

# Lecture 18. Midterm Review

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October 30, 2022

Applied Comparative Genomics



# Exam Topics

## Genomics

- Genomics Technologies
  - Illumina, PacBio, Nanopore
- Genome Assembly
- Whole Genome Alignment
- Read mapping
- Variant Identification
- Gene Finding
- RNA-seq
- Methyl-seq, Chip-Seq, Hi-C
- Genome Annotation
- Single cell vs bulk sequencing

## Quantitative Techniques

- Normal, Poisson, Binomial, P-value
- de Bruijn and overlap graphs
- Minimizers
- Dot plots
- Quality Values (Phred Scale)
- Full text indexing & BWT
- Seed & Extend
- Hidden Markov Models
- PCA / t-SNE / UMAP
- Differential Expression
- Expectation Maximization

**What is the goal? What is the approach? What are the key challenges?**

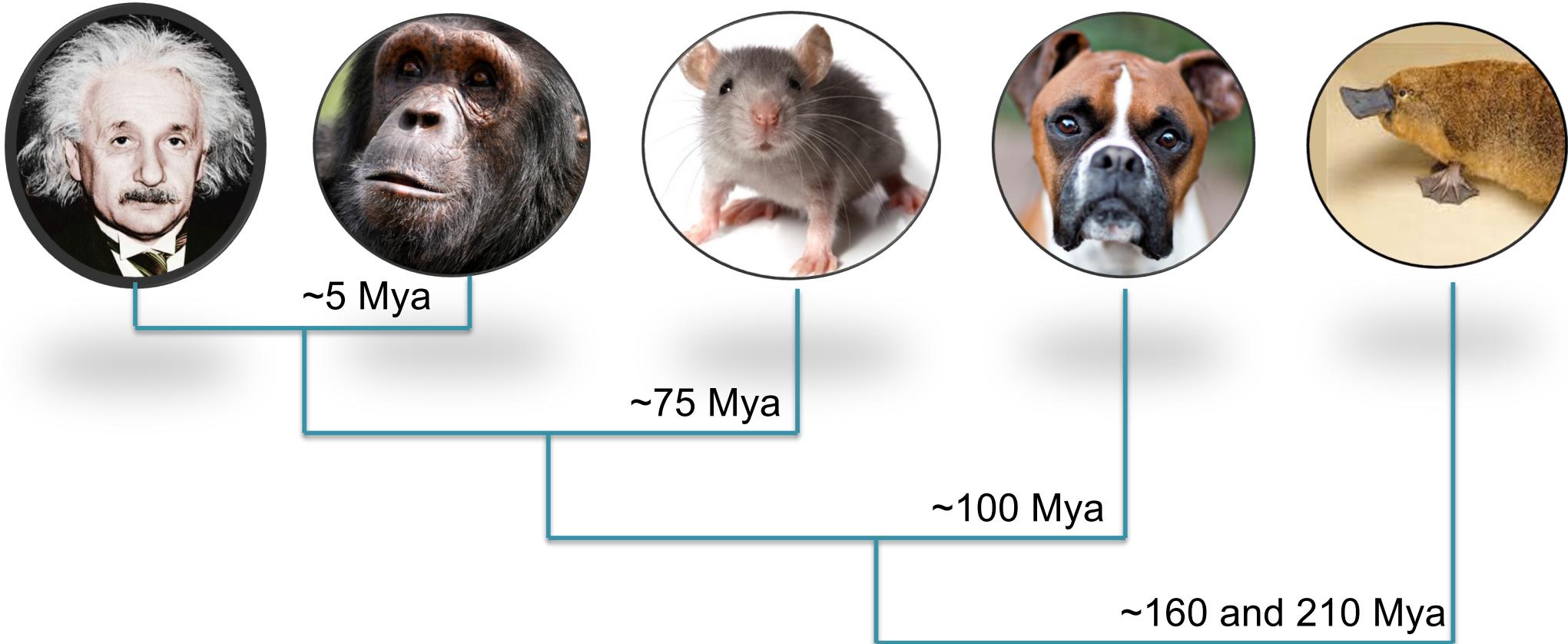
**How did we explore these topics in the homeworks and lectures?**

# Sample Question

**Q3. The Maryland blue crab genome is 1 Gbp in size. Approximately how many 100bp reads should we sequence so that we expect at least 99.85% of the genome will be sequenced at least 40 times? Sketch the expected coverage distribution for this number of reads; be sure to clearly label the mean coverage, and how 40 fold coverage relates to the mean. (Hint: In a normal distribution, 68.2% of the data is within 1 standard deviation of the mean, 95.4% within 2, 99.7% within 3, and 99.9% within 4)**



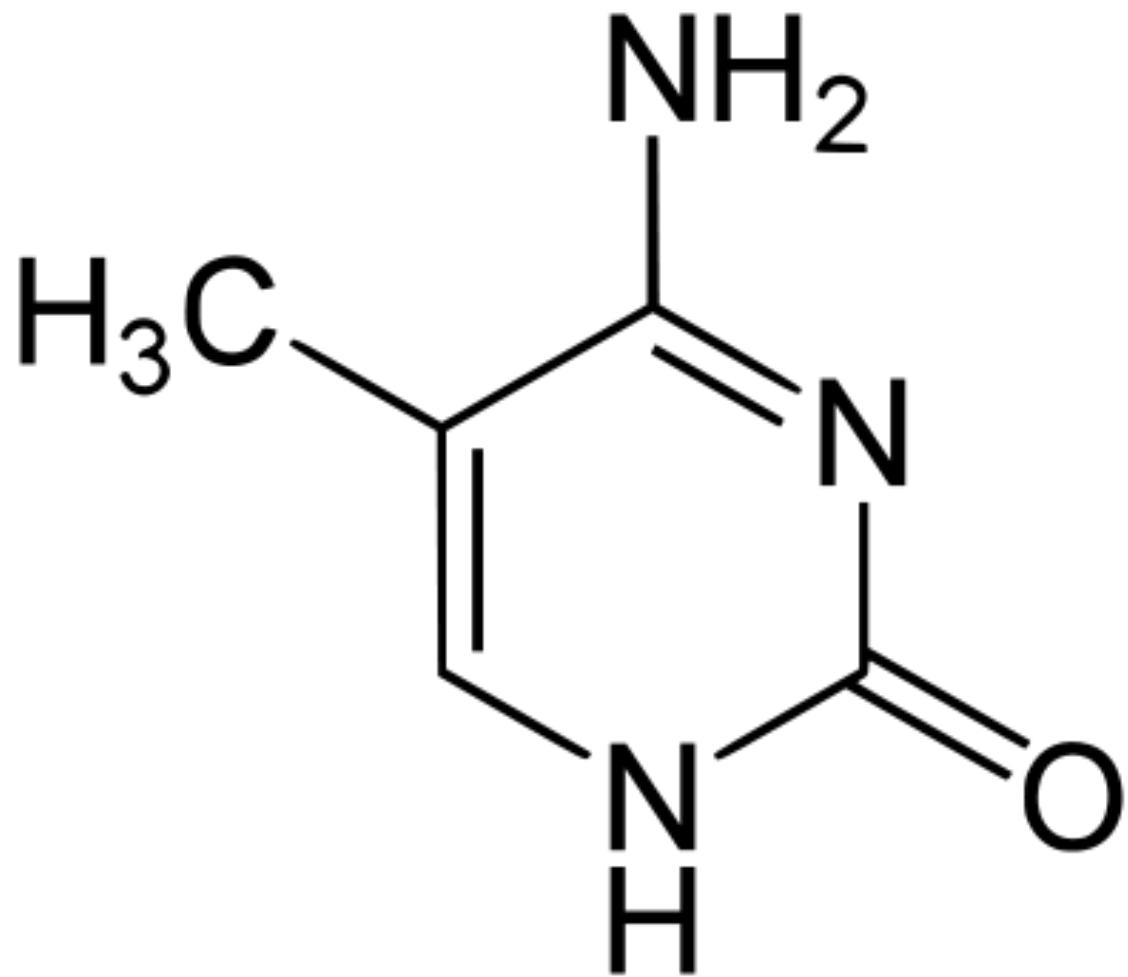
# Human Evolution



**As expected, the majority of platypus genes (82%; 15,312 out of 18,596) have orthologues in these five other amniotes** (Supplementary Table 5). The remaining 'orphan' genes are expected to primarily reflect rapidly evolving genes, for which no other homologues are discernible, erroneous predictions, and true lineage-specific genes that have been lost in each of the other five species under consideration.

**Genome analysis of the platypus reveals unique signatures of evolution**  
(2008) *Nature*. 453, 175-183 doi:10.1038/nature06936

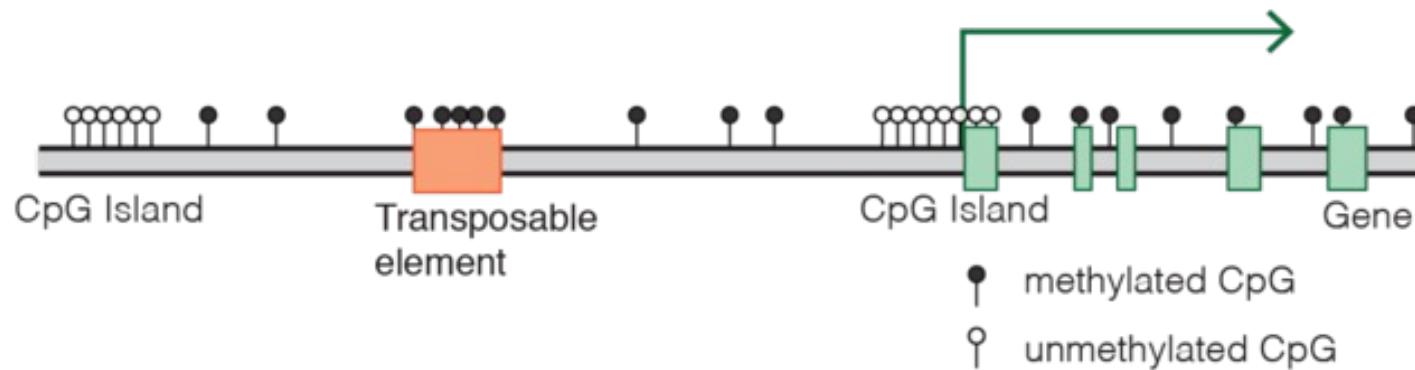
# Methyl-seq



**Finding the fifth base: Genome-wide sequencing of cytosine methylation**  
Lister and Ecker (2009) *Genome Research*. 19: 959-966

# Methylation of CpG Islands

Typical mammalian DNA methylation landscape



**CpG islands are (usually) defined as regions with**

- 1) a length greater than 200bp,
- 2) a G+C content greater than 50%,
- 3) a ratio of observed to expected CpG greater than 0.6

**Methylation in promoter regions correlates negatively with gene expression.**

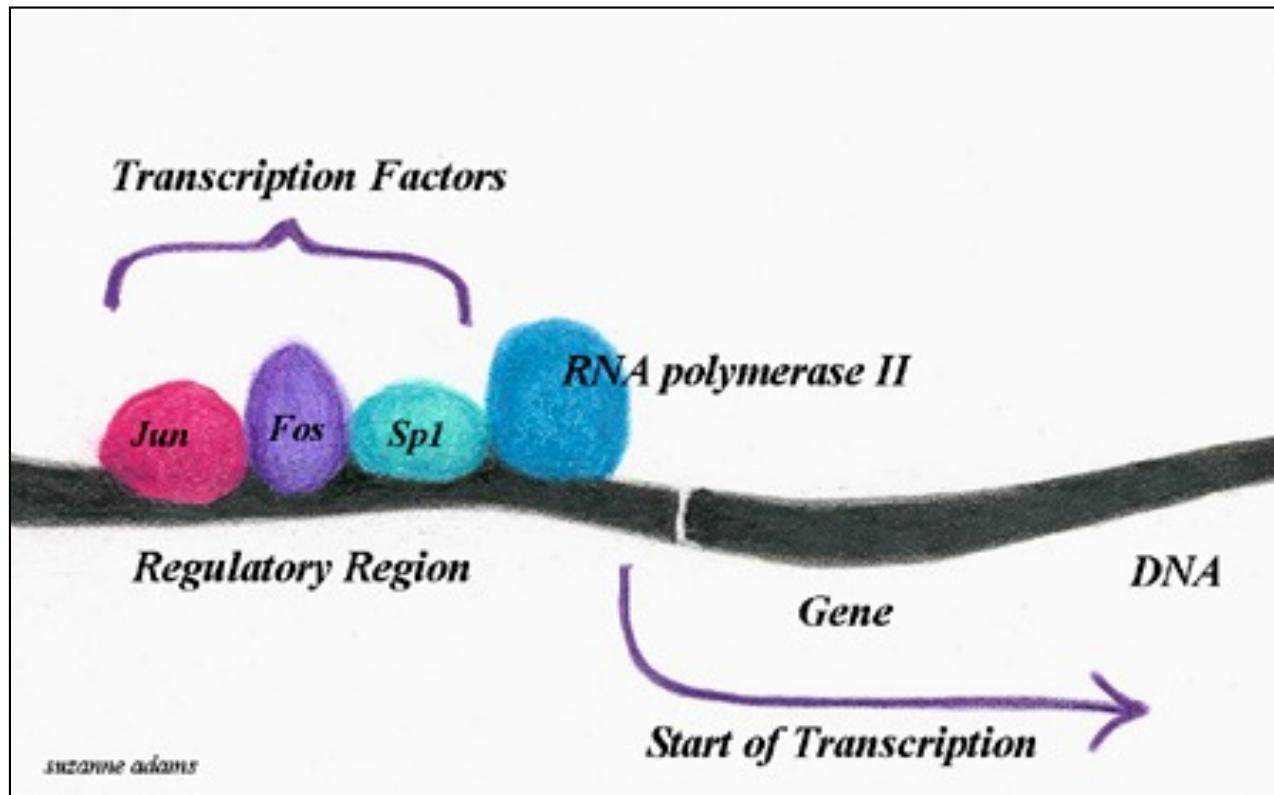
- CpG-dense promoters of actively transcribed genes are never methylated
- In mouse and human, around 60-70% of genes have a CpG island in their promoter region and most of these CpG islands remain unmethylated independently of the transcriptional activity of the gene
- Methylation of DNA itself may physically impede the binding of transcriptional proteins to the gene
- Methylated DNA may be bound by proteins known as methyl-CpG-binding domain proteins (MBDs) that can modify histones, thereby forming compact, inactive chromatin, termed heterochromatin.

# Bisulfite Conversion



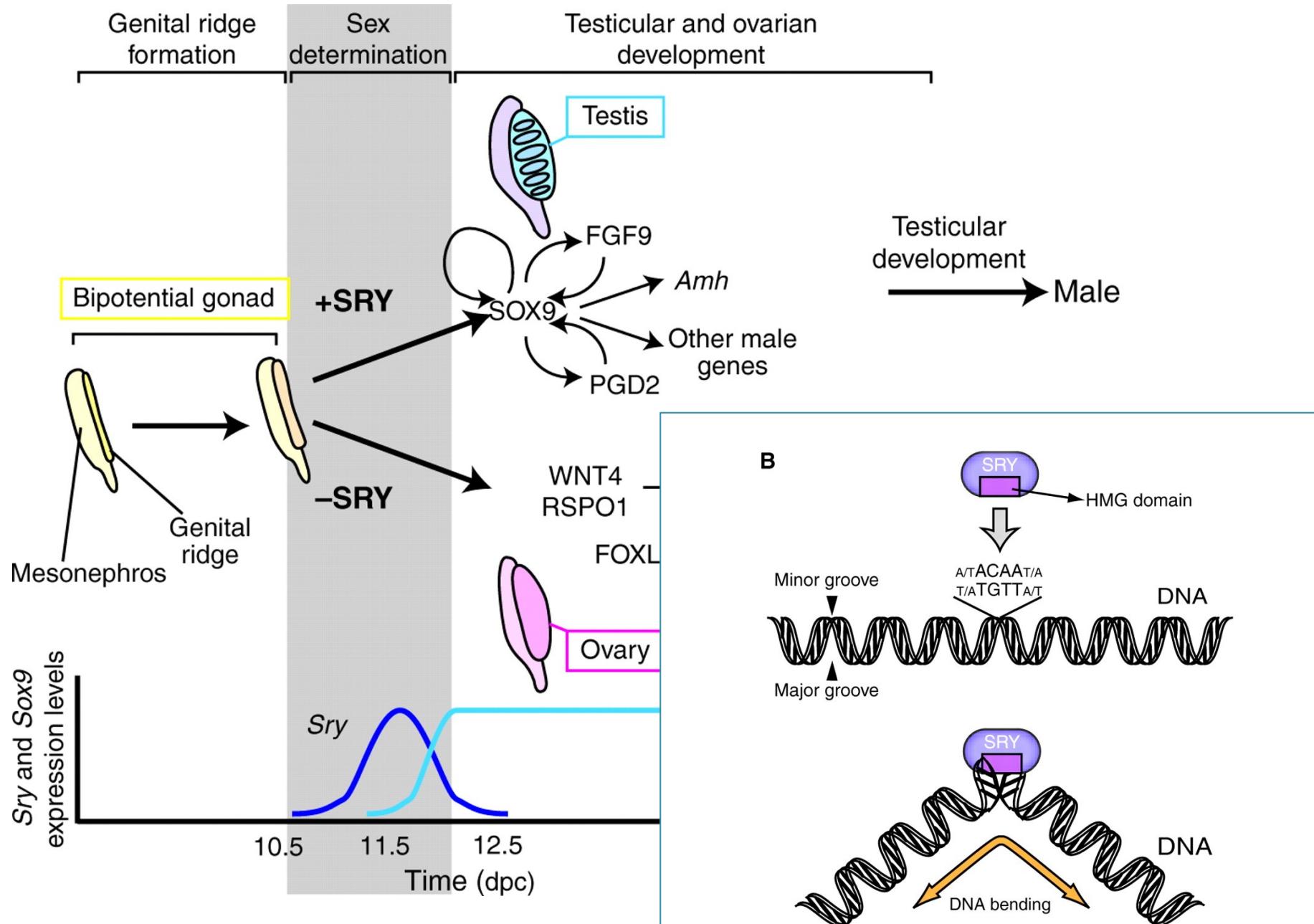
**Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications**  
Krueger and Andrews (2010) *Bioinformatics*. 27 (11): 1571-1572.

