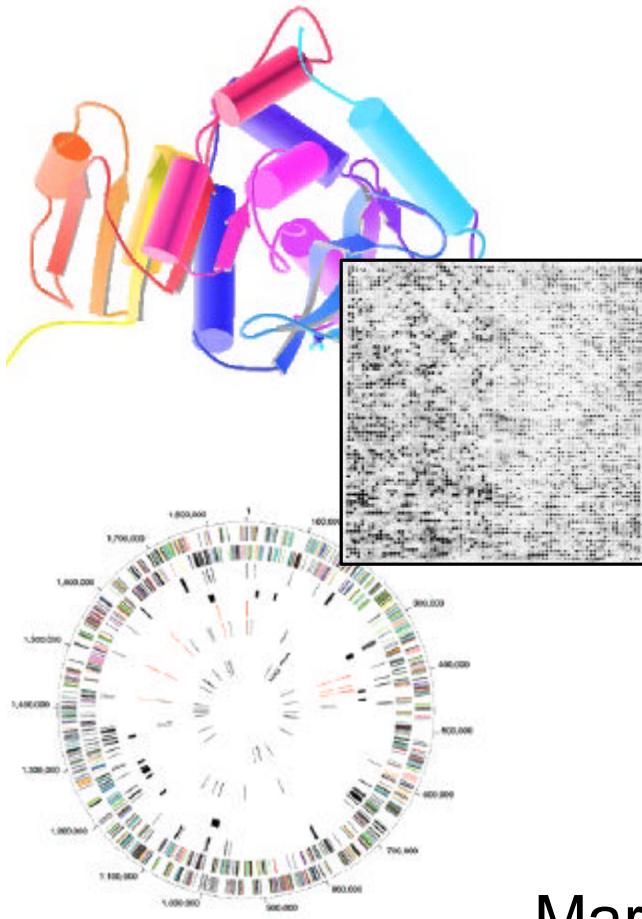


BIOINFORMATICS

Sequences



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Sequence Topics (Contents)

- Basic Alignment via Dynamic Programming
- Suboptimal Alignment
- Gap Penalties
- Similarity (PAM) Matrices
- Multiple Alignment
- Profiles, Motifs, HMMs
- Local Alignment
- Probabilistic Scoring Schemes
- Rapid Similarity Search: Fasta
- Rapid Similarity Search: Blast
- Practical Suggestions on Sequence Searching
- Transmembrane helix predictions
- Secondary Structure Prediction: Basic GOR
- Secondary Structure Prediction: Other Methods
- Assessing Secondary Structure Prediction
- Features of Genomic DNA sequences

Molecular Biology Information: Protein Sequence

- 20 letter alphabet
 - ◊ ACDEF~~GHIKLMNPQRSTVWY~~ but not BJOUXZ
- Strings of ~300 aa in an average protein (in bacteria),
~200 aa in a domain
- ~200 K known protein sequences

d1dhfa_ LNCIVAVSQNM**GIGKNGDLPWPPLRNEFRYFQRMTTSSVEGKQ**-NLVIMGKKTWFSI
d8dfr_ LNSIVAVCQNMG**GIGKDGNLWPPLRNEYKYFQRMTSTSHVEGKQ**-NAVIMGKKTWFSI
d4dfra_ ISLIAALAVDRVIGMENAMPWN-LPADLAWFKRNTL-----NKPVIMGRHTWESI
d3dfr_ TAFLWAQDRDG**LIGKDGHLFWH-LPDDLHYFRAQT**V-----GKIMVVGRRTYESF

d1dhfa_ LNCIVAVSQNM**GIGKNGDLPWPPLRNEFRYFQRMTTSSVEGKQ**-NLVIMGKKTWFSI
d8dfr_ LNSIVAVCQNMG**GIGKDGNLWPPLRNEYKYFQRMTSTSHVEGKQ**-NAVIMGKKTWFSI
d4dfra_ ISLIAALAVDRVIGMENAMPW-NLPADLAWFKRNTLD-----KPVIMGRHTWESI
d3dfr_ TAFLWAQDRNG**LIGKDGHLFWH-LPDDLHYFRAQT**VG-----KIMVVGRRTYESF

d1dhfa_ VPEKNRPL**KGRINLVLSRELKEPPQGAHFLSRSLDDALKTEQPELANKVDMWIVGGSSVYKEAMNHP**
d8dfr_ VPEKNRPL**KDRINIVLSRELKEAPKGAIYLSKSLLDALALLD**SPELKSKVDMWIVGGTAVYKAAMEKP
d4dfra_ ---G-RPLPGRKNIILS-SQPGTDDRV-TWVKSVDEAIAACGDVP-----EIMVIGGGRVYEQFLPKA
d3dfr_ ---PKRPLPERTNVVLTHQEDYQAQGA-VVVDVAAVFAYAKQHLDQ---ELVIAGGAQIFTAFKDDV

d1dhfa_ -PEKNRPL**KGRINLVLSRELKEPPQGAHFLSRSLDDALKTEQPELANKVDMWIVGGSSVYKEAMNHP**
d8dfr_ -PEKNRPL**KDRINIVLSRELKEAPKGAIYLSKSLLDALALLD**SPELKSKVDMWIVGGTAVYKAAMEKP
d4dfra_ -G---RPLPGRKNIILSSSQPGTDDRV-TWVKSVDEAIAACGDVPE-----IMVIGGGRVYEQFLPKA
d3dfr_ -P--KRPLPERTNVVLTHQEDYQAQGA-VVVDVAAVFAYAKQHLD---QELVIAGGAQIFTAFKDDV

Aligning Text Strings

Raw Data ???

T	C	A	T	G
C	A	T	T	G

2 matches, 0 gaps

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

3 matches (2 end gaps)

T	C	A	T	G	.
	-	-	-		
.	C	A	T	T	G

4 matches, 1 insertion

T	C	A	-	T	G
.	C	A	T	T	G

4 matches, 1 insertion

T	C	A	T	-	G
.	C	A	-	T	G

Dynamic Programming

- What to do for Bigger String?

SSDSEREEHVKRFRQALDDTGMKVPMAATTNLFTHPVFKDGGFTANDRDVRYYALRKTIERNIDLAVELGAETYVAWGGREGAESGGAKDVRDALDRMKEAFDLLGEYVTSQGYDIRFAIEPKPNEPRGDILLPTVGHALAFIERLERPELYGVNPEVGHEQMAGLNFPHGIAQALWAGKLFHIDLNGQNGIKYDQLRFGAGDLRAAFWLVDLLESAGYSGPRHFDFKPPRTEDFDGVWAS

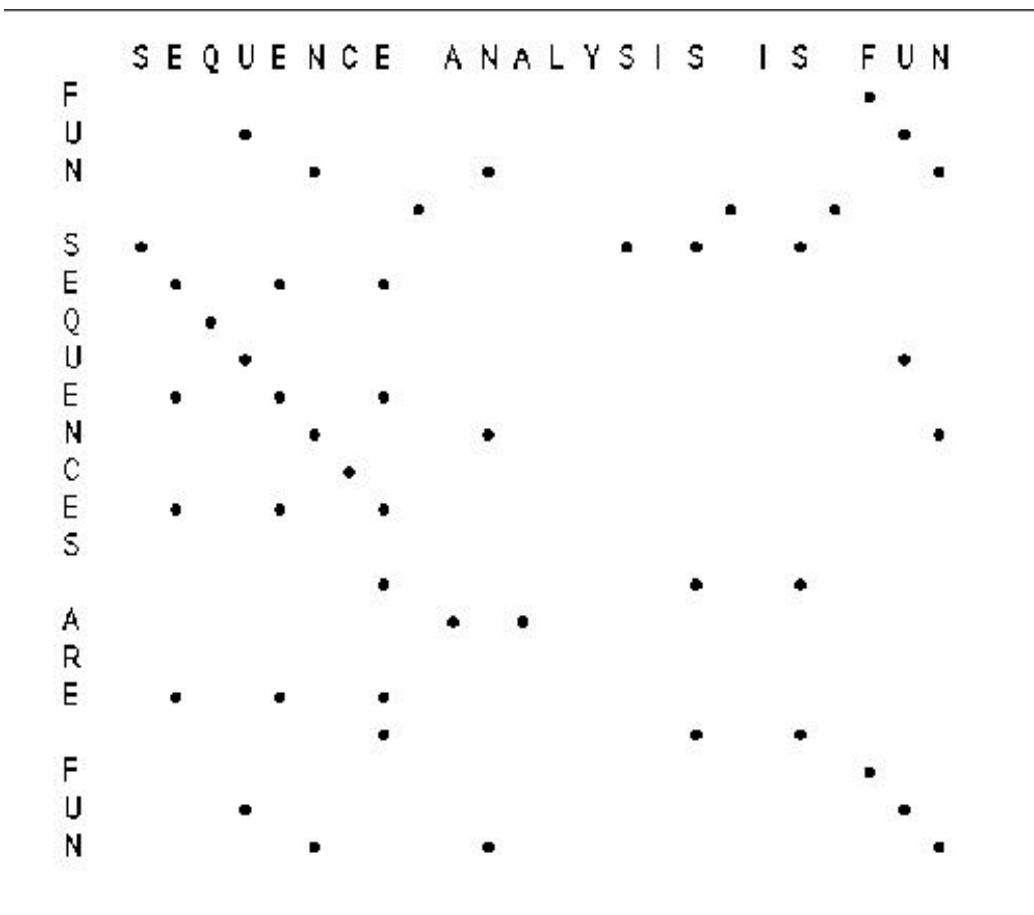
- Needleman-Wunsch (1970) provided first automatic method
 - ◊ Dynamic Programming to Find Global Alignment
- Their Test Data ($X \rightarrow Y$)
 - ◊ ABCNYRQCLCRPM
 - AYCYNRCKCRBP

Step 1 -- Make a Dot Plot (Similarity Matrix)

Put 1's where characters are identical.

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					1		
B		1											
P												1	

A More Interesting Dot Matrix



(adapted from R Altman)

Step 2 --

Start Computing the Sum Matrix

```

new_value_cell(R,C) <=
    cell(R,C)                                { Old value, either 1 or 0      }
    + Max[
        cell (R+1, C+1),                      { Diagonally Down, no gaps   }
        cells(R+1, C+2 to C_max), { Down a row, making col. gap   }
        cells(R+2 to R_max, C+1) { Down a col., making row gap  }
    ]

```

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C		1						1	1				
Y			1										
N			1										
R					1					1			
C		1						1	1				
K													
C		1						1	1				
R					1					1			
B	1												
P										1			

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y						1							
C		1								1	1		
Y			1										
N			1							1			
R					1					1		1	
C		1						1	1				
K													
C		1						1	1				
R					1					1		2	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

Step 3 -- Keep Going

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					2	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					5	4	3
C	3	3	4	3	3	3	3	3	3	3	4	3	3
K	3	3	3	3	3	3	3	3	3	3	3	2	1
C	2	2	3	2	2	2	2	2	2	3	2	3	1
R	2	1	1	1	1	1	2	1	1	1	1	2	0
B	1	2	1	1	1	1	1	1	1	1	1	1	0
P	0	0	0	0	0	0	0	0	0	0	0	0	1

Step 4 -- Sum Matrix All Done

Alignment Score is 8 matches.

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						5	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	4	3	3	1	0	0
K	3	3	3	3	3	3	3	3	3	2	1	0	0
C	2	2	3	2	2	2	2	3	2	3	1	0	0
R	2	1	1	1	1	2	1	1	1	1	2	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	8	7	6	6	5	4	4	3	3	2	1	0	0
Y	7	7	6	6	6	4	4	3	3	2	1	0	0
C	6	6	7	6	5	4	4	4	3	3	1	0	0
Y	6	6	6	5	6	4	4	3	3	2	1	0	0
N	5	5	5	6	5	4	4	3	3	2	1	0	0
R	4	4	4	4	4	5	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	4	3	3	1	0	0
K	3	3	3	3	3	3	3	3	3	3	2	1	0
C	2	2	3	2	2	2	2	2	3	2	3	1	0
R	2	1	1	1	1	2	1	1	1	1	1	2	0
B	1	2	1	1	1	1	1	1	1	1	1	1	0
P	0	0	0	0	0	0	0	0	0	0	0	0	1

Step 5 -- Traceback

Find Best Score (8) and Trace Back

A B C N Y - R Q C L C R - P M
A Y C - Y N R - C K C R B P

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	8	7	6	6	5	4	4	3	3	2	1	0	0
Y	7	7	6	6	6	4	4	3	3	2	1	0	0
C	6	6	7	6	5	4	4	4	3	3	1	0	0
Y	6	6	6	5	6	4	4	3	3	2	1	0	0
N	5	5	5	6	5	4	4	3	3	2	1	0	0
R	4	4	4	4	4	5	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	4	3	3	1	0	0
K	3	3	3	3	3	3	3	3	3	2	1	0	0
C	2	2	3	2	2	2	2	3	2	3	1	0	0
R	2	1	1	1	1	2	1	1	1	1	2	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	1	0	0

Step 5 -- Traceback

A B C N Y - R Q C L C R - P M
A Y C - Y N R - C K C R B P

	A	B	C	N	Y	-	R	Q	C	L	C	R	-	P	M
A	8	7	6	6	5	4	4	3	3	2	1	0	0	0	
Y	7	7	6	6	6	4	4	3	3	2	1	0	0	0	
C	6	6	7	6	5	4	4	4	3	3	1	0	0	0	
Y	6	6	6	5	6	4	4	3	3	2	1	0	0	0	
N	5	5	5	6	5	4	4	3	3	2	1	0	0	0	
R	4	4	4	4	4	5	4	3	3	2	2	0	0	0	
C	3	3	4	3	3	3	3	4	3	3	1	0	0	0	
K	3	3	3	3	3	3	3	3	3	2	1	0	0	0	
C	2	2	3	2	2	2	2	3	2	3	1	0	0	0	
R	2	1	1	1	1	2	1	1	1	1	2	0	0	0	
B	1	2	1	1	1	1	1	1	1	1	1	0	0	0	
P	0	0	0	0	0	0	0	0	0	0	0	1	0	0	

Step 6 -- Alternate Tracebacks

Also,
Suboptimal
Alignments

A B C - N Y R Q C L C R - P M
A Y C Y N - R - C K C R B P

	A	B	C	-	N	Y	R	Q	C	L	C	R	-	P	M
A	8	7	6	6	5	4	4	3	3	2	1	0	0	0	
Y	7	7	6	6	6	4	4	3	3	2	1	0	0	0	
C	6	6	7	6	5	4	4	4	3	3	1	0	0	0	
Y	6	6	6	5	6	4	4	3	3	2	1	0	0	0	
N	5	5	5	6	5	4	4	3	3	2	1	0	0	0	
R	4	4	4	4	4	5	4	3	3	2	2	0	0	0	
C	3	3	4	3	3	3	3	4	3	3	1	0	0	0	
K	3	3	3	3	3	3	3	3	3	2	1	0	0	0	
C	2	2	3	2	2	2	2	3	2	3	1	0	0	0	
R	2	1	1	1	1	2	1	1	1	1	2	0	0	0	
B	1	2	1	1	1	1	1	1	1	1	1	0	0	0	
P	0	0	0	0	0	0	0	0	0	0	1	0	0	0	

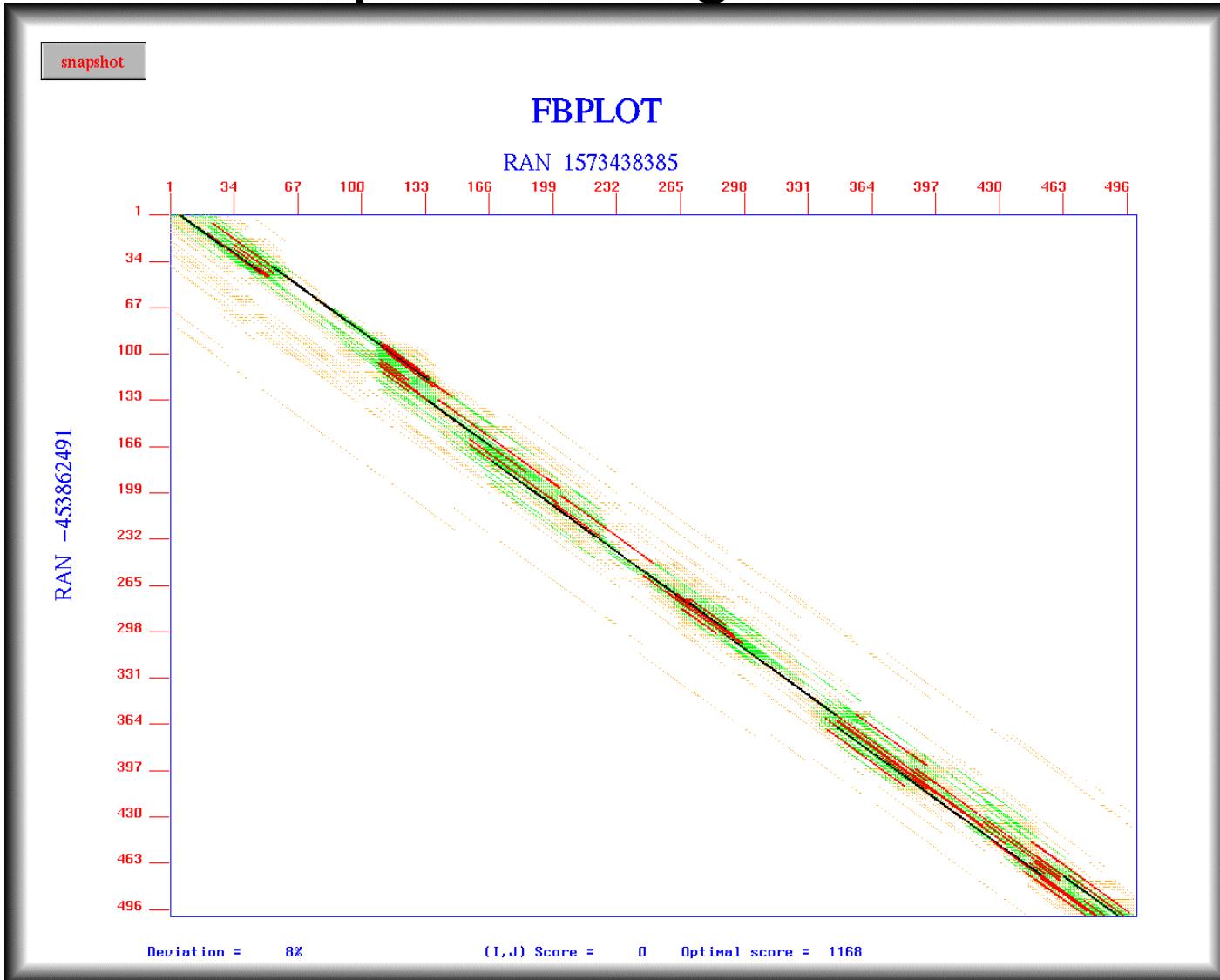
Suboptimal Alignments

```
;
; Random DNA sequence generated using the seed : -453862491
;
; 500 nucleotides
;
; A:C:G:T = 1 : 1 : 1 : 1
;
RAN -453862491
AAATGCCAAA TCATACGAAC AGCCGACGAC GGGAGCAACC CAAAGTCGCAG TTTCGCTTGAG CTAGCGCGCT
CCCACCGGGA TATACTAA TCATTACAGC AGGTCTCCTG GGCGTACAGA CTAGCTGAAC GCGCTGCGCC
AATTCCAAC TCGGTATGAA GGATCGCCTG CGGTTATCGC TGACTTGAGT AACCAAGATCG CTAAGGTTAC
GCTGGGGCAA TGATGGATGT TAACCCCTTA CAGTCTCGGG AGGGACCTTA AGTCGTAATA GATGGCAGCA
TTAATACCTT CGCCGTTAA ATACCTTAA TCCGTTCTTG TCAATGCCGT AGCTGCAGTG AGCCTTCTGT
CACGGGCATA CCGCGGGGTA GCTGCAGCAA CCCTAGGCTG AGCATCAAGA AGACAAACAC TCCTCGCCTA
CCCCGGACAT CATATGACCA GGCAGTCTAG GCGCCGTTAG AGTAAGGAGA CCGGGGGGCC GTGATGATAG
ATGGCGTGTT 1
;
; Random DNA sequence generated using the seed : 1573438385
;
; 500 nucleotides
;
; A:C:G:T = 1 : 1 : 1 : 1
;
RAN 1573438385
CCCTCCATCG CCAGTTCCCTG AAGACATCTC CGTGACGTGA ACTCTCTCCA GCCATATTAA TCGAAGATCC
CCTGTCGTGA CGCGGATTAC GAGGGGATGG TGCTAATCAC ATTGCGAACAA TGTTTCGGTC CAGACTCCAC
CTATGGCATC TTCCGCTATA GGGCACGTAA CTTTCTTCGT GTGGCGGCCG GGCAACTAAA GACGAAAGGA
CCACAACGTG AATAGCCCGT GTCTGTGAGGT AAGGGTCCCG GTGCAAGAGT AGAGGAAGTA CGGGAGTACG
TACGGGGCAT GACGCGGGCT GGAATTTCAC ATCGCAGAAC TTATAGGCAG CCGTGTGCCT GAGGCCGCTA
GAACCTTCAA CGCTAACTAG TGATAACTAC CGTGTGAAAG ACCTGGCCCG TTTTGTCCCT GAGACTAATC
GCTAGTTAGG CCCCATTTGT AGCACTCTGG CGCAGACCTC GCAGAGGGAC CGGCCTGACT TTTTCCGGCT
TCCTCTGAGG 1

Parameters: match weight = 10, transition weight = 1, transversion weight = -3
Gap opening penalty = 50   Gap continuation penalty = 1
Run as a local alignment (Smith-Waterman)
```

(courtesy of Michael Zucker)

Suboptimal Alignments II



(courtesy of Michael Zucker)

Gap Penalties

The score at a position can also factor in a penalty for introducing gaps (i. e., not going from i, j to $i-1, j-1$).

Gap penalties are often of linear form:

$$\text{GAP} = a + bN$$

GAP is the gap penalty

a = cost of opening a gap

b = cost of extending the gap by one (affine)

N = length of the gap

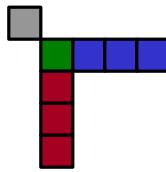
(Here assume $b=0$, $a=1/2$, so $\text{GAP} = 1/2$ regardless of length.)

Step 2 -- Computing the Sum Matrix with Gaps

```

new_value_cell(R,C) <=
    cell(R,C)                                { Old value, either 1 or 0      }
    + Max[
        cell (R+1, C+1),                      { Diagonally Down, no gaps   }
        cells(R+1, C+2 to C_max) - GAP , { Down a row, making col. gap }
        cells(R+2 to R_max, C+1) - GAP { Down a col., making row gap }
    ]

```



	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C		1					1	1					
Y					1								
N			1										
R					1					1			
C		1					1	1					
K													
C		1					1	1					
R					1					1			
B	1												
P										1			

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y						1							
C		1						1	1	1			
Y						1							
N			1					1					
R					1				1		1		
C		1					1	1	1	1			
K													
C		1					1	1	1	1			
R					1			1					
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

GAP
=1/2

All Steps in Aligning a 4-mer

	C	R	P	M
C	1			
R		1		
B				
P			1	

	C	R	P	M
C	1			
R		2	0	0
B	1	1	0	0
P	0	0	1	0

	C	R	P	M
C	3	1	0	0
R	1	2	0	0
B	1	1	0	0
P	0	0	1	0

Bottom right hand corner of previous matrices

C R B P

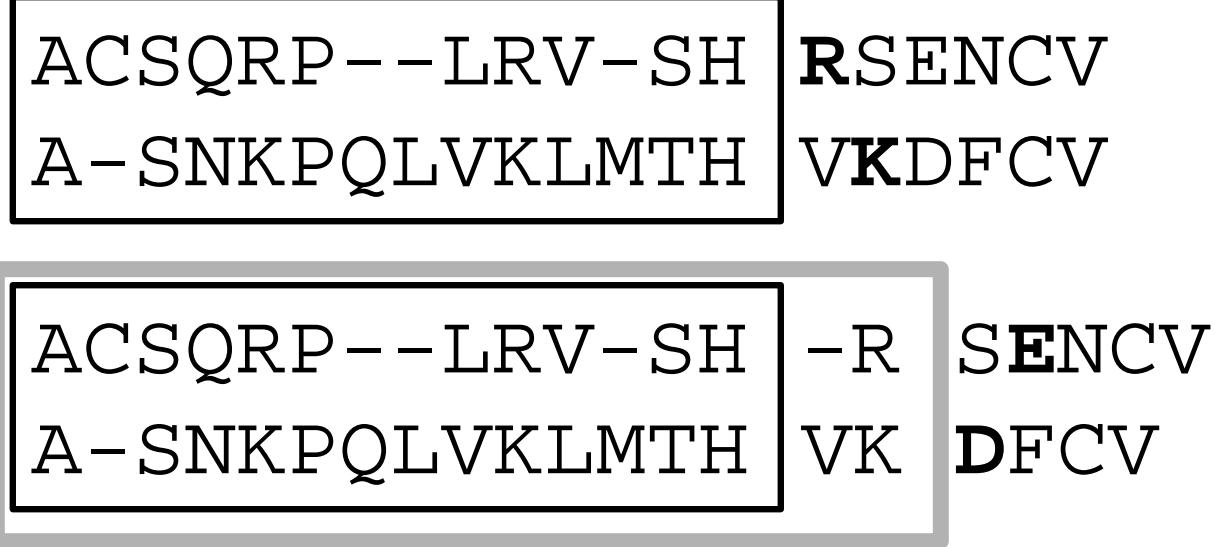
C R P M

- C R P M
C R - P M

	C	R	P	M
C	3	1	0	0
R	1	2	0	0
B	1	1	0	0
P	0	0	1	0

Key Idea in Dynamic Programming

- ◊ The best alignment that ends at a given pair of positions (i and j) in the 2 sequences is the score of the best alignment previous to this position PLUS the score for aligning those two positions.
- ◊ An Example Below
 - Aligning R to K does not affect alignment of previous N-terminal residues. Once this is done it is **fixed**. Then go on to align D to E.
 - How could this be violated?
Aligning R to K changes best alignment in box.



Similarity (Substitution) Matrix

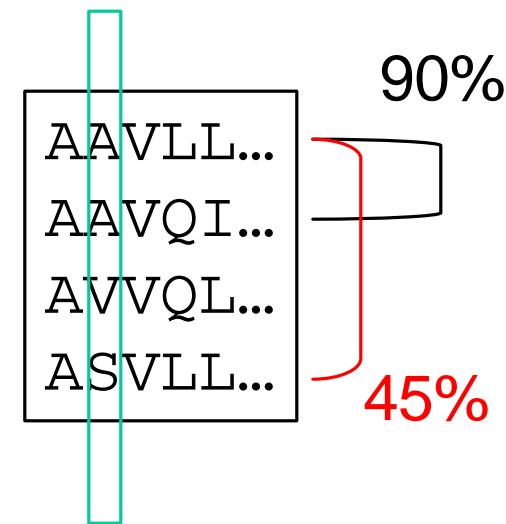
- Identity Matrix
 - ◊ Match L with L => 1
 - Match L with D => 0
 - Match L with V => 0??
- $S(aa-1, aa-2)$
 - ◊ Match L with L => 1
 - Match L with D => 0
 - Match L with V => .5
- Number of Common
Ones
 - ◊ PAM
 - ◊ Blossum
 - ◊ Gonnet

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	8	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	7	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	6	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0	
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	10	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	6	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Where do matrices come from?

- 1 Manually align protein structures (or, more risky, sequences)
- 2 Look at frequency of a.a. substitutions at structurally constant sites. -- i.e. pair i-j exchanges
- 3 Compute log-odds
$$S(aa-1,aa-2) = \log_2 (\text{freq}(O) / \text{freq}(E))$$
O = observed exchanges,
E = expected exchanges
 - odds = freq(observed) / freq(expected)
 - $S_{ij} = \log \text{odds}$
 - freq(expected) = $f(i)*f(j)$
= is the chance of getting amino acid i in a column and then having it change to j
 - e.g. A-R pair observed only a tenth as often as expected

+ → More likely than random
0 → At random base rate
- → Less likely than random



More on this....

To help us understand the knowledge incorporated in amino acid similarity scores we should briefly look at how they are calculated (4). First we compute an amino acid similarity ratio, R_{ij} for every pair of amino acids i and j.

$$R_{ij} = q_{ij} / p_i p_j$$

Where q_{ij} is the relative frequency with which amino acids i and j are observed to replace each other in homologous proteins. p_i and p_j are the frequencies at which amino acids i and j occur in the set of proteins in which the substitutions are observed. Their product, $p_i p_j$, is the frequency at which they would be expected replace each other if the replacements were random. If the observed replacement rate is equal to the theoretical replacement rate, then the ratio is one ($R_{ij} = q_{ij} / p_i p_j = 1.0$). If the replacements are favored during evolution (i.e. a conservative replacement) the ratio will be greater than one and if there is selection against the replacement the ratio will be less than one.

The similarity reported in the evolutionary-based tables for any pair of amino acids i and j, S_{ij} is the logarithm to the base 2 of this ratio, R_{ij} , although it is often scaled by some constant factor.

$$S_{ij} = \log_2(R_{ij}) = \log_2(q_{ij} / p_i p_j)$$

Scores above zero ($S_{ij} > 0.0$) indicate that two amino acids replace each other more often during evolution than we would expect if the replacements were random. Likewise, scores below zero indicate that amino acids replace each other less often than we would expect if the replacements were random. Thus a positive alignment score means that the pattern of identities and substitutions described by an alignment are more likely to result from previously observed evolutionary processes than to result from random replacements.

Amino Acid Frequencies of Occurrence

		1978	1991
L		0.085	0.091
A		0.087	0.077
G		0.089	0.074
S		0.070	0.069
V		0.065	0.066
E		0.050	0.062
T		0.058	0.059
K		0.081	0.059
H		0.037	0.053
D		0.047	0.052
R		0.041	0.051
P		0.051	0.051
N		0.040	0.043
Q		0.038	0.041
F		0.040	0.040
Y		0.030	0.032
M		0.015	0.024
H		0.034	0.023
C		0.033	0.020
W		0.010	0.014

Principles of Scoring Matrix

Construction, in detail

The Dayhoff Matrix: Proteins evolve through a succession of independent point mutations, that are accepted in a population and subsequently can be observed in the sequence pool. (Dayhoff, M.O. *et al.* (1978) Atlas of Protein Sequence and Structure. Vol. 5, Suppl. 3 National Biomedical Research Foundation, Washington D.C. U.S.A).

First step: Pair Exchange Frequencies

A **PAM** (Percent Accepted Mutation) is one accepted point mutation on the path between two sequences, per 100 residues.

$$f_i = \frac{\text{observations of } i}{\text{observations of any amino acid}}$$

Principles of Scoring Matrix

Construction, in detail #2

Third step: Relative Mutabilities

Second step: Frequencies of Occurrence

Amino acid frequencies:

	1978	1991
L	0.085	0.091
A	0.087	0.077
G	0.089	0.074
S	0.070	0.069
V	0.065	0.066
E	0.050	0.062
T	0.058	0.059
K	0.081	0.059
I	0.037	0.053
D	0.047	0.052
R	0.041	0.051
P	0.051	0.051
N	0.040	0.043
Q	0.038	0.041
F	0.040	0.040
Y	0.030	0.032
M	0.015	0.024
H	0.034	0.023
C	0.033	0.020
W	0.010	0.014

$m_i = f_i$ (*number of times i is observed to change*)

Relative mutabilities of amino acids:

	1978	1991
A	100	100
C	20	44
D	106	86
E	102	77
F	41	51
G	49	50
H	66	91
I	96	103
K	56	72
L	40	54
M	94	93
N	134	104
P	56	58
Q	93	84
R	65	83
S	120	117
T	97	107
V	74	98
W	18	25
Y	41	50

All values are taken relative to alanine, which is arbitrarily set at 100.

