Project: Construction of pTALE Library

Team: GreatBay_China

Date: 2018-4-28 to 2018-10-04

SUNDAY, 2018-6-24

1. **Transformation of pSB1A2-BBA_E0240 form 2017 plate 4, 11N.**

   Adding 10μl ddH2O into 11N, and Transformation process sticks to protocol.

MONDAY, 2018-6-25

1.pSB1A2-BBA_E0240 has no colony formed. We decided to wait to one more day.

2. Transformation of RBS-GFP-Terminator from 2017 kit, plate 4, 11N

WEDNESDAY, 2018-6-27

1. **Plasmid extraction of GFP.**

   conc. 158.9ng/μl

THURSDAY, 2018-6-28

1. **Colony PCR of pSB1C3-GFP**

   6 colonies was chosen, in a system of 30μl, yet no band has shown.

   We deduced potential causes might be:

   —microbe contamination

   —too much colonies in the system

   — invalidation of VF，VR

FRIDAY, 2018-6-29

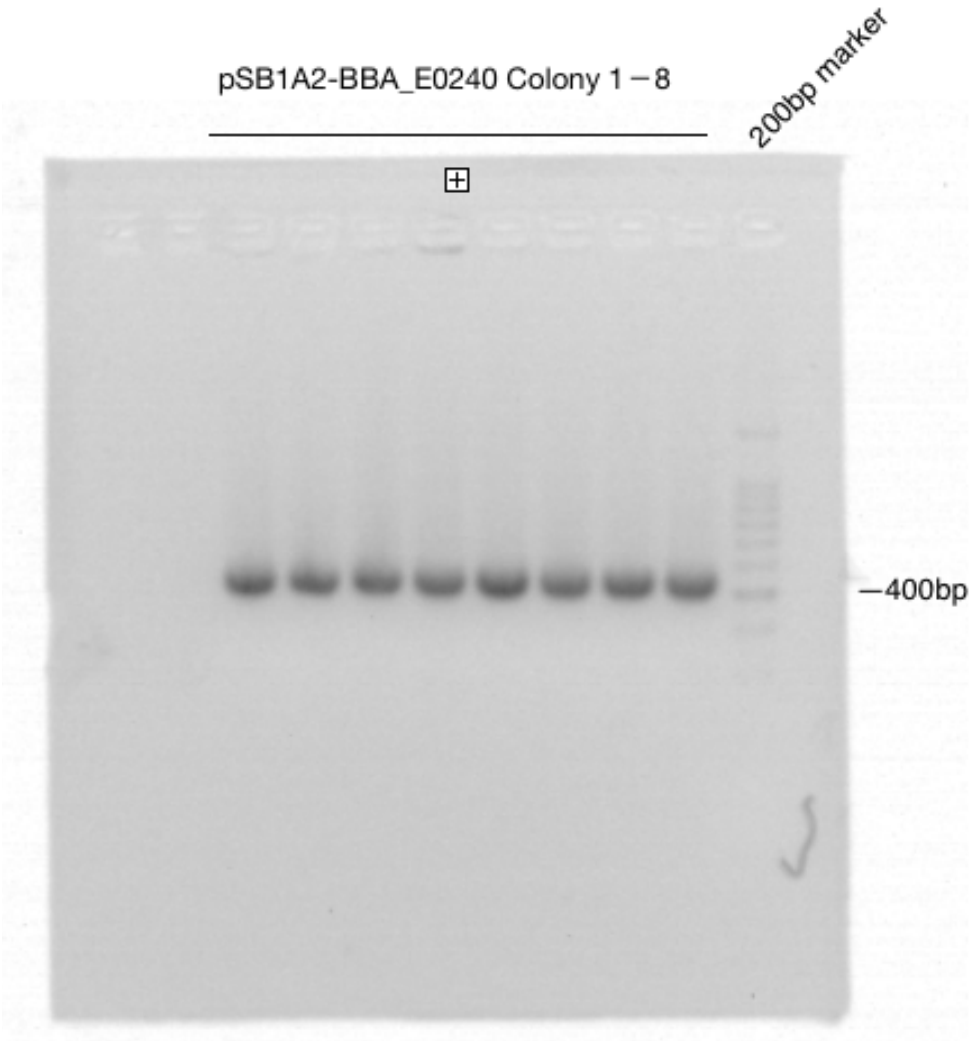
1. Transformation of pSB1A2-BBA_E0240

from 2018 kit, plate 4, 11N

SUNDAY, 2018-7-1

1. Colony PCR of pSB1A2-BBA_E0240

We cloned 6 colonies and PCR results proved they all contain pSB1A2-BBA_E0240. They are inoculated into 5ml LB, overnight 37°C at 220 rpm.



MONDAY, 2018-7-2

1. Plasmid extraction of E0240 No.1-8

Been proved correct by sequencing.

WEDNESDAY, 2018-7-4

1. Restriction enzyme digestion of pTALE sp1, pTALE sp2, GFP.

GFP: EcoRI & xBAI

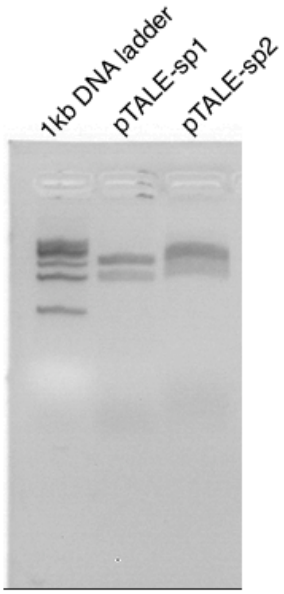
pTALE sp1/sp2: SpeI&EcoRI

Failed with unknown cause. We decide to repeat the Restriction.

THURSDAY, 2018-7-5

1. Restriction of pTALE-sp1/sp2

Follows RD protocol 8. For 1h at 37°C. Bands seemed to be incorrect. Length of bands were strange.

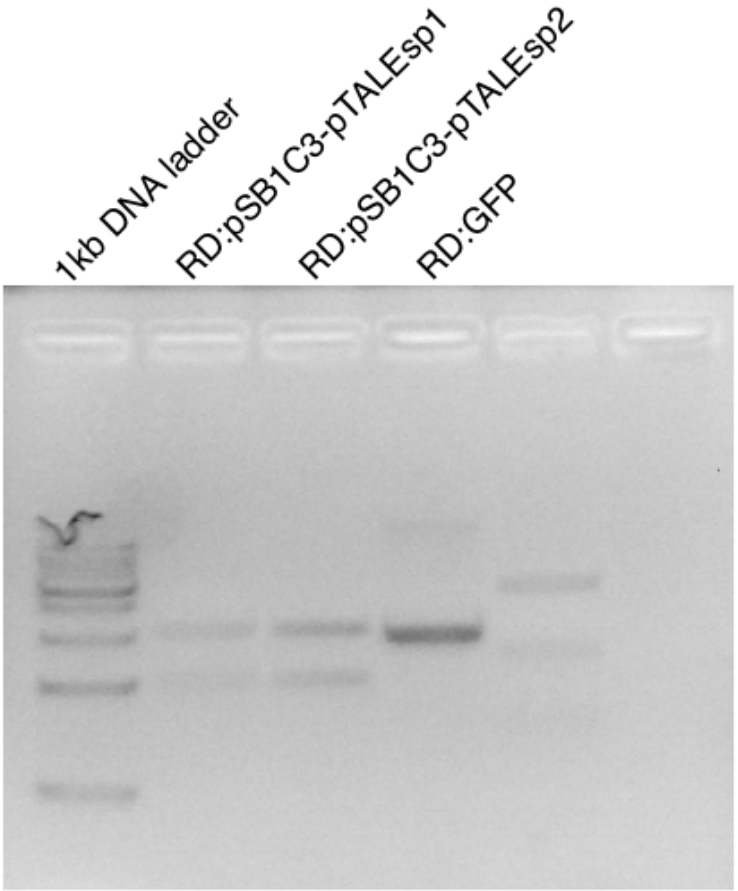


RD mix	30µl
10x cutsmart buffer	3µl
SpeI	1µl
EcoRI	1µl
pSB1C3-pTALEsp1/sp2	500ng
ddH2O	up to 30µl

FRIDAY, 2018-7-6

1. Restriction of pTALE-sp1/sp2 and GFP

Fragments of 3kb and 2kb of pSB1C3-pTALEsp1/sp2 was clearly showed. Purification was performed with TIANgel Midi Purification kit.



	conc. (ng/μl)
pTALEsp1	8
pTALEsp2	18.8
pSB1C3-GFP	73.8

SATURDAY, 2018-7-7

1. sticky-ends ligation of pTALEsp1/pTALEsp2 and pSB1C3-GFP

The ligation sample was prepared as the following:

RD mix	30μl
10x T4 ligase buffer	1.2μl
RD DNA sample	7.2μl/3.2μl/0.8μl
ddH2O	2.3μl/6.6μl/9.0μl

The three fragments was added into 2 different sample. We then added 0.5μl 10x T4 ligase buffer, 1μl ligase, 1μl pSB1C3-GFP linear fragment and 2.5μl ddH2O into the sample of pTALEsp1/pTALEsp2 as remediation. The sample was left in THERMOMIXER, 0r.p.m., 37°C overnight.

