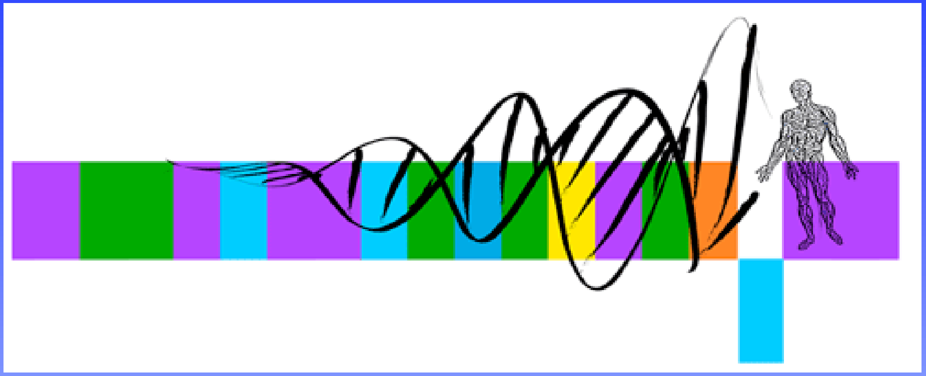


Bioinformatics for Biologists

Sequence Analysis: Part I. Pairwise alignment and database searching

Fran Lewitter, Ph.D.
Head, Biocomputing
Whitehead Institute

Bioinformatics Definitions

"The use of computational methods to make biological discoveries."

Fran Lewitter

Bioinformatics Definitions

"The use of computational methods to make biological discoveries."

Fran Lewitter

"An interdisciplinary field involving biology, computer science, mathematics, and statistics to analyze biological sequence data, genome content, and arrangement, and to predict the function and structure of macromolecules."

David Mount

Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
- Significance of alignments
- Database searching methods

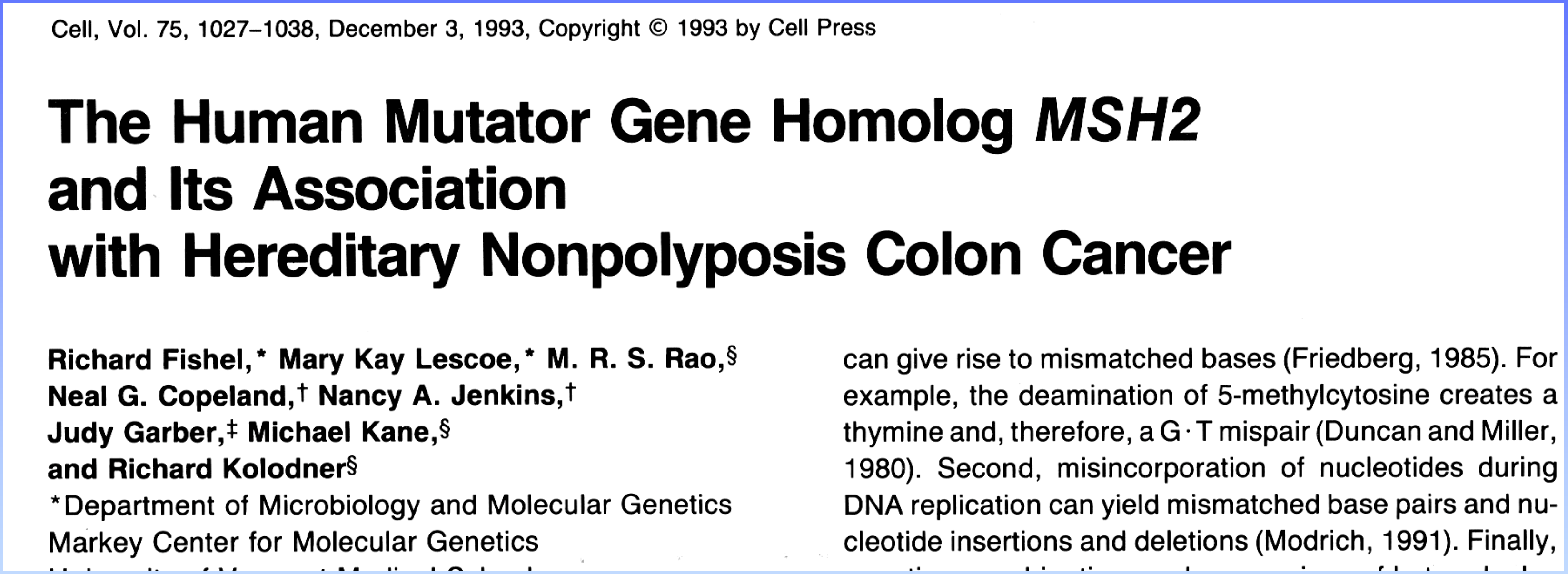
Topics to Cover

- Introduction
  - Why do alignments?
  - A bit of history
  - Definitions
- Scoring alignments
- Alignment methods
- Significance of alignments
- Database searching methods

Simian sarcoma virus onc gene, v-sis, is derived from the gene (or genes) encoding a platelet-derived growth factor.

Doolittle RF, Hunkapiller MW, Hood LE, Devare SG, Robbins KC, Aaronson SA, Antoniades HN. *Science* 221:275-277, 1983.

Cancer Gene Found



Cell, Vol. 75, 1027–1038, December 3, 1993, Copyright © 1993 by Cell Press

The Human Mutator Gene Homolog *MSH2* and Its Association with Hereditary Nonpolyposis Colon Cancer

Richard Fishel,* Mary Kay Lescoe,* M. R. S. Rao,§ Neal G. Copeland,† Nancy A. Jenkins,† Judy Garber,‡ Michael Kane,§ and Richard Kolodner§
*Department of Microbiology and Molecular Genetics
Markey Center for Molecular Genetics

can give rise to mismatched bases (Friedberg, 1985). For example, the deamination of 5-methylcytosine creates a thymine and, therefore, a G · T mispair (Duncan and Miller, 1980). Second, misincorporation of nucleotides during DNA replication can yield mismatched base pairs and nucleotide insertions and deletions (Modrich, 1991). Finally,

Homology to bacterial and yeast genes shed new light on human disease process

Evolutionary Basis of Sequence Alignment

- ***Similarity*** - observable quantity, such as per cent identity
- ***Homology*** - conclusion drawn from data that two genes share a common evolutionary history; no metric is associated with this

Some Definitions

- An ***alignment*** is a mutual arrangement of two sequences, which exhibits where the two sequences are similar, and where they differ.
- An ***optimal alignment*** is one that exhibits the most correspondences and the least differences. It is the alignment with the highest score. May or may not be biologically meaningful.

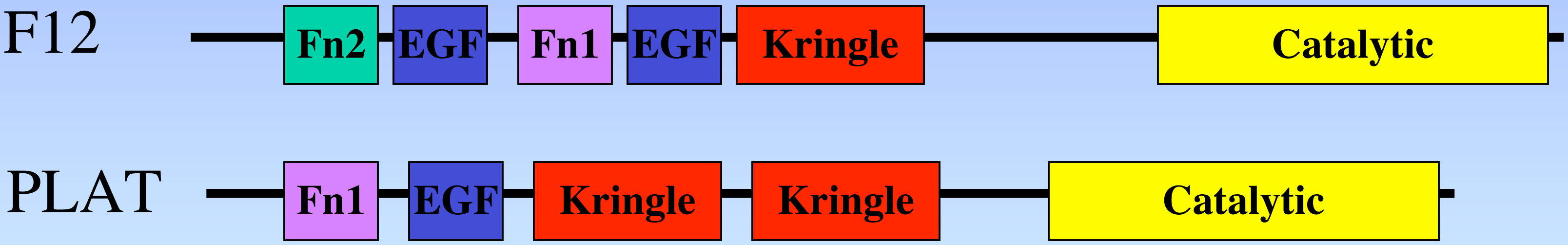
Alignment Methods

- ***Global alignment*** - Needleman-Wunsch (1970) maximizes the number of matches between the sequences along the entire length of the sequences.
- ***Local alignment*** - Smith-Waterman (1981) is a modification of the dynamic programming algorithm gives the highest scoring local match between two sequences.

Alignment Methods

Global vs Local

Modular proteins



Possible Alignments

A: **T C A G A C G A G T G**

B: **T C G G A G C T G**

Possible Alignments

```
A:    T C A G A C G A G T G
B:    T C G G A G C T G

I.    T C A G A C G A G T G

      T C G G A - - G C T G
```

Possible Alignments

```
A:    T C A G A C G A G T G
B:    T C G G A G C T G

I.    T C A G A C G A G T G

      T C G G A - - G C T G

II.   T C A G A C G A G T G

      T C G G A - G C - T G
```

Possible Alignments

```
A:   T C A G A C G A G T G
B:   T C G G A G C T G

I.   T C A G A C G A G T G

     T C G G A - - G C T G

II.  T C A G A C G A G T G

     T C G G A - G C - T G

III. T C A G A C G A G T G

     T C G G A - G - C T G
```

