

Sensing Log Book (August)

MONDAY, 6/12/2017

1. DNA extraction from kit plate
 - pSB1C3-BBa_C0051 (2014 spring distribution kit, plate 2, well 4B)
 - pSB1C3-BBa_T9002 (2013 spring distribution kit, plate 4 well 21I)
2. Transformation of pSB1C3-BBa_C0051, pSB1C3-BBa_T9002 and pSB1A2-BBa_C0261

| | Reagent | Volume (uL) |
|---|----------------|-------------|
| 1 | Competent cell | 50 |
| 2 | DNA template | 1 |
| 3 | Total Volume | 51 |

3. Spread plate
 - pSB1C3-BBa_C0051 (20 uL)
 - pSB1C3-BBa_C0051 (20 uL)
 - pSB1C3-BBa_T9002 (20 uL)
 - pSB1C3-BBa_T9002 (20 uL)
 - pSB1A2-BBa_C0261 (50 uL)

TUESDAY, 6/13/2017

1. Result of 12/6 Transformation plate:
 - pSB1A2-BBa_C0261: 2 colonies
 - pSB1C3-BBa_C0051: 4 colonies
 - pSB1C3-BBa_T9002: no colonies
2. Streaking of pSB1A2-BBa_C0261 and pSB1C3-BBa_T9002
3. Second transformation of pSB1C3-BBa_T9002

| Table11 | | |
|---------|----------------|-------------|
| | Reagent | Volume (uL) |
| 1 | Competent Cell | 50 |
| 2 | DNA Template | 1 |
| 3 | Total volume | 51 |

4. Protocol changed for the 2 transformation sample:
 - o Invitrogen protocol: 200 uL LB for recovery, 45s for heat shock, 2 minutes for cold shock
 - o iGEM protocol: 200 uL LB for recovery, 1 minute for heat shock, 5 minutes for cold shock
5. Spread plate
 - o 200 uL of sample pSB1C3-BBa_T9002 (from 12/6) for 1 hour 2nd recovery
 - o 200 uL of pSB1C3-BBa_T9002 (with 2 different protocol)
 total: 3 plates

WEDNESDAY, 6/14/2017

1. Results from (13/6) transformation plate and spread plate:
 - o pSB1C3-BBa_T9002 (second transformation): no colonies
2. Inoculation of pSB1A2-BBa_C0261 and pSB1C3-BBa_C0051
3. 3rd transformation of pSB1C3-BBa_T9002

| Table12 | | |
|---------|----------------|-------------|
| | Reagent | Volume (uL) |
| 1 | Competent cell | 50 |
| 2 | DNA Template | 1 |
| 3 | Total Volume | 51 |

4. Summer training protocol: 200 uL LB for recovery, 45s for heat shock, 2 minutes for cold shock
iGEM protocol: 200 uL LB for recovery, 1 minute for heat shock, 5 minutes for cold shock
5. Spread plate
 - o 200 uL of pSB1C3-BBa_T9002 (Summer training protocol) incubated on ice for 20 minutes before recovery

- 200 uL of pSB1C3-BBa_T9002 (with 2 different protocol) for 1 and a half hour of recovery
- total: 2 plates

THURSDAY, 6/15/2017

1. Result:
 - pSB1C3-BBa_T9002 (iGEM protocol): 3 colonies
 - pSB1C3-BBa_T9002 (Summer training protocol) ver 1.0: 0 colonies
 - pSB1C3-BBa_T9002 (Summer lab training protocol) ver 2.0: 0 colonies
2. Streak plate of pSB1C3-BBa_T9002 (iGEM protocol)
3. Miniprep of inoculated pSB1C3-BBa_C0051 and pSB1A2-BBa_C0261

| Table13 | | | | |
|---------|------------------|-----------------------|--------------------|-----------------------|
| | Sample | Protein Contamination | Salt Contamination | Concentration (ng/uL) |
| 1 | pSB1C3-BBa_C0261 | 1.807 | 1.548 | 64.94 |
| 2 | pSB1A2-BBa_C0051 | 1.848 | 2.043 | 542.5 |

Note: Abnormal concentration of host DNA due to miniprep error

4. Inoculation of pSB1C3-BBa_C0051 overnight
5. Plate streaking of pSB1C3-BBa_C0051 (2nd streak)

FRIDAY, 6/16/2017

1. Result: successful streaking of pSB1C3-BBa_T9002 and pSB1C3-BBa_C0051
2. Storage of streaked plate into fridge (pSB1C3-BBa_T9002 and pSB1C3-BBa_C0051)
3. Miniprep of pSB1C3-BBa_C0051
 - Protein contamination: 1.849
 - Salt contamination: 2.332
 - Concentration: 411.5 ng/uL
4. Self-electrophoresis: contamination of host DNA

MONDAY, 6/19/2017

1. DNA extraction from kit plate
 - BBa_J61002-J23100 (2010 kit plate 1, 18C)
 - BBa_J61002-J23113 (2009 kit plate 1, 20G)

