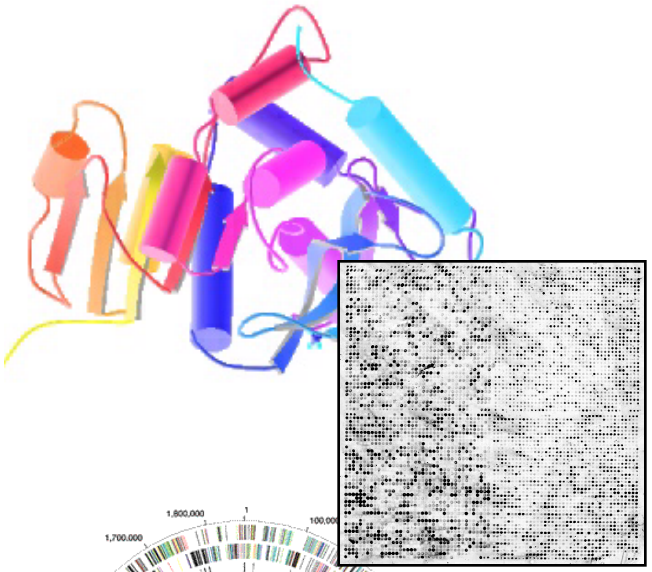
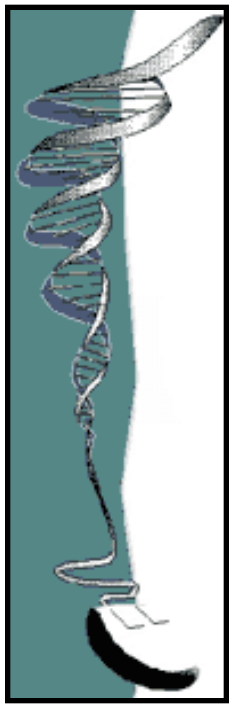
BIOINFORMATICS
Multiple Sequences





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(MG lect. #3, last edit in spring '17)

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

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AGRI_CHICK   154 CVCPAS.........CS....GVa.ESIVCGSDGKDYRSECDLNKHAC.....DK.......QENVFKKFDGAC 201
AGRI_RAT     165 CLCPTT.........CF....GAp.DGTVCGSDGVDYESECQLLSHAC.....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.WDPVCGLNGVAYMSACLA...GCKKFV.GT.......GTNM.VFQDCSC 486
PE60_PIG      37 CEHMTESPD......CS....RI..YDPVCGTDGVTYESECKL....CLARI.EN.......KQDIQIVKDGEC 86
PGT_RAT      444 CRRDCS.........CP....DSf.FHPVCGLNGVEYVSPCHA...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
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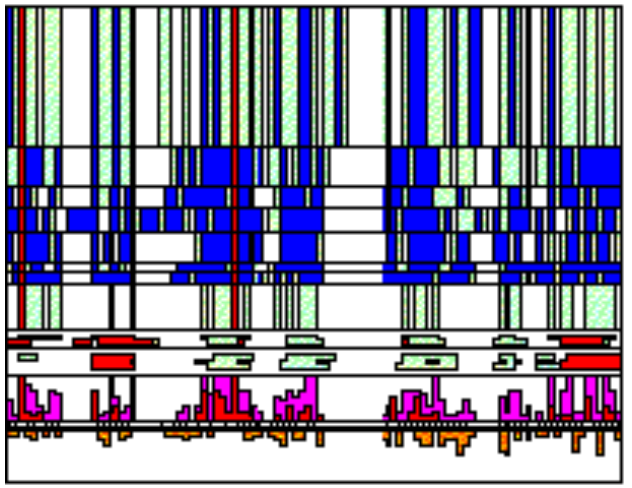
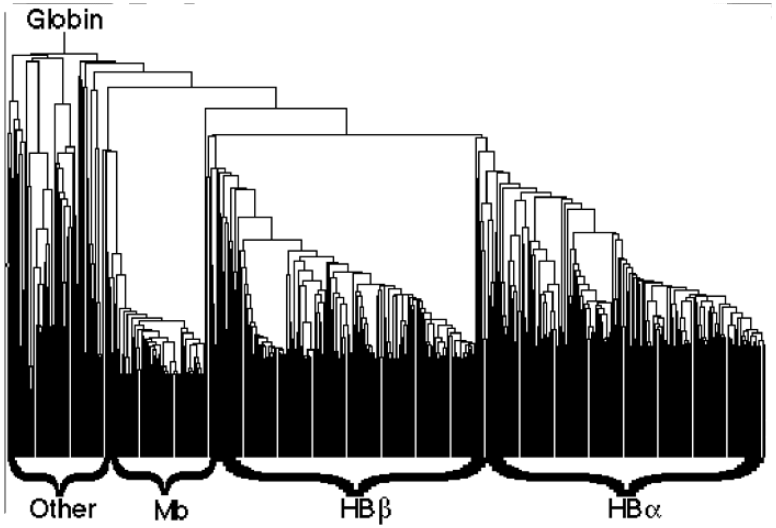


(LEFT, adapted from Sonhammer et al. (1997). "Pfam," Proteins 28:405-20. ABOVE, G Barton AMAS web page)

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

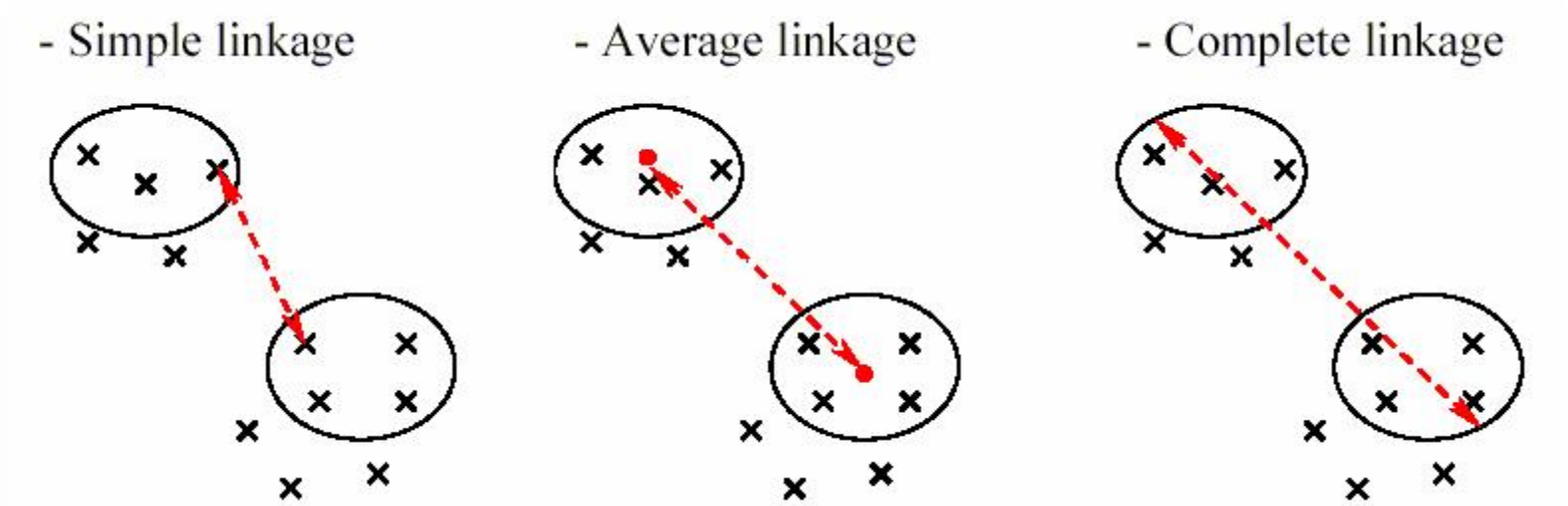


(adapted from Sonhammer et al. (1997). "Pfam," Proteins 28:405-20)

Clustering approaches for multiple sequence alignment

- Clustal uses average linkage clustering
  - ◊ also called UPGMA

    Unweighted Pair Group Method with Arithmetic mean



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

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

C1Q - Example

```
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-------
                     .  :: :   : . :                    * *:*.