Topics to Cover

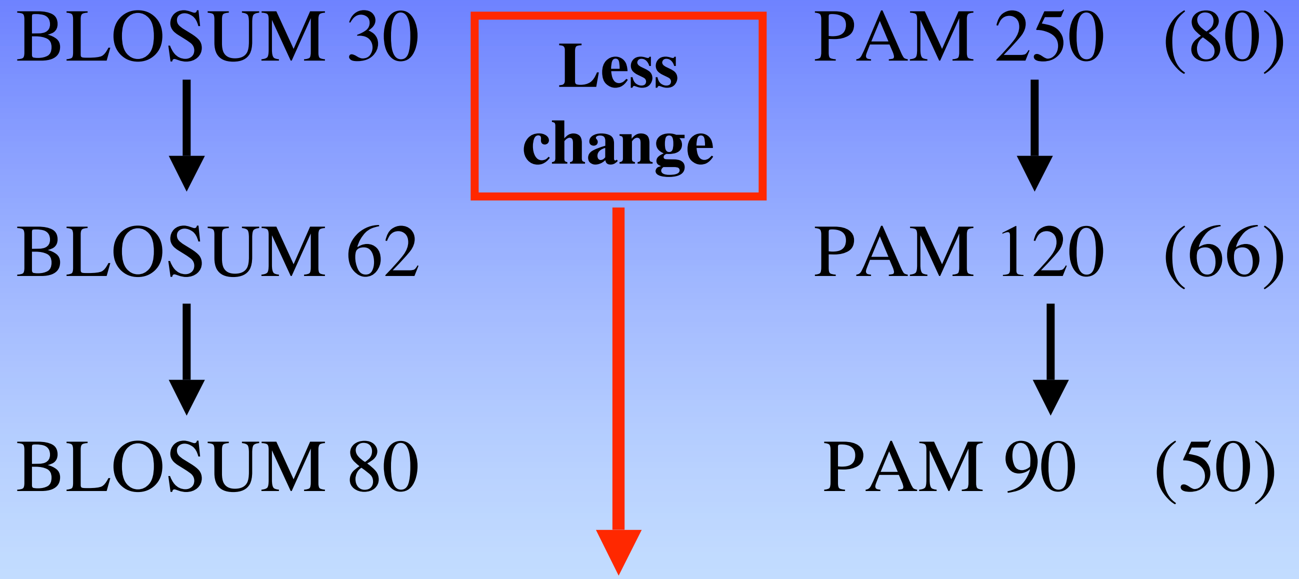
- Introduction
- Scoring alignments
  - Nucleotide vs Proteins
- Alignment methods
- Significance of alignments
- Database searching methods

Amino Acid Substitution Matrices

- ***PAM* -** point accepted mutation based on *global* alignment [evolutionary model]

- ***BLOSUM*** - block substitutions based on *local* alignments [similarity among conserved sequences]

Substitution Matrices



BLOSUM 30

BLOSUM 62

BLOSUM 80

Less change

PAM 250 (80)

PAM 120 (66)

PAM 90 (50)

% identity

% change

Part of BLOSUM 62 Matrix

	C	S	T	P	A	G	N
C	9						
S	-1	4					
T	-1	1	5				
P	-3	-1	-1	7			
A	0	1	0	-1	4		
G	-3	0	-2	-2	0	6	
N	-3	1	0	-2	-2	0	

$$\text{Log-odds} = \frac{\text{obs freq of aa substitutions}}{\text{freq expected by chance}}$$

Part of BLOSUM 62 Matrix

	C	S	T	P	A	G	N
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A	0	1	0	-1	4		
G	-3	0	-2	-2	0	6	
N	-3	1	0	-2	-2	0	

$$\text{Log-odds} = \frac{\text{obs freq of aa substitutions}}{\text{freq expected by chance}}$$

Part of PAM 250 Matrix

	C	S	T	P	A	G	N
C	1	2					
S	0	2					
T	-2	1	3				
P	-3	1	0	6			
A	-2	1	1	1	2		
G	-3	1	0	-1	1	5	
N	-4	1	0	-1	0	0	

$$\text{Log-odds} = \frac{\text{pair in homologous proteins}}{\text{pair in unrelated proteins by chance}}$$

Part of PAM 250 Matrix

	C	S	T	P	A	G	N
C	1	2					
S	0	2					
T	-2	1	3				
P	-3	1	0	6			
A	-2	1	1	1	2		
G	-3	1	0	-1	1	5	
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$$\text{Log-odds} = \frac{\text{pair in homologous proteins}}{\text{pair in unrelated proteins by chance}}$$

Gap Penalties

- ***Insertion and Deletions*** (indels)
- ***Affine gap costs*** - a scoring system for gaps within alignments that charges a penalty for the existence of a gap and an additional per-residue penalty proportional to the gap's length

Example of simple scoring system for nucleic acids

- Match = +1 (ex. A-A, T-T, C-C, G-G)
- Mismatch = -1 (ex. A-T, A-C, etc)
- Gap opening = - 2
- Gap extension = -1

Example of simple scoring system for nucleic acids

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- Gap extension = -1

```
T  C  A  G  A  C  G  A  G  T  G
T  C  G  G  A  -  -  G  C  T  G
```

Example of simple scoring system for nucleic acids

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T  C  A  G  A  C  G  A  G  T  G
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- Gap opening = - 2
- Gap extension = -1

```
 T   C   A   G   A   C   G   A   G   T   G
 T   C   G   G   A   -   -   G   C   T   G
--------------------------------------------
+1  +1  -1  +1  +1  -2  -1  -1  -1  +1  +1  =  0
```

Scoring for BLAST 2 Sequences

```
Score = 94.0 bits (230), Expect = 6e-19
Identities = 45/101 (44%), Positives = 54/101 (52%), Gaps = 7/101 (6%)

Query: 204 YTGPFCDV----DTKASCYDGRGLSYRGLARTTLSGAPCQPWASEATYRNVTAEQ---AR 256
           Y+  FC      +  + CY G G +YRG    T SGA C PW S      V   Q   A+
Sbjct: 198 YSSEFCSTPACSEGNSDCYFGNGSAYRGTHSLTESGASCLPWNSMILIGKVYTAQNPSAQ 257

Query: 257 NWGLGGHAFCRNPDNDIRPWCFVLNRDRLSWEYCDLAQCQT 297
             GLG H +CRNPD D +PWC VL   RL+WEYCD+   C T
Sbjct: 258 ALGLGKHNYCRNPDGDAKPWCHVLKNRRLTWEYCDVPSCST 298
```

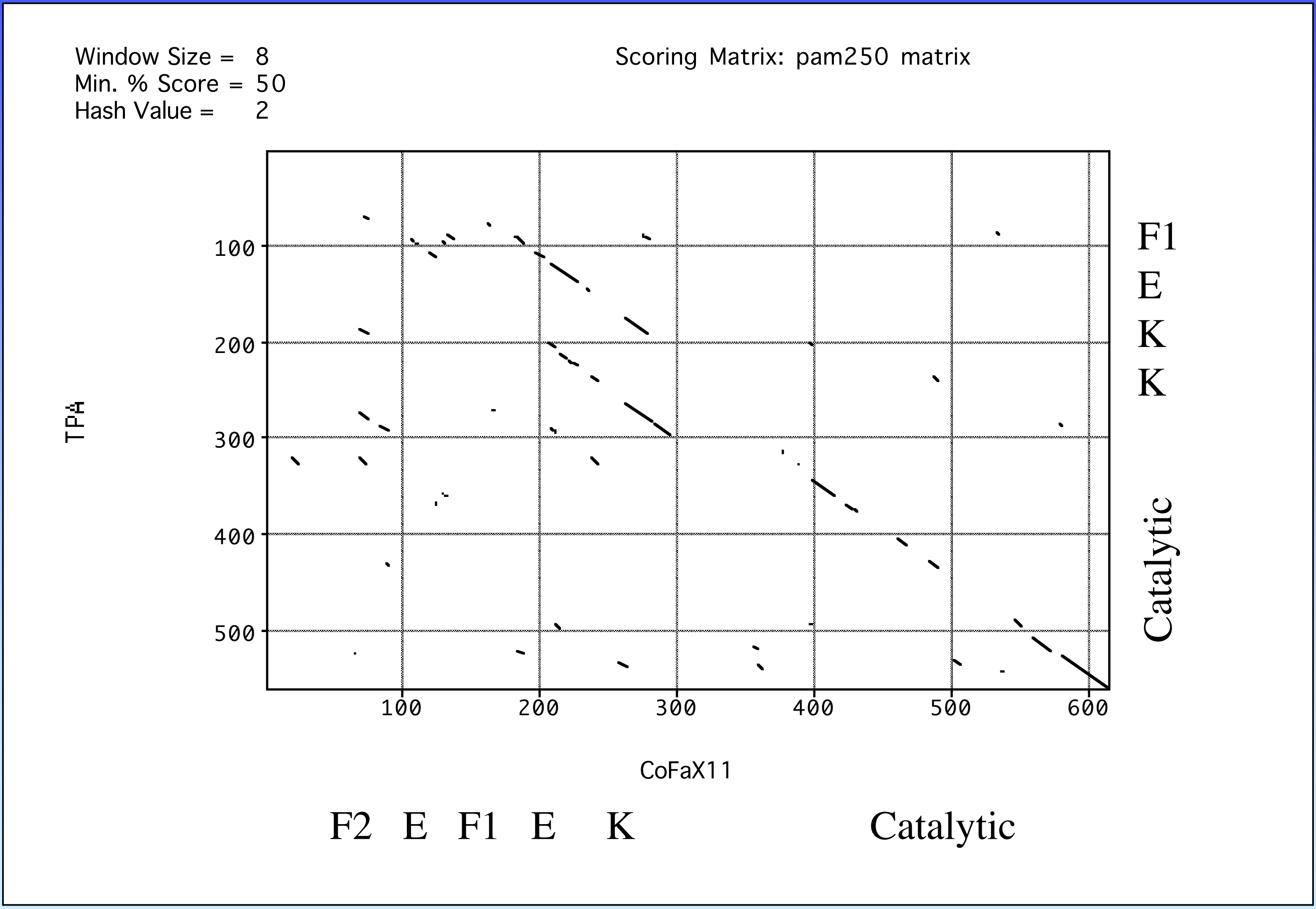
Based on
BLOSUM62

```
Position  1: Y - Y =   7
Position  2: T - S =   1
Position  3: G - S =   0
Position  4: P - E =  -1
        . . .
Position  9: - - P = -11
Position 10: - - A =  -1
        . . .
             Sum     230
```

Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
  - – Dot matrix analysis
  - – Exhaustive methods; Dynamic programming algorithm (Smith-Waterman (Local), Needleman-Wunsch (Global))
  - – Heuristic methods; Approximate methods; word or k-tuple (FASTA, BLAST, BLAT)
- Significance of alignments
- Database searching methods

