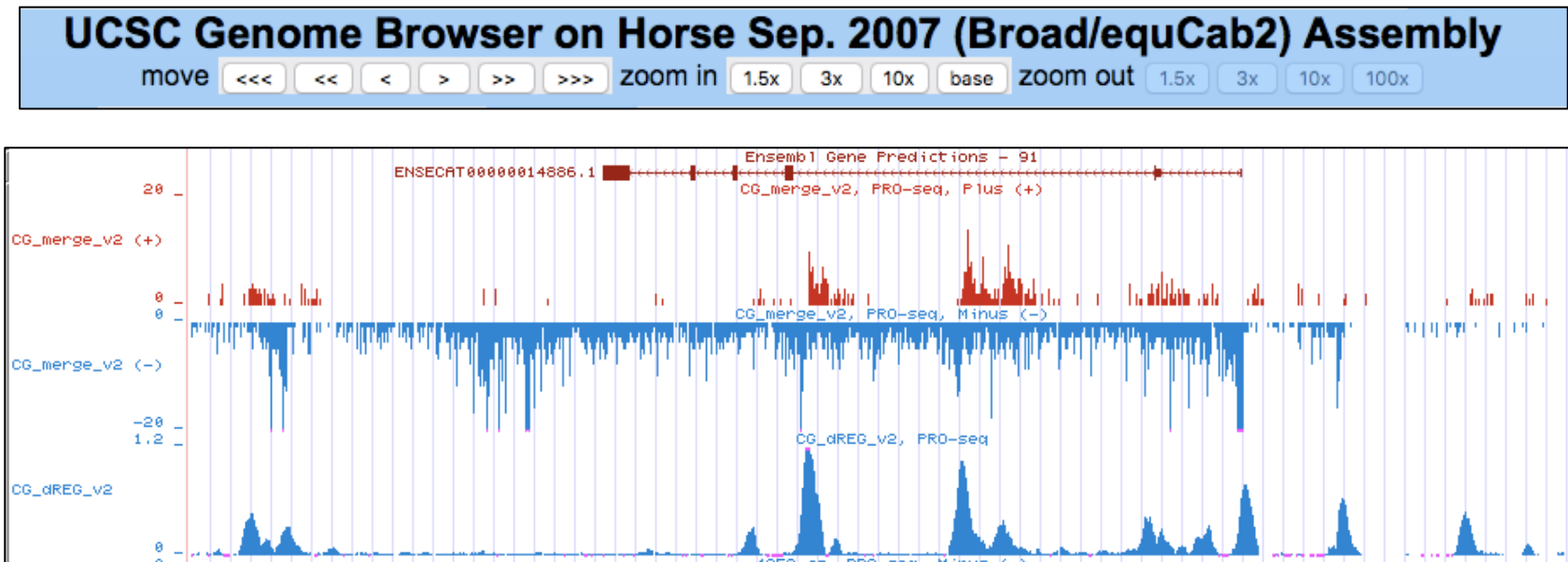




Global Run-on and Sequencing & Computational Pipelines for FAANG





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Outline

Short introduction to ChRO-seq

Our experience with horse tissues

Comparison with horse FAANG data:
ChIP-seq and ATAC-seq

ChRO-seq*

Swiss Army Knife application for epigenomic characterization

ChIP-seq

RNA-seq



miRNA

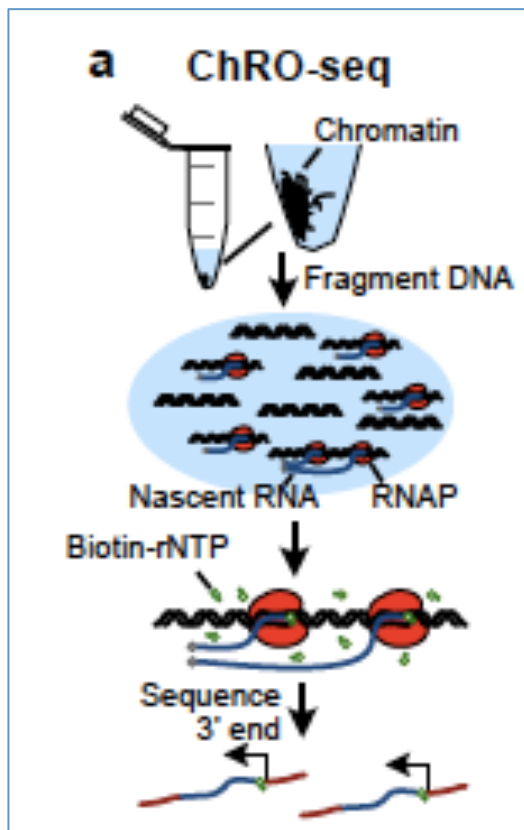
ATAC-seq

lincRNAs

*Chu et al. 2018
Nat. Genet. 50:1553

ChRO-seq, a variant of GRO-seq

(John Lis lab, Cornell, 2008)



Precipitation of insoluble chromatin
(any tissue, any form)

