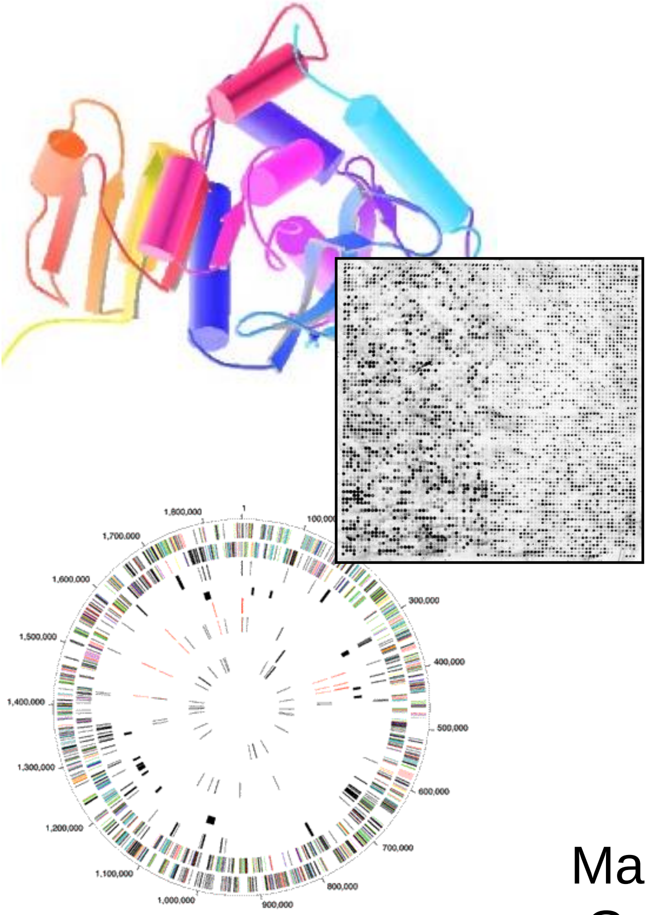
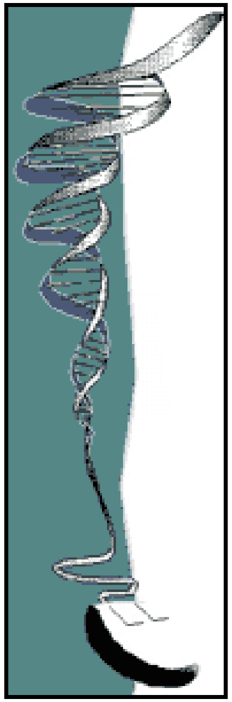
Biomed. Data Sci.
Multiple Sequences





Mark Gerstein, Yale University

GersteinLab.org/courses/452

(Last edit in spring ’22, 22m4. Added slides on agglomerative clustering & HMMs, compared to last year’s M4.)

Multiple Sequence Alignment Topics

- Multiple Sequence Alignment
- Motifs
  - Fast identification methods
- Profile Patterns
  - Refinement via EM
  - Gibbs Sampling
- HMMs
- Applications
  - Protein Domain databases
  - Regression vs expression

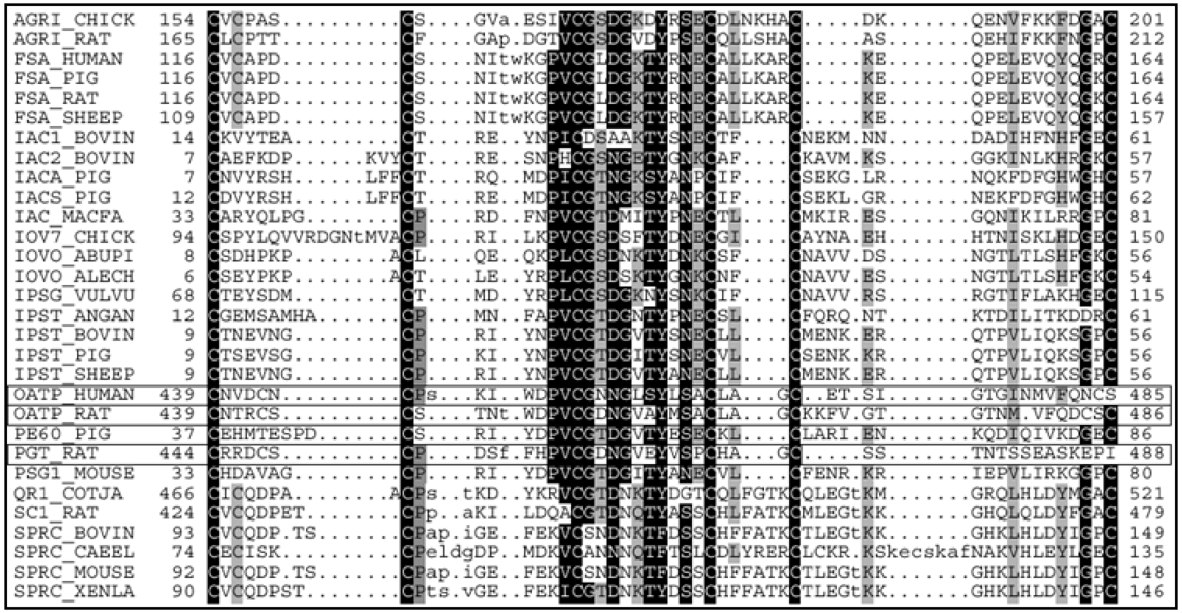
Multiple Sequence Alignments

- One of the most essential tools in molecular biology

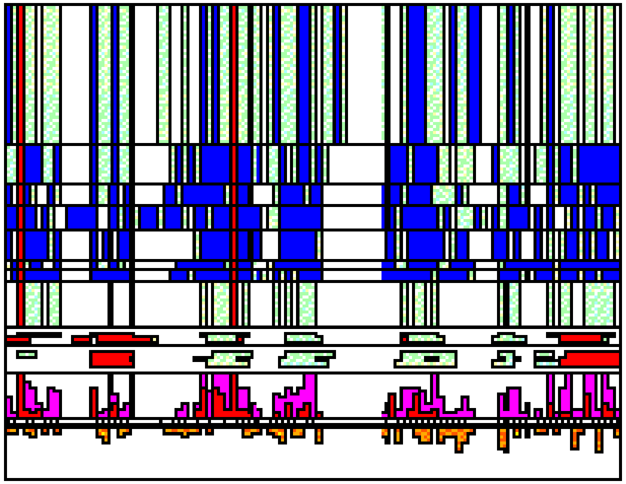
It is widely used in:

- 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

- 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



```
AGRI_CHICK   154 CVCPAS..........CS....GVa.ESIVCGSDGKDYRSECDLNKHAC.....DK.......QENVFKKFDCAC 201
AGRI_RAT     165 CLCPTT..........CF....GAp.DGTVCGSDGVDYPSECQLLSHAC.....AS.......QEHIFKKFNGPC 212
FSA_HUMAN    116 CVCAPD..........CS....NItwKGPVCGLDGKTYRNECALLKARC.....KE.......QPELEVQYQGRC 164
FSA_PIG      116 CVCAPD..........CS....NItwKGPVCGLDGKTYRNECALLKARC.....KE.......QPELEVQYQGKC 164
FSA_RAT      116 CVCAPD..........CS....NItwKGPVCGLDGKTYRNECALLKARC.....KE.......QPELEVQYQGKC 164
FSA_SHEEP    109 CVCAPD..........CS....NItwKGPVCGLDGKTYRNECALLKARC.....KE.......QPELEVQYQGKC 157
IAC1_BOVIN    14 CKVYTEA.........CT....RE..YNPICDSAAKTYSNECTF....CNEKM.NN.......DADIHFNHFGEC 61
IAC2_BOVIN     7 CAEFKDP......KVYCT....RE..SNPHCGSNGETYGNKCAF....CKAVM.KS.......GGKINLKHRGKC 57
IACA_PIG       7 CNVYRSH......LFFCT....RQ..MDPICGTNGKSYANPCIF....CSEKG.LR.......NQKFDFGHWGHC 57
IACS_PIG      12 CDVYRSH......LFFCT....RE..MDPICGTNGKSYANPCIF....CSEKL.GR.......NEKFDFGHWGHC 62
IAC_MACFA     33 CARYQLPG........CP....RD..FNPVCGTDMITYPNECTL....CMKIR.ES.......GQNIKILRRGPC 81
IOV7_CHICK    94 CSPYLQVVRDGNtMVACP....RI..LKPVCGSDSFTYDNECGI....CAYNA.EH.......HTNISKLHDGEC 150
IOVO_ABUPI     8 CSDHPKP........ACL....QE..QKPLCGSDNKTYDNKCSF....CNAVV.DS.......NGTLTLSHFGKC 56
IOVO_ALECH     6 CSEYPKP........ACT....LE..YRPLCGSDSKTYGNKCNF....CNAVV.ES.......NGTLTLSHFGKC 54
IPSG_VULVU    68 CTEYSDM.........CT....MD..YRPLCGSDGKNYSNKCIF....CNAVV.RS.......RGTIFLAKHGEC 115
IPST_ANGAN    12 CGEMSAMHA.......CP....MN..FAPVCGTDGNTYPNECSL....CFQRQ.NT.......KTDILITKDDRC 61
IPST_BOVIN     9 CTNEVNG.........CP....RI..YNPVCGTDGVTYSNECLL....CMENK.ER.......QTPVLIQKSGPC 56
IPST_PIG       9 CTSEVSG.........CP....KI..YNPVCGTDGITYSNECVL....CSENK.KR.......QTPVLIQKSGPC 56
IPST_SHEEP     9 CTNEVNG.........CP....RI..YNPVCGTDGVTYANECLL....CMENK.ER.......QTPVLIQKSGPC 56
OATP_HUMAN   439 CNVDCN..........CPs...KI..WDPVCGNNGLSYLSACLA...GC..ET.SI.......GTGINMVFQNCS 485
OATP_RAT     439 CNTRCS..........CS....TNt.WDPVCGDNGVAYMSACLA...GCKKFV.GT.......GTNM.VFQDCSC 486
PE60_PIG      37 CEHMTESPD.......CS....RI..YDPVCGTDGVTYESECKL....CLARI.EN.......KQDIQIVKDGEC 86
PGT_RAT      444 CRRDCS..........CP....DSf.FHPVCGDNGVEYVSPCHA...GC.....SS.......TNTSSEASKEPI 488
PSG1_MOUSE    33 CHDAVAG.........CP....RI..YDPVCGTDGITYANECVL....CFENR.KR.......IEPVLIRKGGPC 80
QR1_COTJA    466 CICQDPA........ACPs..tKD..YKRVCGTDNKTYDGTCQLFGTKCQLEGtKM.......GRQLHLDYMGAC 521
SC1_RAT      424 CVCQDPET........CPp..aKI..LDQACGTDNQTYASSCHLFATKCMLEGtKK.......GHQLQLDYFGAC 479
SPRC_BOVIN    93 CVCQDP.TS.......CPap.iGE..FEKVCSNDNKTFDSSCHFFATKCTLEGtKK.......GHKLHLDYIGPC 149
SPRC_CAEEL    74 CECISK..........CPeldgDP..MDKVCANNNQTFTSLCDLYRERCLCKR.KSkecskafNAKVHLEYLGEC 135
SPRC_MOUSE    92 CVCQDP.TS.......CPap.iGE..FEKVCSNDNKTFDSSCHFFATKCTLEGtKK.......GHKLHLDYIGPC 148
SPRC_XENLA    90 CVCQDPST........CPts.vGE..FEKICGTDNKTYDSSCHFFATKCTLEGtKK.......GHKLHLDYIGPC 146
```



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

Progressive Multiple Alignments

(quick, simplified overview)

- 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

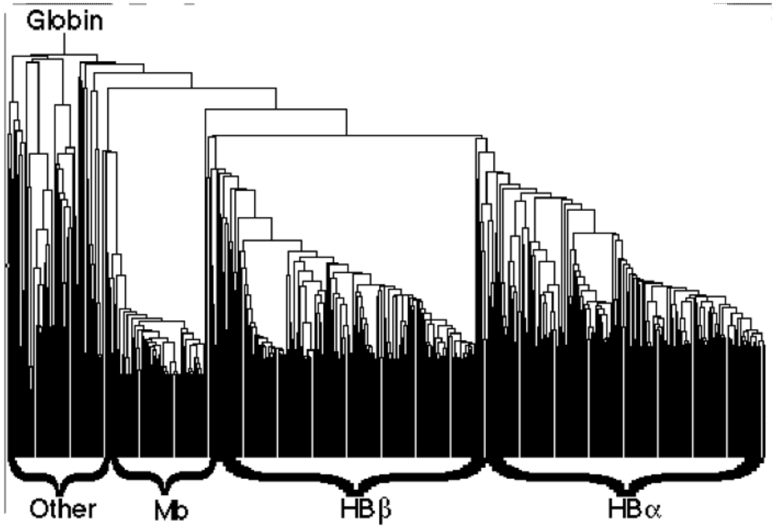
- Follows branching order in tree

- Sufficiently fast

- Sensitive

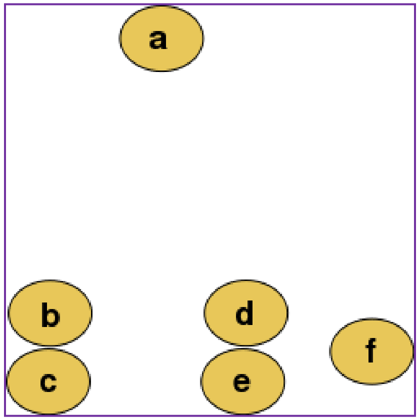
- Algorithmically heuristic, no mathematical property associated with the alignment

- Biologically sound, it is common to derive alignments which are impossible to improve by eye

