Computational analysis of the ENCODE datasets

and other related epigenetic explorations

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Presentation Goals

- FULL understanding of discussed material
- Ask questions along the way!

Outline

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1. Molecular biology in a jiffy
2. A case study
   - Hypothesis formulation
   - Analyzing data
3. More examples

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1. Molecular biology in a jiffy
2. A case study
   - Hypothesis formulation
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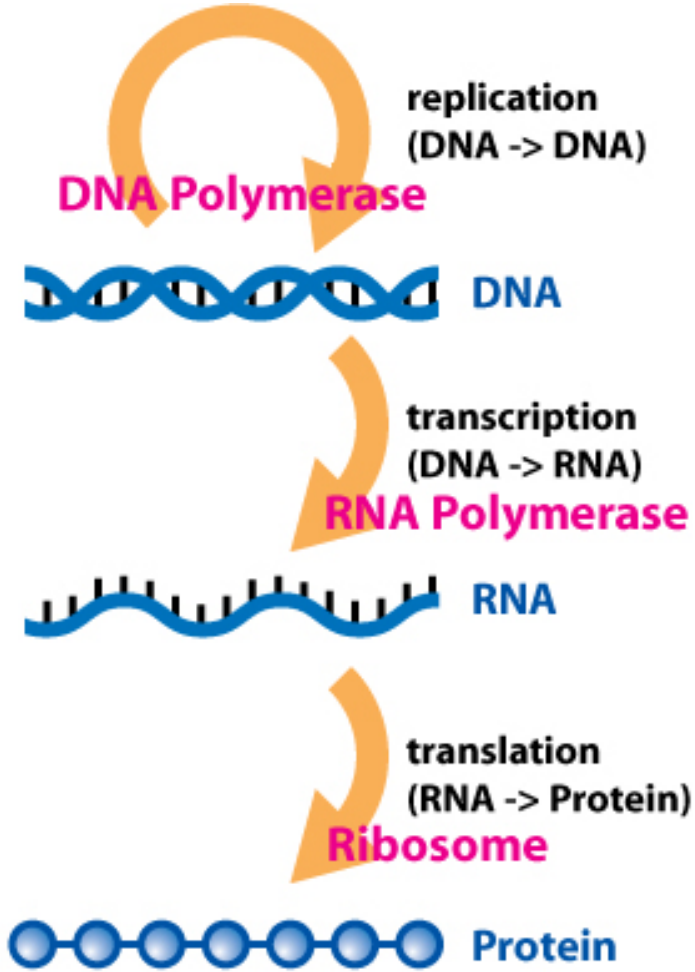
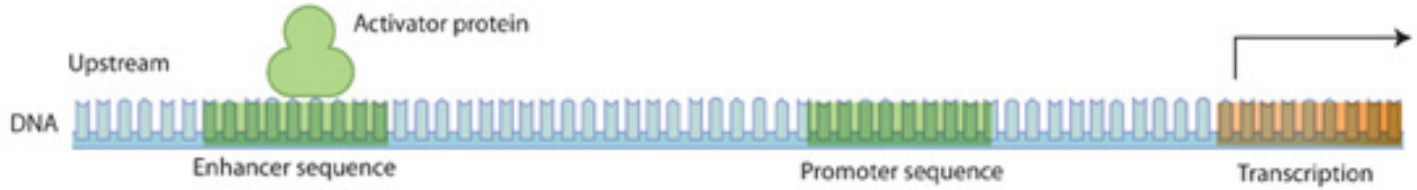
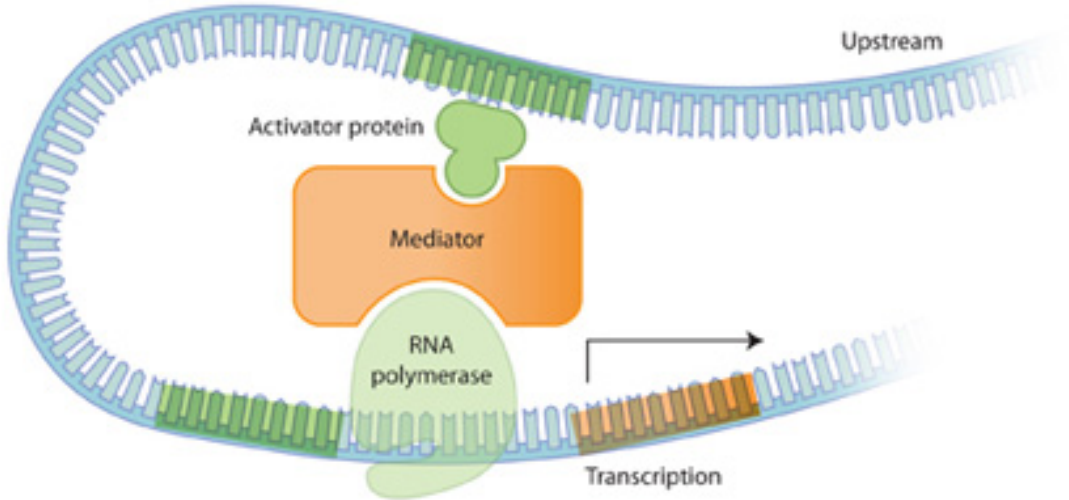
The Cell

