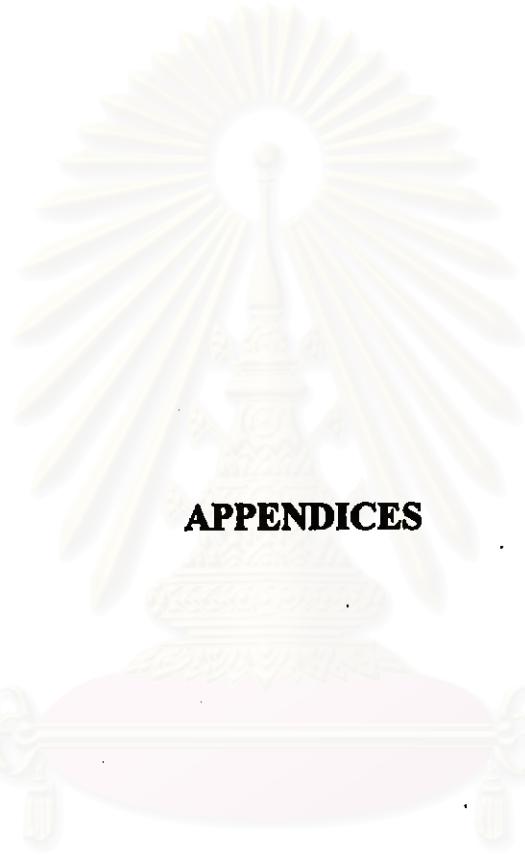


REFERENCES

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990. Basic Local Alignment Search Tool. *J. Mol. Biol.* 215: 403-410.
- Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25: 3389-3402.
- Badet, R., Vermoote, P., Haumont, P.-Y., Lederer, F. and Goffic, F.L. 1987. Glucosamine Synthetase from *Escherichia coli*: Purification, Properties, and Glutamine-Utilizing Site Location. *Biochemistry* 26: 1940-1948.
- Baev, N., Endre, G., Petrovics, G., Banfalvi, Z. and Kondorosi, A. 1991. Six nodulation genes of *nod* box locus 4 in *Rhizobium meliloti* are involved in nodulation signal production: *nodM* codes for D-glucosamine synthetase. *Mol. Gen. Genet.* 228: 113-124.
- Bradford, M.M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* 72: 248-254.
- Crolle, G., D'Este, E. 1980. Glucosamine sulphate for the management of arthrosis: a controlled clinical investigation. *Curr. Med. Res. Opin.* 7: 104-114.
- D'Ambrosio, E., Casa, B., Bompani, R., Scali, G. and Scali, M. 1981. Glucosamine sulphate: a controlled clinical investigation in arthrosis. *Pharmatherapeutica* 2: 504-508.
- Dower, W.J., Miller, J.F. and Ragsdale, C.W. 1988. High efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Res.* 16: 6127-6145.
- Endo, A., Kakiki, K. and Misato, T. 1970. Feedback Inhibition of L-Glutamine D-Fructose 6-Phosphate Amidotransferase by Uridine Diphosphate N-Acetylglucosamine in *Neurospora crassa*. *J. Bacteriol.* 103: 588-594.
- Ghosh, S., Blumenthal, H.J., Davidson, E. and Roseman, S. 1960. Glucosamine Metabolism. *J. Biol. Chem.* 235: 1265-1273.

- Golinelli-Pimpaneau, B. and Badet, B. 1991. Possible involvement of Lys603 from *Escherichia coli* glucosamine-6-phosphate synthase in the binding of its substrate fructose 6-phosphate. *Eur. J. Biochem.* 201: 175-182.
- Higgins, D.G. and Sharp, P.M. 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* 73: 237-244.
- Jung, M.E., Trifunovich, I.D., Gardiner, J.M. and Clevenger, G.L. 1990. Preparation of Modified Nucleosides from Glucosamine: Rapid and Efficient Formal Total Synthesis of Several 2'-Deoxy C-Nucleosides. *J. Chem. Soc. Chem. Commun.* 84-85.
- Kenig, M., Vandamme, E. and Abraham, E.P. 1975. The Mode of Action of Bacilysin and Anticapsin and Biochemical Properties of Bacilysin-resistant Mutants. *J. Gen. Microbiol.* 94: 46-54.
- Kornfeld, R. 1967. Studies on L-Glutamine D-Fructose 6-Phosphate Amidotransferase. *J. Biol. Chem.* 242: 3135-3141.
- Kunst, F. et al. 1997. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* 390: 249-256.
- March, C. 1988. 'DNA Strider': a 'C' program for the fast analysis of DNA and protein sequences on the Apple Macintosh family of computers. *Nucleic Acids Res.* 16: 1829-1836.
- McKnight, G.L., Mudri, S.L., Mathewest, S.L., Traxinger, R.R., Marshall, S., Sheppard, P.O. and O'Hara, P.J. 1992. Molecular Cloning, cDNA Sequence, and Bacterial Expression of Human Glutamine:Fructose-6-phosphate Amidotransferase. *J. Biol. Chem.* 267: 25208-25212.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular Cloning : A Laboratory Manual*, 2nded. Cold Spring Habor Laboratory Press. New York.
- Sanger, F., Nicklen, S. and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* 74: 5463-5467.
- Sayeski, P.P., Paterson, A.J. and Kudlow, J.E. 1994. The murine glutamine:fructose-6-phosphate amidotransferase-encoding cDNA sequence. *Gene* 140 : 289-290.

- Sayeski, P.P., Wang, D., Su, K., Han, I.-O. and Kudlow, J.E. 1997. Cloning and partial characterization of the mouse glutamine:fructose-6-phosphate amidotransferase (GFAT) gene promoter. *Nucleic Acids Res.* 25: 1458-1466.
- Smith, R.J., Milewski, S., Brown, A.J.P. and Gooday, G.W. 1996. Isolation and Characterization of the *GFA1* Gene Encoding the Glutamine:Fructose-6-phosphate Amidotransferase of *Candida albicans*. *J. Bacteriol.* 178: 2320-2327.
- Takami, H., Kobayashi, T., Kobayashi, M., Yamamoto, M., Nakamura, S., Aono, R. and Horikoshi, K. 1992. Molecular Cloning, Nucleotide Sequence, and Expression of the Structural Gene for Alkaline Serine Protease from Alkaliphilic *Bacillus* sp. 221. *Biosci. Biotech. Biochem.* 56: 1455-1460.
- Tanunat, D. 1995. Cloning of protease gene from *Bacillus subtilis* TISTR25. Master's Thesis, Department of Biochemistry, Faculty of Science, Chulalongkorn university.
- Van Der Laan, J.C., Gerritse, G., Mulleners, L.S.M., Van Der Hoek, R.A.C. and Quax, W.J. 1991. Cloning, Characterization, and Multiple Chromosomal Integration of a *Bacillus* alkaline protease gene. *Appl. Environ. Microbiol.* 57: 901-909.
- Walker, J.E., Gay, N.J., Saraste, M. and Eberle, A.N. 1984. DNA sequence around the *Escherichia coli unc* operon. *Biochem. J.* 224: 799-815.
- Watzele, G. and Tanner, W. 1989. Cloning of the Glutamine:Fructose-6-phosphate Amidotransferase Gene from Yeast. *J. Biol. Chem.* 264: 8753-8758.
- Wu, Z.-R., Qi, B.-J., Jiao, R.-Q., Chen, F.-D. and Wang, L.-F. 1991. Development of a novel *Bacillus subtilis* cloning system employing its neutral protease as screen marker. *Gene* 106: 103-107.
- Yanisch-Perron, C., Vieira, J. and Messing, J. 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* 33: 103-119.

