# Insights from integrative analysis of the C. elegans genome:

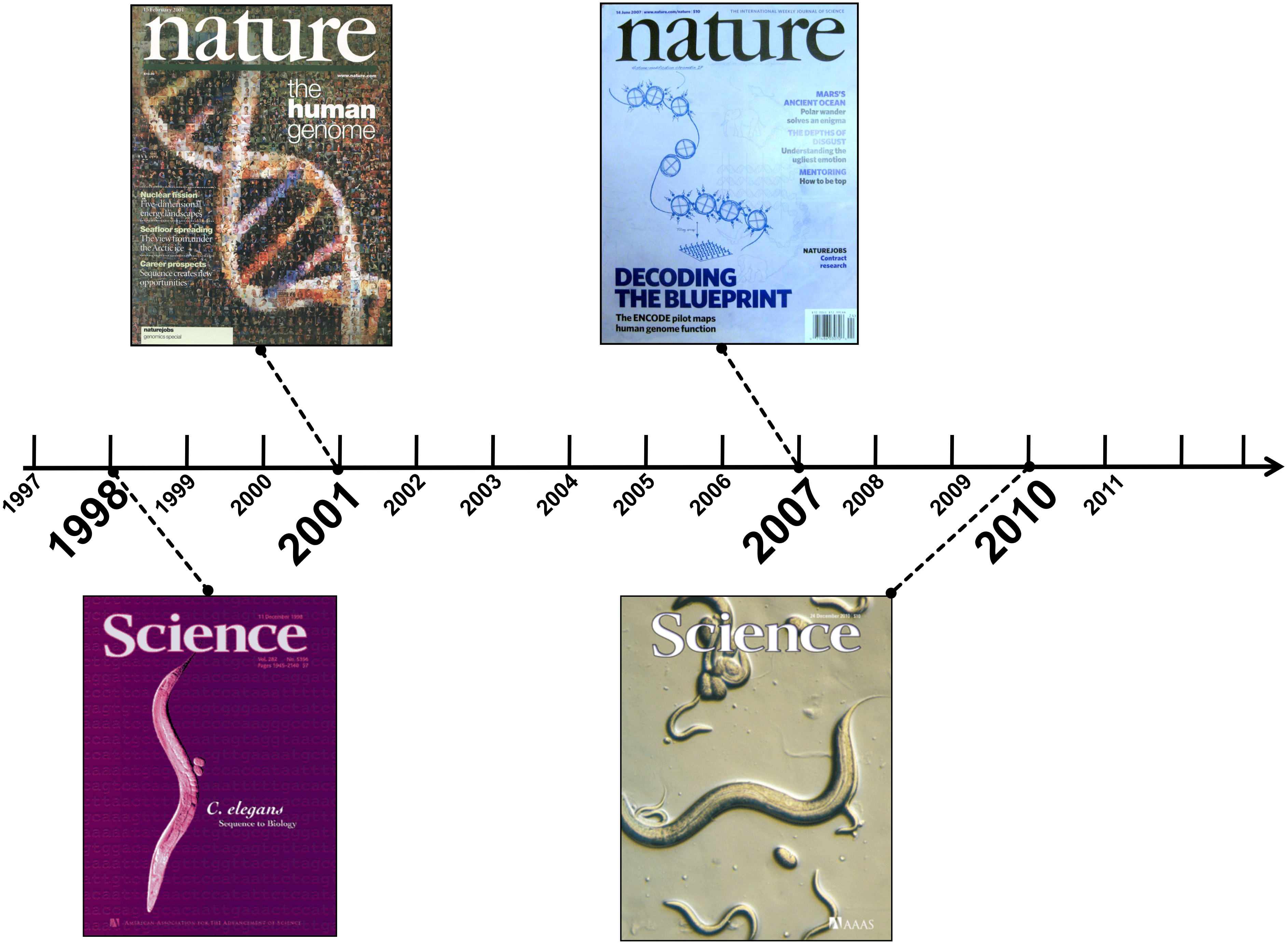
## What approaches we learned that were applicable to annotating the human genome

Mark B Gerstein
Yale

Slides at

Lectures.GersteinLab.org

(See Last Slide for
References & More Info.)



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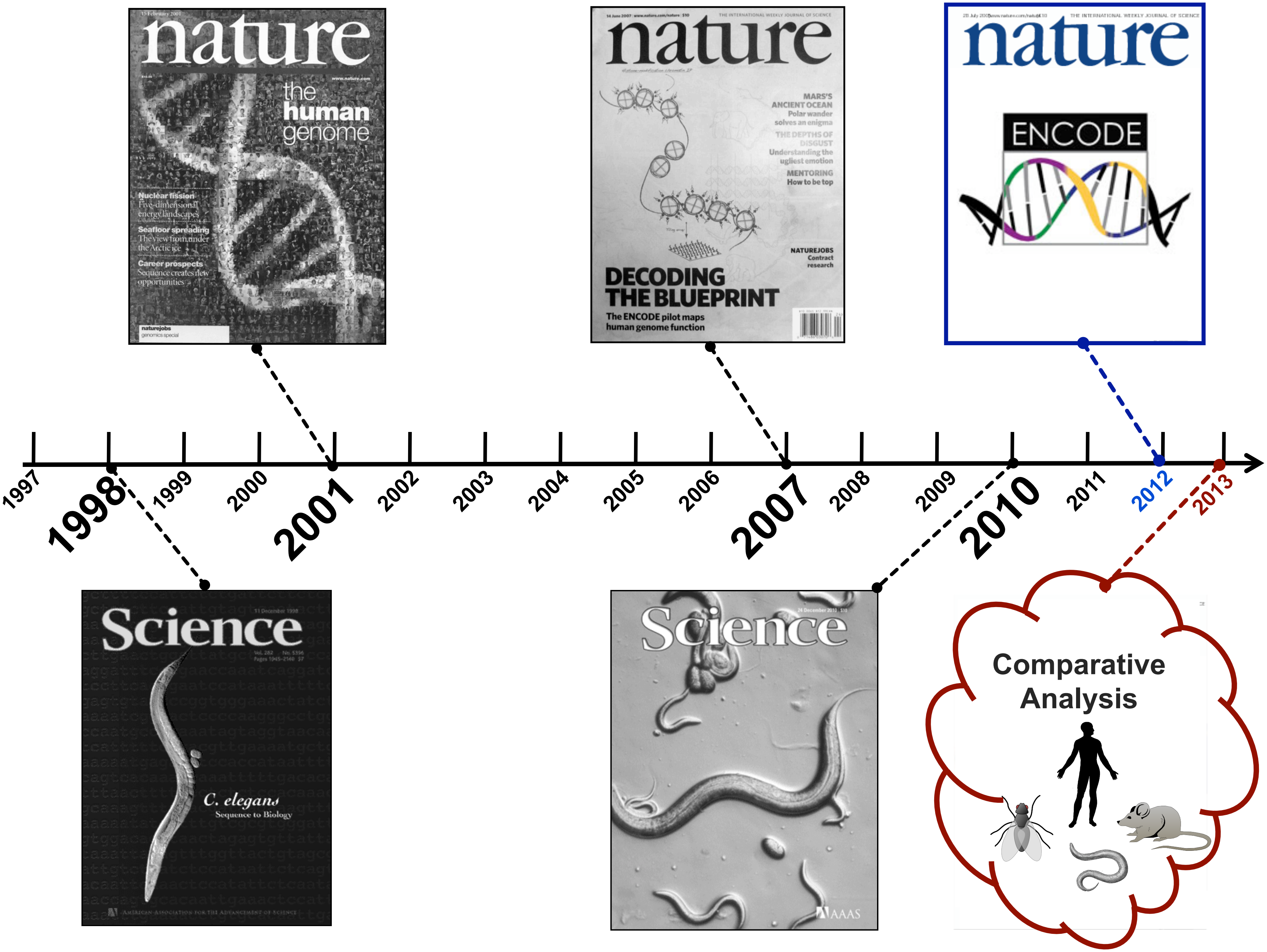
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C. elegans
Sequence to Biology
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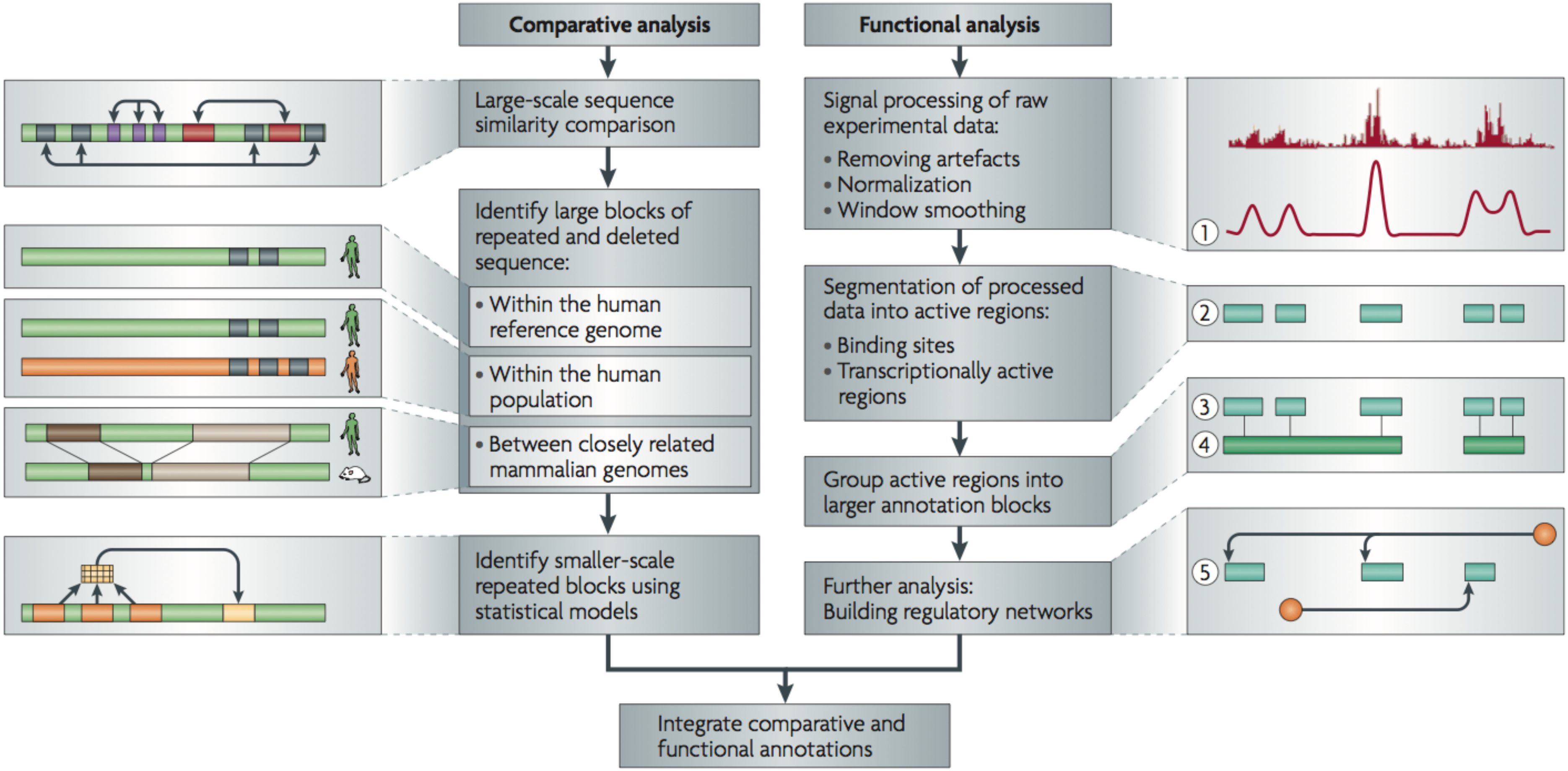
AAAS
Comparative Analysis

# 2 Approaches to Genome Annotation



Comparative analysis

Large-scale sequence similarity comparison

Identify large blocks of repeated and deleted sequence:

- Within the human reference genome
- Within the human population
- Between closely related mammalian genomes

Identify smaller-scale repeated blocks using statistical models

Functional analysis

Signal processing of raw experimental data:

- Removing artefacts
- Normalization
- Window smoothing

Segmentation of processed data into active regions:

- Binding sites
- Transcriptionally active regions

Group active regions into larger annotation blocks

Further analysis: Building regulatory networks

Integrate comparative and functional annotations

[*Nat. Rev. Genet.* (2010) 11: 559]

# Importance of
# Dark Matter of the Genome

- Non-coding regions contain the control elements for coding regions.

- Some non-coding regions are functional & are pervasively transcribed.
- “Molecular Fossils” in the non-coding genome represent a historical record of the genome
- Most disease-associated mutations (e.g. GWAS hits) are in non-coding regions.

[Gravitational lensing by dark matter in Abell 1689 – HST (NASA, ESA)]

# Overview of the Data

- **Worm**
  - Dev. Timecourse:
    E, L1, L2, L3, L4 + more
  - RNA-seq on timecourse + extra stages
    (polyA, small-RNA, 3' UTR selected)
  - Total RNA Tiling Arrays on timecourse + tissues
  - Chip-seq : 22 TFs + Pol2 in a variety of stages
  - Chromatin Chip-chip :
    >12 HMs mostly in EE & L3

- **Human (very briefly!)**
  - ~200 tot. cell lines with lots on tier 1
    (GM12878, K562, H1)
  - ~120 TFs
  - deep RNA-seq
  - ~12 main HMs (chip-seq)

# Insights from worm modencode: Approaches useful for human annotation (outline)

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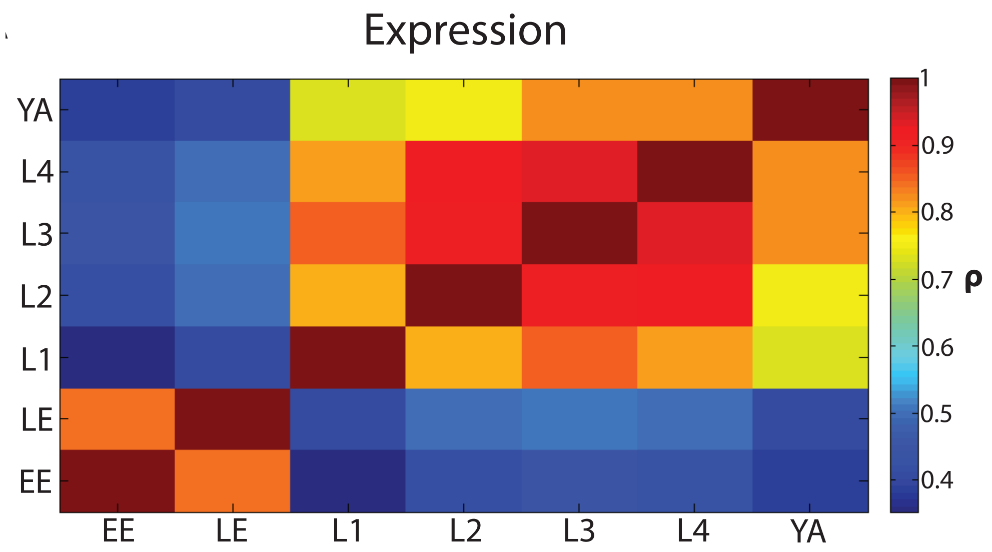
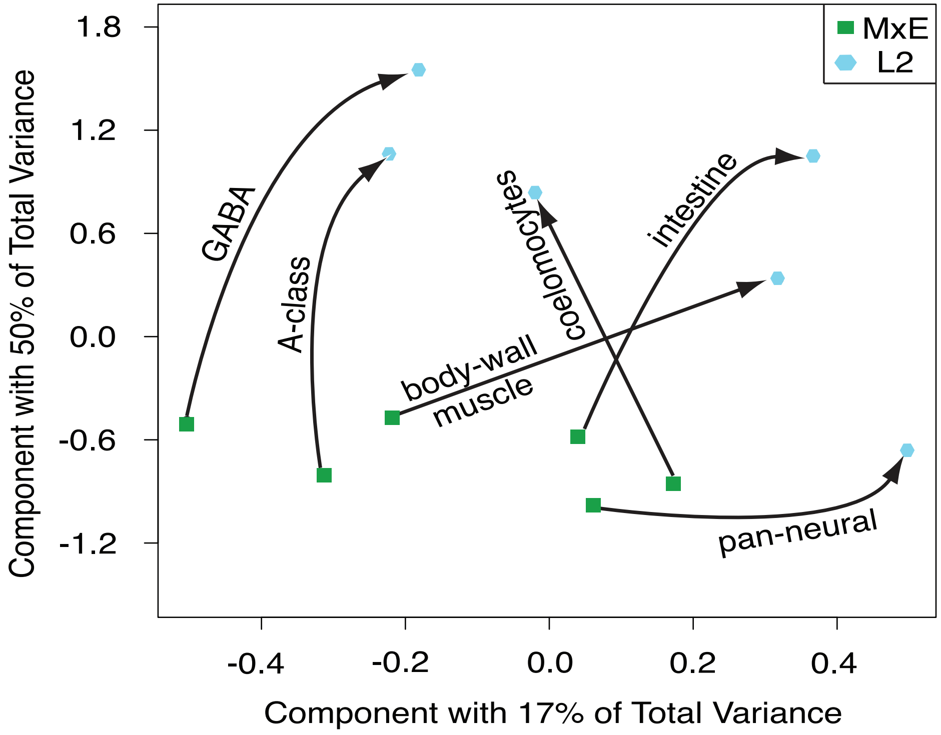
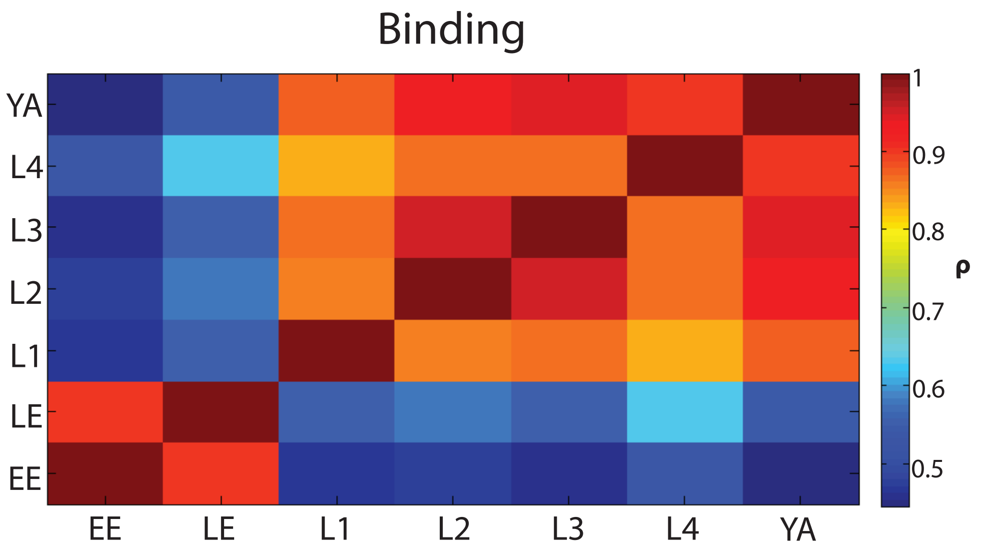
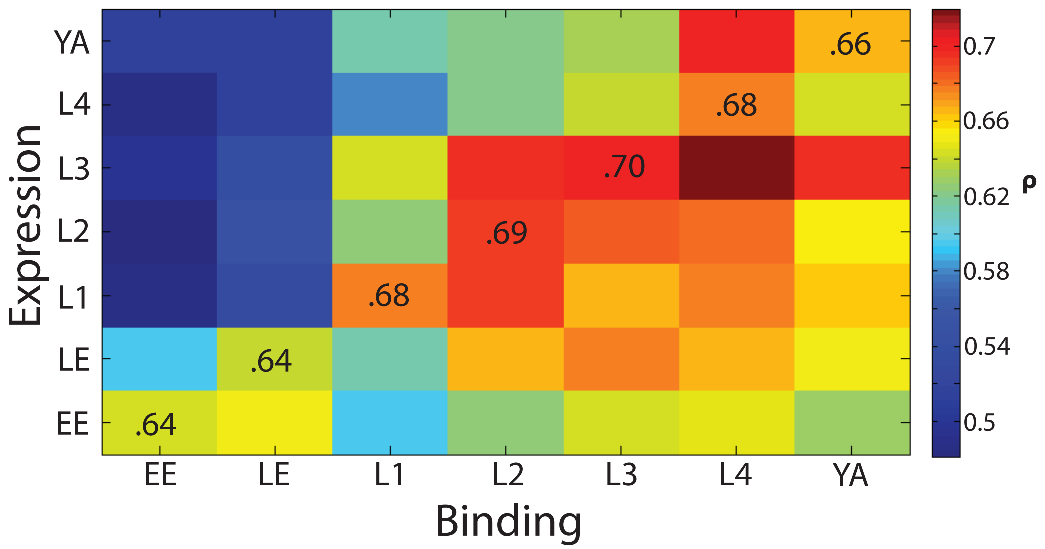
# Dynamics of Correlated Expression Changes Changes over Timecourse



