CSEP 590A

Summer 2006

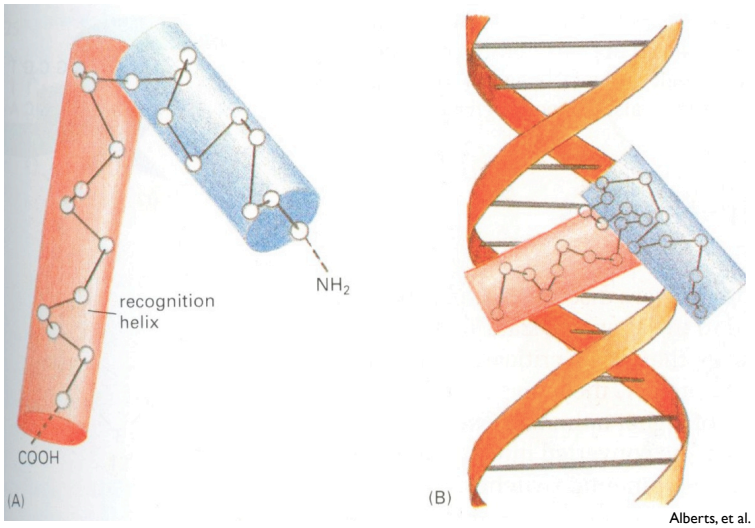
Lecture 5

Motifs: Representation & Discovery

DNA Binding Proteins

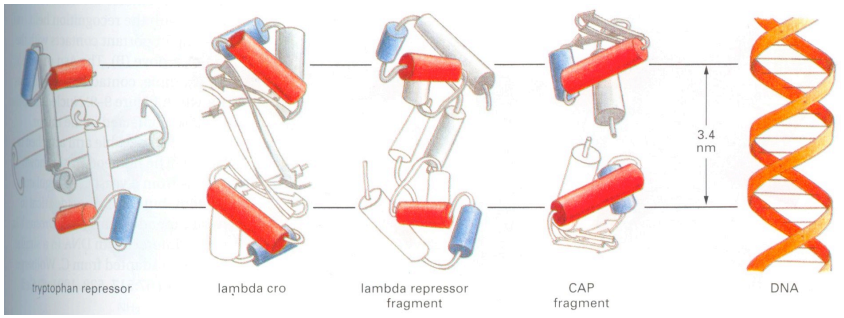
A variety of DNA binding proteins ("transcription factors"; a significant fraction, perhaps 5-10%, of all human proteins) modulate transcription of protein coding genes

Helix-Turn-Helix DNA Binding Motif



Alberts, et al.

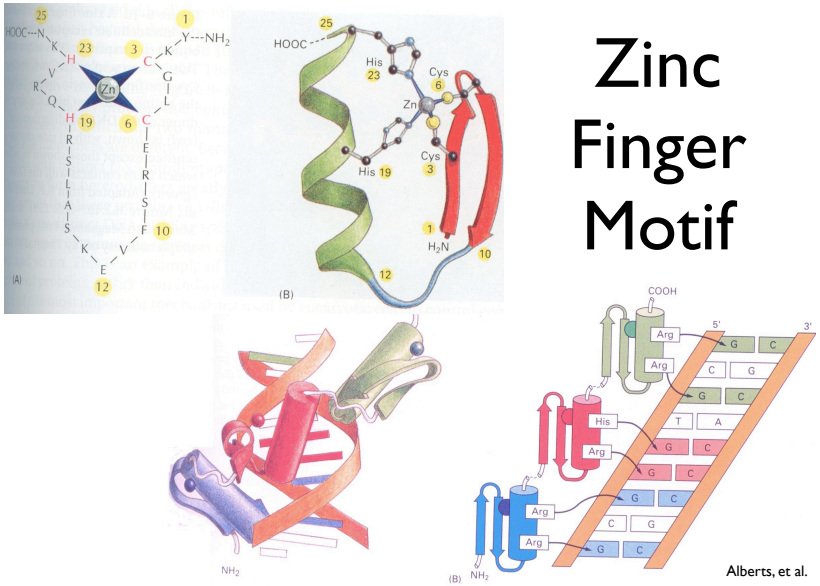
H-T-H Dimers



Alberts, et al.

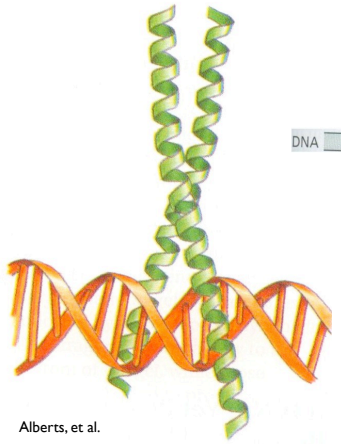
Bind 2 DNA patches, ~ 1 turn apart
Increases both specificity and affinity

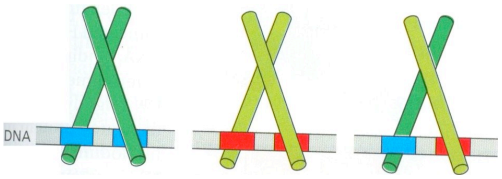
Zinc Finger Motif



Alberts, et al.

