Regulatory sequence analysis

Position-specific scoring matrices (PSSM)

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Alignment of transcription factor binding sites

Binding sites for the yeast Pho4p transcription factor

(Source : Oshima et al. Gene 179, 1996; 171-177)

Gene	Site Name	Sequence	Affinity
PHO5	UASp2	---aCtCaCA**CACGTGGG**ACTAGC-	high
PHO84	Site D	---TTTCCA**GCACGTGGG**GCGGA--	high
PHO81	UAS	----TTATG**GCACGTGCG**AATAA--	high
PHO8	Proximal	GTGATCGCT**GCACGTGGC**CCGA---	high
group 1	consensus	---------**gCACGTGgg**------	high
PHO5	UASp1	--TAAATTA**GCACGTTTT**CGC----	medium
PHO84	Site E	----AATA**CGCACGTTTT**TAATCTA	medium
group 2	consensus	--------**cgCACGTTtt**-------	medium
Degenerate consensus		---------**GCACGTKKk**-------	high-med

Non-binding sites

PHO5	UASp3	--TAATTTG**GCAT GTGCG**ATCTC--	No binding
PHO84	Site C	-----ACGTC**CACGTGGA**ACTAT--	No binding
PHO84	Site A	-----TTTAT**CACGTGA**CACTTTTT	No binding
PHO84	Site B	-----TTAC**GCACGTT G**GTGCTG--	No binding
PHO8	Distal	---TTACCC**GCACGC TT**AATAT---	No binding

IUPAC ambiguous nucleotide code

A	**A**	**A**denine
C	**C**	**C**ytosine
G	**G**	**G**uanine
T	**T**	**T**hymine
R	**A** or **G**	pu**R**ine
Y	**C** or **T**	p**Y**rimidine
W	**A** or **T**	**W**eak hydrogen bonding
S	**G** or **C**	**S**trong hydrogen bonding
M	**A** or **C**	a**M**ino group at common position
K	**G** or **T**	**K**eto group at common position
H	**A**, **C** or **T**	not **G**
B	**G**, **C** or **T**	not **A**
V	**G**, **A**, **C**	not **T**
D	**G**, **A** or **T**	not **C**
N	**G**, **A**, **C** or **T**	a**N**y

Regulatory sequence analysis

From alignments to weights

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Sequence logo

Count matrix (TRANSFAC matrix F$PHO4_01)

Residue\position	1	2	3	4	5	6	7	8	9	10	11	12
A	1	3	2	0	**8**	0	0	0	0	0	1	2
C	2	2	**3**	**8**	0	**8**	0	0	0	2	0	2
G	1	2	**3**	0	0	0	**8**	0	**5**	**4**	**5**	2
T	4	1	0	0	0	0	0	**8**	**3**	2	2	2
Sum	8	8	8	8	8	8	8	8	8	8	8	8

Tom Schneider's sequence logo

(generated with Web Logo http://weblogo.berkeley.edu/logo.cgi)

Frequency matrix

Pos	1	2	3	4	5	6	7	8	9	10	11	12
A	0.13	0.38	0.25	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.13	0.25
C	0.25	0.25	**0.38**	**1.00**	0.00	**1.00**	0.00	0.00	0.00	0.25	0.00	0.25
G	0.13	0.25	**0.38**	0.00	0.00	0.00	**1.00**	0.00	**0.63**	**0.50**	**0.63**	0.25
T	0.50	0.13	0.00	0.00	0.00	0.00	0.00	**1.00**	0.38	0.25	0.25	0.25
Sum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

$$f_{i,j} = \frac{n_{i,j}}{\sum_{i=1}^{A} n_{i,j}}$$

A	*alphabet size (=4)*
$n_{i,j,}$	*occurrences of residue i at position j*
p_i	*prior residue probability for residue i*
$f_{i,j}$	*relative frequency of residue i at position j*

Reference: Hertz (1999). Bioinformatics 15:563-577.

