

# *Assessing the quality of position-specific scoring matrices*

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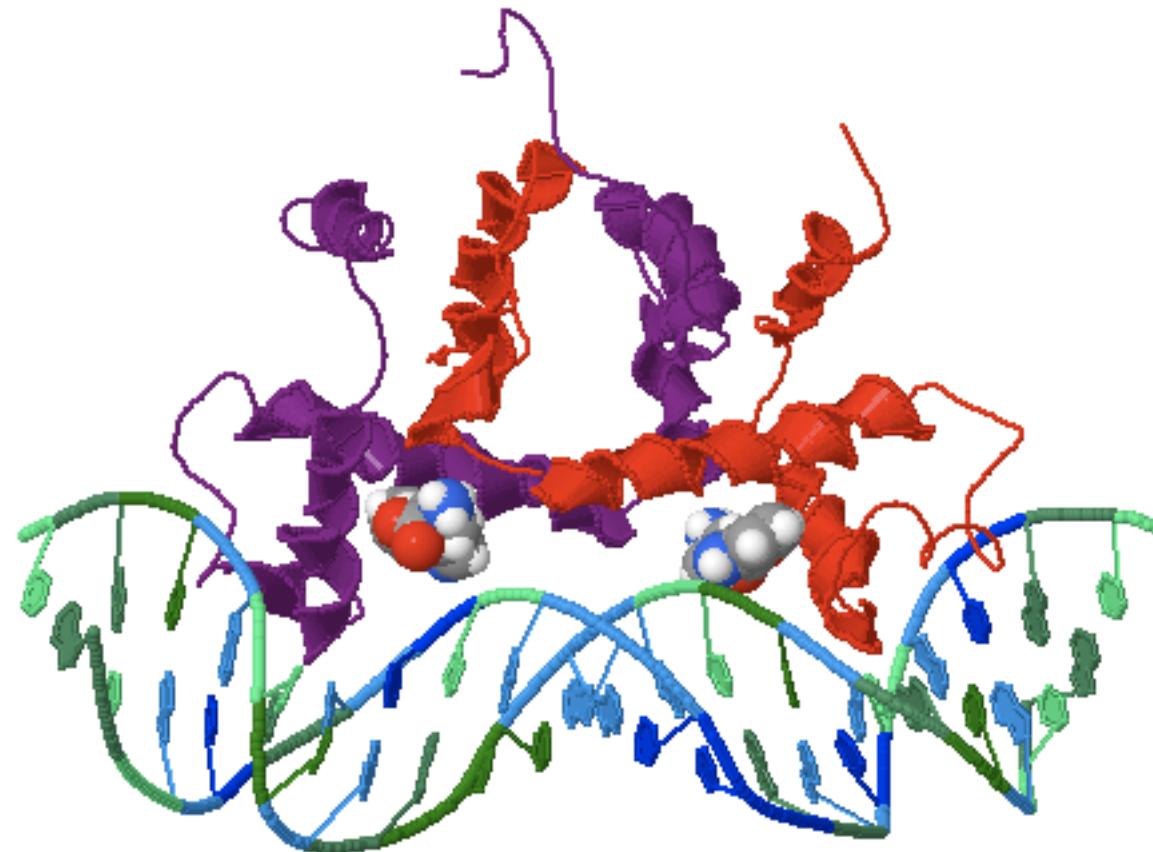
Rekin's Janky  
Ex-PhD student



Eric Vervisch  
Ex-Research fellow

- *Development and application of bioinformatics methods for the analysis of genome function, regulation and evolution.*
- **Regulatory sequences**
  - Pattern discovery algorithms
    - Olivier Sand (Postdoc), Matthieu Defrance (Postdoc), Maud Vidick (Master thesis)
  - Evolution of cis-acting elements in Bacteria
    - Rekin's Janky (PhD student)
  - Regulation of development in Drosophila
    - Jean Valéry Turatsinze (PhD student)
  - Hox regulation in Vertebrates
    - Morgane Thomas-Chollier (PhD student)
  - Work flows on transcriptional regulation
    - Olivier Sand (Postdoc), Eric Vervisch (Research fellow)
- **Molecular networks**
  - Analysis of regulatory networks
    - Rekin's Janky (PhD student), Sylvain Brohée (PhD student)
  - Interactions between membrane-associated proteins
    - Sylvain Brohée (PhD student)
  - Inference of metabolic pathways
    - Karoline Faust (PhD student)
  - Host-virus interaction networks
    - Nicolas Simonis (Postdoc)
- **Mobile genetic elements in prokaryotes**
  - Raphaël Leplae (Postdoc), Gipsi Lima (PhD student), Ariane Toussaint (Professor)
- **Modelling of dynamical systems**
  - Didier Gonze (Premier assistant)

## *NMR study of TrpR*



- Zhang et al (1994). The solution structures of the trp repressor-operator DNA complex. J Mol Biol 238:592-614. PDB entry **1rcs**.

# *TrpR* binding sites and motif in *Escherichia coli* K12

## (a) Annotated TrpR binding sites

### Site ID

Site ID	G	T	A	C	T	A	G	T	T	T	G	A	T	G	G	T	A	T	G	Target Operon
ECK120012644	G	T	A	C	T	A	G	T	T	T	G	A	T	G	G	T	A	T	G	aroL-yaiA-aroM
ECK120012187	G	T	A	C	T	A	G	T	T	T	G	A	T	G	G	T	A	T	G	aroL-yaiA-aroM
ECK120012179	G	A	A	C	T	A	G	T	T	A	A	C	T	A	G	T	A	C	G	trpLEDCBA
ECK120012892	G	A	A	C	T	A	G	T	T	A	A	C	T	A	G	T	A	C	G	trpLEDCBA
ECK120012181	G	A	A	C	T	A	G	T	T	A	A	C	T	A	G	T	A	C	G	trpLEDCBA
ECK120012636	G	T	A	C	T	A	G	A	G	A	A	C	T	A	G	T	T	G	C	aroH
ECK120012183	G	T	A	C	T	A	G	A	G	A	A	C	T	A	G	T	T	G	C	aroH
ECK120012185	G	T	A	C	T	C	G	T	G	T	A	C	T	T	G	G	T	A	C	mtr
ECK120012979	G	T	A	C	T	C	G	T	G	T	A	C	T	G	G	T	T	A	C	mtr
ECK120012894	G	T	A	C	T	C	T	T	T	A	G	C	G	A	G	T	T	A	C	trpR

## (b) Position-specific scoring matrix

A	0	3	10	0	0	7	0	2	0	6	7	2	0	6	0	0	8	0	5	
T	0	7	0	0	10	0	1	8	6	4	0	0	9	0	0	0	10	0	2	0
C	0	0	0	10	0	3	0	0	0	0	0	8	0	0	0	0	0	8	0	
G	10	0	0	0	0	0	9	0	4	0	3	0	1	4	10	0	2	0	5	

## (c) Consensus

G w A C T m G t k w r C t r G T r C r

## (d) Sequence logo



# Background models - Drosophila non-coding upstream sequences

		Drosophila upstream sequences Markov order 2				
		a	c	g	t	
pr\suf		aa	0.37291	0.16877	0.16923	0.28909
		ac	0.35709	0.18621	0.17167	0.28504
		ag	0.26165	0.27237	0.18363	0.28236
		at	0.26257	0.16673	0.20870	0.36201
		ca	0.33684	0.19795	0.20584	0.25936
		cc	0.35835	0.22516	0.19563	0.22085
		cg	0.30620	0.25800	0.21443	0.22137
		ct	0.19334	0.20958	0.26335	0.33373
		ga	0.36047	0.17482	0.20549	0.25922
		gc	0.32151	0.23320	0.18692	0.25838
		gg	0.26746	0.29268	0.22352	0.21634
		gt	0.21600	0.18334	0.26213	0.33853
		ta	0.35342	0.16646	0.15262	0.32750
		tc	0.29702	0.21931	0.22825	0.25543
		tg	0.23549	0.26070	0.23044	0.27338
		tt	0.22564	0.18481	0.21803	0.37151

		Drosophila upstream sequences Markov order 1				
		a	c	g	t	
pr\suf		a	0.35787	0.17609	0.18046	0.28557
		c	0.33195	0.21597	0.19577	0.25632
		g	0.26293	0.26993	0.21370	0.25344
		t	0.22881	0.18378	0.23121	0.35620

# Markov models show strong variations between organisms

**Saccharomyces cerevisiae  
(Fungus)**

P	a	c	g	t
a	0.37000	0.16588	0.17908	0.28504
c	0.32610	0.19058	0.16818	0.31514
g	0.31163	0.21456	0.18957	0.28424
t	0.27256	0.17991	0.17364	0.37389

**Escherichia coli K12  
(Proteobacteria)**

P	a	c	g	t
a	0.34491	0.18156	0.17676	0.29677
c	0.30806	0.21557	0.22129	0.25507
g	0.27123	0.25972	0.21545	0.25360
t	0.24080	0.19176	0.21144	0.35599

**Mycobacterium leprae  
(Actinobacteria)**

P	a	c	g	t
a	0.23239	0.28694	0.25692	0.22375
c	0.24574	0.24601	0.30574	0.20252
g	0.21748	0.29238	0.25535	0.23479
t	0.18806	0.26081	0.31784	0.23329

**Mycoplasma genitalium  
(Firmicute, intracellular)**

P	a	c	g	t
a	0.45565	0.11743	0.13602	0.29091
c	0.39457	0.13008	0.06403	0.41132
g	0.31505	0.18738	0.12047	0.37710
t	0.32450	0.09573	0.11934	0.46044

**Bacillus subtilis  
(Firmicute, extracellular)**

P	a	c	g	t
a	0.38159	0.13935	0.18767	0.29139
c	0.33699	0.19499	0.16508	0.30293
g	0.34249	0.18100	0.23541	0.24110
t	0.25122	0.17199	0.19402	0.38278

**Plasmodium falciparum  
(Apicomplexa, intracellular)**

P	a	c	g	t
a	0.39821	0.06446	0.05206	0.48527
c	0.47798	0.13336	0.06695	0.32171
g	0.36764	0.08587	0.12431	0.42217
t	0.44739	0.05676	0.07673	0.41912

**Anopheles gambiae  
(Insect)**

P	a	c	g	t
a	0.34603	0.21388	0.18890	0.25119
c	0.31499	0.21232	0.24159	0.23109
g	0.26036	0.25414	0.20275	0.28275
t	0.20368	0.20710	0.24970	0.33951

**Homo sapiens  
(Mammalian)**

P	a	c	g	t
a	0.29760	0.19031	0.28856	0.22353
c	0.28019	0.30209	0.11692	0.30080
g	0.24408	0.24738	0.30309	0.20545
t	0.18589	0.23061	0.27491	0.30859

# Scoring a sequence segment with a Markov model

- The example below illustrates the computation of the probability of a sequence segment (CCTACTATATGCCAGAATT) with a Markov chain of order 2, calibrated from 3nt frequencies on the yeast genome.

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

Transition matrix, order 2

Prefix/Suffix	A	C	G	T	P(Prefix)	N(Prefix)
AA	0.388	0.161	0.200	0.251	<b>0.112</b>	<b>525,000</b>
AC	0.339	0.198	0.173	0.290	<b>0.054</b>	<b>251,072</b>
AG	0.345	0.204	0.196	0.255	<b>0.059</b>	<b>274,601</b>
AT	0.311	0.184	0.182	0.323	<b>0.088</b>	<b>413,946</b>
CA	0.347	0.178	0.189	0.286	<b>0.063</b>	<b>293,750</b>
CC	0.341	0.190	0.161	0.309	<b>0.038</b>	<b>178,110</b>
CG	0.293	0.221	0.196	0.290	<b>0.031</b>	<b>145,876</b>
CT	0.229	0.195	0.205	0.371	<b>0.059</b>	<b>275,634</b>
GA	0.394	0.155	0.187	0.264	<b>0.059</b>	<b>277,053</b>
GC	0.330	0.205	0.169	0.297	<b>0.039</b>	<b>184,192</b>
GG	0.318	0.217	0.187	0.277	<b>0.037</b>	<b>173,266</b>
GT	0.285	0.175	0.204	0.336	<b>0.051</b>	<b>239,384</b>
TA	0.300	0.193	0.168	0.339	<b>0.079</b>	<b>369,426</b>
TC	0.313	0.203	0.152	0.332	<b>0.060</b>	<b>280,131</b>
TG	0.302	0.209	0.208	0.282	<b>0.060</b>	<b>279,783</b>
TT	0.210	0.208	0.189	0.392	<b>0.111</b>	<b>520,906</b>
<b>P(Suffix)</b>	<b>0.313</b>	<b>0.191</b>	<b>0.187</b>	<b>0.310</b>		
<b>N(suffix)</b>	<b>1,466,075</b>	<b>893,444</b>	<b>873,260</b>	<b>1,449,351</b>		

pos	<b>P(R W)</b>	<b>wR</b>	<b>S</b>	<b>P(S)</b>	
1	P(CC)	0.038	cc	CC	3.80E-02
2	P(T CC)	0.309	ccT	CCT	1.17E-02
3	P(A CT)	0.229	ctA	CCTA	2.69E-03
4	P(C TA)	0.193	taC	CCTAC	5.19E-04
5	P(T AC)	0.290	acT	CCTACT	1.50E-04
6	P(A CT)	0.229	ctA	CCTACTA	3.45E-05
7	P(T TA)	0.339	taT	CCTACTAT	1.17E-05
8	P(A AT)	0.311	atA	CCTACTATA	3.63E-06
9	P(T TA)	0.339	taT	CCTACTATAT	1.23E-06
10	P(G AT)	0.182	atG	CCTACTATATG	2.25E-07
11	P(C TG)	0.209	tgC	CCTACTATATGC	4.69E-08
12	P(C GC)	0.205	gcC	CCTACTATATGCC	9.61E-09
13	P(C CC)	0.190	ccC	CCTACTATATGCC	1.82E-09
14	P(A CC)	0.341	ccA	CCTACTATATGCCA	6.21E-10
15	P(G CA)	0.189	caG	CCTACTATATGCCAG	1.17E-10
16	P(A AG)	0.345	agA	CCTACTATATGCCAGA	4.04E-11
17	P(A GA)	0.394	gaA	CCTACTATATGCCAGAA	1.59E-11
18	P(T AA)	0.251	aaT	CCTACTATATGCCAGAAT	4.00E-12
19	P(T AT)	0.323	atT	CCTACTATATGCCAGAATT	1.29E-12

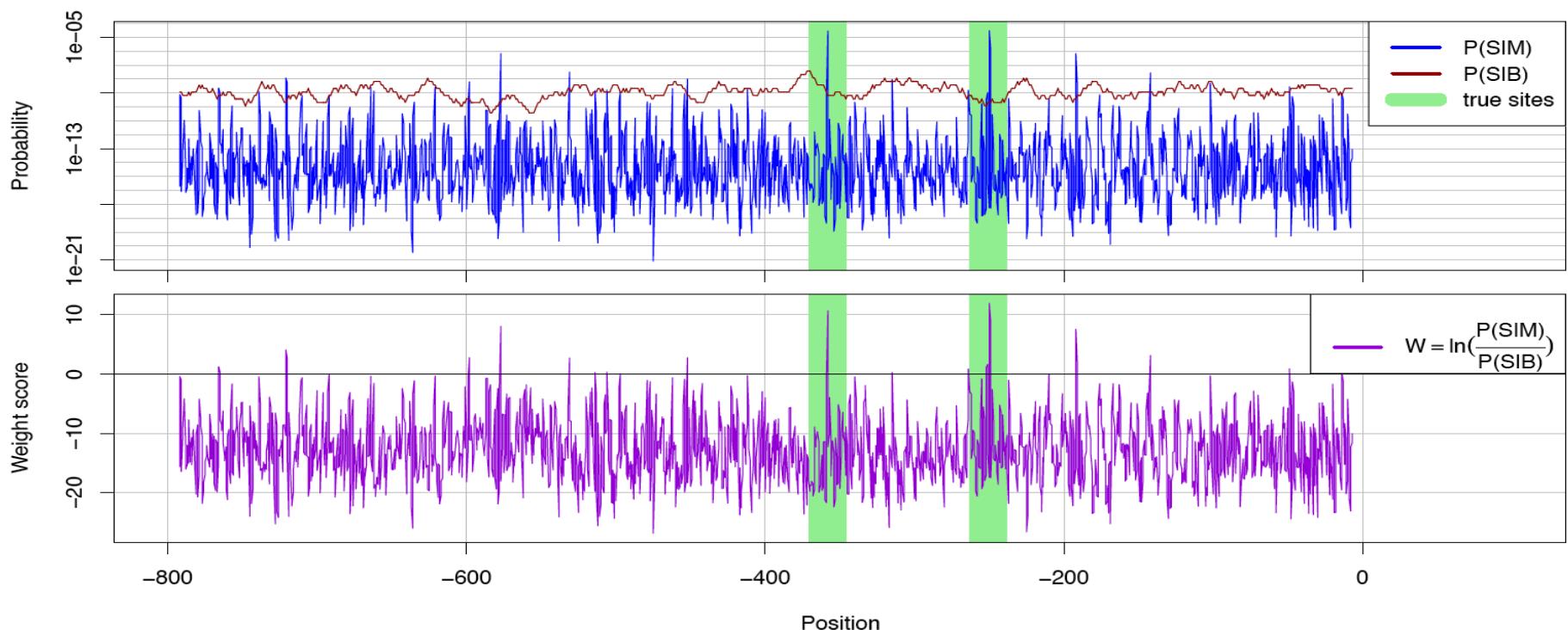
# Scanning a sequence with a position-specific scoring matrix

- $P(S|M)$  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.
- $W$  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.

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

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

$$W_S = \ln\left(\frac{P(S|M)}{P(S|B)}\right)$$



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.

## Question

- If we use a position-specific scoring matrix to predict binding sites for a transcription factor, how good is this matrix to recognize *bona fide* binding sites from other sequences (background) ?
- We will have to measure the trade-off between two **qualities** of a matrix
  - **Sensitivity (=coverage)**: which fraction of the actual binding site are we able to detect ?
  - **Positive predictive power (PPV)**: which fraction of our predictions do correspond to actual binding sites ?
- Several **parameters** have a crucial importance for determining the reliability of the predictions
  - **The matrix**: which is the best matrix that can be built from a collection of annotated binding sites ?
    - Selection of the binding sites to be incorporated
    - Alignment procedure.
    - Matrix width.
    - Background model used for building the matrix
  - Scanning parameters:
    - Background model used for scanning the sequences,
    - Score threshold.

