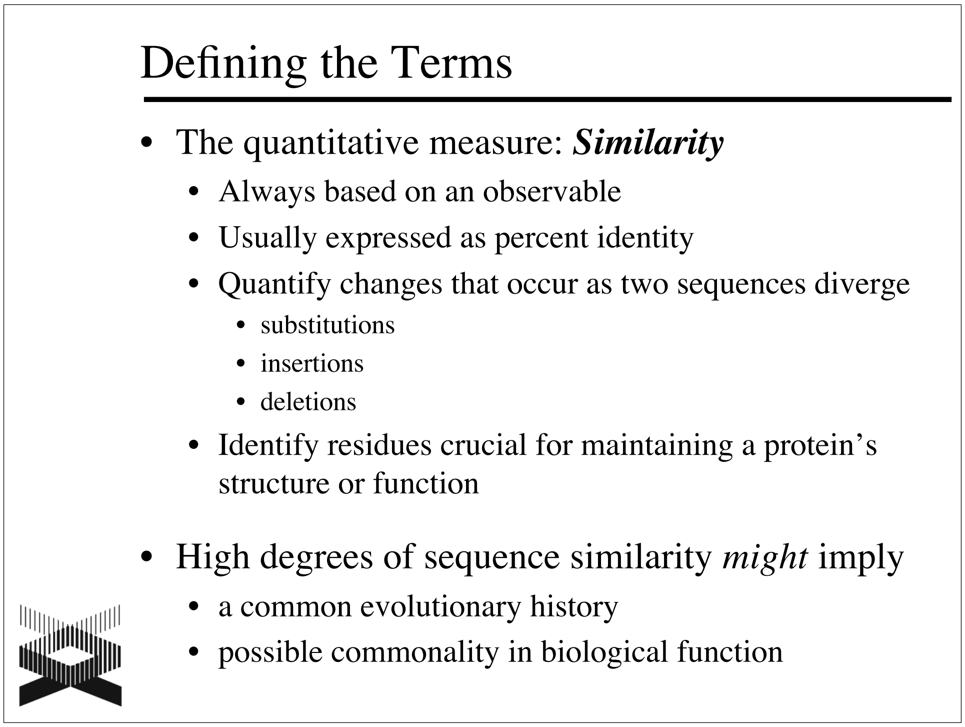
Overview

- Week 2: Comparative methods and concepts
  - Similarity *vs*. Homology
  - Global *vs*. Local Alignments
  - Scoring Matrices
  - BLAST
  - BLAT
- Week 3: Predictive methods and concepts
  - Profiles, patterns, motifs, and domains
  - Secondary structure prediction
  - Structures: VAST, Cn3D, and *de novo* prediction

Why do sequence alignments?

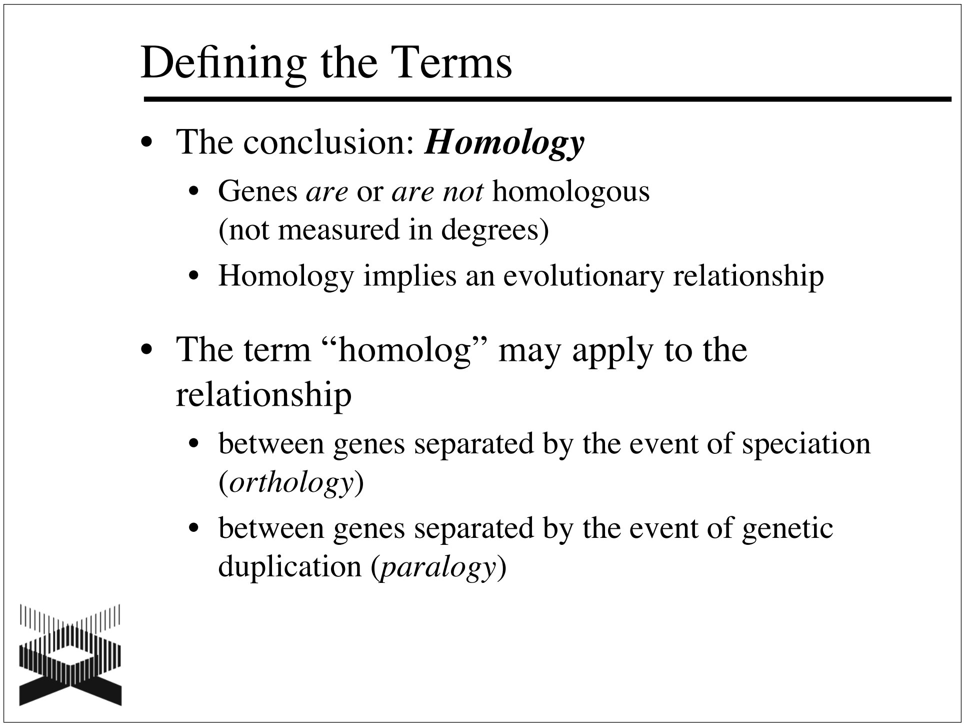
- Provide a measure of relatedness between nucleotide or amino acid sequences
- Determining relatedness allows one to draw biological inferences regarding
  - structural relationships
  - functional relationships
  - evolutionary relationships

→ *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
    - 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



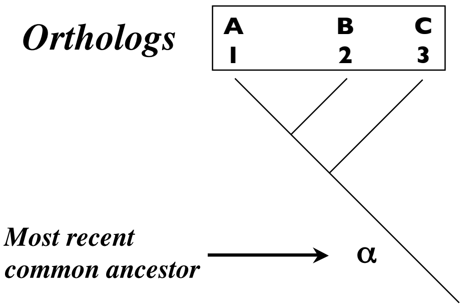
Defining the Terms

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

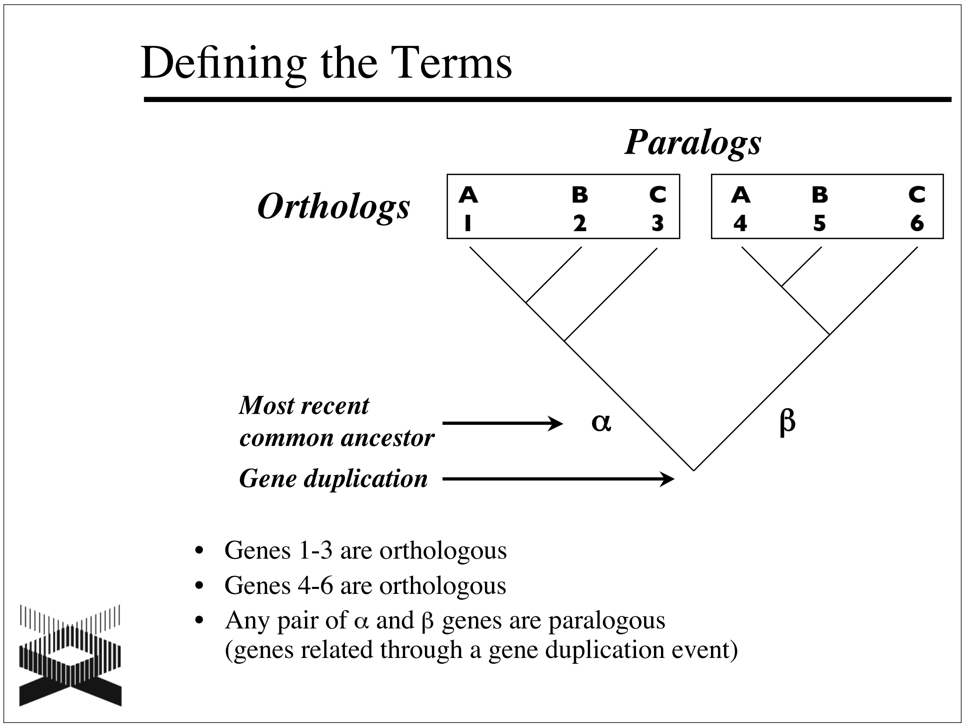
- Orthologs
  - Sequences are direct descendants of a sequence in a common ancestor
  - Most likely have similar domain structure, three-dimensional structure, and biological function
- Paralogs
  - Related through a gene duplication event
  - Provides insight into "evolutionary innovation" (adapting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

Defining the Terms



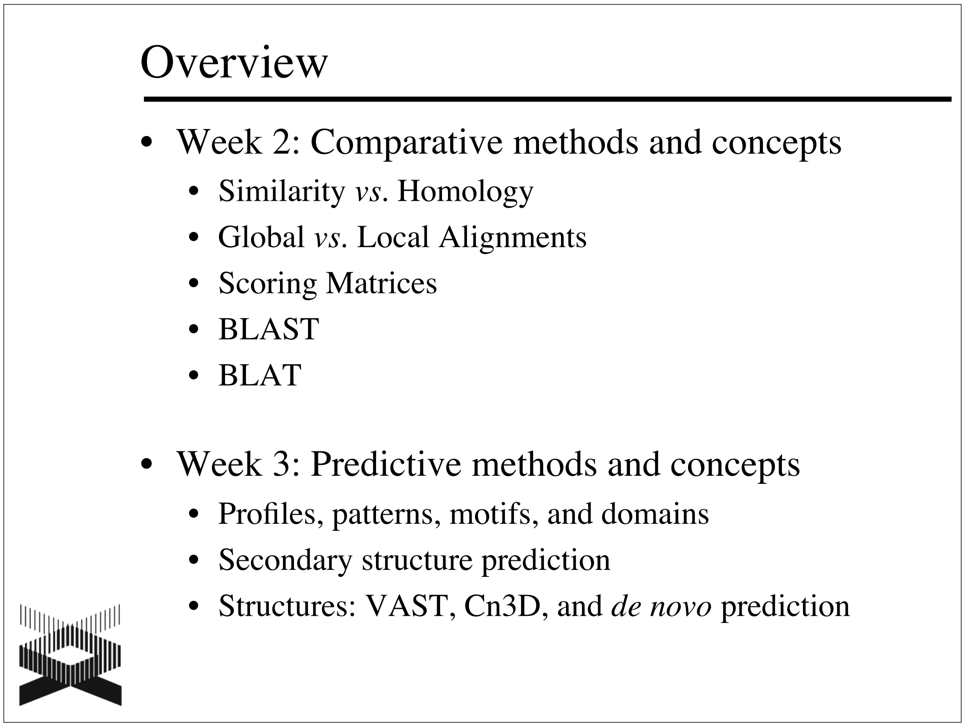
Orthologs

Most recent common ancestor



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)



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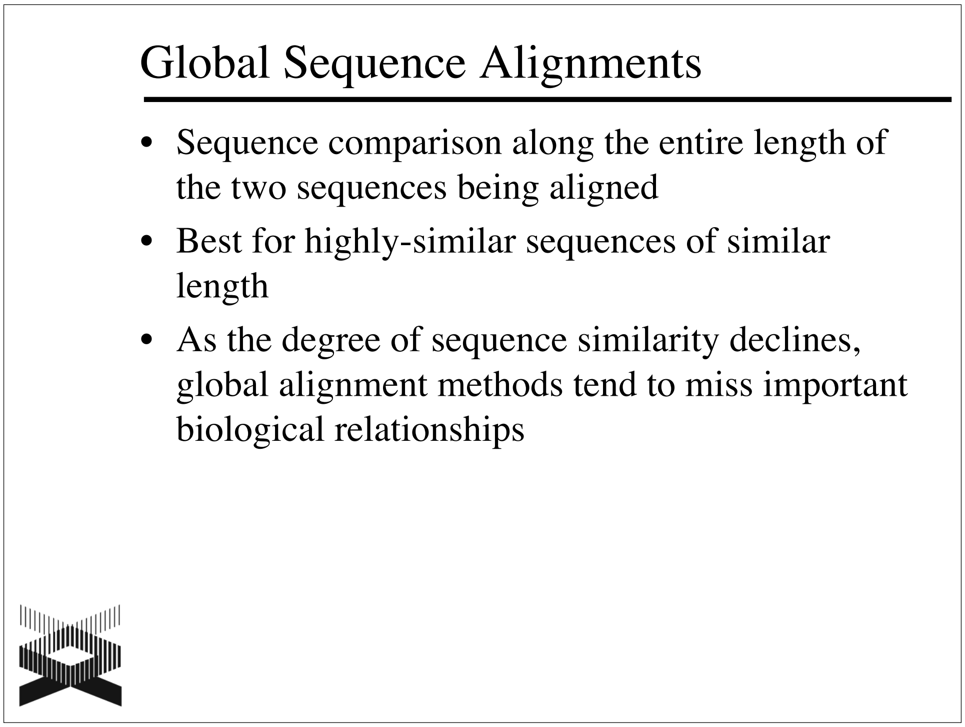
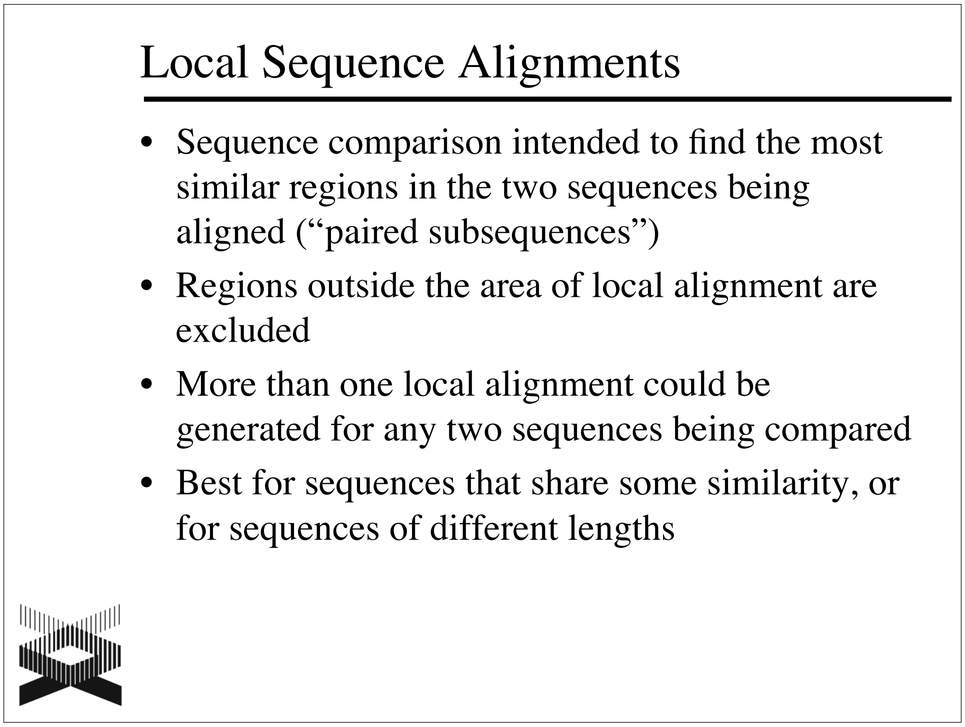


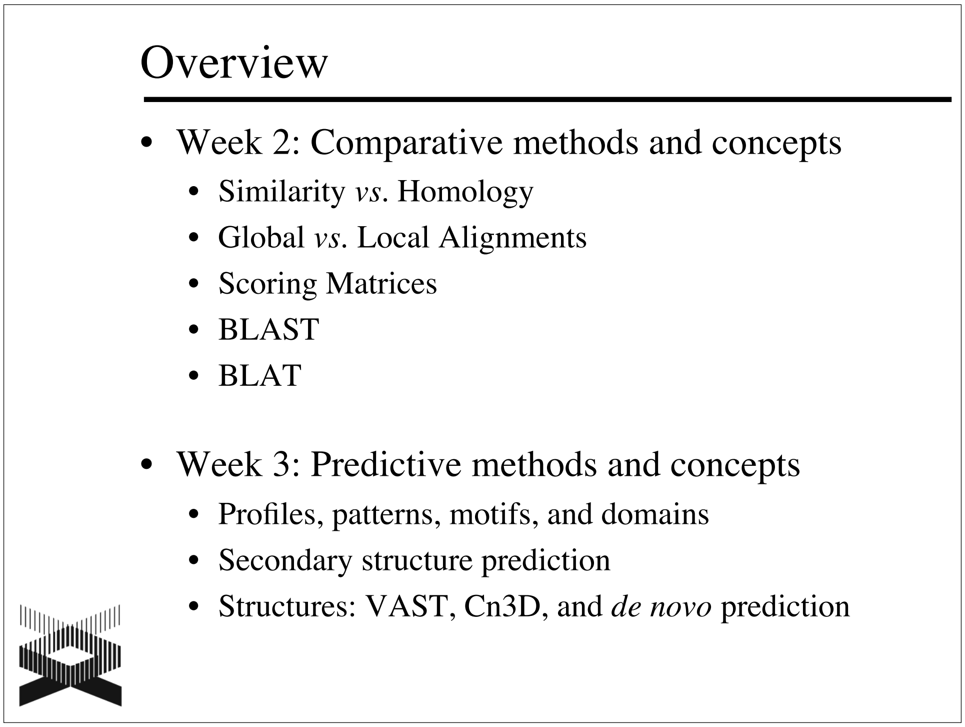
Global Sequence Alignments

- 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



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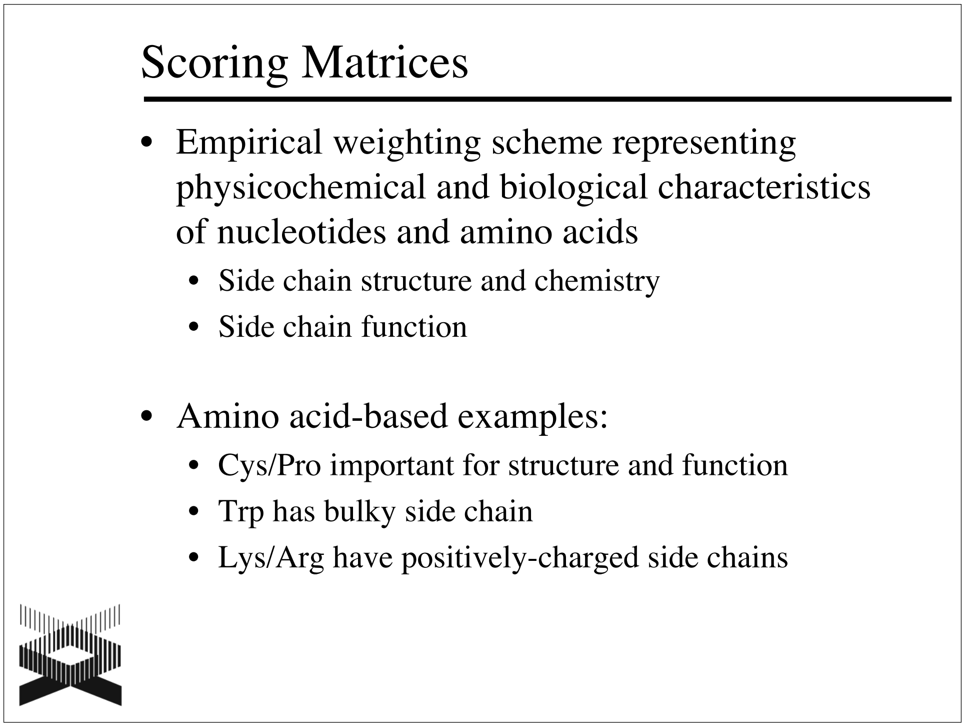
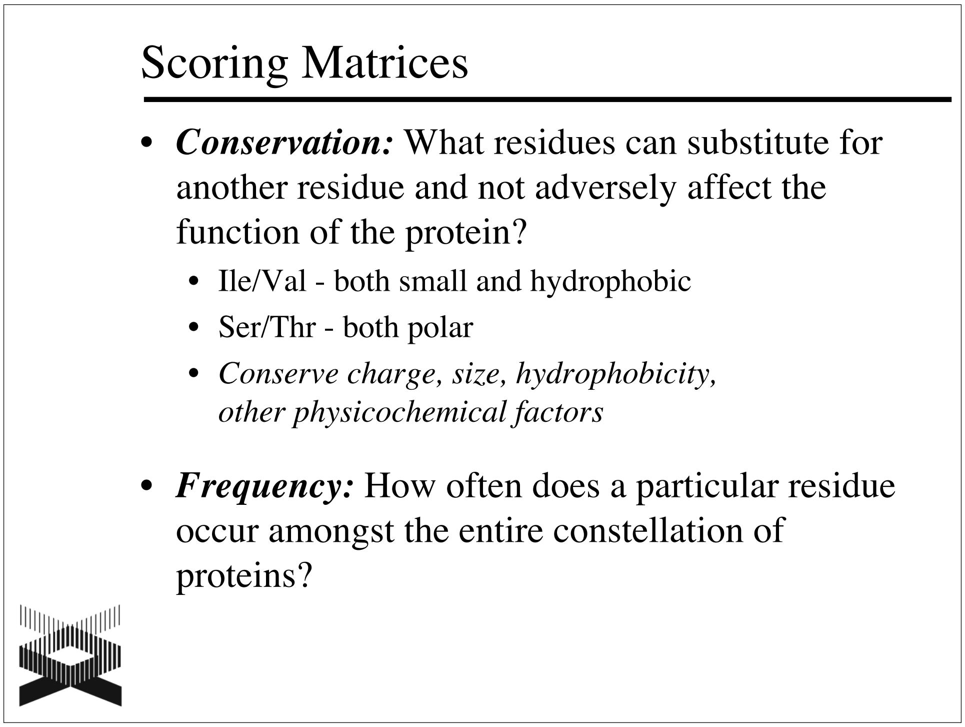


