VIII APPENDIX

1. 20X TBE pH 8.0

Tris base	216.00	g
Boric acid	110.00	g
0.5 M EDTA	8.00	ml
Distilled water	800	ml
Adjust pH 8.0 with 6M HCl		
Adjust volume by DW to final volume	1.00	L

2. Loading buffer 6X

Bromphenol blue	0.25	%
Glyceral	30	%
Mixed thoroughly and kept at -20 °C		

3. Ethidium bromide (10 mg/ml)

Ethidium bromide	1.0	gm
Distilled water	100	ml
Dissolved and kept in a dark bottle at 4 °C		

4. 1.5% Agarose

Nuseive agarose	1.0	gm
Seakem LE agarose	0.5	gm
1X TBE	100	ml
Mixed, boiling until homogeneous melt		

5. 1Kb ladder

Stock 1 Kb	5	μl
6X Loading	10	μl
Dissolved in distilled water	45	μl

6. 10X PCR buffer

KCl	500	mM
Tris-HCl (pH 8.3)	100	mM

7. $MgCl_2$ 50 mM

$MgCl_2$	479	mg
Distilled water	100	ml

Dissolved in distilled water and sterilized by autoclave

8. PBS pH 7.4

NaCl	8.0	gm
KCl	0.2	gm
Na_2HPO_4	1.15	gm
KH_2PO_4	0.2	gm
Dissolved in distilled water		
and adjusted by distilled water to	1.0	L

9. NCBI Blast network programme.

National Center for Biotechnology Information

National Library of Medicine National Institutes of Health

PubMed Entrez BLAST OMIM Books TaxBrowser Structure

Search Nucleotide for tsutsugamushi Go

SITE MAP
Guide to NCBI resources

About NCBI
The science behind our resources. An introduction for researchers, educators and the public.

GenBank
Sequence submission support and software

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PubMed, OMIM, Books and PubMed Central

Molecular databases
Sequences, structures, and taxonomy

Genomic biology
The human genome, whole genomes and related resources

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GenBank is now accepting third party annotation of genomic sequences or computationally derived/assembled sequences. More...

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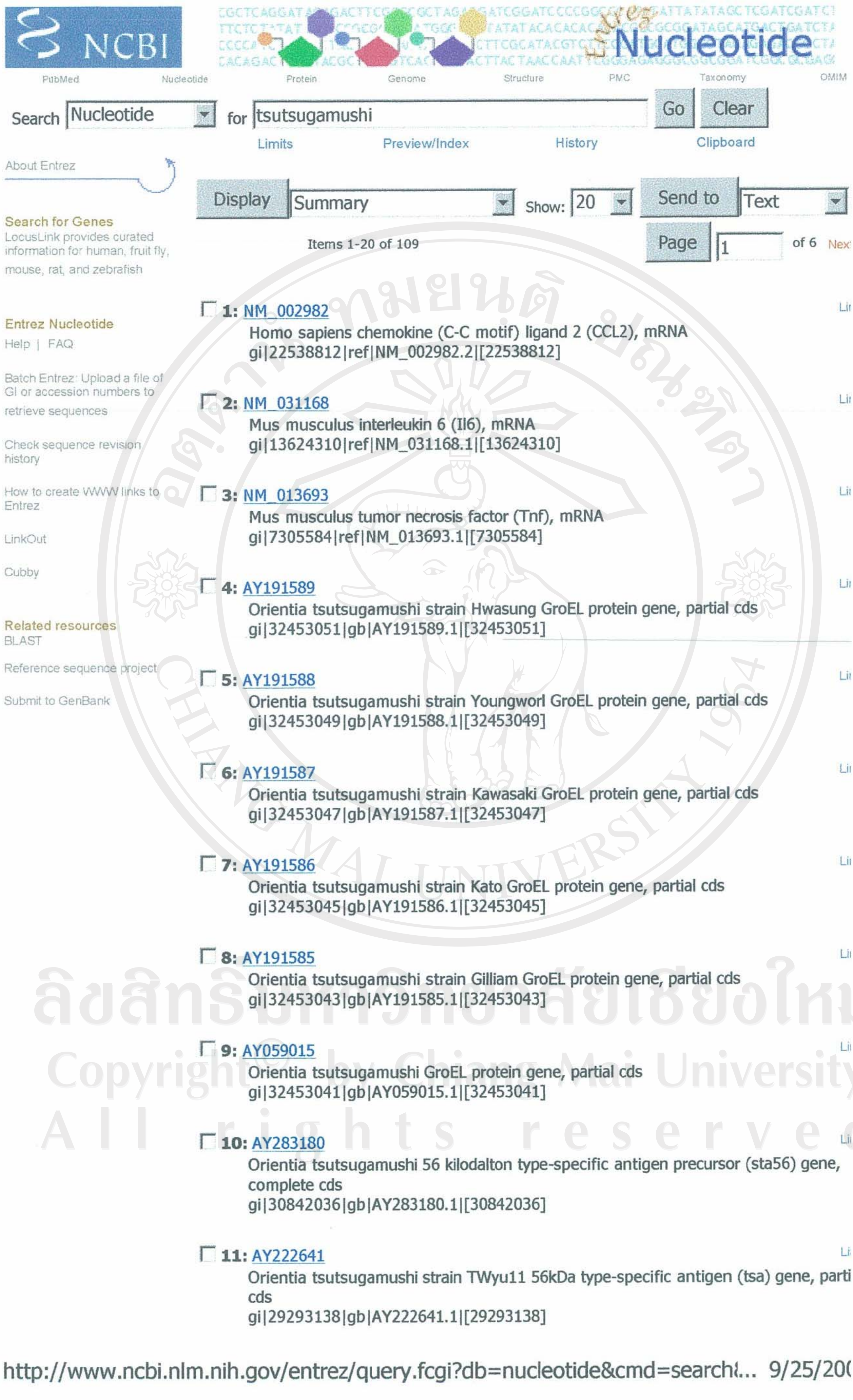
Hot Spots

