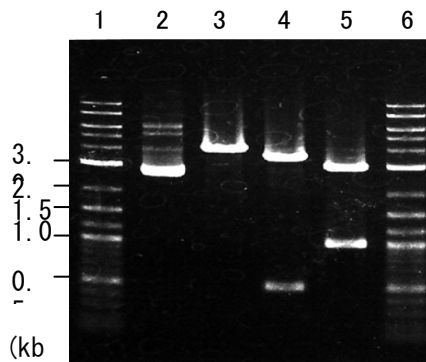


Plasmid name: pBSII_0369 (RDB No. 8949)
Alternative name: pBSII_PF0369
Backbone plasmid: pBluescript II SK (+)
Selection: ampicillin
Plasmid size: 3966 bp
CDS size: 999 bp

1. Restriction enzyme digestion

Working #: BM0175

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
(3966 bp)
- 4: plasmid DNA with *HindIII* digestion
(3461 and 505 bp)
- 5: plasmid DNA with *SmaI* digestion
(2961 and 1005 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

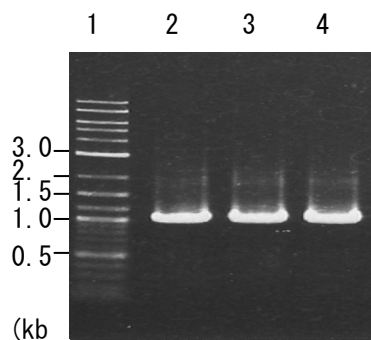
PCR conditons

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

PCR primer set

·PF0369-F1:
ccccctcgaggctcgacATGAAGCTAATTGAAATGCTAAAGG
·PF0369-R1:
tatcgataccgtcgacCTAGATCTCAAATGTTATTGCTTCTATTAAG



PCR product size of target (bp): 1031

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)