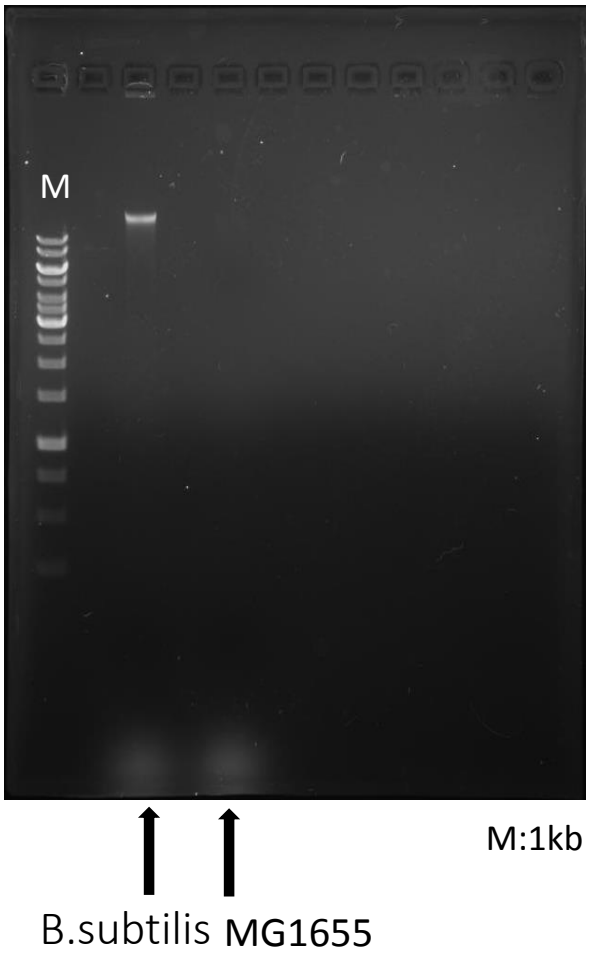
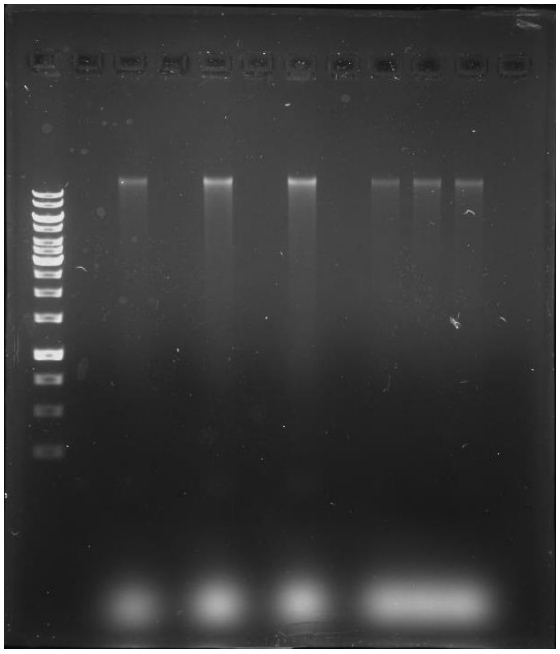
Labnote of the inhibitor

1. Template Quality Check

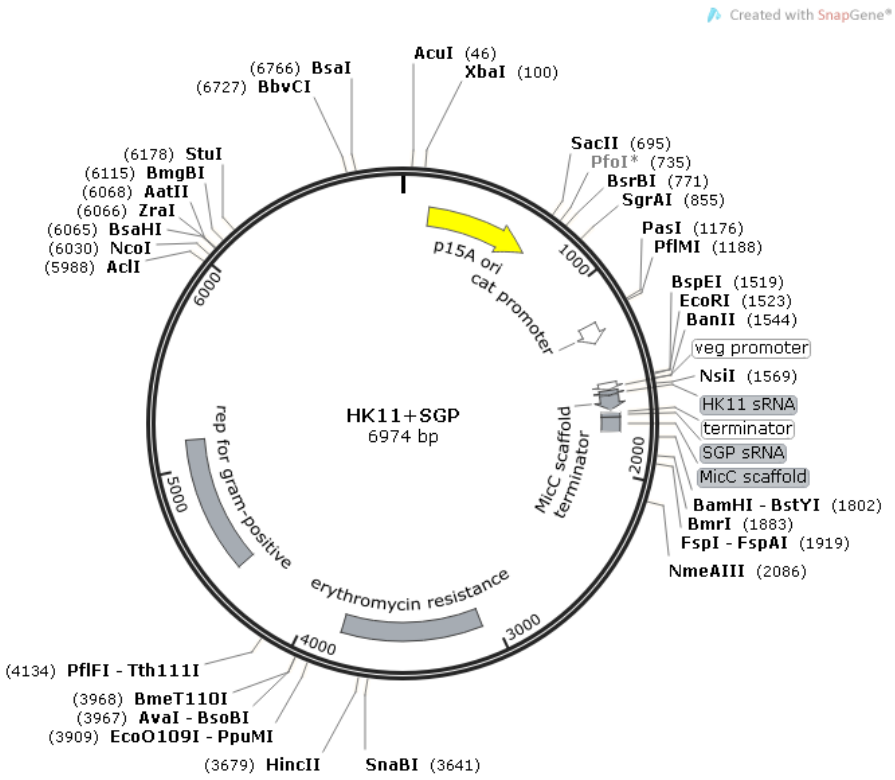


Result: We use Bacillus subtilis as template to PCR our constitutive promoter, and E.ocli MG1655 to PCR the MicC scaffold. The quality of MG1655 is not very well, so we do the MG1655 genomic DNA extraction again.



Result: E.Coli MG1655 genomic DNA quality check, all of the results seems good.

