Regulatory sequence analysis

Matrix-based pattern matching

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Regulatory motif: position-specific scoring matrix (PSSM) Binding motif of the yeast TF Pho4p (TRANSFAC matrix F\$PHO4_01)

Pos	1	2	3	4	5	6	7	8	9	10	11	12
Base												
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
			V	С	Α	С	G	Т	K	В		



Frequency matrix

Pos	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.13	0.38	0.25	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.13	0.25
С	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
Т	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

Pseudo-count correction

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
Т	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
$f_{i,j} \ k$	relative frequency of residue i at position j
k	pseudo weight (arbitrary, 1 in this case)
$f'_{i,i}$	corrected frequency of residue i at position j

Probability of a sequence segment under the matrix model

	Pos	1	2	3	4	5	6	7	8	9	10	11	12
	Α	0.15	0.37	0.26	0.04	0.93	0.04	0.04	0.04	0.04	0.04	0.15	0.26
	С	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
	Т	0.48	0.15	0.04	0.04	0.04	0.04	0.04	0.93	0.37	0.26	0.26	0.26
Sequ	ence S	Α	T	G	С	G	Т	Α	Α	Α	G	С	T
	P(res)												
	P(S M)												

Exercise: estimate the probability of sequence ATGCGTAAAGCT given the motif M

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

- Let
 - ullet M be a frequency matrix of width w
 - $S = \{r_1, r_2, ..., r_w\}$ be a sequence segment of length w (same length as the matrix)
 - \mathbf{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 a sequence segment under the matrix model

	Pos	1	2	3	4	5	6	7	8	9	10	11	12
	Α	0.15	0.37	0.26	0.04	0.93	0.04	0.04	0.04	0.04	0.04	0.15	0.26
	С	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
	Т	0.48	0.15	0.04	0.04	0.04	0.04	0.04	0.93	0.37	0.26	0.26	0.26
Sequ	ence S	Α	Т	G	С	G	Т	Α	Α	Α	G	С	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-1	3										

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

Let

- □ *M* be a frequency matrix of width *w*
- $S = \{r_1, r_2, ..., r_w\}$ be a sequence segment of length w (same length as the matrix)
- \neg 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 highest scoring sequence segment

P	os	1	2	3	4	5	6	7	8	9	10	11	12
	4	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
-	Т	0.48	0.15	0.04	0.04	0.04	0.04	0.04	0.93	0.37	0.26	0.26	0.26
Sequenc	ce S	T	Α	G	С	Α	С	G	T	G	G	G	Т
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	S M)	1.59E-0)3									1.	.,
											$P(S \mid I)$	$M = \prod_{i=1}^{\nu}$	$T_{f'}$
	4		_								1 (511	$m_j - 1$	$\int J r_j J$

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.

Background probability of a sequence segment – Bernoulli model

Pos	Prior
A	0.325
С	0.175
G	0.175
T	0.325

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

Sequence S A T G C G T A A A G C T P(res) 0.325 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
 - Identical and independent distribution (iid): 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 models (treat dependencies between successive nucleotides)
- Under a Bernoulli model, the probability of a sequence motif S is the probability of the prior frequencies of its residues r_i .

Weight of a sequence segment

Pos	1	2	3	4	5	6	7	8	9	10	11	12
Α	-0.79	0.13	-0.23	-2.20	1.05	-2.20	-2.20	-2.20	-2.20	-2.20	-0.79	-0.23
С	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	Α	Т	G	С	G	Т	Α	Α	Α	G	С	Т
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)]			l la dan 4	4laa aaa		. of Don	a III: la	1

$$W_{S} = \ln \left(\frac{P(S \mid M)}{P(S \mid B)} \right)$$

- The weight of a sequence segment is defined as the log-ratio between
 - Arr P(S/M), the sequence probability under the model described by the PSSM, and
 - Arr P(S/B), the sequence probability under the background model.
- The weight W_s represents the likelihood that segment S is an occurrence of the motif M rather than being issued from the background model B.
- Under Bernoulli asumption, the weight matrix W_{ij} can be used to simplify the computation of segment weights.

