

Microbial Genomic DNA

Strain: JCM 10941T
Organism: *Thermus thermophilus* HB8

RIKEN DNA Bank catalog #

JGD 05989

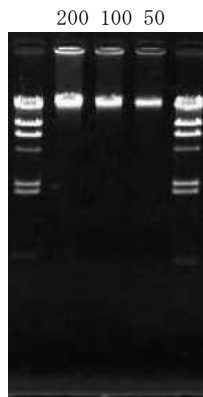
Lot # : 07914-A0Hk

Final concentration	100	ng/ul
Shipping amount	50	ul

1. Electrophoresis of Genomic DNA

Date : 2010/8/19

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)

9.4	23.1
4.4	6.6
2.0	2.3
	0.6

(Kb)

2. PCR Amplification

Date : 2010/8/20

Conditions of PCR ribosomal RNA (rRNA) gene

Template : 100 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 : 2010/8/30

2-2. Conditions of PCR (pTT27 plasmid, TTHB127 gene)

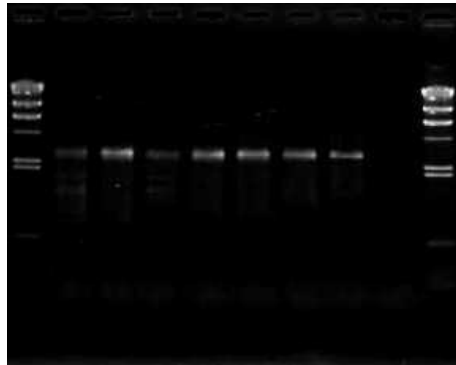
Date: 2010/8/20

Template : 10-10000 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 3 min }
 { 68 degC 2 min }

TTHB127 set	
HB8F	5' GCGATCCCAGCTCTGGTACGCCCGA 3'
HB8R	5' GCGAATTCCTTAGTCAAACCTGTTCTGCT 3'

M (1) (2) (3) (4) (5) (6) (7) (8) M



Sample Number	
Number	fold
(1)	10
(2)	40
(3)	100
(4)	400
(5)	1000
(6)	4000
(7)	10000
(8)	No template

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