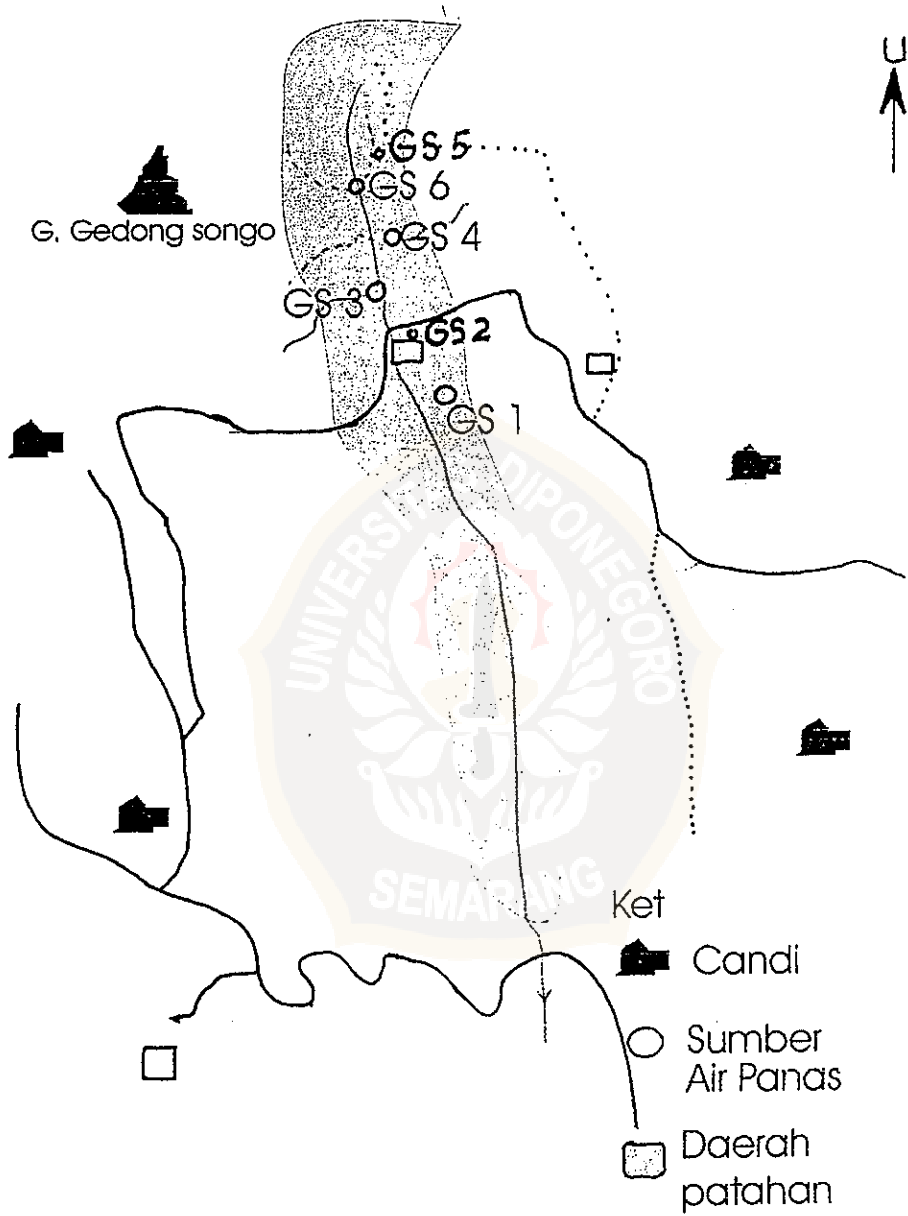


Lampiran A. Denah Lokasi Sumber Air Panas Gedong Songo

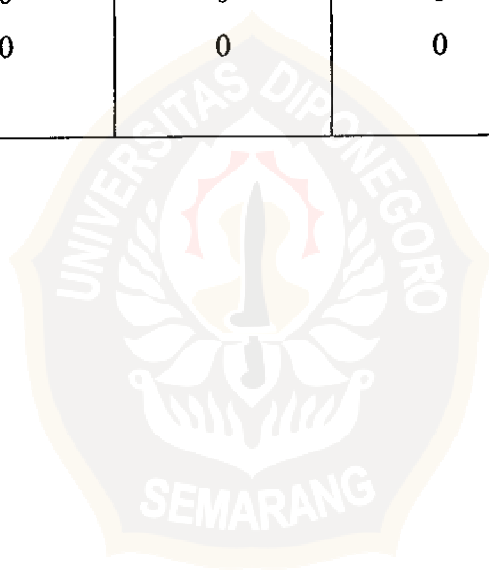


Lampiran B. Data Fisik Sumber-Sumber Air Panas Gedong Songo (GS)

