

Genome-wide ChIP-seq Mapping & Analysis of Butyrate-induced Histone Acetylation in Bovine Cells

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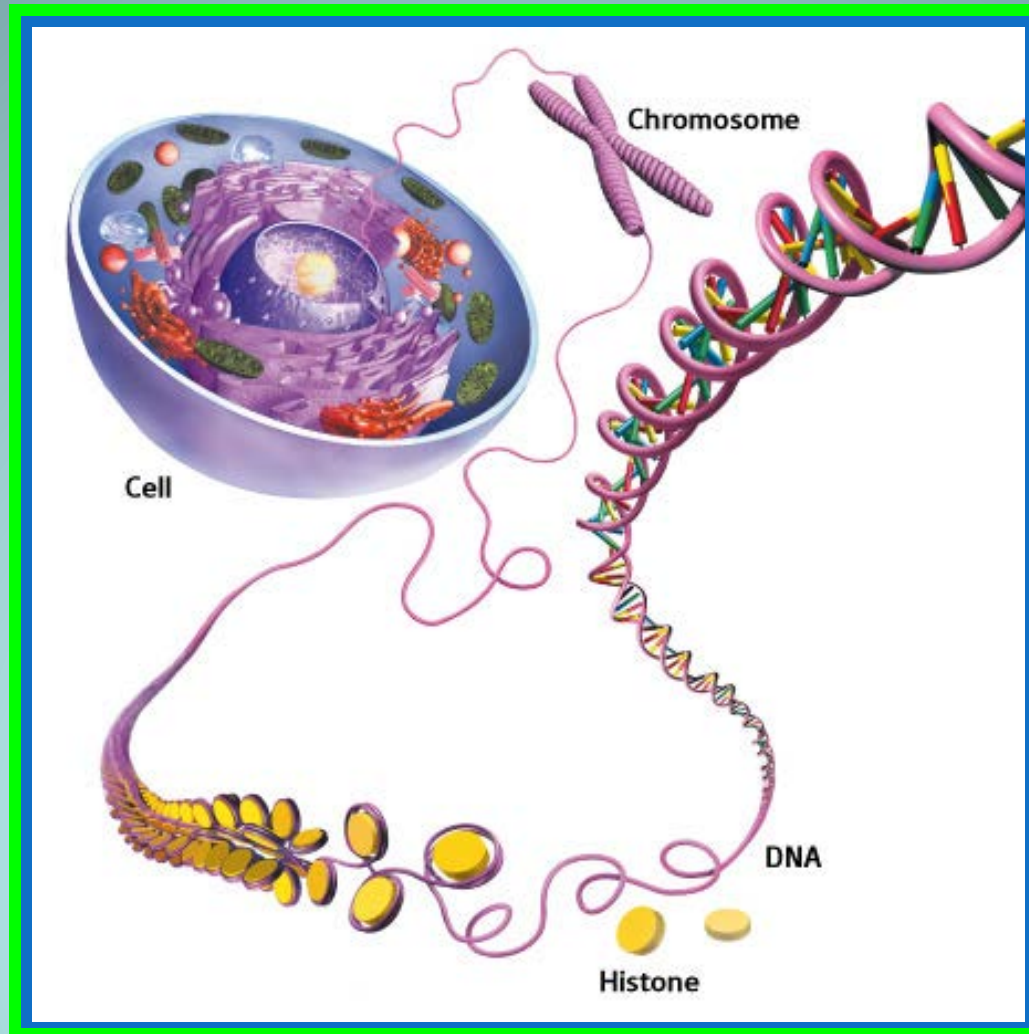


The Sequence of the Bovine Genome Reveals New Insights into the history and Biology of Cattle

