Mini Courses NCBI Resources one course at a time

NCBI Minicourses

BLAST Quick Start

blast-help@ncbi.nlm.nih.gov

NCBI Minicourses

Algorithm Basics
- Introduction
- Words & extensions

Search Basics
- Programs
- Databases
- Submit a search
- Interpret the results
- BLAST options
- Format options
- Examples

NCBI Minicourses

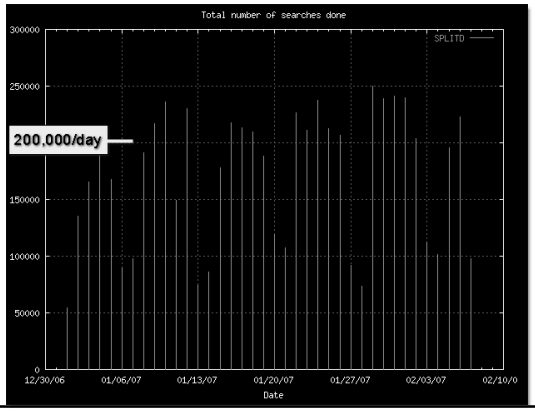
Basic Local Alignment Search Tool

Compare protein or nucleic acid sequences to protein or nucleic acid databases

- in NCBI databases
- in local databases (standalone BLAST)
- to a single protein or nucleotide sequence

(BLAST 2 Sequences, or pairwise BLAST)

BLAST Web Searches, 2007



NCBI Minicourses

Basic Local Alignment Search Tool

- local alignments; isolated regions of similarity
- fast and sensitive
- breaks the query sequence into "words"
- word matches to database sequences are extended in both directions

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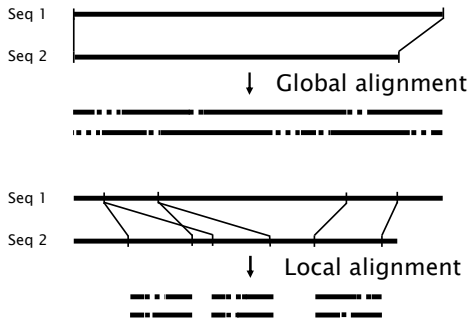
Global vs Local Alignment



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Global vs Local Alignment

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```
Seq1:  WHEREISWALTERNOW      (16aa)
Seq2:  HEWASHEREBUTNOWISHERE (21aa)
```

Global

```
Seq1:  1                 W--HEREISWALTERNOW 16
                         W  HERE
Seq2:  1  HEWASHEREBUTNOWISHERE            21
```

Local

```
Seq1: 1  W--HERE 5        Seq1:  1 W--HERE  5
         W  HERE                  W  HERE
Seq2: 3  WASHERE 9        Seq2: 15 WISHERE 21
```

How BLAST Works

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1. Make lookup table of "words" for query
2. Scan database for hits
3. Extend alignment both directions
   - Ungapped extensions of hits (initial HSPs)
   - Gapped extensions (no traceback)
   - Gapped extensions (traceback – alignment details)

Nucleotide Words

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Make a lookup table based on the word size.

11-mer

ATGCTGCTAGTCGATGACGTAGCTA

ATGCTGCTAGT
 TGCTGCTAGTC
  GCTGCTAGTCG
   . . .

Protein Words

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AIEKCYTGCTLAQEADDTA

AIE
IEK
EKC
KCY
CYT
…

LEK, IDK, IQK, IER, IDR, etc

Neighborhood words

Lookup table, including neighborhood words, is based on word size, score matrix, and threshold.

Scoring Systems - Proteins (BLOSUM62)

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IEK: keep LEK? (threshold = 11)

E / E

I / I

IEK = 14

L / I

LEK = 12

K / K

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	X
A	4																				
R	-1	5																			
N	-2	0	6																		
D	-2	-2	1	6																	
C	0	-3	-3	-3	9																
Q	-1	1	0	0	-3	5															
E	-1	0	0	2	-4	2	5														
G	0	-2	0	-1	-3	-2	-2	6													
H	-2	0	1	-1	-3	0	0	-2	8												
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4											
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4										
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5									
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5								
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6							
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7						
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4					
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5				
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11			
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7		
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4	
X	0	-1	-1	-1	-2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-2	0	0	-2	-1	-1	-1

Word Hits & Extensions

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Nucleotide: one exact match

ATGCTGCTAGTCGATGACGTAGCTA

⇦ GCTGCTAGTCG ⇨

Protein: two matches within 40 residues

AIEKCYTGCTLAQEADDTA

⇦IDK ⇨ ⇦ EAD ⇨

BLASTP Summary

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example query words

```
Query:   IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESILEV…
                          YLS  15 |          | HFL  18
                          YLT  12 |          | HFV  15
                          YVS  12 | Neighborhood | HFS  14
                          YIT  10 |  words   | HWL  13
                          etc  …  |          | NFL  13  Neighborhood
                                                DFL  12  score threshold
                                                HWV  10  T (-f) =11
                                                etc  …

Query   1 IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESI  47
          +E  YA YL K    F+YLSL +SP+ +DVNVHP+K  VHFL+++ I
Sbjct 287 LEETYAKYLHKGASYFVYLSLNMSPEQLDVNVHPSKRIVHFLYDQEI 333
```

Drop-off score =

Highest score - current score

-X X dropoff value for gapped alignment (in bits)
blastn 30, megablast 20, tblastx 0, all others 15

BLASTP Summary

NCBI Minicourses

example query words

```
Query:   IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESILEV…
                          YLS  15 |          | HFL  18
                          YLT  12 |          | HFV  15
                          YVS  12 | Neighborhood | HFS  14
                          YIT  10 |  words   | HWL  13
                          etc  …  |          | NFL  13  Neighborhood
                                                DFL  12  score threshold
                                                HWV  10  T (-f) =11
                                                etc  …

Query   1 IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESI  47
          +E  YA YL K    F+YLSL +SP+ +DVNVHP+K  VHFL+++ I
Sbjct 287 LEETYAKYLHKGASYFVYLSLNMSPEQLDVNVHPSKRIVHFLYDQEI 333
```

High-scoring pair (HSP)

Gapped extension with trace back