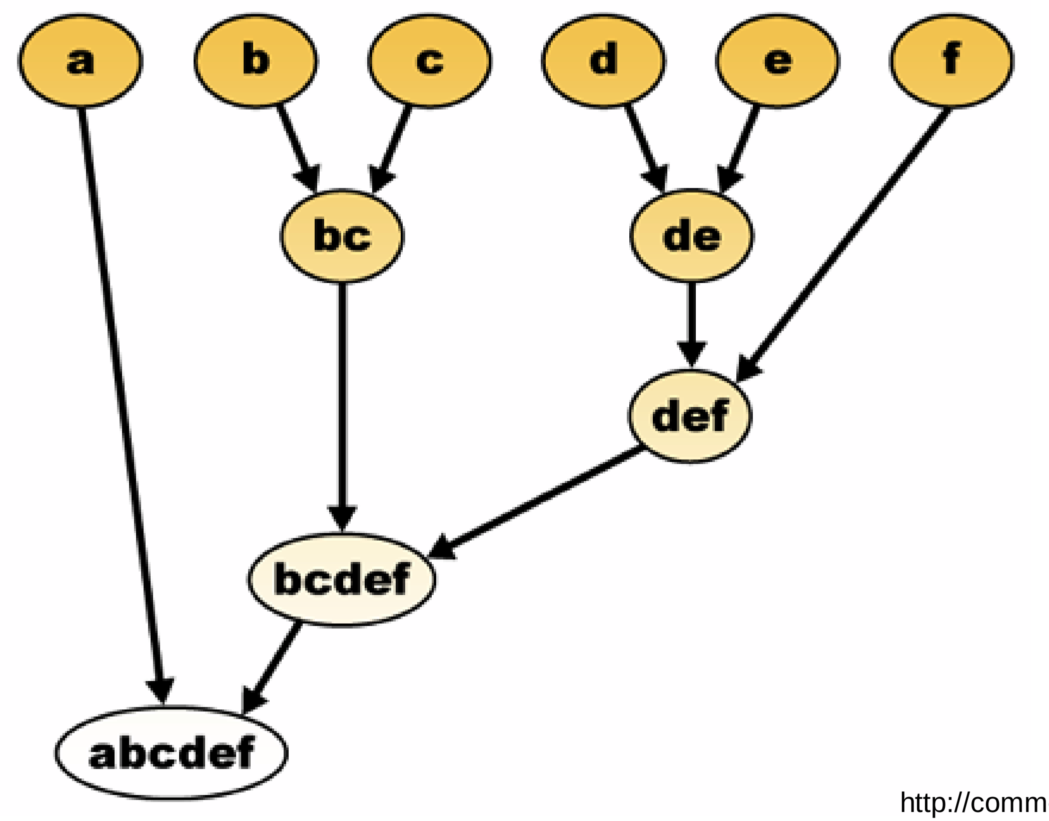


(adapted from Sonhammer et al. (1997). “Pfam,” Proteins 28:405-20)

Agglomerative Clustering



(using Euclidean Dist.)



- Ex. From Wikipedia

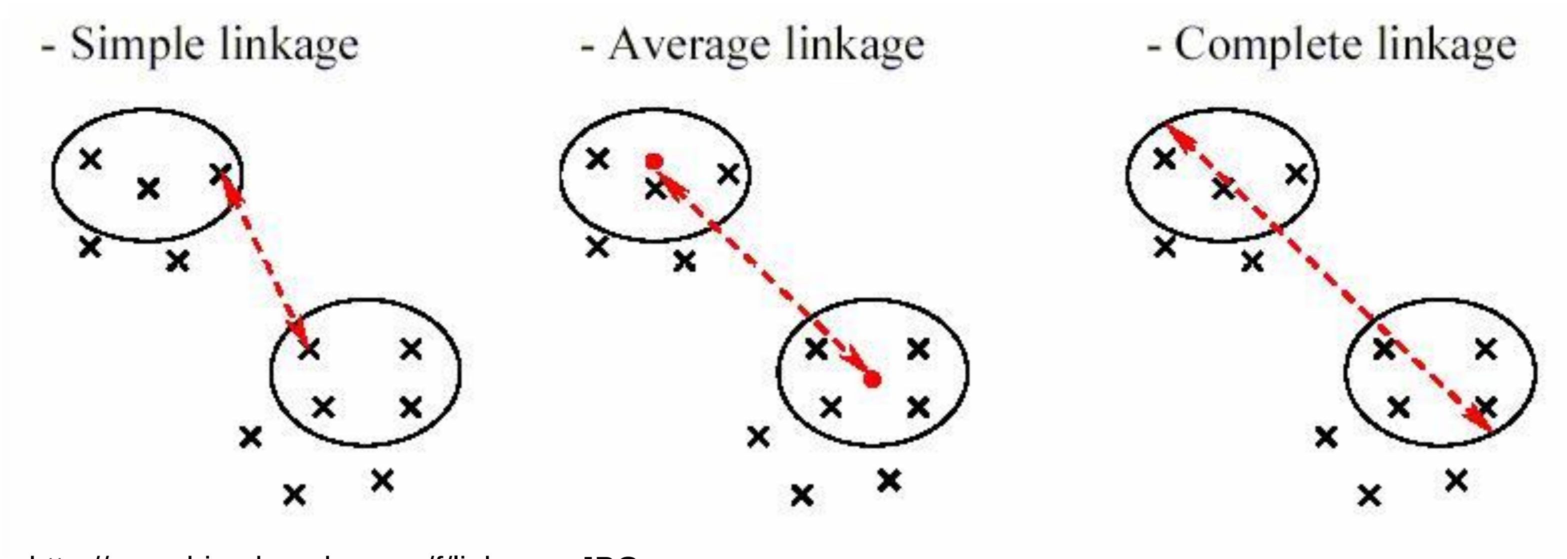
- Suppose we have merged the two closest elements b and c, we now have the following clusters {a}, {b, c}, {d}, {e} and {f}, and want to merge them further. To do that, we need to take the distance between {a} and {b c}, and therefore define the distance between two clusters.

http://commons.wikimedia.org/wiki/File:Hierarchical_clustering_diagram.png

Clustering approaches for multiple sequence alignment

- Clustal uses average linkage clustering
  - ◊ also called UPGMA

Unweighted Pair Group Method with Arithmetic mean



http://compbio.pbworks.com/f/linkages.JPG

Problems with Progressive Alignments

- Local Minimum Problem
  - Parameter Choice Problem

1. Local Minimum Problem

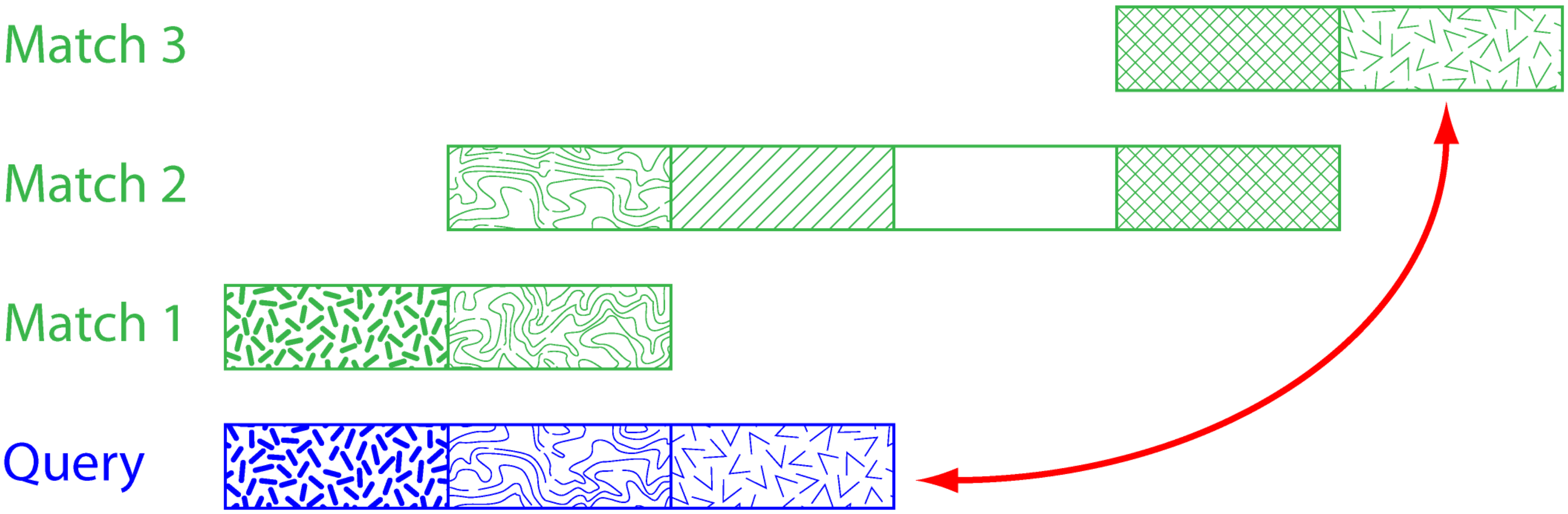
- It stems from greedy nature of alignment (mistakes made early in alignment cannot be corrected later)

- 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)

