Regulatory sequence analysis

Position-specific scoring matrices (PSSM)

Jacques van Helden
Jacques.van.Helden@ulb.ac.be

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	`---aCtCaCACACGTGGGACTAGC-`	high
PHO84	Site D	`---TTTCCAGCACGTGGGGCGGA--`	high
PHO81	UAS	`----TTATGGCACGTGCGAATAA--`	high
PHO8	Proximal	`GTGATCGCTGCACGTGGCCCGA---`	high
PHO5	UASp3	`--TAATTTGGCATGTGCGATCTC--`	low
PHO84	Site C	`-----ACGTCCACGTGGAACTAT--`	low
PHO84	Site A	`-----TTTATCACGTGACACTTTTT`	low
group 1	consensus	`---------gCACGTGggac-----`	high-low
PHO5	UASp1	`--TAAATTAGCACGTTTTCGC----`	medium
PHO84	Site E	`----AATACGCACGTTTTTAATCTA`	medium
PHO84	Site B	`-----TTACGCACGTTGGTGCTG--`	low
PHO8	Distal	`---TTACCCGCACGCTTAATAT---`	low
group 2	consensus	`--------cgCACGTTt--------`	med-low
Degenerate consensus		`---------GCACGTKKk-------`	

Regulatory sequence analysis

From alignments to weights

Jacques van Helden
Jacques.van.Helden@ulb.ac.be

Count matrix

Pos	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

Binding site for the yeast Pho4p transcription factor

(Source : Transfac matrix F$PHO4_01)

$n_{i,j,}$ *occurrences of residue i at position j*

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

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

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.

Probability of a sequence segment under the matrix model

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**
Sequence S	A	T	G	C	G	T	A	A	A	G	C	T
P(res)	**0.15**	**0.15**	**0.35**	**0.91**	**0.02**	**0.04**	**0.04**	**0.04**	**0.04**	**0.46**	**0.02**	**0.26**
P(S|M)	5.32E-13											

$$P(S \mid M) = \prod_{j=1}^{w} f'_{r_j j}$$

- Let
  - M be a frequency matrix of width w
  - $S = \{r_1, r_2, \ldots, r_w\}$ be a sequence segment of length w (same length as the matrix)
  - r_j is the residue found at position j of the sequence segment S.
- The corrected frequencies F'_{ij} can be used to estimate the probability to observe residue i at position j of the motif described by the matrix
- The probability to generate the sequence segment S under the model described by the matrix M is the product of the frequencies of residues at the corresponding columns of the matrix.

Probability of the best sequence segment under the matrix model

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
Sequence S	**T**	**A**	**G**	**C**	**A**	**C**	**G**	**T**	**G**	**G**	**G**	**T**
P(res)	**0.48**	**0.37**	**0.35**	**0.91**	**0.93**	**0.91**	**0.91**	**0.93**	**0.58**	**0.46**	**0.58**	**0.26**

P(S|M) **1.59E-03**

$$P(S \mid M) = \prod_{j=1}^{w} f'_{r_j j}$$

- This segment of sequence is associated to the highest possible probability given the matrix : P(S|M)
- Each nucleotide of the sequence corresponds to the residue with the highest probability in the corresponding column of the matrix.

Probability of a sequence segment under the background model

Pos	Prior
A	0.325
C	0.175
G	0.175
T	0.325

Sequence S	A	T	G	C	G	T	A	A	A	G	C	T
P(res)	0.325	0.325	0.175	0.175	0.175	0.325	0.325	0.325	0.325	0.175	0.175	0.325
P(S|B)	6.29E-08											

- A background model (B) should be defined to estimate the probability of a sequence motif outside of the motif.
- Various possibilities can be envisaged to define the background model
  - Bernoulli model with equiprobable residues (this should generally be avoided, because most biological sequences are biased towards some residues)
  - Bernoulli model with residue-specific probabilities (p_r)
  - Markov chains
- Under a Bernoulli model, the probability of a sequence motif S is the probability of the prior frequencies of its residues r_j.

$$P(S \mid B) = \prod_{j=1}^{w} p_{r_j}$$

Weight of a sequence segment

Pos	1	2	3	4	5	6	7	8	9	10	11	12
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
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
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
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**
residue r	A	T	G	C	G	T	A	A	A	G	C	T
W(r)	-0.79	-0.79	0.70	1.65	-2.20	-2.20	-2.20	-2.20	-2.20	0.97	-2.20	-0.23
Weight	-11.67		=SUM[W(r)]									

$$W_S = \ln\left(\frac{P(S \mid M)}{P(S \mid B)}\right) = \ln\left(\frac{\prod_{j=1}^{w} f'_{r_j j}}{\prod_{j=1}^{w} p_{r_j}}\right) = \ln\left(\prod_{j=1}^{w} \frac{f'_{r_j j}}{p_{r_j}}\right) = \sum_{j=1}^{w} \ln\left(\frac{f'_{r_j j}}{p_{r_j}}\right) = \sum_{j=1}^{w} W_{r_j j}$$

