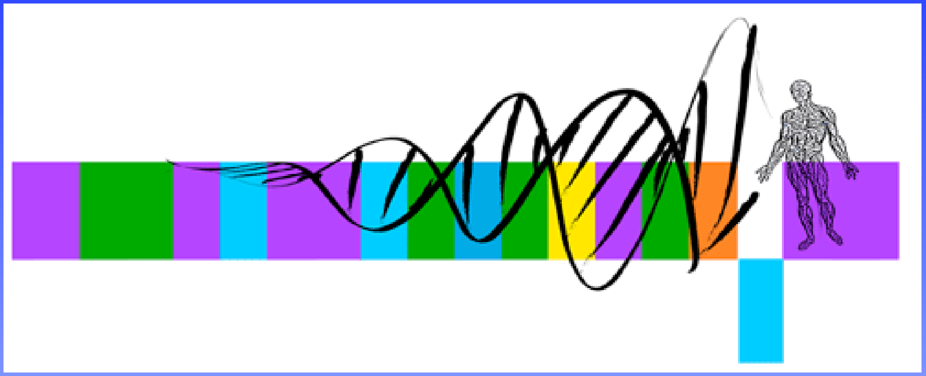

Bioinformatics for Biologists

Sequence Analysis: Part I. Pairwise alignment and database searching

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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.

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.

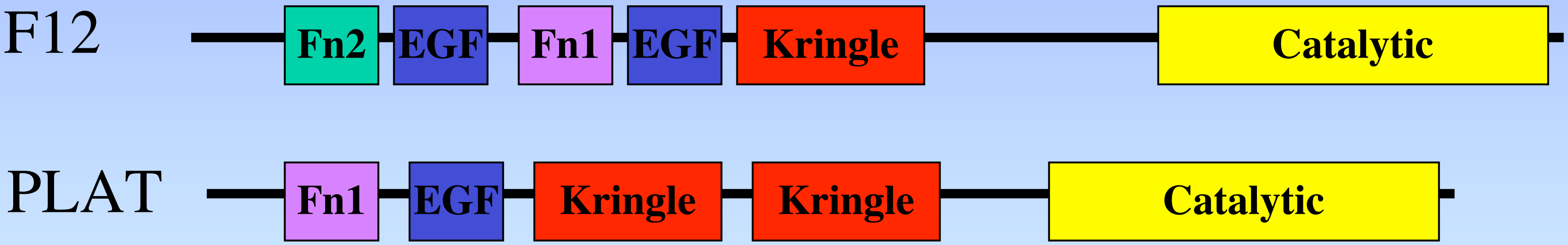
Alignment Methods

- ***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

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

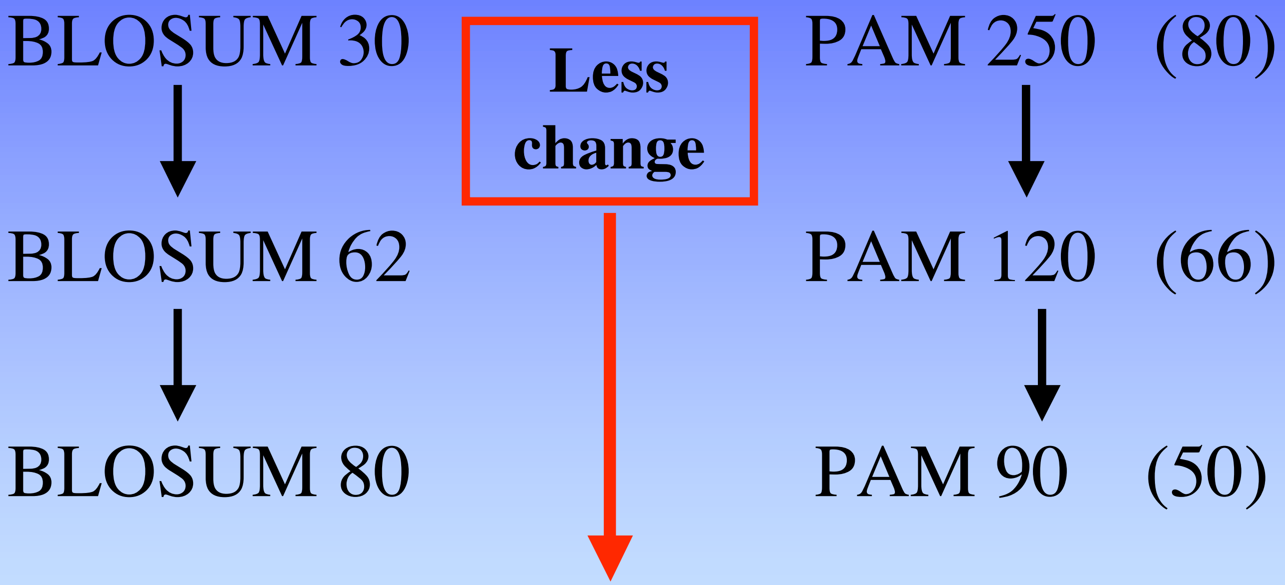
- Introduction
- Scoring alignments
  - Nucleotide vs Proteins
  - Definitions
- Alignment methods
- Significance of alignments
- Database searching methods