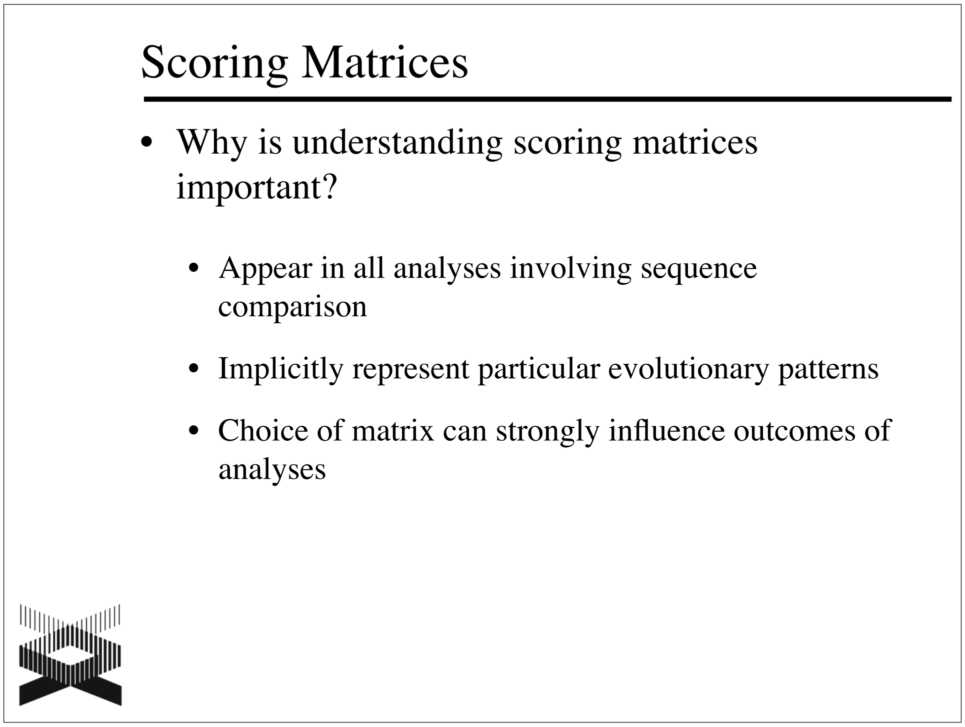
Scoring Matrices

- 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



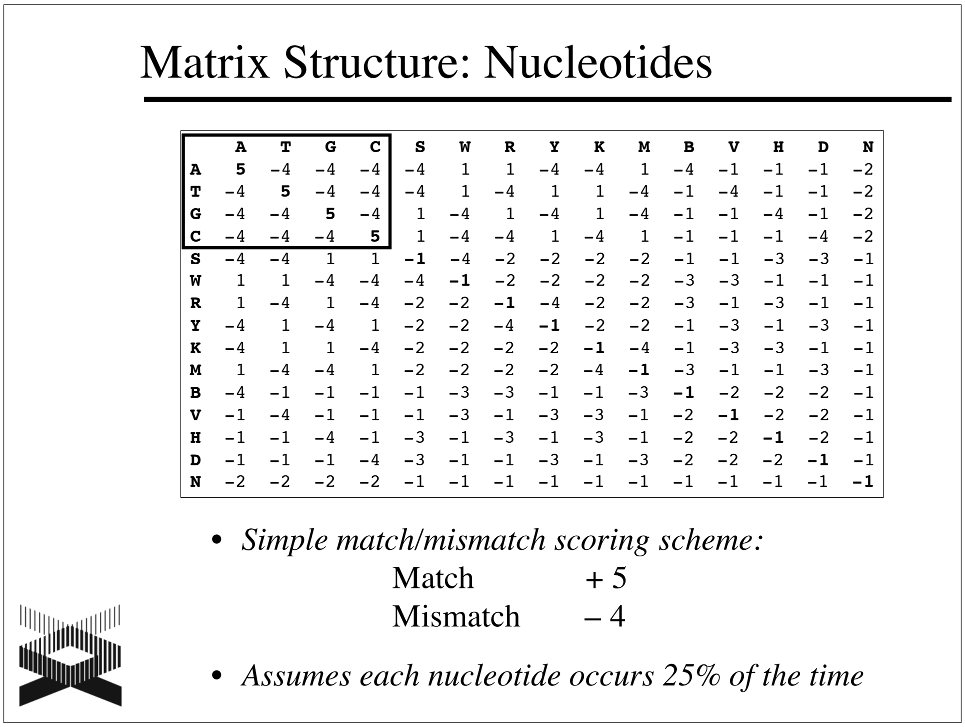
Scoring Matrices

- ***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



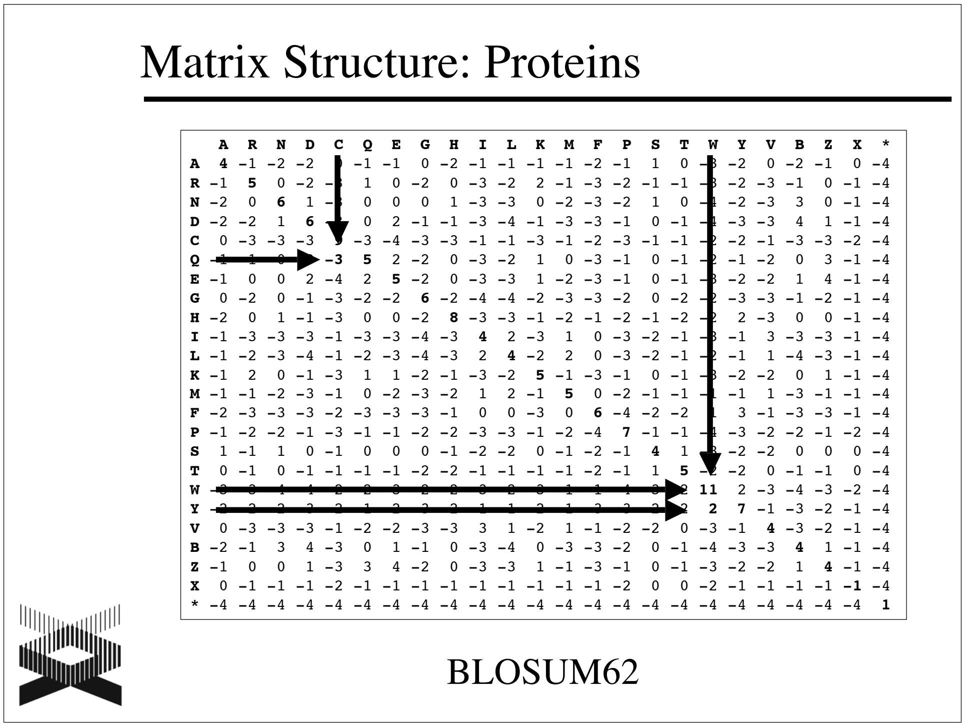
Matrix Structure: Nucleotides

	A	T	G	C	S	W	R	Y	K	M	B	V	H	D	N
A	**5**	−4	−4	−4	−4	1	1	−4	−4	1	−4	−1	−1	−1	−2
T	−4	**5**	−4	−4	−4	1	−4	1	1	−4	−1	−4	−1	−1	−2
G	−4	−4	**5**	−4	1	−4	1	−4	1	−4	−1	−1	−4	−1	−2
C	−4	−4	−4	**5**	1	−4	−4	1	−4	1	−1	−1	−1	−4	−2
S	−4	−4	1	1	**−1**	−4	−2	−2	−2	−2	−1	−1	−3	−3	−1
W	1	1	−4	−4	−4	**−1**	−2	−2	−2	−2	−3	−3	−1	−1	−1
R	1	−4	1	−4	−2	−2	**−1**	−4	−2	−2	−3	−1	−3	−1	−1
Y	−4	1	−4	1	−2	−2	−4	**−1**	−2	−2	−1	−3	−1	−3	−1
K	−4	1	1	−4	−2	−2	−2	−2	**−1**	−4	−1	−3	−3	−1	−1
M	1	−4	−4	1	−2	−2	−2	−2	−4	**−1**	−3	−1	−1	−3	−1
B	−4	−1	−1	−1	−1	−3	−3	−1	−1	−3	**−1**	−2	−2	−2	−1
V	−1	−4	−1	−1	−1	−3	−1	−3	−3	−1	−2	**−1**	−2	−2	−1
H	−1	−1	−4	−1	−3	−1	−3	−1	−3	−1	−2	−2	**−1**	−2	−1
D	−1	−1	−1	−4	−3	−1	−1	−3	−1	−3	−2	−2	−2	**−1**	−1
N	−2	−2	−2	−2	−1	−1	−1	−1	−1	−1	−1	−1	−1	−1	**−1**

- *Simple match/mismatch scoring scheme:*

Match	+ 5
Mismatch	– 4

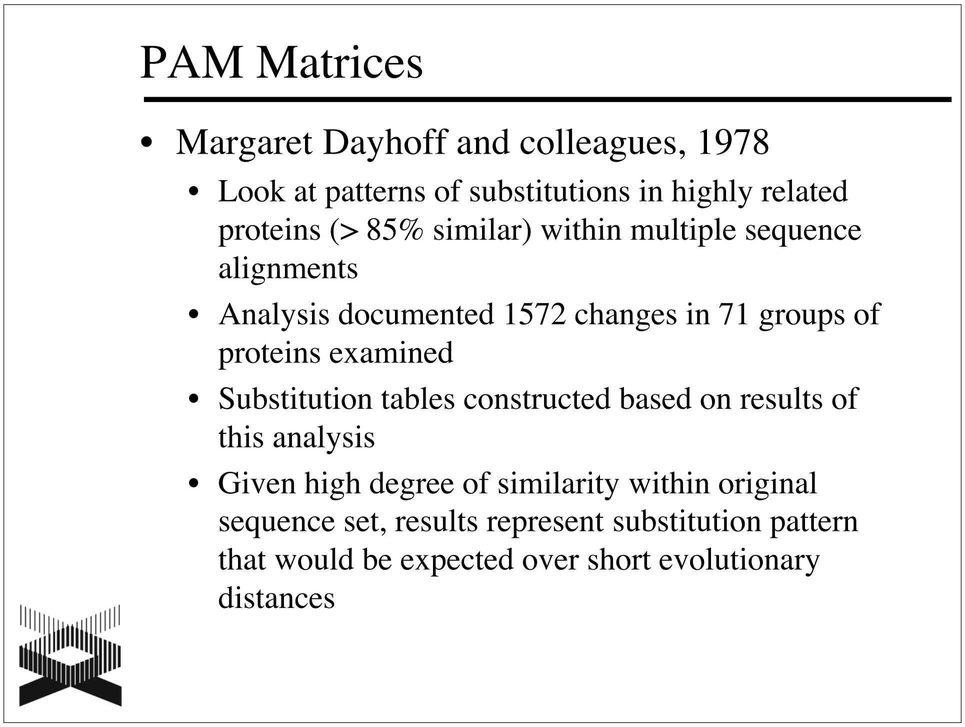
- *Assumes each nucleotide occurs 25% of the time*



Matrix Structure: Proteins

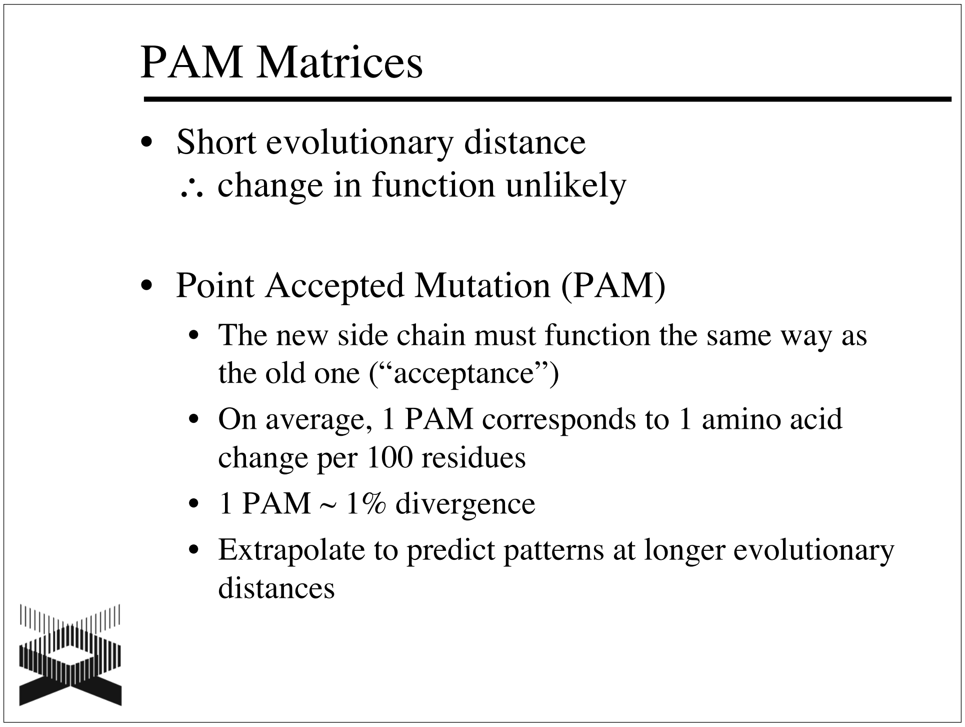
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	*
A	**4**	−1	−2	−2	0	−1	−1	0	−2	−1	−1	−1	−1	−2	−1	1	0	−3	−2	0	−2	−1	0	−4
R	−1	**5**	0	−2	−3	1	0	−2	0	−3	−2	2	−1	−3	−2	−1	−1	−3	−2	−3	−1	0	−1	−4
N	−2	0	**6**	1	−3	0	0	0	1	−3	−3	0	−2	−3	−2	1	0	−4	−2	−3	3	0	−1	−4
D	−2	−2	1	**6**	−3	0	2	−1	−1	−3	−4	−1	−3	−3	−1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



PAM Matrices

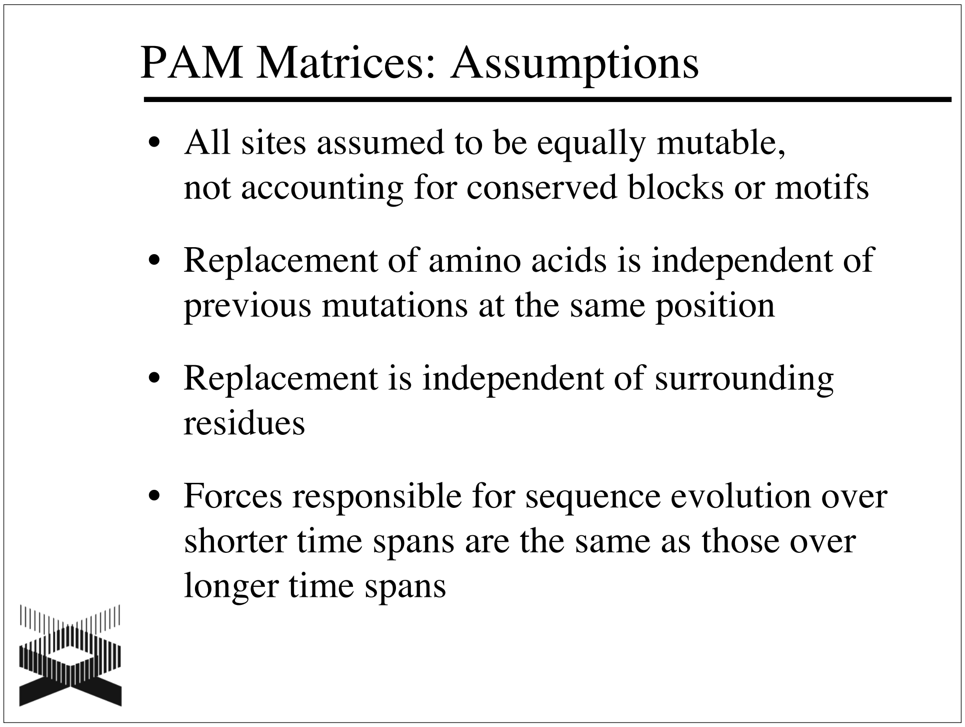
- Margaret Dayhoff and colleagues, 1978
  - Look at patterns of substitutions in highly related proteins (> 85% similar) within multiple sequence alignments
  - Analysis documented 1572 changes in 71 groups of proteins examined
  - Substitution tables constructed based on results of this analysis
  - Given high degree of similarity within original sequence set, results represent substitution pattern that would be expected over short evolutionary distances



PAM Matrices

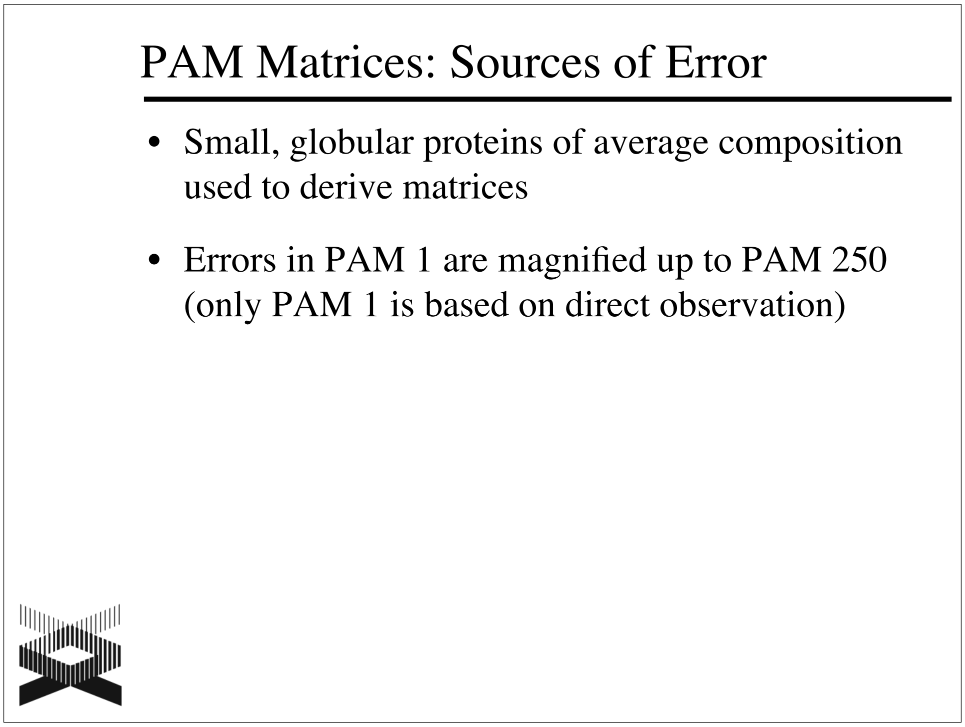
- Short evolutionary distance
  ∴ change in function unlikely

- Point Accepted Mutation (PAM)
  - The new side chain must function the same way as the old one (“acceptance”)
  - On average, 1 PAM corresponds to 1 amino acid change per 100 residues
  - 1 PAM ~ 1% divergence
  - Extrapolate to predict patterns at longer evolutionary distances



PAM Matrices: Assumptions

- All sites assumed to be equally mutable, not accounting for conserved blocks or motifs
- Replacement of amino acids is independent of previous mutations at the same position
- Replacement is independent of surrounding residues
- Forces responsible for sequence evolution over shorter time spans are the same as those over longer time spans



PAM Matrices: Sources of Error

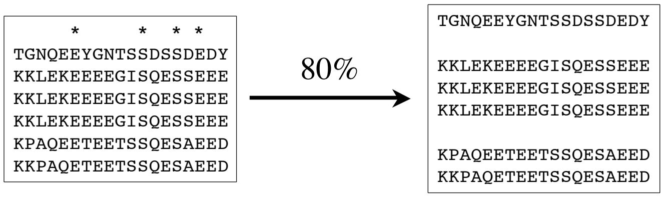
- Small, globular proteins of average composition used to derive matrices
- Errors in PAM 1 are magnified up to PAM 250 (only PAM 1 is based on direct observation)

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



```
     *     *  * *
TGNQEEYGNTSSDSSDEDY
KKLEKEEEEGISQESSEEE
KKLEKEEEEGISQESSEEE
KKLEKEEEEGISQESSEEE
KPAQEETEETSSQESAEED
KKPAQETEETSSQESAEED
```

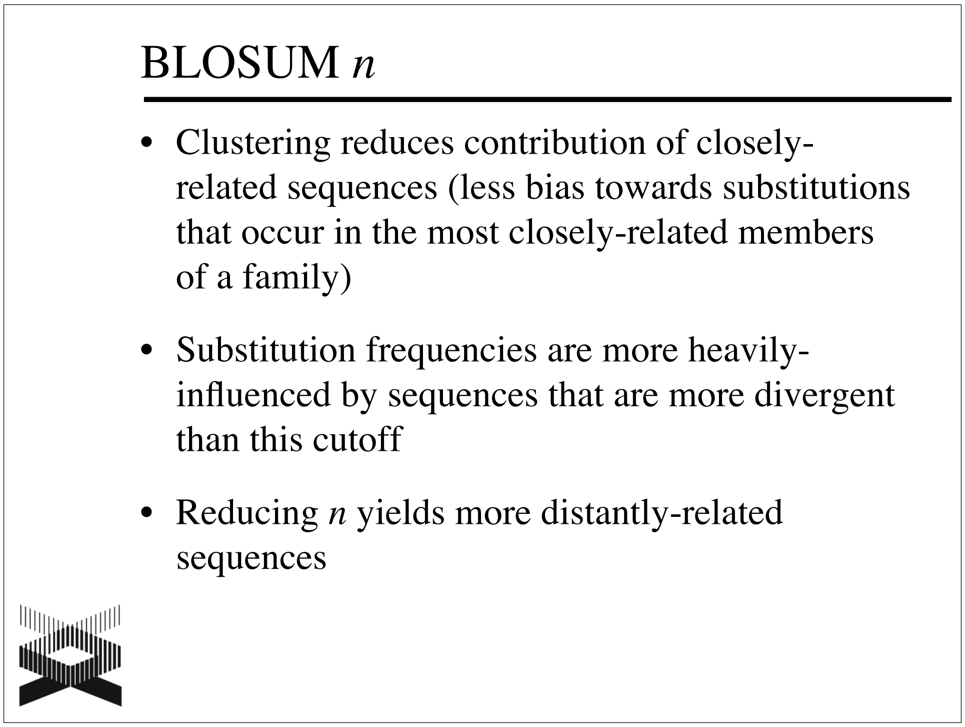
```
TGNQEEYGNTSSDSSDEDY

KKLEKEEEEGISQESSEEE
KKLEKEEEEGISQESSEEE
KKLEKEEEEGISQESSEEE

KPAQEETEETSSQESAEED
KKPAQETEETSSQESAEED
```

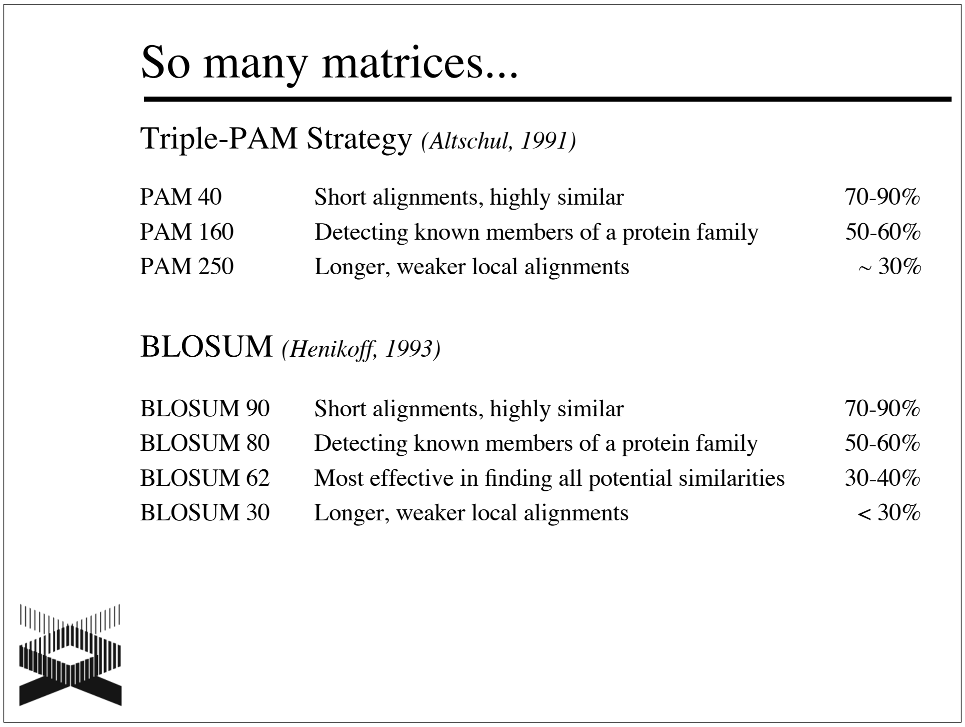
A+T Hook Domain (Block IPB000637B)

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



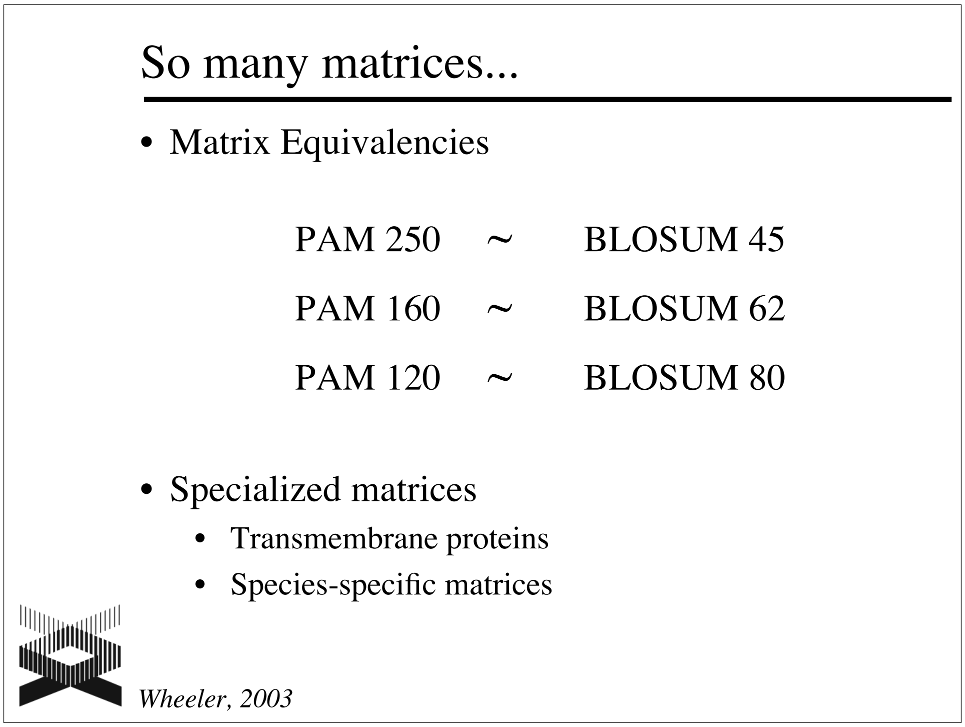
So many matrices...

Triple-PAM Strategy *(Altschul, 1991)*

PAM 40	Short alignments, highly similar	70-90%
PAM 160	Detecting known members of a protein family	50-60%
PAM 250	Longer, weaker local alignments	~ 30%

BLOSUM *(Henikoff, 1993)*

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



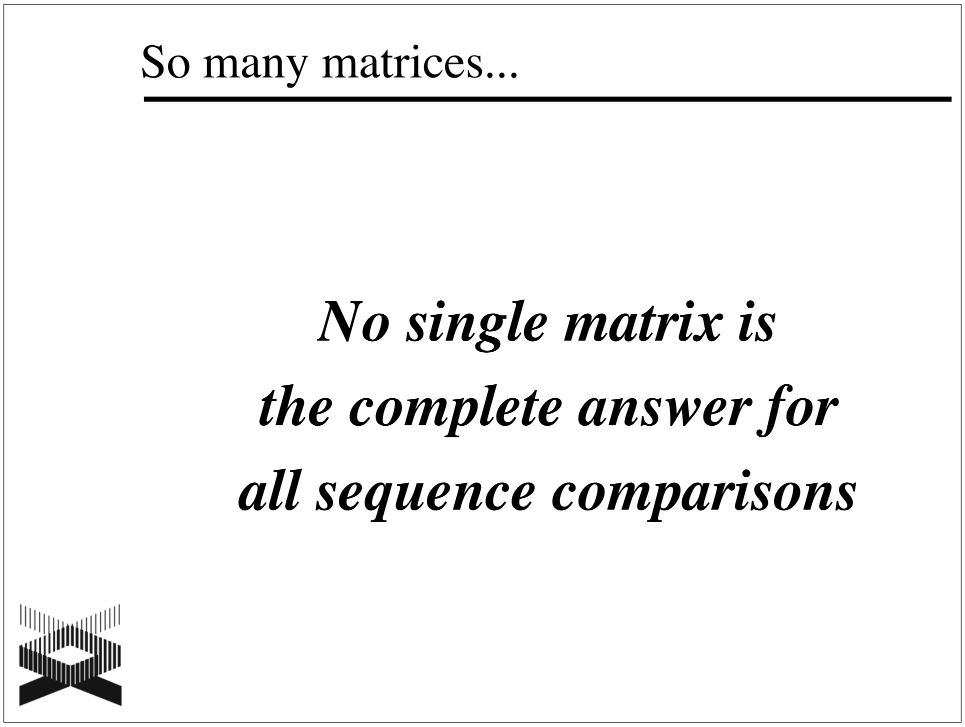
So many matrices...