Leucine Zipper Motif

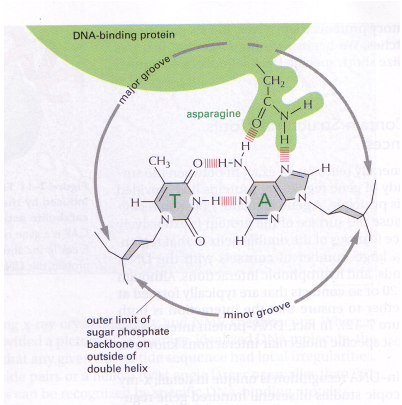


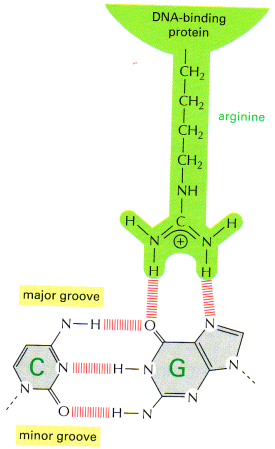


Homo-/hetero-dimers and combinatorial control

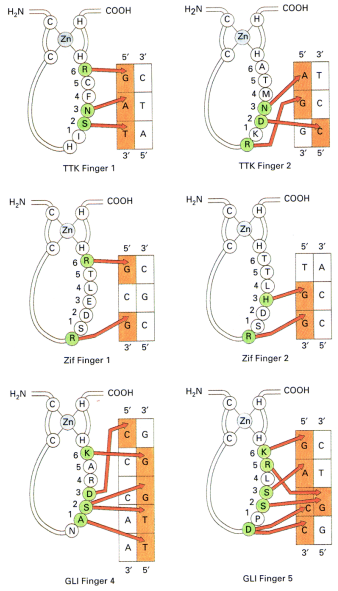
Alberts, et al.

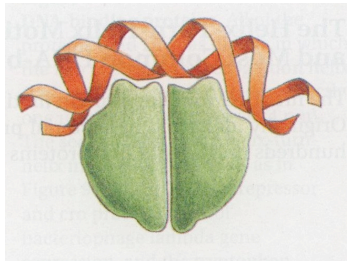
Some Protein/DNA interactions well-understood





But the overall DNA binding "code" still defies prediction





DNA binding site summary

- complex "code"
- short patches (6-8 bp)
- often near each other (1 turn = 10 bp)
- often reverse-complements
- not perfect matches

Sequence Motifs

E. coli Promoters

- "TATA Box" ~ 10bp upstream of transcription start
- How to define it?
  - *Consensus* is TATAAT
  - BUT all differ from it
  - allow k mismatches?
  - equally weighted?
  - wildcards like R,Y? ({A,G}, {C,T}, resp.)

```
TACGAT
TAAAAT
TATACT
GATAAT
TATGAT
TATGTT
```


E. coli Promoters

- "TATA Box" - consensus TATAAT ~ 10bp upstream of transcription start
- *Not* exact: of 168 studied (mid 80's)
  - nearly all had 2/3 of TAxyzT
  - 80-90% had all 3
  - 50% agreed in each of x,y,z
  - no perfect match
- Other common features at -35, etc.

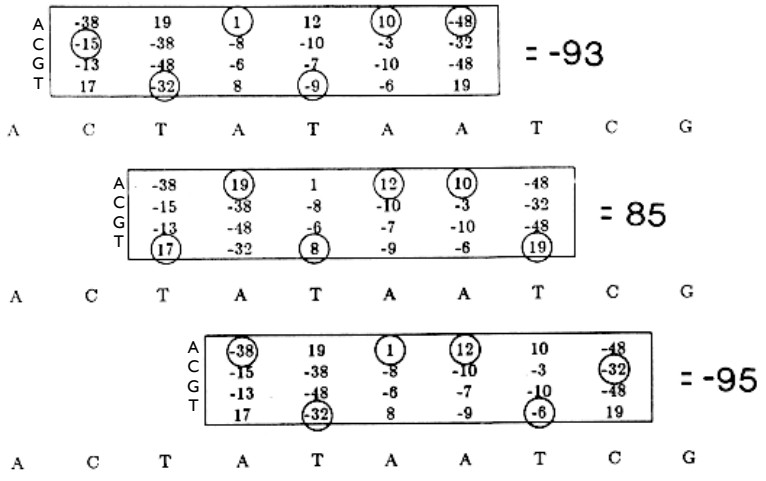
TATA Box Frequencies

pos / base	1	2	3	4	5	6
A	2	95	26	59	51	1
C	9	2	14	13	20	3
G	10	1	16	15	13	0
T	79	3	44	13	17	96

TATA Scores

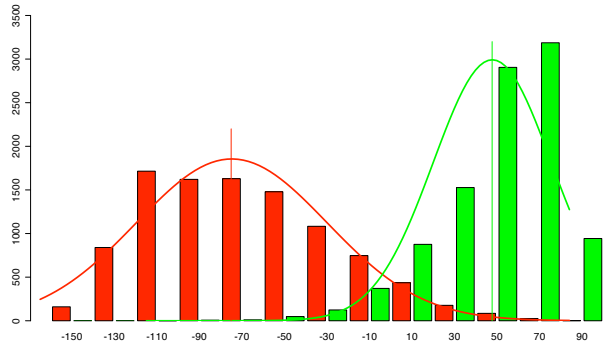
pos / base	1	2	3	4	5	6
A	-36	19	1	12	10	-46
C	-15	-36	-8	-9	-3	-31
G	-13	-46	-6	-7	-9	-46(?)
T	17	-31	8	-9	-6	19

Scanning for TATA



Stormo, Ann. Rev. Biophys. Biophys Chem, 17, 1988, 241-263

Score Distribution
(Simulated)



