LIGATION/RESTRICTION cutting protocol

Clone	Description
1D	**Promoter--BBa_R0010** Part-only sequence
2M	**Ribosome binding site (RBS)--BBa_B0034** Part-only sequence
24C	**Terminator--BBa_B0014** Part-only sequence
6A	**CFP--BBa_E0020** engineered cyan fluorescent protein derived from A. victoria GFP
14K	**GFP--BBa_E0040** green fluorescent protein derived from jellyfish
24E	**YFP--BBa_E0030** enhanced yellow fluorescent protein derived from A. victoria GFP

9 Aug

-Order primers

Suffix	F_BBa_G1002
Prefix	R_BBa_G1003

11 Aug- 30 Aug

Learn the basic technique for lab works(pipetting, autoclave, nanodrop, prepare common medium, transformation, inoculation, PCR, PCR purification, argrose gel electrophoresis, PAGE)

11 Aug- 10 Sep

Test the composite system with the use of fluorescent protein to see if

it is working

23 Aug

Preparation of Competent Cells

-aliquot the competent cells, stored in liquid nitrogen and then -80C refrigerator

Transformation

-Kit1 1A

-Kit1 1C

900ul LB

50ul X-gal

50ul 0.1M IPTG (stock: 0.4M)

50ul cell pellets

Plates:

1. Non-transformed competent cell only w/o Amp
2. Transformed with Kit1 1A w/o Amp
3. Transformed with Kit1 1A with Amp
4. Transformed with Kit1 1C with Amp
5. Transformed with Kit1 1C w/o Amp

24 Aug

Plates Result

1	Non-transformed competent Cells	without Amp	cell grow, no RFP
2	Transformed cell—1A	without Amp	cell grow, no RFP
3	Transformed cell—1A	with Amp	cell grow, WITH RFP
4	Transformed cell—1C	without Amp	cell grow, without RFP
5	Transformed cell—1C	with Amp	little cell grow, without RFP

30 Aug

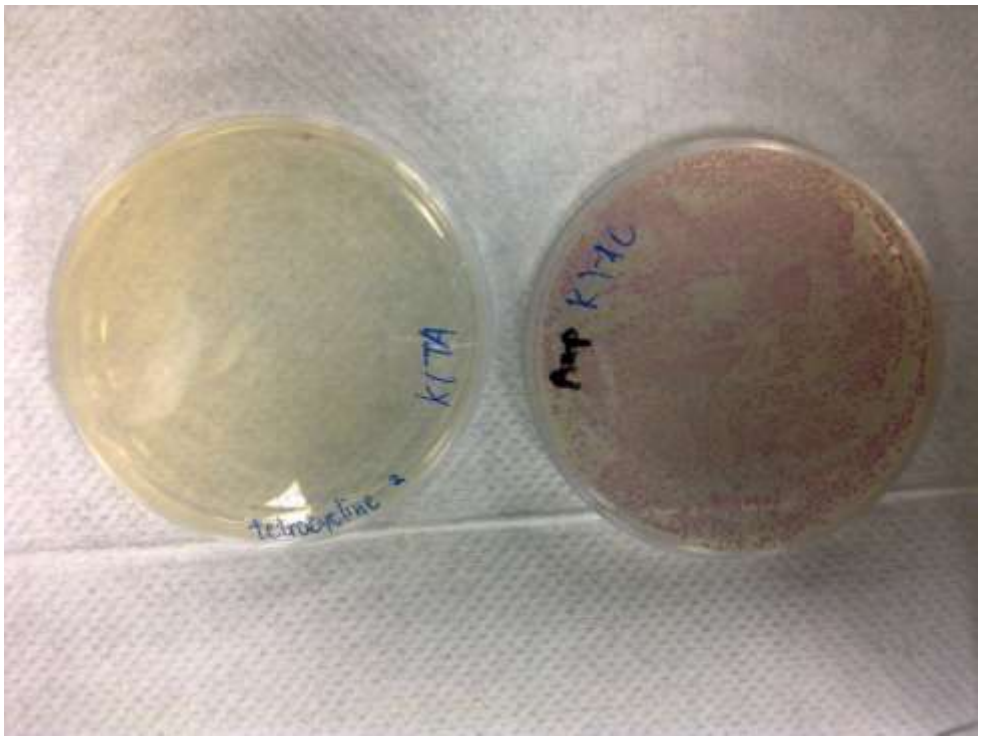
-Receive Rci gene and message gene

1 Sep

-Competent cells (DH-5Alpha) were successfully prepared.

5 Sep

- Transformation of RFP system in pSB1A3 and pSB1T3

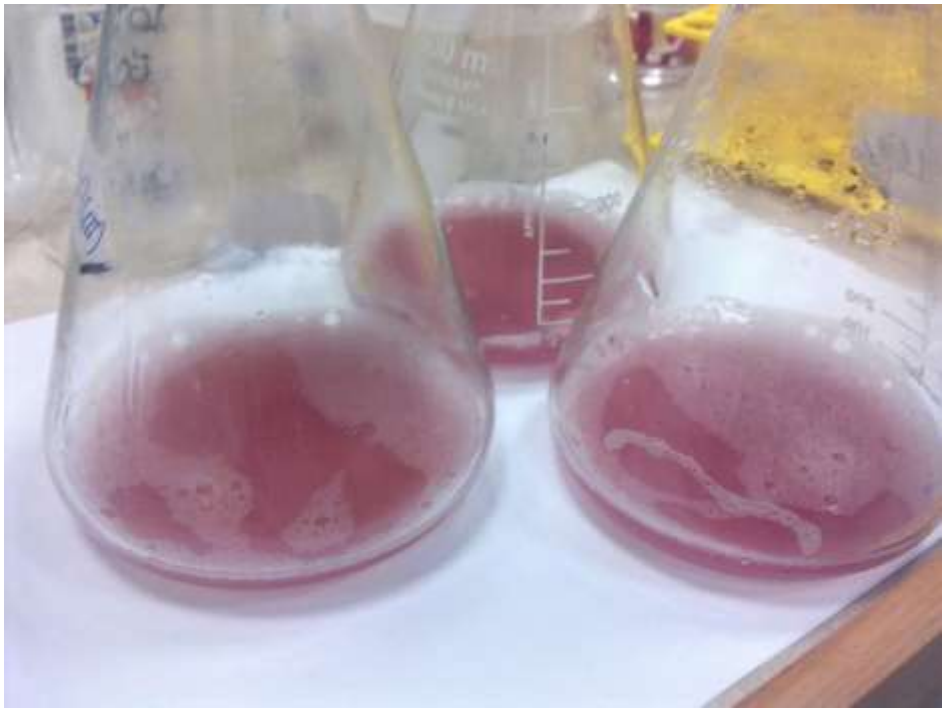


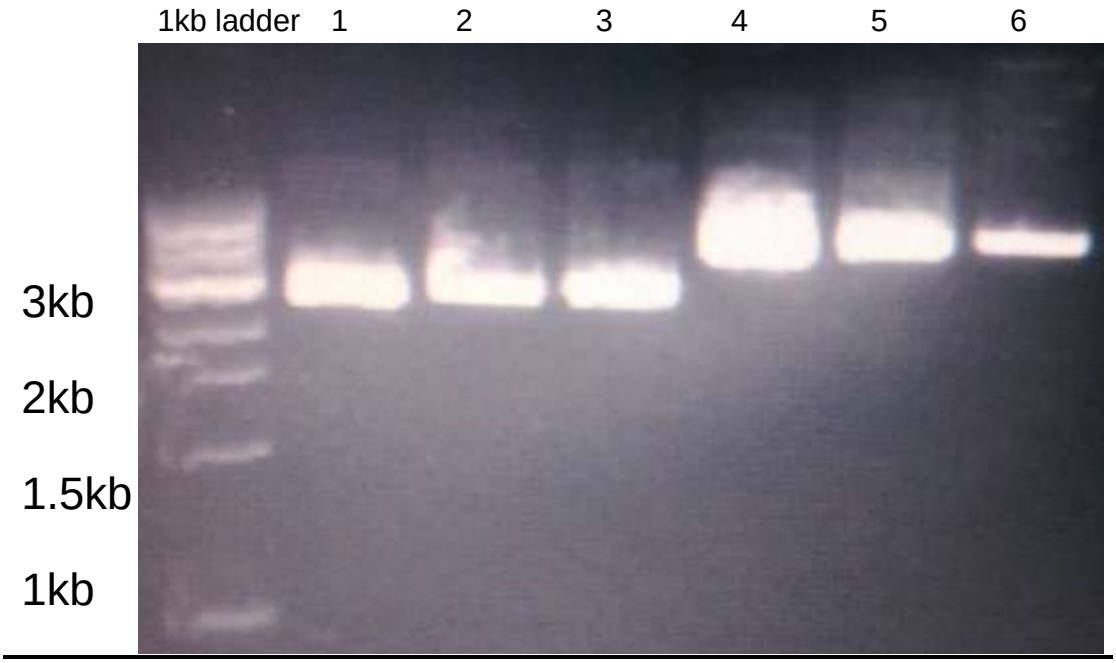
6 Sep

-Pick colonies, inoculation, sub-inoculation from yesterday transformed cells

7 Sep

-Midi-prep of plasmid backbone from transformed cells





12 Sep

- Restriction Cut of the pSB1A3 plasmid

Components	Volume
DNA	1ug
EcoR1	1ul
Pst1	1ul
10X NEBuffer2	5ul
100X BSA	0.5ul
H2O	Make total vol to 48ul