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Clustal Alignment

Problems with Progressive Alignments

- Local Minimum Problem
  - Parameter Choice Problem

1. Local Minimum Problem

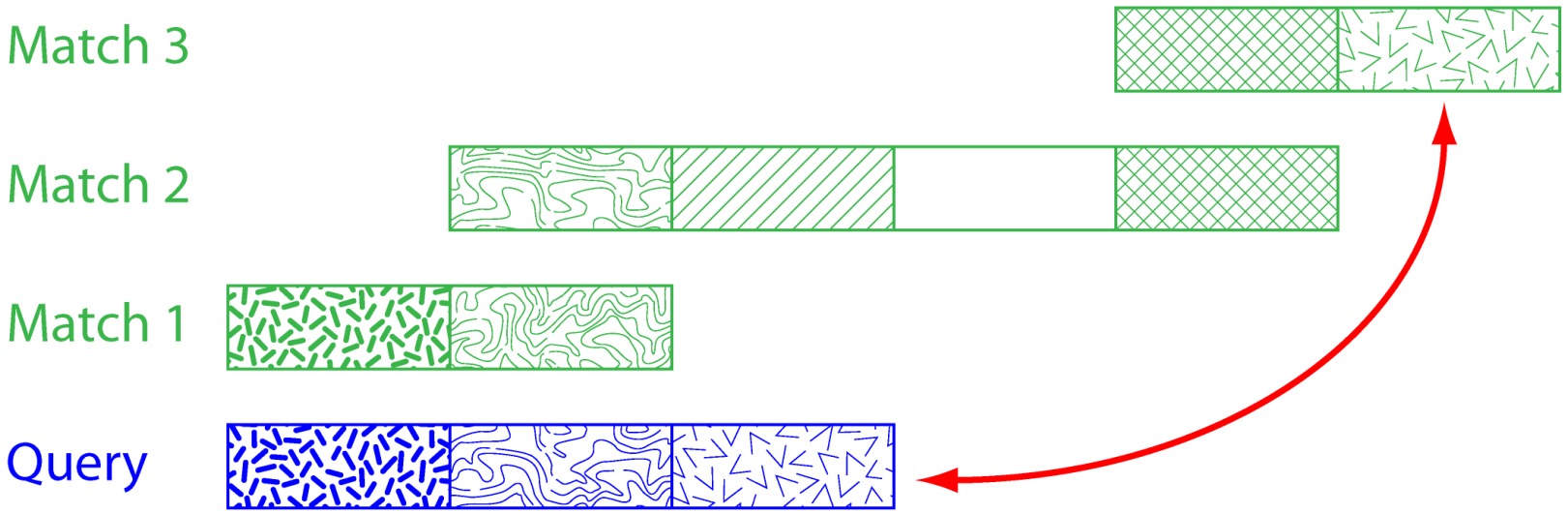
- It stems from greedy nature of alignment (mistakes made early in alignment cannot be corrected later)

- 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 Multiple Alignment



Fuse multiple alignment into:

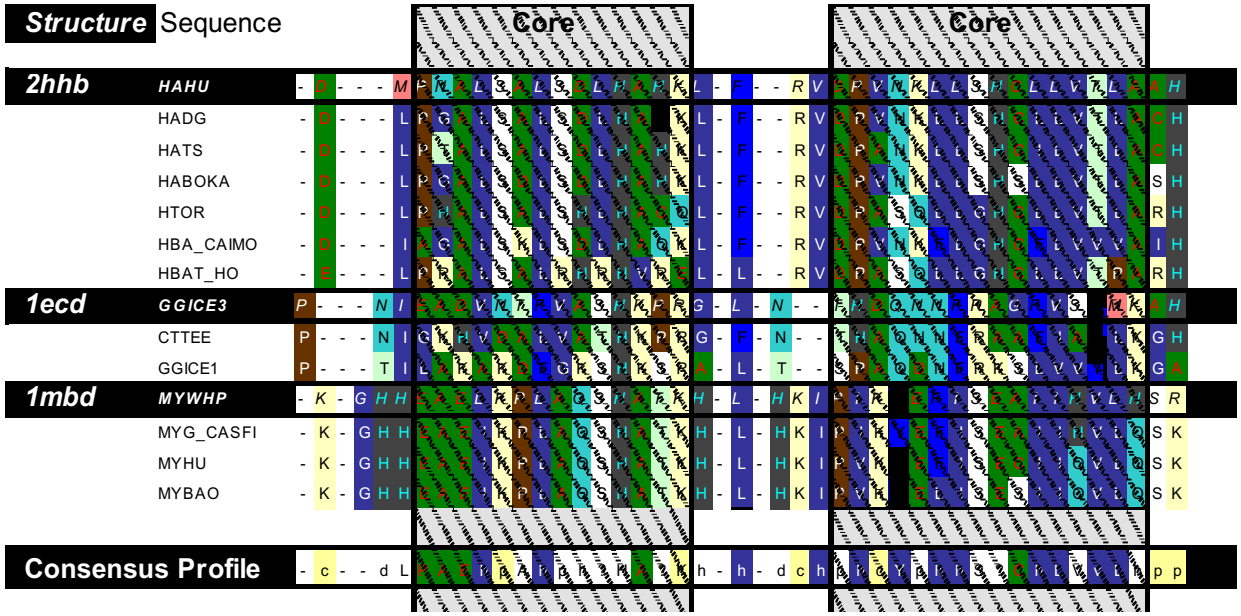
- **Motif**: a short signature pattern identified in the conserved region of the multiple alignment

- **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

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



Multiple Alignment

MOTIFS

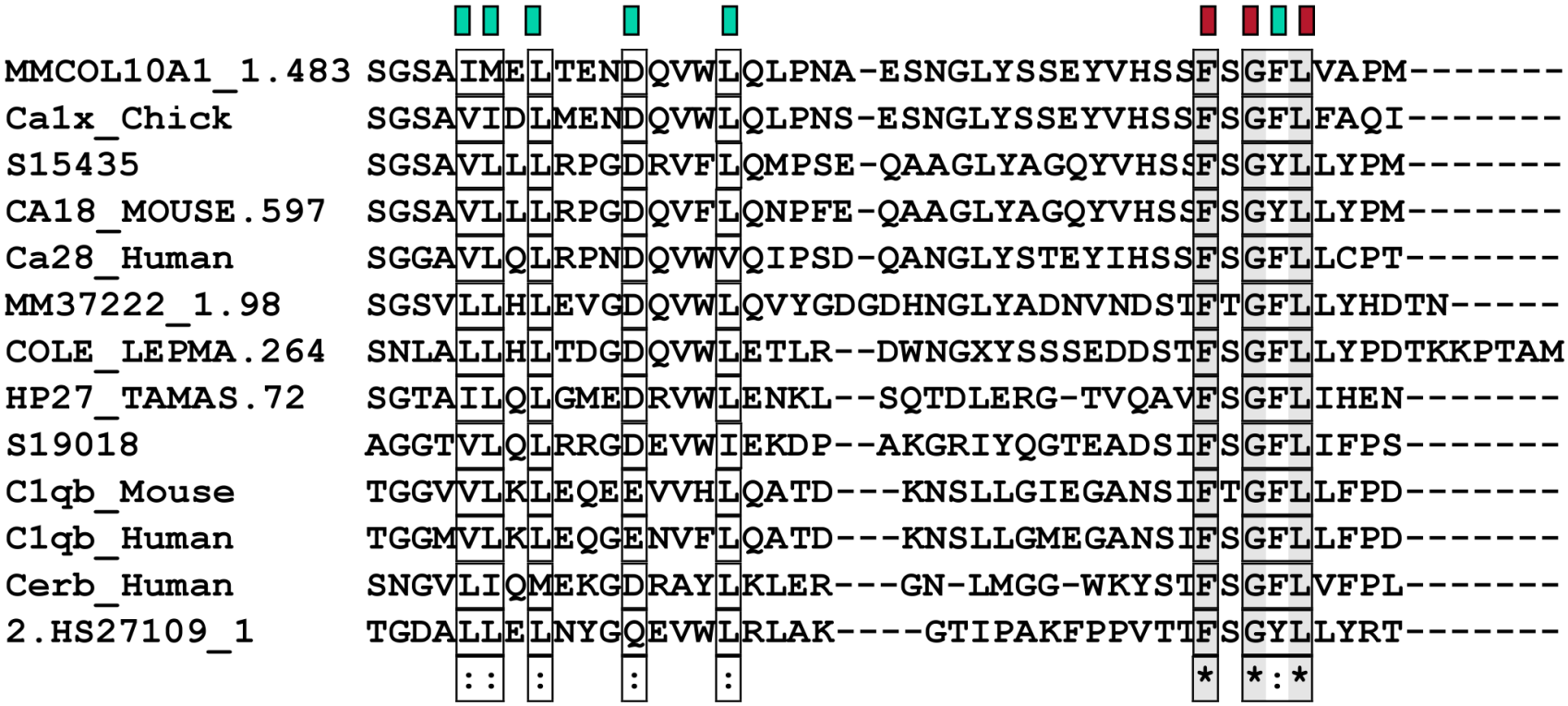
2 different applications for motif analysis

- 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

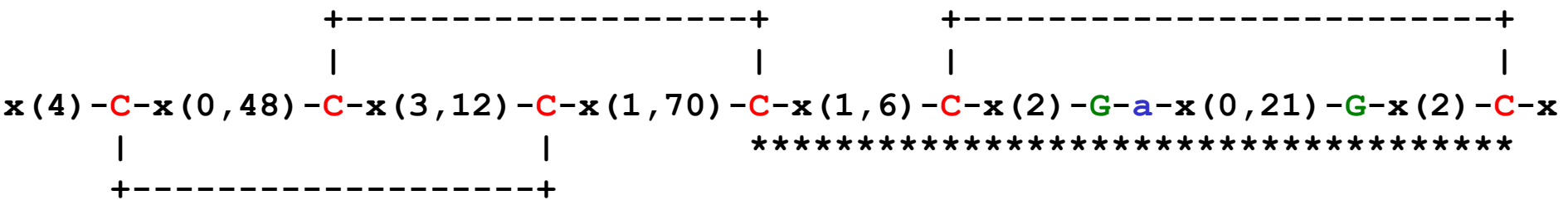
- several proteins are grouped together by similarity searches
