Molecular cloning: 6×RBS+ araC/ lacI

6 ×RBS

results

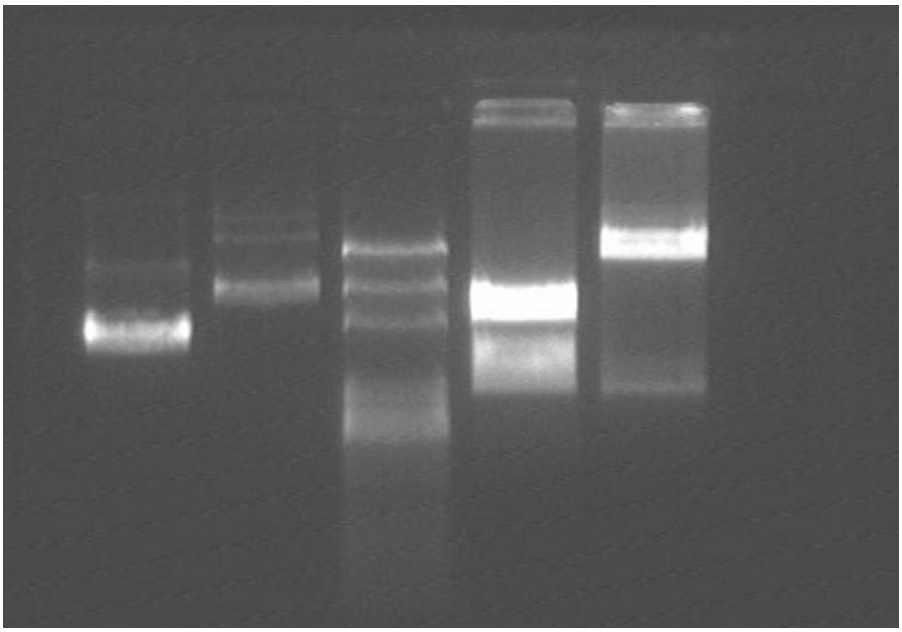
araC/ lacI

Resource:
6 RBS: from the parts: B0030, B0032, B0034, J61100, J61107, J61127; renamed as RBS1 RBS2…RBS6;
araC: C0080
lacI: C0012

July 6th
Plasmid mini prep:
6 RBS;
lacI;
araC;

Double digest:
6 RBS: Spe1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
lacI, araC: Xba1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

July 7th
Gel electrophoresis:
Products of double digest of lacI and araC,
marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
voltage and time: 60V 5min; 120V 15min
lane1: lacI plasmid;
lane2: araC plasmid;
lane3: marker;
lane4: digested product of lacI;
lane5: digested product of araC;



DNA Gel purification:
lacI and araC

PCR product purification:
6 RBS

DNA ligation:
System 10uL: Insert 4uL, vector 1uL, water 3uL, buffer 1uL, T4 DNA ligase 1uL
16℃ 4 hour
Insert: lacI; araC; tetR (from Min Lin);
Vertor: 6 RBS

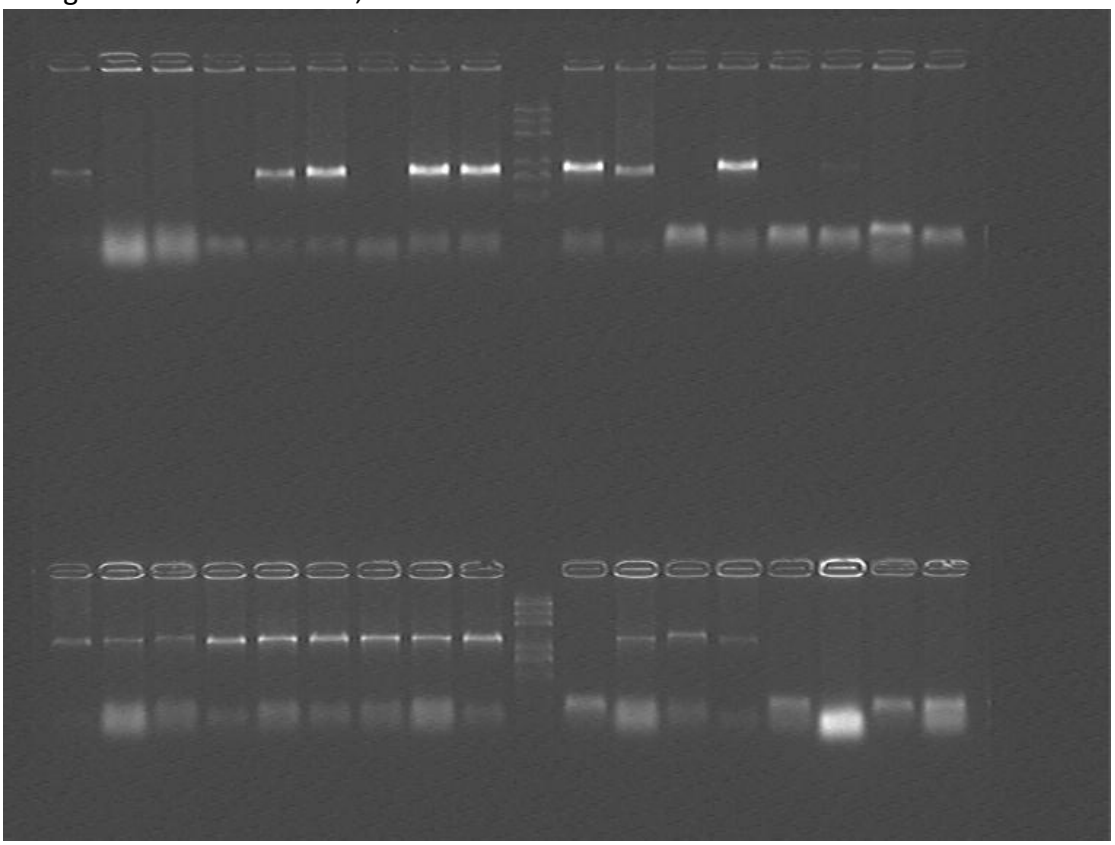
Transformation:
Products of ligation, competent cells 50uL each,
Smear to LB plate with Amp

July 8th
Every plate is very well: more than 100 clones
Waiting for PCR to check the positive clones

July 9th
PCR:
Master mix 5ul, primer (standard primer) 0.5uL each, template;

July 10th
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



Up row:
lane 1~3: tetR+RBS1 1~3;
lane 4~6: tetR+RBS2 1~3;
lane 7~9: tetR+RBS3 1~3;
lane 10: marker;
lane 11~12: tetR+RBS4 2~3;
lane 13~15: tetR+RBS5 1~3;
lane 16~18: tetR+RBS6 1~3;
down row:
lane 1~3: lacI+RBS1 1~3;
lane 4~6: lacI+RBS2 1~3;
lane 7~9: lacI+RBS3 1~3;
lane 10: marker;
lane 11~12: lacI+RBS4 2~3;
lane 13~15: lacI+RBS5 1~3;
lane 16~18: lacI+RBS6 1~3;

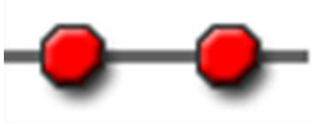
result:
10 clones were successfully constructed: RBS1~5+lacI and tetR;
They are parts: K228801, K228802, K228803, K228804, K228805,

2 clones were failed: RBS6+lacI and tetR.

Molecular cloning: RBS-lacI/tetR + terminator

RBS-lacI/tetR

result



terminator

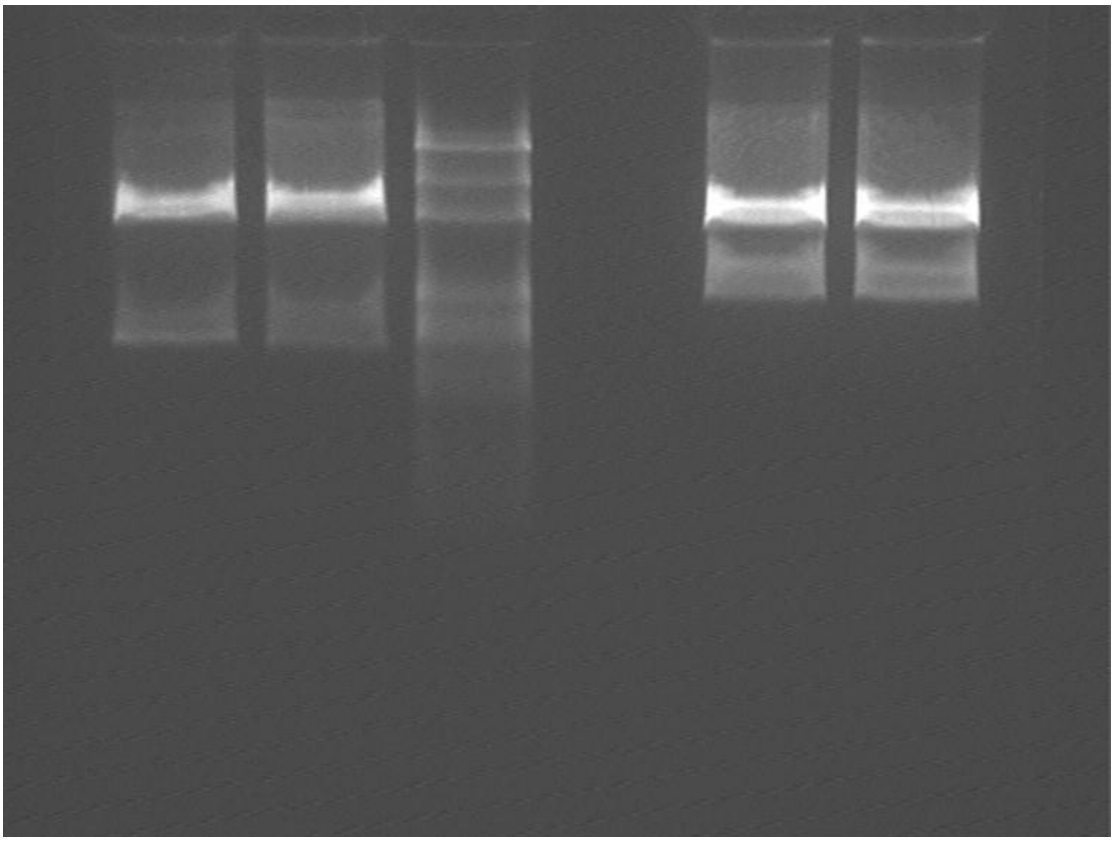
Resource:
RBS-LacI: myself: 2×B0034-C0012, renamed as L1, L2
RBS-tetR: myself: 2×B0034-C0040, renamed as t2, t3
Terminator: Haoqian Zhang & Guosheng Zhang: B0015

July 10th
Plasmid mini prep:
6 RBS-tetR: RBS1-tetR1; RBS2-tetR3; RBS3-tetR2, 3 (t2, t3); RBS4-tetR2; RBS5-tetR2;
6 RBS-lacI: RBS1-lacI1; RBS2-lacI1; RBS3-lacI1, 2 (L1, L2); RBS4-lacI3; RBS5-lacI2;

Double digest:
L1, L2, t2, t3: Spe1 1uL, EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

Gel electrophoresis:
Products of double digest of L1, L2, t2, t3,
Marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
Loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 15min
Lane1: t2: insert 700bp;
Lane2: t3: insert 700bp;
Lane3: marker;
Lane5: L1: insert 1.1kb;

Lane6: L2: insert 1.1kb;



DNA Gel purification:

I made a big mistake here. I purified the brightest ones, which are vectors. So I do the double digest again.

Double digest (again):

L1, L2, t2, t3: Spe1 1uL, EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL

37 ℃ over night.

July 11th

Gel electrophoresis (again):

Products of double digest of L1, L2, t2, t3,

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

Loading buffer and DNA dye: 6×

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

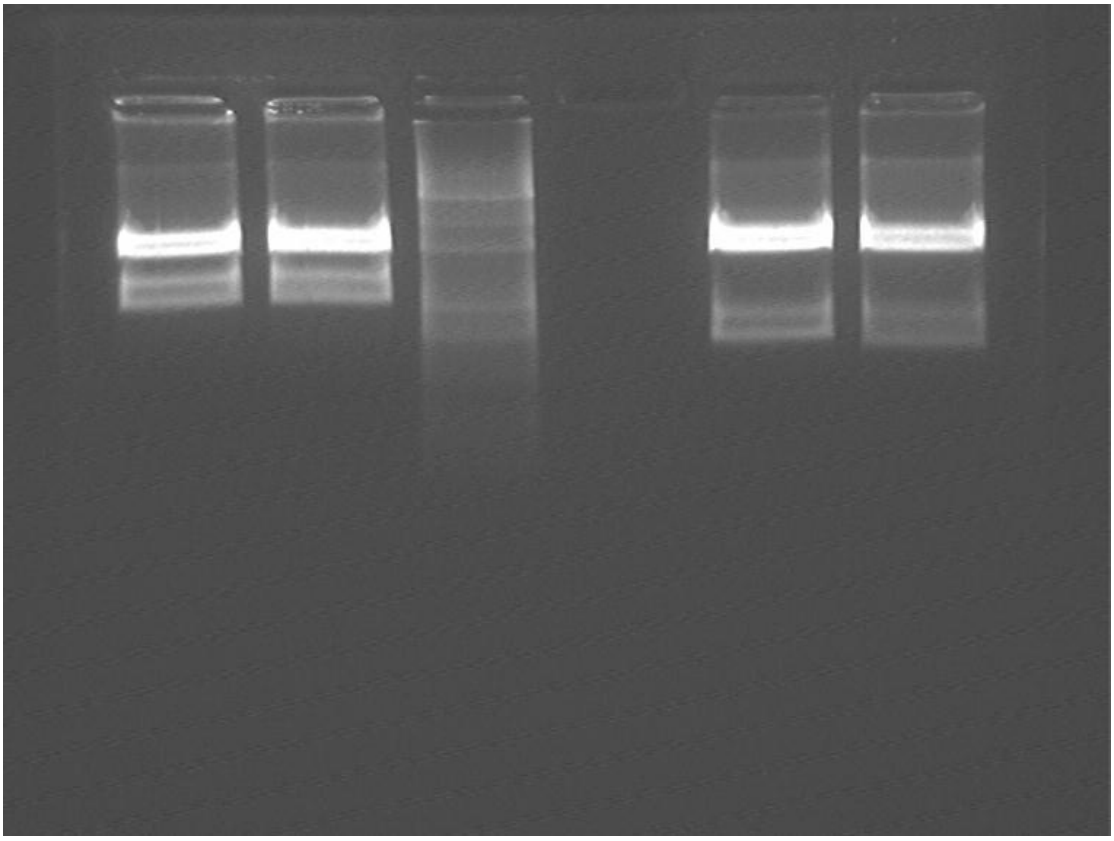
Lane1: L1: insert 1.1kb;

Lane2: L2: insert 1.1kb;

Lane3: marker;

Lane5: t2: insert 700bp;

Lane6: t3: insert 700bp;

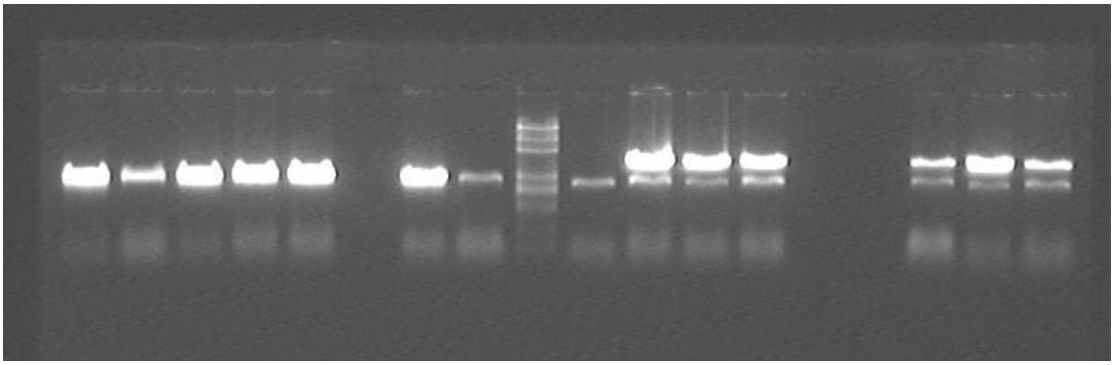


DNA ligation:
System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ 4 hour
Insert: L1 L2 t2 t3;
Vector: terminator digested by EcoR1 and Xba1 (provided by Haoqian Zhang & Guosheng Zhang)

Transformation: (by Min Lin)
Products of ligation, competent cells 50uL each,
Smear to LB plate with Amp

July 12th
Every plate is very well: more than 100 clones
PCR:
14 tubes: L1×3+L2×3+t2×3+t3×3 and 2 negative controls
Master mix 5ul each, primer (standard primer) 0.5uL each, 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



Lane 1: t2-1;
Lane 2~4: t3-3~1;
Lane 5: t2-3;
Lane 7: t2-1+2;
Lane 8: negative control1;
Lane 9: marker;
Lane 10: negative control2;
Lane 11~13: L2-3~1;
Lane 16~18: L1-3~1;

Result:
There is a polluted line at about 1kb place, but it did not confuse us. The right place of L1 & L2 is about 1.3kb and of t2 & t3 is about 800bp.
L1-1~3, L2-1~3, t2-1&3 and t3-1~2 should be the positive clones.
4 clones were successfully constructed:
L1 & L2: 2×B0034-C0012-B0015
T2 & t3: 2×B0034-C0040-B0015

By Shuke Wu

Molecular cloning: 2M-lacI/tetR-term+Pcat

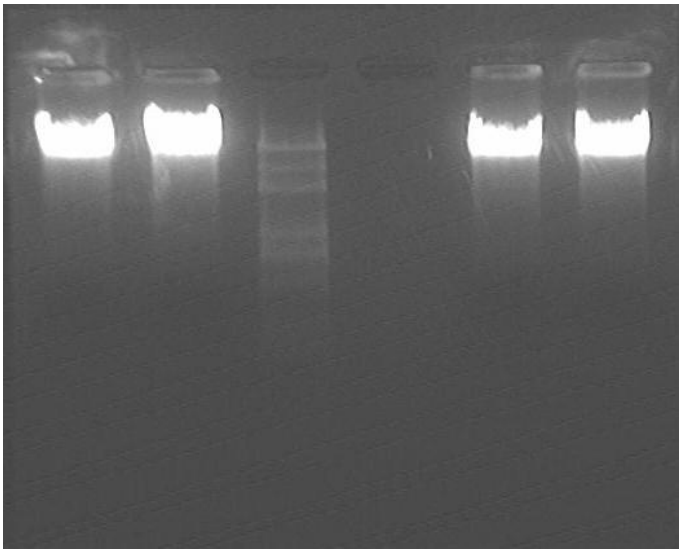
Parts: K228813/14+I14033=K228815/16
Resource:
2m-lacI/tetR-term: myself, rename as L1, L2, T1, T2
Pcat: part I14033

July13 th
Plasmid mini prep:
I14033: Pcat;

K228813: L1, L2;
K22814: T1 T2;

Double digest:
Pcat: Spe1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
L1, L2 T1, T2: Xba1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

Gel electrophoresis:
Products of double digest of L1, L2, T1, T2,
marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
voltage and time: 60V 5min; 120V 15min
lane1: digested product of L1;
lane2: digested product of L2;
lane3: marker;
lane4: digested product of T1;
lane5: digested product of T2;

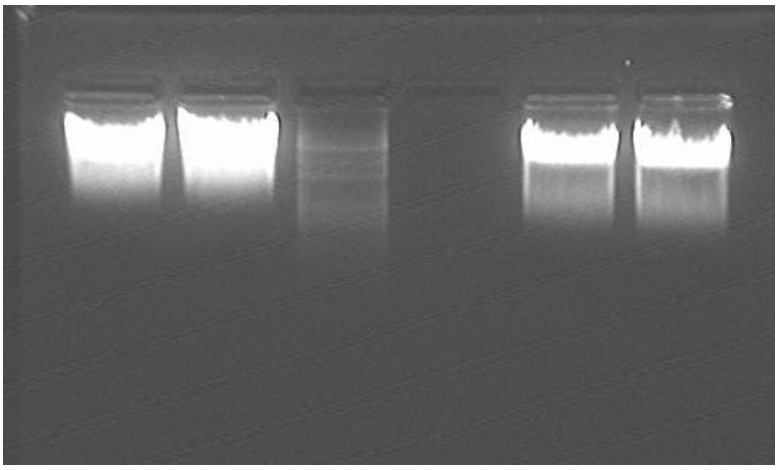


I found that they are not digested well. There are not any insert. There is possibility that I added the wrong enzymes or something wrong in the enzymes.
Repeat digest.

Double digest: (again)
Pcat: Spe1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
L1, L2 T1, T2: Xba1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

Gel electrophoresis: (again)
Products of double digest of L1, L2, T1, T2,
marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb

loading buffer and DNA dye: 6×
Voltage and time: 60V 5min; 120V 15min
lane1: digested product of L1;
lane2: digested product of L2;
lane3: marker;
lane4: digested product of T1;
lane5: digested product of T2;



I can found the inserts of L1, L2, and they about 1.3kb.
I can found the inserts of T1, T2, and they about 800bp.

DNA Gel purification:
Inserts of L1, L2, T1, T2.

PCR product purification:
Product of digest: Pcat.

DNA ligation:
System 10uL: Insert 6uL, vector 2uL, water 3uL, buffer 1uL, T4 DNA ligase 1uL
16℃ overnight.
Insert: L1, L2, T1, T2;
Vertor: Pcat

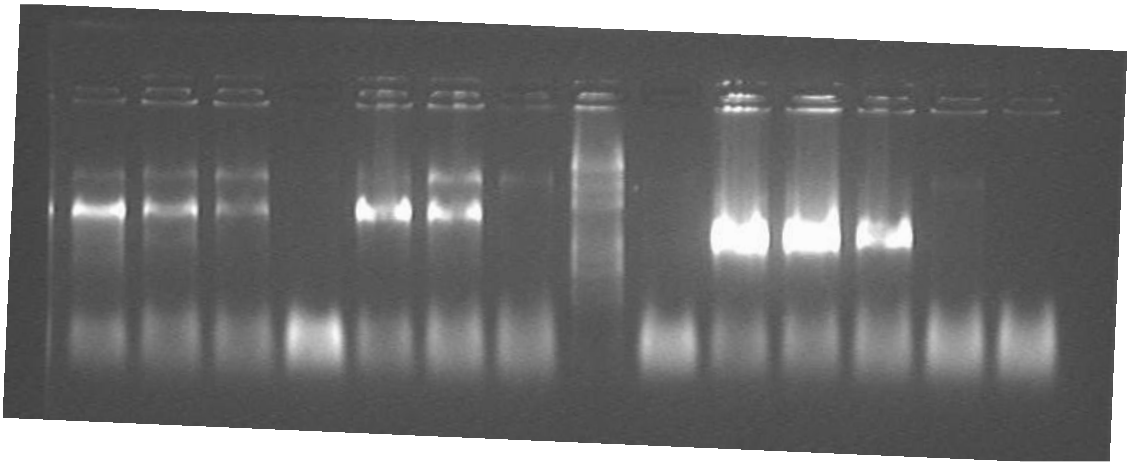
July 14th
Transformation:
Products of ligation, competent cells 50uL each,
Smear to LB plate with Kan
The colonies are too small at night, and let they grow overnight.

July 15th
Every plate is very well: more than 100 clones

PCR: (colony PCR)

System 10 uL: 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



lane 1~3: L1 1~3;
lane 4~6: L2 1~3;
lane 7: PCR control.
lane 8: marker;
lane 9~11: T1 1~3;
lane 12~14: T2 1~3;
Right colonies: L1 L2: 1.4kb; T1, T2 900bp

result:
2 clones were successfully constructed: 2M-tetR-term+Pcat *2
They are parts: K228816.

Unexpected result:
L1, L2 failed, due to reversing PCR from July 16th to July 20th.
Because use the reversing primers can not amplify the plasmid of L1 L2, the clone here should be K228813: 2M-lacI-term, not K228815: Pcat-2M-lacI-term.
For more detail, please refer to my experiment notes "reversing July 16th ~July 21st."

Repeat clone 2M-lacI-term+Pcat:
July 17th
Double digest:
Pcat: Spe1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL

PCR product purification:
Product of digest: Pcat.

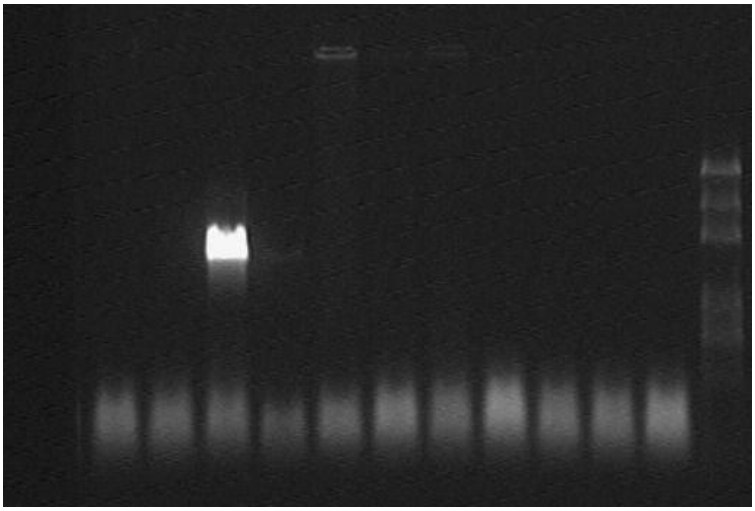
DNA ligation:
System 10uL: Insert 6uL, vector 2uL, water 3uL, buffer 1uL, T4 DNA ligase 1uL
16℃ overnight.
Insert: L1, L2,(use the inserts on the July 13th)
Vector: Pcat.

July 18th
Transformation:
Products of ligation, competent cells 50uL each,
Smear to LB plate with Kan

July 19th
Every plate is very well: more than 100 clones

PCR: (colony PCR)
System 10 uL: Master mix 5ul, primer (reversing primers) 0.5uL each, water: 4uL; template;
Because reversing primers can bind to Pcat and terminator, they can be use to check the right colonies.
For more details, refer to my experiment notes "reversing July 16th ~July 21st."

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 45min



Lane1~8: colonies 1~8;
Lane9, 10: other plasmid controls;

Lane11, 12: no template controls.

Result:

We can found the 3rd colony is right.
So I successfully constructed: 2M-tetR-term+Pcat, it is the part K228815.

BY Shuke Wu