# ChIP-seq



**Genome-wide mapping of in vivo protein-DNA interactions.**

Johnson et al (2007) Science. 316(5830):1497-502



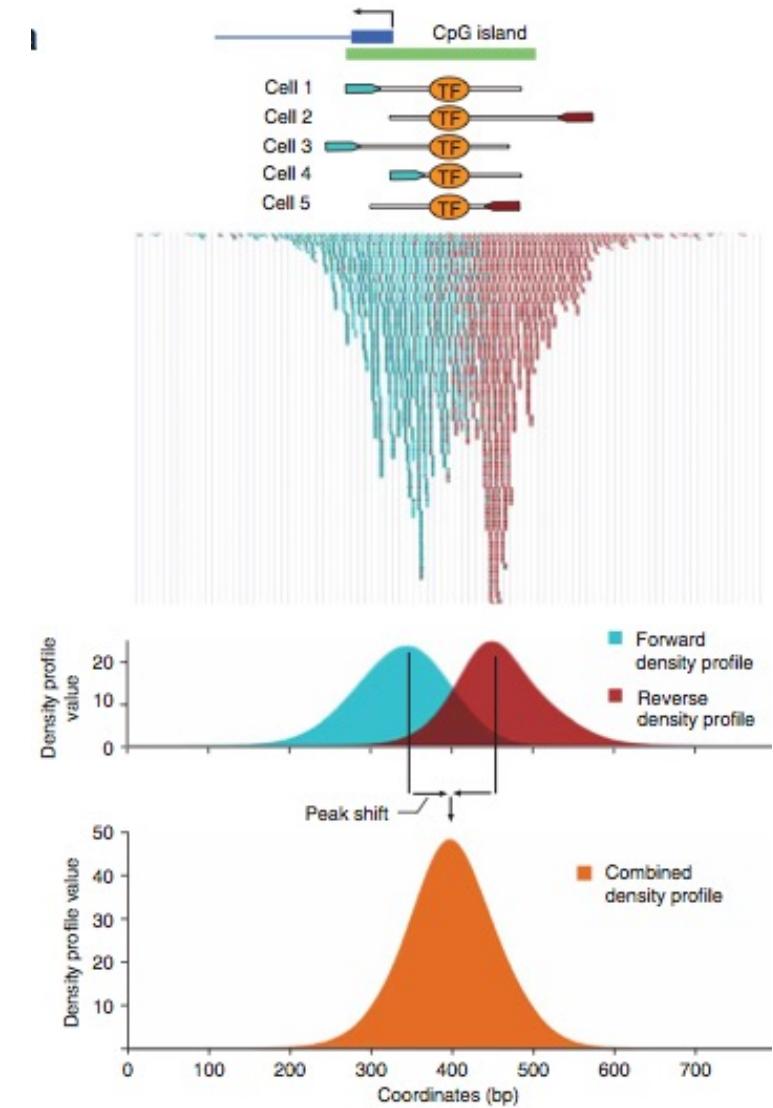
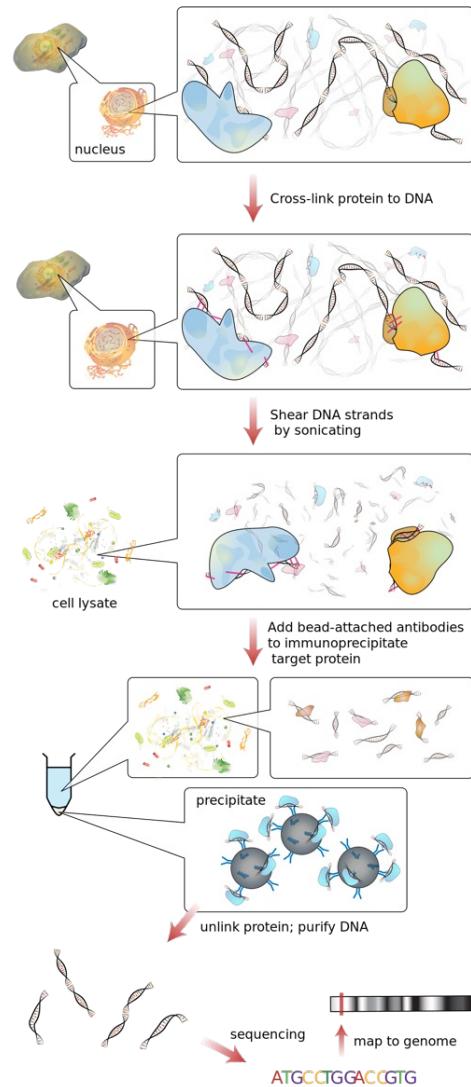
## SRY: The master switch in mammalian sex determination

Kashimada and Koopman (2010) Development 137: 3921-3930; doi: 10.1242/dev.048983

# ChIP-seq: TF Binding

## Goals:

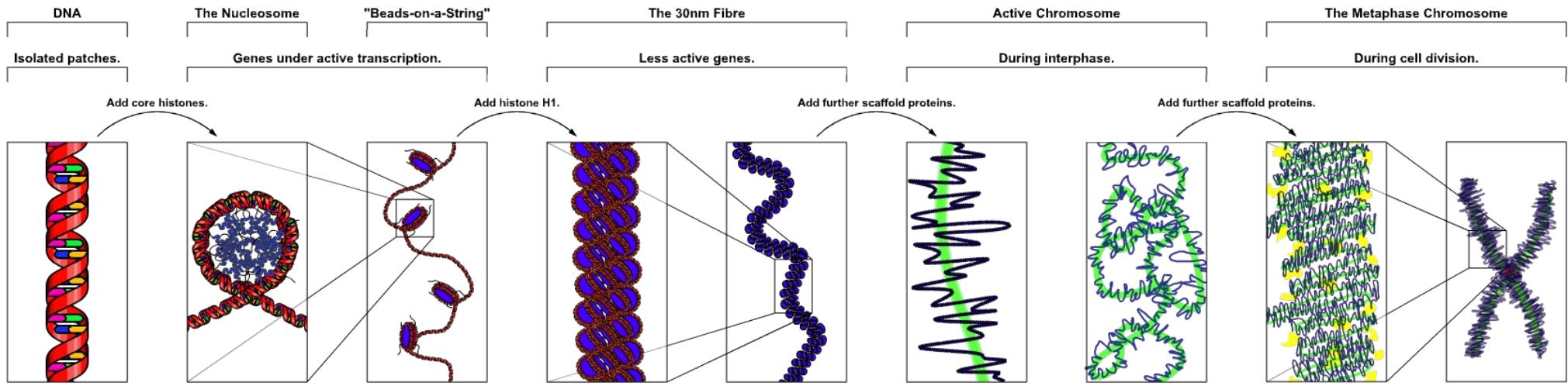
- Where are transcription factors and other proteins binding to the DNA?
- How strongly are they binding?
- Do the protein binding patterns change over developmental stages or when the cells are stressed?



Genome-wide analysis of transcription factor binding sites based on ChIP-Seq data

Valouev et al (2008) *Nature Methods*. 5, 829 - 834

# Chromatin compaction model



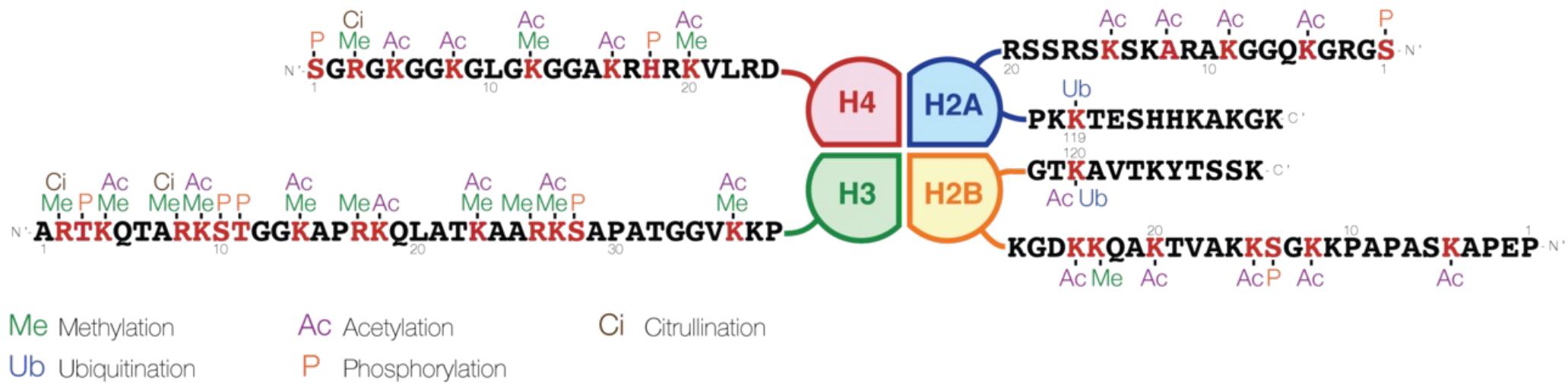
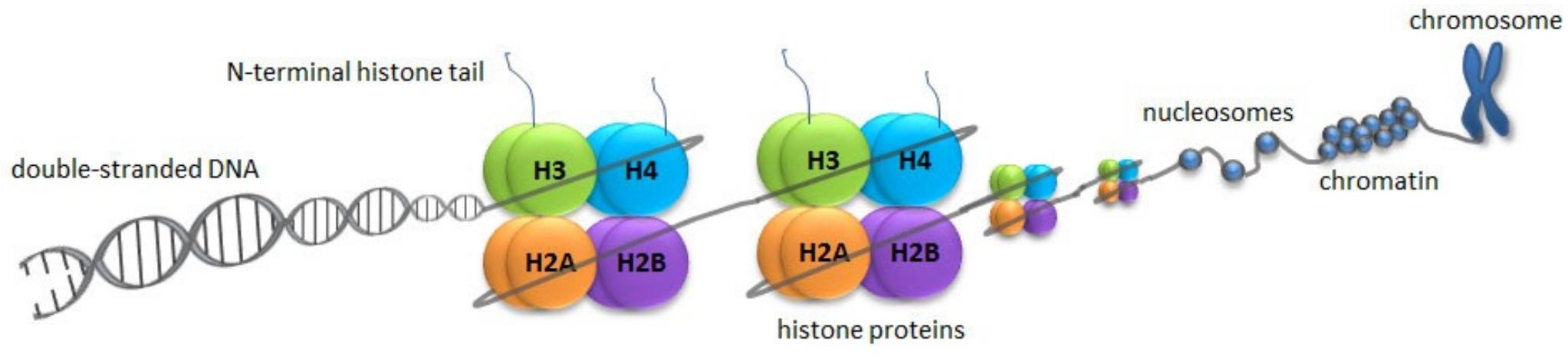
## ***Nucleosome is a basic unit of DNA packaging in eukaryotes***

- Consists of a segment of 146bp DNA wound in sequence around eight histone protein cores (thread wrapped around a spool) followed by a ~38bp linker
- Under active transcription, nucleosomes appear as “beads-on-a-string”, but are more densely packed for less active genes

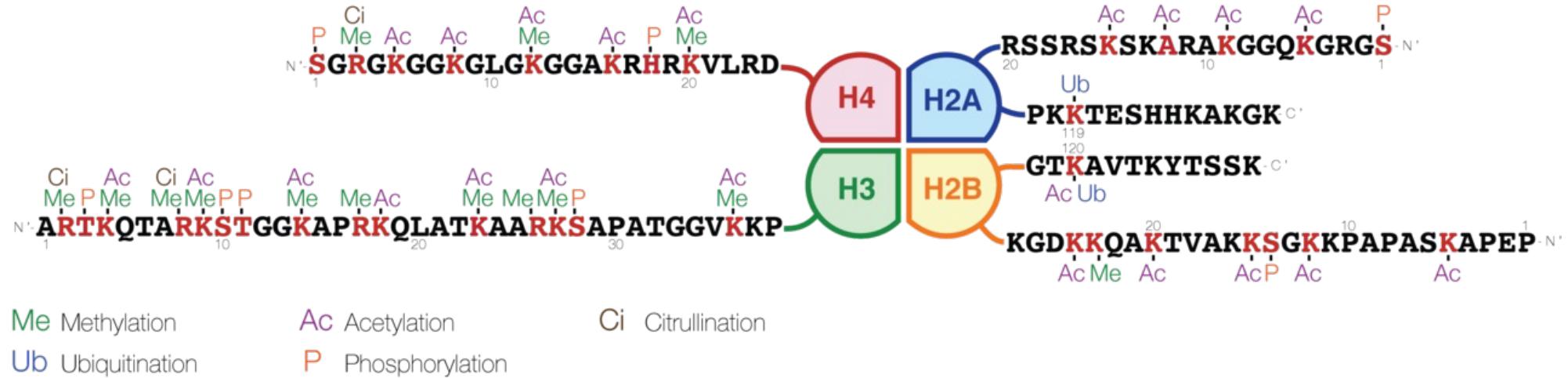
## ***Nucleosomes form the fundamental repeating units of eukaryotic chromatin***

- Used to pack the large eukaryotic genomes into the nucleus while still ensuring appropriate access to it (in mammalian cells approximately 2 m of linear DNA have to be packed into a nucleus of roughly 10  $\mu\text{m}$  diameter).

# ChIP-seq: Histone Modifications



# ChIP-seq: Histone Modifications

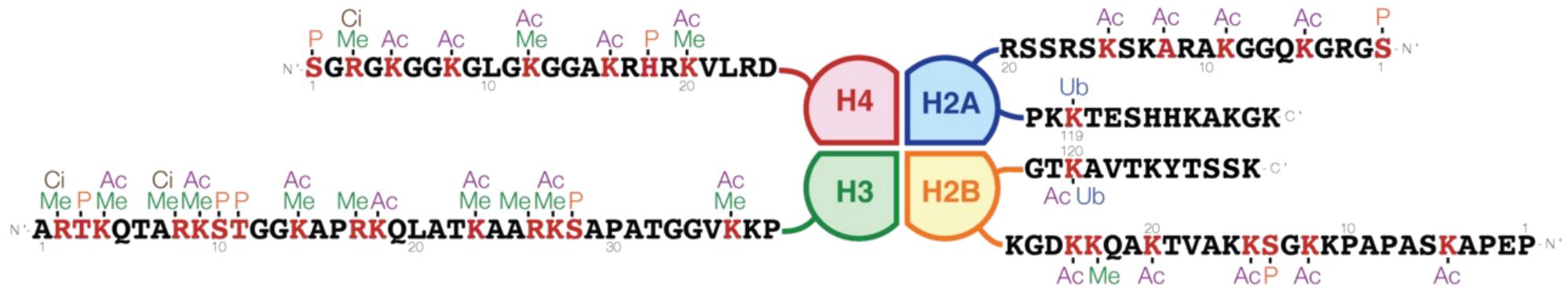


**The common nomenclature of histone modifications is:**

- The name of the histone (e.g., H3)
  - The single-letter amino acid abbreviation (e.g., K for Lysine) and the amino acid position in the protein
  - The type of modification (Me: methyl, P: phosphate, Ac: acetyl, Ub: ubiquitin)
  - The number of modifications (only Me is known to occur in more than one copy per residue. 1, 2 or 3 is mono-, di- or tri-methylation)

**So H3K4me1 denotes the monomethylation of the 4th residue (a lysine) from the start (i.e., the N-terminal) of the H3 protein.**

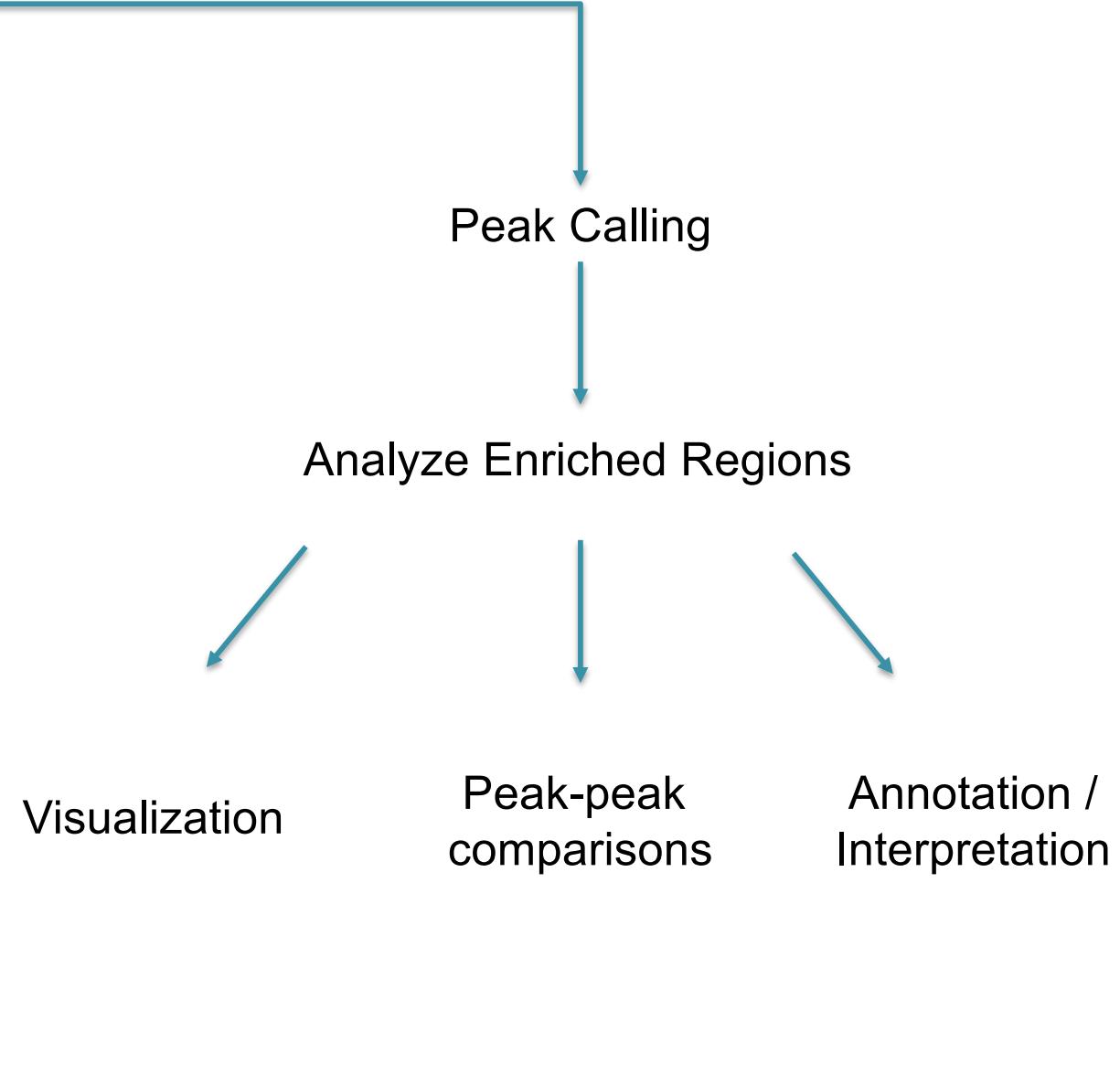
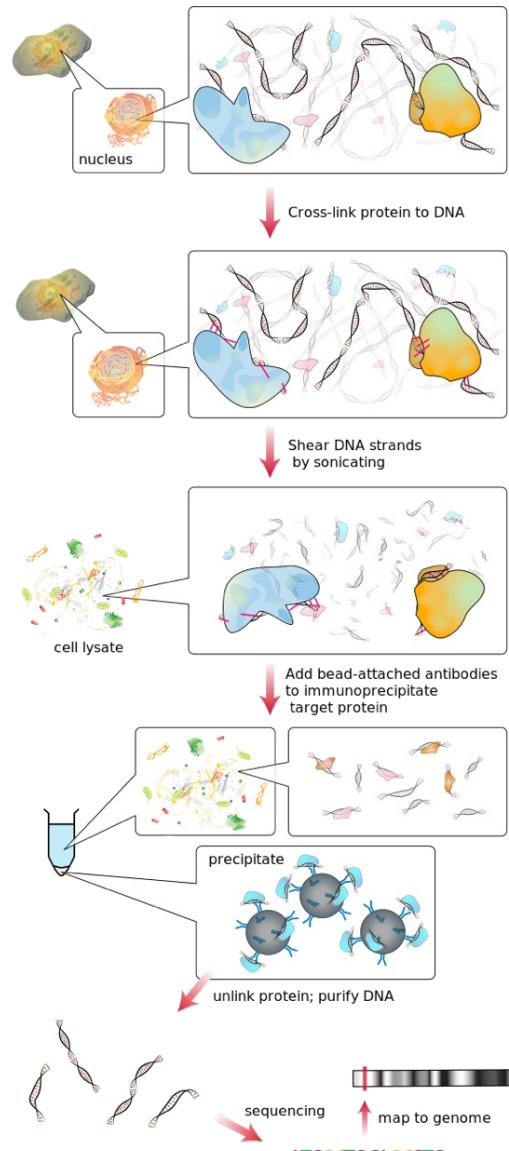
# ChIP-seq: Histone Modifications