Nama Sumber	Suhu (°C)	pH	Keterangan Lain
GS I	54	5	- Air jernih, bau belerang, warna tanah: abu-abu tua, ketinggian: 1390±15 m, ada tanaman.
GS II	74	2	- Warna air abu-abu (campur lumpur), bau belerang, warna tanah: abu-abu tua, ketinggian: 1390±15 m.
GS III	86	5	- Warna air abu-abu tua (campur lumpur), bau belerang, warna tanah: abu-abu tua, ketinggian: 1390±15 m.
GS IV	74	6	- Air jernih, bau belerang, warna tanah: abu-abu, ketinggian: 1390±15 m, ada tanaman lumut warna warni.
GS V	100	1	- Air jernih, bau belerang, warna tanah: hijau, ketinggian: 1390±15 m, ada tanaman lumut warna-warni.
GS VI	56	6	- Air jernih, bau belerang, warna tanah: kuning, ketinggian: 1390±15 m, ada tanaman.

Lampiran C. Absorbansi Kekeruhan Pertumbuhan Bakteri

Temperatur inkubasi (°C)	Absorbansi (Å)			Rerata (Å)
	A ₁	A ₂	A ₃	
40	0,251	0,250	0,251	0,251
60	0,340	0,341	0,340	0,340
65	0,345	0,345	0,345	0,345
70	0,298	0,298	0,298	0,298
75	0,105	0,104	0,104	0,105
77	0	0	0	0
80	0	0	0	0



Lampiran D. Hasil Analisis Urutan Nukleotida Menggunakan Program Primer
Select-DNASTAR

Amplification summary: Primers from Untitled
2005 16:23

Upper Primer: P1 17-mer 5' ATGGCTGTCGTCAGCTC 3'		
Lower Primer: P3 15-mer 5' ACGGGCGGTGTGTAC 3'		
DNA 250 pM, Salt 50 mM	Upper Primer	Lower Primer
Primer Tm	44.3 °C	43.6 °C
Primer Overall Stability	-27.5 kc/m	-30.9 kc/m
Primer Location	1102..1118	1452..1438
Product Tm - Primer Tm	38.6 °C	
Primers Tm Difference	0.7 °C	
Optimal Annealing Temperature	55.7 °C	
Product Length	351 bp	
Product Tm (%GC Method)	82.2 °C	
Product GC Content	59.0%	
Product Tm at 6xSSC	103.8 °C	

Product Melting Temperature (%GC Method)

Salt			Formamide			
mM	xSSC	xSSPE	0%	10%	20%	50%
1	0.005	0.006	54.0	47.5	41.0	21.5
10	0.051	0.062	70.6	64.1	57.6	38.1
50	0.256	0.312	82.2	75.7	69.2	49.7
165	0.846	1.031	90.8	84.3	77.8	58.3
330	1.692	2.062	95.8	89.3	82.8	63.3
500	2.564	3.125	98.8	92.3	85.8	66.3
1000	5.128	6.250	103.8	97.3	90.8	71.3
195	1.000	1.219	+0.0	%formamide = Tm 103.8 °C		

Lampiran E. Hasil Validasi Urutan Nukleotida Menggunakan Program Seqman-DNASTAR

2005 16:43

Project: homologi he-P1.SQD Contig 'Homologi Sampel (he_P1)'

```

      1110      1120      1130      1140      1150
      |-----|-----|-----|-----|-----|
      GTCCCGCAACGAGCGCAaCCCTCGCCTCTAGTTGCCAGCACGAAGG
G.th C.SEQ(1>1561)→ gtccccgcaacgagcgcaaccctcgctctagttgccagcacgaagg
G.th P.SEQ(1>1454)→ gtccccgcaacgagcgcaaccctcgctctagttgccagcacgaagg
he_P1.SEQ(1>399) → ACGAGCGCA-CCCTCGCCTCTAGTTGCCAGCACGAAGG
  