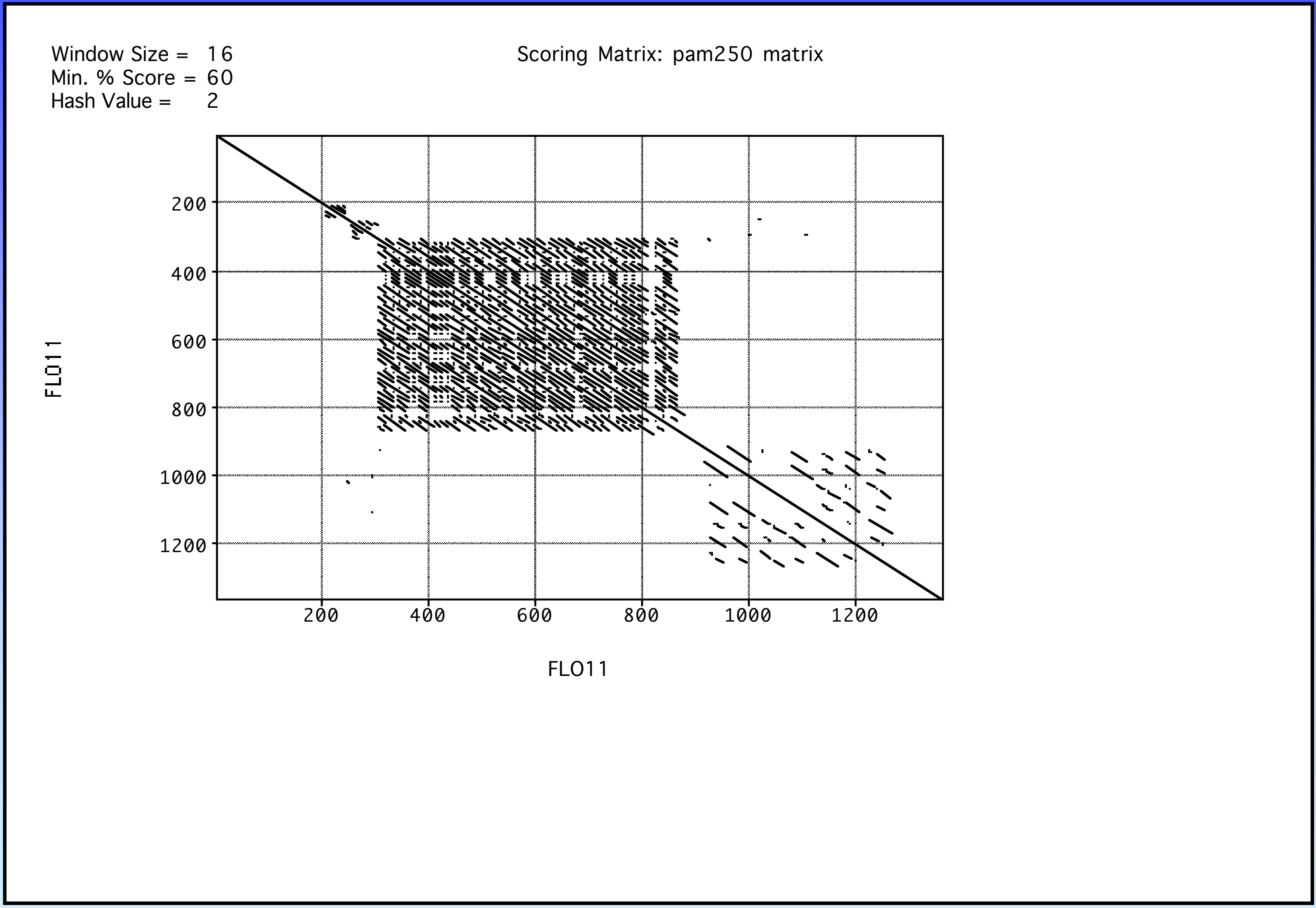
Dot Matrix Comparison



Window Size = 8
Min. % Score = 50
Hash Value = 2

Scoring Matrix: pam250 matrix

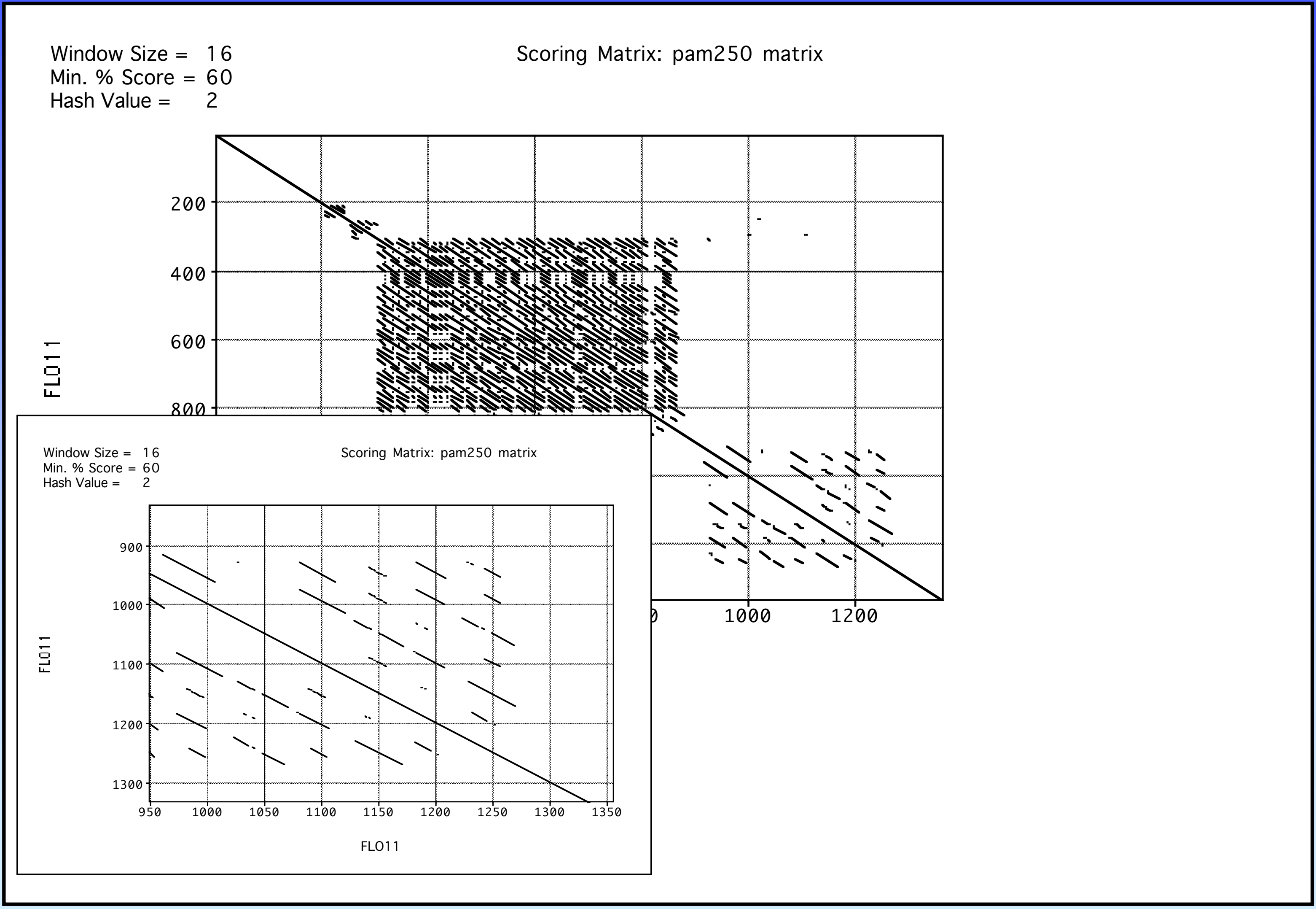
Dot Matrix Comparison



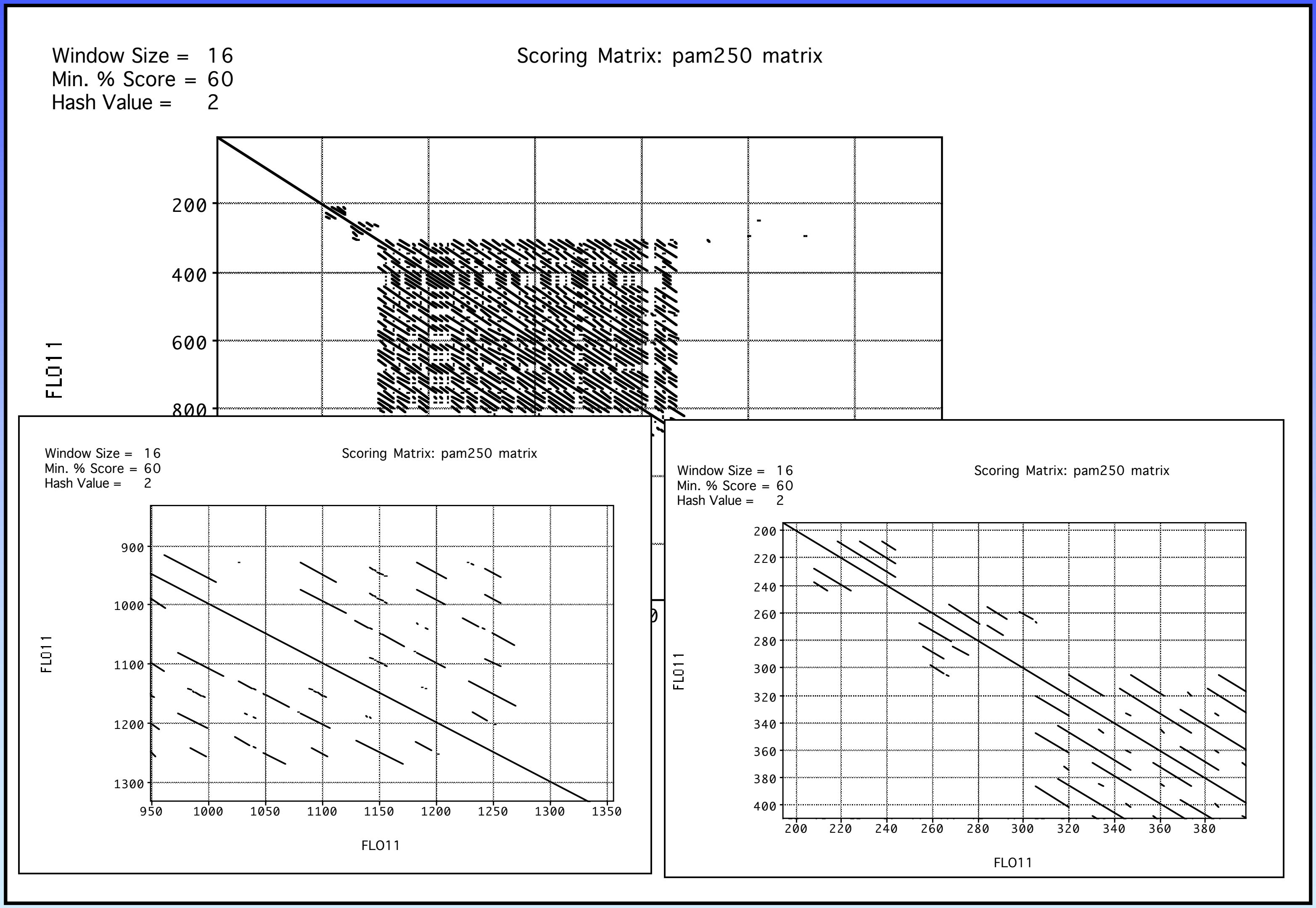
Window Size = 16
Min. % Score = 60
Hash Value = 2

Scoring Matrix: pam250 matrix

Dot Matrix Comparison



Dot Matrix Comparison



Dynamic Programming

Dynamic Programming

- Provides very best or optimal alignment

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- Compares every pair of characters (e.g. bases or amino acids) in the two sequences

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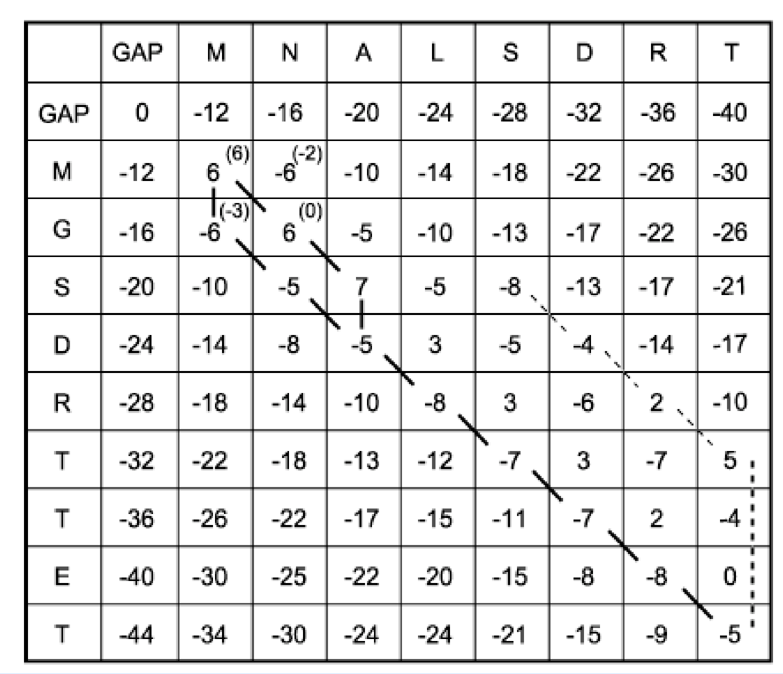
- Compares every pair of characters (e.g. bases or amino acids) in the two sequences
- Puts in gaps and mismatches
- Maximum number of matches between identical or related characters
- Generates a score and statistical assessment

Dynamic Programming

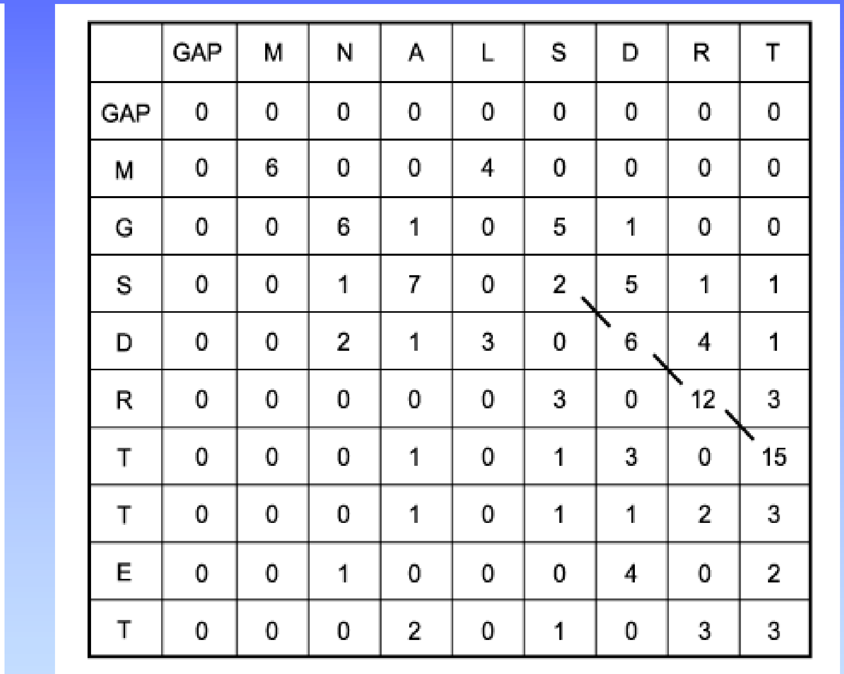
- Provides very best or optimal alignment
- Compares every pair of characters (e.g. bases or amino acids) in the two sequences
- Puts in gaps and mismatches