Weight Matrices: Statistics

- Assume:

  $f_{b,i}$ = frequency of base b in position i *in TATA*

  f_b = frequency of base b in all sequences

- Log likelihood ratio, given $S = B_1B_2...B_6$:

$$\log\left(\frac{P(S|\text{“tata”})}{P(S|\text{“non-tata”})}\right) = \log\frac{\prod_{i=1}^{6} f_{B_i,i}}{\prod_{i=1}^{6} f_{B_i}} = \sum_{i=1}^{6} \log\frac{f_{B_i,i}}{f_{B_i}}$$

Assumes *independence*

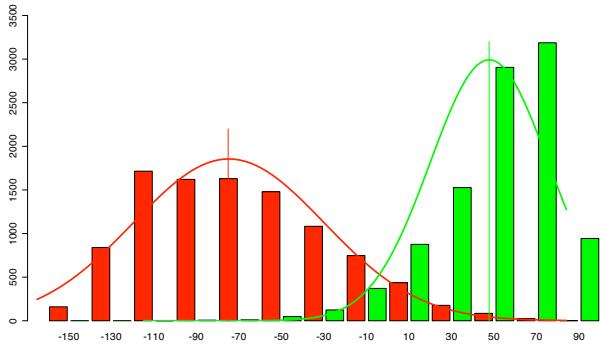
Neyman-Pearson

- Given a sample $x_1, x_2, ..., x_n$, from a distribution $f(...|\Theta)$ with parameter Θ, want to test hypothesis $\Theta = \theta_1$ vs $\Theta = \theta_2$.
- Might as well look at *likelihood ratio:*

$$\frac{f(x_1, x_2, ..., x_n|\theta_1)}{f(x_1, x_2, ..., x_n|\theta_2)} > \tau$$

(or *log likelihood difference*)

Score Distribution (Simulated)



What's best WMM?

- Given 20 sequences $s_1, s_2, ..., s_k$ of length 8, assumed to be generated at random according to a WMM defined by 8 x (4-1) parameters θ, what's the best θ?
- E.g., what's MLE for θ given data $s_1, s_2, ..., s_k$?
- Answer: count frequencies per position.

Weight Matrices: Chemistry

- Experiments show ~80% correlation of log likelihood weight matrix scores to measured binding energy of RNA polymerase to variations on TATAAT consensus [Stormo & Fields]

Another WMM example

8 Sequences:

ATG
ATG
ATG
ATG
ATG
GTG
GTG
TTG

Freq.	Col 1	Col 2	Col3
A	.625	0	0
C	0	0	0
G	.250	0	1
T	.125	1	0

Log-Likelihood Ratio:

$$\log_2 \frac{f_{x_i,i}}{f_{x_i}},\ f_{x_i} = \frac{1}{4}$$

LLR	Col 1	Col 2	Col 3
A	1.32	-∞	-∞
C	-∞	-∞	-∞
G	0	-∞	2.00
T	-1.00	2.00	-∞

Non-uniform Background

- *E. coli* - DNA approximately 25% A, C, G, T
- *M. jannaschi* - 68% A-T, 32% G-C

LLR from previous example, assuming

$$f_A = f_T = 3/8$$
$$f_C = f_G = 1/8$$

LLR	Col 1	Col 2	Col 3
A	.74	-∞	-∞
C	-∞	-∞	-∞
G	1.00	-∞	3.00
T	-1.58	1.42	-∞

e.g., G in col 3 is 8 x more likely via WMM than background, so ($\log_2$) score = 3 (bits).

Relative Entropy

- AKA Kullback-Liebler Distance/Divergence, AKA Information Content
- Given distributions P, Q

$$H(P||Q) = \sum_{x \in \Omega} P(x) \log \frac{P(x)}{Q(x)} \quad \geq 0$$

Notes:

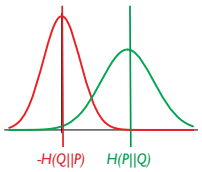
$$\text{Let } P(x) \log \frac{P(x)}{Q(x)} = 0 \text{ if } P(x) = 0 \text{ [since } \lim_{y \to 0} y \log y = 0]$$

$$\text{Undefined if } 0 = Q(x) < P(x)$$

WMM: How "Informative"? Mean score of site vs bkg?

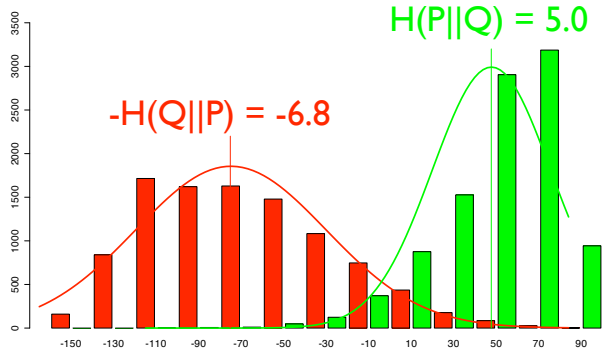
- For any fixed length sequence *x*, let
  P(x) = Prob. of *x* according to WMM
  Q(x) = Prob. of *x* according to background
- Relative Entropy:

$$H(P||Q) = \sum_{x \in \Omega} P(x) \log_2 \frac{P(x)}{Q(x)}$$



- *H(P||Q)* is *expected log likelihood score* of a sequence randomly chosen from *WMM*; *-H(Q||P)* is expected score of *Background*

WMM Scores vs Relative Entropy



For WMM, you can show (based on the assumption of independence between columns), that :

$$H(P||Q) \quad = \quad \sum_i H(P_i||Q_i)$$

where P_i and Q_i are the WMM/background distributions for column i.

