Ⅰ. Standardize Parts

1. Add G-P (Rabies virus strain ERA glycoprotein) to PSB1C3

1.1 PCR amplify G-P (Rabies virus strain ERA glycoprotein) 50ul volume

2013/8/17

TaKaRa LA Taq	0.5ul
dNTP	2ul
Template	1ul
Primer f	1ul
Primer r	1ul
10×buffer	5ul
ddH2O	39.5ul
Total	50ul

Tm=64℃

95℃	5min	
95℃	30S	
64℃	30S	30cycle
72℃	1min40s	
72℃	5min	
4℃	∞	

Lip temperature 105℃

Primer f: GAATTCGCGGCCGCTTCTAGACCATGGTTCCTCAGGCTCT

Primer r: CTGCAGCGGCCGCTACTAGTATCACAGTCTGGTCTCACCCC

Gel Extraction (AxyPrep DNA Gel Extraction Kit)

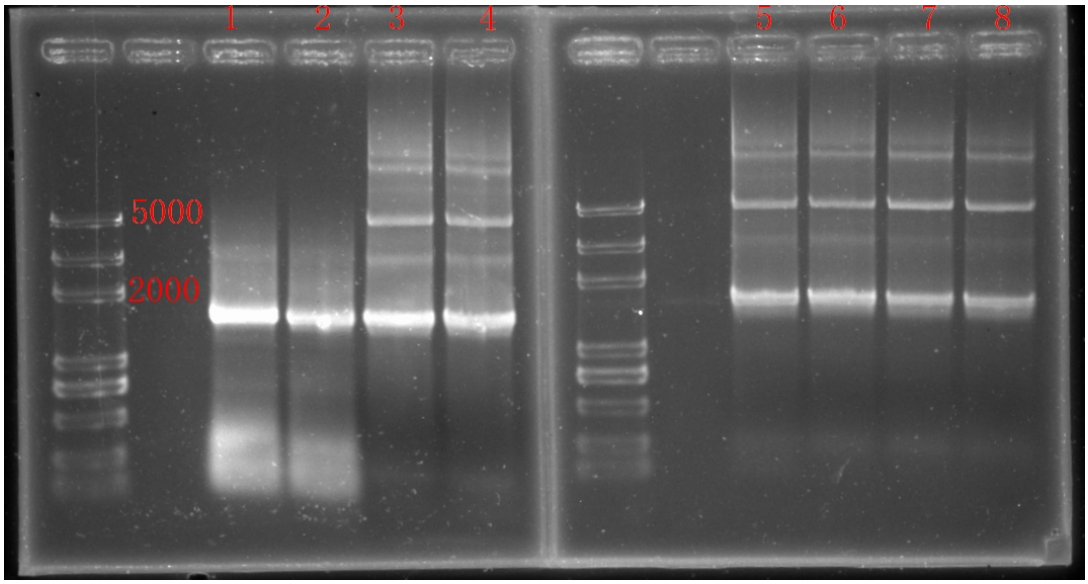


Fig.1 G-P PCR

1.2 PCR products and PSB1C3 double enzyme cutting

2013/8/18

PCR products

EcoRI 1ul
SpeI 1ul
H buffer 3ul
DNA 23ul
ddH_2O up to 30ul

PSB1C3

EcoRI 1ul
SpeI 1ul
H buffer 2ul
DNA 8ul
ddH_2O up to 20ul

1.3 ligation

G-P 10ul
PSB1C3 4ul
T4 DNA ligase 1ul
Buffer 2ul
ddH20 up to 20ul

1.4 Electroporation

Add 1ul ligation products to compement cells

1.5 Validation

2013/8/20
Extract Plasmid DNA by using Plasmid DNA Extract kit
Digest Plasmid DNA by EcoRI PstI

