

Epigenomic Landscapes from Various Cells and Tissues of *Gallus gallus*

R. David Hawkins, Ph.D.

Associate Professor

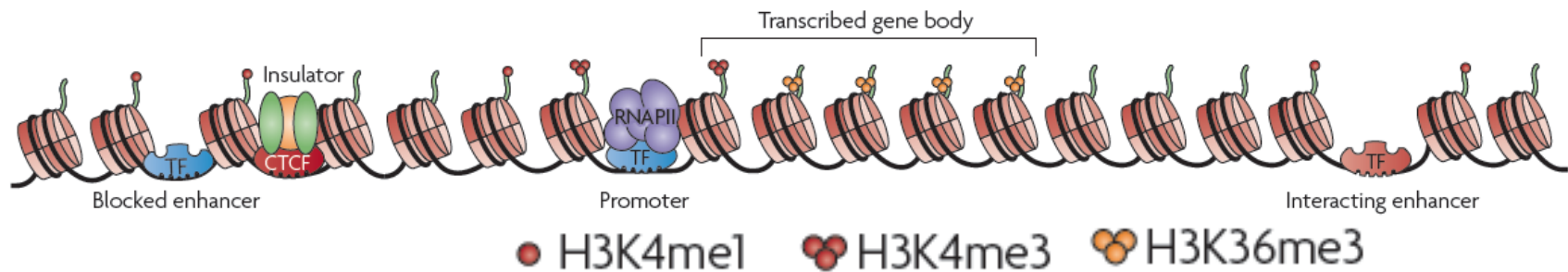
University of Washington School of Medicine

Medicine, Division of Medical Genetics

Genome Sciences

Institute for Stem Cell and Regenerative Medicine

General Overview



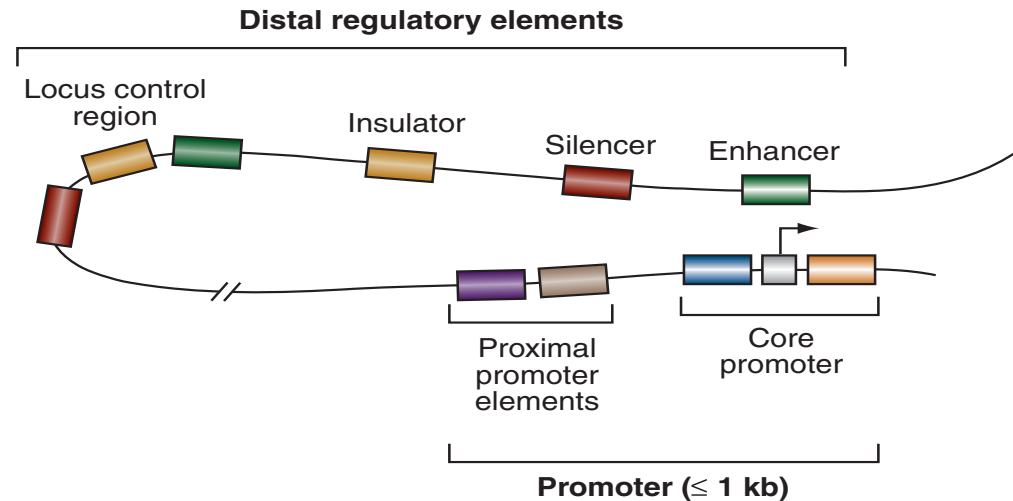
- Annotate *cis*-regulatory elements and novel transcripts in the chicken genome.
- Identify changes in epigenomic states that correspond to differences in *cis*-regulatory activity and expression.

