-B0015 (-)	1.76	0	0	1.8	1 4.44
9	pSB1C3-BBa_B0032-C0051-B0015 (+)	3 .59	0.3	0.3	1 .8	1 2.21
10	pSB1C3-BBa_B0032-C0051-B0015 (-)	0.72	0	0	1.8	1 5.48
11	pSB1A2-BBa_R0051 (+)	1 6.19	0 .3	0.3	1.8	0
12	pSB1A2-BBa_R0051 (-)	4	0	0	1.8	12.15
13	pSB1C3-BBa_F2620 (+)	5 .38	0.3	0.3	1.8	10.42
14	pSB1C3-BBa_F2620 (-)	1.08	0	0	1.8	15.12

- Restriction check
  - pSB1C3-BBa_B0032-C0051-B0015: 2107bp, 896bp (ScaI-HF, CS)

image18.png



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MONDAY, 8/21/2017

- Prepare pSB1C3 backbone

Table164

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C
1		Positive	Negative
2	5X Q5 Buffer	5	5
3	10uM dNTPs	0.5	0.5
4	10uM FWD RFC10suf	1.25	1.25
5	10uM REV RFC10pre	1.25	1.25
6	pSB1C3-BBa_J04450	1	0
7	Q5 DNA polymerase	0.25	0.25
8	MQ	17.5	18.5
9	Total	25	25

Table165

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	95°C	30 seconds
3	30 Cycles	95°C, 68°C, 72°C	15 seconds, 15 seconds, 1 min 15 seconds
4	Final Extension	72°C	5 minutes
5	Hold	10 °C	

Fail: no band is shown. Possible reasons: forget to add stain/only water left in the tube of pSB1C3-BBa_J04450--> innoculation today

- PCR clean-up for T1, T2 (insert of Gibson Assembly)
  - use smaller volume(8ul) to elute in order to get higher concentration
  - T1: 15.59 ng/ul
  - T2: 11 ng/ul
- Innoculation
  - pSB1C3-BBa_J04450
  - P0151
  - P0451

- F2620
- R0051-E0240
-

TUESDAY, 8/22/2017

- Prepare pSB1C3 backbone

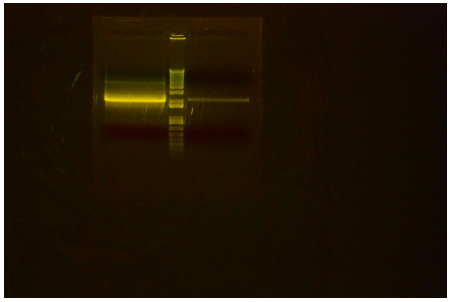
Table166

	A	B	C
1		Positive	Negative
2	5X Q5 Buffer	5	5
3	10uM dNTPs	0.5	0.5
4	10uM FWD RFC10suf	1.25	1.25
5	10uM REV RFC10pre	1.25	1.25
6	pSB1C3-BBa_J04450	1	0
7	Q5 DNA polymerase	0.25	0.25
8	MQ	17.5	18.5
9	Total	25	25

Table167

	A	B	C
1	**STEP**	**TEMP**	**TIME**
2	Initial Denaturation	95°C	30 seconds
3	30 Cycles	95°C, 68°C, 72°C	15 seconds, 15 seconds, 1 min 15 seconds
4	Final Extension	72°C	5 minutes
5	Hold	10 °C	

DSC_0315.jpg



We recovered unsaved changes to your entry. Click here to recover this data.

- Digestion

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Screen Shot 2017-10-

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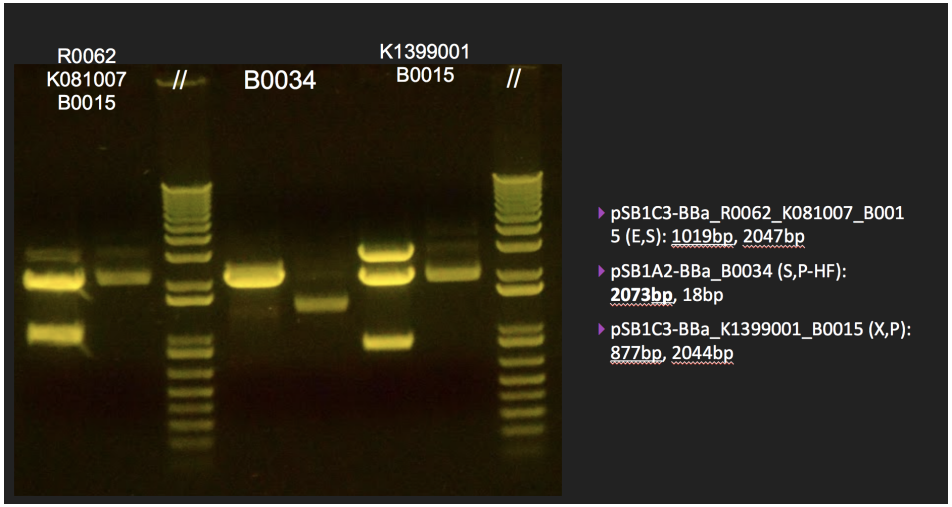