- Matrix Equivalencies

PAM 250	~	BLOSUM 45
PAM 160	~	BLOSUM 62
PAM 120	~	BLOSUM 80

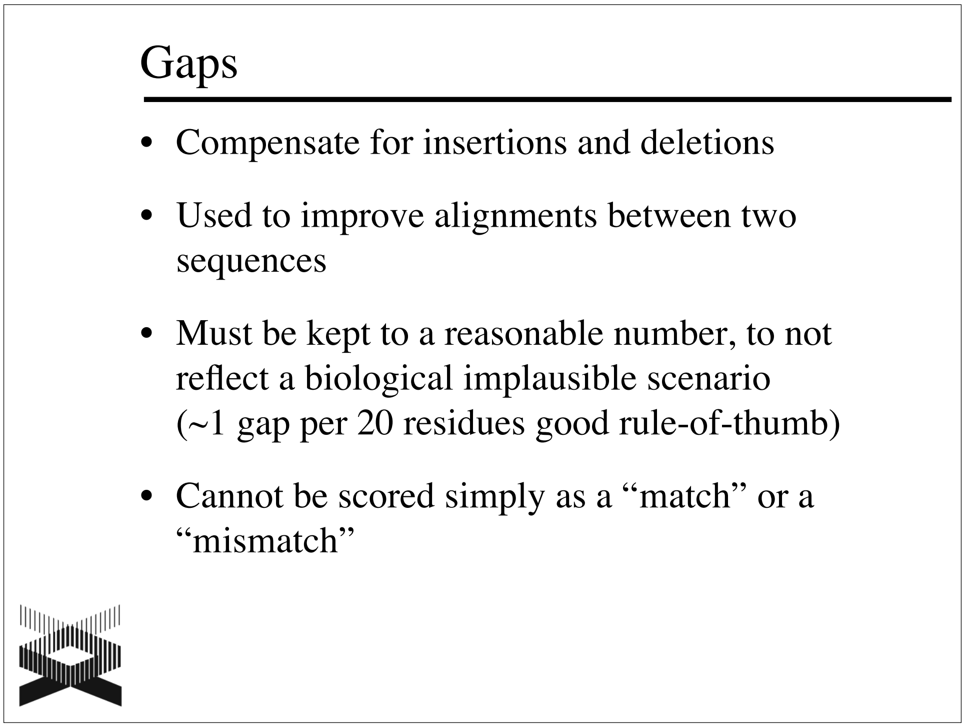
- Specialized matrices
  - Transmembrane proteins
  - Species-specific matrices

Wheeler, 2003



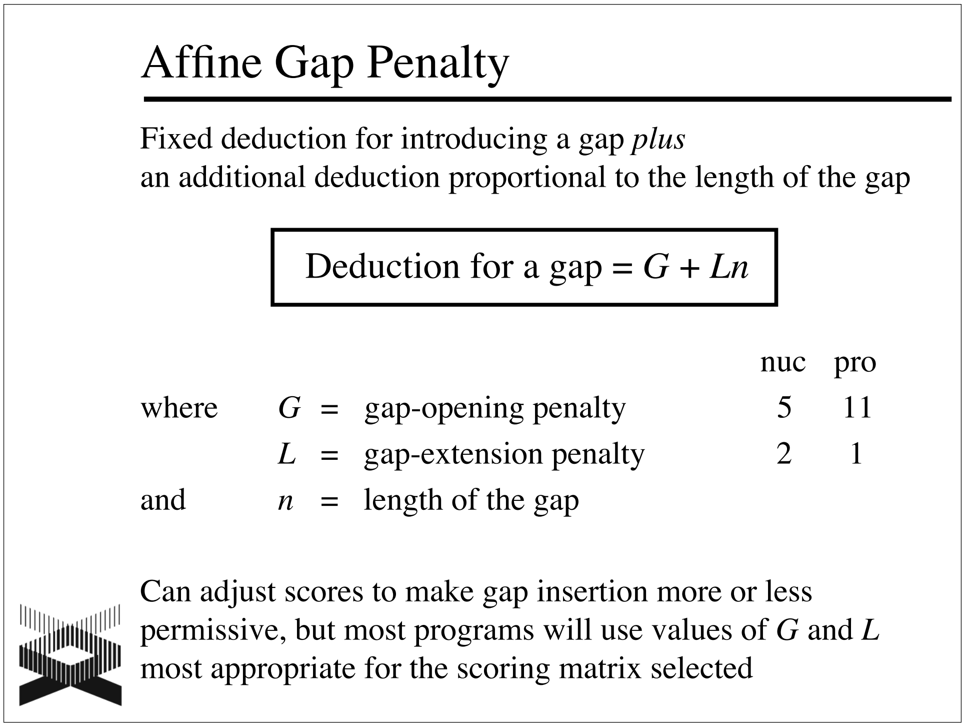
So many matrices...

No single matrix is
the complete answer for
all sequence comparisons



Gaps

- Compensate for insertions and deletions
- Used to improve alignments between two sequences
- 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"



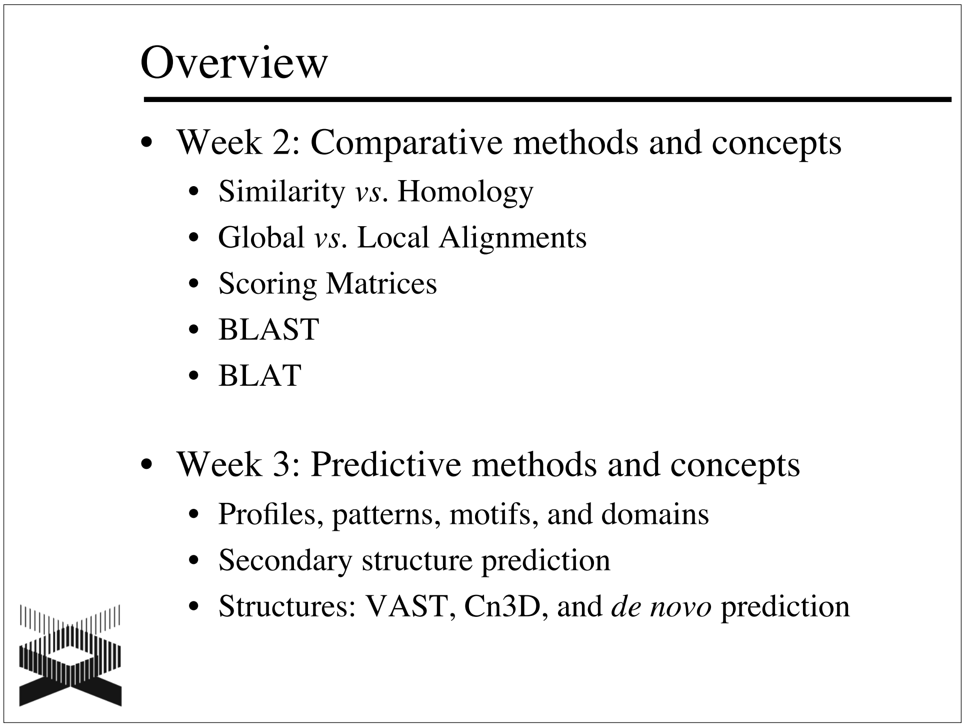
Affine Gap Penalty

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

$$\boxed{\text{Deduction for a gap} = G + Ln}$$

				nuc	pro
where	*G*	=	gap-opening penalty	5	11
	L	=	gap-extension penalty	2	1
and	*n*	=	length of the gap		

Can adjust scores to make gap insertion more or less permissive, but most programs will use values of *G* and *L* most appropriate for the scoring matrix selected



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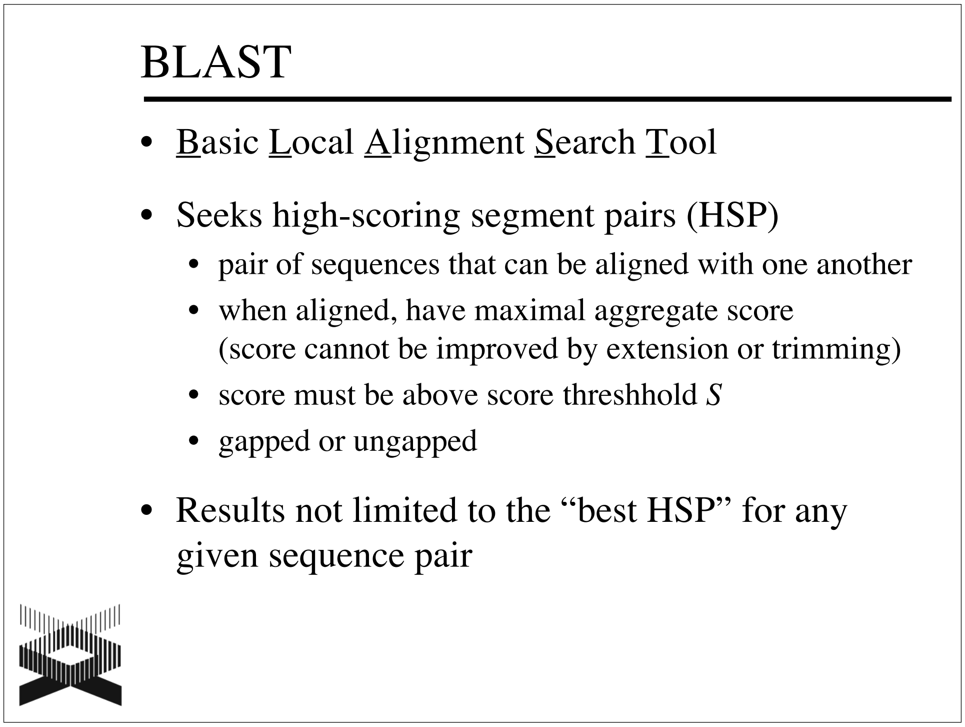


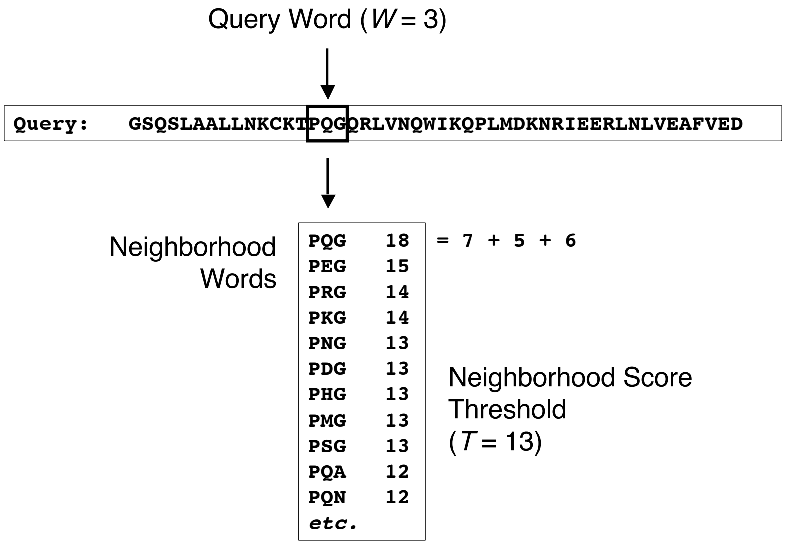
BLAST

- 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 ($W = 3$)

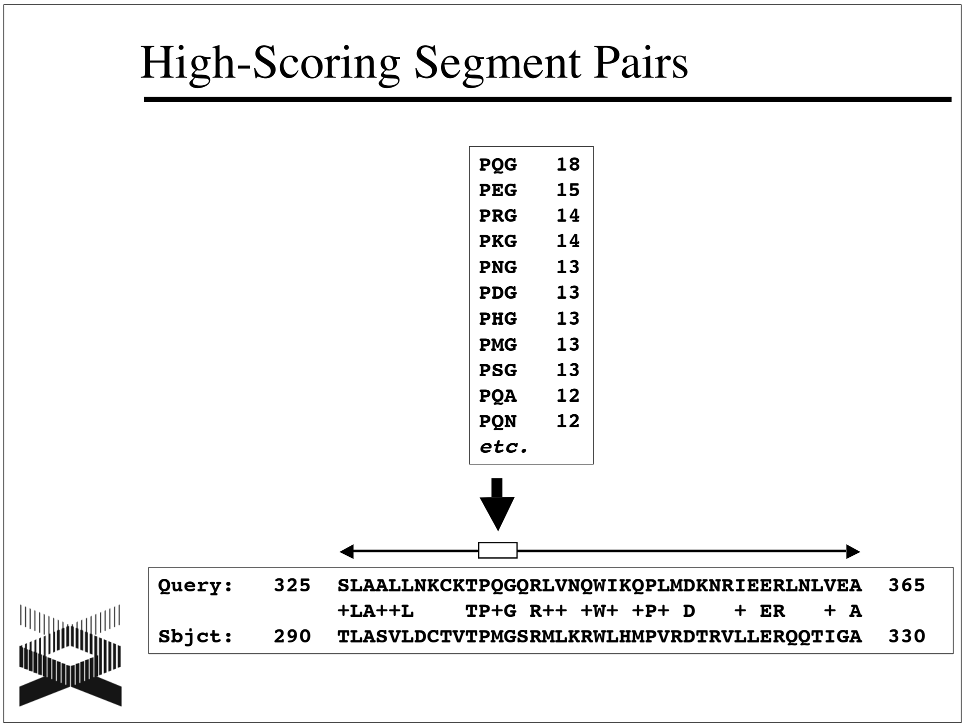
```
Query:   GSQSLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVED
```

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

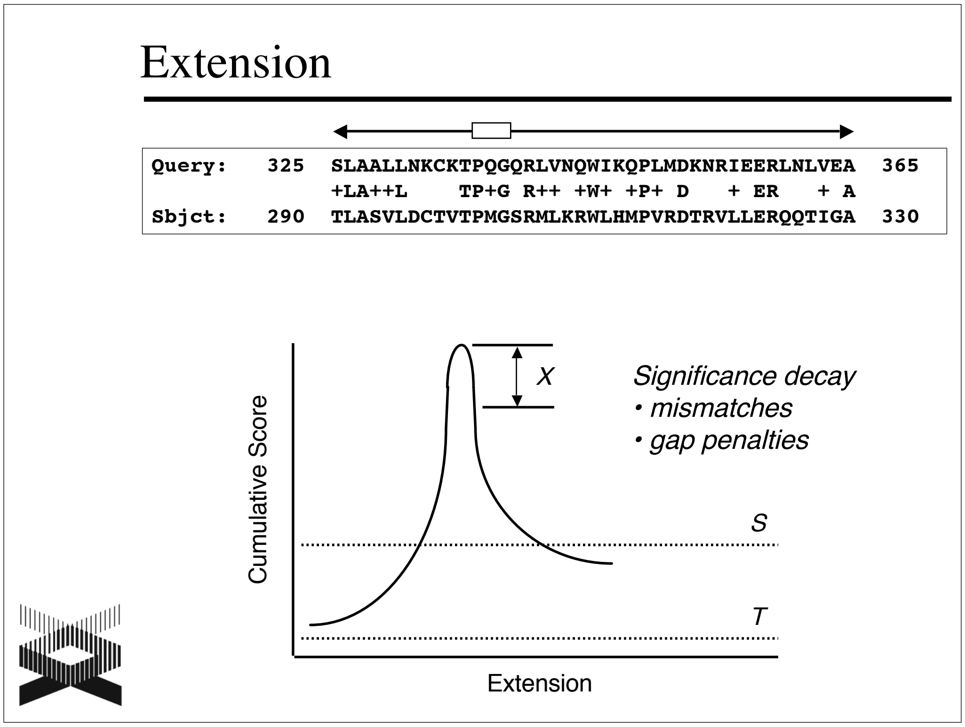
Neighborhood Score Threshold ($T = 13$)



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.	

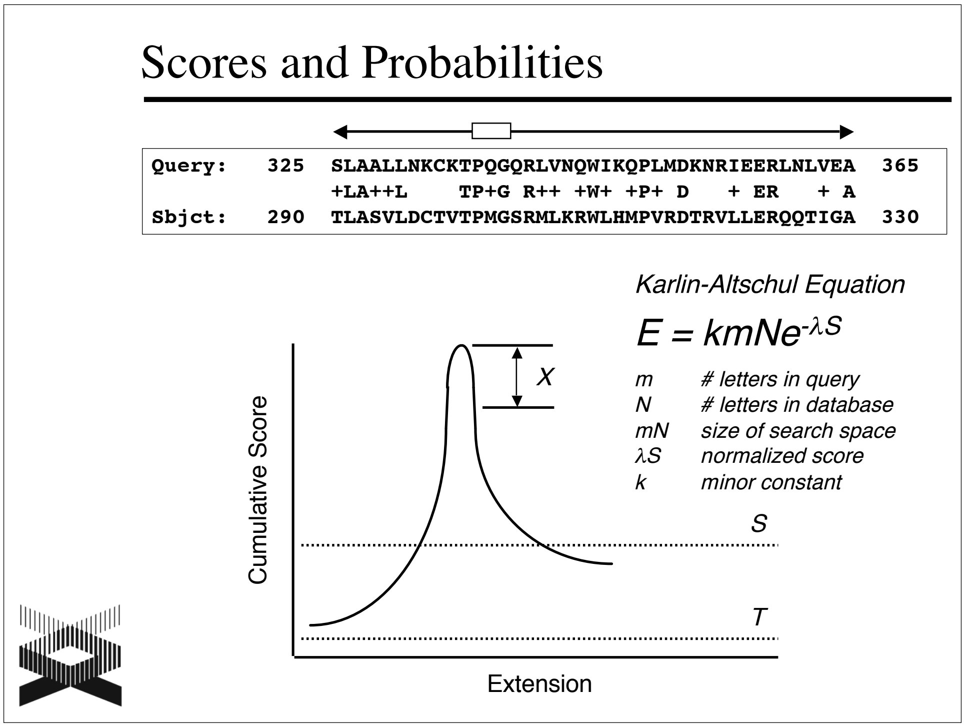
```
Query:   325  SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA  365
              +LA++L     TP+G R++ +W+ +P+ D    + ER    + A
Sbjct:   290  TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA  330
```



Extension

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

Significance decay
- *mismatches*
- *gap penalties*



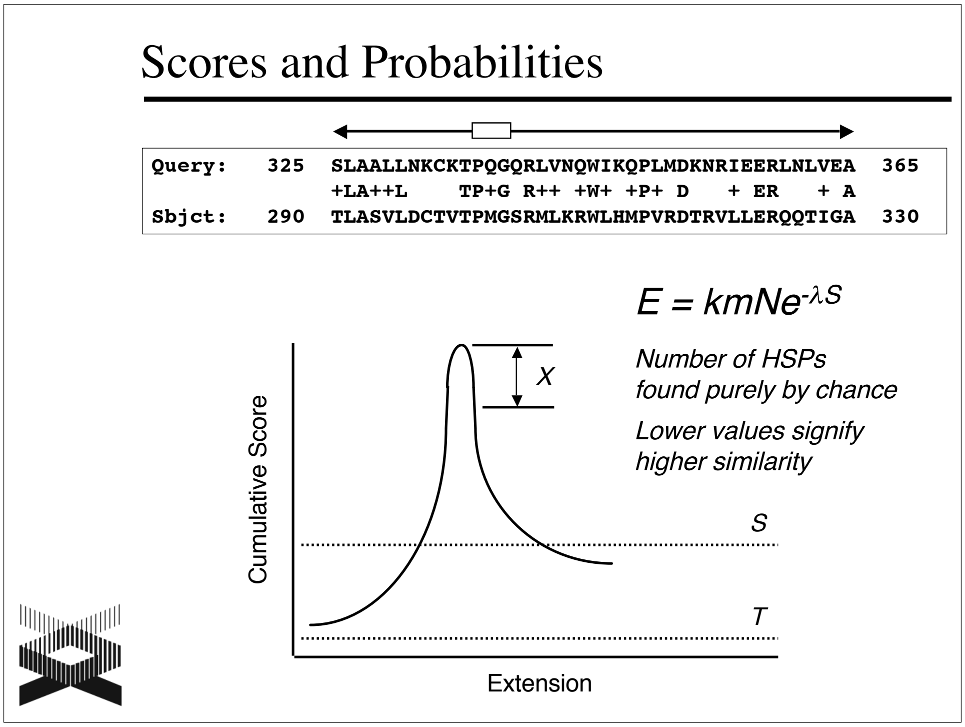
Scores and Probabilities

```
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
```

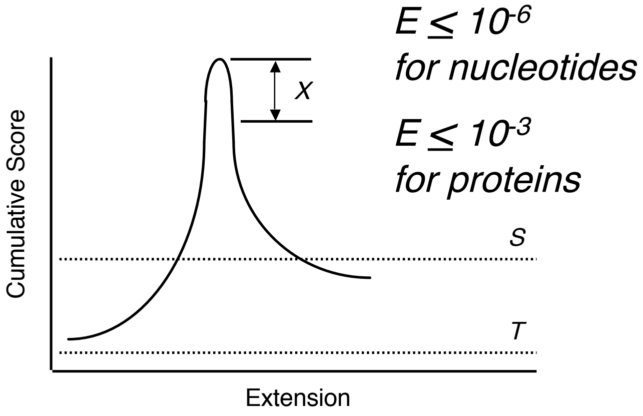
$E = kmNe^{-\lambda S}$

Number of HSPs found purely by chance

Lower values signify higher similarity

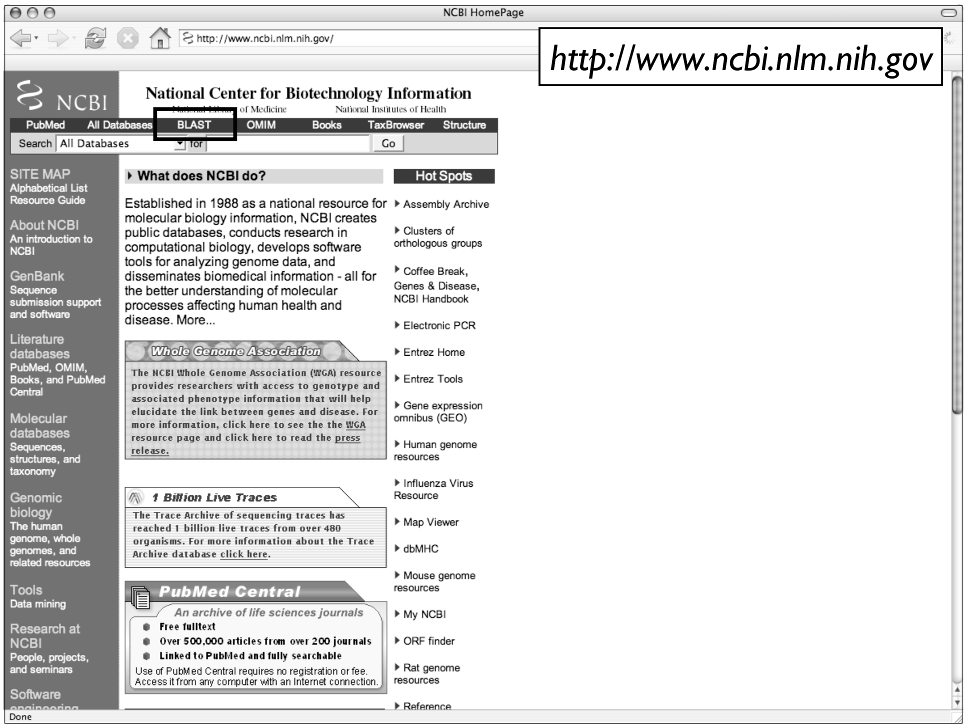
Scores and Probabilities

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



E ≤ 10⁻⁶
for nucleotides

E ≤ 10⁻³
for proteins



http://www.ncbi.nlm.nih.gov

National Center for Biotechnology Information
National Library of Medicine   National Institutes of Health

SITE MAP
Alphabetical List
Resource Guide

About NCBI
An introduction to NCBI

GenBank
Sequence submission support and software

Literature databases
PubMed, OMIM, Books, and PubMed Central

Molecular databases
Sequences, structures, and taxonomy

Genomic biology
The human genome, whole genomes, and related resources

Tools
Data mining

Research at NCBI
People, projects, and seminars

Software engineering

What does NCBI do?

Established in 1988 as a national resource for molecular biology information, NCBI creates public databases, conducts research in computational biology, develops software tools for analyzing genome data, and disseminates biomedical information - all for the better understanding of molecular processes affecting human health and disease. More...

