Gene Engineering Division
RIKEN BioResource Center

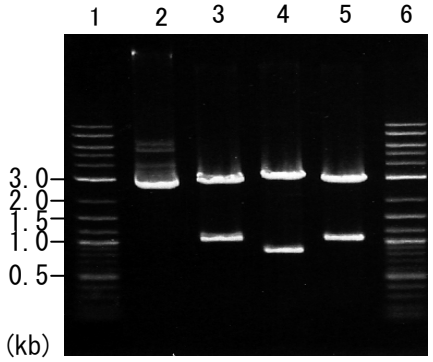
Plasmid name: pBSII_Tr-xyn3 (RDB No. 8139)

Alternative name: pBSII_Tr011
Backbone plasmid: pBluescript II SK (+)
Selection: ampicillin
Plasmid size: 4011 bp
CDS size: 1044 bp

1. Restriction enzyme digestion

Working #: BM0074

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*l digestion (2961 and 1050 bp)
4: plasmid DNA with *Kpn*I digestion (3169 and 842 bp)
5: plasmid DNA with *Sac*I digestion (2964 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)

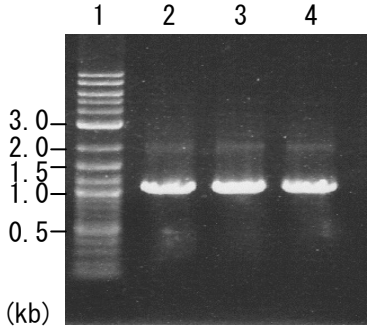
2. PCR Amplification of inserted CDS

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.
64, 66 or 68°C, 30 sec. } x 30 cycles
68°C, 2 min.
68°C, 5 min.

PCR primer set
·Tr011-Fa1:
cccctcgaggtcgac**ATGAAAGCAAACGTCATCTTGTG**
·Tr011-R1:
tatcgataccgtcgac**CTATTGTAAGATGCCAACAATGCTG**



PCR product size of target (bp): 1076

Lane
1: Size marker
2: annealing Temp.: 64°C
3: annealing Temp.: 66°C
4: annealing Temp.: 68°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)