Identifying and representing DNA binding sites

Biol4230 Tues, April 17, 2018
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- Looking for functional sites: promoters, regulatory elements, modification sites
- Products of *convergent*, not divergent evolution
- Weak spacing constraints
- Usually represented as a consensus sequence
- If alignment is given, consensus is obvious
- If consensus is given, alignment is obvious
- Search for consensus and alignment together
- `consensus, meme, gibbs`

To learn more:

- Overview of multiple alignment and motif finding – Mount (2001) Chapter 4
- Schneider et al. (1986) J. Mol. Biol. Information content of binding sites on nucleotide sequences 188:415-431
- Stormo and Hartzell (1989) Identifying protein binding sites from unaligned DNA fragments
- Lawrence and Reilly (1990) An expectation maximization (EM) algorithm for the identification and characterization of common sites in unaligned biopolymer sequences PROTEINS 7:41-51
- Lawrence et al. (1993) Detecting subtle sequence signals: a Gibbs sampling strategy for multiple alignment Science 262:208-214

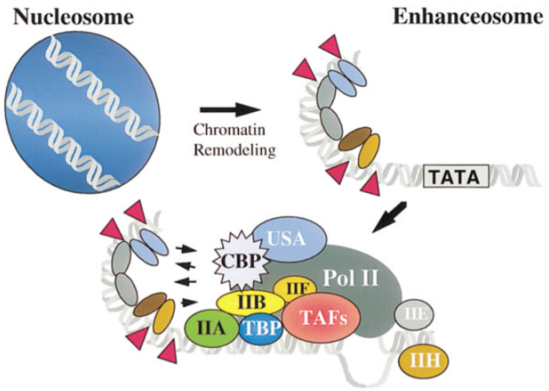
Regulation of transcription: RNA polymerase, promoters, enhancers, transcription factors, DNA binding proteins

- Gene expression is regulated at many levels:
  - production of RNA (transcription)
    - promoter occupancy, transcription rate, termination
  - transcript RNA splicing
  - mRNA stability
  - mRNA translation
  - post-translational processing/stability

Regulation of transcription: RNA polymerase, promoters, enhancers, transcription factors, DNA binding proteins

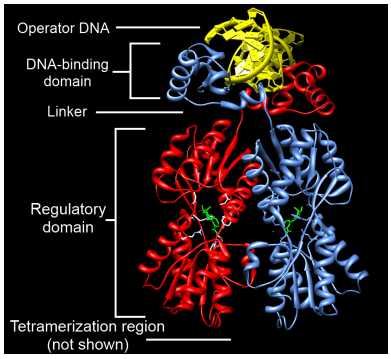
- Transcription factors interact with DNA to increase/decrease transcription levels
  - lacR (bacterial lac Repressor)
  - hundreds of transcription factors modify expression in response to signals
    - FOS/JUN
    - nuclear receptors
    - homeobox proteins
    - Myc
    - etc.,etc.

Regulation of transcription: RNA polymerase, promoters, enhancers, transcription factors, DNA binding proteins

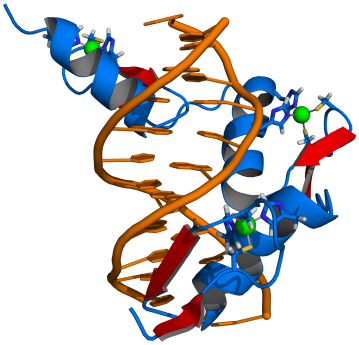


Pol II Preinitiation Complex

Regulation of transcription: RNA polymerase, promoters, enhancers, transcription factors, DNA binding proteins

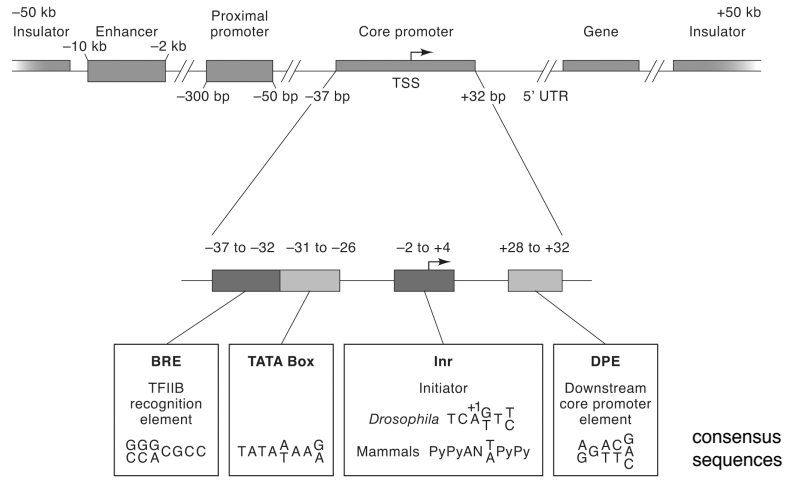


Lac repressor



Zn finger

Regulation of transcription: RNA polymerase, promoters, enhancers, transcription factors, DNA binding proteins



Transcriptional regulation in eukaryotes, concepts, strategies, and techniques. CSHL Press 2009

The "first" promoter motifs: E. coli -10, -35

Harley, C. B. & Reynolds, R. P.. *Nucleic Acids Res* **15,** 2343–2361 (1987).

Oliphant, A. R. & Struhl, K. *Nucleic Acids Res* **16,** 7673–7683 (1988).

Alignment of E. coli Promoter Sequences

SEQUENCE (a)	TYPE (b)	-35 (c)	-10 (d)	SP (e)	PHI (f)	DISCREP. (g)	(h)	TS (i)	REF (j)
aceEF	b	ACGTAGACCTGT GTTATT GAGCTTTC	CGGCGAGAG TTCAAT GGGaCAGGTCCAG	17	-4.3		-4.4	4	24
ada	b	AAGATTGTTGGTTT TTGGGT GATGGTGA	CGCCGCAGC GTAAAG GTaTCGTTAACC	17	-5.5	-3.4	-4.6	4	25,26
alaS	b	AACGCATACGGTAT TTGACC TTCCCAGTC	AAGAAAACT TATGTT ATTCCCaCTTTTCAGT	18	-3.1				9
ampC	b	TGCTATCCCTGACAG TTGTCA GGCTGATT	GCTGTCGT TACAAT GTAaCCCaTCCCCAATG	16	-1.5				9
ampG/C16	b	GCTATC TTGACA GTTGTCAC	GCTGATTGG TATGTT TACAATCTaACGTATCG	17	-1.3			1,3	25
araBAD	b	TTAGCGGATCCTAC CTGACG CTTTTTAT	GCCAACTC TCTACT GTTTCTCCATaCCCGTTT	16	-3.6		-3.7		9
araC	b	GCAAATAATCAATG TGGACT TTTCTGCC	GTGATTATA GACACT TTTGTTAGgCCTTTTTG	17	-3.6				9
araE	b	CTGTTTCCGAC CTGACA CCTGCGTGA	GTTGTTCACG TATTTT TTCACTATgTCTTACTC	19	-3.2			4	28
araI(c)	m	ACCCGATCCTAC CTGCCC CTTTTTAT	CCCAACTC TCTACT GTTTCTCCATaCCCGTTT	16	-4.3			4	29
araI(c)X(c)	m	ACCCGATCCTAC CTGCCC CTTTTTATC	GCAACTCTC TACTAT TTCTCCATaCCCGTTTT	18	-3.8			4	29
argCBH	b	TTTGTTTTTCATTG TTGACA CACCTCTGG	TCATGATAG TATCAA TATTCaCTGCAGTATT	18	-2.4		-2.6		9
argCBH-P1/6-	m	TTTGTTTTTCATTG TTGACA CACCTCT	GGTCATAA TATTAT CAATATTCaCTGCAGTAT	15	-2.0				30
argCBH-P1/LL	m	TTTGTTTTTCATTG TTGACA CACCTCT	GGTCATGA TATTAT CAATATTCaCTGCAGTAT	15	-2.0				30
argE-P1	b	TTAGCGCTGGTGCC TTTTAT TACGCTCA	ACGTTACTG TATTTT TATTCaCTAAATACTGCA	17	-2.6			4	31
argE-P2	b	CCGCATCATTGCTT TCGCGT GAAACAGT	GAAACGCGT TATGTT CATaCTGCGATGCGG	17	-3.9		-3.9	4	31
argE/LL13	m	CCGCATCATTGCTT TCGCCT GAAACAGT	GAAACGCGT TATATT CATaCTGCGATGCGG	17	-3.3				31
argF	b	ATTGTGAAATGCGG TTGCAA ATGAATAA	TTACACATA TAAAGT GAATTTTaATTCAATAA	17	-1.7			4	31,32
argI	b	AGAC TTGCAA ATGAATAA	TCATCCATA TAAATT GAATTTTaaTTCATTGA	17	-1.5			4	31
argR	b	TCGTCGCCCCCC TTGCAG GAGCAAGG	CTTTGACAA TATTAA TCAGTCTaaaGTCTCGG	17	-3.2		-5.9	2,4	31
aroF	b	TACGAAAATATGGA TTGAAA ACTTTACT	TTATGTGT TATGGT TACGTCaCTCCTCGCTG	16	-1.9			2,4	33
aroG	b	AGTGTAAAACCCCC TTTACA CATTCTGA	CGGAAGATA TACATT CGAAGTaTTCCATTCA	17	-1.6			2,4	33
aroH	b	GTACTAGACAACTA GTGCAT TAGCTTAT	TTTTTTGT TATCAT GCTAaccaCCCGCCCAG	16	-3.1				9
bioA	b	GCCTTCTCCAAAAC GTGTTT TTTGTTGTT	AATTCGGTG TAGACT TGTaaaaCCTAAATCT	18	-3.8	-3.4			9
bioB	b	TTGTCATAATCGAC TTGTAA ACCAAATT	GAAAAGATT TAGGTT TACAAGTcCACACCGAA	17	-2.2				9

A.

	-35							-10						
G	1	4	42	2	2	8	...	3	1	11	7	6	2	G
C	0	4	3	15	32	11	...	15	2	14	8	24	1	C
A	0	3	1	33	11	16	...	1	47	13	21	12	2	A
T	57	47	12	8	13	23	...	33	2	14	16	10	47	T

B.