Whole Genome Association

The NCBI Whole Genome Association (WGA) resource provides researchers with access to genotype and associated phenotype information that will help elucidate the link between genes and disease. For more information, click here to see the the WGA resource page and click here to read the press release.

1 Billion Live Traces

The Trace Archive of sequencing traces has reached 1 billion live traces from over 480 organisms. For more information about the Trace Archive database click here.

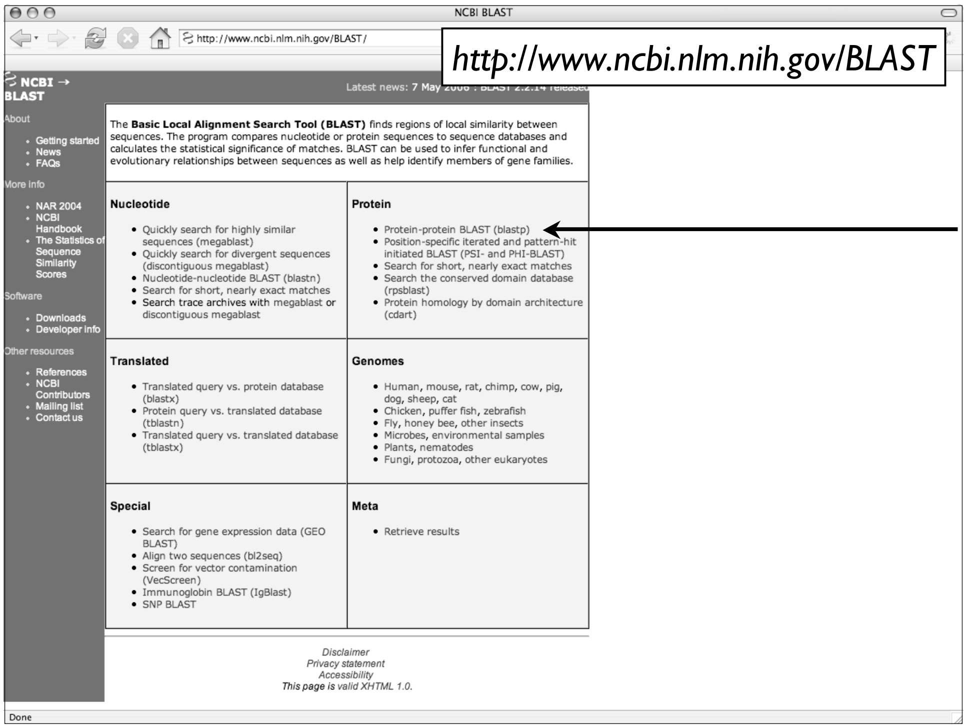
PubMed Central

An archive of life sciences journals

- Free fulltext
- Over 500,000 articles from over 200 journals
- Linked to PubMed and fully searchable

Use of PubMed Central requires no registration or fee. Access it from any computer with an Internet connection.

- Assembly Archive
- Clusters of orthologous groups
- Coffee Break, Genes & Disease, NCBI Handbook
- Electronic PCR
- Entrez Home
- Entrez Tools
- Gene expression omnibus (GEO)
- Human genome resources
- Influenza Virus Resource
- Map Viewer
- dbMHC
- Mouse genome resources
- My NCBI
- ORF finder
- Rat genome resources
- Reference



http://www.ncbi.nlm.nih.gov/BLAST

The **Basic Local Alignment Search Tool (BLAST)** finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Nucleotide

- Quickly search for highly similar sequences (megablast)
- Quickly search for divergent sequences (discontiguous megablast)
- Nucleotide-nucleotide BLAST (blastn)
- Search for short, nearly exact matches
- Search trace archives with megablast or discontiguous megablast

Protein

- Protein-protein BLAST (blastp)
- Position-specific iterated and pattern-hit initiated BLAST (PSI- and PHI-BLAST)
- Search for short, nearly exact matches
- Search the conserved domain database (rpsblast)
- Protein homology by domain architecture (cdart)

Translated

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

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

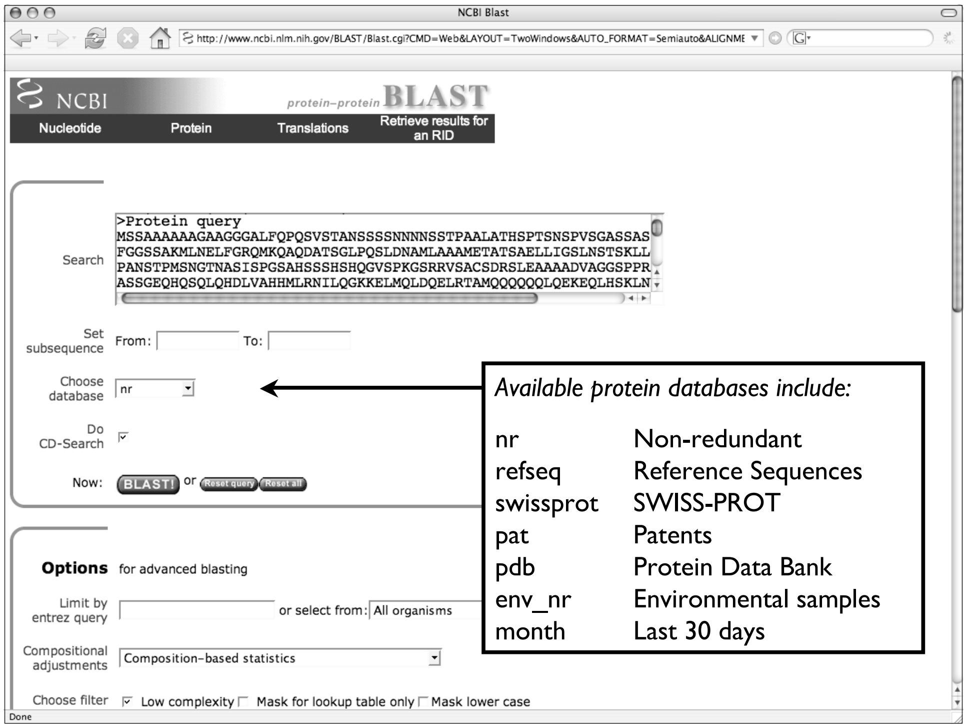
Special

- Search for gene expression data (GEO BLAST)
- Align two sequences (bl2seq)
- Screen for vector contamination (VecScreen)
- Immunoglobin BLAST (IgBlast)
- SNP BLAST

Meta

- Retrieve results

Disclaimer
Privacy statement
Accessibility
This page is valid XHTML 1.0.



NCBI Blast
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&LAYOUT=TwoWindows&AUTO_FORMAT=Semiauto&ALIGNME
NCBI
protein–protein BLAST
Nucleotide
Protein
Translations
Retrieve results for an RID
BLAST!
Reset query
Reset all
Done

Search

```
>Protein query
MSSAAAAAAGAAGGGALFQPQSVSTANSSSSNNNNSSTPAALATHSPTSNSPVSGASSAS
FGGSSAKMLNELFGRQMKQAQDATSGLPQSLDNAMLAAAMETATSAELLIGSLNSTSKLL
PANSTPMSNGTNASISPGSAHSSSHSHQGVSPKGSRRVSACSDRSLEAAAADVAGGSPPR
ASSGEQHQSQLQHDLVAHHMLRNILQGKKELMQLDQELRTAMQQQQQQLQEKEQLHSKLN
```

Set subsequence From: To:

Choose database: nr

Do CD-Search ☑

Now: BLAST! or Reset query Reset all

Options for advanced blasting

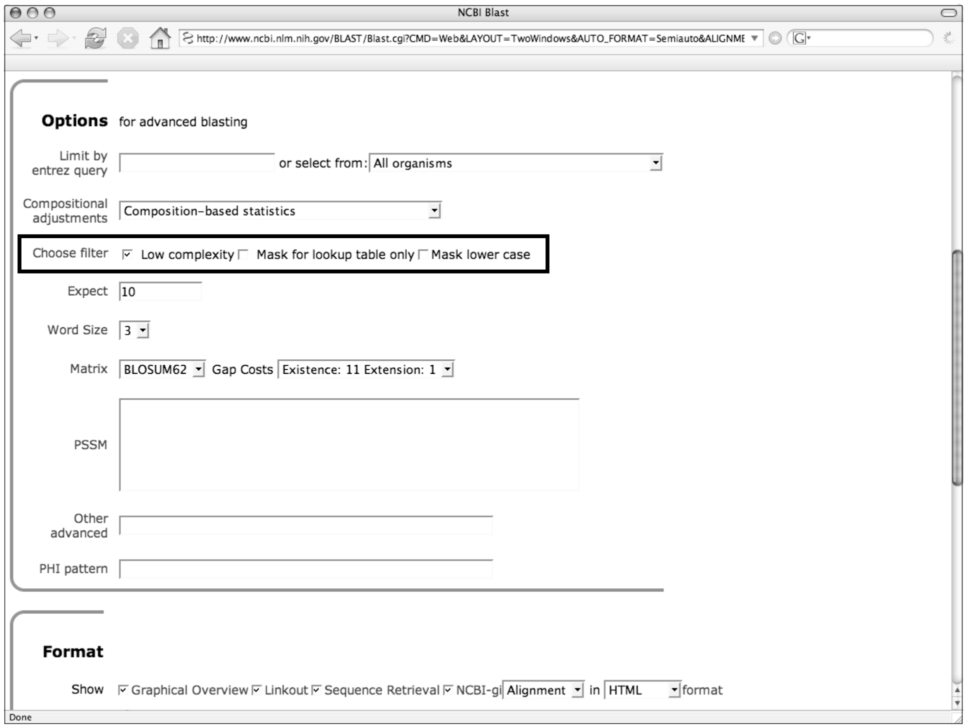
Limit by entrez query — or select from: All organisms

Compositional adjustments: Composition-based statistics

Choose filter ☑ Low complexity □ Mask for lookup table only □ Mask lower case

Available protein databases include:

nr	Non-redundant
refseq	Reference Sequences
swissprot	SWISS-PROT
pat	Patents
pdb	Protein Data Bank
env_nr	Environmental samples
month	Last 30 days



NCBI Blast

http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&LAYOUT=TwoWindows&AUTO_FORMAT=Semiauto&ALIGNME

Options for advanced blasting

Limit by entrez query: ________ or select from: All organisms

Compositional adjustments: Composition-based statistics

Choose filter: ☑ Low complexity □ Mask for lookup table only □ Mask lower case

Expect: 10

Word Size: 3

Matrix: BLOSUM62 Gap Costs: Existence: 11 Extension: 1

PSSM:

Other advanced:

PHI pattern:

Format

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

Done

Low-Complexity Regions

Defined as regions of biased composition

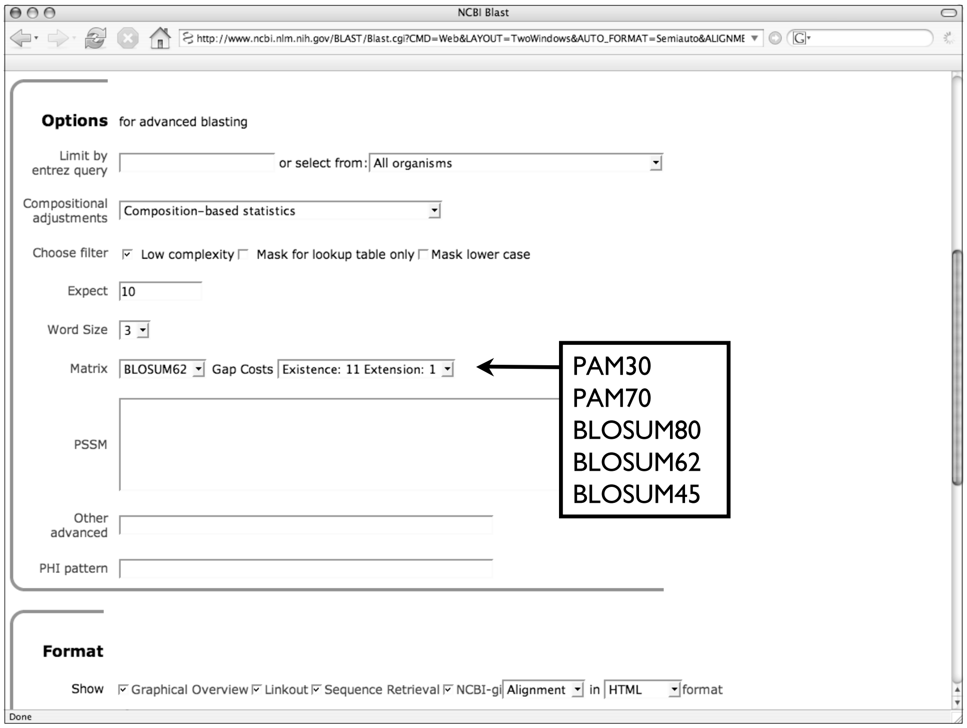
- Homopolymeric runs
- Short-period repeats
- Subtle over-representation of several residues

```
>gi|20455478|sp|P50553|ASC1_HUMAN Achaete-scute homolog 1 (HASH1)
MESSAKMESGGAGQQPQPQPQQPFLPPAACFFATAAAAAAAAAAAAAQSAQQQQQQQQQQQQAPQLRPAA
DGQPSGGGHKSAPKQVKRQRSSSPELMRCKRRLNFSGFGYSLPQQQPAAVARRNERERNRVKLVNLGFAT
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 (and usually enabled by default)


NCBI Blast
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&LAYOUT=TwoWindows&AUTO_FORMAT=Semiauto&ALIGNME
Done

Options for advanced blasting

Limit by entrez query: ____ or select from: All organisms

Compositional adjustments: Composition-based statistics

Choose filter: ☑ Low complexity ☐ Mask for lookup table only ☐ Mask lower case

Expect: 10

Word Size: 3

Matrix: BLOSUM62 Gap Costs: Existence: 11 Extension: 1

PSSM

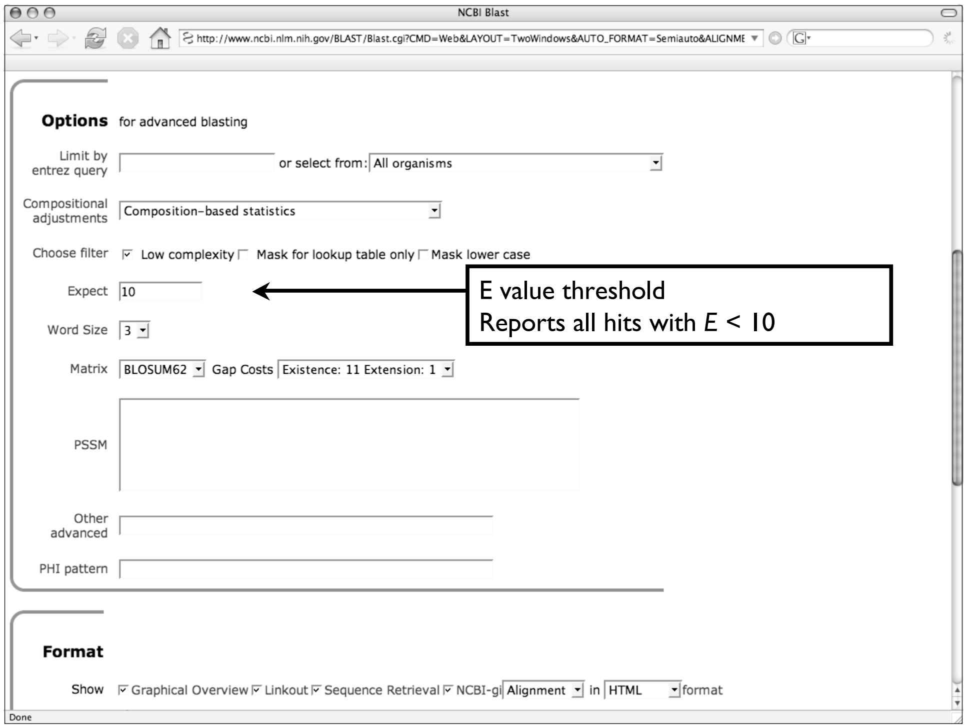
Other advanced

PHI pattern

Format

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

PAM30
PAM70
BLOSUM80
BLOSUM62
BLOSUM45



NCBI Blast
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&LAYOUT=TwoWindows&AUTO_FORMAT=Semiauto&ALIGNME
Done

Options for advanced blasting

Limit by entrez query: or select from: All organisms

Compositional adjustments: Composition-based statistics

Choose filter: ☑ Low complexity □ Mask for lookup table only □ Mask lower case

Expect: 10

E value threshold
Reports all hits with $E < 10$

Word Size: 3

Matrix: BLOSUM62 Gap Costs: Existence: 11 Extension: 1

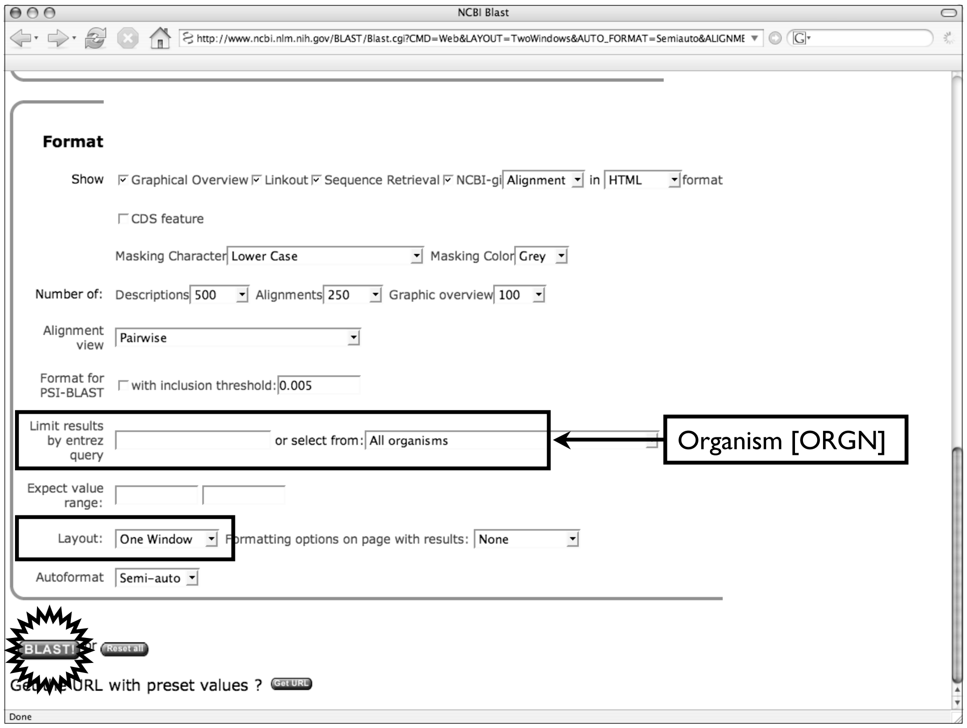
PSSM

Other advanced

PHI pattern

Format

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



NCBI Blast
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&LAYOUT=TwoWindows&AUTO_FORMAT=Semiauto&ALIGNME
BLAST!
Reset all
Get URL
Done

Format

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

□ CDS feature

Masking Character: Lower Case Masking Color: Grey

Number of: Descriptions 500 Alignments 250 Graphic overview 100

Alignment view: Pairwise

Format for PSI-BLAST: □ with inclusion threshold: 0.005

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

Organism [ORGN]

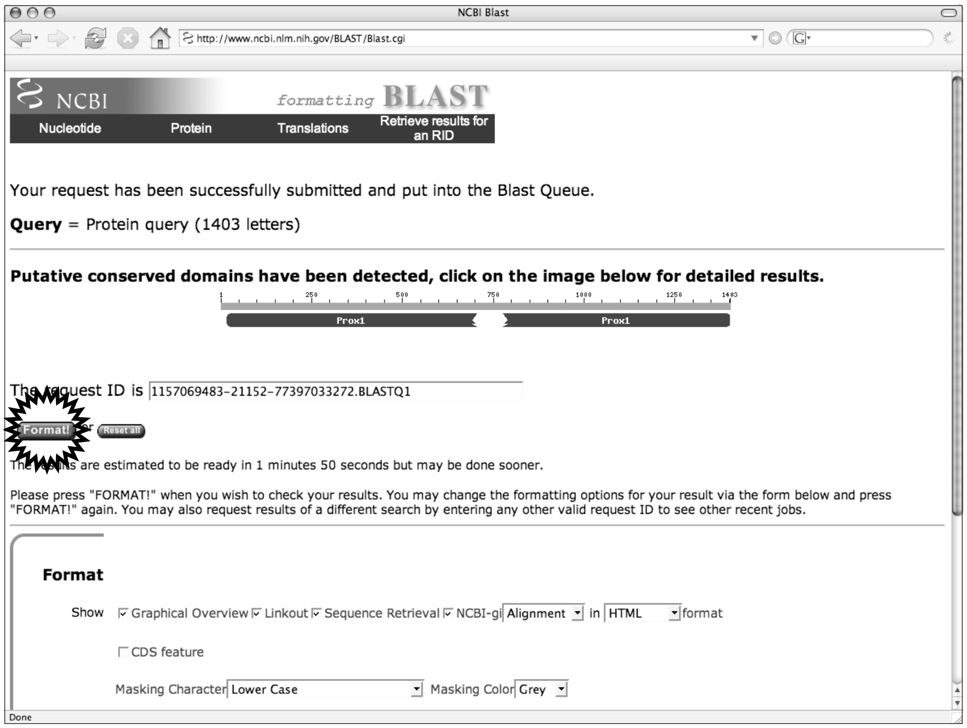
Expect value range:

Layout: One Window Formatting options on page with results: None

Autoformat: Semi-auto

BLAST! or Reset all

Get the URL with preset values ?



Your request has been successfully submitted and put into the Blast Queue.

Query = Protein query (1403 letters)

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

The request ID is 1157069483-21152-77397033272.BLASTQ1

Format! or Reset all

The results are estimated to be ready in 1 minutes 50 seconds but may be done sooner.

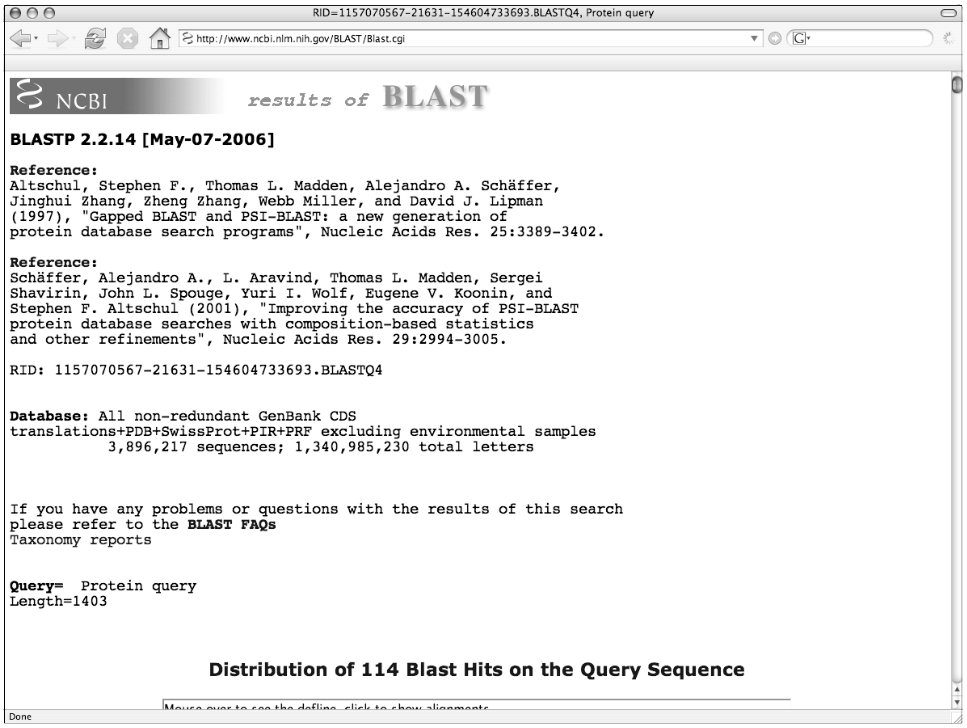
Please press "FORMAT!" when you wish to check your results. You may change the formatting options for your result via the form below and press "FORMAT!" again. You may also request results of a different search by entering any other valid request ID to see other recent jobs.

Format

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

☐ CDS feature

Masking Character Lower Case Masking Color Grey

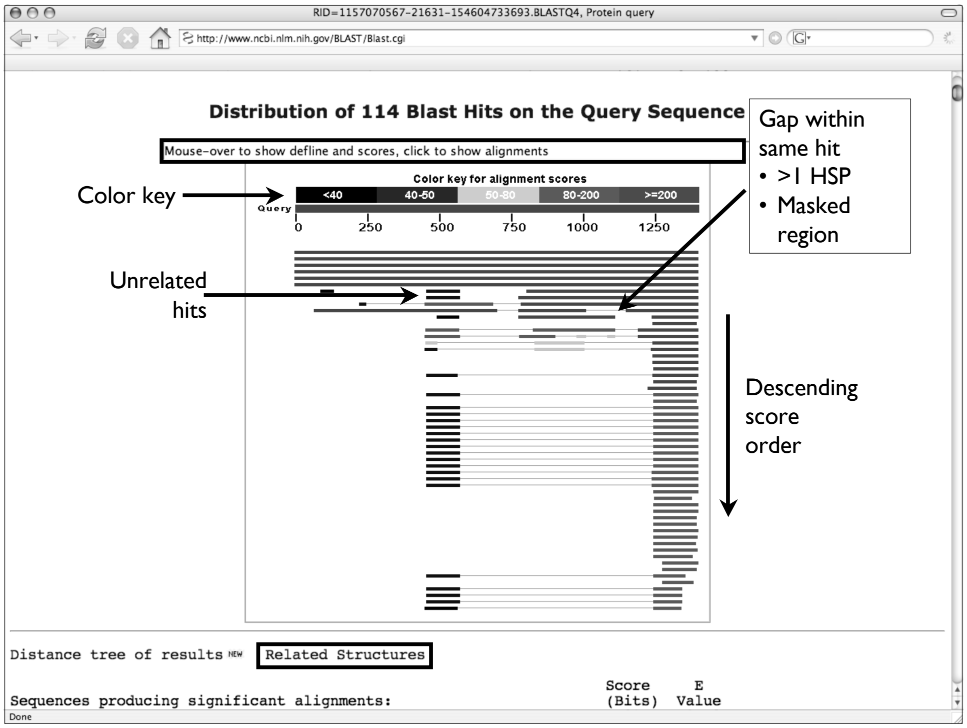

RID=1157070567-21631-154604733693.BLASTQ4, Protein query
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi
NCBI
results of BLAST
Done

BLASTP 2.2.14 [May-07-2006]

