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

Basic Local Alignment Search Tool

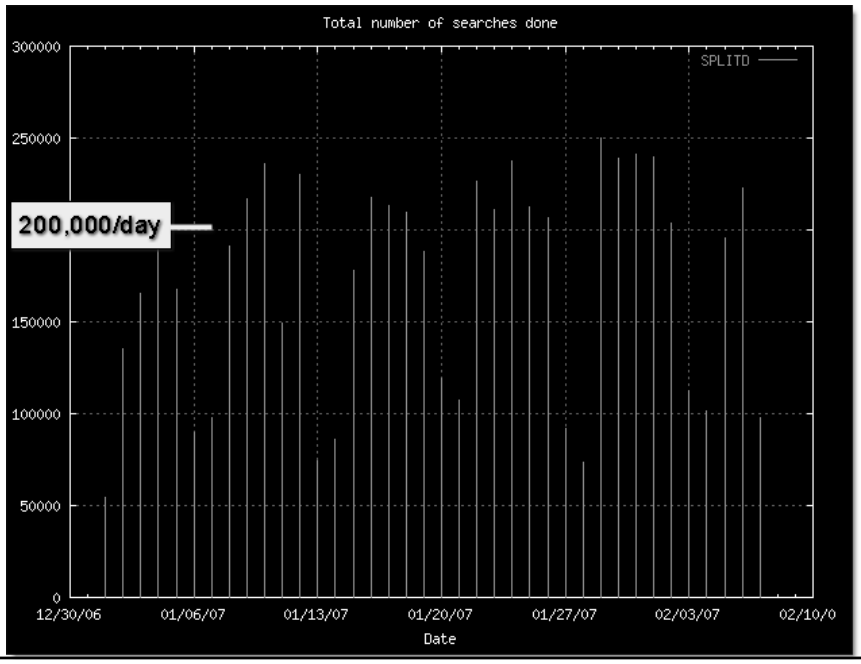
NCBI Minicourses

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



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Basic Local Alignment Search Tool

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- 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

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

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.

Word Hits & Extensions

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Nucleotide: one exact match

ATGCTGCTAGTCGATGACGTAGCTA

⇦ GCTGCTAGTCG ⇨

Protein: two matches within 40 residues

AIEKCYTGCTLAQEADDTA

⇦IDK ⇨ ⇦ EAD ⇨

BLAST

Search Basics

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BLAST Programs

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What is your goal?

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

More BLAST Programs

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

BLAST

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Nucleotide BLAST Databases

- 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

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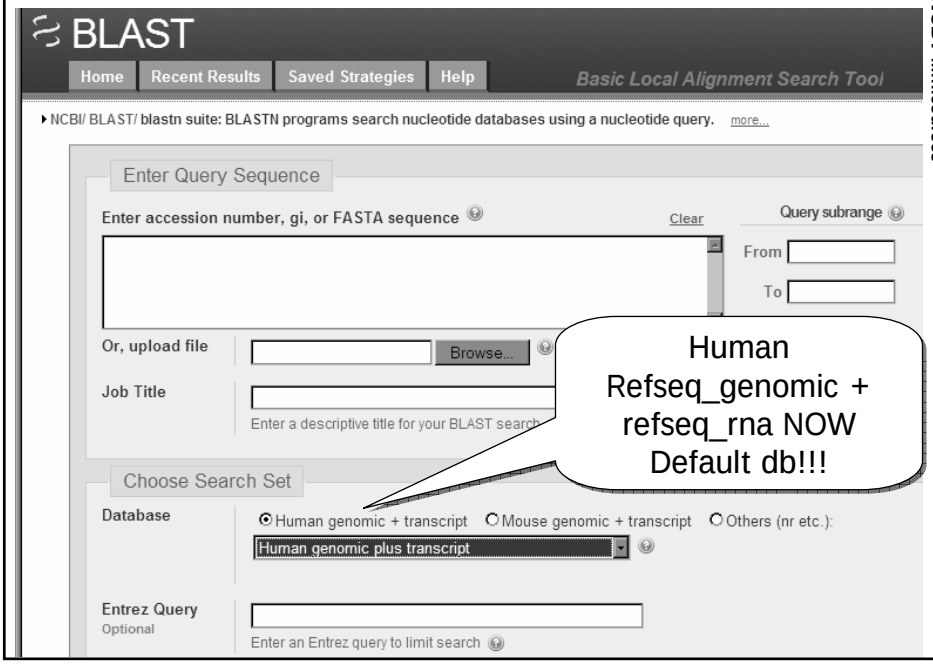
New Nucleotide Databases



