

Patterns of Histone Methylation and Chromatin Organization in Grapevine Leaf



Rachel Schwope

EPIGEN

May 24-27, 2016



**UNIVERSITÀ
DEGLI STUDI
DI UDINE**



Plant of interest: *Vitis vinifera*

Culturally important

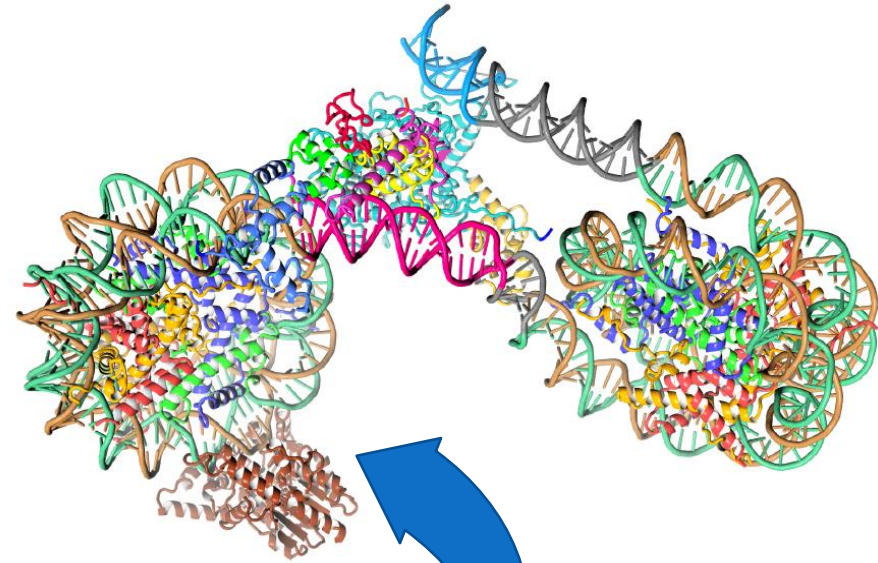
An economically vital organism for Italy and beyond

Scientifically intriguing

A clonal organism whose genome has not undergone meiosis – or its developmental program – in centuries.



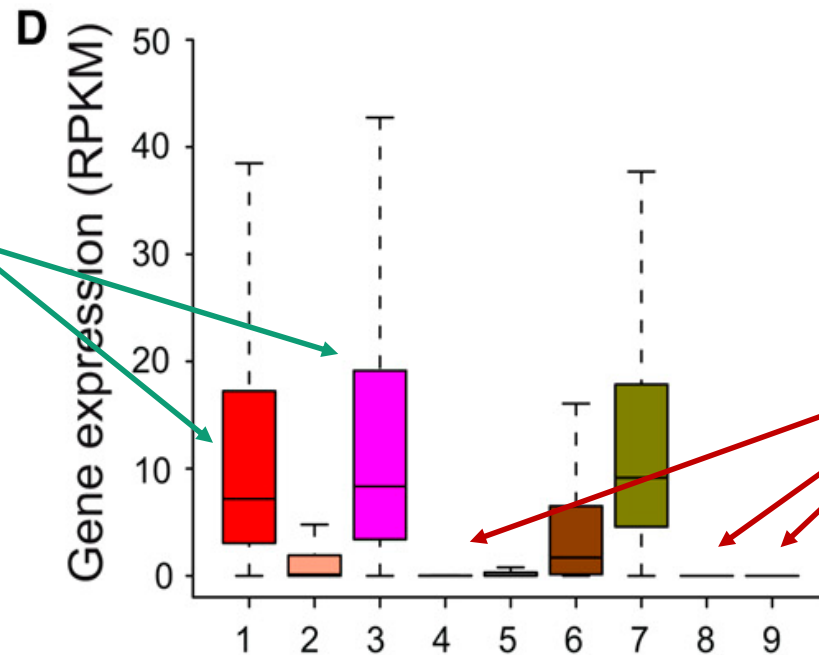
Chromatin – the structural and functional complex of DNA and binding proteins



Chromatin is specialized to maintain genomic states

- A recent ChIP-Seq meta-study identified **9** states of chromatin in *Arabidopsis* (Sequeira-Mendes, *Plant Cell* 2014)

- 4 types = high transcription
- 5 types = low transcription

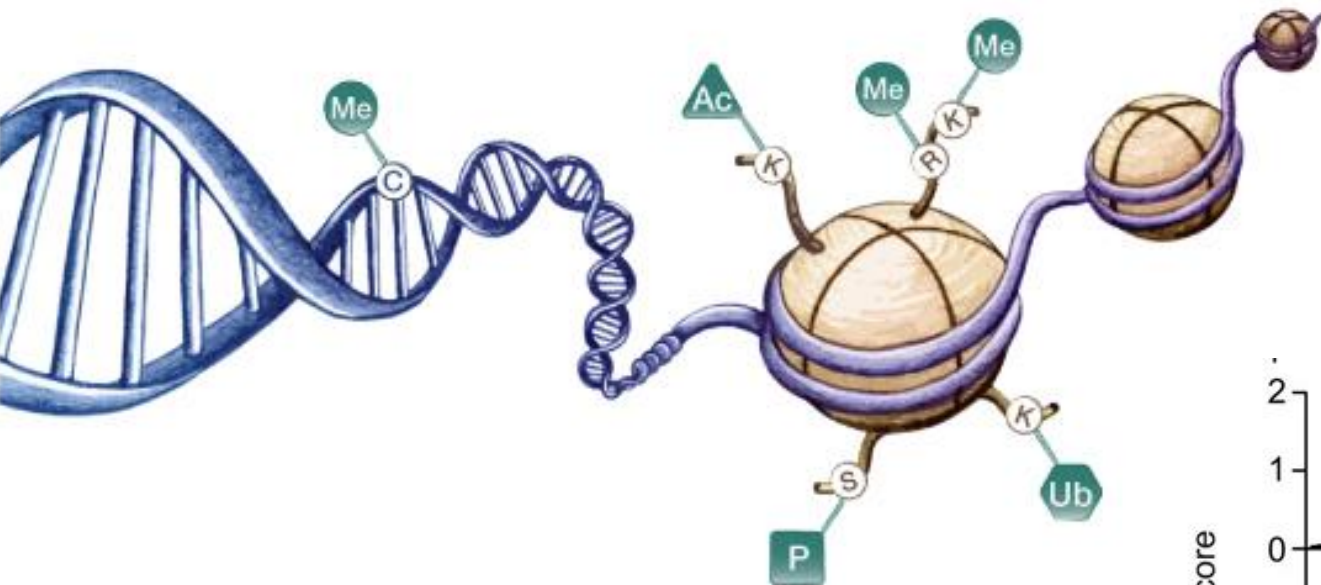


Promoter and
CDS-rich

Intergenic and
TE-rich

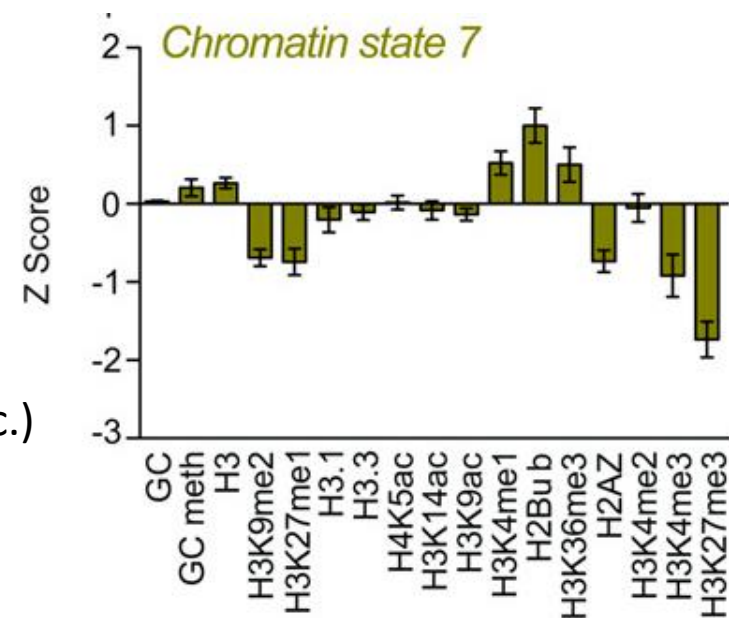
(Sequeira-Mendes, *Plant Cell* 2014)

What defines a Chromatin State?



- 1) DNA methylation (CG, CHG, CHH)
- 2) Post-translational modification of histones (H3K9ac, etc.)
- 3) Histone variants (H3.1, H2AZ, etc.)

From Figure 1B.



Functions of Specific Histone Modifications at H3

H3K4me3

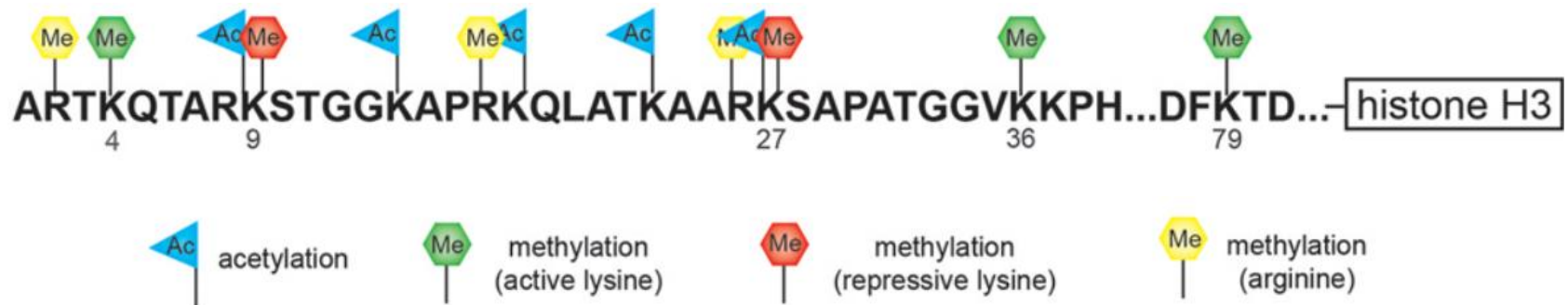
- Recruits **transcription initiation** factors
- Promotes **open** conformation

H3K9me2

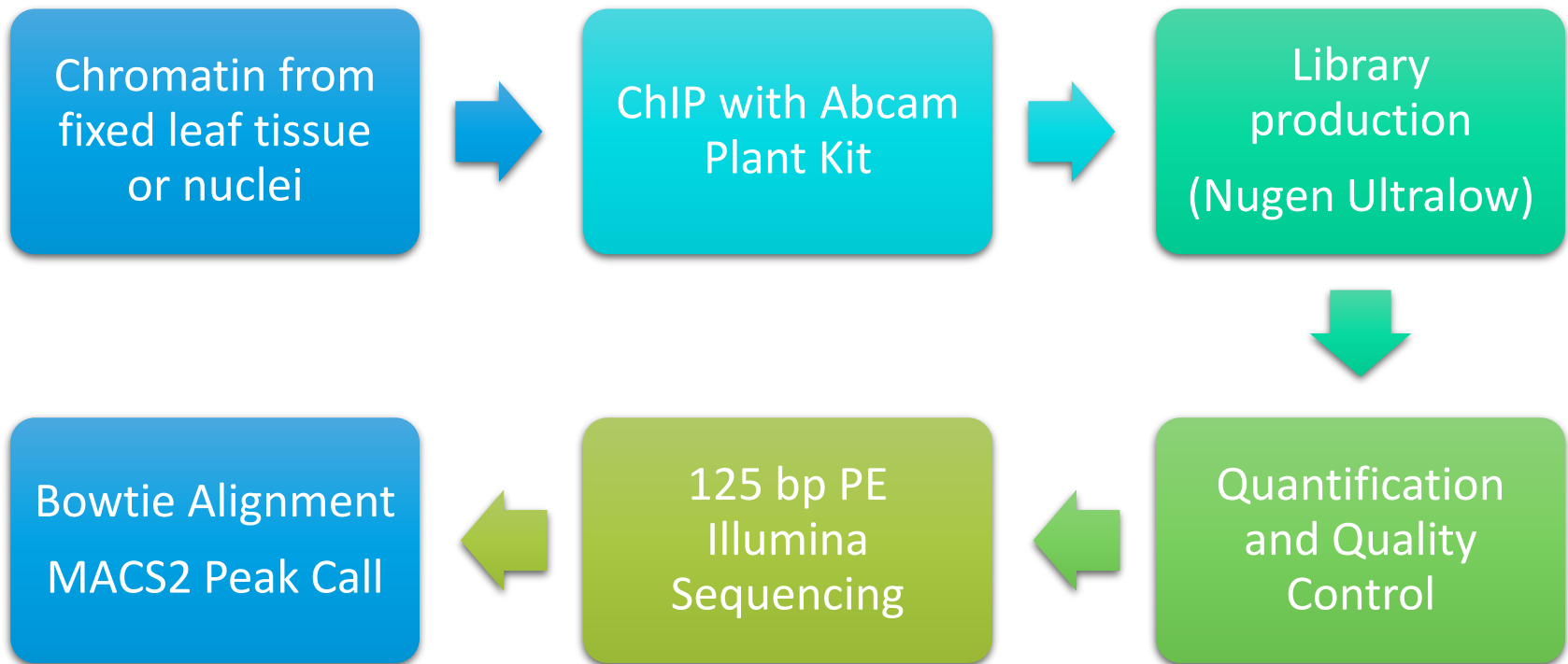
- In *Arabidopsis*, bound by CMT3 DNA methylase
- In maize, increased at **transposons** and some genes

H3K27me3

- Associated with **Polycomb**
- In maize, found in gene-dense chromatin arms, not at pericentric chromatin



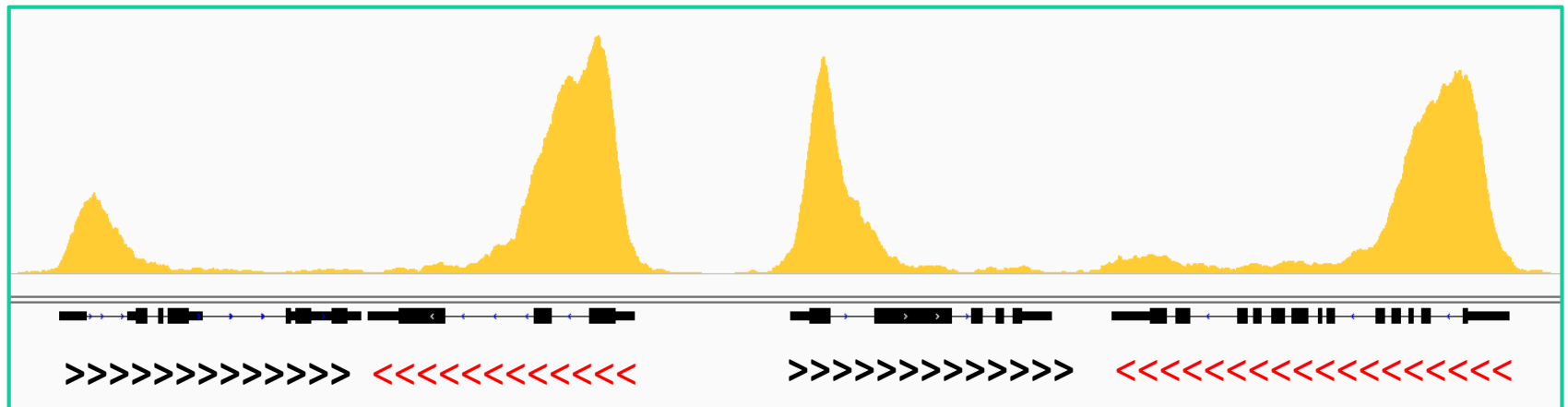
Methodology



Where is H3K4me3 found in grapevine leaves?