Ws	weight of sequence segment S
$P(S \mid M)$	probability of the sequence segment, given the matrix
$P(S \mid B)$	probability of the sequence segment, given the background
j	position within the segment and within the matrix
r_j	residue at position j of the sequence segment
p_{rj}	prior probability of residue r_j
f'_{rjj}	probability of residue r_j at position j of the matrix

- The ***weight*** of a sequence segment is defined as the log-ratio between
  - $P(S|M)$, the sequence probability under the model described by the PSSM, and
  - $P(S|B)$, the sequence probability under the background model.
- The weight represents the likelihood that this segment is an occurrence of the motif rather than being issued from the background model.
- The weight matrix W_{ij} allows to easily calculate segment weights.

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.

Bernoulli asumption

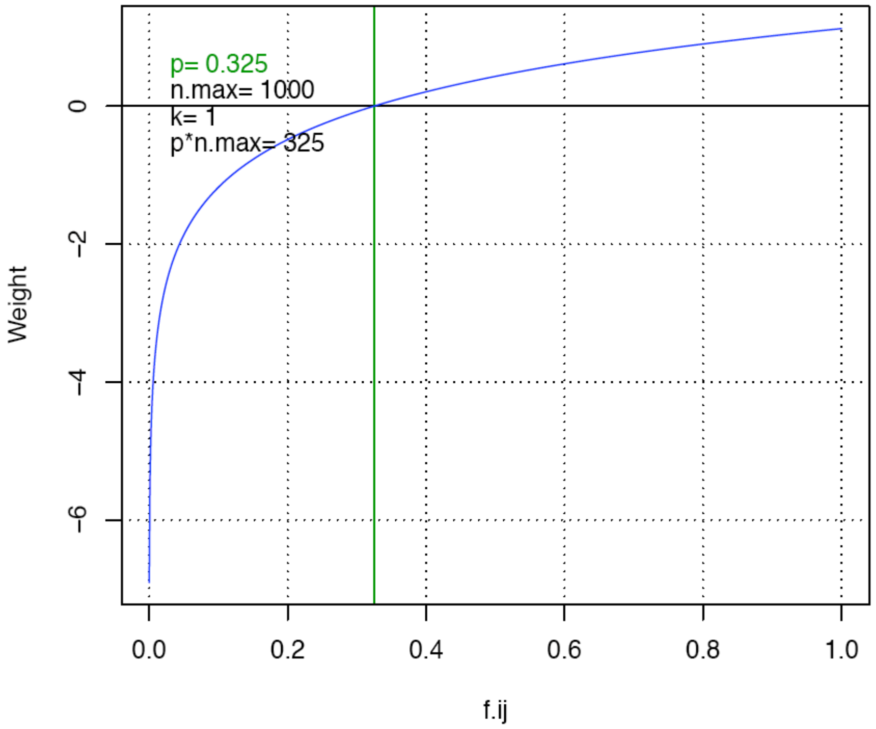
If we assume, for the background model, an independent succession of nucleotides (Bernoulli model), the weight W_S of a sequence segment S is simply the sum of weights of the nucleotides at successive positions of the matrix ($W_{i,j}$).

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

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

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



- 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

Jacques van Helden
Jacques.van.Helden@ulb.ac.be

Shannon uncertainty

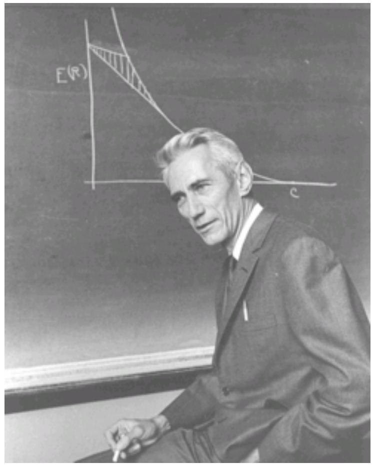
- Shannon uncertainty
  - $H_s(j)$: uncertainty of a column of a PSSM
  - Hg: uncertainty of the background (e.g. a genome)
- Properties of the uncertainty (for a 4 letter alphabet)
  - min(H)=0
    - No uncertainty at all: the nucleotide is completely specified (e.g. p={1,0,0,0})
  - H=1
    - Uncertainty 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.
- Schneider (1986) defines an information content R^*_{seq} based on Shannon's uncertainty.

