

Microbial Genomic DNA

Strain: JCM 9667T
Organism: *Clavibacter michiganensis* subsp. *Sepedonicus*

RIKEN DNA Bank catalog #

JGD 12233

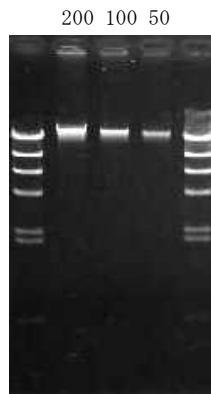
Lot # : 12233-A3I9

Final concentration	50	ng/ul
Shipping amount	20	ul

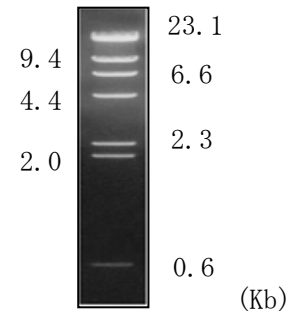
1. Electrophoresis of Genomic DNA

Date : 2013/12/3

Electrophoresis : 1% agarose gel, 1x TAE buffer
Loading DNA amount : 200, 100 and 50 ng/lane
Size marker : Lambda/HindIII (200 ng/lane)



Marker
Lambda/HindIII
(NEB#N3012S)



2. PCR Amplification

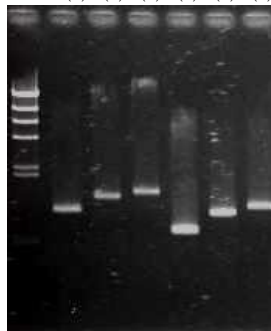
Date : 2013/12/3

Conditions of PCR ribosomal RNA (rRNA) gene

Template : 50 fold diluted DNA, 1 ul
Enzyme : KOD Plus (Toyobo)
Volume : 20 ul

Reaction program: 94 degC 2 min
x30 cycle $\left\{ \begin{array}{l} 94 \text{ degC } 15 \text{ sec} \\ 60 \text{ degC } 30 \text{ sec} \\ 68 \text{ degC } 60 \text{ sec} \end{array} \right.$
68 degC 2 min

(1) (2) (3) (4) (5) (6)



PCR primers

16S rRNA Eubacteria set	
EB-20F	5' AGTTTGATCCTGGCTC 3'
EB-350F	5' TACGGGAGGCAGCAG 3'
EB-1100R	5' AGGTTGCGCTCGTTG 3'
EB-1400R	5' ACGGGCGGTGTGTAC 3'
EB-1530R	5' AAGGAGGTGATCCAGCC 3'

Sample Number

	EB-20F	EB-350F
EB-1100R	(1)	(4)
EB-1400R	(2)	(5)
EB-1530R	(3)	(6)

Electrophoresis : 1% agarose gel, 1x TAE buffer
Loading DNA amount : 5 ul PCR product/lane
Size marker : Lambda/HindIII (200 ng/lane)

3. Nucleotide sequence of rRNA gene

 Confirmed date : 2013/12/4