Mini Courses NCBI Resources one course at a time

NCBI Minicourses

BLAST Quick Start

blast-help@ncbi.nlm.nih.gov

BLAST Quick Start

NCBI Minicourses

Introduction

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

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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)

Why do we need similarity searching?

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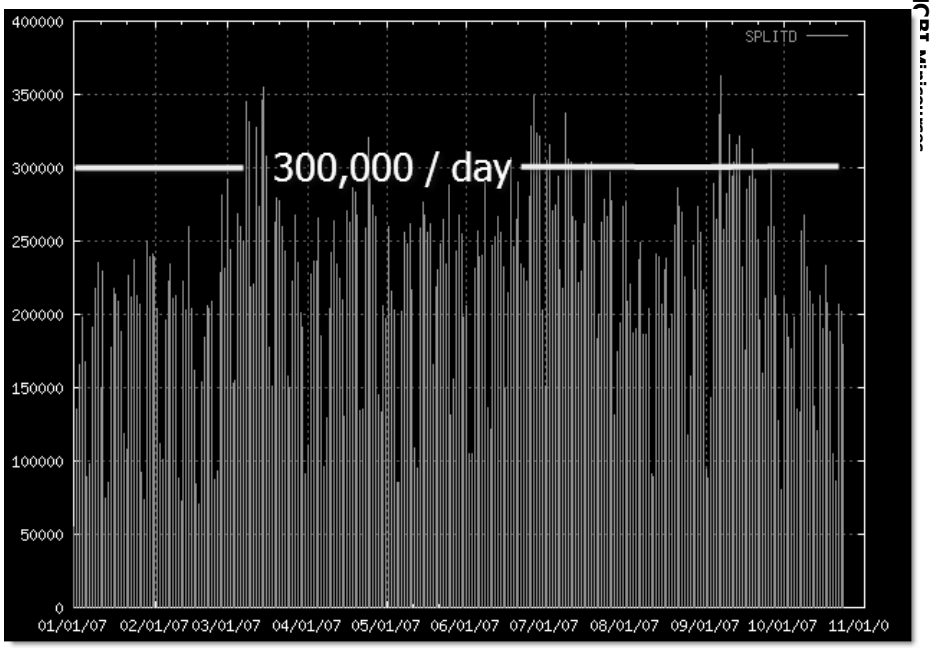
- To identify and annotate sequences with…
  - incomplete, incorrect, or absent annotations
- To assemble genomes
- To explore evolutionary relationships by…
  - finding homologous molecules
  - developing phylogenetic trees

NOTE: Similar sequences may NOT have similar function!

BLAST Web Searches, 2007



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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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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.

Neighborhood Words (BLOSUM62)

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IEK: keep LEK?
(threshold = 11)

LEK = 12

E / E

L / I

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

⇦ TAGTCGATGA ⇨

Protein: two matches within 40 residues

PHAIEKCYTGCTLAQEADDTA

⇦ IDK ⇨ ⇦ EAD ⇨

BLASTP Summary

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example query words

```
Query:   IETVYAAYLPKNTHPFLYLSLEISPQNVDVNVHPTKHEVHFLHEESILEV…
```

YLS	15		HFL	18
YLT	12		HFV	15
YVS	12	Neighborhood words	HFS	14
YIT	10		HWL	13
etc	…		NFL	13
			DFL	12
			HWV	10
			etc	…

Neighborhood score threshold

T (-f) =11

```
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 (gapped extension)

NCBI Minicourses

```
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

Search Basics

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- Programs
- Databases
- Submit a search
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- BLAST options
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- Examples

BLAST Programs

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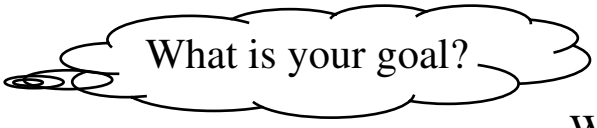

		Word Size
blastn	nucleotide X nucleotide	11
blastp	protein X protein	3

6 frame, translated nucleotide searches

blastx	nucleotide X protein	3
tblastx	nucleotide X nucleotide	3
tblastn	protein X nucleotide	3