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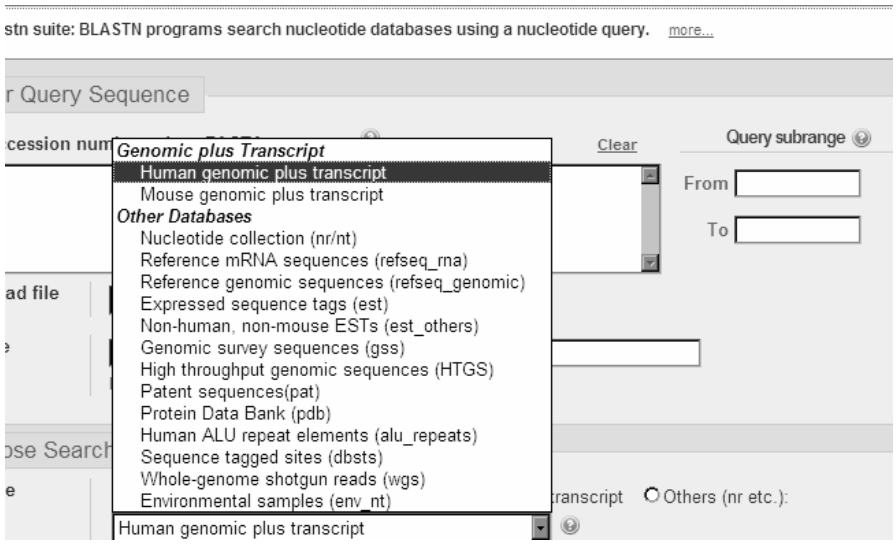
New Nucleotide Databases



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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

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NCBI — NCBI Map Viewer

Genomic Biology | Genome | Taxonomy | Entrez | BLAST | Help

Search - Select Group or Organism - for

Go!

Switch to Graphical View

Click on the organism name to go to the genome view

Vertebrates

Mammals

- BLAST *Bos taurus* (cow) Build 3.1
- BLAST *Bos taurus* (cow) Build 2.1
- BLAST *Canis familiaris* (dog)
- BLAST *Felis catus* (cat)
- BLAST *Homo sapiens* (human) Build 36
- BLAST *Homo sapiens* (human) Build 35
- BLAST *Macaca mulatta* (rhesus macaque)
- BLAST *Mus musculus* (mouse) Build 36
- BLAST *Mus musculus* (mouse) Build 35
- BLAST *Ovis aries* (sheep)
- BLAST *Pan troglodytes* (chimpanzee)
- BLAST *Rattus norvegicus* (rat)
- BLAST *Sus scrofa* (pig)

Other Vertebrates

- BLAST *Danio rerio* (zebrafish)

Plants BLAST Search all plant maps

- BLAST *Aegilops tauschii*
- BLAST *Aegilops umbellulata*
- BLAST *Allium cepa* (onion)
- BLAST *Arabidopsis thaliana* (thale cress)
- BLAST *Avena sativa* (oat)
- BLAST *Beta vulgaris* (beet)
- BLAST *Brassica juncea* (Indian mustard)
- BLAST *Brassica napus* (oilseed rape)
- BLAST *Brassica nigra* (black mustard)
- BLAST *Brassica oleracea*
- BLAST *Brassica rapa* (field mustard)
- BLAST *Capsicum annuum*
- BLAST *Eragrostis tef* (tef)
- BLAST *Glycine max* (soybean)
- BLAST *Hordeum vulgare* (barley)
- BLAST *Lotus japonicus* (lotus)
- BLAST *Manihot esculenta* (cassava)

BLAST

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Home | Recent Results | Saved Strategies | Help

Basic Local Alignment and S

NCBI/ BLAST Hom

BLAST fi ... between biological sequences. more...

Save your searches

What's New?

BLAST

Choose a species genome to search, or list all genomic BLAST databases.

- Human
- Mouse
- Rat
- *Arabadopsis thaliana*
- *Oryza sativa*
- *Bos taurus*
- *Danio rerio*
- *Drosophila melanogaster*
- *Gallus gallus*
- *Pan troglodytes*
- *Escherichia coli*
- *Apis mellifera*

Basic BLAST

Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

Specialized BLAST

Choose a type of specialized search (or database name in parentheses.)

- Search trace archives with within species or among species

News

BLAST 2.2.13 now available
The new release includes a new engine for blastall, changes to statistical parameters, and bug fixes.
2005-12-06 11:55:00

BLAST 2.2.14 now available
BLAST release 2.2.14 offers a universal binary for Mac OS X, and improved performance on some platforms.
2006-06-07 12:05:00

More BLAST news...

Tip of the Day

Checking PCR Primers

A frequent use of nucleotide-nucleotide BLAST is to check the specificity of oligonucleotides for hybridization in PCR. The goal is usually to make sure that the primers will give a unique product from the target genome or cDNA population. Because BLAST