```

```

      1160      1170      1180      1190      1200
      |-----|-----|-----|-----|-----|
      TGGGCACTCTAGAGGGACTGCCGGCGACAAGTCGGAGGAAGGTGGG
G.th C.SEQ(1>1561)→ tgggcactctagagggactgccggcgacaagtcggaggaaggtggg
G.th P.SEQ(1>1454)→ tgggcactctagagggactgccggcgacaagtcggaggaaggtggg
he_P1.SEQ(1>399) → TGGGCACTCTAGAGGGACTGCCGGCGACAAGTCGGAGGAAGGTGGG
  
```

```

      1210      1220      1230      1240
      |-----|-----|-----|-----|-----|
      GATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGT
G.th C.SEQ(1>1561)→ gatgacgtcaaatcatcatgcccttatgacctgggctacacacgt
G.th P.SEQ(1>1454)→ gatgacgtcaaatcatcatgcccttatgacctgggctacacacgt
he_P1.SEQ(1>399) → GATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGT
  
```

```

      1250      1260      1270      1280      1290
      |-----|-----|-----|-----|-----|
      GCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCG
G.th C.SEQ(1>1561)→ gctacaatgggcggtacaaagggctgcgaaaccgagggggagcg
G.th P.SEQ(1>1454)→ gctacaatgggcggtacaaagggctgcgaaaccgagggggagcg
he_P1.SEQ(1>399) → GCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCG
  
```

```

      1300      1310      1320      1330
      |-----|-----|-----|-----|-----|
      AATCCCAAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGC
G.th C.SEQ(1>1561)→ aatcccaaaaagccgctctcagttcggattgcaggctgcaactcgc
G.th P.SEQ(1>1454)→ aatcccaaaaagccgctctcagttcggattgcaggctgcaactcgc
he_P1.SEQ(1>399) → AATCCCAAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGC
  
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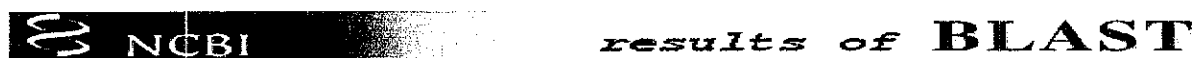
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      1340      1350      1360      1370      1380
      |-----|-----|-----|-----|-----|
      CTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCG
G.th C.SEQ(1>1561)→ ctgcatgaagccggaatcgctagtaatcgcggatcagcatgccgcg
G.th P.SEQ(1>1454)→ ctgcatgaagccggaatcgctagtaatcgcggatcagcatgccgcg
he_P1.SEQ(1>399) → CTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCG
  
```

```

      1390      1400      1410      1420      1430
      |-----|-----|-----|-----|-----|
      GTGAATACGTTCCCGGGCCTTGTTACACACCGCCCGTACACCACGA
G.th C.SEQ(1>1561)→ gtgaatacgttcccgggccttgttacacaccgcccgtcacaccacga
G.th P.SEQ(1>1454)→ gtgaatacgttcccgggccttgttacacaccgcccgtcacaccacga
he_P1.SEQ(1>399) → GTGAATACGTTCCCGGGCCTTGTTACACACCGCCCG
  
```

Lampiran F. Hasil Analisis Homologi (BLAST) GenBank



BLASTN 2.2.12 [Aug-07-2005]

Reference:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402.