2. Circuit construct

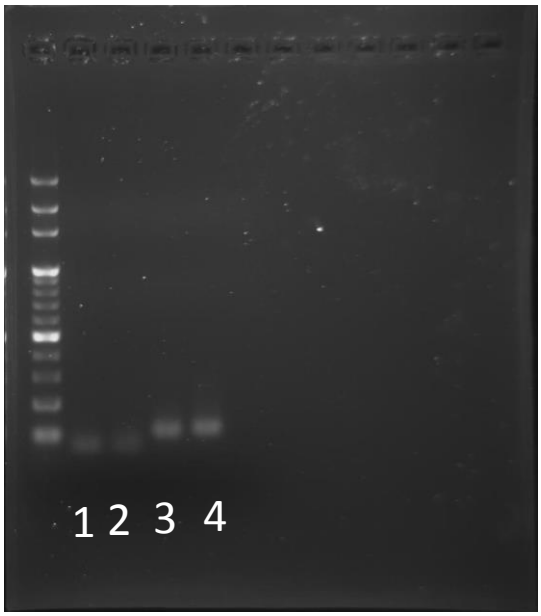


We use PVA838 as our shuttle vector between E.coli and S.mutans. The picture is the overview of our circuit construct.

In the circuit construct, we separate the construct into two parts. One is to PCR constitutive promoter+sRNA and the other is to PCR sRNA+MicC scaffold +terminator. In the below, we use number to represent the part.

1: Constitutive promoter+HK11 sRNA
2: Constitutive promoter+SGP sRNA
3: HK11 sRNA+MicC scaffold +terminator
4:SGP sRNA+MicC
scaffold +terminator

1- FP:CCGGAATTCTTGACAAAAATGGGCT
RP:TTAATTTTAACCGCTTGCATTACATTTATTGTACAACACG
2-FP:CCGGAATTCTTGACAAAAATGGGCT
RP:AATCAGGATTTGTATGCATTACATTTATTGTACAACACG
3-FP:AGTCCACATTTTCTGTTGGGCCATTG
RP:TGTGGATCCCAGCTATCAGCTGAAAAAAAGCCCGGACGACTG
4-FP:TAAATGACATTTTCTGTTGGGCCATT
RP:TGTGGATCCCAGCTATCAGCTGAAAAAAAGCCCGGACGACTG

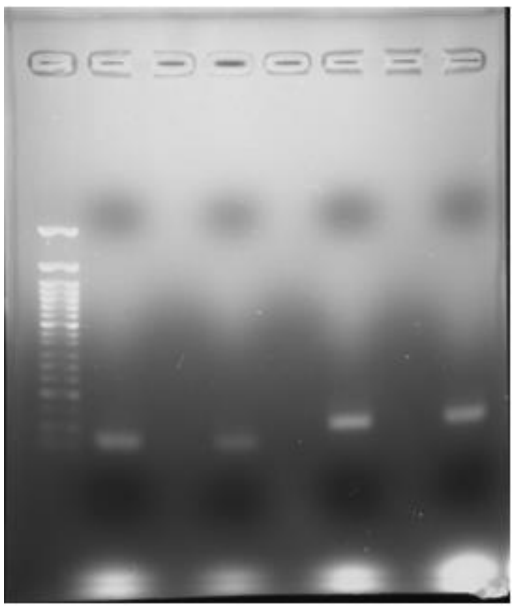


In tube 1 and 2, we use B.sutilis as the template to PCR promoter+HK11 sRNA, HK11 sRNA is synthesized by primer. As for tube 3 and 4, MG1655 is the template and terminator is synthesized by primer.

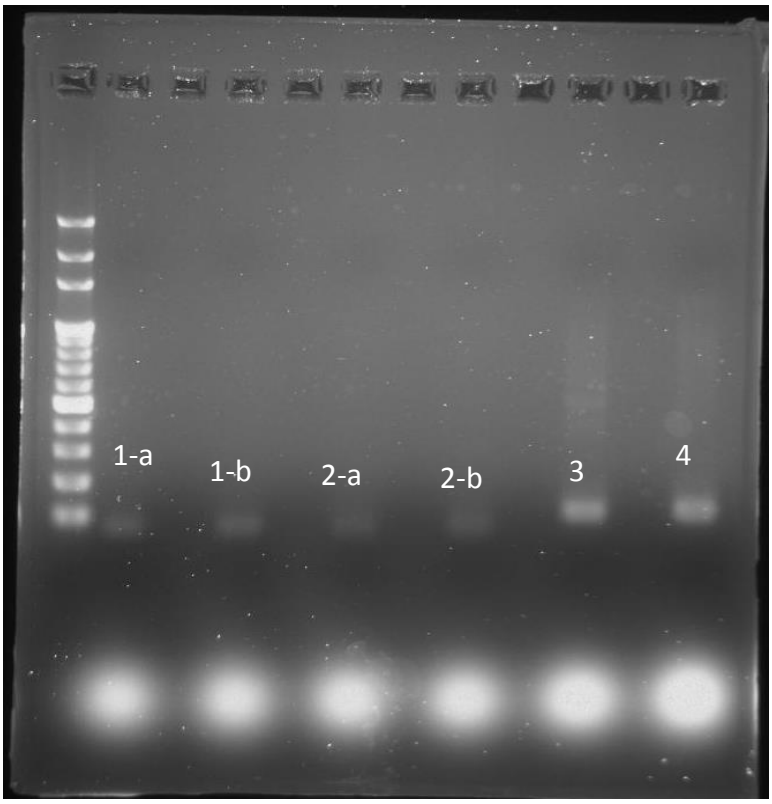
Result: The length of 1 and 2 should be 65bp, 64bp. As for 3.4, it should be 110bp

and 111 bp. All the products are correct

There are two programs to test which efficiency of PCR is better. Final result is program A

programA	programB
Program condition: 1.Hold 95C 3min 2.Cycling 10 repeats step 1 : 95C 15sec step 2 : touchdown over 5 cycles initial temp : 45C final temp : 55C 20sec step 3 : 70C 20sec 3.Cycling 30 repeats step 1 : 95C 20sec step 2 : 55C 20sec step 3 : 70C 20sec 4. Hold : 70C 3min 5. Hold : 25C 10 min	Program condition: 1. Hold 95C 3min 2. Cycling 10 repeats step 1 : 95C 15sec step 2 : touchdown over 1 cycles initial temp : 50C final temp : 60C 20sec step 3 : 70C 20sec 3. Cycling 30 repeats step 1 : 95C 20sec step 2 : 55C 20sec step 3 : 70C 20sec 4. Hold : 70C 3min 5. Hold : 25C 10 min
	