BLAST

Search Basics

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Setting up a BLAST Search

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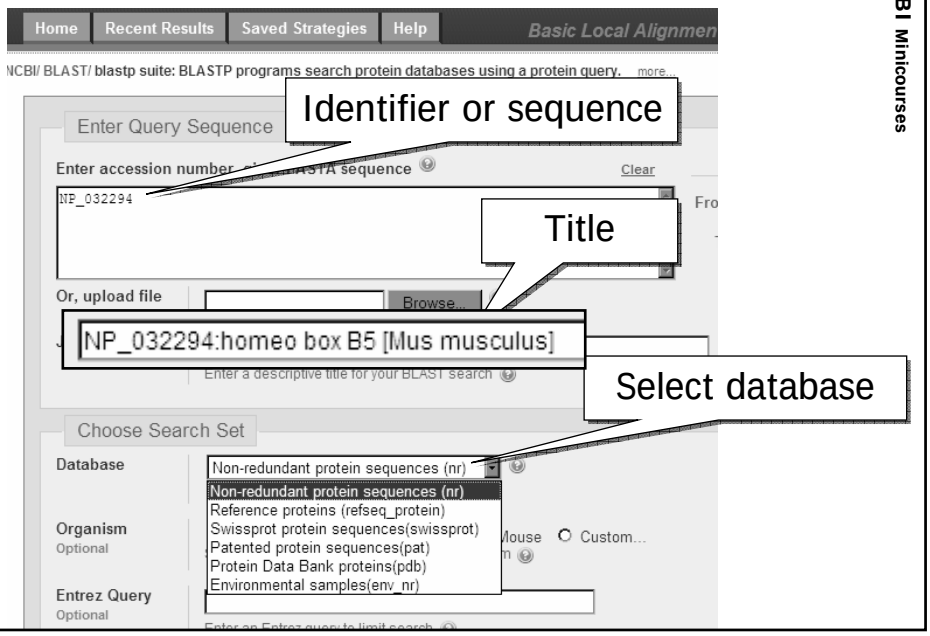
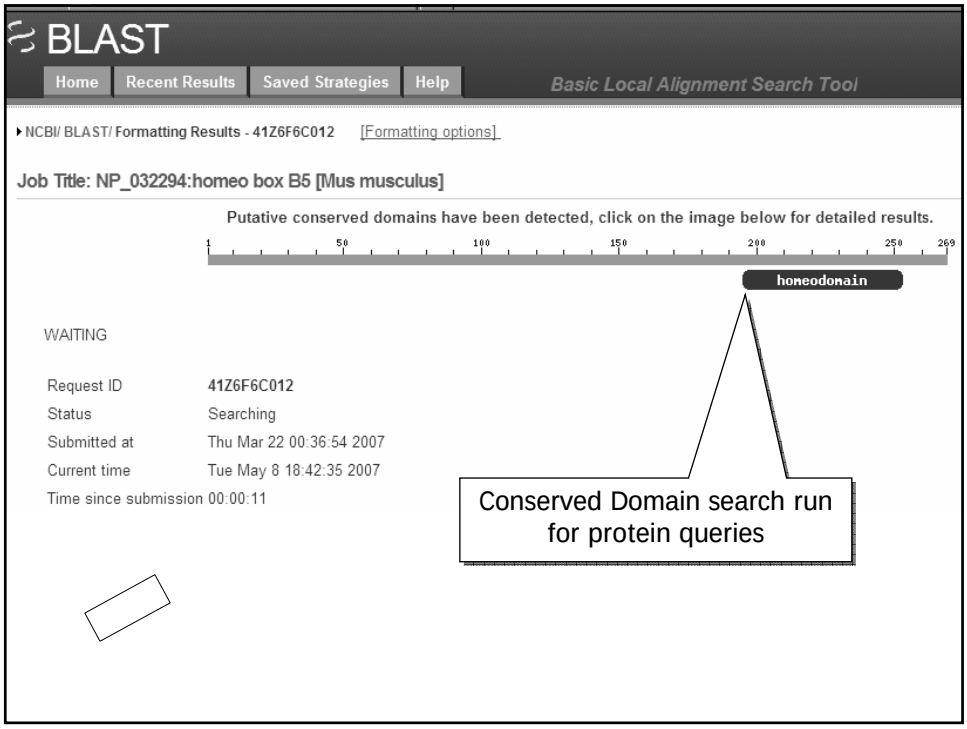
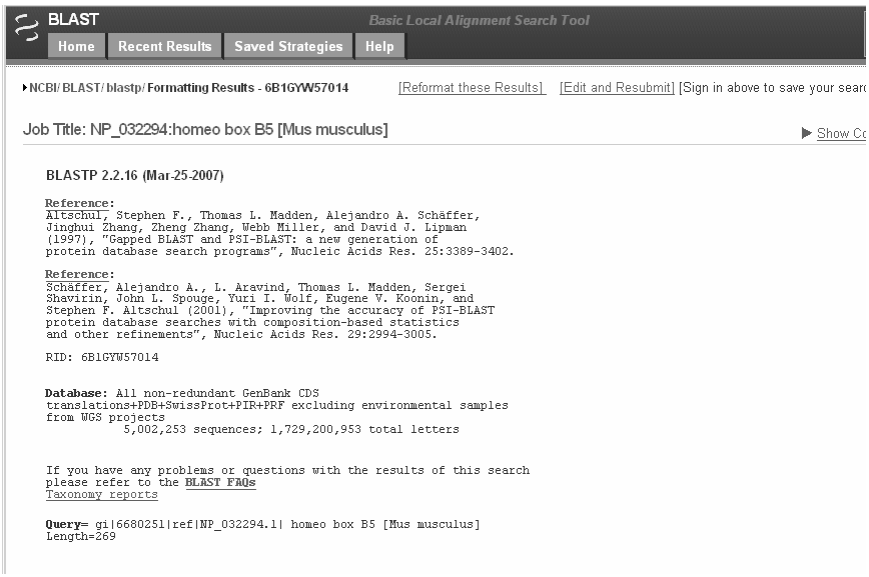