- Maximum number of matches between identical or related characters
- Generates a score and statistical assessment
- Nice example of global alignment using N-W: http://www.sbc.su.se/~per/molbioinfo2001/dynprog/dynamic.html

Global vs Local Alignment

(example from Mount 2001)



	GAP	M	N	A	L	S	D	R	T
GAP	0	-12	-16	-20	-24	-28	-32	-36	-40
M	-12	6 (6)	-6 (-2)	-10	-14	-18	-22	-26	-30
G	-16	-6 (-3)	6 (0)	-5	-10	-13	-17	-22	-26
S	-20	-10	-5	7	-5	-8	-13	-17	-21
D	-24	-14	-8	-5	3	-5	-4	-14	-17
R	-28	-18	-14	-10	-8	3	-6	2	-10
T	-32	-22	-18	-13	-12	-7	3	-7	5
T	-36	-26	-22	-17	-15	-11	-7	2	-4
E	-40	-30	-25	-22	-20	-15	-8	-8	0
T	-44	-34	-30	-24	-24	-21	-15	-9	-5



	GAP	M	N	A	L	S	D	R	T
GAP	0	0	0	0	0	0	0	0	0
M	0	6	0	0	4	0	0	0	0
G	0	0	6	1	0	5	1	0	0
S	0	0	1	7	0	2	5	1	1
D	0	0	2	1	3	0	6	4	1
R	0	0	0	0	0	3	0	12	3
T	0	0	0	1	0	1	3	0	15
T	0	0	0	1	0	1	1	2	3
E	0	0	1	0	0	0	4	0	2
T	0	0	0	2	0	1	0	3	3

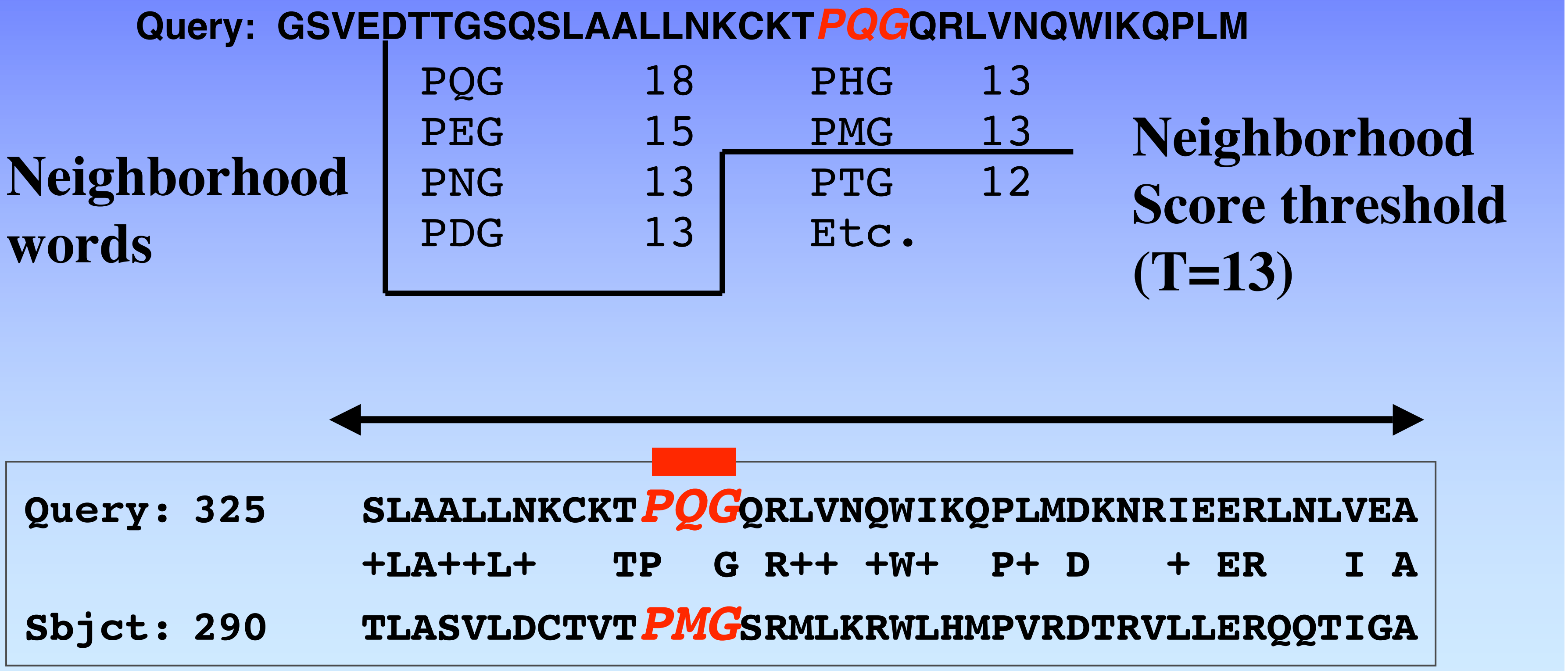
```
sequence 1    M  -   N  A  L  S  D  R  T
sequence 2    M  G   S  D  R  T  T  E  T
score         6 -12  1  0 -3  1  0 -1  3   =  -5
```

```
sequence 1     S D R T
sequence 2     S D R T
score          2 4 6 3 = 15
```

