

## 2020.7.9

1. Preparation of LB medium

- 1) Liquid medium

Tryptone	10g/L
NaCl	10g/L
Yeast Extract	5g/L

- 2) Solid medium

Tryptone	10g/L
NaCl	10g/L
Yeast Extract	5g/L
Agar	20g/L

## 2020.7.15

6 plates streaked of BSU168. Three of them were purely cultured with a single colony.

## 2020.7.16

1. Streak plate of THU B.S.168.
2. Cryopreservation of B.S.168.

Operation at clean bench

1.5ml EP Tube: (1:1)

bacteria liquid	750 $\mu$ m
30% glycerol	750 $\mu$ m

## 2020.7.17

Pure culture THU B.S.168 with a single colony.

## 2020.7.18

Cryopreservation of THU B.S.168.

## 2020.7.25

Activation culture of GFP&RFP.

Incubate at 37 degrees Celsius overnight.

## 2020.7.26

Streak plate of GFP(Cm) & RFP(Amp), and culture.

## 2020.7.29

Pure culture GFP(Cm) & RFP(Amp) with a single colony.

## 2020.7.30

1. Cryopreservation of GFP(Cm) & RFP(Amp).
2. pGFP Plasmid extraction.

3. GFP&RFP part PCR

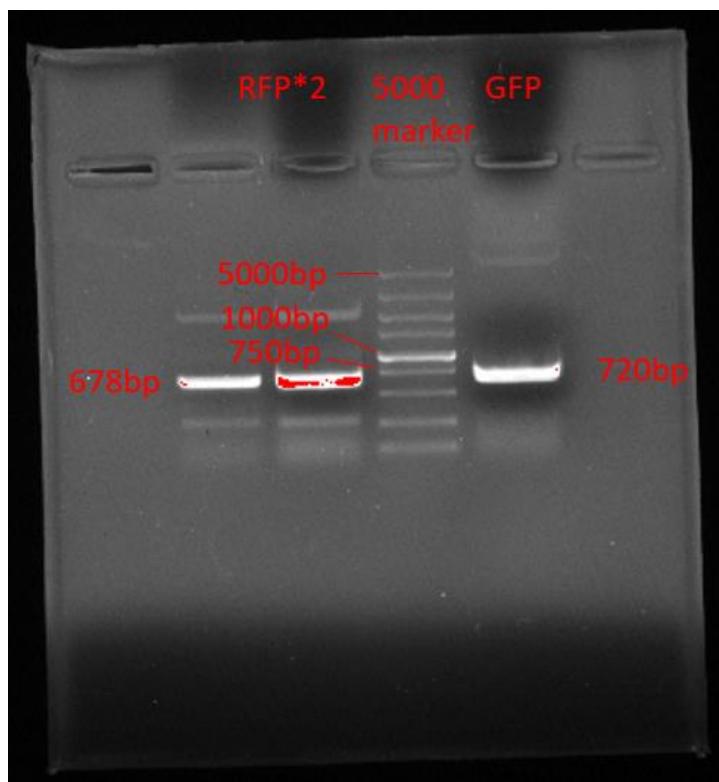
1) RFP(678bp,Tm67°C)

plasmid	1μL
Q5 Reaction Buffer	10μL
dNTPs	1μL
RFP-R	2.5μL
RFP-F	2.5μL
GC Enhancer	10μL
Q5	0.5μL
dd water	22.5μL

2) GFP(720bp,Tm63°C)

plasmid	1μL
Q5 Reaction Buffer	10μL
dNTPs	1μL
GFP-R	2.5μL
GFP-F	2.5μL
GC Enhancer	10μL
Q5	0.5μL
dd water	22.5μL

Result:

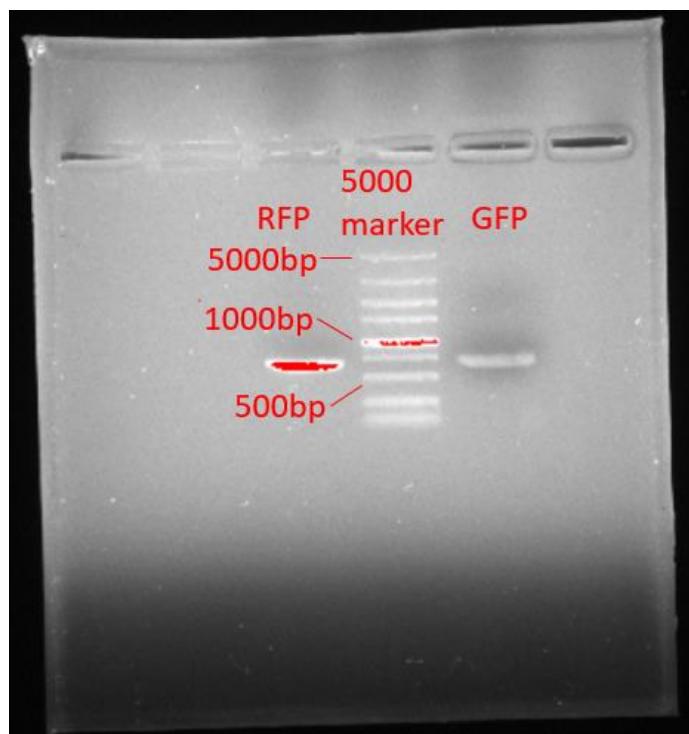


4. Activation culture of piercing fungus with E.coli-galU, E.coli-pgmA, B.S.-galU.

2020.7.31

1. Gel extraction of GFP & RFP part.

Concentration: ①GFP-part 35.35μg/mL ②RFP-part 70.94μg/mL



2. Streak plates of E.coli-galU, E.coli-pgmA, B.S.-galU.

#### 2020.8.1

Pure culture E.coli-galU & E.coli-pgmA & B.S.-galU with a single colony.

#### 2020.8.2

1. Cryopreservation of E.coli-galU & B.S.-galU.
2. Plasmid extraction of pE.coli-galU & B.S.-galU.

Concentration: ①pB.S.galU 574.2μg/mL ②pE.coli-galU 50.79μg/mL

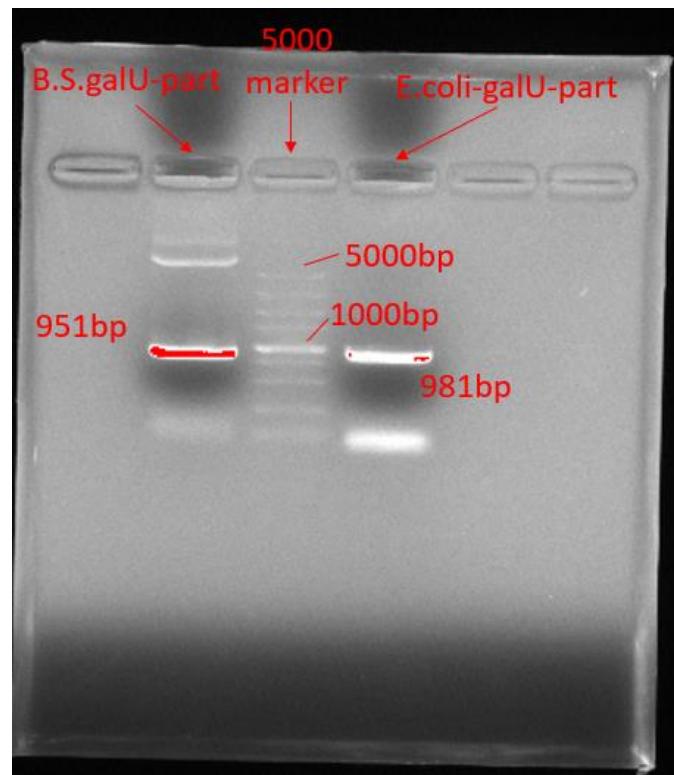
3. B.S.-galU &E.coli-galU part PCR

1) B.S.-galU (951bp, Tm=62°C)

plasmid	1μL
Q5 Reaction Buffer	10μL
dNTPs	1μL
B.S.galU-R	2.5μL
P43-F	2.5μL
Q5	0.5μL
dd water	32.5μL

2) E.coli-galU (981bp, Tm=63°C)

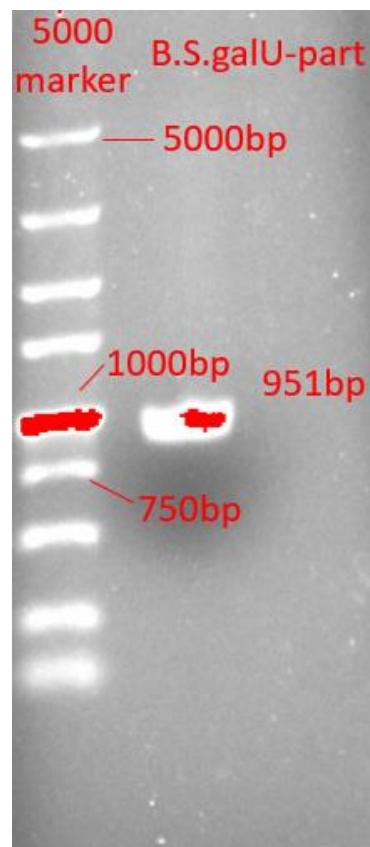
plasmid	1μL
Q5 Reaction Buffer	10μL
dNTPs	1μL
E.coli-R	2.5μL
P43-F	2.5μL
Q5	0.5μL
dd water	32.5μL