Default Output



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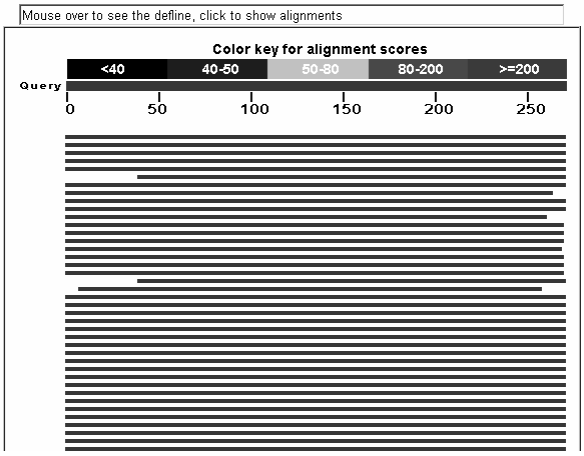
Default Output

Graphic Overview

NCBI Minicourses

Distribution of 104 Blast Hits on the Query Sequence

Mouse over to see the defline, click to show alignments



Summary Table

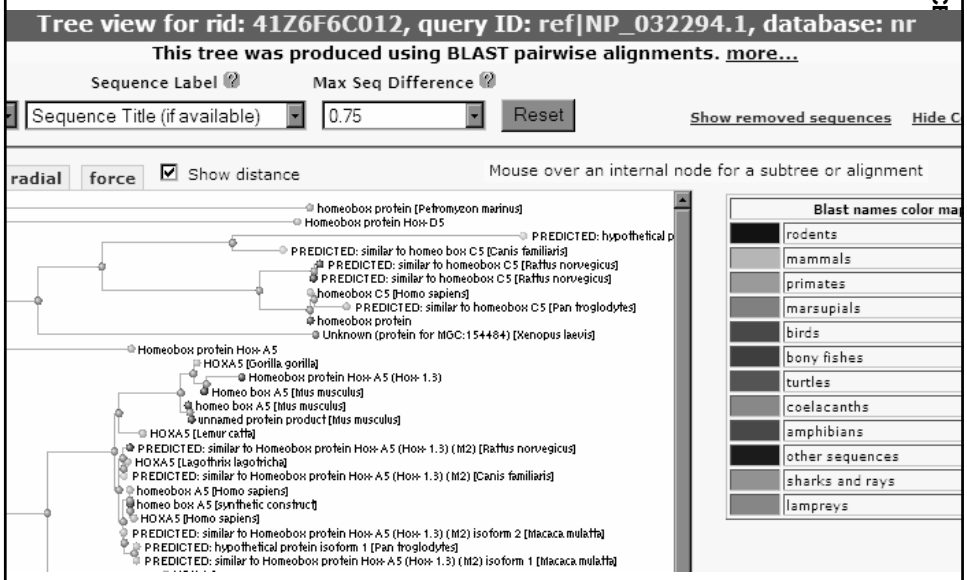
NCBI Minicourses

Distance tree of results NEW

Sequences producing significant alignments:	Score (Bits)	E Value	
ref|NP_032294.1| homeo box B5 [Mus musculus] >gb|AAA37842.1| hom	420	4e-116	U G
ref|XP_573183.1| PREDICTED: similar to Homeobox protein Hox-B...	419	1e-115	U G
ref|XP_548176.2| PREDICTED: similar to Homeobox protein Hox-B...	416	7e-115	U G
ref|NP_002138.1| homeobox B5 [Homo sapiens] >ref|XP_001173004...	412	8e-114	U G
ref|XP_001367145.1| PREDICTED: similar to homeobox protein [Mono	378	2e-103	G
dbj|BAB28059.1| unnamed protein product [Mus musculus]	364	3e-99	U G
ref|NP_001020526.1| homeo box B5 [Gallus gallus] >gb|AAW48484...	347	4e-94	U G
dbj|BAD95556.1| Hoxb-5 [Gallus gallus]	337	4e-91	U G
emb|CAA48320.1| homeodomain protein [Danio rerio] >emb|CAA312...	327	5e-88	G
ref|NP_571176.2| homeo box B5a [Danio rerio] >sp|P09014|HXB5A...	325	1e-87	U G
dbj|BAD95561.1| Hoxb-5 [Pelodiscus sinensis]	320	6e-86	
ref|XP_001336387.1| PREDICTED: similar to homeo box protein B...	315	1e-84	U G
ref|NP_571612.2| homeo box B5b [Danio rerio] >gb|AAH78285.1| ...	315	1e-84	U G
gb|AAD15946.1| homeobox protein [Danio rerio]	312	1e-83	G
gb|ABM73560.1| homeodomain protein [Megalobrama amblycephala]	310	5e-83	