Original "Ungapped" BLAST Algorithm

- To improve speed, use a word based hashing scheme to index database
- Limit search for similarities to only the region near matching words
- Use **T**hreshold parameter to rate neighbor words
- Extend match left and right to search for high scoring alignments

Original BLAST Algorithm (1990)



Query word (W=3)

Query: GSVEDTTGSQSLAALLNKCKT*PQG*QRLVNQWIKQPLM

Neighborhood words

PQG	18	PHG	13
PEG	15	PMG	13
PNG	13	PTG	12
PDG	13	Etc.	

Neighborhood Score threshold (T=13)

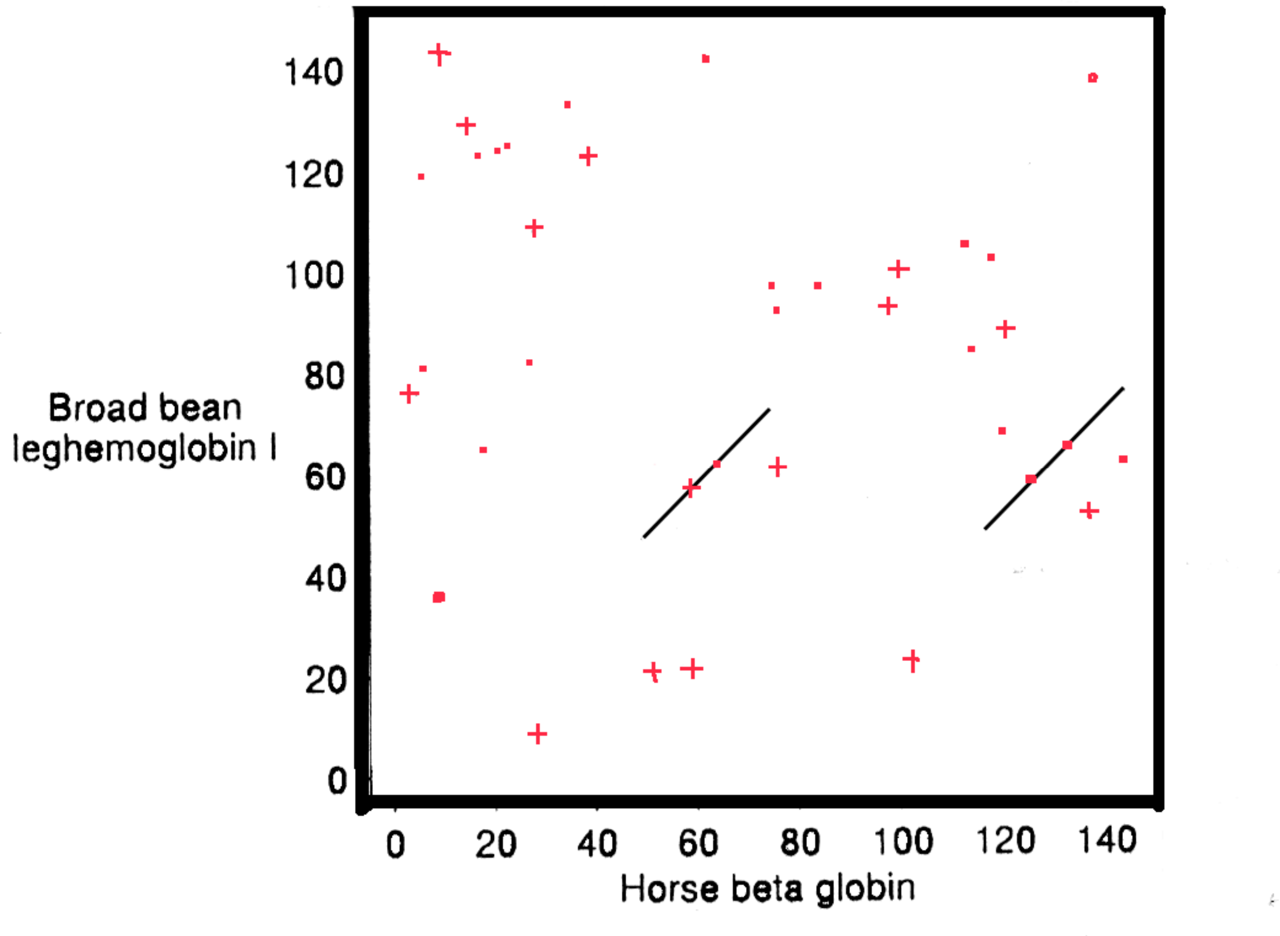
```
Query: 325   SLAALLNKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEA
             +LA++L+   TP G R++ +W+  P+ D   + ER   I A
Sbjct: 290   TLASVLDCTVTPMGSRMLKRWLHMPVRDTRVLLERQQTIGA
```

BLAST Refinements (1997)

- “two-hit” method for extending word pairs
- Gapped alignments
- Iterate with position-specific matrix (PSI-BLAST)
- Pattern-hit initiated BLAST (PHI-BLAST)

Gapped BLAST

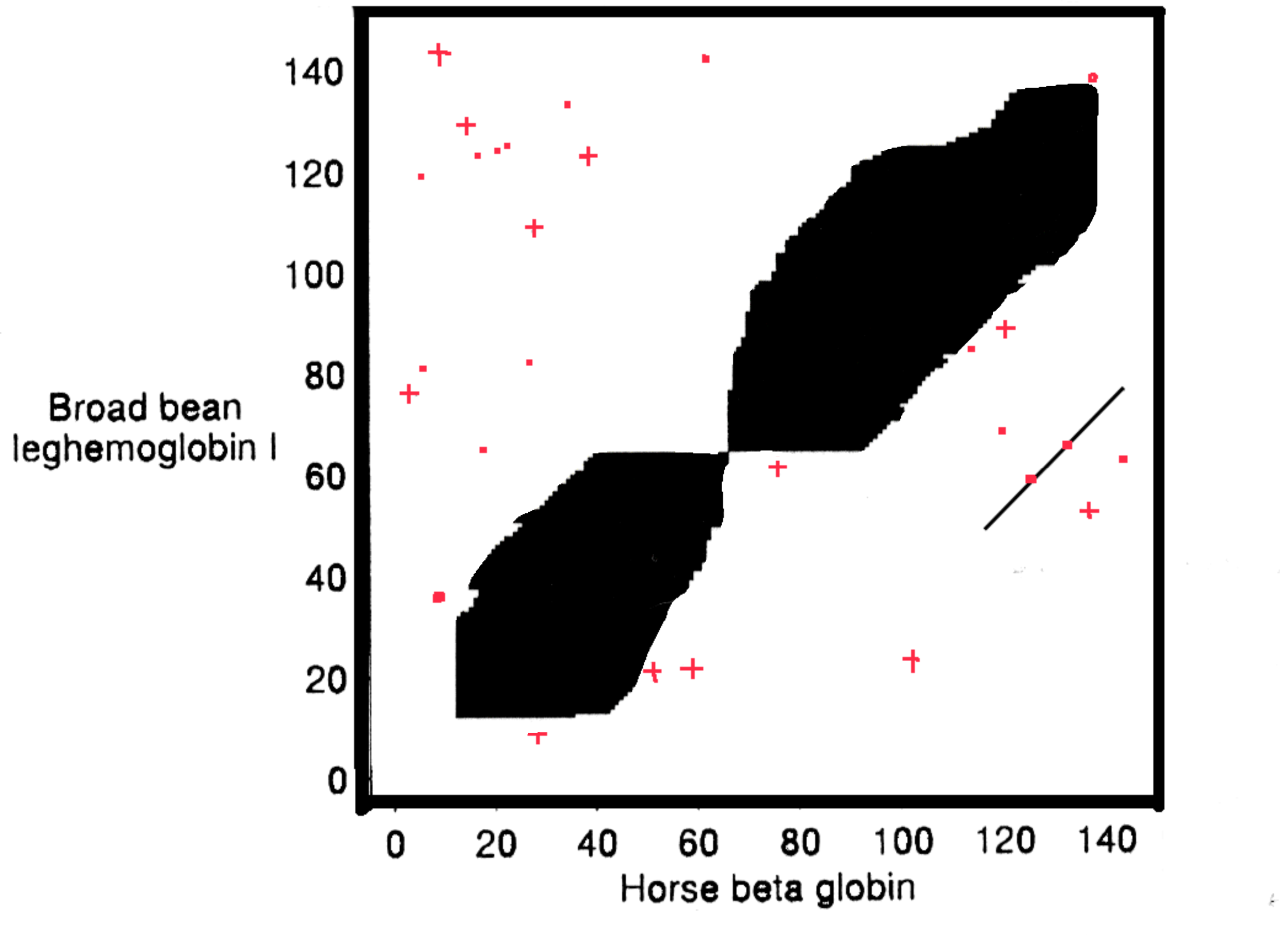
15(+) > 13
22(•) > 11



(Altschul et al 1997)