Under the assumption of Bernoulli background model, this formula becomes

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

\perp		, ,	
	W_S	weight of sequence segment <i>S</i> probability of the sequence segment, given	1
	1 (5 111)	the matrix	•
	P(S B)	probability of the sequence segment, given the background	1
	j	position within the segment and within the	
		matrix	
	r_j	residue at position <i>j</i> of the sequence segment	
	p_{rj}	prior probability of residue r_i	
	f ' _{rjj}	probability of residue r_j at position j of the	
		matrix	9

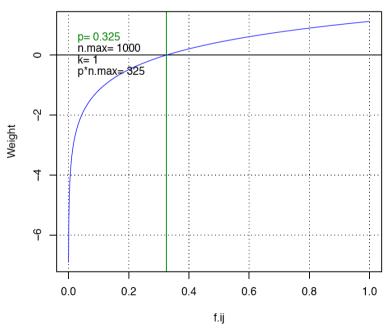
Position-weight matrix

Prior	Pos	1	2	3	4	5	6	7	8	9	10	11	12
0.33	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
0.18	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
0.18	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
0.33	Т	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	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

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

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

A alphabet size (=4) 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



Scanning a sequence with a weight matrix

 The weight matrix is successively aligned to each position of the sequence, and the score is the sum of weights for the letters aligned at each position (Hertz & Stormo, 1999).

Ex: sequenceGCTGCACGTGGCCC..

Weight matrix

	1	2	3	4	5	6	7	8	9	10	11	12
Α	-0.8	0.1	-0.2	-2.2	1.0	-2.2	-2.2	-2.2	-2.2	-2.2	-0.8	-0.2
С	0.3	0.3	0.7	1.6	-2.2	1.6	-2.2	-2.2	-2.2	0.3	-2.2	0.3
G	-0.3	0.3	0.7	-2.2	-2.2	-2.2	1.6	-2.2	1.2	1.0	1.2	0.3
A C G T	0.4	-0.8	-2.2	-2.2	-2.2	-2.2	-2.2	1.0	0.1	-0.2	-0.2	-0.2

Scanning

1	SUM -10.54													С	С
2		С	т	G	С	A	С	G	т	G	G	С	С	С	
	7.55	0.3	-0.8	0.7	1.6	1.0	1.6	1.6	1.0	1.2	1.0	-2.2	0.3		
3		Т	G	С	A	С	G	т	G	G	С	С	С		
	-9.93	0.4	0.3	0.7	-2.2	-2.2	-2.2	-2.2	-2.2	1.2	0.3	-2.2	0.3		

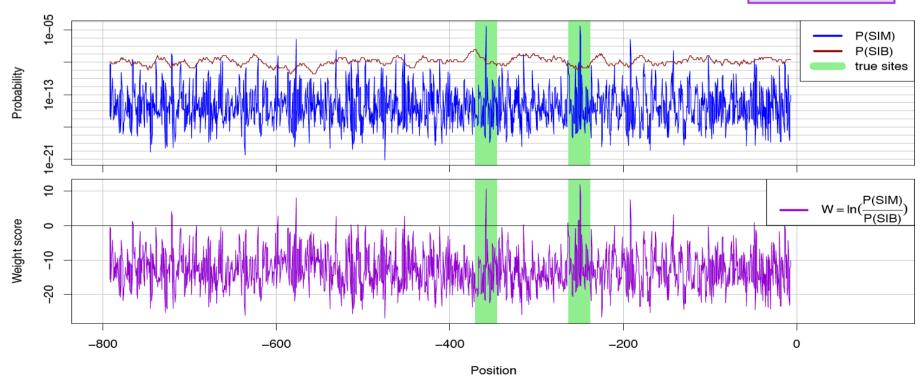
Scanning a sequence with a position-specific scoring matrix

- P(S|M) probability for site S to be generated as an instance of the motif.
- P(S|B) probability for site S to be generated as an instance of the background.
- weight, i.e. the log ratio of the two above probabilities.
 - A positive weight indicates that a site is more likely to be an instance of the motif than of the background.