- BBa_J61002-J23115 (2009 kit plate 1, 20K)
2. Transformation +spread plate
 - BBa_J61002_J23100
 - BBa_J61002_J23113
 - BBa_J61002_J23115
 - BBa_J61002_J23110
 - BBa_J61002_J23117
 3. Inoculation of pSB1C3-BBa_F2620 and pSB1A2-BBa_C0261 using CHL and AMP LB
 4. Gel electrophoresis using 1% gel (0.2g agarose, 0.2 uL midori green, 20 mL buffer)
 5. Digestion of pSB1C3-BBa_F2620 (expected band sizes = 3113 and 18 bp) and pSB1A2-BBa_C0261 (expected band size = 686 and 2053 bp)

| pSB1C3-BBa_F2620 | | |
|------------------|-----------------|-------------|
| | Reagents | Volume (uL) |
| 1 | S | 0.2 |
| 2 | P | 0 |
| 3 | DNA | 15.8 |
| 4 | Cutsmart Buffer | 1.8 |
| 5 | MQ | 0.4 |

| pSB1A2-BBa_C0261 | | |
|------------------|-----------------|-------------|
| | Reagents | Volume (uL) |
| 1 | S | 0.2 |
| 2 | P | 0.2 |
| 3 | DNA | 14.7 |
| 4 | Cutsmart Buffer | 1.8 |
| 5 | MQ | 1.6 |

1. Inoculation and streak plate of J23100 and J23117
2. Second transformation of J23110, J23113, J23115 due to no colonies shown before
3. Gel purification of pSB1C3-BBa_F2620 and BBa_C0261

Table14

| | Sample | Concentration (ng/uL) | Protein contamination | Salt Contamination |
|---|-----------------------------|--------------------------|--------------------------|-----------------------|
| 1 | pSB1C3-BBa_F2620 (digested) | 9.216 | 0.007 | 2.866 |
| 2 | BBa_C0261 | 4.53 | 0.177 | 1.79 |

4. Ligation of digested product

Table15

| | Reagents | Volume (uL) | negative control volume (uL) |
|---|---------------|-------------|------------------------------------|
| 1 | T4 ligase | 0.5 | 0 |
| 2 | Ligase buffer | 1 | 1 |
| 3 | backbone | 3.62 | 3.62 |
| 4 | insert | 4.88 | 4.88 |
| 5 | MQ | 0 | 0.5 |
| 6 | Total Volume | 10 | 10 |

5. Transformation of ligated product and spread plate
6. Miniprep of pSB1C3-BBa_F2620 and pSB1A2-BBa_C0261

Table16

| | Sample | Concentration (ng/uL) | Protein contamination | Salt Contamination |
|---|------------------|--------------------------|--------------------------|-----------------------|
| 1 | pSB1C3-BBa_F2620 | 116.1 | 2.272 | 1.840 |
| 2 | pSB1A2-BBa_C0261 | 86.96 | 2.024 | 1.852 |

WEDNESDAY, 6/21/2017

1. Streaking and inoculation of BBa_J61002-J23110
2. Transformation result of BBa_J61002-J23110: 2 colonies (red)
3. Colony PCR and gel electrophoresis
 - 5 white colonies sample from pSB1C3-BBa_F2620-C0261
 - 1 sample from BBa_J61002-J23100
 - 1 sample from BBa_J61002-J23117

Table17

| | Reagents | Volume (uL) | Mastermix (x15 uL) |
|---|-----------------------------|-------------|-----------------------|
| 1 | MQ | 12.875 | 193.125 |
| 2 | 5x my Taq | 4 | 60 |
| 3 | 10 uM VF2 | 0.5 | 7.5 |
| 4 | 10 uH VR | 0.5 | 7.5 |
| 5 | Jessica's Taq polymerase | 0.125 | 1.875 |
| 6 | Template | 2 | - |
| 7 | Total | 20 | - |

Table18

| | Steps | Temperature (Celcius) | Time |
|---|-------------------------|--------------------------|--------------|
| 1 | Initial Denaturation | 95 | 3 minutes |
| 2 | Denaturation | 95 | 30s |
| 3 | Annealing | 55 | 1 minute |
| 4 | Extension | 68 | 2 minute 15s |
| 5 | Final extension | 68 | 5m ins |
| 6 | holding | 4 | infinity |

4. Transformation + spread plate

- BBa_J61002-J23100 (from prepared stock)
- BBa_J61002-J23100 (newly extracted from 2011 kit plate)
- BBa_J61002-J23117 (from prepared stock)
- BBa_J61002-J23117 (newly extracted from 2011 kit plate)
- BBa_J61002-J23113 (newly extracted from 2011 kit plate)
- BBa_J61002-J23115 (newly extracted from 2011 kit plate)

WEDNESDAY, 8/2/2017

Table7

| | A | B | C |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |

PCR set 1 (pSB1C3-BBa_F2620)

Table49

| | A | B | C |
|---|-----------------------|--------|----------------|
| 1 | Reagent | Volume | MasterMix x3.5 |
| 2 | Water | 12.4 | 43.4 |
| 3 | 5x Q5 reaction buffer | 4 | 14 |
| 4 | dNTP | 0.4 | 1.4 |
| 5 | 10 μ M Fwd | 1 | 3.5 |
| 6 | 10 μ M Rev | 1 | 3.5 |
| 7 | Q5 polymerase | 0.2 | 0.7 |
| 8 | Template (μ l) | 1 / | |
| 9 | Total Volume | 20 | |