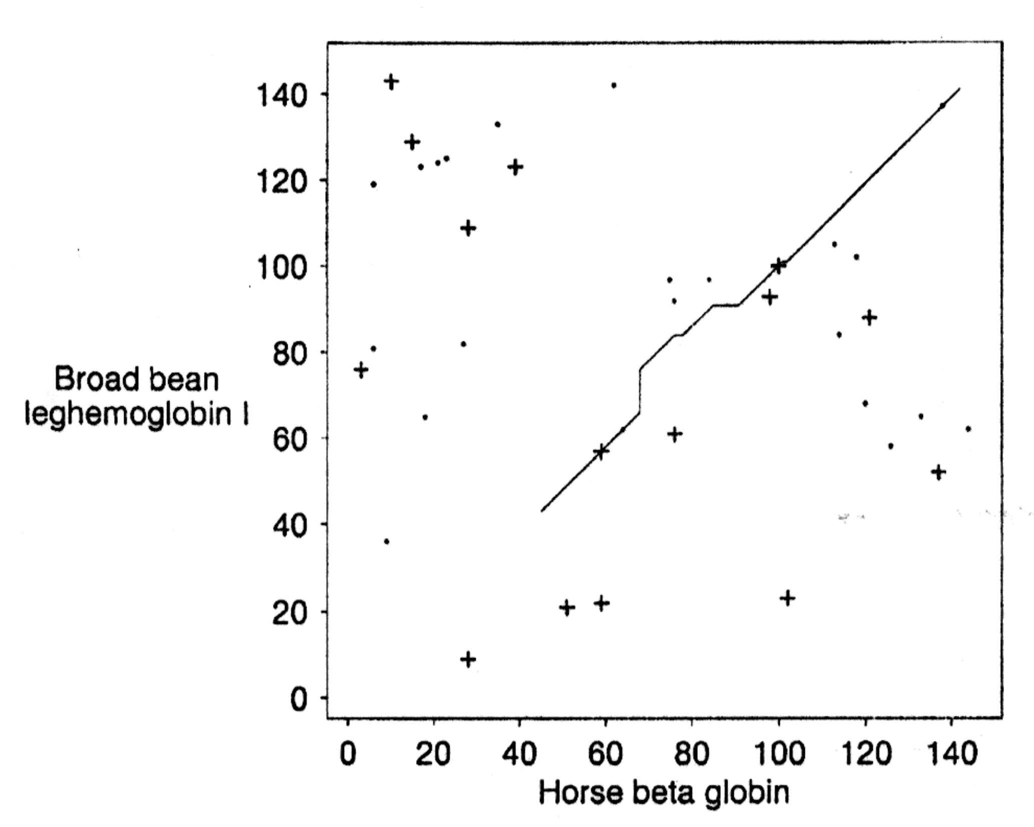
Gapped BLAST

15(+) > 13
22(•) > 11



(Altschul et al 1997)

Gapped BLAST



(Altschul et al 1997)

Programs to Compare two sequences - Unix or Web

NCBI

BLAST 2 Sequences

EMBOSS

water - Smith-Waterman

needle - Needleman -Wunsch

dotmatch (dot plot)

einverted or palindrome (inverted repeats)

equicktandem or etandem (tandem repeats)

Other

lalign (multiple matching subsegments in two sequences)

Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
- Significance of alignments
- Database searching methods
- Demo

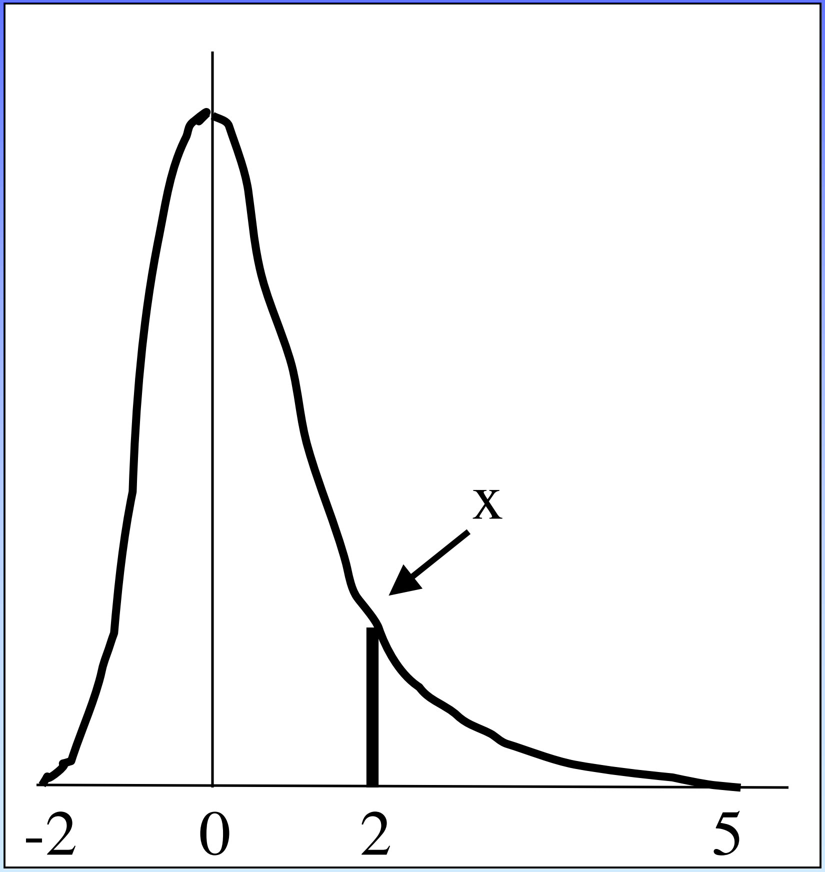
Significance of Alignment

How strong can an alignment be expected by chance alone?

- Real but non-homologous sequences
- Real sequences that are shuffled to preserve compositional properties
- Sequences that are generated randomly based upon a DNA or protein sequence model

Extreme Value Distribution

- When 2 sequences have been aligned optimally, the significance of a local alignment score can be tested on the basis of the distribution of scores expected by aligning two random sequences of the same length and composition as the two test sequences.



Statistical Significance

- ***Raw Scores*** - score of an alignment equal to the sum of substitution and gap scores.
- ***Bit scores*** - scaled version of an alignment's raw score that accounts for the statistical properties of the scoring system used.
- ***E-value*** - expected number of distinct alignments that would achieve a given score by chance. Lower E-value => more significant.

Some formulas

$$E = Kmn\ e^{-\lambda S}$$

This is the Expected number of high-scoring segment pairs (HSPs) with score at least S for sequences of length m and n.

This is the E value for the score S.

Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
- Significance of alignments
- Database searching methods
  - BLAST - ungapped and gapped
  - BLAST vs. FASTA
  - BLAT