RID: 1132093546-18948-147291670881.BLASTQ4

Database: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, environmental samples or phase 0, 1 or 2 HTGS sequences)
3,571,302 sequences; 15,947,463,778 total letters

Query= (400 letters)

Score E

Sequences producing significant alignments:	(Bits)	Value
<u>gi 22654307 gb AY044055.1 </u> Geobacillus lituanicus 16S ribosom...	<u>561</u>	<u>6e-157</u>
<u>gi 18535664 gb AY074879.1 </u> Geobacillus thermoleovorans 16S ri...	<u>561</u>	<u>6e-157</u>
<u>gi 47590298 gb AY603072.1 </u> Geobacillus sp. STB3 16S ribosomal RN	<u>561</u>	<u>6e-157</u>
<u>gi 47590297 gb AY603071.1 </u> Geobacillus sp. STB2 16S ribosomal RN	<u>561</u>	<u>6e-157</u>
<u>gi 47590296 gb AY603070.1 </u> Geobacillus sp. STB1 16S ribosomal RN	<u>561</u>	<u>6e-157</u>
<u>gi 45183557 gb AY550103.1 </u> Geobacillus thermoleovorans isolat...	<u>561</u>	<u>6e-157</u>
<u>gi 56291763 emb AJ704828.1 </u> Geobacillus thermoleovorans subsp...	<u>561</u>	<u>6e-157</u>
<u>gi 72537295 gb DQ143870.1 </u> Bacillus sp. E26312 16S ribosomal RNA	<u>561</u>	<u>6e-157</u>
<u>gi 72537294 gb DQ143869.1 </u> Bacillus sp. E26311 16S ribosomal RNA	<u>561</u>	<u>6e-157</u>
<u>gi 31414371 emb AJ564620.1 GTH564620</u> Geobacillus thermoleovorans	<u>561</u>	<u>6e-157</u>
<u>gi 31414365 emb AJ564614.1 GTH564614</u> Geobacillus thermoleovorans	<u>561</u>	<u>6e-157</u>
<u>gi 31414363 emb AJ564612.1 GTH564612</u> Geobacillus thermoleovorans	<u>561</u>	<u>6e-157</u>
<u>gi 71068493 gb DQ119658.1 </u> Geobacillus sp. LA3 16S ribosomal RNA	<u>561</u>	<u>6e-157</u>
<u>gi 32815087 gb AY323205.1 </u> Geobacillus bogazici 16S ribosomal RN	<u>561</u>	<u>6e-157</u>
<u>gi 6406842 dbj AB034902.1 </u> Geobacillus thermoleovorans gene for	<u>561</u>	<u>6e-157</u>
<u>gi 6425153 dbj AB034836.1 </u> Geobacillus thermoleovorans gene for	<u>561</u>	<u>6e-157</u>
<u>gi 18175428 gb AY062017.1 </u> Bacillus sp. G2 16S ribosomal RNA gen	<u>561</u>	<u>6e-157</u>
<u>gi 66735129 gb DQ055416.1 </u> Geobacillus sp. TibetanG6 16S ribo...	<u>561</u>	<u>6e-157</u>
<u>gi 15004573 gb AF391973.1 AF391973</u> Thermal soil bacterium YNP...	<u>561</u>	<u>6e-157</u>
<u>gi 14485237 gb AF385083.1 AF385083</u> Bacillus thermoleovorans 1...	<u>561</u>	<u>6e-157</u>
<u>gi 56378377 dbj BA000043.1 </u> Geobacillus kaustophilus HTA426 DNA,	<u>561</u>	<u>6e-157</u>
<u>gi 49482191 gb AY608946.1 </u> Bacillus sp. BGSC W9A90 16S riboso...	<u>561</u>	<u>6e-157</u>
<u>gi 49482190 gb AY608945.1 </u> Geobacillus lituanicus strain BGSC...	<u>561</u>	<u>6e-157</u>
<u>gi 49482188 gb AY608943.1 </u> Bacillus sp. BGSC W9A60 16S riboso...	<u>561</u>	<u>6e-157</u>
<u>gi 49482187 gb AY608942.1 </u> Geobacillus stearothermophilus str...	<u>561</u>	<u>6e-157</u>

