Reversing: Pcat-2M-lacI/tetR-term

Parts: reverse K228815/16

Resource:

Pcat-2m-lacI/tetR-term: myself

July 16th.

Plasmid mini prep:

Pcat-2m-lacI-term: K228815: L1, L2; (these two are colonies on July 15th, which are wrong, according to flowing evidence)

Pcat-2m-tetR-term: K228816: T1 T2;

Double digest:

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,

Plasmids 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: plasmid of L1;

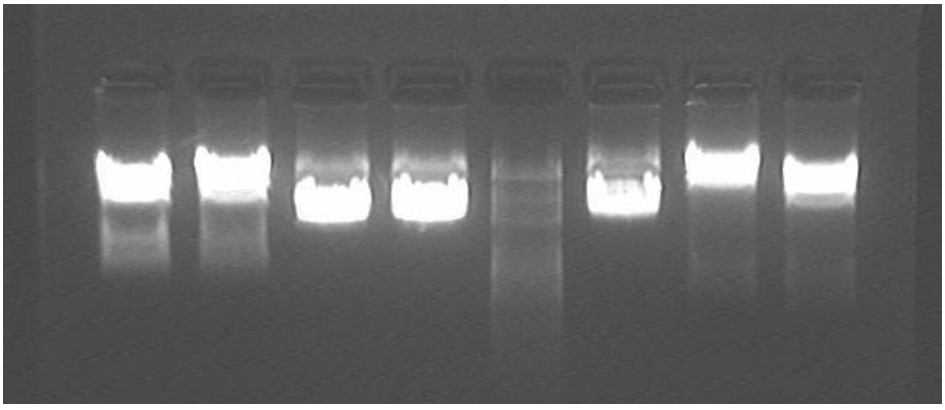
lane4: plasmid of L2;

lane5: marker;

lane6: plasmid of T2;

lane7: digested product of T1;

lane8: digested product of T2;



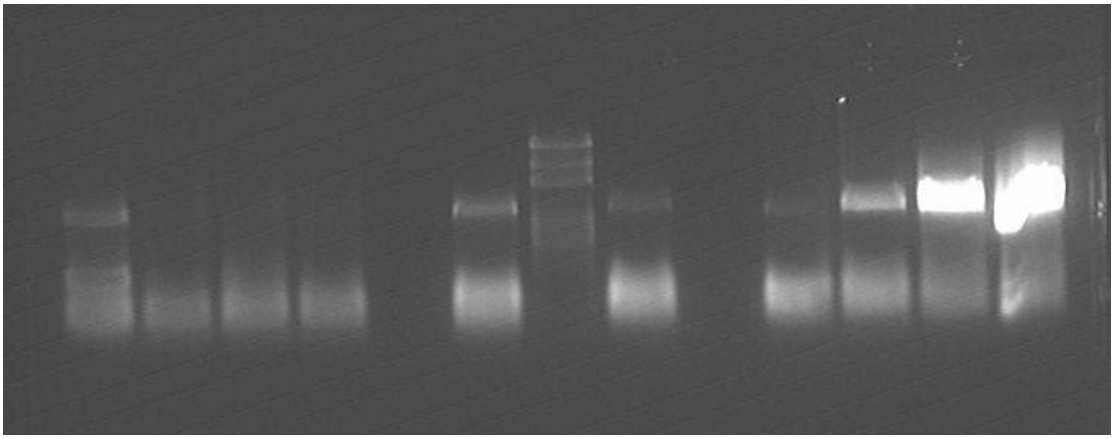
The inserts of L1, L2 should be 1.3kb, of T2, T3 should be 800bp.

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

PCR: (reversing)
System 20 uL: pfu enzyme 1ul, primer (reversing primers) 1uL each, Buffer 2 uL; water 10uL; template (the inserts of L1, L2, T1, T2) 1uL; dNTP 4uL;
Two different annealing temperature: 55C and 60C.
Reversing primers: are a pair of primers designed by me: one of them complement to Pcat, but with a standard suffix tail; another complement to terminator, and with a prefix tail.