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

Search Basics

NCBI Minicourses

- Programs
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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
- **wgs**
  - wgs entries from traditional divisions
- **pdb**
  - Nucleotide sequences from structures
- **env_nt**
  - environmental samples

combined, for human and mouse

New Nucleotide Databases

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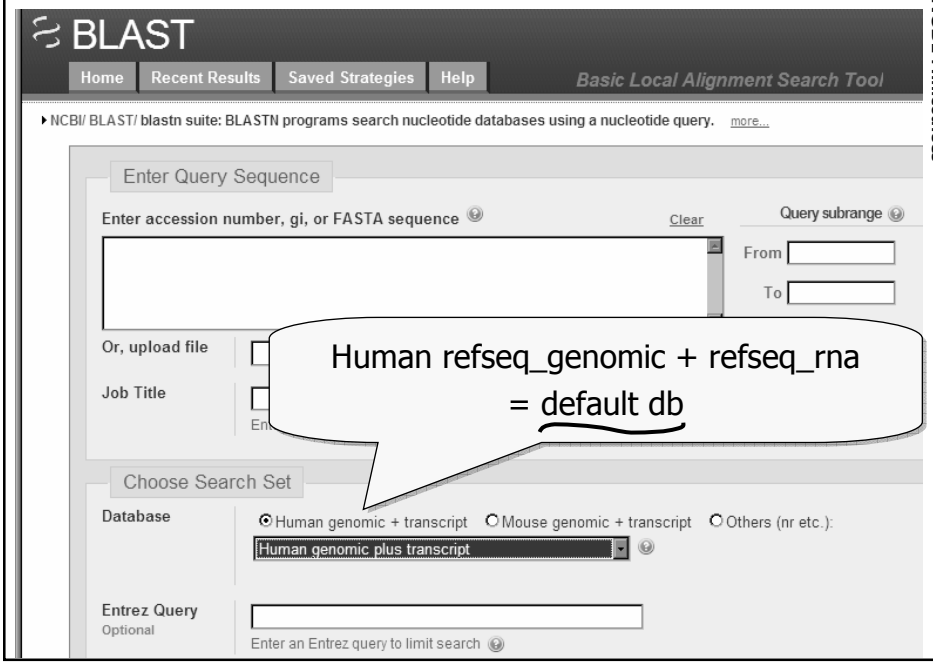
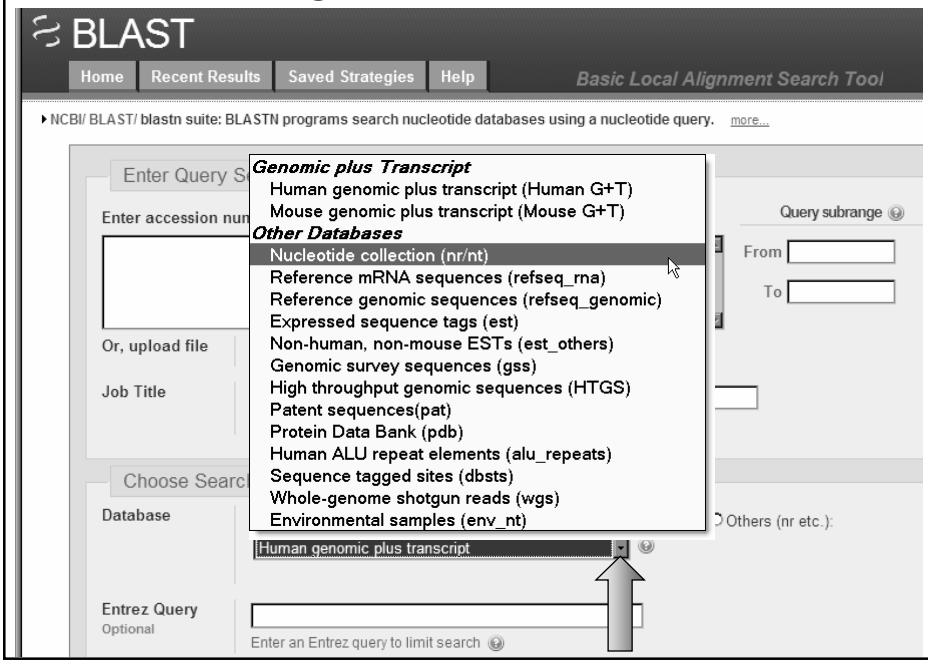


Human refseq_genomic + refseq_rna
= default db

Change Nucleotide Database



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Protein BLAST Databases

Protein

- nr

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

nr ≠ nr

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BLAST Databases: Genome-specific

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NCBI/ BLAST Home

BLAST finds regions of similarity between biological sequences. more...