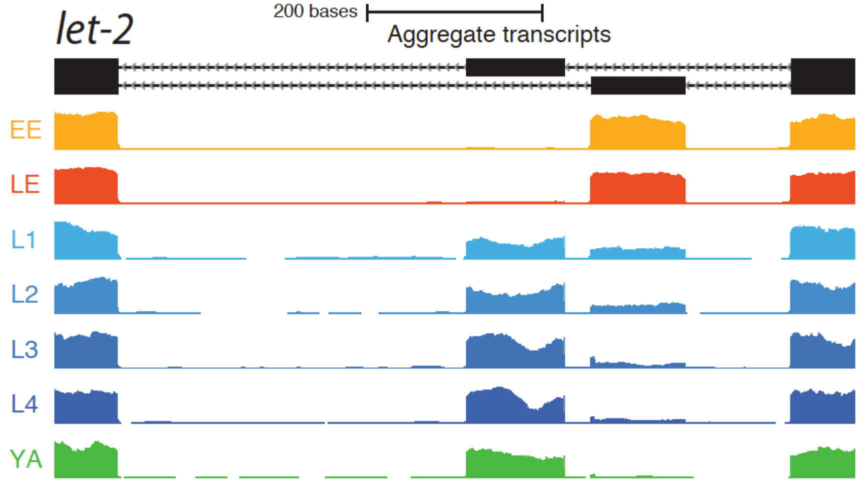


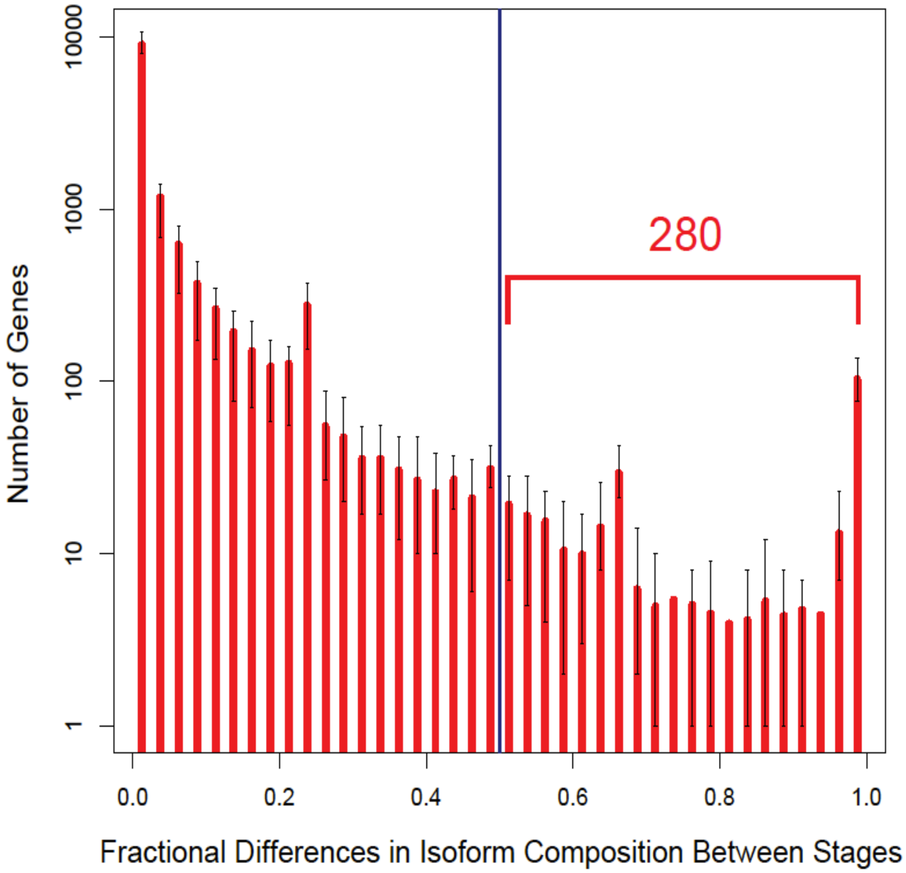
# Dynamics of Correlated Expression & Pol2 Binding Changes Changes over Timecourse





# Splicing Changes over the Timecourse (~280 changes/pair-of-stages)





$$Diff_{gene_i}(\Theta^{(Stage1)}, \Theta^{(Stage2)}) = \frac{\sum_{k=1}^{K} (\theta_k^{(Stage1)} - \theta_k^{(Stage2)})^2}{K}$$

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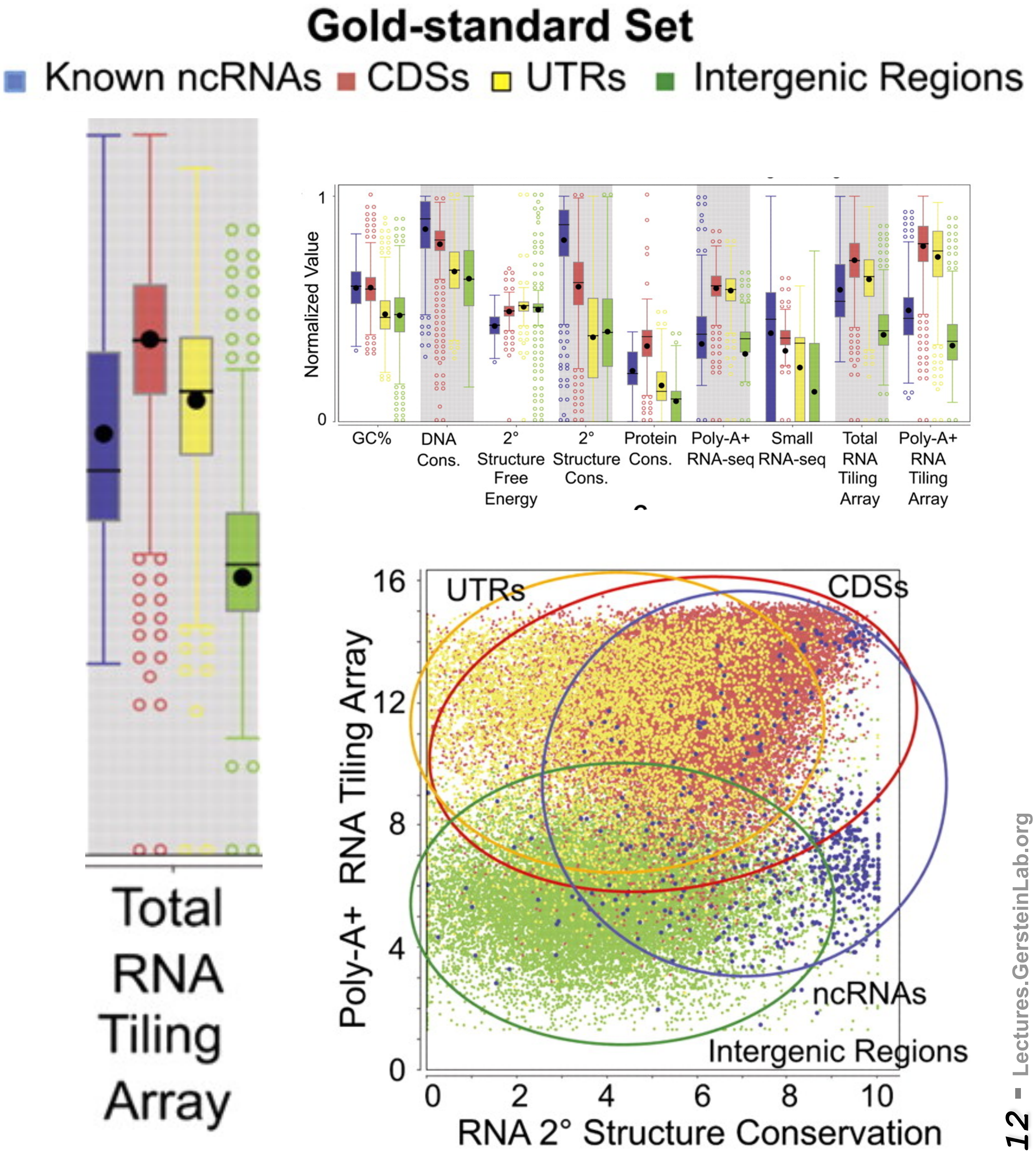
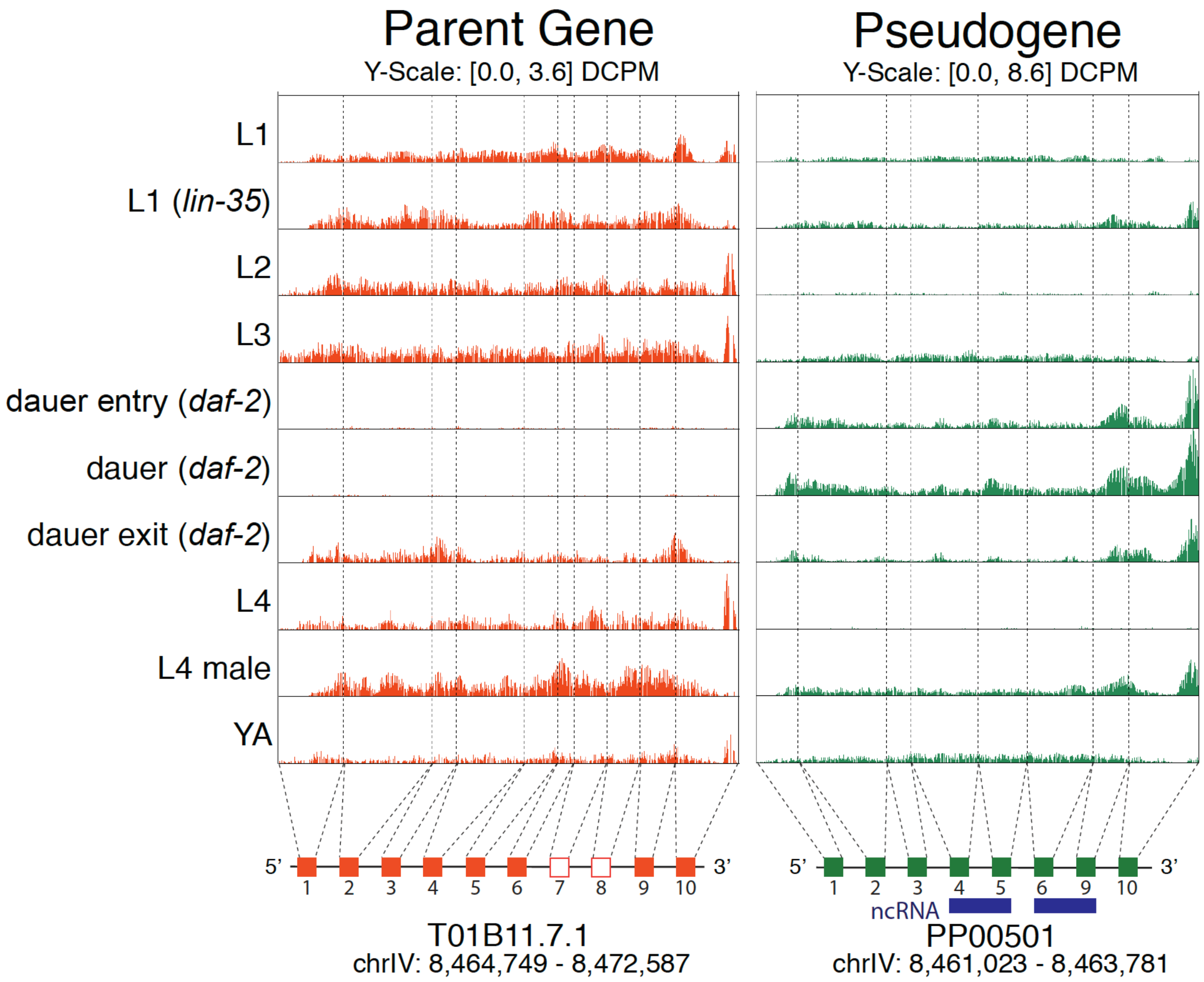
# Identification of many candidate ncRNAs through evidence integration

- ~7k candidates
- No single feature (e.g. expr. expts., conservation, or sec. struc.) finds all known ncRNAs => combine features in stat. model
- 90% PPV, 13 of 15 tested validate



[Lu et al. Genome Res. 2011;21:276-285]

# One type of ncRNA: Transcribed Pseudogenes

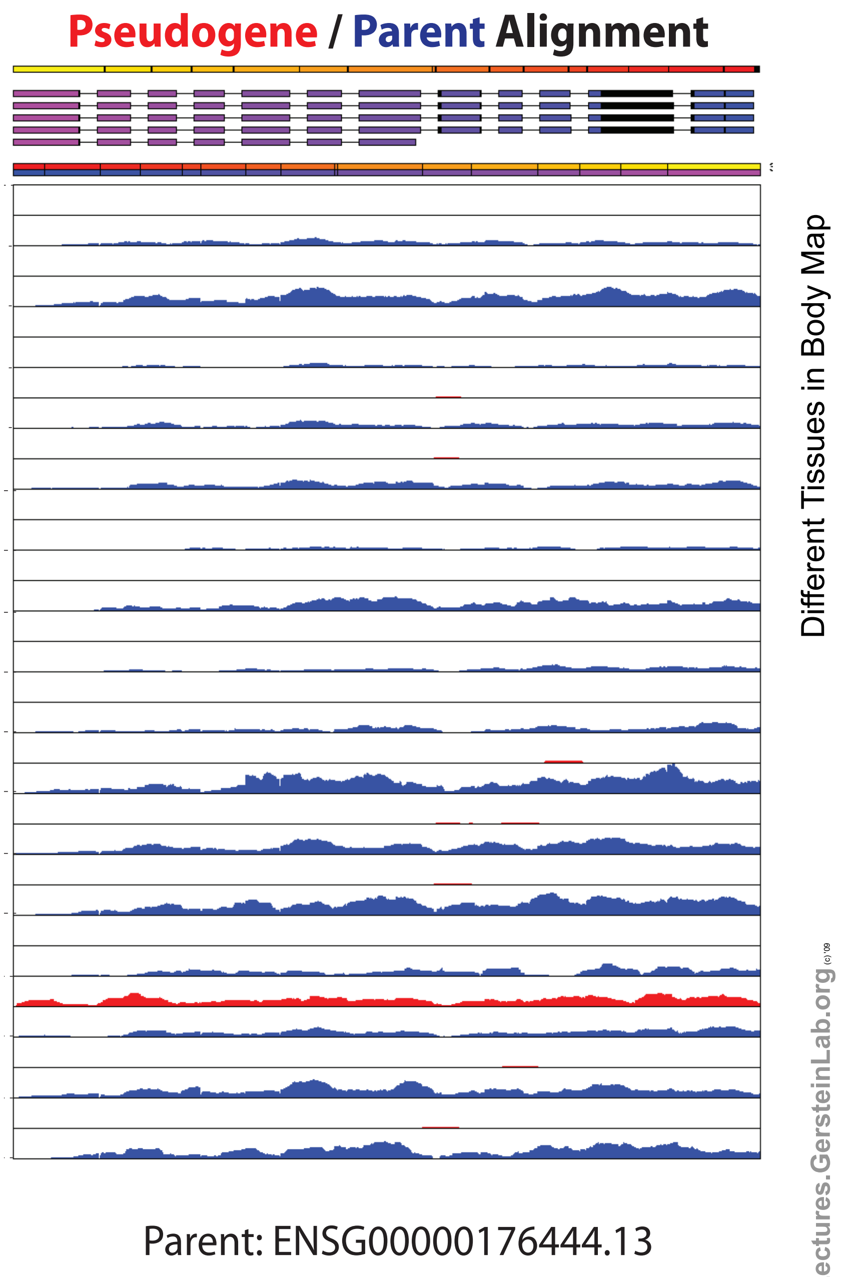


- 1198 total pseudogenes
- 194 (16%) have strong evidence of independent transcription