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	

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

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	

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

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 = - 5
- Gap extension = -2

```
  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  -5  -2  -1  -1  +1  +1  =  -4
```

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
```

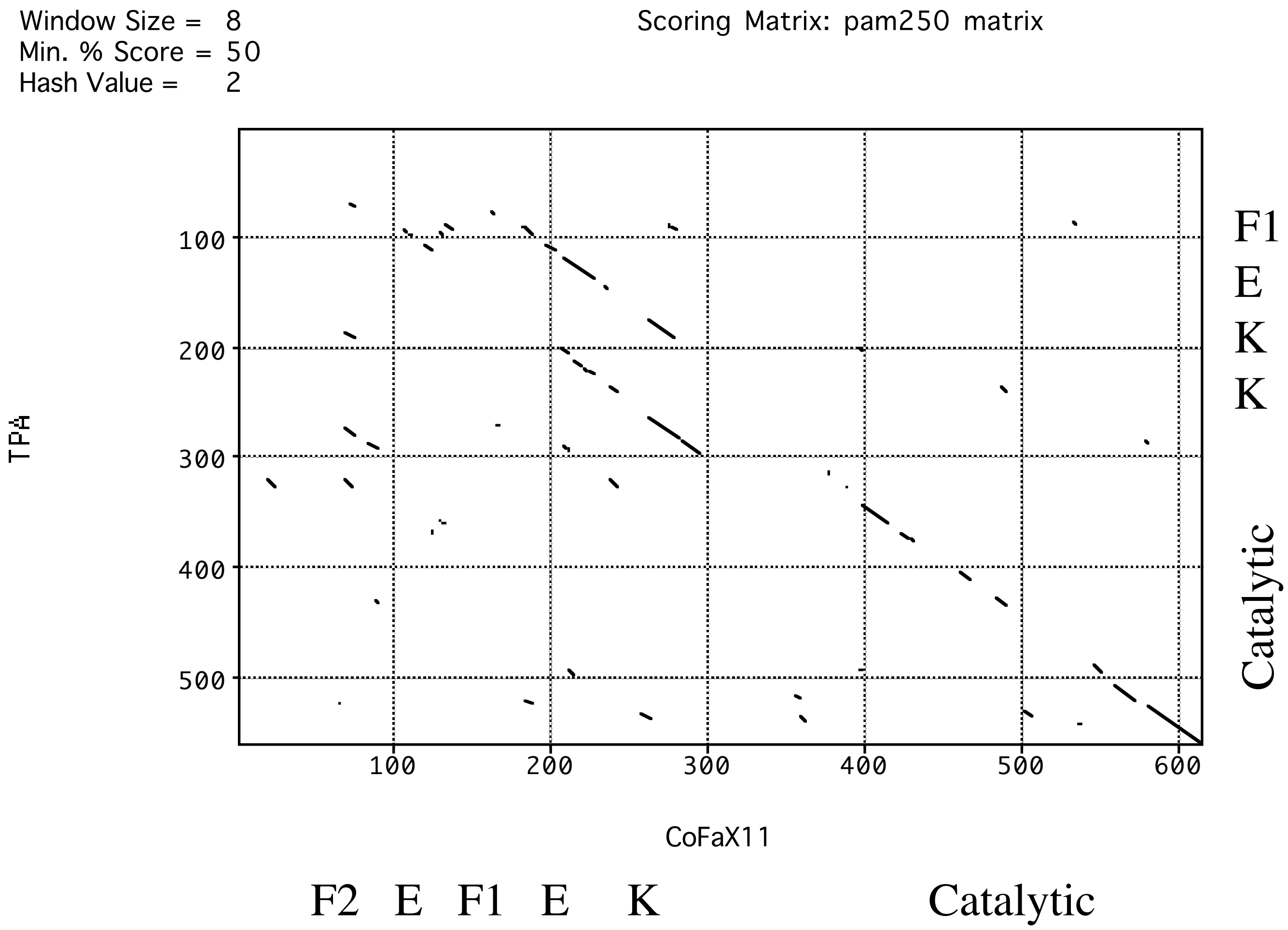
Based on BLOSUM62