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

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

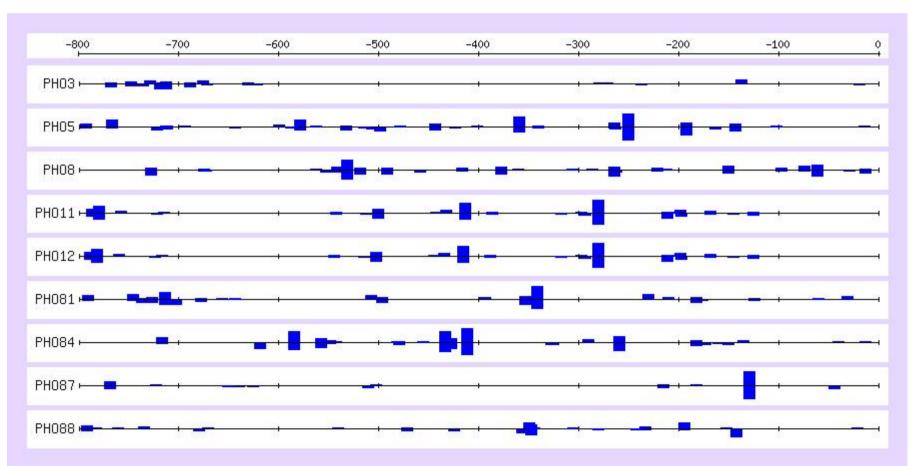
$$W_{S} = \ln \left(\frac{P(S \mid M)}{P(S \mid B)} \right)$$



Sand, O., Turatsinze, J.V. and van Helden, J. (2008). Evaluating the prediction of cis-acting regulatory elements in genome sequences In Frishman, D. and Valencia, A. (eds.), Modern genome annotation: the BioSapiens network. Springer.

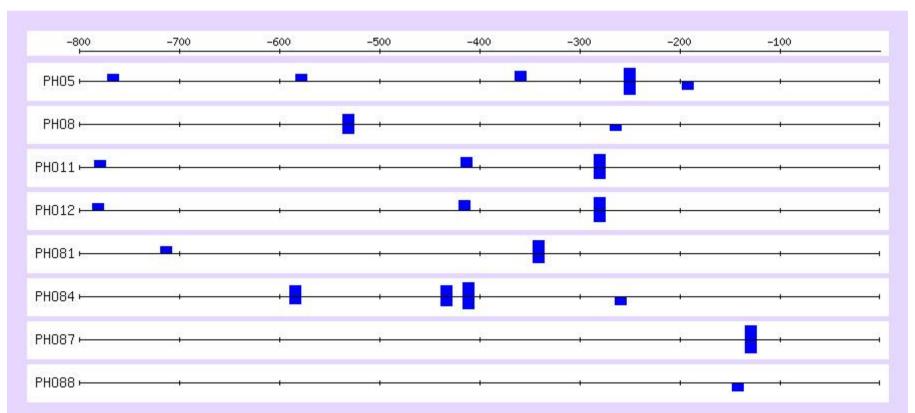
Matrix search : matching positions

- Matrix-based pattern matching is more sensitive than string-based pattern matching.
- How to choose the threshold?



Matrix search: threshold selection

- The program Patser (G. Hertz) includes an option to automatically select a threshold on the basis of
 - the information content of the matrix
 - the length of the sequence to be scanned
- Another approach is to select the threshold on the basis of scores returned when the matrix is used to scan known binding sites for the factor.