WMM Example, cont.

Freq.	Col 1	Col 2	Col3
A	.625	0	0
C	0	0	0
G	.250	0	1
T	.125	1	0

Uniform

LLR	Col 1	Col 2	Col 3	
A	1.32	-∞	-∞	
C	-∞	-∞	-∞	
G	0	-∞	2.00	
T	-1.00	2.00	-∞	
RelEnt	.70	2.00	2.00	4.70

Non-uniform

LLR	Col 1	Col 2	Col 3	
A	.74	-∞	-∞	
C	-∞	-∞	-∞	
G	1.00	-∞	3.00	
T	-1.58	1.42	-∞	
RelEnt	.51	1.42	3.00	4.93

Pseudocounts

- Are the $-\infty$'s a problem?
  - Certain that a given residue *never* occurs in a given position? Then $-\infty$ just right
  - Else, it may be a small-sample artifact
- Typical fix: add a *pseudocount* to each observed count—small constant (e.g., .5, 1)
- Sounds *ad hoc*; there is a Bayesian justification

WMM Summary

- Weight Matrix Model (aka Position Specific Scoring Matrix, PSSM, "possum", 0th order Markov models)
- Simple statistical model assuming independence between adjacent positions
- To build: count (+ pseudocount) letter frequency per position, log likelihood ratio to background
- To scan: add LLRs per position, compare to threshold
- Generalizations to higher order models (i.e., letter frequency per position, conditional on neighbor) also possible, with enough training data

How-to Questions

- Given aligned motif instances, build model?
  - Frequency counts (above, maybe with pseudocounts)
- Given a model, find (probable) instances
  - Scanning, as above
- Given unaligned strings thought to contain a motif, find it? (e.g., upstream regions for co-expressed genes from a microarray experiment)
  - Hard... rest of lecture.

Motif Discovery

Unfortunately, finding a site of max relative entropy in a set of unaligned sequences is NP-hard [Akutsu]

Motif Discovery: 3 example approaches

- Greedy search
- Expectation Maximization
- Gibbs sampler

Greedy Best-First Approach
[Hertz & Stormo]

Input:
- Sequence $s_1, s_2, ..., s_k$; motif length l; "breadth" d

Algorithm:
- create singleton set with each length l subsequence of each $s_1, s_2, ..., s_k$
- for each set, add each possible length l subsequence not already present
- compute relative entropy of each
- discard all but d best
- repeat until all have k sequences

usual "greedy" problems

Expectation Maximization
[MEME, Bailey & Elkan, 1995]

Input (as above):
- Sequence $s_1, s_2, ..., s_k$; motif length l; background model; again assume one instance per sequence (variants possible)

Algorithm: EM
- Visible data: the sequences
- Hidden data: where's the motif

$$Y_{i,j} = \begin{cases} 1 & \text{if motif in sequence } i \text{ begins at position } j \\ 0 & \text{otherwise} \end{cases}$$

- Parameters θ: The WMM

MEME Outline

Typical EM algorithm:
- Parameters θ^t at t^{th} iteration, used to estimate where the motif instances are (the hidden variables)
- Use those estimates to re-estimate the parameters θ to maximize likelihood of observed data, giving θ^{t+1}
- Repeat

Key: given a few good matches to best motif, expect to pick out more

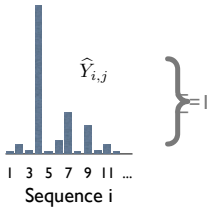
Expectation Step

(where are the motif instances?)

$$\begin{aligned}
\widehat{Y}_{i,j} &= E(Y_{i,j} \mid s_i, \theta^t) \\
&= P(Y_{i,j} = 1 \mid s_i, \theta^t) \\
&= P(s_i \mid Y_{i,j} = 1, \theta^t) \frac{P(Y_{i,j}=1|\theta^t)}{P(s_i|\theta^t)} \\
&= cP(s_i \mid Y_{i,j} = 1, \theta^t) \\
&= c' \prod_{k=1}^{l} P(s_{i,j+k-1} \mid \theta^t)
\end{aligned}$$

$E = 0 \cdot P(0) + 1 \cdot P(1)$

Bayes



$=1$

where c' is chosen so that $\sum_j \widehat{Y}_{i,j} = 1$.

Maximization Step

(what is the motif?)

Find θ maximizing expected value:

$$\begin{aligned}
Q(\theta \mid \theta^t) &= E_{Y \sim \theta^t}[\log P(s, Y \mid \theta)] \\
&= E_{Y \sim \theta^t}[\log \textstyle\prod_{i=1}^{k} P(s_i, Y_i \mid \theta)] \\
&= E_{Y \sim \theta^t}[\textstyle\sum_{i=1}^{k} \log P(s_i, Y_i \mid \theta)] \\
&= E_{Y \sim \theta^t}[\textstyle\sum_{i=1}^{k} \sum_{j=1}^{|s_i|-l+1} Y_{i,j} \log P(s_i, Y_{i,j} = 1 \mid \theta)] \\
&= E_{Y \sim \theta^t}[\textstyle\sum_{i=1}^{k} \sum_{j=1}^{|s_i|-l+1} Y_{i,j} \log(P(s_i \mid Y_{i,j} = 1, \theta) P(Y_{i,j} = 1 \mid \theta))] \\
&= \textstyle\sum_{i=1}^{k} \sum_{j=1}^{|s_i|-l+1} E_{Y \sim \theta^t}[Y_{i,j}] \log P(s_i \mid Y_{i,j} = 1, \theta) + C \\
&= \textstyle\sum_{i=1}^{k} \sum_{j=1}^{|s_i|-l+1} \widehat{Y}_{i,j} \log P(s_i \mid Y_{i,j} = 1, \theta) + C
\end{aligned}$$

