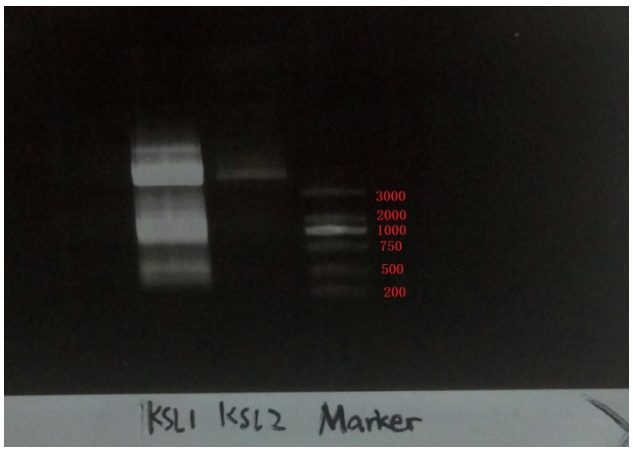
Sep 7th

-PCR Amplification of SmKSL1

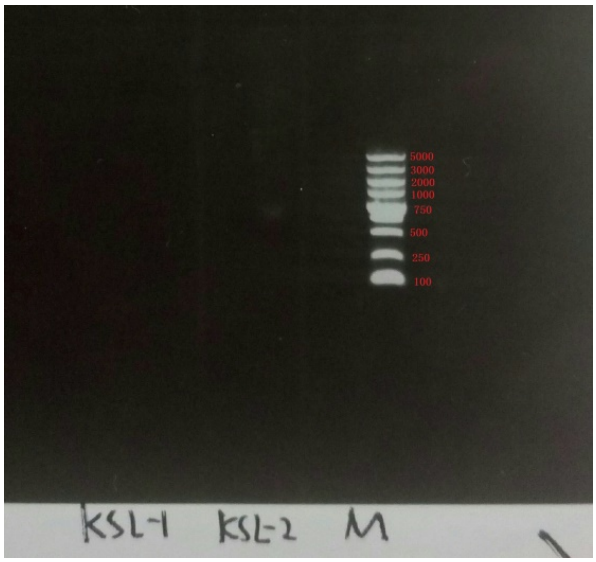
[Volume] (50 μL):
PrimerStar: 25 μL
KSL1-Forward: 1.5 μL
KSL1-Reverse: 1.5 μL
Template (KSL1-32b, 10X): 1
μL ddH2O: 21 μL

[Program] (3 steps 34cycles):
98℃2’
98℃10’’
60℃5’’
72℃1’40’’
72℃5’

-Results:



(failed)



(failed)

Sep 8th

-PCR Amplification of SmKSL1
Program changed to:
98℃2’
98℃10’’
64℃5’’
72℃2’
72℃5’
34 cycles
-Result:

Sep 9th

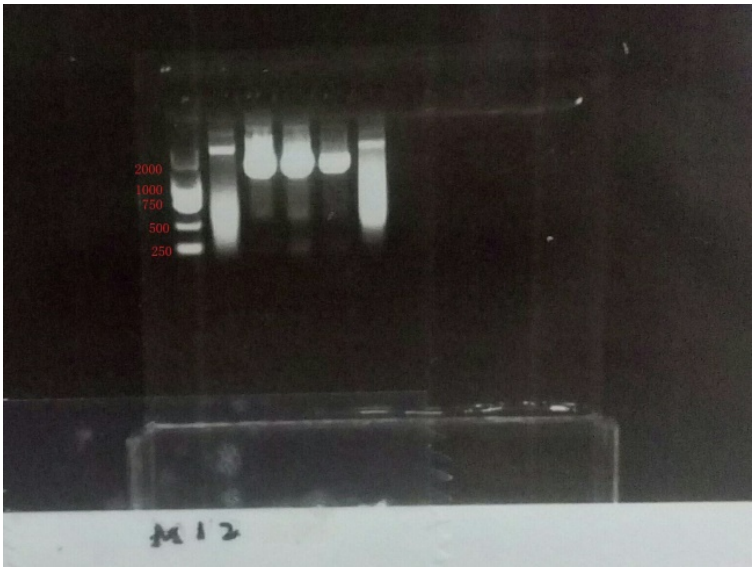
-Connect SmKSL1 to t-vector
-Transformation and spread plate method

Sep 10th

-Two single colonies are selected for TA Cloning Test

Sep 11th

-TA Cloning Test (Blunt-ended t-vector: pEASY- BluntZero Cloning Vector
[Volume](20 μL):
Mix 10 μL
F 0.5 μL
R 0.5 μL
Template 0.5 μL
ddH2O 8.5 μL
-Result: (1-failed, 2-successful)

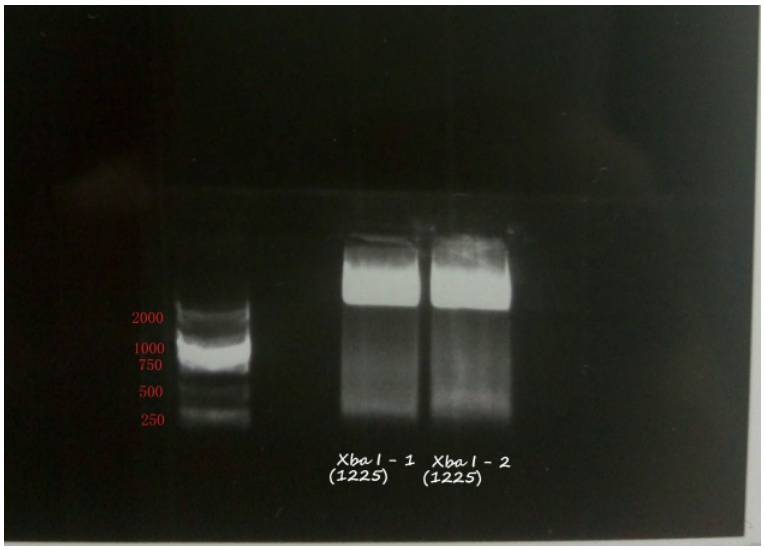


-Mutagenesis of Xba I (1225)
PCR [Volume] (50 μL):
5xPrimeSTAR GXL Buffer 10 μL
dNTP Mix (2.5mm each) 4 μL
Primer1 1.5 μL
Primer2 1.5 μL
Template 0.5 μL
PrimeSTAR GXL DNA Polymerase 1 μL
ddH2O 31.5 μL

[Program] (30cycles, 3-step):
98℃10”
62℃15”

68℃6min (1min/kb)

-PCR Result:



- Enzymatic digestion(enzyme: Takara 1235S Dpn I)
[Volume](20 μL):
Dpn I 1 μL
10x T Buffer 2 μL
DNA 17 μL

Sep 12th

-Transformation & Spread Plate Method

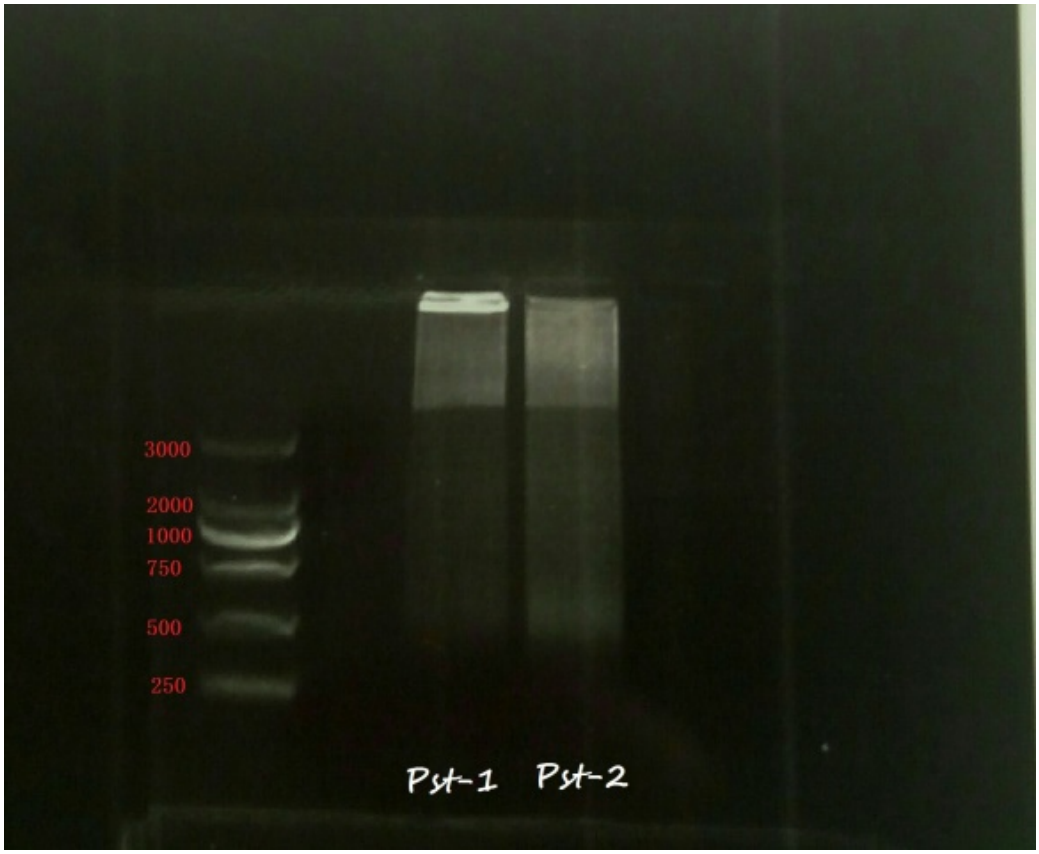
Sep 13th

-Ten single colonies are selected for gene sequencing to check if the mutagenesis is managed

Sep 14th

-Sequencing Result: No.4 & 7 successful

-Mutagenesis of Pst (1316)
PCR Result(unsuccessful)



Sep 15th

-Mutagenesis PCR of EcoR I (1688), Pst I (1316) and Xba I (322)
PCR Result: (Xbal (322) successful)

-Digestion, transformation, and spread plate method

Sep 16th

- Ten single colonies are selected for gene sequencing to check if the mutagenesis is managed

Sep 17th

-Sequencing results show that all colonies failed