image.png

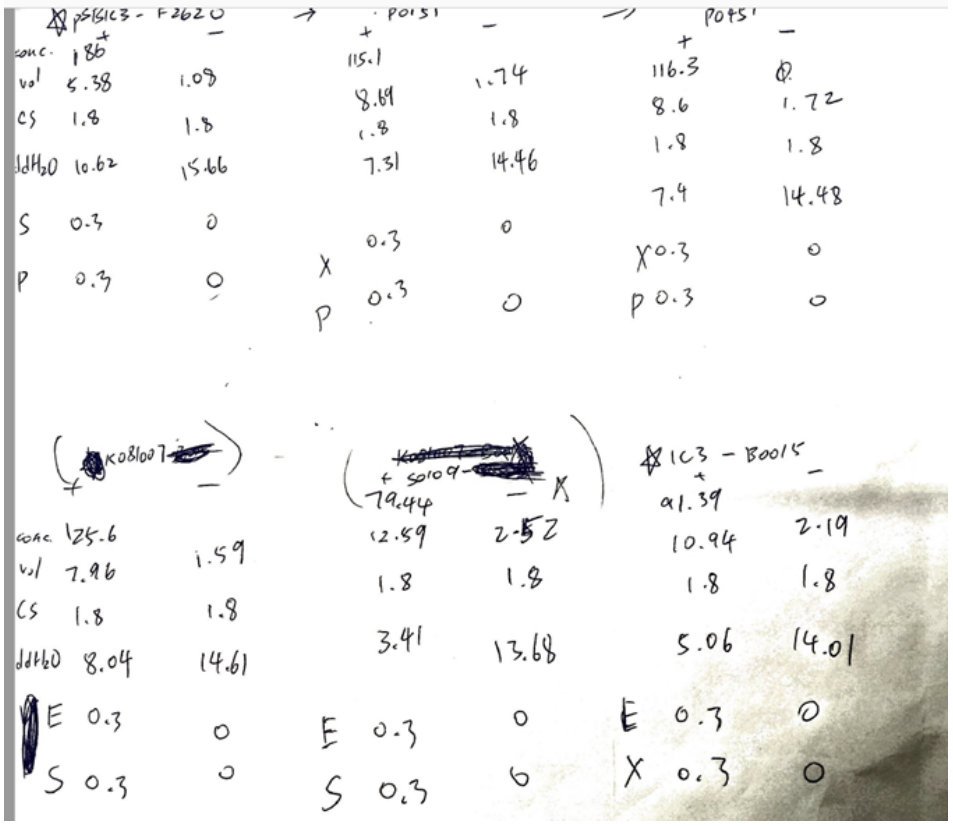
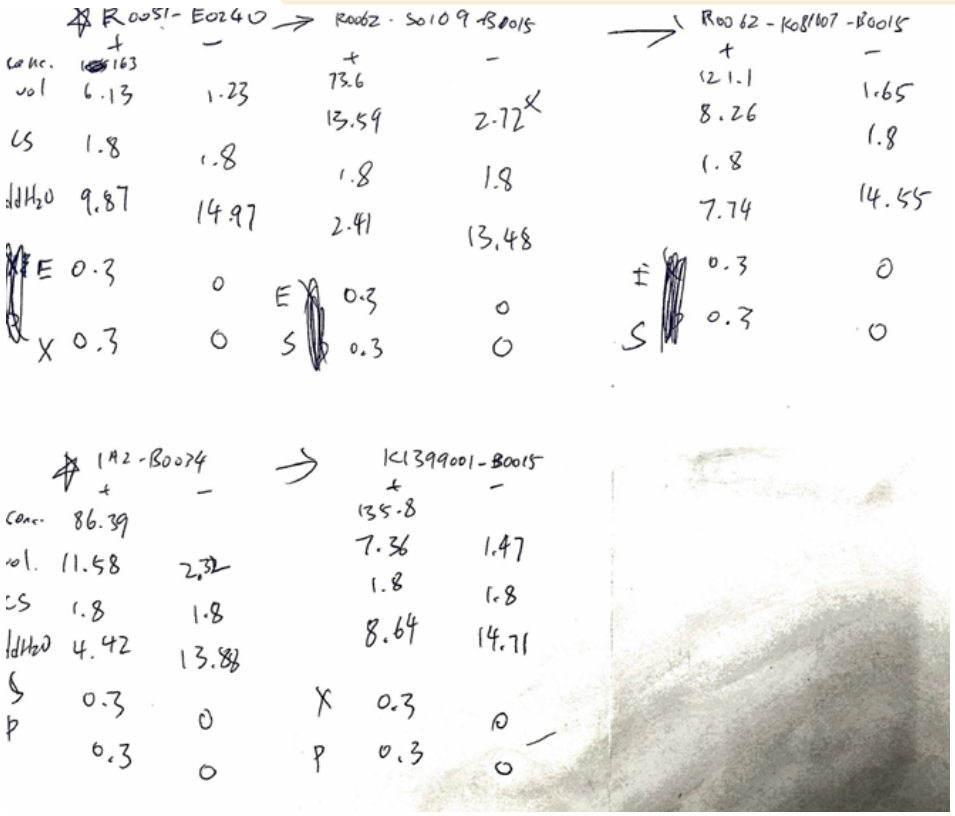