Principles of Scoring Matrix Construction, in detail #3

Fourth step: Mutation Probability Matrix

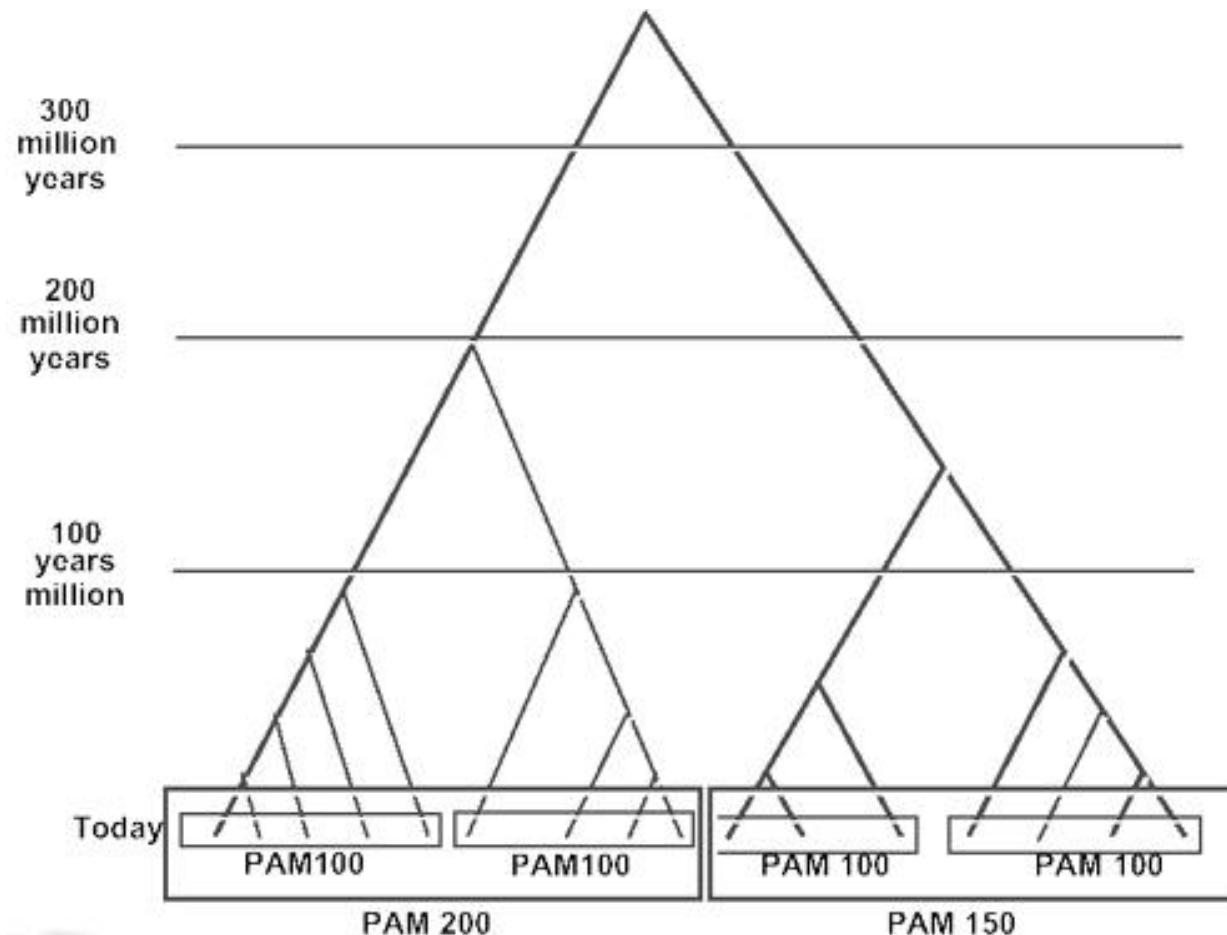
The probability that an amino acid in row i of the matrix will replace the amino acid in column j : the mutability of amino acid j , multiplied by the pair exchange frequency for ij divided by the sum of all pair exchange frequencies for amino acid i :

$$M_{ij} = m_j \frac{A_{ij}}{\sum_{i=1}^{20} A_{ij}}$$

Last step: the log-odds matrix

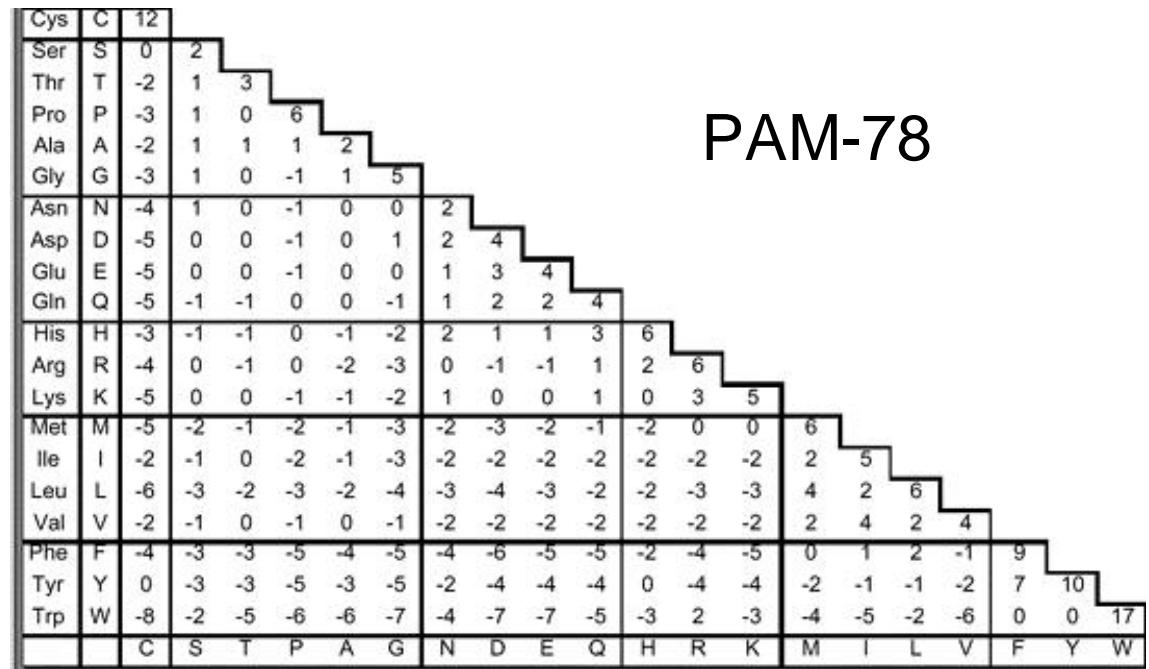
log to base 10: a value of +1 would mean that the corresponding pair has been observed 10 times more frequently than expected by chance. The most commonly used matrix is the matrix from the 1978 edition of the Dayhoff atlas, at PAM 250: this is also frequently referred to as the **MDM78 PAM250** matrix.

Different Matrices are Appropriate at Different Evolutionary Distances

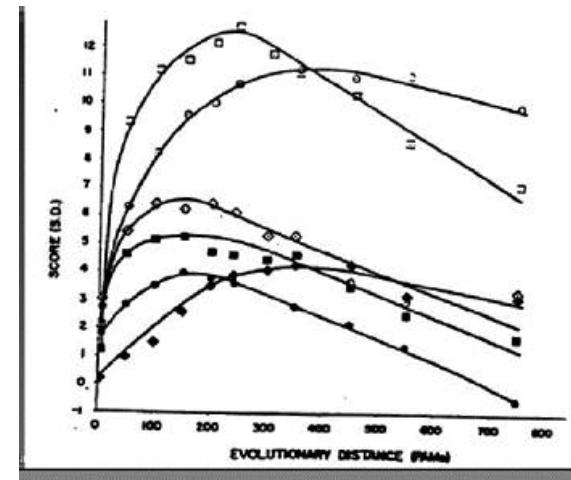


(Adapted from D Brutlag, Stanford)

	PAM-250 (distant)																				
A	Ala	.18																			
R	Arg	-.15	.61																		
N	Asn	.02	0	.20																	
D	Asp	.03	-.13	.21	.39																
C	Cys	-.20	-.36	-.36	-.51	1.19															
Q	Gln	-.04	.13	.08	.16	-.54	.40														
E	Glu	.03	-.11	.14	.34	-.53	.25	.38													
G	Gly	.13	-.26	.03	.06	-.34	-.53	.25	.38												
H	His	-.14	.16	.16	.07	-.34	.29	.07	-.21	.65											
I	Ile	-.05	-.20	-.18	-.24	-.23	-.20	-.20	-.26	-.24	.45										
L	Leu	-.19	-.30	-.29	-.40	-.60	-.18	-.34	-.41	-.21	.24	.59									
K	Lys	-.12	.34	.10	.01	-.54	.07	-.01	-.17	0	-.19	-.29	.47								
M	Met	-.11	-.04	-.17	-.26	-.52	-.10	-.21	-.28	-.21	.22	.37	.04	.64							
F	Phe	-.35	-.45	-.35	-.56	-.43	-.47	-.54	-.48	-.18	.10	.18	-.53	.02	.91						
P	Pro	.11	-.02	-.05	-.10	-.28	.02	-.06	-.05	-.02	-.20	-.25	-.11	-.21	-.46	.59					
S	Ser	.11	-.03	.07	.03	0	-.05	0	.11	-.08	-.14	-.28	-.02	-.16	-.32	.09	.16				
T	Thr	.12	-.09	.04	-.01	-.22	-.08	-.04	0	-.13	.01	-.17	0	-.06	-.31	.03	.13	.26			
W	Trp	-.58	.22	-.42	-.68	-.78	-.48	-.70	-.70	-.28	-.51	-.18	-.35	-.42	.04	-.56	-.25	-.52	1.73		
Y	Tyr	-.35	-.42	-.21	-.43	.03	-.40	-.43	-.52	-.01	-.09	-.09	-.44	-.24	.70	-.49	-.28	-.27	-.02	1.01	
V	Val	.02	-.25	-.17	-.21	-.19	-.19	-.18	-.14	-.22	.37	.19	-.24	.18	-.12	-.12	-.10	.03	-.62	-.25	.43
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	
	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	



Change in Matrix with Ev. Dist.



(Adapted from D Brutlag, Stanford)

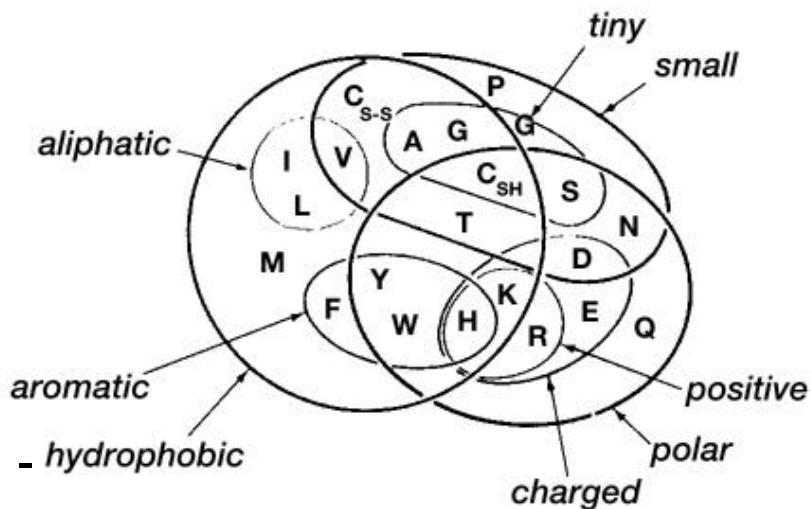
- Simplest way: the identity matrix
- A very crude model : to use the genetic code matrix, the number of point mutations necessary to transform one codon into the other.

Other similarity scoring matrices might be constructed from any property of amino acids that can be quantified -partition coefficients between hydrophobic and hydrophilic phases

- charge
- molecular volume, etc.

Unfortunately, all these biophysical quantities suffer from the fact that they provide only a partial view of the picture - there is no guarantee, that any particular property is a good predictor for conservation of amino acids between related proteins.

Other Matrices: How to score the exchange of two amino acids in an alignment?



(graphic adapted from W Taylor)

Some concepts challenged: Are the evolutionary rates uniform over the whole of the protein sequence?

(No.)

The BLOSUM matrices: Henikoff & Henikoff (Henikoff, S. & Henikoff J.G. (1992) *PNAS* **89**:10915-10919) .

-Use blocks of sequence fragments from different protein families which can be aligned without the introduction of gaps.

Amino acid pair frequencies can be compiled from these blocks

Different evolutionary distances are incorporated into this scheme with a clustering procedure: two sequences that are identical to each other for more than a certain threshold of positions are clustered.

More sequences are added to the cluster if they are identical to any sequence already in the cluster at the same level.

All sequences within a cluster are then simply averaged.

(A consequence of this clustering is that the contribution of closely related sequences to the frequency table is reduced, if the identity requirement is reduced.)

This leads to a series of matrices, analogous to the PAM series of matrices. BLOSUM80: derived at the 80% identity level.

The **BLOSUM** Matrices

BLOSUM62
is the BLAST
default

Local vs. Global Alignment

- GLOBAL
 - = best alignment of entirety of both sequences
 - ◊ For optimum global alignment, we want best score in the final row or final column
 - ◊ Are these sequences generally the same?
 - ◊ Needleman Wunsch
 - ◊ find alignment in which total score is highest, perhaps at expense of areas of great local similarity
- LOCAL
 - = best alignment of segments, without regard to rest of sequence
 - ◊ For optimum local alignment, we want best score anywhere in matrix (will discuss)
 - ◊ Do these two sequences contain high scoring subsequences
 - ◊ Smith Waterman
 - ◊ find alignment in which the highest scoring subsequences are identified, at the expense of the overall score

(Adapted from R Altman)

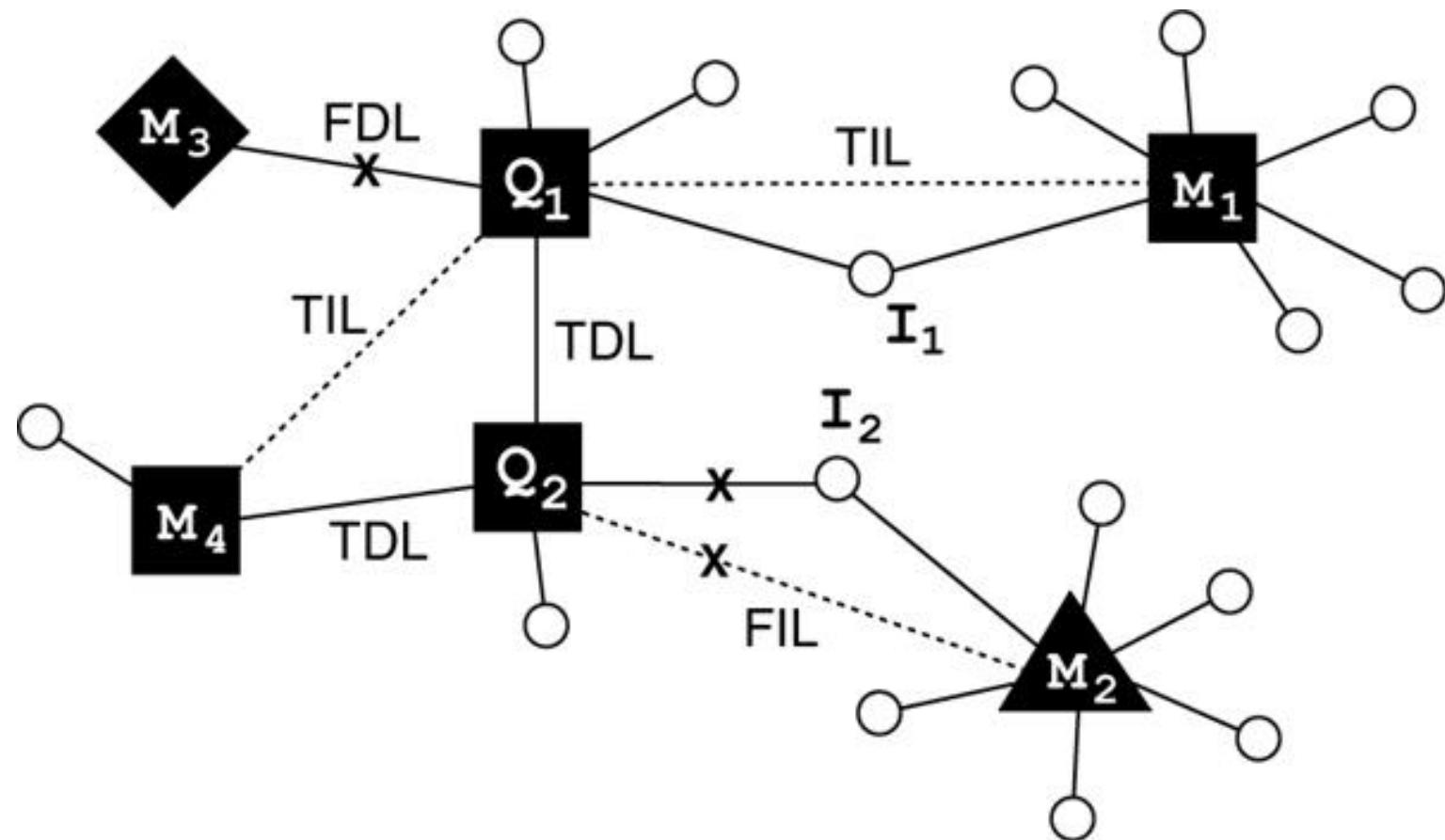
Modifications for Local Alignment

1. The scoring system uses negative scores for mismatches
 2. The minimum score for $[i,j]$ is zero
 3. The best score anywhere in the matrix (not just last column or row)
- These three changes cause the algorithm to seek high scoring subsequences, which are not penalized for their global effects (mod. 1), which don't include areas of poor match (mod. 2), and which can occur anywhere (mod. 3)

(Adapted from R Altman)

End of Class 1

Transitive Sequence Comparison



- One of the most essential tools in molecular biology

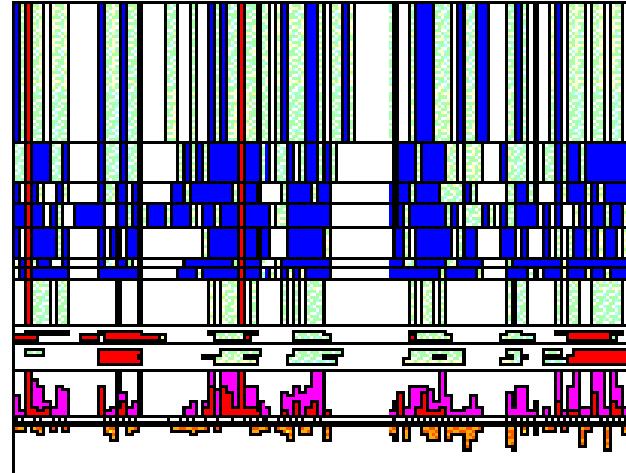
It is widely used in:

- Phylogenetic analysis
- Prediction of protein secondary/tertiary structure
- Finding diagnostic patterns to characterize protein families
- Detecting new homologies between new genes and established sequence families

AGRI_CHICK	154	OVCAPAS.....	CS.....	GVa..ESIVCGSDGKDYRSECDLINKHAC.....	DK.....	QENVFKKFDGAC	201
AGRI_RAT	165	QLCPTT.....	CF.....	GAp..DGTIVCGSDGVDFPSEQOLLSHA.....	AS.....	QEHIIFKKENGPC	212
FSA_HUMAN	116	OVCAPD.....	CS.....	NItwKGPVCGLDGKTYRNECALLKARC.....	KE.....	QPELEVQVQGKC	164
FSA_PIG	116	OVCAPD.....	CS.....	NItwKGPVCGLDGKTYRNECALLKARC.....	KE.....	QPELEVQVQGKC	164
FSA_RAT	116	OVCAPD.....	CS.....	NItwKGPVCGLDGKTYRNECALLKARC.....	KE.....	QPELEVQVQGKC	164
FSA_SHEEP	109	OVCAPD.....	CS.....	NItwKGPVCGLDGKTYRNECALLKARC.....	KE.....	QPELEVQVQGKC	157
IAC1_BOVIN	14	KVYTEA.....	CT.....	RE..YNPICDSAAKTYSNBCTF.....	NEKM.NN.....	DADIFHNHFGECA	61
IAC2_BOVIN	7	QAEFKDP.....	KVYCT.....	RE..SNHHCGSNGETYGNKGAF.....	KAVM.KS.....	GGKINLKHKGKC	57
IACA_PIG	7	QNVYRSH.....	LFFCT.....	RQ..MDPICGTNGKSYANPCIF.....	SEKG.LR.....	NQKDFGHWGHC	57
IACS_PIG	12	ODVYRSH.....	LFFCT.....	RE..MDPICGTNGKSYANPCIF.....	SEKL.GR.....	NEKFDFGEWGHCA	62
IAC_MACFA	33	GARYQLPG.....	CE.....	RD..FNPGCTDMITYPNBCT.....	GMKIR.ES.....	GONIKILRKGPC	81
IOV7_CHICK	94	QSPYLQVRDGNCMVACP.....	RI.....	LKPVGCGSDSFYTDNEGGI.....	CAYNA.EH.....	HTNISKLHDGEC	150
IOVO_ABUPI	8	OSDHPKP.....	ACL.....	QE..QKPLCGSDSKTYDNKGSF.....	CNAV.V.DS.....	NGTLTLSHKGKC	56
IOVO_ALECH	6	SEYPKPK.....	ACT.....	LE..YRPLCGSDSKTYCNKONF.....	CNAV.V.RS.....	NGTLTLSHKGKC	54
IPSG_VULVU	68	QTEYSDM.....	CT.....	MD..YRPLCGSDGKNSNKGIF.....	CNAV.V.RS.....	RTGFLAKHGEC	115
IPST_ANGAN	12	QGEMSAMHA.....	CP.....	MN..FAPVGCTDCNTYPNBSL.....	CFQR.Q.NT.....	KTDLITKDDRC	61
IPST_BOVIN	9	TNEVNG.....	CP.....	RI..YNPVGCGTDGVTYSNBCLL.....	CMENK.ER.....	QTPVLIQKSGPC	56
IPST_PIG	9	QTEVSG.....	CP.....	KI..YNPVGCGTDGVTYSNECVL.....	SENK.KR.....	QTPVLIQKSGPC	56
IPST_SHEEP	9	TNEVNG.....	CP.....	RI..YNPVGCGTDGVTYANBCLL.....	CMENK.ER.....	QTPVLIQKSGPC	56
OATP_HUMAN	439	QNVDCN.....	CP.....	KI..WDPVCGNNGLSISACLA...GC..ET.SI.....	GTGINMVFONCS	485	
OATP_RAT	439	QNTRCS.....	CS.....	Tnt..WDPVCGDNVAYMSACLA...GCKKFV.GT.....	GTNM.VFQDCSC	486	
PE60_PIG	37	QEHMTESPD.....	CS.....	RI..YDPVGCGDGVLYESEBCLL.....	CLARI.EN.....	KQDIQIVKDGEC	86
PGT_RAT	444	QRDRDCS.....	CP.....	DSf..FHPPVGCGDNVEVYVSPCHA...GC.....SS.....	TNTSSEASKEPI	488	
PSG1_MOUSE	33	CHDAVAG.....	CP.....	RI..YDPVGCGDGVLYANEBCV.....	CFENR.KR.....	IEPVLIRKGGPC	80
Q1R_COTJA	466	QICQDPA.....	ACPs..tKD.....	YKRVCGTDNKTVDGTCOLFGTKQLEGT.KM.....	GROLHLDYMGAC	521	
SC1_RAT	424	OVCQDPET.....	CPp..aKI.....	LDQACGTNDQTYASSCHLFATKQMLEGT.KK.....	GHQLQLDYGAC	479	
SPRC_BOVIN	93	OVCQDP_TS.....	CPap.iGE.....	FEKVCSDNKTFDSSCHFFATKQMLEGT.KK.....	GHKLHLDYIGPC	149	
SPRC_CAEEL	74	QECISK.....	CPeldgDP.....	MDKVCAANNCTFTSLCDLYREROLCKR.KSkecskafNAVKHLNIGEC		135	
SPRC_MOUSE	92	OVCQDP_TS.....	CPap.iGE.....	FEKVCSDNKTFDSSCHFFATKQMLEGT.KK.....	GHKLHLDYIGPC	148	
SPRC_XENLA	90	OVCQDPST.....	CPts.vGE.....	FEKICGTDNKTVDSSCHFFATKQMLEGT.KK.....	GHKLHLDYIGPC	146	

Multiple Sequence Alignments

- Practically useful methods only since 1987
- Before 1987 they were constructed by hand
- The basic problem: no dynamic programming approach can be used
- First useful approach by D. Sankoff (1987) based on phylogenetics



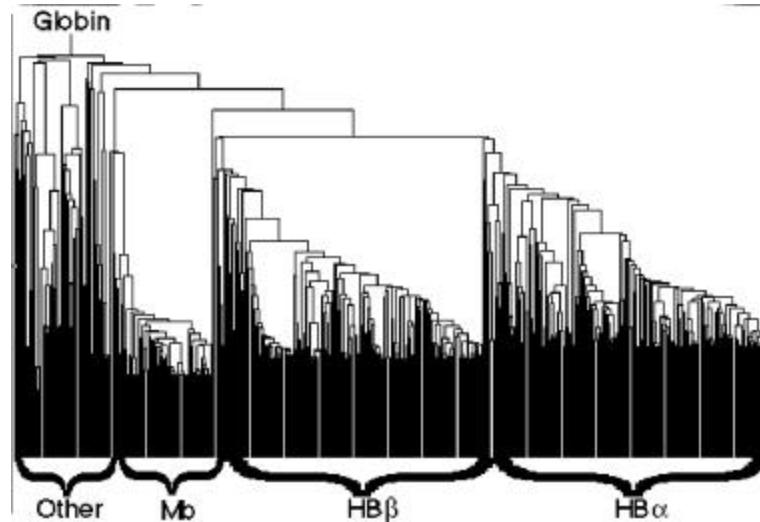
(LEFT, adapted from Sonhammer et al. (1997). "Pfam," Proteins 28:405-20. ABOVE, G Barton AMAS web page)

Progressive Multiple Alignments

- Most multiple alignments based on this approach
- Initial guess for a phylogenetic tree based on pairwise alignments
- Built progressively starting with most closely related sequences
- Follows branching order in phylogenetic tree
- Sufficiently fast
- Sensitive
- Algorithmically heuristic, no mathematical property associated with the alignment
- Biologically sound, it is common to derive alignments which are impossible to improve by eye

AGRI_CHICK	154	CVPAS.....CS...GVA.ESIIVCCSDGRDQFRESEEDLINKHAD.....DK.....QENVFKFKD	IC 201
AGRI RAT	165	CLCPPT.....CF...Gap.DGTVCSSDGVLVPPSCCOLLSHA.....AS.....QEHEIFKKEN	IC 212
FSA_HUMAN	116	CVCAPD.....S...NITwKGPVCCLDGKTYTRNECALLKAR.....KE.....QPELEVQNC	IC 164
FSA_PIG	116	CVCAPD.....S...NITwKGPVCCLDGKTYTRNECALLKAR.....KE.....QPELEVQNC	IC 164
FSA_RAT	116	CVCAPD.....S...NITwKGPVCCLDGKTYTRNECALLKAR.....KE.....QPELEVQNC	IC 164
FSA_SHEEP	109	CVCAPD.....S...NITwKGPVCCLDGKTYTRNECALLKAR.....KE.....QPELEVQNC	IC 157
IAC1_BOVIN	14	KVYTEA.....CT...RE.YNPICDSSAAKTYNSNCTF.....QNEKM.NN.....DADIHFHNFF	IC 61
IAC2_BOVIN	7	CAEFKDP.....KVYCT...RE.SMEHCSSNGETYGNKCAF.....QKAVM.KS.....GGKINLKHRE	IC 57
IACA_PIG	7	CVYRSH.....LFBQT...RQ.MDPICCTNGRSYANPCIF.....GSEKG.LR.....NQKFDPGHMGHC	IC 57
IACS_PIG	12	CDVYRSH.....LFBQT...RQ.MDPICCTNGRSYANPCIF.....GSEKL.GR.....NQKFDPGHMGHC	IC 62
IAC MACFA	33	CAVYOLPG.....C...RD.FNPVCCSTDIMITYPRNEPQTL.....GMKIR.ES.....GONIKLRLRE	IC 81
IOV7_CHICK	94	CGPYLQVVRDGNLNMVACB.....RI..LKPWVCCSDGFTTDNCGGIL.....GAYNA.EH.....HTNISKLHED	IC 150
IOVO_ABUPI	8	CDGHPKP.....ACL...QE.QKPLCCSDNKTVDNCGSF.....QNAVVS.DS.....NGTITLTSHE	IC 56
IOVO_ALBCH	6	CGEYPKP.....ACT...LE.YRPLCCSDGFTKTNQNP.....QNAVVS.ES.....NGTITLTSHE	IC 54
IPSG_VULVU	68	CTEYSDM.....CT...MD.YRPLCCSDGFTKTNQNP.....QNAVVS.RS.....RGTIPLAKH	IC 115
IPST_ANGAN	12	CGEMSAMHA.....C...MN.FAPVCCSDGFTTDNCGGIL.....FPOQ.NT.....KTDJLITKDDR	IC 61
IPST_BOVIN	9	CGENBVNG.....C...RI.YNPVCCSDGFTVSNECOLL.....OMENK.ER.....QTPVLIOKS	IC 56
IPST_PIG	9	CGENBVSG.....C...RI.YNPVCCSDGFTVSNECOLL.....SENK.KR.....QTPVLIOKS	IC 56
IPST_SHEEP	9	CGENBVNG.....C...RI.YNPVCCSDGFTVSNECOLL.....MENK.ER.....QTPVLIOKS	IC 56
OATP_HUMAN	439	GIVDCN.....C...K1.WEPVCCSDGFTVSNECOLL.....C...ET.SI.....GTGINMVQNC	IC 485
OATP RAT	439	GINTRC5.....C...K1.WEPVCCSDGFTVSNECOLL.....C...KRFV.GT.....GTINM.VFDCSC	IC 486
PE60_PIG	37	GHMHTESPQ.....S...RI.WEPVCCSDGFTVSNECOLL.....CLARI.EN.....KODIQIVRUE	IC 86
PGT_RAT	444	GRRDCS.....C...DSF.RFPVCCSDGFTVSNECOLL.....C...SS.....TNTSSEASKEP	IC 488
PSG1_MOUSE	33	GDADAVAG.....C...RI.YLPVCCSDGFTVSNECOLL.....C...FENR.KR.....IEPVLLRKGC	IC 80
QR1_COTJA	466	GCQDPA.....ACps.tKD.YKRVCCEHNRKTYDGTGCOLFGTKQLLEGKMM.....GROHLHDW	IC 521
SCI_RAT	424	GCQCDPET.....Cps.akI.DLOQCCCEHNRKTYDGTGCOLFGTKQLLEGKMM.....GHOLQLDWF	IC 479
SPRC_BOVIN	93	GCVQDP_TS.....Chap.iGE.FEKWCSDNKTBLDSCCHFFATKTCLEGKMM.....GHKLHLDW	IC 149
SPRC_CAEEL	74	GCeICK.....CHeelDGP.MDKVWCANNKTYDGTGCOLFGTKQLLEGKMM.....GKsekskaFNAKVHLEXL	IC 148
SPRC_MOUSE	92	GCVQDP_TS.....Chap.iGE.FEKWCSDNKTBLDSCCHFFATKTCLEGKMM.....GHKLHLDW	IC 148
SPRC_XENLA	90	GCVQDPST.....Cpts.vGE.FEKWCSDNKTBLDSCCHFFATKTCLEGKMM.....GHKLHLDW	IC 146

(adapted from Sonhammer et al. (1997). "Pfam," Proteins 28:405-20)



Problems with Progressive Alignments

- Local Minimum Problem
 - Parameter Choice Problem

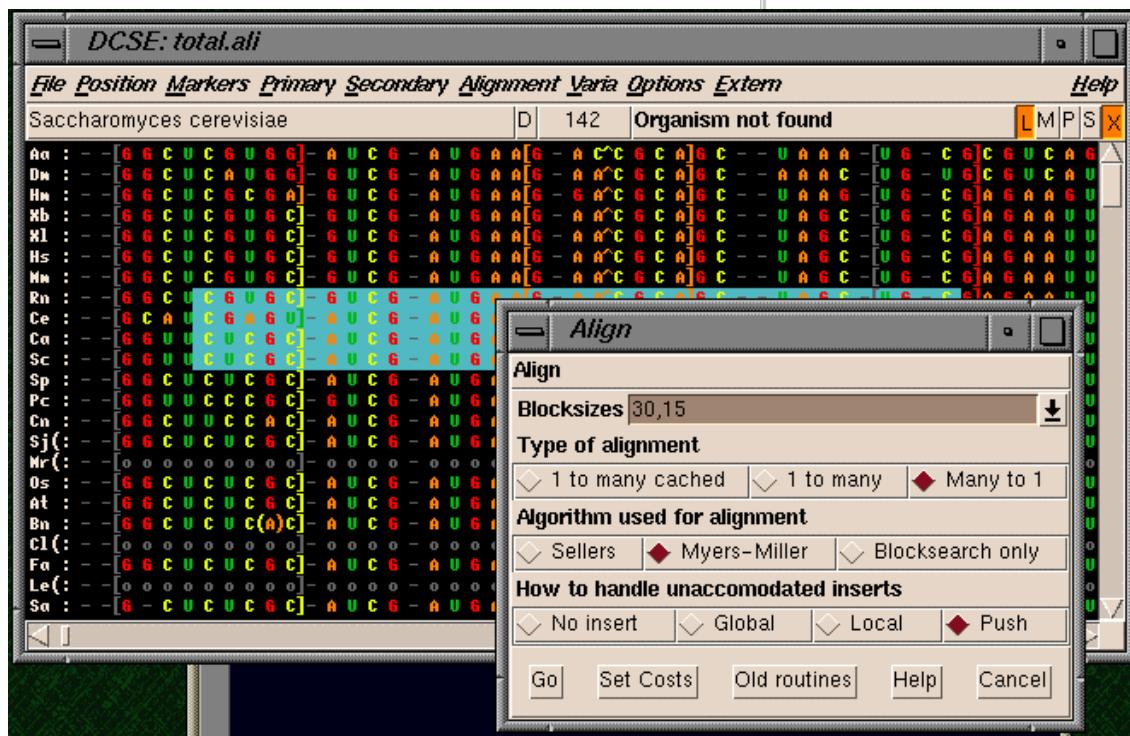
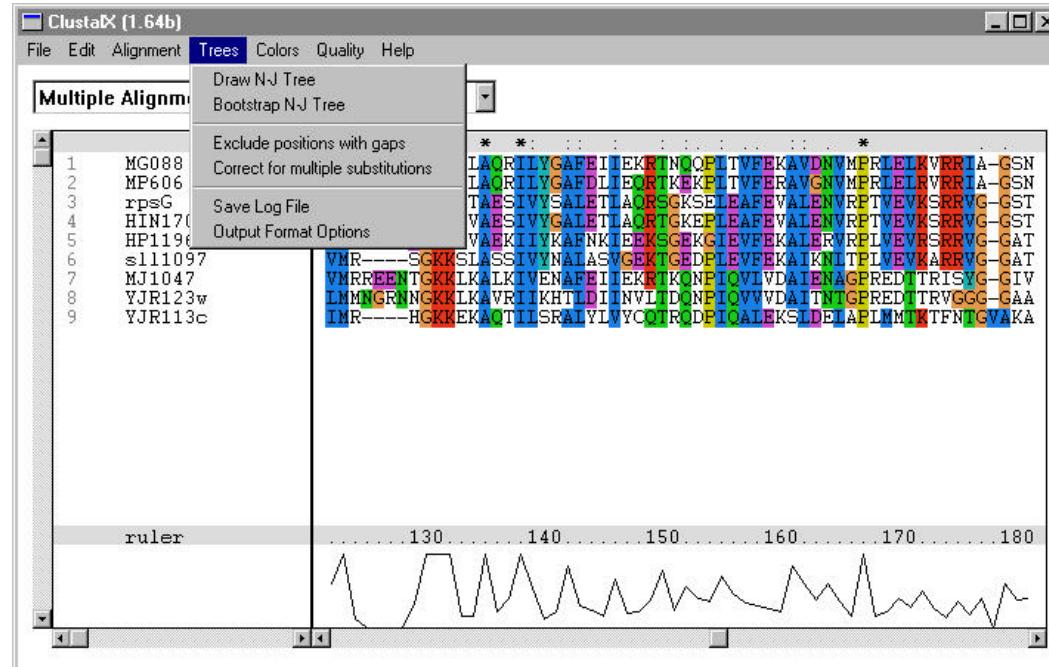
1. Local Minimum Problem

- It stems from greedy nature of alignment
(mistakes made early in alignment cannot be corrected later)
- A better tree gives a better alignment
(UPGMA neighbour-joining tree method)

2. Parameter Choice Problem

- - It stems from using just one set of parameters
(and hoping that they will do for all)

Popular Multiple Alignment Programs

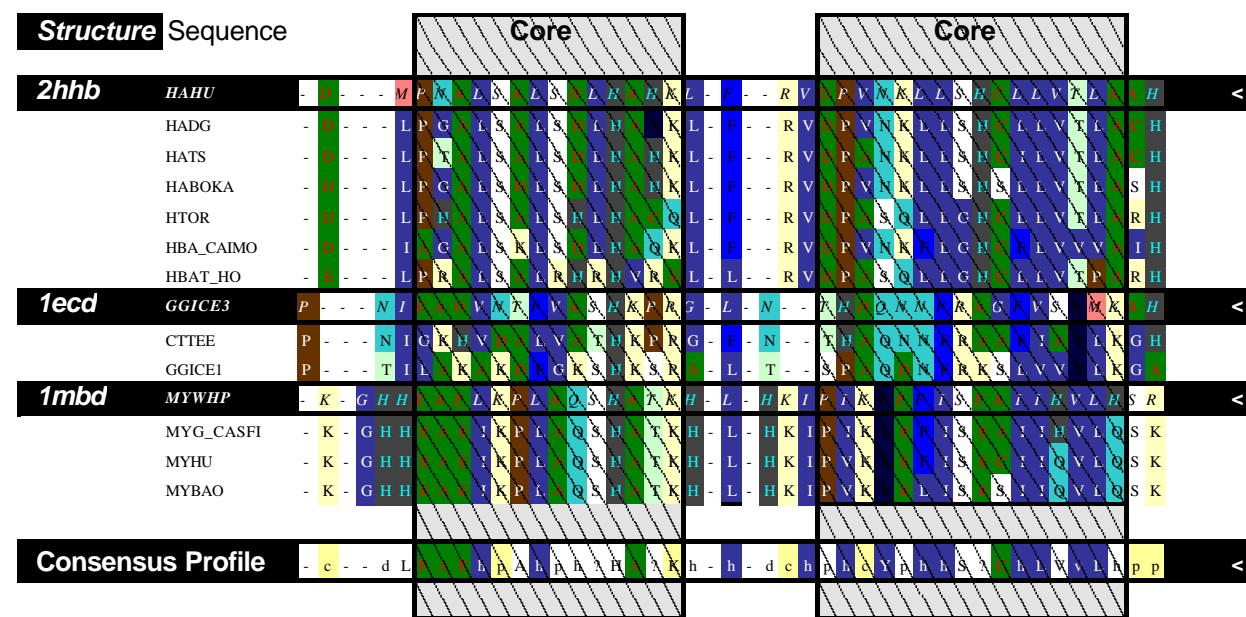


Fuse multiple alignment into:

- **Motif**: a short signature pattern identified in the conserved region of the multiple alignment
- **Profile**: frequency of each amino acid at each position is estimated
- **HMM**: Hidden Markov Model, a generalized profile in rigorous mathematical terms

Profiles Motifs HMMs

Can get more sensitive searches with these multiple alignment representations
(Run the profile against the DB.)