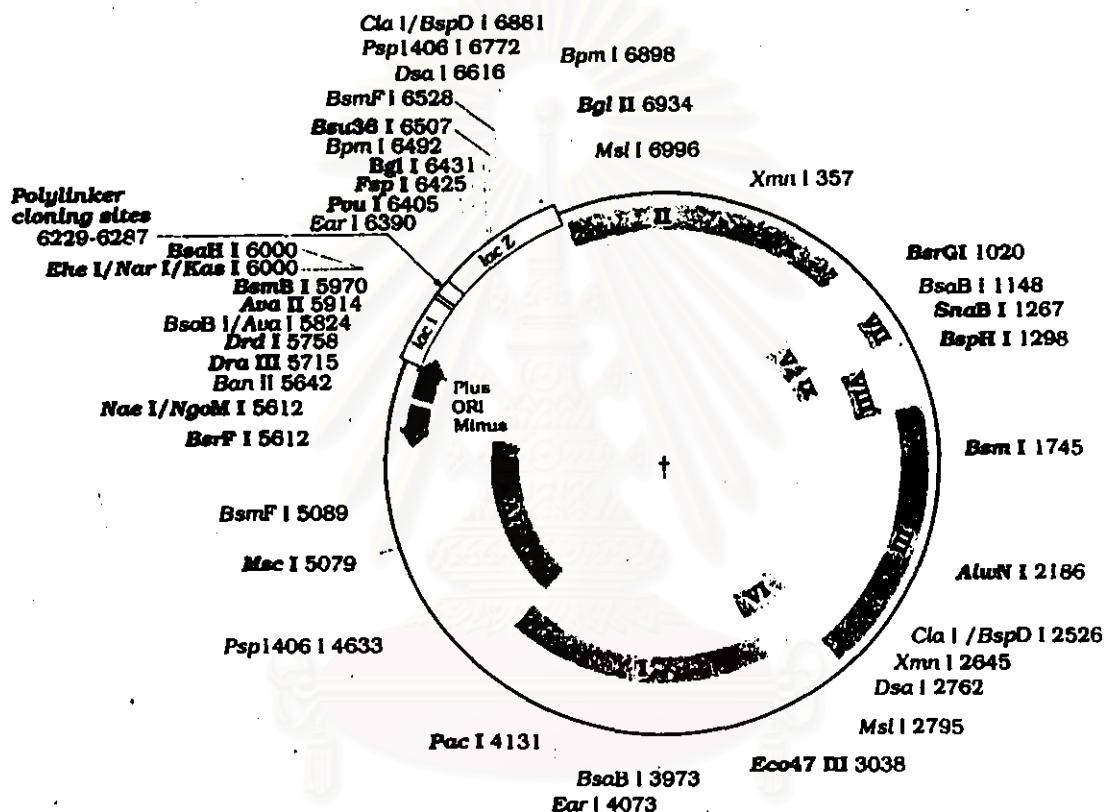


APPENDICES

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX 1

Restriction map of M13mp18



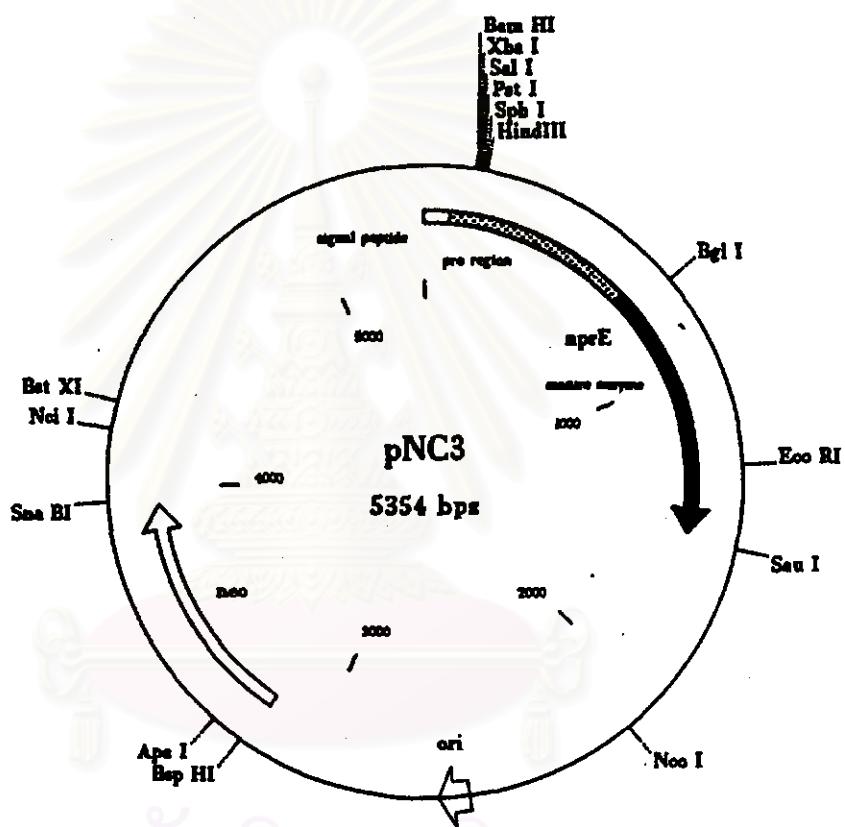
สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

24-base Reverse Oligonucleotide Primer 1-12: F2211 <chem>ACCCGATAACATTTTCAACCAAGA</chem>	17-base Sequencing Primer 1-12: Primer F2211 <chem>TGACCGCAGCAAAATG</chem>	17-base Sequencing Primer 1-12: Primer F222 <chem>CACCACTGACCCCTTTG</chem>
--	--	--

m18/pUC18
0164
TCTATATGGTGGAATTGTAGCGATAAATTTCACACAGGAAACGCTATGACCATGATTACGAAATTCGAGCTCGAGTACCCGGGGATCTCTAGAGTCGAGCTCGAGCTGAGCTGGACTGAGCTGGTTTACACGCTCTGACTGGAAACCCCTGGCG
 Proline Thr Asn Ser Ser Ser Val Pro Gly Asp Pro Glu Glu Ile Ser Leu Ala Val Val Leu Gln Arg Arg Asp Ile Pro Glu Asn Pro Gly -tae2

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 synthetase operon including bacitracin synthetase 1 (bacA), 2 (bacB) and 3 (bacC) genes, complete cds	52	1e-04
emb Z99113 BSUB0010 Bacillus subtilis complete genome (section 10 of 21): from 1781201 to 2014980	48	0.002
emb 234883 BSPEPSYN B.subtilis genes for peptide synthetase and penicillin binding protein	48	0.002
gb L14596 SYCTRPB Synechocystis sp. tryptophan synthase beta subunit	40	0.38
dbj D64006 SYCSLLLH Synechocystis sp. PCC6803 complete genome, section 10 of 21	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|234883|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 aatgaatacggaccgacagaaaacagcgtgg 247
 |||||||||| || ||||||||||| ||||
 Sbjct: 11987 aatgaatacggcccacagaaaacagtgtgg 12017

gb|L14596|SYCTRPS 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 agagtaattattcttttcc 350
 |||||||||||||||||||
 Sbjct: 1833 agagtaattattcttttcc 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 agagtaattattcttttcc 350
 |||||||||||||||||||
 Sbjct: 75984 agagtaattattcttttcc 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 aatgaatacggaccgacagaaaacagcgtgg 247
 |||||||||| || ||||||||||| ||||
 Sbjct: 8171 aatgaatatggccgacagaaaacagtgtgg 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 ggacctgcagcagttaag 82

|||||||||||||||||||

Sbjct: 64 ggacctgcagcagttaag 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 BACGSNDS5	Bacillus subtilis gene for glucosamine syn...	331	2e-88
emb X01631 ECOMC	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 ECOUW82	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 K74895 SMFPOLORF	Simian foamy virus type 3, gag polyprotein ...	38	4.5
emb 268336 CEBF22B3	Caenorhabditis elegans cosmid F22B3, complet...	38	4.5
emb 282547 RPMZ82547	R.prowazekii genomic DNA fragment (clone A1...)	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	atgtgttggaaatttgttaggttatcggtcagttatgcgaagagatTTttaaaaggaa	60
Sbjct: 5613	atgtgttggaaatcgtaggttatatcggtcagttatgcgaaggaaattttataaaaagg	5672
Query: 61	ttagagaagcttgagtaccgcgttatgactctgcgttatcggttatgcgttgccaatggc	120
Sbjct: 5673	ttagagaagcttgagtatcgcgttatgactctgcgttatgttgccacgttatgcgttgccacgcacgc	5732
Query: 121	ggcgtcatgttacaaagaaaaaggccccatcgccgttttatgcgttcaatggatcac	180
Sbjct: 5733	gaaatccatgtttcaaagaaaaaggccatcgccatcgattttcgttcaatggatgc	5792
Query: 181	acgggttaatctcaagcggaaatcgccatacatcgacttgcgtttttttttttttttttttt	240
Sbjct: 5793	aatgtagaagcggaaatggccatcgactcgccatcgattttcgttcaacggcaaccaagg	5852
Query: 241	ttctcgaaacgctcaccccatcaaaggcgactcgccgtttttttttttttttttttttt	300
Sbjct: 5853	tatctcgaaacgctcaccccatcaaaggcgactcgccatcgattttcgttcaacacgg	5912
Query: 301	gtgtatcgagaactatgttcagctgttttttttttttttttttttttttttttttttt	360
Sbjct: 5913	gtgtatcgagaactatgttcagctgttttttttttttttttttttttttttttttttt	5972
Query: 361	gacacgggactatgttcggatcgatcgatcgatcgatcgatcgatcgatcgatcgatcg	420
Sbjct: 5973	gacaccgatatacgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcg	6032
Query: 421	acagaagaagcgttccgcAAAACACTGACTCTGTTAAAGGCTTACGCAATTGCATTA	480
Sbjct: 6033	acagaagaagcgttccgcAAAACACTTACACTGTTAAAGGCTTATGCATTCGTTA	6092
Query: 481	tttgcgttt	540
Sbjct: 6093	ttcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcg	6152
Query: 541	ggccttggatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcg	600
Sbjct: 6153	gttccggatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcg	6212
Query: 601	aatgtatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcg	660
Sbjct: 6213	aacgtatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcgatcg	6272
Query: 661	ataaaaaaaaaaaaaccttgcgggtgaaatgcgcgatcgatcgatcgatcgatcgatcg	720
Sbjct: 6273	atcaaaaaaccttgcgggtgaaatgcgcgatcgatcgatcgatcgatcgatcgatcgatcg	6332