15	16	17	18	19
2	18	59	26	5

Figure 5: Matrix for the consensus of *E.coli* promoter elements. The number of times each base occurs in each position of the -35 and -10 sequence elements selected from random DNA (A), and the number of those elements separated by a spacing of from 15 to 19 base pairs (B).

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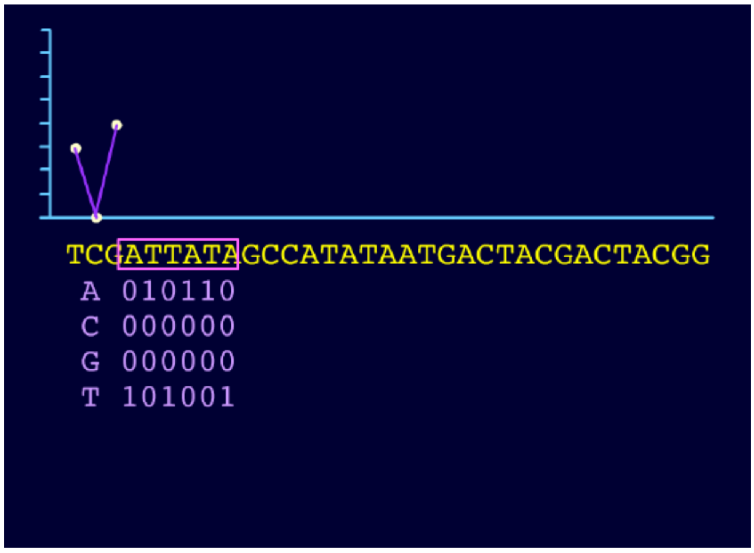
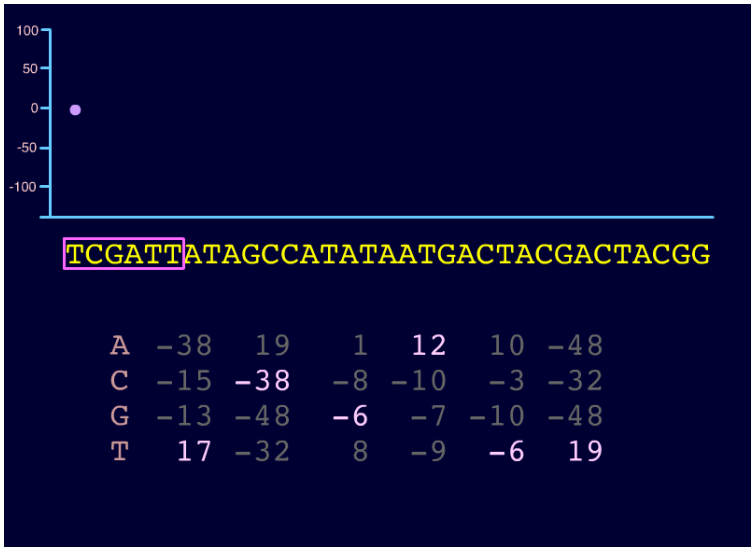
Consensus Patterns and Motifs

- How to represent motifs
  - consensus patterns
  - weight matrices – Position Specific Scoring Matrices
- How to "weight" positions?
  - frequency
  - information content (Σ log odds)
- How to search for motifs
  - Heuristic greedy (consensus, wconsensus)
  - Expectation-Maximization (MEME)
  - Gibbs sampling

Scanning a sequence with PATSER

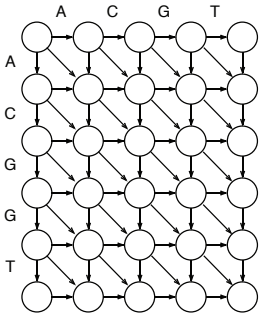


Scanning a sequence with PATSER (PWM, PSSM)

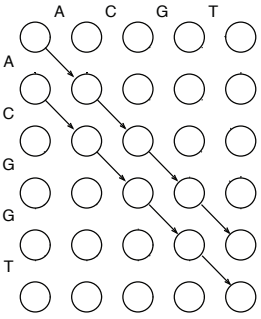


Pattern scanning vs Alignment Computational complexity

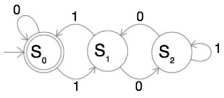
Pairwise alignment
PSSM with gaps
$O(n^2)$



Global alignment
PSSM/PWM no gaps
$O(n^2)$



Exact match
No gaps
$O(n)$



wikipedia.org/wiki/
Deterministic_finite_automaton

Consensus Patterns and Motifs

- How to represent motifs
  - consensus patterns
  - weight matrices
    - do not require identity
    - include consensus (1's, 0's)
    - allow mismatches

Position Specific Scoring Matrix

$$S = \log\left(\frac{f_{pos,b}}{p_b}\right)$$

How to represent motifs – information content

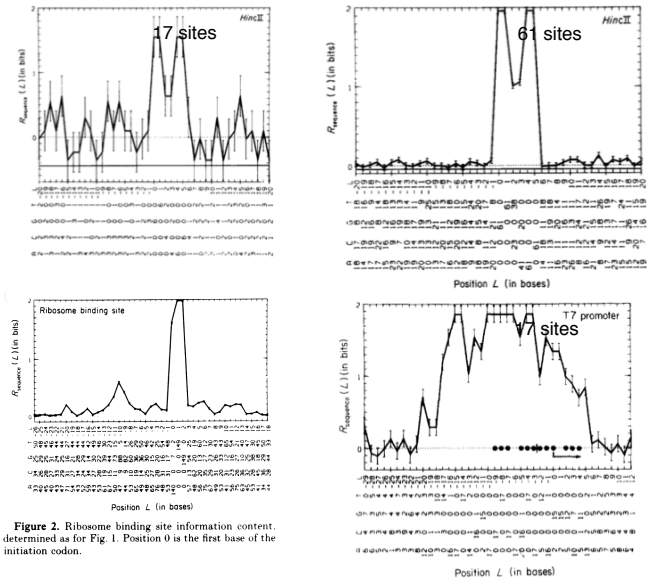


Figure 2. Ribosome binding site information content, determined as for Fig. 1. Position 0 is the first base of the initiation codon.

Schneider TD, Stormo GD, Gold L, Ehrenfeucht A. *J Mol Biol.* (1986) **188**:415-431
Information content of binding sites on nucleotide sequences.

Representing Consensus Sequences ("TATAA" box)

present/absent

A	0	1	0	1	1	0
C	0	0	0	0	0	0
G	0	0	0	0	0	0
T	1	0	1	0	0	1

counts

A	0	11	4	7	9	0
C	1	0	1	2	1	0
G	1	0	1	2	1	0
T	10	1	6	1	1	12

percent

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

$S = f_{pos,b}$

log-odds

A	-38	19	1	12	10	-48
C	-15	-38	-8	-10	-3	-32
G	-13	-48	-6	-7	-10	-48
T	17	-32	8	-9	-6	19

$$S = \log\left(\frac{f_{pos,b}}{p_b}\right)$$

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Consensus Patterns and Motifs

- How to represent motifs
  - consensus patterns
  - weight matrices
- How to "weight" positions?
  - frequency $S = f_{pos,b}$
  - information content Σ (log odds) $S = \log\left(\frac{f_{pos,b}}{p_b}\right)$
- How to search for motifs
  - heuristic greedy
  - Gibbs sampling

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Representing Consensus Sequences

counts

A	9	214	63	142	118	8
C	22	7	26	31	52	13
G	18	2	29	38	29	5
T	193	19	124	31	43	216

frequency

A	0.04	0.88	0.26	0.59	0.49	0.03
C	0.09	0.03	0.11	0.13	0.22	0.05
G	0.07	0.01	0.12	0.16	0.12	0.02
T	0.80	0.08	0.51	0.13	0.18	0.89

log(2)-odds

A	-2.76	1.82	0.06	1.23	0.96	-2.92
C	-1.46	-3.11	-1.22	-1.00	-0.22	-2.21
G	-1.76	-5.00	-1.06	-0.67	-1.06	-3.58
T	1.67	-1.66	1.04	-1.00	-0.49	1.84

$$I_p = \sum_b^{A..T} f_b \log_2\left(\frac{f_b}{p_b}\right)$$

position-specific information content (bits)

I	0.98	1.33	0.28	0.38	0.22	1.35

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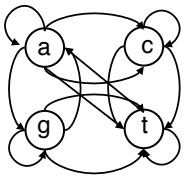
Where do scoring matrices come from?



$$\lambda S = \log\left(\frac{q_{ij}}{p_j}\right)$$

probability of mutation

probability of alignment by chance

q_{ij} = M^20= PAM20

```
{0.828, 0.019, 0.133, 0.019},
{0.019, 0.828, 0.019, 0.133},
{0.133, 0.019, 0.828, 0.019},
{0.019, 0.133, 0.019, 0.828}}
```

p_i(a,c,g,t)=

p_j=0.25

$$\lambda S = 10\log\left(\frac{q_{a,c}}{p_c}\right)$$

$$= 10\log\left(\frac{0.019}{0.25}\right) = -11.2$$

$$\lambda_2 = \frac{\log(2)}{10} = 0.33$$

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Ranking criterion: Information Content

$$\text{Information content} = \frac{1}{\text{N}} \textit{LogLikelihood Ratio}$$

$$I_{total} = \sum_{pos=1}^{n} \sum_{b}^{A..T} f_{pos,b} \log_2 \frac{f_{pos,b}}{p_b}$$

Position independent Scoring Matrix

$$S = \log\left(\frac{f_{ij}}{p_j}\right)$$

Position Specific Scoring Matrix (PSSM)

$$S = \log\left(\frac{f_{pos,b}}{p_b}\right)$$

Information content and binding energy

If we denote H_i the *binding energy* for a DNA site S_i, then the probability that the protein would be bound to S_i (at equilibrium) is given by the Boltzman distribution:

$$P_i = \frac{e^{-Hi}}{Z}$$

where Z is the *partition function* and is defined as the sum of the e^{-Hi} over all possible sites S_x:

$$Z = \sum_x e^{-Hx}$$

The average binding energy for this protein over all sites S_i would be:

$$\langle H \rangle = \sum P_i H_i = -\sum_i (P_i \ln P_i) - \ln Z$$

The sum on the right is called the entropy of the probability distribution.