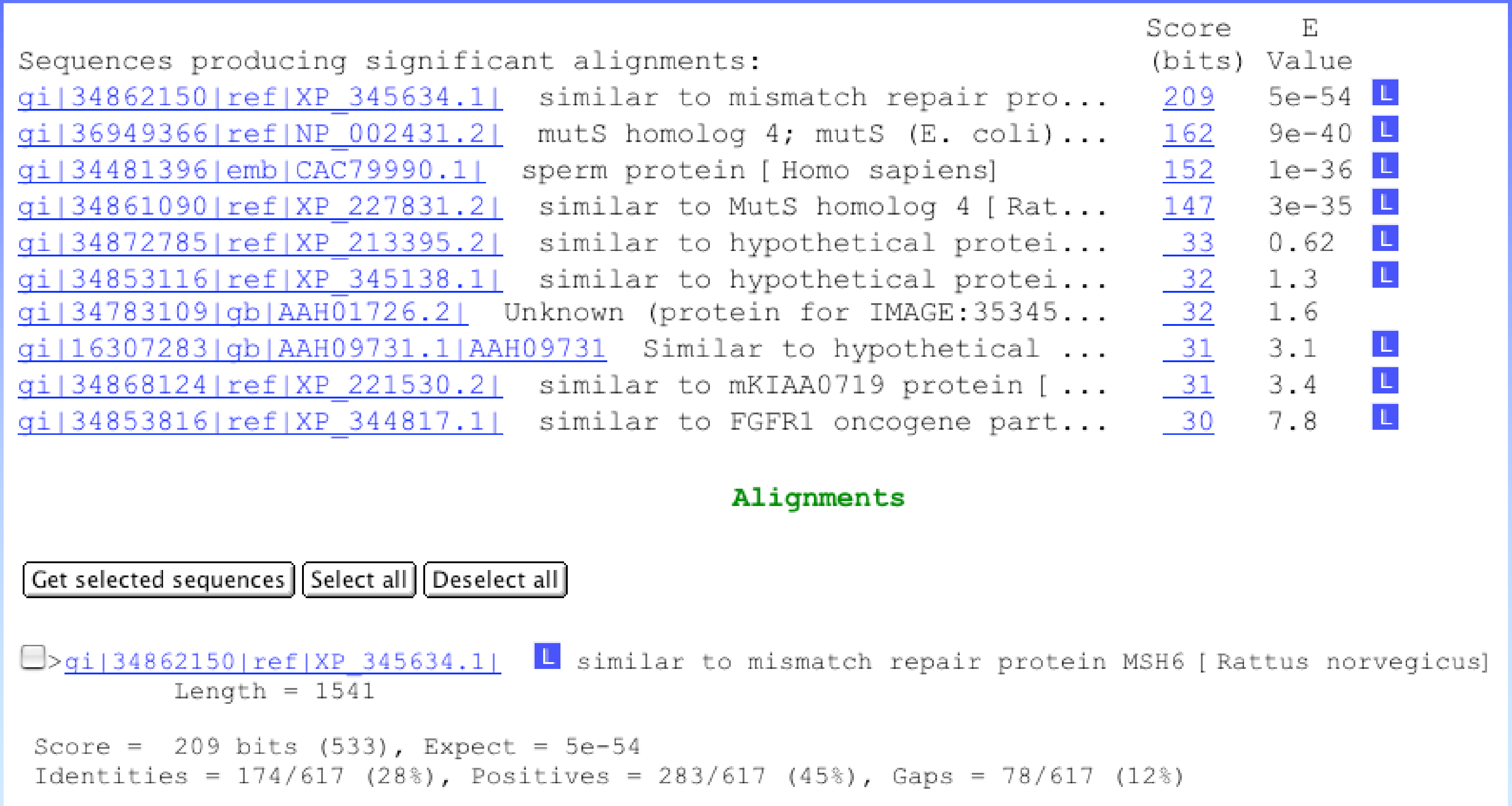
Questions

- Why do a database search?
- What database should be searched?
- What alignment algorithm to use?
- What do the results mean?

Issues affecting DB Search

- Substitution matrices
- Statistical significance
- Filtering
- Database choices

BLASTP Results



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

gi|34862150|ref|XP_345634.1|  similar to mismatch repair pro...   209   5e-54  L
gi|36949366|ref|NP_002431.2|  mutS homolog 4; mutS (E. coli)...   162   9e-40  L
gi|34481396|emb|CAC79990.1|  sperm protein [Homo sapiens]         152   1e-36  L
gi|34861090|ref|XP_227831.2|  similar to MutS homolog 4 [Rat...   147   3e-35  L
gi|34872785|ref|XP_213395.2|  similar to hypothetical protei...    33   0.62   L
gi|34853116|ref|XP_345138.1|  similar to hypothetical protei...    32   1.3    L
gi|34783109|gb|AAH01726.2|  Unknown (protein for IMAGE:35345...    32   1.6
gi|16307283|gb|AAH09731.1|AAH09731  Similar to hypothetical ...    31   3.1    L
gi|34868124|ref|XP_221530.2|  similar to mKIAA0719 protein [...    31   3.4    L
gi|34853816|ref|XP_344817.1|  similar to FGFR1 oncogene part...    30   7.8    L
```

Alignments

```
□>gi|34862150|ref|XP_345634.1|  L similar to mismatch repair protein MSH6 [Rattus norvegicus]
          Length = 1541

 Score =  209 bits (533), Expect = 5e-54
 Identities = 174/617 (28%), Positives = 283/617 (45%), Gaps = 78/617 (12%)
```

Low Complexity Regions

- **Local regions of biased composition**
- **Common in real sequences**
- **Generate false positives on BLAST search**

- **DUST for BLASTN (n’s in sequence)**
- **SEG for other programs (x’s in sequence)**

Filtering is only applied to the query sequence (or its translation products), not to database sequences.

Filtered Sequence

```
>HUMAN MSH2

MAVQPKETLQLESAAEVGFVRFFQGMPEKPTTTVRLFDRGDFYTAHGEDALLAAR
EVFKTQGVIKYMGPAGAKNLQSVVLSKMNFESFVKDLLLVRQYRVEVYKNRAGNK
ASKENDWYLAYKASPGNLSQFEDILFGNNDMSASIGVVGVKMSAVDGQRQVGVGY
VDSIQRKLGLCEFPDNDQFSNLEALLIQIGPKECVLPGGETAGDMGKLRQIIQRG
GILITERKKADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIK
FLELLSDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALL
NKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVEDAELRQTLQEDLLRRFP
DLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQALEKHEGKHQKLLLAVFVTPL
TDLRSDFSKFQEMIETTLDMDQVENHEFLVKPSFDPNLSELREIMNDLEKKMQST
LISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVK
FTNSKLTSLNEEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAV
VSFAHVSNGAPVPYVRPAILEKGQGRIILKASRHACVEVQDEIAFIPNDVYFEKD
KQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAG
DSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQS
FGIHVAELANFPKHVIECAKQKALELEEFQYIGESQGYDIMEPAAKKCYLEREQG
EKIIQEFLSKVKQMPFTEMSEENITIKLKQLKAEVIAKNNSFVNEIISRIKVTT
```

Filtered Sequence

NEEYTKNKTEYEE

```
>HUMAN MSH2
QGMPEKPTTTVRLFDRGDFYTAHGEDALLAAR
VLSKMNFESFVKDLLLVRQYRVEVYKNRAGNK
ILFGNNDMSASIGVVGVKMSAVDGQRQVGVGY
ALLIQIGPKECVLPGGETAGDMGKLRQIIQRG
GILITERKKADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIK
FLELLSDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALL
NKCKTPQGQRLVNQWIKQPLMDKNRIEERLNLVEAFVEDAELRQTLQEDLLRRFP
DLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQALEKHEGKHQKLLLAVFVTPL
TDLRSDFSKFQEMIETTLDMDQVENHEFLVKPSFDPNLSELREIMNDLEKKMQST
LISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVK
FTNSKLTSLNEEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAV
VSFAHVSNGAPVPYVRPAILEKGQGRIILKASRHACVEVQDEIAFIPNDVYFEKD
KQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAG
DSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQS
FGIHVAELANFPKHVIECAKQKALELEEFQYIGESQGYDIMEPAAKKCYLEREQG
EKIIQEFLSKVKQMPFTEMSEENITIKLKQLKAEVIAKNNSFVNEIISRIKVTT
```

Filtered Sequence

```
>HUMAN MSH2
                                                                                                                                  
                    QGMPEKPTTTVRLFDRGDFYTAHGEDALLAAR
                    VLSKMNFESFVKDLLLVRQYRVEVYKNRAGNK
                    ILFGNNDMSASIGVVGVKMSAVDGQRQVGVGY
                    ALLIQIGPKECVLPGGETAGDMGKLRQIIQRG
GILITERKKADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIK
FLELLSDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALL
NKCKTPQGQRLVNQWIKQPLMDKNRIEE
DLNRLAKKFQRQAANLQDCYRLYQGINQ
TDLRSDFSKFQEMIETTLDMDQVENHEF
LISAARDLGLDPGKQIKLDSSAQFGYYF
FTNSKLTSLNEEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAV
VSFAHVSNGAPVPYVRPAILEKGQGRIILKASRHACVEVQDEIAFIPNDVYFEKD
KQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAG
DSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQS
FGIHVAELANFPKHVIECAKQKALELEEFQYIGESQGYDIMEPAAKKCYLEREQG
EKIIQEFLSKVKQMPFTEMSEENITIKLKQLKAEVIAKNNSFVNEIISRIKVTT
```

NEEYTKNKTEYEE

TALTTEETLT

Example Alignment w/o filtering

Score = 29.6 bits (65), Expect = 1.8
Identities = 22/70 (31%), Positives = 32/70 (45%), Gaps = 12/70 (17%)

```
Query: 31   PPPTTQGAPRTSSFTPTTLT------------NGTSHSPTALNGAPSPPNGFS 71
            PPP+ Q   R  S +  T T            NG+S S ++ + + S  +  S
Sbjct: 1221 PPPSVQNQQRWGSSSVITTTCQQRQQSVSPHSNGSSSSSSSSSSSSSSSSSTS 1273

Query: 72   NGPSSSSSSSLANQQLP 88
            +  SSSS+SS    Q P
Sbjct: 1274 SNCSSSSASSCQYFQSP 1290
```

Example BLAST w/ filtering

```
 Score = 36.6 bits (83), Expect = 0.67
 Identities = 21/58 (36%), Positives = 25/58 (42%), Gaps = 1/58 (1%)

Query: 471 AEDALAVINQQEDSSESCWNCGRKASETCSGCNTARYCGSFCQHKDWE-KHHHICGQT 527
           A D   V  Q +  +  C  CG  A  TCS C  A YC    Q  DW+  H   C Q+
Sbjct: 61  ASDTECVCLQLKSGAHLCRVCGCLAPMTCSRCKQAHYCSKEHQTLDWQLGHKQACTQS 118
```

```
 Score = 37.0 bits (84), Expect = 0.55
 Identities = 18/55 (32%), Positives = 22/55 (39%)