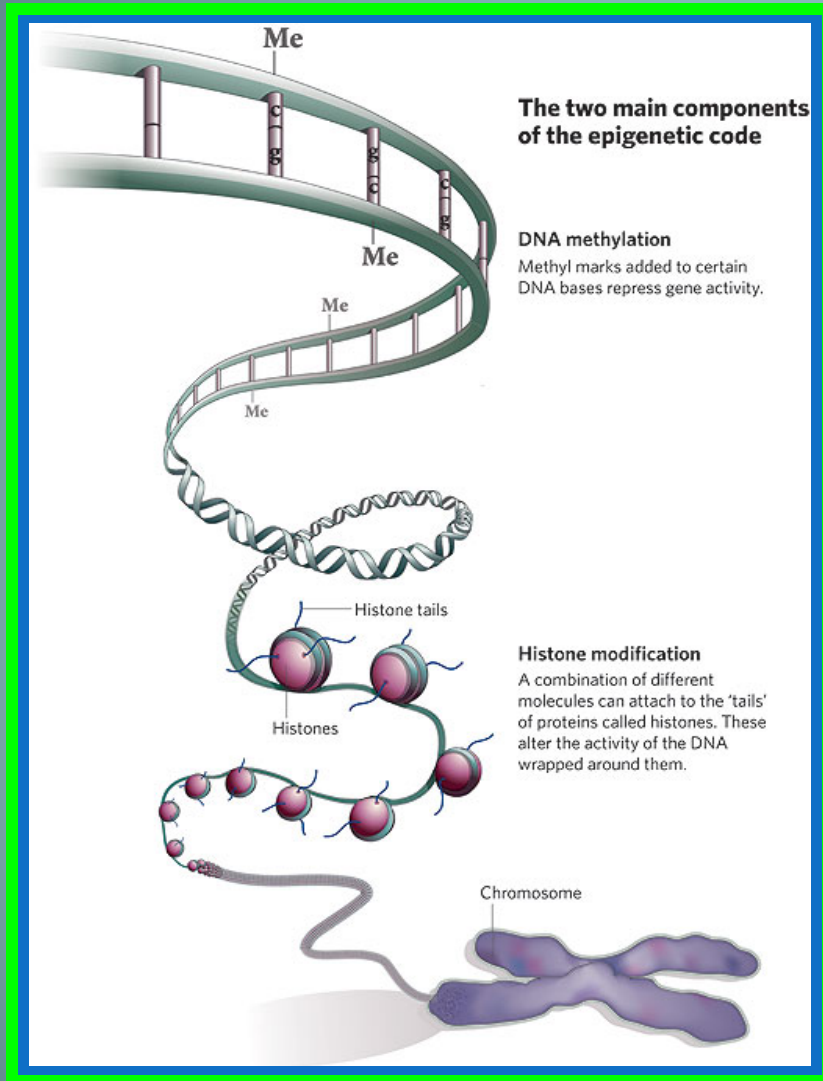


Epigenetics: DNA Isn't Everything

Histone winds up long DNA into a compact state



Molecular basis of epigenetics



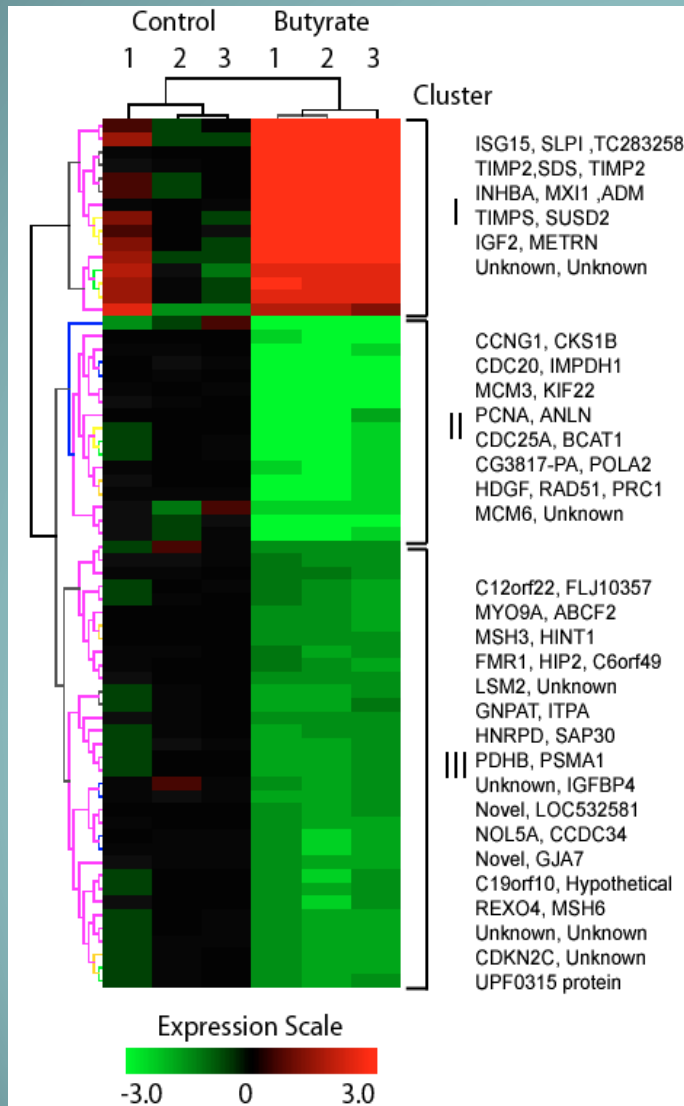
Epigenetic regulations:

- 1) **Histone modifications:**
acetylation
methylation
phosphorylation
ubiquitination
- 2) **DNA methylation**
- 3) **Genomic imprinting**
- 4) **miRNA**
- 5) **Prion proteins**
- 6) *etc. etc. etc...*

Butyrate-induced histone acetylation

- ✓ Ruminant species have evolved to metabolize VFA (acetate, propionate, and butyrate) to fulfill up to 70% of their nutrient energy requirements.
- ✓ VFA have important implication for ruminant physiology
- ✓ Butyrate links nutrition to histone acetylation and epigenetic regulation