Markov chains and transition matrices

 The two tables below show the transition matrices for a Markov model of order 1 (top) and 2 (bottom), respectively.

$$P(r_i \mid S_{i-m,i-1})$$

- The two models were trained with yeast noncoding upstream sequences.
- Notice the strong probability of transitions from AA to A and TT to T.

Transition matrix, order 1

Prefix/Suffix	Α	С	G	Т	N(Suffix)
а	0.369	0.163	0.176	0.293	0.323
С	0.329	0.189	0.165	0.317	0.181
g	0.315	0.211	0.188	0.286	0.174
t	0.279	0.177	0.171	0.373	0.322
; P_res	0.323	0.181	0.174	0.322	

Transition matrix, order 2

Prefix/Suffix	Α	С	G	Т	P(Prefix)
aa	0.411	0.150	0.184	0.255	0.119
ac	0.353	0.179	0.170	0.298	0.053
ag	0.339	0.199	0.193	0.269	0.057
at	0.353	0.163	0.160	0.325	0.095
ca	0.344	0.183	0.178	0.295	0.059
cc	0.307	0.198	0.169	0.326	0.034
cg	0.283	0.228	0.193	0.296	0.030
ct	0.246	0.188	0.183	0.383	0.057
ga	0.410	0.142	0.186	0.261	0.055
gc	0.335	0.191	0.179	0.295	0.037
gg	0.323	0.215	0.193	0.270	0.033
gt	0.310	0.154	0.198	0.338	0.050
ta	0.304	0.179	0.157	0.360	0.090
tc	0.316	0.193	0.149	0.342	0.057
tg	0.304	0.210	0.177	0.309	0.055
tt	0.224	0.193	0.163	0.419	0.120
P(Suffix)	0.323	0.181	0.174	0.322	

ቯ	æ	ပ	0.0	ب
a	0.369	0.163	0.176	0.293
С	0.329	0.189	0.165	0.317
g	0.315	0.211	0.188	0.286
t	0.279	0.177	0.171	0.373

aa 0.411 0.150 0.184 0.255 ac 0.353 0.179 0.170 0.298 ag 0.339 0.199 0.193 0.269 at 0.353 0.163 0.160 0.325 ca 0.344 0.183 0.178 0.295 cc 0.307 0.198 0.169 0.326 cg 0.283 0.228 0.193 0.296 ct 0.246 0.188 0.183 0.383 ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309 tt 0.224 0.193 0.163 0.419	ם	æ	ပ	0.0	4
ag 0.339 0.199 0.193 0.269 at 0.353 0.163 0.160 0.325 ca 0.344 0.183 0.178 0.295 cc 0.307 0.198 0.169 0.326 cg 0.283 0.228 0.193 0.296 ct 0.246 0.188 0.183 0.383 ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	aa	0.411	0.150	0.184	0.255
at 0.353 0.163 0.160 0.325 ca 0.344 0.183 0.178 0.295 cc 0.307 0.198 0.169 0.326 cg 0.283 0.228 0.193 0.296 ct 0.246 0.188 0.183 0.383 ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	ac	0.353	0.179	0.170	0.298
ca 0.344 0.183 0.178 0.295 cc 0.307 0.198 0.169 0.326 cg 0.283 0.228 0.193 0.296 ct 0.246 0.188 0.183 0.383 ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	ag	0.339	0.199	0.193	0.269
cc 0.307 0.198 0.169 0.326 cg 0.283 0.228 0.193 0.296 ct 0.246 0.188 0.183 0.383 ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	at	0.353	0.163	0.160	0.325
cg 0.283 0.228 0.193 0.296 ct 0.246 0.188 0.183 0.383 ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	ca	0.344	0.183	0.178	0.295
ct 0.246 0.188 0.183 0.383 ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	cc	0.307	0.198	0.169	0.326
ga 0.410 0.142 0.186 0.261 gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	cg	0.283	0.228	0.193	0.296
gc 0.335 0.191 0.179 0.295 gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	ct	0.246	0.188	0.183	0.383
gg 0.323 0.215 0.193 0.270 gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	ga	0.410	0.142	0.186	0.261
gt 0.310 0.154 0.198 0.338 ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	gc	0.335	0.191	0.179	0.295
ta 0.304 0.179 0.157 0.360 tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	gg	0.323	0.215	0.193	0.270
tc 0.316 0.193 0.149 0.342 tg 0.304 0.210 0.177 0.309	gt	0.310	0.154	0.198	0.338
tg 0.304 0.210 0.177 0.309	ta	0.304	0.179	0.157	0.360
	tc	0.316	0.193	0.149	0.342
tt 0.224 0.193 0.163 0.419	tg	0.304	0.210	0.177	0.309
	tt	0.224	0.193	0.163	0.419