Query: 483 DSSESCWNCGRKASETCSGCNTARYCGSFCQHKDWEKHHHICGQTLQAQQQGDTP 537
           D    C  CG  A++ C+ C  ARYC    Q  DW  H   C +        D P
Sbjct: 75  DGPGLCRICGCSAAKKCAKCQVARYCSQAHQVIDWPAHKLECAKAATDGSITDEP 129
```

WU-BLAST vs NCBI BLAST

- WU-BLAST first for gapped alignments
- Use different scoring system for gaps
- Report different statistics
- WU-BLAST does not filter low-complexity by default
- WU-BLAST looks for and reports multiple regions of similarity
- Results will be different

BLAT

- ***B***last-***L***ike ***A***lignment ***T***ool
- Developed by Jim Kent at UCSC
- For DNA it is designed to quickly find sequences of >= 95% similarity of length 40 bases or more.
- For proteins it finds sequences of >= 80% similarity of length 20 amino acids or more.
- DNA BLAT works by keeping an index of the entire genome in memory - non-overlapping 11-mers (< 1 GB of RAM)
- Protein BLAT uses 4-mers (~ 2 GB)

FASTA

- Index "words" and locate identities
- Rescore best 10 regions
- Find optimal subset of initial regions that can be joined to form single alignment
- Align highest scoring sequences using Smith-Waterman

NCBI Programs for nt vs nt

- Discontiguous megablast
- Megablast
- Nucleotide-nucleotide BLAST (blastn)
- Search for short, nearly exact matches

NCBI Programs for proteins

- Protein-protein BLAST (blastp)
- PHI- and PSI-BLAST
- Search for short, nearly exact matches
- Search the conserved domain database (rpsblast)
- Search by domain architecture (cdart)

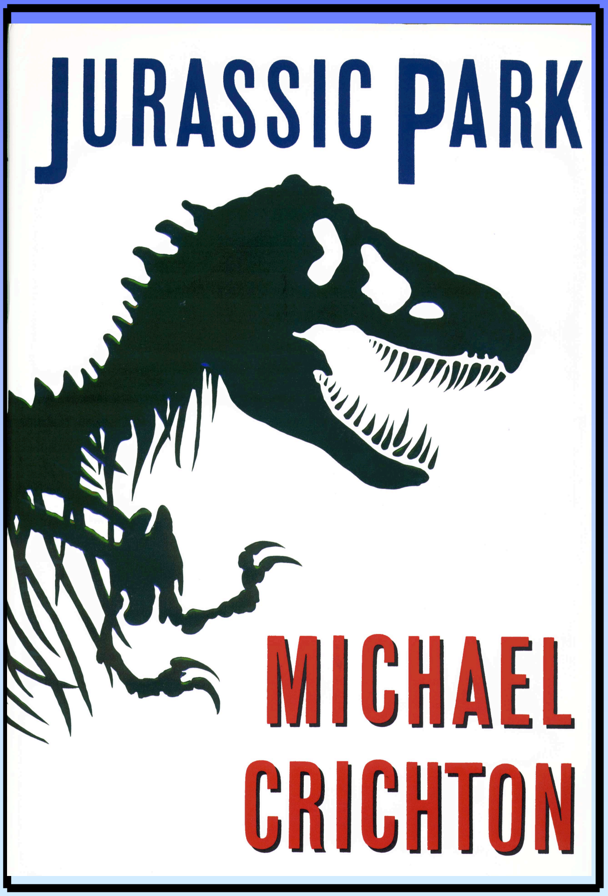
NCBI Programs w/ translations

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

Basic Searching Strategies

- Search early and often
- Use specialized databases
- Use multiple matrices
- Use filters
- Consider Biology

Sequence of Note



```
   1 GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC
  31 CCCCCTGACG AGCATCACAA AAATCGACGC
  61 GGTGGCGAAA CCCGACAGGA CTATAAAGAT
…………..
1371 GTAAAGTCTG GAAACGCGGA AGTCAGCGCC
```

"Here you see the actual structure of a small fragment of dinosaur DNA," Wu said. "Notice the sequence is made up of four compounds - adenine, guanine, thymine and cytosine. This amount of DNA probably contains instructions to make a single protein - say, a hormone or an enzyme. The full DNA molecule contains *three billion* of these bases. If we looked at a screen like this once a second, for eight hours a day, it'd still take more than two years to look at the entire DNA strand. It's that big." (page 103)

DinoDNA "Dinosaur DNA" from Crichton's THE LOST WORLD p. 135

```
GAATTCCGGAAGCGAGCAAGAGATAAGTCCTGGCATCAGA
TACAGTTGGAGATAAGGACGACGTGTGGCAGCTCCCGCAG
AGGATTCACTGGAAGTGCATTACCTATCCCATGGGAGCCA
TGGAGTTCGTGGCGCTGGGGGGGCCGGATGCGGGCTCCCC
CACTCCGTTCCCTGATGAAGCCGGAGCCTTCCTGGGGCTG
GGGGGGGGCGAGAGGACGGAGGCGGGGGGGCTGCTGGCCT
CCTACCCCCCCTCAGGCCGCGTGTCCCTGGTGCCGTGGCA
GACACGGGTACTTTGGGGACCCCCCAGTGGGTGCCGCCCG
CCACCCAAATGGAGCCCCCCCACTACCTGGAGCTGCTGCA
ACCCCCCCGGGGCAGCCCCCCCCATCCCTCCTCCGGGCCC
CTACTGCCACTCAGCAGCGCCTGCGGCCTCTACTACAAAC
```

....

```
>Erythroid transcription factor (NF-E1 DNA-binding protein)

Query: 121  MEFVALGGPDAGSPTPFPDEAGAFLGLGGGERTEAGGLLASYPPSGRVSLVPWADTGTLG 300
            MEFVALGGPDAGSPTPFPDEAGAFLGLGGGERTEAGGLLASYPPSGRVSLVPWADTGTLG
Sbjct: 1    MEFVALGGPDAGSPTPFPDEAGAFLGLGGGERTEAGGLLASYPPSGRVSLVPWADTGTLG 60

Query: 301  TPQWVPPATQMEPPHYLELLQPPRGSPPHPSSGPLLPLSSGPPPCEARECVMARKNCGAT 480
            TPQWVPPATQMEPPHYLELLQPPRGSPPHPSSGPLLPLSSGPPPCEARECV    NCGAT
Sbjct: 61   TPQWVPPATQMEPPHYLELLQPPRGSPPHPSSGPLLPLSSGPPPCEARECV----NCGAT 116

Query: 481  ATPLWRRDGTGHYLCNWASACGLYHRLNGQNRPLIRPKKRLLVSKRAGTVCSHERENCQT 660
            ATPLWRRDGTGHYLCN   ACGLYHRLNGQNRPLIRPKKRLLVSKRAGTVCS    NCQT
Sbjct: 117  ATPLWRRDGTGHYLCN---ACGLYHRLNGQNRPLIRPKKRLLVSKRAGTVCS----NCQT 169

Query: 661  STTTLWRRSPMGDPVCNNIHACGLYYKLHQVNRPLTMRKDGIQTRNRKVSSKGKKRRPPG 840
            STTTLWRRSPMGDPVCN   ACGLYYKLHQVNRPLTMRKDGIQTRNRKVSSKGKKRRPPG
Sbjct: 170  STTTLWRRSPMGDPVCN---ACGLYYKLHQVNRPLTMRKDGIQTRNRKVSSKGKKRRPPG 226

Query: 841  GGNPSATAGGGAPMGGGGDPSMPPPPPPPAAAPPQSDALYALGPVVLSGHFLPFGNSGGF 1020
            GGNPSATAGGGAPMGGGGDPSMPPPPPPPAAAPPQSDALYALGPVVLSGHFLPFGNSGGF
Sbjct: 227  GGNPSATAGGGAPMGGGGDPSMPPPPPPPAAAPPQSDALYALGPVVLSGHFLPFGNSGGF 286

Query: 1021 FGGGAGGYTAPPGLSPQI 1074
            FGGGAGGYTAPPGLSPQI
Sbjct: 287  FGGGAGGYTAPPGLSPQI 304
```

>Erythroid transcription factor (NF-E1 DNA-binding protein)

```
Query: 121   MEFVALGGPDAGSPTPFPDEAGAFLGLGGGERTEAGGLLASYPPSGRVSLVPWADTGTLG 300
             MEFVALGGPDAGSPTPFPDEAGAFLGLGGGERTEAGGLLASYPPSGRVSLVPWADTGTLG
Sbjct: 1     MEFVALGGPDAGSPTPFPDEAGAFLGLGGGERTEAGGLLASYPPSGRVSLVPWADTGTLG 60

Query: 301   TPQWVPPATQMEPPHYLELLQPPRGSPPHPSSGPLLPLSSGPPPCEARECYMARKNCGAT 480
             TPQWVPPATQMEPPHYLELLQPPRGSPPHPSSGPLLPLSSGPPPCEARECY    NCGAT
Sbjct: 61    TPQWVPPATQMEPPHYLELLQPPRGSPPHPSSGPLLPLSSGPPPCEARECY----NCGAT 116

Query: 481   ATPLWRRDGTGHYLCNWASACGLYHRLNGQNRPLIRPKKRLLVSKRAGTVCSHERENCQT 660
             ATPLWRRDGTGHYLCN   ACGLYHRLNGQNRPLIRPKKRLLVSKRAGTVCS    NCQT
Sbjct: 117   ATPLWRRDGTGHYLCN---ACGLYHRLNGQNRPLIRPKKRLLVSKRAGTVCS----NCQT 169

Query: 661   STTTLWRRSPMGDPVCNNIHACGLYYKLHQVNRPLTMRKDGIQTRNRKVSSKGKKRRPPG 840
             STTTLWRRSPMGDPVCN   ACGLYYKLHQVNRPLTMRKDGIQTRNRKVSSKGKKRRPPG
Sbjct: 170   STTTLWRRSPMGDPVCN---ACGLYYKLHQVNRPLTMRKDGIQTRNRKVSSKGKKRRPPG 226

Query: 841   GGNPSATAGGGAPMGGGGDPSMPPPPPPPAAAPPQSDALYALGPVVLSGHFLPFGNSGGF 1020
             GGNPSATAGGGAPMGGGGDPSMPPPPPPPAAAPPQSDALYALGPVVLSGHFLPFGNSGGF
Sbjct: 227   GGNPSATAGGGAPMGGGGDPSMPPPPPPPAAAPPQSDALYALGPVVLSGHFLPFGNSGGF 286

Query: 1021  FGGGAGGYTAPPGLSPQI 1074
             FGGGAGGYTAPPGLSPQI
Sbjct: 287   FGGGAGGYTAPPGLSPQI 304
```

Useful Web Links

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

http://www.ebi.ac.uk/blast2/

http://www2.ebi.ac.uk/fasta33/

http://www2.ebi.ac.uk/bic_sw/

http://genome-test.cse.ucsc.edu/cgi-bin/hgBlat