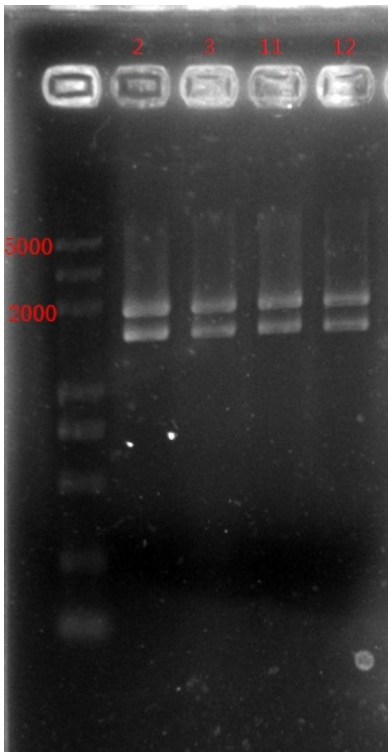


Fig.2 8.20 G-P+PSB1C3 double enzyme cutting
G-P-2/3/11/12 are sequenced
http://igem.org/File:8-20_G-P-2,3,11,12.zip

2. Assembly standard part

2.1 Add G-P behind 1-11E

2013/8/31

2.1.1 G-P digest by XbaI、PstI

XbaI	2ul
PstI	2ul
M buffer	4ul
BSA	4ul
DNA	16ul
ddH2O up to	40ul

2.1.2 1-11E digest by SpeI、PstI

DNA	20ul
SpeI	2ul
PstI	2ul
H Buffer	4ul
ddH2O up to	40ul

2.2 ligation

G-P	14ul
1-11E	3ul
T4 DNA ligase	1ul
Buffer	2ul

ddH20 up to 20ul

2.3 Electroporation

Add 1ul ligation products to compement cells

2.4 Validation

Extract Plasmid DNA by using Plasmid DNA Extract kit

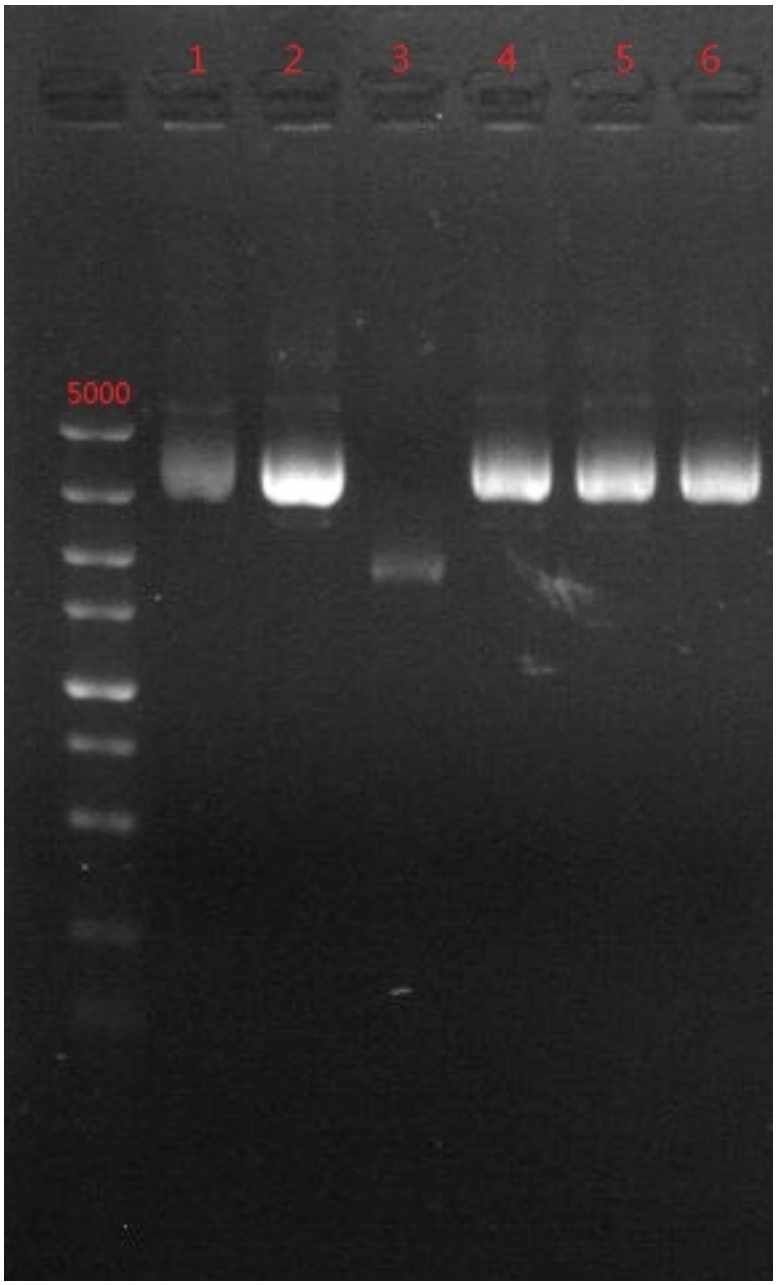


Fig.3 9.2 G-P+1-11E

G-P-E-2/4/5/6 are sequenced, no problem.

http://igem.org/File:9-2_G-P-E-2,4,5,6.zip