Learn more about how to use the new BLAST design

BLAST Assembled Genomes

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

- Human
- Mouse
- Rat
- *Arabidopsis thaliana*
- *Oryza sativa*
- *Bos taurus*
- *Danio rerio*
- *Drosophila melanogaster*
- *Gallus gallus*
- *Pan troglodytes*
- *Microbes*
- *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

BLAST

My NCBI [Sign In] [Register]

Home | Recent Results | Saved Strategies | Help

Basic Local Alignment and Search Tool

Save your searches

What else is new?

NCBI/ BLAST Hom

BLAST fi etween biological sequences. more...

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

Search Basics

NCBI Minicourses

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Setting up a BLAST Search

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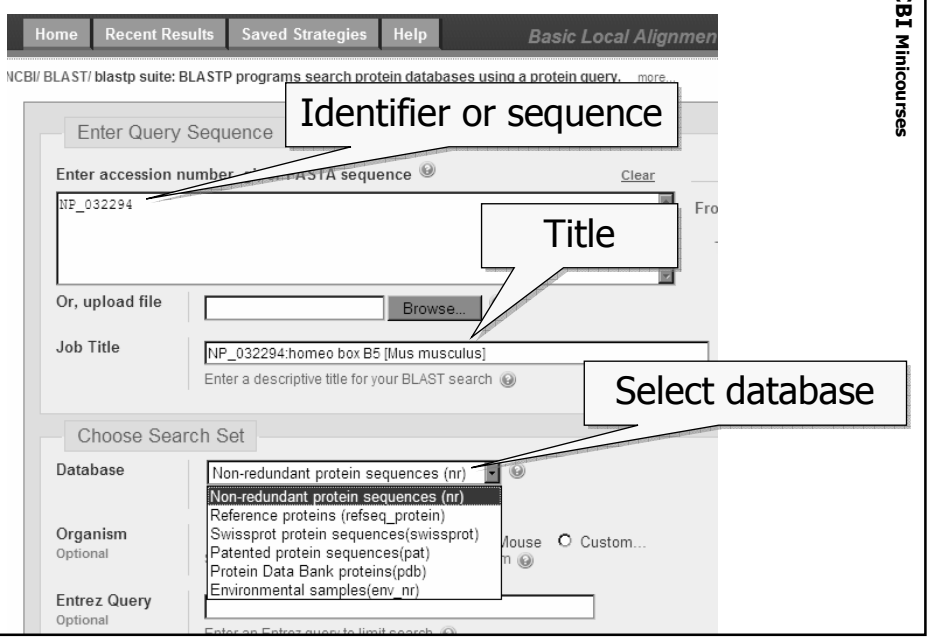

BLAST

Home | Recent Results | Saved Strategies | Help

Basic Local Alignment Search Tool

NCBI/ BLAST/ Formatting Results - 41Z6F6C012 [Formatting options]

Job Title: NP_032294:homeo box B5 [Mus musculus]

Putative conserved domains have been detected, click on the image below for detailed results.

1 50 100 150 200 250 269

homeodomain

WAITING

Request ID	41Z6F6C012
Status	Searching
Submitted at	Thu Mar 22 00:36:54 2007
Current time	Tue May 8 18:42:35 2007
Time since submission	00:00:11

Conserved Domain search run for protein queries

Default Output

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BLAST

Basic Local Alignment Search Tool

Home | Recent Results | Saved Strategies | Help

NCBI/ BLAST/ blastp/ Formatting Results - 6B1GYW57014 [Reformat these Results] [Edit and Resubmit] [Sign in above to save your searc