THURSDAY, 8/3/2017

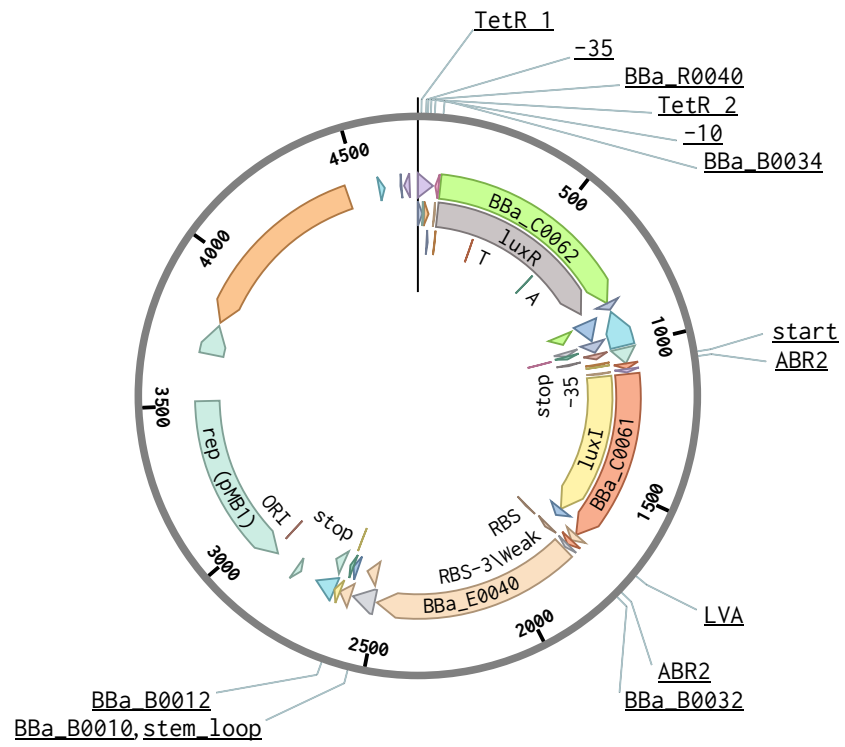
1. Gel electrophoresis of set 1a, 1b, 1 (-) and pSB1A2-BBa_R0063
 - 120V, 30 minutes
 - 1% agarose gel

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

Table50

| | A | B | C | D |
|---|---------------------------------|-------|-------|------------------|
| 1 | | 1a | 1b | pSB1A2-BBa_R0063 |
| 2 | DNA Concentration (μ g/ml) | 15.54 | 5.201 | 2.147 |
| 3 | A260/A280 | 1.629 | 2.363 | 1.872 |
| 4 | A260/A230 | 0.689 | 1.000 | 1.000 |

🔄 pSB1C3-BBa_F2620-C0261-E0240 (ABR2)



FRIDAY, 8/4/2017

1. Gibson assembly of
 - a) pSB1C3-BBa_F2620, BBa_C0261 (ABR1), BBa_E0240(ABR1)
 - b) pSB1C3-BBa_F2620, BBa_C0261 (ABR1), BBa_E0240(ABR2)

2. Ligate pSB1A2-BBa_R0063 iwth antisense 1 and 2

3. Transformation of

- a) pSB1C3-BBa_F2620-C0261-E0240 (ABR 1)
- b) pSB1C3-BBa_F2620-C0261-E0240 (ABR 1) (-)
- c) pSB1C3-BBa_F2620-C0261-E0240 (ABR2)
- d) pSB1C3-BBa_F2620-C0261-E0240 (ABR 2) (-)
- e) pSB1A2-BBa_R0063-Anti 1
- f) pSB1A2-BBa_R0063-Anti 1 (-)
- g) pSB1A2-BBa_R0063-Anti 2
- h) pSB1A2-BBa_R0063-Anti 2 (-)

SATURDAY, 8/5/2017

Result:

- 1. pSB1A2-BBa_R0063-Anit 1 (+) :Presence of colonies
- 2. pSB1A2-BBa_R0063-Anti 2 (+): Presence of colonies
- 3. pSB1A2-BBa_R0063- Anti (-): 3 colonies
- 4. Others have no colonies

Colony PCR: Replace 20ul of saline to MQ

| | A | B | C |
|---|------------------|------------------|------------|
| 1 | | Each volume (ul) | MX*13 (ul) |
| 2 | MQ | 14.35 | 186.55 |
| 3 | 5X mg Taq Buffer | 4 | 52 |
| 4 | dNTP | 0.5 | 6.5 |
| 5 | 10 uM VF2 | 0.5 | 6.5 |
| 6 | 10 uM VR | 0.5 | 6.5 |
| 7 | Jessica's Taq | 0.15 | 1.95 |
| 8 | Template DNA | 0.5 | / |
| 9 | Total | 20 | |

Table2

| | A | B |
|---|--------|-------------------------|
| 1 | Sample | |
| 2 | 1-3 | Antisense RNA 1 |
| 3 | 4-6 | Antisense RNA 2 |
| 4 | m+ | Mili Q with B0030-phlf |
| 5 | m- | Mili Q pSB1A2-BBa_R0063 |
| 6 | s+ | Saline with B0030-phlf |
| 7 | s- | Saline pSB1A2-BBa_R0063 |
| 8 | s | Saline only |
| 9 | m | MQ only |

