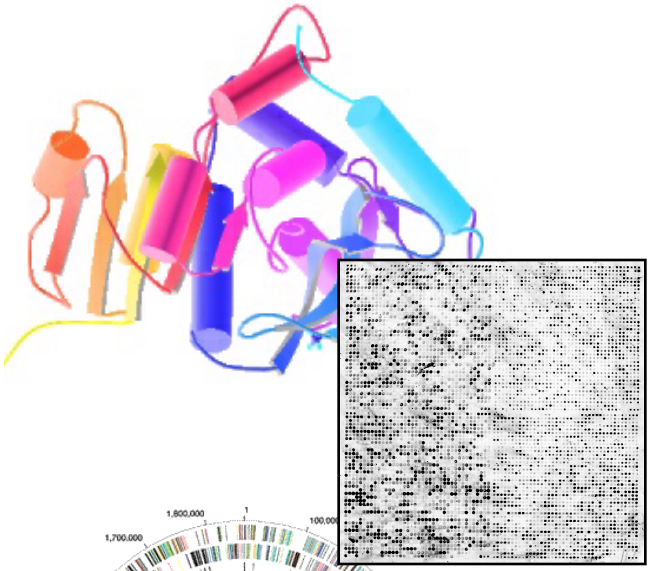
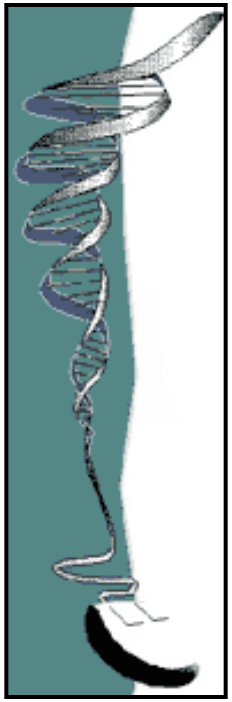
BIOINFORMATICS
Multiple Sequences





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(last edit in spring '11, not including in-class edits)

Multiple Alignment Topics

- Multiple Alignment
- Motifs
  - Fast identification methods
- Profile Patterns
  - Refinement via EM
  - Gibbs Sampling
- HMMs
- Applications
  - Module DBs
  - Regression vs expression
- Issues: site independence
  - BoCaTFBS

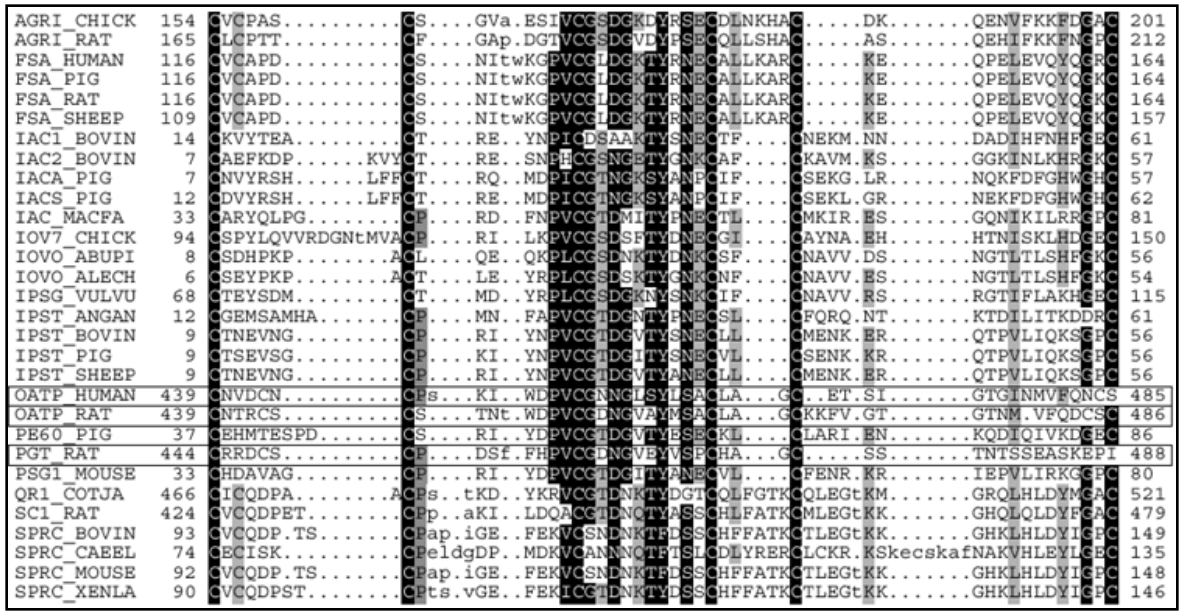
Multiple Sequence Alignments

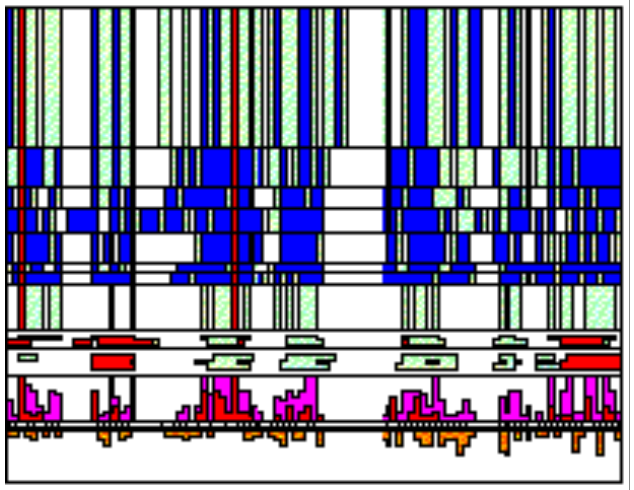
- One of the most essential tools in molecular biology

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

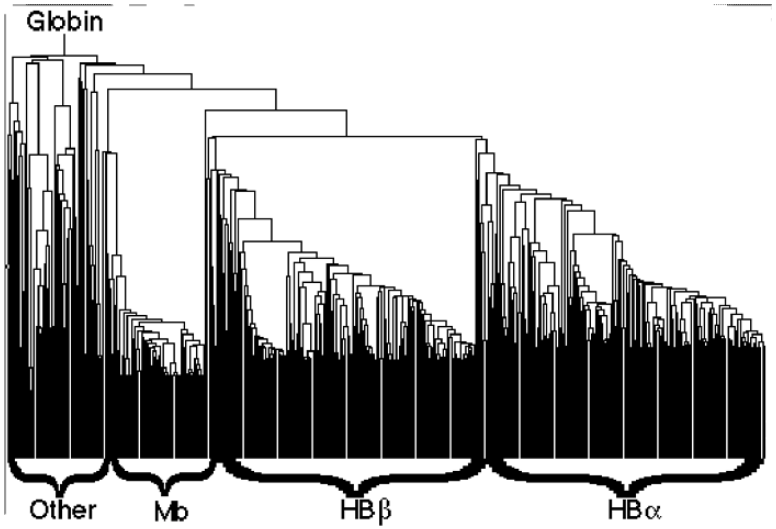




(LEFT, adapted from Sonhammer et al. (1997). "Pfam," Proteins 28:405-20. ABOVE, G Barton AMAS web page)