Domain Problem in Multiple Alignment



Profiles
Motifs
HMMs

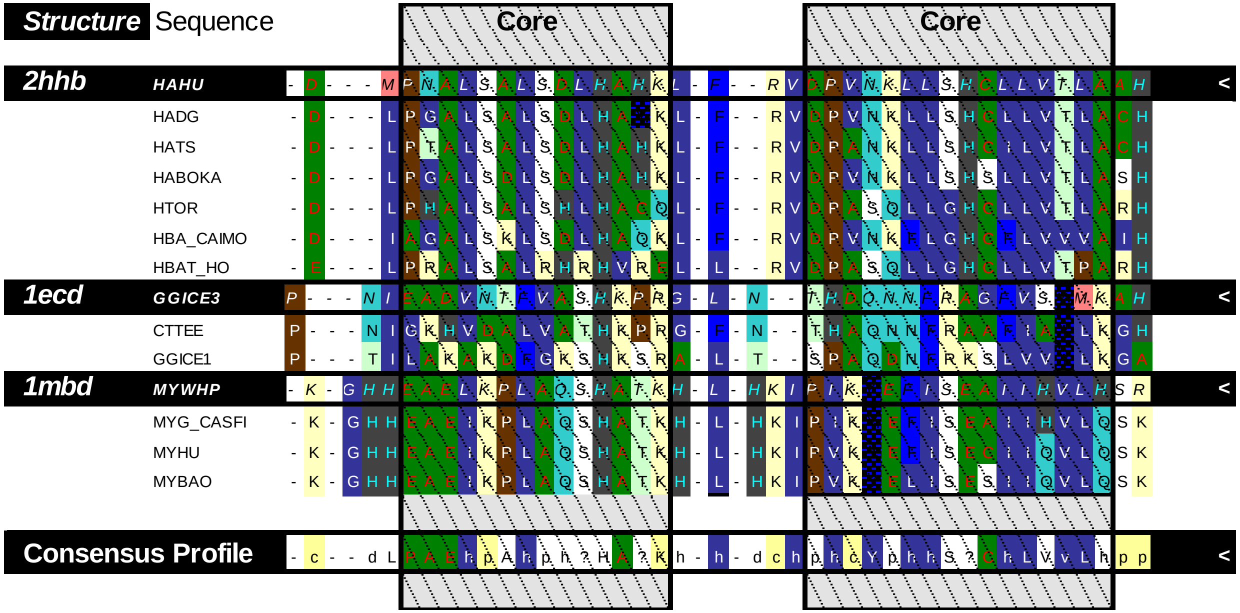
Fuse multiple alignment into:

- **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

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



Multiple Alignment

MOTIFS

2 different applications for motif analysis

- Given a collection of binding sites (or protein sequences with binding motifs), develop a representation of those sites that can be used to search new sites and reliably predict where additional binding sites occur.
- Given a set of sequences known to contain binding sites for a common factor, but not knowing where the sites are, discover the location of the sites in each sequence and a representation of the protein.

[Adapted from C Bruce, CBB752 '09]

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)

```
                      🟩🟩 🟩   🟩   🟩                    🟥 🟥🟩🟥
MMCOL10A1_1.483   SGSAIMELTENDQVWLQLPNA-ESNGLYSSEYVHSSFSGFLVAPM-------
Ca1x_Chick        SGSAVIDLMENDQVWLQLPNS-ESNGLYSSEYVHSSFSGFLFAQI-------
S15435            SGSAVLLLRPGDRVFLQMPSE-QAAGLYAGQYVHSSFSGYLLYPM-------
CA18_MOUSE.597    SGSAVLLLRPGDQVFLQNPFE-QAAGLYAGQYVHSSFSGYLLYPM-------
Ca28_Human        SGGAVLQLRPNDQVWVQIPSD-QANGLYSTEYIHSSFSGFLLCPT-------
MM37222_1.98      SGSVLLHLEVGDQVWLQVYGDGDHNGLYADNVNDSTFTGFLLYHDTN-----
COLE_LEPMA.264    SNLALLHLTDGDQVWLETLR--DWNGXYSSSEDDSTFSGFLLYPDTKKPTAM
HP27_TAMAS.72     SGTAILQLGMEDRVWLENKL--SQTDLERG-TVQAVFSGFLIHEN-------
S19018            AGGTVLQLRRGDEVWIEKDP--AKGRIYQGTEADSIFSGFLIFPS-------
C1qb_Mouse        TGGVVLKLEQEEVVHLQATD---KNSLLGIEGANSIFTGFLLFPD-------
C1qb_Human        TGGMVLKLEQGENVFLQATD---KNSLLGMEGANSIFSGFLLFPD-------
Cerb_Human        SNGVLIQMEKGDRAYLKLER---GN-LMGG-WKYSTFSGFLVFPL-------
2.HS27109_1       TGDALLELNYGQEVWLRLAK----GTIPAKFPPVTTFSGYLLYRT-------
                      :: :   :   :                    * *:*
```

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).

```
               +-------------------+        +--------------------------+
               |                   |        |                          |
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
     |                   |             *************************************
     +-------------------+
```

'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

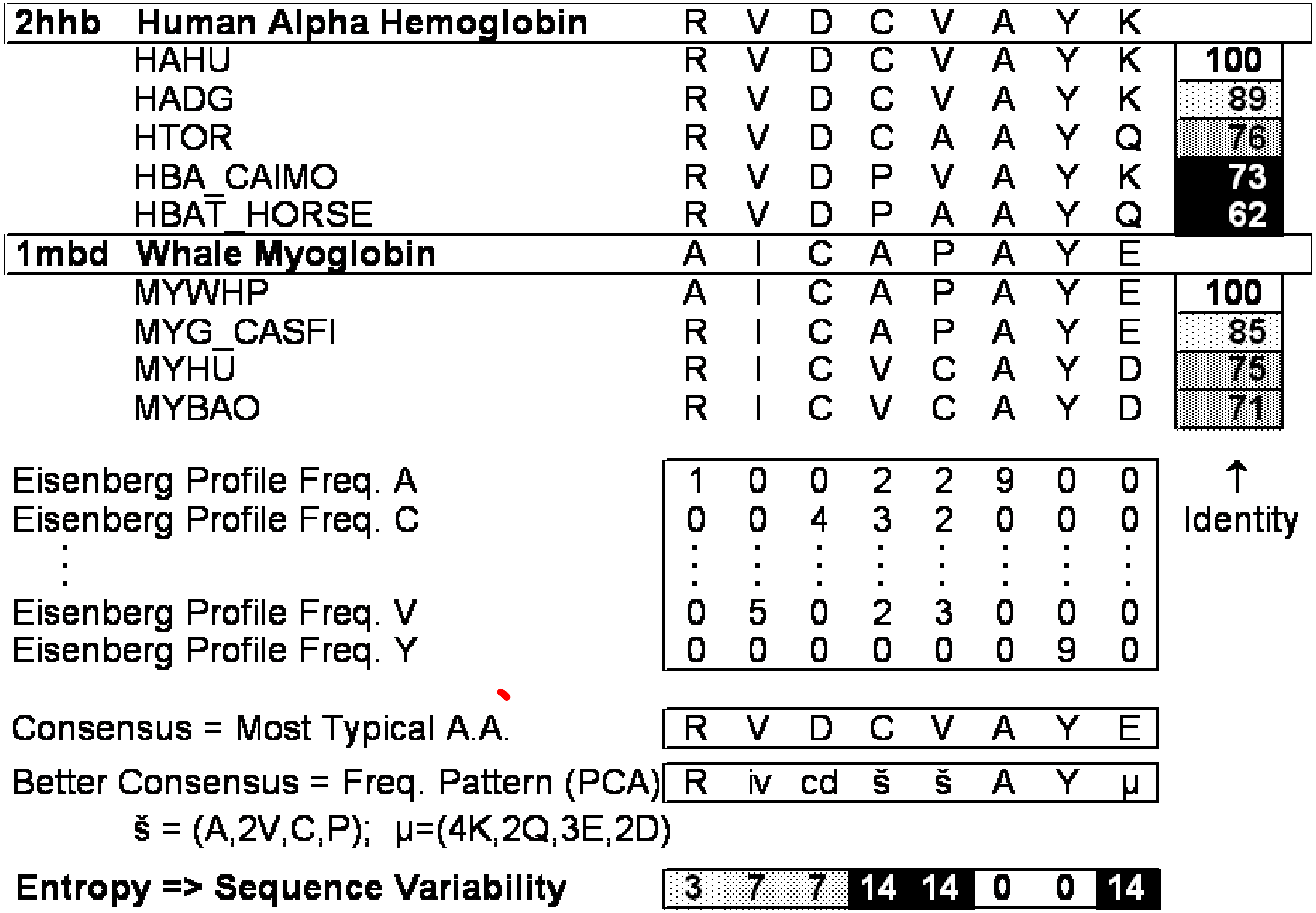
[The 3 C's are involved in disulfide bonds]

http://www.expasy.ch/sprot/prosite.html

Multiple Alignment

PROFILES

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_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