Job Title: NP_032294:homeo box B5 [Mus musculus] Show Co

```
BLASTP 2.2.16 (Mar-25-2007)

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.

Reference:
Schäffer, Alejandro A., L. Aravind, Thomas L. Madden, Sergei
Shavirin, John L. Spouge, Yuri I. Wolf, Eugene V. Koonin, and
Stephen F. Altschul (2001), "Improving the accuracy of PSI-BLAST
protein database searches with composition-based statistics
and other refinements", Nucleic Acids Res. 29:2994-3005.

RID: 6B1GYW57014


Database: All non-redundant GenBank CDS
translations+PDB+SwissProt+PIR+PRF excluding environmental samples
from WGS projects
          5,002,253 sequences; 1,729,200,953 total letters


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


Query= gi|6680251|ref|NP_032294.1| homeo box B5 [Mus musculus]
Length=269
```

header

Default Output con't

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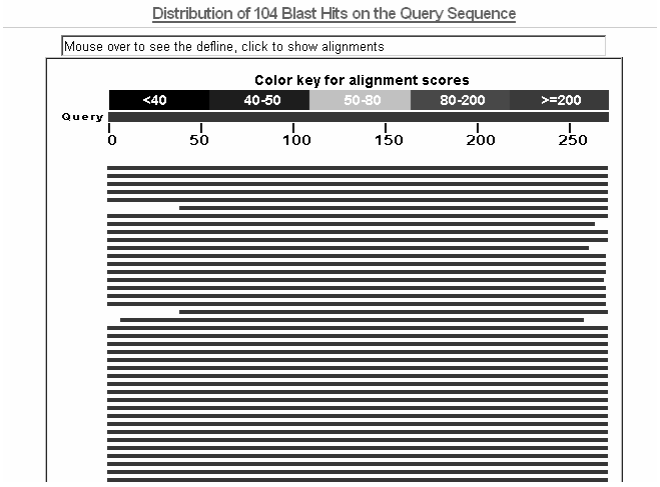
Graphical Overview



Default Output con't

NCBI Minicourses

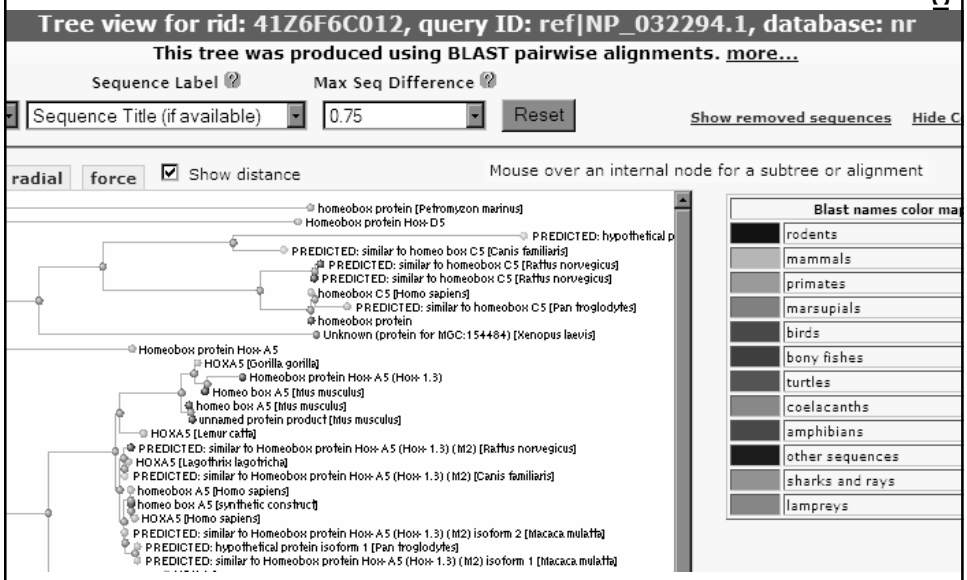
One line descriptions (default view)