Nuclear run-on reaction to label
nascent RNA associated with Pol II

Illumina sequencing

Downstream computational programs
for data analysis & interpretation

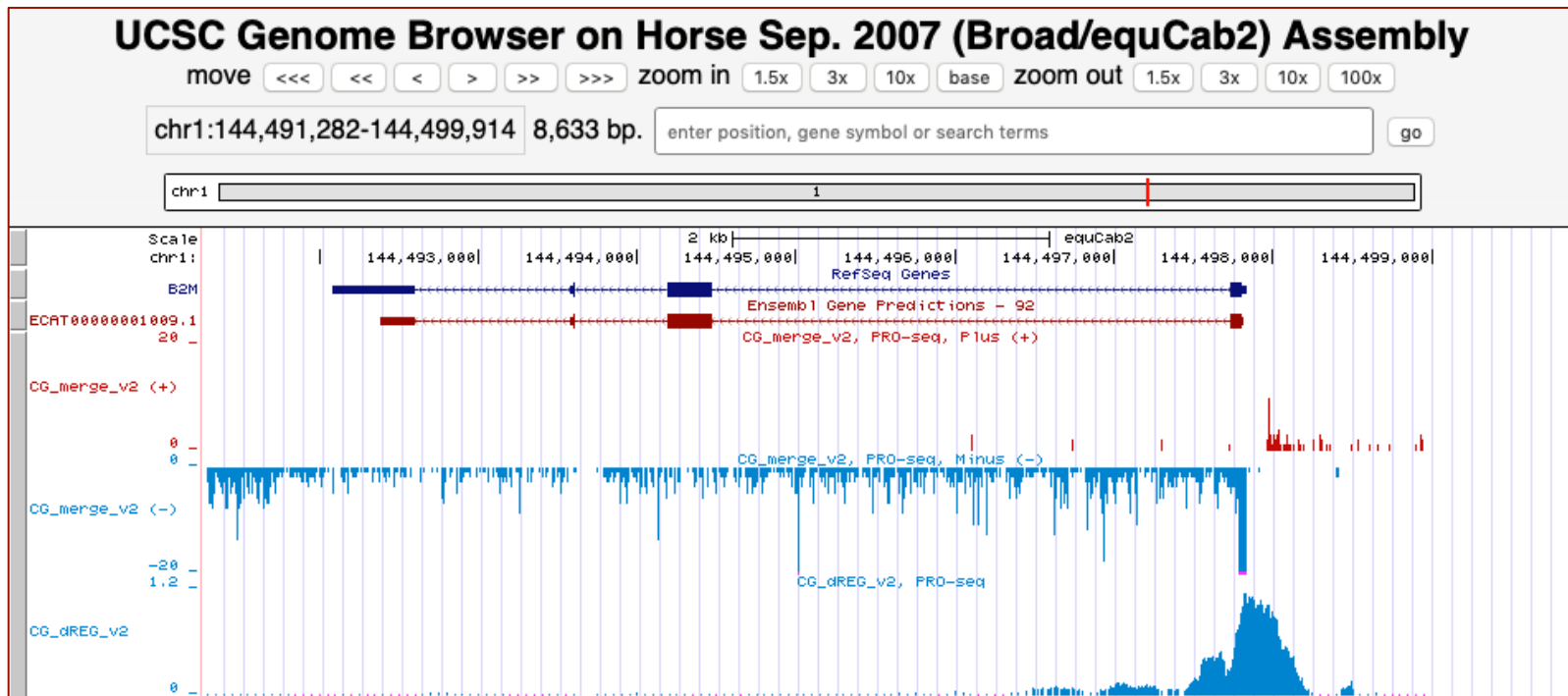
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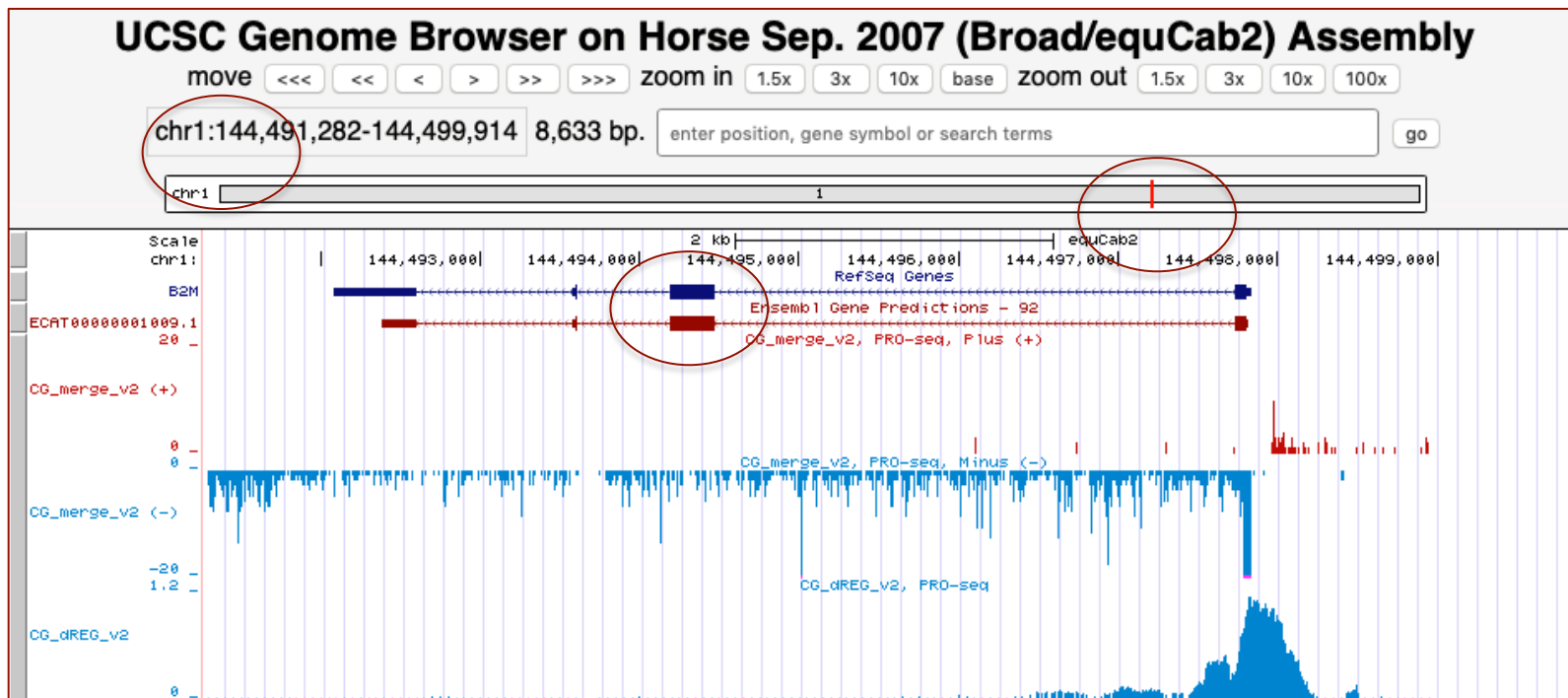
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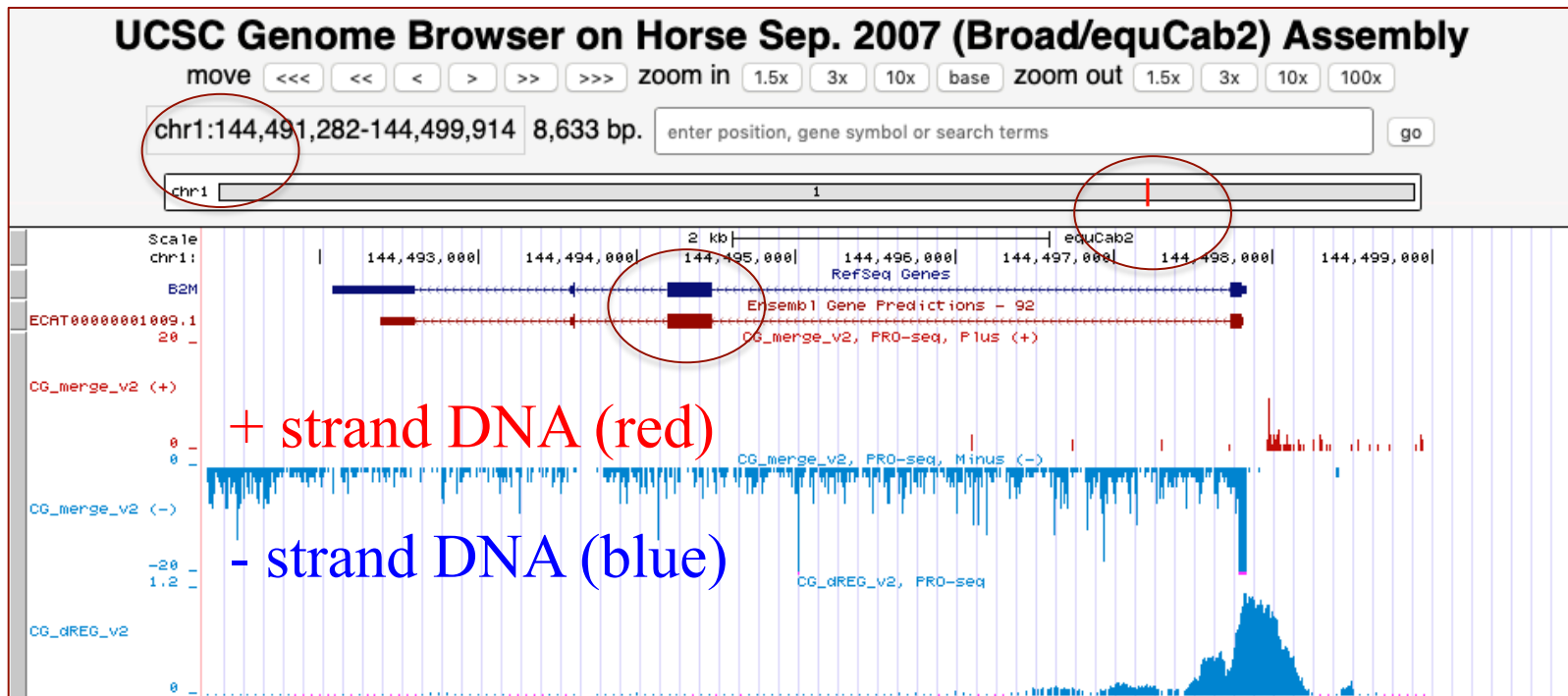
How to read the traces of ChRO-seq: Equine Beta 2 Microglobulin