Profiles

2hhb	Human Alpha Hemoglobin	R	V	D	C	V	A	Y	K	
	HAHU	R	V	D	C	V	A	Y	K	100
	HADG	R	V	D	C	V	A	Y	K	89
	HTOR	R	V	D	C	A	A	Y	Q	76
	HBA_CAIMO	R	V	D	P	V	A	Y	K	73
	HBAT_HORSE	R	V	D	P	A	A	Y	Q	62

1mbd	Whale Myoglobin	A	I	C	A	P	A	Y	E	
	MYWHP	A	I	C	A	P	A	Y	E	100
	MYG_CASF1	R	I	C	A	P	A	Y	E	85
	MYHU	R	I	C	V	C	A	Y	D	75
	MYBAO	R	I	C	V	C	A	Y	D	71

Eisenberg Profile Freq. A

Eisenberg Profile Freq. C

⋮

Eisenberg Profile Freq. V

Eisenberg Profile Freq. Y

1	0	0	2	2	9	0	0	-
0	0	4	3	2	0	0	0	Identity
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
0	5	0	2	3	0	0	0	
0	0	0	0	0	0	9	0	

Consensus = Most Typical A.A.

R	V	D	C	V	A	Y	E
---	---	---	---	---	---	---	---

Better Consensus = Freq. Pattern (PCA)

R	iv	cd	š	š	A	Y	μ
---	----	----	---	---	---	---	---

š = (A,2V,C,P); μ =(4K,2Q,3E,2D)

Entropy => Sequence Variability

3	7	7	14	14	0	0	14
---	---	---	----	----	---	---	----

Profile : a position-specific scoring matrix composed of 21 columns and N rows (N=length of sequences in multiple alignment)

2hhb	Human Alpha Hemoglobin	R	V	D	C	V	A	Y	K	
	HAHU	R	V	D	C	V	A	Y	K	100
	HADG	R	V	D	C	V	A	Y	K	89
	HTOR	R	V	D	C	A	A	Y	Q	76
	HBA_CAIMO	R	V	D	P	V	A	Y	K	73
	HBAT_HORSE	R	V	D	P	A	A	Y	Q	62
1mbd	Whale Myoglobin	A	I	C	A	P	A	Y	E	
	MYWHP	A	I	C	A	P	A	Y	E	100
	MYG_CASFI	R	I	C	A	P	A	Y	E	85
	MYHU	R	I	C	V	C	A	Y	D	75
	MYBAO	R	I	C	V	C	A	Y	D	71

Eisenberg Profile Freq. A	1	0	0	2	2	9	0	0	-
Eisenberg Profile Freq. C	0	0	4	3	2	0	0	0	Identity
.	
Eisenberg Profile Freq. V	0	5	0	2	3	0	0	0	
Eisenberg Profile Freq. Y	0	0	0	0	0	0	9	0	

Consensus = Most Typical A.A.	R V D C V A Y E
Better Consensus = Freq. Pattern (PCA)	R iv cd š š A Y μ
š = (A,2V,C,P); μ = (4K,2Q,3E,2D)	
Entropy => Sequence Variability	3 7 7 14 14 0 0 14

Profiles formula for position M(p,a)

M(p,a) = chance of finding amino acid a at position p

$M_{simp}(p,a)$ = number of times a occurs at p divided by number of sequences

However, what if don't have many sequences in alignment? $M_{simp}(p,a)$ might be biased. Zeros for rare amino acids. Thus:

$$M_{cplx}(p,a) = \sum_{b=1 \text{ to } 20} M_{simp}(p,b) \times Y(b,a)$$

$\mathbf{Y}(b,a)$: Dayhoff matrix for a and b amino acids

$$S(p,a) \sim \sum_{a=1 \text{ to } 20} M_{simp}(p,a) \ln M_{simp}(p,a)$$

2hhb	Human Alpha Hemoglobin	R V D C V A Y K	
	HAHU	R V D C V A Y K	100
	HADG	R V D C V A Y K	89
	HTOR	R V D C A A Y Q	76
	HBA_CAIMO	R V D P V A Y K	73
	HBAT_HORSE	R V D P A A Y Q	62
1mbd	Whale Myoglobin	A I C A P A Y E	
	MYWHP	A I C A P A Y E	100
	MYG_CASFI	R I C A P A Y E	85
	MYHÜ	R I C V C A Y D	75
	MYBAO	R I C V C A Y D	71
Eisenberg Profile Freq. A	1 0 0 2 2 9 0 0	-	
Eisenberg Profile Freq. C	0 0 4 3 2 0 0 0	Identity	
:		
Eisenberg Profile Freq. V	0 5 0 2 3 0 0 0		
Eisenberg Profile Freq. Y	0 0 0 0 0 0 9 0		
Consensus = Most Typical A.A.	R V D C V A Y E		
Better Consensus = Freq. Pattern (PCA)	R iv cd š š A Y μ		
š = (A,2V,C,P); μ=(4K,2Q,3E,2D)			
Entropy => Sequence Variability	3 7 7 14 14 0 0 14		

Profiles

formula for

entropy

H(p,a)

$H(p,a) = - \sum_{a=1 \text{ to } 20} f(p,a) \log_2 f(p,a),$
 where $f(p,a) = \text{frequency of amino acid } a \text{ occurs at position } p$ ($M_{\text{simp}}(p,a)$)

Say column only has one aa (AAAAA):

$$H(p,a) = 1 \log_2 1 + 0 \log_2 0 + 0 \log_2 0 + \dots = 0 + 0 + 0 + \dots = 0$$

Say column is random with all aa equiprobable (ACD..ACD..ACD..):

$$H_{\text{rand}}(p,a) = .05 \log_2 .05 + .05 \log_2 .05 + \dots = -.22 + -.22 + \dots = -4.3$$

Say column is random with aa occurring according to probability found in the sequence databases (ACAAAAADAADDAAA....):

$$H_{\text{db}}(a) = - \sum_{a=1 \text{ to } 20} F(a) \log_2 F(a),$$

where $F(a)$ is freq. of occurrence of a in DB

$$H_{\text{corrected}}(p,a) = H(p,a) - H_{\text{db}}(a)$$

C1Q - Example

Ca28_Human

ELSAHATPAFTAVLTSPLPASGMPVKFDRTLYNGHSGYNPATGIFTCPVGGVYYFAYHVH
VKGTNVWVALYKNNVPATYTYDEYKKGYLDQASGGAVLQLRPNDQVWVQIPSDQANGLYS
TEYIHSSFGFLCPT

C1qb_Human

DYKATQKIAFSATRTINVPLRRDQTIRFDHVITNMNNNYEPRSGKFTCKVPGLYYFTYHA
SSRGNLCVNLMRGRERAQKVVTFCDYAYNTFQVTTGGMVLKLEQGENVFLQATDKNSLLG
MEGANSIFSGFLFPD

Cerb_Human

VRSGSAKVAFAIRSTNHEPSEMSNRTMIIYFDQVLVNIGNNFDSERSTFIAPRKGIYSF
NFHVVKVYNRQTIQVSMLNGWPVISAFLAGDQDVTRREAASNGVLIQMEKGDRAYLKLERG
NLMGGWKYSTFSGFLVFPL

COLE_LEPMA. 264

RGPKGPPGESVEQIRSAFSVGLFPSRSFPPPSLPVKFDKVFYNGEGHWDPTLNKFNVTYP
GVYLFSYHITVRNRPVRAALVVNGVRKLRTRDLSYGQDIDQASNLLALLHTDGDQVWLET
LRDWNGXYSSSEDDSTFSGFLYPDTKKPTAM

HP27_TAMAS. 72

CPPGPPGMTVNCHSKGTSAFAVKANELPPAPSQPVIFKEALHDAQGHFDLATGVFTCPVP
GLYQFGFHIEAVQRAVKVSLMRNGTQVMEREAEAQDGYESHISGTAILQLGMEDRVWLENK
LSQTDLERGTVQAVFSGFLIHEN

HSUPST2_1.95

GIQGRKGEPEGAYVYRSAFSVGLETYVTIPNMPIRFTKIFYNQQNHGDGSTGKFHCNIP
GLYYFAYHITVYMKDVKVSFKDKAMLFTYDQYQENNVDQASGSVLLHLEVGDQVWLQV
YGEGERNGLYADNDNDSTFTGFLLYHDTN

2.HS27109_1

ENALAPDFS KGSYRYAPMVAFFASHTYGMTIPGPILFNNLDVNYGASYTPRTGKFRIPYL
GVYVFKYTIESFSAHISGFLVVDGIDKLAFESENINSEIHCDRVLTGDALLENYGQEWW
LRLAKGTIPAKFPPVTTFSGYLLYRT

4.YQCC_BACSU

VVHGWTWPQKISGFAHANIGTTGVQYLKKIDHTKIAFNRVIKDSHNAFDTKNNRFIAFPND
GMYLIGASIYTLYNTSYINFHLKVYLNKGAYKTLHHVRGDFQEKDNGMNLGLNGNATVPM
NKGDYVEIWCYCNYGGDETLKRAVDDKNGVFNFFD

5.BSPBSXSE_25

ADSGWTAWQKISGFAHANIGTTGRQALIKGENNKIKYNRIIKDSHKLFDTKNNRFVASHA
GMHLVSASLYIENTERYSNFELYVYVNGTKYKLMNQFRMPTPSNNSDNEFNATVTGSVTV
PLDAGDYVEIYVYVGYSGDVTRYVTDNGALNYFD

MMCOL10A1_1.483
 Calx_Chick
 S15435
 CA18_MOUSE.597
 Ca28_Human
 MM37222_1.98
 COLE_LEPMA.264
 HP27_TAMAS.72
 S19018
 C1qb_Mouse
 C1qb_Human
 Cerb_Human
 2.HS27109_1

```

SGMPLVSANHGVGTG-----MPVSAFTVILS--KAYPA---VGCPhPIYEILYNRQQHY
-----ALTG-----MPVSAFTVILS--KAYPG---ATVPIKFDKILYNRQQHY
-----GGPA-----YEMPAFTAELT--APFPP---VGGPVKFNKLLYNGRQNY
HAYAGKKGKHGGPA-----YEMPAFTAELT--VPFPP---VGAPVKFDKLLYNGRQNY
-----ELSA-----HATPAFTAFLT--SPLPA---SGMPVKFDRTLYNGHSGY
----GTPGRKGEPEGE---AAEMYRSAFSVGLFPSRSFPP---NVPIRFTKIFYNQONHY
----RGPKGPPGE---SVEQIRSAFSVGLFPSRSFPP---PSLPVKFDKVFYNGEGHW
----GPPGPPGMTVNCHSKGTSAFKAN--ELPPA---PSQPVIFKEALHDAQGHF
----NIRD-----QPRPAFSAIRQ---NPMT---LGNVVIFDKVLTNQESPY
----D---YRATQKVAFSALRTINSPLR---PNQVIRFEKVITNANENY
----D---YKATQKIAFSATRTINVPLR---RDQTIRFDHVITNMNNNY
----V--RSGSAKVAFAIRSTNHEPSEMSNRTMIIFYFDQVLVNIGNNF
---ENALAPDFSKGS---YRYAPMVAFFASHTYGMTIP----GPILFNNLDVNYGASY
* * . : : :

```

MMCOL10A1_1.483
 Calx_Chick
 S15435
 CA18_MOUSE.597
 Ca28_Human
 MM37222_1.98
 COLE_LEPMA.264
 HP27_TAMAS.72
 S19018
 C1qb_Mouse
 C1qb_Human
 Cerb_Human
 2.HS27109_1

```

DPRSGIFTCKIPGIYYFSYHVHKGT--HVVVGLYKNGTP-TMYTY---DEYSKGYLDTA
DPRTGIFTCRIPGLYYFSYHVHAKGT--NVWVALYKNGSP-VMYTY---DEYQKGYLDQA
NPQTGIFTCEVPGVYYFAYHVHCKGG--NVWVALFKNNEP-VMYTY---DEYKKGFLDQA
NPQTGIFTCEVPGVYYFAYHVHCKGG--NVWVALFKNNEP-MMYTY---DEYKKGFLDQA
NPATGIFTCPVGGVYYFAYHVHKGT--NVWVALYKNNVP-ATYTY---DEYKKGFLDQA
DGSTGKFYCNIPLGLYYFSYHITVYMK--DVKVSLSFKDKA-VLFTY---DQYQEKNVDQA
DPTLNKFNVTPGVYLFSYHITVRNR--PVRAALVVNGVR-KLRTR---DSLYGQDIDQA
DLATGVFTCPVPGLYQFGFHIEAVQR--AVKVSLMRNGTQ-VMERE---AEAQDG-YEHI
QNHTGRFICAVPGFYYFNQVISKWD--LCLFIKSSSGGQ-PRDSLFSNTNNKGLFQVL
EPRNGKFTCKVPGLYYYFTYHASSRGN--LCVNLVRGRDRDSMQKVVTFCDYAQNTFQVT
EPRSGKFTCKVPGLYYYFTYHASSRGN--LCVNLMRGRER--AQKVVTFCDYAYNTFQVT
DSERSTFIAPRKIYISFNFHVVVKVYNRQTIQVSLMLNGWP---VISAFAQGDQDVTRREAA
TPRTGKFRIPIYLGVYVFKYTIESFSA--HISGFLVVDGIDKLAFASEN-INSEIHCDRV
* * * * :

```

MMCOL10A1_1.483
 Calx_Chick
 S15435
 CA18_MOUSE.597
 Ca28_Human
 MM37222_1.98
 COLE_LEPMA.264
 HP27_TAMAS.72
 S19018
 C1qb_Mouse
 C1qb_Human
 Cerb_Human
 2.HS27109_1

```

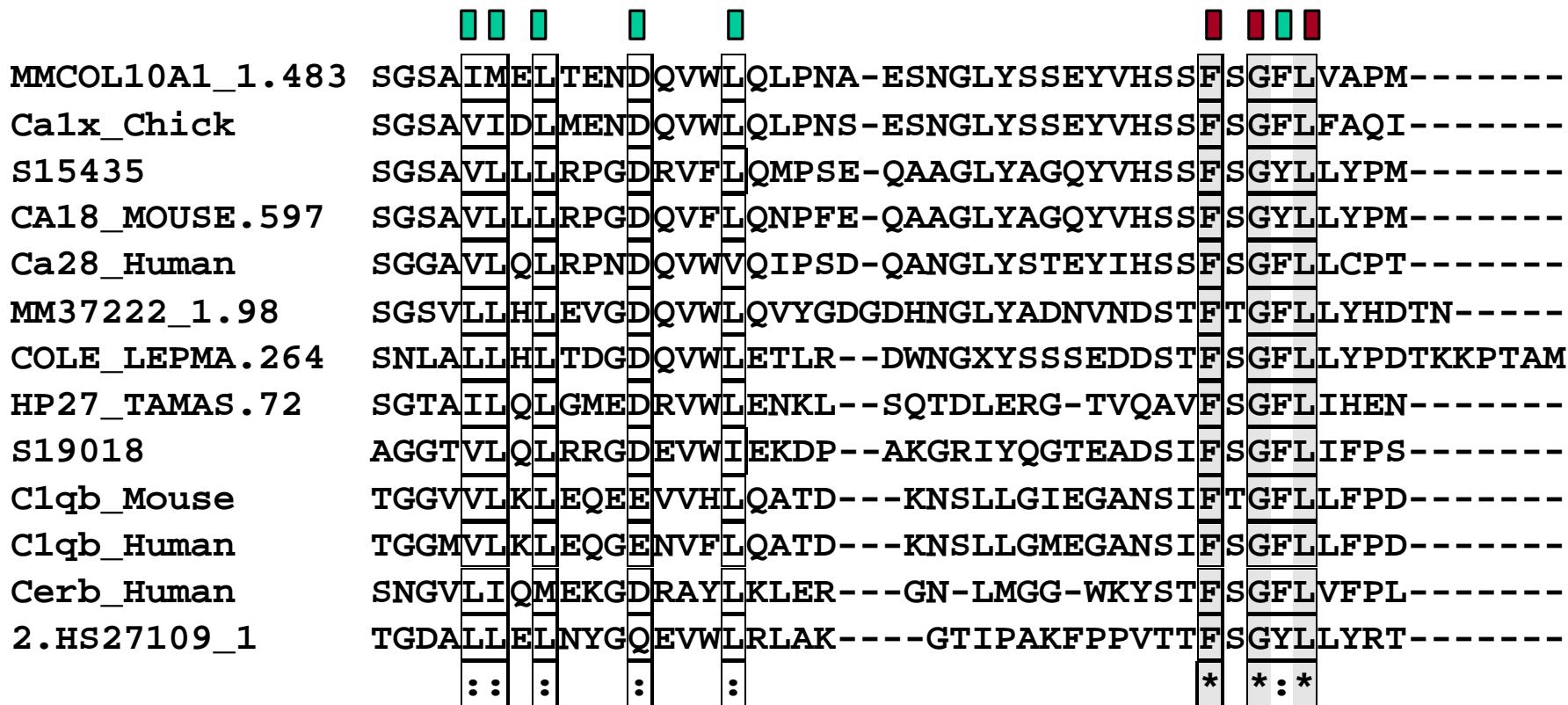
SGSAIMELTENDQVWLQLPNA-ESNGLYSSEYVHSSFSGFLVAPM-----
SGSAVIDLMENDQVWLQLPNS-ESNGLYSSEYVHSSFSGFLFAQI-----
SGSAVLLLRPGDRVFLQMPSE-QAAGLYAGQYVHSSFSGFLYLYPM-----
SGSAVLLLRPGDQVFLQNPFE-QAAGLYAGQYVHSSFSGFLYLYPM-----
SGGAVLQLRPNDQVWVQIPSD-QANGLYSTEYIHSSFSGFLLCPT-----
SGSVLLHLEVGDOVWLQVYGDGDHNGLYADNVNDSTFTGFLLYHDTN-----
SNLALLHLDGDQVWLETLR--DWNGXYSSSEDDSTFSGFLLYPDTKKPTAM
SGTAILQLGMEDRVWLENKL--SQTDLERG-TVQAVFSGFLIHEN-----
AGGTVLQLRRGDEVWIEKD--AKGRIYQGTEADSIFSGFLIFPS-----
TGGVVLKLEQEEVVHLQATD--KNSLLGIEGANSIFTGFLLFPD-----
TGGMVLKLEQGENVFLQATD--KNSLLGMEGANSIFSGFLFPD-----
SNGVLIQMEKGDRAYLKER--GN-LMGG-WKYSTFSGFLVFPL-----
TGDALLELNYGQEVLRLAK---GTIPAKFPPVTTFSGFLYRT-----
* * : *

```

Clustal Alignment

Motifs

- several proteins are grouped together by similarity searches
- they share a conserved motif
- motif is stringent enough to retrieve the family members from the complete protein database
- PROSITE: a collection of motifs (1135 different motifs)



Prosite Pattern -- EGF like pattern

A sequence of about thirty to forty amino-acid residues long found in the sequence of epidermal growth factor (EGF) has been shown [1 to 6] to be present, in a more or less conserved form, in a large number of other, mostly animal proteins. The proteins currently known to contain one or more copies of an EGF-like pattern are listed below.

- Bone morphogenic protein 1 (BMP-1), a protein which induces cartilage and bone formation.
- *Caenorhabditis elegans* developmental proteins lin-12 (13 copies) and glp-1 (10 copies).
- Calcium-dependent serine proteinase (CASP) which degrades the extracellular matrix proteins type
- Cell surface antigen 114/A10 (3 copies).
- Cell surface glycoprotein complex transmembrane subunit .
- Coagulation associated proteins C, Z (2 copies) and S (4 copies).
- Coagulation factors VII, IX, X and XII (2 copies).
- Complement C1r/C1s components (1 copy).
- Complement-activating component of Ra-reactive factor (RARF) (1 copy).
- Complement components C6, C7, C8 alpha and beta chains, and C9 (1 copy).
- Epidermal growth factor precursor (7-9 copies).

The diagram shows a sequence of amino acids represented by a string of characters. Above the sequence, there are three pairs of vertical lines connecting specific positions to three horizontal dashed lines above them. Below the sequence, there is a row of asterisks (*). The sequence itself consists of the following characters: x(4)-C-x(0,48)-C-x(3,12)-C-x(1,70)-C-x(1,6)-C-x(2)-G-a-x(0,21)-G-x(2)-C-x. The 'C' characters are red, while the other characters (x, G, a) are black. The 'x' characters are enclosed in parentheses with ranges, such as x(0,48) or x(1,6). The 'G' character is green, and the 'a' character is blue. The asterisks are black and aligned under the sequence.

'C': conserved cysteine involved in a disulfide bond.

'G': often conserved glycine

'a': often conserved aromatic amino acid

'*': position of both patterns.

'x': any residue

-Consensus pattern: C-x-C-x(5)-G-x(2)-C

[The 3 C's are involved in disulfide bonds]

EGF Profile Generated for SEARCHWISE

Cons	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Gap
V	-1	-2	-9	-5	-13	-18	-2	-5	-2	-7	-4	-3	-5	-1	-3	0	0	-1	-24	-10	100
D	0	-14	-1	-1	-16	-10	0	-12	0	-13	-8	1	-3	0	-2	0	0	-8	-26	-9	100
V	0	-13	-9	-7	-15	-10	-6	-5	-5	-7	-5	-6	-4	-4	-6	-1	0	-1	-27	-14	100
D	0	-20	18	11	-34	0	4	-26	7	-27	-20	15	0	7	4	6	2	-19	-38	-21	100
P	3	-18	1	3	-26	-9	-5	-14	-1	-14	-12	-1	12	1	-4	2	0	-9	-37	-22	100
C	5	115	-32	-30	-8	-20	-13	-11	-28	-15	-9	-18	-31	-24	-22	1	-5	0	-10	-5	100
A	2	-7	-2	-2	-21	-5	-4	-12	-2	-13	-9	0	-1	0	-3	2	1	-7	-30	-17	100
s	2	-12	3	2	-25	0	0	-18	0	-18	-13	4	3	1	-1	7	4	-12	-30	-16	25
n	-1	-15	4	4	-19	-7	3	-16	2	-16	-10	7	-6	3	0	2	0	-11	-23	-10	25
p	0	-18	-7	-6	-17	-11	0	-17	-5	-15	-14	-5	28	-2	-5	0	-1	-13	-26	-9	25
c	5	115	-32	-30	-8	-20	-13	-11	-28	-15	-9	-18	-31	-24	-22	1	-5	0	-10	-5	25
L	-5	-14	-17	-9	0	-25	-5	4	-5	8	8	-12	-14	-1	-5	-7	-5	2	-15	-5	100
N	-4	-16	12	5	-20	0	24	-24	5	-25	-18	25	-10	6	2	4	1	-19	-26	-2	100
g	1	-16	7	1	-35	29	0	-31	-1	-31	-23	12	-10	0	-1	4	-3	-23	-32	-23	50
G	6	-17	0	-7	-49	59	-13	-41	-10	-41	-32	3	-14	-9	-9	5	-9	-29	-39	-38	100
T	3	-10	0	2	-21	-12	-3	-5	1	-11	-5	1	-4	1	-1	6	11	0	-33	-18	100
C	5	115	-32	-30	-8	-20	-13	-11	-28	-15	-9	-18	-31	-24	-22	1	-5	0	-10	-5	100
I	-6	-13	-19	-11	0	-28	-5	8	-4	6	8	-12	-17	-4	-5	-9	-4	6	-12	-1	100
d	-4	-19	8	6	-15	-13	5	-17	0	-16	-12	5	-9	2	-2	-1	-1	-13	-24	-5	31
i	0	-6	-8	-6	-4	-11	-5	3	-5	1	2	-5	-8	-4	-6	-2	0	4	-14	-6	31
g	1	-13	0	0	-20	-3	-3	-12	-3	-13	-8	0	-7	0	-5	2	0	-7	-29	-16	31
L	-5	-11	-20	-14	0	-23	-9	9	-11	8	7	-14	-17	-9	-14	-8	-4	7	-17	-5	100
E	0	-20	14	10	-33	5	0	-25	2	-26	-19	11	-9	4	0	3	0	-19	-34	-22	100
S	3	-13	4	3	-28	3	0	-18	2	-20	-13	6	-6	3	1	6	3	-12	-32	-20	100
Y	-14	-9	-25	-22	31	-34	10	-5	-17	0	-1	-14	-13	-13	-15	-14	-13	-7	17	44	100
T	0	-10	-6	-1	-11	-16	-2	-7	-1	-9	-5	-3	-9	0	-1	1	3	-4	-16	-8	100
C	5	115	-32	-30	-8	-20	-13	-11	-28	-15	-9	-18	-31	-24	-22	1	-5	0	-10	-5	100
R	0	-13	0	2	-19	-11	1	-12	4	-13	-8	3	-8	4	5	1	1	-8	-23	-13	100
C	5	115	-32	-30	-8	-20	-13	-11	-28	-15	-9	-18	-31	-24	-22	1	-5	0	-10	-5	100
P	0	-14	-8	-4	-15	-17	0	-7	-1	-7	-5	-4	6	0	-2	0	1	-3	-26	-10	100
P	1	-18	-3	0	-24	-13	-3	-12	1	-13	-10	-2	15	2	0	2	1	-8	-33	-19	100
G	4	-19	3	-4	-48	53	-11	-40	-7	-40	-31	5	-13	-7	-7	4	-7	-29	-39	-36	100
y	-22	-6	-35	-31	55	-43	11	-1	-25	6	4	-21	-34	-20	-21	-22	-20	-7	43	63	50
S	1	-9	-3	-1	-14	-7	0	-10	-2	-12	-7	0	-7	0	-4	4	4	-5	-24	-9	100
G	5	-20	1	-8	-52	66	-14	-45	-11	-44	-35	4	-16	-10	-10	4	-11	-33	-40	-40	100
E	2	-20	10	12	-31	-7	0	-19	6	-20	-15	5	4	7	2	4	2	-13	-38	-22	100
R	-5	-17	0	1	-16	-13	8	-16	9	-16	-11	5	-11	7	15	-1	-1	-13	-18	-6	100
C	5	115	-32	-30	-8	-20	-13	-11	-28	-15	-9	-18	-31	-24	-22	1	-5	0	-10	-5	100
E	0	-26	20	25	-34	-5	6	-25	10	-25	-17	9	-4	16	5	3	0	-18	-38	-23	100
T	-4	-11	-13	-8	-1	-21	2	0	-4	-1	0	-6	-14	-3	-5	-4	0	0	-15	0	100
D	0	-18	5	4	-24	-11	-1	-11	2	-14	-9	1	-6	2	0	0	0	-6	-34	-18	100
I	0	-10	-2	-1	-17	-14	-3	-4	-1	-9	-4	0	-11	0	-4	0	2	-1	-29	-14	100
D	-4	-15	-1	-2	-13	-16	-3	-8	-5	-6	-4	-1	-7	-2	-7	-3	-2	-6	-27	-12	100

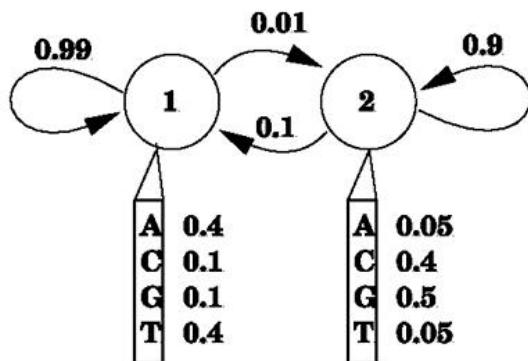
Cons.
Cys

Hidden Markov Model:

- a composition of finite number of states,
- each corresponding to a column in a multiple alignment
- each state emits symbols, according to symbol-emission probabilities

HMMs

Starting from an initial state, a sequence of symbols is generated by moving from state to state until an end state is reached.



state sequence (hidden):

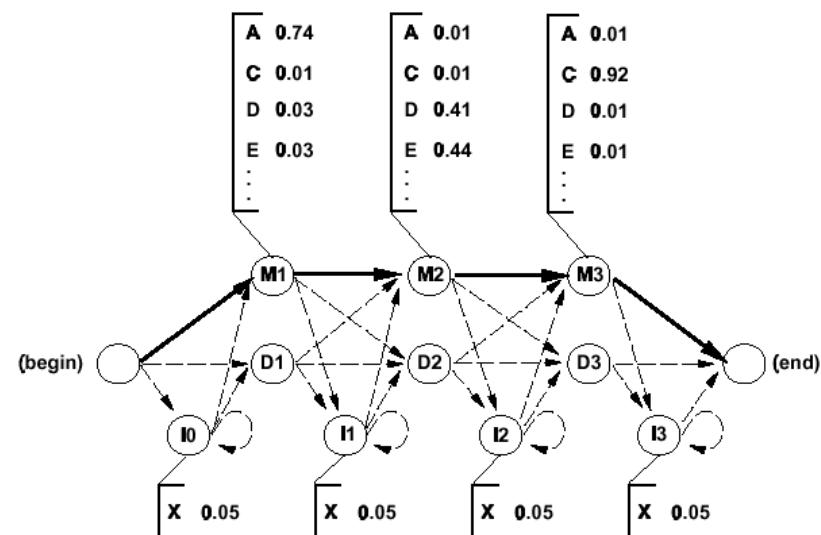
... (1) (1) (1) (1) (1) (2) (2) (2) (1) (1) ...

transitions: ? 0.99 0.99 0.99 0.99 0.01 0.9 0.9 0.1 0.99

symbol sequence (observable):

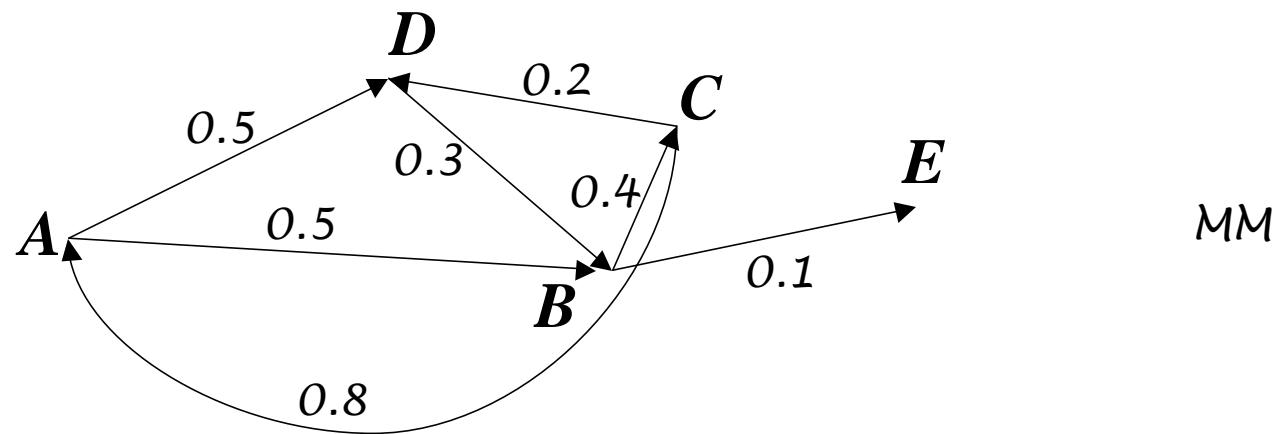
... A T C A A G G C G A T ...

emissions: 0.4 0.4 0.1 0.4 0.4 0.5 0.5 0.4 0.5 0.4 0.4



(Figures from Eddy, Curr. Opin. Struct. Biol.)

Markov Models



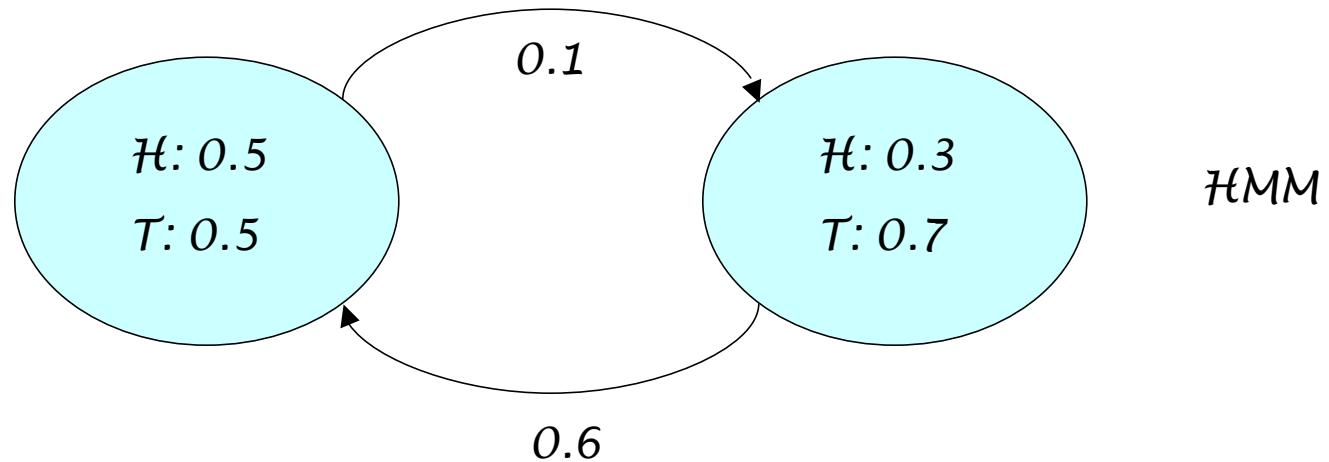
Path: A D B C

Probability = Init(A)*0.5*0.3*0.4

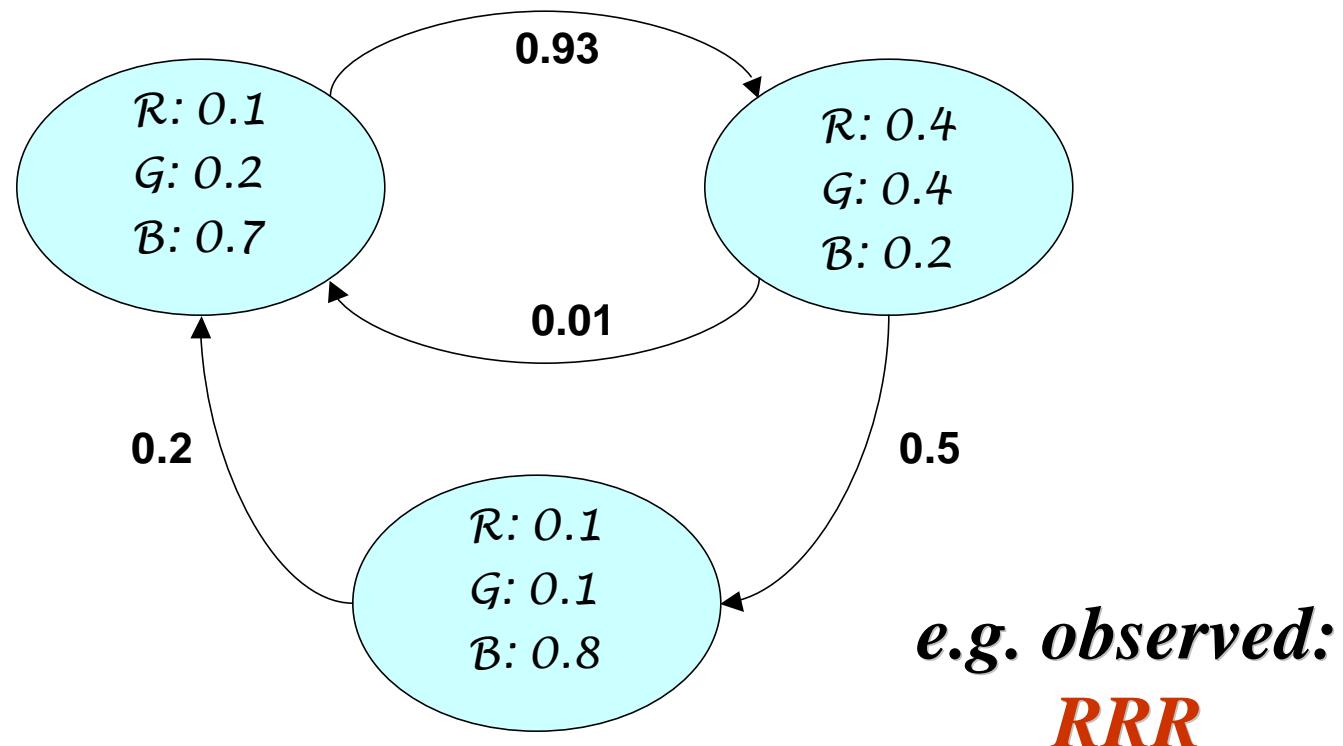
Hidden Markov models

The path is unknown (hidden): H H T H T T H T T T

Probability = ?



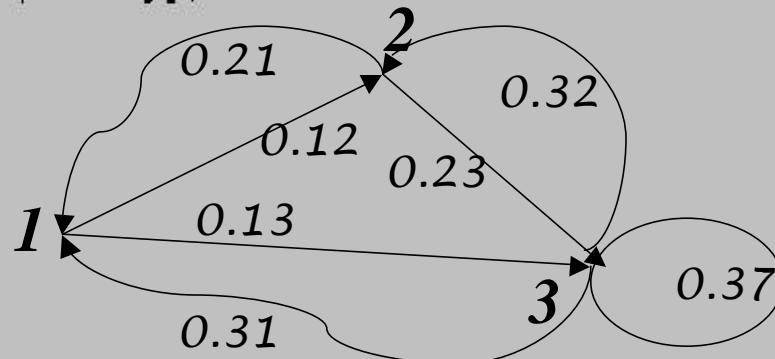
More HMMs



Probability of a given sequence =
Sum probability over ALL paths giving that sequence

Example:
simple fully interconnected model (N=3)

```
Nmax = 3;
Mmax = 3;
Tmax = 3;
a = Table[0.0, {i, 1, Nmax}, {j, 1, Nmax}];
b = Table[0.0, {i, 1, Nmax}, {j, 1, Mmax}];
init = Table[0.0, {i, 1, Nmax}];
mobs = Table[0.0, {i, 1, Tmax}];
a = {
  {0.75, 0.12, 0.13},
  {0.21, 0.56, 0.23},
  {0.31, 0.32, 0.37}
};
b = {
  {0.10, 0.45, 0.45},
  {0.20, 0.40, 0.40},
  {0.30, 0.35, 0.35}
};
init = {0.5, 0.2, 0.3};
mobs = {1, 1, 1};
Print["HMM and observation sequence have been initialized"];
```



Scoring by Brute Force method:

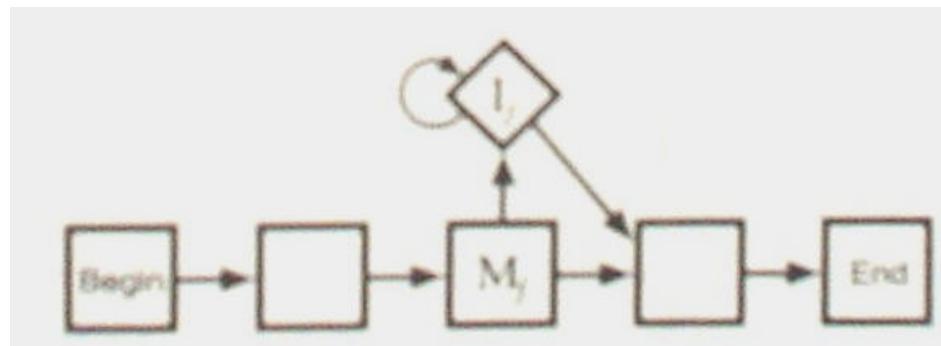
0.5*0.1*0.75*0.1*0.75*0.1	0.2*0.2*0.56*0.2*0.56*0.2
0.5*0.1*0.75*0.1*0.12*0.2	0.2*0.2*0.56*0.2*0.23*0.3
0.5*0.1*0.75*0.1*0.13*0.3	0.2*0.2*0.23*0.3*0.31*0.1
0.5*0.1*0.12*0.2*0.21*0.1	0.2*0.2*0.23*0.3*0.32*0.2
0.5*0.1*0.12*0.2*0.56*0.2	0.2*0.2*0.23*0.3*0.37*0.3
0.5*0.1*0.12*0.2*0.23*0.3	0.3*0.3*0.31*0.1*0.75*0.1
0.5*0.1*0.13*0.3*0.31*0.1	0.3*0.3*0.31*0.1*0.12*0.2
0.5*0.1*0.13*0.3*0.32*0.2	0.3*0.3*0.31*0.1*0.13*0.3
0.5*0.1*0.13*0.3*0.37*0.3	0.3*0.3*0.32*0.2*0.21*0.1
0.2*0.2*0.21*0.1*0.75*0.1	0.3*0.3*0.32*0.2*0.56*0.2
0.2*0.2*0.21*0.1*0.12*0.2	0.3*0.3*0.32*0.2*0.23*0.3
0.2*0.2*0.21*0.1*0.13*0.3	0.3*0.3*0.37*0.3*0.31*0.1
0.2*0.2*0.56*0.2*0.21*0.1	0.3*0.3*0.37*0.3*0.32*0.2
0.2*0.2*0.56*0.2*0.56*0.2	0.3*0.3*0.37*0.3*0.37*0.3

Brute Force Method Score=0.00635752

Sequence profile elements

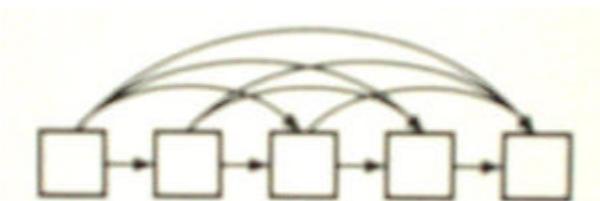
- Insertions:

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

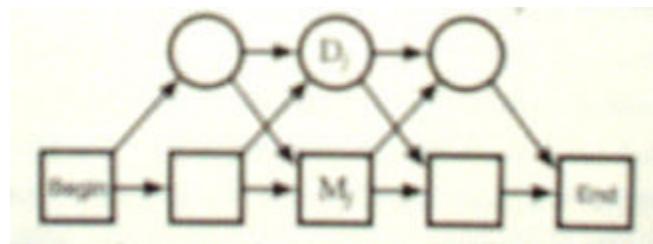


Sequence profile elements

- Deletions:

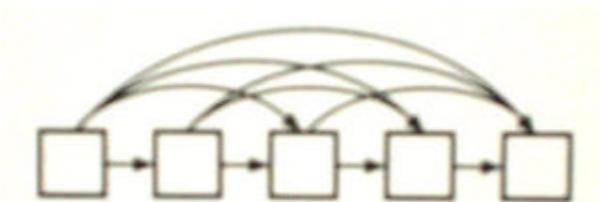


C	A	-	T	G	
-	-	-	-	-	
C	A	T	T	G	
-	-	-	-	-	
C	A	A	T	T	G
			-	-	
			?	?	

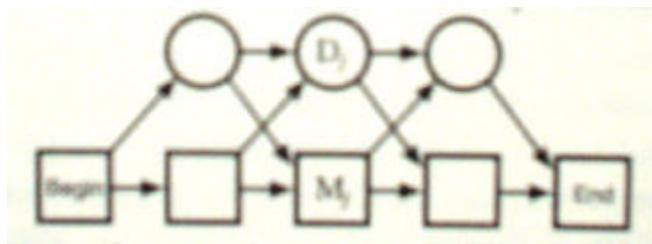


HMM sequence profiles

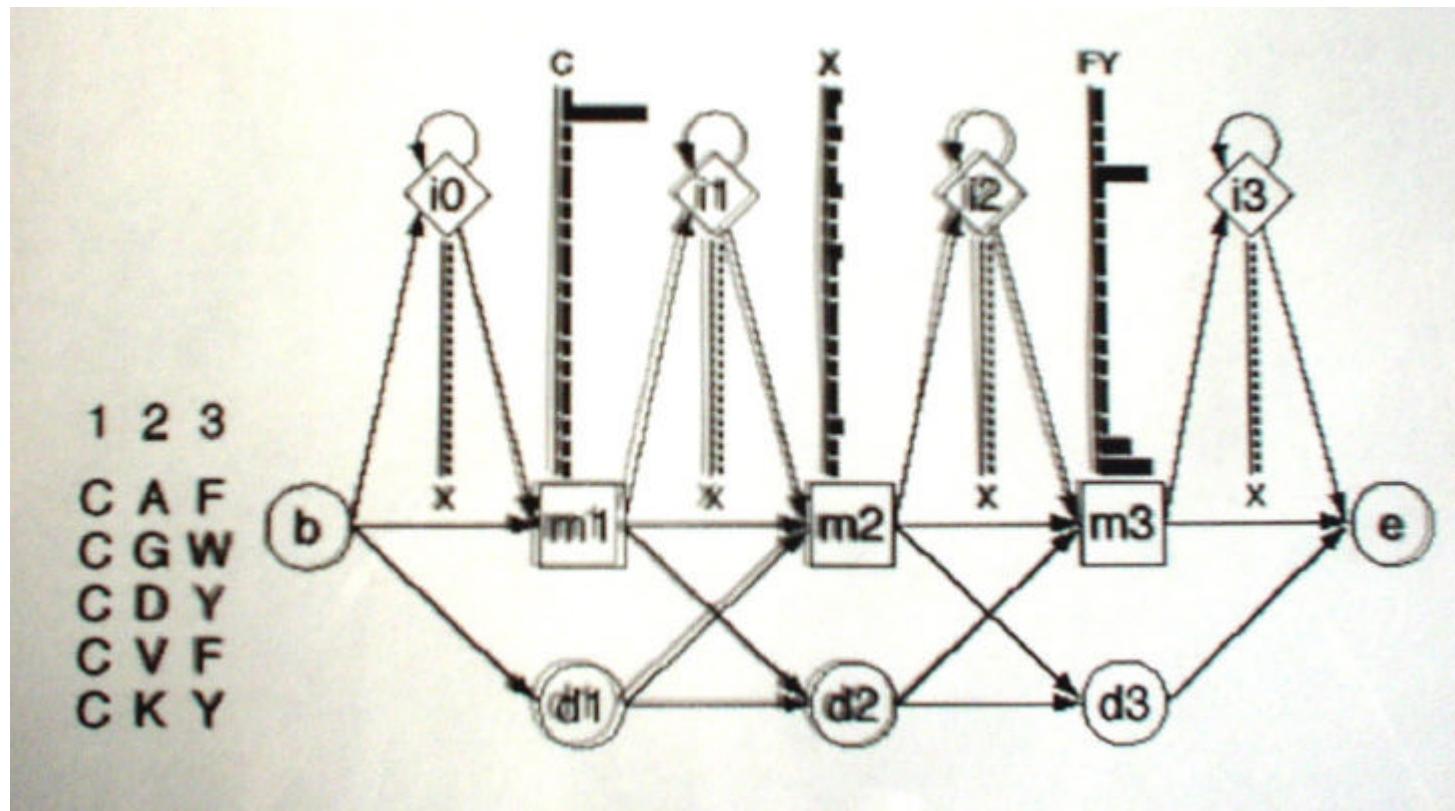
- Deletions:



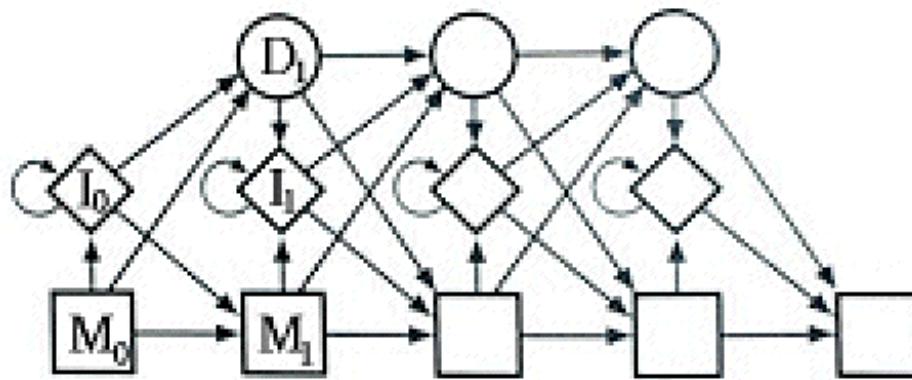
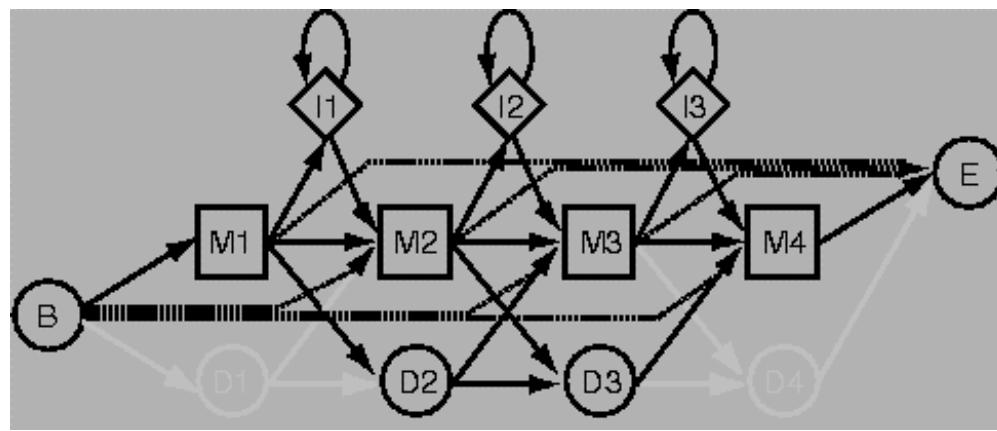
C	A	-	T	G	
-	-	-	-	-	
C	A	T	T	G	
-	-	-	-	-	
C	A	A	T	T	G
			-	-	
			?	?	



Result: HMM sequence profile



Different topologies:



Algorithms

$$P(O, \lambda) = \sum_{i=1}^N \alpha_t(i) \quad \alpha_1(i) = \pi_i b_i(O_1)$$

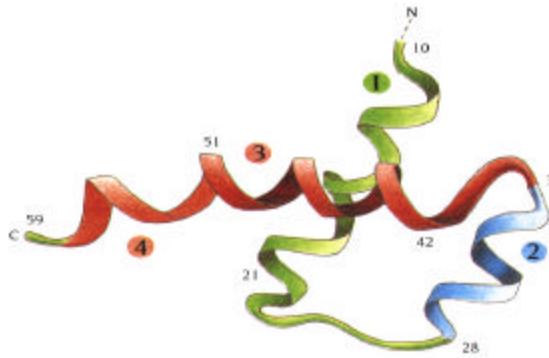
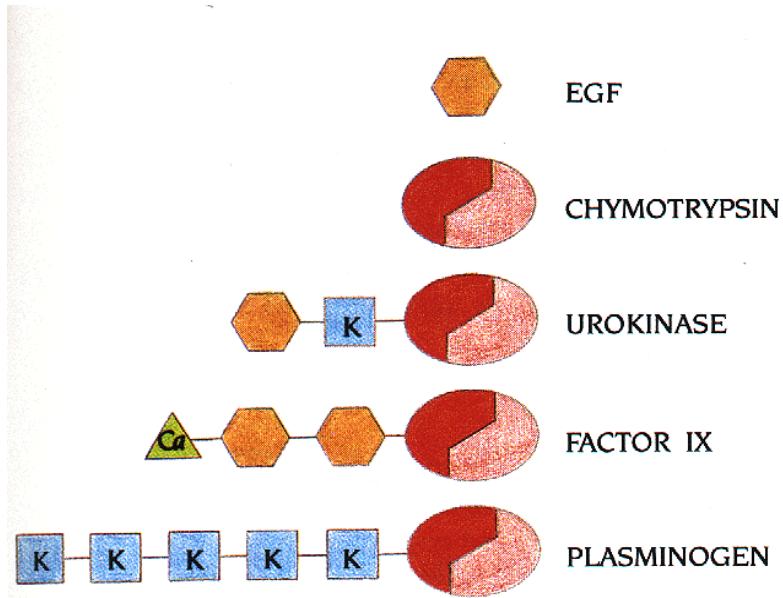
$$\alpha_{t+1}(j) = b_j(O_{t+1}) \left(\sum_{i=1}^N \alpha_t(j) a_{i,j} \right)$$

Forward Algorithm – finds probability P that a model λ emits a given sequence O by summing over all paths that emit the sequence the probability of that path

Viterbi Algorithm – finds the most probable path through the model for a given sequence
(both usually just boil down to simple applications of dynamic programming)

Modules

(Figures from Branden & Tooze)



- Another example of the helix-loop-helix motif is seen within several DNA binding domains including the homeobox proteins which are the master regulators of development

Figure 2.19 Organization of polypeptide chains into domains. Small protein molecules like the epidermal growth factor, EGF, comprise only one domain. Others like the serine proteinase chymotrypsin are arranged in two domains that are both required to form a functional unit (Chapter 15). Many of the proteins that are involved in blood coagulation and fibrinolysis, such as urokinase, factor IX, and plasminogen have long polypeptide chains that comprise different combinations of domains homologous to EGF and serine proteinases and, in addition, calcium-binding domains and Kringle domains.

- Domains that are homologous to the epidermal growth factor, EGF, which is a small polypeptide chain of 53 amino acids;
- Serine proteinase domains that are homologous to chymotrypsin, which has about 245 amino acids arranged in two domains;
- Kringle domains that have a characteristic pattern of three internal disulphide bridges within a region of about 85 amino acid residues;
- Calcium-binding domain (see Figure 2.13).

- Several motifs (β -sheet, beta-alpha-beta, helix-loop-helix) combine to form a compact globular structure termed a domain or tertiary structure
- A domain is defined as a polypeptide chain or part of a chain that can independently fold into a stable tertiary structure
- Domains are also units of function (DNA binding domain, antigen binding domain, ATPase domain, etc.)

The Score

Simplest score
(for identity
matrix) is $S = \#$
matches

What does a
Score of 10
mean? What is
the Right Cutoff?

$$S = \sum_{i,j} S(i, j) - nG$$

S = Total Score

$S(i,j)$ = similarity matrix
score for aligning i and j

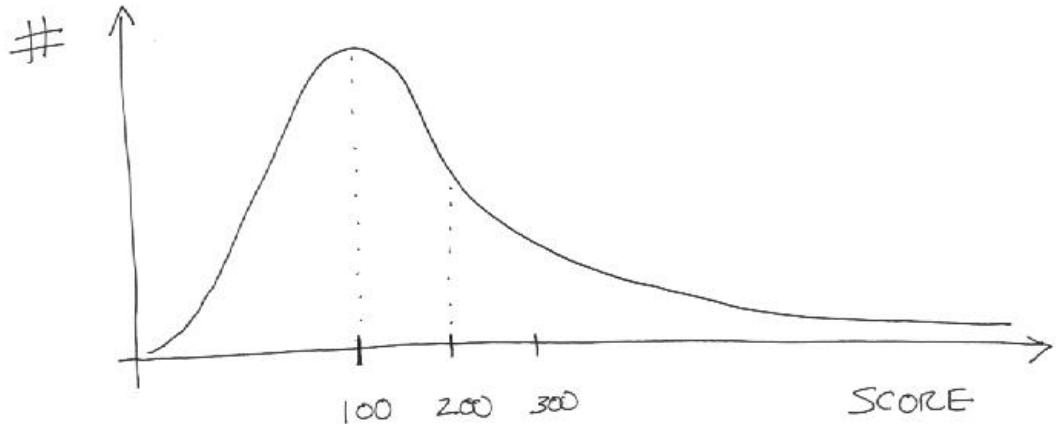
Sum is carried out over all
aligned i and j

n = number of gaps
(assuming no gap ext.
penalty)

G = gap penalty

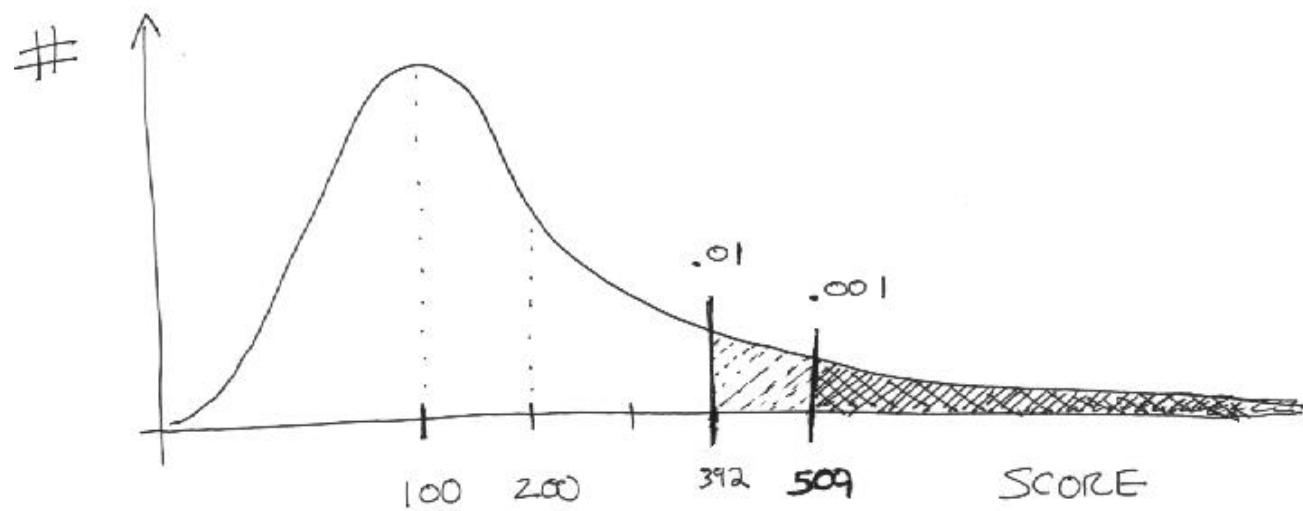
Score in Context of Other Scores

- How does Score Rank Relative to all the Other Possible Scores
 - ◊ P-value
 - ◊ Percentile Test Score Rank
- All-vs-All comparison of the Database (100K x 100K)
 - ◊ Graph Distribution of Scores
 - ◊ $\sim 10^{10}$ scores much smaller number of true positives
 - ◊ N dependence



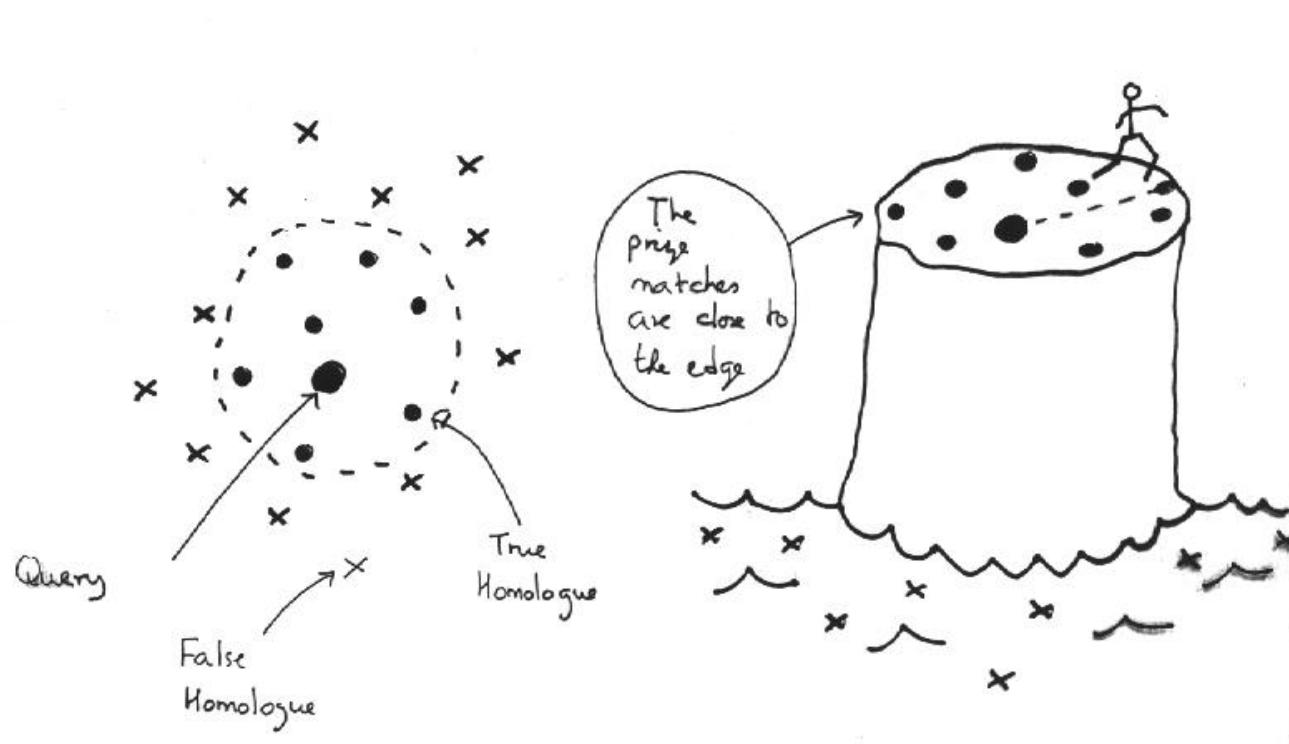
P-value in Sequence Matching

- $P(s > S) = .01$
 - ◊ P-value of .01 occurs at score threshold S (392 below) where score s from random comparison is greater than this threshold 1% of the time
- Likewise for $P=.001$ and so on.



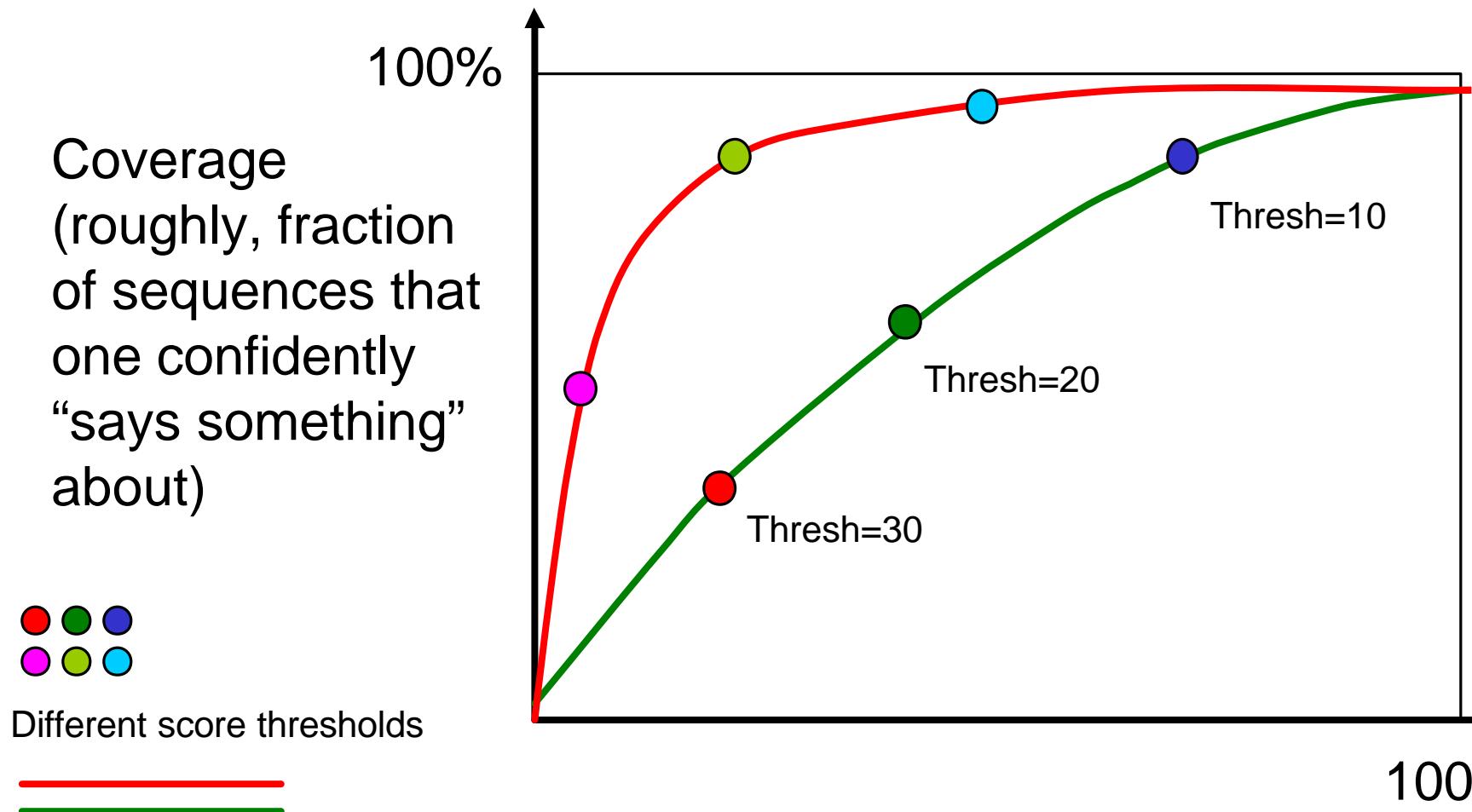
Objective is to Find Distant Homologues

- Score (Significance) Threshold
- Maximize Coverage with an Acceptable Error Rate



(graphic adapted from M Levitt)

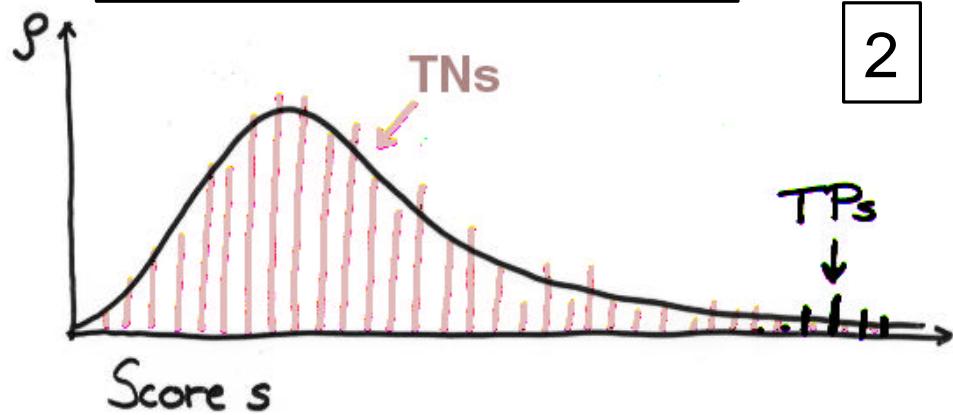
Coverage v Error Rate



d1ahn	*	■■■■■
d1wab	0.7 TP	■■■■■
d5lima	4.9	■■■■■
d4lima	7.1	■■■■■
d1hila	5.1	■■■■■
d1igbt1	6.2	■■■■■
	1.2 TP	■■■■■
	2.1 FP	■■■■■
	4.4	■■■■■

P-values

1

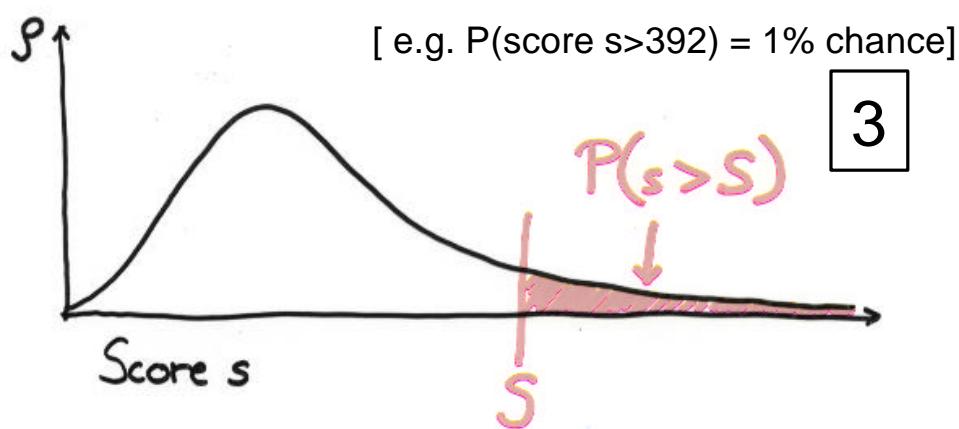


•Significance Statistics

- ◊ For sequences, originally used in Blast (Karlin-Altschul). Then in FASTA, &c.
- ◊ Extrapolated Percentile Rank: How does a Score Rank Relative to all Other Scores?

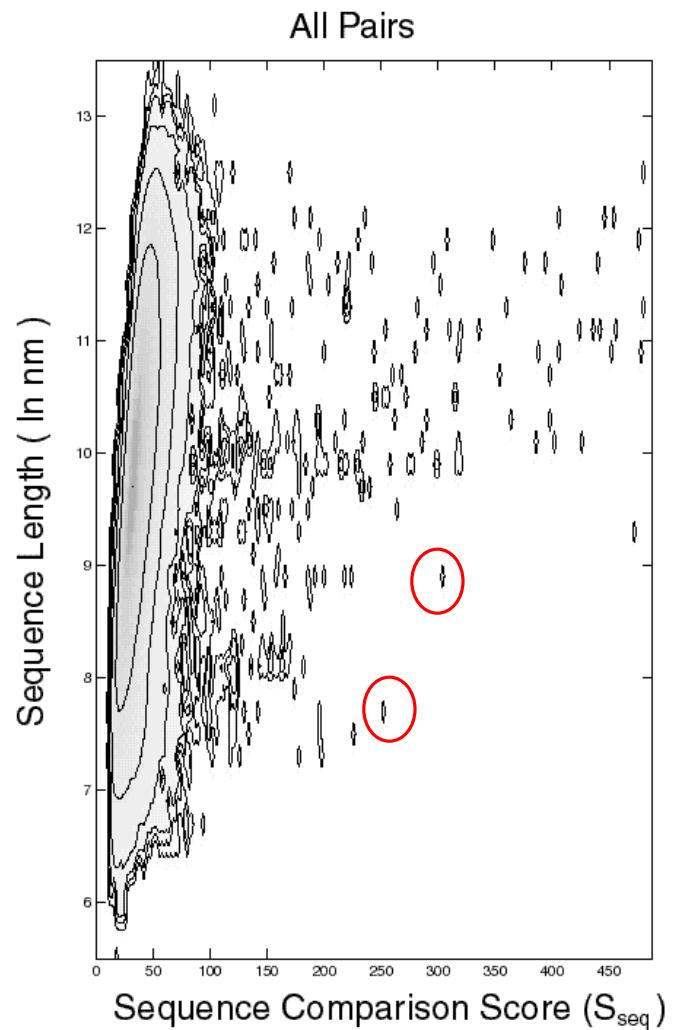
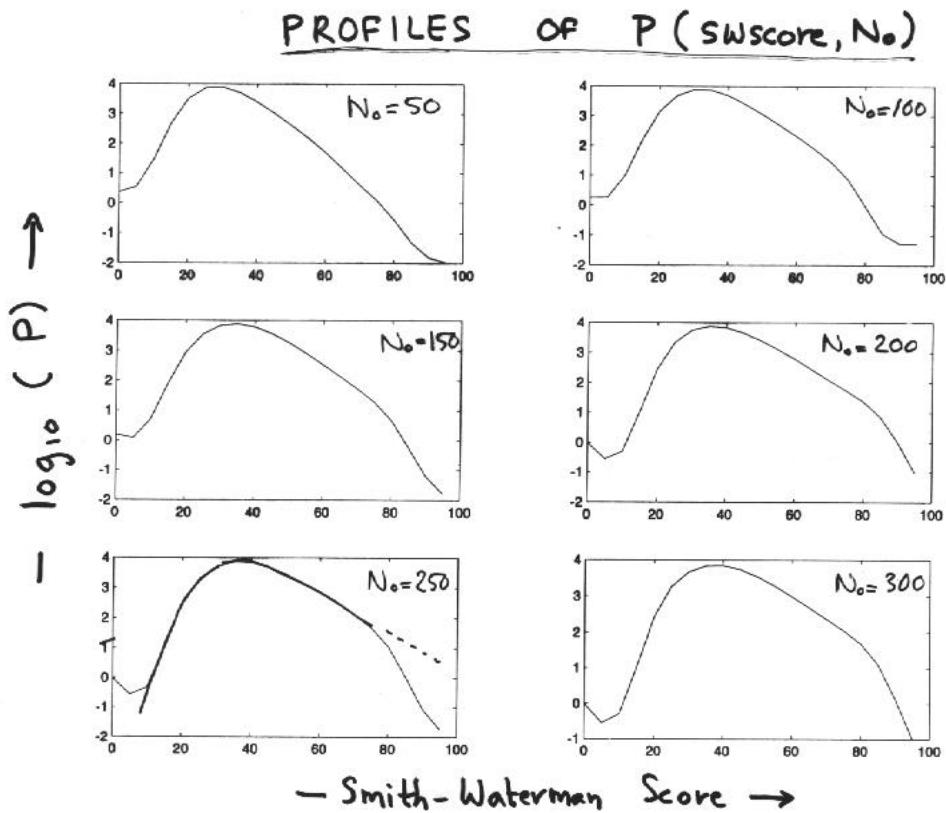
•Our Strategy: Fit to Observed Distribution

- 1) All-vs-All comparison
- 2) Graph Distribution of Scores in 2D (N dependence); $1K \times 1K$ families $\rightarrow \sim 1M$ scores; $\sim 2K$ included TPs
- 3) Fit a function $p(S)$ to TN distribution (TNs from scop); Integrating p gives $P(s>S)$, the CDF, chance of getting a score better than threshold S randomly
- 4) Use same formalism for sequence & structure



What Distribution Really Looks Like

- N Dependence
- True Positives ○



EVD Fits

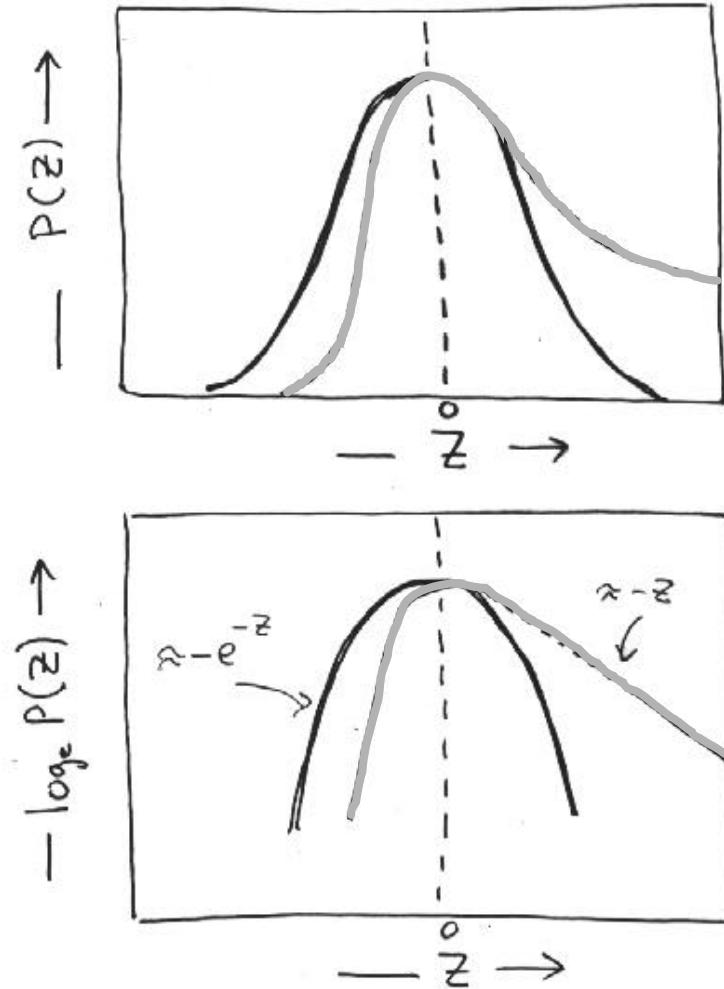
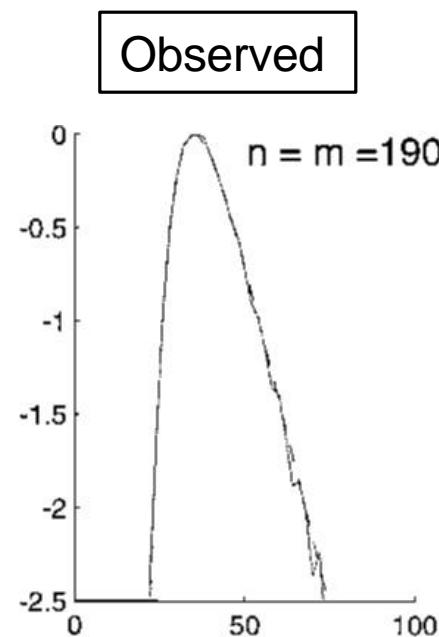
$$r(z) = \exp(-z - e^{-z})$$

$$(\ln r(z) = -z - e^{-z})$$

- Reasonable as Dyn. Prog. maximizes over pseudo-random variables
- EVD is **Max**(indep. random variables);
- Normal is **Sum**(indep. random variables)

$$\rho(z) = \exp(-z^2)$$

$$\ln \rho(z) = -z^2$$

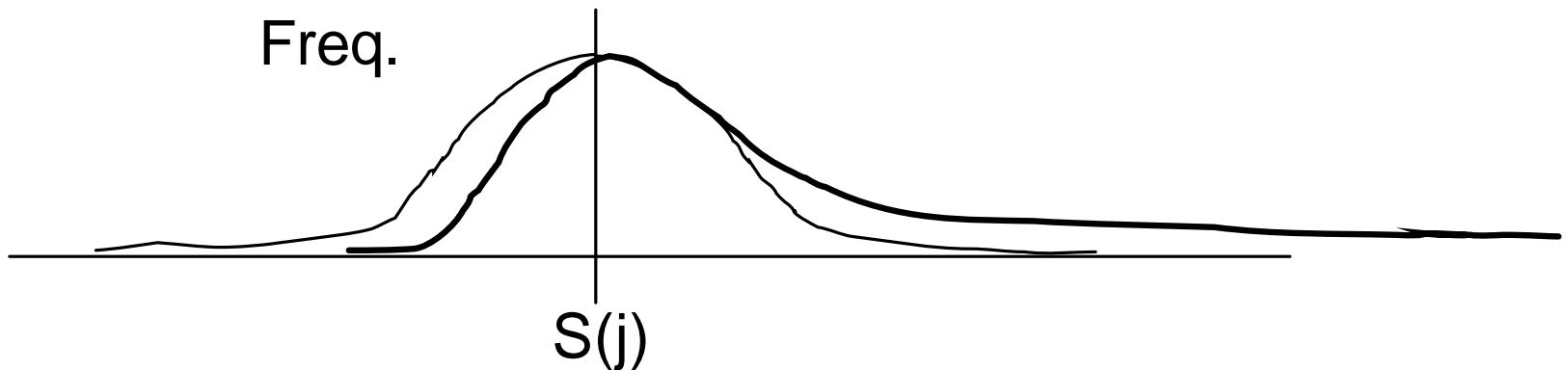


Extreme Value Distribution (EVD, long-tailed) fits the observed distributions best. The corresponding formula for the P-value:

$$P(z > Z) = \int r(z) dz = 1 - \exp(-e^{-Z})$$

Extreme Value vs. Gaussian

- $X = \text{set of random numbers}$
Each set indexed by j
 - ◊ $j=1: 1,4,9,1,3,1$
 - ◊ $j=2: 2,7,3,11,22,1,22$
- Gaussian $S(j) = \sum_j X_i$ [central limit]
- EVD $S(j) = \max(X_i)$



EVD #2

3 Free Parm. fit to EVD involving: a, b, s .

These are the only difference betw. sequence and structure.

$$Z = \frac{S - (a \ln N + b)}{s}$$

$$S = \sum_{i,j} M(i,j) - G$$

$$r(z) = \exp(-z - e^{-z})$$

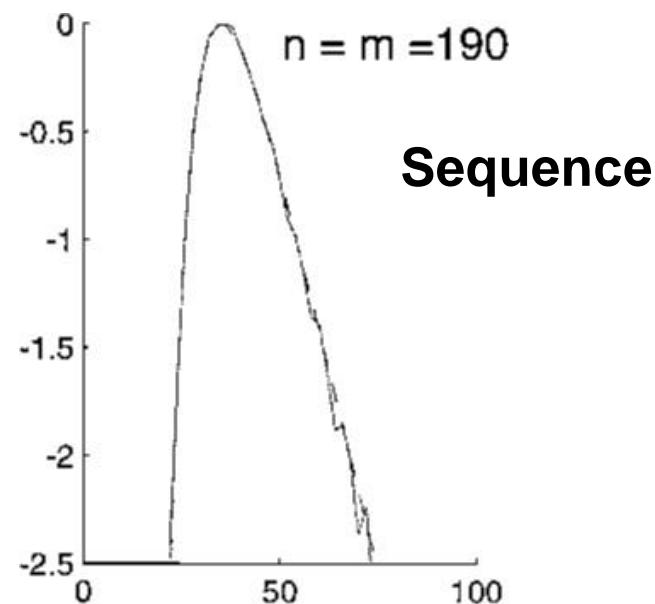
N, G, M also defined differently for sequence and structure.

N = number of residues matched.

G = total gap penalty.

$M(i,j)$ = similarity matrix

(Blossum for seq. or $M_{str}(i,j)$, struc.)



End of Class 2

Explicit Form of the P-value in terms of Extreme Value Distribution

$F(s) = \text{E.V.D of scores}$

$$F(s) = \exp(-Z(s)) - \exp(-Z(s)))$$

$$\begin{aligned} Z(s) &= s/A + \ln(NM) + B \\ &= (s' - L)/W \end{aligned}$$

s = Score from random S-W
Alignment

L = most common one (mode)

W = width parameter (like SD)

N & M are lengths of 2 seq.

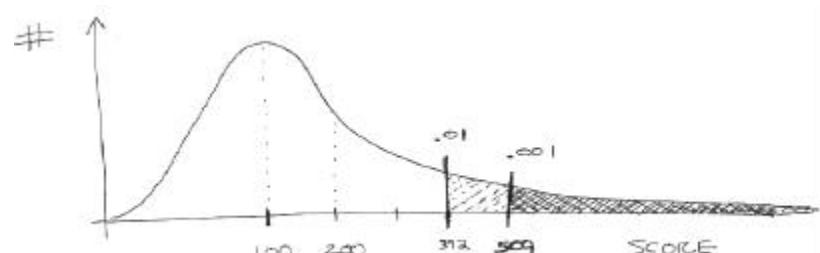
A & B are fit parameters

$$P(s>S) = \text{CDF} = \int F(s) \, ds$$

$$P(s>S) = 1 - \exp(-\exp(-Z(s)))$$

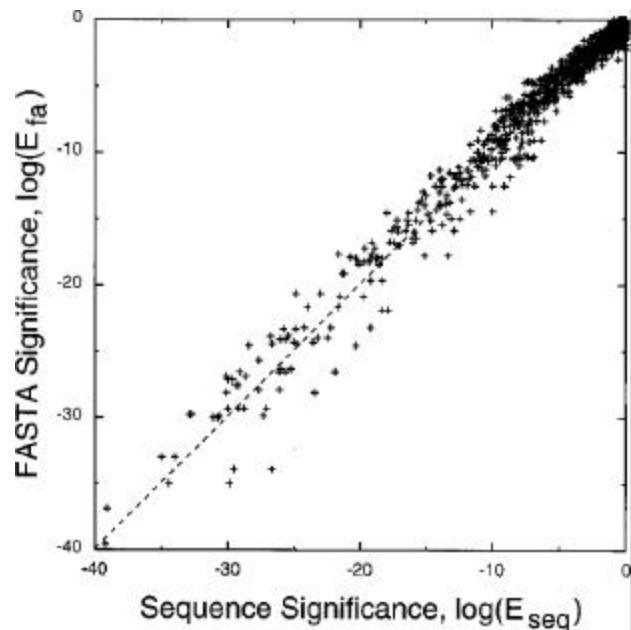
Given Score Threshold S (1%),

$P(s > S)$ is the chance that a given random score s is greater than the threshold



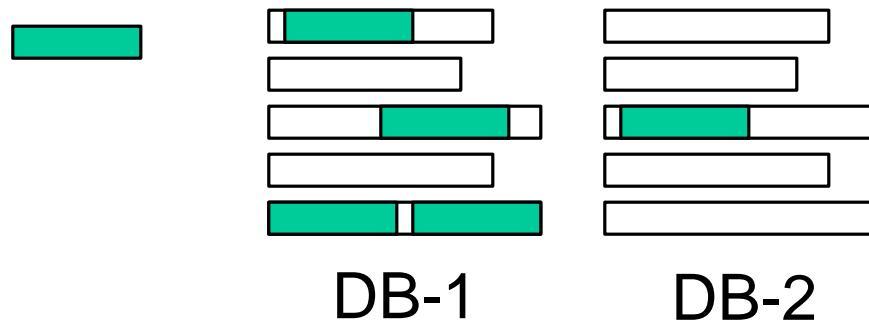
Use Sequence Scores to Validate

- Sequence P-value perfectly tracks FASTA e-value
 - ◊ Validates approach
 - ◊ Added Benefit: allows computation of an e-value without doing a db run
- Significance computation can be applied to **any** existing sequence or structure alignment



Significance Depends on Database Size

- The Significance of Similarity Scores Decreases with Database Growth
 - ◊ The score between any pair of sequence pair is constant
 - ◊ The number of database entries grows exponentially
 - ◊ The number of nonhomologous entries >> homologous entries
 - ◊ Greater sensitivity is required to detect homologies
Greater s
- Score of 100 might rank as best in database of 1000 but only in top-100 of database of 1000000



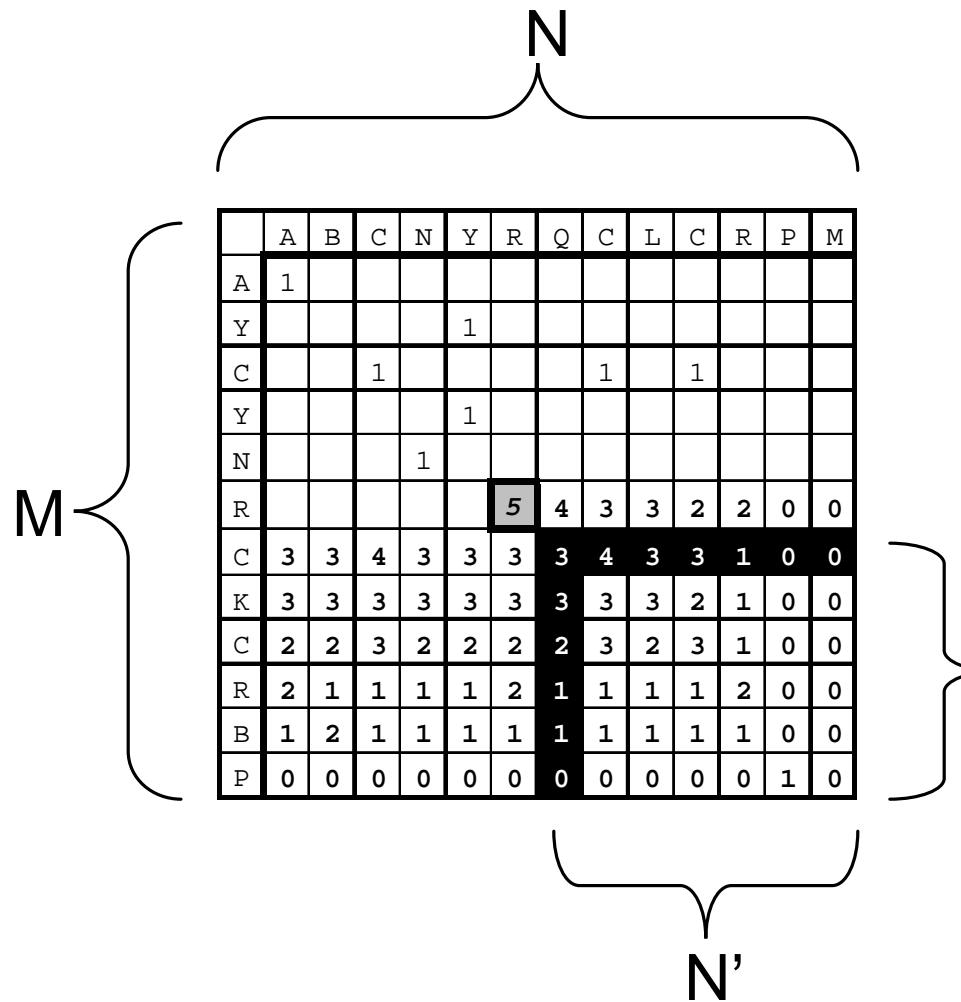
Low-Complexity Regions

- Low Complexity Regions
 - ◊ Different Statistics for matching
AAATTTAAATTAAATTAAATTAAATTAAATT
than
ACSRPLRVSHRSENCVASNKPQLVKLMTHVKDFCV
 - ◊ Automatic Programs Screen These Out (SEG)
 - ◊ Identify through computation of sequence entropy in a window of a given size
 $H = \sum f(a) \log_2 f(a)$
- Also, Compositional Bias
 - ◊ Matching A-rich query to A-rich DB vs. A-poor DB



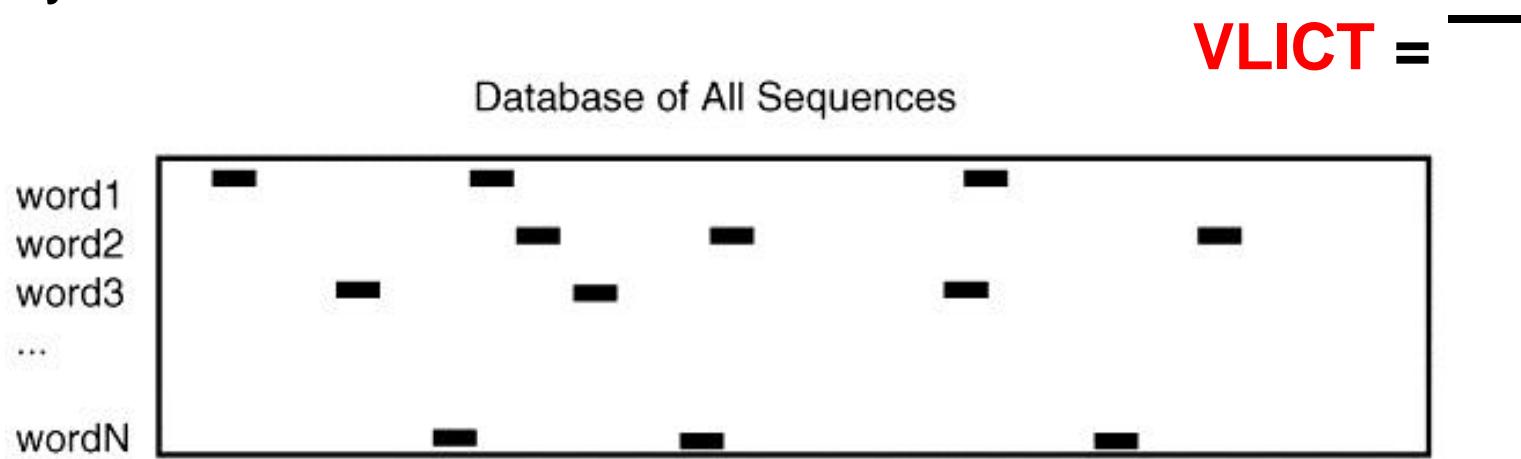
Computational Complexity

- Basic NW Algorithm is $O(n^2)$ (in speed)
 - ◊ $M \times N$ squares to fill
 - ◊ At each square need to look back $(M'+N')$ “black” squares to find max in block
 - ◊ $M \times N \times (M'+N') \rightarrow O(n^3)$
 - ◊ However, max values in block can be **cached**, so algorithm is really only $O(n^2)$
- $O(n^2)$ in memory too!
- Improvements can (effectively) reduce sequence comparison to $O(n)$ in both



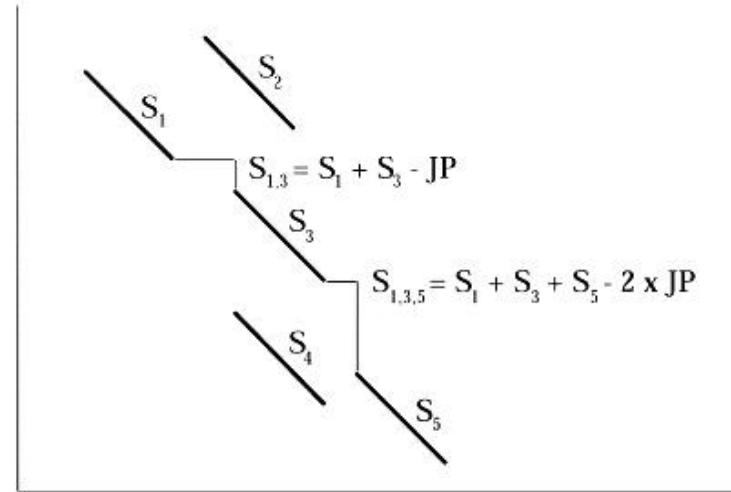
FASTA

- Hash table of short words in the query sequence
- Go through DB and look for matches in the query hash (linear in size of DB)
- perl: \$where{“ACT”} = 1,45,67,23....
- K-tuple determines word size (k-tup 1 is single aa)
- by Bill Pearson

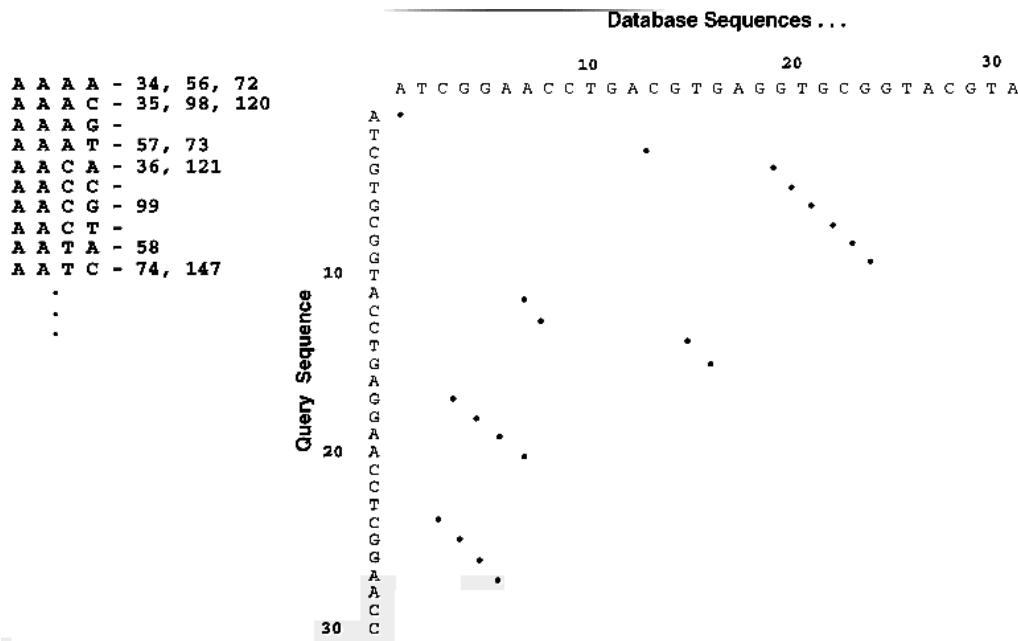


VLICTAVLMVLICTAAAVLICTMSDFFD

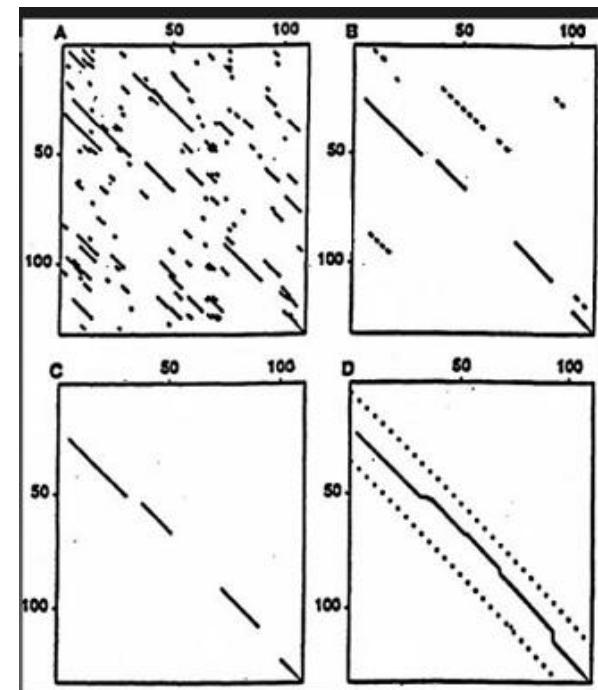
Join together query lookups into diagonals and then a full alignment



JP = Joining penalty



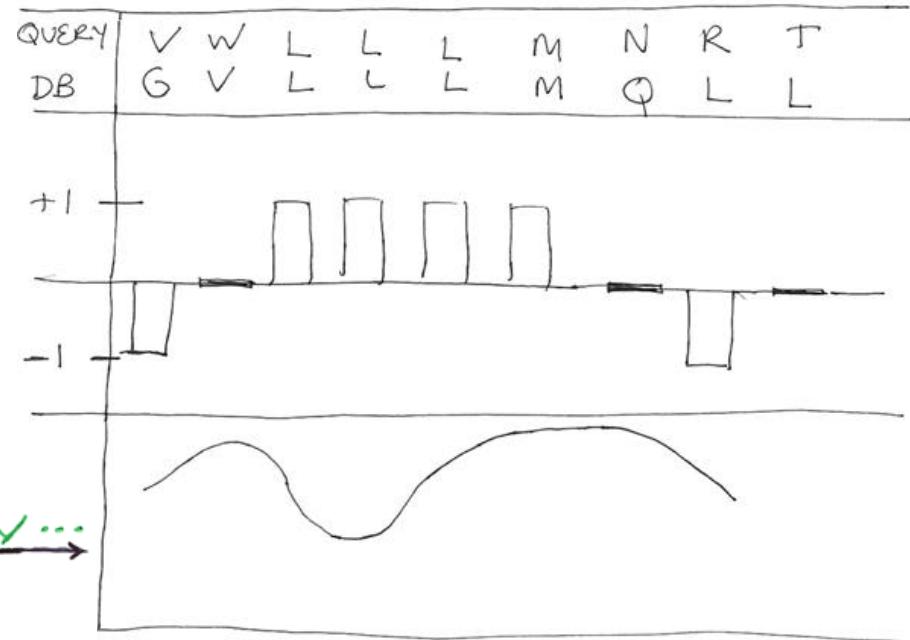
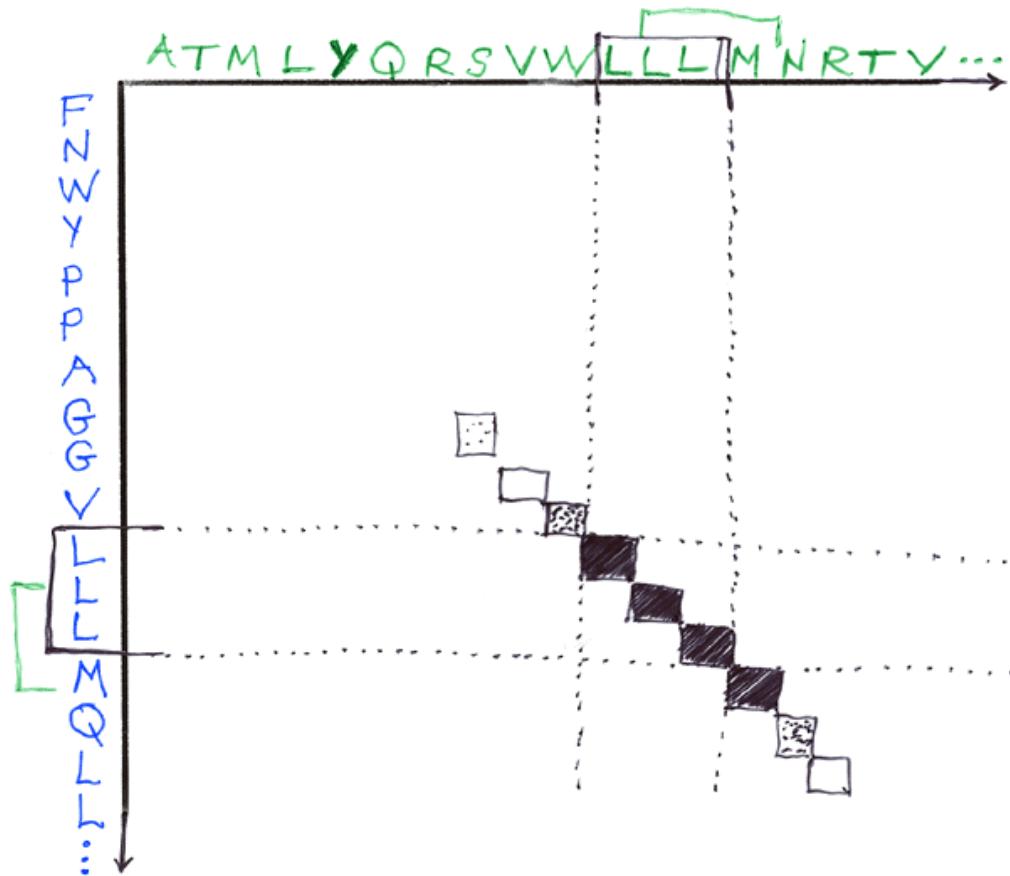
(Adapted from D Brutlag)



- Altschul, S., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410
- Indexes query (also tried indexing DB)
- Starts with all overlapping words from query
- Calculates “neighborhood” of each word using PAM matrix and probability threshold matrix and probability threshold
- Looks up all words and neighbors from query in database index
- Extends High Scoring Pairs (HSPs) left and right to maximal length
- Finds Maximal Segment Pairs (MSPs) between query and database
- Blast 1 does not permit gaps in alignments

Basic Blast

Blast: Extension of Hash Hits

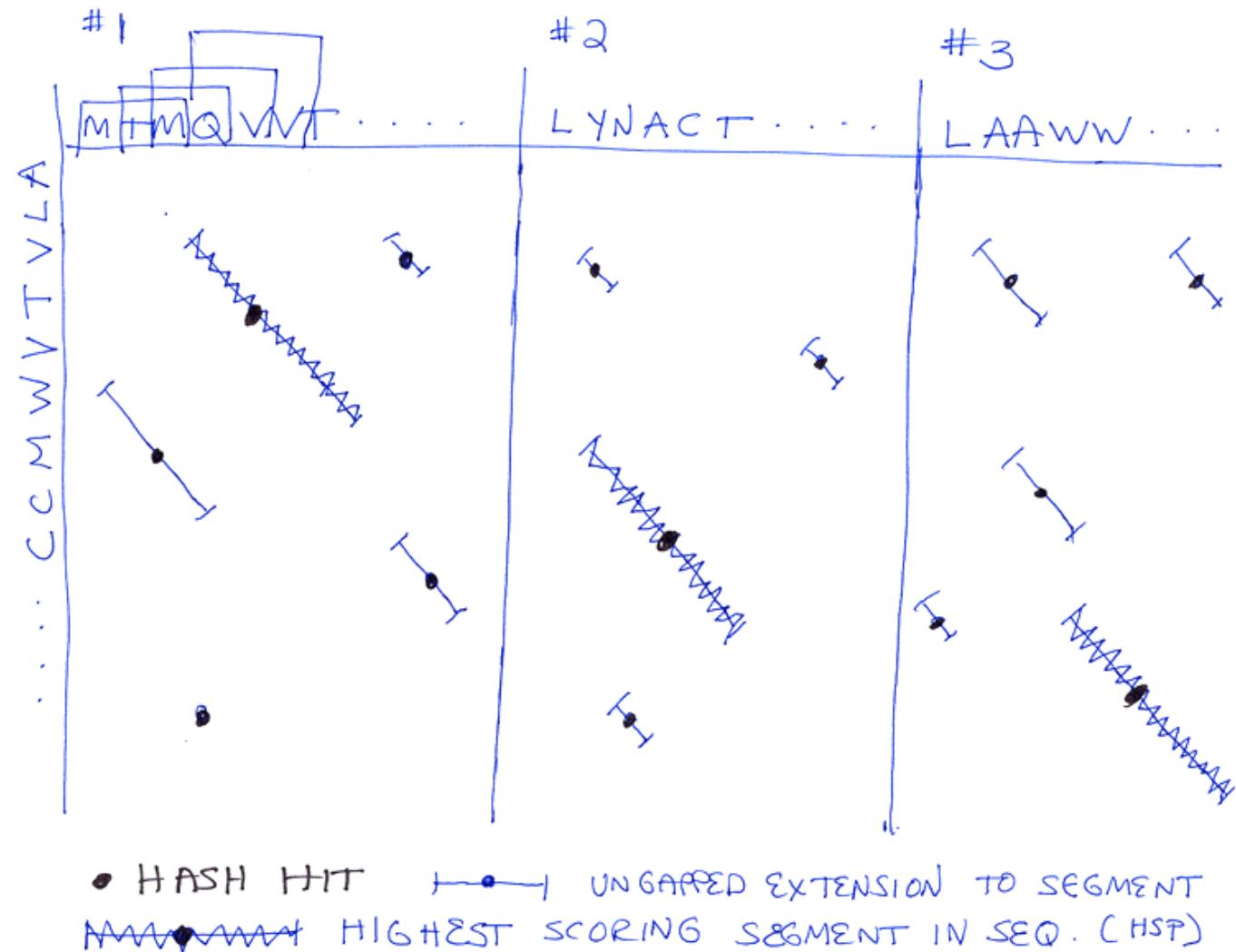


- Extend hash hits into High Scoring Segment Pairs (HSPs)
- Stop extension when total score doesn't increase
- Extension is $O(N)$. This takes most of the time in Blast

- In simplest Blast algorithm, find best scoring segment in each DB sequence
- Statistics of these scores determine significance

Blasting against the DB

Number of hash hits is proportional to $O(N^*M^*D)$, where N is the query size, M is the average DB seq. size, and D is the size of the DB



Analytic Score Formalism for Blast

Karlin-Altschul statistics for occurrence of high-scoring segments (HSPs) in random sequences

$$\text{Prob}(S > X) \approx 1 - \exp\{-Ke^{-\lambda X}\}$$

where λ is the root of the equation:

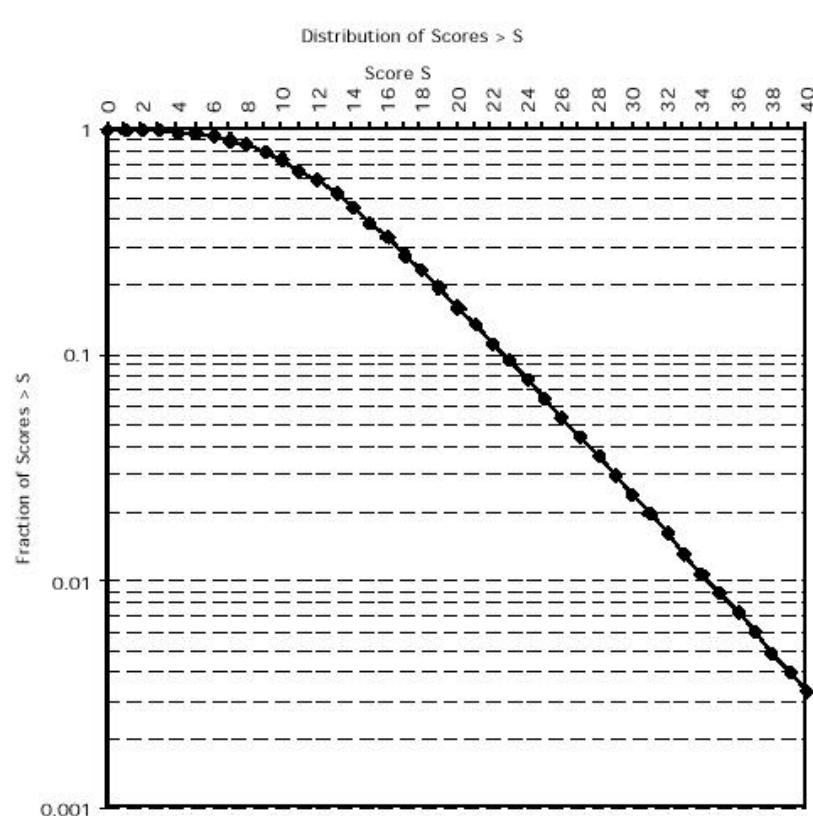
$$\sum_{i=1}^r \sum_{j=1}^r p_i p_j \exp\{\lambda s_{ij}\} = 1$$

p_i and p_j are the probabilities of each residue in each sequence,
 s_{ij} are the similarity scores of two residues.

If the expected value of the scores for random sequences is

$$< 0, \text{ i. e. } \left(\sum_{i=1}^r \sum_{j=1}^r p_i p_j s_{ij} < 0 \right)$$

then there are two solutions for λ , zero and one other positive root.



Blast2: Gapped Blast

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Gapped BLAST and PSI-BLAST: a new generation of protein database search programs

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Zheng Zhang², Webb Miller² and David J. Lipman

National Center
Bethesda, MD
Institute, Natio
Engineering, P

Received June 20

ABSTRACT

The BLAST searching pr
similarities.
definitional,

3392 Nucleic Acids Research, 1997, Vol. 25, No. 17

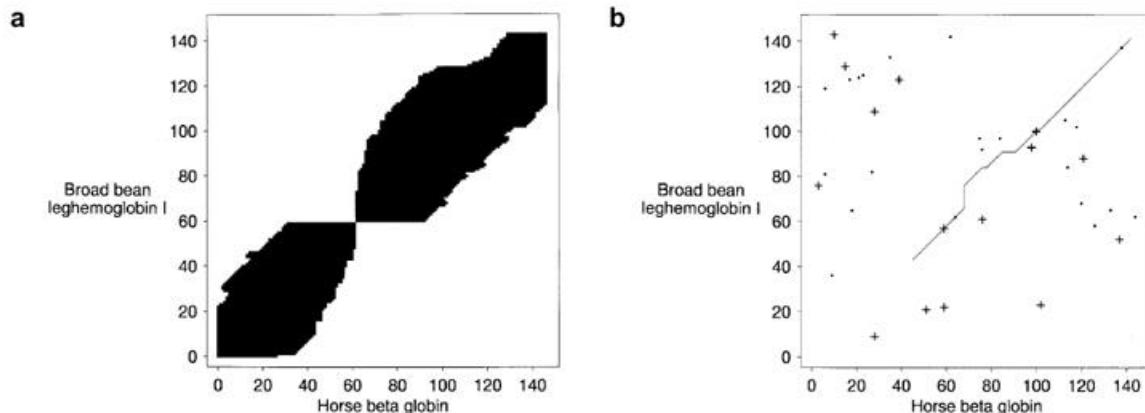
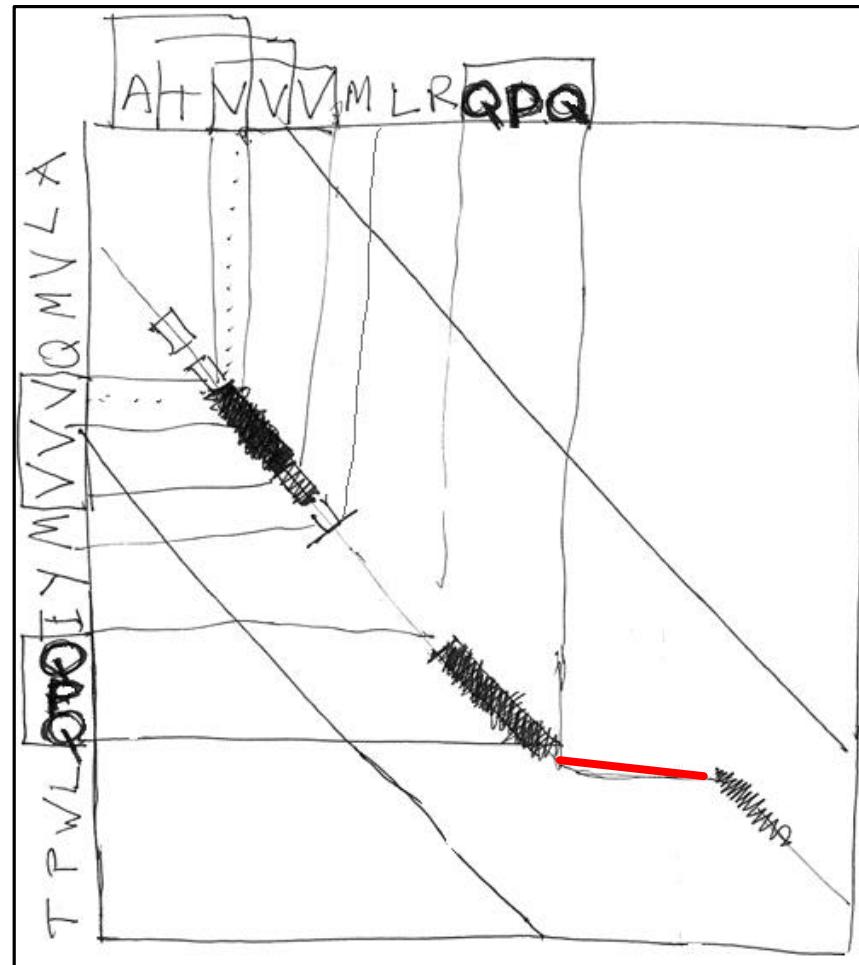


Figure 3. A gapped extension generated by BLAST for the comparison of broad bean leghemoglobin I (87) and horse β -globin (88). (a) The region of the path graph explored when seeded by the alignment of alanine residues at respective positions 60 and 62. This seed derives from the HSP generated by the leftward of the two ungapped extensions illustrated in Figure 2. The X_k dropoff parameter is the nominal score 40, used in conjunction with BLOSUM-62 substitution scores and a cost of $10 + k$ for gaps of length k . (b) The path corresponding to the optimal local alignment generated, superimposed on the hits described in Figure 2. The original BLAST program, using the one-hit heuristic with $T = 11$, is able to locate three of the five HSPs included in this alignment, but only the first and last achieve a score sufficient to be reported. (c) The optimal local alignment, with nominal score 75 and normalized score 32.4 bits. In the context of a search of SWISS-PROT (26), release 34 (21 219 450 residues), using the leghemoglobin sequence (143 residues) as query, the E -value is 0.54 if no edge-effect correction (22) is invoked. The original BLAST program locates the first and last ungapped segments of this alignment. Using sum-statistics with no edge-effect correction, this combined result has an E -value of 31 (21,22). On the central lines of the alignment, identities are echoed and substitutions to which the BLOSUM-62 matrix (18) gives a positive score are indicated by a '+' symbol.

Blast2: Gapped Blast

- Gapped Extension on Diagonals with two Hash Hits
- Statistics of Gapped Alignments follows EVD empirically



Ψ -Blast

Parameters: overall threshold, inclusion threshold, iterations

- Automatically builds profile and then searches with this
- Also PHI-blast

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Gapped BLAST and PSI-BLAST: a new generation of protein database search programs

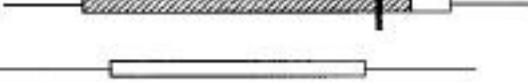
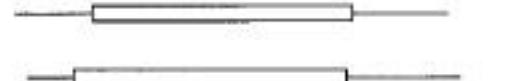
Stephen F. Altschul*, Thomas Madden†, Alejandro A. Schäffer‡, Linahui Zhang‡,
Zheng Zhang‡, Webb Miller‡ and

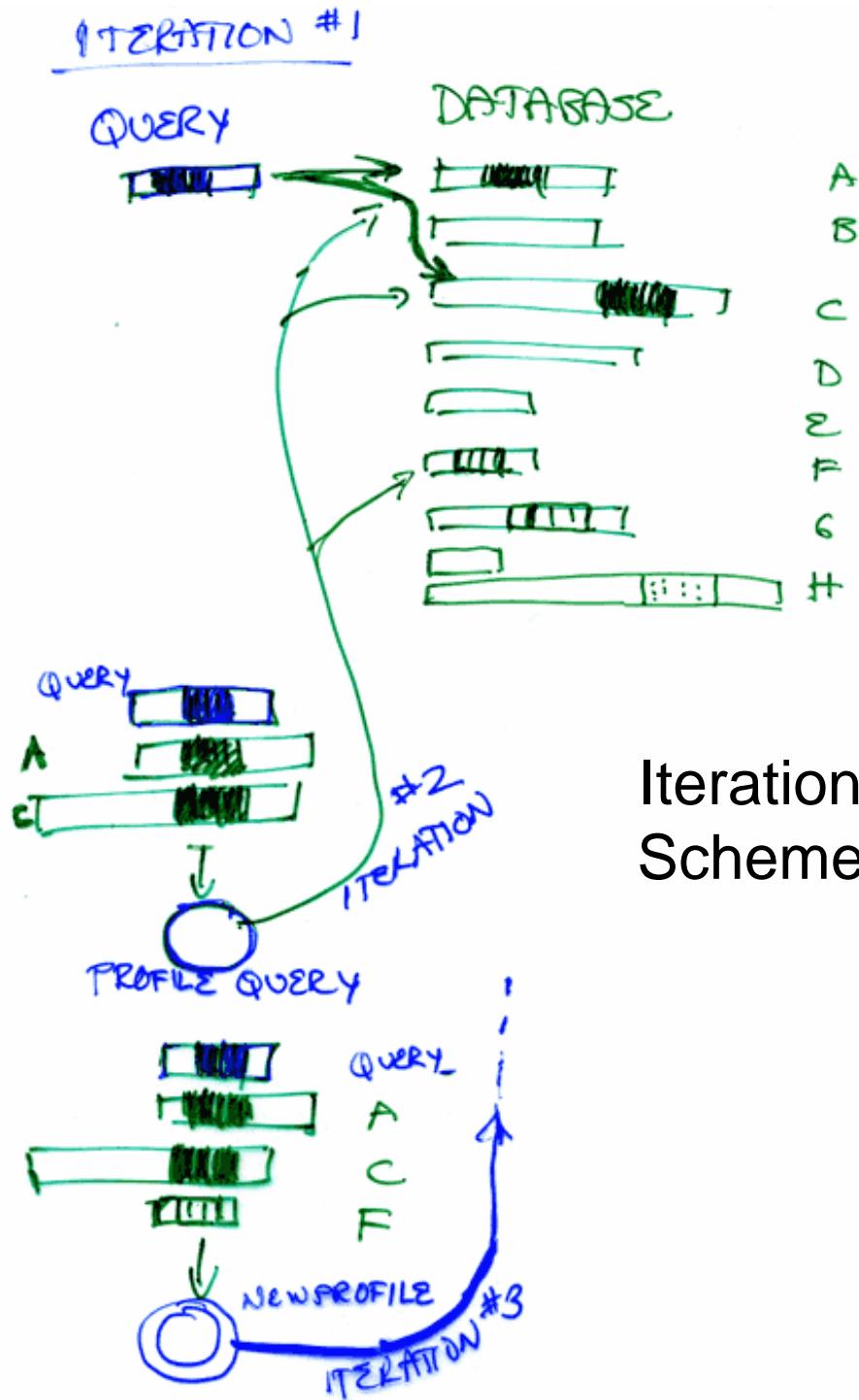
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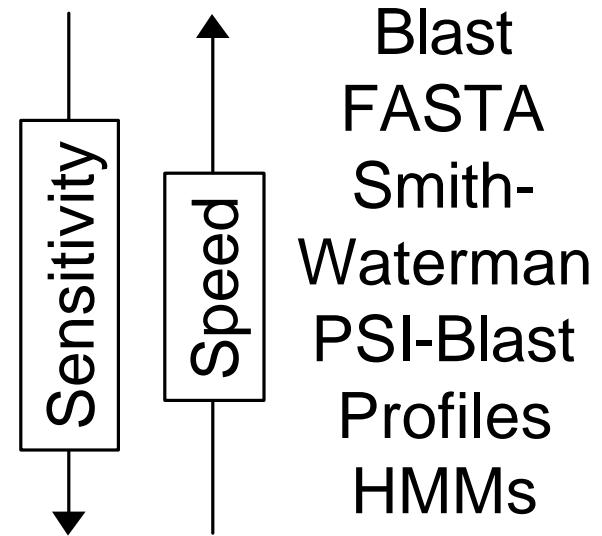
ABSTRACT

The BLAST programs are widely used for quickly searching protein and DNA databases for regions of local sequence similarity. For protein comparison, BLAST uses a heuristic search algorithm based on a probabilistic model of local alignments, combined with a statistical measure of significance. The algorithm is highly sensitive, yet computationally efficient.

Accession	Alignment	E-value
P49789		
P49779		8e-27
P49775		6e-18
Q11066		3e-07
Q09344		4e-05
P49378		0.001
P32084		0.002

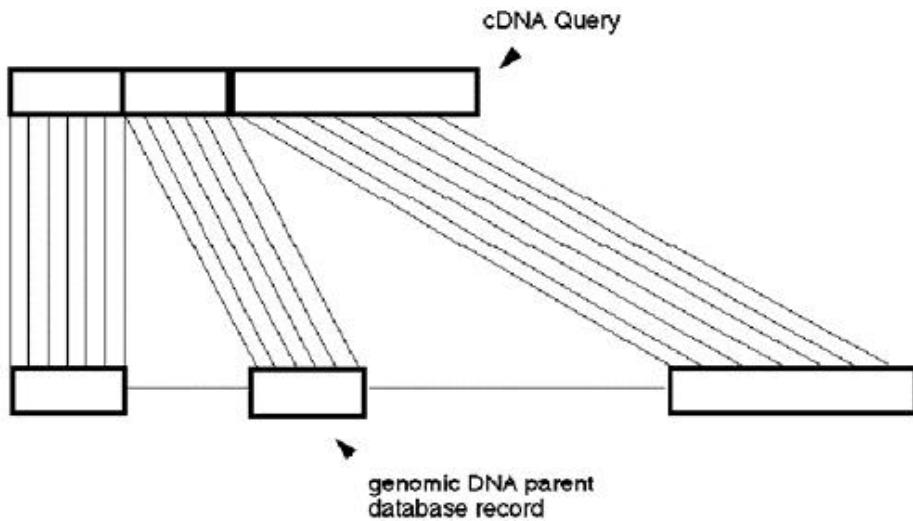


PSI-Blast



Practical Issues on DNA Searching

(graphic and some text adapted from D Brutlag)



- Examine results with exp. between 0.05 and 10
- Reevaluate results of borderline significance using limited query
- Beware of hits on long sequences
- Limit query length to 1,000 bases
- Segment query if more than 1,000 bases
 - Search both strands
 - Protein search is more sensitive, Translate ORFs
 - BLAST for infinite gap penalty
 - Smith-Waterman for cDNA/genome comparisons
 - cDNA =>Zero gap- Transition matrices Consider transition matrices
 - Ensure that expected value of score is negative

General Protein Search Principles

- Choose between **local or global** search algorithms
- Use most sensitive search algorithm available
- Original BLAST for no gaps
- Smith-Waterman for most sensitivity
- FASTA with k-tuple 1 is a good compromise
- Gapped BLAST for well delimited regions
- PSI-BLAST for families
- Initially BLOSUM62 and default gap penalties
- If no significant results, use BLOSUM30 and lower gap penalties
- FASTA cutoff of **.01**
- Blast cutoff of **.0001**
- Examine results between exp. 0.05 and 10 for biological significance
- Ensure expected score is negative
- Beware of hits on long sequences or hits with unusual aa composition
- Reevaluate results of borderline significance using limited query region
- Segment long queries ≥ 300 amino acids
- Segment around known motifs

(some text adapted from D Brutlag)

Overview

- Why interesting?
 - ◊ Not tremendous success, but many methods brought to bear.
 - ◊ What does difficulty tell about protein structure?
- Start with TM Prediction (Simpler)
- Basic GOR Sec. Struc. Prediction
- Better GOR
 - ◊ GOR III, IV, semi-parametric improvements, DSC
- Other Methods
 - ◊ NN, nearest nbr.

What secondary structure prediction tries to accomplish?

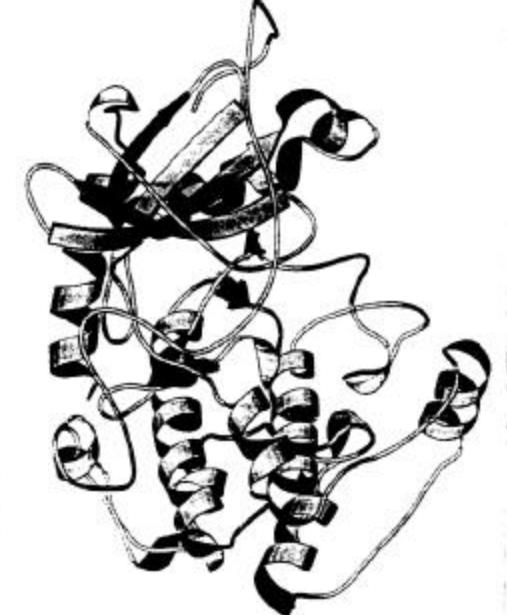
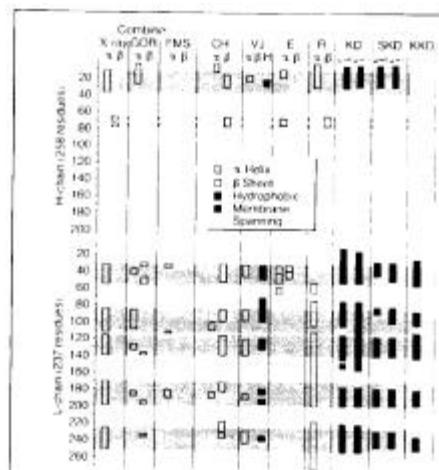
Credits: Rost et al. 1993;
Fasman & Gilbert, 1990

- Not Same as Tertiary Structure Prediction -- no coordinates
 - Need torsion angles of terms + slight diff. in torsions of sec. str.

Sequence RPDFCLEPPYTGPCKARIIRYFYNAKAGLVQTFVYGGCRAKRNNFKSAEDAMRTCGGA
Structure CCGGGGCCCGCCCGCCCEEEEEETTTTEEEEEECCCCTTTBTTHHHHHHHHCC



[a] Residue-by-residue comparison of experimentally observed (OBS) and predicted [COM¹⁰, ETH¹⁸, PHD (Ref. 35 and B. Rest and C. Sonder, submitted)] structures of the catalytic subunit of the cAMP-dependent protein kinase Iα (cpk). AA_i is the amino acid sequence taken from Protein Data Bank entry 1ckp (residues 27–287). Secondary structure: H = α -helix, E = β -sheet (extended), blank = loop. Predicted α -helices and β -strands that have insufficient overlap with an observed segment of the same type are underlined. Note the relatively good prediction of the location of segments for the ETH and PHD methods and overprediction of α -helices for the COM method.



(b) Ribbon view of the domain used in this blind test. The X-ray structure of catalytic subunit of the cAMP-dependent protein kinase. Drawn using Molscript.²⁴

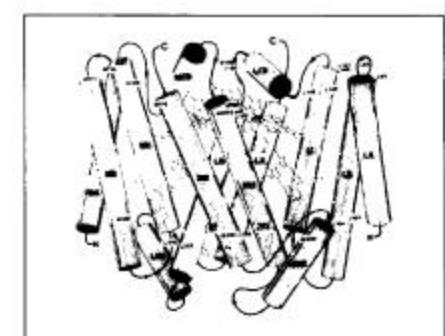


Figure 1
Column model for the core of the reaction center from *Rsp. viridis*. Reproduced, with permission, from Ref. 18.

Some TM scales:

GES

F -3.7

M -3.4

I -3.1

L -2.8

Goldman, Engleman, Steiz

C 0.0

W -1.9

A -1.6

T -1.2

G -1.0

S -0.6

P +0.2

Y +0.7

H +3.0

Q +4.1

N +4.8

E +8.2

K +8.8

D +9.2

R +12.3

KD

I 4.5

V 4.2

L 3.8

F 2.8

C 2.5

M 1.9

A 1.8

G -0.4

T -0.7

W -0.9

S -0.8

Y -1.3

P -1.6

H -3.2

E -3.5

Q -3.5

D -3.5

N -3.5

K -3.9

R -4.5

For instance, ΔG from
transfer of a Phe
amino acid from water
to hexane

How to use GES to predict proteins

- Transmembrane segments can be identified by using the GES hydrophobicity scale (Engelman et al., 1986). The values from the scale for amino acids in a window of size 20 (the typical size of a transmembrane helix) were averaged and then compared against a cutoff of -1 kcal/mole. A value under this cutoff was taken to indicate the existence of a transmembrane helix.
- $H-19(i) = [H(i-9)+H(i-8)+\dots+H(i) + H(i+1) + H(i+2) + \dots + H(i+9)] / 19$

Graph showing Peaks in scales

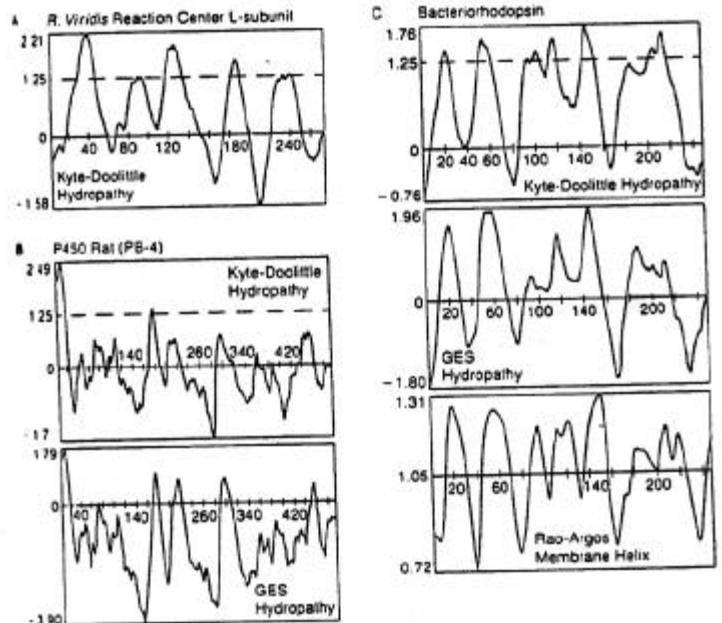
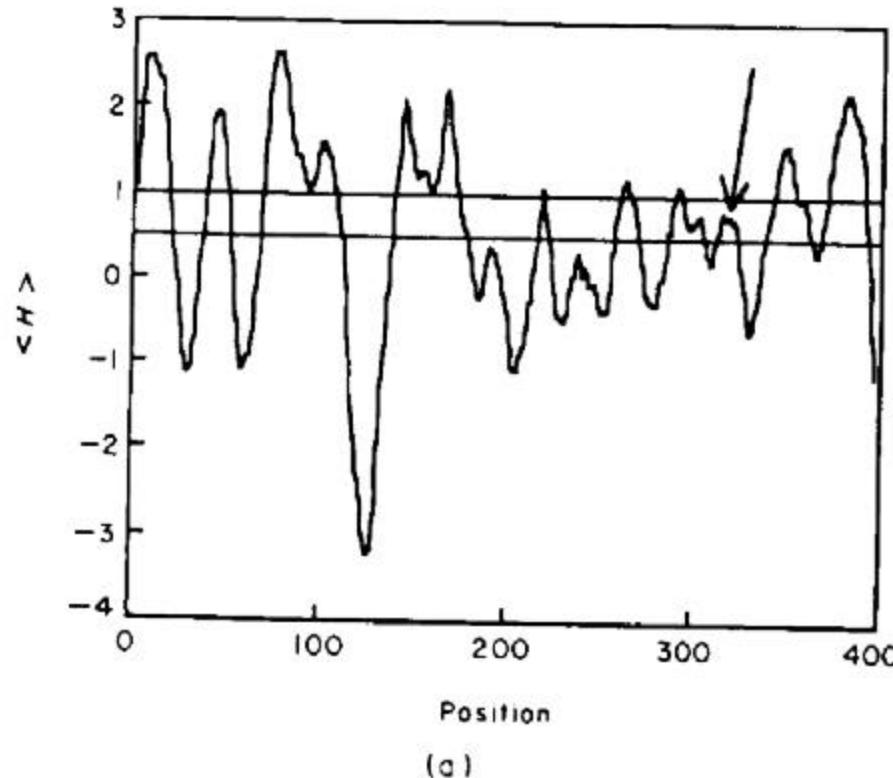


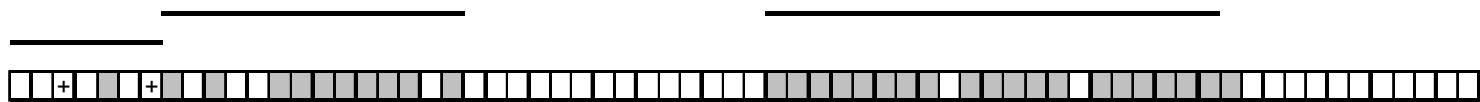
Figure 3.12. Representative profiles of three membrane proteins used to predict membrane-spanning helices. The amino acid scales of Kyte-Doolittle (804), Goldstein-Engelman-Steitz (GES) (389), and Rao-Argos (1194) were used. A computer software package (SEQANAL) provided by Dr. A. Croft (Univ. of Illinois) was used to generate these profiles. For comparative purposes, the Kyte-Doolittle and GES plots were obtained using a window of 19 residues and then smoothed using a second pass with a window of 7. The average value at each residue position is plotted as a function of residue number starting with the amino terminus on the left in each case. The values plotted for the Kyte-Doolittle and GES scales represent average hydrophobicity and transfer free energy per residue (kcal/mol). The Rao-Argos plot used a span of 7 residues and was smoothed used two additional passes with the same span of 7, as recommended by the authors. The scale values reflect the relative preference for being in a membrane-spanning helix. Note that the version of the GES algorithm which was used does not take into account possible ion pair formation. See text for details.