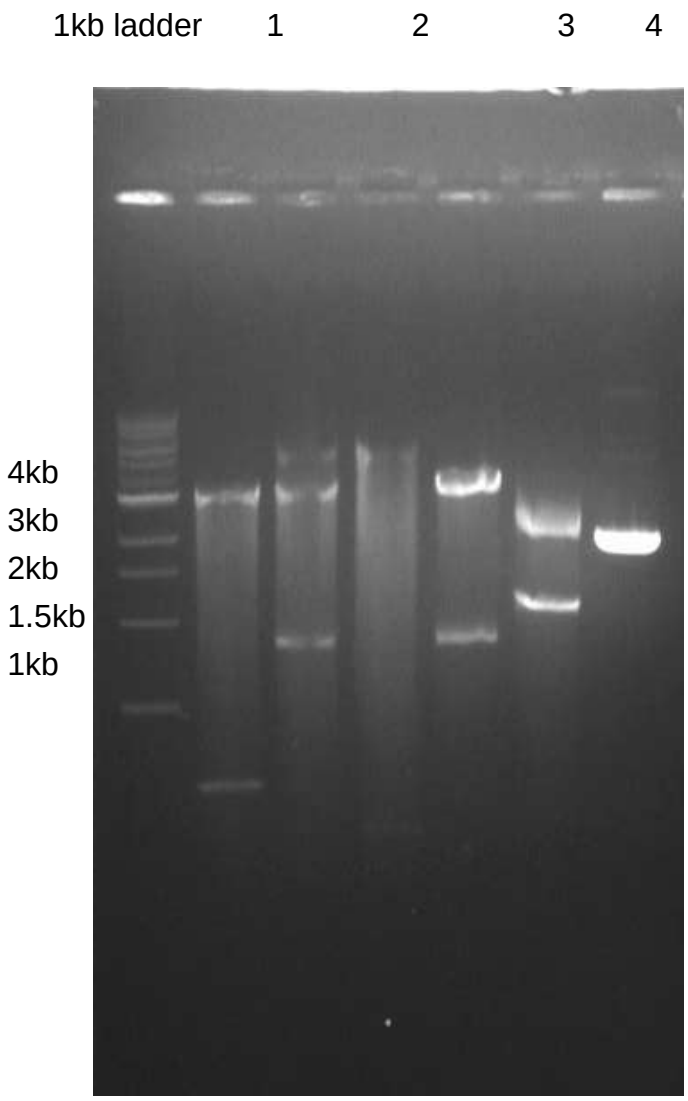
- Restriction Cut of the following part at 37C for 2.5hours

1. Promoter + RBS
2. Different Florescence proteins + terminator

Components	Volume
DNA	1ug
Enzyme A*	1ul
Enzyme B*	1ul
10X NEBuffer2	5ul
100X BSA	0.5ul
H2O	Make total vol to 50ul

DNA sample	Enzyme used*
Plasmid backbone(pSB1A3)	EcoRI, Pst1
Promoter+RBS	EcoR1, Spe1
Fluorescent protein + terminator	Xba1, Pst1

-Gel photos after restriction cut:



Lane	Description	Size
1	1D+2M	=223bp+70bp =293bp
2	6A+24C	=746bp+117bp =863bp
3	14K+24C	=743bp+117bp

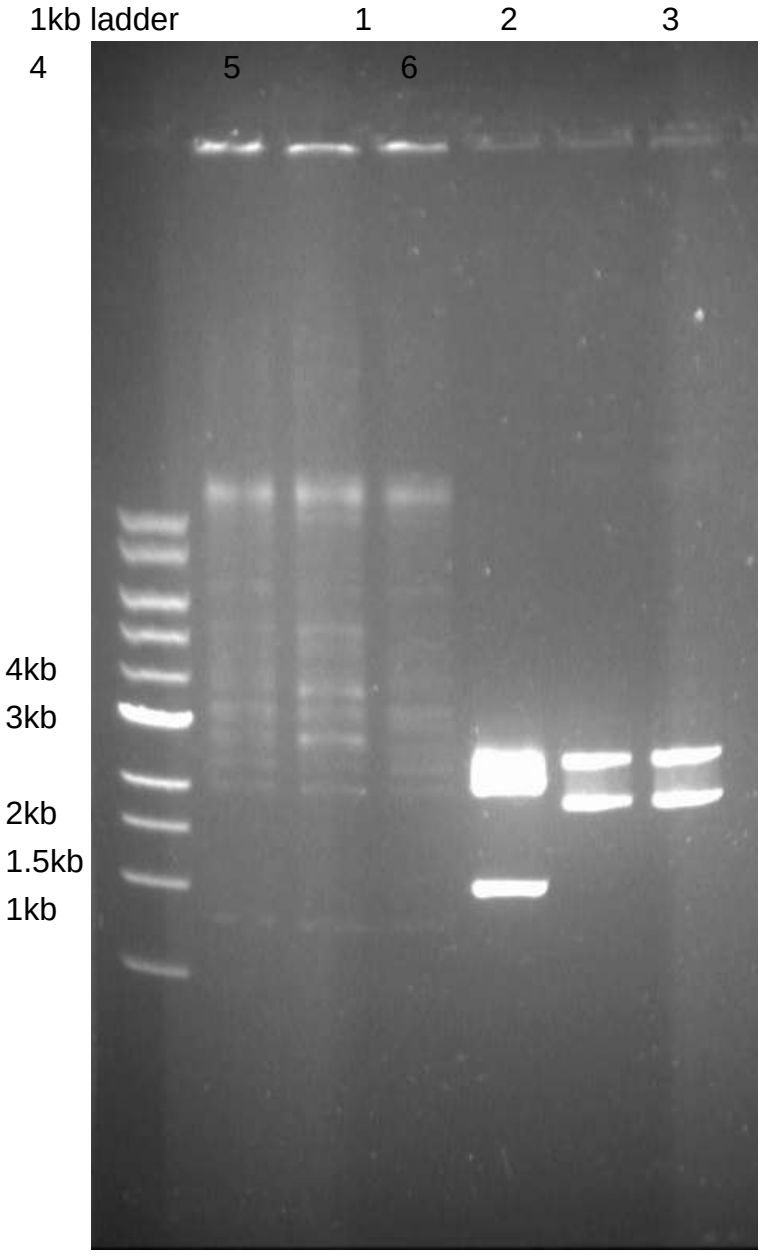
		=860bp
4	24E+24C	=746bp+117bp =863bp
5	Restricted pSB1A3	2157bp
6	Non-restricted pSB1A3	3226bp

-Ligation of following parts overnight at 16C

Prefix: [Promoter + RBS]

Suffix: [Fluorescent protein + terminator]

	Volume
Prefix /suffix	4ul
Plasmid backbone(pSB1A3)	2ul
T4 DNA ligase	1ul
10X T4 DNA Ligase Buffer	2ul
H2O	7ul
Total:	20ul



-Ligation Gel Photo:

Lane	Description	Size
1	[1D+2M]+[6A+24C]+pSB1A3	=223bp+70bp+746bp+117bp+2157bp =3313bp
2	[1D+2M]+[14K+24C]+pSB1A3	=223bp+70bp+743bp+117bp+2157bp =3310bp
3	[1D+2M]+[24E+24C]+pSB1A3	=223bp+70bp+746bp+117bp+2157bp =3313bp

4	Restricted pSB1A3	2157bp
5	Non-restricted pSB1A3 (1C_2)	3226bp
6	Non-restricted pSB1A3 (1C_3)	3226bp

21 Sep

-Restriction cut of:

1. pSB1A3 (gel purification, remove fluorescent protein)
2. Promoter + RBS
3. Rci gene + Terminator

Combination of enzymes is shown here:

DNA sample	Enzyme used*
Plasmid backbone(pSB1A3)	EcoRI, Pst1
Promoter+RBS	EcoR1, Spe1
Rci gene+terminator	Xba1, Pst1

Volume added:

Components	Volume (X4 for pSB1A3)
DNA	1ug
Enzyme A*	1ul
Enzyme B*	1ul
10X NEBuffer2	5ul
100X BSA	0.5ul
H2O	Make total vol to 50ul

37C for 2.5hours

-Ligation at 16C for overnight

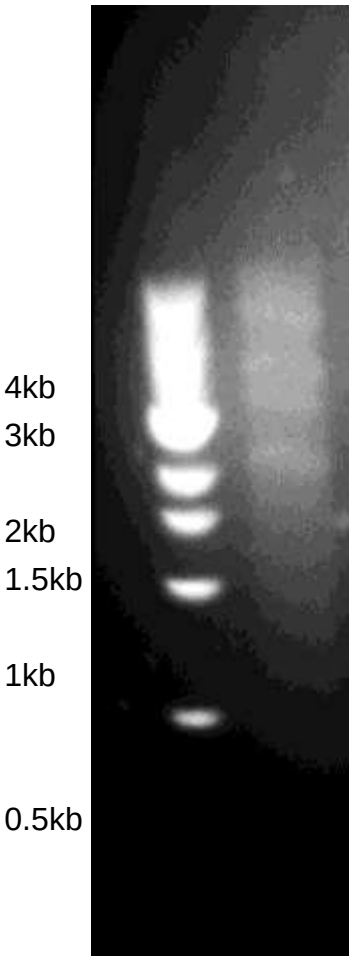
[Promoter+RBS] prefix + [rci gene +terminator] suffix