```
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

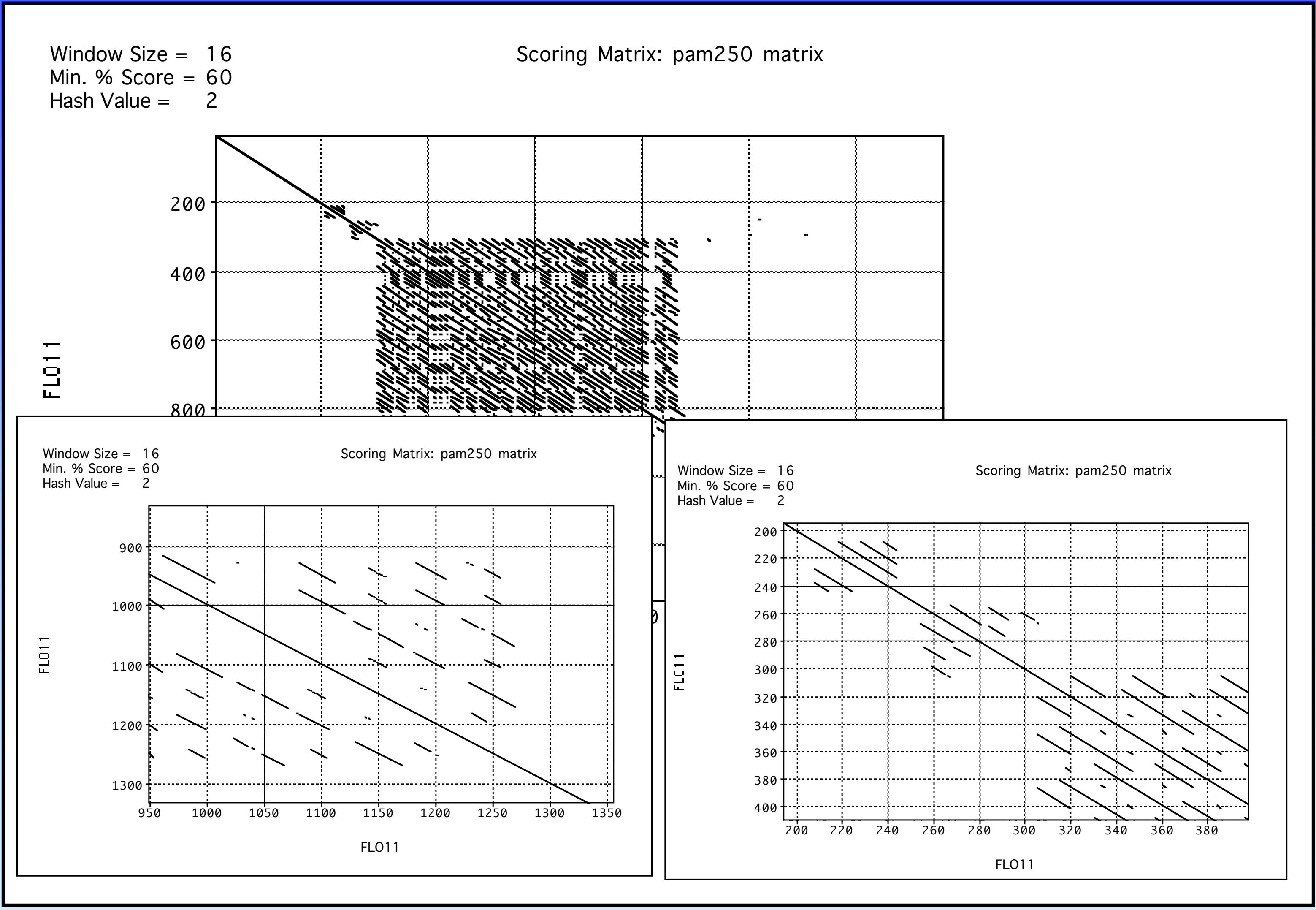
Dot Matrix Comparison



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

Scoring Matrix: pam250 matrix

Dot Matrix Comparison

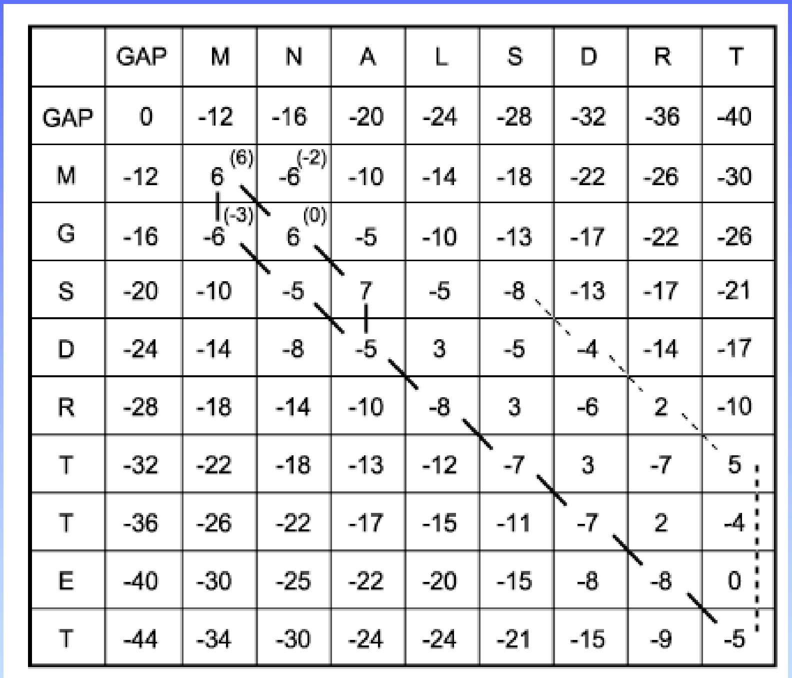


Dynamic Programming

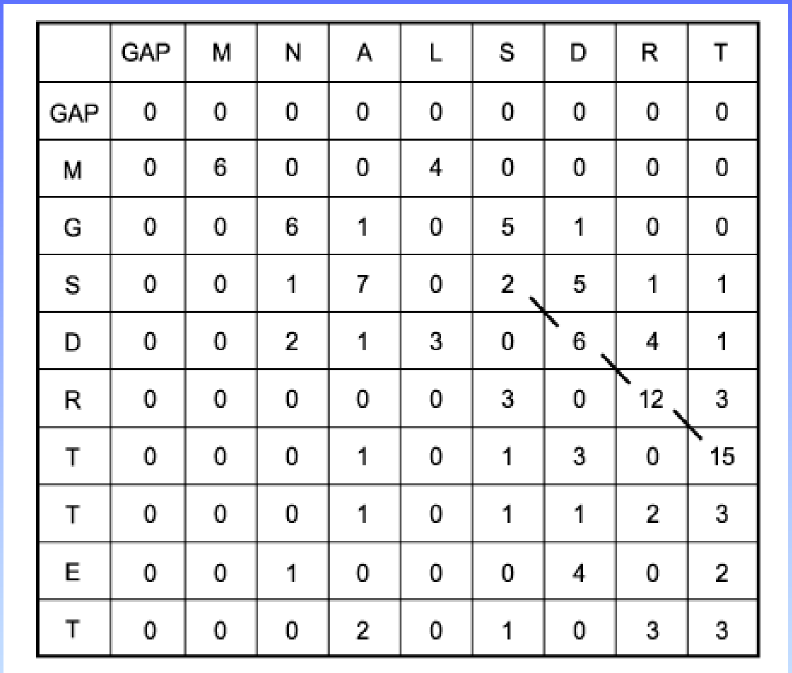
- Provides very best or optimal alignment
- Compares every pair of characters (e.g. bases or amino acids) in the two sequences
- Puts in gaps and mismatches
- Maximum number of matches between identical or related characters
- Generates a score and statistical assessment
- Nice example of global alignment 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
```

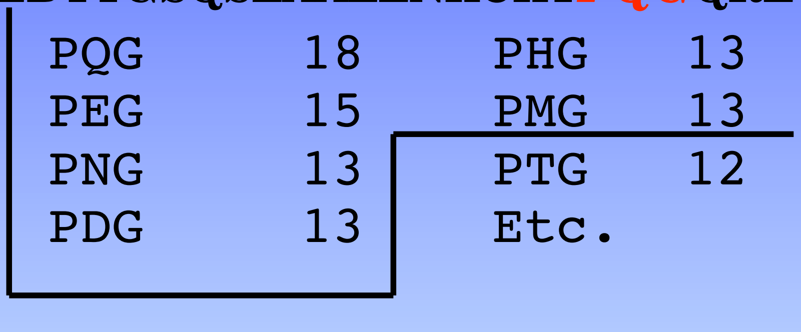
Original "Ungapped" BLAST Algorithm (1990)

- 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
```