General Overview

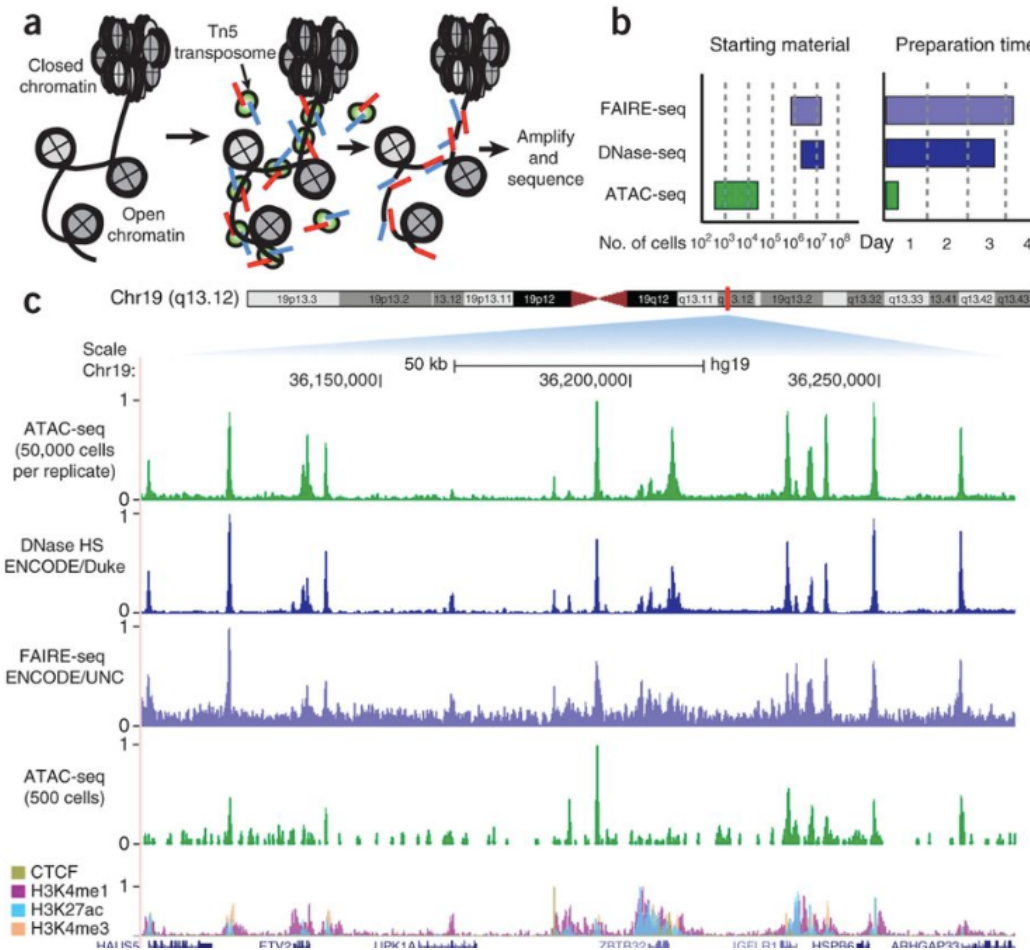
- *Gallus gallus* reference/data production ADOL line 6x7 F1.
 - assay optimization: SPF.
- Targeted tissues and cells for Phase 1:
 - Blood cells: macrophage, B cells, polarized T cells; lung macrophages.
 - Reproductive tissues: ovaries, oviduct, shell gland.
- Assays
 - RNA-seq
 - ATAC-seq
 - ChIP-seq
 - WGBS
 - 3D genome architecture