4. Gel extraction of PCR productions.

Concentration: B.S.galU-part 42.51 $\mu$ g/mL  
E.coli-galU-part 36.89 $\mu$ g/ml

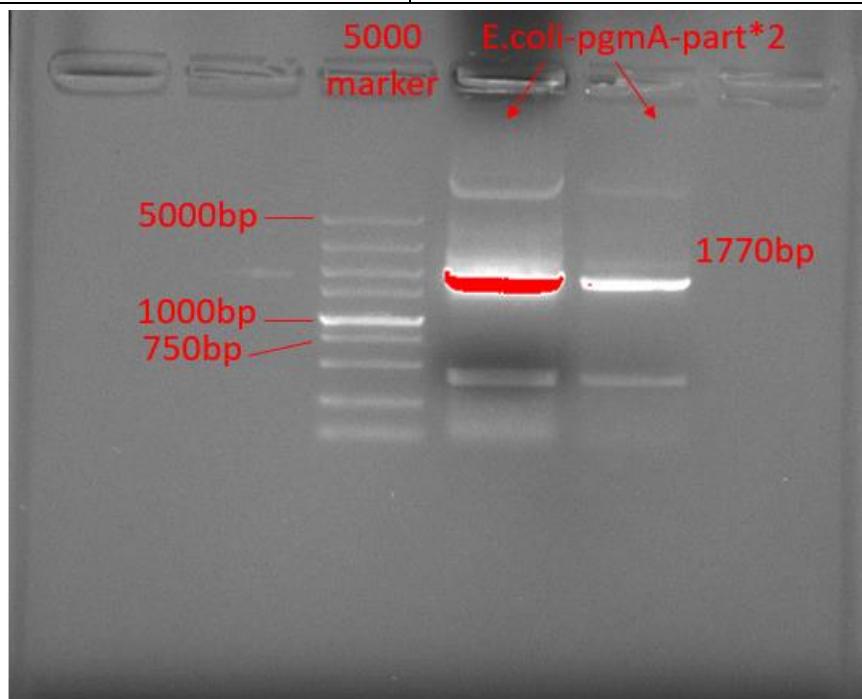
5. The size of the B.S-Galu Part is questionable, so we redid the gel electrophoresis.



### 2020.8.3

1. Restreak plates of E.coli-pgmA, and cryopreservation.
2. Plasmid extraction of pE.coli-pgmA.  
Concentration: pE.coli-pgmA 336.1 $\mu$ g/mL
3. E.coli-pgmA PCR (1770bp, Tm=64°C)

plasmid	1 $\mu$ L
Q5 Reaction Buffer	10 $\mu$ L
dNTPs	1 $\mu$ L
Terminator-R	2.5 $\mu$ L
E.coli-pgmA-F	2.5 $\mu$ L
GC Enhancer	10 $\mu$ L
Q5	0.5 $\mu$ L
dd water	22.5 $\mu$ L



4. Gel extraction.  
Concentration: E.coli-pgmA-part 39.29 $\mu$ g/mL.
5. Activation culture of piercing fungus with B.S. pgmA.

### 2020.8.4

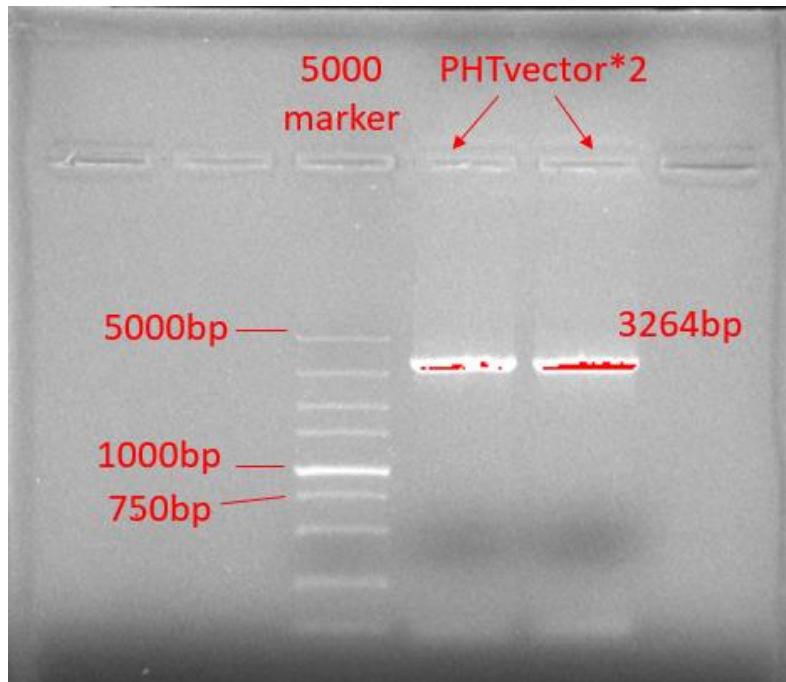
1. Streak plates of B.S.pgmA.
2. Try to make competent cells of Bacillus subtilis.

### 2020.8.5

1. Try to transfer pH43 into the competent cells of Bacillus subtilis.
2. pH43 vehicle PCR (3300bp, Tm=63°C)

plasmid	1 $\mu$ L
Q5 Reaction Buffer	10 $\mu$ L

dNTPs	1μL
PHT vehicle-R	2.5μL
PHT vehicle-F	2.5μL
GC Enhancer	10μL
Q5	0.5μL
dd water	22.5μL



### 3. Gel extraction:

Concentration of pHT vector: 20.37 μg/ml.

### 4. Goden Gate Assembly: pHT vehicle +B.S. galU +E.coli-pgmA

pHT vehicle-part(3246bp)	35.24ng	1.7μL
B.S. galU-part(951bp)	20.54ng	0.5μL
E.coli-pgmA(1776bp)	38.35ng	1μL
T4 ligase buffer		2μL
Sap I		1μL
Bca I		1μL
T4 ligase		1μL
dd water		11.8μL

Reaction Condition:

37°C 15min      } 10 cycles  
 16°C 10min  
 37°C 10min  
 65°C 10min  
 80°C 10min  
 12°C ∞

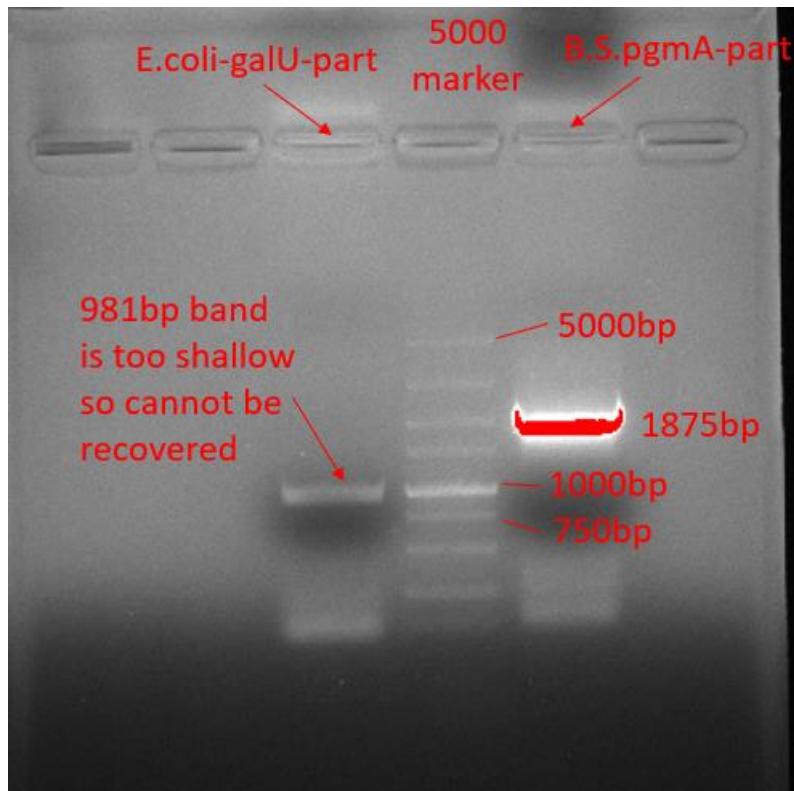
2020.8.7

### 1. Plasmid extraction of B.S. pgmA. Concentration: 157.9μg/ml.

2. Cryopreservation of B.S. pgmA.
  3. E.coli-galU part & B.S. pgmA part PCR
- B.S. pgmA part PCR

plasmid	1µL
Q5 Reaction Buffer	10µL
dNTPs	1µL
B.S. pgmA -R	2.5µL
P43-F	2.5µL
Q5	0.5µL
dd water	32.5µL

Result: The strip is shallow, and cannot be extracted.