[*Science* 330:6012]

# Human ncRNAs and Pseudogene Transcription

- Gencode 10 :
  manual annotation
  + a variety of pipelines
  - ~5500 lincRNAs
  - 11216 high-qual. pseudogenes (from ~14K total)
    - Total transcribed pseudogene: 876 (RTPCR validated: 57 of 76)



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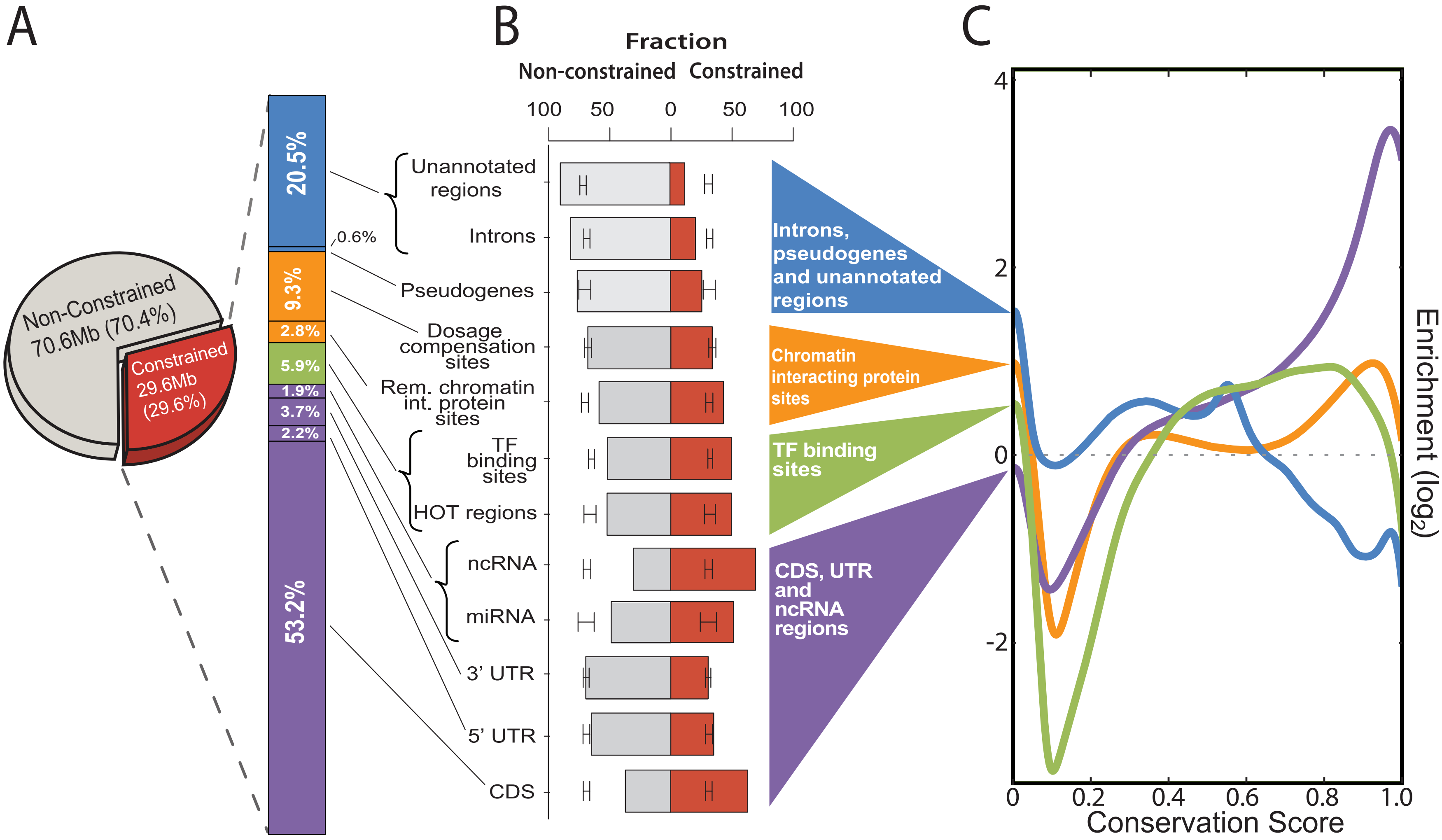
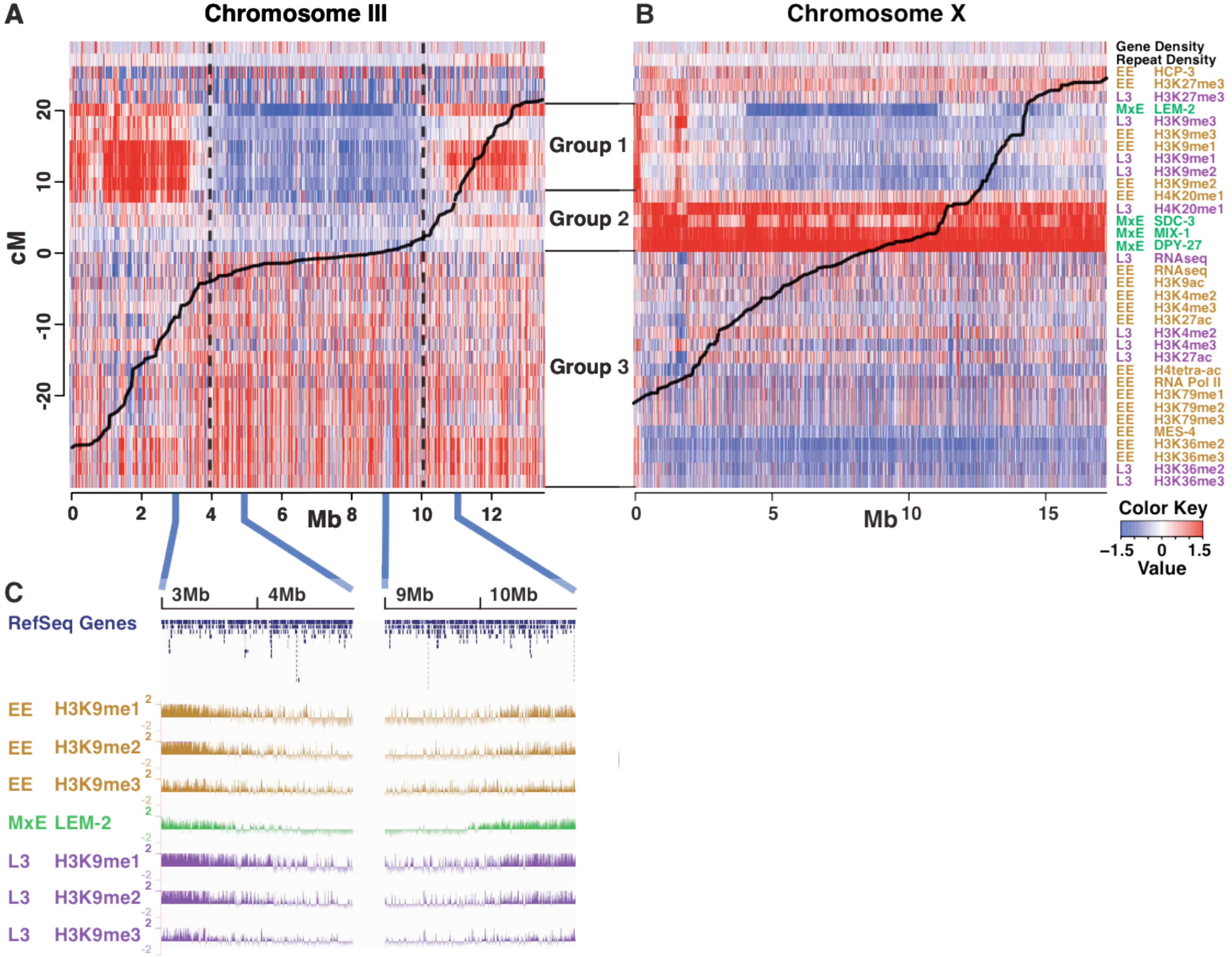
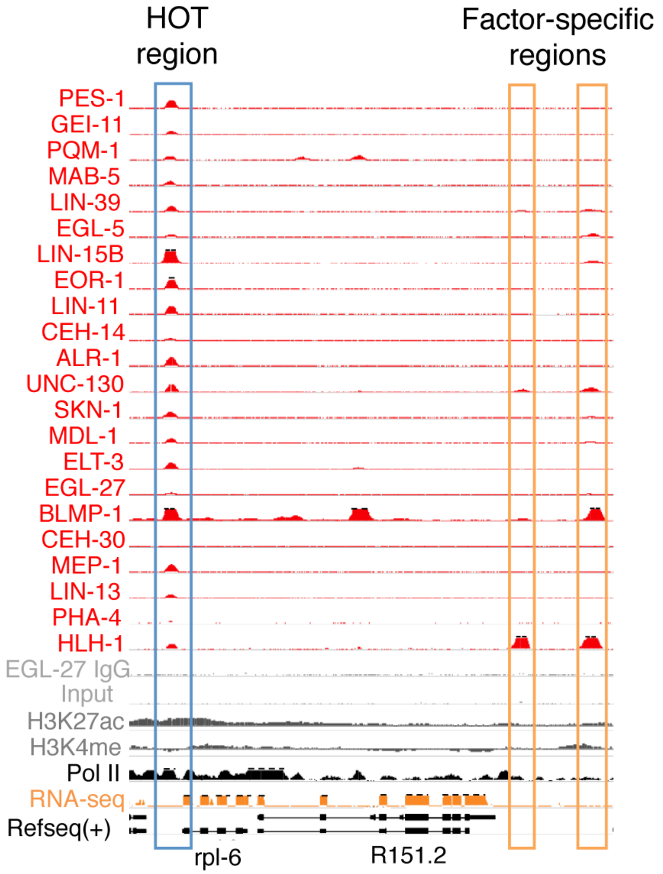
# Conservation of Functional Elements: Most Constrained Bases are Annotated

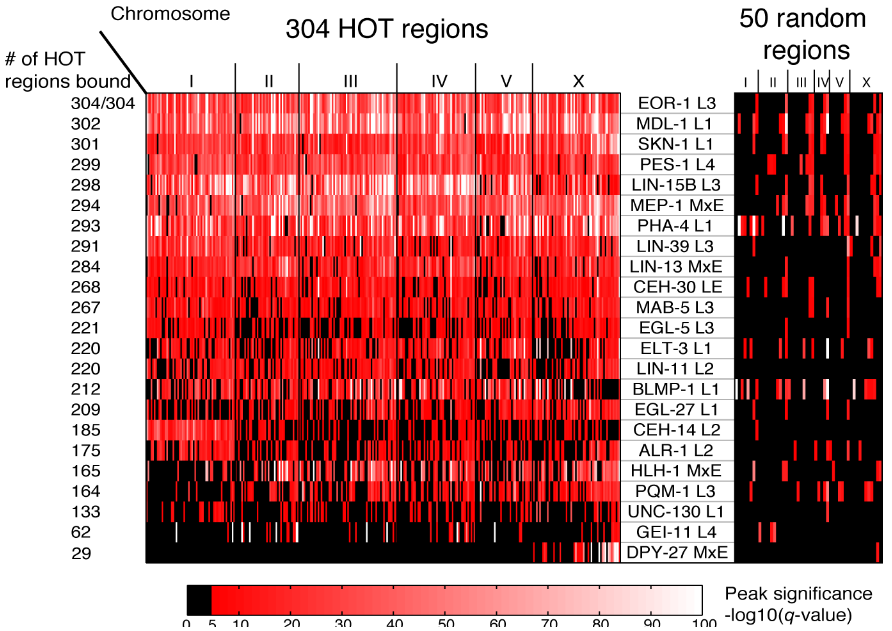


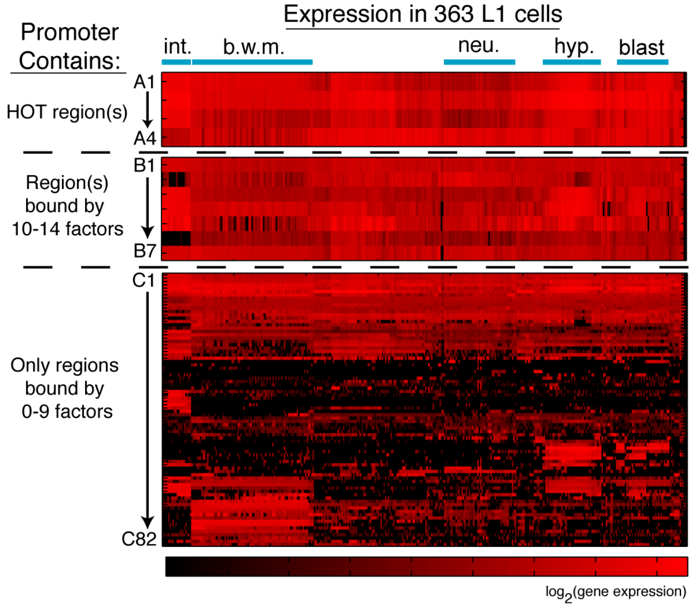
# Large-scale Chromatin Structure

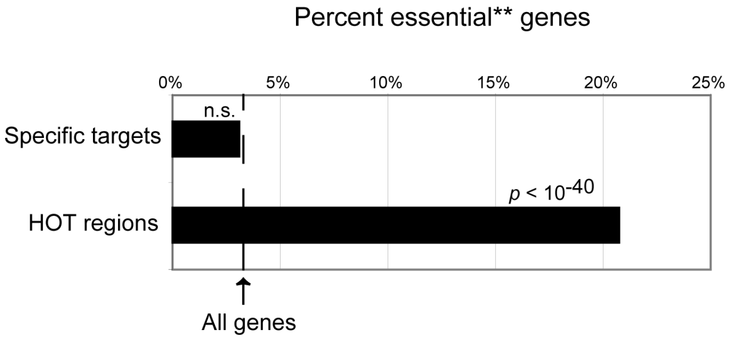


# HOT regions of clustered binding









**Similar approach in human ~81K HOT Regions (K5672)**

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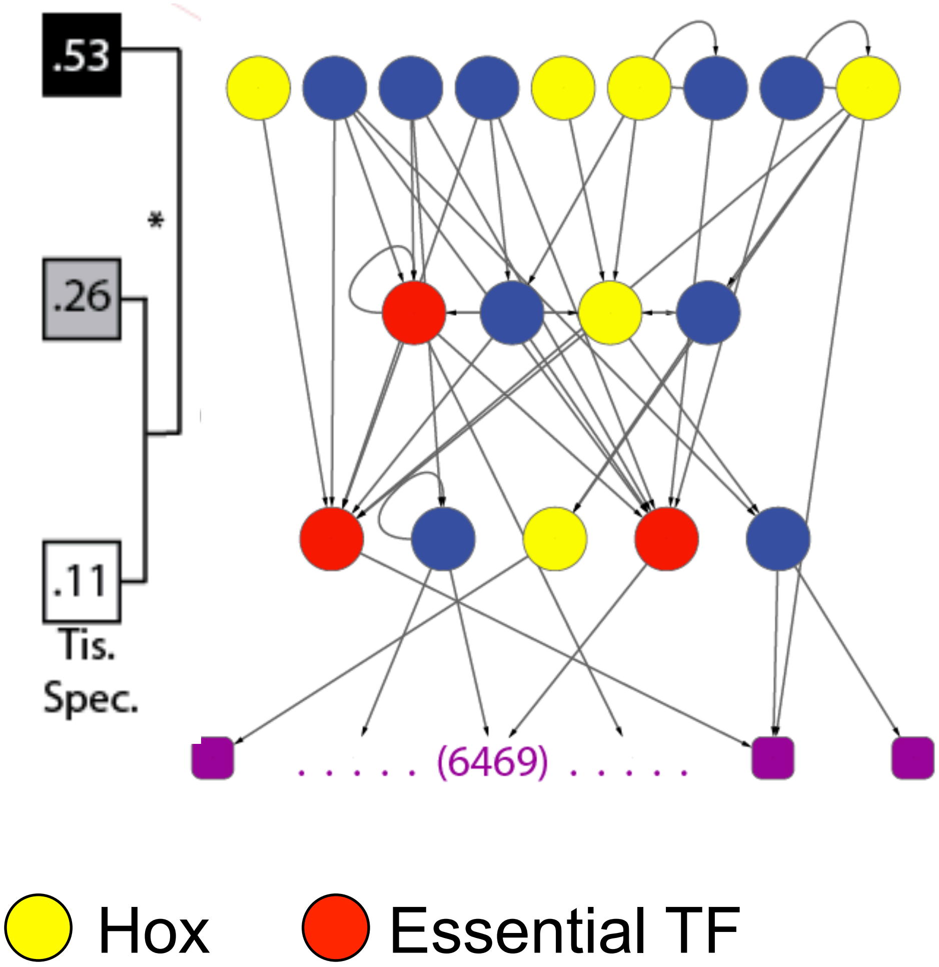
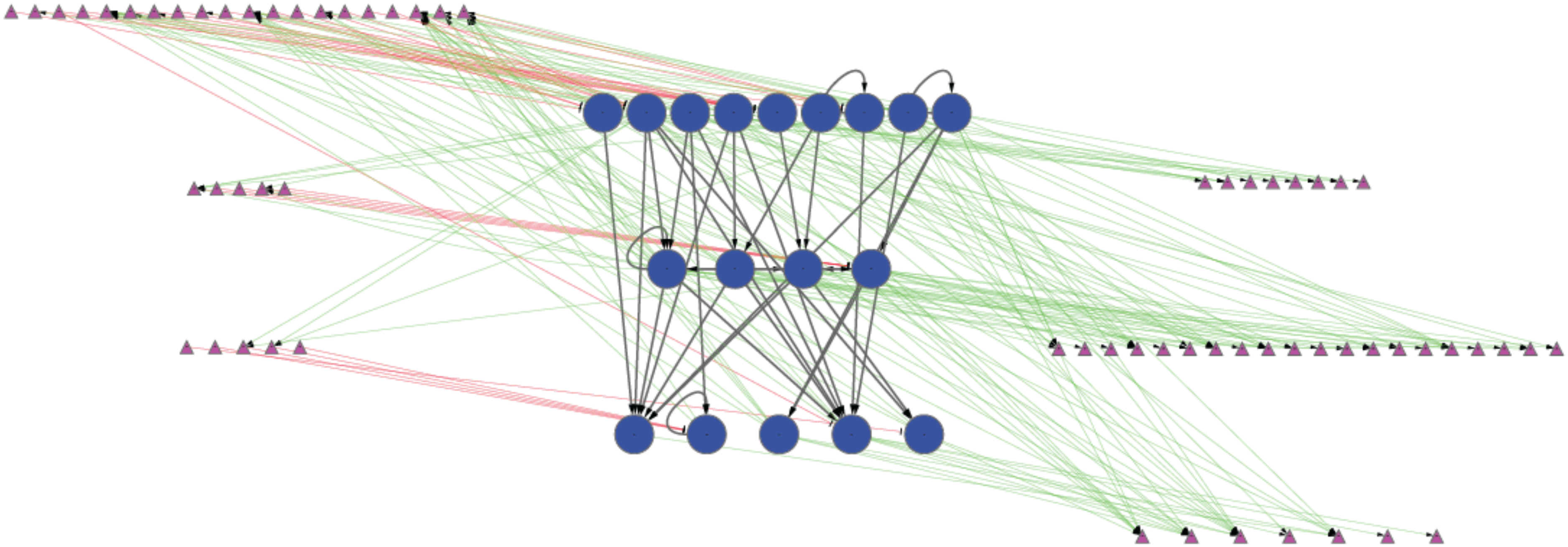
# Worm TF Hierarchy & Gene Properties