Regulation of gene expression

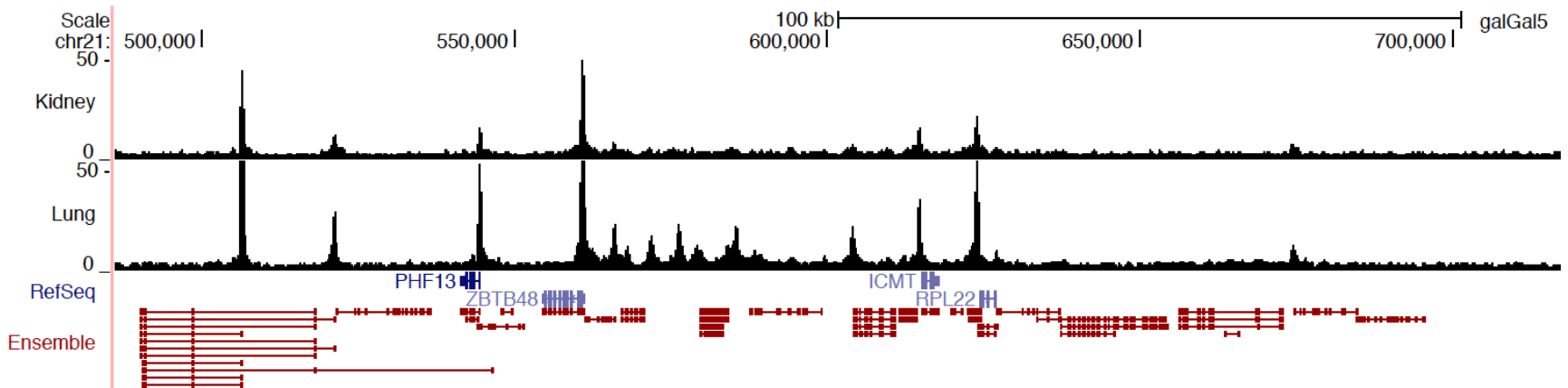
- Transcriptional regulatory elements
 - Promoters
 - Enhancers
 - Insulators
 - Silencers



ATAC-seq



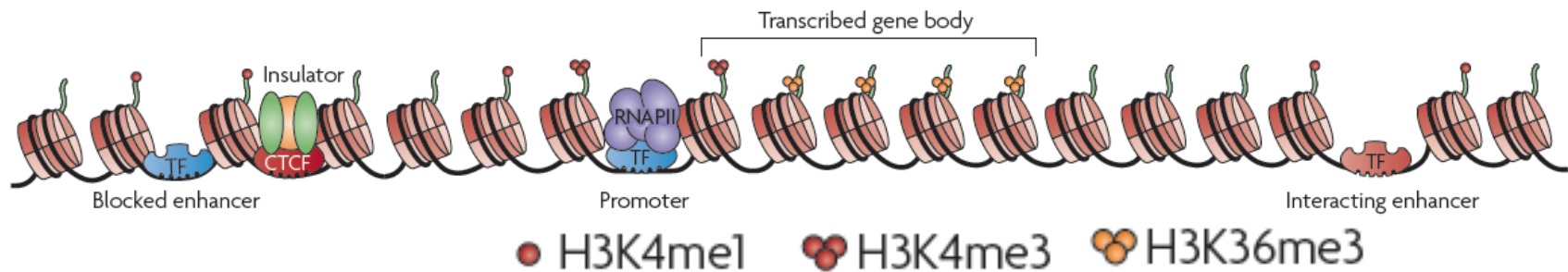
ATAC-seq



- ATAC-seq on lung and kidney tissue.
- ~72,000 peaks called per tissue.
- ~4% reads map to chrM.

Analysis Step	Read Count
Original	50,204,006
After trimming	50,204,006
Remove Unmapped	43,122,572 (14.1% loss)
Remove Orphaned Reads & non-unique reads	43,122,450
Remove Duplicates	31,789,256 (22.6% loss)