MONDAY, 8/7/2017

1. Gel electrophoresis of digested PSB1C3-BBa_F2620, set 1a, digested pSB1A2-BBa_C0261, set 2a & 2b, digested pSB1A2-BBa_E0240 and set 3a & 3b
2. Inoculation of pSB1A2-BBa_R0063, antisense RNA 1 & 2

TUESDAY, 8/8/2017

1. Digestion of pSB1C3-BBa_F2620, pSB1A2-BBa_C0261 and pSB1A2-BBa_E0240

Table3

| | A | B | C | D |
|---|---------------------------|------------------|------------------|------------------|
| 1 | | pSB1C3-BBa_F2620 | pSB1A2-BBa_C0261 | pSB1A2-BBa_E0240 |
| 2 | DNA Concentration (ng/ul) | 116.1 | 86.96 | 21.72 |
| 3 | DNA Mass | 60 | 60 | 60 |
| 4 | Water (ul) | 15.5 | 15.1 | 13.0 |
| 5 | DNA (ul) | 0.51 | 0.69 | 2.76 |
| 6 | Ecol | 0.2 | 0.2 | 0.2 |
| 7 | PstI | 0 | 0.2 | 0.2 |
| 8 | Cutsmart (ul) | 1.8 | 1.8 | 1.8 |
| 9 | Total Volume (ul) | 18 | 18 | 18 |

2. Gel electrophoresis of:

Table4

| | A | B |
|---|-------|---------------------------------|
| 1 | F2620 | Digested pSB1C3-BBa_F2620 (E) |
| 2 | 1a | PCR product of 1a from 1/8 |
| 3 | C0261 | Digested pSB1A2-BBa_C0261 (E,P) |
| 4 | 2a | PCR product of 2a from 31/7 |
| 5 | 2b | PCR product of 2b from 31/7 |
| 6 | E0240 | Digested pSB1A2-BBa_E0240 (E,P) |
| 7 | 3a | PCR product 3a from 14/7 |
| 8 | 3b | PCR product 3b from 14/7 |

3. PCR of set 1,2a,2b,3a,3b using previous PCR products as template (MasterMix recipe is the same as before)

Table5

| | A | B | C | D | E | F | G | H | I | J |
|---|----------------------|------------------|--------|--------|------------------|--------|--------|------------------|--------|--------|
| 1 | | 1 | | | 2a,2b | | | 3a, 3b | | |
| 2 | | Temperature (°C) | Time | Cycles | Temperature (°C) | Time | Cycles | Temperature (°C) | Time | Cycles |
| 3 | Initial denaturation | 98 | 30s | 1 | 98 | 30s | 1 | 98 | 30s | 1 |
| 4 | Denaturation | 98 | 10s | 33 | 98 | 10s | 33 | 98 | 10s | 33 |
| 5 | Annealing | 60 | 15s | 33 | 56.2 | 15s | 33 | 58.7 | 15s | 33 |
| 6 | Extension | 72 | 30s | 33 | 72 | 30s | 33 | 72 | 30s | 33 |
| 7 | Final extension | 72 | 2 mins | 1 | 72 | 2 mins | 1 | 72 | 2 mins | 1 |

4. Inoculation of pSB1A2-BBa_R0063-Anti 2

WEDNESDAY, 8/9/2017

1. Gel electrophoresis of PCR products :

F2620: digested pSB1C3-BBa_F2620 (E) from 8/8

1a: PCR of set 1 using 1a PCR product (1/8) as template

C0261: digested pSB1A2-BBa_C0261 (E,P) from 8/8

2a: PCR of 2a using 2a PCR product (31/7) as template

2b: PCR of 2a using 2b PCR product (31/7) as template

E0240: digested pSB1A2-BBa_E0240 (E,P)

3a: PCR of 3a using 3a PCR product (14/7) as template

3b: PCR of 3b using 3b PCR product (14/7) as template

2. Gel purification

Table6

| | A | B | C | D | E |
|---|-----------|-------|-------|-------|-------|
| 1 | | 2a | 2b | 3a | 3b |
| 2 | DNA | 12.86 | 11.58 | 13.56 | 12.41 |
| 3 | A260/A280 | 1.874 | 2.529 | 2.438 | 1.937 |
| 4 | A260/A230 | 0.404 | 1.350 | 0.258 | 0.106 |

3. Miniprep of pSB1A2-BBa_R0063-Anti1 and Anti2

Table8

| | A | B | C |
|---|-----------|--------------------------------|--------------------------------|
| 1 | | pSB1A2- BBa_R0063- Anti1 | pSB1A2- BBa_R0063- Anti2 |
| 2 | DNA | 95.47 | 121.8 |
| 3 | A260/A280 | 1.819 | 1.823 |
| 4 | A260/A230 | 2.196 | 2.037 |

THURSDAY, 8/10/2017

1. Gibson assembly of

a. Old: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(31/7), BBa_E0240(ABR1)(14/7)

b. New: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(9/8), BBa_E0240(ABR1)(9/8)