A useful measure of difference between two probability distributions is the relative entropy, which is defined as:

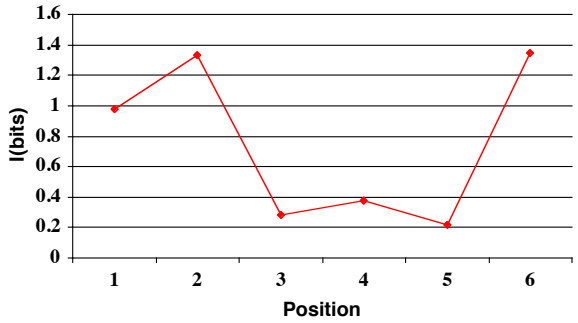
$$H(P,Q) \equiv \sum_i P_i \ln \frac{P_i}{Q_i}$$

Benos, P. V., Lapedes, A. S. & Stormo, G. D. *Bioessays* **24,** 466–475 (2002).

Conensus Information Content

$$I_p = \sum_{b}^{A..T} f_b \log\left(\frac{f_b}{p_b}\right)$$

I	0.98	1.33	0.28	0.38	0.22	1.35



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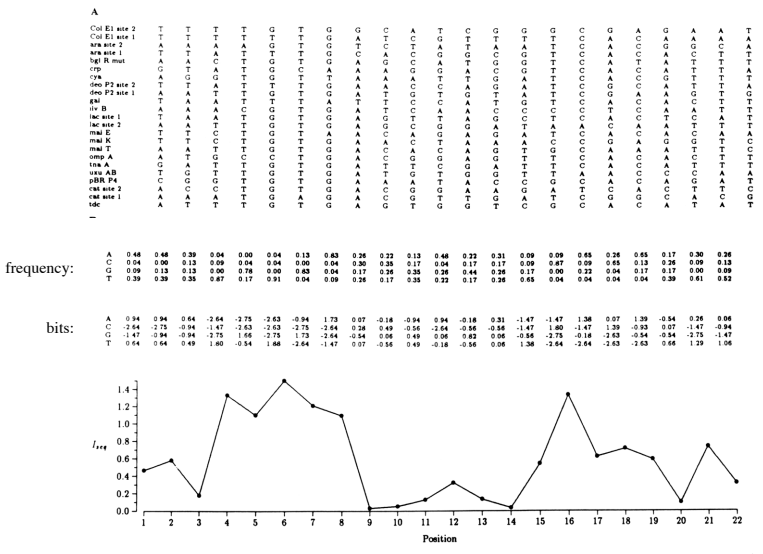

FIG. 1. CRP binding sites. (*A*) Twenty-three sites identified as binding to CRP (8, 9). (*B*) The frequency with which each base occurs at each position in the CRP binding sites. (*C*) The specificity matrix for the protein, based on the binding sites, which is calculated as $\log_2(f_b/p_b)$, where f_b is the observed frequency of each base (from the matrix above) and p_b is the *a priori* probability of obtaining base *b*. In this example p_b = 0.25 for all *b*, approximating the *E. coli* genomic composition. At positions for which $f_b = 0$, an estimated frequency of 0.5/23 is used in the calculation. (*D*) The "information content" at each position of the CRP binding sites is plotted (10). The sum of all positions is 13.06 bits.

Stormo GD, Hartzell GW (1989) Identifying protein-binding sites from unaligned DNA fragments.Proc Natl Acad Sci U S A. 86:1183-1187.

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Identifying Functional Domains in Biological Sequences

A problem in:
Feature Detection
or
Multiple Alignment

Two parts to the problem:

1. Can't look at all possible alignments, pick a subset likely to contain the answer
2. Need criterion for ranking alignments that is reasonable and efficient

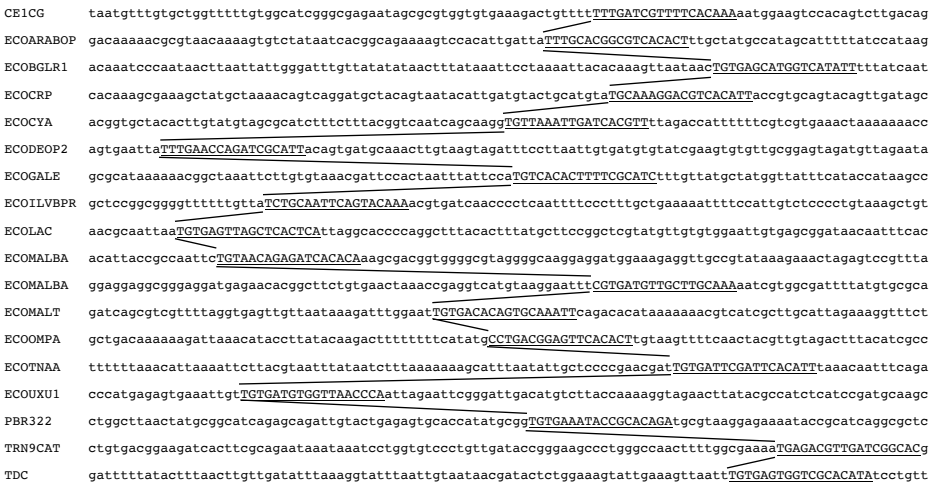
E. coli CRP binding regions

```
CE1CG      taatgtttgtgctggtttttgtggcatcgggcgagaatagcgcgtggtgtgaaagactgttttttgatcgttttcacaaaaatggaagtccacagtcttgacag
ECOARABOP  gacaaaaacgcgtaacaaaagtgtctataatcacggcagaaaagtccacattgattatttgcacggcgtcacactttgctatgccatagcatttttatccataag
ECOBGLR1   acaaatcccaataacttaattattgggatttgttatatataactttataaattcctaaaattacacaaagttaataactgtgagcatggtcatatttttatcaat
ECOCRP     cacaaagcgaaagctatgctaaaacagtcaggatgctacagtaatacattgatgtactgcatgtatgcaaaggacgtcacattaccgtgcagtacagttgatagc
ECOCYA     acggtgctacacttgtatgtagcgcatctttctttacggtcaatcagcaaggtgttaaattgatcacgttttagaccatttttcgtcgtgaaactaaaaaaacc
ECODEOP2   agtgaattatttgaaccagatcgcattacagtgatgcaaacttgtaagtagatttccttaattgtgatgtgtatcgaagtgtgttgcggagtagatgttagaata
ECOGALE    gcgcataaaaaacggctaaattcttgtgtaaacgattccactaatttattccatgtcacacttttcgcatctttgttatgctatggttatttcataccataagcc
ECOILVBPR  gctccggcggggttttttgttatctgcaattcagtacaaaacgtgatcaacccctcaattttccctttgctgaaaaattttccattgtctcccctgtaaagctgt
ECOLAC     aacgcaattaatgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcac
ECOMALBA   acattaccgccaattctgtaacagagatcacacaaagcgacggtggggcgtaggggcaaggaggatggaaagaggttgccgtataaagaaactagagtccgttta
ECOMALBA   ggaggaggcgggaggatgagaacacggcttctgtgaactaaaccgaggtcatgtaaggaatttcgtgatgttgcttgcaaaaatcgtggcgattttatgtgcgca
ECOMALT    gatcagcgtcgttttaggtgagttgttaataaagatttggaattgtgacacagtgcaaattcagacacataaaaaaacgtcatcgcttgcattagaaaggtttct
ECOOMPA    gctgacaaaaaagattaaacatacctatacaagactttttttcatatgcctgacggagttcacacttgtaagttttcaactacgttgtagactttacatcgcc
ECOTNAA    ttttttaaacattaaaattcttacgtaatttataatctttaaaaaaagcatttaatattgctccccgaacgattgtgattcgattcacatttaaacaatttcaga
ECOUXU1    cccatgagagtgaaattgttgtgatgtggttaacccaattagaattcgggattgacatgtcttaccaaaaggtagaacttatacgccatctcatccgatgcaagc
PBR322     ctggcttaactatgcggcatcagagcagattgtactgagagtgcaccatatgcggtgtgaaataccgcacagatgcgtaaggagaaaataccgcatcaggcgctc
TRN9CAT    ctgtgacggaagatcacttcgcagaataaataaatcctggtgtccctgttgataccgggaagccctgggccaacttttggcgaaaatgagacgttgatcggcacg
TDC        gattttatactttaacttgttgatatttaaaggtatttaattgtaataacgatactctggaaagtattgaaagttaatttgtgagtggtcgcacatatcctgtt
```


