Computational Systems Biology
Deep Learning in the Life Sciences

6.802 6.874 20.390 20.490 HST.506

David Gifford

Lecture 8

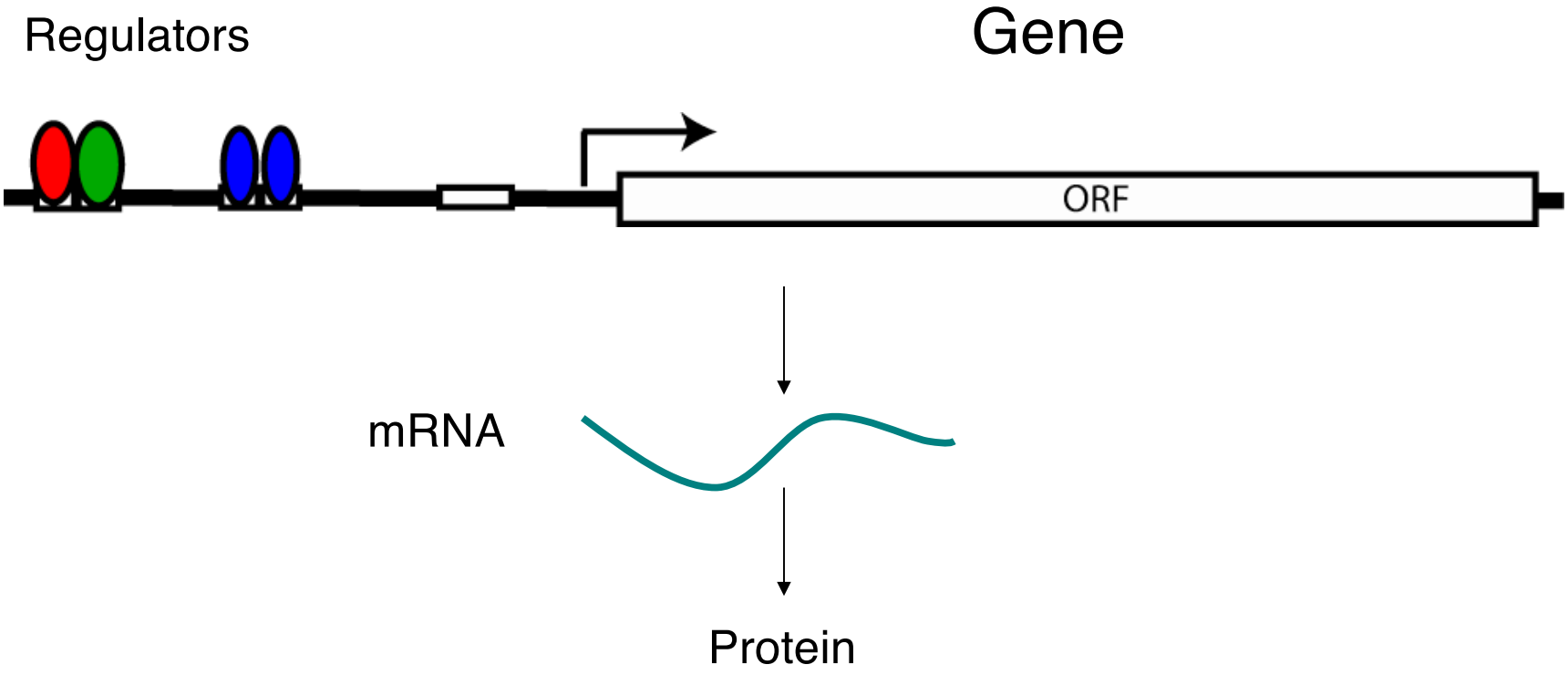
February 27, 2020

Decoding the regulatory genome

http://mit6874.github.io

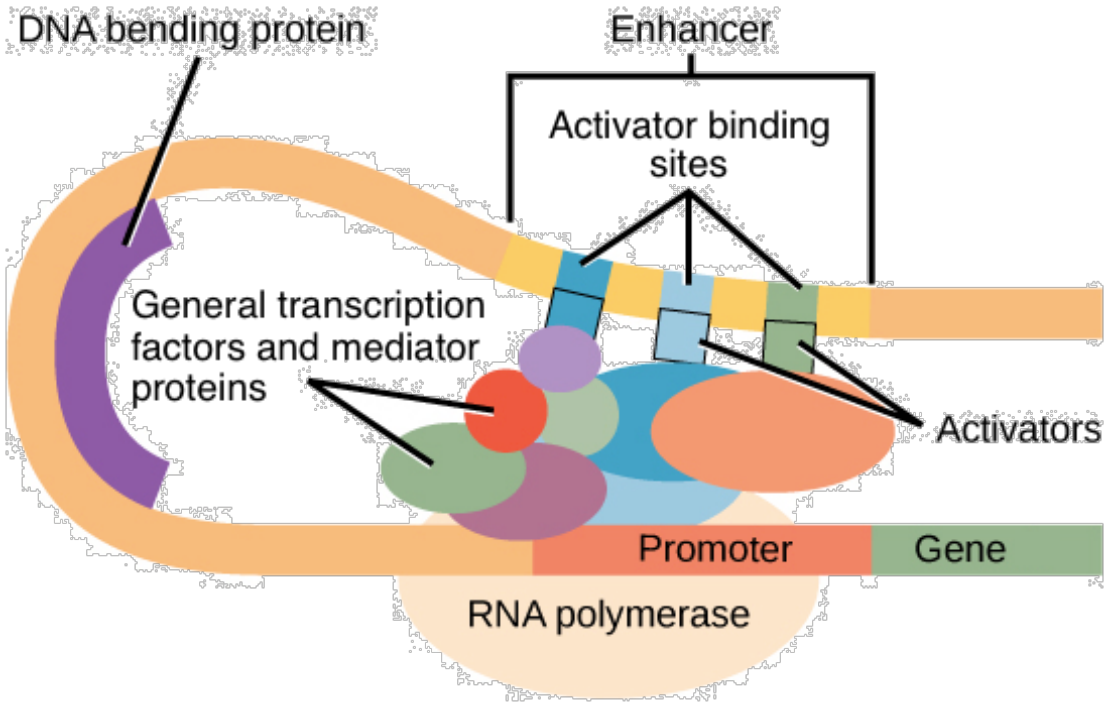
Transcription factors implement genomic regulation

Gene Regulation: DNA > RNA > Protein

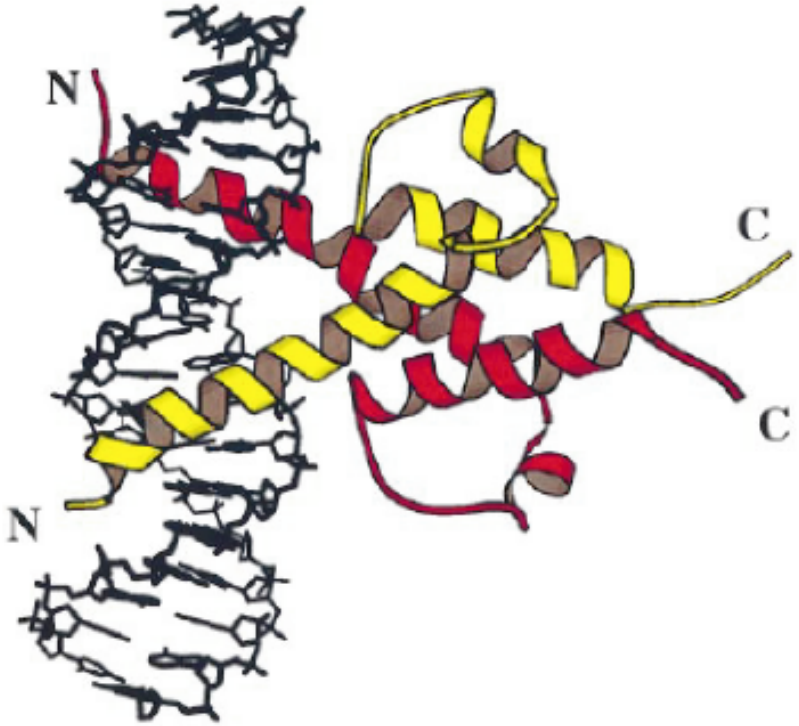


What are the gene regulators that control gene expression?
At what genes do these regulators operate?

DNA-protein binding is essential to cellular function



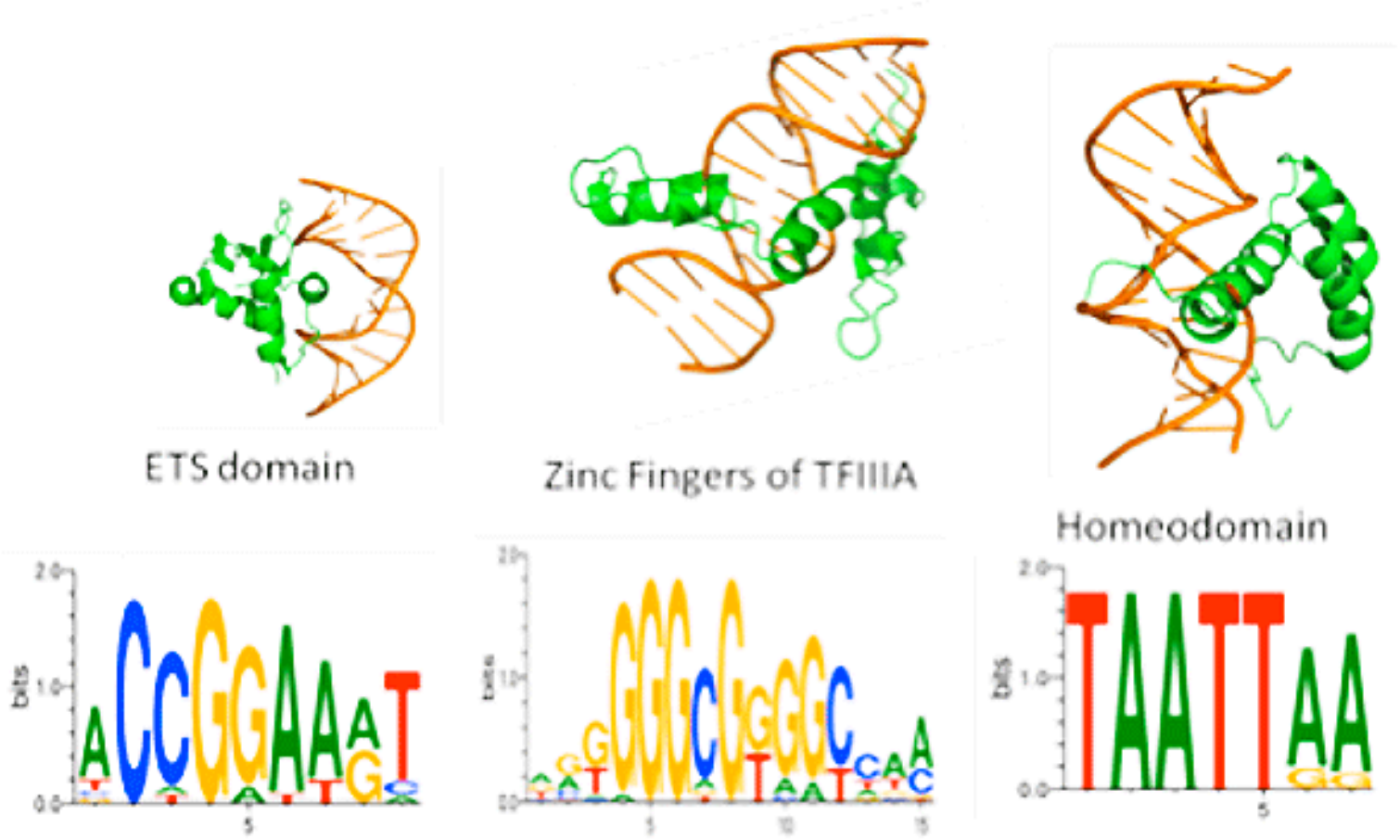
Transcription factors bind specific sequences



Protein molecules that bind to specific DNA sequences and act as molecular switches to turn genes on or off.

Humans have ~2000 transcription factors.

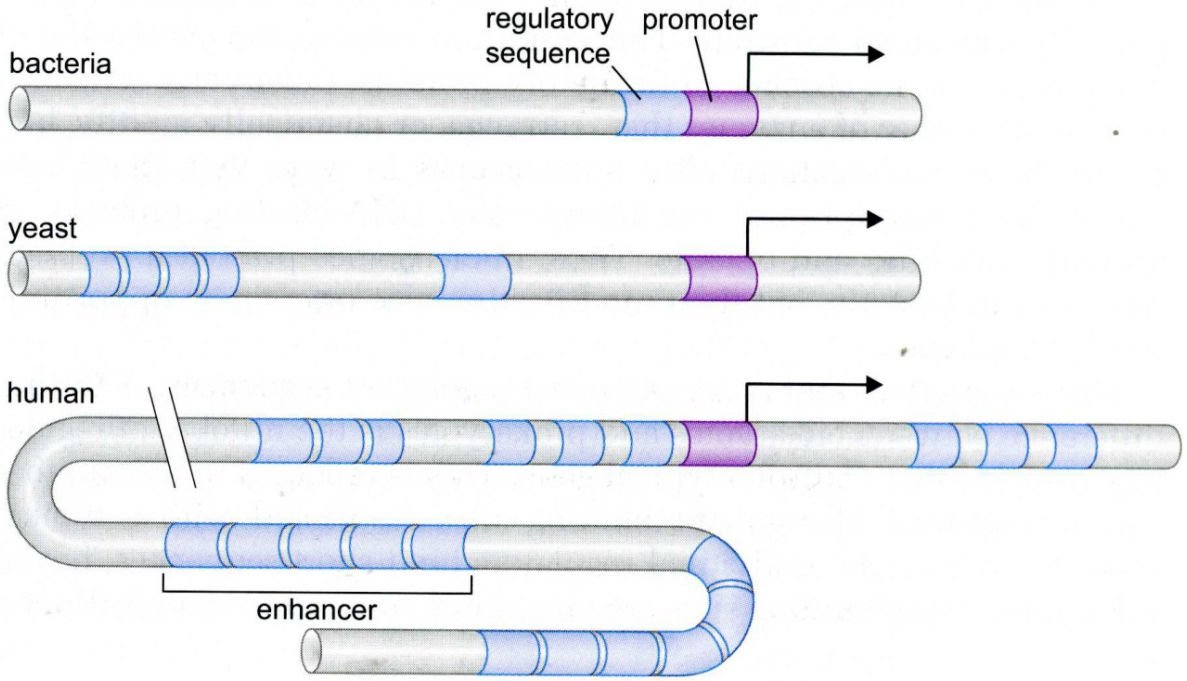
Transcription factors bind specific sequences



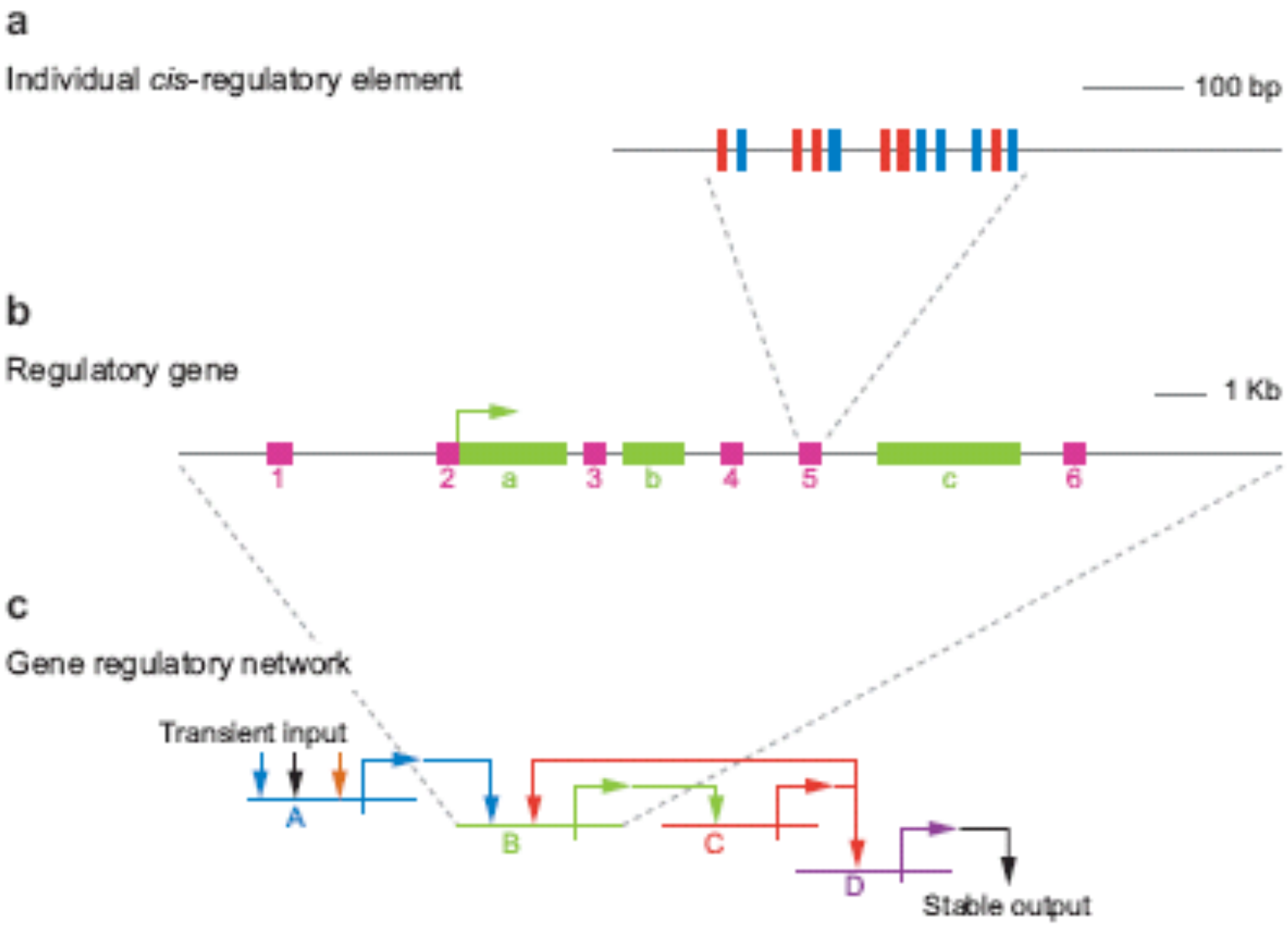
Protein molecules that bind to specific DNA sequences and act as molecular switches to turn genes on or off.

Humans have ~2000 transcription factors.

Combinatorial control lies at the heart of the complexity and diversity of eukaryotes



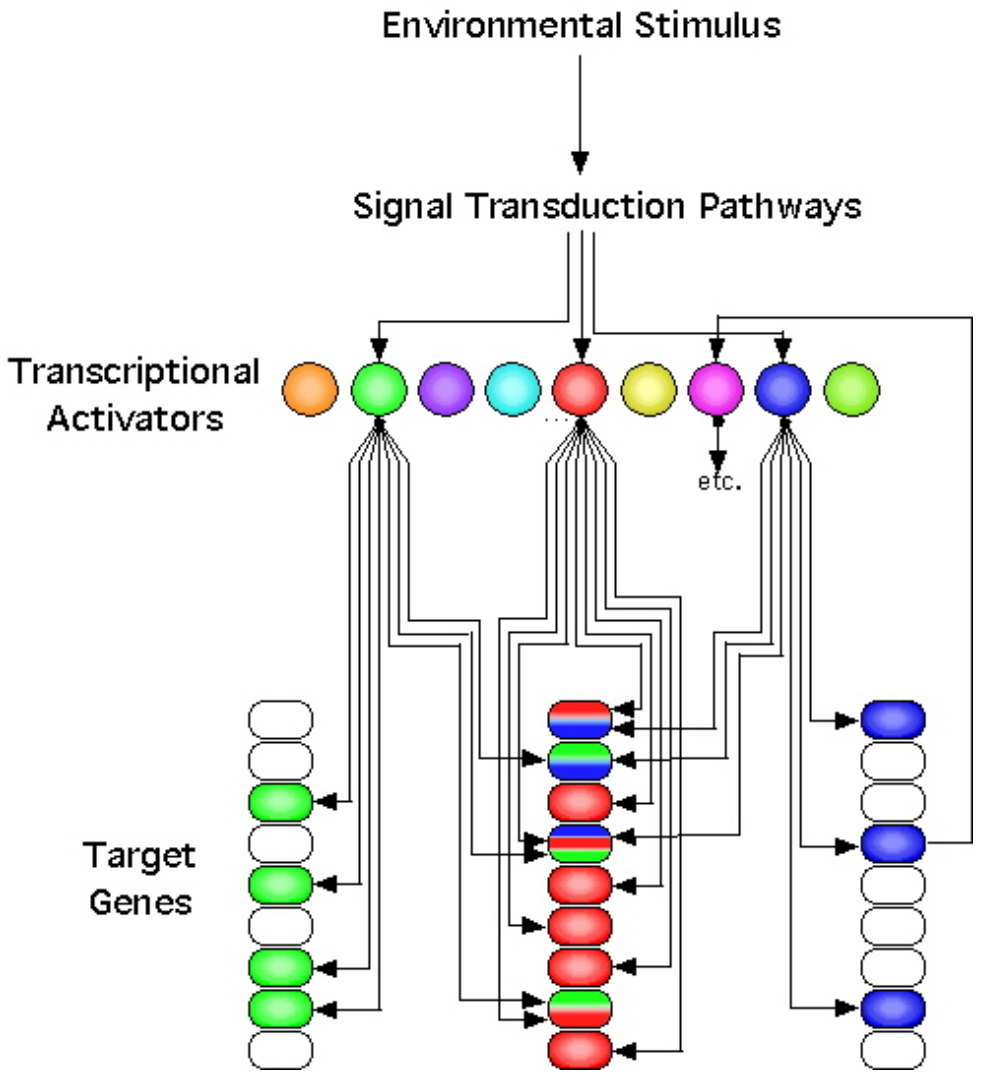
(Molecular biology of the gene, 6ed)



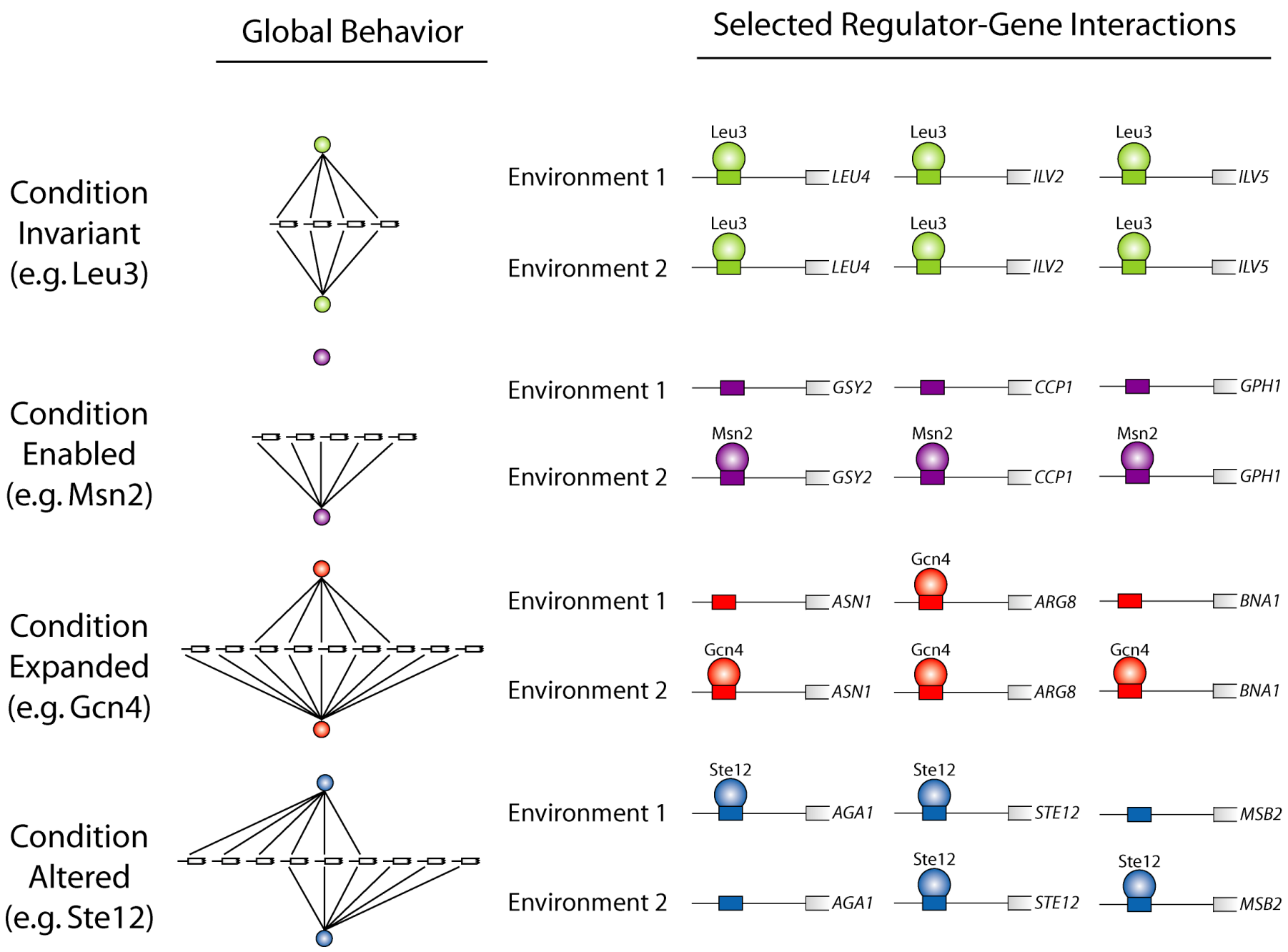
Why Map Transcriptional Regulatory Networks?

Transcriptional regulatory network information will:

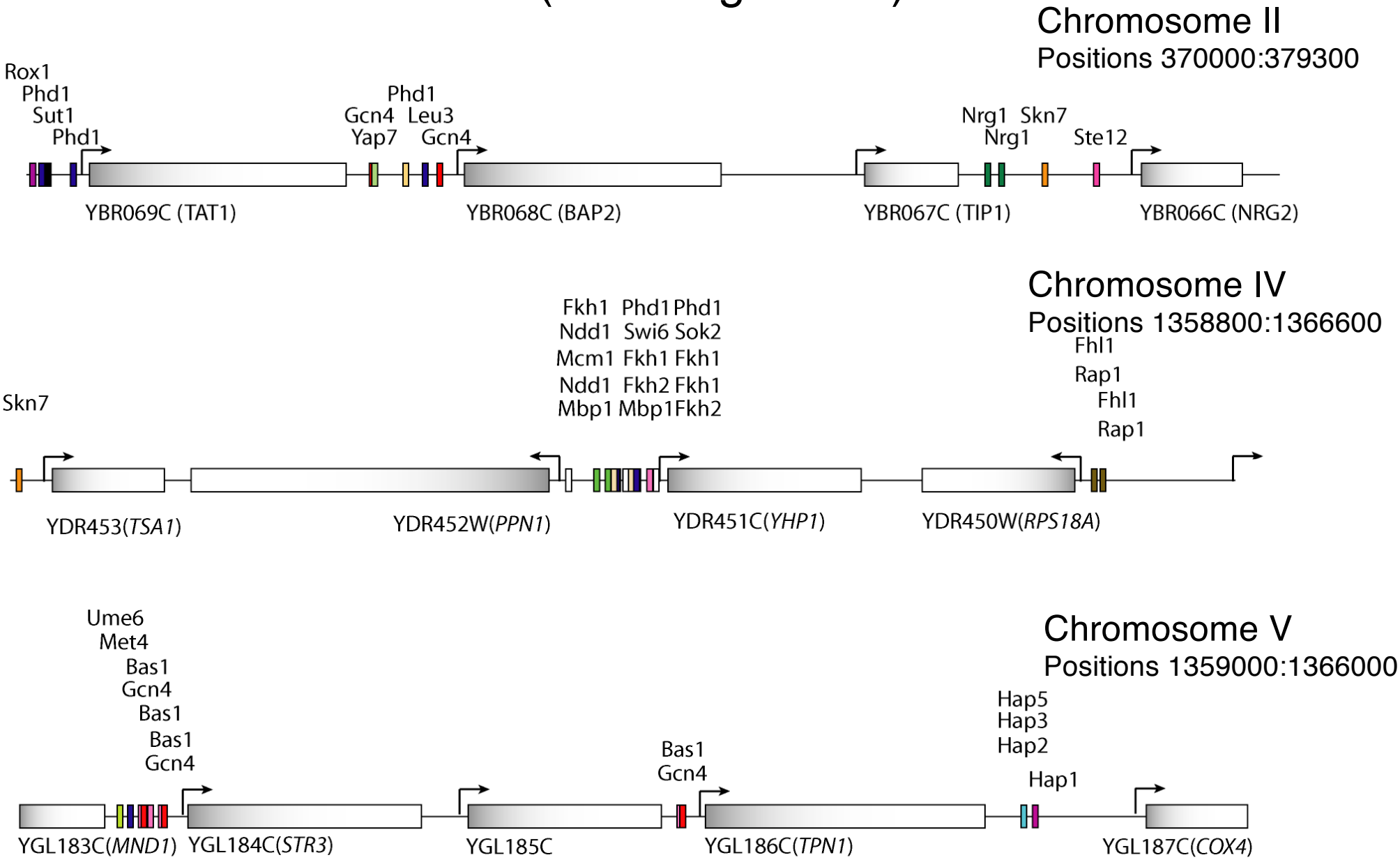
- reveal how cellular processes are connected and coordinated
- suggest new strategies to manipulate phenotypes and combat disease



Environment-Specific Regulator Behaviors



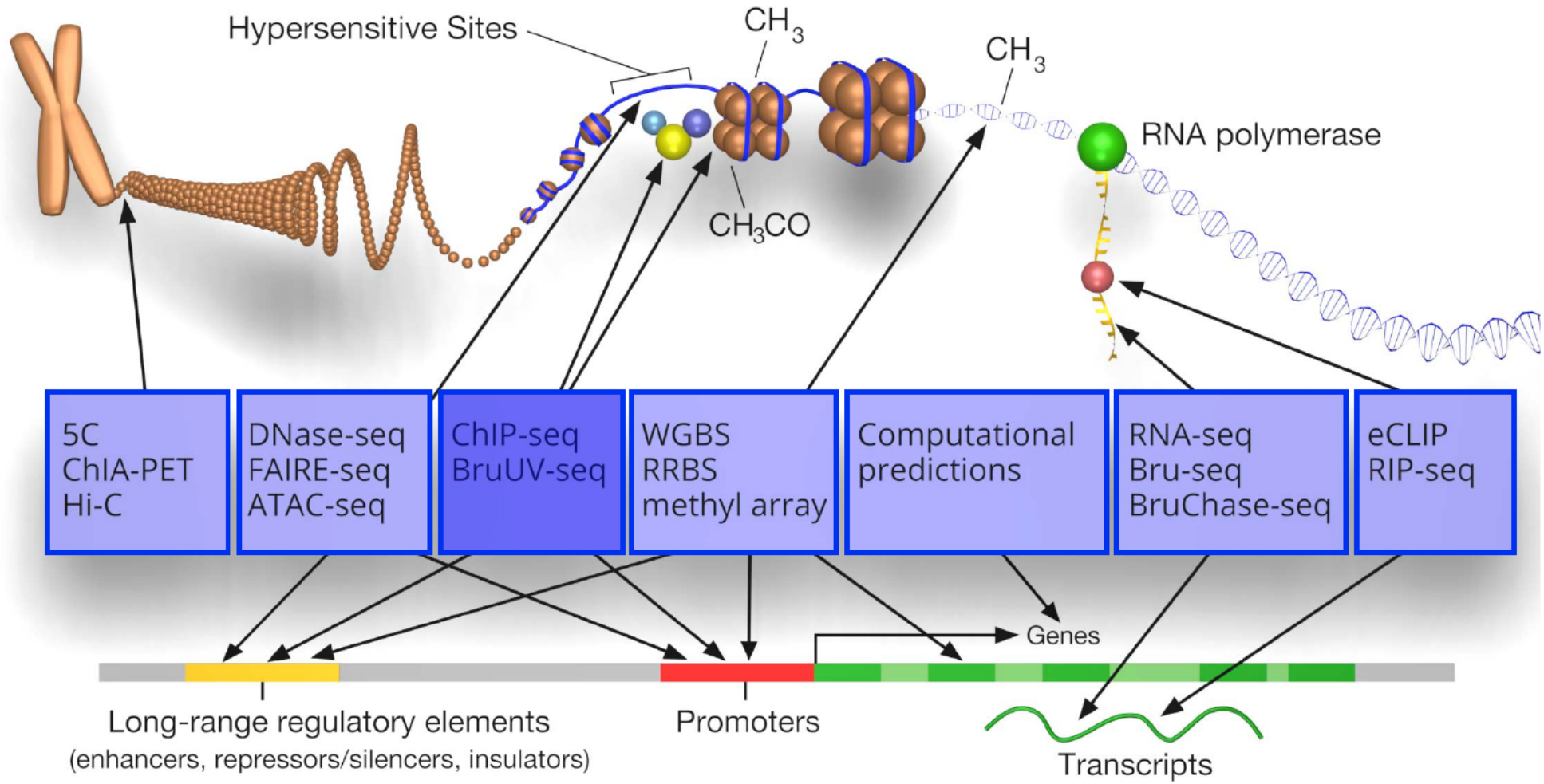
Sample of the Yeast Draft Transcriptional Regulatory Code (~150 regulators)