- ~25K edges
- Top:
  more tissue specific & HOX (& more miRNA reg.)
- Bottom:
  more essential
- Stats weak but pattern consistent with that in yeast, human…

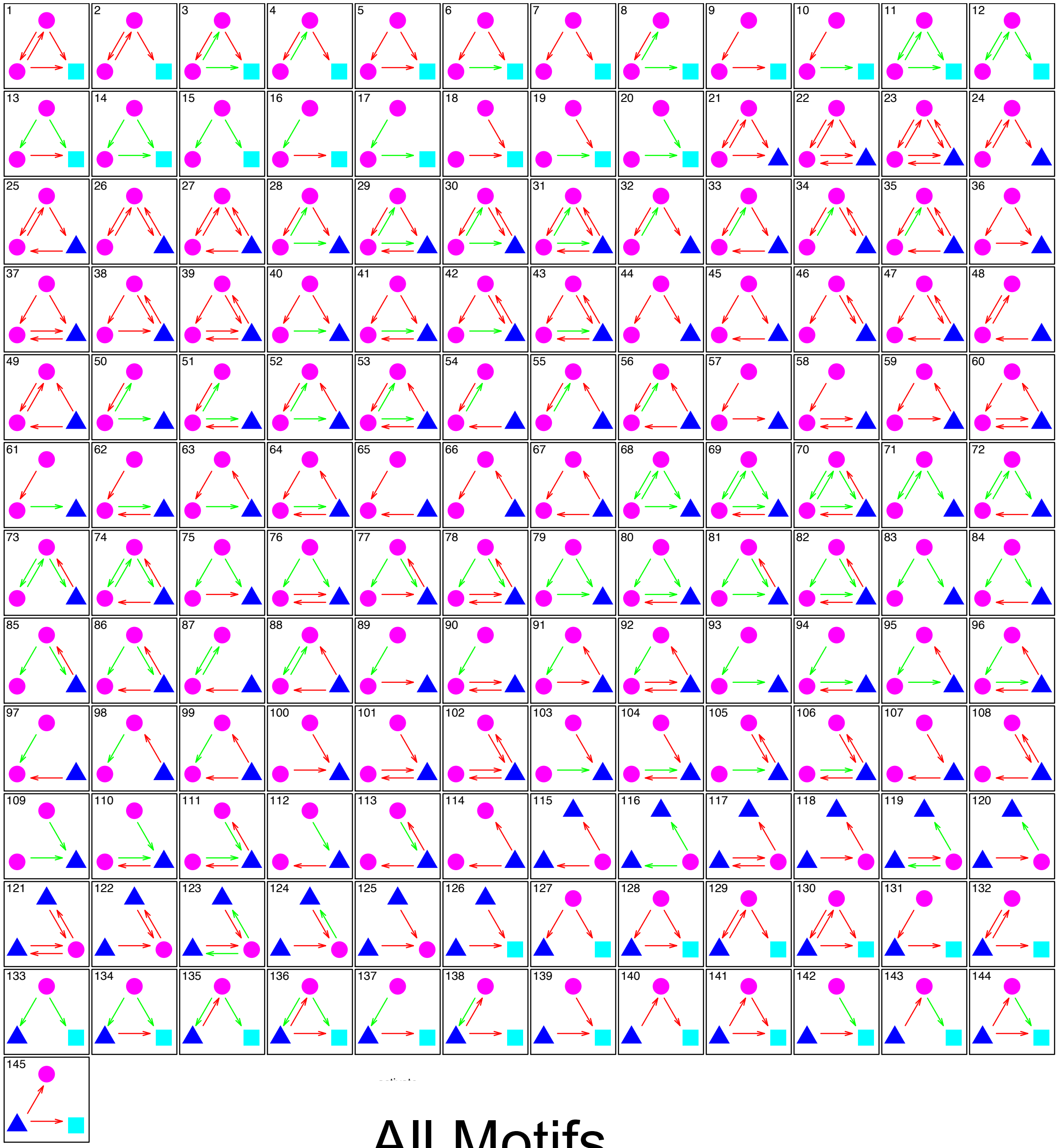
[*Science* 330:6012]

# Relating Worm TF Hierarchy with miRNAs



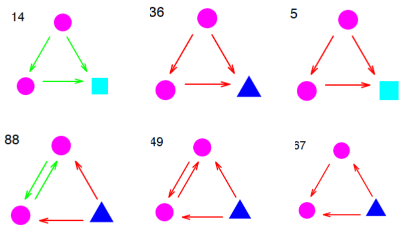
[*Science* 330:6012]

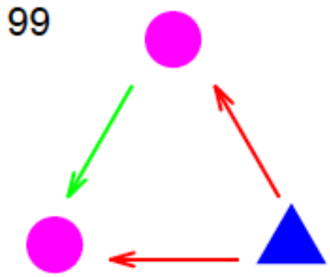
# Network Motifs



All Motifs

7 Motifs
Over-represented



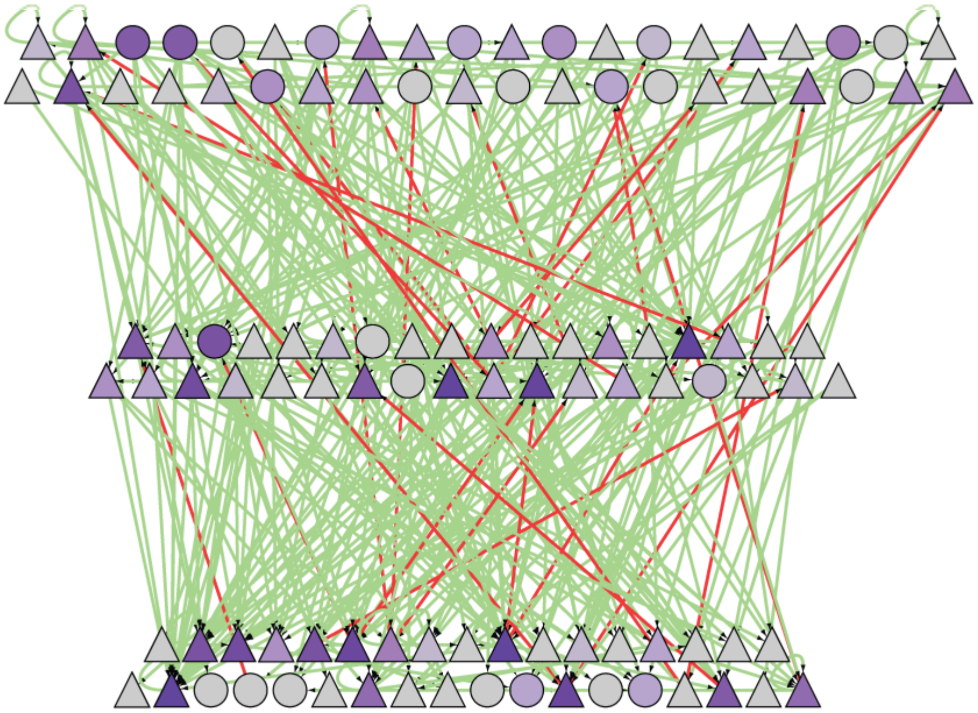


FFL involving
miRNA & 2 TFs

● TF ▲ MIR ■ GENE

activate →
repress →

[*Science* 330:6012]



# Human: Strongest Proximal Regulatory Edges Can be Arranged into a Hierarchy

Optimally arrange TFs into 3 levels by sim. annealing, maximizing downward-pointing edges

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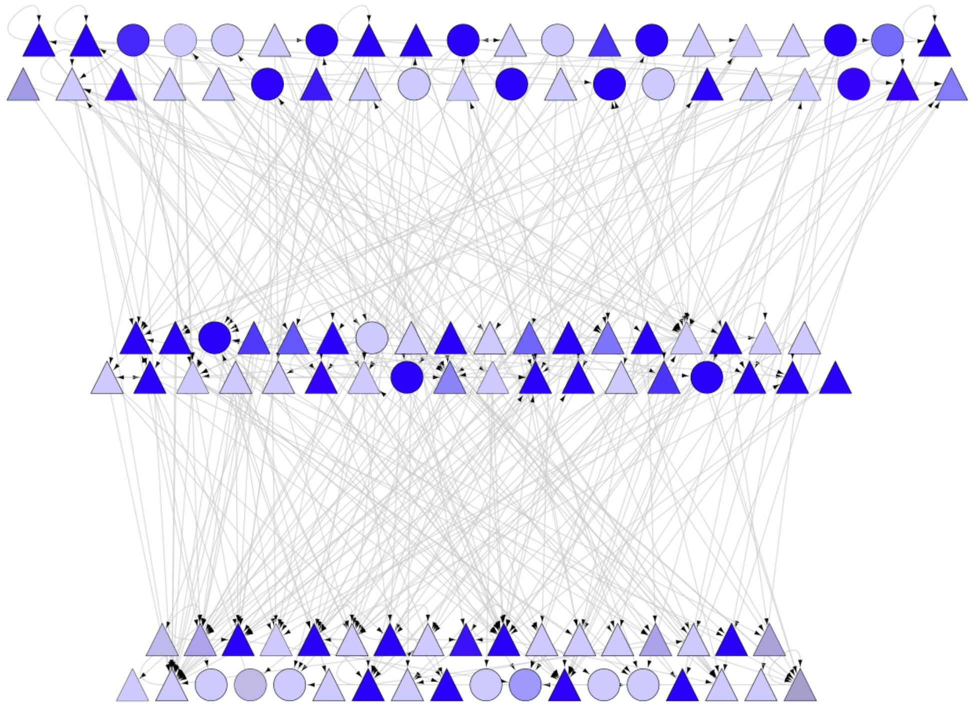
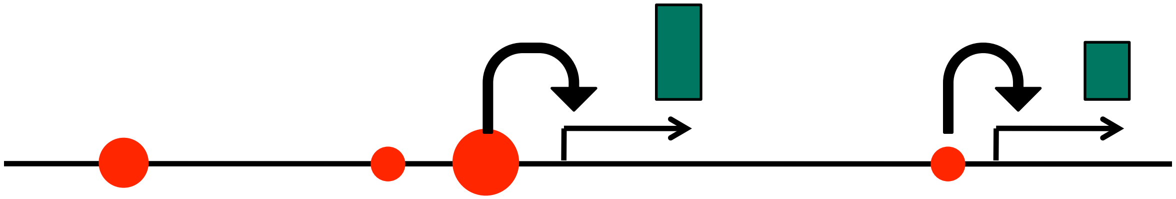
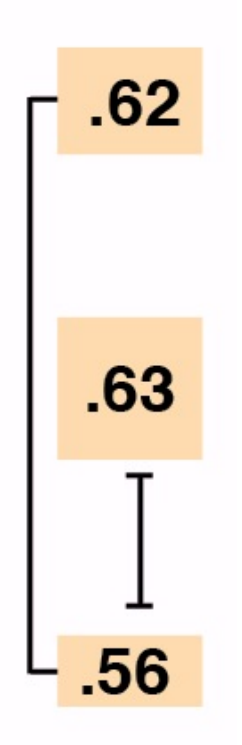


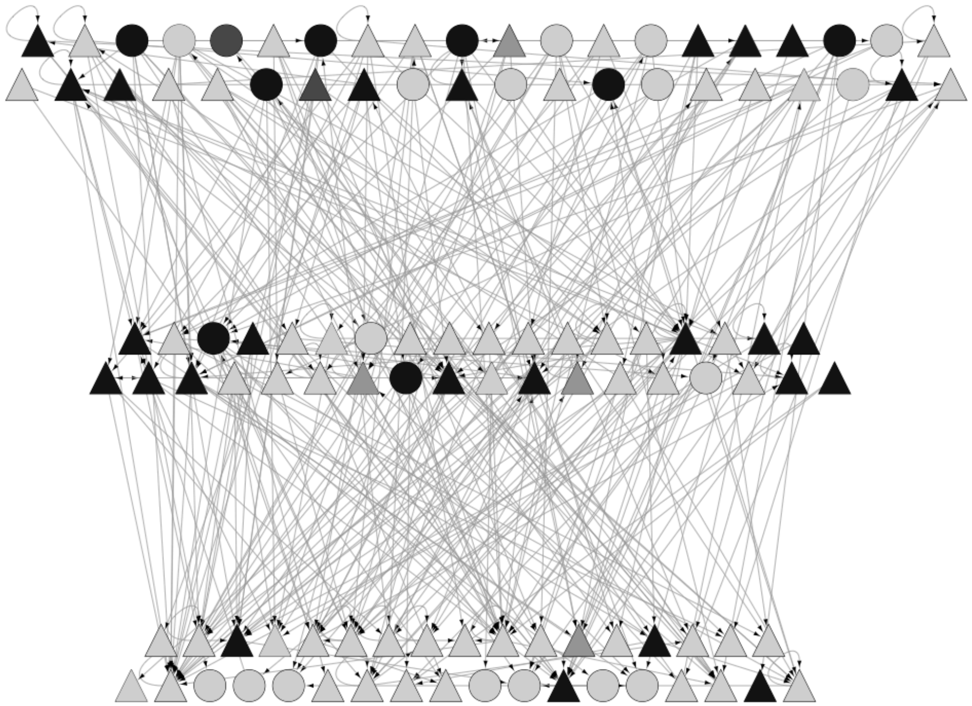


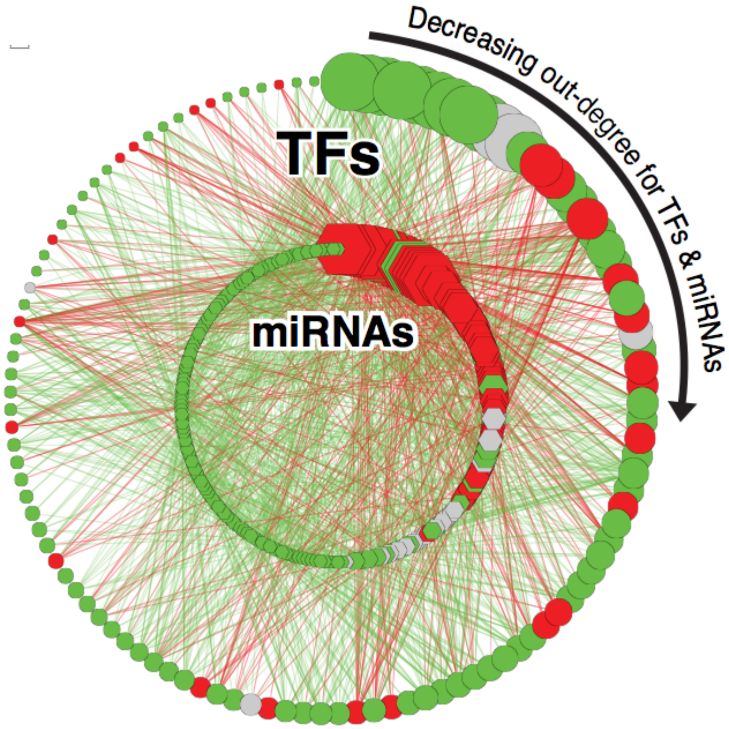


Avg. correlation betw. binding signal of TF & gene expr. of its target

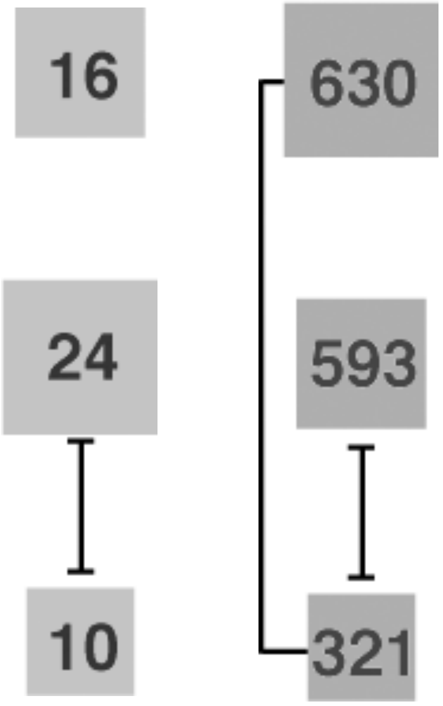
# Integration of TF hierarchy with other ‘omic information :