#### 2020.8.9

1. Piercing fungus with B.S. pgmA Plasmid Extraction.  
Concentration: 74.07µg/ml.  
Then streak plates and culture.
2. Pure culture E . coli-galU with a single colony.
3. Activation culture of piercing fungus with E.coli-pgmA / E.coli-galU / B.S. galU.(Failed)
4. Make competent cells of Bacillus subtilis.

#### 2020.8.10

1. Activation culture of piercing fungus with E.coli-pgmA / E.coli-galU / B.S. galU / B.S. pgmA.
2. Culture pHT vehicle+ B.S. galU +E.coli-pgmA in LB.
3. Transfer pHT vehicle into competent cells to test conversion efficiency.

2020.8.11

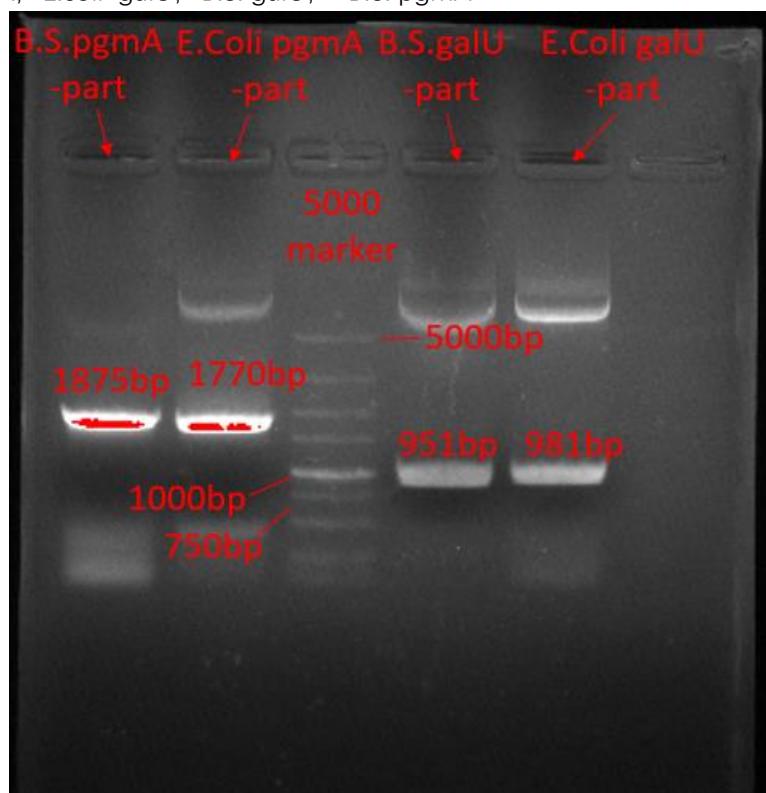
1. Plasmid extraction.

Concentration:

p. B.S. galU	857.5 $\mu$ g/ml
p. B.S. pgmA	336.2 $\mu$ g/ml
p. E.coli-galU	304.5 $\mu$ g/ml
p. E.coli-pgmA	829.5 $\mu$ g/ml

2. PCR:

E.coli-pgmA, E.coli-galU, B.S. galU, B.S. pgmA



Concentration of gel extraction production:

B.S. galU-part	23.48 $\mu$ g/ml
B.S. pgmA-part	26.86 $\mu$ g/ml
E . coli-galU-part	32.79 $\mu$ g/ml
E . coli- pgmA-part	23.94 $\mu$ g/ml

3. Linking pieces using Golden Gate

Tube 1: pHT vehicle +B.S. galU + B.S. pgmA

pHT vehicle-part(3246bp)	35.24ng	1.7 $\mu$ L
B.S. galU-part(951bp)	20.54ng	0.5 $\mu$ L
B.S. pgmA (1875bp)	40.49ng	1.6 $\mu$ L
T4 ligase buffer		2 $\mu$ L

Sap I		1μL
Bca I		1μL
T4 ligase		1μL
dd water		11.2μL

Tube 2: pHT vehicle + E.coli-galU + B.S. pgmA

pHT vehicle-part(3246bp)	35.24ng	1.7μL
B.S. galU-part(951bp)	20.54ng	0.5μL
B.S. pgmA (1875bp)	40.49ng	1.6μL
T4 ligase buffer		2μL
Sap I		1μL
Bca I		1μL
T4 ligase		1μL
dd water		11.2μL

Tube 3: pHT vehicle + E.coli-galU + E.coli- pgmA

pHT vehicle-part(3246bp)	35.24ng	1.7μL
E . coli-galU -part(981bp)	21.18ng	0.7μL
E . coli- pgmA -part (1770bp)	38.22ng	1.6μL
T4 ligase buffer		2μL
Sap I		1μL
Bca I		1μL
T4 ligase		1μL
dd water		11μL

## 2020.8.12

- Identify Golden Gate production (pHT vehicle +B.S. galU + E . coli pgmA Gogen) by doing colony PCR.

2xTaq	200μL
P43-F	16μL
Terminator-R	16μL
dd water	160μL

Result: Failed.

## 2020.8.13

- Golden Gate Assembly: pHT vehicle +B.S. galU +E.coli-pgmA

pHT vehicle-part(3246bp)	35.24ng	1.72μL
B.S. galU-part(951bp)	20.54ng	0.875μL
E.coli-pgmA(1776bp)	38.35ng	1.6μL
T4 ligase buffer		2μL
Sap I		1μL
Bca I		1μL

T4 ligase		1μL
dd water		10.8μL

2. Identify Golden Gate production by doing colony PCR.

1) pHT vehicle + B.S. galU + B.S. pgmA, Total: 240μL

Tube1s:

2xTaq	60μL
P43-F	4.8μL
B.S. galU -R	4.8μL
dd water	38.4μL

Tube2s:

2xTaq	60μL
B.S. pgmA -F	4.8μL
Terminator-R	4.8μL
dd water	38.4μL

2) pHT vehicle + E . coli-galU + B.S. pgmA, Total: 240μL

Tube3s:

2xTaq	60μL
P43-F	4.8μL
E.coli-galU -R	4.8μL
dd water	38.4μL

Tube4s:

2xTaq	60μL
B.S. pgmA -F	4.8μL
Terminator-R	4.8μL
dd water	38.4μL

3) pHT vehicle + E . coli-galU + E . coli- pgmA, Total: 240μL

Tube5s:

2xTaq	60μL
P43-F	4.8μL
E.coli-galU -R	4.8μL
dd water	38.4μL

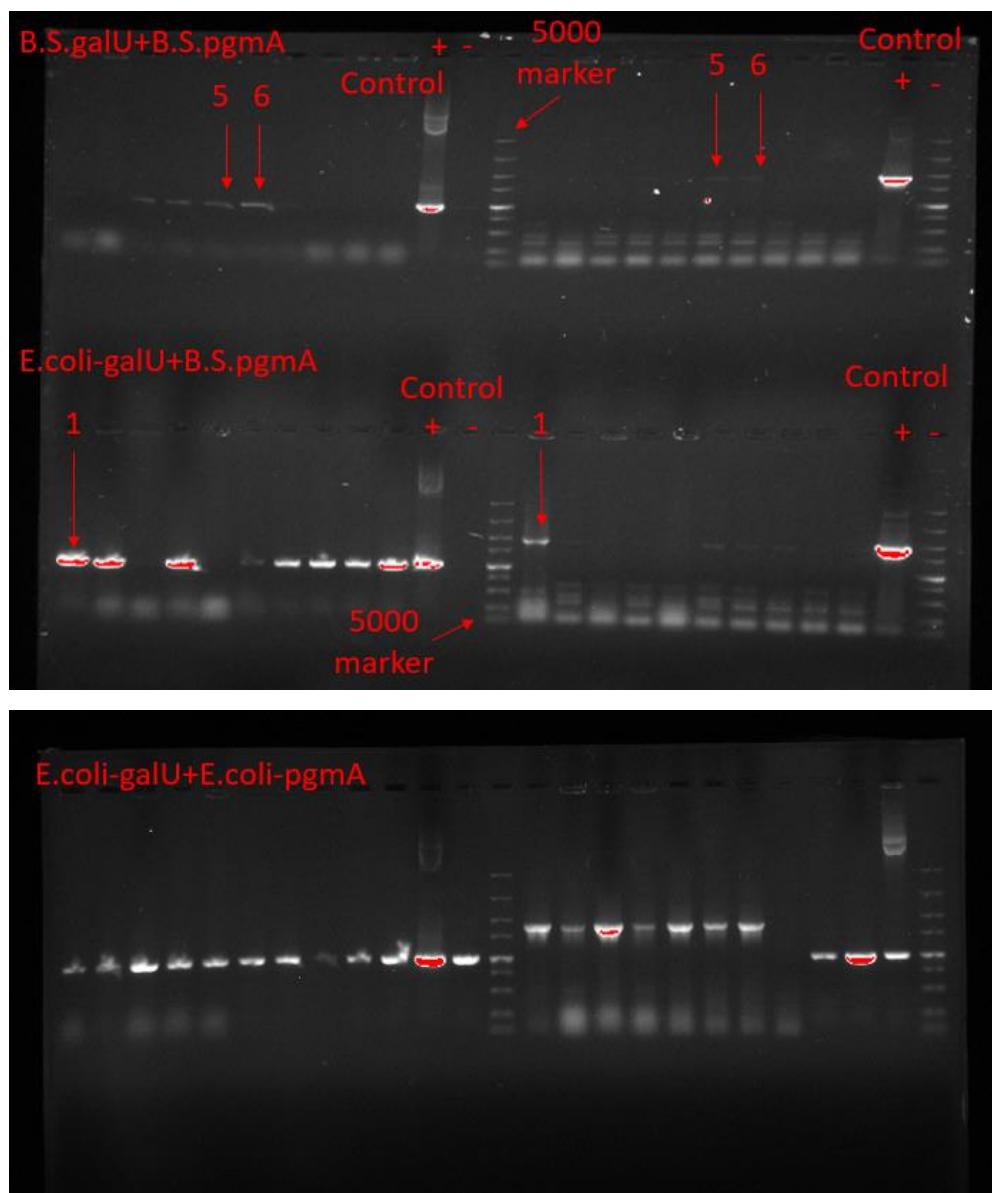
Tube6s:

2xTaq	60μL
B.S. pgmA -F	4.8μL
E.coli- pgmA -R	4.8μL
dd water	38.4μL

Result:

E.coli-galU+E.coli-pgmA	Select NO. 3, 5 and 7 colonies cultures to culture, extract plasmids and sequence.
B.S.galU+B.S.pgmA	Select NO.5 and 6 colonies cultures to culture, extract plasmids and sequence.
E. coli-galU+B.S.pgmA	Select NO.1 colony cultures to culture, extract plasmids and sequence.

The sequencing results do not match the genetic map, so we plan to reconstruct the plasmid.



2020.8.14

1. Enzyme digestion validation of bacteria selected by colony PCR for identification.  
Then do plasmid extraction and cryopreservation.

Concentration of plasmid:

B.S. galU + B.S. pgmA	660.6µg/ml
E.coli-galU + E.coli-pgmA	815.1µg/ml
E.coli-galU + B.S. pgmA	265.0µg/ml

- Identify Golden Gate production (pHT vehicle +B.S. galU +E.coli-pgmA) by doing colony PCR. Select 4 colonies cultures to culture, extract plasmids and sequence.

#### 2020.8.15

Enzyme digestion validation of 4 groups of bacteria from yesterday.

Result: Failed. The reason may be the long incubation time.

#### 2020.8.17

- Preparation 0.1% methylene blue.

methylene blue	0.1g
dd water	100ml

- Identification of *Nostoc* sp. survival status.

Result: surviving.

- Linking pieces using Golden Gate

- ①pHT vehicle +B.S. galU + B.S. pgmA
- ②pHT vehicle + E . coli-galU + E . coli- pgmA
- ③pHT vehicle + E . coli-galU + B.S. pgmA

#### 2020.8.18

- Linking pieces using Golden Gate

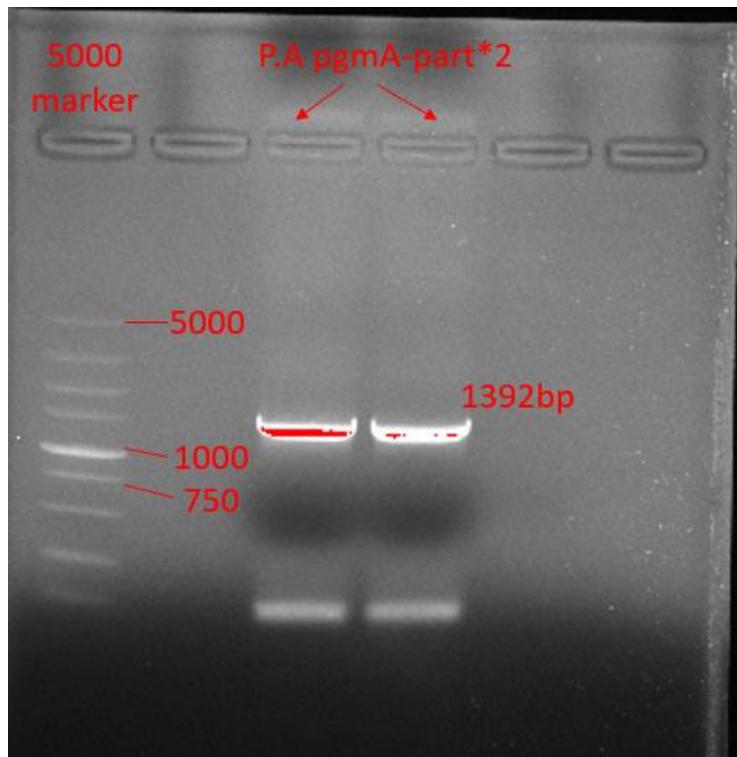
- ①pHT vehicle +B.S. galU +E.coli-pgmA

- Transformation B.S. galU +E.coli-pgmA.

- P.A. pgmA PCR,

Total: 50µL

plasmid	1µL
Q5 Reaction Buffer	10µL
dNTPs	1µL
P.A. pgmA -R	2.5µL
P43-F	2.5µL
Q5	0.5µL
dd water	32.5µL



2020.8.19

1. Linking pieces using Golden Gate

①pHT vehicle +B.S. galU + P.A. pgmA, total: 20 $\mu$ L

pHT vehicle-part(3246bp)	35.24ng	1.7 $\mu$ L
B.S. galU-part(951bp)	20.54ng	0.5 $\mu$ L
P.A. pgmA (1875bp)	40.49ng	0.8 $\mu$ L
T4 ligase buffer		2 $\mu$ L
Sap I		1 $\mu$ L
Bca I		1 $\mu$ L
T4 ligase		1 $\mu$ L
dd water		12 $\mu$ L

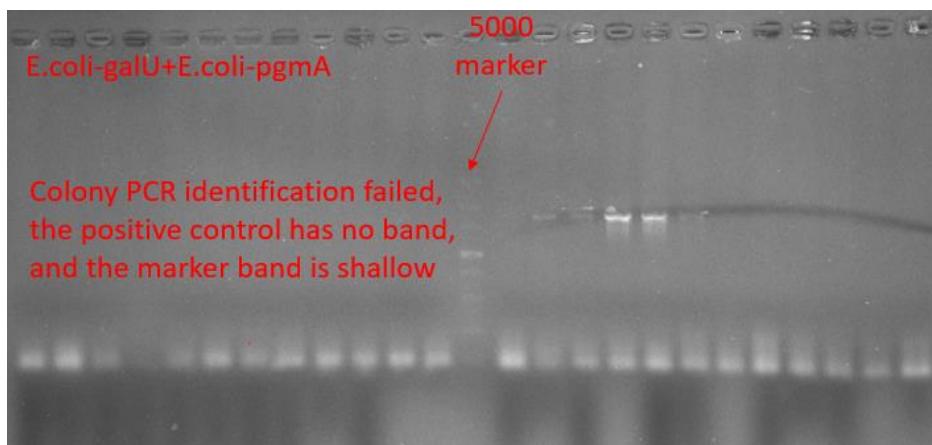
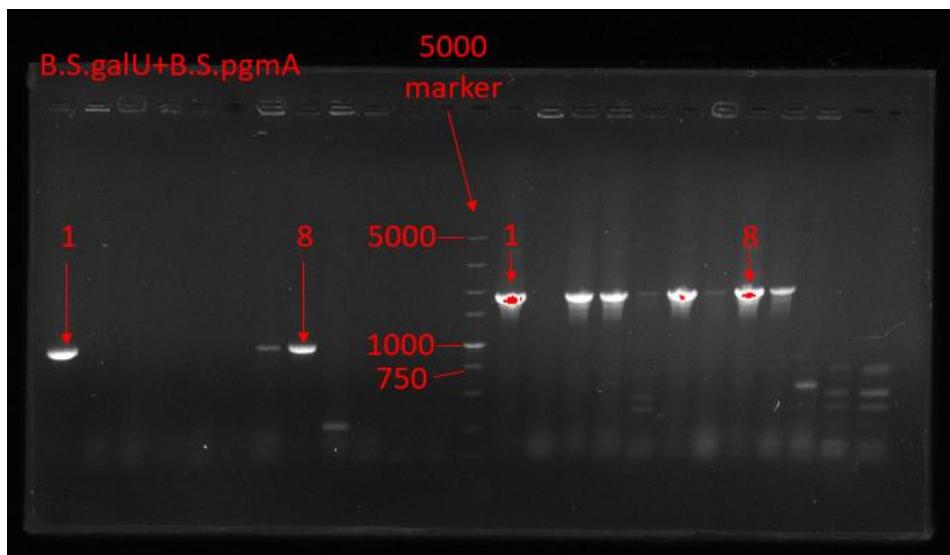
②pHT vehicle + E . coli-galU + P.A. pgmA, total 20 $\mu$ L