image.png



THURSDAY, 8/24/2017

- Ligation (overnight)
  - pSB1C3-BBa_F2620-P0151
  - pSB1C3-BBa_F2620-P0451
  - pSB1C2-BBa_S0109-B0015
  - pSb1C3-BBa_K081007-B0015

- 6th Gibson assembly
  - T1: 15.59ng/ul
  - T2: 11.5ng/ul
  - pSB1C3: 16.62 ng/ul

- backbone:inser

FRIDAY, 8/25/2017

- Transformation (of ligation products)
  - pSB1C3-BBa_F2620-P0151
  - pSB1C3-BBa_F2620-P0451
  - 2pSB1C2-BBa_S0109-B0015
  - pSb1C3-BBa_K081007-B0015

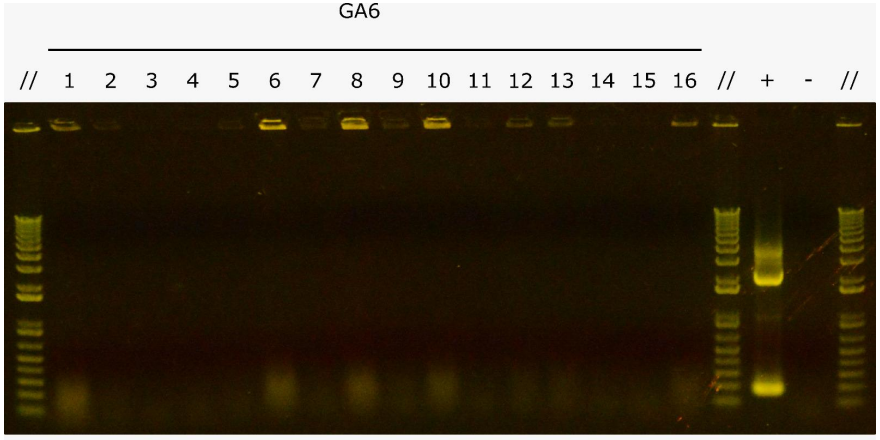
MONDAY, 8/28/2017

Innoculation

TUESDAY, 8/29/2017

- Colony PCR (for 6th Gibson assembly)
  - VF2 (Tm: 55)
  - Time Consruct part 1 REV (Tm: 58.6)
  - 1279bp
  - +ve control: pSB1C3-C0051

WhatsApp Image 2017-08-29 at 4.20.03 PM.jpeg



+: pSB1C3-BBa_C0051
-: No template control
//: 1kb plus DNA marker (Life Technologies)

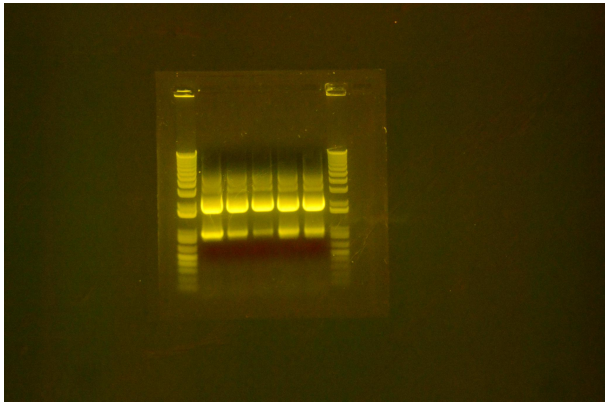
Q5 10uL reaction, extension 45 sec, annealing 63C. 25 cyc. 1% agarose/1XTAE, 7V/ cm

- Gradient PCR
  - Time Construct part2 FWD & VR
  - pSB1C3-BBa_C0051 (848bp)
  - (add DMSO when needed)

Table168

	Temperature	Time
1	95	30s
2	95	15s
3	52-60	30s
4	68	56s
5	go to 2 (24 repeats)	
6	68	5mins
7	4	infinity

