Computational Systems Biology
Deep Learning in the Life Sciences

6.802 6.874 20.390 20.490 HST.506

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Lecture 5
March 2, 2017

Characterizing Cellular Regulation

http://mit6874.github.io

Overall goal for today

- Understand models of the two levels of cellular regulation: chromatin accessibility and transcription factor cis-effects
- Recall issues in model selection given limited data

Today's lecture

- Model selection
  - Model performance as a function of capacity
- Models of chromatin accessibility
  - Log-linear models based on k-mers
  - What can we learn about biology from models?
- Models of gene expression
  - Regression trees provide interpretable models

Part 1 - Model Capacity

Model capacity

- Capacity - ability to fit a wide range of functions (hypothesis space)
- Vapnik-Chervonenkis dimension - size of largest unique training set of examples a binary classifier can label arbitrarily is a measure of capacity

Generalization and Capacity

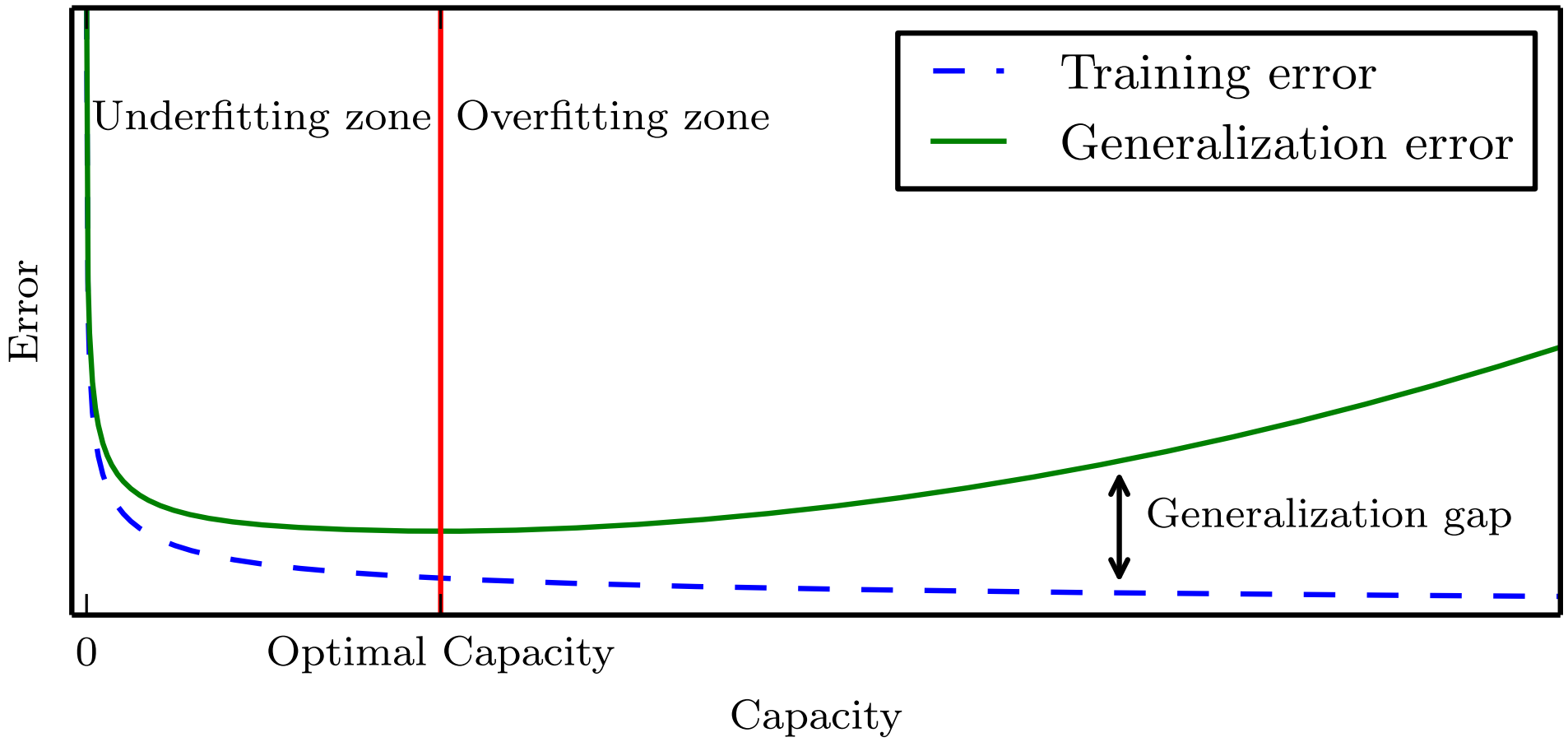


Figure 5.3

The capacity of non-parametric models is defined by the size of their training set

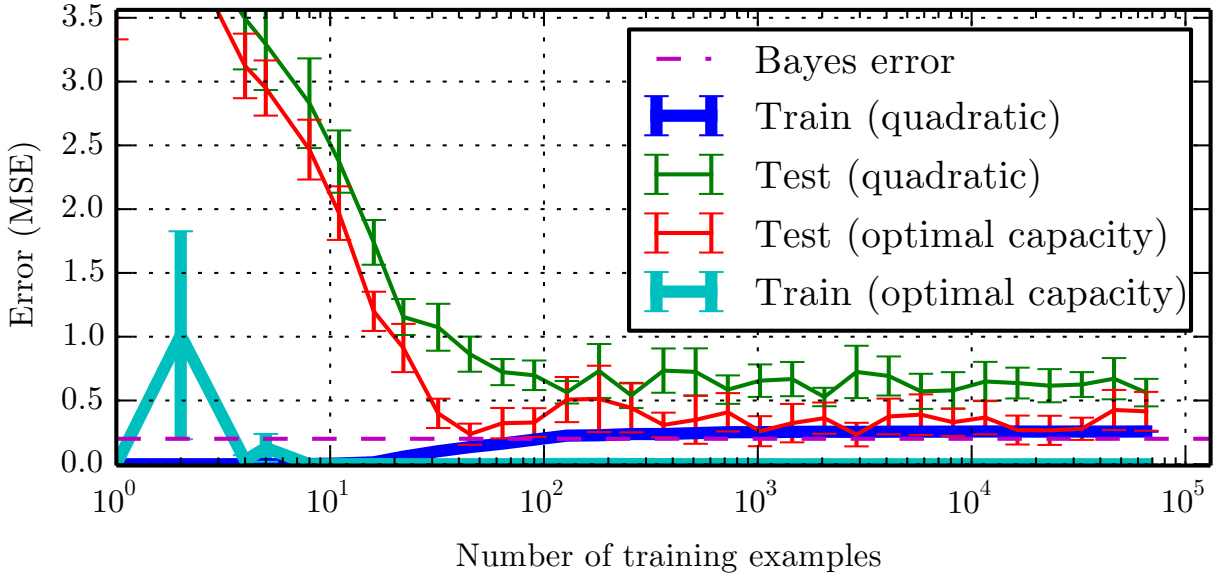
- k-nearest neighbor (KNN) regression computes its output based upon the k “nearest” training examples
- Often the best method, and certainly a baseline to beat

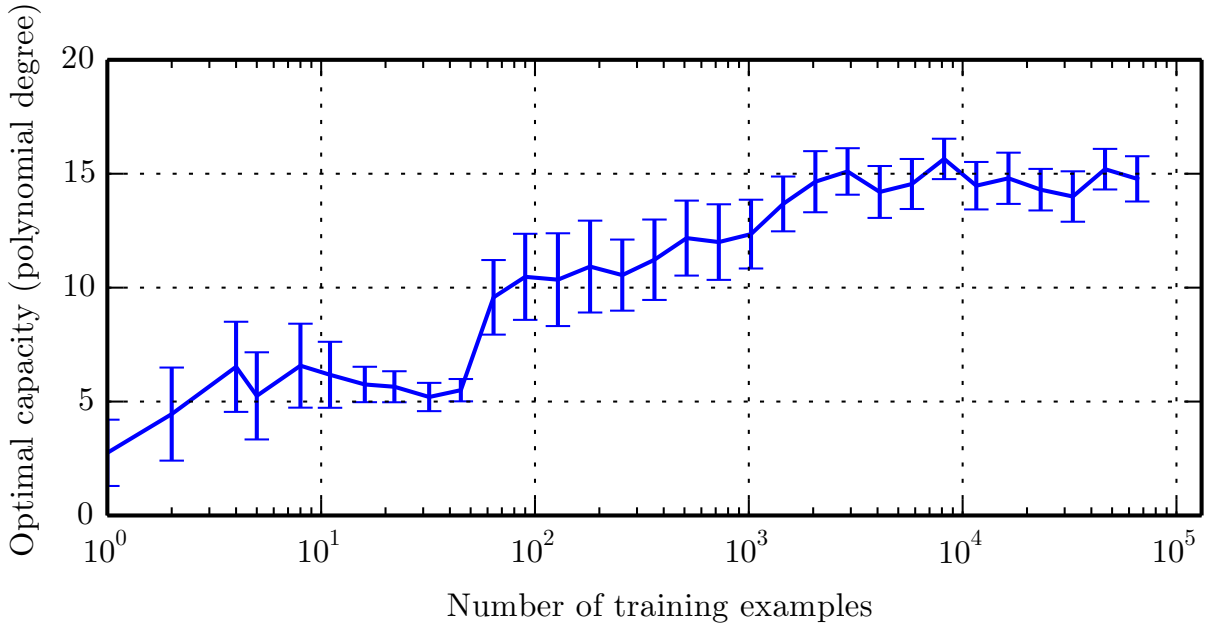
Bayes error is residual noise from confounders and observation noise

- Bayes error is the error made by an Oracle given the training set
- For example, if there is an unobserved variable that affects the labels the Oracle's predictions will be noisy

Sufficient training data are necessary to generalize well

Figure 5.4



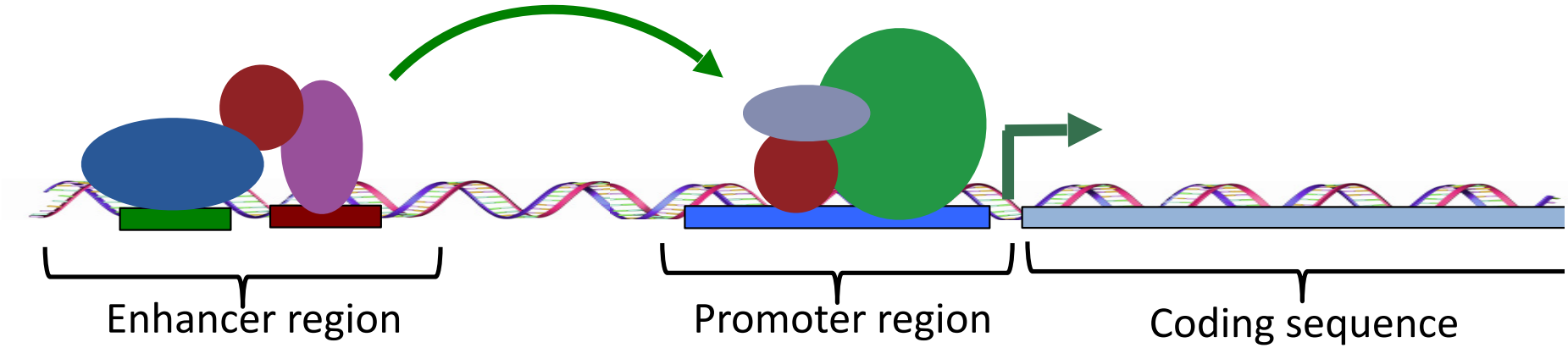


Machine Learning has limits

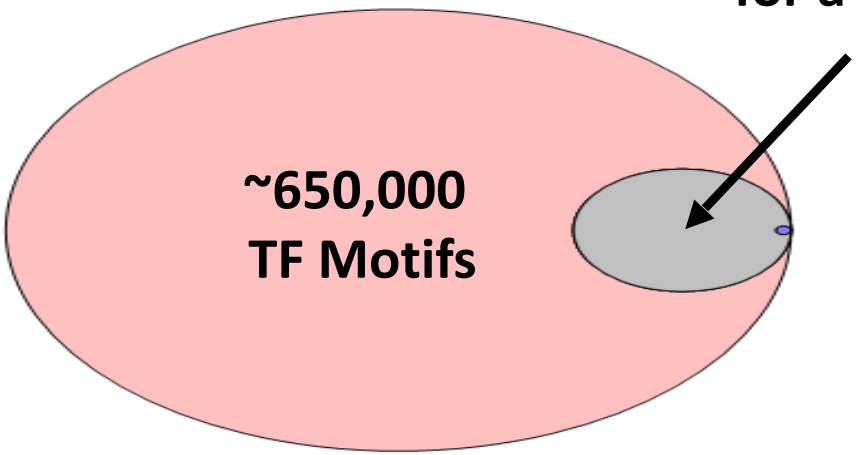
- The *no free lunch* theorem: You can only obtain generalization from finitely many training examples if the algorithm searches a limited hypothesis space.
- We desire a learning algorithm to perform *generalization* and to be *stable*. It generalize if the training error on a data set will converge to the expected error. It is stable if small perturbations in the data result in only small perturbations in the output hypothesis.

Part 2 - Modeling Genome Accessibility

How do transcription factors control activation of cell-type-specific promoters and enhancers?

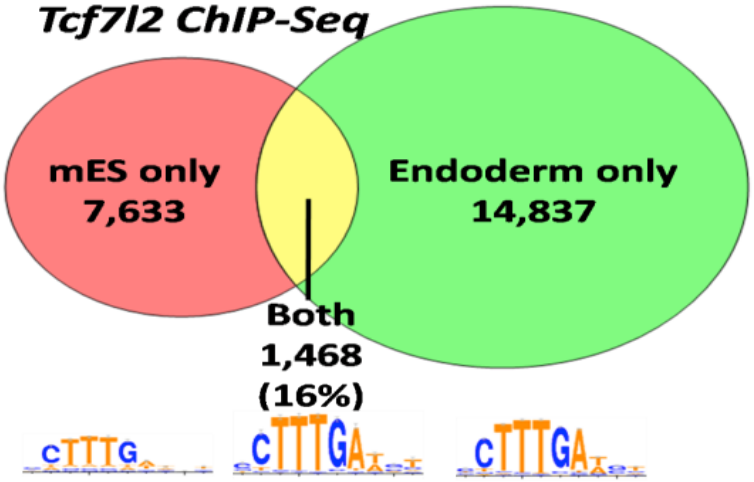


Motifs are insufficient to predict TF binding

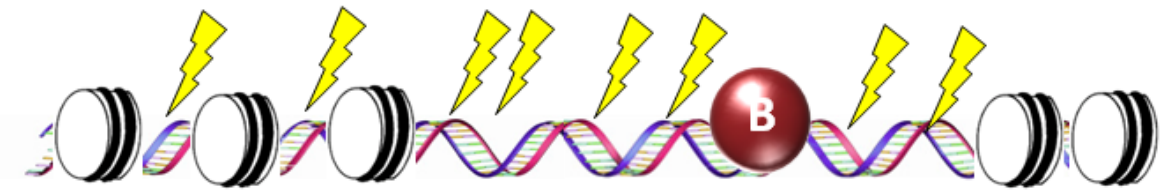


~50,000 binding sites for a typical TF

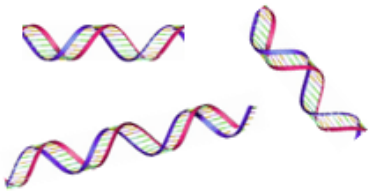
Binding sites change across time



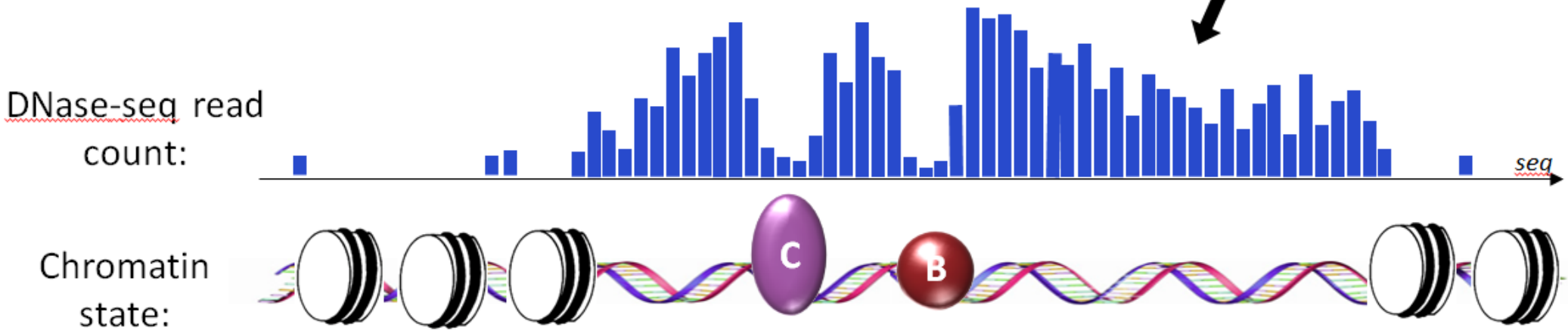
DNase-seq reveals genome protection profiles



Digest nuclei with DNase-I
(concentration/exposure specific)

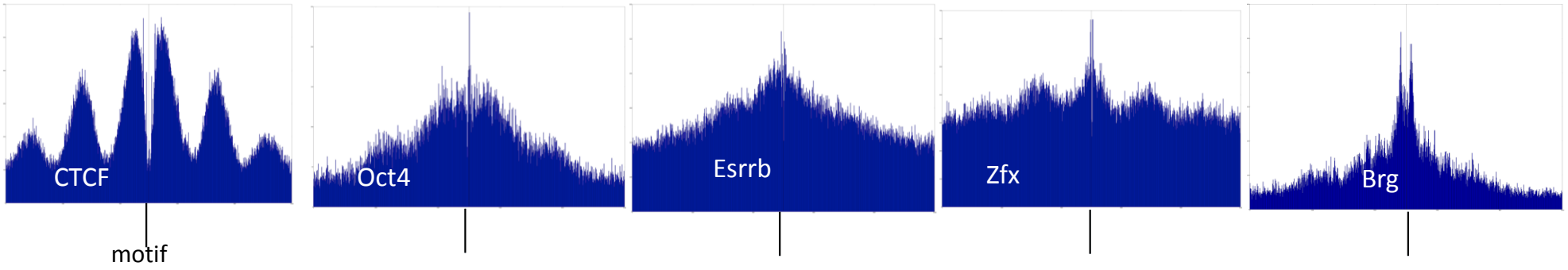


Collect DNA, size
separate(100-300bp)



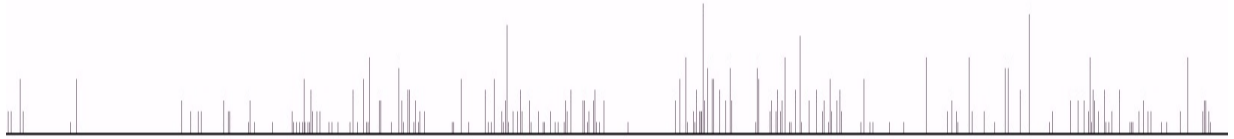
Sequence (60-100M reads)

Bound factors leave distinct DNase-seq profiles

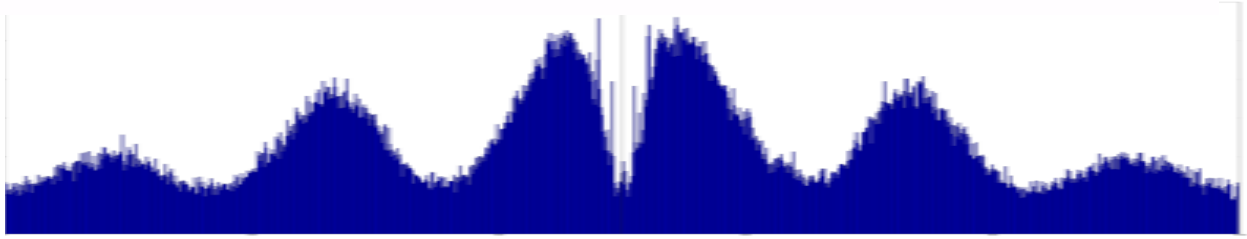


Individual binding site prediction is difficult

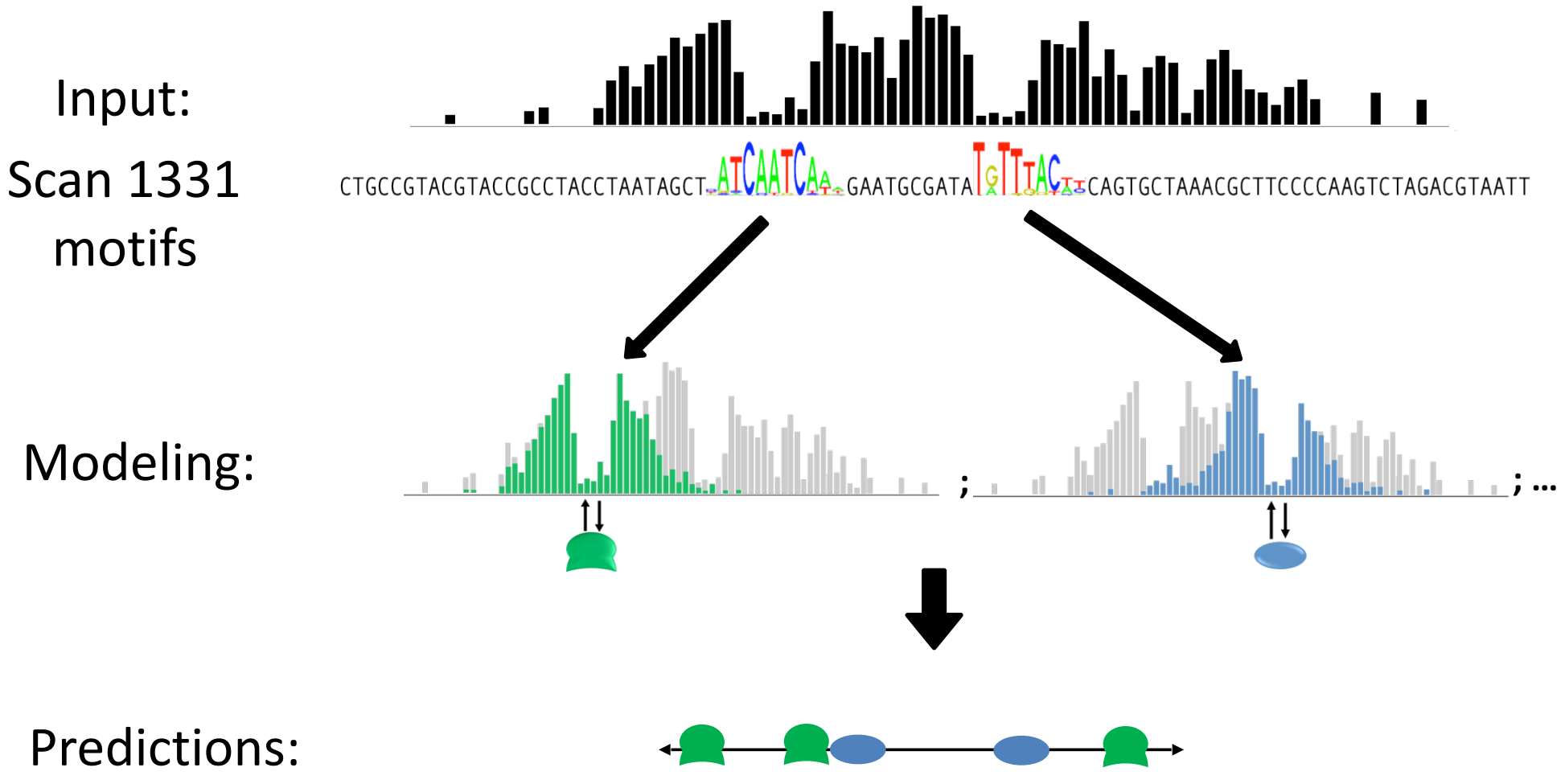
Individual CTCF:



Aggregate CTCF:

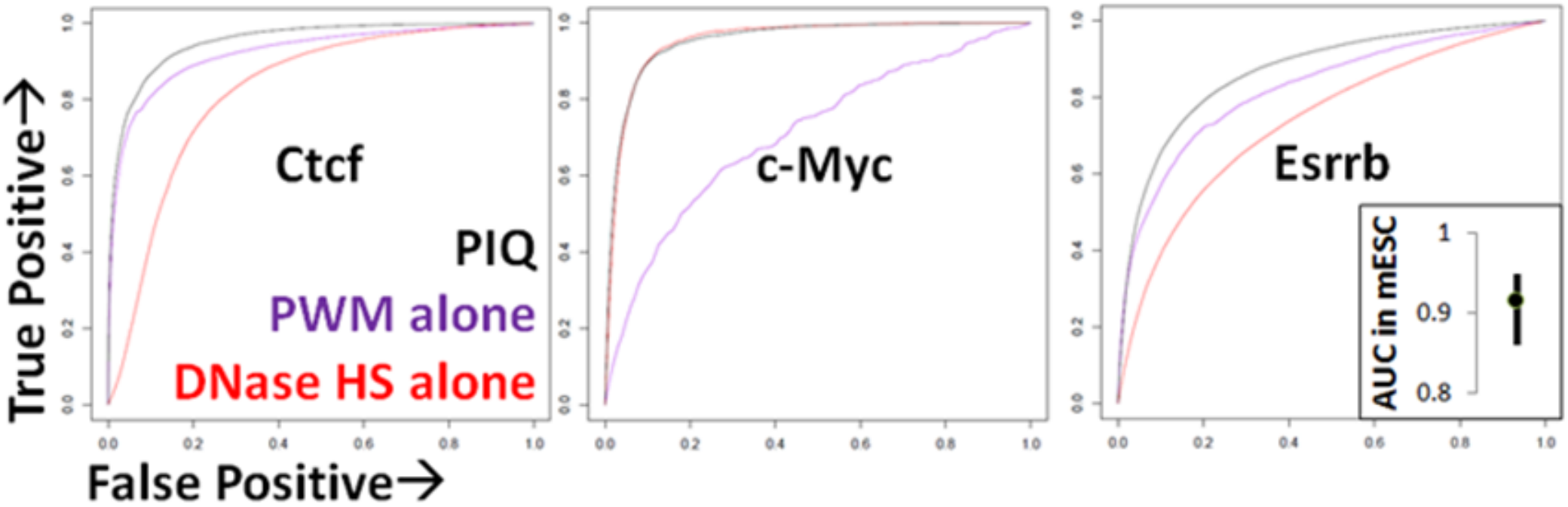


PIQ: algorithm to predictively model TF binding from DNase-seq + Sequence



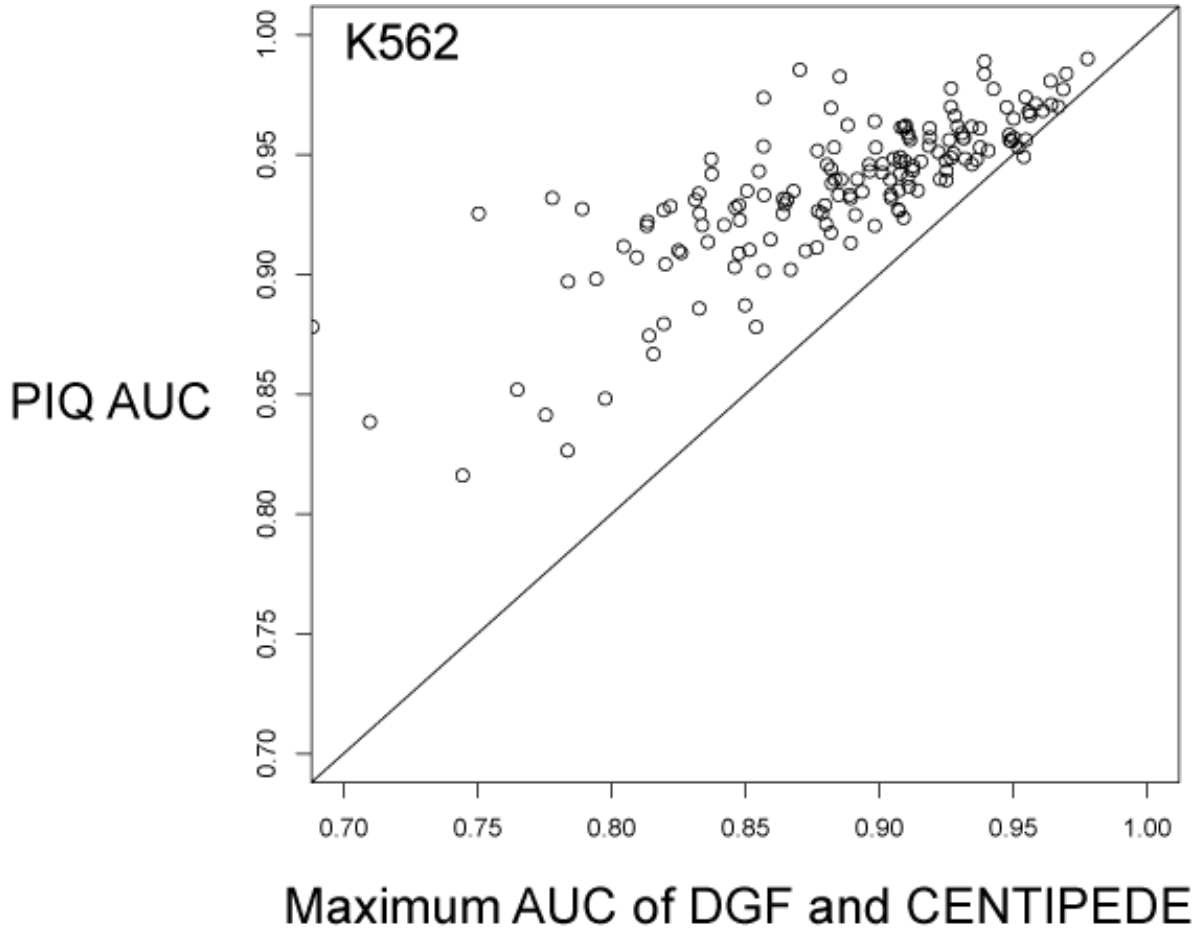
PIQ accurately predicts mESC TF binding

Receiver operating characteristic (ROC) curves show PIQ matches closely with ChIP-seq data.