- Clusters of orthologous groups
- Electronic PCR
- Gene expression omnibus
- Genes and disease
- Human genome resources
- Human/mouse homology maps
- LocusLink
- Malaria genetics & genomics
- Map Viewer
- MHC
- Mouse genome resources
- NCBI Handbook
- ORF finder
- Reference sequence project
- Retrovirus resources
- Serial analysis of gene expression
- SKY/CGH database
- SNP
- Trace archive
- UniGene
- VecScreen
- NCI-CGAP



Search Nucleotide for tsutsugamushi

About Entrez

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LocusLink provides curated information for human, fruit fly, mouse, rat, and zebrafish

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Check sequence revision history

How to create WWW links to Entrez

LinkOut

Cubby

Related resources
BLAST

Reference sequence project

Submit to GenBank

☐ **1:** NM_002982
Homo sapiens chemokine (C-C motif) ligand 2 (CCL2), mRNA
gi|22538812|ref|NM_002982.2|[22538812]

☐ **2:** NM_031168
Mus musculus interleukin 6 (Il6), mRNA
gi|13624310|ref|NM_031168.1|[13624310]

☐ **3:** NM_013693
Mus musculus tumor necrosis factor (Tnf), mRNA
gi|7305584|ref|NM_013693.1|[7305584]

☐ **4:** AY191589
Orientia tsutsugamushi strain Hwasung GroEL protein gene, partial cds
gi|32453051|gb|AY191589.1|[32453051]

☐ **5:** AY191588
Orientia tsutsugamushi strain Youngworl GroEL protein gene, partial cds
gi|32453049|gb|AY191588.1|[32453049]

☐ **6:** AY191587
Orientia tsutsugamushi strain Kawasaki GroEL protein gene, partial cds
gi|32453047|gb|AY191587.1|[32453047]

☐ **7:** AY191586
Orientia tsutsugamushi strain Kato GroEL protein gene, partial cds
gi|32453045|gb|AY191586.1|[32453045]

☐ **8:** AY191585
Orientia tsutsugamushi strain Gilliam GroEL protein gene, partial cds
gi|32453043|gb|AY191585.1|[32453043]

☐ **9:** AY059015
Orientia tsutsugamushi GroEL protein gene, partial cds
gi|32453041|gb|AY059015.1|[32453041]

☐ **10:** AY283180
Orientia tsutsugamushi 56 kilodalton type-specific antigen precursor (sta56) gene, complete cds
gi|30842036|gb|AY283180.1|[30842036]