- Peaks are generally at the 5' ends of genes
- Have a broader "shoulder" at the 3' side of the peak

Chromosome 2: 3,198,150..3,225,396

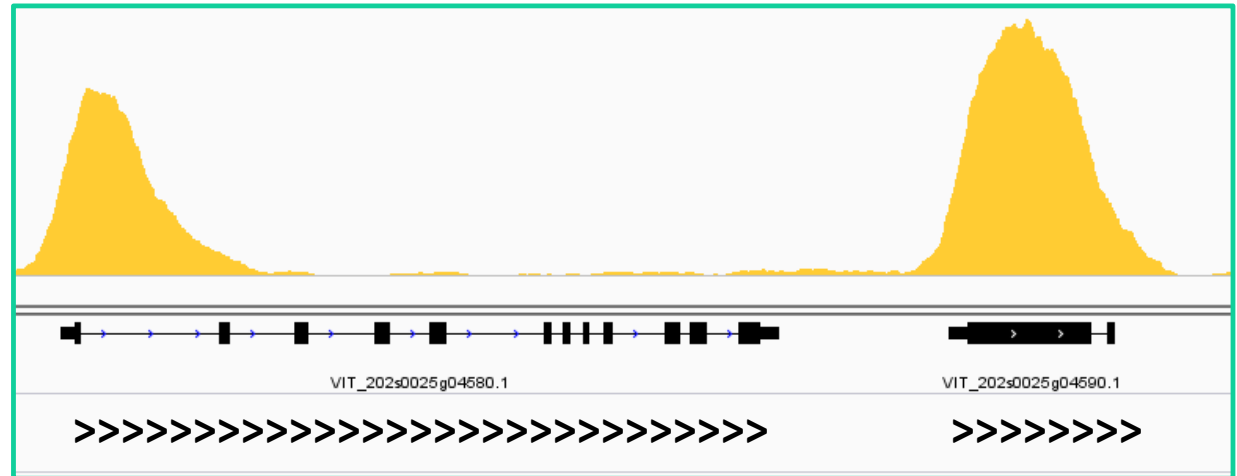
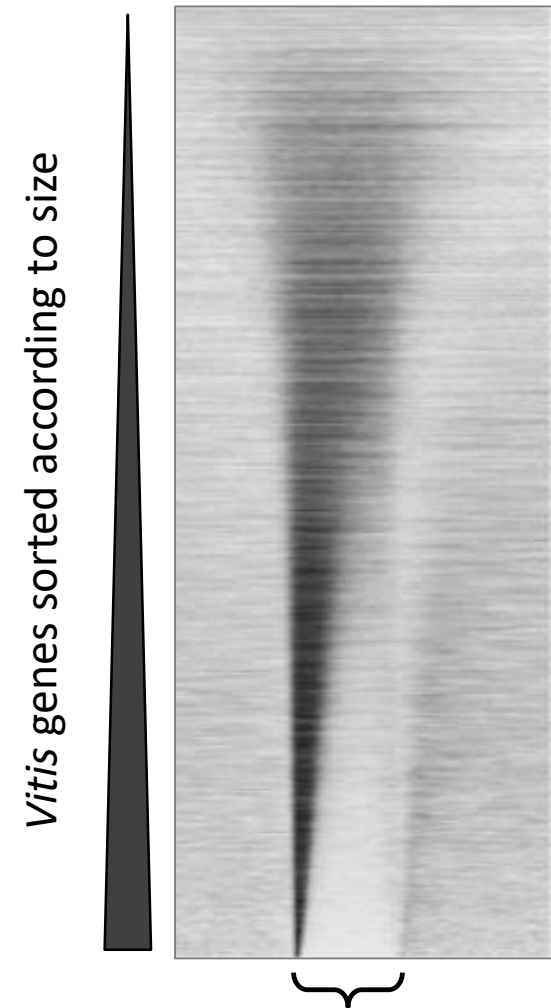


27 kb

H3K4me3 distribution across genes

H3K4me3 coverage over gene body

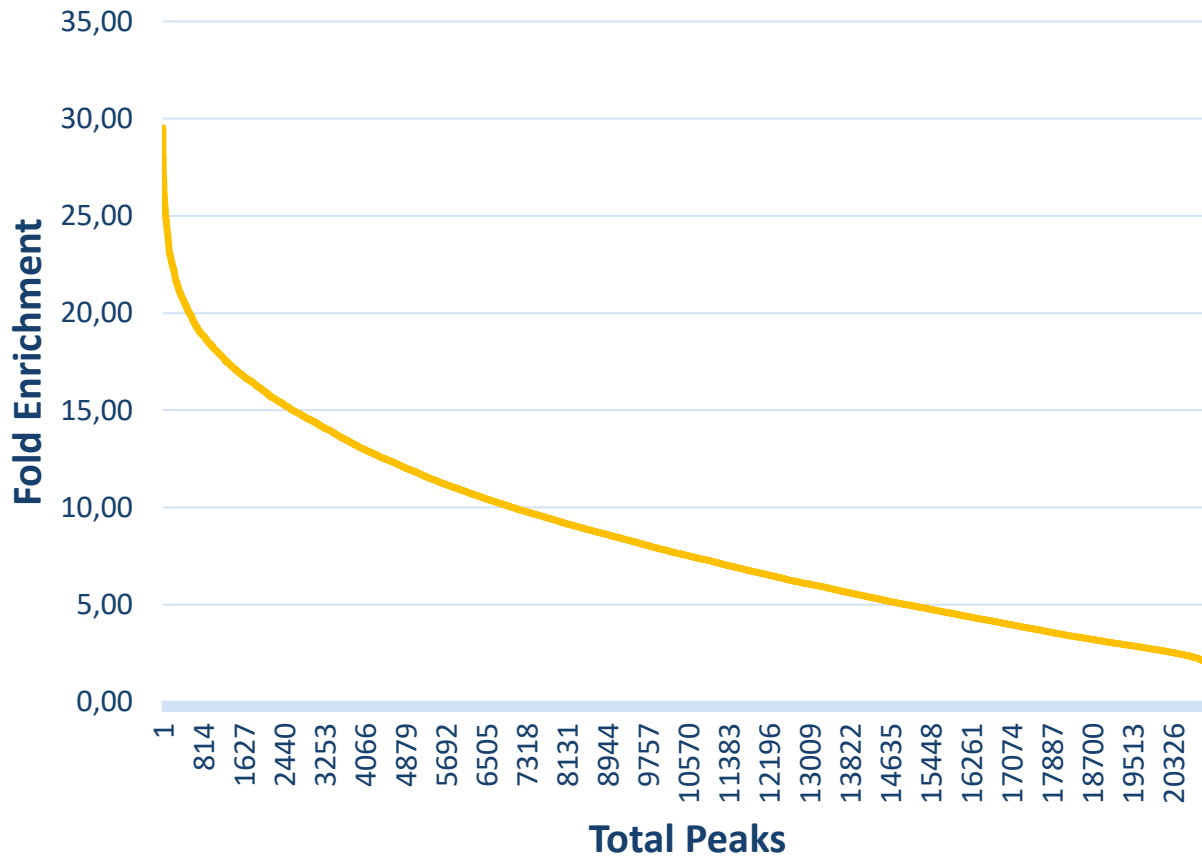
- As gene size increases, H3K4me3 covers a proportionally smaller amount of the gene



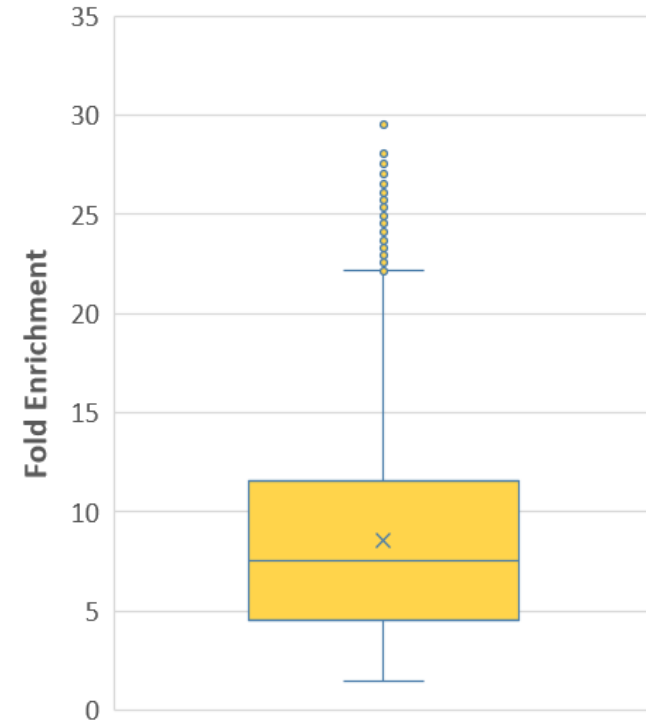
100% of gene length

How Enriched is H3K4me3 in Grapevine leaf?