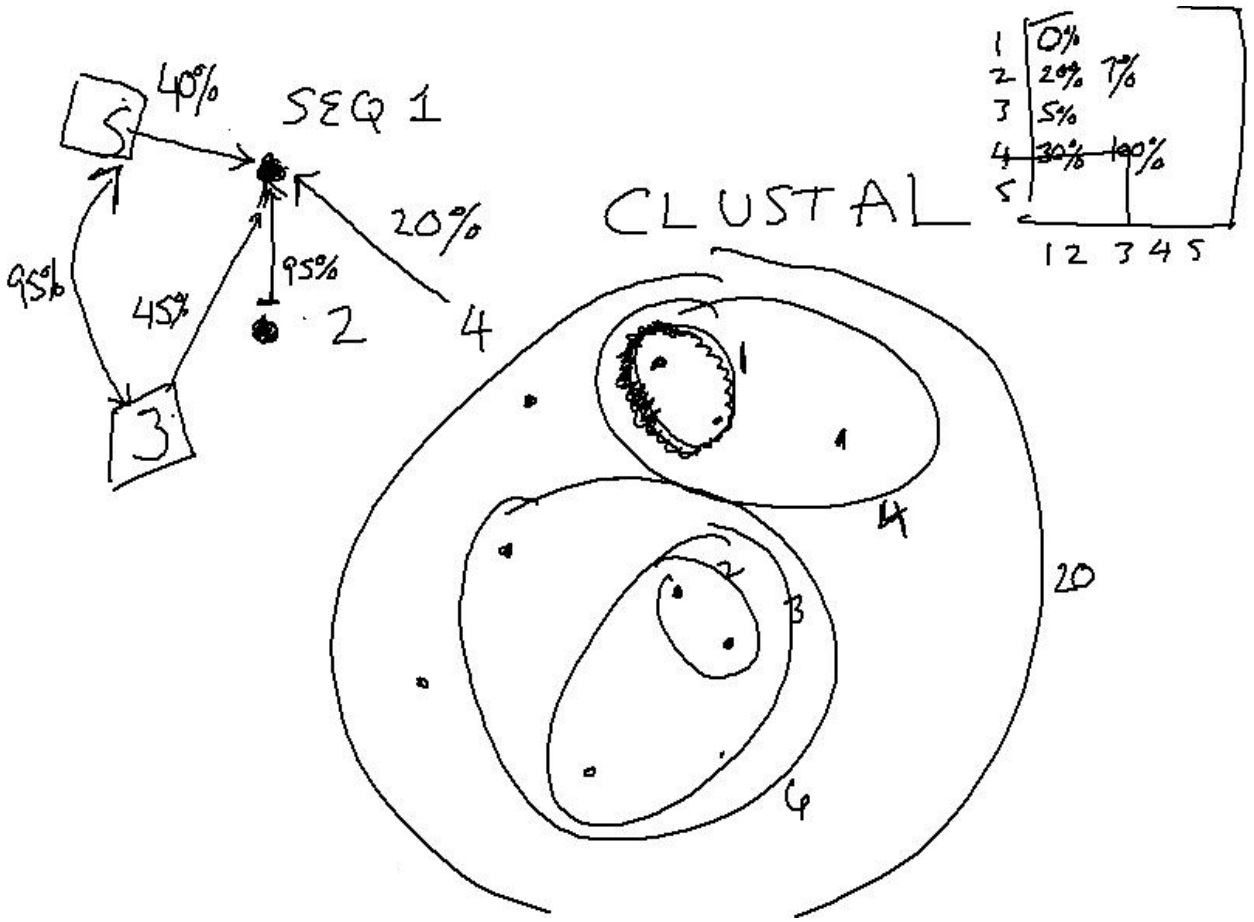
Progressive Multiple Alignments

- 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



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

Clustering approaches for multiple sequence alignment



C1Q - Example

```
Ca28_Human
ELSAHATPAFTAVLTSPLPASGMPVKFDRTLYNGHSGYNPATGIFTCPVGGVYYFAYHVH
VKGTNVWVALYKNNVPATYTYDEYKKGYLDQASGGAVLQLRPNDQVWVQIPSDQANGLYS
TEYIHSSFSGFLLCPT
C1qb_Human
DYKATQKIAFSATRTINVPLRRDQTIRFDHVITNMNNNYEPRSGKFTCKVPGLYYFTYHA
SSRGNLCVNLMRGRERAQKVVTFCDYAYNTFQVTTGGMVLKLEQGENVFLQATDKNSLLG
MEGANSIFSGFLLFPD
Cerb_Human
VRSGSAKVAFSAIRSTNHEPSEMSNRTMIIYFDQVLVNIGNNFDSERSTFIAPRKGIYSF
NFHVVKVYNRQTIQVSLMLNGWPVISAFAGDQDVTREAASNGVLIQMEKGDRAYLKLERG
NLMGGWKYSTFSGFLVFPL
COLE_LEPMA.264
RGPKGPPGESVEQIRSAFSVGLFPSRSFPPPSLPVKFDKVFYNGEGHWDPTLNKFNVTYP
GVYLFSYHITVRNRPVRAALVVNGVRKLRTRDSLYGQDIDQASNLALLHLTDGDQVWLET
LRDWNGXYSSSEDDSTFSGFLLYPDTKKPTAM
HP27_TAMAS.72
GPPGPPGMTVNCHSKGTSAFAVKANELPPAPSQPVIFKEALHDAQGHFDLATGVFTCPVP
GLYQFGFHIEAVQRAVKVSLMRNGTQVMEREAEAQDGYEHISGTAILQLGMEDRVWLENK
LSQTDLERGTVQAVFSGFLIHEN
HSUPST2_1.95
GIQGRKGEPGEGAYVYRSAFSVGLETYVTIPNMPIRFTKIFYNQQNHYDGSTGKFHCNIP
GLYYFAYHITVYMKDVKVSLFKKDKAMLFTYDQYQENNVDQASGSVLLHLEVGDQVWLQV
YGEGERNGLYADNDNDSTFTGFLLYHDTN
2.HS27109_1
ENALAPDFSKGSYRYAPMVAFFASHTYGMTIPGPILFNNLDVNYGASYTPRTGKFRIPYL
GVYVFKYTIESFSAHISGFLVVDGIDKLAFESENINSEIHCDRVLTGDALLELNYGQEVW
LRLAKGTIPAKFPPVTTFSGYLLYRT
4.YQCC_BACSU
VVHGWTPWQKISGFAHANIGTTGVQYLKKIDHTKIAFNRVIKDSHNAFDTKNNRFIAPND
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NKGDYVEIWCYCNYGGDETLKRAVDDKNGVFNFFD
5.BSPBSXSE_25
ADSGWTAWQKISGFAHANIGTTGRQALIKGENNKIKYNRIIKDSHKLFDTKNNRFVASHA
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PLDAGDYVEIYVYVGYSGDVTRYVTDSNGALNYFD
```

```
MMCOL10A1_1.483     SGMPLVSANHGVTG-------MPVSAFTVILS--KAYPA---VGCPHPIYEILYNRQQHY
Ca1x_Chick          ----------ALTG-------MPVSAFTVILS--KAYPG---ATVPIKFDKILYNRQQHY
S15435              ----------GGPA-------YEMPAFTAELT--APFPP---VGGPVKFNKLLYNGRQNY
CA18_MOUSE.597      HAYAGKKGKHGGPA-------YEMPAFTAELT--VPFPP---VGAPVKFDKLLYNGRQNY
Ca28_Human          ----------ELSA-------HATPAFTAVLT--SPLPA---SGMPVKFDRTLYNGHSGY
MM37222_1.98        ----GTPGRKGEPGE---AAYMYRSAFSVGLETRVTVP-----NVPIRFTKIFYNQQNHY
COLE_LEPMA.264      ------RGPKGPPGE---SVEQIRSAFSVGLFPSRSFPP---PSLPVKFDKVFYNGEGHW
HP27_TAMAS.72       -------GPPGPPGMTVNCHSKGTSAFAVKAN--ELPPA---PSQPVIFKEALHDAQGHF
S19018              ----------NIRD-------QPRPAFSAIRQ---NPMT---LGNVVIFDKVLTNQESPY
C1qb_Mouse          --------------D---YRATQKVAFSALRTINSPLR----PNQVIRFEKVITNANENY
C1qb_Human          --------------D---YKATQKIAFSATRTINVPLR----RDQTIRFDHVITNMNNNY
Cerb_Human          --------------V---RSGSAKVAFSAIRSTNHEPSEMSNRTMIIYFDQVLVNIGNNF
2.HS27109_1         ---ENALAPDFSKGS---YRYAPMVAFFASHTYGMTIP------GPILFNNLDVNYGASY
                                        .* .                  :     :

MMCOL10A1_1.483     DPRSGIFTCKIPGIYYFSYHVHVKGT--HVWVGLYKNGTP-TMYTY---DEYSKGYLDTA
Ca1x_Chick          DPRTGIFTCRIPGLYYFSYHVHAKGT--NVWVALYKNGSP-VMYTY---DEYQKGYLDQA
S15435              NPQTGIFTCEVPGVYYFAYHVHCKGG--NVWVALFKNNEP-VMYTY---DEYKKGFLDQA
CA18_MOUSE.597      NPQTGIFTCEVPGVYYFAYHVHCKGG--NVWVALFKNNEP-MMYTY---DEYKKGFLDQA
Ca28_Human          NPATGIFTCPVGGVYYFAYHVHVKGT--NVWVALYKNNVP-ATYTY---DEYKKGYLDQA
MM37222_1.98        DGSTGKFYCNIPGLYYFSYHITVYMK--DVKVSLFKKDKA-VLFTY---DQYQEKNVDQA
COLE_LEPMA.264      DPTLNKFNVTYPGVYLFSYHITVRNR--PVRAALVVNGVR-KLRTR---DSLYGQDIDQA
HP27_TAMAS.72       DLATGVFTCPVPGLYQFGFHIEAVQR--AVKVSLMRNGTQ-VMERE---AEAQDG-YEHI
S19018              QNHTGRFICAVPGFYYFNFQVISKWD--LCLFIKSSSGGQ-PRDSLSFSNTNNKGLFQVL
C1qb_Mouse          EPRNGKFTCKVPGLYYFTYHASSRGN---LCVNLVRGRDRDSMQKVVTFCDYAQNTFQVT
C1qb_Human          EPRSGKFTCKVPGLYYFTYHASSRGN---LCVNLMRGRER--AQKVVTFCDYAYNTFQVT
Cerb_Human          DSERSTFIAPRKGIYSFNFHVVKVYNRQTIQVSLMLNGWP----VISAFAGDQDVTREAA
2.HS27109_1         TPRTGKFRIPYLGVYVFKYTIESFSA--HISGFLVVDGIDKLAFESEN-INSEIHCDRVL
                        . *     * * * :

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-------
                     .  :: :   : . :                    * *:*.
```

Clustal Alignment

Problems with Progressive Alignments

- Local Minimum Problem
  - Parameter Choice Problem

1. Local Minimum Problem

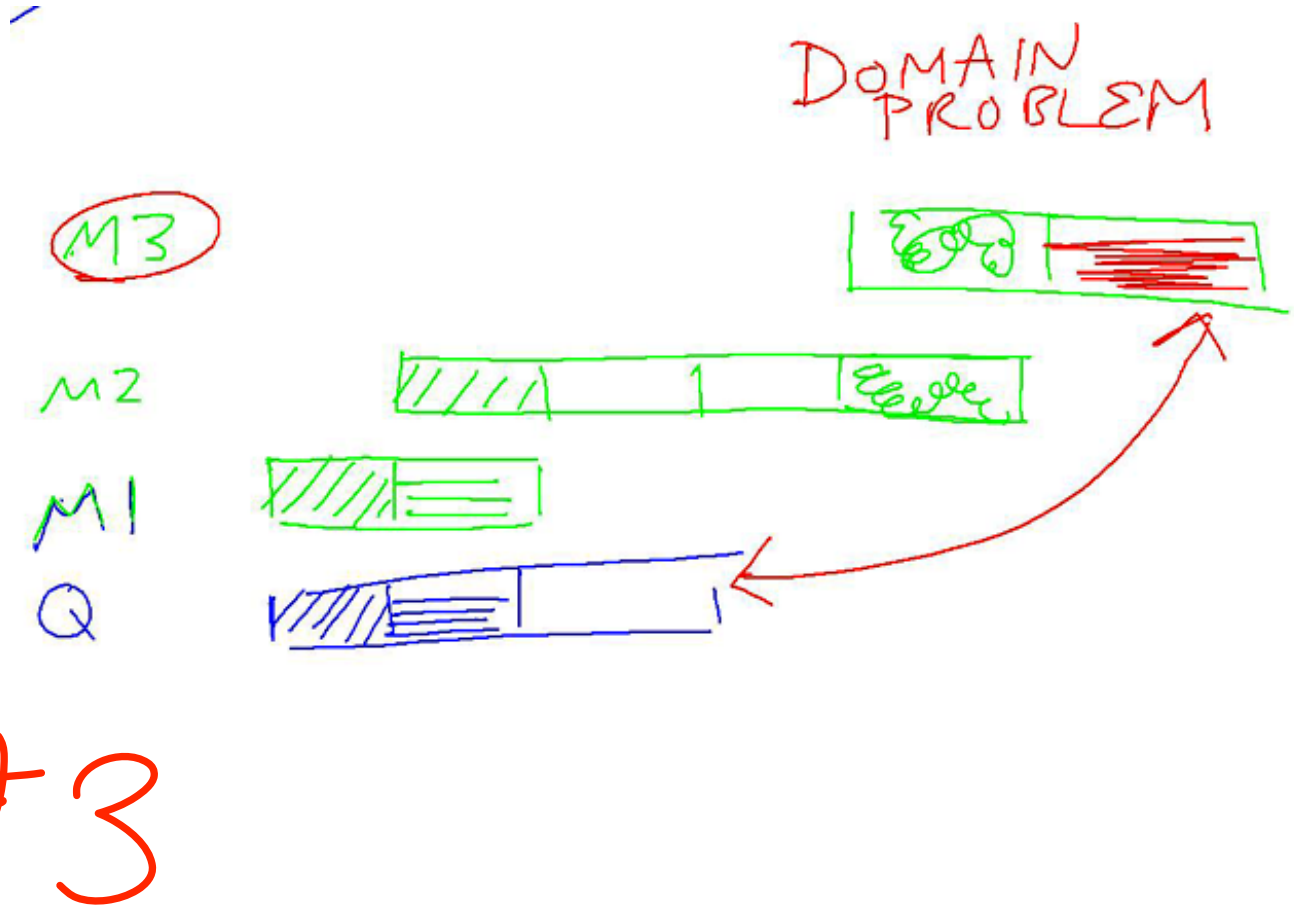
- It stems from greedy nature of alignment (mistakes made early in alignment cannot be corrected later)

- 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 Mult. Alignment



Fuse multiple alignment into:

- **Motif**: a short signature pattern identified in the conserved region of the multiple alignment

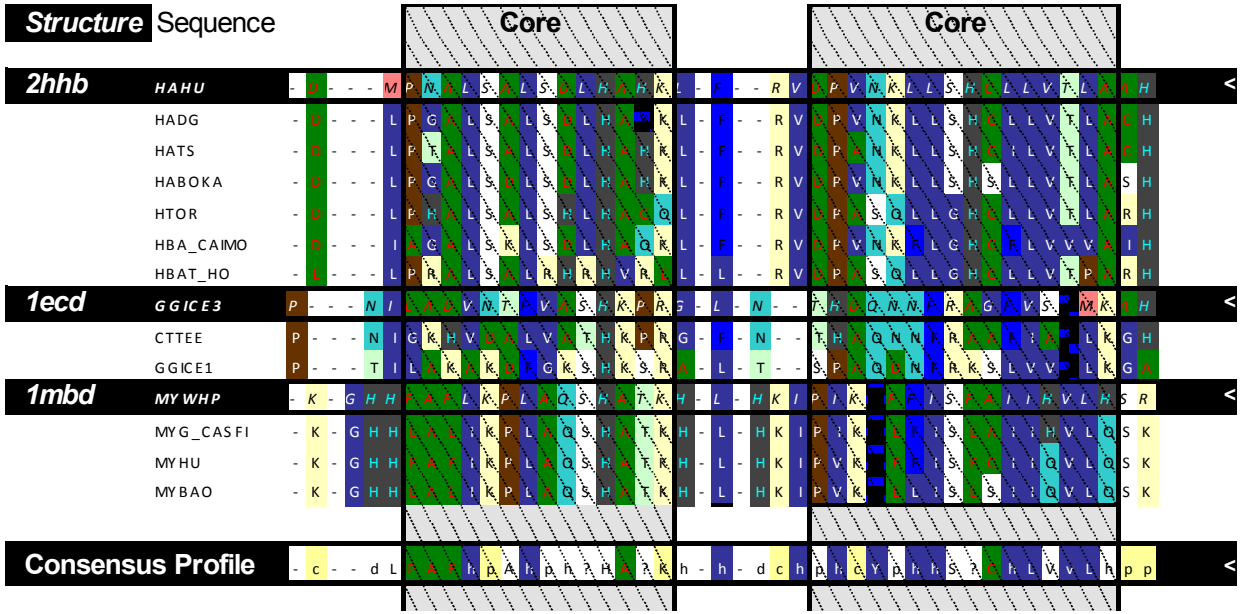
- **Profile**: frequency of each amino acid at each position is estimated

- **HMM**: Hidden Markov Model, a generalized profile in rigorous mathematical terms

Profiles
Motifs
HMMs

Cor

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



Multiple Alignment

motifs

Examples of when you would want to find motifs -- Finding TF-binding sequences

- ChIP-on-chip or ChIP-seq: Immunoprecipitate DNA-TF complexes, then either hybridize them to a microarray chip or sequence them.
- List promoter regions of co-regulated genes.
- SELEX: Systematic Evolution of Ligands by Exponential Enrichment (or in vitro selection). A library of random oligonucleotides are bound to a purified protein, then the bound ones are identified.