- they share a conserved motif
- motif is stringent enough to retrieve the family members from the complete protein database
- PROSITE: a collection of motifs (1135 different motifs)



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

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	↑ 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	
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	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

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

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 ($M_{simp}(p,a)$)

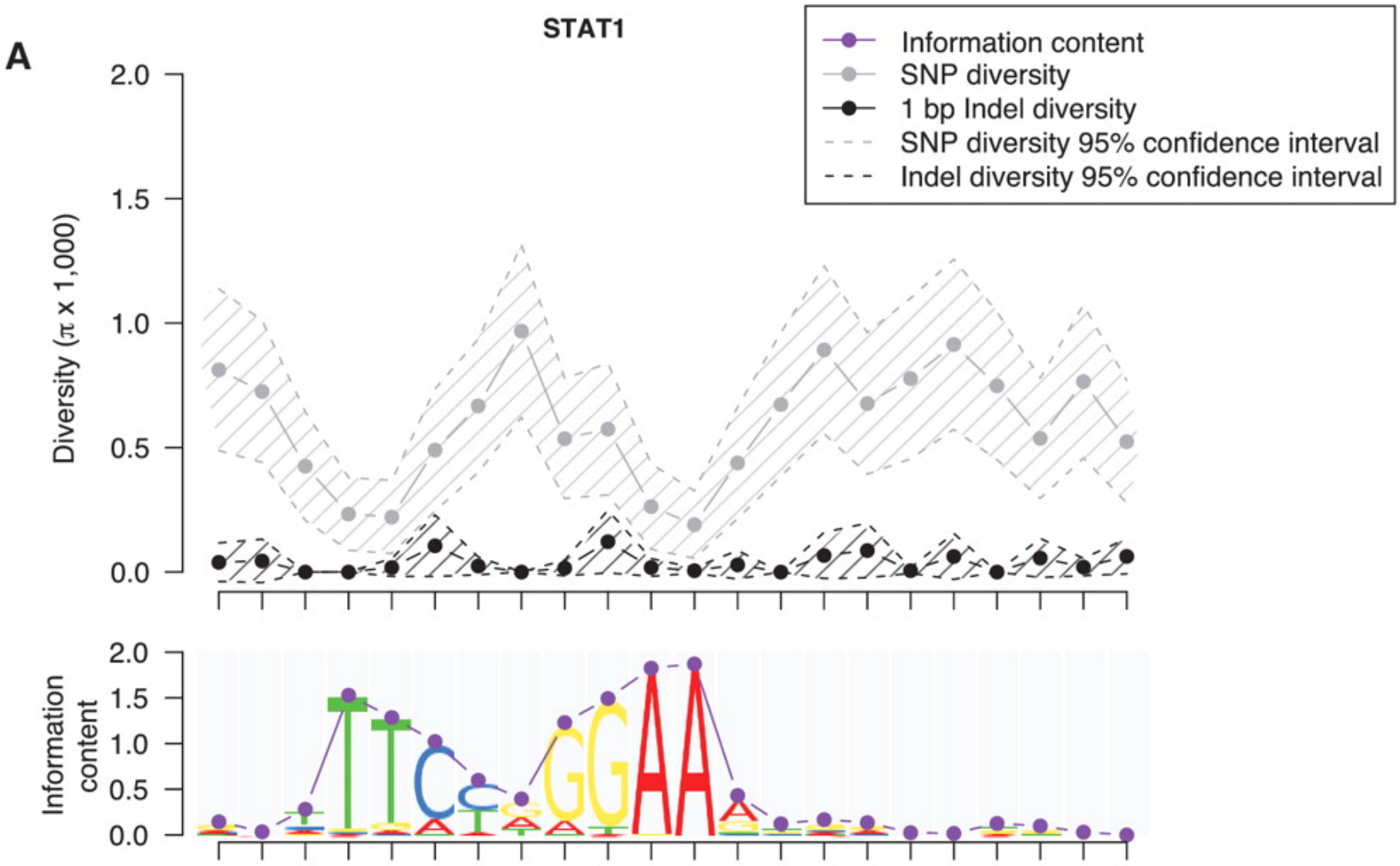
Say column only has one aa (AAAAA):
$H(p,a) = 1 \log_2 1 + 0 \log_2 0 + 0 \log_2 0 + \ldots = 0 + 0 + 0 + \ldots = 0$

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

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

(A) Aggregation of nucleotide diversity across STAT1 motifs.