Corrected frequency matrix

Pos	1	2	3	4	5	6	7	8	9	10	11	12
A	0.15	0.37	0.26	0.04	**0.93**	0.04	0.04	0.04	0.04	0.04	0.15	0.26
C	0.24	0.24	**0.35**	**0.91**	0.02	**0.91**	0.02	0.02	0.02	0.24	0.02	0.24
G	0.13	0.24	**0.35**	0.02	0.02	0.02	**0.91**	0.02	**0.58**	**0.46**	**0.58**	0.24
T	0.48	0.15	0.04	0.04	0.04	0.04	0.04	**0.93**	0.37	0.26	0.26	0.26
Sum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

1st option: identically distributed pseudo-weight

$$f'_{i,j} = \frac{n_{i,j} + k/A}{\sum_{i=1}^{A} n_{i,j} + k}$$

2nd option: pseudo-weight distributed according to residue priors

$$f'_{i,j} = \frac{n_{i,j} + p_i k}{\sum_{i=1}^{A} n_{i,j} + k}$$

A	*alphabet size (=4)*
$n_{i,j,}$	*occurrences of residue i at position j*
p_i	*prior residue probability for residue i*
$f_{i,j}$	*relative frequency of residue i at position j*
k	*pseudo weight (arbitrary, 1 in this case)*
$f'_{i,j}$	*corrected frequency of residue i at position j*

Reference: Hertz (1999). Bioinformatics 15:563-577.

Weight matrix (Bernoulli model)

Prior	Pos	1	2	3	4	5	6	7	8	9	10	11	12
0.325	A	-0.79	0.13	-0.23	-2.20	**1.05**	-2.20	-2.20	-2.20	-2.20	-2.20	-0.79	-0.23
0.175	C	0.32	0.32	**0.70**	**1.65**	-2.20	**1.65**	-2.20	-2.20	-2.20	0.32	-2.20	0.32
0.175	G	-0.29	0.32	**0.70**	-2.20	-2.20	-2.20	**1.65**	-2.20	**1.19**	**0.97**	**1.19**	0.32
0.325	T	0.39	-0.79	-2.20	-2.20	-2.20	-2.20	-2.20	**1.05**	0.13	-0.23	-0.23	-0.23
1.000	Sum	-0.37	-0.02	-1.02	-4.94	-5.55	-4.94	-4.94	-5.55	-3.08	-1.13	-2.03	0.19

$$f'_{i,j} = \frac{n_{i,j} + p_i k}{\sum_{r=1}^{A} n_{r,j} + k}$$

$$W_{i,j} = \ln\left(\frac{f'_{i,j}}{p_i}\right)$$

A	*alphabet size (=4)*
$n_{i,j,}$	*occurrences of residue i at position j*
p_i	*prior residue probability for residue i*
$f_{i,j}$	*relative frequency of residue i at position j*
k	*pseudo weight (arbitrary, 1 in this case)*
$f'_{i,j}$	*corrected frequency of residue i at position j*
$W_{i,j}$	*weight of residue i at position j*

Reference: Hertz (1999). Bioinformatics 15:563-577.

