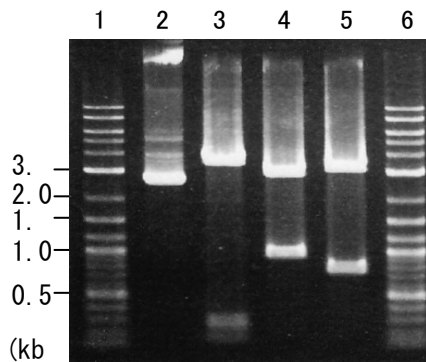


Plasmid name: pBSII\_PF0854 (RDB No. 8945)  
Alternative name: pBSII\_PF0854  
Backbone plasmid: pBluescript II SK (+)  
Selection: ampicillin  
Plasmid size: 3927 bp  
CDS size: 960 bp

## 1. Restriction enzyme digestion

Working #: BM0147

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 *Hind*III digestion  
(3502, 265 and 160 bp)  
4: plasmid DNA with *Sa*I digestion  
(2961 and 966 bp)  
5: plasmid DNA with *Eco*RI digestion  
(3165 and 762 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

Working #: BM0195

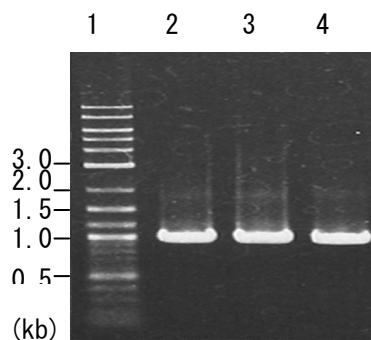
PCR conditons

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.  
61, 63 or 65°C, 30 sec. } x 30 cycles  
68°C, 1 min  
68°C, 5 min.

PCR primer set

·PF0854-F1:  
ccccctcgaggctcgacATGAGCAAGAAAAAGTTCGTCA  
·PF0854-R1:  
tatcgataccgtcgacTTAGGAAATAAGAGGTCTATCTAGAGGAGTT



PCR product size of target (bp): 992

Lane

1: Size marker  
2: annealing Temp. : 61°C  
3: annealing Temp. : 63°C  
4: annealing Temp. : 65°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)