N55: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(31/7), BBa_E0240(ABR1)(14/7)(55°C)

N45: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(31/7), BBa_E0240(ABR1)(14/7)(45°C)

N35: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(31/7), BBa_E0240(ABR1)(14/7)(35°C)

O55: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(9/8), BBa_E0240(ABR1)(9/8)(55°C)

O45: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(9/8), BBa_E0240(ABR1)(9/8)(45°C)

O35: pSB1C3-BBa_F2620 (2/8), BBa_CO261(ABR1)(9/8), BBa_E0240(ABR1)(9/8)(35°C)

(-): pSB1C3-BBa_F2620 (2/8), MQ

2. Digestion of pSB1A2-BBa_R0063-Anti1 and 2 and pSB1A2-BBa_R0063 as negative control at (X,S) to check the band size of R0063-Anti1 and 2

| | A | B | C | D |
|---|--------------------------|------------------------|------------------------|------------------|
| 1 | | pSB1A2-BBa_R0063-Anti1 | pSB1A2-BBa_R0063-Anti2 | pSB1A2-BBa_R0063 |
| 2 | Exptected band size (bp) | 253 | 254 | 159 |

FRIDAY, 8/11/2017

1. Digestion of pSB1A2-BBa_R0063-andit 1 & 2 and pSB1C3-BBa_R0063

| | A | B | C | D |
|----|---------------|-------------------------|-------------------------|------------------|
| 1 | | psb1A2-BBa_R0063 anti 1 | psb1A2-BBa-R0063 anti 2 | psb1A2-BBa-R0063 |
| 2 | DNA mass | 300 | 300 | 300 |
| 3 | water | 13.2 | 12.4 | 11.4 |
| 4 | DNA | 2.58 | 3.45 | 4.41 |
| 5 | EcoRI | 0.2 | 0.2 | 0.2 |
| 6 | Pst1 | 0.2 | 0.2 | 0.2 |
| 7 | Cutsmart | 1.8 | 1.8 | 1.8 |
| 8 | Total volume | 18 | 18 | 18 |
| 9 | expected size | 286 | 287 | 192 |
| 10 | DNA conc | 116.1 | | 67.97 |

Negative control:

| | A | B | C | D |
|---|-------|--------------------------------|--------------------------------|----------------------|
| 1 | | psb1A2- BBa_R0063 anti 1 | psb1A2- BBa_R0063 anti 2 | psb1A2- BBa_R0063 |
| 2 | water | 15.4 | 14.6 | 13.6 |
| 3 | DNA | 2.58 | 3.45 | 4.41 |

MONDAY, 8/14/2017

1. colony PCR of psb1A2-BBa_R0063 antisense RNA 1 and 2, psb1A2-BBa_R0063

| | A | B |
|---|----------------------|------------|
| 1 | | master mix |
| 2 | 5x my tag | 18 |
| 3 | dNTP | 2.25 |
| 4 | 10mM VF | 2.25 |
| 5 | 10mM VR | 2.25 |
| 6 | Taq polymerase | 0.675 |
| 7 | diluted tempelate | 1 |
| 8 | water | 62.325 |
| 9 | total volume | 20 |

2. colony checking of gibson assembly products

| Table22 | | |
|---------|--------|-----|
| | A | B |
| 1 | sample | |
| 2 | 1-3 | N55 |
| 3 | 4-6 | N45 |
| 4 | 7-8 | N35 |
| 5 | 9-12 | 55 |
| 6 | 13-15 | 45 |
| 7 | 16-18 | 35 |
| 8 | 19-21 | - |

3. Gel electrophoresis of colony PCR product and colony cracking product from (14/8)

TUESDAY, 8/15/2017

1. Colony PCR of psb1A2-BBa_R0063 anti 1 and anti 2, psb1A2-BBa_R0063

| Table23 | | |
|---------|--------|-------------------------|
| | A | B |
| 1 | sample | |
| 2 | 1-5 | psb1A2-BBa_R0063 anti 1 |
| 3 | 6-10 | psb1A2-BBa_R0063 anti 2 |
| 4 | R | psb1A2-BBa_R0063 |
| 5 | (-) | MQ |

2. gel electrophoresis and colony pcr from (15/8)

3. inoculation of pSB1C3-BBa-F2620-C0261-E0240 (ABR1)(GA sample 14,15)

WEDNESDAY, 8/16/2017

1. colony pcr of psb1A2-BBa_R0063 anti 1 and anti 2, psb1A2-BBa_R0063

Table24

| | A | B |
|---|--------|-------------------------|
| 1 | sample | |
| 2 | 1-5 | psb1A2-BBa_R0063 anti 1 |
| 3 | 6-10 | psb1A2-BBa_R0063 anti 2 |
| 4 | R | psb1A2-BBa_R0063 |
| 5 | (-) | MQ |

2. Miniprep of pSB1C3-BBa-F2620-C0261-E0240 (ABR1)(GA sample 14,15)

Table25

| | A | B | C |
|---|-----------|-----------|-----------|
| 1 | | sample 14 | sample 15 |
| 2 | DNA conc | 188.8 | 191.5 |
| 3 | A260/A280 | 1.819 | 1.815 |
| 4 | A260/230 | 2.524 | 2.471 |