```
Query   1 IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESI-LEV…  50
          +E  YA YL K    F+YLSL +SP+ +DVNVHP+K  VHFL+++ I + +
Sbjct 287 LEETYAKYLHKGASYFVYLSLNMSPEQLDVNVHPSKRIVHFLYDQEIATSI… 337
```

Final HSP

BLAST

Search Basics

NCBI Minicourses

- Programs
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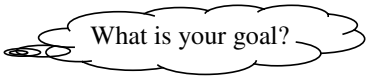
BLAST Programs



blastn nucleotide X nucleotide
blastp protein X protein

6 frame, translated nucleotide searches

blastx nucleotide X protein
tblastx nucleotide X nucleotide
tblastn protein X nucleotide

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Word Size

Trade-off: sensitivity vs speed

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More BLAST Programs

	Word Size
MegaBLAST - batch nucleotide queries - very similar sequences	28
Discontiguous MegaBLAST - batch nucleotide queries - divergent sequences	11 matches out of 18

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Templates for Discontiguous Words

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```
W = 11, t = 16, coding:       1101101101101101
W = 11, t = 16, non-coding:   1110010110110111
W = 12, t = 16, coding:       1111101101101101
W = 12, t = 16, non-coding:   1110110110110111
W = 11, t = 18, coding:       101101100101101101
W = 11, t = 18, non-coding:   111010010110010111
W = 12, t = 18, coding:       101101101101101101
W = 12, t = 18, non-coding:   111010110010110111
W = 11, t = 21, coding:       100101100101100101101
W = 11, t = 21, non-coding:   111010010100010010111
W = 12, t = 21, coding:       100101101101100101101
W = 12, t = 21, non-coding:   111010010110010010111
```

W = word size; # matches in template
t = template length

Reference: Ma, B, Tromp, J, Li, M. PatternHunter: faster and more sensitive homology search. Bioinformatics March, 2002; 18(3):440-5

BLAST

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NCBI Minicourses

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Nucleotide BLAST Databases

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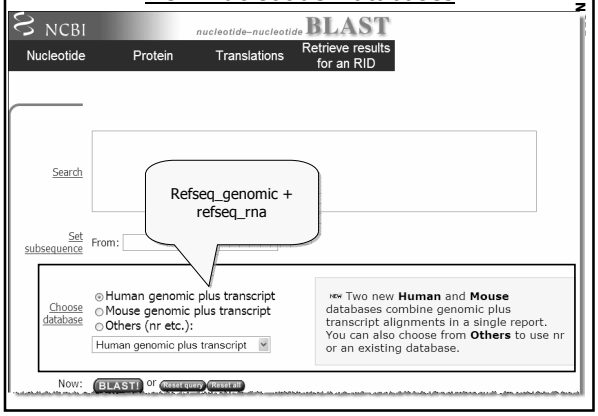
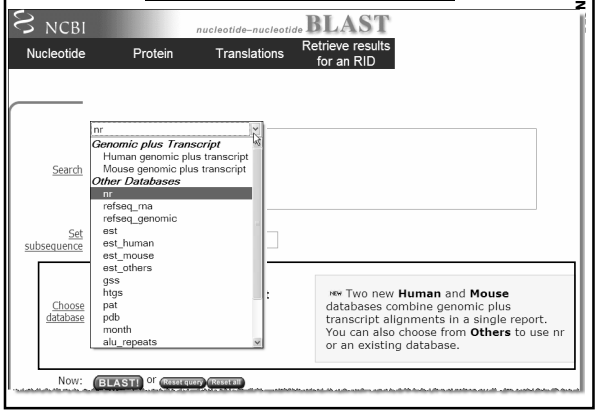
- **nr (nt)**
  - Traditional GenBank Divisions
  - NM_ and XM_ RefSeqs
- **refseq_rna**
  - NM_ , XM_ , NR_
- **refseq_genomic**
  - NC_ , NT_ , NG_
- **est**
  - EST Division
- **htgs**
  - HTG division
- **dbsts**
  - STS Division
- **chromosome**
  - NC genomic records
- **gss**
  - GSS division
- **pat**
  - PAT Division
- **wgs**
  - wgs entries from traditional divisions
- **pdb**
  - Nucleotide sequences from structures
- **env_nt**
  - environmental samples

New Nucleotide Databases



New Nucleotide Databases



Protein BLAST Databases

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Protein

- nr
  traditional GenBank records
- refseq = NP_, XP_
- swissprot
- pdb
- pat
- env_nr

nr ≠ nr

BLAST Databases: Genome-specific

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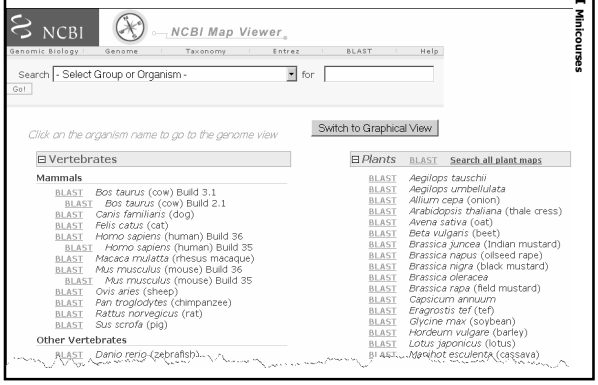
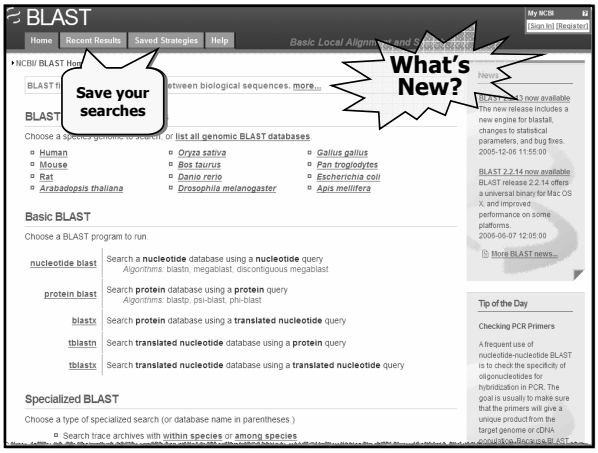
Genomes