BLAST Output – tree view

NCBI



Alignment View - pairwise

NCBI Minicourses

```
> emb|CAA48320.1| homeodomain protein [Danio rerio]
 emb|CAA31290.1| ZF-21 gene product [Danio rerio]
Length=275

 Score =  327 bits (837),  Expect = 5e-88, Method: Composition-based stats.
 Identities = 200/278 (71%), Positives = 227/278 (81%), Gaps = 12/278 (4%)

Query  1    MSSYFVNSFSGRYPNGPDYQLLNYGSGSS-LSGSYRDPAAMHTGSYGYNYNGMDLSVNRS  59
            MSSYFVNSFSGRYPNGPDYQLLNYG+ SS ++ SYRD   MH+GSYGYNYNGMDLSVNRS
Sbjct  1    MSSYFVNSFSGRYPNGPDYQLLNYGTSSSAMNASYRDSGTMHSGSYGYNYNGMDLSVNRS  60

Query  60   SASSSHFGAVGESSRAFPASAKEPRFRQATSSCSLSSPESLPCTNGDSHGAKPSASSPSD  119
            + S+ HFGAVG++SR F + A E RFRQ  SSCSL+SPE LPC+N +S G +    +PSD
Sbjct  61   T-STGHFGAVGDNSRVFQSPAPETRFRQP-SSCSLASPEPLPCSNSESFGTQ-RLFAPSD  117

Query  120  QATPAS-----SSANFTEIDEASASSEPEEAASQLSSPSLARAQPEPMATSTAAP---EG  171
            Q+T  +     S+ +FTEIDEASASSE EEA+ + ++ +    Q +    ++      +G
Sbjct  118  QSTTTAGNNLNSNTHFTEIDEASASSETEEASHRANNSAPRTQQKQETTATSTTSATSDG  177

Query  172  QTPQIFPWMRKLHISHDMTGPDGKRARTAYTRYQTLELEKEFHFNRYLTRRRRIEIAHAL  231
            Q PQIFPWMRKLHISHDMTGPDGKRARTAYTRYQTLELEKEFHFNRYLTRRRRIEIAHAL
Sbjct  178  QAPQIFPWMRKLHISHDMTGPDGKRARTAYTRYQTLELEKEFHFNRYLTRRRRIEIAHAL  237

Query  232  CLSERQIKIWFQNRRMKWKKDNKLKSMSLATAGSAFQP  269
            CLSERQIKIWFQNRRMKWKKDNKLKSMSLATAGSAFQP
Sbjct  238  CLSERQIKIWFQNRRMKWKKDNKLKSMSLATAGSAFQP  275
```

New Output View

NCBI Minicourses

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

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

Accession	Description	Max score	Total score	Query coverage	E value	Links
NP_032294.1	homeo box B5 [Mus musculus] >gb|AAA37842.1| homeobox protein	420	420	100%	4e-116	U G
XP_573183.1	PREDICTED: similar to Homeobox protein Hox-B5 (Hox-2.1) (MU-1) (H24.1) [Rattus norveg	419	419	100%	1e-115	U G
XP_548176.2	PREDICTED: similar to Homeobox protein Hox-B5 (Hox-2A) (HHO.C10) (HU-1) isoform 1 [C	416	416	100%	7e-115	U G
NP_002139.1	homeobox B5 [Homo sapiens] >ref|XP_001173004.1| PREDICTED: homeobox B5 [Pan tro	412	412	100%	8e-114	U G
XP_001367145.1	PREDICTED: similar to homeobox protein [Monodelphis domestica]	370	370	100%	2e-103	G
BAB28059.1	unnamed protein product [Mus musculus]	364	364	85%	3e-99	U G
NP_001020526.1	homeo box B5 [Gallus gallus] >gb|AAW40484.1| homeodomain transcription factor [Gallus	347	347	100%	4e-94	U G
BAD95556.1	Hoxb-5 [Gallus gallus]	337	337	97%	4e-91	U G
CAA48320.1	homeodomain protein [Danio rerio] >emb|CAA31290.1| ZF-21 gene product [Danio rerio]	327	327	100%	5e-88	G
NP_571176.2	homeo box B5a [Danio rerio] >sp|P09014|HXB5A_BRARE Homeobox protein Hox-B5a (Ho	325	325	100%	2e-87	U G
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XP_001336387.1	PREDICTED: similar to homeo box protein B5b [Danio rerio] >sp|P09013|HXB5B_BRARE H	315	315	99%	1e-84	U G
NP_571612.2	homeo box B5b [Danio rerio] >gb|AAH78285.1| Homeo box B5b [Danio rerio]	315	315	99%	1e-84	U G
AAD15946.1	homeobox protein [Danio rerio]	312	312	99%	1e-83	G
ABM73560.1	homeodomain protein [Megalobrama amblycephala]	310	310	99%	5e-83	
BAE44275.1	hoxB5b [Oryzias latipes] >dbj|BAE53482.1| hoxB5b [Oryzias latipes]	303	303	99%	5e-81	
Q1KKX0	Homeobox protein Hox-B5b >gb|ABF22424.1| homeobox protein HoxB5b [Takifugu rubripe	302	302	99%	1e-80	
AAQ72845.1	Hoxb5b [Sphoeroides nephelus]	301	301	99%	3e-80	
AAQ43020.1	HoxB5 [Latimeria menadoensis]	292	292	85%	1e-77	
AAX63757.1	Hoxb5bi [Oncorhynchus mykiss]	284	284	92%	3e-75	
AAI35756.1	Unknown (protein for MGC:122098) [Xenopus tropicalis]	278	278	100%	2e-73	
CAG01627.1	unnamed protein product [Tetraodon nigroviridis]	260	260	100%	5e-68	
Q1KKX9	Homeobox protein Hox-B5a >gb|ABF22415.1| homeobox protein HoxB5a [Takifugu rubripe	259	259	100%	1e-67	
Q6B3N0	Homeobox protein Hox-A5 >gb|AAT90845.1| homeodomain transcription factor [Gallus gall	253	253	100%	5e-66	
XP_001362736.1	PREDICTED: similar to homeodomain transcription factor [Monodelphis domestica]	249	249	100%	2e-64	G
XP_001064984.1	PREDICTED: similar to Homeobox protein Hox-A5 (Hox-1.3) (M2) [Rattus norvegicus] >ref	248	248	100%	2e-64	U G
AAH11063.1	Homeo box A5 [Mus musculus]	248	248	100%	3e-64	U G
A2DSK9	Homeobox protein Hox-A5 >gb|ABM68201.1| HOXA5 [Lagothrix lagotricha]	248	248	100%	3e-64	
A2DSY4	Homeobox protein Hox-A5 >gb|ABM68948.1| HOXA5 [Lemur catta]	247	247	100%	4e-64	