Scoring a sequence segment with a Markov model

 The example below illustrates the computation of the probability of a short sequence (ATGCGTAAAGCT) with a Markov chain of order 2, estimated from 3nt frequencies on the yeast genome.

Transition matrix, order 2

Prefix/Suffix	Α	С	G	Т	P(Prefix)
aa	0.411	0.150	0.184	0.255	0.119
ac	0.353	0.179	0.170	0.298	0.053
ag	0.339	0.199	0.193	0.269	0.057
at	0.353	0.163	0.160	0.325	0.095
ca	0.344	0.183	0.178	0.295	0.059
cc	0.307	0.198	0.169	0.326	0.034
cg	0.283	0.228	0.193	0.296	0.030
ct	0.246	0.188	0.183	0.383	0.057
ga	0.410	0.142	0.186	0.261	0.055
gc	0.335	0.191	0.179	0.295	0.037
gg	0.323	0.215	0.193	0.270	0.033
gt	0.310	0.154	0.198	0.338	0.050
ta	0.304	0.179	0.157	0.360	0.090
tc	0.316	0.193	0.149	0.342	0.057
tg	0.304	0.210	0.177	0.309	0.055
tt	0.224	0.193	0.163	0.419	0.120
P(Suffix)	0.323	0.181	0.174	0.322	

$$P(S) = P(S_{1,m}) \prod_{i=m+1}^{L} P(r_i \mid S_{i-m,i-1})$$

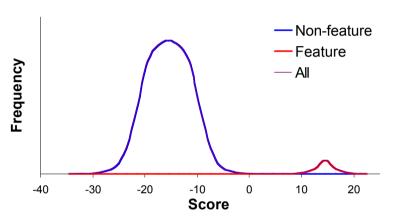
pos	P(R W)		wR	S	P(S)
1	P(at)	0.094	at	at	9.42E-02
3	P(g at)	0.161	atG	atg	1.52E-02
4	P(c tg)	0.210	tgC	atgc	3.19E-03
5	P(g gc)	0.180	gcG	atgcg	5.74E-04
6	P(t cg)	0.295	cgT	atgcgt	1.69E-04
7	P(a gt)	0.309	gtA	atgcgta	5.23E-05
8	P(a ta)	0.304	taA	atgcgtaa	1.59E-05
9	P(a aa)	0.409	aaA	atgcgtaaa	6.50E-06
10	P(g aa)	0.184	aaG	atgcgtaaag	1.20E-06
11	P(c ag)	0.200	agC	atgcgtaaagc	2.39E-07
12	P(t gc)	0.294	gcT	atgcgtaaagct	7.04E-08

Sensitivity / selectivity tradeoff

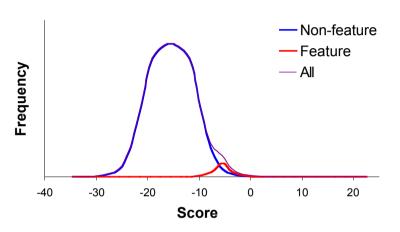
- The sequence is scanned with the matrix, and a score is assigned to each position.
- The highest score reflects the highest probability of having a functional site.
- How to define the threshold? There is a tradeoff:
 - high selectivity ⇔ low sensitivity
 - high confidence in the predicted sites, but many real sites are missed
 - low selectivity ⇔ high sensitivity
 the real sites are drawn in a see of false positive