```
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: 1157070567-21631-154604733693.BLASTQ4


Database: All non-redundant GenBank CDS
translations+PDB+SwissProt+PIR+PRF excluding environmental samples
           3,896,217 sequences; 1,340,985,230 total letters



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


Query=  Protein query
Length=1403
```

Distribution of 114 Blast Hits on the Query Sequence

Mouse over to see the defline, click to show alignments



RID=1157070567-21631-154604733693.BLASTQ4, Protein query

http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi

Distribution of 114 Blast Hits on the Query Sequence

Mouse-over to show defline and scores, click to show alignments

Color key

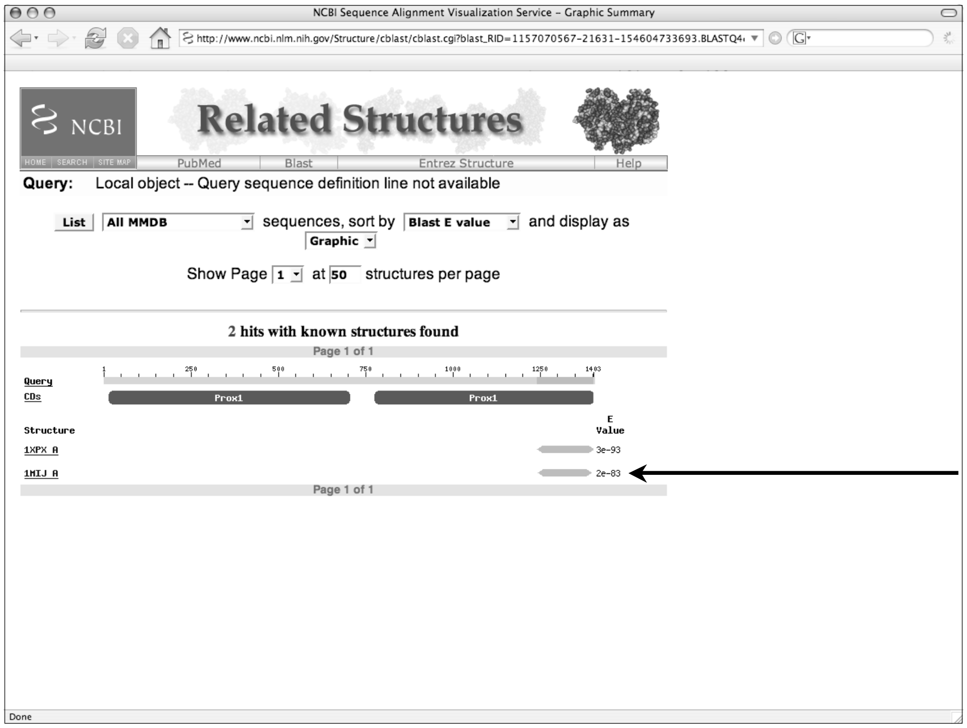
Unrelated hits

Gap within same hit
- >1 HSP
- Masked region

Descending score order

```
Distance tree of results NEW   Related Structures

                                                   Score     E
Sequences producing significant alignments:        (Bits)  Value
```

Done



NCBI Sequence Alignment Visualization Service – Graphic Summary

http://www.ncbi.nlm.nih.gov/Structure/cblast/cblast.cgi?blast_RID=1157070567-21631-154604733693.BLASTQ4

Query: Local object -- Query sequence definition line not available

List | All MMDB | sequences, sort by | Blast E value | and display as | Graphic

Show Page 1 at 50 structures per page

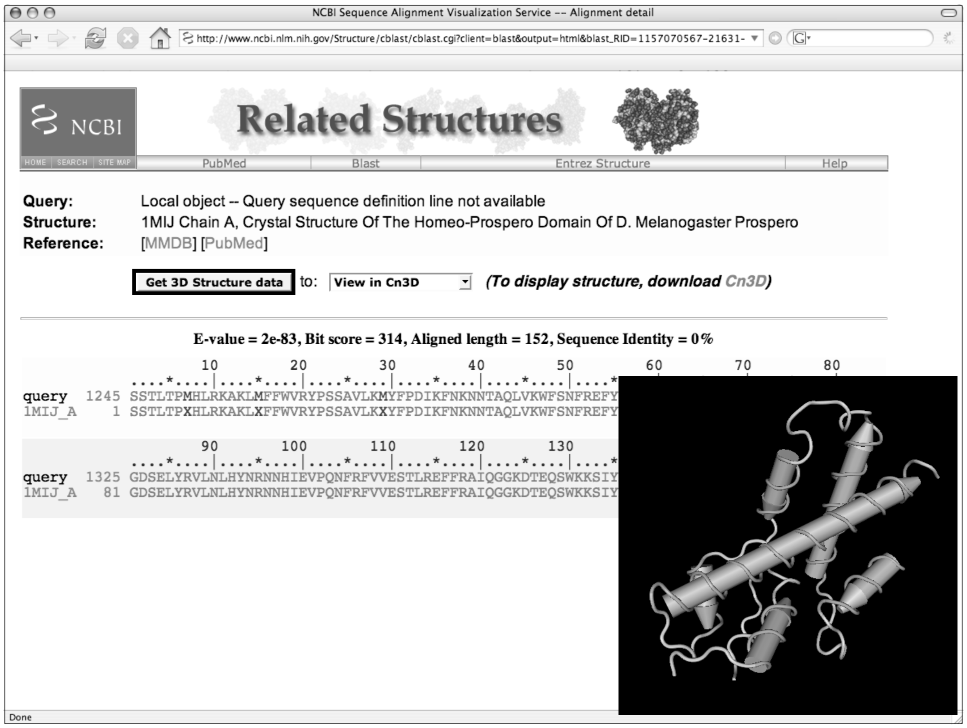
2 hits with known structures found

Page 1 of 1

Structure	E Value
1XPX_A	3e-93
1MIJ_A	2e-83

Page 1 of 1

Done



NCBI Sequence Alignment Visualization Service -- Alignment detail

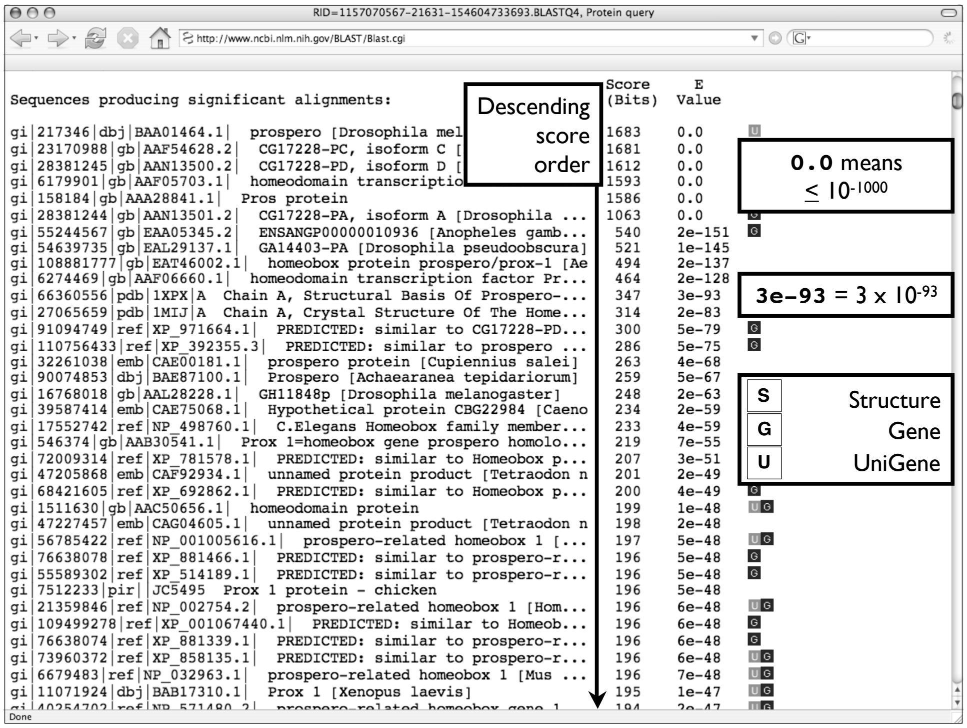
http://www.ncbi.nlm.nih.gov/Structure/cblast/cblast.cgi?client=blast&output=html&blast_RID=1157070567-21631-

Query: Local object -- Query sequence definition line not available
Structure: 1MIJ Chain A, Crystal Structure Of The Homeo-Prospero Domain Of D. Melanogaster Prospero
Reference: [MMDB] [PubMed]

(To display structure, download Cn3D)

E-value = 2e-83, Bit score = 314, Aligned length = 152, Sequence Identity = 0%

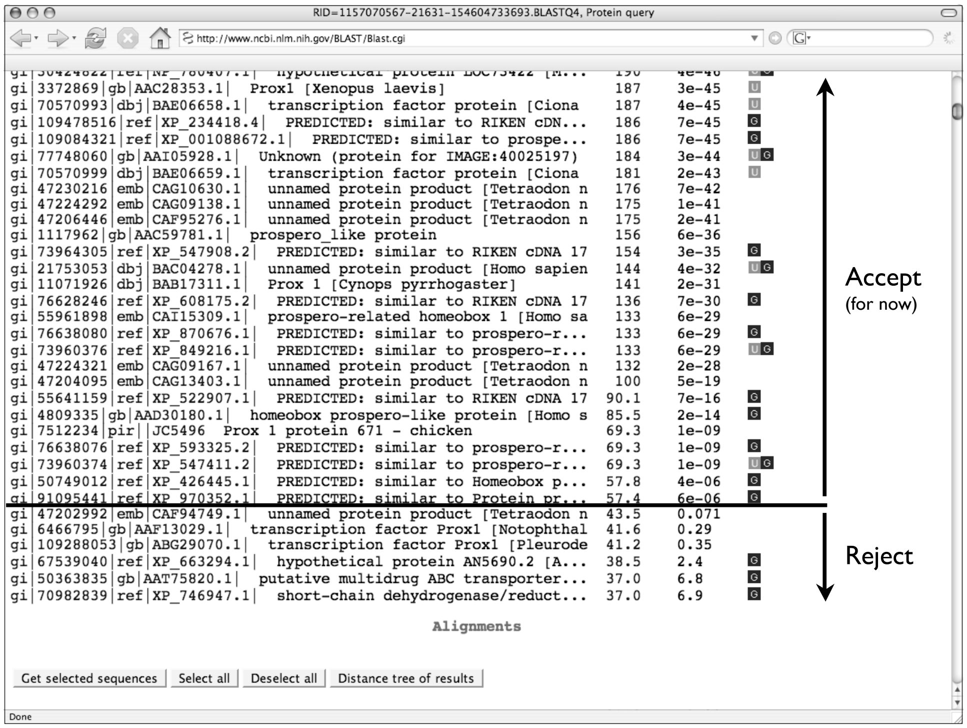
```
                  10        20        30        40        50        60        70        80
          ....*....|....*....|....*....|....*....|....*....|....*
query  1245 SSTLTPMHLRKAKLMFFWVRYPSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFY
1MIJ_A    1 SSTLTPXHLRKAKLXFFWVRYPSSAVLKXYFPDIKFNKNNTAQLVKWFSNFREFY

                  90       100       110       120       130
          ....*....|....*....|....*....|....*....|....*....|....*
query  1325 GDSELYRVLNLHYNRNNHIEVPQNFRFVVESTLREFFRAIQGGKDTEQSWKKSIY
1MIJ_A   81 GDSELYRVLNLHYNRNNHIEVPQNFRFVVESTLREFFRAIQGGKDTEQSWKKSIY
```



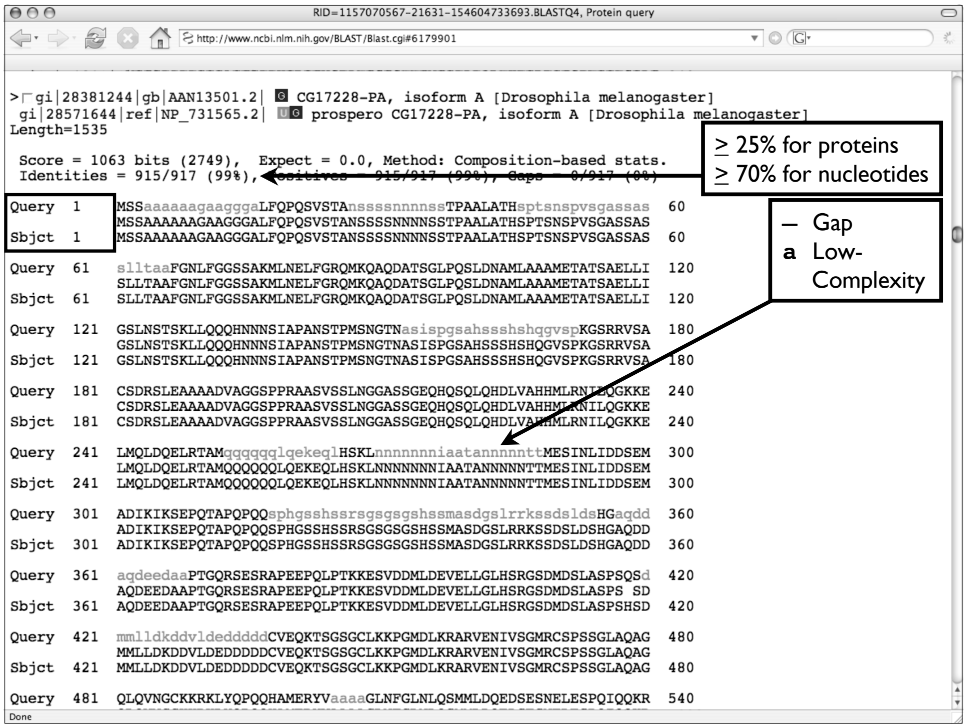
RID=1157070567-21631-154604733693.BLASTQ4, Protein query

http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi

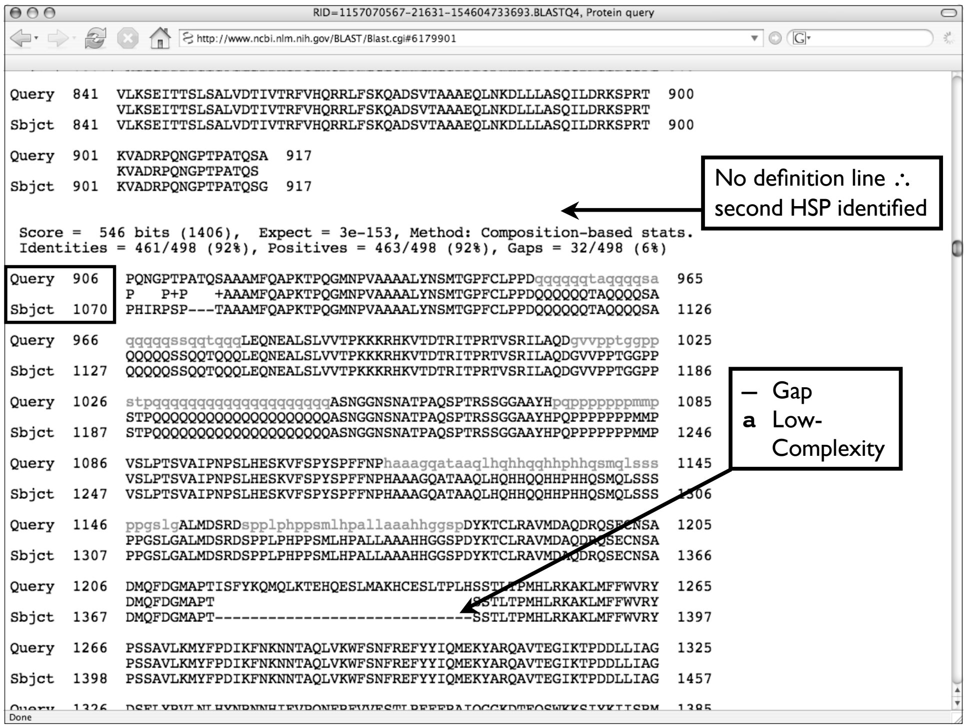
```
                                                                Score    E
Sequences producing significant alignments:                     (Bits)  Value

gi|217346|dbj|BAA01464.1|  prospero [Drosophila mel              1683    0.0     U
gi|23170988|gb|AAF54628.2|  CG17228-PC, isoform C [              1681    0.0
gi|28381245|gb|AAN13500.2|  CG17228-PD, isoform D [              1612    0.0
gi|6179901|gb|AAF05703.1|  homeodomain transcriptio              1593    0.0
gi|158184|gb|AAA28841.1|  Pros protein                           1586    0.0
gi|28381244|gb|AAN13501.2|  CG17228-PA, isoform A [Drosophila ...  1063    0.0     G
gi|55244567|gb|EAA05345.2|  ENSANGP00000010936 [Anopheles gamb...   540    2e-151  G
gi|54639735|gb|EAL29137.1|  GA14403-PA [Drosophila pseudoobscura]   521    1e-145
gi|108881777|gb|EAT46002.1|  homeobox protein prospero/prox-1 [Ae   494    2e-137
gi|6274469|gb|AAF06660.1|  homeodomain transcription factor Pr...   464    2e-128
gi|66360556|pdb|1XPX|A  Chain A, Structural Basis Of Prospero-...   347    3e-93
gi|27065659|pdb|1MIJ|A  Chain A, Crystal Structure Of The Home...   314    2e-83
gi|91094749|ref|XP_971664.1|  PREDICTED: similar to CG17228-PD...   300    5e-79   G
gi|110756433|ref|XP_392355.3|  PREDICTED: similar to prospero ...   286    5e-75   G
gi|32261038|emb|CAE00181.1|  prospero protein [Cupiennius salei]    263    4e-68
gi|90074853|dbj|BAE87100.1|  Prospero [Achaearanea tepidariorum]    259    5e-67
gi|16768018|gb|AAL28228.1|  GH11848p [Drosophila melanogaster]      248    2e-63
gi|39587414|emb|CAE75068.1|  Hypothetical protein CBG22984 [Caeno   234    2e-59
gi|17552742|ref|NP_498760.1|  C.Elegans Homeobox family member...   233    4e-59
gi|546374|gb|AAB30541.1|  Prox 1=homeobox gene prospero homolo...   219    7e-55
gi|72009314|ref|XP_781578.1|  PREDICTED: similar to Homeobox p...   207    3e-51
gi|47205868|emb|CAF92934.1|  unnamed protein product [Tetraodon n   201    2e-49
gi|68421605|ref|XP_692862.1|  PREDICTED: similar to Homeobox p...   200    4e-49   G
gi|1511630|gb|AAC50656.1|  homeodomain protein                      199    1e-48   UG
gi|47227457|emb|CAG04605.1|  unnamed protein product [Tetraodon n   198    2e-48
gi|56785422|ref|NP_001005616.1|  prospero-related homeobox 1 [...   197    5e-48   UG
gi|76638078|ref|XP_881466.1|  PREDICTED: similar to prospero-r...   196    5e-48   G
gi|55589302|ref|XP_514189.1|  PREDICTED: similar to prospero-r...   196    5e-48   G
gi|7512233|pir||JC5495  Prox 1 protein - chicken                    196    5e-48
gi|21359846|ref|NP_002754.2|  prospero-related homeobox 1 [Hom...   196    6e-48   UG
gi|109499278|ref|XP_001067440.1|  PREDICTED: similar to Homeob...   196    6e-48   G
gi|76638074|ref|XP_881339.1|  PREDICTED: similar to prospero-r...   196    6e-48   G
gi|73960372|ref|XP_858135.1|  PREDICTED: similar to prospero-r...   196    6e-48   UG
gi|6679483|ref|NP_032963.1|  prospero-related homeobox 1 [Mus ...   196    7e-48   UG
gi|11071924|dbj|BAB17310.1|  Prox 1 [Xenopus laevis]                195    1e-47   UG
gi|40254702|ref|NP_571480.2|  prospero-related homeobox gene 1      194    2e-47   UG
```


RID=1157070567-21631-154604733693.BLASTQ4, Protein query
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi
Accept
(for now)
Reject
Get selected sequences
Select all
Deselect all
Distance tree of results
Done

```
gi|30424822|ref|NP_780407.1|  hypothetical protein LOC73422 [M...   190    4e-46   U G
gi|3372869|gb|AAC28353.1|  Prox1 [Xenopus laevis]                   187    3e-45   U
gi|70570993|dbj|BAE06658.1|  transcription factor protein [Ciona    187    4e-45   U
gi|109478516|ref|XP_234418.4|  PREDICTED: similar to RIKEN cDN...   186    7e-45   G
gi|109084321|ref|XP_001088672.1|  PREDICTED: similar to prospe...   186    7e-45   G
gi|77748060|gb|AAI05928.1|  Unknown (protein for IMAGE:40025197)    184    3e-44   U G
gi|70570999|dbj|BAE06659.1|  transcription factor protein [Ciona    181    2e-43   U
gi|47230216|emb|CAG10630.1|  unnamed protein product [Tetraodon n   176    7e-42
gi|47224292|emb|CAG09138.1|  unnamed protein product [Tetraodon n   175    1e-41
gi|47206446|emb|CAF95276.1|  unnamed protein product [Tetraodon n   175    2e-41
gi|1117962|gb|AAC59781.1|  prospero_like protein                    156    6e-36
gi|73964305|ref|XP_547908.2|  PREDICTED: similar to RIKEN cDNA 17   154    3e-35   G
gi|21753053|dbj|BAC04278.1|  unnamed protein product [Homo sapien   144    4e-32   U G
gi|11071926|dbj|BAB17311.1|  Prox 1 [Cynops pyrrhogaster]           141    2e-31
gi|76628246|ref|XP_608175.2|  PREDICTED: similar to RIKEN cDNA 17   136    7e-30   G
gi|55961898|emb|CAI15309.1|  prospero-related homeobox 1 [Homo sa   133    6e-29
gi|76638080|ref|XP_870676.1|  PREDICTED: similar to prospero-r...   133    6e-29   G
gi|73960376|ref|XP_849216.1|  PREDICTED: similar to prospero-r...   133    6e-29   U G
gi|47224321|emb|CAG09167.1|  unnamed protein product [Tetraodon n   132    2e-28
gi|47204095|emb|CAG13403.1|  unnamed protein product [Tetraodon n   100    5e-19
gi|55641159|ref|XP_522907.1|  PREDICTED: similar to RIKEN cDNA 17  90.1    7e-16   G
gi|4809335|gb|AAD30180.1|  homeobox prospero-like protein [Homo s  85.5    2e-14   G
gi|7512234|pir||JC5496  Prox 1 protein 671 - chicken               69.3    1e-09
gi|76638076|ref|XP_593325.2|  PREDICTED: similar to prospero-r...  69.3    1e-09   G
gi|73960374|ref|XP_547411.2|  PREDICTED: similar to prospero-r...  69.3    1e-09   U G
gi|50749012|ref|XP_426445.1|  PREDICTED: similar to Homeobox p...  57.8    4e-06   G
gi|91095441|ref|XP_970352.1|  PREDICTED: similar to Protein pr...  57.4    6e-06   G
gi|47202992|emb|CAF94749.1|  unnamed protein product [Tetraodon n  43.5    0.071
gi|6466795|gb|AAF13029.1|  transcription factor Prox1 [Notophthal  41.6    0.29
gi|109288053|gb|ABG29070.1|  transcription factor Prox1 [Pleurode  41.2    0.35
gi|67539040|ref|XP_663294.1|  hypothetical protein AN5690.2 [A...  38.5    2.4     G
gi|50363835|gb|AAT75820.1|  putative multidrug ABC transporter...  37.0    6.8     G
gi|70982839|ref|XP_746947.1|  short-chain dehydrogenase/reduct...  37.0    6.9     G

