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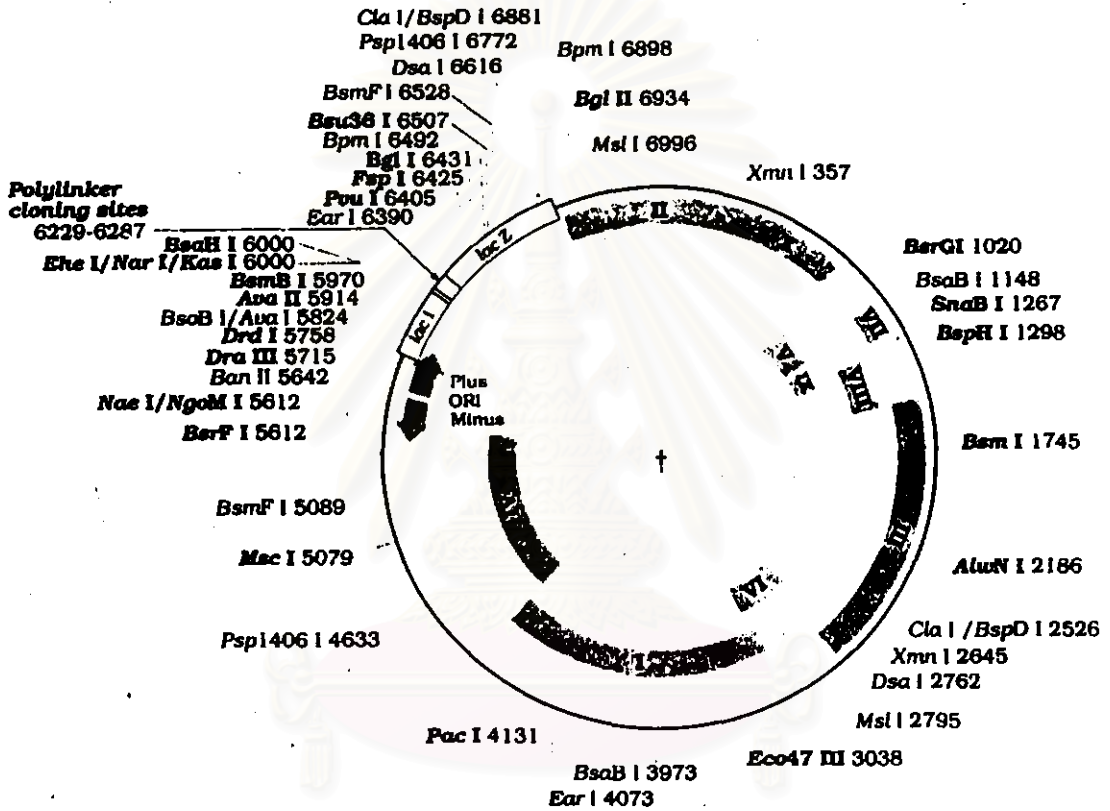


APPENDICES

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX 1

Restriction map of M13mp18

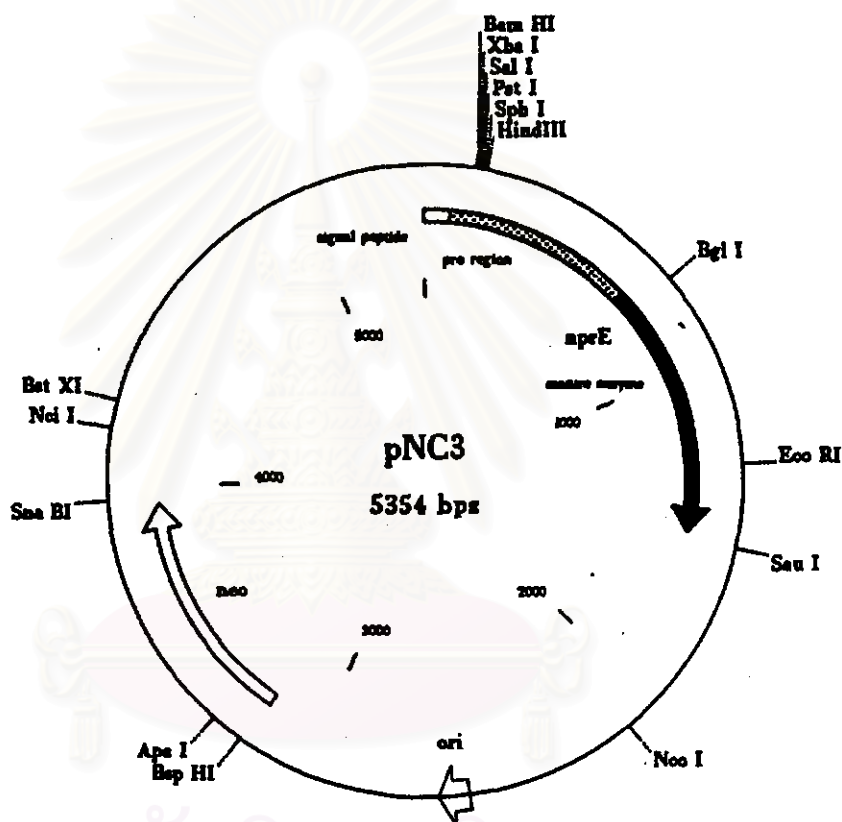


สถาบันวิทยบริการ
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3'-end Reverse Sequencing Primer (-35) P1291
3'-end Sequencing Primer (-35) Primer P1291
3'-end Sequencing Primer (-35) Primer P1292

mp18/pUC18
 6164
 TCCTATGTTGTTGTAATTGTGACGCBATAACAATTTACACAGBAACAGACTATGACCATGATTACGAATTCGAGCTCGATACCCGCGGATCCTCTAGAGTCAAGCTCAGGCATGCAAGCTTGGCACTGBCCTGCTTTTACAAGCTCTGACTGGGAAAACCCCTGCGC
 notThrMetIleThrAsnSerSerSerValProIlyAspProLeuGluSerThrTysArgHisAlaSerLeuAlaLeuAlaValValLeuGlnArgArgAspIleProGluAsnProGly →locZ

APPENDIX 2

Restriction map of pNC3 (Wu *et al.*, 1991)

DESCRIPTION

size: 5354 bp
 construction: Wu, Z. R., *et al.* 1991. Gene 106:103-107
 replication: rolling circle (pUB-like)
 copy number: high

FEATURES

<i>neo</i>	kanamycin/neomycin resistance
<i>nprE</i>	neutral protease structural gene
SP	signal peptide portion of <i>nprE</i>
PRO	pro-peptide portion of <i>nprE</i>
<i>ori</i>	pUB110 positive origin of replication

APPENDIX 3

BLAST result of 5' sequence of pCSBC14

Query= (600 letters)

Sequences producing significant alignments:	Score (bits)	E Value
gb AF007865 AF007865 Bacillus licheniformis bacitracin syntheta...	52	1e-04
emb Z99113 BSUB0010 Bacillus subtilis complete genome (section ...	48	0.002
emb Z34883 BSPEPSYN B.subtilis genes for peptide synthetase and...	48	0.002
gb L14596 SYCTRPB Synechocystis sp. tryptophan synthase beta su...	40	0.38
dbj D64006 SYCSLLH Synechocystis sp. PCC6803 complete genome, ...	40	0.38
gb U60581 HSU60581 Human c-jun gene, promoter region with flank...	38	1.5
gb AF023464 AF023464 Bacillus subtilis fengycin synthetase FenA...	38	1.5
emb X59744 HSCJUNU H.sapiens c-jun upstream region	38	1.5

gb|AF007865|AF007865 Bacillus licheniformis bacitracin synthetase operon including bacitracin synthetase 1 (bacA), 2 (bacB) and 3 (bacC) genes, complete cds
Length = 43297