SUNDAY, 2018-7-8

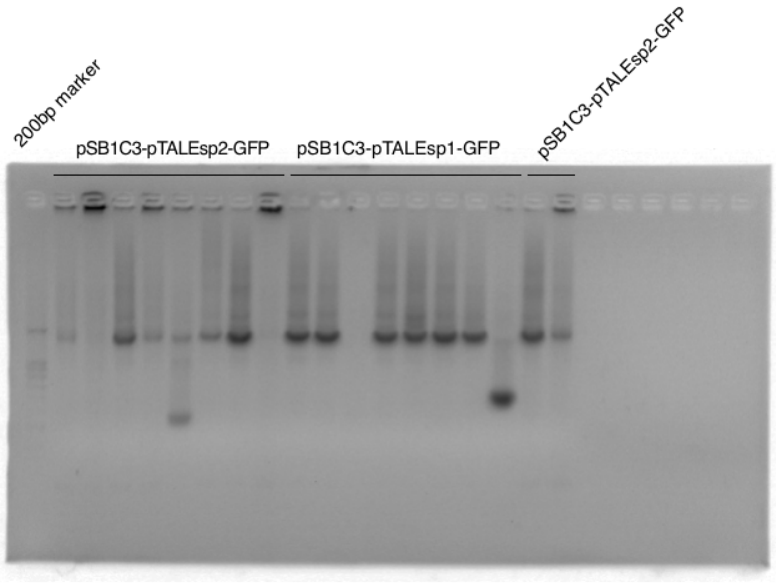
1. Transformation of pSB1C3-pTALEsp1/sp2-GFP liagtion sample.

A negative control was carried out. Three samples were plated on Chl LB dish and placed at 37°C overnight.

Notation: “7.8 pTALEsp1-GFP”, “7.8 pTALEsp2-GFP”, “7.8 ctrl”

TUESDAY, 2018-7-10

1. Colony PCR of pSB1C3-pTALEsp1/sp2-GFP



2 plate of pSB1C3-pTALEsp2-GFP, 8 colonies were picked from the plate of date 7.8 and 2 colonies were picked from the plate of 7.8. Only 1 plate of pSB1C3-pTALEsp1-GFP has colonies on, 8 colonies were picked. VF and VR were used, with the annealing temperature of 55°C and extension of 60s.

Thus, we deduced that undetectable Green fluorescence is due to the low leaking expression of pTALE, and the construction of pTALE-GFP is considered as a success.

WEDNESDAY, 2018-7-11

1. Prep for plasmid miniprep

pSB1C3-pTALEsp2-GFP: Colony # 1 2 3 4

pSB1C3-pTALEsp2-GFP: Colony #1 2

Inoculate into 5ml LB broth with 5μl Chl. Overnight shaking at 37°C.

THURSDAY, 2018-7-12

1. **Plasmid miniprep**

   ready for sequencing

WEDNESDAY, 2018-7-18

1. **Plasmid miniprep**

   pR6k-J23119-GFP 145.5ng/μl

   pSC101-J23119-GFP 170.15ng/μl

THURSDAY, 2018-7-19

1. **Amplification of “pTALEsp1”and”pTALEsp2” for backbone substitution.**

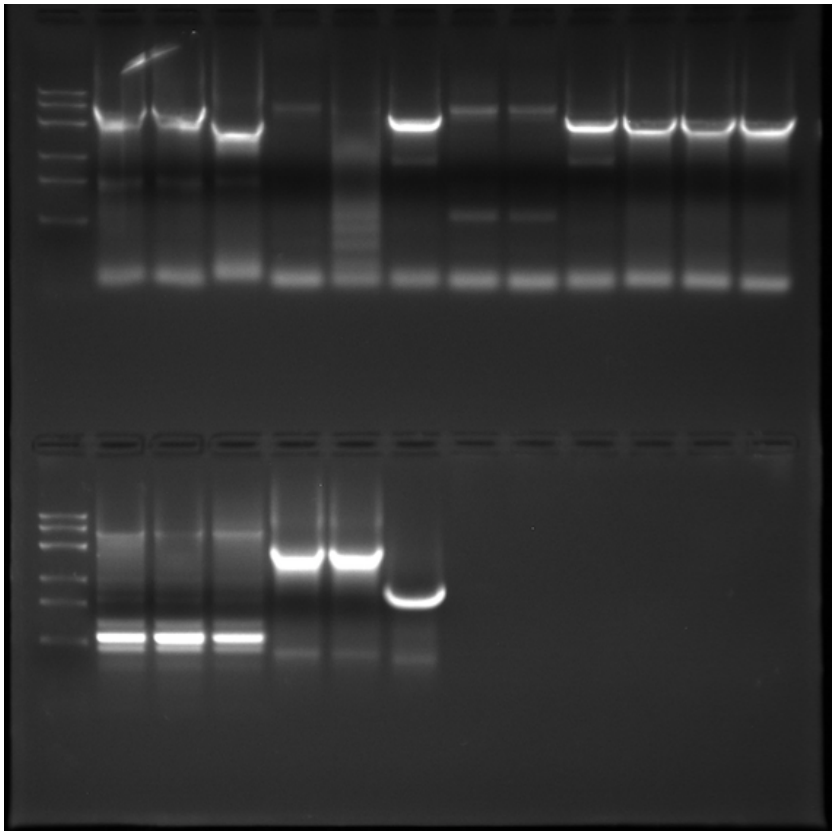
   50μl rTaq system was used, with primers BBpioF/RiboJR.

   Failed. Might due to a modicum of template.

FRIDAY, 2018-7-20

1. **Amplification of pSC101, pR6K, pUC20 for backbone substitution.**

	pTALEsp1	pTALEsp2	ptac	J23119
pUC20	RiboJ F/R24_R BBpio_F/RiboJ_R	RiboJ F/R24_R BBpio_F/RiboJ_R	existing	F24_F/R24_R R24_F/F24_R
pR6k	RiboJ F/R24_R BBpio_F/RiboJ_R	RiboJ F/R24_R BBpio_F/RiboJ_R	F24_F/R24_R R24_F/F24_R	existing
pSC101	RiboJ F/R24_R BBpio_F/RiboJ_R	RiboJ F/R24_R BBpio_F/RiboJ_R	F24_F/R24_R R24_F/F24_R	existing



20180720 Gibson assembly fragments

Trans8k ladder; pR6Kbackbone 1-3; pSC101backbone 1-3; pUC20backbone 1-3; pTALEsp1 1-3;

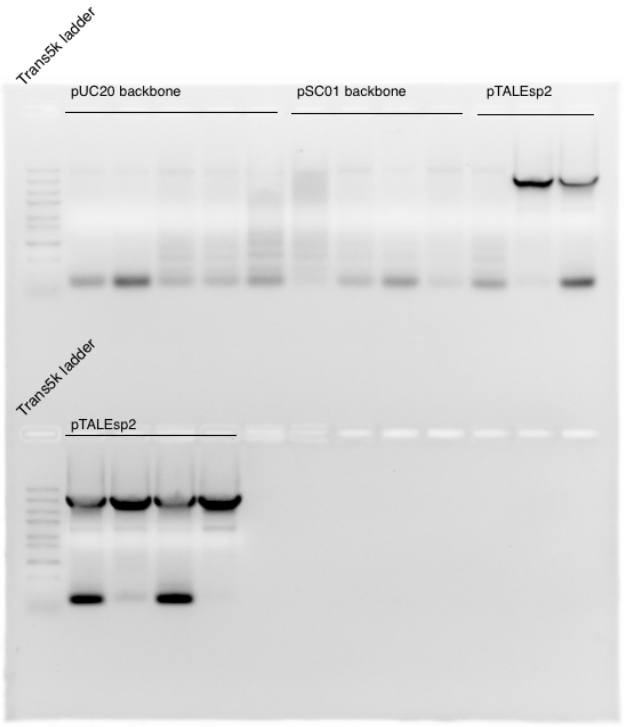
Trans8k ladder; pTALEsp2 1-2; J23119 1-2; ptac

Gel extraction was performed.