**more influential & connected TFs on the top**





Sig. corr. w/ TF hubbiness
(.24 & .62)



Avg. values

# regulating miRNAs & # regulated miRNAs

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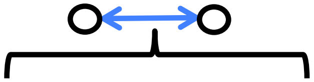
# Network Motif Analysis: Enrichment of FFLs

6 pairs of toggle switches



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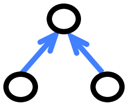
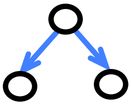
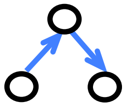
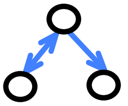
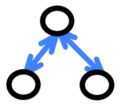
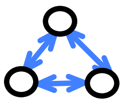
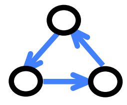
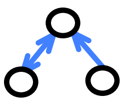
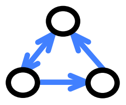
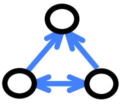
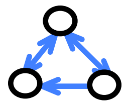
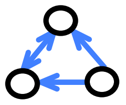
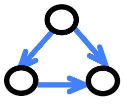
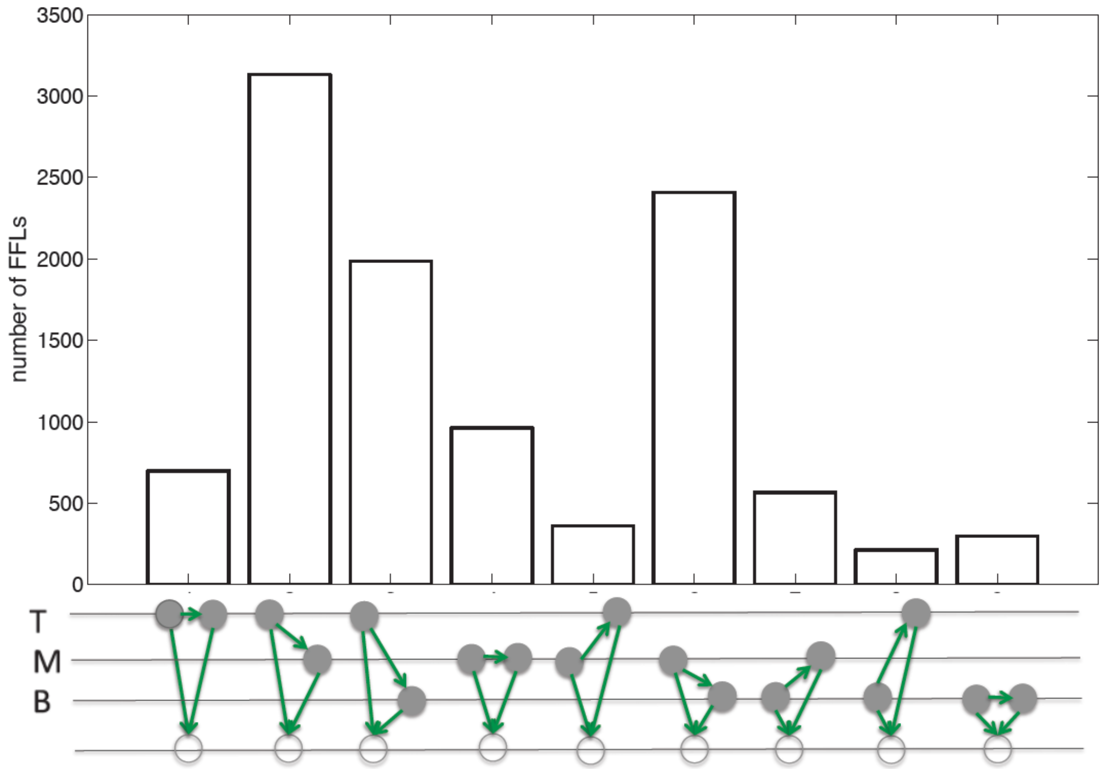
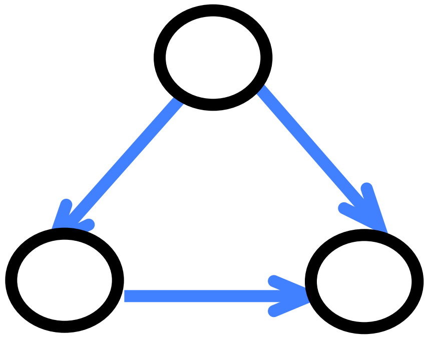
| 3-node motifs |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Freq. N | 868<br>(0.84) | 490<br>(0.81) | 729<br>(0.72) | 26<br>(0.62) | 0<br>(0) | 0<br>(0) | 8<br>(0.70) | 64<br>(0.91) | 6<br>(1.8) | 6<br>(2.9) | 2<br>(5.6) | 16<br>(4.8) | 122<br>(1.4) |





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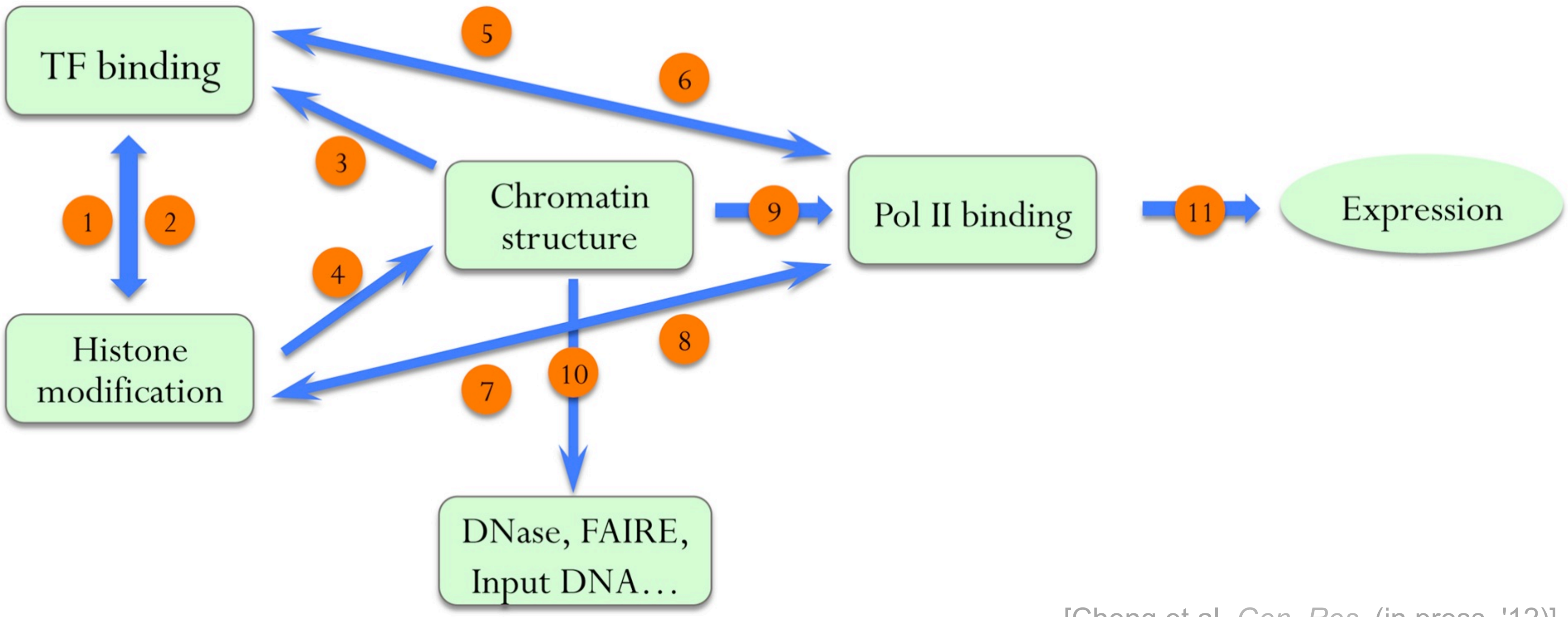
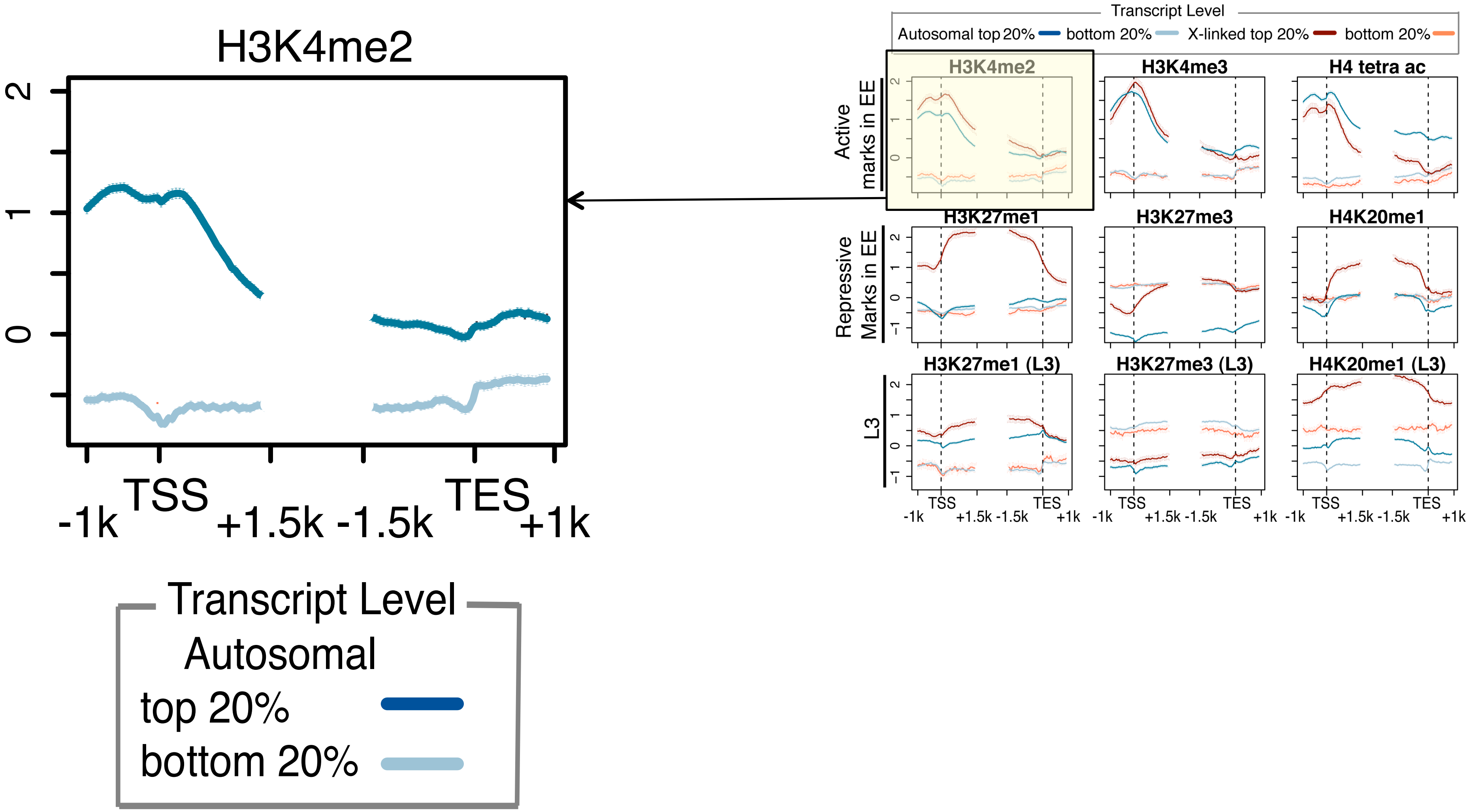
# Modeling Transcription: Connecting Inputs & Outputs

- Models
  - HMs+TFs => gene expression
  - HMs => TFs



[Cheng et al. *Gen. Res.* (in press, '12)]

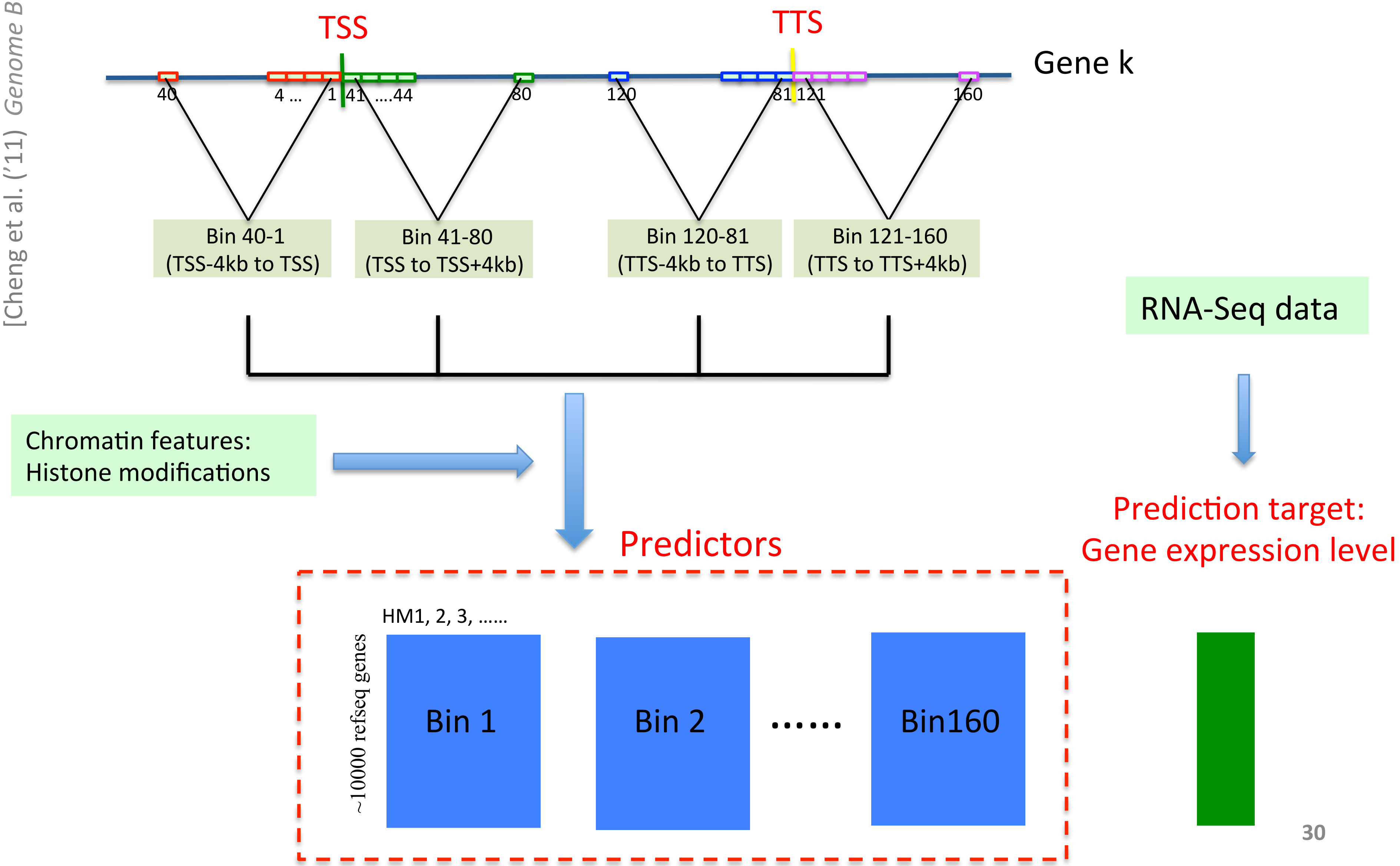
# His. mods around TSS are related to level of gene expression



[*Science* 330:6012]

[Cheng et al. ('11) *Genome Biol.* 12: R15]

# Histone Modification model



Bin 40-1 (TSS-4kb to TSS)

Bin 41-80 (TSS to TSS+4kb)

Bin 120-81 (TTS-4kb to TTS)

Bin 121-160 (TTS to TTS+4kb)