M-Step (cont.)

$$Q(\theta \mid \theta^t) = \textstyle\sum_{i=1}^{k} \sum_{j=1}^{|s_i|-l+1} \widehat{Y}_{i,j} \log P(s_i \mid Y_{i,j} = 1, \theta) + C$$

Exercise: Show this is maximized by "counting" letter frequencies over all possible motif instances, with counts weighted by $\widehat{Y}_{i,j}$, again the "obvious" thing.

s_1 : ACGGATT...

...

s_k : GC...TCGGAC

$\widehat{Y}_{1,1}$	ACGG
$\widehat{Y}_{1,2}$	CGGA
$\widehat{Y}_{1,3}$	GGAT
⋮	⋮
$\widehat{Y}_{k,l-1}$	CGGA
$\widehat{Y}_{k,l}$	GGAC

Initialization

1. Try *every* motif-length substring, and use as initial θ a WMM with, say 80% of weight on that sequence, rest uniform
2. Run a few iterations of each
3. Run best few to convergence

(Having a supercomputer helps)

Another Motif Discovery Approach

The Gibbs Sampler

Lawrence, *et al.* "Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Sequence Alignment," *Science* 1993

```
Sigma-37        223 IIDLTYIQNK SQKETGDILGISQMHVSR LQRKAVKKLR  240    A25944
SpoIIIC          94 RFGLDLKKEK TQREIAKELGISRSYVSR IEKRALMKMF  111    A28627
NahR             22 VVFNQLLVDR RVSITAENLGLTQPAVSN ALKRLRTSLQ   39    A32837
Antennapedia    326 FHFNRYLTRR RRIEIAHALCLTERQIKI WFQNRRMKWK  343    A23450
NtrC (Brady.)   449 LTAALAATRG NQIRAADLLGLNRNTLRK KIRDLDIQVY  466    B26499
DicA             22 IRYRRKNLKH TQRSLAKALKISHVSVSQ WERGDSEPTG   39    B24328 (BVECDA)
MerD              5       MNAY TVSRLALDAGVSVHIVRD YLLRGLLRPV   22    C29010
Fis              73 LDMVMQYTRG NQTRAALMMGINRGTLRK KLKKYGMN    90    A32142 (DNECFS)
MAT a1           99 FRRKQSLNSK EKEEVAKKCGITPLQVRV WFINKRMRSK  116    A90983 (JEBY1)
Lambda cII       25 SALLNKIAML GTEKTAEAVGVDKSQISR WKRDWIPKFS   42    A03579 (QCBP2L)
Crp (CAP)       169 THPDGMQIKI TRQEIGQIVGCSRETVGR ILKMLEDQNL  186    A03553 (QRECC)
Lambda Cro       15 ITLKDYAMRF GQTKTAKDLGVYQSAINK AIHAGRKIFL   32    A03577 (RCBPL)
P22 Cro          12 YKKDVIDHFG TQRAVAKALGISDAAVSQ WKEVIPEKDA   29    A25867 (RGBP22)
AraC            196 ISDHLADSNF DIASVAQHVCLSPSRLSH LFRQQLGISV  213    A03554 (RGECA)
Fnr             196 FSPREFRLTM TRGDIGNYLGLTVETISR LLGRFQKSGM  213    A03552 (RGECF)
HtpR            252 ARWLDEDNKS TLQELADRYGVSAERVRQ LEKNAMKKLR  269    A00700 (RGECH)
NtrC (K.a.)     444 LTTALRHTQG HKQEAARLLGWGRNTLTR KLKELGME    461    A03564 (RGKBCP)
CytR             11 MKAKKQETAA TMKDVALKAKVSTATVSR ALMNPDKVSQ   28    A24963 (RPECCT)
DeoR             23 LQELKRSDKL HLKDAAALLGVSEMTIRR DLNNHSAPVV   40    A24076 (RPECDO)
GalR              3          MA TIKDVARLAGVSVATVSR VINNSPKASE  20    A03559 (RPECG)
LacI              5        MKPV TLYDVAEYAGVSYQTVSR VVNQASHVSA  22    A03558 (RPECL)
TetR             26 LLNEVGIEGL TTRKLAQKLGVEQPTLYW HVKNKRALLD   43    A03576 (RPECTN)
TrpR             67 IVEELLRGEM SQRELKNELGAGIATITR GSNSLKAAPV   84    A03568 (RPECW)
NifA            495 LIAALEKAGW VQAKAARLLGMTPRQVAY RIQIMDITMP  512    S02513
SpoIIG          205 RFGLVGEEEK TQKDVADMMGISQSYISR LEKRIIKRLR  222    S07337
Pin             160 QAGRLIAAGT PRQKVAIIYDVGVSTLYK TFPAGDK     177    S07958
PurR              3          MA TIKDVAKRANVSTTTVSH VINKTRFVAE  20    S08477
EbgR              3          MA TLKDIAIEAGVSLATVSR VLNDDPTLNV  20    S09205
LexA             27 DHISQTGMPP TRAEIAQRLGFRSPNAAE EHLKALARKG  44    S11945
P22 cI           25 SSILNRIAIR GQRKVADALGINESQISR WKGDFIPKMG  42    B25867 (Z1BPC2)
                                ****************** ***
```