The Central Dogma

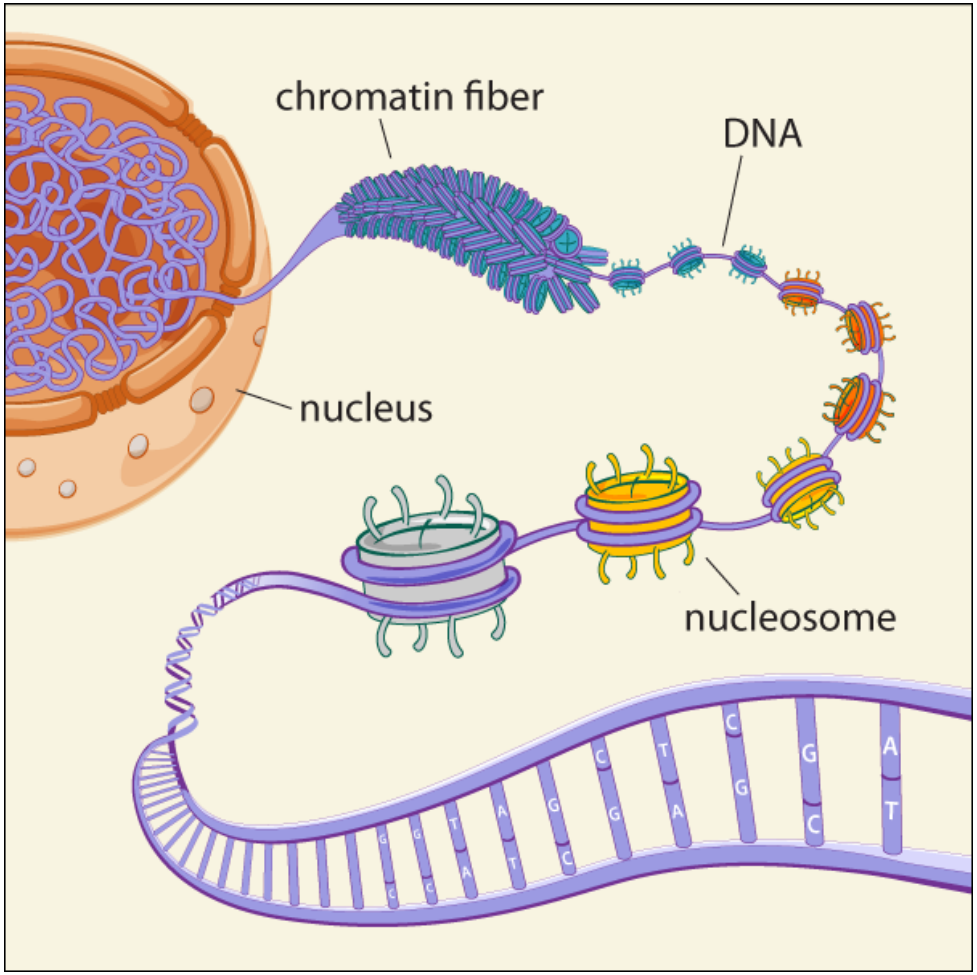


Transcriptional Regulation





Transcriptional Access



Epigenetics and Gene Expression

Things beyond just the base pairs in DNA matter $\rightarrow$ gene expression

The Question

Analyze the ENCODE dataset

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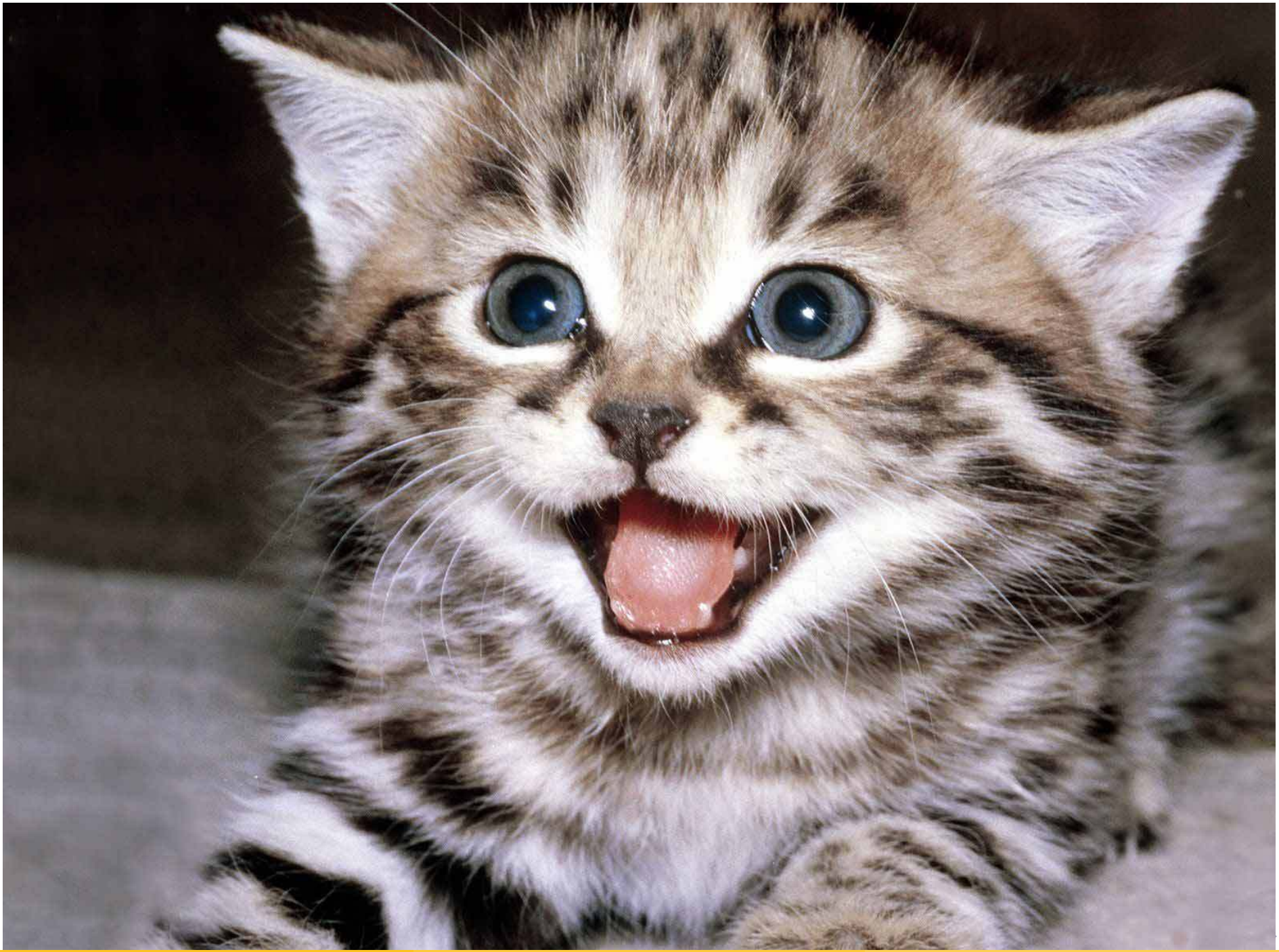
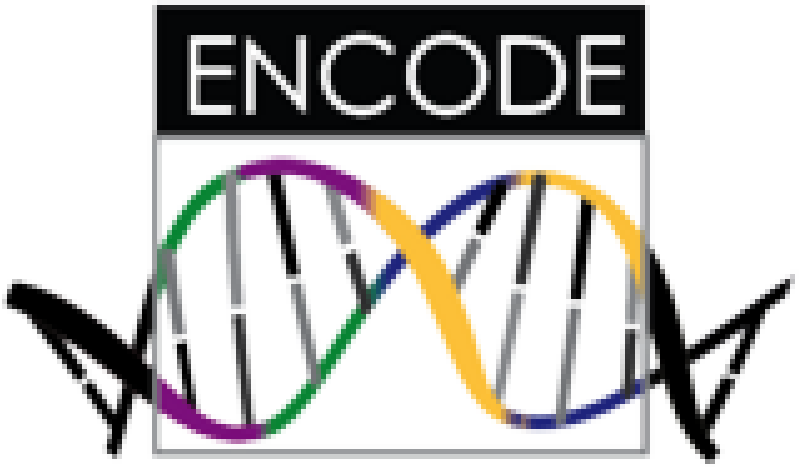


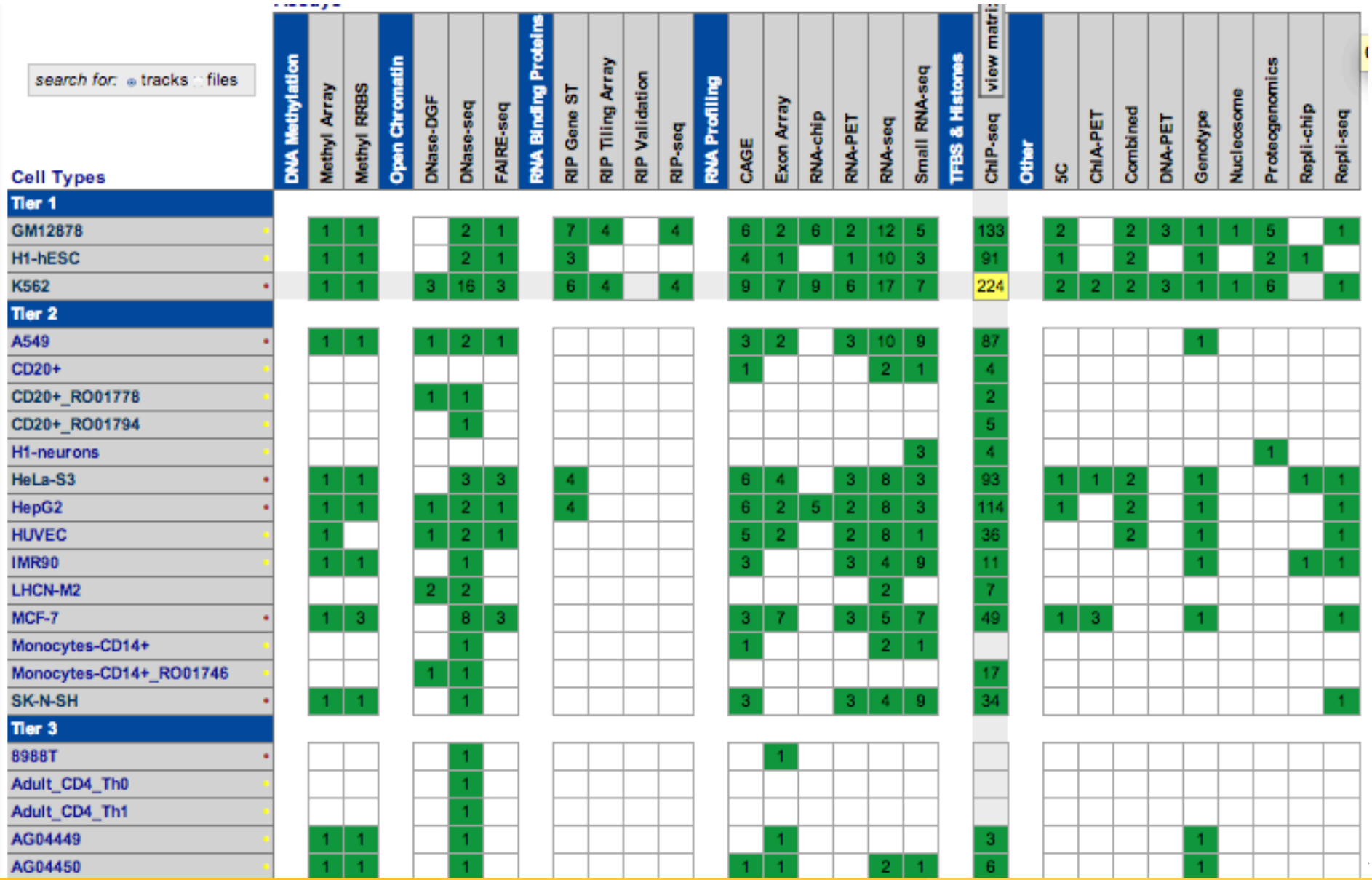
ENCODE (Overview)