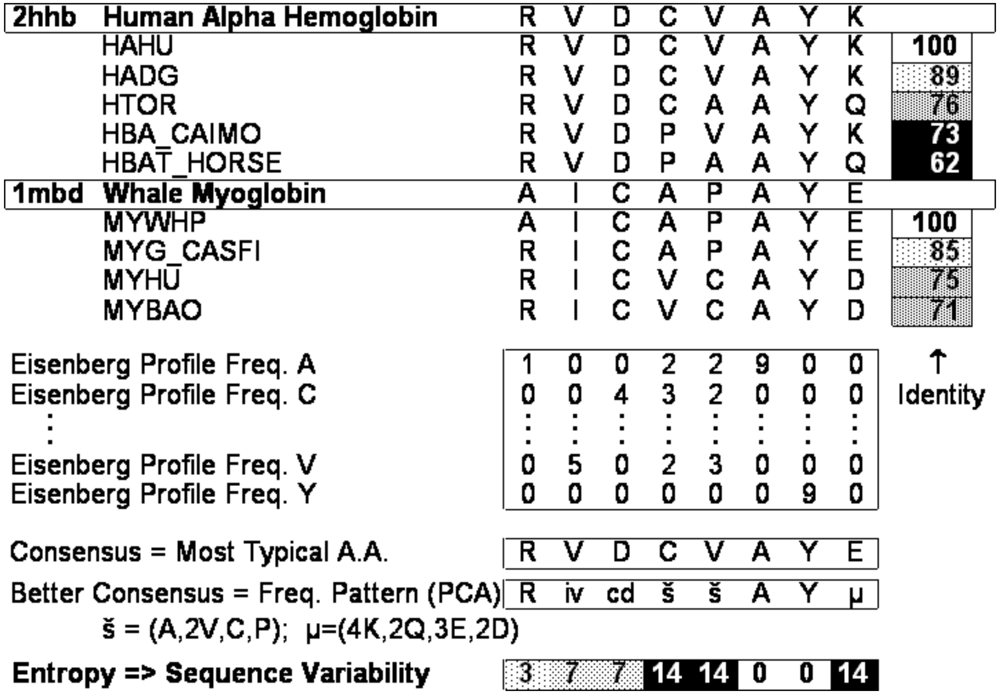
Profile : a position-specific scoring matrix composed of 21 columns and N rows (N=length of sequences in multiple alignment)

What happens with gaps?

EGF Profile Generated for SEARCHWISE

Cons.
Cys

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



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	
Entropy => Sequence Variability		3	7	7	14	14	0	0	14	

š = (A,2V,C,P); μ=(4K,2Q,3E,2D)

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

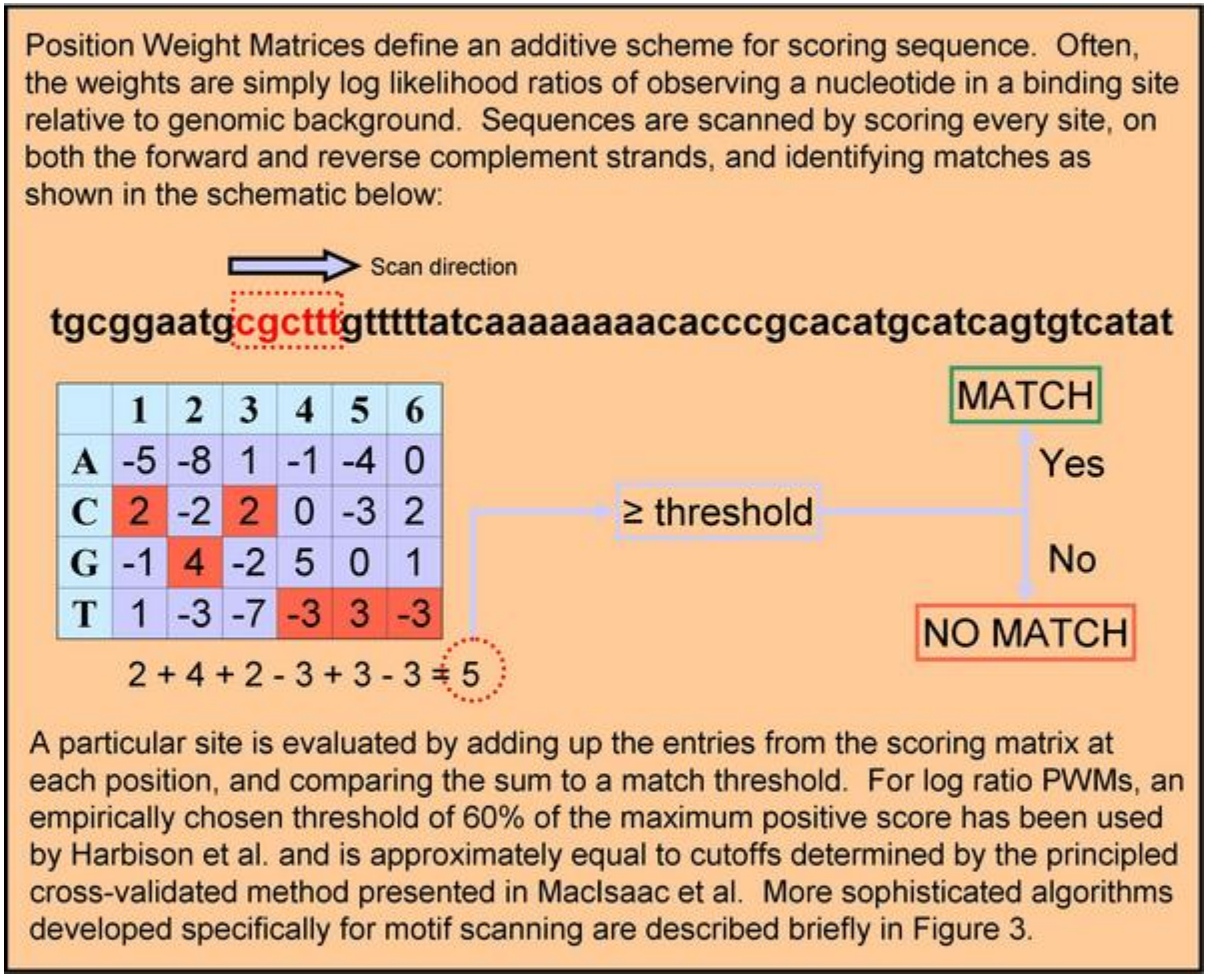
However, what if don't have many sequences in alignment? $M_{simp}(p,a)$ might be baised. Zeros for rare amino acids. Thus:

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

Y(b,a): Dayhoff matrix for *a* and *b* amino acids

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

Scanning for Motifs with PWMs



Position Weight Matrices define an additive scheme for scoring sequence. Often, the weights are simply log likelihood ratios of observing a nucleotide in a binding site relative to genomic background. Sequences are scanned by scoring every site, on both the forward and reverse complement strands, and identifying matches as shown in the schematic below:

	1	2	3	4	5	6
A	-5	-8	1	-1	-4	0
C	2	-2	2	0	-3	2
G	-1	4	-2	5	0	1
T	1	-3	-7	-3	3	-3

2 + 4 + 2 - 3 + 3 - 3 = 5

A particular site is evaluated by adding up the entries from the scoring matrix at each position, and comparing the sum to a match threshold. For log ratio PWMs, an empirically chosen threshold of 60% of the maximum positive score has been used by Harbison et al. and is approximately equal to cutoffs determined by the principled cross-validated method presented in MacIsaac et al. More sophisticated algorithms developed specifically for motif scanning are described briefly in Figure 3.

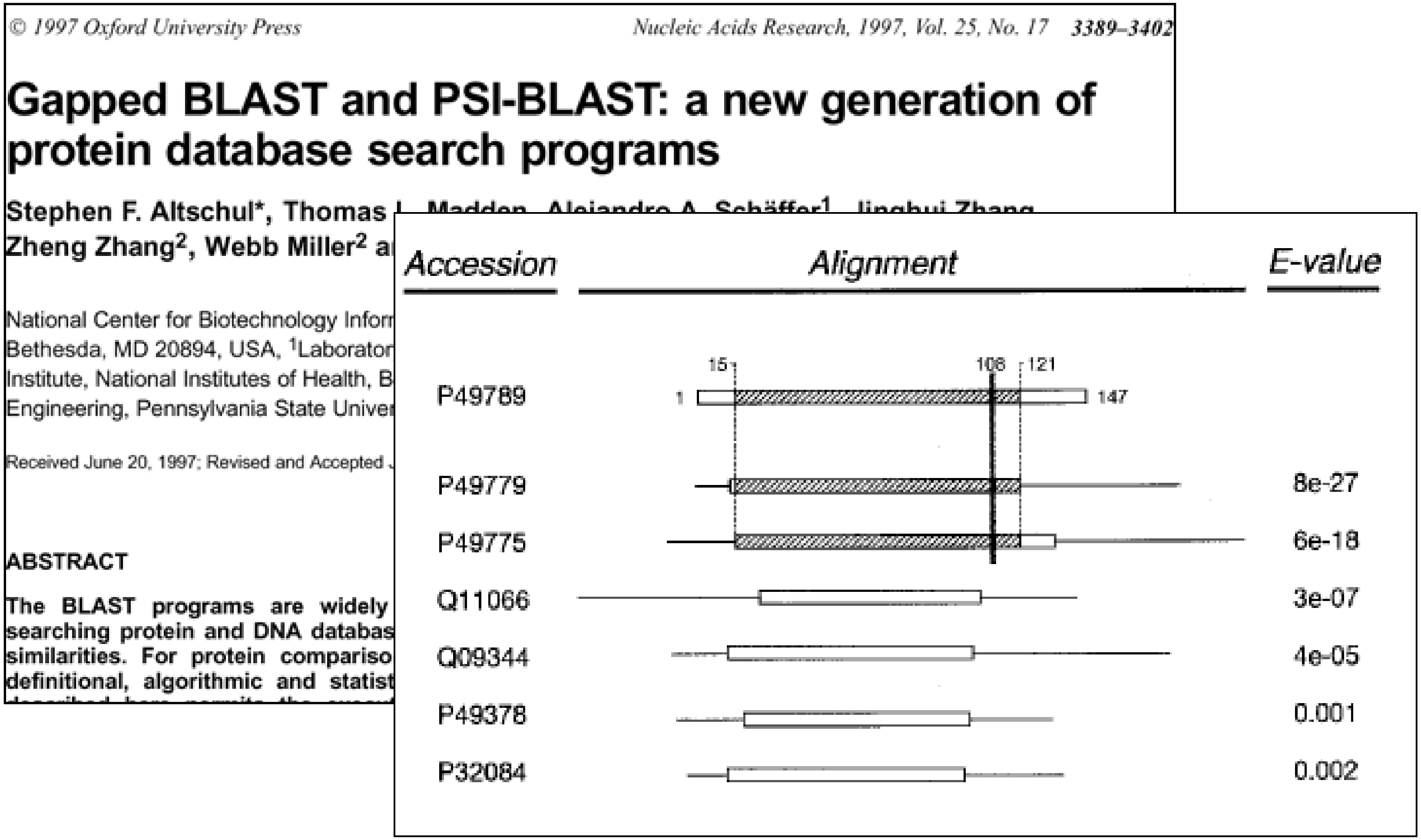
[Adapted from C Bruce, CBB752 '09]

MacIsaac & Fraenkel, 2006

Ψ-Blast

Parameters: overall threshold, inclusion threshold, interations

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



© 1997 Oxford University Press Nucleic Acids Research, 1997, Vol. 25, No. 17 3389–3402

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

Stephen F. Altschul*, Thomas L. Madden, Alejandro A. Schäffer[1], Jinghui Zhang,
Zheng Zhang[2], Webb Miller[2] a

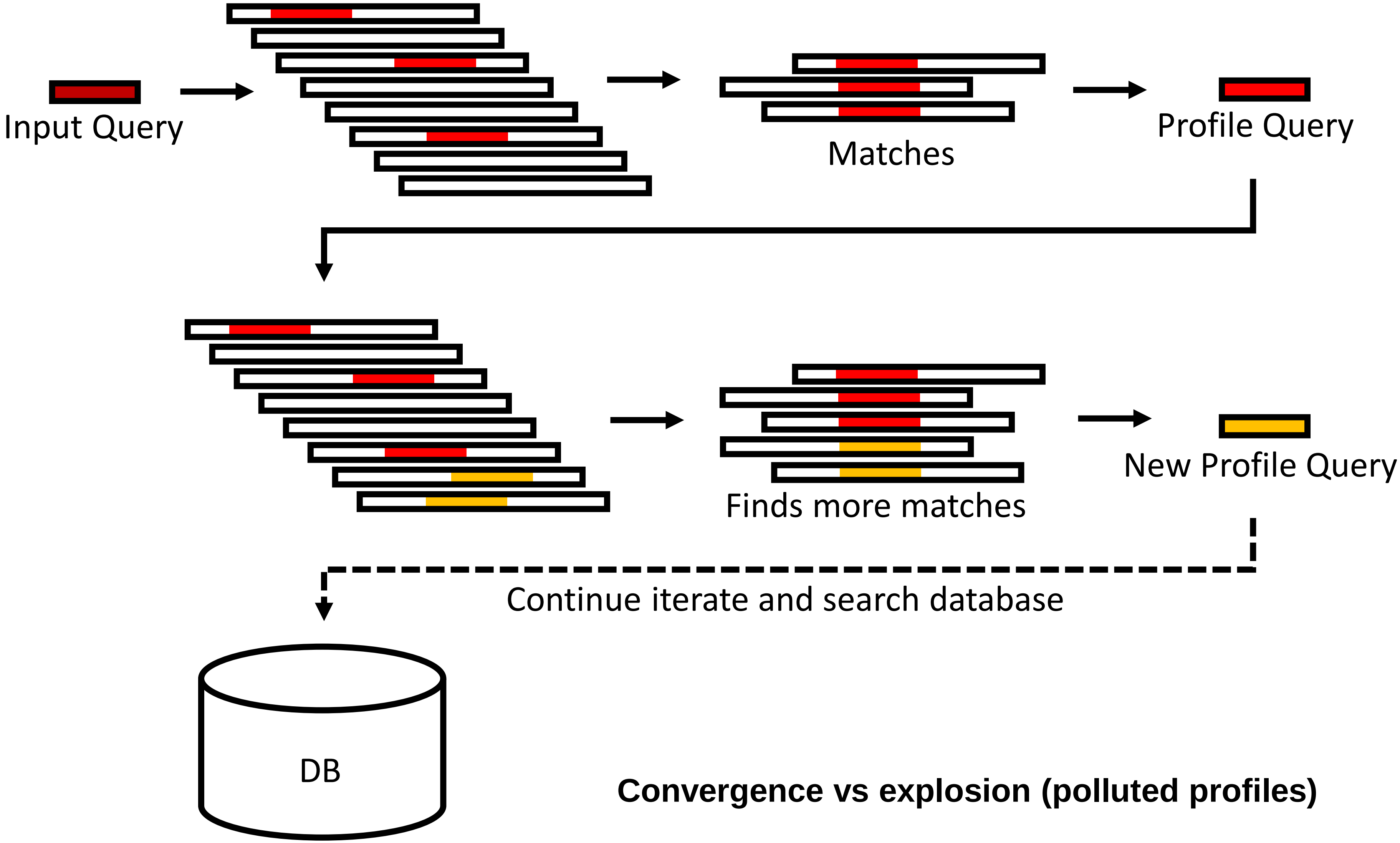
National Center for Biotechnology Infor
Bethesda, MD 20894, USA, [1]Laborator
Institute, National Institutes of Health, B
Engineering, Pennsylvania State Univer