The ENCODE project

- After the Human Genome Project, ENCODE was established (the DECODE name was taken…)
- The National Institutes of Health (NIH) funded project to characterize the function of the human genome
- ~$400M cost, > 32 labs, > 440 scientists
- Mostly based upon the technologies from Tuesday and Today

ENCODE: Encyclopedia of DNA Elements



ENCODE

Based on an image by Darryl Leja (NHGRI), Ian Dunham (EBI), Michael Pazin (NHGRI)

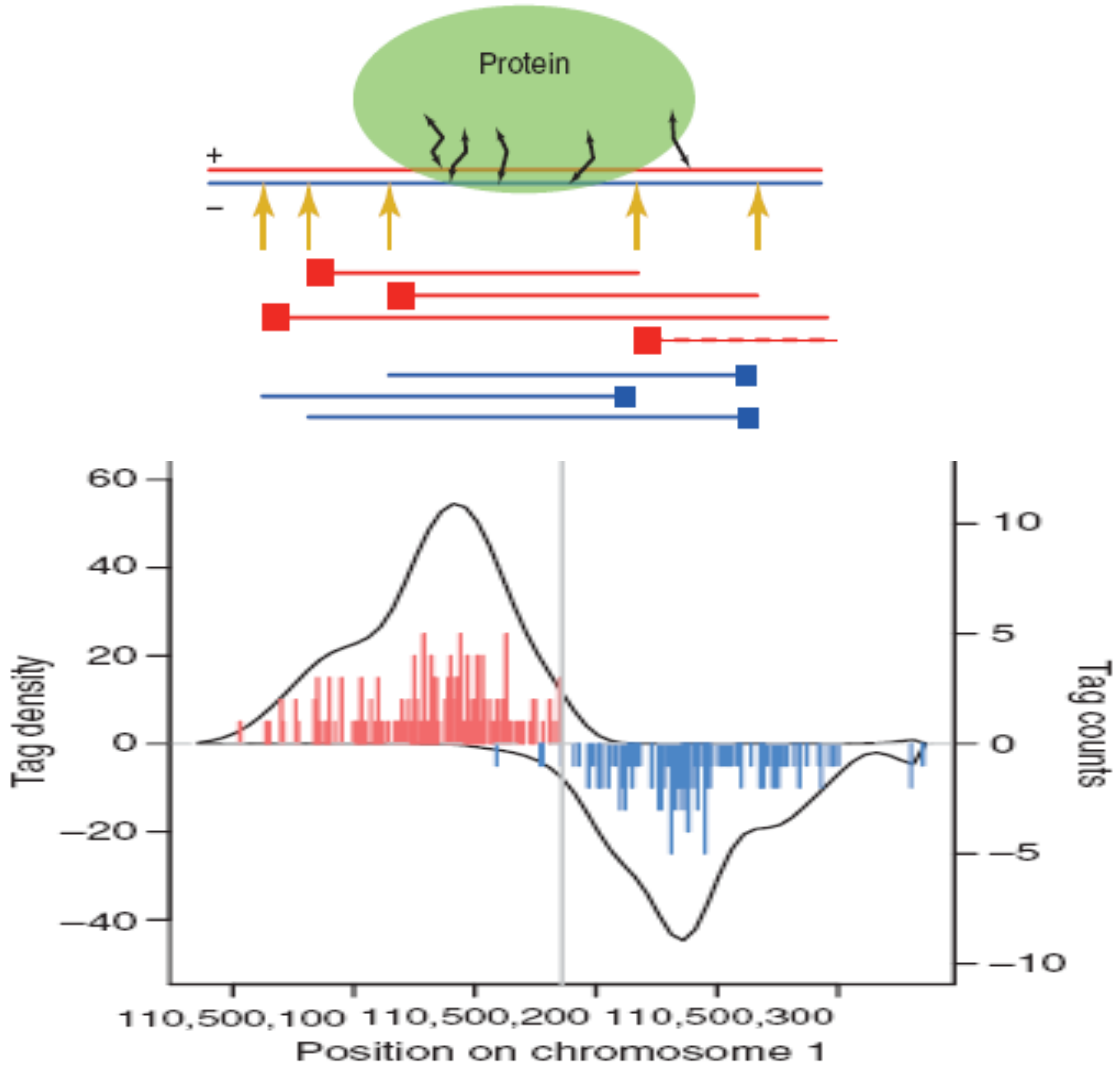
encodeproject.org

Discovering the genomic regulatory code

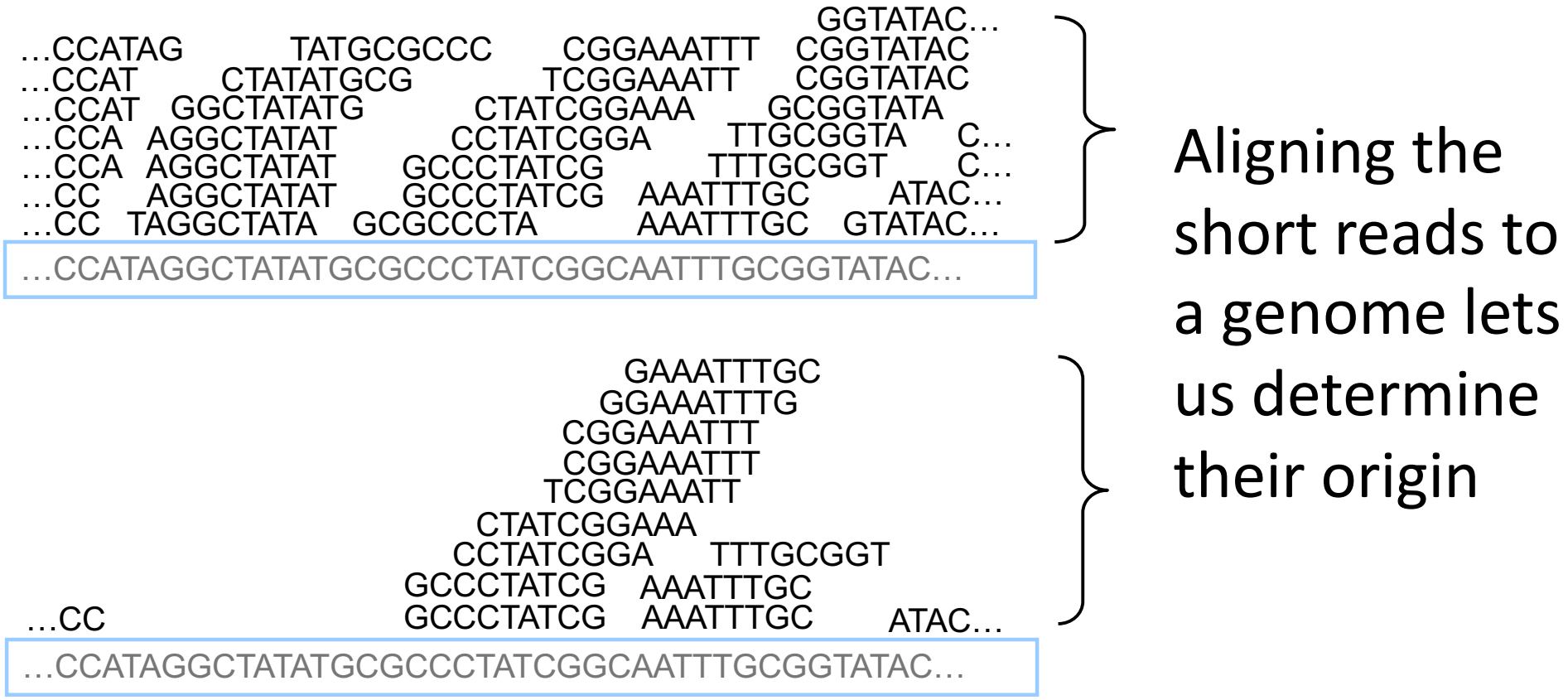
Need you to think critically about claimed results

- How replicable?
- How interpretable?
- What could go wrong?
- At what spatial resolution? (1bp? 50bp? 500bp?)
- How generalizable from “typical best” example to whole genome function

Chromatin Immunoprecipitation (ChIP) sequencing (ChIP-seq) reveals genome-protein interactions



Short Read Mapping

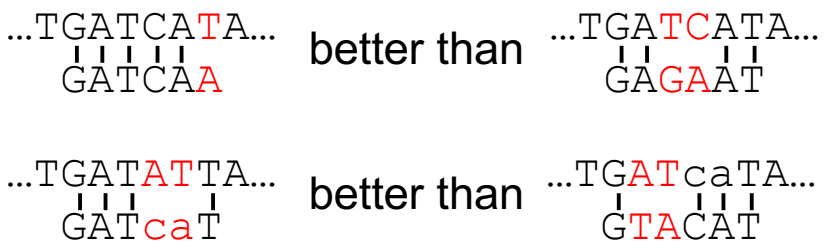


Aligning the short reads to a genome lets us determine their origin

http://www.cbcb.umd.edu/~langmead/NCBI_Nov2008.ppt

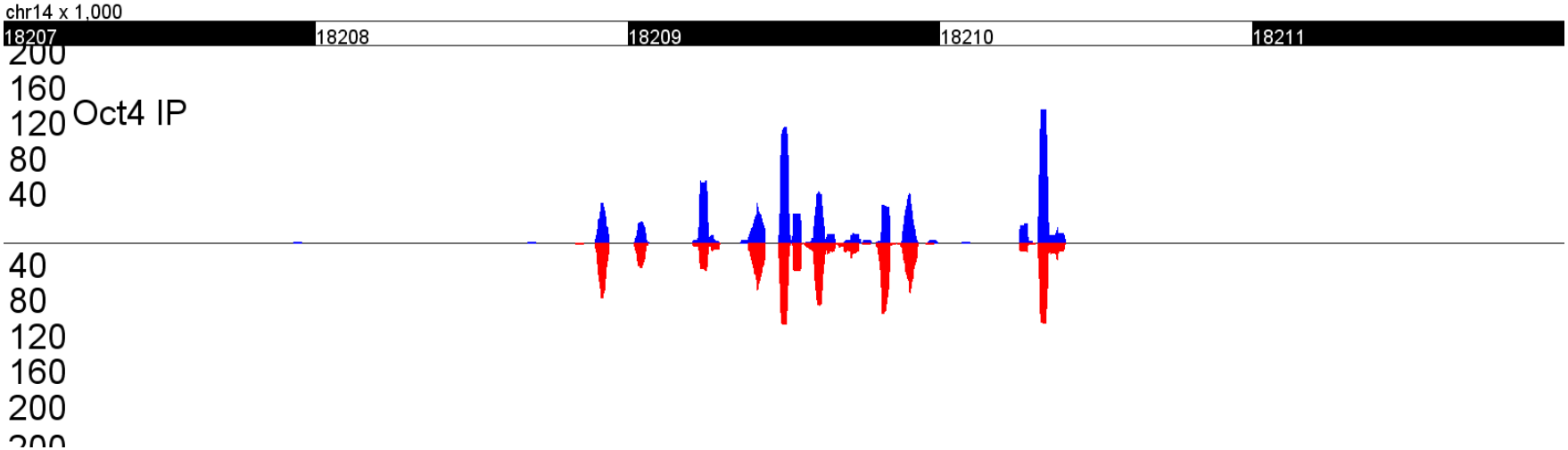
Short Read Alignment Task

- Given a reference and a set of reads, report at least one "good" local alignment for each read to a genome if the alignment exists
- Reads may match in multiple places ("multimapping")
- What is "good"? We concentrate on:
  - Fewer mismatches is better
  - Failing to align a low-quality base is better than failing to align a high-quality base

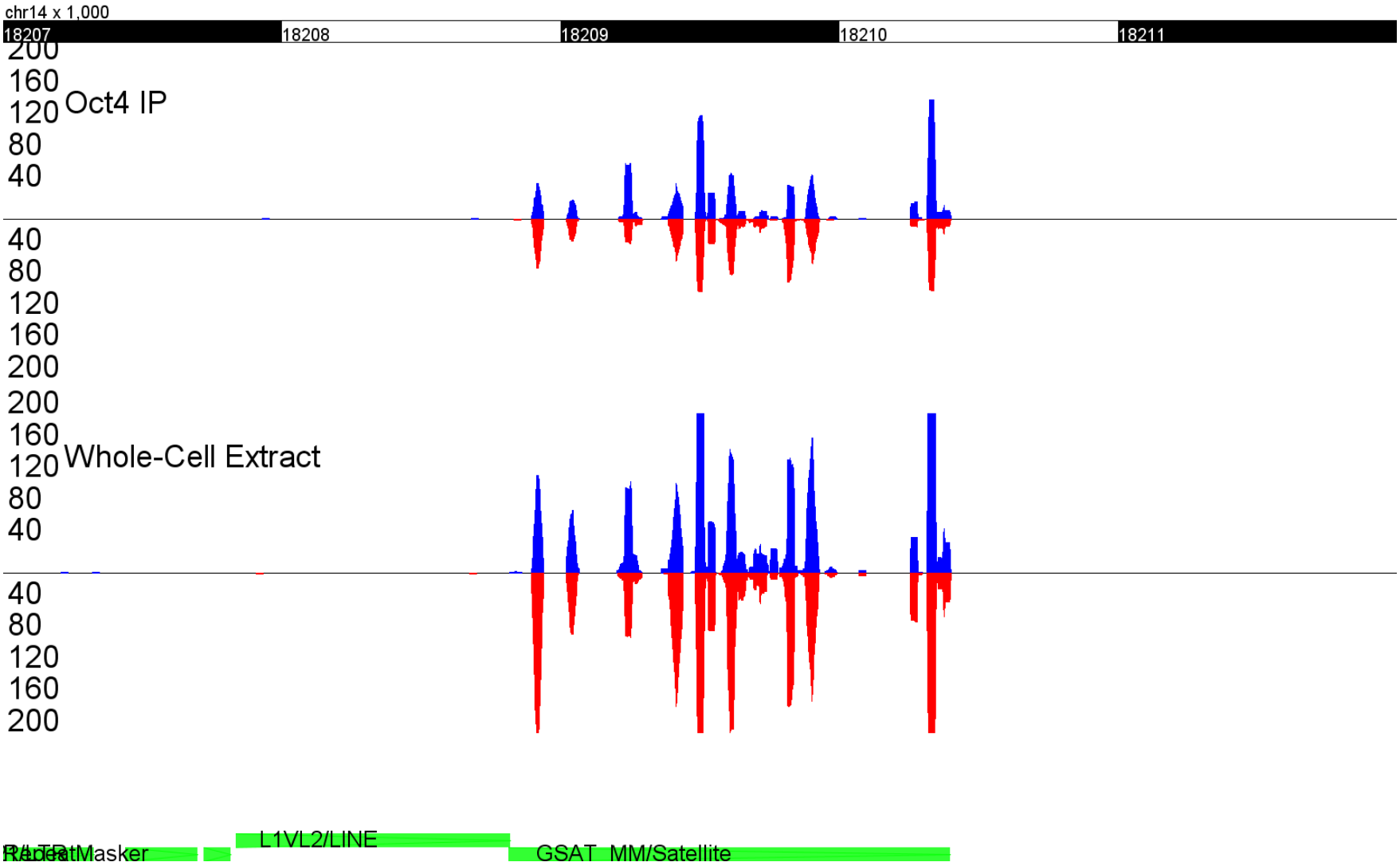


http://www.cbcb.umd.edu/~langmead/NCBI_Nov2008.ppt

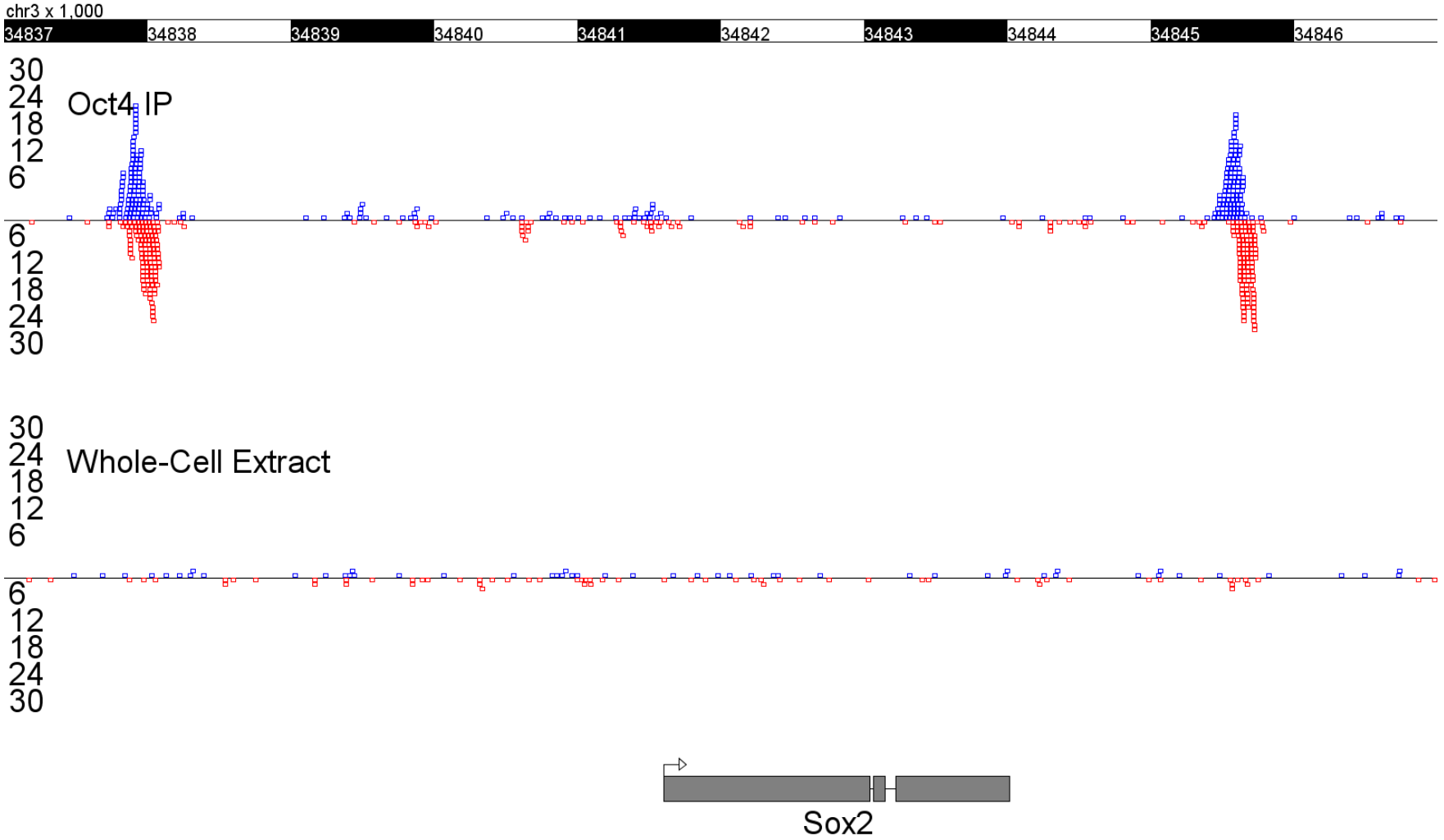
Where are the Oct4 binding events in these data?
(x axis genome location, y axis read count observed)
(black bar = 1000bp)



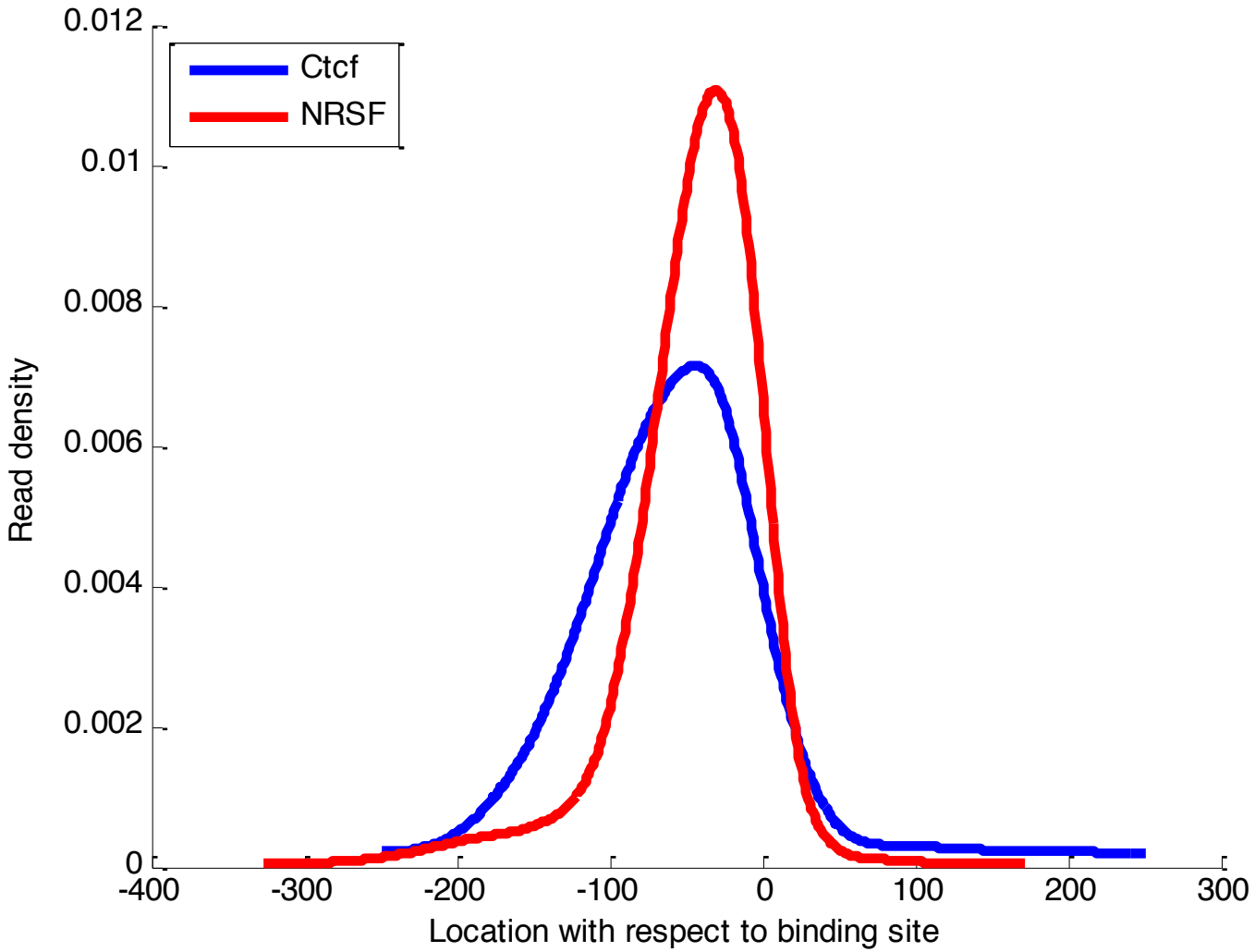
Repetitive “blacklisted” regions are typically not considered and are gaps in our knowledge of genomic function



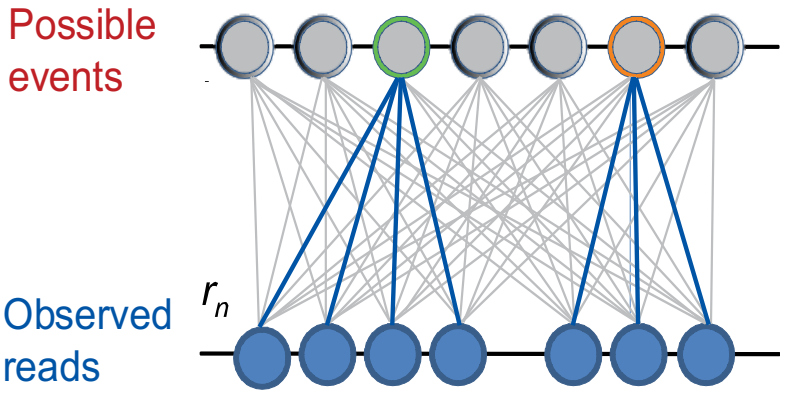
mES cell Oct4 ChIP Seq displays distinct binding events



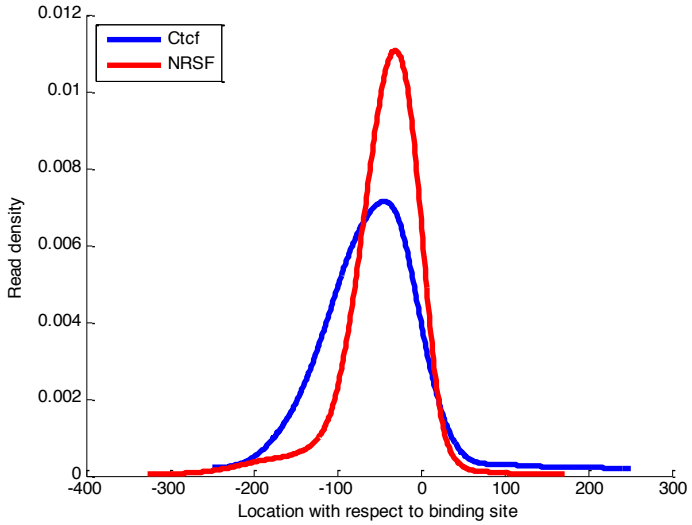
The read spatial distribution can be learned



A mixture model can describe which genomic locations are bound



Mixture model

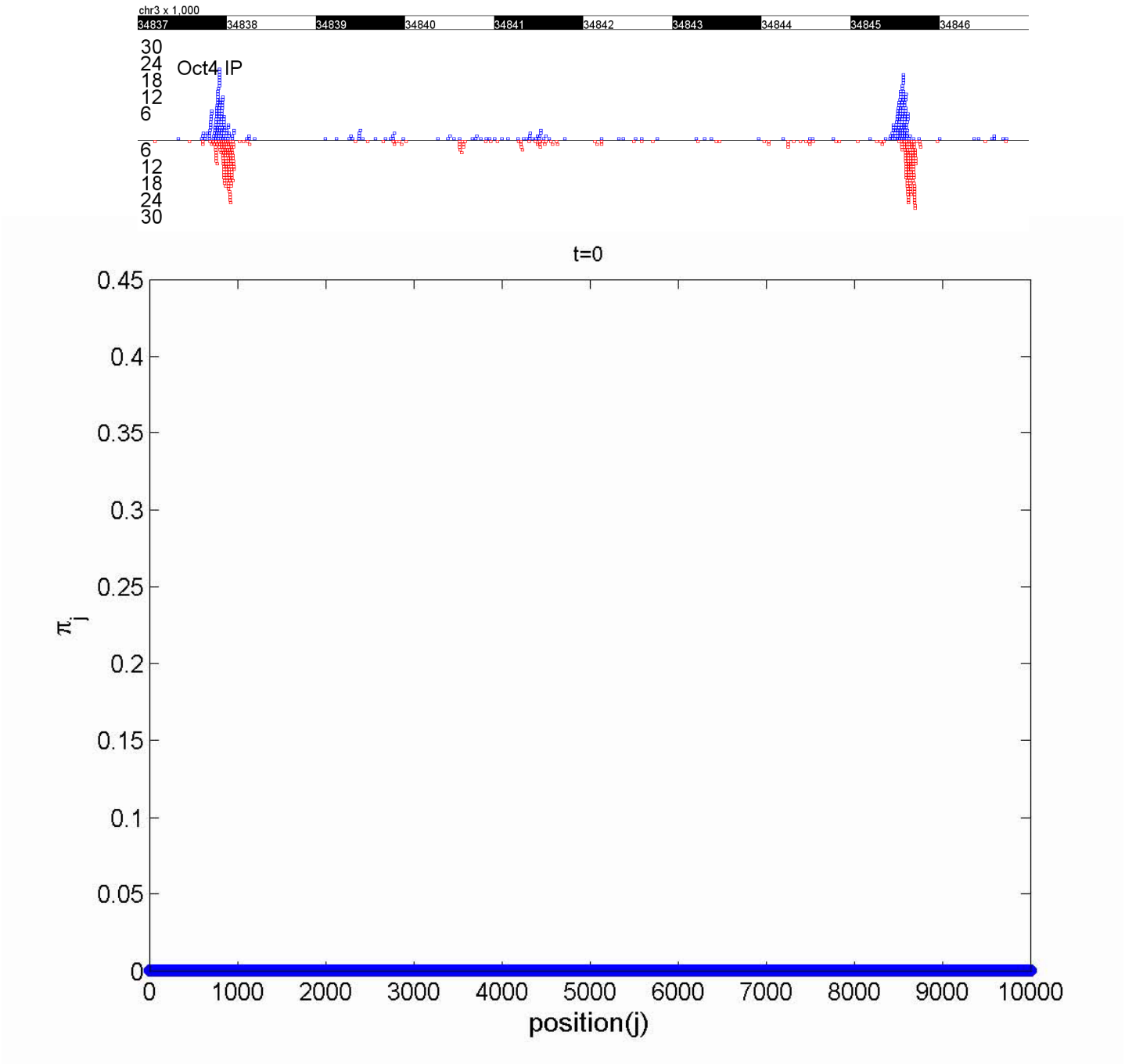


p(r_n | m offset)

$$p(R|\pi) = \prod_{n=1}^{N} \sum_{m=1}^{M} \pi_m p(r_n|m)$$

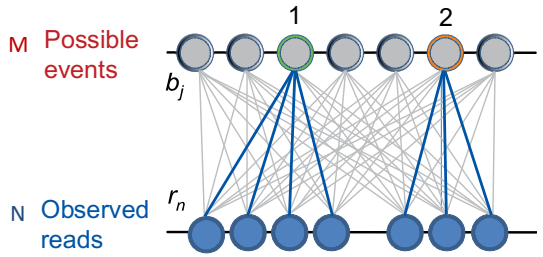
$$\sum_{m=1}^{M} \pi_m = 1$$

EM –
no prior


chr3 x 1,000
34837
34838
34839
34840
34841
34842
34843
34844
34845
34846
Oct4 IP
30
24
18
12
6
6
12
18
24
30
t=0
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
π_j
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
10000
position(j)

Motif-based positional prior biases the binding event prediction

Mixture model



$$p(R \mid \pi) = \prod_{n=1}^{N} \sum_{m=1}^{M} \pi_m p(r_n \mid j), \quad \sum_{m=1}^{M} \pi_m = 1$$

Position specific priors

- Events are sparse
- Events occurs more likely at motif positions

$$p(\pi) \propto \prod_{m=1}^{M} (\pi_m)^{-\alpha_s + \alpha_m}$$

α_s : uniform sparse prior parameter governing the degree of sparseness, $\alpha_s > 0$;
α_m : position specific motif-based prior

Mixture model of protein-genome interaction

- π_m is fraction of all reads N produced by location m
- M is the total number of locations
- $\sum_{m=1}^{M} \pi_m = 1$
- γ_{mn} is fractional responsibility of location m for read n
- N_m is number of reads produced by location m
- $p(r_n|m)$ is probability location m produced read r_n using our shear distribution