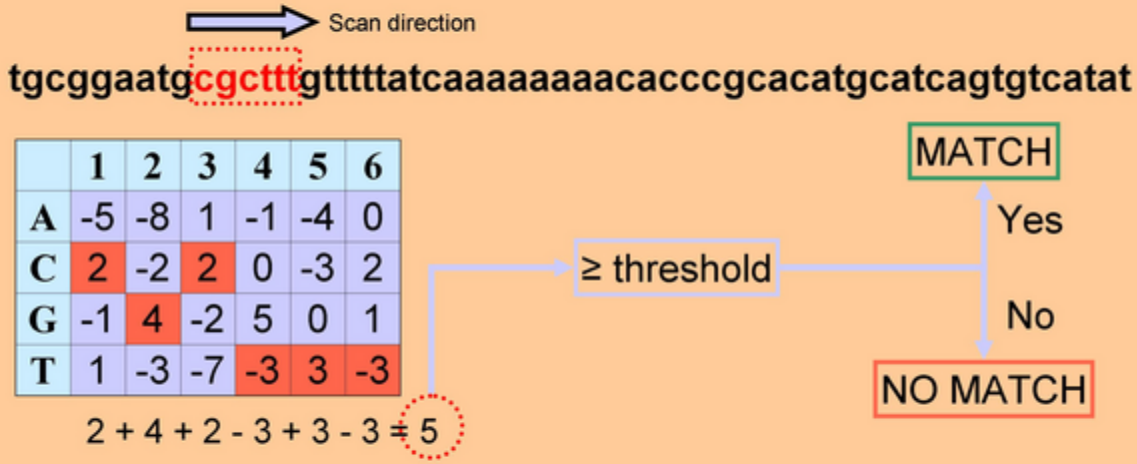


Mu X J et al. Nucl. Acids Res. 2011;39:7058-7076

Nucleic Acids Research

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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Zheng Zhang[2], Webb Miller[2] a

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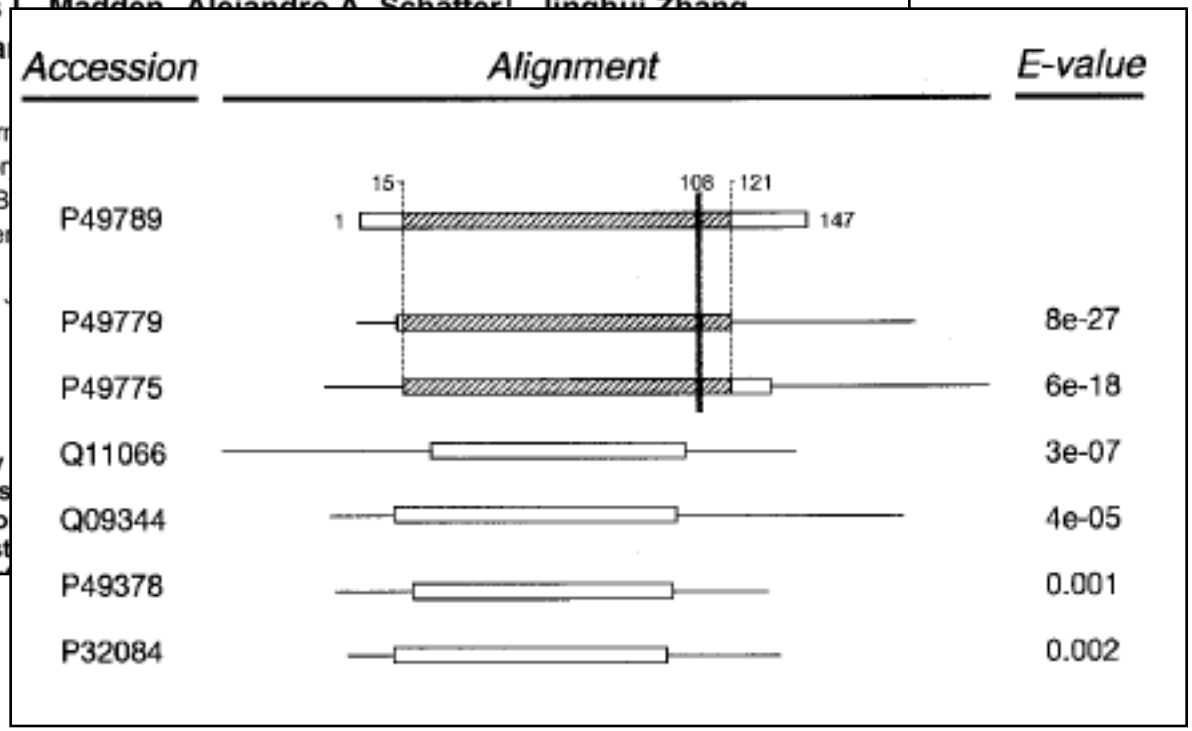
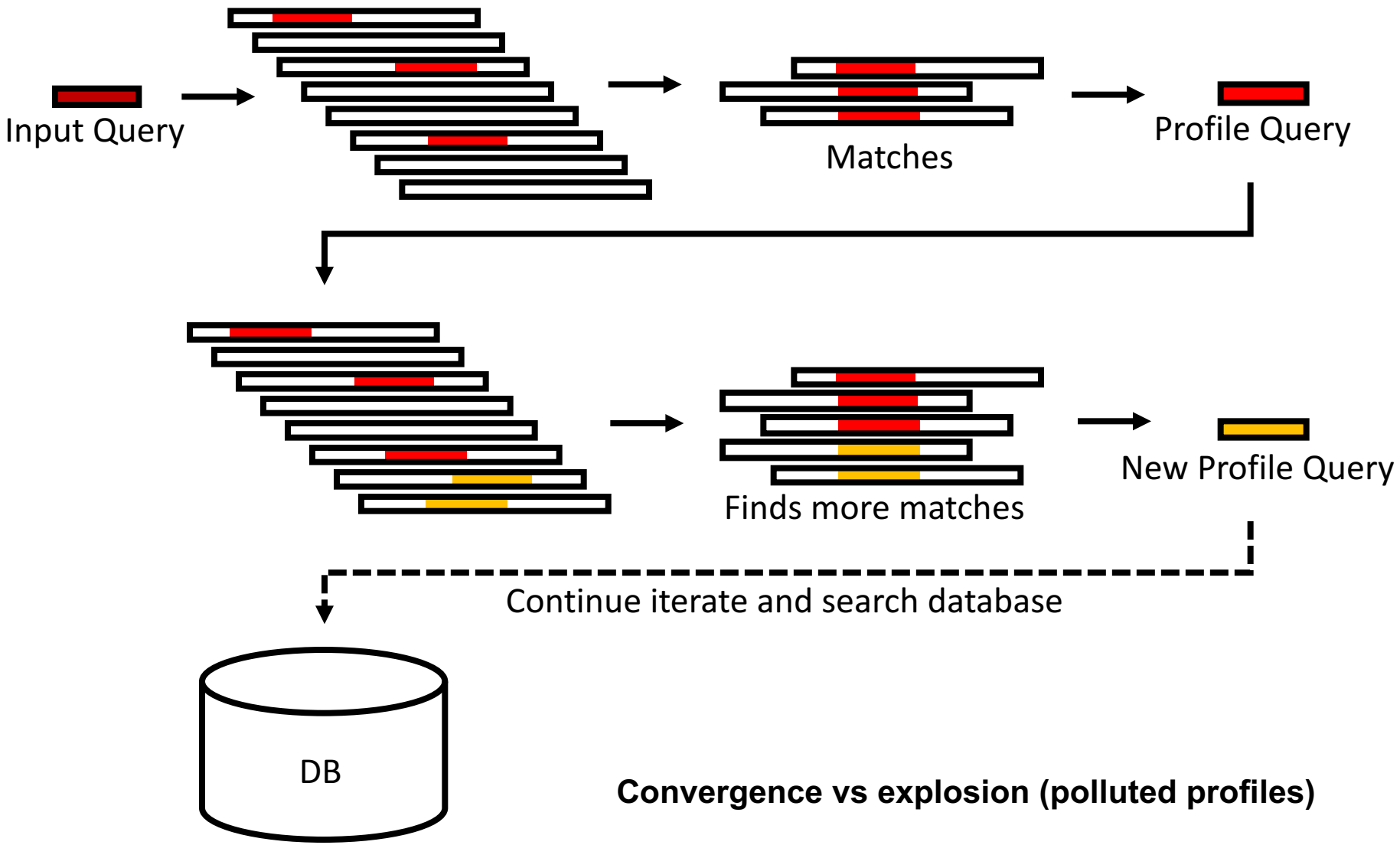
Received June 20, 1997; Revised and Accepted

ABSTRACT

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