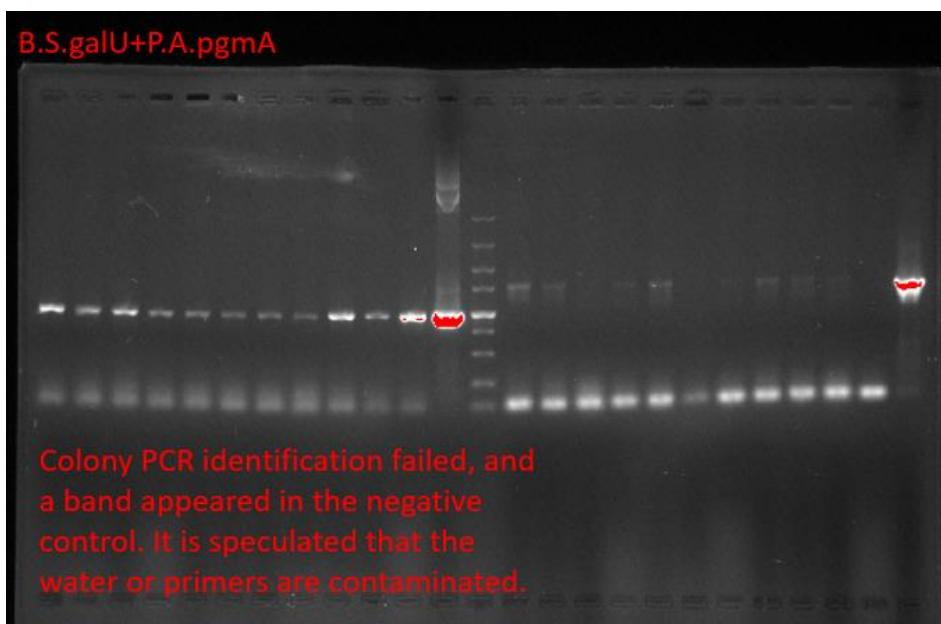
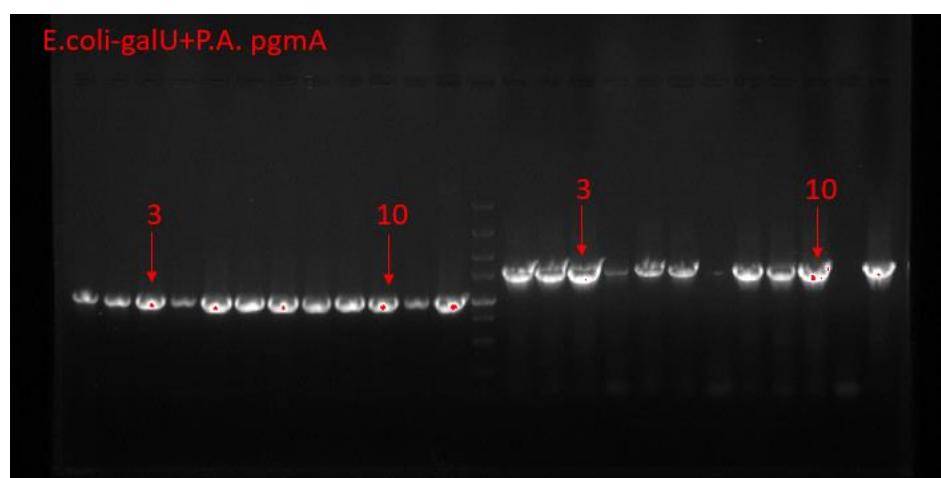
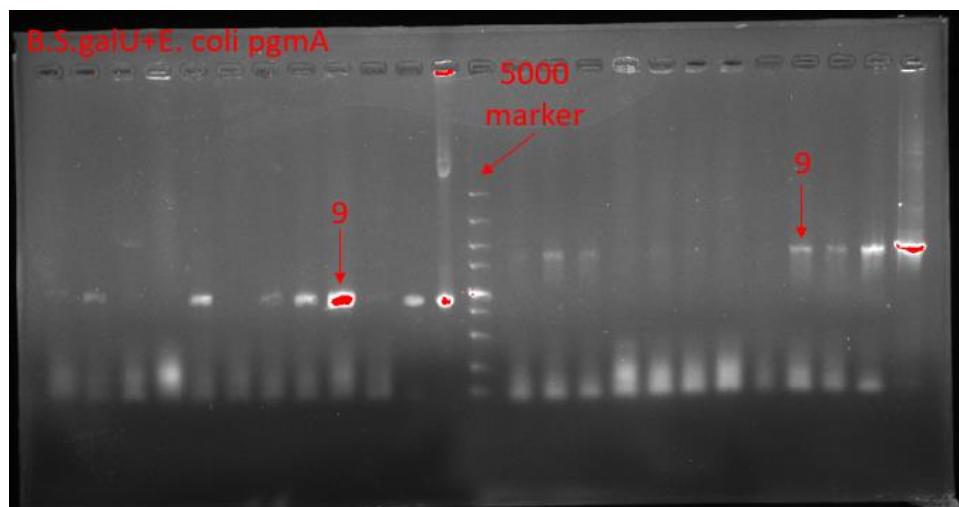
pHT vehicle-part(3246bp)	35.24ng	1.7 $\mu$ L
E.coli-galU-part(981bp)	21.18ng	0.7 $\mu$ L
P.A. pgmA-part (1521bp)	32.85ng	0.8 $\mu$ L
T4 ligase buffer		2 $\mu$ L
Sap I		1 $\mu$ L
Bca I		1 $\mu$ L
T4 ligase		1 $\mu$ L
dd water		11.8 $\mu$ L

2. Identify Golden Gate production by doing colony PCR.

Result:

B.S.galU+B.S.pgmA	Select NO. 1 and 8 colonies cultures to culture, extract plasmids and sequence.
E.coli-galU+E.coli-pgmA	Colony PCR identification failed. The positive control group has no strip, and the marker strip is shallow. Speculate the reason is due to wrong PCR condition or wrong system preparation, and operation error cannot be ruled out, either.
B.S.galU+ E.coli-pgmA	Select NO.9 colony cultures to culture, extract plasmids and sequence.
E.coli-galU+P.A. pgmA	Select NO. 3 and 10 colonies cultures to culture, extract plasmids and sequence.
B.S.galU+P.A.pgmA	Colony PCR identification failed. A strip appears in the negative control group. Speculate that the water or primers are contaminated. Plan to pack the dissolved primer dry powder separately. But the negative control was brighter than the sample strip, for unknown reasons.



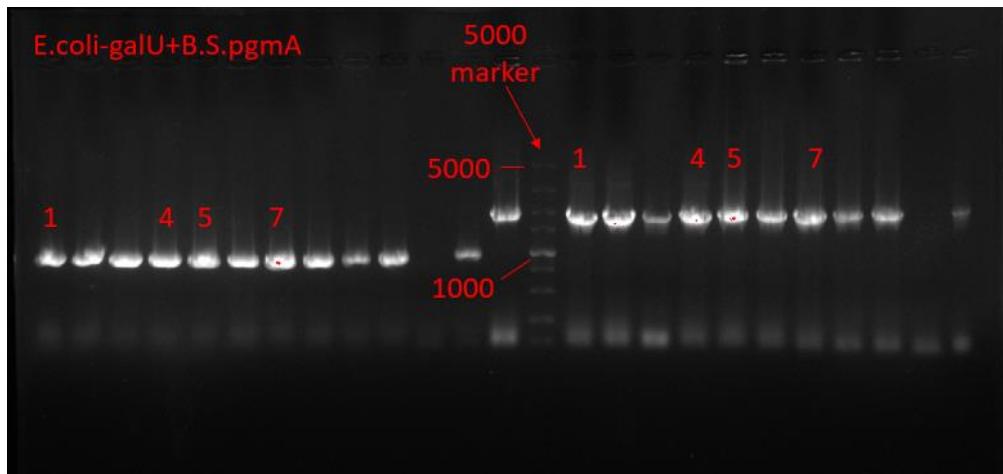


2020.8.20

1. Linking pieces using Golden Gate pHT vehicle + E . coli-galU + B.S. pgmA

2. Identify Golden Gate production by doing colony PCR.

Result: Select NO. 1,4,5 and 7 colonies cultures to culture, extract plasmids and sequence.