Score = 52.0 bits (26), Expect = 1e-04
Identities = 29/30 (96%), Positives = 29/30 (96%)

Query: 216 taatgaatacggaccgacagaaaacagcgt 245
|||||
Sbjct: 33964 taatgaatacggaccgacggaaaacagcgt 33993

emb|Z99113|BSUB0010 Bacillus subtilis complete genome (section 10 of 21): from 1781201 to 2014980
Length = 233780

Score = 48.1 bits (24), Expect = 0.002
Identities = 27/28 (96%), Positives = 27/28 (96%)

Query: 220 gaatacggaccgacagaaaacagcgtgg 247
|||||
Sbjct: 184454 gaatatggaccgacagaaaacagcgtgg 184427

emb|Z34883|BSPEPSYN B.subtilis genes for peptide synthetase and penicillin binding protein
Length = 39822

Score = 48.1 bits (24), Expect = 0.002
Identities = 27/28 (96%), Positives = 27/28 (96%)

Query: 220 gaatacggaccgacagaaaacagcgtgg 247
|||||
Sbjct: 33579 gaatatggaccgacagaaaacagcgtgg 33606

Score = 38.2 bits (19), Expect = 1.5
Identities = 28/31 (90%), Positives = 28/31 (90%)

BLAST result of 5' sequence of pCSBC14 (continued)

Query: 217 aatgaatacggaccgacagaaaaacagcgtgg 247
 |||
 Sbjct: 11987 aatgaatacggggccacagaaaaacagtgtgg 12017

gb|L14596|SYCTRPB *Synechocystis* sp. tryptophan synthase beta subunit
 (trpB) gene, complete cds.
 Length = 3231

Score = 40.1 bits (20), Expect = 0.38
 Identities = 20/20 (100%), Positives = 20/20 (100%)

Query: 331 agagtaattattctttttcc 350
 |||
 Sbjct: 1833 agagtaattattctttttcc 1814

dbj|D64006|SYCSLLLH *Synechocystis* sp. PCC6803 complete genome,
 25/27, 3138604-3270709
 Length = 132106

Score = 40.1 bits (20), Expect = 0.38
 Identities = 20/20 (100%), Positives = 20/20 (100%)

Query: 331 agagtaattattctttttcc 350
 |||
 Sbjct: 75984 agagtaattattctttttcc 75965

gb|U60581|HSU60581 Human c-jun gene, promoter region with flanking
 evolutionary conserved sequences
 Length = 3198

Score = 38.2 bits (19), Expect = 1.5
 Identities = 19/19 (100%), Positives = 19/19 (100%)

Query: 64 ggacctgcagcagtttaag 82
 |||
 Sbjct: 1618 ggacctgcagcagtttaag 1600

gb|AF023464|AF023464 *Bacillus subtilis* fengycin synthetase FenA
 (fenA) gene, complete cds
 Length = 10513

Score = 38.2 bits (19), Expect = 1.5
 Identities = 28/31 (90%), Positives = 28/31 (90%)

Query: 217 aatgaatacggaccgacagaaaaacagcgtgg 247
 |||
 Sbjct: 8171 aatgaatatggccgacagaaaaacagtgtgg 8201

BLAST result of 5' sequence of pCSBC14 (continued)

emb|X59744|HSCJUNU H.sapiens c-jun upstream region
Length = 1498

Score = 38.2 bits (19), Expect = 1.5
Identities = 19/19 (100%), Positives = 19/19 (100%)

Query: 64 ggacctgcagcagtttaag 82
 |||||
Sbjct: 64 ggacctgcagcagtttaag 46

CPU time: 20.88 user secs. 0.81 sys. secs 21.69
total secs.

Database: Non-redundant GenBank+EMBL+DDBJ+PDB sequences
Posted date: Sep 2, 1998 10:09 AM
Number of letters in database: 807,979,516
Number of sequences in database: 363,368

Lambda K H
1.37 0.711 1.31

Gapped
Lambda K H
1.37 0.711 1.31

Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 265157
Number of Sequences: 363368
Number of extensions: 265157
Number of successful extensions: 21128
Number of sequences better than 10.0: 9
length of query: 600
length of database: 807979516
effective HSP length: 20
effective length of query: 580
effective length of database: 800712156
effective search space: 464413050480
effective search space used: 464413050480
T: 0
A: 0
X1: 6 (11.9 bits)
X2: 25 (49.6 bits)
S1: 0 (0.5 bits)
S2: 18 (36.2 bits)

APPENDIX 4

BLAST result of *B. subtilis* TISTR25 *gcaA* gene

Query= (1803 letters)

Sequences producing significant alignments:	Score (bits)	E Value
emb Z99105 BSUB0002 Bacillus subtilis complete genome (section ...	1043	0.0
emb Z99104 BSUB0001 Bacillus subtilis complete genome (section ...	1043	0.0
gb U21932 BSU21932 Bacillus subtilis L-glutamine-D-fructose-6-p...	1043	0.0
dbj D21199 BACGSND85 Bacillus subtilis gene for glucosamine syn...	331	2e-88
emb X01631 ECUNC E. coli origin of replication oriC and genes g...	40	1.1
gb AE000450 ECAE000450 Escherichia coli K-12 MG1655 section 340...	40	1.1
dbj D13558 HPCJ483 Hepatitis C virus genome, complete sequence	40	1.1
dbj D00826 HPCNS5J4 Hepatitis C virus gene for NS5 protein, par...	40	1.1
gb L10328 ECOOW82 E. coli; the region from 81.5 to 84.5 minutes	40	1.1
emb Y11778 RPNTH R.prowazekii nth, grlA, glyA, lipA, tlyC, tlcD...	38	4.5
gb M74895 SMFPOLORF Simian foamy virus type 3, gag polyprotein ...	38	4.5
emb Z68336 CEF22B3 Caenorhabditis elegans cosmid F22B3, ccomplet...	38	4.5
emb Z82547 RPZ82547 R.prowazekii genomic DNA fragment (clone Al...	38	4.5

emb|Z99105|BSUB0002 Bacillus subtilis complete genome (section 2 of 21): from 194651 to 415810
Length = 221160

Score = 1043 bits (526), Expect = 0.0
Identities = 1033/1202 (85%), Positives = 1033/1202 (85%)

Query: 1 atgtgtggaattgtaggttacatcggtcagcttgatgcaagagattttgttaaaggga 60
|||||
Sbjct: 5613 atgtgtggaatcgtaggttatcctcggtcagcttgatgcaaggaattttatttaaaggga 5672

Query: 61 ttagagaagcttgagtaccggttatgactctgcccgtatcgctgtggcgaatgagcag 120
|||||
Sbjct: 5673 ttagagaagcttgagtaccggttatgactctgcccgtatcgctgtggcgaatgagcag 5732

Query: 121 ggcgtgcatgtgtacaaagaaaaaggccgcacatcgccgaccttcgtgaagtgtggatcac 180
|||
Sbjct: 5733 ggaatccatgtgttcaaagaaaaaggacgcattgcagatcttcgtgaagtgtggatgcc 5792

Query: 181 acggttgaatctcaagcgggaatcgccatcacgctgggagactcacggtgaaccaagc 240
|||
Sbjct: 5793 aatgtagaagcgaagcgggaatcgccatcacgctgggagactcacggtgaaccaagc 5852