Enrichment of H3K4me3 peaks in grapevine leaf

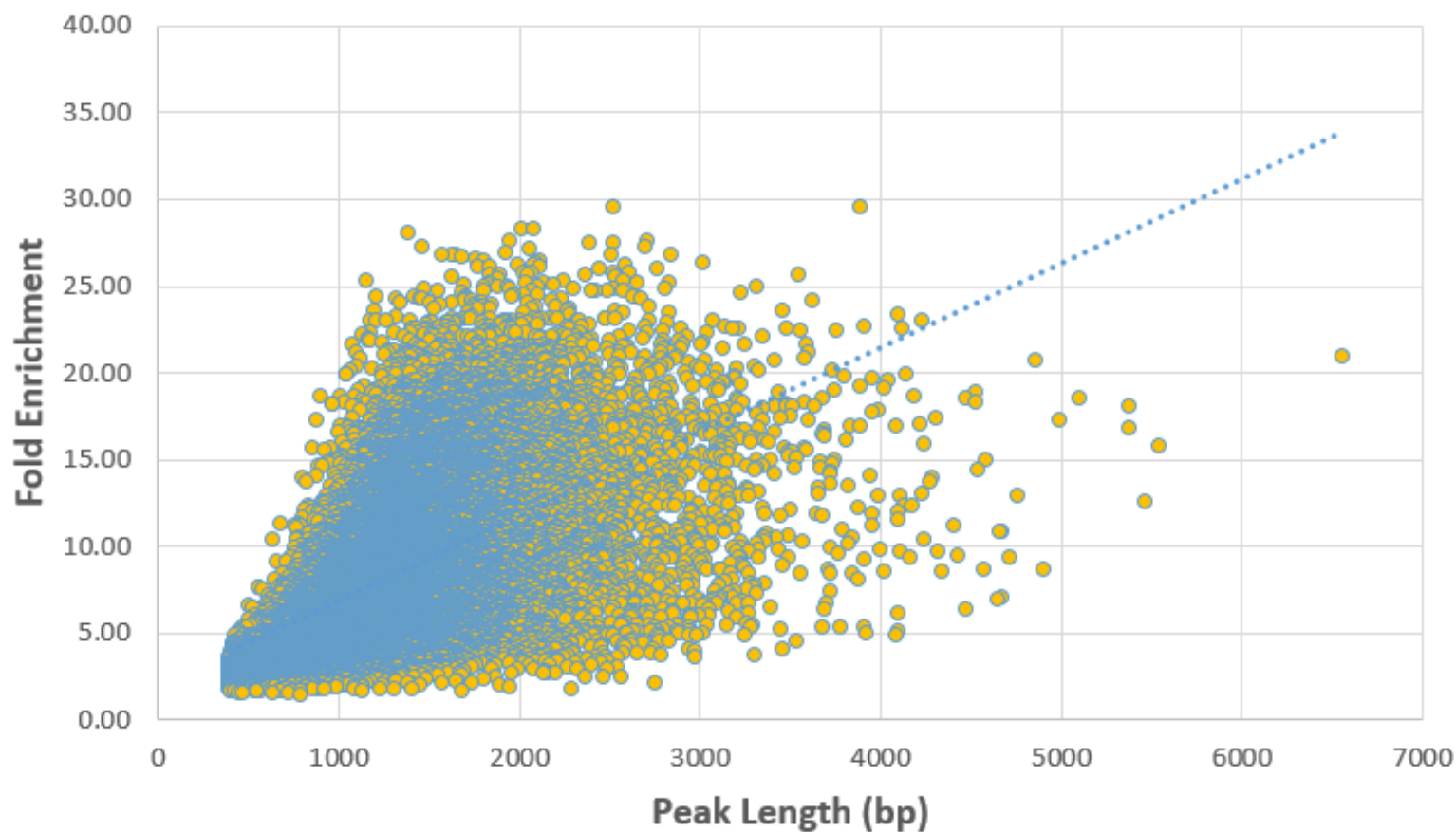


Enrichment level Distribution

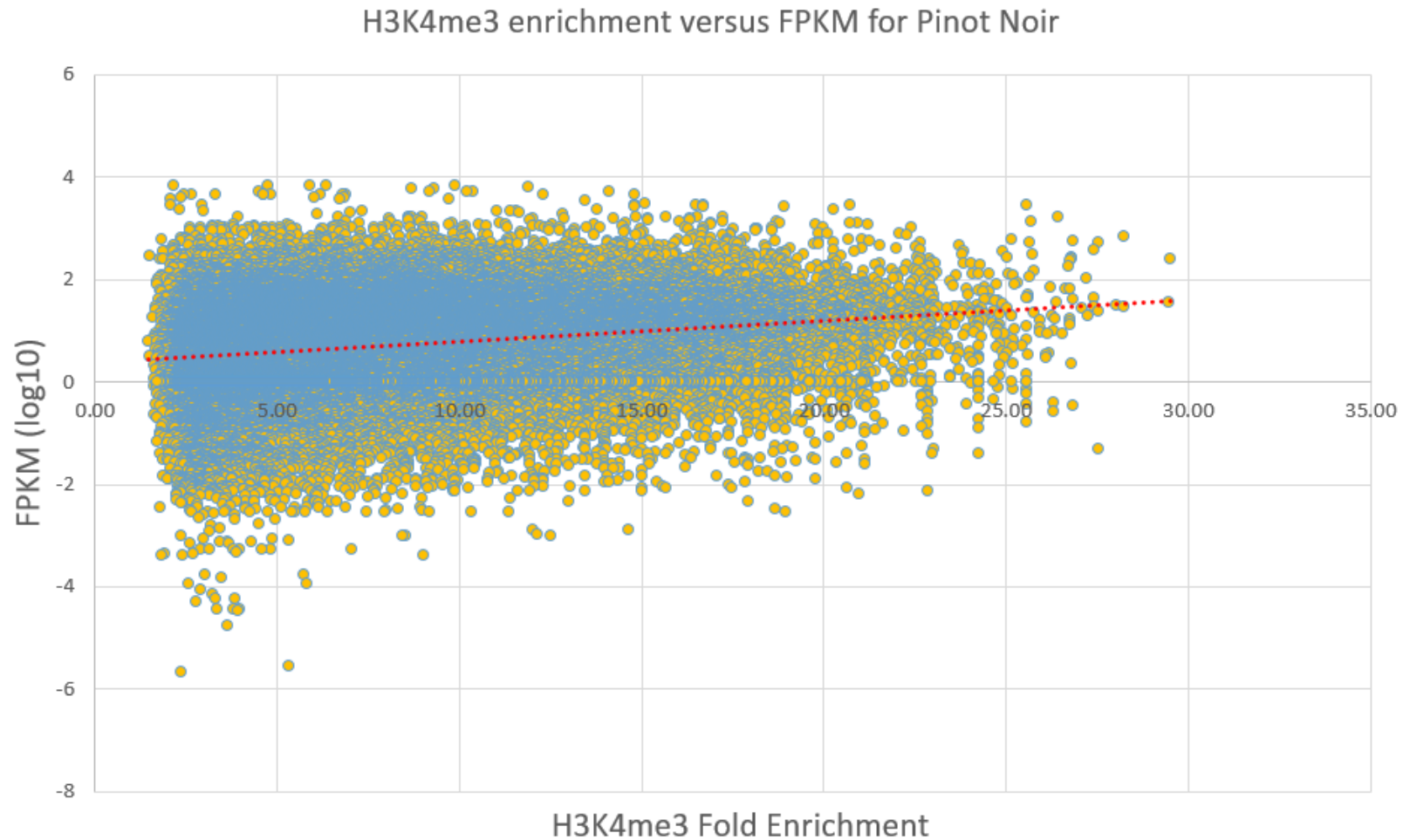


H3K4me3 Enrichment generally increases with Peak Length

H3K4me3 Peak Enrichment vs. peak length

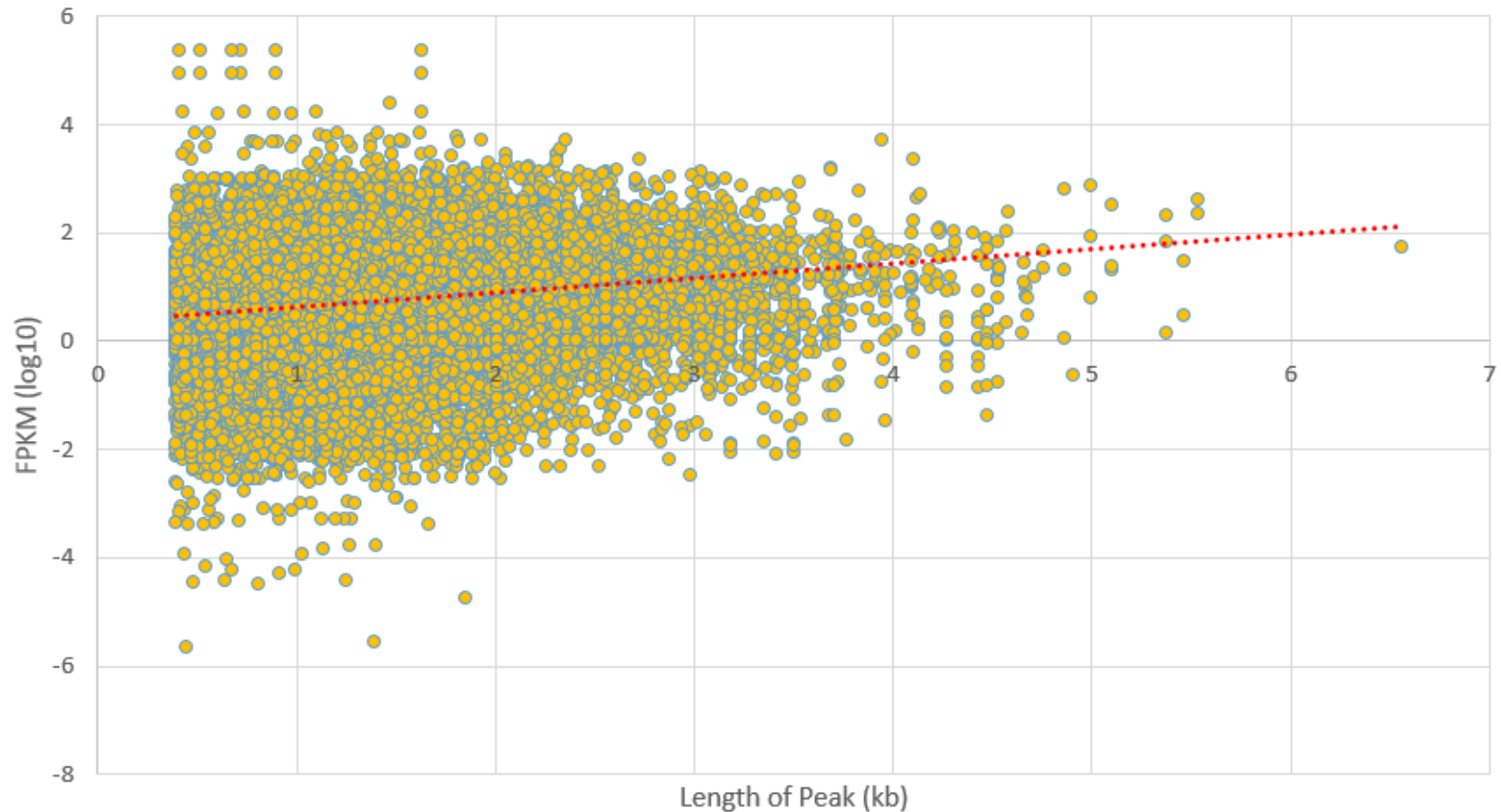


...But only correlates weakly with Transcript Accumulation



Peak length also correlates weakly with Transcript Accumulation

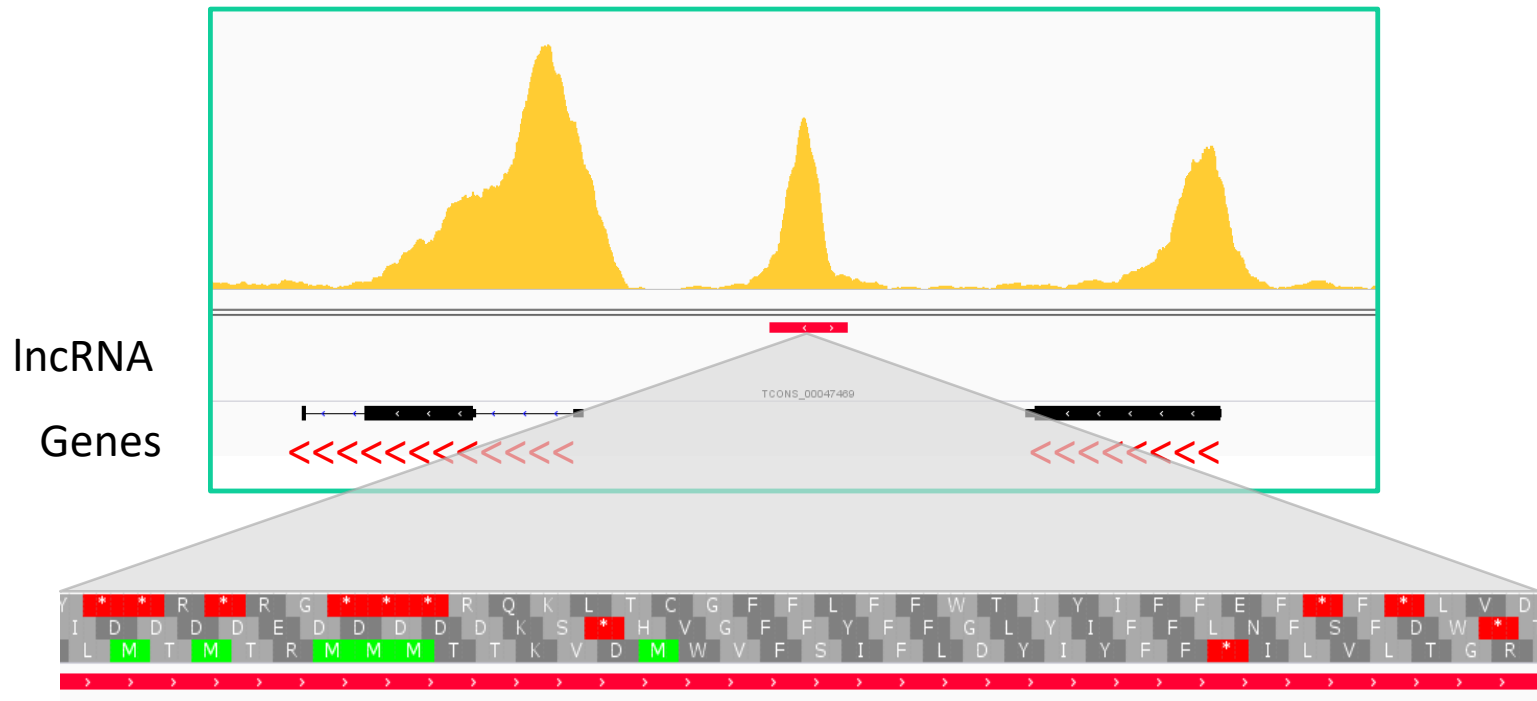
H3K4me3 Peak length versus FPKM for Pinot Noir



Where else do we find H3K4me3 enrichment?

Long non-coding RNAs: at least 200 bp long

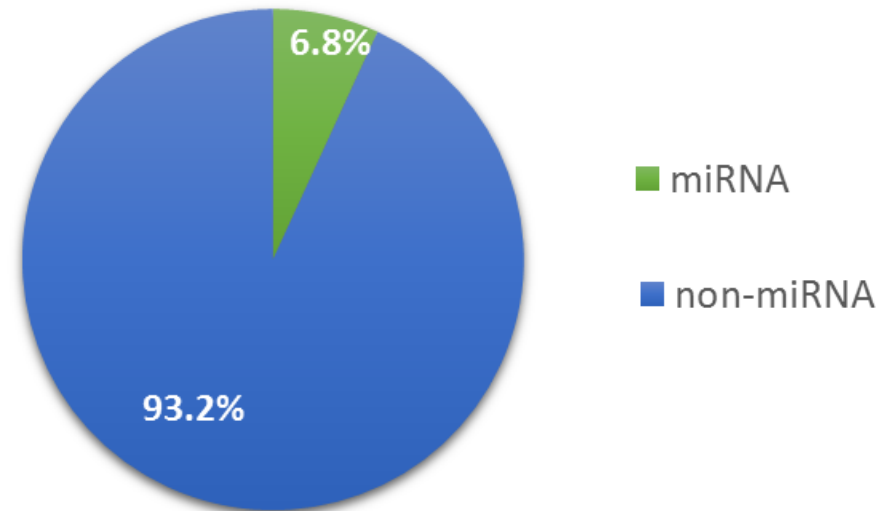
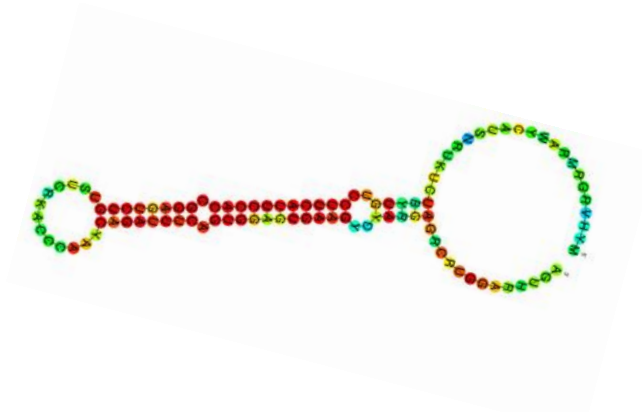
- 2044 intergenic long-ncRNAs in *Vitis* genome (*Genoscope*)
- In leaf, 219 of these (11%) correlate with H3K4me3 signal



What are the lncRNA species co-occur with H3K4me3?

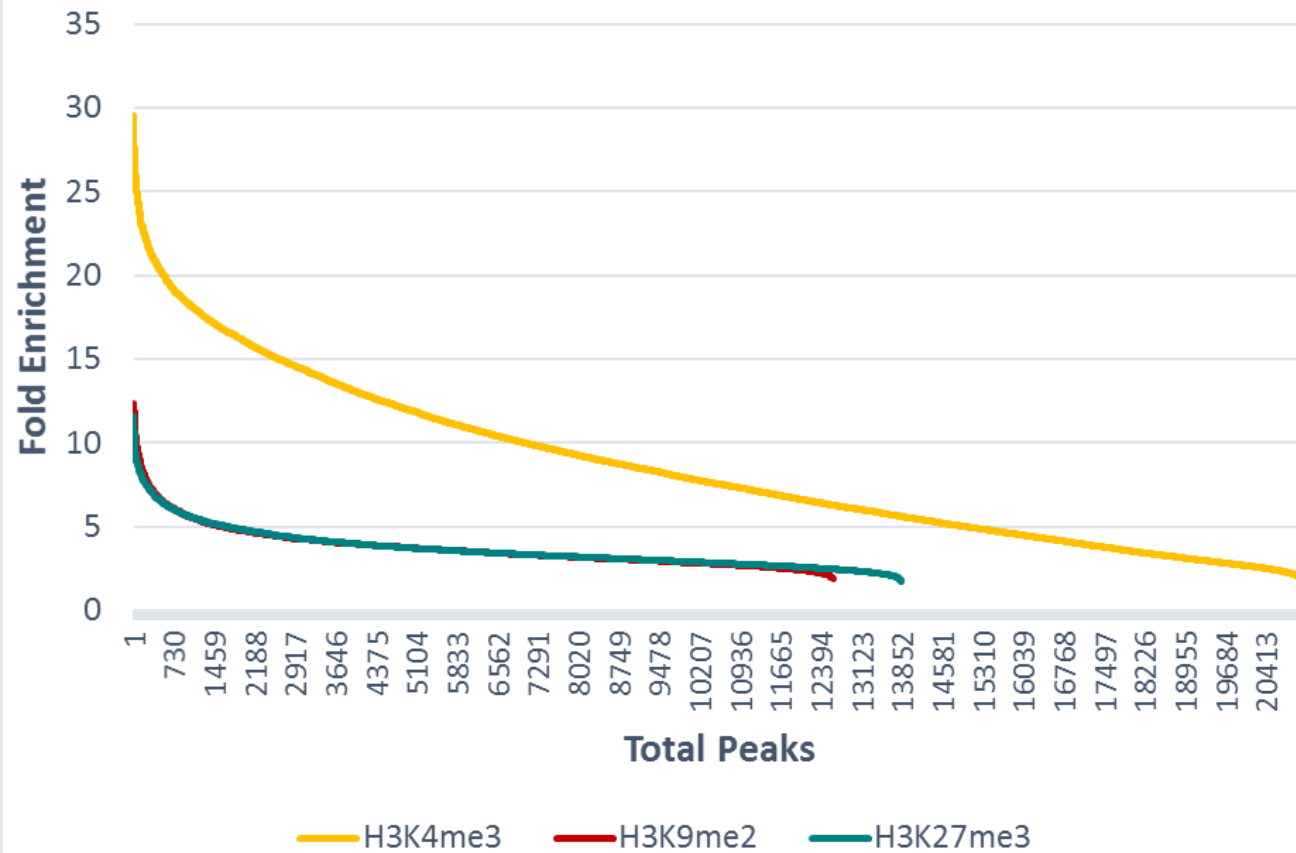
Total: 219 lncRNAs

- 15 of these encode miRNAs (*MIRbase.org*)
- 204 have no discernable miRNA structure

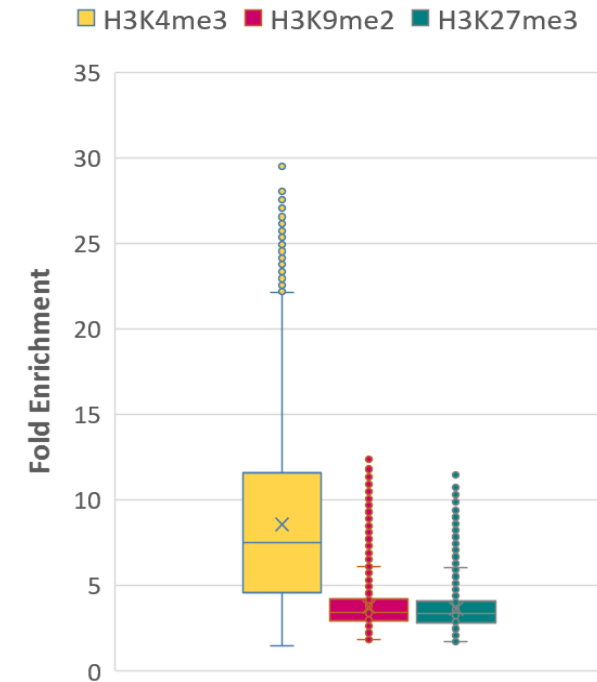


How do Heterochromatic modifications compare?

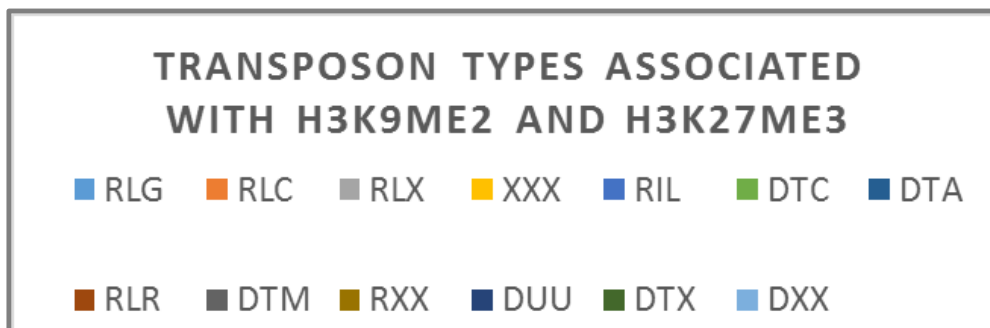
Distribution of Enrichment in grapevine leaf



Enrichment level Distribution



Where do we find H3K9me2 and H3K27me3?



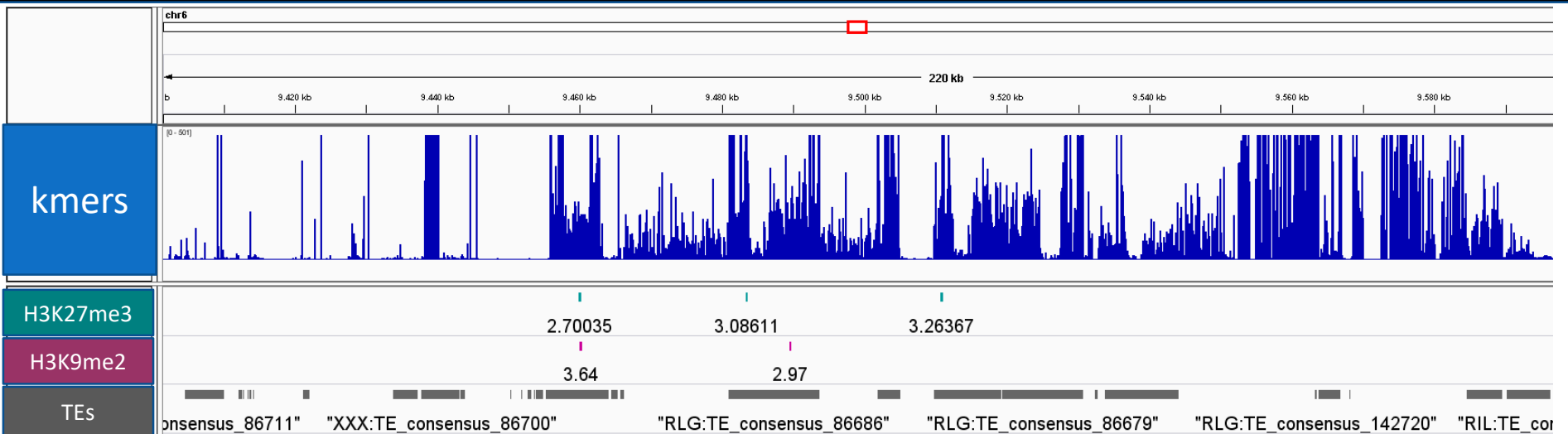
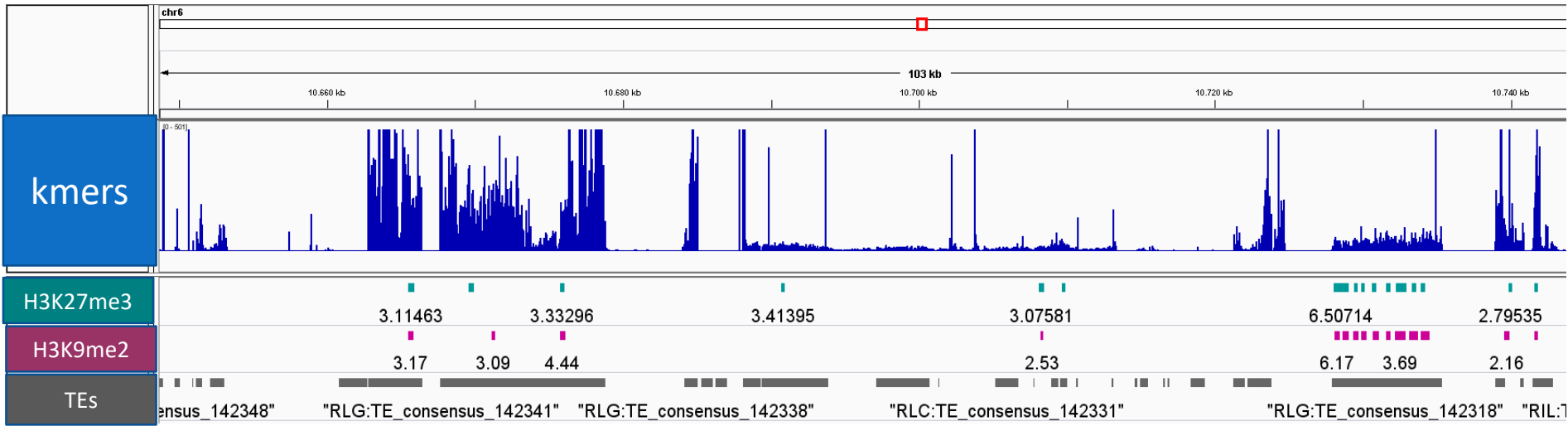
LTR-Gypsy