Distance tree of results NEW

Sequences producing significant alignments:	Score (Bits)	E Value	
ref|NP 032294.2| homeo box B5 [Mus musculus] >gi|62656894|ref...	553	4e-156	G
gb|AAA37842.1| homeobox protein	551	1e-155	U G
ref|XP 548176.2| PREDICTED: similar to Homeobox protein Hox-B...	548	1e-154	U G
ref|XP 001502124.1| PREDICTED: hypothetical protein [Equus cabal	546	4e-154	G
ref|NP 002138.1| homeobox B5 [Homo sapiens] >ref|XP_001173004...	546	7e-154	U G
dbj|BAB28059.1| unnamed protein product [Mus musculus]	474	3e-132	U G
ref|XP 001367145.1| PREDICTED: similar to homeobox protein [Mono	462	8e-129	G
ref|NP 001020526.1| homeo box B5 [Gallus gallus] >gb|AAW48484...	431	3e-119	U G
dbj|BAD95556.1| Hoxb-5 [Gallus gallus]	417	4e-115	U G
ref|NP 571176.2| homeo box B5a [Danio rerio] >sp|P09014|HXB5A...	394	3e-108	U G
emb|CAA48320.1| homeodomain protein [Danio rerio] >emb|CAA312...	392	1e-107	G
dbj|BAD95561.1| Hoxb-5 [Pelodiscus sinensis]	385	2e-105	