SATURDAY, 2018-7-21

1. Amplification of pSC101 backbone, pUC20 backbone and pTALEsp2 insertion.

pSC101 backbone needs to be re-amplified bucause the only correct band was polluted when doing gel extraction.



After gel extraction, Gibson assembly was carried to assemble the following:

1. pUC20-pTALEsp1
2. pUC20-pTALEsp2
3. pUC20-J23119
4. pR6k-pTALEsp1
5. pR6k-pTALEsp2
6. pR6k-ptac
7. pSC101-pTALEsp1
8. pSC101-pTALEsp2
9. pSC101-ptac

#5 & #8 used backbone with wrong sticky ends, the assay should be repeated.

SATURDAY, 2018-7-28

1. **Amplification of fragments of Gibson assembly**
2. **Plated pUC20-ptac-GFP on Amp LB plate**

MONDAY, 2018-7-30

1. **Gibson assembly ——backbone substitution**
    1. pUC20α1-pTALEsp1-1
    2. pUC20α-pTALEsp2-1
    3. pUC20ß1-J23119-2
    4. pR6k-α2-pTALEsp1-1
    5. pR6k-α2-pTALEsp2-1
    6. pR6k-ß2-ptac-1
    7. pSC101α1-pTALEsp1-2
    8. pSC101α2-pTALEsp2-2
    9. pSC101ß2-ptac1

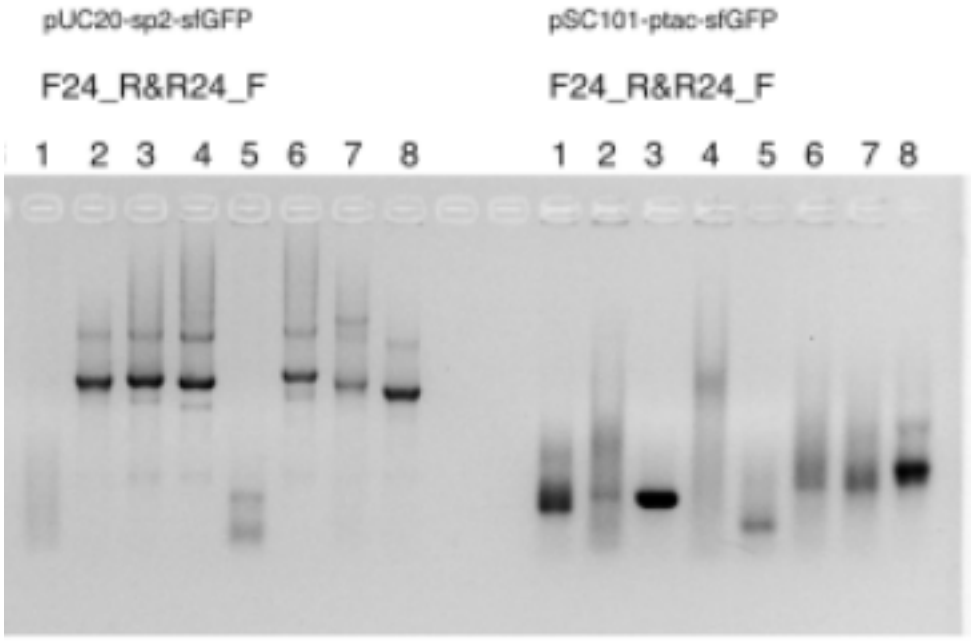
SUNDAY, 2018-7-31

1. **Transformation of gibson assembly product on date 7.30**
2. **Plasmid extraction of pUC20-ptac-GFP**

WEDNESDAY,2018-8-1

1. Plasmid PCR of pUC20-pTALE2-sp2-GFP and pSC101-ptac-GFP

Using primers F24_R/R24_F, with corresponding length of 4315bp and 2561bp.



FRIDAY, 2018-8-3

1. Making glycerol stock at -20°C and -80°C freezer, and plasmid miniprep. for following strains/ plasmids.

DH5a	pUC20-J23119－GFP-7	98.5ng/μl
DH5a	pUC20-J23119－GFP-6	102.7ng/μl
DH5a	pUC20-J23119－GFP-3	112.15ng/μl
DH5a	pUC20-pTALEsp1－GFP-4	90.15ng/μl
DH5a	pUC20-pTALEsp1－GFP-3	74.55ng/μl
DH5a	pUC20-pTALEsp1-GFP-1	99.15ng/μl
DH5a	pSC101-pTALEps2-GFP-5	64.65ng/μl
DH5a	pR6k－ptac-GFP－3	72.4ng/μl
DH5a	pR6k－ptac-GFP-1	66.3ng/μl
DH5a	pR6k－pTALEsp2-GFP-2	66.15ng/μl
DH5a	pR6k－pTALEsp2-GFP-1	69.65ng/μl
DH5a	pR6k－pTALEsp1-GFP-5	68.45ng/μl
DH5a	pSC101-pTALEsp2-GFP-2	74.4ng/μl
DH5a	pSC101-pTALEsp2-GFP-1	90.8ng/μl
DH5a	pSC101-pTALEsp1-GFP-5	71.7ng/μl
DH5a	pUC20-pTALEsp1-GFP-7	98.5ng/μl

SATURDAY, 2018-8-4

1. Plasmid extraction of pUC20-pTALEsp1-GFP-7

MONDAY, 2018-8-6

1. Colony PCR of pSC101-ptac-GFP and pUC20-TALE sp2-GFP

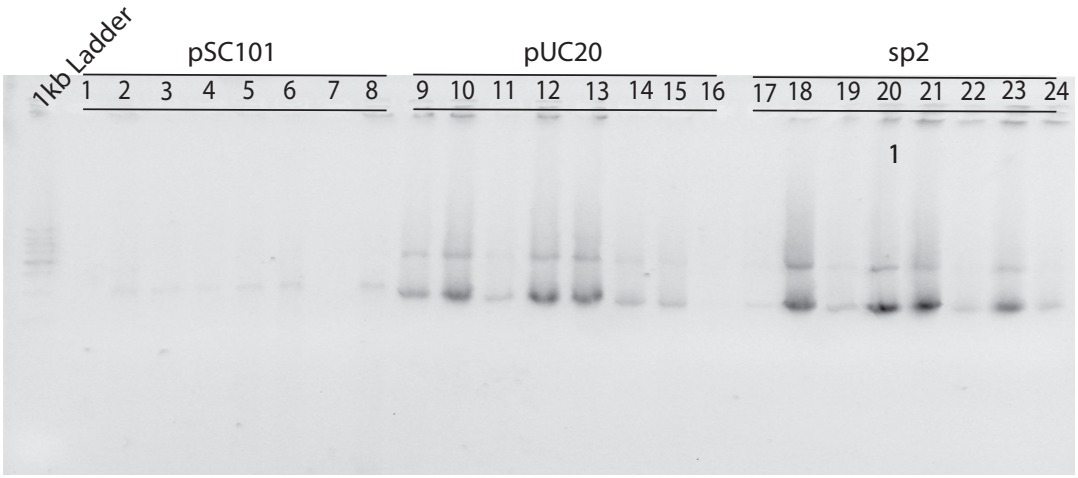


Figure: 1-8 is the backbone: pSC101, 9-16 is the backbone: pUC20, 17-24 is the promoter sp2