How to read the traces of ChRO-seq: Equine Beta 2 Microglobulin



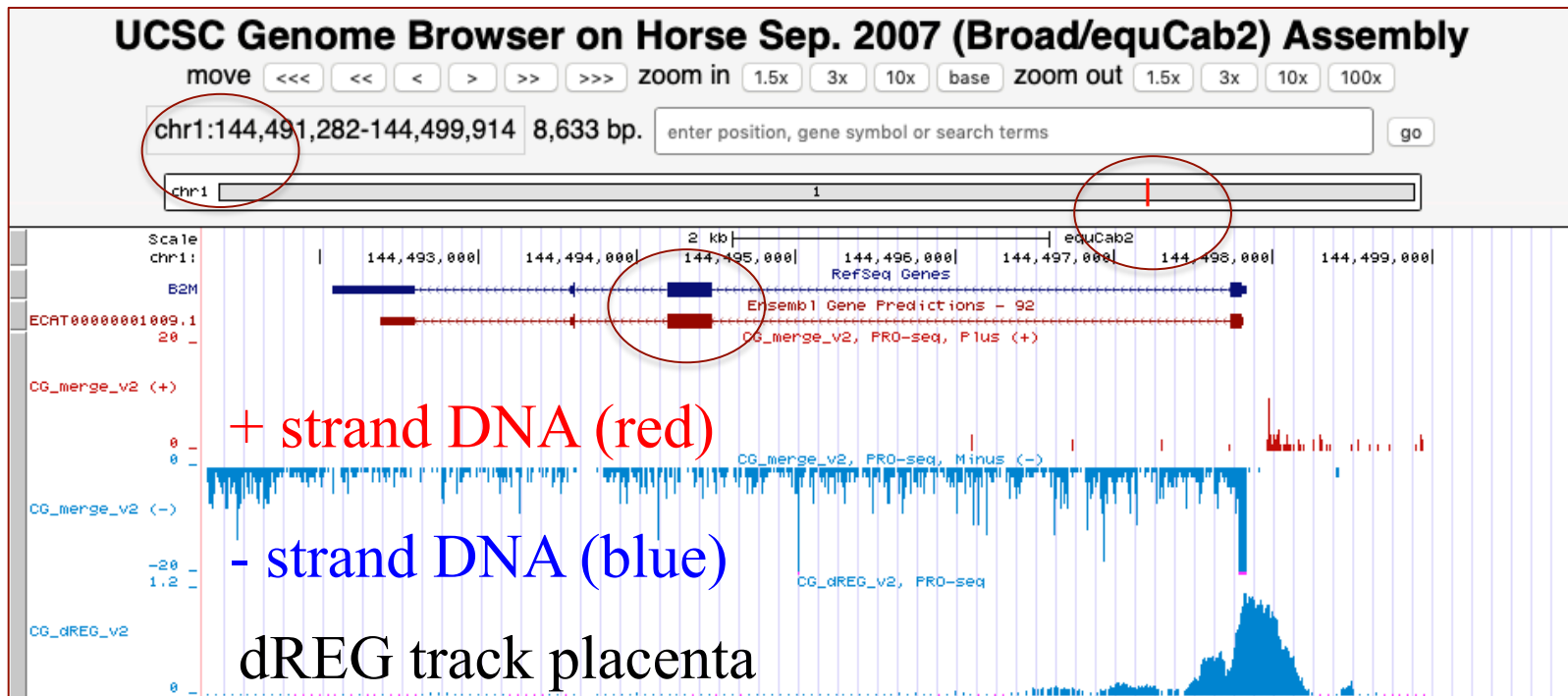
How to read the traces of ChRO-seq: Equine Beta 2 Microglobulin



Unprocessed, nascent RNA transcript

← Direction of Transcription

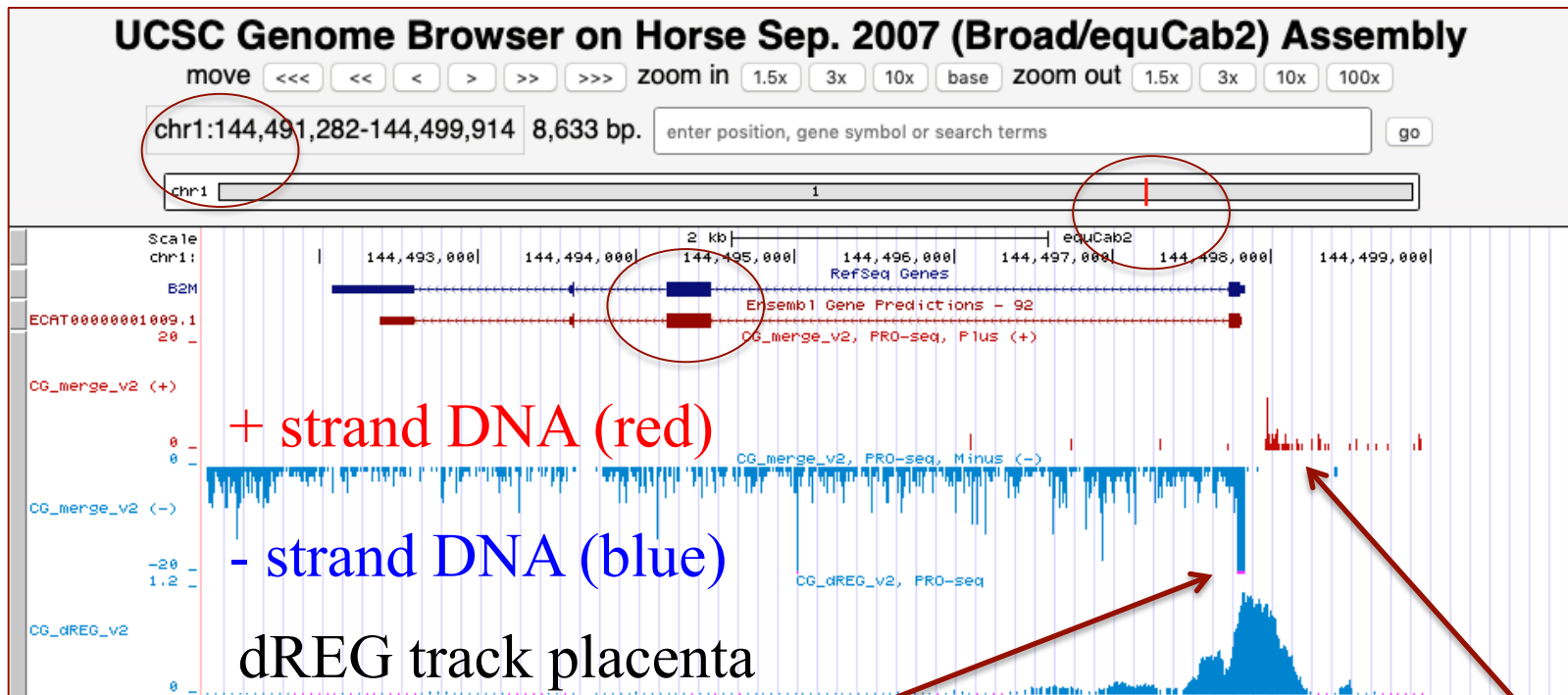
How to read the traces of ChRO-seq: Equine Beta 2 Microglobulin



dREG = Detection of Regulatory Elements
Machine Learning Imputation, Danko lab

← Direction of Transcription

How to read the traces of ChRO-seq: Equine Beta 2 Microglobulin



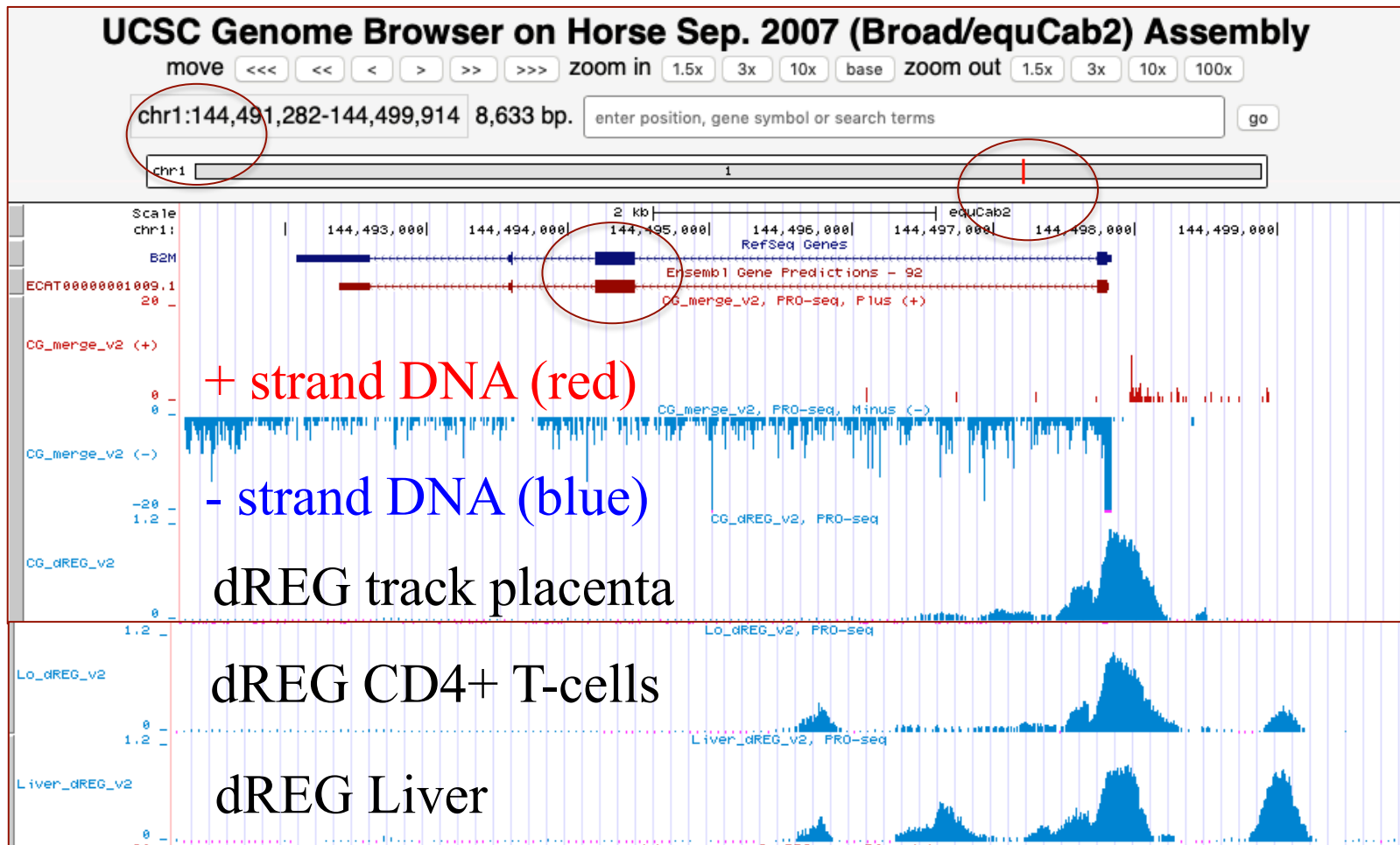
Transcriptional Start Site (TSS)

Regulatory Sites (dREG peaks)

Transcription on
non-coding strand

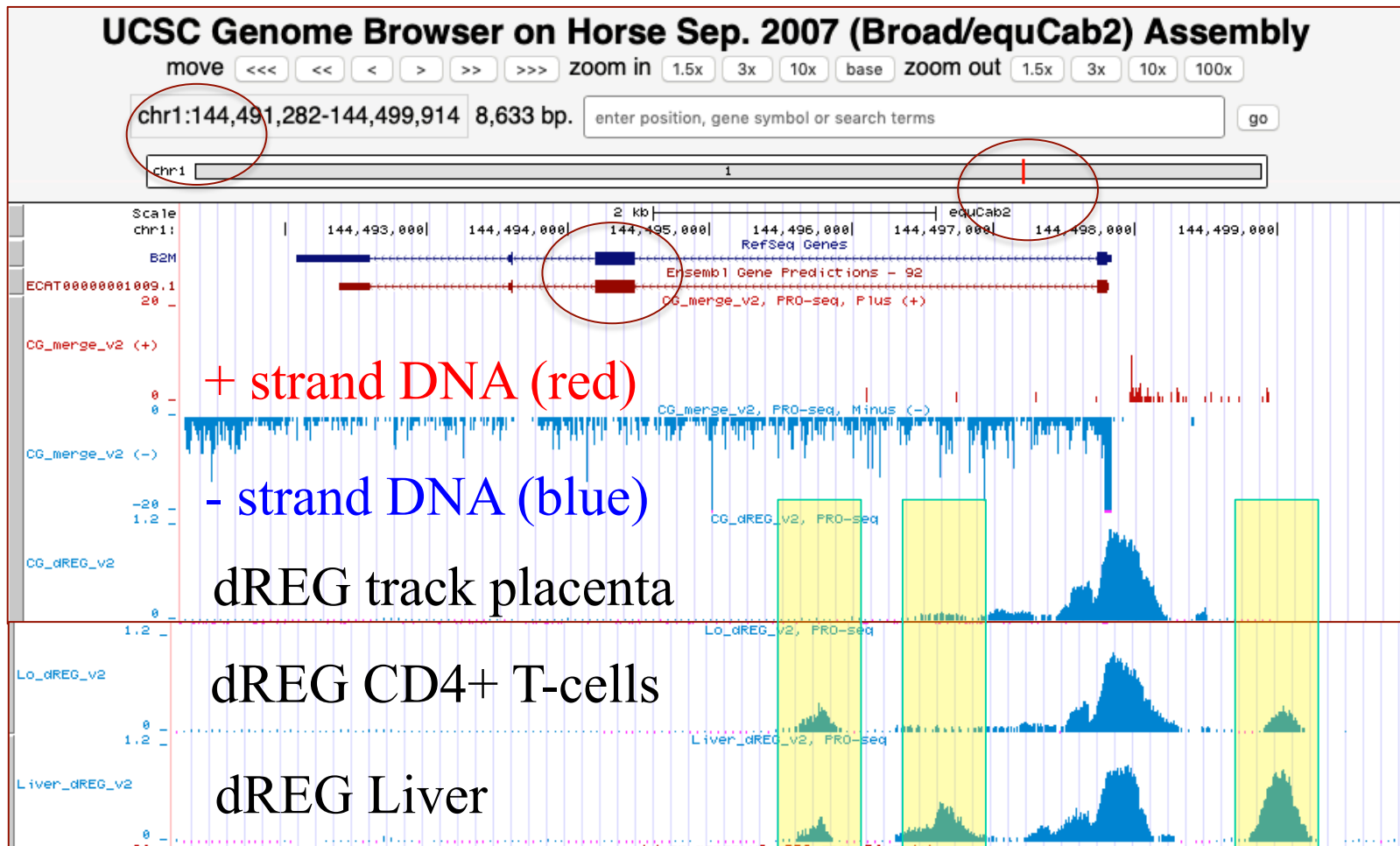
← Direction of Transcription

How to read the traces of ChRO-seq: Equine Beta 2 Microglobulin



← Direction of Transcription

How to read the traces of ChRO-seq: Equine Beta 2 Microglobulin



+ strand DNA (red)

- strand DNA (blue)

dREG track placenta

dREG CD4+ T-cells

dREG Liver

Putative Enhancers

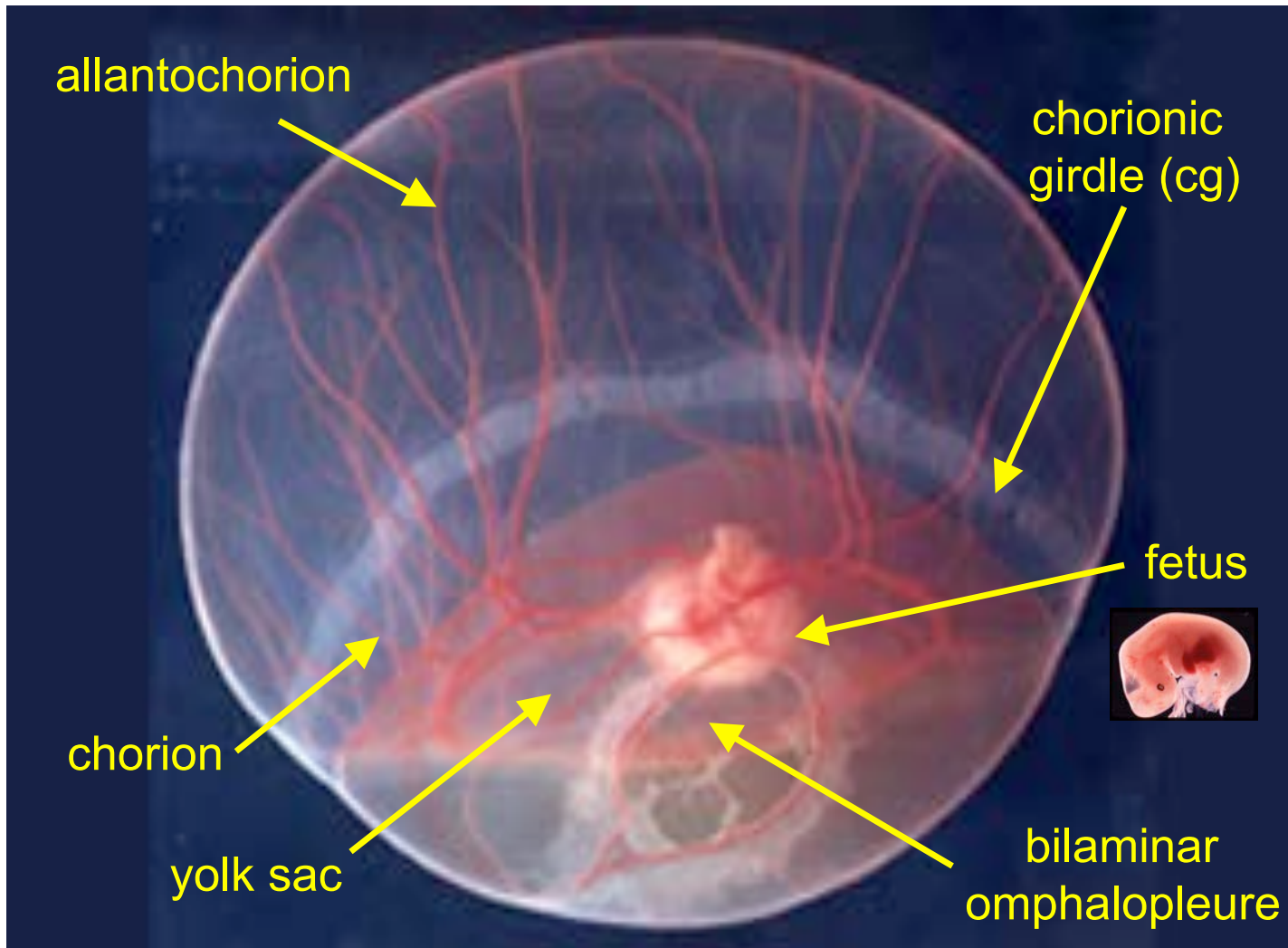
dREG peaks differ between tissues

What tissues have we tested?

- 1) Placenta: Chorionic Girdle and Chorion (Twilight x Bravo)
- 2) FACS purified CD4+ T-cells (lymphocytes from Twilight)
- 3) Liver (UCD FAANG samples)

Two biological replicates each

Day 32-36 Equine Conceptus



Data Summary: Horse Tissues

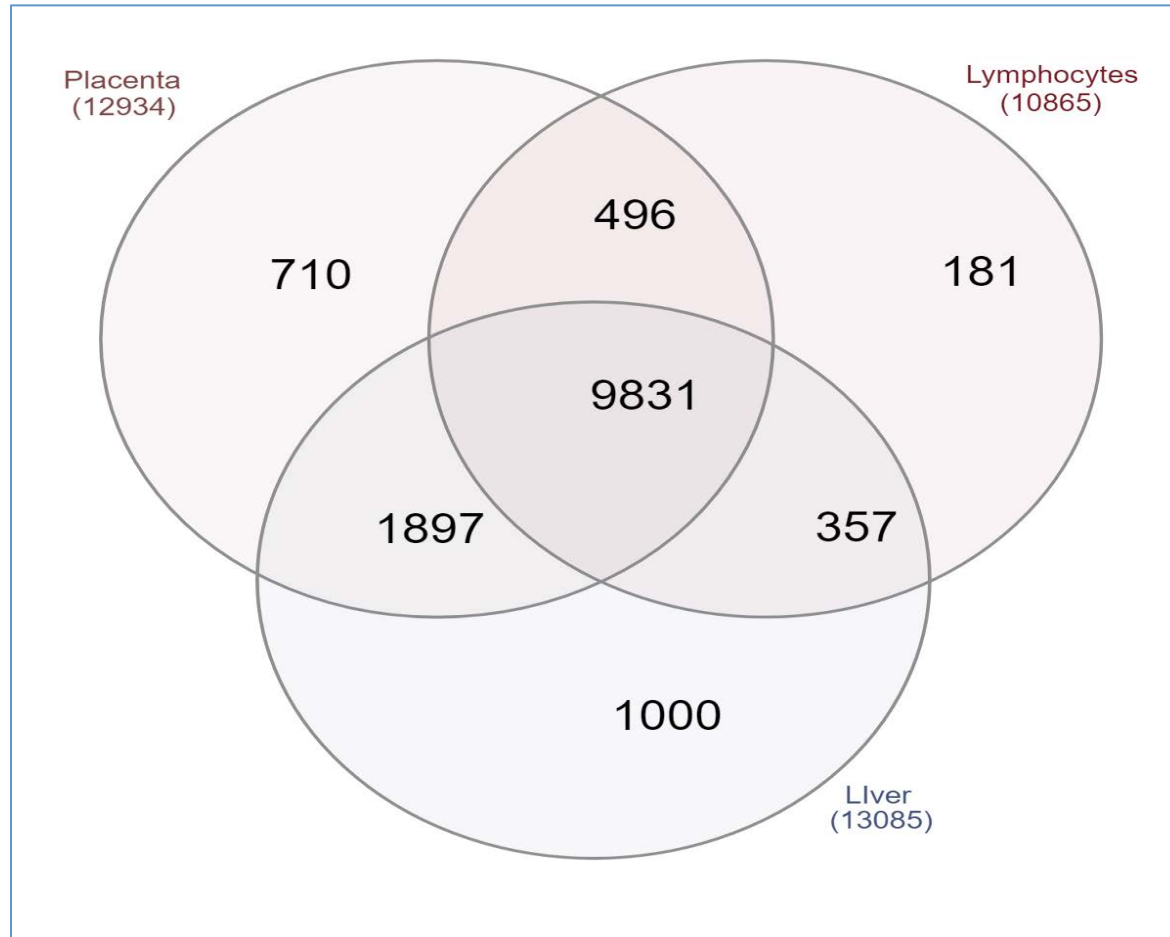
Tissue	Illumina Reads	Genes / Tissue	Body Counts / Gene
CG 1	50,549,287	11,855	330
CG 2	42,388,046		
Chorion 1	47,664,138	11,488	218
Chorion 2	42,445,334		
CD4+ T-cell 1	51,269,738	10,865	334
CD4+ T-cell 2	57,009,792		
FAANG Liver 1	54,238,882	13,085	461
FAANG Liver 2	58,664,361		

Differential Gene Expression (DGE) R script

(Danko Lab)

Placenta

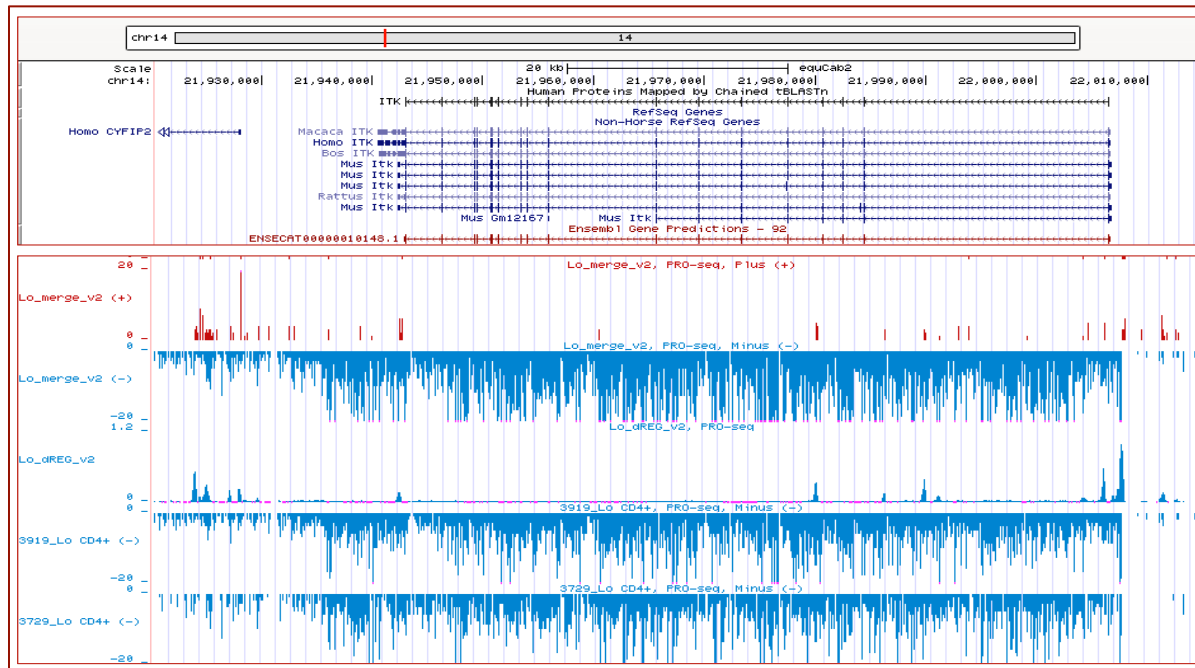
Lymphocytes



Liver

Rigor and Reproducibility of ChRO-seq

	cg_4223	cg_4258	chr_4223	chr_4258	lo_3729	lo_3919	liver_4989	liver_4990
cg_4223	1.00	0.97	0.88	0.89	0.77	0.77	0.69	0.76
cg_4258	0.97	1.00	0.87	0.89	0.76	0.77	0.70	0.76
chr_4223	0.88	0.87	1.00	0.92	0.72	0.73	0.71	0.76
chr_4258	0.89	0.89	0.92	1.00	0.74	0.75	0.74	0.80
lo_3729	0.77	0.76	0.72	0.74	1.00	0.95	0.66	0.72
lo_3919	0.77	0.77	0.73	0.75	0.95	1.00	0.66	0.72
liver_4989	0.69	0.70	0.71	0.74	0.66	0.66	1.00	0.93
liver_4990	0.76	0.76	0.76	0.80	0.72	0.72	0.93	1.00



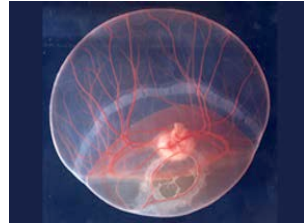
ITK gene
CD4+ T-cells

Transcription Factor Target Program (Danko Lab script)

- 1) Upregulated expression of Transcription Factor (TF)
- 2) Enriched TF binding site motifs (dREG output) associated with the same TF
- 3) Differentially expressed (or upregulated) downstream target genes affected by the regulatory regions associated with those TFs

Transcription Factor Target Program

Chorionic Girdle vs. Chorion



TF	Prior Association with Trophoblast?
GATA2	Yes
GATA3	Yes
NFAT5	Yes
BCL6	Yes
RXRB	No, but + in embryogenesis

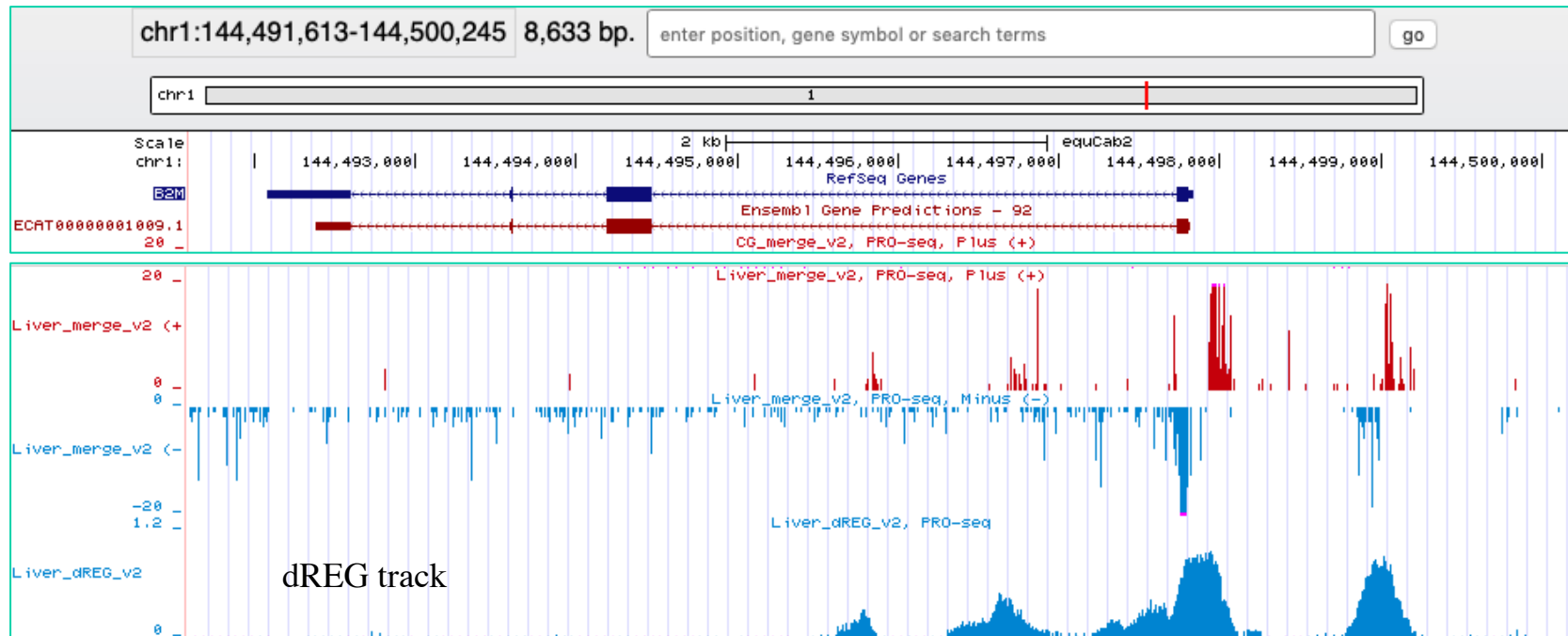
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**Comparison with horse FAANG data:
ChIP-seq and ATAC-seq**