[Adapted from C Bruce, CBB752 '09]

Two problems in motif analysis

- Given a collection of binding sites, 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]

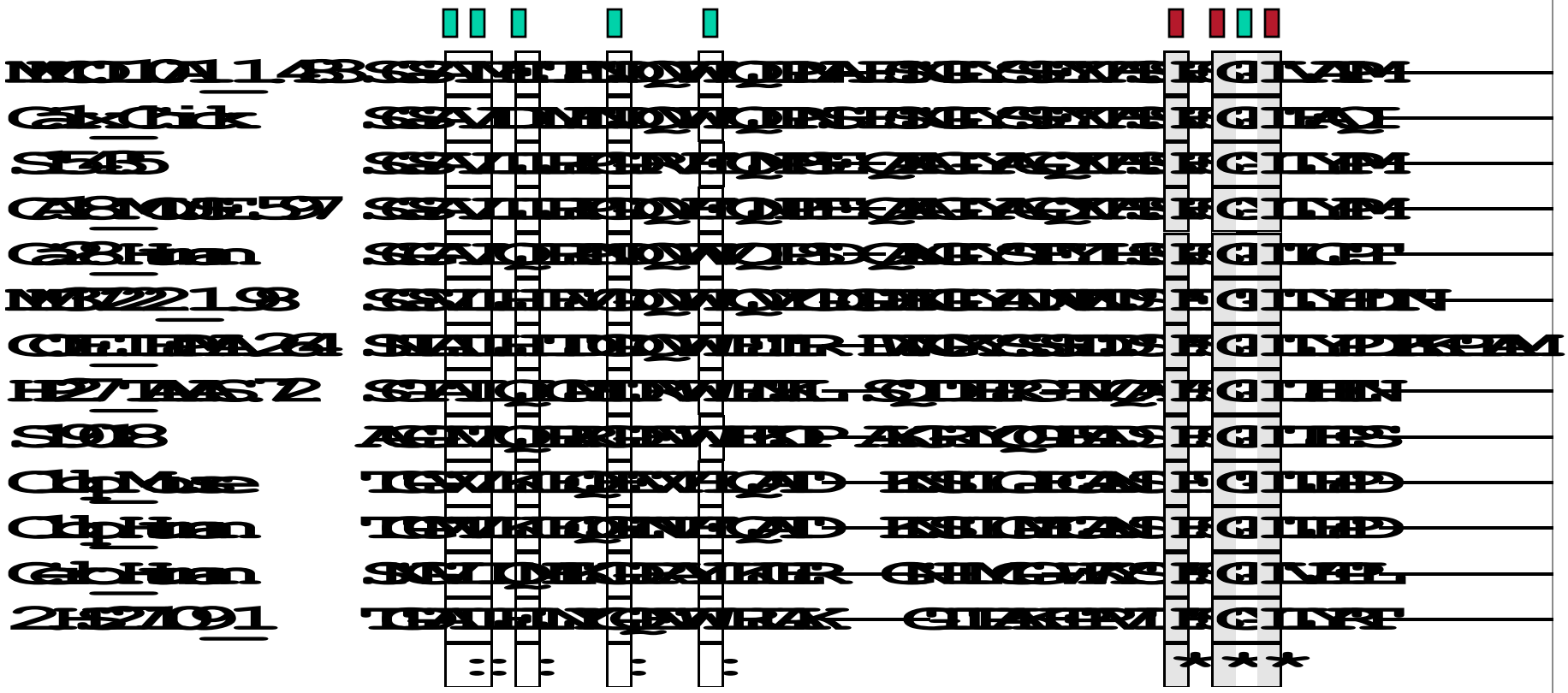
Two classes of motif discovery algorithms

- Multiple alignment methods.
  - Return PWM; use local search techniques such as Gibbs sampling or EM
- Deterministic combinatorial algorithms based on word frequency counts.
  - Search for various sized sequences exhaustively and evaluate significance.

[Adapted from C Bruce, CBB752 '09]

Motifs

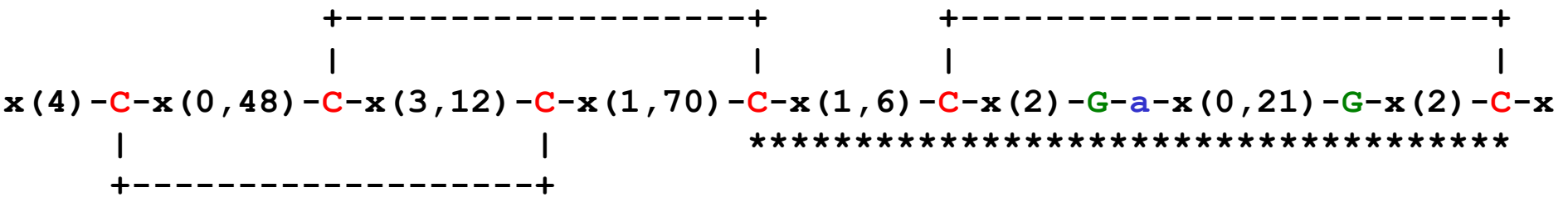
- several proteins are grouped together by similarity searches
- they share a conserved motif
- motif is stringent enough to retrieve the family members from the complete protein database
- PROSITE: a collection of motifs (1135 different motifs)



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).



'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

Motifs

· Each element in a pattern is separated from its neighbor by a "-".
· The symbol "x" is used for a position where any amino acid is accepted.
· Ambiguities are indicated by listing the acceptable amino acids for a given position, between brackets "[]".
· Ambiguities are also indicated by listing between a pair of braces "{}" the amino acids that are not accepted at a given position.
· Repetition of an element of the pattern is indicated by with a numerical value or a numerical range between parentheses following that element.

PKC_PHOSPHO_SITE	Protein kinase C phosphorylation site	[ST]-x-[RK]	Post-translational modifications
RGD	Cell attachment sequence	R-G-D	Domains
SOD_CU_ZN_1	Copper/Zinc superoxide dismutase	[GA]-[IMFAT]-H-[LIVF]-H-x(2)-[GP]-[SDG]-x-[STAGDE]	Enzymes_Oxidoreductases
THIOL_PROTEASE_ASN	Eukaryotic thiol (cysteine) proteases active site	[FYCH]-[WI]-[LIVT]-x-[KRQAG]-N-[ST]-W-x(3)-[FYW]-G-x(2)-G-[LFYW]-[LIVMFYG]-x-[LIVMF]	Enzymes_Hydrolases
TNFR_NGFR_1	TNFR/CD27/30/40/95 cysteine-rich region	C-x(4,6)-[FYH]-x(5,10)-C-x(0,2)-C-x(2,3)-C-x(7,11)-C-x(4,6)-[DNEQSKP]-x(2)-C	Receptors

Enumerative techniques

- dictionary-based methods count the number of occurrences of all n-mers in the target sequences, and calculate which ones are most overrepresented.
- a number of similar overrepresented words may be combined into a more flexible motif description.
- Alternatively, one can search the space of all degenerate consensus sequences up to a given length, for example, using IUPAC codes for 2-nucleotide or 3-nucleotide degenerate positions in the motif
- WEEDER describes a motif as a consensus sequence and an allowed number of mismatches, and uses an efficient suffix tree representation to find all such motifs in the target sequences

IUPAC Code	Meaning
G	G
A	A
T	T
C	C
R	G or A
Y	T or C
M	A or C
K	G or T
S	G or C
W	A or T
H	A or C or T
B	G or T or C
V	G or C or A
D	G or A or T
N	G or A or T or C

[Adapted from C Bruce, CBB752 '09]

Consensus-based methods

- Enumerate all the oligos of (or up to) a given length, in order to determine which ones appear, with possible substitutions, in a significant fraction of the input sequences, and finally to rank them according to statistical measure of significance.
- Drawbacks:
  - For motif length of m, there are 4^m candidates to enumerate. $O(4^m)$ execution time.
  - Too slow.
- Motif search can be accelerated by pre-processing the data in an indexing structure, such as a suffix tree.

[Adapted from C Bruce, CBB752 '09]

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

š = (A,2V,C,P); µ=(4K,2Q,3E,2D)

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	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

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
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
Eisenberg Profile Freq. V	0	5	0	2	3	0	0	0
Eisenberg Profile Freq. Y	0	0	0	0	0	0	9	0

↑ Identity

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 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

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

Profiles formula for entropy H(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
	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	↑ Identity
Eisenberg Profile Freq. C	0	0	4	3	2	0	0	0	
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	
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	

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

Say column only has one aa (AAAAA):
$H(p,a) = 1 \log_2 1 + 0 \log_2 0 + 0 \log_2 0 + \ldots = 0 + 0 + 0 + \ldots = 0$

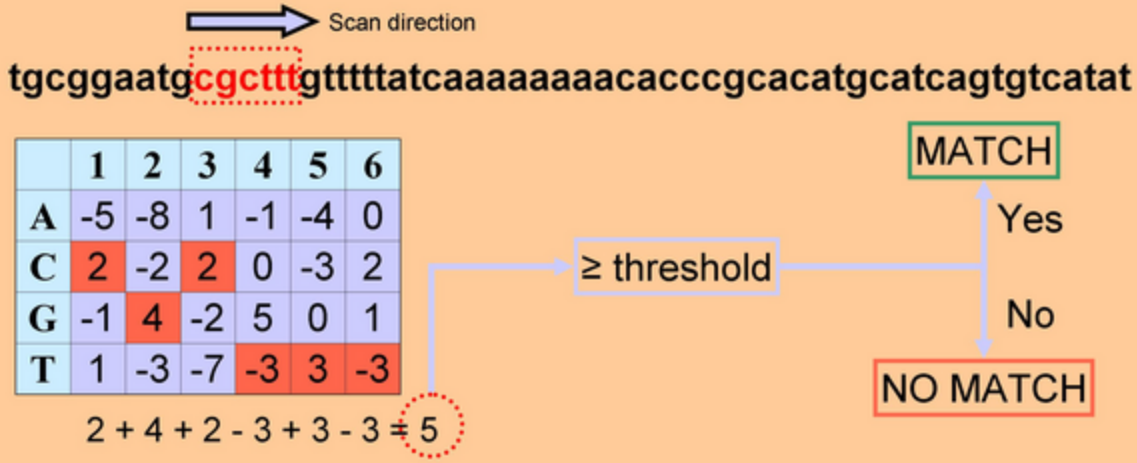
Say column is random with all aa equiprobable (ACD..ACD..ACD..):
$H_{rand}(p,a) = .05 \log_2 .05 + .05 \log_2 .05 + \ldots = -.22 + -.22 + \ldots = -4.3$

Say column is random with aa occurring according to probability found in the sequence databases (ACAAAADAADDDDAAAA….):
$H_{db}(a) = - \Sigma_{a=1 \text{ to } 20} F(a) \log_2 F(a)$,
where F(a) is freq. of occurrence of a in DB

$H_{corrected}(p,a) = H(p,a) – H_{db}(a)$

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:



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

Nucleic Acids Research, 1997, Vol. 25, No. 17 3389–3402

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

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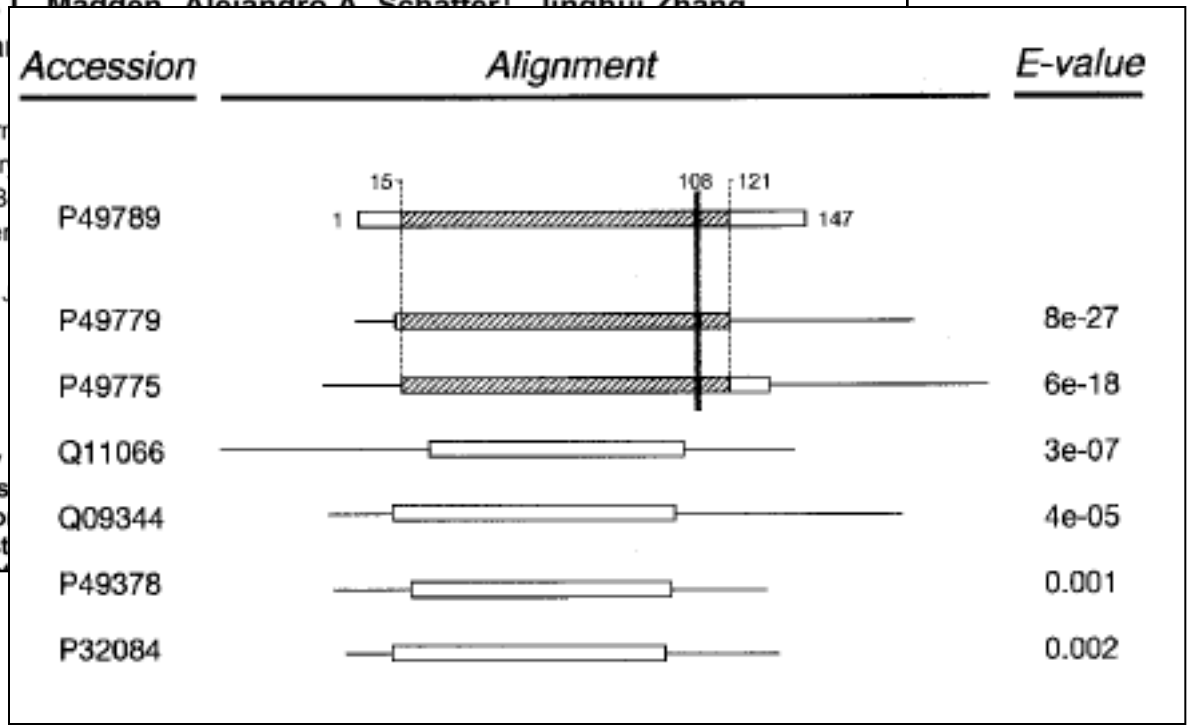
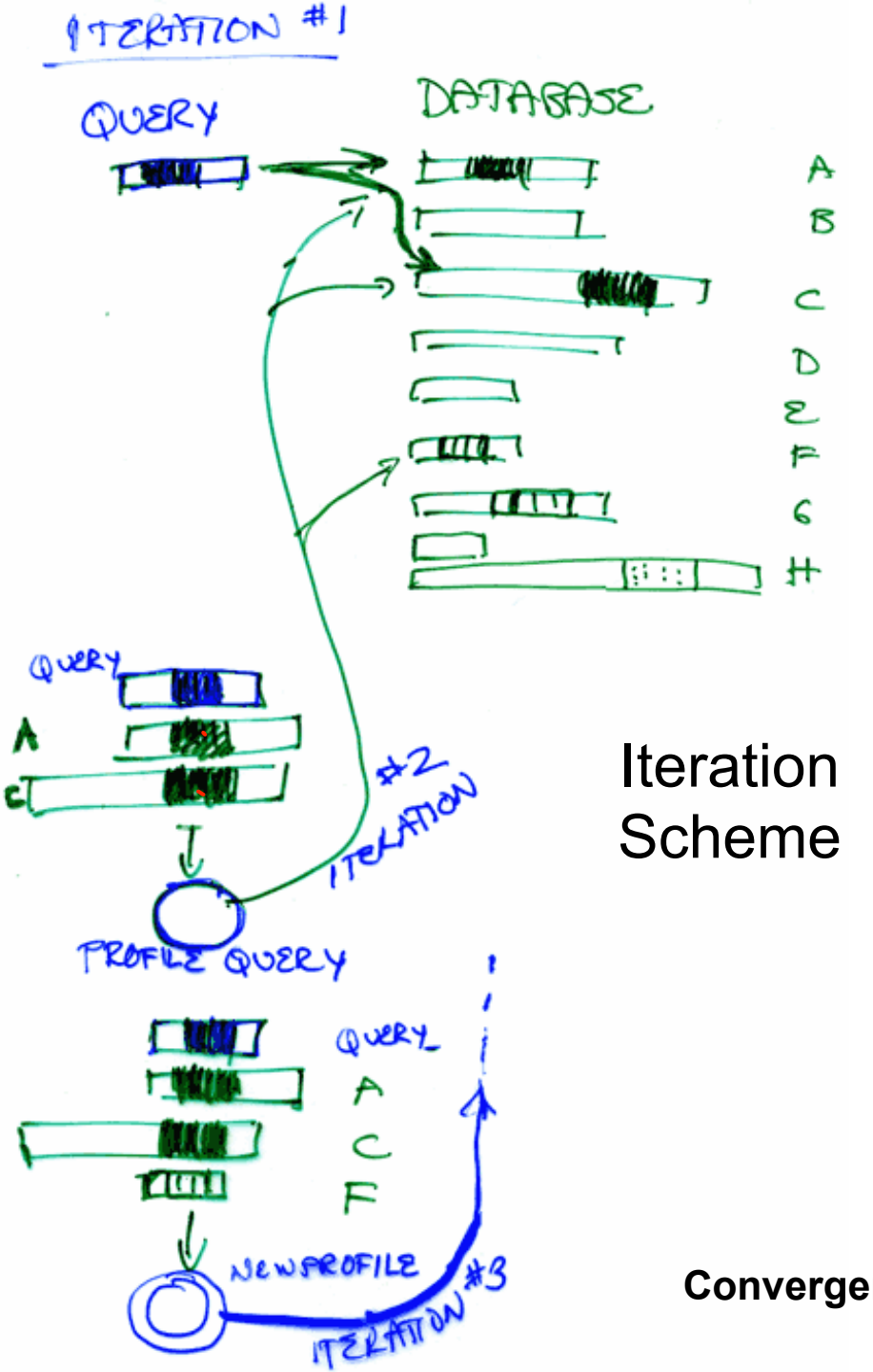
Received June 20, 1997; Revised and Accepted

ABSTRACT

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





PSI-Blast

Cor

Semi-supervised learning

Iteration Scheme

Sensitivity

Speed

Blast
FASTA
Smith-Waterman
PSI-Blast
Profiles
HMMs

Convergence vs explosion (polluted profiles)

Multiple Alignment

EM

Probabilistic Approaches

- 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.

[Adapted from C Bruce, CBB752 '09]

Alternating approach

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 & 3 until satisfied.

Examples: Gibbs sampler (Lawrence et al.)
MEME (expectation max. / Bailey, Elkan)
ANN-Spec (neural net / Workman, Stormo)

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

Expectation-maximization

EM

```
foreach subsequence of width W
  convert subsequence to a matrix
  do {
    re-estimate motif occurrences from matrix
    re-estimate matrix model from motif occurrences
  } until (matrix model stops changing)
end
select matrix with highest score
```

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

Sample DNA sequences

```
>ce1cg
TAATGTTTGTGCTGGTTTTTGTGGCATCGGGCGAGAATA
GCGCGTGGTGTGAAAGACTGTTTTTTTGATCGTTTTCAC
AAAAATGGAAGTCCACAGTCTTGACAG
>ara
GACAAAAACGCGTAACAAAAGTGTCTATAATCACGGCAG
AAAAGTCCACATTGATTATTTGCACGGCGTCACACTTTG
CTATGCCATAGCATTTTTATCCATAAG
>bglr1
ACAAATCCCAATAACTTAATTATTGGGATTTGTTATATA
TAACTTTATAAATTCCTAAAATTACACAAAGTTAATAAC
TGTGAGCATGGTCATATTTTTATCAAT
>crp
CACAAAGCGAAAGCTATGCTAAAACAGTCAGGATGCTAC
AGTAATACATTGATGTACTGCATGTATGCAAAGGACGTC
ACATTACCGTGCAGTACAGTTGATAGC
```

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

Motif occurrences

>ce1cg
taatgtttgtgctggtttttgtggcatcgggcgagaata
gcgcgtggtgtgaaagactgtttt**TTTGATCGTTTTCAC**
aaaaatggaagtccacagtcttgacag

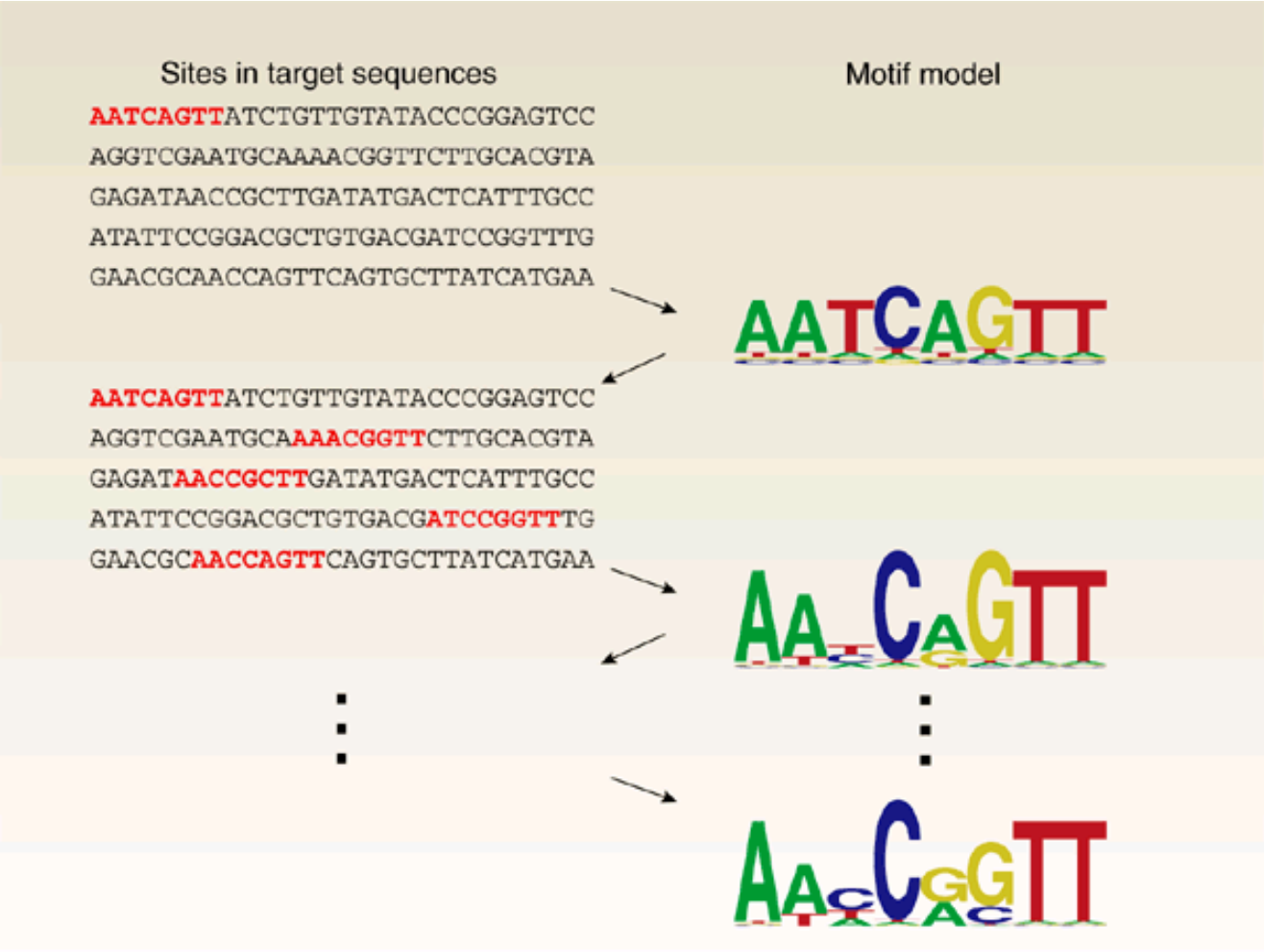
>ara
gacaaaaacgcgtaacaaaagtgtctataatcacggcag
aaaagtccacattgatta**TTTGCACGGCGTCAC**actttg
ctatgccatagcatttttatccataag

>bglr1
acaaatcccaataacttaattattgggatttgttatata
taactttataaattcctaaaattacacaaagttaataac
TGTGAGCATGGTCATatttttatcaat

>crp
cacaaagcgaaagctatgctaaaacagtcaggatgctac
agtaatacattgatgtactgcatgta**TGCAAAGGACGTC**
ACattaccgtgcagtacagttgatagc

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

How does EM algorithms work?



