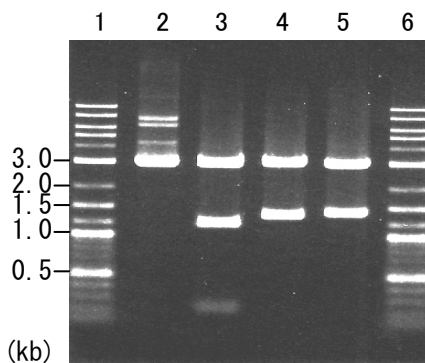


Plasmid name: pBSII_Tr-cbh2 (RDB No. 8132)
Alternative name: pBSII_Tr002
Backbone plasmid: pBluescript II SK (+)
Selection: ampicillin
Plasmid size: 4383 bp
CDS size: 1416 bp

1. Restriction enzyme digestion

Working #: BM0073

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, 1192 and 230 bp)
4: plasmid DNA with *Hind*III digestion
(3025 and 1358 bp)
5: plasmid DNA with *Xba*I digestion
(2969 and 1414 bp)
6: Size marker

Electrophoresis: 1% agarose gel, 1x TAE buffer
Size marker: 2-Log DNA Ladder (200 ng/lane; Cat. # N3200L, NEB)

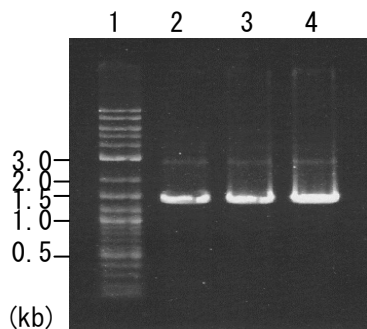
2. PCR Amplification of inserted CDS

Working #: BM0118

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, 2 min. }
68°C, 5 min. }

PCR primer set
·Tr002-F1:
ccccctcgaggctcgacATGATTGTGGCATTCTCA
·Tr002-Rd1:
tatcgataccgtcgacTTACAGGAACGATGGGTTTG



PCR product size of target (bp): 1448

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)