DSC_0321.jpg



left to right: 52-60
SYBR safe stained, 1% gel, 1 kb plus ladder

- Miniprep
  - B0034-phlf

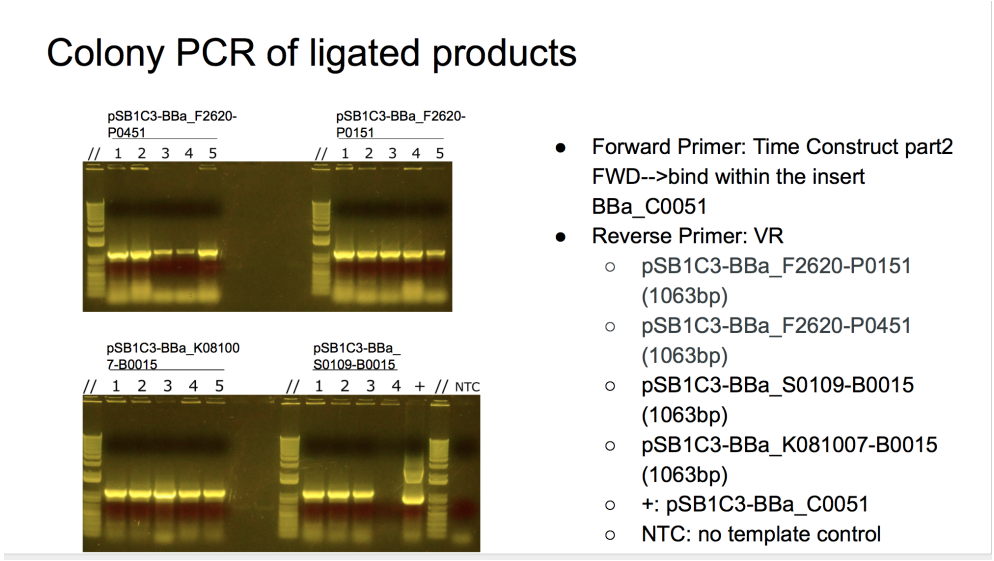
- pSB1C3-BBa_R

- Transformation
  - pSB1C3-BBa_E0420 (2016 kitplate 3)

WEDNESDAY, 8/30/2017

- Colony PCR (of ligation products)
  - pSB1C3-BBa_F2620-P0151 (1063bp)
  - pSB1C3-BBa_F2620-P0451 (1063bp)
  - pSB1C3-BBa_S0109-B0015 (1063bp)
  - pSB1C3-BBa_K081007-B0015 (1063bp)
  - Forward Primer
    - Time Construct part2 FWD (Tm: 60.4)-->bind within the insert BBa_C0051
  - Reverse Primer
    - VR: ATTACCGCCTTTGAGTGAGC (Tm: 51.6)

Screen Shot 2017-10-31 at 5.01.50 PM.png



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THURSDAY, 8/31/2017

- Transformation
  - pSB1C3-BBa_E0420 (2016 kitplate 3)
  - pSB1A2-BBa_E0420 7L (2016 kitplate 4)
- Digestion:
  - pSB1A2-BBa_B0034-K1725040 (E, S)
  - pSB1C-BBa_B0015 (E, X)

FRIDAY, 9/1/2017

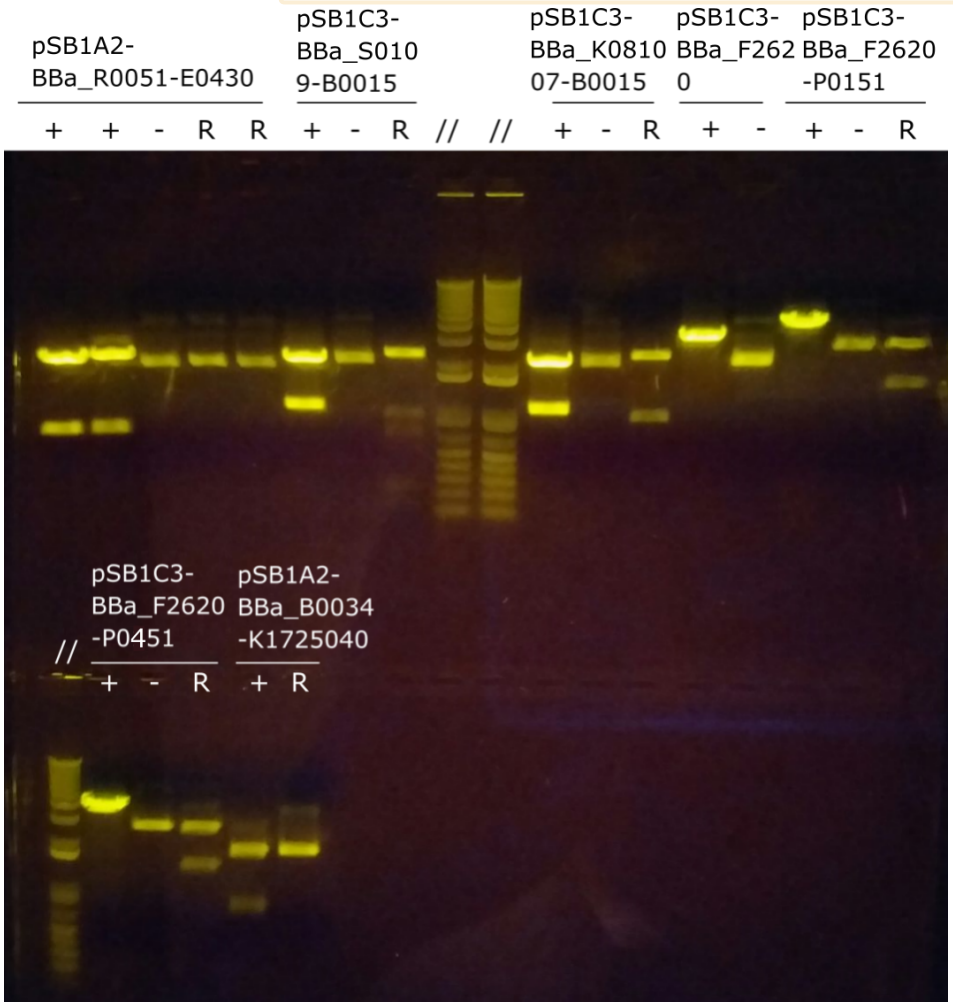
- Transformation
  - pSB1C3-BBa_E0420 (2016 kitplate 3)
  - pSB1A2-BBa_E0420 7L (2016 kitplate 4)
- Digestion
  - pSB1A2-BBa_B0034-K1725040 (E, S) (done, waiting for digestion)
  - pSB1C-BBa_B0015 (E, X) (done, waiting for digestion)
- Ligation
  - pSB1A2-BBa_B0034-K1725040-B0015
  - pSB1A2-BBa_K1725000 (PphlF) (S,P)+ E0240 (X,P) (test phlfp) (in freezer)