gi 49482185 gb AY608940.1 	Bacillus vulcani strain BGSC 97A1 ...	<u>561</u>	6e-157
gi 49482184 gb AY608939.1 	Geobacillus thermoleovorans strain...	<u>561</u>	6e-157
gi 49482183 gb AY608938.1 	Bacillus caldovelox strain BGSC 96...	<u>561</u>	6e-157
gi 49482182 gb AY608937.1 	Bacillus caldotenax strain BGSC 96...	<u>561</u>	6e-157
gi 49482181 gb AY608936.1 	Geobacillus thermoleovorans strain...	<u>561</u>	6e-157
gi 49482179 gb AY608934.1 	Geobacillus kaustophilus strain BG...	<u>561</u>	6e-157
gi 62530447 gb AY986796.1 	Bacillus sp. WPD616 16S ribosomal RNA	<u>561</u>	6e-157
gi 511095 emb Z26925.1 BC16SRRND	B.caldovelox gene for 16S ribo	<u>561</u>	6e-157
gi 511109 emb Z26923.1 BT16SRRNF	B.thermoleovorans gene for 16S	<u>561</u>	6e-157
gi 511093 emb Z26922.1 BC16SRRNE	B.caldotenax gene for 16S ribo	<u>561</u>	6e-157
gi 21615541 emb AJ489330.1 GTH489330	Geobacillus thermoleovorans	<u>561</u>	6e-157
gi 21615540 emb AJ489329.1 GTH489329	Geobacillus thermoleovorans	<u>561</u>	6e-157
gi 21615539 emb AJ489328.1 GTH489328	Geobacillus thermoleovorans	<u>561</u>	6e-157
gi 23495579 dbj AB089224.1 	Bacillus sp. G62 gene for 16S riboso	<u>561</u>	6e-157
gi 23495568 dbj AB089213.1 	Bacillus sp. B7 gene for 16S ribosom	<u>561</u>	6e-157
gi 23495567 dbj AB089212.1 	Bacillus sp. B6 gene for 16S ribosom	<u>561</u>	6e-157
gi 23495566 dbj AB089211.1 	Bacillus sp. B5 gene for 16S ribosom	<u>561</u>	6e-157
gi 23495564 dbj AB089209.1 	Bacillus sp. B3 gene for 16S ribosom	<u>561</u>	6e-157
gi 23495563 dbj AB089208.1 	Bacillus sp. B2 gene for 16S ribosom	<u>561</u>	6e-157
gi 23495562 dbj AB089207.1 	Bacillus sp. B1 gene for 16S ribosom	<u>561</u>	6e-157
gi 61676049 gb AY952967.1 	Bacillus caldotenax strain CCSD 39...	<u>561</u>	6e-157
gi 39549 emb X60618.1 BKAU16S	B.kaustophilus 16S ribosomal RNA	<u>561</u>	6e-157
gi 25052800 gb AY166603.1 	Geobacillus sp. T1 16S ribosomal RNA	<u>561</u>	6e-157
gi 27764186 emb AJ536599.1 BTH536599	Bacillus thermoleovorans 16	<u>561</u>	6e-157
gi 1209243 gb M77485.1 BACRRSSB	Bacillus caldovelox (DSM 411) ri	<u>561</u>	6e-157
gi 23495565 dbj AB089210.1 	Bacillus sp. B4 gene for 16S ribosom	<u>557</u>	9e-156
gi 49482186 gb AY608941.1 	Geobacillus stearothermophilus str...	<u>555</u>	3e-155
gi 511094 emb Z26924.1 BC16SRRNC	B.caldolyticus gene for 16S ri	<u>555</u>	3e-155
gi 46371097 gb AY583457.1 	Bacillus sp. W13 16S ribosomal RNA ge	<u>553</u>	1e-154
gi 11877135 emb AJ293805.1 BVU293805	Bacillus vulcani 16S rRNA g	<u>553</u>	1e-154
gi 1209246 gb M77488.1 BACRRSSE	Bacillus thermoleovorans (ATC...	<u>551</u>	5e-154
gi 2337751 dbj AB002645.1 	Unidentified low G+C gram-positive...	<u>549</u>	2e-153
gi 1209242 gb M77484.1 BACRRSSA	Bacillus caldolyticus (DSM 405)	<u>549</u>	2e-153
gi 37694431 gb AY397768.1 	Geobacillus sp. R-7160 16S ribosomal	<u>545</u>	3e-152
gi 28395524 gb AY193888.1 	Geobacillus gargensis 16S ribosomal R	<u>537</u>	8e-150
gi 45183565 gb AY550104.1 	Geobacillus thermocatenulatus isol...	<u>537</u>	8e-150
gi 45183549 gb AY550102.1 	Geobacillus thermoleovorans isolat...	<u>537</u>	8e-150
gi 49482180 gb AY608935.1 	Geobacillus thermocatenulatus stra...	<u>537</u>	8e-150
gi 511110 emb Z26926.1 BT16SRRNG	B.thermocatenulatus gene for 1	<u>537</u>	8e-150
gi 30026029 gb AY248717.1 	Geobacillus thermoleovorans strain...	<u>535</u>	3e-149
gi 49482192 gb AY608947.1 	Bacillus sp. BGSC W9A93 16S riboso...	<u>533</u>	1e-148
gi 7637528 gb AF228764.1 AF228764	Bacillus sp. Papandayan 16S...	<u>533</u>	1e-148
gi 47590299 gb AY603073.1 	Geobacillus sp. STB4 16S ribosomal RN	<u>531</u>	5e-148
gi 45386026 gb AY551908.1 	Geobacillus stearothermophilus str...	<u>529</u>	2e-147
gi 45386025 gb AY551907.1 	Bacillus sp. B31 16S ribosomal RNA ge	<u>529</u>	2e-147
gi 21686514 gb AF517644.1 	Bacillus sp. PS3D 16S ribosomal RNA g	<u>529</u>	2e-147
gi 49482189 gb AY608944.1 	Bacillus sp. BGSC W9A88 16S riboso...	<u>529</u>	2e-147
gi 46578141 gb AY297092.2 	Geobacillus stearothermophilus str...	<u>529</u>	2e-147