Discrimination power of a matrix

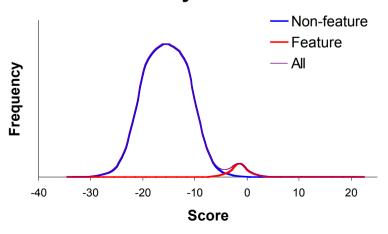




Poorly discriminant



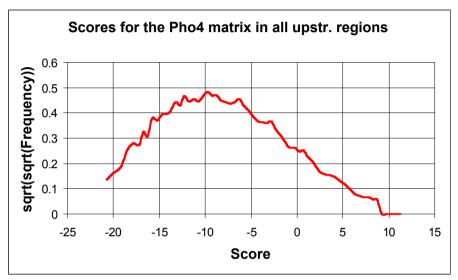
Reasonably discriminant

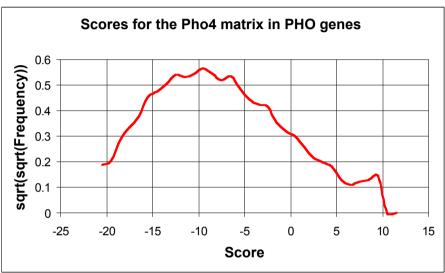


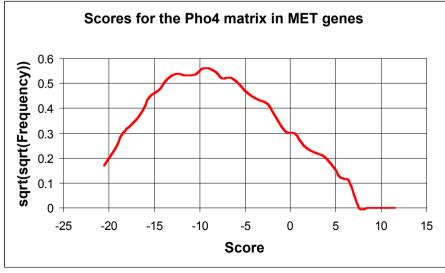
Regulatory Sequence Analysis

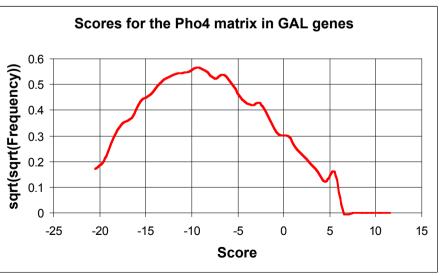
Old slides

Pho4p matrix: score distribution in all upstream sequences









Genome-scale pattern matching: top scoring upstream sequences for the Pho4p matrix

ORF	strand	start	end	score	gene description
YMR255W	D	-326	-315	10.2	hypothetical protein
YKR050W	D	-744	-733	9.83	TRK2; moderate-affinity potassium transport protein
YKR048C	D	-442	-431	9.83	NAP1; nucleosome assembly protein I
YKR047W	D	-689	-678	9.83	questionable ORF
YKR031C	D	-597	-586	9.83	SPO14; phospholipase D
YCR037C	D	-135	-124	9.83	PHO87; member of the phosphate permease family
YAL038W	D	-525	-514	9.83	CDC19; pyruvate kinase
YML123C	D	-417	-406	9.56	PHO84; high-affinity inorganic phosphate/H+ symporter
YJL067W	D	-442	-4 31	9.56	questionable ORF
YCR051W	D	-298	-287	9.56	weak similarity to ankyrins
YBR296C	D	-326	-315	9.56	strong similarity to phosphate-repressible phosphate permease
YHR215W	D	-286	-275	9.46	PHO12; secreted acid phosphatase
YDR281C	D	-282	-271	9.46	hypothetical protein
YBR093C	D	-256	-245	9.46	PHO5; repressible acid phosphatase precursor
YAR071W	D	-286	-275	9.46	PHO11; secreted acid phosphatase
YNR050C	D	-516	-505	9.35	LYS9; saccharopine dehydrogenase (NADP+, L-glutamate forming)
YNL062C	D	-68	-57	9.35	GCD10; translation initiation factor eIF3 RNA-binding subunit
YJR059W	D	-246	-235	9.19	PTK2; involved in polyamine uptake
YHR107C	D	-506	-495	9.19	CDC12; septin
YGR233C	D	-347	-336	9.19	PHO81; cyclin-dependent kinase inhibitor
YFR040W	D	-81	-70	9.19	SAP155; Sit4p-associated protein
YJR067C	D	-505	-494	9.08	YAE1; protein of unknown function
YDR163W	D	-668	-657	9.08	hypothetical protein