**2020.8.21**

Enzyme digestion validation of Golden Gate production.

**2020.8.25**

Spread plate:

- ①B.S. galU + B.S. pgmA
- ②E.coli-galU + E . coli- pgmA
- ③E.coli-galU + P.A. pgmA

**2020.8.26**

1. Spread plate again:
2. Golden Gate: pH'T vehicle +B.S. galU + P.A. pgmA

**2020.8.27**

1. Identify Golden Gate production (B.S. galU + P.A. pgmA) by doing colony PCR.

Tube1:

2xTaq	75µL
P43-F	7.5µL
B.S. galU -R	7.5µL
dd water	45µL

Tube2:

2xTaq	75µL
P.A. pgmA -F	7.5µL
Terminator-R	7.5µL
dd water	45µL

The above system was evenly divided into 9 tubes, and a negative control group and a positive control group were also set.

negative control	1µL dd water	9µL dd water
positive control	1µL pB.S. galU	9µL dd water

- Transfer E.coli-galU + B.S. pgmA into competent cells of Bacillus subtilis. Then culture and do colony identification.

### 2020.8.28

- Colony PCR: B.S. galU + P.A. pgmA
- Golden Gate Assembly: B.S. galU + P.A. pgmA
- Transfer B.S. galU + P.A. pgmA into competent cells of Bacillus subtilis.

### 2020.8.29

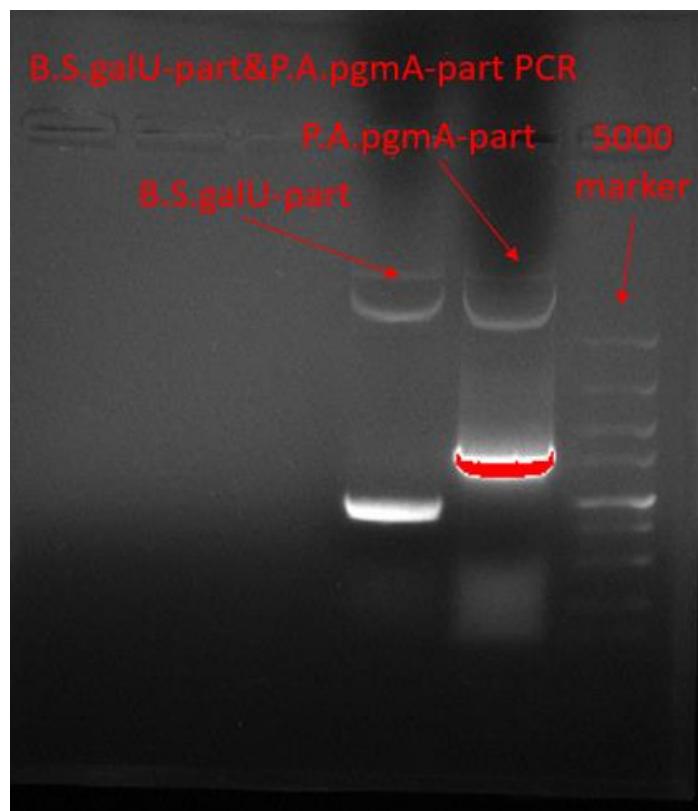
- Colony PCR: pHT vector
  - negative control: dd water
  - positive control: ①E.coli-galU + E.coli-pgmA;  
②E.coli-galU + E.coli-pgmA;  
③B.S. galU + B.S. pgmA

Result: Failed.

- Cryopreservation of bacteria:
  - ①E . coli-galU + E . coli- pgmA
  - ②E . coli-galU + E . coli- pgmA
  - ③B.S. galU + B.S. pgmA

### 2020.8.30

- Repeat experiment 1 from yesterday.
- PCR: ①B.S. galU ②P.A. pgmA



2020.8.30

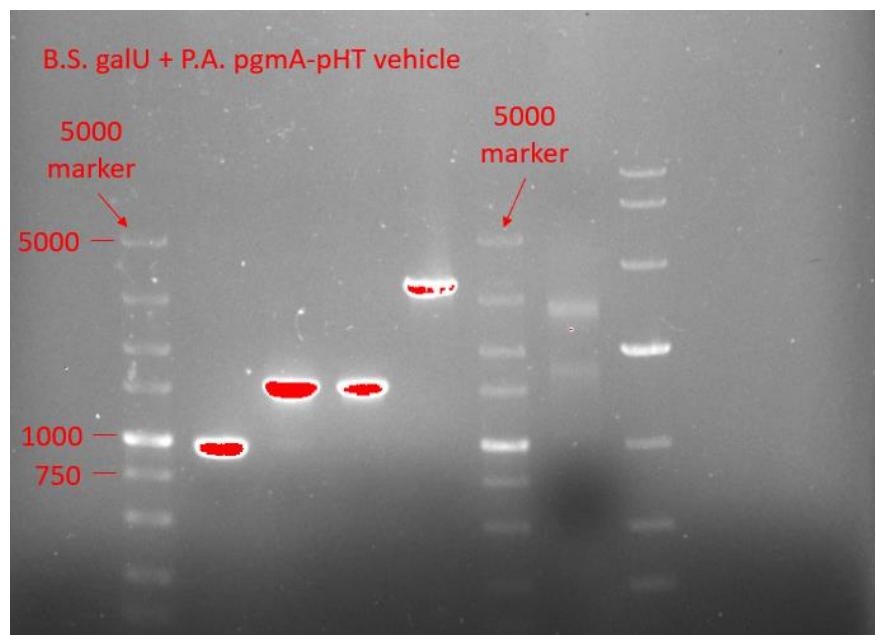
Golden Gate Assembly: B.S. galU + P.A. pgmA

2020.09.01

1. pH PCR, verify whether the plasmid is valid
2. Gel Extraction.  
Concentration: 33.00 $\mu$ g/ml
3. pH vehicle +B.S. galU + P.A. pgmA Godengate
4. pH vehicle +B.S. galU + P.A. pgmA Transformation

2020.09.03

1. Colony PCR verification: pH vehicle +B.S. galU + P.A. pgmA



2. Measuring growth curve: B.S. E. coli-galU + B.S. pgmA

2020.09.04

1. Preliminary experiment of 168+Nsp co-cultivation

Inoculation amount:

168	1 $\mu$ L
Nsp	100 $\mu$ L
LB	10ml

2. Plasmid Extraction: B.S. galU + P.A. , total three groups.

Concentration: 276.7 $\mu$ g/ml; 307.4 $\mu$ g/ml; 343.3 $\mu$ g/ml

2020.09.06

1. MazF-Plasmid transformation
2. B.S. galU +E.coli-pgmA Golden Gate
3. Activation culture:

- ① B.S. galU + B.S. pgmA
- ② E. coli galU + E. coli pgmA
- ③ E. coli galU + B.S. pgmA
- ④ B.S. galU + E. coli pgmA

**2020.09.07**

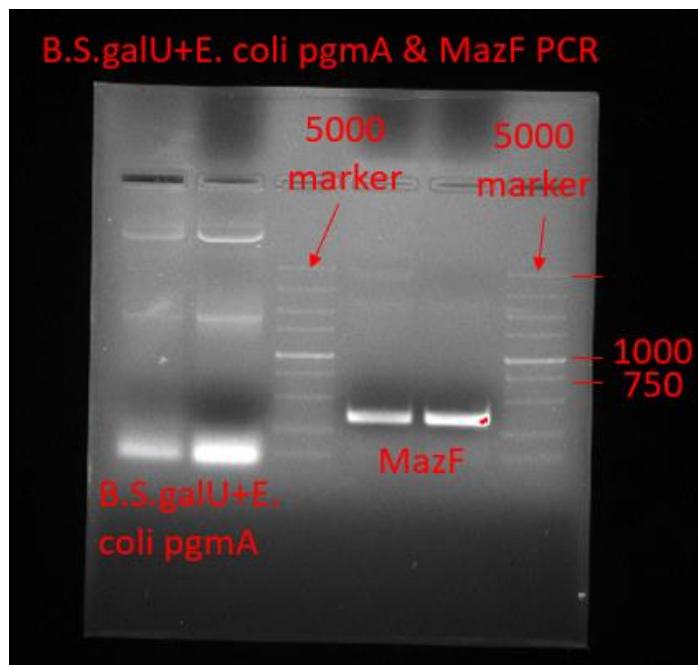
After 24 hours of cultivation, determine the OD value of the bacterial solution:

E. coli-galU + P.A. pgmA	A=0.780
B.S. galU + B.S. pgmA	A=0.710
E. coli-galU + E . coli- pgmA	A=0.766
E. coli-galU + B.S. pgmA	A=0.753

**2020.09.08**

1. Colony PCR: B.S. galU + E. coli-pgmA
2. E. coli-pgmA part PCR.  
Result: The band is too shallow to do gel extraction.
3. MazF plasmid extraction
4. MazF part PCR:

plasmid	1µL
Q5 Reaction Buffer	10µL
dNTPs	1µL
MazF -R	2.5µL
MazF -F	2.5µL
GC Enhancer	10µL
Q5 Enzyme	0.5µL
dd water	22.5µL

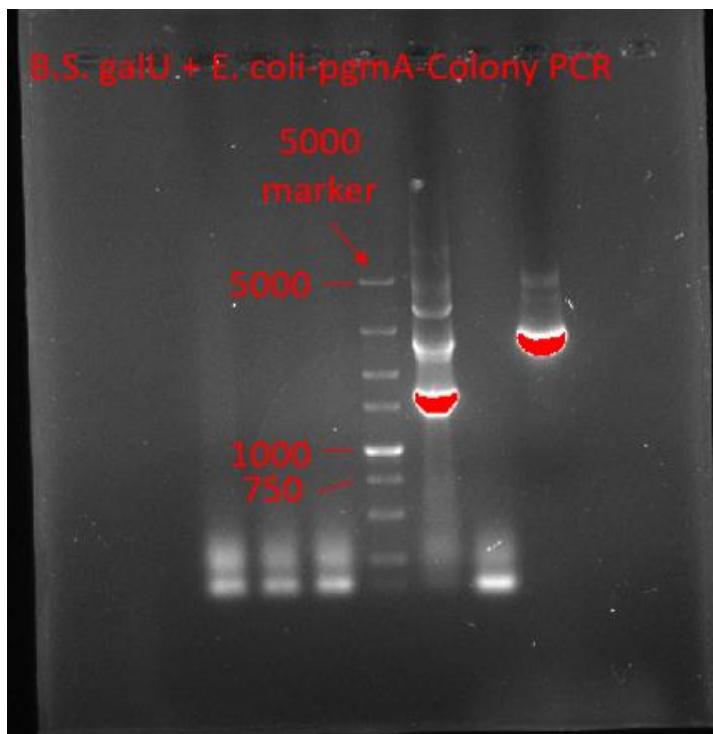


**2020.09.09**

B.S. galU + E. coli-pgmA: plasmid extraction and colony identification

**2020.09.10**

1. B.S. galU + P.A. pgmA: colony PCR:



2. MazF: Plasmid extraction, Cryopreservation of bacteria and part PCR

**2020.10.04**

EP, EE, BE, BP, BB in E. coli plasmid extraction and RNA extraction:

	Plasmid concentration / µg·ml	RNA concentration / µg·ml
EP	2872.2	351.1
EE	2717.2	351.9
BE	2553.0	612.6
BP	2542.9	627.3
BB	2510.8	421.0

**2020.10.05**

RT-PCR:

1	EP	0.35µL	RNase free water	7.65µL
2	EE	0.37µL	RNase free water	7.63µL
3	BE	0.39µL	RNase free water	7.61µL
4	BP	0.39µL	RNase free water	7.61µL
5	BB	0.40µL	RNase free water	7.60µL

## 2020.10.13

1. Culture E. coli with pBAD.
2. Culture DH5 $\alpha$  with EP, EE, BE, BP, BB, EB.

## 2020.10.14

1. Q5 PCR: ①PHT 43 (7868bp, 64°C) ②MazF (376bp, 63°C)
2. pBAD-His B Enzyme digestion verification

## 2020.10.15

pBAD, EP, EE, BE, BP, BB, EB-Single colony activation

## 2020.10.16

1. pBAD, EP, EE, BE, BP, BB, EB-Cryopreservation of bacteria
2. pBAD-His B: Plasmid extraction

## 2020.10.17

Measuring growth curve: EP, EE, BE, BP, BB, EB in DH5 $\alpha$   
(Positive control DH5 $\alpha$ , negative control LB)

## 2020.10.18

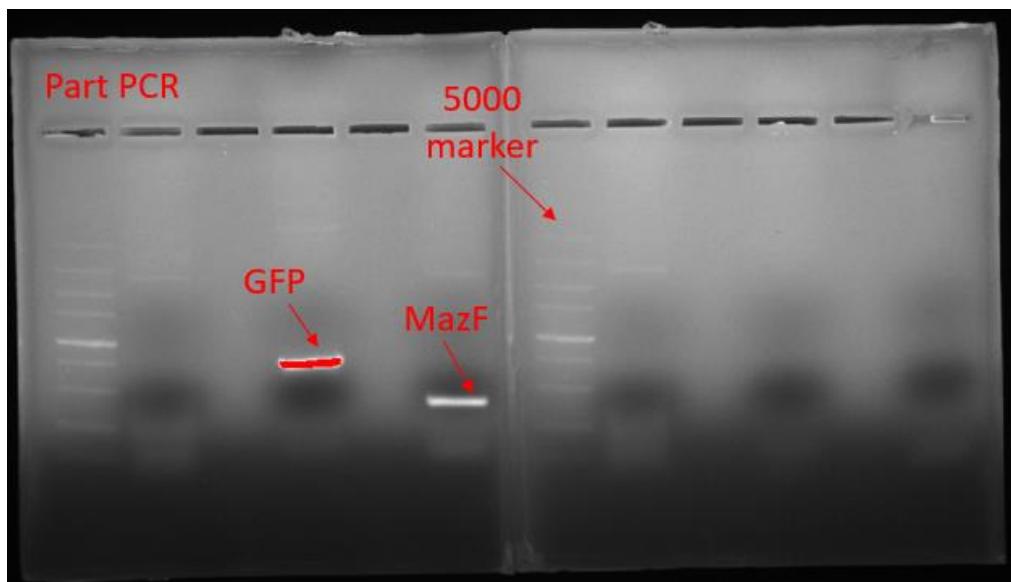
1. Part PCR:

Part	Annealing temperature / extension time	Primer
①E.coli pBAD vector	67°C/2min 10s	ara-F1
		ara-R1/2
②B.S. pBAD vector	64°C/1min 30s	ara-F2
		ara-R1/2
③GFP	64°C/30s	araGFP-F
		araGFP-R
④MazF	63°C/15s	ara MazF -F
		ara MazF -R
⑤ara 1	65°C/15s	ara BS -F
		inter -R
⑥ara 2	69°C/30s	ara BS -F
		inter -R

Result: Only ③ and ④ groups succeeded.

2. Gel Extraction of ③ and ④

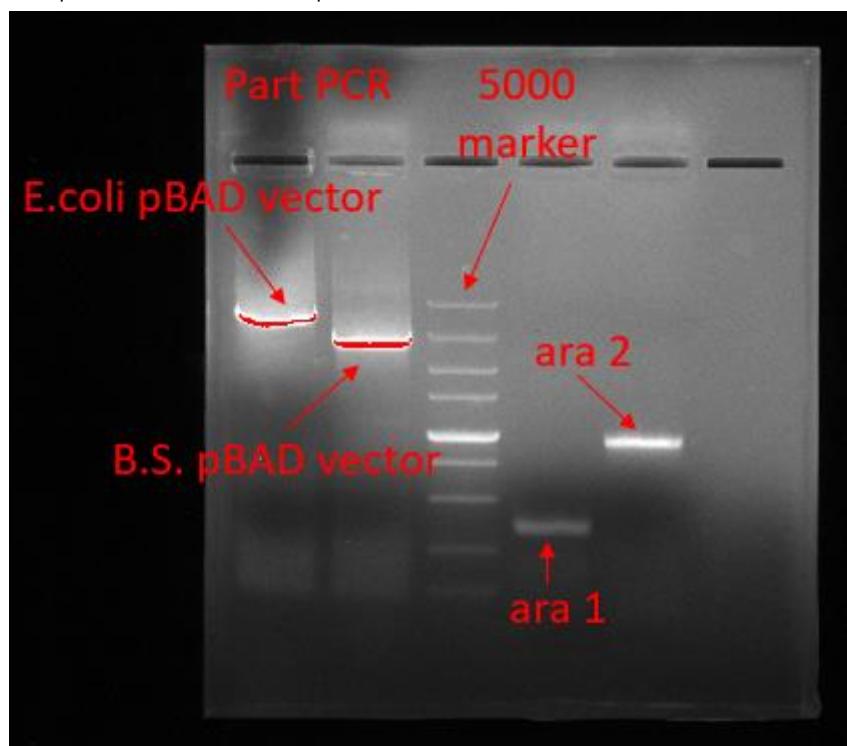
The concentrations are respectively: 28.11 $\mu$ g/ml, 34.19 $\mu$ g/ml



2020.10.19

1. Part PCR:

- ① E.coli pBAD vector; ② B.S. pBAD vector; ③ ara 1; ④ ara 2



2. Gel Extraction

Concentrations:

- ① 9.284 $\mu$ g/ml; ② 17.73 $\mu$ g/ml; ③ 15.10 $\mu$ g/ml; ④ 11.05 $\mu$ g/ml

2020.10.20

Linking pieces using Golden Gate:

- ① pBAD + GFP
- ② pBAD + araR + GFP (B.S.)