TUESDAY, 9/5/2017

- Restriction check
  - pSB1C3-BBa_F2620-P0151 (HindIII: 1436, 2635)
  - pSB1C3-BBa_F2620-P0451 (HindIII: 1434, 2635)
  - pSB1C3-BBa_S0109-B0015 (HindIII, PvuII: 859, 2140)
  - pSB1C3-BBa_K081007-B0015 (HindIII, PvuII: 863, 2140)
  - pSB1A2-BBa_B0034-K1725040 (PvuII: 24, 153, 2523)
  - pSB1A2-R0051-E0430 (ScaI-HF: 3014bp)
- Digestion
  - pSB1A2-R0051-E0430 (X,P): 2053, 961
  - pSB1C2-BBa_S0109-B0015 (X,P): 2044, 955
  - pSB1C3-BBa_K081007-B0015 (X,P): 2044, 959
  - psB1C3-BBa_F2620 (S,P): 3113, 18
  - pSB1C3-BBa_F2620-P0151 (S,P): 4053, 18
  - pSB1C3-BBa_F2620-P0451 (S,P): 4051, 18

We recovered unsaved changes to your entry. Click here to recover this data.

text16.png



130V, 30mins, SYBR-safe stained

- Digestion
  - pSB1C3-BBa_R0062-P0151 (S,P) 500ng
  - pSB1C3-BBa_R0062-P0451 (S,P) 500ng
  - pSB1C3-BBa_R0062-S0109-B0015 (S,P) 500ng (Can't be done, out of stock) **
  - pSB1C3-BBa_R0062-K081007-B0015 (S,P) 500ng (Soon will be out of stock)
  - pSB1A2-BBa_R0051-E0430 (X,P) 1500ng (404 Not Found) **

    - pSB1A2-BBa_R
- Ligation
    - pSB1C3-BBa_R0062-P0151-R0051-E0430**
    - pSB1C3-BBa_R0062-P0451-R0051-E0430**
    - pSB1C3-BBa_R0062-S0109-B0015-R0051-E0430 **
    - pSB1C3-BBa_R0062-K081007-B0015-R0051-E0430 **
    - pSB1C3-BBa_R0062-P0151-R0051-E0240
    - pSB1C3-BBa_R0062-P0451-R0051-E0240
    - pSB1C3-BBa_R0062-S0109-B0015-R0051-E0240 **
    - pSB1C3-BBa_R0062-K081007-B0015-R0051-E0240
    - pSB1A2-BBa_B0034-K1725040 (E, S)
    - pSB1C-BBa_B0015 (E, X)
    - **hold
- Inoculation
    - pSB1C3-BBa_R0062-S0109-B0015
    - pSB1C3-BBa_R0062-K081007-B0015
    - pSB1A2-BBa_R0051-E0430

- Max's
- Transformation
    - pSB1C3-BBa_E0420 (2016 kitplate 3)
    - pSB1A2-BBa_E0420 7L (2016 kitplate 4)
- Digestion and Ligation
    - Aim: test the funtionality of E0420, phlf and phlfp
    - DAPG to induce phlf