BLAST

Search Basics

NCBI Minicourses

- Programs
- Databases
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...con't

Where do Scores Come From?

NCBI Minicourses

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

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Scoring Matrices

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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

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Protein search: substitution matrix

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

PAM Matrices

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- 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

BLOSUM Matrices

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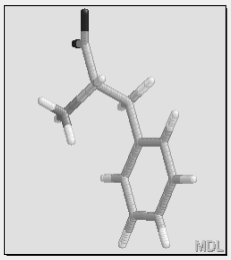
- local alignments
- all matrices based on observed alignments; not extrapolated
- BLOSUM 62 calculated from sequences with no more than 62% identity
- Examples: BLOSUM45, BLOSUM62 and BLOSUM80
- BLOSUM62: very good for detecting weak protein similarities
- BLOSUM62 is the default matrix in BLAST

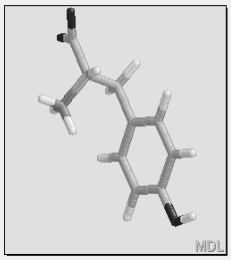
BLOSUM62

NCBI Minicourses

L-phenylalanine (F)



L-tyrosine (Y)



	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	-2	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	[illegible]	[illegible]	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4					
T	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-1	-1	-2	-1	1	5				
W	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	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	[illegible]	[illegible]	-1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	1	-2	-2	0	-3	-1	4	
X	0	-1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1	-2	0	0	-2	-1	-1	-1

Negative for less likely substitutions

Positive for more likely substitutions

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*

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Where do Expect Values Come From?

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%	

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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**

E is dependent on m x n (search space)

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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