1-a;1-b:
Promoter+HK11 sRNA:
2-a;2-b:
Promoter+
SGP sRNA
3.HK11-f2+sca+ter
4.SGP-f2+sca+ter



Result: The length of 1 and 2 should be 65bp, 64bp.
As for 3.4, it should be 110bp and 111 bp.
All the products are correct

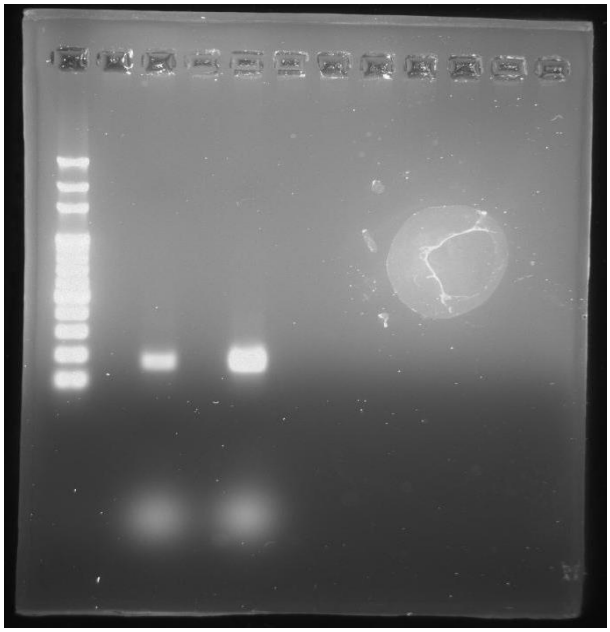
After taq PCR, the length of the product are correct, so we continue to do KOD PCR to ensure the accuracy and less mutation.

After KOD PCR, we do gel extraction to purify our product, and the concentration is in below:

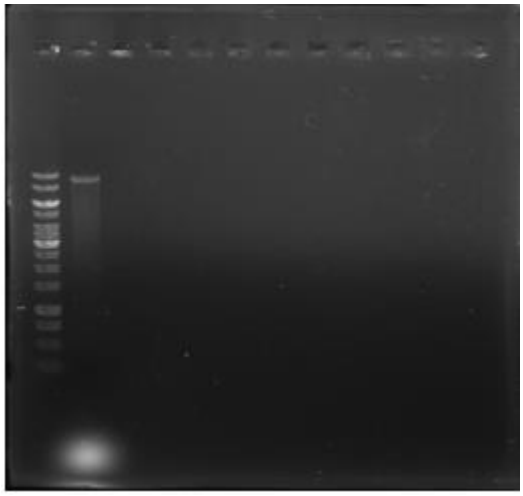
1.Promoter+HK11 sRNA : 23.11 ng/ul
2.Promoter+SGP sRNA : 26.31 ng/ul
3.HK11 sRNA+sca+ter: 25.94 ng/ul
4.SGP sRNA+sca+ter : 26.47 ng/ul

Later, we use gel extraction product as our template to assembly our circuit. The length of 1+3 and 2+4 are both

1+3: Promoter+HK11 sRNA-f2+sca+ter
Junction primer:
AGCGGTTAAAATTAAAGTCCACAT
2+4:Promoter+SGP sRNA-f2+sca+ter
Junction primer:
AATCCTGATTTAAATGACATTTTC

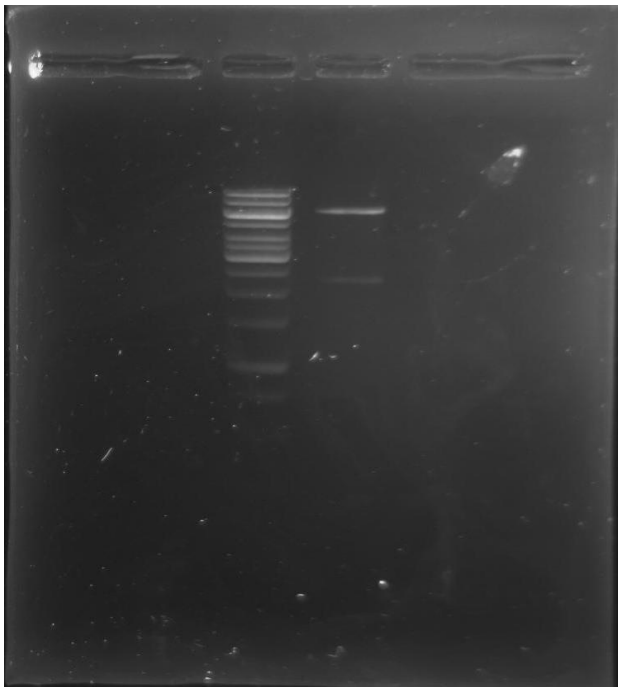


Result: The length of 1+3 and 2+4 are both 175bp. The results are correct.



PVA838: 9.1 bp

We liquid culture E.coli that has PVA838 vector and do plasmid extraction. To ensure we have extracted the right vector PVA838, we use restriction enzyme EcoRI to do digestion check. The result is correct.