PSI-BLAST (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

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]

EM (again!)

```
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
```

EM

[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

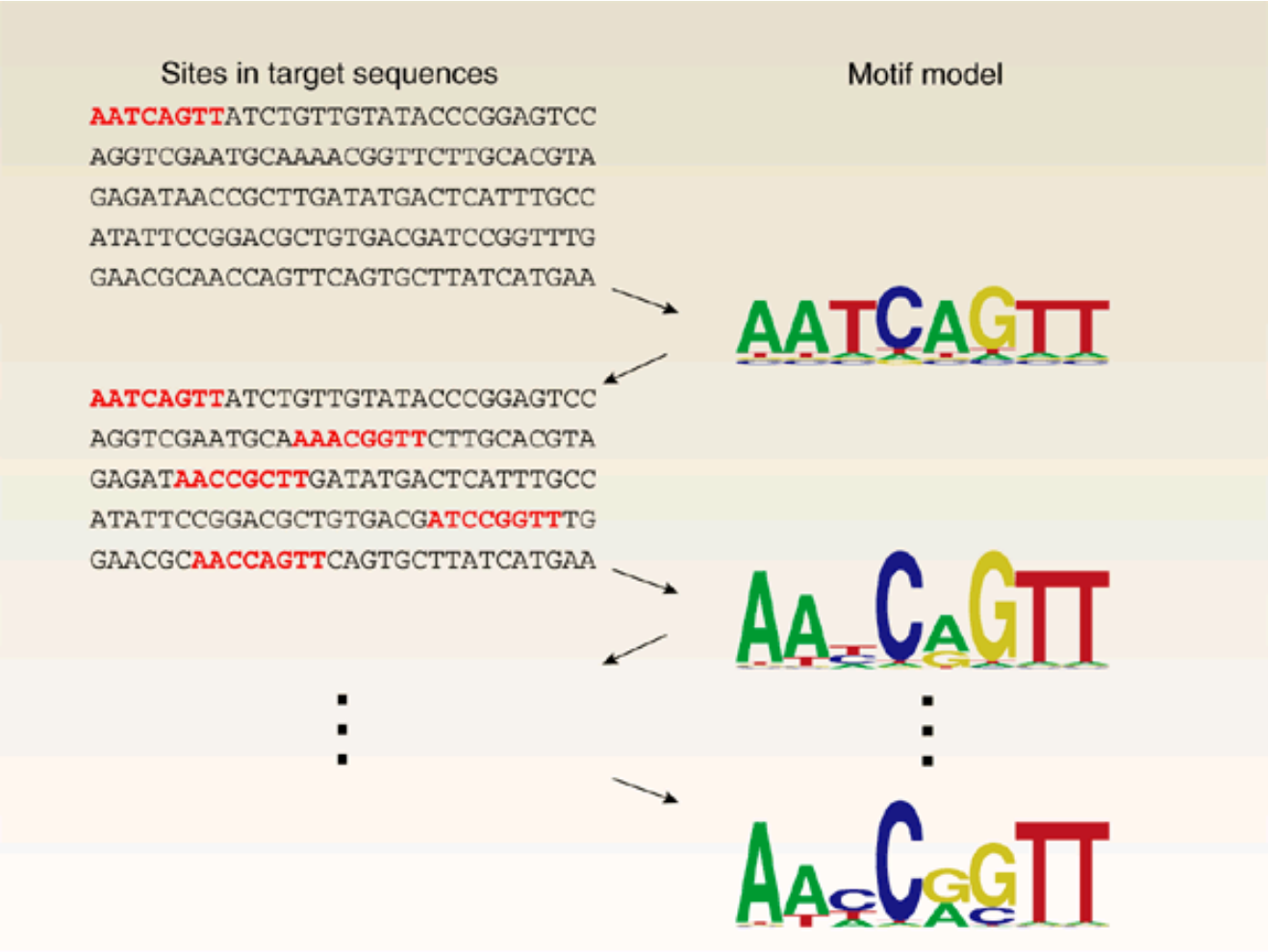
>ara
gacaaaaacgcgtaacaaaagtgtctataatcacggcag
aaaagtccacattgatta**TTTGCACGGCGTCAC**actttg
ctatgccatagcatttttatccataag

>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 algorithm work?