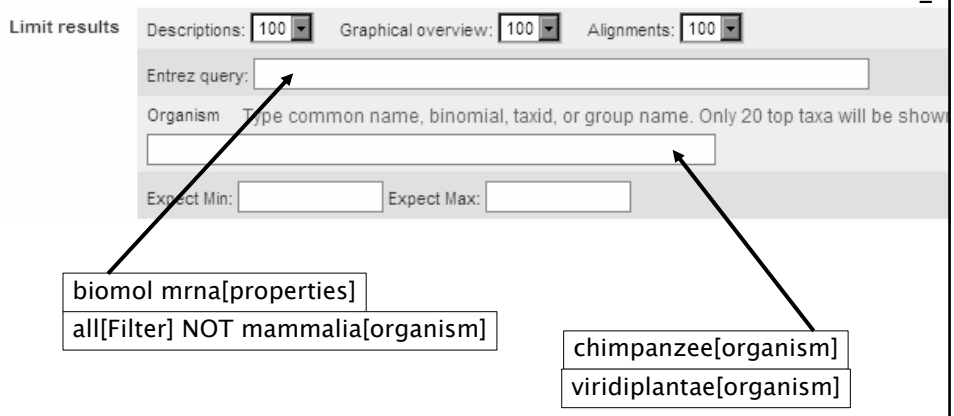
Search Basics

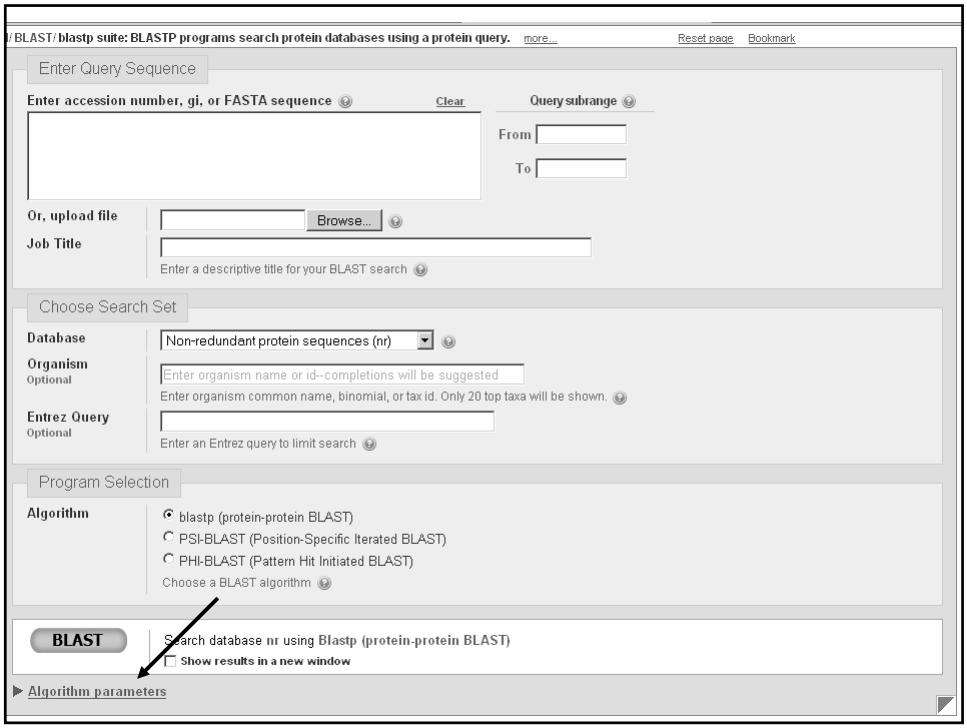
NCBI Minicourses

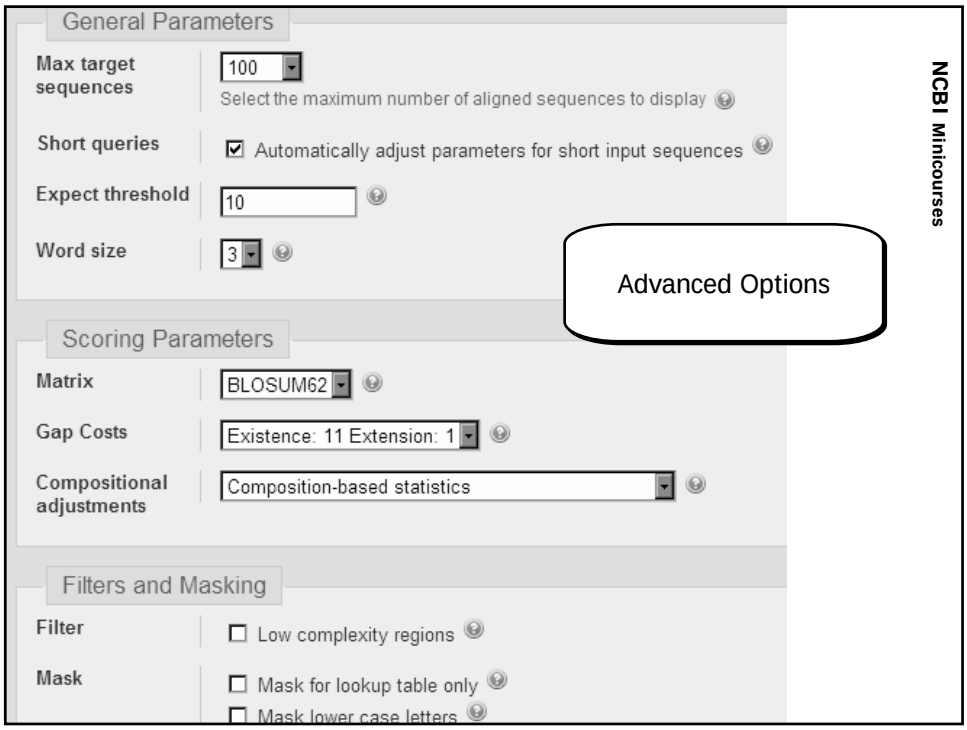
- Programs
- Databases
- Submit a search
- Interpret the results
- BLAST options
- Format options
- Examples

Initial Options

NCBI







NCBI Minicourses

BLAST

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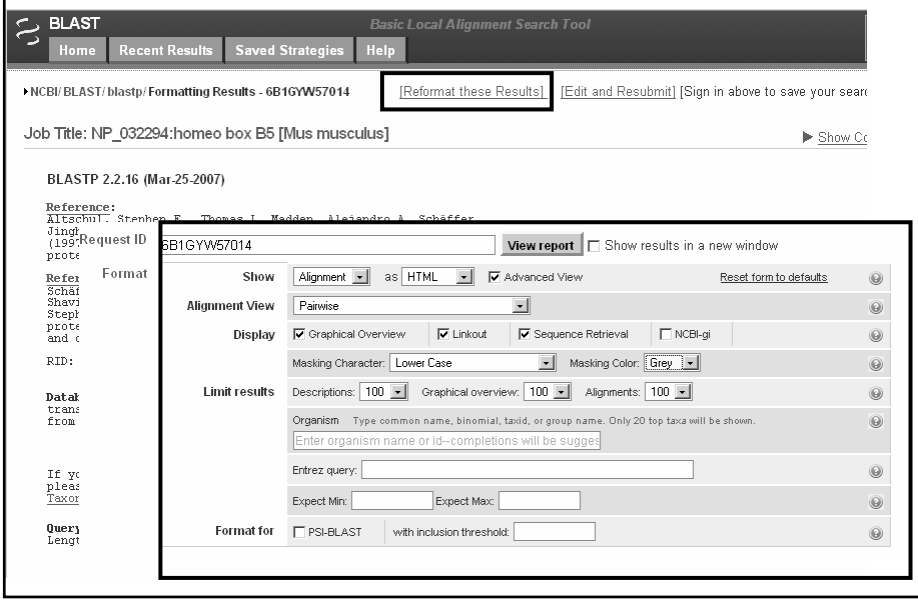
Default Output

