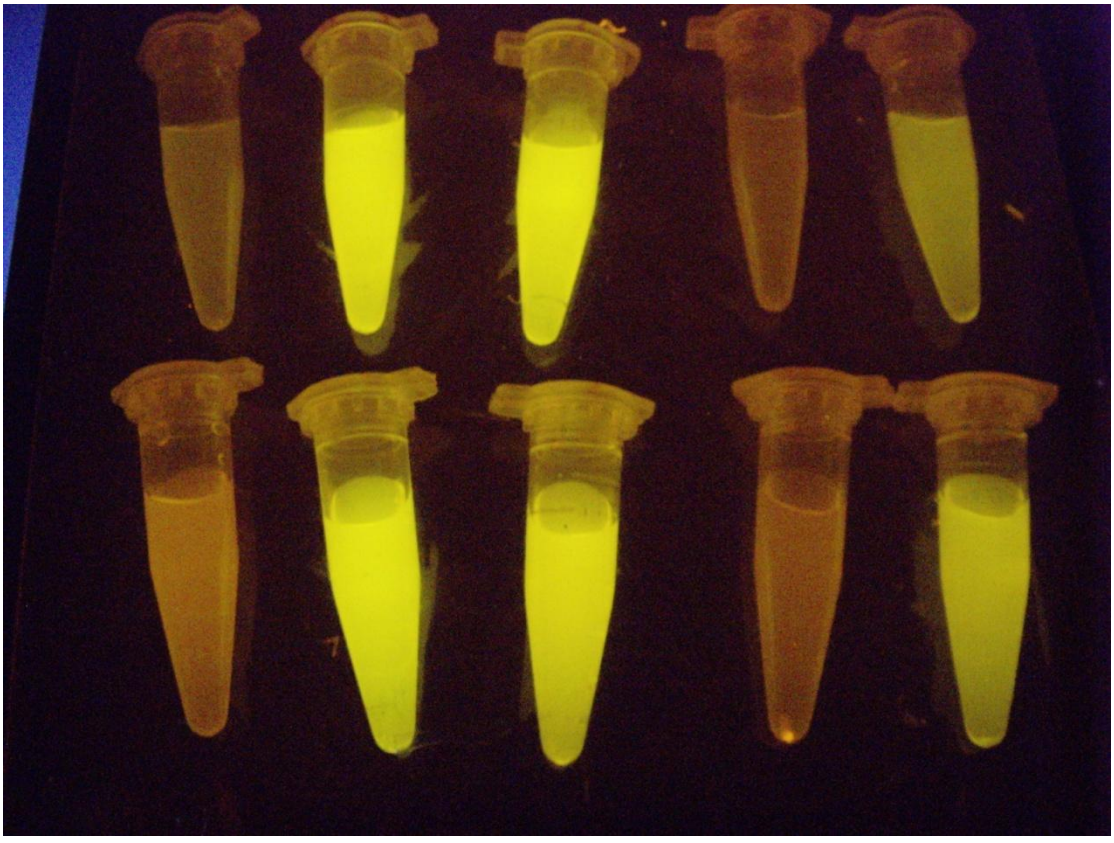
Some inducing data

Resource:
Delete LVA reverse tetR-tetP-GFP, colony, myself, renamed as DRTPG
Reverse tetR-tetP-GFP, colony, myself, RTPG
TetR-tetP-GFP, colony, myself (K228820), TPG
LacI-lacP-GFP, colony, myself (K228819), LPG

August 17^th
Inducing:
Transfer five different colonies (DRTPG, RTPG, TPG, LPG, control) into 5 different tubes of 5mL LB.
When they grow to OD 0.4~0.6, we transfer 2mL LB of each tube into 2 ep tubes. Add some IPTG (1mM) or aTc (1mM) to one ep tube to induce, and the other one without any IPTG, as a control.
After 4 hours, spin down the Ecoli and pour out LB, and then suspend the Ecoli with PBS buffer.
Now we can put them under blue light to see the GFP.