Overview

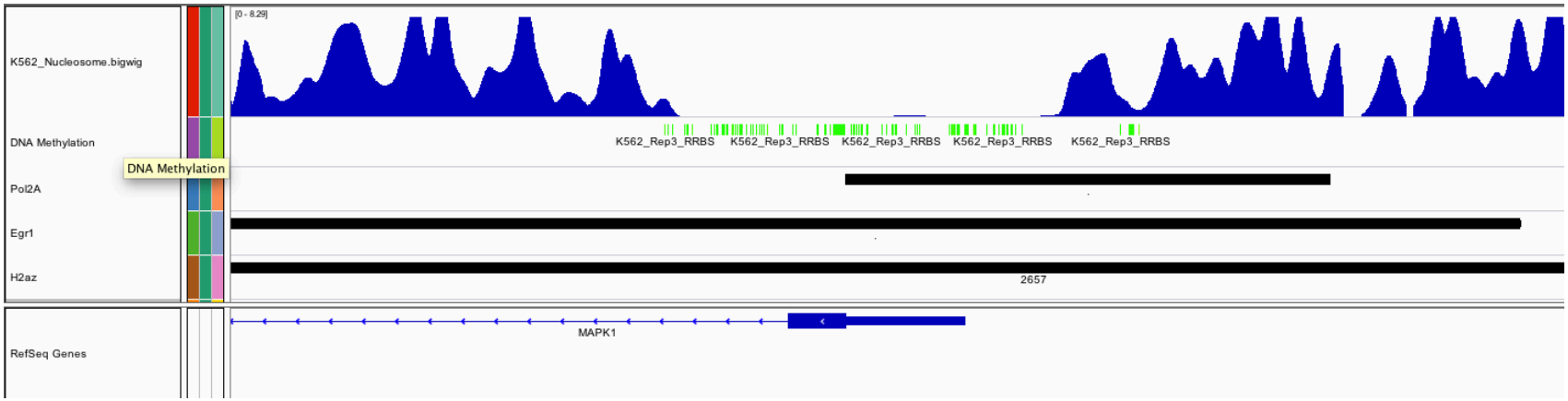
- National Human Genome Institute: Encyclopedia of DNA Elements (ENCODE)
- Nearly 600 collaborating labs post HGP



The Data Set


search for: tracks files
DNA Methylation
Methyl Array
Methyl RRBS
Open Chromatin
DNase-DGF
DNase-seq
FAIRE-seq
RNA Binding Proteins
RIP Gene ST
RIP Tiling Array
RIP Validation
RIP-seq
RNA Profiling
CAGE
Exon Array
RNA-chip
RNA-PET
RNA-seq
Small RNA-seq
TFBS & Histones
ChIP-seq
view matrix
Other
5C
ChIA-PET
Combined
DNA-PET
Genotype
Nucleosome
Proteogenomics
Repli-chip
Repli-seq
Cell Types
Tier 1
GM12878 1 1 2 1 7 4 4 6 2 6 2 12 5 133 2 2 3 1 1 5 1
H1-hESC 1 1 2 1 3 4 1 1 10 3 91 1 2 1 2 1
K562 1 1 3 16 3 6 4 4 9 7 9 6 17 7 224 2 2 2 3 1 1 6 1
Tier 2
A549 1 1 1 2 1 3 2 3 10 9 87 1
CD20+ 1 2 1 4
CD20+_RO01778 1 1 2
CD20+_RO01794 1 5
H1-neurons 3 4 1
HeLa-S3 1 1 3 3 4 6 4 3 8 3 93 1 1 2 1 1 1
HepG2 1 1 1 2 1 4 6 2 5 2 8 3 114 1 2 1 1
HUVEC 1 1 2 1 5 2 2 8 1 36 2 1 1
IMR90 1 1 1 3 3 4 9 11 1 1 1
LHCN-M2 2 2 2 7
MCF-7 1 3 8 3 3 7 3 5 7 49 1 3 1 1
Monocytes-CD14+ 1 1 2 1
Monocytes-CD14+_RO01746 1 1 17
SK-N-SH 1 1 1 3 3 4 9 34 1
Tier 3
8988T 1 1
Adult_CD4_Th0 1
Adult_CD4_Th1 1
AG04449 1 1 1 1 3 1
AG04450 1 1 1 1 1 2 1 6 1

Data Types



- Raw signals
- Raw signal peak calling outputs (e.g. PeakSeq results)
- Relatively course-grain peak data

The Game Plan

Can we reduce transcription factor binding landscapes into categories?

- Scan across genome, looking for promoters
- Bin promoters appropriately
- Score binding at each promoter
- Clustering analysis

RefSeq

Overview

- Curated database of genes
- New versions released as frequently as Firefox
- Includes pseudogenes, haplotype variations, and predicted genes

Defining Promoter?

- Only upstream from TSS?
- Incredibly far regulatory regions?
- Intronic regulation?
- Post termination regulatory elements?
- **1000 bp upstream**

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Binning

- How do we quantitatively analyze promoter presence?
- Break promoter regions into bins for a finer metric?
- Do we give weights to bins as a function of their position?
- **Single, unweighted 1000 bp bin of counts**