Query: 241 ttctgaaagcctcaccgcatcaaagcgcactcggccgctttacacttgttcacaatggt 300
|||
Sbjct: 5853 tatctgaaagcctcaccgcatcaaagcgcactcggccgctttacacttgttcacaagcgc 5912

Query: 301 gtgatcgagaactatgttcagctgaagcgcgaatattctgaaaacgttgaaactgaaaagc 360
|||||
Sbjct: 5913 gtgatcgagaactatgttcagctgaagcgcgaatattctgaaaacgttgaaactgaaaagc 5972

Query: 361 gacacggacactgaagtagtctgaaatgatcgagcaatttggtggcggaggactcagc 420
|||||
Sbjct: 5973 gacacggacactgaagtagtctgaaatgatcgagcaatttggtggcggaggactcagc 6032

Query: 421 acagaagaagcgtttccgcaaaacactgactctgttaaaggctcttacgcaattgcatta 480
|||||
Sbjct: 6033 acagaagaagcgtttccgcaaaacactgactctgttaaaggctcttacgcaattgcatta 6092

Query: 481 tttgacggtgaaaacacagacaccatttacgttgcaaaaaacaaaagccctctgttaac 540
|||
Sbjct: 6093 ttcgataacgacaacagagaaacgattttgttagcgaaaaaacaaaagccctctattagta 6152

Query: 541 ggccttgagatagctttaaactcgtgcatctgacgcatggtatgcttcaagtaacg 600
|||
Sbjct: 6153 ggtcttgagatagctttaaactcgtgcatctgacgcatggtatgcttcaagtaacc 6212

Query: 601 aatgaatacgttgagcttttgcaaaagaaatggtgatcgtaaaaagatgaagccgtg 660
|||
Sbjct: 6213 aacgaatacgttagctgatgataaagaaatggtgatcgtaaaaagatgaagccgtg 6272

Query: 661 attaaaaaccttgacggtgaaagtcagacacgtgctcttataatcgctgagcttgacgcc 720
|||
Sbjct: 6273 atcaaaaaccttgacggtgaaagtcagacacgtgctcttataatcgctgagcttgacgcc 6332

BLAST result of *B. subtilis* TISTR25 *gcaA* gene (continued)

Query: 721 agtgatatcgaaaaaggcacataccctcactacatgtttaaagaacggatgagcagccg 780
 |||
 Sbjct: 6333 agtgatatcgaaaaaggcacgtaccctcactacatgtttaaagaacggatgagcagcc 6392

Query: 781 cttgttatcgcaaaatcatccaacgtatcaggacgaaaacggcagactggccgtggcc 840
 |||
 Sbjct: 6393 gttgttatcgcaaaatcatccaacgtatcaagatgaaaacggcaagctgtctgtgccc 6452

Query: 841 ggcgatgtcgtgacgcccgtggcgaagcggaccgcatttatatcgtggcttgcggaacg 900
 |||
 Sbjct: 6453 ggcgatatcgtgccgctgtagcgaagcggaccgcatttatatcattggctgcggaaca 6512

Query: 901 agctaccacgcccgtcttctcgggaaacaatatattgaaatgtgggcaaacgtaccggt 960
 |||
 Sbjct: 6513 agctaccatgcaggacttctcggtaaacatatattgaaatgtgggcaaacgtaccggt 6572

Query: 961 gaagtgcattgtagcagtggaattctcttacaacatgccgcttctgtctaagaagccgctc 1020
 |||
 Sbjct: 6573 gaagtgcattgtagcagtggaattctcttacaacatgccgcttctgtctaagaaccgctc 6632

Query: 1021 tttatcttctttctcaaagcggagaaacagcggacagccgcgctgcttgttcaagtc 1080
 |||
 Sbjct: 6633 ttcatttcttcttctcaaagcggagaaacagcagacagccgcgctgactcgttcaagtc 6692

Query: 1081 aaagcgtgggtcacaagcgtgacgattacaacgttccgggatcaacgcttctccgt 1140
 |||
 Sbjct: 6693 aaagcgtcggacacaagccctgacaatcacaacgtacctggatcaacgcttctcgt 6752

Query: 1141 gaagcggattacacattgcttctgcacgcagccctgagatgccgctggcatcaacaaa 1200
 |||
 Sbjct: 6753 gaagctgactatacatgctgcttcatgcagccctgagatcgtgtgctgcaacgaaa 6812

Query: 1201 gc 1202
 ||
 Sbjct: 6813 gc 6814

Score = 460 bits (232), Expect = e-127

Identities = 457/532 (85%), Positives = 457/532 (85%)

Query: 1268 atatcggttttgatttagtcaagaattaggtatcgcggcaaacgccatggaagccctct 1327
 |||
 Sbjct: 6880 atatcggttttgacctcgtcaagaactcggatcgcgcaaacgcaatggaagctctat 6939

Query: 1328 gcgaccagaaggacgaaatggaatgatcgcacgtgagtagctgtttcaagaacg 1387
 |||
 Sbjct: 6940 gcgaccagaaggacgaaatggaatgatcgcgctggaatcctgactgtatccagaatg 6999

Query: 1388 ctttcttcatcgccgcccgttactactcgtgtgtgctgaaggcgcctgaaactga 1447
 |||
 Sbjct: 7000 ctttcttcatcgccgcccgttactactcgtatgtgctgaaggcgcactgaaactga 7059

Query: 1448 aagagatttcttaccatccaggcgaaggcttgcggcggcgagctgaagcatggaacaa 1507
 |||
 Sbjct: 7060 aagagatttcttaccatccaggcgaaggcttgcggcgggtgagctaaagcaggaacga 7119

Query: 1508 tcgctctgattgaagaaggaacaccggtcttgcgcttgcgacacaagaacacgtcaacc 1567
 |||
 Sbjct: 7120 ttgccttgatcgaacaaggaacaccagtattcgcactggcaactcaagagcatgtaacc 7179

Query: 1568 tgagcatccgcggtaatgtgaaggaagtcgagcccggcgccatcactgcatcatct 1627
 |||
 Sbjct: 7180 taagcatccgcggaacgtcaagaagttgctgctcgggagcaaacacatgcatcatct 7239

Query: 1628 cgctgaaaggcttagaagcgcagacgacagattcatcctgccggaagtcaaccctgccc 1687
 |||
 Sbjct: 7240 cactgaaaggcctagacgatcggatgacagattcgtattgccggaagtcaaccagcgc 7299

Query: 1688 ttgctccgctggtttctgttggcattgcagctgatcgcttactacgctgactgcacc 1747
 |||
 Sbjct: 7300 ttgctccgctggatctgttggcattgcagctgatcgcttactatgctgactgcacc 7359

Query: 1748 gcgctgtgacgttgataaacgcgcaaccttgcaagagtggttacggtgga 1799
 |||
 Sbjct: 7360 gcgctgtgacgttgataaacctcgtaaccttgcaagagtggttacggtgga 7411

Score = 60.0 bits (30), Expect = 1e-06

Identities = 66/78 (84%), Positives = 66/78 (84%)

BLAST result of *B. subtilis* TISTR25 *gcaA* gene (continued)

Query: 841 ggcgatgtcgtgacgccgtggcggaaagcggaccgcatttatatcgtggcttgcggaacg 900
 Sbjct: 201103 ggcgatatcgtgcccgtgtagcggaaagcggaccgcatttatatcattggctgcggaaca 201162

Query: 901 agctaccacgccggtcttgcgggaacaatatattgaaatgtgggcaaacgtaccgggt 960
 Sbjct: 201163 agctaccatgcaggacttgcggtaacaatatattgaaatgtgggcaaacgtgccggt 201222