Improving genome-scale predictions

- The prediction of single binding sites is notoriously noisy. However, one can try to combine different types of information in order to predict the regulation of a gene as a whole, rather than each potential binding site.
- Include as much biological information as possible
 - Site repetition (e.g. GATA boxes)
 - Combination of heterologous sites (e.g. Met4p and Met31p)
 - Information about site positions: distribution of position for known sites and impose a constraint on putative site positions
- Supervised classification: train a program with
 - a set of reliable positive sites (from known target genes)
 - a set of reliable negative sites
 (genes known not to respond to the factor)

Regulatory Sequence Analysis

Matching a sequence with a library of patterns

Match a sequence with a library of patterns

- Goal: given a sequence, find matches for any known regulatory site
 - → identify transcription factors that could regulate the gene
- Strategy: apply systematically pattern search with all patterns stored in the library
- Problem: how to set the threshold for the different patterns?
- Warning : generates many false positive

Transfac Matsearch result - PHO5 upstream region

Inspecting sequence	PHO5_4 [?] (1 - 81	6):		
F\$NIT2_01	141 (+)	1.000	0.995	TATCtc
F\$PHO4_01	561 (+)	1.000	0.990	tcaCACGtggga
F\$PHO4_01	561 (-)	1.000	0.982	tccCACGtgtga
F\$NIT2_01	634 (+)	1.000	0.972	TATCaa
F\$NIT2_01	543 (-)	1.000	0.967	TATCga
F\$NIT2_01	676 (-)	1.000	0.945	TATCCC
F\$NIT2_01	31 (-)	1.000	0.937	TATCag
F\$PHO4_01	452 (+)	1.000	0.935	tagCACGttttc
F\$MCM1_01	666 (-)	0.961	0.929	tatCCCAaatgggtat
F\$MATA1_01	202 (+)	1.000	0.926	tGATG tcagt
F\$GCR1_01	323 (-)	1.000	0.922	gaCTTCcaa
F\$GCN4_C	536 (+)	0.837	0.902	aaaTGAATcg
F\$ABAA_01	292 (-)	1.000	0.889	atttgcgCATTcttgttga
F\$ABF_C	205 (+)	0.887	0.885	tgtcagtccccACGC
F\$MATA1_01	727 (+)	1.000	0.882	tGATGttttg
F\$MIG1_01	210 (-)	1.000	0.881	gctattagcgtGGGGac
F\$GCR1_01	69 (+)	0.826	0.880	ggCATCcaa
F\$PHO4_01	90 (-)	1.000	0.879	ggtCACGtttct
F\$MAT1MC_02	696 (+)	1.000	0.875	tgaaTTGTcg
F\$GCN4_C	589 (+)	0.882	0.862	ttaTGATTct
F\$STE11_01	415 (+)	1.000	0.860	ctttttCTTTgtctgcac
F\$GCR1_01	249 (-)	0.783	0.859	ggCGTCctg
F\$STE11_01	425 (-)	1.000	0.859	atatttCTTTgtgcagac
F\$MCM1_01	484 (+)	0.831	0.855	atgCCAAaaaaagtaa

Transfac Matsearch result - random sequence (mkv 5)