No correct result

WEDNESDAY, 2018-8-8

1. Colony PCR of pSC101-ptac-2

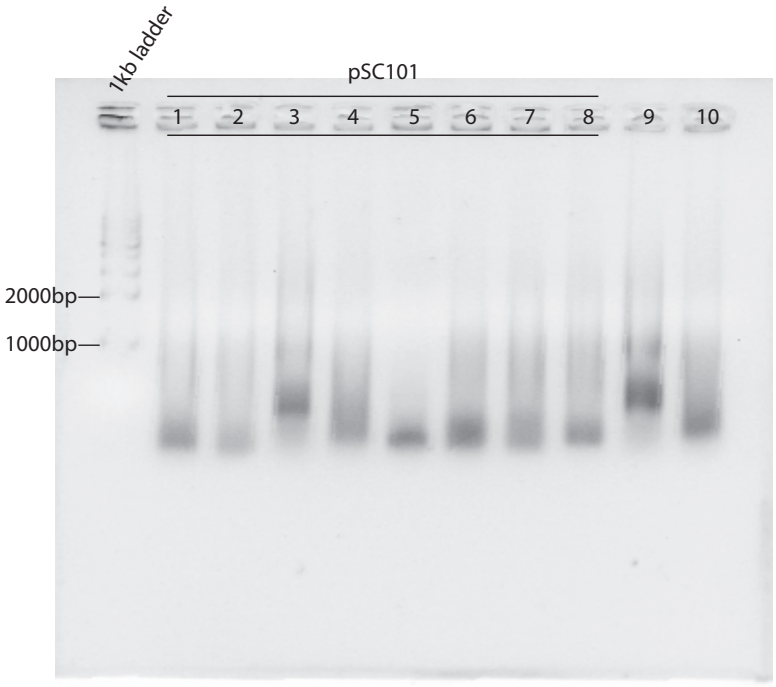


Figure: 1-8 is pSC101-ptac-GFP; 9 is the repeat of 3; 10 is the repeat of 4

No correct result

THURSDAY, 2018-8-9

1. Plasmid extraction of pSC101-ptac-GFP and pUC20-pTALEsp2-GFP

DH5a	pSC101-ptac-GFP-1	117.75ng/μl
DH5a	pSC101-ptac-GFP-2	75.85ng/μl
DH5a	pSC101-ptac-GFP-3	85.8ng/μl
DH5a	pSC101-ptac-GFP-4	91.9ng/μl

DH5a	pSC101-ptac-GFP-5	124.05ng/μl
DH5a	pSC101-ptac-GFP-6	128.15ng/μl
DH5a	pUC20-pTALEsp2-GFP-a4	146.4ng/μl
DH5a	pUC20-pTALEsp2-GFP-b2	233.85ng/μl
DH5a	pUC20-pTALEsp2-GFP-b3	256.35ng/μl

2. Colony PCR of pSC101-ptac-sfGFP

Using primers F24_R/R24_F, with corresponding length of 4315bp and 2561bp.

3. Plasmid PCR of pUC20-GPPS-GES

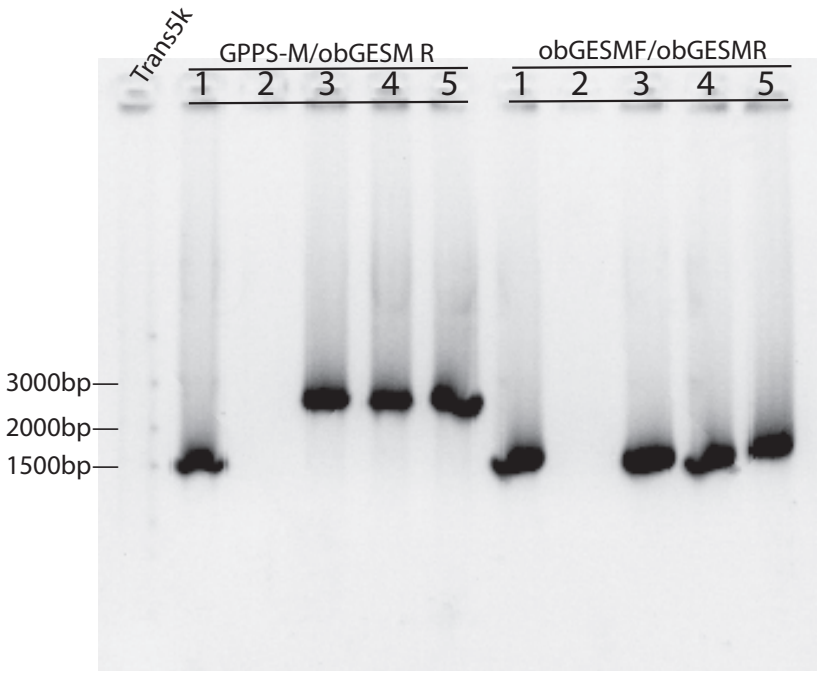


Figure:the correct length of GPPS-M/obGESM R is 2469bp; the correct length of obGESMF/obGESMR is 1543bp; sample 3-5 are the correct result

FRIDAY, 2018-8-10

1. Colony PCR of pSC101-ptac-GFP

TUESDAY , 2018-8-14

1. Plasmid extraction of pUC20-ptac-GPPS-GES 4&5

DH5a	pUC20-ptac-GPPS-GES-4	234.85ng/μl
DH5a	pUC20-ptac-GPPS-GES-5	255.1ng/μl

2. Amplification of pSC101, pR6K, pUC20 for backbone substitution of pUC20-ptac-GPPS-GES.

	Sequence
ptac-GPPS-GES a **(from pUC20-ptac-GPPS-GES)**	R24_F/GPP R1 (1995bp)
ptac-GPPS-GES b **(from pUC20-ptac-GPPS-GES)**	LSF/F24_R (2521bp)
pR6k **(backbone only)**	F24_F/R24_R (3196bp)
pSC101 **(backbone only)**	F24_F/R24_R (3023bp)

WEDNESDAY, 2018-8-15

1. Gibson assembly ——backbone substitution

1. pR6K-GPPS-GES
2. pSC101-GPPS-GES

2. **Transformation of gibson assembly product and plated it on Kan LB plate**

THURSDAY, 2018-8-16

1. **Colony PCR of pR5K-GPPS-GES and pSC101-GPPS-GES**

	Sequence 1	Sequence 2
pR6K-ptac-GPPS-GES 1	R24_F/GPP R1 (1995bp)	LSF/F24_R (2521bp)
pSC101-ptac-GPPS-GES 1	R24_F/GPP R1 (1995bp)	LSF/F24_R (2521bp)
pSC101-ptac-GPPS-GES 2	R24_F/GPP R1 (1995bp)	LSF/F24_R (2521bp)

2.

FRIDAY, 2018-8-17

1. **Amplification of pUC20/pR6K/pSC101-GFP without promoter**

	Sequence
pUC20-GFP	Dqdz_F/Dqdz_R (3501bp)
pR6K-GFP	Dqdz_F/Dqdz_R (4403bp)
pSC101-GFP	Dqdz_F/Dqdz_R (4230bp)

2.