- Human, mouse, rat, chimp, cow, pig, dog, sheep, cat
- Chicken, puffer fish, zebrafish
- Fly, honey bee, other insects
- Microbes, environmental samples
- Plants, nematodes
- Fungi, protozoa, other eukaryotes

Genome-specific: Map Viewer

NCBI Minicourses





BLAST

Search Basics

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- Programs
- Databases
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Setting up a BLAST Search

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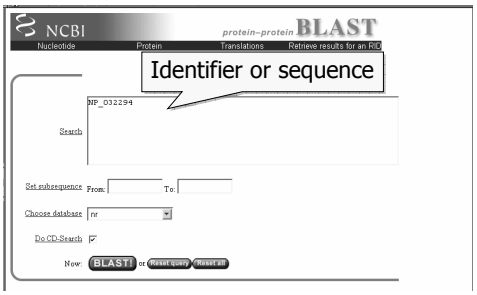

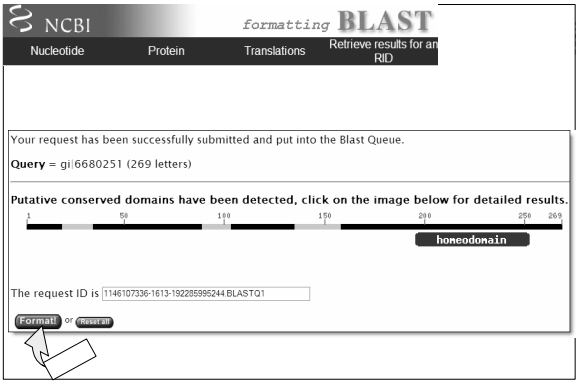

BLAST Output

NCBI results of BLAST

BLASTP 2.2.13 [Nov-27-2005]

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

RID: 1146107336-1613-192285995244.BLASTQ1

Database: All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples
3,605,113 sequences; 1,238,276,235 total letters

If you have any problems or questions with the results of this search please refer to the **BLAST FAQs**
Taxonomy reports

Tax BLAST Report

Index

- Lineage Report
- Organism Report
- Taxonomy Report
- Help

Lineage Report

```
root
. Bilateria          [animals]
. . Coelomata          [animals]
. . . Deuterostomia    [animals]
. . . . Chordata         [chordates]
. . . . . Vertebrata       [vertebrates]
. . . . . . Gnathostomata    [vertebrates]
. . . . . . . Euteleostomi     [vertebrates]
. . . . . . . . Sarcopterygii    [vertebrates]
. . . . . . . . . Tetrapoda        [vertebrates]
. . . . . . . . . . Amniota          [vertebrates]
. . . . . . . . . . . Eutheria         [mammals]
. . . . . . . . . . . . Euarchontoglires [mammals]
. . . . . . . . . . . . . Hominea          [mammals]
. . . . . . . . . . . . . . Mus              [mammals]
. . . . . . . . . . . . . . . Mus musculus (mouse) ---------------  419 46 hits [mammals]        Homeobox protein Hox-B5 (Hox-2.1) (MU-1)
. . . . . . . . . . . . . . . Mus sp. (mice) ......................  148  4 hits [mammals]        Hoxa-4 [Mus sp.] >gi|8322211|gb|AAB26662.2
. . . . . . . . . . . . . . . Rattus norvegicus (brown rat) ---------  195 11 hits [mammals]        Homeobox protein Hox-A5 (Hox-1.3) >gi|2046
. . . . . . . . . . . . . . . Rattus sp. (rats) ....................  117  2 hits [mammals]        Hox-B1Hox-2 [Rattus sp.]
. . . . . . . . . . . . . . . Macaca fascicularis (cynomolgus monkey) -  416  1 hit  [mammals]        hypothetical protein [Macaca fascicularis]
. . . . . . . . . . . . . . . Homo sapiens (man) ..................  412 43 hits [mammals]        homeobox B5 [Homo sapiens] >gi|4504469|ref
. . . . . . . . . . . . . . . Pan troglodytes .....................  240  9 hits [mammals]        PREDICTED: similar to homeobox protein A7
. . . . . . . . . . . . . . Bos taurus (cow) ----------------------  365 17 hits [mammals]        PREDICTED: similar to Homeobox protein Hox
. . . . . . . . . . . . . . Canis familiaris (dogs) ................  257 15 hits [mammals]        PREDICTED: similar to Homeobox protein Hox
. . . . . . . . . . . . . . Ovis aries (domestic sheep) ............  124  2 hits [mammals]        Homeobox protein Hox-A7 >gi|1455055|gb|AAA
. . . . . . . . . . . . Gallus gallus (chickens) ------------------  353 23 hits [birds]          homeodomain transcription factor [Gallus g
. . . . . . . . . . . . Pelodiscus sinensis .......................  316  2 hits [turtles]         Hoxb-5 [Pelodiscus sinensis]
. . . . . . . . . . . . Coturnix japonica .........................  132  1 hit  [birds]          Homeobox protein Hox-A7 (Quox-1) >gi|2136
. . . . . . . . . . . Xenopus tropicalis --------------------------  249  3 hits [amphibians]      Homeo box A5 [Xenopus tropicalis] >gi|5833
. . . . . . . . . . . Xenopus laevis (clawed frog) ................  221 18 hits [amphibians]      unnamed protein product [Xenopus laevis]
. . . . . . . . . . . Ambystoma mexicanum .........................  185  2 hits [amphibians]      homeobox protein [Ambystoma mexicanum] >gi
. . . . . . . . . . . Pleurodeles waltl ............................  125  1 hit  [amphibians]      transcription factor HoxA4 [Pleurodeles wa
. . . . . . . . . . . Notophthalmus viridescens (red-spotted newt) ..  125  4 hits [amphibians]      Homeobox protein Hox-C5 (DrHox-3.4)
. . . . . . . . . . Latimeria menadoensis (king of the sea) --------  312 10 hits [coelacanths]     HoxB5 [Latimeria menadoensis]
```