Mixture model of protein-genome interaction

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E Step (Step 1)

$$\gamma_{mn} = \frac{\pi_m p(r_n|m)}{\sum_{j=1}^{M} \pi_j p(r_n|j)}$$

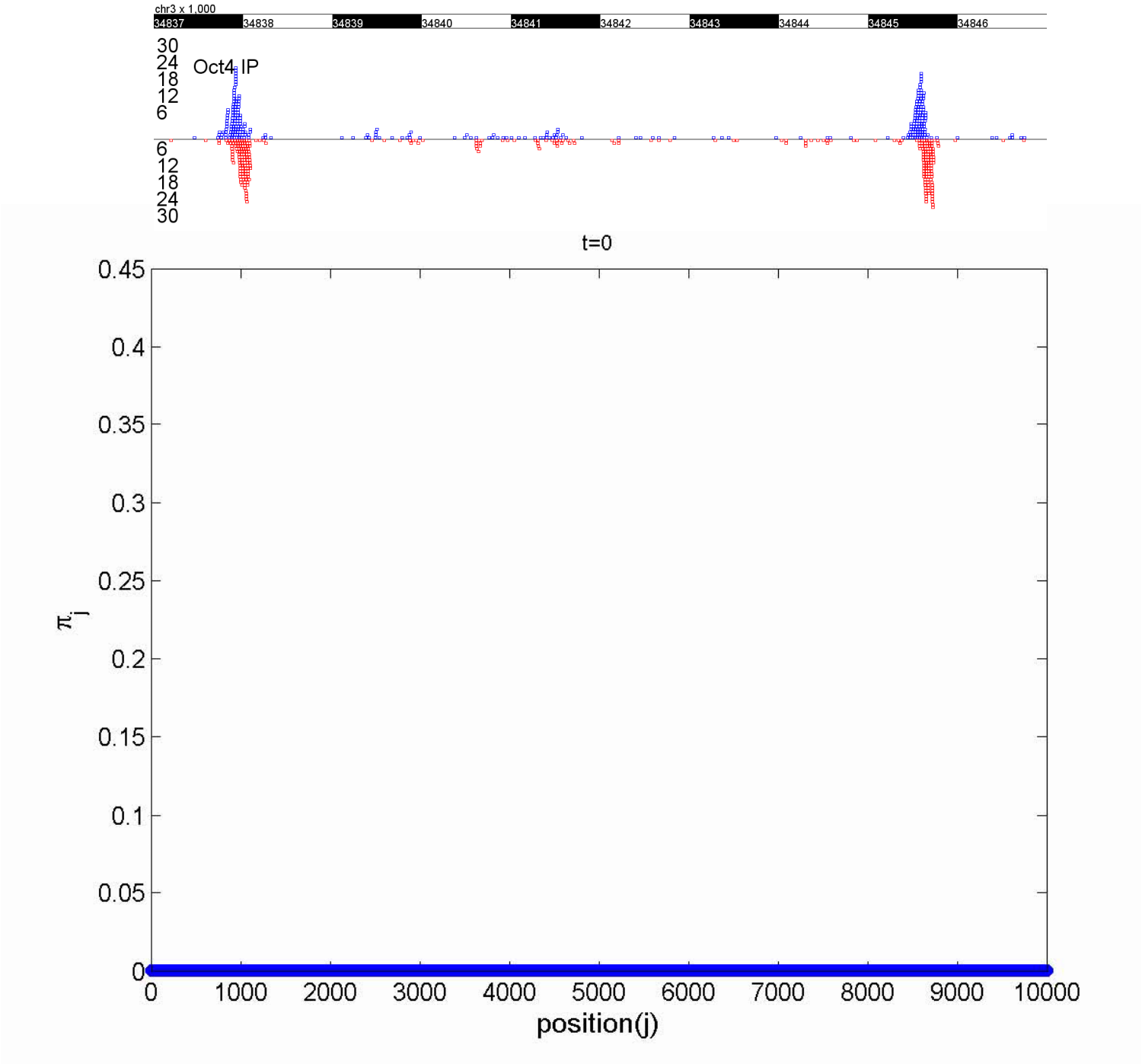
M Step (Step 2, then iterate)

$$N_m = \sum_{n=1}^{N} \gamma_{mn}$$

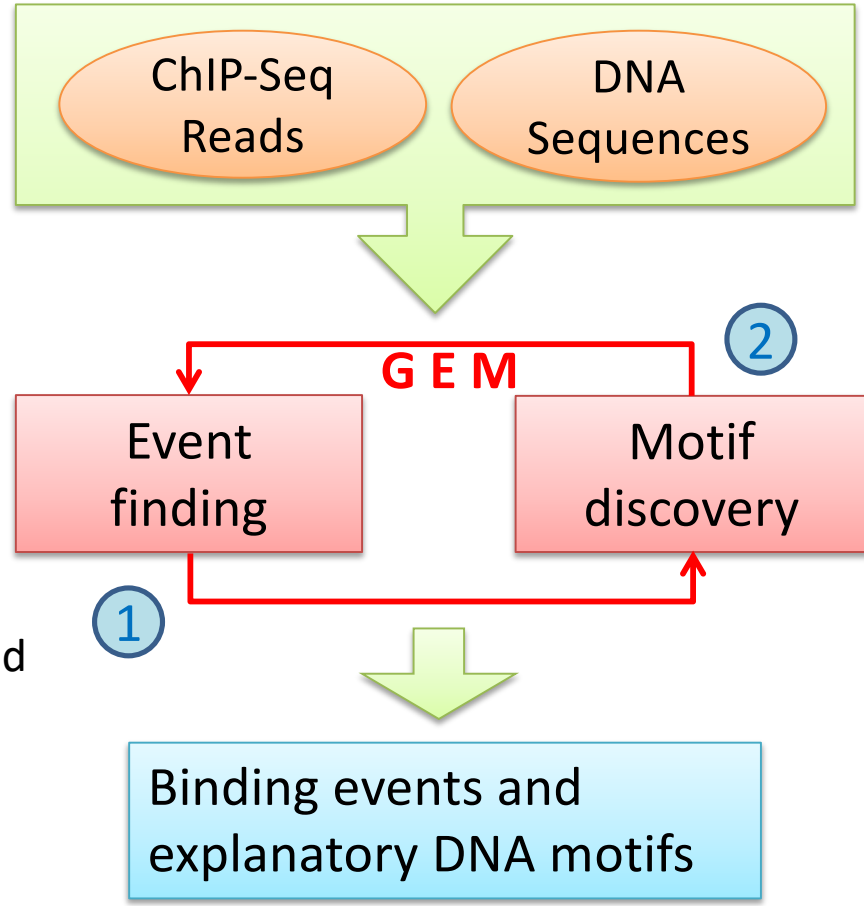
$$\pi_m = \frac{max(0, N_m - \alpha_s + \alpha_m)}{\sum_{j=1}^{M} max(0, N_j - \alpha_s + \alpha_m)}$$

max performs component elimination to simplify solution

EM – Sparse prior



Genome-wide Event finding and Motif discovery



Sequence logos describe what is bound

$S_{b,i} =$

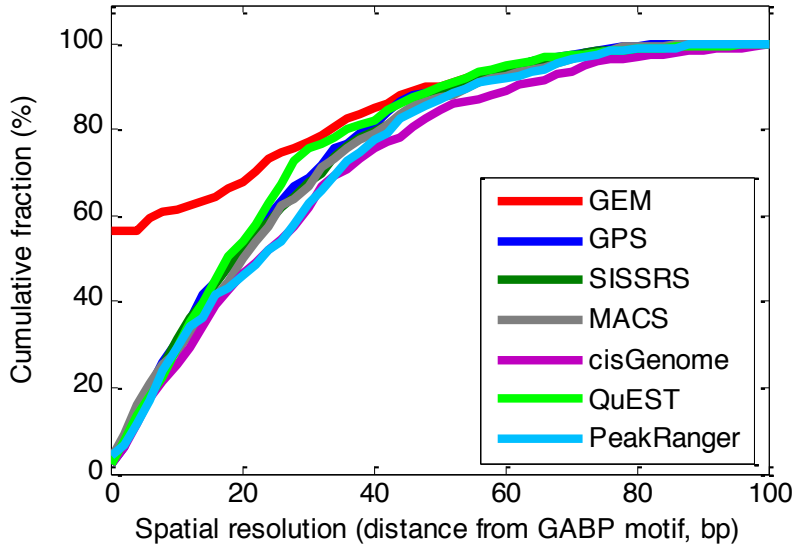
Logo height of base b at position i

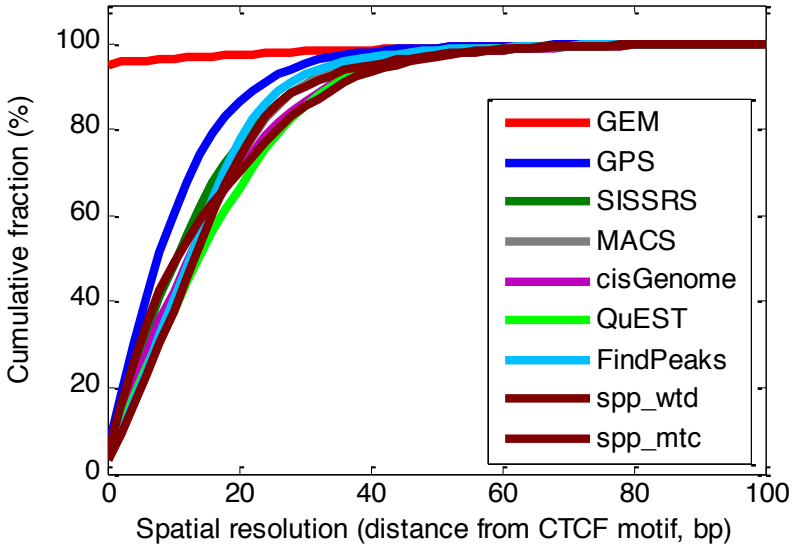
$f_{b,i} =$ Fraction of base b at position i

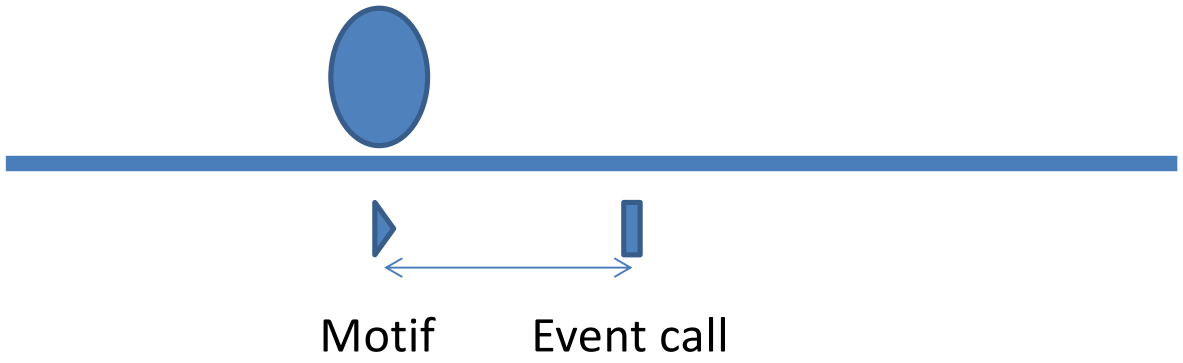
$$I_i = 2 + \sum_{b \in \{A,C,G,T\}} f_{b,i} log_2 f_{b,i}$$

$$S_{b,i} = f_{b,i}\, I_i$$

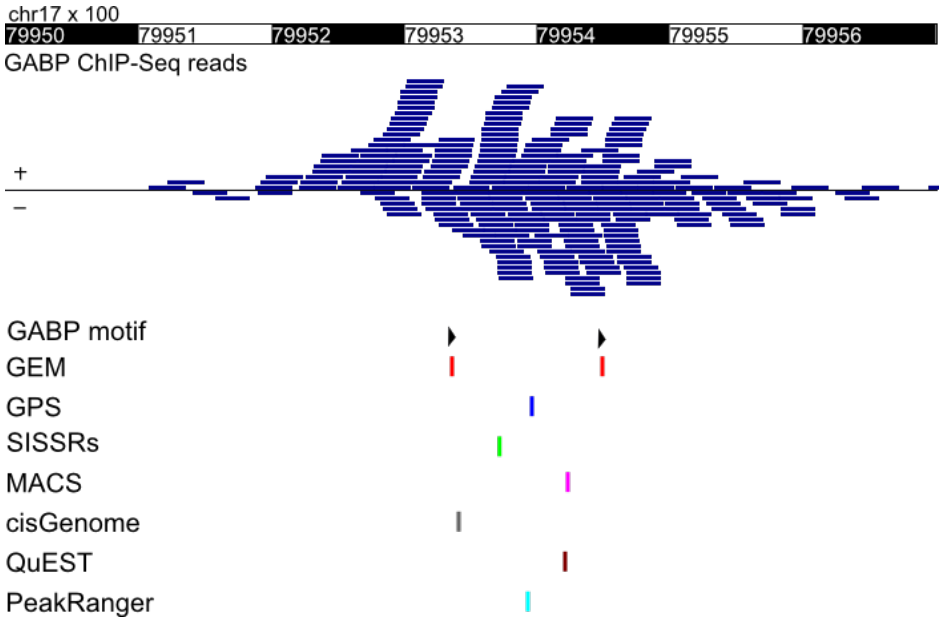
GEM improves spatial accuracy in binding event prediction

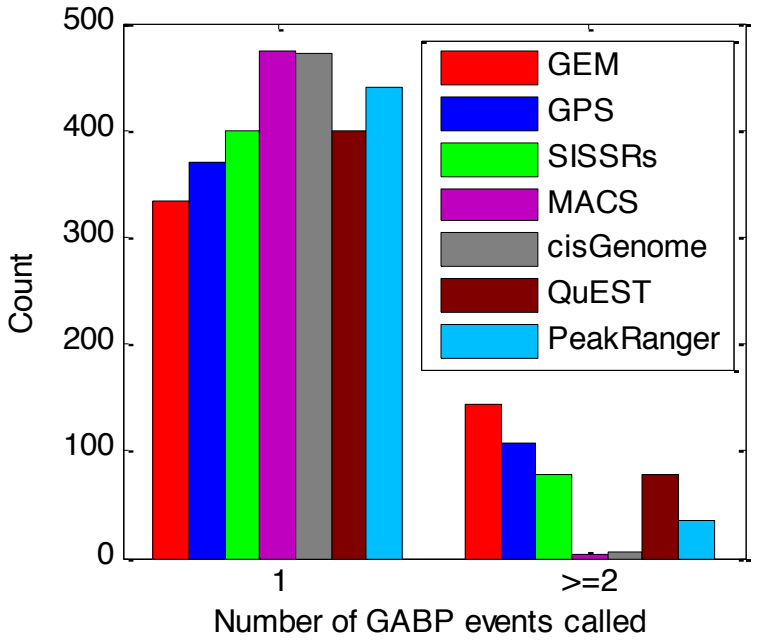


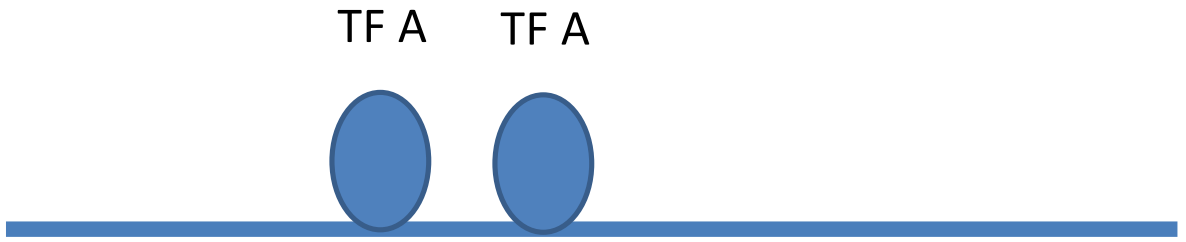




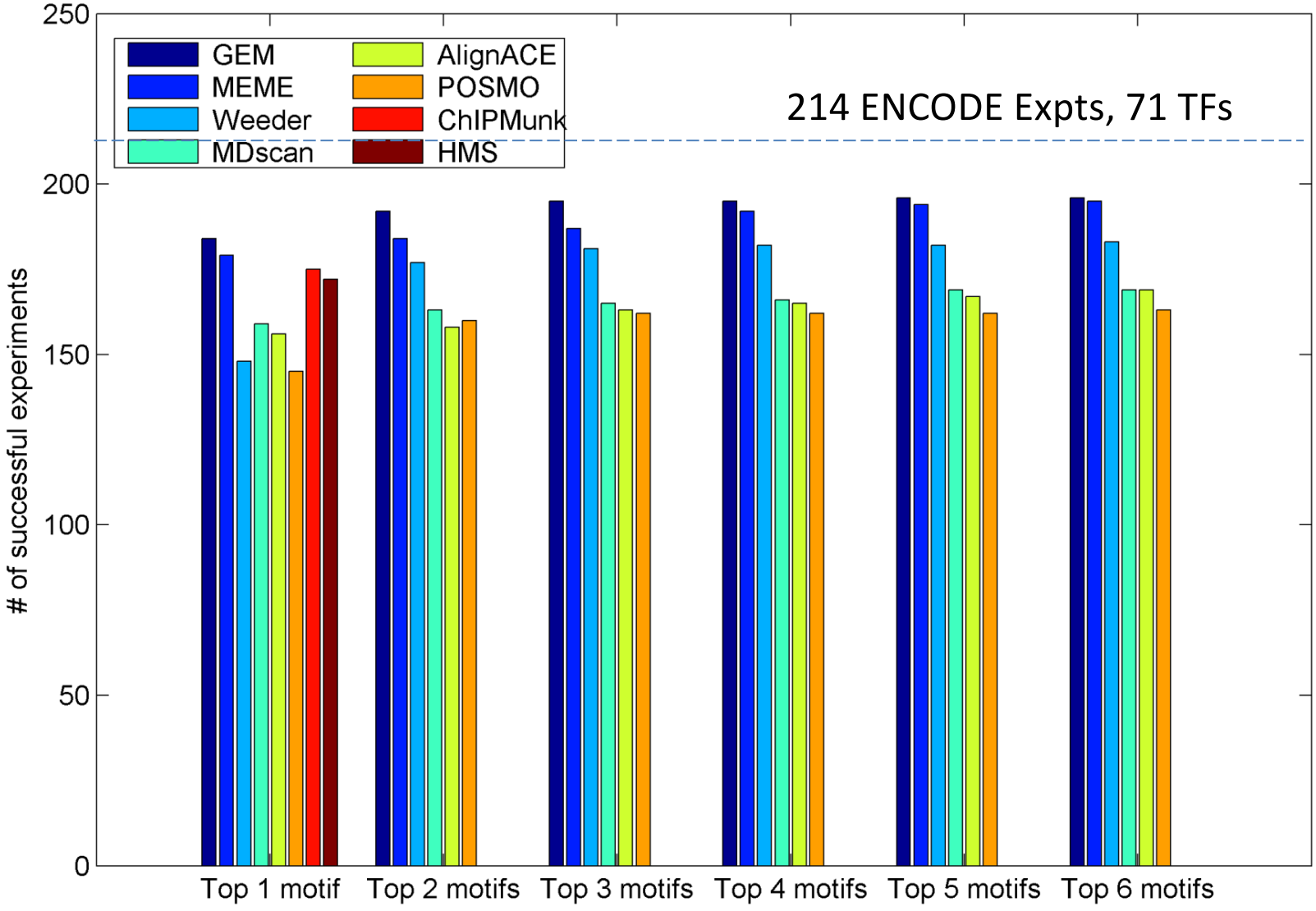
GEM improves the spatial accuracy in resolving proximal binding events.





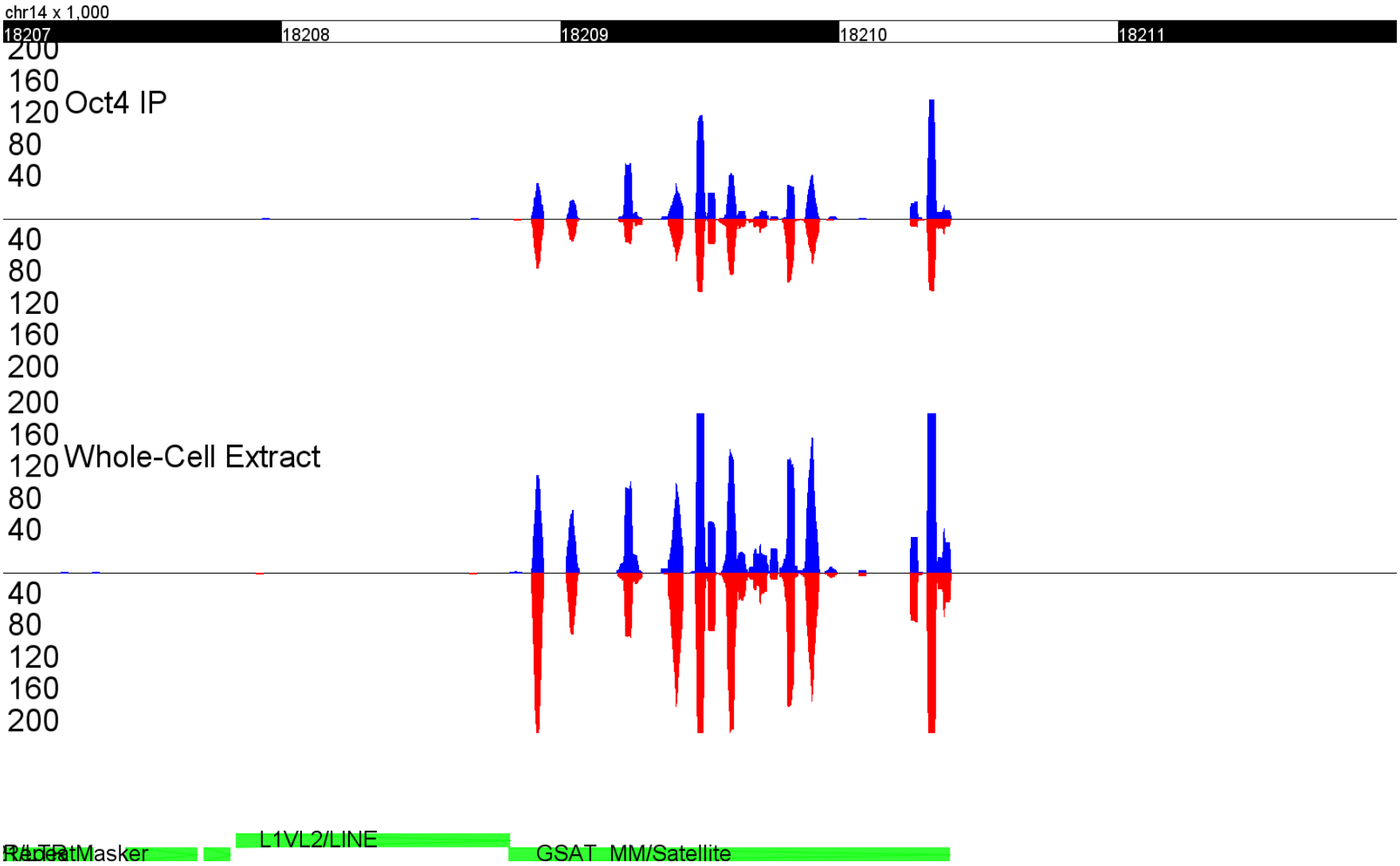


GEM motif discovery outperforms other methods when detecting motifs in ChIP-Seq data



What discovered genome binding events are significant?

How can we compute the significance of an event in the context of background noise?

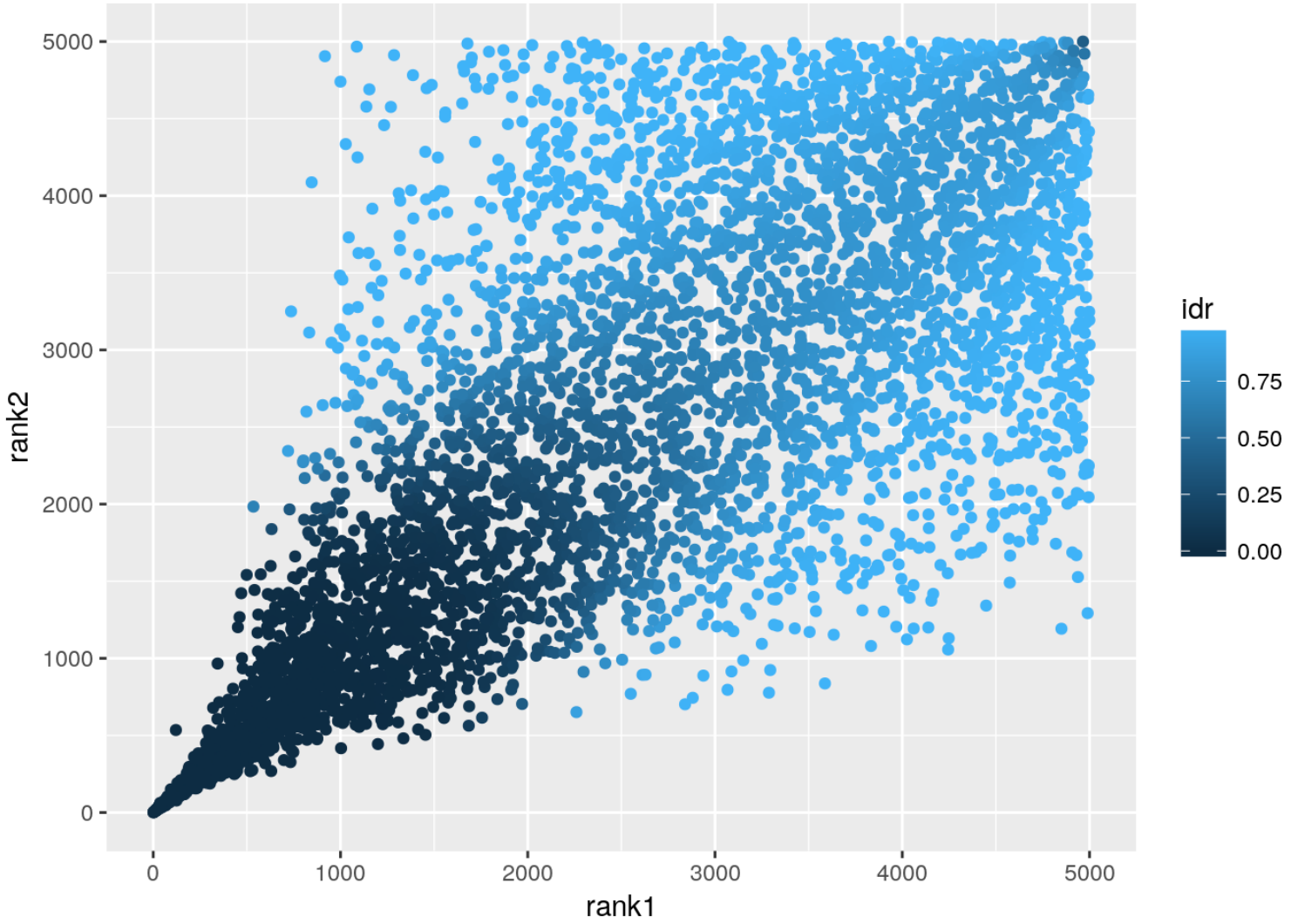


Computing the significance of an event

- N_e is the number of reads assigned to an event
- N_c is the scaled number of reads in the same genomic region in the control channel
- $N = N_e + N_c$
- For our null we use the Cumulative Distribution Function for the Binomial distribution with $P = 0.5$ This assumes that reads go to either the IP or control channel with equal probability
- We will need to do multiple hypothesis correction on the total number of events found using the Benjamani-Hochberg FDR method (Lecture 1)

$$p_{value} = \sum_{i=0}^{N_c} \binom{N}{i} P^i (1-P)^{N-i}$$

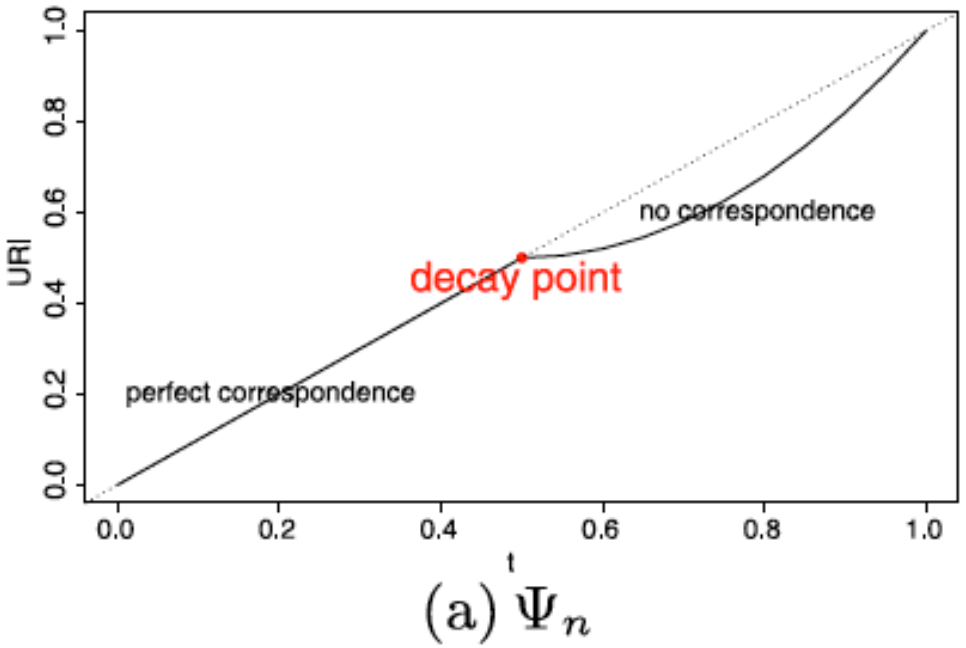
The Irreproducible Data Rate (IDR) identifies significant events by consistent replicate ranks



https://biodatascience.github.io/compbio/test/IDR.html

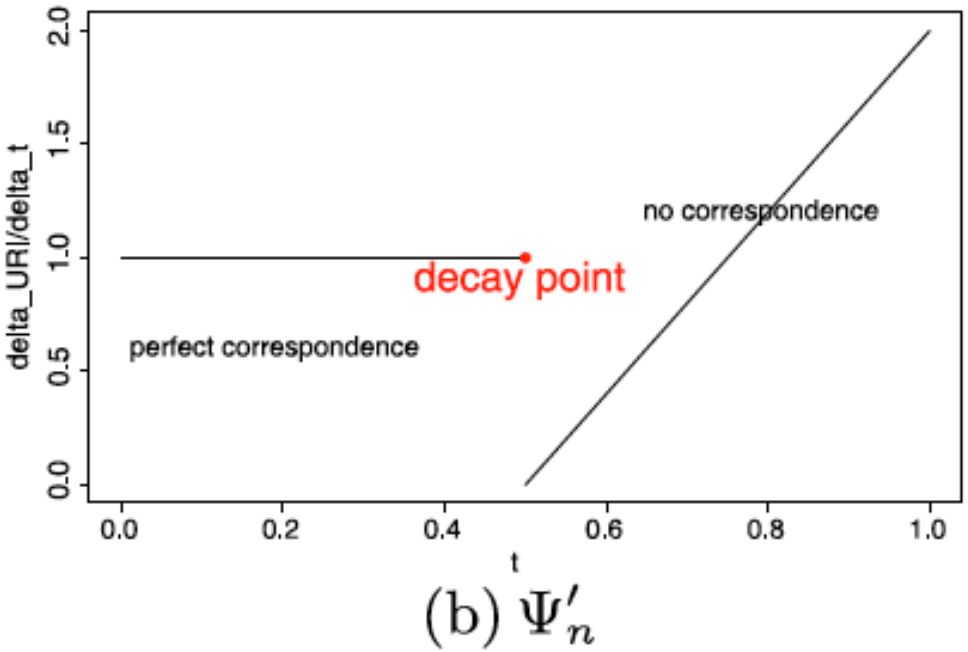
The Irreproducible Data Rate (IDR) fits two mixture components to a rank correspondence function

Ψ is % of pairs ranked in upper t% of R1 and R2



(a) Ψ_n

Derivative Ψ' shows decay point between components



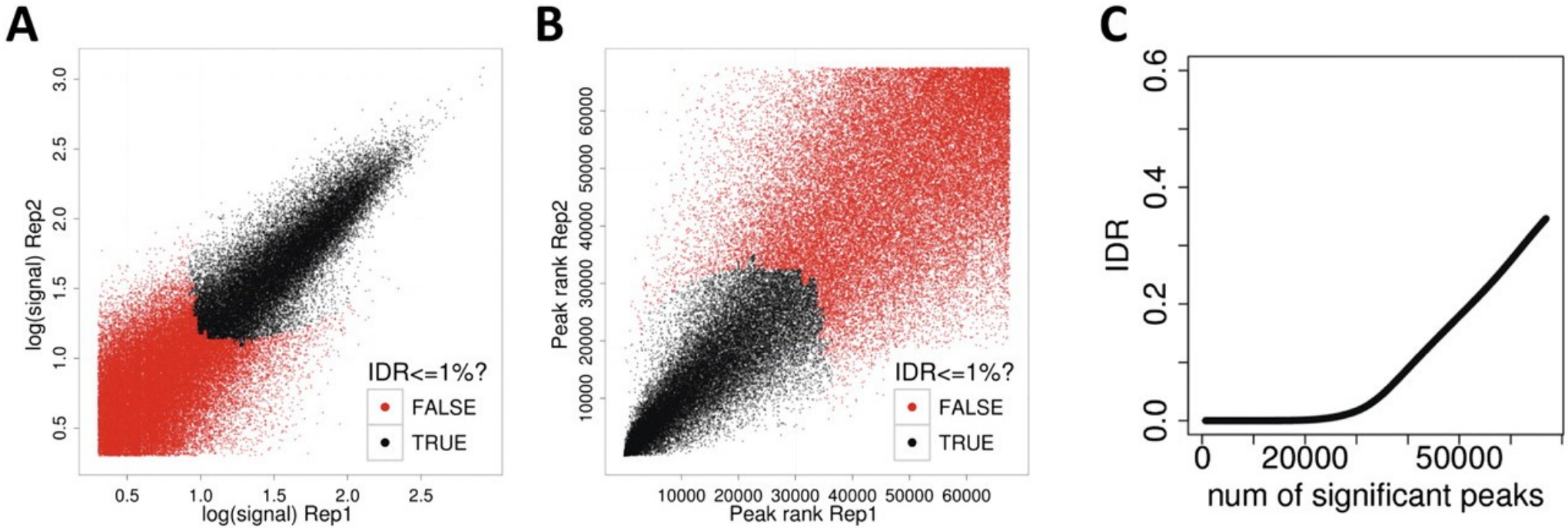
(b) Ψ'_n

Idea – construct mixture model containing two components to model the ranks of the replicates, reproducible and irreproducible.

