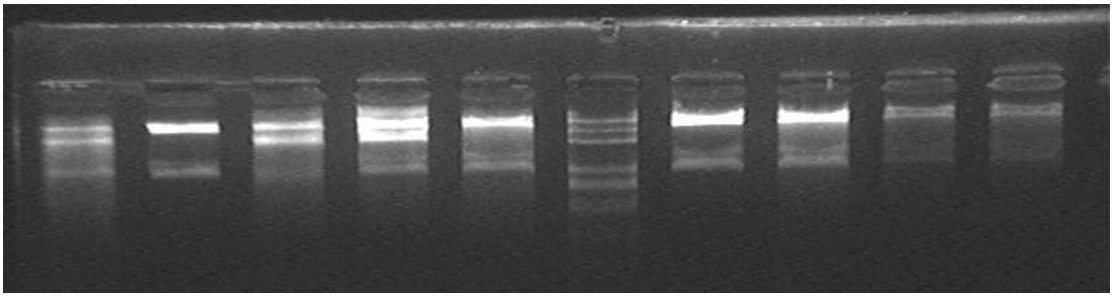
2009 09 02

Enzyme digestion of the T7 promoter-9xrbs – CI –terminator with EcoRI and SpeI.

GEL:

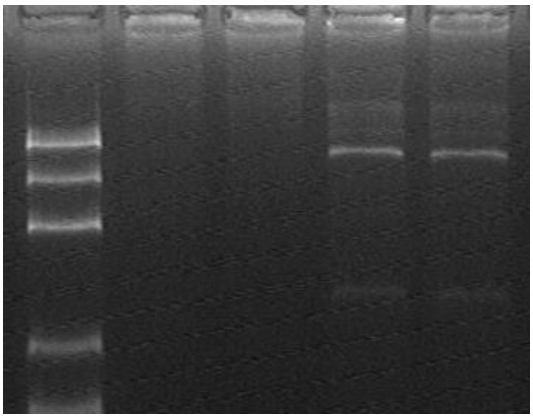


Cut GEL and purify the insert.

MIniprep 1-7G, 1-7E and Bistable plasmid.

Digest the 1-7G and 1-7E with EcoRI and PstI.

GEL:



Purify the backbone.

Ligation:

9 x T7promoter-CI insert (ES)

pSB1A2 vector(ES)

Transformation.

0903

PCR T3polymerase

Phusion 0.5ul

Primer F: 1.25ul

Primer R: 1.25ul

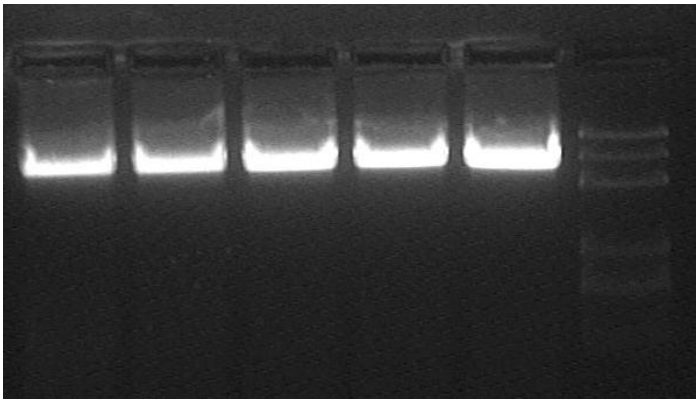
Template: 1ul

HF Buffer 10ul

ddH2O: 32ul

dNTP: 4ul

PCR a gradient 58, 60, 62, 65, 67



GEL Purification.

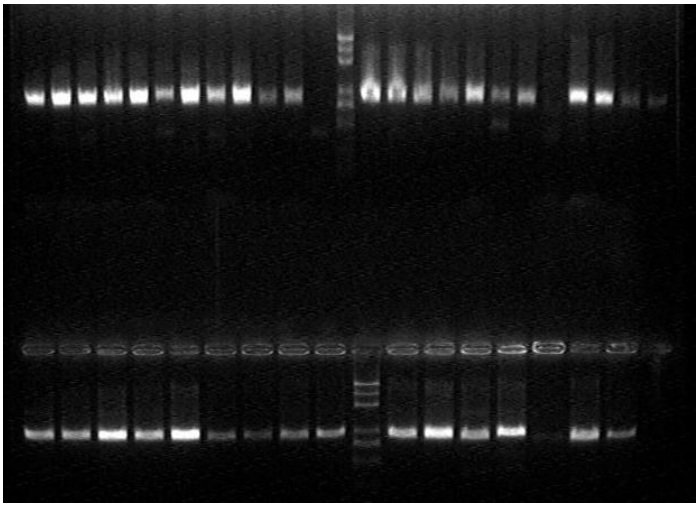
Enzyme Digestion

PCR product 10ul
EcoRI 1ul
PstI 1ul
10xH Buffer 2ul
ddH2O 6ul

0904
Purify the product of enzyme digestion.

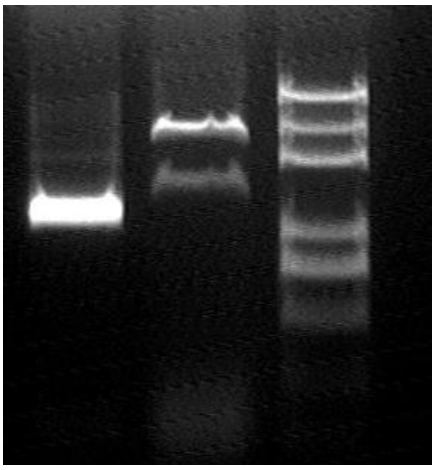
Ligation:
pSB1K3 backbone
T3 polymerase

Pick colonies of the T7p-CI → 1-1M.
PCR assessment:

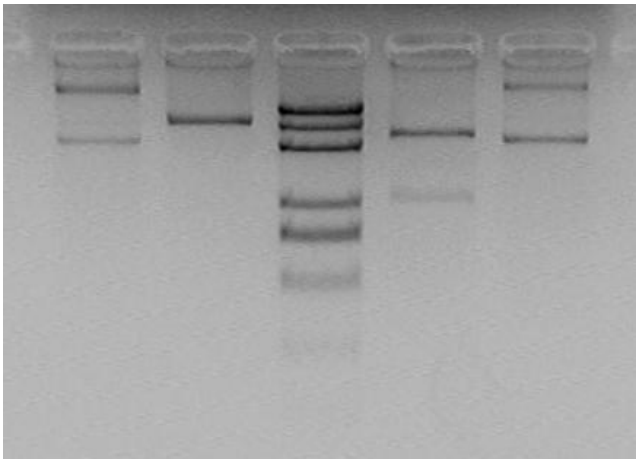


Shake each one of the correct colonies in the incubator.

0905
Miniprep 6 of the T3polymerase colonies.
5 of them are red.
Enzyme Digestion assessment of the remaining one.
At the same time PCR with the Sequencing primer for assessment.

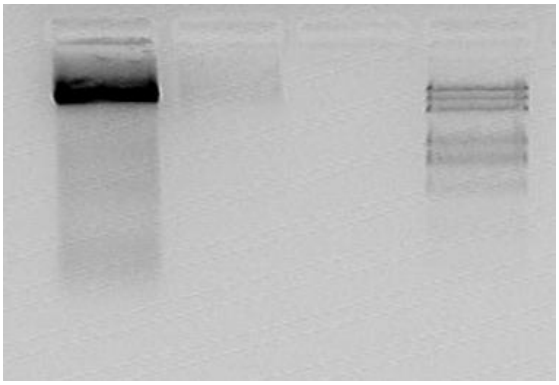


Enzyme Digestion again to confirm:
XhoI, NotI, (XbaI, SpeI), (XbaI, SpeI, HindIII) for assessment



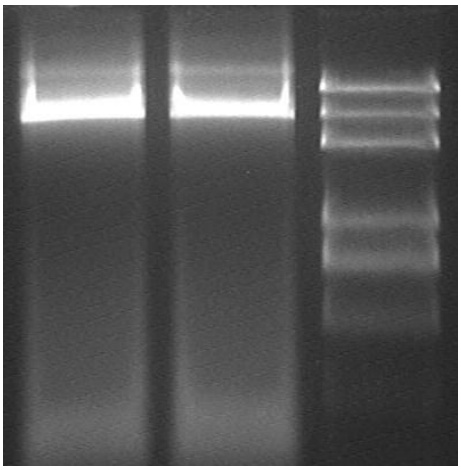
It is not a correct colony.

0906
PCR again the 3 counter plasmid. Same protocol.
At the same time use the PCR product of last time to do nested PCR with Standard primer.



No result for the nested PCR.

Again PCR 3 counter plasmid

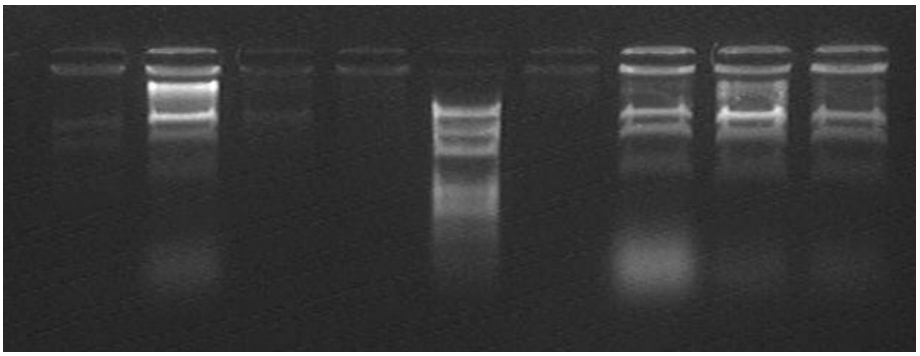


Purification;
Enzyme Digestion one the PCR products with EcoRI and SpeI.

Ligation:
T3 polymerase PCR product into pEASY-BLUNT.
T3 polymerase PCR product after digestion into pSB1A2(ES).

Transformation.

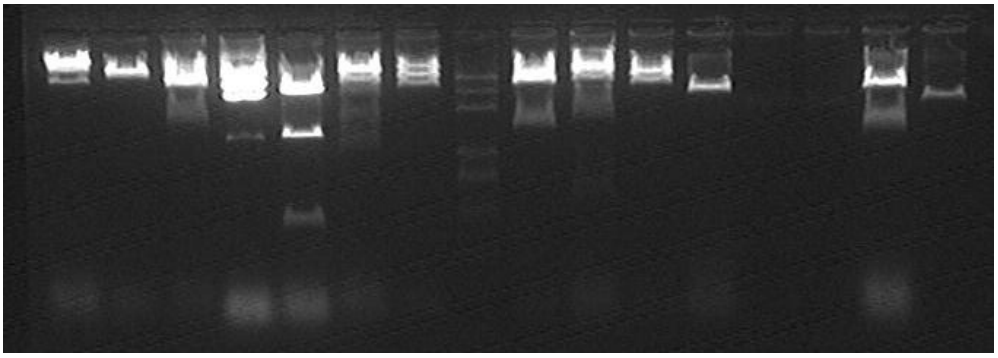
Enzyme Digestion of the T7p-CI into 1-1M for Enzyme digestion assessment:



Looks like no one is correct in size.

0908
Pick 10 colonies from the pEASY-BLUNT plate for assessment.
Pick 5 colonies from the pSB1A2 plate.

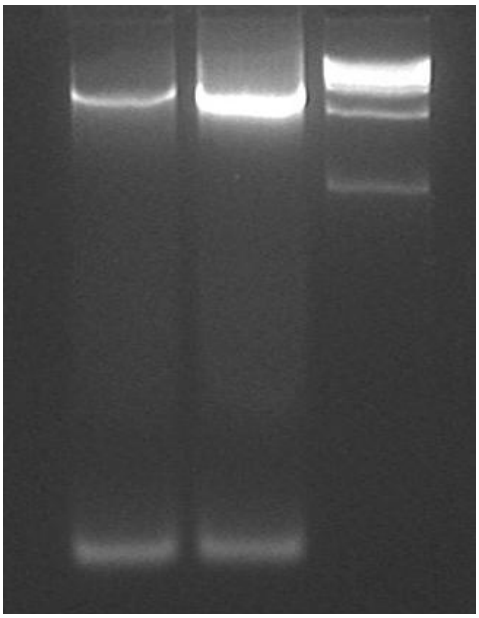
Miniprep
Enzyme Digestion with EcoRI and PstI



None is correct!!!

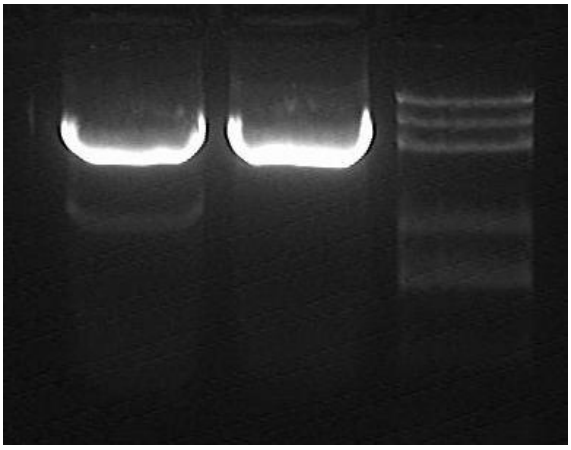
0909
Enzyme Digestion of the 3 Counter plasmid with EcoRI and NheI, Gel purification of the correct insert that contains the T3 pol.

PCR using the purified insert as a template.
PCR 2 tubes with Phusion, one without DMSO the other with DMSO.

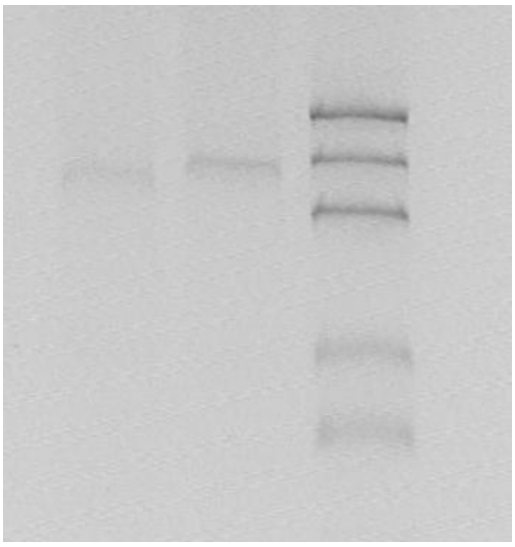


Gel purification of the two bands.

PCR again using the Standard primer and product of the first cycle of PCR as template.
GEL:



It seems that the size is not correct.
Purification and use some to confirm the size.
It turns out that the size is actually correct.

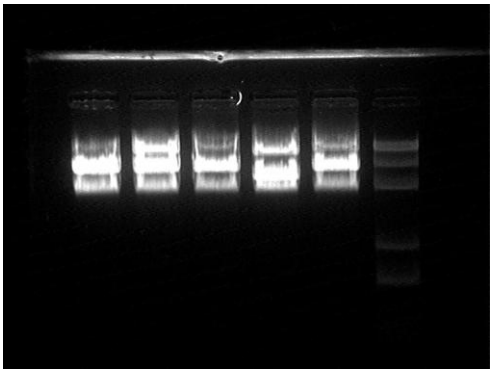


The product is digested with by XbaI and PstI directly.

Ligation:
T3 pol(XP) into pSB1A2

Transformation.

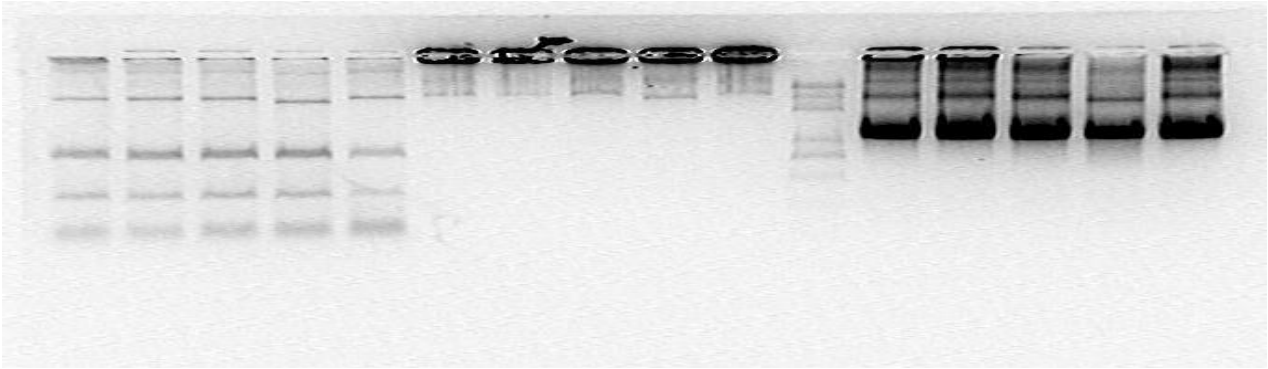
0911
Pick 10 colonies of the T3pol clone. No band
Mniprep 5 plasmids.
Digest with EcoRI and PstI for assessment



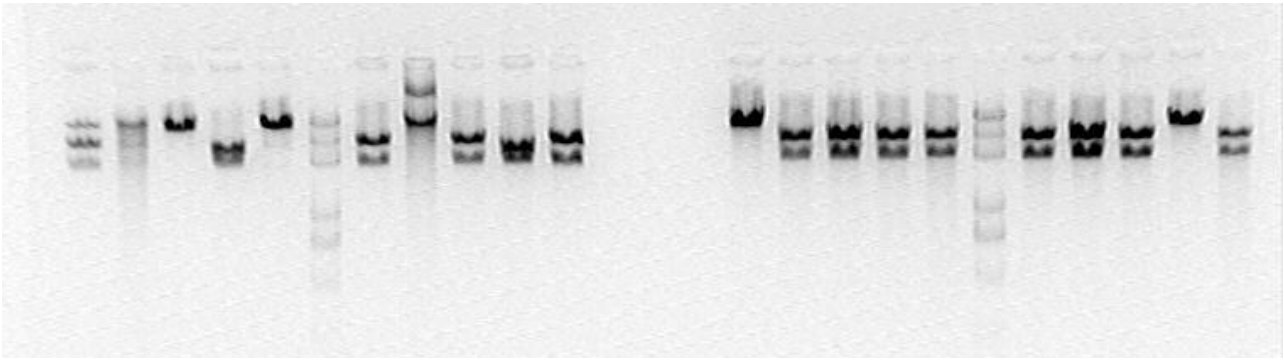
It seems right for NO. 1,2,3,5.

Digest with:
(ExoRI and PstI)
(EcoRI and SpeI)
(XbaI and PstI)
(XbaI and SpeI)
PCR with:
Sequencing primer
Standarlize primer
Universal Primer

For assessment
PCR



Enzyme Digestion



All phenomenon shows that this time it is correct.

Send for sequence, However, the forward primer has no signal, and the reverse primer shows more than one binding site. It is weird.