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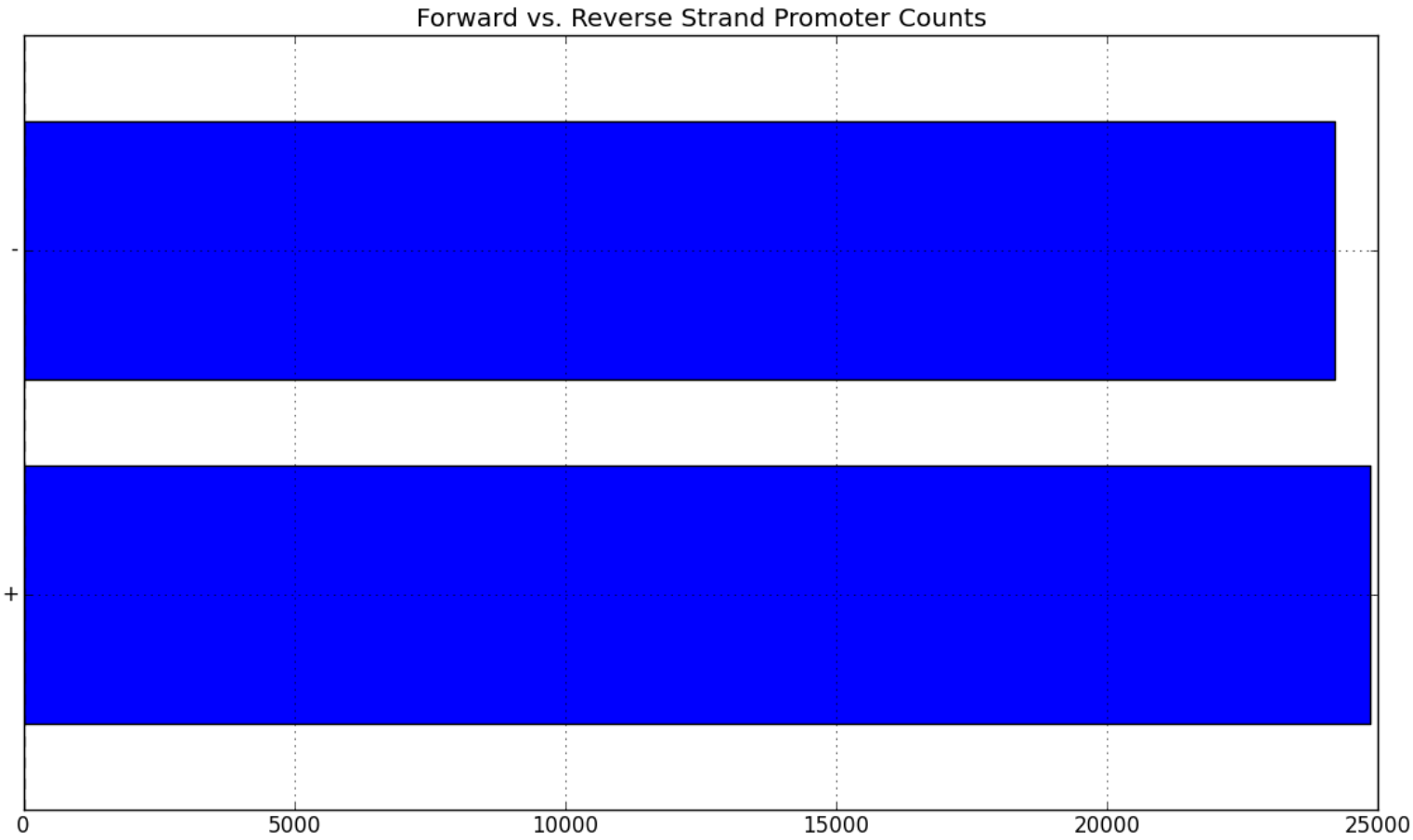
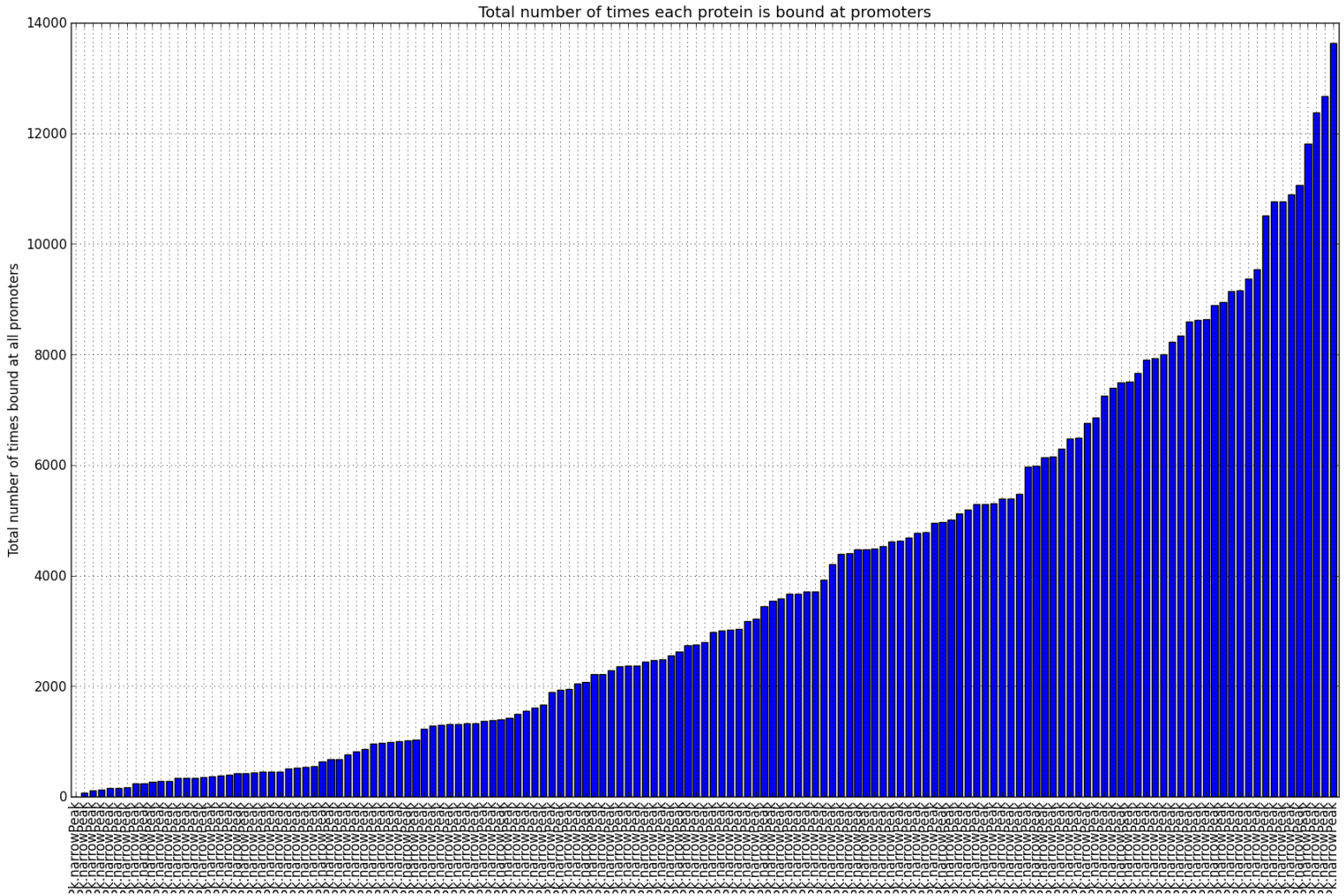
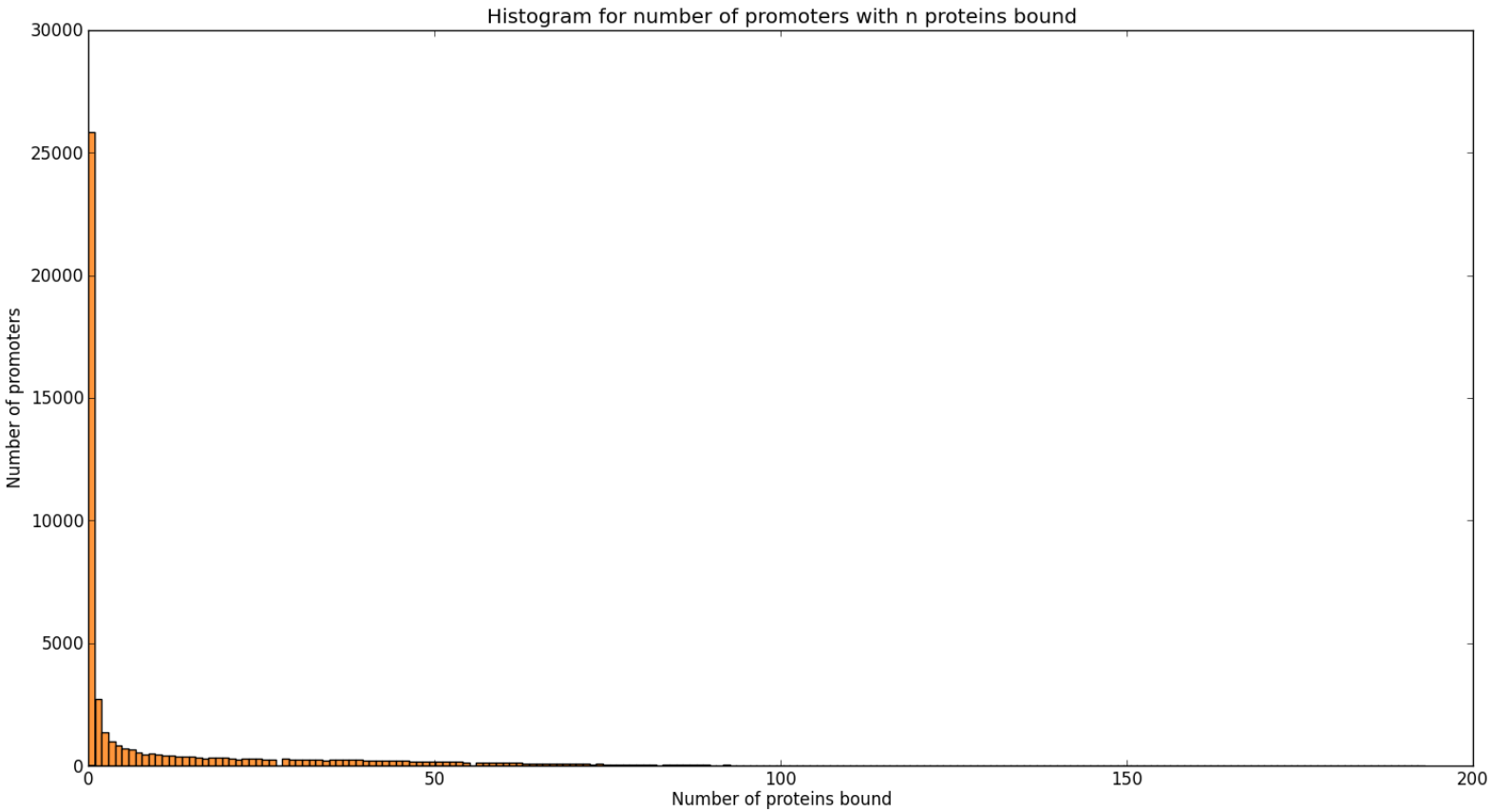
Forward vs. Backward Strand