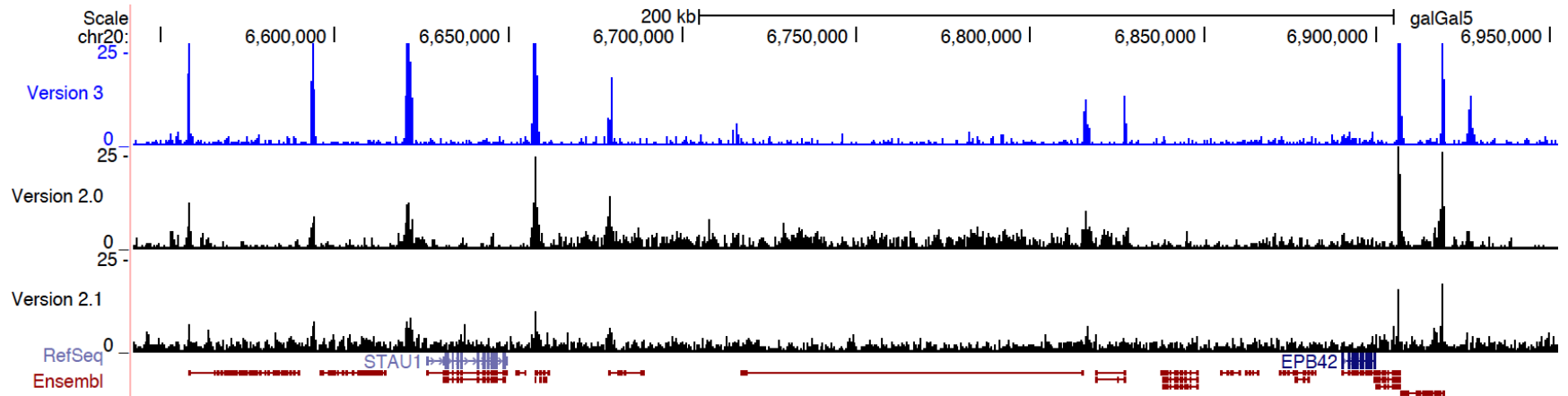
ChIP-seq



- H3K4me3: novel & alternative promoters
- H3K4me1: enhancer elements
- H3K36me3: validate novel transcripts from RNA-seq
- CTCF: insulators
- H3K27ac: indicative of active promoters/enhancers
- H3K27me3: repressed, bivalent promoters