Lampiran G. Perbedaan Urutan Nukleotida Sampel Dengan *Geobacillus lituanicus*

gi|22654307|gb|AY044055.1| *Geobacillus lituanicus* 16S ribosomal RNA gene,
complete sequence
Length=1523

Score = 561 bits (283), Expect = 6e-157
Identities = 299/303 (98%), Gaps = 1/303 (0%)
Strand=Plus/Plus

Sampl	19	ACGAGCGCA-CCCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACTCTAGAGGGACTGCC	77
Bltns	1111	ACGAGCGCA-CCCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACTCTAGAGGGACTGCC	1170
Sampl	78	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGG	137
Bltns	1171	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGG	1230
Sampl	138	CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCCAACC CGAGGGGGAGCGAATC	197
Bltns	1231	CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCCAACC CGAGGGGGAGCGAATC	1290
Sampl	198	CCAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	257
Bltns	1291	CCAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	1350
Sampl	258	GCTAGTAATCGCGGATCATCCTGCCGCGGCGAATACGTTCCCGGGCCTTGTAACACCCGC	317
Bltns	1351	GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTAACACCCGC	1410
Sampl	318	CCG 320	
Bltns	1411	CCG 1413	

Lampiran H. Perbedaan Urutan Nukleotida Sampel Dengan *Geobacillus thermoleovorans* Parsial Sekuen

>gi|18535664|gb|AY074879.1| *Geobacillus thermoleovorans* 16S ribosomal RNA gene, partial sequence
Length=1454

Score = 561 bits (283), Expect = 6e-157
Identities = 299/303 (98%), Gaps = 1/303 (0%)
Strand=Plus/Plus

Sampl	19	ACGAGCGCA-CCCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACTCTAGAGGGACTGCC	77
Gther	1074	ACGAGCGCAACCCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACTCTAGAGGGACTGCC	1133
Sampl	78	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGG	137
Gther	1134	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGG	1193
Sampl	138	CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCGAATC	197
Gther	1194	CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCGAATC	1253
Sampl	198	CCAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	257
Gther	1254	CCAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	1313
Sampl	258	GCTAGTAATCGCGGATCATCCTGCCGCGGCGAATACGTTCCCGGGCCTTGACACACCGC	317
Gther	1314	GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGACACACCGC	1373
Sampl	318	CCG 320	
Gther	1374	CCG 1376	

Lampiran I. Perbedaan Urutan Nukleotida Sampel Dengan *Geobacillus thermoleovorans* Komplit Sekuen

gi|49482184|gb|AY608939.1| *Geobacillus thermoleovorans* strain BGSC 96A6 16S
 ribosomal RNA
 gene, complete sequence
 Length=1561

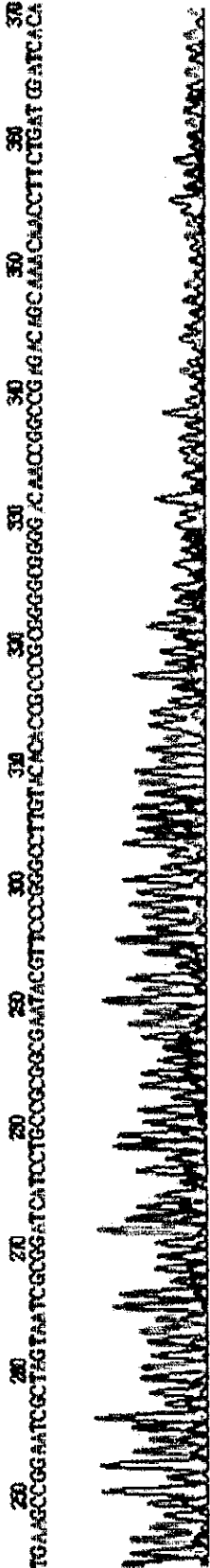
Score = 561 bits (283), Expect = 6e-157
 Identities = 299/303 (98%), Gaps = 1/303 (0%)
 Strand=Plus/Plus

Sampl	19	ACGAGCGCA-CCCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACTCTAGAGGGACTGCC	77
Gth.C	1118	ACGAGCGCA-CCCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACTCTAGAGGGACTGCC	1177
Sampl	78	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGG	137
Gth.C	1178	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGG	1237
Sampl	138	CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCGAATC	197
Gth.C	1238	CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCGAATC	1297
Sampl	198	CCAAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	257
Gth.C	1298	CCAAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	1357
Sampl	258	GCTAGTAATCGCGGATCATCCTGCCGCGGCGAATACGTTCCCGGGCCTTGTACACACCGC	317
Gth.C	1358	GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGC	1417
Sampl	318	CCG 320	
Gth.C	1418	CCG 1420	

Lampiran J. Elektroforegram Hasil Sekuensing



File: 051107-02_K01_He-P1.ab1 Run Ended: 2005/11/17 21:21:55 Signal G:878 A:459 C:565 T:521
Sample: He_P1_Lane: 5 Base spacing: 14.03 352 bases in 10896 scans Page 1 of 1



Lampiran K. Preparasi Bahan

Bufer Tris-Cl pH 8

Sebanyak 121,1 g Tris-base dilarutkan kedalam 800 mL H₂O. Kemudian pH diatur dengan penambahan HCl pekat sampai pH 8. Setelah pH yang dibutuhkan tercapai, ditambahkan H₂O hingga volume 1 L. Larutan kemudian disterilisasi dengan diautoklav (Sambrook dan Russell, 2001).

Bufer Pospat pH 8

Sejumlah K₂HPO₄ 1 M ditambahkan dengan KH₂PO₄ 1 M sampai pH 8. Kemudian H₂O ditambahkan sehingga volume larutan menjadi 1L (Sambrook dan Russell, 2001).

0,5 M EDTA pH 8

Sebanyak 18,61 g Na₂EDTA.H₂O ditambahkan kedalam H₂O, kemudian diaduk dengan pengaduk magnet. Pengaturan pH 8 dilakukan dengan menambahkan pelet NaOH kemudian ditambahkan H₂O sehingga volume larutan menjadi 100 mL. Larutan kemudian disterilisasi dengan diautoklav (Sambrook dan Russell, 2001).

Pembuatan media cair ½ LB

Media cair dibuat dengan mencampurkan tripton, yeast ekstrak, NaCl (0,5%; 0,25%; 0,5%) dan air sumber. Kemudian larutan di sterilisasi selama 45 menit.

Pembuatan media ½ LB padat

Media padat dibuat dengan mencampurkan tripton, yeast ekstrak, NaCl dan bakto agar dengan komposisi masing-masing 0,5 %; 0,25 %; 0,5 % dan 3 %. Setelah tercampur, larutan di sterilisasi selama ± 45 menit, didiamkan beberapa saat. Dalam keadaan hangat larutan kemudian dituang ke cawan petri steril masing-masing sebanyak 20 mL secara aseptis. Cawan petri ditutup setelah uap air hilang dan dibiarkan memadat (Zharkov, 1998).