	Volume
Prefix /suffix	4ul
Plasmid backbone(pSB1A3)	2ul

T4 DNA ligase	1ul
10X T4 DNA Ligase Buffer	2ul
H2O	7ul
Total:	20ul

22 Sep

-Ligation Gel photos of 21 Sep

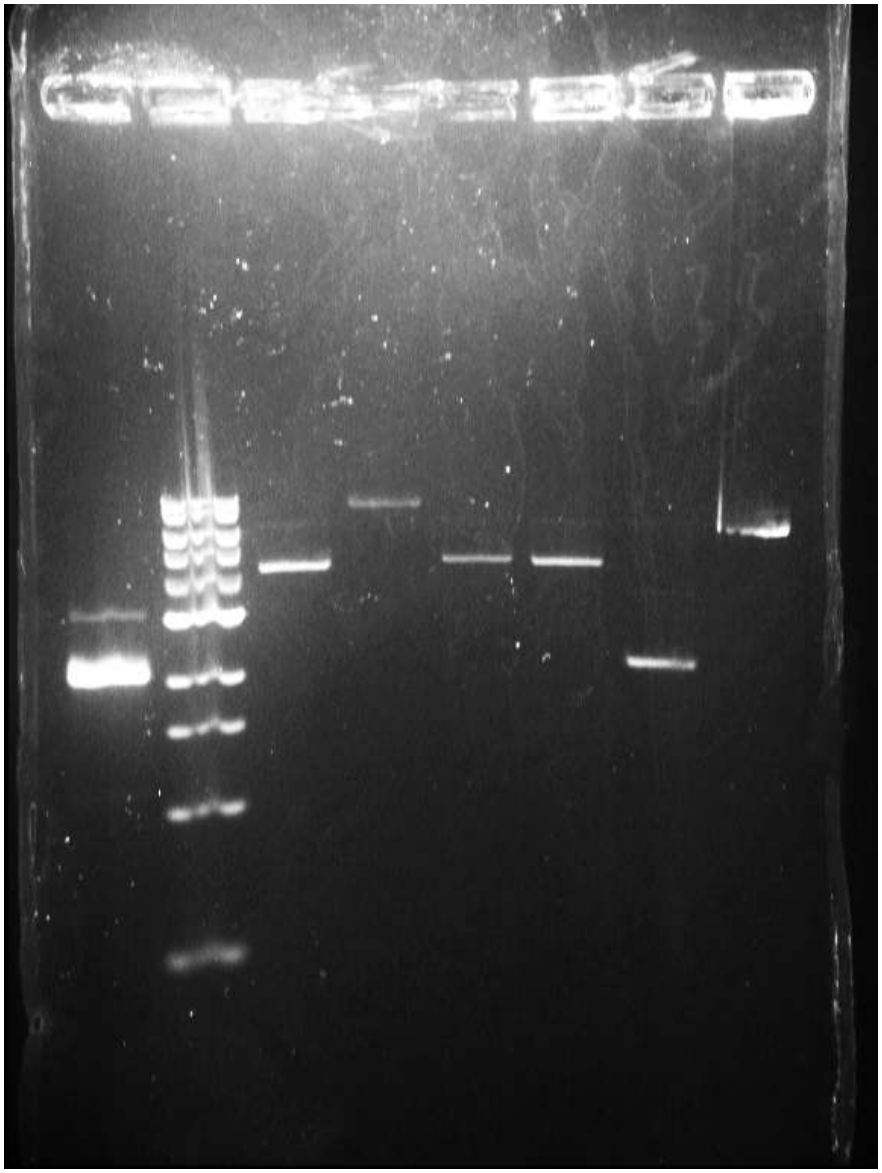


5ul from each sameple was added for running gel

Description	Size
[1D+2M]+[rci+24C]+pSB1A3	=223bp+70bp+~1200bp+117bp+2157bp =~3700bp

-After Ligation, mini-prep was carried out to get the DNA

1 2 3 4 5 6 7 8



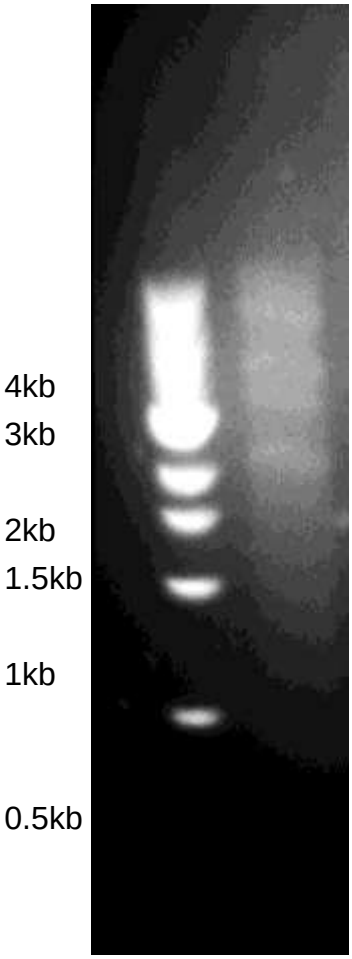
Lane	Description	Remarks
1	pSB1A3 backbone	2157bp
2	1kb ladder	*
3	Rci+PRT_1	~3700bp
4	Rci+PRT_2	
5	Rci+PRT_3	
6	Rci+PRT_4	
7	1D+2M+pSB1T3_2	2760bp
8	6A+24C+pSB1T3_1	3330bp

23 Sep

-Result of ligation:

Lane	Description	Size
1	[1D+2M]+[rci+24C] + pSB1A3	=223bp+70bp+~1200bp+117bp+2157bp =~3700bp

5ul from each sameple was added for running gel



-Changed to use 3A assembly instead of Standard Assembly

24 Sep

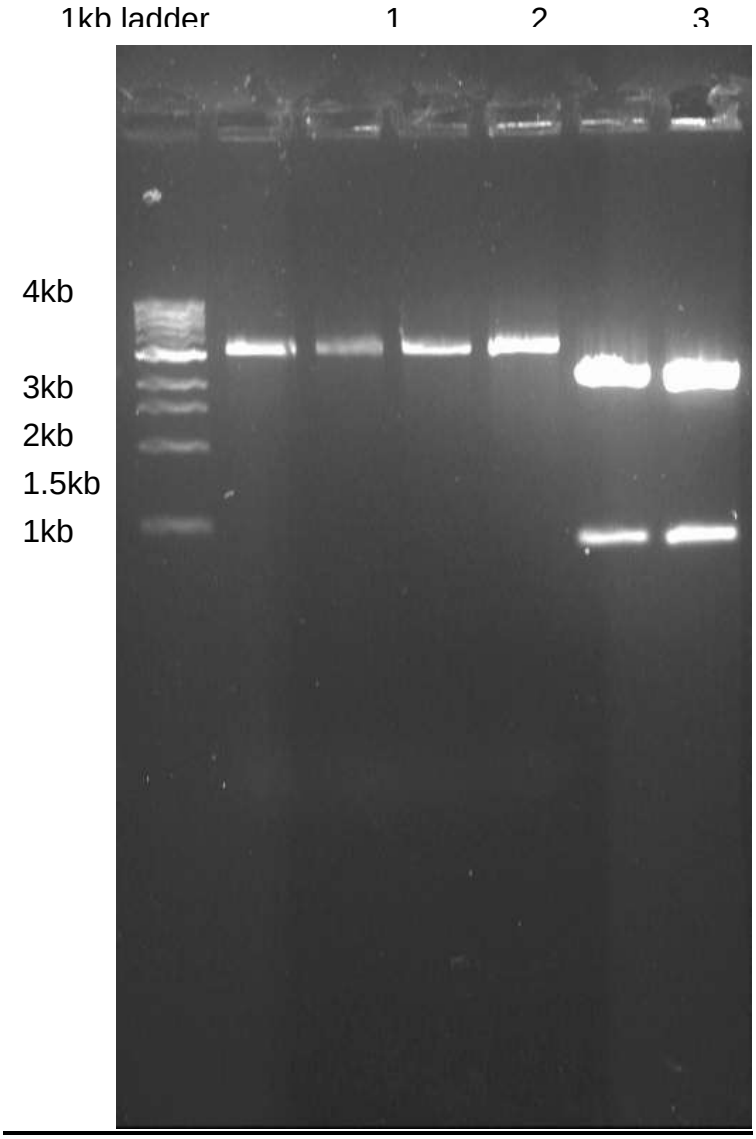
-Restriction of following parts

1. Rci system
2. Message

Components	Volume
DNA	1ug
Enzyme A*	1ul
Enzyme B*	1ul
10X NEBuffer2	5ul
100X BSA	0.5ul
H2O	Make total vol to 50ul

DNA sample	Enzyme used*
message	EcoR1, Spe1
Rci system	Xba1, Pst1

Gel photos after restriction cut:
10ul from each sameple was added for running gel



Lane	Description	Remarks
1	Restricted rci+PRT_1	Should have two bands: 1610bp(rci system) 2157bp(pSB1A3)
2	Restricted rci+PRT_2	
3	Restricted rci+PRT_3	
4	Restricted rci+PRT_4	
5	Message	/
6	Message	/

27 Sep

-Order following primers for sequencing

BBa_G00100_VR2
BBa_G00100_VR

The gap in the middle!?????