# Approaches

- Theoretical estimates
  - Various statistics have been used to estimate the relevance of a motif
    - Relative entropy (Schneider, 1986)
    - Information Content (Hertz & Stormo, 1990; 1999)
    - P-value (Staden, 1989) or E-value (Hertz & Stormo, 1999; Bailey, 1994)
    - Consensus score (Thijs et al., 2001)
    - Log-likelihood (Thijs et al., 2001)
    - Alpha- and beta-riks (Rahmann et al, 2003)
  - All those statistics rely on theoretical assumption, which might fail to reproduce the characteristics of real biological sequences.
- We propose here a complementary approach, combining theoretical and empirical measurements of matrix quality.
  - Compute the theoretical distributions of scores for all possible threshold values.
  - Compare theoretical distributions with those obtained in several reference data sets.
    - Positive control
      - Annotated binding sites for the transcription factor of interest
      - Cis-regulatory regions of the target genes
    - Negative control
      - DNA sequences supposed to be devoid of binding sites.

## ***Sensitivity, PPV and Accuracy curves***

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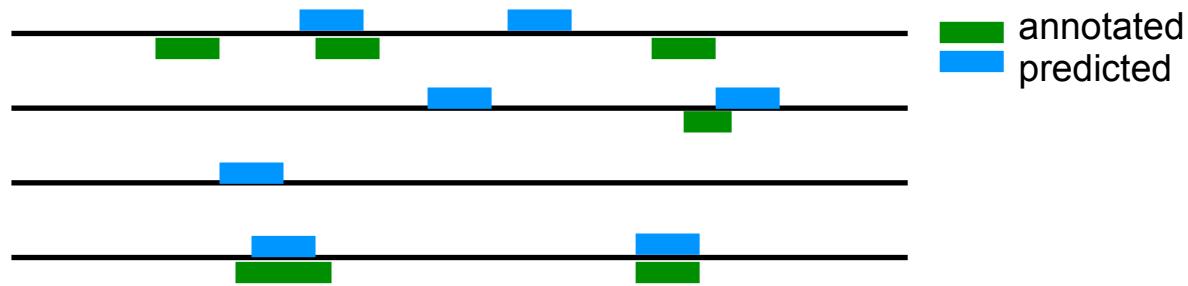
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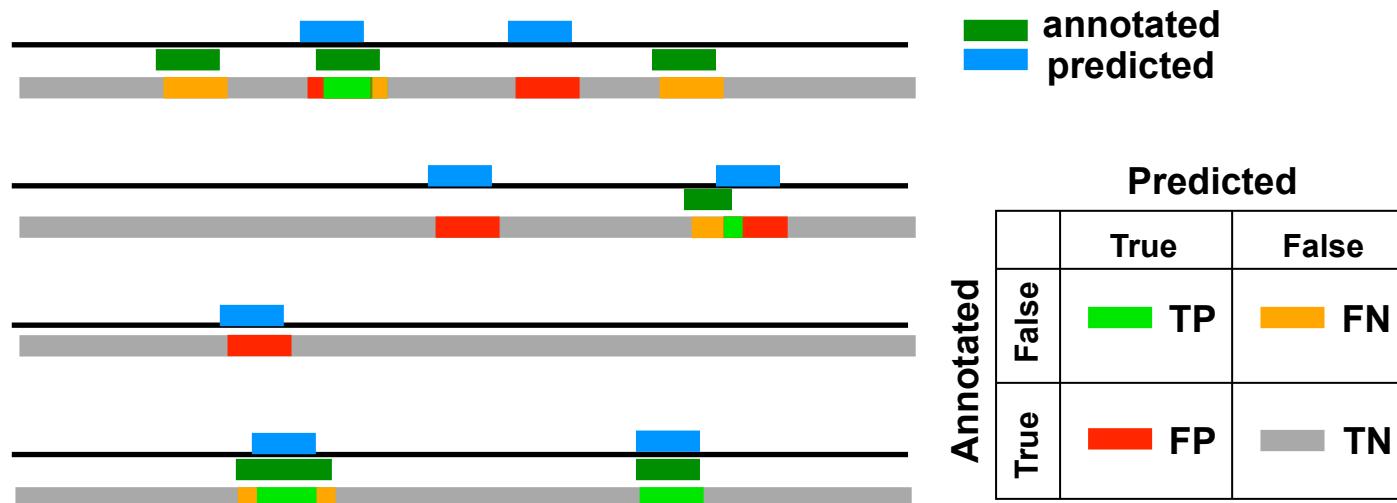
## *Comparison between annotated and predicted sites*

- The annotated and predicted sites are compared.



## Comparison at the nucleotide level

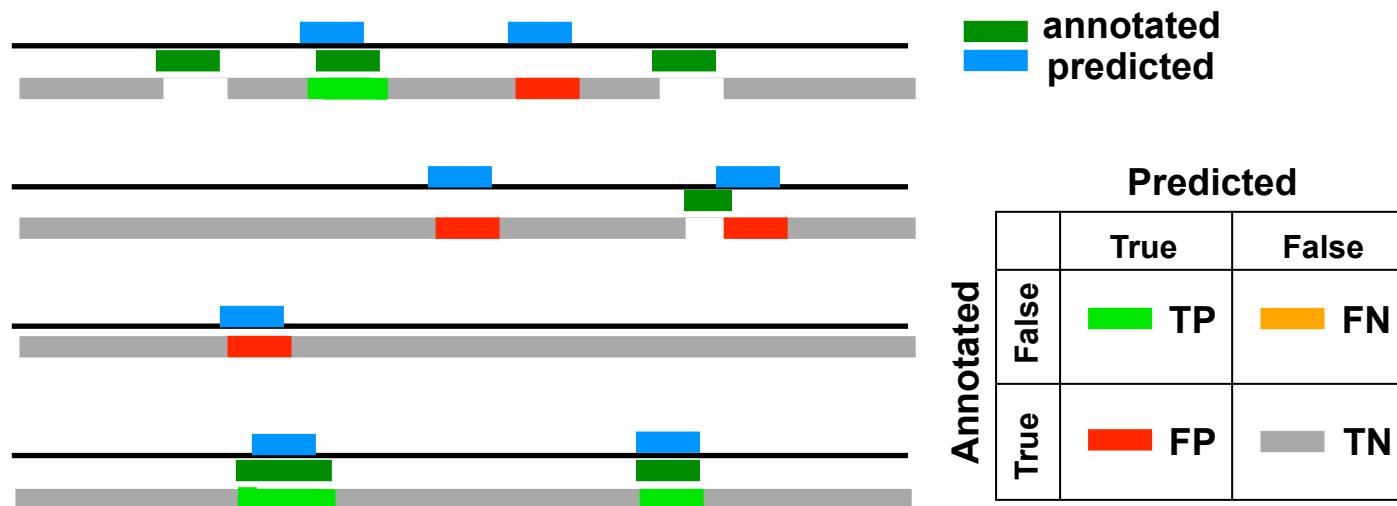
- Annotated and predicted sites can be compared **at the nucleotide level**.
- Each predicted nucleotide is considered as a match if it falls within an annotated site.
- A predicted site can thus contain a mixture of false positive and true positive nucleotides.
- An annotated site can thus contain a mixture of true positive and false negative nucleotides.



<b>TP</b>	<b>True Positive</b>	<b>Annotated and predicted.</b>
<b>FP</b>	<b>False Positive</b>	<b>Predicted but not annotated</b>
<b>TN</b>	<b>True Negative</b>	<b>Neither annotated nor predicted</b>
<b>FN</b>	<b>False Negative</b>	<b>Annotated but not predicted</b>

## Comparison at the site level

- Annotated and predicted sites can be compared **at the site level**.
- Each predicted site is considered as a match (**as a whole**) if it overlaps with an annotated site.
- Each annotated site is considered as detected (**as a whole**) if it is matched by at least one predicted site.
- A threshold can be imposed on the minimal number of overlapping nucleotides in order to consider that a predicted site does or not match an annotated site.



TP	True Positive	Annotated and predicted.
FP	False Positive	Predicted but not annotated
TN	True Negative	Neither annotated nor predicted
FN	False Negative	Annotated but not predicted

# Validation statistics

- Various statistics can be derived from the 4 elements of a contingency table.

Abbrev	Name	Formula
TP	True positive	TP
FP	False positive	FP
FN	False negative	FN
TN	True negative	TN
KP	Known Positive	TP+FN
KN	Known Negative	TN+FP
PP	Predicted Positive	TP+FP
PN	Predicted Negative	FN+TN
N	Total	TP + FP + FN + TN
Prev	Prevalence	(TP + FN)/N
ODP	Overall Diagnostic Power	(FP + TN)/N
CCR	Correct Classification Rate	(TP + TN)/N
<b>Sn</b>	<b>Sensitivity</b>	<b>TP/(TP + FN)</b>
Sp	Specificity	TN/(FP + TN)
FPR	False Positive Rate	FP/(FP + TN)
FNR	False Negative Rate	FN/(TP + FN)
<b>PPV</b>	<b>Positive Predictive Value</b>	<b>TP/(TP + FP)</b>
FDR	False Discovery Rate	FP/(FP+TP)
NPV	Negative Predictive Value	TN/(FN + TN)
Mis	Misclassification Rate	(FP + FN)/N
Odds	Odds-ratio	(TP + TN)/(FN + FP)
Kappa	Kappa	((TP + TN) - (((TP + FN)*(TP + FP) + (FP + TN)*(FN + TN))/N))/((N - (((TP + FN)*(TP + FP) + (FP + TN)*(FN + TN))/N)))
NMI	NMI n(s)	(1 - -TP*log(TP)-FP*log(FP)-FN*log(FN)-TN*log(TN)+(TP+FP)*log(TP+FP)+(FN+TN)*log(FN+TN))/(N*log(N) - ((TP+FN)*log(TP+FN) + (FP+TN)*log(FP+TN)))
ACP	Average Conditional Probability	0.25*(Sn+ PPV + Sp + NPV)
MCC	Matthews correlation coefficient	(TP*TN - FP*FN) / sqrt[(TP+FP)*(TP+FN)*(TN+FP)*(TN+FN)]
Acc.a	Arithmetic accuracy	(Sn + PPV)/2
Acc.a2	Accuracy (alternative)	(Sn + Sp)/2
Acc.g	Accuracy (Sn+PPV)/2	sqrt(Sn*PPV)
Hit.noTN	A sort of hit rate without TN (to avoid the effect of their large number)	TP/(TP+FP+FN)

		Predicted	
		True	False
Known	True	TP	FN
	False	FP	TN

$$Sn = TP/(TP+FN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$PPV=TP/(TP+FP)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$Sp=TN/(FP+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$NPV=TN/(FN+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FPR=FP/(FP+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FDR=FP/(FP+TP)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FN/(FN+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FNR=FN/(TP+FN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

# Validation statistics including TN should be avoided

- For the predictions of TF binding sites, TN can represent >99.9%
- > All the statistics including TN are misleading (e.g. Sp can be very high)

Abbrev	Name	Formula
TP	True positive	TP
FP	False positive	FP
FN	False negative	FN
TN	True negative	TN
KP	Known Positive	TP+FN
KN	Known Negative	TN+FP
PP	Predicted Positive	TP+FP
PN	Predicted Negative	FN+TN
N	Total	TP + FP + FN + TN
Prev	Prevalence	(TP + FN)/N
ODP	Overall Diagnostic Power	(FP + TN)/N
CCR	Correct Classification Rate	(TP + TN)/N
<b>Sn</b>	<b>Sensitivity</b>	<b>TP/(TP + FN)</b>
Sp	Specificity	TN/(FP + TN)
FPR	False Positive Rate	FP/(FP + TN)
FNR	False Negative Rate	FN/(TP + FN)
<b>PPV</b>	<b>Positive Predictive Value</b>	<b>TP/(TP + FP)</b>
FDR	False Discovery Rate	FP/(FP+TP)
NPV	Negative Predictive Value	TN/(FN + TN)
Mis	Misclassification Rate	(FP + FN)/N
Odds	Odds-ratio	(TP + TN)/(FN + FP)
Kappa	Kappa	((TP + TN) - (((TP + FN)*(TP + FP) + (FP + TN)*(FN + TN))/N))/((N - (((TP + FN)*(TP + FP) + (FP + TN)*(FN + TN))/N)))
NMI	NMI n(s)	(1 - -TP*log(TP)-FP*log(FP)-FN*log(FN)-TN*log(TN)+(TP+FP)*log(TP+FP)+(FN+TN)*log(FN+TN))/(N*log(N) - ((TP+FN)*log(TP+FN) + (FP+TN)*log(FP+TN)))
ACP	Average Conditional Probability	0.25*(Sn+ PPV + Sp + NPV)
MCC	Matthews correlation coefficient	(TP*TN - FP*FN) / sqrt[(TP+FP)*(TP+FN)*(TN+FP)*(TN+FN)]
Acc.a	Arithmetic accuracy	(Sn + PPV)/2
Acc.a2	Accuracy (alternative)	(Sn + Sp)/2
Acc.g	Accuracy (Sn+PPV)/2	sqrt(Sn*PPV)
Hit.noTN	A sort of hit rate without TN (to avoid the effect of their large number)	TP/(TP+FP+FN)

		Predicted	
		True	False
Known	True	TP	FN
	False	FP	TN

$$Sn = TP/(TP+FN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$PPV=TP/(TP+FP)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$Sp=TN/(FP+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$NPV=TN/(FN+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FPR=FP/(FP+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FDR=FP/(FP+TP)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FN/(FN+TN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

$$FNR=FN/(TP+FN)$$

Predicted

		True	False
Known	True	TP	FN
	False	FP	TN

# The arithmetic accuracy should be avoided

Abbrev	Name	Formula
TP	True positive	TP
FP	False positive	FP
FN	False negative	FN
TN	True negative	TN
KP	Known Positive	TP+FN
KN	Known Negative	TN+FP
PP	Predicted Positive	TP+FP
PN	Predicted Negative	FN+TN
N	Total	TP + FP + FN + TN
Prev	Prevalence	(TP + FN)/N
ODP	Overall Diagnostic Power	(FP + TN)/N
CCR	Correct Classification Rate	(TP + TN)/N
<b>Sn</b>	<b>Sensitivity</b>	$TP/(TP + FN)$
Sp	Specificity	$TN/(FP + TN)$
FPR	False Positive Rate	$FP/(FP + TN)$
FNR	False Negative Rate	$FN/(TP + FN)$
<b>PPV</b>	<b>Positive Predictive Value</b>	$TP/(TP + FP)$
NPV	Negative Predictive Value	$TN/(FN + TN)$
Mis	Misclassification Rate	$(FP + FN)/N$
Odds	Odds-ratio	$(TP + TN)/(FN + FP)$
Kappa	Kappa	$((TP + TN) - (((TP + FN)*(TP + FP) + (FP + TN)*(FN + TN))/N))/(N - (((TP + FN)*(TP + FP) + (FP + TN)*(FN + TN))/N))$
NMI	NMI n(s)	$(1 - TP*\log(TP) - FP*\log(FP) - FN*\log(FN) - TN*\log(TN) + (TP+FP)*\log(TP+FP) + (FN+TN)*\log(FN+TN))/(N*\log(N) - ((TP+FN)*\log(TP+FN) + (FP+TN)*\log(FP+TN)))$
ACP	Average Conditional Probability	$0.25*(Sn + PPV + Sp + NPV)$
MCC	Matthews correlation coefficient	$(TP*TN - FP*FN) / \sqrt{[ (TP+FP)*(TP+FN)*(TN+FP)*(TN+FN) ]}$
Acc.a	Arithmetic accuracy	$(Sn + PPV)/2$
Acc.a2	Accuracy (alternative)	$(Sn + Sp)/2$
<b>Acc.g</b>	<b>Accuracy (<math>Sn+PPV)/2</math></b>	$\sqrt{Sn*PPV}$
Hit.noTN	A sort of hit rate without TN (to avoid the effect of their large number)	$TP/(TP+FP+FN)$

		Predicted	
		True	False
Known	True	TP	FN
	False	FP	TN

$$Sn = TP/(TP+FN)$$

Predicted

		Predicted	
		True	False
Known	True	TP	FN
	False	FP	TN

$$PPV=TP/(TP+FP)$$

Predicted

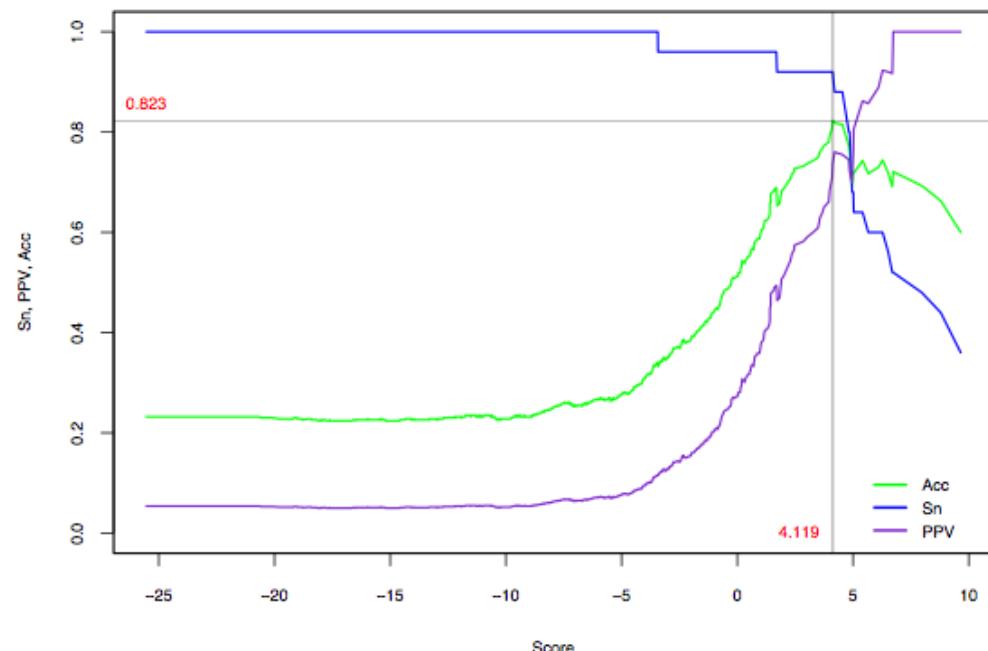
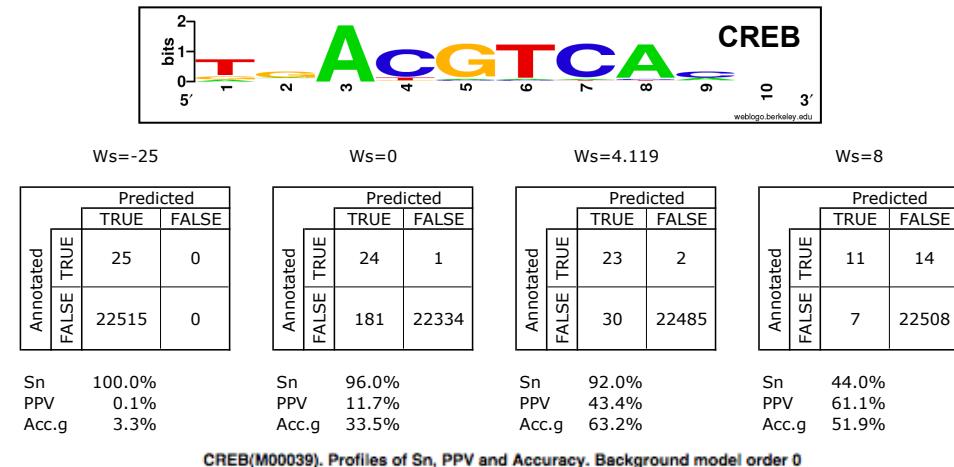
		Predicted	
		True	False
Known	True	TP	FN
	False	FP	TN

- It is easy to be fooled by the arithmetic accuracy: let us imagine that a trivial program predicts all features as positive
  - $\rightarrow Sn$  guaranteed to be 100%
  - Of course, you have a poor PPV
  - $Acc_a = (Sn + PPV)/2$ 
    - $\rightarrow$  guaranteed to be >50%

- The geometric accuracy circumvents this problem
  - $Acc_g = \sqrt{Sn*PPV}$
  - Requires for both  $Sn$  and  $PPV$  to be high.