Here is the result:



The upper row is control group (without inducing): from left to right is control, LPG, TPG, RTPG, DRTPG;

The under row is induced group: from left to right is control, LPG, TPG, RTPG, DRTPG;

Result & discussion:
The result is consistent to the result of colonies on the plate (for more detail, refer to my prior experiment notes). The four devices LPG, TPG, RTPG, and DRTPG do not work very well, because the repressors (lacI & tetR) can not significantly repress the promoter lacP and tetP (or pLac and pTet).
We do not have too much time waste on them, so we decide to give up these four devices.

BY Shuke Wu

Molecular cloning: lacP+SupD/GFP, Sal+SupD/GFP

Parts:
lacP (R0010)+SupD-term (K228001+B0015)=lacP-SupD-term (K228822)
lacP (R0010)+GFP (E0840)=lacP-GFP (K228821)
Sal+SupD-term (K228001+B0015)=Sal-SupD-term
Sal+GFP (E0840)=Sal-GFP

Resource:
lacP: from parts, myself,
SupD-term: from Lin Min, has digested by EcoR1 and Xba1,
GFP: from Lin Min, has digested by EcoR1 and Xba1;
Sal: from a plasmid from Lin Min;

August 17^th
Plasmid mini prep:
lacP

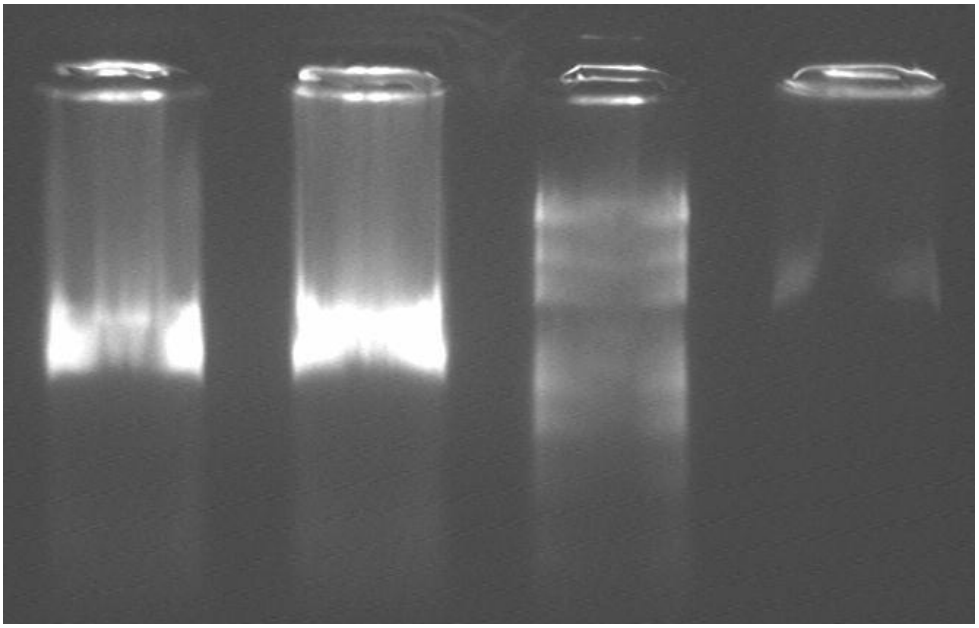
Double digest:
LacP: Spe1 1uL, EcoR1 1uL, plasmid 10uL, Buffer 2uL, water 6uL
37 ℃ 4 hour

PCR: (sal)
System 20 uL: pfu enzyme 1ul, primer (with standard prefix and suffix) 1uL each, Buffer 2 uL; water 10uL; template (the plasmid content Sal) 1uL; dNTP 4uL;

Gel electrophoresis:
Products of double digest of L and PCR
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
Loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 15min

Lane 1&2: Sal, PCR product,
Lane 3: Marker,
Lane 4: lacP, digest product

Sal should be about 1.4kb, and the result is correct!
After digested, lacP should have a 200bp one, but I can not see it. So I decided to repeat to amplify the Ecoli and mini prep.