Query: 961 gaagtgcattgtagcagtggaattctcttacaacatgccgcttctgtctaagaagccgctc 1020
 Sbjct: 201223 gaagtgcattgtagcagtggaattctcttacaacatgccgcttctgtctaagaagccgctc 201282

Query: 1021 tttatcttcttctcCaagcgggagaacagcggacagccgcgctgtgttcaagtc 1080
 Sbjct: 201283 ttcattttcttctcCaagcgggagaacagcagacagccgcgctgtgttcaagtc 201342

Query: 1081 aaagcgtggtgcacaagcgtgacgattacaacgttccgggatcaacgcttcccgt 1140
 Sbjct: 201343 aaagcgtcggacacaagccctgacaatcacaacgtacctgatcaacgcttctcgt 201402

Query: 1141 gaagcggattacacattgcttctgcacgcagccctgagatcggcgtggcatcaacaaaa 1200
 Sbjct: 201403 gaagctgactatacattgctgcttcatgcagccctgagatcgtgttgcgtcaacgaaa 201462

Query: 1201 gc 1202
 Sbjct: 201463 gc 201464

Score = 460 bits (232), Expect = e-127
 Identities = 457/532 (85%), Positives = 457/532 (85%)

Query: 1268 atatcggttttgatttagtcaagaattaggtatcgcggcaaacgccatggaagccctct 1327
 Sbjct: 201530 atatcggttttgacctcgtcaagaactcggatcgcgtgcaaacgcaatggaagctctat 201589

Query: 1328 gcgaccagaaggacgaaatgaaatgatcgcacgtgagctgactgtttcaagaaacg 1387
 Sbjct: 201590 gcgaccagaagaagcgaatgaaatgatcgcctgtaaacctgactgtatccagaaatg 201649

Query: 1388 ctttcttcatcgcccgcccttgactacttcggtgtgtgctgaaggcgcctgaagctga 1447
 Sbjct: 201650 ctttcttcatcgacgcggccttgactacttcggtatgtgtcgaaggcgcactgaagctga 201709

Query: 1448 aagagatttcttacatccaggcgaaggcttcgcccggcggagctgaagcatggaacaa 1507
 Sbjct: 201710 aagagatttcttacatccaggcagaaggctttgccggcggtagctaaagcagcgaacga 201769

Query: 1508 tcgctctgattgaagaaggaacaccggtctttgcttgcgacacaagaacacgtcaacc 1567
 Sbjct: 201770 ttgccttgatcgaacaaggaacaccagttatcgcactggcaactcaagagcatgtaaac 201829

Query: 1568 tgagcatccgcggtaatgtgaaggaagtcgcagcccgcggcgaacacttgcatcatct 1627
 Sbjct: 201830 taagcatccgcggaaacgtcaagaagttgctgctcgcggagcaaacacatgcatcatct 201889

Query: 1628 cgctgaaaggcttagaagacgcagacgagattcctcctgcgggaagtcaaccctgcgc 1687
 Sbjct: 201890 cactgaaaggcctagacgatgaggatgacagattcgtattgccggaagttaaccagcgc 201949

Query: 1688 ttcgtccgctggtttctgttgcattgcagctgatcgttactacgtgcactgcacc 1747
 Sbjct: 201950 ttgctccgctggttatctgttgcattgcagctgatcgttactacgtgcactgcacc 202009

Query: 1748 gggctgtgacgcttgataaacccgcgaaccttgcgaagagtgttacgggtgga 1799
 Sbjct: 202010 gggctgtgatgtggataaacctcgtaaccttgcgaagagtgttactgtgga 202061

gb|U21932|BSU21932 *Bacillus subtilis* L-glutamine-D-fructose-6-phosphate
 amidotransferase (*gcaA*) gene, complete cds.

Length = 2581

Score = 1043 bits (526), Expect = 0.0

Identities = 1033/1202 (85%), Positives = 1033/1202 (85%)

Query: 1 atgtgtggaattgtaggttacatcggtcagcttgatgcaagagattttgttaaaggga 60
 Sbjct: 312 atgtgtggaatcgttaggttatatcggtcagcttgatgcaaggaattttattaaaggga 371

BLAST result of *B. subtilis* TISTR25 *gcaA* gene (continued)

Query: 76 taccgcggttatgactctgccggt 99
 ||||| |||||
 Sbjct: 12444 taccgcggtatgactctgccggt 12467
 Score = 38.2 bits (19), Expect = 4.5
 Identities = 22/23 (95%), Positives = 22/23 (95%)

Query: 214 cgctgggcgactcacggtgaacc 236
 ||||| |||||
 Sbjct: 12588 cgctgggcgacccacggtgaacc 12610

gb|AE000450|ECAE000450 *Escherichia coli* K-12 MG1655 section 340 of 400 of the complete genome
 Length = 11414

Score = 40.1 bits (20), Expect = 1.1
 Identities = 23/24 (95%), Positives = 23/24 (95%)

Query: 76 taccgcggttatgactctgccggt 99
 ||||| |||||
 Sbjct: 2046 taccgcggtatgactctgccggt 2023
 Score = 38.2 bits (19), Expect = 4.5
 Identities = 22/23 (95%), Positives = 22/23 (95%)

Query: 214 cgctgggcgactcacggtgaacc 236
 ||||| |||||
 Sbjct: 1902 cgctgggcgacccacggtgaacc 1880

dbj|D13558|HPCJ483 *Hepatitis C virus* genome, complete sequence
 Length = 9448

Score = 40.1 bits (20), Expect = 1.1
 Identities = 20/20 (100%), Positives = 20/20 (100%)

Query: 1693 ccgctggtttctgtgtgcc 1712
 ||||| |||||
 Sbjct: 9305 ccgctggtttctgtgtgcc 9324

dbj|D00826|HPCNS5J4 *Hepatitis C virus* gene for NS5 protein, partial cds, isolate:HC-J4
 Length = 1173

Score = 40.1 bits (20), Expect = 1.1
 Identities = 20/20 (100%), Positives = 20/20 (100%)

Query: 1693 ccgctggtttctgtgtgcc 1712
 ||||| |||||
 Sbjct: 1030 ccgctggtttctgtgtgcc 1049

gb|L10328|ECOUW82 *E. coli*; the region from 81.5 to 84.5 minutes
 Length = 136254

Score = 40.1 bits (20), Expect = 1.1
 Identities = 23/24 (95%), Positives = 23/24 (95%)

Query: 76 taccgcggttatgactctgccggt 99
 ||||| |||||
 Sbjct: 102984 taccgcggtatgactctgccggt 102961

Score = 38.2 bits (19), Expect = 4.5
 Identities = 22/23 (95%), Positives = 22/23 (95%)

Query: 214 cgctgggcgactcacggtgaacc 236
 ||||| |||||
 Sbjct: 102840 cgctgggcgacccacggtgaacc 102818

emb|Y11778|RPNTH *R. prowazekii* nth, grlA, glyA, lipA, tlyC, tlcD genes
 Length = 7653

Score = 38.2 bits (19), Expect = 4.5
 Identities = 19/19 (100%), Positives = 19/19 (100%)

Query: 1512 tctgattgaagaaggaaca 1530
 ||||| |||||
 Sbjct: 580 tctgattgaagaaggaaca 598

BLAST result of *B. subtilis* TISTR25 *gca4* gene (continued)

gb|M74895|SMFPOLORF Simian foamy virus type 3, gag polyprotein 3' end, pol polyprotein 3' end, env protein 3' end, orf1 3' end, orf2 partial cds, and orf3 3' end.
Length = 13111