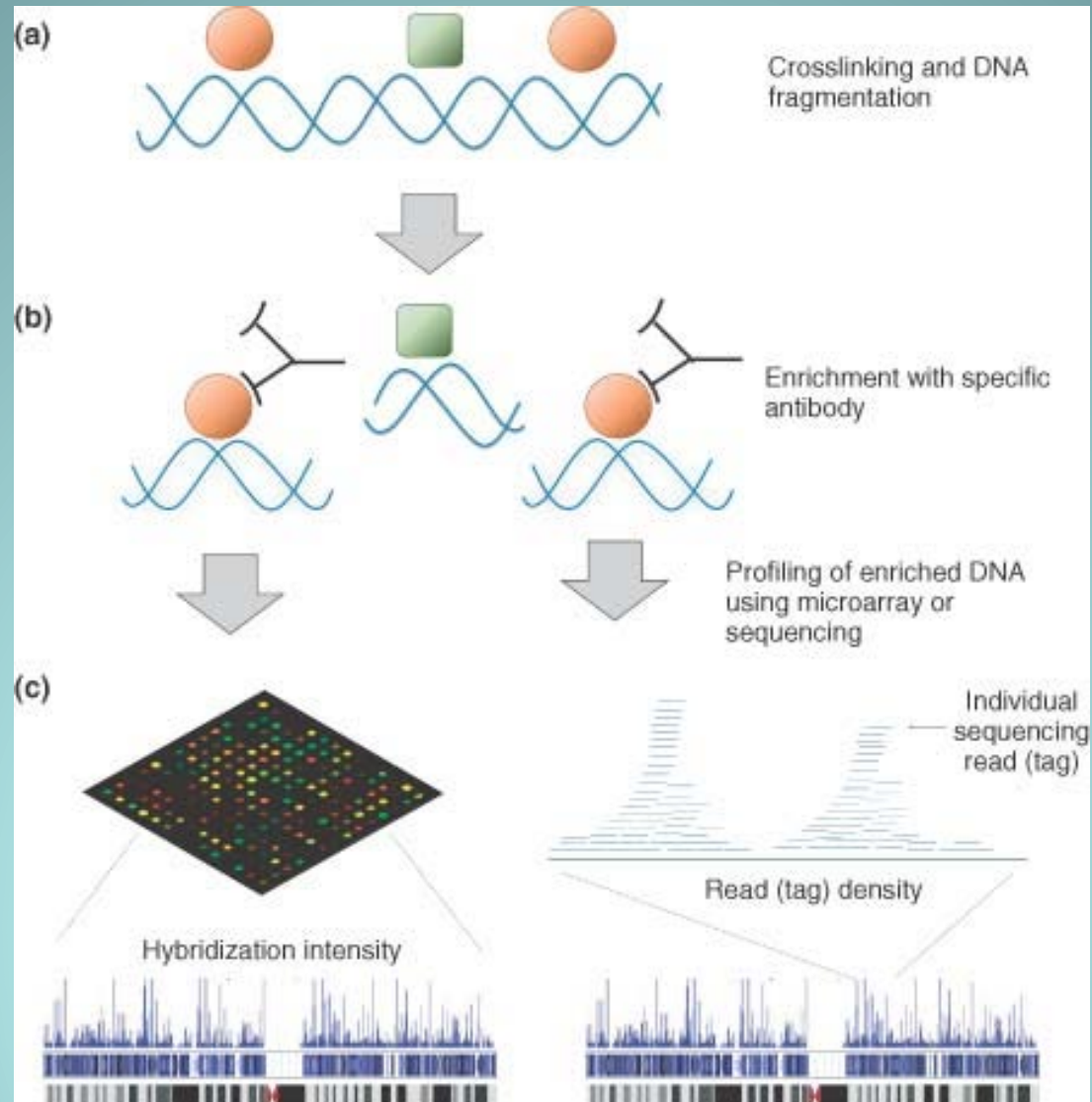
Butyrate-induced histone acetylation regulates gene expression



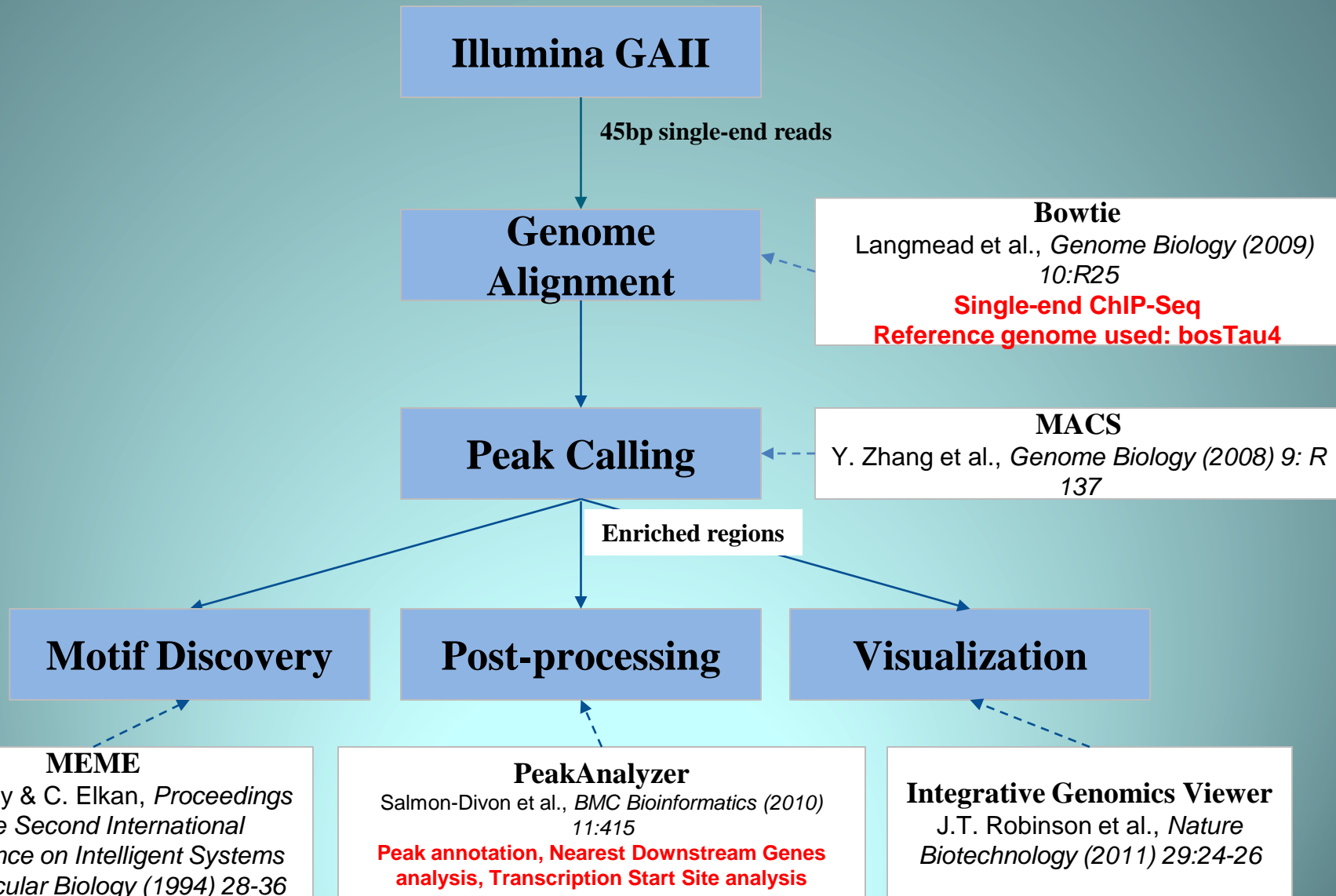
Global gene expression profiles indicated:

- 1) Only 2~3% of genes are regulated by butyrate
- 2) There are more genes down regulated than up-regulated by butyrate
- 3) Other regulatory pathway may be involved too

Workflow of ChIP-CHIP/ChIP-seq



Overall Processing Procedure



Bowtie Alignment Results

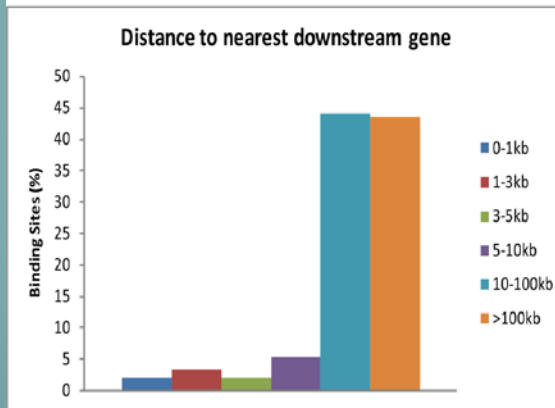
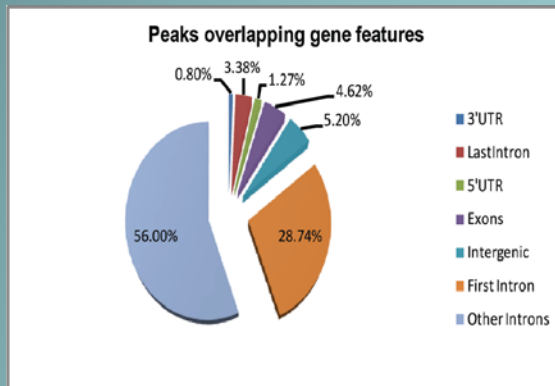
Bowtie alignment with 2 mismatch		
Sample	Total reads	Mapped
H3	29,780,699	27,078,053 (90.92%)
H3K9	23,326,139	21,368,435 (91.61%)
H3K27	30,901,336	28,318,383 (91.64%)

MACS Peak Detection Results

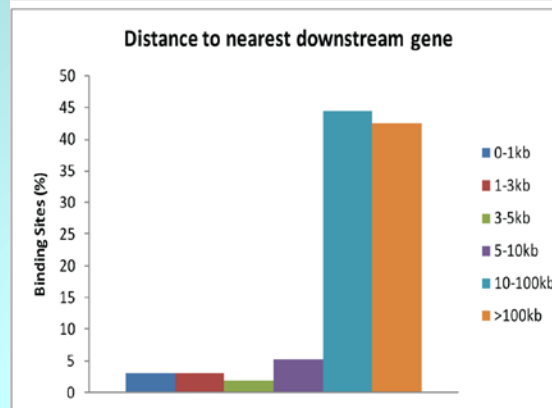
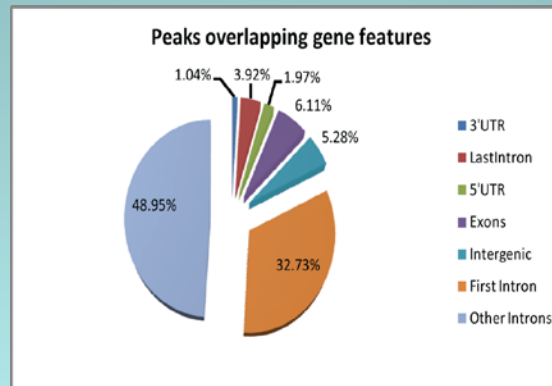
Sample	# peaks detected
H3	16,927
H3K9	9,160
H3K27	50,754

Nearest Downstream Gene (NDG) Analysis

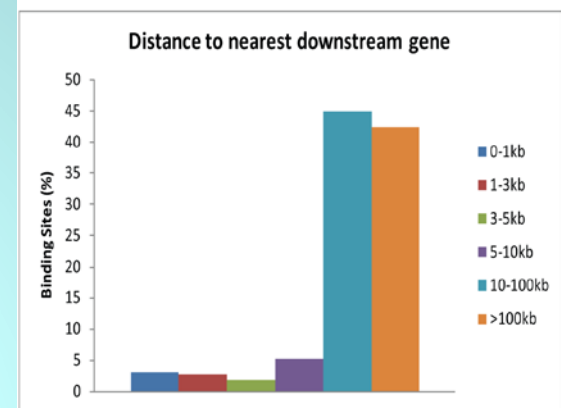
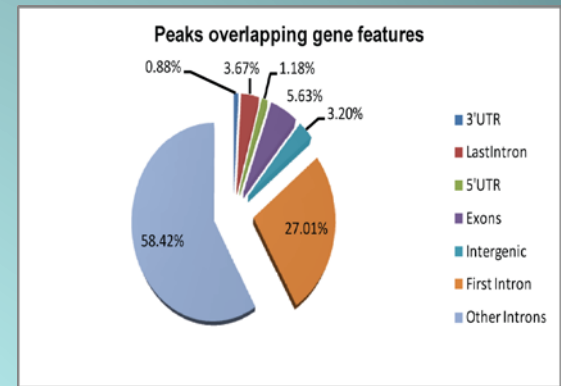
H3