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

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

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

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



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

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

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

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

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

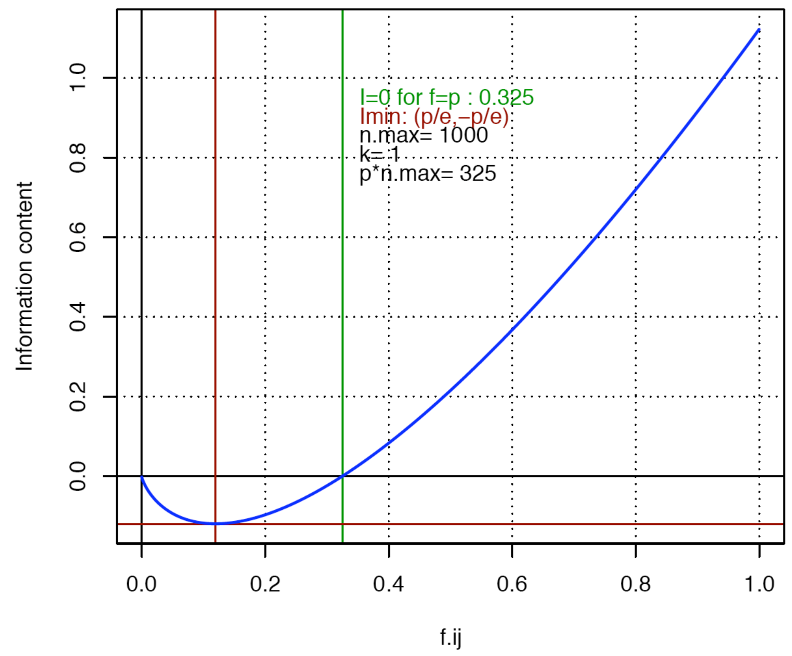
A	*alphabet size (=4)*
$n_{i,j,}$	*occurrences of residue i at position j*
w	*matrix width (=12)*
p_i	*prior residue probability for residue i*
$f_{i,j}$	*relative frequency of residue i at position j*
k	*pseudo weight (arbitrary, 1 in this case)*
$f'_{i,j}$	*corrected frequency of residue i at position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

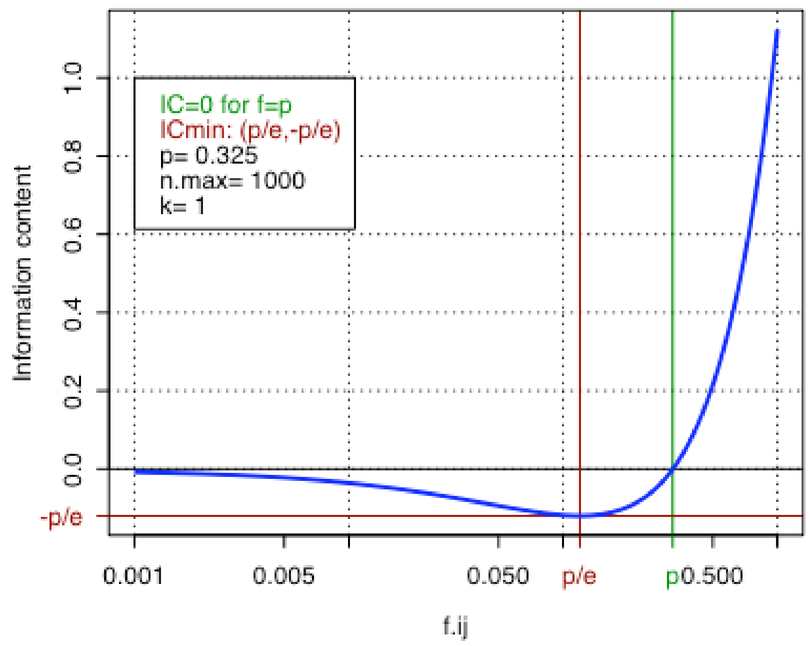
Reference: Hertz (1999).

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} -> 0 (because $limit_{x\text{->}0}\ x*ln(x) = 0$)





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.