Score = 38.2 bits (19), Expect = 4.5
Identities = 22/23 (95%), Positives = 22/23 (95%)

Query: 1260 cggcggttgatcgggttttgatt 1282
|||||
Sbjct: 3623 cggcggttgattcgggttttgatt 3601

emb|Z68336|CEF22B3 *Caenorhabditis elegans* cosmid F22B3, complete sequence
[*Caenorhabditis elegans*]
Length = 30480

Score = 38.2 bits (19), Expect = 4.5
Identities = 22/23 (95%), Positives = 22/23 (95%)

Query: 82 ggttatgactctgccggtatcgc 104
|||||
Sbjct: 5228 ggttacgactctgccggtatcgc 5250

emb|Z82547|RPZ82547 *R. prowazekii* genomic DNA fragment (clone A195F)
Length = 468

Score = 38.2 bits (19), Expect = 4.5
Identities = 19/19 (100%), Positives = 19/19 (100%)

Query: 1512 tctgattgaagaaggaaca 1530
|||||
Sbjct: 277 tctgattgaagaaggaaca 295

CPU time: 10.55 user secs. 0.66 sys. secs 11.21 total secs.

Database: Non-redundant GenBank+EMBL+DDBJ+PDB sequences
Posted date: Aug 9, 1998 7:11 AM
Number of letters in database: 788,862,056
Number of sequences in database: 358,864

Lambda	K	H
1.37	0.711	1.31

Gapped Lambda	K	H
1.37	0.711	1.31

Matrix: blastn matrix:1 -3
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 597843
Number of Sequences: 358864
Number of extensions: 597843
Number of successful extensions: 41074
Number of sequences better than 10: 16
length of query: 1803
length of database: 788862056
effective HSP length: 21
effective length of query: 1782
effective length of database: 781325912
effective search space: 1792322775184
T: 0
A: 0
X1: 6 (11.9 bits)
X2: 25 (49.6 bits)
S1: 0 (0.5 bits)
S2: 19 (38.2 bits)

APPENDIX 5

Position of restriction endonucleases sites of *B. subtilis* TISTR25 *gcaA* gene

Mnl I

Nla III
Nsp7524 I

Mbo II
Sau3A I

Mbo II
EcoN I

GATCTTCCAAAAAACATGTGGGAGGGGACGATTGAAAGTCCCTTGTAAATTTGACTTCTCTCGTCTCCTTTTGCACCTT 80
CTAGAAGGTTTTTTGTACACCCTCCCTGCTAACTTTTACGGGGAACATTAAACTGAAAGAAGCAGAGGAAAACGTGGAA

15 59 77

3 15 16 23

SfaN I

Mbo II
Mnl I

Mae III
Cvi JI
Alu I

Mse I

TAGGAGGAAGAAAATATGTGTGGAATTGTAGGTTACATCGGTCAGCTTGATGCGAAAGAGATTTTGTAAAAGGATTAGA 160
ATCTCTCTTTTATACACACCTTAACATCCAATGTAGCCAGTCGAACTACGCTTCTCTAAAACAATTTTCTTAATCT

84 112 124 129 147

87 124

BstU I
Sec I
Sac II

Cfr10 I
Ple I Hpa II

Rsa I
Nla III

Hae

Hind III
NspB II
Hinf I

Nsp7524 I
Cvi JI

GAAGCTTGAAGTACCGCGGTTATGACTCTGCCGGTATCGCTTGGCGAATGAGCAGGGCGTGCATGTGTACAAAGAAAAAG 240
CTTCGAACTCATGGCCCACTACTGAGACGGCCATAGCGACACCGCTTACTCGTCCCGCACGTACACATGTTTCTTTTTC

162 173 183 190 221 240
163 170 173 183 190 221 240
163 173 174 189 227 240

Hae III
Cvi JI
Gdi II

Eae I

SfaN I
Fnu4H I

Sau3A I
Alw I

Hinf I
Hinf I

Ple I
Hinf I

GCCGCATCGCCGACCTTCGTGAAGTGGTGGATCACACGGTTGAATCTCAAGCGGGAATCGGCCATACACGCTGGGCGACT 320
CGGCGTAGCGGCTGGAAGCACTTCACCACCTAGTGTGCCACTTAGAGTTCGCCCTTAGCGCGGTATGTGCGACCCGCTGA

241 269 282 295 317
244 270 299 317
299 300 317

Fnu4H I
Hae III
Cvi JI
Gdi II

Eaq I

Taq I
Sau3A I

Hph I
Hind III

Hph I
SfaN I

Hha I
Eae I

CACGGTGAACCAAGCTTCCTGAACCGCTCACCCGATCAAGCGCAGTCCGGCGCTTTACACTTGTTTACAATGGTGTGAT 400
GTGCCACTTGGTTCGAAGGACTTCCGAGTGGGCGTAGTTCCGGTGAGCCGGCGAAATGTGAACAAGTGTACCACACTA

324 332 347 353 361 368 368 368 370 398 400
333 369 369 370

Position of restriction endonucleases sites of *B. subtilis* TISTR25 *gcaA* gene
(continued)

Cvi JI
Alu I
Pvu II Hha I
NspB II BstU I Mae II

CGAGAACTATGTTTACGCTGAAGCGCGAATATCTTGAACAACGTTGAACCTGAAAAGCGACACGGACACTGAAGTAGTCGTTTC 480
GCTCTTGATACAAGTCGACTTCGGCGTTATAGAACCTTTGCAACTTGACTTTTCGCTGTGCCTGTGACTTCATCAGCAAG
Y | | Y | | V | | Y | | Y | | Y | | Y | | Y | |

414 423 439
414 422
415
415

Dde I
Ple I
Hinf I
Taq I
Sau3A I Mnl I Mbo II Ple I Mae I
Hinf I Cvi JI

AAATGATCGAGCAATTTGTGGCGGGAGGACTCAGCACAGAAGAGCGTTCCGCAAACACTGACTCTGTTAAAAGGCTCT 560
TTTACTAGCTCGTTAAACACCGCCCTCCTGAGTCGTCTTCTTCGCAAGGCGTTTTGTGACTGAGACAATTTCCGAGA
| | Y | | Y | | | | V | | V | | V | | V | |

485 505 519 542 555
487 508 508 510 542 549

Sty I
Hae III
Cvi JI
Mnl I Mae I Sec I
Hph I Mae II Cvi JI Mae I

TACGCAATTGCATTATTTGACGGTGAAAACACAGACACCATTACGTTGCAAAAAACAAAAGCCCTCTGTTAATCGGCCT 640
ATGCGTTAACGTAATAAAGTCCACTTTTGTGCTGTGGTAAATGCAACGTTTTTGTTCGGGAGACAATTAGCCGGA
Y | | V | | Y | | Y | | V | | V | | V | | V | |

582 604 621 630 636 638
624 636 636 638

Mae II Cvi JI
Mae I Alu I
Mae II SfaN I BstU I Mae III Mae II
Hga I Cvi JI

TGGAGATACGTTAACGTCGTGGCATCTGACCGGATGGCTATGCTTCAAGTAACGAATGAATACGTTGAGCTTTTGGACA 720
ACCTCTATGCAAATTCAGCACCGTAGACTGCGCTACCGATACGAAGTTCATTGCTTACTTATGCAACTCGAAAACCTGT
| V | | V | | | | V | | Y | | V | | V | | V | |

648 652 663 669 677 690 703 709 709
655 709

Mae II
Pml I
Afl III
Sau3A I Nla III
Hph I Mae III Cvi JI Mae I Hph I BspH I Hga I