B

	Position in site																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Arg	94	222	265	137	9	9	137	137	9	9	9	52	222	94	94	9	265	606
Lys	9	133	442	380	9	71	380	194	9	133	9	9	71	9	9	9	71	256
Glu	53	9	96	401	9	9	140	140	9	9	9	53	140	140	9	9	9	53
Asp	67	9	9	473	9	9	299	125	9	67	9	67	67	9	9	9	9	67
Gln	9	600	224	9	9	9	224	9	9	9	9	9	278	63	278	9	9	170
His	240	9	9	9	9	9	125	125	9	9	9	9	125	125	125	9	9	240
Asn	168	9	9	9	9	9	168	89	9	89	9	248	9	168	89	9	89	89
Ser	117	9	117	117	9	9	9	9	9	9	9	819	63	387	63	9	819	9
Gly	151	9	56	9	9	151	9	9	9	1141	9	151	9	56	9	9	56	9
Ala	9	9	112	43	181	901	43	181	215	9	43	9	43	181	112	43	78	9
Thr	915	130	130	9	251	9	9	9	9	9	9	311	130	70	855	9	130	9
Pro	76	9	9	9	9	9	9	9	9	9	9	9	210	210	9	9	9	9
Cys	9	9	9	9	9	9	9	9	295	581	295	9	9	9	9	9	9	9
Val	58	107	9	9	500	9	9	9	156	9	598	9	205	58	9	746	9	58
Leu	9	121	9	9	149	9	93	149	458	9	149	9	37	37	9	177	9	9
Ile	9	166	114	61	323	9	114	166	9	9	427	9	61	9	61	427	9	61
Met	9	104	9	9	9	9	9	198	198	9	104	9	9	198	9	9	9	9
Tyr	9	9	136	9	9	9	9	262	262	9	9	136	136	9	262	9	262	136
Phe	9	9	9	9	9	9	9	9	9	9	108	9	9	9	9	9	9	9
Trp	9	9	9	9	9	9	9	9	9	9	366	9	9	9	9	9	9	366

Some History

- Geman & Geman, IEEE PAMI 1984
- Hastings, Biometrika, 1970
- Metropolis, Rosenbluth, Rosenbluth, Teller, & Teller, "Equations of State Calculations by Fast Computing Machines," J. Chem. Phys. 1953
- Josiah Williard Gibbs, 1839-1903, American physicist, a pioneer of thermodynamics

How to Average

An old problem:

- n random variables: $x_1, x_2, \ldots, x_k$
- Joint distribution (p.d.f.): $P(x_1, x_2, \ldots, x_k)$
- Some function: $f(x_1, x_2, \ldots, x_k)$
- Want Expected Value: $E(f(x_1, x_2, \ldots, x_k))$

How to Average

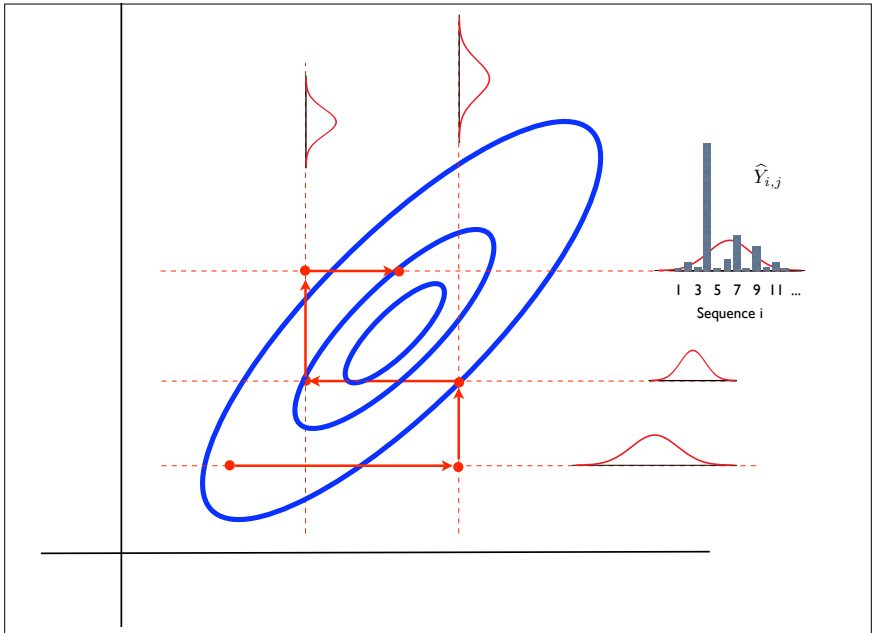
$$E(f(x_1, x_2, \ldots, x_k)) = \int_{x_1}\int_{x_2}\cdots\int_{x_k} f(x_1, x_2, \ldots, x_k) \cdot P(x_1, x_2, \ldots, x_k) dx_1 dx_2 \ldots dx_k$$

- Approach 1: direct integration
  (rarely solvable analytically, esp. in high dim)
- Approach 2: numerical integration
  (often difficult, e.g., unstable, esp. in high dim)
- Approach 3: Monte Carlo integration
  sample $\vec{x}^{(1)}, \vec{x}^{(2)}, \ldots \vec{x}^{(n)} \sim P(\vec{x})$ and average:
  $E(f(\vec{x})) \approx \frac{1}{n}\sum_{i=1}^{n} f(\vec{x}^{(i)})$