H3K9

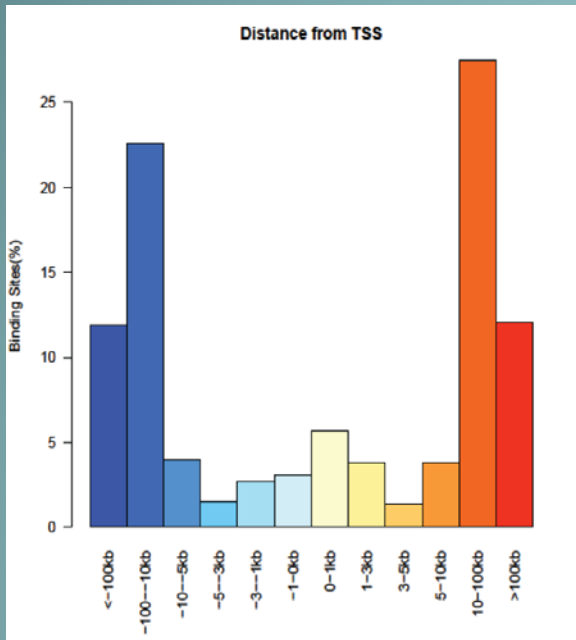


H3K27

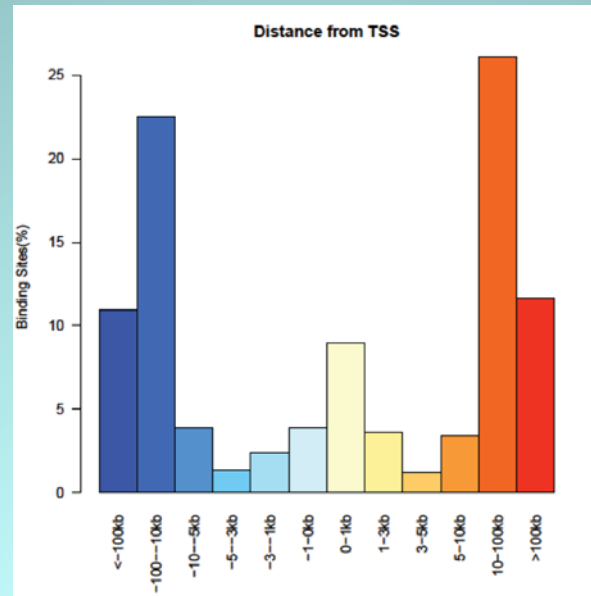


Transcription Start Site (TSS) Analysis

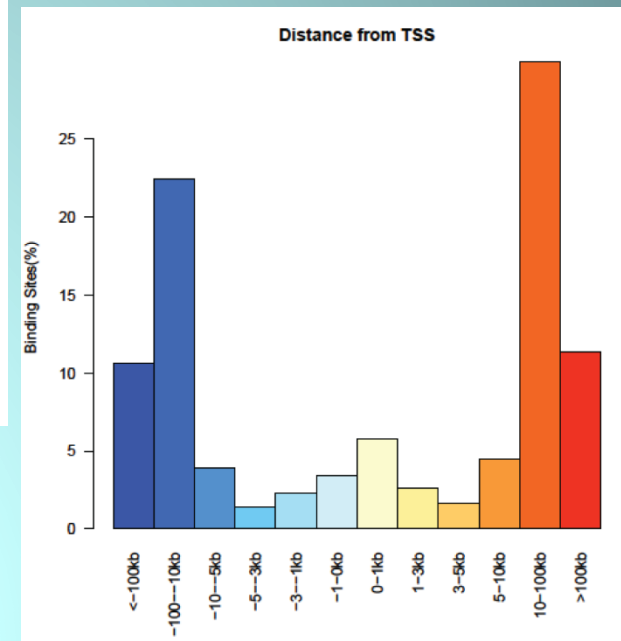
