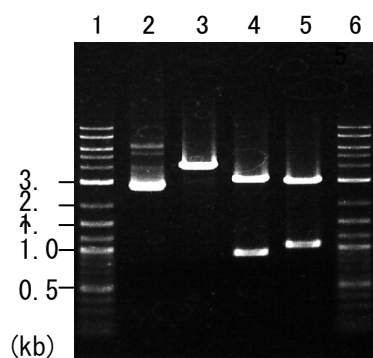


Plasmid name: pBSII_PF1861 (RDB No. 8947)
Alternative name: pBSII_PF1861
Backbone plasmid: pBluescript II SK (+)
Selection: ampicillin
Plasmid size: 4008 bp
CDS size: 1041 bp

1. Restriction enzyme digestion

Working #: BM0148

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 *EcoRI* digestion
(4008 bp)
4: plasmid DNA with *HindIII* digestion
(3078 and 930 bp)
5: plasmid DNA with *SaI* digestion
(2961 and 1047 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

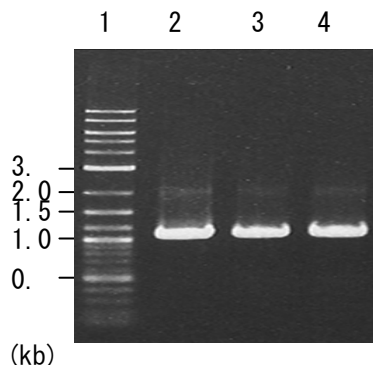
PCR conditons

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

PCR primer set

·PF1861-F1:
cccctcgaggctcgacATGGTAGATTGGGAAC TAATGAAA
·PF1861-R1:
tatcgataccgtcgacTCACGGTG TAAAGTCCATTG



PCR product size of target (bp): 1073

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)