E. coli CRP binding sites

```
CE1CG      taatgtttgtgctggtttttgtggcatcgggcgagaatagcgcgtggtgtgaaagactgttttTTTGATCGTTTTCACAAAaatggaagtccacagtcttgacag
ECOARABOP  gacaaaaacgcgtaacaaaagtgtctataatcacggcagaaaagtccacattgattaTTTGCACGGCGTCACACTttgctatgccatagcatttttatccataag
ECOBGLR1   acaaatcccaataacttaattattgggatttgttatatataactttataaattcctaaaattacacaaagttaataacTGTGAGCATGGTCATATTtttatcaat
ECOCRP     cacaaagcgaaagctatgctaaaacagtcaggatgctacagtaatacattgatgtactgcatgtaTGCAAAGGACGTCACATTaccgtgcagtacagttgatagc
ECOCYA     acggtgctacacttgtatgtagcgcatctttctttacggtcaatcagcaaggTGTTAAATTGATCACGTTttagaccatttttcgtcgtgaaactaaaaaaacc
ECODEOP2   agtgaattaTTTGAACCAGATCGCATTacagtgatgcaaacttgtaagtagatttccttaattgtgatgtgtatcgaagtgtgttgcggagtagatgttagaata
ECOGALE    gcgcataaaaaacggctaaattcttgtgtaaacgattccactaatttattccaTGTCACACTTTTCGCATCtttgttatgctatggttatttcataccataagcc
ECOILVBPR  gctccggcggggttttttgttaTCTGCAATTCAGTACAAAacgtgatcaacccctcaattttccctttgctgaaaaattttccattgtctcccctgtaaagctgt
ECOLAC     aacgcaattaaTGTGAGTTAGCTCACTCAttaggcaccccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcac
ECOMALBA   acattaccgccaattcTGTAACAGAGATCACACAaagcgacggtggggcgtaggggcaaggaggatggaaagaggttgccgtataaagaaactagagtccgttta
ECOMALBA   ggaggaggcgggaggatgagaacacggcttctgtgaactaaaccgaggtcatgtaaggaatttCGTGATGTTGCTTGCAAAaatcgtggcgattttatgtgcgca
ECOMALT    gatcagcgtcgttttaggtgagttgttaataaagatttggaatTGTGACACAGTGCAAATTcagacacataaaaaaacgtcatcgcttgcattagaaaggtttct
ECOOMPA    gctgacaaaaaagattaaacatacctatacaagactttttttcatatgCCTGACGGAGTTCACACTtgtaagttttcaactacgttgtagactttacatcgcc
ECOTNAA    ttttttaaacattaaaattcttacgtaatttataatctttaaaaaaagcatttaatattgctccccgaacgatTGTGATTCGATTCACATTtaaacaatttcaga
ECOUXU1    cccatgagagtgaaattgtTGTGATGTGGTTAACCCAattagaattcgggattgacatgtcttaccaaaaggtagaacttatacgccatctcatccgatgcaagc
PBR322     ctggcttaactatgcggcatcagagcagattgtactgagagtgcaccatatgcggTGTGAAATACCGCACAGAtgcgtaaggagaaaataccgcatcaggcgctc
TRN9CAT    ctgtgacggaagatcacttcgcagaataaataaatcctggtgtccctgttgataccgggaagccctgggccaacttttggcgaaaaTGAGACGTTGATCGGCACg
TDC        gattttatactttaacttgttgatatttaaaggtatttaattgtaataacgatactctggaaagtattgaaagttaattTGTGAGTGGTCGCACATAtcctgtt
```

E. coli CRP binding sites – location gives alignment



Aligned CRP binding sites

```
CE1CG     cgcgtggtgtgaaagactgtttttTTTGATCGTTTTCACAAAaatggaagtccacagtcttgacag
ECOARABOP gcagaaaagtccacattgattaTTTGCACGGCGTCACACTttgctatgccatagcatttttatccataag
ECOBGLR1 taaaattacacaaagttaataacTGTGAGCATGGTCATATTtttatcaat
ECOCRP   aatacattgatgtactgcatgtaTGCAAAGGACGTCACATTaccgtgcagtacagttgatagc
ECOCYA   ttctttacggtcaatcagcaaggTGTTAAATTGATCACGTTttagaccatttttcgtcgtgaaactaaaa
ECODEOP2              agtgaattaTTTGAACCAGATCGCATTacagtgatgcaaacttgtaagtagatttccttaat
ECOGALE  aacgattccactaatttattccaTGTCACACTTTTCGCATCtttgttatgctatggttatttcataccata
ECOILVBPR gctccggcgggggtttttgttaTCTGCAATTCAGTACAAAacgtgatcaacccctcaattttcccttgctg
ECOLAC               aacgcaattaaTGTGAGTTAGCTCACTCAttaggcaccccaggctttacactttatgcttccggct
ECOMALBA        acattaccgccaattcTGTAACAGAGATCACACAaagcgacggtggggcgtaggggcaaggaggatggaaa
ECOMALBA aaccgaggtcatgtaaggaatttCGTGATGTTGCTTGCAAAaatcgtggcgattttatgtgcgca
ECOMALT gagttgttaataaagatttggaatTGTGACACAGTGCAAATTcagacacataaaaaaacgtcatcgcttgcattagaaa
ECOOMPA tatacaagactttttttcatatgCCTGACGGAGTTCACACTtgtaagttttcaactacgttgtagactttacatcgcc
ECOT    atttaatattgctccccgaacgatTGTGATTCGATTCACATTtaaacaatttcaga
ECOUXU1      cccatgagagtgaaattgtTGTGATGTGGTTAACCCAattagaattcgggattgacatgtcttaccaaaaggta
PBR322  gtactgagagtgcaccatatgcggTGTGAAATACCGCACAGAtgcgtaaggagaaaatacccgcatcaggcgctc
TRN9CAT ccctgggccaacttttggcgaaaaTGAGACGTTGATCGGCACg
TDC     tctggaaagtattgaaagttaatttGTGAGTGGTCGCACATAtcctgtt
```

Ranking criterion: Information Content

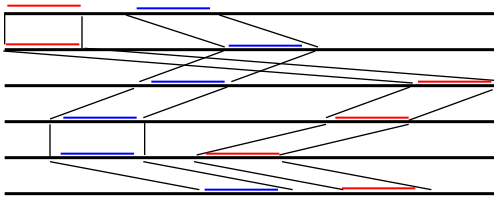
$$\text{Information content} = \frac{1}{N} LogLikelihood\ \ Ratio$$

$$I_{total} = \sum_{pos=1}^{n} \sum_{b}^{A..T} f_{pos,b} \log_2 \frac{f_{pos,b}}{p_b}$$

Consensus Patterns and Motifs

- How to represent motifs
  - consensus patterns
  - weight matrices
- How to "weight" positions?
  - frequency
  - information content (Σ log odds)
- How to search for motifs
  - heuristic greedy (consensus, wconsensus)
  - Expectation-Maximization (MEME)
  - Gibbs sampling

Finding a consensus sequence: `consensus`



Finding a consensus sequence: `consensus`

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

l=12.0

	A	C	T	G	A	A
A	1	0	0	0	1	1
C	0	1	0	0	0	0
G	0	0	0	1	0	0
T	0	0	1	0	0	0

l=12.0

	C	T	G	A	A	T
A	0	0	0	1	1	0
C	1	0	0	0	0	0
G	0	0	1	0	0	0
T	0	1	0	0	0	1

l=8.0

	A	C	T	G	A	A
	A	G	C	G	T	C
A	2	0	0	0	1	1
C	0	1	1	0	0	1
G	0	1	0	2	0	0
T	0	0	1	0	1	0

l=7.0

	A	C	T	G	A	A
	G	C	G	T	C	C
A	1	0	0	0	1	1
C	0	2	0	0	1	0
G	1	0	1	1	0	1
T	0	0	1	1	0	0

l=6.0

	C	T	G	A	A	T
	A	G	C	G	T	C
A	1	0	0	1	1	0
C	1	0	1	0	0	1
G	0	1	1	1	0	0
T	0	1	0	0	1	1

l=7.0

	C	T	G	A	A	T
	G	C	G	T	C	C
A	0	0	0	1	1	0
C	1	1	0	0	1	1
G	1	0	2	0	0	0
T	0	1	0	1	0	1

l=6.1

	A	C	T	G	A	A
	A	G	C	G	T	C
	C	T	T	G	C	C
A	2	0	0	0	1	1
C	1	1	1	0	1	2
G	0	1	0	3	0	0
T	0	1	2	0	1	0

l=3.8

	A	C	T	G	A	A
	A	G	C	G	T	C
	T	T	G	C	C	G
A	2	0	0	0	1	1
C	0	1	1	1	1	1
G	0	1	1	2	0	1
T	1	1	1	0	1	0

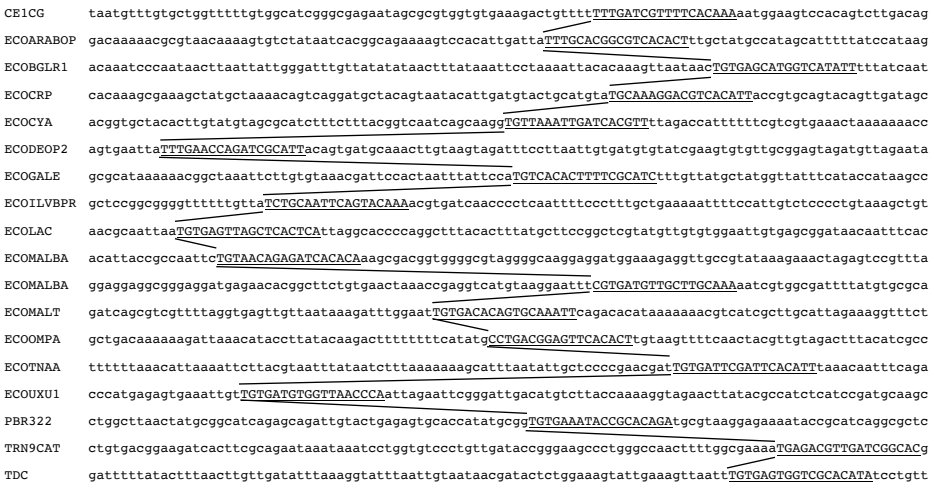
l=5.8

	C	T	G	A	A	T
	G	C	G	T	C	C
	C	T	T	G	C	C
A	0	0	0	1	1	0
C	2	1	0	0	2	2
G	1	0	2	1	0	0
T	0	2	1	1	0	1

l=5.4

	C	T	G	A	A	T
	G	C	G	T	C	C
	T	T	G	C	C	G
A	0	0	0	1	1	0
C	1	1	0	1	2	1
G	1	0	3	0	0	1
T	1	2	0	1	0	1

E. coli CRP binding sites



Aligned CRP binding sites

```
CE1CG    cgcgtggtgtgaaagactgtttttTTTGATCGTTTTCACAAAaatggaagtccacagtcttgacag
ECOARABOP gcagaaaagtccacattgattaTTTGCACGGCGTCACACTttgctatgccatagcatttttatccataag
ECOBGLR1 taaaattacacaaagttaataacTGTGAGCATGGTCATATTtttatcaat
ECOCRP   aatacattgatgtactgcatgtaTGCAAAGGACGTCACATTaccgtgcagtacagttgatagc
ECOCYA   ttctttacggtcaatcagcaaggTGTTAAATTGATCACGTTttagaccatttttcgtcgtgaaactaaaa
ECODEOP2              agtgaattaTTTGAACCAGATCGCATTacagtgatgcaaacttgtaagtagatttccttaat
ECOGALE  aacgattccactaatttattccaTGTCACACTTTTCGCATCtttgttatgctatggttatttcataccata
ECOILVBPR gctccggcggggttttttgttaTCTGCAATTCAGTACAAAacgtgatcaacccctcaattttcccttgctg
ECOLAC               aacgcaattaaTGTGAGTTAGCTCACTCAttaggcaccccaggctttacactttatgcttccggct
ECOMALBA        acattaccgccaattcTGTAACAGAGATCACACAaagcgacggtggggcgtaggggcaaggaggatggaaa
ECOMALBA aaccgaggtcatgtaaggaatttCGTGATGTTGCTTGCAAAaatcgtggcgattttatgtgcgca
ECOMALT gagttgttaataaagatttggaatTGTGACACAGTGCAAATTcagacacataaaaaaacgtcatcgcttgcattagaaa
ECOOMPA tatacaagacttttttttcatatgCCTGACGGAGTTCACACTtgtaagttttcaactacgttgtagactttacatcgcc
ECOT    atttaatattgctccccgaacgatTGTGATTCGATTCACATTtaaacaatttcaga
ECOUXU1      cccatgagagtgaaattgtTGTGATGTGGTTAACCCAattagaattcgggattgacatgtcttaccaaaaggta
PBR322  gtactgagagtgcaccatatgcggTGTGAAATACCGCACAGAtgcgtaaggagaaaatacccgcatcaggcgctc
TRN9CAT ccctgggccaacttttggcgaaaaTGAGACGTTGATCGGCACg
TDC     tctggaaagtattgaaagttaattTGTGAGTGGTCGCACATAtcctgtt
```

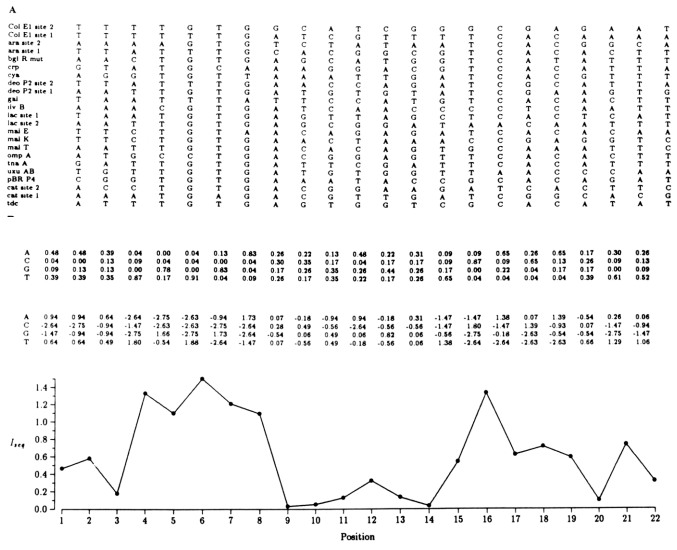


FIG. 1. CRP binding sites. (*A*) Twenty-three sites identified as binding to CRP (8, 9). (*B*) The frequency with which each base occurs at each position in the CRP binding sites. (*C*) The specificity matrix for the protein, based on the binding sites, which is calculated as $\log_2(f_b/p_b)$, where f_b is the observed frequency of each base (from the matrix above) and p_b is the *a priori* probability of obtaining base *b*. In this example p_b = 0.25 for all *b*, approximating the *E. coli* genomic composition. At positions for which $f_b = 0$, an estimated frequency of 0.5/23 is used in the calculation. (*D*) The "information content" at each position of the CRP binding sites is plotted (10). The sum of all positions is 13.06 bits.

Stormo GD, Hartzell GW (1989) Identifying protein-binding sites from unaligned DNA fragments.Proc Natl Acad Sci U S A. 86:1183-1187.

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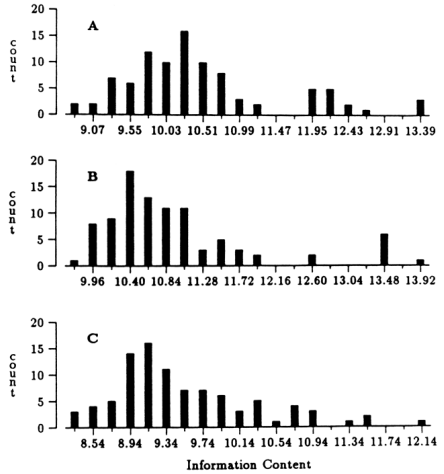

FIG. 3. Distribution of "information content" of the matrices at the end of each analysis, described in the text. Count is the number of matrices with "information content" in the interval shown. (*A*) The 94 20-wide matrices. (*B*) The 93 22-wide matrices. (*C*) The 93 16-wide matrices.

Stormo GD, Hartzell GW (1989) Identifying protein-binding sites from unaligned DNA fragments.Proc Natl Acad Sci U S A. 86:1183-1187.

fasta.bioch.virginia.edu/biol4230

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1186 Biochemistry: Stormo and Hartzell *Proc. Natl. Acad. Sci. USA 86 (1989)*

A

A	0.00	0.00	0.00	0.17	0.78	0.28	0.17	0.11	0.50	0.06	0.11	0.00	0.06	0.63	0.11	0.89
C	0.22	0.06	0.06	0.00	0.06	0.22	0.50	0.28	0.00	0.28	0.22	0.06	0.72	0.00	0.72	0.06
G	0.06	0.61	0.00	0.72	0.00	0.11	0.22	0.33	0.17	0.56	0.28	0.11	0.17	0.11	0.00	0.00
T	0.72	0.33	0.94	0.11	0.17	0.39	0.11	0.28	0.33	0.11	0.39	0.83	0.06	0.06	0.17	0.06

B

A	-3.43	-3.43	-3.43	-0.82	1.38	-0.10	-0.82	-1.45	0.74	-2.32	-1.45	-3.43	-2.32	1.47	-1.45	1.57
C	0.29	-1.59	-1.59	-0.97	-1.59	0.29	1.47	0.64	-2.70	0.64	0.29	-1.59	2.00	-2.70	2.00	-1.59
G	-1.81	1.54	-2.92	1.78	-2.92	-0.93	0.07	0.65	-0.31	1.42	0.42	-0.93	-0.31	-0.93	-2.92	-2.92
T	1.22	0.09	1.60	-1.50	-0.87	0.33	-1.50	-0.15	0.09	-1.50	0.33	1.42	-2.37	-2.37	-0.87	-2.37

C

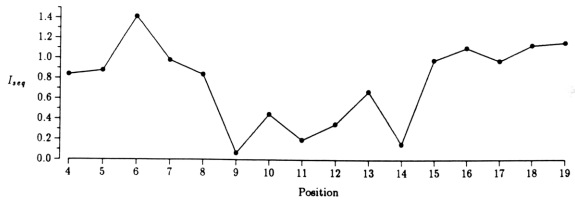


FIG. 4. The best 16-wide matrix. The positions are numbered 4–19, corresponding to the central positions of Fig. 1. (*A*) The frequency of each base for the sites included in the best matrix, as in Fig. 1*B*. (*B*) The specificity matrix determined from the frequency matrix, as in Fig. 1*C*. In this case the *a priori* values were determined from the data set shown in Fig. 2: $p_A = 0.30$; $p_C = 0.18$; $p_G = 0.21$; and $p_T = 0.31$. Analyses were also performed using $p_b = 0.25$ for all b. In that case, essentially the same matrix remained the best, but the distribution had more high-scoring matrices due to the high probability of adenine and thymine matches. The specificity matrix values for positions with $f_b = 0$ were estimated using $f_b = 0.5/18$ from the 18 sequences in the data set. (*C*) The "information content" at each position of the matrix is plotted. The sum from all positions is 12.15 bits.

Stormo GD, Hartzell GW (1989) Identifying protein-binding sites from unaligned DNA fragments.Proc Natl Acad Sci U S A. 86:1183-1187.

Consensus (CRP)

```
consensus -L 16 -q 1000 -c0 -pr2 -pt 4 -pf 4

L-mer Width: 16

Top Matrices saved from each cycle: 4
    Matrices Saved from the last cycle: 4

sequence 1: CE1CG     fragments: 1-105
sequence 18: TDC      fragments: 1-105
Total number of sequences: 18.
Total number of sequence fragments: 18.

# Observed frequency and occurrence of each letter.
#anumber of letters in the input sequences = 1890
A  0.302646; observed occurrence =   572 (letter   1)
C  0.182540; observed occurrence =   345 (letter   2)
G  0.208995; observed occurrence =   395 (letter   3)
T  0.305820; observed occurrence =   578 (letter   4)

PRIOR FREQUENCIES DETERMINED BY OBSERVED FREQUENCIES.
* Information for the alphabet from the command line.
letter   1: A (complement: T)  prior freq = 0.302646 CYCLE
letter   2: C (complement: G)  prior freq = 0.182540
letter   3: G (complement: C)  prior freq = 0.208995
letter   4: T (complement: A)  prior freq = 0.305820

INFORMATION CONTENT IS CALCULATED USING NATURAL
LOGARITHMS (i.e. BASE e). DIVIDE BY ln(2) = 0.693 TO
CONVERT TO BASE 2, WHICH WAS USED INPREVIOUS VERSIONS
OF THIS PROGRAM.
```

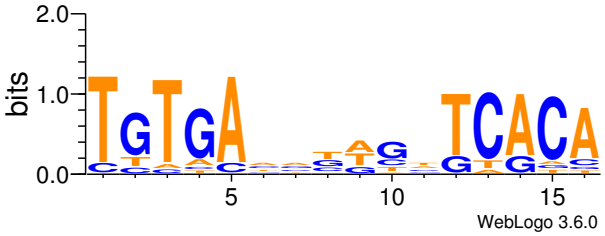
MATRICES SAVED FOR NEXT CYCLE

number	total	top adjusted information	ln top p-value	ln expected frequency
1	1620	2.9492	0.0000	7.3902
2	748	6.8222	-13.5825	0.4475
3	849	8.2051	-20.1461	0.0577
4	832	8.7882	-26.3882	-0.3628
5	848	8.9275	-31.8728	-0.3179
6	857	9.1860	-38.8902	-2.0624
7	877	9.2908	-45.6680	-3.8014
8	864	9.1929	-51.3100	-4.6251
9	876	9.1152	-57.2498	-5.9597
10	879	9.0644	-63.5596	-7.8751
11	864	8.8973	-68.7012	-8.8353
12	854	8.8738	-75.4184	-11.5917
13	850	8.6817	-80.0738	-12.5205
14	873	8.5267	-85.0113	-13.9878
15	852	8.2955	-88.6578	-14.4562
16	865	8.1066	-92.6288	-15.6014
17	857	7.8793	-95.7075	-16.3204
18	936	7.6066	-97.6650	-16.6684

```
  1|11  :   1/64    TTTGATCGTTTTCACA
  2|9   :   2/58    TTTGCACGGCGTCACA
  3|14  :   3/79    TGTGAGCATGGTCATA
  4|8   :   4/66    TGCAAAGGACGTCACA
  5|18  :   5/53    TGTTAAATTGATCACG
  6|10  :   6/10    TTTGAACCAGATCGCA
  7|13  :   7/54    TGTCACACTTTTCGCA
  8|1   :   8/23    TCTGCAATTCAGTACA
  9|4   :   9/12    TGTGAGTTAGCTCACT
 10|5   :  10/17    TGTAACAGAGATCACA
 11|15  :  11/64    CGTGATGTTGCTTGCA
 12|6   :  12/44    TGTGACACAGTGCAAA
 13|7   :  13/51    CCTGACGGAGTTCACA
 14|12  :  14/74    TGTGATTCGATTCACA
 15|16  :  15/20    TGTGATGTGGTTAACC
 16|2   :  16/56    TGTGAAATACCGCACA
 17|17  :  17/87    TGAGACGTTGATCGGC
 18|3   :  18/81    TGTGAGTGGTCGCACA
```

```
THE LIST OF TOP MATRICES FROM EACH CYCLE-- (total of 17):

MATRIX 1
number of sequences = 18
unadjusted information = 9.03227
sample size adjusted information = 7.60657
ln(p-value) = -97.665   p-value = 3.84279E-43
ln(expected frequency) = -16.6684   expected frequency = 5.76782E-08
A |   0   0   1   2  16   6   6   1   7   1   5   0   1  14   1  14
C |   2   2   1   1   2   5   4   4   0   4   4   0  15   0  15   2
G |   0  13   0  14   0   3   5   6   4  10   3   4   0   4   1   1
T |  16   3  16   1   0   4   3   7   7   3   6  14   2   0   1   1
```




```
consensus -L 16 -q 1000 -c0 -pr2 -pt 4 -pf 4

L-mer Width: 16

Top Matrices saved from each cycle: 4
    Matrices Saved from the last cycle: 4

sequence 1: CE1CG     fragments: 1-105
sequence 18: TDC      fragments: 1-105
Total number of sequences: 18.
Total number of sequence fragments: 18.

# Observed frequency and occurrence of each letter.
#anumber of letters in the input sequences = 1890
A  0.302646; observed occurrence =   572 (letter   1)
C  0.182540; observed occurrence =   345 (letter   2)
G  0.208995; observed occurrence =   395 (letter   3)
T  0.305820; observed occurrence =   578 (letter   4)

PRIOR FREQUENCIES DETERMINED BY OBSERVED FREQUENCIES.
* Information for the alphabet from the command line.
letter   1: A (complement: T)  prior freq = 0.302646 CYCLE
letter   2: C (complement: G)  prior freq = 0.182540
letter   3: G (complement: C)  prior freq = 0.208995
letter   4: T (complement: A)  prior freq = 0.305820

INFORMATION CONTENT IS CALCULATED USING NATURAL
LOGARITHMS (i.e. BASE e). DIVIDE BY ln(2) = 0.693 TO
CONVERT TO BASE 2, WHICH WAS USED INPREVIOUS VERSIONS
OF THIS PROGRAM.
```

Statistics for Consensus

MATRICES SAVED FOR NEXT CYCLE

CYCLE	total number	top adjusted information	ln top p-value	ln expected frequency
1	1620	2.9492	0.0000	7.3902
2	748	6.8222	-13.5825	0.4475
3	849	8.2051	-20.1461	0.0577
4	832	8.7882	-26.3882	-0.3628
5	848	8.9275	-31.8728	-0.3179
6	857	9.1860	-38.8902	-2.0624
7	877	9.2908	-45.6680	-3.8014
8	864	9.1929	-51.3100	-4.6251
9	876	9.1152	-57.2498	-5.9597
10	879	9.0644	-63.5596	-7.8751
11	864	8.8973	-68.7012	-8.8353
12	854	8.8738	-75.4184	-11.5917
13	850	8.6817	-80.0738	-12.5205
14	873	8.5267	-85.0113	-13.9878
15	852	8.2955	-88.6578	-14.4562
16	865	8.1066	-92.6288	-15.6014
17	857	7.8793	-95.7075	-16.3204
18	936	7.6066	-97.6650	-16.6684

Consensus from random sequences

```
      []            MATRICES SAVED FOR NEXT CYCLE             []
      []------------------------------------------------------[]
      []  total    | top adjusted |   ln top    [] ln expected []
CYCLE []  number   | information  |   p-value   []  frequency  []
------[]----------|--------------|-------------[]-------------[]
    1 []     2040 |       1.4133 |      0.0000 []      7.6207 []
    2 []      648 |       6.5863 |    -14.0510 []      0.4547 []
    3 []      866 |       8.2103 |    -21.0667 []     -0.1259 []
    4 []      819 |       8.6626 |    -26.3960 []      0.6457 []
    5 []      849 |       8.6417 |    -30.6613 []      2.2093 []
    6 []      871 |       8.7530 |    -36.3388 []      2.1271 []
    7 []      872 |       8.7389 |    -41.7409 []      2.1122 []
    8 []      878 |       8.6112 |    -46.5558 []      2.4937 []
    9 []      875 |       8.4596 |    -51.2305 []      2.8370 []
   20 []      937 |       6.4446 |    -88.4984 []      9.6257 []
   21 []      943 |       6.3042 |    -91.2183 []      9.6902 []
   22 []      955 |       6.1507 |    -93.4632 []      9.8955 []
   23 []      947 |       5.9713 |    -94.9290 []     10.4300 []
   24 []      969 |       5.8093 |    -96.4855 []     10.1381 []
```

```
MATRIX 1    number of sequences = 3
unadjusted information = 18.0219
sample size adjusted information = 8.21031
ln(p-value) = -21.0667   p-value = 7.09314E-10
ln(expected frequency) = -0.125938   expected frequency = 0.88167
A |   0   0   0   3   1   0   0   3   0   0   0   0   3   0   1   0
C |   0   0   3   0   0   2   0   0   0   3   1   0   0   1   2   0
G |   0   3   0   0   0   0   1   0   3   0   1   0   0   2   0   3
T |   3   0   0   0   2   1   2   0   0   0   1   3   0   0   0   0
```


`consensus`
greedy search for information content

- No longer used (good for teaching)
- Measure for success – information content
- Greedy strategy:
  - consider all windows of length(n) vs all windows in second sequence
  - take best pairs of windows (most information content), continue to next sequence, repeat
  - order dependent
  - time kN^2 rather than N^k

Statistical Strategies for Consensus Alignment - EM and Gibbs

- A problem of estimation with hidden data - the positions are easy to find if the consensus is known, and the consensus is easy to find if the positions are known
- Start with random positions, build a consensus estimate
- Apply consensus to sequences, assign probability of being a consensus, repeat
- Gibbs is similar, but a target sequence is left out and scanned at each stage

Expectation Maximization for Consensus Alignment

(1) Begin with a set of sequences:

```
CE1CG     taatgtttgtgctggtttttgtggc
ECOARABOP gacaaaaacgcgtaacaaaagtgtc
ECOBGLR1  acaaatcccaataacttaattattg
ECOCRP    cacaaagcgaaagctatgctaaaac
ECOCYA    acggtgctacacttgtatgtagcgc
ECODEOP2  agtgaattatttgaaccagatcgca
ECOGALE   gcgcataaaaaacggctaaattctt
ECOILVBPR gctccggcggggttttttgttatct
```

(2) Select consensus sites at random:

```
CE1CG     taatGTTTGtgctggtttttgtggc
ECOARABOP gacaaaaacgcgTAACAaaagtgtc
ECOBGLR1  acaaatcccAATAActtaattattg
ECOCRP    cacaaagcgaaagctatgctAAAAC
ECOCYA    ACGGTgctacacttgtatgtagcgc
ECODEOP2  agtgAATTAtttgaaccagatcgca
ECOGALE   gcgcataaAAAACggctaaattctt
ECOILVBPR gctccggcggggttttttgtTATCT
```

(3) Use the consensus to build a matrix

```
CE1CG     GTTTG
ECOARABOP TAACA
ECOBGLR1  AATAA
ECOCRP    AAAAC
ECOCYA    ACGGT
ECODEOP2  AATTA
ECOGALE   AAAAC
ECOILVBPR TATCT
```

A	5	6	3	3	3
C	0	1	0	2	2
G	1	0	1	1	1
T	2	1	4	2	2

f_{ns}		f_1	f_2	f_3	f_4	f_5
0.3	A	0.6	0.8	0.4	0.4	0.4
0.2	C	0.0	0.1	0.0	0.2	0.3
0.2	G	0.2	0.0	0.1	0.1	0.1
0.3	T	0.2	0.1	0.5	0.3	0.2

(4) Use the consensus to weight each position

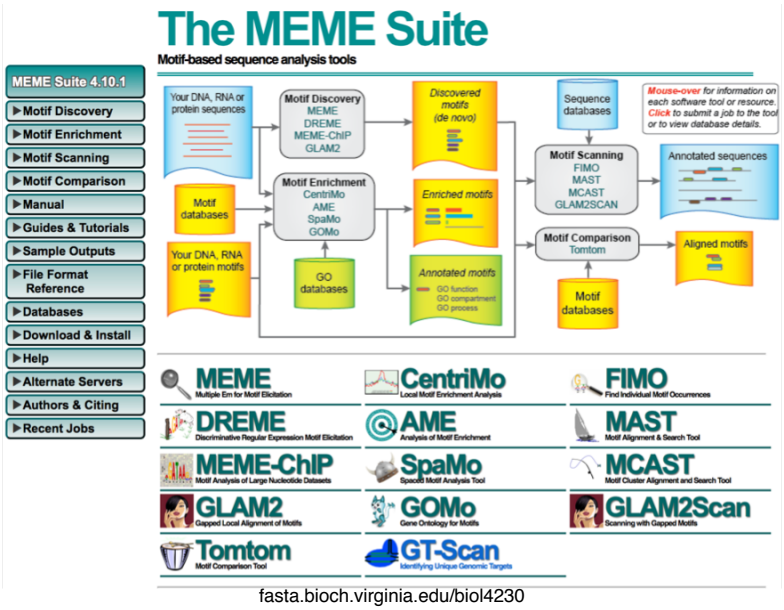
$$p_i = \sum_{j=0..5} f_{sb} + \sum_{non-site} f_{ns}$$

(5) Use the weighted site to build a new consensus

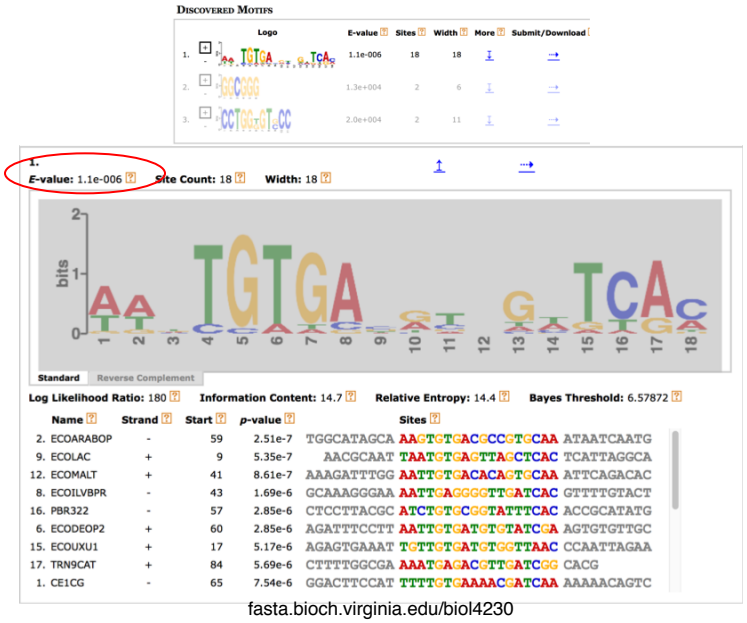
(6) Repeat (3..5)

Lawrence, C. E. & Reilly, A. A. *PROTEINS* **7,** 41–51 (1990).

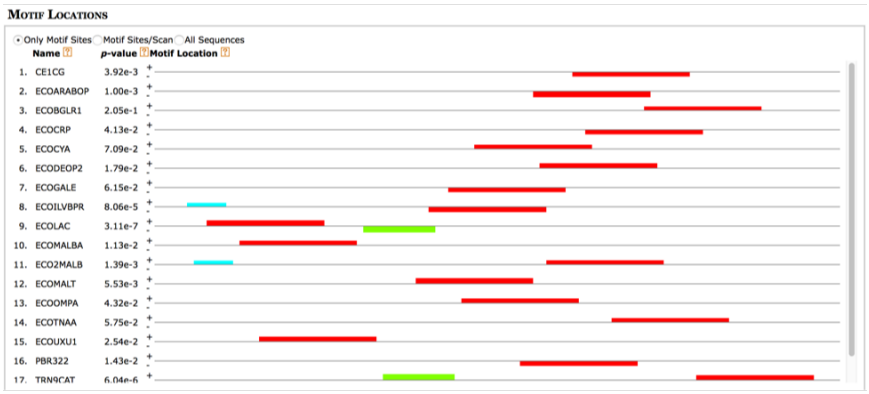
MEME: http://meme-suite.org



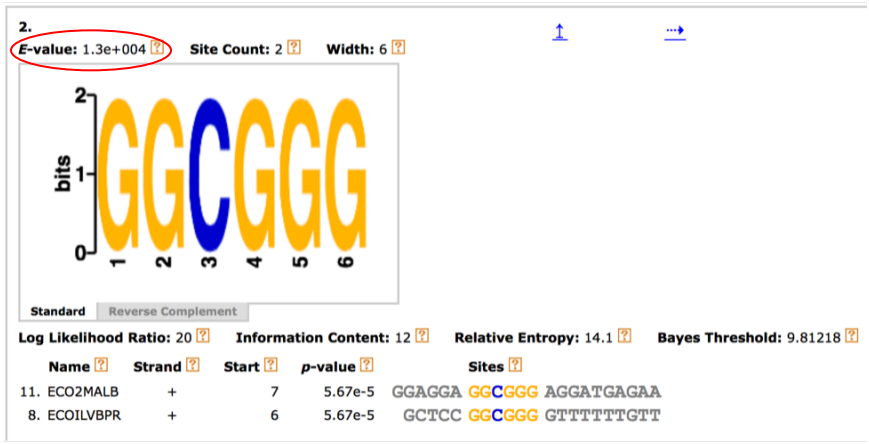
MEME Results: CRP sites



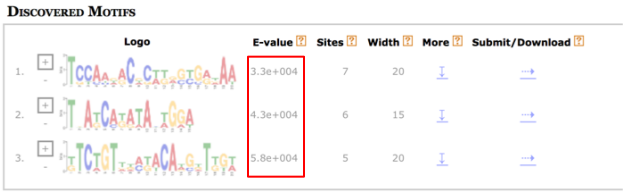
MEME Results: CRP site positions

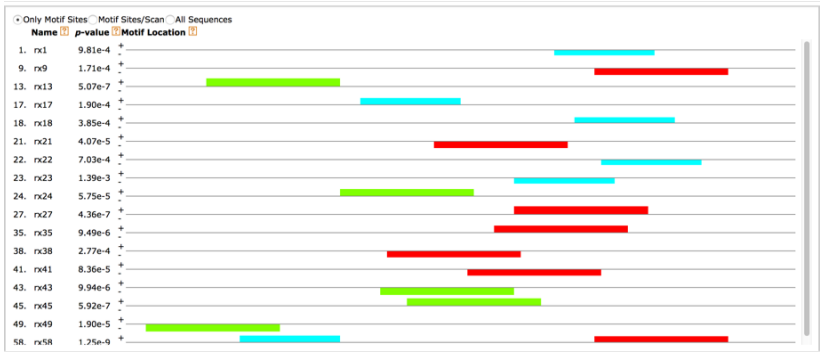


MEME Results: CRP - Motif 2



MEME Results: Random





MEME sensitivity (recall) and specificity (precision)

	Output of MEME+		*Analysis of discovered motifs*				
dataset name	*pass*	*log likelihood of discovered motif* (d)	*motif name*	*recall*	*precision*	*log likelihood of known motif* (k)	*difference* ($d-k$)
lipocalin	1	-55013	lipA	1.000	0.357	-55090	77
	2	-55057	lipB	0.400	0.200	-55092	35
hth	1	-496332	hth	0.933	0.571	-496346	14
farn	1	-92518	farnL	0.917	0.880	-92525	7
	2	-92585	farnB	0.615	0.842	-92517	-68
	3	-92569	farnA	0.733	0.647	-92566	-3
crp	1	-60547	crp	0.792	0.905	-60590	43
lexa	1	-109155	lexa	0.842	0.615	-109147	-8
crplexa	1	-169923	lexa	0.842	0.696	-169918	-5
	2	-170048	crp	0.667	0.471	-170116	68

Table 2: Overview of results of MEME+ on test datasets. MEME+ was run with W set to the values shown in Table 1 and $\beta = 0.01$. The *log likelihood* values are base-2 logarithms.

Bailey and Elkan, (1994) "Fitting a mixture model by expectation maximization to discover motifs in biopolymers", *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*, pp. 28-36

Gibb's sampling for Consensus Alignment

(1) Begin with a set of sequences with randomly located motifs:

```
CE1CG      taatGTTTGtgctggtttttgtggc
ECOARABOP gacaaaaacgcgTAACAaaagtgtc
ECOBGLR1  acaaatcccAATAActtaattattg
ECOCRP    cacaaagcgaaagctatgctAAAAC
ECOCYA    ACGGTgctacacttgtatgtagcgc
ECODEOP2  agtgAATTAtttgaaccagatcgca
ECOGALE   gcgcataaAAAACggctaaattctt
ECOILVBPR gctccggcggggttttttgtTATCT
```

(2) Exclude one of the sequences at random, and build a consensus matrix from the other motifs

```
CE1CG      taatGTTTGtgctggtttttgtggc
ECOARABOP gacaaaaacgcgTAACAaaagtgtc
ECOBGLR1  acaaatcccAATAActtaattattg
ECOCRP    cacaaagcgaaagctatgctaaaac
ECOCYA    ACGGTgctacacttgtatgtagcgc
ECODEOP2  agtgAATTAtttgaaccagatcgca
ECOGALE   gcgcataaAAAACggctaaattctt
ECOILVBPR gctccggcggggttttttgtTATCT
```

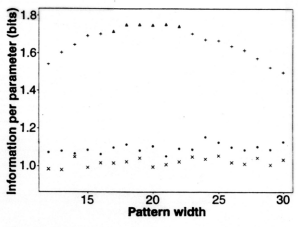
(3) Using the probability matrix from the included sequences, calculate the probability of each site on the excluded sequence

```
ECOCRP    cacaaagcgaaagctatgctaaaac
```

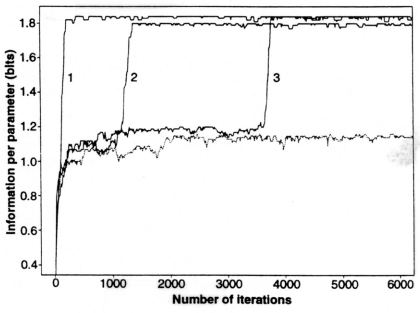
(4) Select a site at random, using weights from the probabilities in (3)

(5) Repeat steps (2) - (4)

g. 2. Information per parameter as the criterion pattern width for helix-turn-helix (HTH) pro- ns. The points indicate the maximum values of ormation per parameter found by the algo- nm. The upper points (▲ and +) used the mplete sequences of the 30 HTH proteins ted in Fig. 1A. (▲) All of the sequences in the ta set were aligned in the correct register (as Fig. 1A). (+) One or more of the sequences in data set were incorrectly aligned. All com- tely correct alignments in the width range m 17 to 22 residues gave greater values of ormation per parameter than any incorrect gnments outside this width range. (●) The onsites" sequence data of the 30 HTH proteins, constructed by deleting the 18 residues of the H pattern itself (Fig. 1A) from each of the sequences. (×) A shuffled data set (*46*) of the 30 HTH quences. The alignments from the nonsites background of the HTH proteins give values slightly ater than random expectation.