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

Query: 721 agtgatatcgaaaaaggcacataccctcaactacatgttaaaaagaacggatgagcagccg 780
 Sbjct: 6333 agtgatatcgaaaaaggcacgtaccctcaactacatgttgaaaaaacggatgagcagcc 6392

Query: 781 ctttgttatgcgcasaatcatccaaacgttatcccgagcggacactggccgtggcc 840
 Sbjct: 6393 gtttgttatgcgcasaatcatccaaacgttatcaagatgaaaacggcaagctgtgtgcct 6452

Query: 841 ggcgatgtcgctgacgcgtggcgaaagcgacccgtttatatcggttcggaaacg 900
 Sbjct: 6453 ggcgatgtcgctgacgttgcggaaacggacccgttatatcatggctgcggaca 6512

Query: 901 agtaccacgcggcttcgtcgaaacaaatattgaaatgtggcaacgttaccggtt 960
 Sbjct: 6513 agtaccatgcaggacttgcgttgcggaaacaaatattgaaatgtggcaacgttaccggtt 6572

Query: 961 gaagtgcgttagcgagtgttgcatttacaacatgcgcgttgcgtcaagaaggccgtc 1020
 Sbjct: 6573 gaagtgcgttagcgagtgttgcatttacaacatgcgcgttgcgtcaagaaggccgtc 6632

Query: 1021 ttatcttccttctcaaagcgagaaacagcgacccgcggctgttgcgtcaagt 1080
 Sbjct: 6633 ttatcttccttctcaaagcgagaaacagcgacccgcggctgttgcgtcaagt 6692

Query: 1081 aaagcgctgggtcacaaagcgctgacgattacaacgttccggatcaacgcgtttccgt 1140
 Sbjct: 6693 aaagcgctcgacacaaagccctgacaatcacaaacgttacccgttgcgtcaacgcgttccgt 6752

Query: 1141 gaagcggttgcgttgcacgcggccctgagatcgccgtggcatcaacaaaa 1200
 Sbjct: 6753 gaagctgtactatacttgcgttgcgttgcggccctgagatcgccgtggcatcaacaaaa 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 atatcggtttgatttatgtcaagaattatgttatcgccggaaacgcgttatggaaaggccctct 1327
 Sbjct: 6880 atatcggtttgacctcgtaaagaactcggttatcgctgcaacgcgttatggaaaggcttat 6939

Query: 1328 ggcgaccagaaggacgaaatggaaatgtatcgacgtgttgcgttgcgttatccaaacgcgttt 1387
 Sbjct: 6940 ggcgaccagaaggacgaaatggaaatgtatcgacgtgttgcgttgcgttatccaaacgcgttt 6999

Query: 1388 ctttcttcatcgccgcggccctgactacttcgtgtgtcgaaaggccctgaaagctga 1447
 Sbjct: 7000 ctttcttcatcgccgcggccctgactacttcgtgtgtcgaaaggccctgaaagctga 7059

Query: 1448 aagagatttttacatccaggcgaaaggcttcggccggcgagctgttgcgttatccaaacgcgttt 1507
 Sbjct: 7060 aagagatttttacatccaggcgaaaggcttcggccggcgagctgttgcgttatccaaacgcgttt 7119

Query: 1508 tgcgtctgttatgttgcgttatccaggcgaaacccgtttcgccgttatccaaacgcgttatccaaacgcgttt 1567
 Sbjct: 7120 ttgcgttatgttgcgttatccaggcgaaacccgttatccaaacgcgttatccaaacgcgttt 7179

Query: 1568 tgagcatcccggtaatgttgcgttatccaggcgaaacccgtttcgccgttatccaaacgcgttatccaaacgcgttt 1627
 Sbjct: 7180 taagcatcccggtaatgttgcgttatccaggcgaaacccgttatccaaacgcgttatccaaacgcgttt 7239

Query: 1628 cgctgaaaggcttagaagacgcgcacgcacatccatcgccggaaatgcgttatccatccatcc 1687
 Sbjct: 7240 cactgaaaggccctagacgtcggttatccatccatccatccatccatccatccatccatcc 7299

Query: 1688 ttgcgtccgtgtttctgtgtccattgcgttatccatccatccatccatccatccatccatcc 1747
 Sbjct: 7300 ttgcgtccgtgttatccatccatccatccatccatccatccatccatccatccatccatcc 7359

Query: 1748 gcccgtgttatccatccatccatccatccatccatccatccatccatccatccatccatcc 1799
 Sbjct: 7360 gcccgtgttatccatccatccatccatccatccatccatccatccatccatccatccatcc 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: 1612 aacacattgcataatctcgctgaaaggcttagaagacgcacgacagattcattccgg 1671
Sbjct: 18623 aacacattgcataatctcgctgaaaggcttagaacatgcggatgacagattcgatgccg 18682

Query: 1672 gaagtcaaccctcgctt 1689
Sbjct: 18683 gaagttaaacacagcgctt 18700

Score = 52.0 bits (26), Expect = 3e-04
Identities = 35/38 (92%), Positives = 35/38 (92%)

Query: 1423 tgggtcgaaaggcgccctgaaagctgaaagagatttttta 1460
Sbjct: 18466 tgggtcgaaaggcgactgaaagctgaatgagatttttta 18503

emb|z99104|BSU80001 Bacillus subtilis complete genome (section 1 of 21): from 1 to 213080
Length = 213080

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

Query: 1 atgtgttggaaattttaggttatcatcggtcagcttgcgaaagagattttttaaaaaggaa 60
Sbjct: 200263 atgtgttggaaatcgtaggttatcatcggtcagcttgcgaaagggattttattaaaaagggg 200322

Query: 61 ttagagaagctttagtaccgcggttatgactctgcggatcgatgcgttgcggaaatggatgg 120
Sbjct: 200323 ttagagaagctttagtaccgcggttatgactctgcgttgcggatggatgg 200382

Query: 121 ggcgtgcattgttcaaaaagaaaaggccgcattgcggacatcttcgttgcgaaatggatggatcac 180
Sbjct: 200383 ggaatccatgttcaaaaagaaaaggccgcattgcgatcttcgttgcgaaatggatggatgcc 200442

Query: 181 acgggttgcatttcgcggaaatcgccatcacaatcgatgcggccacttcgttgcgaaatggatgg 240
Sbjct: 200443 aatgttgcggaaatggccatctgcgttgcggccatcgatgcggatggatgg 200502

Query: 241 ttccgttgcatttcgcggatcgatgcgttgcggatcgatgcgttgcggatggatggatgg 300
Sbjct: 200503 tatcttgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatggatgg 200562

Query: 301 gtgtatgcggatcgatgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatggatgg 360
Sbjct: 200563 gtgtatgcggatcgatgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatgg 200622

Query: 361 gacacggacactgaaatgttcgttgcggatcgatgcgttgcggatcgatgcgttgcggatgg 420
Sbjct: 200623 gacacccgttgcggatcgatgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatgg 200682

Query: 421 acagaagaacgcgttccgcaaaaactgcgttgcggatcgatgcgttgcggatcgatgcgttgcatta 480
Sbjct: 200683 acagaagaacgcgttccgcaaaaactgcgttgcggatcgatgcgttgcggatcgatgcgttgcatta 200742

Query: 481 ttgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatgg 540
Sbjct: 200743 ttgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatgg 200802

Query: 541 ggcgttgcggatcgatgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatgg 600
Sbjct: 200803 ggcgttgcggatcgatgcgttgcggatcgatgcgttgcggatgg 200862

Query: 601 aatgttgcgttgcggatcgatgcgttgcggatcgatgcgttgcggatggatggatggatgg 660
Sbjct: 200863 aatgttgcgttgcggatcgatgcgttgcggatggatggatggatggatggatgg 200922

Query: 661 attaaaaacccgttgcggatcgatgcgttgcggatcgatgcgttgcggatggatggatgg 720
Sbjct: 200923 attaaaaacccgttgcggatcgatgcgttgcggatggatggatggatggatggatgg 200982