AAGAAATGGTGATCGTGACAAAAGATGAAGCCGTGATTA AAAACCTTGACGGTGAAGTCATGACACGTCGCTTATATC 800
TTCTTTACCAGTACGACTGTTTCTACTTCGGCACTAATTTTGGAACTGCCACTTCAGTACTGTGCACGCAGAAATATAG
| V | | V | | V | | V | | V | | V | | V | | V | |

728 735 749 757 771 778 789
731 779 783 784 785

Cvi JI Mae I Fnu4H I
Alu I Nla III Fnu4H I
Dde I Hga I Taq I Nsp7524 I Bbv I
Esp I Aha II EcoR V Mnl I Afl III Fok I Cvi JI

GCTGAGCTTGAGCCAGTGATATCGAAAAGGCACATACCCCTCACTACATGTTAAAAGAAACGGATGAGCAGCCGCTTGT 880
CGACTCGAAGTCGGGCTACTATAGCTTTTCCGTGTATGGGAGTGATGTACAATTTTCTTTGCCTACTCGTCGGCGAACA
| | | | V | | | | V | | V | | V | | V | |

801 810 819 823 840 847 863 871
802 810 823 847 869 869
805 848 852 869
805 872

Position of restriction endonucleases sites of *B. subtilis* TISTR25 *gcaA* gene
(continued)

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                                     Nae I
                                     Hae III
                                     Gdi II
                                     Eae I
                                     Sec I
                                     Hae III
                                     Cvi JI Hpa II
                                     Gdi II Cfr10 I
                                     Eae I Cvi JI
                                     || | |||
TATGCGCAAATCATCCAAACGTATCAGGACGAAAACGGCAGACTGGCCGTGGCCGGCGATGTCGCTGACGCCGTGGCGG 960
ATACGGCTTTTAGTAGGTTGCATAGTCCCTGCTTTTGCCGTCGTGACCGGCCACCGGCCGTACAGCGACTGGCCGCCCGCC
|| V | | V | | V | | V | | V |
883 893 900 925 932 948 948
884 925 933 948
926 934 952
926
928
931
931
932
933

Sau96 I
Ava II
Rsr II
Cvi JI
Cvi JI
Alu I
Hpa II
Cfr10 I
AAGCGGACCGCATTATATCGTGGCTTGGCGAAGCAGCTACCACGCCGGTCTTGTGCGGAACAATATATTGAAATGTGG 1040
TTCCGCTGGCGTAAATATAGCACCGAACGCCCTTGCTCGATGGTGGCCGAGAACAGCCCTTTGTTATATAACTTTACACC
|| V | V | V | V | V | || V | V | V |
964 983 996 1005 1006
965 996
965

Hpa II
Cfr10 I
Rsa I
Mae II
Nla III
Nsp7524 I
EcoR I
Nla III
Nsp7524 I
Dde I
Fnu4H I
Cvi JI
Fnu4H I
Mbo II
GCAAACGTACCGGTTGAAGTGCATGTAGCGAGTGAATTCTCTTACAACATGCCGCTTCTGTCTAAGAAGCCGCTCTTTAT 1120
CGTTTGCATGGCCAACCTCACGTACATCGCTCACTTAAGAGAATGTTGTACGGCGAAGACAGATTCTTCGGCGAGAAATA
|| | | V | | V | | V | | V | | V |
1045 1061 1074 1087 1102 1109 1120
1047 1062 1088 1108
1049 1091
1050

Hha I
BstU I
Cvi JI
NspB II
Fnu4H I
Tth111 II
Hha I
Hae II
Mae III
Eco47 III
Eco47 III
CTTCCTTCTCAAAGCGGAGAAACAGCGGACAGCCGCGCGTGTGTTCAAGTCAAAGCGCTGGGTCACAAGCGCTGA 1200
GAAGGAAAGAGTTTCGCTCTTTGTCGCTGTCCGGCGGCACGAACAAGTTCAGTTTCGGGACCCAGTGTTCGCGACT
V | V | V | V | V | || V | V | V |
1144 1153 1162 1178 1186 1193
1152 1178 1186
1155 1179 1193
1156 1194

Sau3A I
ScrF I
Nci I
Hpa II
Bcn I
Mae II
Alw I
CGATTACAACGTTCCGGGATCAACGCTTCCCGTGAAGCGGATTACACATTGCTTCTGCACGCAGGCCCTGAGATCGCC 1280
GCTAATGTTTGAAGGCCCTAGTTGCGAAAGGGCACTTCGCTAATGTGTACGAAGACGTGCGTCCGGGACTCTAGCGG
|| | || V | V | V | || | | | V |
1210 1218 1264 1274
1215 1265 1279
1215 1266
1215 1266
1215 1266
1219 1270
1219

```


Position of restriction endonucleases sites of *B. subtilis* TISTR25 *gcaA* gene (continued)

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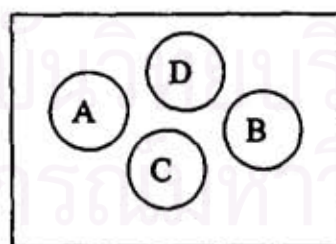
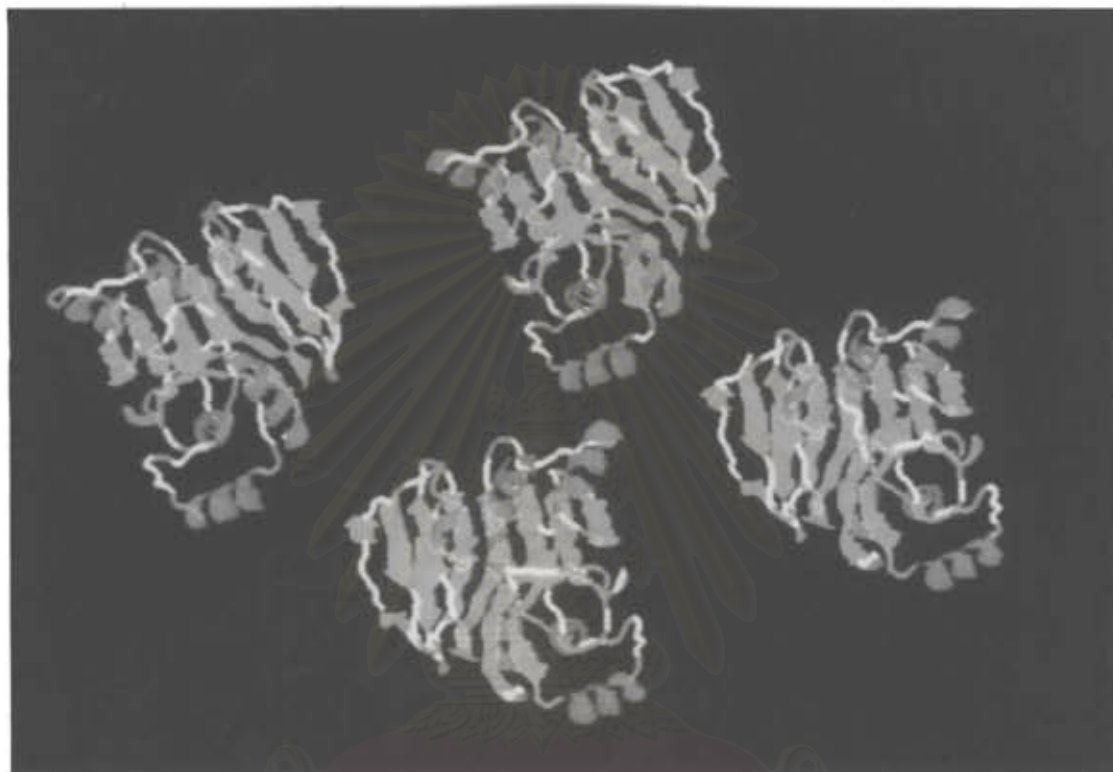
                Mse I      BstU I      Mse I      BstU I
                Dra I      Nru I      Dra I      Nru I
                ||        ||        ||        ||
TAGTGTGAATTTAAATAAAGTCGGGATGTTTATAAAAAAGATCGGATGTTTAAATAAAGTCGGGANGTTTATAAAAAA 2000
ATCACACTTAAATTTTATTTTCAGCGCTACAAATATTTTTTCTACGCTACAAATTTTATTTCAGCGCTNCAAAATATTTTT
                ||        Y ||        V |        ||        Y ||        Y
                1930      1942      1961      1970      1982
                1931      1943      1971      1983

Cvi JI
    Alu I
    Hind III
    ||
GTTGCGAAGCTTACCCCTTTGGATATGATTATCTAAAGGGGTGTTTTTGTGTCGAAAAGAAAAGAACATCTAAATTGA 2080
CAACGCTTCGAATGGGGAACCTATACTAATAGATTTCCCCACAAAACACAGCTTTTCTTTTCTGTAGATTTTAACT
    || Y      Y      Y      Y      Y |      Y      Y      Y
    2007
    2008
    2008
                                Sau3A I
                                BstY I
                                Bgl II
                                Mnl I
                                Hinc II
                                ||
TAAGTGGATTAAGAGGGCTCGAGGAACCTGGCAGTGGGGCATGATTATCAAGATCTTTCCTCATTAGGTCGGTCAACAAGA 2160
ATTACCTAATTTCTCCAGCTCCTTGACCGTCAACCCGTAATAAGTCTAGAAAGGAGTAATCCAGCCAGTTGTTCT
    |Y |      |Y|      V      |V |      ||      |V |      V|      V
    2089      2099      2119      2130      2138      2151
    2094      2101
                                2130
                                2131

Mse I      Hpa II      Nla III      Mse I
|          ||          |          |
TTAAAAGGTATAAAAACCGGCAGACACATGAGTTTTTATCGGATTTGGAACGAACTACTTTTATTTAACTGAATTTTC 2240
AATTTTCCATATTTTGGCCGTCGTGTAAGTCTCAAAAATAGCCCTAAACCTTGCCTTGTGAAATAAATGACTTAAAG
|          V      || V      |V |      V      V      V      |V |      V
2161      2176      2188      2227
                2177

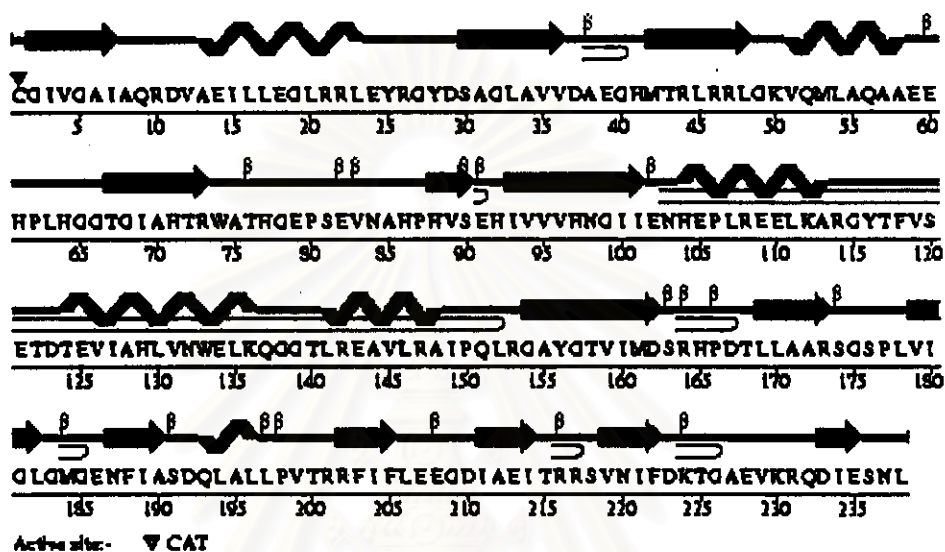
                                Xmn I      Mbo II      Mae II
                                |          |          |
TGATGTTATTTTAGATATTCGTGAACAATTCCTTTATTACCACAAGAAGAGACGTTGCCATTGCTG 2308
ACTACAATAAAATCTATAAGCACTTGTAAAGGAAATAATGGTGTCTTCTCTGCAAACGGTAACGAC
    Y      Y |      Y      Y      |Y |      Y
                2263      2287
                2293
    
```

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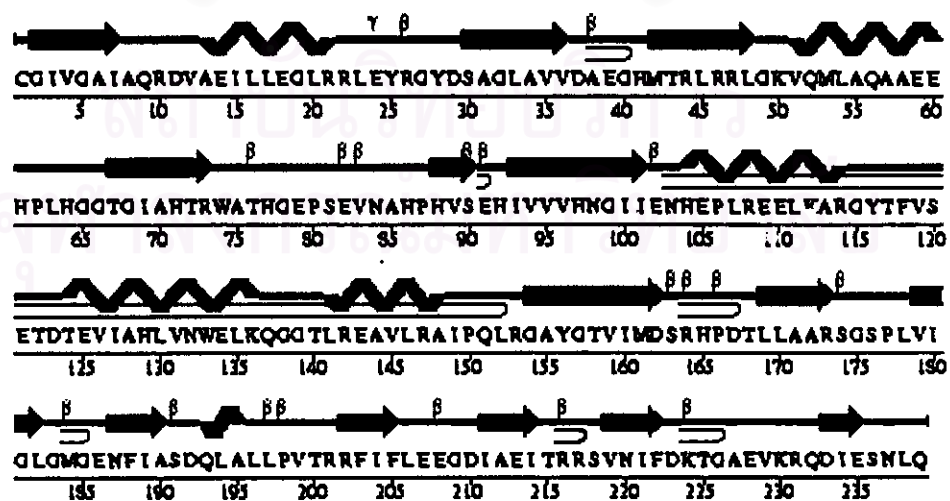
APPENDIX 6**Three-dimensional structure of *E. coli* *gcaA* protein in chain A, B, C and D**

Three-dimensional structure of *E. coli gcaA* protein in chain A, B, C and D (continued)

- Chain A (238 residues)

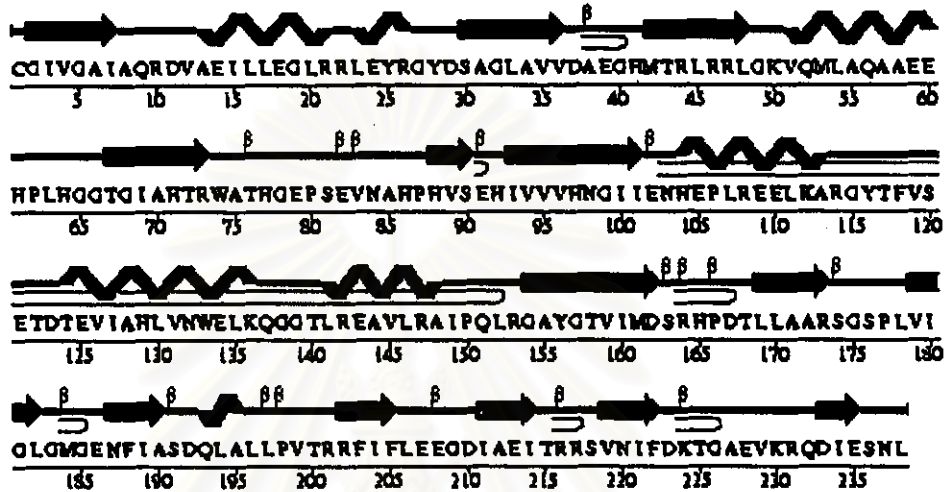


- Chain B (239 residues)

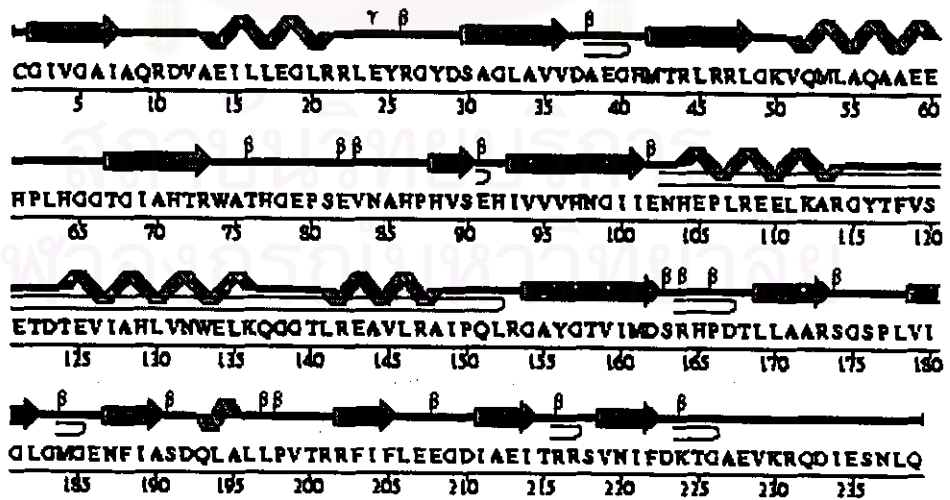


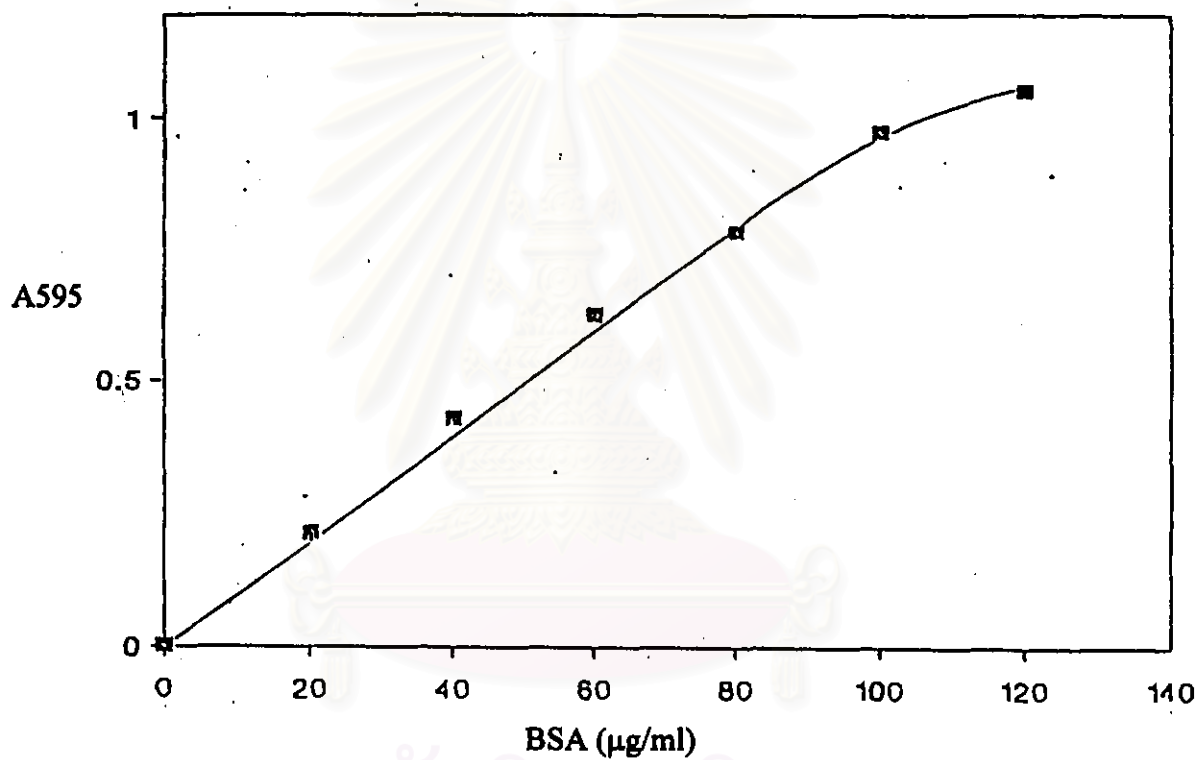
**Three-dimensional structure of *E. coli gcaA* protein in chain A, B, C and D
(continued)**

- Chain C (238 residues)

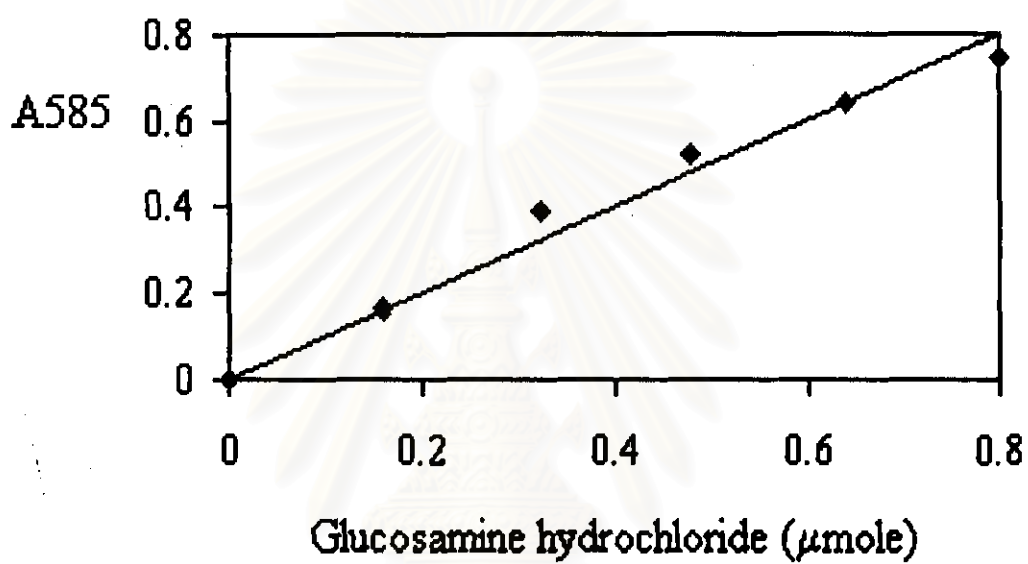


- Chain D (239 residues)



APPENDIX 7**Standard curve for protein determination by Bradford's method**

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APPENDIX 8**Standard curve for glucosamine determination**

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BIOGRAPHY

Miss Jarunee Vanichtanankul was born on October 16, 1973. She graduated with the Bachelor degree of Science in Medical Technology from Chulalongkorn University in 1994. She has further study in Master's degree of Science, department of Biochemistry, Chulalongkorn University in 1995.



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