ChRO-seq vs. ChIP-seq & ATAC-seq

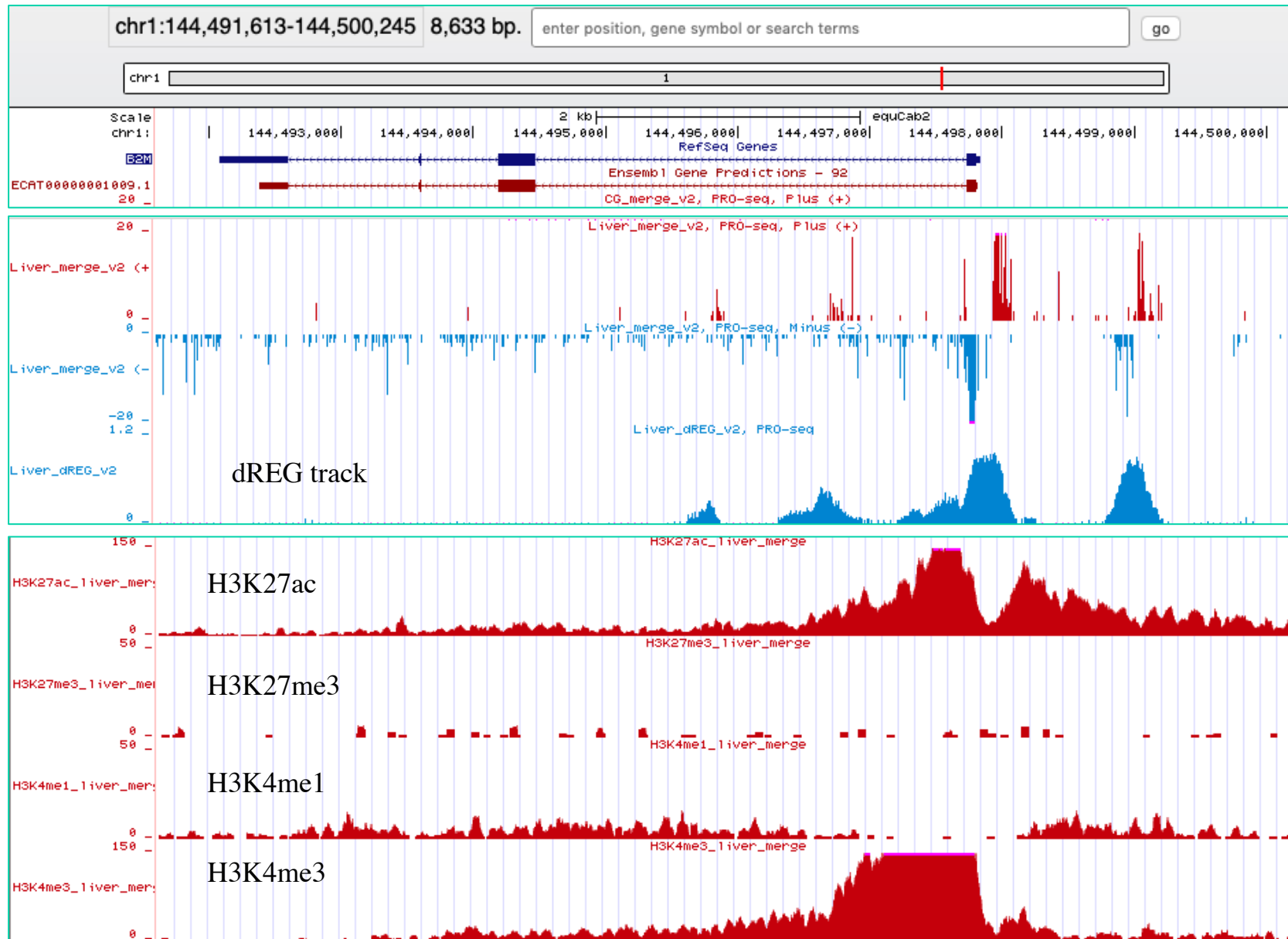


Beta 2 Microglobulin – FAANG Horse Liver Samples

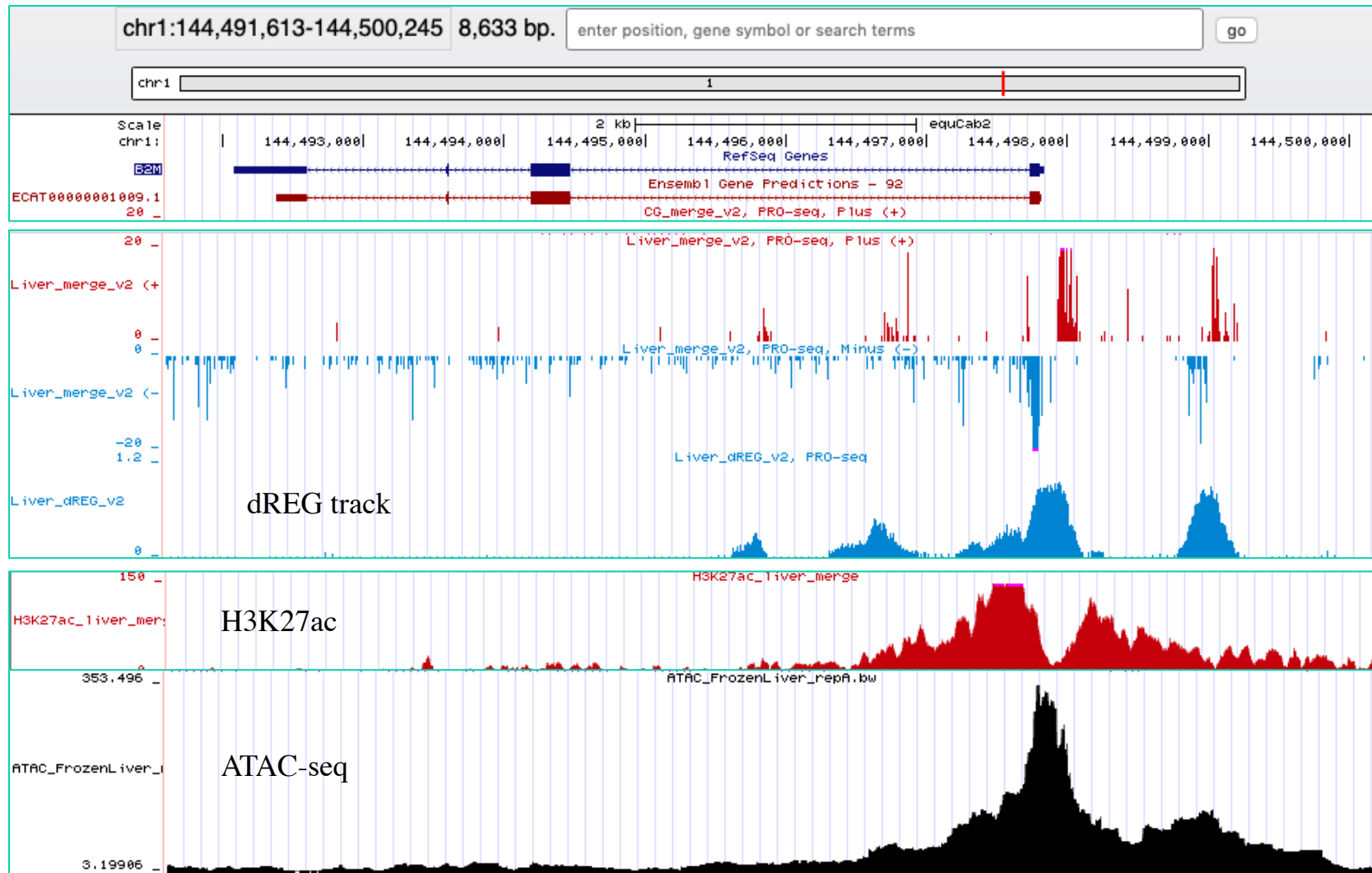


Direction of Transcription

ChRO-seq vs. ChIP-seq & ATAC-seq



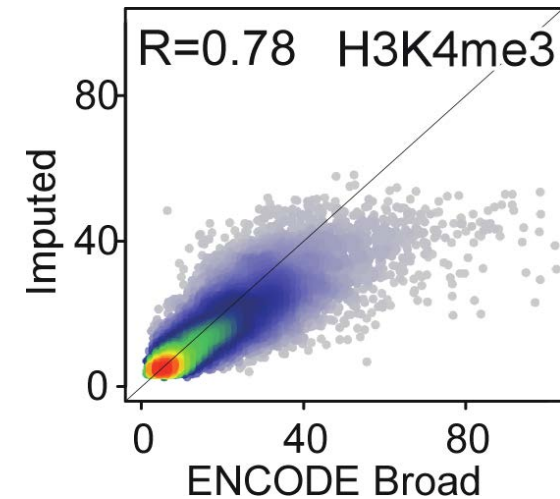
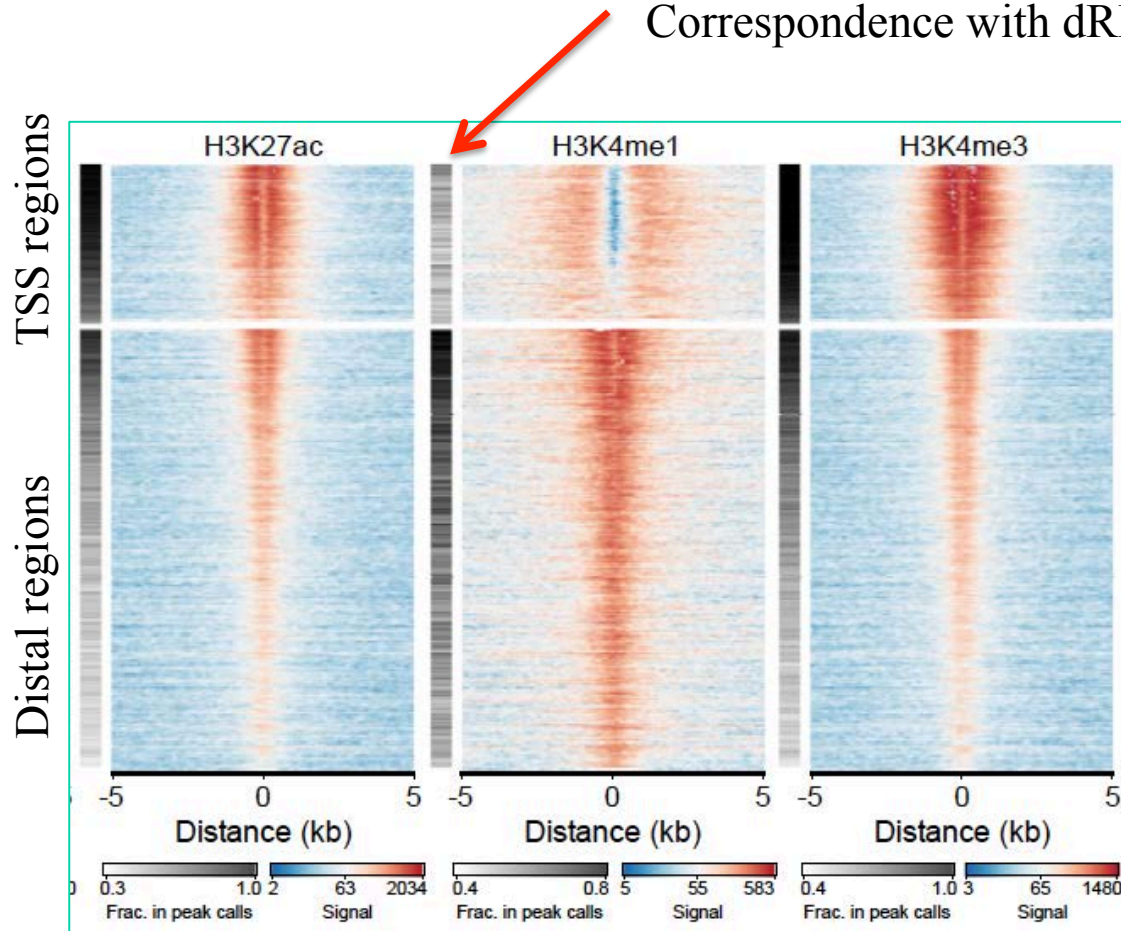
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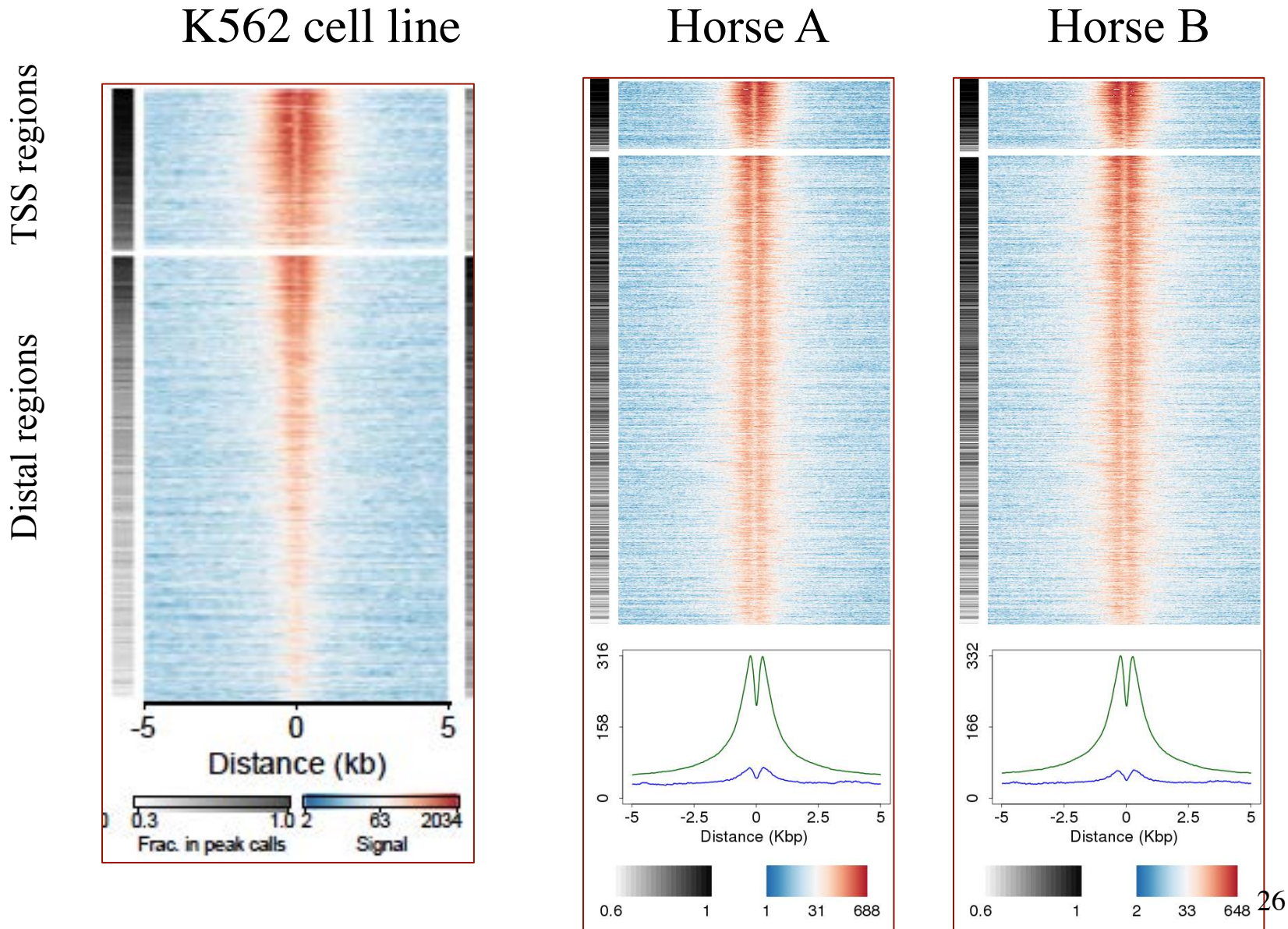
Whole genome analysis – 3 marks

K562 cell line

Correspondence with dREG peaks



H3K27ac vs. ChRO-seq whole genome analysis



Outline

An introduction to ChRO-seq

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Conclusion

ChRO-seq holds promise
for rapid identification
of regulatory elements -
often with higher precision
than ENCODE methods