- ③ pBAD + MazF
- ④ pBAD + araR+ MazF (B.S.)

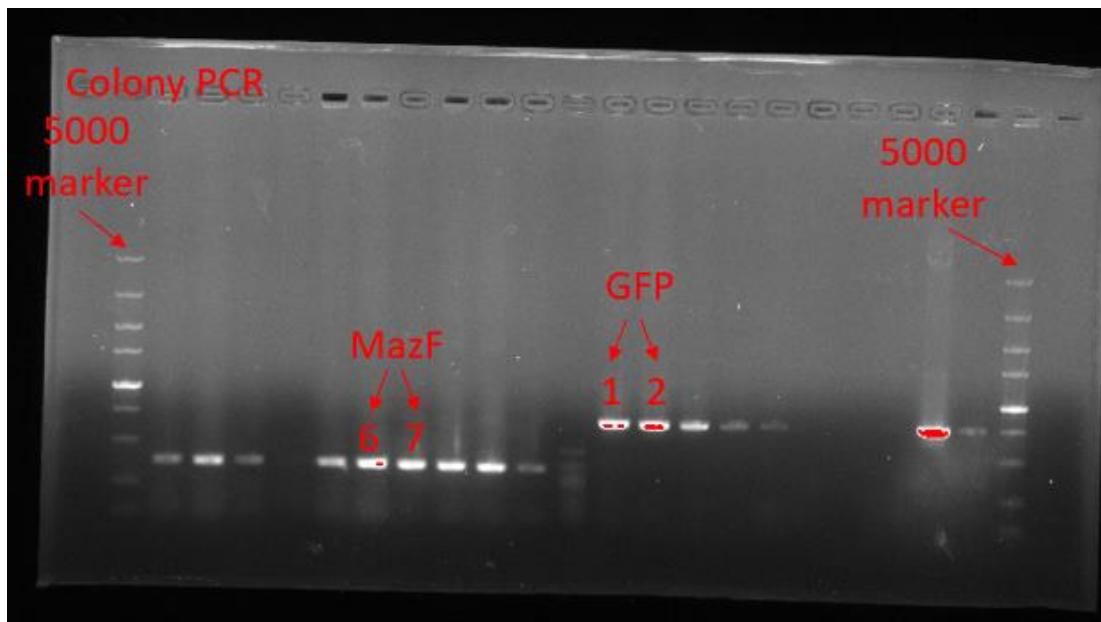
Result: ①②④ failed. ③ succeed.

**2020.10.21**

1. Linking pieces using Golden Gate:

- ① pBAD + araR+ MazF (B.S.)
- ② pBAD + araR + GFP (B.S.)

2. Do colony PCR verification after transformation with the above products.



Result:

① pBAD + araR+ MazF (B.S.)	Select NO.1 and 2 colonies cultures to culture, extract plasmids and sequence.
② pBAD + araR + GFP (B.S.)	Select NO. 6 and 7 colonies cultures to culture, extract plasmids and sequence.

The sequencing result of ② does not match the genetic map, so we plan to reconstruct the plasmid.

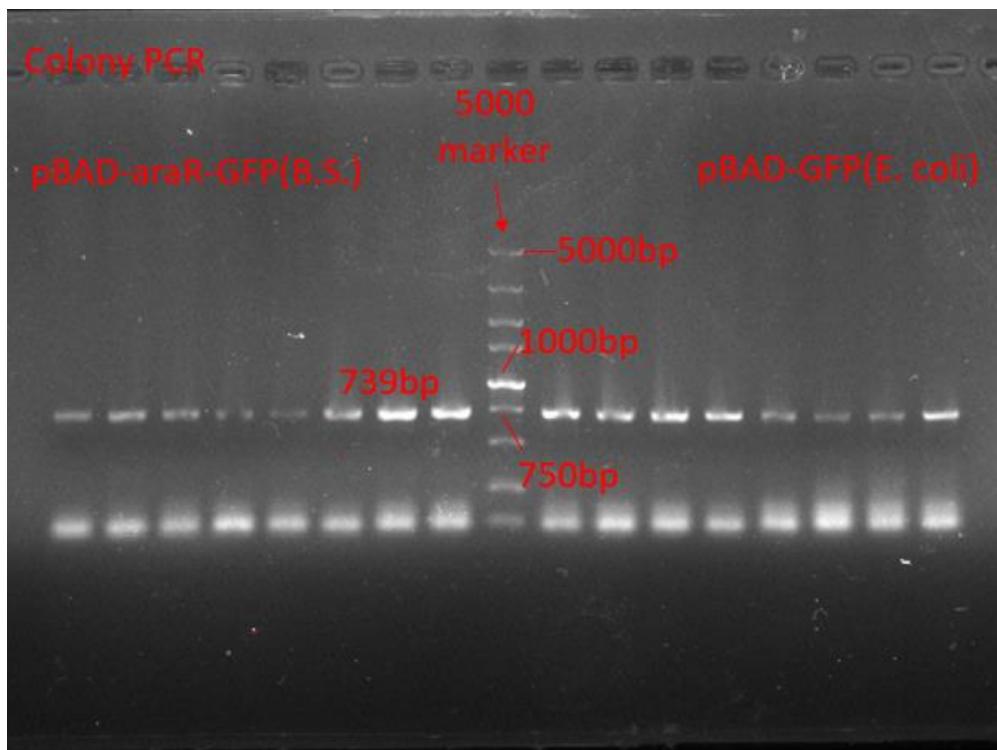
**2020.10.24**

1. Linking pieces using Golden Gate:

- ① pBAD + araR + GFP (B.S.)
- ② pBAD + GFP

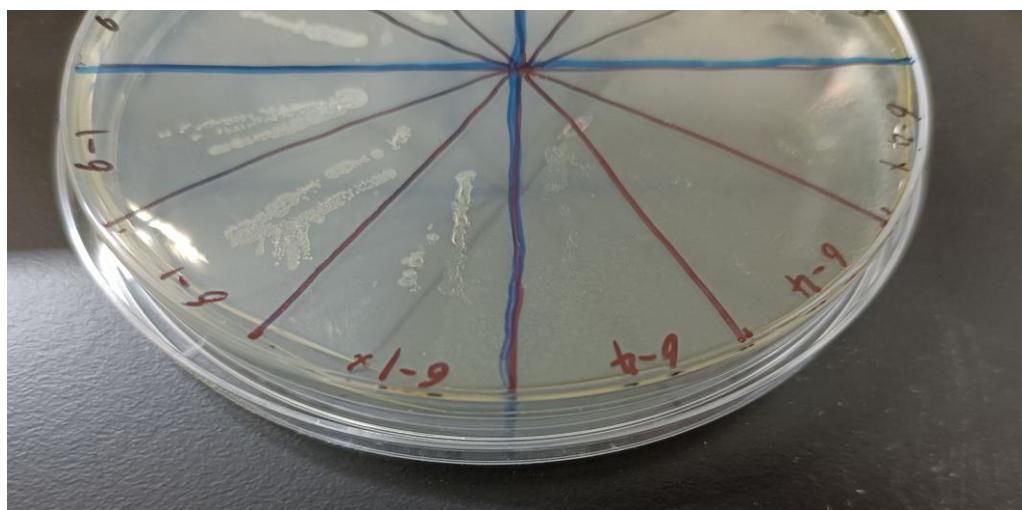
2. Do colony PCR verification after transformation with the above products.

Result: The resulting bacteria contain the target gene fragment.



2020.10.27

Verify that arabinose can induce MazF expression of suicide gene.



In the figure, the bacteria in this plate contained plasmids of pBAD-MazF. Three of 6-1 groups were the control group without arabinose induction, and three of 6-4 groups were the experimental group with 1.5 M/L arabinose induction. Where, the dilution factor of the group with \* is  $10^4$ , and the dilution factor of the group without \* is  $10^6$ .

Result: The bacteria without arabinose induction grow normally, while with arabinose induction, the bacteria suicide.

2020.10.28

Verify the expression of GFP induced by arabinose to compare the efficiency of arabinose operon in B.S. and E.coli.