SATURDAY, 2018-8-18

1. Amplification of Gibson Assembly (pSB1C3-TALEsp1-LacZ and pSB1C3-TALEsp2-LacZ)

	Sequence
sp1 a (from pSB1C3-TALEsp1)	lLac_1F/BBp_R (2175bp)
sp1 b **(from pSB1C3-TALEsp1)**	BBp_F/lLac_2R (3004bp)
sp2 a **(from pSB1C3-TALEsp2)**	lLac_1F/BBp_R (2175bp)
sp2 b **(from pSB1C3-TALEsp2)**	BBp_F/lLac_2R (3004bp)
LacZ **(from pCRCT)**	lLac_2F/lLac_1R (485bp)

2. Gibson assembly

1. pSB1C3-TALEsp1-LacZ
2. pSB1C3-TALEsp2-LacZ

3. Transformation of gibson assembly product

SUNDAY, 2018-8-19

1. Inculcated the colonies into 96-well plates and added IPTG(0μM, 25μM, 100μM and 500μM)

2. Sampling of pTALE characterization (6h, 12h and 24h)

FRIDAY, 2018-8-20

1. **Flow cytometry of pTALE characterization**

TUESDAY, 2018-8-21

1. **Plasmid extraction of pSB1C3-TALEsp1-LacZ and pSB1C3-TALEsp2-LacZ**

2. **Plasmid PCR of pSB1C3-TALEsp1-LacZ and pSB1C3-TALEsp2-LacZ**

	Sequence 1	Sequence 2
pSB1C3-TALEsp1-LacZ	BBp_F/lLac_2R (2990bp)	lLac_2F/lLac_1R (485bp)
pSB1C3-TALEsp2-LacZ	BBp_F/lLac_2R (2990bp)	lLac_2F/lLac_1R (485bp)

3. **Colony PCR of non-promoter pR6K and non-promoter pSC101**

	Sequence
non-promoter pR6K	R24_F/Riboj_R (71bp)
non-promoter pSC101	R24_F/Riboj_R (115bp)

4. **Streak the cultures of pTALE characterization on plates**

WEDNESDAY, 2018-8-22

1. **Amplification of fragments of Gibson Assembly (pSB1C3-pTALE-GFP-LacZ)**

	Fragment 1	Fragment 2	Fragment 3
pSB1C3-sp1-GFP-LacZ	Ndr_2F/BBp_R (2159bp)	BBp_F/Ndr_1R (2976bp)	Ndr_1F/Ndr_2R (497bp)
pSB1C3-sp2-GFP-LacZ	Ndr_2F/BBp_R (2159bp)	BBp_F/Ndr_1R (2976bp)	Ndr_1F/Ndr_2R (497bp)

2. **Gibson Assembly**

    1. pUC20-GFP only

    2. pSB1C3-sp1-GFP-LacZ

    3. pSB1C3-sp2-GFP-LacZ

3. **Transformation of gibson assembly product**

4. **Streak a culture of pUC20-sp1-sfGFP and pUC20-sp2-sfGFP on plate**

THURSDAY, 2018-8-30

1. **Amplification of Fragments for Gibson Assembly**

2. **Gibson Assembly**

    pUC20/pR6K/pSC101-TALEsp1/TALEsp2-GPPSGES

3. **Transformation of gibson assembly product**

FRIDAY, 2018-8-31

1. Amplification of Fragments for Gibson Assembly

	Sequence
sp1	R24_F/Riboj_R
sp2	R24_F/Riboj_R
pR6K(backbone)	F24_F/R24_R
pR6K-sp1-GFP	Gsp1F/R24_R
pR6K-sp2-GFP	Gsp2F/R24_R
pSC101-sp1-GFP	Gsp1F/R24_R
pSC101-sp2-GFP	Gsp2F/R24_R
pUC20-sp2-GFP	Gsp2F/R24_R

2.

MONDAY, 2018-9-3

1. Amplification of Fragments for Gibson Assembly

	Sequence
pUC20-J23119	rep119F/rep119R (3593bp)
pR6K-J23120	rep119F/rep119R (4319bp)
pSC101-J23121	rep119F/rep119R (4492bp)
pUC20-sp1-GFP	Gsp1F/R24_R (3691bp)
pUC20-sp2-GFP	Gsp2F/R24_R (3691bp)

2. Gibson Assembly

1. pUC20-J23119-GFP
2. pR6K-J23119-GFP
3. pSC101-J23119-GFP
4. pUC20-sp1-GFP
5. pUC20-sp2-GFP

3. Transformation of gibson assembly product

WEDNESDAY, 2018-9-5

1. **Golden Gate Assembly**
    1. pSB1C3-TALEsp1-J23119-CP
    2. pSB1C3-TALEsp2-J23119-CP
2. **Transformation of Golden Gate assembly product**
3. **Plasmid extraction of pSB1C3-TALE1/2-CP 1/2/3**

THURSDAY, 2018-9-6

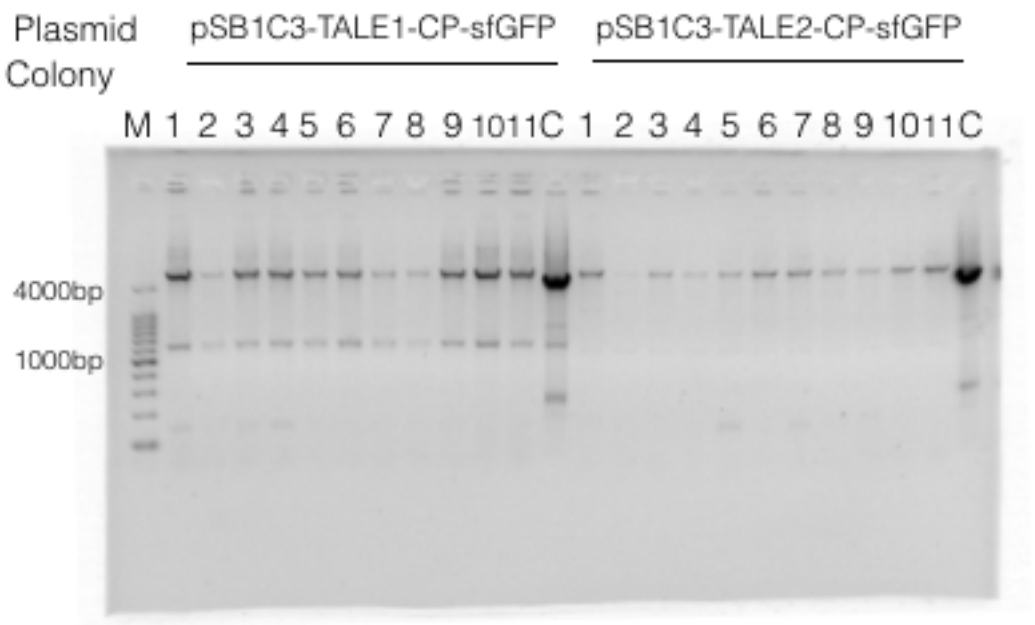
1. **Plasmid extraction of pSB1C3-TALEsp1/TALEsp2 only, pR6K-GPPS-GES and pSC101-GPPS-GES**

FRIDAY, 2018-9-7

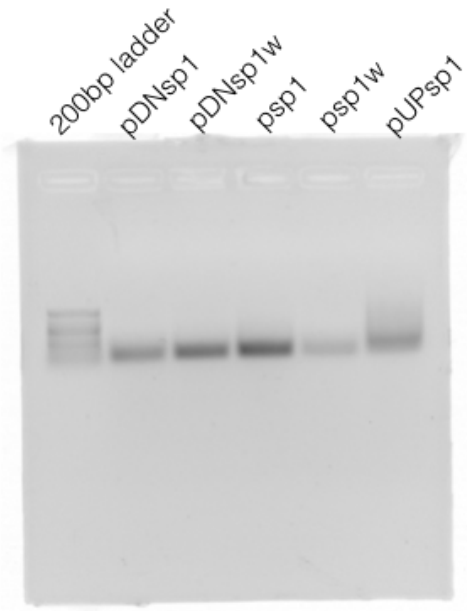
1. **Plasmid extraction of pSB1C3-sp2-sfGFP and pUC20-J23101-GFP**