- Digestion:
    - pSB1A2-BBa_R0051 (S,P)**
    - pSB1A2-BBa_K1725000 (PphlF) (S,P)**
    - pSB1A2-BBa_B0034-K1725040 (X,P)**
    - pSB1C3-BBa_E0420 (X,P)**
    - pSB1C3-BBa_E0240 (X,P)**
- Ligation: (not yet done)
    - R0051+ E0240 (test our positive control)**
    - R0051+ E0420 (test our E0420)**
    - phlfp+ E0420 (for subsequent ligation)**

**hold

WEDNESDAY, 9/6/2017

Gel purification

Table169

	A	Concentration (ng/uL)	Salt contamination	Protein contamination
1	pSB1C3-BBa_R0062-S0109-B0015	55.73	0.024	2.085
2	pSB1C3-BBa_R0062-P0451	51.32	0.162	1.812
3	pSB1C3-BBa_R0062-P0151	31.80	0.027	1.893
4	pSB1C3-BBa_R0062-K081007-B0015	62.31	0.033	1.929

Miniprep

Table170

	A	Concentration (ng/uL)	Salt contamination	Protein contamination
1	pSB1A2-BBa_E0240	76.43	2 .098	1 .845

THURSDAY, 9/7/2017

- Ligation
  - pSB1C3-BBa_F2620-P0151-R0051-E0430
  - pSB1C3-BBa_F2620-P0451-R0051-E0430
  - pSB1C3-BBa_F2620-K081007-B0015
  - pSB1C3-BBa_F2620-S0109-B0015
- Transformation
  - pSB1C3-BBa_F2620-P0151-R0051-E0430
  - pSB1C3-BBa_F2620-P0451-R0051-E0430
  - pSB1C3-BBa_F2620-K081007-B0015

- pSB1C3-BBa_F
- pSB1A2-BBa_E

- Inoculation
  - pSB1A2-BBa_R0051-E0240

FRIDAY, 9/8/2017

- Digestion
  - pSB1C3-BBa_R0062-P0451 (S,P) 1000ng
  - pSB1A2-BBa_R0051-E0240 (X,P) 1000ng
  - pSB1C3-BBa_R0062-P0151 (S,P) 1000ng
  - pSB1C3-BBa_R0062-K081007-B0015 (S,P) 1000ng

Screen Shot 2017-10-31 at 5.06.50 PM.png

MONDAY, 9/11/2017

- Colony pcr of ligation products
  - pSB1C3-BBa_F2620-P0151-R0051-E0430
    - fwd primer: FW for full costruct part 2 (Tm: 60.4)
    - rev primer: ydjM-RBS-RV (Tm: 57.1) [primer box2 7C]
    - expected band size: 1875bp
  - pSB1C3-BBa_F2620-P0451-R0051-E0430

        - fwd prim
        - rev prim
        - expected band size: 1875bp
    - pSB1C3-BBa_F2620-K081007-B0015
        - fwd primer: 1st FWD (Tm: 56.6) [primer box6 9A]
        - rev primer: ydjM-RBS-RV (Tm: 57.1) [primer box2 7C]
        - expected band size: 1289bp
    - pSB1C3-BBa_F2620-S0109-B0015
        - fwd primer: 1st FWD (Tm: 56.6) [primer box6 9A]
        - rev primer: ydjM-RBS-RV (Tm: 57.1) [primer box2 7C]
        - expected band size: 1289bp

- Ligation
    - pSB1C3-BBa_R0062-P0151-R0051-E0430
    - pSB1C3-BBa_R0062-P0451-R0051-E0430
    - pSB1C3-BBa_R0062-S0109-B0015-R0051-E0430
    - pSB1C3-BBa_R0062-K081007-B0015-R0051-E0430
    - pSB1C3-BBa_R0062-P0151-R0051-E0240
    - pSB1C3-BBa_R0062-P0451-R0051-E0240
    - pSB1C3-BBa_R0062-S0109-B0015-R0051-E0240
    - pSB1C3-BBa_R0062-K081007-B0015-R0051-E0240