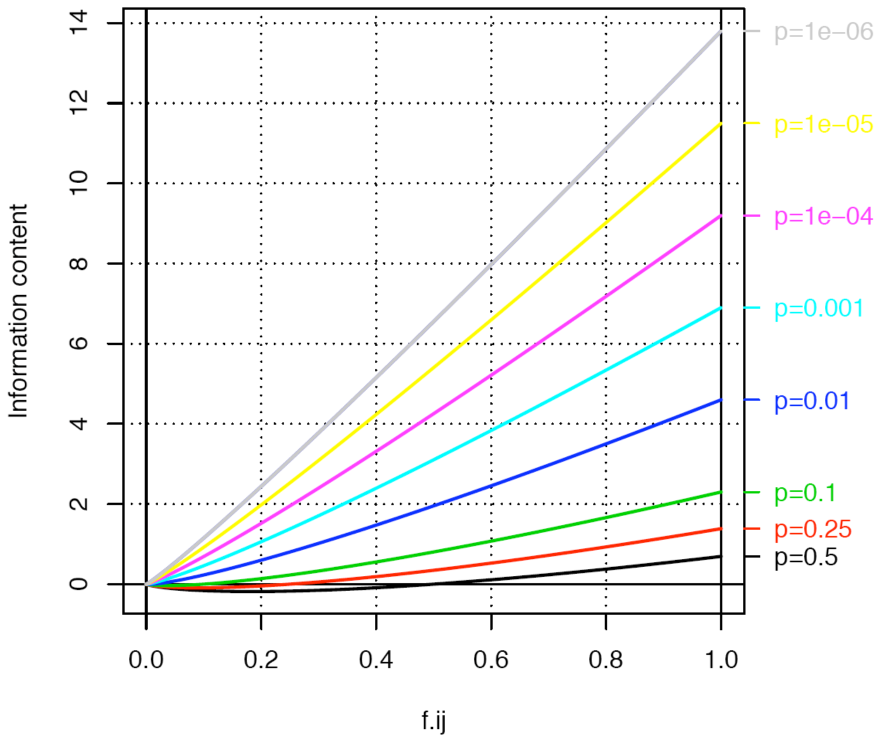
$$I_{matrix} = \sum_{j=1}^{w} \sum_{i=1}^{A} I_{i,j}$$

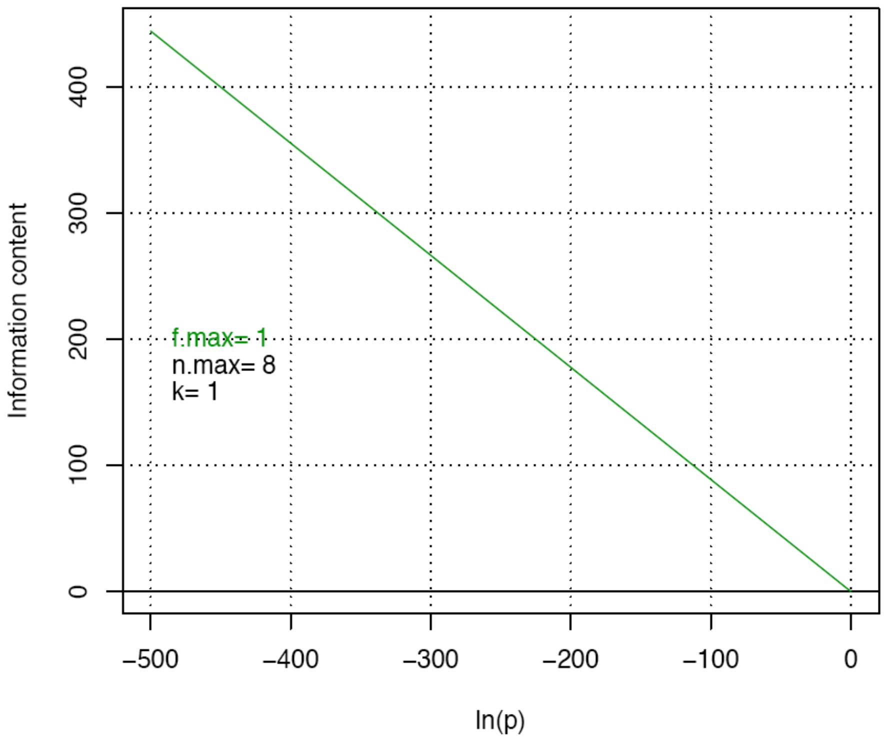
$$P(site) \leq e^{-I_{matrix}}$$

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

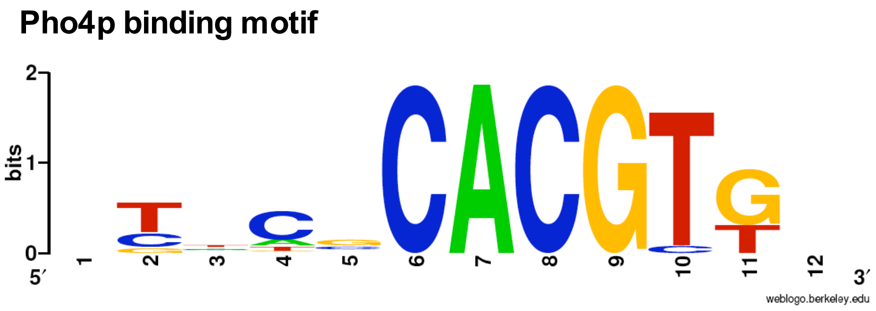
Jacques van Helden
Jacques.van.Helden@ulb.ac.be