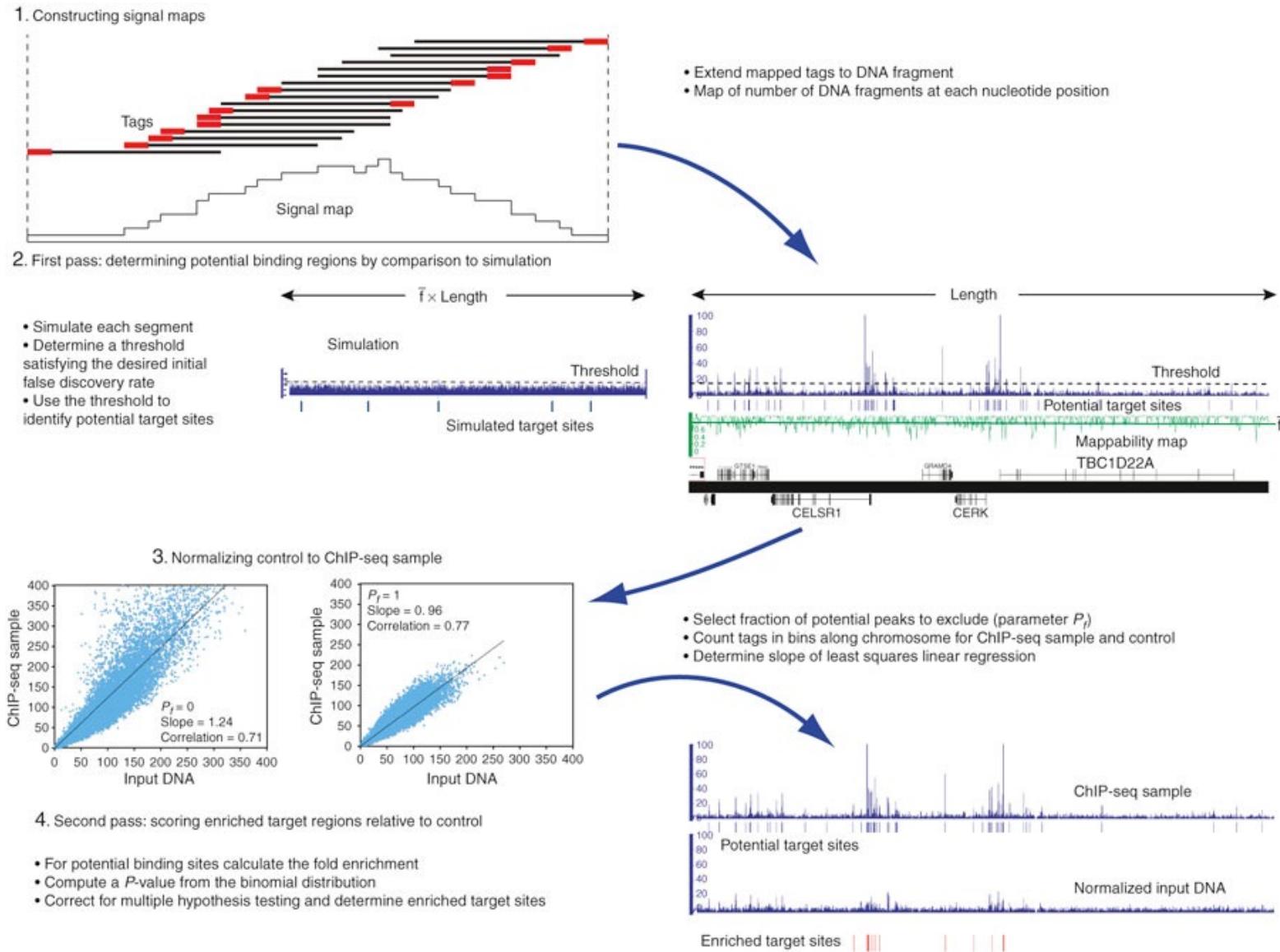
Type of modification	Histone							
	H3K4	H3K9	H3K14	H3K27	H3K79	H3K122	H4K20	H2BK5
mono-methylation	activation <sup>[6]</sup>	activation <sup>[7]</sup>		activation <sup>[7]</sup>	activation <sup>[7][8]</sup>		activation <sup>[7]</sup>	activation <sup>[7]</sup>
di-methylation	activation	repression <sup>[3]</sup>		repression <sup>[3]</sup>	activation <sup>[8]</sup>			
tri-methylation	activation <sup>[9]</sup>	repression <sup>[7]</sup>		repression <sup>[7]</sup>	activation, repression <sup>[7]</sup>			repression <sup>[3]</sup>
acetylation		activation <sup>[9]</sup>	activation <sup>[9]</sup>	activation <sup>[10]</sup>		activation <sup>[11]</sup>		

- H3K4me3 is enriched in transcriptionally active promoters.<sup>[12]</sup>
- H3K9me3 is found in constitutively repressed genes.
- H3K27me is found in facultatively repressed genes.<sup>[7]</sup>
- H3K36me3 is found in actively transcribed gene bodies.
- H3K9ac is found in actively transcribed promoters.
- H3K14ac is found in actively transcribed promoters.
- H3K27ac distinguishes active enhancers from poised enhancers.
- H3K122ac is enriched in poised promoters and also found in a different type of putative enhancer that lacks H3K27ac.

# General Flow of ChIP-seq Analysis



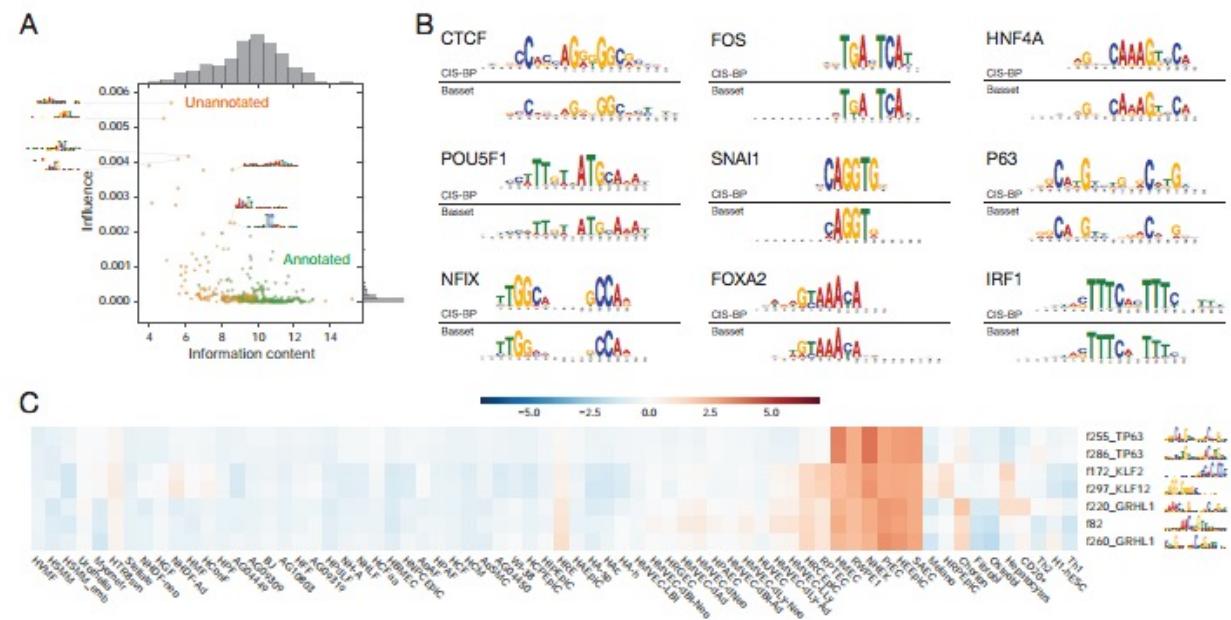
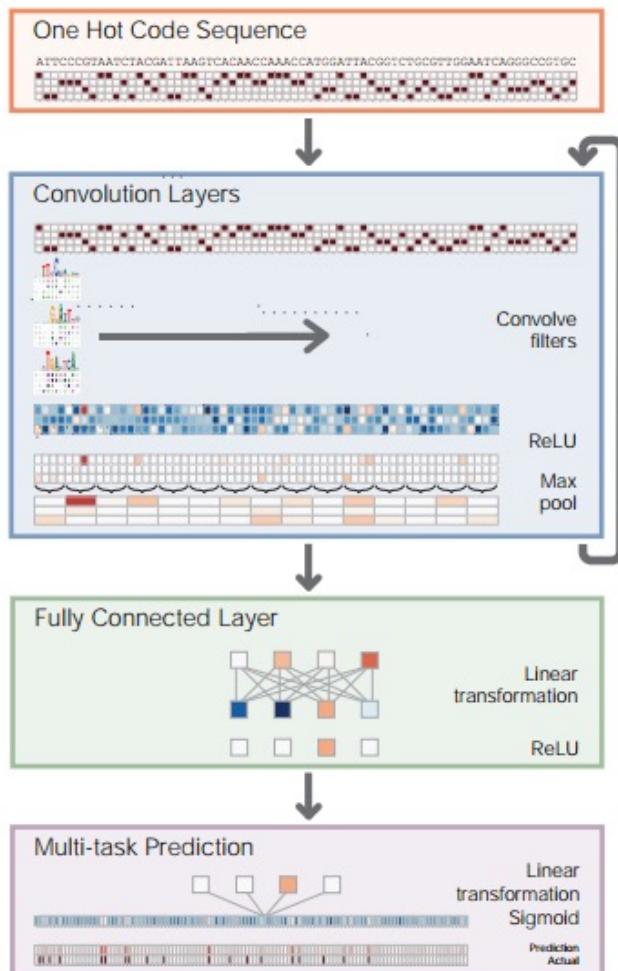
# PeakSeq



**PeakSeq enables systematic scoring of ChIP-seq experiments relative to controls**

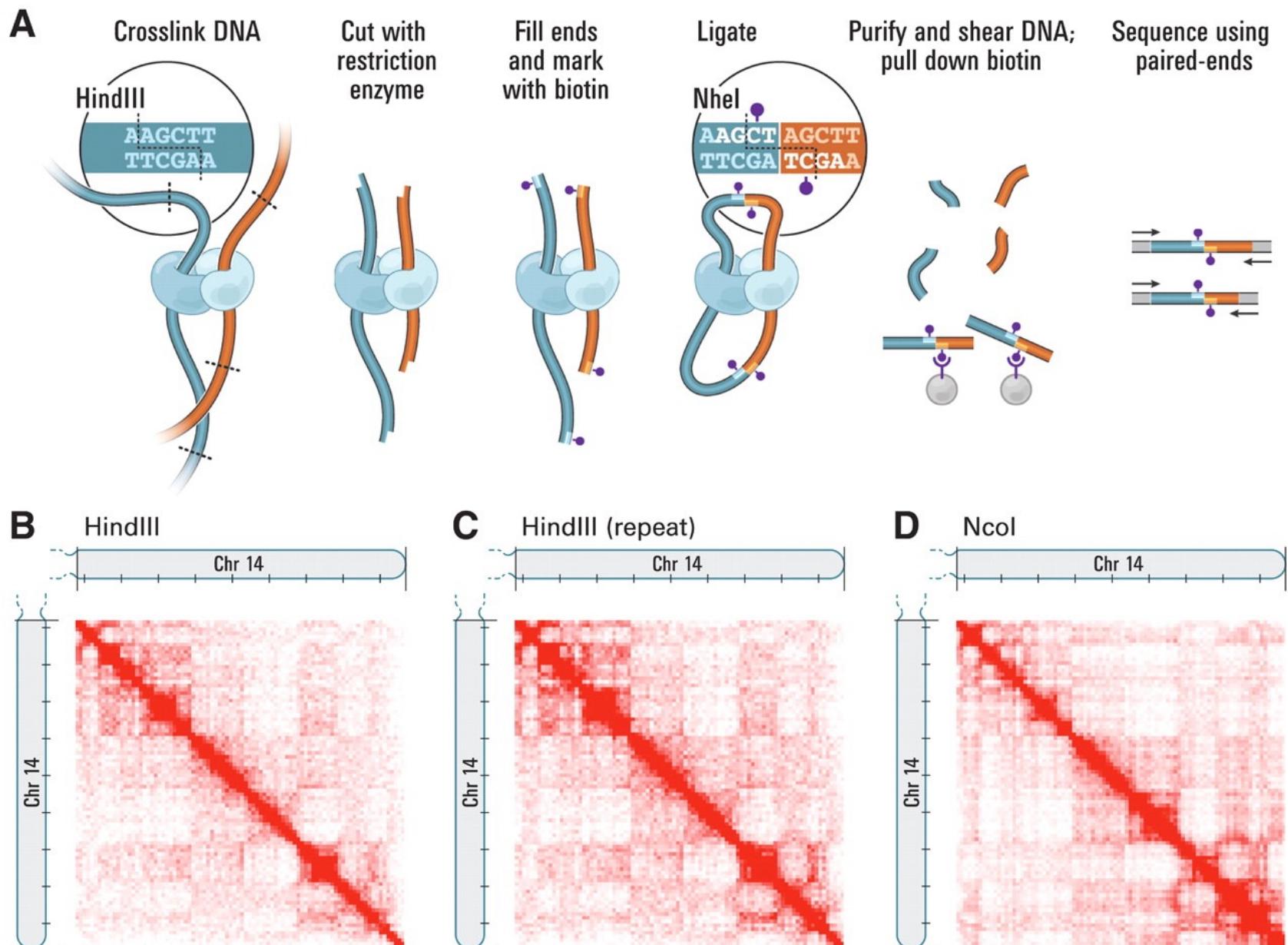
Rozowsky et al (2009) Nature Biotechnology 27, 66 - 75

# Basset



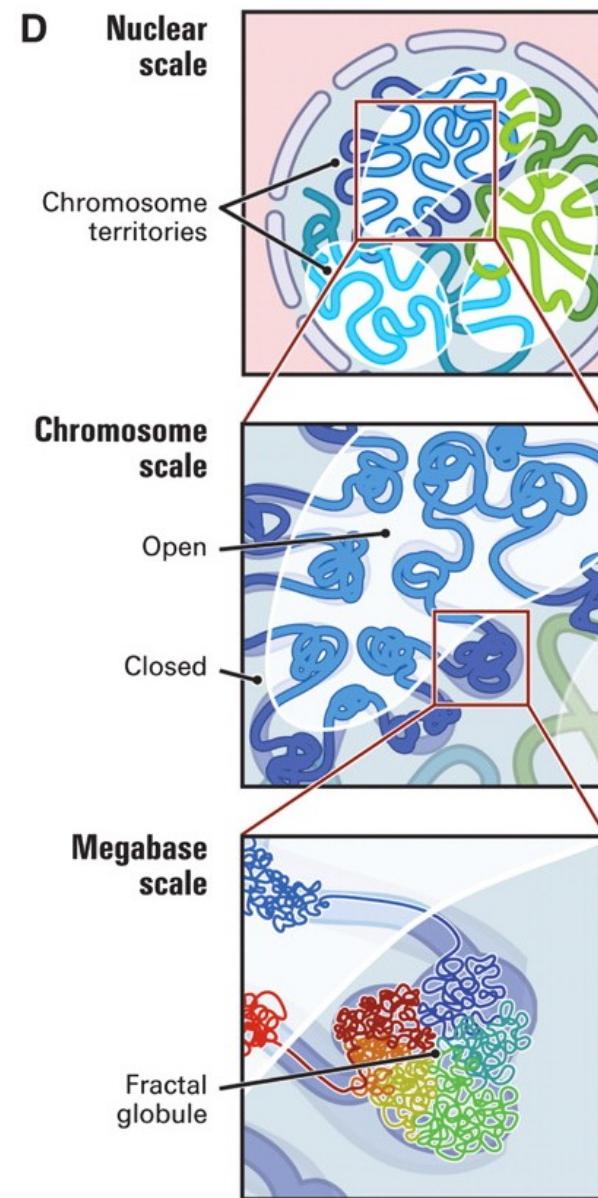
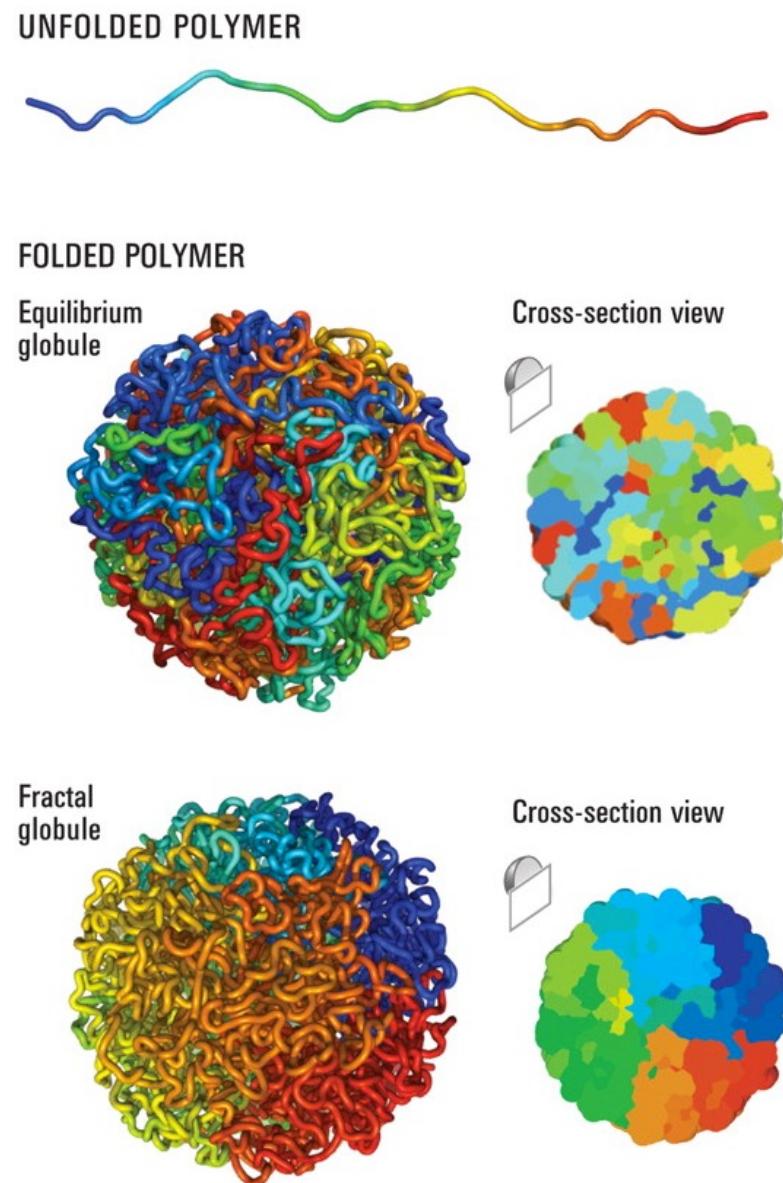
**Basset: Learning the regulatory code of the accessible genome with deep convolutional neural networks**  
Kelley et al. (2016) Genome Research doi: 10.1101/gr.200535.115

# Hi-C: Mapping the folding of DNA



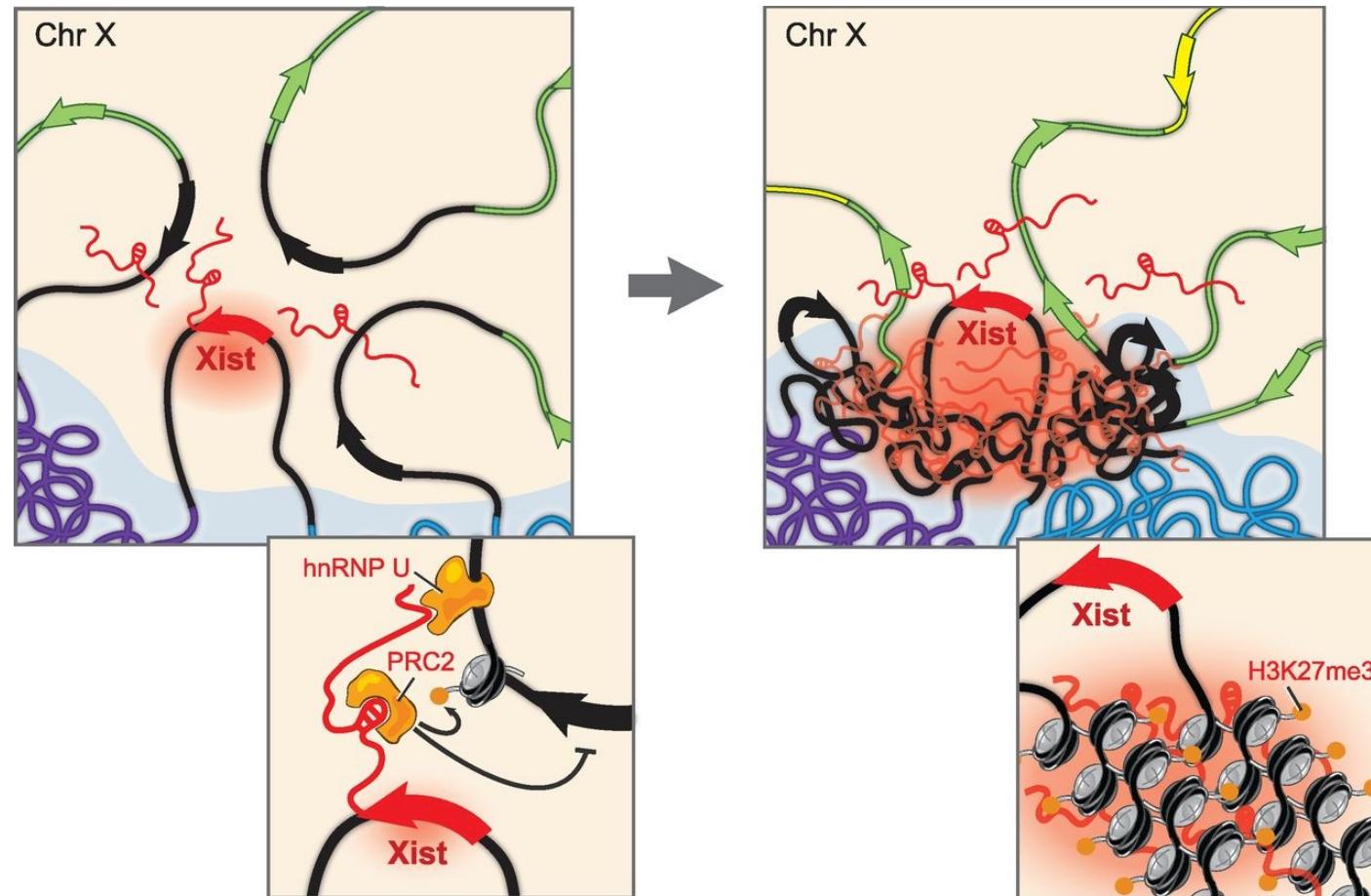
Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome  
Lieberman-Aiden et al. (2009) Science. 326 (5950): 289-293

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Lieberman-Aiden et al. (2009) Science. 326 (5950): 289-293

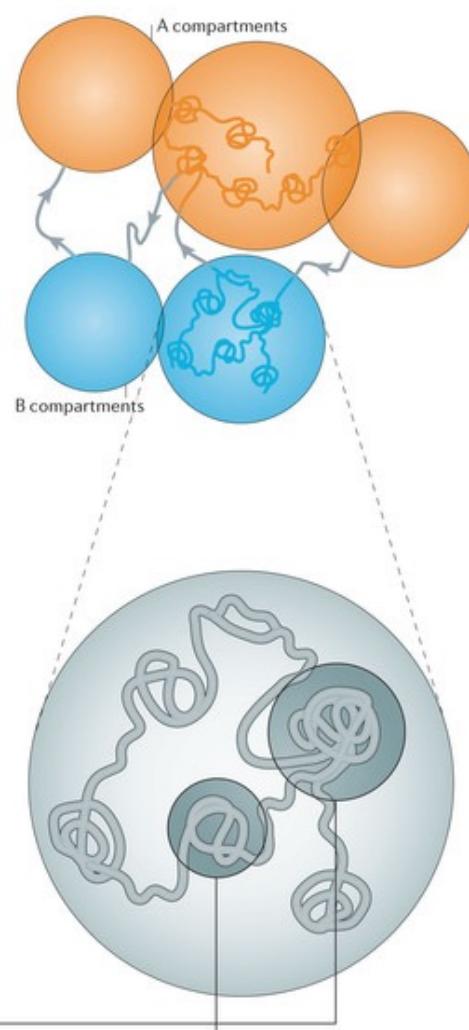
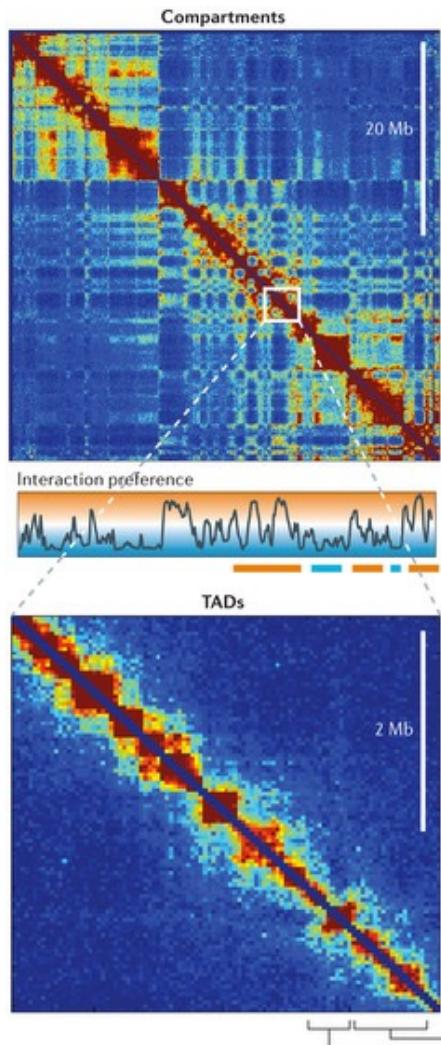
# Gene Regulation in 3-dimensions



**Fig 6. A model for how *Xist* exploits and alters three-dimensional genome architecture to spread across the *X* chromosome.**

The *Xist* lncRNA Exploits Three-Dimensional Genome Architecture to Spread Across the *X* Chromosome  
Engreitz et al. (2013) Science. 341 (6147)

# Genome compartments & TADs



**Mammalian genomes have a pattern of interactions that can be approximated by two compartments called A and B**

- alternate along chromosomes and have a characteristic size of ~5 Mb each.
- A compartments (orange) preferentially interact with other A compartments; B compartments (blue) associate with other B compartments.
- A compartments are largely euchromatic, transcriptionally active regions.

**Topologically associating domains (TADs)**

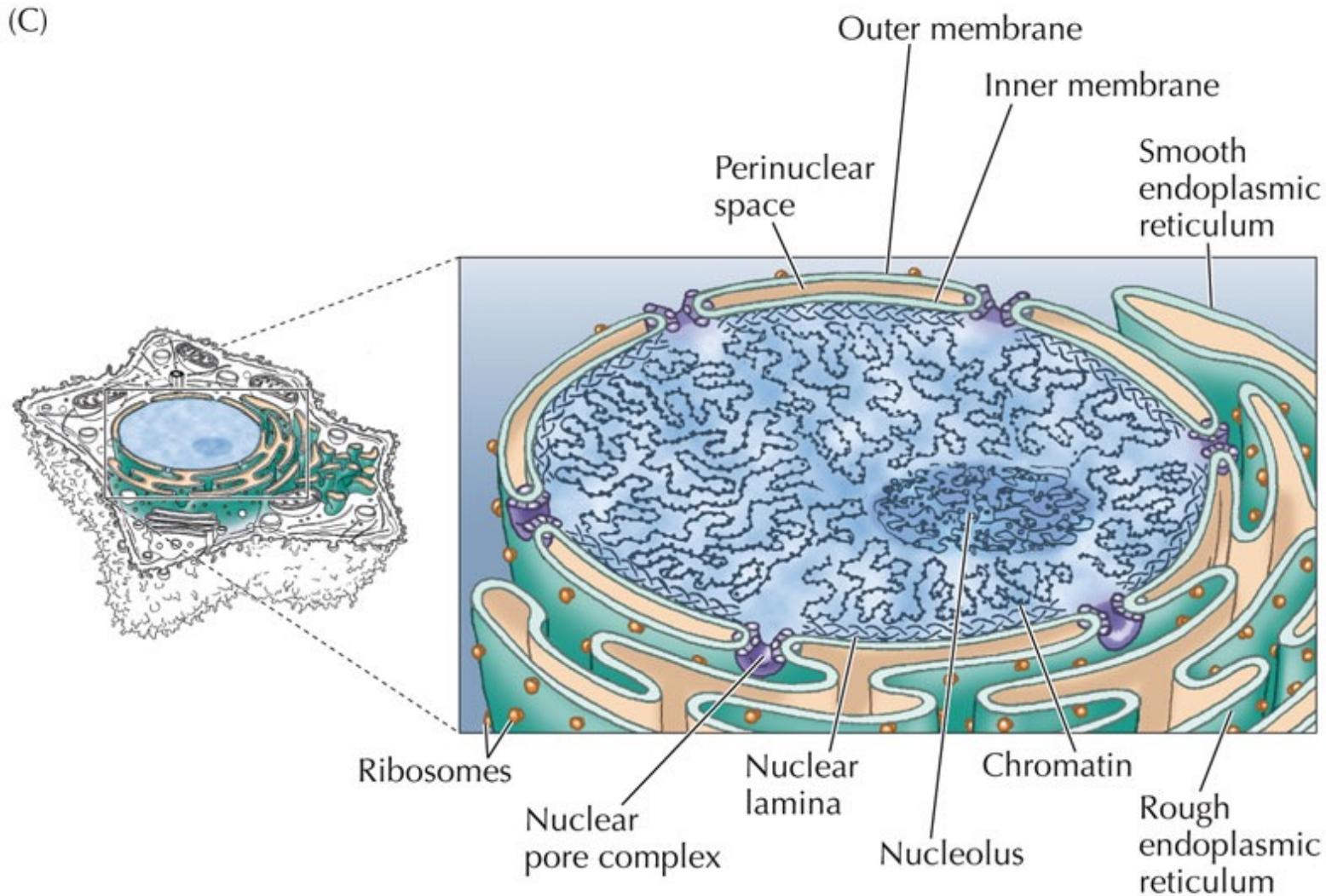
- TADs are smaller (~400–500 kb)
- Can be active or inactive, and adjacent TADs are not necessarily of opposite chromatin status.
- TADs are hard-wired features of chromosomes, and groups of adjacent TADs can organize in A and B compartments

Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data

Dekker et al. (2013) *Nature Reviews Genetics* 14, 390–403

Nature Reviews | Genetics

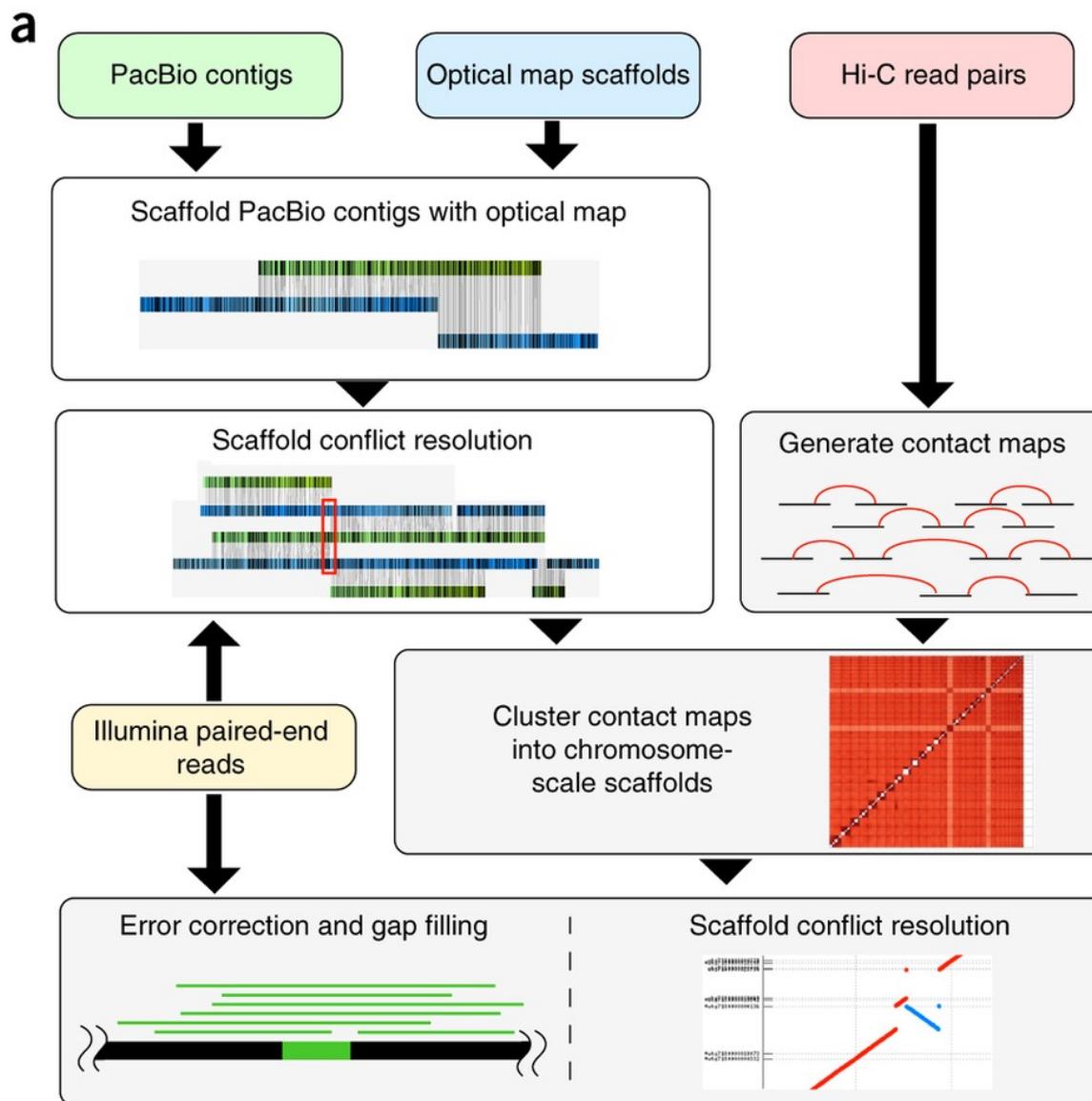
# “Lamina-Associated Domains are the B compartment”



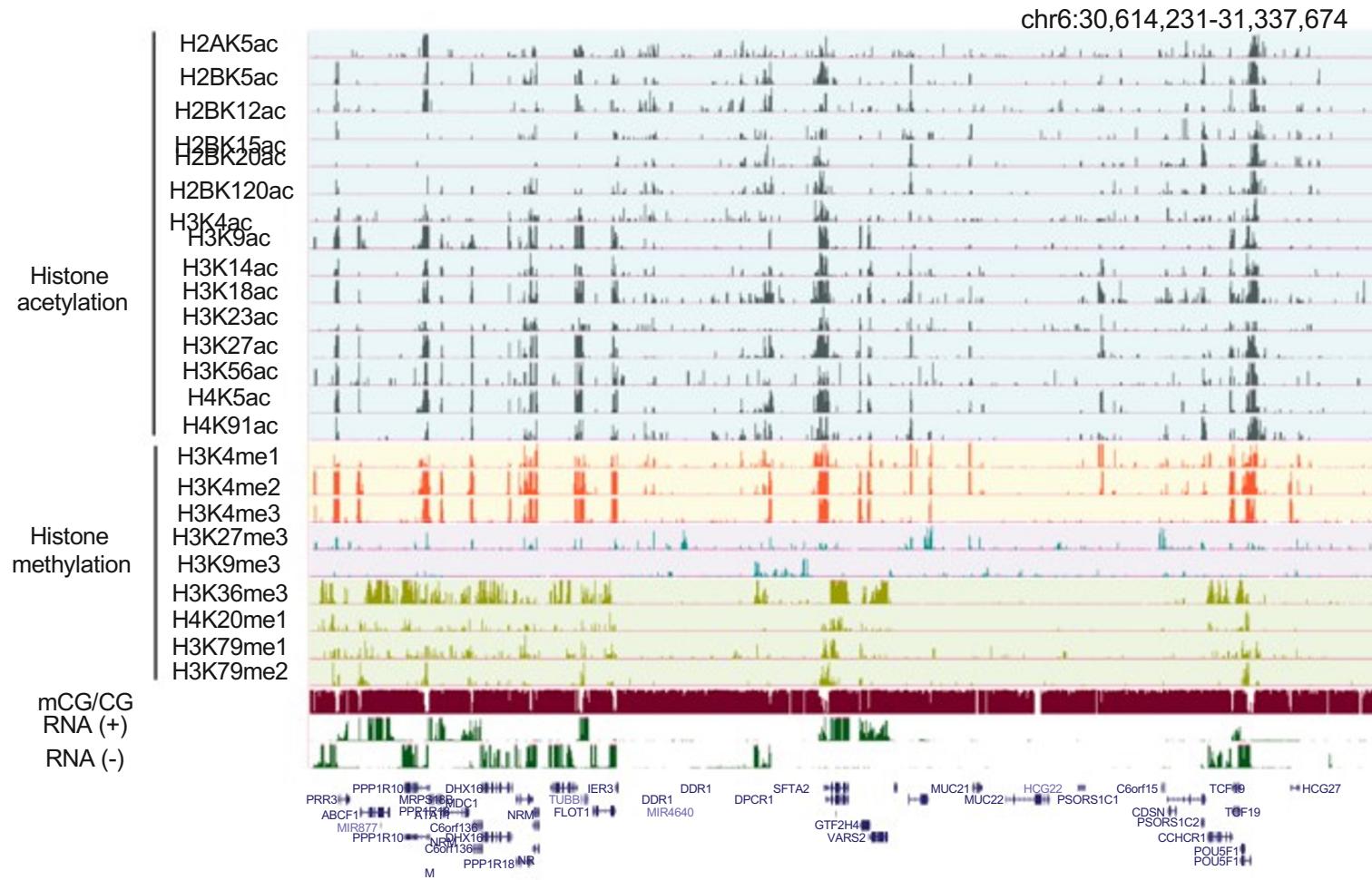
*THE CELL, Fourth Edition, Figure 9.1 (Part 3) © 2006 ASM Press and Sinauer Associates, Inc.*

**Chromosome Conformation Paints Reveal the Role of Lamina Association in Genome Organization and Regulation**  
Luperchio et al. (2017) bioRxiv. doi: <https://doi.org/10.1101/122226>

# Scaffolding with Hi-C

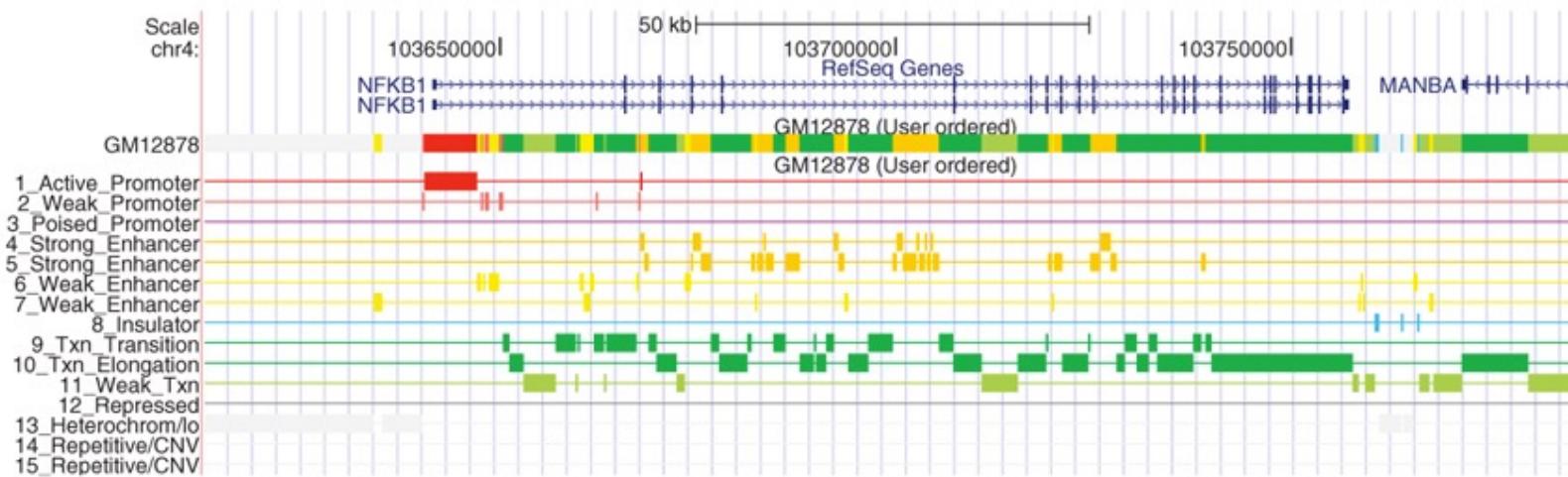


# We can call peaks, but...



*We need a way to summarize the combinatorial patterns of multiple histone marks into meaningful biological units*

# ChromHMM



***ChromHMM is software for learning and characterizing chromatin states.***

- ChromHMM can integrate multiple chromatin datasets such as ChIP-seq data of various histone modifications to discover de novo the major re-occurring combinatorial and spatial patterns of marks.
- ChromHMM is based on a multivariate Hidden Markov Model that explicitly models the presence or absence of each chromatin mark.
- The resulting model can then be used to systematically annotate a genome in one or more cell types.

**ChromHMM: automating chromatin-state discovery and characterization**

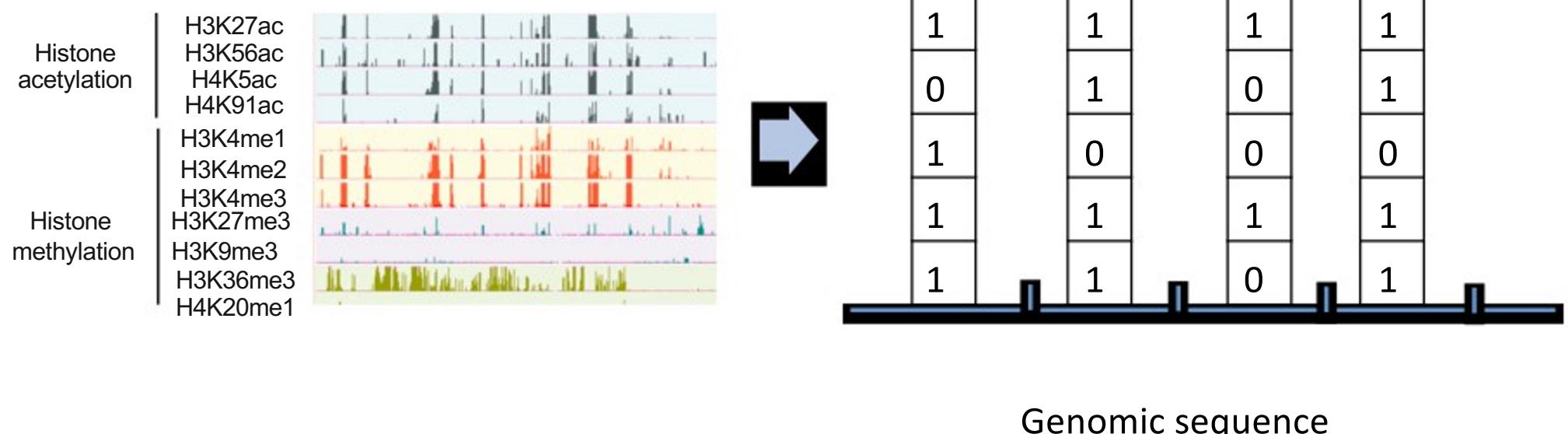
Ernst & Kellis (2012) Nature Methods 9, 215–216. doi:10.1038/nmeth.1906

# The Workflow

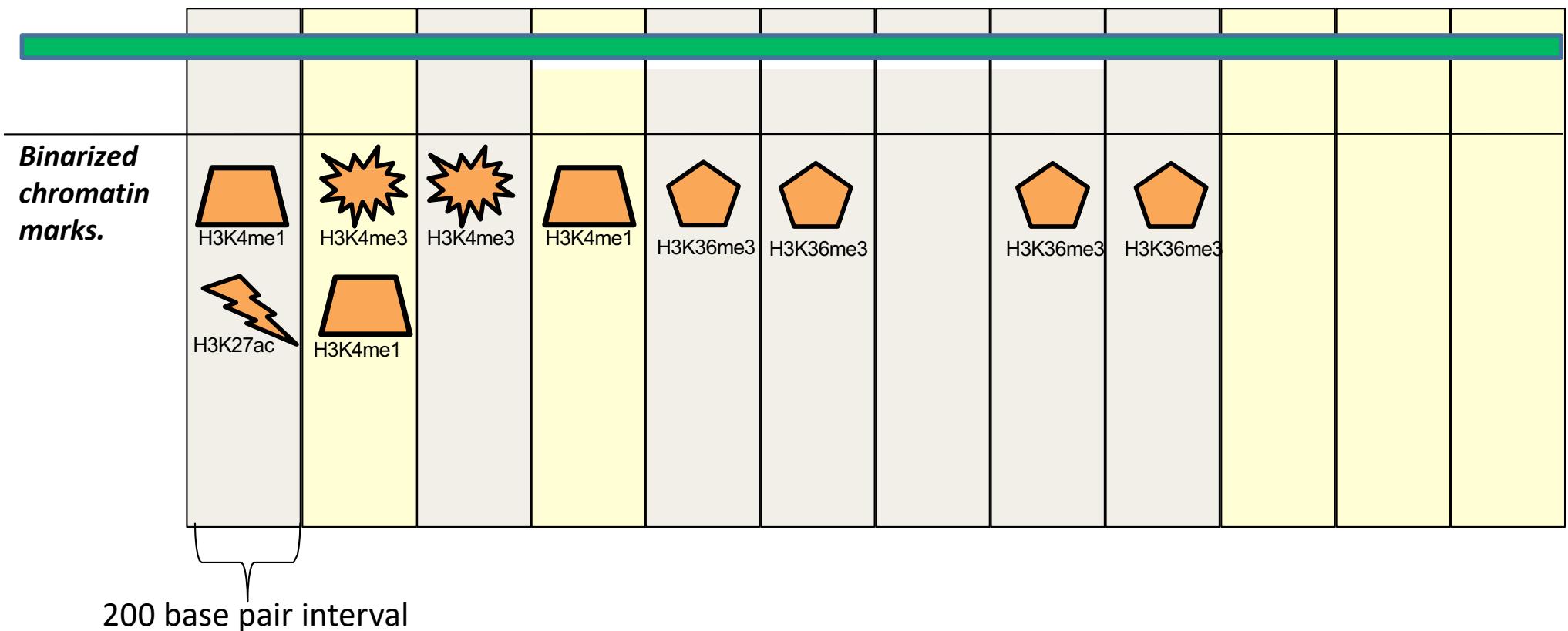
1. Get ChIP-seq raw reads for different histone modifications
2. Align the reads to a reference genome
3. Convert aligned reads in bed format
4. Create Binned and Binarized Tracks
5. Train the model
6. Infer the states
7. Interpretation

# Create Binned and Binarized Tracks

- ChromHMM quantify the presence or absence of each mark in bins of fixed size



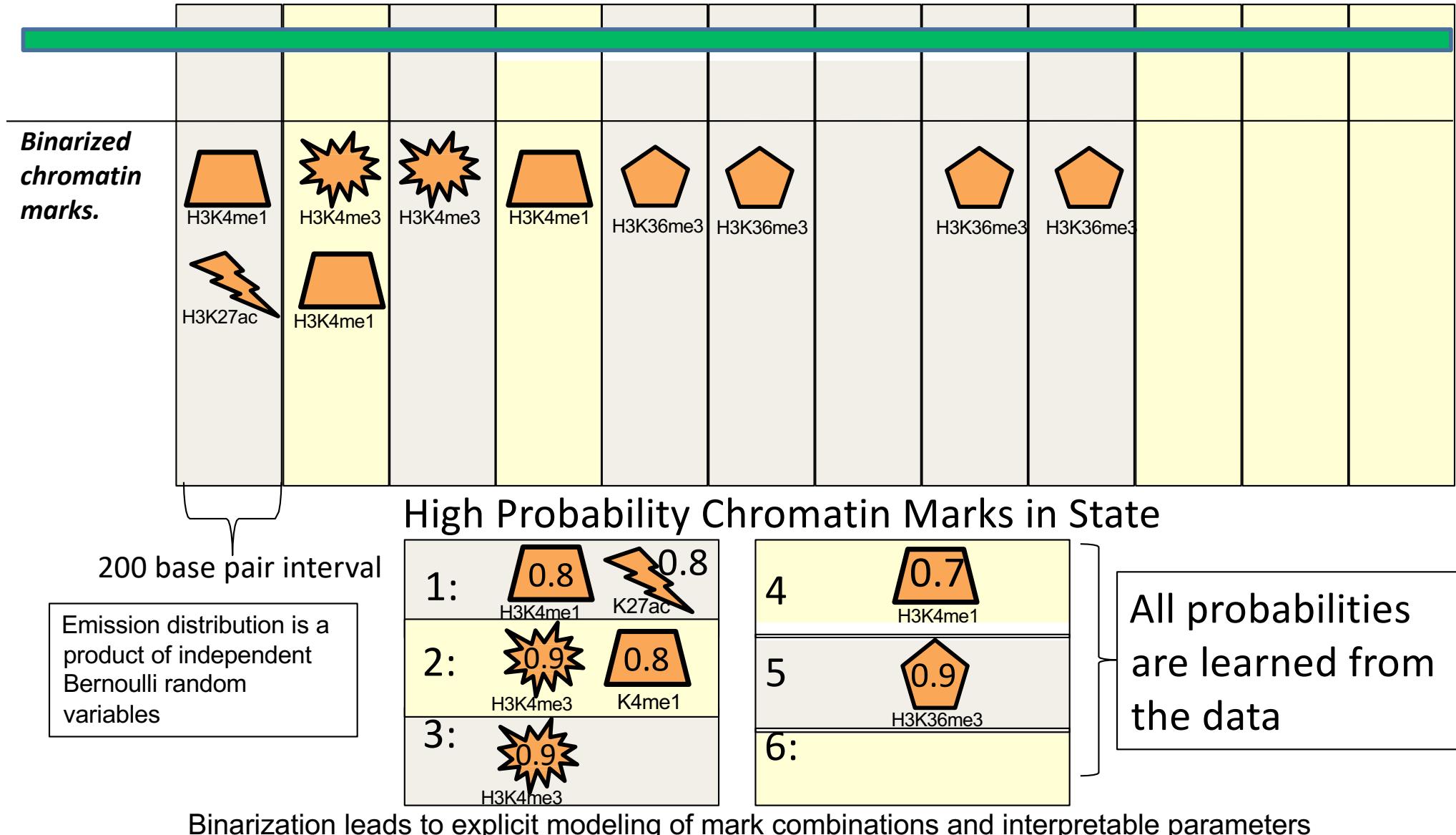
# ChromHMM : Multivariate Hidden Markov Model



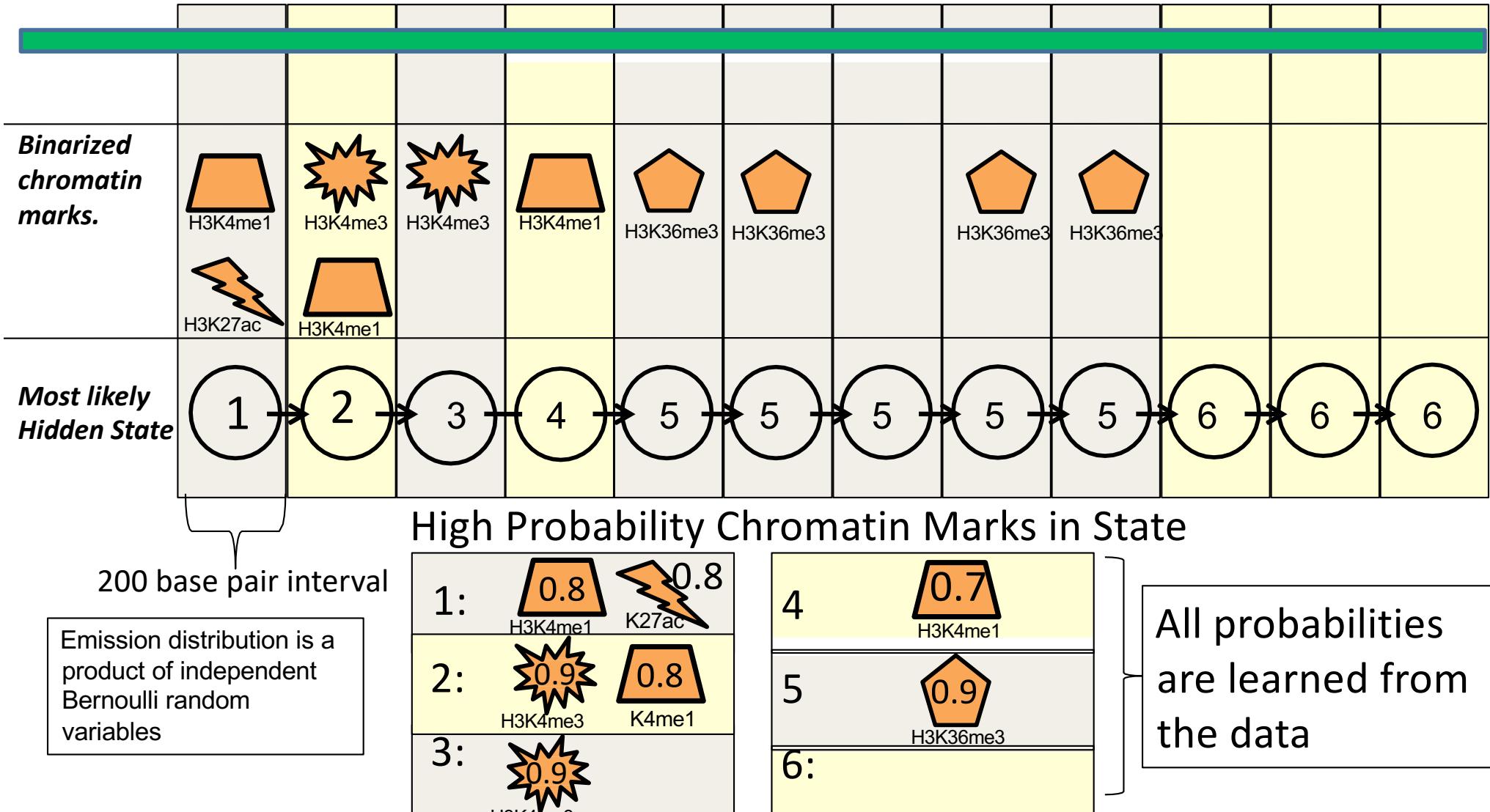
Emission distribution is a product of independent Bernoulli random variables

Binarization leads to explicit modeling of mark combinations and interpretable parameters

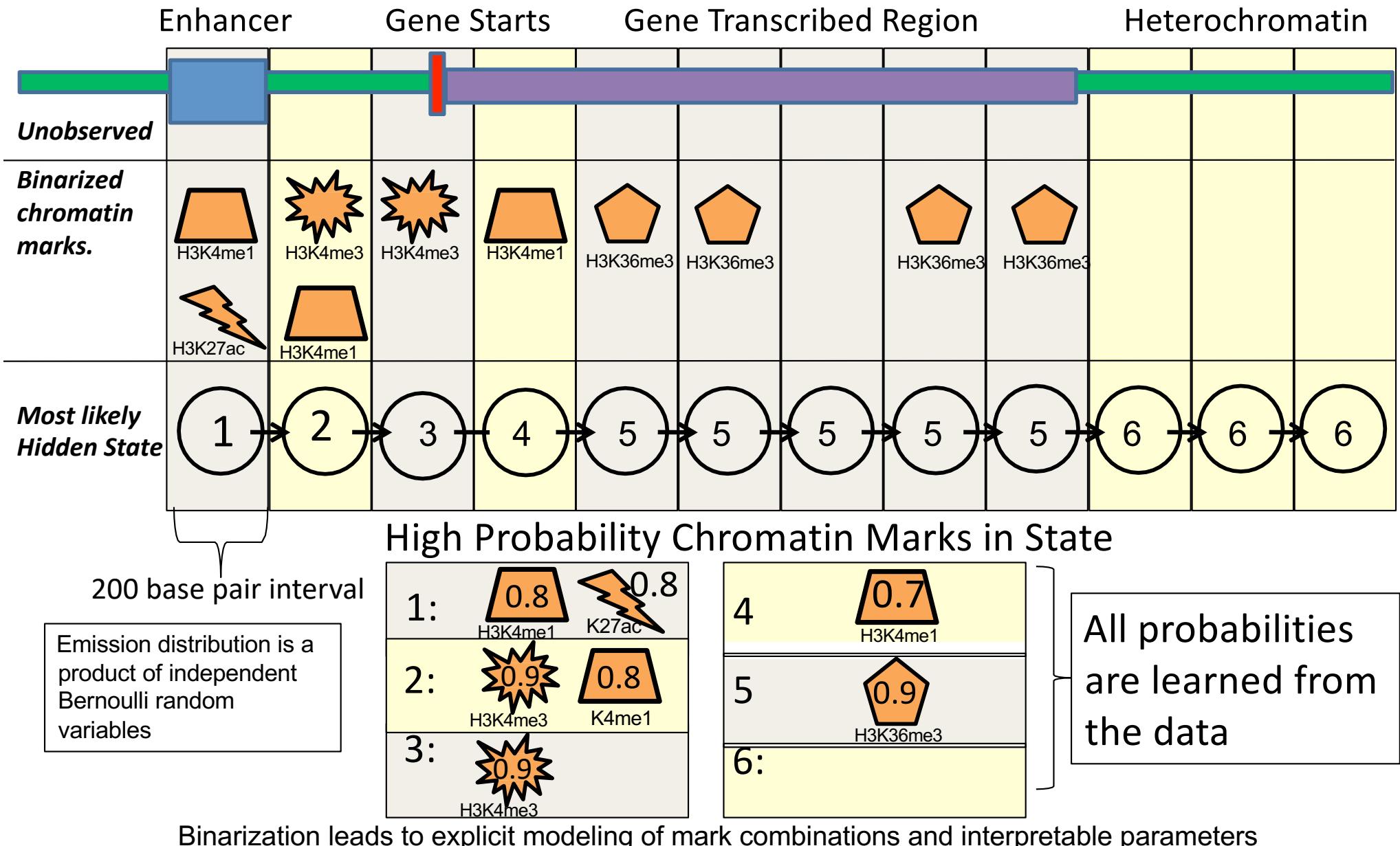
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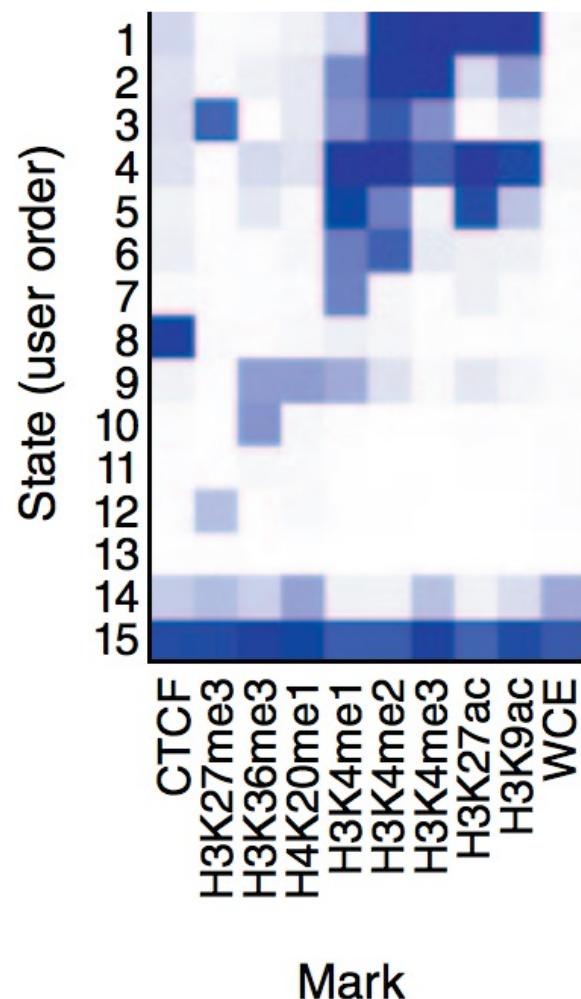
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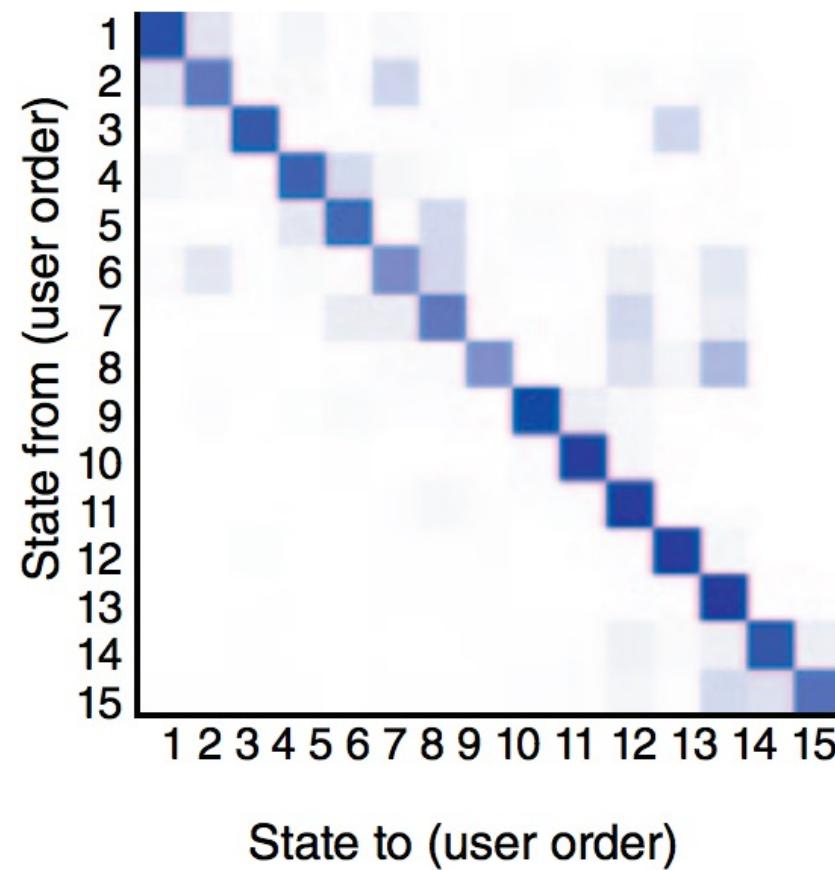
Ernst and Kellis, Nat Biotech 2010 : Ernst and Kellis, Nature Methods 2012

# Transition and Emission Parameters

Emission parameters



Transition parameters



# Enriched functional category

b

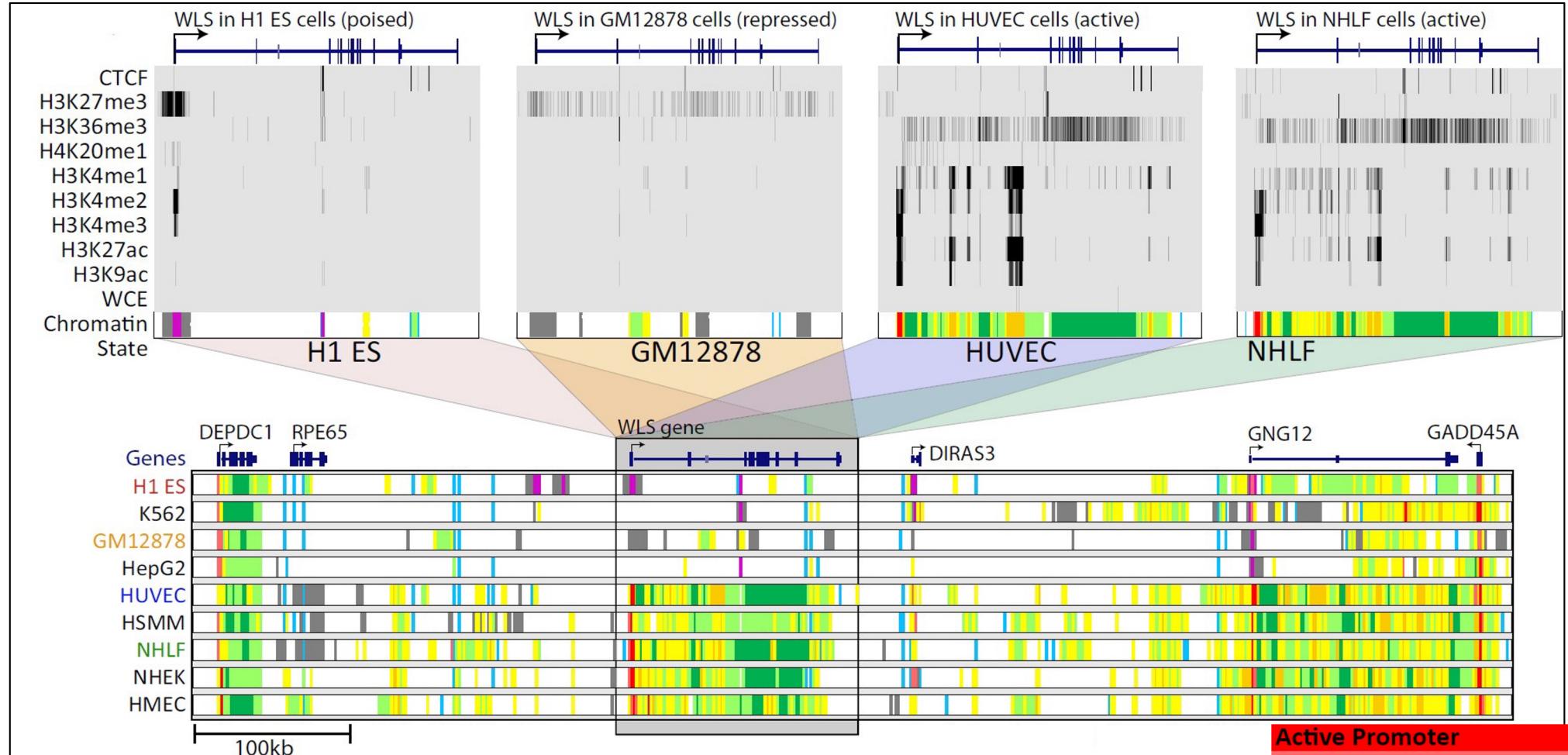
State	CTCF	H3K27me3	H3K36me3	H4K20me1	H3K4me1	H3K4me2	H3K4me3	H3K27ac	H3K9ac	WCE	Coverage			±2 kb TSS	Conserved non-exon	DNase (K562)	c-Myc (K562)	NF-κB (GM12878)	Transcript	Nuclear lamina (NHLF)	Candidate state annotation	
											Median	H1 ES	GM									
1	16	2	2	6	17	93	99	96	98	2	0.6	0.5	1.2	1.0	83	3.8	23.3	82.0	40.7	0.2	0.15	Active promoter
2	12	2	6	9	53	94	95	14	44	1	0.5	1.2	1.3	0.4	58	2.8	15.3	12.6	5.8	0.6	0.30	Weak promoter
3	13	72	0	9	48	78	49	1	10	1	0.2	4.0	1.0	0.6	49	4.3	10.8	3.1	1.0	0.4	0.68	Inactive/poised promoter
4	11	1	15	11	96	99	75	97	86	4	0.7	0.1	1.1	0.6	23	2.7	23.1	31.8	49.0	1.3	0.05	Strong enhancer
5	5	0	10	3	88	57	5	84	25	1	1.2	0.2	0.7	0.6	3	1.8	13.6	6.3	15.8	1.4	0.10	Strong enhancer
6	7	1	1	3	58	75	8	6	5	1	0.9	1.3	1.0	0.2	17	2.4	11.9	5.7	7.0	1.1	0.31	Weak/poised enhancer
7	2	1	2	1	56	3	0	6	2	1	1.9	1.2	1.1	0.4	4	1.5	5.1	0.6	2.4	1.3	0.20	Weak/poised enhancer
8	92	2	1	3	6	3	0	0	1	1	0.5	1.4	1.0	0.4	3	1.5	12.8	2.5	1.2	1.1	0.61	Insulator
9	5	0	43	43	37	11	2	9	4	1	0.7	1.3	1.0	0.8	4	1.1	4.5	0.7	0.8	2.4	0.02	Transcriptional transition
10	1	0	47	3	0	0	0	0	0	1	4.3	0.6	1.2	3.0	1	0.9	0.3	0.0	0.0	2.5	0.11	Transcriptional elongation
11	0	0	3	2	0	0	0	0	0	0	12.5	1.3	0.8	2.6	2	0.9	0.3	0.0	0.1	1.9	0.24	Weak transcribed
12	1	27	0	2	0	0	0	0	0	0	4.1	0.3	0.7	2.8	5	1.4	0.3	0.0	0.1	0.8	0.63	Polycomb repressed
13	0	0	0	0	0	0	0	0	0	0	71.4	1.0	1.0	10.0	1	0.9	0.1	0.0	0.0	0.7	1.30	Heterochrom; low signal
14	22	28	19	41	6	5	26	5	13	37	0.1	0.9	1.2	0.6	3	0.4	1.9	0.3	0.2	0.4	1.44	Repetitive/CNV
15	85	85	91	88	76	77	91	73	85	78	0.1	0.9	1.0	0.2	1	0.2	5.9	9.5	7.4	0.4	1.30	Repetitive/CNV

The states predicted by the HMM are **statistical** entities (#1 – #15)

The states we want are **biological** entities (Active/Weak/Poised promoter)

Investigate the properties of the statistical entities to label them with biological functions  
=> Supervised learning problem ☺

# Chromatin states dynamics across nine cell types



- Single annotation track for each cell type
- Summarize cell-type activity at a glance
- Can study 9-cell activity pattern across ↓

# ARTICLE

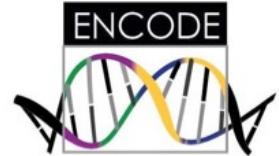
doi:10.1038/nature11247

# An integrated encyclopedia of DNA elements in the human genome

The ENCODE Project Consortium\*

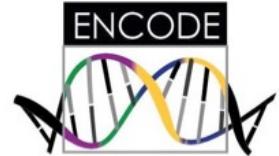
The human genome encodes the blueprint of life, but the function of the vast majority of its nearly three billion bases is unknown. The Encyclopedia of DNA Elements (ENCODE) project has systematically mapped regions of transcription, transcription factor association, chromatin structure and histone modification. These data enabled us to assign biochemical functions for 80% of the genome, in particular outside of the well-studied protein-coding regions. Many discovered candidate regulatory elements are physically associated with one another and with expressed genes, providing new insights into the mechanisms of gene regulation. The newly identified elements also show a statistical correspondence to sequence variants linked to human disease, and can thereby guide interpretation of this variation. Overall, the project provides new insights into the organization and regulation of our genes and genome, and is an expansive resource of functional annotations for biomedical research.

# Major Findings



1. *The vast majority (80.4%) of the human genome participates in at least one biochemical RNA- and/or chromatin-associated event in at least one cell type.*
2. *Primate-specific elements as well as elements without detectable mammalian constraint show, in aggregate, evidence of negative selection; thus, some of them are expected to be functional.*
3. *Classifying the genome into seven chromatin states indicates an initial set of 399,124 regions with enhancer-like features and 70,292 regions with promoter-like features, as well as hundreds of thousands of quiescent regions. High-resolution analyses further subdivide the genome into thousands of narrow states with distinct functional properties.*
4. *It is possible to correlate quantitatively RNA sequence production and processing with both chromatin marks and transcription factor binding at promoters, indicating that promoter functionality can explain most of the variation in RNA expression.*
5. *Many non-coding variants in individual genome sequences lie in ENCODE-annotated functional regions; this number is at least as large as those that lie in protein-coding genes.*
6. *Single nucleotide polymorphisms (SNPs) associated with disease by GWAS are enriched within non-coding functional elements, with a majority residing in or near ENCODE-defined regions that are outside of protein-coding genes. In many cases, the disease phenotypes can be associated with a specific cell type or transcription factor.*

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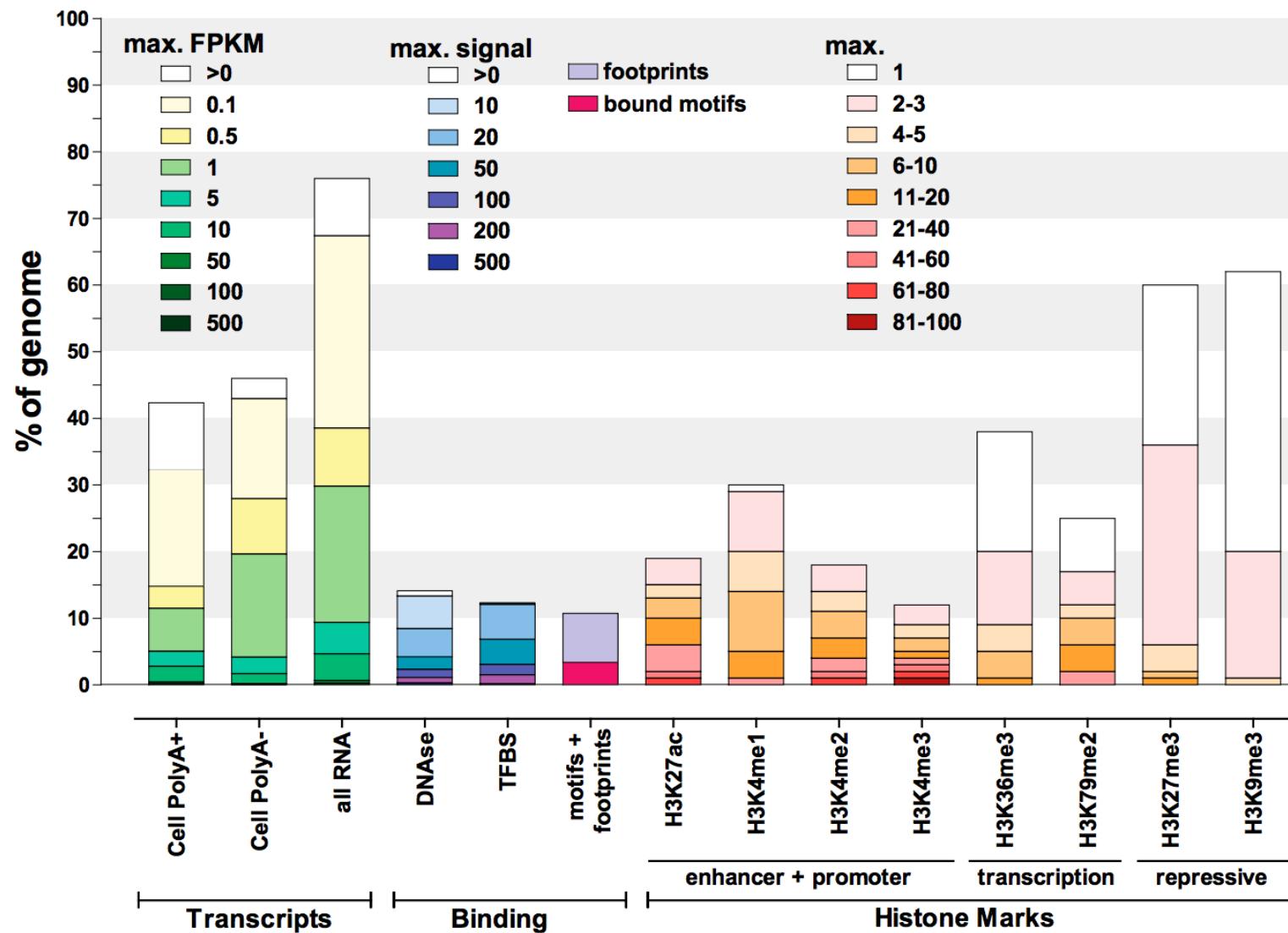
# Summary of ENCODE elements

*“Accounting for all these elements, a surprisingly large amount of the human genome, 80.4%, is covered by at least one ENCODE-identified element”*

- 62% transcribed
- 56% enriched for histone marks
- 15% open chromatin
- 8% TF binding
- 19% At least one DHS or TF Chip-seq peak
- 4% TF binding site motif
- (Note protein coding genes comprise ~2.94% of the genome)

*“Given that the ENCODE project did not assay all cell types, or all transcription factors, and in particular has sampled few specialized or developmentally restricted cell lineages, **these proportions must be underestimates of the total amount of functional bases.**”*

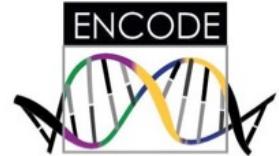
# Pervasive Transcription and Regulation



**Defining functional DNA elements in the human genome**

Kellis et al (2014). PNAS 6131–6138, doi: 10.1073/pnas.1318948111

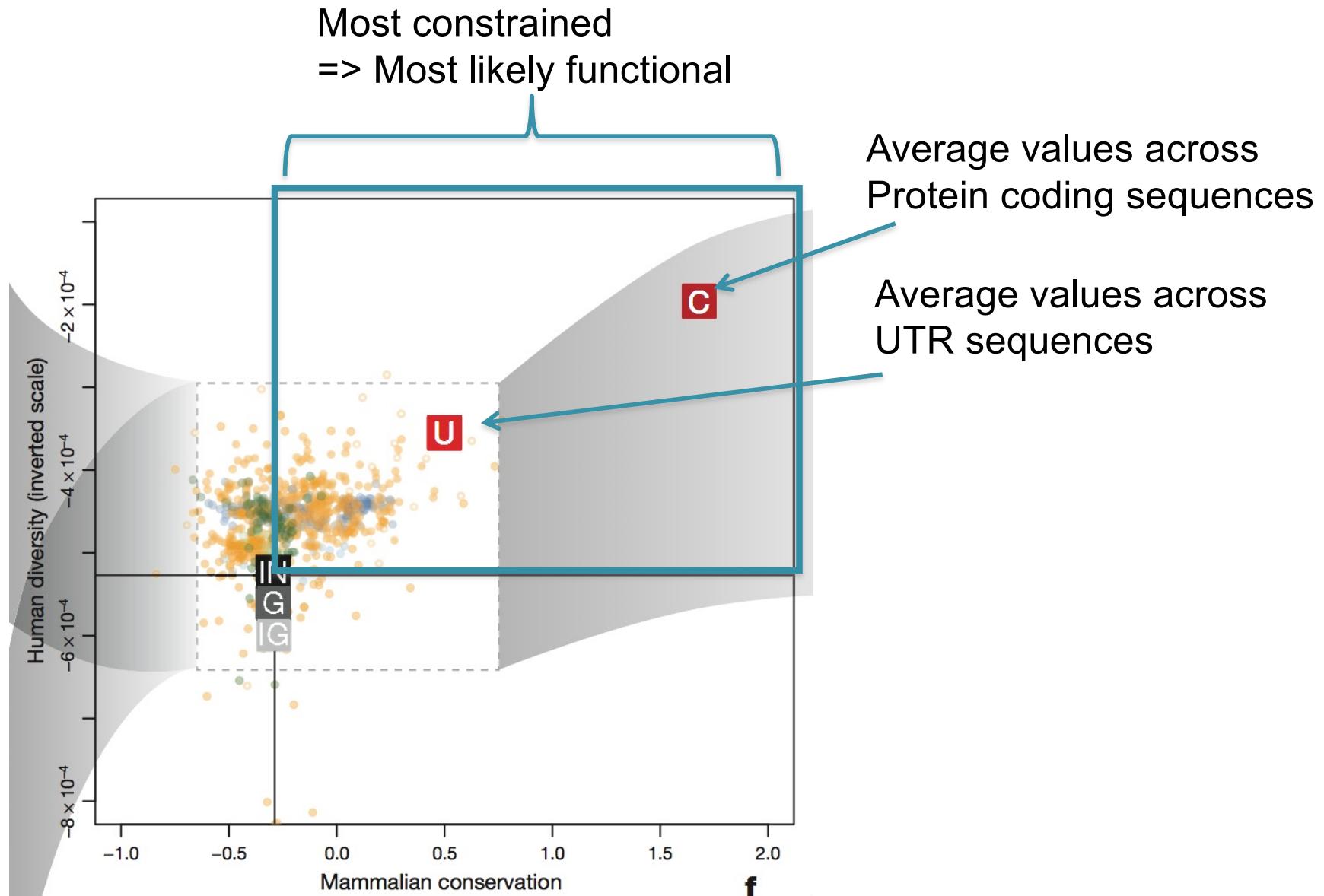
# Major Findings



1. *The vast majority (80.4%) of the human genome participates in at least one biochemical RNA- and/or chromatin-associated event in at least one cell type.*
2. ***Primate-specific elements as well as elements without detectable mammalian constraint show, in aggregate, evidence of negative selection; thus, some of them are expected to be functional.***
3. *Classifying the genome into seven chromatin states indicates an initial set of 399,124 regions with enhancer-like features and 70,292 regions with promoter-like features, as well as hundreds of thousands of quiescent regions. High-resolution analyses further subdivide the genome into thousands of narrow states with distinct functional properties.*
4. *It is possible to correlate quantitatively RNA sequence production and processing with both chromatin marks and transcription factor binding at promoters, indicating that promoter functionality can explain most of the variation in RNA expression.*
5. *Many non-coding variants in individual genome sequences lie in ENCODE-annotated functional regions; this number is at least as large as those that lie in protein-coding genes.*
6. *Single nucleotide polymorphisms (SNPs) associated with disease by GWAS are enriched within non-coding functional elements, with a majority residing in or near ENCODE-defined regions that are outside of protein-coding genes. In many cases, the disease phenotypes can be associated with a specific cell type or transcription factor.*

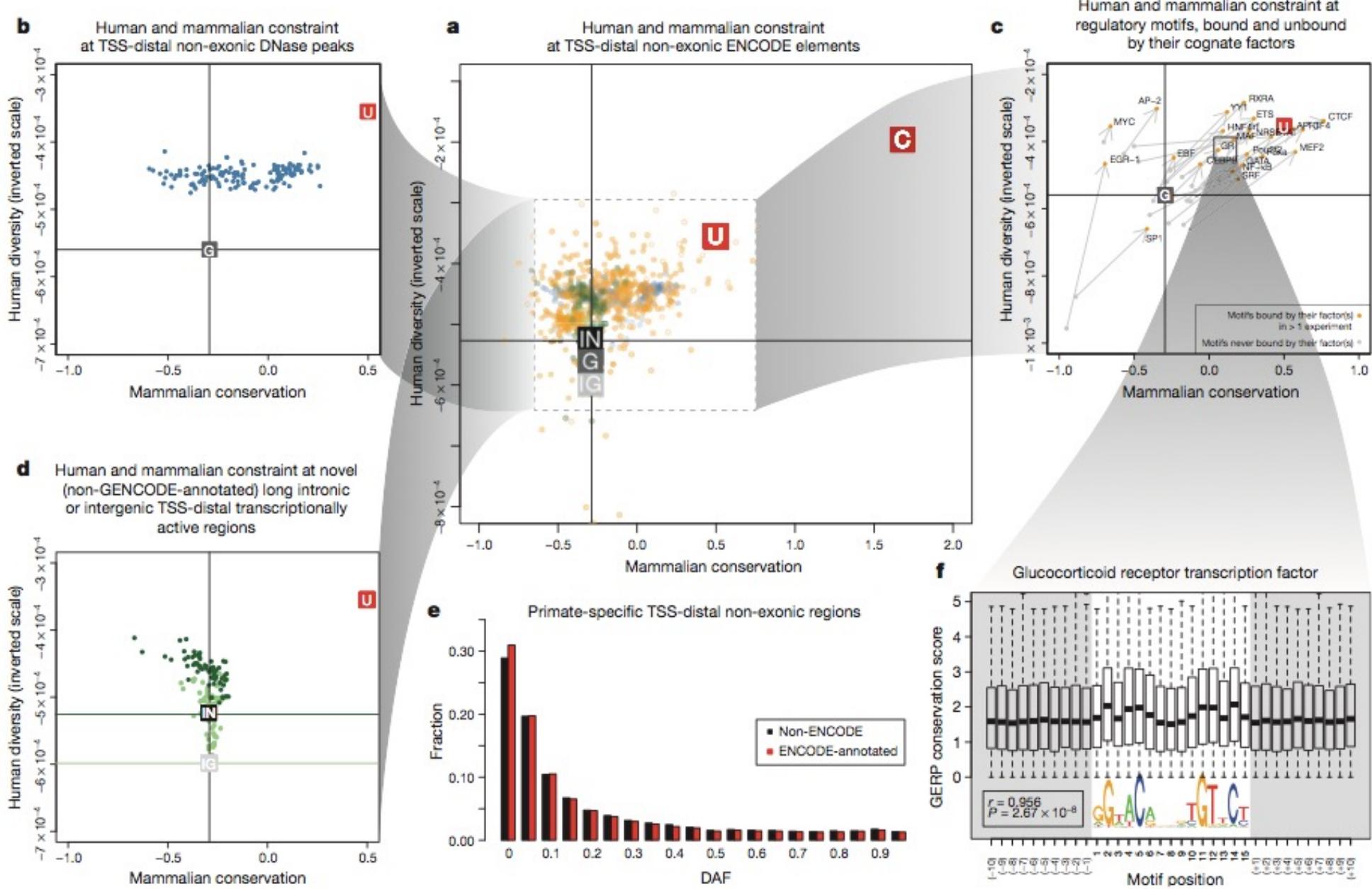
# Impact and Evidence of Selection

For a given ENCODE region, how much conservation do we see across modern humans (1000 genomes project)

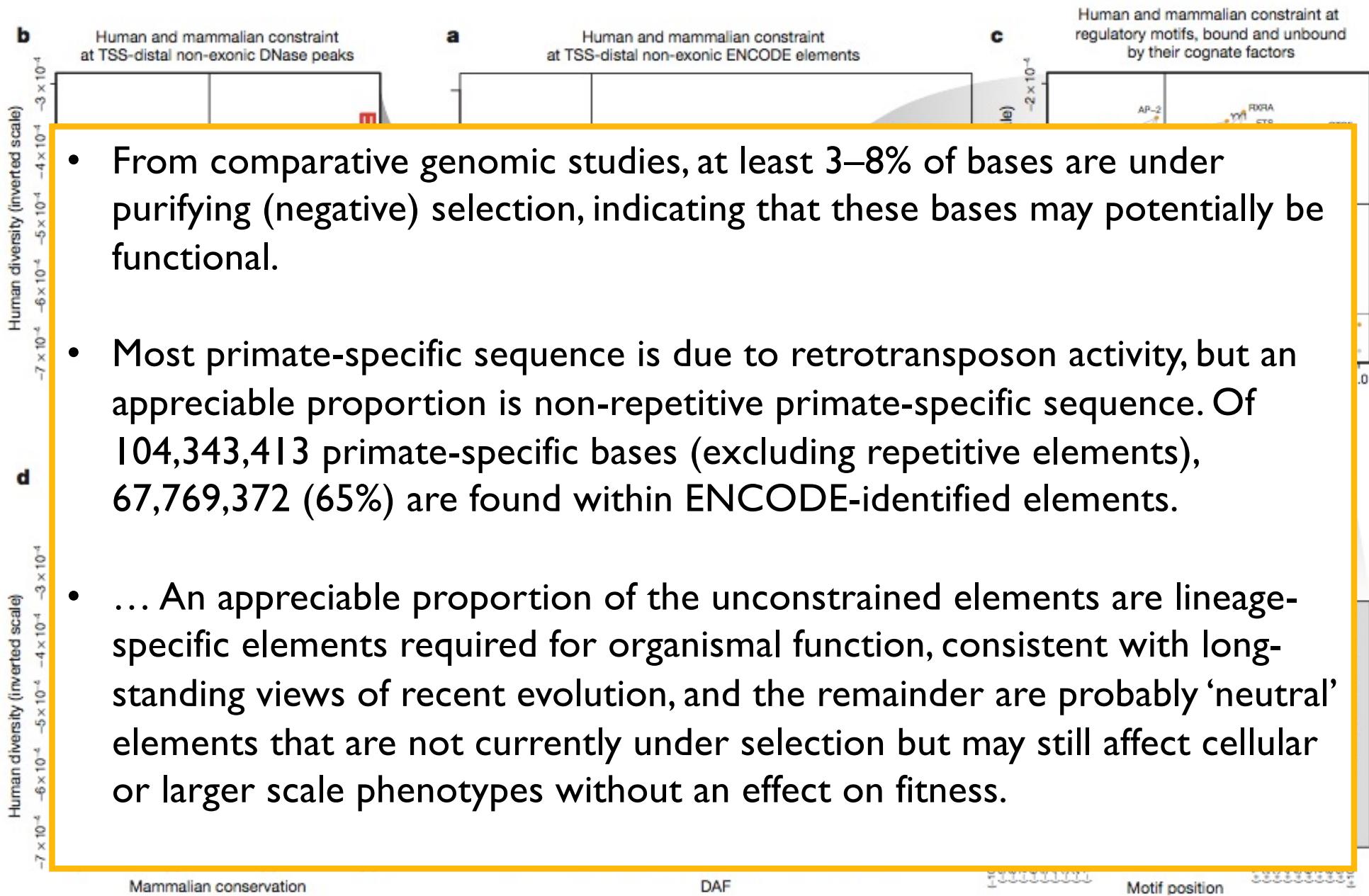


For a given ENCODE region, how much conservation do we see across 24 sequenced mammalian genomes?

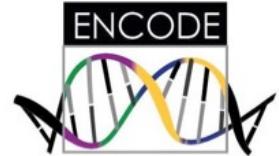
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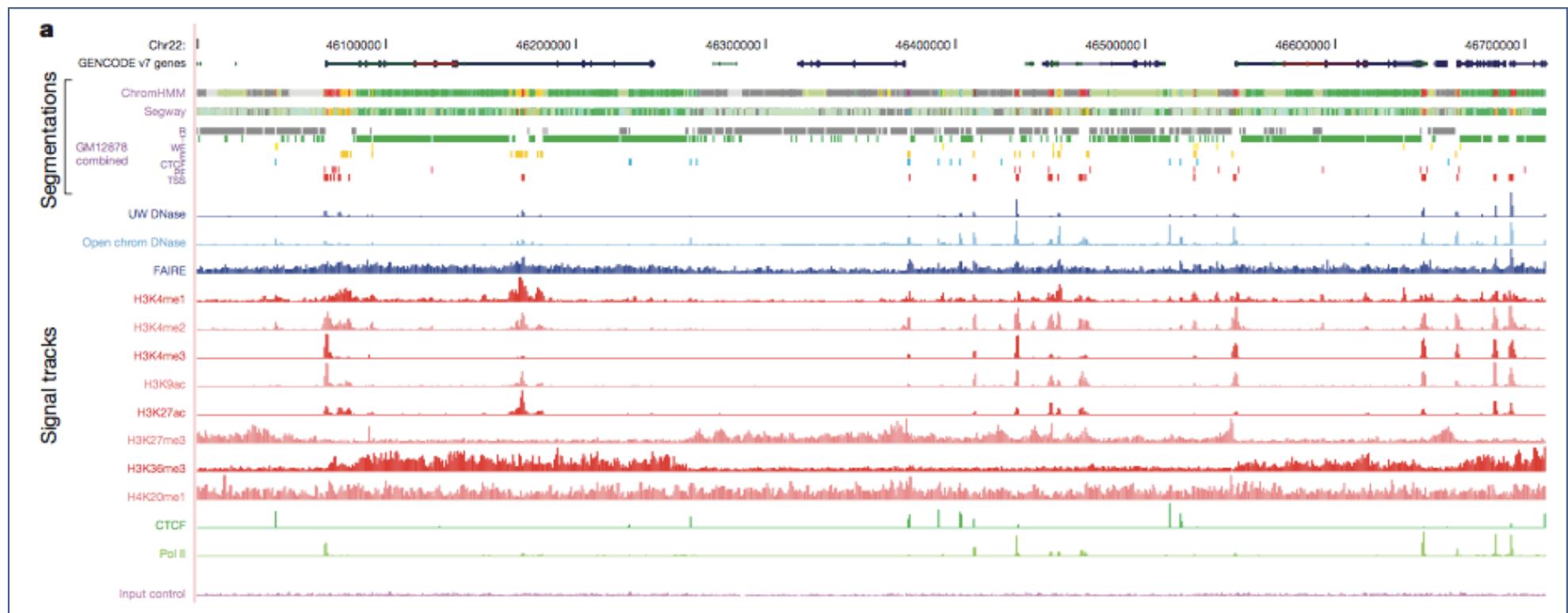


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# Signal Integration

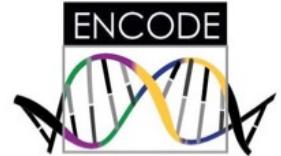


**Table 3 | Summary of the combined state types**

Label	Description	Details*	Colour
CTCF	CTCF-enriched element	Sites of CTCF signal lacking histone modifications, often associated with open chromatin. Many probably have a function in insulator assays, but because of the multifunctional nature of CTCF, we are conservative in our description. Also enriched for the cohesin components RAD21 and SMC3; CTCF is known to recruit the cohesin complex.	Turquoise
E	Predicted enhancer	Regions of open chromatin associated with H3K4me1 signal. Enriched for other enhancer-associated marks, including transcription factors known to act at enhancers. In enhancer assays, many of these (>50%) function as enhancers. A more conservative alternative would be <i>cis</i> -regulatory regions. Enriched for sites for the proteins encoded by <i>EP300</i> , <i>FOS</i> , <i>FOSL1</i> , <i>GATA2</i> , <i>HDAC8</i> , <i>JUNB</i> , <i>JUND</i> , <i>NFE2</i> , <i>SMARCA4</i> , <i>SMARCB1</i> , <i>SIRT6</i> and <i>TAL1</i> genes in K562 cells. Have nuclear and whole-cell RNA signal, particularly poly(A) <sup>+</sup> fraction.	Orange
PF R	Predicted promoter flanking region Predicted repressed or low-activity region	Regions that generally surround TSS segments (see below). This is a merged state that includes H3K27me3 polycomb-enriched regions, along with regions that are silent in terms of observed signal for the input assays to the segmentations (low or no signal). They may have other signals (for example, RNA, not in the segmentation input data). Enriched for sites for the proteins encoded by <i>REST</i> and some other factors (for example, proteins encoded by <i>BRF2</i> , <i>CEBPB</i> , <i>MAFK</i> , <i>TRIM28</i> , <i>ZNF274</i> and <i>SETDB1</i> genes in K562 cells).	Light red Grey
TSS	Predicted promoter region including TSS	Found close to or overlapping GENCODE TSS sites. High precision/recall for TSSs. Enriched for H3K4me3. Sites of open chromatin. Enriched for transcription factors known to act close to promoters and polymerases Pol II and Pol III. Short RNAs are most enriched in these segments.	Bright red
T	Predicted transcribed region	Overlap gene bodies with H3K36me3 transcriptional elongation signal. Enriched for phosphorylated form of Pol II signal (elongating polymerase) and poly(A) <sup>+</sup> RNA, especially cytoplasmic.	Dark green
WE	Predicted weak enhancer or open chromatin <i>cis</i> -regulatory element	Similar to the E state, but weaker signals and weaker enrichments.	Yellow

- Use ChromHMM and Segway to Summarize the individual assays into 7 functional/regulatory states

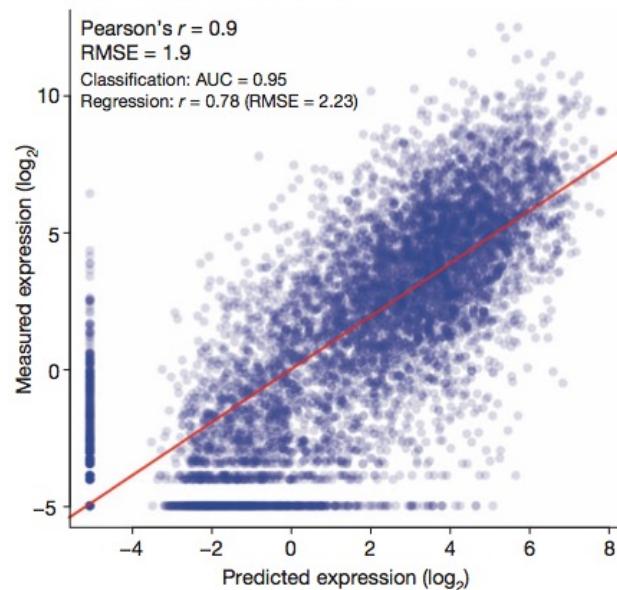
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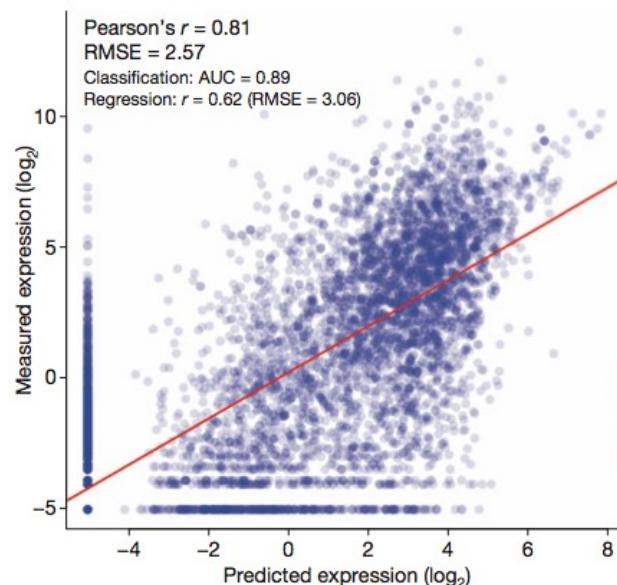
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# Expression Modeling

a CAGE poly(A)<sup>+</sup> K562 whole cell



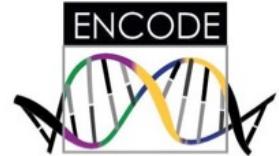
b CAGE poly(A)<sup>+</sup> K562 whole cell



- Developed predictive models to explore the interaction between histone modifications and transcription factor binding towards level of transcription
- The best models had two components: an initial classification component (on/off) and a second quantitative model component
- Together, these correlation models indicate both that a limited set of chromatin marks are sufficient to 'explain' transcription and that a variety of transcription factors might have broad roles in general transcription levels across many genes

**Modeling gene expression using chromatin features in various cellular context**  
Dong et al. (2012) *Genome Biology*. 12:R53