30 Sep

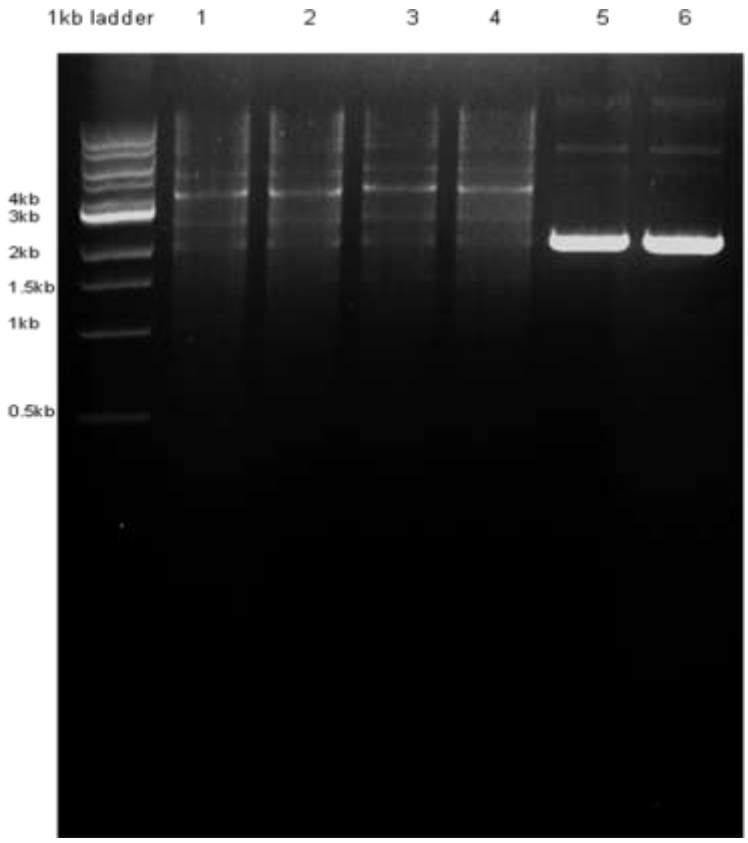
-Send following parts for sequencing

BBa_R0010 Part only sequence promoter
BBa_B0034 Part only sequence RBS
BBa_B0014 Part only sequence terminator

1 Oct

-in Lab: Sophie, Cathy

Work: Ligation of Rci and Terminator (BBa_B0014) in pSB1T3 and pSB1C3 for 12 hours 16C



Lane	Description	Remarks
1	Rci_1a+terminator+pSB1T3	=1200bp+117bp+2467bp =3784bp
2	Rci_2a+terminator+pSB1T3	
3	Rci_1b+terminator+pSB1T3	
4	Rci_2b+terminator+pSB1T3	
5	Rci_1+pSB1C3(after midi-prep)	=1200bp+2072bp =3272bp
6	Rci_2+pSB1C3(after midi-prep)	=1200bp+2072bp =3272bp

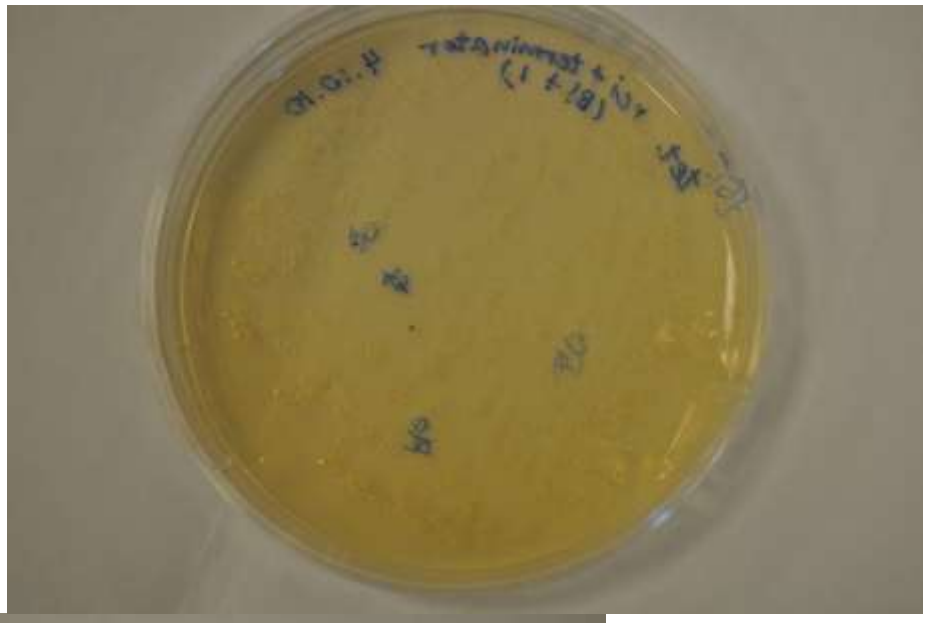
Lane 1-4: 10ul from each sameple was added for running gel
Lane 5-6: 1ul sample added

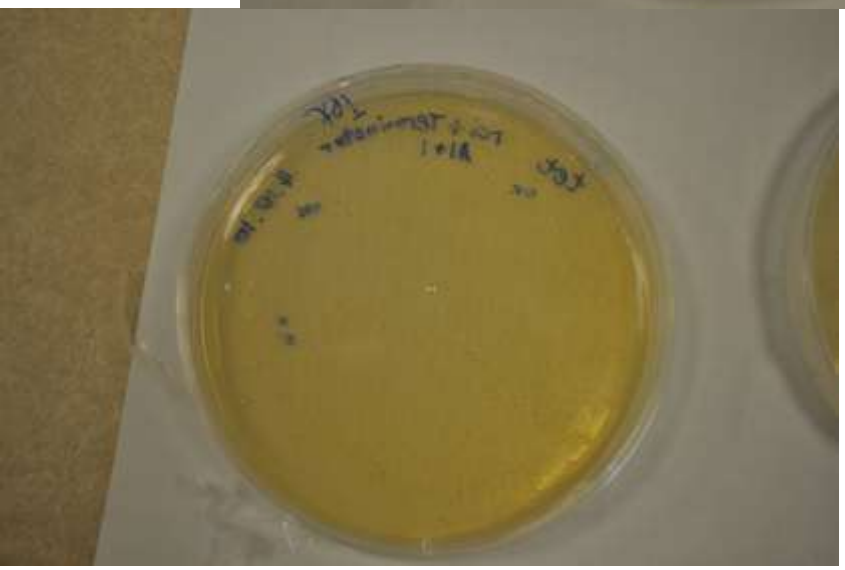
4 Oct

-in lab: VV

Work:

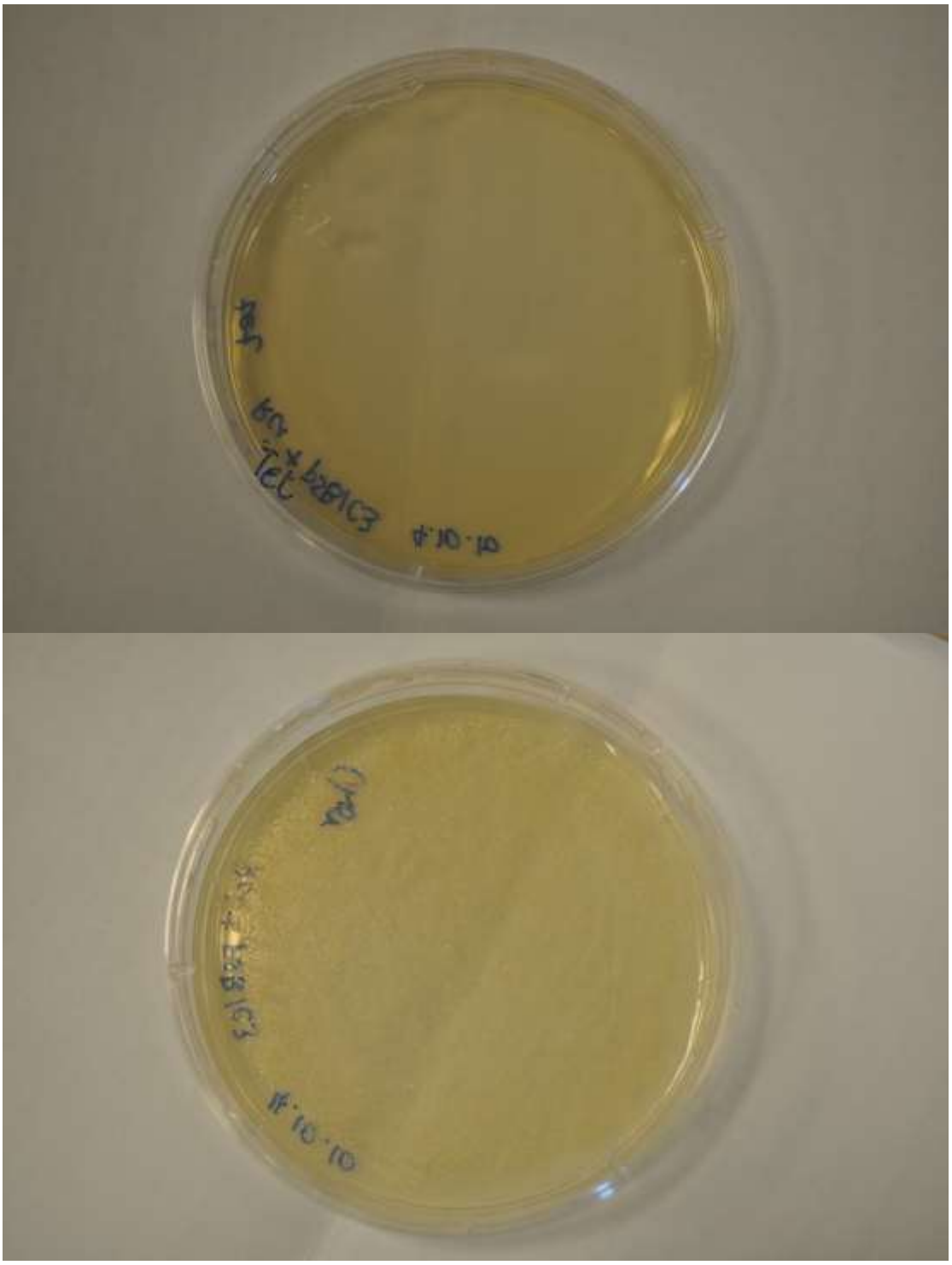
1. Transformation of Rci and Terminator in pSB1T3





2. **Transform rci in pSB1C3 and spread on tetracycline,** chloramohenicol **plate to ensure** only pSB1C3 but not other plasmids present in our sample sent for part registry