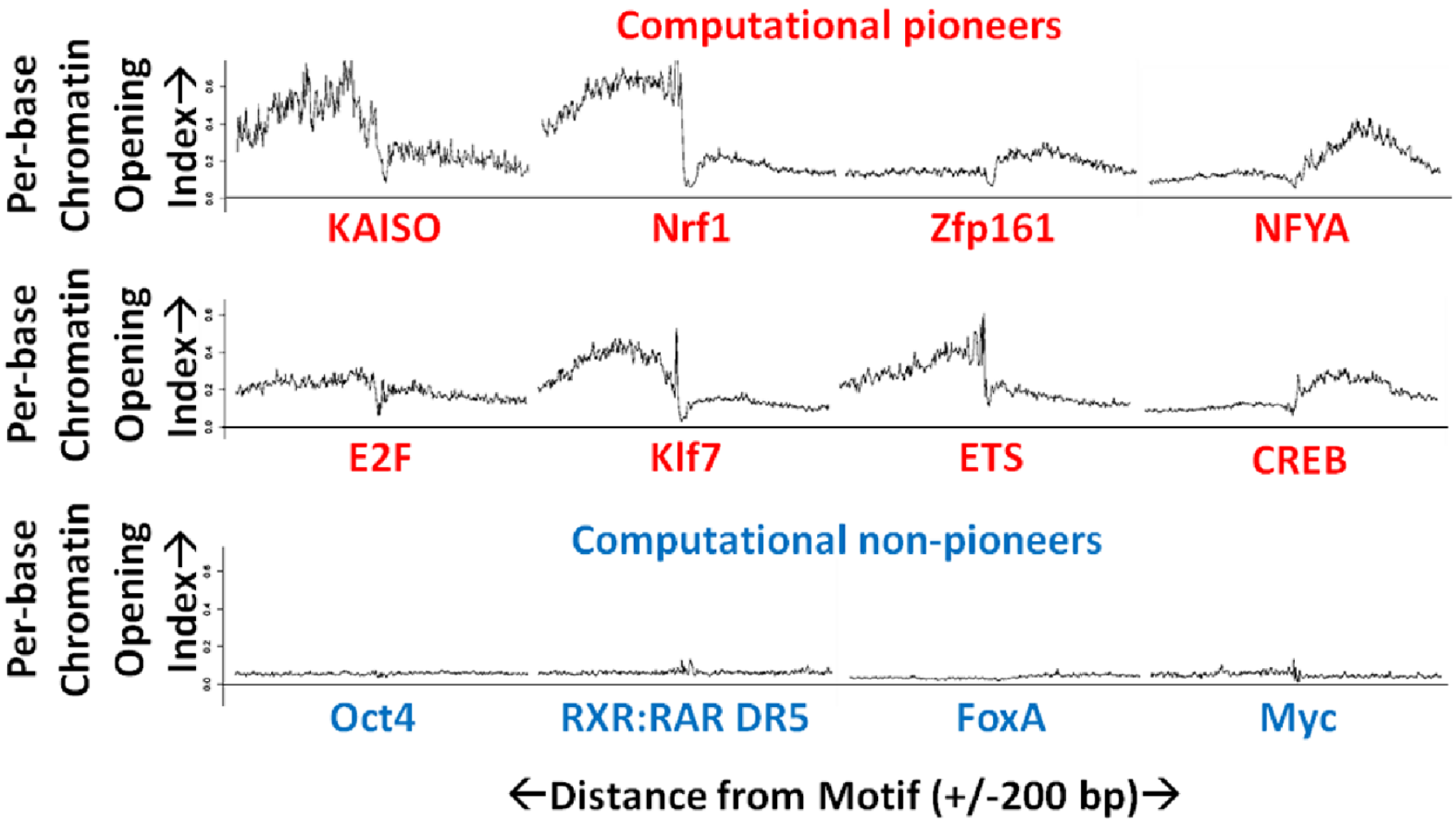


PIQ outperforms existing methods when predicting binding for 313 ENCODE ChIP-seq experiments

PIQ (.93 Mean AUC); Centipede (.87); DGF (.65)

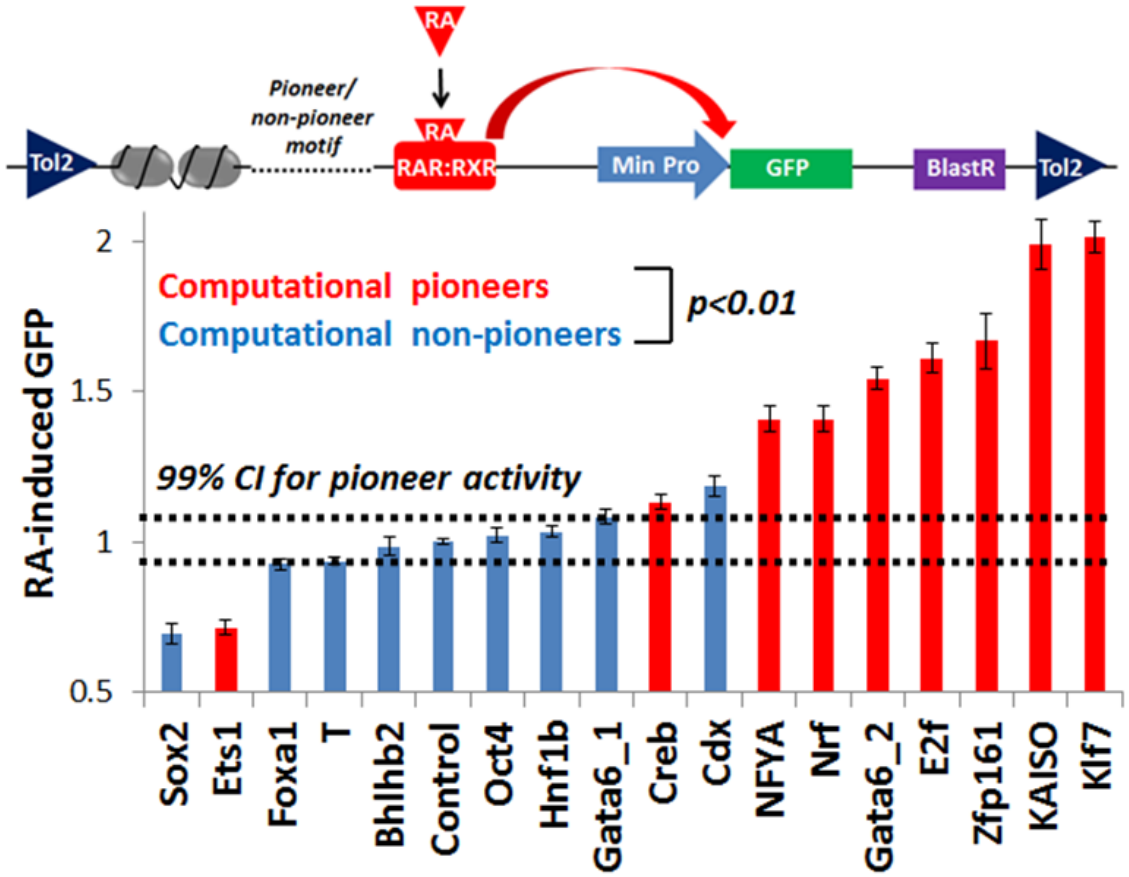


Pioneer TFs have identifiable profiles

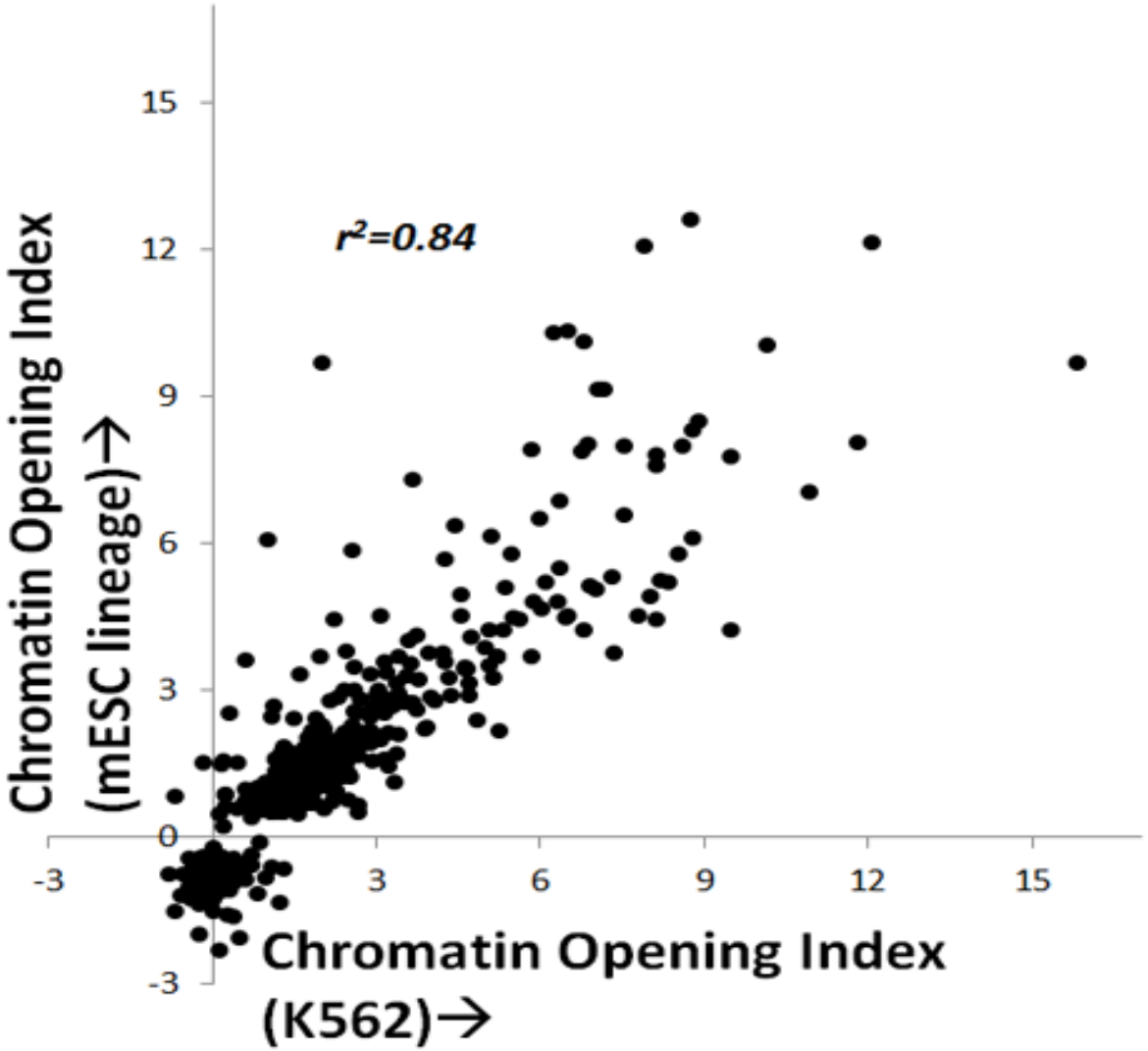


In vitro reporter assays recapitulate computational predictions

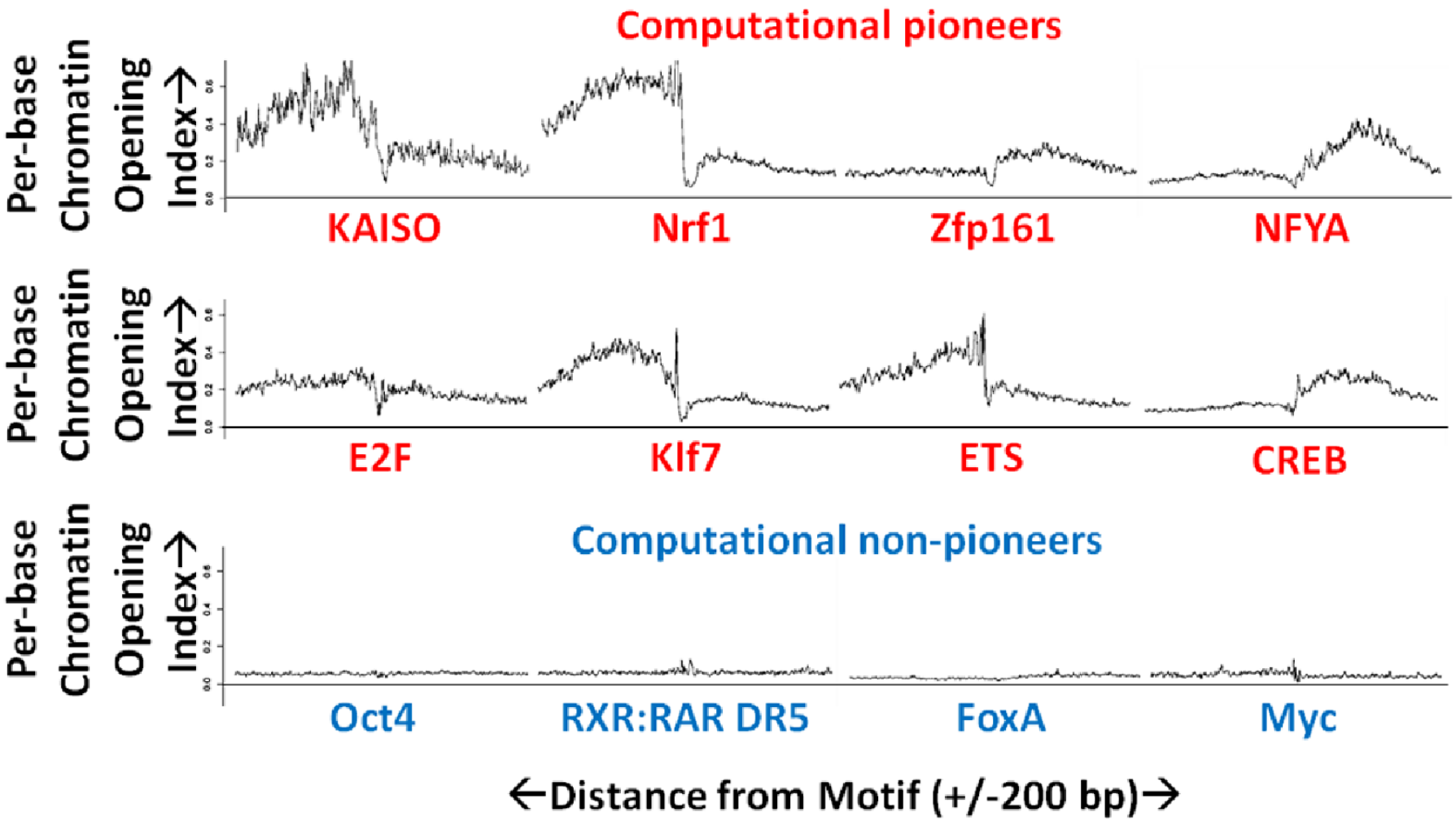
Using a Tol2 based GFP reporter, we confirm finding that these pioneers create new enhancers.



Pioneers appear to be conserved between human/mouse

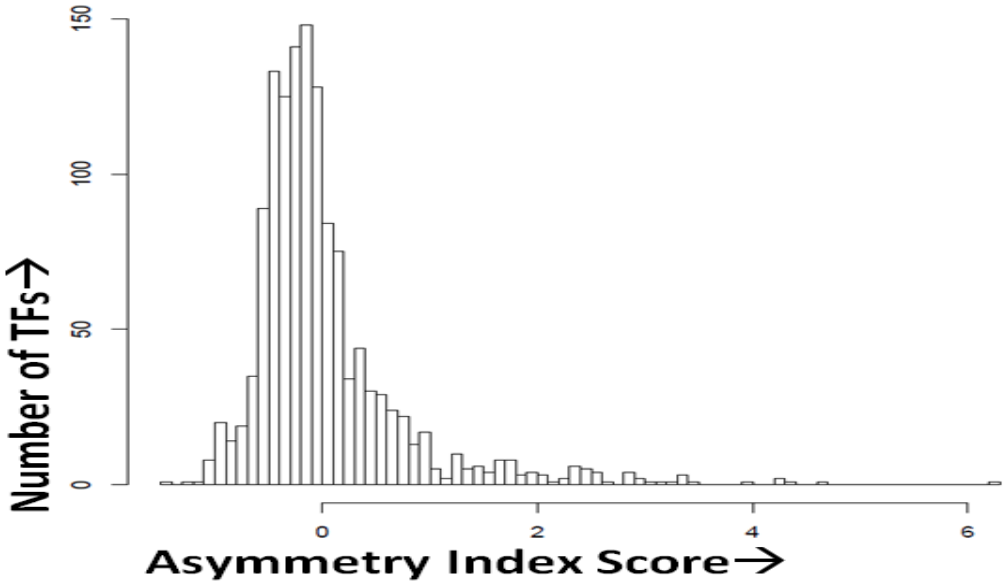


Pioneer TFs have identifiable profiles

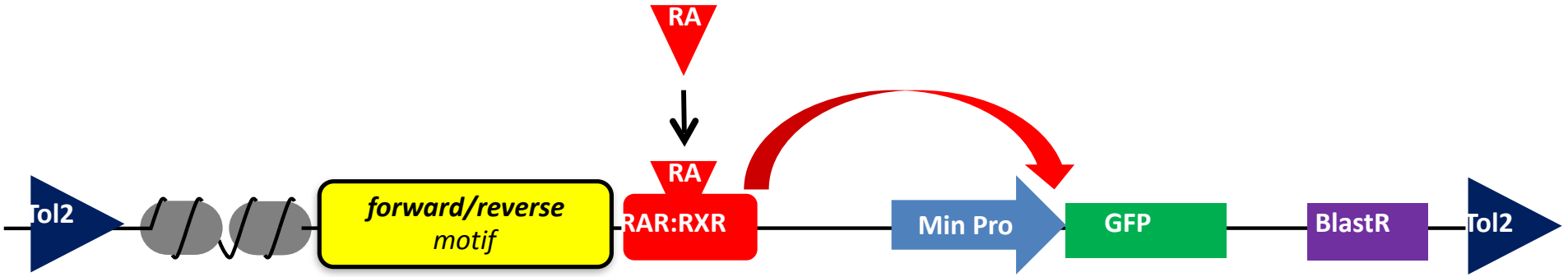


Certain pioneer TFs are directional

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

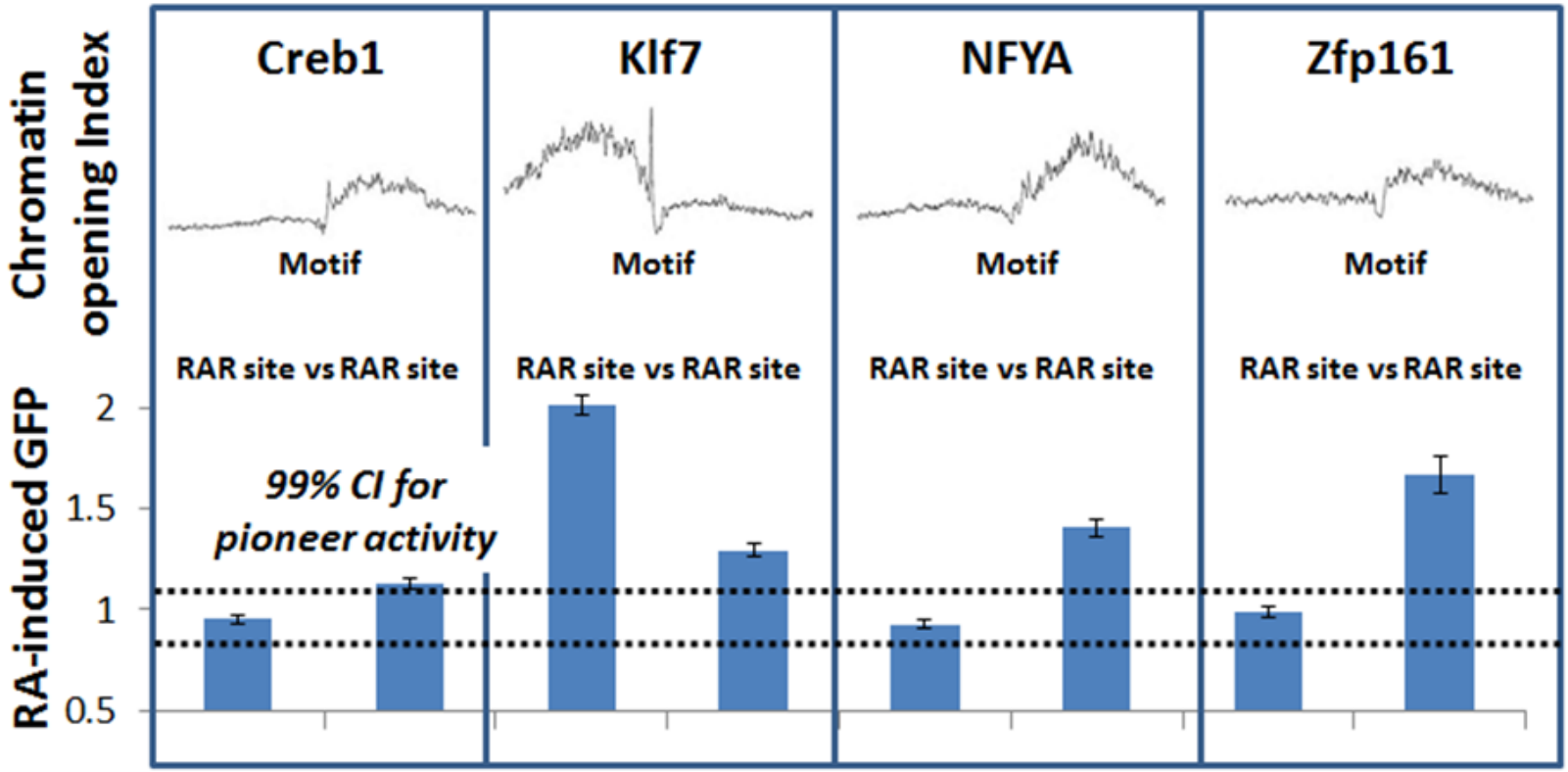


- Biological validation by testing both motif orientations



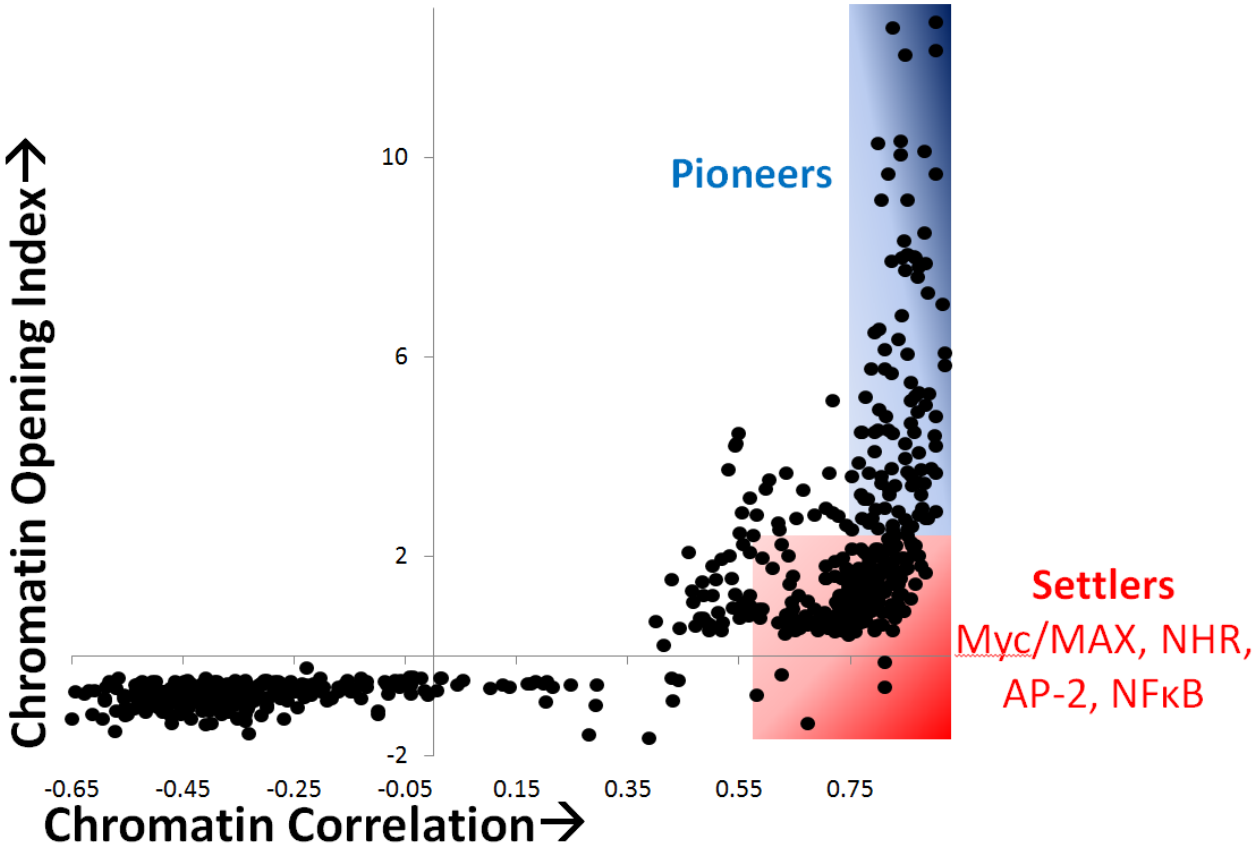
Certain pioneer TFs are directional

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



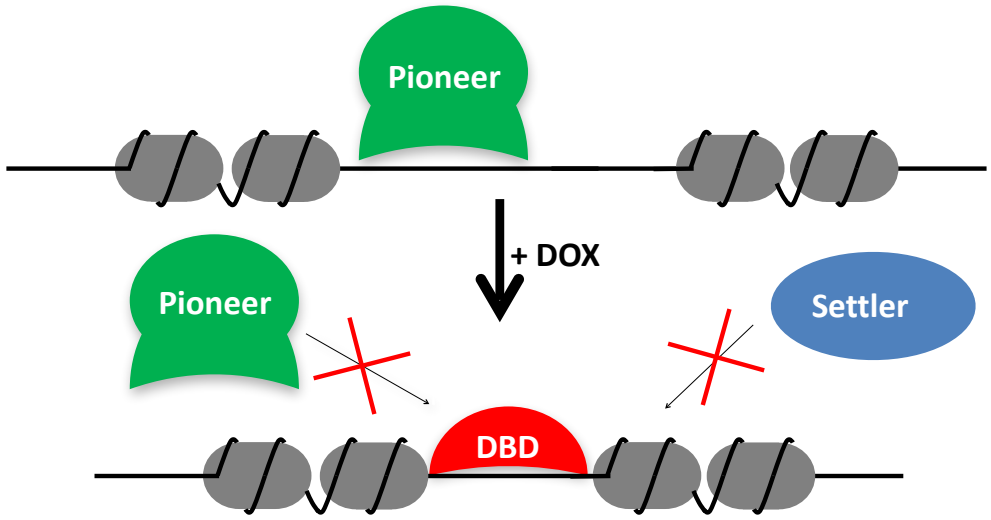
Settler factors follow pioneer factor binding and loss of pioneer binding causes chromatin to return to a closed state