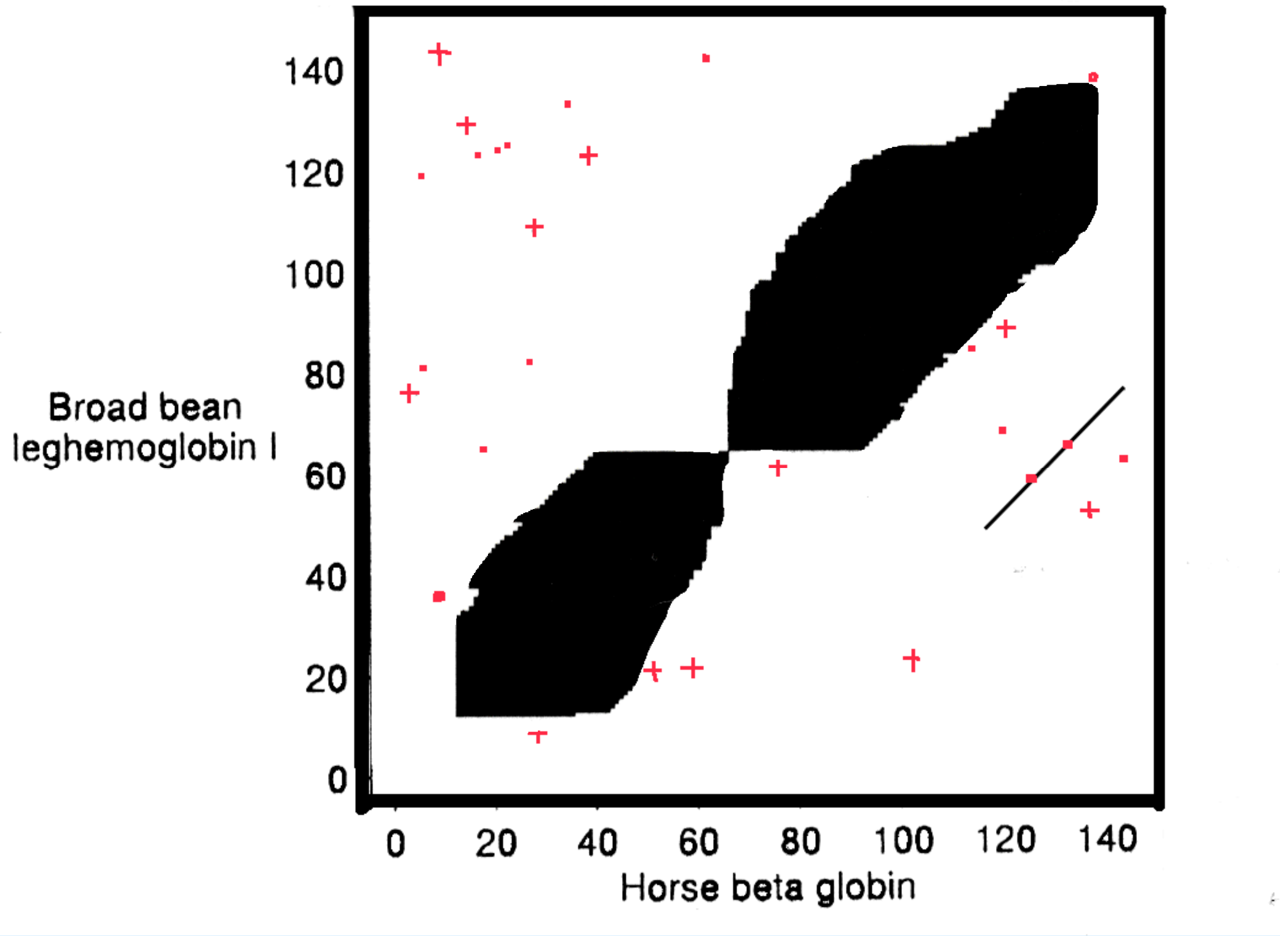
BLAST Refinements (1997)