☐ **11:** AY222641
Orientia tsutsugamushi strain TWyu11 56kDa type-specific antigen (tsa) gene, parti
cds
gi|29293138|gb|AY222641.1|[29293138]

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nucleotide&cmd=search&... 9/25/20

NCBI Nucleotide

PubMed Nucleotide Protein Genome Structure PMC Taxonomy OMIM

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☐ **101:** M63383 Li
Rickettsia tsutsugamushi 56 kDa type-specific antigen (tsw56) gene, complete cds
gi|152512|gb|M63383.1|RIRTSW56A[152512]

☐ **102:** M63382 Li
Rickettsia tsutsugamushi 56 kDa type-specific antigen (tst56) gene, complete cds
gi|152510|gb|M63382.1|RIRTST56A[152510]

☐ **103:** M63381 Li
Rickettsia tsutsugamushi 56 kDa type-specific antigen (tss56) gene, complete cds
gi|152508|gb|M63381.1|RIRTSS56A[152508]

☐ **104:** M63380 Li
Rickettsia tsutsugamushi 56 kDa type-specific antigen (tsr56) gene, complete cds
gi|152506|gb|M63380.1|RIRTSR56A[152506]

☐ **105:** M31887 Li
R.tsutsugamushi heat shock protein 11 (stp11) and major antigen 58 (sta58) genes complete cds
gi|152501|gb|M31887.1|RIRSTPSTA[152501]

☐ **106:** M33004 Li
R.tsutsugamushi scrub typhus antigen 56 (sta 56) gene, complete cds
gi|152499|gb|M33004.1|RIRSTA56[152499]

☐ **107:** L04956 Li
Rickettsia tsutsugamushi 56 kDa outher membrane protein gene, complete cds
gi|152453|gb|L04956.1|RIR56OMPA[152453]

☐ **108:** L11697 Li
Rickettsia tsutsugamushi (clone Pkt5) 47 kDa protein gene, complete cds
gi|152452|gb|L11697.1|RIR47KDA[152452]

☐ **109:** M63076 Li
R.tsutsugamushi Sta22 protein gene, complete cds
gi|152480|gb|M63076.1|RIRKARPAA[152480]

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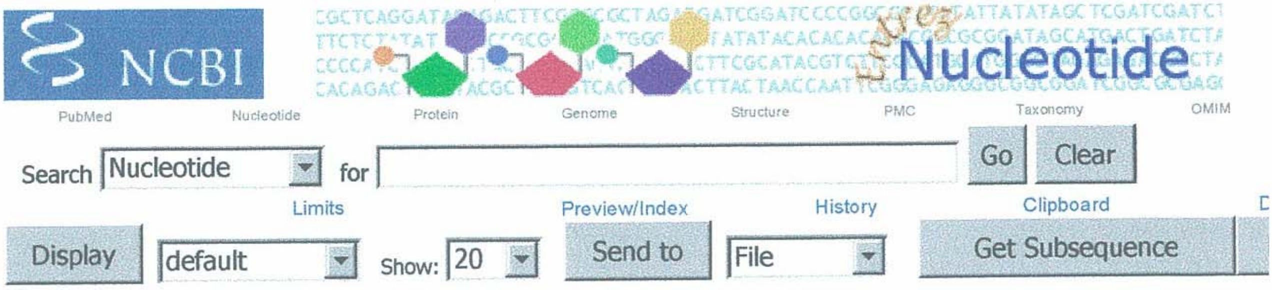

☐ **1:** M33004. R.tsutsugamushi s...[gi:152499]