Markov Chain Monte Carlo (MCMC)

- *Independent* sampling also often hard, but *not required* for expectation
- MCMC $\vec{X}_{t+1} \sim P(\vec{X}_{t+1} \mid \vec{X}_t)$ w/ stationary dist = P
- Simplest & most common: Gibbs Sampling
  $P(x_i \mid x_1, x_2, \ldots, x_{i-1}, x_{i+1}, \ldots, x_k)$
- Algorithm
  for t = 1 to ∞
    for i = 1 to k do :

$$x_{t+1,i} \sim P(x_{t+1,i} \mid \underbrace{x_{t+1,1}, x_{t+1,2}, \ldots, x_{t+1,i-1}}_{t+1}, \underbrace{x_{t,i+1}, \ldots, x_{t,k}}_{t})$$



- Input: again assume sequences $s_1, s_2, ..., s_k$ with one length w motif per sequence
- Motif model: WMM
- Parameters: Where are the motifs? for $1 \le i \le k$, have $1 \le x_i \le |s_i|$-w+1
- "Full conditional": to calc $P(x_i = j \mid x_1, x_2, \ldots, x_{i-1}, x_{i+1}, \ldots, x_k)$ build WMM from motifs in all sequences except i, then calc prob that motif in i^{th} seq occurs at j by usual "scanning" alg.

Overall Gibbs Alg

Randomly initialize x_i's

for t = 1 to ∞

  for i = 1 to k

    discard motif instance from s_i;

    recalc WMM from rest

    for j = 1 ... $|s_i|$-w+1

      calculate prob that i^{th} motif is at j: $P(x_i = j \mid x_1, x_2, \ldots, x_{i-1}, x_{i+1}, \ldots, x_k)$

    pick new x_i according to that distribution

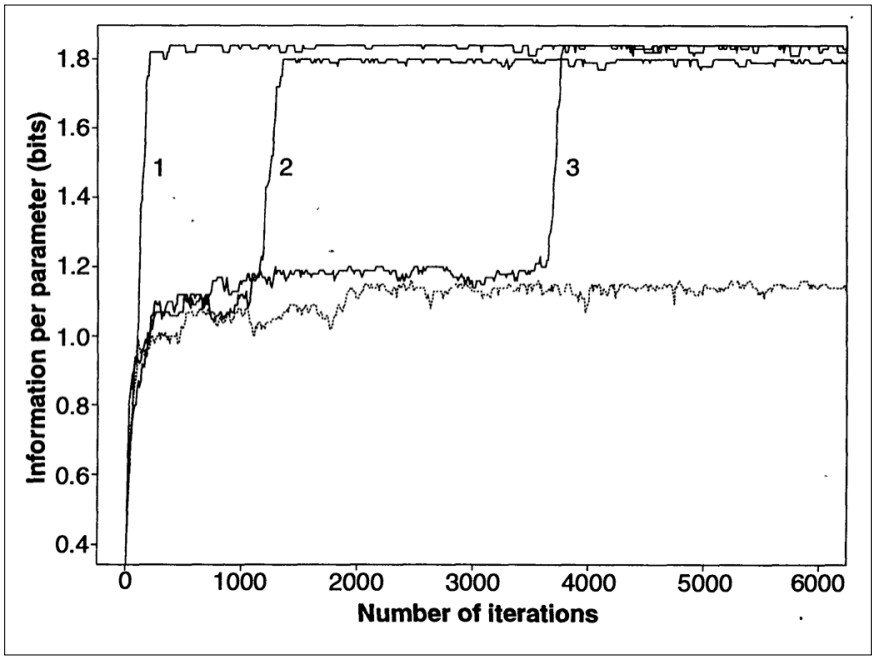
Similar to MEME, but it would average over, rather than sample from

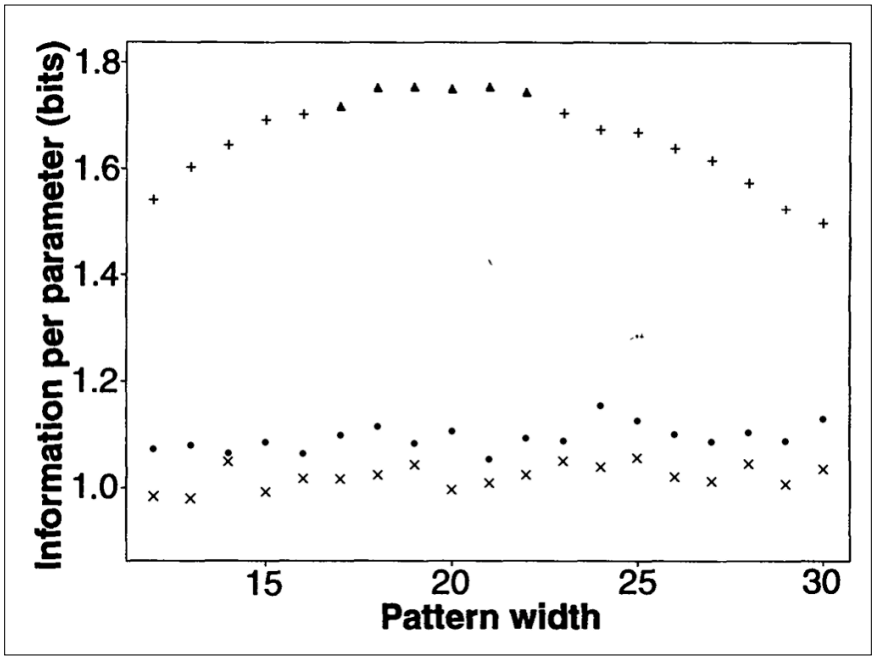
Issues

- Burnin - how long must we run the chain to reach stationarity?
- Mixing - how long a post-burnin sample must we take to get a good sample of the stationary distribution? (Recall that individual samples are not independent, and may not "move" freely through the sample space. Also, many isolated modes.)