Received June 20, 1997; Revised and Accepted J

ABSTRACT

The BLAST programs are widely
searching protein and DNA databas
similarities. For protein compariso
definitional, algorithmic and statist

PSI-BLAST (Position-Specific Iterative Basic Local Alignment Search Tool)



Convergence vs explosion (polluted profiles)

Low-Complexity Regions

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

LLLLLLLLLLLLL

Multiple Alignment: Probabilistic Approaches for Determining PWMs

- Expectation Maximization: Search the PWM space randomly
- Gibbs sampling: Search sequence space randomly.

[Adapted from C Bruce, CBB752 '09]

Expectation-Maximization (EM) algorithm

- Used in statistics for finding maximum likelihood estimates of parameters in probabilistic models, where the model depends on unobserved latent variables.
- EM alternates between performing
  - an expectation (E) step, which computes an expectation of the likelihood by including the latent variables as if they were observed, and
  - a maximization (M) step, which computes the maximum likelihood estimates of the parameters by maximizing the expected likelihood found on the E step.
- The parameters found on the M step are then used to begin another E step, and the process is repeated.

1. Guess an initial weight matrix
2. Use weight matrix to predict instances in the input sequences
3. Use instances to predict a weight matrix
4. Repeat 2 [E-step] & 3 [M-step] until satisfied.

Another good source is Wes Craven's 776 course: https://www.biostat.wisc.edu/~craven/776/lecture9.pdf

[Adapted from B Noble, GS 541 at UW, http://noble.gs.washington.edu/~wnoble/genome541/]

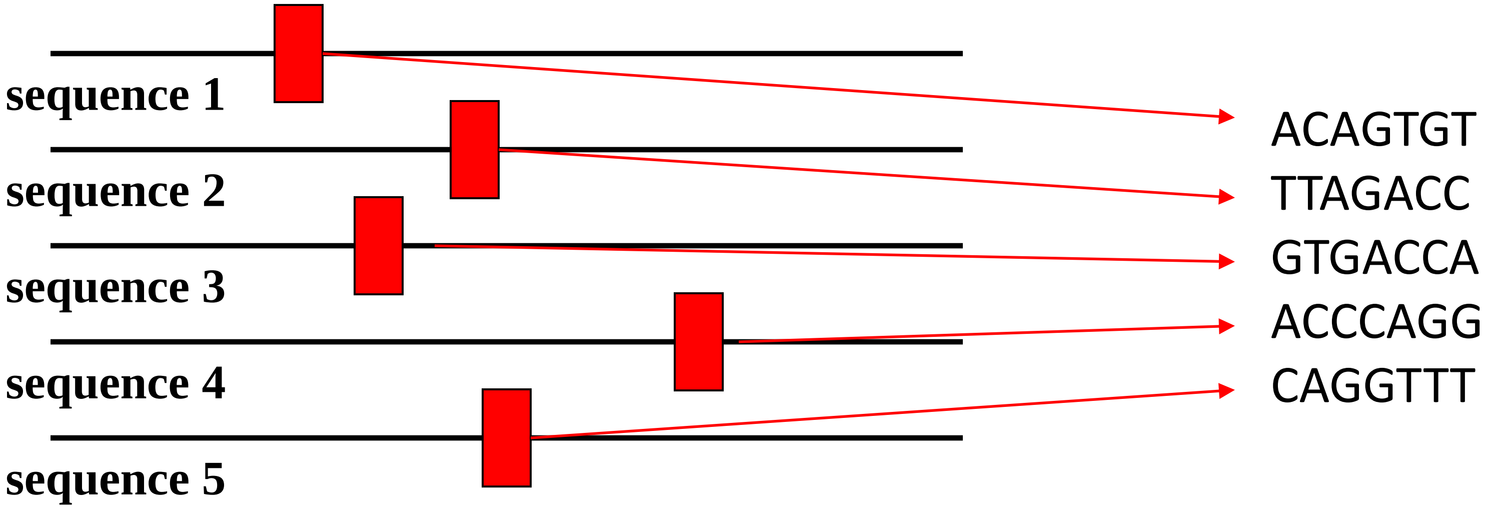
[Also Adapted from C Bruce, CBB752 '09]

Multiple Alignment

Gibbs Sampling

Initialization

- Step 1: Randomly guess an instance s_i from each of t input sequences $\{S_1, ..., S_t\}$.



[Adapted from B Noble, GS 541 at UW, http://noble.gs.washington.edu/~wnoble/genome541/]

Gibbs sampler

- Steps 2 & 3 (search):
  - Throw away an instance s_i: remaining (t - 1) instances define weight matrix.
  - Weight matrix defines instance probability at each position of input string S_i
  - Pick new s_i according to probability distribution (not necessarily always the s_i giving the highest prob.)
- Return highest-scoring motif seen

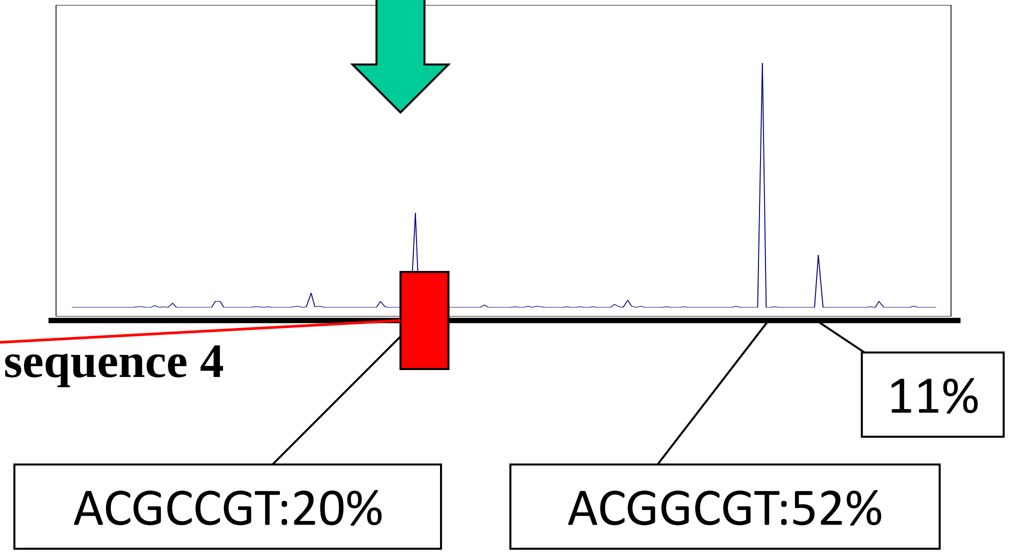
[Adapted from B Noble, GS 541 at UW, http://noble.gs.washington.edu/~wnoble/genome541/]

Sampler step illustration:

ACAGTGT
TAGGCGT
ACACCGT
???????
CAGGTTT

A	.45	.45	.45	.05	.05	.05	.05
C	.25	.45	.05	.25	.45	.05	.05
G	.05	.05	.45	.65	.05	.65	.05
T	.25	.05	.05	.05	.45	.25	.85

ACAGTGT
TAGGCGT
ACACCGT
ACGCCGT
CAGGTTT



sequence 4

11%

ACGCCGT:20%

ACGGCGT:52%

[Adapted from B Noble, GS 541 at UW, http://noble.gs.washington.edu/~wnoble/genome541/]

Comparison

- Both EM and Gibbs sampling involve iterating over two steps
- Convergence:
  - EM converges when the PSSM stops changing.
  - Gibbs sampling runs until you ask it to stop.
- Solution:
  - EM may not find the motif with the highest score.
  - Gibbs sampling will provably find the motif with the highest score, if you let it run long enough.

[Adapted from B Noble, GS 541 at UW, http://noble.gs.washington.edu/~wnoble/genome541/]

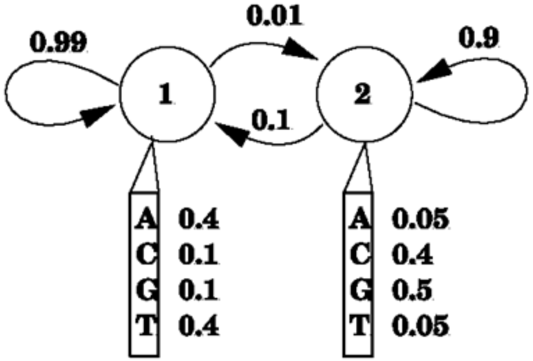
Multiple Alignment

HMMs

HMMs

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

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

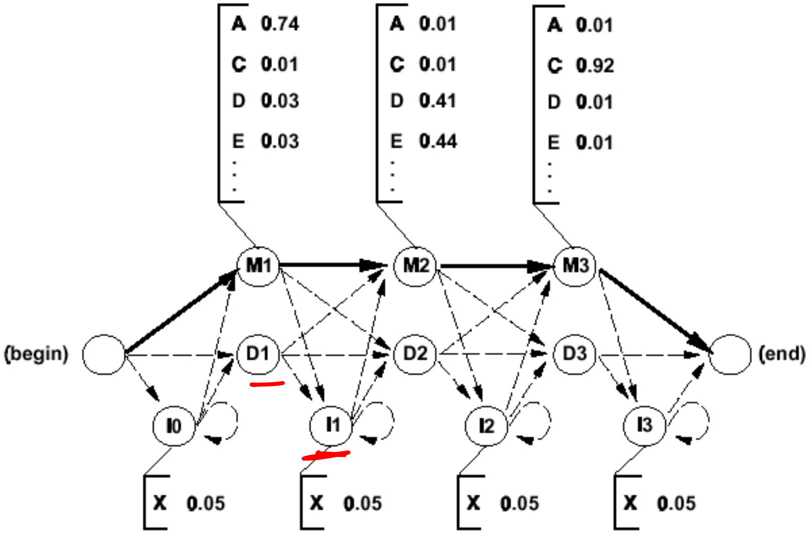


state sequence (hidden):

	...	1	1	1	1	1	2	2	2	2	1	1	...
transitions:		?	0.99	0.99	0.99	0.99	0.01	0.9	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.)

Algorithms

Probability of a path through the model

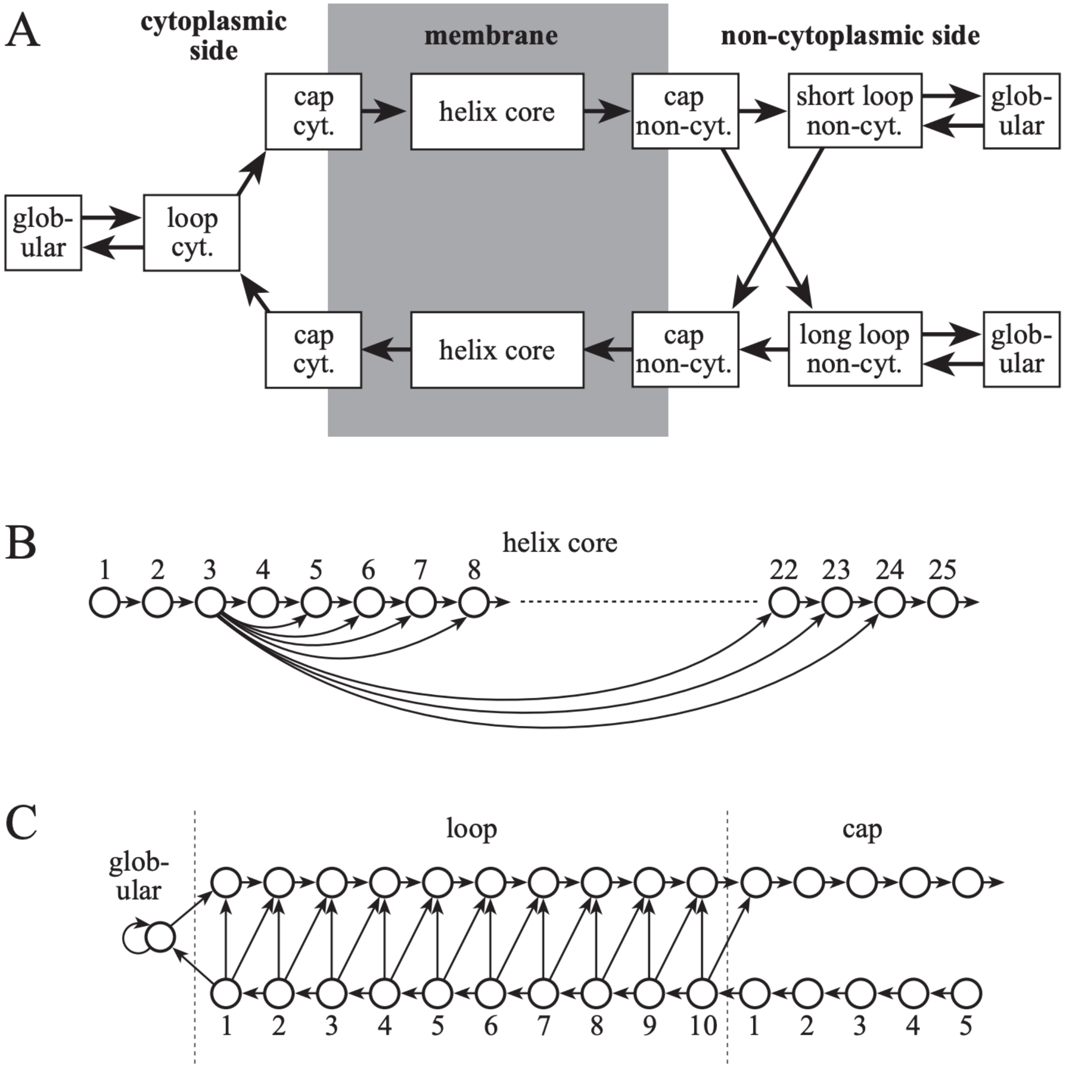
Viterbi maximizes for seq

Forward sums of all possible paths

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)

EX of Richness of the HMM Modelling Framework: Predicting Membrane Proteins



Sonnhammer et al. Proc Int Conf Intell Syst Mol Biol. 1998;6:175-82.