                              Alignments
```


RID=1157070567-21631-154604733693.BLASTQ4, Protein query
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi#6179901
≥ 25% for proteins
≥ 70% for nucleotides
– Gap
a Low-Complexity
Done

```
>gi|28381244|gb|AAN13501.2| G CG17228-PA, isoform A [Drosophila melanogaster]
 gi|28571644|ref|NP_731565.2| U G prospero CG17228-PA, isoform A [Drosophila melanogaster]
Length=1535

 Score = 1063 bits (2749),  Expect = 0.0, Method: Composition-based stats.
 Identities = 915/917 (99%), Positives = 915/917 (99%), Gaps = 0/917 (0%)

Query  1    MSSaaaaaagaaggaLFQPQSVSTAnssssnnnnssTPAALATHsptsnspvsgassas  60
            MSSAAAAAAGAAGGALFQPQSVSTANSSSSNNNNSSTPAALATHSPTSNSPVSGASSAS
Sbjct  1    MSSAAAAAAGAAGGALFQPQSVSTANSSSSNNNNSSTPAALATHSPTSNSPVSGASSAS  60

Query  61   slltaaFGNLFGGSSAKMLNELFGRQMKQAQDATSGLPQSLDNAMLAAAMETATSAELLI  120
            SLLTAAFGNLFGGSSAKMLNELFGRQMKQAQDATSGLPQSLDNAMLAAAMETATSAELLI
Sbjct  61   SLLTAAFGNLFGGSSAKMLNELFGRQMKQAQDATSGLPQSLDNAMLAAAMETATSAELLI  120

Query  121  GSLNSTSKLLQQQHNNNSIAPANSTPMSNGTNasispgsahssshshqgvspKGSRRVSA  180
            GSLNSTSKLLQQQHNNNSIAPANSTPMSNGTNASISPGSAHSSSHSHQGVSPKGSRRVSA
Sbjct  121  GSLNSTSKLLQQQHNNNSIAPANSTPMSNGTNASISPGSAHSSSHSHQGVSPKGSRRVSA  180

Query  181  CSDRSLEAAAADVAGGSPPRAASVSSLNGGASSGEQHQSQLQHDLVAHHMLRNILQGKKE  240
            CSDRSLEAAAADVAGGSPPRAASVSSLNGGASSGEQHQSQLQHDLVAHHMLRNILQGKKE
Sbjct  181  CSDRSLEAAAADVAGGSPPRAASVSSLNGGASSGEQHQSQLQHDLVAHHMLRNILQGKKE  240

Query  241  LMQLDQELRTAMqqqqqqlqekeqlHSKLnnnnnnniaatannnnnttMESINLIDDSEM  300
            LMQLDQELRTAMQQQQQQLQEKEQLHSKLNNNNNNNIAATANNNNNTTMESINLIDDSEM
Sbjct  241  LMQLDQELRTAMQQQQQQLQEKEQLHSKLNNNNNNNIAATANNNNNTTMESINLIDDSEM  300

Query  301  ADIKIKSEPQTAPQPQQsphgsshssrsgsgsgshssmasdgslrrkssdsldsHGaqdd  360
            ADIKIKSEPQTAPQPQQSPHGSSHSSRSGSGSGSHSSMASDGSLRRKSSDSLDSHGAQDD
Sbjct  301  ADIKIKSEPQTAPQPQQSPHGSSHSSRSGSGSGSHSSMASDGSLRRKSSDSLDSHGAQDD  360

Query  361  aqdeedaaPTGQRSESRAPEEPQLPTKKESVDDMLDEVELLGLHSRGSDMDSLASPSQSd  420
            AQDEEDAAPTGQRSESRAPEEPQLPTKKESVDDMLDEVELLGLHSRGSDMDSLASPS SD
Sbjct  361  AQDEEDAAPTGQRSESRAPEEPQLPTKKESVDDMLDEVELLGLHSRGSDMDSLASPSHSD  420

Query  421  mmlldkddvldedddddCVEQKTSGSGCLKKPGMDLKRARVENIVSGMRCSPSSGLAQAG  480
            MMLLDKDDVLDEDDDDDCVEQKTSGSGCLKKPGMDLKRARVENIVSGMRCSPSSGLAQAG
Sbjct  421  MMLLDKDDVLDEDDDDDCVEQKTSGSGCLKKPGMDLKRARVENIVSGMRCSPSSGLAQAG  480

Query  481  QLQVNGCKKRKLYQPQQHAMERYVaaaaGLNFGLNLQSMMLDQEDSESNELESPQIQQKR  540
```



RID=1157070567-21631-154604733693.BLASTQ4, Protein query
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi#6179901
Done

```
Query  841   VLKSEITTSLSALVDTIVTRFVHQRRLFSKQADSVTAAAEQLNKDLLLASQILDRKSPRT  900
             VLKSEITTSLSALVDTIVTRFVHQRRLFSKQADSVTAAAEQLNKDLLLASQILDRKSPRT
Sbjct  841   VLKSEITTSLSALVDTIVTRFVHQRRLFSKQADSVTAAAEQLNKDLLLASQILDRKSPRT  900

Query  901   KVADRPQNGPTPATQSA  917
             KVADRPQNGPTPATQS
Sbjct  901   KVADRPQNGPTPATQSG  917


 Score =  546 bits (1406),  Expect = 3e-153, Method: Composition-based stats.
 Identities = 461/498 (92%), Positives = 463/498 (92%), Gaps = 32/498 (6%)

Query  906   PQNGPTPATQSAAAMFQAPKTPQGMNPVAAAALYNSMTGPFCLPPDqqqqqqtaqqqqsa  965
             P   P+P   +AAAMFQAPKTPQGMNPVAAAALYNSMTGPFCLPPDQQQQQQTAQQQQSA
Sbjct  1070  PHIRPSP---TAAAMFQAPKTPQGMNPVAAAALYNSMTGPFCLPPDQQQQQQTAQQQQSA  1126

Query  966   qqqqqssqqtqqqLEQNEALSLVVTPKKKRHKVTDTRITPRTVSRILAQDgvvpptggpp  1025
             QQQQQSSQQTQQQLEQNEALSLVVTPKKKRHKVTDTRITPRTVSRILAQDGVVPPTGGPP
Sbjct  1127  QQQQQSSQQTQQQLEQNEALSLVVTPKKKRHKVTDTRITPRTVSRILAQDGVVPPTGGPP  1186

Query  1026  stpqqqqqqqqqqqqqqqqqqqqASNGGNSNATPAQSPTRSSGGAAYHpqppppppppmmp  1085
             STPQQQQQQQQQQQQQQQQQQQQASNGGNSNATPAQSPTRSSGGAAYHPQPPPPPPPPMMP
Sbjct  1187  STPQQQQQQQQQQQQQQQQQQQQASNGGNSNATPAQSPTRSSGGAAYHPQPPPPPPPPMMP  1246

Query  1086  VSLPTSVAIPNPSLHESKVFSPYSPFFNPhaaagqataaqlhqhhqqhhphhqsmqlsss  1145
             VSLPTSVAIPNPSLHESKVFSPYSPFFNPHAAAGQATAAQLHQHHQQHHPHHQSMQLSSS
Sbjct  1247  VSLPTSVAIPNPSLHESKVFSPYSPFFNPHAAAGQATAAQLHQHHQQHHPHHQSMQLSSS  1306

Query  1146  ppgslgALMDSRDspplphppsmlhpallaaahhggspDYKTCLRAVMDAQDRQSECNSA  1205
             PPGSLGALMDSRDSPPLPHPPSMLHPALLAAAHHGGSPDYKTCLRAVMDAQDRQSECNSA
Sbjct  1307  PPGSLGALMDSRDSPPLPHPPSMLHPALLAAAHHGGSPDYKTCLRAVMDAQDRQSECNSA  1366

Query  1206  DMQFDGMAPTISFYKQMQLKTEHQESLMAKHCESLTPLHSSTLTPMHLRKAKLMFFWVRY  1265
             DMQFDGMAPT                            SSTLTPMHLRKAKLMFFWVRY
Sbjct  1367  DMQFDGMAPT----------------------------SSTLTPMHLRKAKLMFFWVRY  1397

Query  1266  PSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQMEKYARQAVTEGIKTPDDLLIAG  1325
             PSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQMEKYARQAVTEGIKTPDDLLIAG
Sbjct  1398  PSSAVLKMYFPDIKFNKNNTAQLVKWFSNFREFYYIQMEKYARQAVTEGIKTPDDLLIAG  1457

Query  1326  DSFLYRVLNLHYNRPNNHIFVPQNFREVVESTLREFFRAIQCGKDTFQSWKKSIYKLISRM  1385
```

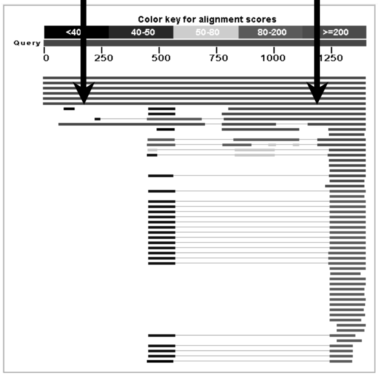
No definition line ∴ second HSP identified

– Gap
a Low-Complexity

```
>gi|28381244|gb|AAN13501.2| G CG17228-PA, isoform A [Drosophila melanogaster]
 gi|28571644|ref|NP_731565.2| UG prospero CG17228-PA, isoform A [Drosophila melanogaster]
Length=1535

 Score = 1063 bits (2749),  Expect = 0.0✓ Method: Composition-based stats.
 Identities = 915/917 (99%)✓ Positives = 915/917 (99%), Gaps = 0/917 (0%)


 Score =  546 bits (1406),  Expect = 3e-153✓ Method: Composition-based stats.
 Identities = 461/498 (92%)✓ Positives = 463/498 (92%), Gaps = 32/498 (6%)
```

HSP 1
Q: 1-917
S: 1-917

HSP 2
Q: 906-1403
S: 1070-1535



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

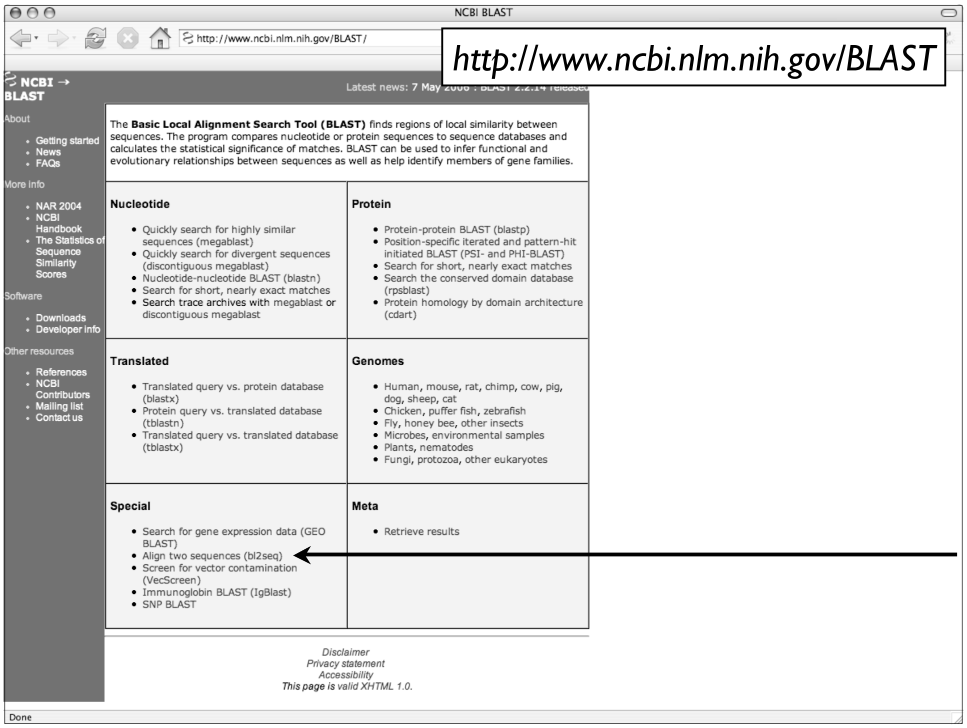
- Low-complexity regions
  - Nucleotide searches: removed with DUST (➔ N)
  - Protein searches: removed with SEG (➔ X)
- Repetitive elements
  - LINEs, SINEs, retroviral repeats
  - Choose “Filter: Human Repeats” when using BLASTN
  - RepeatMasker
    http://www.repeatmasker.org

Database Searching Artifacts

- 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



NCBI BLAST

http://www.ncbi.nlm.nih.gov/BLAST/

http://www.ncbi.nlm.nih.gov/BLAST

NCBI → BLAST

Latest news: 7 May 2006 : BLAST 2.2.14 released

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- Getting started
- News
- FAQs

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- NCBI Handbook
- The Statistics of Sequence Similarity Scores

Software
- Downloads
- Developer info

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The **Basic Local Alignment Search Tool (BLAST)** finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Nucleotide
- Quickly search for highly similar sequences (megablast)
- Quickly search for divergent sequences (discontiguous megablast)
- Nucleotide-nucleotide BLAST (blastn)
- Search for short, nearly exact matches
- Search trace archives with megablast or discontiguous megablast

Protein
- Protein-protein BLAST (blastp)
- Position-specific iterated and pattern-hit initiated BLAST (PSI- and PHI-BLAST)
- Search for short, nearly exact matches
- Search the conserved domain database (rpsblast)
- Protein homology by domain architecture (cdart)

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

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

Special
- Search for gene expression data (GEO BLAST)
- Align two sequences (bl2seq)
- Screen for vector contamination (VecScreen)
- Immunoglobin BLAST (IgBlast)
- SNP BLAST

Meta
- Retrieve results

Disclaimer
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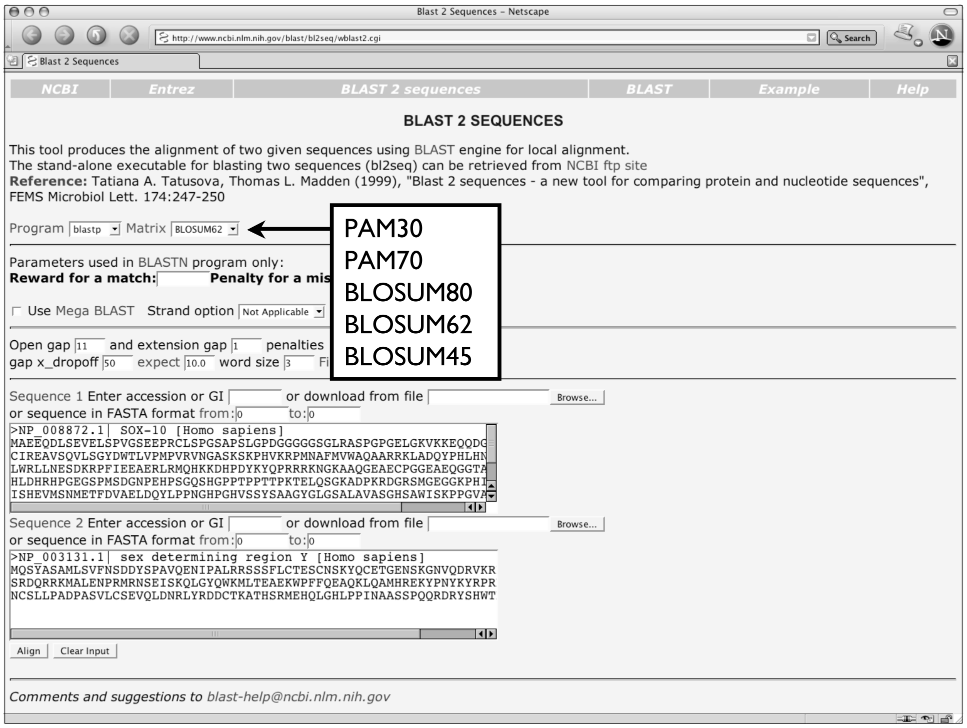
Accessibility
This page is valid XHTML 1.0.

Done



Blast 2 Sequences – Netscape

http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi

Blast 2 Sequences

NCBI | *Entrez* | *BLAST 2 sequences* | *BLAST* | *Example* | *Help*

BLAST 2 SEQUENCES

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

Program blastp Matrix BLOSUM62

PAM30
PAM70
BLOSUM80
BLOSUM62
BLOSUM45

Parameters used in BLASTN program only:
Reward for a match: **Penalty for a mis**

□ Use Mega BLAST Strand option Not Applicable

Open gap 11 and extension gap 1 penalties
gap x_dropoff 50 expect 10.0 word size 3 Fi

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

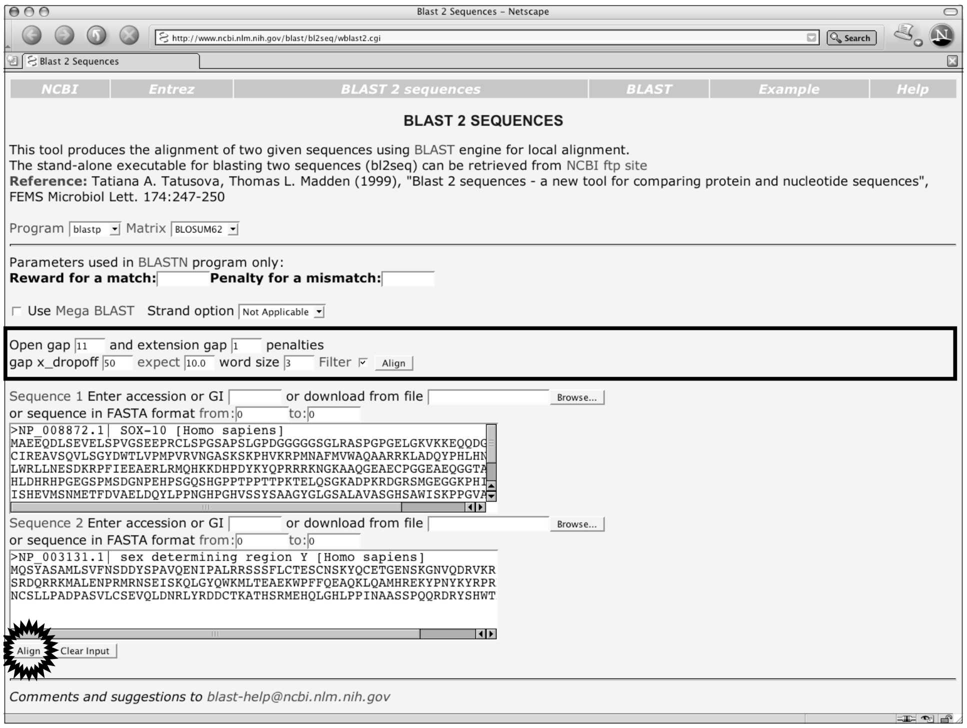
```
>NP_008872.1| SOX-10 [Homo sapiens]
MAEEQDLSEVELSPVGSEEPRCLSPGSAPSLGPDGGGGGSGLRASPGPGELGKVKKEQQDG
CIREAVSQVLSGYDWTLVPMPVRVNGASKSKPHVKRPMNAFMVWAQAARRKLADQYPHLHN
LWRLLNESDKRPFIEEAERLRMQHKKDHPDYKYQPRRRKNGKAAQGEAECPGGEAEQGGTA
HLDHRHPGEGSPMSDGNPEHPSGQSHGPPTPPTTPKTELQSGKADPKRDGRSMGEGGKPHI
ISHEVMSNMETFDVAELDQYLPPNGHPGHVSSYSAAGYGLGSALAVASGHSAWISKPPGVA
```

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

```
>NP_003131.1| sex determining region Y [Homo sapiens]
MQSYASAMLSVFNSDDYSPAVQENIPALRRSSSFLCTESCNSKYQCETGENSKGNVQDRVKR
SRDQRRKMALENPRMRNSEISKQLGYQWKMLTEAEKWPFFQEAQKLQAMHREKYPNYKYRPR
NCSLLPADPASVLCSEVQLDNRLYRDDCTKATHSRMEHQLGHLPPINAASSPQQRDRYSHWT
```

Align | Clear Input

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


Blast 2 Sequences – Netscape
http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi
Search
Blast 2 Sequences
NCBI
Entrez
BLAST 2 sequences
BLAST
Example
Help

BLAST 2 SEQUENCES

This tool produces the alignment of two given sequences using BLAST engine for local alignment.
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Program blastp Matrix BLOSUM62

Parameters used in BLASTN program only:
Reward for a match: **Penalty for a mismatch:**

□ Use Mega BLAST Strand option Not Applicable

Open gap 11 and extension gap 1 penalties
gap x_dropoff 50 expect 10.0 word size 3 Filter ☑ Align

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

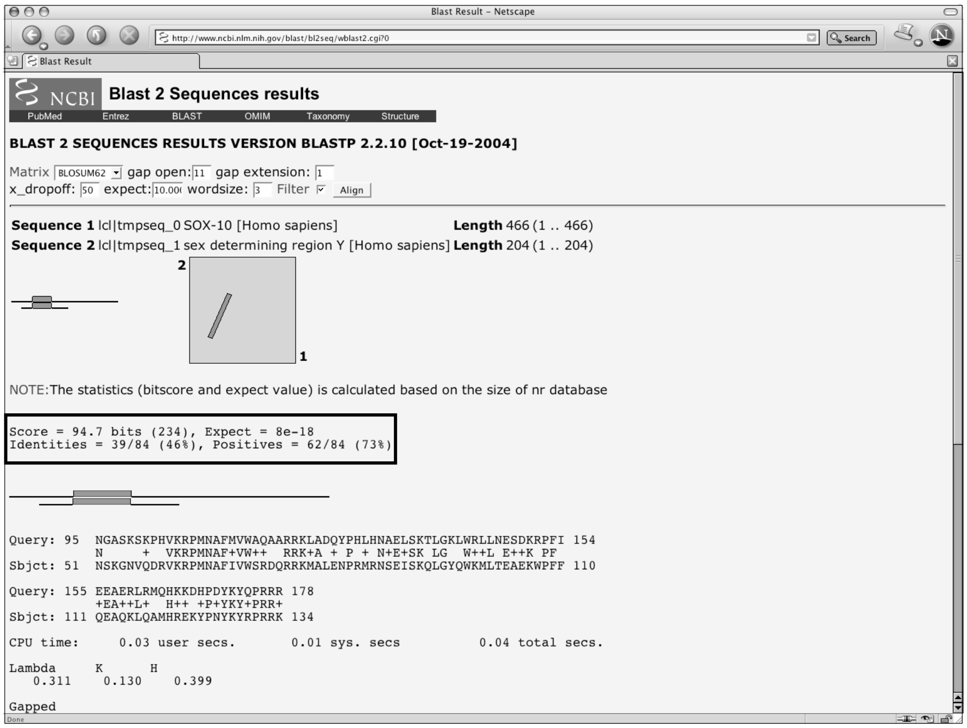
```
>NP_008872.1| SOX-10 [Homo sapiens]
MAEEQDLSEVELSPVGSEEPRCLSPGSAPSLGPDGGGGGSGLRASPGPGELGKVKKEQQDG
CIREAVSQVLSGYDWTLVPMPVRVNGASKSKPHVKRPMNAFMVWAQAARRKLADQYPHLHN
LWRLLNESDKRPFIEEAERLRMQHKKDHPDYKYQPRRRKNGKAAQGEAECPGGEAEQGGTA
HLDHRHPGEGSPMSDGNPEHPSGQSHGPPTPPTTPKTELQSGKADPKRDGRSMGEGGKPHI
ISHEVMSNMETFDVAELDQYLPPNGHPGHVSSYSAAGYGLGSALAVASGHSAWISKPPGVA
```

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

