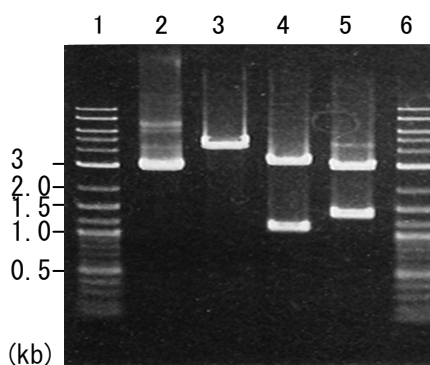


Plasmid name: pBSII_PH1171 (RDB No. 8948)
Alternative name: pBSII_PH1171
Backbone plasmid: pBluescript II SK (+)
Selection: ampicillin
Plasmid size: 4344 bp
CDS size: 1377 bp

1. Restriction enzyme digestion

Working #: BM0164

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
(4344 bp)
4: plasmid DNA with *HindIII* digestion
(3233 and 1111 bp)
5: plasmid DNA with *SmaI* digestion
(2961 and 1383 bp)
6: Size marker

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

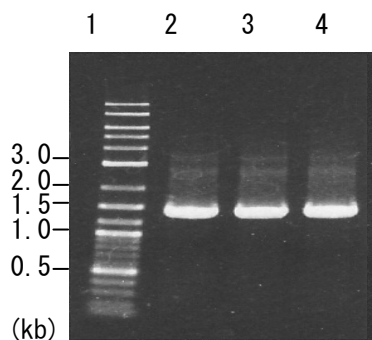
2. PCR Amplification

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.
62, 64 or 66°C, 30 sec. } x 30 cycles
68°C, 90 sec.
68°C, 5 min.

PCR primer set
·PH1171-F1:
ccccctcgaggtcgacATGGAGGGGAATACTATTCTTAAAATC
·PH1171-R1:
tatcgataccgtcgacCTACCTGGGAGCCCTTCTTA



PCR product size of target (bp): 1409

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)