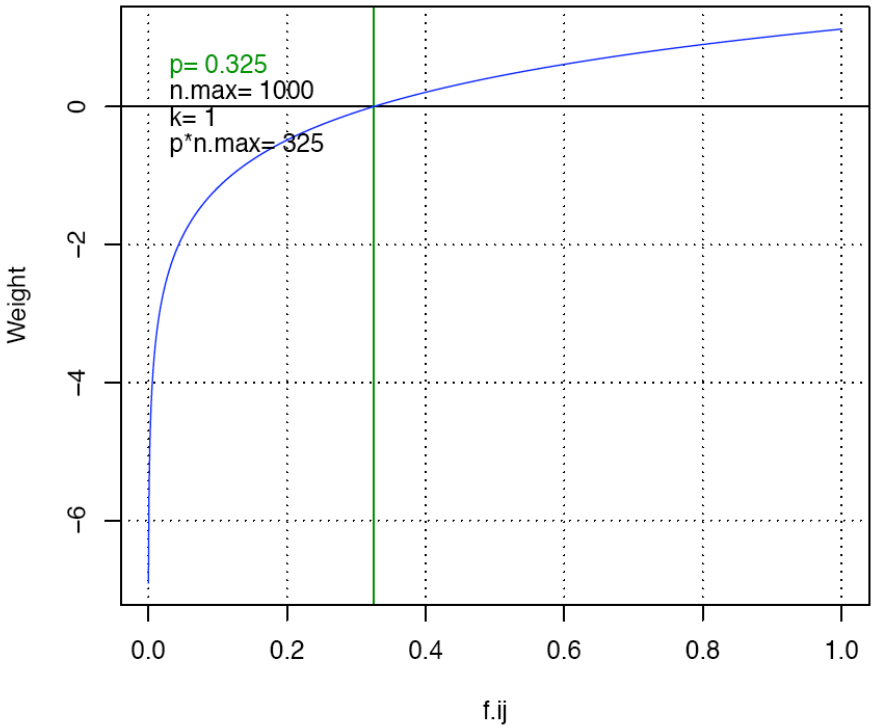
The use of a weight matrix relies on Bernoulli assumption

If we assume, for the background model, an independent succession of nucleotides (Bernoulli model), the weight W_S of a sequence segment S is simply the sum of weights of the nucleotides at successive positions of the matrix ($W_{i,j}$).

In this case, it is convenient to convert the PSSM into a weight matrix, which can then be used to assign a score to each position of a given sequence.

Properties of the weight function

$$W_{i,j} = \ln\left(\frac{f'_{i,j}}{p_i}\right) \qquad f'_{i,j} = \frac{n_{i,j} + p_i k}{\sum_{i=1}^{A} n_{i,j} + k} \qquad \sum_{i=1}^{A} f'_{i,j} = 1$$



- The weight is
  - *positive* when $f'_{i,j} > p_i$ (*favourable* positions for the binding of the transcription factor)
  - *negative* when $f'_{i,j} < p_i$ (*unfavourable* positions)

Regulatory sequence analysis

Information content

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Shannon uncertainty

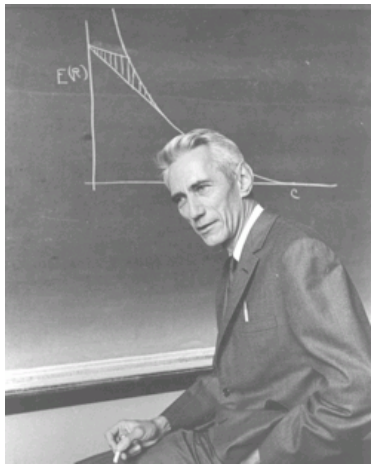
- Shannon uncertainty
  - $H_s(j)$: uncertainty of a column of a PSSM
  - Hg: uncertainty of the background (e.g. a genome)
- Special cases of uncertainty (for a 4 letter alphabet)
  - *min(H)=0*
    - No uncertainty at all: the nucleotide is completely specified (e.g. p={1,0,0,0})
  - *H=1*
    - Uncertainty between two letters (e.g. p={0.5,0,0,0.5})
  - *max(H) = 2 (Complete uncertainty)*
    - One bit of information is required to specify the choice between each alternative (e.g. p={0.25,0.25,0.25,0.25}).
    - Two bits are required to specify a letter in a 4-letter alphabet.
- R_{seq}
  - Schneider (1986) defines an ***information content*** based on Shannon's uncertainty.
- R^*_{seq}
  - For skewed genomes (i.e. unequal residue probabilities), Schneider recommends an alternative formula for the information content . This is the formula that is nowadays used.

$$H_s(j) = -\sum_{i=1}^{A} f_{i,j} \log_2(f_{i,j})$$

$$H_g = -\sum_{i=1}^{A} p_i \log_2(p_i)$$

$$R_{seq}(j) = H_g - H_s(j) \qquad R_{seq} = \sum_{j=1}^{w} R_{seq}(j)$$

$$R^*_{seq}(j) = \sum_{i=1}^{A} f_{i,j} \log_2\left(\frac{f_{i,j}}{p_i}\right) \qquad R^*_{seq} = \sum_{j=1}^{w} R^*_{seq}(j)$$



Adapted from Schneider (1986)

Information content

Prior	Pos	1	2	3	4	5	6	7	8	9	10	11	12
0.325	A	-0.12	0.05	-0.06	-0.08	**0.97**	-0.08	-0.08	-0.08	-0.08	-0.08	-0.12	-0.06
0.175	C	0.08	0.08	**0.25**	**1.50**	-0.04	**1.50**	-0.04	-0.04	-0.04	0.08	-0.04	0.08
0.175	G	-0.04	0.08	**0.25**	-0.04	-0.04	-0.04	**1.50**	-0.04	**0.68**	**0.45**	**0.68**	0.08
0.325	T	0.19	-0.12	-0.08	-0.08	-0.08	-0.08	-0.08	**0.97**	0.05	-0.06	-0.06	-0.06
1.000	Sum	0.11	0.09	**0.36**	**1.29**	**0.80**	**1.29**	**1.29**	**0.80**	**0.61**	**0.39**	**0.47**	0.04

$$f'_{i,j} = \frac{n_{i,j} + p_i k}{\sum_{i=1}^{A} n_{i,j} + k}$$

$$I_{i,j} = f'_{i,j} \ln\left(\frac{f'_{i,j}}{p_i}\right)$$

$$I_j = \sum_{i=1}^{A} I_{i,j}$$

$$I_{matrix} = \sum_{j=1}^{w} \sum_{i=1}^{A} I_{i,j}$$

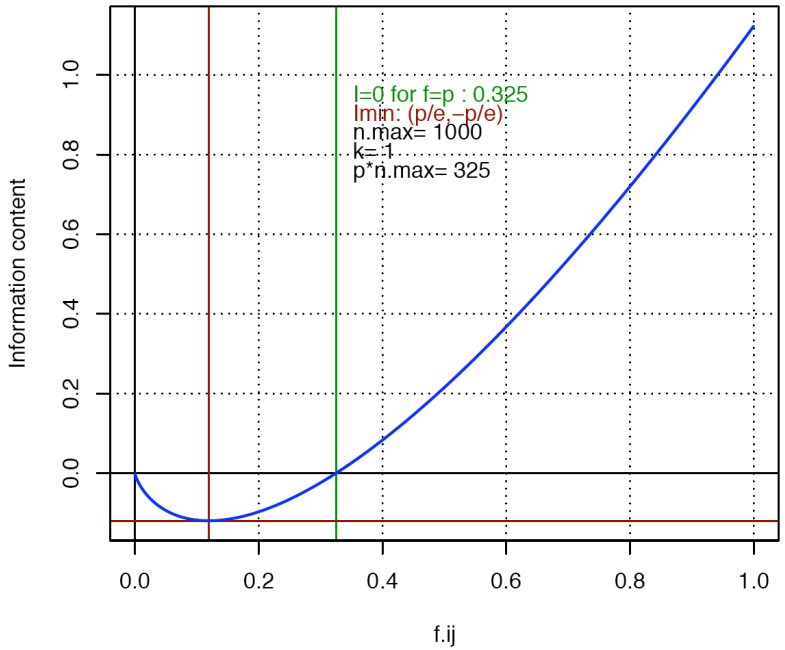
A	*alphabet size (=4)*
$n_{i,j,}$	*occurrences of residue i at position j*
w	*matrix width (=12)*
p_i	*prior residue probability for residue i*
$f_{i,j}$	*relative frequency of residue i at position j*
k	*pseudo weight (arbitrary, 1 in this case)*
$f'_{i,j}$	*corrected frequency of residue i at position j*
$W_{i,j}$	*weight of residue i at position j*
$I_{i,j}$	*information of residue i at position j*

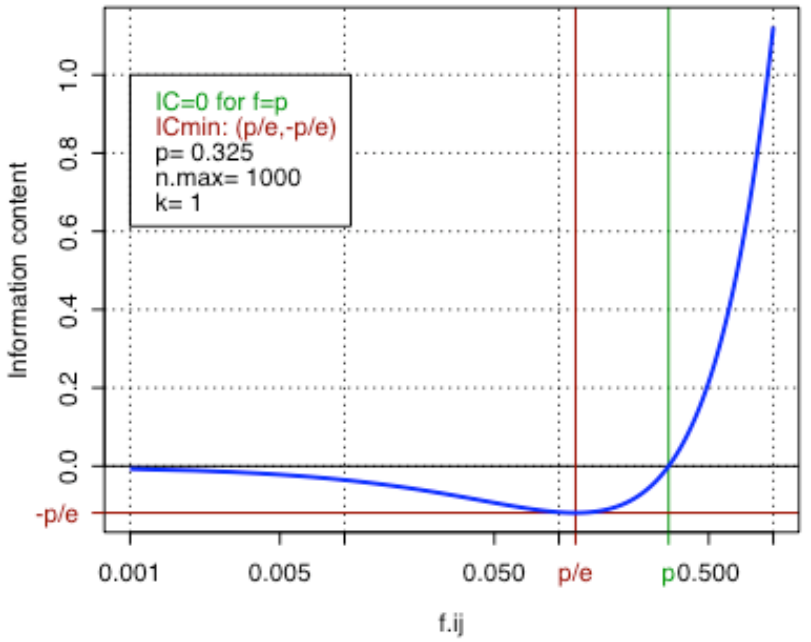
Reference: Hertz (1999).

Bioinformatics 15:563-577.

Information content I_{ij} of a cell of the matrix

- For a given cell of the matrix
  - I_{ij} is positive when $f'_{ij} > p_i$
    (i.e. when residue *i* is more frequent at position *j* than expected by chance)
  - I_{ij} is negative when $f'_{ij} < p_i$
  - I_{ij} tends towards 0 when $f'_{ij} \to 0$ (because $limit_{x->0}\ x*ln(x) = 0$)





Information content of a column of the matrix

- For a given column i of the matrix
  - The information of the column (I_j) is the sum of information of its cells.
  - I_j is always positive
  - I_j is always positive
  - I_j is 0 when the frequency of all residues equal their prior probability ($f_{ij}=p_i$)
  - I_j is maximal when
    - the residue i_m with the lowest prior probability has a frequency of 1 (all other residues have a frequency of 0)
    - and the pseudo-weight is 0

$$I_j = \sum_{i=1}^{A} I_{i,j} = \sum_{i=1}^{A} f'_{i,j} \ln\left(\frac{f'_{i,j}}{p_i}\right)$$

$$i_m = \arg\min_i(p_i) \qquad k = 0$$

$$\max(I_j) = 1 * \ln(\frac{1}{p_i}) = -\ln(p_i)$$

Information content of the matrix

- The total information content represents the capability of the matrix to make the distinction between a binding site (represented by the matrix) and the background model.
- The information content also allows to estimate an upper limit for the expected frequency of the binding sites in random sequences.
- The pattern discovery program *consensus* (developed by Jerry Hertz) optimises the information content in order to detect over-represented motifs.
- Note that this is not the case of all pattern discovery programs: the gibbs sampler algorithm optimizes a log-likelihood.

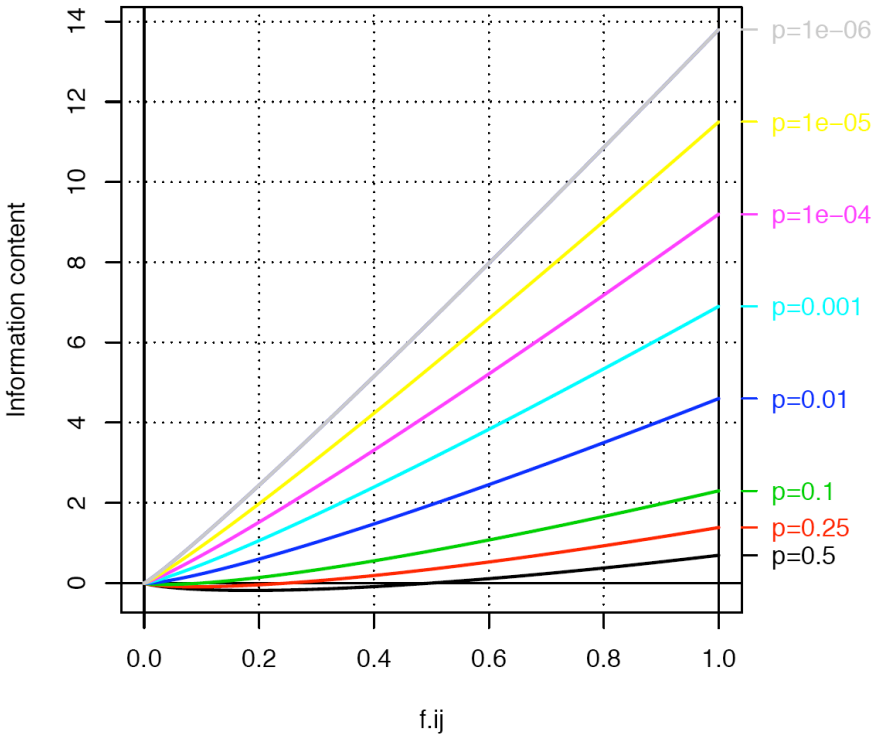
$$I_{matrix} = \sum_{j=1}^{w} \sum_{i=1}^{A} I_{i,j}$$

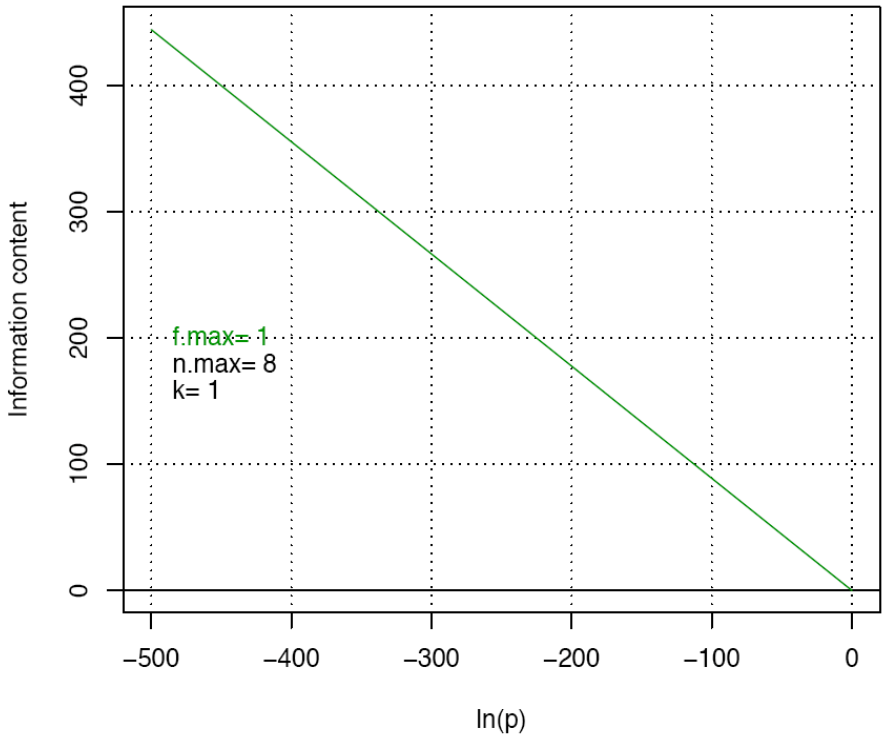
$$P(site) \leq e^{-I_{matrix}}$$

Reference: Hertz (1999). Bioinformatics 15:563-577.

