

**Microbial Genomic DNA**

Strain: JCM 20277

Organism: *Gluconobacter frateurii* M-2

RIKEN DNA Bank catalog #

JGD 08235

Lot # : 110811

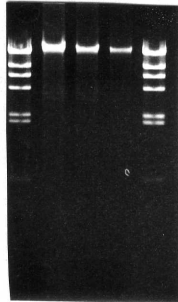
Final concentration : 100 ng/ul

Shipping amount : 100 ul

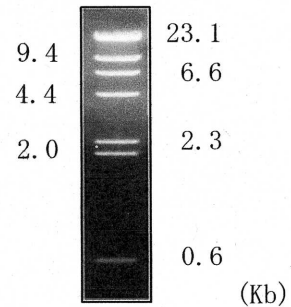
## 1. Electrophoresis of Genomic DNA

Date : 2011/8/12

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 : 2011/8/16

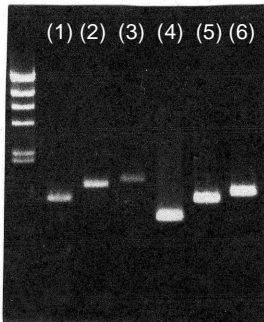
Conditions of PCR (16S ribosomal RNA genes)

Template : 10 fold diluted DNA, 1 ul  
 Enzyme : KOD Plus (Toyobo)  
 Volume : 20 ul

Reaction program: 94 degC 2 min  
 x30 cycle ( 94 degC 15 sec )  
 ( 60 degC 30 sec )  
 ( 68 degC 60 sec )  
 68 degC 2 min

PCR primers

Eubacteria set	
EB-20F	5' AGTTTGATCCTGGCTC 3'
EB-350F	5' TACGGGAGGCAGCAG 3'
EB-1100R	5' AGGGTTGCGCTCGTTG 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 16S rRNA gene

Confirmed date : 2011/8/19