Tax BLAST Report


```
. . . . . Mus musculus (mouse) ----------------  419 46 hits [mammals]
. . . . . Mus sp. (mice) ......................  148  4 hits [mammals]
. . . . . Rattus norvegicus (brown rat) ---------  195 11 hits [mammals]
. . . . . Rattus sp. (rats) ....................  117  2 hits [mammals]
. . . . Macaca fascicularis (cynomolgus monkey) -  416  1 hit  [mammals]
. . . . Homo sapiens (man) ....................  412 43 hits [mammals]
. . . . Pan troglodytes .......................  240  9 hits [mammals]
. . . Bos taurus (cow) ----------------------  365 17 hits [mammals]
. . . Canis familiaris (dogs) ................  257 15 hits [mammals]
. . . Ovis aries (domestic sheep) ............  124  2 hits [mammals]
. . Gallus gallus (chickens) ------------------  353 23 hits [birds]
. . Pelodiscus sinensis .......................  316  2 hits [turtles]
. . Coturnix japonica .........................  132  1 hit  [birds]
. Xenopus tropicalis --------------------------  249  3 hits [amphibians]
. Xenopus laevis (clawed frog) ................  221 18 hits [amphibians]
. Ambystoma mexicanum .........................  185  2 hits [amphibians]
. Pleurodeles waltl ...........................  125  1 hit  [amphibians]
. Notophthalmus viridescens (red-spotted newt) ..  125  4 hits [amphibians]
Latimeria menadoensis (king of the sea) ---------  312 10 hits [coelacanths]
```

New Output View

NCBI Minicourses

Format

Show ☑ Graphical Overview ☑ Linkout ☑ Sequence Retrieval ☐ NCBI-gi Alignment in HTML format

☑ CDS feature

☑ New View

Masking Character Lower Case Masking Color Red

Number of: Descriptions 100 Alignments 50 Graphic overview 100

Alignment view Pairwise

Limit results by entrez query AND All organisms

Graphic Overview

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Distribution of 65 Blast Hits on the Query Sequence

NM_001255 Homo sapiens CDC20 cell division cycle 20 homolog (S. c.. S=3172 E=0

Color key for alignment scores

<40	40-50	50-80	80-200	>=200

Query 0 300 600 900 1200 1500

New Output View

NCBI Minico

Results can be sorted

Transcript & genomic hits separated

Legend f ... es: U UniGene E GEO ... Map

Sequences producing significa alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
Transcripts							
NM_001255.1	Homo sapiens CDC20 cell divisio	2876	2876	95%	0.0	97%	U E G M
XM_9449		40.1	40.1	1%	8.2	100%	G M
XM_9330		40.1	40.1	1%	8.2	100%	G M
Genomic sequences [show first]							
NT_023935.17	Homo sapiens chromosome 9 ge	2629	2673	94%	0.0	100%	
NW_924484.1	Homo sapiens chromosome 9 ge	2601	2645	94%	0.0	100%	
NT_032977.8	Homo sapiens chromosome 1 ge	428	3002	95%	9e-117	100%	
NW_921351.1	Homo sapiens chromosome 1 ge	428	3010	95%	9e-117	100%	
NT_009237.17	Homo sapiens chromosome 11 g	44.1	44.1	1%	0.53	100%	

Pseudogene, chr 9

Functional gene, chr 1

Sorting Results

NCBI Minicourses

Resorted by Total score

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
Transcripts							
NM_001255.1	Homo sapiens CDC20 cell divisi	2876	2876	95%	0.0	97%	U E G M
XM_933013.2	PREDICTED: Homo sapiens chrc	40.1	40.1	1%	8.2	100%	G M
XM_944935.2	PREDICTED: Homo sapiens chrc	40.1	40.1	1%	8.2	100%	G M
Genomic sequences [show first]							
NW_921351.1	Homo sapiens chromosome 1 ge	428	3010	95%	9e-117	100%	
NT_032977.8	Homo sapiens chromosome 1 ge	428	3002	95%	9e-117	100%	
NT_023935.17	Homo sapiens chromosome 9 ge	2629	2673	94%	0.0	100%	
NW_924484.1	Homo sapiens chromosome 9 ge	2601	2645	94%	0.0	100%	
NW_921807.1	Homo sapiens chromosome 3 ge	44.1	44.1	1%	0.53	100%	

Functional gene now first

Sorting Hits: by Score

NCBI Minic

```
> ref|NT_032977.8|Hs1_33153  Homo sapiens chromosome 1 genomic contig, reference assembly
Length=73835825

                                                    Sort alignments for this subject sequence by:
                                                      E value  Score  Percent identity
                                                      Query start position  Subject start position

 Features in this part of subject sequence:
   cell division cycle 20

 Score =  428 bits (216),  Expect = 9e-117
 Identities = 231/236 (97%), Gaps = 0/236 (0%)
 Strand=Plus/Plus

Query  965       CTCCAGTGGTTCACGTTCTGGCCACATCCACCACCATGATGTTCGGGTAGCAGAACACCA  1024
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  13798316  CTCCAGTGGTTCACGTTCTGGCCACATCCACCACCATGATGTTCGGGTAGCAGAACACCA  13798375

Query  1025      TGTGGCTACACTGAGTGGCCACAGCCAGGAAGTGTGTGGGCTGCGCTGGGCCCCAGATGG  1084
```

Sort by Score: longest exon usually first

Sorting Hits: by Query Start

NCBI Mincou

```
> ref|NT_032977.8|Hs1_33153  Homo sapiens chromosome 1 genomic contig, reference assembly
Length=73835825

                                                    Sort alignments for this subject sequence by:
                                                      E value  Score  Percent identity
                                                      Query start position  Subject start position

 Features flanking this part of subject sequence:
   6169 bp at 5' side: myeloproliferative leukemia virus oncogene
   223 bp at 3' side: cell division cycle 20

 Score = 89.7 bits (45),  Expect = 1e-14
 Identities = 51/53 (96%), Gaps = 0/53 (0%)
 Strand=Plus/Plus

Query  1         AGCGGAGAGTTTAAGAGGCGTAAGCGAGGCGTGTTAAACCCGGTCGGAACTGC  53
                 ||||||||||||||||||||||||||| |||||||||||| |||||||||||||
Sbjct  13796530  AGCGGAGAGTTTAAGAGGCGTAAGCCAGGCGTGTTAAAGCCGGTCGGAACTGC  13796582
```

Sort by Query start: Proper exon order

BLAST

Search Basics

NCBI Minicourses

- Programs
- Databases
- Submit a search
- Interpret the results
- BLAST options
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- Examples

...con't

Where do Scores Come From?