Information content: effect of prior probabilities

- The upper bound of I_j increases when p_i decreases
  - I_j -> *Inf* when p_i -> *0*
- The information content, as defined by Gerald Hertz, has thus no upper bound.





Regulatory sequence analysis

Sequence logos

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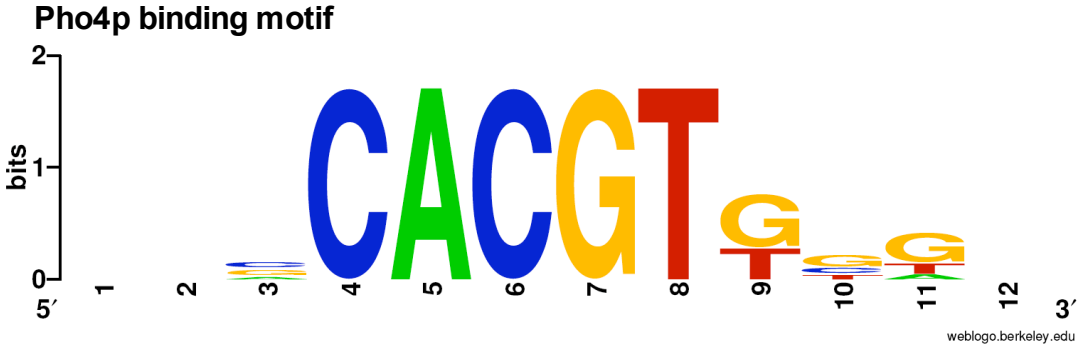
Schneider logos

$$H_s(j) = -\sum_{i=1}^{A} f_{ij} \log_2(f_{ij})$$

$$R_{seq}(j) = 2 - H_s(j) + e(n)$$

$$h_{ij} = f_{ij} R_{seq}(j)$$



- Schneider (1990) proposes a graphical representation based on his previous entropy (H) for representing the importance of each residue at each position of an alignment. He provides a new formula for R_{seq}
  - $H_s(j)$ uncertainty of column j
  - $R_{seq}(j)$ "information content" of column j (beware, this definition differs from Hertz' information content)
  - $e(n)$ correction for small samples (pseudo-weight)
- Remarks
  - This information content does not include any correction for the prior residue probabilities (p_i)
  - This information content is expressed in bits.
- Boundaries
  - $min(Rseq)=0$ equiprobable residues
  - $max(Rseq)=2$ perfect conservation of 1 residue with a pseudo-weight of 0,
- Sequence logos can be generated from aligned sequences on the *Weblogo* server
  - http://weblogo.berkeley.edu/

Sequence logo

References - PSSM information content

- Papers by Tom Schneider
  - Schneider, T.D., G.D. Stormo, L. Gold, and A. Ehrenfeucht. 1986. Information content of binding sites on nucleotide sequences. J Mol Biol 188: 415-431.
  - Schneider, T.D. and R.M. Stephens. 1990. Sequence logos: a new way to display consensus sequences. Nucleic Acids Res 18: 6097-6100.
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    - http://www.lecb.ncifcrf.gov/~toms/paper/index.html
- Papers by Gerald Hertz
  - Hertz, G.Z. and G.D. Stormo. 1999. Identifying DNA and protein patterns with statistically significant alignments of multiple sequences. Bioinformatics 15: 563-577.