Result: clones formed only in chl plate



Oct 5

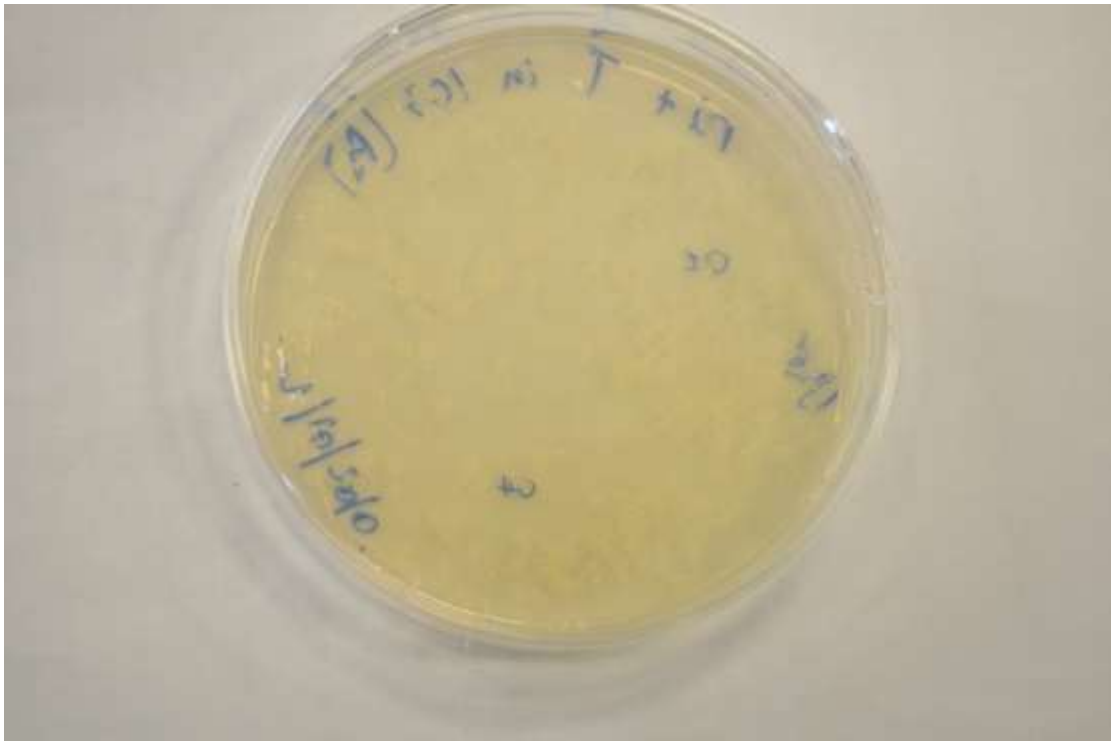
-Receive results of sequencing of 30Sep

Result: all successes

-in lab: Jacky

Work:

1. pick clone of transformed product yesterday and inoculation for 12 hours, then do mini-prep
2. Work : transformation of the rci +terminator (in pSB1C3)



6 Oct

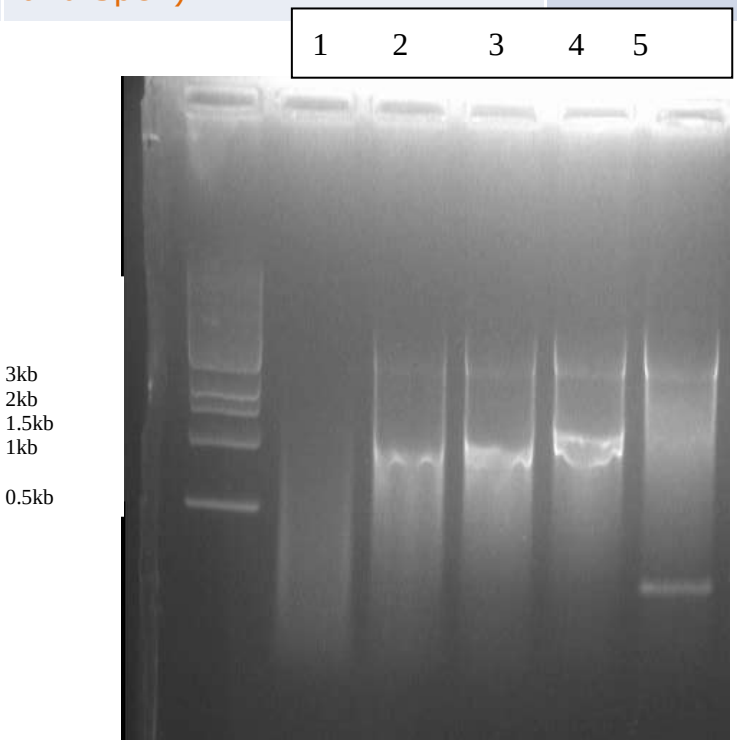
-in lab: VV

Work:

1. pick clone, inoculate and subinoculate rci +terminator (in pSB1C3)
2.midi-prep of Rci and Terminator in pSB1T3
3.Restriction of midi-prep product with XbaI+PstI and EcoRI+SpeI for 2 hours 37C

Lane	Description	Remarks
1	Rci_A+Terminator+pSB1T3(cut with EcoRI and Pst1)	~1300bp(rci gene+terminator)
2	Rci_B+Terminator+pSB1T3(cut with EcoRI and Pst1)	~2500bp(pSB1T3)
3	Rci_A+Terminator+pSB1T3(cut with Xba1 and Pst1)	
4	Rci_B+Terminator+pSB1T3(cut with Xba1 and Pst1)	

5	Promoter+Ribosome binding site+pSB1T3(cut with EcoR1 and Spe1)	~200bp(promoter+ribosome binding site)



3. Ligation of promoter+ribosome binding site with rci+terminator and pSB1C3 with 3A assembly
4. Ligation of promoter+ribosome binding site to pSB1C3
5. Ligation of rci+terminator to pSB1C3

All ligation take place for 12 hours 16C

7 Oct

-Send the following parts for sequencing

BBa_R0010 Part only sequence promoter + BBa_B0032 Part only sequence RBS
Rci gene
Rci gene + BBa_B0014 Part only sequence terminator

-in Lab: Ada, Ying

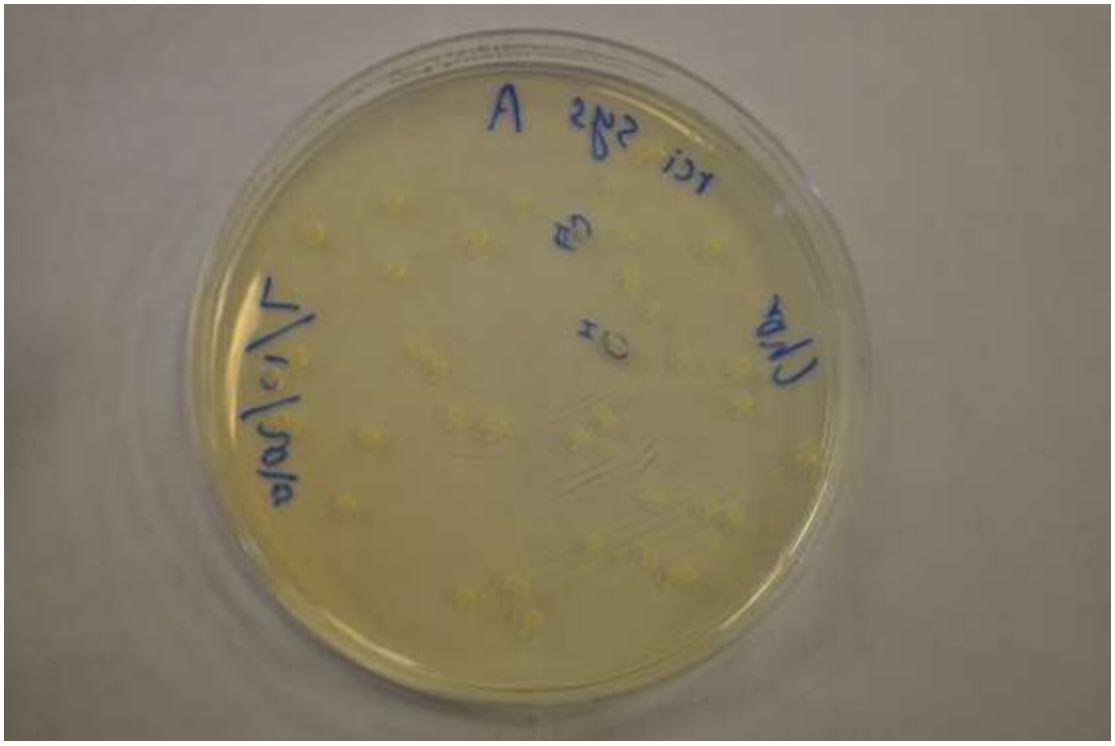
Work:

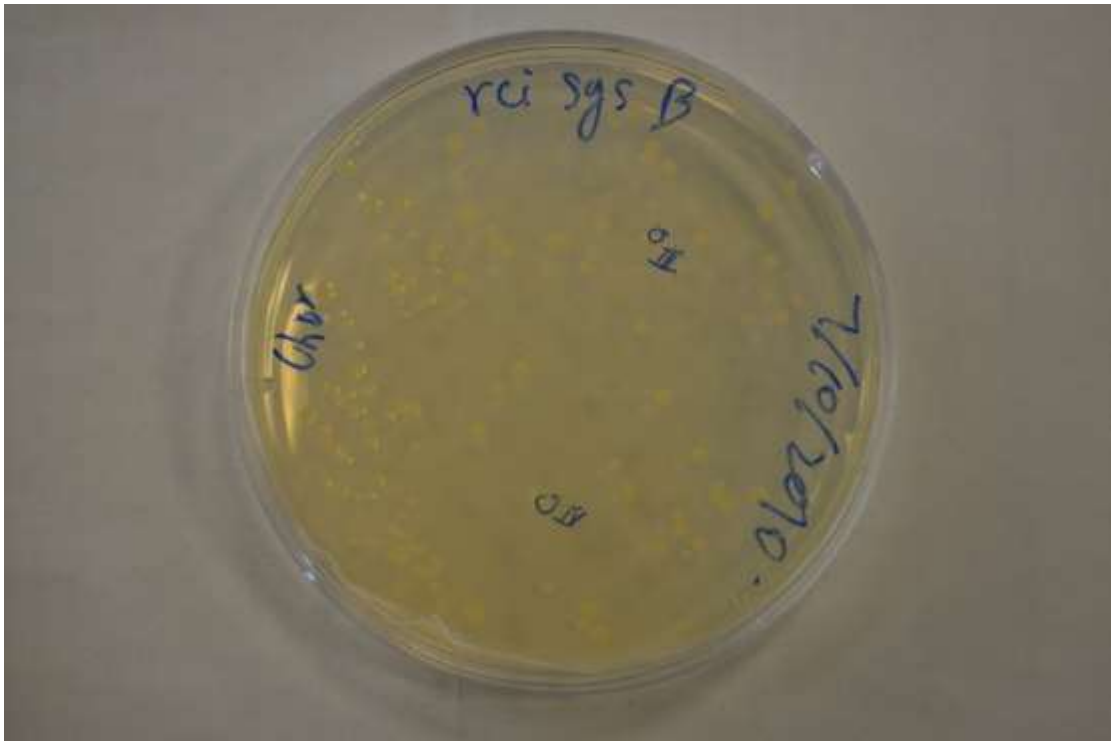
Transform the parts promoter+ribosome binding site, rci system rci+terminator in pSB1C3, transform message

Rci system: promoter+ribosome binding site+rci gene+terminator

1D + 2M in 1C3

rci + T in 1C3
(B)





8 Oct

-Receive sequencing result of 7 Oct

Result: Successes

-in Lab: Jacky

Work: pick clone, inoculation, Sub-inoculation

9 Oct

-in lab: Cathy, RT

Work:

1.midi-prep of 7 Oct transformed product

2.Restriction of message binding site with EcoRI+SpeI for 2 hours 37C

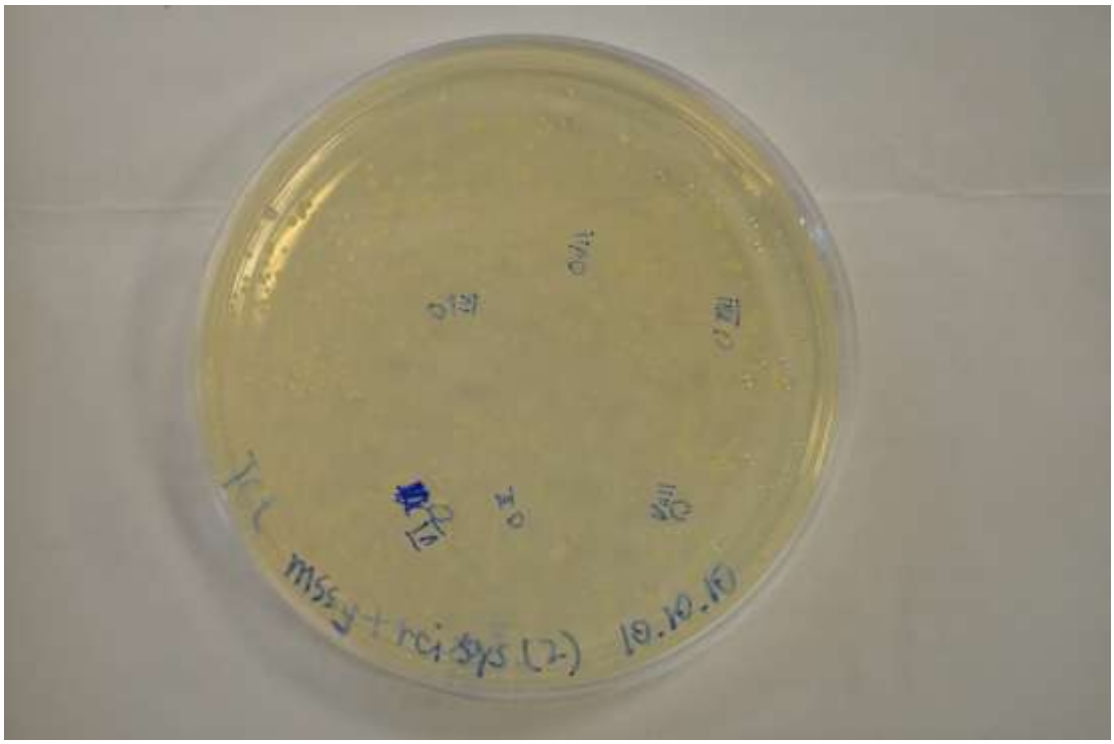
3. Restriction of rci system with XbaI+PstI for 2 hours 37C

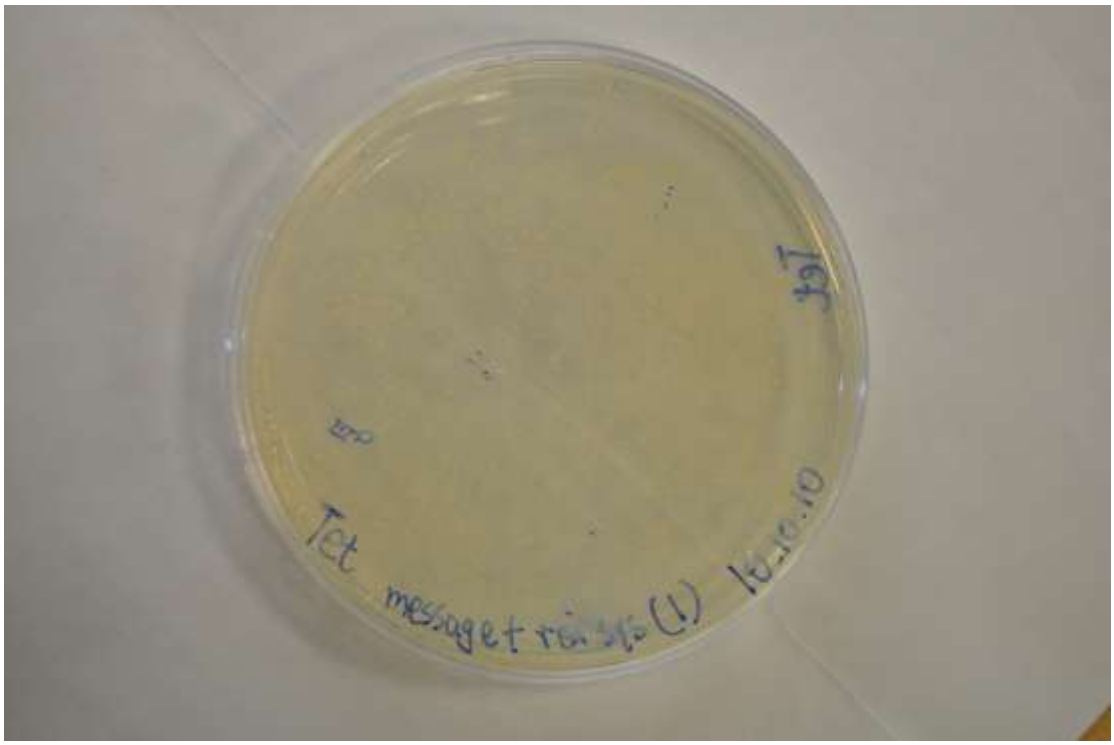
4. Ligation of restricted message and rci system with pSB1T3 for 12 hours, 16C

10 Oct

-in Lab: VV

Work: Transformation of message + rci system in pSB1T3





11 Oct

-in lab: Jacky

- Send the following parts for sequencing

BBa_R0010 Part only sequence promoter + BBa_B0032 Part only sequence RBS in

pSB1C3
Rci gene in pSB1C3
Rci gene + BBa_B0014 Part only sequence terminator in pSB1C3

Work:
1.pick clone, Inoculation of transformed cells for 12 hours
2. Mini-prep to extract message + rci system
3. Restriction of mini-prep product to confirm if it is desirable product for 2 hours 37C

(NO GEL PHOTOS!!!!!!)

