

Microbial Genomic DNA

Strain : **JCM 21484^T**
 Organism : ***Paracoccus denitrificans***

RIKEN DNA Bank catalog #
 JGD : **12662**
 Lot # : **12662_A7Kf**

1. Shipping

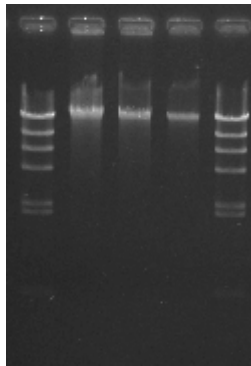
Volume : **40** μ L

2. Concentration of Genomic DNA

Concentration (OD 260) : **45** ng/ μ L
 A260 / A280 : **1.8**

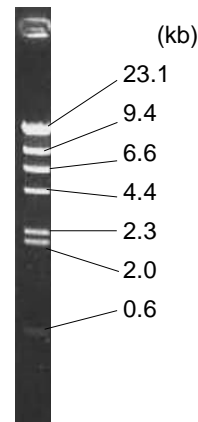
3. Electrophoresis of Genomic DNA

Electrophoresis : 1% agarose gel, 1x TAE buffer
 Loading DNA amount : **10, 5, 2.5** μ L/lane
 size marker : Lambda / HindIII (200 ng/Lane)
10, 5, 2.5 (μ L)



Date : **2017/11/16**

Marker (Lambda / HindIII, NEB#3012S)

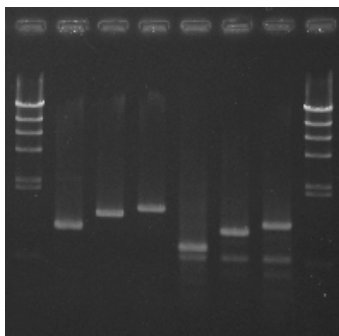


4. PCR Amplification

Conditions of PCR Ribosomal RNA (rRNA) gene
 Template : 100 fold diluted DNA, 1 μ L
 Enzyme : KOD plus (TOYOBO)
 Volume : 20 μ L

Reaction program :
 x 30 cycle $\left[\begin{array}{ll} 94 \text{ degC} & 2 \text{ min} \\ 94 \text{ degC} & 15 \text{ sec} \\ 60 \text{ degC} & 30 \text{ sec} \\ 68 \text{ degC} & 60 \text{ sec} \\ 68 \text{ degC} & 2 \text{ min} \end{array} \right]$

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



Date : **2017/11/16**

PCR primers for 16S rRNA

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 : 3 μ L PCR product/Lane
 Size marker : Lambda / HindIII (200ng/Lane)

5. Nucleotide sequence of rRNA gene

Confirmed Date : **2017/11/17**