Gene Engineering Division
RIKEN BioResource Center

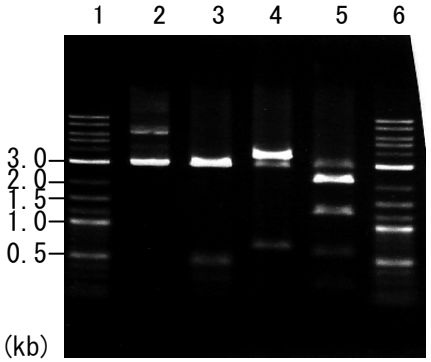
Plasmid name: pBSII_Tr-egl1 (RDB No. 8133)

Alternative name: pBSII_Tr003
Backbone plasmid: pBluescript II SK (+)
Selection: ampicillin
Plasmid size: 4347 bp
CDS size: 1380 bp

1. Restriction enzyme digestion

Working #: BM0063

reaction volume: 10 ul
DNA: 300 ng of plasmid DNA
restriction enzyme: 10 to 15 units



Lane
1: Size marker
2: uncut plasmid DNA
3: plasmid DNA with *Sa*/I digestion (2961, 483, 438, 294 and 171 bp)
4: plasmid DNA with *Xho*I digestion (3661 and 686 bp)
5: plasmid DNA with *Bg*/I digestion (2238, 1267, 595 and 247 bp)
6: Size marker

Electrophoresis: 1% agarose gel, 1x TAE buffer
Size marker: 2-Log DNA Ladder (250 ng/lane; Cat.# N3200L, NEB)

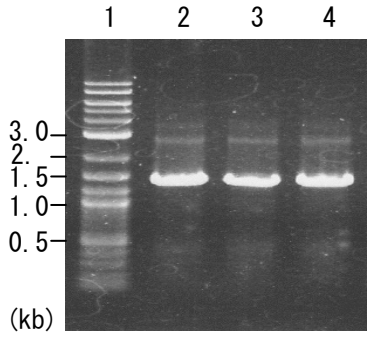
2. PCR Amplification of inserted CDS

Working #: BM0118

PCR conditons
Template: 1 ng of plasmid DNA
Enzyme: KOD Plus (Cat.# KOD-201, Toyobo)
Volume: 25 ul

Reaction program:
95°C, 5 min.
95°C, 30 sec.
64, 66 or 68°C, 30 sec. } x 30 cycles
68°C, 2 min.
68°C, 5 min.

PCR primer set
·Tr003-Fa1:
ccccctcgaggtcgac**ATGGCGCCCTCAGTTACAC**
·Tr003-R1fixed:
tatcgataccgtcgac**CTAAAGGCATTGCGAGTAGTAGTCG**



PCR product size of target (bp): 1412

Lane
1: Size marker
2: annealing Temp.: 64°C
3: annealing Temp.: 66°C
4: annealing Temp.: 68°C

Electrophoresis: 1% agarose gel, 1x TAE buffer
Loading DNA amount: 2 ul of PCR product per lane
Size marker: 2-Log DNA Ladder (250 ng/lane)