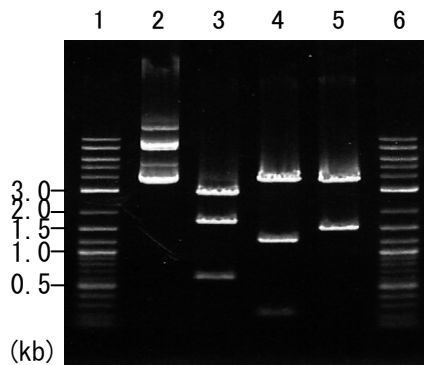


Plasmid name: pBSII_Tr-bx11 (RDB No. 8140)
Alternative name: pBSII_Tr012
Backbone plasmid: pBluescript II SK (+)
Selection: ampicillin
Plasmid size: 5361 bp
CDS size: 2394 bp

1. Restriction enzyme digestion

Working #: BM0048

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, 1755 and 645 bp)
4: plasmid DNA with *Pst*I digestion
(3806 and 1554 bp)
5: plasmid DNA with *Bg*II digestion
(3853, 1267 and 241 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 of inserted CDS

Working #: BM0119

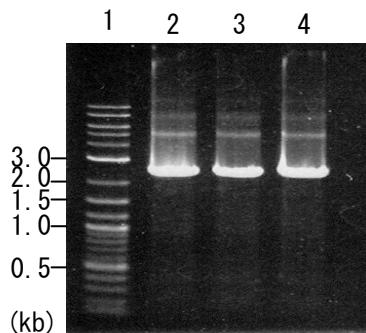
PCR conditons

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
60, 62 or 64°C, 30 sec. }
68°C, 3 min. }
68°C, 5 min. }

PCR primer set

·Tr012-F1:
ccccctcgaggctcgacATGGTGAATAACGCAGCTCTT
·Tr012-R1:
tatcgataccgtcgacTTATGCGTCAGGTGTAGCATC



PCR product size of target (bp): 2426

Lane

1: Size marker
2: annealing Temp. : 60°C
3: annealing Temp. : 62°C
4: annealing Temp. : 64°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)