Links

```
LOCUS       RIRSTA56       2280 bp    DNA     linear   BCT 26-APR-1993
DEFINITION  R.tsutsugamushi scrub typhus antigen 56 (sta 56) gene, complete
            cds.
ACCESSION   M33004
VERSION     M33004.1  GI:152499
KEYWORDS    antigen; outer membrane protein; scrub typhus antigen 56.
SOURCE      Orientia tsutsugamushi
  ORGANISM  Orientia tsutsugamushi
            Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales;
            Rickettsiaceae; Rickettsieae; Orientia.
REFERENCE   1  (bases 1 to 2280)
  AUTHORS   Stover,C.K., Marana,D.P., Carter,J.M., Roe,B.A., Mardis,E. and
            Oaks,E.V.
  TITLE     The 56 kilodalton major protein antigen of Rickettsia
            tsutsugamushi: Molecular cloning and sequence analysis of sta 56
            gene, and precise identification of a strain specific epitope
  JOURNAL   Unpublished (1990)
COMMENT     Original source text: R.tsutsugamushi (strain Karp) DNA, clone
            pRTS56H2.3.
            Draft entry and computer-readable sequence for [1] kindly submitted
            by E.V.Oaks, 16-MAR-1990. Walter Reed Institute, Dept. of Enteric
            Infections, Walter Reed Army Institute of Research, Washington,
            D.C. 20307.
FEATURES             Location/Qualifiers
     source          1..2280
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                     /mol_type="genomic DNA"
                     /db_xref="taxon:784"
     -35_signal      77..82
     -10_signal      99..103
     RBS             545..550
                     /note="ribosome binding site"
     CDS             556..2154
                     /note="scrub typhus antigen 56 precursor (sta 56)"
                     /codon_start=1
                     /transl_table=11
                     /protein_id="AAA26391.1"
                     /db_xref="GI:152500"
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                     EEGKVKADSVGETKADSVGGKDAPIRKRFKLTPPQPTIMPISIAVRDFGIDIPNQTSA
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                     FVMPQQAQQQGQGQQQQAQATAQEAVAAAAVRLLNGNDQIAQLYKDLVKLQRHAGIKK
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                     TESVSIYAGVGAGLAYTSGKIDNKDIKGHTGMVASGALGVAINAAEGVYVDIEGSYMY
                     SFSKIEEKYSINPLMASVSVRYNF"
     sig_peptide     556..621
                     /note="scrub typhus antigen 56 signal peptide"
     mat_peptide     622..2151
                     /product="scrub typhus antigen 56"
BASE COUNT      764 a    319 c    489 g    708 t
ORIGIN
        1 aagcttgttc attttttatg tgggctaatt ttagataatg caatgttagt ataattatgt
       61 ggttaattaa tgtatcttga tttaagattt tatataaata taataagatt tatgtagggc
      121 ttaattatta gcttaaaaaa ctgttgctat tttagctaaa aataaaagtt tgggcaagaa
      181 aaattattaa taattgaagg tagttgttgc gtaaaaagct gtgttatgct atctaaggtt
      241 aaatgtagca aggtgctaat agataattaa tgtattttcg aacgtgtctt taagctatat
      301 ataagagcag tattctattg aatattgttt ctaagtatat aaaaaataaa aatacatttt
      361 acaattgata aaacgctttg agcacatttt taacacagtg ttttatagat tgtttaaatt
```

```
 421 attttacaag tactattaaa tattagtata ctaaataata gtttttgat ataaaactaa
 481 agttagtgtg gctaaataat tagtttagaa tggttaccac taaaaataa atttaattct
 541 tttaaggaga ttagaatgaa aaaaattatg ttaattgcta gtgcaatgtc tgcgttgtcg
 601 ttgccatttt cagctagtgc aatagaattg ggggaagaag gattagagtg tggtccttat
 661 gctaaagttg gagttgttgg aggaatgatt actggcgtag aatctgctcg cttggatcca
 721 gctgatgctg aaggcaaaaa acacttgtca ttaacaaatg ggctgccatt tggtggaacg
 781 ttggctgcag gtatgacaat cgctccagga tttagagcag agataggtgt tatgtacctt
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 901 aaggcagatt ctgtaggtgg gaaagatgct cctatacgta agcggtttaa acttacacct
 961 cctcagccta ctataatgcc tataagtata gctgtacgtg actttgggat tgatattcct
1021 aaccagacct cagcagcaag cacaagccgc agcctcaggc ttaatgatga gcaacgtgct
1081 gcagctagga tcgcttggtt aaagaattgt gctggtattg actatagggt aaaaaaccct
1141 aatgatccta atgggcctat ggttataaat ccgatattgt taaatattcc acagggtaac
1201 cctaatcctg ttggaaatcc accgcagcga gcaaatccgc ctgcaggttt tgcgatacat
1261 aaccatgagc aatggaggca tttggtagtt gggcttgctg cattatcaaa tgctaataaa
1321 cctagcgctt ctcctgtcaa agtattaagt gataaaatta ctcagatata tagtgatata
1381 aagcatttgg ctgatatagc tggtattgat gttcctgata ctagtttgcc taatagtgca
1441 tctgtcgaac agatacagaa taaaatgcaa gaattaaacg atctattgga agagctcaga
1501 gaatcttttg atgggtatct tggtggtaat gcttttgcta atcagataca gttgaatttt
1561 gtcatgccgc agcaagcaca gcagcagggg caagggcagc aacagcaagc tcaagctaca
1621 gcgcaagaag cagtagcagc agcagctgtt aggcttttaa atggcaatga tcagattgcg
1681 cagttatata aagatcttgt taaattgcag cgtcatgcag gaattaagaa agcgatggaa
1741 aaattagctg cccaacaaga agaagatgca aagaatcaag gtgaaggtga ctgcaagcag
1801 caacaaggaa catctgaaaa atctaaaaaa ggaaaagaca aagaggcaga gtttgatctg
1861 agtatgattg tcggccaagt taaactctat gctgacgtaa tgataactga atcagtctca
1921 atatatgctg gtgttggtgc agggttagct tatacttctg gaaaaataga taataaggat
1981 attaaagggc atacaggcat ggttgcatca ggagcacttg gtgtagcaat taatgctgct
2041 gaaggtgtgt atgtggacat agaaggtagt tatatgtact cattcagtaa aatagaagag
2101 aagtattcaa taaatcctct tatggcaagt gtaagtgtac gctataactt ctagtttttt
2161 ctttaaaatt ataaaaaaag cagctaaaag ttctttacag ggtttttagc tgctttttca
2221 gagttttttt ataataataa aaataacttt attctttgct atttaattag cttgaagctt
//
```

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Sep 16 2003 13:15:1

NCBI BL

PubMed Entrez BLAST OMIM Taxonomy

Info

- FAQs
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- References
- Credits

Education

- Program selection guide
- Tutorial
- URL API guide

Download

- Executables
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- Source code

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NEW **15 August 2003** We will be reorganizing the executables/ directory of our FTP site. Read more...

Nucleotide

- Discontiguous megablast
- Megablast
- Nucleotide-nucleotide BLAST (blastn)
- Search for short, nearly exact matches
- Search trace archives with megablast or discontiguous megablast

Protein

- Protein-protein BLAST (blastp)
- PHI- and PSI-BLAST
- Search for short, nearly exact matc
- Search the conserved domain data (rpsblast)
- Search by domain architecture (cd

Translated

- Translated query vs. protein database (blastx)
- Protein query vs. translated database (tblastn)
- Translated query vs. translated database (tblastx)

Genomes

- Human, mouse, rat
- Fugu rubripes, zebrafish
- Flies, nematodes, plants, yeasts, m
- Microbial genomes, other eukaryot

Special

- Align two sequences (bl2seq)
- Screen for vector contamination (VecScreen)
- Immunoglobin BLAST (IgBlast)

Meta

- Retrieve results by RID
- Get this page with javascript-free l

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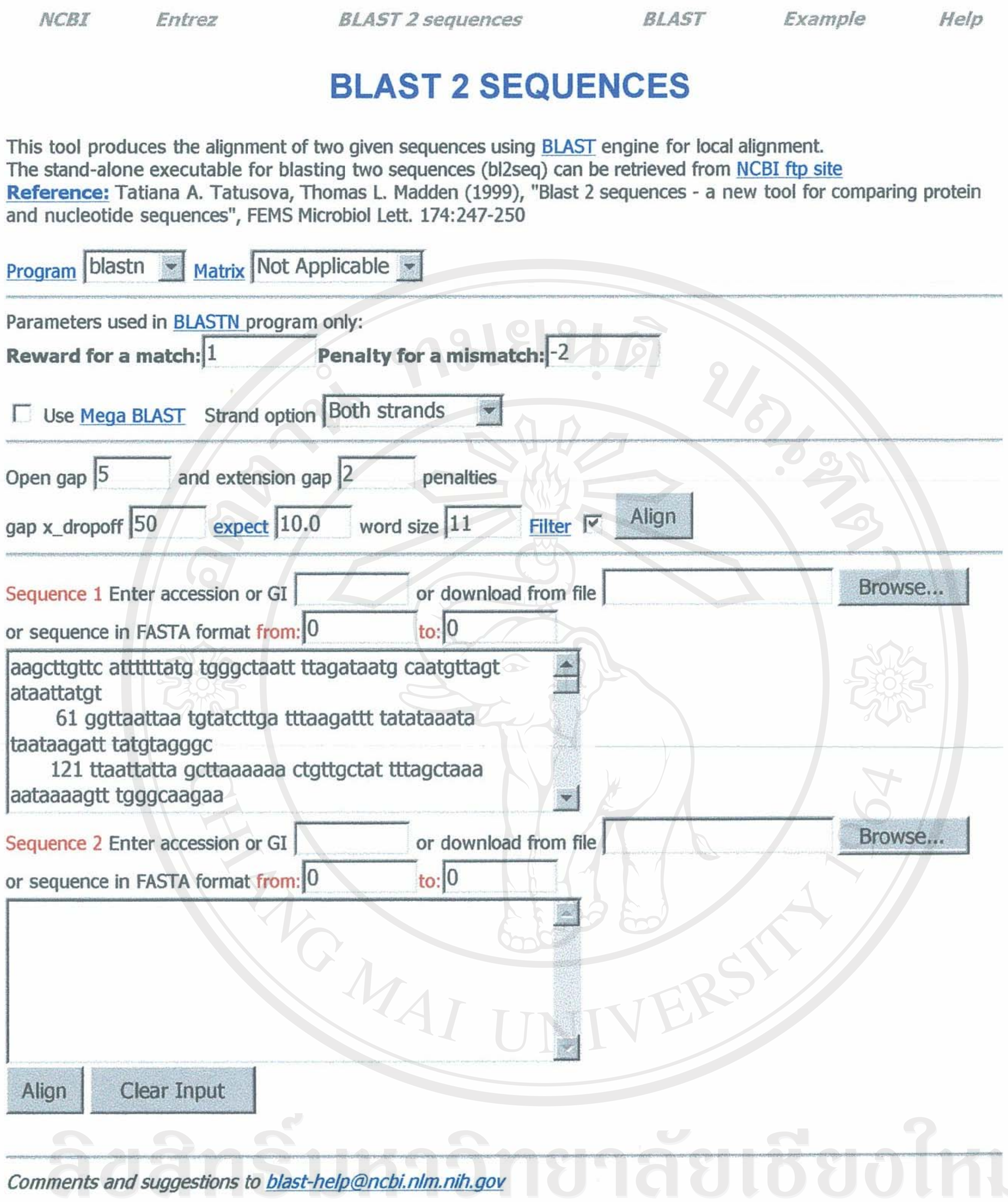


BLAST 2 SEQUENCES

This tool produces the alignment of two given sequences using BLAST engine for local alignment.
The stand-alone executable for blasting two sequences (bl2seq) can be retrieved from NCBI ftp site
Reference: Tatiana A. Tatusova, Thomas L. Madden (1999), "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250

Program: blastn
Matrix: Not Applicable

Parameters used in BLASTN program only:
Reward for a match: 1
Penalty for a mismatch: -2

☐ Use Mega BLAST
Strand option: Both strands

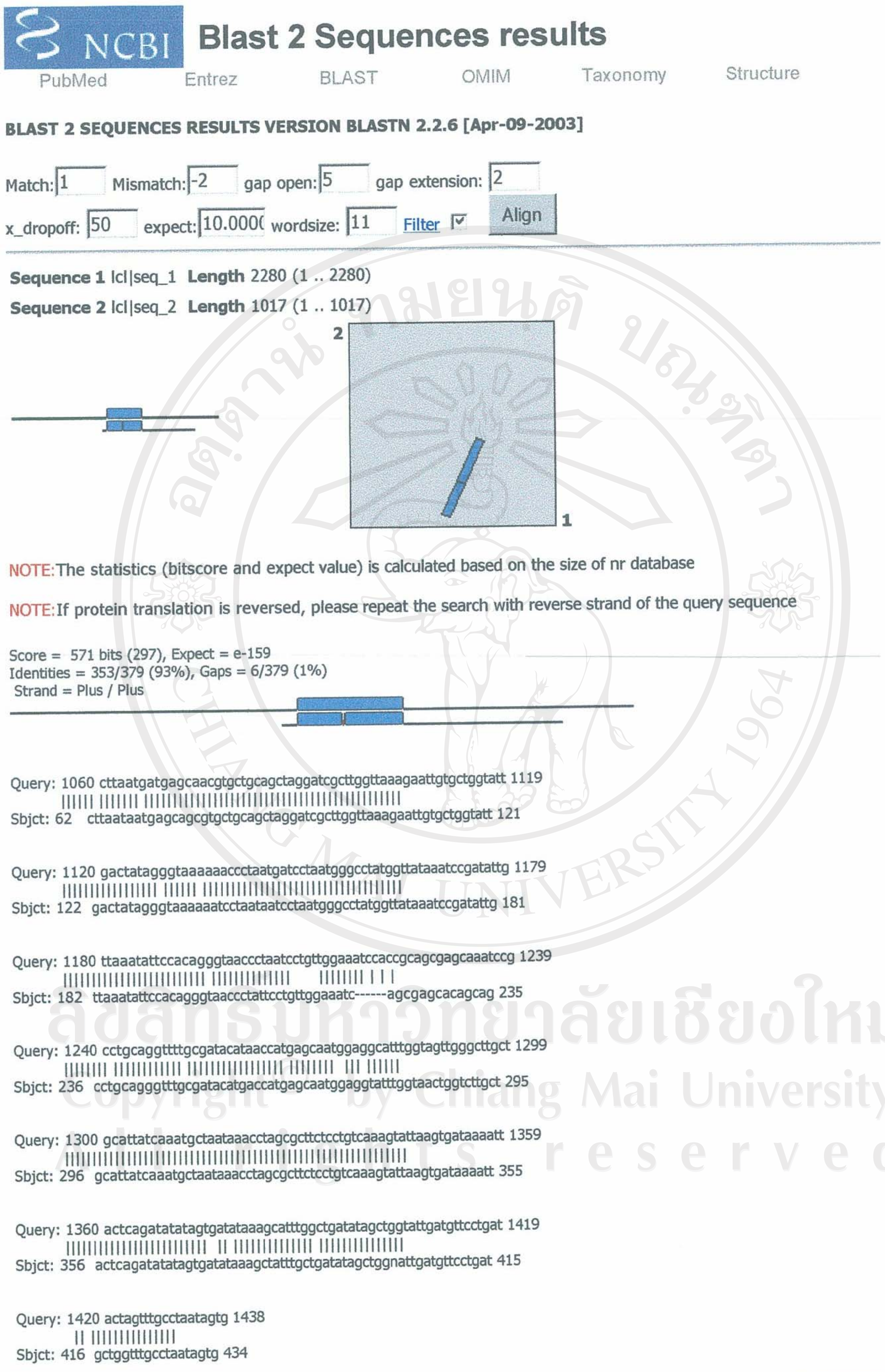
Open gap 5 and extension gap 2 penalties
gap x_dropoff 50
expect 10.0
word size 11
Filter ☑

Sequence 1 Enter accession or GI
or download from file
or sequence in FASTA format from: 0 to: 0

```
aagcttgttc attttttatg tgggctaatt ttagataatg caatgttagt
ataattatgt
      61 ggttaattaa tgtatcttga tttaagattt tatataaata
taataagatt tatgtagggc
     121 ttaattatta gcttaaaaaa ctgttgctat tttagctaaa
aataaaagtt tgggcaagaa
```

Sequence 2 Enter accession or GI
or download from file
or sequence in FASTA format from: 0 to: 0

Comments and suggestions to blast-help@ncbi.nlm.nih.gov



Blast 2 Sequences results

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.6 [Apr-09-2003]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2
x_dropoff: 50 expect: 10.000(wordsize: 11 Filter ☑

Sequence 1 lcl|seq_1 **Length** 2280 (1 .. 2280)
Sequence 2 lcl|seq_2 **Length** 1017 (1 .. 1017)

NOTE:The statistics (bitscore and expect value) is calculated based on the size of nr database

NOTE:If protein translation is reversed, please repeat the search with reverse strand of the query sequence

```
Score =  571 bits (297), Expect = e-159
Identities = 353/379 (93%), Gaps = 6/379 (1%)
 Strand = Plus / Plus


Query: 1060 cttaatgatgagcaacgtgctgcagctaggatcgcttggttaaagaattgtgctggtatt 1119
            |||||| ||||||| |||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 62   cttaataatgagcagcgtgctgcagctaggatcgcttggttaaagaattgtgctggtatt 121


Query: 1120 gactatagggtaaaaaccctaatgatcctaatgggcctatggttataaatccgatattg 1179
            |||||||||||||||| |||||| ||||||||||||||||||||||||||||||||||||
Sbjct: 122  gactatagggtaaaaatcctaataatcctaatgggcctatggttataaatccgatattg 181


Query: 1180 ttaaatattccacagggtaaccctaatcctgttggaaatccaccgcagcgagcaaatccg 1239
            |||||||||||||||||||||||| |||||||||||||||      |||||||| | | |
Sbjct: 182  ttaaatattccacagggtaaccctattcctgttggaaatc------agcgagcacagcag 235


Query: 1240 cctgcaggttttgcgatacataaccatgagcaatggaggcatttggtagttgggcttgct 1299
            |||||||| |||||||||||| ||||||||||||||||| ||||||||  ||| ||||||
Sbjct: 236  cctgcagggtttgcgatacatgaccatgagcaatggaggtatttggtaactggtcttgct 295


Query: 1300 gcattatcaaatgctaataaacctagcgcttctcctgtcaaagtattaagtgataaaatt 1359
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 296  gcattatcaaatgctaataaacctagcgcttctcctgtcaaagtattaagtgataaaatt 355


Query: 1360 actcagatatatagtgatataaagcatttggctgatatagctggtattgatgttcctgat 1419
            |||||||||||||||||||||||||  || |||||||||||||| |||||||||||||||
Sbjct: 356  actcagatatatagtgatataaagctatttgctgatatagctggnattgatgttcctgat 415


Query: 1420 actagtttgcctaatagtg 1438
             || |||||||||||||||
Sbjct: 416  gctggtttgcctaatagtg 434
```

```
CPU time:     0.02 user secs.              0.00 sys. secs            0.02 total secs.

Lambda     K      H
   1.33    0.621    1.12

Gapped
Lambda     K      H
   1.33    0.621    1.12


Matrix: blastn matrix:1 -2
Gap Penalties: Existence: 5, Extension: 2
Number of Hits to DB: 4
Number of Sequences: 0
Number of extensions: 4
Number of successful extensions: 3
Number of sequences better than 10.0: 1
Number of HSP's better than 10.0 without gapping: 1
Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 2
length of query: 2280
length of database: 9,114,278,828
effective HSP length: 26
effective length of query: 2254
effective length of database: 9,114,278,802
effective search space: 20543584419708
effective search space used: 20543584419708
T: 0
A: 0
X1: 6 (11.5 bits)
X2: 26 (50.0 bits)
S1: 12 (23.8 bits)
S2: 21 (41.1 bits)
```

IX CURRICULUM VITAE

Name	Mrs. Salakchit Chutipongvivate
Date of Birth	October 25, 1957

Education

March 1979	Bachelor of Science in Medical Technology B.Sc (Med.Tech) Faculty of Associated Medical Sciences, Chiang Mai University
March 1982	Master of Science in Microbiology M.Sc (Microbiology) Graduate school, Chiang Mai University

Publications

1. Chutipongvivate S. Research Abstract Publication. Study of hepatitis B virus and transmission of hepatitis B virus from mother to infant in Chiang Mai. Department of Medical Sciences, Thailand. 1985; 107
2. Chutipongvivate S and Bunyarukyothin G. Co- incidence of Viral hepatitis B and Syphilis in HIV positive person. Bull Chiang Mai Assoc Med Sci. 1995; 28: 2- 7
3. Chutipongvivate S, Choasaun U and Jinorod S. Prevalence of Hepatitis C Virus Antibody in the upper north of Thailand. Bull Chiang Mai Assoc Med Sci. 1995; 27: 2- 8
4. Chutipongvivate S and Wanaratwijit T. Detection of Rotavirus in Children with Diarrhea by Polyacrylamide Gel Electrophoresis. Bull Chiang Mai Assoc Med Sci. 1995; 28: 2- 7
5. Thichak S and Chutipongvivate S. Detection of HIV Infection Among Infants in the Upper North of Thailand using Nested PCR. J Med Tech Assoc Thailand. 2002; 30:88-93

Working experiences

1. Head of bacteriology section, Regional Medical Sciences Center, Chiang Mai.
2. Head of clinical pathology section, Regional Medical Sciences Center, Chiang Mai.