NCBI Minicourses

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
Transcripts							
NM_001255.1	Homo sapiens CDC20 cell divisi	2876	2876	95%	0.0	97%	U E G M
XM_933013.2	PREDICTED: Homo sapiens chro	40.1	40.1	1%	8.2	100%	G M
XM_944935.2	PREDICTED: Homo sapiens chro	40.1	40.1	1%	8.2	100%	G M
Genomic sequences [show first]							
NW_921351.1	Homo sapiens chromosome 1 ge	428	3010	95%	9e-117	100%	
NT_032977.8	Homo sapiens chromosome 1 ge	428	3002	95%	9e-117	100%	
NT_023935.17	Homo sapiens chromosome 9 ge	2629	2673	94%	0.0	100%	
NW_924484.1	Homo sapiens chromosome 9 ge	2601	2645	94%	0.0	100%	
NW_921807.1	Homo sapiens chromosome 3 ge	44.1	44.1	1%	0.53	100%	

Scoring Matrices

NCBI Minicourses

Nucleotide search: identity matrix

	A	T	C	G
A	1	-3	-3	-3
T	-3	1	-3	-3
C	-3	-3	1	-3
G	-3	-3	-3	1

```
CAGGTAGCAAGCTTGCATGTCA
|| |||||||||||  |||||    raw score = 19-9* = 10*
CACGTAGCAAGCTTG-GTGTCA
```

* ignores gap costs

Scoring Matrices

Protein search: substitution matrix

- Percent Accepted Mutation (PAM)
- Blocks Substitution Matrix (BLOSUM)

NCBI Minicourses

PAM Matrices

- global alignments of closely related proteins
- PAM1 calculated from sequences with no more than 1% divergence
- Other PAM matrices are extrapolated from PAM1
- Examples: PAM30 and PAM70

NCBI Minicourses

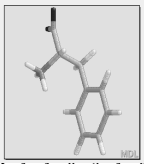
BLOSUM Matrices

- local alignments
- all matrices based on observed alignments; not extrapolated
- BLOSUM 62 calculated from sequences with no more than 62% identity
- Examples: BLOSUM45, BLOSUM62 and BLOSUM80
- BLOSUM62: very good for detecting weak protein similarities
- BLOSUM62 is the default matrix in BLAST

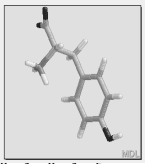
NCBI Minicourses

BLOSUM62

L-phenylalanine (F)



L-tyrosine (Y)



```
A  4
R -1  5
N -2  0  6
D -2 -2  1  6
C  0 -3 -3 -3  9
Q -1  1  0  0 -3  5
E -1  0  0  2 -4  2  5
G  0 -2  0 -1 -3 -2 -2  6
H -2  0  1 -1 -3  0  0 -2  8
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4
X  0 -1 -1 -1 -2 -1 -1 -1 -1 -1 -1 -1 -1 -1 -2  0  0 -2 -1 -1 -1
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  X
```

Negative for less likely substitutions

Positive for more likely substitutions

BLOSUM62

J (leucine or isoleucine) and O (pyrrolysine)

```
A  4
R -1  5
N -2  0  6
D -2 -2  1  6
C  0 -3 -3 -3  9
Q -1  1  0  0 -3  5
E -1  0  0  2 -4  2  5
G  0 -2  0 -1 -3 -2 -2  6
H -2  0  1 -1 -3  0  0 -2  8
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4  7
S  1 -1  1  0 -1  0  0  0 -1 -2 -2  0 -1 -2 -1  4
T  0 -1  0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1  1  5
W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1  1 -4 -3 -2 11
Y -2 -2 -2 -3 -2 -1 -2 -3  2 -1 -1 -2 -1  3 -3 -2 -2  2  7
V  0 -3 -3 -3 -1 -2 -2 -3 -3  3  1 -2  1 -1 -2 -2  0 -3 -1  4
X  0 -1 -1 -1 -2 -1 -1 -1 -1 -1 -1 -1 -1 -1 -2  0  0 -2 -1 -1 -1
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  X
```

Substitution Matrices

A Provisional Table

Query Length	Substitution Matrix
<35	PAM30
35-50	PAM70
50-85	BLOSUM80
>85	BLOSUM62

BLOSUM 80	BLOSUM 62	BLOSUM 45
PAM 1	PAM 120	PAM 250

Less divergent ⟷ *More divergent*

Where do Expect Values Come From?

NCBI Minicourses

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
Transcripts							
NM_001255.1	Homo sapiens CDC20 cell divisi	2876	2876	95%	0.0	97%	U E G M
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NW_924484.1	Homo sapiens chromosome 9 ge	2601	2645	94%	0.0	100%	
NW_921807.1	Homo sapiens chromosome 3 ge	44.1	44.1	1%	0.53	100%	

NCBI Minicourses

Expect Value

E = number of database hits you expect to find by chance, ≥ S

$$E = Kmne^{-\lambda S} \quad \text{or} \quad E = mn2^{-S'}$$

K = scale for search space
λ = scale for scoring system
λS' = bitscore = (λS – lnK)/ln2
m = query length
n = database length

E is dependent on m x n (search space)

More info: The Statistics of Sequence Similarity Scores

NCBI Minicourses

E is dependent on m x n (search space)

Short query =

low score

high Expect

```
 Score = 30.2 bits (15),  Expect =     19
 Identities = 15/15 (100%), Gaps = 0/15 (0%)
 Strand=Plus/Plus

Query  4         GTAGCAAGCTTGCAT  18
                 |||||||||||||||
Sbjct  40347983  GTAGCAAGCTTGCAT  40347997
```

```
CAGGTAGCAAGCTTGCATGTCA
|| |||||||||||  |||||    raw score = 19-9 = 10
CACGTAGCAAGCTTG-GTGTCA
```

More info: The Statistics of Sequence Similarity Scores

BLAST

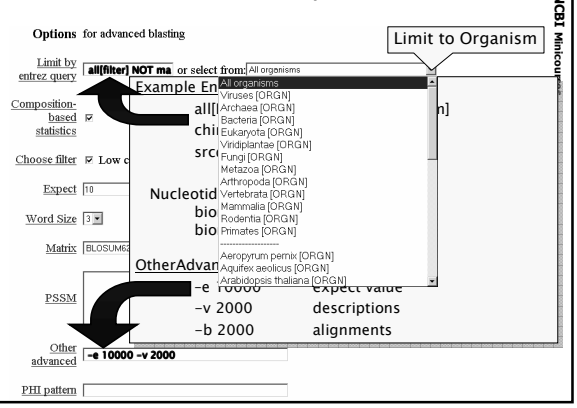
Search Basics

NCBI Minicourses

- Programs
- Databases
- Submit a search
- Interpret the results
- BLAST options
- Format options
- Examples

Advanced Options

NCBI Minicourses



Other Advanced Options

NCBI Minicourses

For short queries: increase e and lower W

Other advanced **– e 10000 –W 7 –v 2000 –b 1500**

-e expect value
-W word size
-v descriptions
-b alignments

BLASTP – "…short, nearly exact matches"

NCBI Minico

Options for advanced blasting

Limit by entrez query | or select from: All organisms

Compositional adjustments | No adjustment

Choose filter | Low complexity | Mask for lookup table only | Mask lower case

Expect | 20000

Word Size | 2

Matrix | PAM30 | Gap Costs | Existence: 9 Extension: 1

BLAST Search Basics

NCBI Minicourses

- Programs
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- Format options
- Examples

'New' Formatter

NCBI Minicourses

Format

Show | Graphical Overview | Linkout | Sequence Retrieval | NCBI-gi | Alignment | in | HTML | format

Default(X for protein, n for nucleotide) | Masking | Black

Default(X for protein, n for nucleotide)
Lower Case

Black
Grey
Red

Select lower case

Select red

Alignment view

Format for PSI-BLAST | with inclusion threshold: 0.005

Limit results by entrez query | or select from: All organisms

Expect value range:

Layout: Two Windows | Formatting options on page with results: None

Autoformat | Semi-auto

BLAST Output: Alignments & Filter

NCBI

```
>gi|466462|gb|AAA17374.1| human homolog of E. coli mutL gene product, Swiss-Prot
          Number P23367
        Length=756

 Score = 219.935 bits (559),  Expect = 8.711
 Identities = 120/131 (91%), Positives = 125

Query  1    IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKH    EESILE-VQQHIESKLL  59
            IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKH    HEESILE VQQHIESKLL
Sbjct  276  IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKH    LHEESILERVQQHIESKLL  335

Query  60   GSNSSRMYFTQTLLPGLAGPSGEMVK-sttsltsssT/gsDKVYAHQMVRTDSREQKLDA  118
            GSNSSRMYFTQTLLPGLAGPSGEMVK +T+  +SS++  SDKVYAHQMVRTDSREQKLDA
Sbjct  336  GSNSSRMYFTQTLLPGLAGPSGEMVKSTTSLTSSSTSGSSDKVYAHQMVRTDSREQKLDA  395

Query  119  FLQPLSKPLSS  129
            FLQPLSKPLSS
Sbjct  396  FLQPLSKPLSS  406
```

positives, from score matrix

low complexity sequence filtered

Alignment View Options

NCBI Mi

Format

Show ☑ Graphical Overview ☑ Linkout ☑ Sequence Retrieval ☑ NCBI-gi Alignment in HTML format

☐ CDS feature

Masking Character Lower Case Masking Color Grey

Number of: Descriptions 500 Alignments 250

Alignment view: Pairwise

- Pairwise
- Pairwise with identities
- query-anchored with identities
- query-anchored without identities
- flat query-anchored with identities
- flat query-anchored without identities
- Hit Table

Format for PSI-BLAST

Limit results by entrez query

Alignment View: Flat Query-Anchored with Identities

NCBI Mi

Get selected sequences | Select all | Deselect all | Tree View

```
Query     1     MSSYFVNSF-SGRYPN-GPDyqllnygsgsslsgsy--RDP--AAMH---TGSY-----G  46
1708354   1     .........-......-...................--...-....---....-----.  46
74225740  1     .........-......-...................--...-....---....-----.  46
84579185  1     .........-......-...................--...-....---....-----.  46
11138933  1     .........-......-...................--...-....---....-----.  46
12848710  1                                                  ..---....-----.  7
76644496  1     .........-......-...................--...-....---....-----.  46
57235483  1     .........-......-.........TS..MN...--..S--ST..---SS..-----.  46
62526079  1     .........-......-.........TS..MN...--..S--ST..---SS..-----.  46
62526089  1     .........-......-..E.......TS.AMN...--..S--GT..---SS..-----A  46
28629649  1                                                  ..---SS..-----.  7
54261740  1     .........-......-.........TS..AMNASY-..S--GT..---S...-----.  47
62537     1     .........-......-.........TS..AMNASY-..S--GT..---S...-----.  47
53749656  1     .....L...-......-.S........TNGAMNA..--..S--TS..---S..F-----.  46
22316136  1     .....L...-......-.S........TNGAMNA..--..S--TS..---S..F-----.  46
4322074   1     .....L...-......-.S........TNGAMNA..--..S--TS..---S..F-----.  46
74267553  1     .........-......-.S........TNGAV...F--...--GT..---S..F-----.  46
34484445  1     .........-......-.S........TNGAV...F--...--GT..---S..F-----.  46
50956652  1     .........-C.....-......H...DH..V.EQ.--..S--.S..---S.R.-----.  46
32879831  1     .........-C.....-......H...DH..V.EQF--..S--.S..---S.R.-----.  46
32879829  1     .........-C.....-......H...DH..V.EQF--..S--.S..---S.R.-----.  46
```

Alignment View: Hit Table

```
# BLASTP 2.2.13 [Nov-27-2005]
# Query: gi|6680251|ref|NP_032294.1| homeo box B5 [Mus musculus]
# Database: nr
# Fields: query id, subject ids, % identity, % positives, alignment length, mismatches, gap
# 514 hits found
gi|6680251|ref|NP_032294.1|     gi|1708354|sp|P09079|HXB5_MOUSE;gi|6680251|ref|NP_032294.1|;
gi|6680251|ref|NP_032294.1|     gi|74225740|dbj|BAE21695.1|;gi|73695452|gb|AAI03605.1|;gi|73
gi|6680251|ref|NP_032294.1|     gi|84579185|dbj|BAE73026.1|;gi|73966238|ref|XP_548176.2|
gi|6680251|ref|NP_032294.1|     gi|11138933|gb|AAG31553.1|;gi|4504469|ref|NP_002138.1|;gi|40
gi|6680251|ref|NP_032294.1|     gi|12848710|dbj|BAB28059.1|     99.57   100.00  230     1
gi|6680251|ref|NP_032294.1|     gi|76644496|ref|XP_584774.2|    90.18   92.73   275     21
gi|6680251|ref|NP_032294.1|     gi|57235483|gb|AAW48484.1|;gi|70778859|ref|NP_001020526.1|
gi|6680251|ref|NP_032294.1|     gi|62526079|dbj|BAD95556.1|     77.86   84.35   262     53
gi|6680251|ref|NP_032294.1|     gi|62526089|dbj|BAD95561.1|     73.85   80.38   260     63
gi|6680251|ref|NP_032294.1|     gi|28629649|gb|AAO43030.1|     77.83   84.35   230     46
gi|6680251|ref|NP_032294.1|     gi|54261740|ref|NP_571176.2|;gi|47938028|gb|AAH71493.1|;gi|2
gi|6680251|ref|NP_032294.1|     gi|62537|emb|CAA48320.1|;gi|62555|emb|CAA31290.1|       70.14
```

Fields: query id, subject ids, % identity, % positives, alignment length, mismatches, gap opens, q. start, q. end, s. start, s. end, evalue, bit score

NCBI Minicourses

BLAST

Search Basics

- Programs
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- Submit a search
- Interpret the results
- BLAST options
- Format options
- Examples

NCBI Minicourses

Examples

- Protein searches more sensitive than nucleotide searches
  - redundancy of the genetic code
- blastn, megablast best when searching within same organism

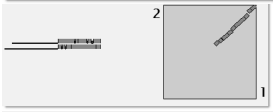
NCBI Minicourses

BLASTN vs BLASTP

NCBI Minicourses

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.14 [Apr-09-2006]

Matrix BLOSUM62 gap open: 11 gap extension: 1
x_dropoff: 50 expect: 10.0000 wordsize: 3 Filter ☐ View option Standard
Masking character option X for protein, n for nucleotide Masking color option Black
☐ Show CDS translation Align



BLASTP

NOTE: Bitscore and expect value are calculated based on the size of the nr database.

```
Score = 48.5 bits (114),  Expect = 7e-04
Identities = 50/181 (27%), Positives = 80/181 (44%), Gaps = 27/181 (14%)
```

BLAST is a shortcut . . .

NCBI Minicourses

An alignment BLAST cannot make:

```
  1 GAATATATGAAGACCAAGATTGCAGTCCTGCTGGCCTGAACCACGCTATTCTTGCTGTTG
    || | || || || |  || || ||   ||  |  ||| |||||| | | || | ||| |
  1 GAGTGTACGATGAGCCCGAGTGTAGCAGTGAAGATCTGGACCACGGTGTACTCGTTGTCG

 61 GTTACGGAACCGAGAATGGTAAAGACTACTGGATCATTAAGAACTCCTGGGGAGCCAGTT
    | || ||     ||  ||| ||  | |||||| || | ||||||  |||||  |     |
 61 GCTATGGTGTTAAGGGTGGGAAGAAGTACTGGCTCGTCAAGAACAGCTGGGCTGAATCCT

121 GGGGTGAACAAGGTTATTTCAGGCTTGCTCGTGGTAAAAAC
    |||| || ||||| ||  ||    |  | ||||  || |||
121 GGGGAGACCAAGGCTACATCCTTATGTCCCGTGACAACAAC
```

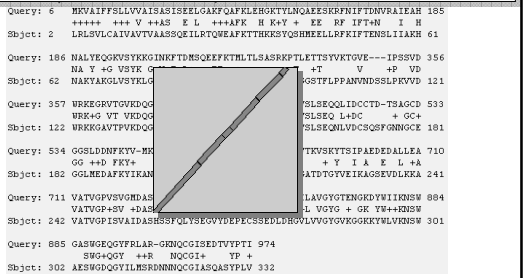
Reason:

no contiguous exact match of 7 bp.

An Alignment BLAST Can Make

NCBI M

```
Score = 290 bits (741), Expect = 7e-77
Identities = 147/331 (44%), Positives = 206/331 (61%), Gaps = 8/331 (2%)
Frame = +3
```



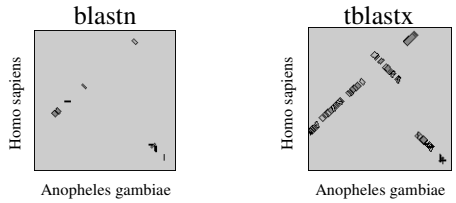
```
Query: 6   MKVAIFFSLLVVAISASISEELGAKFQAFKLEHGKTYLNQAEESKRFNIFTDNVRAIEAH 185
           +++++  +++ V ++AS  E L  +++AFK  H K+Y +  EE  RF IFT+N   I  H
Sbjct: 2   LRLSVLCAIVAVTVAASSQEILRTQWEAFKTTHKKSYQSHMEELLRFKIFTENSLIIAKH 61

Query: 186 NALYEQGKVSYKKGINKFTDMSQEEFKTMLTLSASRKPTLETTSYVKTGVE---IPSSVD 356
           NA Y +G VSYK G                          +T      V    +P  VD
Sbjct: 62  NAKYAKGLVSYKLG                          GSTFLPPANVNDSSLPKVVD 121

Query: 357 WRKEGRVTGVKDQG                          SLSEQQLIDCCTD-TSAGCD 533
           WRK+G VT VKDQG                          SLSEQ L+DC     + GC+
Sbjct: 122 WRKKGAVTPVKDQG                          SLSEQNLVDCSQSFGNNGCE 181

Query: 534 GGSLDDNFKYV-MK                          TKVSKYTSIPAEDEDALLEA 710
           GG ++D FKY+                              + Y  I A  E  L +A
Sbjct: 182 GGLMEDAFKYIKAN                          ATDTGYVEIKAGSEVDLKKA 241

Query: 711 VATVGPVSVGMDAS                          LAVGYGTENGKDYWIIKNSW 884
           VATVGP+SV +DAS                          L VGYG + GK YW++KNSW
Sbjct: 242 VATVGPISVAIDASHSSFQLYSEGVYDEPECSSEDLDHGVLVVGYGVKGGKKYWLVKNSW 301

Query: 885 GASWGEQGYFRLAR-GKNQCGISEDTVYPTI 974
           SWG+QGY  ++R  NQCGI+    YP +
Sbjct: 302 AESWGDQGYILMSRDNNNQCGIASQASYPLV 332
```

BLASTN vs TBLASTX

NCBI Minicourses

Anopheles gambiae mitochondrion, complete genome.
ACCESSION NC_002084 GI:5834911

Homo sapiens mitochondrion, complete genome.
ACCESSION NC_001807 GI:17981852



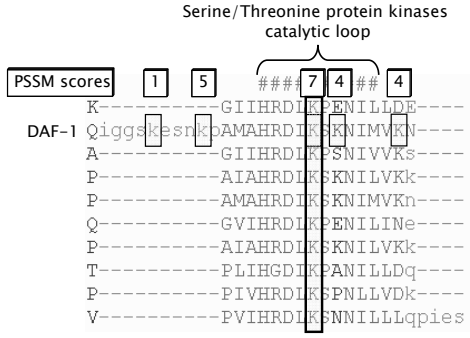
Hands-On

NCBI Minicourses

www.ncbi.nlm.nih.gov/Class/minicourses/quickblast.html

Position-Specific Score Matrix

NCBI Minicourses



Position-Specific Score Matrix

NCBI Minicourses

catalytic loop

		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
435	K	-1	0	0	-1	-2	3	0	3	0	-2	-2	1	-1	-1	-1	-1	-1	-1	-1	-2
436	E	0	1	0	2	-1	0	2	-1	0	-1	-1	0	0	0	-1	0	0	-1	-1	-1
437	S	0	0	-1	0	1	1	0	1	1	0	-1	0	0	0	2	0	-1	-1	0	-1
438	N	-1	0	-1	-1	1	0	-1	3	3	-1	-1	1	-1	0	0	-1	-1	1	1	-1
439	K	-2	1	1	-1	-2	0	-1	-2	-2	-1	-2	5	1	-2	-2	-1	-1	-2	-2	-1
440	P	-2	-2	-2	-2	-3	-2	-2	-2	-2	-1	-2	-1	0	-3	7	-1	-2	-3	-1	-1
441	A	3	-2	1	-2	0	-1	0	1	-2	-2	-2	0	-1	-2	3	1	0	-3	-3	0
442	M	-3	-4	-4	-4	-3	-4	-4	-5	-4	7	0	-4	1	0	-4	-4	-2	-4	-1	2
443	A	4	-4	-4	-4	0	-4	-4	-3	-4	4	-1	-4	-2	-3	-4	-1	-2	-4	-3	4
444	H	-4	-2	-1	-3	-5	-2	-2	-4	10	-6	-5	-3	-4	-3	-2	-3	-4	-5	0	-5
445	R	-4	8	-3	-4	0	-1	-2	-3	-2	-5	-4	0	-3	-2	-4	-3	-3	0	-4	-5
446	D	-4	-4	-1	8	-6	-2	0	-3	-3	-5	-6	-3	-5	-6	-4	-2	-3	-7	-5	-5
447	I	-4	-5	-6	-6	-3	-4	-5	-6	-5	3	5	-5	1	1	-5	-5	-3	-4	-3	1
448	K	0	0	1	-3	-5	-1	-1	-3	-3	-5	-5	7	-4	-5	-3	-1	-2	-5	-4	-4
449	S	0	-3	-2	-3	0	-2	-2	-3	-3	-4	-4	-2	-4	-5	2	6	2	-5	-4	-4
450	K	0	3	0	1	-5	0	0	-4	-1	-4	-3	4	-3	-2	2	1	-1	-5	-4	-4
451	N	-4	-3	8	-1	-5	-2	-2	-3	-1	-6	-6	-2	-4	-5	-4	-1	-2	-6	-4	-5
452	I	-3	-5	-5	-6	0	-5	-5	-6	-5	6	2	-5	2	-2	-5	-4	-3	-5	-3	3
453	M	-4	-4	-6	-6	-3	-4	-5	-6	-5	0	6	-5	1	0	-5	-4	-3	-4	-3	0
454	V	-3	-3	-5	-6	-3	-4	-5	-6	-5	3	3	-4	2	-2	-5	-4	-3	-5	-3	5
455	K	-2	1	1	4	-5	0	-1	-2	1	-4	-2	4	-3	-2	-3	0	-1	-5	-2	-3
456	N	1	1	3	0	-4	-1	1	0	-3	-4	-4	3	-2	-5	-2	2	-2	-5	-4	-4
457	D	-3	-2	5	5	-1	-1	1	-1	0	-5	-4	0	-2	-5	-1	0	-2	-6	-4	-5
458	L	-3	-1	0	-3	0	-3	-2	3	-4	-2	3	0	1	1	-2	-2	-3	5	-1	-3