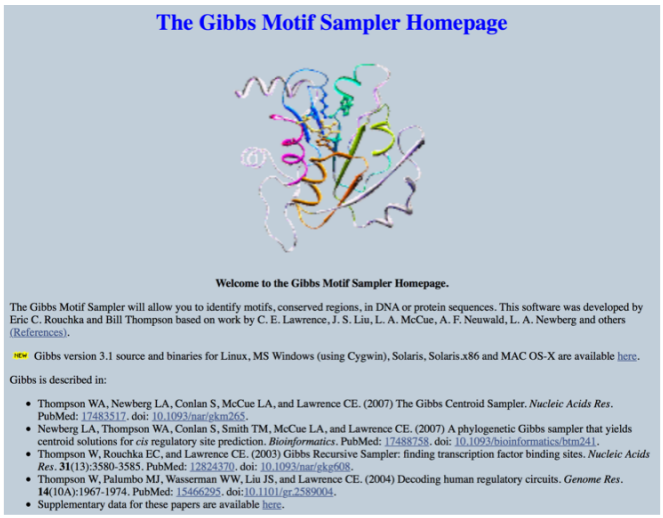


. 3. Convergence behav- of the Gibbs sampling orithm. Because the bs sampler, when run for e time, is a heuristic rath- han a rigorous optimiza- procedure, one cannot arantee the optimality of results it produces. refore, the best solution nd in a series of runs will called "maximal." A sin- pattern of width 18 resi- s was sought in the data of 30 HTH proteins wn in Fig. 1A. Solid lines w the course of three pendent runs with dif- nt random seeds. Evolv-



Lawrence CE, Altschul SF, Boguski MS, Liu JS, Neuwald AF, Wootton JC. (1993) Detecting subtle sequence signals: a Gibbs sampling strategy for multiple alignment.Science 262:208-214.

Gibbs: ccmbweb.ccv.brown.edu/gibbs/gibbs.html



The Gibbs Motif Sampler Homepage

Welcome to the Gibbs Motif Sampler Homepage.

The Gibbs Motif Sampler will allow you to identify motifs, conserved regions, in DNA or protein sequences. This software was developed by Eric C. Rouchka and Bill Thompson based on work by C. E. Lawrence, J. S. Liu, L. A. McCue, A. F. Neuwald, L. A. Newberg and others (References).

NEW Gibbs version 3.1 source and binaries for Linux, MS Windows (using Cygwin), Solaris, Solaris.x86 and MAC OS-X are available here.

Gibbs is described in:

- Thompson WA, Newberg LA, Conlan S, McCue LA, and Lawrence CE. (2007) The Gibbs Centroid Sampler. *Nucleic Acids Res*. PubMed: 17483517. doi: 10.1093/nar/gkm265.
- Newberg LA, Thompson WA, Conlan S, Smith TM, McCue LA, and Lawrence CE. (2007) A phylogenetic Gibbs sampler that yields centroid solutions for *cis* regulatory site prediction. *Bioinformatics*. PubMed: 17488758. doi: 10.1093/bioinformatics/btm241.
- Thompson W, Rouchka EC, and Lawrence CE. (2003) Gibbs Recursive Sampler: finding transcription factor binding sites. *Nucleic Acids Res*. **31**(13):3580-3585. PubMed: 12824370. doi: 10.1093/nar/gkg608.
- Thompson W, Palumbo MJ, Wasserman WW, Liu JS, and Lawrence CE. (2004) Decoding human regulatory circuits. *Genome Res*. **14**(10A):1967-1974. PubMed: 15466295. doi:10.1101/gr.2589004.
- Supplementary data for these papers are available here.

GIBBS sampler of CRP sites: (1000 iterations)

```
16 columns
Num Motifs: 17
  1,  1      61 actgt TTTTTTGATCGTTTTCACAAAA atgga      82   0.93 F CE1CG
  2,  1      55 attga TTATTTGCACGGCGTCACACTT tgcta      76   0.99 F ECOARABOP
  3,  1      76 ttaat AACTGTGAGCATGGTCATATTT ttatc      97   0.97 F ECOBGLR1
  4,  1      63 tgcat GTATGCAAAGGACGTCACATTA ccgtg      84   0.99 F ECOCRP
  5,  1      50 cagca AGGTGTTAAATTGATCACGTTT tagac      71   0.89 F ECOCYA
  6,  1       7 gtgaa TTATTTGAACCAGATCGCATTA cagtg      28   1.00 F ECODEOP2
  7,  1      42 tccac TAATTTATTCCATGTCACACTT ttcgc      63   0.72 F ECOGALE
  8,  1      20 ttttg TTATCTGCAATTCAGTACAAAA cgtga      41   0.74 F ECOILVBPR
  9,  1       9 gcaat TAATGTGAGTTAGCTCACTCAT taggc      30   1.00 F ECOLAC
 10,  1      14 gccaa TTCTGTAACAGAGATCACACAA agcga      35   1.00 F ECOMALBA
 11,  1      61 aggaa TTTCGTGATGTTGCTTGCAAAA atcgt      82   0.93 F ECO2MALB
 12,  1      41 tttgg AATTGTGACACAGTGCAAATTC agaca      62   0.97 F ECOMALT
 13,  1      48 ttcat ATGCCTGACGGAGTTCACACTT gtaag      69   0.98 F ECOOMPA
 14,  1      71 cgaac GATTGTGATTCGATTCACATTT aaaca      92   1.00 F ECOTNAA
 15,  1      17 gaaat TGTTGTGATGTGGTTAACCCAA ttaga      38   0.50 F ECOUXU1
 16,  1      53 atatg CGGTGTGAAATACCGCACAGAT gcgta      74   0.91 F PBR322
 18,  1      78 agtta ATTTGTGAGTGGTCGCACATAT cctgt      99   1.00 F TDC
                      ********      ********
```

GIBBS sampler of CRP sites: (5000 iterations)

```
16 columns
Num Motifs: 19
  1,  1      60 gactg TTTTTTTGATCGTTTTCACAAAAA tggaa      83   0.94 F CE1CG
  2,  1      54 cattg ATTATTTGCACGGCGTCACACTTT gctat      77   1.00 F ECOARABOP
  3,  1      75 gttaa TAACTGTGAGCATGGTCATATTTT tatca      98   0.98 F ECOBGLR1
  4,  1      62 ctgca TGTATGCAAAGGACGTCACATTAC cgtgc      85   0.98 F ECOCRP
  5,  1      49 tcagc AAGGTGTTAAATTGATCACGTTTT agacc      72   0.94 F ECOCYA
  6,  1       6 agtga ATTATTTGAACCAGATCGCATTAC agtga      29   0.95 F ECODEOP2
  6,  2      59 ttcct TAATTGTGATGTGTATCGAAGTGT gttgc      82   0.56 F ECODEOP2
  7,  1      41 ttcca CTAATTTATTCCATGTCACACTTT tcgca      64   0.45 F ECOGALE
  8,  1      38 agtac AAAACGTGATCAACCCCTCAATTT tccct      61   0.73 F ECOILVBPR
  9,  1       8 cgcaa TTAATGTGAGTTAGCTCACTCATT aggca      31   1.00 F ECOLAC
 10,  1      13 cgcca ATTCTGTAACAGAGATCACACAAA gcgac      36   1.00 F ECOMALBA
 11,  1      60 aagga ATTTCGTGATGTTGCTTGCAAAAA tcgtg      83   0.97 F ECO2MALB
 12,  1      40 atttg GAATTGTGACACAGTGCAAATTCA gacac      63   0.94 F ECOMALT
 13,  1      47 tttca TATGCCTGACGGAGTTCACACTTG taagt      70   0.95 F ECOOMPA
 14,  1      70 ccgaa CGATTGTGATTCGATTCACATTTA aacaa      93   1.00 F ECOTNAA
 15,  1      16 tgaaa TTGTTGTGATGTGGTTAACCCAAT tagaa      39   0.55 F ECOUXU1
 16,  1      52 catat GCGGTGTGAAATACCGCACAGATG cgtaa      75   0.88 F PBR322
 17,  1       4   ctg TGACGGAAGATCACTTCGCAGAAT aaata      27   0.05 F TRN9CAT
 18,  1      77 aagtt AATTTGTGAGTGGTCGCACATATC ctgtt     100   1.00 F TDC
                     *** *****      ***** ***
```

fasta.bioch.virginia.edu/biol4230

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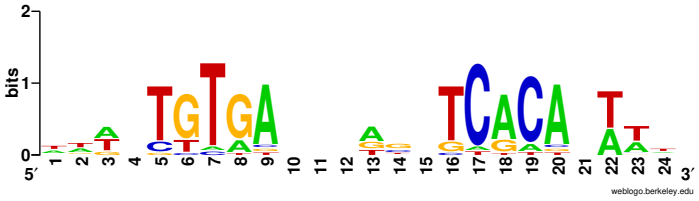
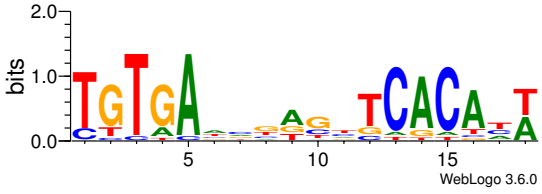
GIBBS sampler of CRP sites: (5000 iterations)



MEME



consensus

fasta.bioch.virginia.edu/biol4230

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Finding consensus regions in unaligned sequences

- Some introduction: regulation of transcription
- Looking for functional sites: promoters, regulatory elements, modification sites
- Products of *convergent*, not divergent evolution
- Weak spacing constraints
- Usually represented as a consensus sequence
- If alignment is given, consensus is obvious
- If consensus is given, alignment is obvious
- Search for consensus and alignment together
- `consensus, meme, gibbs`