For 5' GAATTC GCGGCCGC T TCTAGA G TATAAACGCAAAGGCCCA 3'
Rev 5' CTGCAG CGGCCGC T ACTAGT A GGCACGTAAGAGGTTCCAA 3'

July 17th.
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



lane1~4: L1 55C, 60C, L2 55C 60C;
lane5, 7: negative control;
Lane6: marker;
Lane8~11: T1 55C 60C, T2 55C 60C;

Rsult & discussion:
We can easily find that T1is right , but L1,L2 and T1 are wrong.

It is possible that L1, L2 and T1 are not Pcat-2m-lacI/tetR-term (K228815/16), but 2m-lacI/tetR-term (K228813/14). And this means that I was failed at the last cloning!
If they are Pcat-2m-lacI/tetR-term (K228815/16), the plasmid backbone is pSB1AK3 about 3.2kb, while if they are 2m-lacI/tetR-term (K228813/14), the plasmid backbone is pSB2K3 about 4.4kb. From the gel of July 16th, we can find that the backbone (vector) of T1 is a little smaller than T2 (compare the lane7 and lane8).

Furthermore, 2m-lacI/tetR-term (K228813/14) are double resistant Amp and Kan, and thereby the plasmid can grow on Kan plate, which is use for growing the Pcat-2m-lacI/tetR-term (K228815/16). So all this evidence supports the assumption: L1, L2 and T1 are not Pcat-2m-lacI/tetR-term (K228815/16), but 2m-lacI/tetR-term (K228813/14)!!!

Repeat clone 2M-lacI-term+Pcat: (refer to my experiment notes July 13th to July 20th)

July 18th

PCR: (colony and plasmid PCR to confirm that reversing primers can check right clone)

System 10 uL: Master mix 5ul, primer (reversing primers) 0.5uL each, water: 4uL; template; annealing temperature 55C.

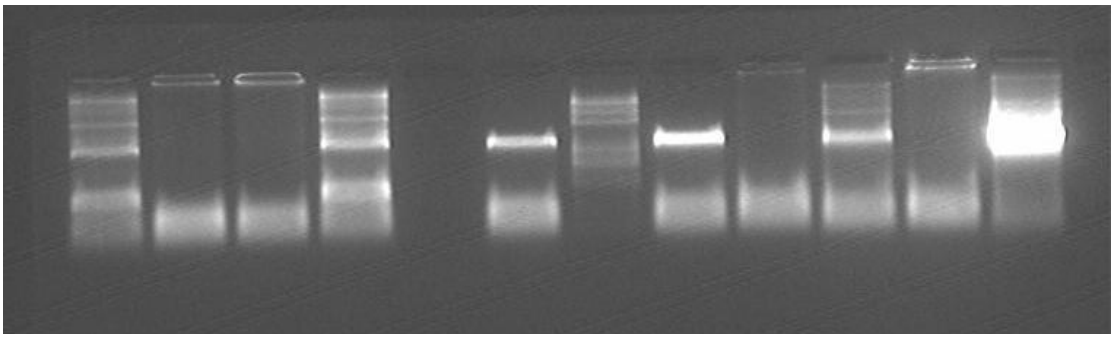
Gel electrophoresis:

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



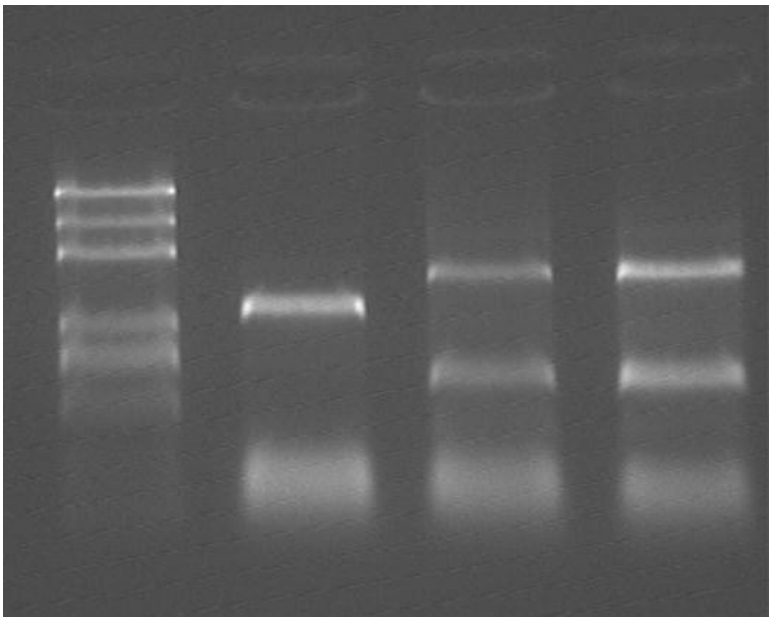
Lane1: plasmid L1-1;
Lane2: colony L1-2;
Lane3: colony L1-3;
Lane4: plasmid L2-1;
Lane5&7: negative control;
Lane6: Marker;
Lane8: colony L2-2;
Lane9: plasmid T1-1;
Lane10: colony T1-2;
Lane11: plasmid T2;

We can find that the last one is very different from others, so the reversing primers can be used to check right colonies.

July 20th

PCR: (reversing the right colony of July 19th 2M-tetR-term+Pcat, K228815.)
System 20 uL: pfu enzyme 1ul, primer (reversing primers) 1uL each, Buffer 2 uL; water 10uL; template (the inserts of L1, L2, T1, T2) 1uL; dNTP 4uL;

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

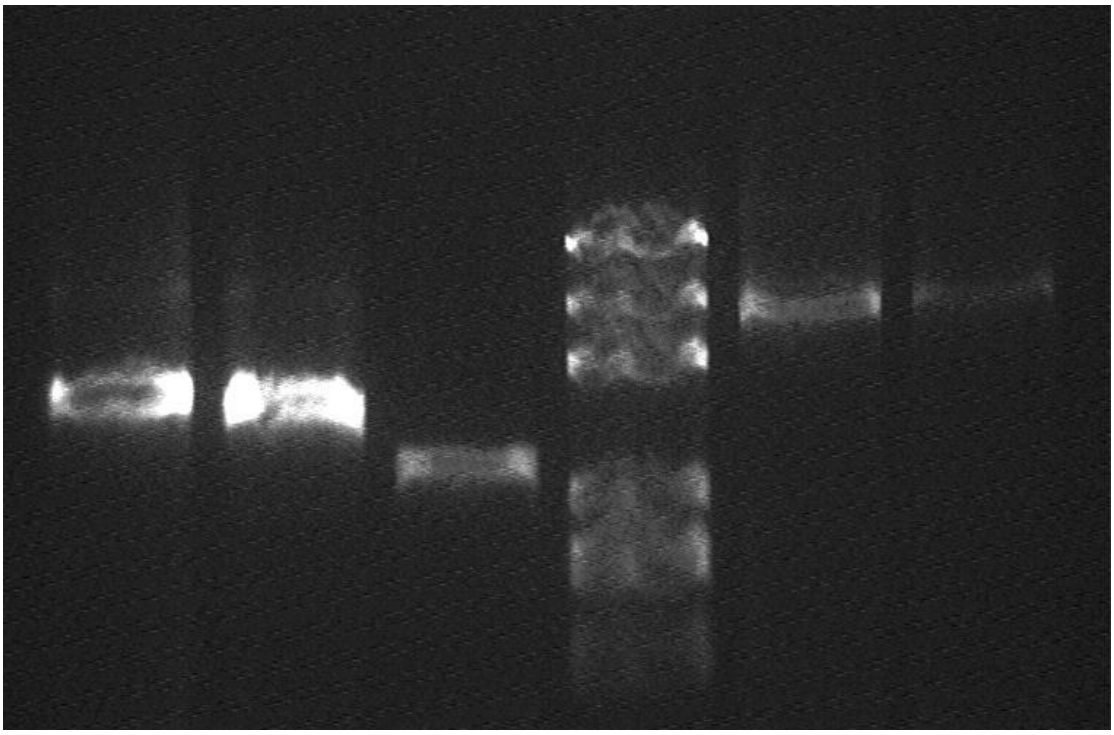


Lane1: Marker;
Lane2: negative control;
Lane3&4: right colony.