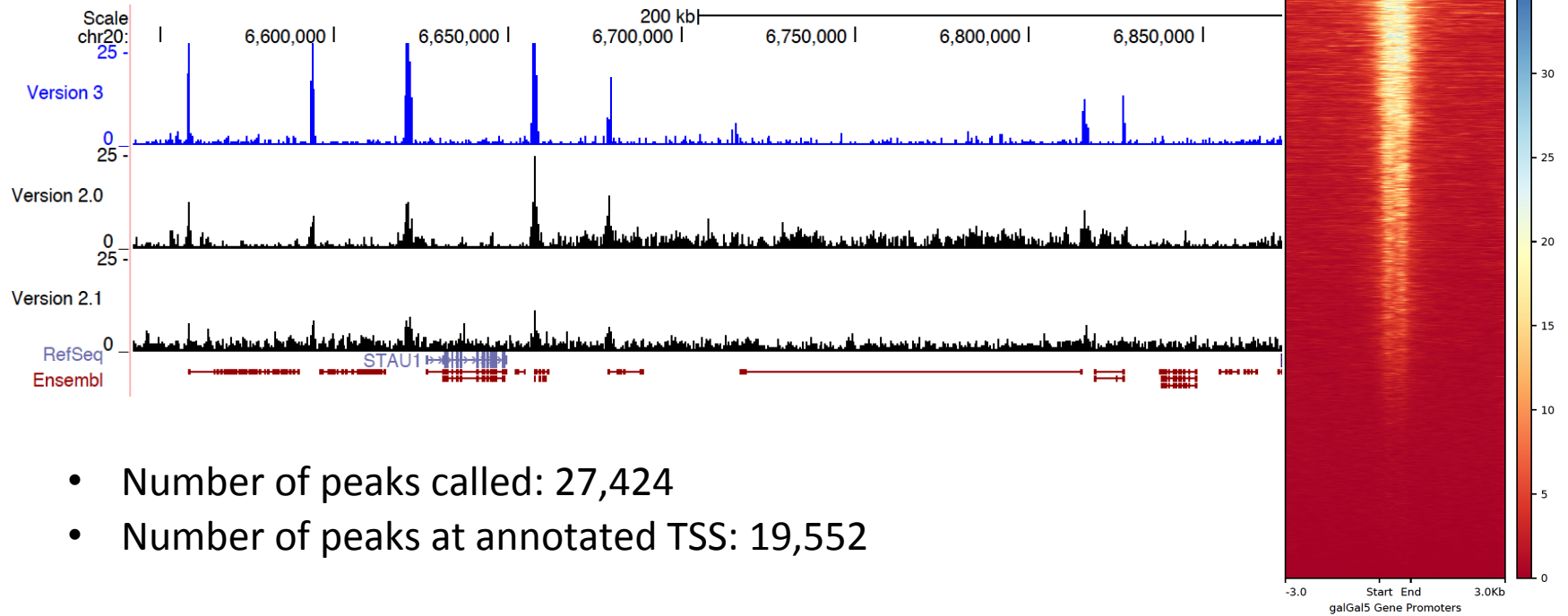
ChIP-seq

H3K4me3 from lung



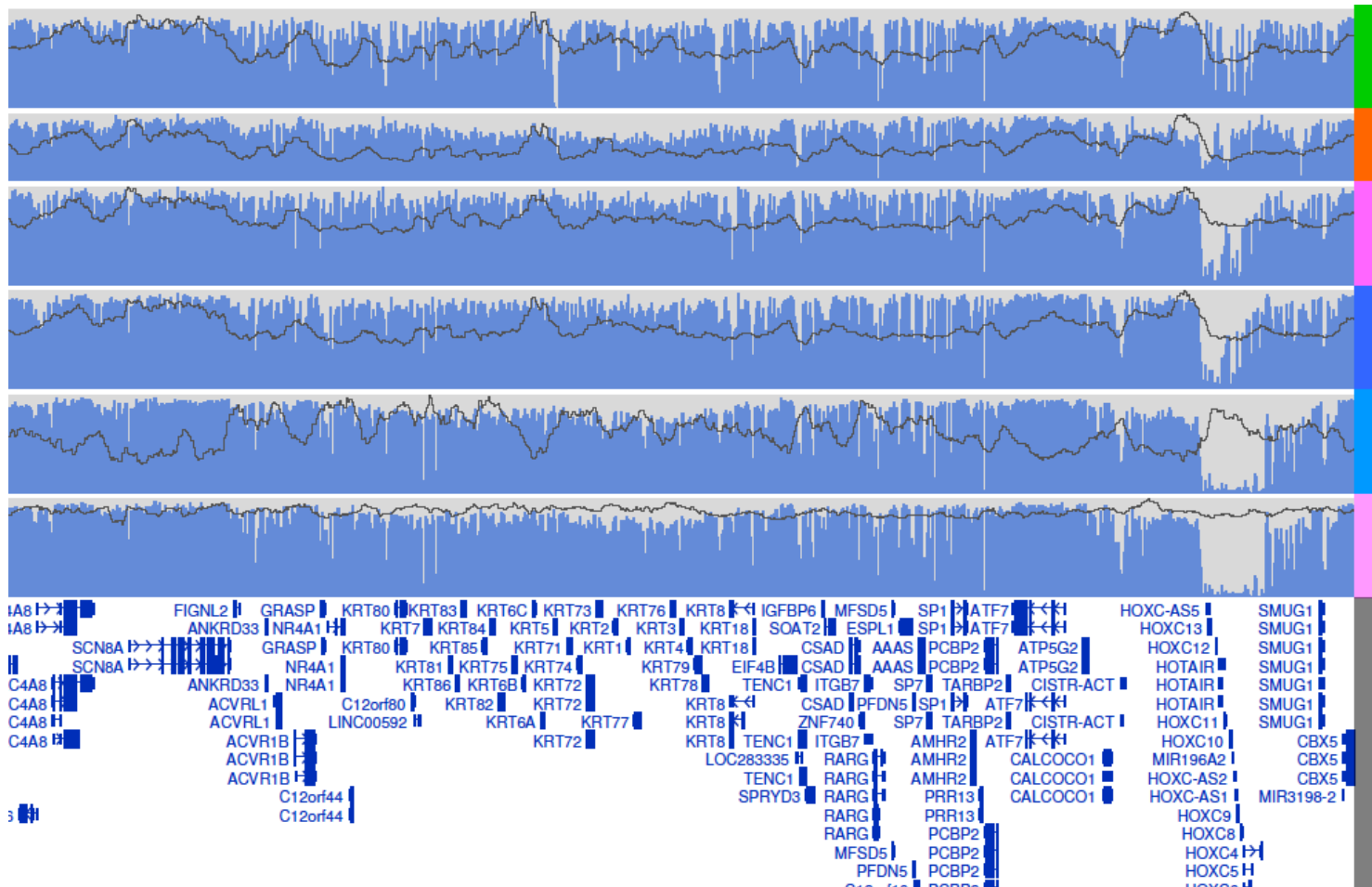
ChIP-seq

H3K4me3

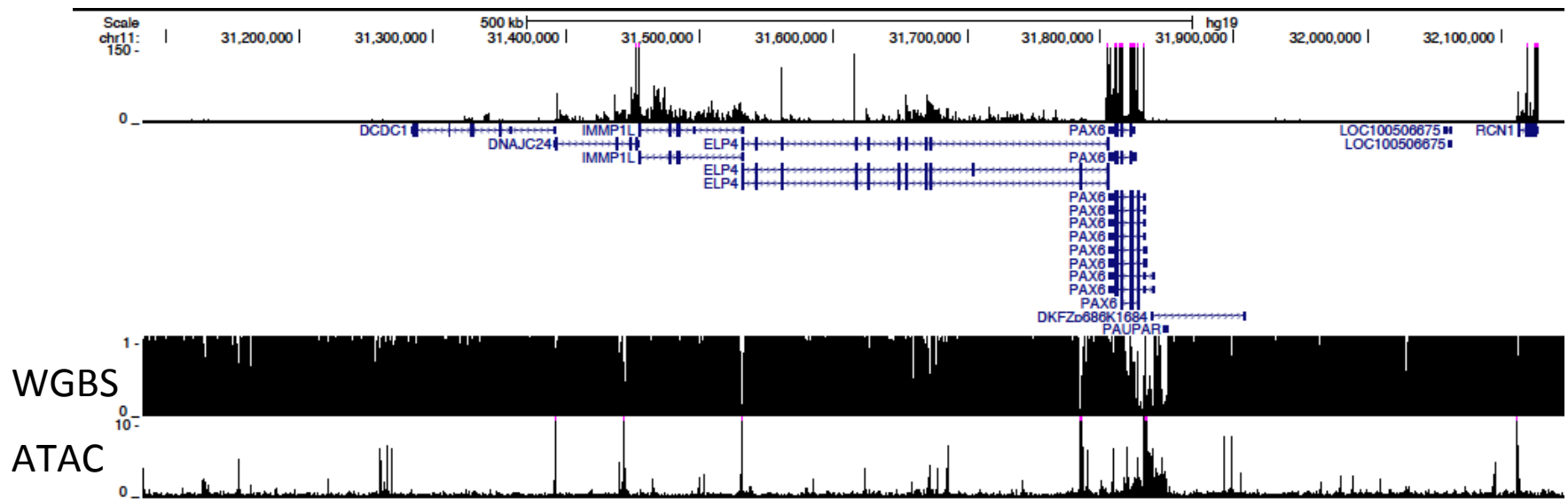


- Number of peaks called: 27,424