Ⅱ. Express of G-P (Rabies virus strain ERA glycoprotein)

1. Add G-P (Rabies virus strain ERA glycoprotein) to PSB1C3

1.1 PCR amplify G-P 50ul volume

2013/7/23

TaKaRa LA Taq	0.5ul
dNTP	2ul
Template	1ul
Primer f	1ul
Primer r	1ul
10×buffer	5ul
ddH2O	39.5ul
Total	50ul

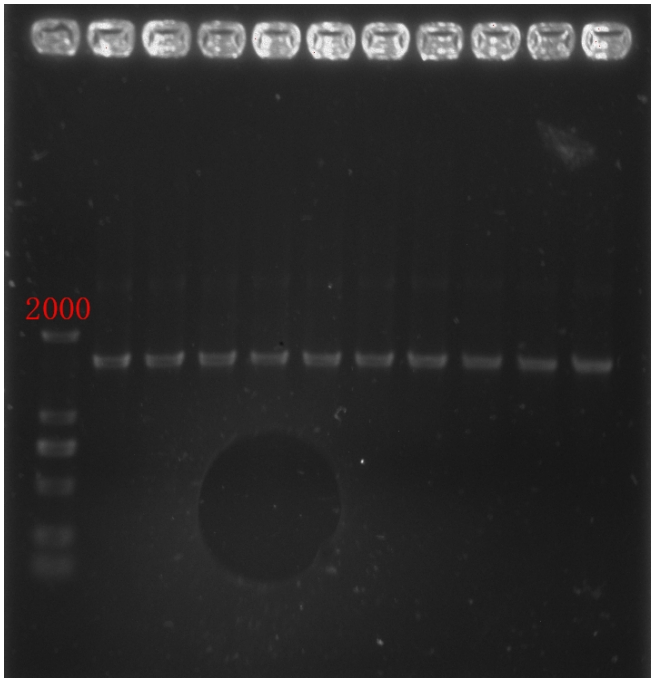
Tm=64℃

95 ℃	5min	
95 ℃	30S	
64 ℃	30S	↓ 30cycle
72 ℃	1min40s	
72 ℃	5min	
4 ℃	∞	

Lip temperature 105 ℃

Primer f: GAATTCGCGGCCGCTTCTAGACCATGGTTCCTCAGGCTCT

Primer r: TACTAGTAGCGGCCGCTGCAGTCACAGTCTGGTCTCACCCC



Fig,4 G-P PCR products

Gel Extraction (AxyPrep DNA Gel Extraction Kit)

1.2 PCR products and PSB1C3 double enzyme cutting

2013/7/28

PCR products

EcoRI	1ul

PstI 1ul
H buffer 4ul
DNA 25ul
ddH_2O up to 40ul
PSB1C3
EcoRI 1ul
PstI 1ul
H buffer 2ul
DNA 9ul
ddH_2O up to 20ul