Starting from a single site, expectation maximization algorithms 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.

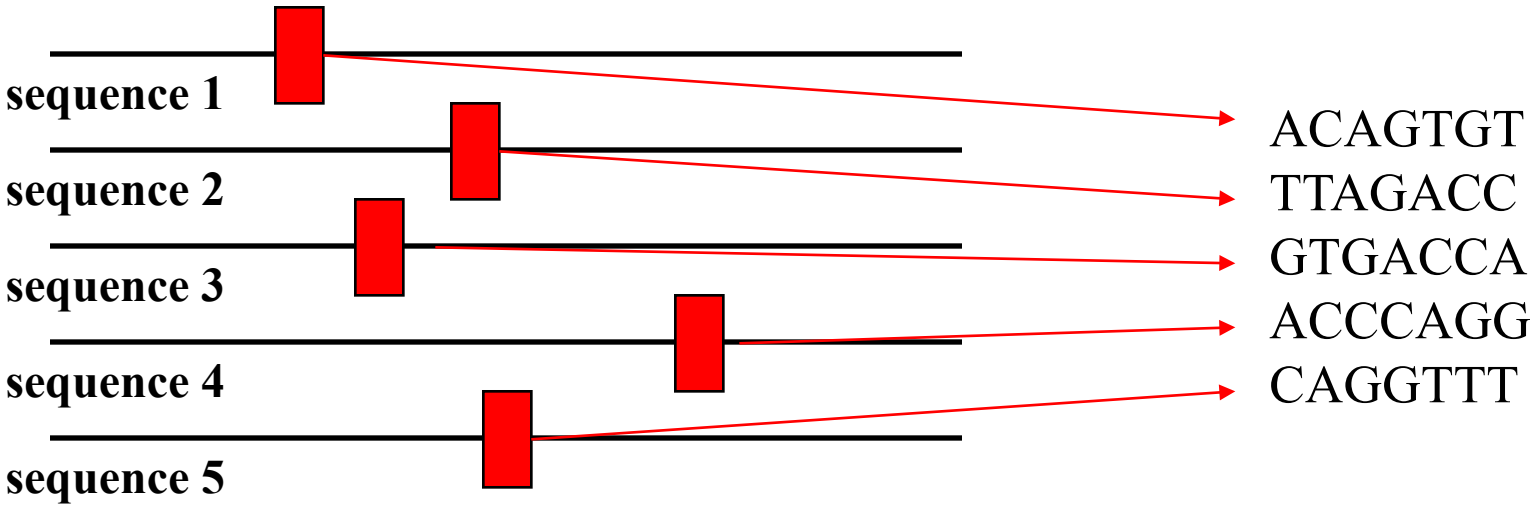
Specifically, in E step, estimate location of motif match. In M step, find most likely parameters of motif model given the locations.

Multiple Alignment

Gibbs Sampling

Initialization

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



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

Gibbs sampler

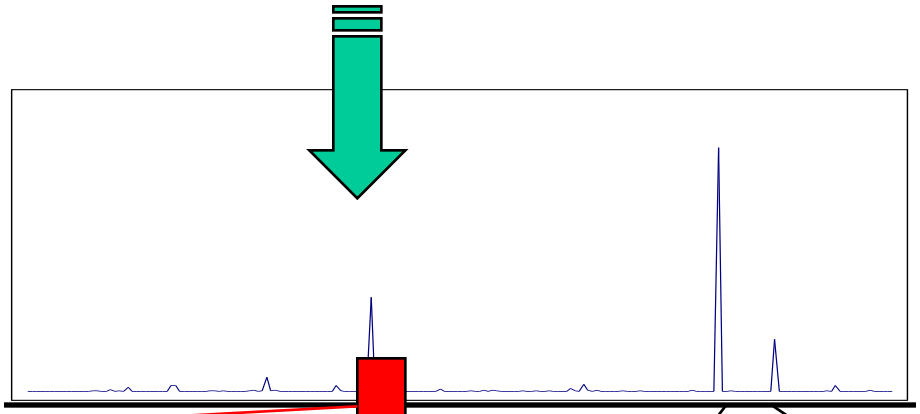
- Steps 2 & 3 (search):
  - Throw away an instance s_i: remaining (t - 1) instances define weight matrix.
  - Weight matrix defines instance probability at each position of input string S_i
  - Pick new s_i according to probability distribution (not necessarily always the s_i giving the highest prob.)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

[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/]

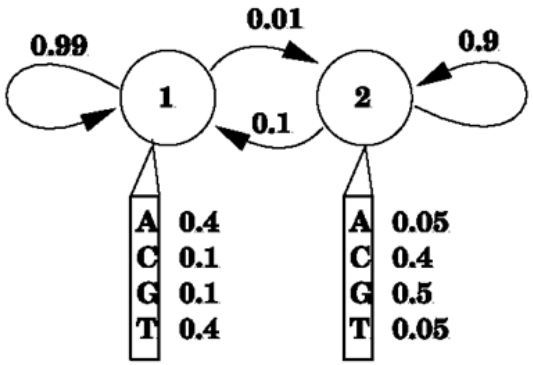
Multiple Alignment

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):

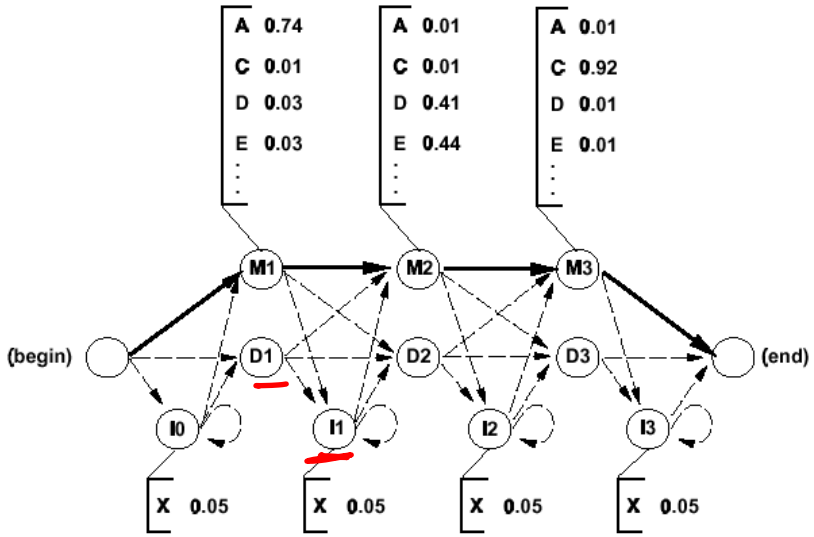
... 1 1 1 1 1 2 2 2 2 1 1 ...

