

LAMPIRAN A**PREPARASI LARUTAN**

1. Pembuatan larutan tris-HCl 100 X, pH 8

Sebanyak 12,1 g tris HCl dilarutkan dalam 80 mL akuades dan pH diatur dengan penambahan 4,2 mL HCl pekat. Kemudian ditambahkan akuades hingga volume 100 mL. Setelah itu disterilisasi dengan autoklaf.

2. Bufer TAE (Tris Acetat EDTA) 50 X

Sebanyak 24,2 g Tris HCl ditambah dengan 5,71 mL asam asetat glasial dan 100 mL EDTA 0,5 M (pH 8).

3. Lisozim (10 mg/mL)

Lisozim dilarutkan dalam 10 mM tris-HCl (pH 8) hingga diperoleh konsentrasi akhir 10 mg/mL.

4. Bufer pencuci

Bufer pencuci dibuat dengan menambahkan 100 mM Tris-HCL pada 70 % etanol absolut.

5. SSC 20 X

Sebanyak 17,53 g natrium klorida ditambah dengan 8,82 g natrium sitrat kemudian dilarutkan dalam 80 mL akuades dan pH diatur menjadi 7 dengan penambahan 14 N HCl. Selanjutnya ditambahkan akuades hingga volume menjadi 100 mL. Setelah itu disterilisasi dengan autoklaf.

6. *Loading Buffer*

Sukrosa 40 % (w/v) ditambahkan dengan brom fenol blue 0,25 % (w/v).

7. Natrium asetat 3 M, pH 4,8

Sebanyak 40,8 g natrium asetat dilarutkan dalam 80 mL akuades. pH diatur dengan menambahkan asam asetat glasial, kemudian tambahkan akuades hingga volume 100 mL. Larutan disterilisasi dengan autoklaf.

9. Bufer fosfat

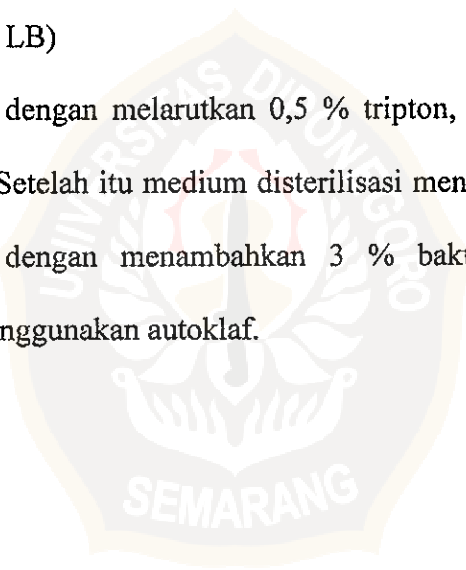
Larutan 0,1 M K_2HPO_4 ditambah larutan HCl hingga pH bufer mencapai 8.

10. Bufer proteinase-K 10 X

Larutan dibuat dengan mencampurkan 100 mM tris-Cl pH 8, 50 mM EDTA pH 8, dan 500 mM NaCl.

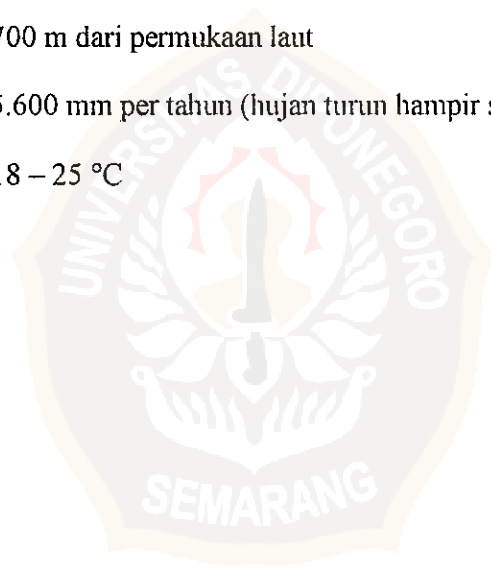
11. Pembuatan Media (1/2 LB)

Media cair dibuat dengan melarutkan 0,5 % tripton, 0,5 % NaCl, serta 0,25 % yeast ekstrak. Setelah itu medium disterilisasi menggunakan autoklaf. Media padat dibuat dengan menambahkan 3 % bakto agar kemudian disterilisasi dengan menggunakan autoklaf.



LAMPIRAN B**DATA FISIK SUMBER AIR PANAS BATURADEN**

Nama Sumber	: Pancuran 7, Baturaden, Purwokerto
Suhu	: 52 °C
PH	: 7
Keterangan lain	: Warna air jernih, batuan: kuning (endapan belerang), hijau (lumut), ada tanaman.
Letak	: 700 m dari permukaan laut
Curah hujan	: 5.600 mm per tahun (hujan turun hampir setiap hari)
Suhu udara	: 18 – 25 °C



LAMPIRAN C

**TABEL SUHU PERTUMBUHAN BAKTERI
DAN ABSORBANSINYA**

NO	SUHU PERTUMBUHAN (°C)	ABSRBANSI (A)
1	40	0,093
2	45	0,141
3	50	0,171
4	55	0,190
5	60	0,275
6	65	0,321
7	70	0,217
8	75	0,122
9	80	0,058

LAMPIRAN D

**HASIL PERBANDINGAN URUTAN NUKLEOTIDA
DENGAN METODE BLAST**

Sequences producing significant alignments:	Score (Bits)	E Value
gi 22654307 gb AY044055.1 Geobacillus lituanicus 16S ribosom...	561	3e-158
gi 18535664 gb AY074879.1 Geobacillus thermoleovorans 16S ri...	561	3e-158
gi 47590298 gb AY603072.1 Geobacillus sp. STB3 16S ribosomal RN	561	3e-158
gi 47590297 gb AY603071.1 Geobacillus sp. STB2 16S ribosomal RN	561	3e-158
gi 47590296 gb AY603070.1 Geobacillus sp. STB1 16S ribosomal RN	561	3e-158
gi 45183557 gb AY550103.1 Geobacillus thermoleovorans isolat...	561	3e-158
gi 56291763 emb AJ704828.1 Geobacillus thermoleovorans subsp...	561	3e-158
gi 31414371 emb AJ564620.1 GTH564620 Geobacillus thermoleovorans	561	3e-158
gi 31414365 emb AJ564614.1 GTH564614 Geobacillus thermoleovorans	561	3e-158
gi 31414363 emb AJ564612.1 GTH564612 Geobacillus thermoleovorans	561	3e-158
gi 72537295 gb DQ143870.1 Bacillus sp. E26312 16S ribosomal RNA	561	3e-158
gi 72537294 gb DQ143869.1 Bacillus sp. E26311 16S ribosomal RNA	561	3e-158
gi 71068493 gb DQ119658.1 Geobacillus sp. LA3 16S ribosomal RNA	561	3e-158
gi 32815087 gb AY323205.1 Geobacillus bogazici 16S ribosomal RN	561	3e-158
gi 6406842 dbj AB034902.1 Geobacillus thermoleovorans gene for	561	3e-158
gi 6425153 dbj AB034836.1 Geobacillus thermoleovorans gene for	561	3e-158
gi 18175428 gb AY062017.1 Bacillus sp. G2 16S ribosomal RNA gen	561	3e-158
gi 66735129 gb DQ055416.1 Geobacillus sp. TibetanG6 16S ribo...	561	3e-158
gi 15004573 gb AF391973.1 AF391973 Thermal soil bacterium YNP...	561	3e-158
gi 14485237 gb AF385083.1 AF385083 Bacillus thermoleovorans I...	561	3e-158
gi 56378377 dbj BA000043.1 Geobacillus kaustophilus HTA426 DNA,	561	3e-158
gi 49482191 gb AY608946.1 Bacillus sp. BGSC W9A90 16S riboso...	561	3e-158
gi 49482188 gb AY608943.1 Bacillus sp. BGSC W9A60 16S riboso...	561	3e-158
gi 49482187 gb AY608942.1 Geobacillus stearothermophilus str...	561	3e-158
gi 49482185 gb AY608940.1 Bacillus vulcani strain BGSC 97A1 ...	561	3e-158
gi 49482184 gb AY608939.1 Geobacillus thermoleovorans strain...	561	3e-158
gi 49482183 gb AY608938.1 Bacillus caldovelox strain BGSC 96...	561	3e-158
gi 49482182 gb AY608937.1 Bacillus caldotenax strain BGSC 96...	561	3e-158
gi 49482181 gb AY608936.1 Geobacillus thermoleovorans strain...	561	3e-158
gi 49482179 gb AY608934.1 Geobacillus kaustophilus strain BG...	561	3e-158
gi 62530447 gb AY986796.1 Bacillus sp. WPD616 16S ribosomal RNA	561	3e-158
gi 511095 emb Z26925.1 BC16SRRND B.caldovelox gene for 16S ribo	561	3e-158
gi 511109 emb Z26923.1 BT16SRRNF B.thermoleovorans gene for 16S	561	3e-158
gi 511093 emb Z26922.1 BC16SRRNB B.caldotenax gene for 16S ribo	561	3e-158
gi 21615541 emb AJ489330.1 GTH489330 Geobacillus thermoleovorans	561	3e-158
gi 21615540 emb AJ489329.1 GTH489329 Geobacillus thermoleovorans	561	3e-158
gi 21615539 emb AJ489328.1 GTH489328 Geobacillus thermoleovorans	561	3e-158
gi 61676049 gb AY952967.1 Bacillus caldotenax strain CCSD 39...	561	3e-158
gi 23495579 dbj AB089224.1 Bacillus sp. G62 gene for 16S riboso	561	3e-158
gi 23495568 dbj AB089213.1 Bacillus sp. B7 gene for 16S ribosom	561	3e-158

gi 23495567 dbj AB089212.1	Bacillus sp. B6 gene for 16S ribosom	<u>561</u>	3e-158
gi 23495566 dbj AB089211.1	Bacillus sp. B5 gene for 16S ribosom	<u>561</u>	3e-158
gi 23495564 dbj AB089209.1	Bacillus sp. B3 gene for 16S ribosom	<u>561</u>	3e-158
gi 23495563 dbj AB089208.1	Bacillus sp. B2 gene for 16S ribosom	<u>561</u>	3e-158
gi 23495562 dbj AB089207.1	Bacillus sp. B1 gene for 16S ribosom	<u>561</u>	3e-158
gi 39549 emb X60618.1 BKAU16S	B.kaustophilus 16S ribosomal RNA	<u>561</u>	3e-158
gi 25052800 gb AY166603.1	Geobacillus sp. T1 16S ribosomal RNA	<u>561</u>	3e-158
gi 27764186 emb AJ536599.1 BTH536599	Bacillus thermoleovorans 16	<u>561</u>	3e-158
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gi 30026029 gb AY248717.1	Geobacillus thermoleovorans strain...	<u>559</u>	1e-157
gi 23495565 dbj AB089210.1	Bacillus sp. B4 gene for 16S ribosom	<u>557</u>	5e-157
gi 49482186 gb AY608941.1	Geobacillus stearothermophilus str...	<u>555</u>	2e-156
gi 511094 emb Z26924.1 BC16SRRNC	B.caldolyticus gene for 16S ri	<u>555</u>	2e-156
gi 46371097 gb AY583457.1	Bacillus sp. W13 16S ribosomal RNA ge	<u>553</u>	8e-156
gi 49482190 gb AY608945.1	Geobacillus lituanicus strain BGSC...	<u>553</u>	8e-156
gi 11877135 emb AJ293805.1 BVU293805	Bacillus vulcani 16S rRNA g	<u>553</u>	8e-156
gi 1209246 gb M77488.1 BACRRSSE	Bacillus thermoleovorans (ATC...	<u>551</u>	3e-155
gi 2337751 dbj AB002645.1	Unidentified low G+C gram-positive...	<u>549</u>	1e-154
gi 1209242 gb M77484.1 BACRRSSA	Bacillus caldolyticus (DSM 405)	<u>549</u>	1e-154
gi 47590299 gb AY603073.1	Geobacillus sp. STB4 16S ribosomal RN	<u>545</u>	2e-153
gi 37694431 gb AY397768.1	Geobacillus sp. R-7160 16S ribosomal	<u>545</u>	2e-153
gi 28395524 gb AY193888.1	Geobacillus gargensis 16S ribosomal R	<u>537</u>	5e-151
gi 45183565 gb AY550104.1	Geobacillus thermocatenulatus isol...	<u>537</u>	5e-151
gi 45183549 gb AY550102.1	Geobacillus thermoleovorans isolat...	<u>537</u>	5e-151
gi 49482180 gb AY608935.1	Geobacillus thermocatenulatus stra...	<u>537</u>	5e-151
gi 511110 emb Z26926.1 BT16SRRNG	B.thermocatenulatus gene for 1	<u>537</u>	5e-151
gi 38492162 gb AY450926.1	Geobacillus thermoleovorans strain...	<u>535</u>	2e-150
gi 49482192 gb AY608947.1	Bacillus sp. BGSC W9A93 16S riboso...	<u>533</u>	7e-150
gi 7637528 gb AF228764.1 AF228764	Bacillus sp. Papandayan 16S...	<u>533</u>	7e-150
gi 45386026 gb AY551908.1	Geobacillus stearothermophilus str...	<u>529</u>	1e-148
gi 45386025 gb AY551907.1	Bacillus sp. B31 16S ribosomal RNA ge	<u>529</u>	1e-148
gi 21686514 gb AF517644.1	Bacillus sp. PS3D 16S ribosomal RNA g	<u>529</u>	1e-148
gi 49482189 gb AY608944.1	Bacillus sp. BGSC W9A88 16S riboso...	<u>529</u>	1e-148
gi 46578141 gb AY297092.2	Geobacillus stearothermophilus str...	<u>529</u>	1e-148
gi 23495595 dbj AB089240.1	Bacillus sp. D1 gene for 16S ribosom	<u>529</u>	1e-148
gi 23495594 dbj AB089239.1	Bacillus sp. G6 gene for 16S ribosom	<u>529</u>	1e-148
gi 23495593 dbj AB089238.1	Bacillus sp. VA2 gene for 16S riboso	<u>529</u>	1e-148
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gi 23495570 dbj AB089215.1	Bacillus sp. E2 gene for 16S ribosom	<u>529</u>	1e-148
gi 11231160 dbj AB051200.1	Bacillus sp. S gene for 16S rRNA	<u>529</u>	1e-148
gi 7637529 gb AF228765.1 AF228765	Bacillus sp. Cimanggu 16S ribo	<u>527</u>	5e-148
gi 49482174 gb AY608929.1	Geobacillus stearothermophilus str...	<u>525</u>	2e-147
gi 53645719 gb AY738089.1	Geobacillus stearothermophilus 16S...	<u>521</u>	3e-146
gi 51980855 gb AY632569.1	Geobacillus stearothermophilus 16S...	<u>521</u>	3e-146
gi 47590302 gb AY603076.1	Geobacillus sp. STB7 16S ribosomal RN	<u>521</u>	3e-146
gi 47590301 gb AY603075.1	Geobacillus sp. STB6 16S ribosomal RN	<u>521</u>	3e-146
gi 47590300 gb AY603074.1	Geobacillus sp. STB5 16S ribosomal RN	<u>521</u>	3e-146
gi 18496669 gb AF465646.1	Bacillus sp. YNPRH2P-2 16S ribosomal	<u>521</u>	3e-146
gi 40457246 gb AY491496.1	Geobacillus stearothermophilus 16S...	<u>521</u>	3e-146
gi 39988172 gb AY466708.1	Geobacillus sp. NS3-2 16S ribosomal R	<u>521</u>	3e-146

gi 71068497 gb DQ119662.1	Geobacillus sp. LA6 16S ribosomal RNA	<u>521</u>	3e-146
gi 71068495 gb DQ119660.1	Geobacillus sp. LA1 16S ribosomal RNA	<u>521</u>	3e-146
gi 22651495 gb AY079151.1	Geobacillus uralicus 16S ribosomal RN	<u>521</u>	3e-146
gi 22654306 gb AY044054.1	Geobacillus stearothermophilus str...	<u>521</u>	3e-146
gi 22654304 gb AY044053.1	Geobacillus stearothermophilus str...	<u>521</u>	3e-146
gi 15004575 gb AF391975.1 AF391975	Thermal soil bacterium YNP...	<u>521</u>	3e-146
gi 8575825 gb AF276304.1 AF276304	Geobacillus uzenensis strai...	<u>521</u>	3e-146
gi 10443141 emb AJ294817.1 BST294817	Bacillus stearothermophilus	<u>521</u>	3e-146
gi 50295605 gb AY682096.1	Geobacillus stearothermophilus str...	<u>521</u>	3e-146
gi 50295606 gb AY682097.1	Geobacillus stearothermophilus str...	<u>517</u>	4e-145



Perbandingan Urutan Nukleotida Bakteri Sampel dengan
Geobacillus lituanicus

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

Score = 561 bits (283), Expect = 3e-158
Identities = 290/291 (99%), Gaps = 1/291 (0%)
Strand=Plus/Plus

Query	1	ACGAGCGCA-CCCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACCTCTAGAGGGACTGCC	59
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Query	60	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGG	119
Sbjct	1171	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCCTTATGACCTGGG	1230
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Sbjct	1231	CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCGAATC	1290
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Sbjct	1291	CCAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	1350
Query	240	GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGT	290
Sbjct	1351	GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGT	1401



**Perbandingan Urutan Nukleotida bakteri Sampel dengan
*Geobacillus thermoleovorans***

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

Score = 561 bits (283), Expect = 3e-158
Identities = 290/291 (99%), Gaps = 1/291 (0%)
Strand=Plus/Plus

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Sbjct 1074   ACGAGCGCAACCTCGCCTCTAGTTGCCAGCACGAAGGTGGGCACTCTAGAGGGACTGCC 1133

Query 60     GGCACAAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGG 119
             |||
Sbjct 1134   GGCACAAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGG 1193

Query 120    CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCGAATC 179
             |||
Sbjct 1194   CTACACACGTGCTACAATGGGCGGTACAAAGGGCTGCGAACCCGCGAGGGGGAGCGAATC 1253

Query 180    CCAAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC 239
             |||
Sbjct 1254   CCAAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC 1313

Query 240    GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGT 290
             |||
Sbjct 1314   GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGT 1364

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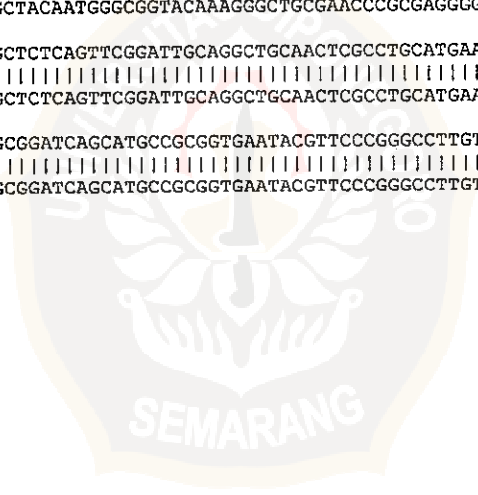
Perbandingan urutan Nukleotida Bakteri Sampel dengan

Bacillus thermoleovorans

> gi|14485237|gb|AF385083.1|AF385083 *Bacillus thermoleovorans* 16S ribosomal
RNA gene, complete sequence
Length=1478

Score = 561 bits (283), Expect = 3e-158
Identities = 290/291 (99%), Gaps = 1/291 (0%)
Strand=Plus/Plus

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Sbjct	1089	ACGAGCGCAACCCCTCGCCTCTAGTTGCCAGCACGAAGTGGGCACTCTAGAGGGACTGCC	1148
Query	60	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGG	119
Sbjct	1149	GGCGACAAGTCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGG	1208
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Sbjct	1269	CCAAAAGCCGCTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATC	1328
Query	240	GCTAGTAATCGCGGATCAGCATGCCGCGTGAATACGTTCCCGGGCCTTGT	290
Sbjct	1329	GCTAGTAATCGCGGATCAGCATGCCGCGTGAATACGTTCCCGGGCCTTGT	1379



LAMPIRANE

PERKIRAAN PANJANG BASA TERAMPLIFIKASI PADA PROSES PCR DENGAN PRIMER SELECT

Upper Primer: P1 16-mer 5' ATGGCTGTCGTCAGCT 3'		
Lower Primer: P2 15-mer 5' ACGGGCGGTGTGTAC 3'		
DNA 250 pM, Salt 50 mM	Upper Primer	Lower Primer
Primer Tm	40.8 °C	43.6 °C
Primer Overall Stability	-25.9 kc/m	-30.9 kc/m
Primer Location	1042..1057	1392..1378
Product Tm - Primer Tm	41.4 °C	
Primers Tm Difference	2.8 °C	
Optimal Annealing Temperature	54.8 °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 F

ELEKTROFOREGRAM HASIL SEKUENSING

MACROGEN
ITS SURVEY CENTER

File: 051107-02_1001_Mfhw_P1.mf1 Run Date: 2005/11/7 21:21:55 Signal C: 13447 A: 3532 C: 11750 T: 7372
Sample: Mfhw_P1 Lane: 3 Base spacing: 14.03 348 bases in 4171 scans Page 1 of 1

10 20 30 40 50 60 70 80 90 100 110 120
:NN FT: AGRSE TFR GCME AFDCALITTCCTTC TTECAFCGCE AAGG TGGCCTC TAAAGGACTG CCG GCG AGLAS TCG EAG AAGGCTG DGG AT EAC TCGAATCA TCA TCGCC



130 140 150 160 170 180 190 200 210 220 230 240 250
TTA TACTTCG TACACAG TGC TACA TGCGGGTGGGAGG GGTTCGACCTGGG AGGGGATTCG AATCCAAAAGCCCTCTCTGATTCGACTTCGACTCGCTGCTA TGAAGC



350 360 370 380 390 400 410 420 430 440 450
CGGATCTACTAG TAAATCCCGATCAGCATVDCGGCTG AATACGTTCCGGATCCTTGTG NC CCG CCGGTA YNHTNNNNNNNNNNNNNNNNNT