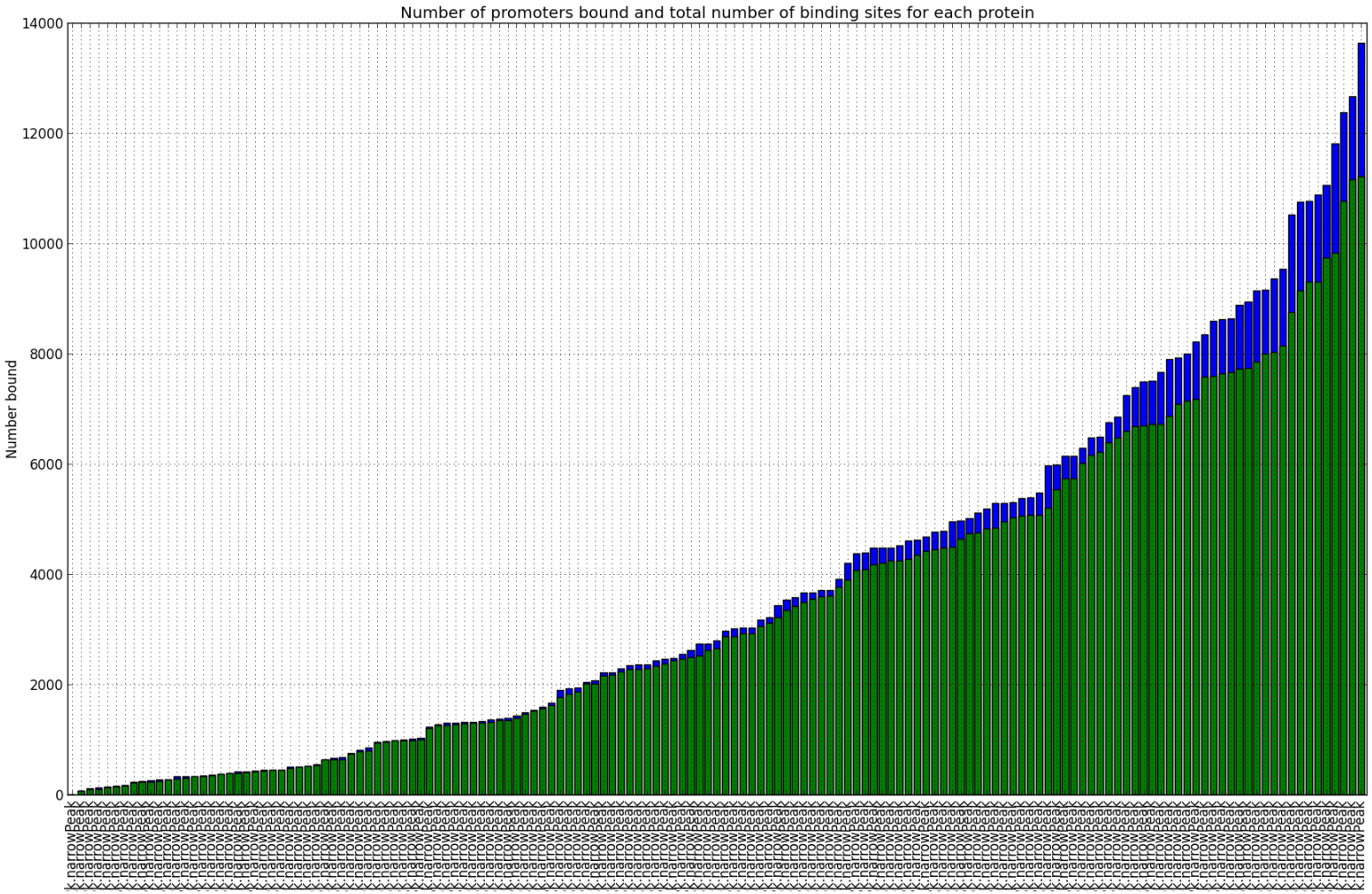
TF Binding Frequency



Histogram of promoter binding

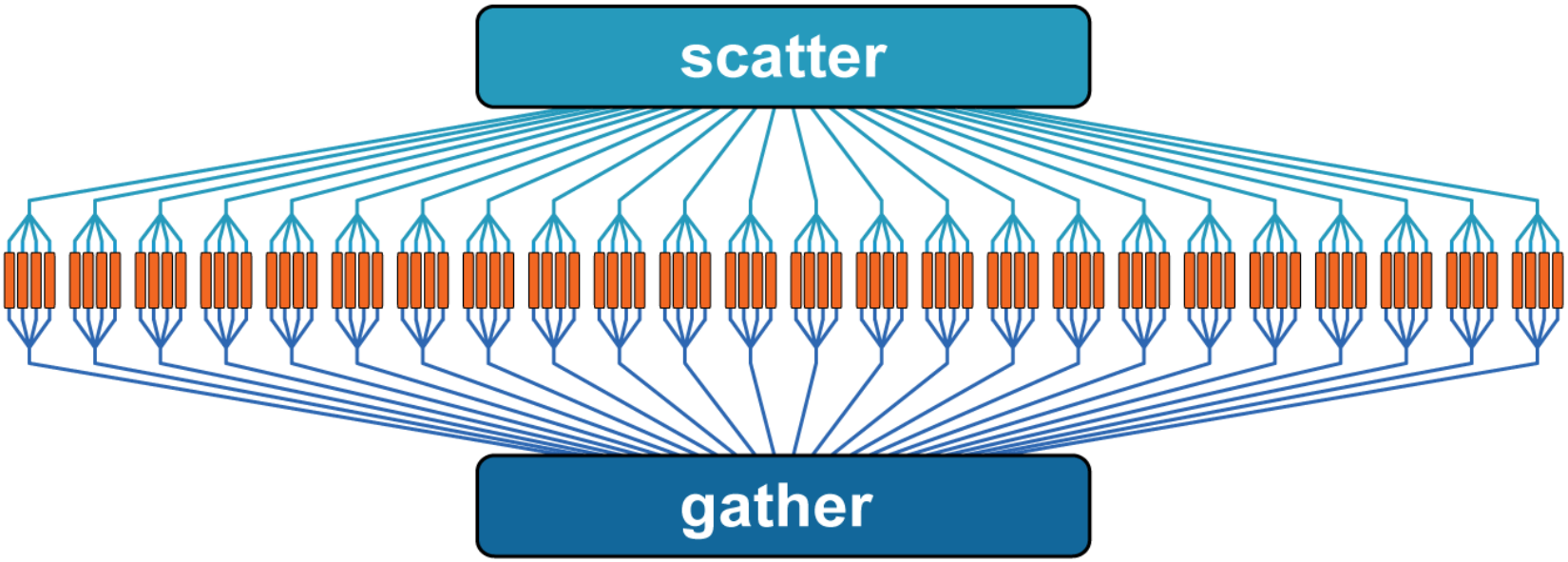


Promoter/TFBS intersections



Computational Efficiency

- This was an exercise in program optimization
- Original algorithm took about 5 days, optimized/parallelized algorithm took just a few hours