Illustrations Adapted From: von Heijne, 1992; Smith notes, 1997



Removing Signal sequences

- Initial hydrophobic stretches corresponding to signal sequences for membrane insertion were excluded. (These have the pattern of a charged residue within the first 7, followed by a stretch of 14 with an average hydrophobicity under the cutoff).



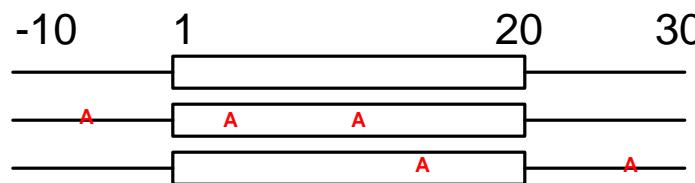
Ex. $P(i, \alpha)$ probability that residue i has secondary structure α

- Problem of DB Bias
- $f(A) =$ frequency of residue A to have a TM-helical conf. in db
- $f(A,i) = f(A)$ at position i in a particular sequence
- $E(\alpha) =$ statistical energy of helix over a window
- $p(i, \alpha) =$ probability that residue i is in a TM-helix

$$E_a = \sum_i^N \ln f_a^i$$

$$p_a^i = \frac{e^{-E_a/RT}}{\sum_j^N e^{-E_j/RT}}$$

$$F_{\text{in-DB}}(A) = 5/120$$



$$F_{\text{in-TM}}(A) = 3/60$$

End of Class 3

Statistics Based Methods:

Persson & Argos

- Propensity $P(A)$ for amino acid A to be in the middle of a TM helix or near the edge of a TM helix

$$P(A) = \frac{\frac{n(A, \text{TM})}{\sum_A n(A, \text{TM})}}{\frac{n(A, \text{everywhere})}{\sum_A n(A, \text{everywhere})}}$$

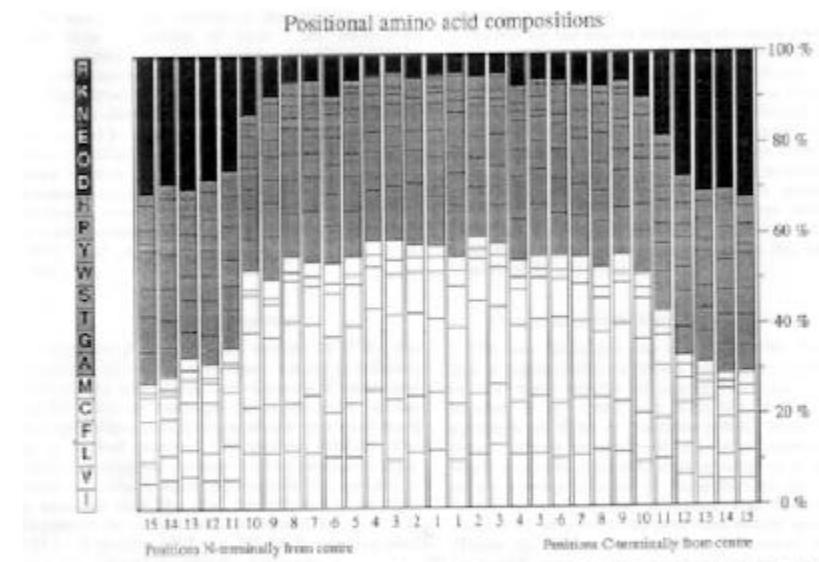


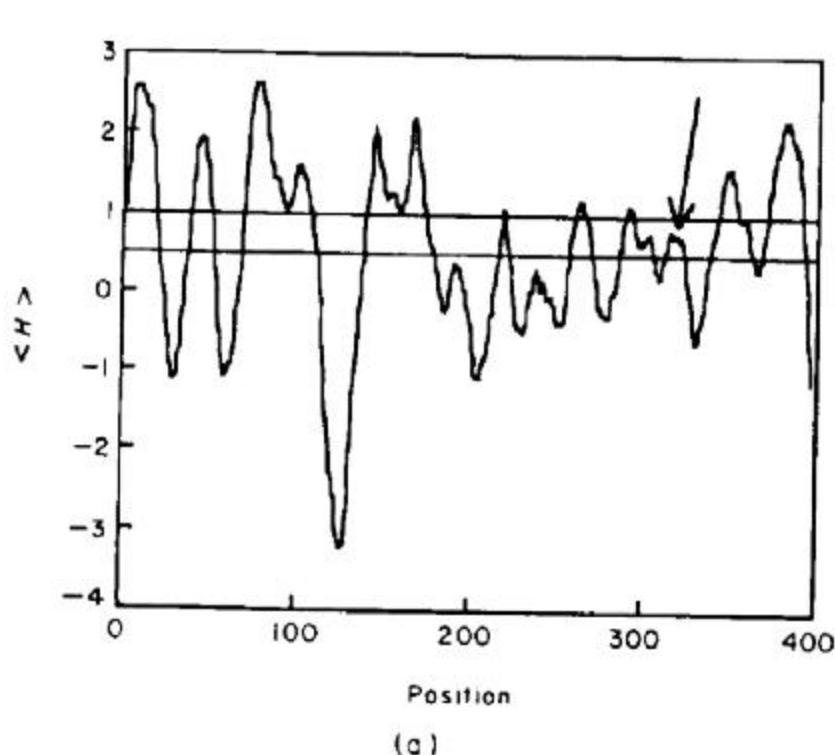
Figure 1. Positional amino acid compositions of transmembrane segments. Bar chart showing the amino acid compositions for 15 N- and C-terminal positions relative to the centre of putative transmembrane segments listed in feature tables of the Swiss-Prot database. For each position, the percentage contribution of each amino acid type is shown according to the hydrophilic (top) to hydrophobic (bottom) order, given in the ruler bar at the left. The hydrophilic residue contributions are illustrated in white, the hydrophobic in dark-gray, and intermediate in light-gray. The compositions of positions 11 to 15 at the N-terminal side and 1 to 15 at the C-terminal side differ significantly from the others, especially for the most hydrophobic and charged hydrophilic residues. These results suggest that in general transmembrane spans consist of a hydrophobic portion 21 residues in length.

Illustration Credits: Persson & Argos, 1994

$$P(A) = f_{\text{TM}}(A)/f_{\text{SwissProt}}(A)$$

Refinements: Charge on the Outside, Positive Inside Rule

- for marginal helices, decide on basis of R+K inside (cytoplasmic)



Credits: von Heijne, 1992

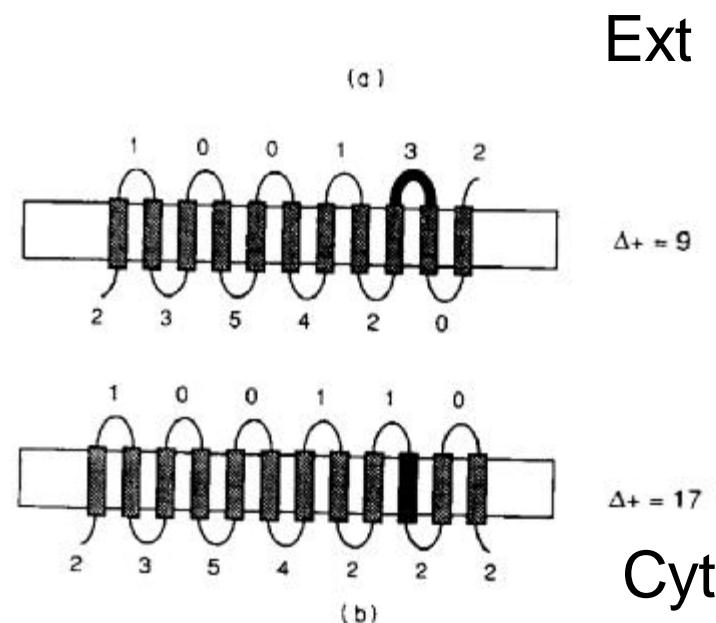
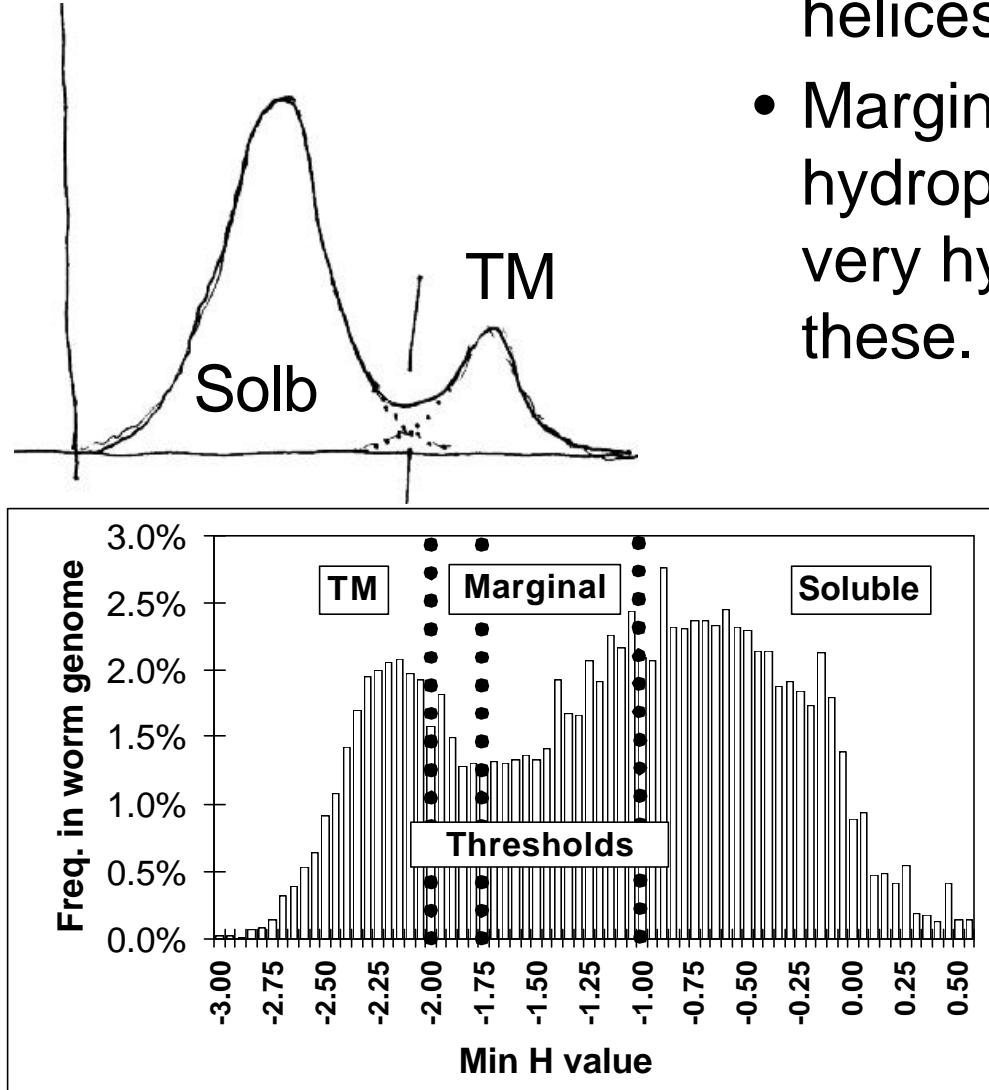
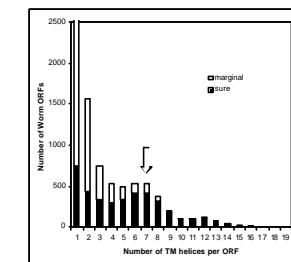


Figure 4. (a) Hydrophobicity plot for the SecY protein. The upper and lower cutoffs are marked. A tentative transmembrane segment with a mean hydrophobicity falling between the 2 cutoffs is marked by an arrow. (b) Two possible topologies for the SecY protein based on the hydrophobicity plot. The putative transmembrane segment is shown in black. The number of Arg+Lys residues is shown next to each polar segment. Note that the correct alternative (bottom, including the putative transmembrane segment) has a much higher charge-bias than the incorrect one.

Refinements: MaxH



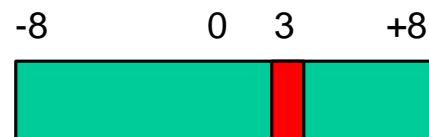
- How to train to find right threshold? Not that many TM helices
- Marginal TM helices are not that hydrophobic but 1/3 of TM's are very hydrophobic, so focus on these.
- Sosui, Klein & Delisi, Boyd
- Discriminant analysis: set threshold to be best partition of dataset



GOR: Simplifications

- For independent events just add up the information
- $I(S_j ; R_1, R_2, R_3, \dots, R_{last})$ = Information that first through last residue of protein has on the conformation of residue j (S_j)
 - ◊ Could get this just from sequence sim. or if same struc. in DB (homology best way to predict sec. struc.!)
- Simplify using a 17 residue window:
 $I(S_j=H ; R[j-8], R[j-7], \dots, R[j], \dots, R[j+8])$
- Difference of information for residue to be in helix relative to not: $I(dSj;y) = I(Sj=H;y) - I(Sj=\sim H;y)$
 - ◊ odds ratio: $I(dSj;y) = \ln P(Sj;y)/P(\sim Sj;y)$
 - ◊ I determined by observing counts in the DB, essentially a lod value

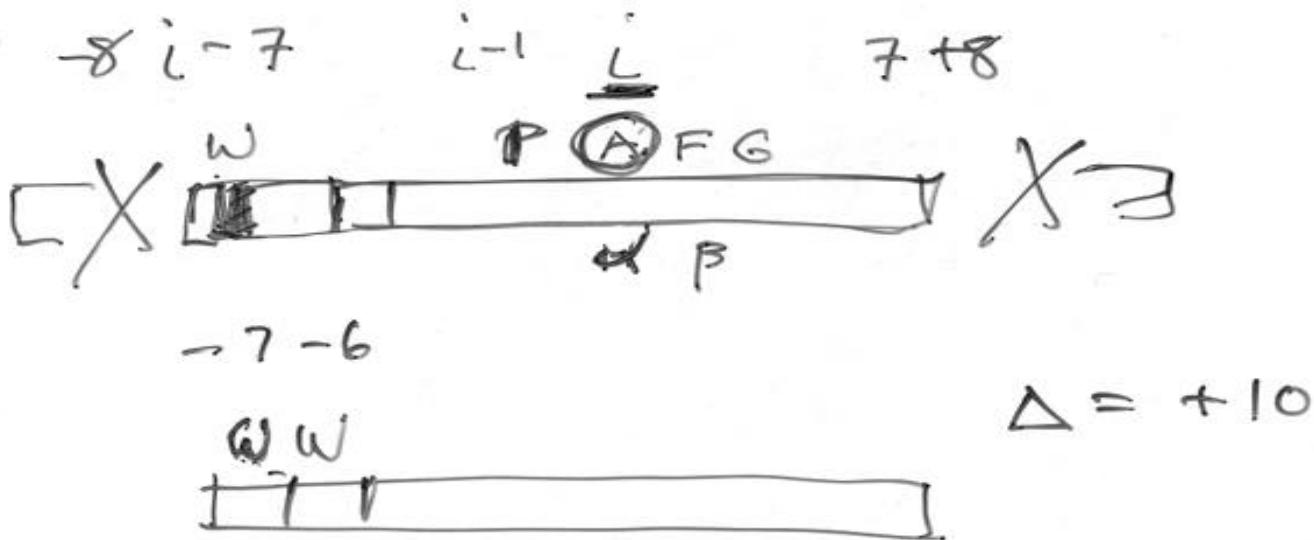
Basic GOR



$f(H,+3)/f(\sim H,+3)$

- Pain & Robson, 1971;
Garnier, Osguthorpe, Robson, 1978
- $I \sim \text{sum of } I(S_j, R[j+m]) \text{ over 17 residue window}$
centered on j and indexed by m
 - ◊ $I(S_j, R[j+m])$ = information that residue at position m in window has about conformation of protein at position j
 - ◊ 1020 bins = $17 * 20 * 3$
- In Words
 - ◊ Secondary structure prediction can be done using the GOR program (Garnier et al., 1996; Garnier et al., 1978; Gibrat et al., 1987). This is a well-established and commonly used method. It is statistically based so that the prediction for a particular residue (say Ala) to be in a given state (i.e. helix) is directly based on the frequency that this residue (and taking into account neighbors at +/- 1, +/- 2, and so forth) occurs in this state in a database of solved structures. Specifically, for version II of the GOR program (Garnier et al., 1978), the prediction for residue i is based on a window from $i-8$ to $i+8$ around i , and within this window, the 17 individual residue frequencies (singlets).

More GOR



$f(A \rightarrow b \text{ in a helix at position } i-4)$
centered at i
 $f(A \text{ in the db})$

Directional Information

OBS
 LOD= ln -----
 EXP helix
 strand
 coil

i-8	i-7	i-6	i-5	i-4	i-3	i-2	i-1	i	i+1	i+2	i+3	i+4	i+5	i+6	i+7	i+8
a	-12	-15	-12	-12	-17	-13	-25	-24	-32	-35	-32	-29	-24	-20	-12	-5
c	36	2d	41	50	43	31	29	19	7	5	27	29	38	48	41	45
d	-8	-10	-13	-8	-13	-10	12	25	50	43	39	27	7	-7	-6	-9
e	-3	-11	-19	-11	-10	-7	-5	-23	-26	-23	-2	-5	-1	-3	3	-5
f	22	25	28	25	21	9	-23	-34	-49	-40	-29	-12	9	30	13	18
g	-3	-8	-18	-17	-7	2	26	68	97	58	19	-2	-18	-14	-18	-11
h	15	9	-4	-7	8	-2	12	8	8	5	-4	1	-3	-5	-5	-10
i	7	12	19	14	7	1	-21	-42	-66	-55	-26	-14	14	18	4	2
k	-12	-7	-10	-9	-1	5	11	5	0	9	5	-8	-20	-15	-7	-10
l	2	8	11	11	11	2	-23	-42	-65	-63	-52	-39	-15	-11	-10	-6
m	11	14	4	3	-9	-16	-33	-52	-62	-77	-71	-54	-32	-7	3	9
n	-2	-8	-11	1	8	12	32	51	61	31	18	6	-6	-8	-4	2
p	4	8	4	-1	5	15	39	76	120	159	98	59	32	17	11	3
q	-1	-11	-12	-15	-17	-4	5	-5	-13	1	1	2	-2	-5	-1	-9
r	-4	-9	-8	-10	-10	-13	-18	-16	-14	-9	-14	-16	-14	-11	-5	-2
s	-3	-4	-4	-4	4	11	22	26	41	31	20	13	3	5	4	8
t	-5	-5	-4	-4	-7	-5	0	2	15	21	29	30	19	7	3	-4
v	3	17	20	20	8	-2	-26	-46	-68	-51	-20	3	25	24	23	15
w	5	9	28	28	12	-16	-32	-46	-53	-38	-20	5	13	30	9	2
y	10	7	12	7	6	3	7	-1	-31	-14	-11	11	13	1	3	12

Credits: King & Sternberg, 1996

Table 3. Directional informational parameters: $I(Sj = x:x'; Rj + m)$ for residue position versus residue type for α -helices^a

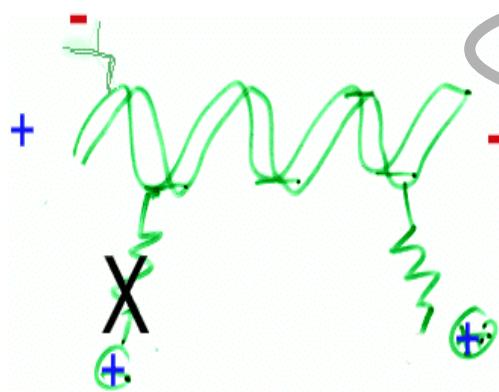
	i-8	i-7	i-6	i-5	i-4	i-3	i-2	i-1	i	i+1	i+2	i+3	i+4	i+5	i+6	i+7	i+8
a	19	21	22	24	34	36	44	47	60	60	53	50	44	40	31	23	24
c	-47	-45	-44	-47	-44	-36	-44	-55	-56	-58	-54	-55	-58	-58	-59	-53	-66
d	14	15	14	15	17	21	15	17	-7	-11	-31	-42	-28	-12	-8	1	-5
e	14	16	15	20	26	27	34	52	62	57	32	15	19	12	6	7	9
f	-19	-14	-10	-4	-2	-1	6	-1	10	10	12	12	-4	-5	2	0	2
g	5	2	1	-5	-22	-30	-50	-70	-92	-52	-28	-21	-13	-17	-8	-6	-6
h	-22	-20	-9	-10	-19	-10	-14	-7	-11	-4	0	-3	-2	2	6	11	12
i	7	7	0	0	1	1	2	-5	1	2	1	7	-6	-3	10	8	6
k	-2	-1	-1	-1	-6	-9	-6	5	17	17	21	27	35	33	21	22	23
l	0	-1	0	6	9	16	30	33	45	47	51	53	37	32	30	25	18
m	4	3	15	23	30	30	39	36	45	54	57	53	44	29	30	14	1
n	2	3	2	-5	-9	-10	-16	-17	-31	-16	-17	-16	-9	-8	-9	-10	-5
p	-12	-15	-14	-19	-23	-25	-30	-48	-82	-195	-145	-104	-67	-49	-43	-33	-17
q	-4	3	7	4	13	8	10	24	35	32	31	21	18	18	9	8	6
r	5	3	6	13	7	13	19	27	34	32	36	41	33	29	23	21	18
s	-10	-7	-10	-10	-16	-17	-25	-21	-39	-35	-39	-41	-32	-35	-34	-35	-33
t	1	-1	-6	-8	-6	-11	-16	-25	-48	-47	-48	-46	-34	-31	-34	-26	-24
v	-5	-12	-13	-14	-13	-19	-17	-20	-15	-22	-22	-20	-26	-19	-15	-10	-5
w	0	-4	-12	-19	-7	14	16	12	18	17	12	8	1	-6	1	3	-13
y	-22	-19	-17	-20	-16	-21	-30	-32	-8	-10	-4	-12	-17	-9	-10	-14	-15

^aNote that the convention used is the reverse of that adopted by (Garnier et al., 1978), for example the first entry for alanine at position j-8 is the amount of information that an alanine residue eight positions toward the N terminus has for predicting an α -helix

Table 4. Directional informational parameters for residue position versus residue type for β -strands

	i-8	i-7	i-6	i-5	i-4	i-3	i-2	i-1	i	i+1	i+2	i+3	i+4	i+5	i+6	i+7	i+8
a	-8	-7	-13	-17	-23	-33	-26	-32	-43	-37	-30	-30	-26	-27	-26	-25	-25
c	3	13	-9	-20	-15	-3	9	33	47	51	21	19	9	-5	7	-5	-14
d	-7	-5	0	-9	-4	-14	-42	-73	-83	-59	-21	10	22	24	16	11	13
e	-14	-5	-5	-11	-21	-27	-45	-44	-57	-54	-46	-29	-25	-12	-12	0	0
f	-9	-20	-32	-34	-30	-12	24	44	49	39	24	2	-9	-23	-24	-29	-23
g	-3	9	24	29	34	30	18	-23	-48	-27	6	27	39	38	33	23	23
h	6	11	17	22	12	16	0	-2	3	-2	5	3	8	4	-1	1	-3
i	-21	-30	-31	-21	-12	-3	26	58	76	64	33	11	-14	-24	-20	-14	-11
k	20	12	15	14	8	4	-8	-14	-25	-40	-39	-27	-20	-24	-20	-15	-15
l	-2	-10	-18	-27	-30	-27	-6	15	27	21	2	-19	-31	-29	-28	-26	-25
m	-22	-26	-29	-40	-31	-17	-7	23	24	28	17	2	-15	-31	-53	-36	-16
n	1	8	14	5	0	-6	-30	-65	-62	-28	-6	11	18	21	16	10	3
p	9	7	12	24	20	8	-22	-65	-108	-64	-8	17	25	30	32	31	21
q	6	12	8	16	8	-5	-22	-27	-30	-52	-49	-34	-22	-17	-9	2	20
r	0	8	3	-3	5	2	1	-14	-26	-32	-30	-35	-27	-26	-25	-25	-21
s	16	14	17	19	14	5	-3	-13	-15	-4	15	27	32	32	31	28	21
t	6	8	14	15	16	21	19	25	31	22	13	9	12	25	34	34	34
v	1	-11	-15	-11	4	25	51	75	91	81	49	19	-6	-12	-16	-11	-11
w	-8	-8	-28	-19	-9	5	23	44	45	30	13	-18	-22	-40	-15	-7	-9
y	13	13	4	14	12	20	24	37	48	31	20	-1	2	11	7	0	-4

Types of Residues



Credits: King & Sternberg, 1996

Table 3. Directional informational parameters: $I(Sj = x|x': Rj + m)$ for residue position versus residue type for α -helices^a

	i-8	i-7	i-6	i-5	i-4	i-3	i-2	i-1	i	i+1	i+2	i+3	i+4	i+5	i+6	i+7	i+8
a	19	21	22	24	34	36	44	47	60	60	53	50	44	40	31	23	24
c	-47	-45	-44	-47	-44	-36	-44	-53	-56	-58	-54	-55	-58	-58	-59	-53	-66
d	14	15	14	15	17	21	15	18	-7	-11	-31	-42	-28	-12	-8	1	-5
e	14	16	15	20	26	27	34	52	62	57	32	15	19	12	6	7	9
f	-19	-14	-10	-4	-2	-1	6	-1	16	10	12	12	-4	-5	2	0	2
g	5	2	1	-5	-22	-30	-50	-70	-92	-52	-28	-21	-13	-17	-8	-6	-6
h	-22	-20	-9	10	-19	-10	-14	-7	-11	-4	0	-3	-2	2	6	11	12
i	7	7	0	0	1	1	2	-5	1	2	1	7	0	-3	10	0	6
k	-2	-1	-1	-1	-6	-9	-6	5	17	17	21	27	35	33	21	22	23
l	0	-1	0	6	9	16	30	33	45	47	51	53	57	32	30	25	18
m	4	3	15	23	30	30	39	36	45	54	57	53	44	29	30	14	1
n	2	3	2	-5	-9	-10	-16	-17	-31	-11	-17	-16	-9	-8	-9	-10	-5
p	-12	-15	-14	-19	-23	-25	-30	-48	-82	-195	-145	-104	-67	-49	-43	-33	-17
q	-4	3	7	4	13	8	10	24	35	32	31	21	18	18	9	8	6
r	5	3	6	13	7	13	19	27	34	32	36	41	33	29	23	21	18
s	-10	-7	-10	-10	-16	-17	-25	-21	-39	-35	-39	-41	-32	-35	-34	-35	-33
t	1	-1	-6	-8	-6	-11	-16	-25	-48	-47	-48	-46	-34	-31	-34	-26	-24
v	-5	-12	-13	-14	-13	-19	-17	-20	-15	-22	-22	-20	-26	-19	-15	-10	-5
w	0	-4	-12	-19	-7	14	16	12	18	17	12	8	1	-6	1	3	-13
y	-22	-19	-17	-20	-16	-21	-30	-32	-8	-10	-4	-12	-17	-9	-10	-14	-15

^aNote that the convention used is the reverse of that adopted by (Garnier et al., 1978), for example the first entry for alanine at position j-8 is the amount of information that an alanine residue eight positions toward the N terminus has for predicting an α -helix at position j.

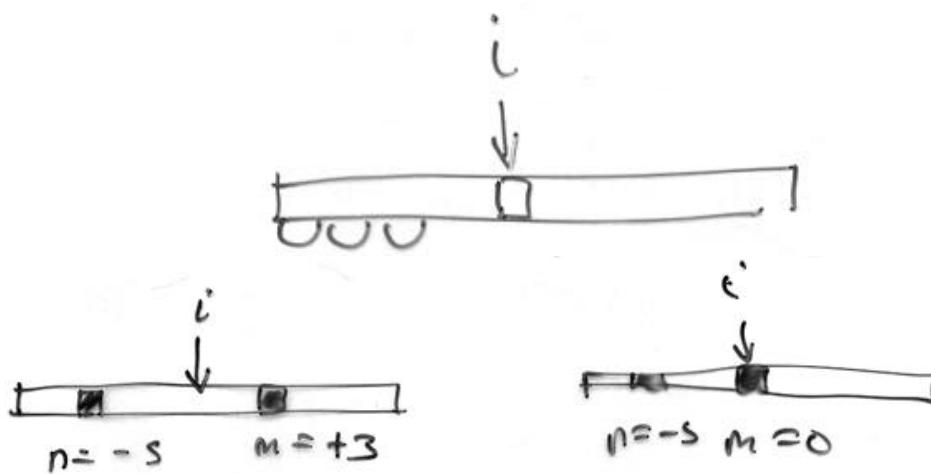
- Group I favorable residues and Group II unfavorable one:
- A, E, L -> H; V, I, Y, W, C -> E; G, N, D, S -> C
- P complex; largest effect on proceeding residue
- Some residues favorable at only one terminus (K)

GOR IV

- $I(S_j; R[j+m], R[j+n])$ = the frequencies of all 136 ($=16 \cdot 17 / 2$) possible di-residue pairs (doublets) in the window.
 - ◊ $20 \cdot 20 \cdot 3 \cdot 16 \cdot 17 / 2 = 163200$ pairs
- Parameter Explosion Problem: 1000 dom. struc. * 100 res./dom. = 100k counts, over how many bins
- Dummy counts for low values (Bayes)

All Singletons in 17 residue window

All Pairs



Assessment

- Q3 + other assess, 3x3
- Q3 = total number of residues predicted correctly over total number of residues
- GOR gets 65%
 - ◊ sum of diagonal over total number of residue -- $(14K+5K+21K)/64K$
- Under predict strands & to a lesser degree, helices: 5.9 v 4.1, 10.9 v 10.6

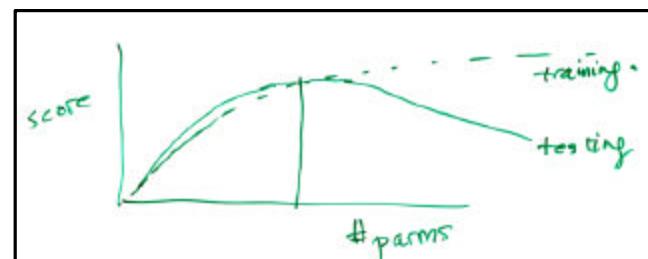
		Observed			
		H	E	C	Total
Predicted	Observed				
H	14,460	3094	4790	22,344	
E	1124	4965	2089	8178	
C	6002	5546	21,496	33,044	
Total	21,586	13,605	28,375	63,566	
Q_{prd}^a	64.7	60.7	65.1		
Q_{obs}^b	67.0	36.5	75.8		
$Q_3^c = 64.4\%$					

^a Number of correctly predicted residues/number of predicted residues.

^b Number of correctly predicted residues/number of observed residues

^c Total number of correctly predicted residues/total number of residues.

Credits: Garnier et al., 1996



Training and Testing Set

- Cross Validation:
Leave one out,
seven-fold

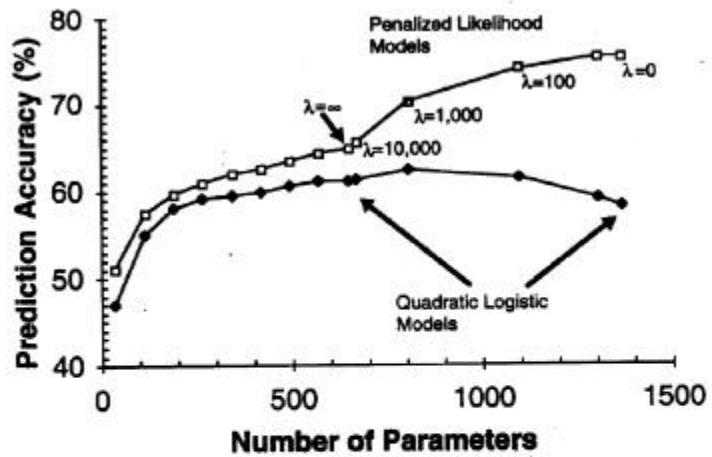
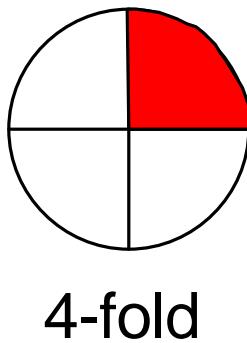


Figure 2. Comparison of prediction accuracy (correctly predicted residues as a proportion of total residues) versus effective number of parameters for linear-logistic models (number of parameters ≤ 640) and penalized likelihood models for crossvalidated (\blacklozenge) and uncrossvalidated (\square) results. The values of the penalty parameter λ are shown.

Credits: Munson,
1995;
Garnier et al., 1996

TABLE I
DATABASE PROTEINS^a

1aa.j.x	1aa.k.x	1aa.p.a	1ab.a.x	1abk.x	1abm.a	1add.x
1ads.x	1alk.a	1aoz.a	1apa.x	1apm.e	1arb.x	1atr.x
1avh.a	1ayh.x	1bab.a	1bbh.a	1bbp.a	1bet.x	1bge.a
1bll.e	1bmd.a	1bov.a	1bpb.x	1brs.d	1btc.x	1c2r.a
1caj.x	1cau.a	1cau.b	1cde.x	1cdt.a	1cew.i	1cgt.x
1chm.a	1cmb.a	1cob.a	1col.a	1cpc.a	1cpc.b	1cpt.x
1crl.x	1cse.i	1ctf.x	1ctm.x	1cus.x	1ddt.x	1dhr.x
1dog.x	1dsb.a	1leaf.x	1eco.x	1ede.x	1end.x	1epa.a
1fsa.a	1fdx.x	1fha.x	1fia.a	1fkf.x	1fnax	1fnr.x
1fxi.a	1gal.x	1gd1.o	1gda.h	1gky.x	1glx.x	1gmaf.a
1gof.x	1gox.x	1gp1.a	1gbp.x	1gpr.x	1gsr.a	1hbq.x
1hdx.a	1hiv.a	1hlb.x	1hle.a	1hm.y	1hoe.x	1hpl.a
1hrh.a	1hs1.a	1huw.x	1ifc.x	1ipd.x	1isu.a	1ith.a
1l29.x	1le4.x	1len.a	1lga.a	1lis.x	1lla.x	1lmb.3
1lts.a	1lts.d	1mdc.x	1mgn.x	1min.a	1min.b	1mjcx
1mpx.x	1mup.x	1nar.x	1nba.a	1ndk.x	1noax	1nsb.a
1nxb.x	1ofv.x	1olb.a	1omf.x	1omp.x	1onc.x	1osa.x
1pda.x	1pfk.a	1pgb.x	1pgd.x	1phh.x	1php.x	1piix
1plf.a	1poc.x	1poh.x	1pox.a	1ppa.x	1ppfe	1ppfi
1ppn.x	1prc.c	1prc.h	1prc.l	1prc.m	1pts.a	1pya.a
1pya.b	1pyd.a	1rcb.x	1rec.x	1rib.a	1rnd.x	1rop.a
1rve.a	1s01.x	1sac.a	1sbp.x	1ses.a	1sgt.x	1sha.a
1shf.a	1sim.x	1slt.b	1snc.x	1spa.x	1stf.i	1tbe.a
1tca.x	1tie.x	1tml.x	1tna.d	1tpl.a	1trbx	1trka
1tro.a	1ttb.a	1utg.x	1vaa.a	1vaa.b	1vmo.a	1whta
1wht.b	1wsy.a	1wsy.b	1yhb.x	1zaa.c	256b.a	2aaib
2aza.a	2bop.a	2ccy.a	2cdv.x	2chs.a	2cmd.x	2cp4x
2cpl.x	2cro.x	2ctc.x	2cts.x	2cyp.x	2dnj.a	2er7.e
2hbq.x	2hhm.a	2hip.a	2hpda	2ih1.x	2lh2.x	2liv.x
2mhr.x	2mnrx	2msb.a	2mta.c	2mtah	2mtal	2pfl.x
2pia.x	2pol.a	2por.x	2reb.x	2rn2.x	2rs1.a	2sar.a
2sas.x	2scp.a	2sga.x	2sn3.x	2spc.a	2tgi.x	2tmd.a
2tpr.a	2tsc.a	3aah.a	3aah.b	3adk.x	3b5c.x	3cd4.x
3chy.x	3cla.x	3cox.x	3dfr.x	3eca.a	3gap.a	3gbpx
3ink.c	3rub.l	3rub.s	3sdh.a	3tgt.x	451c.x	4blma
4enl.x	4fgf.x	4gcr.x	4ts1.a	4xis.x	5fbp.a	5p21.x
5tim.a	6fab.h	6fab.l	6taa.x	8abp.x	8acn.x	8atc.a
8atc.b	8cat.a	8ilb.x	8rxn.a	8tlne	9ldt.a	9rnt.x

^aThe database was prepared by J. M. Levin and checked for homologous sequences with the help of V. Di Francesco. This database has been modified to restore the total length of the sequences as defined in the SEQRES field of the Protein Data Bank (PDB) file (the DSSP program omits residues whose coordinates are missing in the PDB file, and thus if this occurs in the middle of the polypeptide chain it is split into two or more chains). Residues having no coordinates were assigned the conformation X and were not taken into account for the prediction accuracy although the prediction was done with the whole sequence length. The PDB code is followed by the chain name a, b, c, d, h (heavy), l (light), x (one chain only), e (enzyme), or i (inhibitor).

Is 100% Accuracy Possible?

Quoted from Barton (1995):

One problem that has arisen is how to evaluate secondary structure predictions. For prediction of a single protein sequence one might expect the best residue by residue accuracy to be 100%. It is not possible to define the secondary structure of a protein exactly, however. There is always room for alternative interpretations of where a helix or strand begins or ends so failure of a prediction to match exactly the secondary structure definition is not a disaster [24]. The problem of evaluation is more complicated for prediction from multiple sequences, as the prediction is a consensus for the family and so is not expected to be 100% in agreement with any single family member. The expected range in accuracy for a perfect consensus prediction is a function of the number, diversity and length of the sequences. Russell and I have calculated estimates of this range [11].

Simple residue by residue percentage accuracy has long been the standard method of assessment of secondary structure predictions. Although a useful guide, high percentage accuracies can be obtained for predictions of structures that are unlike proteins. For example, predicting myoglobin to be entirely helical (no strand or coil) will give over 80% accuracy but the prediction is of little practical use. Rost *et al.* [25] and Wang [26] explore these problems and suggest some alternative measures of predictive success based on secondary structure segment overlap. Although such measures help in an objective assessment of the prediction, there is no complete substitute for visual inspection. By eye, serious errors stand out and predictions of structures that are unlike proteins are usually recognizable. By eye, it is also straightforward to weight the importance of individual secondary structures. For example, prediction of what is in fact a core strand to be a helix would seriously hamper attempts to generate the correct tertiary structure of the protein from the predicted secondary structure, whereas prediction of a non-core helix as coil may have little impact on the integrity of the tertiary structure.

Types of Secondary Structure

Prediction Methods

- Parametric Statistical
 - ◊ struc. = explicit numerical func. of the data (GOR)
- Non-parametric
 - ◊ struc. = NON- explicit numerical func. of the data
 - ◊ generalize Neural Net, seq patterns, nearest nbr, &c.
- Semi-parametric: combine both
- single sequence
- multi sequence
 - ◊ with or without multiple-alignment

GOR Semi-parametric Improvements

- Filtering GOR to regularize

[$\neg a, \neg a, c, b, *, \neg b$] $\rightarrow c$
[$\neg a, *, *, a, b$] $\rightarrow b$
[$\neg a, *, *, a, c$] $\rightarrow c$
[$a, *, *, a, c, *, \neg c$] $\rightarrow c$
[$\neg a, \neg a, a, a, c, \neg a$] $\rightarrow c$

[$\neg a, c, \neg c, a, a, c, \neg a$] $\rightarrow c$
[$\neg a, c, c, a, a, \neg b, \neg a$] $\rightarrow c$
[$a, c, *, a, a, a, \neg a$] $\rightarrow c$
[* , c, *, a, a, b, $\neg a$] $\rightarrow c$
[c, b, t a, a, *, a] $\rightarrow b$
[c, * a, a, $\neg a$, a] $\rightarrow c$

a = α -helix, b = β -strand, c = coil, * = wildcard (α -helix or β -strand or coil) \neg = not.

If the pattern c on the left is met in a prediction, then the secondary structure in c on the left is rewritten as the secondary structure on the right of the rule. For example:

[b, b, b, a, c] \rightarrow [b, b, b, c, c]
[b, b, c, a, c] \rightarrow [b, b, c, c, c]
[b, b, b, a, b, b, b] \rightarrow [b, b, b, b, b, b, b].

Illustration Credits: King & Sternberg, 1996

Multiple Sequence Methods

- Average GOR over multiple seq. Alignment
- The GOR method only uses single sequence information and because of this achieves lower accuracy (65 versus >71 %) than the current "state-of-the-art" methods that incorporate multiple sequence information (e.g. King & Sternberg, 1996; Rost, 1996; Rost & Sander, 1993).

Illustration Credits: Livingston & Barton, 1996

[29]

MULTIPLE ALIGNMENT AND SECONDARY STRUCTURE

505

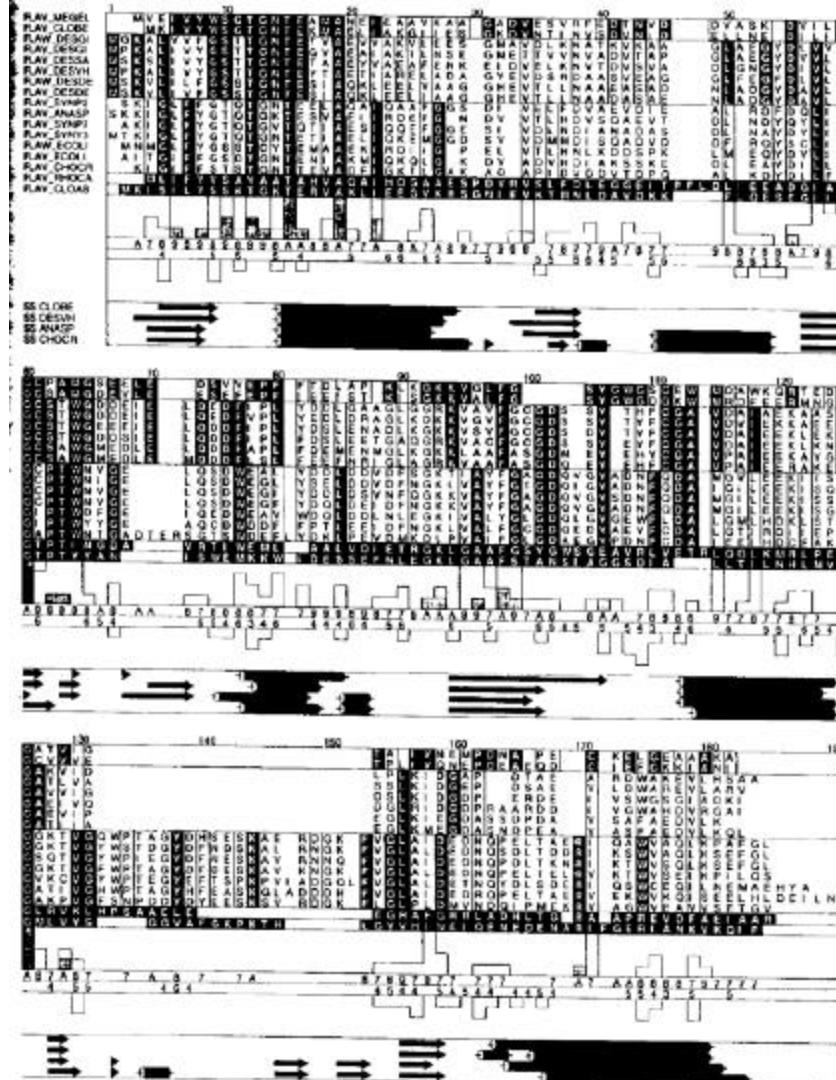


FIG. 5. Conservation analysis of the 17 flavodoxin sequences clustered in Fig. 3. The Taylor Venn diagram was used (Fig. 1) with a threshold of $T = 7$. See text for details.

DSC -- an improvement on GOR

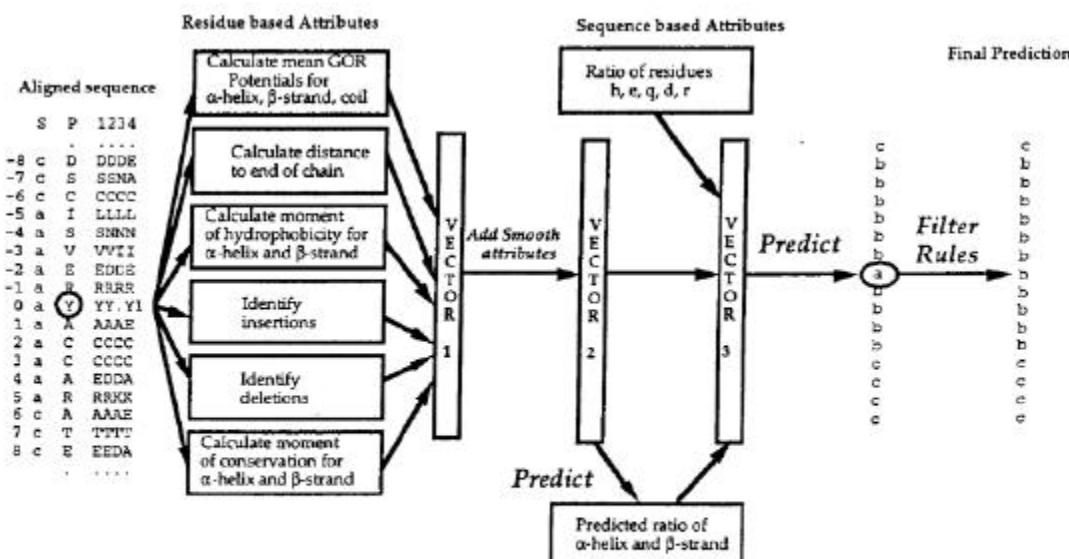


Fig. 1. DSC prediction method. For the aligned sequence: S is the observed secondary structure of the primary sequence, P. The residue at position 0 is predicted (circled).

- GOR parms
- + simple linear discriminant analysis on:

- ◊ dist from C-term, N-term
- ◊ insertions/deletes
- ◊ overall composition
- ◊ hydrophobic moments
- ◊ autocorrelate: helices
- ◊ conservation moment

Conservation, k-nn

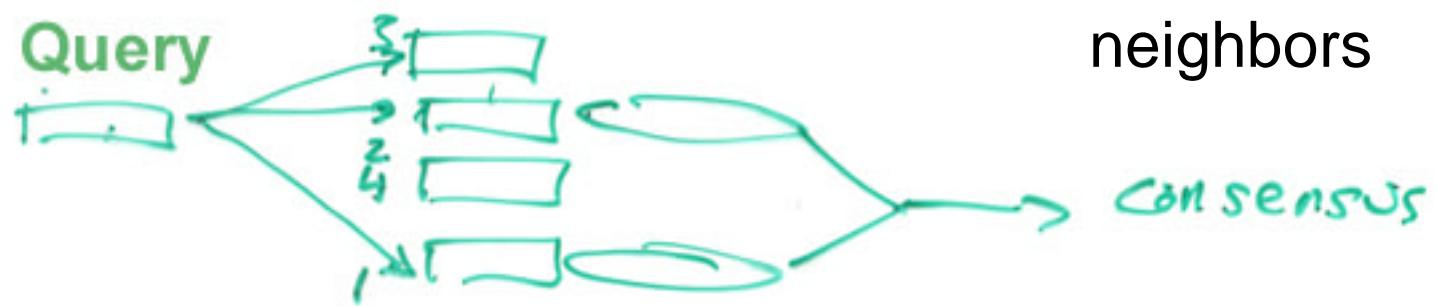
ATLUMSQ
ATLUMTQ
ATLUMTQ
C V V
i ⊗ ⊗

i i⁺⁴
i i⁺²

Patterns of
Conservation

k - nearest nbr

Query



k-nearest
neighbors

Neural Networks

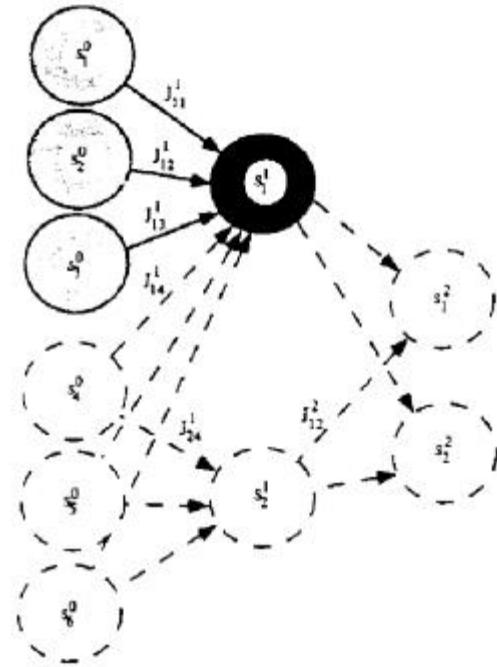
Figure 1. Function of a perceptron, the simplest neural network. A simple perceptron has only 1 output unit (black). Each of the left nodes receives a certain input signal (e.g. binary, i.e. =0 or 1). All units are connected to the output node by the junctions J^1_{ij} , with e.g. J^1_{1j} connecting input unit j with output unit 1. The contribution of each left node (e.g. the j th) to the signal arriving at the right one is a product of the strength of the junction connecting the 2 units, and the input: e.g. $J^1_{1j}s_j^0$. All products (here 3) are summed by the right node (here s_1^1). This sum is then evaluated by a non-linear trigger function. The resulting map of the sum onto an interval between 0 and 1 is the actual output of the network. The broken-line nodes show a potential extension of the perceptron to a 2-layered feed-forward network. Stippled circles, input units, signal = 1 or 0. Black circle, output unit. Step 1, the input to this unit is summed according to:

$$h_i^1 = \sum_{j=1}^{N^0+1} J_{ij}s_j^0 \quad (\text{here, } i=1).$$

Step 2, the output from this unit is computed by a sigmoid trigger function:

$$s_i^1 = \frac{1}{1 + \exp(-h_i^1)}$$

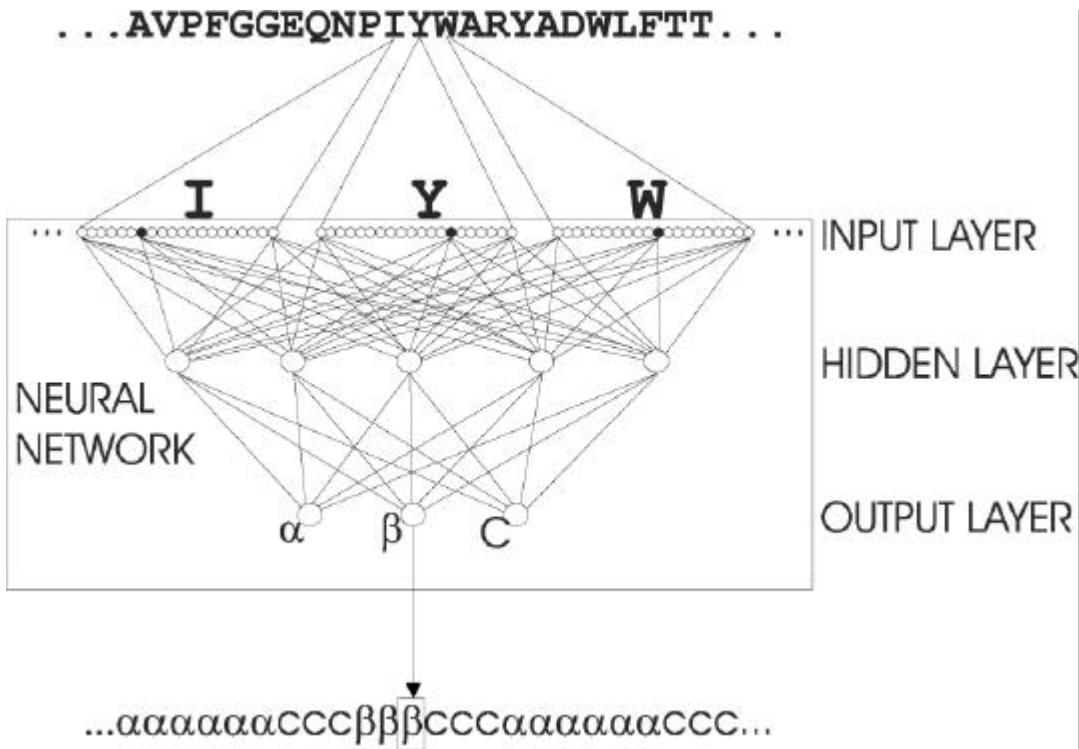
Broken-line circles, the potential extension to a 2-layered feed-forward network.



- Somehow generalize and learn patterns
- Black Box
- Rost, Kneller, Qian....
- Perceptron (above) is Simplest network
 - ◊ Multiply junction * input, sum, and threshold

Illustration Credits: Rost & Sander, 1993

More NN



- Hidden Layer
- Learning
 - ◊ Steepest descent to minimize an error function
- Jury Decision
 - ◊ Combine methods
 - ◊ Escape initial conditions

Illustration Credits: D Frishman handout

Yet more methods....

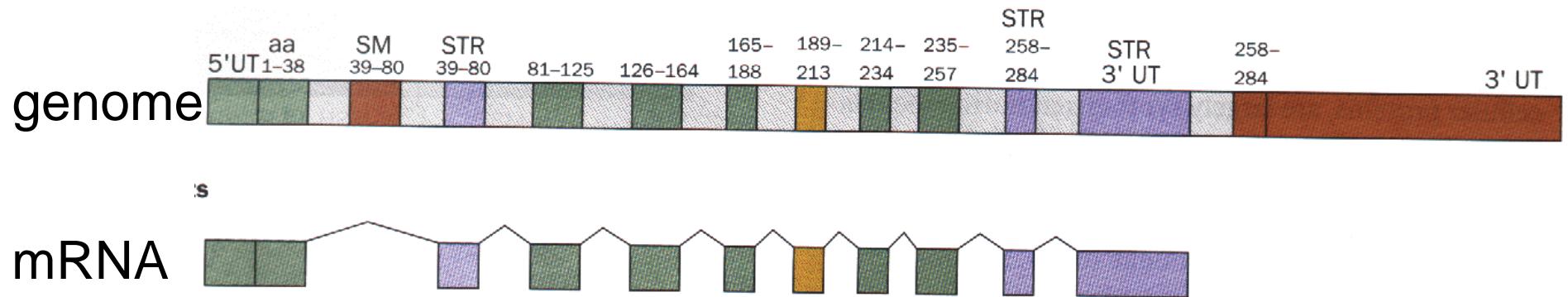
- struc class predict
 - ◊ Vect dist. between composition vectors
- threading via pair pot
- seq comparison
- ab initio from md
- ab initio from pair pot.

Mail Servers and Web Forms

Method	URL	Institution	Source code Availability
ANTHE-PROT	http://www.ibcp.fr/antheprot.html (currently unreachable)	Institute of Biology and Chemistry of Proteins (Lyon)	YES
PSSP	http://dot.imgen.bcm.tmc.edu:9331/pssprediction/pssp.html	Baylor College of Medicine (Houston)	NO
DSC	http://bonsai.lif.icnet.uk/bmm/dsc/dsc_form_align.html	Imperial Cancer Research Center (London)	YES
GOR	http://molbiol.soton.ac.uk/compute/GOR.html	University of Southampton	NO
nnPredict	http://www.cmpharm.ucsf.edu/~nomi/nnpredict.html	University of California (San Francisco)	NO
Predict-Protein	http://www.embl-heidelberg.de/predictprotein/predictprotein.html	EMBL (Heidelberg)	NO
PREDATOR	http://www.embl-heidelberg.de/argos/predator/predator_form.html	EMBL (Heidelberg)	YES
PSA	http://bmerc-www.bu.edu/psa/	BioMolecular Engineering Research Center, Boston	NO
SSPRED	http://www.embl-heidelberg.de/sspred/sspred_info.html	EMBL (Heidelberg)	NO
GOR and DSC	http://genome.imb-jena.de/cgi-bin/GDEWWW/menu.cgi	IMB (Jena)	NO
GOR	http://absalpha.dcrt.nih.gov:8008/gor.html	DCRT/NIH (Washington)	NO
GOR	ftp://ftp.virginia.edu/pub/fasta	University of Virginia	YES
Mult-Predict	http://kestrel.ludwig.ucl.ac.uk/zpred.html	Ludwig Institute for Cancer Research (London)	NO

Illustration Credits: D Frishman handout

Additional Features of DNA sequences in Genomes



Gene finding

- composition of codons, nts
- Splice site finding

Genetic Code

CAI

TABLE 30-2. THE "STANDARD" GENETIC CODE^a

First position (5' end)	Second position				Third position (3' end)
	U	C	A	G	
U	UUU Phe	UCU	UAU Tyr	UGU Cys	U
	UUC	UCC Ser	UAC	UGC	C
	UUA Leu	UCA	UAA Stop	UGA Stop	A
	UUG Leu	UCG	UAG Stop	UGG Trp	G
C	CUU	CCU	CAU His	CGU	U
	CUC Leu	CCC Pro	CAC	CGC Arg	C
	CUA	CCA	CAA Gln	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU	ACU	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC	AGC	C
	AUA	ACA	AAA Lys	AGA Arg	A
	AUG Met ^b	ACG	AAG	AGG	G
G	GUU	GCU	GAU Asp	GGU	U
	GUC Val	GCC Ala	GAC	GGC Gly	C
	GUA	GCA	GAA Glu	GGA	A
	GUG	GCG	GAG	GGG	G

- Codons with second position pyrimidines encode mostly hydrophobic amino acids (tan), while those with second position purines encode mostly polar amino acids (blue, red, and purple)

- The genetic code is nonambiguous. Each codon encodes a single amino acid. The only exception is GUG which in some mRNAs is used as a start codon to encode Met
- The genetic code includes three stop codons, UAG, UAA, and UGA which are termed amber, ochre, and opal codons
- The genetic code is nearly but not absolutely universal. The genetic code in mitochondria and some ciliates use a slightly modified version of the code

(Page adapted from S Strobel, Biochemistry Lecture Notes)

Splicing

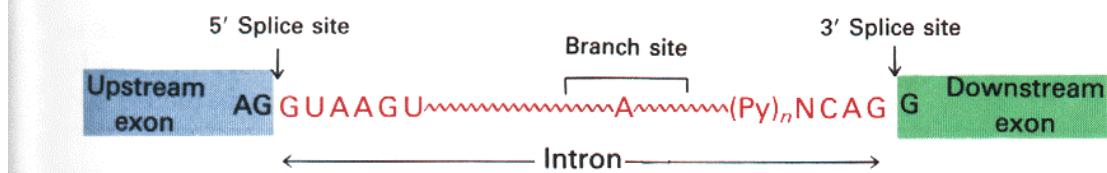


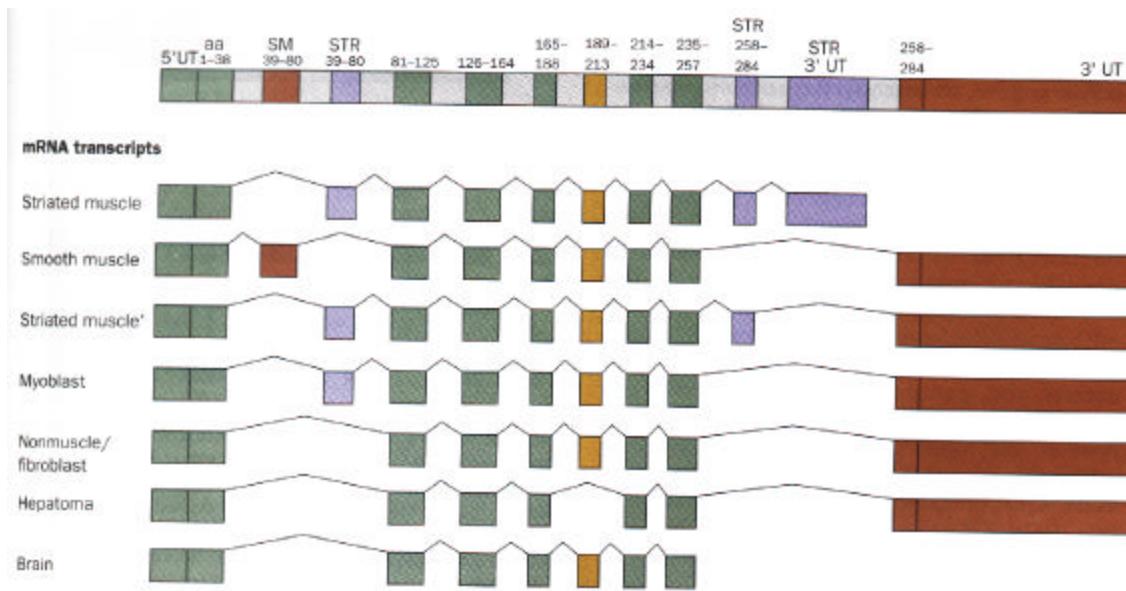
Figure 33-34

Splicing signals. Consensus sequences for the 5' splice site and the 3' splice site are shown.

- Splicing must be done accurately. Missplicing by even one nucleotide would result in a frameshift mutation throughout the remainder of the message
- The splice sites are defined largely by sequences within the intron
- The intron begins with the sequence GU and ends with AG and is part of a larger consensus sequence at both the 5' and 3' splice sites (see figure)
- 30-50 nucleotides upstream of the 3' splice site is the branch site which includes an A that serves as the nucleophile in the reaction

(Page adapted from S Strobel, Biochemistry Lecture Notes)

Alternative Splicing: Multiple Proteins from One Gene



- A single transcript can be processed to include or not include specific exons within the gene. This is termed alternative splicing
- This makes it possible to generate multiple proteins from a single gene
- For example a single gene encodes seven tissue-specific variants of the muscle protein α -tropomyosin through the process of alternative splicing
- Sex determination in *Drosophila* is largely controlled by a series of alternative splicing events

(Page adapted from S Strobel, Biochemistry Lecture Notes)

Promotors

- The RNA polymerase recognizes a promoter sequence within the DNA
- The consensus promoter includes two six base pair regions upstream of the transcription start site (defined as nucleotide +1)
- The Pribnow box (consensus sequence of TATAAT) is 10 nt upstream
- There is second element 35 nt upstream (consensus sequence TTGACA)

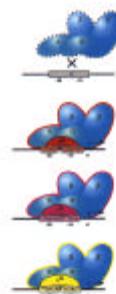


Figure 1. Different Prokaryotic RNA Polymerases. Prokaryotic RNA polymerases, consisting of four subunits, are recruited to specific transcription initiation. The association of one of the sigma prokaryotic RNA polymerases results in an RNA polymerase holoenzyme that can recognize specific DNA sequences. The sigma subunit is shown in red, the α subunit in blue, the β subunit in yellow, and the β' subunit in green. The σ core σ^E , and the σ core σ^H subunits program RNA polymerase holoenzymes to initiate transcription from promoters with different DNA sequences.

(Page adapted from S Strobel, Biochemistry Lecture Notes)

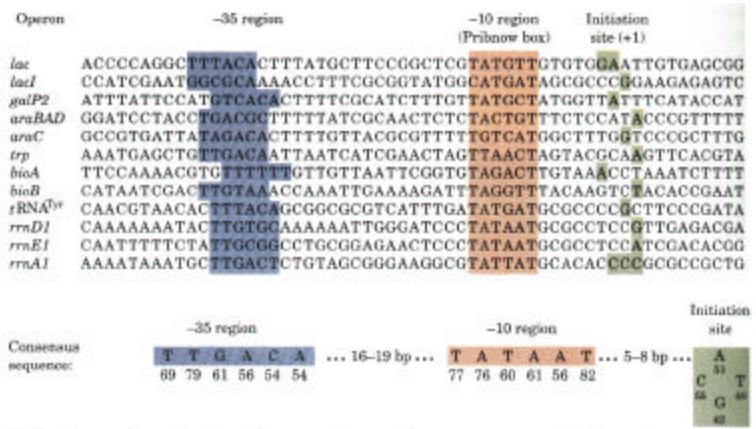


FIGURE 29-10. The sense (noncoding) strand sequences of selected *E. coli* promoters. A 6-bp region centered around the -10 position (red shading) and a 6-bp sequence around the -35 region (blue shading) are both conserved. The transcription initiation sites (+1), which in most promoters occurs at a single purine nucleotide, are shaded in green. The bottom row shows

the consensus sequence of 298 *E. coli* promoters with the number below each base indicating its percentage occurrence. [After Rosenberg, M., and Court, D., *Annu. Rev. Genet.* 13, 321–323 (1979). Consensus sequence from Lissner, S., and Margalit, H., *Nucleic Acids Res.* 21, 1512 (1993).]

- The rates at which genes are transcribed vary directly with the rate that their promoters form stable initiation complexes with the holoenzyme
- The -10 and -35 regions of the promoter sequence are recognized by the sigma subunit of the RNA polymerase holoenzyme (which also includes two α and two β subunits
- Without the sigma subunit the RNA polymerase has no affinity for the DNA
- After entering the elongation phase of transcription, the sigma factor is removed from the polymerase complex
- Expression of different sigma factors makes it possible for a bacteria to efficiently respond to external stimuli (turn on sporulation genes, heat shock genes, etc.)

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