I found that the extending time is only 2mins, which is too short for pfu to amply 1.3kb. So I did PCR again.

PCR: (again, reversing the right colony of July 19th 2M-tetR-term+Pcat, K228815.)
System 20 uL: pfu enzyme 1ul, primer (reversing primers) 1uL each, Buffer 2 uL; water 10uL; template (the inserts of L1, L2, T1, T2) 1uL; dNTP 4uL;
Extending time: 3 min

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&2: right colony,
Lane3: control;
Lane4: Marker;

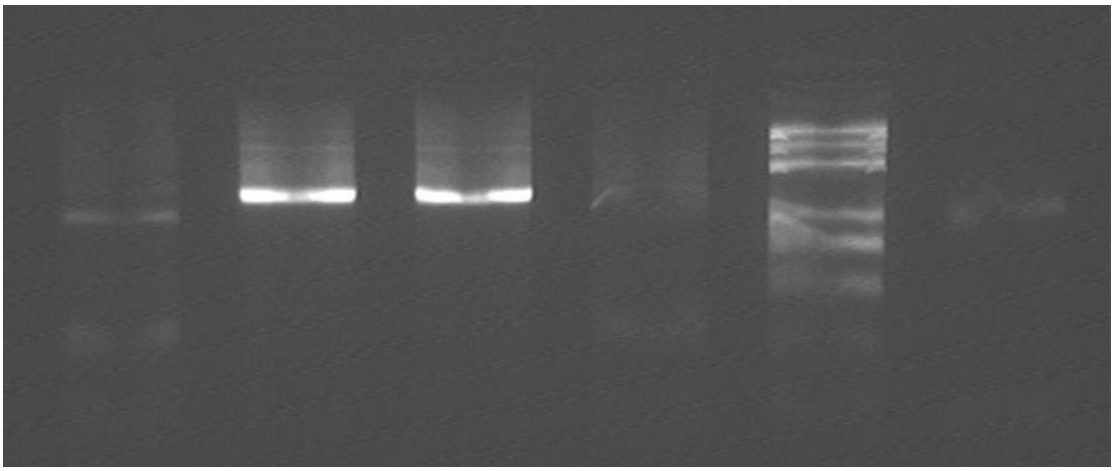


This time, the result is correct, but I use colony PCR and pfu, which may lead to some mutations. So I decided to mini prep the plasmid of 2M-tetR-term+Pcat (K228815). Then did PCR.

Plasmid mini prep:
The right plasmid of 2M-tetR-term+Pcat (K228815) and other two wrong plasmids

PCR: (again, reversing the right plasmid of July 19th 2M-tetR-term+Pcat, K228815.)
System 20 uL: pfu enzyme 1ul, primer (reversing primers) 1uL each, Buffer 2 uL; water 10uL; template (the inserts of L1, L2, T1, T2) 1uL; dNTP 4uL;
Extending time: 3 min

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 15min
Lane2&3: right plasmid,
Lane1&4: other plasmid control;
Lane5: Marker;
Lane6: negative control of PCR.



Result:
I successfully reverse the Pcat-2m-lacI/tetR-term (K228815/16).

BY Shuke Wu

Molecular cloning: reversed (K228815/16)+lacP/tetP

Resource:
Reversed Pcat-2m-lacI/tetR-term (K228815/16): myself, PCR product;
lacP: parts R0010, from Lin Min, plasmid,
tetP: parts R0040, from Lin Min, plasmid,

July 17th
DNA Gel purification:
Reversed Pcat-2m-tetR-term (K228816), from the gel of July17th, refer to my experiment note: July 16th-21st
Products renamed as T1, T2.

July 19th
Double digest:
T1, T2: Spe1 1uL, EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour
lacP, tetP: Xba1 1uL, EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

PCR product purification:

Product of digest: T1, T2, lacP and tetP.

July 20th

DNA ligation:

System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ overnight.
Insert: T1, T2;
Vector: tetP

July 21st

Transformation:

2 Products of ligation (T1, T2), competent cells 50uL each,
Smear to LB plate with Amp

DNA Gel purification:

Reversed Pcat-2m-lacI-term (K228815), from the gel of July 20th and 21st, refer to my experiment note: July 16th-21st
Products renamed as L1 (July 20th), L2 (July 21st).

Double digest:

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

PCR product purification:

Product of digest: L1, L2,

DNA ligation:

System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ overnight.
Insert: L1, L2;
Vector: lacP

July 22nd

Result of transformation (T1, T2):

No any colony!!!!!
Repeat!!!

DNA ligation (again):

System 10uL: Insert 6uL, vector 2uL, buffer 1uL, T4 DNA ligase 1uL
16℃ overnight.
Insert: T1, T2;
Vector: tetP

Double digest (again):

T1, T2: Spe1 1uL, EcoR1 1uL, plasmid 10uL, Buffer 2uL, water 6uL
37 ℃ 4 hour
tetP: Xba1 1uL, EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

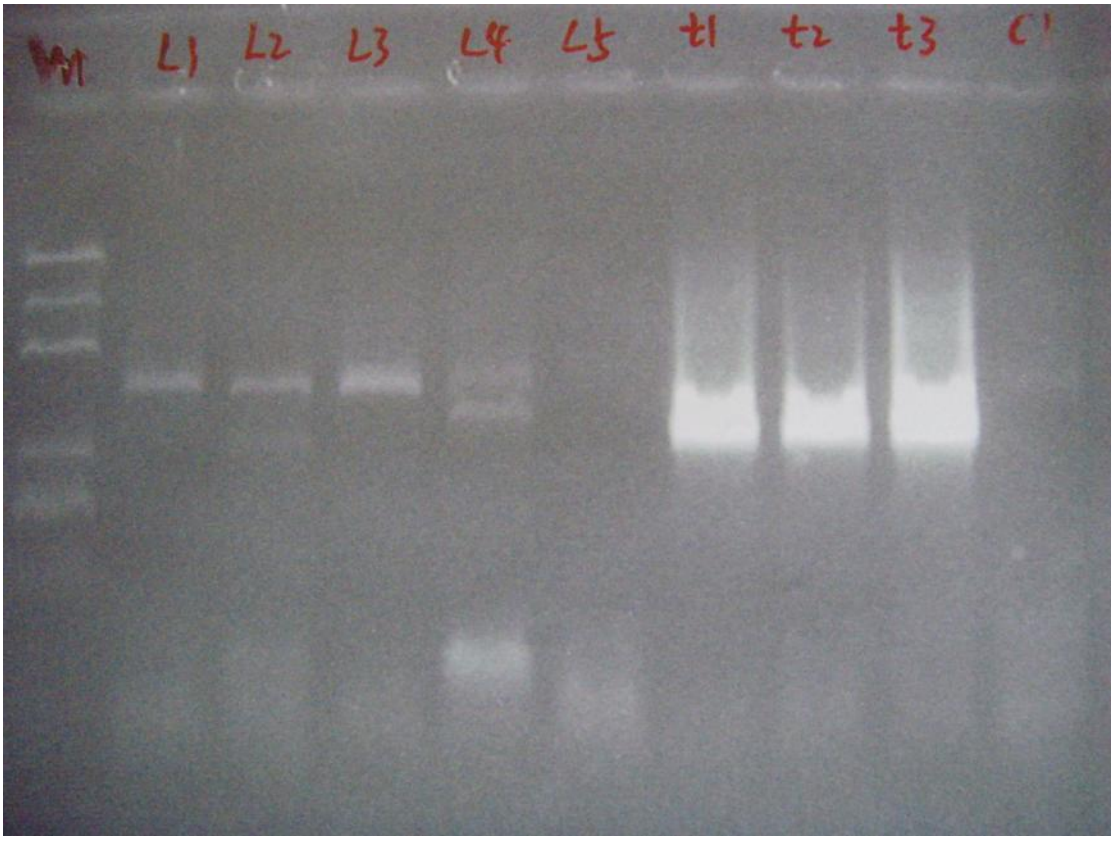
Transformation:
2 Products of ligation (L1, L2), competent cells 50uL each,
Smear to LB plate with Amp

Transformation (again): helped by Siheng He
2 Products of ligation (T1, T2), competent cells 50uL each,
Smear to LB plate with Amp

July 23th
Result of transformation (T1, T2 again) and L1 L2:
Only a few colonies on each plate, fewer than 5!

PCR: (colony PCR)
System 10 uL: Master mix 5ul, primer (standard primer) 0.5uL each, water: 4uL template;

Gel electrophoresis: Helped by Siheng He
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: Marker;
Lane2~6: colonies of L1/L2+lacP;
Lane7~10: colonies of T1/T2+tetP;

Result:
I successfully constructed the clone: Reversed Pcat-2m-tetR-term-tetP;
But failed to construct the clone: Reversed Pcat-2m-lacI-term-lacP

Work transfer:
The remanding work "Reversed Pcat-2 m-tetR-term-tetP + GFP" and failed work "Reversed Pcat-2 m-lacI-term-lacP" transferred to **He Siheng** and **Lin Min.**

By Shuke Wu

Confirm a clone

Reversed Pcat-2 m-tetR-term-tetP-GFP
Resource:
Plasmid: Reversed Pcat-2 m-tetR-term-tetP-GFP, from Lin Min *2 renamed as G2, G5

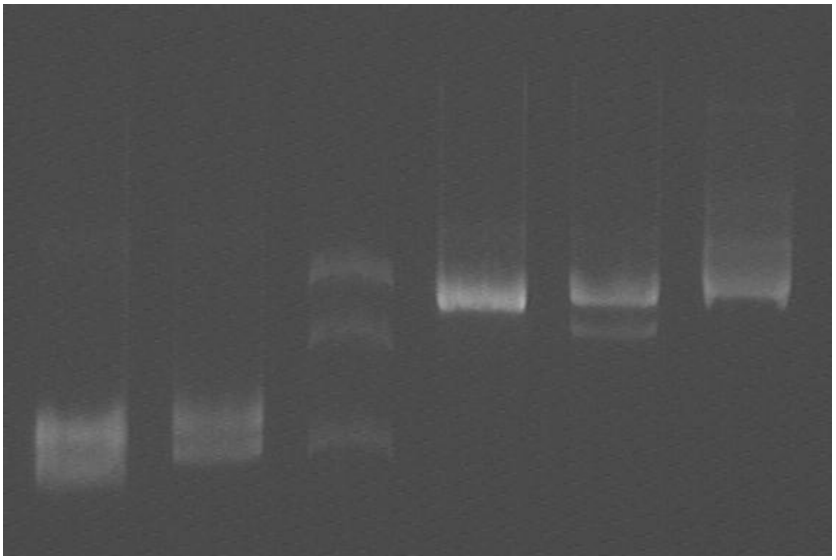
July30 th

Digest:

Double: G2, G5: EcoR1 1uL, Pst1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
Single: G2, G5: EcoR1 1uL, plasmid 4uL, Buffer 2uL, water 12uL
37 ℃ 4 hour

Gel electrophoresis:

Products of digest of G2, G5
marker: 100bp 250bp 500bp 750bp 1kb 2kb 3kb 5kb
loading buffer and DNA dye: 6×
voltage and time: 60V 5min; 120V 45min
lane1: Double digested product of G2;
lane2: Double digested product of G5;
lane3: marker;
lane4: Single digested product of G2;
lane5: Single digested product of G5;
lane6: plasmid of G2



Result:

The insert Reversed Pcat-2 m-tetR-term-tetP-GFP should be 1.8kb, the backbone is pSB1A2, 2.1kb. So, both G2 and G5 are correct!!!

BY Shuke Wu