Clustering

The unsupervised grouping of information such that groups have similar elements that are dissimilar from elements in other groups

Clustering

Minkowski Distance ($p = 2 \rightarrow$ Euclidean Distance)

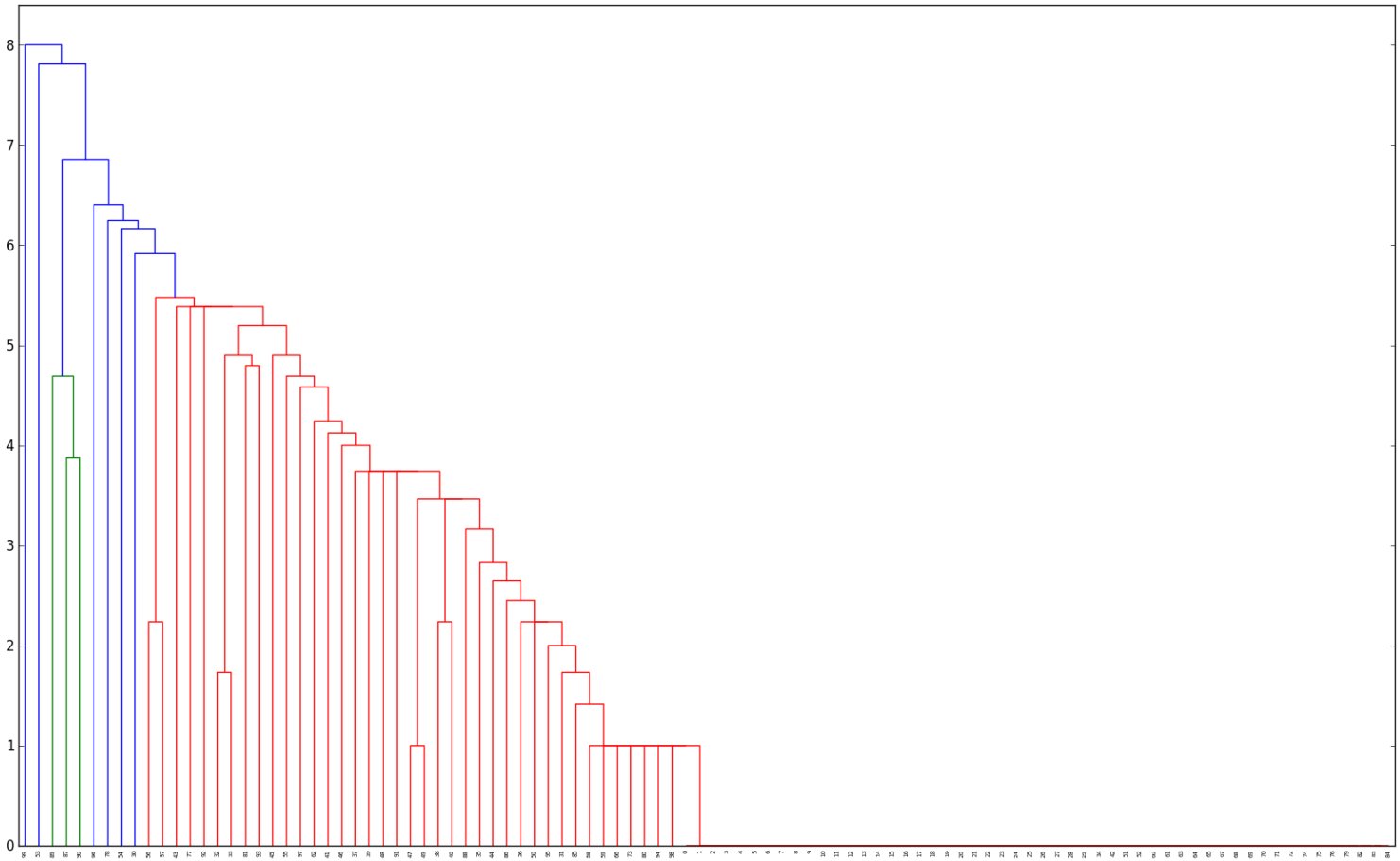
$$\left(\sum_{i=1}^{n} |x_i - y_i|^p \right)^{\frac{1}{p}}$$

Clustering

Simple Linkage Clustering

$$D(X, Y) = min(d(x, y)) \; \forall \; (x, y) \epsilon (X, Y)$$

Clustering (simple linkage exhibiting chaining)