```
>NP_003131.1| sex determining region Y [Homo sapiens]
MQSYASAMLSVFNSDDYSPAVQENIPALRRSSSFLCTESCNSKYQCETGENSKGNVQDRVKR
SRDQRRKMALENPRMRNSEISKQLGYQWKMLTEAEKWPFFQEAQKLQAMHREKYPNYKYRPR
NCSLLPADPASVLCSEVQLDNRLYRDDCTKATHSRMEHQLGHLPPINAASSPQQRDRYSHWT
```

Align Clear Input

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



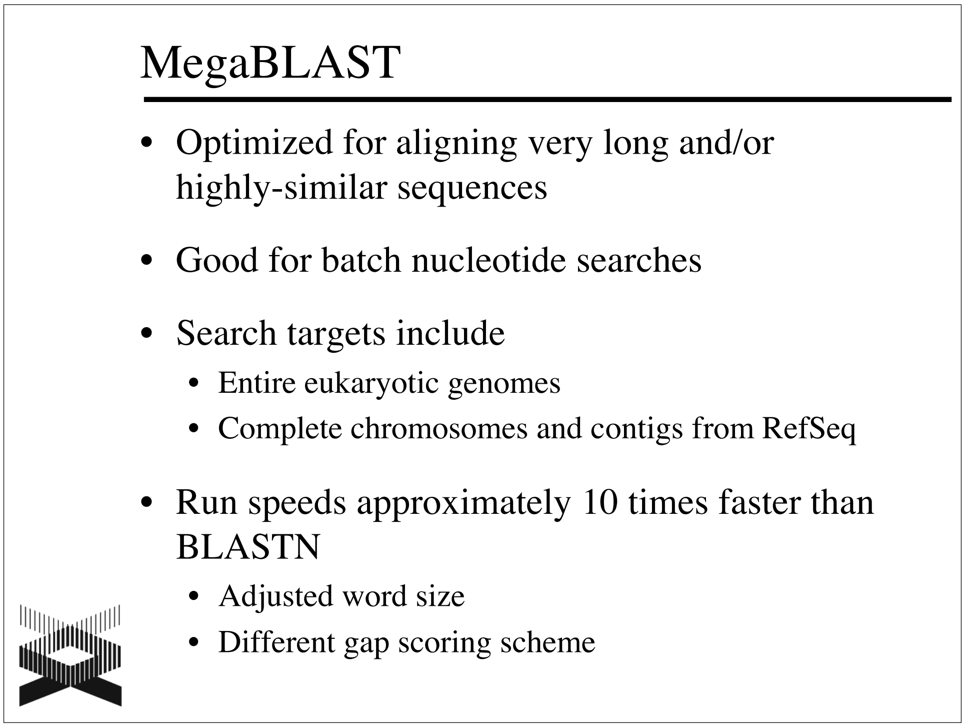
BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.2.10 [Oct-19-2004]

Matrix BLOSUM62 gap open: 11 gap extension: 1
x_dropoff: 50 expect: 10.000 wordsize: 3 Filter ☑ Align

Sequence 1 lcl|tmpseq_0 SOX-10 [Homo sapiens] **Length** 466 (1 .. 466)
Sequence 2 lcl|tmpseq_1 sex determining region Y [Homo sapiens] **Length** 204 (1 .. 204)

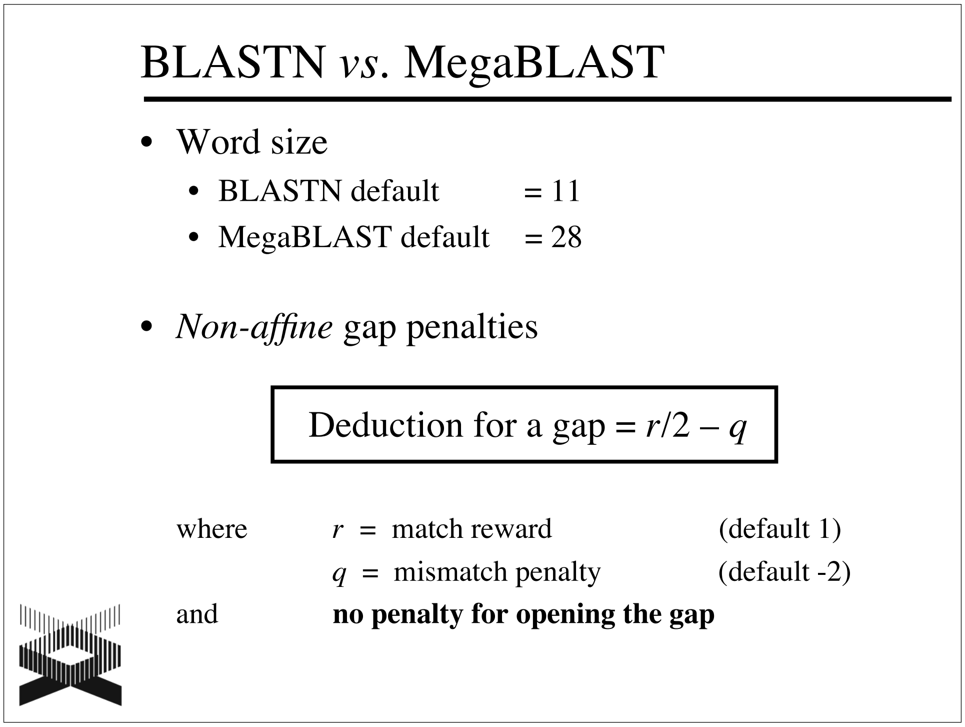
NOTE:The statistics (bitscore and expect value) is calculated based on the size of nr database

```
Score = 94.7 bits (234), Expect = 8e-18
Identities = 39/84 (46%), Positives = 62/84 (73%)


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

CPU time:     0.03 user secs.       0.01 sys. secs          0.04 total secs.

Lambda     K      H
   0.311    0.130    0.399

Gapped
```



MegaBLAST

- Optimized for aligning very long and/or highly-similar sequences
- Good for batch nucleotide searches
- Search targets include
  - Entire eukaryotic genomes
  - Complete chromosomes and contigs from RefSeq
- Run speeds approximately 10 times faster than BLASTN
  - Adjusted word size
  - Different gap scoring scheme

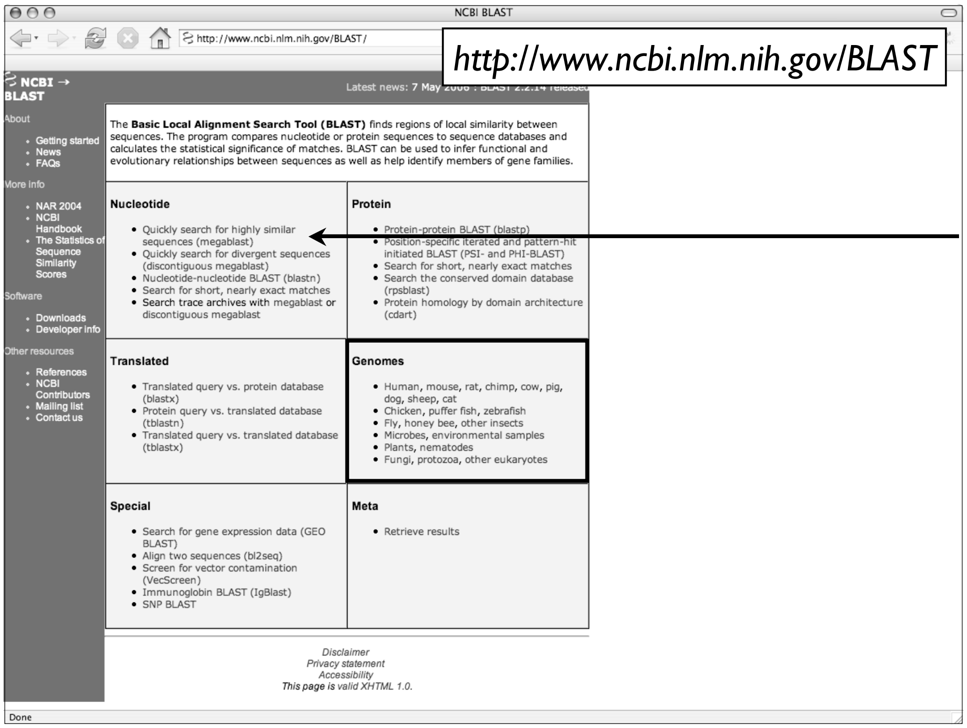


BLASTN *vs*. MegaBLAST

- Word size
  - BLASTN default = 11
  - MegaBLAST default = 28
- *Non-affine* gap penalties

$$\boxed{\text{Deduction for a gap} = r/2 - q}$$

where	r = match reward	(default 1)
	q = mismatch penalty	(default -2)
and	**no penalty for opening the gap**	



NCBI BLAST

http://www.ncbi.nlm.nih.gov/BLAST/

http://www.ncbi.nlm.nih.gov/BLAST

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Latest news: **7 May 2006 : BLAST 2.2.14 released**

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- NCBI Handbook
- The Statistics of Sequence Similarity Scores

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- Search for short, nearly exact matches
- Search trace archives with megablast or discontiguous megablast

Protein
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- Position-specific iterated and pattern-hit initiated BLAST (PSI- and PHI-BLAST)
- Search for short, nearly exact matches
- Search the conserved domain database (rpsblast)
- Protein homology by domain architecture (cdart)

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

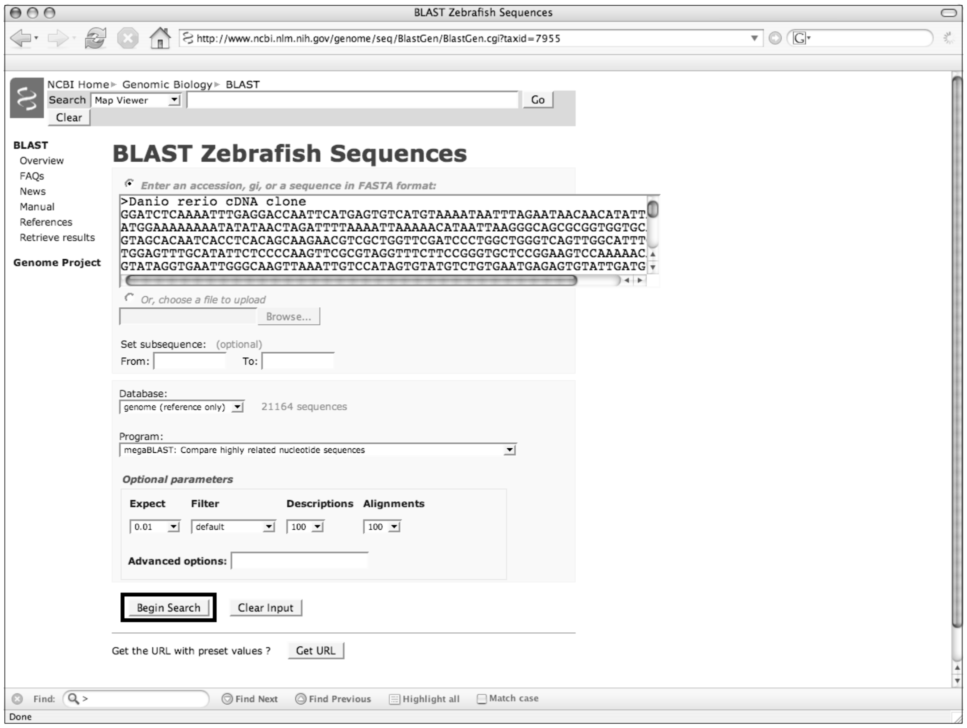
Genomes
- Human, mouse, rat, chimp, cow, pig, dog, sheep, cat
- Chicken, puffer fish, zebrafish
- Fly, honey bee, other insects
- Microbes, environmental samples
- Plants, nematodes
- Fungi, protozoa, other eukaryotes

Special
- Search for gene expression data (GEO BLAST)
- Align two sequences (bl2seq)
- Screen for vector contamination (VecScreen)
- Immunoglobin BLAST (IgBlast)
- SNP BLAST

Meta
- Retrieve results

Disclaimer
Privacy statement
Accessibility
This page is valid XHTML 1.0.

Done



BLAST Zebrafish Sequences

http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=7955

NCBI Home ▸ Genomic Biology ▸ BLAST

Search: Map Viewer

BLAST
- Overview
- FAQs
- News
- Manual
- References
- Retrieve results

Genome Project

BLAST Zebrafish Sequences

● *Enter an accession, gi, or a sequence in FASTA format:*

```
>Danio rerio cDNA clone
GGATCTCAAAATTTGAGGACCAATTCATGAGTGTCATGTAAAATAATTTAGAATAACAACATATT
ATGGAAAAAAAATATATAACTAGATTTTAAAATTAAAAACATAATTAAGGGCAGCGCGGTGGTGC
GTAGCACAATCACCTCACAGCAAGAACGTCGCTGGTTCGATCCCTGGCTGGGTCAGTTGGCATTT
TGGAGTTTGCATATTCTCCCCAAGTTCGCGTAGGTTTCTTCCGGGTGCTCCGGAAGTCCAAAAAC
GTATAGGTGAATTGGGCAAGTTAAATTGTCCATAGTGTATGTCTGTGAATGAGAGTGTATTGATG
```

○ *Or, choose a file to upload*

Set subsequence: (optional)
From: To:

Database: genome (reference only) 21164 sequences

Program: megaBLAST: Compare highly related nucleotide sequences

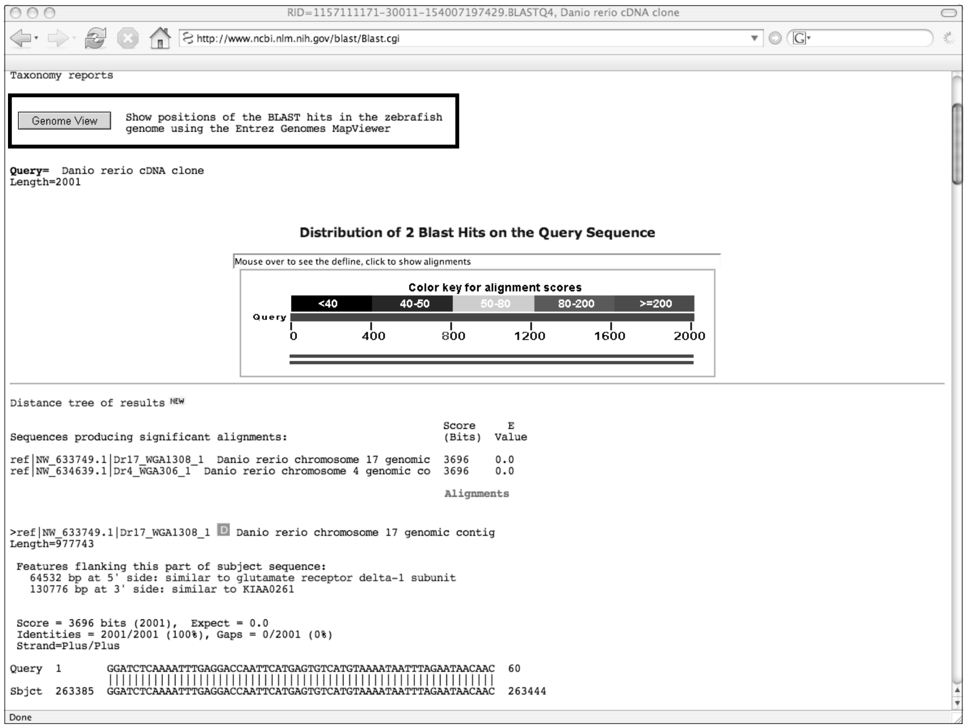
Optional parameters

Expect	Filter	Descriptions	Alignments
0.01	default	100	100

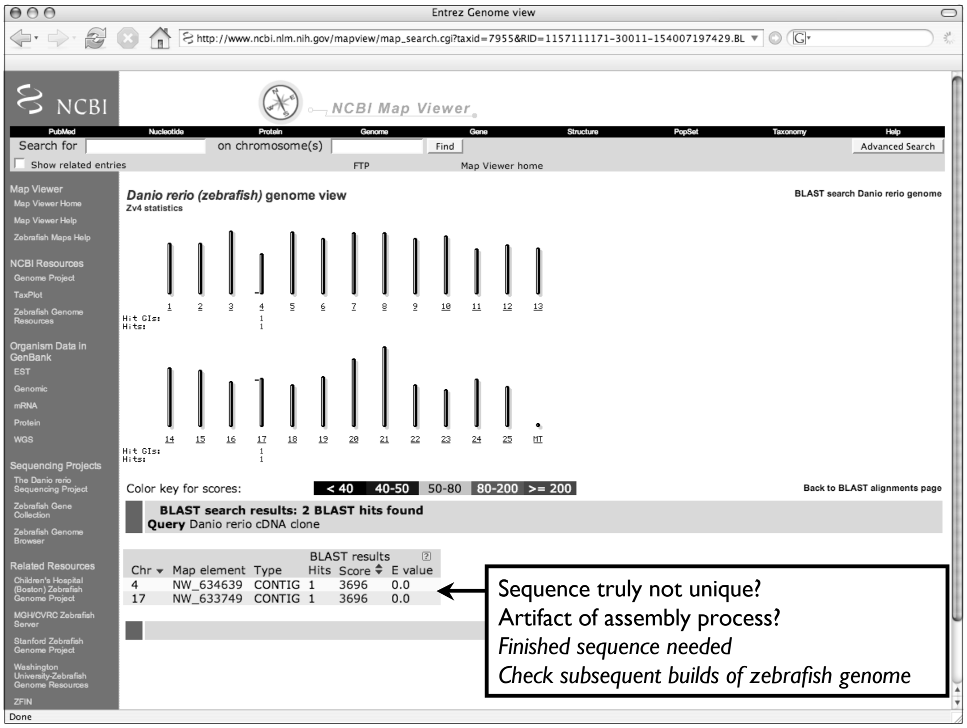
Advanced options:

Get the URL with preset values ?

Done


RID=1157111171-30011-154007197429.BLASTQ4, Danio rerio cDNA clone
http://www.ncbi.nlm.nih.gov/blast/Blast.cgi
Genome View
Distribution of 2 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
0
400
800
1200
1600
2000
Done

```
Taxonomy reports

Genome View   Show positions of the BLAST hits in the zebrafish
              genome using the Entrez Genomes MapViewer

Query=  Danio rerio cDNA clone
Length=2001

Distance tree of results NEW

                                                                 Score    E
Sequences producing significant alignments:                      (Bits)  Value

ref|NW_633749.1|Dr17_WGA1308_1  Danio rerio chromosome 17 genomic  3696    0.0
ref|NW_634639.1|Dr4_WGA306_1  Danio rerio chromosome 4 genomic co  3696    0.0

                                                                 Alignments

>ref|NW_633749.1|Dr17_WGA1308_1 D Danio rerio chromosome 17 genomic contig
Length=977743

 Features flanking this part of subject sequence:
   64532 bp at 5' side: similar to glutamate receptor delta-1 subunit
   130776 bp at 3' side: similar to KIAA0261

 Score = 3696 bits (2001),  Expect = 0.0
 Identities = 2001/2001 (100%), Gaps = 0/2001 (0%)
 Strand=Plus/Plus

Query  1       GGATCTCAAAATTTGAGGACCAATTCATGAGTGTCATGTAAAATAATTTAGAATAACAAC  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  263385  GGATCTCAAAATTTGAGGACCAATTCATGAGTGTCATGTAAAATAATTTAGAATAACAAC  263444
```

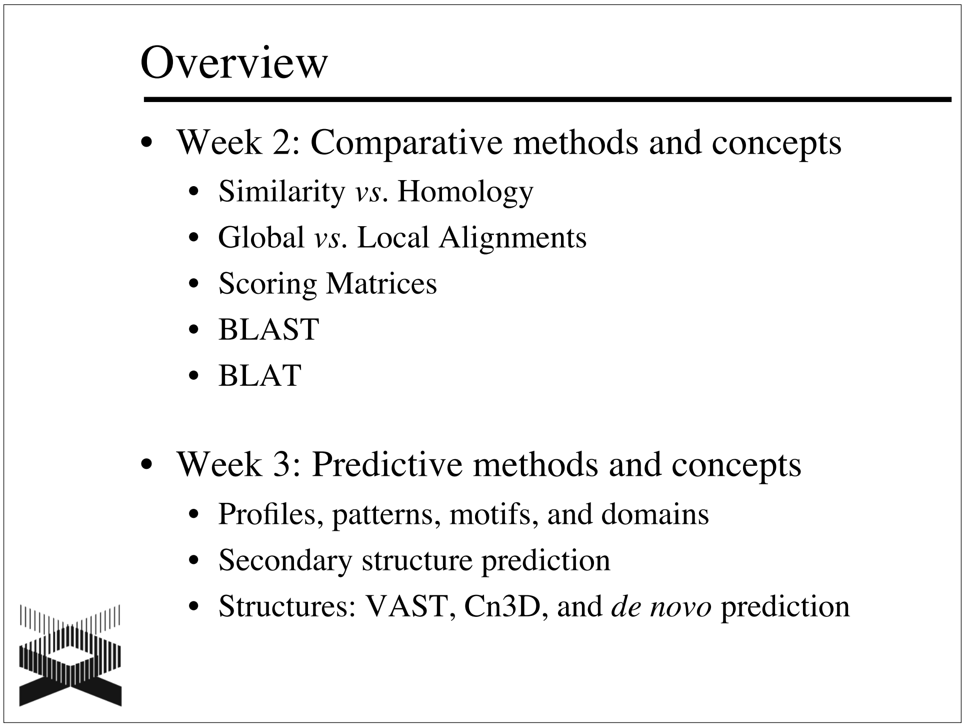


Danio rerio (zebrafish) genome view
Zv4 statistics

BLAST search results: 2 BLAST hits found
Query Danio rerio cDNA clone

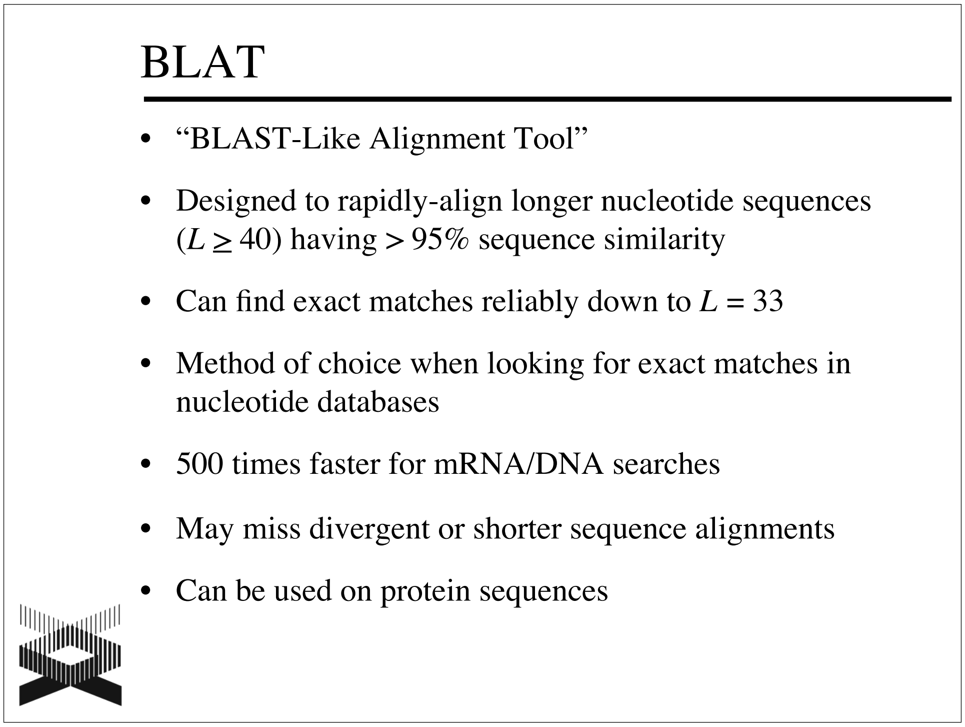
			BLAST results		
Chr	Map element	Type	Hits	Score	E value
4	NW_634639	CONTIG	1	3696	0.0
17	NW_633749	CONTIG	1	3696	0.0

Sequence truly not unique?
Artifact of assembly process?
Finished sequence needed
Check subsequent builds of zebrafish genome



Overview

- Week 2: Comparative methods and concepts
  - Similarity *vs*. Homology
  - Global *vs*. Local Alignments
  - Scoring Matrices
  - BLAST
  - BLAT
- Week 3: Predictive methods and concepts
  - Profiles, patterns, motifs, and domains
  - Secondary structure prediction
  - Structures: VAST, Cn3D, and *de novo* prediction

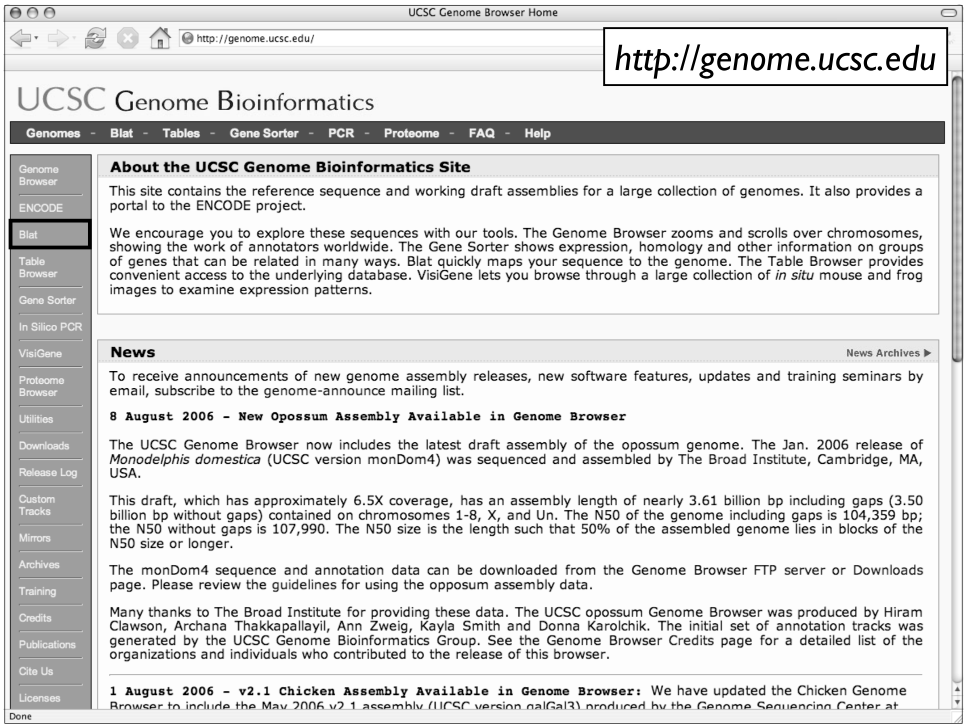


BLAT

- "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



About the UCSC Genome Bioinformatics Site

This site contains the reference sequence and working draft assemblies for a large collection of genomes. It also provides a portal to the ENCODE project.

We encourage you to explore these sequences with our tools. The Genome Browser zooms and scrolls over chromosomes, showing the work of annotators worldwide. The Gene Sorter shows expression, homology and other information on groups of genes that can be related in many ways. Blat quickly maps your sequence to the genome. The Table Browser provides convenient access to the underlying database. VisiGene lets you browse through a large collection of *in situ* mouse and frog images to examine expression patterns.

News

To receive announcements of new genome assembly releases, new software features, updates and training seminars by email, subscribe to the genome-announce mailing list.

8 August 2006 - New Opossum Assembly Available in Genome Browser

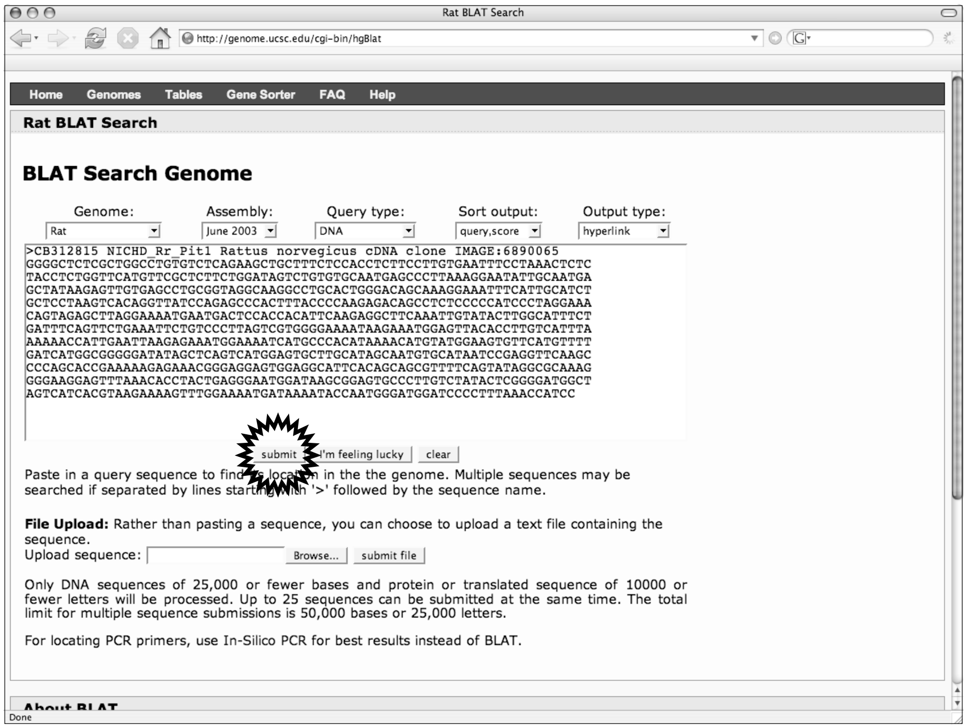
The UCSC Genome Browser now includes the latest draft assembly of the opossum genome. The Jan. 2006 release of *Monodelphis domestica* (UCSC version monDom4) was sequenced and assembled by The Broad Institute, Cambridge, MA, USA.

This draft, which has approximately 6.5X coverage, has an assembly length of nearly 3.61 billion bp including gaps (3.50 billion bp without gaps) contained on chromosomes 1-8, X, and Un. The N50 of the genome including gaps is 104,359 bp; the N50 without gaps is 107,990. The N50 size is the length such that 50% of the assembled genome lies in blocks of the N50 size or longer.

The monDom4 sequence and annotation data can be downloaded from the Genome Browser FTP server or Downloads page. Please review the guidelines for using the opposum assembly data.

Many thanks to The Broad Institute for providing these data. The UCSC opossum Genome Browser was produced by Hiram Clawson, Archana Thakkapallayil, Ann Zweig, Kayla Smith and Donna Karolchik. The initial set of annotation tracks was generated by the UCSC Genome Bioinformatics Group. See the Genome Browser Credits page for a detailed list of the organizations and individuals who contributed to the release of this browser.

1 August 2006 - v2.1 Chicken Assembly Available in Genome Browser: We have updated the Chicken Genome Browser to include the May 2006 v2.1 assembly (UCSC version galGal3) produced by the Genome Sequencing Center at



Rat BLAT Search

BLAT Search Genome

Genome: Rat | Assembly: June 2003 | Query type: DNA | Sort output: query,score | Output type: hyperlink