NCBI Minicourses



BLAST Output: Alignments & Filter

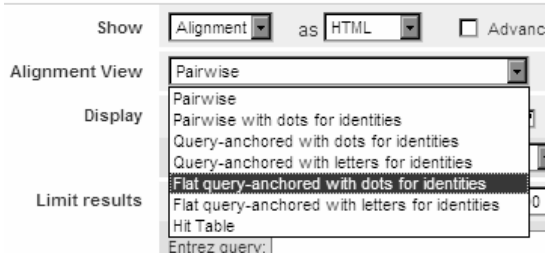
NCBI

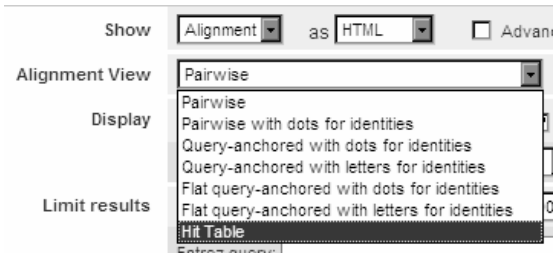
```
>gi|466462|gb|AAA17374.1| human homolog of E. coli mutL gene product, Swiss-Prot
          Number P23367
        Length=756

 Score = 219.935 bits (559),  Expect = 8.71148e-57
 Identities = 120/131 (91%), Positives = 125/131 (95%), Gaps = 2/131 (1%)

Query  1    IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESILE-VQQHIESKLL  59
            IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESILE VQQHIESKLL
Sbjct  276  IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESILERVQQHIESKLL  335

Query  60   GSNSSRMYFTQTLLPGLAGPSGEMVK-sttsltssstsgsDKVYAHQMVRTDSREQKLDA  118
            GSNSSRMYFTQTLLPGLAGPSGEMVK +T+  +SS++  SDKVYAHQMVRTDSREQKLDA
Sbjct  336  GSNSSRMYFTQTLLPGLAGPSGEMVKSTTSLTSSSTSGSSDKVYAHQMVRTDSREQKLDA  395

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

low complexity sequence filtered

Alignment View Options

NCBI Minicourses





Alignment View: Flat Query-Anchored with Dots for 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

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```
# 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 o
# 514 hits found
gi|6680251|ref|NP_032294.1|     gi|1708354|sp|P09079|HXB5_MOUSE;gi|6680251|ref|NP_032294.1|;2
gi|6680251|ref|NP_032294.1|     gi|74225740|dbj|BAE21695.1|;gi|73695452|gb|AAI03605.1|;gi|730
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|400
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|26
gi|6680251|ref|NP_032294.1|     gi|62537|emb|CAA48320.1|;gi|62555|emb|CAA31290.1|       70.14
gi|6680251|ref|NP_032294.1|     gi|15029897|gb|AAH11083.1|      58.93   68.21   280     94
gi|6680251|ref|NP_032294.1|     gi|47216999|emb|CAG01627.1|    55.35   61.64   318     92
```

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

BLAST

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NCBI Minicourses

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Examples

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- Protein searches more sensitive than nucleotide searches

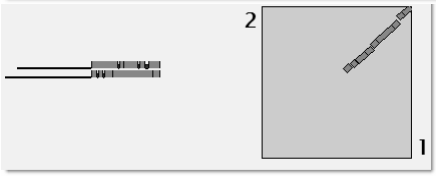
  - redundancy of the genetic code

- blastn, megablast best when searching within same organism

BLASTN vs BLASTP

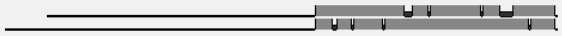
BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.14 [Apr-09-2006]

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 . . .

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

```
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                                YSLSEQQLIDCCTD-TSAGCD 533
           WRK+G VT VKDQG                                YSLSEQ L+DC     + GC+
Sbjct: 122 WRKKGAVTPVKDQG                                YSLSEQNLVDCSQSFGNNGCE 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

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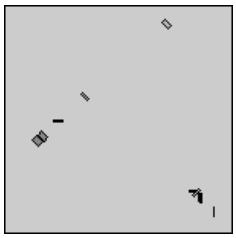
Anopheles gambiae mitochondrion, complete genome.
ACCESSION NC_002084 GI:5834911

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

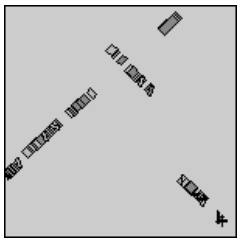
blastn



Homo sapiens

Anopheles gambiae

tblastx



Homo sapiens

Anopheles gambiae

Hands-On

NCBI Minicourses

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