Inspecting sequence ra	ndom mkv5 [?] (1	- 817):		
F\$NIT2_01	176 (+)	1.000	1.000	TATCta
F\$NIT2_01	656 (+)	1.000	1.000	TATCta
F\$NIT2_01	275 (+)	1.000	0.995	TATCtc
F\$NIT2_01	455 (+)	1.000	0.995	TATCtc
F\$NIT2_01	298 (-)	1.000	0.980	TATCtt
F\$MATA1_01	506 (-)	1.000	0.980	tGATGtatgt
F\$ABF_C	84 (+)	0.991	0.973	aatcattcttgACGT
F\$MIG1_01	264 (-)	1.000	0.958	gagataaaactGGGGtt
F\$NIT2_01	701 (+)	1.000	0.947	TATCgt
F\$NIT2_01	802 (-)	1.000	0.947	TATCgt
F\$ABF1_01	81 (+)	0.976	0.944	gtaaatcattcttgACGTtttt
F\$MAT1MC_02	665 (-)	1.000	0.918	cctaTTGTga
F\$NIT2_01	280 (-)	1.000	0.915	TATCcg
F\$ABAA_01	42 (+)	1.000	0.902	tccccatCATTctaacagt
F\$PACC_01	331 (-)	1.000	0.897	acgaGCCAagaaaagtt
F\$ABAA_01	201 (+)	1.000	0.883	accatagCATTctggatct
F\$MAT1MC_02	442 (-)	1.000	0.882	tataTTGTat
F\$ABF_C	638 (-)	0.991	0.882	agtcaaatgaaACGT
F\$ABF_C	609 (-)	0.949	0.874	tttctttaaacACGG
F\$MATA1_01	558 (-)	1.000	0.868	tGATGgaaga
F\$HSF_03	713 (-)	1.000	0.859	AGAAttgaaattttt
F\$MAT1MC_02	134 (-)	1.000	0.858	cacaTTGTgt
F\$ABAA_01	80 (+)	1.000	0.856	agtaaatCATTcttgacgt
F\$HAP234_01	332 (-)	1.000	0.851	acgagCCAAgaaaagt

Transfac Matsearch result - random sequence (iid)

Inspecting sequence r	andom iid [2] (1	_ 817)•		
F\$NIT2 01	534 (-)	· .	1.000	TATCta
F\$NIT2_01	294 (+)		0.995	TATCtc
_	! ` '!			
F\$NIT2_01	634 (-)		0.972	TATCaa
F\$NIT2_01	216 (-)	1.000	0.965	TATCtg
F\$STUAP_01	808 (-)	1.000	0.959	attCGCGtct
F\$NIT2_01	24 (+)	1.000	0.952	TATCat
F\$NIT2_01	343 (+)	1.000	0.952	TATCat
F\$NIT2_01	413 (-)	1.000	0.952	TATCat
F\$STUAP_01	441 (+)	1.000	0.930	aagCGCGcct
F\$NIT2_01	244 (-)	1.000	0.930	TATCct
F\$STUAP_01	808 (+)	1.000	0.926	agaCGCGaat
F\$GCR1_01	499 (+)	1.000	0.922	gaCTTCcta
F\$PACC_01	647 (-)	1.000	0.920	ctccGCCAggcactgaa
F\$NIT2_01	475 (+)	1.000	0.915	TATCcg
F\$ABF_C	235 (-)	0.949	0.904	tatcctgcaacACGG
F\$PHO4_01	246 (-)	1.000	0.882	gctCACGttatc
F\$GCR1_01	763 (-)	1.000	0.866	acCTTCcgc
F\$STUAP_01	441 (-)	1.000	0.859	aggCGCGctt
F\$MIG1_01	371 (+)	1.000	0.857	accgaaacagtGGGGtt
F\$MAT1MC_02	375 (-)	0.769	0.855	cccaCTGTtt