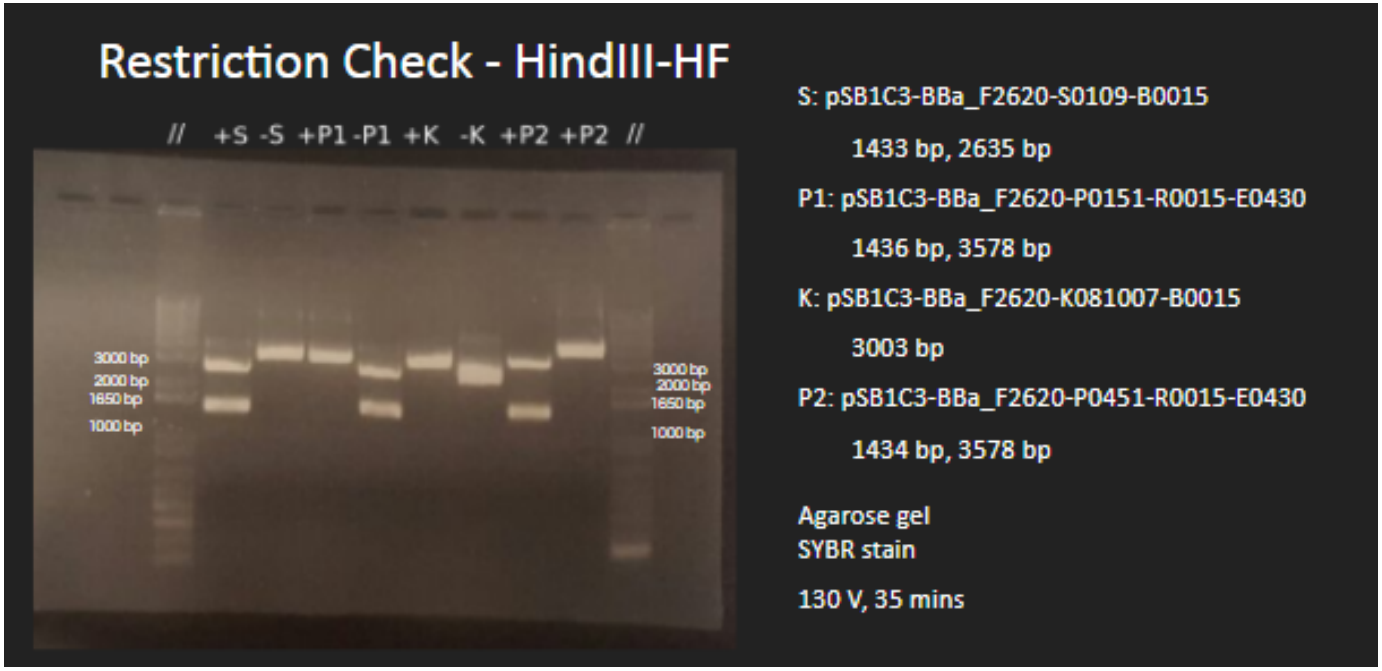
TUESDAY, 9/12/2017

Restriction check for ligated products

- pSB1C3-BBa_F2620-P0151-R0051-E0430
- pSB1C3-BBa_F2620-P0451-R0051-E0430
- pSB1C3-BBa_F2620-K081007-B0015
- pSB1C3-BBa_F2620-S0109-B0015

image.png

We recovered unsaved changes to your entry. Click here to recover this data.



Correct band size: S, K, P2-->sequencing

Table172

	A	HindIII	CutSmart	ddH2O	DNA Amount
1	pSB1C3-BBa_F2620-S0109-B0015	0.2	1.8	13.7	2.3
2	pSB1C3-BBa_F2620-P0151-R0051-E0430	0.2	1.8	12.83	3.17
3	pSB1C3_F2620-K081007-B0015	0.2	1.8	13.54	2.46
4	pSB1C3-BBA_F2620-P0451-R0051-E0430	0.2	1.8	13.74	2.26

Table173

We recovered unsaved changes to your entry. Click here to recover this data.

	A	HindIII	CutSmart	ddH2O	DNA Amount
1	pSB1C3-BBa_F2620-S0109-B0015	0	1.8	13.9	2.3
2	pSB1C3-BBa_F2620-P0151-R0051-E0430	0	1.8	13.03	3.17
3	pSB1C3_F2620-K081007-B0015	0	1.8	13.74	2.46
4	pSB1C3-BBA_F2620-P0451-R0051-E0430	0	1.8	13.94	2.26

WEDNESDAY, 9/13/2017

Inoculation
pSB1A2-R0051-E0240

THURSDAY, 9/14/2017

Miniprep
pSB1A2-R0051-E0240

Table171

	A	Concentration (ng/uL)	Salt contamination	Protein contamination
1	pSB1A2-R0051-E0240	52.19	2.027	1.919