Query: 721 agtgatatcgaaaaaggccatccctcaactacatgttgcggatcgatgcgttgcggatggatgg 780
Sbjct: 200983 agtgatatcgaaaaaggccatccctcaactacatgttgcggatcgatgcgttgcggatgg 201042

Query: 781 cttgttatgcgcggatcgatgcgttgcggatcgatgcgttgcggatggatggatggatgg 840
Sbjct: 201043 gttgttatgcgcggatcgatgcgttgcggatcgatgcgttgcggatggatggatgg 201102

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

Query: 841 ggcgatgtcgctgacccgtggcggaaagcgaccgcatttatatcgctggctgcggaaacg 900
 Sbjct: 201103 ggcgatatecgctgccgtgtacggaaagcgaccgcatttatatcgctgcggaaaca 201162
 Query: 901 agtacccacccgggtttgtcgaaaacaataatattgaaatgtggcggaaacgtaccggtt 960
 Sbjct: 201163 agtacccatcgaggacttgcgttaacaataatattgaaatgtggcggaaacgtgcgggtt 201222
 Query: 961 gaagtgcgttagcgagtgaattcttacaacatgcgcctctgtctaagaagccgctc 1020
 Sbjct: 201223 gaagtgcgttagcgagtgaattcttacaacatgcgcctctgtctaagaacccgctc 201282
 Query: 1021 ttatcttccttctcaaagcgaggaaacagcgacagccgcgcgtgctgttcaagtc 1080
 Sbjct: 201283 ttcattttccttctcaaagcgaggaaacagcgacagccgcgcgtactgttcaagtc 201342
 Query: 1081 aaagcgctgggtcacaaagcgctgacgattacaaacgttccggatcaacgtttccgt 1140
 Sbjct: 201343 aaagcgctggacacaaagccctgacaatcacaaacgtacccgtatcaacgtttccgt 201402
 Query: 1141 gaagcggttacacatgtttctgcacgcaggccctgagatgcgcgtggatcaacaaaa 1200
 Sbjct: 201403 gaagctgactatacatgtgttcatgcaggccctgagatgcgtgtgcgtcaacgaaa 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 atatcggtttgatttagtcaaagaatttaggtatcgccggaaacgcctatggaaagccctct 1327
 Sbjct: 201530 atatcggtttgacccgtcaaaagaactcggtatcgctgcggaaacgcgttat 201589
 Query: 1328 gcgaccagaaggacgaaatggaaatgatcgacgtgagttactgtactgttcaagaaacg 1387
 Sbjct: 201590 gcgaccagaaggacgaaatggaaatgatcgctgtgaaatccactgtatccagaaatg 201649
 Query: 1388 ctttcttcatcgccgcggccttactacttcgtgtgtcgaaaggccgttgcggactgtga 1447
 Sbjct: 201650 ctttcttcatcgacgcggccttactacttcgtatgtgtcgaaaggccgttgcggactgtga 201709
 Query: 1448 aagagatttcttacatccaggcggaaaggcttcgcggccggcgagctgaaacatggaaacaa 1507
 Sbjct: 201710 aagagatttcttacatccaggcggaaaggcttcgcggccggcgagctgaaacatggaaacga 201769
 Query: 1508 tcgtctgattgaaaggaaacaccggctttgcgtgcacacaaggaaacacgtcaacc 1567
 Sbjct: 201770 ttgccttgatcgaaacaggaaacaccgttattcgactggcaactcaagagcatgtaaacc 201829
 Query: 1568 tgagcatcccggtaatgtgaaggaaatcgccggccaaacactgtcatcatct 1627
 Sbjct: 201830 taagcatcccggtaaacgtcaaaaggatgtgtcgccggagcaacacatgtcatcatct 201889
 Query: 1628 cgctgaaaggcttagaaaggacgcacgcacatccgtccggaaatgcacccctgcgc 1687
 Sbjct: 201890 cactgaaaggccatgcgtcgatgcggatgcacatccgttattgcggaaatgcacccgcgc 201949
 Query: 1688 ttgcgtccgtggtttctgtgtgcattgcagctgtatcgcttactacgtgtactgcacc 1747
 Sbjct: 201950 ttgcgtccgtggatctgtgttccattgcagctgtatcgcttactatgtgtactgcacc 202009
 Query: 1748 gcccgtgtgacgttataacccgcacccgttgcggaaatgttacgggttgc 1799
 Sbjct: 202010 gcccgtgtgatgtggataaacctcgtaacccgttgcggaaatgttactgttgc 202061
 gbr|B21932|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 atgtgtggattgttagttacatcggtcagcttgcgtatgcggaaagagatttgttaaaagg 60
 Sbjct: 312 atgtgtggattgttagttacatcggtcagcttgcgtatgcggaaagagattttattaaaagg 371

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

Query: 61 ttagagaagttgagtaccgcggattatgactctgcggatcgctgtggcgaatgagcag 120
 Sbjct: 372 ttagagaagttgagtatcgccgttatgactctgcgttgttgcgttgcacacgaa 431
 Query: 121 ggcgtgcgtgtacaagaaaaaggcccatcgccgacccctgtgaagtggatcac 180
 Sbjct: 432 ggaatccatgttcaaagaaaaaggacgcattgcagatcttcgtgaagtggatccc 491
 Query: 181 acgggtgaatctcaagcggaatcgccatacgcgtggcactcaggtgaaccaagc 240
 Sbjct: 492 aatgtgaaagcggaaattggcatactgcgtggcacaacacggcgaaccaagc 551
 Query: 241 ttccctgaacgcgtcaccgcataaagcgcactcgccgtttacacttgcgttgcac 300
 Sbjct: 552 tatctgaacgcgtcaccgcataaagcgcactcgccgtttacacttgcgttgcac 611
 Query: 301 gtgatcgagaactatgttcgttgaaatgcgttgcac 360
 Sbjct: 612 gtgatcgagaactatgttcgttgaaatgcgttgcac 671
 Query: 361 gacacggacactgaagttagtgcgttgcac 420
 Sbjct: 672 gacacggatcagaagtagtgcgttgcac 731
 Query: 421 acagaagaagcgttccgaaaaactgactctgttgcac 480
 Sbjct: 732 acagaagaagcgttccgaaaaacttacactgttgcac 791
 Query: 481 ttgcgttgcac 540
 Sbjct: 792 ttgcgttgcac 851
 Query: 541 ggccgttgcac 600
 Sbjct: 852 ggccgttgcac 911
 Query: 601 aatgaatacggttgcac 660
 Sbjct: 912 aacgaatacggttgcac 971
 Query: 661 atcaaaaacccgttgcac 720
 Sbjct: 972 atcaaaaacccgttgcac 1031
 Query: 721 agtgcgttgcac 780
 Sbjct: 1032 agtgcgttgcac 1091
 Query: 781 ctgttatgcgttgcac 840
 Sbjct: 1092 ctgttatgcgttgcac 1151
 Query: 841 ggcgtatgcgttgcac 900
 Sbjct: 1152 ggcgtatgcgttgcac 1211
 Query: 901 agtaccacgcggcttgcgttgcac 960
 Sbjct: 1212 agtaccacgcggcttgcgttgcac 1271
 Query: 961 gaagtgcgttgcgttgcac 1020
 Sbjct: 1272 gaagtgcgttgcgttgcac 1331
 Query: 1021 ttatcccttgcgttgcac 1080
 Sbjct: 1332 ttatcccttgcgttgcac 1391
 Query: 1081 aaagcgctgggttgcgttgcac 1140
 Sbjct: 1392 aaagcgctgggttgcgttgcac 1451
 Query: 1141 gaagcggttgcgttgcac 1200
 Sbjct: 1452 gaagcggttgcgttgcac 1511
 Query: 1201 gc 1202
 Sbjct: 1512 gc 1513