M: 1kb
EcoRI + BamHI
Digestion
6689+2.4kbp

After the digestion check, we cut PVA838 with RE EcoRI+BamHI and do gel extraction of 6689bp band to use as our backbone.
Concentration: 23.53 ng/ul

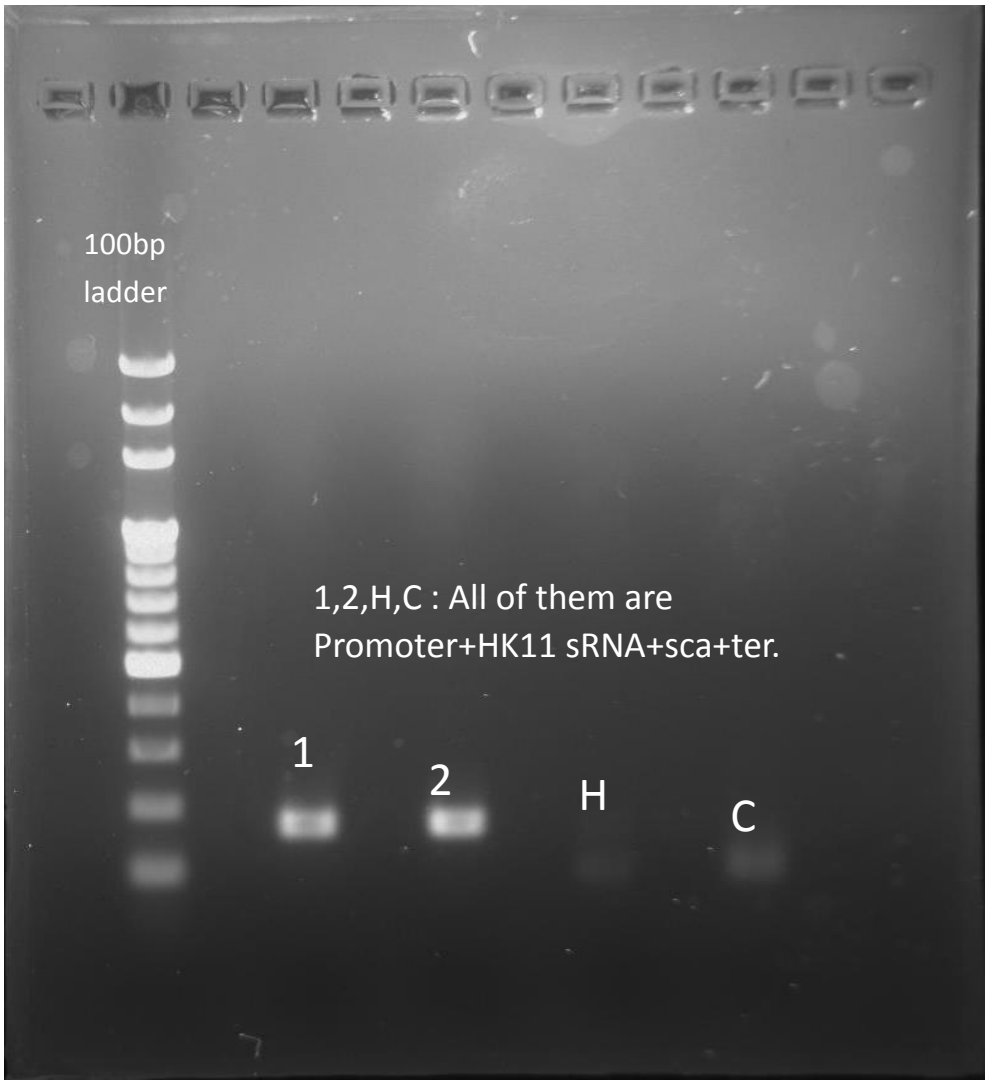
To joint PVA838 and our circuit, we both cut them with EcoRI+BamHI, then we do ligation and transform to E.coli to choose an single colony.

HK11 colony PCR

To joint PVA838 and our circuit, we both cut them with EcoRI+BamHI, then we do overnight ligation and transform to E.coli to choose an single colony .

To test which concentration of erythromycin is suitable to pick single colony. We have tried a series to find out the best concentration. 25ng/ul is easy to pick single colony but have to wait about 20 hours.

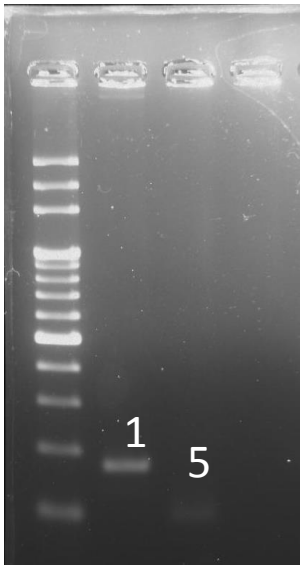
We chose three colonies to do PCR, two of them are succeed.



Result: our products should be 175bp, 1 and 2 are succeed.

SGP plasmid check

After about one week of test, we finally pick a successful SGP circuit colony and do liquid culture. Then we do plasmid extraction and do plasmid PCR.



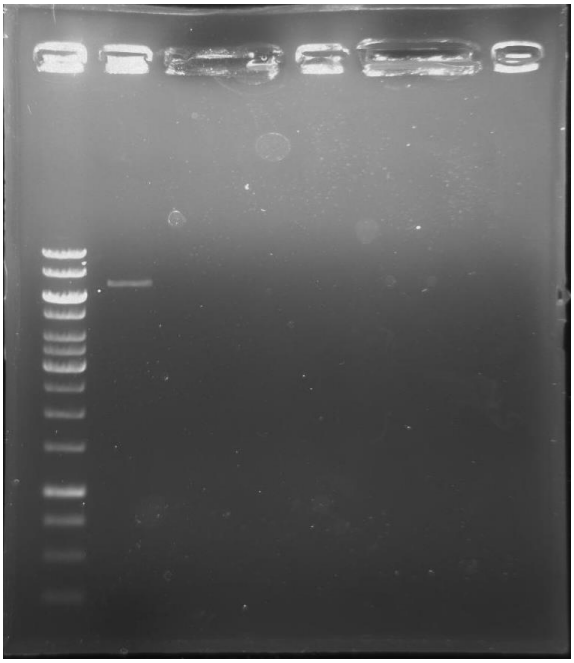
Result: The product should be 175bp. Colony 1 is succeed.