The IDR of an event is the probability that it belongs to the irreproducible component

https://arxiv.org/pdf/1110.4705.pdf

The Irreproducible Data Rate (IDR) identifies significant events by consistent replicate ranks



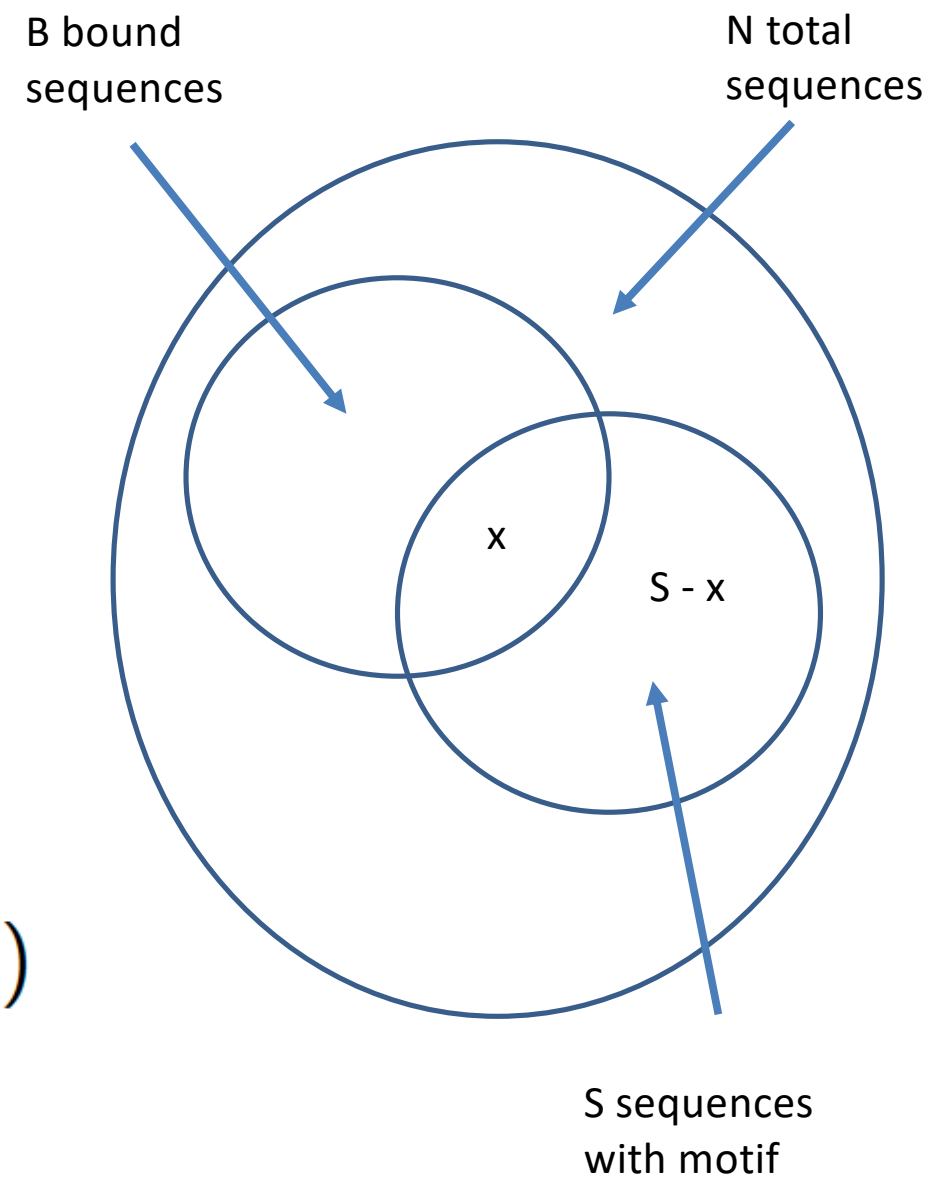
https://ccg.epfl.ch//var/sib_april15/cases/landt12/idr.html

Chance of a motif occurring x times in bound set by chance (hypergeometric)

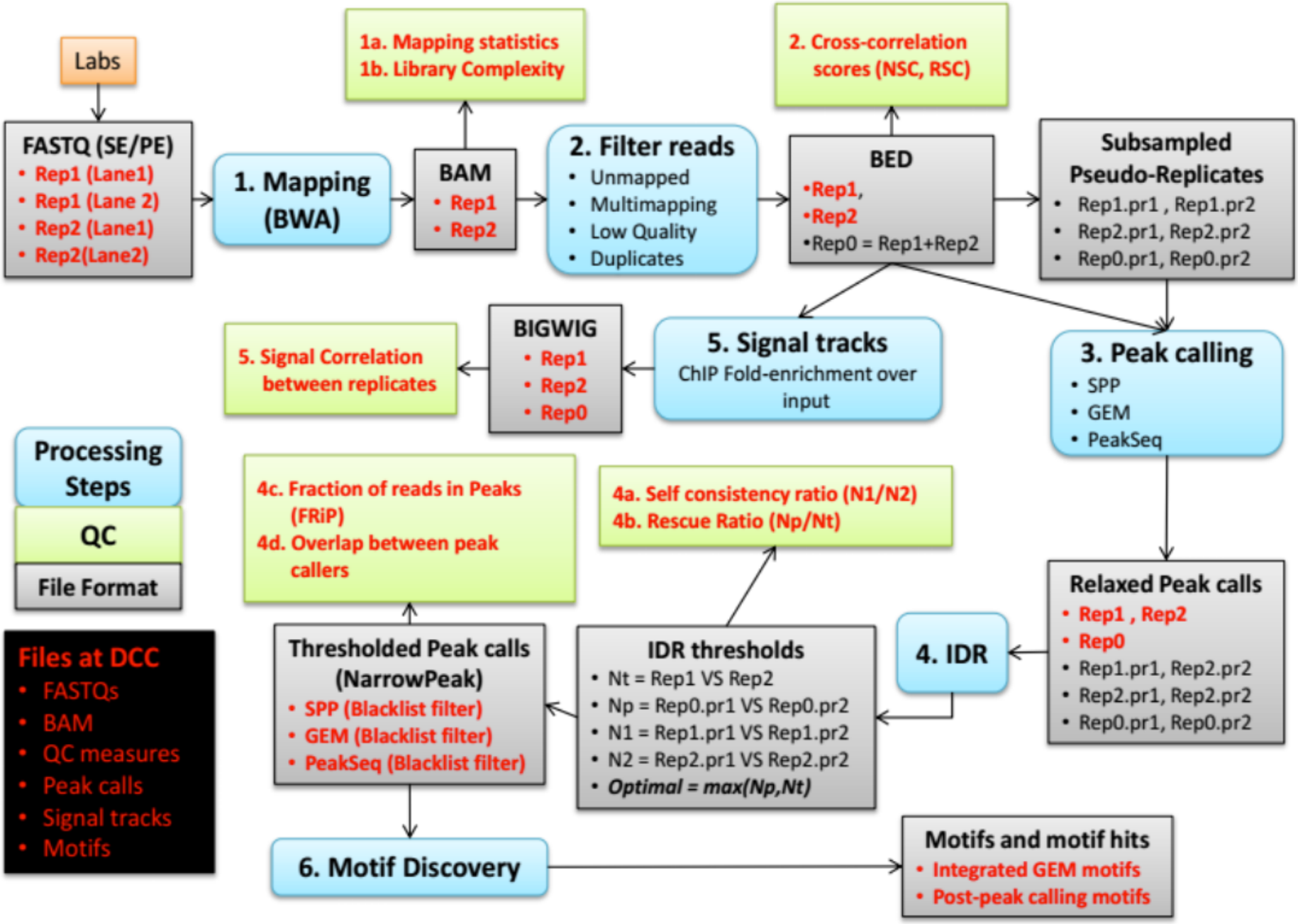
$$\binom{N}{x} = \frac{N!}{(N-x)!x!}$$

$$p(x) = \frac{\binom{B}{x}\binom{N-B}{S-x}}{\binom{N}{S}}$$

$$p_{value}(x) = \sum_{i=x}^{min(S,B)} p(i)$$



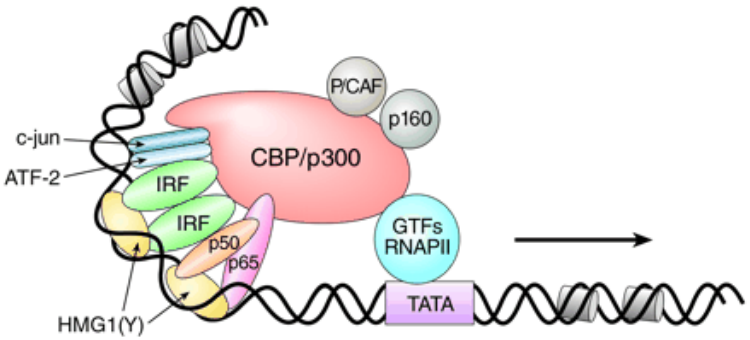
Encode ChiP-seq Processing Pipeline



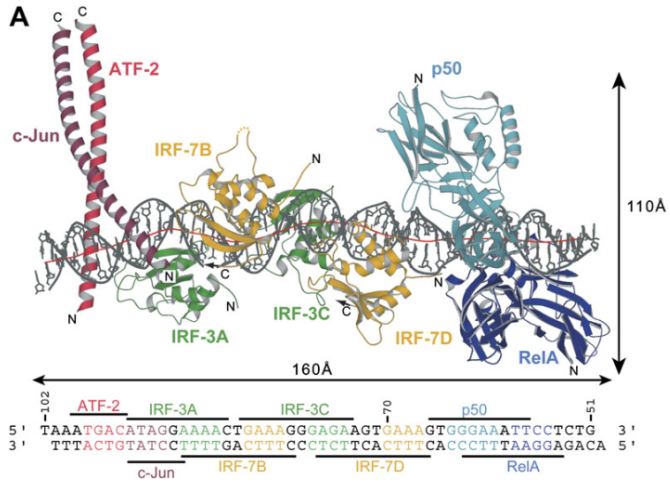
Transcription Factors Interact

The spatial arrangement of transcription factor binding is critical

The IFN-β enhanceosome



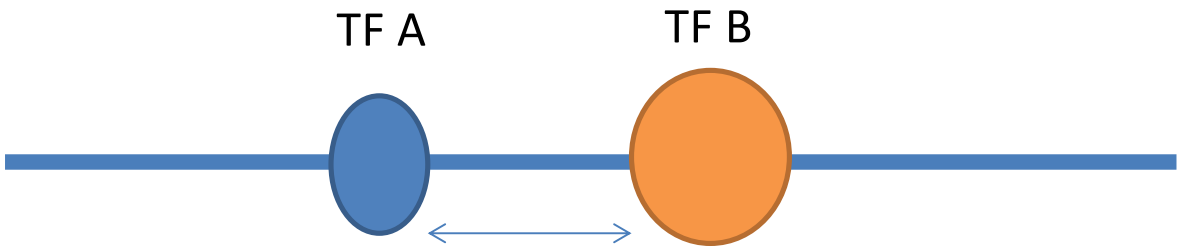
(Vo and Goodman, JBC, 2001)

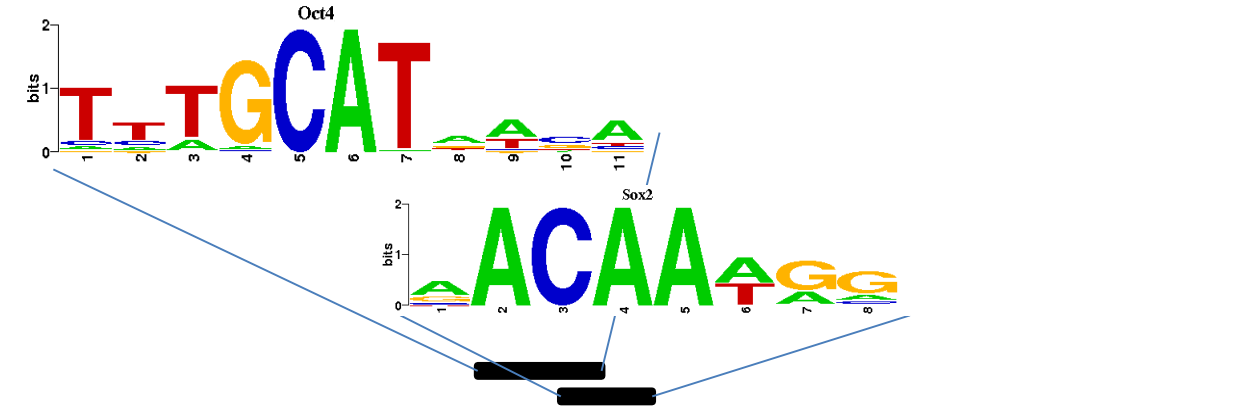


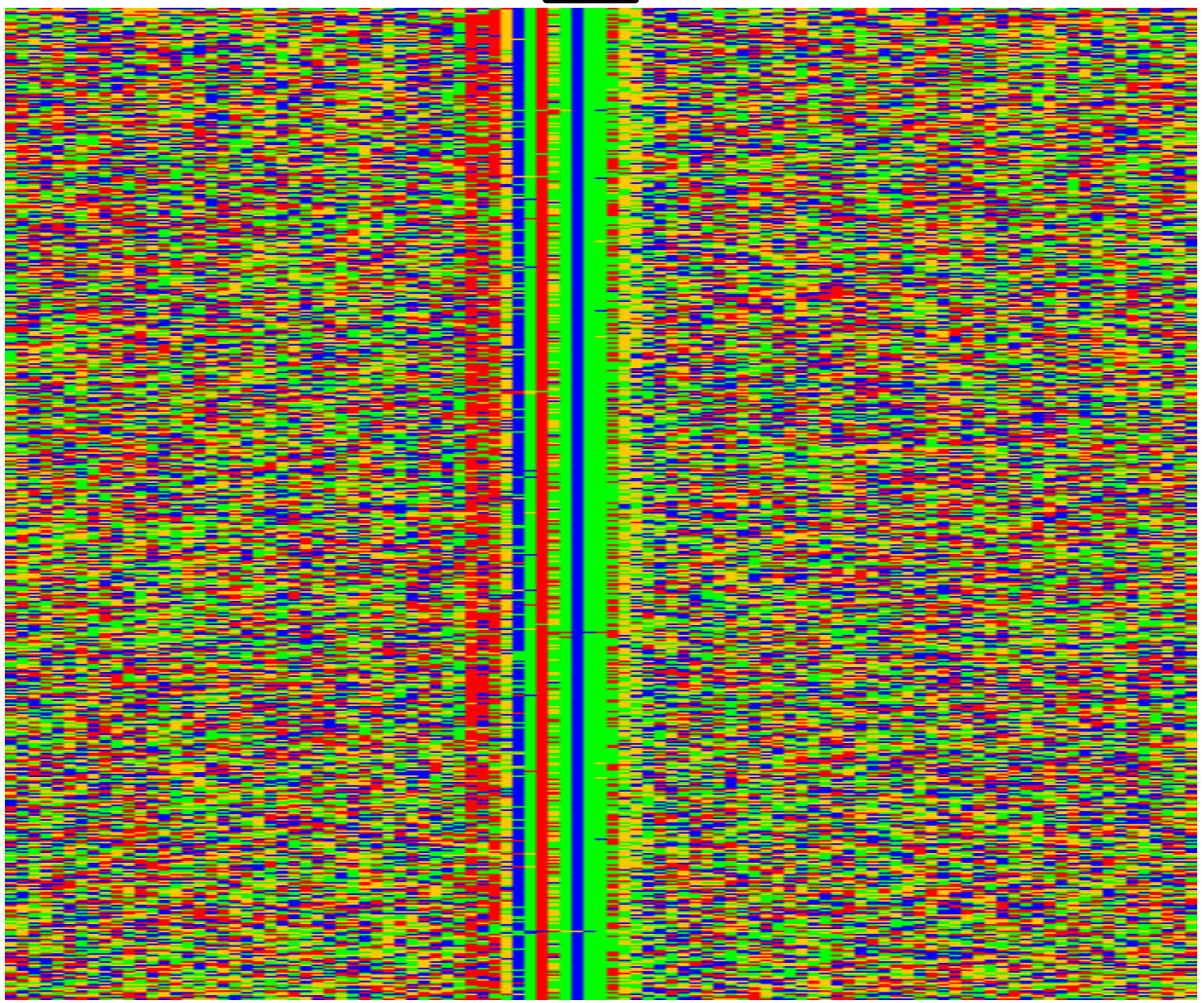
(Panne, Cell, 2007)

- Single point mutations disable the enhancer
- No major protein-protein interaction

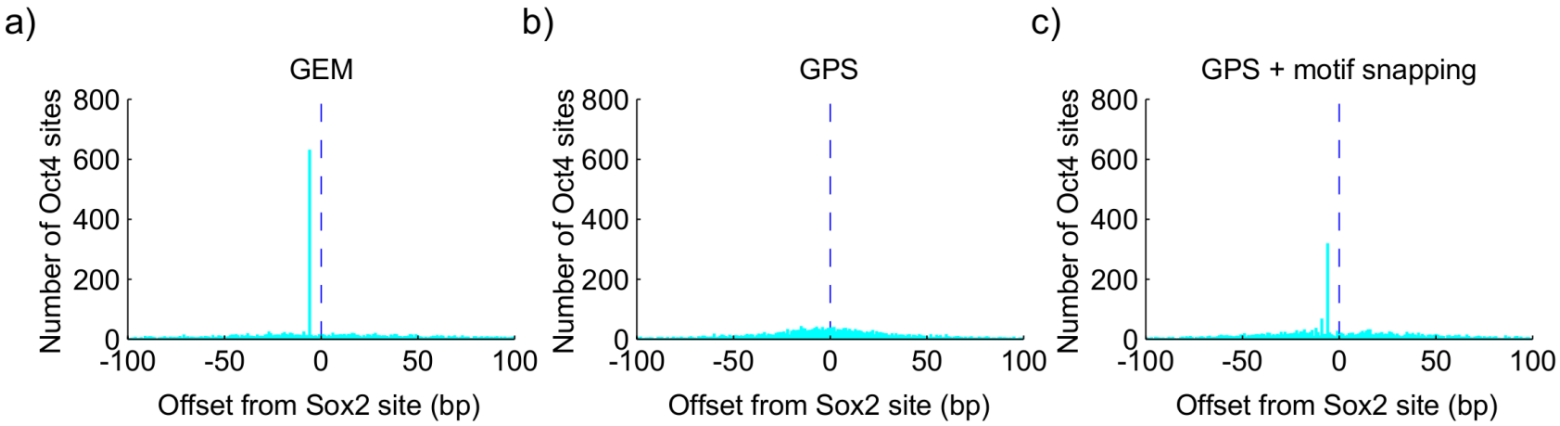
A precise genome wide characterization of *in vivo* spacing constraints between key transcription factors would reveal key aspects of the gene regulation.

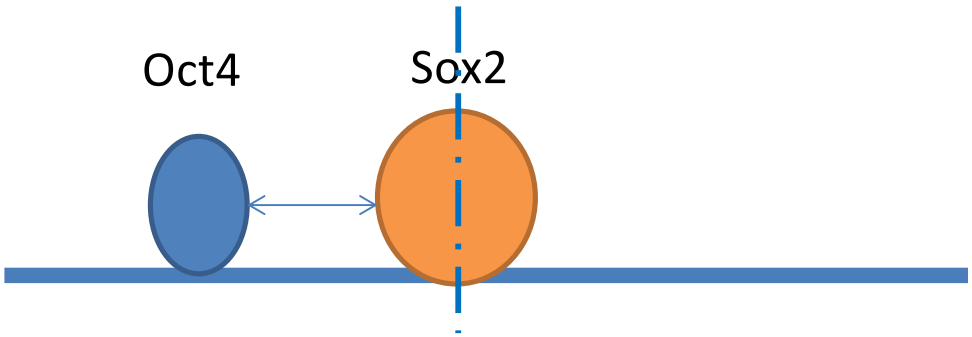



Oct4
bits
Sox2
bits



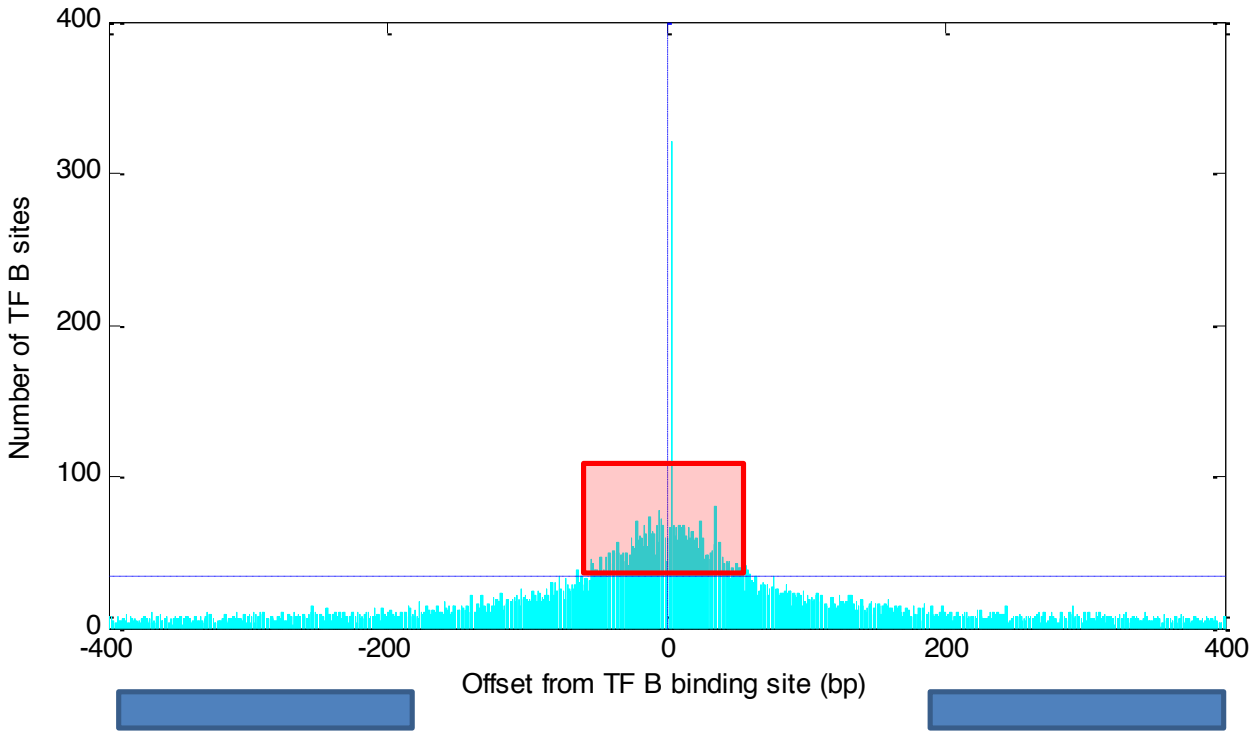
GEM reveals transcription factor spatial binding constraints





What are significant spacings?

- Compute average number of motifs in background region [200bp 400bp] and [-400bp -200bp]
- Use Poisson CDF to compute p-value of number of occurrences at each location in [-100bp 100bp]
- Bonferroni correct each p-value (p-value x 201)
- We choose that a corrected p-value is significant at 10^-8



Spatial binding constraints detected from ENCODE ChIP-Seq datasets

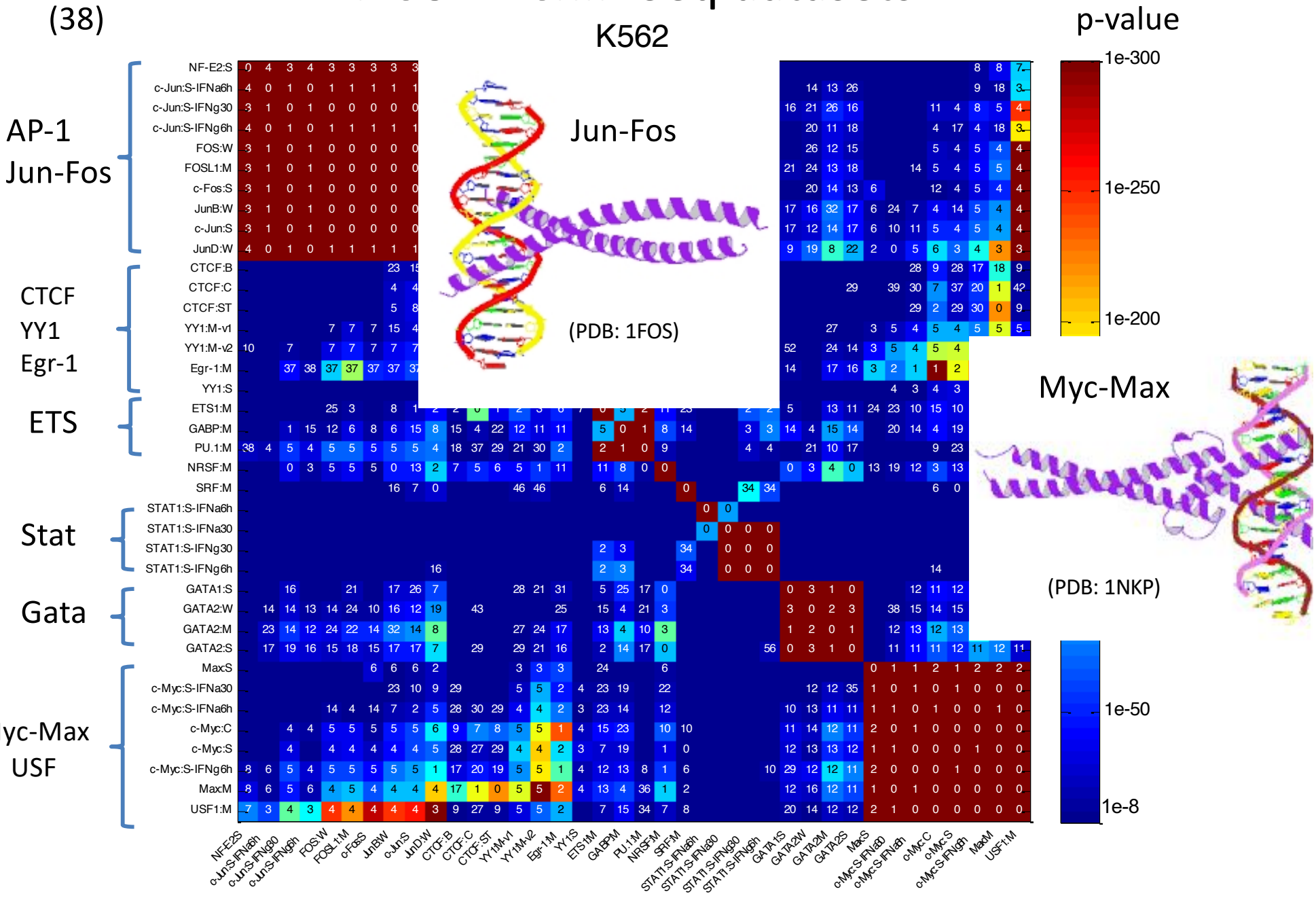
Cell type	Description	Expts	Constraint pairs
K562	leukemia	38	154
GM12878	lymphoblastoid	29	134
HepG2	liver carcinoma	21	86
HeLa-S3	cervical carcinoma	13	34
H1-hESC	embryonic stem cells	11	19

(355 distinct TF pairs)

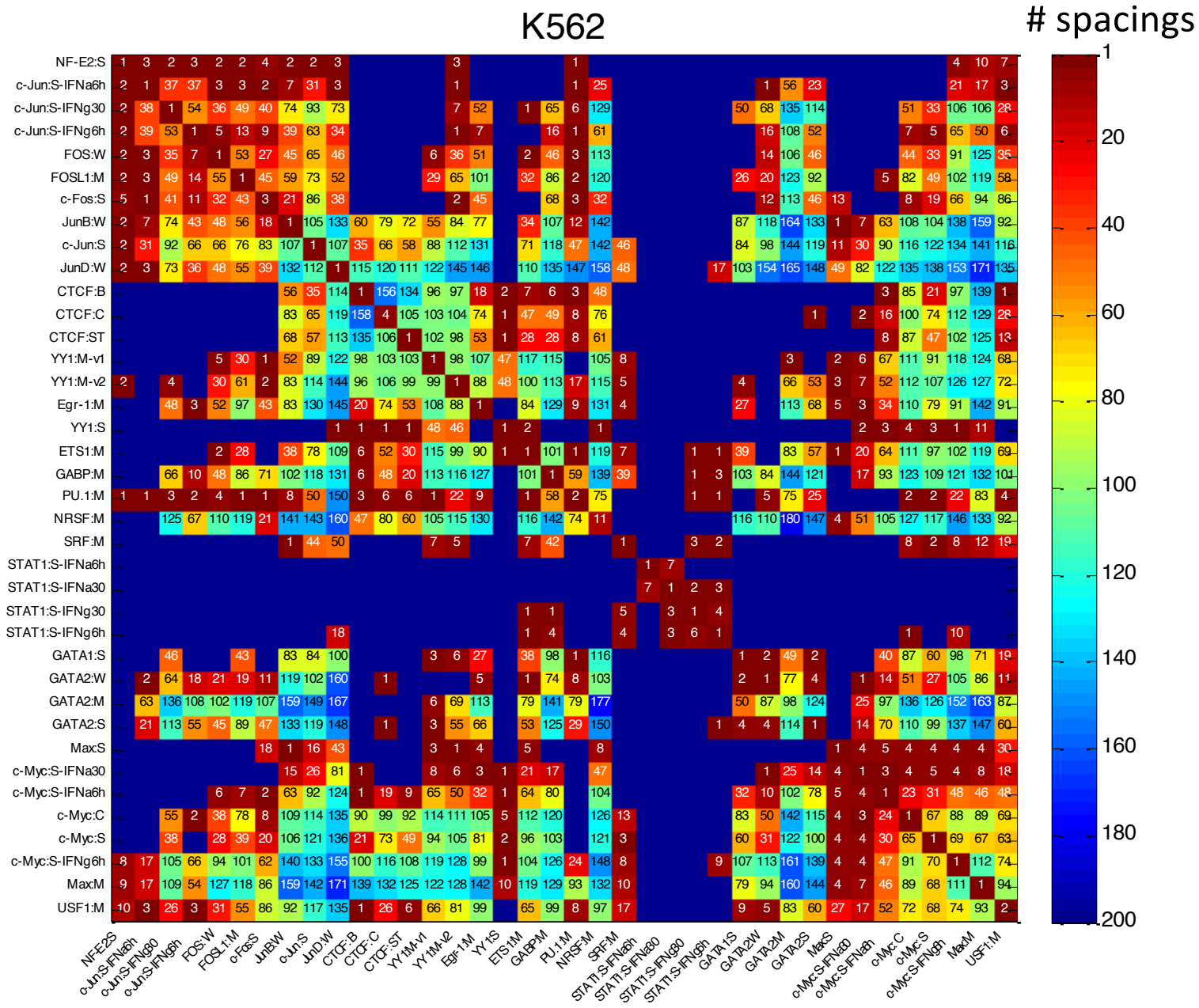
Spatial binding constraints detected from ENCODE ChIP-Seq datasets

(38)

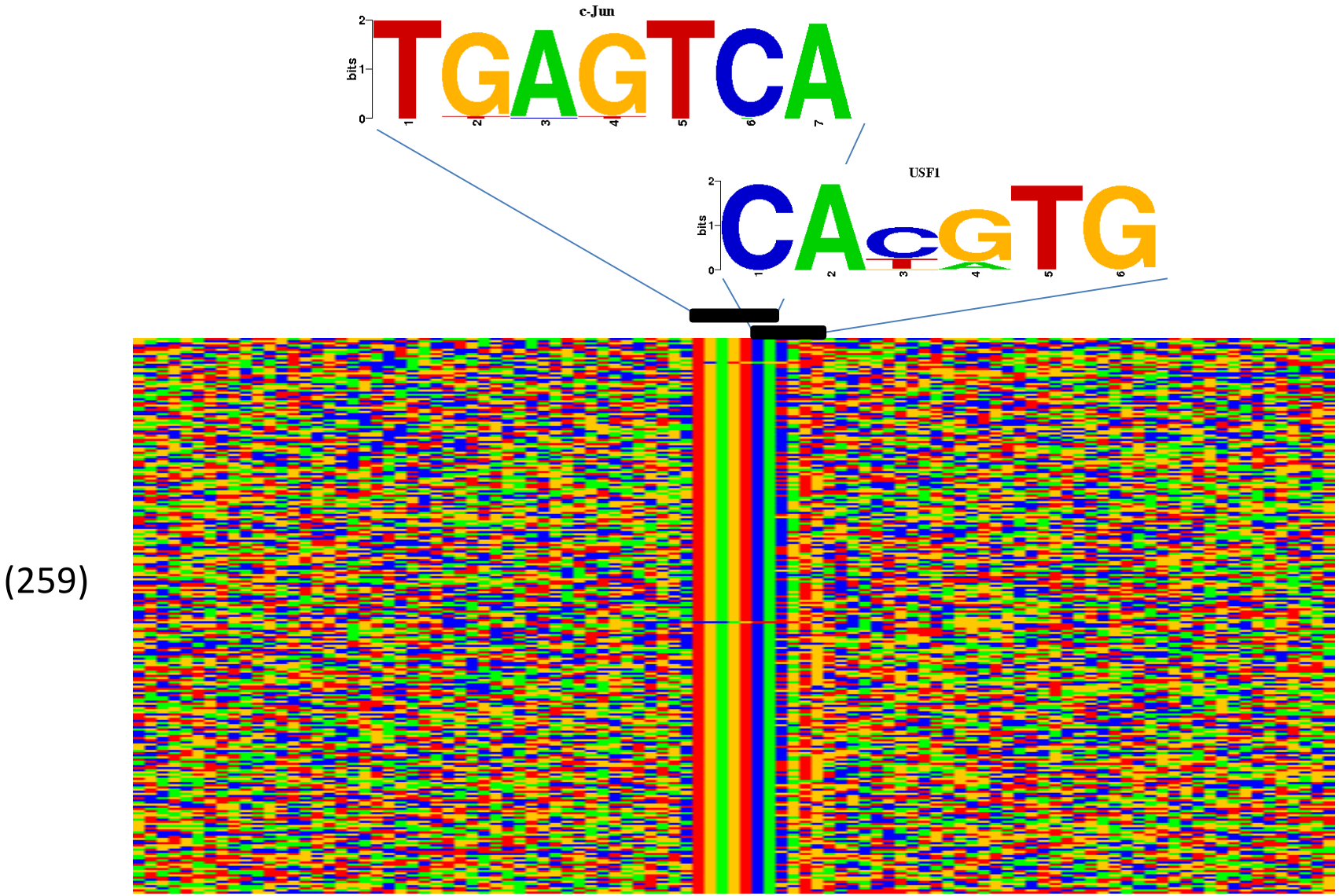
K562



Spatial binding constraints detected from ENCODE ChIP-Seq datasets

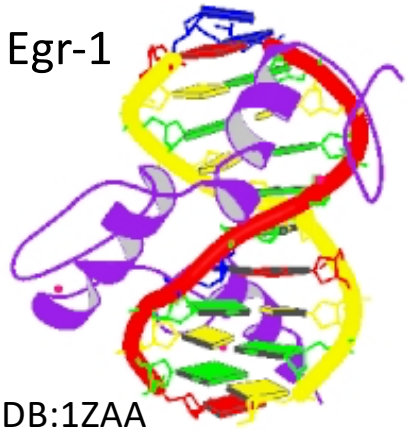


Cooperative binding: c-Jun/USF-1

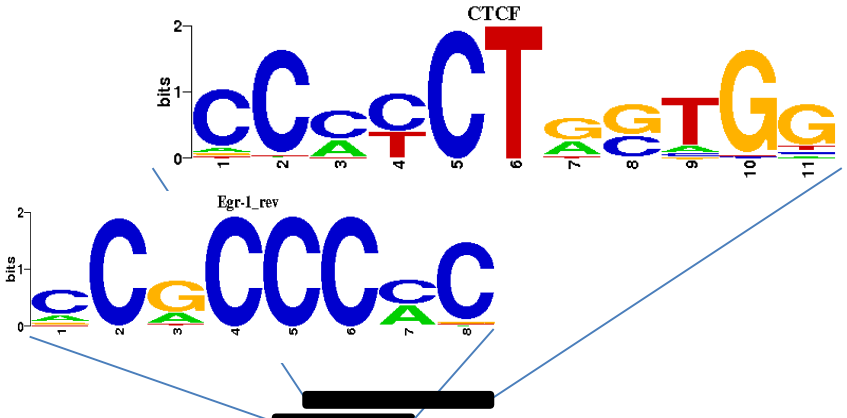


Competitive binding: CTCF/Egr-1

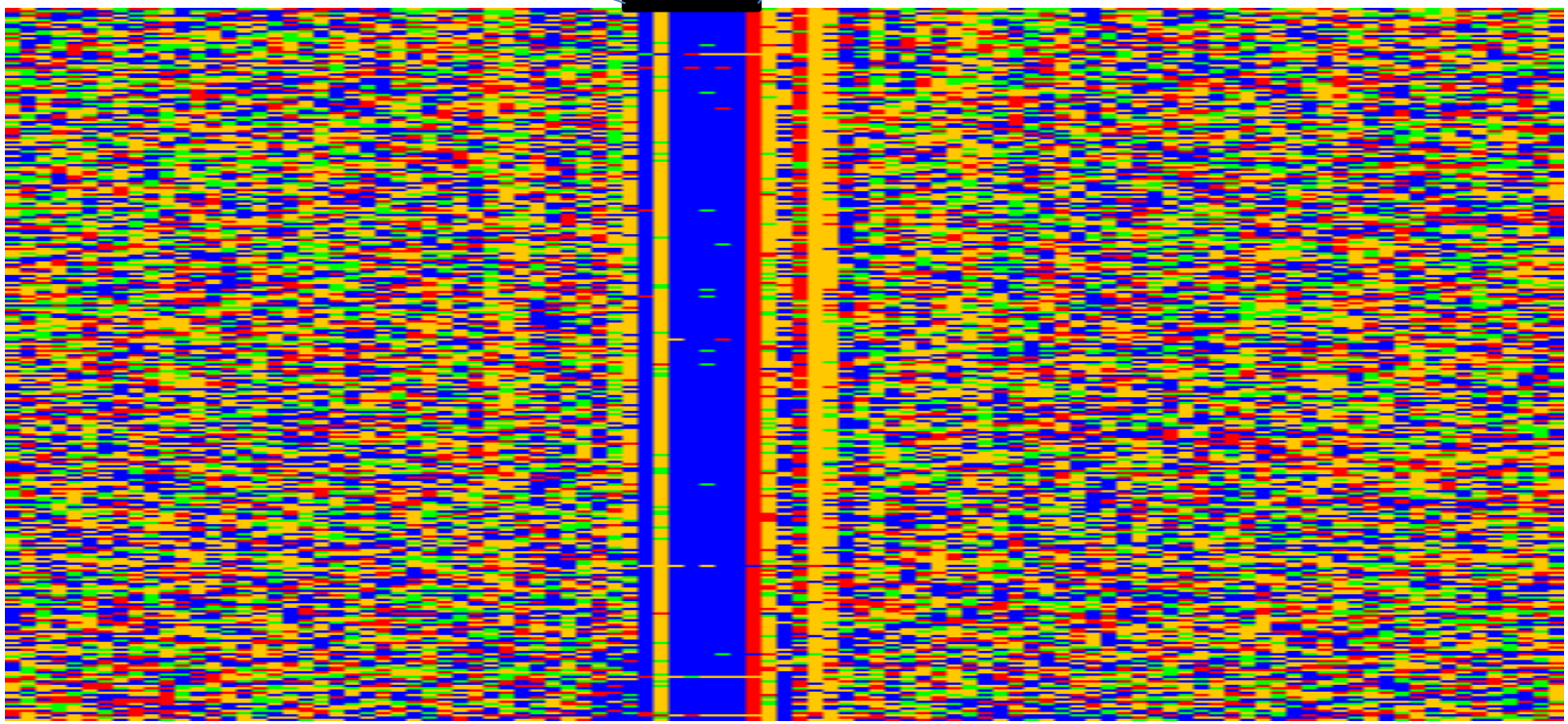
Egr-1



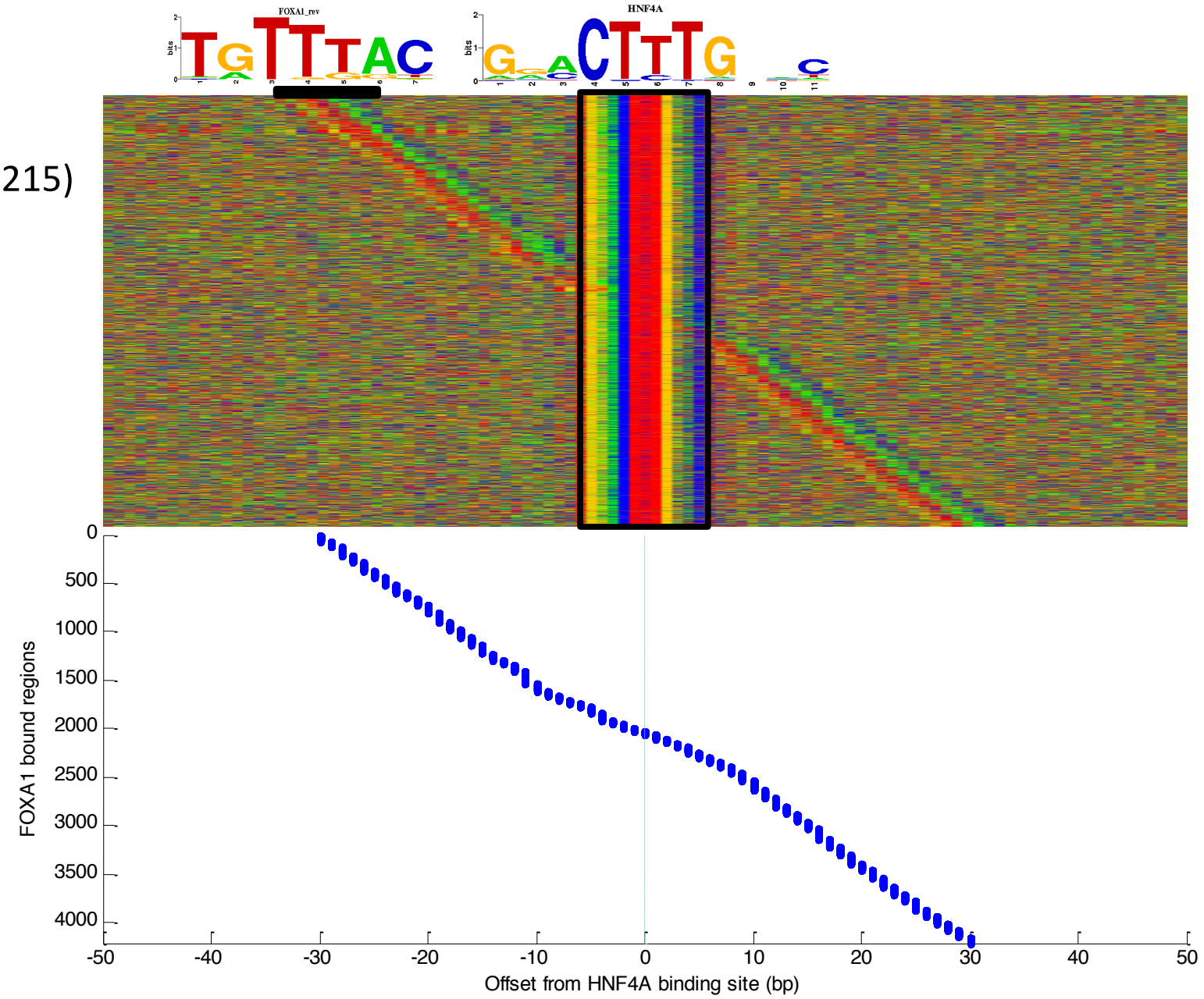
PDB:1ZAA



(315)

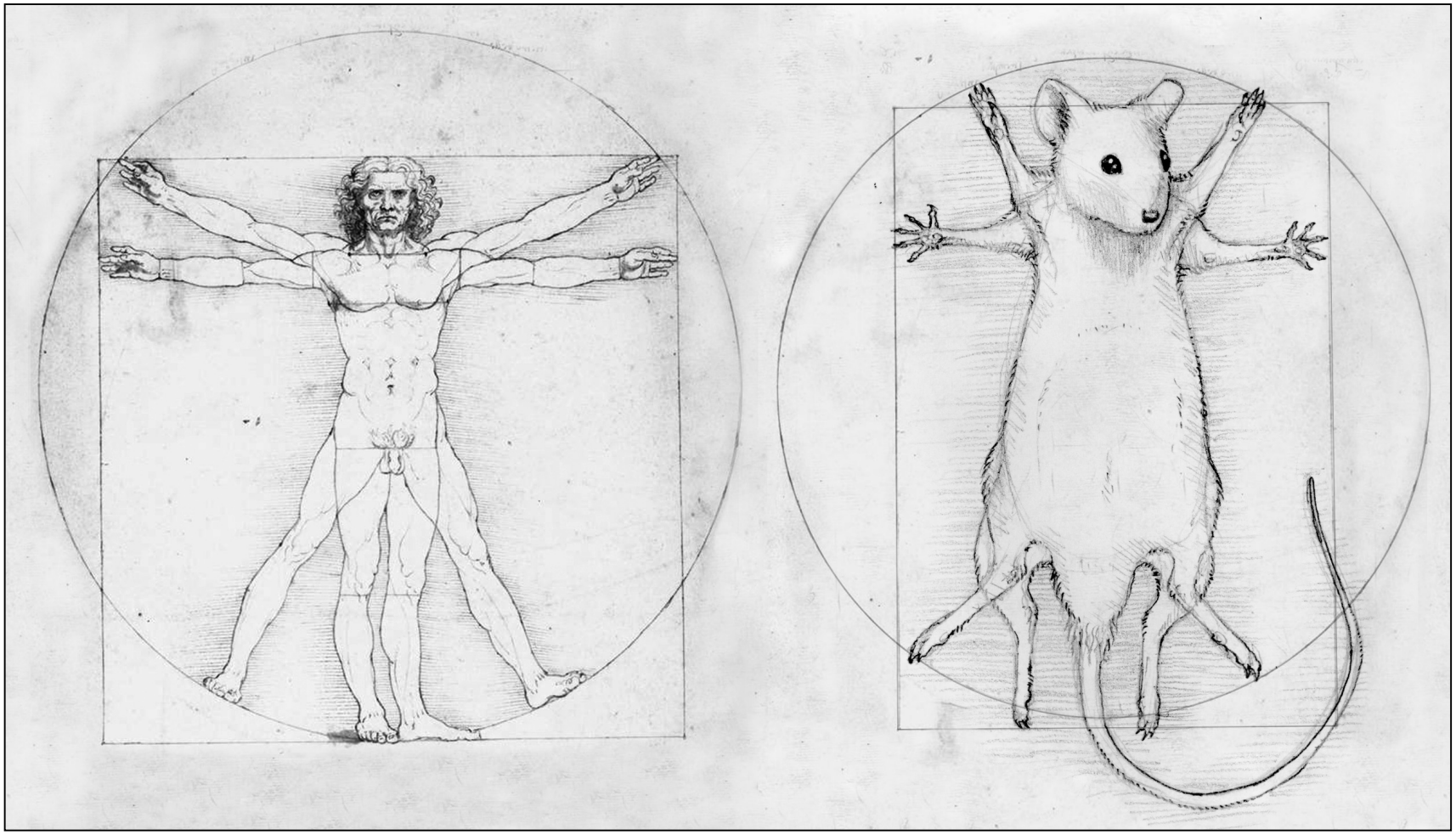


Collaborative binding: HNF4-α/FOXA1

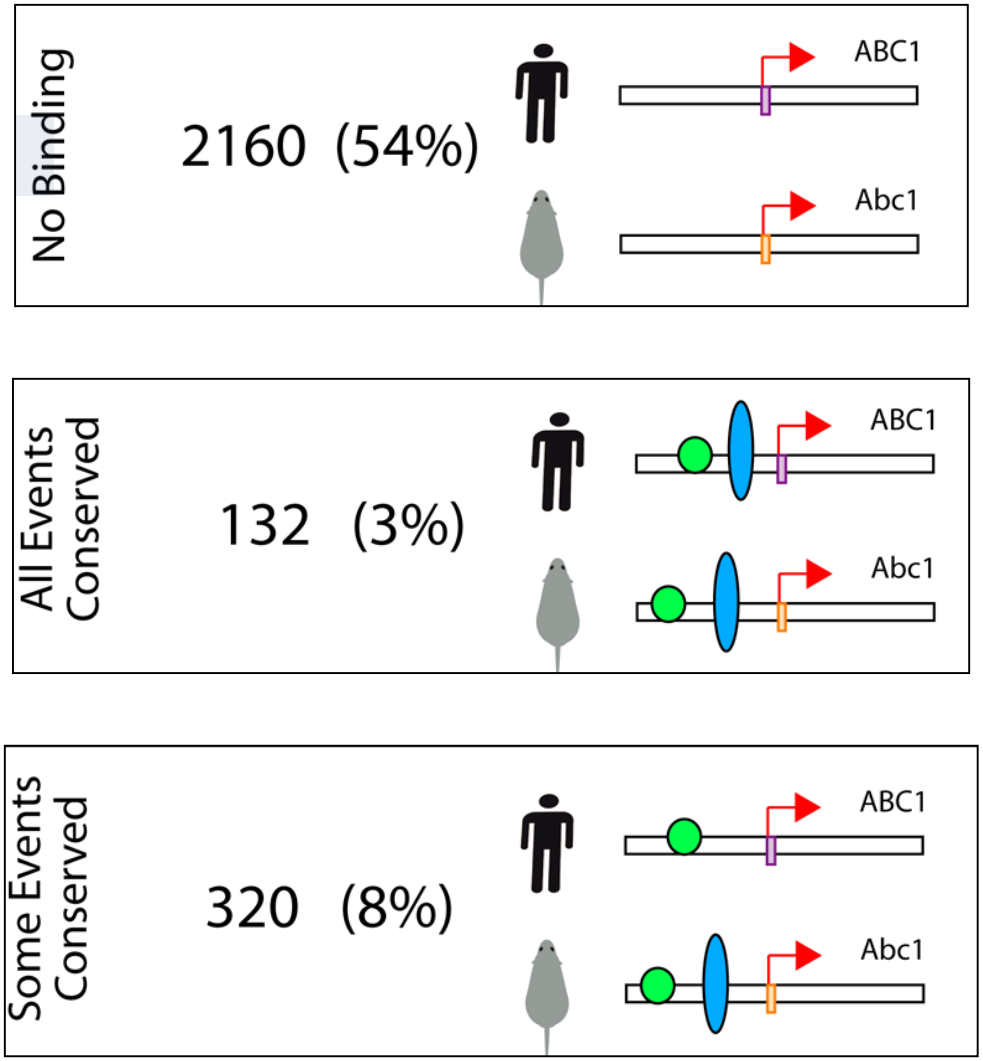


Binding is not necessarily evolutionarily conserved

Is conservation a good predictor of conserved binding events across species?



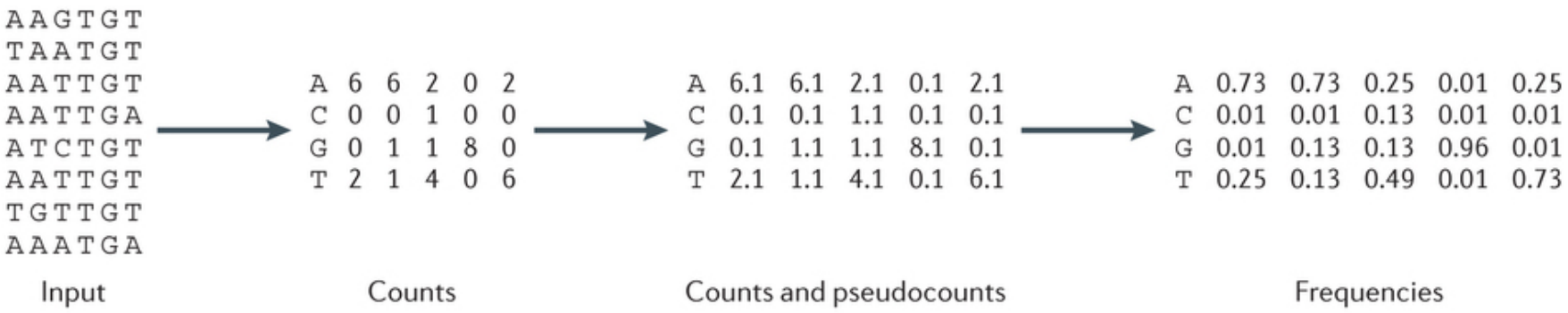
Promoter proximal binding is not well conserved in liver (FOXA2, HNF1A, HNF4A, HNF6)



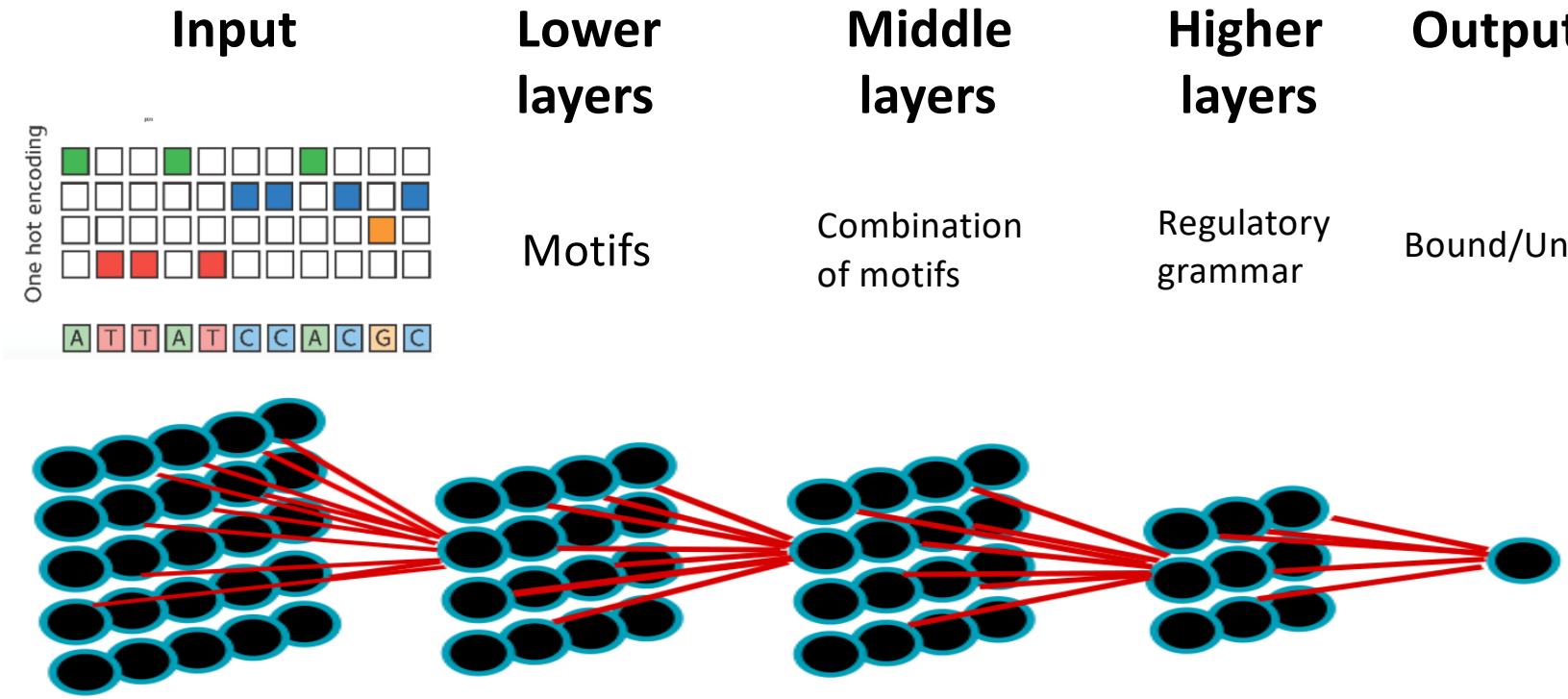
D. Odom, R. Dowell E. Fraenkel, D. Gifford Labs
Nature Genetics, 2007

Evaluating CNNs for binding prediction – what makes a good architecture?

Traditional DNA-protein binding models



One possible learned network structure



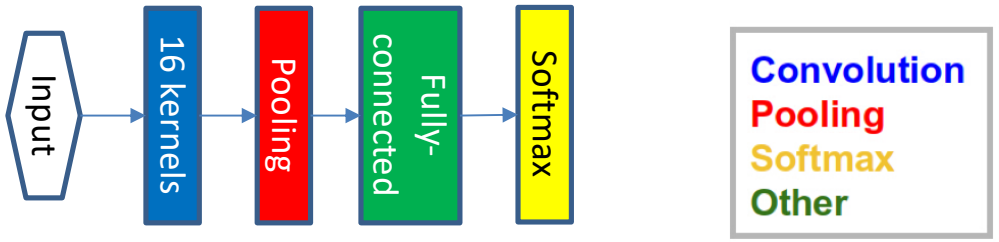
CNNs can outperform conventional approaches in modeling DNA-protein binding

Predicting the sequence specificities of DNA- and RNA-binding proteins by deep learning

Babak Alipanahi[1,2,6], Andrew Delong[1,6], Matthew T Weirauch[3–5] & Brendan J Frey[1–3]

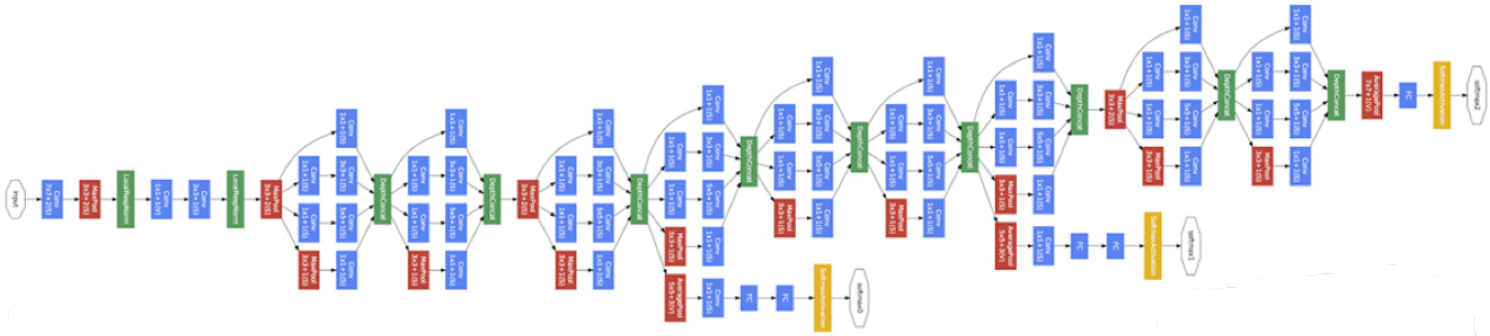
DeepBind (2015):

One convolutional layer with 16 kernels, maximum pooling window



DeepBind is “shallow learning” compared with other CNNs

GoogLeNet[1]
(Computer Vision)



DeepBind

Convolution
Pooling
Softmax
Other

[1] Szegedy et al. Going Deeper with Convolutions.

Open questions about deep learning for genomics

- What architectures work best to model DNA-protein binding?
- How “deep” should a network be?
- What components of the network contribute most to overall performance?
- Is the optimum network design specific to the task / experiment / TF?

Today's approach

- Use a framework to systematically benchmark CNN architectures on genomics tasks
- Analyze the contribution of different network components
- Explore if the optimum architecture is task-specific
- Evaluate training data requirements

Systematic benchmarking is important

- Task should be meaningful
  - *Real vs. artificial sequences (DeepBind): motif discovery*
    - Simple
    - Learn motif from similar nucleotide background
    - Not generalizable to classify real bound sequences

Systematic benchmarking is important

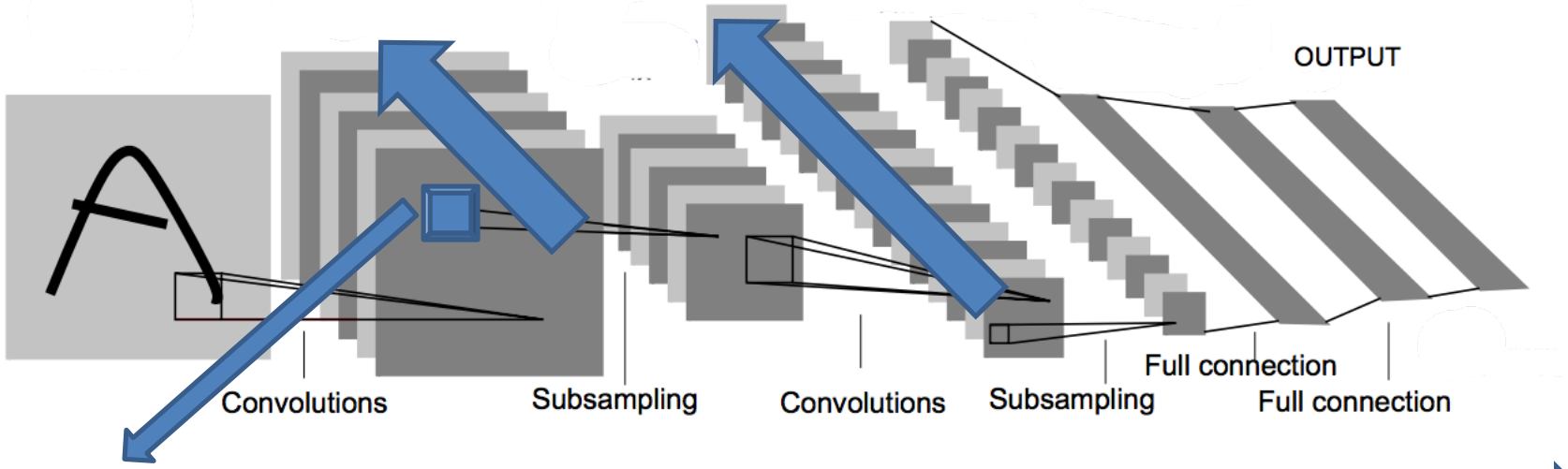
- Task should be meaningful
  - *Real vs. artificial sequences (DeepBind): motif discovery*
  - *Bound motif vs. unbound motif*: *motif occupancy*
    - Hard
    - Forces the model to learn better and higher-level sequence determinants

Systematic benchmarking is important

- Task should be meaningful
- Balance the number of positive and negative samples
- Control any artificial bias, location of the motif in the sample
- Conclusion should be the consensus across diverse TF ChIP-seq experiments (we use 690 from ENCODE)

CNNs have three important architectural dimensions to vary

More convolution kernels better capture feature diversity



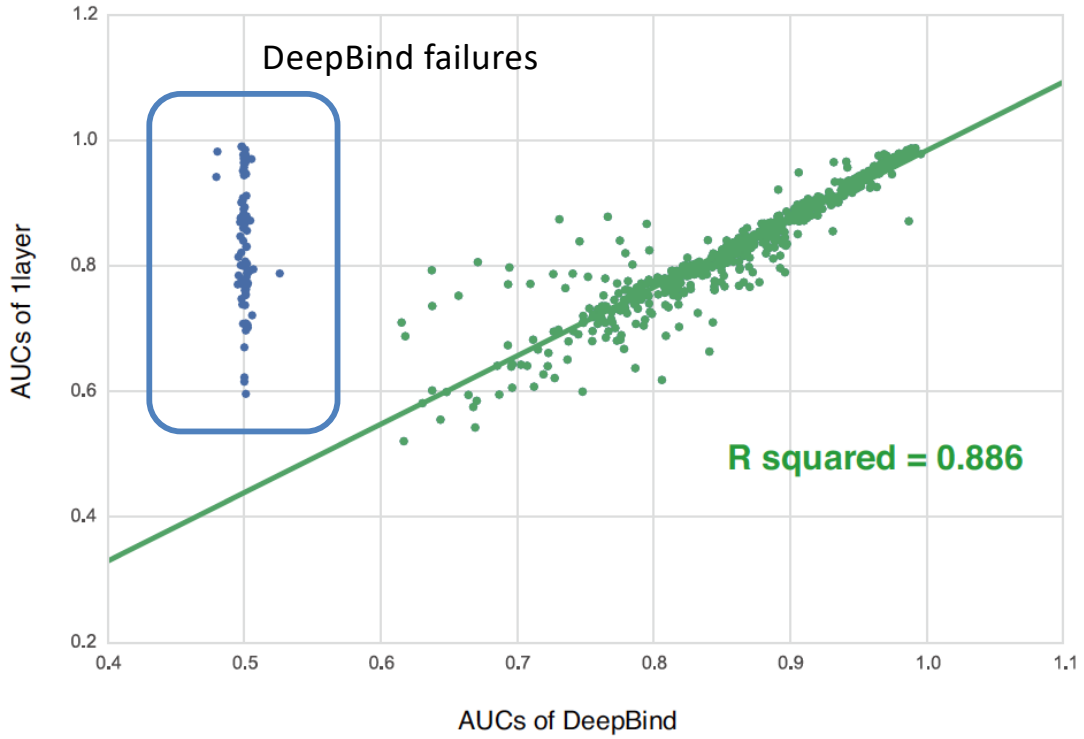
Smaller pooling window retains more location information

More layers can capture higher level features

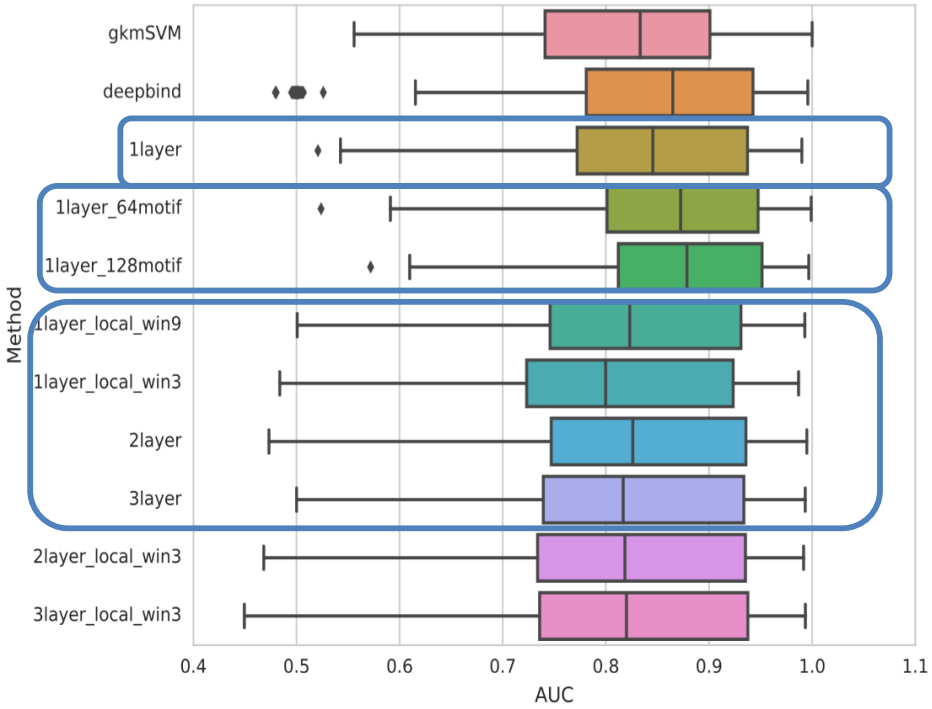
CNN architectures compared

Our Name	More Conv. Kernels	Deeper	Smaller pooling size
1layer (DeepBind)	-	-	-
1layer_64motif	✓	-	-
1layer_128motif	✓✓	-	-
1layer_local_win9	-	-	✓
1layer_local_win3	-	-	✓✓
2layer	-	✓	-
3layer	-	✓✓	-
2layer_local_win3	-	✓	✓✓
3layer_local_win3	-	✓✓	✓✓

Baseline model reproduces DeepBind



Simple models are best for a **motif discovery task**



baseline

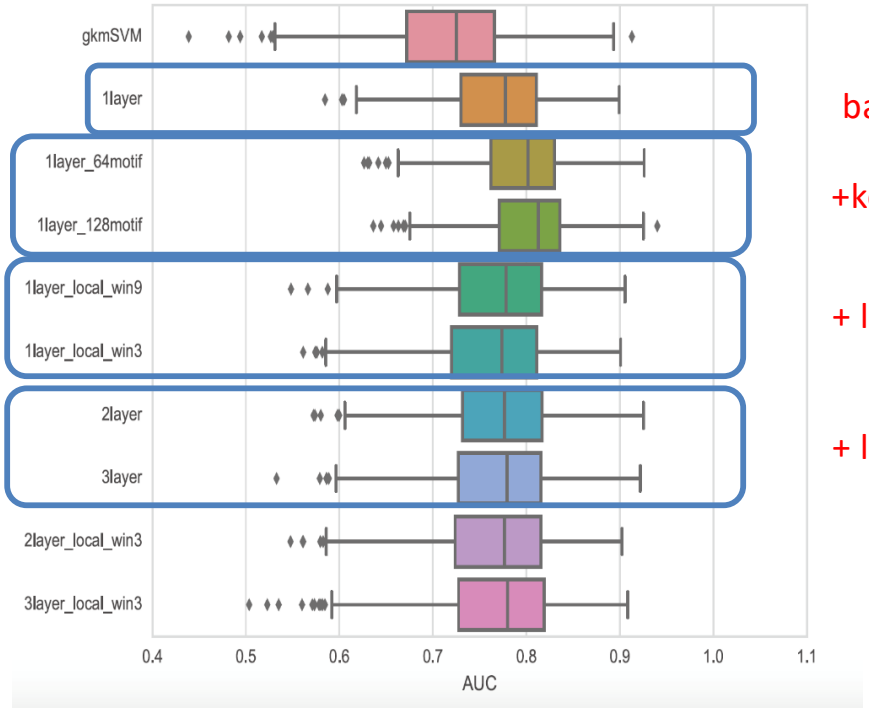
+kernels

+ loc. info

+ layers

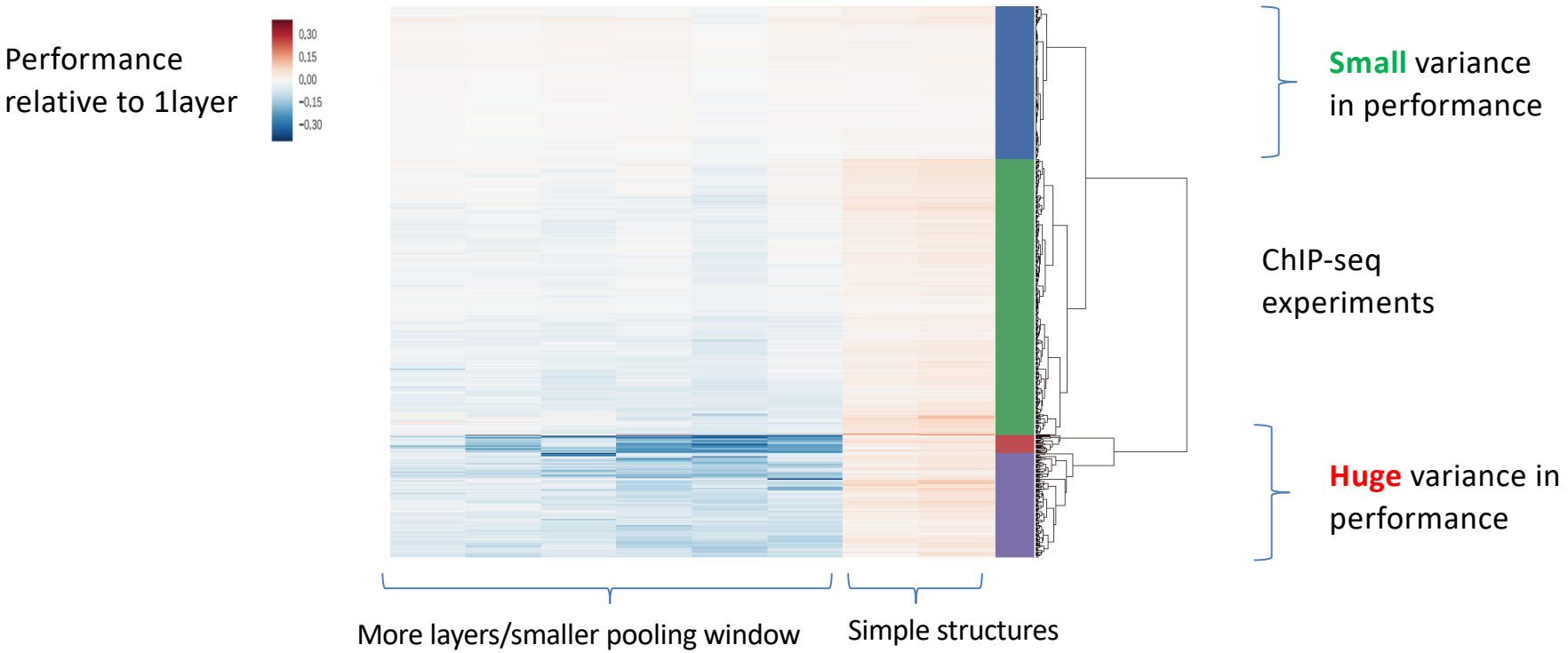
- More convolutional kernels helps model motif diversity
- Smaller pooling size, more layers monotonically decrease performance
  - possibly because most determinants are low-level (motifs) and position-independent

Depth improves performance in a **motif occupancy task**

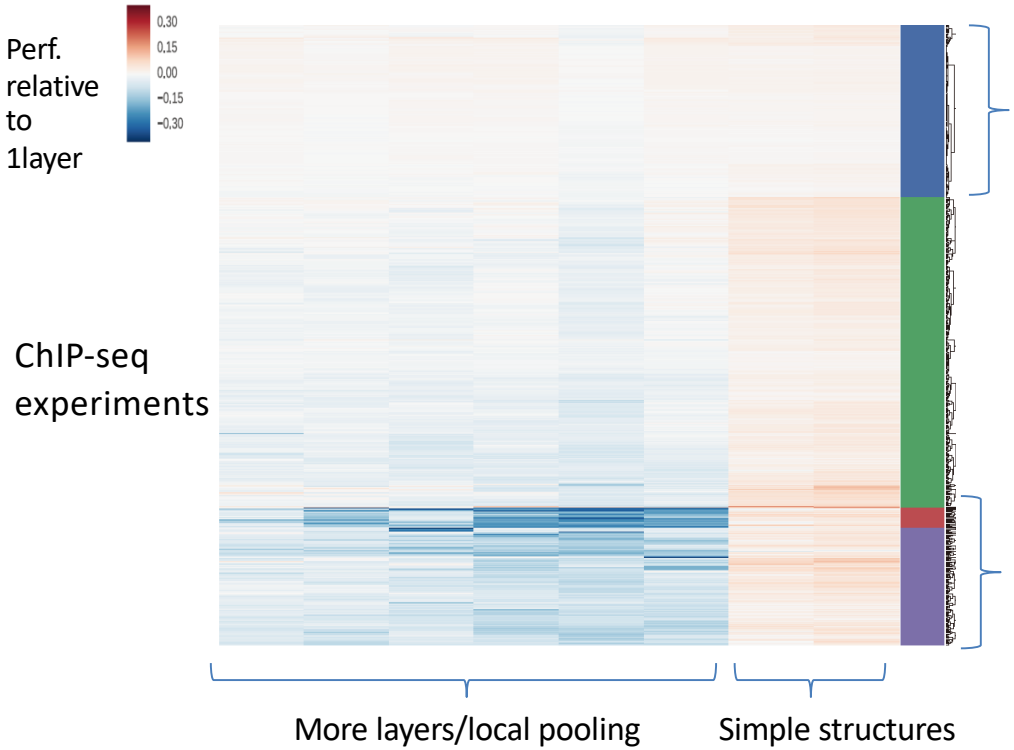


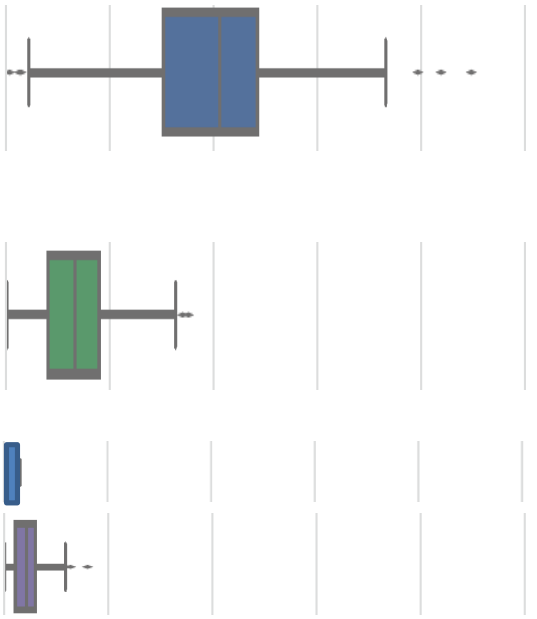
- AUC decreases for all architectures
- More convolutional kernels help model the motif diversity
- Smaller pooling size slightly decreases the performance
- Deeper networks have slightly better performance
  - There are more high-level determinants that can be better modeled by deeper layers, consistent with the task design

Observed performance is experiment-specific



More complex networks require more training data

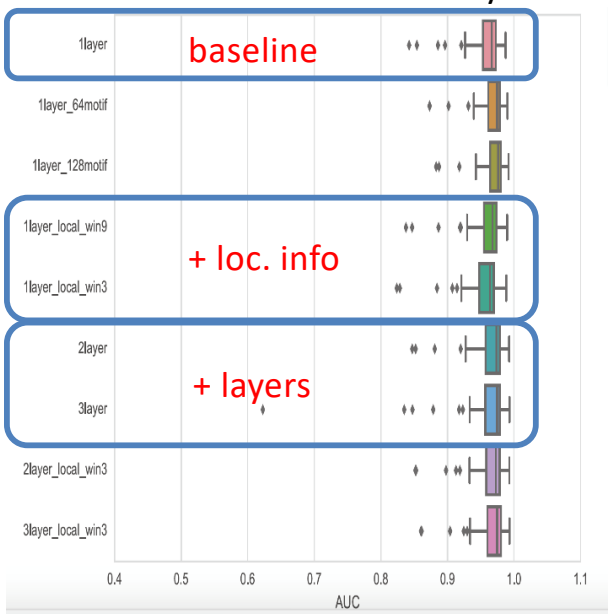




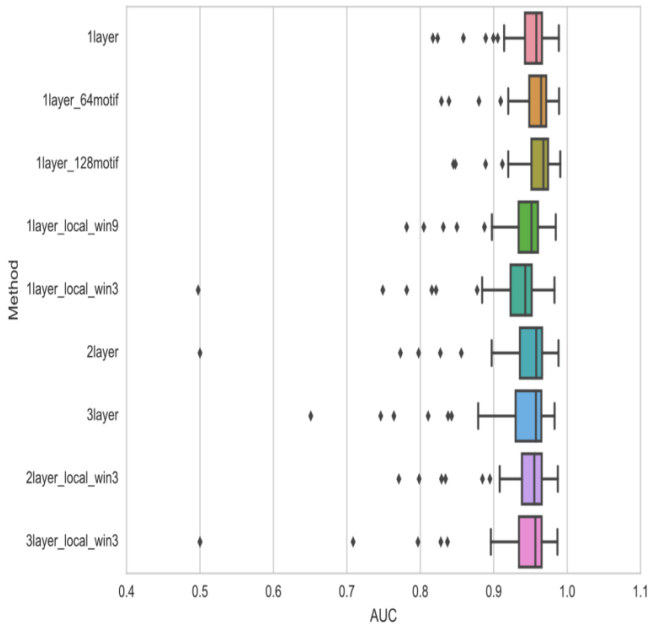
Training Example Count

Variance increases with fewer training examples

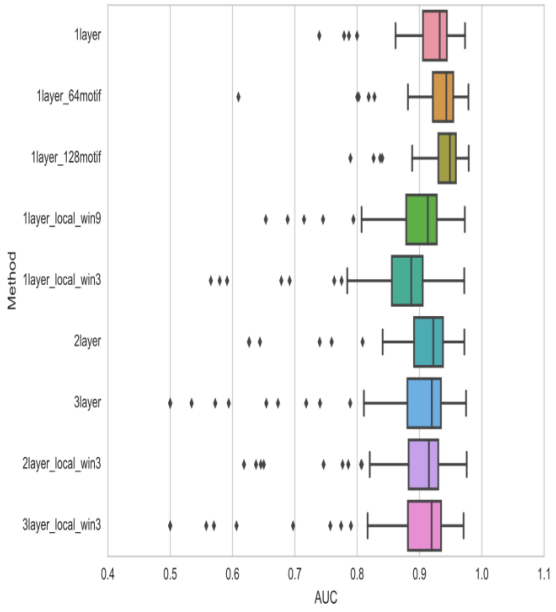
Performance on motif discovery task



80,000 training examples



20,000 training examples



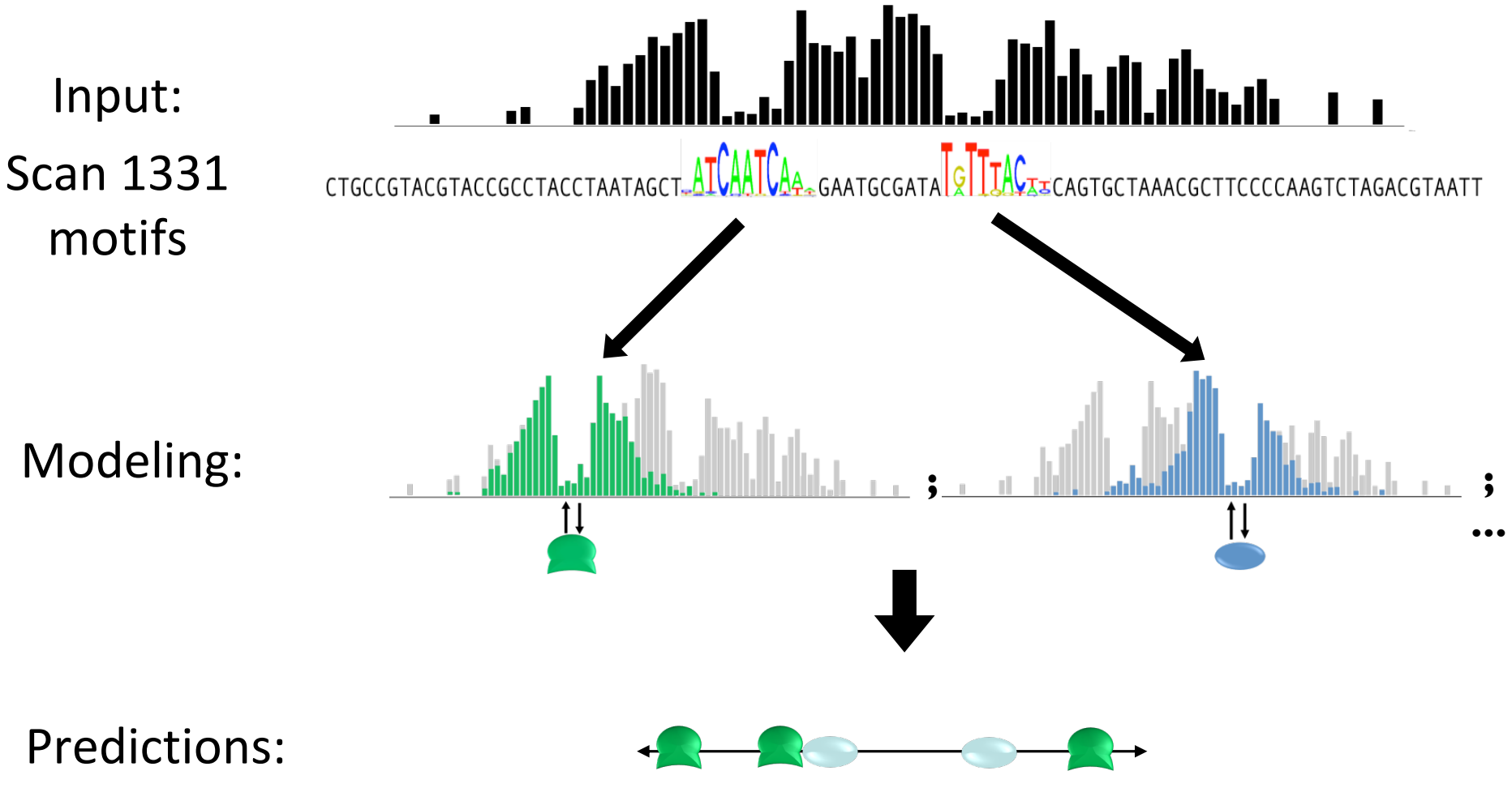
5,000 training examples

CNNs can outperform conventional methods

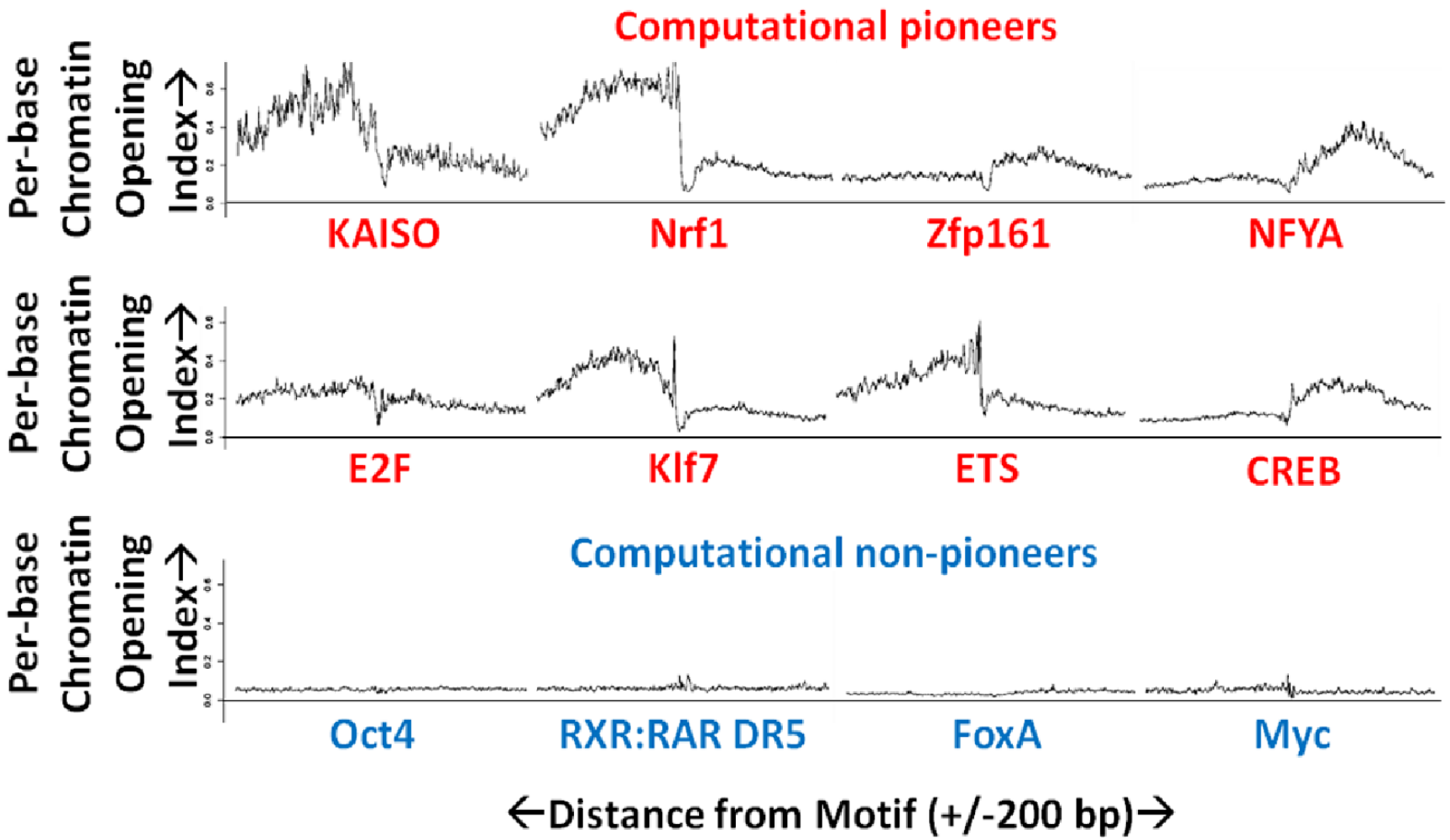
- CNNs outperform conventional methods with the right structure
- The optimum structure is different from that in computer vision
- Different biological tasks and data yield different conclusions
- Understanding the problem at hand and comparing different structures is important to design a good CNN model for biology applications (http://cnn.csail.mit.edu)

”Pioneer Factors” can have directional effects

PIQ: algorithm to predictively model TF binding from DNase-seq + Sequence

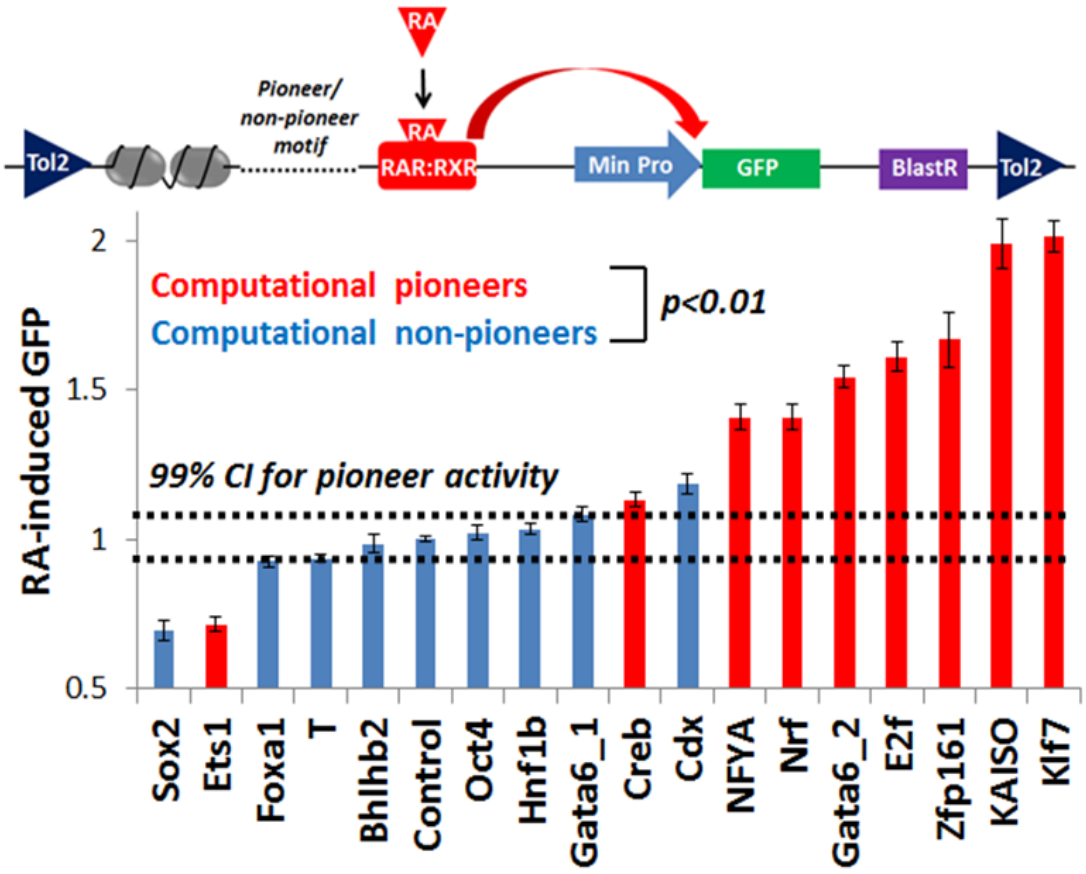


Pioneer TFs have identifiable profiles

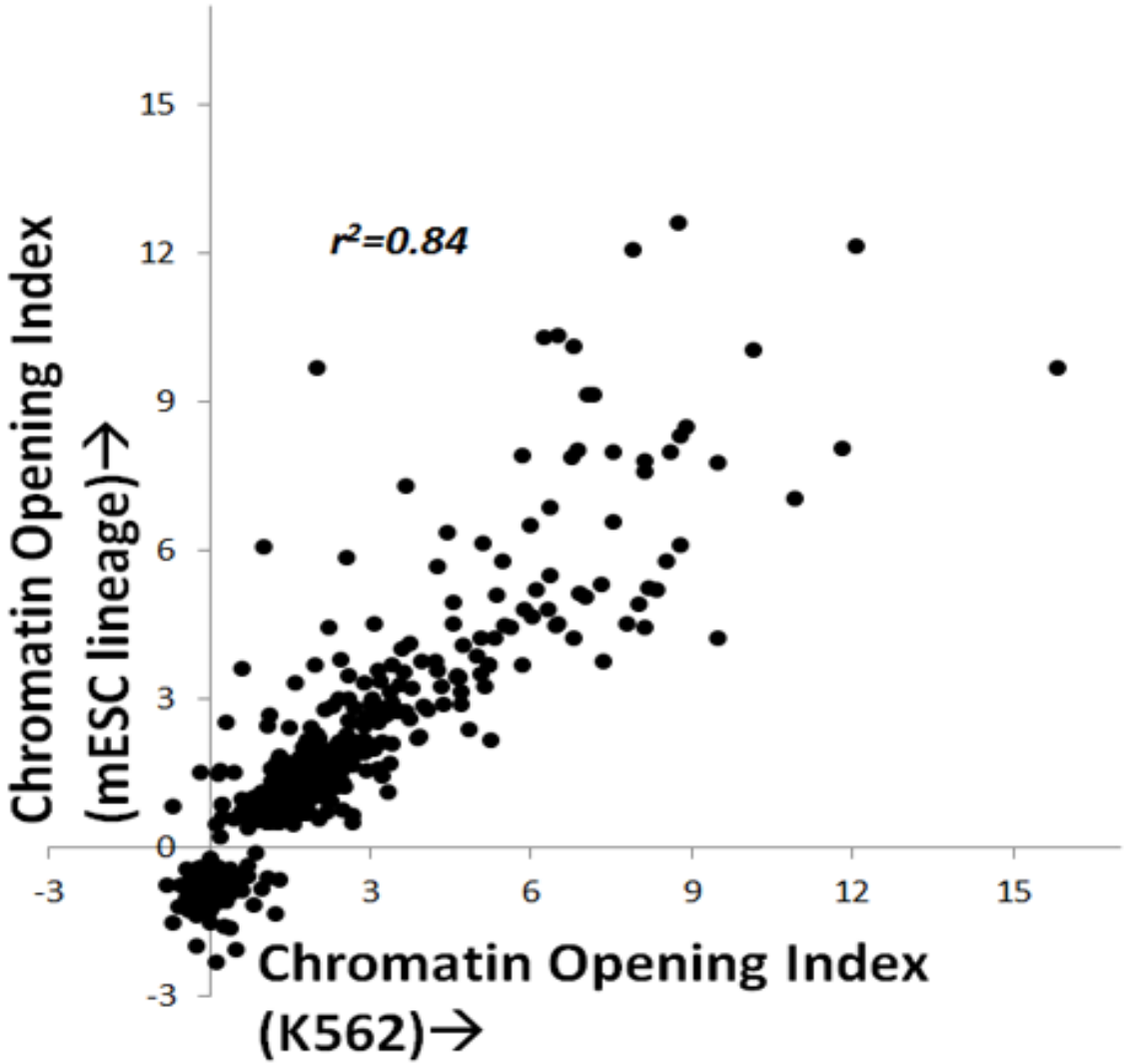


In vitro reporter assays recapitulate computational predictions

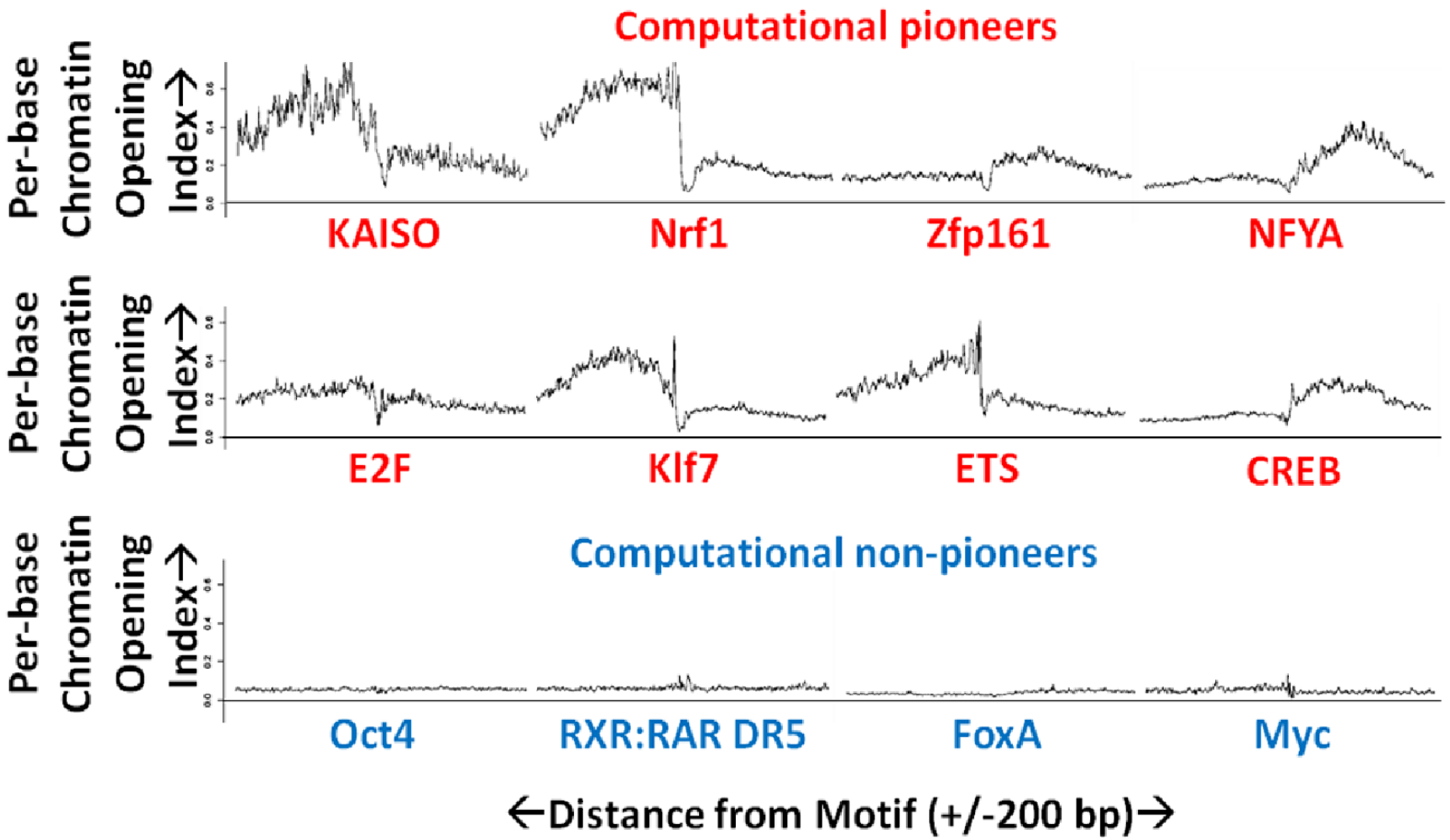
Using a Tol2 based GFP reporter, we confirm finding that these pioneers create new enhancers.