LTR-Copia

RIL

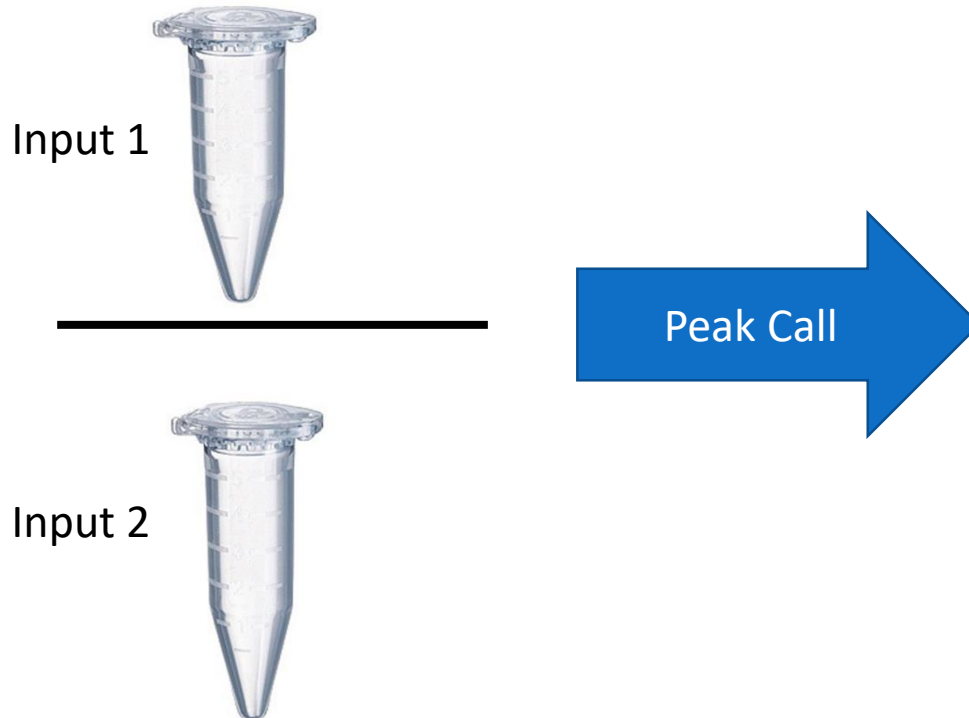


Many, but Not All Highly Repetitive TEs are Enriched

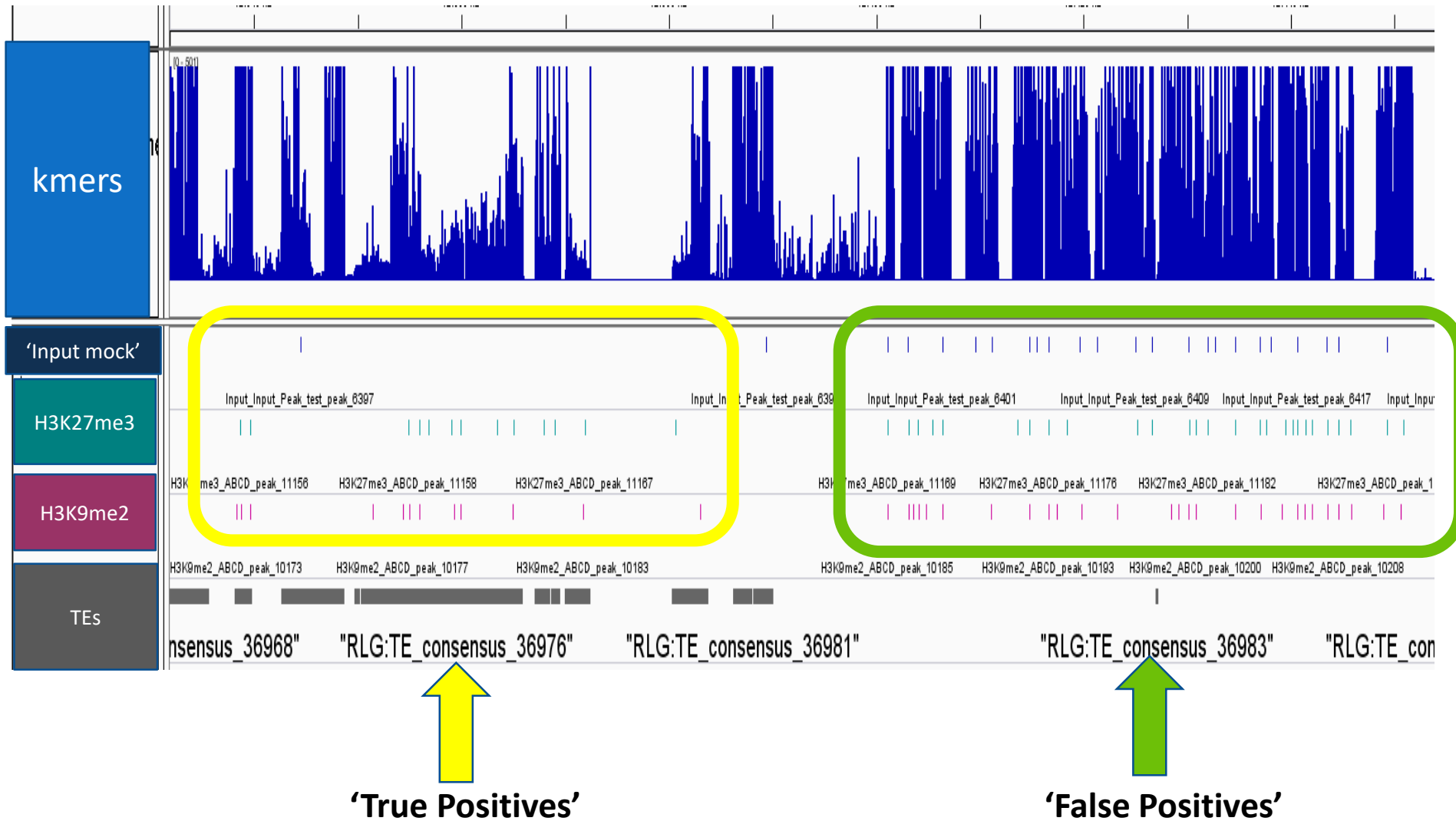


Computational Background Test

What happens when I ask MACS2 to find peaks from an **unenriched** alignment?

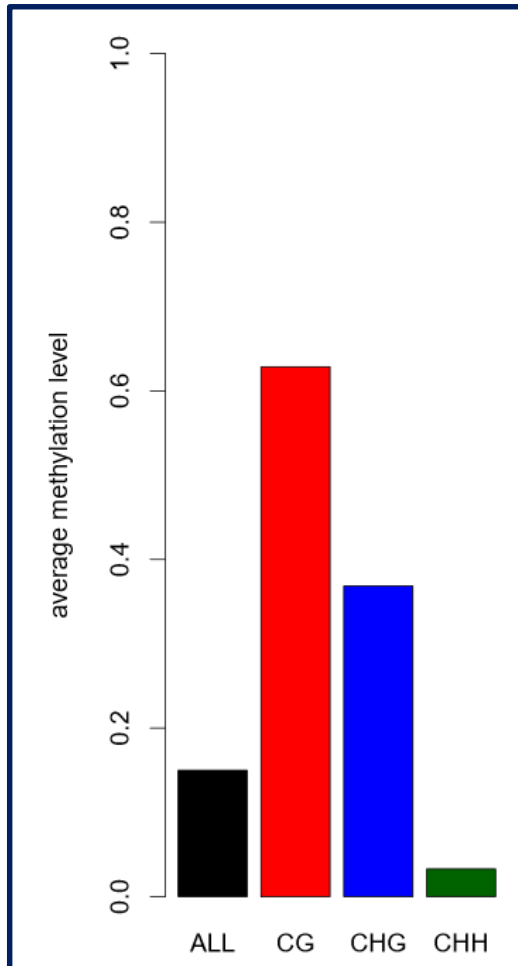


Separating Signal from Noise

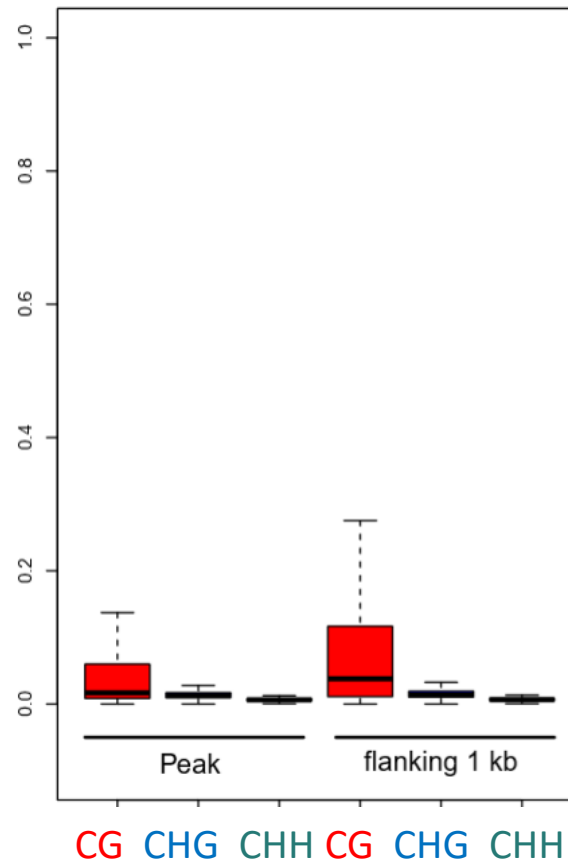


What is the DNA methylation state at loci with modified histones?

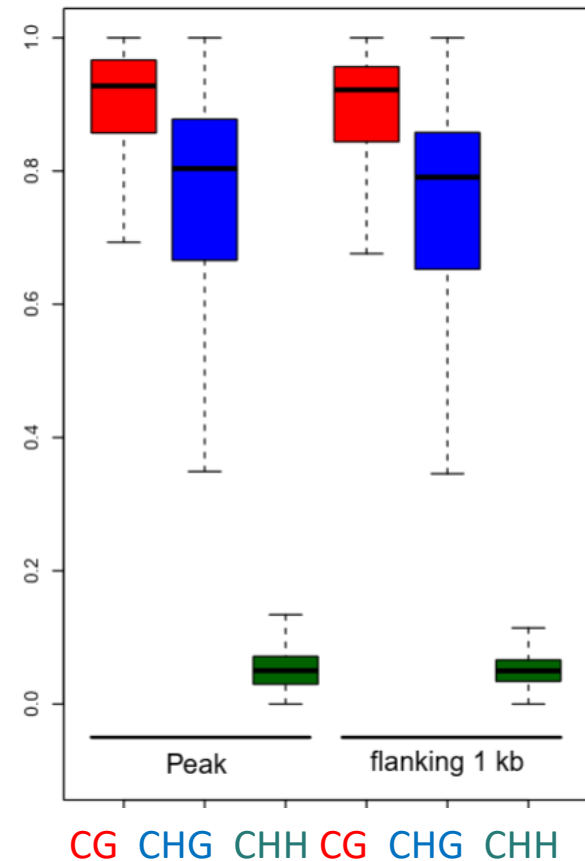
Genome-wide average methylation



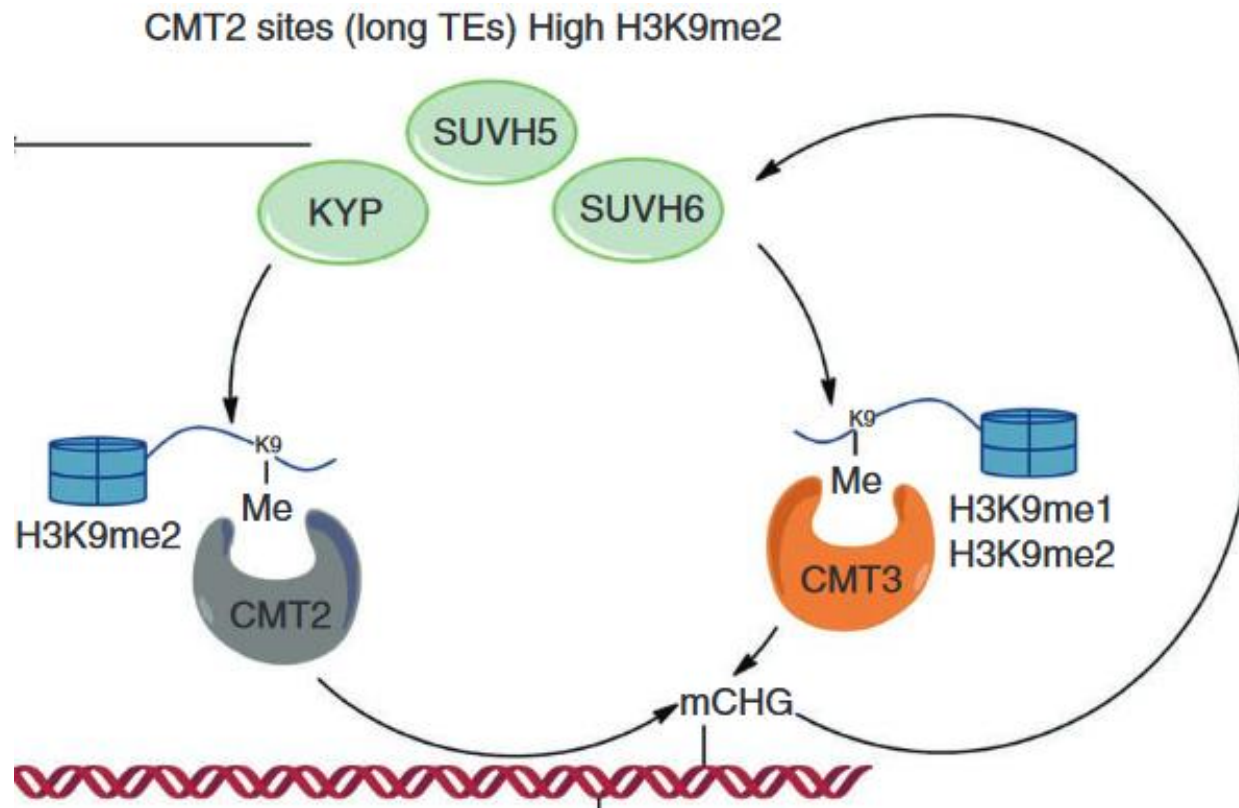
H3K4me3



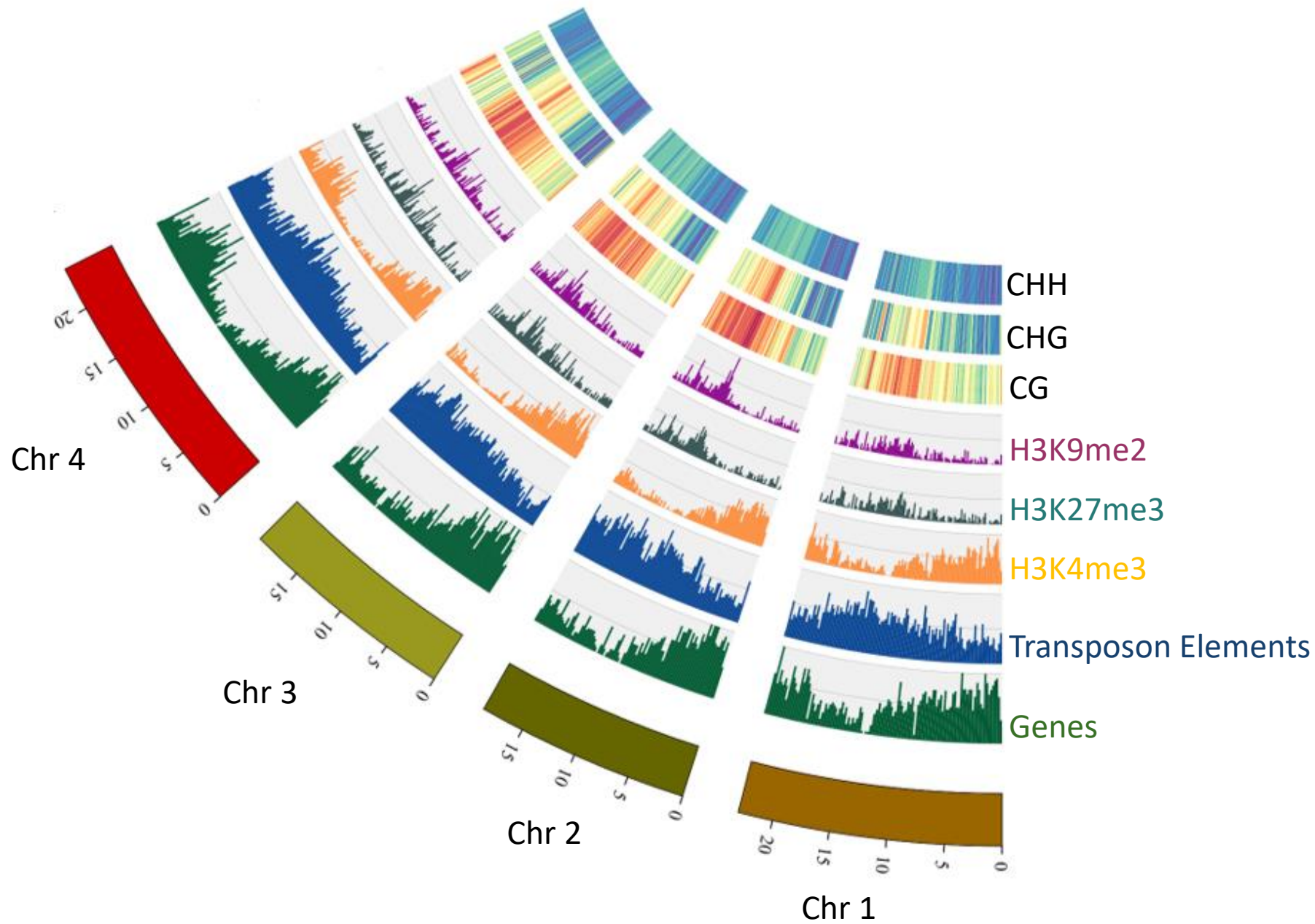
H3K9me2



Vitis results support *Arabidopsis* data that DNA and histone methylation form feedback loop



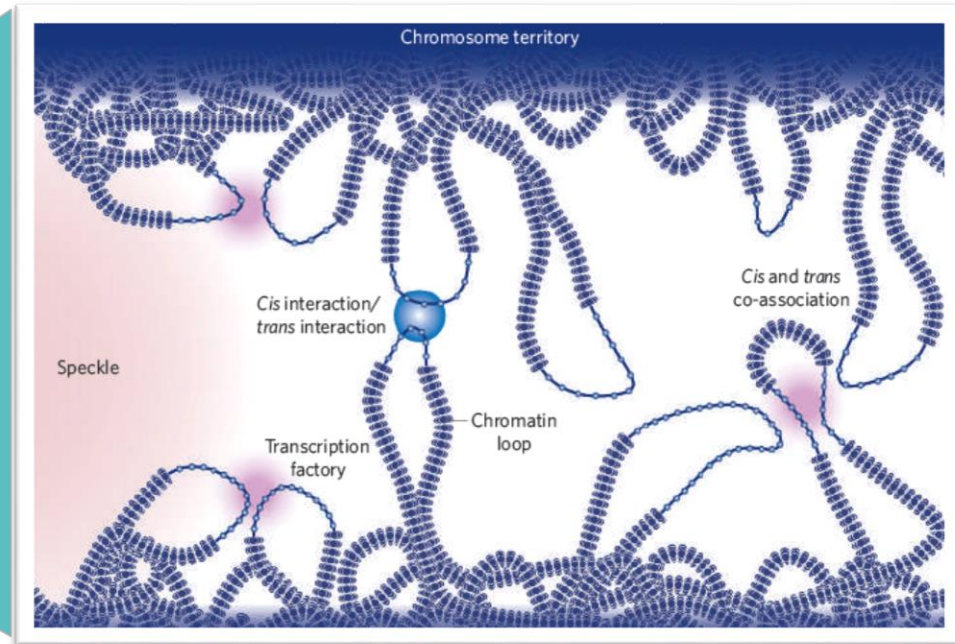
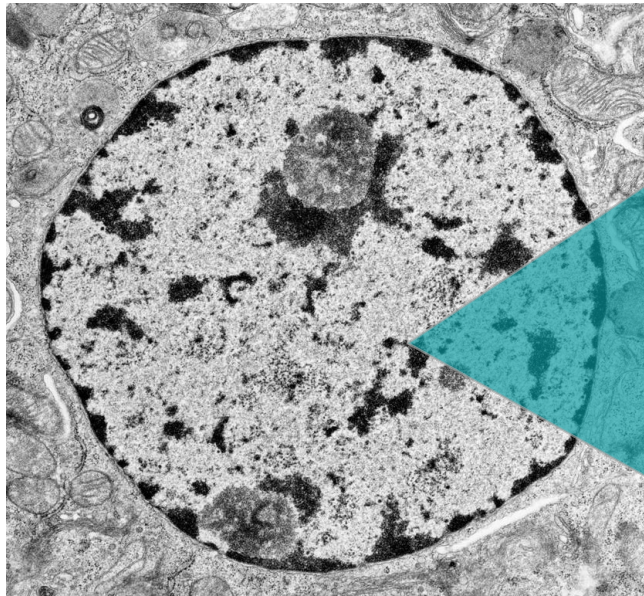
Adapted from Stroud et al., 2013