SGP plasmid check

After doing plasmid extraction, we do plasmid digestion to check if the plasmid is correct. We use restriction enzyme EcoRI and BamHI to cut the plasmid into 6689 bp and 175bp
fragments.

Ladder:1Kbp

plasmid:6689 bp



Result: plasmid length is correct. Success! And below are our plasmid maps

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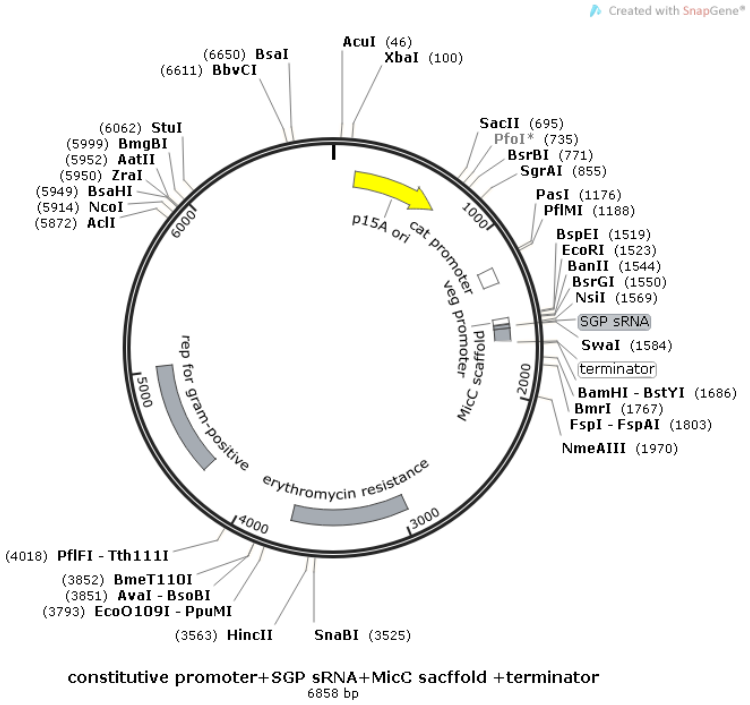
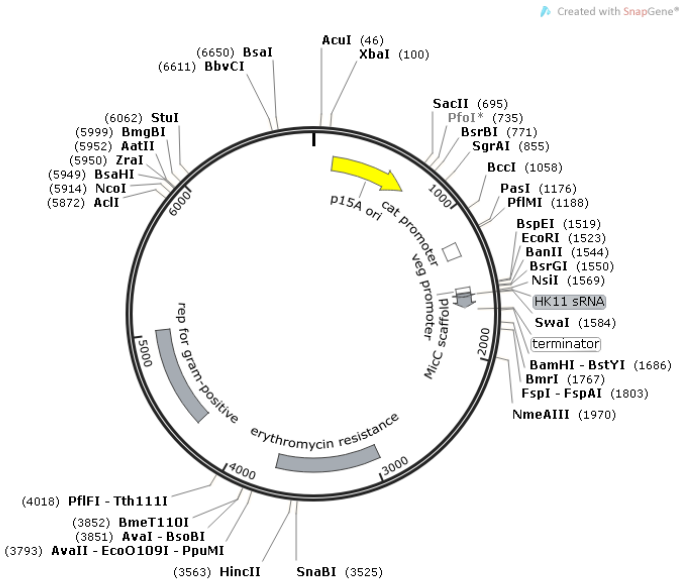
BHI plate + erythromycin for S.mutans

BHI is a commercial medium for S.mutans to grow. We use 14.8g BHI powder +1.5% agar and dissolve in 400mL ddwater. We make two different concentration of erythromycin, one is 5ug/mL and the other is 10ug/mL.



constitutive promoter+SGP sRNA+MicC sacffold +terminator
6858 bp



constitutive promoter+HK11 sRNA+MicC sacffold+terminator
6858 bp

S.mutans transformation

Following is the transformation protocol for S.mutans modified from M.Nilsson et al.[1]

1. 250uL S.mutans overnight culture grown in BHI medium is added to 10mL BHI containing 25% horse serum.
2. Incubate at 37°C
3. When the culture reach OD600 between 0.15~0.25, left at room temperature for 10 min.
4. Add plasmid (~200ng) into 2ml culture, and incubated at 37°C for 1 hour.
5. New culture is diluted with 1 ml BHI containing 25% horse serum, and incubated for another 1 hour.
6. S.mutans is harvest by centrifugation. (10K 1min)
7. Discard the supernatant and resuspended in 100ul BHI(without horse serum)
8. 15 ul culture is spread to one BHI plate containing 5ul/ml of 10ul/ml erythromycin.

S.mutans liquid culture and plasmid extraction.