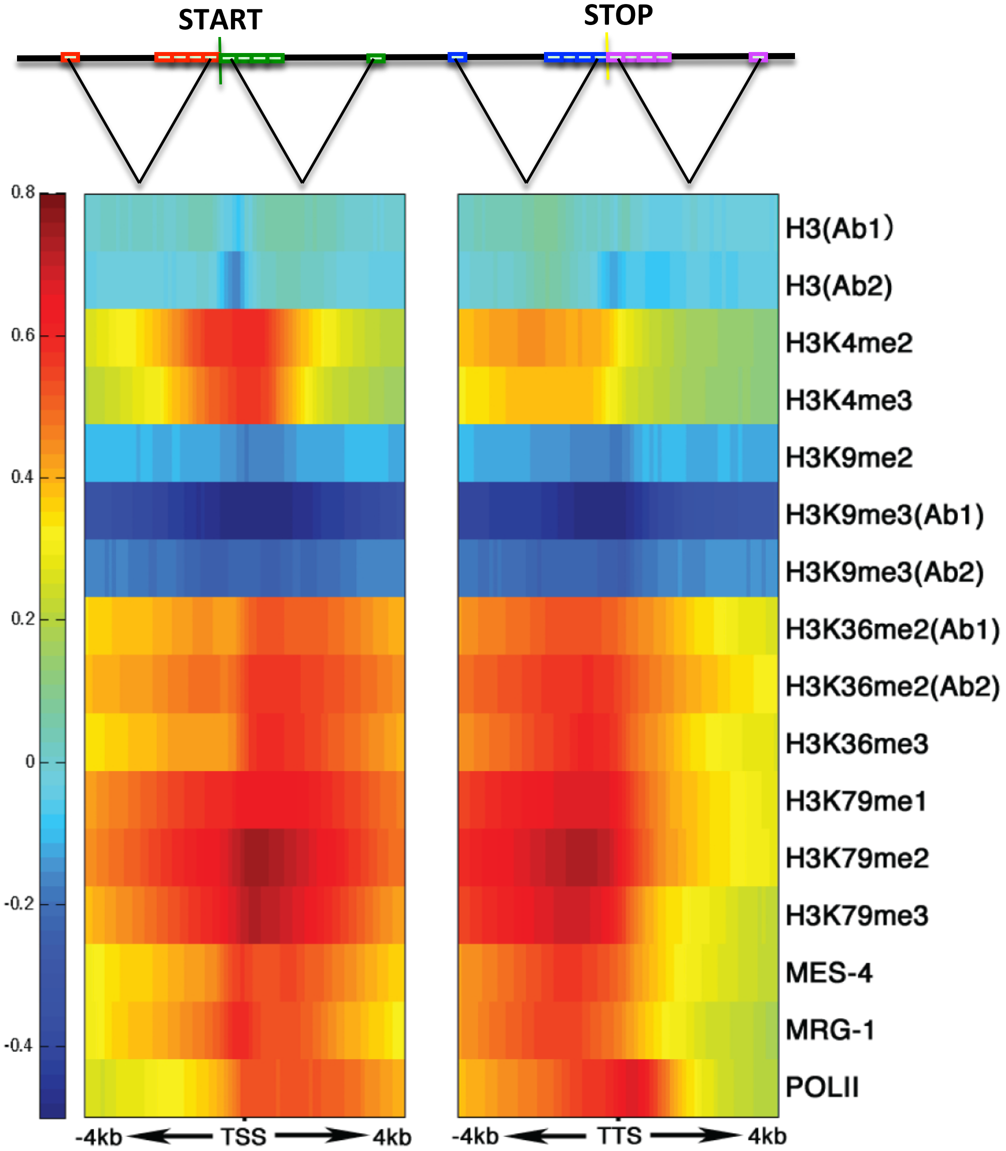
RNA-Seq data

Chromatin features: Histone modifications

Predictors

Prediction target: Gene expression level

# His. mods around TSS & TTS are clearly related to level of gene expression, in a position-dependent fashion

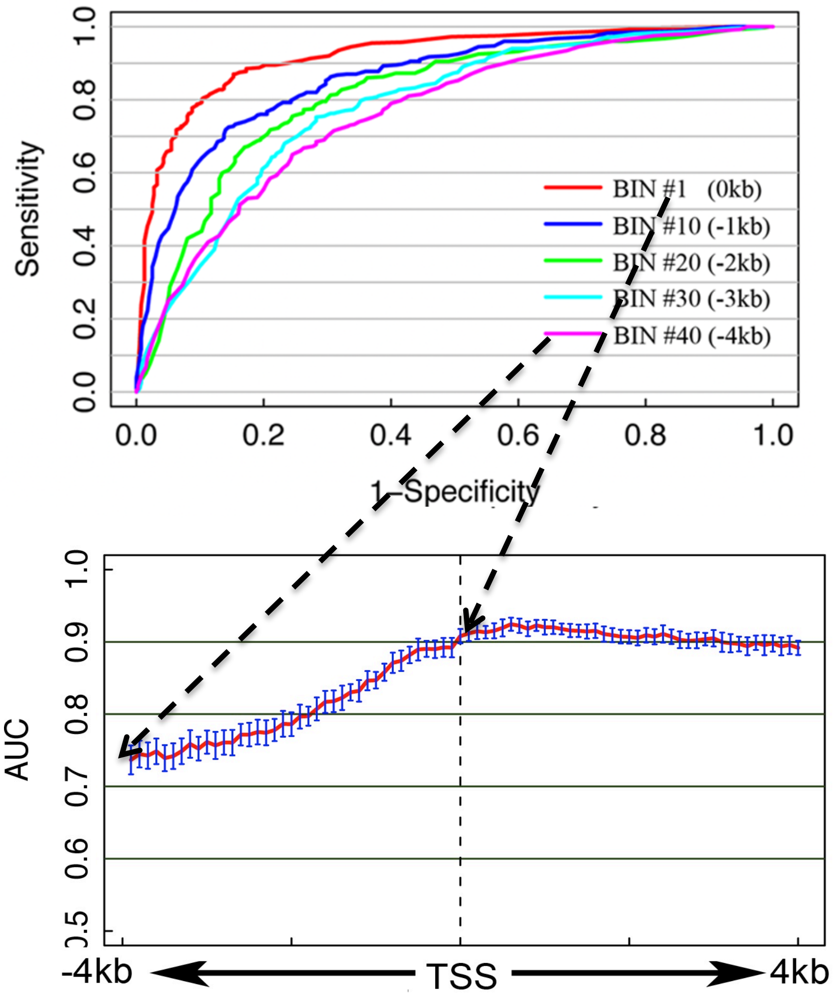


[*Science* 330:6012] [Related work: Ouyang et al. ('09) *PNAS*; Karlic et al. ('10) *PNAS*]

[Cheng et al. ('11) *Genome Biol.* 12: R15]

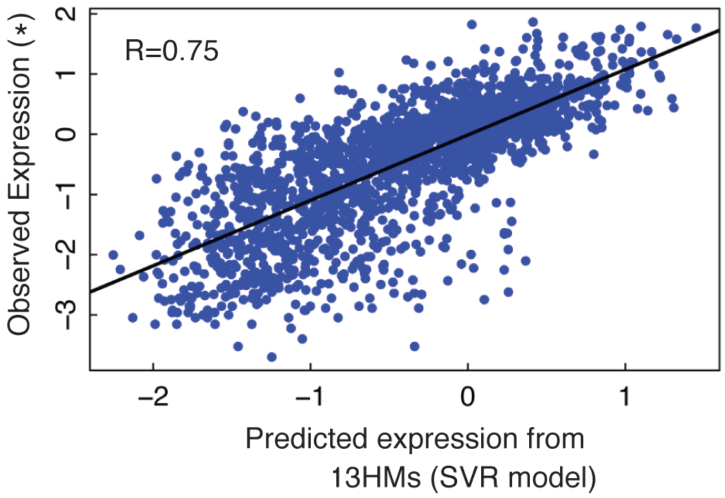
# Integrate all histone modifications to predict gene expression levels

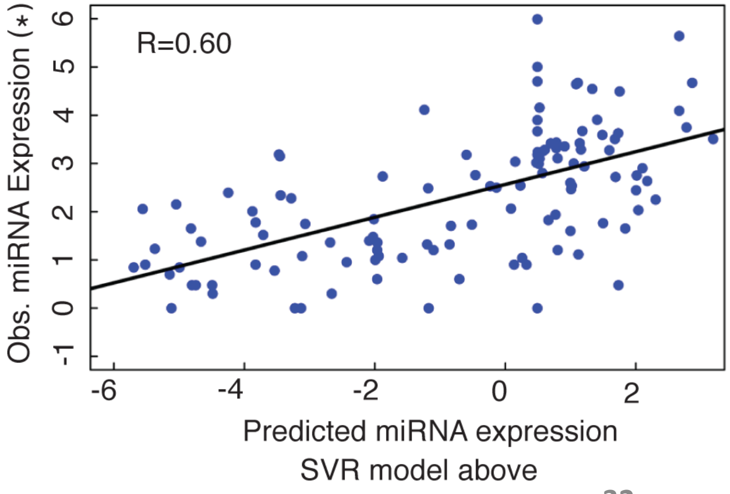
### Classify H/L genes (SVM)



Magnitude of Prediction from a "bin" around the TSS

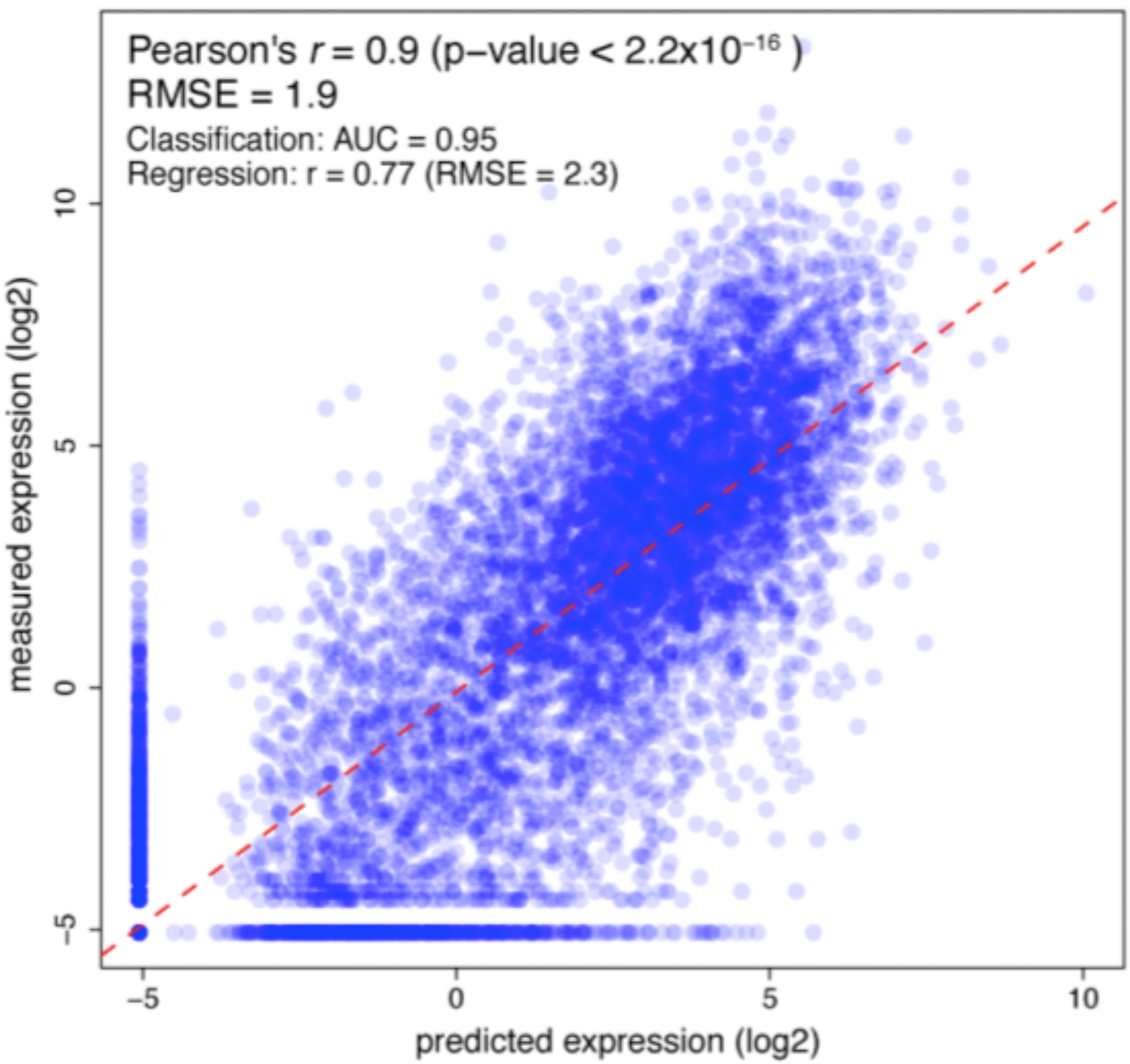
### Predict expression values

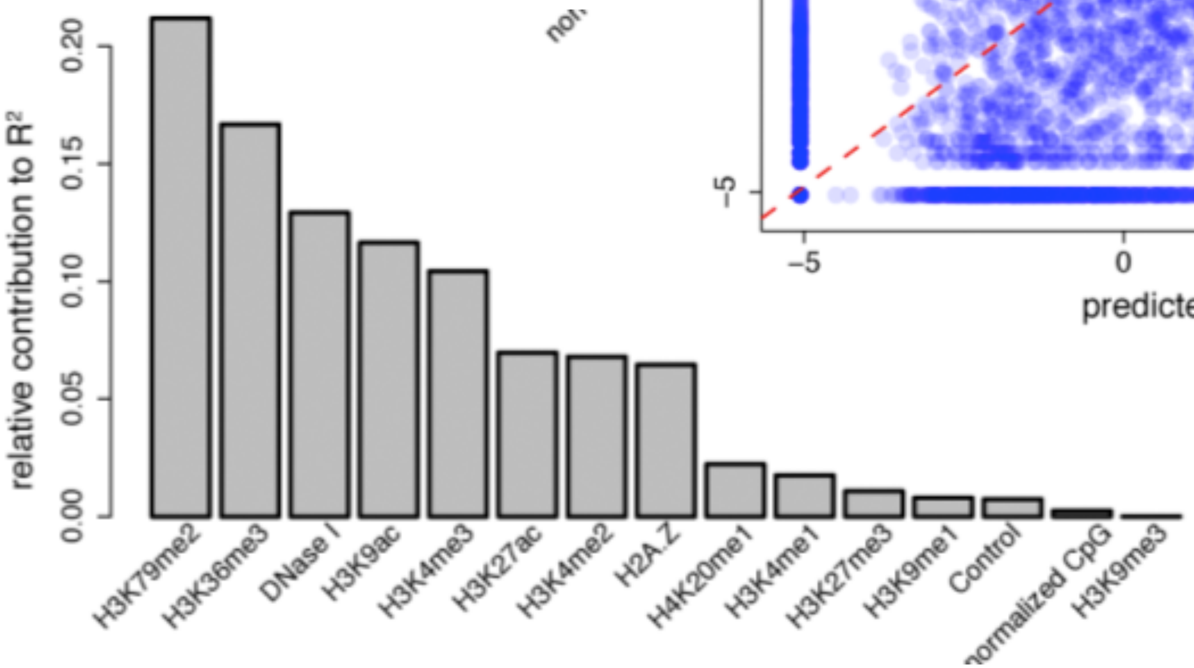




$*$ = LOG$_{10}$RPKM

# Scale up to Human & Mouse





# Insights from worm modencode: Approaches useful for human annotation (outline)

- **Expression Timecourse Analysis**
  - Coordinated binding & expression; E v L separation; ~280 large splicing changes
- **ncRNAs [Hum]**
  - Importance of evidence integration
  - Large numbers of transcribed pseudogenes (8-15%)
- **Chromosomal activity distribution [Hum]**
  - Most constrained regions active
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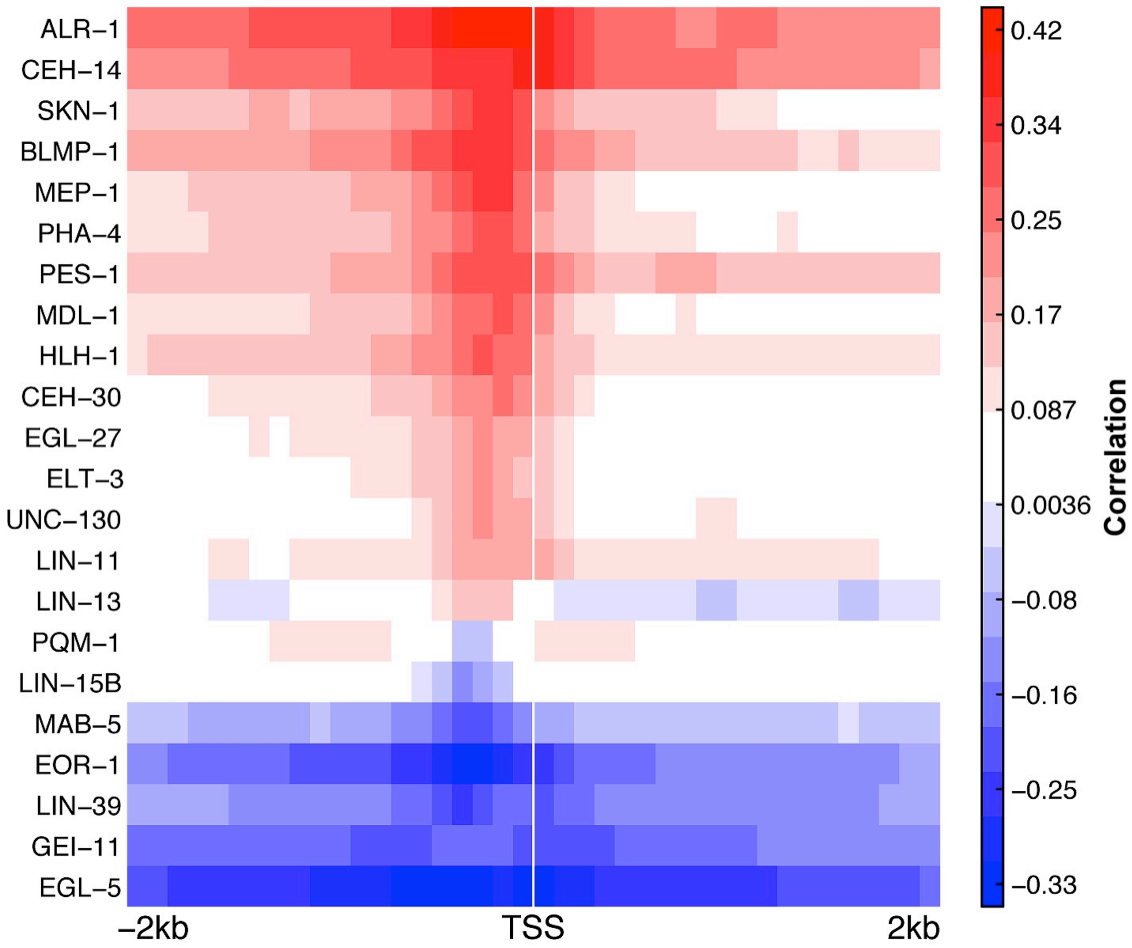
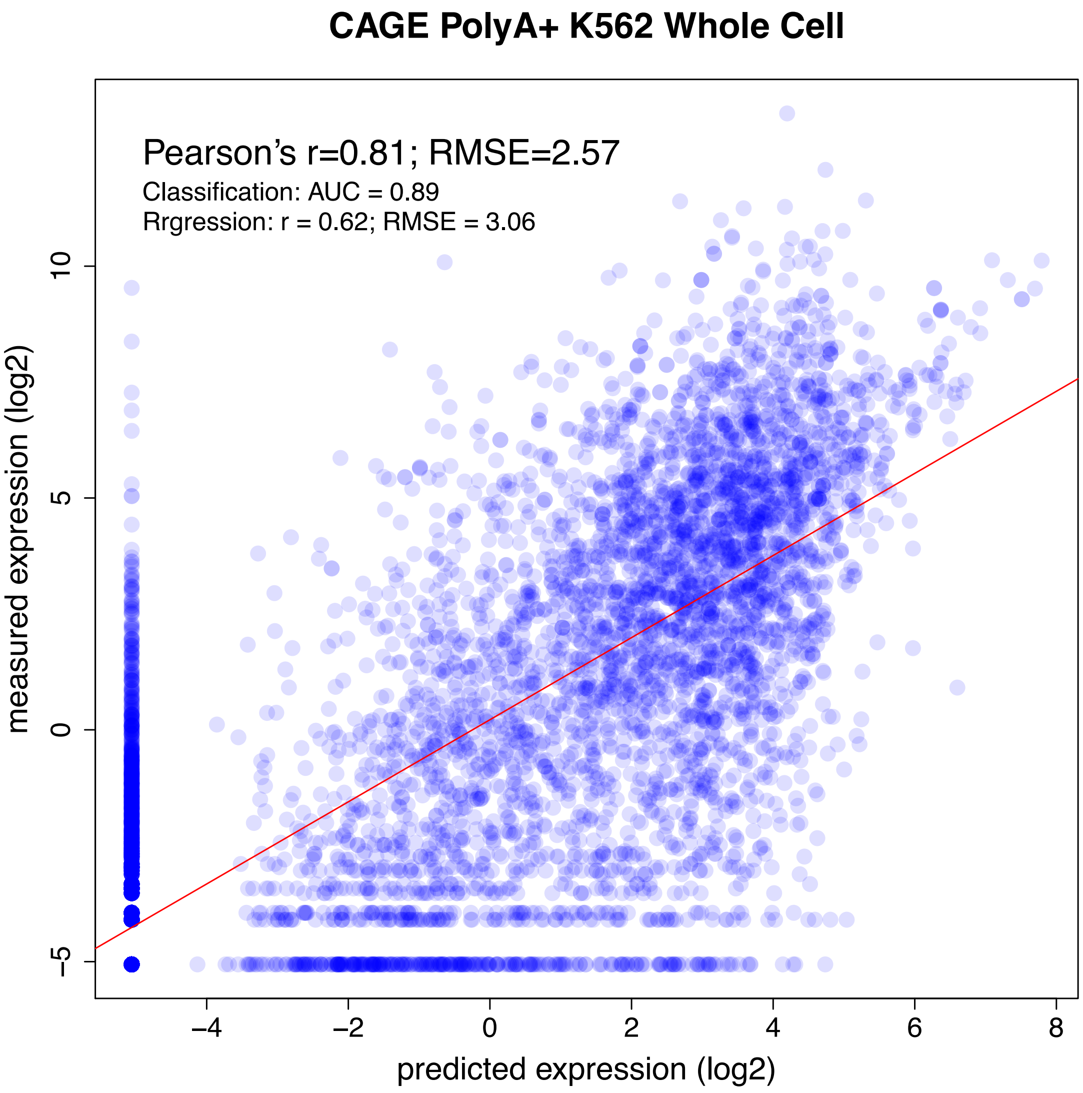
- **Stat Models relating HMs, TFs & Expression [Hum]**
  - HMs statistically predict expression for protein-coding genes and miRNAs
  - **Similar results for TFs**, highlighting predictive power of a few TFs.
  - Chromatin model (+ PWM) effective in predicting TF sites -- useful in identifying enhancers.

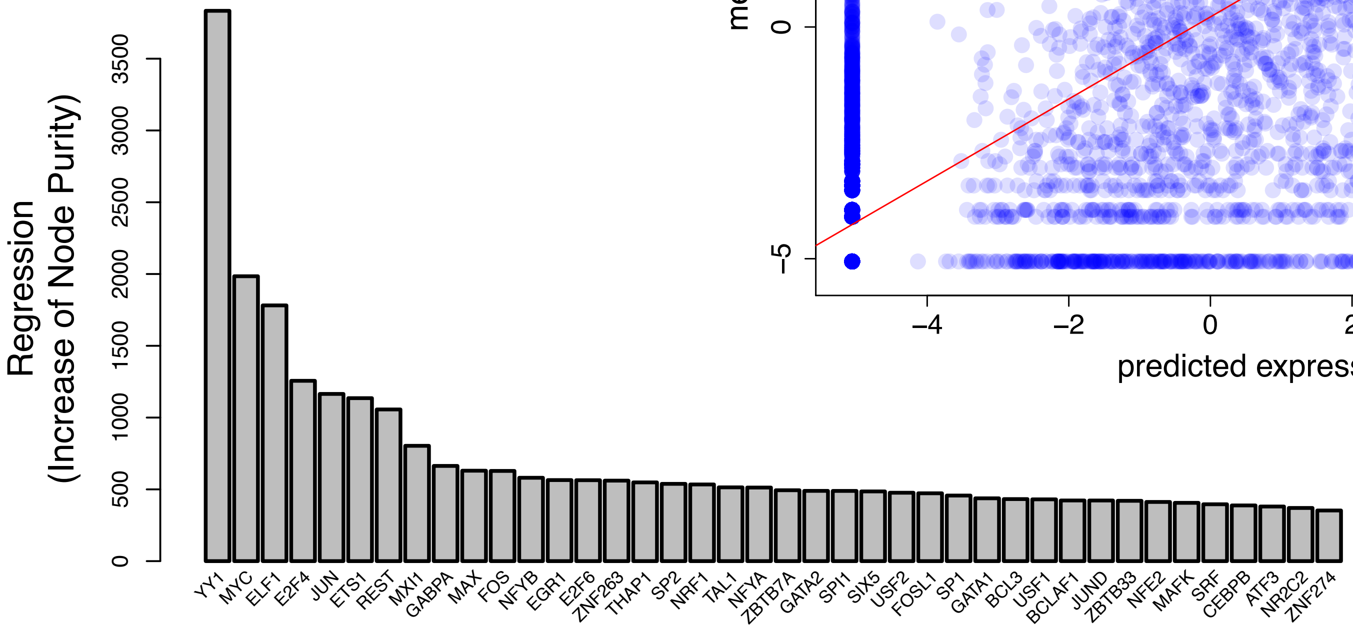
# Modeling with Worm TFs: Positive and negative regulators from correlating TF signal at TSS with gene expression



[ ]

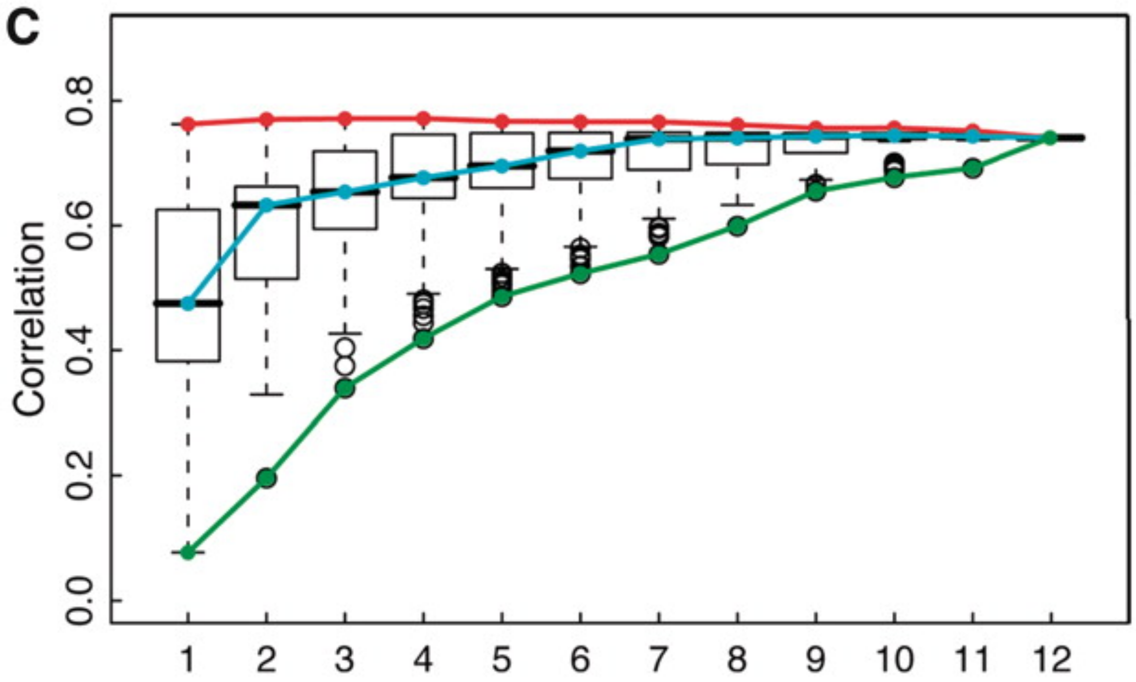
# Scale up to Human: TFs



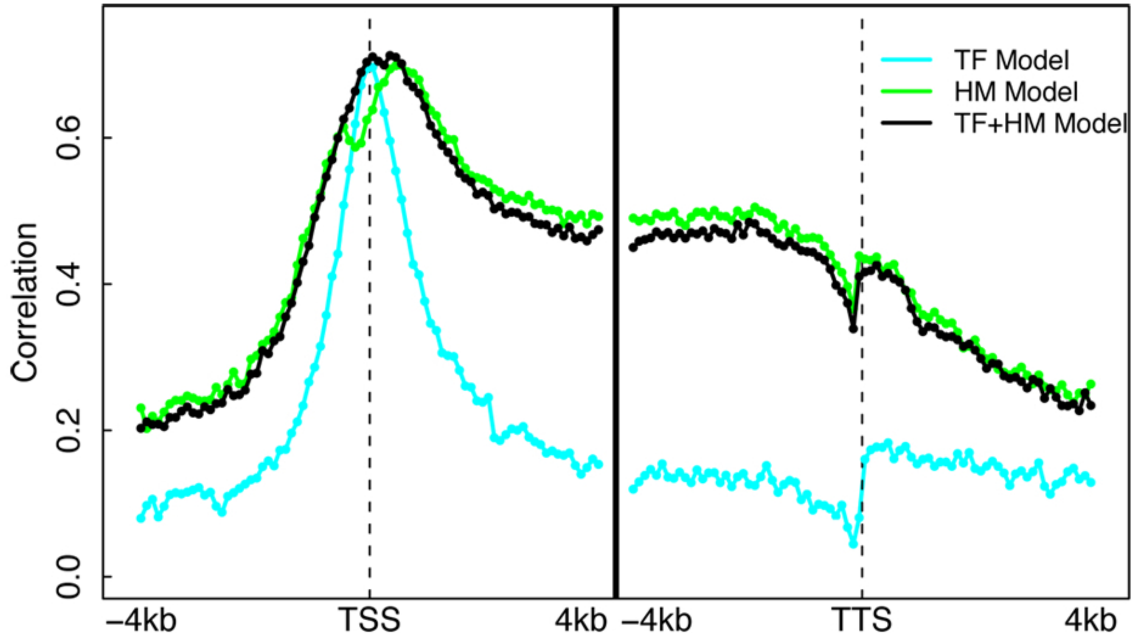


# Aspects of Mammalian (Human & Mouse) TF Model

- Model with only a few of the 1000s of total TFs is able to predict well



- Different Regions of Influence for TFs vs HMs



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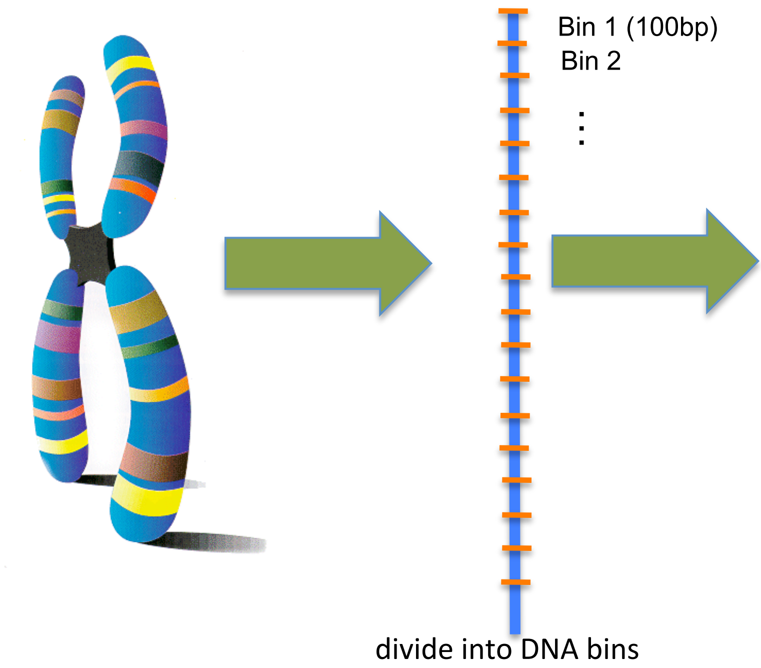
# Chromatin model: link histone modification patterns to TF binding



ChIP-seq Data

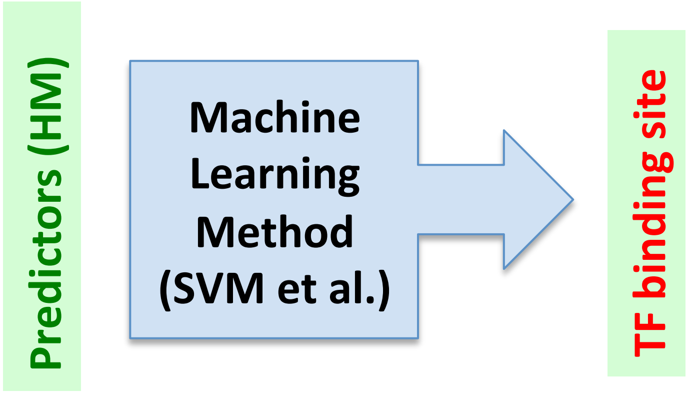
Predictors (HM)

|  | HM1 | HM2 | HM3 | …… |
|---|---|---|---|---|
| **Bin1** | 0.2 | 0.4 | 0.6 | … |
| **Bin2** | 0.3 | 0.3 | 0.2 | … |
| **Bin3** | 0 | 0.2 | 2.1 | … |
| **Bin4** | 0.4 | 0.4 | 0 | … |
| **Bin5** | 0.3 | 1.2 | 0.5 | … |
| **Bin6** | 1.2 | 3.1 | 2.1 | … |
| **Bin7** | 3.4 | 2.4 | 0.8 | … |
| **Bin8** | 1.5 | 1.2 | 0.9 | … |
| **…….** | … | … | … | … |

+

TF binding site?

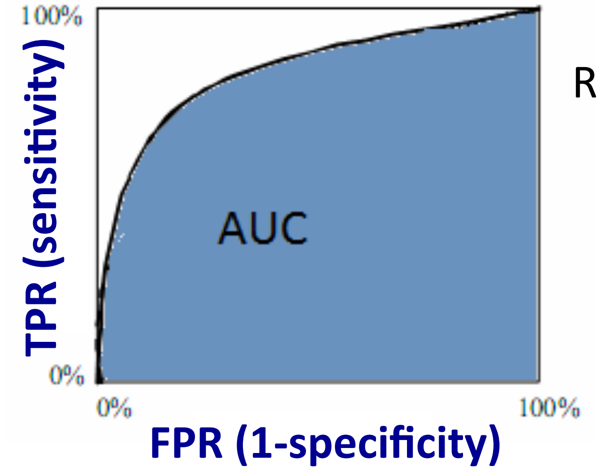
|  | TF1 | TF2 | …… |
|---|---|---|---|
| **Bin1** | 0 | 0 | … |
| **Bin2** | 0 | 0 | … |
| **Bin3** | 1 | 0 | … |
| **Bin4** | 1 | 0 | … |
| **Bin5** | 0 | 1 | … |
| **Bin6** | 0 | 0 | … |
| **Bin7** | 0 | 1 | … |
| **Bin8** | 1 | 0 | … |
| **…….** | … | … | … |



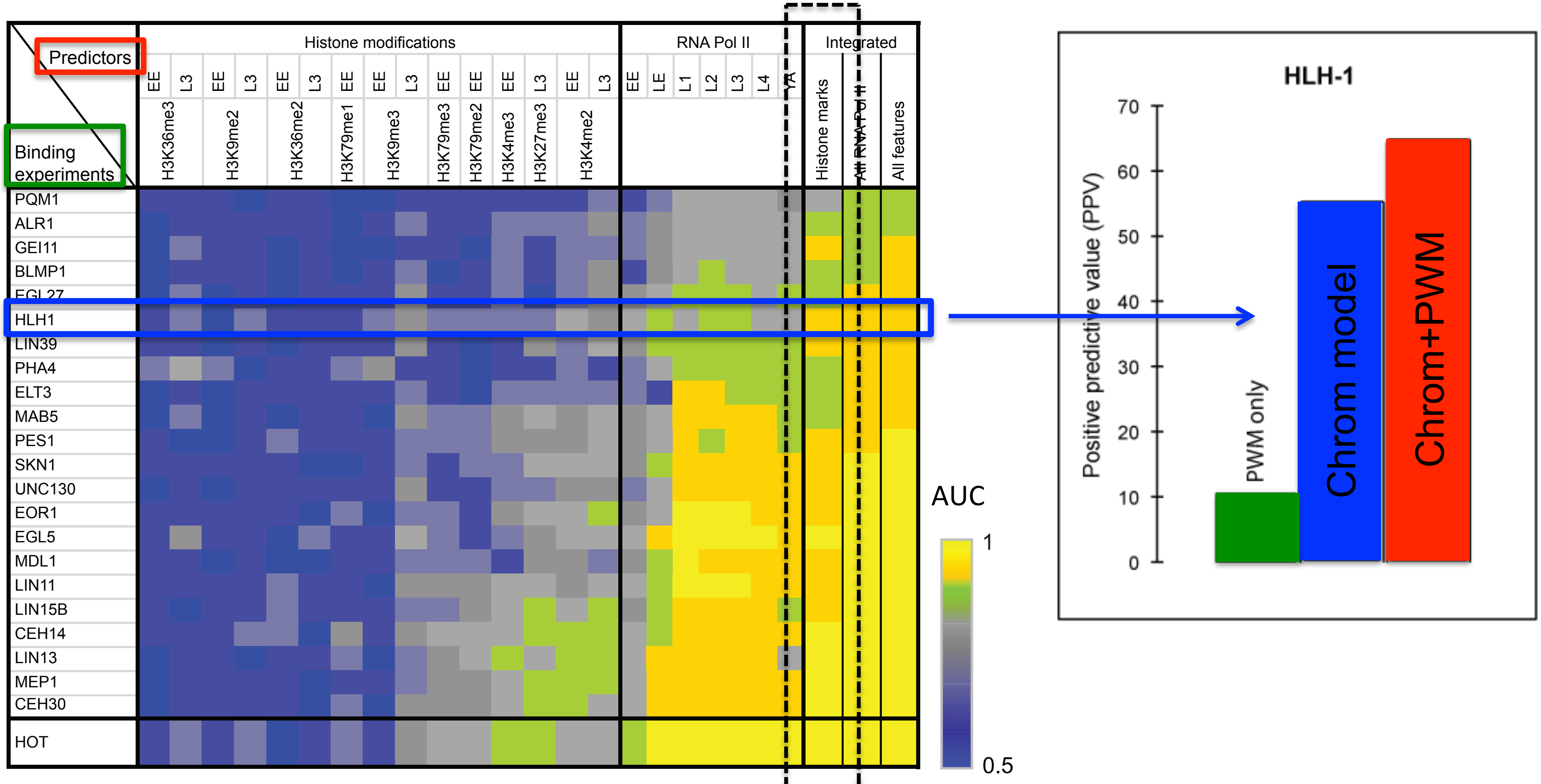
Predictors (HM)

Machine Learning Method (SVM et al.)