- Number of peaks at annotated TSS: 19,552

DNA methylation: WGBS

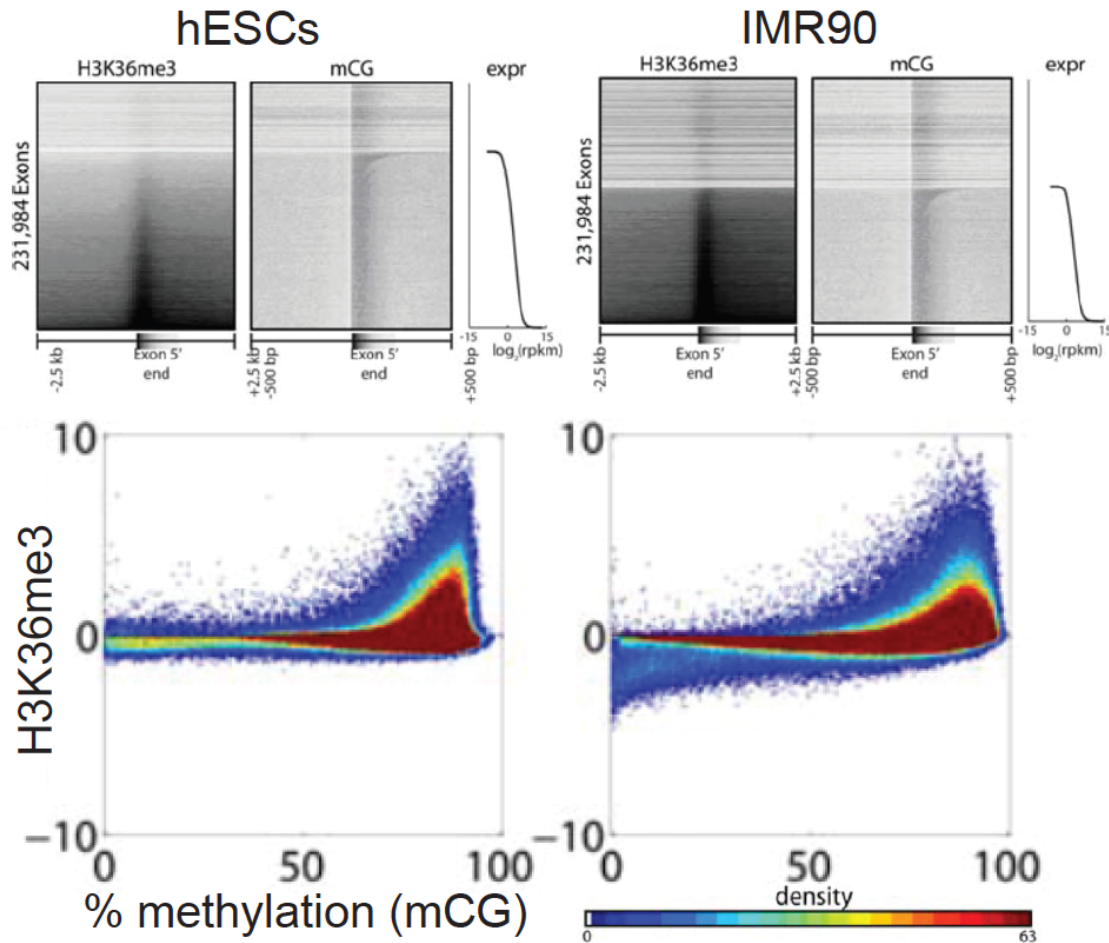


DNA methylation: WGBS



- Unmethylated regions (UMRs) – broad regions largely devoid of DNA methylation
 - UMRs: Largely indicative of promoters (and CGIs)
- Lowly methylated regions (LMRs) – narrow regions of low/modest DNA methylation
 - LMRs = Largely indicative of enhancers