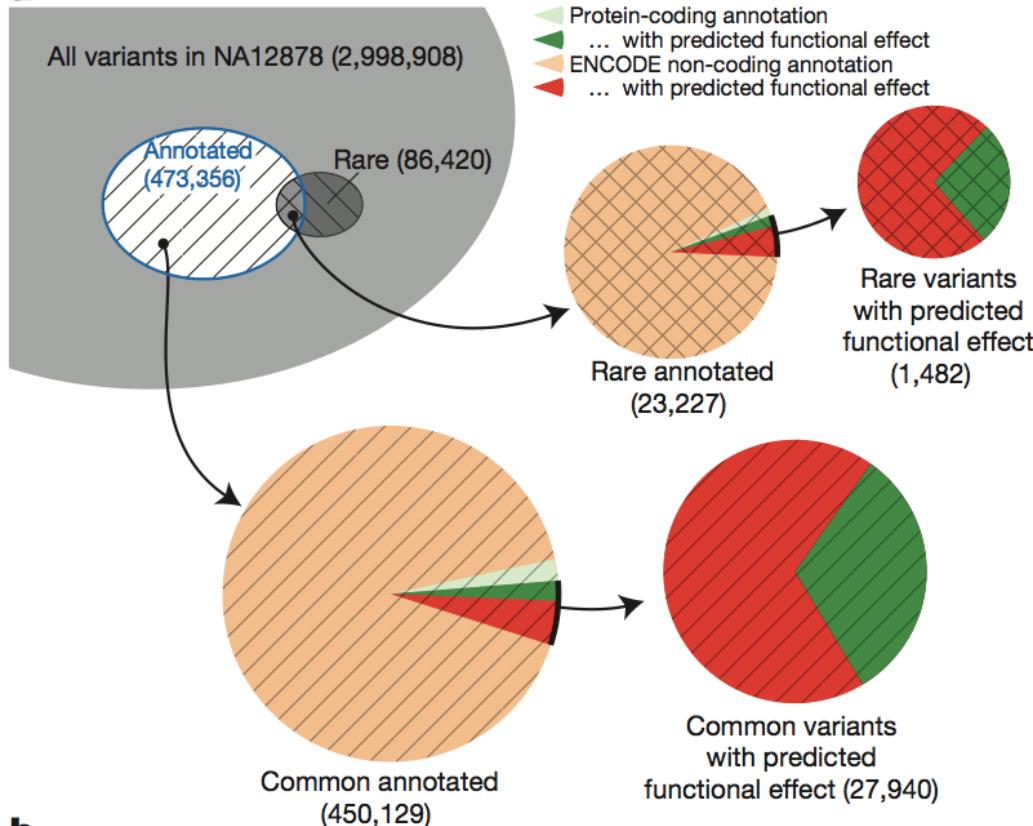
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# Many variants in ENCODE-regions

a



b

**Figure 9 | Examining ENCODE elements on a per individual basis in the normal and cancer genome.** a, Breakdown of variants in a single genome (NA12878) by both frequency (common or rare (that is, variants not present in the low-coverage sequencing of 179 individuals in the pilot 1 European panel of the 1000 Genomes project<sup>55</sup>) and by ENCODE annotation, including protein-coding gene and non-coding elements (GENCODE annotations for protein-coding genes, pseudogenes and other ncRNAs, as well as transcription-factor binding sites from ChIP-seq data sets, excluding broad annotations such as histone modifications, segmentations and RNA-seq). Annotation status is further subdivided by predicted functional effect, being non-synonymous and missense mutations for protein-coding regions and variants overlapping bound transcription factor motifs for non-coding element annotations. A substantial proportion of variants are annotated as having predicted functional effects in the non-coding category. b, One of several relatively rare occurrences, where

## Breakdown of variants by frequency

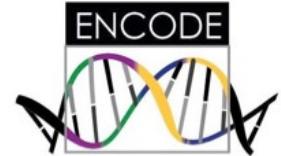
- Common or Rare (that is, variants not present in the low-coverage sequencing of 179 individuals in the pilot 1 European panel of the 1000 Genomes project)
- ENCODE annotation, including protein-coding gene and non-coding elements

Annotation status is further subdivided by predicted functional effect

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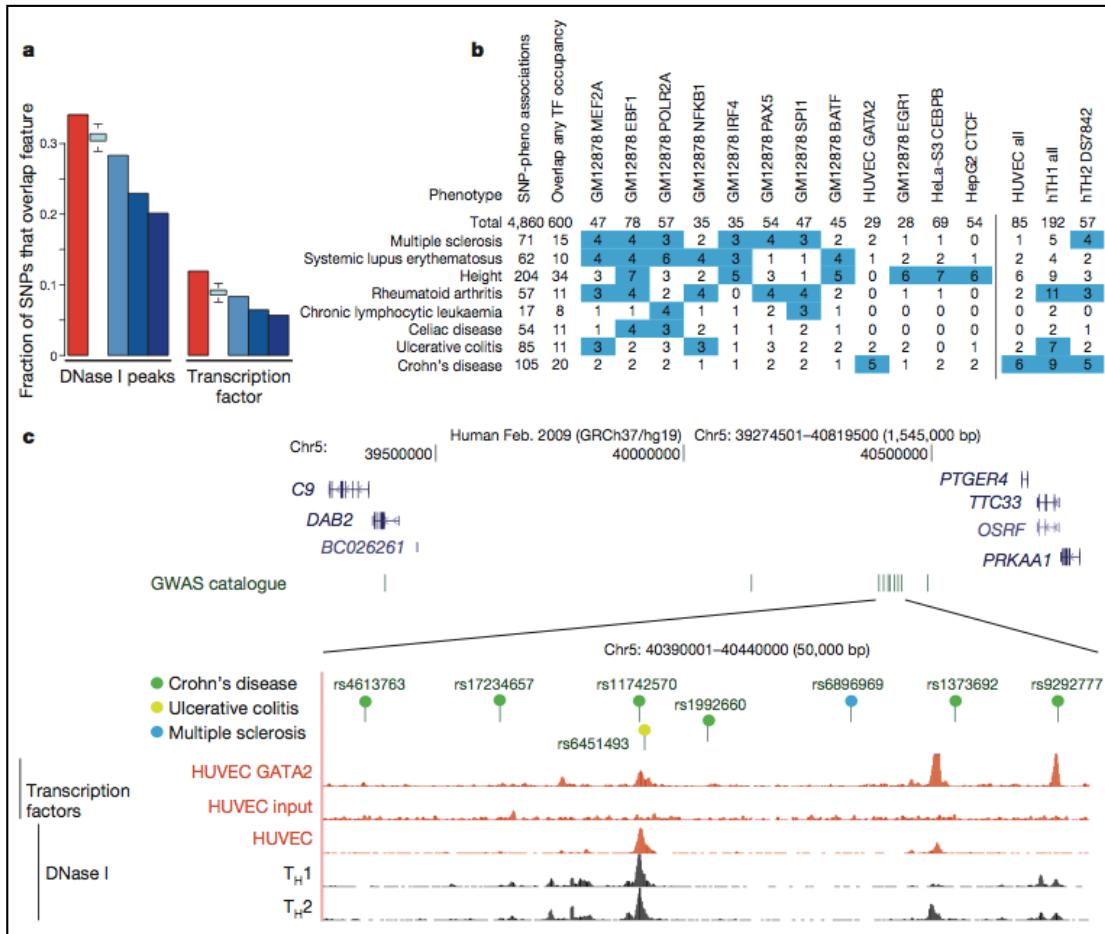
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# Major Findings



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# ENCODE and Disease



**Figure 10 | Comparison of genome-wide association study-identified loci with ENCODE data.** **a**, Overlap of lead SNPs in the NHGRI GWAS SNP catalogue (June 2011) with DHSs (left) or transcription-factor-binding sites (right) as red bars compared with various control SNP sets in blue. The control SNP sets are (from left to right): SNPs on the Illumina 2.5M chip as an example of a widely used GWAS SNP typing panel; SNPs from the 1000 Genomes project; SNPs extracted from 24 personal genomes (see personal genome variants track at <http://main.genome-browser.bx.psu.edu> (ref. 80)), all shown as blue bars. In addition, a further control used 1,000 randomizations from the genotyping SNP panel, matching the SNPs with each NHGRI catalogue SNP for allele frequency and distance to the nearest TSS (light blue bars with bounds at 1.5 times the interquartile range). For both DHSs and transcription-factor-binding regions, a larger proportion of overlaps with GWAS-implicated SNPs is found compared to any of the controls sets. **b**, Aggregate overlap of phenotypes to selected transcription-factor-binding sites (left matrix) or DHSs in selected cell lines (right matrix), with a count of overlaps between the phenotype and the cell line/factor. Values in blue squares pass an empirical *P*-value threshold  $\leq 0.01$  (based on the same analysis of overlaps between randomly chosen, GWAS-matched SNPs and these epigenetic features) and have at least a count of three overlaps. The *P* value for the total number of phenotype–transcription factor associations is  $< 0.001$ . **c**, Several SNPs associated with Crohn's disease and other inflammatory diseases that reside in a large gene desert on chromosome 5, along with some epigenetic features indicative of function. The SNP (rs11742570) strongly associated to Crohn's disease overlaps a GATA2 transcription-factor-binding signal determined in HUVECs. This region is also DNase I hypersensitive in HUVECs and T-helper T<sub>H</sub>1 and T<sub>H</sub>2 cells. An interactive version of this figure is available in the online version of the paper.

- 88% of GWAS SNPs are intronic or intergenic of unknown function
- We found that 12% of these GWAS-SNPs overlap transcription-factor-occupied regions whereas 34% overlap DHSs
- GWAS SNPs are particularly enriched in the segmentation classes associated with enhancers and TSSs across several cell types

# ENCODE Studies

ENCODE: Encyclopedia of DNA x Michael  
Secure https://www.encodeproject.org  
JHUMail Daily schatzlab SL cshl jhu Media edit Rm Cookies GoPerf Other Bookmarks

ENCODE Data Encyclopedia Materials & Methods Help Search...

## ENCODE: Encyclopedia of DNA Elements

The ENCODE (Encyclopedia of DNA Elements) Consortium is an international collaboration of research groups funded by the National Human Genome Research Institute (NHGRI). The goal of ENCODE is to build a comprehensive parts list of functional elements in the human genome, including elements that act at the protein and RNA levels, and regulatory elements that control cells and circumstances in which a gene is active.

[Get Started](#)

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

ENCODE

HUMAN MOUSE WORM FLY

[View Assay Matrix](#)

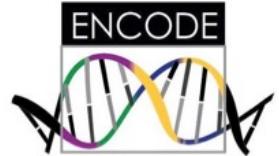
Project Biosample Type Assay Categories

13223

13223

>5000 Citations for main paper; >>10k for all papers

# Summary & Critique



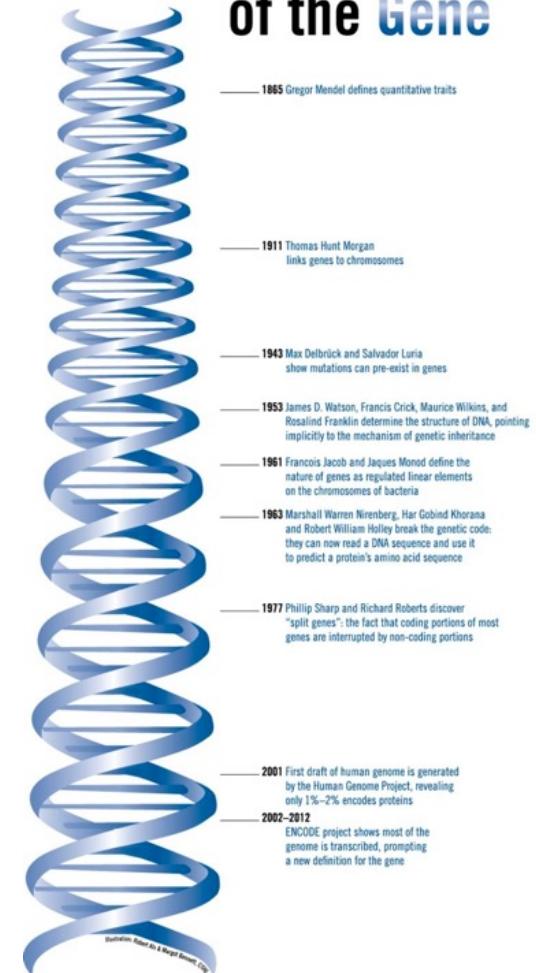
- **Summary**

- *The unprecedented number of functional elements identified in this study provides a valuable resource to the scientific community as well as significantly enhances our understanding of the human genome.*

- **Critique**

- Was it correct?
- What is functional?
- What is conservation?
- What was the control?
- What are the tradeoffs of organizing so much funding (\$288M!) around a single project; will other groups successfully use these data?

## Redefining the Nature of the Gene

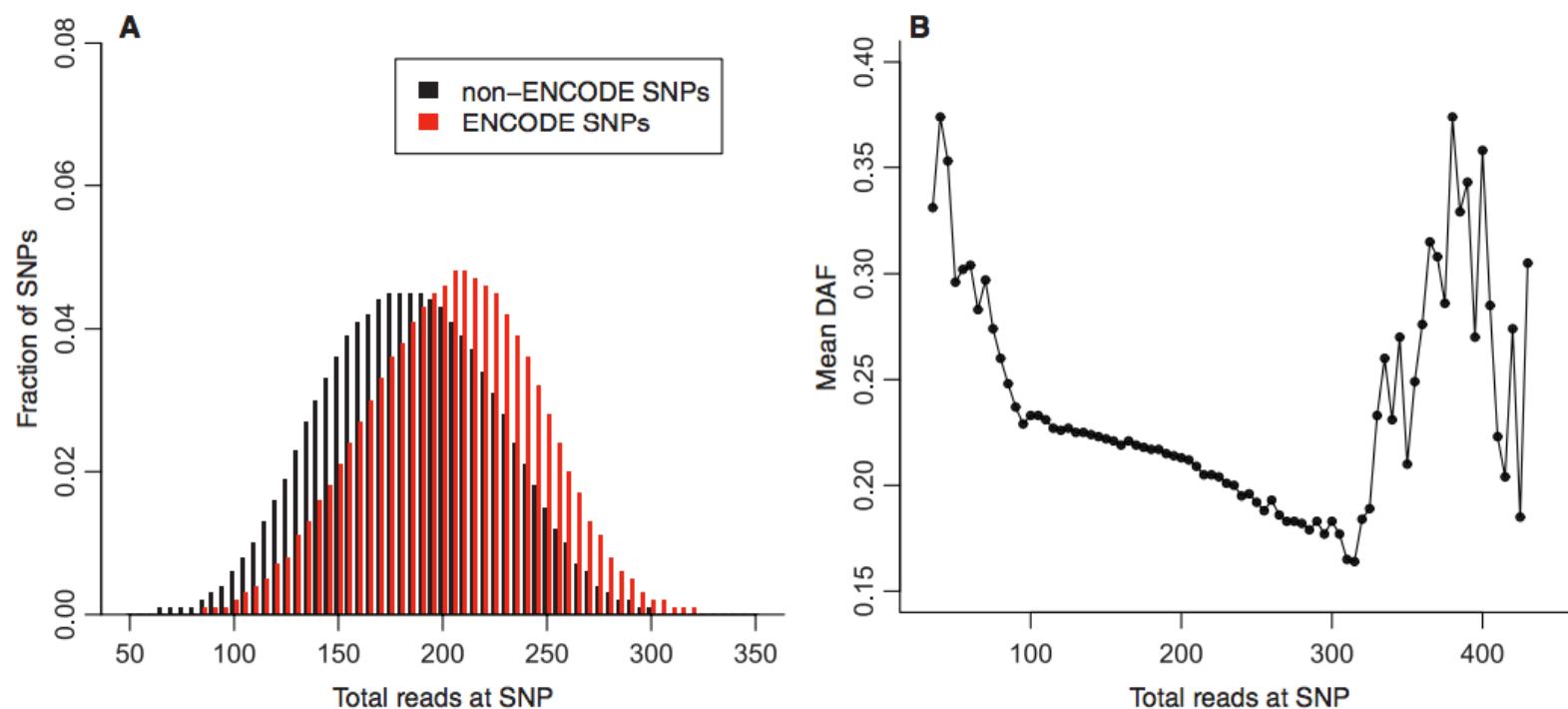


# Comment on “Evidence of Abundant Purifying Selection in Humans for Recently Acquired Regulatory Functions”



Phil Green\* and Brent Ewing

Ward and Kellis (Reports, 28 September 2012, p. 1675; published online 5 September 2012) found altered patterns of human polymorphism in biochemically active but non-mammalian-conserved genomic regions relative to control regions and interpreted this as due to lineage-specific purifying selection. We find on closer inspection of their data that the polymorphism trends are primarily attributable to mutational variation and technical artifacts rather than selection.



**Fig. 1.** Variation in 1000 Genomes read depth (totaled over 59 Yoruban individuals) and its impact on DAF. (A) Read-depth distribution for SNPs in neutral control (non-ENCODE) and ENCODE target regions. (B) DAF as a function

of read depth, for non-ENCODE SNPs. DAF decreases with increasing depth, due to increasing sensitivity to detect rare variants; the reverse trend at depths above 300 likely reflects the presence of spurious “parologue-collapse” SNPs.

# On the Immortality of Television Sets: “Function” in the Human Genome According to the Evolution-Free Gospel of ENCODE

Dan Graur<sup>1,\*</sup>, Yichen Zheng<sup>1</sup>, Nicholas Price<sup>1</sup>, Ricardo B.R. Azevedo<sup>1</sup>, Rebecca A. Zufall<sup>1</sup>, and Eran Elhaik<sup>2</sup>

<sup>1</sup>Department of Biology and Biochemistry, University of Houston

<sup>2</sup>Department of Mental Health, Johns Hopkins University Bloomberg School of Public Health

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Accepted: February 16, 2013

## Abstract

A recent slew of ENCYclopedia Of DNA Elements (ENCODE) Consortium publications, specifically the article signed by all Consortium members, put forward the idea that more than 80% of the human genome is functional. This claim flies in the face of current estimates according to which the fraction of the genome that is evolutionarily conserved through purifying selection is less than 10%. Thus, according to the ENCODE Consortium, a biological function can be maintained indefinitely without selection, which implies that at least  $80 - 10 = 70\%$  of the genome is perfectly invulnerable to deleterious mutations, either because no mutation can ever occur in these “functional” regions or because no mutation in these regions can ever be deleterious. This absurd conclusion was reached through various means, chiefly by employing the seldom used “causal role” definition of biological function and then applying it inconsistently to different biochemical properties, by committing a logical fallacy known as “affirming the consequent,” by failing to appreciate the crucial difference between “junk DNA” and “garbage DNA,” by using analytical methods that yield biased errors and inflate estimates of functionality, by favoring statistical sensitivity over specificity, and by emphasizing statistical significance rather than the magnitude of the effect. Here, we detail the many logical and methodological transgressions involved in assigning functionality to almost every nucleotide in the human genome. The ENCODE results were predicted by one of its authors to necessitate the rewriting of textbooks. We agree, many textbooks dealing with marketing, mass-media hype, and public relations may well have to be rewritten.

**Key words:** junk DNA, genome functionality, selection, ENCODE project.

## The ENCODE project: Missteps overshadowing a success

Two clichés of science journalism have now played out around the ENCODE project. ENCODE's publicity first presented a misleading "all the textbooks"

*"To clarify what noise means, I propose the **Random Genome Project**. Suppose we put a few million bases of entirely random synthetic DNA into a human cell, and do an ENCODE project on it. Will it be reproducibly transcribed into mRNA-like transcripts, reproducibly bound by DNA-binding proteins, and reproducibly wrapped around histones marked by specific chromatin modifications? I think yes.*

*A striking feature of genetic regulation is that regulatory factors (proteins or RNAs) generally recognize and bind to small sites, small enough that any given factor will find specific binding sites even in random DNA. Promoters, enhancers, splice sites, poly-A addition sites, and other functional features in the genome all have substantial random occurrence frequencies. These sites are not nonspecific in a random genome. They are specific sequences, albeit randomly occurring and not under selection for any function.*

*Would biochemical activities in the random genome be regulated under different conditions? For example, would they be cell type-specific? Surely yes, because the regulatory factors themselves (such as transcription factors) are regulated and expressed in specific cell types and conditions."*



Bruce Alberts is Editor-in-Chief of *Science*.

# The End of “Small Science”?

I AM PROMPTED TO WRITE THIS EDITORIAL BY THE RELEASE OF 30 PAPERS THIS MONTH FROM THE ENCODE Project Consortium. This decade-long project involved an international team of 442 scientists who have compiled what is being called an “encyclopedia of DNA elements,” a comprehensive list of functional elements in the human genome. The detailed overview is expected to spur further research on the fundamentals of life, health, and disease. ENCODE exemplifies a “big-science” style of research that continues to sweep the headlines, and the increased efficiency of data production by such projects is impressive. Does this mean that the highly successful “small-science” era of biological research will soon be over? Will government funding increasingly favor big-science projects? I certainly hope that the answer is no.

...

Each year, the amount of factual information that scientists acquire about cells increases and, stimulated by -omics projects, the compilations of data expand at a tremendous rate. But the grand challenges that remain in attaining a deep understanding of the chemistry of life will require going beyond detailed catalogs. Ensuring a successful future for the biological sciences will require restraint in the growth of large centers and -omics-like projects, so as to provide more financial support for the critical work of innovative small laboratories striving to understand the wonderful complexity of living systems.

– Bruce Alberts

10.1126/science.1230529