TF binding site



ROC curve

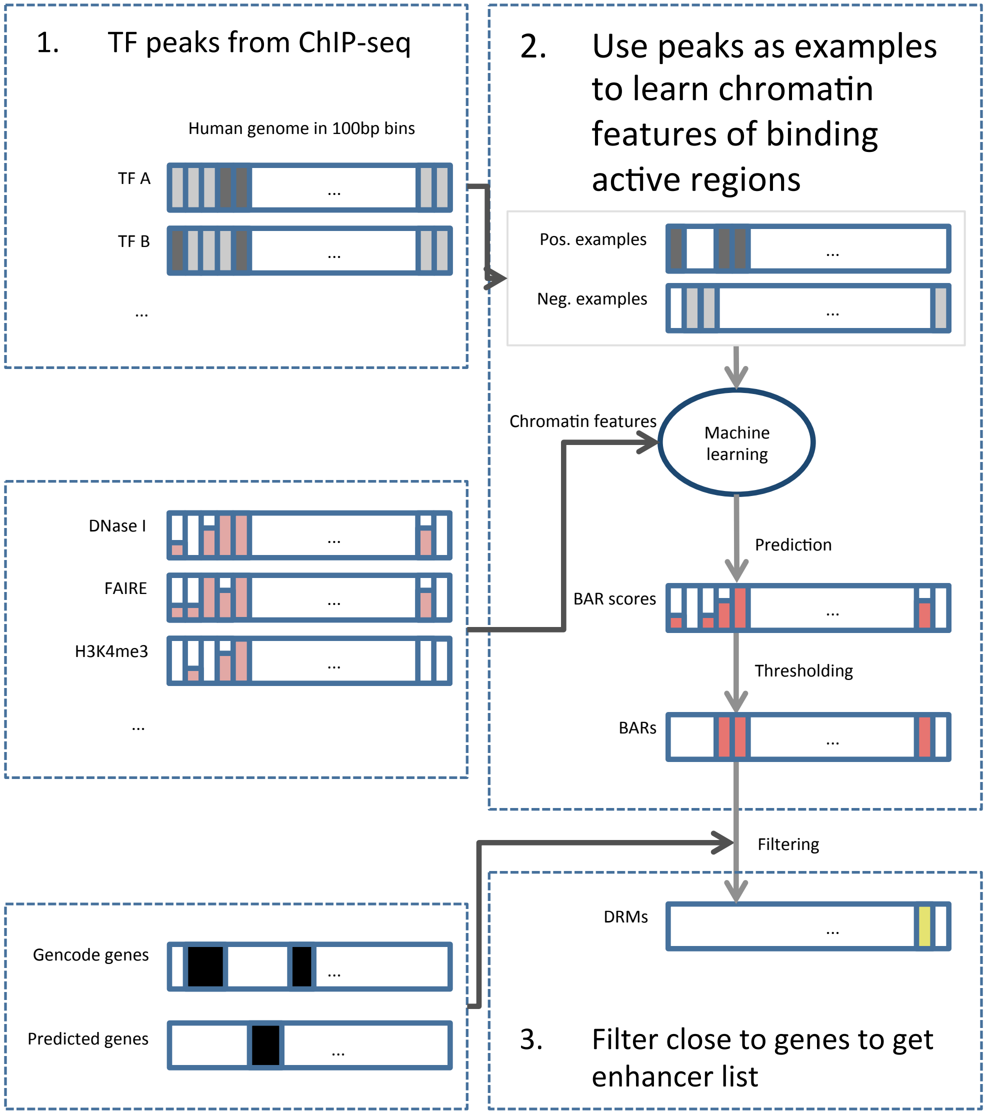


# Relating the Chromatin Model to TF Binding Sites in worm

- Model Predicts Sites Fairly Accuracy
- Accuracy improved when coupled with PWM

[ ]

## Identifying potential enhancers

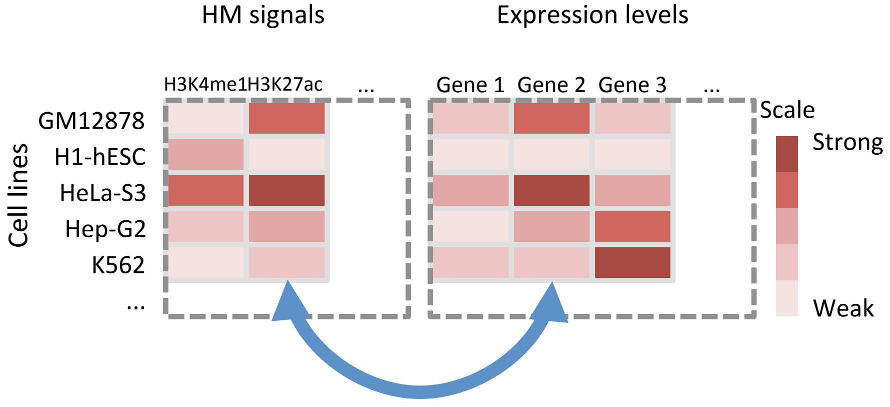


1. TF peaks from ChIP-seq

2. Use peaks as examples to learn chromatin features of binding active regions

3. Filter close to genes to get enhancer list

## Finding potential target genes



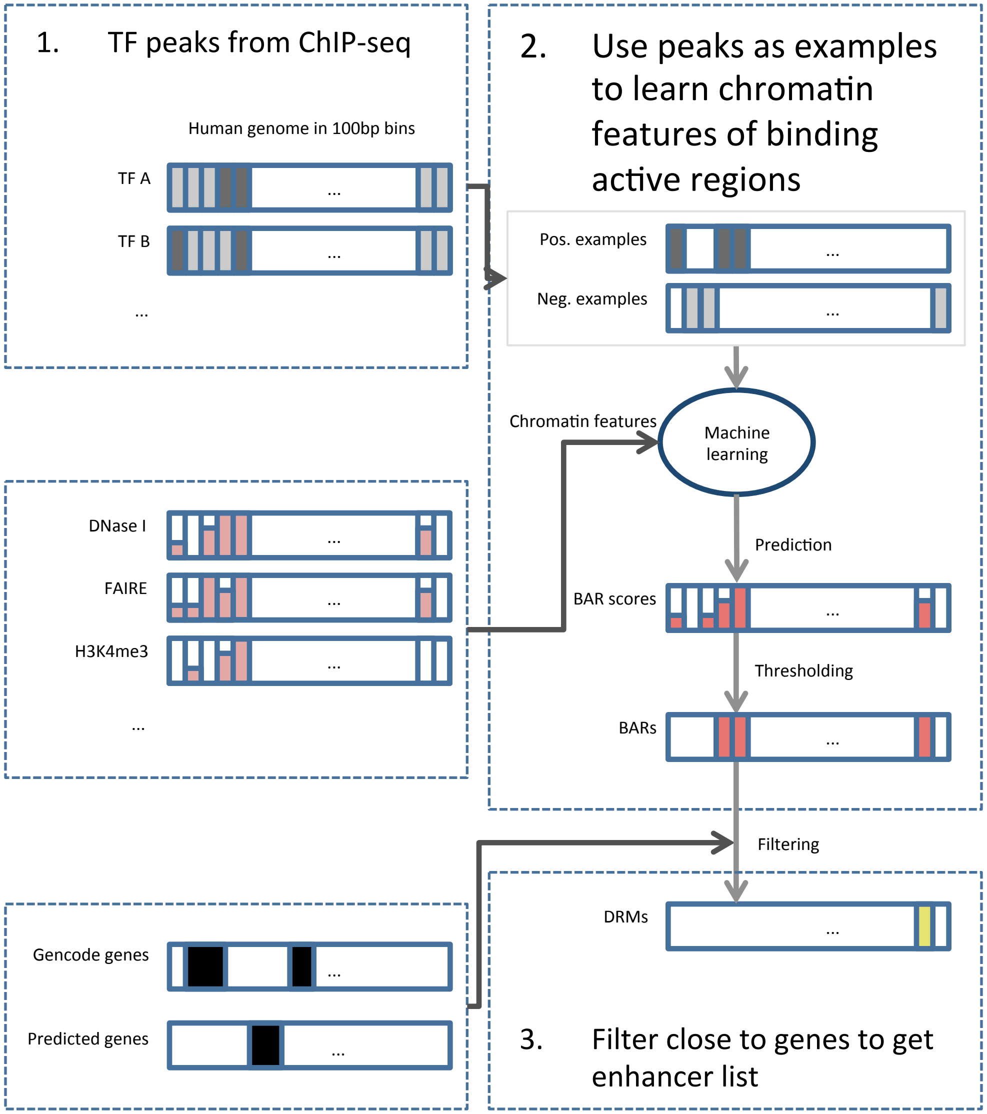
4. Find correlated enhancer-target pairs

~20K distal edges tot. from
~130K enhancer-like elements

(Related to but more “targeted” than enhancer “states” from unsupervised segmentation,
M Hoffman et al. & J Ernst et al.)

## Identifying Potential Enhancer-like Elements from Discriminative HM Model & then Linking these to Targets (via cell-line correlations) to Create Distal Edges

## Identifying potential enhancers



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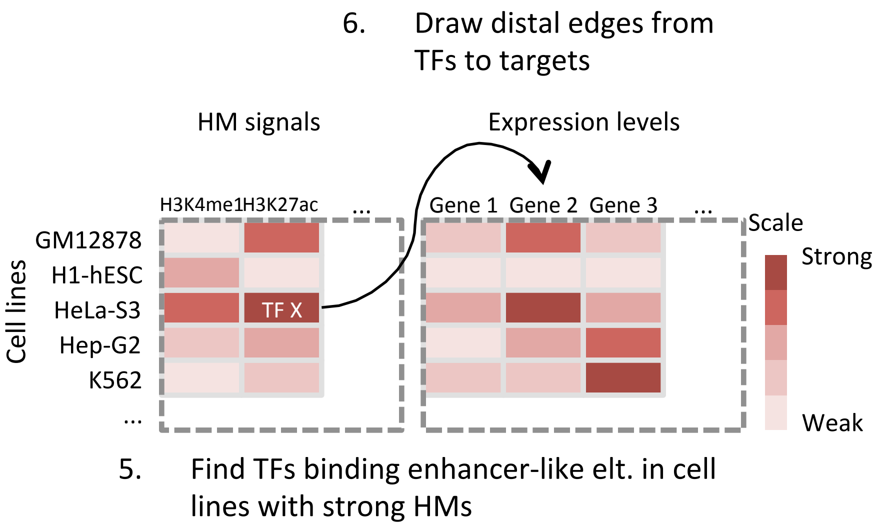

6. Draw distal edges from TFs to targets

5. Find TFs binding enhancer-like elt. in cell lines with strong HMs

~20K distal edges tot. from ~130K enhancer-like elements

(Related to but more “targeted” than enhancer “states” from unsupervised segmentation,
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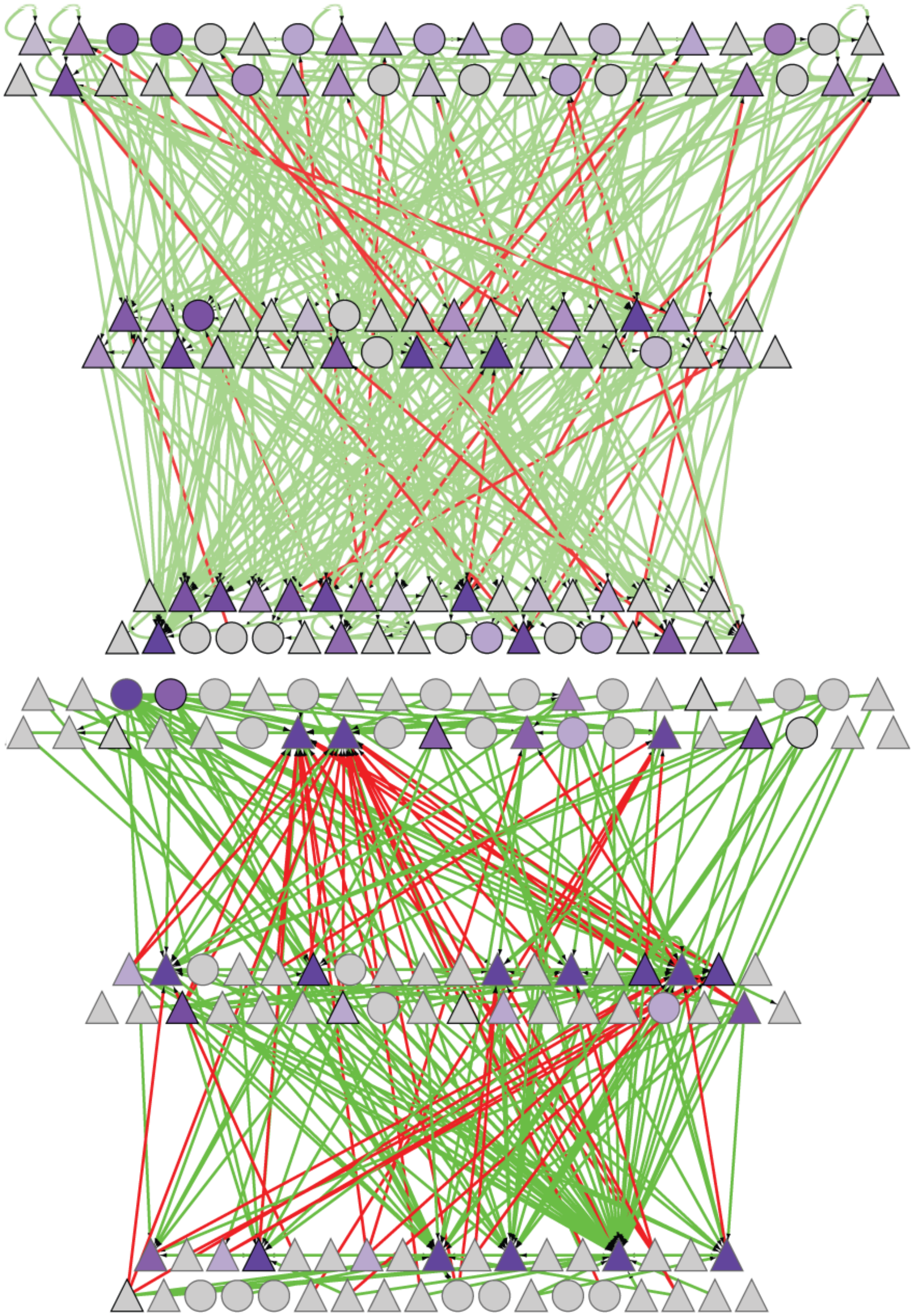
## Identifying Potential Enhancer-like Elements from Discriminative HM Model & then Linking these to Targets (via cell-line correlations) to Create Distal Edges

[ ]



# Comparing

# Proximal

# &

# Distal

# Networks

[ ]

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ENCODE

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Nets/ Elements

GENCODE

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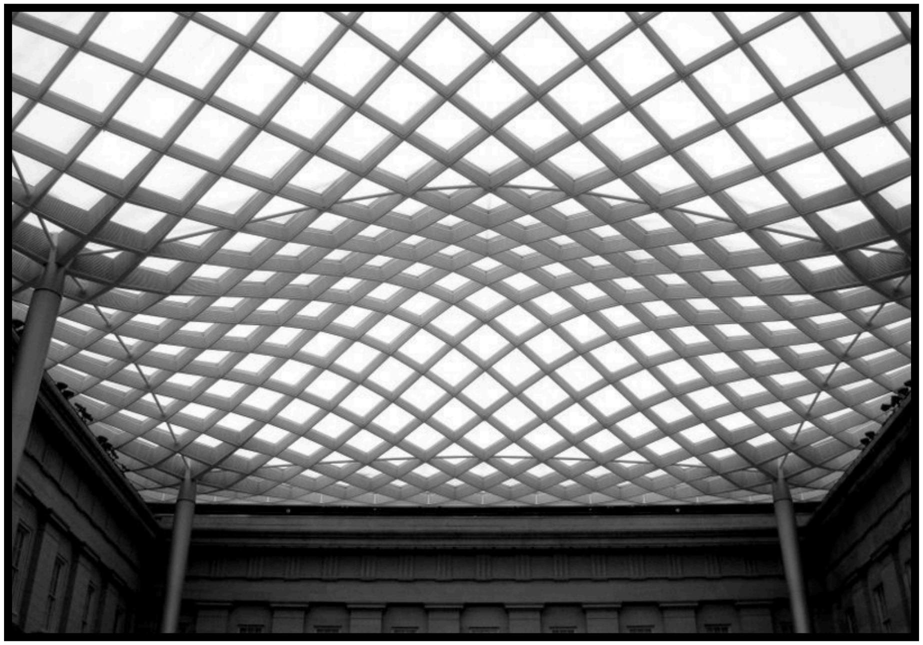

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