DNA Gel purification:
Sal

Double digest:
Sal: Spe1 1uL, EcoR1 1uL, plasmid 10uL, Buffer 2uL, water 6uL
37 ℃ overnight

August 18th
PCR product purification:
Sal

DNA ligation:
System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ 4 hour
Insert: Sal;
Vector: GFP and SupD

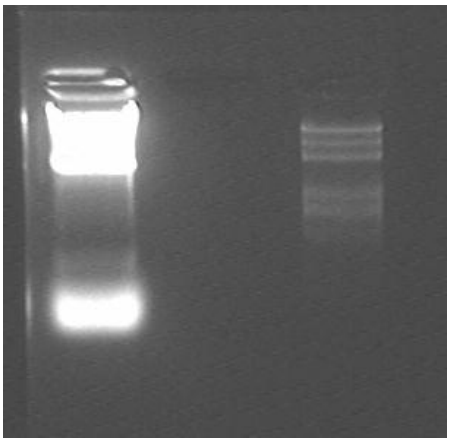
Transformation:
Products of ligation (Sal-SupD, Sal-GFP), competent cells 50uL each,

Smear to LB plate with Amp

Plasmid mini prep: (again)
lacP

Double digest: (again)
LacP: Spe1 1uL, EcoR1 1uL, plasmid 10uL, Buffer 2uL, water 6uL
37 ℃ overnight

August 19th
Gel electrophoresis:
Products of double digest of lacP,
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
Loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 15min
Lane1: lacP: insert 200bp;
Lane2: Marker



We can find that the smallest but bright one may be RNA, and a little larger and dim one is the insert.

DNA Gel purification:
Insert of lacP

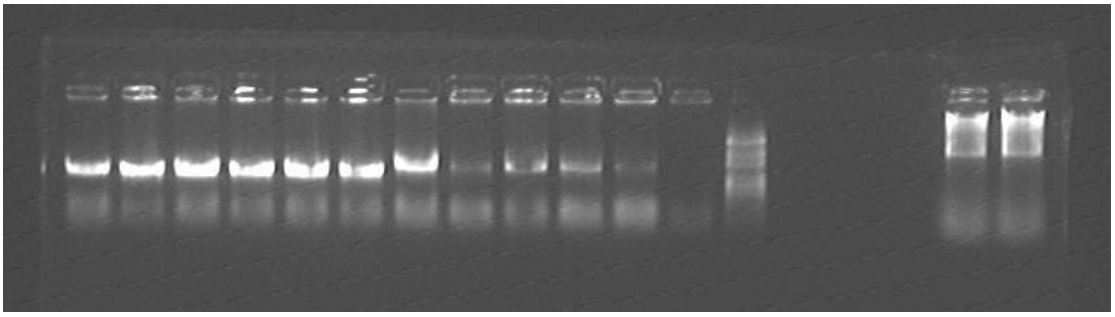
DNA ligation:
System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ 4 hour
Insert: lacP;
Vector: GFP and SupD

Transformation:
Products of ligation (lacP-SupD, lacP-GFP), competent cells 50uL each,
Smear to LB plate with Amp

PCR: (colony PCR to check the Sal-GFP and Sal-SupD)
System 10 uL: Master mix 5ul, primer (standard primers) 0.5uL each, water: 4uL; template

Double digest: (prepare lacP as a vector)
LacP: Spe1 1uL, Pst1 1uL, plasmid 10uL, Buffer 2uL, water 6uL
37 ℃ 4 hour

Gel electrophoresis:
Products of double digest of lacP, and PCR Sal
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
Loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 15min
Lane1~6: Sal-GFP1~6, should be 2.4kb, all are wrong;
Lane7~11: Sal-SupD1~5, should be 1.7kb, all are correct;
Lane12: negative control;
Lane13: Marker;
Lane14,15: lacP vector, should be 2.3kb, but there are some pollution plasmids?



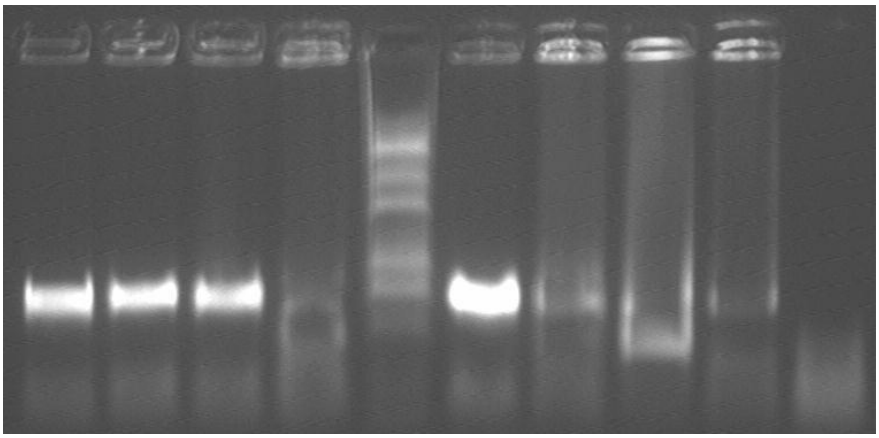
August 20^th
Plasmid mini prep:
Sal-SupD

DNA Gel purification:
lacP vector;

The plates of lacP-SupD and lacP-GFP are very good, and the colonies on the lacP-GFP are green. So I do not need to confirm it.
PCR: (colony PCR to check the lacP-SupD)
System 10 uL: Master mix 5ul, primer (standard primers) 0.5uL each, water: 4uL; template

Gel electrophoresis:
Products of PCR lacP-SupD
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
Loading buffer and DNA dye: 6×

Voltage and time: 60V 5min; 120V 15min
Lane1~4, 6~9: lacP-SupD 1~7;
Lane5: Marker;
Lane10: negative control;
The correct one should about 600bp, and we can find that 1, 2, 3 and 5 are correct.



Result:
I successfully constructed the clone: lacP-SupD-term (K228822), lacP-GFP (K2288221) and Sal-SupD-term (which turn out to be wrong after a few days, for more detail, refer to Haoqian Zhang's notes).
I failed to construct Sal-GFP.

Work transfer:
Sal transfers to Haoqian Zhang to do, and lacP vector transfers to ShenShan.

By Shuke Wu

Transfer backbone: lacP+SupD/GFP to low copy pSB4K5

Motivation:
In order to make use of lacIQ of the F plasmid of JM109 (more detail refer to my notes 0817), the AND gates should be build on low copy plasmid. And transfer at this time can save a lot of workload.

Resource:
lacP-SupD-term (K228822): plasmid, from myself; renamed as LS;
lacP-GFP (K228821): plasmid, from myself; LG;
low copy backbone, pSB4K5: vector, has already digested by EcoR1 and Pst1, from

ShenShan.

August 21st

Plasmid mini prep:

LG, LS1, LS2;

Double digest:

LG, LS1, LS2: Pst1 1uL, EcoR1 1uL, plasmid 10uL, Buffer 2uL, water 6uL

37 ℃ 4 hour

Gel electrophoresis:

Products of double digest of LG, LS1 and LS2;

marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb

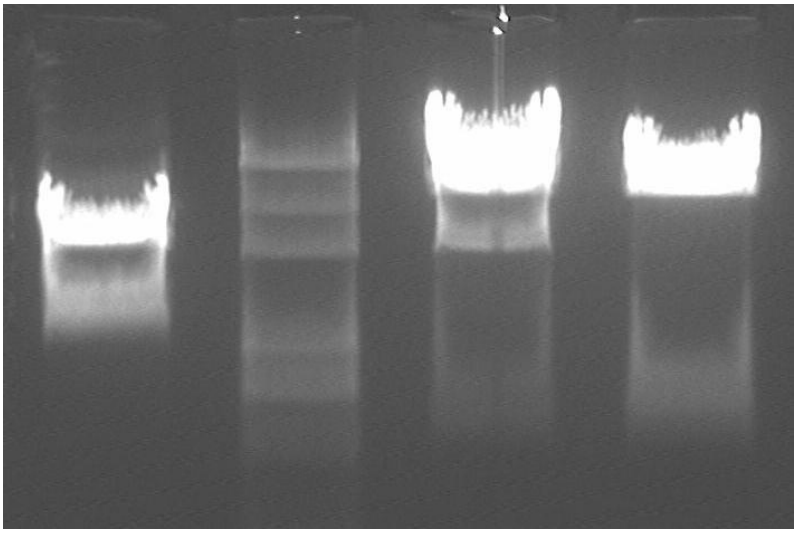
loading buffer and DNA dye: 6×

voltage and time: 60V 5min; 120V 15min

lane1: LG;

lane3,4: LS1, LS2;

lane2: marker;



The insert of LG is 1.1k, correct;

The insert of LS is 600bp, correct;

DNA Gel purification:

Insert of LG, LS1 and LS2.

DNA ligation:

System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL

16℃ 4 hour

Insert: LG, LS1 and LS2;

Vertor: pSB4K5

Transformation:

Products of ligation, competent cells 50uL each,
Smear to LB plate with Kan.

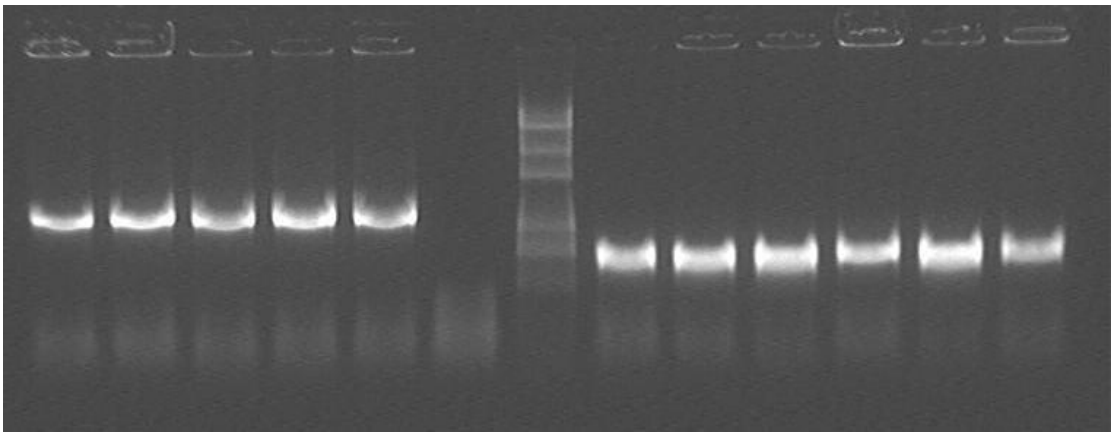
August 21st

PCR: (colony PCR)

Master mix 5ul, primer (standard primer) 0.5uL each, water 4uL; template;

Gel electrophoresis:

Products of PCR
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 15min



Lane1~5: LG1~5;
Lane6: PCR negative control;
Lane7: Marker
Lane8~13: LS1~6
All colonies are correct!!!

result:

I successfully transferred lacP-SupD-term (K228822) and lacP-GFP (K228821) to low copy plasmid pSB4K5.

BY Shuke Wu

Molecular cloning: AND gate LSAT*9

LAST CLONING of me this summer!!

Parts:

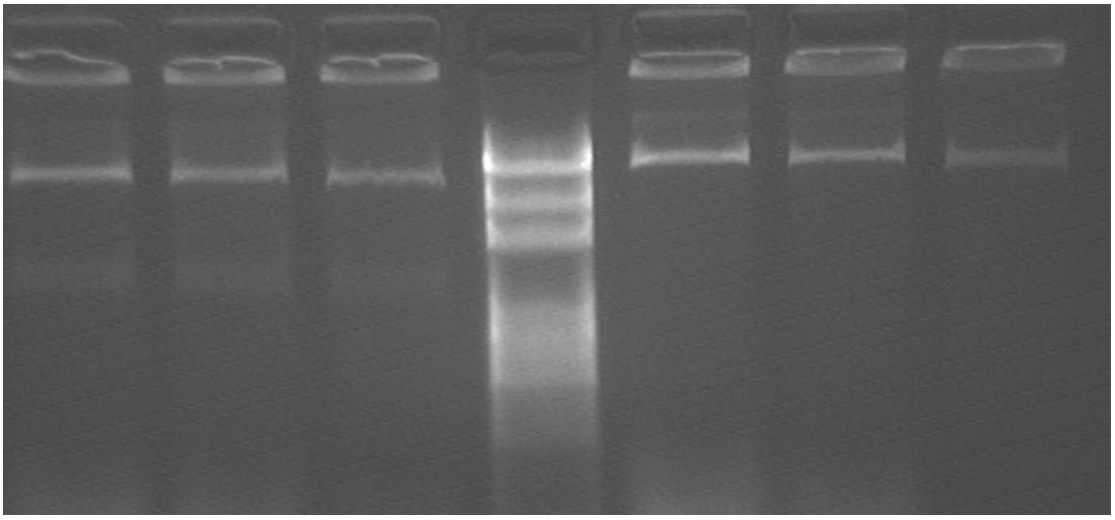
lacP-SupD (K228822)+araC-T7ptag=lacP-SupD-AraO-T7ptag, LSAT, (K228823~31)

Resource:
lacP-SupD: plasmid, from myself; renamed as LS;
araC-T7ptag: insert (digested by Xba1 and Pst1), from GuoSheng Zhang.

August 23rd
Plasmid mini prep:
LG*3, LS*3;

Double digest:
LG1, 2 and 3: EcoR1 1uL, Pst1 1uL, plasmid 6uL, Buffer 2uL, water 10uL
LS1, 2 and 3: Spe1 1uL, Pst1 1uL, plasmid 6uL, Buffer 2uL, water 10uL
37 ℃ 4 hour

Gel electrophoresis:
Products of double digest of LG and LS,
marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
voltage and time: 60V 5min; 120V 15min



lane1~3: LG1~3, should has insert 1.1k and vector 3.4k, all are correct!!
Lane5~7: LS1~3, should be 4.1k, all are correct.
Lane4: marker;

DNA Gel purification:
LS1, 2 and 3;

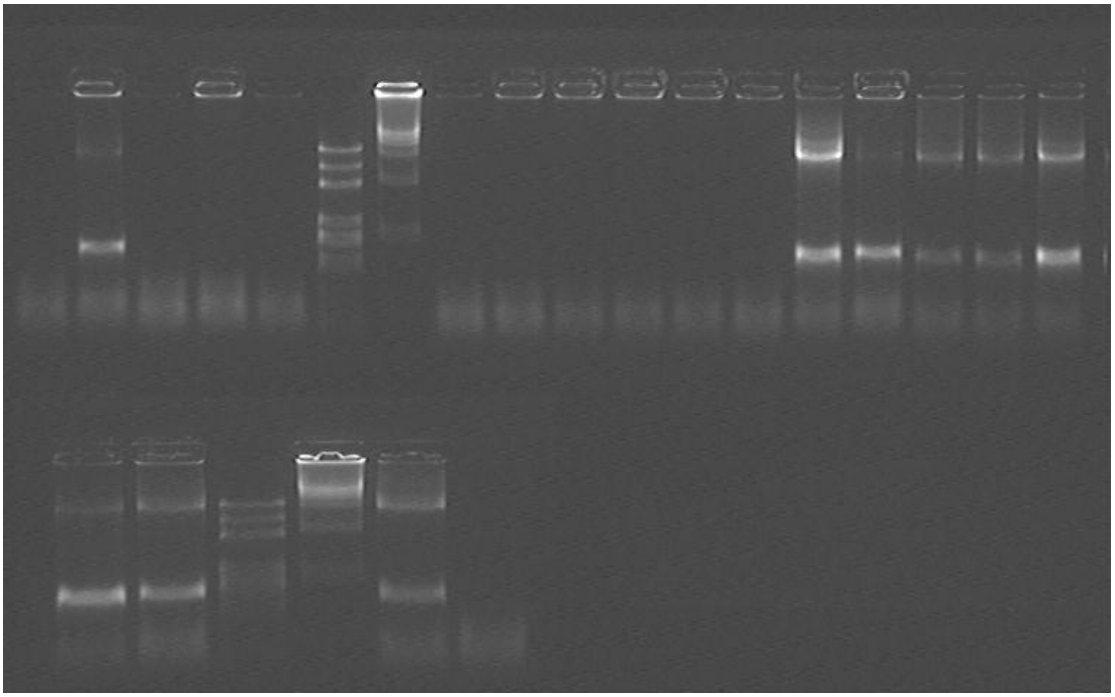
DNA ligation:
System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ 4 hour
Insert: araC-T7ptag*9;
Vertor: LS1;

Transformation:
Products of ligation, competent cells 50uL each,
Smear to LB plate with Kan.

August 24^th
Every plate is not very good: less than ten clones each plate; and LSAT-2G plate has no clone!!

PCR: (colony PCR)
Master mix 5ul, primer (standard primer) 0.5uL each, water 4uL, template;

Gel electrophoresis:
Products of PCR
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 30min



lane1~4: LSAT-2M (2M means the RBS);
lane5: 5J;
lane6, 7: Marker
lane8: 5N;
lane9, 10: 1H;
lane11, 12: 2k;
lane13: 11N;
lane14~17: 2I;
lane18+under row lane1, 2, 5: 1J;

Under row lane3, 4: Marker
Under row lane6: negative control;

If the colony is correct, its insert should be 4.6kb. So we can find only 2I and 1J are correct! REPEAT!!

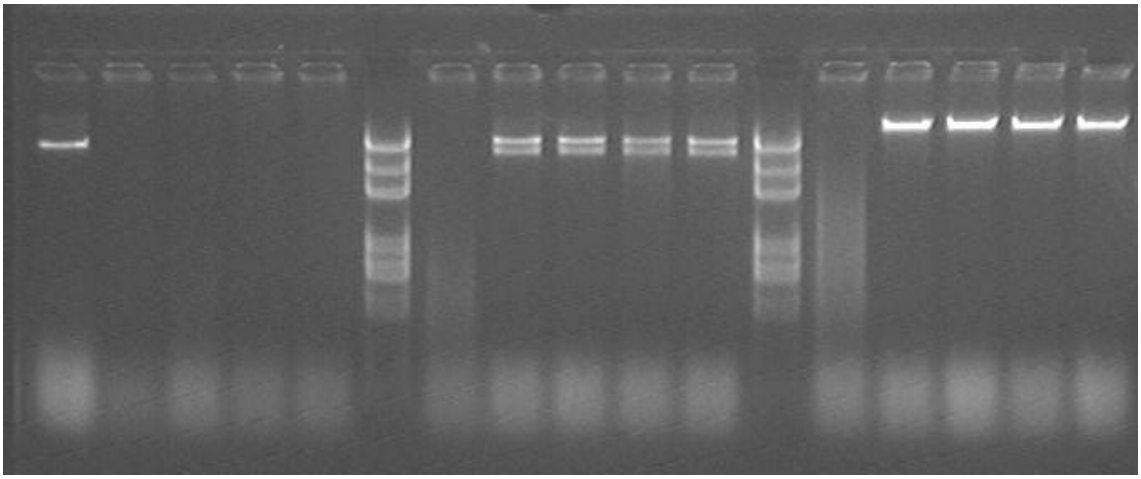
Because the insert is large, sometimes can not be amplified by taq enzyme, I decided to mini prep some of them.
Transfer the colonies: 2M(2), 5J, 5N, 1H, 2K, 2I(1, 3), 1J(3, 4) into 5 mL LB each.

Double digest (2nd time):
LS1: Spe1 1uL, Pst1 1uL, plasmid 16uL, Buffer 2uL.
37 ℃ overnight

August 25th
Plasmid mini prep:
2M(2), 5J, 5N, 1H, 2K, 2I(1, 3), 1J(3, 4);

Digest:
Single digest: 2M(2), 5J, 5N, 1H, 2K, 2I(1, 3), 1J(3, 4): EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
Double digest: 2M(2); 2I(1, 3); 1J (3, 4): EcoR1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

Gel electrophoresis:
Products of digest
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 30min
Lane1: lacP-SupD digested by Spe1 and Pst1
Lane2~5: Single digest:, 5J, 5N, 1H, 2K,
Lane6, 12: Marker;
Lane7~11: Double digest: 2M(2); 2I(1, 3); 1J (3, 4);
Lane13~17: single digest: 2M(2); 2I(1, 3); 1J (3, 4);



Correct colony should have 3.4k vector and 4.6k insert, and the whole plasmid is 8k. And from the gel: we found that only 2I(1, 3), 1J (3, 4) are correct, which consistent with the result of PCR.

DNA Gel purification: (2nd time)
lacP-supD (LS1),

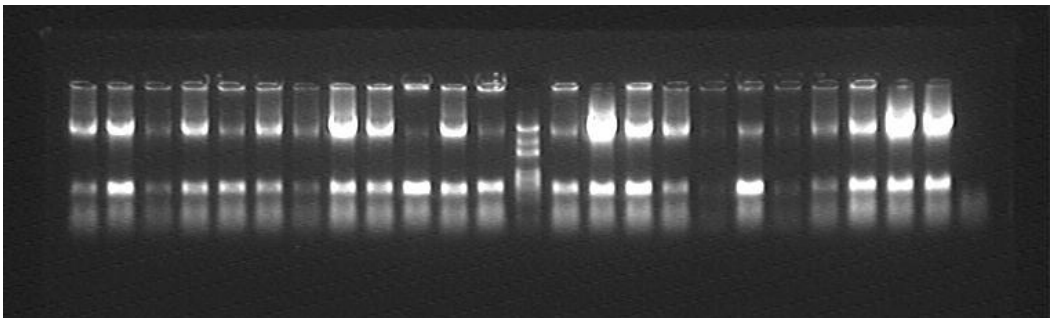
DNA ligation: (2nd time)
System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ 4 hour
Insert: araC-T7ptag*7 (without 2I and 1J);
Vertor: LS1;

Transformation: (2nd time) (Helped by Haoqian Zhang)
Products of ligation, competent cells 50uL each,
Smear to LB plate with Kan.

August 26th
Every plate is not very good, but better than the first time: more than ten clones each plate.

PCR: (colony PCR) (2nd time)
Master mix 5ul, primer (standard primer) 0.5uL each, water 4uL, template;

Gel electrophoresis:
Products of PCR
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 30min



Lane1~3: LSAT-11N
Lane4~6: 5N
Lane7~9: 1H
Lane10~12: 5J
Lane13: Marker
Lane14~16: 2K
Lane17~19: 2G
Lane20~24: 2M
Lane25: PCR negative control
We can find that every clone has some correct one.

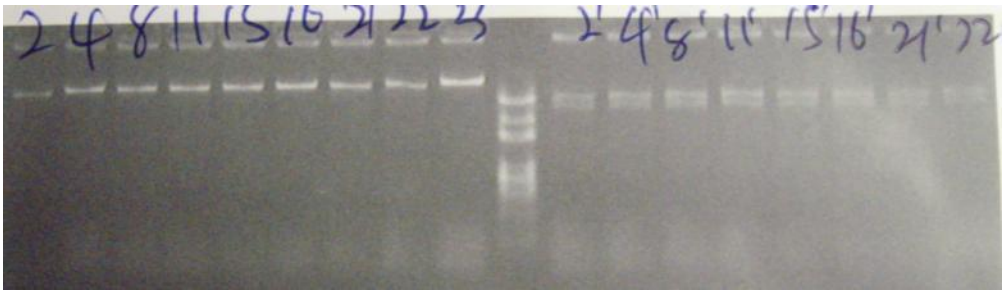
Transfer the colonies:11N-2, 5N-1, 1H-2, 5J-2, 2K-3, 2G-3, 2M-3,4,5 into 5 mL LB each.

August 27th
Plasmid mini prep: (2nd time)
11N-2, 5N-1, 1H-2, 5J-2, 2K-3, 2G-3, 2M-3,4,5

Digest: (2nd time)
Single digest: 11N-2, 5N-1, 1H-2, 5J-2, 2K-3, 2G-3, 2M-3,4,5: EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
Double digest: 211N-2, 5N-1, 1H-2, 5J-2, 2K-3, 2G-3, 2M-3,4,5: EcoR1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

Gel electrophoresis:
Products of digest
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 30min
Lane 1~9: Single digest: 11N-2, 5N-1, 1H-2, 5J-2, 2K-3, 2G-3, 2M-3,4,5
Lane 10: Marker
Lane 11~18: Double digest: 211N-2, 5N-1, 1H-2, 5J-2, 2K-3, 2G-3, 2M-3,4
Correct colony should have 3.4k vector and 4.6k insert, and the whole plasmid is 8k.

And from the gel: we found that all of they are **CORRECT!!!**



Result:

I have already successfully constructed all the night AND gates:

LSAT lacP-SupD-AraC-T7ptag, and they are parts K228823~31.

Now I finished all the cloning!!!

Appreciate every member of our iGEM group!!!

Go on to test my AND gates~~~

By Shuke Wu