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



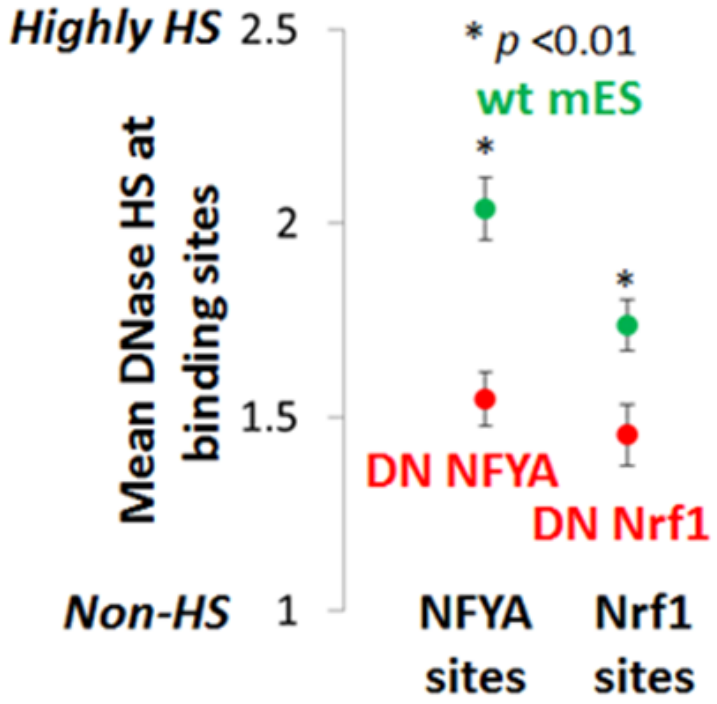
Validate pioneer/settler model via dominant-negative competition assay

- 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

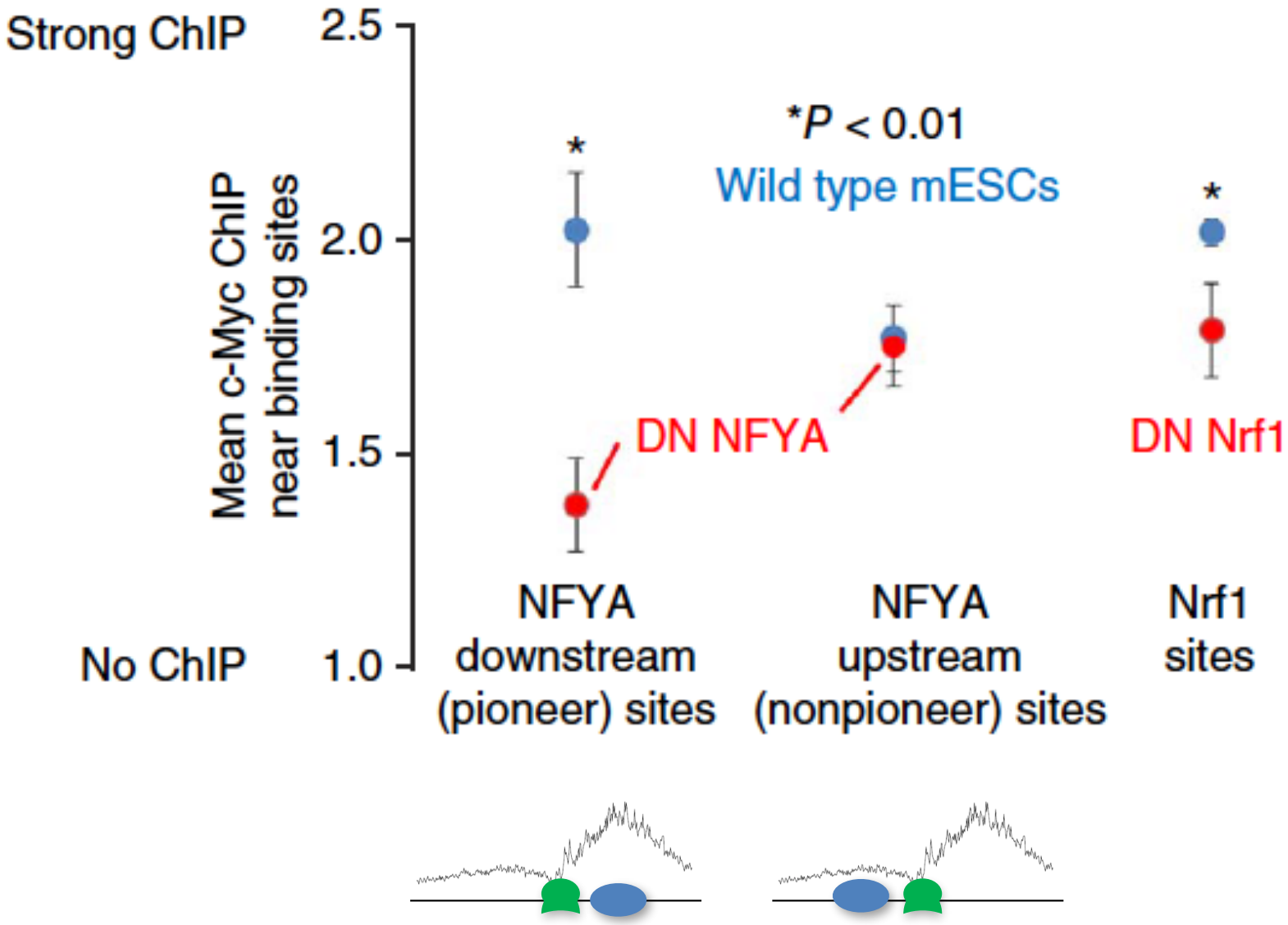


Dominant negative pioneers reduce proximal DNase HS

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

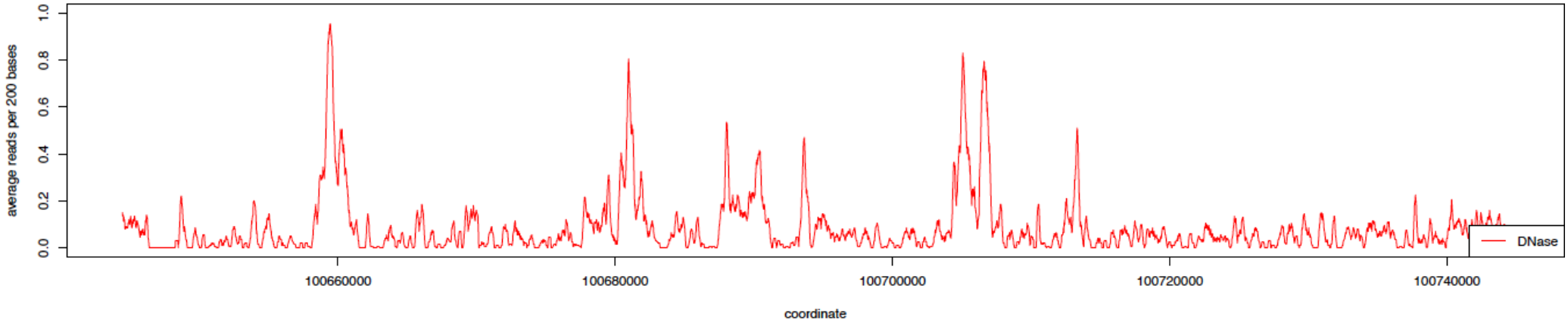


Chromatin accessibly influences transcription factor binding

- Modeling accessibility profiles yields binding predictions and pioneer factor discovery
- Asymmetric accessibility is induced by *directional pioneers*
- The binding of *settler factors* can be enabled by proximal pioneer factor binding

Sherwood, RI, et al. "**Discovery of directional and nondirectional pioneer transcription factors by modeling DNase profile magnitude and shape**" *Nat. Biotech 2014.*

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

We model accessibility read counts with a sum of k-mer influences

The input variable *c* is a vector of length *N* representing counts and c_i represents the read-count observed at base *i*.

The latent variable λ is a vector of length *N* representing the current estimate for *c* using θ.

θ^k is the parameter matrix of size $4^k \times 2M$ associated with the set of all *k*-mers.

The variable g^k is a mapping from genomic coordinate *i* to the *k* mer starting at *i*. The *k*-mer for g^k is represented as an integer that maps to rows of θ such that the g^kth row of θ^k is the effect of a *k*-mer starting at coordinate *i*.

For instance, g_i^4 is the 4-mer starting at coordinate *i*. If this is **ATCG**, then the row $\theta^k_{g_i^4}$ must be the effect that **ATCG** exerts on its neighbors.

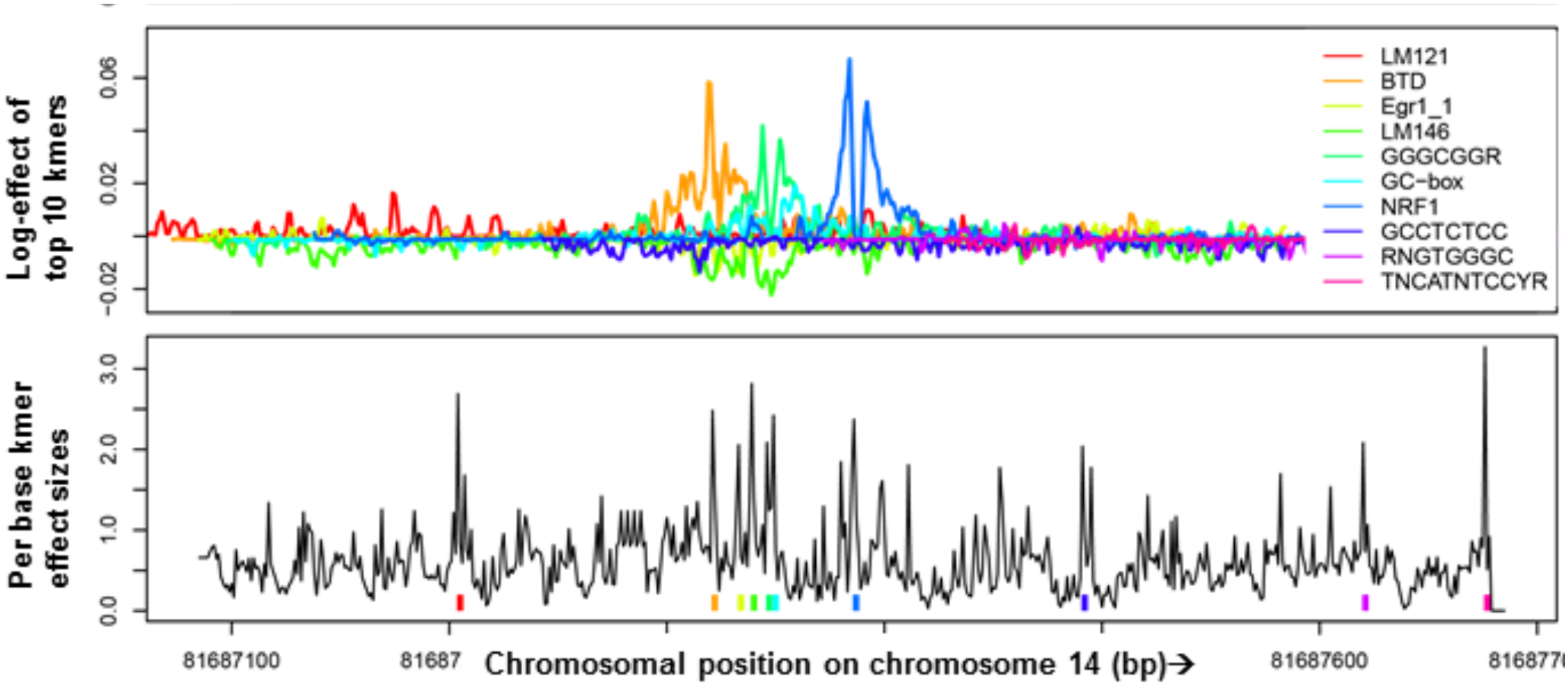
The special parameter θ_0 is used to set the average read rate of the genome globally.

We model accessibility read counts with a sum of k-mer influences

$$\max_{\theta}\left(\sum_{i} c_i log(\lambda_i) - \lambda_i\right) - \eta \sum \left|\theta^k\right|_1$$

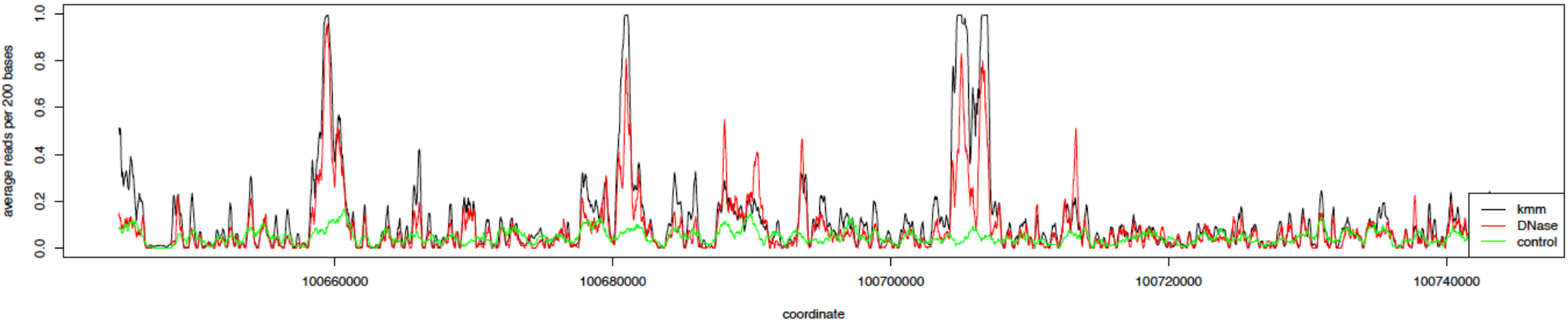
$$\lambda_i = exp\left(\left(\sum_{k\in[1..K]} \sum_{j\in[-M,M-1]} \theta^k_{\left(g^k_{i+j},-j\right)}\right) - \theta_0\right)$$

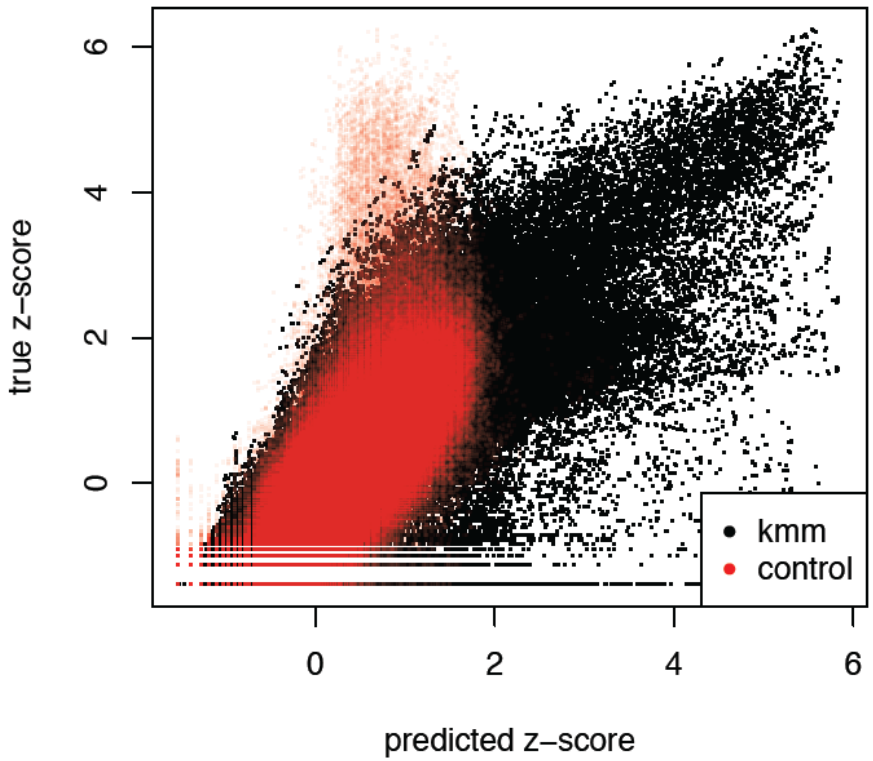
The Synergistic Chromatin Model (SCM) is a K-mer model



~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)

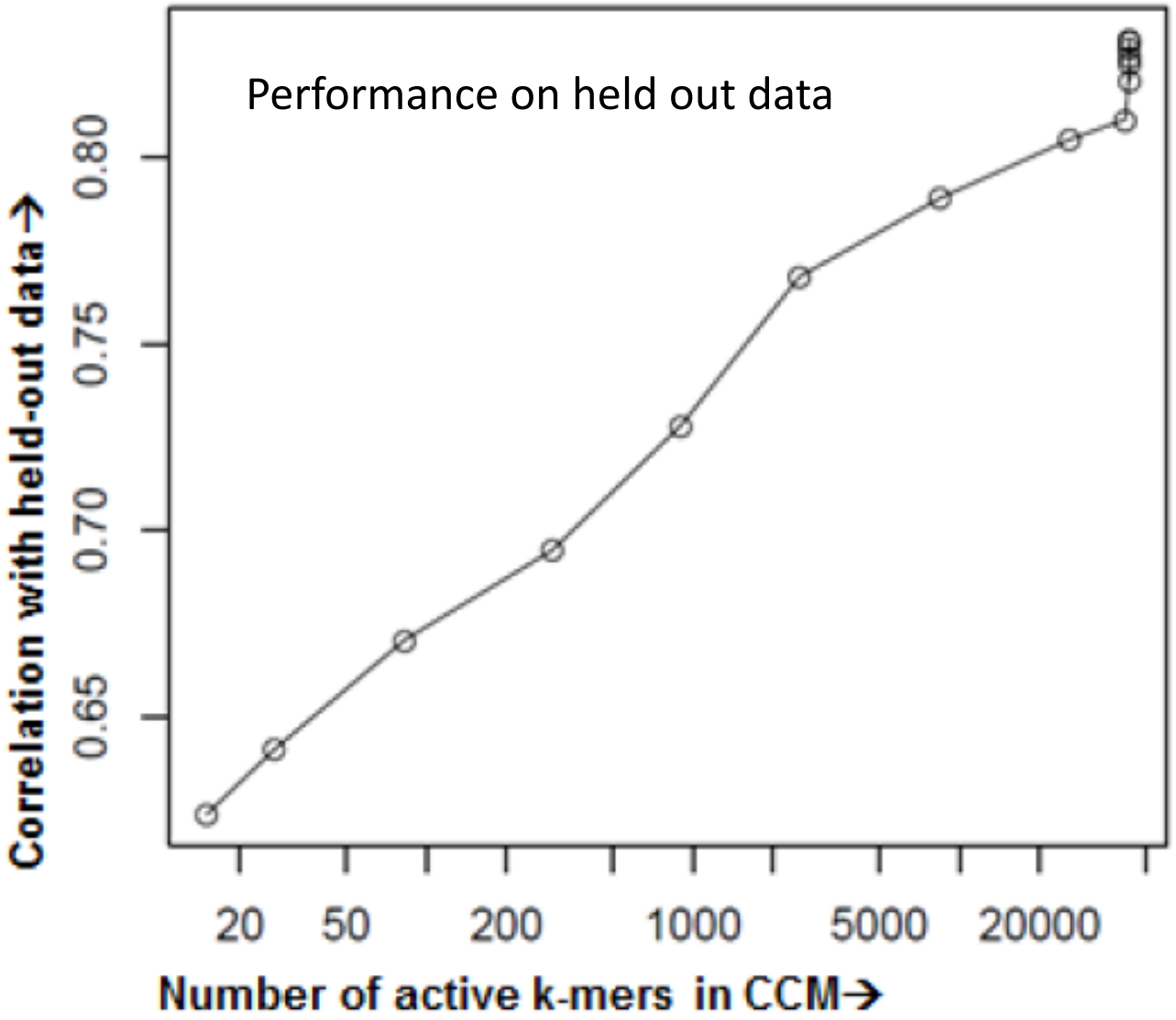




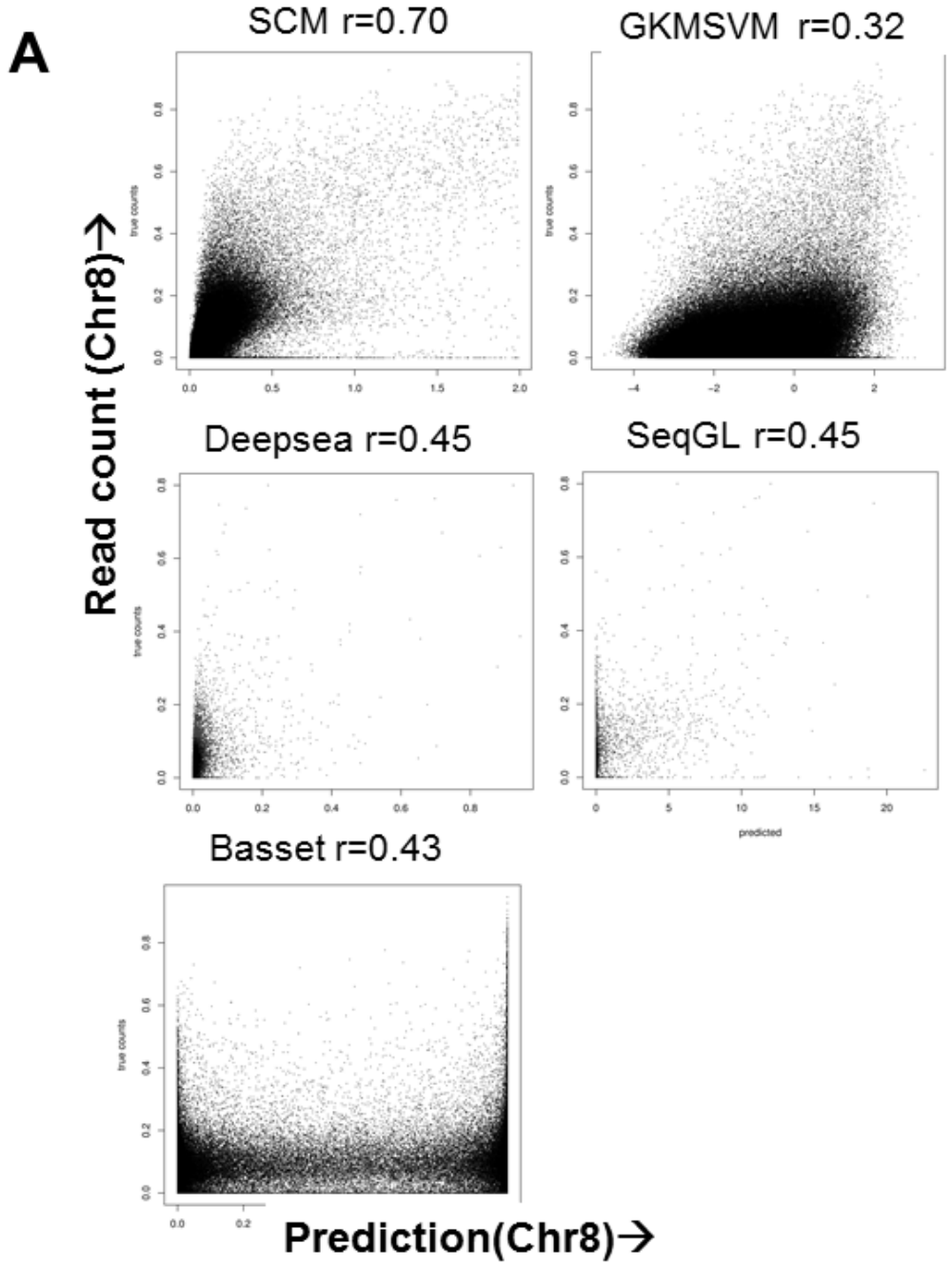
KMM R^2 0.80

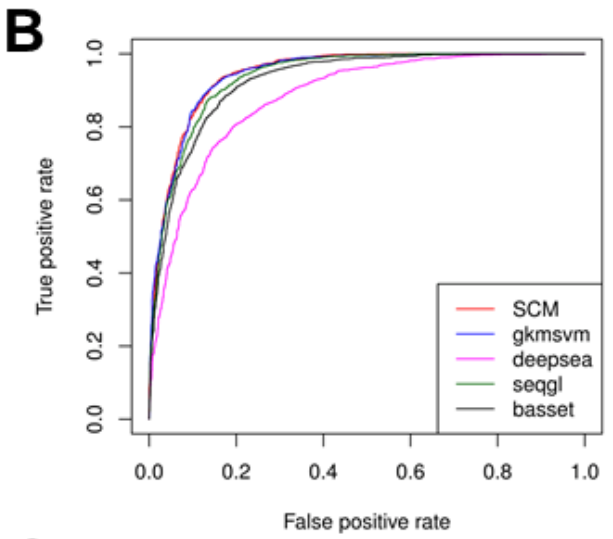
Control R^2 0.47

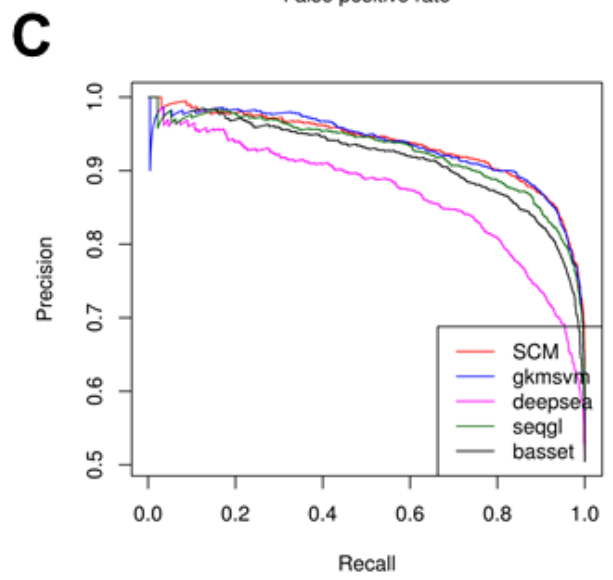
SCM model performance improves with more k-mer features



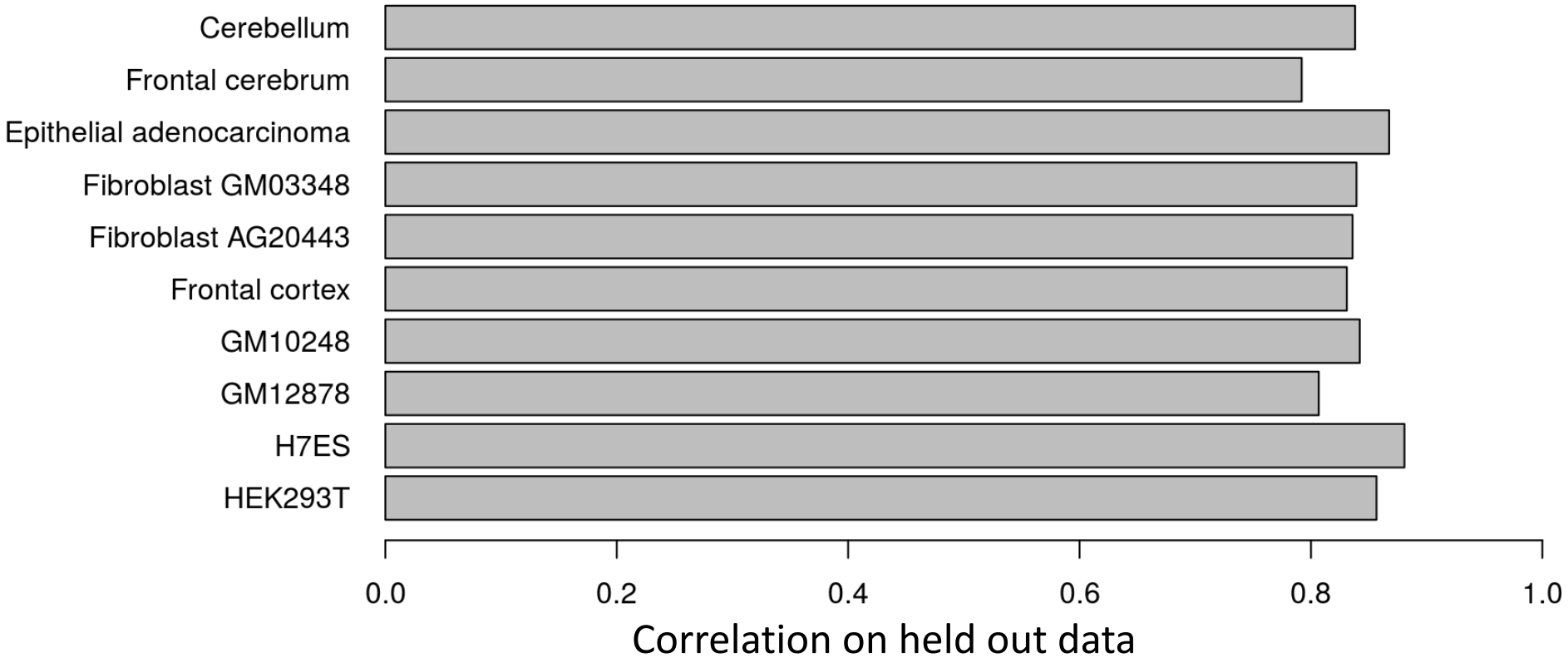
SCM outperforms contemporary models at predicting chromatin accessibility from sequence (K562)