Starting from a single site, expectation maximization algorithms such as MEME[4] alternate between assigning sites to a motif (left) and updating the motif model (right). Note that only the best hit per sequence is shown here, although lesser hits in the same sequence can have an effect as well.

Specifically, in E step, estimate location of motif match. In M step, find most likely parameters of motif model given the locations.

MEME - a practical program using EM

- Subsequences which occur in the input DNA sequence are used as the starting points from which EM converges iteratively to locally optimal motifs. This increases the likelihood of finding globally optimal motifs.
- Multiple occurrences of a motif are allowed. Algorithm is allowed to ignore sequences with no appearance of a shared motif. So, more resistance to noisy data.
- Motifs are probabilistically erased after they are found, so more than one motif can be found.

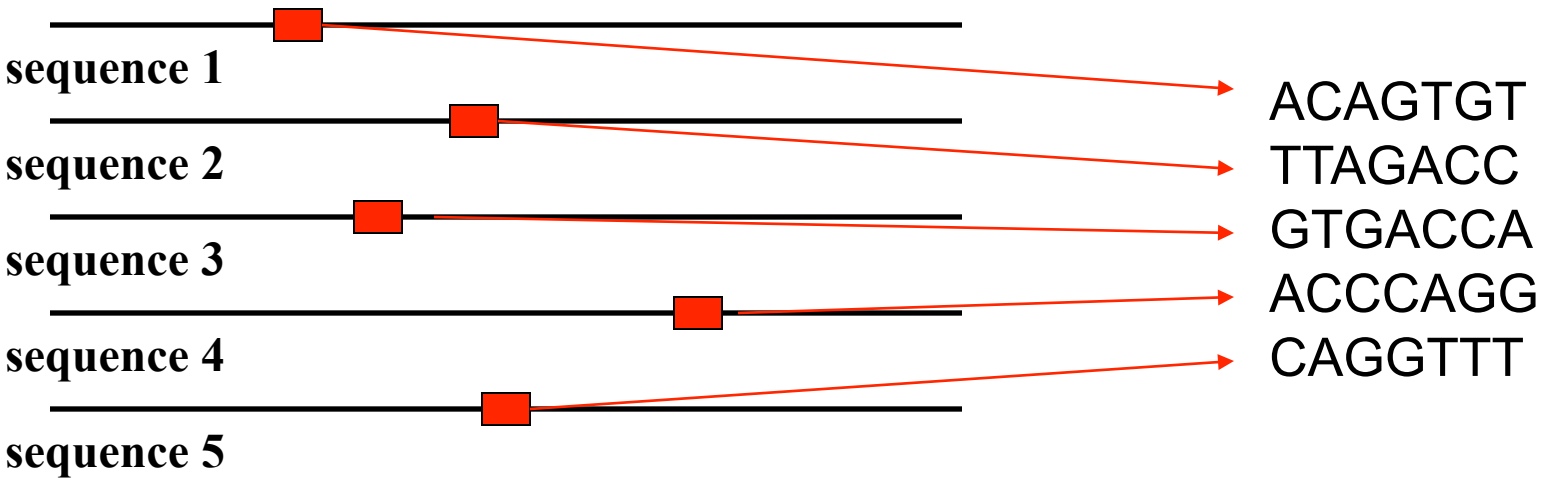
[Adapted from C Bruce, CBB752 '09]

Multiple Alignment

Gibbs Sampling

Initialization

- 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

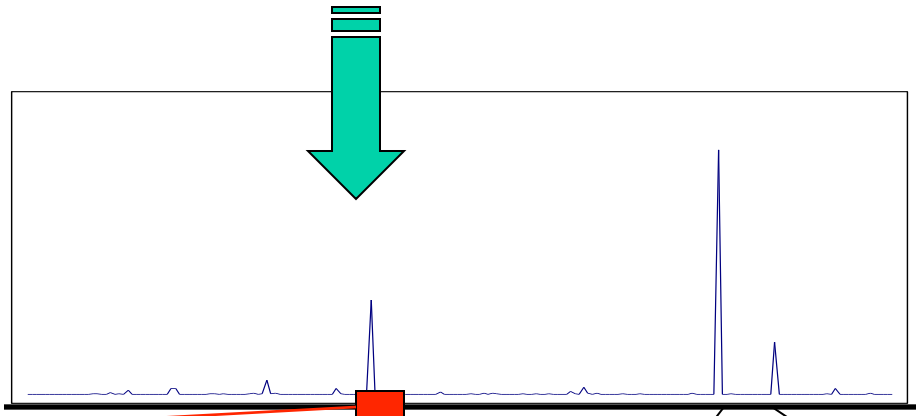
- Initially: randomly guess an instance s_i from each of t input sequences $\{S_1, ..., S_t\}$.
- Steps 2 & 3 (search):
  - Throw away an instance s_i: remaining $(t - 1)$ instances define weight matrix.
  - Weight matrix defines instance probability at each position of input string S_i
  - Pick new s_i according to probability distribution
- 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

[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/]

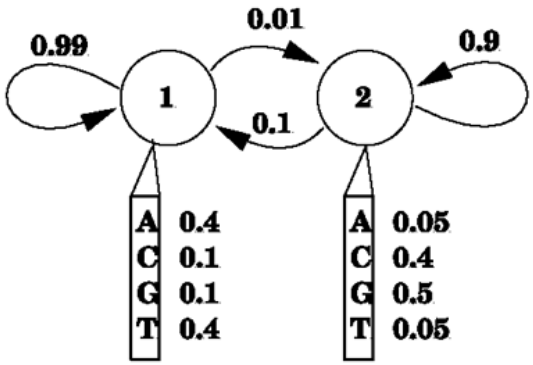
Multiple Alignment

HMMs

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):

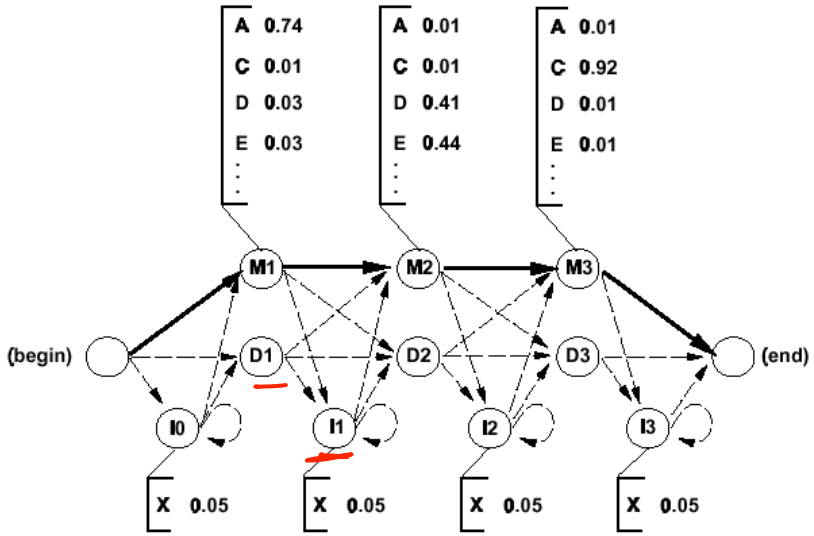
... 1 1 1 1 1 2 2 2 2 1 1 ...

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

symbol sequence (observable):

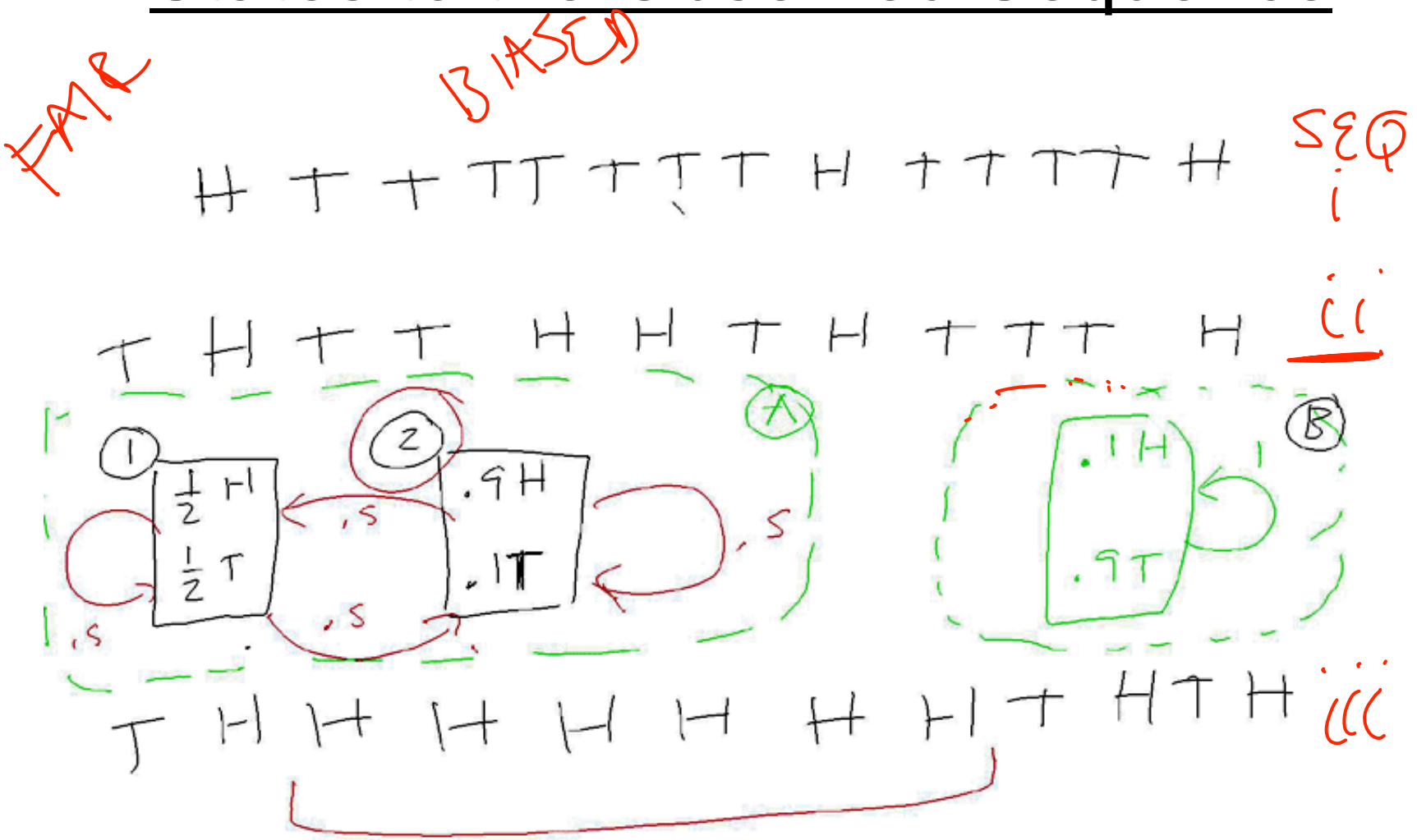
... A T C A A G G C G A T ...

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

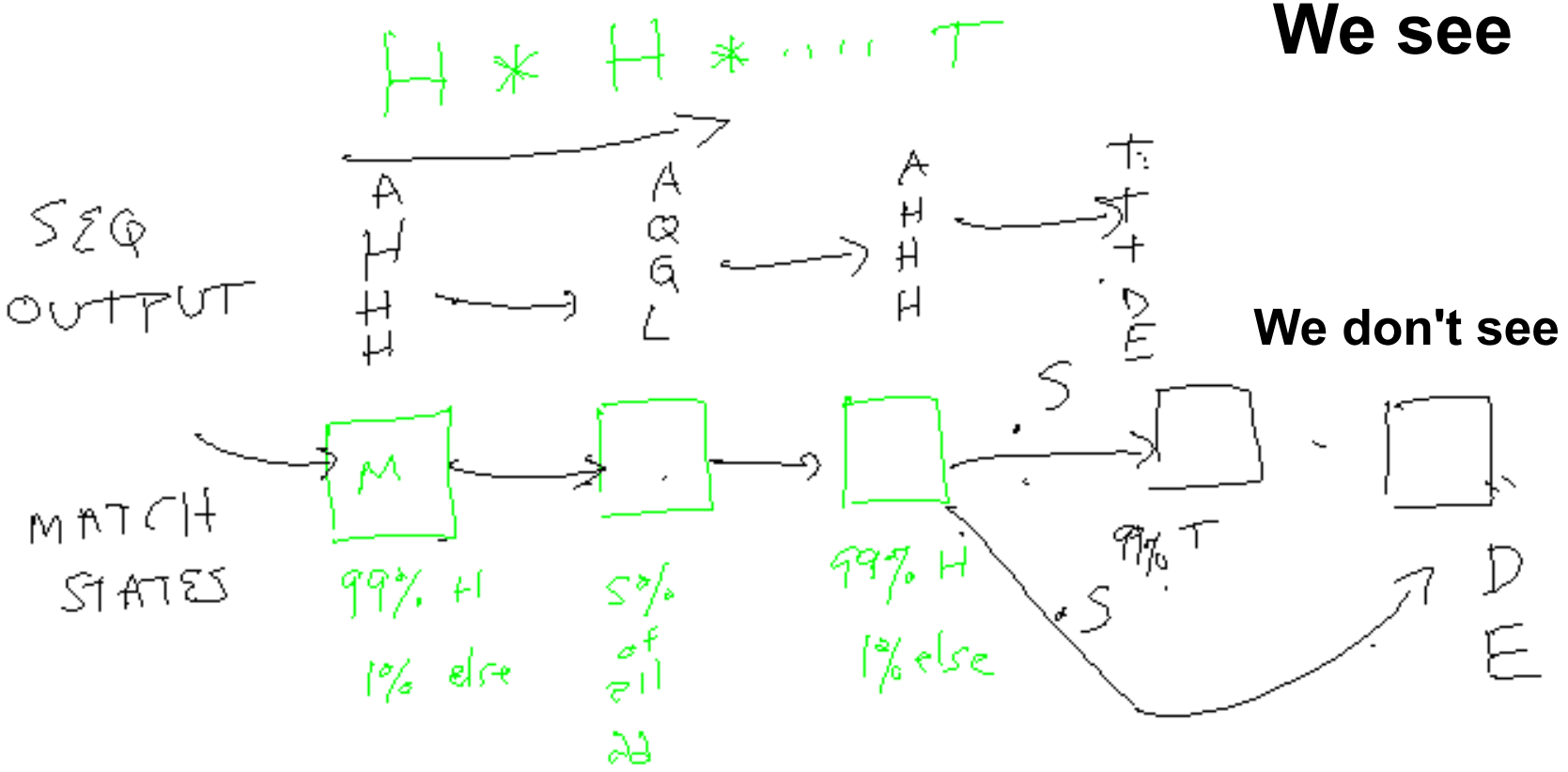


(Figures from Eddy, Curr. Opin. Struct. Biol.)

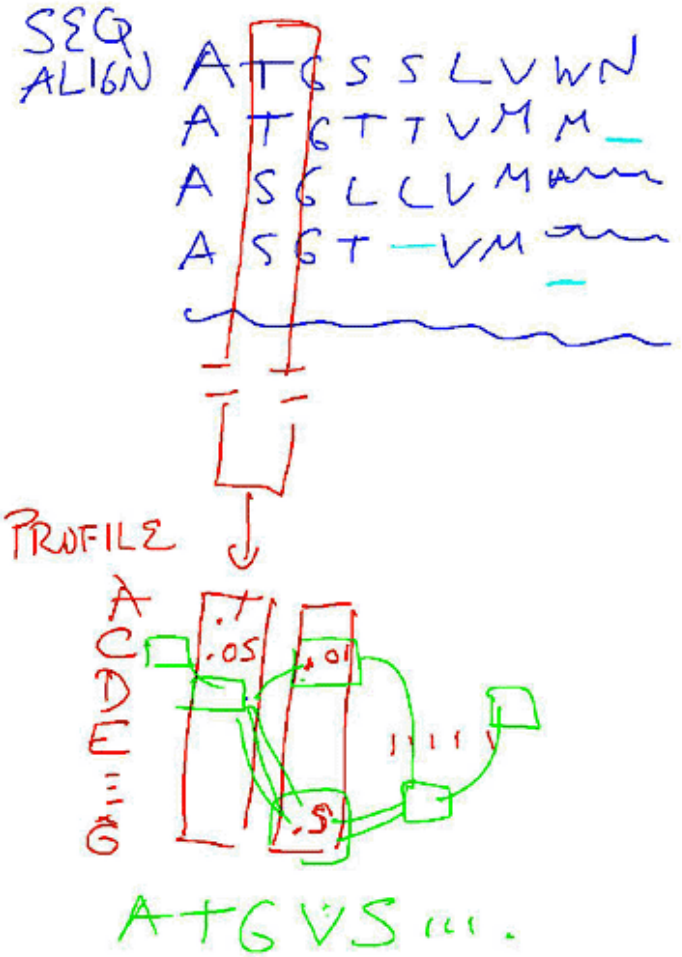
Relating Different Hidden Match States to the Observed Sequence

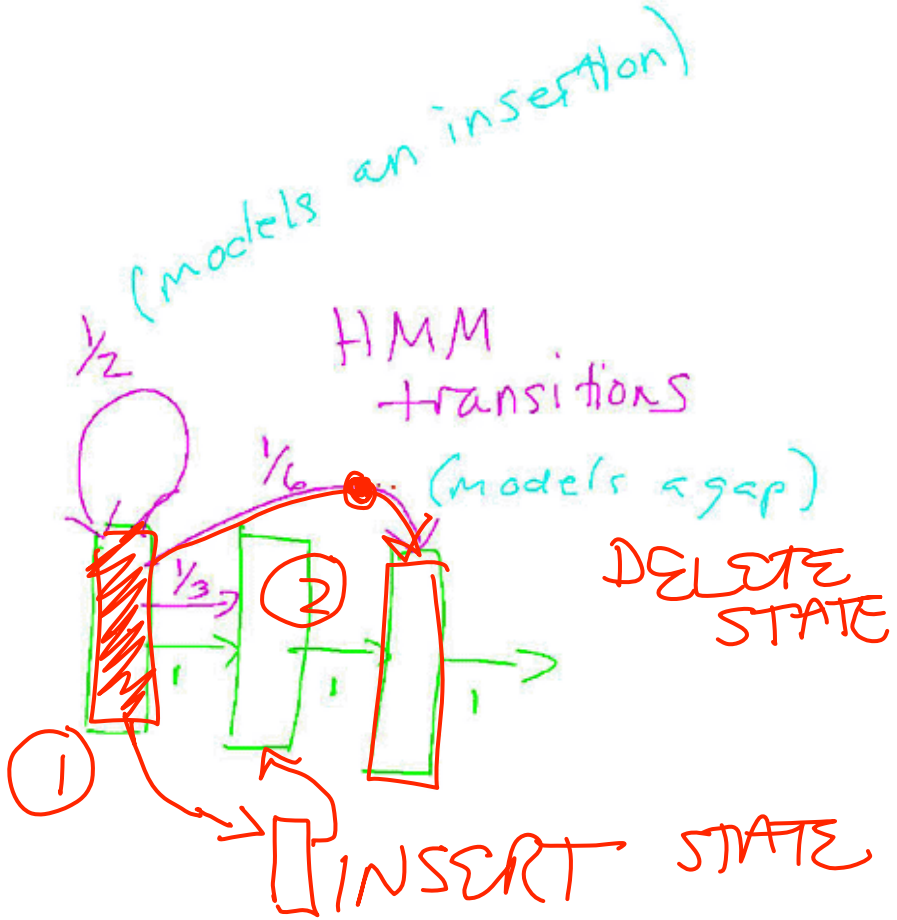


The Hidden Part of HMMs



Comparison of HMMs to Profiles

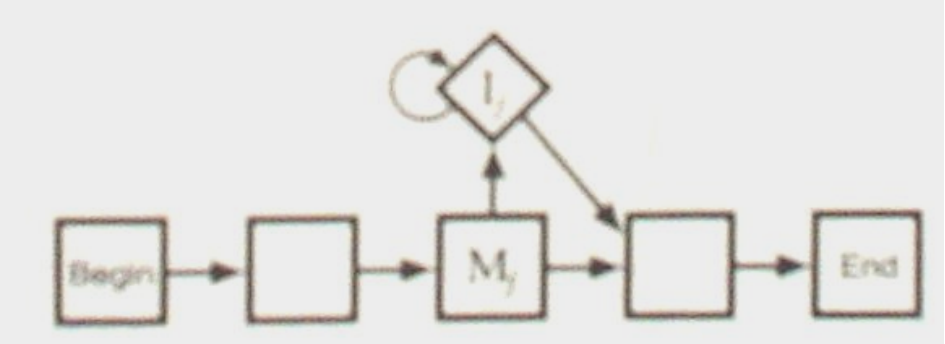




Sequence profile elements

- Insertions:

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



Algorithms

Probability of a path through the model

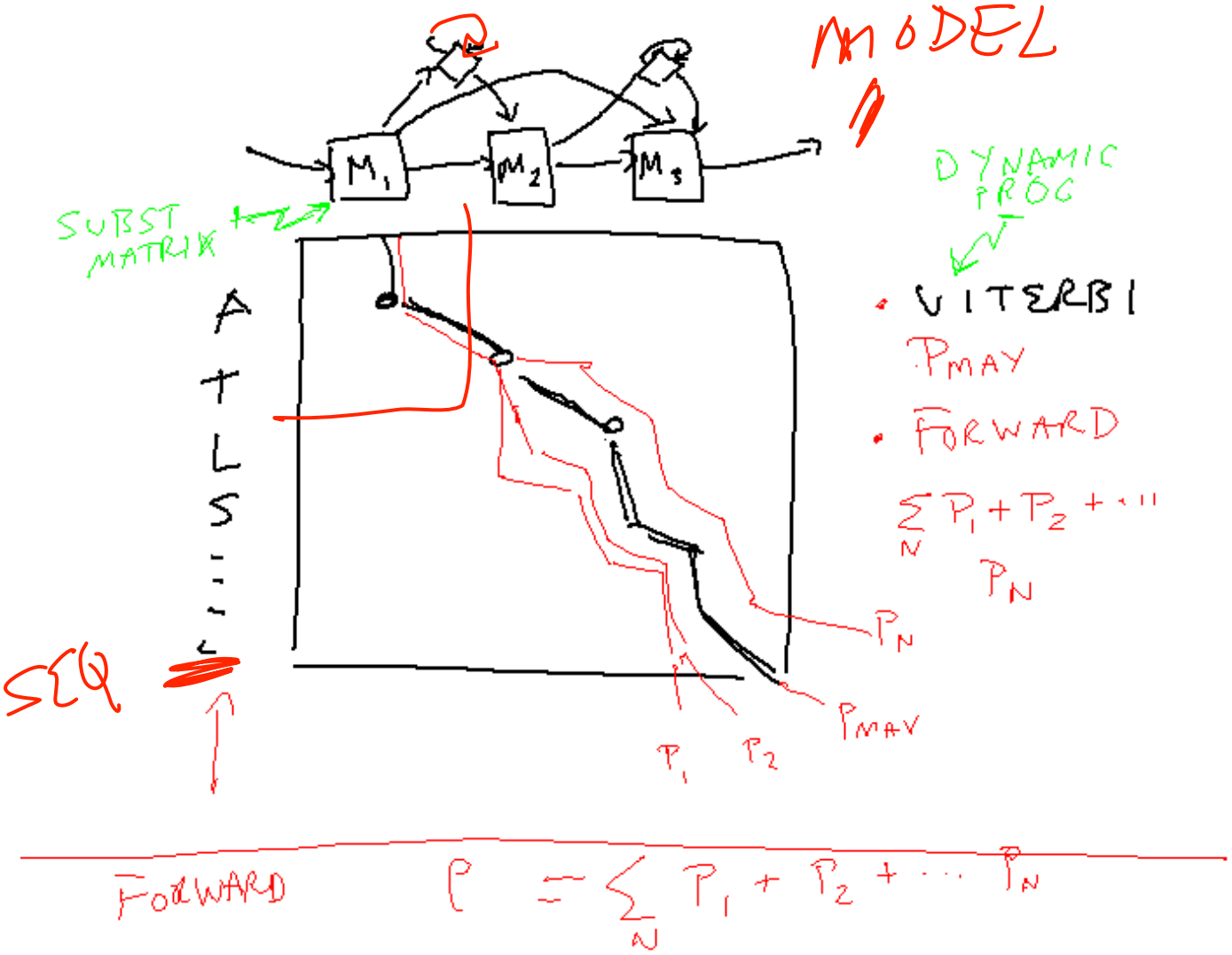
Viterbi maximizes for seq

Forward sums of all possible paths

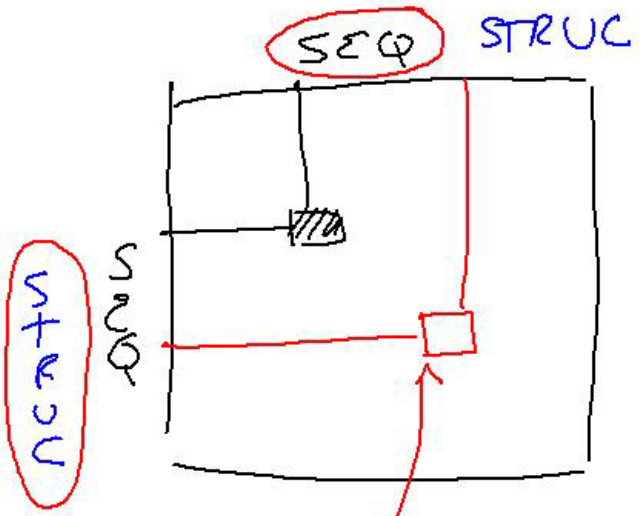
Forward Algorithm – finds probability P that a model λ emits a given sequence O by summing over all paths that emit the sequence the probability of that path

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)

HMM algorithms similar to those in sequence alignment



Seq. Alignment, Struc. Alignment, Threading



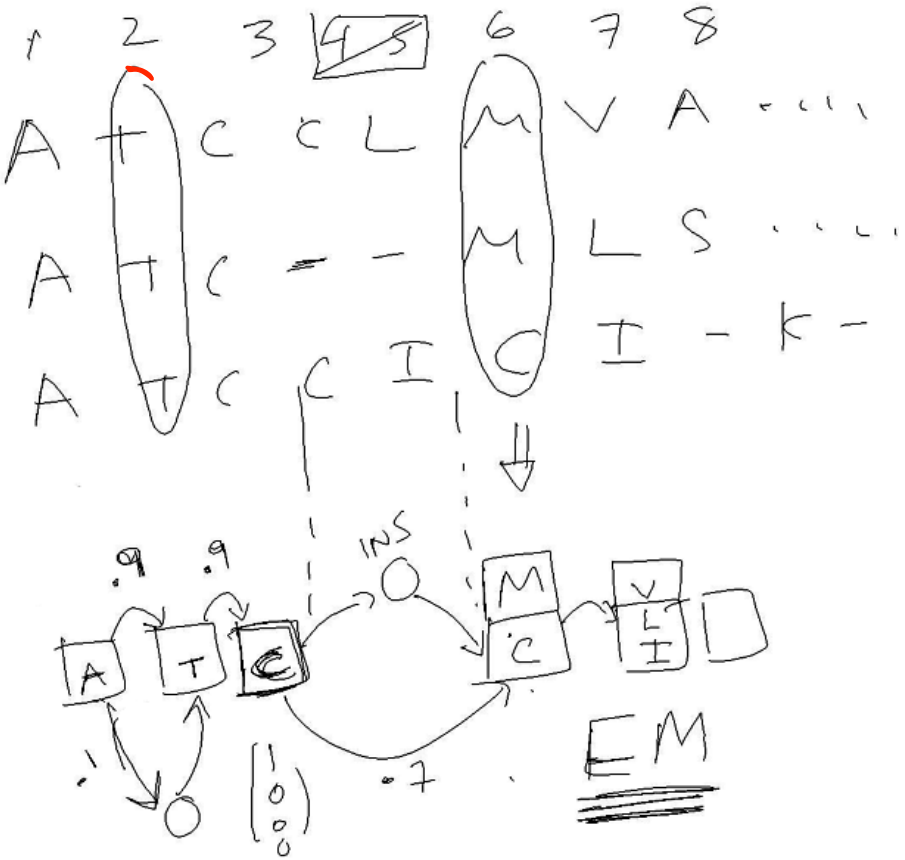
▨ = SEQ IDENTITY FOR SEQ ALIGNMENT

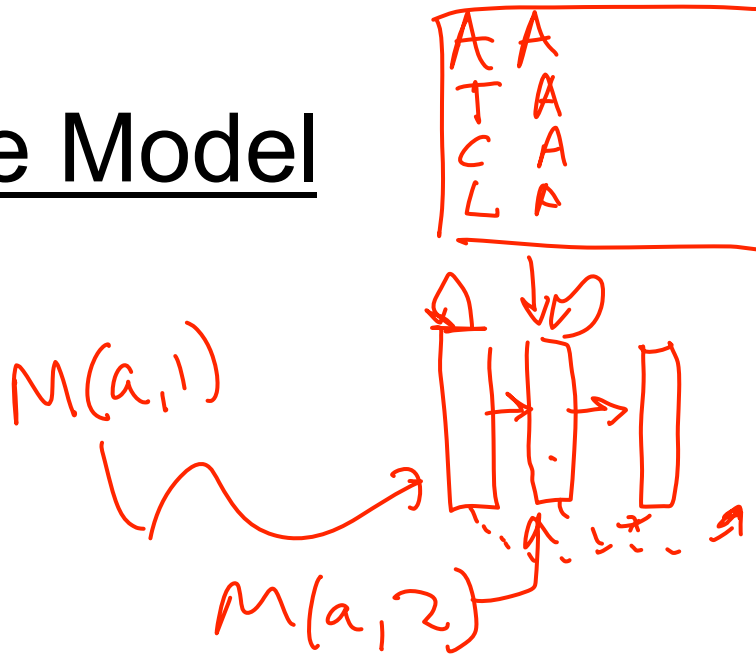
= STRUC COORD SIM. FOR STRUC ALIGNMENT

= MATCH OF SEQ TO 3D STRUC FOR THREADING

DEGREE THAT RES i IN TOP SEQ MATCHES STRUC ENVIRON. OF J IN LEFT STRUC

Building the Model

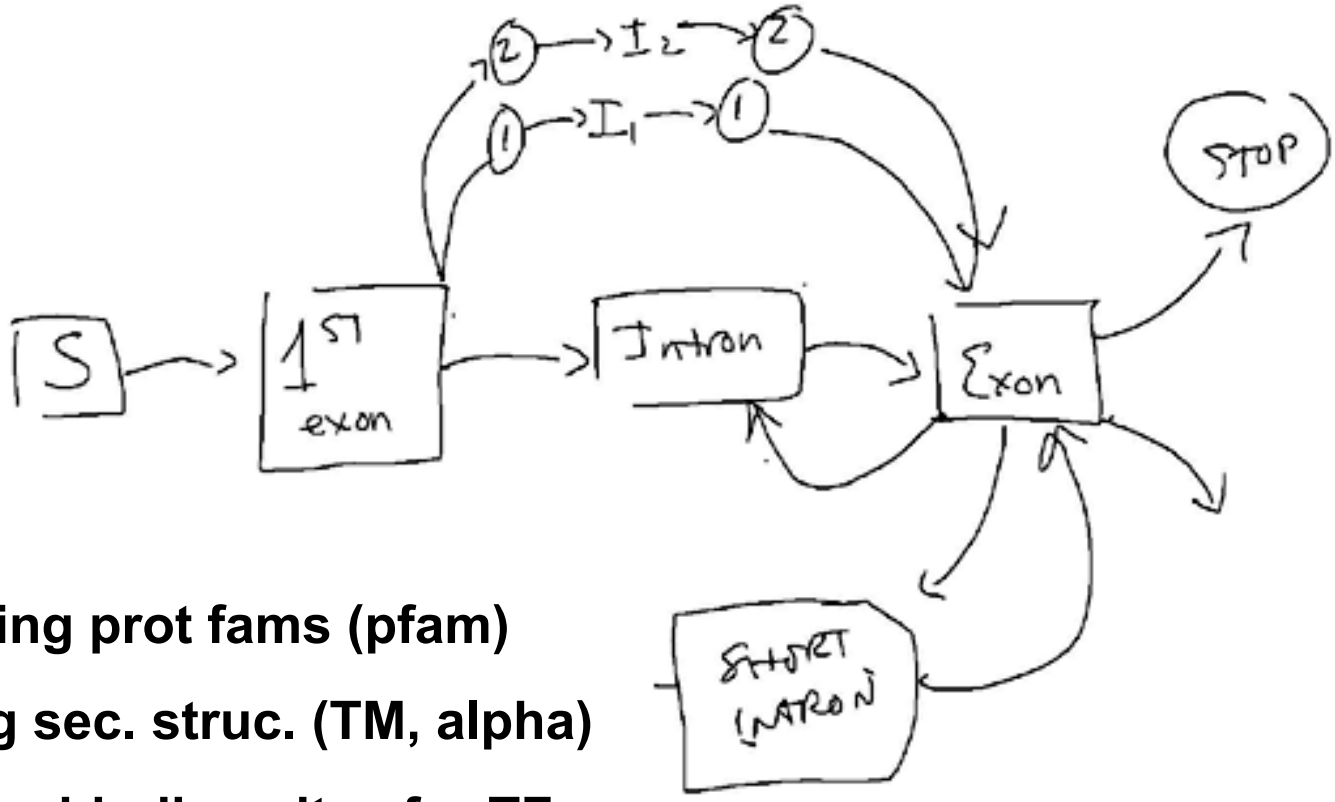




EM - expectation maximization

"roll your own" model -- dialing in probabilities

Applications of HMMs (Gene Finding)



Matching prot fams (pfam)

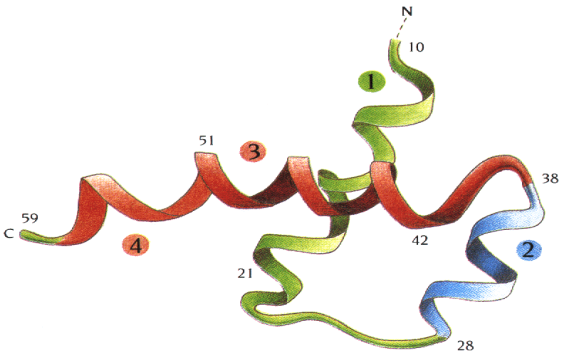
Predicting sec. struc. (TM, alpha)

Modelling binding sites for TF

(speech recognition)

Modules

HMMs, Profiles, Motifs, and Multiple Alignments used to define modules



•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

(Figures from Branden & Tooze)

CDD

INTERPRO, Pfam, SMART

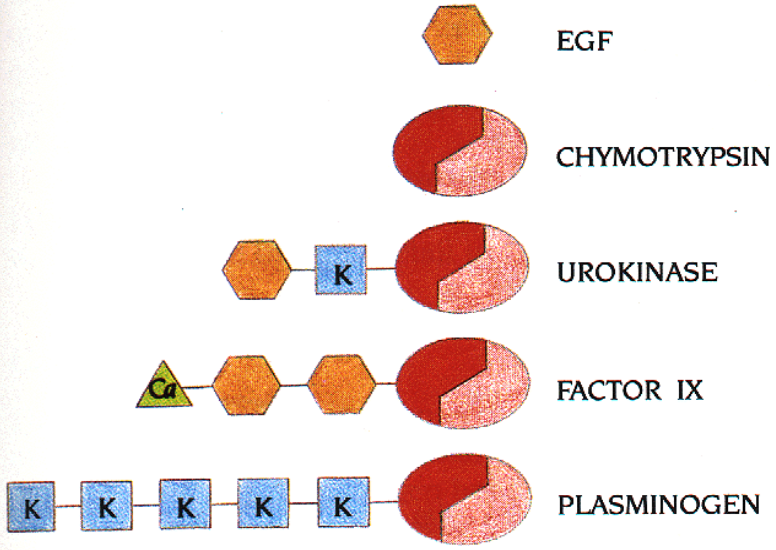


Figure 2.19 Organization of polypeptide chains into domains. Small protein molecules like the epidermal growth factor, EGF, comprise only one domain. Others like the serine proteinase chymotrypsin are arranged in two domains that are both required to form a functional unit (Chapter 15). Many of the proteins 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;

K Kringle domains that have a characteristic pattern of three internal disulphide bridges within a region of about 85 amino acid residues;

Ca 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.)

Multiple Alignment

Positions Independent

Independence of bases within motif

- Limitation of position weight matrix is the assumption that the positions in the site contribute additively to the total binding activity.
- Statistical methods (e.g. neural networks) used to identify which pairs of sites are dependent on each other.

[Adapted from C Bruce, CBB752 '09]

Correlated bases

(a)

(b)

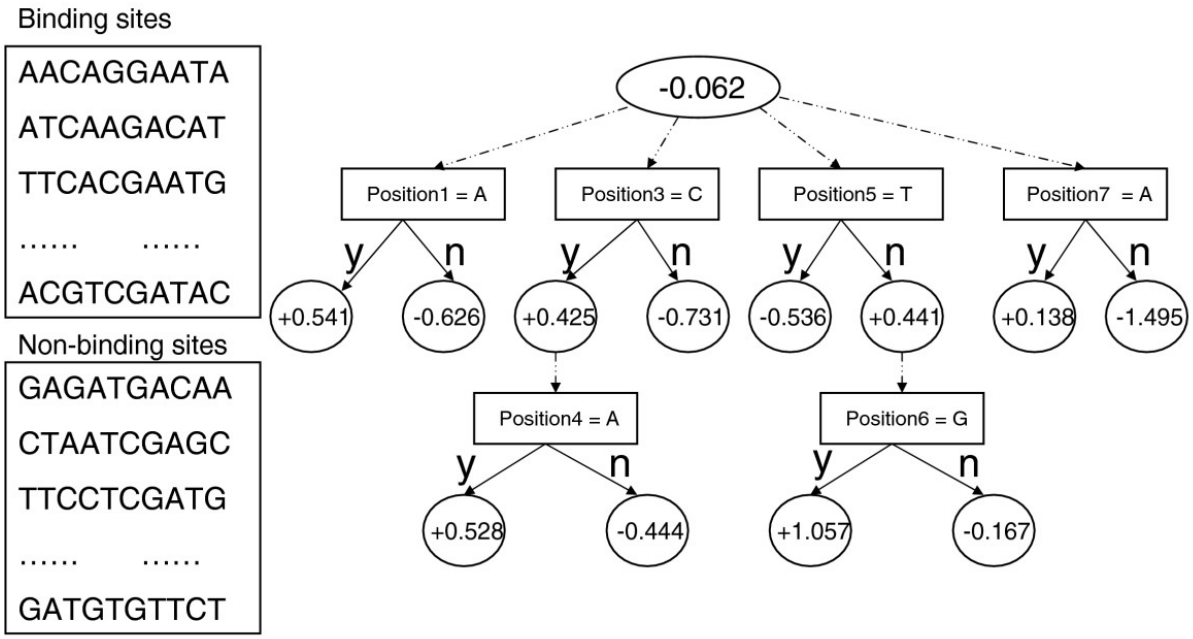
1\2	C	G	T
A		0.19	
C		0.16	0.06
G	0.09		
T	0.44	0.06	

Fig. 2. (**a**) Sequence logo plot for the E2F sites predicted by the GMS-MP. The traditional consensus for the E2F motif is the one from positions 2 to 10. (**b**) The joint distribution of the position pair (1, 2), which has been found to be significantly correlated by the GMS-MP.

[Adapted from C Bruce, CBB752 '09]

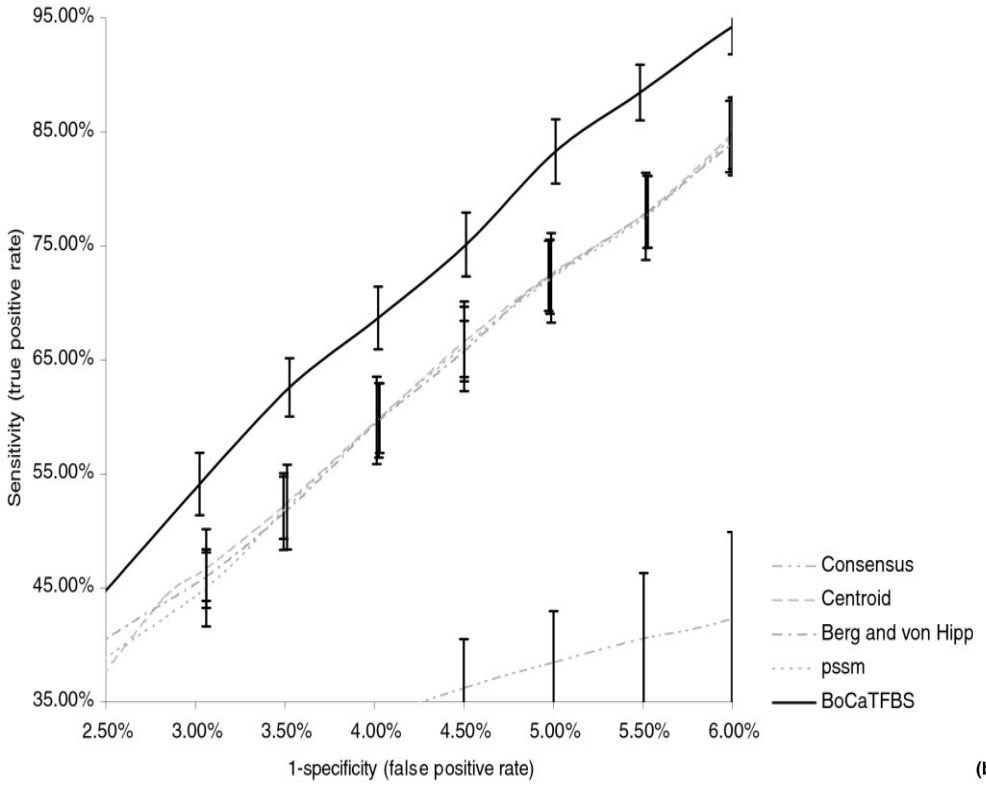
Using Binding Site Regions Found by ChIP-chip to refine motifs: BoCaTFBS

- Traditional motif learners (e.g. consensus sequences, profile methods, and HMMs) only use positive information
- ChIP-chip & Chip-seq give vast amount of negative information (regions not bound)
- Explicitly use this in constructing classifier that **refines** known positive motif seeds
- Use sequence of **Alternating Decision Trees (ADTboost),** which allow explicit inter-positional correlations between nucleotide positions

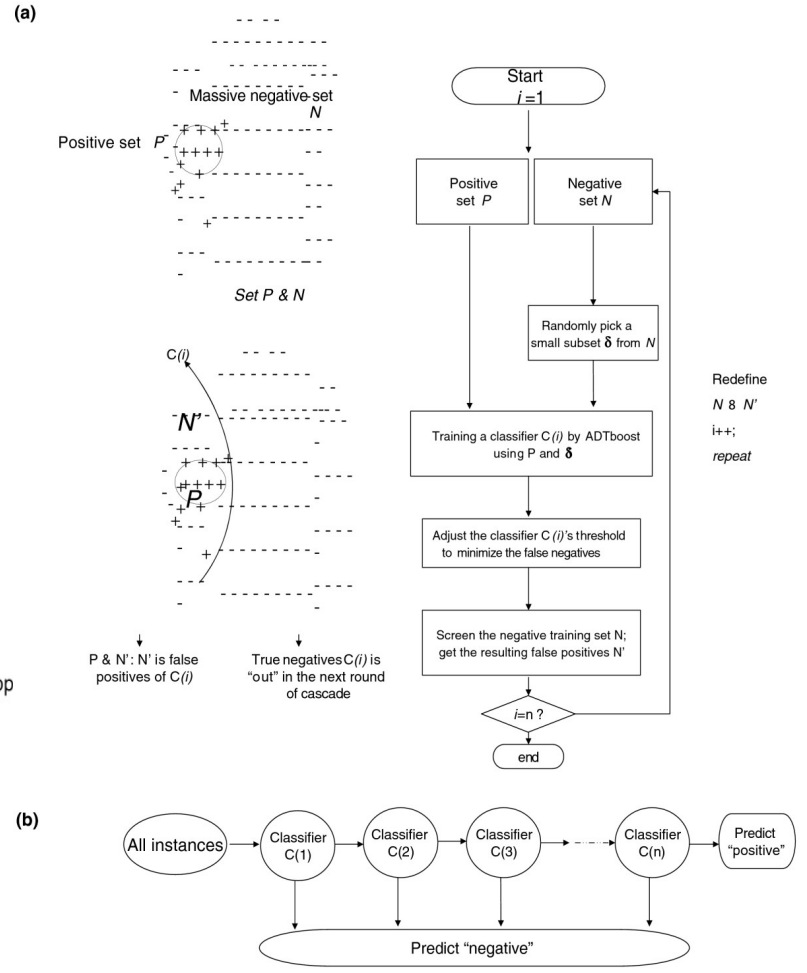


[Wang et al., GenomeBiology, '06]

Good performance compared to traditional motif-finders but large negative set requires training and detection cascade for efficiency and balance



[Wang et al., GenomeBiology, '06]



Multiple Alignment Topics

- Multiple Alignment
- Motifs
  - ◊ Fast identification methods
- Profile Patterns
  - ◊ Refinement via EM
  - ◊ Gibbs Sampling
- HMMs
- Applications
  - ◊ Module DBs
  - ◊ Regression vs expression
- Issues: site independence
  - ◊ BoCaTFBS