Schneider logos

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

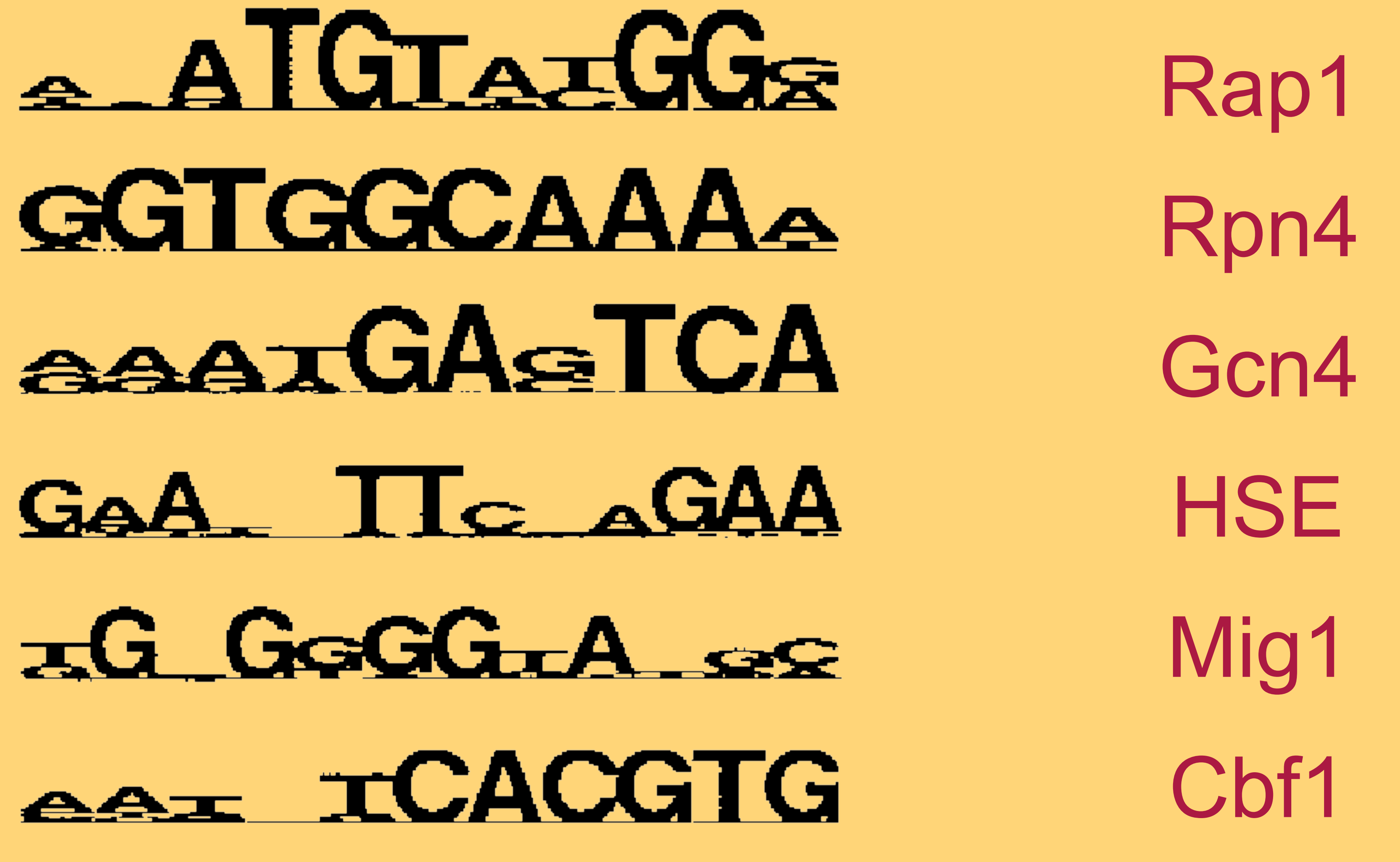
$$R_{seq}(j) = 2 - H_s(j) + e(n)$$

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


Pho4p binding motif

- 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
  - $e(n)$ 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, all the others are forbidden
- 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.
  - Tom Schneider’s publications online
    - 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.