M. Celi

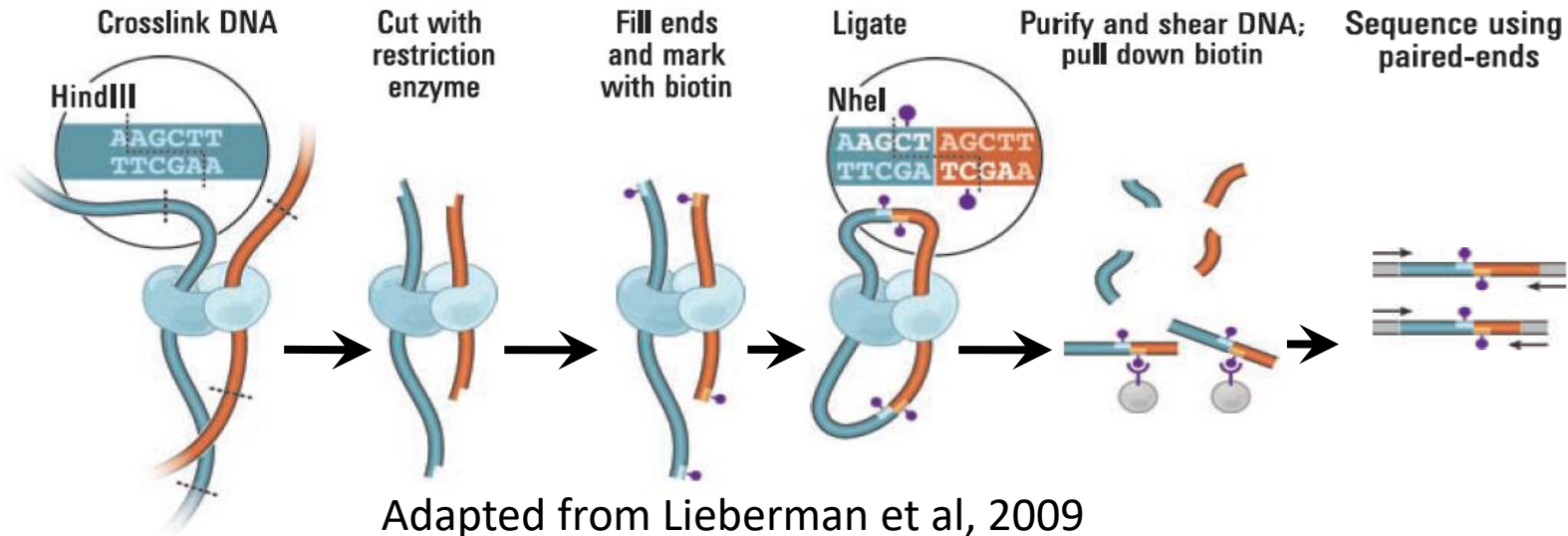
Looking at the Global Chromatin Environment with Hi-C

Hi-C: a method of inferring the global **three-dimensional** relationships from paired-end sequence data



Hi-C Methodology

In the lab:



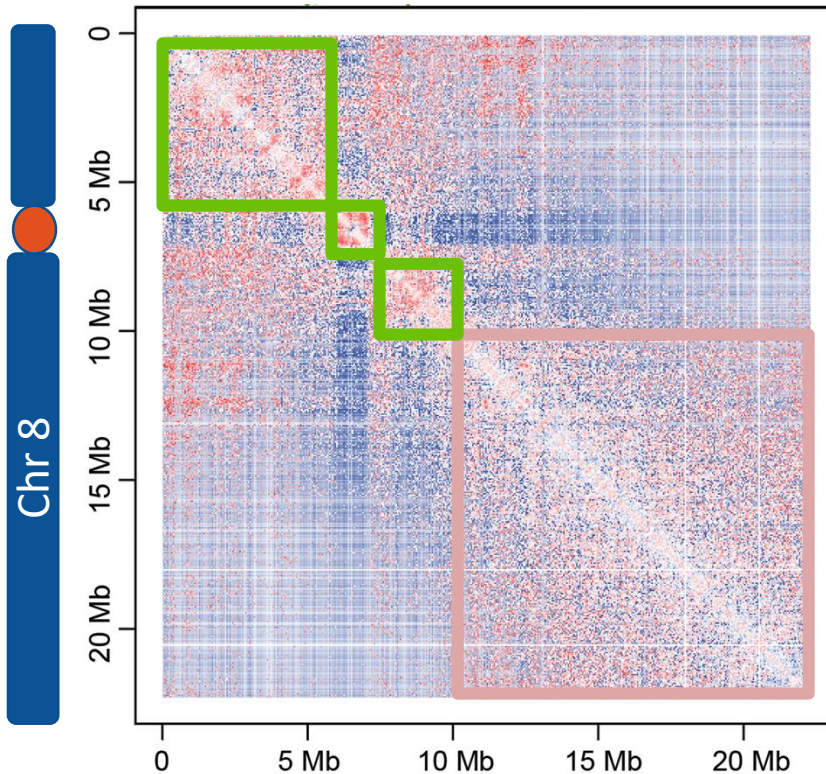
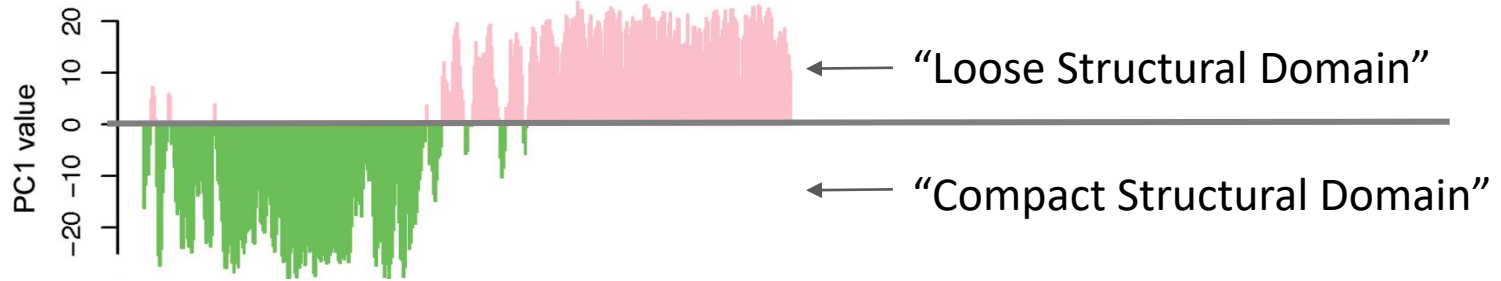
In silico:



HOMER (v4.8, 1-13-2016)

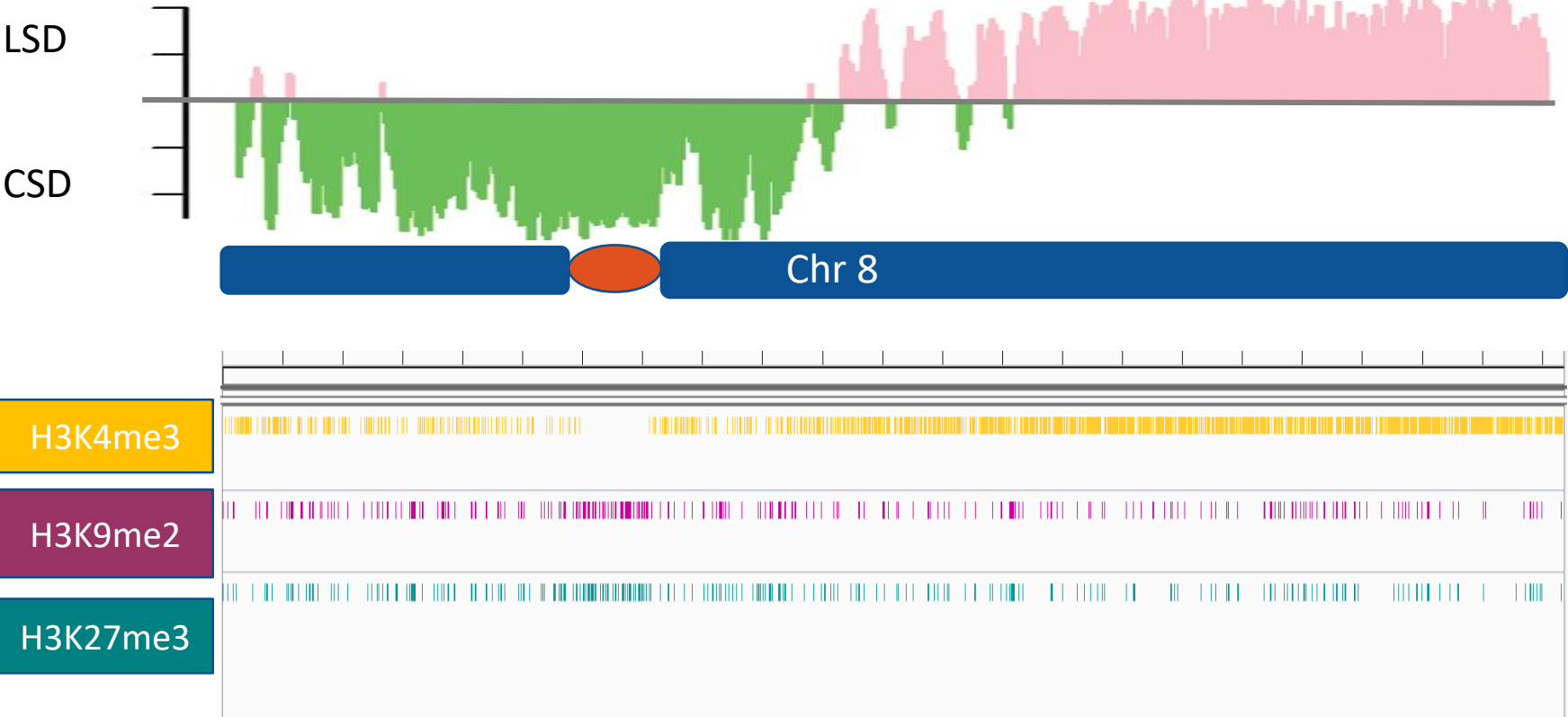
Software for motif discovery and next generation sequencing analysis

PCA identifies Structural Domains in Vitis chromosomes

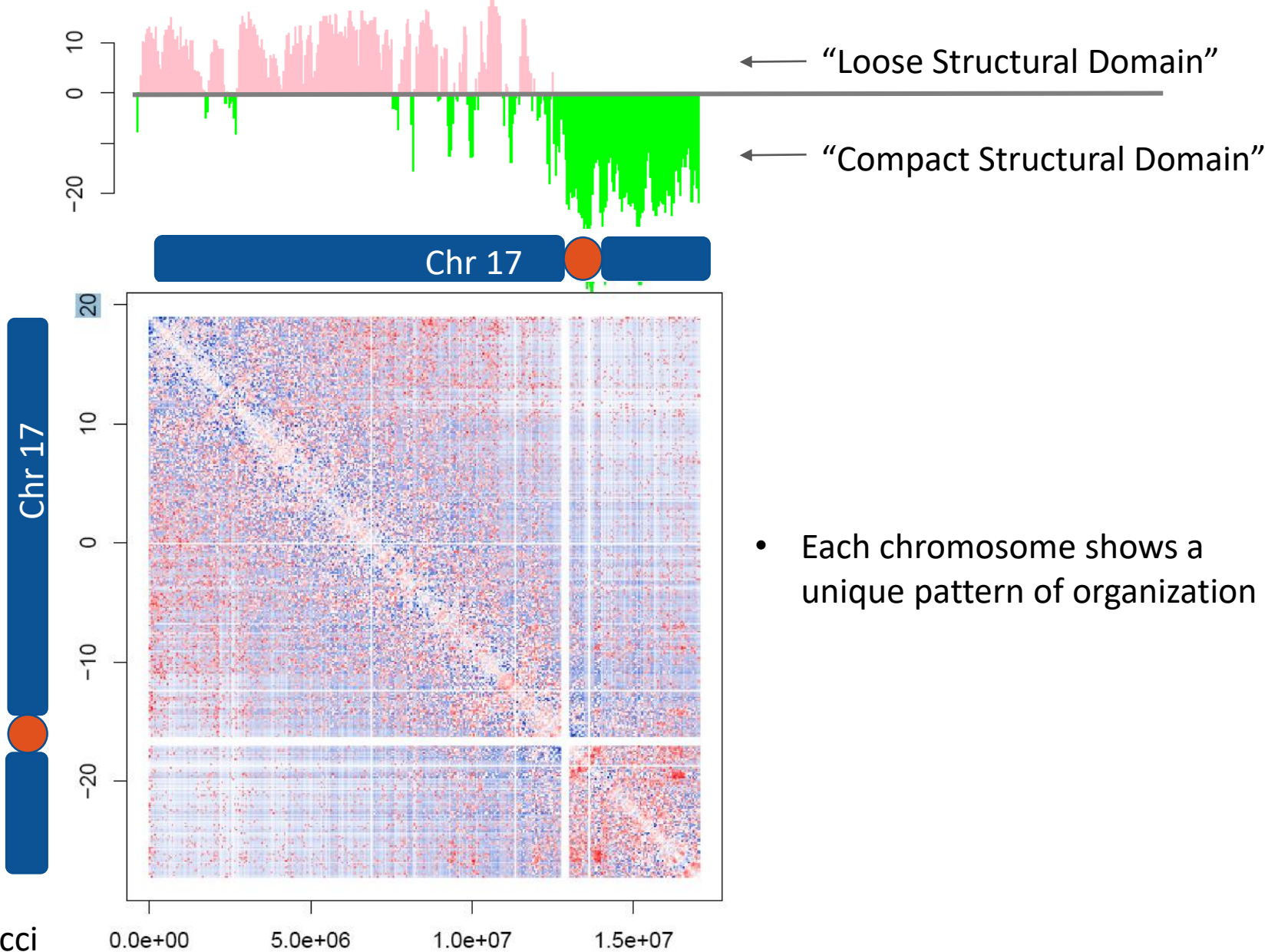


- Each chromosome shows a unique pattern of organization

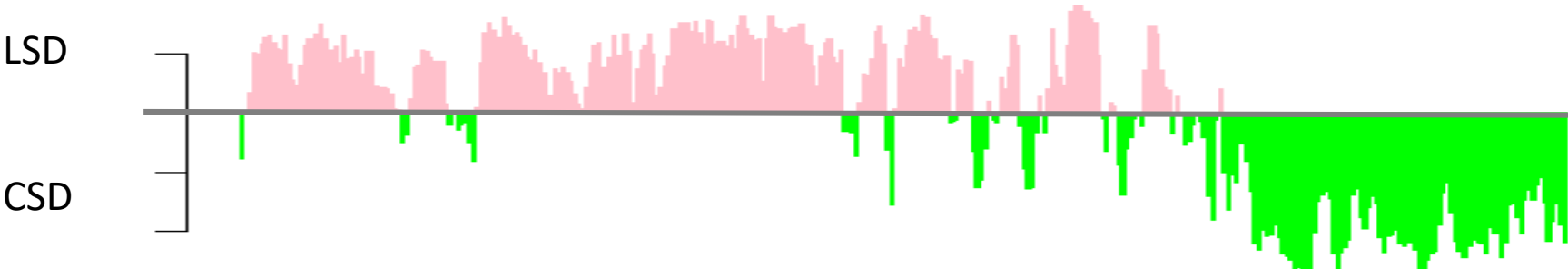
ChIP data corresponds with 3D structure



PCA vs Heatmap vitis chr17



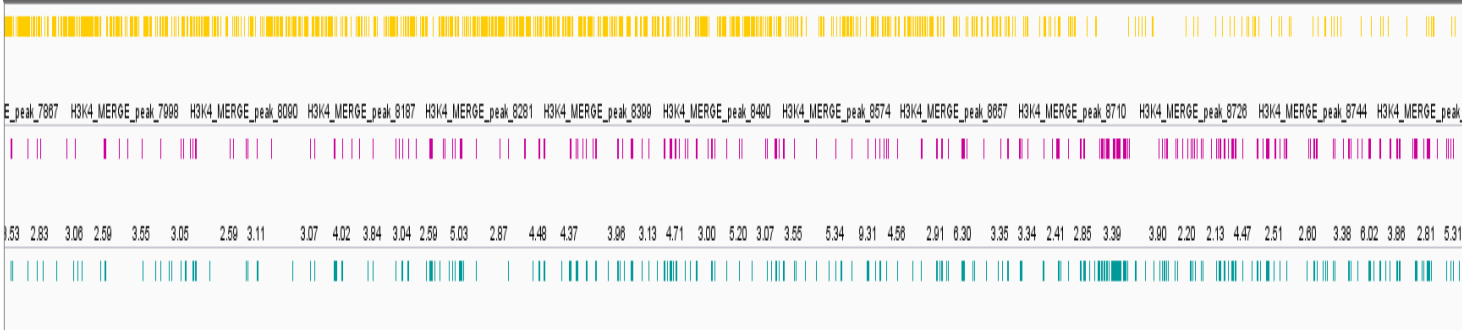
ChIP data corresponds with 3D structure

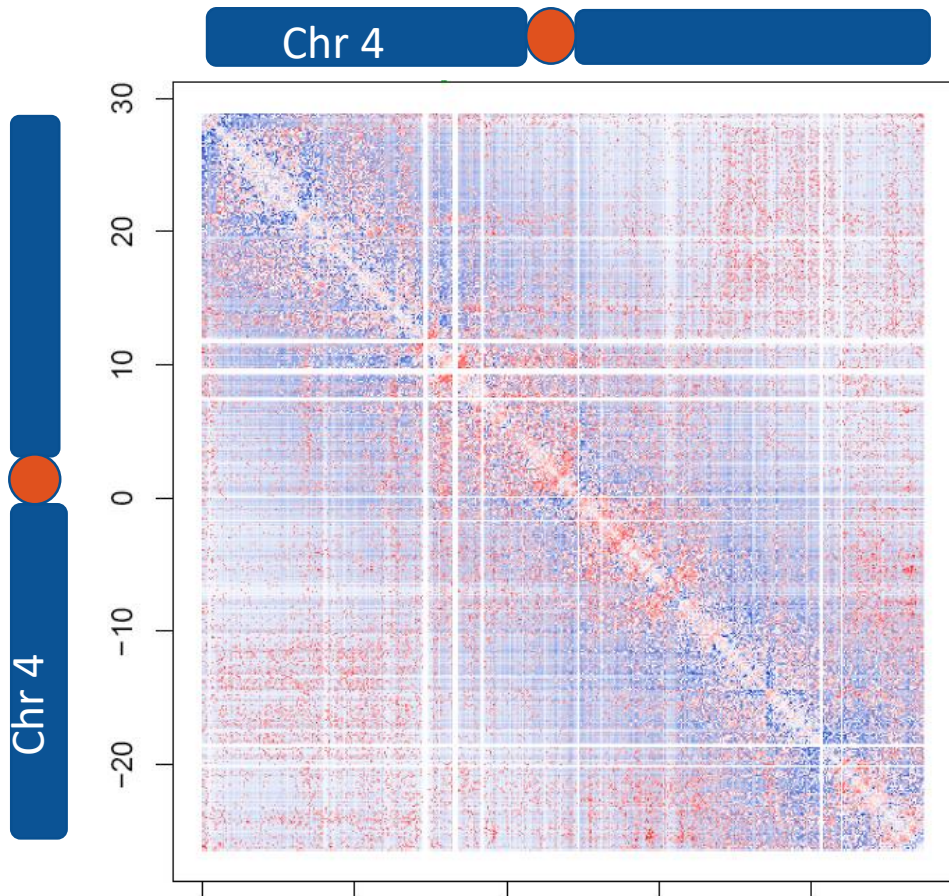
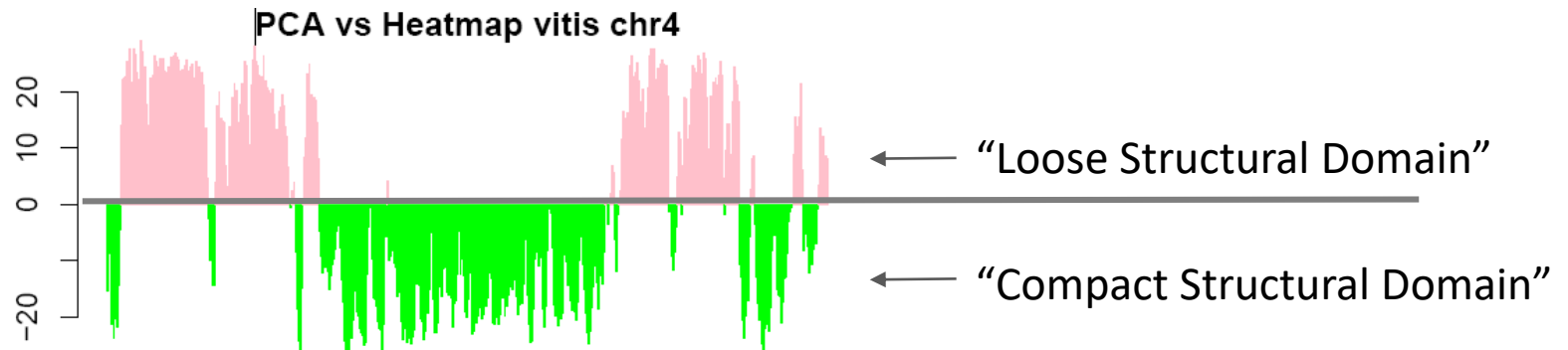


H3K4me3

H3K9me2

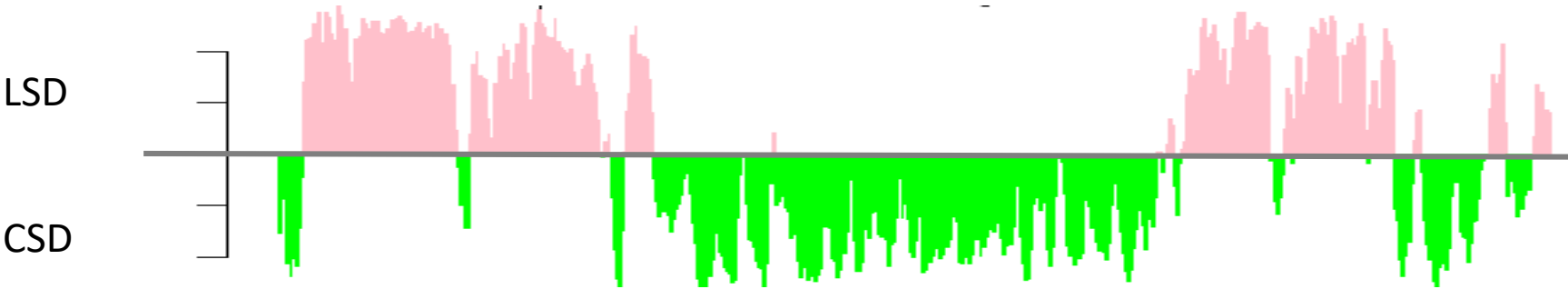
H3K27me3





- Each chromosome shows a unique pattern of organization

ChIP data corresponds with 3D structure

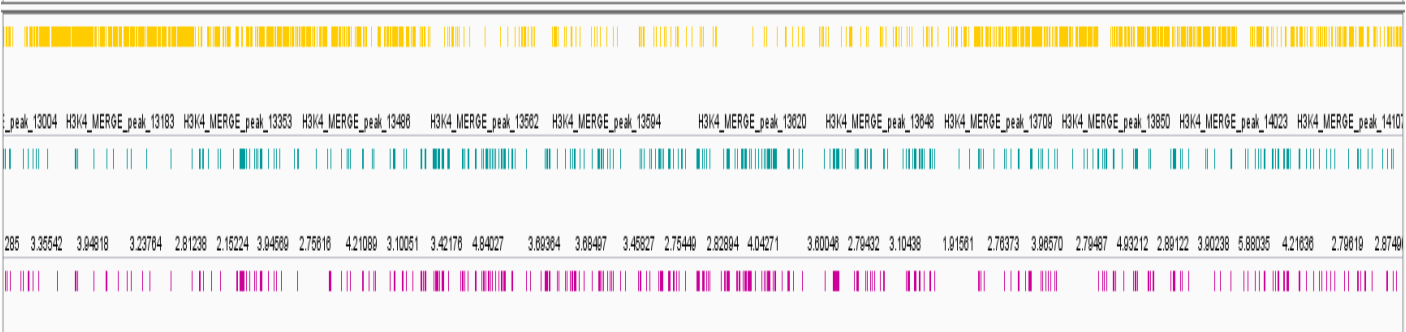


Chr 4

H3K4me3

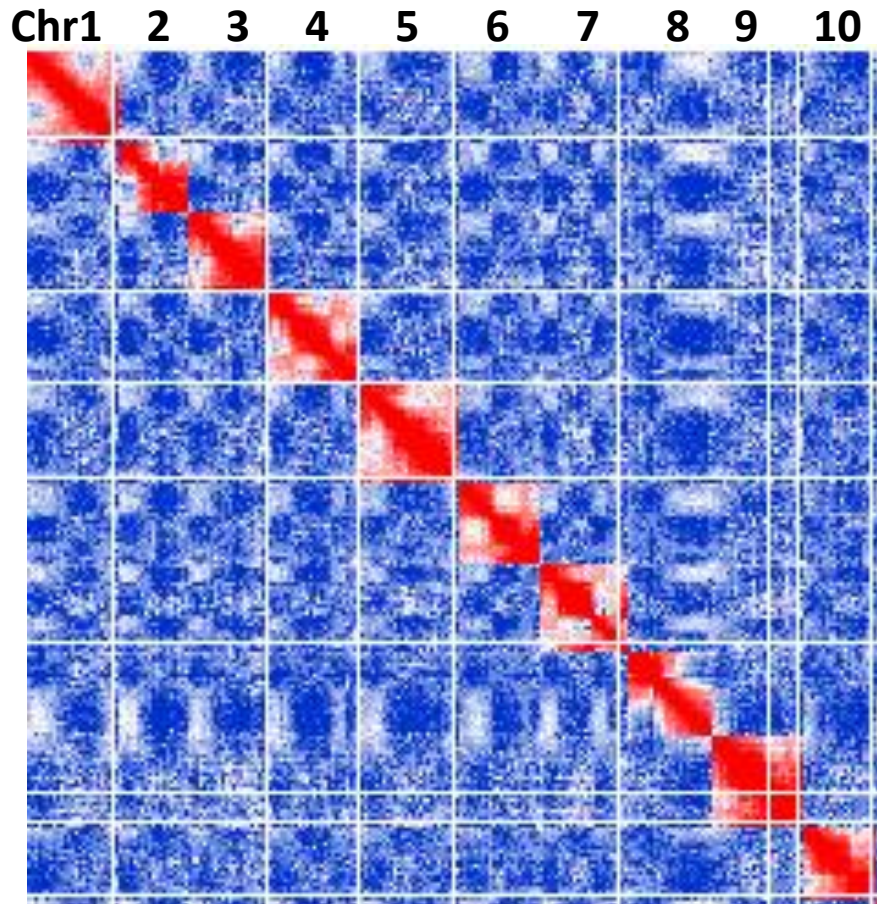
H3K27me3

H3K9me2

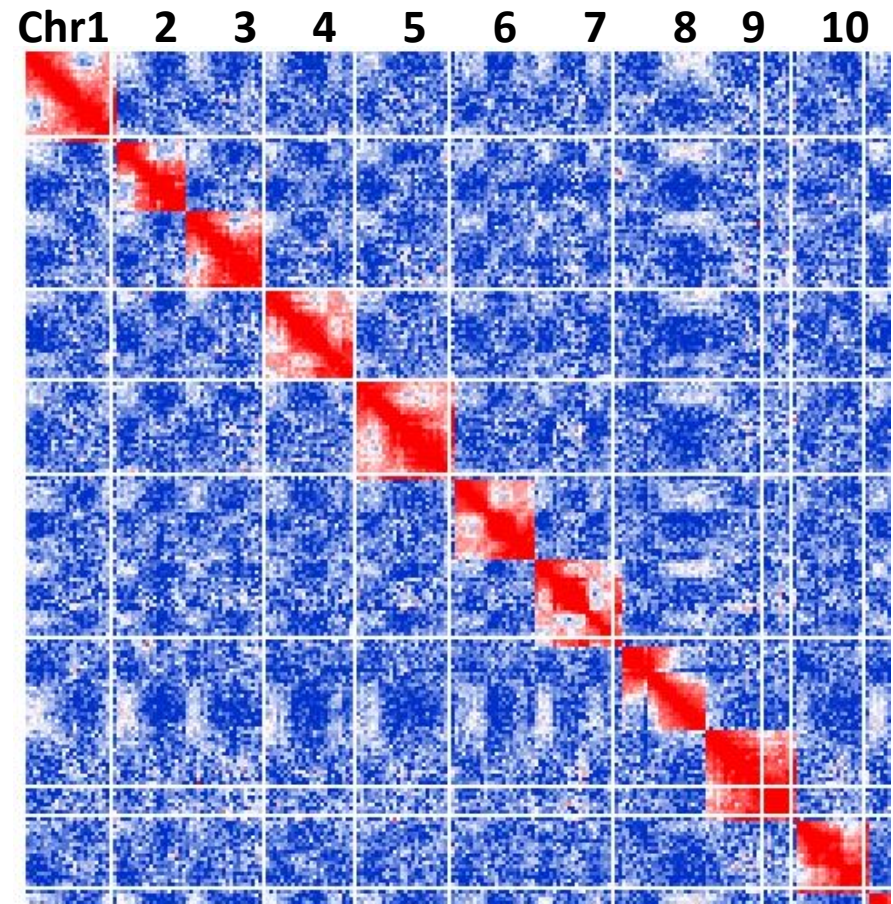


Chromatin Organization is Highly Stable

Biological Replicate 1

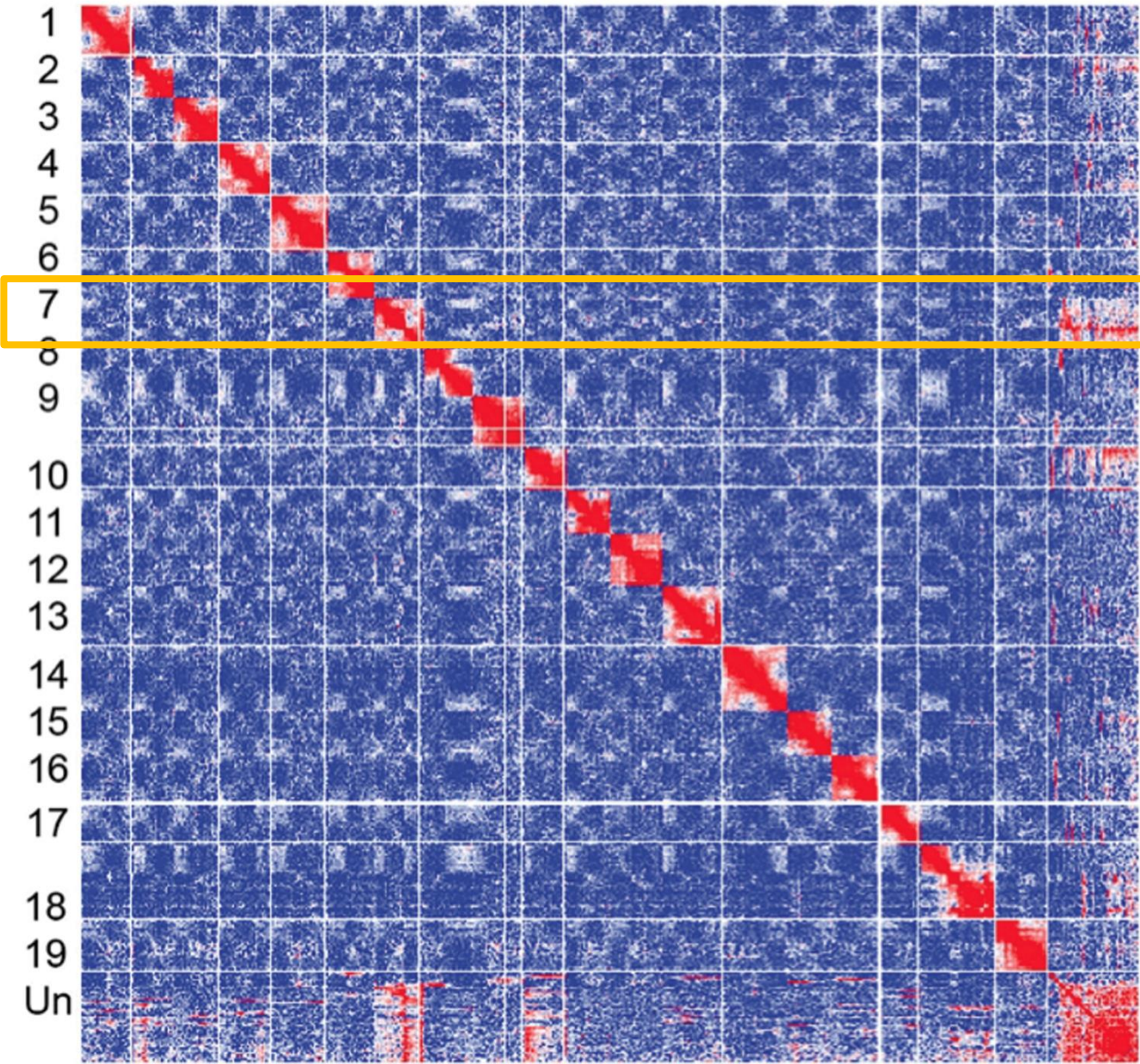


Biological Replicate 2



Pinot Noir Genomic Contact Map

Chr 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | Un



Easily visualize the actual locations of unassigned regions of the genome assembly

Conclusions and Future Directions

1. Much to uncover with ChIP-Seq

- H3K4me3 results = high confidence
- Allele specific analysis in progress
- Other histone modifications will require finer analyses

2. Hi-C shows chromatin structure very stable

- Try varieties other than Pinot Noir – do they have the same organization?
- Use Hi-C to improve genome assembly (in progress - Aldo Tocci)



Acknowledgements



IGA

Prof. Michele Morgante
Emanuele de Paoli
Fabio Marroni
Aldo Tocci
Mirko Celi
Mara Miculan
Gabriele Magris