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

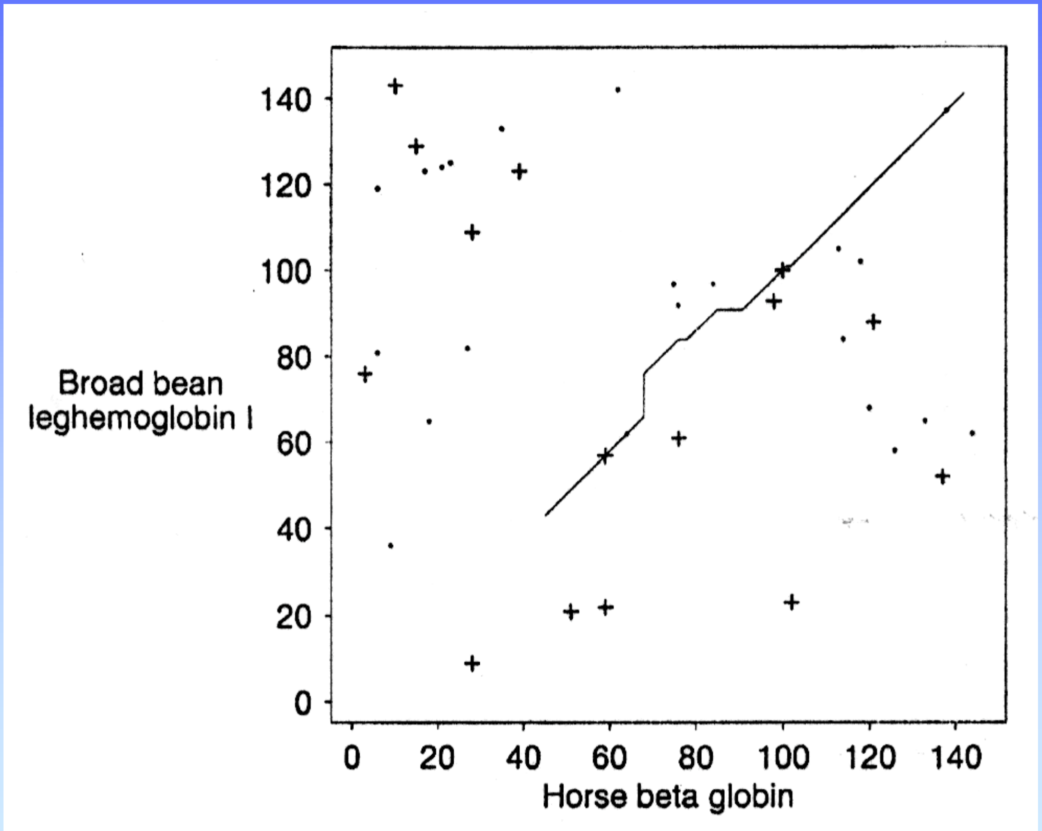
Gapped BLAST

15(+) > 13
22(•) > 11

(Altschul et al 1997)



Gapped BLAST



(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
  - How strong can alignment be by chance alone?
- Database searching methods

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.

