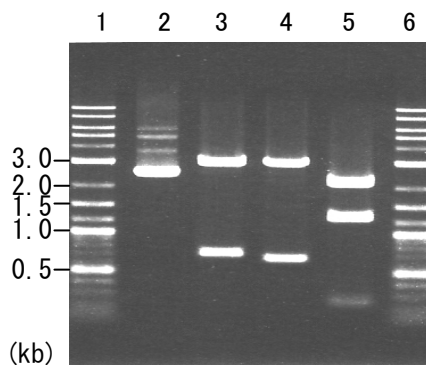


Plasmid name: pBSII\_Tr-egl3 (RDB No. 8135)  
Alternative name: pBSII\_Tr005  
Backbone plasmid: pBluescript II SK (+)  
Selection: ampicillin  
Plasmid size: 3672 bp  
CDS size: 705 bp

## 1. Restriction enzyme digestion

Working #: BM0071

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 and 711 bp)  
4: plasmid DNA with *Kpn*/I digestion  
(3006 and 666 bp)  
5: plasmid DNA with *Bg*/II digestion  
(2155, 1267 and 250 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 #: BM0111

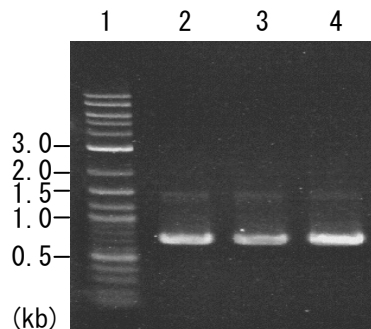
PCR conditons

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. } x 30 cycles  
62, 64 or 66°C, 30 sec. }  
68°C, 1 min }  
68°C, 5 min.

PCR primer set

·Tr005-Fa1:  
ccccctcgaggtcgacATGAAGTTCCTTCAAGTCCTCC  
·Tr005-R1:  
tatcgataccgtcgacTTAGTTGATAGATGCGGTCCAG



PCR product size of target (bp): 737

Lane

1: Size marker  
2: annealing Temp. : 62°C  
3: annealing Temp. : 64°C  
4: annealing Temp. : 66°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)