H3



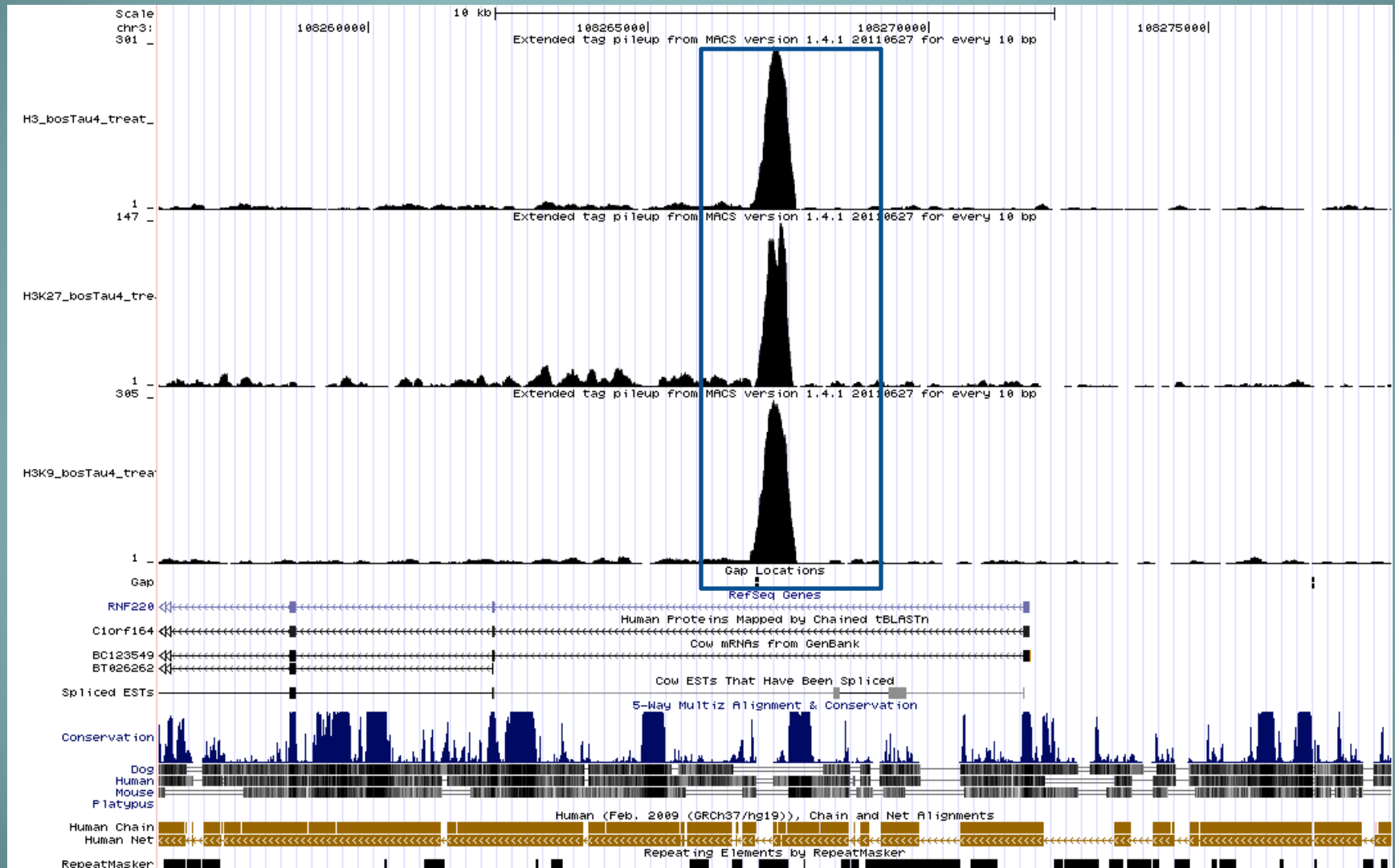
H3K9



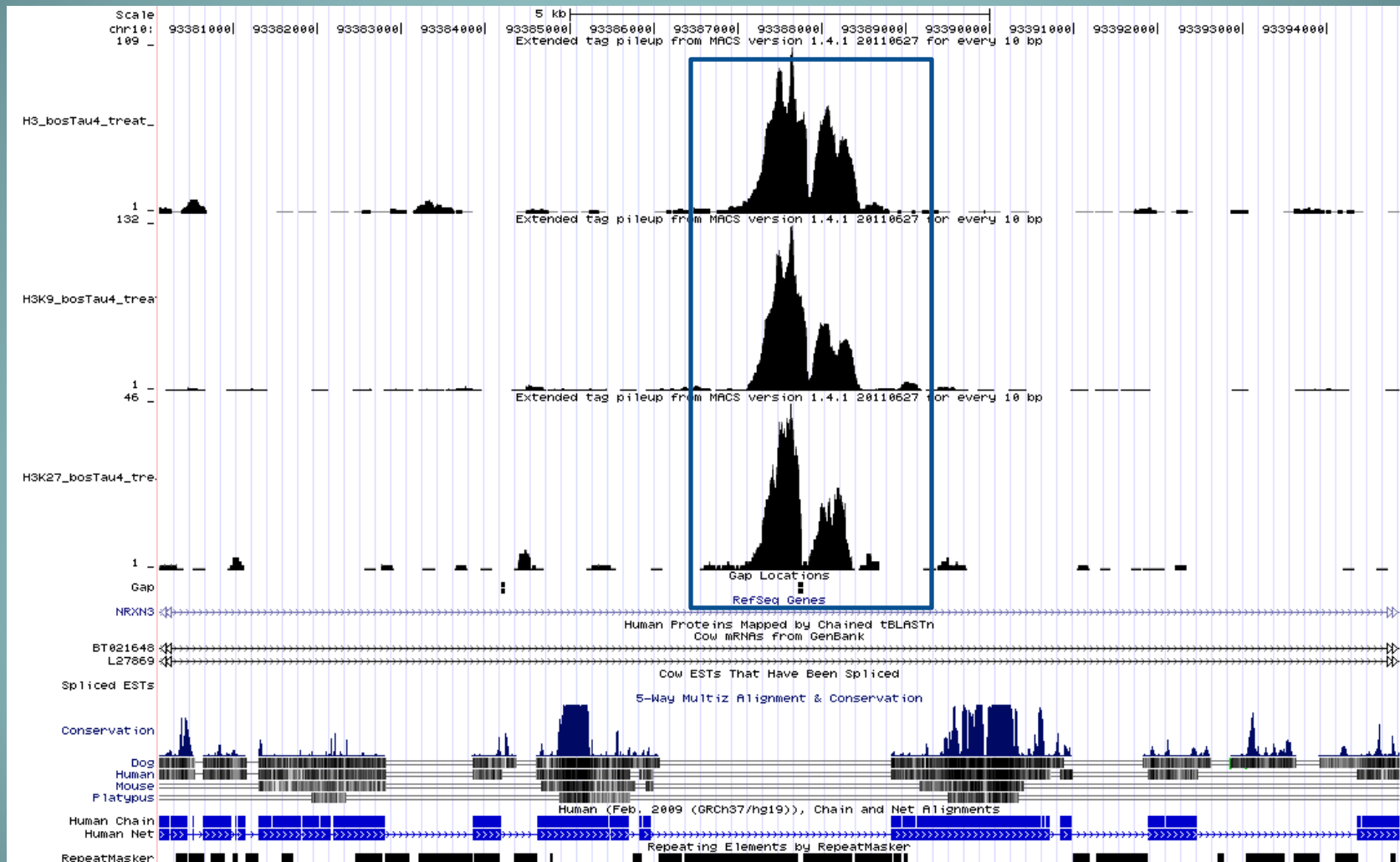
H3K27



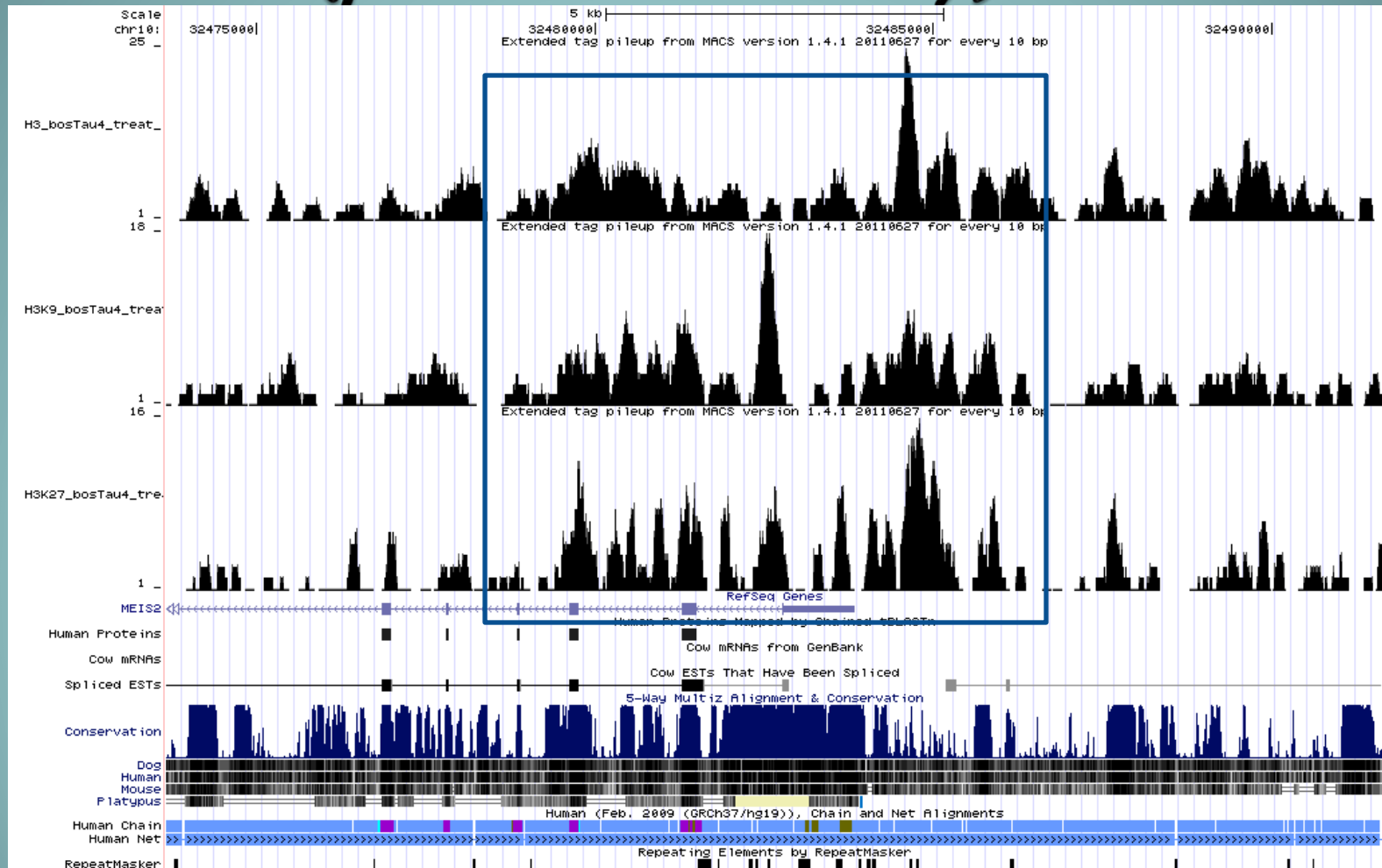
UCSC browser view of Genomic data of representative chromosome regions (peak similarity)



UCSC browser view of Genomic data of representative chromosome regions (peak similarity)