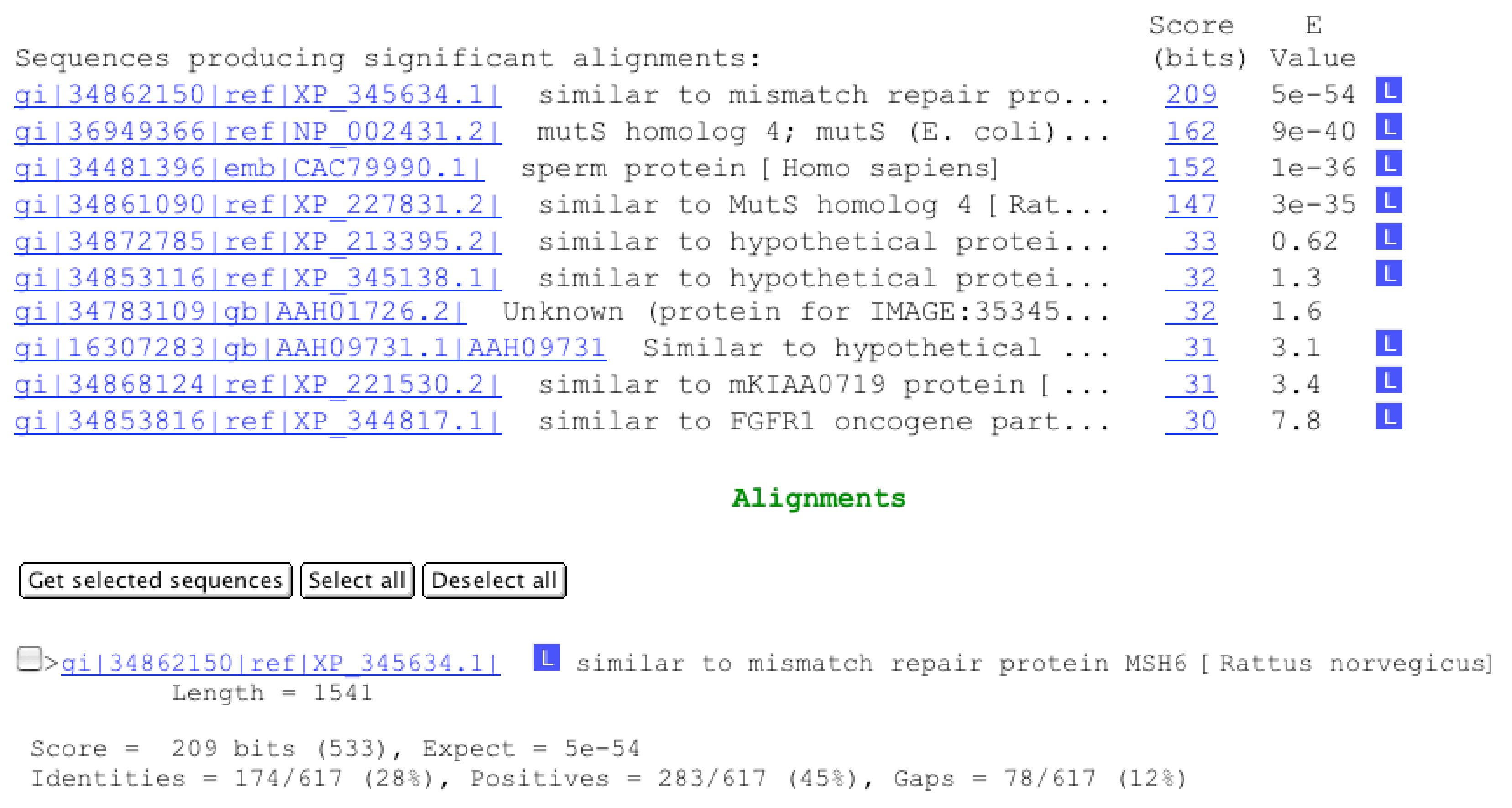
Topics to Cover

- Introduction
- Scoring alignments
- Alignment methods
- Significance of alignments
- Database searching methods
  - BLAST
  - 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?
- What parameters can be changed?
  - Substitution matrices
  - Statistical significance
  - Filtering for low complexity