BLAST Output – tree view

NCI



Alignment View - pairwise

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```
> emb|CAA48320.1| G homeodomain protein [Danio rerio]
 emb|CAA31290.1| G 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
```

Where do Scores Come From?

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One line descriptions
(Advanced view)

Distance tree of results NEW

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 average	E value	Links
NP_032294.2	homeo box B5 [Mus musculus] >ref|XP_573183.1| PREDICTED: similar to	553	553	.00%	4e-156	G
AAA37842.1	homeobox protein	551	551	.00%	1e-155	U G
XP_548176.2	PREDICTED: similar to Homeobox protein Hox-B5 (Hox-2A) (HHO.C10) (H	548	548	.00%	1e-154	U G
XP_001502124.1	PREDICTED: hypothetical protein [Equus caballus]	546	546	.00%	4e-154	G
NP_002138.1	homeobox B5 [Homo sapiens] >ref|XP_001173004.1| PREDICTED: home	546	546	.00%	7e-154	U G
BAB28059.1	unnamed protein product [Mus musculus]	474	474	85%	3e-132	U G
XP_001367145.1	PREDICTED: similar to homeobox protein [Monodelphis domestica]	462	462	.00%	8e-129	G
NP_001020526.1	homeo box B5 [Gallus gallus] >gb|AAW48484.1| homeodomain transcrip	431	431	.00%	3e-119	U G
BAD95556.1	Hoxb-5 [Gallus gallus]	417	417	97%	4e-115	U G
NP_571176.2	homeo box B5a [Danio rerio] >sp|P09014|HXB5A_BRARE Homeobox prot	394	394	.00%	3e-108	U G
CAA48320.1	homeodomain protein [Danio rerio] >emb|CAA31290.1| ZF-21 gene prod	392	392	.00%	1e-107	G
BAD95561.1	Hoxb-5 [Pelodiscus sinensis]	385	385	96%	2e-105	

Scoring Matrices

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Nucleotide = 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 = substitution matrix

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

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

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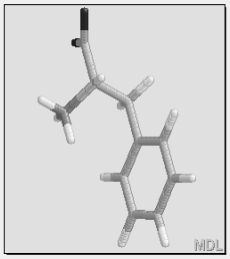
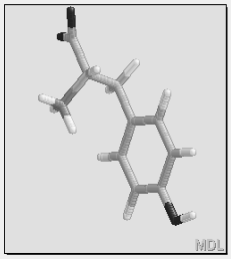
L-phenylalanine (F)



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	-3	-3	-3	-3	-1	-3	-3	-4	-3	4											
L	-2	-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

Negative for less likely substitutions

Positive for more likely substitutions

Where do Expect Values Come From?

NCBI Minicourses

Distance tree of results NEW

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
NP_032294.2	homeo box B5 [Mus musculus] >ref|XP_573183.1| PREDICTED: similar to H	553	553	100%	4e-156
AAA37842.1	homeobox protein	551	551	100%	1e-155
XP_548176.2	PREDICTED: similar to Homeobox protein Hox-B5 (Hox-2A) (HHO.C10) (HU-	548	548	100%	1e-154
XP_001502124.1	PREDICTED: hypothetical protein [Equus caballus]	546	546	100%	4e-154
NP_002138.1	homeobox B5 [Homo sapiens] >ref|XP_001173004.1| PREDICTED: homeob	546	546	100%	7e-154
BAB28059.1	unnamed protein product [Mus musculus]	474	474	85%	3e-132
XP_001367145.1	PREDICTED: similar to homeobox protein [Monodelphis domestica]	462	462	100%	8e-129
NP_001020526.1	homeo box B5 [Gallus gallus] >gb|AAW48484.1| homeodomain transcriptio	431	431	100%	3e-119
BAD95556.1	Hoxb-5 [Gallus gallus]	417	417	97%	4e-115
NP_571176.2	homeo box B5a [Danio rerio] >sp|P09014|HXB5A_BRARE Homeobox protein	394	394	100%	3e-108
CAA48320.1	homeodomain protein [Danio rerio] >emb|CAA31290.1| ZF-21 gene produc	392	392	100%	1e-107
BAD95561.1	Hoxb-5 [Pelodiscus sinensis]	385	385	96%	2e-105

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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**

NCBI Minicourses

Search Basics

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

Initial Options – Search Set

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Choose Search Set

Database: Non-redundant protein sequences (nr)

Organism Optional: mamm

- mammals (taxid:40674)
- Mammalia (taxid:40674)
- placental mammals (taxid:9347)
- eutherian mammals (taxid:9347)
- egg-laying mammals (taxid:9255)
- mammalian hepatitis B-type viruses (taxid:10405)
- Mammalian virus group (taxid:353212)
- Mammalian orthoreovirus (taxid:351073)
- Mouse mammary tumor virus (taxid:11757)
- Mammillaria (taxid:130139)

be shown.

Entrez Query Optional

Program Selectio

Algorithm

BLAST

Search database nr using Blastp (protein-protein BLAST)

Show results in a new window

Algorithm parameters

Initial Options – Search Set

NCBI Minicourses

Choose Search Set

Database: Non-redundant protein sequences (nr)

Organism Optional: Enter organism name or id--completions will be suggested

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Entrez Query Optional

Enter an Entrez query to limit search

all[filter] NOT mammalia[organism]

Program Selection

Algorithm

- blastp (protein-protein BLAST)
- PSI-BLAST (Position-Specific Iterated BLAST)
- PHI-BLAST (Pattern Hit Initiated BLAST)

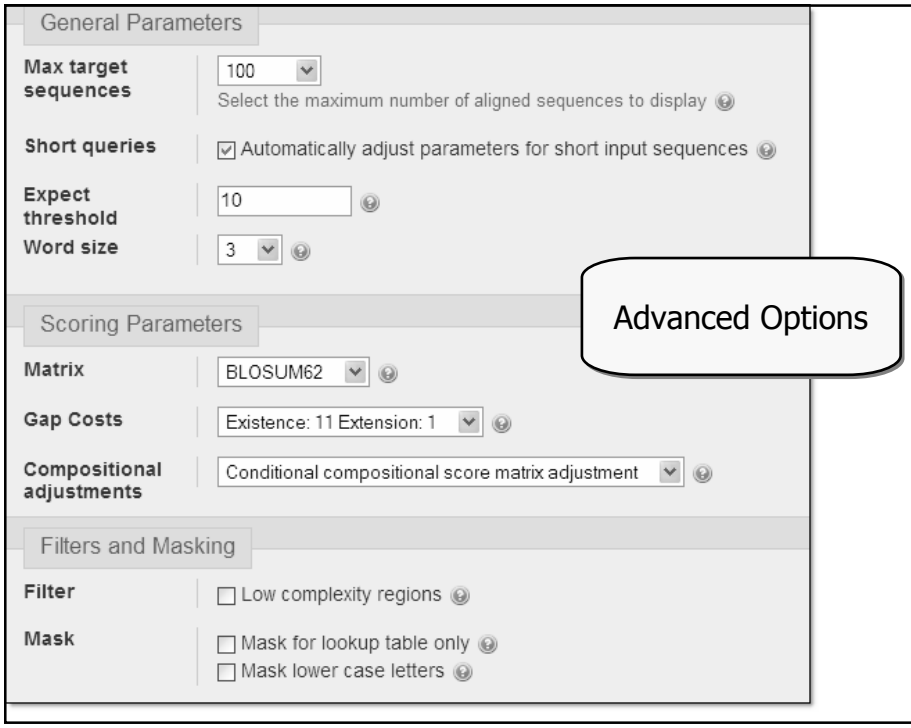
Choose a BLAST algorithm

BLAST

Search database nr using Blastp (protein-protein BLAST)

Show results in a new window

Algorithm parameters



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Reformat

NCBI Minicourses

NCBI/ BLAST/ blastp/ Formatting Results - N5W6JP2F013 [Reformat these Results] [Edit and Resubmit] [Sign in

Job Title: NP_032294:homeo box B5 [Mus musculus]

BLASTP 2.2.17 (Aug-26-2007)

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.

Reference:
Stephen F. Altschul, John C. Wootton, E. Michael Gertz, Richa Agarwala, Aleksandr Morgulis, Alejandro A. Schäffer, and Yi-Kuo Yu "Protein database searches using compositionally adjusted substitution matrices", FEBS J. 272:5101-5109.

RID: N5W6JP2F013

Database: All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects
5,678,482 sequences; 1,961,803,296 total letters

Reformat

NCBI

Query	ref|np_032294| (269 letters)
Database	nr
Job title	NP_032294:homeo box B5 [Mus musculus]
Request ID	N5W6JP2F013 View report ☐ Show results in a new window
Format	
Show	Alignment as HTML ☑ Advanced View Reset form to defaults
Alignment View	Pairwise
Display	☑ Graphical Overview ☑ Linkout ☑ Sequence Retrieval ☐ NCBI-gi
Limit results	Masking Character: Lower Case / X for protein, n for nucleotide / Lower Case; Descriptions: 100; Organism; Entrez query; Expect Min; Expect Max
Format for	☐ PSI-BLAST with inclusion threshold

BLAST Output: Low Complexity Filter

NCBI

```
>gi|466462|gb|AAA17374.1| L 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: Pairwise

- Pairwise
- Pairwise with dots for identities
- Query-anchored with dots for identities
- Query-anchored with letters for identities
- Flat query-anchored with dots for identities
- Flat query-anchored with letters for identities
- Hit Table

Alignment View: Pairwise

- Pairwise
- Pairwise with dots for identities
- Query-anchored with dots for identities
- Query-anchored with letters for identities
- Flat query-anchored with dots for identities
- Flat query-anchored with letters for identities
- Hit Table

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

NCBI Minicourses

```
# 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|;
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|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|15029697|gb|AAH11063.1|      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

Search Basics

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Examples

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

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.

Nucleotide vs. Protein BLAST

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Comparing ADSS from *H. sapiens* and *A. thaliana*

```
         aaccggtgacggtggtgctcggtgcgcagtggggcgacgaaggc
Human:    N  R  V  T  V  V  L  G  A  Q  W  G  D  E  G
          +  +  V  +     V  L  G     Q  W  G  D  E  G
A.th.:    S  Q  V  S  G  V  L  G  C  Q  W  G  D  E  G
         agtcaagtatctggtgtactcggttgccaatggggagatgaaggt
```

BLASTp finds three matching words

BLASTn finds no match, because there are no 7 bp words

Protein searches are generally more sensitive than nucleotide searches.

BLASTN vs TBLASTX

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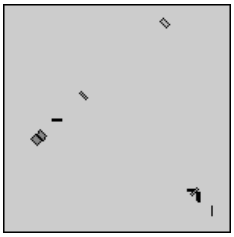
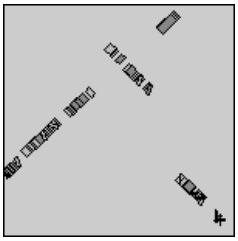
Anopheles gambiae mitochondrion, complete genome.
ACCESSION NC_002084 GI:5834911

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

blastn



tblastx



Hands-On

NCBI Minicourses

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