MONDAY, 2018-9-10

1. **Colony PCR of pSB1C3-TALE1-CP-sfGFP and pSB1C3-TALE2-CP-sfGFP**



2. Amplification of sp1-series promoter



Gel extractions were performed, following by ligation with pSB1C3 backbone

Following plasmids were assembled:

pSB1C3-TALE sp1-pDNsp1-sfGFP

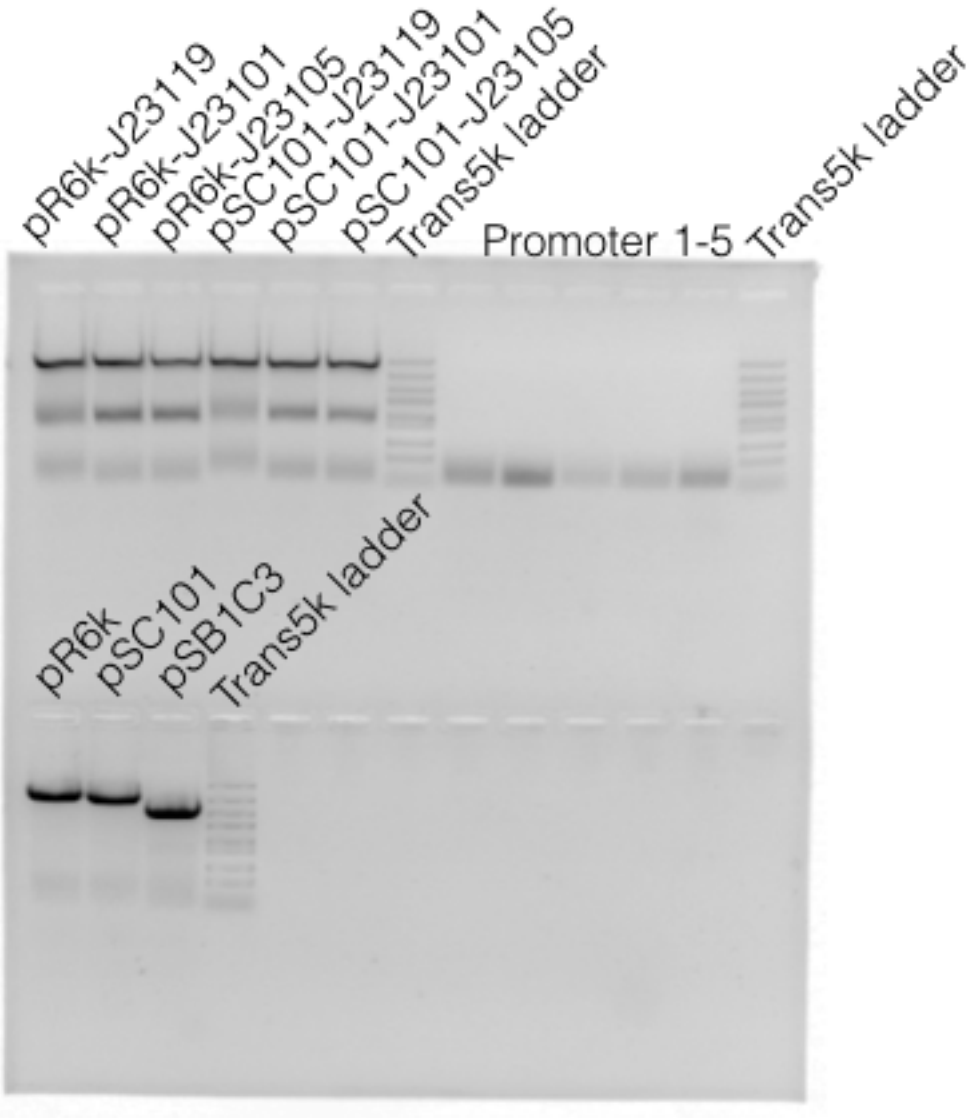
pSB1C3-TALE sp1-pDNsp1w-sfGFP

pSB1C3-TALE sp1-psp1w-sfGFP

pSB1C3-TALE sp1-pDNsp1-sfGFP

Transformation of DH5α chemically competent cell was carried out.

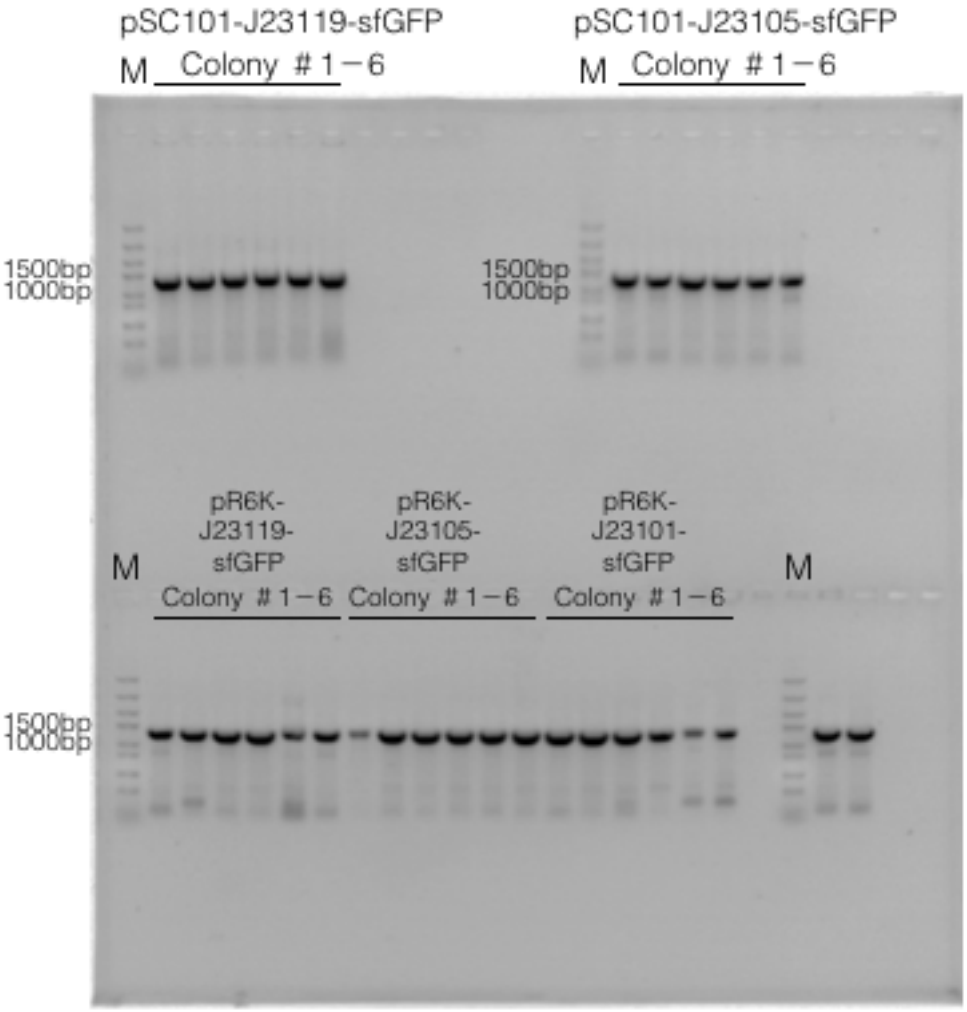
3. Amplification



Series of amplifications were carried out to prepare the backbone substitution of constitutive promoters.

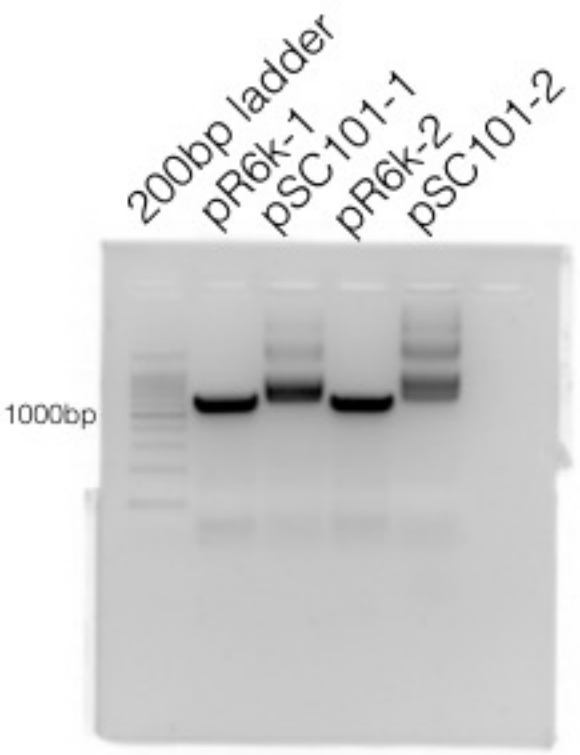
TUESDAY, 2018-9-11

1. **Colony PCR of pR6k/pSC101-J23119/J23101/J23105-sfGFP**



THURSDAY, 2018-9-13

1. **Transformation of pR6K-promoter1~6-sfGFP, pSC101-promoter1~6-sfGFP,pSB1C3-J23105/J23101/J23119 and pSB1C3-TALE1-Amicp-sfGFP**
2. **Amplification of pR6K-1, pR6K-2, pSC101-1, pSC101-2**



FRIDAY, 2018-9-14

1. **Transformation of pSB1C3-J23105 and pSB1C3-J23114**
2. **Amplification of fragments for Gibson Assembly**

	Sequence
pSB1C3	1C3F24_F/1C3R24_R
sp1-GFP	R24_F/F24_R
sp2-GFP	R24_F/F24_R

WEEK 3, SEPT.

#9.17

1. Prep. of Standard Flow Cytometry assay

Strains(*DH5α*) containing the plasmids of interest were plated or streaked on plates containing LB + 2% agar and grown overnight at 37 °C. Strains are listed below:

1. pSB1C3-J23119-sfGFP
2. pR6k-J23119-sfGFP
3. pSC101-J23119-sfGFP
4. pSB1C3-sp1only-sfGFP
5. pR6k-sp1only-sfGFP
6. pSC101-sp1only-sfGFP
7. pSB1C3-sp2only-sfGFP
8. pSC101-sp2only-sfGFP
9. pR6k-sp2only-sfGFP
10. pSB1C3-TALE1sp1-sfGFP
11. pSC101-TALE1sp1-sfGFP
12. pR6k-TALE1sp1-sfGFP
13. pSB1C3-psp1mut-sfGFP
14. pSC101-psp1mut-sfGFP
15. pR6k-psp1mut-sfGFP
16. pSB1C3-pUpsp1mut-sfGFP
17. pSC101-pUpsp1mut-sfGFP

18. pR6k-pUpsp1mut-sfGFP
19. pSB1C3-TALE2sp2-sfGFP
20. pSC101-TALE2sp2-sfGFP
21. pR6k-TALE2sp2-sfGFP
22. pSB1C3-pUPAAsp2-sfGFP
23. pSC101-pUPAAsp2-sfGFP
24. pR6k-pUPAAsp2-sfGFP
25. pSB1C3-pUPAsp2-sfGFP
26. pSC101-pUPAsp2-sfGFP
27. pR6k-pUPAsp2-sfGFP

2. Prep. of constructing backbone-sp1 series of promoter-sfGFP

Inoculation of pSB1C3-TALE1-Psp1mut-sfGFP and pSB1C3-TALE1-Pupsp1mut-sfGFP in 5ml LB broth.

Overhnight culture at 37°C, 220rpm

#9.18

1. Prep. of Standard Flow Cytometry assay

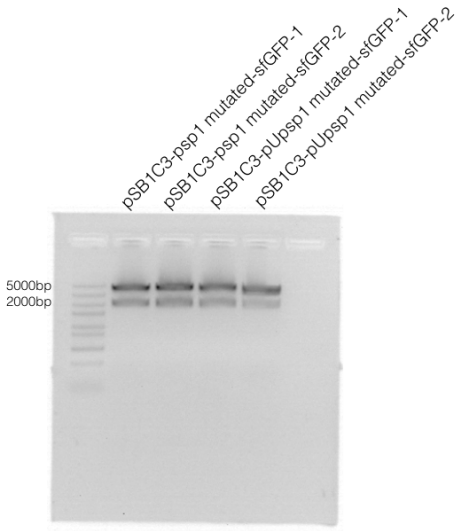
Single colonies were inoculated into 0.5 ml LB medium with an appropri- ate combination of the antibiotics Kan or Cam for plasmid maintenance in 2-ml 96-well deep-well plates,and the plates were sealed with film and incubated at 37 °C and 900 r.p.m. overnight.

Experiment failed as no parallel sample was made when dilution was performed.

2.Construction of backbone-sp1 series of promoter-sfGFP

Plasmid extraction

RD



Gibson assembly

Transformation into DH5a chemically competent cell.

#9.19

1. Streaked single colonies from 9.18 transformation on LB plate

#9.20

2. Prep. of Standard Flow Cytometry assay

Single colony inoculation for overnight culture in 2-ml 96-well deep-well plates.

#9.21

1. Dilution follows protocol:

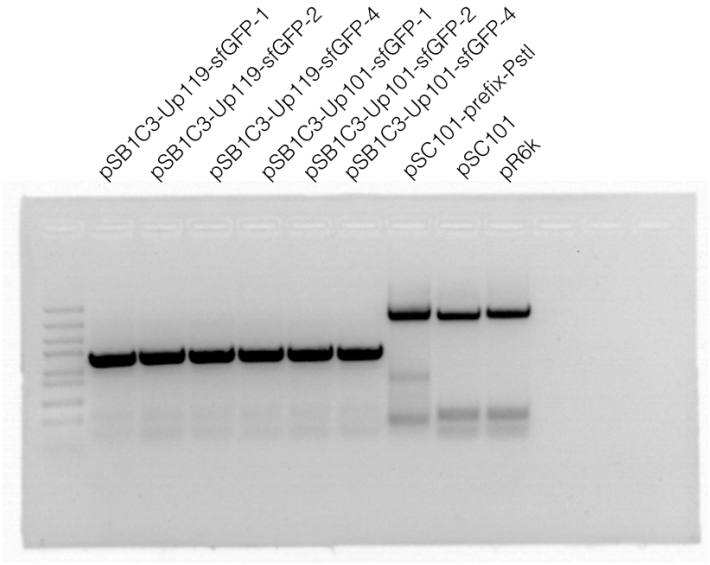
Dilute the bacterium solution in 150μL of LB with antibiotic in the ratio of 1:225. Grow inside a 96-well plate, filmed, 37℃, 1000 r.p.m, 2 hours

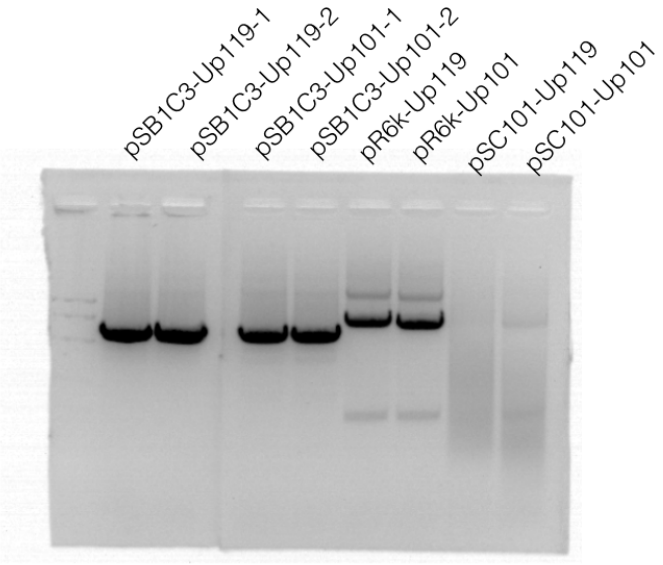
3 paralleled samples for each bacteria. Dilute the bacterium solution in 150μL of LB with antibiotics. Grow in a 96-well plate, filmed, 37℃, 1000 r.p.m. Sample after 6 hours.

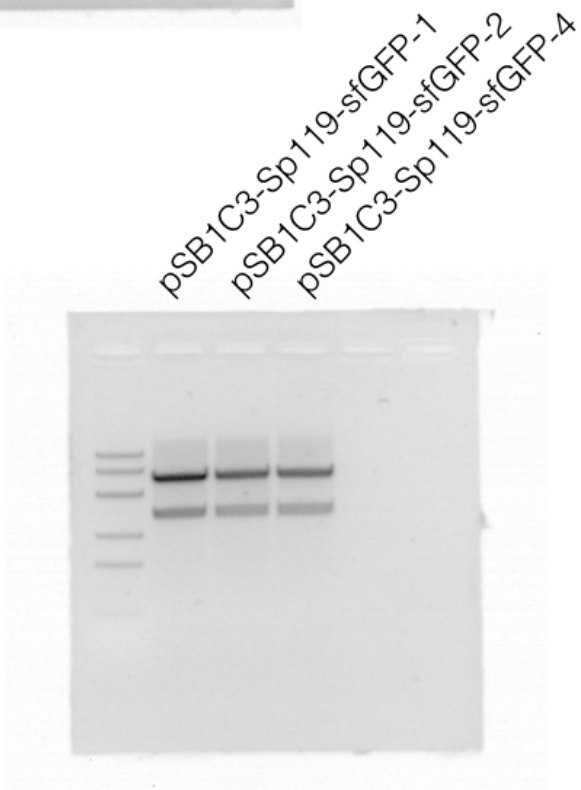
Samples were diluted in 1X PBS + Kan and characterized immediately.

WEEK 4, SEPT.

1. Construction and characterization of Sp119&Up119 promoter





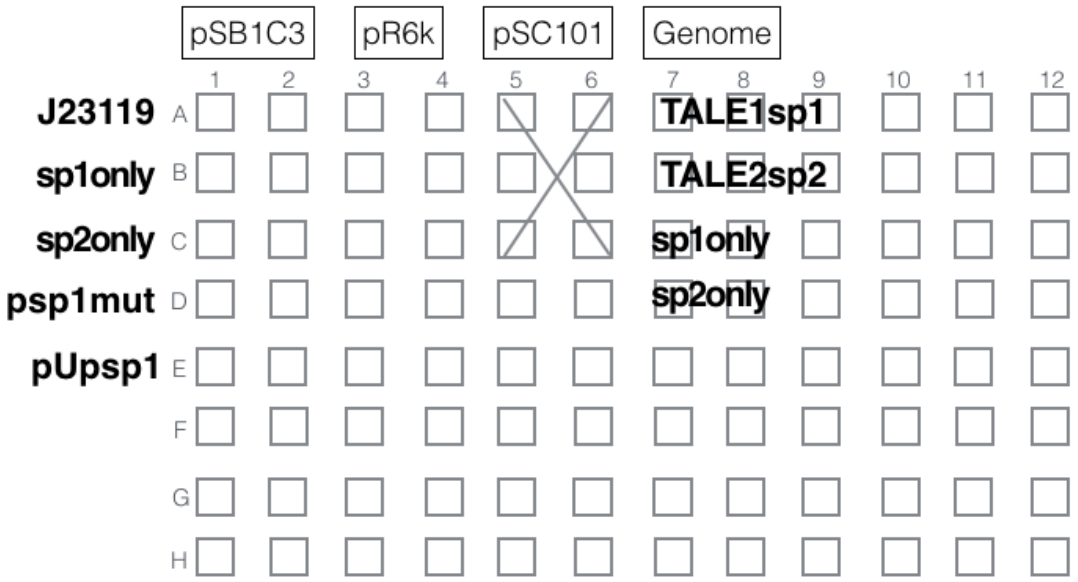


Only pR6k/pSC101/pSB1C3-Up119/Sp119-sfGFP were characterized, and we forgo the rest.

Single colonies were picked and streaked on LB plate.

2. Characterization of "promoter-sfGFP genome"

We also redo some experiments to achieve a more reliable date.



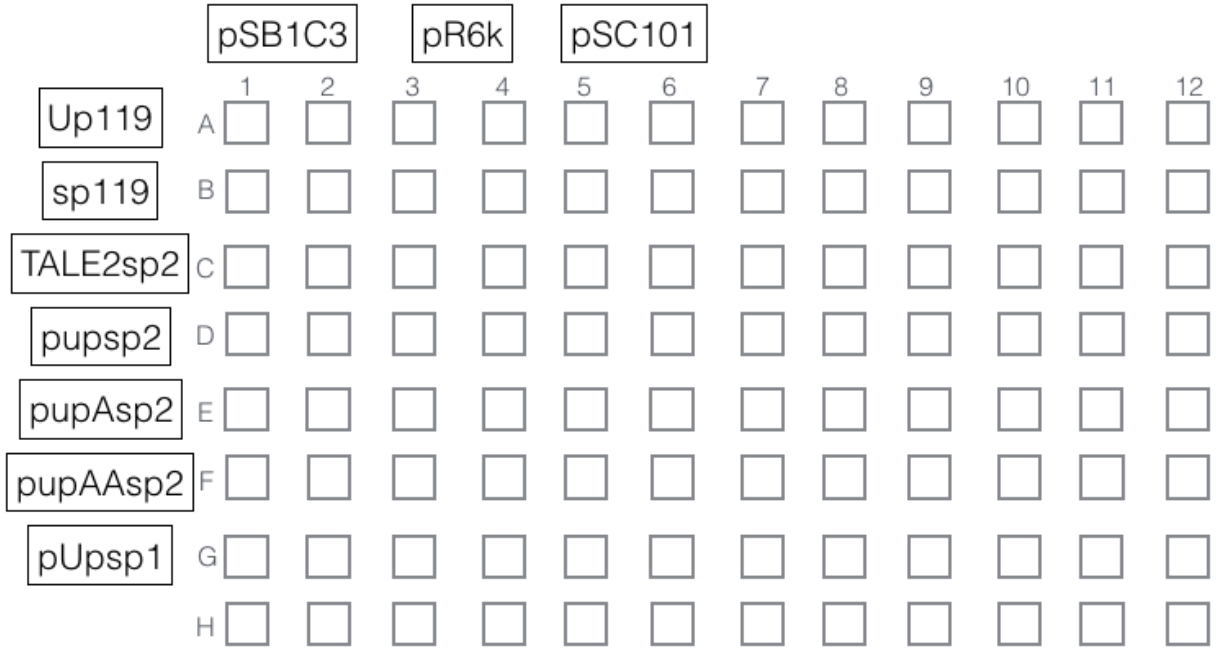
	Colony 1			Colony 2		
TALEsp1 genome	440000	427000	431000	283656	292429	281178
TALEsp2 genome	116527	273421	266922	309854	281603	283942
Sp1 only genome	50730	59190	51005	63460	66281	75916
Sp2 only genome	25495	28569	26911	29827	30922	27218
	J23119	Sp1only	Sp2 only	pSp1mut	pUPsp1mut	
	2290000	1480000	2090000	978000	1190000	
	2230000	1590000	2060000	971000	1210000	
	2310000	1490000	2070000	972000	1210000	
1C3	2230000	1530000	2110000	975000	1180000	
	1490000	1470000	1310000	1130000	1270000	
	1560000	1500000	1290000	1100000	1340000	
	1480000	1420000	1310000	1100000	1260000	
R6K	1520000	1490000	1300000	1070000	1320000	

WEEK 1, OCT.

1. Standard Flow Cytometry assay of Up119/Sp119

Single colonies were picked from the newly streaked plate.

2 colonies were chosen for each strain



	TALE2sp1	SP119	UP-119	TALE1sp1			TALE2sp1	SP119	UP-119	TALE1sp1
	233914	85770	1840000	901000			157756	93158	279889	117063
1C3	205069	81440	1800000	930000		1C3	176779	85711	312708	97881
colony1	204635	78351	1790000	895000		colony1	140435	82004	264003	103889
	229147	88219	1660000	736000			203758	76126	277698	102850
1C3	240966	85373	1600000	765000		1C3	193320	82371	300428	140264
colony2	231622	84030	1600000	792000		colony2	154687	73319	330647	155358
	319309	157377	1660000	642000			292745	85253	1740000	336231
R6K	305557	155164	1590000	618000		R6K	264544	91539	1720000	334331
colony1	284850	153333	1570000	601000		colony1	245725	87091	1690000	348177
	305242	148509	1630000	515000			338336	103573	1670000	344350
R6K	307985	154041	1610000	508000		R6K	261791	82576	1760000	349946
colony2	297001	154991	1550000	476000		colony2	254162	82777	1650000	324871
	262857	111179	1220000	849000			178387	71870	1070000	362574
PSC101	280901	139425	1190000	829000		PSC101	191563	64482	1090000	422000
colony1	290580	135839	1230000	846000		colony1	190745	67582	1120000	422000
	261652	146234	1170000	769000			186891	71808	1030000	468000
PSC101	271858	133614	1210000	735000		PSC101	195307	71222	1030000	424000
colony2	213284	115662	1220000	745000		colony2	191292	69273	1050000	496000