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.)

Profile HMMs

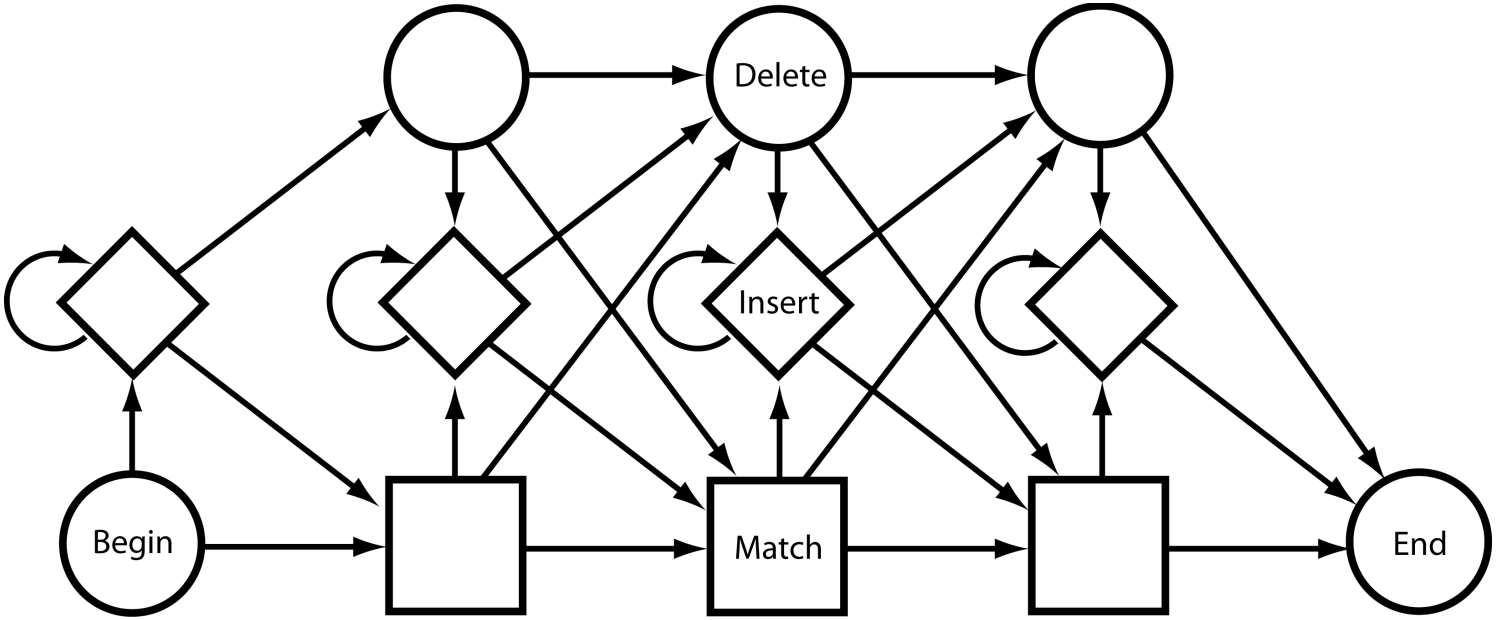
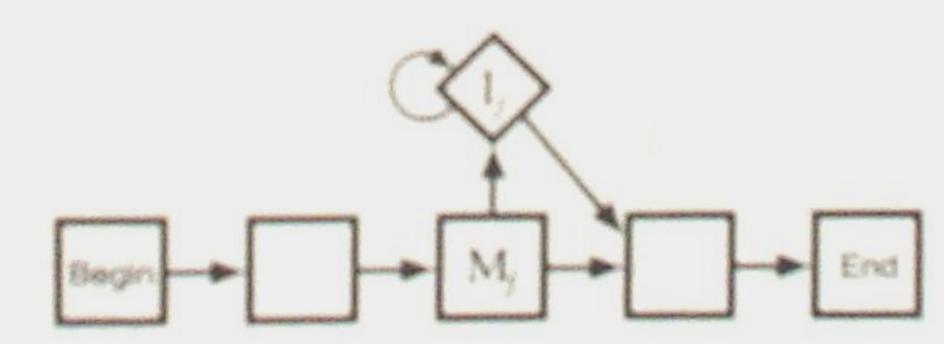


Figure 5.2, Durbin, Eddy, Krogh, and Mitchison, Biological Sequence Analysis (1998)

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

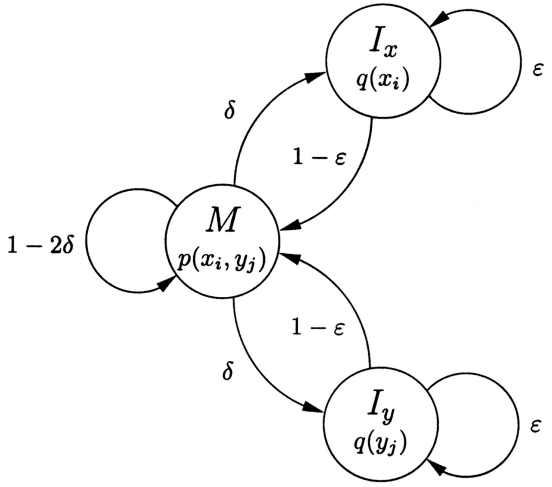
Forward sums of all possible paths

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

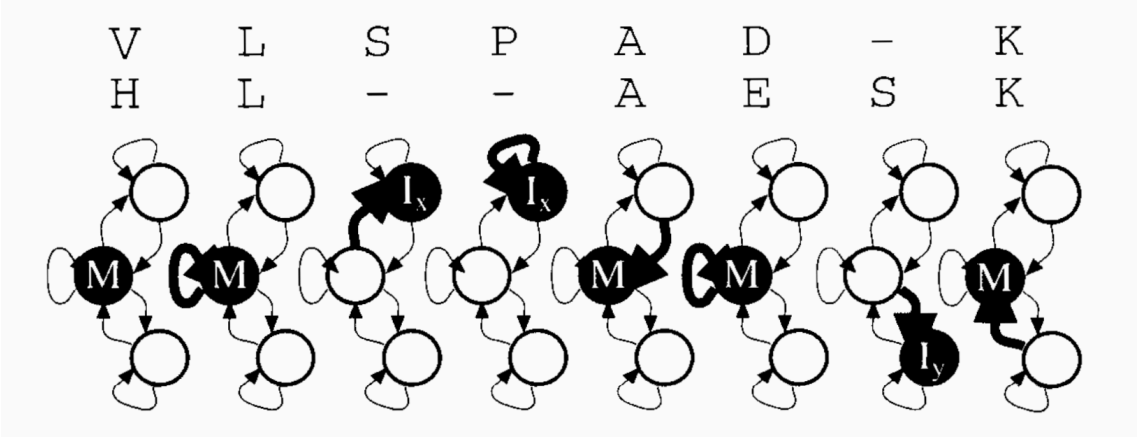
Viterbi Algorithm – finds the most probable path through the model for a given sequence
(both usually just boil down to simple applications of dynamic programming)

Pair Hidden Markov Models and Sequence Alignment

HMM for pairwise sequence alignment



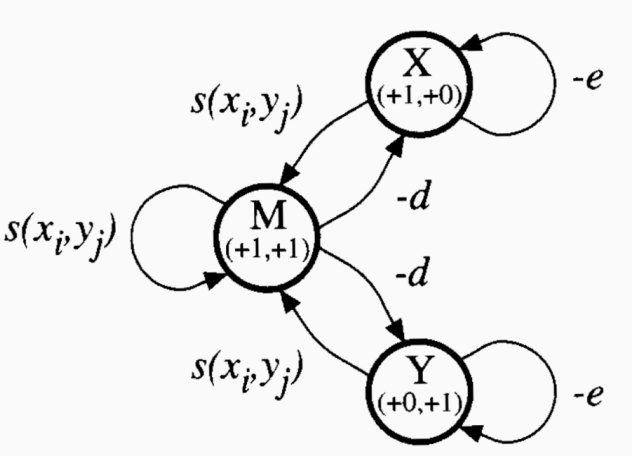
Example alignment using HMM and corresponding states



δ and ε determine gap creation extension penalties

Durbin et al. 1998

HMM algorithms are similar to those in sequence alignment



Algorithm: Optimal log-odds alignment

Initialisation:

$V^{\mathrm{M}}(0,0) = 2\log\eta, V^{\mathrm{X}}(0,0) = V^{\mathrm{Y}}(0,0) = -\infty.$

All $V^{\bullet}(i,-1), V^{\bullet}(-1,j)$ are set to $-\infty$.