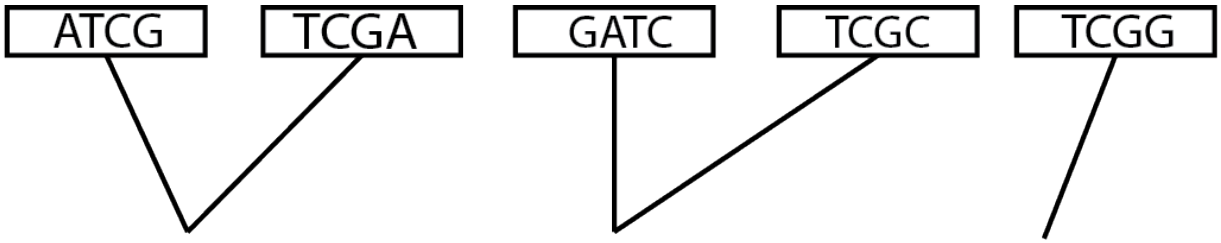


SCM models have similar predictive power for other cell types

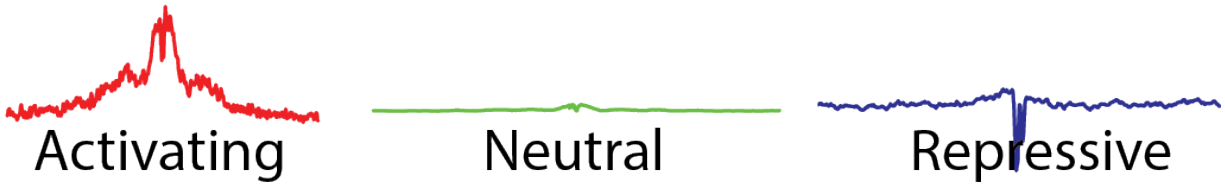


We created synthetic “phrases” each of which contains k-mers that are similar in chromatin opening score

All K-mers



Sort into classes

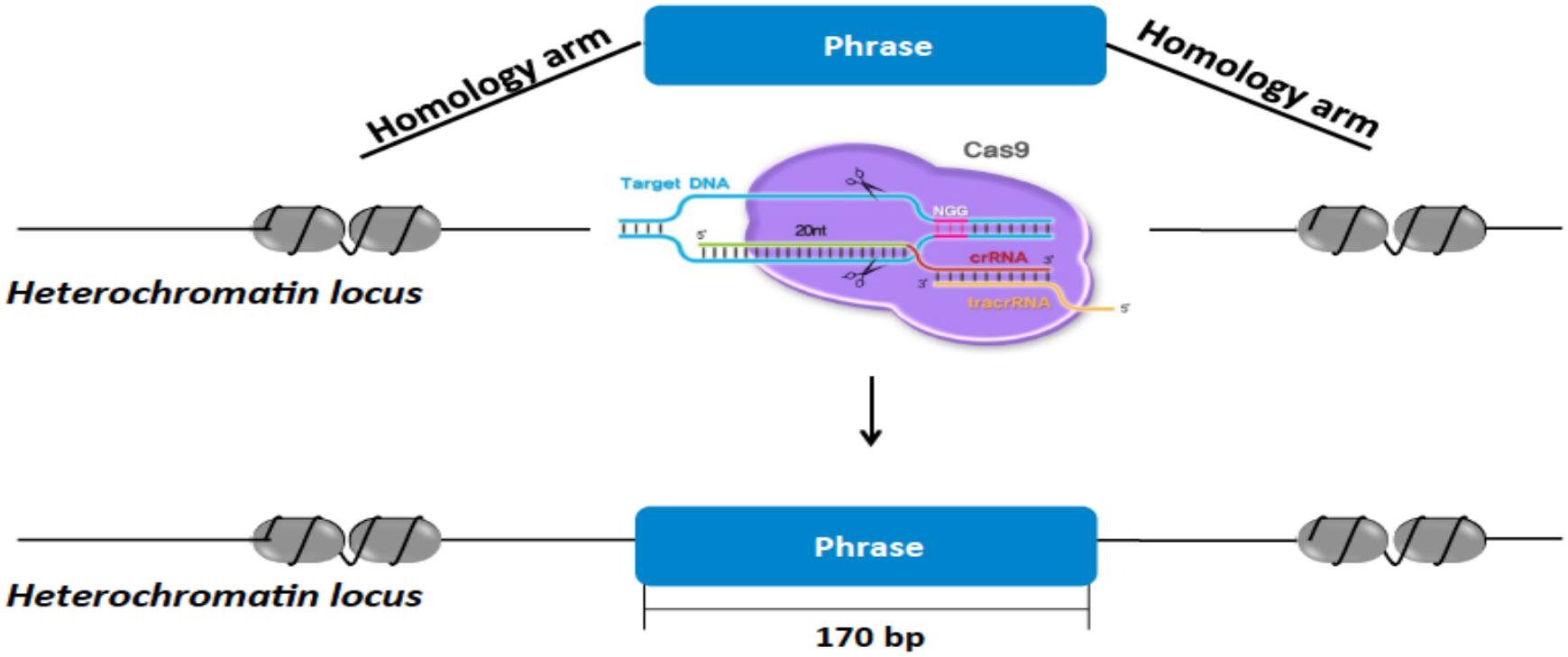


Construct Debrujin Graph

Biased random walk

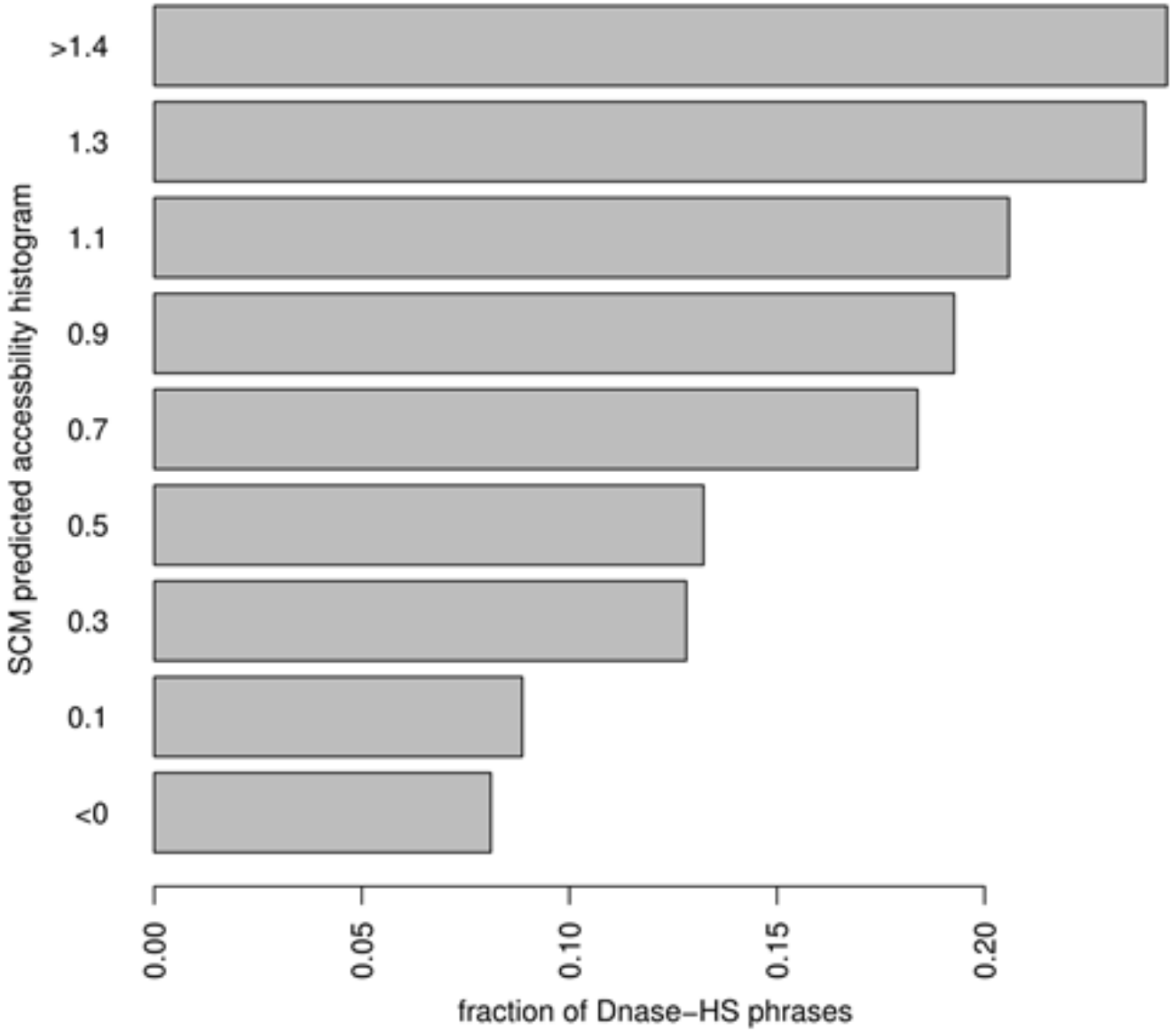
1. GATCGC
2. GATCGA

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

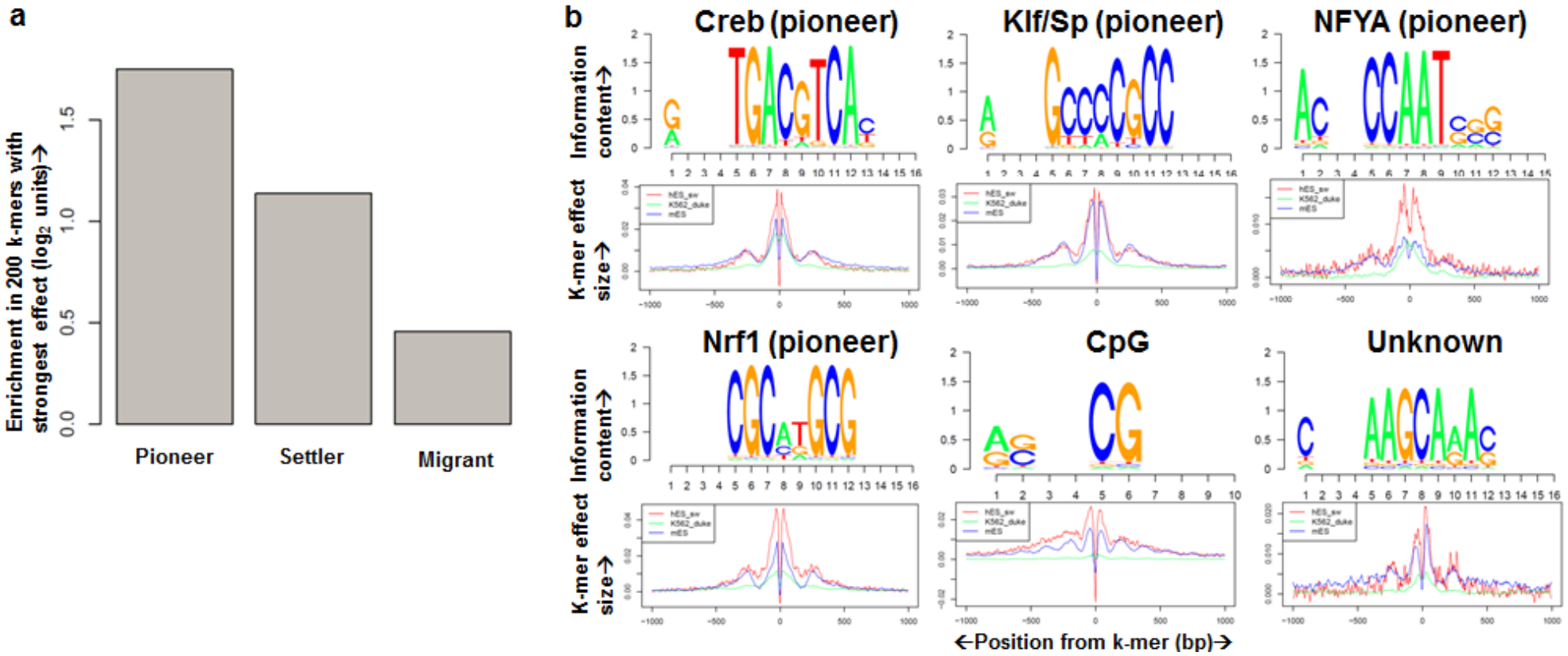


Heterochromatin locus	A	B	C
% alleles with phrase integration	35	15	15
# unique integrations	350,000	150,000	150,000

Predicted accessibility matches measured accessibility

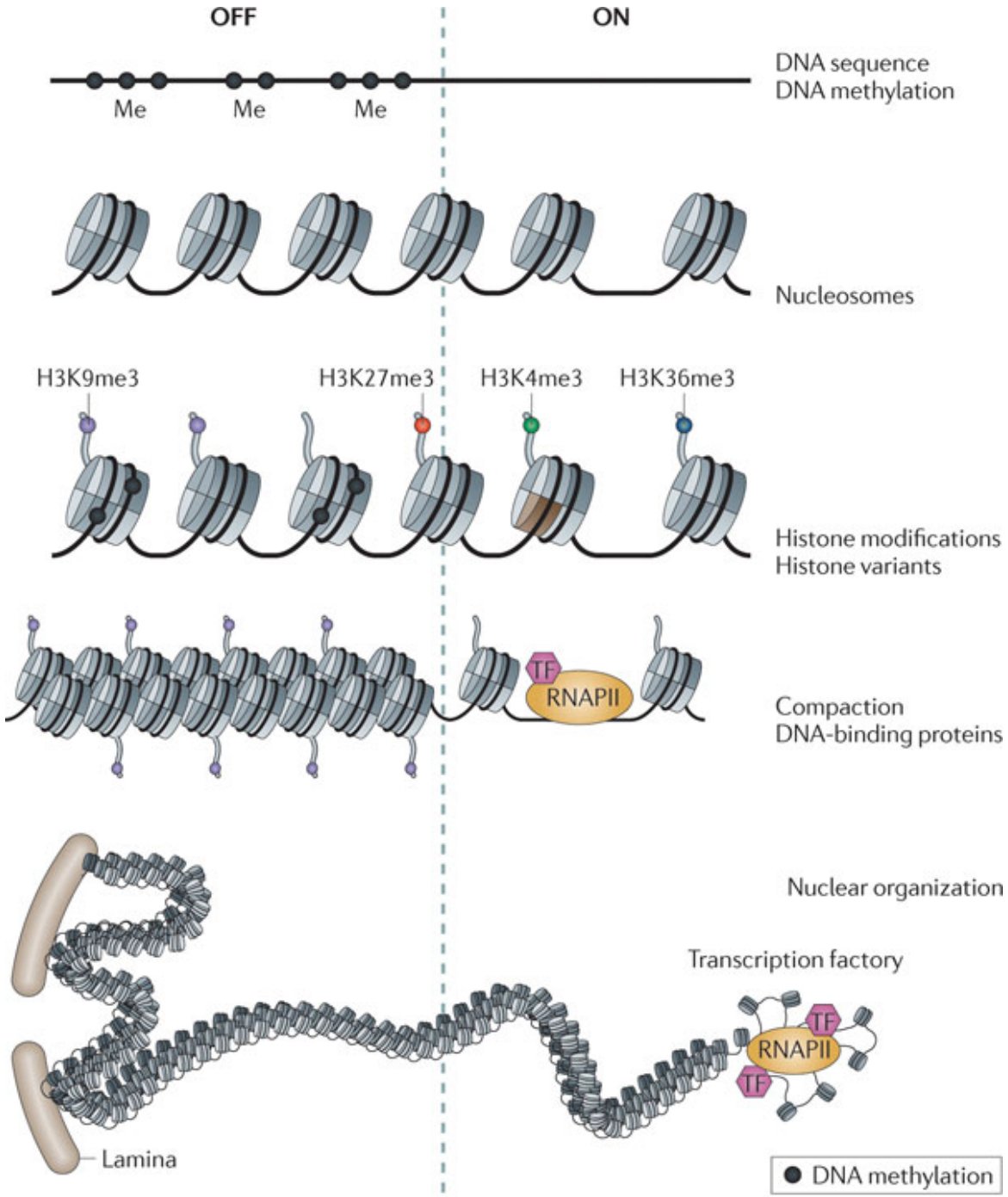


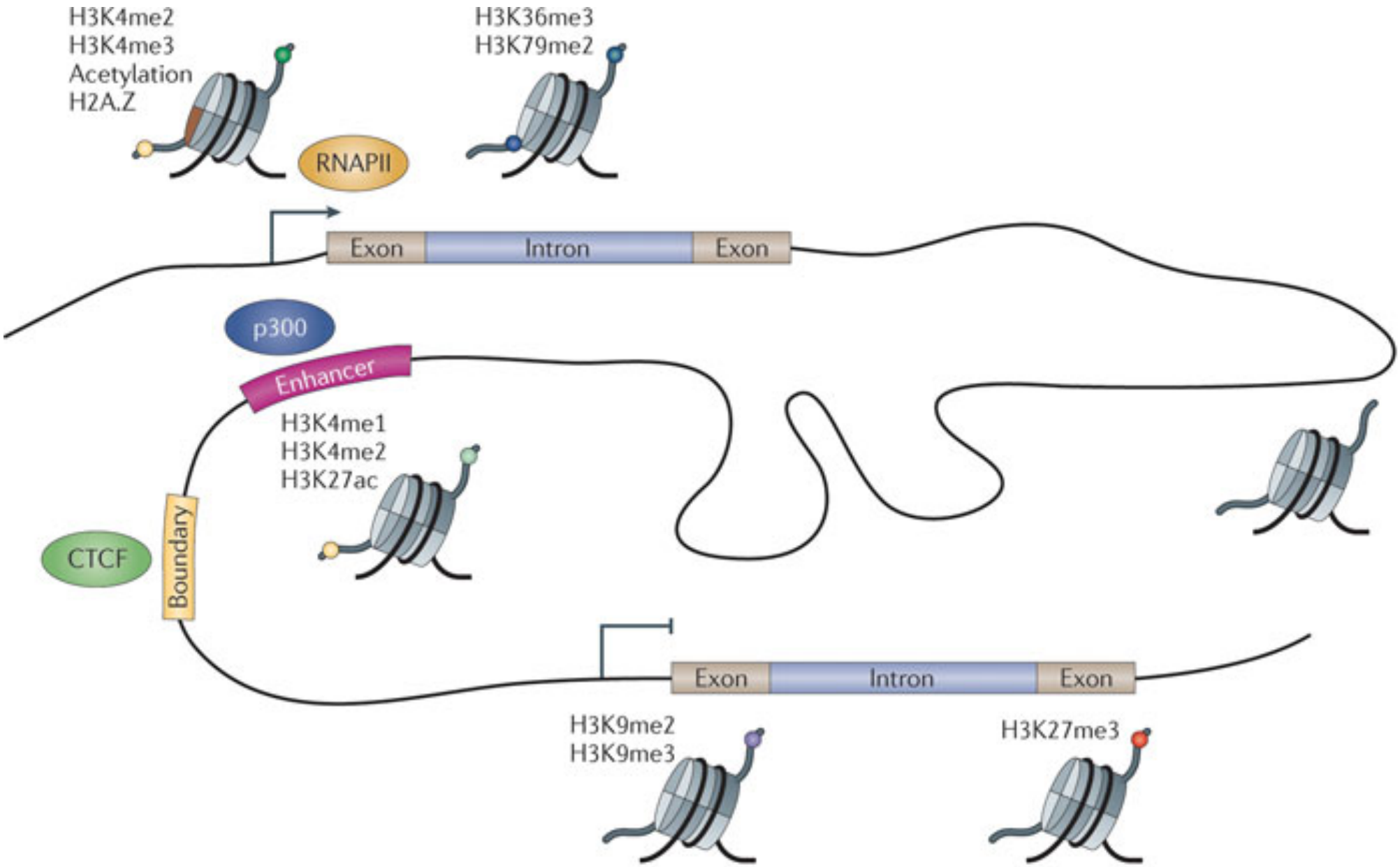
Chromatin accessibility arises from interactions, largely among pioneer TFs



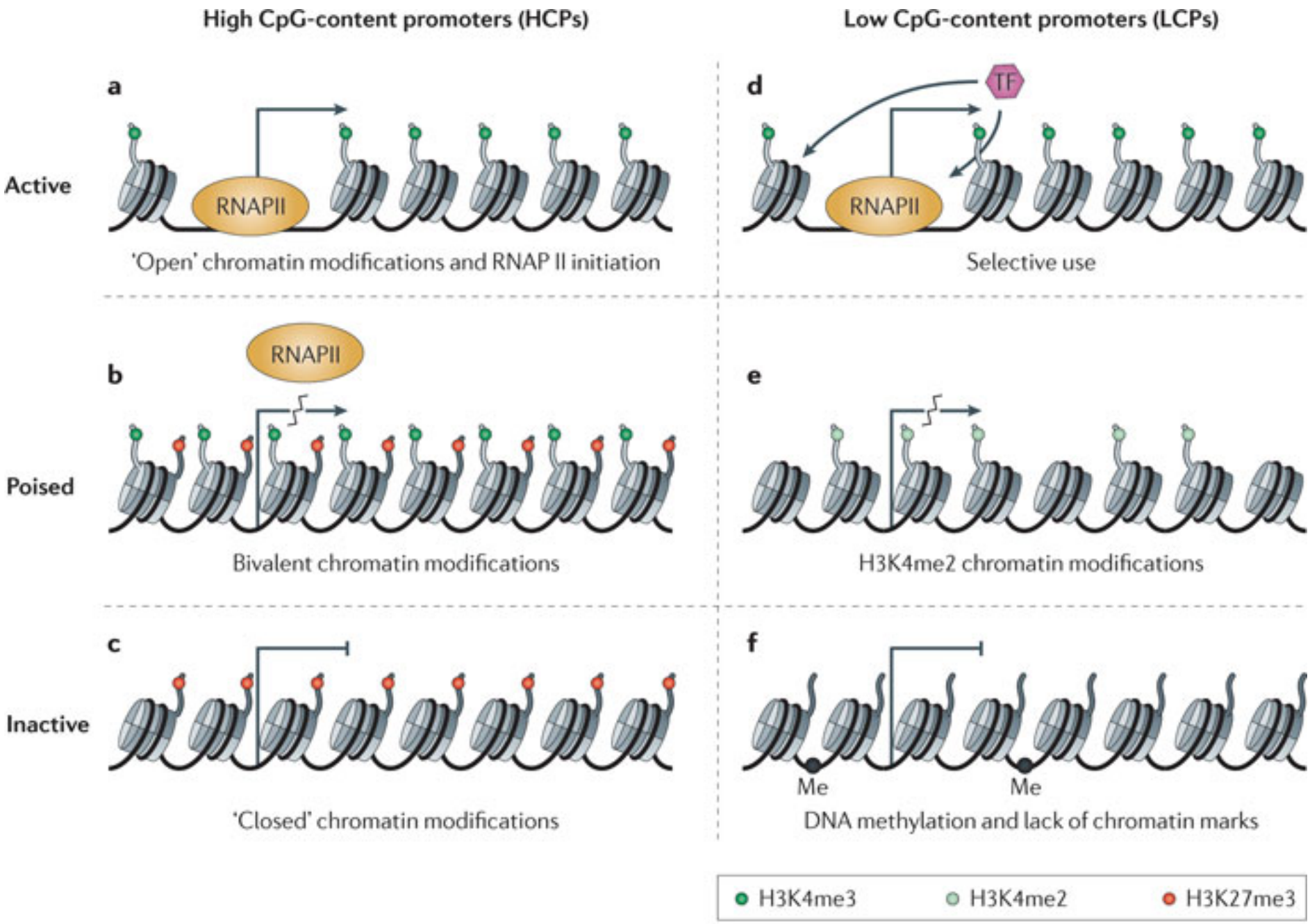
Chromatin organization has multiple structural layers and organizes chromatin into "domains"

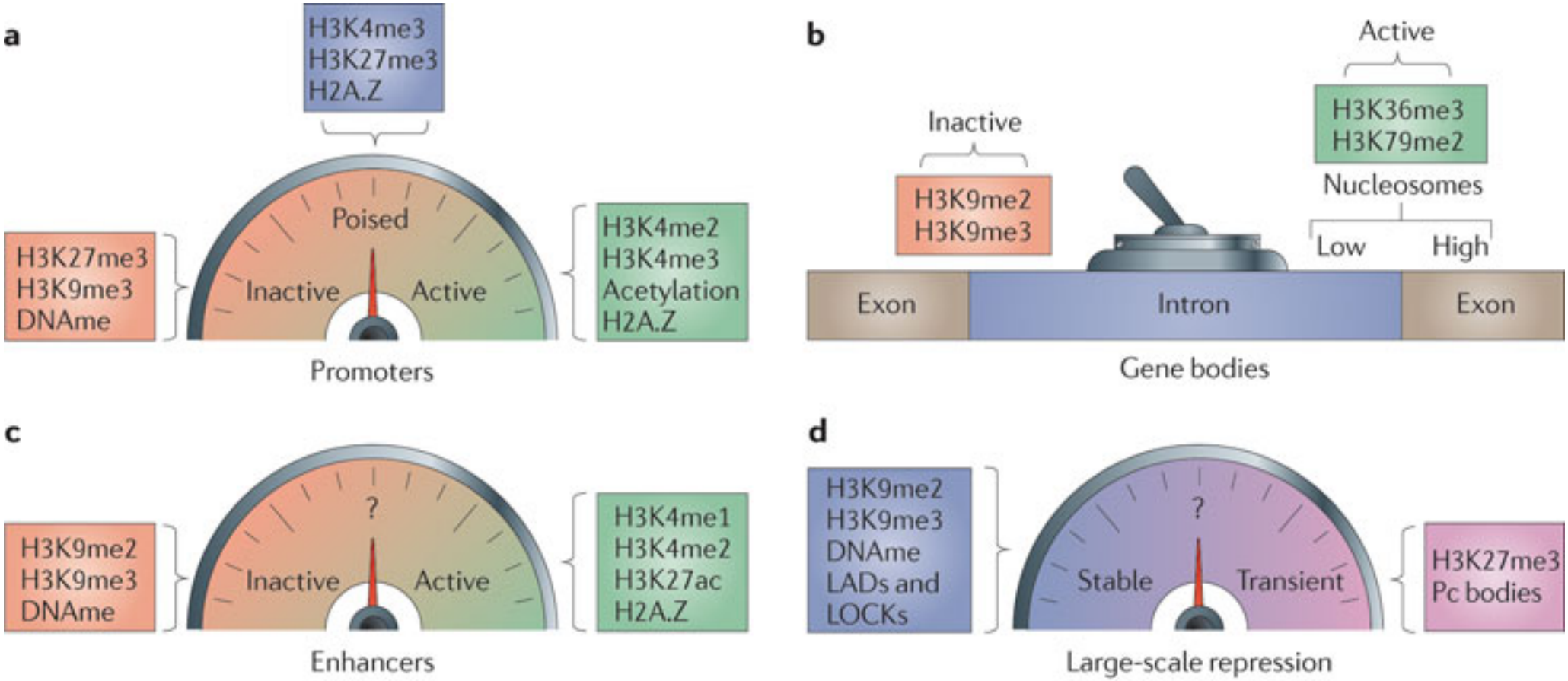
Both DNA methylation and chromatin marks contain important functional information