TUESDAY, 9/19/2017

image.png

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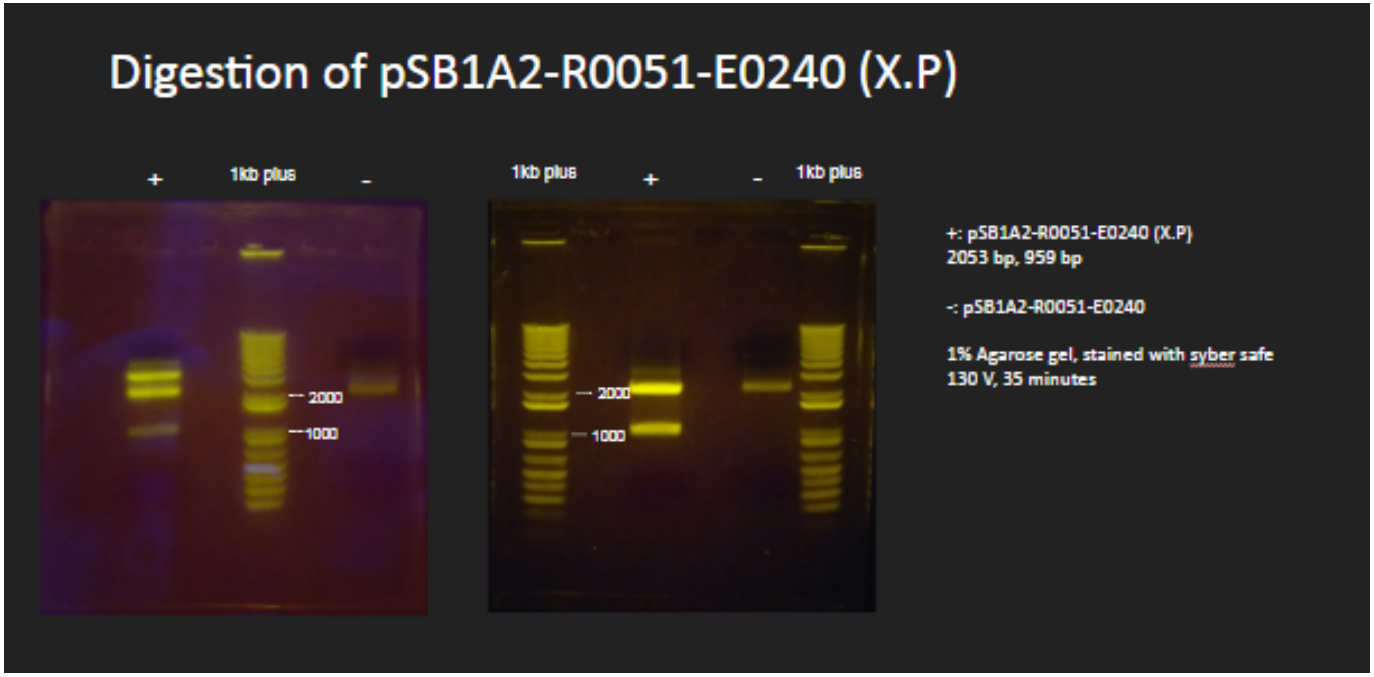

wrong band size: a bit larger

MONDAY, 9/25/2017

- Colony pcr of ligation products
  - pSB1C3-BBa_F2620-P0151-R0051-E0430
    - fwd primer: FW for full costruct part 2 (Tm: 60.4)
    - rev primer: ydjM-RBS-RV (Tm: 57.1) [primer box2 7C]
    - expected band size: 1875bp
  - pSB1C3-BBa_F2620-P0451-R0051-E0430
    - fwd primer: FW for full costruct part 2 (Tm: 60.4)
    - rev primer: ydjM-RBS-RV (Tm: 57.1) [primer box2 7C]
    - expected band size: 1875bp
  - +ve control: pSB1C3-BBa_F2620-P0151 (932bp)
  - NTC: no template control

  - pSB1C3-BBa_F2620-K081007-B0015

- fwd prim
- rev prim
- expected band size: 1289bp

- pSB1C3-BBa_F2620-S0109-B0015
  - fwd primer: 1st FWD (Tm: 56.6) [primer box6 9A]
  - rev primer: ydjM-RBS-RV (Tm: 57.1) [primer box2 7C]
  - expected band size: 1289bp
- +ve control: pSB1C3-F2620 (328bp)
- NTC: no template control

Table174

	A	B	C	D
1	Reagents (ul)	Volume (ul)	MasterMix (13X)	MasterMix (13X)
2	MQ	5.25	68.5	68.5
3	5X MyTaq Reaction Buffer	2	26	26
4	fwd	0.25	3.25	3.25
5	rev	0.25	3.25	3.25
6	Jessica's Taq	0.25	3	3
7	Template	dip	dip	
8	Total	8 uL		

Table175

We recovered unsaved changes to your entry. Click here to recover this data.

	A	B	C	D
1	Steps	Temperature (°C)	Time	
2	Initial denaturation	95	3 min	
3	Denaturation	95	15 s	
4	Annealing	55	30 s	2-4: 24 cycles
5	Extension	68	1min 23s	
6	Final extension	68	5 min	
7	Holding	10	infinity	

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- Ligation of pSB1C3-BBa_R0062-P0151-R0051-E0430
  P. S. pSB1C3-R0051-E0402

FRIDAY, 10/6/2017

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Miniprep of pSB1A2-BBa_R(

Table176

	Name	Concentration (ng/uL)	Protein contamination	Salt Contamination
1	pSB1A2-BBa_R0051-E0430 (set 1)	11.96	2.41	0.749
2	pSB1A2-BBa_R0051-E0430 (set 2)	24.73	1.801	0.925