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

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

```

Query: 1268 atatcggtttatggatcaaagaattttaggtatcgccgaaacgccatggaaagccctct 1327
        ||||| ||||| | | | | | | | | | | | | | | | |
Sbjct: 1579 atatcggtttatggatcaaagaactcggatcgctgcggaaacgcattggaaagcttat 1638
        ||| | | | | | | | | | | | | | | | | | | | | |
Query: 1328 gcgaccagaaggacgaaatggaaatgatcgcacgtggatcgactgtttcaagaaaacg 1387
        ||| | | | | | | | | | | | | | | | | | | | | |
Sbjct: 1639 gcgaccagaaggacgaaatggaaatgatcgctgcggatcgactgtttcaagaaaacg 1698
        ||| | | | | | | | | | | | | | | | | | | | | |
Query: 1388 ctttcattcatcgccgcggccttgcactacttcgtgtgtcgaaaggcgccctgaaagtga 1447
        ||| | | | | | | | | | | | | | | | | | | | | |
Sbjct: 1699 ctttcattcatcgccgcggccttgcactacttcgtgtgtcgaaaggcgccctgaaagtga 1758
        ||| | | | | | | | | | | | | | | | | | | | | |
Query: 1448 aagagatttccatccaggcggaaaggcttcgcggcgaaatggatcgactgtttcaagaaa 1507
        ||| | | | | | | | | | | | | | | | | | | | | |
Sbjct: 1759 aagagatttccatccaggcggaaatggatcgactgtttcaagaaaacatgttttttttt 1818
        ||| | | | | | | | | | | | | | | | | | | | | |
Query: 1508 tcgcgttgcattttttttttttttttttttttttttttttttttttttttttttttttttt 1567
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 1819 ttgccttgatcgaaacaggaaacaccgttattcgactggacttcaagagcatgtttttt 1878
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1568 ttagcatccgcggtaatgtttttttttttttttttttttttttttttttttttttttttt 1627
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 1879 taagcatccgcggaaacgtttttttttttttttttttttttttttttttttttttttttt 1938
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1628 cgctgaaaggctttagaaagacgcggcggcggcggcggcggcggcggcggcggcggc 1687
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 1939 cactgaaaggcttagacgtttttttttttttttttttttttttttttttttttttttttt 1998
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1688 ttgcgtccgtgggtttttttttttttttttttttttttttttttttttttttttttttt 1747
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 1999 ttgcgtccgtgggtttttttttttttttttttttttttttttttttttttttttttttt 2058
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1748 gcggctgtgacgttataaccgcgcacccttttttttttttttttttttttttttttttt 1799
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 2059 gcggctgtgatgtggataaacctcgtaaccccttgcggaaatgtttttttttttttt 2110
        ||| | | | | | | | | | | | | | | | | | | | |

```

dbj|D21199|BACGSNDS5 Bacillus subtilis gene for glucosamine synthetase and NADH dehydrogenase subunit 5, partial sequence

Length = 1444

Score = 331 bits (167), Expect = 2e-88
 Identities = 335/391 (85%), Positives = 335/391 (85%)

```

Query: 1409 ttgactacttcgtgtgtcgaaaggcgccctttttttttttttttttttttttttttttt 1468
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 1 ttgactactttttttttttttttttttttttttttttttttttttttttttttttttttttt 60
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1469 cggaggcttcgcggcgagctttttttttttttttttttttttttttttttttttttttt 1528
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 61 cagaaggctttttttttttttttttttttttttttttttttttttttttttttttttttt 120
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1529 caccggctttttttttttttttttttttttttttttttttttttttttttttttttttt 1588
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 121 caccatgtttttttttttttttttttttttttttttttttttttttttttttttttttt 180
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1589 aggaagtgcgcggccggccggccggccggccggccggccggccggccggccggccggc 1648
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 181 aagaagtgcgcggccggccggccggccggccggccggccggccggccggccggccggc 240
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1649 cagacgcacagattttttttttttttttttttttttttttttttttttttttttttttt 1708
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 241 cggatgcacagattttttttttttttttttttttttttttttttttttttttttttttt 300
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1709 tgccatgtgcgttttttttttttttttttttttttttttttttttttttttttttttt 1768
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 301 ttccatgtgcgtttttttttttttttttttttttttttttttttttttttttttttttt 360
        ||| | | | | | | | | | | | | | | | | | | | |
Query: 1769 cgcgcaaccccttgcggaaatgtttttttttttttttttttttttttttttttttttt 1799
        ||| | | | | | | | | | | | | | | | | | | | |
Sbjct: 361 cgcgtaaccccttgcggaaatgtttttttttttttttttttttttttttttttttttt 391
        ||| | | | | | | | | | | | | | | | | | | | |

```

emb|X01631|ECUNC E. coli origin of replication oriC and genes gid, unc, EcoURF-1 and glmS

Length = 14526

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

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

Query: 76 taccgcggtatgactctgccgt 99
 ||||||| |||||||||||||
 Sbjct: 12444 taccgcggatatgactctgccgt 12467

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

Query: 214 cgctggcgactcacggtaacc 236
 ||||||| ||||| |||||||
 Sbjct: 12588 cgctggcgacccacggtaacc 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 taccgcggtatgactctgccgt 99
 ||||||| |||||||||||||
 Sbjct: 2046 taccgcggatatgactctgccgt 2023

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

Query: 214 cgctggcgactcacggtaacc 236
 ||||||| ||||| |||||||
 Sbjct: 1902 cgctggcgacccacggtaacc 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 ccgctggttctgttgtgcc 1712
 ||||||| ||||| |||||||
 Sbjct: 9305 ccgctggttctgttgtgcc 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 ccgctggttctgttgtgcc 1712
 ||||||| ||||| |||||||
 Sbjct: 1030 ccgctggttctgttgtgcc 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 taccgcggtatgactctgccgt 99
 ||||||| |||||||||||||
 Sbjct: 102984 taccgcggatatgactctgccgt 102961

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

Query: 214 cgctggcgactcacggtaacc 236
 ||||||| ||||| |||||||
 Sbjct: 102840 cgctggcgacccacggtaacc 102818

emb|Y11778|RPNTB 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 gcaA gene (continued)

gb|M74895|SMPPOLORF 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 cggcggttgcataatcggtttgatt 1282

||||||| ||||| ||||| |||||

Sbjct: 3623 cggcggttgcattcggtttgatt 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 ggtagactctgcggtatcgc 104

||||| ||||||| ||||||| |||||

Sbjct: 5228 ggtagactctgcggtatcgc 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: 1^92322775184
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
 Mbo II Nla III
 Sau3A I Nsp7524 I
 Afl III
 GATCTCCAAAAAAACATGTGGGAGGGGACGATTGAAAGTCCCCTTGTAATTGACTTCTCGTCCTTTGCACCTT 80
 CTAGAAGGTTTTTTGTACACCCCTCCCTGCTAACCTTCAGGGAACTTAAACTGAAAGAACAGAGGAAAACGTGGAA
 1 15 15 16 59 77
 3 15 15 16 59 77
 23

SfaN I
 Mbo II Cvi JI
 Mnl I Mae III Alu I Mse I
 TAGGAGGAAGAAAATATGTTGGAATTGTAGGTTACATCGGTCAAGCTTGATGCCRAAGAGAGATTTGTTAAAGGATTAGA 160
 ATCCTCCTTCTTTATACACACCTTAACATCCATGTAGCCAGTCGAACACTCGCTTCTCTAAACAATTTCTAATCT
 84 112 124 147
 87 124 129

BstU I
 Sec I
 Cvi JI Sac II Cfr10 I Rsa I
 Alu I Rsa I Ple I Hpa II Nla III Hae
 III HinD III NspB II HinF I Nsp7524 I Cvi JI
 162 173 183 183 190 221 240
 163 170 183 190 222 240
 163 173 189 227
 173 174

Hae III
 Cvi JI
 Gdi II
 Eae I Ple I
 SfaN I Sau3A I Alw I HinF I HinF I HinF I
 Fnu4H I 241 269 282 295 317
 244 270 299 300 300
 320

Fnu4H I
 Hae III
 Cvi JI
 Gdi II
 Eag I Taq I
 Sau3A I
 Cvi JI
 Alu I SfaN I Hha I Eae I
 Hph I HinD III Hph I Hha I Eae I
 324 332 347 361 368 398
 333 353 368 369
 333 368 369
 370 369 400