Nature Reviews | Genetics


High CpG-content promoters (HCPs)
Low CpG-content promoters (LCPs)
Active
Poised
Inactive
a
RNAPII
'Open' chromatin modifications and RNAP II initiation
d
TF
RNAPII
Selective use
b
RNAPII
Bivalent chromatin modifications
e
H3K4me2 chromatin modifications
c
'Closed' chromatin modifications
f
Me
Me
DNA methylation and lack of chromatin marks
H3K4me3
H3K4me2
H3K27me3

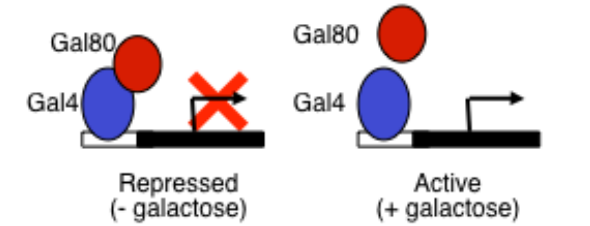

a
H3K4me3
H3K27me3
H2A.Z
Poised
Inactive
Active
H3K27me3
H3K9me3
DNAme
H3K4me2
H3K4me3
Acetylation
H2A.Z
Promoters
b
Active
H3K36me3
H3K79me2
Inactive
H3K9me2
H3K9me3
Nucleosomes
Low
High
Exon
Intron
Exon
Gene bodies
c
?
Inactive
Active
H3K9me2
H3K9me3
DNAme
H3K4me1
H3K4me2
H3K27ac
H2A.Z
Enhancers
d
?
Stable
Transient
H3K9me2
H3K9me3
DNAme
LADs and
LOCKs
H3K27me3
Pc bodies
Large-scale repression

Part 3 - Modeling Gene Expression

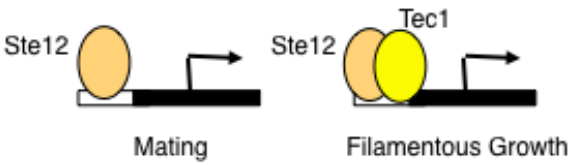
Modules and modularity

- Modularity guides most engineering systems
  - separates implementation from interface/function
  - creates building blocks, easy combination
- Biological systems are more "integrated" and involve greater robustness (compensation)
- Finding (abstracting) units of (partial) autonomy in cells is therefore a more challenging task
  - the focus on larger groups of genes nevertheless gives us some statistical power

TF expression, mechanisms

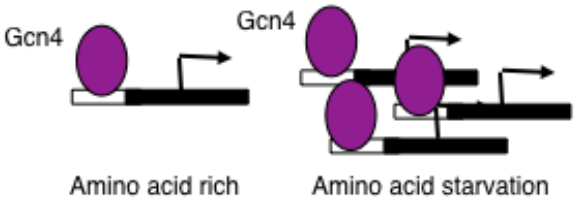


Regulator is bound under both conditions but is modified upon activation



Regulator alters binding site preference under second condition

Regulator binds only in activating conditions due to nuclear exclusion



Regulator binds expanded set of targets in activating conditions

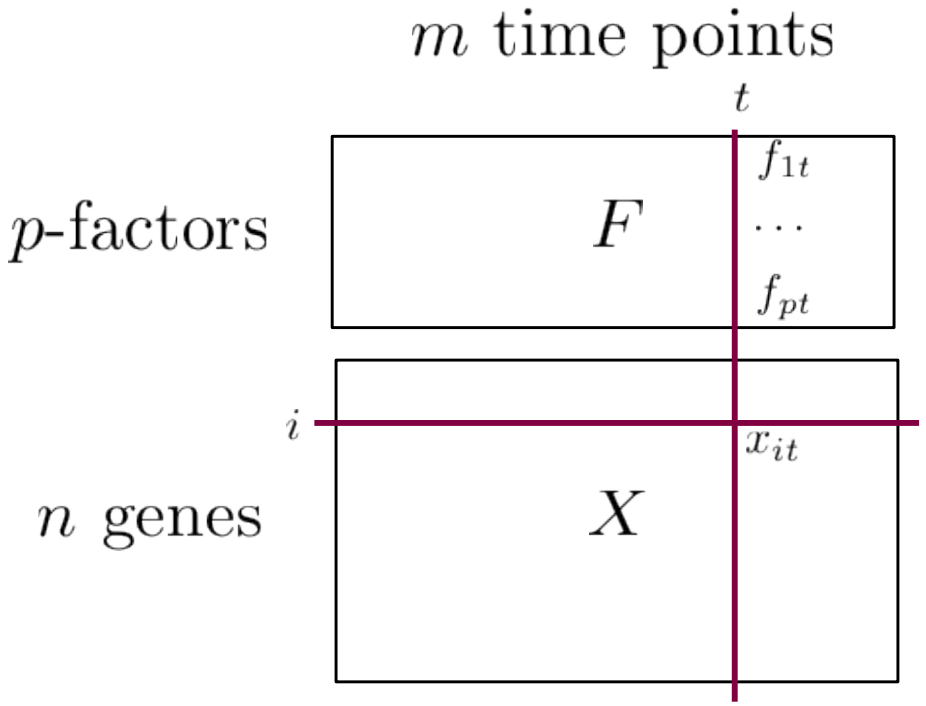
(Young, 2003)

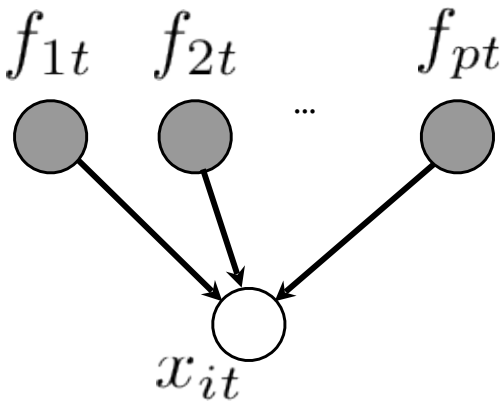
Expression programs

- We can try to relate the expression of transcription factors (TFs) and genes they regulate
- The key assumption we have to make is that TFs are themselves transcriptionally regulated, i.e., their mRNA levels are indicative of their activity
- The problem:

  Find a subset of TFs and the genes they regulate along with the function (program) that relates their expression

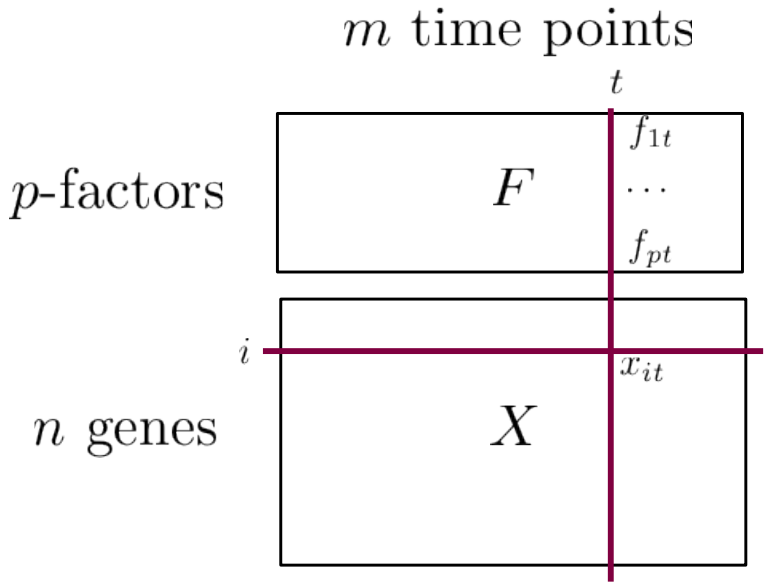
A regression model

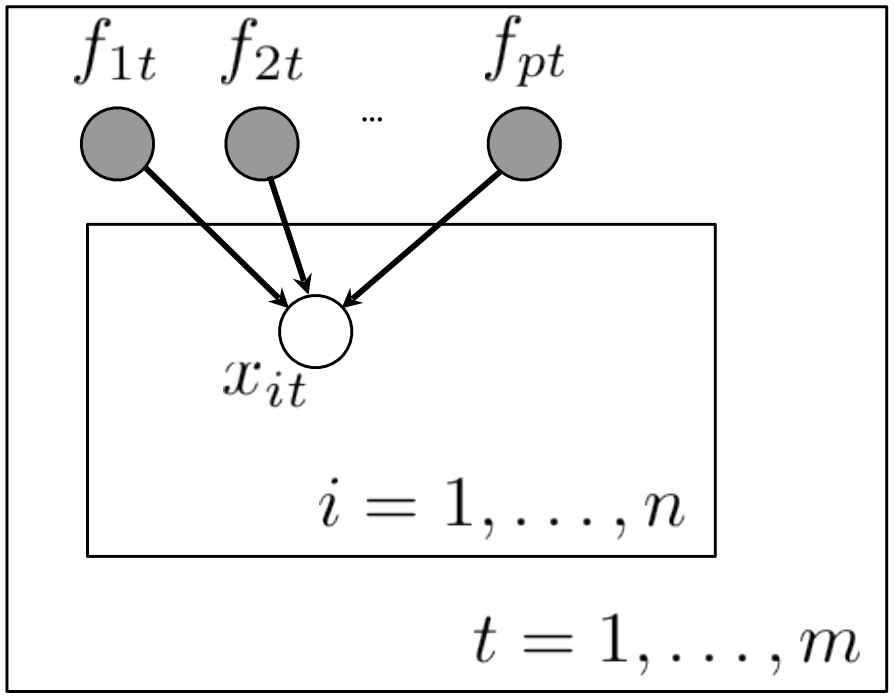




$$P(x_{it}|f_{1t},\dots,f_{pt},\Theta)$$

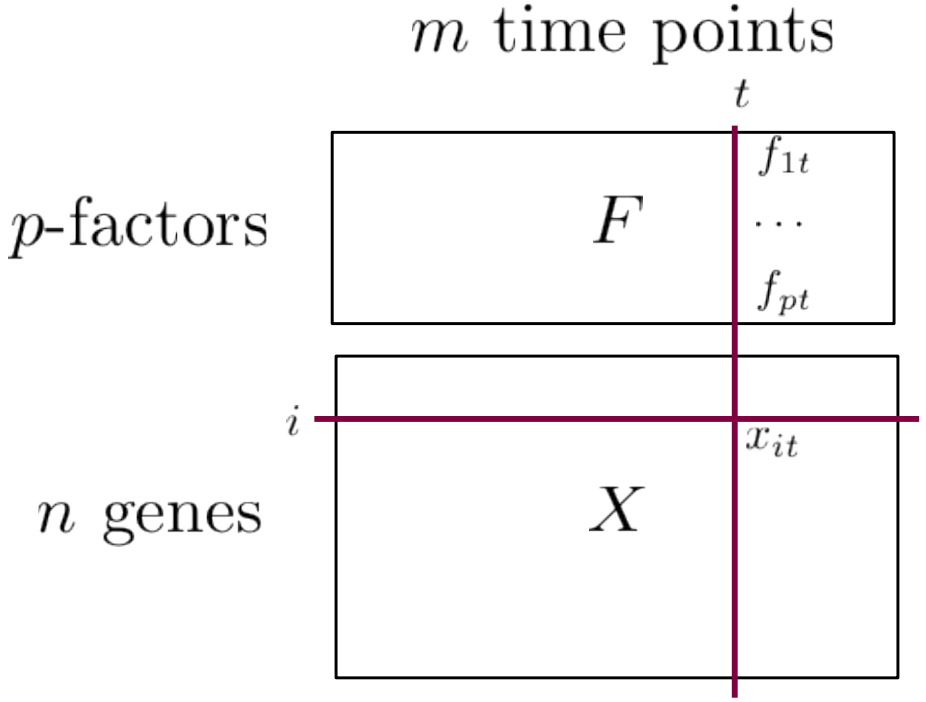
A regression model: sampling

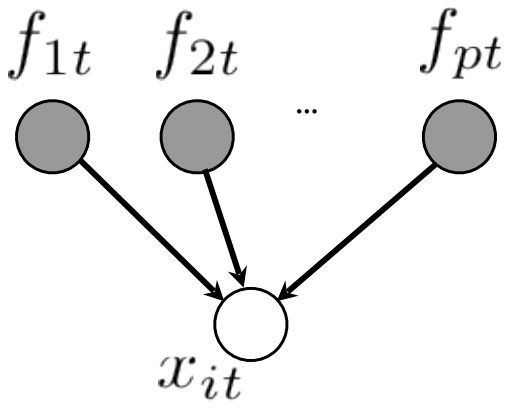




$$P(X|F,\Theta_1) = \prod_{t=1}^{m} \left[\prod_{i=1}^{n} P(x_{it}|f_{1t},\ldots,f_{pt},\Theta_1) \right]$$

Possible regression models



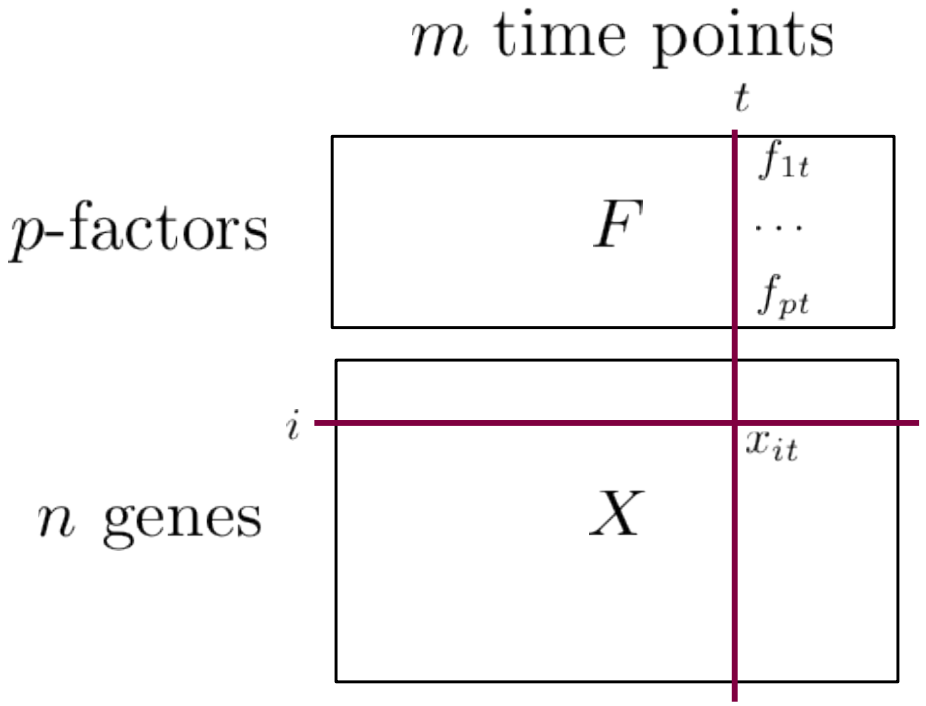


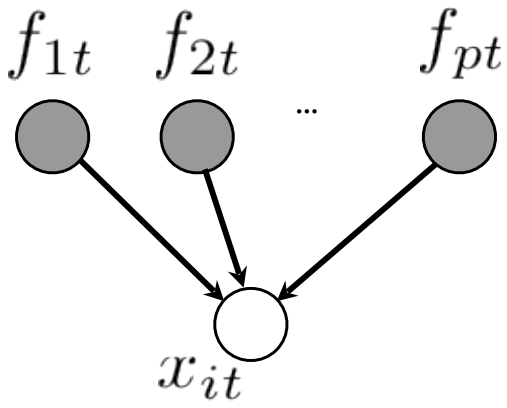
$$P(x_{it}|f_{1t},\dots,f_{pt},\Theta_1) =$$

- Linear regression (with feature selection)
- Regression tree
- Discretization + conditional table

etc.

A regression tree





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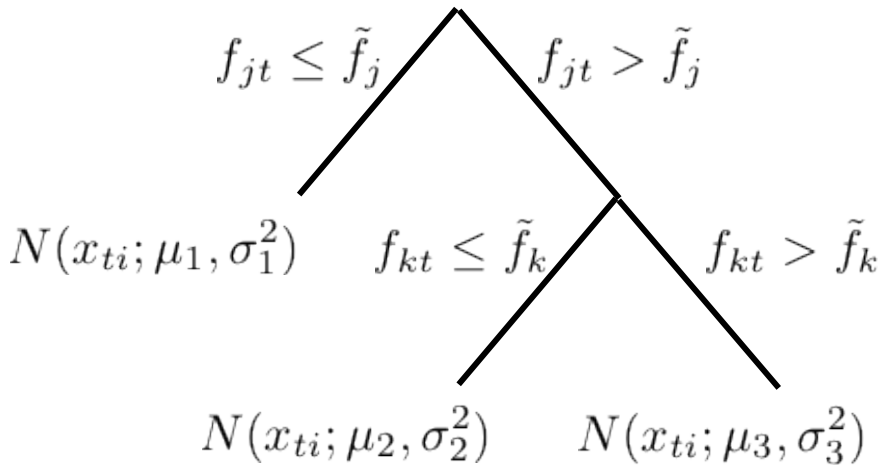
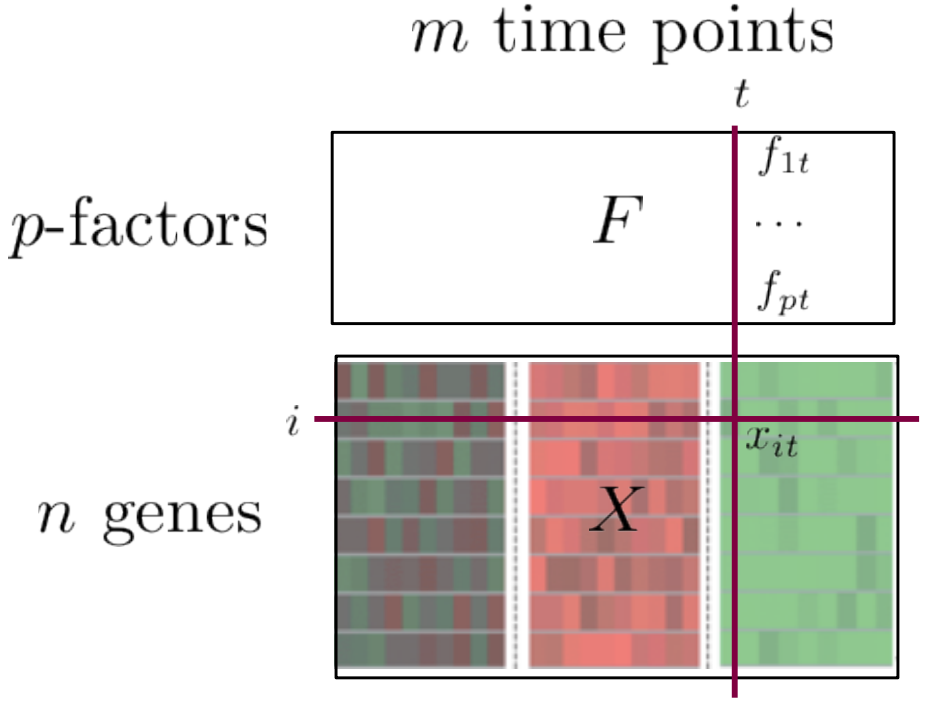
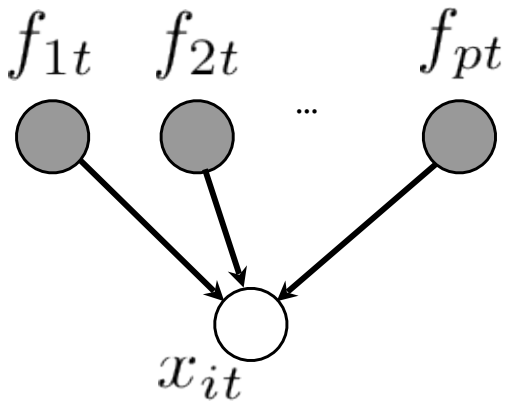


A regression tree





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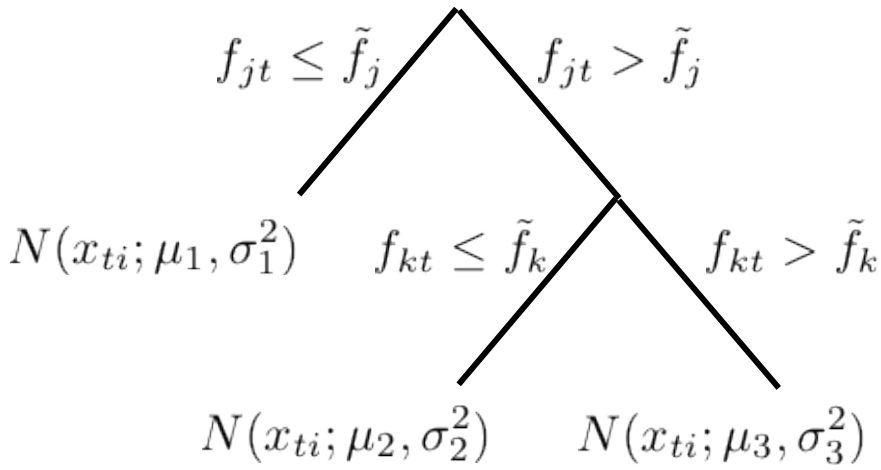
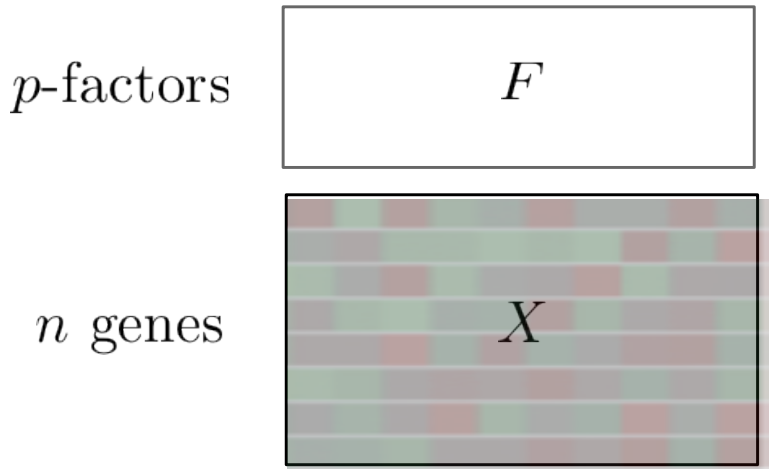


Learning regression trees: null model

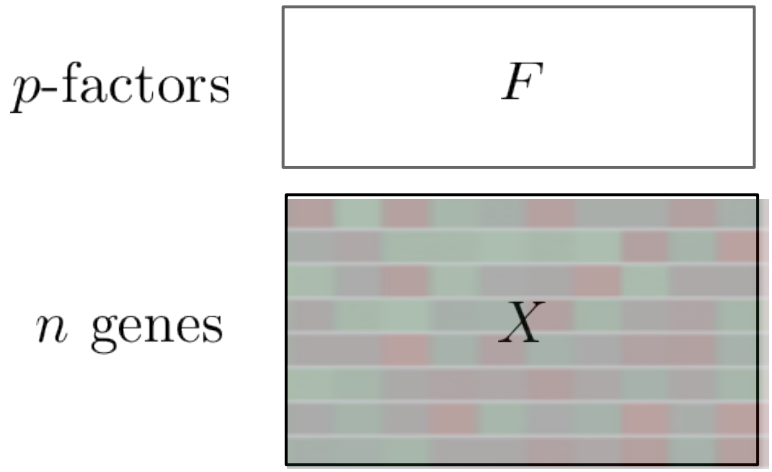


- The simplest case (null model): no dependence on the factors

$$P(X;\theta) = \prod_{i=1}^{n}\prod_{t=1}^{m} N(x_{it};\, \mu, \sigma^2)$$

where $\theta = \{\mu, \sigma^2\}$.

Learning regression trees: null model



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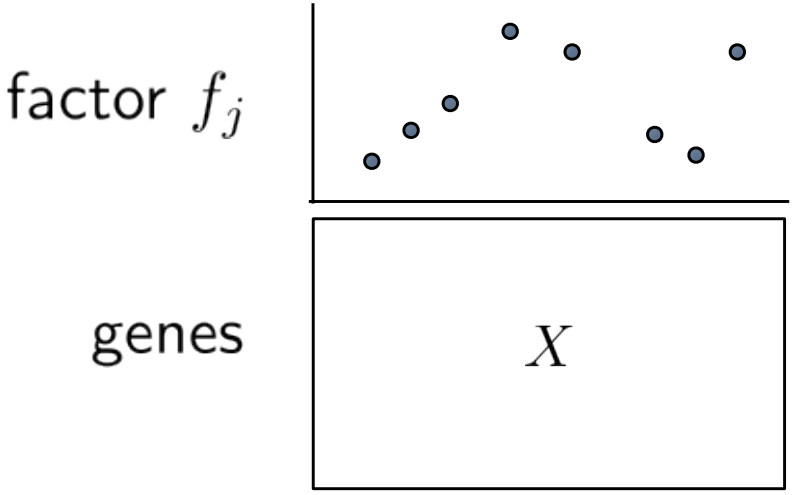
where $\theta = \{\mu, \sigma^2\}$.

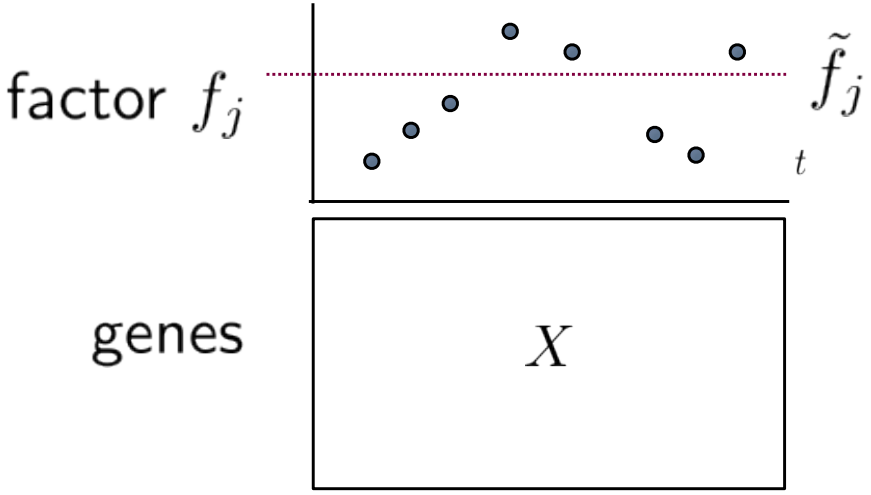
- By maximizing $l(X;\theta) = \log P(X;\theta)$ with respect to the parameters (mean and variance), we get

$$l(X;\hat{\theta}) = nm\left[-\frac{1}{2} - \frac{1}{2}\log(2\pi\hat{\sigma}^2)\right]$$

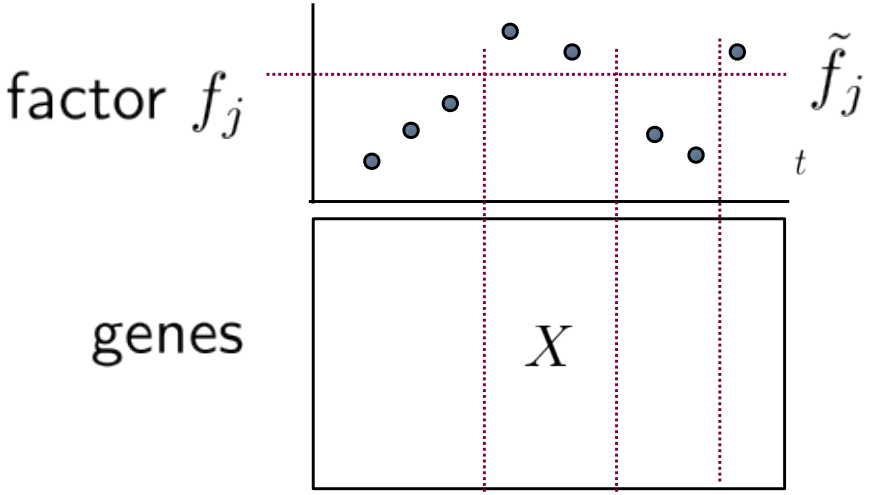
Learning regression trees: one factor



Learning regression trees: one factor



Learning regression trees: one factor



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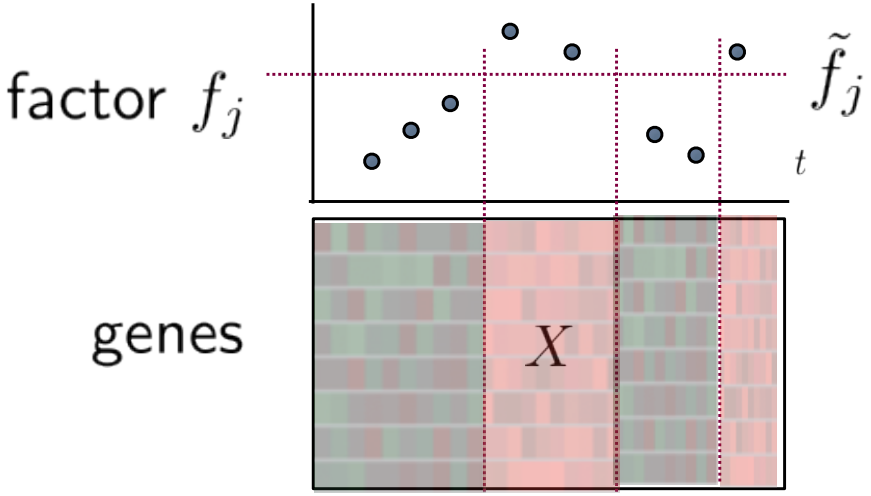
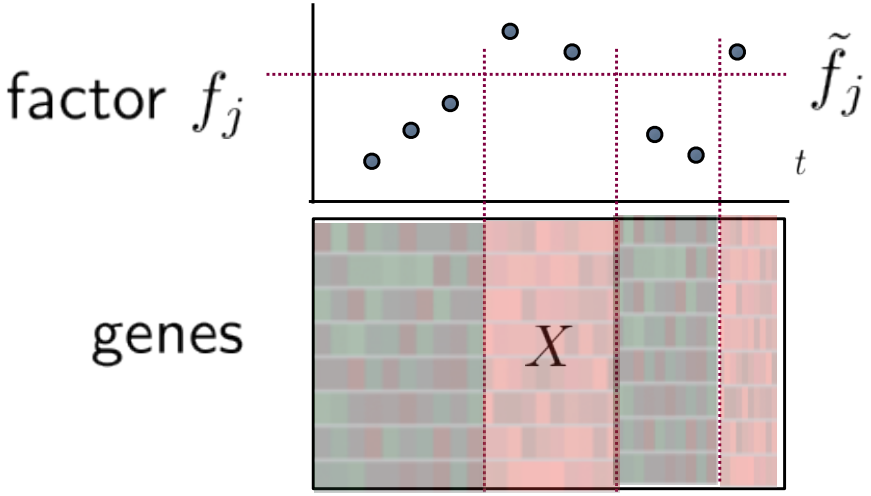


Learning regression trees: one factor



partitions

$I_1 = \{t : f_{jt} \leq \tilde{f}_{jt}\},$

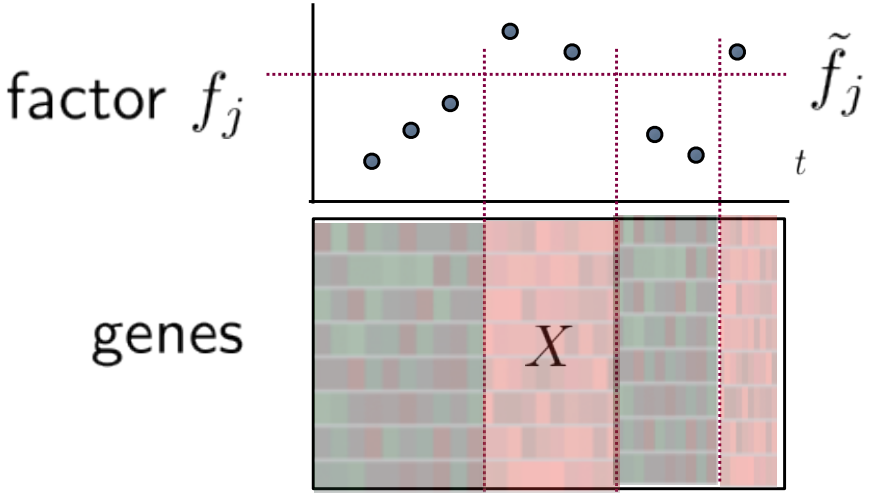
$I_2 = \{t : f_{jt} > \tilde{f}_{jt}\}$

- Conditioning on the factor value partitions the expression matrix into two types of segments

$$P(X|f_j;\Theta) = \left[\prod_{t\in I_1}\prod_{i=1}^{n} N(x_{it};\,\mu_1,\sigma_1^2)\right]\left[\prod_{t\in I_2}\prod_{i=1}^{n} N(x_{it};\,\mu_2,\sigma_2^2)\right]$$

where $\Theta = \{\tilde{f}_j, \mu_1, \mu_2, \sigma_1^2, \sigma_2^2\}$.

Learning regression trees: one factor



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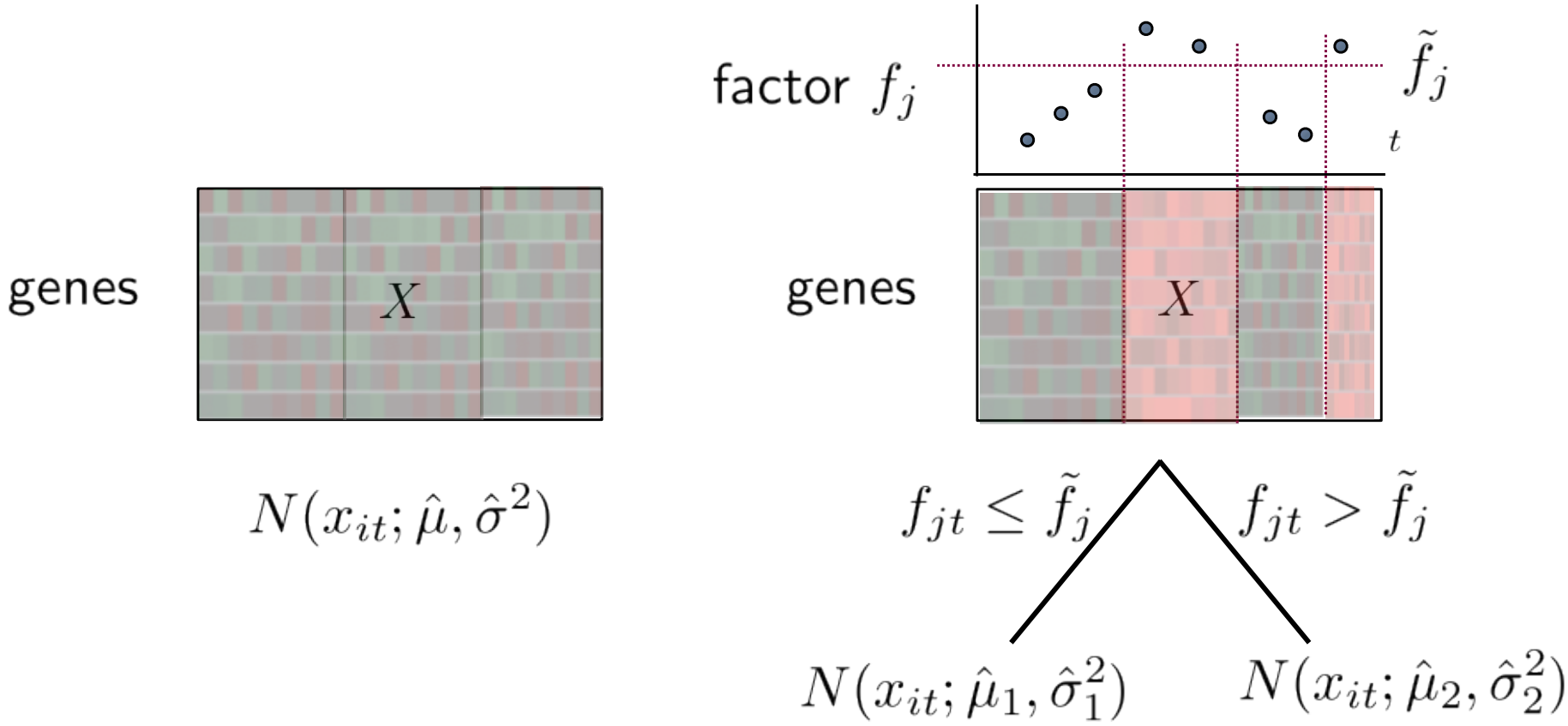
where $\Theta = \{\tilde{f}_j, \mu_1, \mu_2, \sigma_1^2, \sigma_2^2\}$.

- By maximizing $l(X|f_j;\Theta) = \log P(X|f_j;\Theta)$ with respect to the parameters (threshold, means and variances), we get

$$l(X|f_j;\hat{\Theta}) = n|\hat{I}_1|\left[-\frac{1}{2} - \frac{1}{2}\log(2\pi\hat{\sigma}_1^2)\right] + n|\hat{I}_2|\left[-\frac{1}{2} - \frac{1}{2}\log(2\pi\hat{\sigma}_2^2)\right]$$

Competing "programs"



We need to penalize our likelihood models based on their complexity

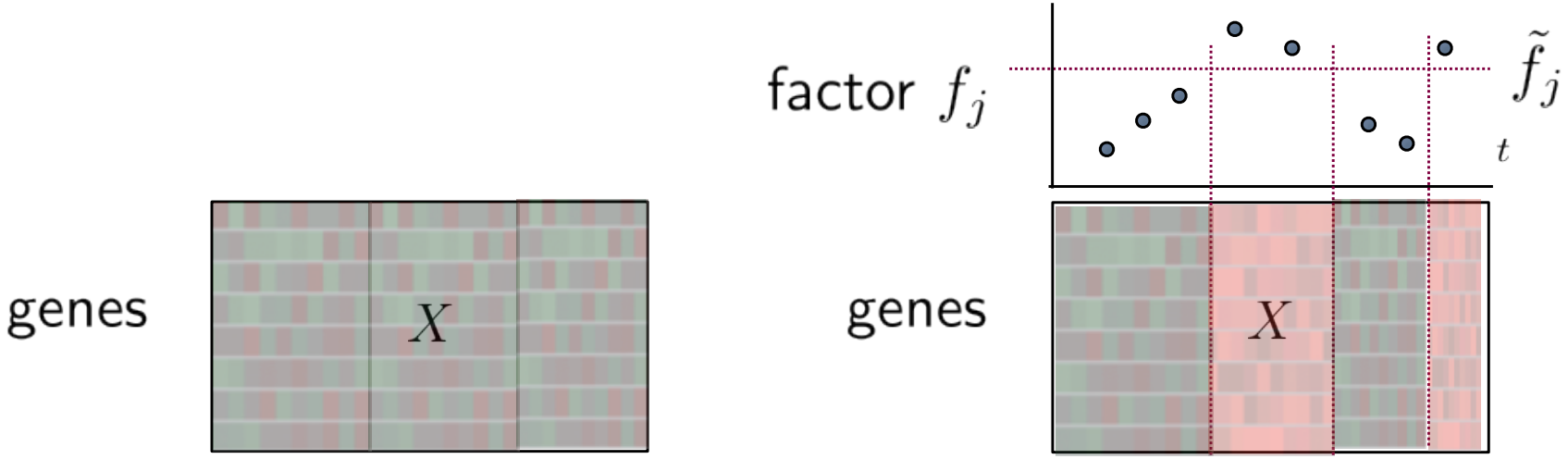
Number of model parameters (2)

BIC score =

$$l(X;\hat{\theta}) - \frac{2}{2}\log(nm)$$

Number of observations

Competing "programs"



$$N(x_{it}; \hat{\mu}, \hat{\sigma}^2)$$

BIC score =

$$l(X; \hat{\theta}) - \tfrac{2}{2} \log(nm)$$

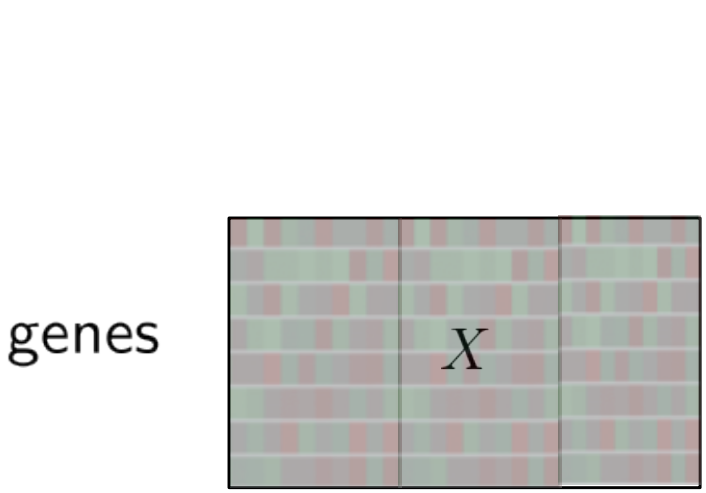
$$f_{jt} \leq \tilde{f}_j \qquad f_{jt} > \tilde{f}_j$$

$$N(x_{it}; \hat{\mu}_1, \hat{\sigma}_1^2) \qquad N(x_{it}; \hat{\mu}_2, \hat{\sigma}_2^2)$$

BIC score =

$$l(X|f_j; \hat{\Theta}) - \tfrac{5}{2} \log(nm)$$

The algorithm



$$N(x_{it}; \hat{\mu}, \hat{\sigma}^2)$$

BIC score =
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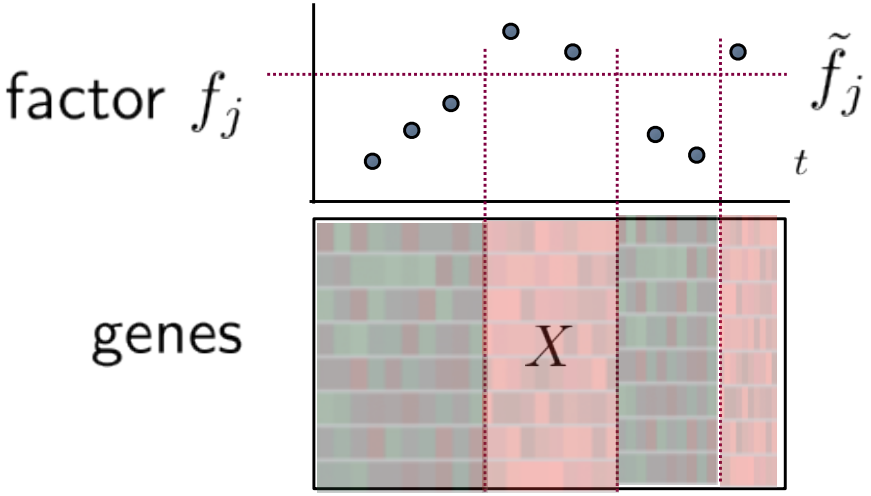

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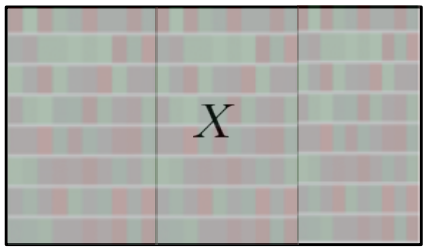
BIC score =
$$l(X|f_j; \hat{\Theta}) - \tfrac{5}{2}\log(nm)$$

Algorithm:

1) Calculate BIC scores with and without factor
2) If null model wins, stop, otherwise recurse

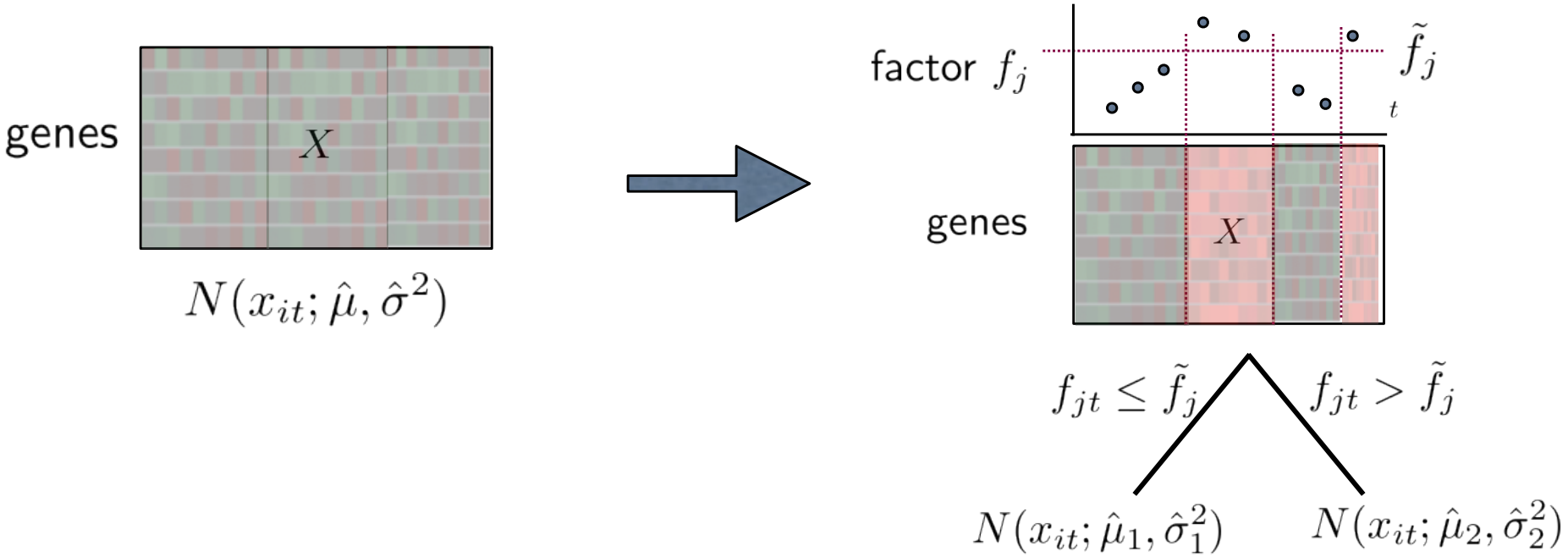
Recursive estimation

genes

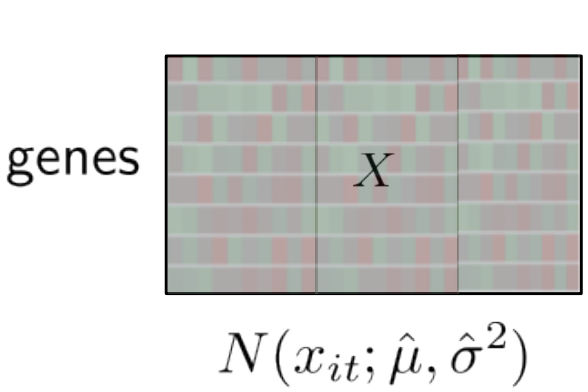


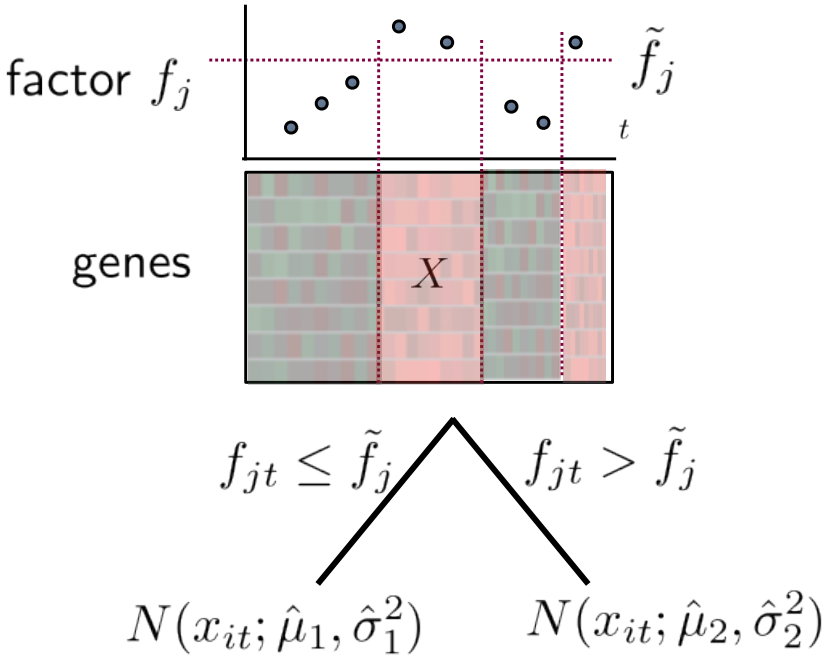
$N(x_{it}; \hat{\mu}, \hat{\sigma}^2)$

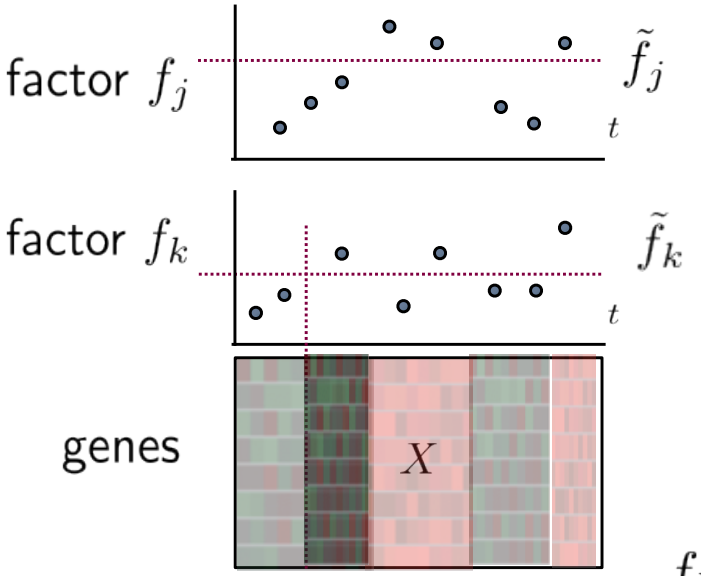
Recursive estimation

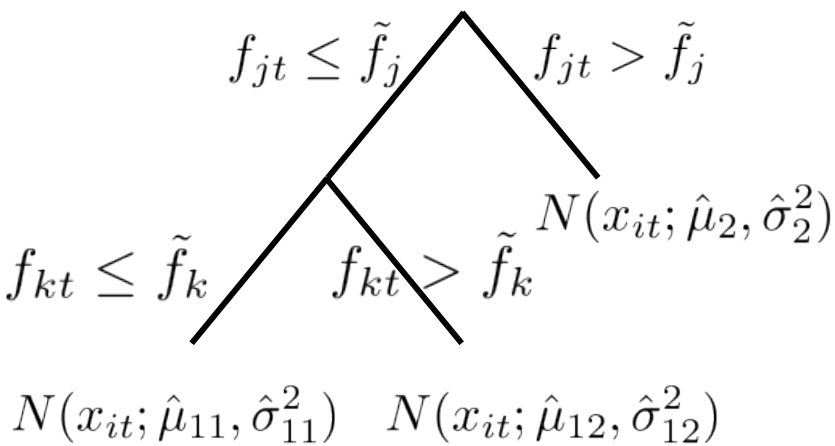


Recursive estimation

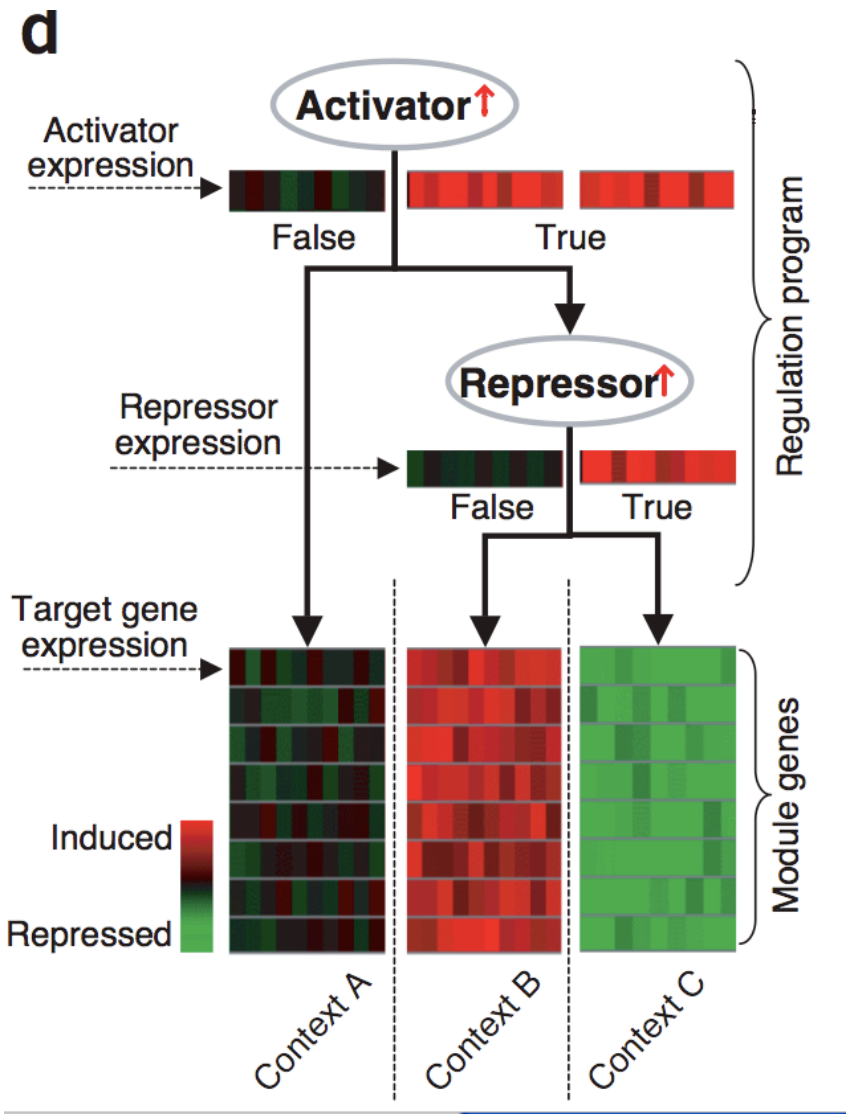








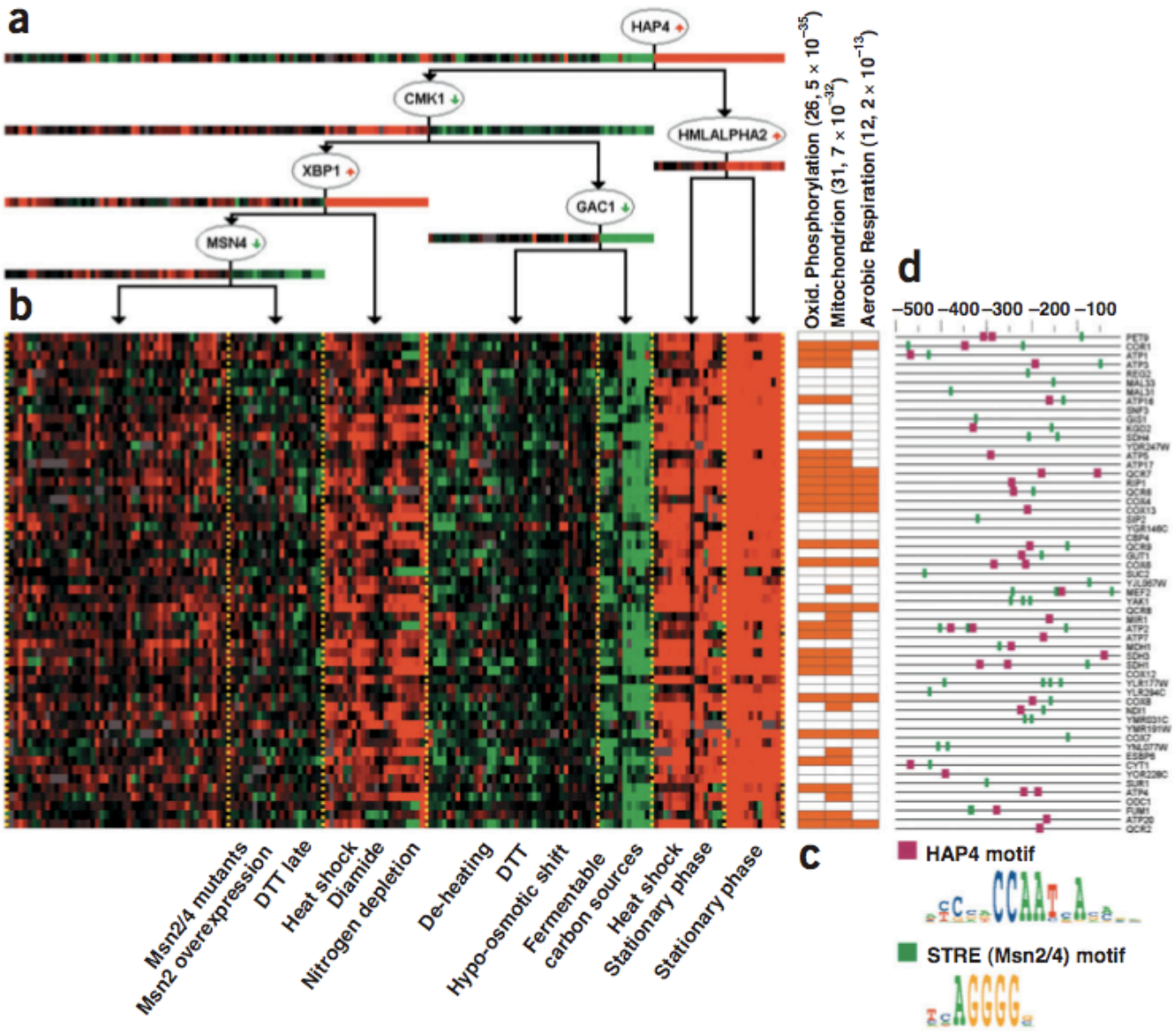
An ideal regression tree



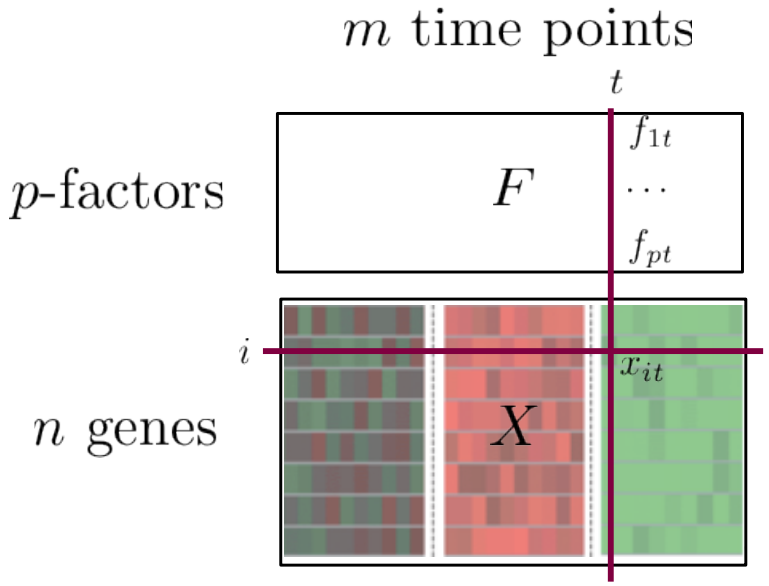
(Segal et al. 2003)