3. restriction check of pSB1C3-BBa-F2620-C0261-E0240 (ABR1)(GA sample 14,15)

enzymes Hind III and NdeI

expected size: 1879, 2827 bp

THURSDAY, 8/17/2017

1. colony pcr of psb1A2-BBa_R0063 anti 1 and 1, psb1A2-BBa_R0063

FRIDAY, 8/18/2017

1. colony pcr of psb1A2-BBa_R0063 anti 1 and 2, psb1A2-BBa_R0063

Table26

| | A | B |
|---|----------------|------------|
| 1 | | master mix |
| 2 | 5x my tag | 4 |
| 3 | dNTP | 0.5 |
| 4 | 10mM VF | 0.5 |
| 5 | 10mM VR | 0.5 |
| 6 | Taq polymerase | 0.15 |
| 7 | tempelate | 0 |
| 8 | water | 14.85 |
| 9 | total volume | 20 |

MONDAY, 8/21/2017

1. digestoin of IDT antisense RNA 1 and 2, pSB1C3-BBa-R0063 (for ligation)

Table27

| | A | B | C | D |
|----|----------|------------|------------|------------------|
| 1 | | IDT anti 1 | IDT anti 2 | pSB1C2-BBa-R0063 |
| 2 | DNA conc | 10 | 10 | 67.97 |
| 3 | DNA msas | 100 | 100 | 200 |
| 4 | water | 5.8 | 5.8 | 13.1 |
| 5 | DNA | 10 | 10 | 2.94 |
| 6 | Xbal | 0.2 | 0.2 | 0 |
| 7 | SpeI | 0 | 0 | 0.2 |
| 8 | PstI | 0.2 | 0.2 | 0.2 |
| 9 | Cutsmart | 1.8 | 1.8 | 1.8 |
| 10 | total | 18 | 18 | 18 |

negative control

Table28

| | A | B | C | D |
|---|-------|-------|-------|------------------|
| 1 | | IDT 1 | IDT 2 | pSB1C3-BBa-R0063 |
| 2 | water | 16 | 16 | 16 |
| 3 | DNA | 2 | 2 | 2 |

2. gibson assembly of pSB1C3-BBa-F2620 (2/8), BBa_C0261(ABR2)(31/7), BBa-E0240 (ABR2) (14/7), PSB1C3-BBa-F2620(2/8), BBa_C0261(ABR2) (9/8), BBa_E0240 (9/8)

Table29

| | A | B | C | D | E |
|---|------------------|-------|--------|--------|------------|
| 1 | | conc | length | volume | mole ratio |
| 2 | PSB1C3-BBa-F2620 | 15.54 | 3131 | 0.748 | 1 |
| 3 | BBa-CO261 | 6.195 | 700 | 2.10 | 5 |
| 4 | BBa_E0240 | 7.901 | 916 | 2.15 | 5 |

TUESDAY, 8/22/2017

1. Amplification of antisense RNA 1&2

Table30

| | A | B | C |
|---|--------------------|-------------------|-------------------|
| 1 | | Volume (μ L) | Master mix (*3.5) |
| 2 | MQ | 32.5 | 113.75 |
| 3 | Q5 reaction buffer | 10 | 35 |
| 4 | 10 mM dNTP | 1 | 3.5 |
| 5 | VF2 | 2.5 | 8.75 |
| 6 | VR | 2.5 | 8.75 |
| 7 | Q5 | 0.5 | 1.75 |
| 8 | Template | 1 | |
| 9 | Total | 50 | |

Condition :

Table31

| | A | B | C | D |
|---|-------------------------|--------------------------|--------|-------|
| 1 | | Temperature (Celcius) | Time | Cycle |
| 2 | Initial Denaturation | 98 | 30 s | 1 |
| 3 | Denaturation | 98 | 10 s | 35 |
| 4 | Annealing | 66 | 15 s | 35 |
| 5 | Extension | 72 | 15 s | 35 |
| 6 | Final Extension | 72 | 2 mins | 1 |
| 7 | Holding | | | |

2. PCR Cleanup of Antisense RNA 1&2

Table32

| | A | B | C |
|---|----------------------|-----------------|--------------------|
| 1 | | Antisense RNA 1 | Antisense RNA 2 |
| 2 | DNA Conc. (µg/ml) | 34.92 | 32.27 |
| 3 | A260/A280 | 1.846 | 1.766 |
| 4 | A260/A230 | 1.347 | 1.066 |

3. Ligation of pSB1A2-BBa_R0063 with antisense rna 1&2

Table33

| | A | B | C | D | E |
|----|---------------------------|-------------------------------------|------------------------------------|------------------------------------|-------------------------------------|
| 1 | | pSB1A2- BBa_R0063- anti 1 (+) | pSB1A2- BBa_R0063- anti 1(-) | pSB1A2- BBa_R0063- anti 2(+) | pSB1A2- BBa_R0063- anti 2 (-) |
| 2 | Insert length | 169 | 169 | 170 | 170 |
| 3 | Backbone length | 2221 | 2221 | 2221 | 2221 |
| 4 | Insert concentration | 2.108 | 2.109 | 3.26 | 3.27 |
| 5 | Backbone concentration | 7.156 | 7.157 | 7.156 | 7.157 |
| 6 | Insert volume | 4.68 | 0 | 4.07 | 0 |
| 7 | Backbone volume | 1.81 | 1.81 | 2.43 | 2.44 |
| 8 | m μ | 2 | 8.18 | 2 | 7.57 |
| 9 | t4 ligase | 0.5 | - | 0.5 | - |
| 10 | Ligase buffer | 1 | - | 1 | - |