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					
CGAGAACTATGTTCAGCTGAGCGCGAATATCTGAAAACGTTGACTGAAAAGCGACACGGACACTGAAGTAGTCGTTT	480								
GCTCTTGATAACAGTCGACTTCGGCTTATAGAACTTTGCAACTTGCACATTGACTTTCCGTGTGCCGTGTGACTTCAGCAAG									
V		V		V		Y	V	V	V
414	423			439					
414	422								
415									
415									
Dde I									
Taq I	Ple I								
Sau3A I	Hinf I								
	Mnl I			Mbo II					
AAATGATCGAGCAATTGTCGCGGGAGGACTCACCGCACAGAAGAACGCGTTCCGAAAAACACTGACTCTGTTAAAGGCTCT	560								
TTTACTAGCTCGTTAACACCGCCCTCCGTGAGTCGTCTTCTCCGACAGGCGTTTGTGACTGAGACAATTCCGAGA									
	V	V				V	V	V	V
485	505			519			542		555
487	508			508			542		549
508									
510									
Sty I									
	Hae III								
	Cvi JI								
Hph I									
	Mae II								
	Cvi JI								
	Mae I								
	Cvi JI								
TACGCCATTGATTGGCAGGGATGAAAACACAGACACCATTTACGTTGAAAAACAAAAGCCCTCTGTTAACGTTGGCCT	640								
ATGCGTTAACGTAATAAACACTGCCACTTTGTGTCTGTTAACATGCAAGTTTGTGTGTTGGAGACAATTAGCCCGA									
V	V	V	V	V	V	V	V	V	V
582			604			621		630	638
						624		636	
								636	
									638
Mae II									
Mae I	Hga I	Cvi JI							
	SfaN I	BstU I							
TGGAGATACGTTAACGTCGCGCATCTGACCGCATGGCTATGCTCAAGTAACGAATGAAATCGTTGAGCTTGGACA	720								
ACCTCTATGCAATTGCAAGCACCGTAGACTGCGCTACCGATAACGAAAGTTCATTGTTACTTATGCAACTCGAACCTGT									
	V	V		V		V	V	V	V
648	663		671			690		703	
652	669		677					709	
655									709
Mae II									
Hph I	Pml I								
	Afl III								
	Nla III								
Sau3A I									
Hph I	Mae III	Cvi JI	Mse I						
	Hph I	BspH I							
AAGAAATGGTATCGTGACAAAAGATGAAAGCCGTGATTAAAAACCTTGACCGTGAAAGTCATGACACGTGCGCTTATATC	800								
TTCTTTACCACTAGCACTGTTCTACTTCGGCACTAAATTGAACTGCCACTTCAGTACTGTGACCGAGAATATAG									
	V	V		V		V	V	V	V
728	735		749		757		771	778	789
731							779		
								783	
								784	
								785	
Cvi JI									
Alu I									
Dde I	Hga I	Taq I							
Esp I	Aha II	EcoR V							
	Mnl I	Afl III							
	Nla III								
	Nsp7524 I								
GCTGAGCTTGGCGCAGTGATATGCAAAGGACACATACCCCTCACTACATGTTAAAAGAACGGATGAGCAGCCGCTTGT	880								
CGACTCGAACCTGGGTCACTATAGCTTTCCGTGTAGTACAATTTCCTACTCGTCGGCGAAC									
	V	V	V	V	V	V	V	V	V
801	810		819			840	847	863	871
802	810		823				847		869
805							848		869
805								852	872

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

Hha I	Fsp I	Fok I	Mae II	Nae I	Hae III	Gdi II	Eae I
				Sec I	Hae III	Cvi JI	Hpa II
863	893	900		Gdi II	Cfr10 I	Eae I	Cvi JI
884							
				V	V	V	V
				925	932	948	
				925	933	948	
				926	934		952
				926			
				928			
						931	
						931	
						932	
						933	
Sau96 I	Ava II	Rsr II	Cvi JI	Cvi JI	Hpa II	Dde I	Fnu4H I
				Alu I	Cfr10 I	Fnu4H I	Cvi JI
964			983	996	1005	1102	1109
965				996	1006		
965						1108	
	Hpa II	Cfr10 I					
Rsa I	Mae II	Nsp7524 I	EcoR I	Nsp7524 I	Dde I	Fnu4H I	Mbo II
GCAAACGTACCGGTTGAAGTCATGTAGCGAGTGAATTCTCTTACAACATGCCGCTCTGTCTAAAGAACAGCCCTTTAT							
CGTTTGCATGGCCAACCTCACGTACATCGCTACTTAAGAGAAATGTTGTACGGCGAAGACAGATTCTCGGGAGAAAATA							
1045.		V	V	V	V	V	V
1047		1061	1074	1087	1102	1109	1120
1049		1062		1088			
1050				1091			
Hha I	BstU I	Cvi JI	NspB II	Fnu4H I	Tth111 II	Eco47 III	Eco47 III
CTTCCCTTCTAAAGCGGAGAACAGCGGACAGCGCGCCGTGCTGTTCAAGTCAAAGCGCTGGGTACAAAGCGCTGA							
GAAGGAAAGAGTTCGCCTTTGTCGCGCTGTCGGCGGACGAAACAAGTTCAAGTTTCGCGACCCCCAGTGTTCGCGACT							
V	V	V	V	V	V	V	V
1144	1153	1162		1178		1186	
	1152			1178			
		1155		1179		1193	
		1156				1194	
Sau3A I	SceF I	Nci I	Hpa II	Bcl I	Mae II	Hae III	EcoO109 I
Mae II	Alw I						Sec I
CGATTACAAACGTTCCGGGATCAACGCTTCCGTGAAGCGGATTACACATTGCTCTGCACGCAGGCCCTGAGATGCC							
GCTAATGTTGCAAGGCCCTAGTTGCGAAAGGGCACTTCGCCCTAATGTGTAACCGAAGACGTGCGTCCGGGACTCTAGCGG							
1210	1218				1264	1274	
1215					1265		1279
1215						1266	
1215						1266	
1215						1266	
1219						1270	

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

SfaN I Xba I Cvi JI Xba I Sma I BstU I Nru I Mn1 I Hpa I Fnu4H I Bbv I	Alw I Acc I Dde I	GTCGCATCAACAAAAGCGTATAACGGCTCAGATTGCCGTCTCGCGATCCCTGGCTCCGGTTGCAGCAGAACGCAACGGCGT 1360							
1284	1298	1306	1319	1332	1341	CACCGTAGTTGTTTCGCATATGCCGAGTCAACGGCAGGAGCGCTAGGAACGCGAGGCAACGTCGTCTGGCGTTGCCA			
1298			1321		1341				
		1304	1322						
				1325					
				1325					
							Nla III		
							Sty I		
EcoR V	Fnu4H I	BstU I	Sec I	Mn1 I					
1362	1397	1408	1415						
	1398	1408	1418						
		1408							
		1409							
							Fnu4H I		
							BstU I		
							Sec I		
							Sac II		
							NspB II		
							Fnu4H I		
							Hae III		
							Gdi II		
							Eag I	Hae III	
							Eae I	Cvi JI	
Sau3A I	Rsa I		Mbo II	Cvi JI					
1448	1460		1486	1494					
1453				1493	1500				
1454				1493	1500				
				1493					
				1494					
				1495					
				1496					
				1496					
				1496					
				1497					
				1498					
							Hha I		
							Nla IV		
							Nar I		
							Hae II		
							Ban I		
							Aha II	Cvi JI	
Taq I	Alu I		Fok I	Cvi JI	Fnu4H I	Nla III			
1522	1537		1556	1569	1579	1593	GTCGAAGGGCCCTGAAAGCTGAAAGAGTTCTTACATCCAGGCGGAAGGCTTCGGCGGGCGAGCTGAAGCATGGAC 1600		
1527	1537		1559		1575	1585	CAGCTCCCGGGACTTCGACTTCTCTAAAGAATGTAGGTCCGCCTCCGAAGCGGCCGCGCTCGACTTCGTACCTG		
1527			1559		1575	1585			
1527					1576				
1527									
1527									
1528									

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

Hpa II Cfr10 I Hha I 1614 1625 1635 Nla IV Nar I Hae II Ban I BstU I Sec I Sac II NspB II Cvi JI Hha I Fnu4H I Aha II Bbv I Fnu4H I 1692 1699 1714 1731 1740 1753 1692 1701 1733 1737 1737 1757 1694 1702 1697 1697 1697 1698 1701 1701 1701 1701 1701 1701 Hpa II Hha I NspB II 1764 1779 1788 Cvi JI Fnu4H I BstU I Sec I Sac II Mae III Hha I Mae III NspB II Mae II BstU I Mbo II Mae I 1841 1852 1864 1877 1884 1904 1841 1849 1865 1841 1842 1843 1845	BatU I Sec I Sac II Hinc II Fok I Mae II SfaN I Acl III Dde I NspB II 1652 1662 1666 1654 1667 1670 1670 1671 Mbo II Bbv II Dde I Cvi JI Hga I Fok I 1760 TGAAGGAAAGTCGCRCCCGCGGCCAACACTTGCATCATCTCGCTGAAAGGCTTAGAAGACGCCAGACGACAGATTCACTC ACTTCCTTCAGCGTCGGCGCCGGTTGTGAACGTAGTAGAGCGACTTCCGAATCTCTCGCTCTGCTGTCAAGTAG 1692 1699 1714 1731 1740 1753 1692 1701 1733 1737 1737 1757 1694 1702 1697 1697 1697 1698 1701 1701 1701 1701 1701 1701 Sau3A I Cvi JI Alu I Pvu II NspB II Fnu4H I Bbv I 1840 CTGCCGGAAGTCAACCCCTGGCTTCTGGCTGGTTCTGGTGTGCCATTGCAGCTGATCGCTTAACCGCTGCACGTGCA GACGGCCTTCAGTTGGGACGCCAGCGACCCAAACACGGTAACGTGACTAGCGAATGATGCGACGTGACCGT 1764 1779 1788 1811 1830 1770 1811 1830 1812 1812 1813 1813 1817 Fnu4H I Bbv I 1830 1812 1812 1813 1813 1817 Cvi JI Fnu4H I BstU I Sec I Sac II Mae III Hha I Mae III NspB II Mae II BstU I Mbo II Mae I 1920 CGCGGGCTGTGACGTTGATAAACCGCGAACCTTGCAGAGAGTGTTACGGTGGAAATAATATATTAAACCCCTTGTTAT GCGGGCGACACTGCAACTATTGGCGCGTTGGAAACGCTCTCACAAATGCCACCTTATTATATAATTGGGGAAACCAATA 1841 1852 1864 1877 1884 1904 1841 1849 1865 1841 1842 1843 1845
---	--