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%)
```

WU-BLAST vs NCBI BLAST

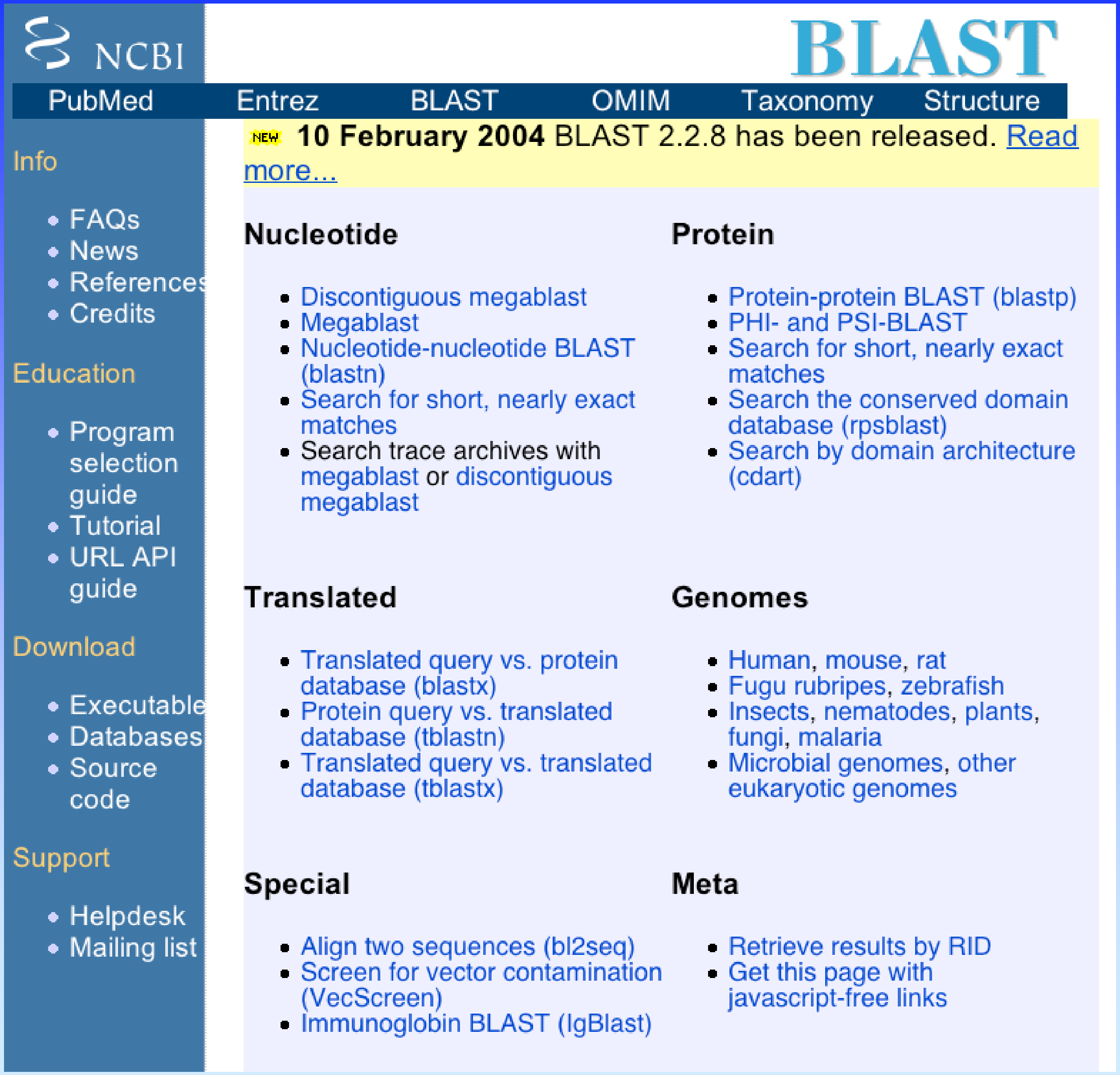
- WU-BLAST first for gapped alignments
- Use different scoring system for gaps
- Report different statistics
- WU-BLAST does not filter low-complexity by default
- WU-BLAST looks for and reports multiple regions of similarity
- Results will be different!

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



10 February 2004 BLAST 2.2.8 has been released. Read more...

Nucleotide

- Discontiguous megablast
- Megablast
- Nucleotide-nucleotide BLAST (blastn)
- Search for short, nearly exact matches
- Search trace archives with megablast or discontiguous megablast

Protein

- 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)

Translated

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

Genomes

- Human, mouse, rat
- Fugu rubripes, zebrafish
- Insects, nematodes, plants, fungi, malaria
- Microbial genomes, other eukaryotic genomes

Special

- Align two sequences (bl2seq)
- Screen for vector contamination (VecScreen)
- Immunoglobin BLAST (IgBlast)

Meta

- Retrieve results by RID
- Get this page with javascript-free links

Basic Searching Strategies

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