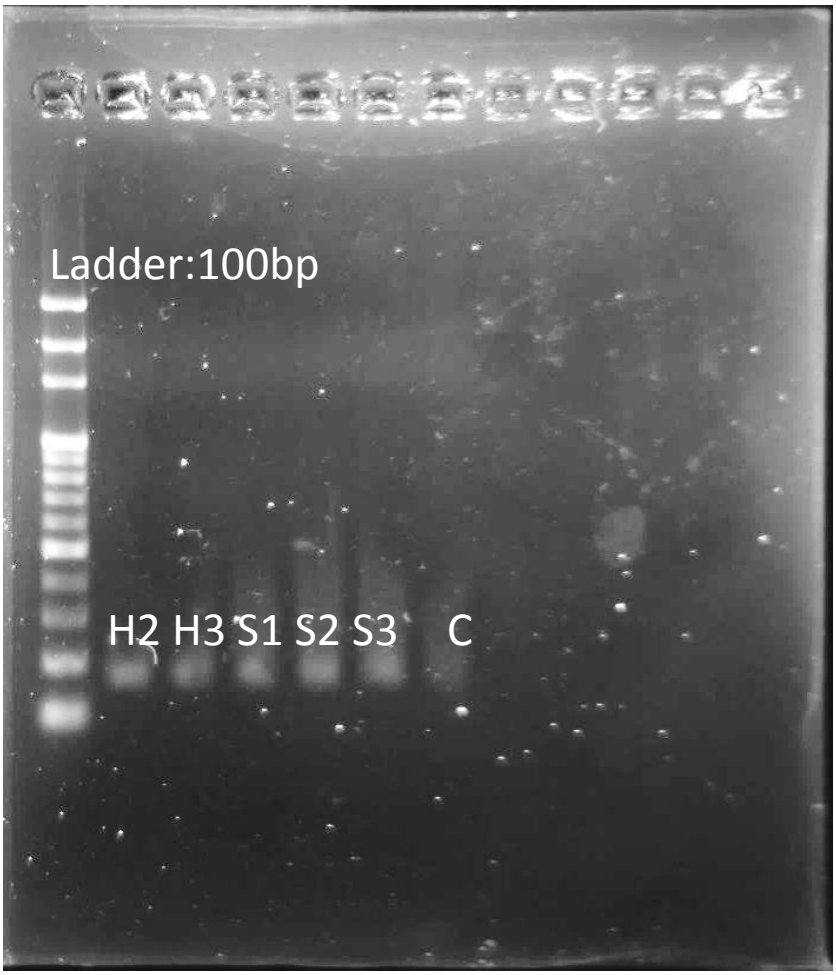
After incubating the plate at room temperature for 3 days, we pick several colonies in both HK11 and SGP plate. Because S.mutans is a gram-positive bacteria, we need to do additional step before normal plasmid extraction protocol. First, add 200ul lysozyme buffer and resuspend. Then, incubate at room temperature and invert the tube every 2-3min. After completing, go on to the lysis step.

The final concentration of plasmid :

HK11-2 : 199.68ng/ul
HK11-3 : 128.12ng/ul
SGP -1 : 81.66ng/ul
SGP -2 : 135.54ng/ul
SGP -3 : 114.33ng/ul

S.mutans plasmid PCR check

After S.mutans plasmid extraction, we do plasmid PCR to check if the vector have been successfully transform into S.mutans.



Result: All of the product should be 175bp, all of them are correct.

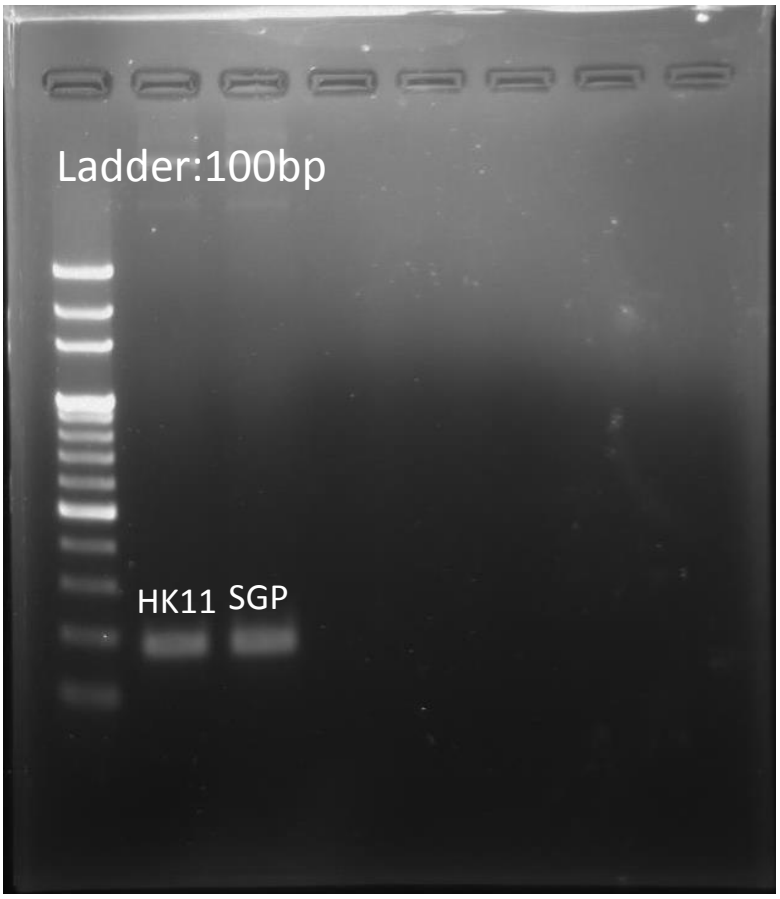
1. http://onlinelibrary.wiley.com/doi/10.1002/mbo3.171/full

send part plasmid PCR

To fulfill the standard part qualification, we use our PVA838+HK11, PVA838+SGP as template to do another plasmid PCR.

Forward primer : gaattcgcggccgcttctagagttgacaaaaatgggctcgt

Reverse primer : actagtatcccagctatcagctgaa



Result: Both of them are 189bp, success!