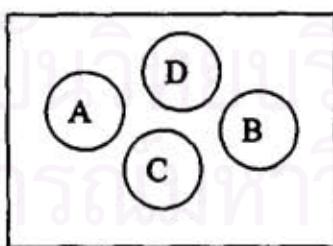
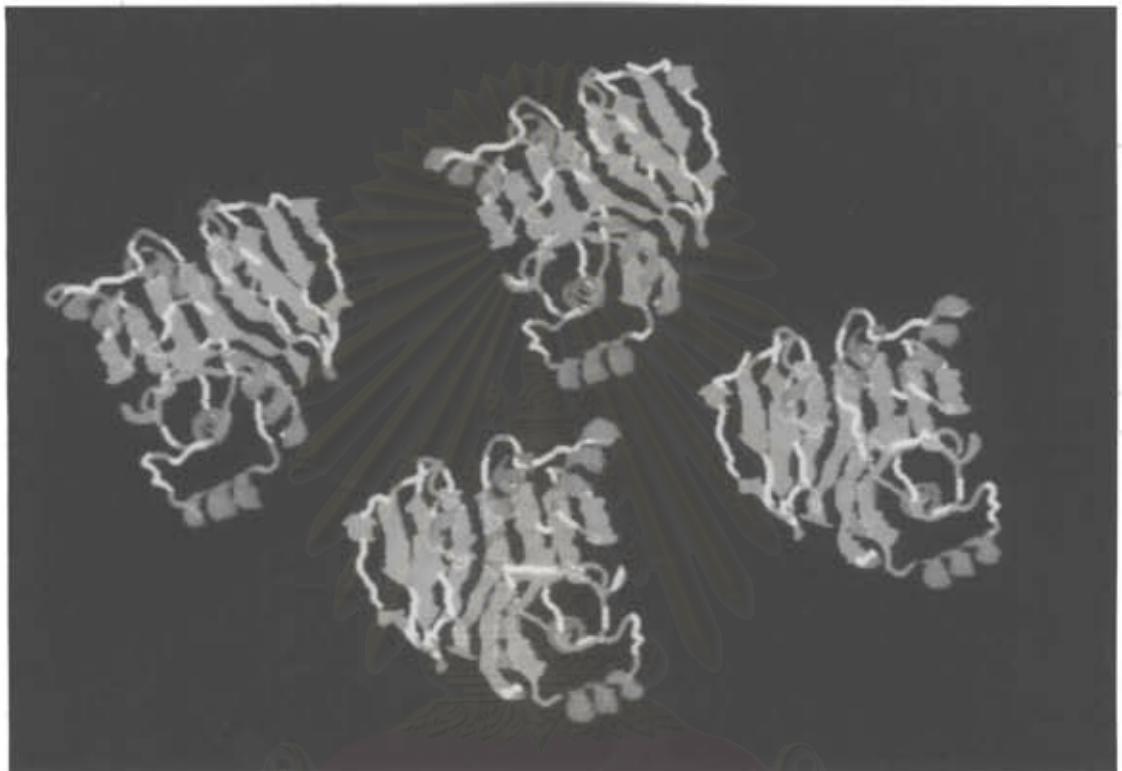
**Position of restriction endonucleases sites of *B. subtilis* TISTR25 *gcaA* gene
(continued)**

Mse I	BstU I	SfaN I	Dra I	Mse I	BstU I	
Dra I	Nru I			Dra I	Nru I	
TAGTGTGAATTAAAAATAAAAGTCGCATGTTTATAAAAAGATGCCATGTTAAAATAAAAGTCGCCANGTTATAAAAATCACA CTTAACTTAACTTATTCAGCGCTACAAATATTTTCTACGCTACAAATTATTCAGCGCTNCAAATATTTTT						2000
1930	1942		1961	1970	1982	
1931	1943			1971	1983	
Cvi JI						
Alu I				Taq I		
HinD III						
GTTGCAGCTTACCCCTTTGGATATGATTATCTAAAGGGGTGTTTGTGTCGAAAAGAAAAAGAACATCTAAATTGA CAACGCTTCGAATGGGAAACCTATACTAATAGATTTCCCCACAAAAACAGCTTCTTGTAGATTAACT						2080
V	V	V	V	V	V	
2007				2052		
2008						
2008						
Mnl I	Mnl I			Sau3A I		
Mse I	Taq I			BstY I		
				Bgl II	Mnl I	HinC II
TAAGTGGATTAAAGAGGGTCGAGGAACCTGGCAGTGGCATGATTATCAAGATCTTCCTCATTAGGTGGTCACAAAGA ATTCACTTAATTCTCCCAGCTCCTGACCGTCACCCCGACTAATAGTTCTAGAAAGGAGTAATCCAGCCAGTTGTC						2160
V	V	V	V	V	V	
2089	2099		2119	2130	2138	
2094	2101			2130		
				2131		
Hpa II				Mae II		
Mse I	Cfr10 I	Nla III		Mse I		
TTAAAGGTATAAAACCGGCAGACATGAGTTTATCGGATTGGACGAAACTACTTTATTAACGAAATTTC AATTTCCATATTTGGCCGTCTGTGACTCAAAATAGCCTAACCTTGATGAAAAAATTGACTAAAG						2240
V	V	V	V	V	V	
2161	2176	2188				
2177						
Xba I				Mbo II		
TGATGTTATTTAGATATTCTGAACAATTCTTATTACACAGAACAGAGACGTTGCCATTGCTG ACTACATAAATCTATAAGCACTTGTAAAGGAAATAATGGTGTCTCTGCAAACGGTAACGAC						2308
V	V	V	V	V	V	
2263				2287		
					2293	

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

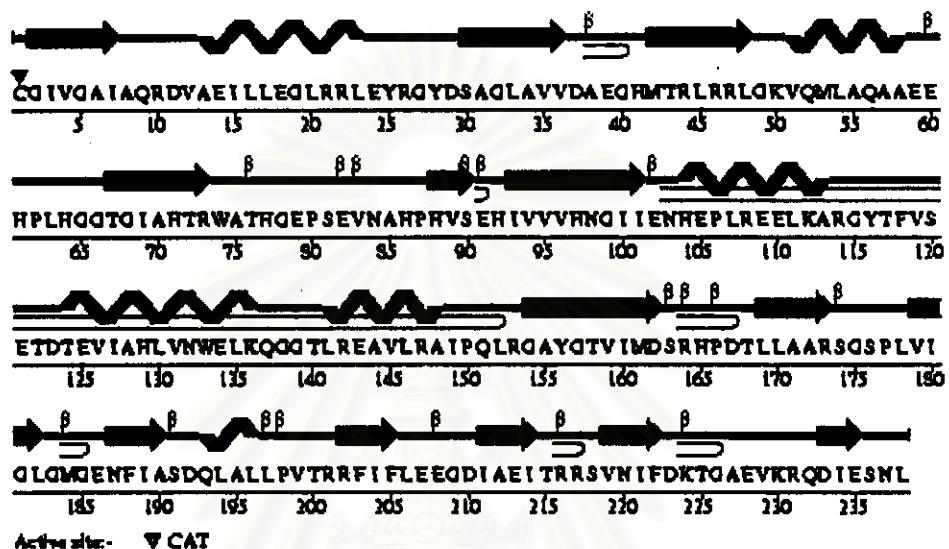
APPENDIX 6

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

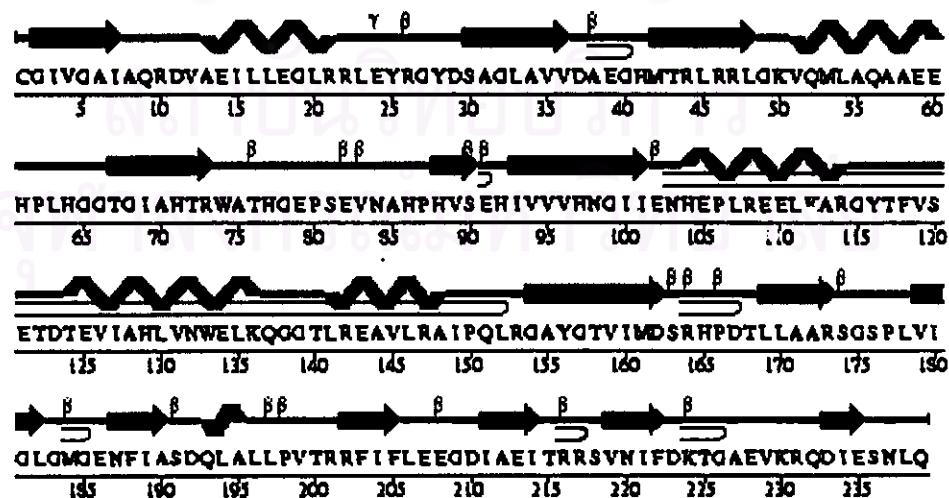


**Three-dimensional structure of *E. coli* gcaA protein in chain A, B, C and D
(continued)**

- Chain A (238 residues)