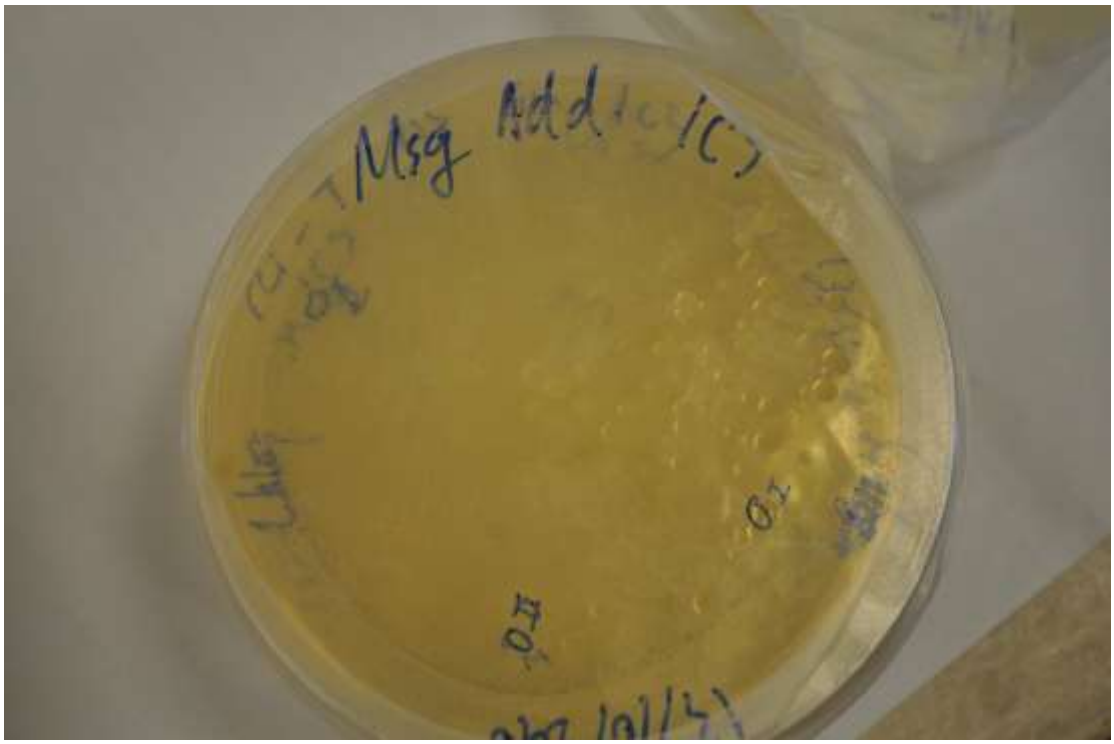
12 Oct

- Receive sequencing result for 11 Oct
  Result: Some failed

-Other primers for testing of rearranged message
-in lab: Cathy
Work:

1. Restriction of promoter+ribosome binding site in pSB1T3 with EcoRI and PstI
2. Restriction of rci+terminator in pSB1T3 with EcoRI and PstI
3. Restriction of message with EcoRI and PstI
4. Ligation of the above products to pSB1C3

13 Oct
-in lab: Jacky
Work: transform promoter+ribosome binding site in pSB1C3, rci+terminator in pSB1C3 and message in pSB1C3 for part registry



14 Oct

-in lab: VV

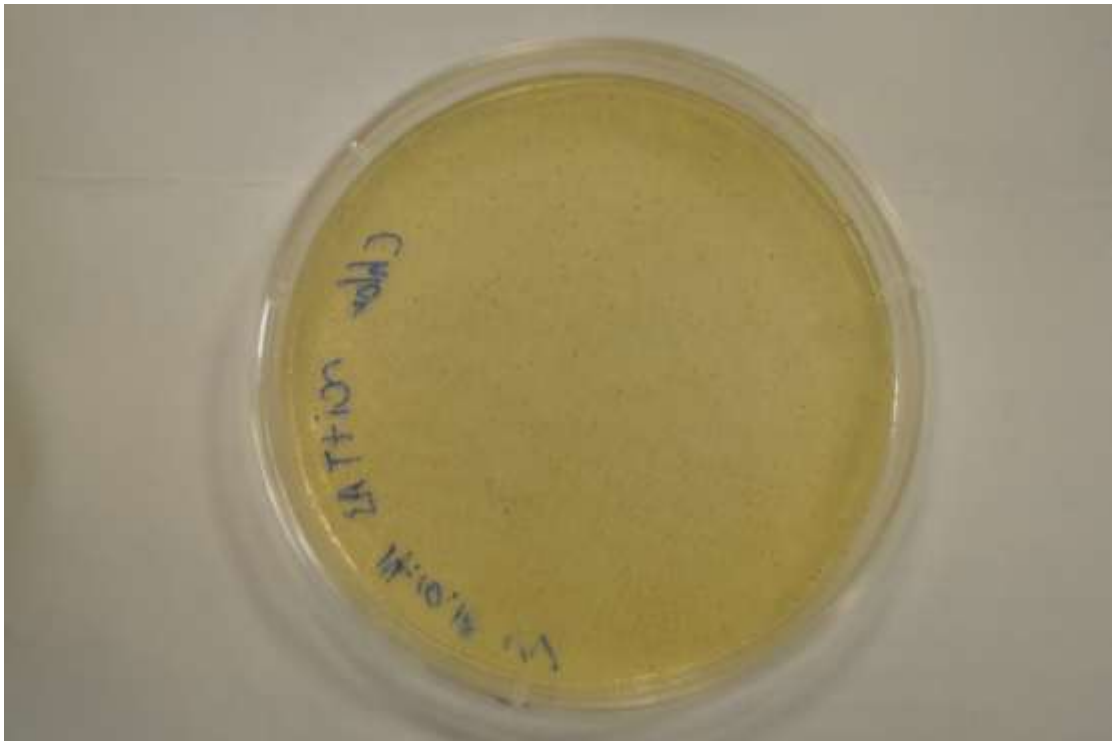
- Send the following parts for sequencing:

Message in pSB1C3
Recombinated Message

3. Work: Transformation of rci+terminator in pSB1C3 and promoter+ribosome binding site in pSB1C3 to ensure all the parts submitted to igem is in pSB1C3

Result:

	Tet	Chl
rci+terminator in pSB1C3	No clones	Clones formed
promoter+ribosome binding site in pSB1C3	No clones	Clones formed


Cip
14.10.10


Tet

15 Oct

-Receive sequencing result from 14 Oct

Result: Some success

-in Lab: Tinyi, VV

Work:

4. Rci expression test:

Protocol:

Overproduction and purification of Rci protein:

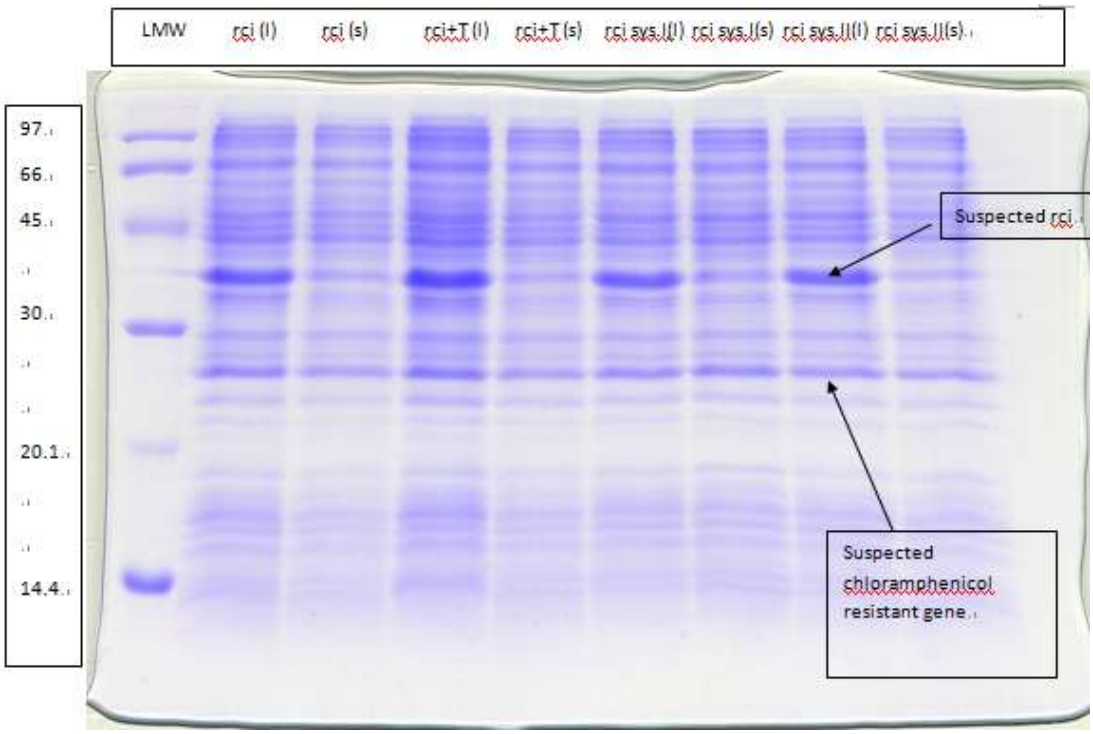
1. Grown in M9 glucose medium containing 5 mg of thiamine/ ml, 0.1% Casamino Acid, and 100 mg of ampicillin/ml at 37°C
2. When the A600 of the culture reached approximately 0.4, isopropyl-b-D-thiogalactopyranoside (IPTG) was added to 0.4 mM (final concentration)\
3. After 2 h, the cells were collected by centrifugation, washed with 50 mM Tris-HCl (pH 7.4), and resuspended in 80 ml of ice-cold buffer Y (50 mM Tris-HCl [pH 7.4], 0.8MKCl, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 10% glycerol) supplemented with 1 mg of lysozyme/ml.
4. After 30 min at 0°C, the suspension was frozen at -280°C.
5. The frozen cells were thawed at room temperature and lysed by sonication.

The following purification steps were performed at 4°C.

6. The lysate was centrifuged at 100,000 3 g for 90 min, and the supernatant was loaded onto a 25-ml phosphocellulose P11 (Whatman) column equilibrated with buffer Y.
7. The column was eluted with 50 ml of buffer Y with a linear gradient (from 0.8 to 1.8 M) of KCl.
8. Every fraction was analyzed by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE).
9. The Rci-containing fractions were concentrated by ammonium sulfate precipitation.
10. The pellet was dissolved in 2 ml of buffer Y and loaded onto a Sephacryl S-200HR (Pharmacia LKB) column (1.6 by 60 cm) equilibrated with buffer G (50 mM Tris-HCl [pH 7.4], 0.5 M KCl, and 1 mM EDTA).
11. The Rci-containing fractions were concentrated, dialyzed against buffer Y containing 50% glycerol, and stored at 280°C.