```
>CB312815 NICHD_Rr_Pit1 Rattus norvegicus cDNA clone IMAGE:6890065
GGGGCTCTCGCTGGCCTGTGTCTCAGAAGCTGCTTTCTCCACCTCTTCCTTGTGAATTTCCTAAACTCTC
TACCTCTGGTTCATGTTCGCTCTTCTGGATAGTCTGTGTGCAATGAGCCCTTAAAGGAATATTGCAATGA
GCTATAAGAGTTGTGAGCCTGCGGTAGGCAAGGCCTGCACTGGGACAGCAAAGGAAATTTCATTGCATCT
GCTCCTAAGTCACAGGTTATCCAGAGCCCACTTTACCCCAAGAGACAGCCTCTCCCCCATCCCTAGGAAA
CAGTAGAGCTTAGGAAAATGAATGACTCCACCACATTCAAGAGGCTTCAAATTGTATACTTGGCATTTCT
GATTTCAGTTCTGAAATTCTGTCCCTTAGTCGTGGGGAAAATAAGAAATGGAGTTACACCTTGTCATTTA
AAAAACCATTGAATTAAGAGAAATGGAAAATCATGCCCACATAAAACATGTATGGAAGTGTTCATGTTTT
GATCATGGCGGGGGATATAGCTCAGTCATGGAGTGCTTGCATAGCAATGTGCATAATCCGAGGTTCAAGC
CCCAGCACCGAAAAAGAGAAACGGGAGGAGTGGAGGCATTCACAGCAGCGTTTTCAGTATAGGCGCAAAG
GGGAAGGAGTTTAAACACCTACTGAGGGAATGGATAAGCGGAGTGCCCTTGTCTATACTCGGGGATGGCT
AGTCATCACGTAAGAAAAGTTTGGAAAATGATAAAATACCAATGGGATGGATCCCCTTTAAACCATCC
```

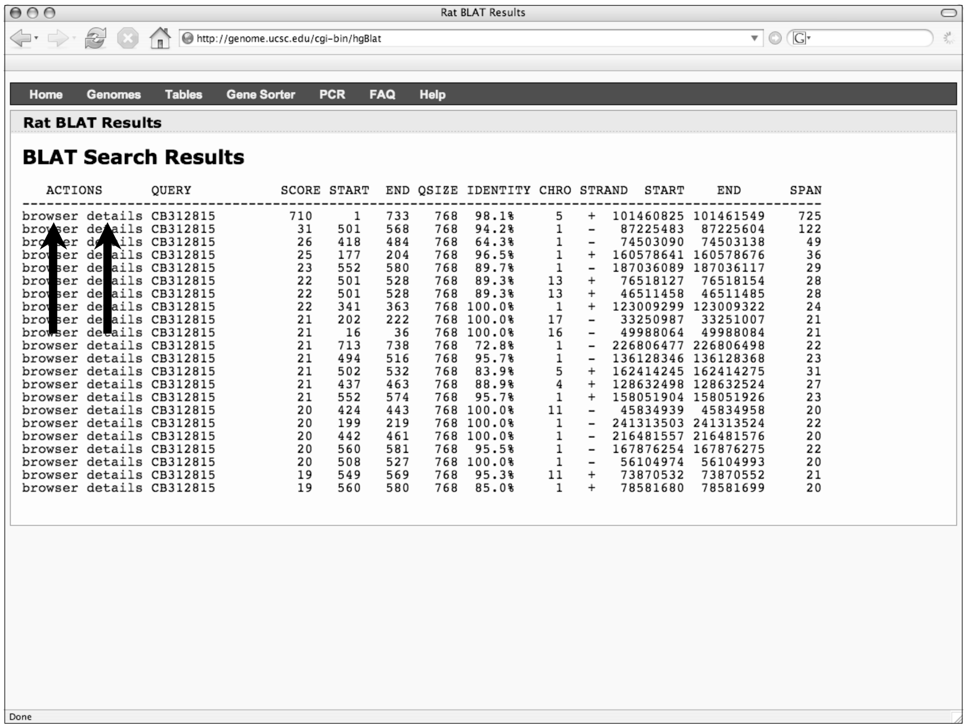
Paste in a query sequence to find its location in the the genome. Multiple sequences may be searched if separated by lines starting with '>' followed by the sequence name.

File Upload: Rather than pasting a sequence, you can choose to upload a text file containing the sequence.
Upload sequence:

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.

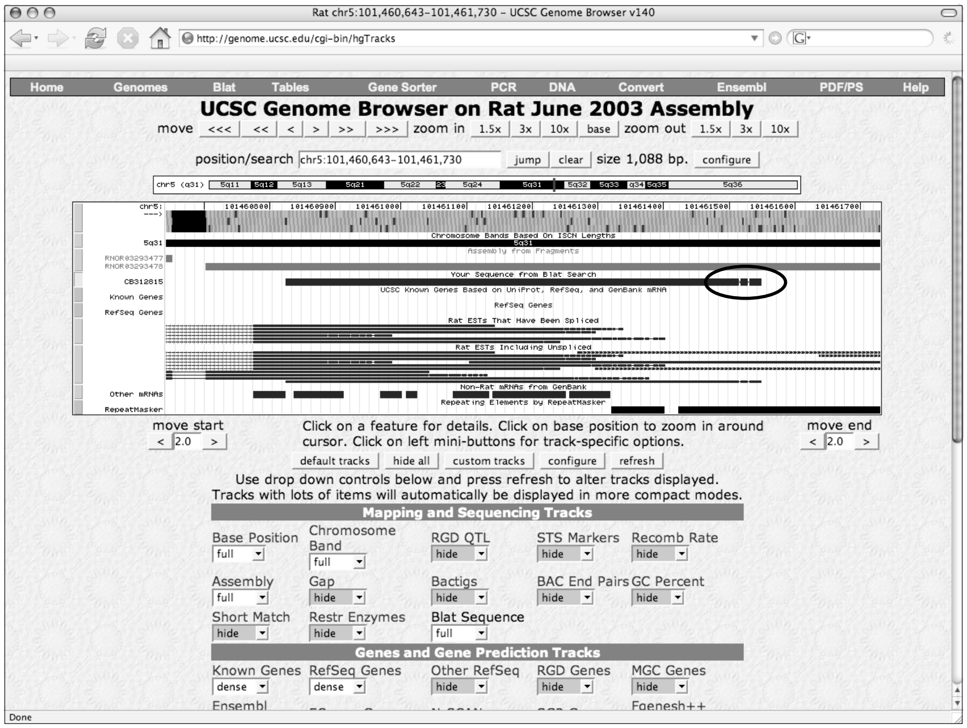
About BLAT



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   +  101460825 101461549    725
browser details CB312815           31   501   568   768  94.2%     1   -   87225483  87225604    122
browser details CB312815           26   418   484   768  64.3%     1   -   74503090  74503138     49
browser details CB312815           25   177   204   768  96.5%     1   +  160578641 160578676     36
browser details CB312815           23   552   580   768  89.7%     1   -  187036089 187036117     29
browser details CB312815           22   501   528   768  89.3%    13   +   76518127  76518154     28
browser details CB312815           22   501   528   768  89.3%    13   +   46511458  46511485     28
browser details CB312815           22   341   363   768 100.0%     1   +  123009299 123009322     24
browser details CB312815           21   202   222   768 100.0%    17   -   33250987  33251007     21
browser details CB312815           21    16    36   768 100.0%    16   -   49988064  49988084     21
browser details CB312815           21   713   738   768  72.8%     1   -  226806477 226806498     22
browser details CB312815           21   494   516   768  95.7%     1   -  136128346 136128368     23
browser details CB312815           21   502   532   768  83.9%     5   +  162414245 162414275     31
browser details CB312815           21   437   463   768  88.9%     4   +  128632498 128632524     27
browser details CB312815           21   552   574   768  95.7%     1   +  158051904 158051926     23
browser details CB312815           20   424   443   768 100.0%    11   -   45834939  45834958     20
browser details CB312815           20   199   219   768 100.0%     1   -  241313503 241313524     22
browser details CB312815           20   442   461   768 100.0%     1   -  216481557 216481576     20
browser details CB312815           20   560   581   768  95.5%     1   -  167876254 167876275     22
browser details CB312815           20   508   527   768 100.0%     1   -   56104974  56104993     20
browser details CB312815           19   549   569   768  95.3%    11   +   73870532  73870552     21
browser details CB312815           19   560   580   768  85.0%     1   +   78581680  78581699     20
```



Rat chr5:101,460,643-101,461,730 - UCSC Genome Browser v140
http://genome.ucsc.edu/cgi-bin/hgTracks
Home Genomes Blat Tables Gene Sorter PCR DNA Convert Ensembl PDF/PS Help
chr5 (q31) 5q11 5q12 5q13 5q21 5q22 23 5q24 5q31 5q32 5q33 q34 5q35 5q36
chr5: 101460800 101460900 101461000 101461100 101461200 101461300 101461400 101461500 101461600 101461700
--->
Chromosome Bands Based On ISCN Lengths
5q31
Assembly from Fragments
RNOR03293477
RNOR03293478
Your Sequence from Blat Search
CB312815
UCSC Known Genes Based on UniProt, RefSeq, and GenBank mRNA
Known Genes
RefSeq Genes
Rat ESTs That Have Been Spliced
Rat ESTs Including Unspliced
Non-Rat mRNAs from GenBank
Other mRNAs
Repeating Elements by RepeatMasker
RepeatMasker
Done

UCSC Genome Browser on Rat June 2003 Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position/search chr5:101,460,643-101,461,730 jump clear size 1,088 bp. configure

move start < 2.0 >

Click on a feature for details. Click on base position to zoom in around cursor. Click on left mini-buttons for track-specific options.

move end < 2.0 >

default tracks | hide all | custom tracks | configure | refresh

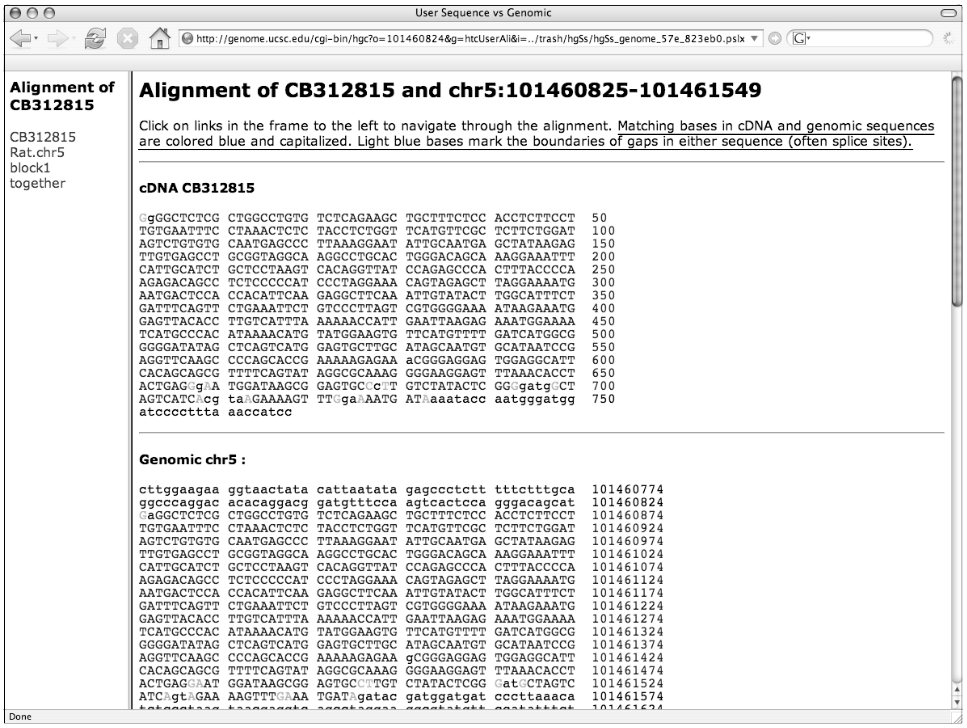
Use drop down controls below and press refresh to alter tracks displayed.
Tracks with lots of items will automatically be displayed in more compact modes.

Mapping and Sequencing Tracks

Base Position: full
Chromosome Band: full
RGD QTL: hide
STS Markers: hide
Recomb Rate: hide
Assembly: full
Gap: hide
Bactigs: hide
BAC End Pairs: hide
GC Percent: hide
Short Match: hide
Restr Enzymes: hide
Blat Sequence: full

Genes and Gene Prediction Tracks

Known Genes: dense
RefSeq Genes: dense
Other RefSeq: hide
RGD Genes: hide
MGC Genes: hide
Ensembl
Fgenesh++



User Sequence vs Genomic
http://genome.ucsc.edu/cgi-bin/hgc?o=101460824&g=htcUserAli&i=../trash/hgSs/hgSs_genome_57e_823eb0.pslx
Done

Alignment of CB312815

CB312815
Rat.chr5
block1
together

Alignment of CB312815 and chr5:101460825-101461549

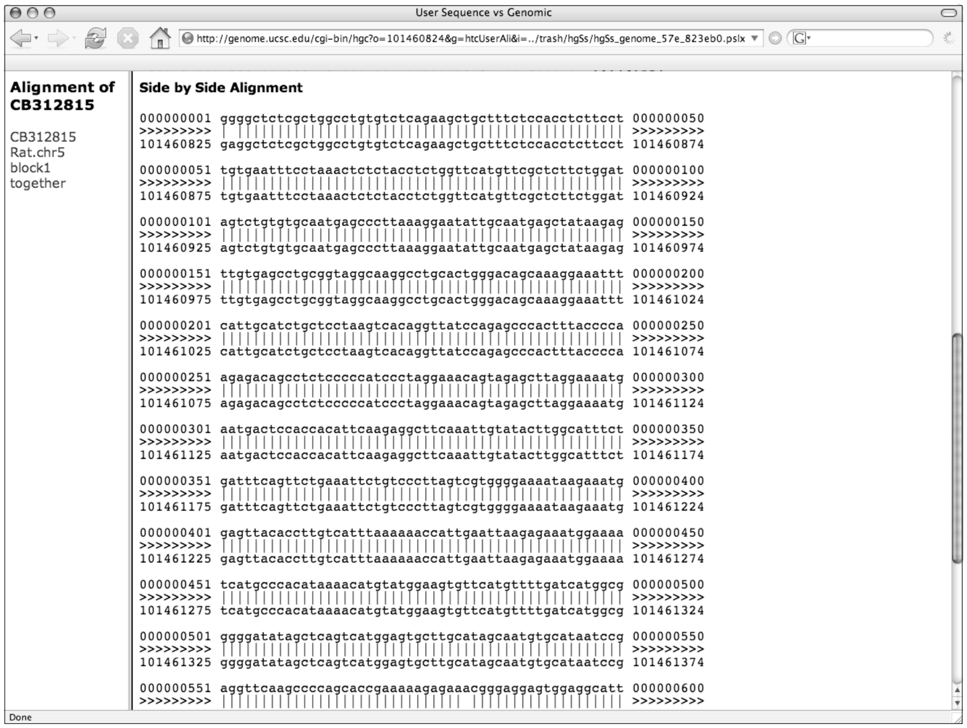
Click on links in the frame to the left to navigate through the alignment. Matching bases in cDNA and genomic sequences are colored blue and capitalized. Light blue bases mark the boundaries of gaps in either sequence (often splice sites).

cDNA CB312815

```
GgGGCTCTCG 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
atcccettta aaccatcc
```

Genomic chr5 :

```
cttggaagaa ggtaactata cattaatata gagccctctt tttctttgca  101460774
ggcccaggac acacaggacg gatgtttcca agtcactcca gggacagcat  101460824
GaGGCTCTCG CTGGCCTGTG TCTCAGAAGC TGCTTTCTCC ACCTCTTCCT  101460874
TGTGAATTTC CTAAACTCTC TACCTCTGGT TCATGTTCGC TCTTCTGGAT  101460924
AGTCTGTGTG CAATGAGCCC TTAAAGGAAT ATTGCAATGA GCTATAAGAG  101460974
TTGTGAGCCT GCGGTAGGCA AGGCCTGCAC TGGGACAGCA AAGGAAATTT  101461024
CATTGCATCT GCTCCTAAGT CACAGGTTAT CCAGAGCCCA CTTTACCCCA  101461074
AGAGACAGCC TCTCCCCCAT CCCTAGGAAA CAGTAGAGCT TAGGAAAATG  101461124
AATGACTCCA CCACATTCAA GAGGCTTCAA ATTGTATACT TGGCATTTCT  101461174
GATTTCAGTT CTGAAATTCT GTCCCTTAGT CGTGGGGAAA ATAAGAAATG  101461224
GAGTTACACC TTGTCATTTA AAAAACCATT GAATTAAGAG AAATGGAAAA  101461274
TCATGCCCAC ATAAAACATG TATGGAAGTG TTCATGTTTT GATCATGGCG  101461324
GGGGATATAG CTCAGTCATG GAGTGCTTGC ATAGCAATGT GCATAATCCG  101461374
AGGTTCAAGC CCCAGCACCG AAAAAGAGAA gCGGGAGGAG TGGAGGCATT  101461424
CACAGCAGCG TTTTCAGTAT AGGCGCAAAG GGGAAGGAGT TTAAACACCT  101461474
ACTGAGGAAT GGATAAGCGG AGTGCCTTGT CTATACTCGG GatGCTAGTC  101461524
ATCAgtAGAA AAGTTTGAAA TGATAgatac gatggatgat cccttaaaca  101461574
```


User Sequence vs Genomic
http://genome.ucsc.edu/cgi-bin/hgc?o=101460824&g=htcUserAli&i=../trash/hgSs/hgSs_genome_57e_823eb0.pslx
Done

Alignment of CB312815

CB312815
Rat.chr5
block1
together

Side by Side Alignment

```
000000001 ggggctctcgctggcctgtgtctcagaagctgctttctccacctcttcct 000000050
>>>>>>>>> | |||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101460825 gaggctctcgctggcctgtgtctcagaagctgctttctccacctcttcct 101460874

000000051 tgtgaatttcctaaactctctacctctggttcatgttcgctcttctggat 000000100
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101460875 tgtgaatttcctaaactctctacctctggttcatgttcgctcttctggat 101460924

000000101 agtctgtgtgcaatgagcccttaaaggaatattgcaatgagctataagag 000000150
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101460925 agtctgtgtgcaatgagcccttaaaggaatattgcaatgagctataagag 101460974

000000151 ttgtgagcctgcggtaggcaaggcctgcactgggacagcaaaggaaattt 000000200
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101460975 ttgtgagcctgcggtaggcaaggcctgcactgggacagcaaaggaaattt 101461024

000000201 cattgcatctgctcctaagtcacaggttatccagagcccactttacccca 000000250
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101461025 cattgcatctgctcctaagtcacaggttatccagagcccactttacccca 101461074

000000251 agagacagcctctcccccatccctaggaaacagtagagcttaggaaaatg 000000300
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101461075 agagacagcctctcccccatccctaggaaacagtagagcttaggaaaatg 101461124

000000301 aatgactccaccacattcaagaggcttcaaattgtatacttggcatttct 000000350
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101461125 aatgactccaccacattcaagaggcttcaaattgtatacttggcatttct 101461174

000000351 gatttcagttctgaaattctgtcccttagtcgtggggaaaataagaaatg 000000400
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101461175 gatttcagttctgaaattctgtcccttagtcgtggggaaaataagaaatg 101461224

000000401 gagttacaccttgtcatttaaaaaaccattgaattaagagaaatggaaaa 000000450
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101461225 gagttacaccttgtcatttaaaaaaccattgaattaagagaaatggaaaa 101461274

000000451 tcatgcccacataaaacatgtatggaagtgttcatgttttgatcatggcg 000000500
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101461275 tcatgcccacataaaacatgtatggaagtgttcatgttttgatcatggcg 101461324

000000501 ggggatatagctcagtcatggagtgcttgcatagcaatgtgcataatccg 000000550
>>>>>>>>> |||||||||||||||||||||||||||||||||||||||||||||||||| >>>>>>>>>
101461325 ggggatatagctcagtcatggagtgcttgcatagcaatgtgcataatccg 101461374

000000551 aggttcaagccccagcaccgaaaaagagaaacgggaggagtggaggcatt 000000600
>>>>>>>>> |||||||||||||||||||||||||||||| ||||||||||||||||||| >>>>>>>>>
```

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*