HK11, SGP circuit gel extraction + digestion

After successfully PCR our products, we do gel extraction and digest with EcoRI+Spel restriction enzyme. The final concentration is:

HK11:46.84 ng/ul
SGP: 51.32 ng/ul

HK11+pSB1C3 , SGP+pSB1C3 ligation

	HK11	SGP
10X buffer	1ul	1ul
Vector(pSB1C3)	2.04ul	2.04ul
Insert	0.13ul	0.14ul
Ligase	0.5ul	0.5ul
ddH_2O	6.33ul	6.32ul
total	10ul	10ul

E.coli transformation

After ligate for 2 hours at room temperature, we use 2 ul plasmid + 20 ul competent cell and do transformation protocol. Then spread the E.coli on chloramphenicol plate.

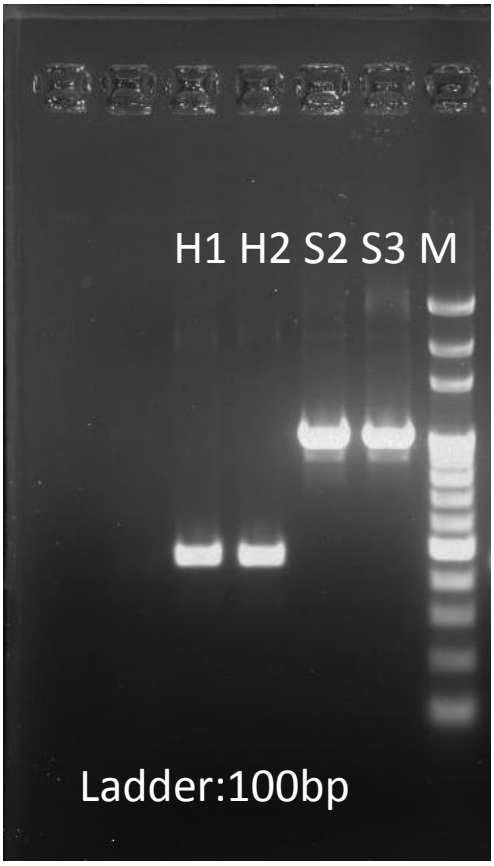
Send part plasmid extraction

We pick several colonies after 14 hours incubation at 37^{o}C, and do liquid culture. Another 14hours, we do plasmid extraction. The final concentration is in the below:

HK11-1: 55.31ng/ul
HK11-2: 27.50ng/ul
SGP-2: 42.89ng/ul
SGP-3: 56.77ng/ul

send part plasmid PCR

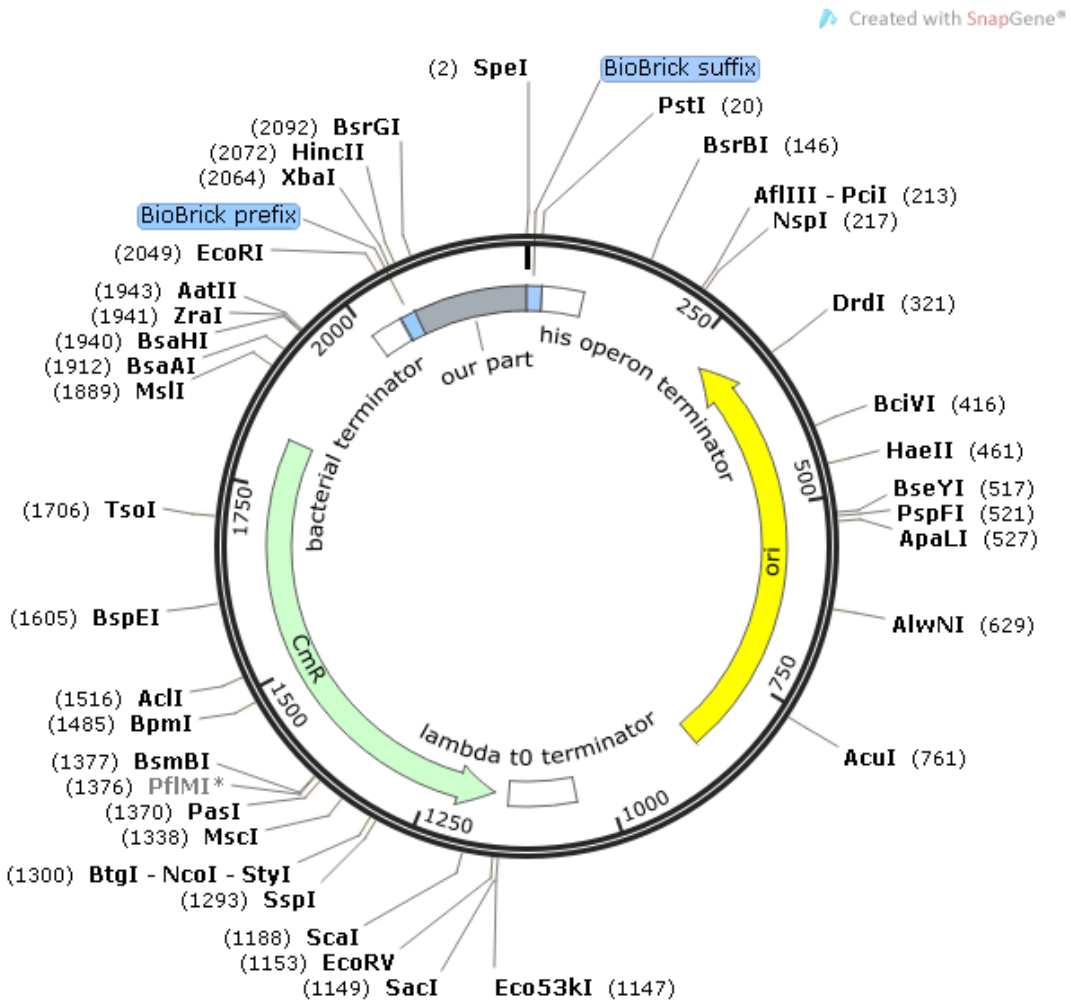
We use VR+CF2 as our colony PCR primer.