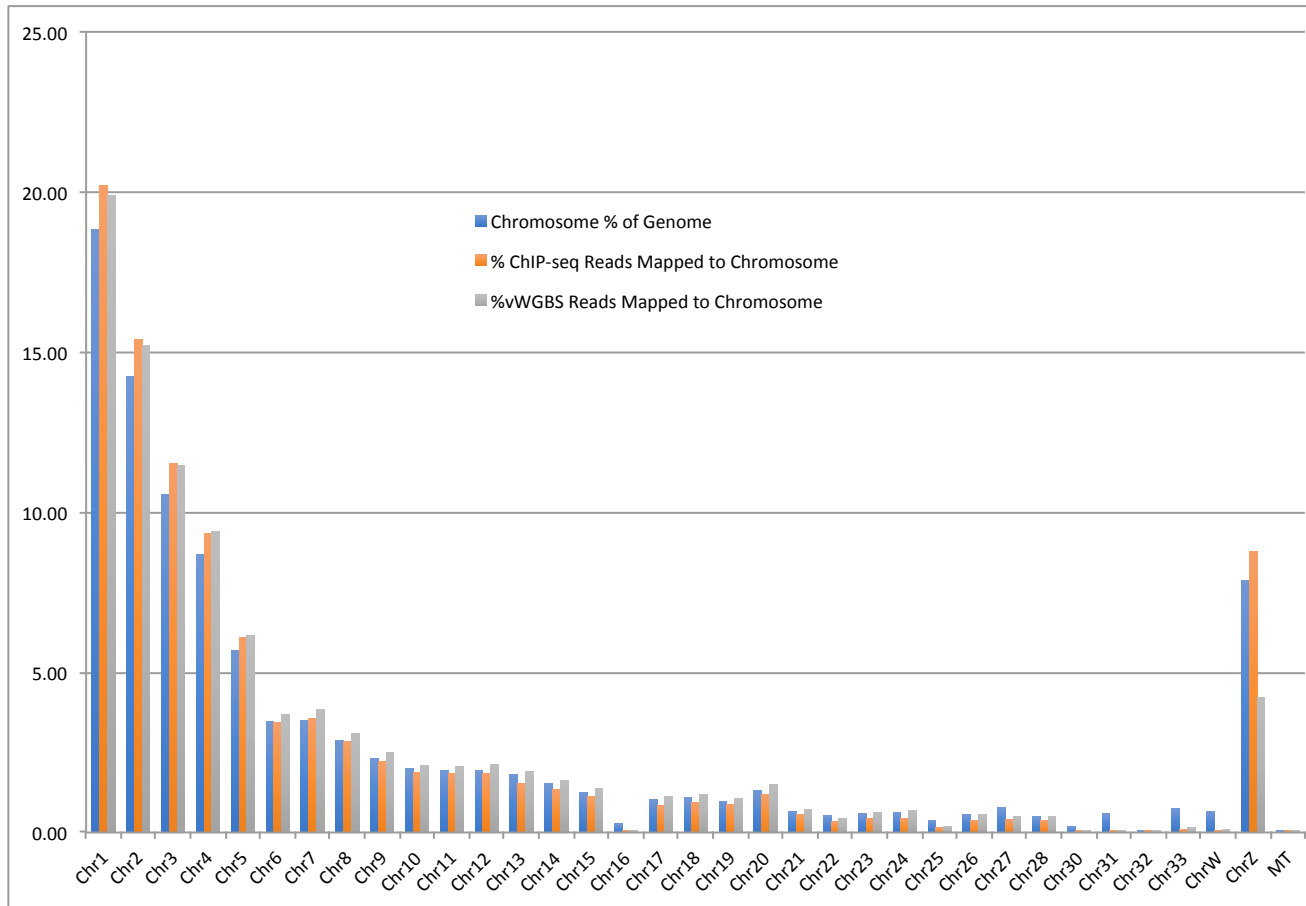
WGBS validation of transcription



WGBS QC from 6x7 F1 chickens

Cell Type	BSC rate (%) lambda	Mapping efficiency (%)	CpG methylation (%)
Macrophage rep1	99.7	81.2	62.0
Macrophage rep2	98.7	77.5	65.0
B cells rep1	99.6	81.8	63.8
B cells rep2	99.4	77.9	66.9
Th cells rep1	99.1	82.5	72.6
Th cells rep2	99.9	81.7	71.5

QC: mapping bias



Summary

- Expression and Epigenomic assays have been optimized for data production.
- Blood cells and reproductive tissue have been isolated from production/reference line.
- PLAC-seq is being optimized to map promoter-enhancer interactions.

The Lab

Stephanie Battle

Naresh Doni Jayavelu

Elyn Fritz-Waters*

Ajay Jajodia

Arpit Mishra

Elijah Overbey

Thomas Smith*

Cristina Valensisi

Eric Waddell*

Hans Cheng MSU

Huaijun Zhou UCD

Western University

Yvonne Drechsler

Pietro Catini

Suzana Tkalcic

**STRAIGHT
OUTTA
THE NUCLEUS**

\$\$\$\$

AFRI-USDA

NIH NIDDK

NIH NIAMS

WA-LSDF

ChIP sonication bio reps