# Accuracy profiles for the human CREB factor

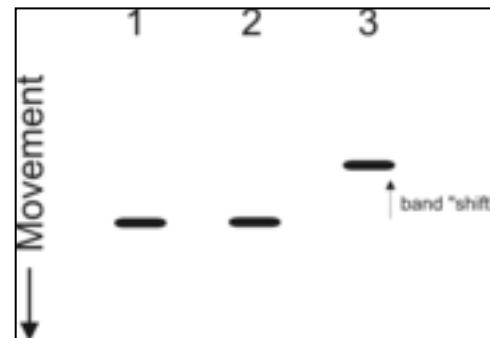
- We can measure for each score value
  - $Sn = TP/(TP+FN)$
  - $PPV=TP/(TP+FP)$
  - $Acc.g =\sqrt{Sn*PPV}$
- With increasing scores
  - $Sn$  decreases when score increases (we are progressively loosing sites)
  - If the matrix + annotations are good, the  $PPV$  should increase.
  - The accuracy shows some optimum (peak)
- Beware
  - There is a **bias** in these evaluations.
  - The “optimal” accuracy may be **catastrophic** choice for whole-genome predictions.
  - Indeed, even an apparently low risk of false positive ( $FPR=0.1\%$ ) represents a high number of false predictions, since it is multiplied by the size of the genome.



# Difficulties for estimating site predictions

- Annotations are far from complete
  - > what we consider as false positive can reveal non-annotated binding sites
- The boundaries of binding sites are not defined in an all-or-none fashion.
  - Only a few residues are involved in the direct contact between transcription factor and its binding site, but the flanking residues affect the binding by conformation effects.
- Site boundaries in databases depend much on the experimental evidence
  - Footprint: typically 6-20bp
  - Gel shift: sometimes 50bp or more
- The concept of “binding site” itself can be questioned.
  - Transcription factors have a higher affinity for DNA than for the nucleoplasm.
  - According to some models, they can bind anywhere on DNA, but they spend more time on some sites than on other ones.
  - One could thus consider a continuum of binding affinities.

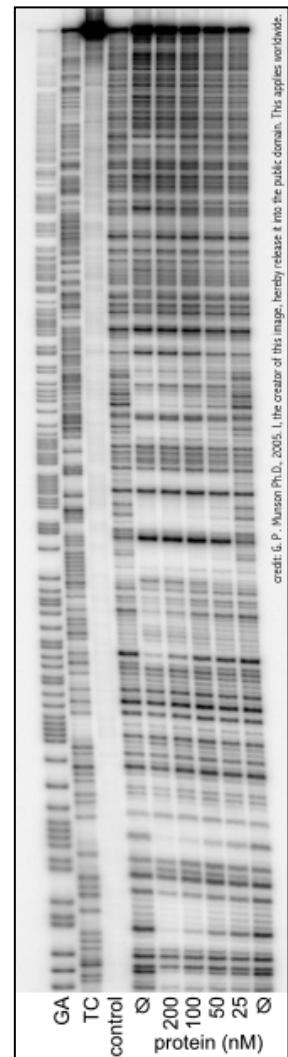
Gel shift assay



Lane 1 is a negative control, and contains only DNA. Lane 2 contains protein as well as a DNA fragment that, based on its sequence, does not interact. Lane 3 contains protein and a DNA fragment that does react; the resulting complex is larger, heavier, and slower-moving. The pattern shown in lane 3 is the one that would result if all the DNA were bound and no dissociation of complex occurred during electrophoresis. When these conditions are not met a second band might be seen in lane 3 reflecting the presence of free DNA or the dissociation of the DNA-protein complex.

Source: Wikipedia

DNAse footprint



*Regulatory sequence analysis*

# *Distributions of scores in biological sequences*

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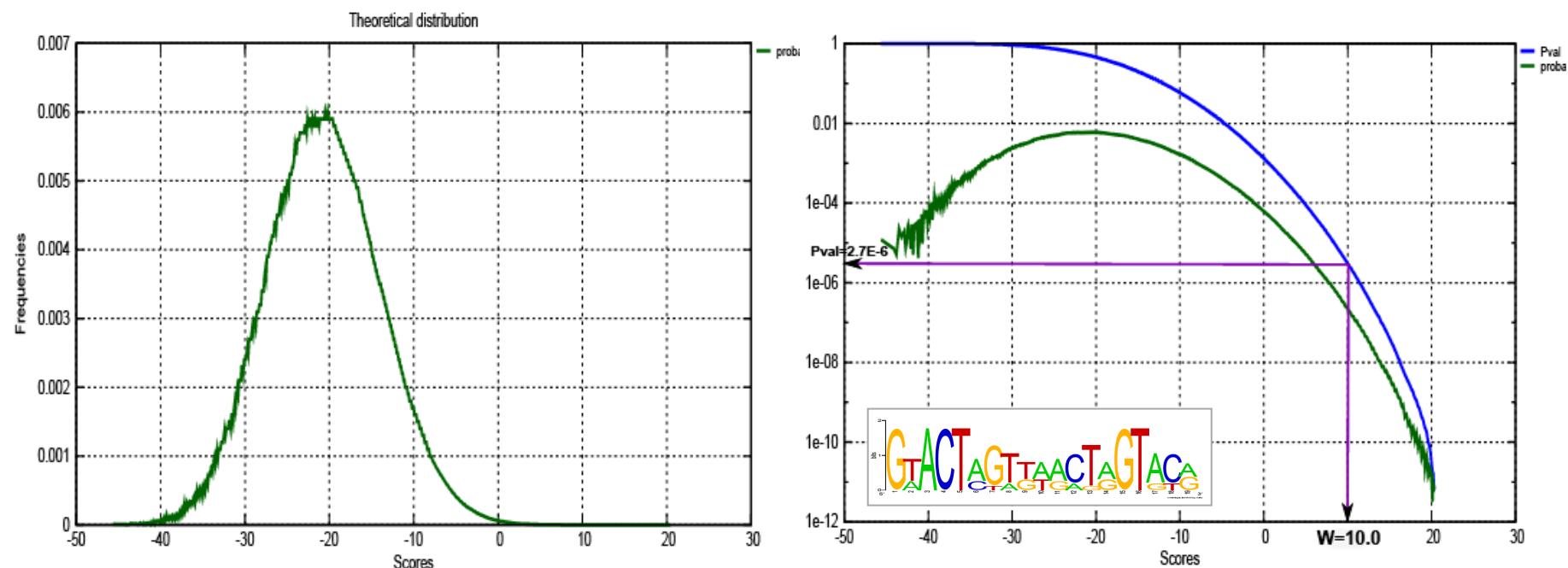
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# Theoretical distribution of scores for the TrpR matrix

- The RSAT program *matrix-distrib* allows to compute the theoretical distribution of the weight score according to Markovian background models (by extension of the algorithm from Staden, 1989).
- **Green curve**
  - Probability  $P(W=w)$  to observe **exactly** a given weight score at a given position of a random sequence.
- **Blue curve**
  - Probability  $P(W>=w)$  to observe **at least** a given weight score at a given position of a random sequence.
  - Note: the Y axis of the right figure is logarithmic.
  - This is an estimate of the **risk of false positive (FPR)**: probability to consider a position of the sequence as a binding site whereas it is not.
  - Ex: for the TrpR matrix, the probability to observe a score  $W>=10.0$  at a given position is 2.7E-6.



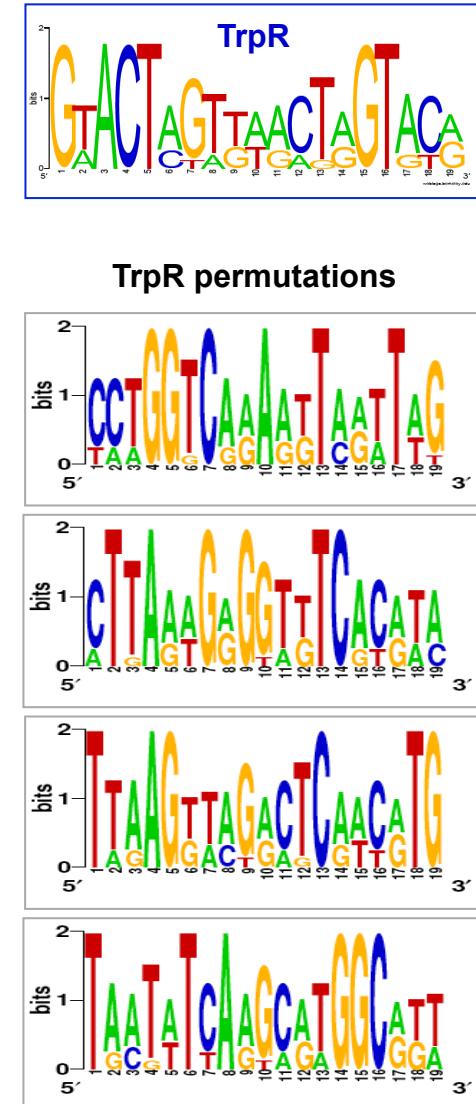
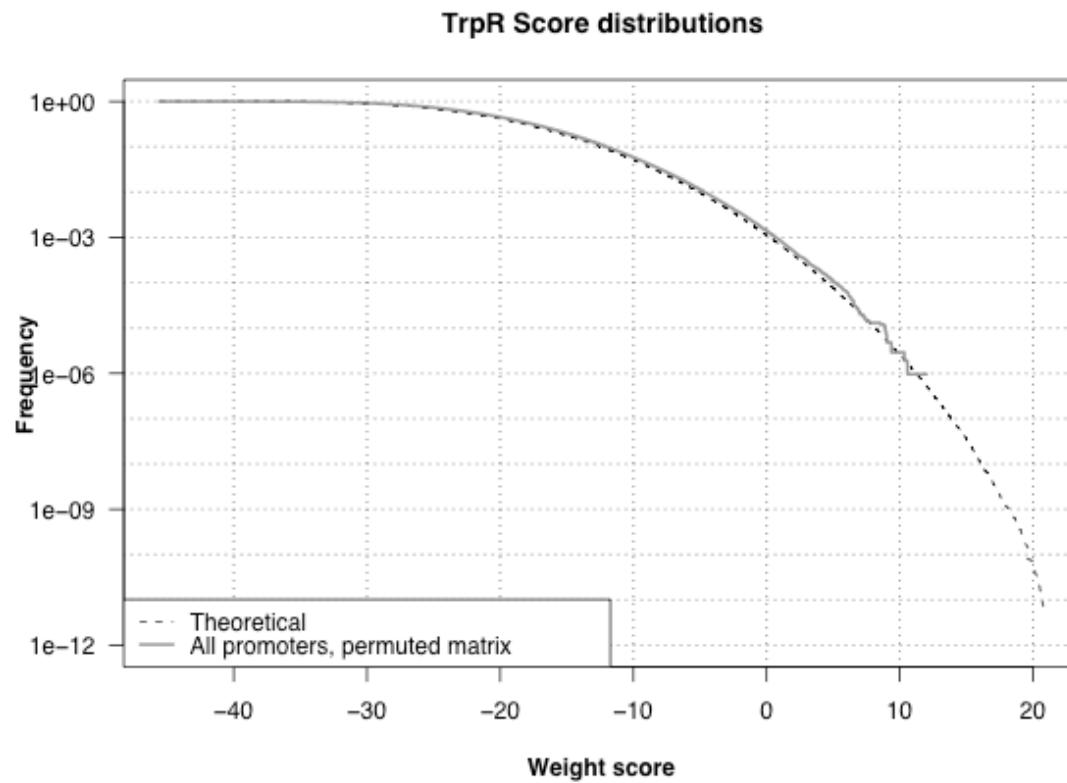
## *The negative control for the FPR*

- How reliable is this theoretical distribution ?  
Does it provide a good estimate of the risk of false positives in real conditions ?
- Approach: scan sequences supposedly devoid of binding sites (“negative” set), and check if the empirical distribution of scores fits the theoretical one.
- Problem: how can we obtain a collection of sequences without binding sites ?
  - Perform experiments to check the non-binding of several thousands/millions of sites ?
    - **Problem:** costly
  - Generate random sequences ?
    - **Problem:** this test is trivial, because those random sequences will by definition reflect our theoretical background, and thus fit the theoretical curve.
    - Nothing guarantees that the background model is appropriate to model real biological sequences.
  - Select random sets of genome sequences ?
    - **Problem:** the selection might include some actual binding sites (bad luck).
  - Scan genome sequences with shuffled matrices.
    - We can shuffle the columns of the matrix, so that the motif becomes uninformative.

# Distribution of matrix scores in *E.coli* promoters

## TrpR matrix from RegulonDB - permuted

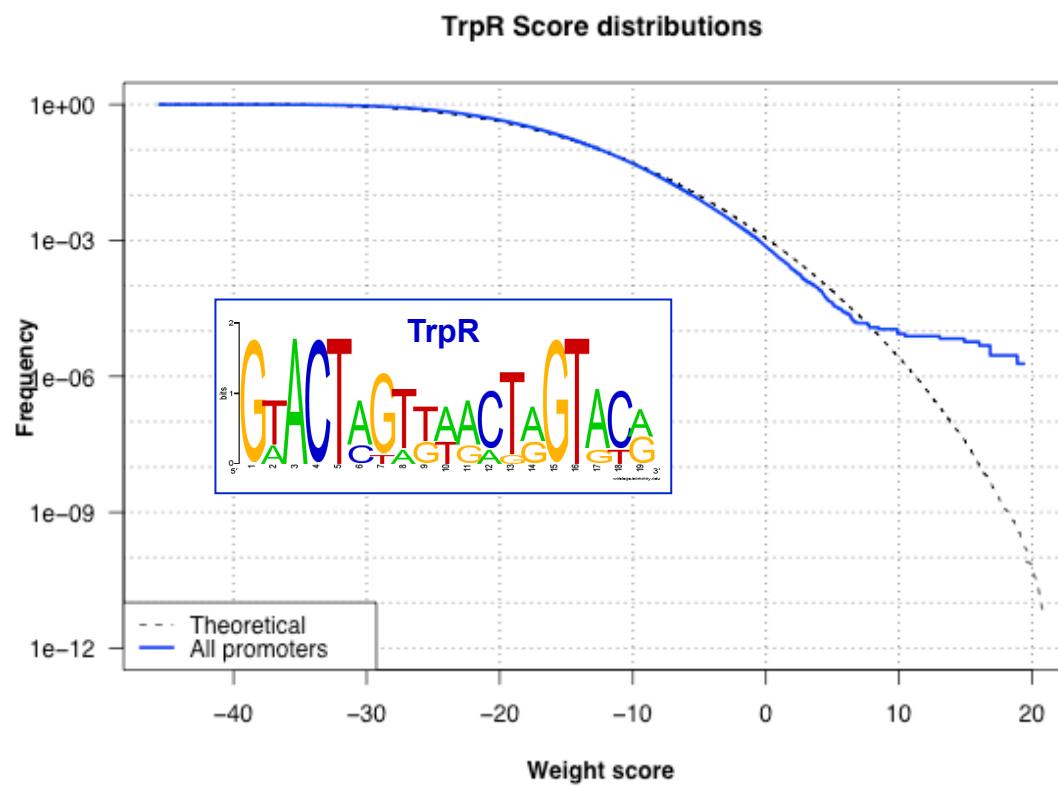
- To control the validity of the theoretical distribution: scan a reference sequence set (e.g. all promoters) with shuffled motifs.
  - For this, we perform a random permutation of the columns of the original PSSM, using the program *convert-matrix* (RSAT).
  - This permuted matrix is not supposed to correspond to any existing transcription factor, and we thus expect to find no specific high-scoring site.
  - Indeed, the score distribution observed with the permuted matrix fits the theoretical distribution.



# Distribution of matrix scores in *E.coli* promoters

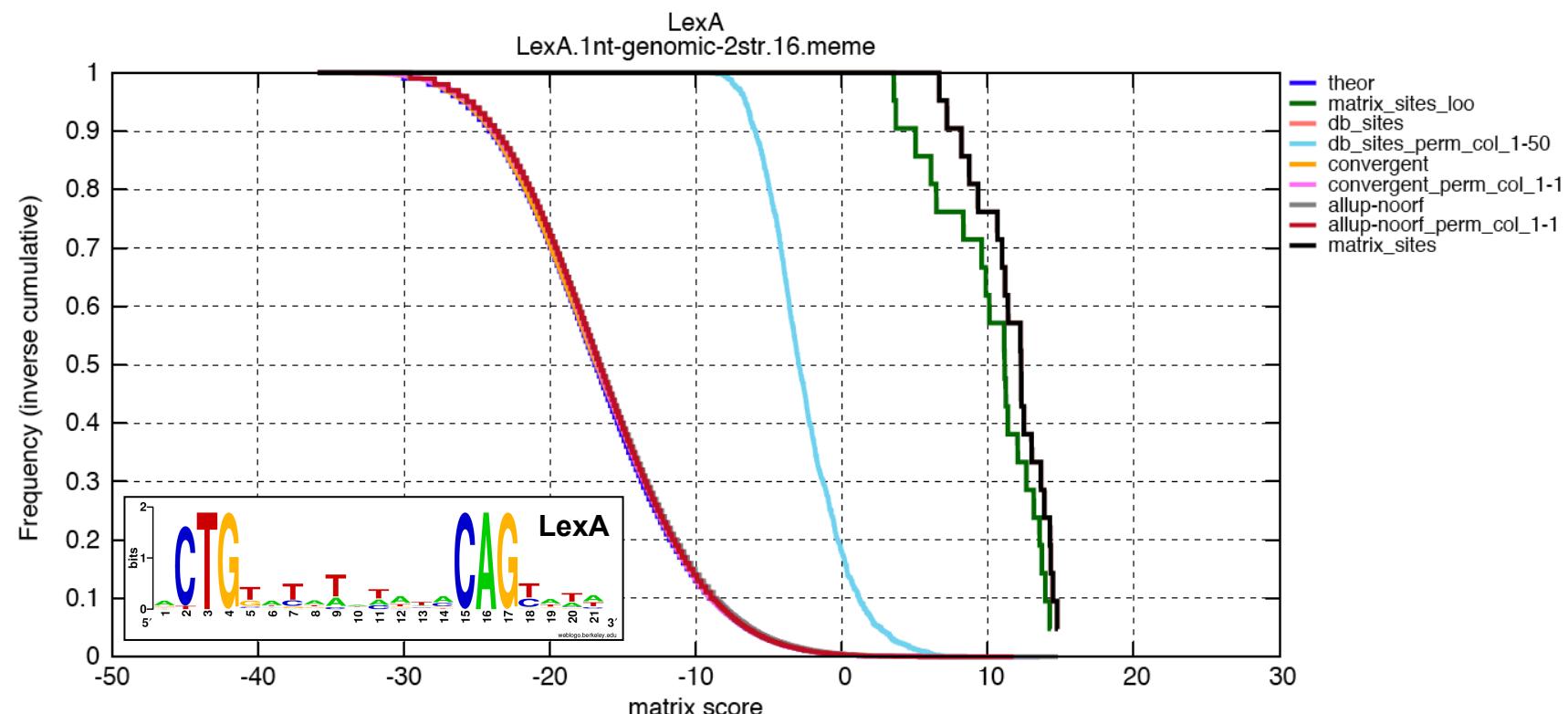
## TrpR matrix from RegulonDB

- We compare the distribution observed in all the *E.coli* promoters (blue) with the theoretical distribution, computed by matrix-distrib (blue).
  - For low score values ( $W \leq 8$ ) observed distribution follows pretty well the theoretical one
    - (the small difference is due to a particular of this motif, which contains the GTAC tetramer).
  - We also observe a “plateau” corresponding to a few sites having a very high score. These high-scoring sites are much more frequent than expected by chance (remind, the axis is logarithmic).
- Do these high-scoring sites correspond to the actual binding sites ?
  - We can partly check this with the collection of annotated binding sites.



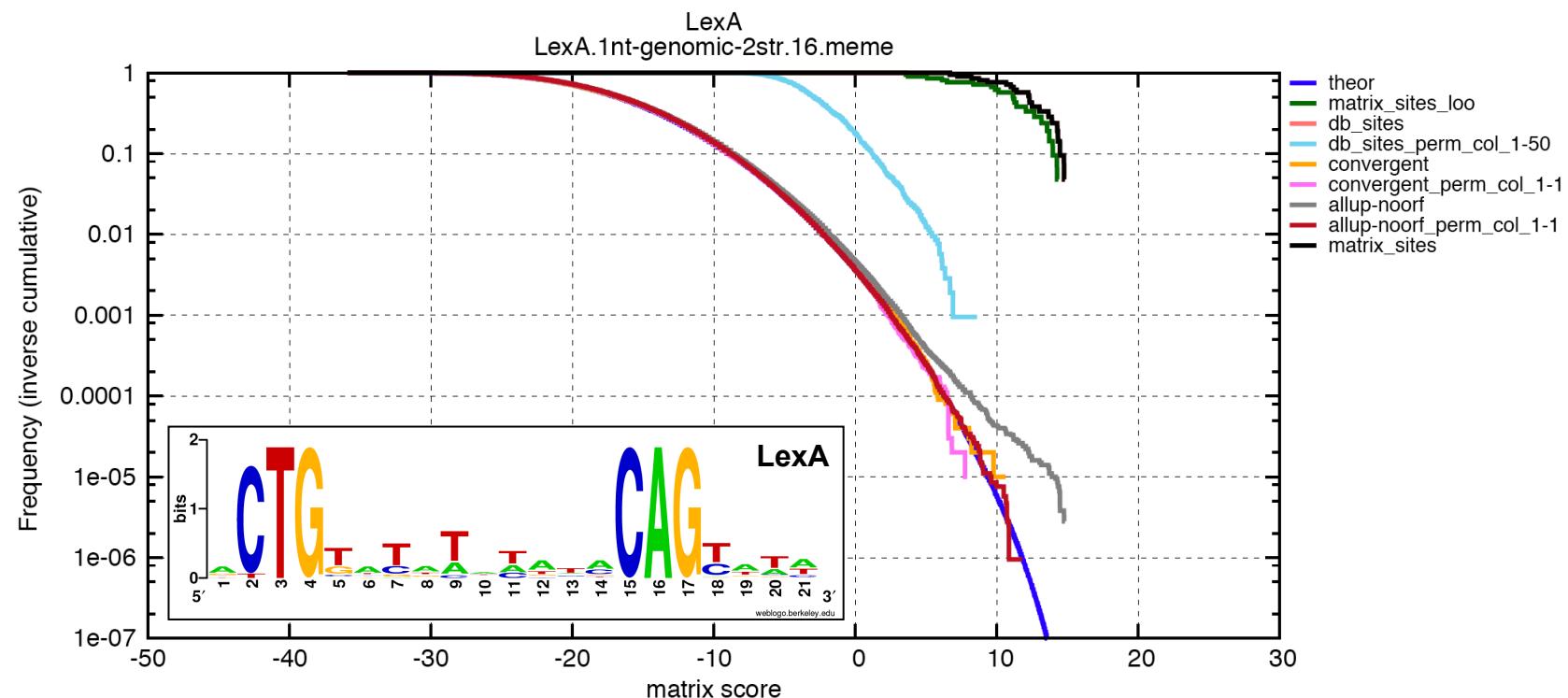
# *Escherichia coli LexA - Score distributions (logarithmic Y axis)*

- + **Black**: annotated binding sites that served to build the matrix (**this is biased !**)
- + **Green**: leave-one-out (LOO) test with annotated binding sites
- - **Blue** : theoretical distribution
- - **Cyan**: scores obtained with 50 permuted matrices in the annotated sites
- - **Brownish red**: scores obtained with 5 permuted matrices in a random selection of promoters
- -/+ **Gray**: distribution in all promoters

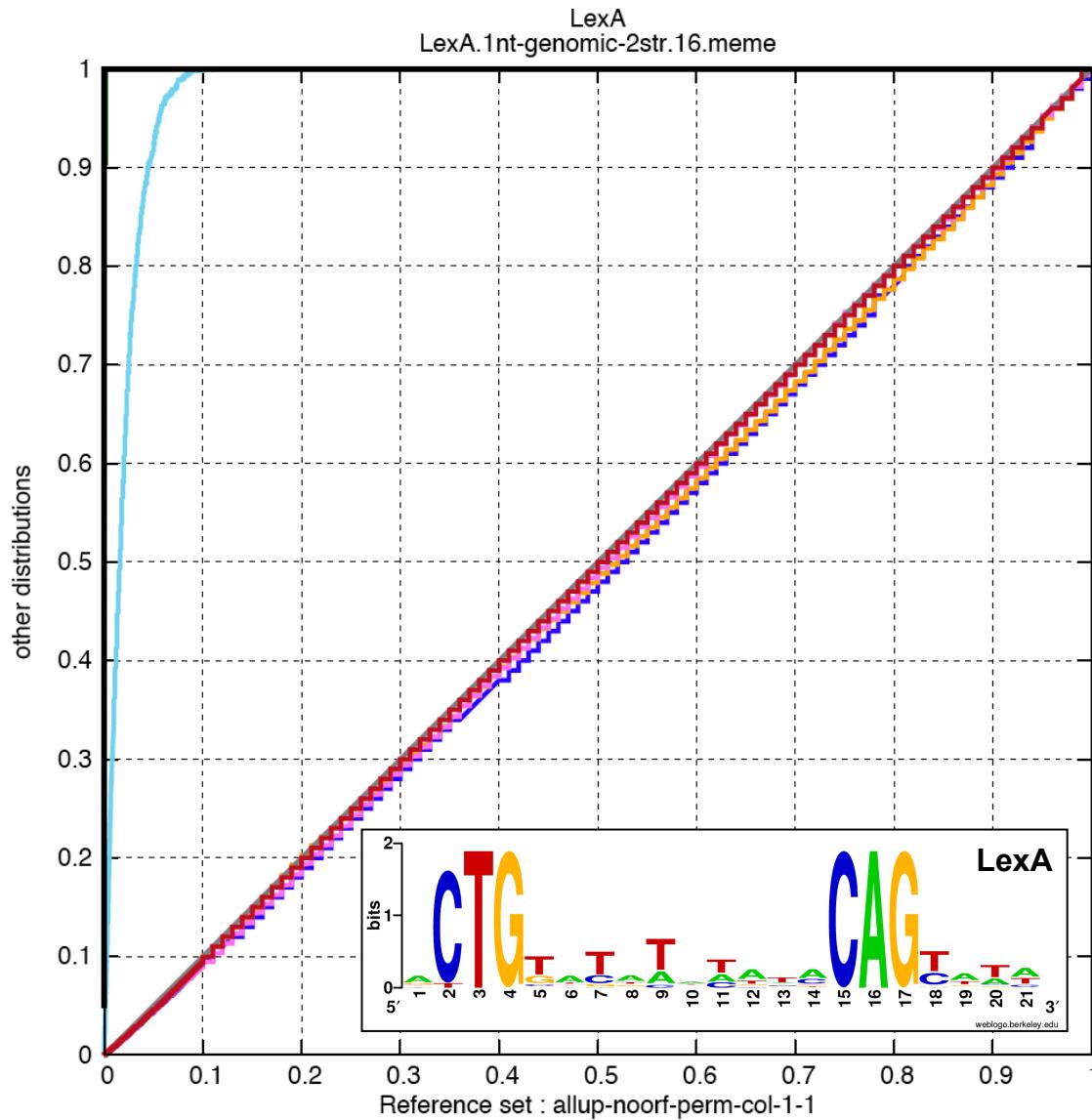


# *Escherichia coli LexA - Score distributions (logarithmic Y axis)*

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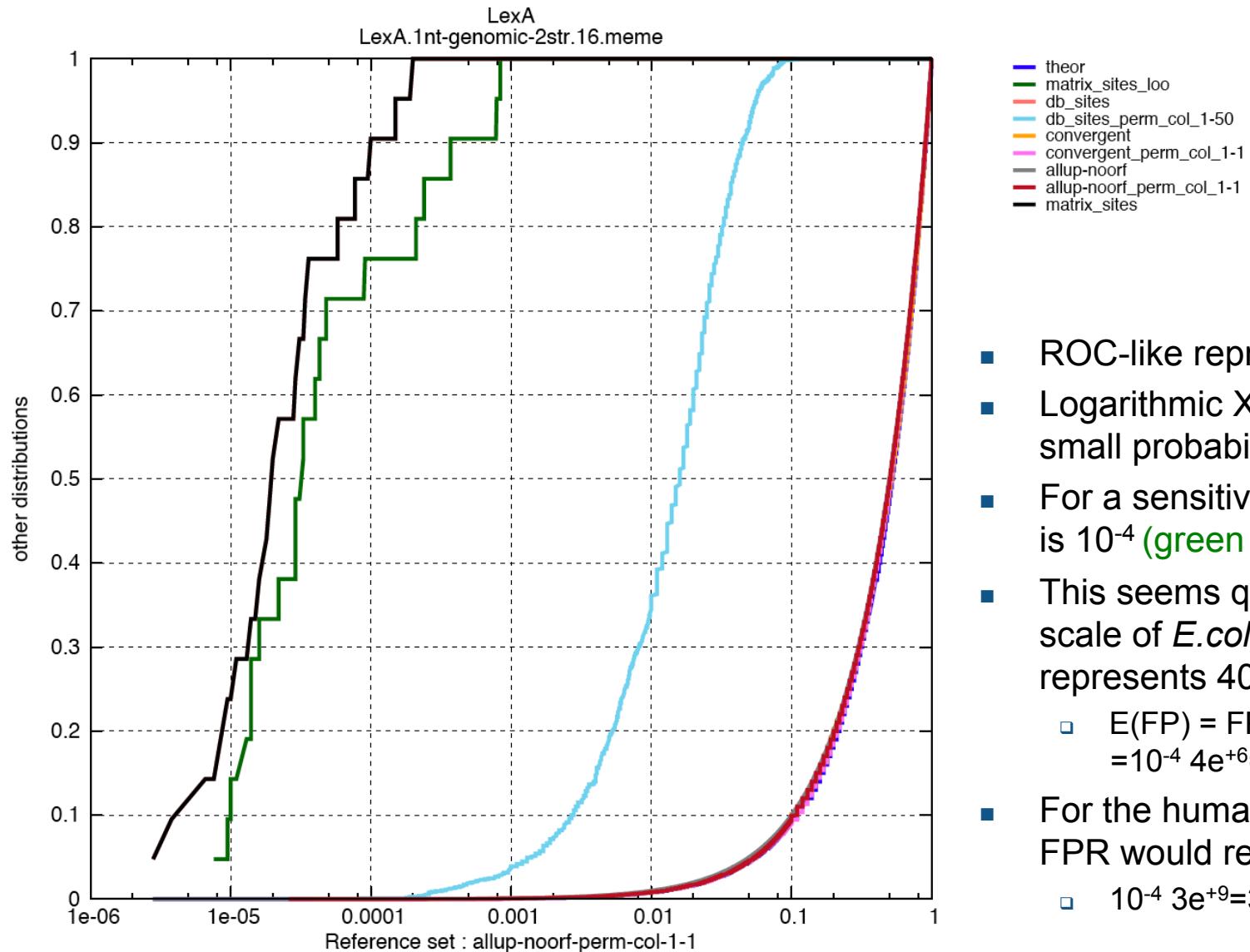


# *ROC-like curves can be misleading !*



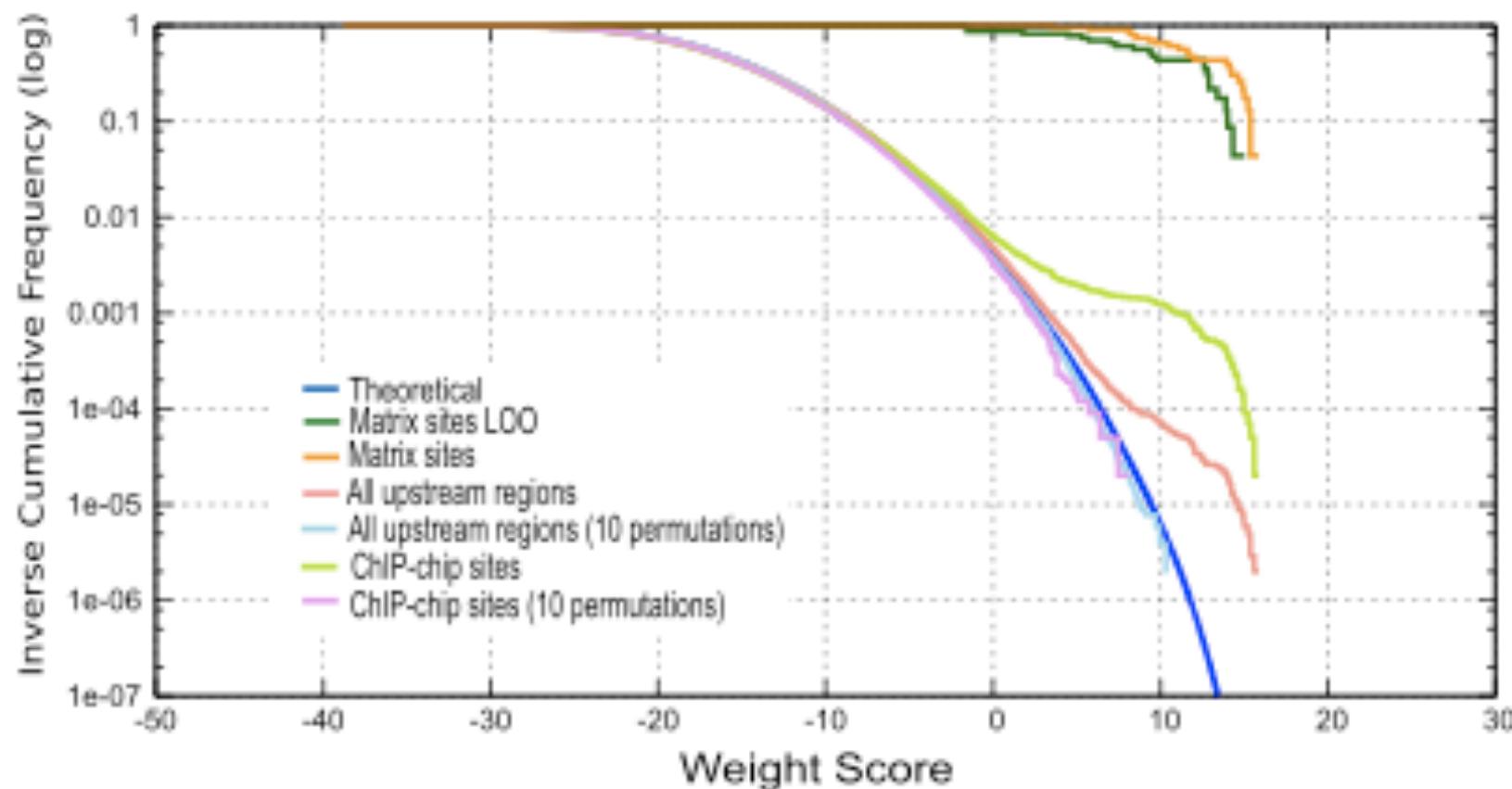
- ROC-like representation
  - X axis
    - reference negative set
    - = all promoters scanned with permuted matrices
  - Y axis: each other set tested
  - The curves seem perfect
    - Negative sets are on the diagonal
      - Only exception: permuted sites.
    - This Positive sets are in the top left corner
  - However, this is misleading.
  - Why ?
    - Because the rate of false positive has to be multiplied by genome scale -> a very small percentage can represent thousands of hits.

## Log-representation of the FPR is more relevant



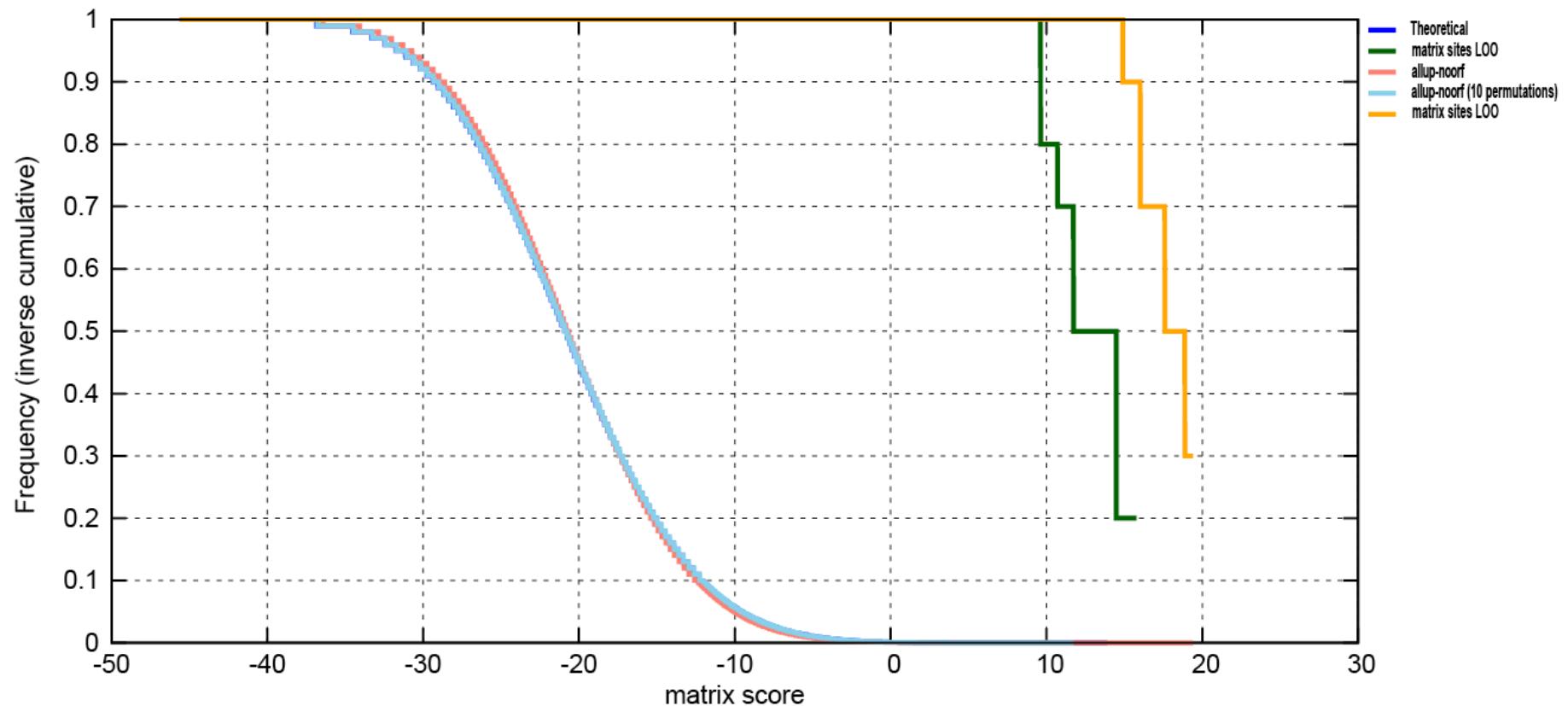
- ROC-like representation
- Logarithmic X axis emphasizes small probabilities
- For a sensitivity of 75%, the FPR is  $10^{-4}$  (green curve)
- This seems quite good, but at the scale of *E.coli* genome, it represents 400 false predictions !
  - $E(FP) = FPR \cdot N = 10^{-4} \cdot 4e^{+6} = 400$
- For the human genome, the same FPR would represent
  - $10^{-4} \cdot 3e^{+9} = 300,000$  FP !

## LexA

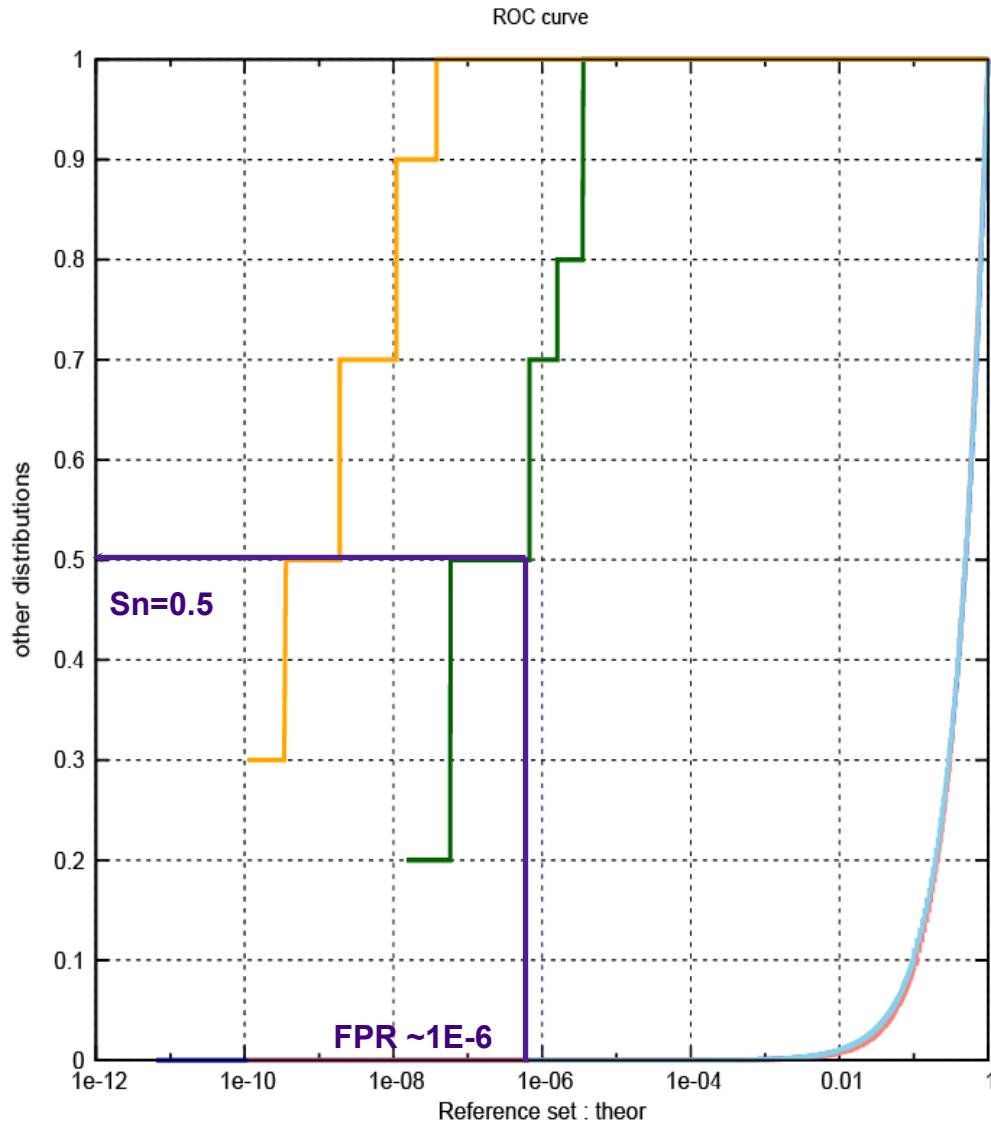


# *Escherichia coli TrpR* - Score distributions in annotated sites

- We compute score distributions in annotated sites
  - with the **full matrix (BIASED)**
  - with **LOO-corrected matrices**

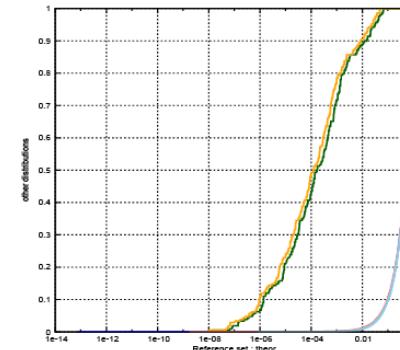
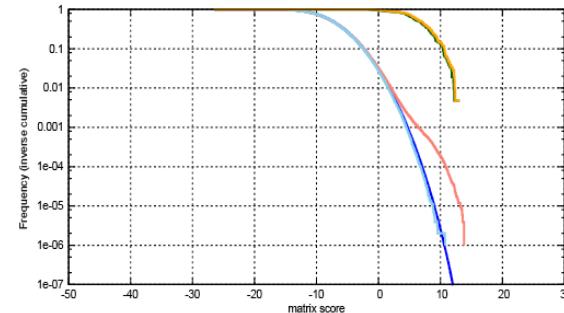
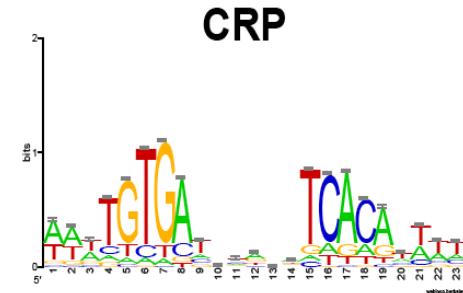


# *Escherichia coli TrpR* - ROC curve



- This representation is derived from the ROC curve, but we display the X axis on a logarithmic scale, in order to emphasize the small ranges of FPR.
- This is particularly important, because for genome-scale searches, the FPR is multiplied by the size of the genome !

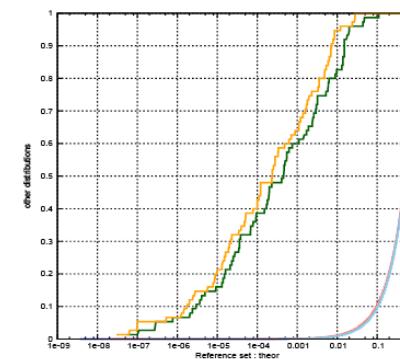
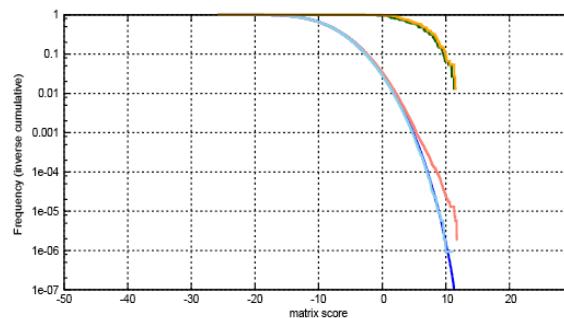
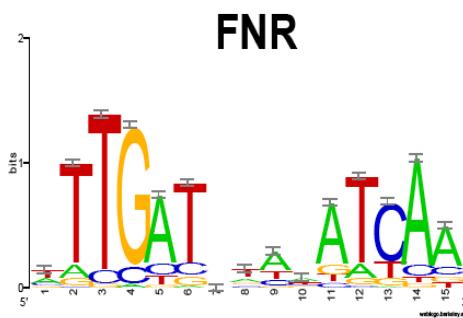
# Generic versus specific factors



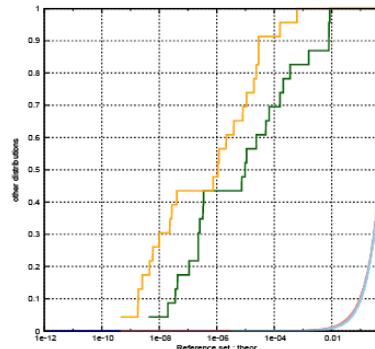
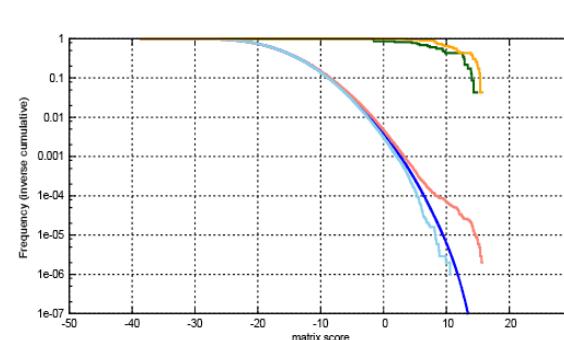
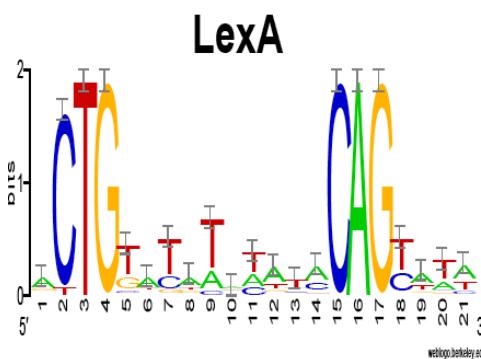
CRP matrix was built from ~200 sites.

The distribution in all promoters discards very early from the theoretical one.

The LOO and biased site distributions are almost identical.



More or less the same effect is observed for FNR.

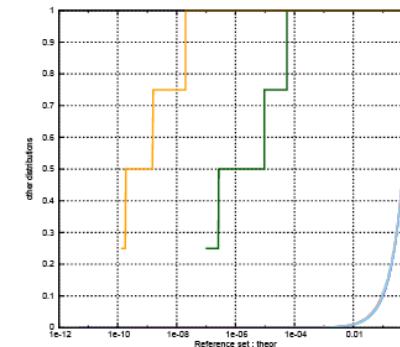
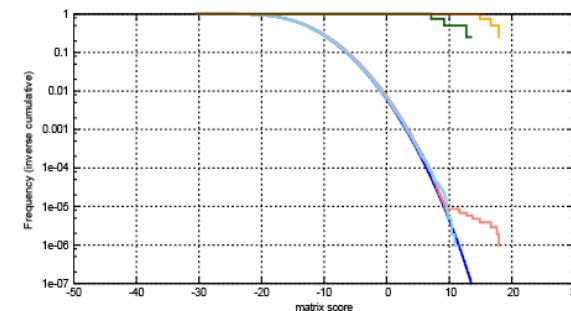
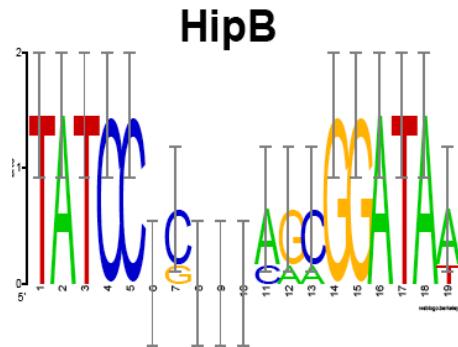


LexA is quite specific, but collecting all sites would represent a high cost:

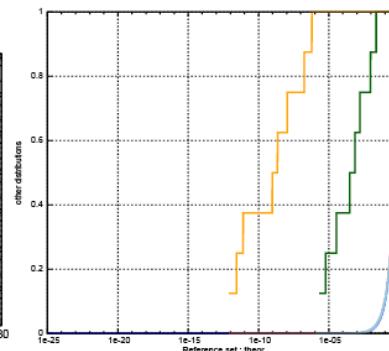
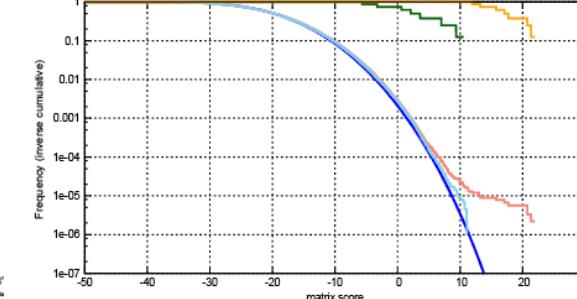
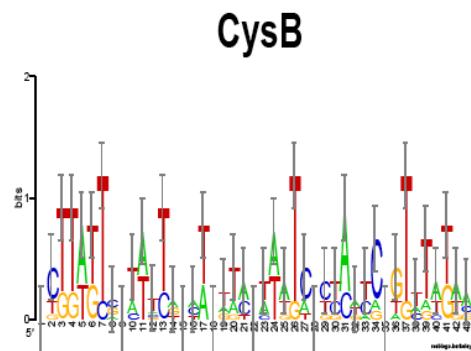
$$FPR_{0.5} = 1.3E^{-5}$$

$$FPR_{0.9} = 8.3E^{-3}$$

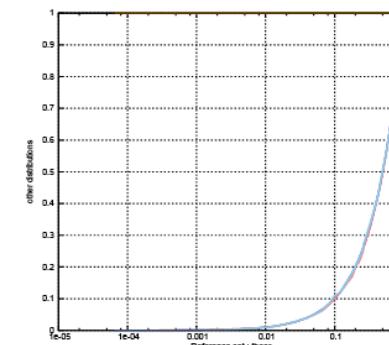
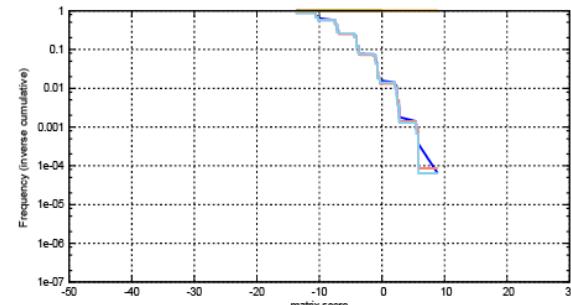
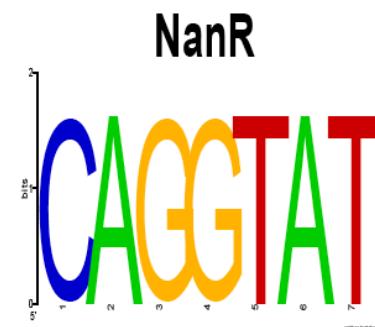
## *Problematic matrices*



The HipB matrix was built from 3 sites only -> overfitting.  
The biased and LOO FPR curves differ by a factor 10.000

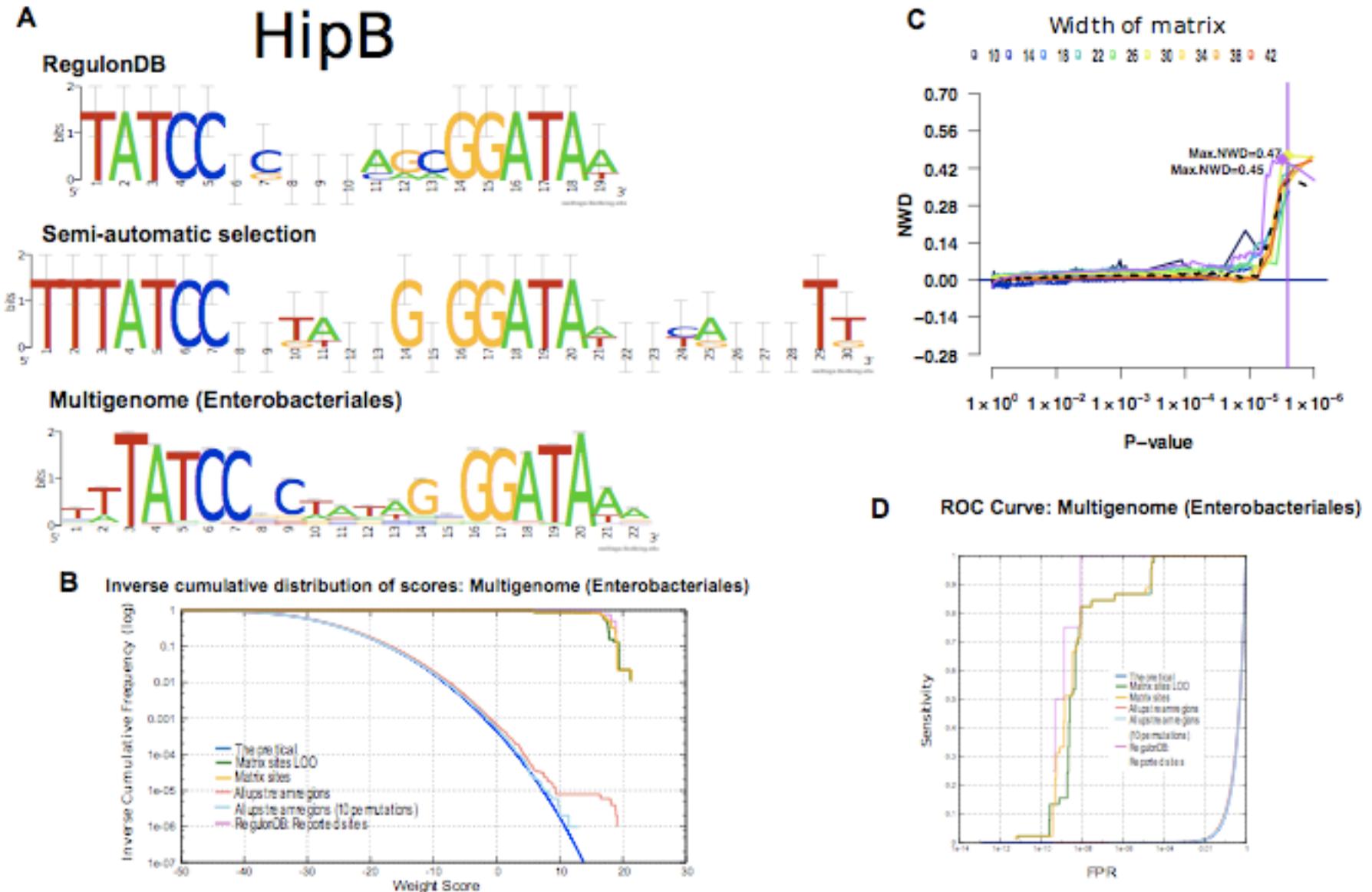


The CysB matrix was built from very large sites (42 columns).  
The sites are very far away from the theoretical min Pval (1e-25).  
Biased and LOO FPR curves differ by a factor of 100.000.



NanR matrix was built from 6 identical heptanucleotides. The P-value distribution almost fits a binomial. The LOO test is impossible (all sites are twins).

# Using multigenome pattern discovery to enrich a matrix



## *Selected study cases*

### *Transcription factors from metazoans*

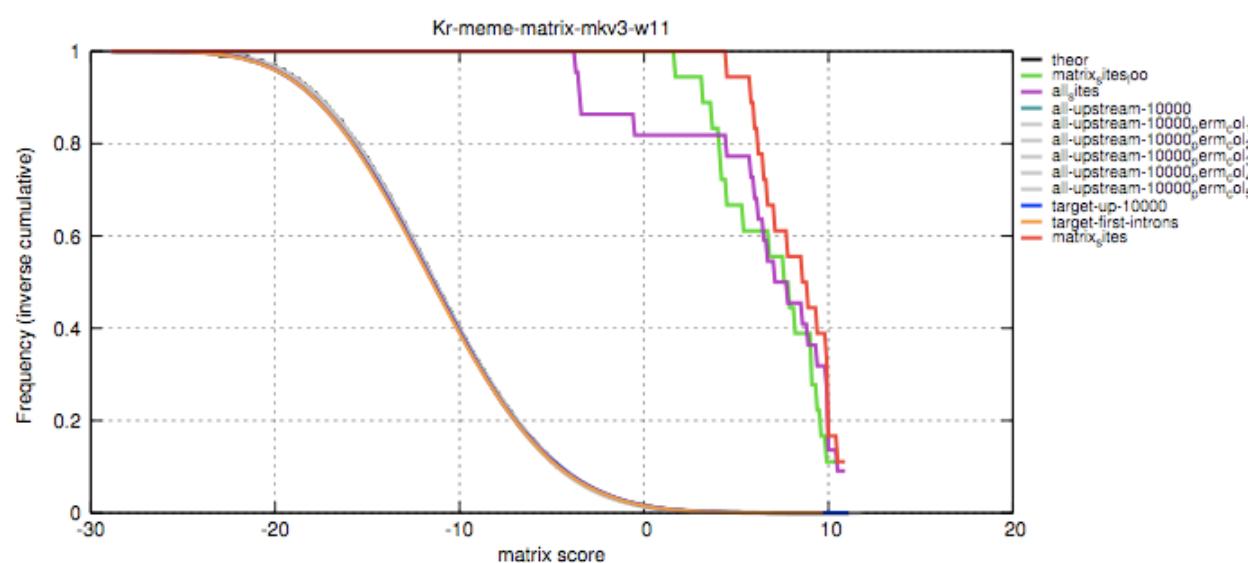
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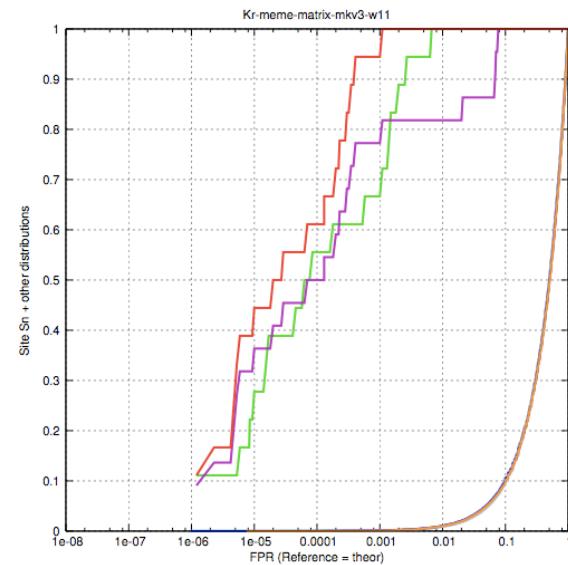
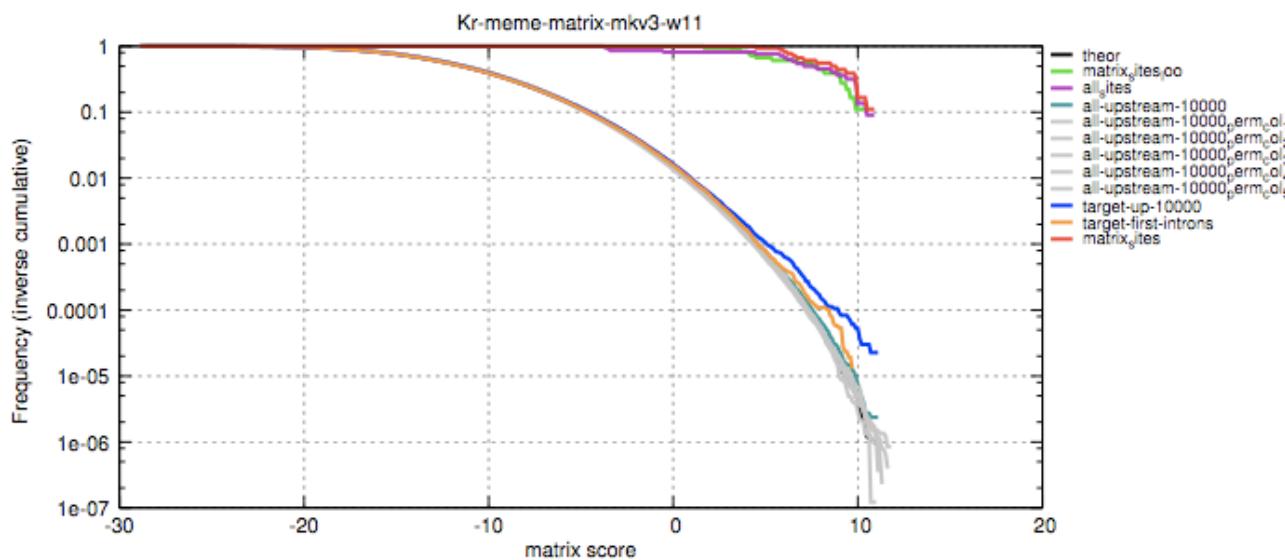
Laboratoire de Bioinformatique des Génomes et des Réseaux (BiGRe)

<http://www.bigré.ulb.ac.be/>

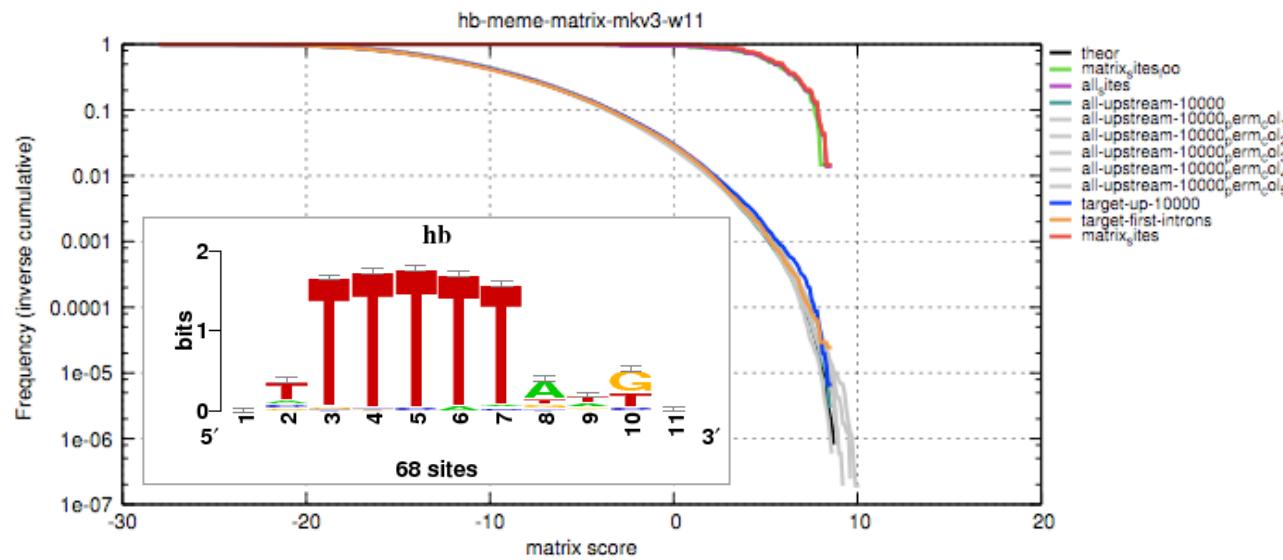
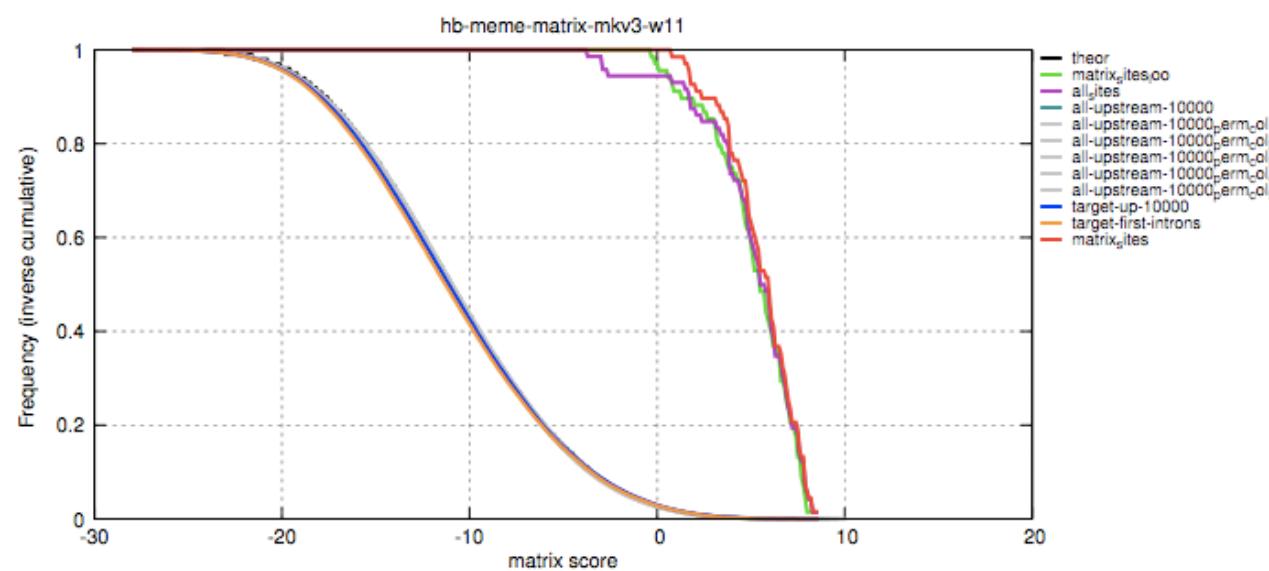
# Matrix-quality for *Drosophila Krüppel* (*Kr*)



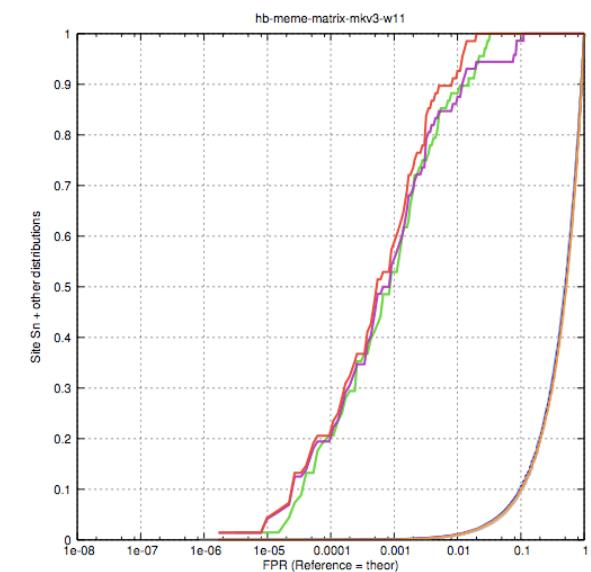
- Score distributions
  - Permutation tests fit theoretical distribution.
  - The upstream regions of Kr target genes show an enrichment in predicted sites (blue curve)
- ROC curve (green)
  - The  $FPR_{0.50}$  is  $\sim 10^{-4}$ , indicating achieving a Sn of 50% costs 1FP every 10kb.
  - To achieve a Sn of 70%, the cost raises to 1FP/kb.



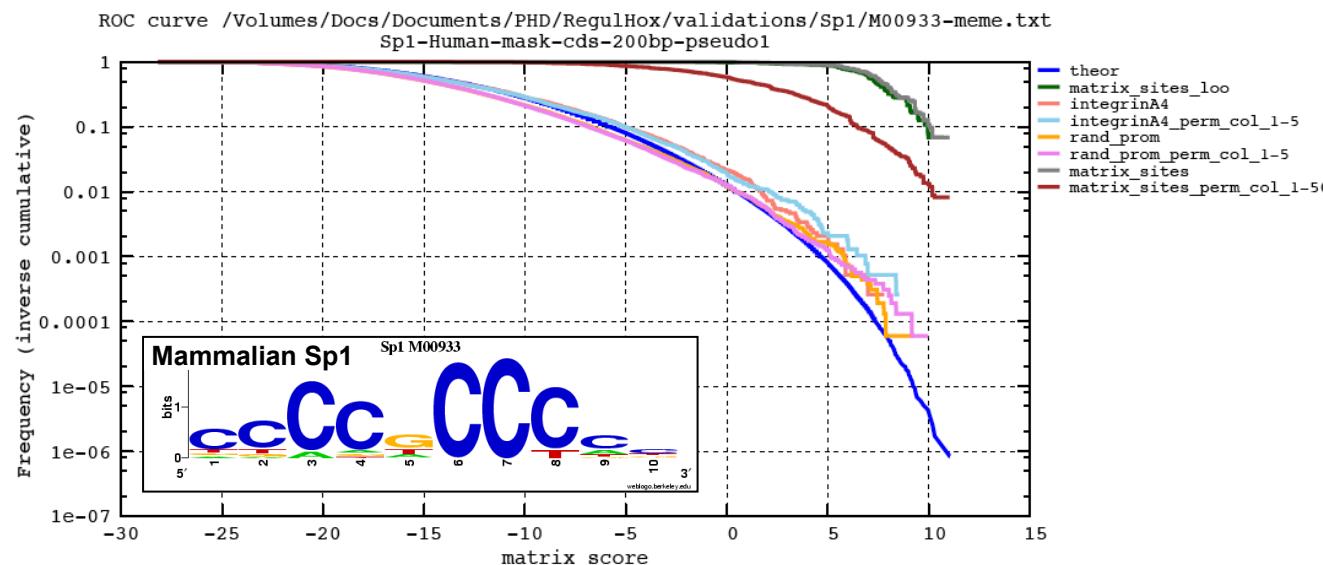
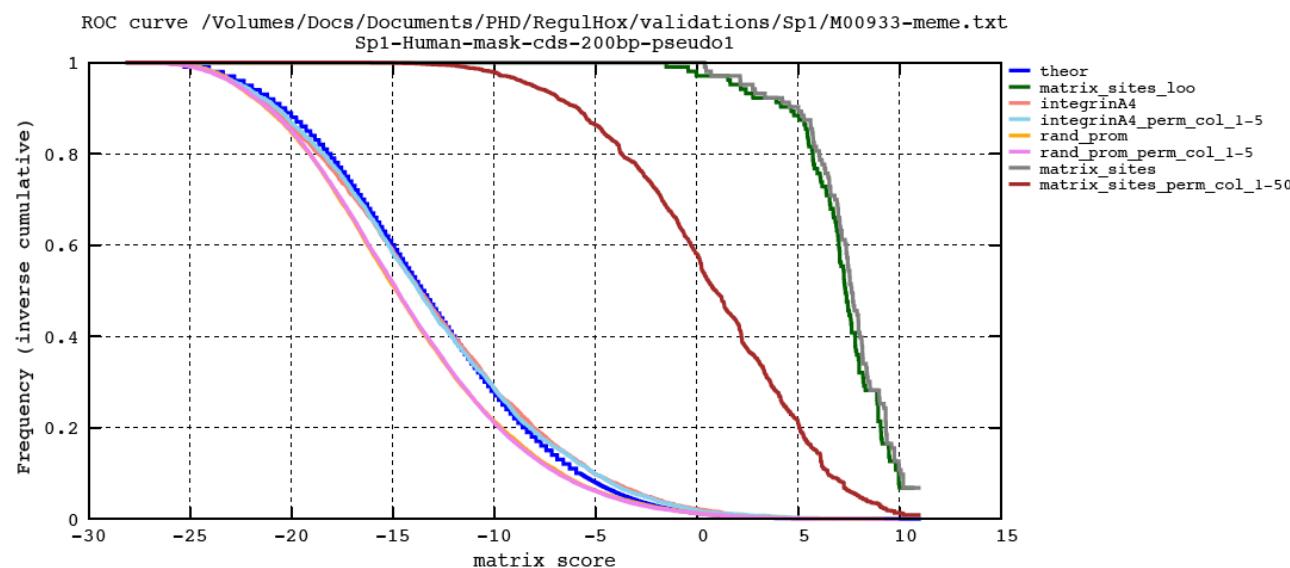
# Matrix-quality for *Drosophila* Hunchback



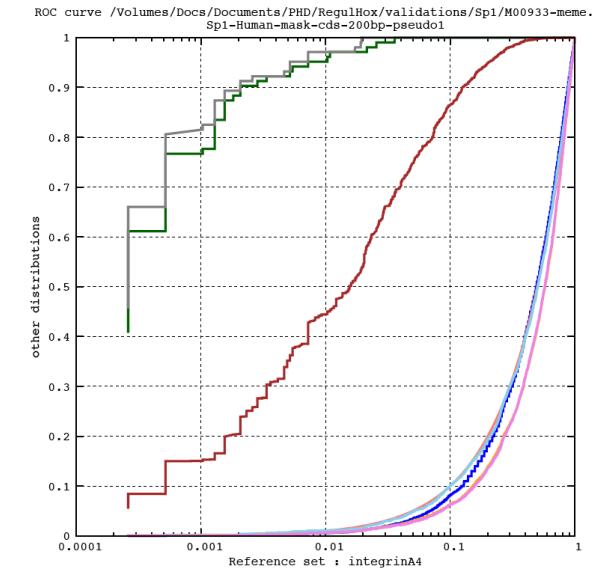
- The drosophila factor hunchback (hb) binds a T-repetitive motif.
- The motif is poorly informative, it is found ubiquitously in the genome.
- The FPR<sub>0.50</sub> is ~10<sup>-3</sup>, indicating achieving a Sn of 50% costs 1FP every kb.
- The target genes (blue) show no particular enrichment relative to the theoretical distribution (black).



# Matrix-quality result for Human Sp1



- The human transcription factor Sp1 binds a C-rich motif.
- The permuted motif is almost identical to the original motif.
- The matrix column-shuffling test is thus inoperant in this case.



## *Points for discussion*

- Assessment based on binding sites
  - Is it possible to develop datasets with full characterization of binding elements?
  - Using artificial data (implanted motifs) ?
- Distribution-based assessment
  - One could consider to assess a motif on the basis of its whole distribution
  - Simple visual inspection already gives us informative clues
  - How to quantify this systematically ?
- Matrix parameters
  - Various parameters can be computed on a matrix
    - Information content
    - E-value of a matrix
    - MAP
  - Do these parameter give us reliable indication on the biological relevance of a motif ? On the specificity of the TF ?
  - Note: some of these parameters are used for optimizing matrices in pattern discovery (more details tomorrow)

## *Supplementary material*

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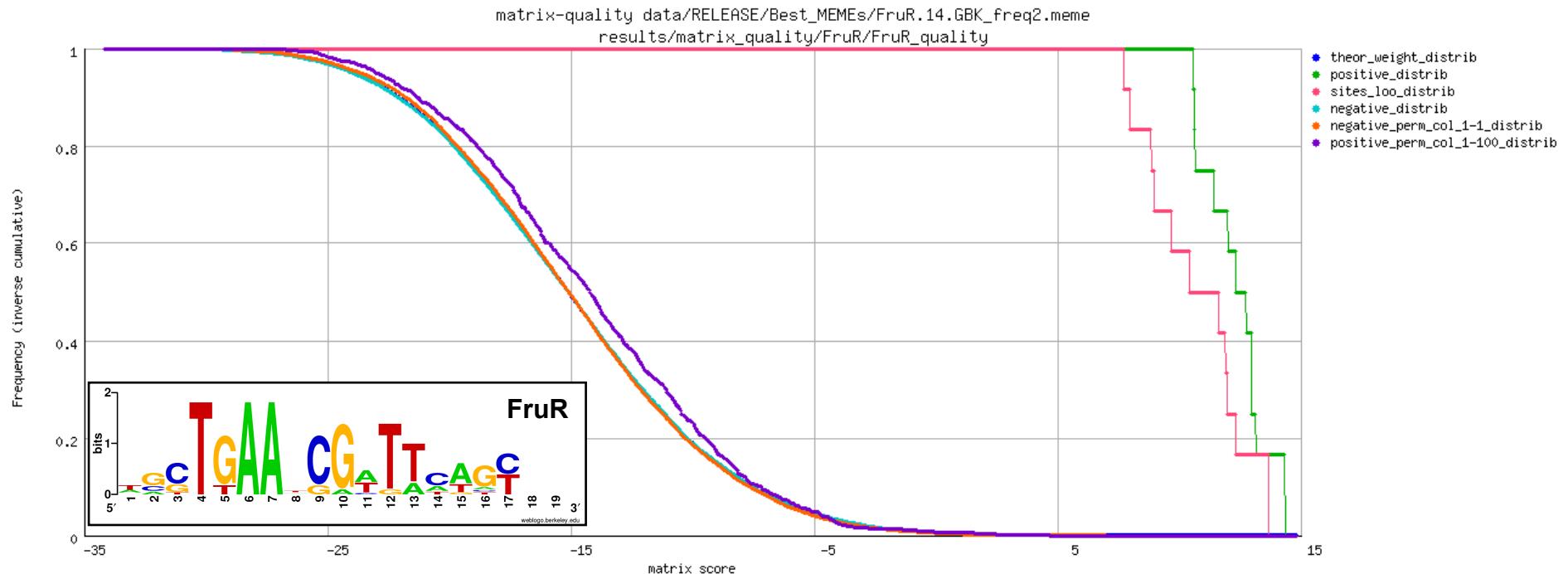
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<http://www.bigré.ulb.ac.be/>

# Estimating matrix quality FruR from RegulonDB

- We compare the following inverse cumulative distributions of scores
  - Blue : theoretical distribution
  - Green: annotated binding sites that served to build the matrix (this is biased !)
  - Pink: leave-one-out (LOO) test with annotated binding sites
  - Violet: scores obtained with 100 permuted matrices in the annotated sites
  - Cyan: distribution in a random selection of promoters
  - Orange: scores obtained with 5 permuted matrices in a random selection of promoters



# PurR

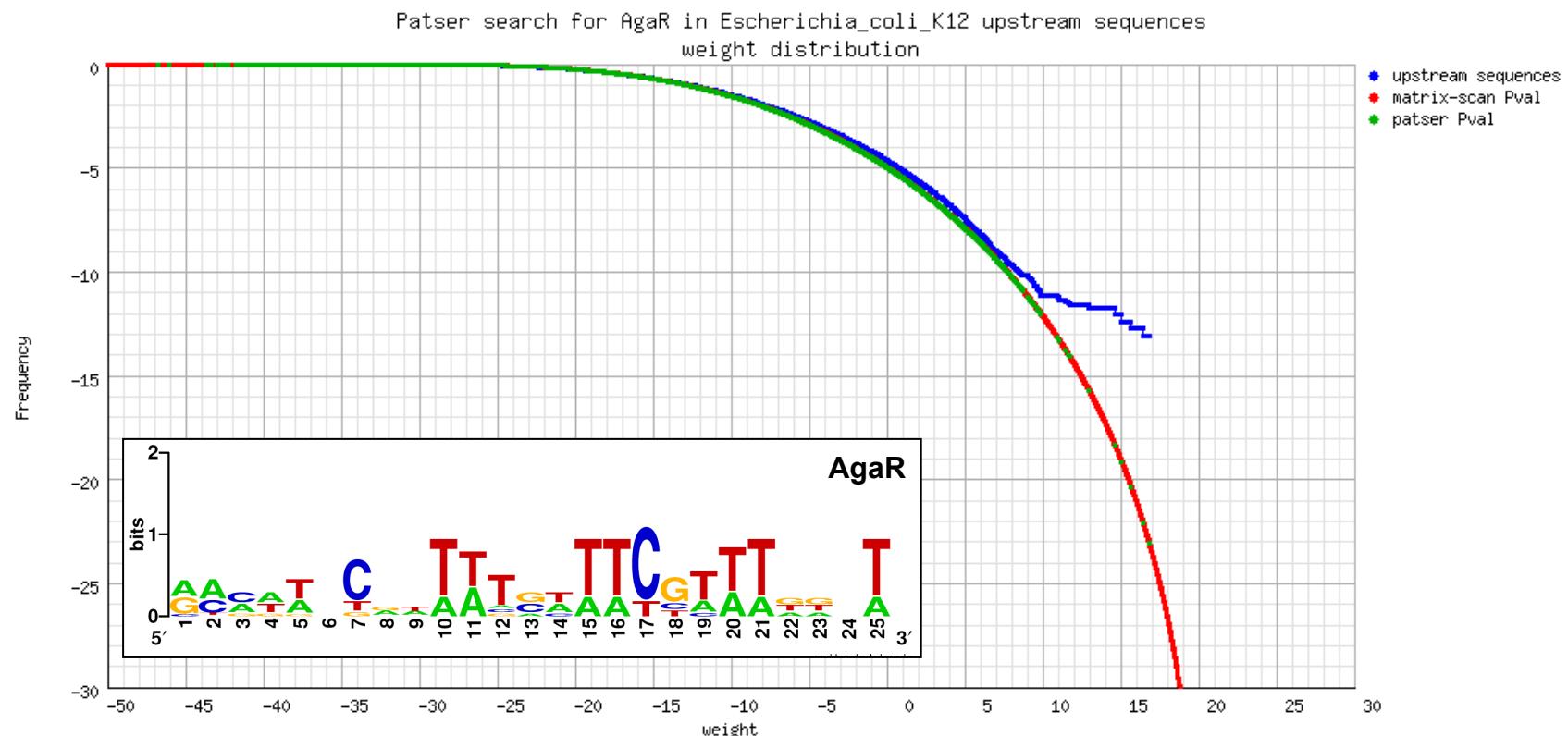
A	11	1	0	3	17	17	13	2	1	3	0	1	3	0	2	3	1
T	1	3	0	1	0	0	2	0	0	11	15	15	6	0	3	10	11
C	2	8	0	13	0	0	1	15	0	1	0	0	2	17	4	3	3
G	3	5	17	0	0	0	1	0	16	2	2	1	6	0	8	1	2
G	C	G	C	A	A	G	C	G	T	T	T	T	C	C	A	G	
A	C	G	A	A	A	A	C	G	A	T	T	T	G	C	T	T	
A	C	G	C	A	A	C	C	G	T	T	T	T	T	C	C	T	
C	G	G	A	A	A	A	C	G	T	T	T	T	G	C	G	T	
A	G	G	A	A	A	A	C	G	T	T	T	T	C	C	G	T	
C	T	G	C	A	A	A	A	A	C	T	G	G	C	C	G	C	
A	G	G	C	A	A	A	C	G	T	T	T	T	A	C	C	T	
G	A	G	C	A	A	A	C	G	T	T	T	T	C	C	A	T	
A	C	G	C	A	A	A	C	G	T	T	T	T	T	C	T	T	
A	C	G	C	A	A	A	C	G	T	T	G	T	G	C	G	T	
T	C	G	C	A	A	A	C	G	T	T	T	T	G	C	T	T	
A	T	G	C	A	A	T	C	G	G	T	T	T	A	C	G	C	
A	G	G	T	A	A	T	C	G	T	T	T	G	C	G	T	A	
A	T	G	C	A	A	A	C	G	A	T	T	T	C	A	A	G	
A	C	G	C	A	A	A	C	G	G	T	T	T	C	G	T	C	
A	G	G	C	A	A	A	A	G	A	G	A	A	C	G	A	T	
G	C	G	C	A	A	A	C	G	T	T	T	T	C	G	T	T	

2



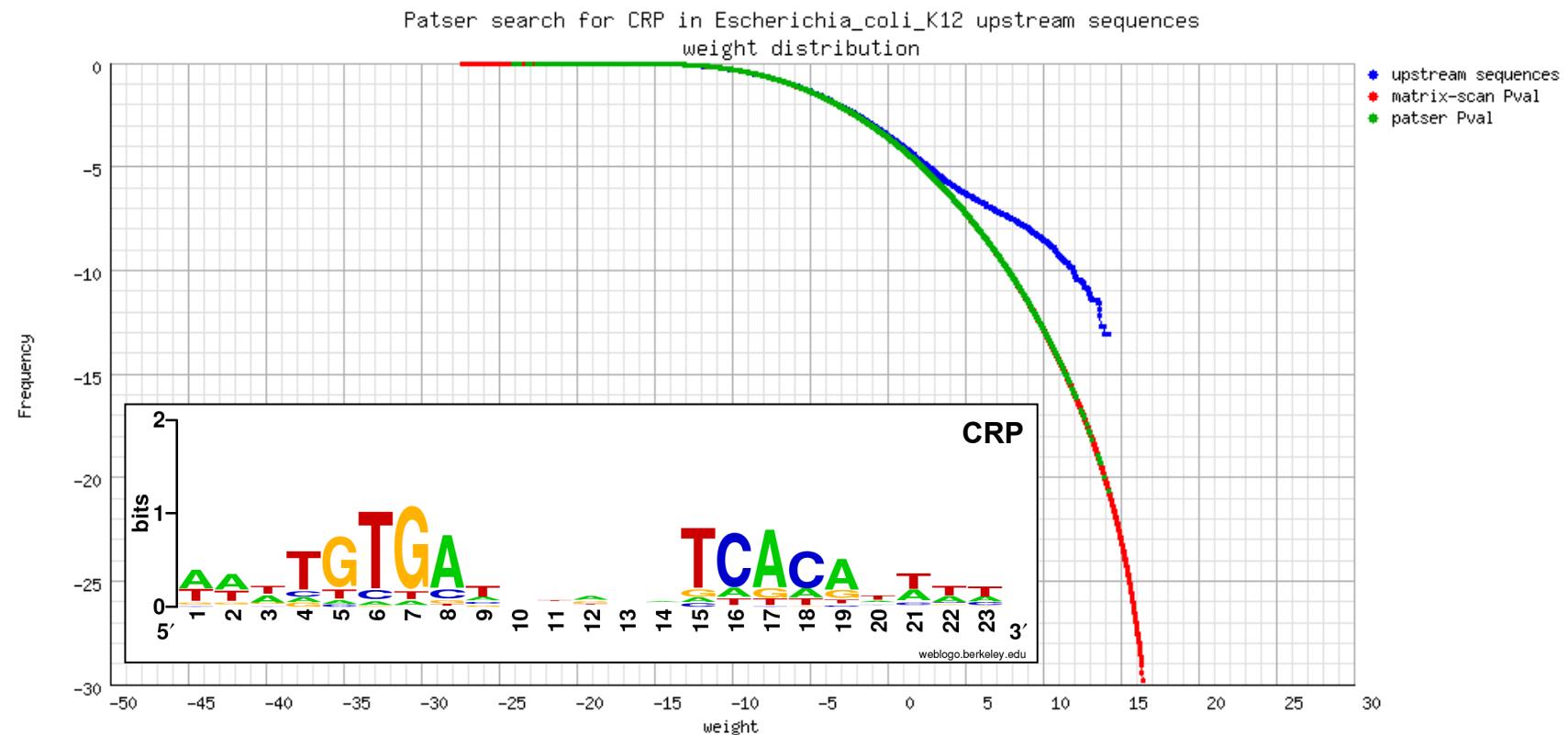
# *Distribution of matrix scores in E.coli promoters AgaR matrix from RegulonDB*

- We compare the distribution observed in all the E.coli promoters (blue) with the theoretical distribution, computed by patser (green) or matrix-scan (blue).
    - For low score values ( $W \leq 5$ ) observed distribution follows pretty well the theoretical one.
    - We also observe a few sites with a very high score(remind, the axis is logarithmic). These high scoring sites are more frequent than expected by chance.
    - These high-scoring sites hopefully correspond to the binding sites of ArgR, but we would of course like to check this.



# Distribution of matrix scores in *E.coli* promoters CRP matrix from RegulonDB

- CRP is a general transcription factor in *E.coli*
- RegulonDB contains annotations about ~130 target genes and ~200 binding sites
- Questions:
  - does the distribution reflect the genericity of the TF ?
  - Is this genericity related to
    - a weak specificity of the matrix ?
    - a large number of specific sites ?



## Example of position-specific scoring matrix

- Binding sites and motif of the human cAMP-response element-binding protein (CREB)

Alignment of CREB binding sites (TRANSFAC annotations)

R01508	T	G	A	C	G	T	C	A	C	G
R15485	T	G	A	C	G	T	C	A	A	G
R04356	T	G	A	C	G	T	C	A	A	G
R16066	T	G	A	C	G	T	C	A	C	C
R00592	T	G	A	C	G	T	C	A	T	G
R14792	T	G	A	T	G	T	C	A	C	T
R12540	T	A	A	C	G	T	C	A	C	A
R04029	T	G	A	C	A	T	C	A	C	G
R16774	G	T	A	C	G	T	C	A	C	G
R02906	G	T	A	C	G	T	C	A	C	G
R09519	T	G	A	C	G	T	C	C	A	T
R14826	G	C	A	C	G	T	C	A	A	G
R16161	T	G	A	C	G	A	C	A	A	C
R14781	T	C	A	C	G	T	A	A	C	T
R17209	T	G	A	C	G	C	T	A	C	G
R08102	T	G	A	G	C	T	C	A	C	T
R04963	T	G	A	C	G	T	C	T	G	A
R12331	A	A	A	C	G	T	C	A	T	C
R14791	A	A	A	T	G	T	C	A	C	A

Count matrix (TRANSFAC matrix CREB)

residue\position	1	2	3	4	5	6	7	8	9	10
A	2	3	19	0	1	1	1	17	5	3
C	0	2	0	16	1	1	17	1	11	3
G	3	12	0	1	17	0	0	0	1	9
T	14	2	0	2	0	17	1	1	2	4
Sum	19	19	19	19	19	19	19	19	19	19

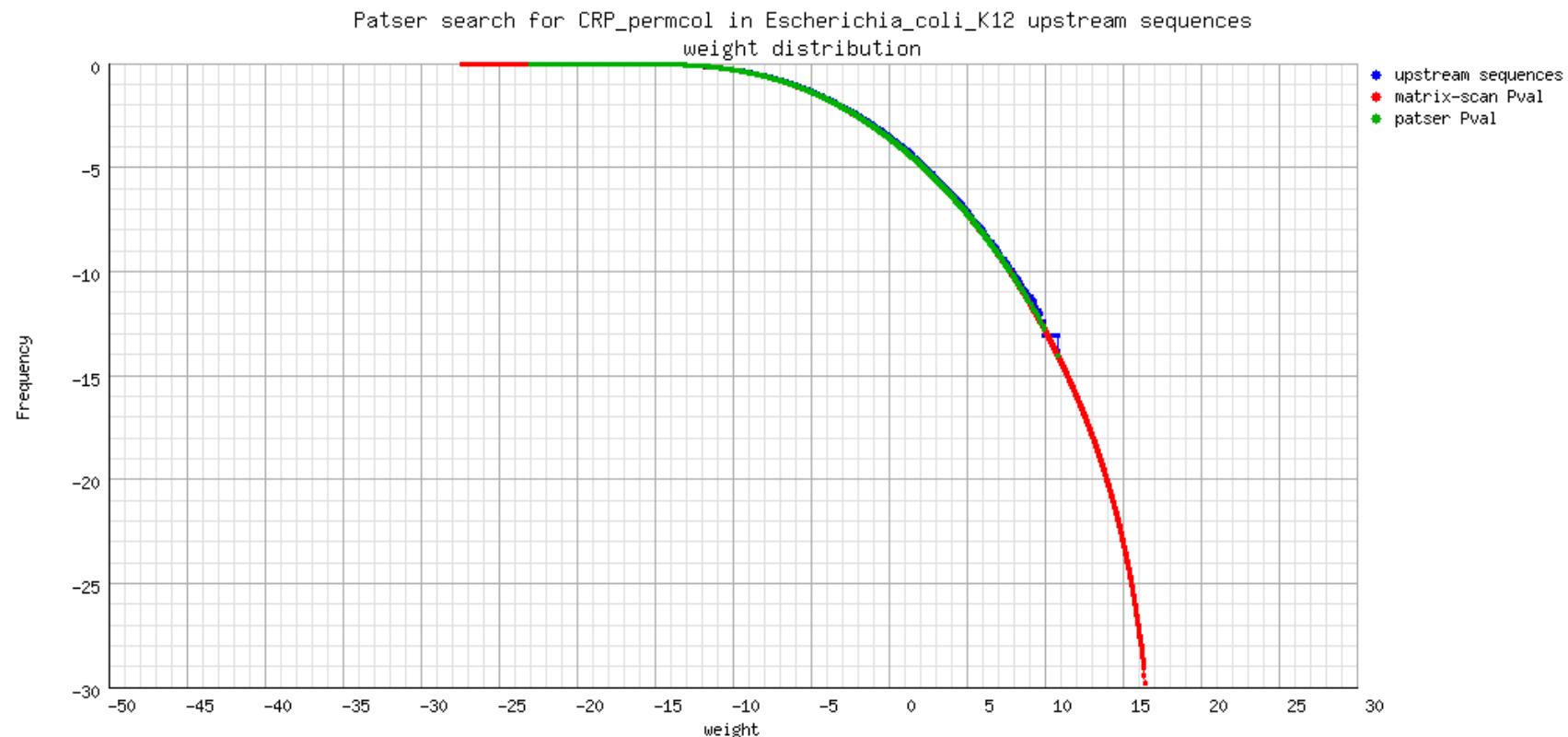


Figure from  
Jean Valéry Turatsinze

# *Distribution of matrix scores in E.coli promoters*

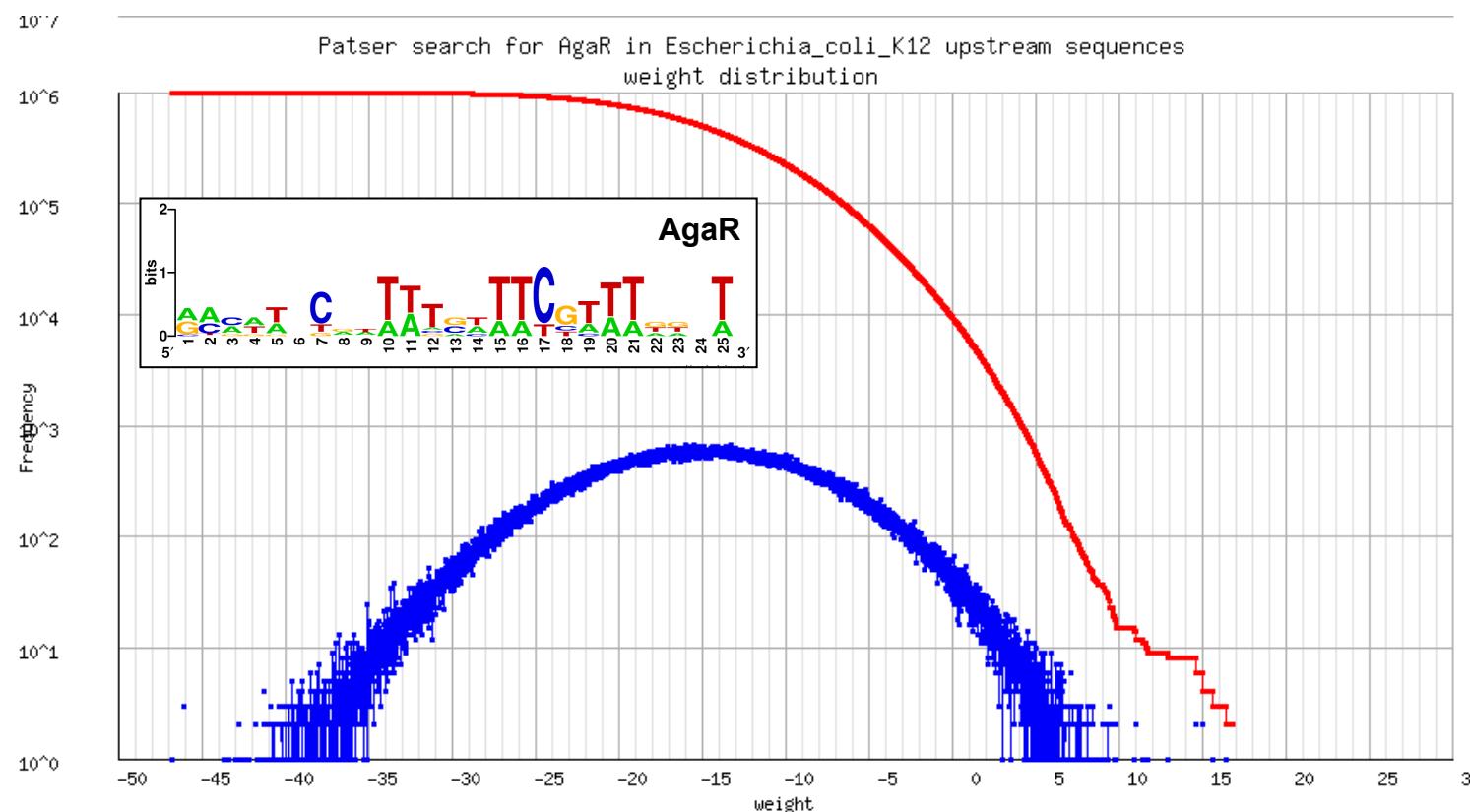
## *CRP matrix from RegulonDB - permuted*

- CRP is a general transcription factor in E.coli
- RegulonDB contains annotations about ~130 target genes and ~200 binding sites
- Questions:
  - does the distribution reflect the genericity of the TF ?
  - Is this genericity related to
    - a weak specificity of the matrix ?
    - a large number of specific sites ?

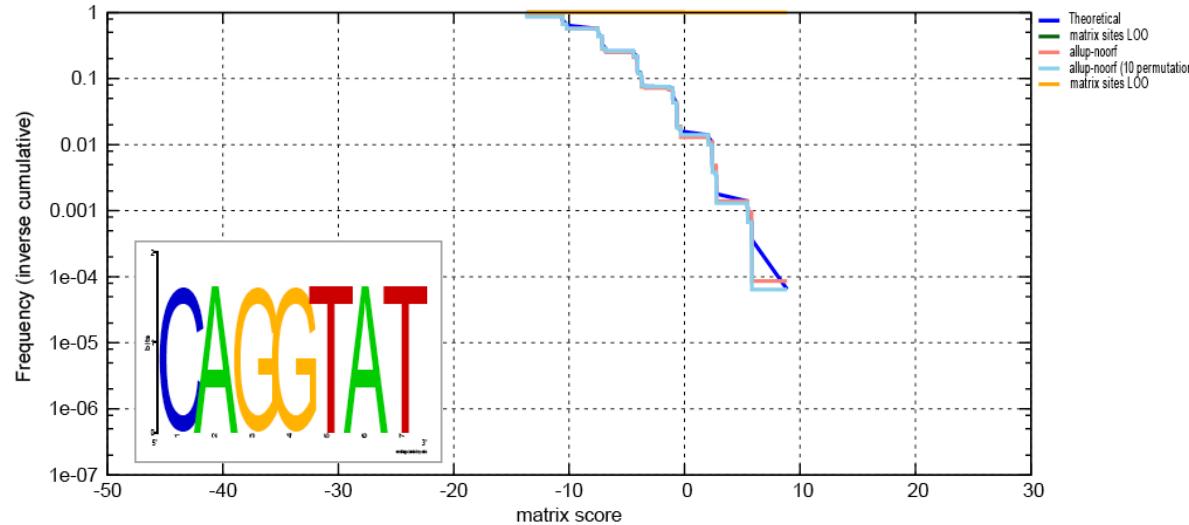


# Distribution of matrix scores in *E.coli* promoters

- We scanned upstream sequences for all the genes of *Escherichia coli K12*, with each matrix of RegulonDB.
- This is illustrated below with the distribution of scores assigned with the matrix AgaR for all the positions in the complete collection of *E.coli* promoters.
  - Blue: distribution of scores in all promoters (beware: Y axis is logarithmic)
  - Red: inverse cumulative distribution.

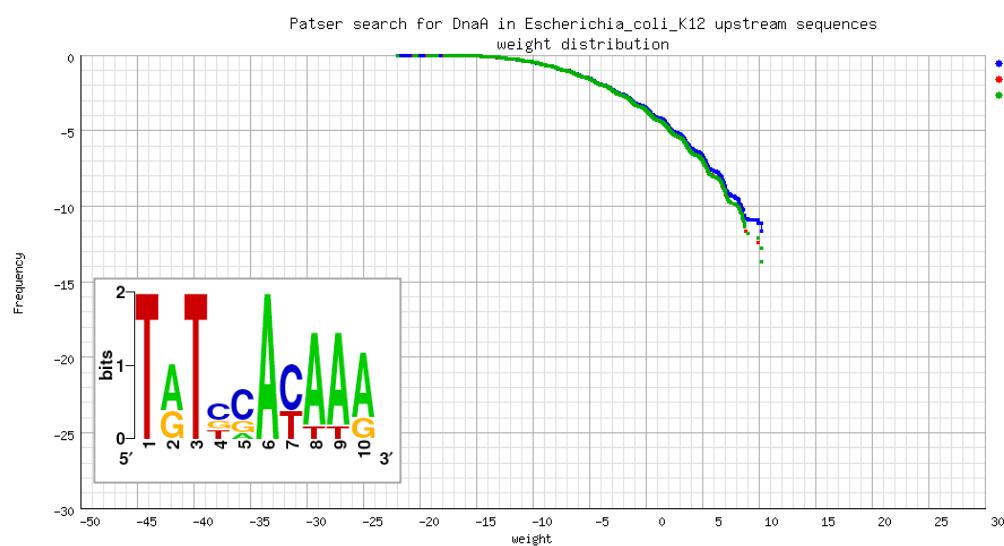


# Some tricky cases



**NanR matrix**

A	0	<b>6</b>	0	0	0	<b>6</b>	0
T	0	0	0	0	<b>6</b>	0	<b>6</b>
C	<b>6</b>	0	0	0	0	0	0
G	0	0	<b>6</b>	<b>6</b>	0	0	0



**DnaA matrix**

A	0	5	0	0	1	<b>8</b>	0	7	7	<b>6</b>
T	<b>8</b>	0	<b>8</b>	2	0	0	3	1	1	0
C	0	0	0	4	5	0	5	0	0	0
G	0	3	0	2	2	0	0	0	0	2

J. Mol. Biol. (1987) 193, 723–750

## Selection of DNA Binding Sites by Regulatory Proteins Statistical-mechanical Theory and Application to Operators and Promoters

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We present a statistical-mechanical selection theory for the sequence analysis of a set of specific DNA regulatory sites that makes it possible to predict the relationship between individual base-pair choices in the site and specific activity (affinity). The theory is based on the assumption that specific DNA sequences have been selected to conform to some requirement for protein binding (or activity), and that all sequences that can fulfil this requirement are equally likely to occur. In most cases, the number of specific DNA sequences that are known for a certain DNA-binding protein is very small, and we discuss in detail the small-sample uncertainties that this leads to. When applied to the binding sites for *cro* repressor in phage lambda, the theory can predict, from the sequence statistics alone, their rank order binding affinities in reasonable agreement with measured values. However, the statistical uncertainty generated by such a small sample (only 6 sites known) limits the result to order-of-magnitude comparisons. When applied to the much larger sample of *Escherichia coli* promoter sequences, the theory predicts the correlation between *in vitro* activity ( $k_2 K_B$  values) and homology score (closeness to the consensus sequence) observed by Mulligan *et al.* (1984). The analysis of base-pair frequencies in the promoter sample is consistent with the assumption that base-pairs at different positions in the sites contribute independently to the specific activity, except in a few marginal cases that are discussed. When the promoter sites are ordered according to predicted activities, they seem

- In 1987, Otto G. Berg and Peter H. von Hippel propose a theoretical model to predict binding affinity between regulatory proteins and their DNA binding sites.
- They compare binding affinity and score

# Quality of the CRP matrix