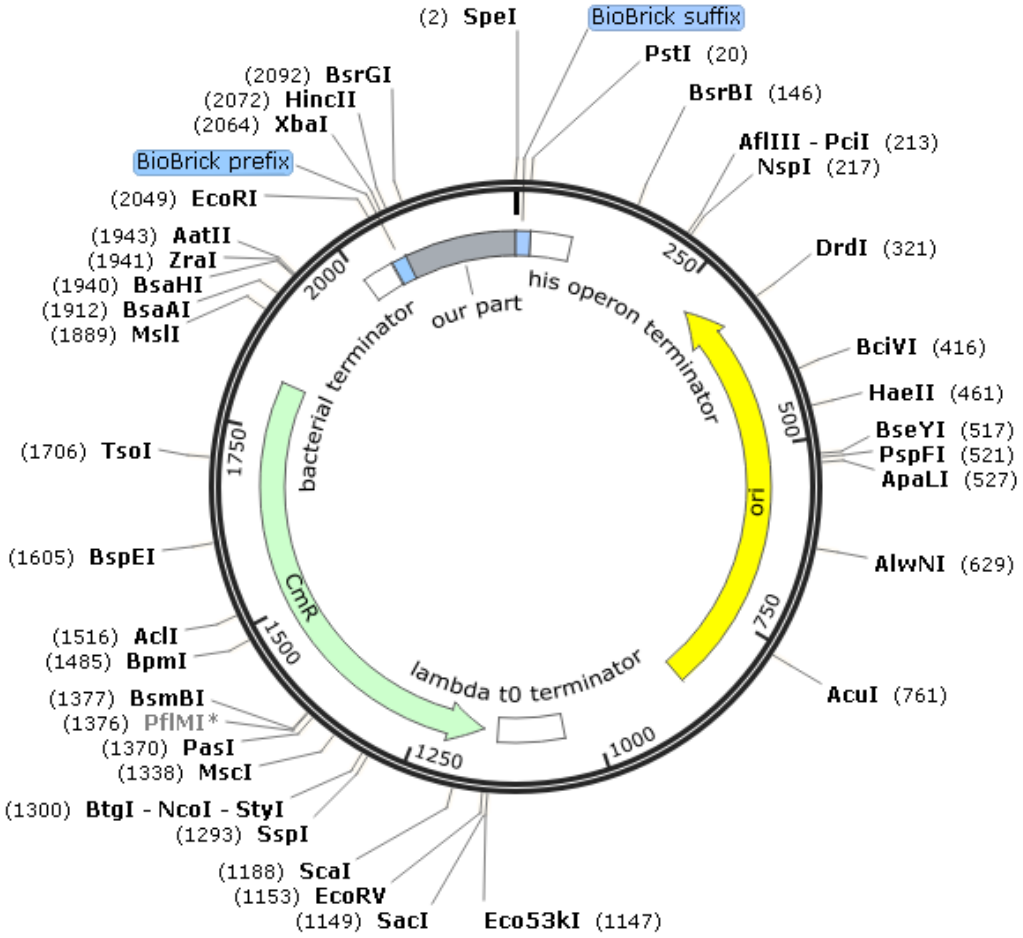
Result: HK11(H1, H2) and SGP(S2,S3) should be 480bp, only HK11 is correct, so we chose another tube of SGP plasmid to do again.

Plasmid map

The complete plasmid map is shown below:

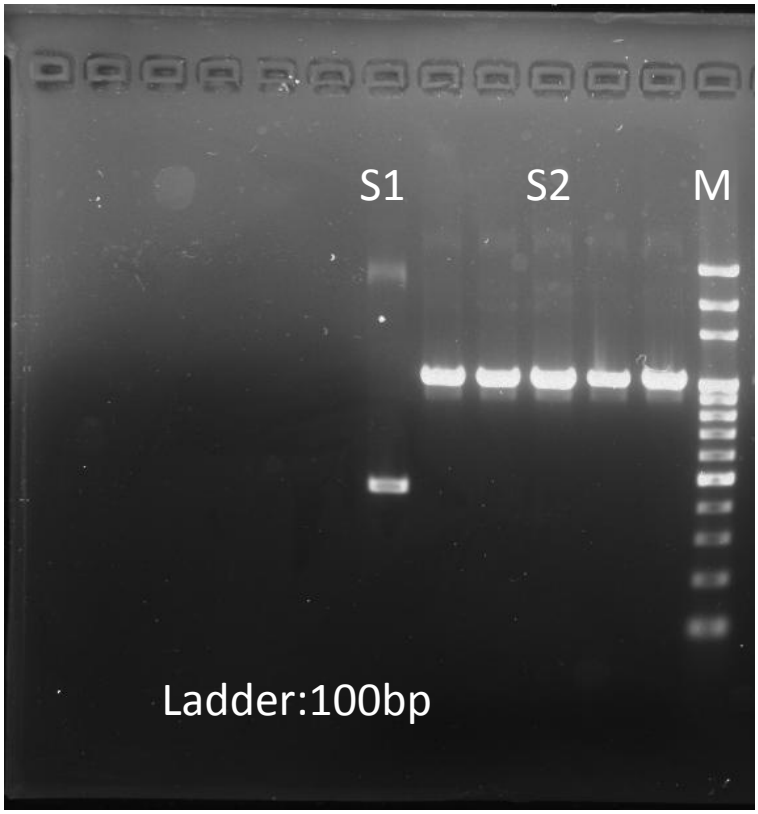


pSB1C3+constitutive promoter + HK11 sRNA+ MicC scaffold + terminator
2227 bp



pSB1C3+constitutive promoter +SGP sRNA+ MicC scaffold + terminator

2227 bp



Result: The product should be 480bp. SGP(S1) is correct, too.