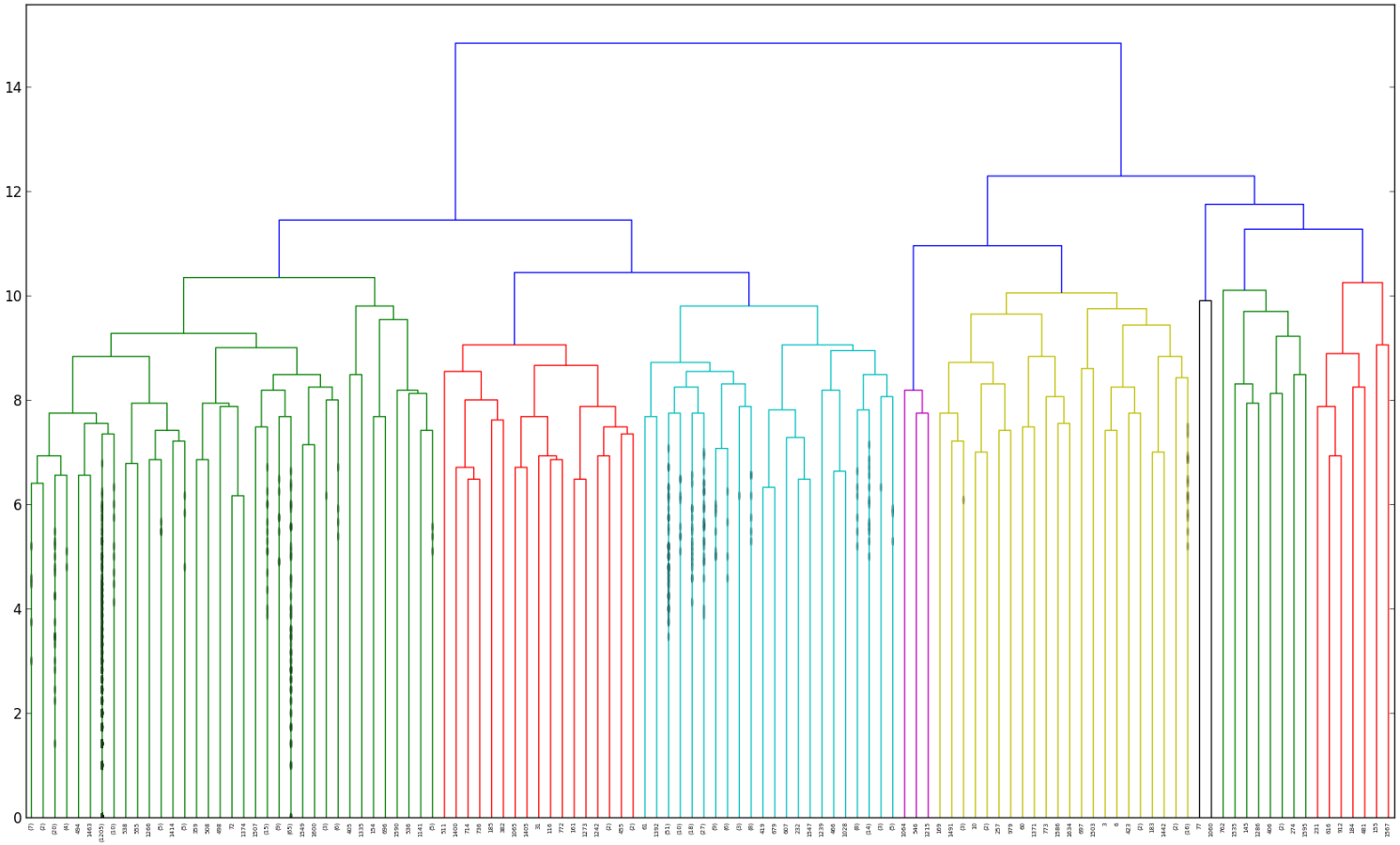


Clustering

Complete Linkage Clustering

$$D(X, Y) = max(d(x, y)) \; \forall \; (x, y) \epsilon (X, Y)$$

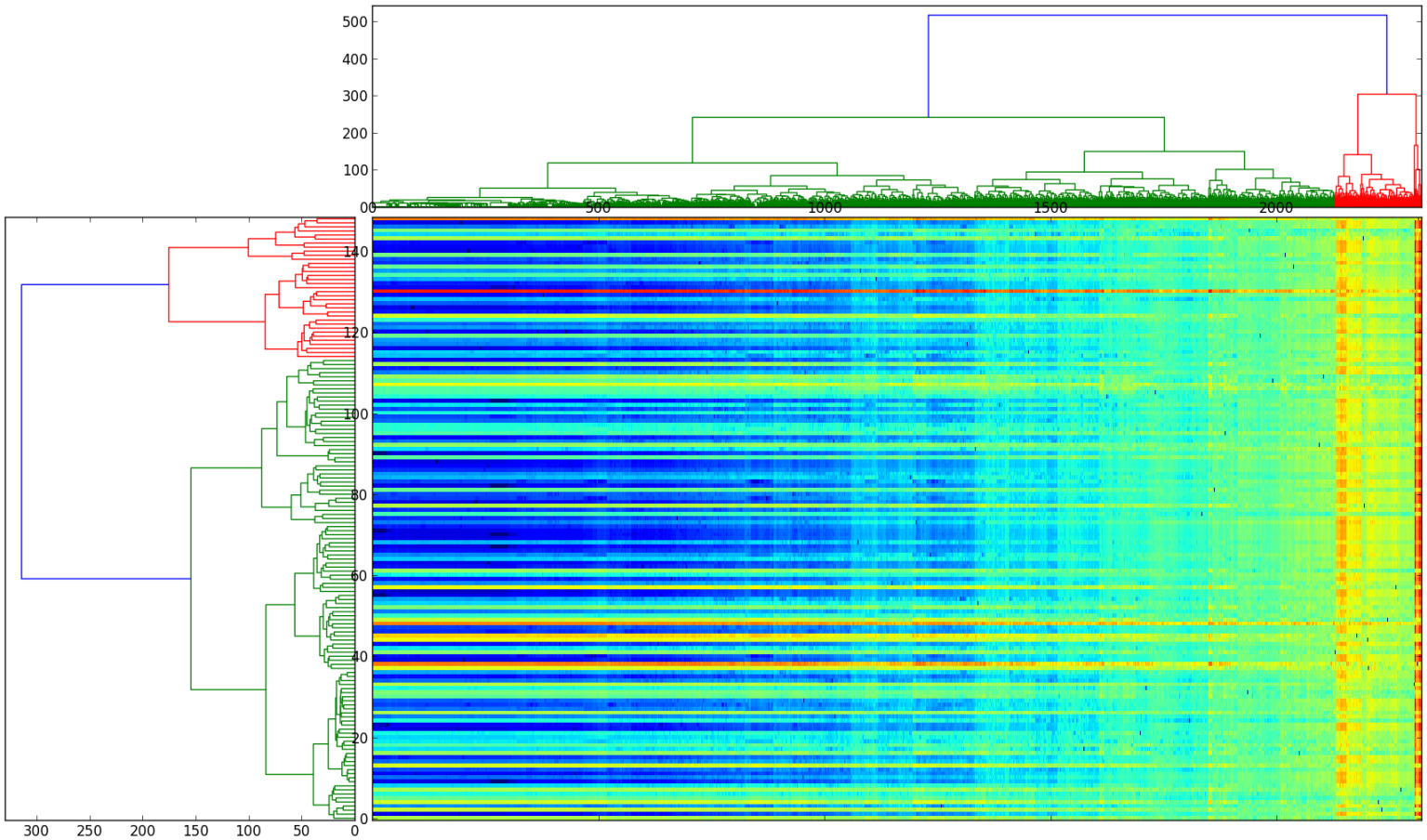
Clustering (complete linkage)



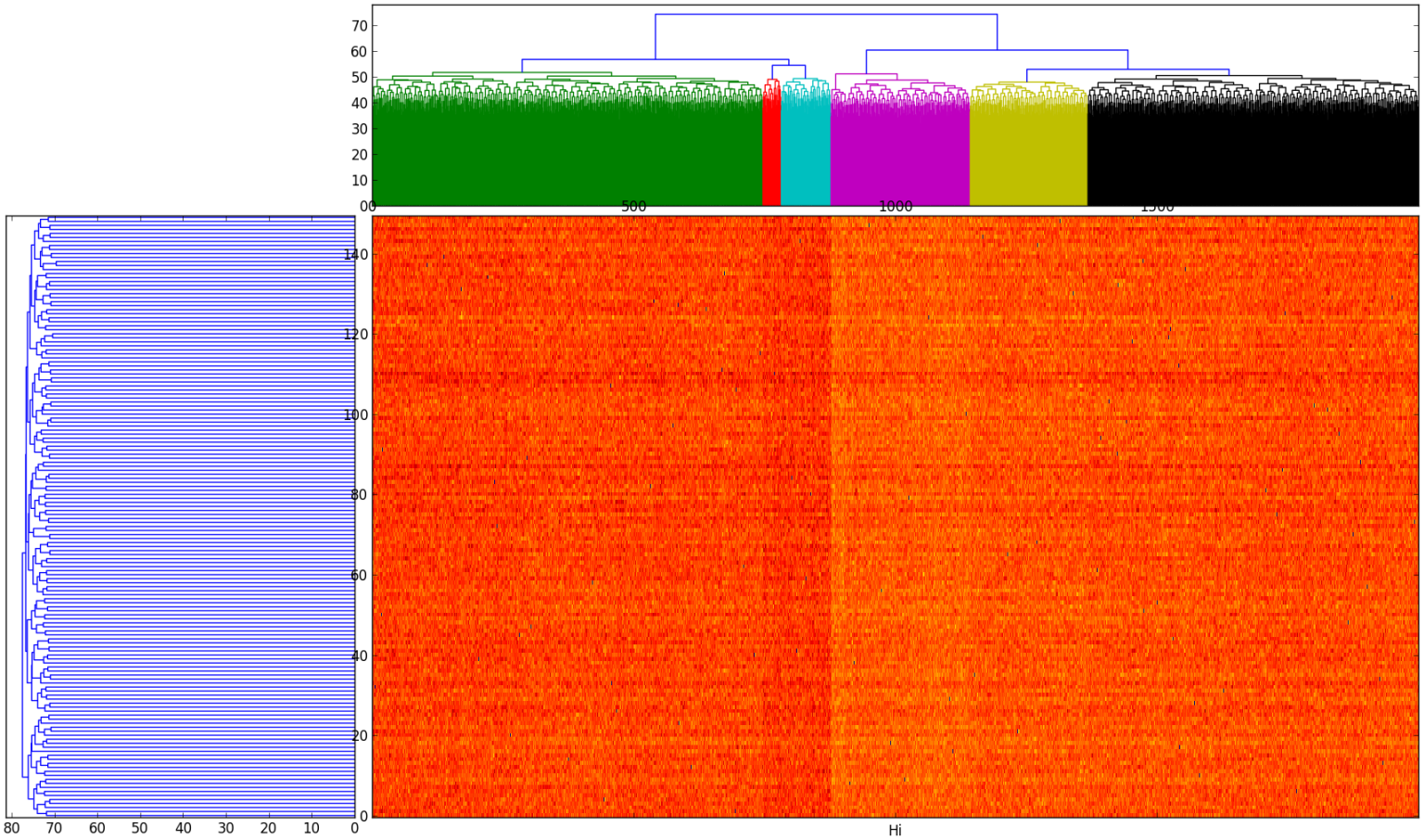
Computational Efficiency

- Pairwise distance calculations require a LOT of RAM

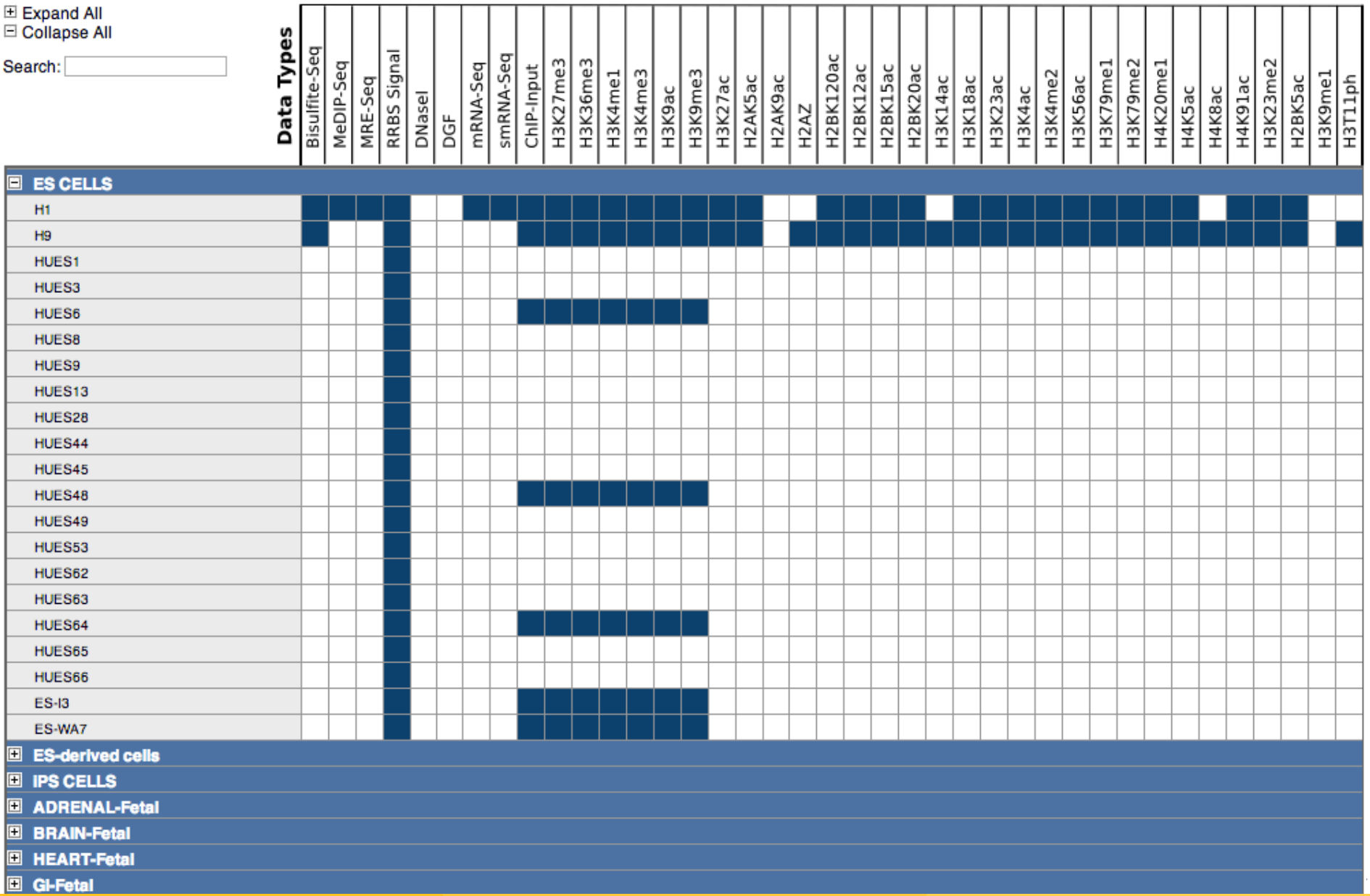
2-way clustering heatmap



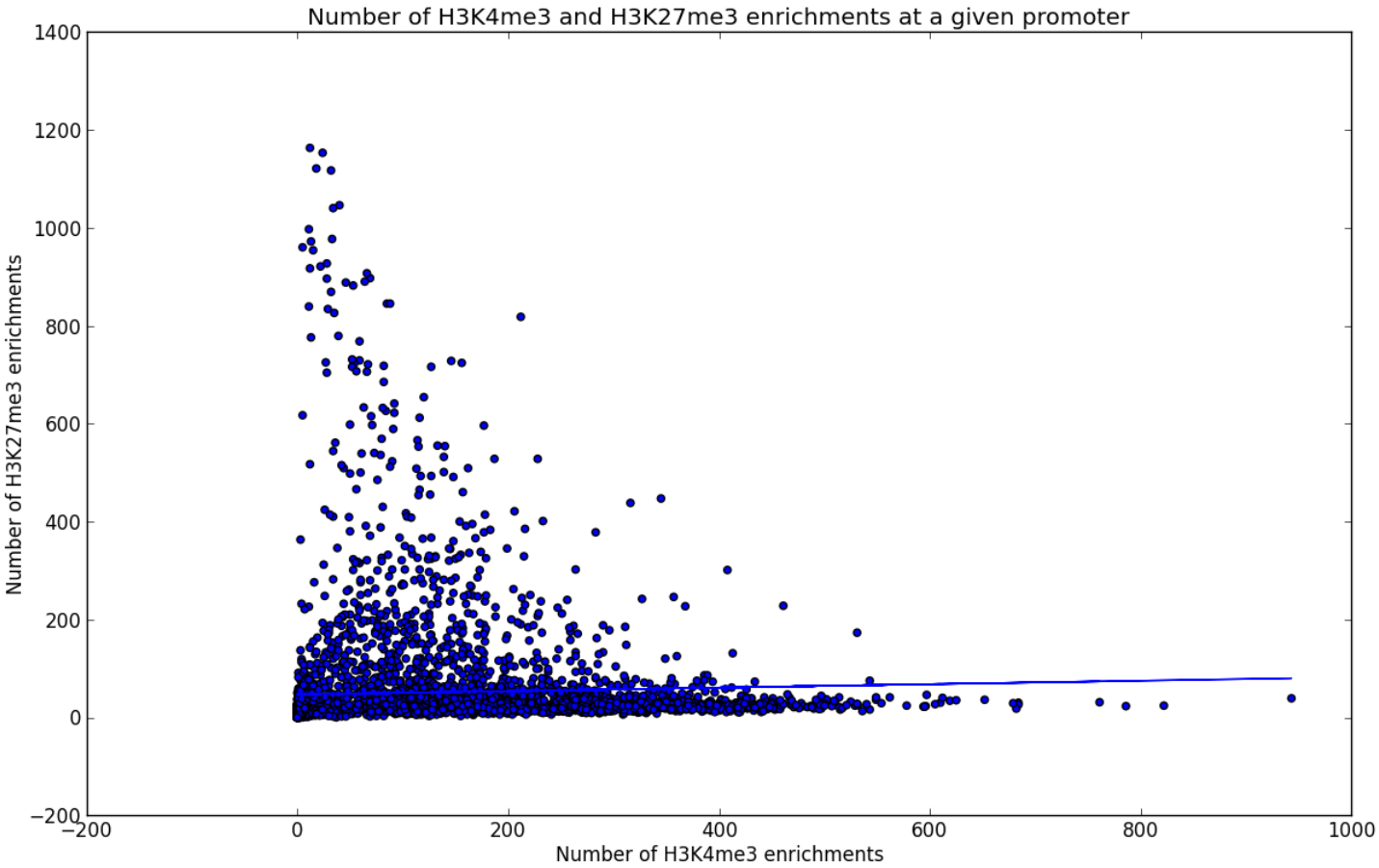
2-way clustering heatmap (with random data arrays)



Histone Modifications (Roadmap Dataset)



A quick correlation test



$R = 0.042$

Conclusions

- There are numerous methods of promoter binning that prove useful for complexity reduction
- Unsupervised clustering of preprocessed promoter data yield results with biological significance

Next Steps

- Incorporation of nucleosome enrichment, methylation, and histone modification data in a more meaningful way
- Further refining of cluster analysis pipeline to uncover more unknown biology

Thanks!

- Jeremy Gunawardena and the HMS Department of Systems Biology!
- My collaborator Tobias Ahsendorf!
- PRISE and Greg Llacer!
- The lovely PRISE staff!
- This audience!