Rci: ~44KDa

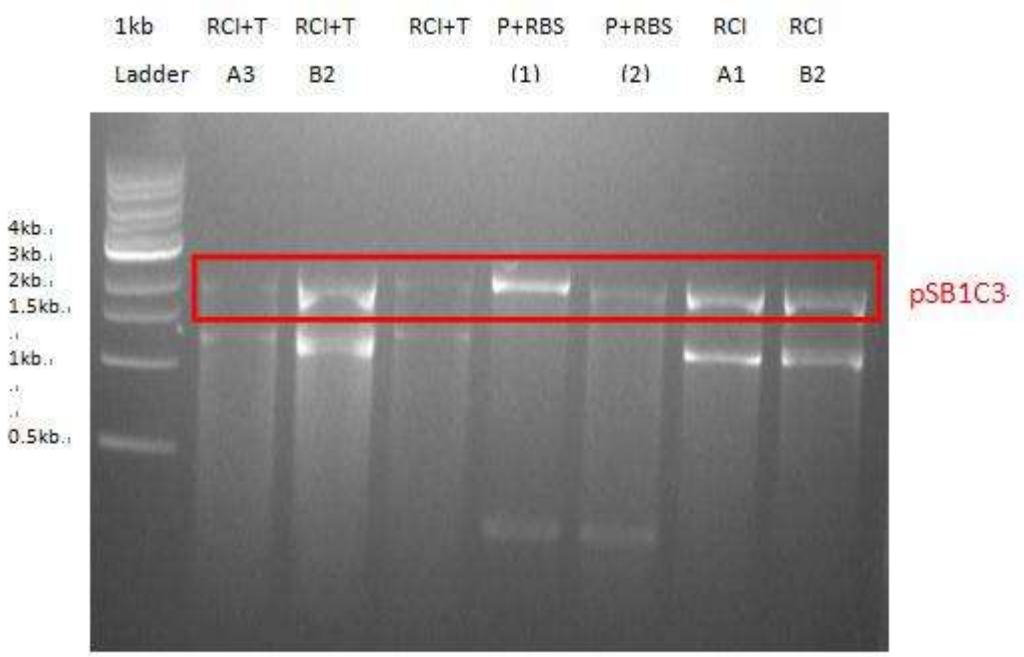
37 deagree 3hr 1/1000 IPTG
Buffer: 10mM Tris 200mM NaCl 1% Triton
15% SDS-PAGE



5. Pick clone of transformed product yesterday, inoculate for 12 hours, Mini-prep of rci + terminator in pSB1C3 and promoter + ribosome binding site in pSB1C3 and nano-drop to measure DNA concentration

	DNA conc(ug/ml)	260:280
rci + terminator in pSB1C3	117.36	1.84
ribosome binding site in pSB1C3	57.43	1.88

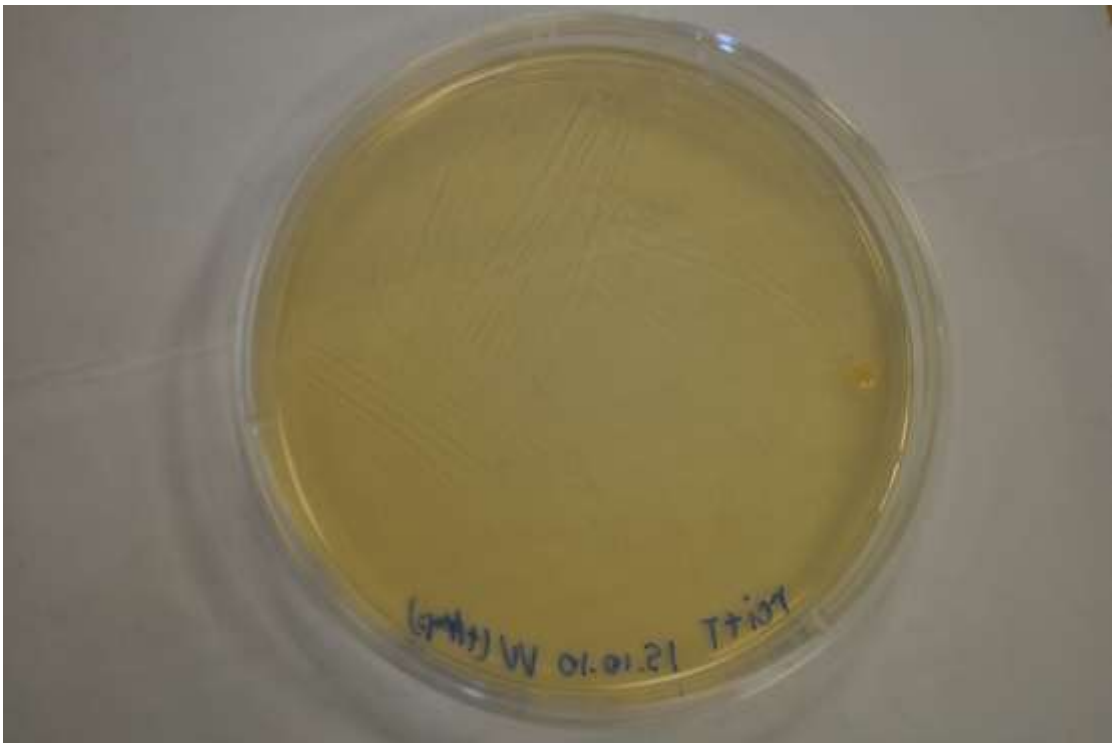
6. do enzyme restriction with transformed product yesterday to ensure all the parts submitted to igem is in pSB1C3
7. Run gel of the mini-prep sample(rci+terminator in pSB1C3, promoter+ribosome binding site in pSB1C3 on 14 Oct and rci in pSB1C3 on 4 Oct)

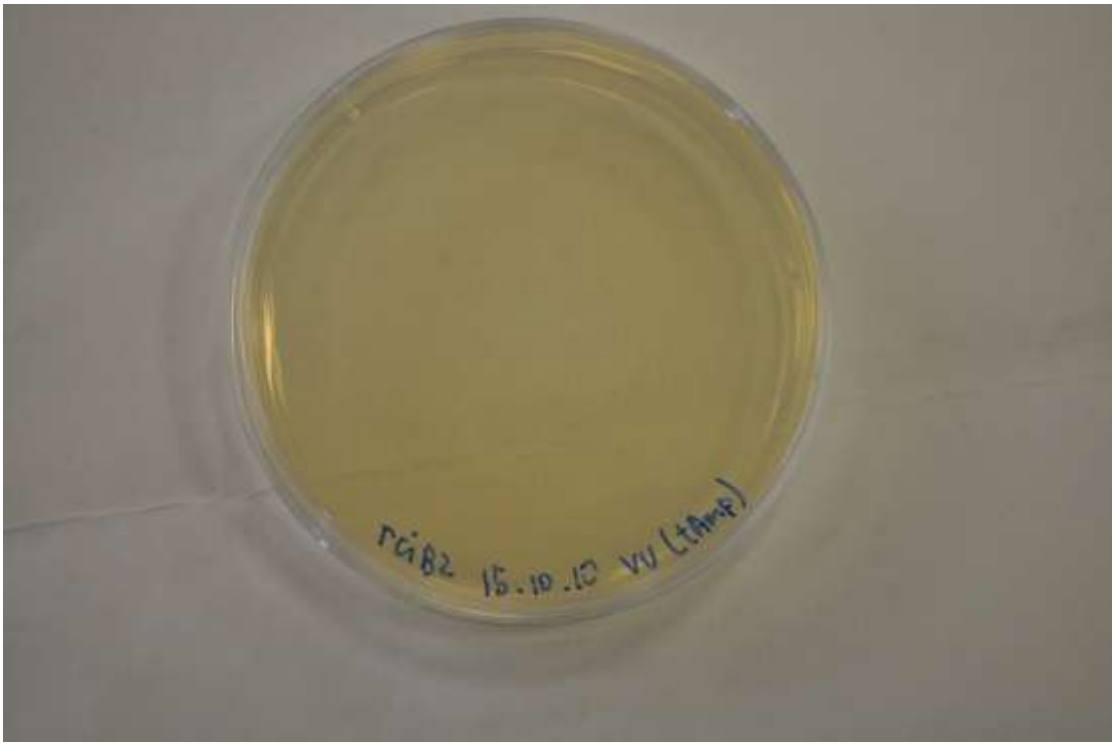


8. Transform rci in pSB1C3, rci + terminator in pSB1C3 and promoter+ribosome binding site in pSB1C3 in plates with Amp to ensure only pSB1C3 but not other plasmids present in our sample sent for part registry

Results

rci+terminator in pSB1C3	promoter+ribosome binding site in pSB1C3	Rci
No clones	No clones	No clones


(gMM) W 01.01.21 T+107


rGB2 15.10.20 W (tAmp)

18 Oct

-Sending the following parts for sequencing:

BBa_R0010 Part only sequence promoter + BBa_B0032 Part only sequence RBS in pSB1C3
Rci gene in pSB1C3
Rci + BBa_B0014 Part only sequence terminator in pSB1C3

19 Oct

- Receive sequencing results from 18 Oct
  Result: all success

21 Oct

-Sending the following parts for sequencing:

Rci system in pSB1C3

Rci System= BBa_R0010 Part only sequence promoter + BBa_B0032 Part only sequence RBS in pSB1C3 + Rci gene + BBa_B0014 Part only sequence terminator in pSB1C3

22 Oct

-Receiving sequencing results from 21 Oct
  Result: some success

25ct

-in lab: RT

Work:

1. transform message in pSB1C3, rci system in pSB1C3 to ensure only pSB1C3 but not other plasmids present in our sample sent for part registry
2. Restriction of message in pSB1C3 and rci system in pSB1C3 to ensure only pSB1C3 but not other plasmids present in our sample sent for part registry

GEL photos: JACKY plate photos: ADA