1.3 ligation

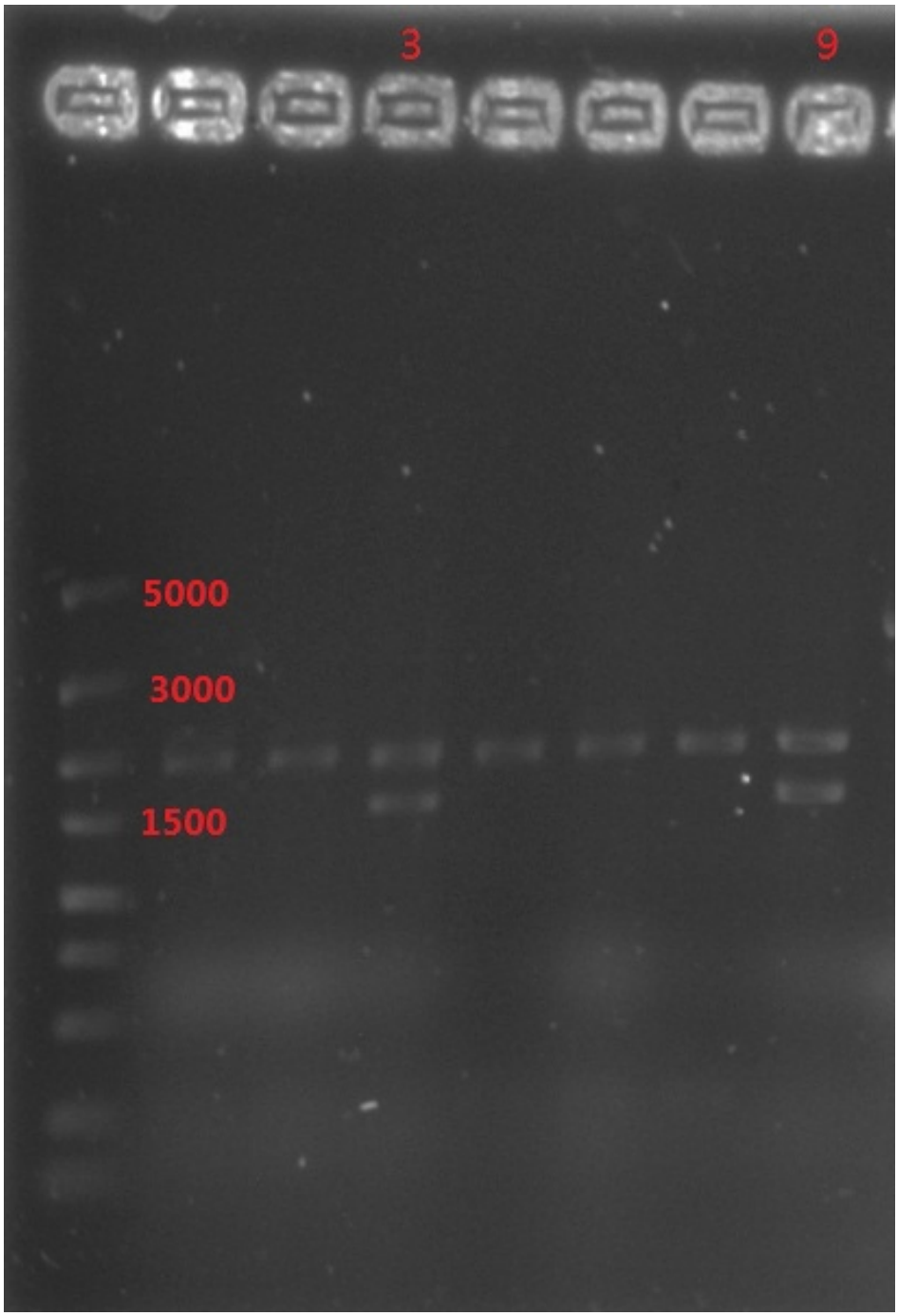
g-p 9ul
PSB1C3 3ul
T4 DNA ligase 1ul
Buffer 1ul
ddH20 up to 20ul

1.4 Electroporation

Add 1ul ligation products to compement cells

1.5 Validation

2013/7/30
Extract Plasmid DNA by using Plasmid DNA Extract kit
Digest Plasmid DNA by EcoRI PstI



Fig,5 G-P+PSB1C3 double enzyme cutting

G-P-3/9 are sequenced

http://igem.org/File:7-30_G-P-3,9.zip

2. Add G-P behind 1-11E

2013/8/9

2.1 G-P digest by XbaI、PstI

XbaI 1ul

PstI 1ul

M buffer 3ul
BSA 1ul
DNA 20ul
ddH2O up to 30ul

2.2 1-11E digest by SpeI、PstI

DNA 16ul
SpeI 1ul
PstI 1ul
H Buffer 2ul
ddH2O up to 20ul

2.3 ligation

G-P 12ul
1-11E 6ul
T4 DNA ligase 1ul
Buffer 2ul
ddH20 up to 20ul

2.4 Electroporation

Add 1ul ligation products to compement cells

2.5 Validation

Extract Plasmid DNA by using Plasmid DNA Extract kit
Digest Plasmid DNA by EcoRI PstI

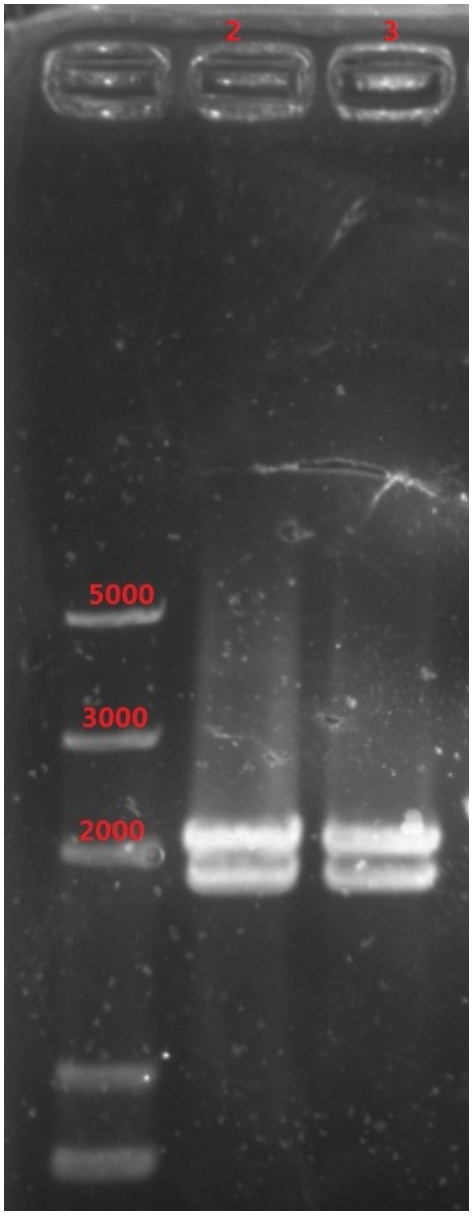


Fig.6 g-p+1-11e double enzyme cutting
g-p-e-2/3 are sequenced
http://igem.org/File:8-13_g-p-e-2,3.zip

3. Ligate G-P+1-11E to PHT304

3.1 G-P with 1-11E digest by EcoRI PstI

DNA	16ul
EcoRI	1ul
PstI	1ul
H Buffer	2ul
ddH2O up to	20ul

3.2 PHT304 digest by EcoRI PstI and gel extraction

DNA	16ul
EcoRI	1ul
PstI	1ul

H Buffer 2ul
ddH2O up to 20ul
Gel Extraction (AxyPrep DNA Gel Extraction Kit)

3.3 ligation

2013/9/11
G-P+1-11E 10ul
Pht304 7ul
T4 DNA ligase 1ul
Buffer 2ul
ddH20 up to 20ul

3.4 Electroporation

Add 2ul ligation products to competent cells

3.5 Validation

2013/9/13
Extract Plasmid DNA by using Plasmid DNA Extract kit
Digest Plasmid DNA by EcoRI PstI

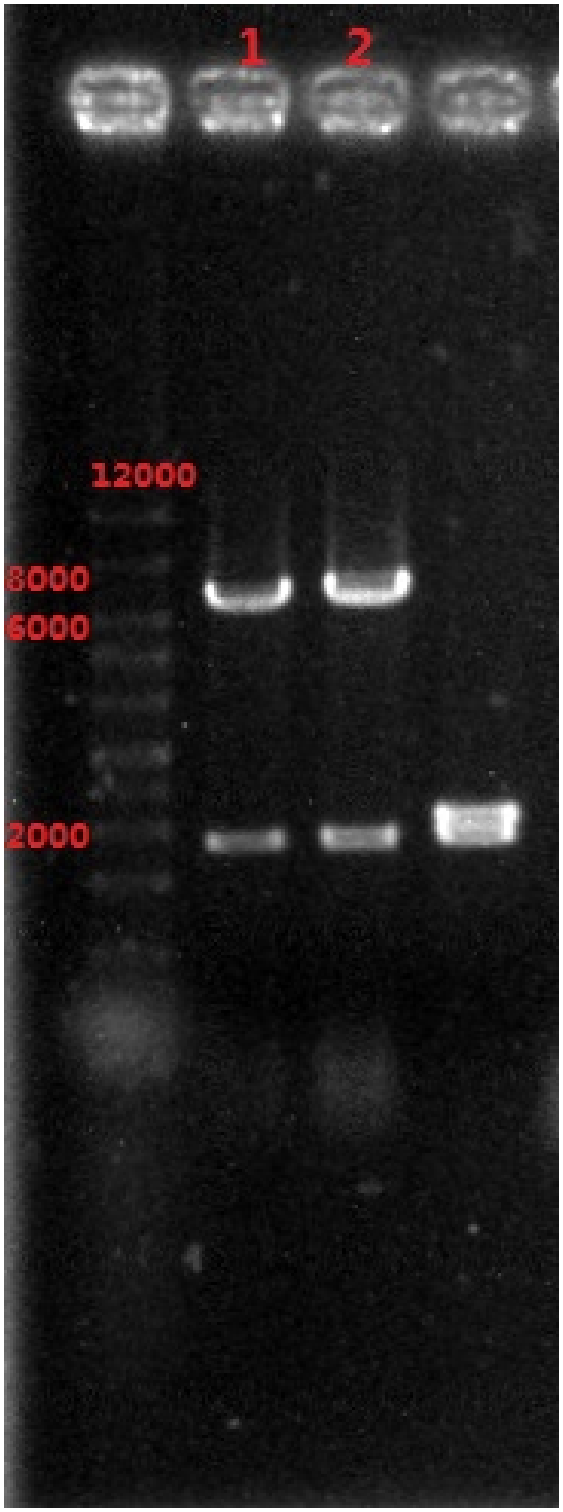


Fig.7 g-p+1-11e_pht304 double enzyme cutting
g-304-1/2 are sequenced
http://igem.org/File:9-13_G-304-1,2.zip

4. Electroporation PHT304 to BS

2013/9/15
Add 5ul g-p+1-11E+pht304 plasmid to competent cells.

5. Western blotting

2013/9/20

1. The recombinant strains which were trans formed with the plasmid pht-304 were inoculated into 5 ml LB broth containing 25μg erythromycin ml^{-1}, shaken at 37° C overnight, and then 100 ml LB medium supplemented with erythromycin was inoculated with 1 ml recombinant B.subtilis overnight culture. The cultures were grown at 37°C with shaking at 200 rpm for 10h,
2. The strains carrying only the pht-304 shuttle vector with no insert were used as controls.
3. The liquid culture was centrifuged at 10000× g for 10 min , and the cell-free supernatant was then concentrated with Millipore.
4. Add 20 ul 5XSDS sample buffer to 80 ul concentrated culture and heat 95°C for 10 minutes;
5. Load 20 μl onto 12% SDS-PAGE gel，80V for stacking gel , 120V for separation gel .
6. Make the gel for transfer in transfer buffer: 12V overnight, on ice.
7. Block the filter with blocking buffer for 1 hour at room temperature with gentle agitation on a platform shaker.
8. Discard blocking solution and immediately incubate filter with primary antibody.
9. Add 0.005 ml of primary antibody (1:5000) in to blocking solution. Incubate 2 hour at room temperature. with gentle agitation on a platform shaker.
10. Discard blocking solution and wash filter 3 times (5 minutes each time) with TBST.
11. Immediately incubate the filter with secondary antibody, add 0.003ml of secondary antibody solution (1:3000).
12. Incubate 1 – 2 hours at room temperature with gentle agitation.
13. Discard blocking solution and wash filter 3 times (5 minutes each time) with TBST.
14. Chemiluminescence for 1minute.

Results: