Current Topics in Genome Analysis 2012

Week 2: Biological Sequence Analysis I

Andy Baxevanis, Ph.D.

NATIONAL HUMAN GENOME RESEARCH INSTITUTE
Division of Intramural Research

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Current Topics in Genome Analysis 2012

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No Relevant Financial Relationships with Commercial Interests

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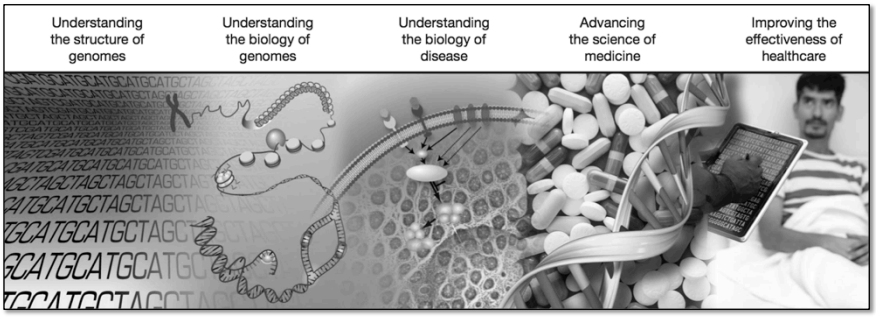

Bioinformatics and Computational Biology

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Overview

- **Week 2**
  - **Similarity *vs.* Homology**
  - **Global *vs.* Local Alignments**
  - **Scoring Matrices**
  - **BLAST**
  - **BLAT**
- **Week 4**
  - Profiles, Patterns, Motifs, and Domains
  - Structures: VAST, Cn3D, and ***de novo*** Prediction
  - Multiple Sequence Alignment

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Why do sequence alignments?

- Provide a measure of relatedness between nucleotide or amino acid sequences
- Determining relatedness allows one to draw biological inferences regarding
  - structural relationships
  - functional relationships
  - evolutionary relationships

  → *importance of using correct terminology*

Defining the Terms

- The quantitative measure: ***Similarity***
  - Always based on an observable
  - Usually expressed as percent identity
  - Quantify changes that occur as two sequences diverge (substitutions, insertions, or deletions)
  - Identify residues crucial for maintaining a protein's structure or function
- High degrees of sequence similarity *might* imply
  - a common evolutionary history
  - possible commonality in biological function

Defining the Terms

- The conclusion: ***Homology***
  - Genes *are* or *are not* homologous (not measured in degrees)
  - Homology implies an evolutionary relationship

> It is worth repeating here that homology, like pregnancy, is indivisible[8]. You either are homologous (pregnant) or you are not. Thus, if what one means to assert is that 80% of the character states are identical one should speak of 80% identity, and not 80% homology.

Fitch, Trends Genet. 16: 227-231, 2000

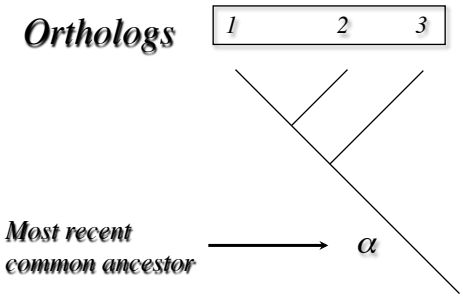
Defining the Terms

- The term "homolog" may apply to the relationship:
  - between genes separated by the event of speciation (*orthology*)
  - between genes separated by the event of genetic duplication (*paralogy*)

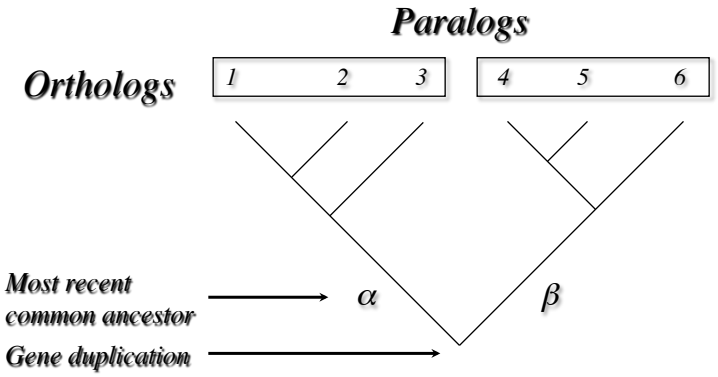
Defining the Terms

- Orthologs
  - Sequences are direct descendants of a sequence in a common ancestor
  - Most likely have similar domain structure, three-dimensional structure, and biological function
- Paralogs
  - Related through a gene duplication event
  - Provides insight into "evolutionary innovation" (adapting a pre-existing gene product for a new function)

Defining the Terms



Defining the Terms



- Genes 1-3 are orthologous
- Genes 4-6 are orthologous
- Any pair of α and β genes are paralogous (genes related through a gene duplication event)

Global Sequence Alignments

- Sequence comparison along the entire length of the two sequences being aligned
- Best for highly-similar sequences of similar length
- As the degree of sequence similarity declines, global alignment methods tend to miss important biological relationships

Local Sequence Alignments

- Sequence comparison intended to find the most similar regions in the two sequences being aligned ("paired subsequences")
- Regions outside the area of local alignment are excluded
- More than one local alignment could be generated for any two sequences being compared
- Best for sequences that share some similarity, or for sequences of different lengths

Scoring Matrices

- Empirical weighting scheme representing physicochemical and biological characteristics of nucleotides and amino acids
  - Side chain structure and chemistry
  - Side chain function
- Amino acid-based examples:
  - Cys/Pro important for structure and function
  - Trp has bulky side chain
  - Lys/Arg have positively charged side chains

Scoring Matrices

- ***Conservation:*** What residues can substitute for another residue and not adversely affect the function of the protein?
  - Ile/Val - both small and hydrophobic
  - Ser/Thr - both polar
  - *Conserve charge, size, hydrophobicity, other physicochemical factors*
- ***Frequency:*** How often does a particular residue occur amongst the entire constellation of proteins?

Scoring Matrices

- Why is understanding scoring matrices important?
  - Appear in all analyses involving sequence comparison
  - Implicitly represent particular evolutionary patterns
  - Choice of matrix can strongly influence outcomes of analyses

Matrix Structure: Nucleotides

- ***Simple match/mismatch scoring scheme:***

  Match +2
  Mismatch – 3

- ***Assumes each nucleotide occurs 25% of the time***

	A	T	G	C
A	2	-3	-3	-3
T	-3	2	-3	-3
G	-3	-3	2	-3
C	-3	-3	-3	2

Matrix Structure: Proteins

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

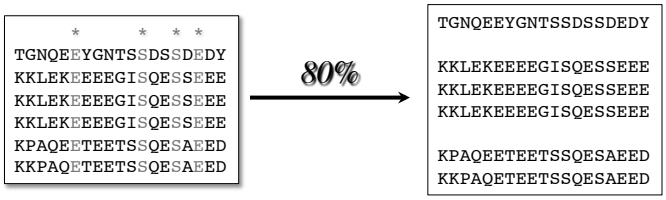
BLOSUM62

BLOSUM Matrices

- Henikoff and Henikoff, 1992
- Blocks Substitution Matrix
  - Look only for differences in conserved, ungapped regions of a protein family ("blocks")
  - Directly calculated, using no extrapolations
  - More sensitive to detecting structural or functional substitutions
  - Generally perform better than PAM matrices for local similarity searches *(Henikoff and Henikoff, 1993)*

BLOSUM *n*

- Calculated from sequences sharing no more than *n%* identity
- Contribution of sequences > *n%* identical clustered and weighted to 1



A+T Hook Domain (Block IPB000637B)

2,000 blocks representing > 500 groups of related proteins

BLOSUM *n*

- Clustering reduces contribution of closely-related sequences (less bias towards substitutions that occur in the most closely-related members of a family)
- Substitution frequencies are more heavily-influenced by sequences that are more divergent than this cutoff
- Reducing *n* yields more distantly related sequences

Which one to choose?

BLOSUM		% Similarity
90	Short alignments, highly similar	70-90
80	Best for detecting known members of a protein family	50-60
62	Most effective in finding all potential similarities	30-40
30	Longer, weaker local alignments	< 30

So many matrices...

No single matrix is the complete answer for all sequence comparisons

Further Reading

Unit 3.5
Current Protocols in Bioinformatics

- ***PAM Matrices***
- ***BLOSUM Matrices***
- ***Specialized Scoring Matrices***

Selecting the Right Protein-Scoring Matrix

UNIT 3.5

OVERVIEW

Every program for searching protein sequences against a database includes a choice of a "protein-scoring matrix," also called a "weight matrix." Weight matrices add sensitivity to the search, while statistical significance adds selectivity (see *UNIT 4.1*). Virtually every user chooses the default, typically PAM 250 or BLOSUM62. Despite the fact that the choice of matrix can strongly influence the outcome of the analysis, most users do not know why a particular matrix should be used. In general, scoring matrices implicitly represent a particular theory of protein sequence evolution. This unit provides guidance in the choice of a scoring matrix, as understanding the assumptions underlying the PAM and BLOSUM scoring matrices can aid in making the proper choice. The selection of PAM matrices is covered first, after which the selection of BLOSUM matrices is discussed, and finally a brief overview of the wide variety of specialized scoring matrices is provided.

PAM MATRICES

PAM, a rearranged acronym derived from Accepted Point Mutation (Dayhoff, 1978) is a probabilistic model for amino acid replacement derived by comparing the frequencies of replacement in closely related sequences to the frequency expected from the completely random replacement of amino acids. The basis of this scoring system is the observation that the evolution of protein sequences is a nonrandom process—i.e., some amino acid replacements occur much more frequently than others, especially in related sequences. Amino acid substitutions tend to conserve charge, size, and hydrophobicity among other characteristics. One would expect that the substitution of glycine for alanine (CH_3 versus H) would have less of an effect on a protein's structure and function than the substitution of alanine for threonine (CH_3 versus substituted indole ring). The inference is that if two aligned sequences manifest a higher than expected prevalence of these characteristic replacements, the sequences are related. An excellent discussion of the derivation and use of the PAM matrices is given in George et al. (1990).

PAM matrices are the result of computing the probability of one substitution per 100 amino acids, called the PAM 1 matrix. Higher PAM matrices are derived by multiplying the PAM 1 matrix by itself a defined number of times. Thus, a PAM 160 matrix is the result of performing 160 matrix multiplications of the PAM 1 matrix against itself. Similarly, the PAM 250 matrix is derived by multiplying the PAM 1 matrix against itself 250 times.

Biologically, the PAM 50 matrix means that in 100 amino acids there have been 50 substitutions, while the PAM 250 matrix means there have been 2.5 amino acid replacements at each site (see *UNIT 4*) regarding insertions and deletions). This sounds unusual, but remember that over evolutionary time, it is possible that an alanine was changed to a glycine, then to a valine, and then back to an alanine. These silent substitutions are derived from observed amino acid frequency data in protein families and superfamilies.

Choosing a PAM Matrix

It is extremely important to note that PAM matrices are derived from protein sequence data available in the late 1960s and early 1970s. Most proteins known at that time were small, globular, and hydrophilic. If the researcher believes their protein contains substantial hydrophobic regions, such as membrane-spanning helices or sheets, the PAM matrices are less useful than others described in this unit. Dayhoff et al. (1978) were the first to define the terms protein family and superfamily. A protein *family* is defined as sequences 85% identical or greater to each other. A protein *superfamily* is defined as sequences related from 30% identical or greater to each other. A protein superfamily may contain many protein families. The user should be aware that while the terms "family" and "superfamily" are widely used in biology, most of the time the original definition of Dayhoff and collaborators is not being used (see below).

Locating all potential similarities: PAM 250

The most widely used PAM matrix is PAM 250 (Fig. 3.5.1). It has been chosen because it is capable of accurately detecting similarities in the 30% range (i.e., superfamilies), that is, when the two proteins are up to 70% different from each other (George et al., 1990). Another way to think about this is that the PAM 250

Contributed by David Wheeler

Current Protocols in Bioinformatics (2003) 3.5.1-3.5.6

Finding Similarities and Inferring Homologies
3.5.1

Gaps

- Used to improve alignments between two sequences
- Compensate for insertions and deletions → *gaps represent biological events*
- Must be kept to a reasonable number, to not reflect a biological implausible scenario (~1 gap per 20 residues good rule-of-thumb)
- Cannot be scored simply as a "match" or a "mismatch"

Affine Gap Penalty

Fixed deduction for introducing a gap *plus*
an additional deduction proportional to the length of the gap

Deduction for a gap = $G + Ln$

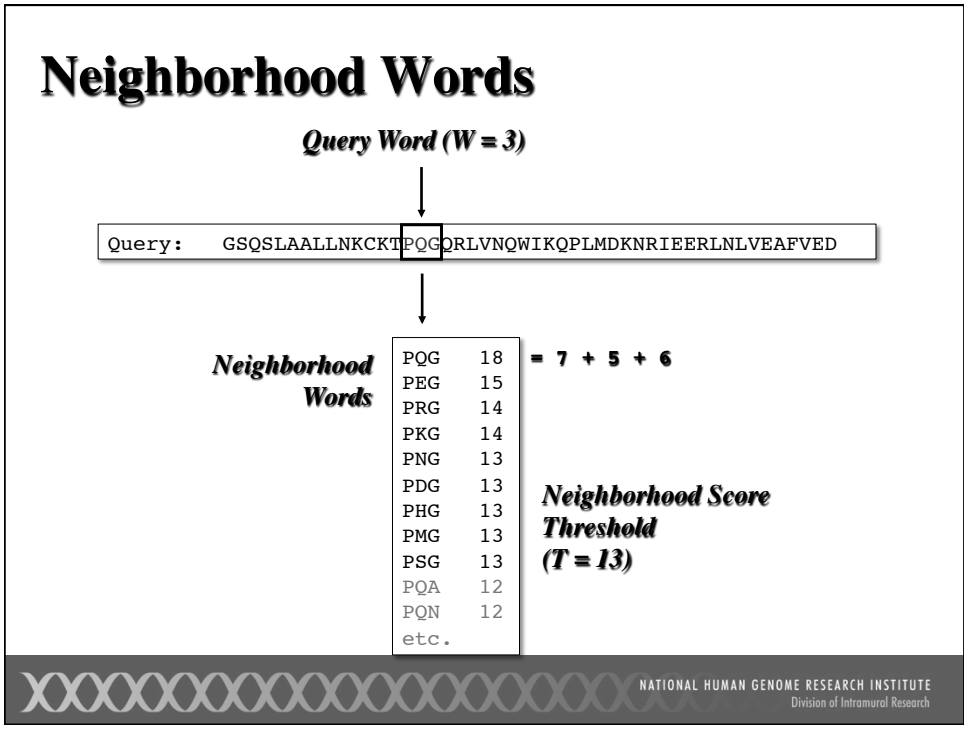
			nucleotide	protein
where	G	= gap-opening penalty	5	11
	L	= gap-extension penalty	2	1
	n	= length of the gap		
and	G	> L		

BLAST

- Basic Local Alignment Search Tool
- Seeks high-scoring segment pairs (HSP)
  - Pair of sequences that can be aligned with one another
  - When aligned, have maximal aggregate score (score cannot be improved by extension or trimming)
  - Score must be above score threshhold *S*
  - Gapped or ungapped
- Results not limited to the "best HSP" for any given sequence pair

BLAST Algorithms

Program	*Query Sequence*	*Target Sequence*
BLASTN	Nucleotide	Nucleotide
BLASTP	Protein	Protein
BLASTX	Nucleotide, six-frame translation	Protein
TBLASTN	Protein	Nucleotide, six-frame translation
TBLASTX	Nucleotide, six-frame translation	Nucleotide, six-frame translation


Neighborhood Words
Query Word (W = 3)
Query: GSQSLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVED
Neighborhood Words
PQG 18
PEG 15
PRG 14
PKG 14
PNG 13
PDG 13
PHG 13
PMG 13
PSG 13
PQA 12
PQN 12
etc.
= 7 + 5 + 6
Neighborhood Score Threshold (T = 13)
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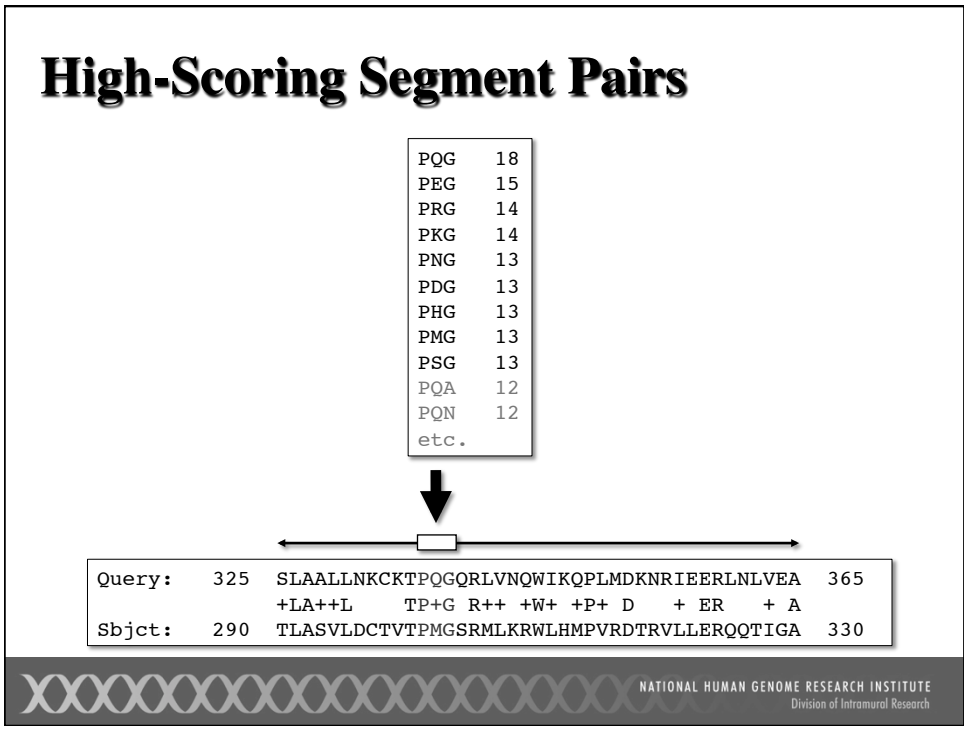
High-Scoring Segment Pairs
PQG 18
PEG 15
PRG 14
PKG 14
PNG 13
PDG 13
PHG 13
PMG 13
PSG 13
PQA 12
PQN 12
etc.
Query: 325 SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA 365
+LA++L TP+G R++ +W+ +P+ D + ER + A
Sbjct: 290 TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA 330
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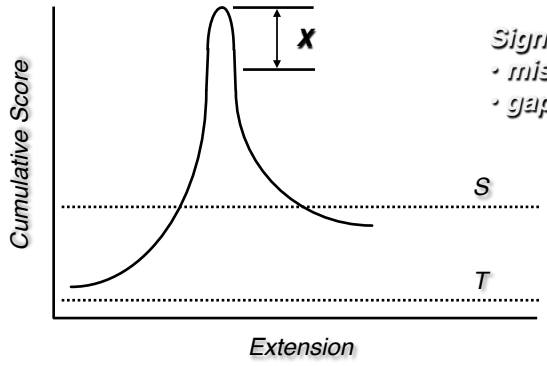
Extension

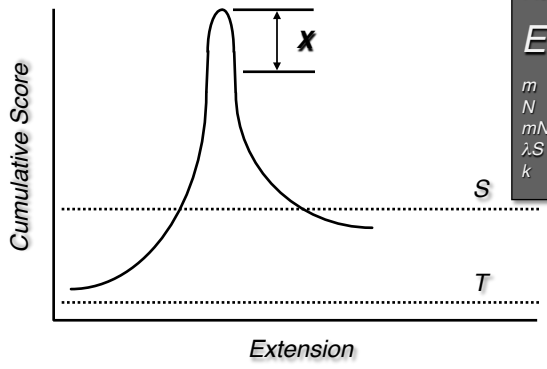
```
Query:   325  SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA  365
              +LA++L    TP+G R++ +W+ +P+ D   + ER   + A
Sbjct:   290  TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA  330
```



Significance decay
- *mismatches*
- *gap penalties*

Scores and Probabilities

```
Query:   325  SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA  365
              +LA++L    TP+G R++ +W+ +P+ D   + ER   + A
Sbjct:   290  TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA  330
```



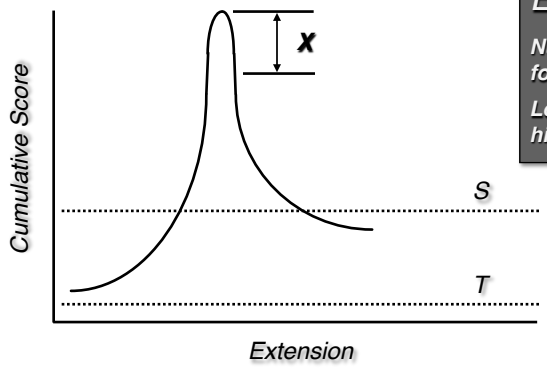
Karlin-Altschul Equation

$$E = kmNe^{-\lambda S}$$

m	*# letters in query*
N	*# letters in database*
mN	*size of search space*
λS	*normalized score*
k	*minor constant*

Scores and Probabilities

```
Query:   325  SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA  365
              +LA++L    TP+G R++ +W+ +P+ D   + ER   + A
Sbjct:   290  TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA  330
```



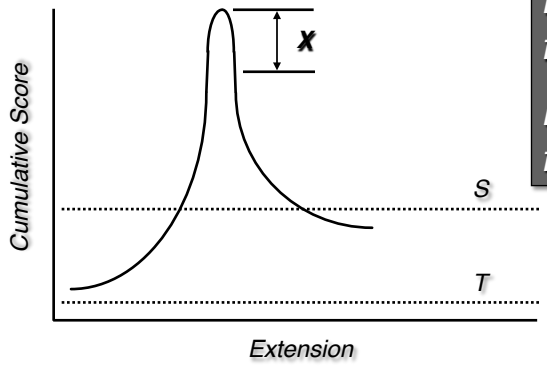
$E = kmNe^{-\lambda S}$

Number of HSPs found purely by chance

Lower values signify higher similarity

Scores and Probabilities

```
Query:   325  SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA  365
              +LA++L    TP+G R++ +W+ +P+ D   + ER   + A
Sbjct:   290  TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA  330
```



$E \leq 10^{-6}$ for nucleotides

$E \leq 10^{-3}$ for proteins

Protein BLAST
BLASTP

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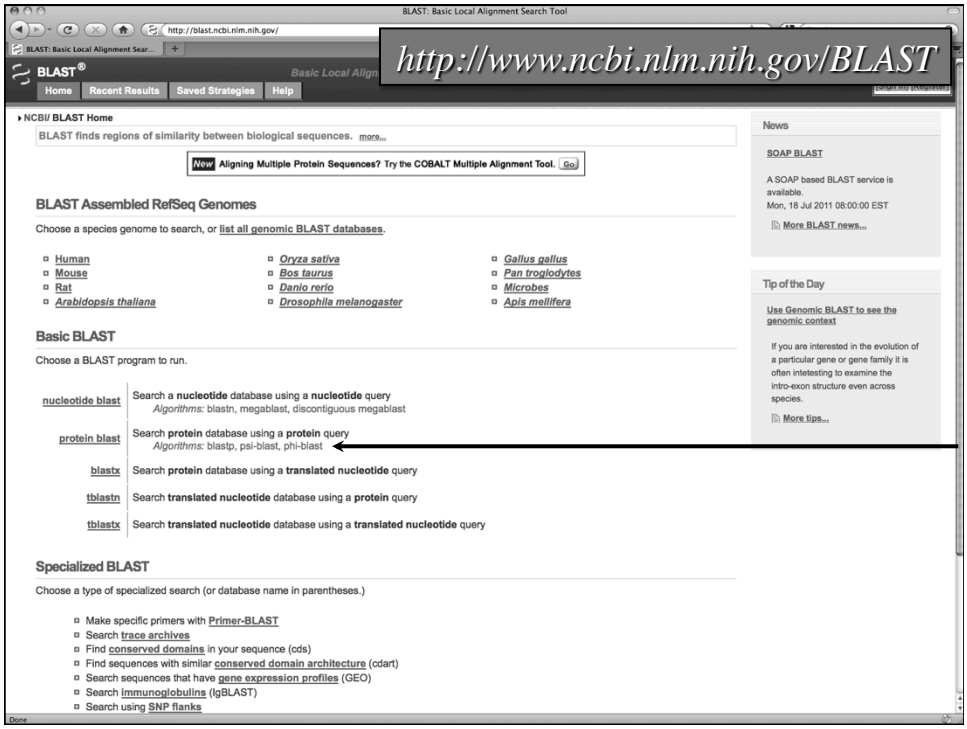
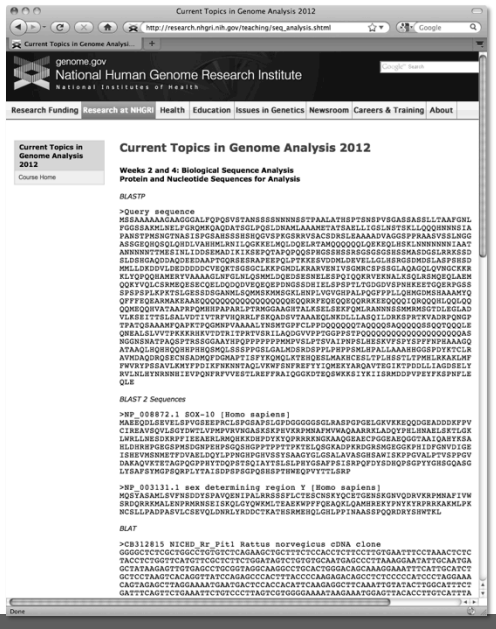
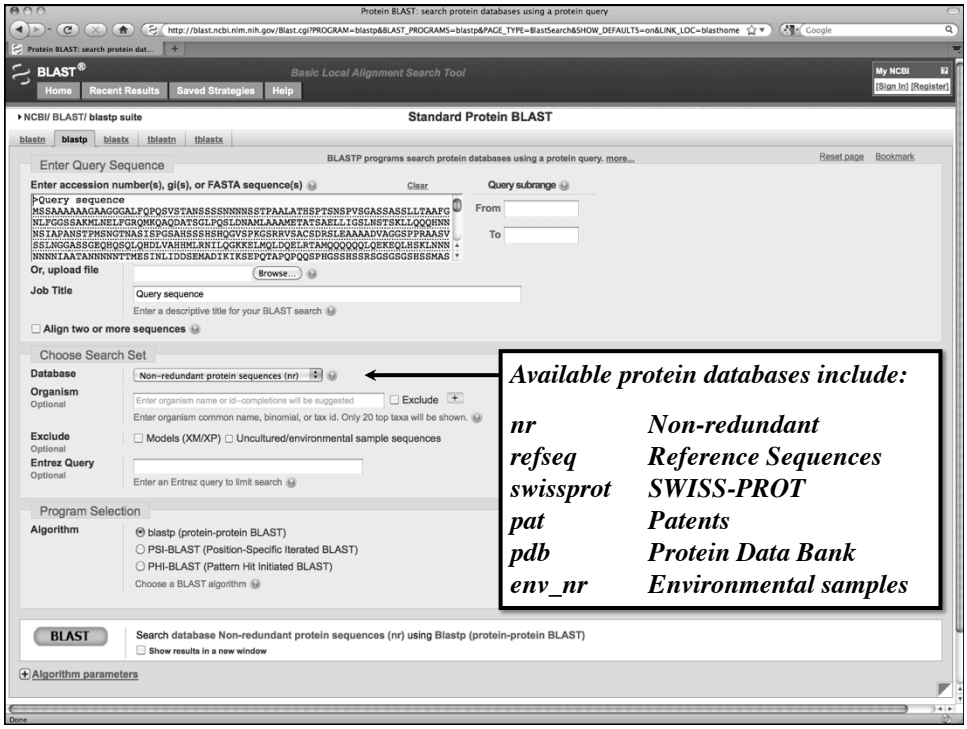


Sequences Used in Examples

http://research.nhgri.nih.gov/teaching/seq_analysis.shtml





RefSeq

- *Goal:* Provide a single reference sequence for each molecule of the central dogma (DNA, mRNA, protein)

- Distinguishing Features
  - Non-redundancy
  - Updates to reflect the current knowledge of sequence data and biology
  - Ongoing curation by NCBI staff and collaborators, with review status indicated on each record

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RefSeq Accesion Format

From curation of GenBank entries:

NT_123456	Genomic contigs
NM_123456	mRNAs
NP_123456	Proteins
NR_123456	Non-coding transcripts

From genome annotation:

XM_123456	Model mRNA
XP_123456	Model proteins

Complete key at
http://www.ncbi.nlm.nih.gov/RefSeq/key.html

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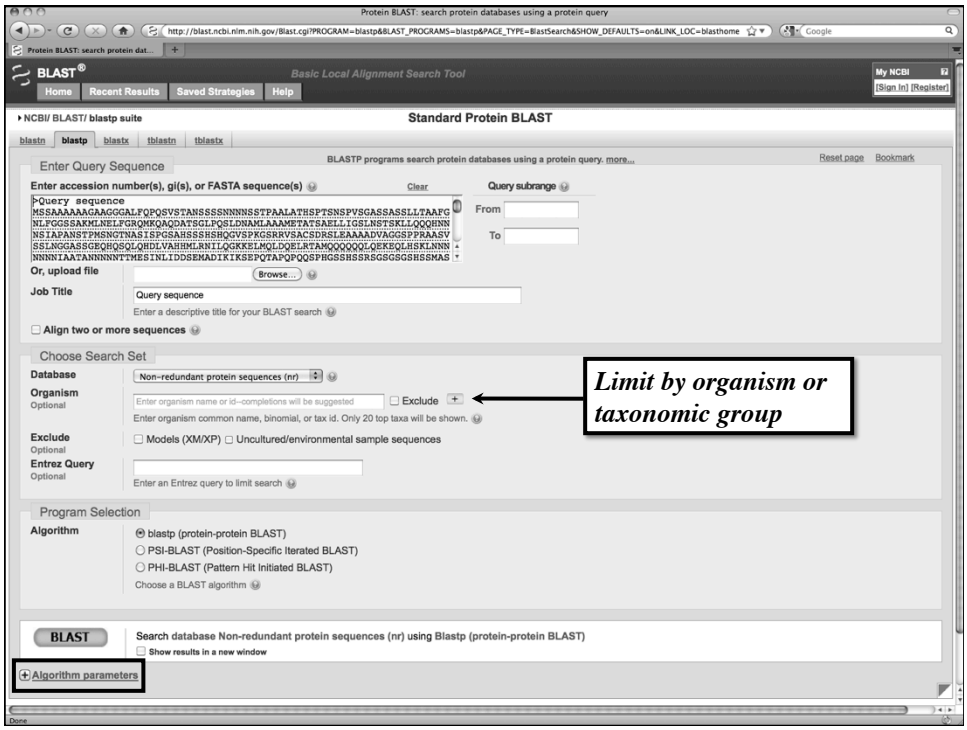
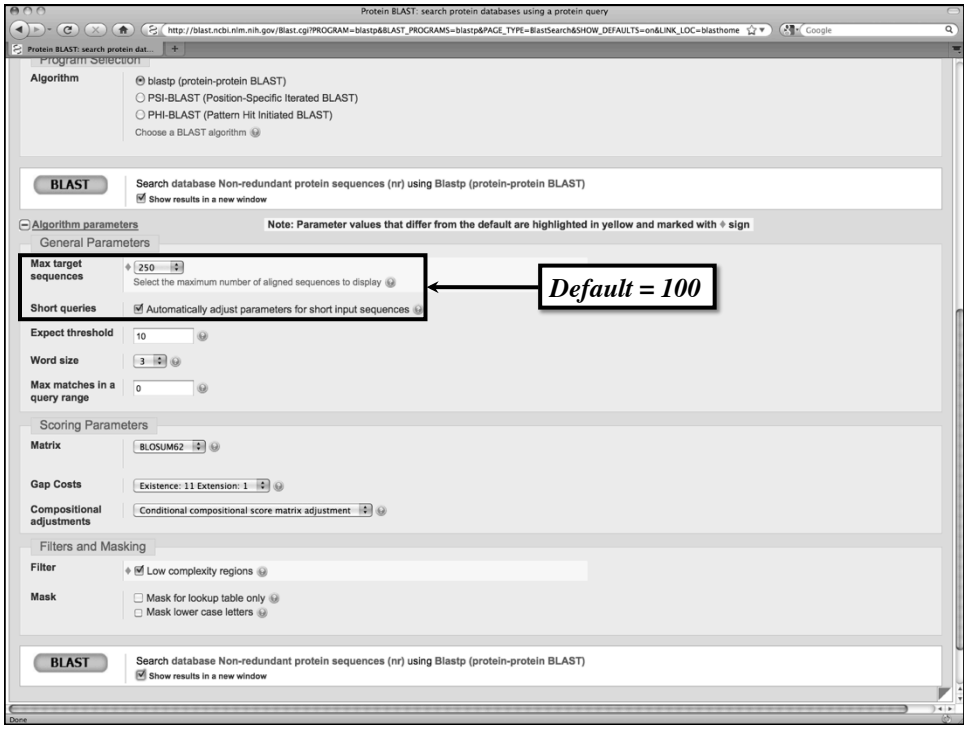
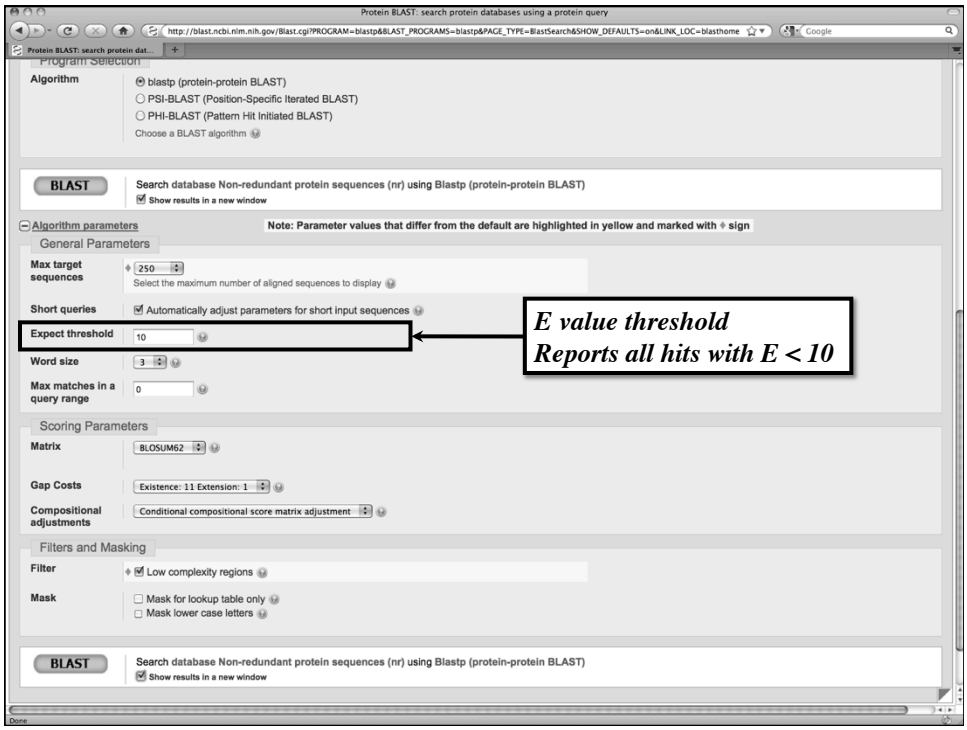



Protein BLAST: search protein databases using a protein query
Program Selection
Algorithm
blastp (protein-protein BLAST)
PSI-BLAST (Position-Specific Iterated BLAST)
PHI-BLAST (Pattern Hit Initiated BLAST)
Choose a BLAST algorithm
BLAST
Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)
Show results in a new window
Algorithm parameters
Note: Parameter values that differ from the default are highlighted in yellow and marked with ♦ sign
General Parameters
Max target sequences
250
Select the maximum number of aligned sequences to display
Short queries
Automatically adjust parameters for short input sequences
Default = 100
Expect threshold
10
Word size
3
Max matches in a query range
0
Scoring Parameters
Matrix
BLOSUM62
Gap Costs
Existence: 11 Extension: 1
Compositional adjustments
Conditional compositional score matrix adjustment
Filters and Masking
Filter
Low complexity regions
Mask
Mask for lookup table only
Mask lower case letters
BLAST
Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)
Show results in a new window


Protein BLAST: search protein databases using a protein query
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 Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)
Show results in a new window
Algorithm parameters
Note: Parameter values that differ from the default are highlighted in yellow and marked with ♦ sign
General Parameters
Max target sequences
250
Select the maximum number of aligned sequences to display
Short queries
Automatically adjust parameters for short input sequences
Expect threshold
10
E value threshold
Reports all hits with E < 10
Word size
3
Max matches in a query range
0
Scoring Parameters
Matrix
BLOSUM62
Gap Costs
Existence: 11 Extension: 1
Compositional adjustments
Conditional compositional score matrix adjustment
Filters and Masking
Filter
Low complexity regions
Mask
Mask for lookup table only
Mask lower case letters
BLAST
Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)
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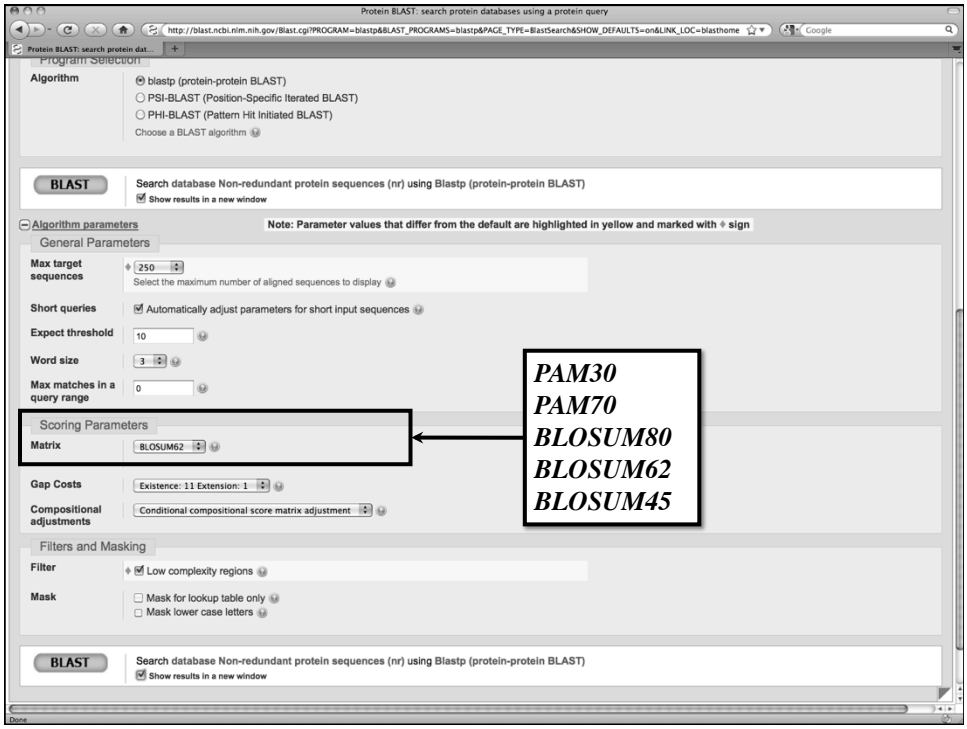
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BLAST
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Word size
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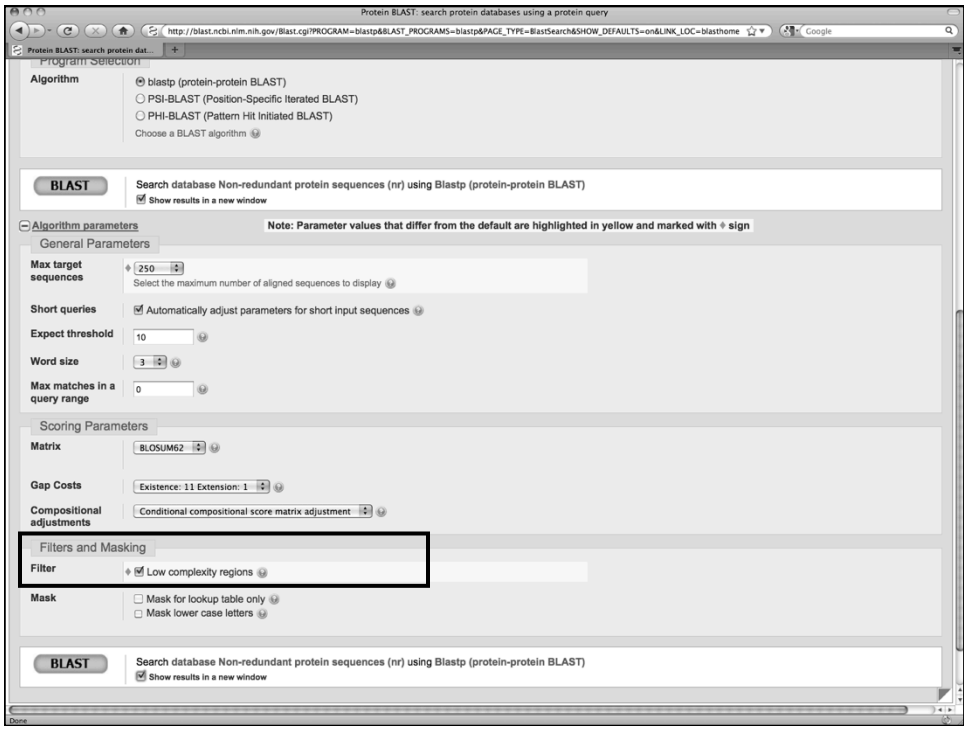
Scoring Parameters
Matrix
BLOSUM62
Gap Costs
Compositional adjustments
Filters and Masking
Filter
Low complexity regions
Mask
PAM30
PAM70
BLOSUM80
BLOSUM62
BLOSUM45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Gap Costs
Compositional adjustments
Filters and Masking
Filter
Low complexity regions
Mask

Low-Complexity Regions

Defined as regions of biased composition

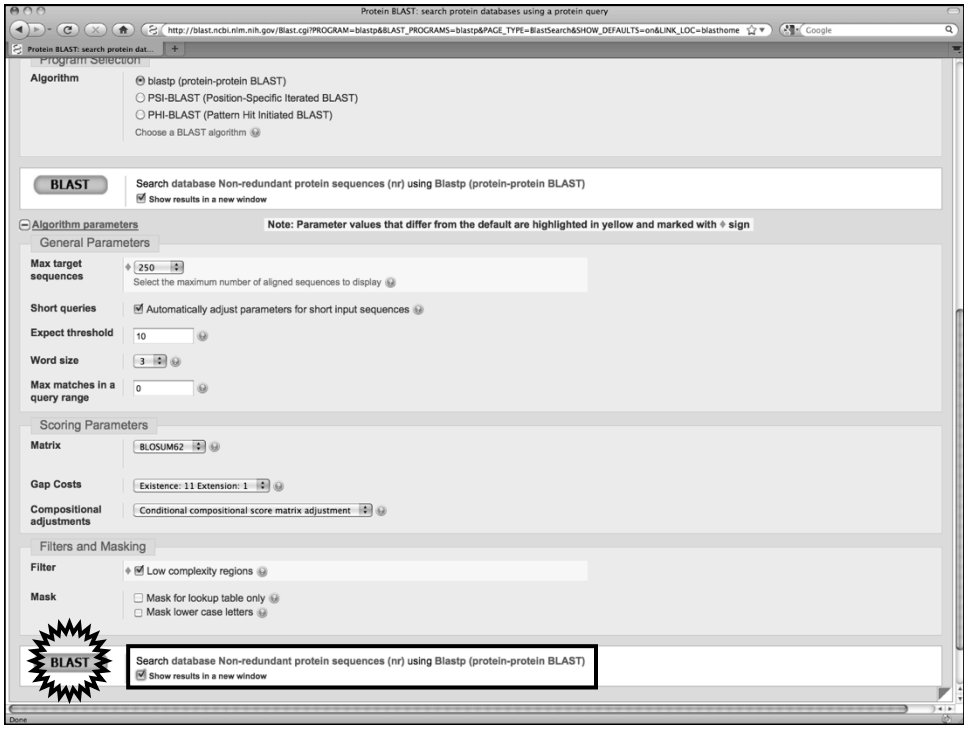
- Homopolymeric runs
- Short-period repeats
- Subtle over-representation of several residues

```
>gi|20455478|sp|P50553|ASC1_HUMAN Achaete-scute homolog 1 (HASH1)
MESSAKMESGGAGQQPQPQPQQPFLPPAACFFATAAAAAAAAAAAAAQSAQQQQQQQQQQQQAPQLRPAA
DGQPSGGGHKSAPKQVKRQRSSSPELMRCKRRLNFSGFGYSLPQQQAAAVARRNERERNRVKLVNLGFAT
LREHVPNGAANKKMSKVETLRSAVEYIRALQQLLDEHDAVSAAFQAGVLSPTISPNYSNDLNSMAGSPVS
SYSSDEGSYDPLSPEEQELLDFTNWF
```

Homopolymeric alanine-glutamine tract

Identifying Low-Complexity Regions

- Biological origins and role not well-understood
  - DNA replication errors (polymerase slippage)?
  - Unequal crossing-over?
- May confound sequence analysis
  - BLAST relies on uniformly-distributed amino acid frequencies
  - Often lead to false positives
  - Filtering is advised (but *not* enabled by default)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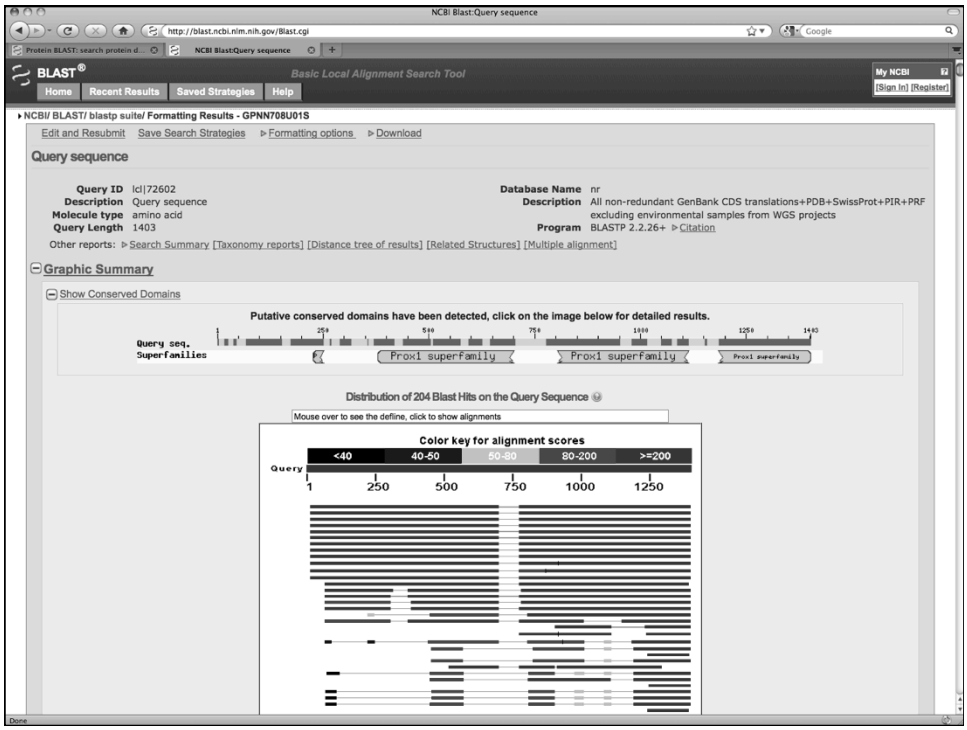
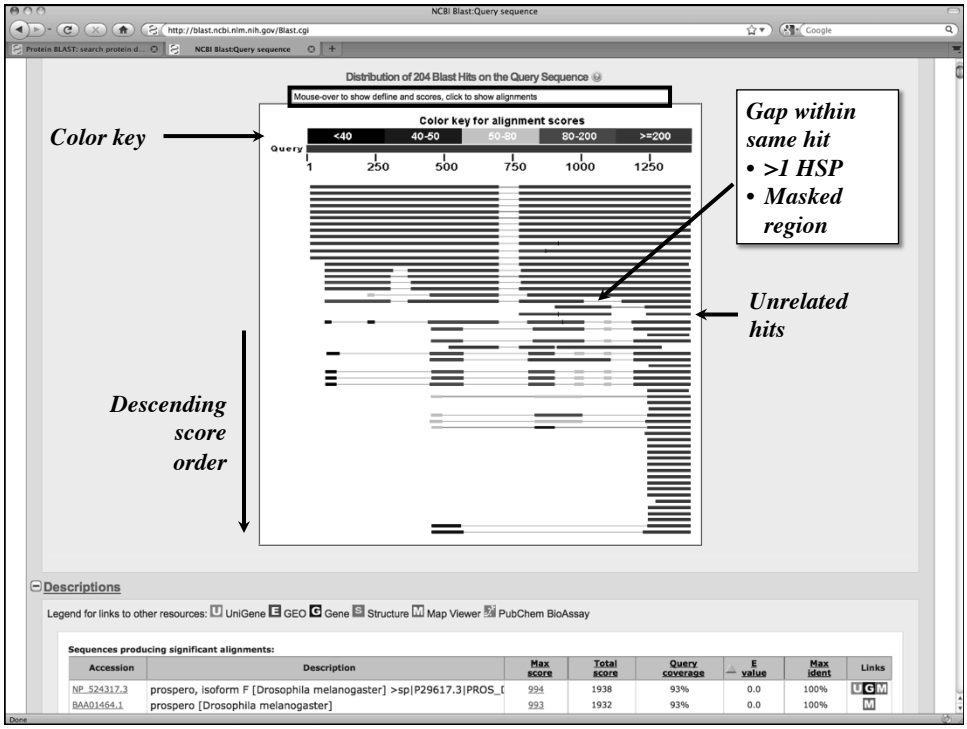
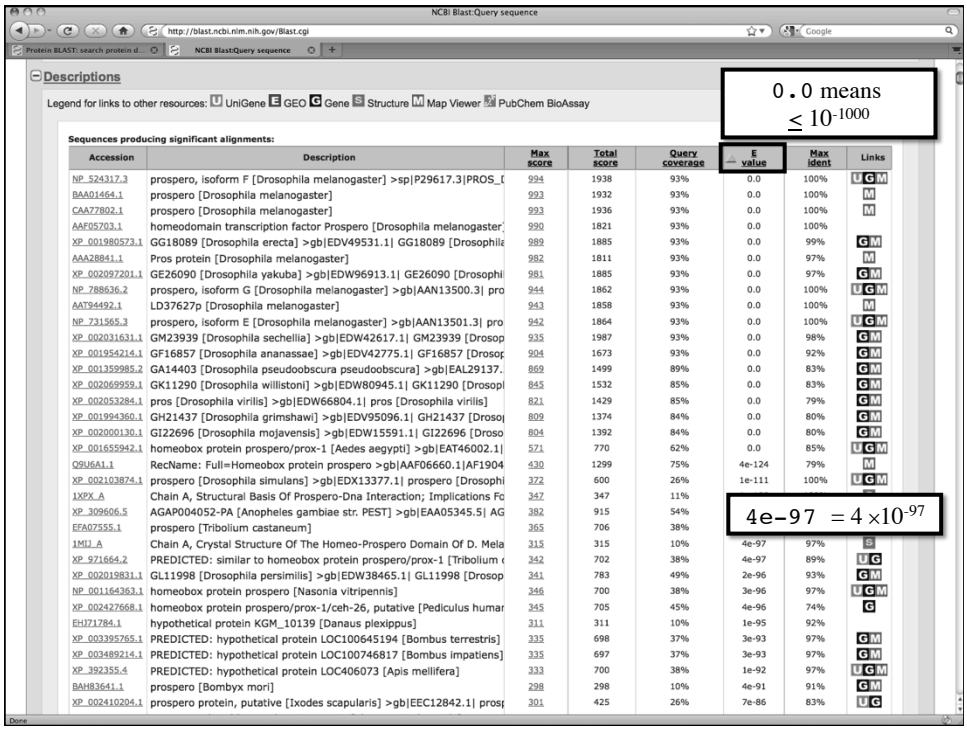
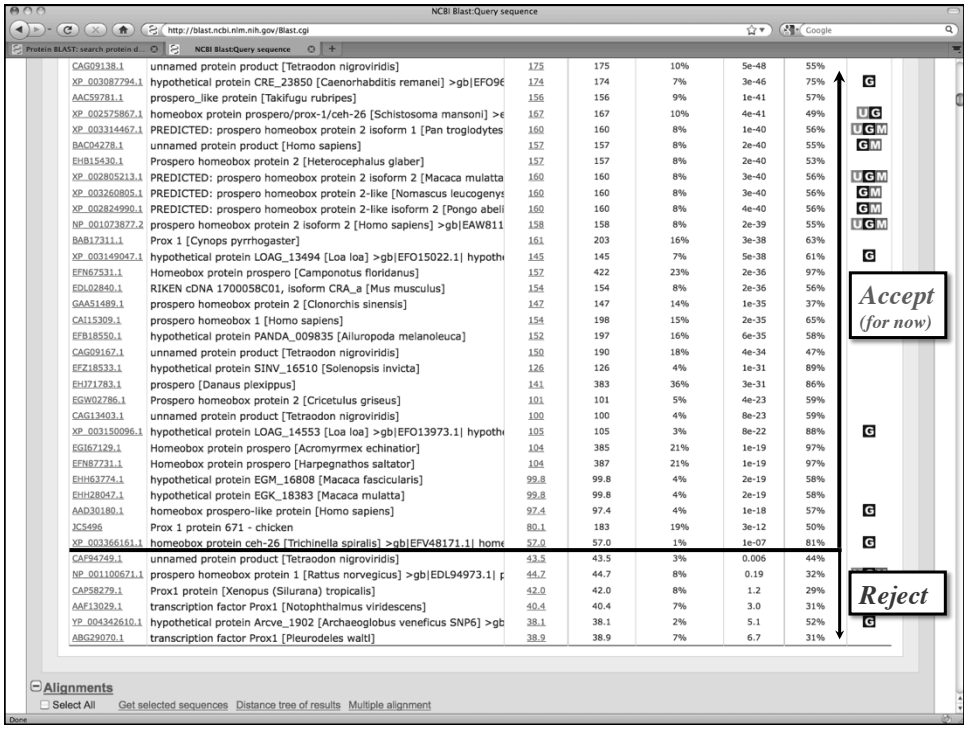
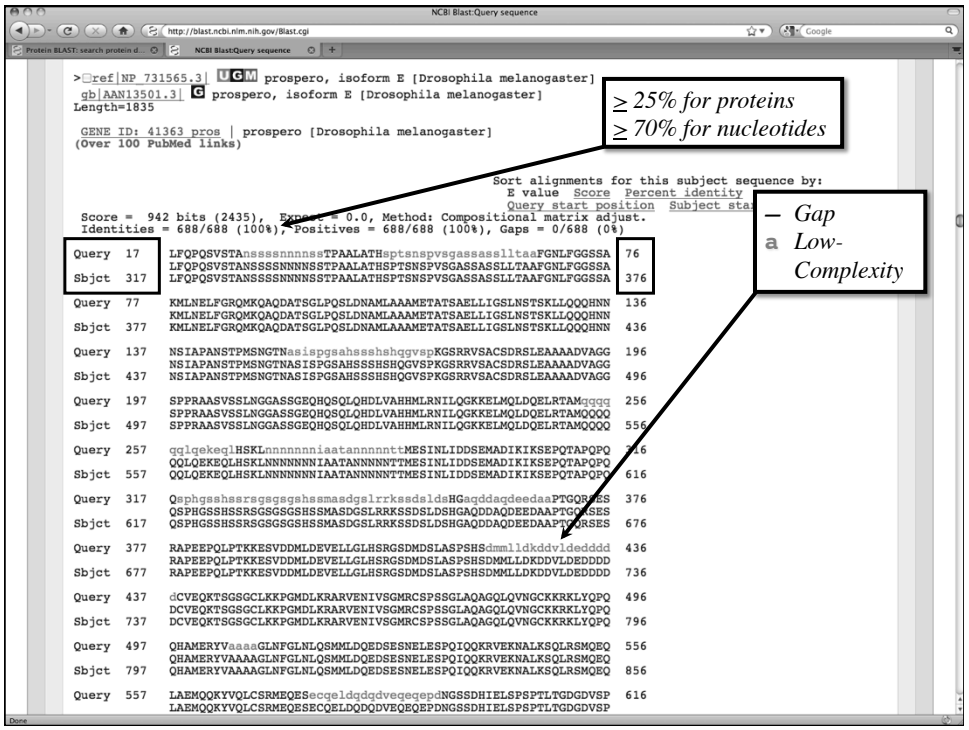
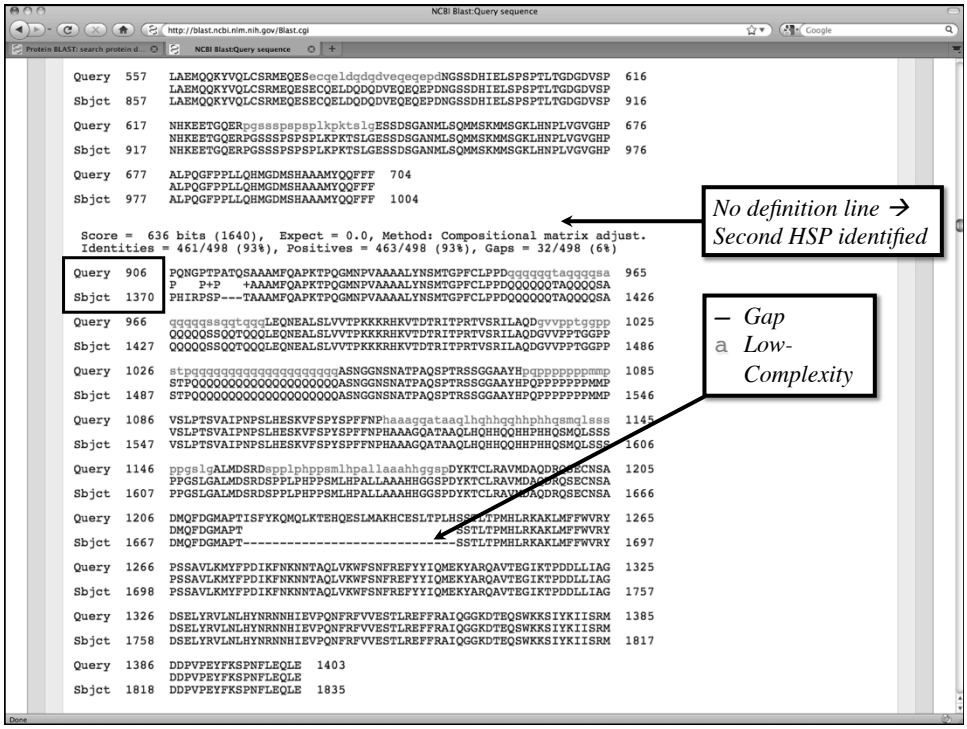

NCBI Blast:Query sequence
BLAST®
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
My NCBI
[Sign In] [Register]
NCBI/ BLAST/ blastp suite/ Formatting Results - GPNN708U01S
Edit and Resubmit
Save Search Strategies
Formatting options
Download
Query sequence
Query ID lcl|72602
Description Query sequence
Molecule type amino acid
Query Length 1403
Database Name nr
Description All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects
Program BLASTP 2.2.26+ Citation
Other reports: Search Summary [Taxonomy reports] [Distance tree of results] [Related Structures] [Multiple alignment]
Graphic Summary
Show Conserved Domains
Putative conserved domains have been detected, click on the image below for detailed results.
Query seq.
Superfamilies
Prox1 superfamily
Prox1 superfamily
Distribution of 204 Blast Hits on the Query Sequence
Mouse over to see the defline, click to show alignments
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
250
500
750
1000
1250


NCBI Blast:Query sequence
http://blast.ncbi.nlm.nih.gov/Blast.cgi
Distribution of 204 Blast Hits on the Query Sequence
Mouse-over to show define and scores, click to show alignments
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
250
500
750
1000
1250
Color key
Gap within same hit
• >1 HSP
• Masked region
Unrelated hits
Descending score order
Descriptions
Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay
Sequences producing significant alignments:
Accession
Description
Max score
Total score
Query coverage
E value
Max ident
Links
NP_524317.3
prospero, isoform F [Drosophila melanogaster] >sp|P29617.3|PROS_[
994
1938
93%
0.0
100%
U G M
BAA01464.1
prospero [Drosophila melanogaster]
993
1932
93%
0.0
100%
M


NCBI Blast:Query sequence
http://blast.ncbi.nlm.nih.gov/Blast.cgi
Descriptions
Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer PubChem BioAssay
0.0 means ≤ 10-1000
4e-97 = 4 ×10-97
Sequences producing significant alignments:
Accession
Description
Max score
Total score
Query coverage
E value
Max ident
Links
NP_524317.3 prospero, isoform F [Drosophila melanogaster] >sp|P29617.3|PROS_[994 1938 93% 0.0 100% U G M
BAA01464.1 prospero [Drosophila melanogaster] 993 1932 93% 0.0 100% M
CAA77802.1 prospero [Drosophila melanogaster] 993 1936 93% 0.0 100% M
AAF05703.1 homeodomain transcription factor Prospero [Drosophila melanogaster] 990 1821 93% 0.0 100%
XP_001980573.1 GG18089 [Drosophila erecta] >gb|EDV49531.1| GG18089 [Drosophila 989 1885 93% 0.0 99% G M
AAA28841.1 Pros protein [Drosophila melanogaster] 982 1811 93% 0.0 97% M
XP_002097201.1 GE26090 [Drosophila yakuba] >gb|EDW96913.1| GE26090 [Drosophil 981 1885 93% 0.0 97% G M
NP_788636.2 prospero, isoform G [Drosophila melanogaster] >gb|AAN13500.3| pro 944 1862 93% 0.0 100% U G M
AAT94492.1 LD37627p [Drosophila melanogaster] 943 1858 93% 0.0 100% M
NP_731565.3 prospero, isoform E [Drosophila melanogaster] >gb|AAN13501.3| pro 942 1864 93% 0.0 100% U G M
XP_002031631.1 GM23939 [Drosophila sechellia] >gb|EDW42617.1| GM23939 [Drosop 935 1987 93% 0.0 98% G M
XP_001954214.1 GF16857 [Drosophila ananassae] >gb|EDV42775.1| GF16857 [Drosop 904 1673 93% 0.0 92% G M
XP_001359985.2 GA14403 [Drosophila pseudoobscura pseudoobscura] >gb|EAL29137. 869 1499 89% 0.0 83% G M
XP_002069959.1 GK11290 [Drosophila willistoni] >gb|EDW80945.1| GK11290 [Drosopl 845 1532 85% 0.0 83% G M
XP_002053284.1 pros [Drosophila virilis] >gb|EDW66804.1| pros [Drosophila virilis] 821 1429 85% 0.0 79% G M
XP_001994360.1 GH21437 [Drosophila grimshawi] >gb|EDV95096.1| GH21437 [Droso 809 1374 84% 0.0 80% G M
XP_002000130.1 GI22696 [Drosophila mojavensis] >gb|EDW15591.1| GI22696 [Droso 804 1392 84% 0.0 80% G M
XP_001655942.1 homeobox protein prospero/prox-1 [Aedes aegypti] >gb|EAT46002.1| 571 770 62% 0.0 85% U G M
Q9U6A1.1 RecName: Full=Homeobox protein prospero >gb|AAF06660.1|AF1904 430 1299 75% 4e-124 79% M
XP_002103874.1 prospero [Drosophila simulans] >gb|EDX13377.1| prospero [Drosophi 372 600 26% 1e-111 100% U G M
1XPX_A Chain A, Structural Basis Of Prospero-Dna Interaction; Implications Fo 347 347 11%
XP_309606.5 AGAP004052-PA [Anopheles gambiae str. PEST] >gb|EAA05345.5| AG 382 915 54%
EFA07555.1 prospero [Tribolium castaneum] 365 706 38%
1MIJ_A Chain A, Crystal Structure Of The Homeo-Prospero Domain Of D. Mela 315 315 10% 4e-97 97% S
XP_971664.2 PREDICTED: similar to homeobox protein prospero/prox-1 [Tribolium c 342 702 38% 4e-97 89% U G
XP_002019831.1 GL11998 [Drosophila persimilis] >gb|EDW38465.1| GL11998 [Drosop 341 783 49% 2e-96 93% G M
NP_001164363.1 homeobox protein prospero [Nasonia vitripennis] 346 700 38% 3e-96 97% U G M
XP_002427668.1 homeobox protein prospero/prox-1/ceh-26, putative [Pediculus humar 345 705 45% 4e-96 74% G
EHJ71784.1 hypothetical protein KGM_10139 [Danaus plexippus] 311 311 10% 1e-95 92%
XP_003395765.1 PREDICTED: hypothetical protein LOC100645194 [Bombus terrestris] 335 698 37% 3e-93 97% G M
XP_003489214.1 PREDICTED: hypothetical protein LOC100746187 [Bombus impatiens] 335 697 37% 3e-93 97% G M
XP_392355.4 PREDICTED: hypothetical protein LOC406073 [Apis mellifera] 333 700 38% 1e-92 97% U G M
BAH83641.1 prospero [Bombyx mori] 298 298 10% 4e-91 91% G M
XP_002410204.1 prospero protein, putative [Ixodes scapularis] >gb|EEC12842.1| prosp 301 425 26% 7e-86 83% U G


NCBI Blast:Query sequence
Accept
(for now)
Reject
Alignments
Select All
Get selected sequences
Distance tree of results
Multiple alignment


NCBI Blast:Query sequence
≥ 25% for proteins
≥ 70% for nucleotides
– Gap
a Low-Complexity


NCBI Blast:Query sequence
http://blast.ncbi.nim.nih.gov/Blast.cgi
Query 557 LAEMQQKYVQLCSRMEQESecqeldqdqdvecqecqepdNGSSDHIELSPSPTLTGDGDVSP 616
LAEMQQKYVQLCSRMEQESECQELDQDQDVEQECQEPDNGSSDHIELSPSPTLTGDGDVSP
Sbjct 857 LAEMQQKYVQLCSRMEQESECQELDQDQDVEQECQEPDNGSSDHIELSPSPTLTGDGDVSP 916
Query 617 NHKEETGQERpgsssspspsplkpktslgESSDSGANMLSQMMSKMMSGKLHNPLVGVGHP 676
NHKEETGQERPGSSSPSPSPLKPKTSLGESSDSGANMLSQMMSKMMSGKLHNPLVGVGHP
Sbjct 917 NHKEETGQERPGSSSPSPSPLKPKTSLGESSDSGANMLSQMMSKMMSGKLHNPLVGVGHP 976
Query 677 ALPQGFPPLLQHMGDMSHAAAMYQQFFF 704
ALPQGFPPLLQHMGDMSHAAAMYQQFFF
Sbjct 977 ALPQGFPPLLQHMGDMSHAAAMYQQFFF 1004
Score = 636 bits (1640), Expect = 0.0, Method: Compositional matrix adjust.
Identities = 461/498 (93%), Positives = 463/498 (93%), Gaps = 32/498 (6%)
Query 906 PQNGPTPATQSAAAMFQAPKTPQGMNPVAAAALYNSMTGPFCLPPDqqqqqqtaqqqqsa 965
P P+P +AAAMFQAPKTPQGMNPVAAAALYNSMTGPFCLPPDQQQQQQTAQQQQSA
Sbjct 1370 PHIRPSP---TAAAMFQAPKTPQGMNPVAAAALYNSMTGPFCLPPDQQQQQQTAQQQQSA 1426
Query 966 qqqqqssqqtqqqLEQNEALSLVVTPKKKRHKVTDTRITPRTVSRILAQDgvvpptggpp 1025
QQQQQSSQQTQQQLEQNEALSLVVTPKKKRHKVTDTRITPRTVSRILAQDGVVPPTGGPP
Sbjct 1427 QQQQQSSQQTQQQLEQNEALSLVVTPKKKRHKVTDTRITPRTVSRILAQDGVVPPTGGPP 1486
Query 1026 stpqqqqqqqqqqqqqqqqqqqqqASNGGNSNATPAQSPTRSSGGAAYHpgpppppppmmp 1085
STPQQQQQQQQQQQQQQQQQQQQASNGGNSNATPAQSPTRSSGGAAYHPQPPPPPPPMMP
Sbjct 1487 STPQQQQQQQQQQQQQQQQQQQQASNGGNSNATPAQSPTRSSGGAAYHPQPPPPPPPMMP 1546
Query 1086 VSLPTSVAIPNPSLHESKVFSPYSPFFNPhaaaqqataaqlhqhhqqhhphhqsmqlsss 1145
VSLPTSVAIPNPSLHESKVFSPYSPFFNPHAAAGQATAAQLHQHHQQHHPHHQSMQLSSS
Sbjct 1547 VSLPTSVAIPNPSLHESKVFSPYSPFFNPHAAAGQATAAQLHQHHQQHHPHHQSMQLSSS 1606
Query 1146 ppgslgALMDSRDspplphppsmlhpallaaahhggspDYKTCLRAVMDAQDRQSECNSA 1205
PPGSLGALMDSRDSPPLPHPPSMLHPALLAAAHHGGSPDYKTCLRAVMDAQDRQSECNSA
Sbjct 1607 PPGSLGALMDSRDSPPLPHPPSMLHPALLAAAHHGGSPDYKTCLRAVMDAQDRQSECNSA 1666
Query 1206 DMQFDGMAPTISFYKQMQLKTEHQESLMAKHCESLTPLHSSLTPMHLRKAKLMFFWVRY 1265
DMQFDGMAPT SSTLTPMHLRKAKLMFFWVRY
Sbjct 1667 DMQFDGMAPT----------SSTLTPMHLRKAKLMFFWVRY 1697
Query 1266 PSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQMEKYARQAVTEGIKTPDDLLIAG 1325
PSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQMEKYARQAVTEGIKTPDDLLIAG
Sbjct 1698 PSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQMEKYARQAVTEGIKTPDDLLIAG 1757
Query 1326 DSELYRVLNLHYNRNNHIEVPQNFRFVVESTLREFFRAIQGGKDTEQSWKKSIYKIISRM 1385
DSELYRVLNLHYNRNNHIEVPQNFRFVVESTLREFFRAIQGGKDTEQSWKKSIYKIISRM
Sbjct 1758 DSELYRVLNLHYNRNNHIEVPQNFRFVVESTLREFFRAIQGGKDTEQSWKKSIYKIISRM 1817
Query 1386 DDPVPEYFKSPNFLEQLE 1403
DDPVPEYFKSPNFLEQLE
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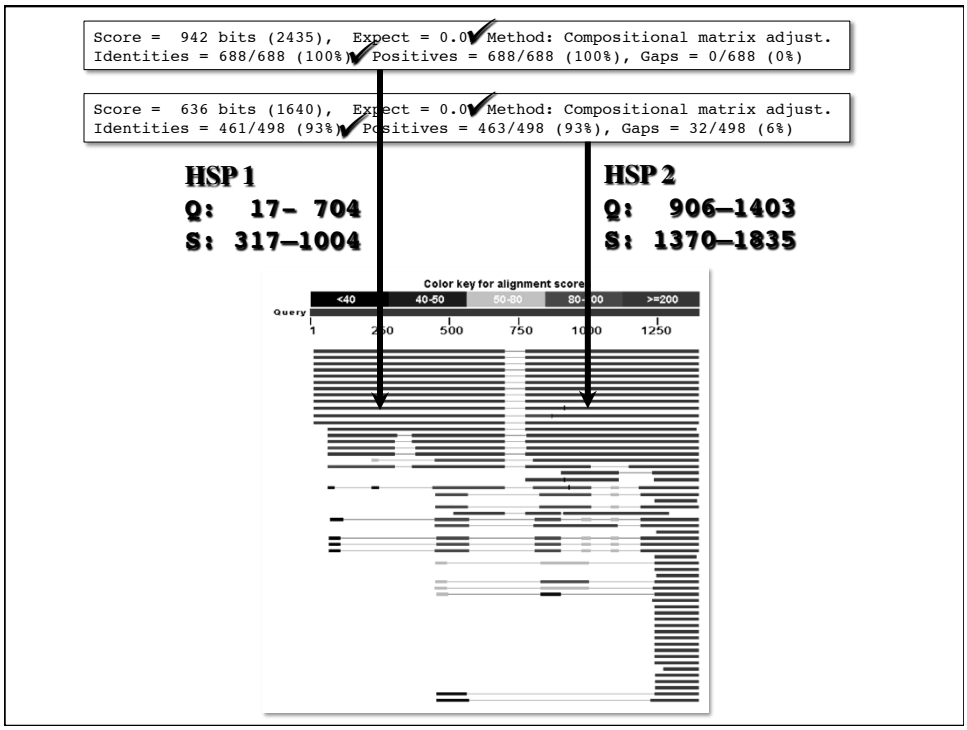
– Gap
a Low-Complexity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Score = 636 bits (1640), Expect = 0.0, Method: Compositional matrix adjust.
Identities = 461/498 (93%), Positives = 463/498 (93%), Gaps = 32/498 (6%)
HSP 1
Q: 17– 704
S: 317–1004
HSP 2
Q: 906–1403
S: 1370–1835
Color key for alignment score
<40
40-50
50-80
80-200
>=200
Query
1
250
500
750
1000
1250

Suggested BLAST Cutoffs

	E-value	Sequence Identity
Nucleotide	$\leq 10^{-6}$	$\geq 70\%$
Protein	$\leq 10^{-3}$	$\geq 25\%$

- ***Do not use these cutoffs blindly!***
- ***Pay attention to alignments on either side of the dividing line***
- ***Do not ignore biology!***

Database Searching Artifacts

- Low-complexity regions
- Repetitive elements
  - LINEs, SINEs, retroviral repeats
  - Choose "Filter: Species-Specific Repeats" with BLASTN
  - RepeatMasker
    http://www.repeatmasker.org
- Low-quality sequence hits
  - Expressed sequence tags (ESTs)
  - Single-pass sequence reads from large-scale sequencing (possibly with vector contaminants)

BLAST 2 Sequences

- Finds local alignments between two protein or nucleotide sequences of interest
  - All BLAST programs available
  - Select BLOSUM and PAM matrices available for protein comparisons
  - Same affine gap costs (adjustable)
  - Input sequences can be masked

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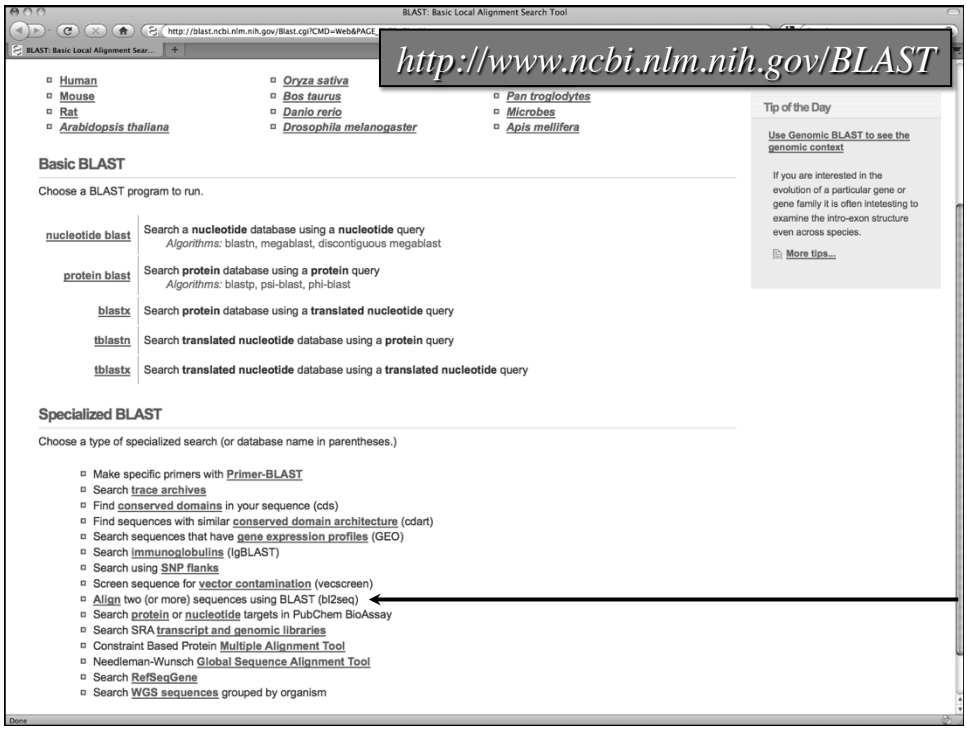
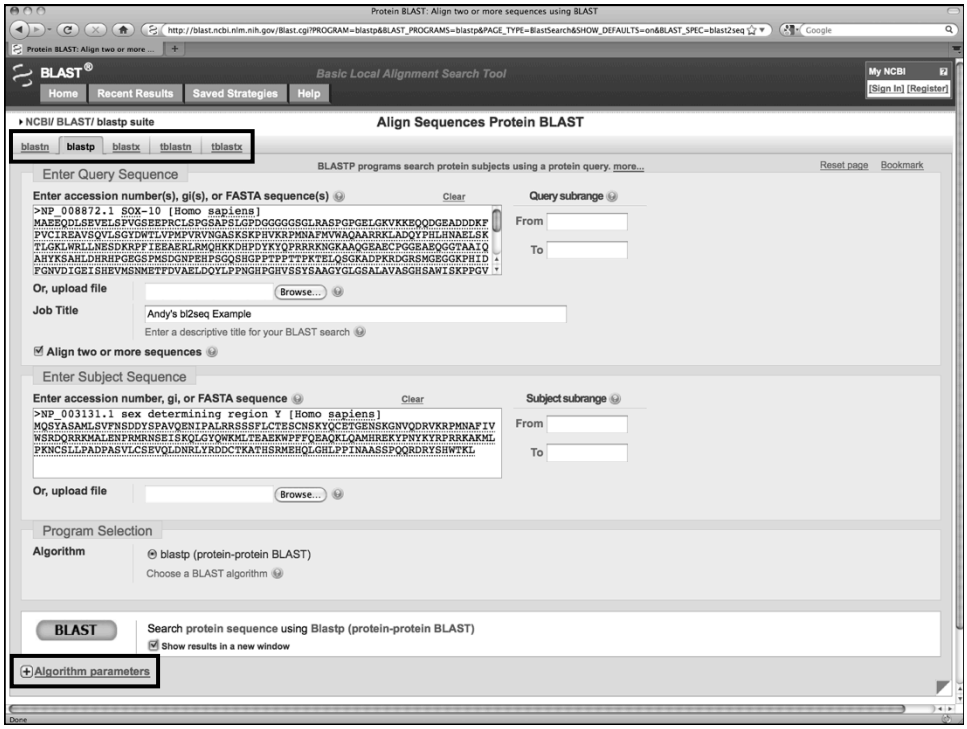
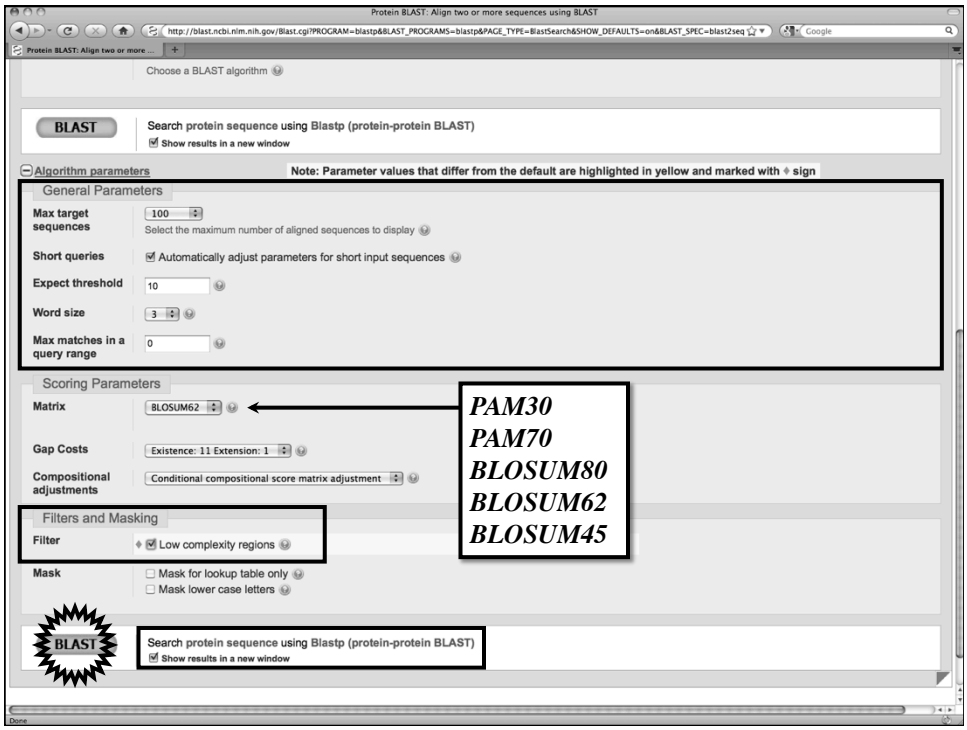
Division of Intramural Research




Protein BLAST: Align two or more sequences using BLAST
BLAST®
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
My NCBI
[Sign In] [Register]
NCBI/ BLAST/ blastp suite
Align Sequences Protein BLAST
blastn
blastp
blastx
tblastn
tblastx
BLASTP programs search protein subjects using a protein query. more...
Reset page
Bookmark
Enter Query Sequence
Enter accession number(s), gi(s), or FASTA sequence(s)
Clear
Query subrange
From
To
Or, upload file
Browse...
Job Title
Andy's bl2seq Example
Enter a descriptive title for your BLAST search
Align two or more sequences
Enter Subject Sequence
Enter accession number, gi, or FASTA sequence
Subject subrange
Program Selection
Algorithm
blastp (protein-protein BLAST)
Choose a BLAST algorithm
BLAST
Search protein sequence using Blastp (protein-protein BLAST)
Show results in a new window
Algorithm parameters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Max matches in a query range
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Scoring Parameters
Matrix
BLOSUM62
Gap Costs
Existence: 11 Extension: 1
Compositional adjustments
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BLAST
Search protein sequence using Blastp (protein-protein BLAST)
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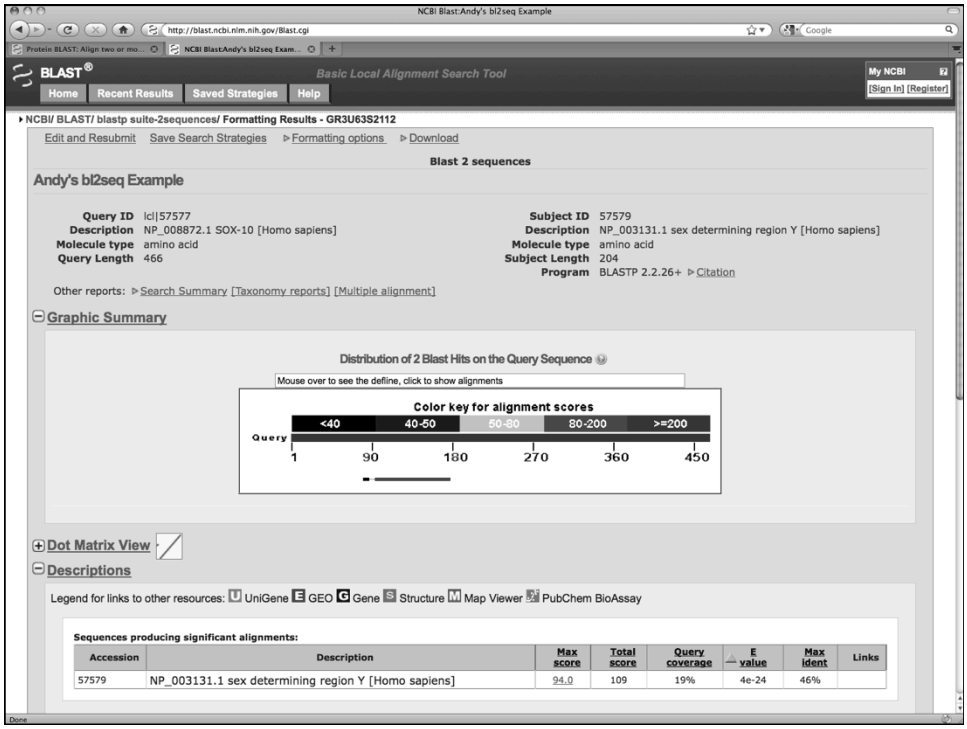
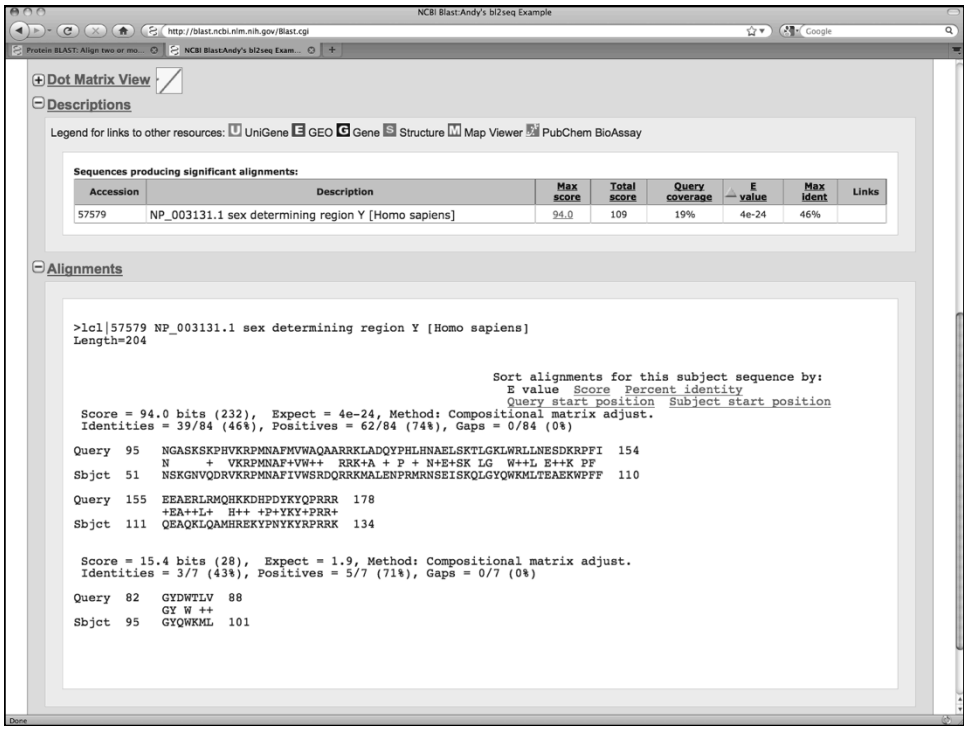
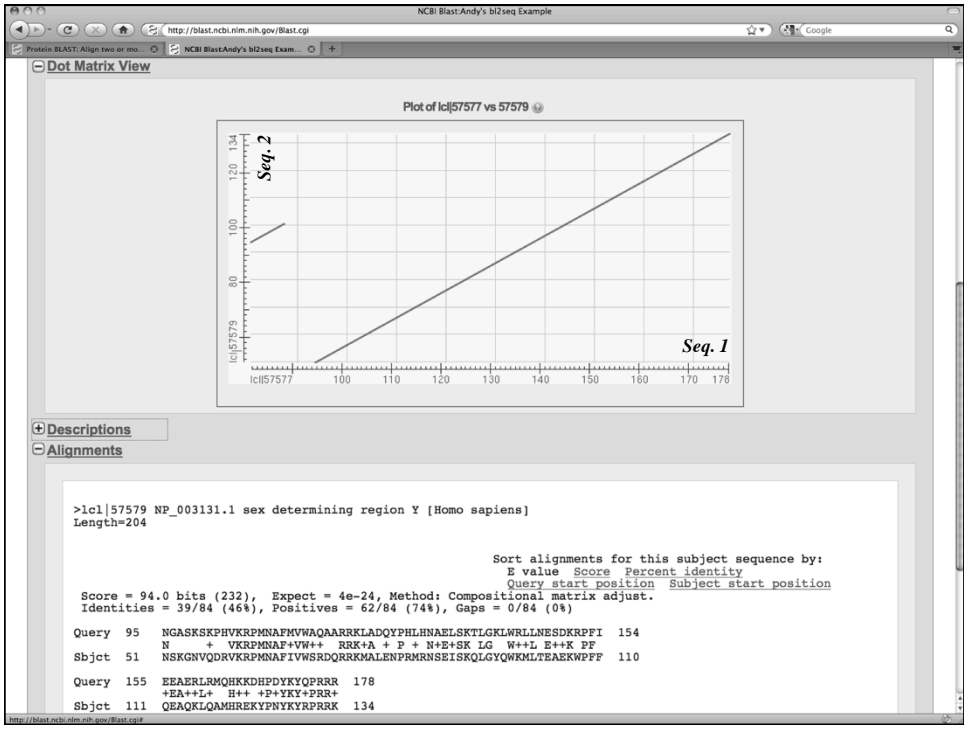
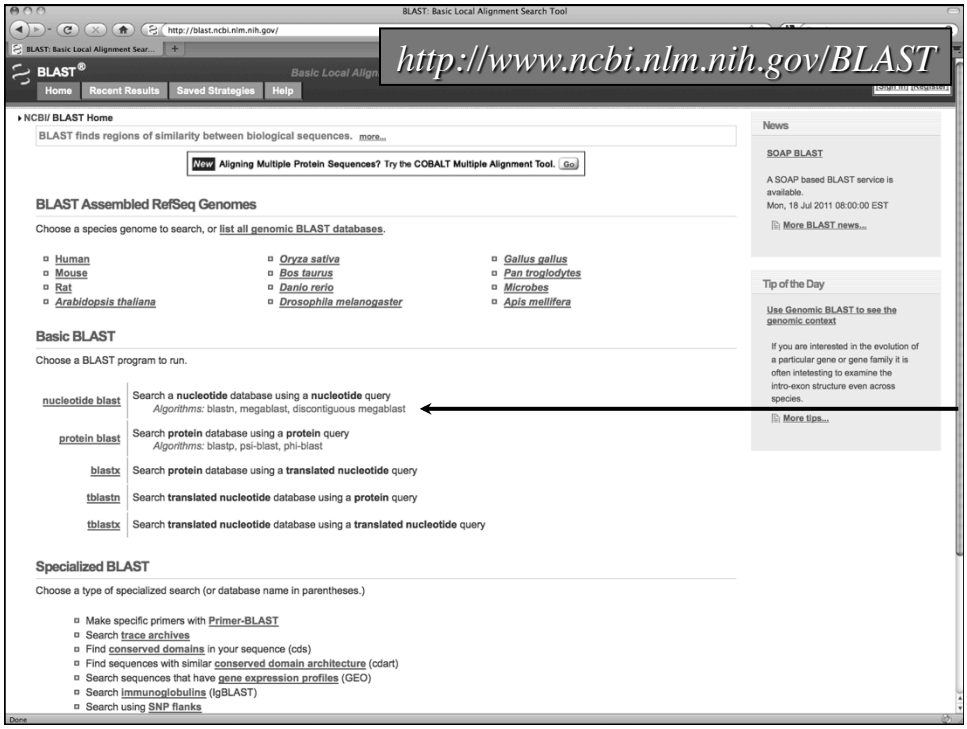
PAM30
PAM70
BLOSUM80
BLOSUM62
BLOSUM45

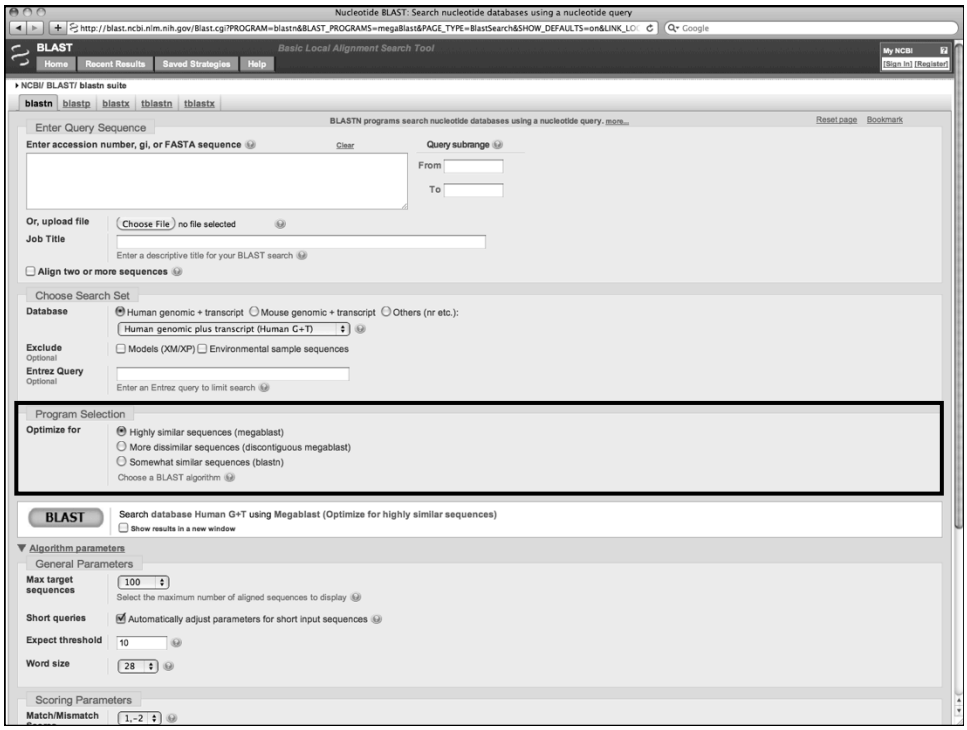

NCBI Blast:Andy's bl2seq Example
http://blast.ncbi.nlm.nih.gov/Blast.cgi
BLAST®
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
My NCBI
[Sign In] [Register]
NCBI/ BLAST/ blastp suite-2sequences/ Formatting Results - GR3U63S2112
Edit and Resubmit
Save Search Strategies
Formatting options
Download
Blast 2 sequences
Andy's bl2seq Example
Query ID lcl|57577
Description NP_008872.1 SOX-10 [Homo sapiens]
Molecule type amino acid
Query Length 466
Subject ID 57579
Description NP_003131.1 sex determining region Y [Homo sapiens]
Molecule type amino acid
Subject Length 204
Program BLASTP 2.2.26+
Citation
Other reports: Search Summary [Taxonomy reports] [Multiple alignment]
Graphic Summary
Distribution of 2 Blast Hits on the Query Sequence
Mouse over to see the define, click to show alignments
Color key for alignment scores
<40
40-50
50-80
80-200
>=200
Query
1
90
180
270
360
450
Dot Matrix View
Descriptions
Legend for links to other resources: UniGene GEO Gene Structure Map Viewer PubChem BioAssay
Sequences producing significant alignments:
Accession
Description
Max score
Total score
Query coverage
E value
Max ident
Links
57579
NP_003131.1 sex determining region Y [Homo sapiens]
94.0
109
19%
4e-24
46%
Done


NCBI Blast:Andy's bl2seq Example
http://blast.ncbi.nlm.nih.gov/Blast.cgi
Dot Matrix View
Descriptions
Legend for links to other resources: UniGene GEO Gene Structure Map Viewer PubChem BioAssay
Sequences producing significant alignments:
Accession
Description
Max score
Total score
Query coverage
E value
Max ident
Links
57579
NP_003131.1 sex determining region Y [Homo sapiens]
94.0
109
19%
4e-24
46%
Alignments
>lcl|57579 NP_003131.1 sex determining region Y [Homo sapiens]
Length=204
Sort alignments for this subject sequence by:
E value Score Percent identity
Query start position Subject start position
Score = 94.0 bits (232), Expect = 4e-24, Method: Compositional matrix adjust.
Identities = 39/84 (46%), Positives = 62/84 (74%), Gaps = 0/84 (0%)
Query 95 NGASKSKPHVKRPMNAFMVWAQAARRKLADQYPHLHNAELSKTLGKLWRLLNESDKRPFI 154
N + VKRPMNAF+VW++ RRK+A + P + N+E+SK LG W++L E++K PF
Sbjct 51 NSKGNVQDRVKRPMNAFIVWSRDQRRKMALENPRMRNSEISKQLGYQWKMLTEAEKWPFF 110
Query 155 EEAERLRMQHKKDHPDYKYQPRRR 178
+EA++L+ H++ +P+YKY+PRR+
Sbjct 111 QEAQKLQAMHREKYPNYKYRPRRK 134
Score = 15.4 bits (28), Expect = 1.9, Method: Compositional matrix adjust.
Identities = 3/7 (43%), Positives = 5/7 (71%), Gaps = 0/7 (0%)
Query 82 GYDWTLV 88
GY W ++
Sbjct 95 GYQWKML 101
Done



Nucleotide BLAST
MegaBLAST and BLASTN


BLAST: Basic Local Alignment Search Tool
http://www.ncbi.nlm.nih.gov/BLAST
BLAST®
Basic Local Align
Home
Recent Results
Saved Strategies
Help
NCBI/ BLAST Home
BLAST finds regions of similarity between biological sequences. more...
New Aligning Multiple Protein Sequences? Try the COBALT Multiple Alignment Tool. Go
BLAST Assembled RefSeq 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
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.)
Make specific primers with Primer-BLAST
Search trace archives
Find conserved domains in your sequence (cds)
Find sequences with similar conserved domain architecture (cdart)
Search sequences that have gene expression profiles (GEO)
Search immunoglobulins (IgBLAST)
Search using SNP flanks
News
SOAP BLAST
A SOAP based BLAST service is available.
Mon, 18 Jul 2011 08:00:00 EST
More BLAST news...
Tip of the Day
Use Genomic BLAST to see the genomic context
If you are interested in the evolution of a particular gene or gene family it is often intetesting to examine the intro-exon structure even across species.
More tips...


Nucleotide BLAST: Search nucleotide databases using a nucleotide query
BLAST
Basic Local Alignment Search Tool
Home
Recent Results
Saved Strategies
Help
NCBI/ BLAST/ blastn suite
blastn
blastp
blastx
tblastn
tblastx
BLASTN programs search nucleotide databases using a nucleotide query. more...
Reset page
Bookmark
Enter Query Sequence
Enter accession number, gi, or FASTA sequence
Clear
Query subrange
From
To
Or, upload file
Choose File
no file selected
Job Title
Enter a descriptive title for your BLAST search
Align two or more sequences
Choose Search Set
Database
Human genomic + transcript
Mouse genomic + transcript
Others (nr etc.):
Human genomic plus transcript (Human G+T)
Exclude
Optional
Models (XM/XP)
Environmental sample sequences
Entrez Query
Optional
Enter an Entrez query to limit search
Program Selection
Optimize for
Highly similar sequences (megablast)
More dissimilar sequences (discontiguous megablast)
Somewhat similar sequences (blastn)
Choose a BLAST algorithm
BLAST
Search database Human G+T using Megablast (Optimize for highly similar sequences)
Show results in a new window
Algorithm parameters
General Parameters
Max target sequences
100
Select the maximum number of aligned sequences to display
Short queries
Automatically adjust parameters for short input sequences
Expect threshold
10
Word size
28
Scoring Parameters
Match/Mismatch
1,-2

Nucleotide-Based BLAST Algorithms

	W	*+/–*	*Gaps*
Optimized for aligning very long and/or highly similar sequences (> 95%)			
MegaBLAST *(default)*	28	1, –2	Linear
Better for diverged sequences and/or cross-species comparisons (< 80%)			
Discontiguous MegaBLAST	11	2, –3	Affine
BLASTN	11	2, –3	Affine
Finding short, nearly exact matches (< 20 bases)			
BLASTN *E = 1000, all filtering off*	7	2, –3	Affine

BLAT

- "BLAST-Like Alignment Tool"
- Designed to rapidly-align longer nucleotide sequences ($L \geq 40$) having > 95% sequence similarity
- Can find exact matches reliably down to $L = 33$
- Method of choice when looking for exact matches in nucleotide databases
- 500 times faster for mRNA/DNA searches
- May miss divergent or shorter sequence alignments
- Can be used on protein sequences

When to Use BLAT

- To characterize an unknown gene or sequence fragment
  - Find its genomic coordinates
  - Determine gene structure (the presence and position of exons)
  - Identify markers of interest in the vicinity of a sequence
- To find highly-similar sequences
  - Identify gene family members
  - Identify putative homologs
- To display a specific sequence as a separate track

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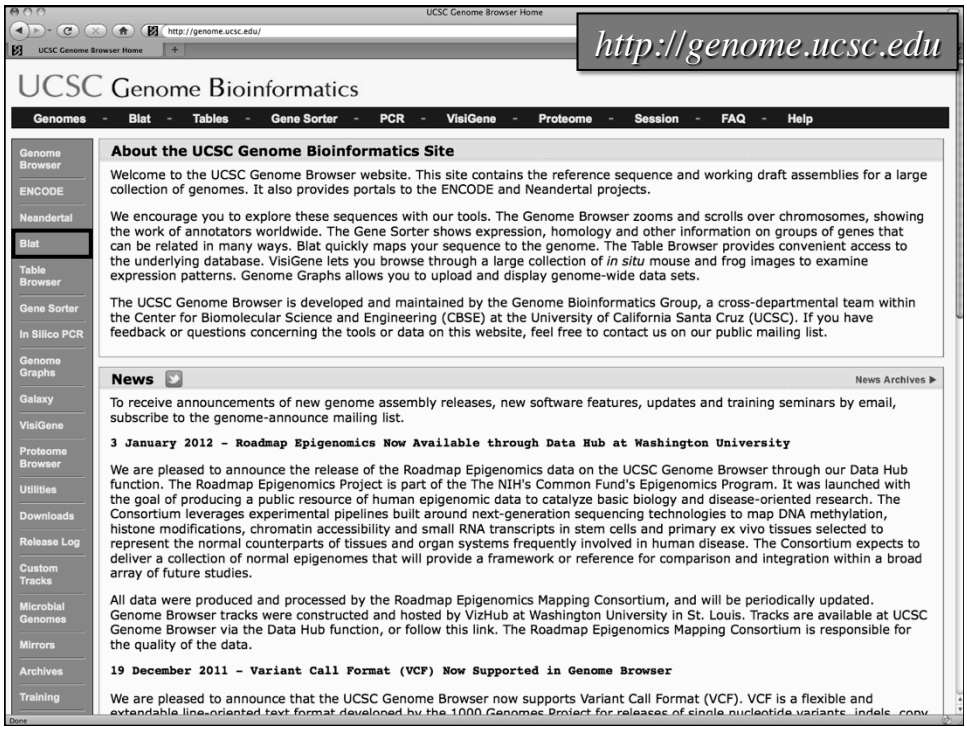

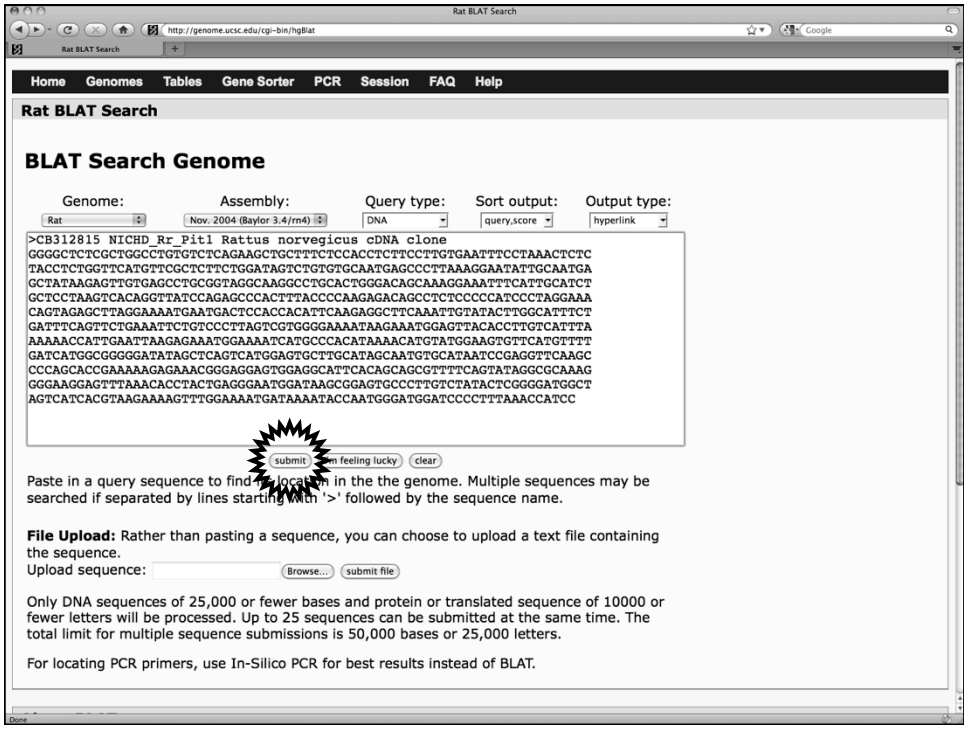
Rat BLAT Search
http://genome.ucsc.edu/cgi-bin/hgBlat
Home Genomes Tables Gene Sorter PCR Session FAQ Help
Rat BLAT Search
BLAT Search Genome
Genome: Assembly: Query type: Sort output: Output type:
Rat
Nov. 2004 (Baylor 3.4/rn4)
DNA
query,score
hyperlink
>CB312815 NICHD_Rr_Pit1 Rattus norvegicus cDNA clone
GGGGCTCTCGCTGGCCTGTGTCTCAGAAGCTGCTTTCTCCACCTCTTCCTTGTGAATTTCCTAAACTCTC
TACCTCTGGTTCATGTTCGCTCTTCTGGATAGTCTGTGTGCAATGAGCCCTTAAAGGAATATTGCAATGA
GCTATAAGAGTTGTGAGCCTGCGGTAGGCAAGGCCTGCACTGGGACAGCAAAGGAAATTTCATTGCATCT
GCTCCTAAGTCACAGGTTATCCAGAGCCCACTTTACCCCAAGAGACAGCCTCTCCCCCATCCCTAGGAAA
CAGTAGAGCTTAGGAAAATGAATGACTCCACCACATTCAAGAGGCTTCAAATTGTATACTTGGCATTTCT
GATTTCAGTTCTGAAATTCTGTCCCTTAGTCGTGGGGAAAATAAGAAATGGAGTTACACCTTGTCATTTA
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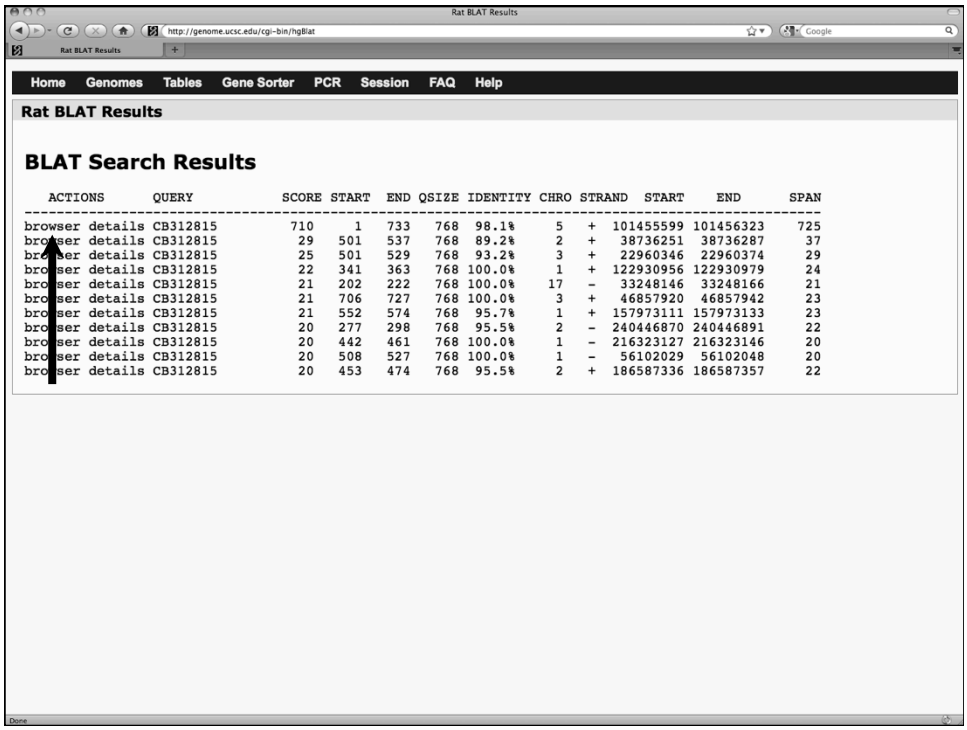
GGGAAGGAGTTTAAACACCTACTGAGGGAATGGATAAGCGGAGTGCCCTTGTCTATACTCGGGGATGGCT
AGTCATCACGTAAGAAAAGTTTGGAAAATGATAAAATACCAATGGGATGGATCCCCTTTAAACCATCC
submit
clear
Paste in a query sequence to find out location in the the genome. Multiple sequences may be searched if separated by lines starting with '>' followed by the sequence name.
File Upload: Rather than pasting a sequence, you can choose to upload a text file containing the sequence.
Upload sequence:
Browse...
submit file
Only DNA sequences of 25,000 or fewer bases and protein or translated sequence of 10000 or fewer letters will be processed. Up to 25 sequences can be submitted at the same time. The total limit for multiple sequence submissions is 50,000 bases or 25,000 letters.
For locating PCR primers, use In-Silico PCR for best results instead of BLAT.


Rat BLAT Results
http://genome.ucsc.edu/cgi-bin/hgBlat
Home Genomes Tables Gene Sorter PCR Session FAQ Help
Rat BLAT Results
BLAT Search Results
ACTIONS QUERY SCORE START END QSIZE IDENTITY CHRO STRAND START END SPAN
browser details CB312815 710 1 733 768 98.1% 5 + 101455599 101456323 725
browser details CB312815 29 501 537 768 89.2% 2 + 38736251 38736287 37
browser details CB312815 25 501 529 768 93.2% 3 + 22960346 22960374 29
browser details CB312815 22 341 363 768 100.0% 1 + 122930956 122930979 24
browser details CB312815 21 202 222 768 100.0% 17 - 33248146 33248166 21
browser details CB312815 21 706 727 768 100.0% 3 + 46857920 46857942 23
browser details CB312815 21 552 574 768 95.7% 1 + 157973111 157973133 23
browser details CB312815 20 277 298 768 95.5% 2 - 240446870 240446891 22
browser details CB312815 20 442 461 768 100.0% 1 - 216323127 216323146 20
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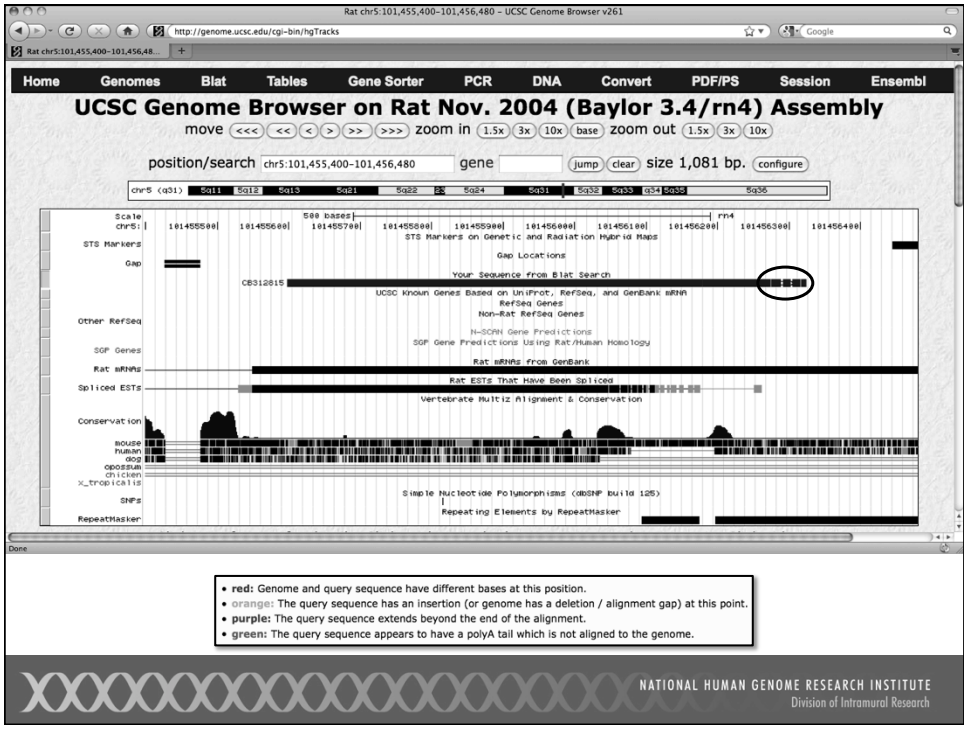
browser details CB312815 20 453 474 768 95.5% 2 + 186587336 186587357 22


Rat chr5:101,455,400-101,456,480 - UCSC Genome Browser v261
http://genome.ucsc.edu/cgi-bin/hgTracks
Home Genomes Blat Tables Gene Sorter PCR DNA Convert PDF/PS Session Ensembl
UCSC Genome Browser on Rat Nov. 2004 (Baylor 3.4/rn4) Assembly
move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x
position/search chr5:101,455,400-101,456,480 gene jump clear size 1,081 bp. configure
STS Markers
Gap
Gap Locations
Your Sequence from Blat Search
CB312815
UCSC Known Genes Based on UniProt, RefSeq, and GenBank mRNA
RefSeq Genes
Non-Rat RefSeq Genes
Other RefSeq
N-SCAN Gene Predictions
SGP Gene Predictions Using Rat/Human Homology
SGP Genes
Rat mRNAs from GenBank
Rat mRNAs
Rat ESTs That Have Been Spliced
Spliced ESTs
Vertebrate Multiz Alignment & Conservation
Conservation
mouse
human
dog
opossum
chicken
X_tropicalis
Simple Nucleotide Polymorphisms (dbSNP build 125)
SNPs
Repeating Elements by RepeatMasker
RepeatMasker
red: Genome and query sequence have different bases at this position.
orange: The query sequence has an insertion (or genome has a deletion / alignment gap) at this point.
purple: The query sequence extends beyond the end of the alignment.
green: The query sequence appears to have a polyA tail which is not aligned to the genome.
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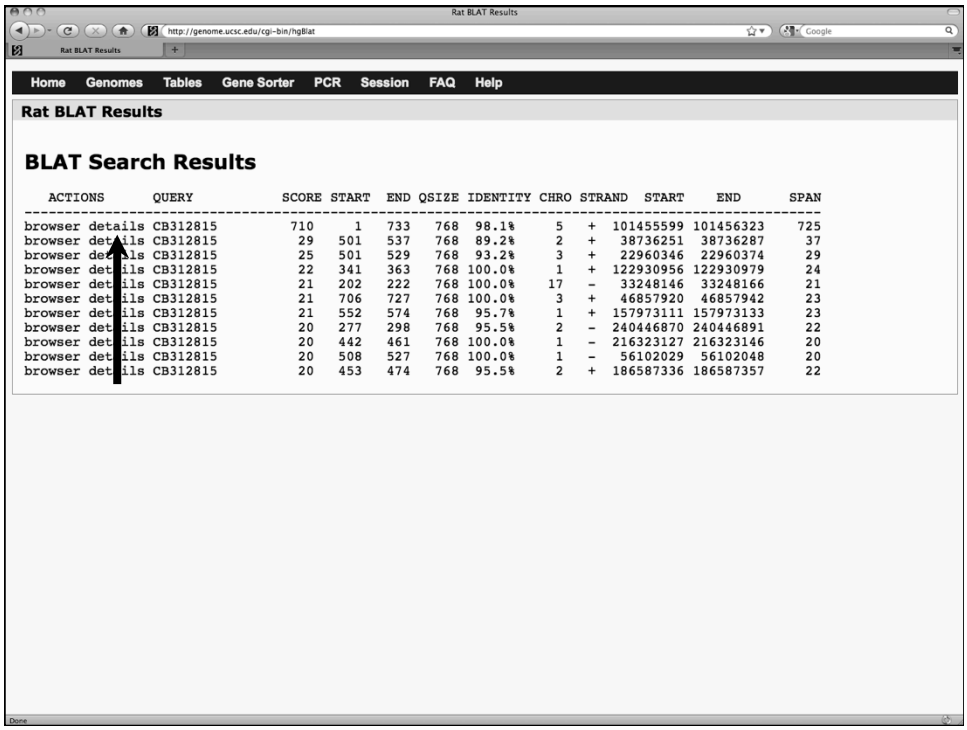
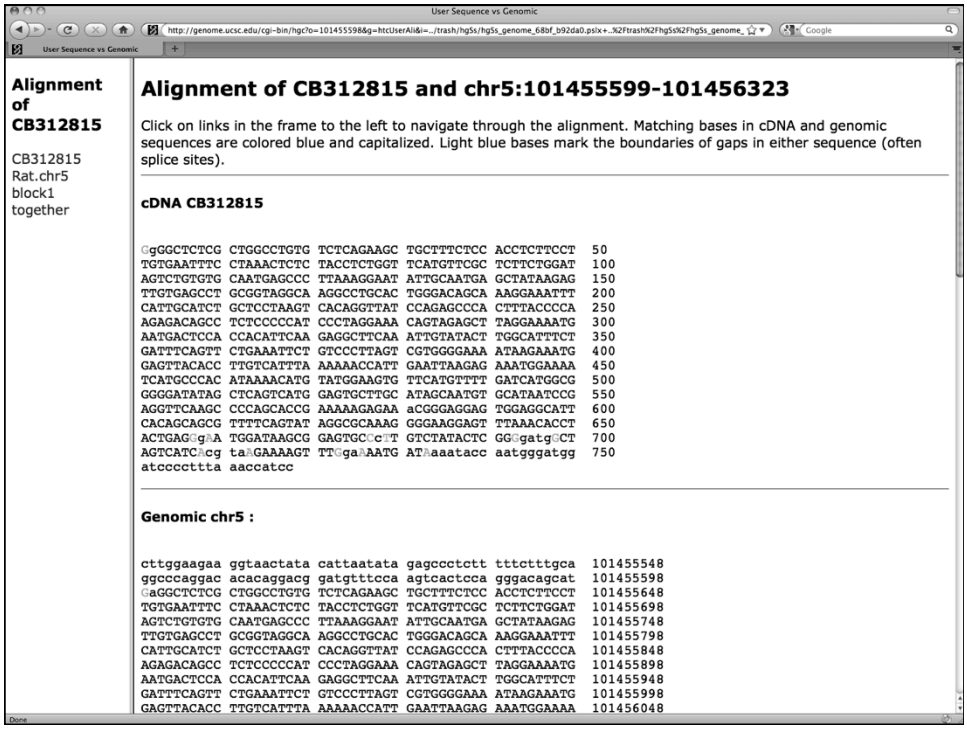
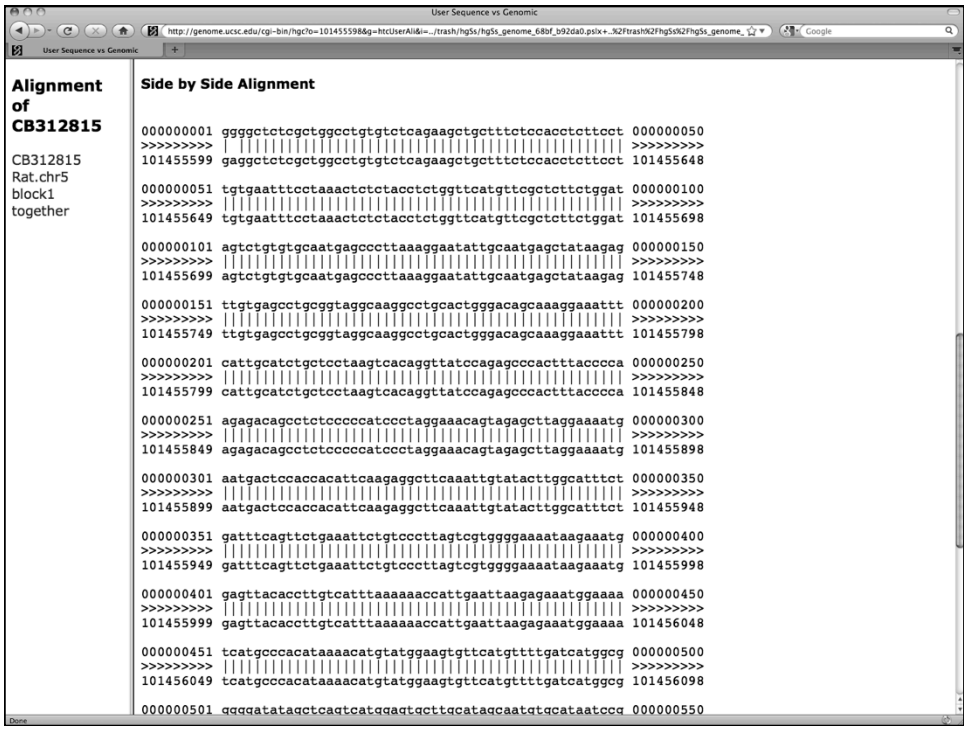

Rat BLAT Results
http://genome.ucsc.edu/cgi-bin/hgBlat
Home Genomes Tables Gene Sorter PCR Session FAQ Help
Rat BLAT Results
BLAT Search Results
ACTIONS QUERY SCORE START END QSIZE IDENTITY CHRO STRAND START END SPAN
browser details CB312815 710 1 733 768 98.1% 5 + 101455599 101456323 725
browser details CB312815 29 501 537 768 89.2% 2 + 38736251 38736287 37
browser details CB312815 25 501 529 768 93.2% 3 + 22960346 22960374 29
browser details CB312815 22 341 363 768 100.0% 1 + 122930956 122930979 24
browser details CB312815 21 202 222 768 100.0% 17 - 33248146 33248166 21
browser details CB312815 21 706 727 768 100.0% 3 + 46857920 46857942 23
browser details CB312815 21 552 574 768 95.7% 1 + 157973111 157973133 23
browser details CB312815 20 277 298 768 95.5% 2 - 240446870 240446891 22
browser details CB312815 20 442 461 768 100.0% 1 - 216323127 216323146 20
browser details CB312815 20 508 527 768 100.0% 1 - 56102029 56102048 20
browser details CB312815 20 453 474 768 95.5% 2 + 186587336 186587357 22


User Sequence vs Genomic
http://genome.ucsc.edu/cgi-bin/hgc?o=101455598&g=htcUserAli&i=../trash/hgSs/hgSs_genome_68bf_b92da0.psix+..%2Ftrash%2FhgSs%2FhgSs_genome_
Google
User Sequence vs Genomic
Alignment of CB312815
CB312815
Rat.chr5
block1
together
Alignment of CB312815 and chr5:101455599-101456323
Click on links in the frame to the left to navigate through the alignment. Matching bases in cDNA and genomic sequences are colored blue and capitalized. Light blue bases mark the boundaries of gaps in either sequence (often splice sites).
cDNA CB312815
gGGCTCTCG CTGGCCTGTG TCTCAGAAGC TGCTTTCTCC ACCTCTTCCT 50
TGTGAATTTC CTAAACTCTC TACCTCTGGT TCATGTTCGC TCTTCTGGAT 100
AGTCTGTGTG CAATGAGCCC TTAAAGGAAT ATTGCAATGA GCTATAAGAG 150
TTGTGAGCCT GCGGTAGGCA AGGCCTGCAC TGGGACAGCA AAGGAAATTT 200
CATTGCATCT GCTCCTAAGT CACAGGTTAT CCAGAGCCCA CTTTACCCCA 250
AGAGACAGCC TCTCCCCCAT CCCTAGGAAA CAGTAGAGCT TAGGAAAATG 300
AATGACTCCA CCACATTCAA GAGGCTTCAA ATTGTATACT TGGCATTTCT 350
GATTTCAGTT CTGAAATTCT GTCCCTTAGT CGTGGGGAAA ATAAGAAATG 400
GAGTTACACC TTGTCATTTA AAAAACCATT GAATTAAGAG AAATGGAAAA 450
TCATGCCCAC ATAAAACATG TATGGAAGTG TTCATGTTTT GATCATGGCG 500
GGGGATATAG CTCAGTCATG GAGTGCTTGC ATAGCAATGT GCATAATCCG 550
AGGTTCAAGC CCCAGCACCG AAAAAGAGAA aCGGGAGGAG TGGAGGCATT 600
CACAGCAGCG TTTTCAGTAT AGGCGCAAAG GGGAAGGAGT TTAAACACCT 650
ACTGAGGgAA TGGATAAGCG GAGTGCCcTT GTCTATACTC GGGgatgGCT 700
AGTCATCAcg taAGAAAAGT TTGgaAAATG ATAaaatacc aatgggatgg 750
atccccttta aaccatcc
Genomic chr5 :
cttggaagaa ggtaactata cattaatata gagccctctt tttctttgca 101455548
ggccccaggac acacaggacg gatgtttcca agtcactcca gggacagcat 101455598
GaGGCTCTCG CTGGCCTGTG TCTCAGAAGC TGCTTTCTCC ACCTCTTCCT 101455648
TGTGAATTTC CTAAACTCTC TACCTCTGGT TCATGTTCGC TCTTCTGGAT 101455698
AGTCTGTGTG CAATGAGCCC TTAAAGGAAT ATTGCAATGA GCTATAAGAG 101455748
TTGTGAGCCT GCGGTAGGCA AGGCCTGCAC TGGGACAGCA AAGGAAATTT 101455798
CATTGCATCT GCTCCTAAGT CACAGGTTAT CCAGAGCCCA CTTTACCCCA 101455848
AGAGACAGCC TCTCCCCCAT CCCTAGGAAA CAGTAGAGCT TAGGAAAATG 101455898
AATGACTCCA CCACATTCAA GAGGCTTCAA ATTGTATACT TGGCATTTCT 101455948
GATTTCAGTT CTGAAATTCT GTCCCTTAGT CGTGGGGAAA ATAAGAAATG 101455998
GAGTTACACC TTGTCATTTA AAAAACCATT GAATTAAGAG AAATGGAAAA 101456048
Done


User Sequence vs Genomic
http://genome.ucsc.edu/cgi-bin/hgc?o=101455598&g=htcUserAli&i=../trash/hgSs/hgSs_genome_68bf_b92da0.psix+..%2Ftrash%2FhgSs%2FhgSs_genome_
Google
User Sequence vs Genomic
Alignment of CB312815
CB312815
Rat.chr5
block1
together
Side by Side Alignment
000000001 ggggctctcgctggcctgtgtctcagaagctgctttctccacctcttcct 000000050
>>>>>>>>> | ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455599 gaggctctcgctggcctgtgtctcagaagctgctttctccacctcttcct 101455648
000000051 tgtgaatttcctaaactctctacctctggttcatgttcgctcttctggat 000000100
>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455649 tgtgaatttcctaaactctctacctctggttcatgttcgctcttctggat 101455698
000000101 agtctgtgtgcaatgagcccttaaaggaatattgcaatgagctataagag 000000150
>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455699 agtctgtgtgcaatgagcccttaaaggaatattgcaatgagctataagag 101455748
000000151 ttgtgagcctgcggtaggcaaggcctgcactgggacagcaaaggaaattt 000000200
>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455749 ttgtgagcctgcggtaggcaaggcctgcactgggacagcaaaggaaattt 101455798
000000201 cattgcatctgctcctaagtcacaggttatccagagcccactttacccca 000000250
>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455799 cattgcatctgctcctaagtcacaggttatccagagcccactttacccca 101455848
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>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455849 agagacagcctctcccccatccctaggaaacagtagagcttaggaaaatg 101455898
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>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455899 aatgactccaccacattcaagaggcttcaaattgtatacttggcatttct 101455948
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>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
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000000401 gagttacaccttgtcatttaaaaaaccattgaattaagagaaatggaaaa 000000450
>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101455999 gagttacaccttgtcatttaaaaaaccattgaattaagagaaatggaaaa 101456048
000000451 tcatgcccacataaaacatgtatggaagtgttcatgttttgatcatggcg 000000500
>>>>>>>>> ||||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101456049 tcatgcccacataaaacatgtatggaagtgttcatgttttgatcatggcg 101456098
000000501 ggggatatagctcagtcatggagtgcttgcataggcaatgtgcataatccg 000000550
Done

FASTA

- Identifies regions of local alignment
- Employs an approximation of the Smith-Waterman algorithm to determine the best alignment between two sequences
- Method is significantly different from that used by BLAST
- Online implementations at
  http://fasta.bioch.virginia.edu
  http://www.ebi.ac.uk/fasta33

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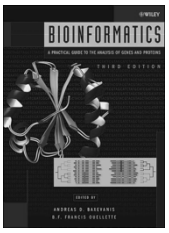
Further Reading



Chapter 11
Assessing Pairwise Sequence Similarity: BLAST and FASTA

Chapter 6
Sequence Database Searching for Similar Sequences

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