Recursion: $i = 0,\ldots,n, j = 0,\ldots,m$ except $(0,0)$;

$$V^{\mathrm{M}}(i,j) = s(x_i,y_j) + \max\begin{cases} V^{\mathrm{M}}(i-1,j-1), \\ V^{\mathrm{X}}(i-1,j-1), \\ V^{\mathrm{Y}}(i-1,j-1); \end{cases}$$

$$V^{\mathrm{X}}(i,j) = \max\begin{cases} V^{\mathrm{M}}(i-1,j) - d, \\ V^{\mathrm{X}}(i-1,j) - e; \end{cases}$$

$$V^{\mathrm{Y}}(i,j) = \max\begin{cases} V^{\mathrm{M}}(i,j-1) - d, \\ V^{\mathrm{Y}}(i,j-1) - e. \end{cases}$$

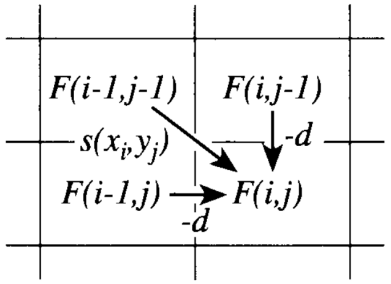
Termination:

$V = \max(V^{\mathrm{M}}(n,m), V^{\mathrm{X}}(n,m)+c, V^{\mathrm{Y}}(n,m)+c).$

		H	E	A	G	A	W	G	H	E	E
	0	**-8**	**-16**	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-9	**-17**	**-25**	-33	-42	-49	-57	-65	-73
A	-16	-10	-3	-4	-12	**-20**	-28	-36	-44	-52	-60
W	-24	-18	-11	-6	-7	-15	**-5**	**-13**	-21	-29	-37
H	-32	-14	-18	-13	-8	-9	-13	-7	**-3**	-11	-19
E	-40	-22	-8	-16	-16	-9	-12	-15	-7	**3**	-5
A	-48	-30	-16	-3	-11	-11	-12	-12	-15	**-5**	2
E	-56	-38	-24	-11	-6	-12	-14	-15	-12	-9	**1**

HEAGAWGHE-E

--P-AW-HEAE

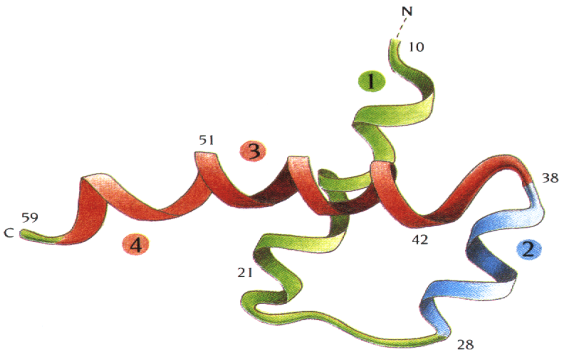


$$F(i,j) = \max\begin{cases} F(i-1,j-1) + s(x_i,y_j), \\ F(i-1,j) - d, \\ F(i,j-1) - d. \end{cases}$$

Durbin et al. 1998

Domains

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



•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)

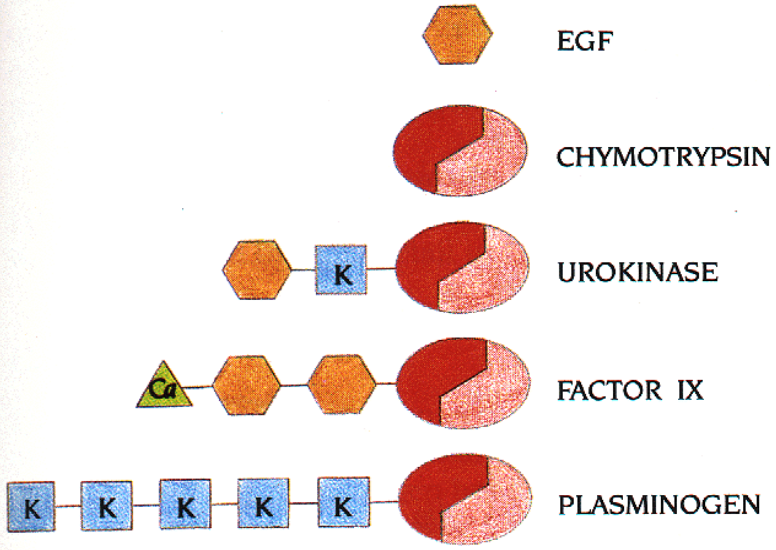


Figure 2.19 Organization of polypeptide chains into domains. Small protein molecules like the epidermal growth factor, EGF, comprise only one domain. Others like the serine proteinase chymotrypsin are arranged in two domains that are both required to form a functional unit (Chapter 15). Many of the proteins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

Domain Databases

Pfam	http://pfam.sanger.ac.uk/
SMART	http://smart.embl-heidelberg.de/
CDD, Interpro	