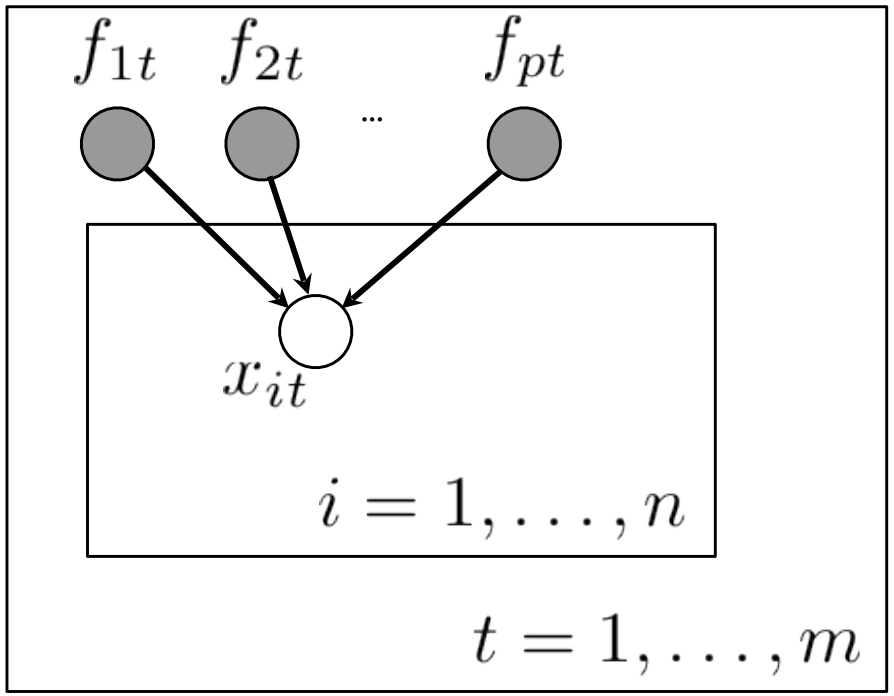
Expression programs

- An example expression program found in yeast



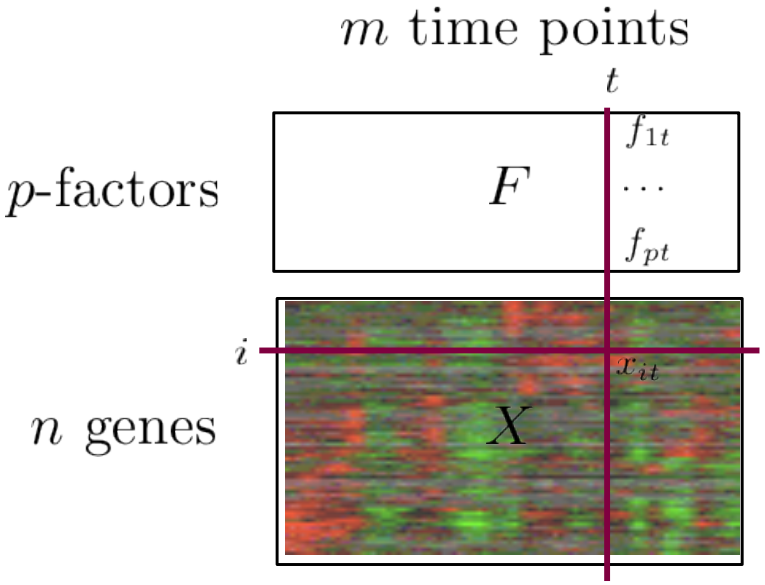
A single regression tree: sampling model

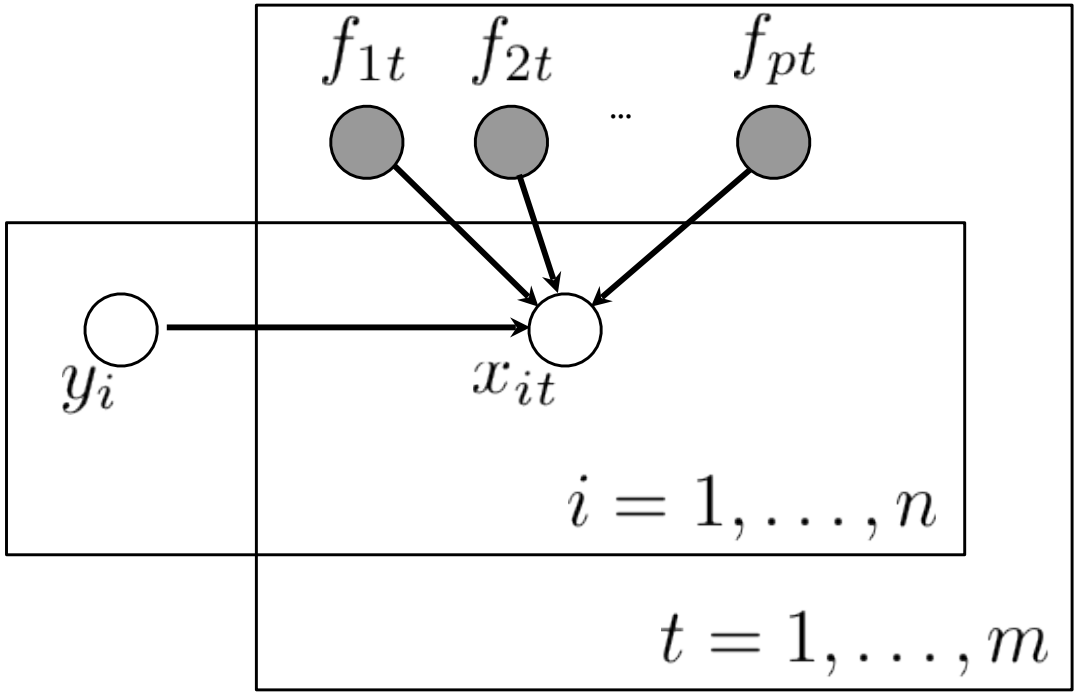




$$P(X|F,\Theta_1) = \prod_{t=1}^{m} \left[\prod_{i=1}^{n} P(x_{it}|f_{1t},\ldots,f_{pt},\Theta_1) \right]$$

We can allow different regulation programs for k different "modules" of genes

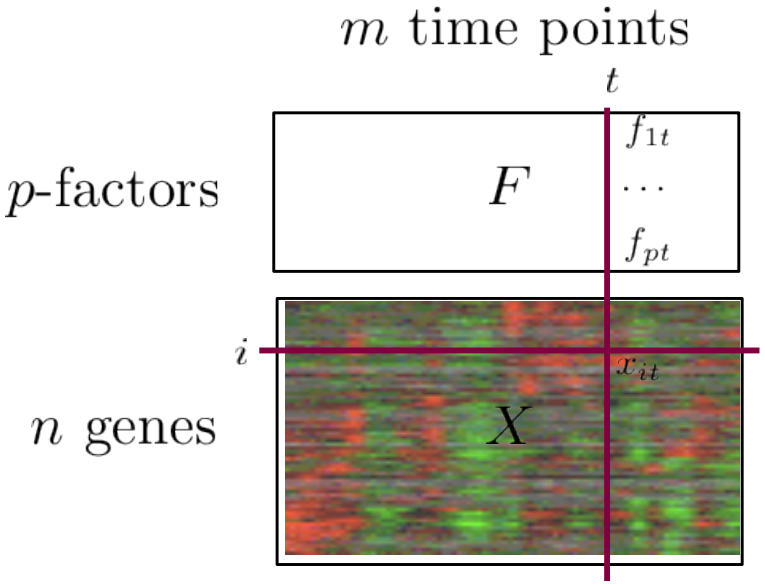


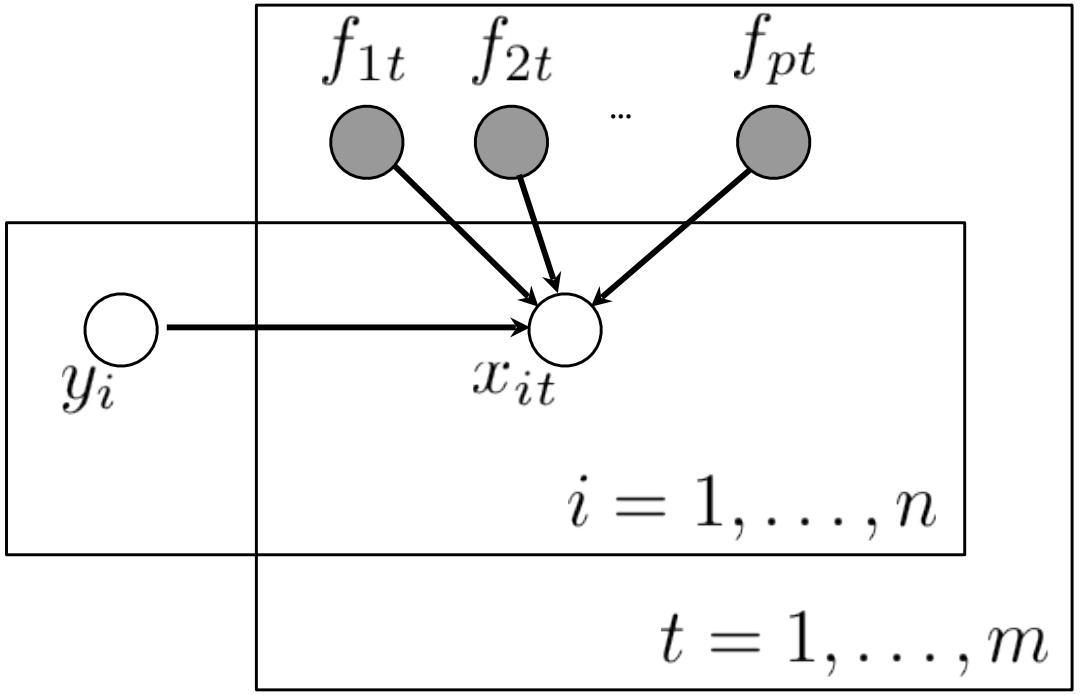


- k trees $\Theta_1, \ldots, \Theta_k$

$$P(x_{it}|f_{1t}, \ldots, f_{pt}, y) = P(x_{it}|f_{1t}, \ldots, f_{pt}, \Theta_y)$$

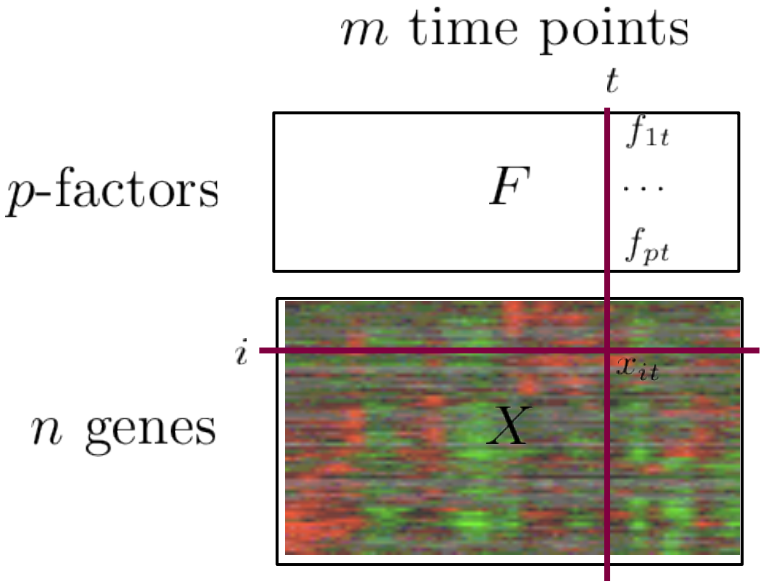
A mixture of trees: sampling model

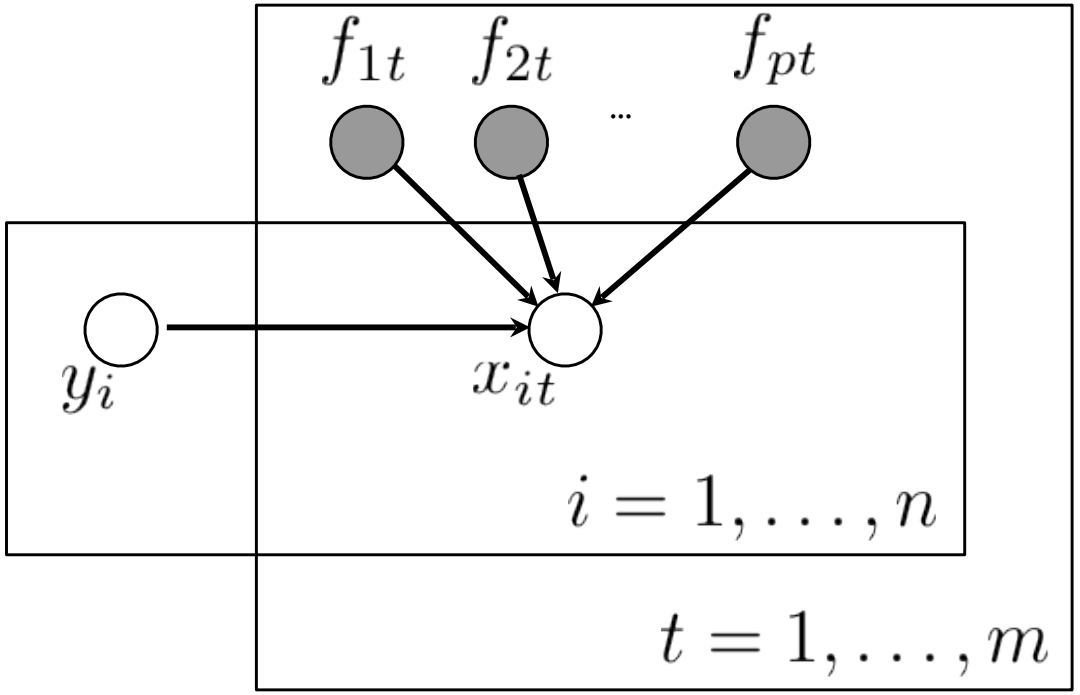




$$P(X|F,\Theta) = \prod_{i=1}^{n} \sum_{y_i=1}^{k} P(y_i) \prod_{t=1}^{m} P(x_{it}|f_{1t},\ldots,f_{pt},\Theta_{y_i})$$

A mixture of trees: sampling model





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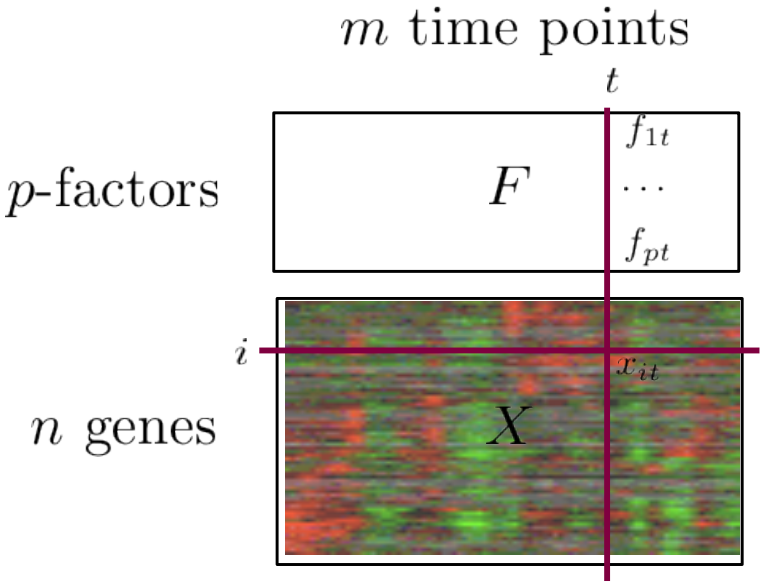
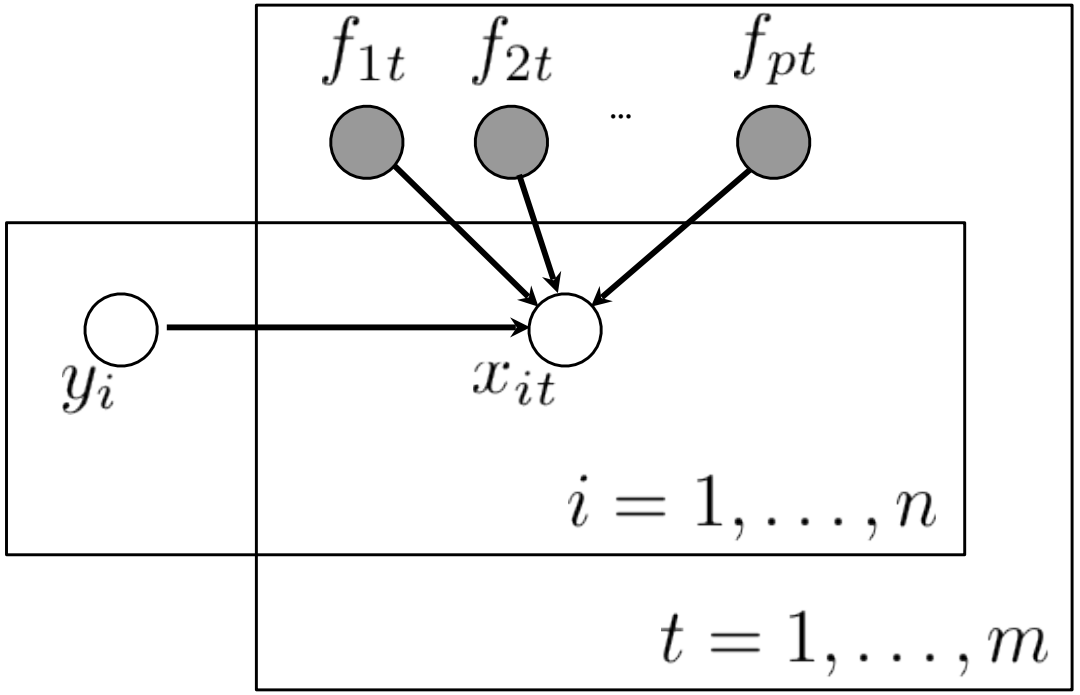
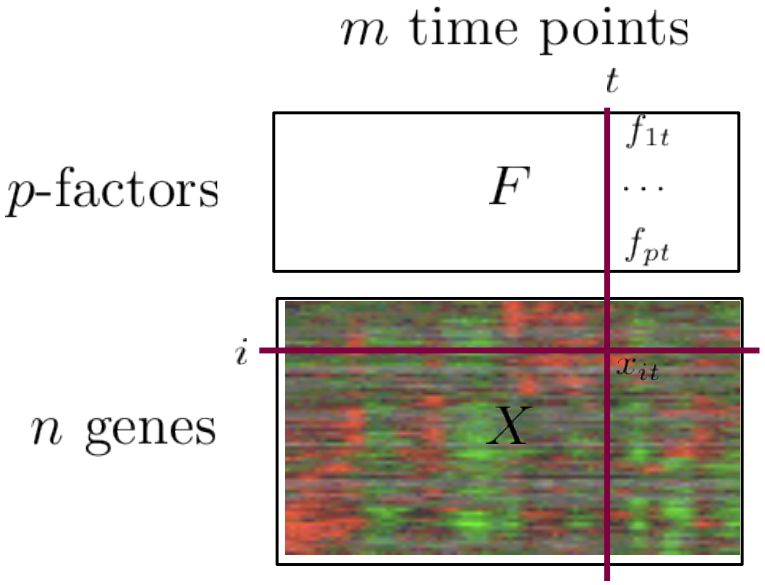
A mixture of trees: sampling model

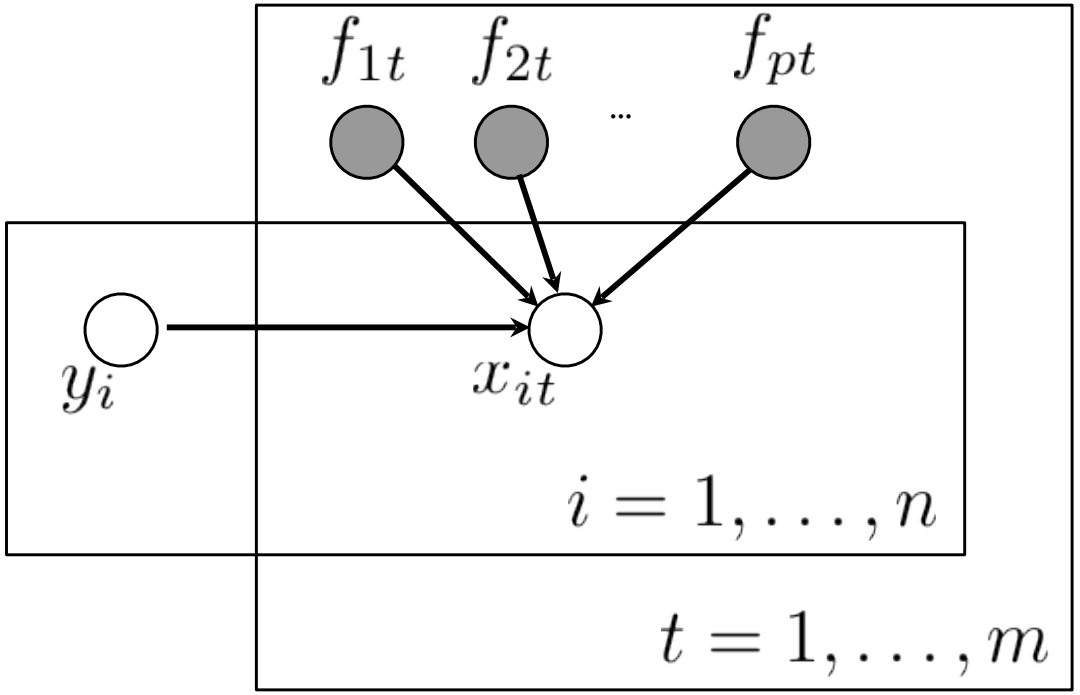




$$P(X|F,\Theta) = \prod_{i=1}^{n} \sum_{y_i=1}^{k} P(y_i) \prod_{t=1}^{m} P(x_{it}|f_{1t},\ldots,f_{pt},\Theta_{y_i})$$

A mixture of trees: sampling model

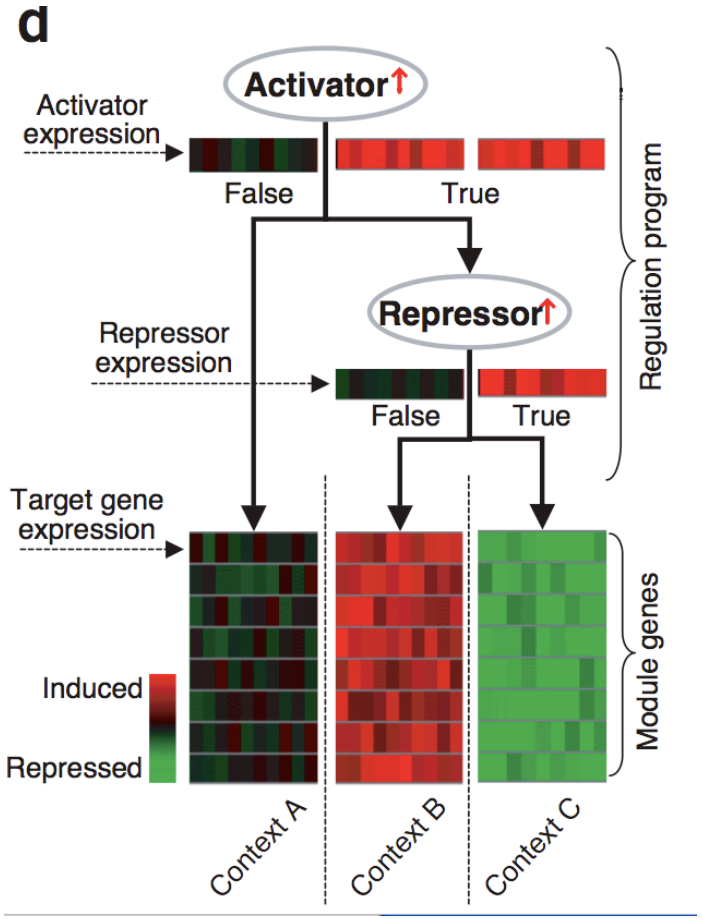




$$P(X|F,\Theta) = \prod_{i=1}^{n} \sum_{y_i=1}^{k} P(y_i) P(\underline{x}_i|F,\Theta_{y_i})$$

Finding expression programs

- We can jointly search for transcriptionally regulated subsets of genes and the corresponding programs (Segal et al., 2003)



EM algorithm

- We model the program assignments and expression profiles as

$$P(y_1, \ldots, y_n, \underline{x}_1, \ldots, \underline{x}_n | F, \Theta) = \prod_{i=1}^{n} P(y_i)\, P(\underline{x}_i | F, \Theta_{y_i})$$

- We can optimize the parameters of this model via the EM algorithm

(0) cluster genes into k clusters
(1) re-estimate regression trees for each cluster (program)
(2) (softly) re-assign each gene to the best program

$$p(y = j|i) \propto P(y) P(\underline{x}_i | F, \Theta_y)$$

Regression trees as programs

- Each regression tree can be represented as an "if-then-else" program

Cond. distribution $P(x|f_1, \ldots, f_p) = P(x|F)$

if $f_j \leq \tilde{f}_j$

$x \sim N(x; \mu_1, \sigma_1^2)$

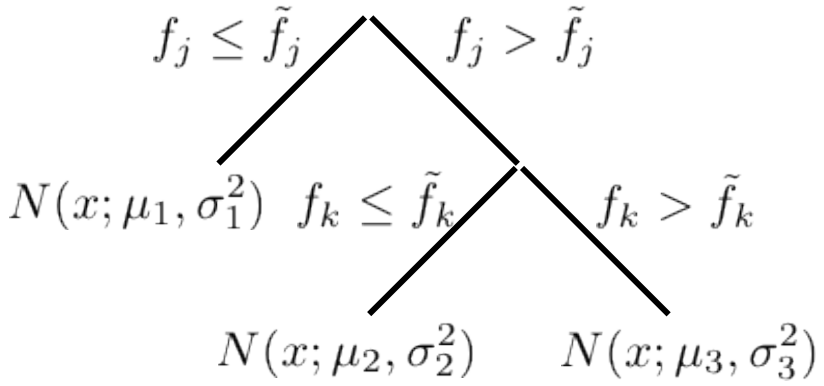
else

if $f_k \leq \tilde{f}_k$

$x \sim N(x; \mu_2, \sigma_2^2)$

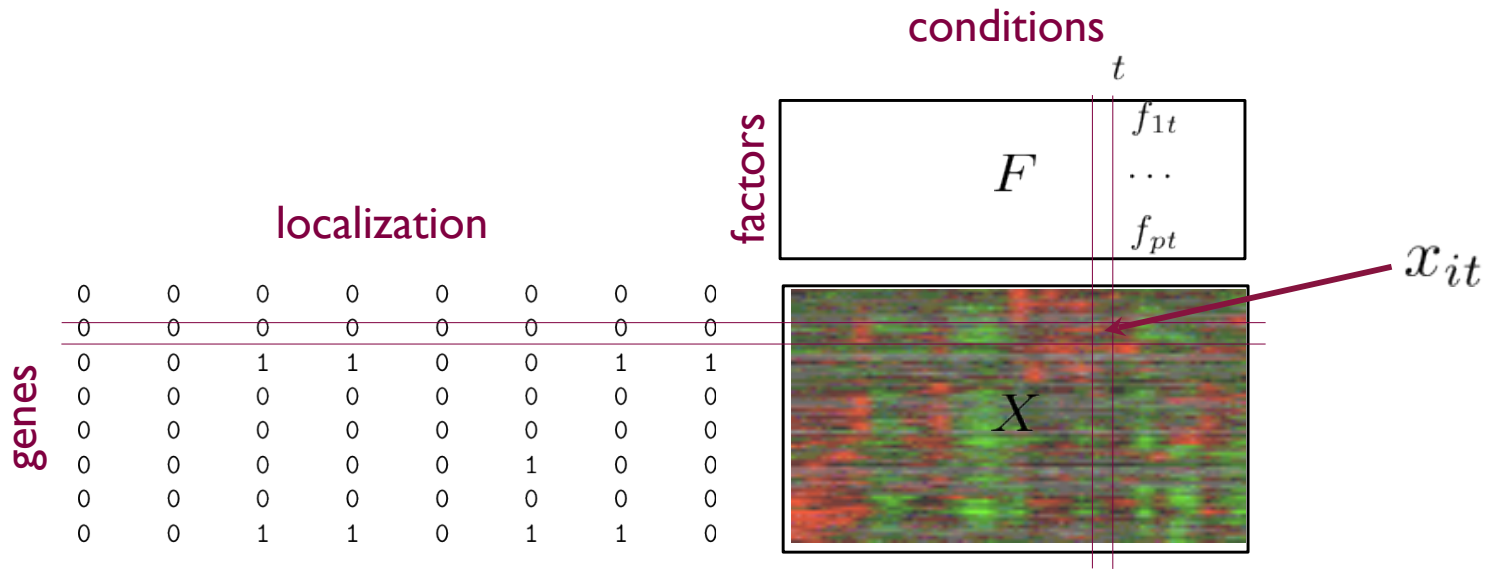
else

$x \sim N(x; \mu_3, \sigma_3^2)$



Extensions...

- The basic regression trees formulation permits us to estimate various types of "programs"
  - e,g, TF expression & localization, etc.

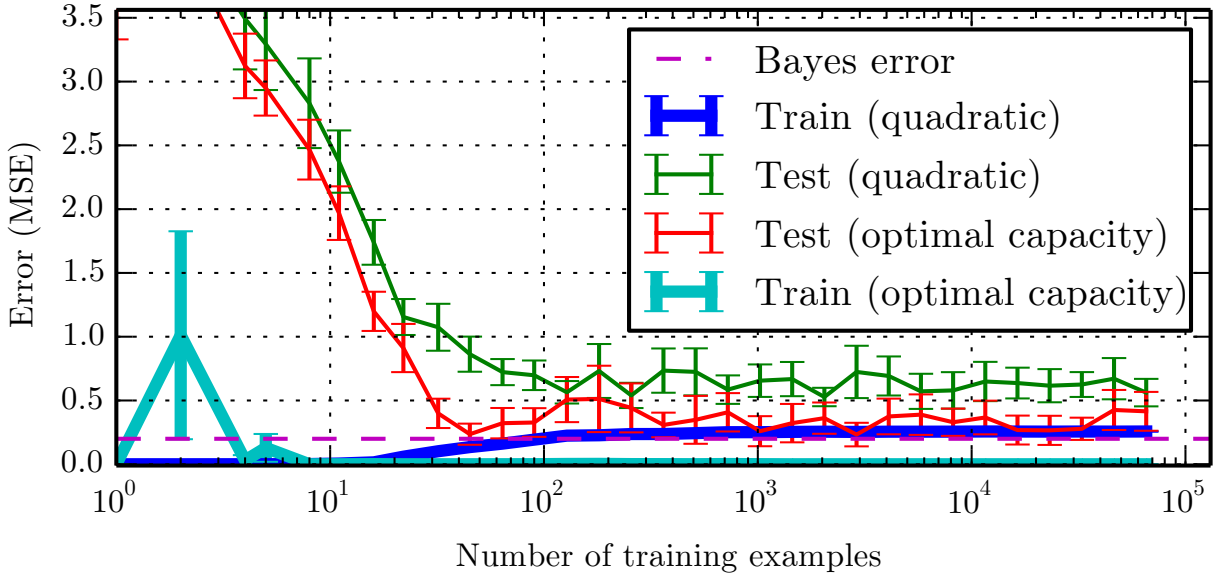


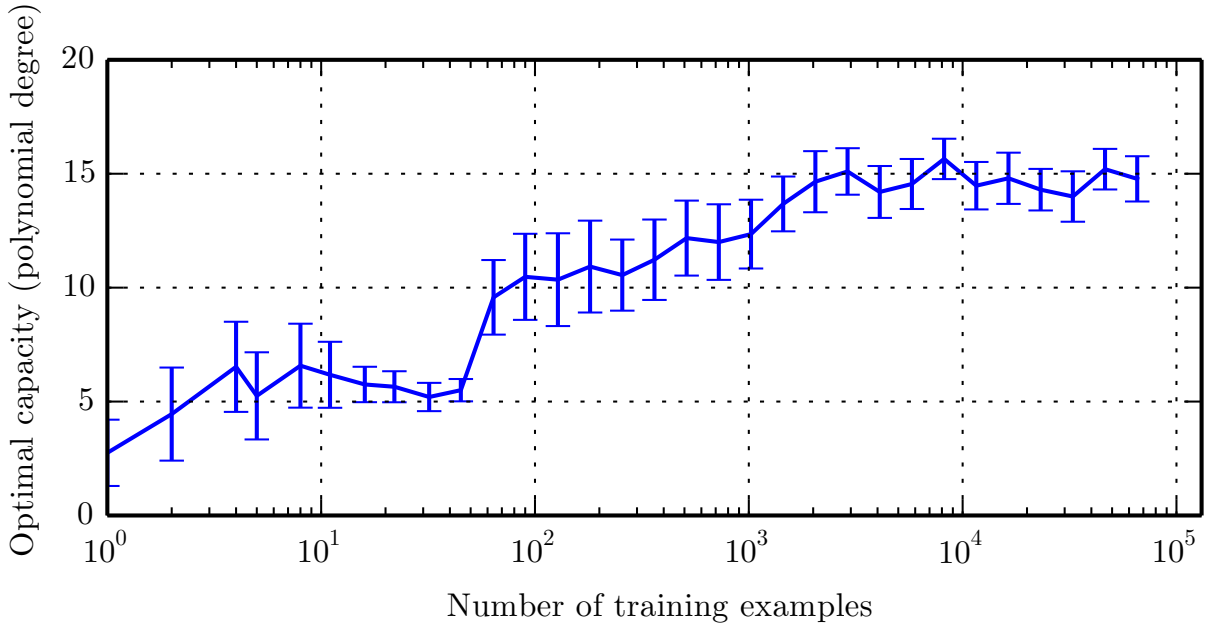
FIN - Thank You

Part 1 - Model Capacity

Sufficient training data are necessary to generalize well

Figure 5.4

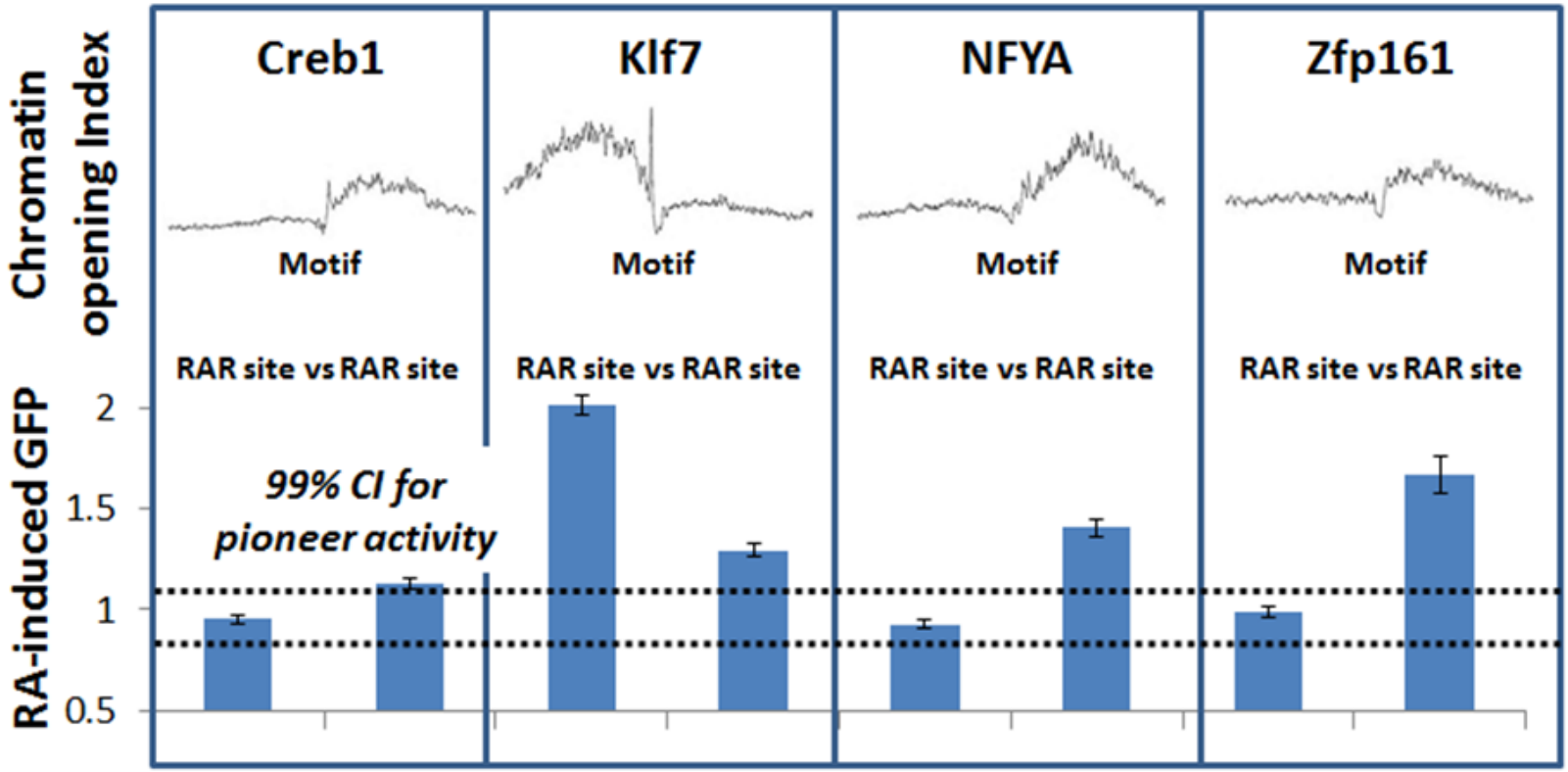




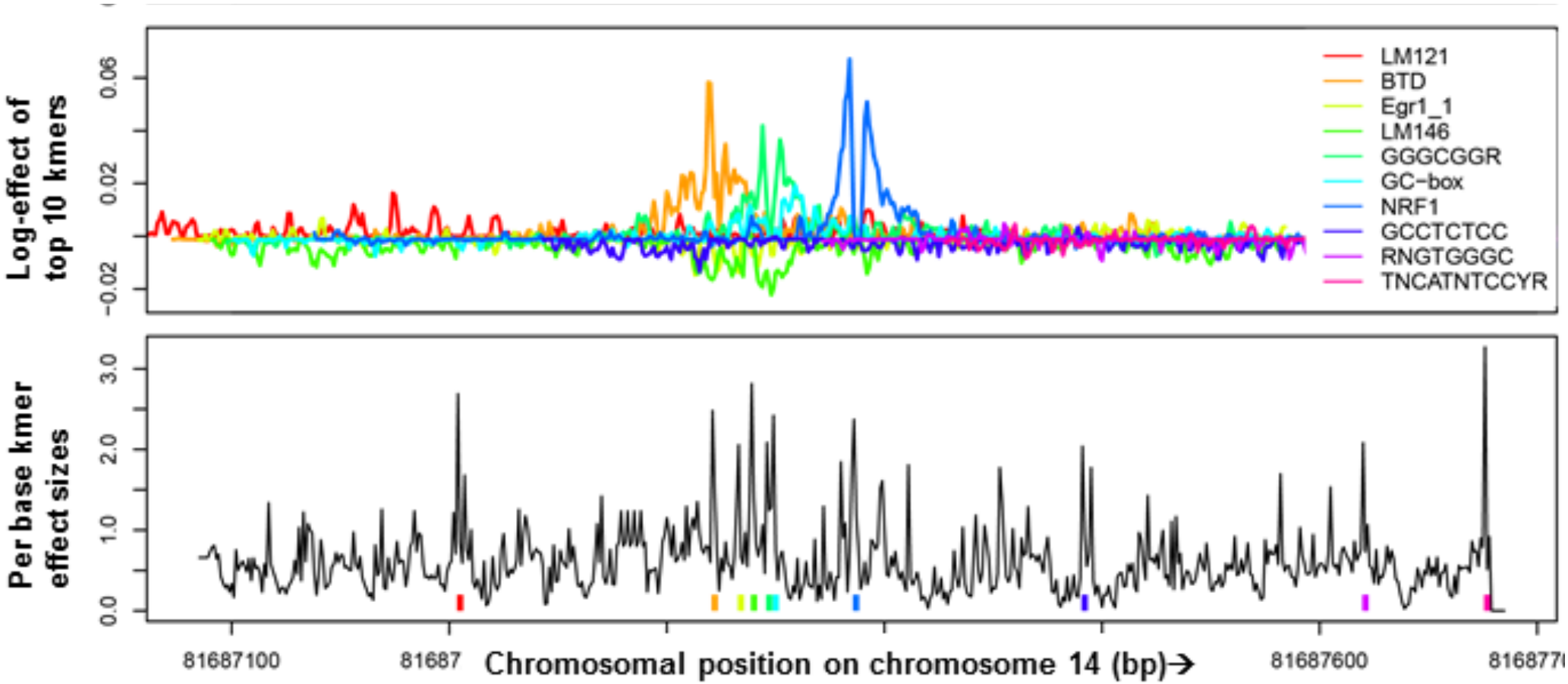
Part 2 - Modeling Genome Accessibility

Certain pioneer TFs are directional

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

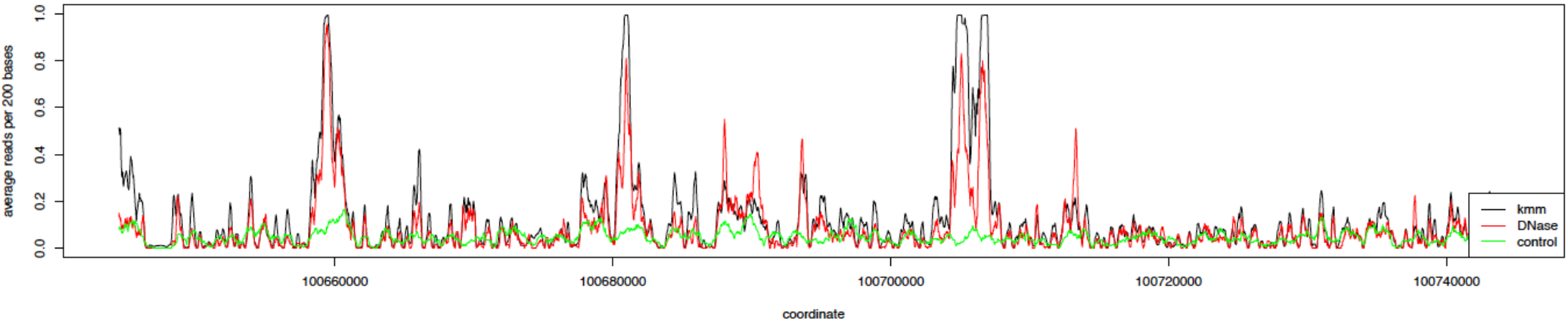


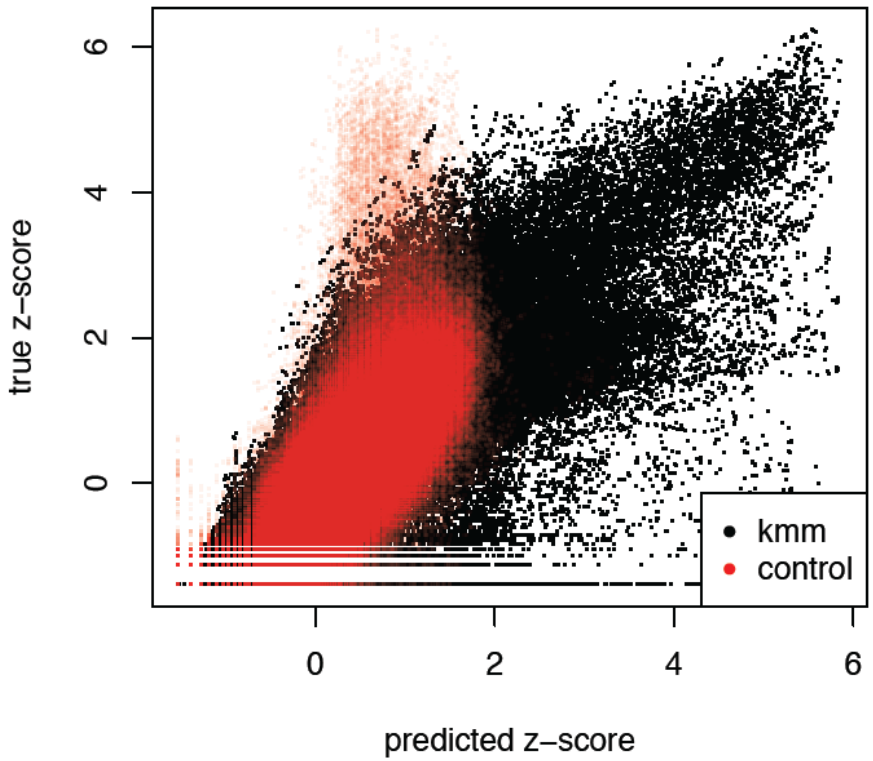
The Synergistic Chromatin Model (SCM) is a K-mer model



~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)

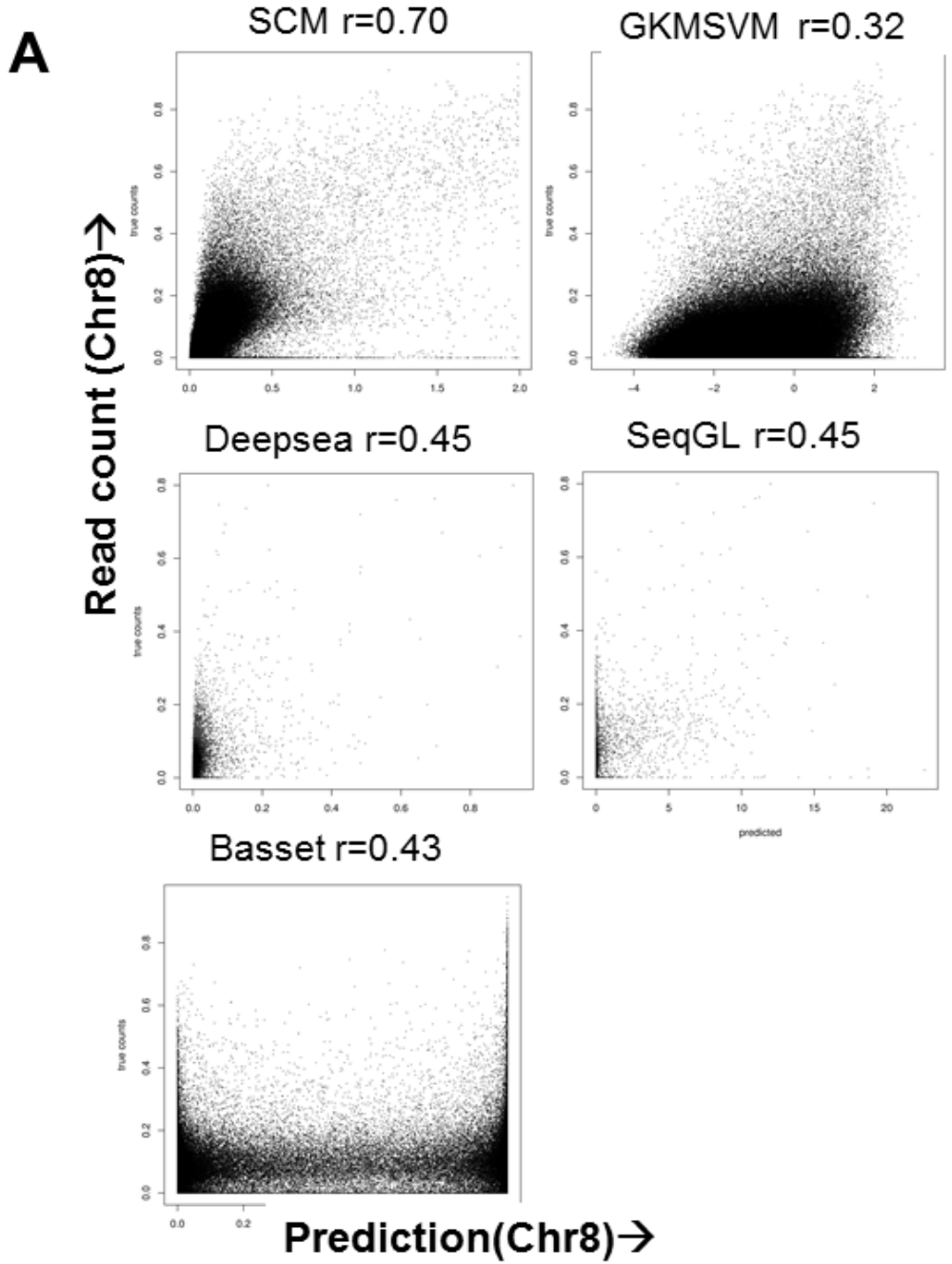


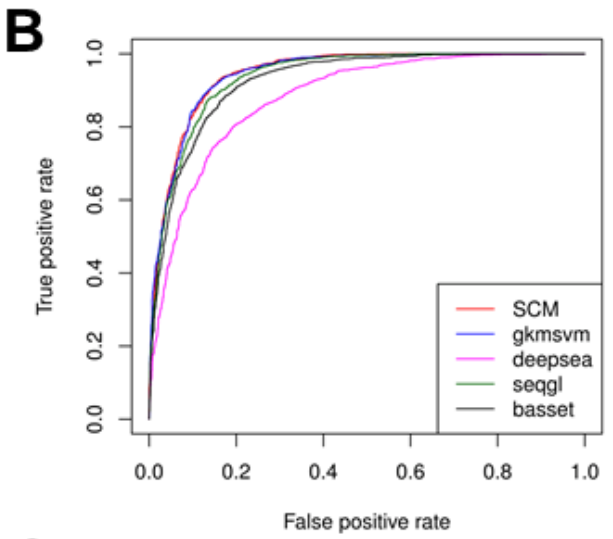


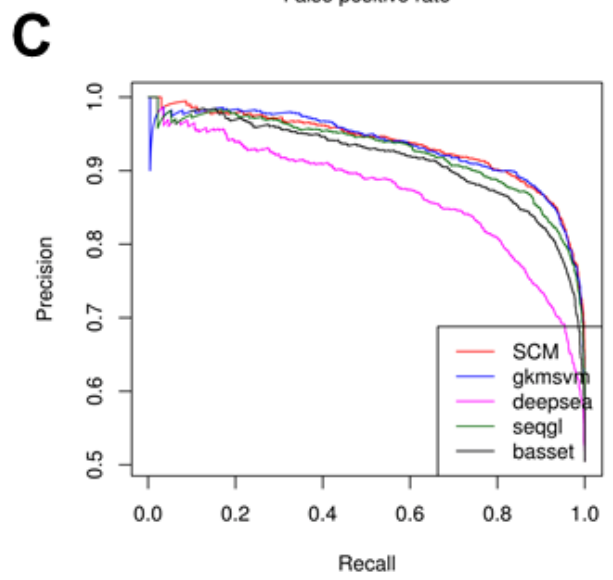
KMM R^2 0.80

Control R^2 0.47

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

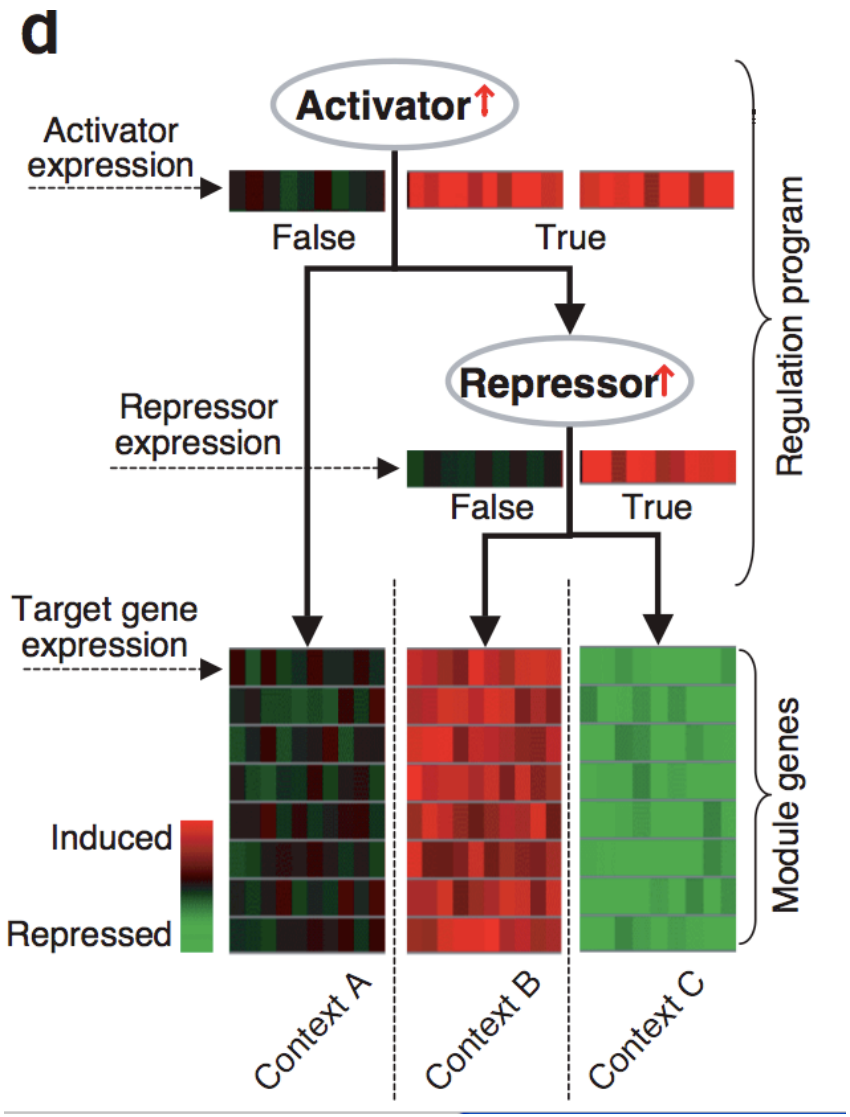






Part 3 - Modeling Gene Expression

An ideal regression tree



(Segal et al. 2003)

Expression programs

- An example expression program found in yeast