Analysis of human and mouse expressed genes enriched by H3K9me3 histone modification

Sidorov Svyatoslav

Scientific advisor:

Alexander Predeus, Bioinformatics Institute

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Outline

1. Introduction.
2. My master thesis in brief.
3. The current project.
4. Current results.
5. Perspective.

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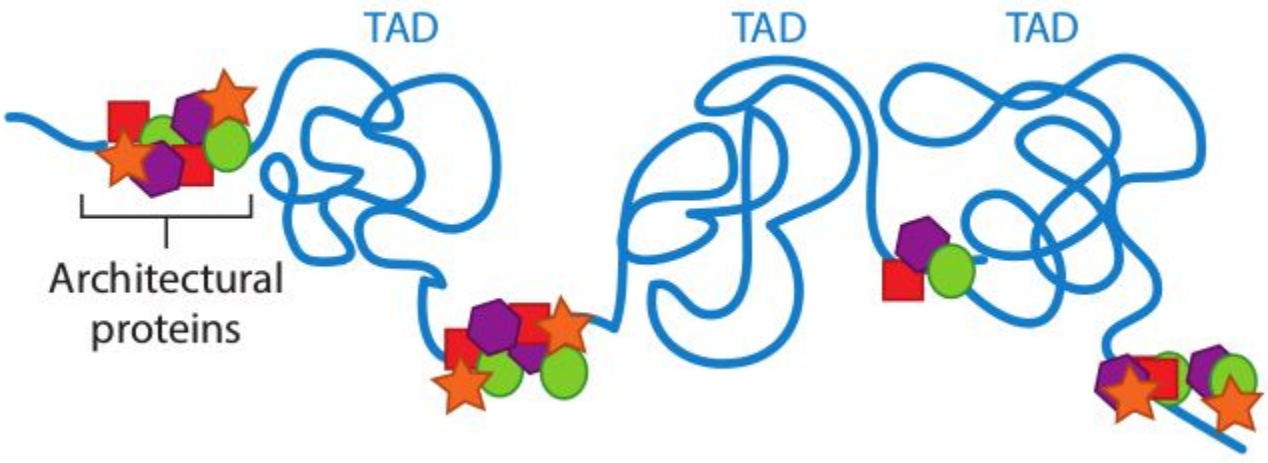
Topologically associating domains

Topologically associating domains (TADs) are such regions of chromatin that frequency of intra-TAD interactions is higher than frequency of inter-TAD interactions.



Adapted from *Nguyen H. Q.* and *Bosco G.*, Annual Review of Genetics. 2015, 49:627-646.

Topologically associating domains

TADs as functional domains in mammals:

- TAD borders are enriched in active transcription, housekeeping genes, tRNA genes and SINE repeats, as well as binding sites for the architectural proteins CTCF and cohesin.

See *Dekker J. and Heard E.,* FEBS Letters. 2015, 589(20 Pt A):2877-2884.

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- TADs are units of coordinated gene expression.
- Series of adjacent TADs correspond to replication domains.
- TAD borders are to a significant extent conserved between different cell types, and even between mouse and human.

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Chromatin modifications

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Some of these modifications are well established markers of specific genomic loci, e. g.,

- **H3K36me3** and **H3K79me2** mark actively transcribed genes;
- **H3K9me3** and **H3K27me3** mark heterochromatin;
- **H3K4me3** marks promoters of transcribed genes.

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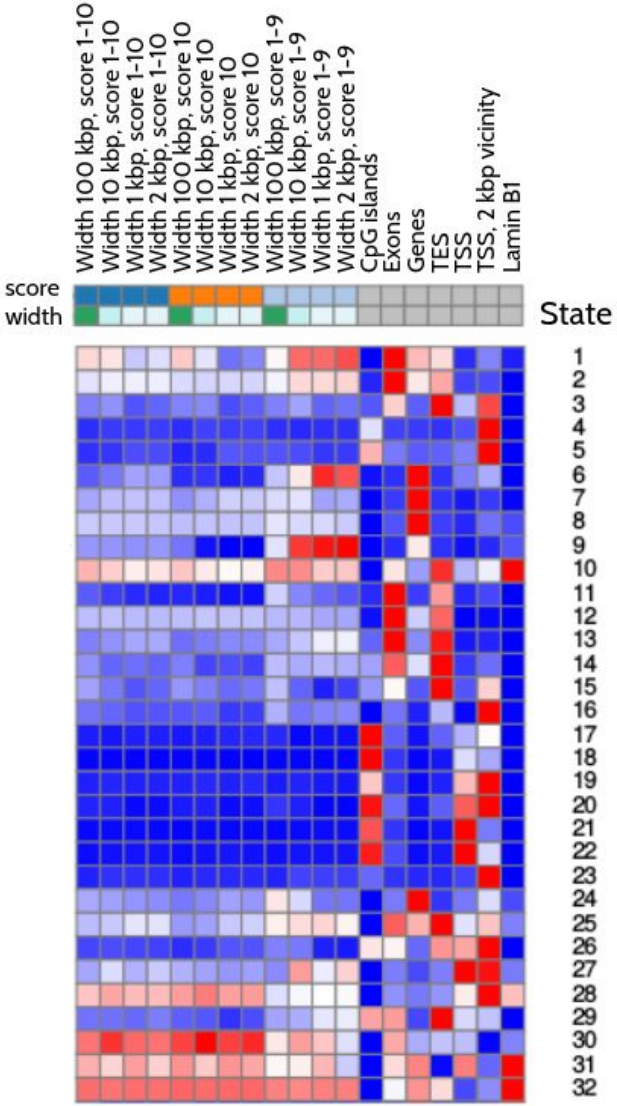
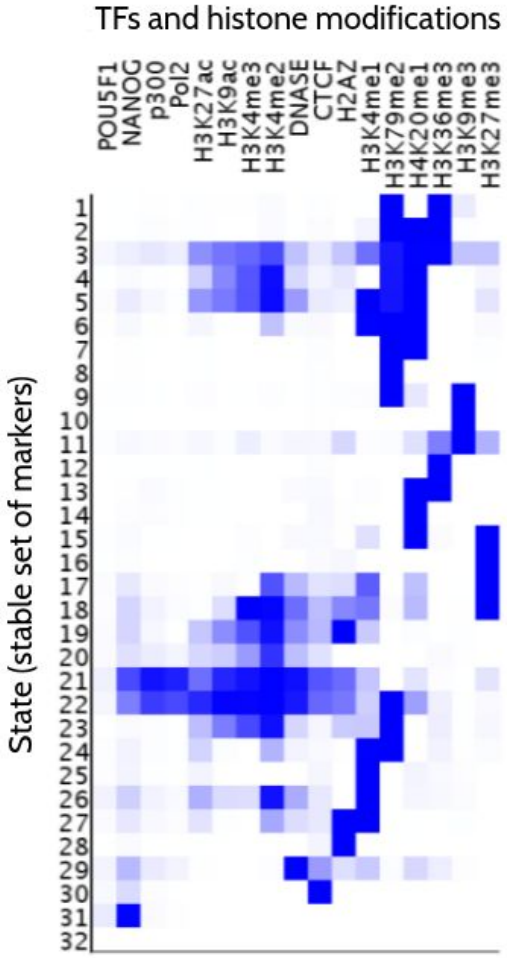
- Process Hi-C data to obtain genome segmentation into TADs and TAD borders.
- Find TAD border enrichments by the stable combinations and some genomic features.

The main result





TAD border is strong if it nearly prohibits contacts over itself, and weak otherwise. Border strength is assessed with a score from 1 (the weakest) to 10 (the strongest).

Here we call borders with score 10 ‘strong’ and all other borders - ‘weak’.

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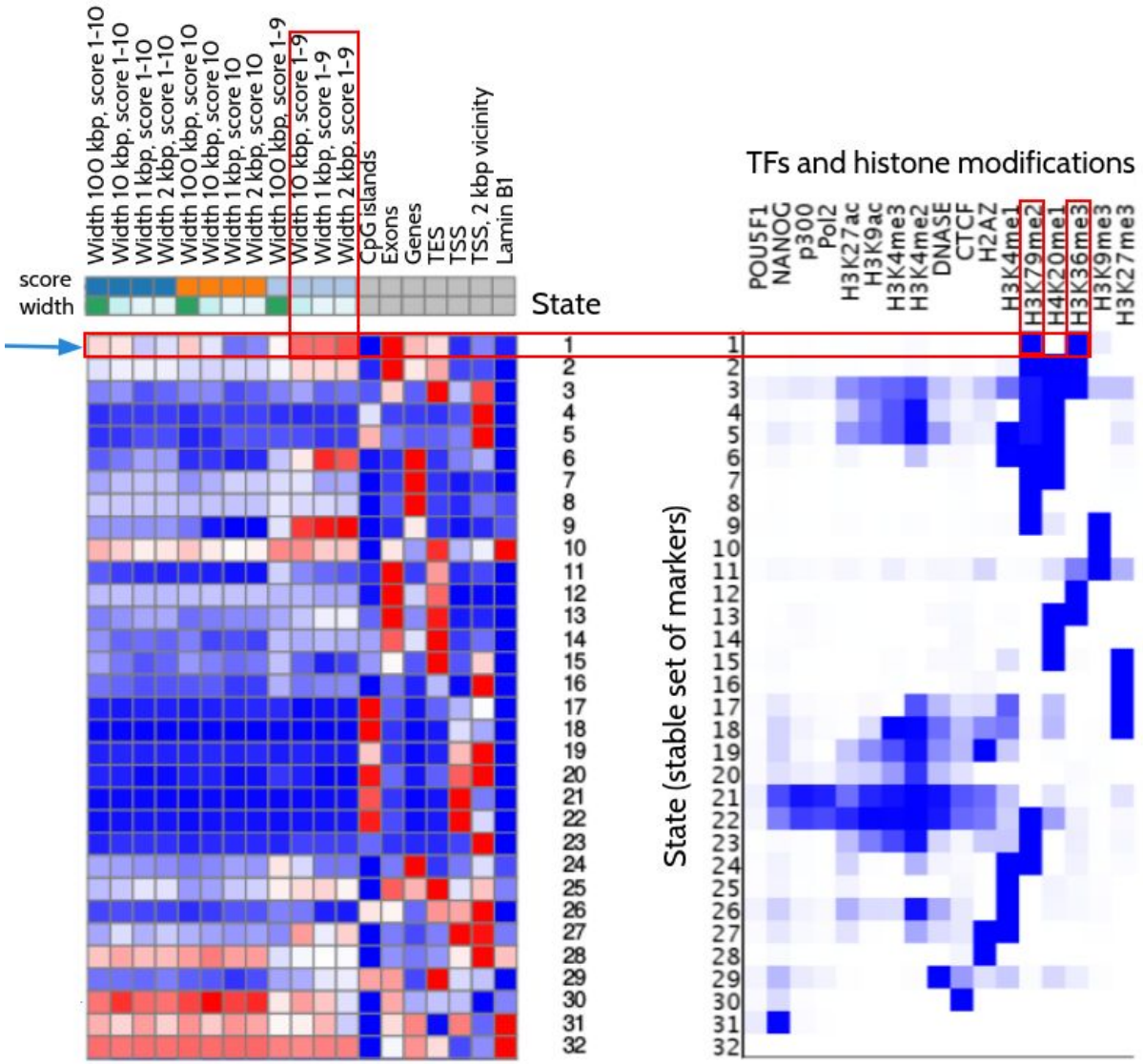

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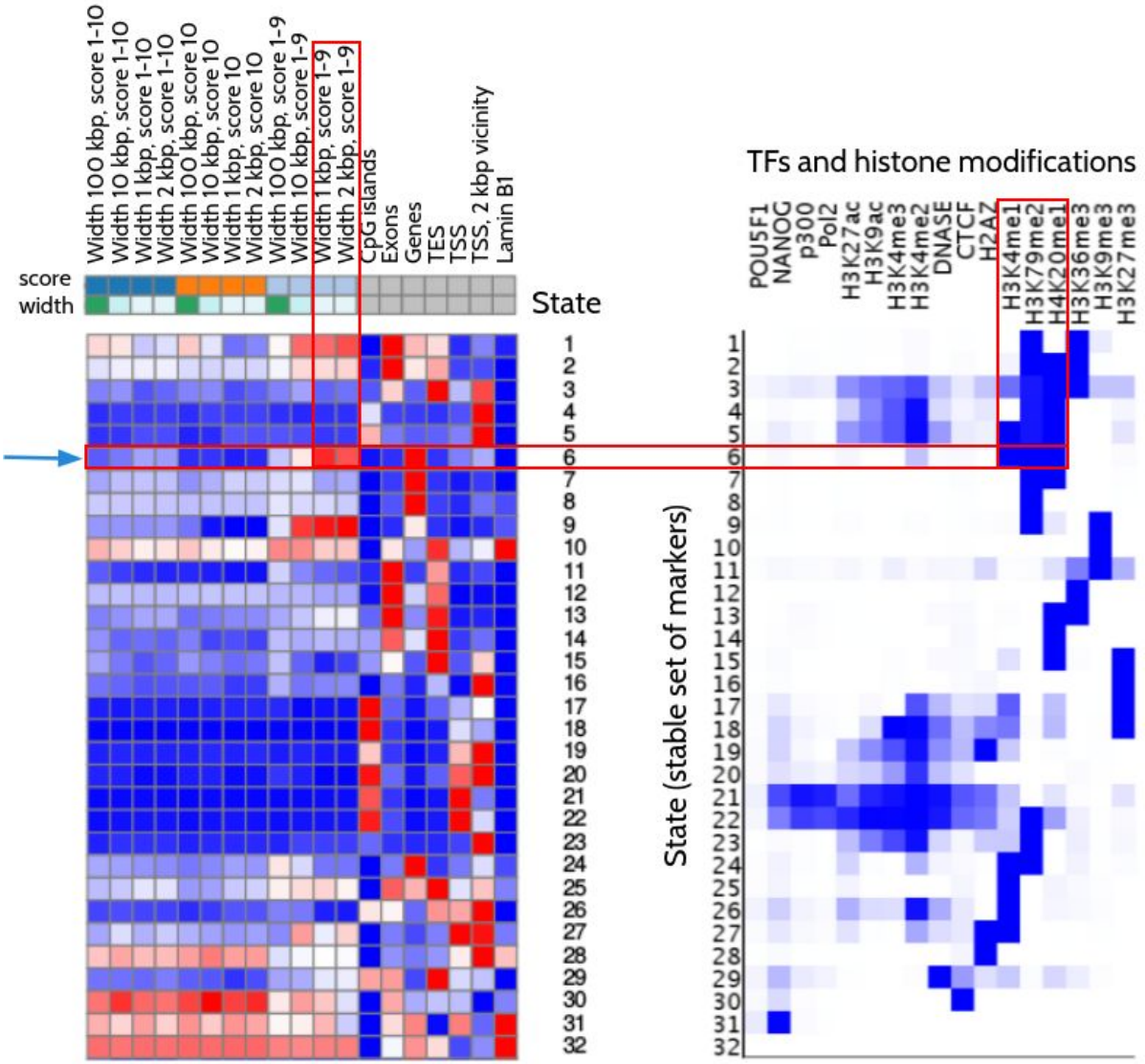

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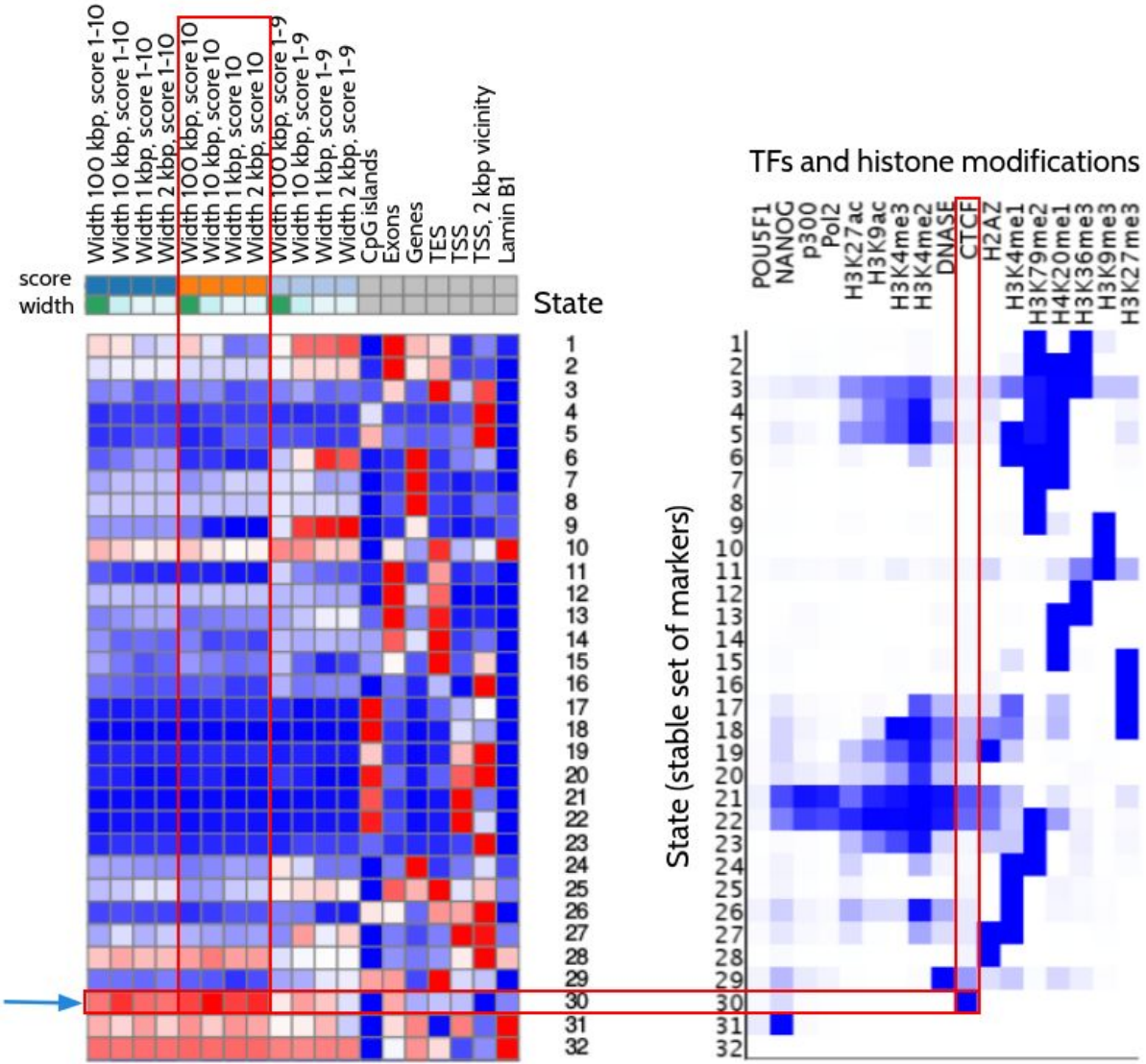

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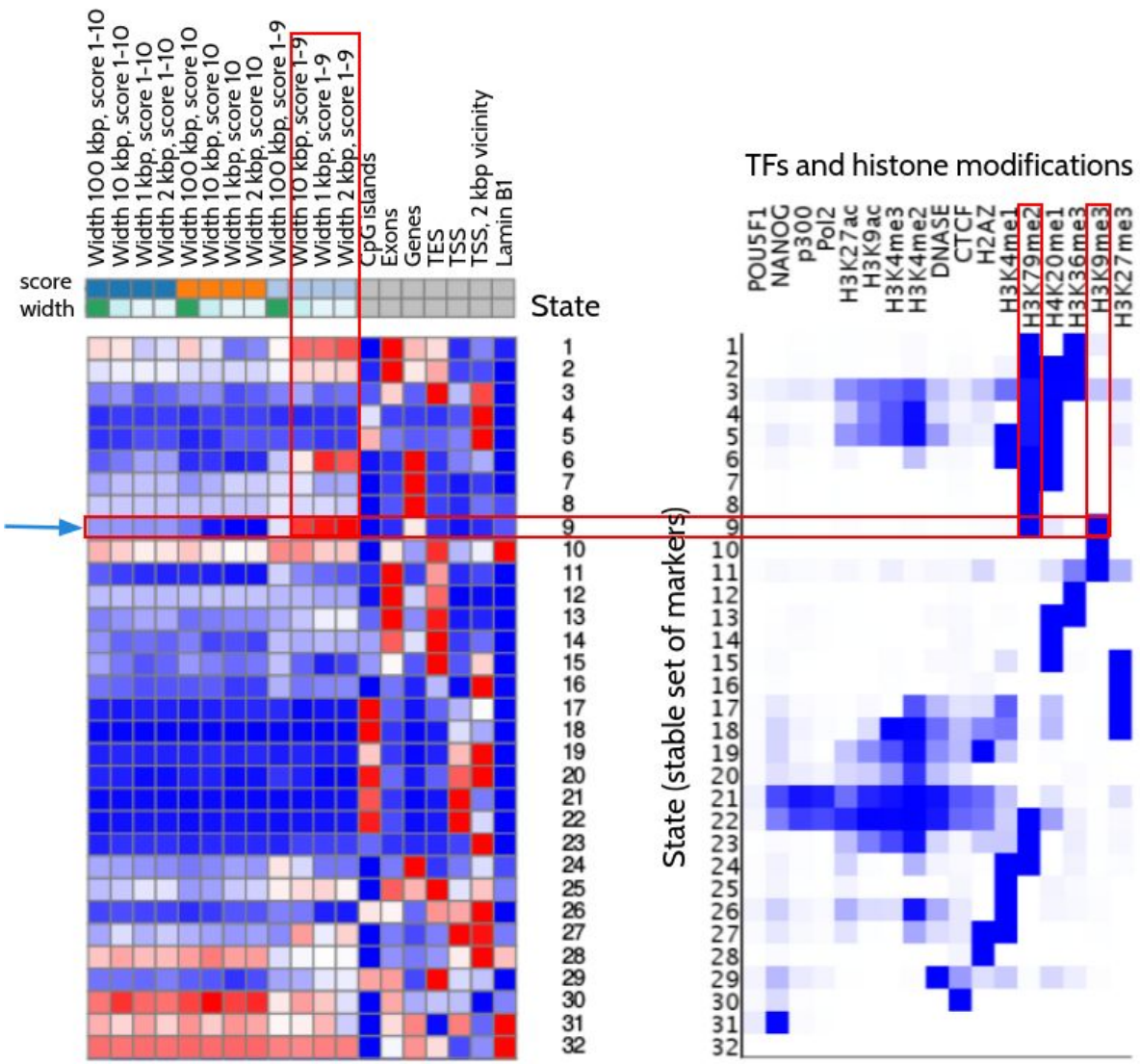

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Also, weak borders are enriched by state 9 that contains H3K9me3 and H3K79me2 at the same time.

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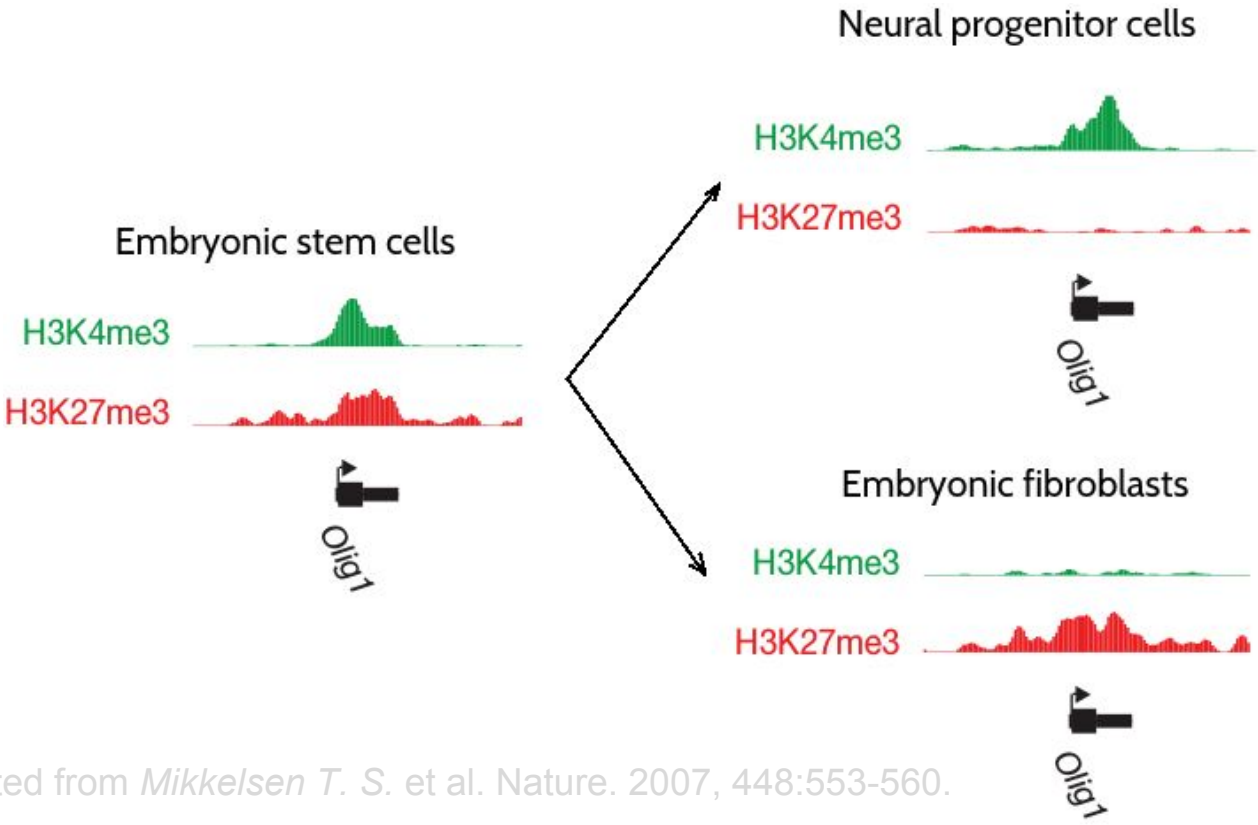
First hypothesis

Since H3K79me2 is an active modification, and H3K9me3 is a repressive modification, we considered the possibility that the combination **H3K79me2 + H3K9me3 marks a kind of bivalent promoters** (similar to the well known combination H3K4me3 + H3K27me3):



Adapted from *Mikkelsen T. S.* et al. Nature. 2007, 448:553-560.

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So, we selected one cell line (with ChiP-seq and Hi-C data) from each of the primary germ layers:

- **HeLa-S3** (a clonal derivative of the parent HeLa line; cervix adenocarcinoma) from ectoderm;
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Also we found data for murine embryonic stem cells (**mESC**) to compare them with human embryonic stem cells (**hESC**) regarding H3K79me2 + H3K9me3 combination.

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ChIP-seq data for hESC-H1, HeLa-S3, HUVEC, IMR90: H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K27ac, H3K36me3, H3K79me2, H3K27me3, H3K9me3, RNA Pol II, H2A.Z, DNase, P300, CTCF. For mESC: the same except H3K4me2 and H2A.Z.

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5. Find stable sets of marks (states) with ChromHMM.

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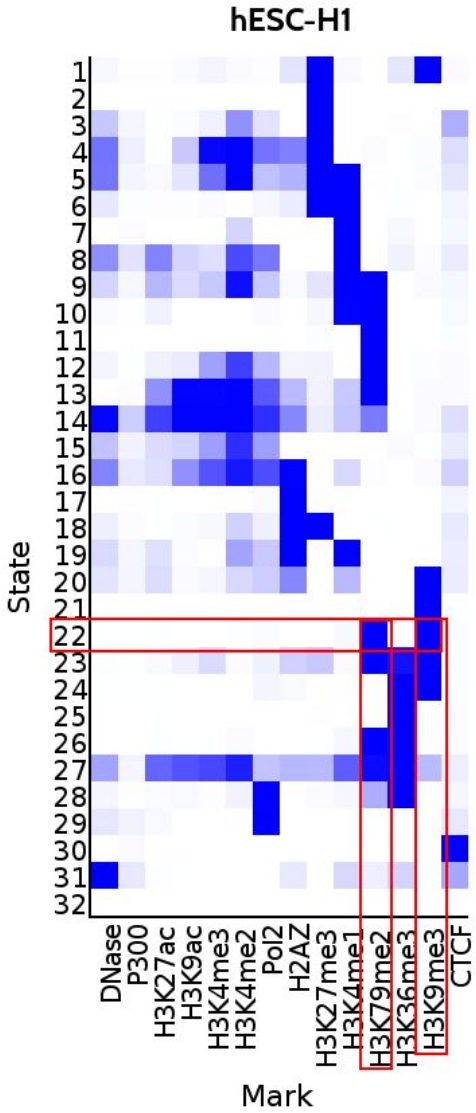

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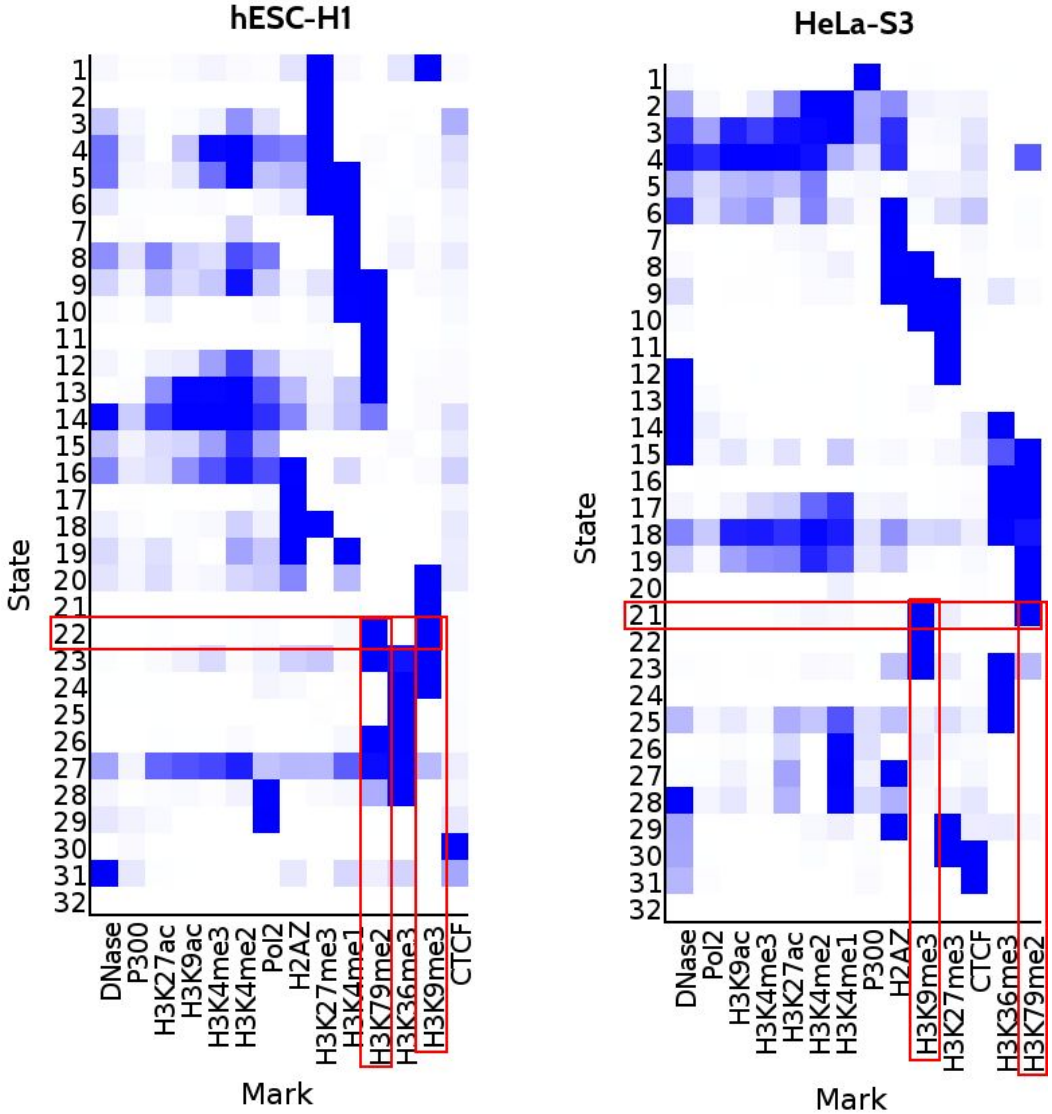

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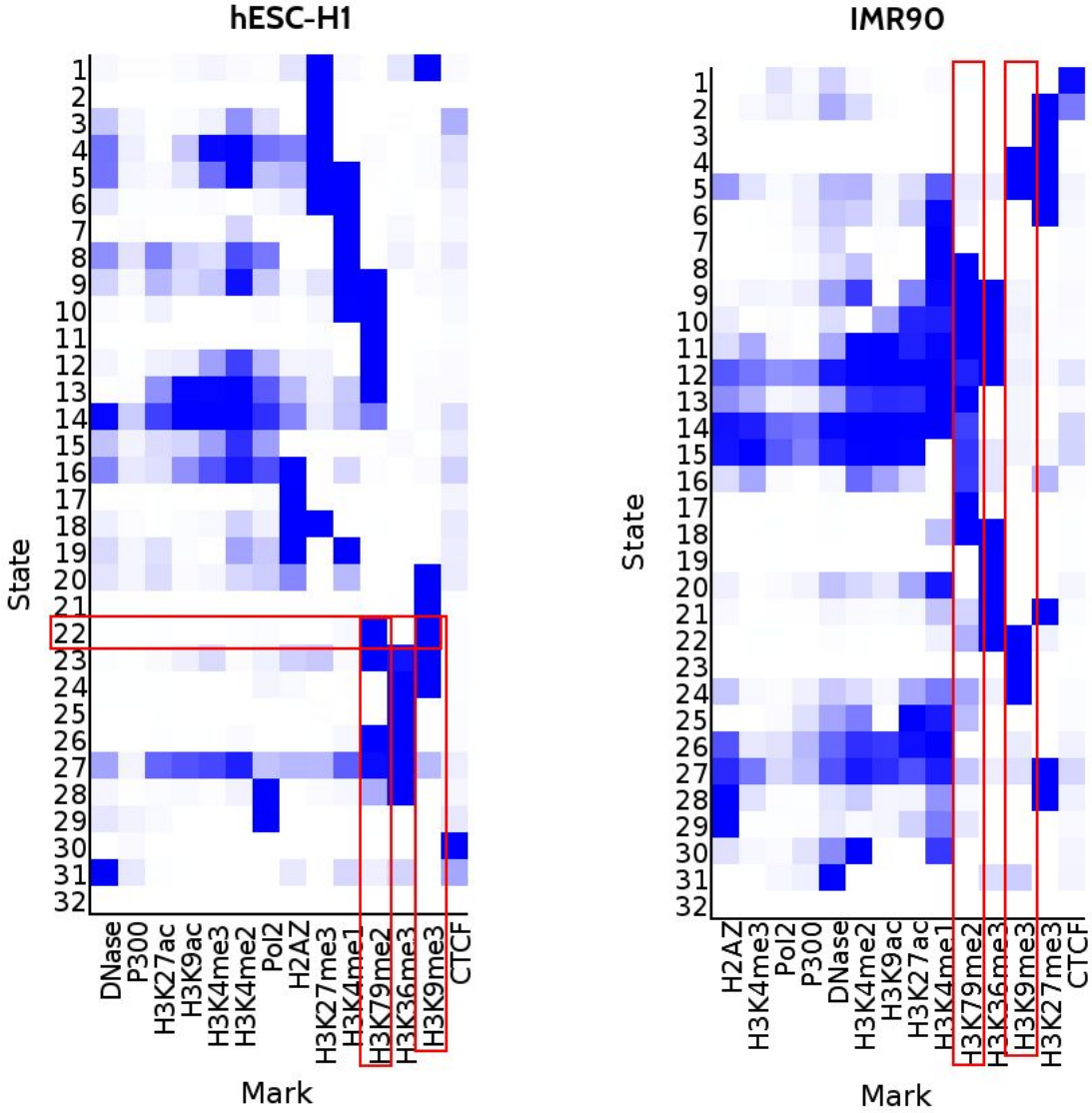

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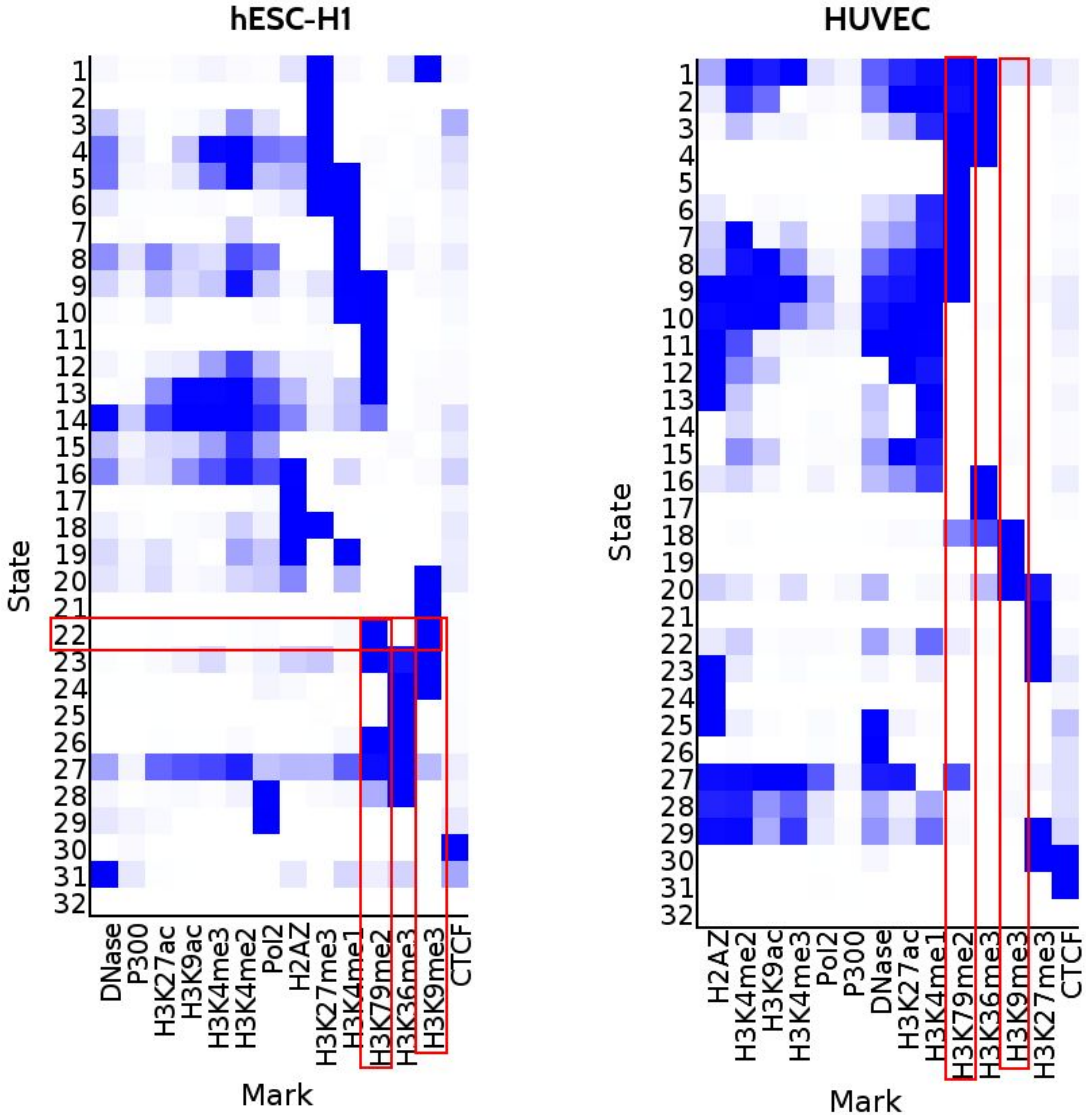

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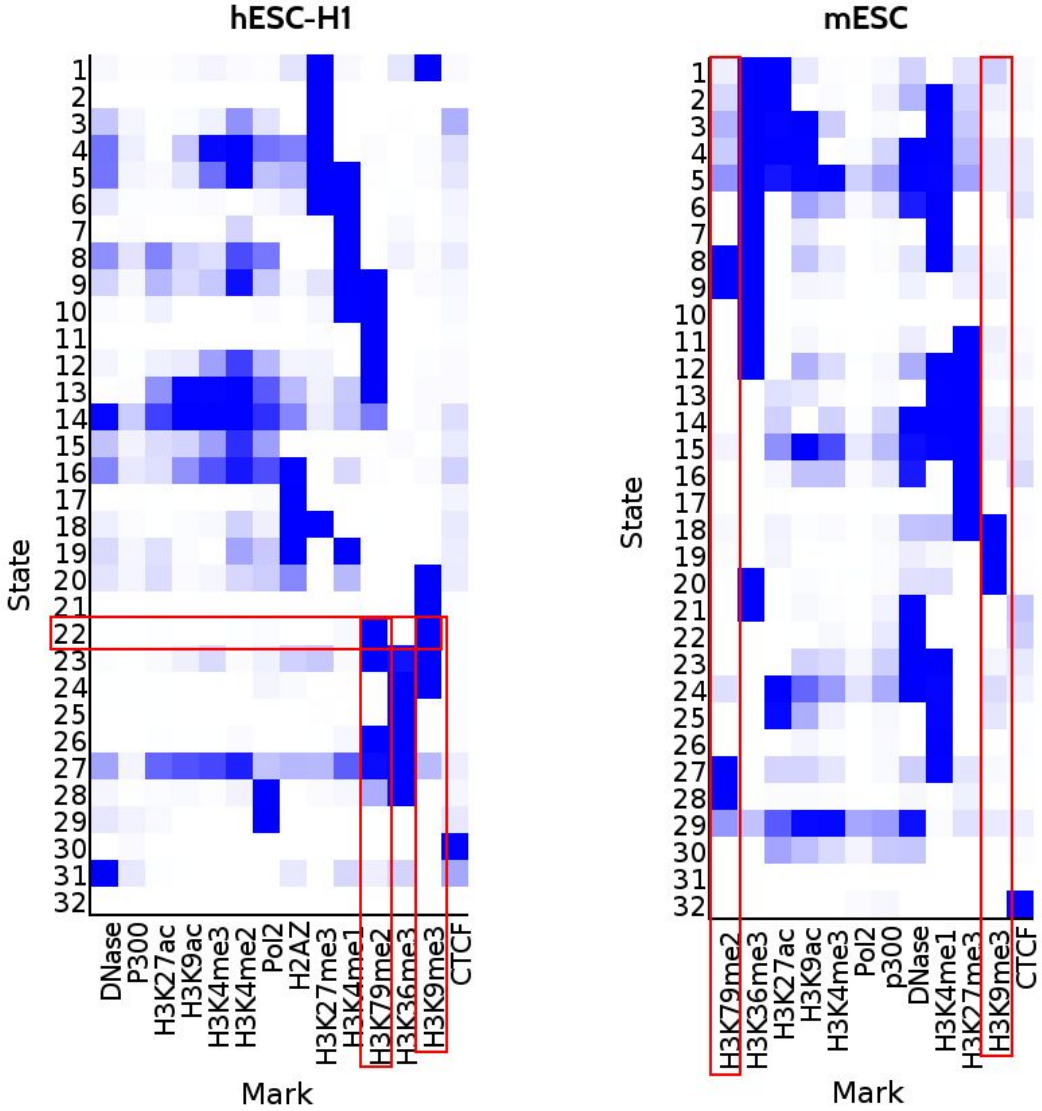

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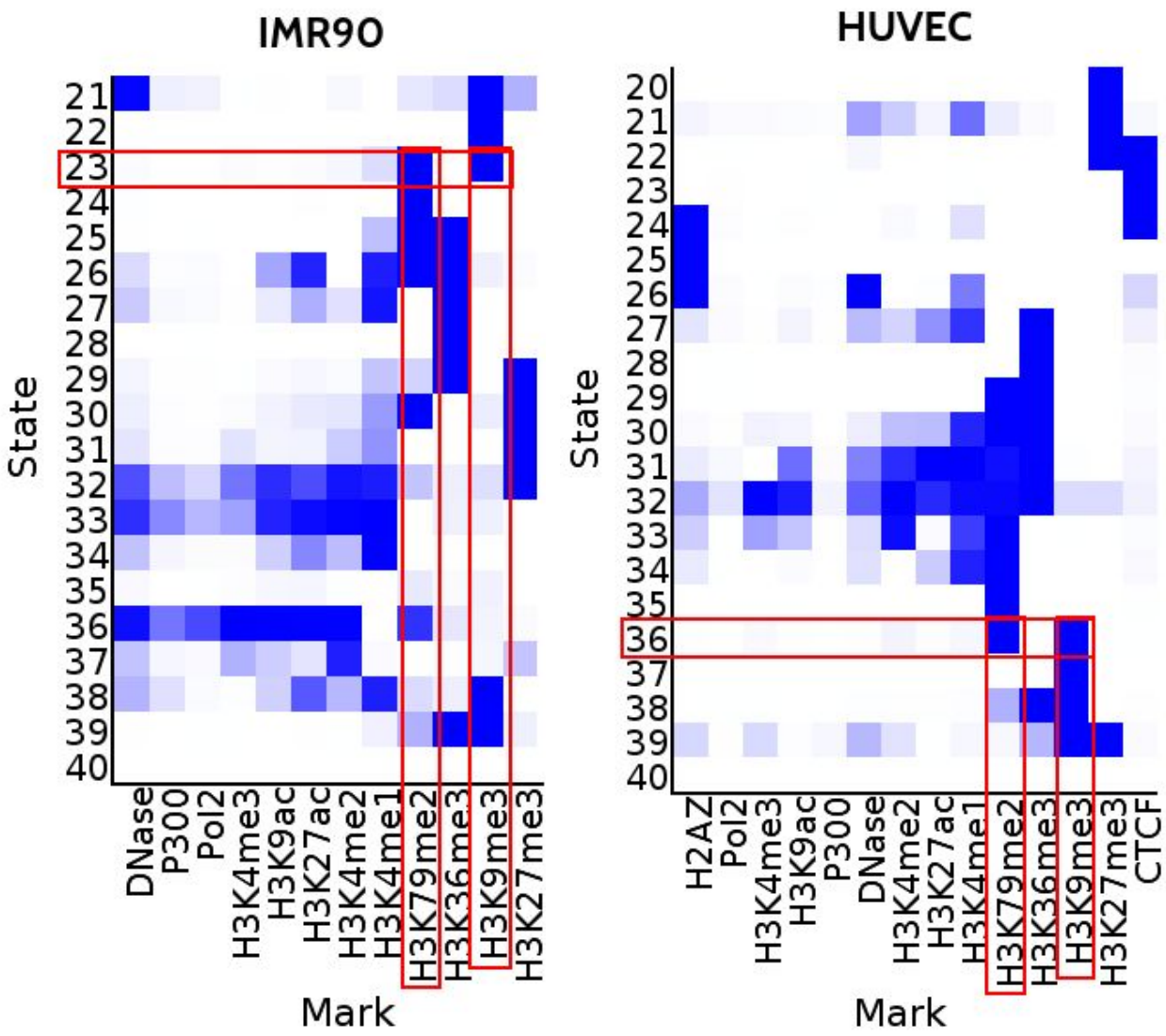

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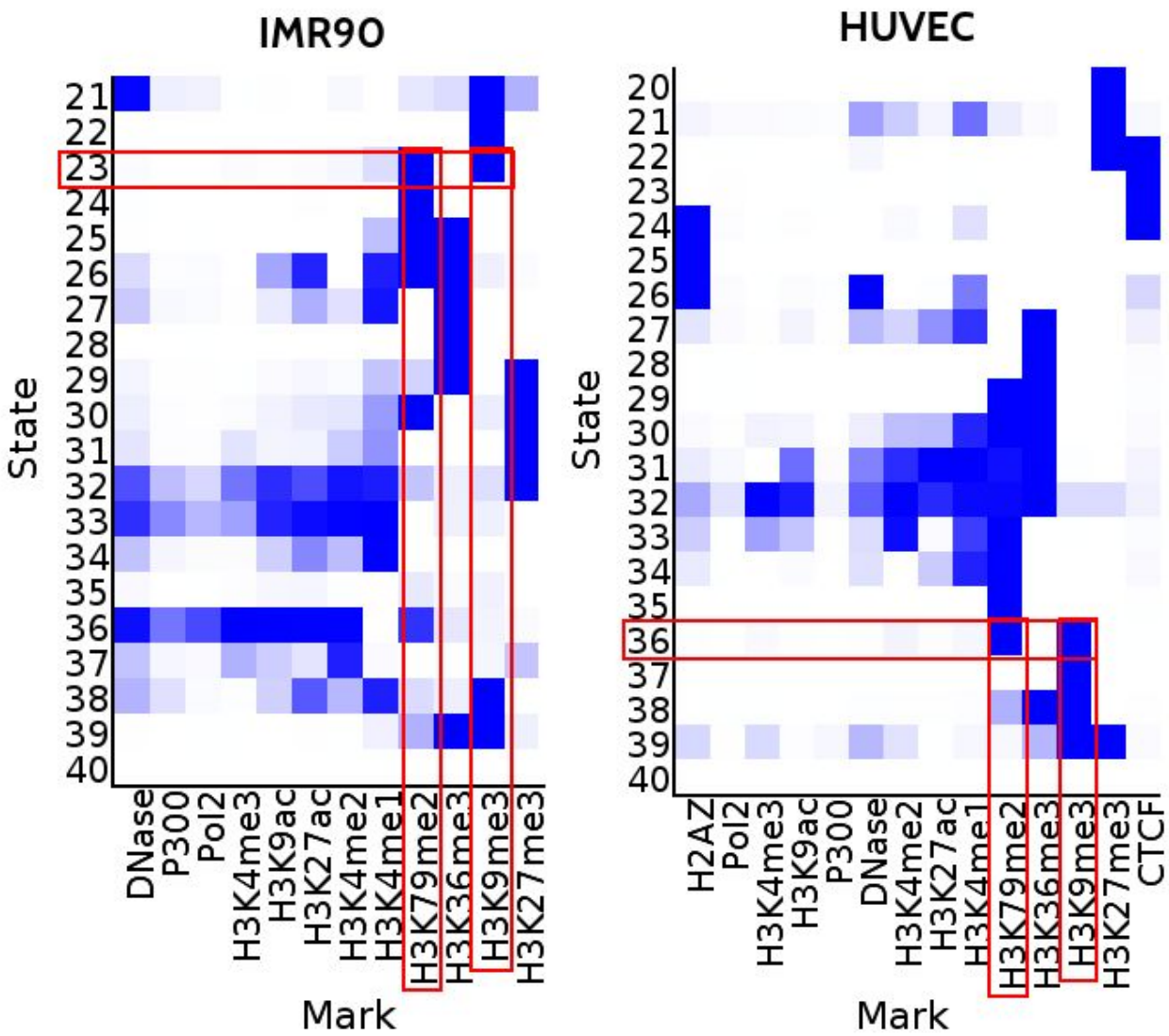

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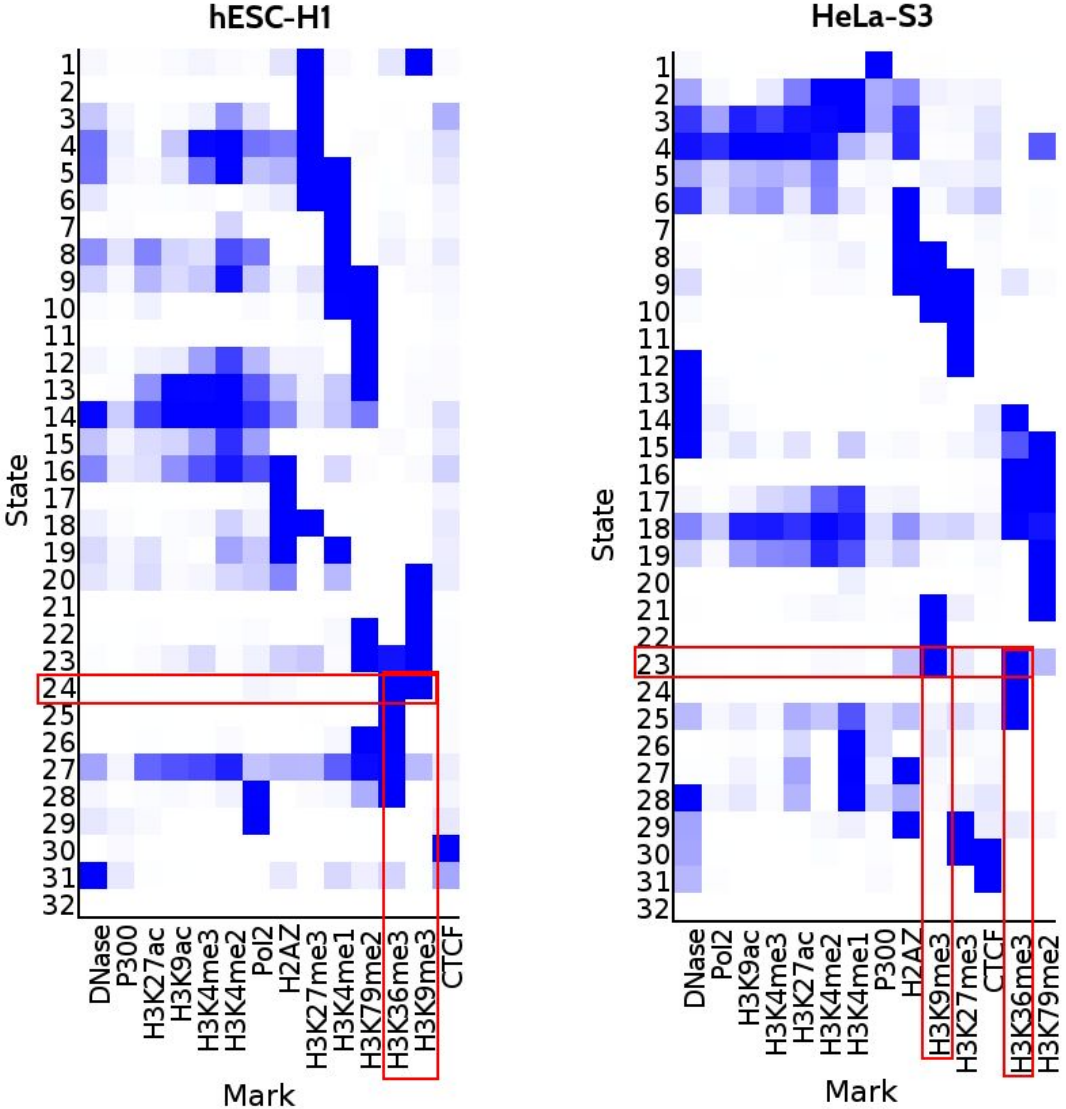
Thus, it can't be a mechanism like that of bivalent promoters.

Two observations



(1) A combination **H3K36me3 + H3K9me3** is presented as a clear separate state in all considered cell lines: hESC-H1, HeLa-S3, HUVEC, IMR90, and mESC.

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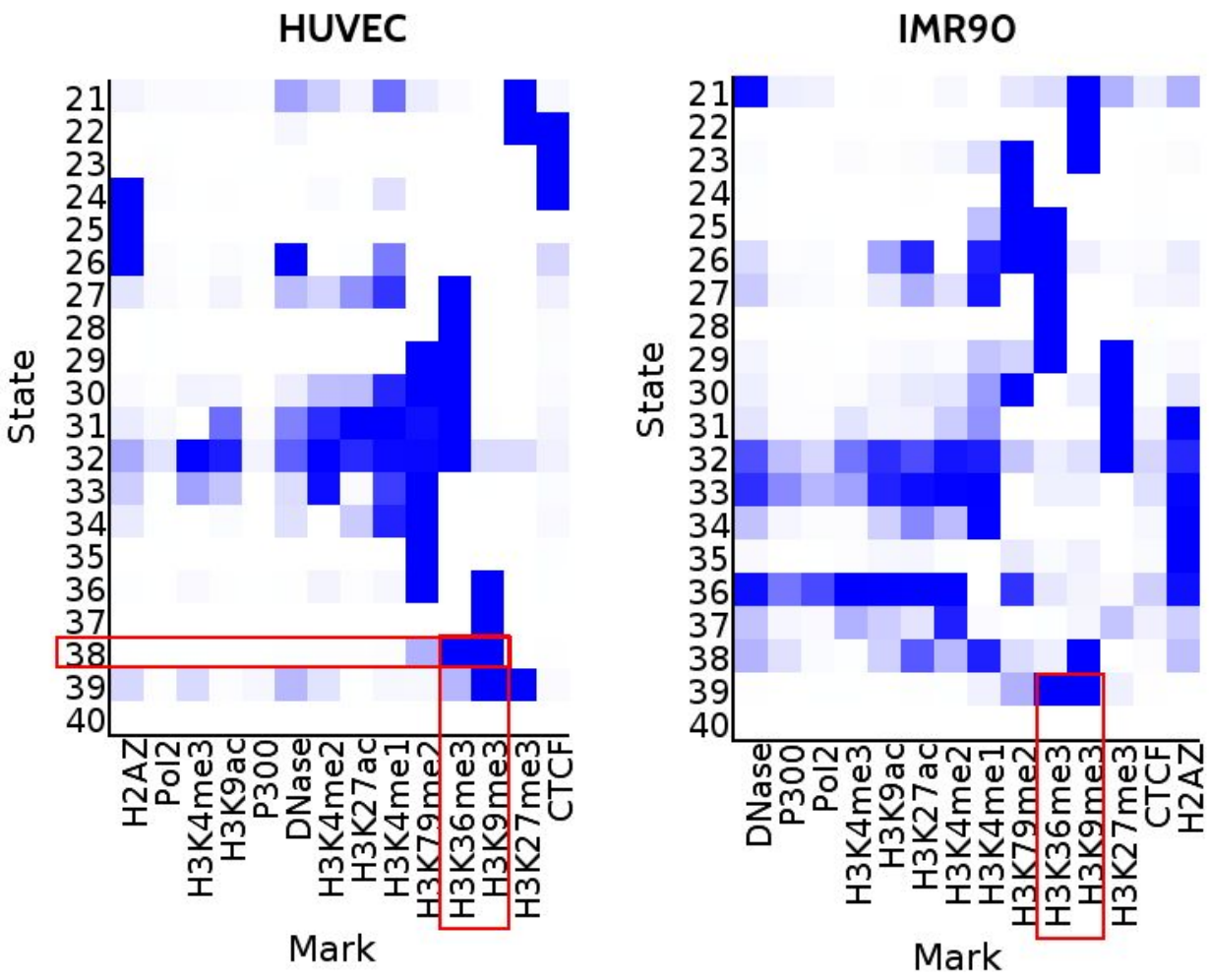

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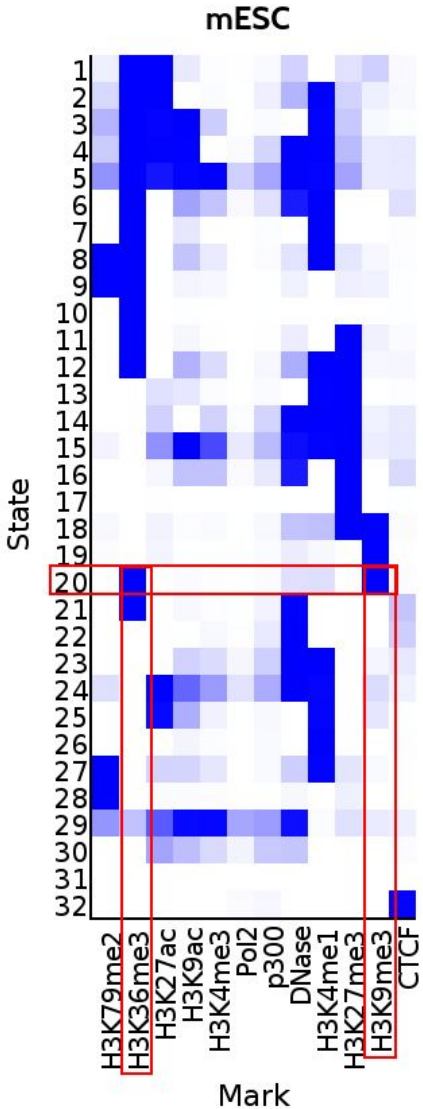

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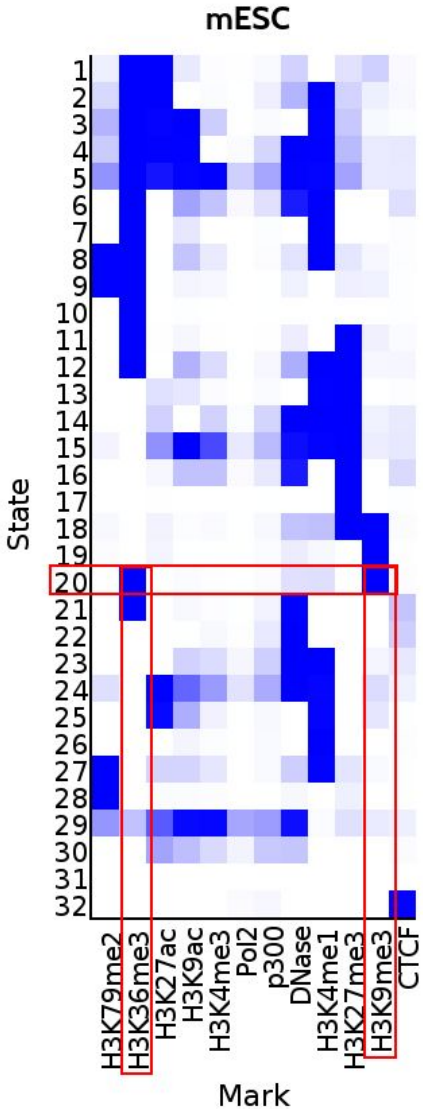

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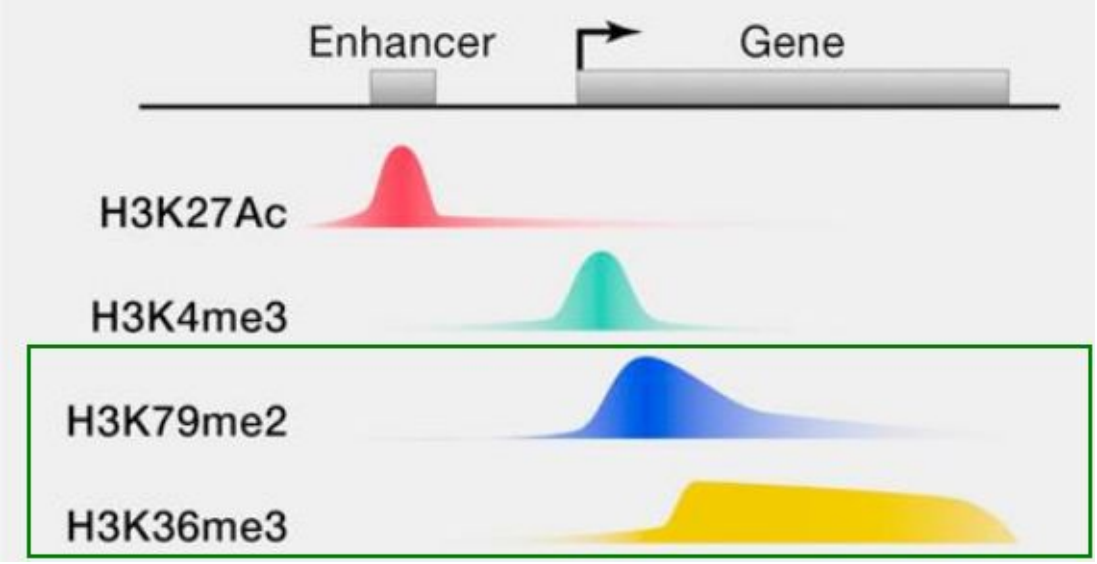

Adapted from *Lee T. I.* and *Young R. A.* Cell. 2013, 152(6):1237-1251.

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Thus, **the most interesting thing is** not an existence of H3K79me2 + H3K9me3 and H3K36me3 + H3K9me3 combinations themselves, but **the presence of H3K9me3 marks in supposedly transcribed genes!**

Study of H3K9me3-enriched genes

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4. Select genes with TPM >= 5 (they are considered to be transcribed).

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Gene counts within these sets are as follows:

hESC-H1	635
HeLa-S3	1273
HUVEC	110
IMR90	1686
mESC	158

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To study this cases more formally, we produced classification tables.

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Such table was produced for each cell line. It has the following columns:

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Active genes enriched by H3K9me3

Let’s find transcribed genes with H3K9me3 peaks strictly within introns:

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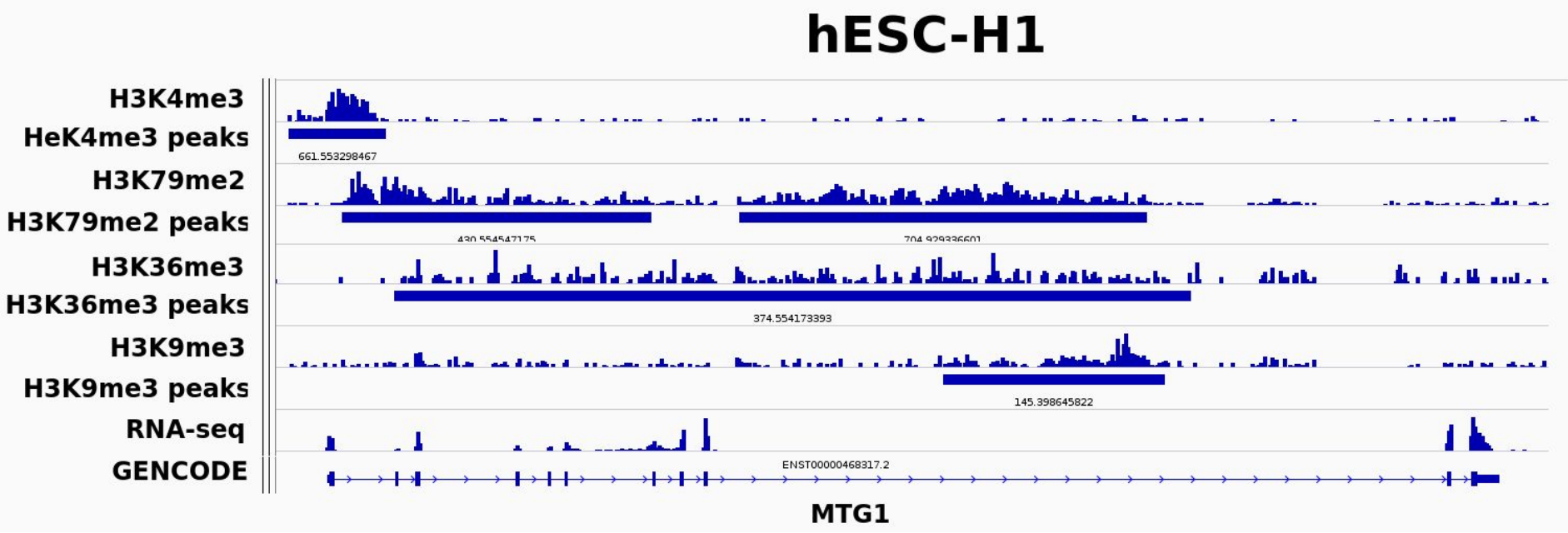

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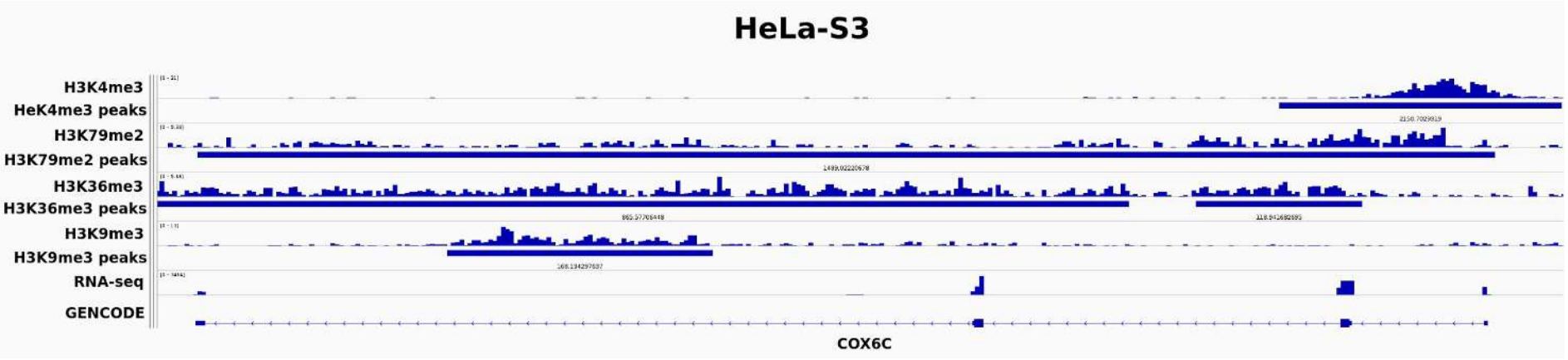

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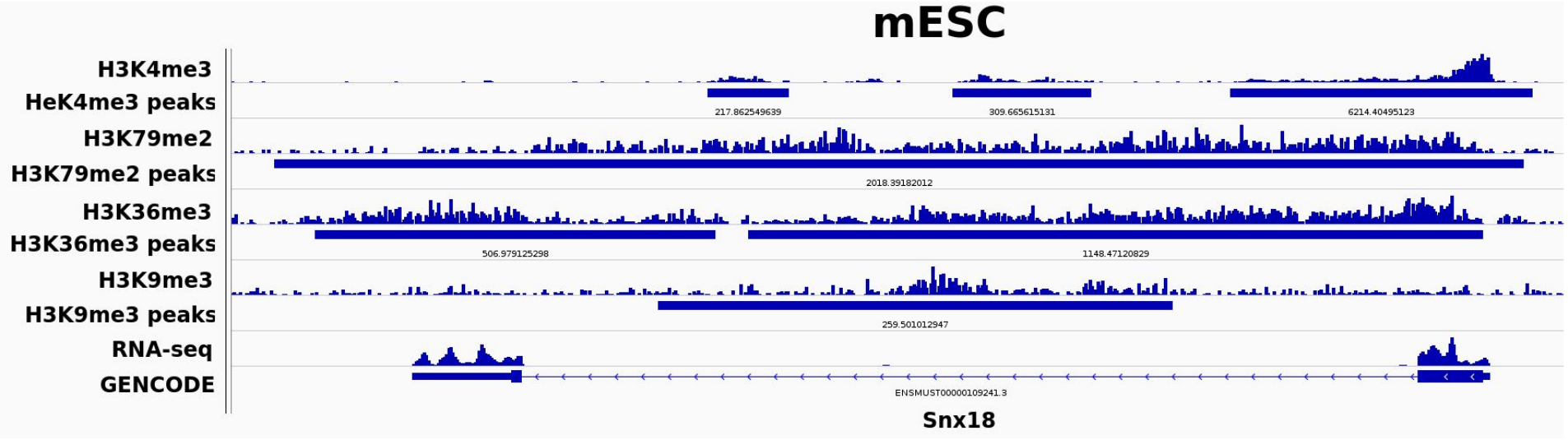

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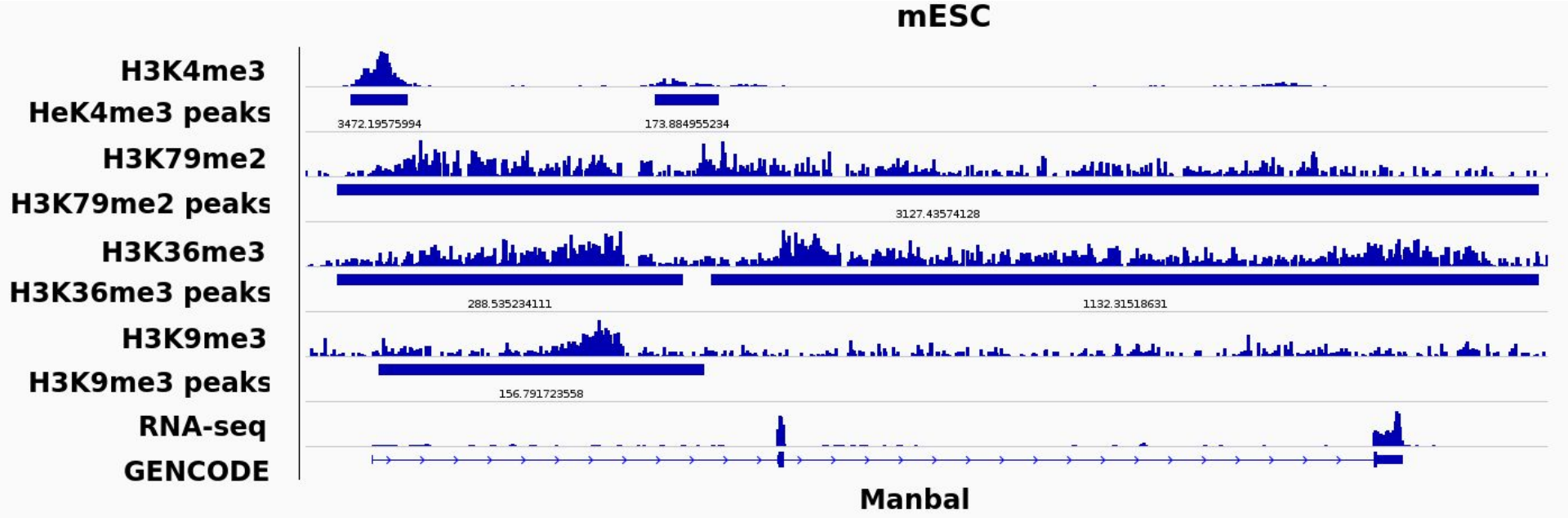
Also, for some genes we could see the following:



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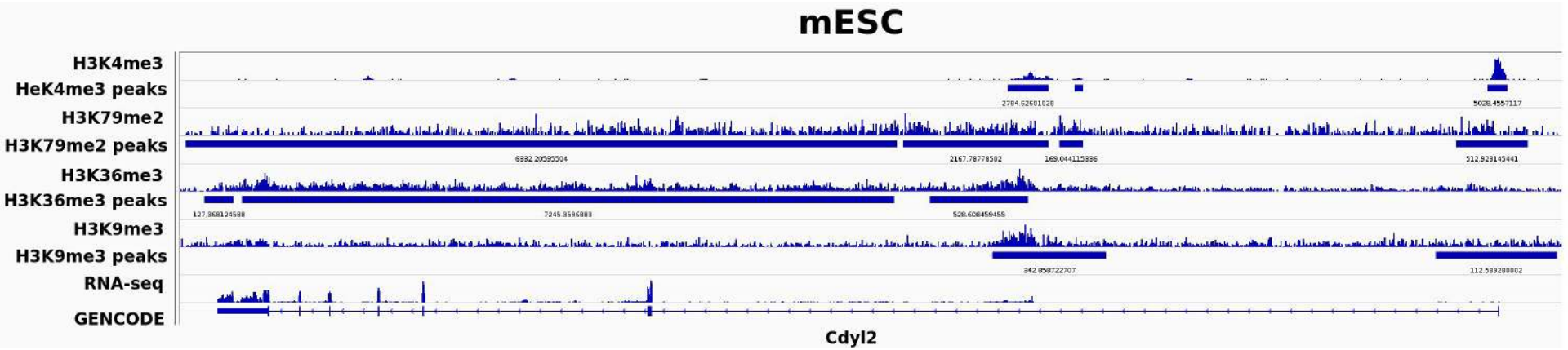

So, we suggest that such H3K9me3 peaks may mark alternative promoters.

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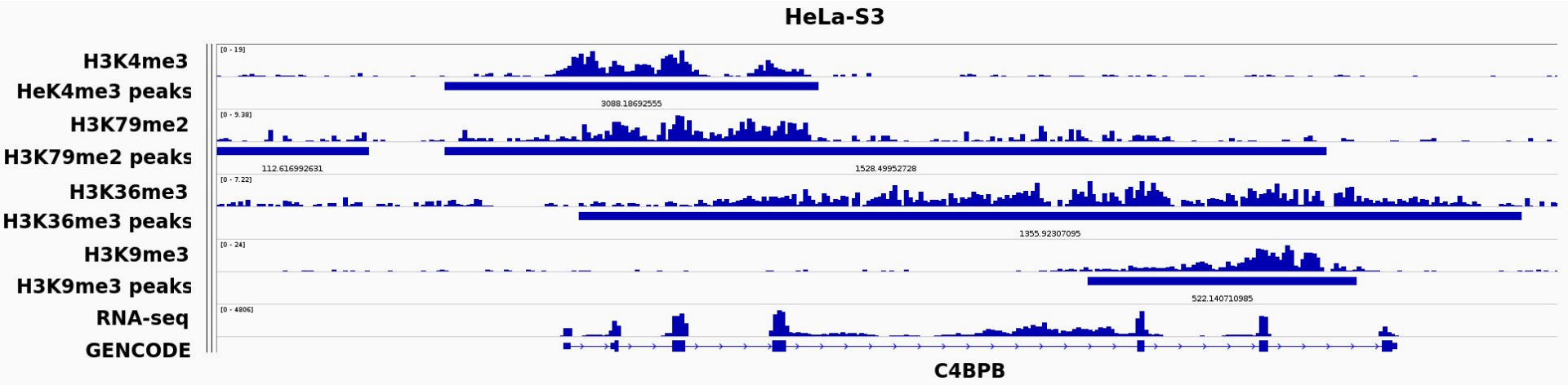
Additionally, we can observe through visual inspection H3K9me3 peaks that overlap exons and introns but are situated within active genes:



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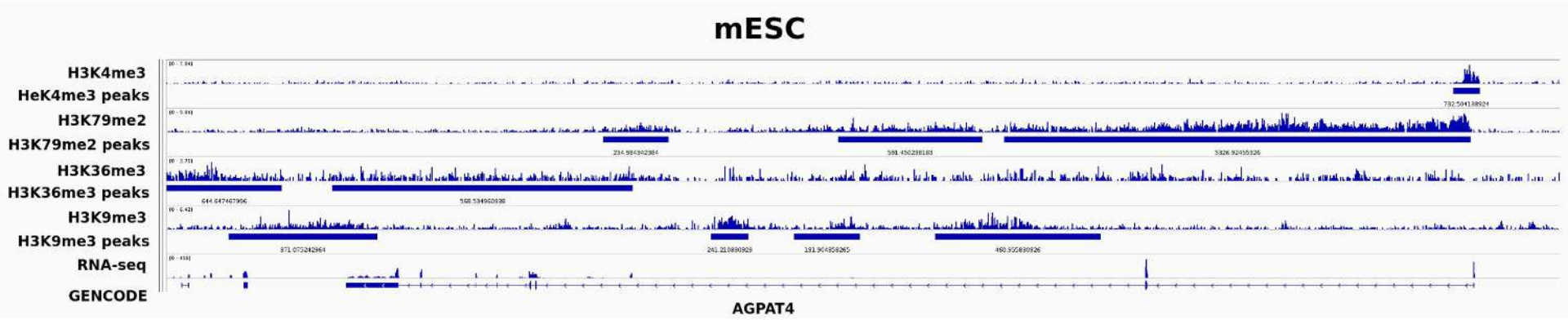
Additionally, we can observe through visual inspection H3K9me3 peaks that overlap several exons and introns but are situated within active genes or overlap with active genes from intergenic regions:

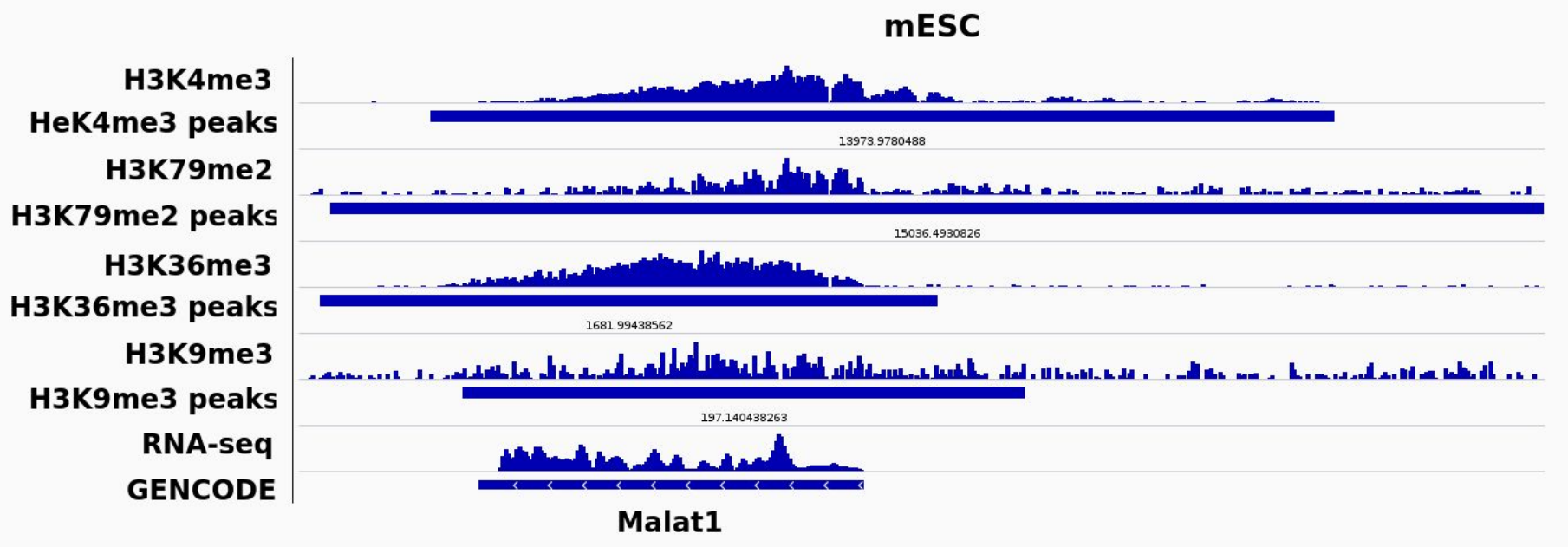


Active genes enriched by H3K9me3

Let's select active genes that overlap with HeK9me3 peaks only with their exons:

```
if_overlaps_exon == 1 && if_overlaps_intron == 0
```

Among them we could see genes with a long last exons overlapped with H3K9me3, but not always:



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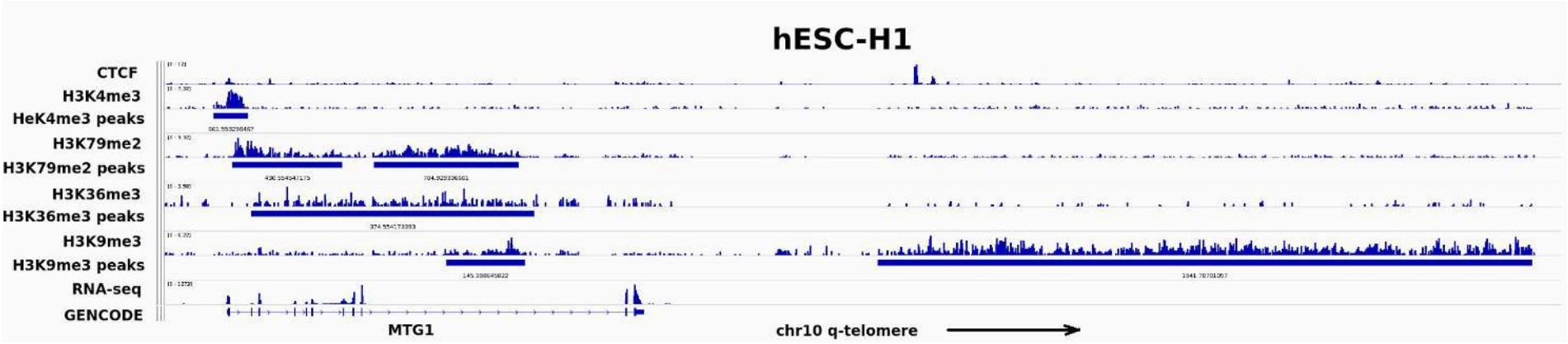

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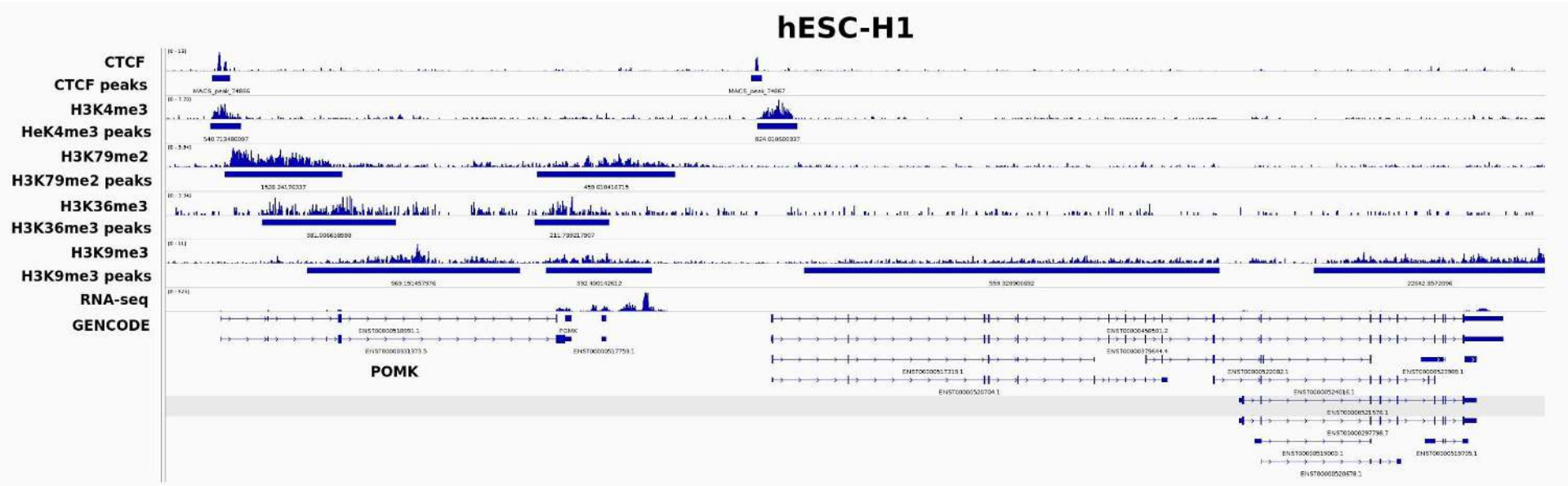

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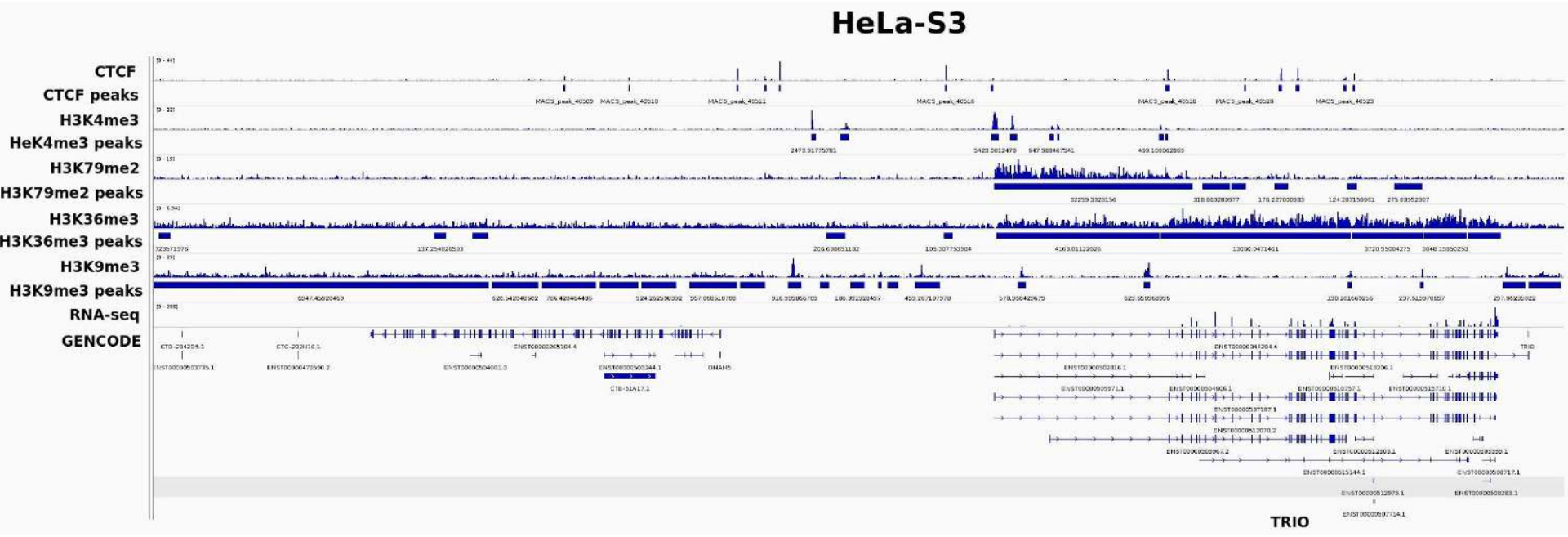

Enrichments

Next we decided to calculate enrichments for H3K9me3 + H3K79me2/H3K36me3 combinations (approximately marking H3K9me3 regions in active genes) in the following genome elements:

1. Genes, exons, promoters, TSS, TES.
2. CpG-islands, lamina-associated domains.
3. Origins of replication.
4. High-occupancy target (HOT) regions, super-enhancers.
5. Known repeat families.
6. Strong and weak TAD borders 1 kbp, 2 kbp, 10 kbp, 100 kbp wide.

TAD calling

We took Hi-C data for hESC-H1, HeLa-S3, HUVEC, IMR90, and mESC and processed them using TADbit:

1. Mapped paired-end reads to hg19 or mm10 reference genome with GEM-mapper.
2. Produced contact matrices.
3. Found TADs (back-to-back segmentation) and scored borders between them (from 1 that is the weakest border possible to 10 that is the strongest one).
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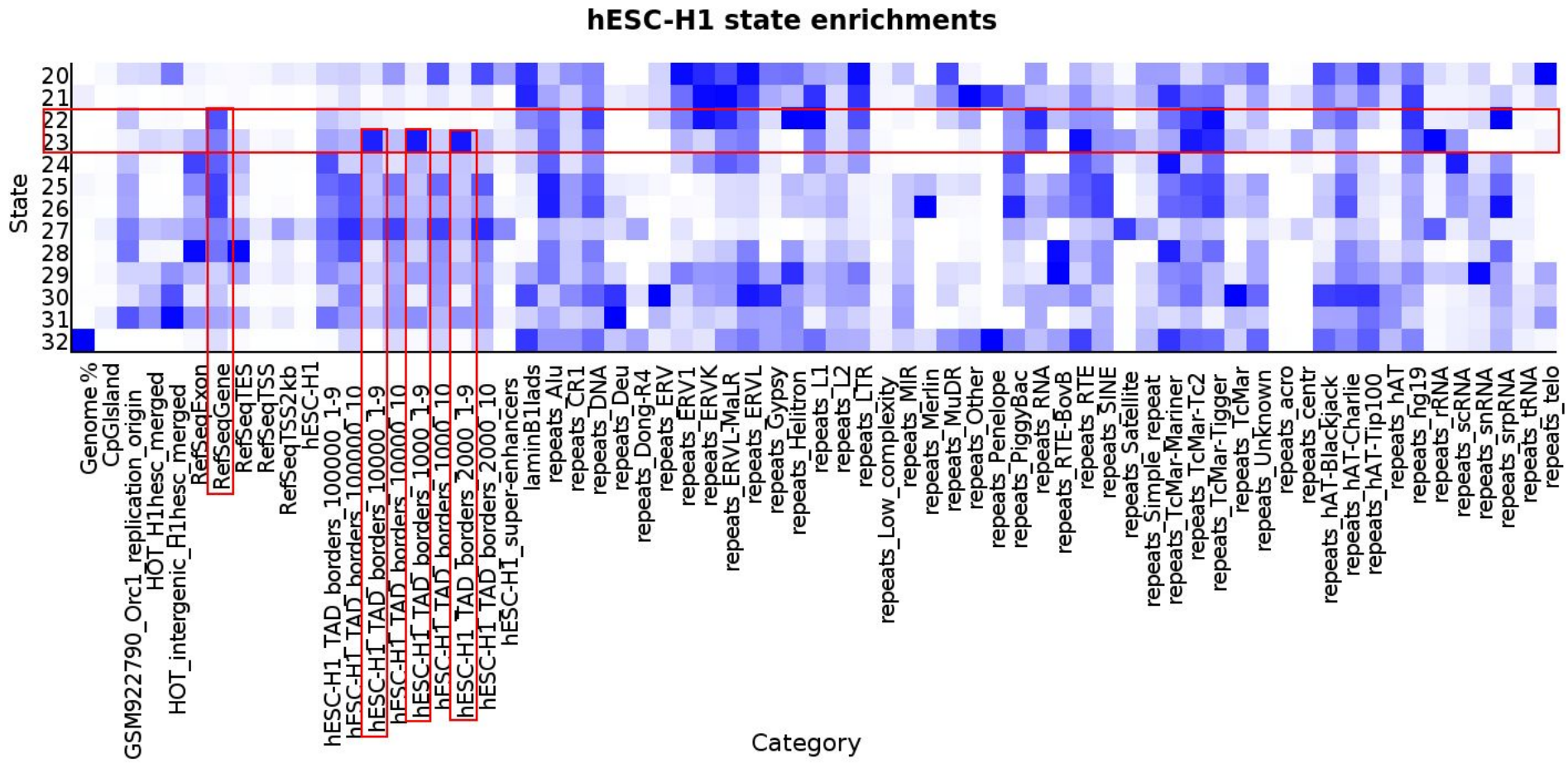
We obtained 2627 borders for hESC-H1, 2014 borders for HeLa-S3, 2353 borders for HUVEC, 2244 borders for IMR90, and 1922 borders for mESC.

Enrichments in human cell-lines

Weak TAD borders of **hESC-H1** and **HUVEC** are enriched by H3K9me3 + H3K79m2/H3K36me3:



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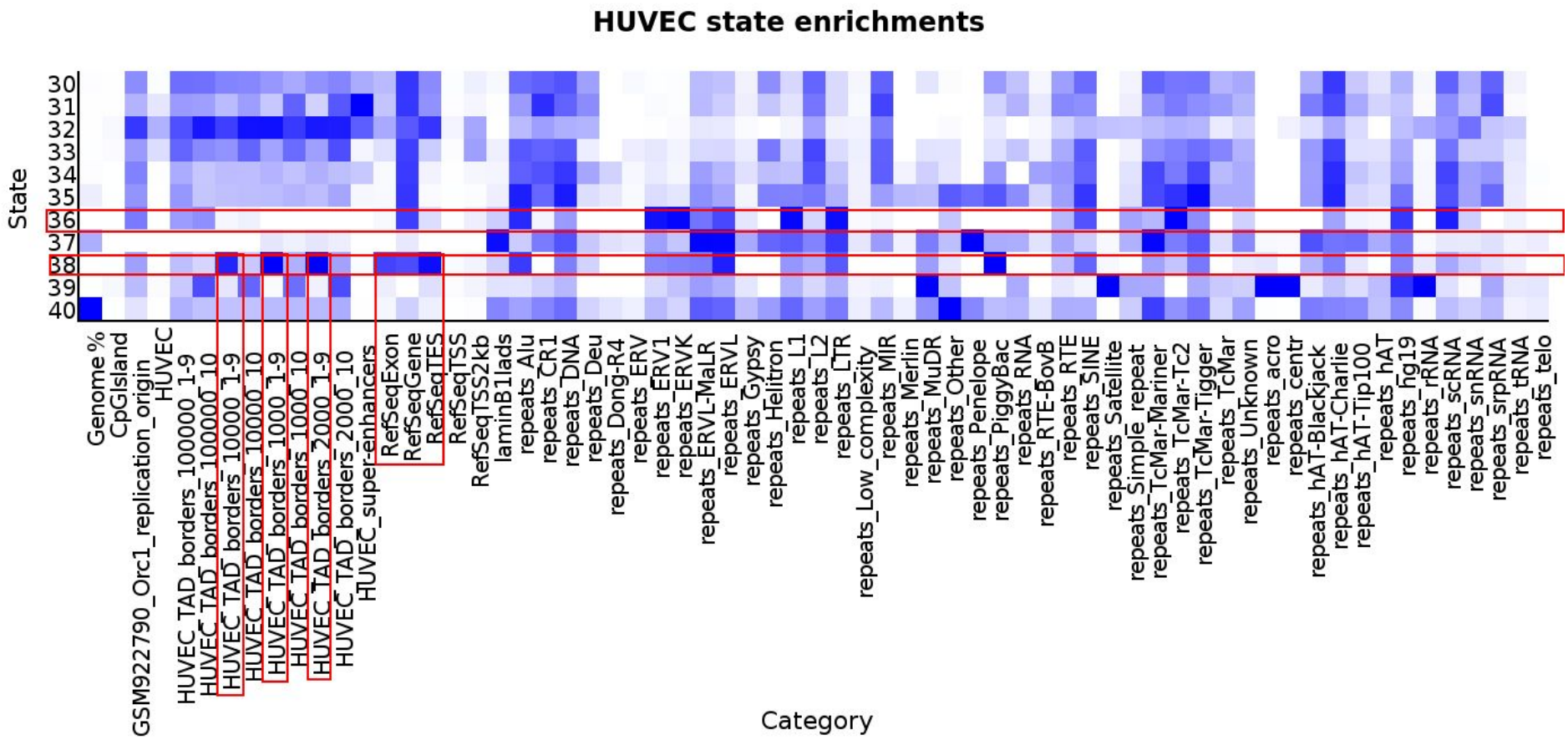

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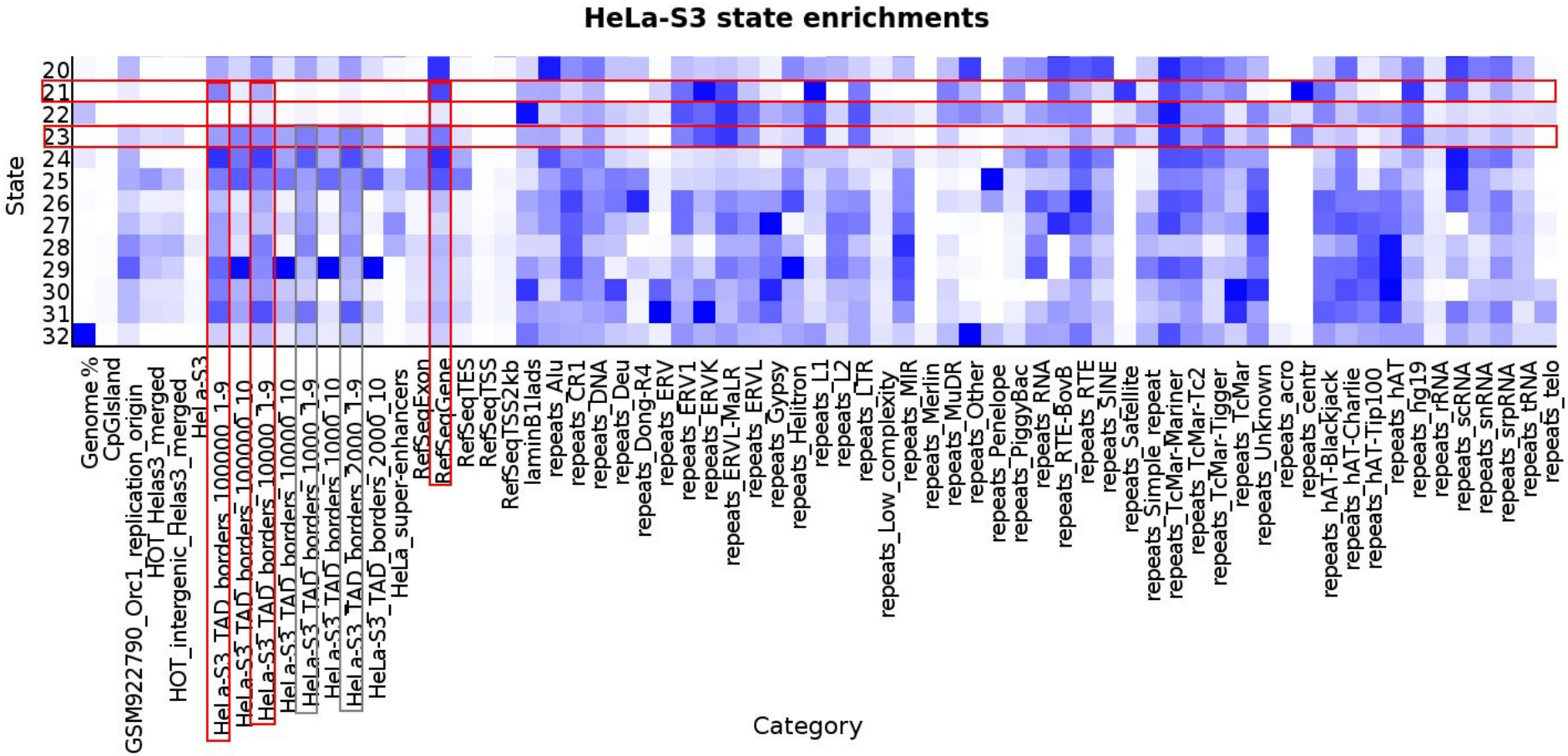

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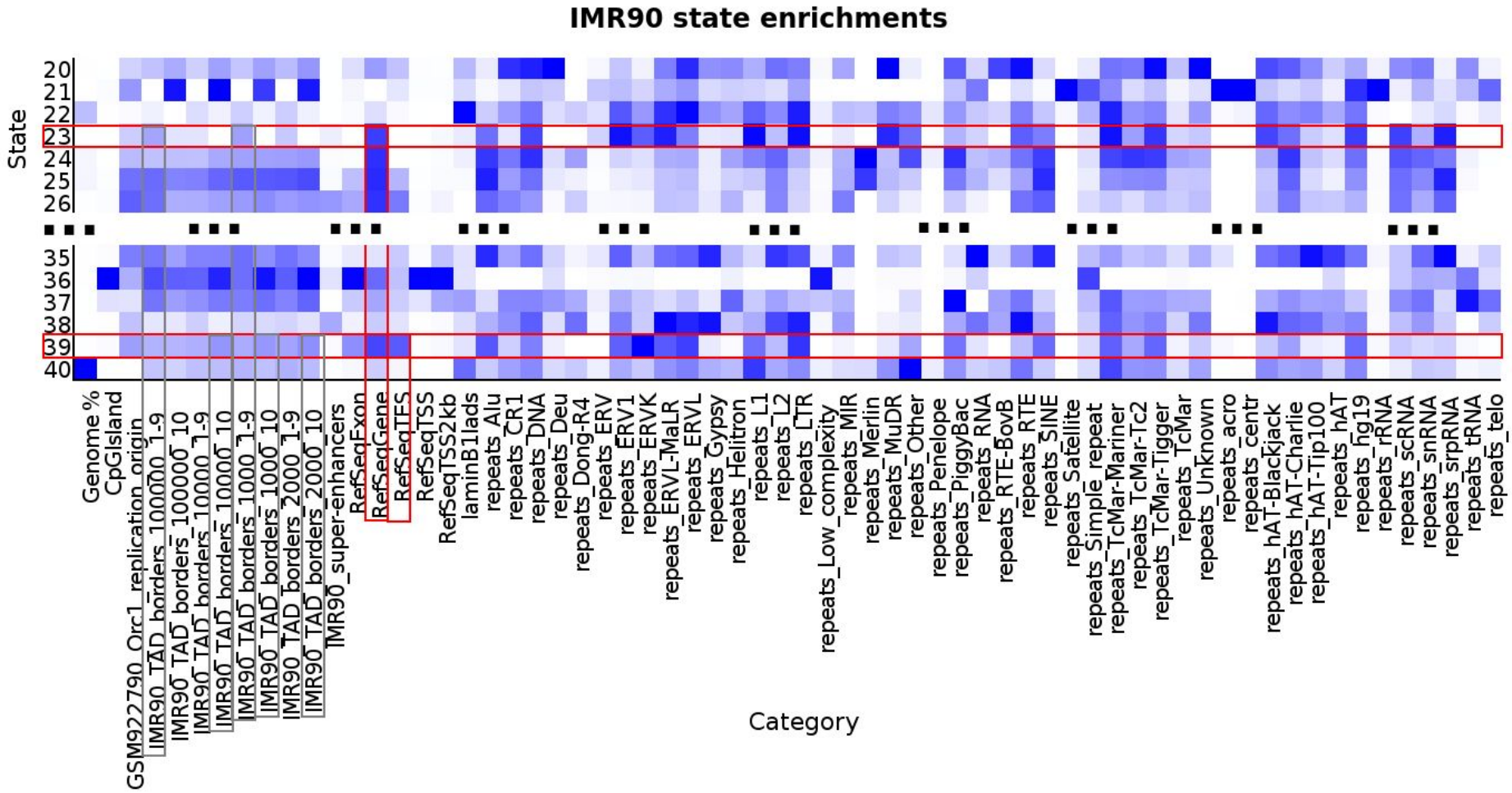

Outline

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2. My master thesis in brief.
3. The current project.
4. Current results.
5. Perspective.

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3. We calculated enrichments of various genome elements by H3K9me3 + H3K79me2/H3K36me3 combination (which approximately marks H3K9me3 regions within active genes).

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Perspective

1. Finish enrichment analysis.
2. Analyse the sequences of the H3K9me3-enriched regions within active genes.
3. Analyse literature on H3K9me3 in active genes.
4. Select a subset of such genes and check proteins from the chromatin of their H3K9me3-enriched regions.

Thank you!

TAGGTGCAGGAGCCTGCGTGACCGGTGCACTAACGGTGGGCAGCGCCAGCACATCTTGAGTCTCATGCCAGAGCTGTGGT
CGCTGGGCTAGGGCGTGCAGGAGTTGGCAATAACACTGGAACATCTGATCCATTAGCGCCGGTTCAAACAATTGTTCCAC
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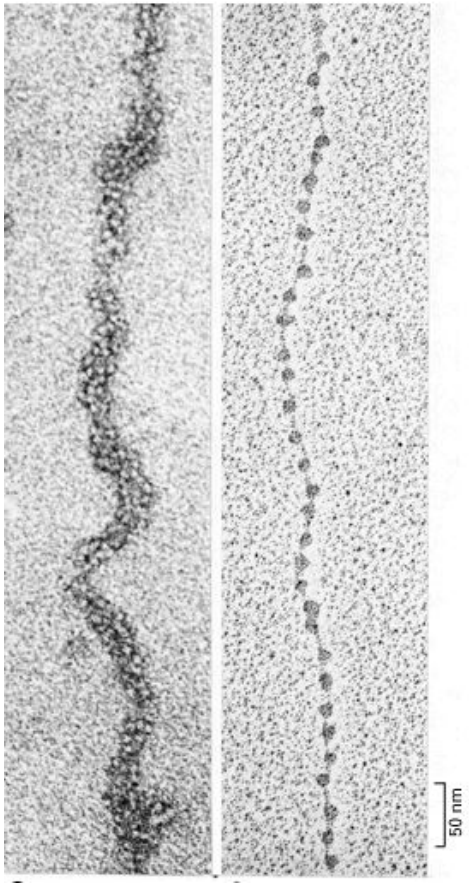
AAAATGGTTGACGCTAACAGCGAATGCCCACCCAGTTCGAAGAAGCTGGCGTGGTGATCATCAATGCTGACACCAAGCGT

What a bioinformatics specialist thinks the genome is like.



And what it’s really like...