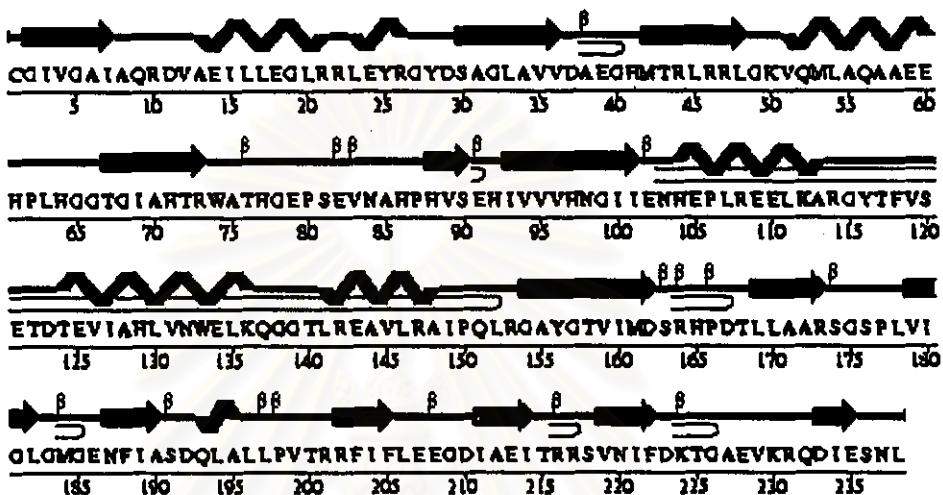


- Chain B (239 residues)

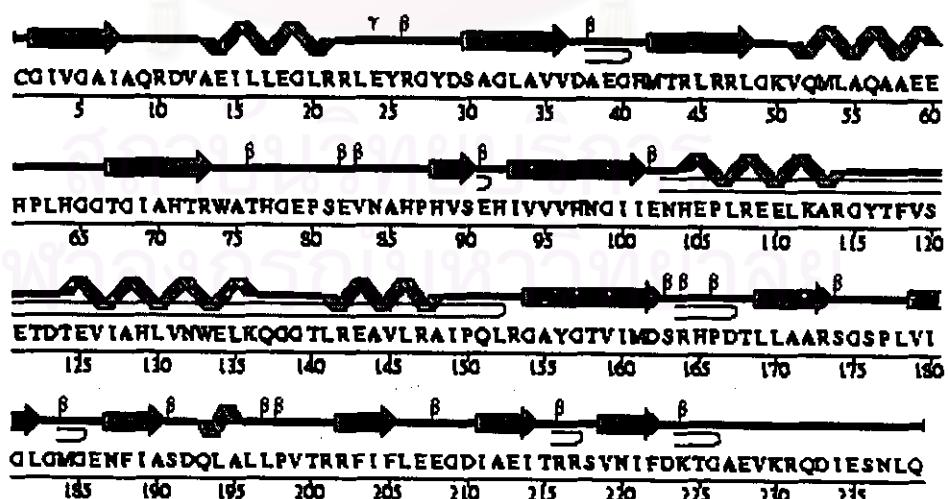


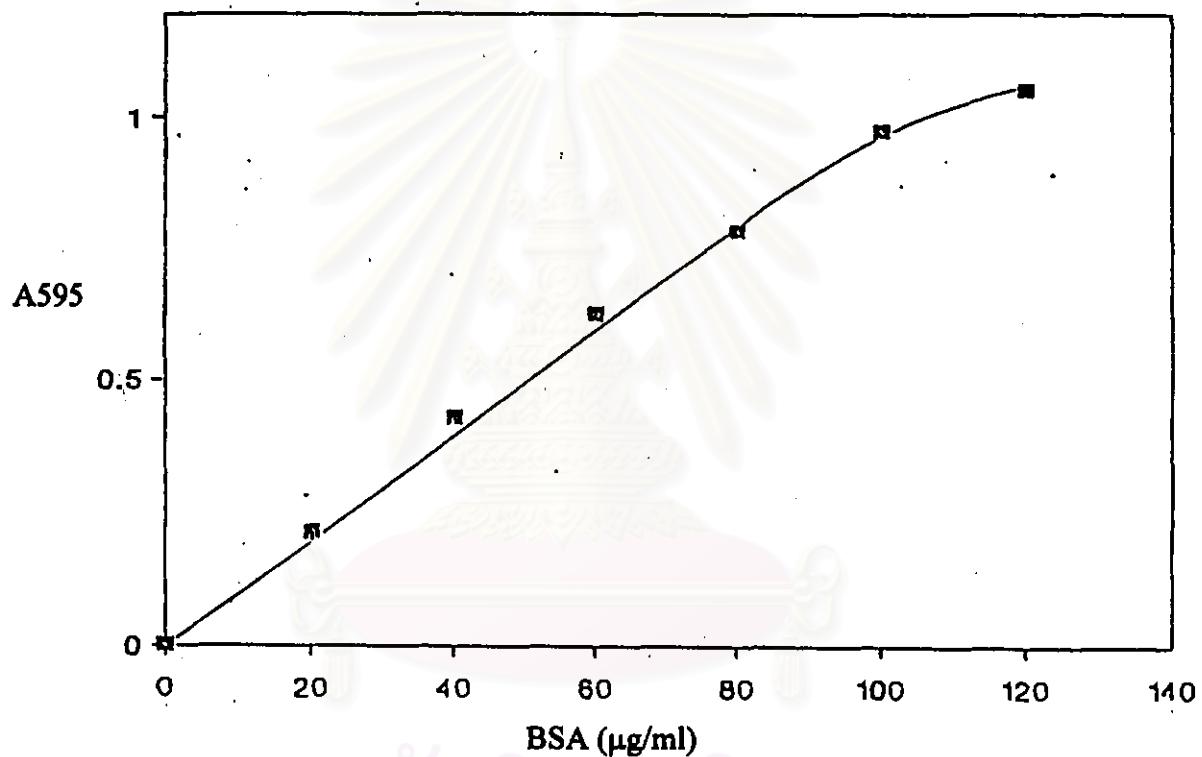
**Three-dimensional structure of *E. coli* gcaA protein in chain A, B, C and D
(continued)**

- Chain C (238 residues)

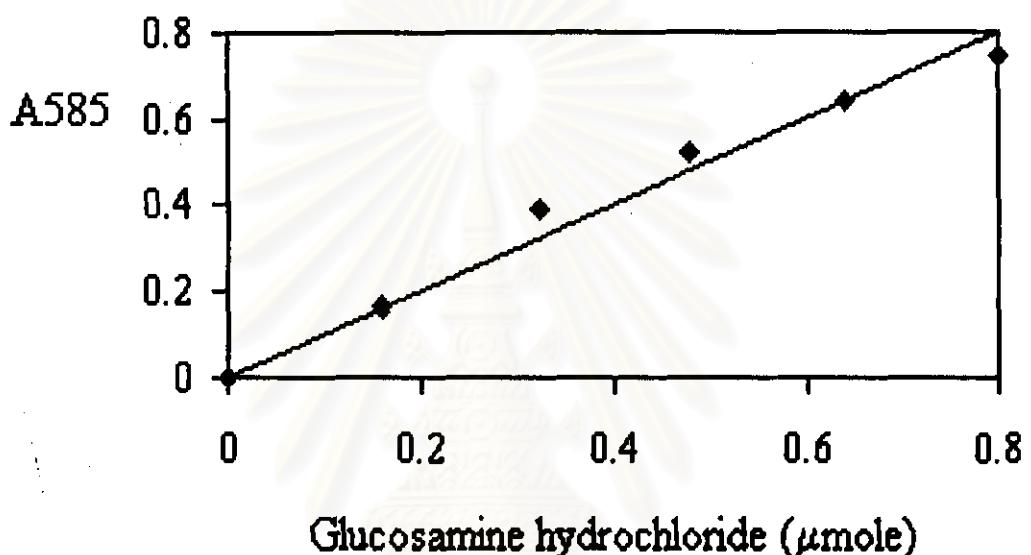


- Chain D (239 residues)



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

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX 8**Standard curve for glucosamine determination**

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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.



สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย