2. Binding of transcription factors

- protein-DNA interactions
- experimental approaches to determine binding sites (BS)
- representing binding specificity of TFs
- databases
- comparing binding profiles

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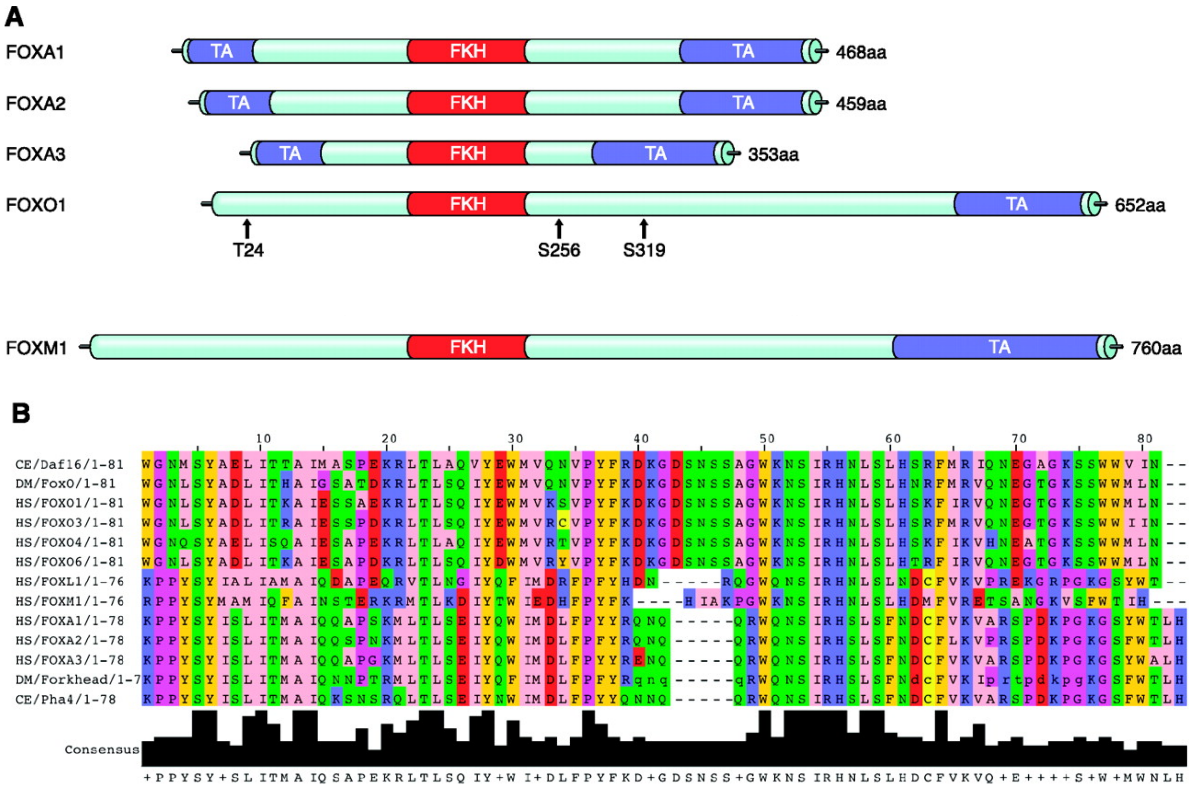
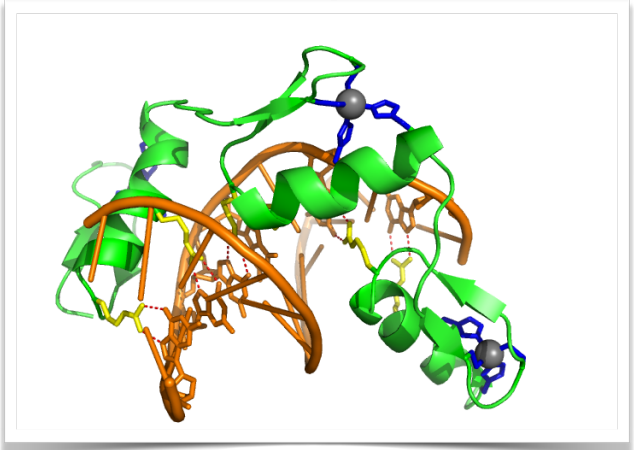
DNA binding domains

- Transcription factors contain a **DNA binding domain** (DBD) and a **transcriptional activator** (TA)
- Homologous TFs share similar DBDs (here: forkhead)



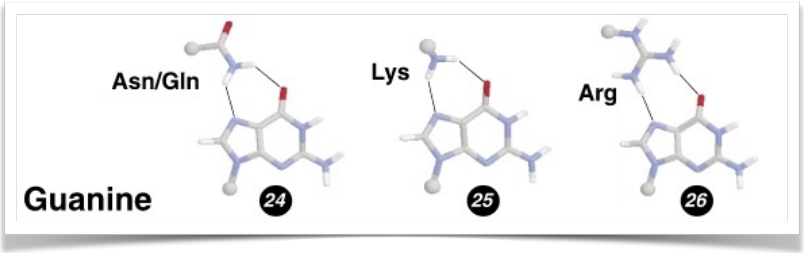
[Luscombe 2010]

Protein DNA interactions



Amino acids	Mode of interaction	Recognised base
Hydrogen bond		
[ARG, LYS]	Multiple-donor	G/complex
[HIS]	Multiple-donor (bifurcate)	G
[SER]	Multiple-donor (bifurcate)	G
	Acceptor+donor	complex
[ASN, GLN]	Acceptor+donor	A/complex
[ASP, GLU]	Multiple-acceptor	complex
van der Waals contacts		
[PHE, PRO]	Ring-stacking	A, T
[THR]	Methyl contact	T
[GLY, ALA, VAL, LEU, ISO, TYR]	-	many (non-specific)
No base contact		
[CYS, MET, TRP]	-	-

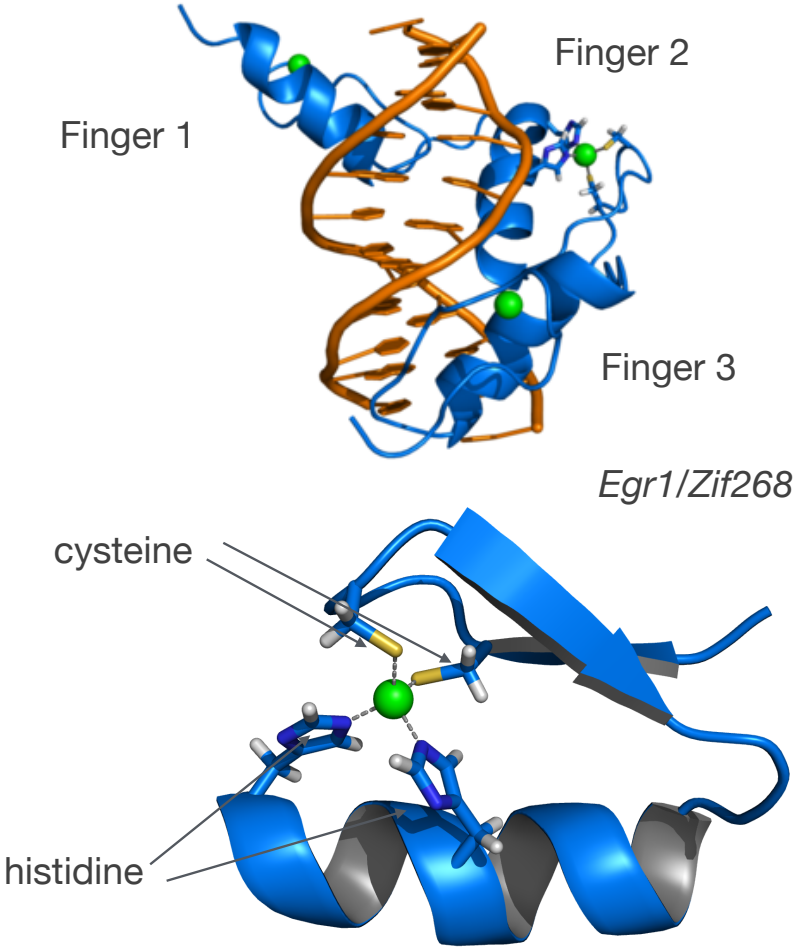
- majority of protein-DNA interactions for TF occur through a **alpha-helix** fitting into the major groove (=DNA binding domain)
- **hydrogen bonds** with specific bases
- stabilization of the protein-DNA complex is ensured by additional structures (helix, beta-sheet) via **van der Walls** interactions



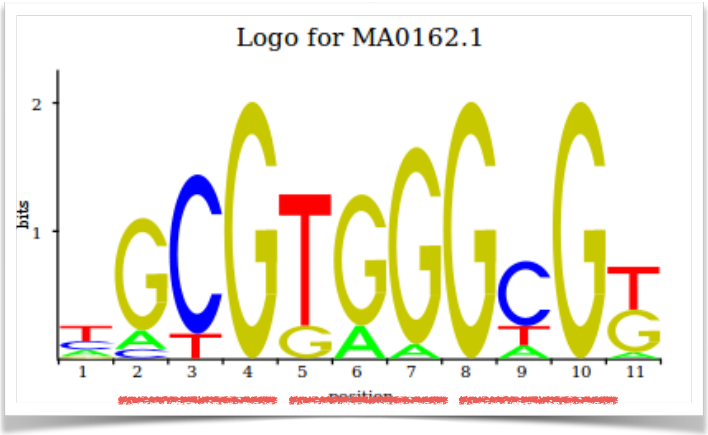
[...] while there appear to be favoured interactions, the specificity for the entire DNA sequence can rarely be explained by one-to-one correspondences between amino acids and bases.

[Luscombe et al., NAR (2001)]

Structural family: Zinc coordinating



Cys2His2 Fold (“Zinc finger”)
→ one of the most common family
of transcription factors in mammalians

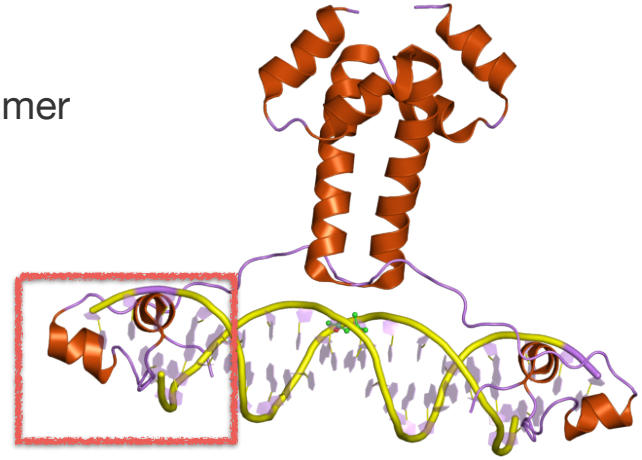


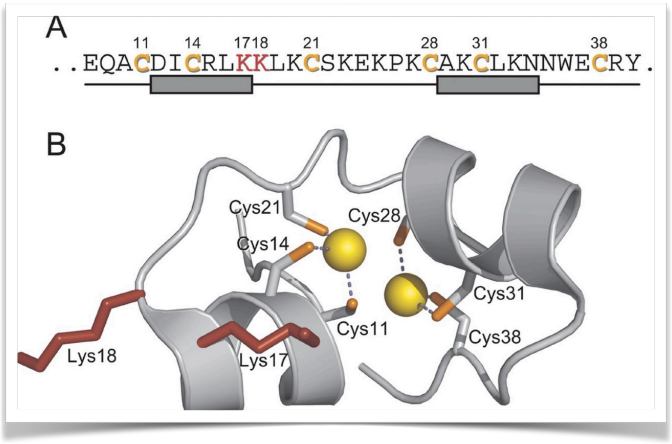
Finger 1 Finger 2 Finger 3

Structural family: Zinc coordinating

Gal4 *S. cerevisae*

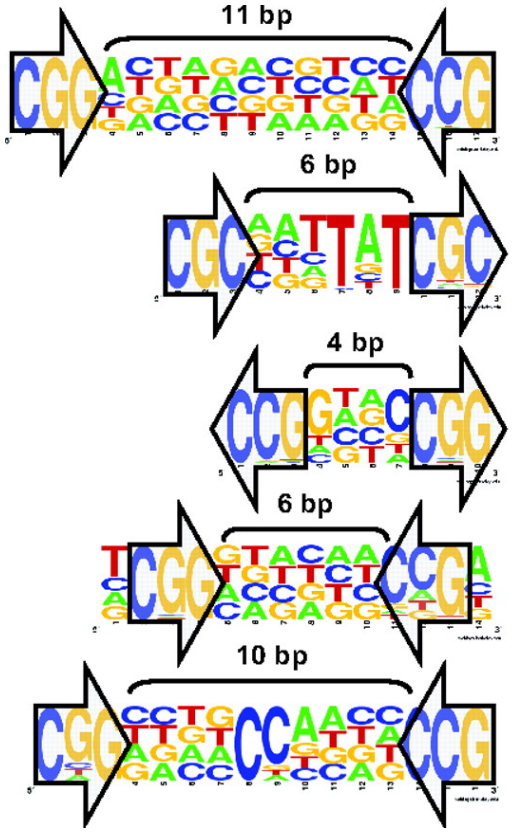
→ binds as homodimer





[Campbell (2008)]

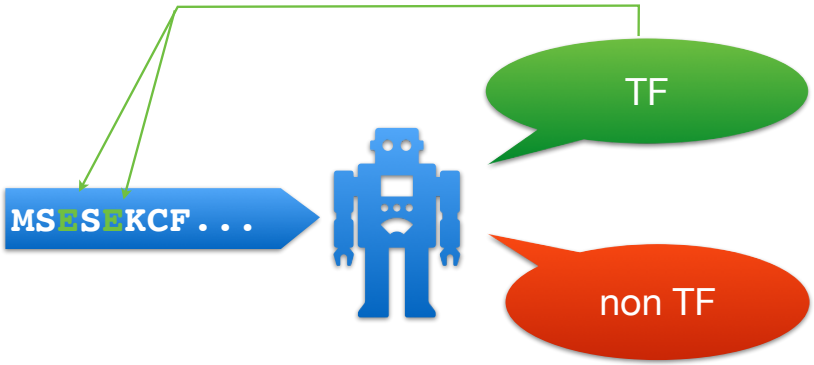
- 6 cysteins
- 2 Zinc atoms
= **Cys6Zn2 zinc-fingers**

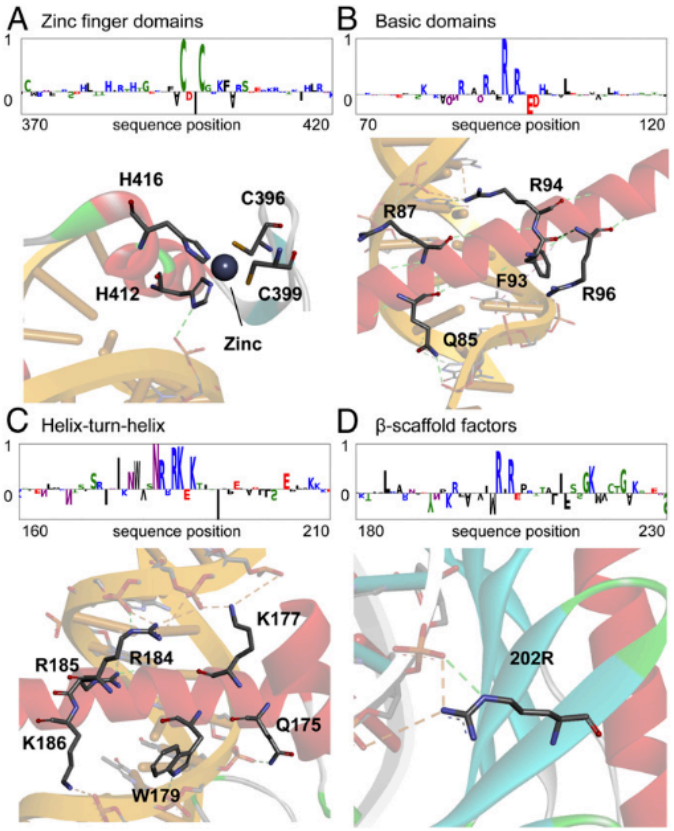


TF binding site is a **dyad** (short repeated motif with spacer)

Predicting TFs from protein sequence

- No prediction of binding site from protein sequence (yet...)!
- However, one can **predict if a protein is a TF** using only the protein sequence using deep learning approaches: **DeepTFactor**

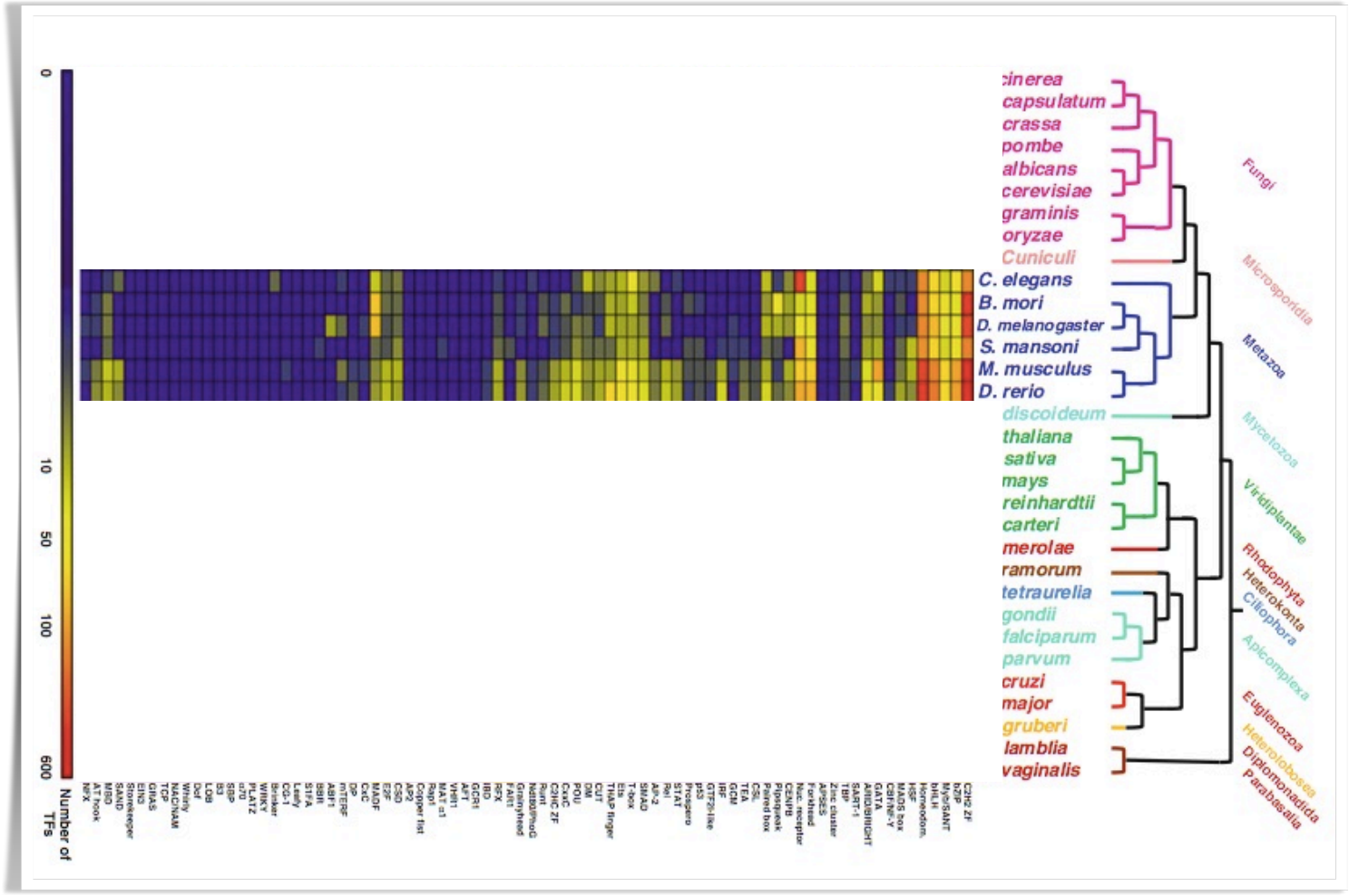




prediction of residues which are contributing highly to the classification of TFs

[Kim et al., PNAS (2020)]

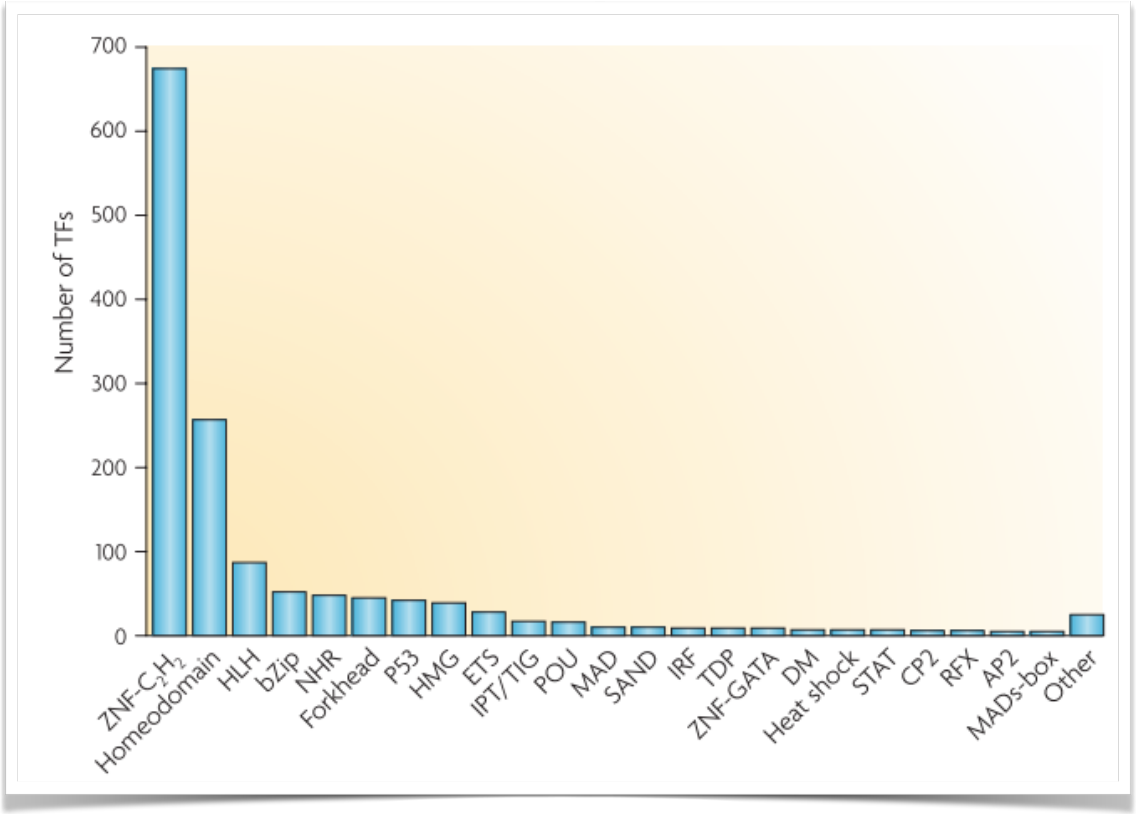
Structural families of TFs



[Weirauch, Hughes (2010)]

Structural families of TFs

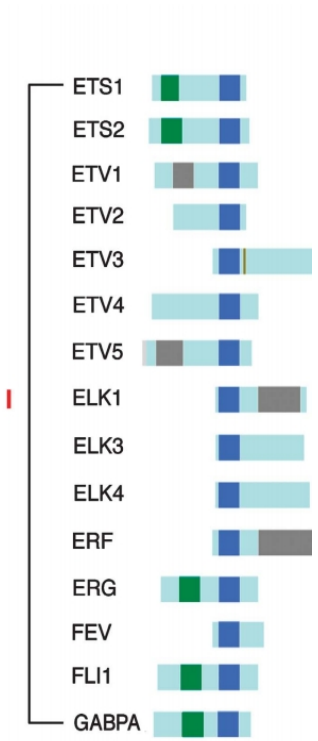
- in human, 3 classes account for > 80% of known transcription factors
  - Zinc-finger **C2H2**
    (→ Zinc finger nucleases)
  - **homeodomain**
    (e.g. Hox TFs)
  - **helix-loop-helix factors**
    (e.g. c-Myc, N-Myc,...)



[Vaquerizas et al. Nat.Rev.Gen (2009)]

From protein to DNA motif

- Transcription factors belonging to the same structural family have **similar binding profiles**
- Highly conserved accross species
- conserved **core motif**
- variable flanking regions
  → specificity



[Wei et al. EMBO Journal 2010]

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Proc. Nat. Acad. Sci. USA
Vol. 70, No. 12, Part I, pp. 3581–3584, December 1973

The Nucleotide Sequence of the *lac* Operator

(regulation/protein–nucleic acid interaction/DNA–RNA sequencing/oligonucleotide priming)

WALTER GILBERT AND ALLAN MAXAM

Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138

Communicated by J. D. Watson, August 9, 1973

ABSTRACT **The *lac* repressor protects the *lac* operator against digestion with deoxyribonuclease. The protected fragment is double-stranded and about 27 base-pairs long. We determined the sequence of RNA transcription copies of this fragment and present a sequence for 24 base pairs. It is:**

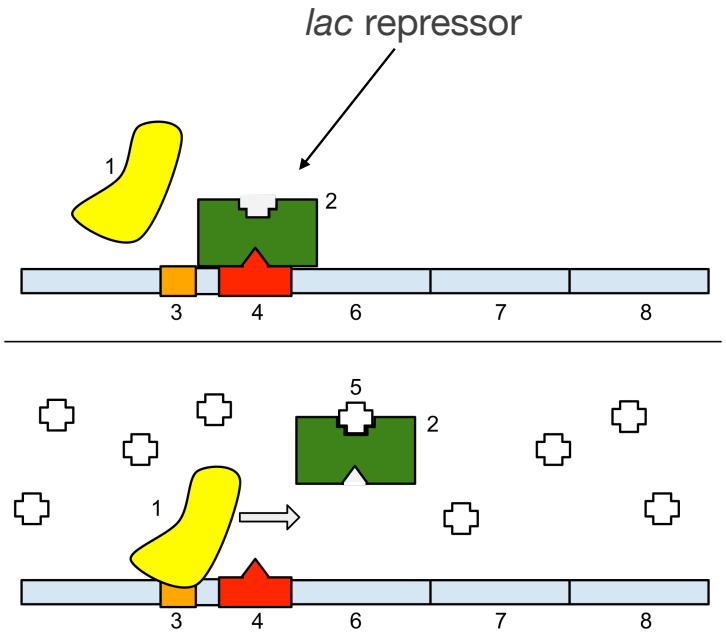
5′--T G G A A T T G T G A G C G G A T A A C A A T T 3′
3′--A C C T T A A C A C T C G C C T A T T G T T A A 5′

The sequence has 2-fold symmetry regions; the two longest are separated by one turn of the DNA double helix.

The lactose repressor selects one out of six million nucleotide sequences in the *Escherichia coli* genome and binds to it to prevent the expression of the genes for lactose metabolism. bind again to the repressor, and is about 27 base-pairs long. Here we shall describe its sequence.

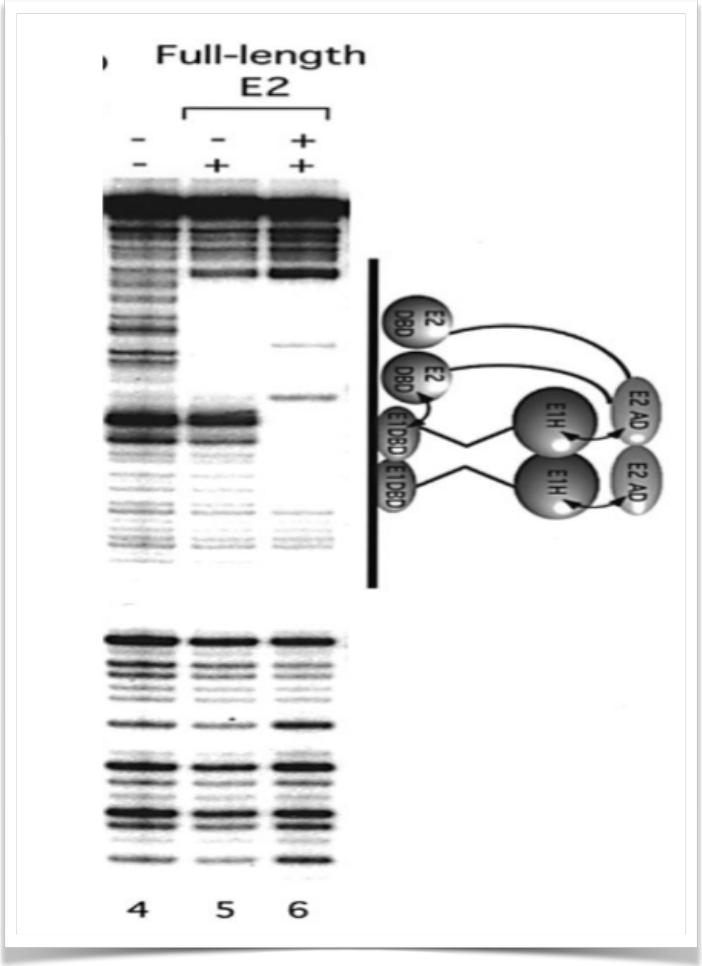
METHODS

Sonicated DNA Fragments. Sonicated [^{32}P]DNA fragments were made by growing a temperature-inducible lysogen of λc1857p*lac*5S7 at 34° in a glucose–50 mM Tris·HCl or TES (pH 7.4) medium in 3 mM phosphate, heating at 42° for 15 min at a cell density of 4×10^8/ml, then washing and resuspending the cells at a density of 8×10^8/ml in the same medium with 0.1 mM phosphate. 100 mCi of neutralized $H_3{}^{32}PO_4$ was added to 10 ml of cells, and the incorporation was continued for 2 hr at 34°. The cells were washed, suspended in 2



Experimental identification of binding sites

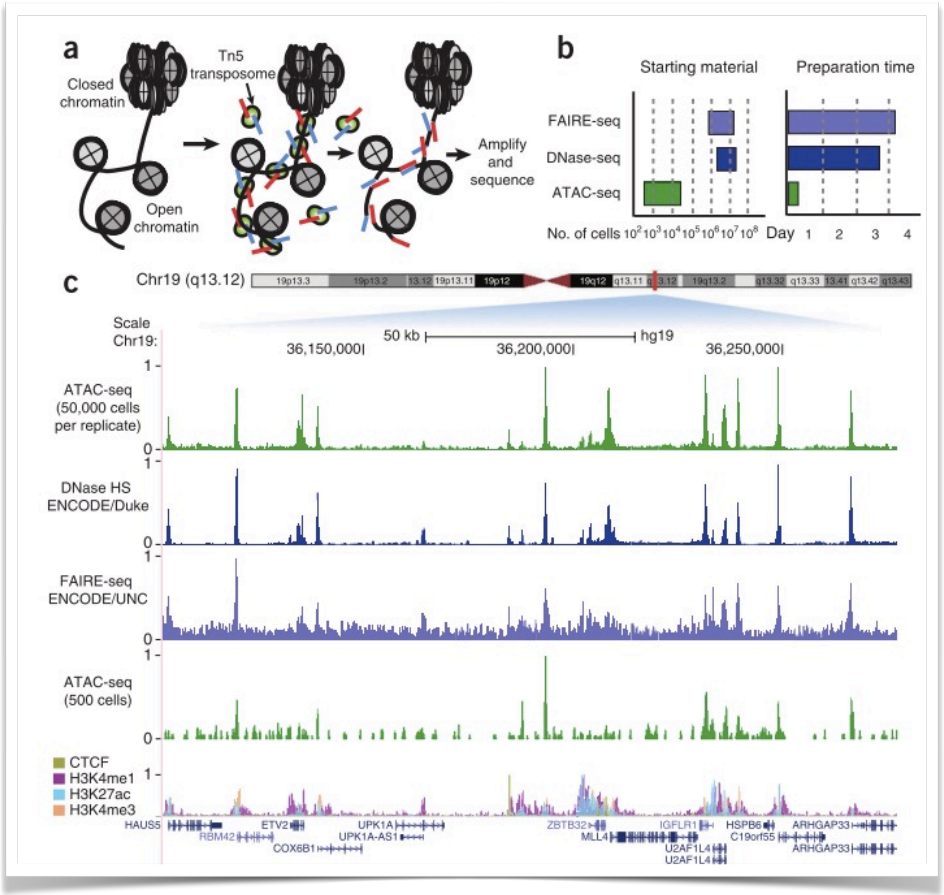
- **DNAse1 footprint:**
  protein-bound DNA is protected from digestion by the DNAse1 enzyme
- gel electrophoresis allows identification of the digested / undigested fragments
- if whole sequence is known, binding sites can be identified
- in vitro / low-throughput



[A.Stenlund The EMBO Journal (2003)]

Experimental identification of binding sites

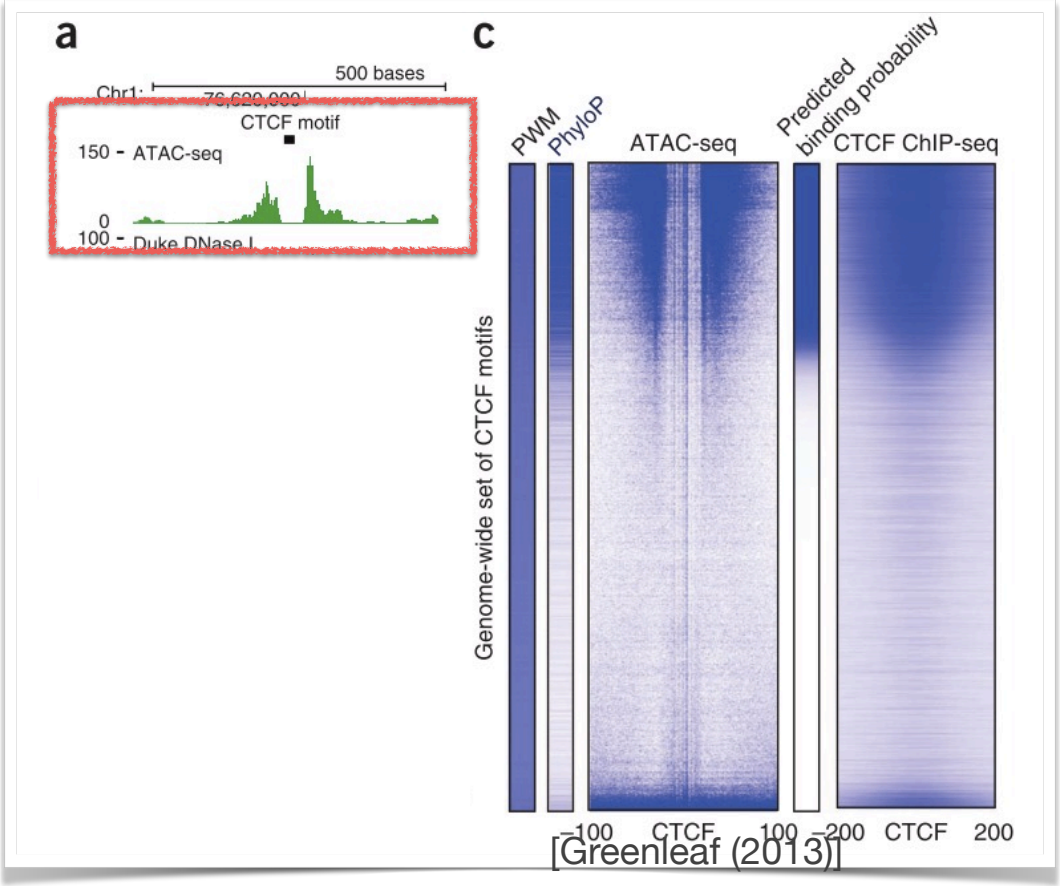
- **ATAC-seq**: using Tn5 transposase prepared with sequencing primers
- requires a small number of input material (~10,000 cells)
- identification of open chromatin regions (peaks)



[Greenleaf (2013)]

Experimental identification of binding sites

- From open regions to transcription factor binding sites
  → **footprinting**
- Zooming into the peaks (open regions) : valleys of undigested / un-transposed DNA
  → **TF binding sites (TFBS)**
- binding sequence can be identified with base-pair resolution

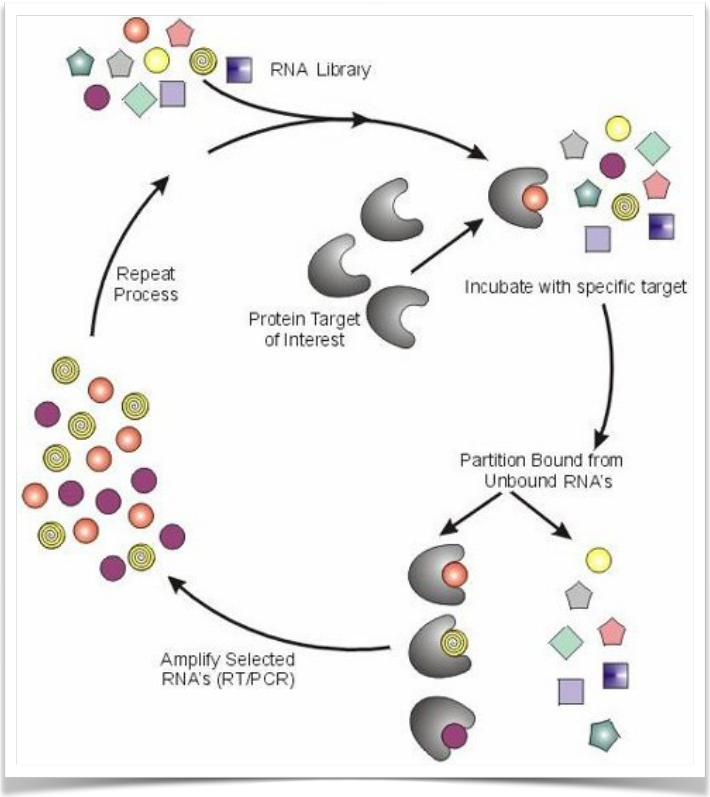


[Greenleaf (2013)]

Experimental identification of binding sites

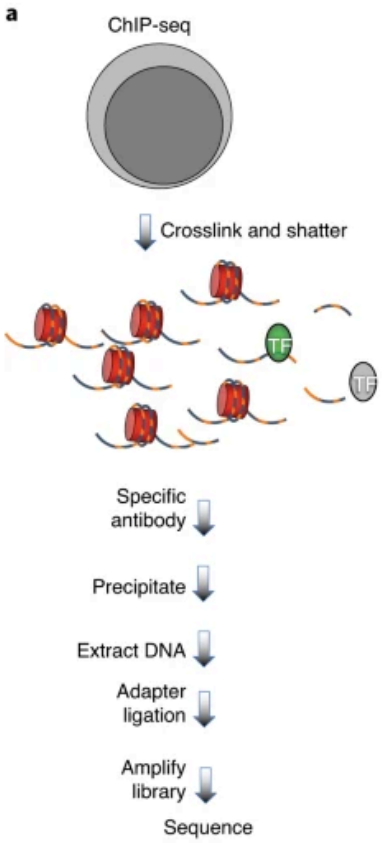
- **SELEX** (= *systematic evolution of ligands by exponential enrichment*)
  - random library of k-mers generated
  - incubated with TF of interest
  - elution to separate bound from unbound targets
  - PCR (=enrichment)
  - next cycle

- in-vitro / medium throughput
- increasing number of cycles leads to **over-select high affinity binding sites**



[A.Stenlund The EMBO Journal (2003)]

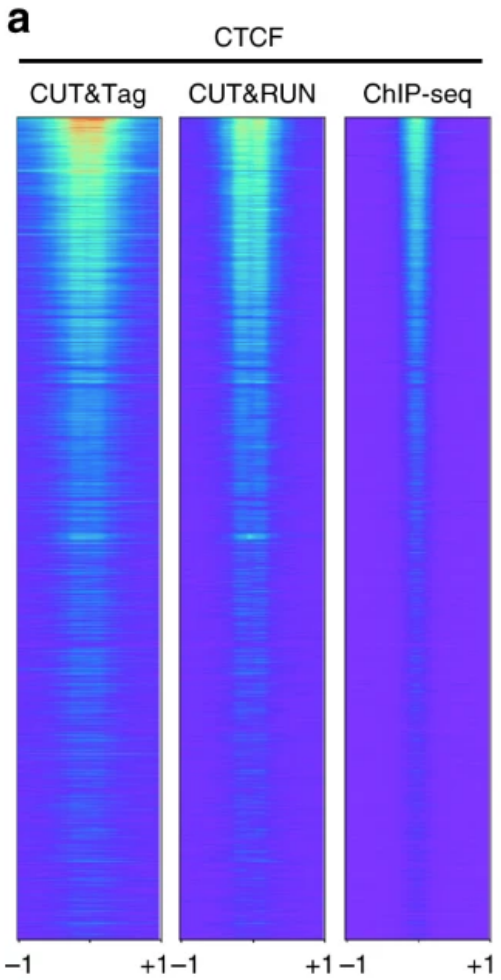
Antibody-based assays



[Kaya-Okur et al., Nat.Prot. (2020)]

Antibody-based assay

- Cut & Tag has **higher signal-to-noise** ratio compared to ChIP-seq
- Can be performed under **native conditions** → adaptable to single-cell
- **Greater resolution** to detect the actual binding site of the TF



[Kaya-Okur et al., Nat.Com. (2019)]

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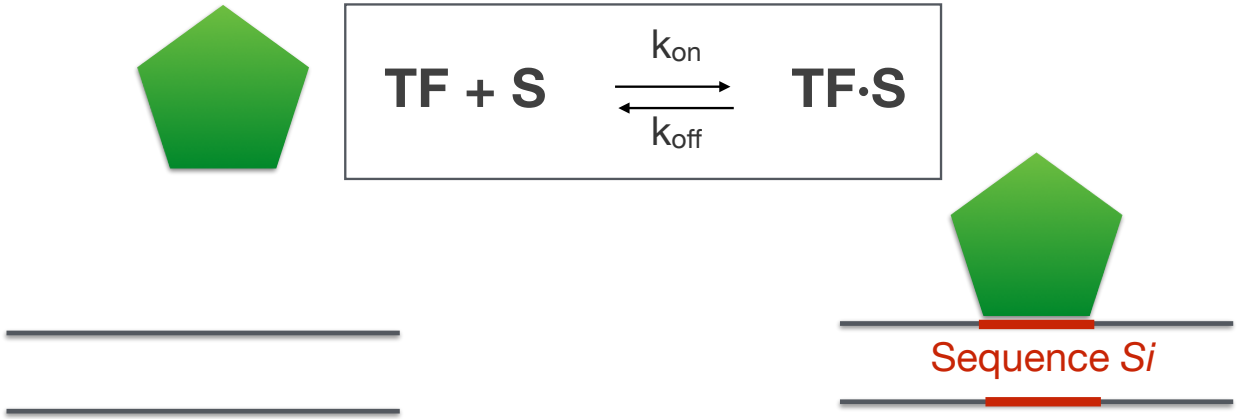
DNA transcription factor interactions



Equilibrium constant

$$K_i = \frac{k_{on}}{k_{off}} = \frac{[TF \cdot S_i]}{[TF][S_i]}$$

Binding probability

$$P(s=1|S_i) = \frac{[TF \cdot S_i]}{[TF \cdot S_i] + [S_i]} = \frac{1}{1 + e^{E_i - \mu}}$$

$$E_i = -\ln K_i$$

free energy

$$\mu = \ln[TF]$$

chemical potential

DNA transcription factor interactions

- How many parameters are needed to describe the DNA-protein interaction ?

Naive approach

- if TF binds regions of size w, then if one K_i per possible sequence
  - 4^w parameters
  - w = 6 : 4096 parameters
  - w = 8 : 65536 parameters

Model :

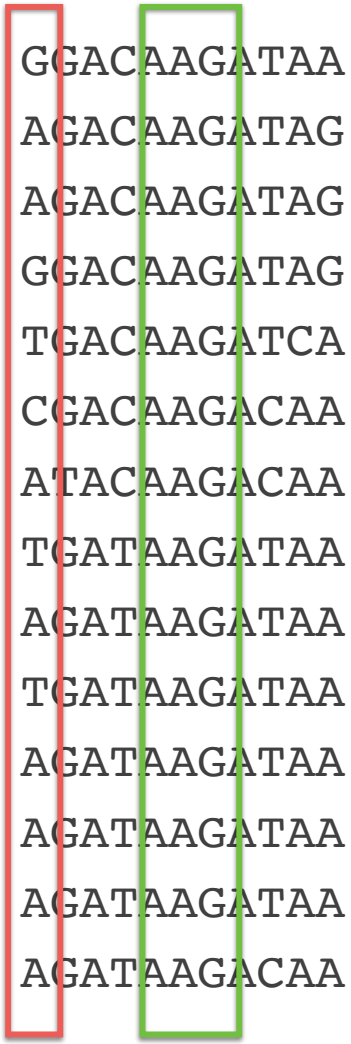
- Assume independent contribution of each position of S_i to the binding energy
- contribution proportional to **log(freq at position j)**
  → 3w parameters
- w = 6 : 18 parameters
- w = 8 : 24 parameters

[Berg, von Hippel (1987)]
[Stormo, Bioinformatics (2000)]

Characterizing binding affinities

- **Example** :
  - Evi-1 mouse transcription factor
  - 14 binding sites obtained using SELEX

- Binding sites of transcription factors are not fixed strings !



Some positions are very degenerate

Some positions are well conserved

Characterizing binding affinities

- **Solution 1:**
  represent binding sites as **consensus sequence**
- **IUPAC conventions**
  - `W = A or T ; R = A or G (puRines)`
  - `K = G or T ; S = C or G`
  - `Y = C or T (pYrimidine) ; M = A or C`
  - `B = C, G or T ; D = A,G or T`
  - `H = A,C or T ; V = A,C or G`
  - `N = any nucleotide`
- **Transfac conventions**
  - for a given position, order nucleotides by frequency : f1>f2>f3>f4
  - **single** nucleotide if f1 > 50% AND f1>2*f2
  - **double** if f1 + f2 > 75% AND f1<50 %; f2<50 %
  - **triple** if one nucleotide never appears and previous rules don't apply
  - N in all other cases

Characterizing binding affinities

- **Solution 1:**
  represent binding sites as consensus sequence
- **Weakness 1:**
  - column 1 has 8/14 A's
  - column 10 has 13/14 A's
  - both are represented by A
- **Weakness 2:**
  - other nucleotides in column 1 are not taken into account

Characterizing binding affinities

- **Solution 2 :**
- count frequencies of nucleotides at each position
- obtain a count matrix
- represent the **matrix** as a **logo**

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

a	8	0	14	0	14	14	0	14	0	13	11
c	1	0	0	7	0	0	0	0	3	1	0
g	2	13	0	0	0	0	14	0	0	0	3
t	3	1	0	7	0	0	0	0	11	0	0

Characterizing binding affinities

- **Solution 2 :**
- count frequencies of nucleotides at each position
- normalize to obtain **position frequency matrix (PFM)**

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

$f_{i,j}$

a|	0.57	0.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	0.93	0.79
c|	0.07	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.21	0.07	0.00
g|	0.14	0.93	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.21
t|	0.21	0.07	0.00	0.50	0.00	0.00	0.00	0.00	0.79	0.00	0.00

Characterizing binding affinities

- ***Beware of sampling effects !***
- Two different sets of BS for the same TF will give different PFM
- Example : Evi-1
  - set 1 : 14 BS (selex)
  - set 2 : 47 BS (selex)

a|	0.57	0.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	0.93	0.79
c|	0.07	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.21	0.07	0.00
g|	0.14	0.93	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.21
t|	0.21	0.07	0.00	0.50	0.00	0.00	0.00	0.00	0.79	0.00	0.00

a|	0.55	0.74	0.00	1.00	0.02	1.00	0.98	0.00	1.00	0.00	0.87	0.85	0.23	0.64
c|	0.09	0.04	0.02	0.00	0.43	0.00	0.00	0.00	0.00	0.13	0.04	0.00	0.23	0.21
g|	0.19	0.09	0.94	0.00	0.00	0.00	0.02	1.00	0.00	0.00	0.00	0.15	0.30	0.06
t|	0.17	0.13	0.04	0.00	0.55	0.00	0.00	0.00	0.00	0.87	0.09	0.00	0.23	0.09

Increasing the sample of binding sites makes some frequencies become > 0

What if we would further increase the dataset ?

Characterizing binding affinities

- Sampling effects are attenuated by adding **pseudo-counts**
- at each position, a pseudo-count (usually 1) is distributed among the nucleotides
  - equal amount (0.25)
  - OR : fraction proportional to nucleotide prior frequency

a|	8	0	14	0	14	14	0	14	0	13	11
c|	1	0	0	7	0	0	0	0	3	1	0
g|	2	13	0	0	0	0	14	0	0	0	3
t|	3	1	0	7	0	0	0	0	11	0	0

$n_{i,j}$

add 1 using priors
a:t = 0.2 ; c:g = 0.3

a|	8.2	0.2	14.2	0.2	14.2	14.2	0.2	14.2	0.2	13.2	11.2
c|	1.3	0.3	0.3	7.3	0.3	0.3	0.3	0.3	3.3	1.3	0.3
g|	2.3	13.3	0.3	0.3	0.3	0.3	14.3	0.3	0.3	0.3	3.3
t|	3.2	1.2	0.2	7.2	0.2	0.2	0.2	0.2	11.2	0.2	0.2

$n'_{i,j}$

normalize to 1 by dividing by 14+1

a|	0.55	0.02	0.94	0.02	0.94	0.94	0.02	0.94	0.02	0.88	0.75
c|	0.08	0.02	0.02	0.48	0.02	0.02	0.02	0.02	0.22	0.08	0.02
g|	0.15	0.88	0.02	0.02	0.02	0.02	0.95	0.02	0.02	0.02	0.22
t|	0.22	0.08	0.02	0.48	0.02	0.02	0.02	0.02	0.75	0.02	0.02

$f'_{i,j}$

Polls and pseudo-counts

Polls

Résumé des intentions

	Ifop R 17-20 avril	BVA 18-19 avril	CSA 18-19 avril	Harris 18-19 avril	Ipsos 18-19 avril
Nathalie Arthaud	0,5	0	1	0,5	0
Philippe Poutou	1	1,5	1,5	1,5	1,5
Jean-Luc Mélenchon	13,5	14	14,5	12	14
François Hollande	**27**	**30**	**28**	**27,5**	**29**
Eva Joly	3	2	2	3	2
François Bayrou	10,5	10	10,5	11	10
Nicolas Sarkozy	**27**	**26,5**	**25**	**26,5**	**25,5**
Nicolas Dupont-Aignan	1,5	2	1,5	2	1,5
Marine Le Pen	16	14	16	16	16
Jacques Cheminade	0	0	0	0	0,5
	Détails	*Détails*	*Détails*	*Détails*	*Détails*

They should have added pseudo counts !

Final results

Characterizing binding affinities

- why is it so important to get rid of 0 frequencies ?
- compute the probability of a sequence to represent a binding site for Evi-1

a|	0.57	0.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	0.93	0.79
c|	0.07	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.21	0.07	0.00
g|	0.14	0.93	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.21
t|	0.21	0.07	0.00	0.50	0.00	0.00	0.00	0.00	0.79	0.00	0.00
	C	C	A	C	A	A	G	A	C	A	A

P = 0.07 * 0 * 1 * 0.5 * 1 * 1 * 1 * 1 * 1 * 0.21 * 0.93 * 0.79 = 0

a|	0.55	0.02	0.94	0.02	0.94	0.94	0.02	0.94	0.02	0.88	0.75
c|	0.08	0.02	0.02	0.48	0.02	0.02	0.02	0.02	0.22	0.08	0.02
g|	0.15	0.88	0.02	0.02	0.02	0.02	0.95	0.02	0.02	0.02	0.22
t|	0.22	0.08	0.02	0.48	0.02	0.02	0.02	0.02	0.75	0.02	0.02
	C	C	A	C	A	A	G	A	C	A	A

P = 0.08* 0.02* 0.94*0.48* 0.95* 0.95* 0.95*0.95*0.22 *0.88*0.75 = 8.6e-5

This sequence can NEVER be a BS → this sequence has non-0 probability of being a BS

Comparing two models

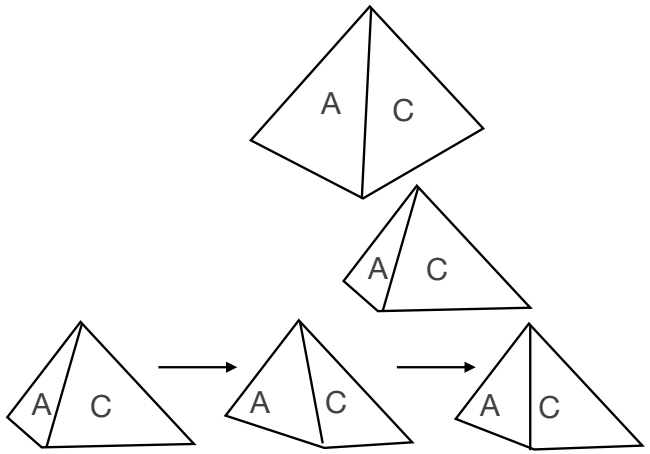
- We want to use the PFM model to find potential binding sites in a DNA sequence
- How well does the model discriminate between
  - True binding sites ("**Binding site model**")
  - Random background sequences ("**Background model**")
- Same as identikit procedure: how well does an identikit distinguish between
  - the real criminal searched for
  - a random person picked in the crowd

Not really: many average features

Probably more discriminative: many specific features!

Generating background sequences

- We often need to bioinformatically **generate sequences according a a specific model** *("rolling a dice")*
- this model gives the emission probabilities, i.e. the probability *P(X,j)* at each position *j* of the sequence to obtain one of the four bases A,C,G,T
- possible models
  - equiprobable model: $P(A)=P(C)=P(G)=P(T)=0.25$
  - more realistic model: $P(A)=P(T)=p_{A,T}$; $P(C)=P(G)=p_{C,G}$
  - position dependent model: $P(A,j) = f_{A,j}$



Information content of a matrix

Would I get the same PFM if I would consider 14 random sequences?

- Generate 14 sequences of length L=11 according to the emission probabilities of the PFM; probability of obtaining exactly the same count matrix:

$$P_{mat} = \prod_{j=1}^{L} \prod_{i \in A,C,G,T} C_{i,j} f_{i,j}'^{n'_{i,j}}$$

- Generate 14 **random sequences** on length L=11; probability of obtaining exactly the same count matrix:

$$P_{rand} = \prod_{j=1}^{L} \prod_{i \in A,C,G,T} C_{i,j}\ p_i^{n'_{i,j}}$$

$p_A = p_T = 0.2 \quad p_C = p_G = 0.3$

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

$n'_{i,j}$

a|	8.2	0.2	14.2	0.2	14.2	14.2	0.2	14.2	0.2	13.2	11.2
c|	1.3	0.3	0.3	7.3	0.3	0.3	0.3	0.3	3.3	1.3	0.3
g|	2.3	13.3	0.3	0.3	0.3	0.3	14.3	0.3	0.3	0.3	3.3
t|	3.2	1.2	0.2	7.2	0.2	0.2	0.2	0.2	11.2	0.2	0.2

p_i

a|	0.2
c|	0.3
g|	0.3
t|	0.2

$f'_{i,j}$

a|	0.55	0.02	0.94	0.02	0.94	0.94	0.02	0.94	0.02	0.88	0.75
c|	0.08	0.02	0.02	0.48	0.02	0.02	0.02	0.02	0.22	0.08	0.02
g|	0.15	0.88	0.02	0.02	0.02	0.02	0.95	0.02	0.02	0.02	0.22
t|	0.22	0.08	0.02	0.48	0.02	0.02	0.02	0.02	0.75	0.02	0.02

Information content of a matrix

- Log-likelihood ratio

$$LLR = \log_2\left(\frac{P_{mat}}{P_{rand}}\right) = \sum_{j=1}^{L}\sum_{i\in A,C,G,T} n'_{i,j}\log_2\frac{f'_{i,j}}{p_i}$$

- Information content (bits)

$$IC = \frac{1}{n}\log_2\left(\frac{P_{mat}}{P_{rand}}\right) = \sum_{j=1}^{L}\sum_{i\in A,C,G,T} f'_{i,j}\log_2\frac{f'_{i,j}}{p_i}$$

- Properties
  - if $f'_{ij} \rightarrow p_i$: IC → 0 : **zero information content** if the frequencies in the alignment are equal to the background frequencies ("random matrix")
  - IC is **maximal** if the least abundant nucleotide (smallest p_i) is the most represented in the matrix (largest f'_{ij})
  - information content is related to the **Shannon entropy**, mesuring the uncertainty : high information content → low Shannon entropy

[Hertz, Stormo 1999]

Matrix logos

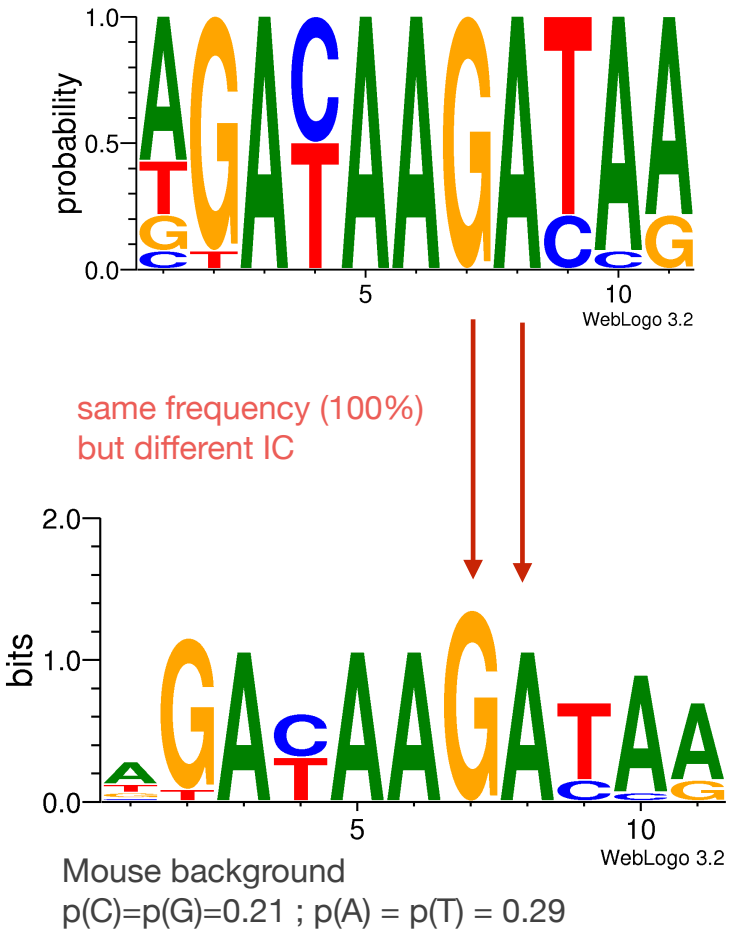
- Information content of the matrix:

$$IC = \sum_{j=1}^{L} \sum_{i \in A,C,G,T} f'_{i,j} \log_2 \frac{f'_{i,j}}{p_i}$$

- Information content of a column:

$$IC^j = \sum_{i \in A,C,G,T} f'_{i,j} \log_2 \frac{f'_{i,j}}{p_i}$$

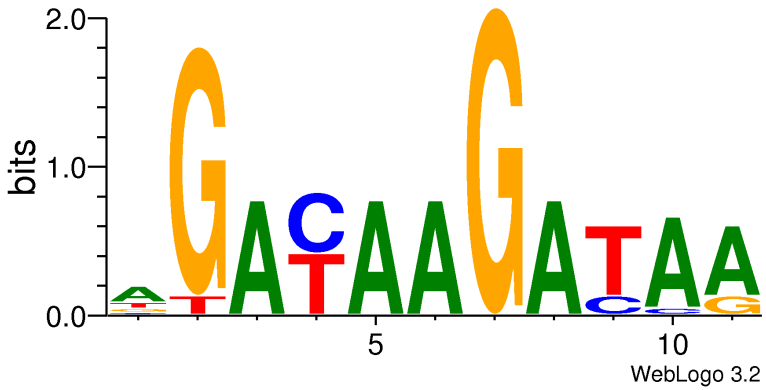
- Conventions:
  - height of column represents IC
  - relative sizes proportional to frequencies



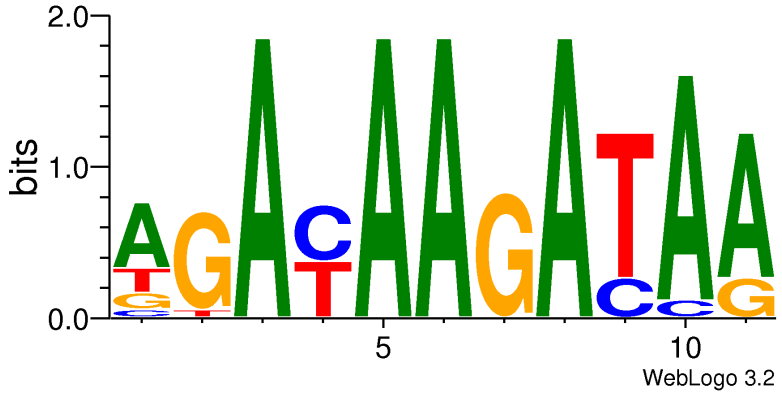
Mouse background
p(C)=p(G)=0.21 ; p(A) = p(T) = 0.29

Matrix logos

a	8	0	14	0	14	14	0	14	0	13	11
c	1	0	0	7	0	0	0	0	3	1	0
g	2	13	0	0	0	0	14	0	0	0	3
t	3	1	0	7	0	0	0	0	11	0	0



P. falciparum background
p(C)=p(G)=0.1 ; p(A) = p(T) = 0.4



Anaeromyxobacter background
p(C)=p(G)=0.37 ; p(A) = p(T) = 0.13

From frequencies (PFM) to weights (PWM)

- position frequency matrices do not contain information about the background distribution
- define **position-weight matrices (PWM)** as
  (or Position Specific Scoring Matrix PSSM)

$$PWM_{i,j} = \ln \frac{f'_{ij}}{p_i}$$

a|	8	0	14	0	14	14	0	14	0	13	11
c|	1	0	0	7	0	0	0	0	3	1	0
g|	2	13	0	0	0	0	14	0	0	0	3
t|	3	1	0	7	0	0	0	0	11	0	0

Count matrix

a|	0.55	0.02	0.94	0.02	0.95	0.95	0.02	0.95	0.02	0.88	0.75
c|	0.08	0.02	0.02	0.48	0.02	0.02	0.02	0.02	0.22	0.08	0.02
g|	0.15	0.88	0.02	0.02	0.02	0.02	0.95	0.02	0.02	0.02	0.22
t|	0.22	0.08	0.02	0.48	0.02	0.02	0.02	0.02	0.75	0.02	0.02

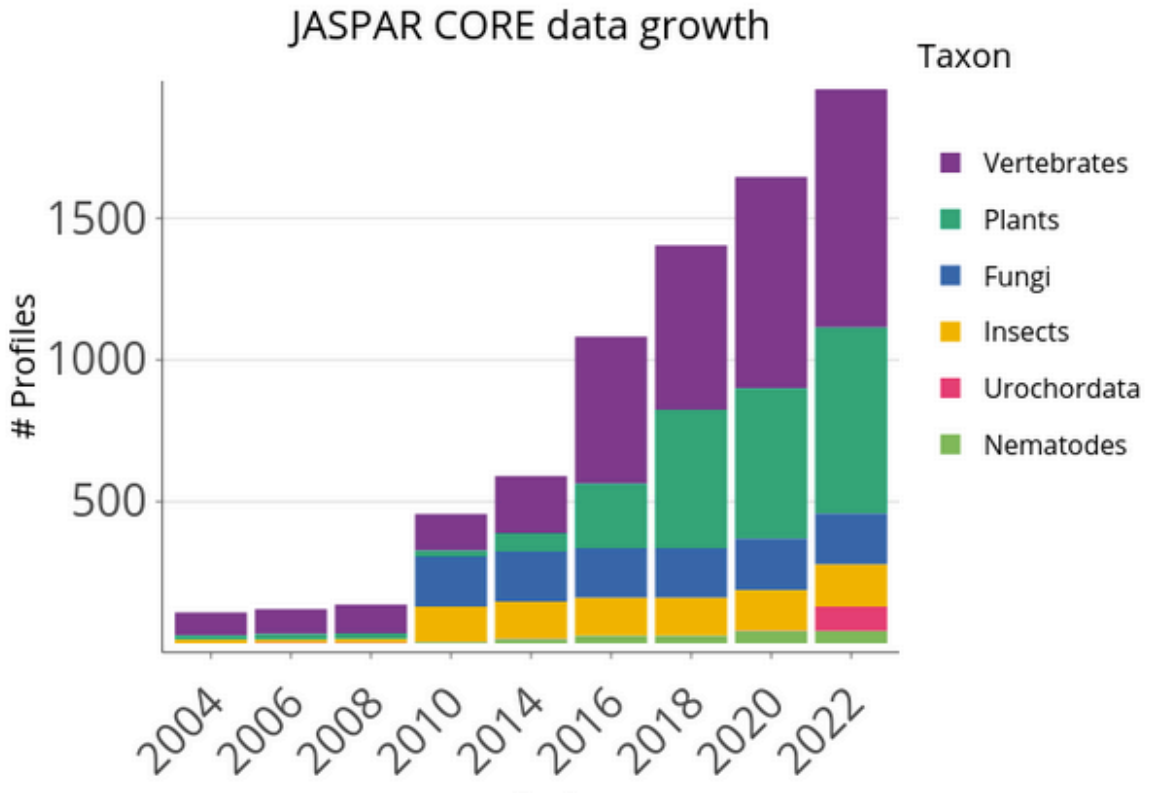
PFM

a|	0.75	-2.71	1.30	-2.71	1.30	1.30	-2.71	1.30	-2.71	1.22	1.06
c|	-1.04	-2.71	-2.71	0.73	-2.71	-2.71	-2.71	-2.71	-0.07	-1.04	-2.71
g|	-0.46	1.31	-2.71	-2.71	-2.71	-2.71	1.39	-2.71	-2.71	-2.71	-0.10
t|	-0.22	-1.16	-2.71	0.58	-2.71	-2.71	-2.71	-2.71	1.02	-2.71	-2.71

PWM

Motif databases

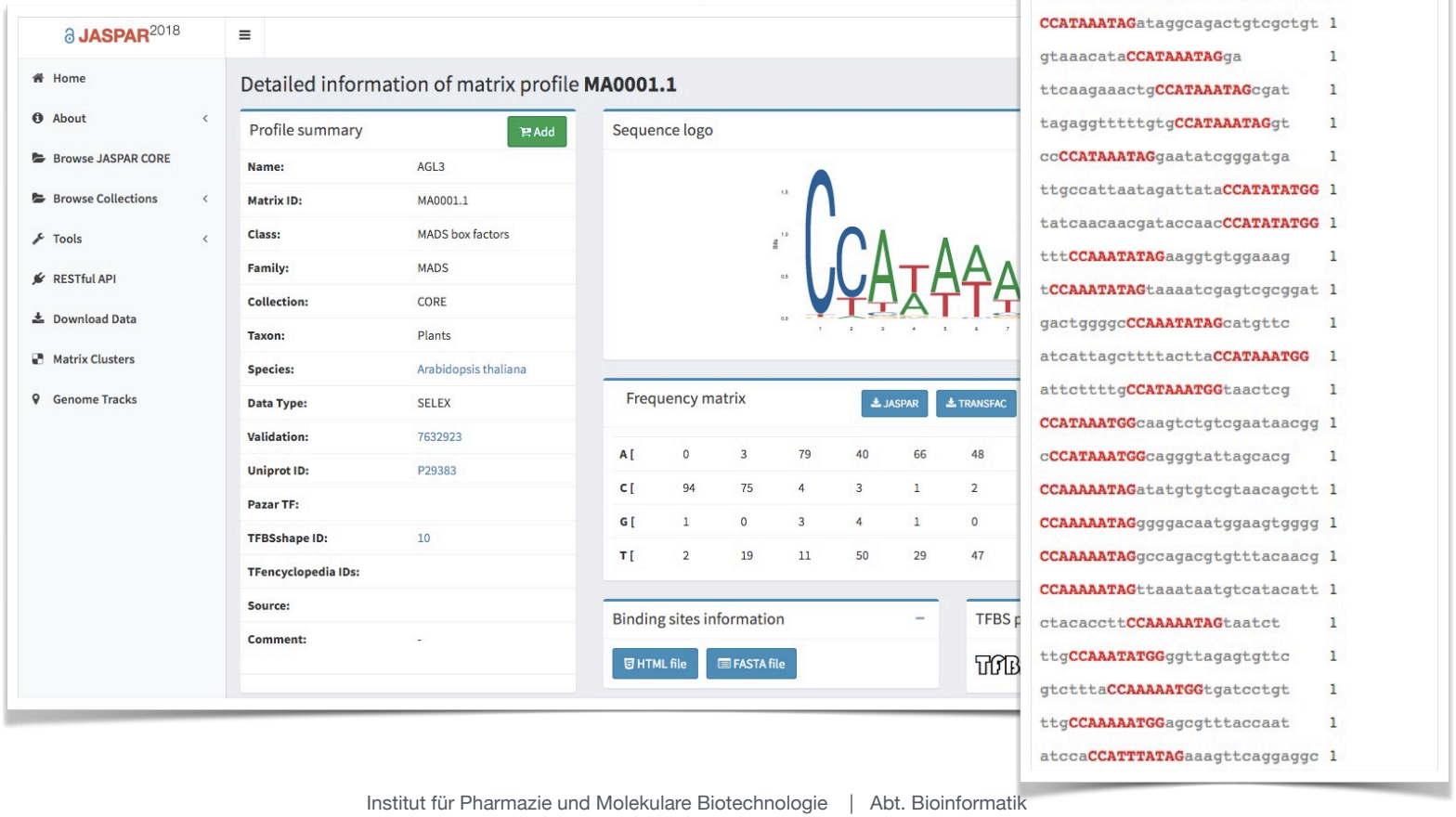
- JASPAR is an open database of binding profiles



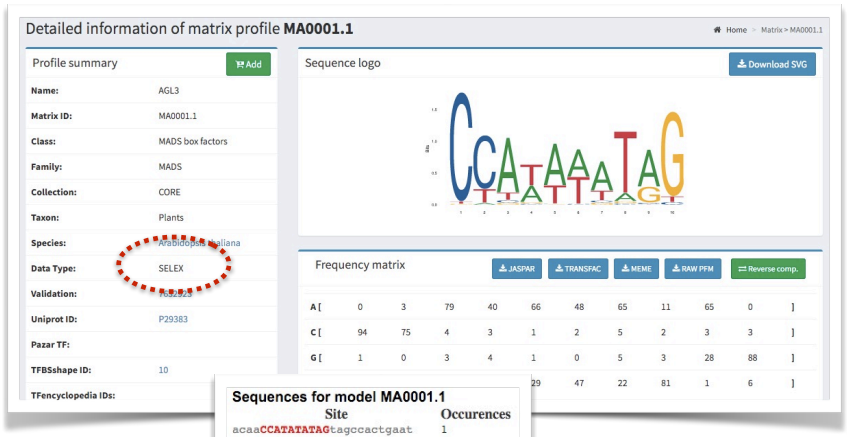
http://jaspar.genereg.net/

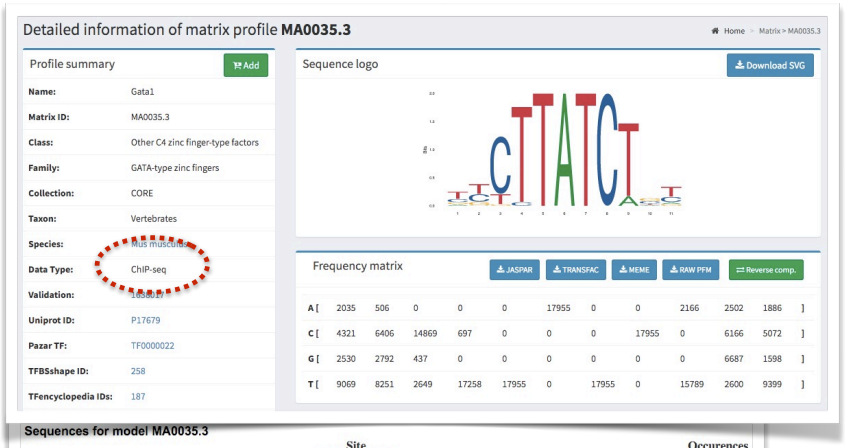
Motif databases

- JASPAR is an open database of binding profiles



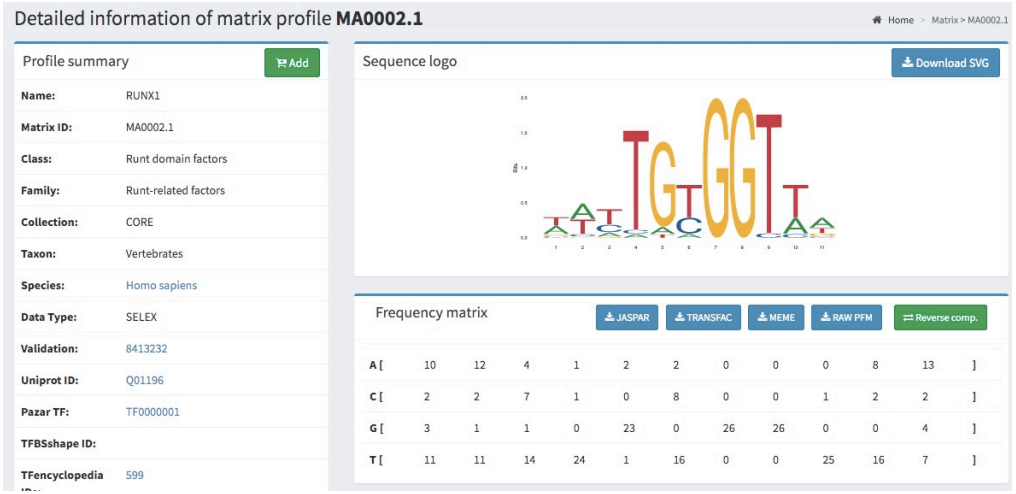
Different sources



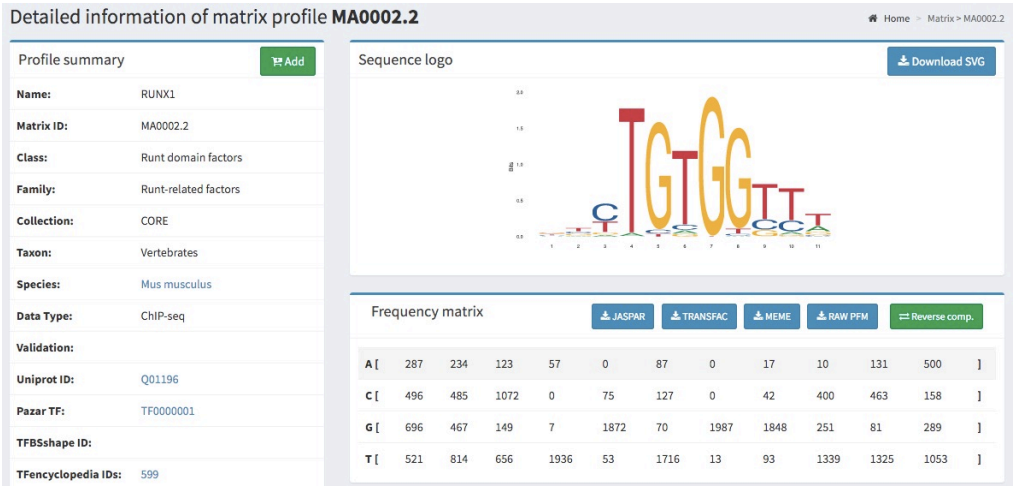


ChIP-seq: real binding site is hidden in much longer sequence
→ lower resolution

Redundant profiles: RUNX1



SELEX
26 binding sites



ChIP-seq
2000 binding sites