Variants & Extensions

- "Phase Shift" - may settle on suboptimal solution that overlaps part of motif. Periodically try moving all motif instances a few spaces left or right.
- Algorithmic adjustment of pattern width: Periodically add/remove flanking positions to maximize (roughly) average relative entropy per position
- Multiple patterns per string



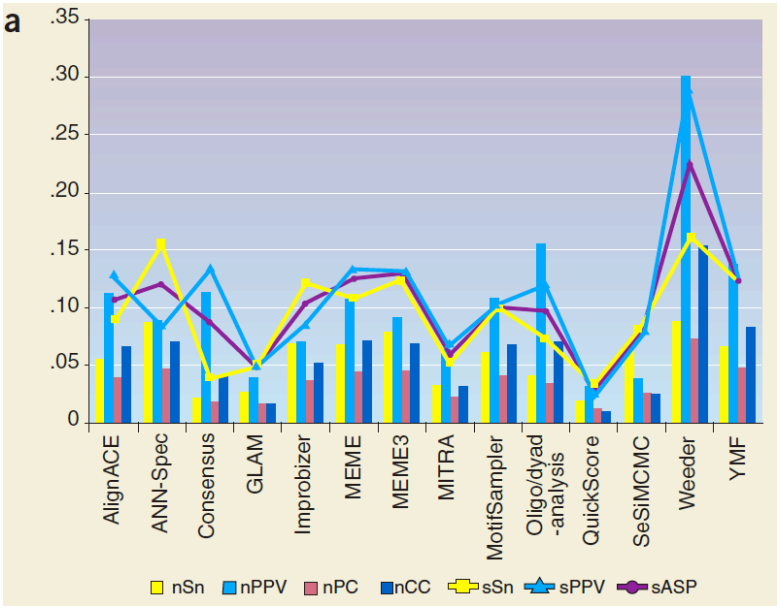


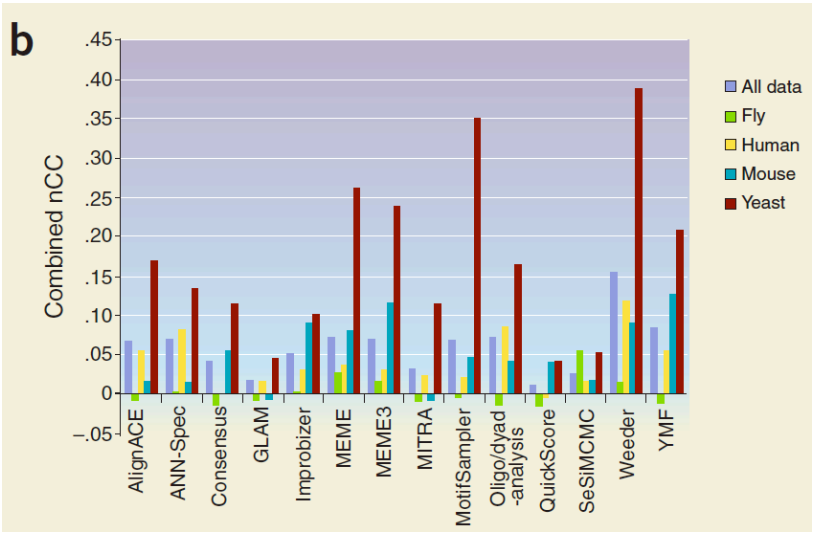
NATURE BIOTECHNOLOGY VOLUME 23 NUMBER 1 JANUARY 2005

Assessing computational tools for the discovery of transcription factor binding sites

Martin Tompa[1,2], Nan Li[1], Timothy L Bailey[3], George M Church[4], Bart De Moor[5], Eleazar Eskin[6], Alexander V Favorov[7,8], Martin C Frith[9], Yutao Fu[9], W James Kent[10], Vsevolod J Makeev[7,8], Andrei A Mironov[7,11], William Stafford Noble[1,2], Giulio Pavesi[12], Graziano Pesole[13], Mireille Régnier[14], Nicolas Simonis[15], Saurabh Sinha[16], Gert Thijs[5], Jacques van Helden[15], Mathias Vandenbogaert[14], Zhiping Weng[9], Christopher Workman[17], Chun Ye[18] & Zhou Zhu[4]

- dozen tools
- Real 'motifs' (Transfac)
- 56 data sets (human, mouse, fly, yeast)
- 'real', 'generic', 'Markov'
- expert users, top prediction only





Summary

- Important problem: a key to understanding gene regulation
- Hard problem: short, degenerate signals amidst much noise
- *Many* variants have been tried, for representation, search, and discovery. We looked at only a few:
  - Weight matrix models for representation & search
  - greedy, MEME and Gibbs for discovery
- Still much room for improvement. *Comparative genomics*, i.e. cross-species comparison is very promising