IGA Technology Services

Emanuela Aleo
Federica Magni
Giacomo Prete

Lab and Administration

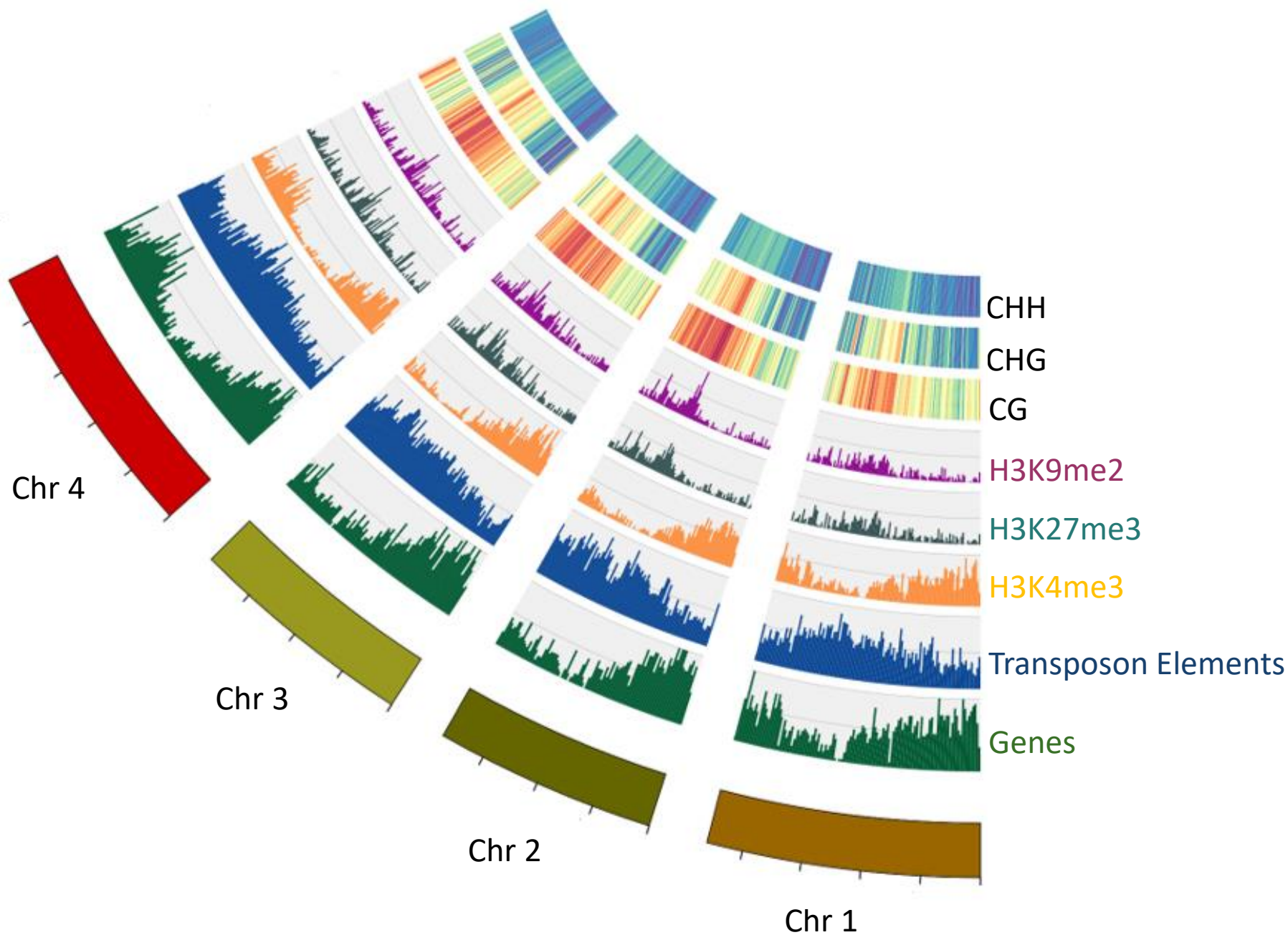
Giusi Zaina
Nicoletta Felice

Stefano Grasso

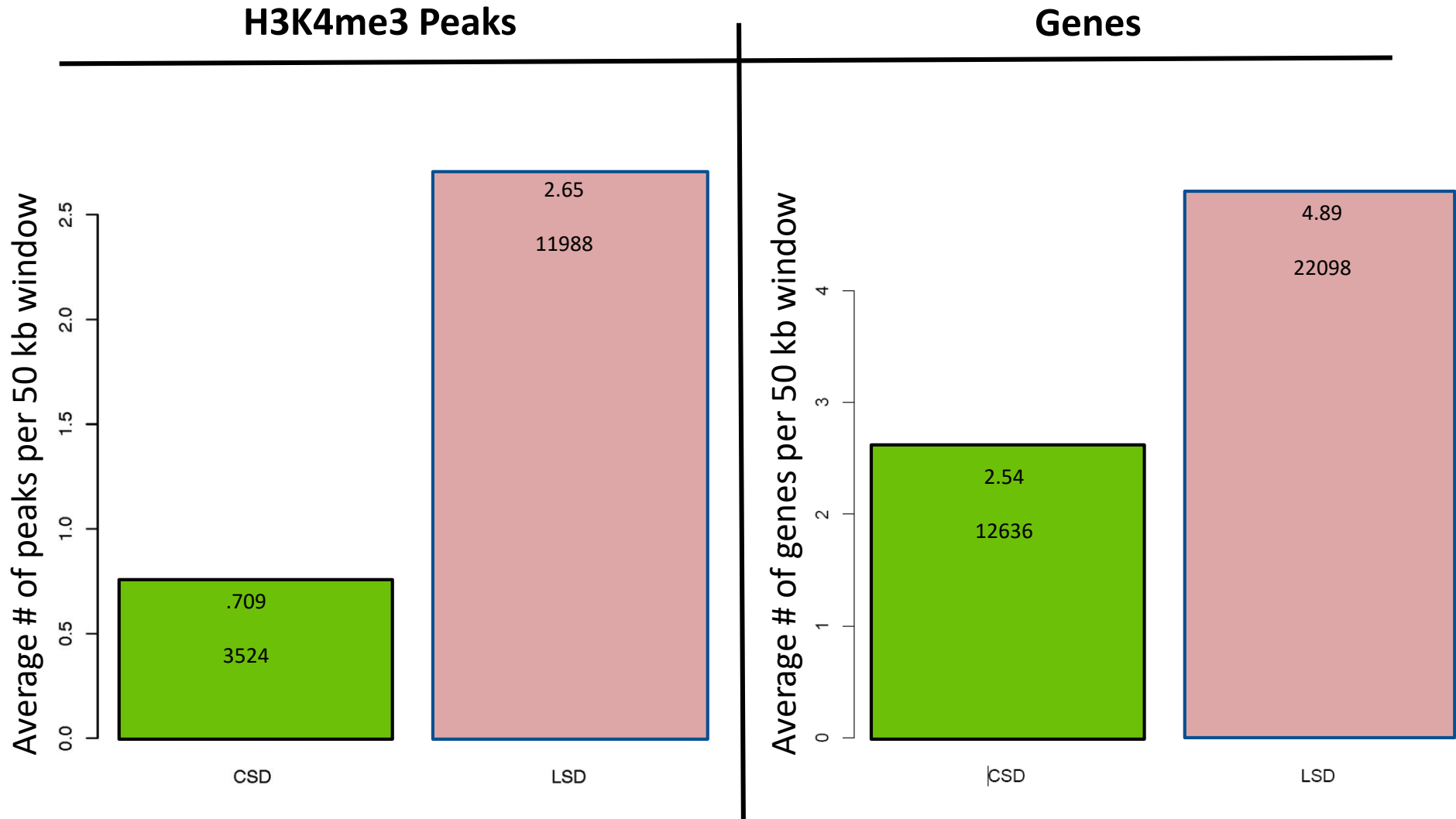


European Research Council

Supported by the ERC project NOVABREED - Novel variation in plant breeding and the plant pan-genomes (Grant agreement no.: 294780)

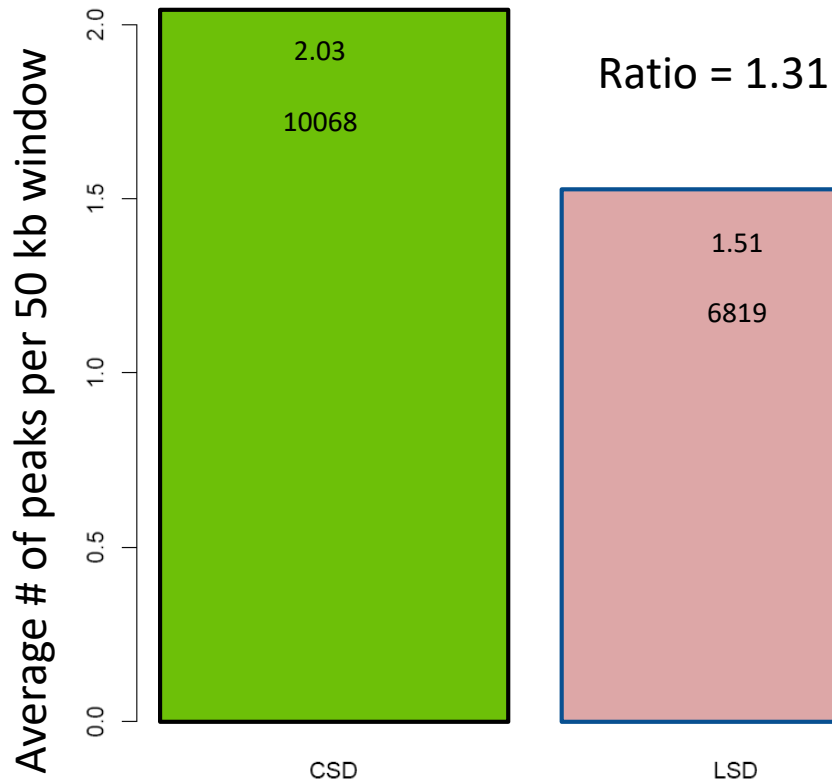


Hi-C shows H3K4me3 is located preferentially in Loose Structural Domains

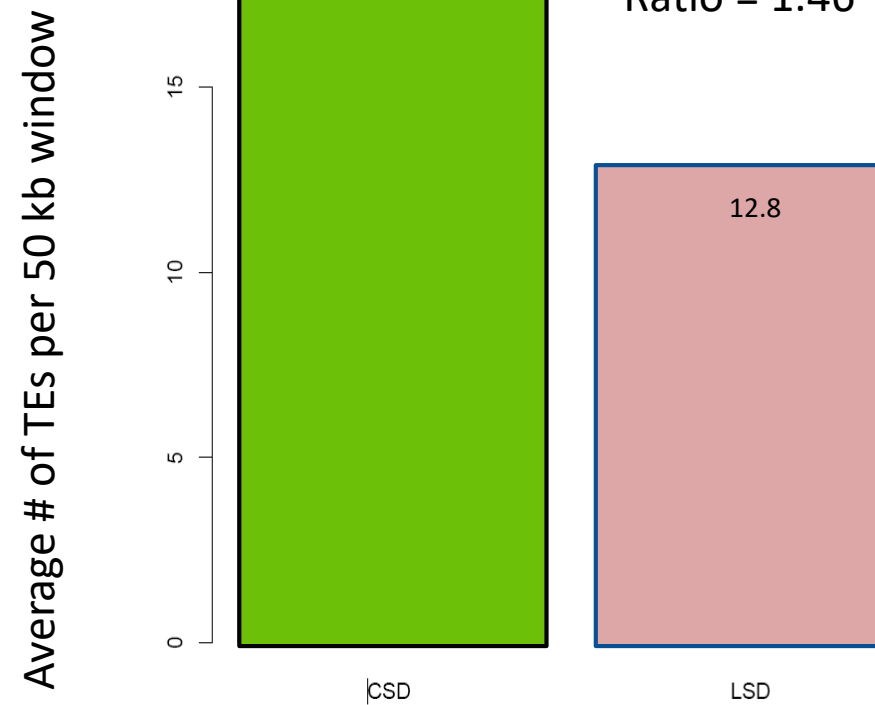


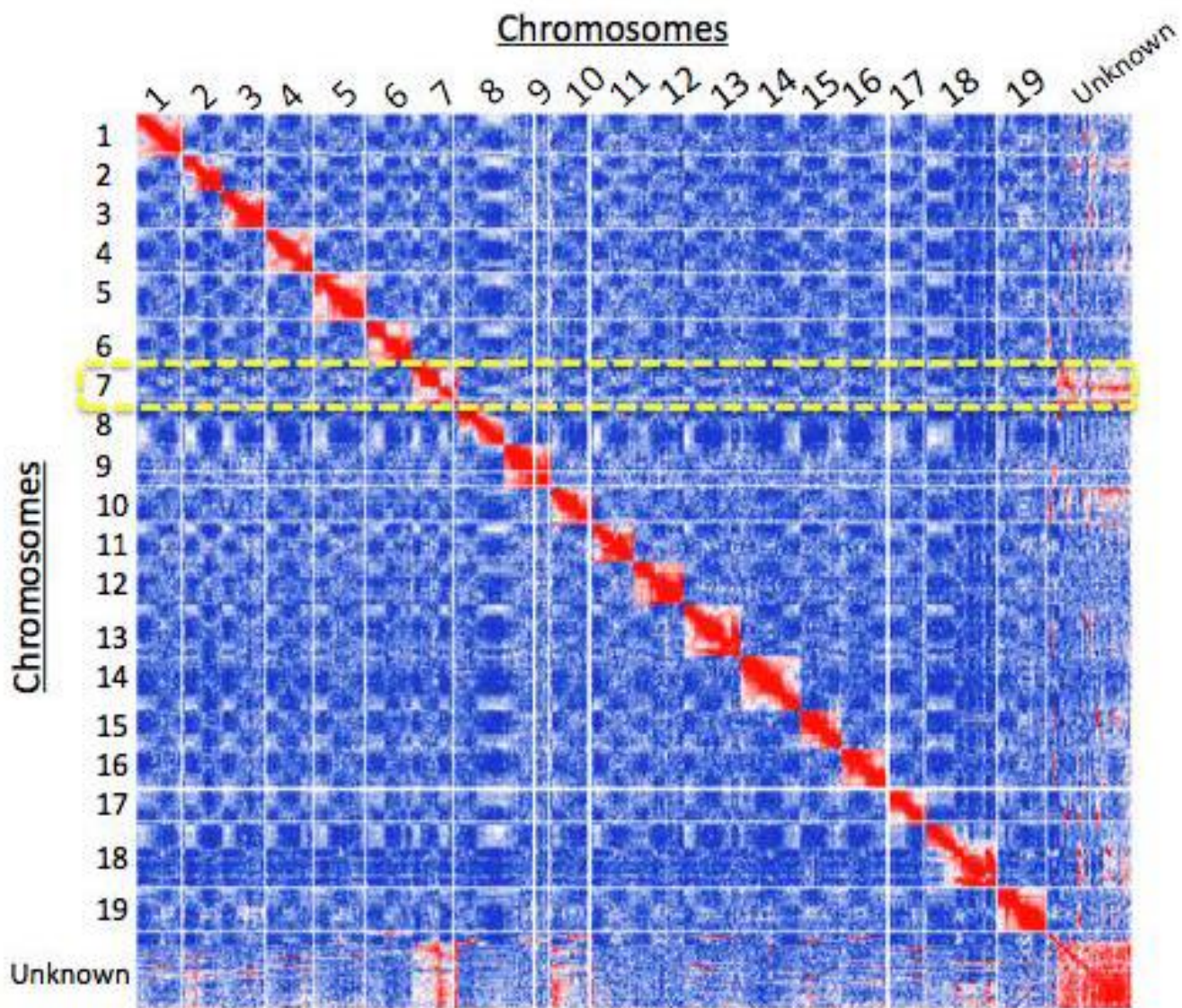
H3K9me2 distribution mimics that of total TEs

H3K9me2 Peaks



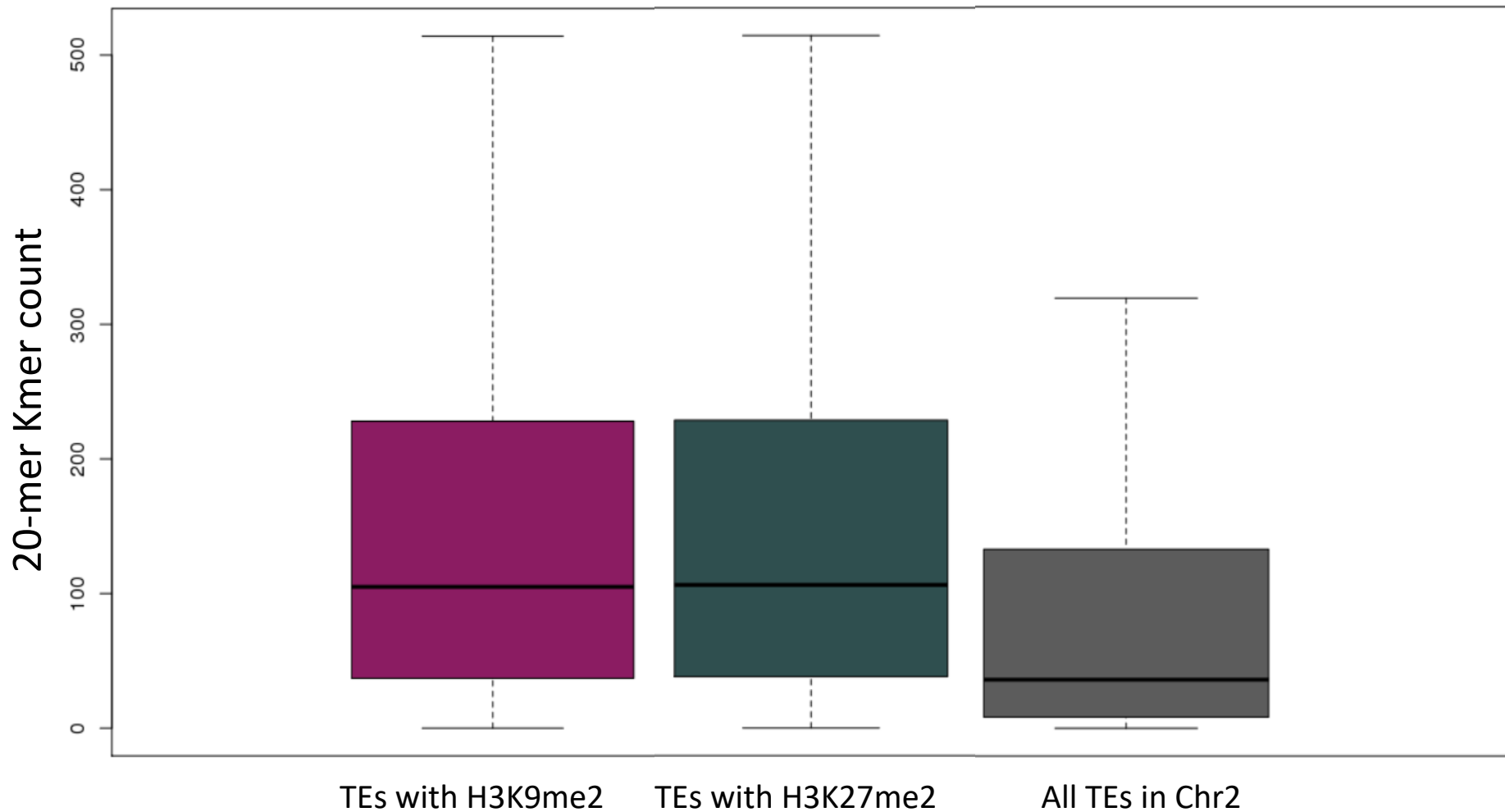
Transposon Elements



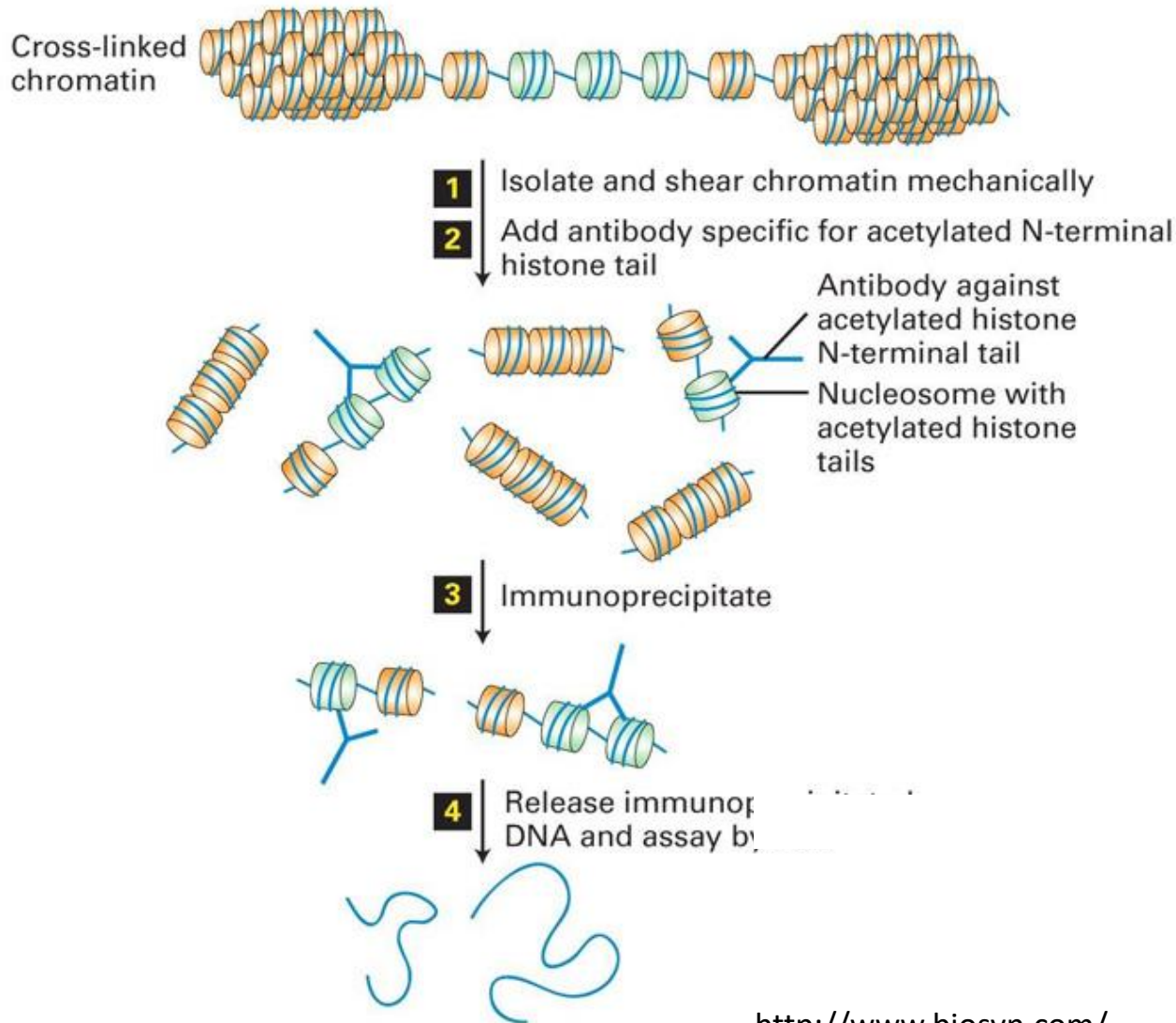


Transposons with K9me2 or K27me3 have higher k-mer count

K-mer count of TEs from Chromosome 2

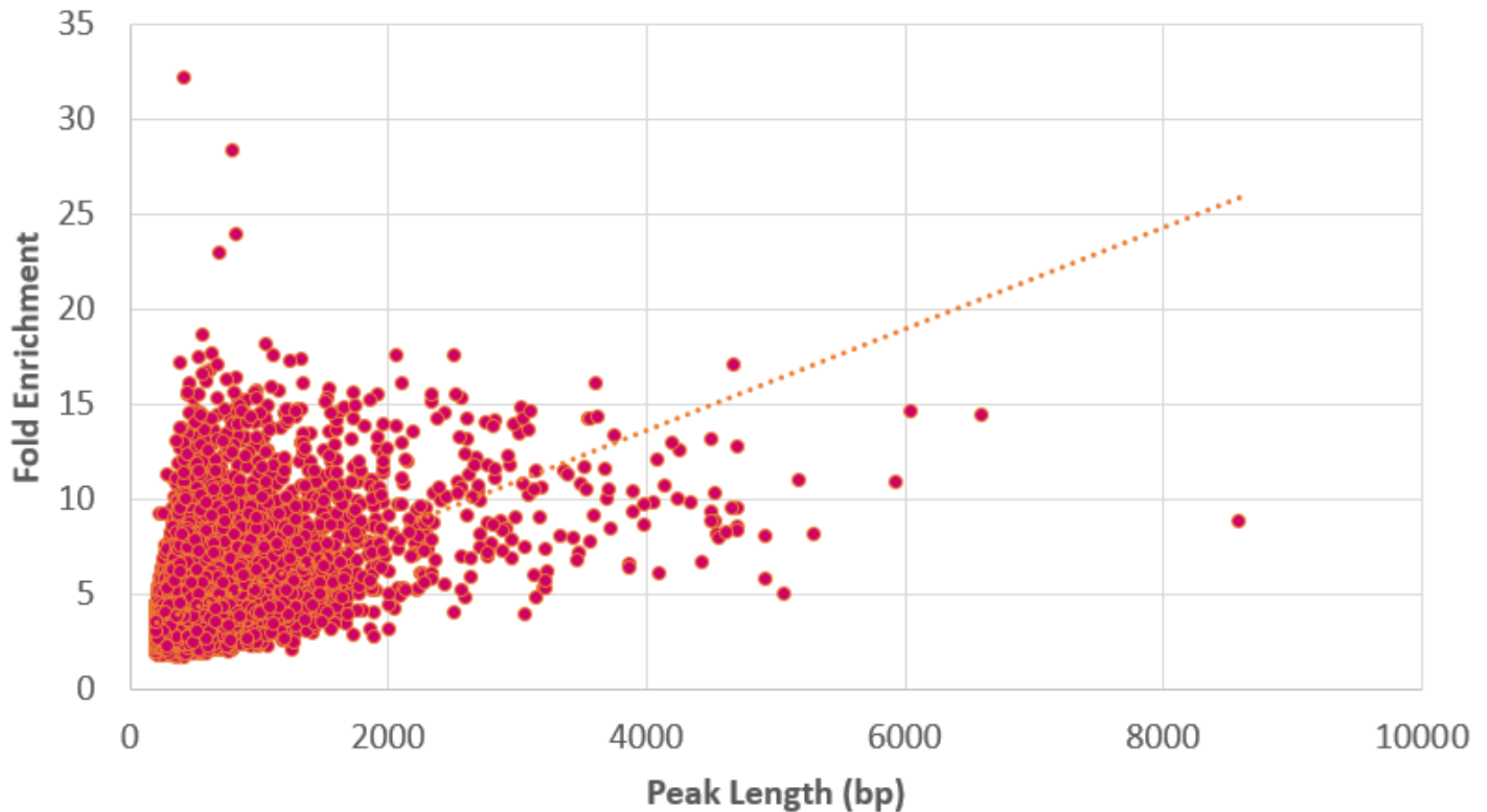


Enriching for Antibody-associated DNA with Chromatin Immunoprecipitation



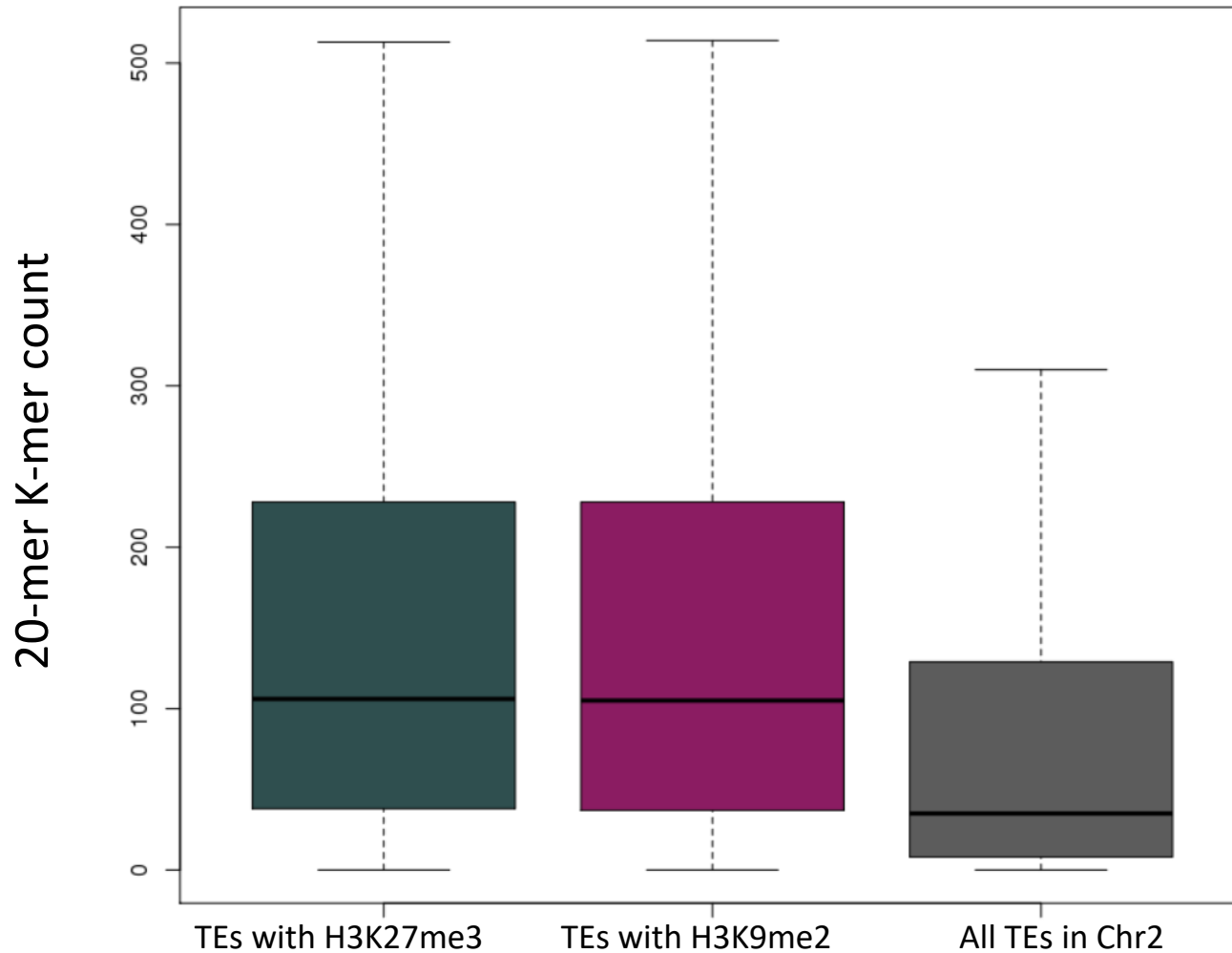
H3K9me2 Enrichment generally increases with Peak Size

H3K9me2 Peak Fold Enrichment vs. Peak Length

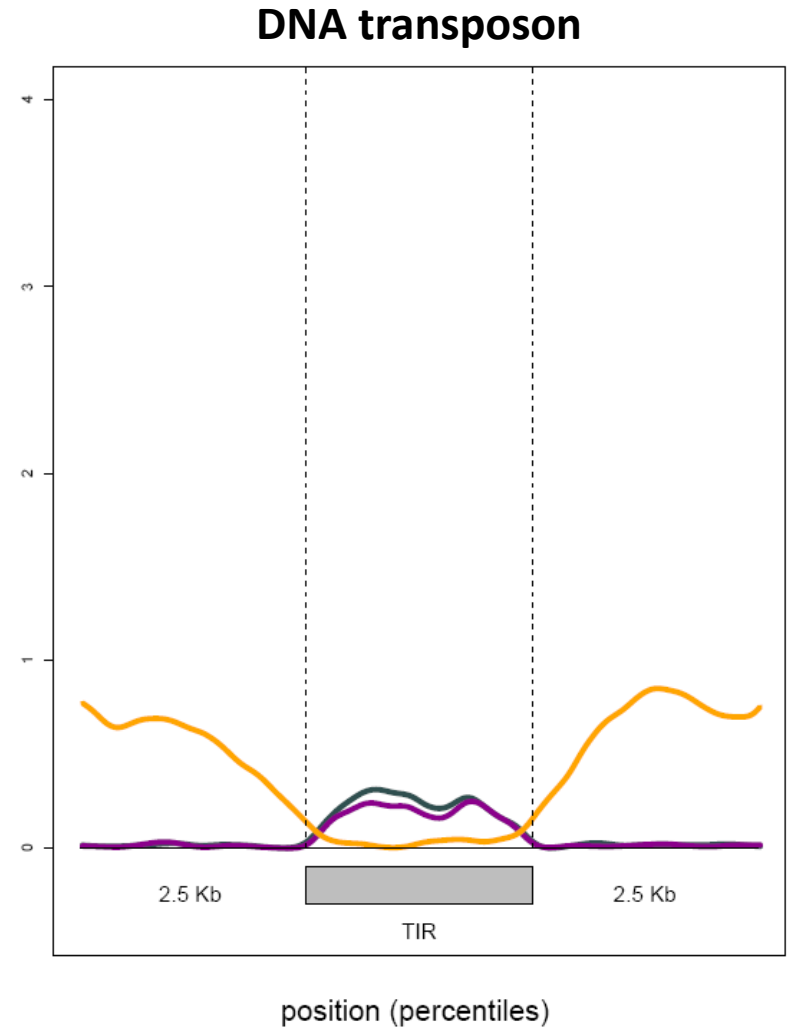
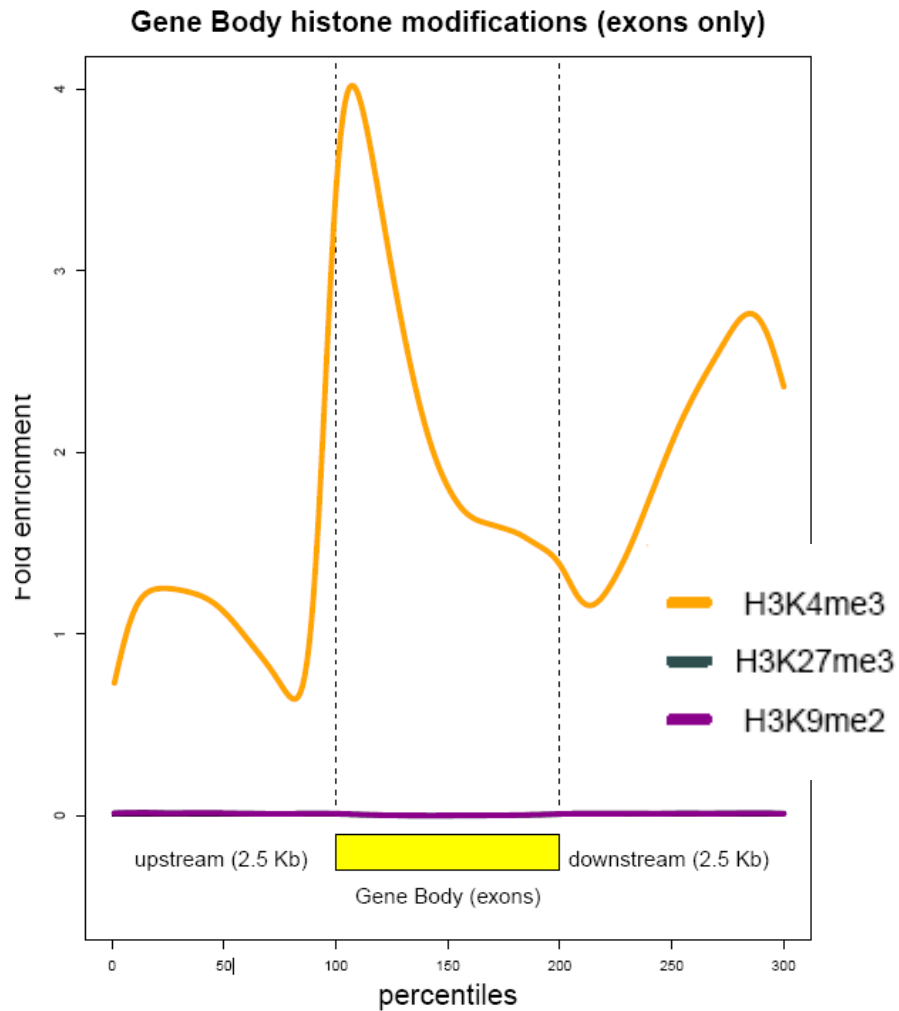


Transposons with K9me2 or K27me3 have higher k-mer count

K-mer count of TEs in Chromosome 2



How do heterochromatic modifications at histones relate to DNA methylation?



How do heterochromatic modifications at histones relate to DNA methylation?