Pioneers appear to be conserved between human/mouse

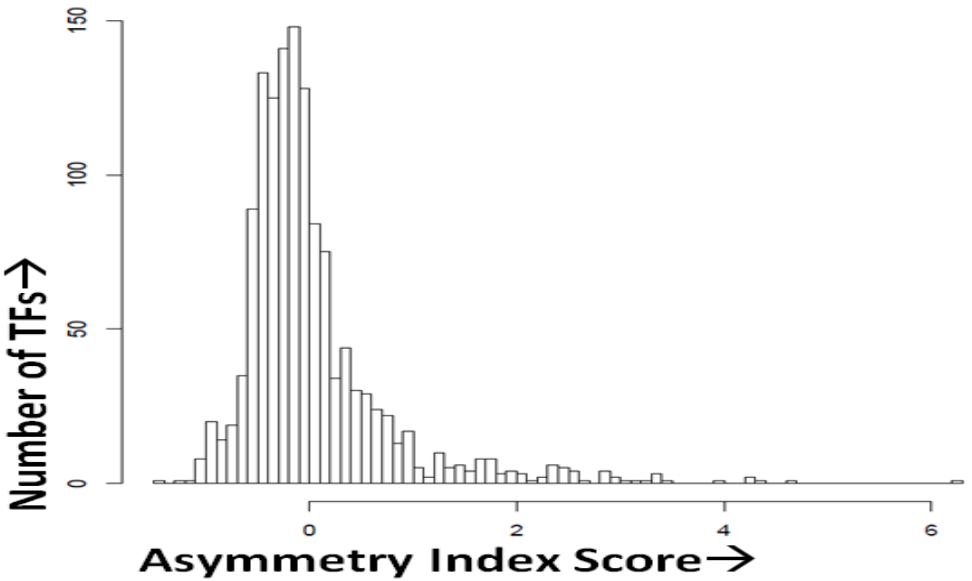


Pioneer TFs have identifiable profiles

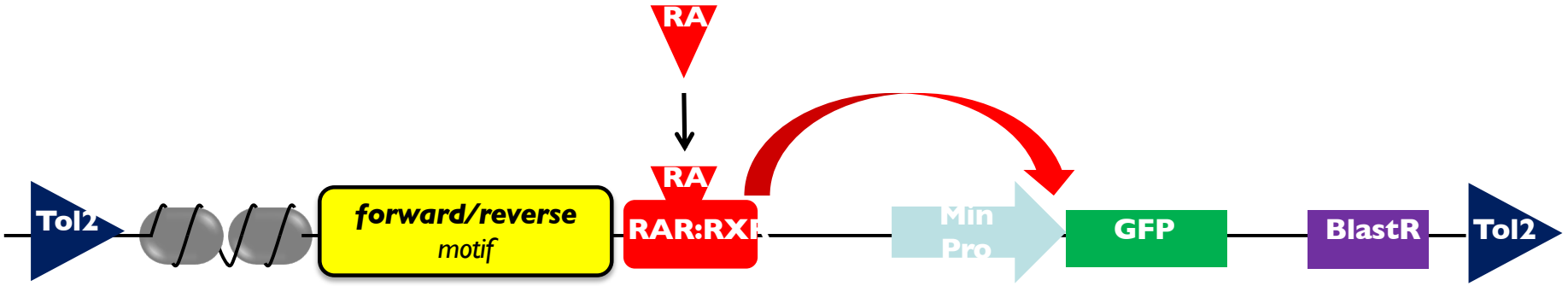


Certain pioneer TFs are directional

- We define asymmetry index as the expected change between left and right sides in (squared) chromatin opening index score

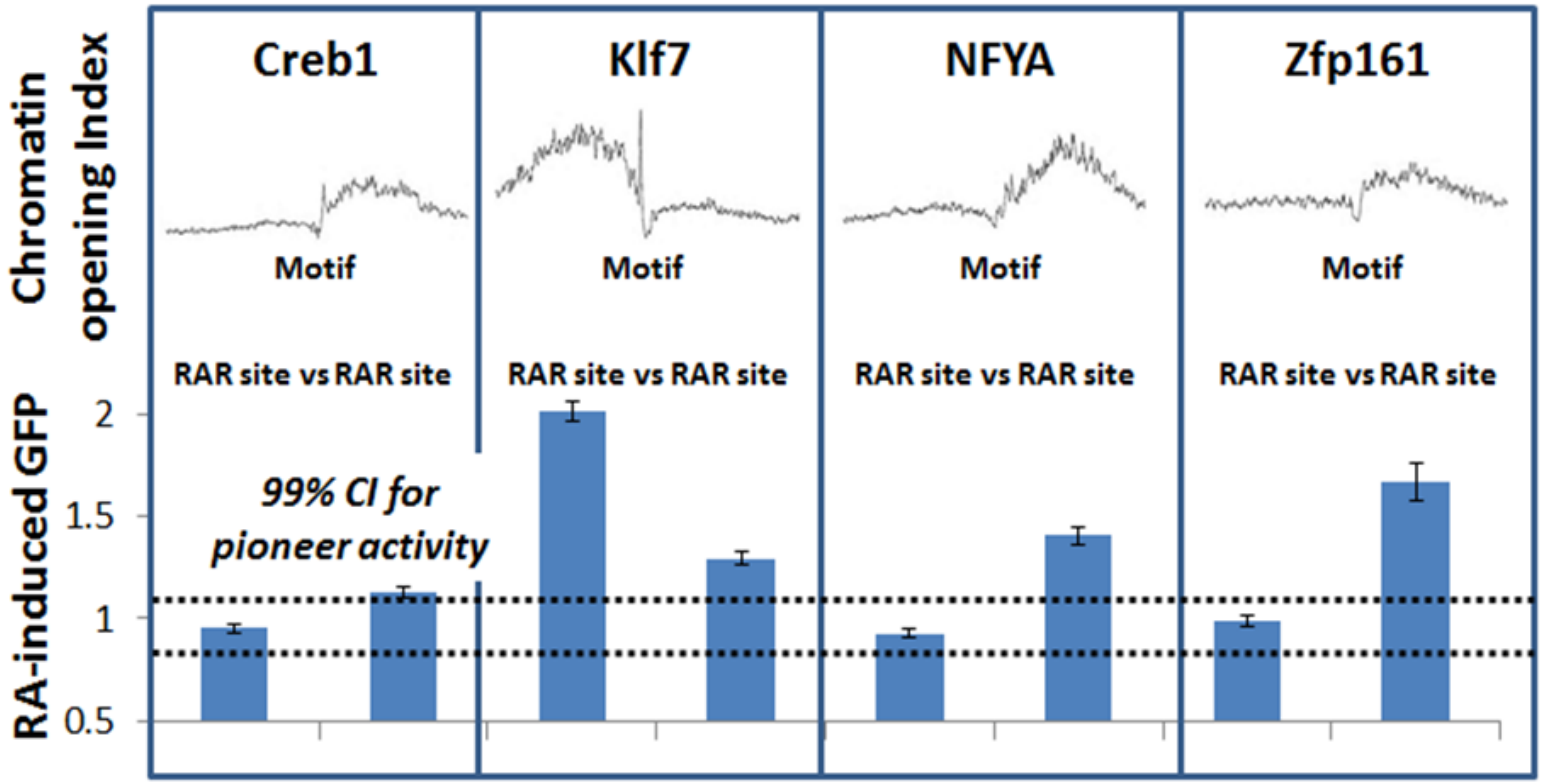


- Biological validation by testing both motif orientations



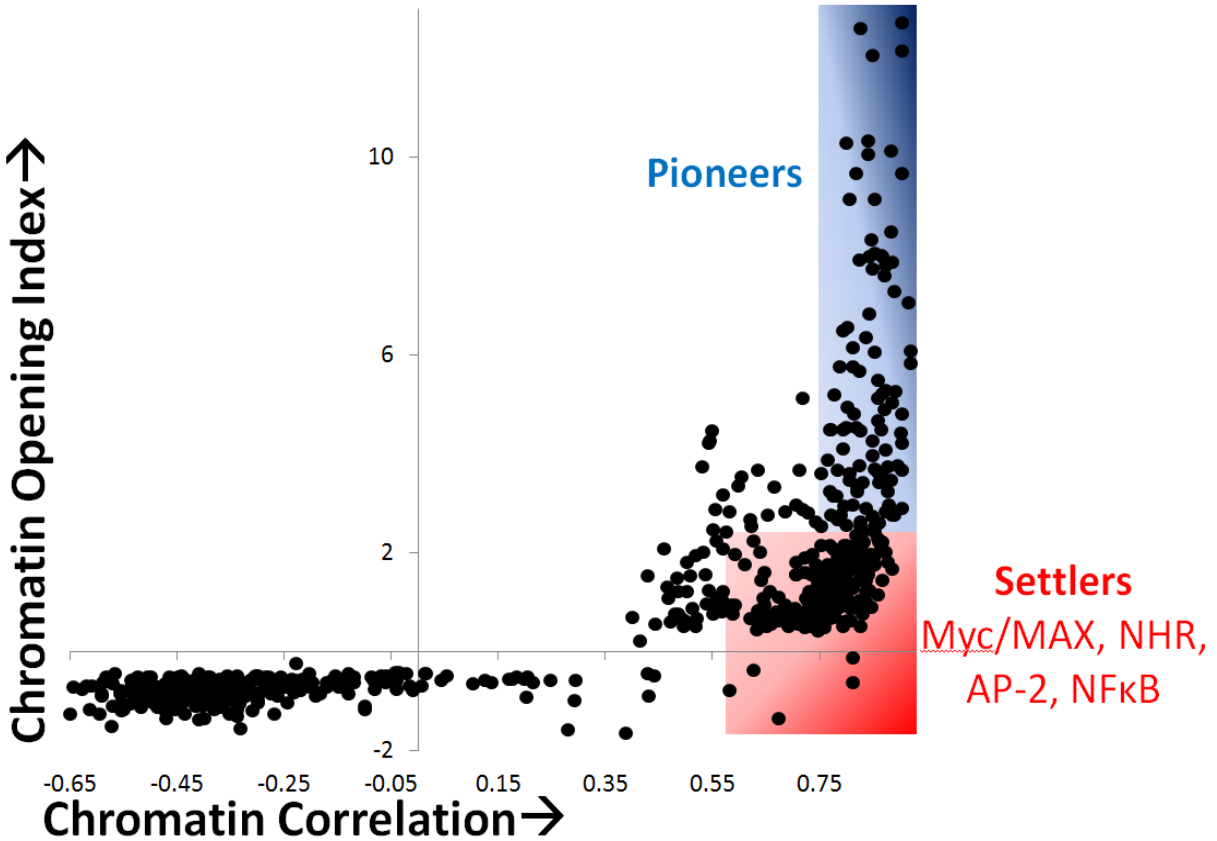
Certain pioneer TFs are directional

Orienting the motif direction in the reporter recapitulates expected directional behaviors.

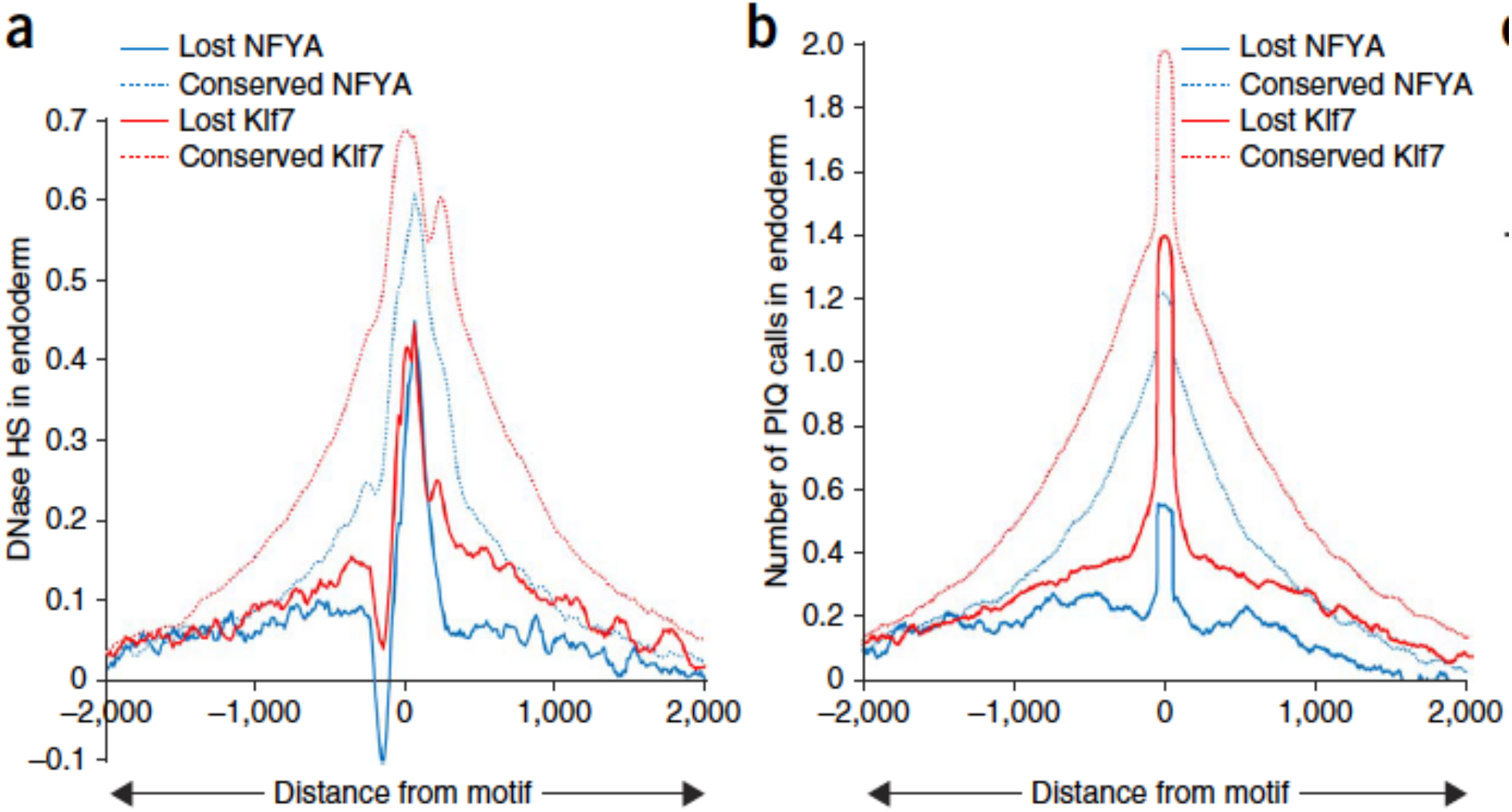


Settler factors follow pioneer factor binding and loss of pioneer binding causes chromatin to return to a closed state

Pioneers (chromatin opening and dependent) are rare and distinct, while there exists a class of chromatin dependent, but non-opening factors.

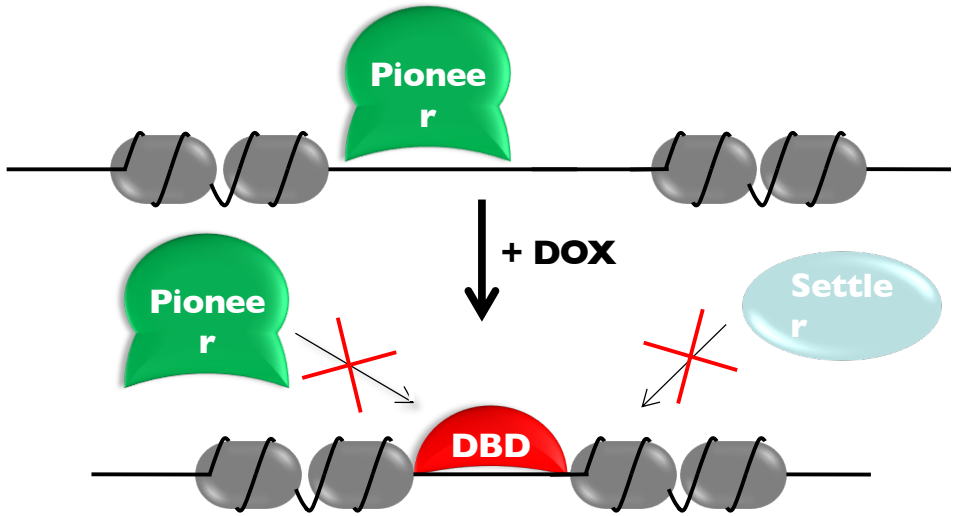


Loss of pioneer binding causes chromatin to return to closed states



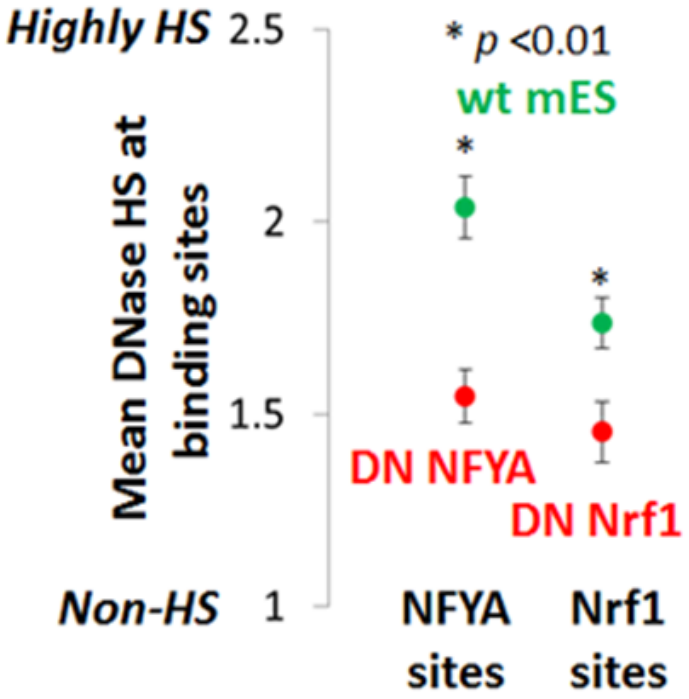
Validate pioneer/settler model via dominant-negative competition assay

- Construct pioneer DBD protein that retains no pioneering function
- Induction of DBD protein competes for genomic binding, reducing local chromatin accessibility settlers rely on
- Compare proximal chromatin openness
- Compare ChIP levels for neighboring settler binding

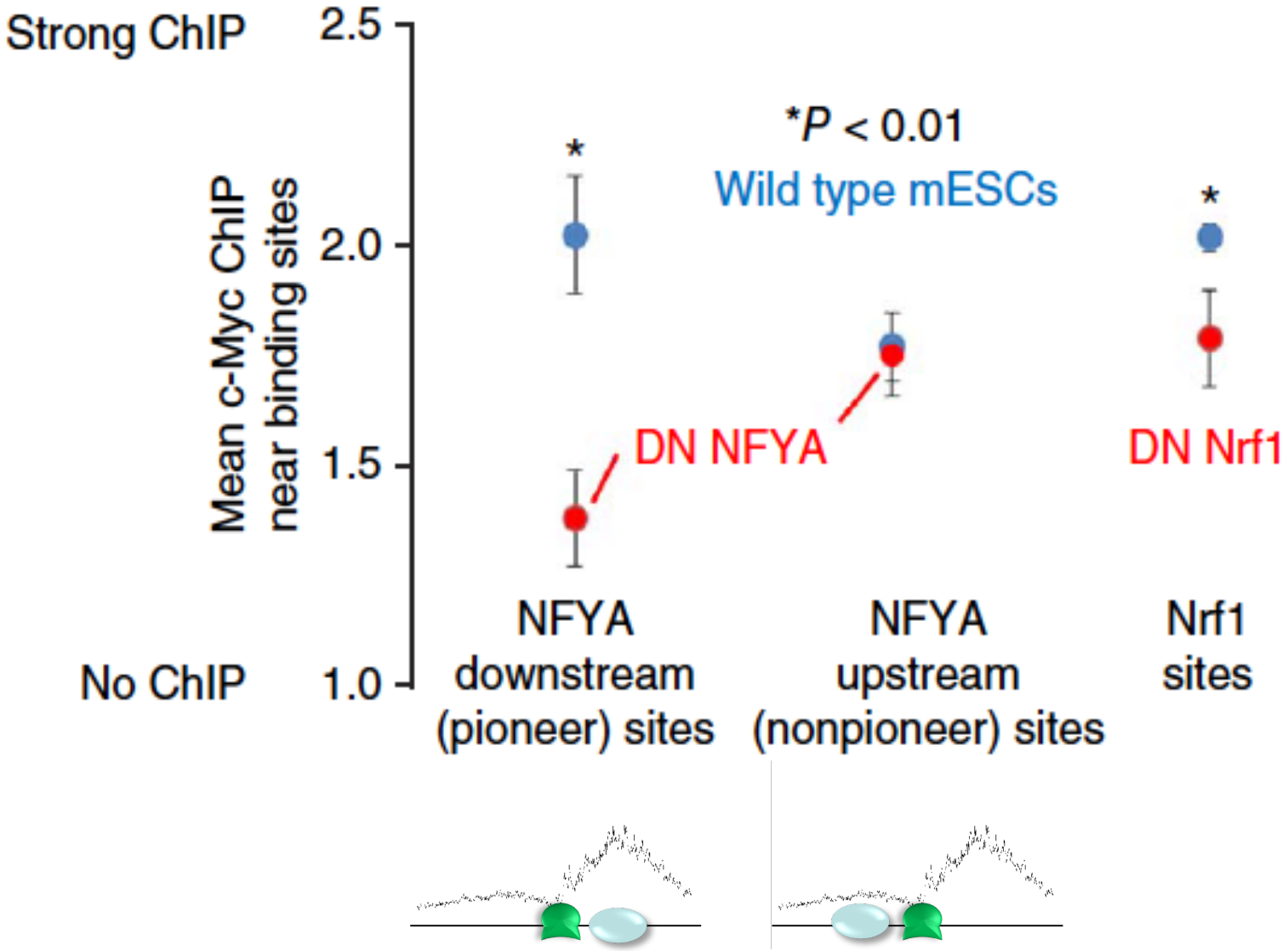


Dominant negative pioneers reduce proximal DNase HS

We created dominant negative versions of the NFYA and Nrf1 pioneers and measured DNase accessibility at native NFYA and Nrf1 sites after induction of dominant negatives.



Dominant negative pioneers reduce proximal binding of c-Myc

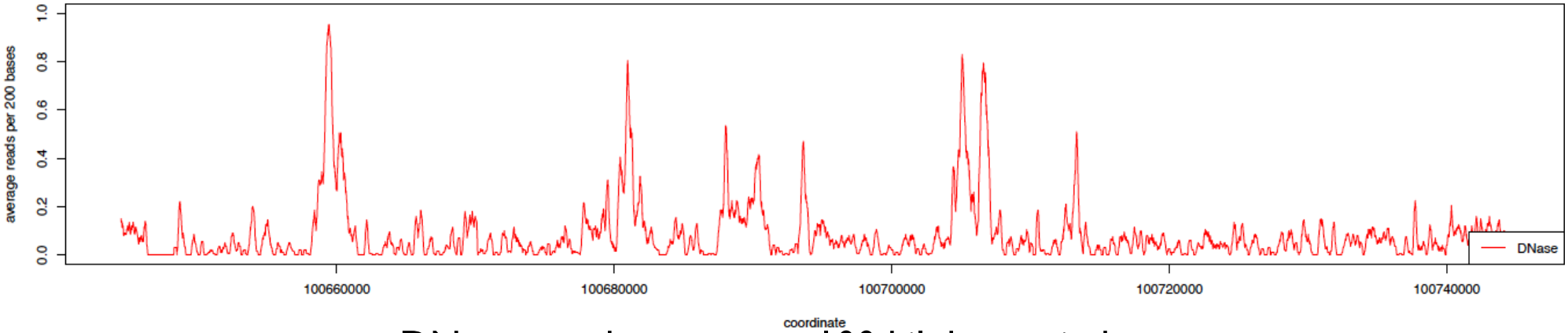


Predicting chromatin accessibility

How genome sequence determines cell-type specific chromatin accessibility

Hashimoto TB, et al. "**A Synergistic DNA Logic Predicts Genome-wide Chromatin Accessibility"** *Genome Research* 2016

Can we predict chromatin accessibility directly from DNA sequence?



DNase-seq data across a 100 kilobase window
(Chromosome 14 K562 cells)

Motivation –

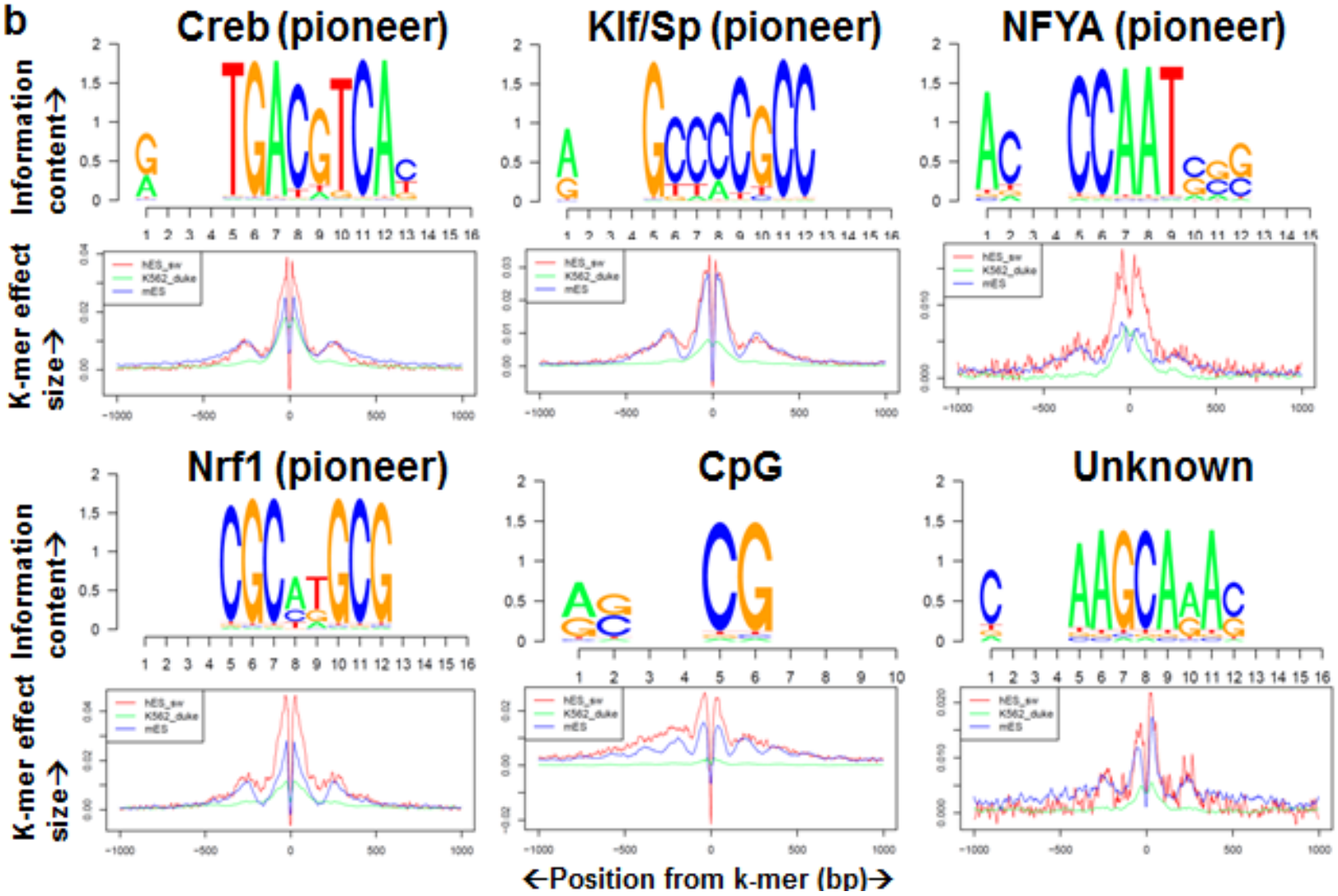
1. Understand the fundamental biology of chromatin accessibility

2. Predict how genomic variants change chromatin accessibility

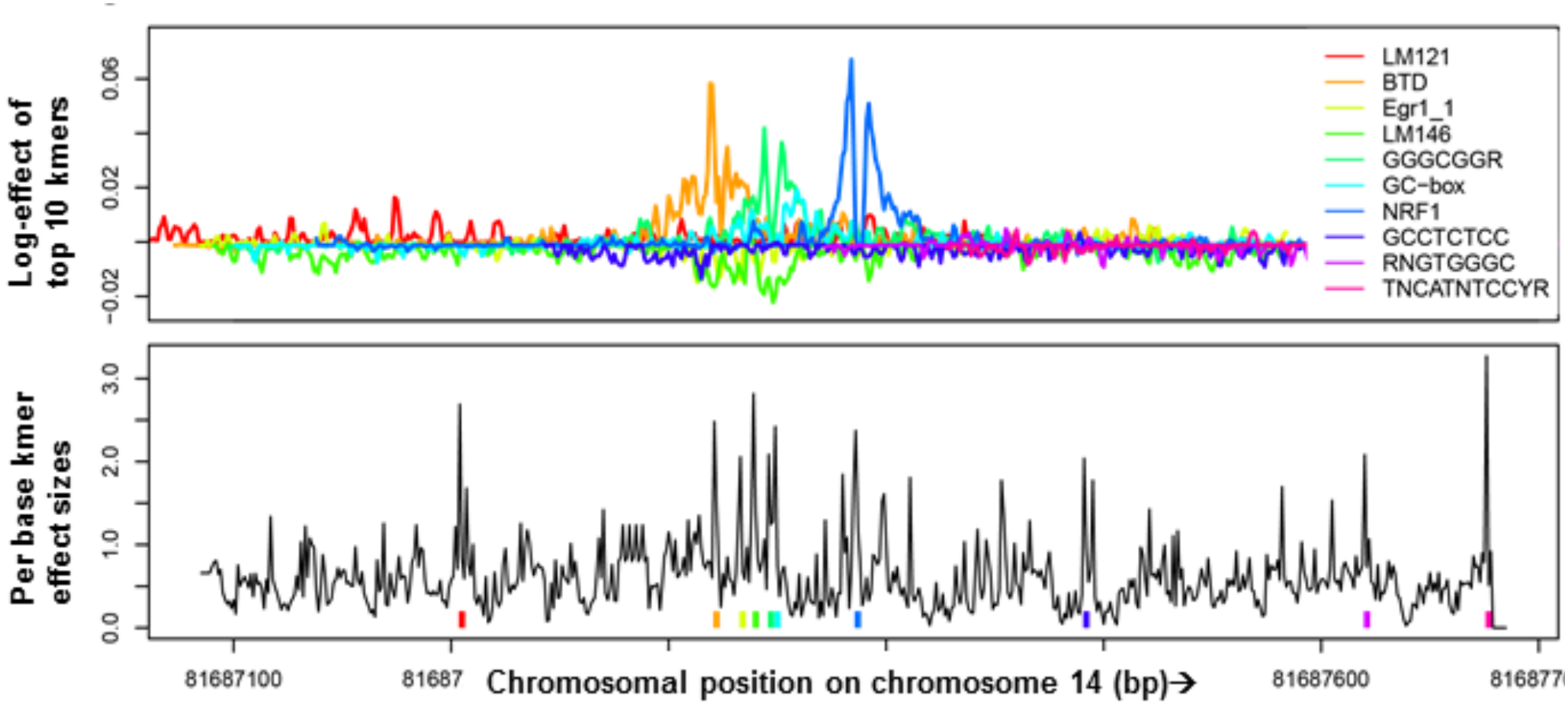
Can we discover DNA “code words” encoding chromatin accessibility?

- The DNA “code words” encoding chromatin accessibility can be represented by k-mers (k <= 8)
- K-mers affect chromatin accessibility locally within +/- 1 kb with a fixed spatial profile
- A particular k-mer produces the same effect wherever it occurs

Chromatin accessibility arises from interactions, largely among pioneer TFs

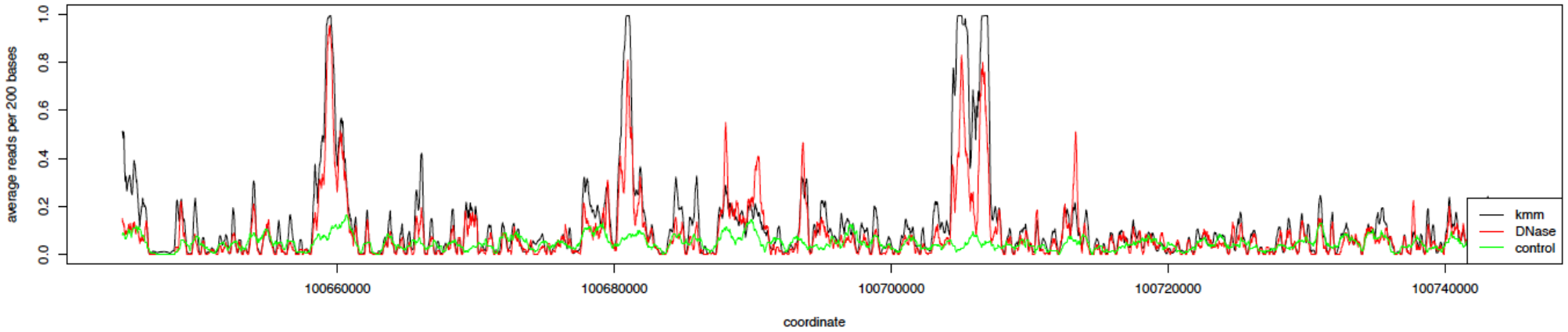


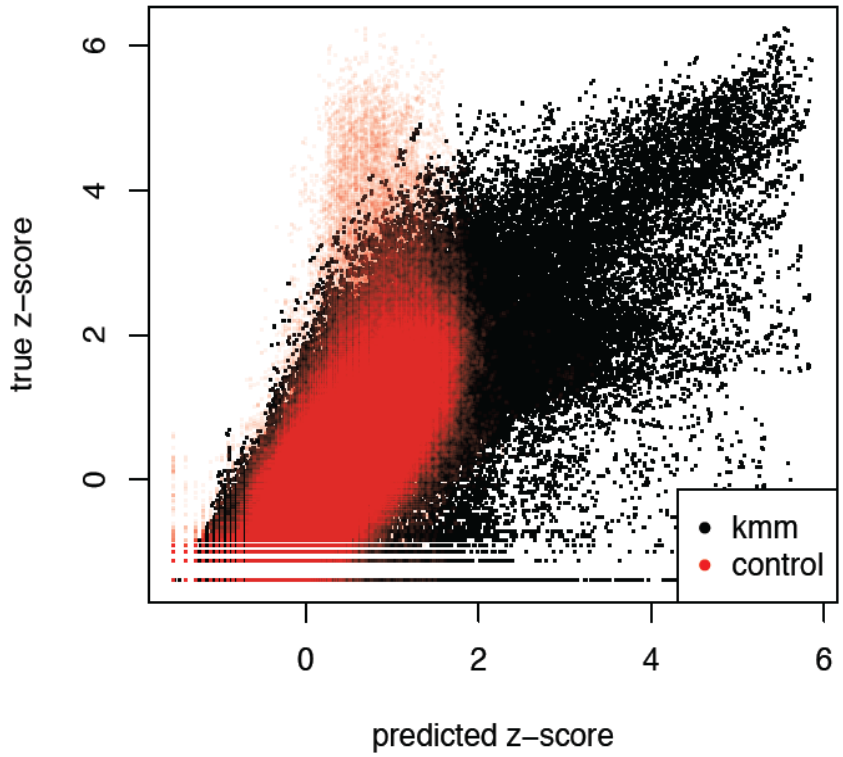
The Synergistic Chromatin Model (SCM) is a K-mer model



~40,000 K-mers in model
~5,000,000 parameters
543 iterations * 360 seconds / iteration * 40 cores
= ~ 90 days

Training on K562 DNase-seq data from chromosomes 1 – 13 predicts chromosome 14 (black line)

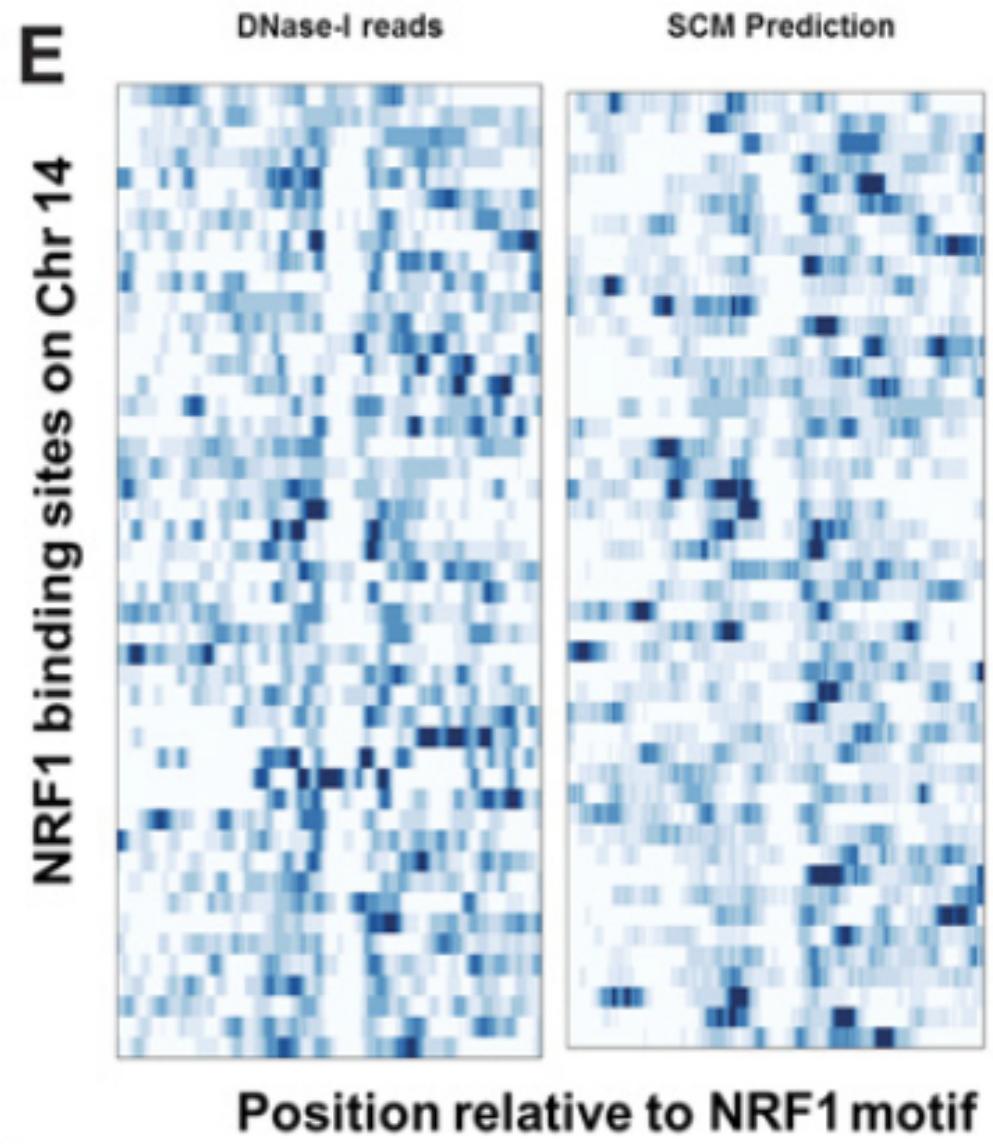




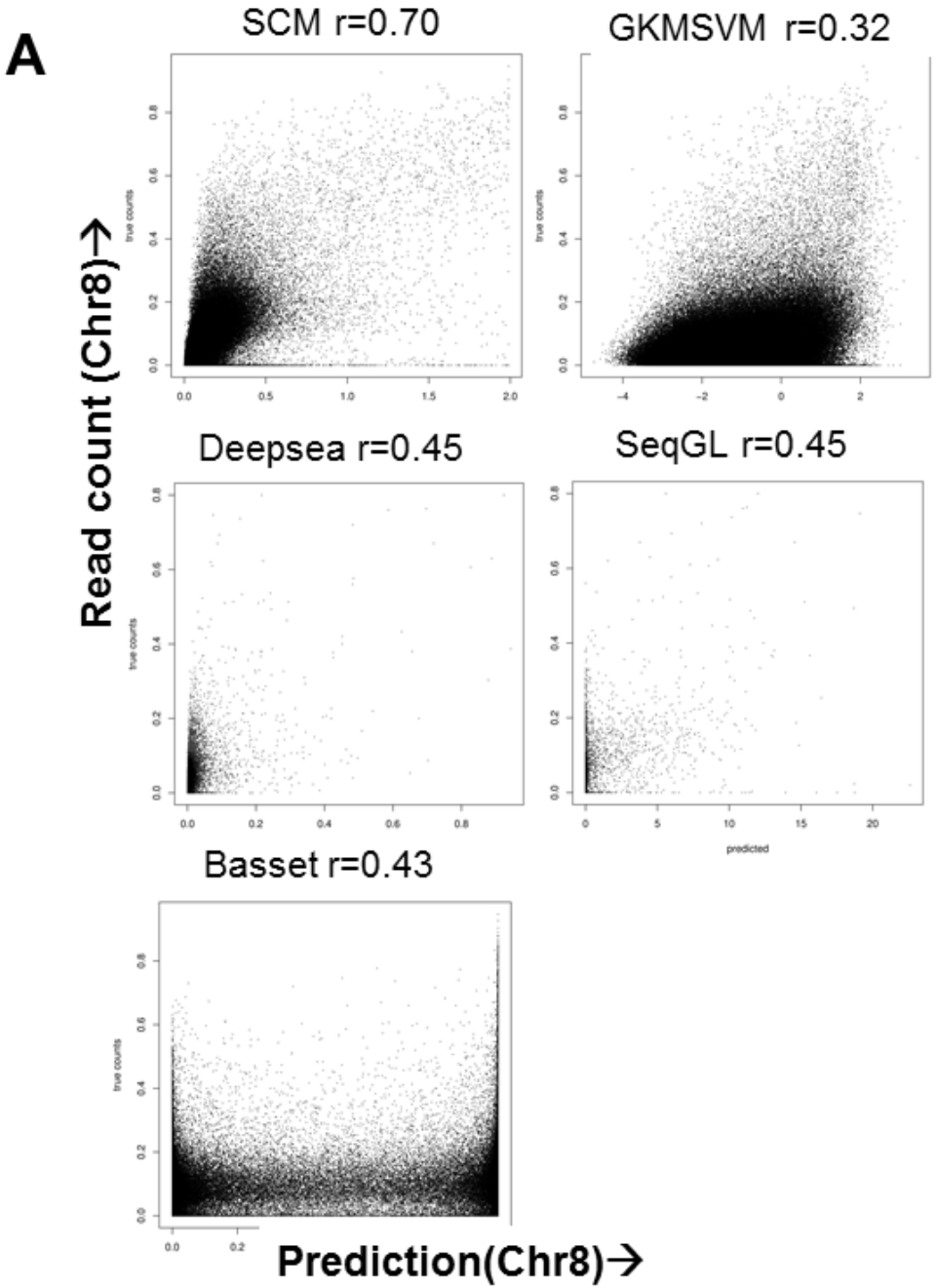
KMM R^2 0.80

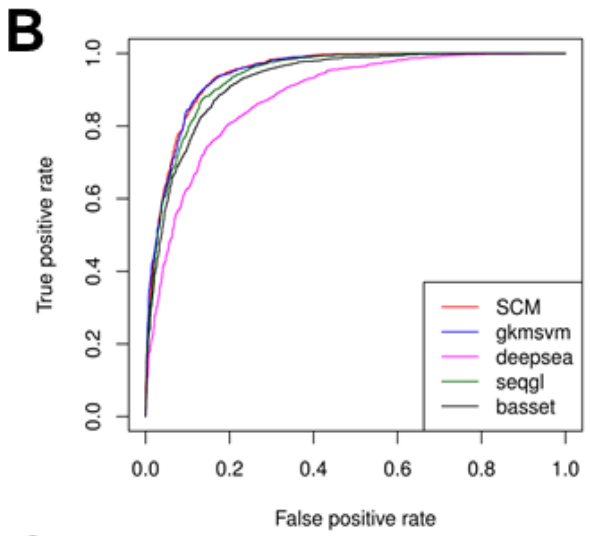
Control R^2 0.47

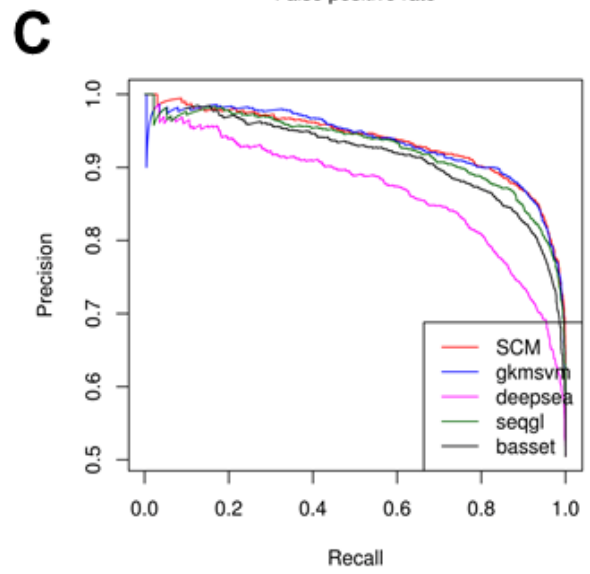
SCM predicts accessibility data from a NRF1 binding site



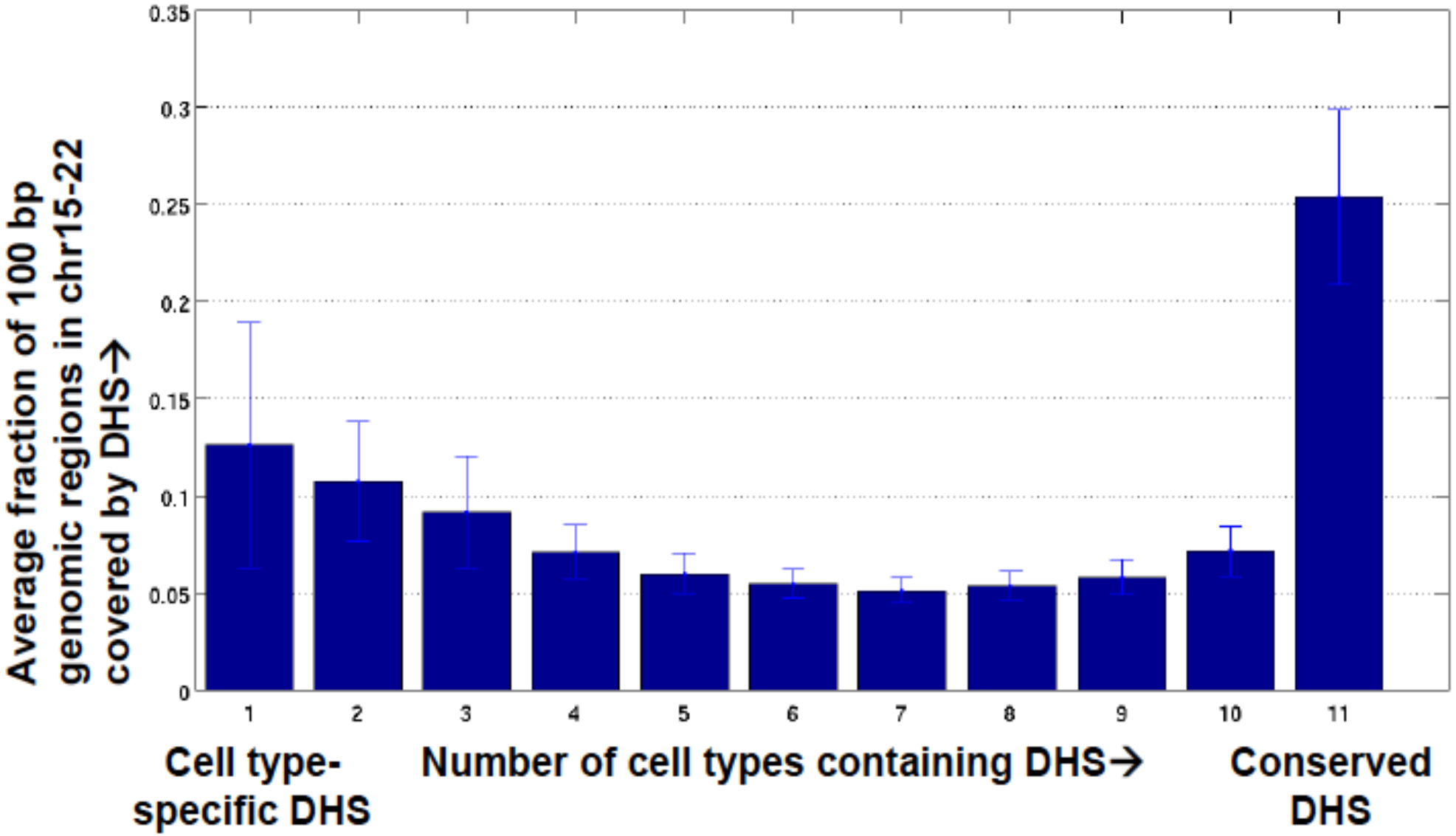
SCM outperforms contemporary models at predicting chromatin accessibility from sequence (K562)



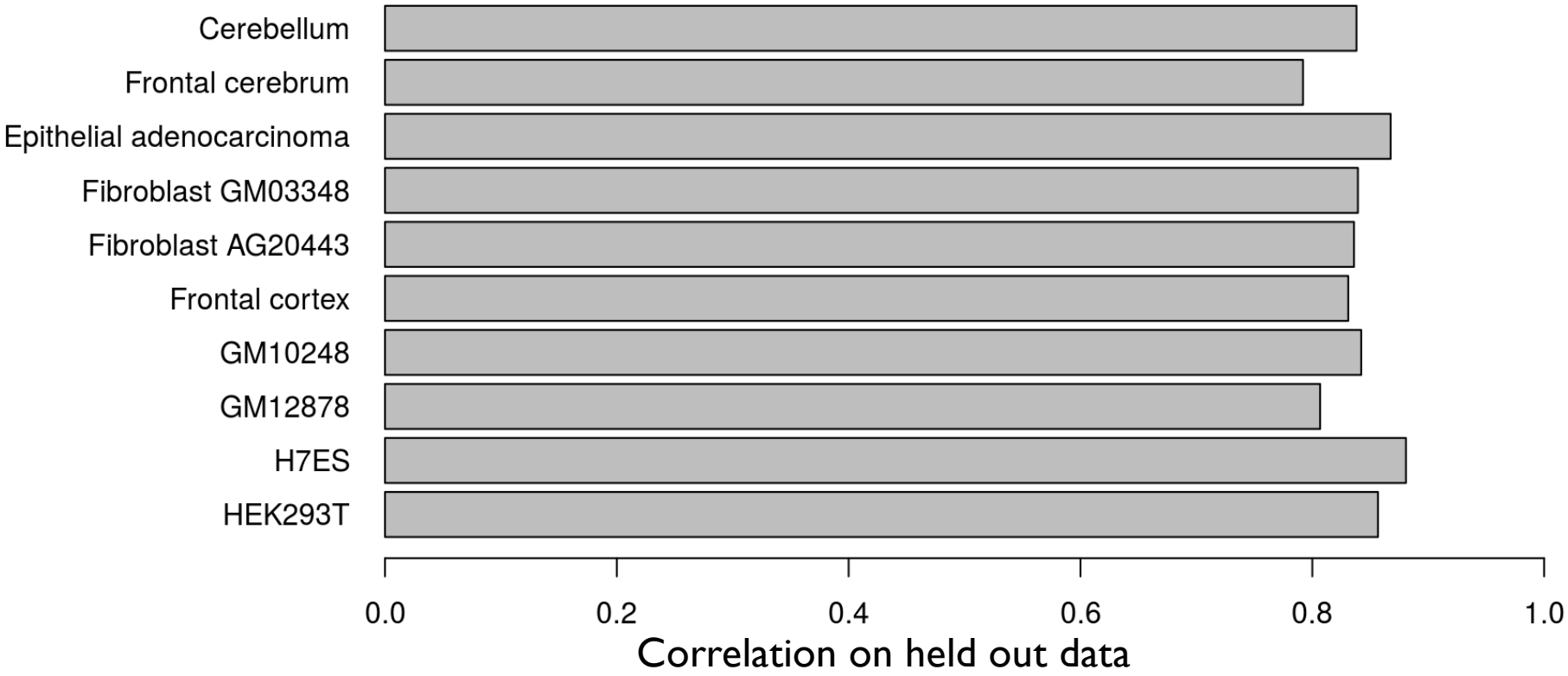




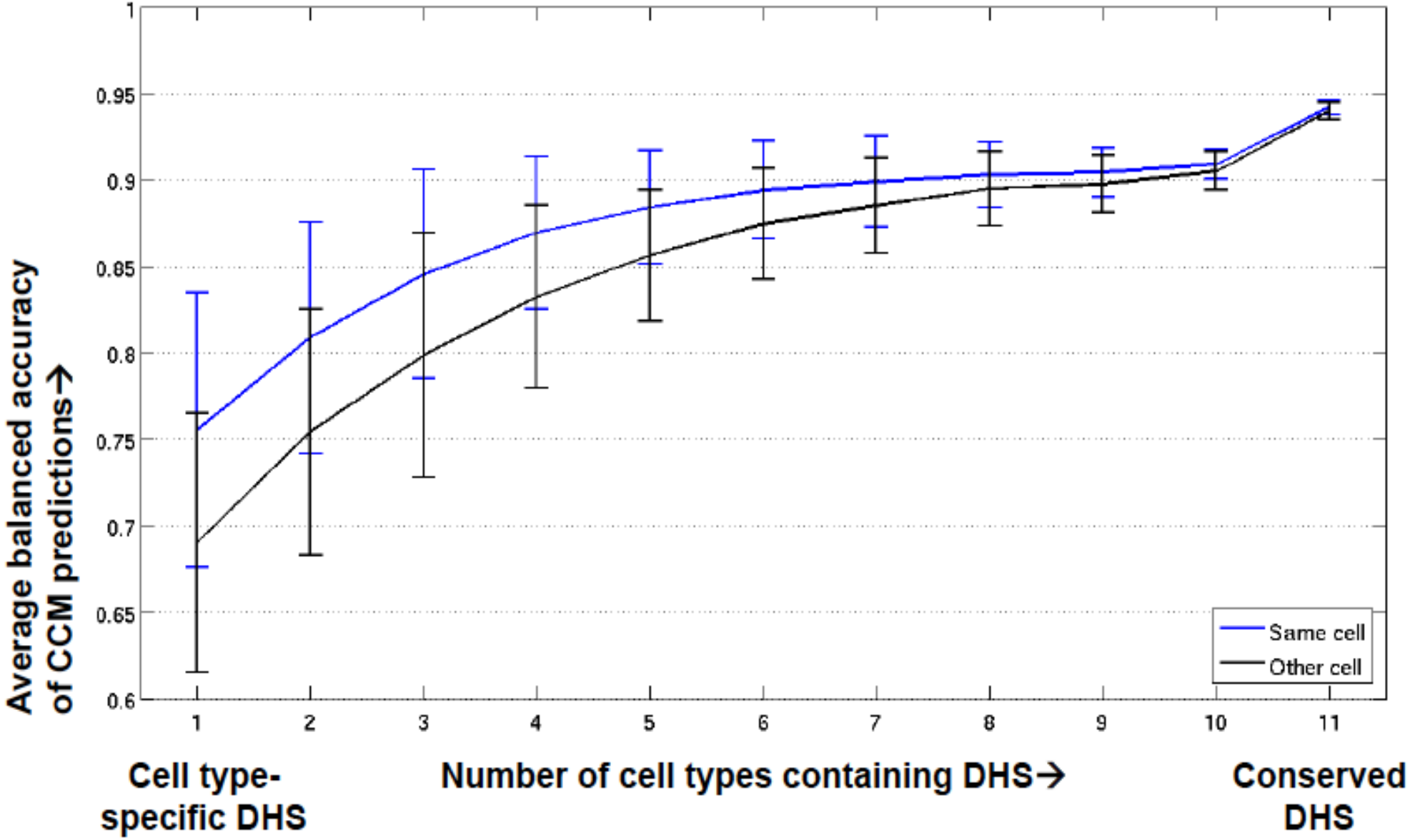
Accessibility contains cell type specific and cell type independent components (11 cell types, Chr 15-22)



SCM models have similar predictive power for other cell types

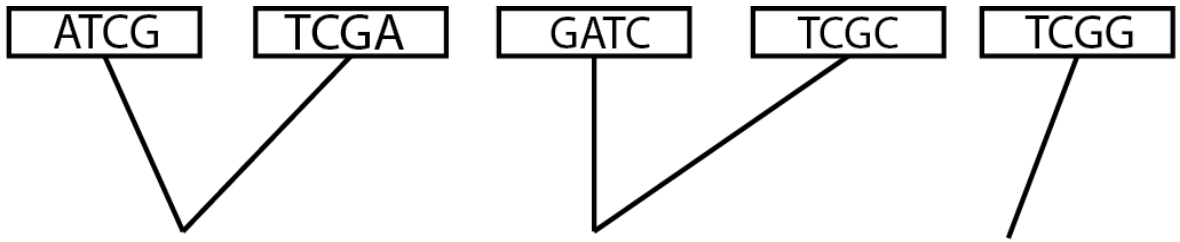


SCM model trained on ES data performs better on shared DNase hot spots (Chr 15 – 22)

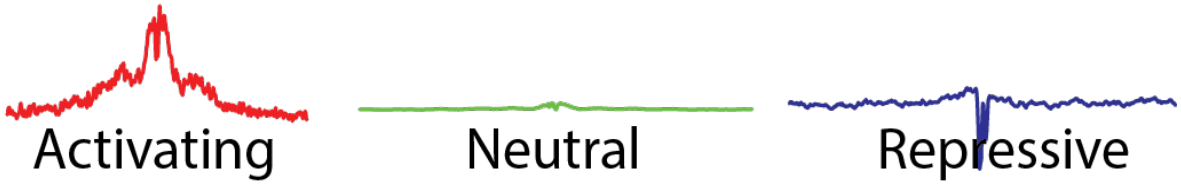


We created synthetic "phrases" each of which contains k-mers that are similar in chromatin opening score

All K-mers



Sort into classes

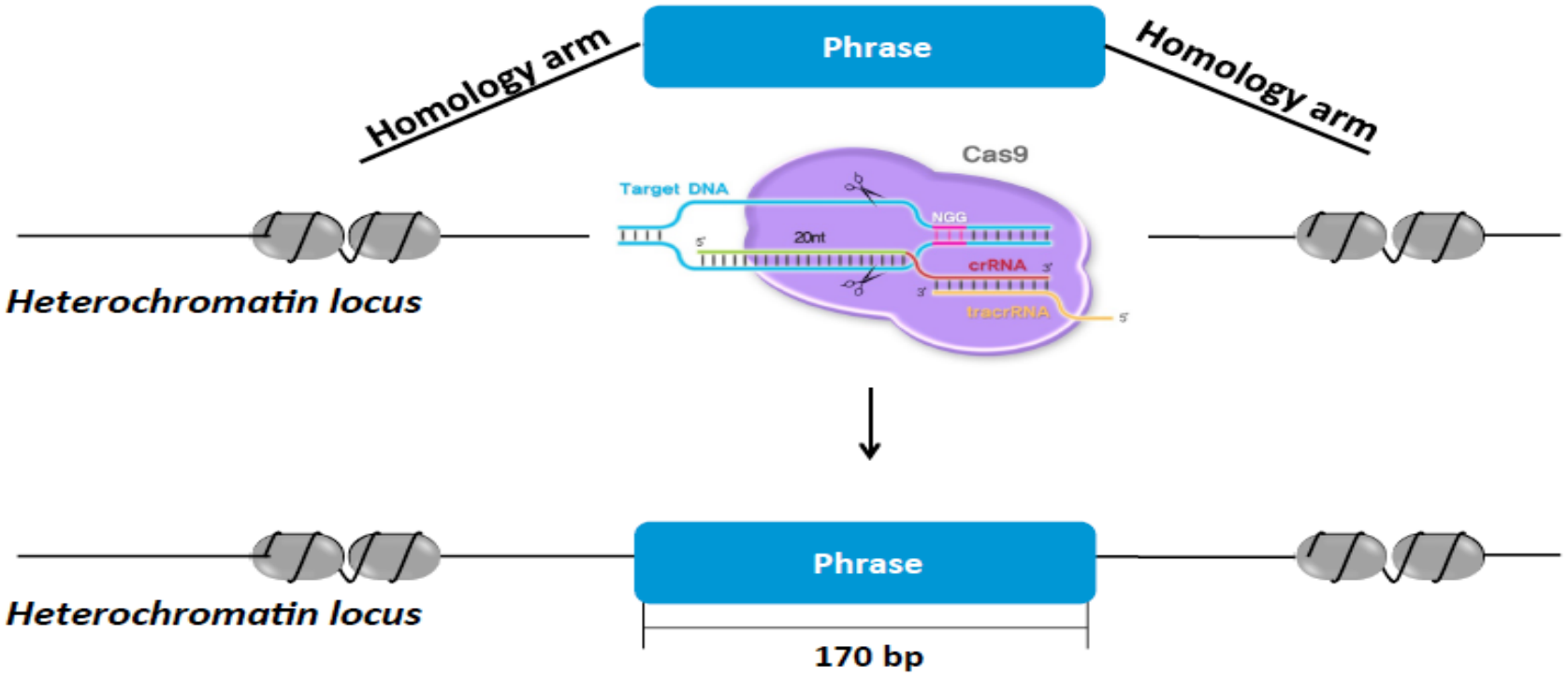


Construct Debrujin Graph

Biased random walk

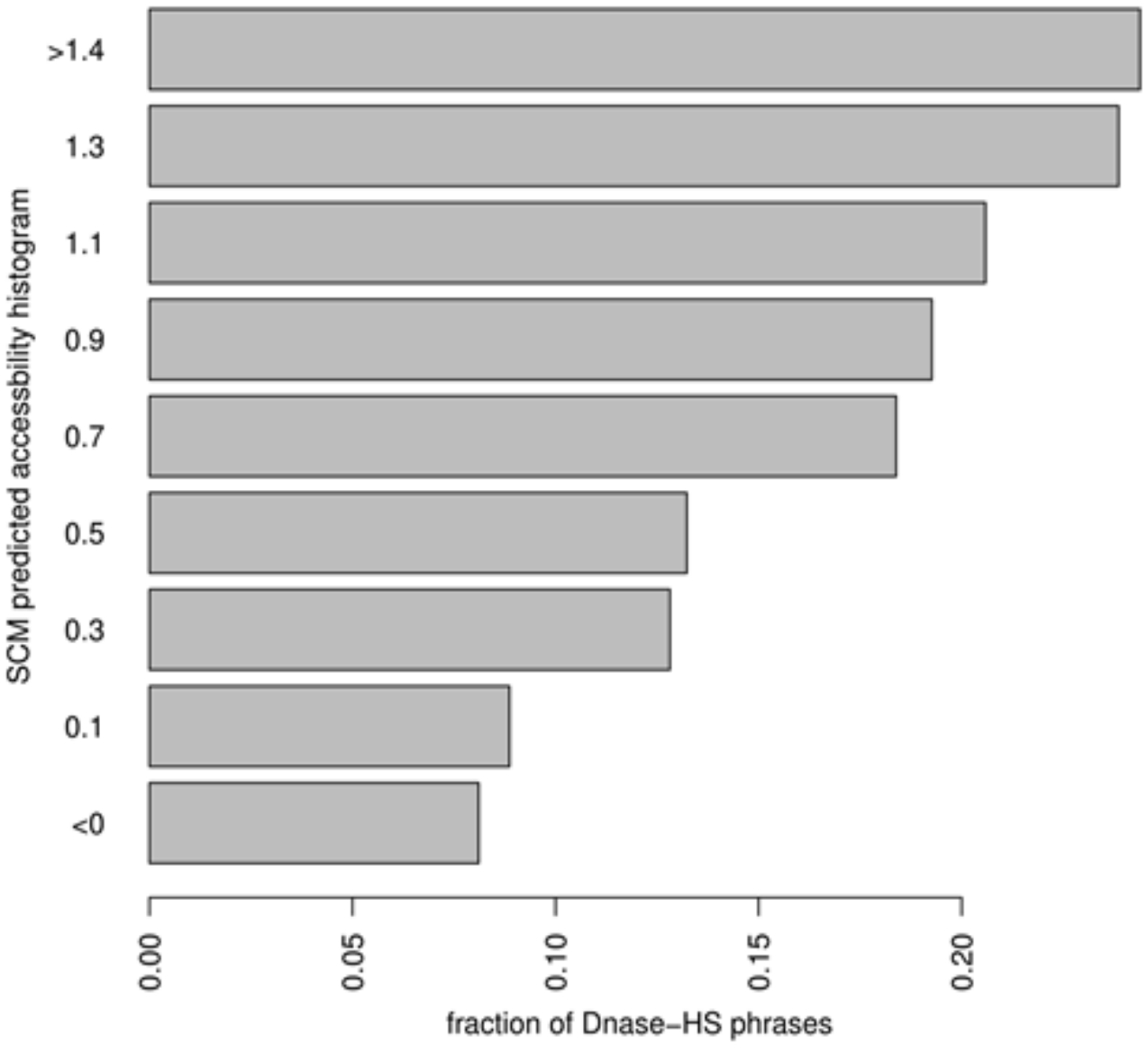
1. GATCGC
2. GATCGA

Single Locus Oligonucleotide Transfer
>6,000 designed phrases into a chromosomal locus



Heterochromatin locus	A	B	C
% alleles with phrase integration	35	15	15
# unique integrations	350,000	150,000	150,000

Predicted accessibility matches measured accessibility



FIN