4. Gel Purification of digested R0063, Antisense 1&2

Table34

| | A | B | C | D |
|---|-----------|----------------|-------------|-------------|
| 1 | | Digested R0063 | Antisense 1 | Antisense 2 |
| 2 | DNA conc. | 7.156 | 2.108 | 3.260 |
| 3 | A260/A280 | 17.22 | 1.903 | 2.587 |
| 4 | A260/A230 | 0.705 | 0.297 | 0.318 |

5. Digestion of antisense RNA 1 & 2 as inserts

Table35

| | A | B | C | D | E |
|----|----------------------|-------|-----|-------|-----|
| 1 | | RNA 1 | (-) | RNA 2 | (-) |
| 2 | DNA conc. (ng/ul) | 34.92 | / | 32.27 | / |
| 3 | DNA mass (g) | 230 | / | 230 | / |
| 4 | Water (ul) | 9.214 | 17 | 8.67 | 17 |
| 5 | DNA (ul) | 6.39 | 1 | 7.13 | 1 |
| 6 | Xbal | 0.2 | / | 0.2 | / |
| 7 | SpeI | 0.2 | / | 0.2 | / |
| 8 | PstI | 0.2 | / | 0.2 | / |
| 9 | cutsmart | 1.8 | / | 1.8 | / |
| 10 | total volume | 18 | 18 | 18 | 18 |

6. Transformation of pSB1A2_BBa_R0063-anti1 & pSB1A2-BBa_R0063-anti2

THURSDAY, 8/24/2017

1. Colony RPC of pSB1A2_BBa_R0063-anti1 & pSB1A2-BBa_R0063-anti2

Table36

| | A | B | C |
|---|-----------------------------|-------------|---------|
| 1 | | Volume (ul) | MX*16.5 |
| 2 | Water | 14.85 | 245.025 |
| 3 | 5X MyTaq Reaction buffer | 4 | 66 |
| 4 | dNTP | 0.5 | 8.25 |
| 5 | 10uM VF | 0.5 | 8.25 |
| 6 | 10uM VR | 0.5 | 8.25 |
| 7 | Taq polymerase | 0.15 | 2.475 |
| 8 | DNA template | 0.5 | |
| 9 | Total volume | 20 | |

Sample 1-7: pSB1A2-BBa_R0063-ant1

Sample 8-14: pSB1A2-BBa_R0063-anti2

R: pSB1A2-BBa_R0063

(-): MQ only

2. Colony cracking of pSB1C3-BBa_F2620-C0261-E0240 (ABR2)

Sample 1-22: pSB1C3-BBa_F2620-C0261-E0240 (ABR2)

F: pSB1C3-BBa_F2620

FCE: pSB1C3-BBa_F2620-C0261-E0240

3. Inoculation of pSB1C3-BBa_F2620-C0261-E0240 (ABR2) and pSB1A2-BBa_R0063-anti2

FRIDAY, 8/25/2017

1. Colony PCR of pSB1A2-BBa_R0063-anti1 as (24/8)

Sample 1-14: pSB1A2-BBa_R0063-anti1

2. Miniprep of pSB1A2-BBa_R0063-anti2 (Sample 10) and pSB1C3-BBa_F2620-C0261-E0240 (ABR2)

Result:

Table37

| | A | B | C |
|---|----------------------|--------------------------------|--|
| 1 | | pSB1A2- BBa_R0063- anti2 | pSB1C3- BBa_F2620- C0261-E0240 (ABR2) |
| 2 | DNA conc. (ug/ml) | 114.9 | 43.88 |
| 3 | A260/A280 | 1.828 | 1.837 |
| 4 | A260/A230 | 1.918 | 1.918 |

3. Restriction check of pSB1C3-BBa_F2620-C0261-E0240 (ABR2)

Table38

| | A | B | C |
|----|-----------------------|--|-----|
| 1 | | pSB1C3- BBa_F2620- C0261-E0240 (ABR2) | (-) |
| 2 | DNA conc. (ng/ul) | 43.88 | / |
| 3 | DNA mass (g) | 100 | / |
| 4 | Water (ul) | 13.5 | 17 |
| 5 | DNA (ul) | 2.28 | 1 |
| 6 | HindIII-HF | 0.2 | / |
| 7 | NdeI | 0.2 | / |
| 8 | cutsmart (ul) | 1.8 | / |
| 9 | total volume | 18 | / |
| 10 | Expected size (bp) | 1881, 2827 | / |

MONDAY, 8/28/2017

1. Inoculation of pSB1C3-BBa_F2620-C0261-E0240 and pSB1A2-BBa_R0063-anti1 (Sample 4)

TUESDAY, 8/29/2017

1. Miniprep of pSB1A2-BBa_R0063-anti1 and pSB1C3-BBa_F2620-C0261-E0240

Result:

Table39

| | A | B | C |
|---|----------------------|--------------------------------|--------------------------------------|
| 1 | | pSB1A2- BBa_R0063- anti1 | pSB1C3- BBa_F2620- C0261-E0240 |
| 2 | DNA conc. (ug/ml) | 38.77 | 71.17 |
| 3 | A260/A280 | 1.781 | 1.817 |
| 4 | A260/A230 | 1.781 | 1.968 |

2. Digestion of pSB1C3-BBa_F2620-C0261-E0240, pSB1C3-BBa_F2620-C0261-E0240(ABR1), pSB1C3-BBa_F2620-C0261-E0240(ABR2), pSB1A2-BBa_R0063-anti1 & pSB1A2-BBa_R0063-anti2

Table40

| | A | B | C | D | E | F |
|----|----------------------|-------|-----------|-----------|---------------------|---------------------|
| 1 | | FCE | FCE(ABR1) | FCE(ABR2) | BBa_R0063- anti1 | BBa_R0063- anti2 |
| 2 | DNA conc. (ng/ul) | 71.17 | 188.8 | 43.88 | 38.77 | 114.9 |
| 3 | DNA mass (g) | 500 | 500 | 500 | 500 | 500 |
| 4 | Water (ul) | 8,77 | 13.2 | 4.41 | 2.9 | 11.3 |
| 5 | DNA (ul) | 7.03 | 2.65 | 11.4 | 12.9 | 4.35 |
| 6 | Xbal | 0 | 0 | 0 | 0.2 | 0.2 |
| 7 | SpeI | 0.2 | 0.2 | 0.2 | 0 | 0 |
| 8 | PstI | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 9 | cutsmart | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| 10 | total volume | 18 | 18 | | 18 | 18 |

Negative control:

Table41

| | A | B | C | D | E | F |
|---|-----------|-----|-----------|-----------|-----------------|-----------------|
| 1 | | FCE | FCE(ABR1) | FCE(ABR2) | BBa_R0063-anti1 | BBa_R0063-anti2 |
| 2 | DNA(ul) | 16 | 16 | 16 | 16 | 16 |
| 3 | Water(ul) | 2 | 2 | 2 | 2 | 2 |

WEDNESDAY, 8/30/2017

1. Digestion of pSB1C3-BBa_F2620-C0261-E0240, pSB1C3-BBa_F2620-C0261-E0240(ABR1), pSB1C3-BBa_F2620-C0261-E0240(ABR2), pSB1A2-BBa_R0063-anti1 & pSB1A2-BBa_R0063-anti2
2. Gel purification of pSB1C3-BBa_F2620-C0261-E0240, pSB1C3-BBa_F2620-C0261-E0240(ABR1), pSB1C3-BBa_F2620-C0261-E0240(ABR2), pSB1A2-BBa_R0063-anti1 & pSB1A2-BBa_R0063-anti2

Result:

Table42

| | A | B | C | D | E | F |
|---|-----------|-------|-----------|-----------|-----------------|-----------------|
| 1 | | FCE | FCE(ABR1) | FCE(ABR2) | BBa_R0063-anti1 | BBa_R0063-anti2 |
| 2 | DNA(ul) | 10.58 | 15.02 | 15.79 | 13.38 | 11.95 |
| 3 | A260/A280 | 1.608 | 1.665 | 1.796 | 1.813 | 1.72 |
| 4 | A260/A230 | 0.779 | 0.556 | 0.378 | 0.817 | 0.599 |

3. Inoculation of pSB1C3-BBa_F2620-C0261-E0240(ABR2)

THURSDAY, 8/31/2017

1. Make

- a) pSB1C3-BBa_F2620-C0261-E0240-BBa_R0063-anti1
- b) pSB1C3-BBa_F2620-C0261-E0240-BBa_R0063-anti2
- c) pSB1C3-BBa_F2620-C0261-E0240(ABR1)-BBa_R0063-anti1
- d) pSB1C3-BBa_F2620-C0261-E0240(ABR2)-BBa_R0063-anti2

Table43

| | A | B | C | D | E | F | G |
|----|------------------------|---|------|-------|---|------|-------|
| 1 | | a) pSB1C3-BBa_F2620-C0261-E0240-BBa_R0063-anti1 | | | b) pSB1C3-BBa_F2620-C0261-E0240-BBa_R0063-anti2 | | |
| 2 | | (+) | (-) | Ratio | (+) | (-) | Ratio |
| 3 | insert length | 253 | | | 254 | | |
| 4 | backbone length | 4666 | | | 4666 | | |
| 5 | insert conc. (ng/ul) | 13.38 | | | 11.95 | | |
| 6 | backbone conc. (ng/ul) | 10.58 | | | 10.58 | | |
| 7 | insert volume | 1.95 / | | 10 | 2.11 / | | 10 |
| 8 | backbone volume | 4.55 | 4.55 | 1 | 4.39 | 4.39 | 1 |
| 9 | MQ | 2 | 5.45 | | 2 | 5.61 | |
| 10 | T4 ligase | 0.5 / | | | 0.5 / | | |
| 11 | Ligase buffer | 1 / | | | 1 / | | |
| 12 | | | | | | | |