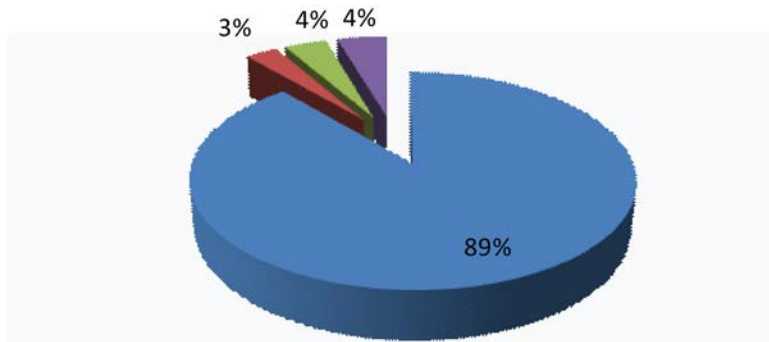


UCSC browser view of Genomic data of representative chromosome regions (peak dissimilarity)



Correlation of gene perturbed by butyrate & ChIP-seq detected binding peaks

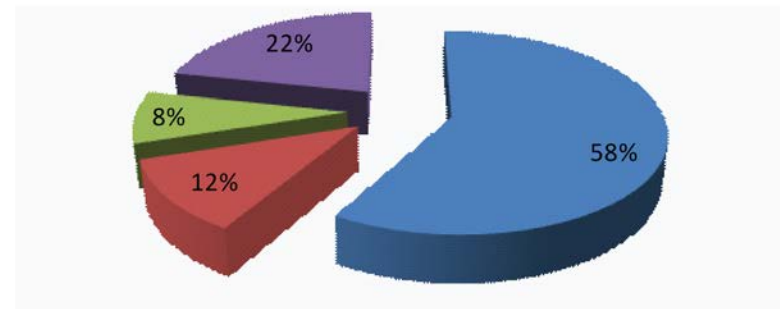
■ No peak detected ■ H3 ■ Acetyl-H3H9 ■ Acetyl-H3H27



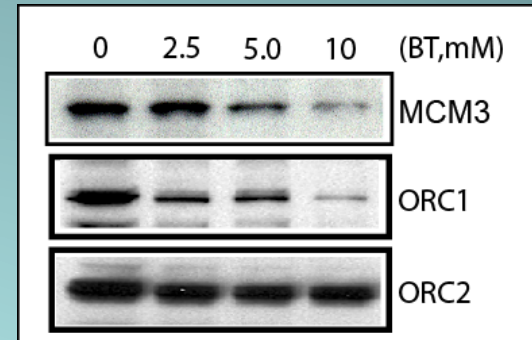
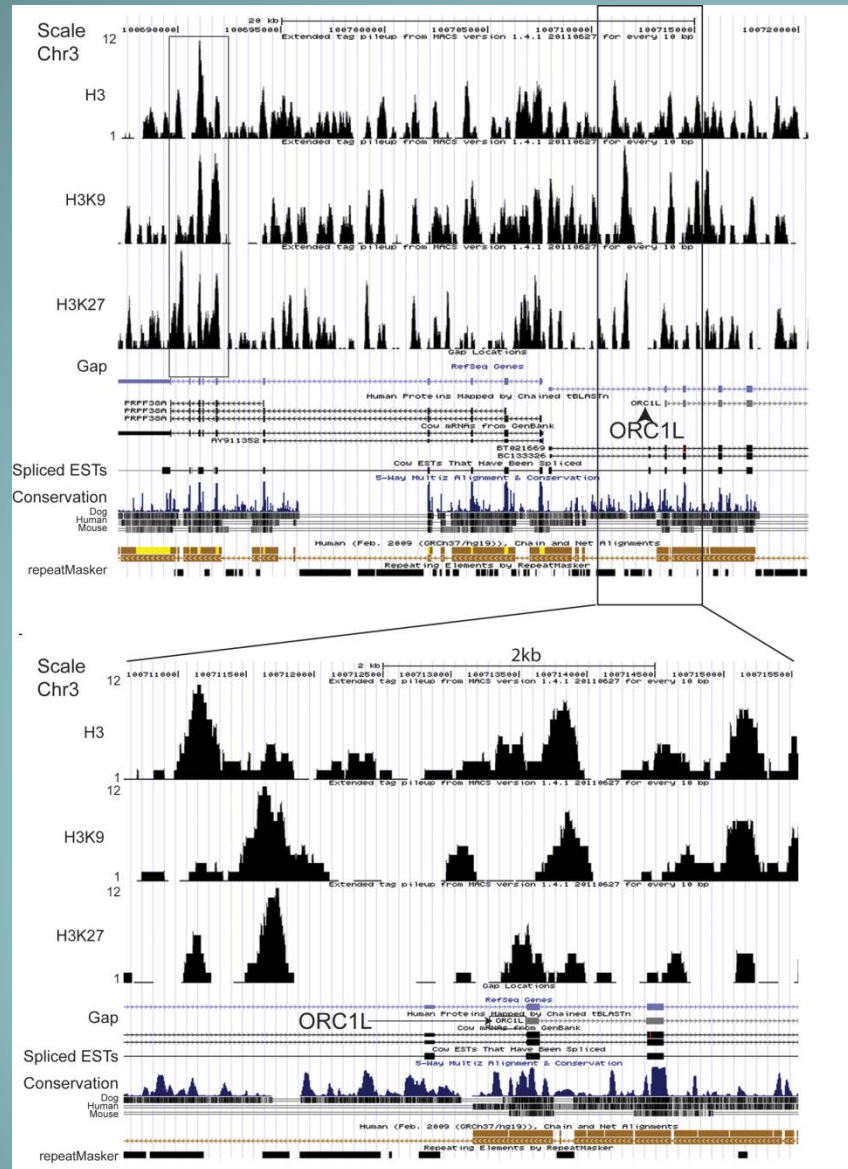
ChIP-seq peaks located up-stream of the genes

ChIP-seq peaks overlapping the gene structure

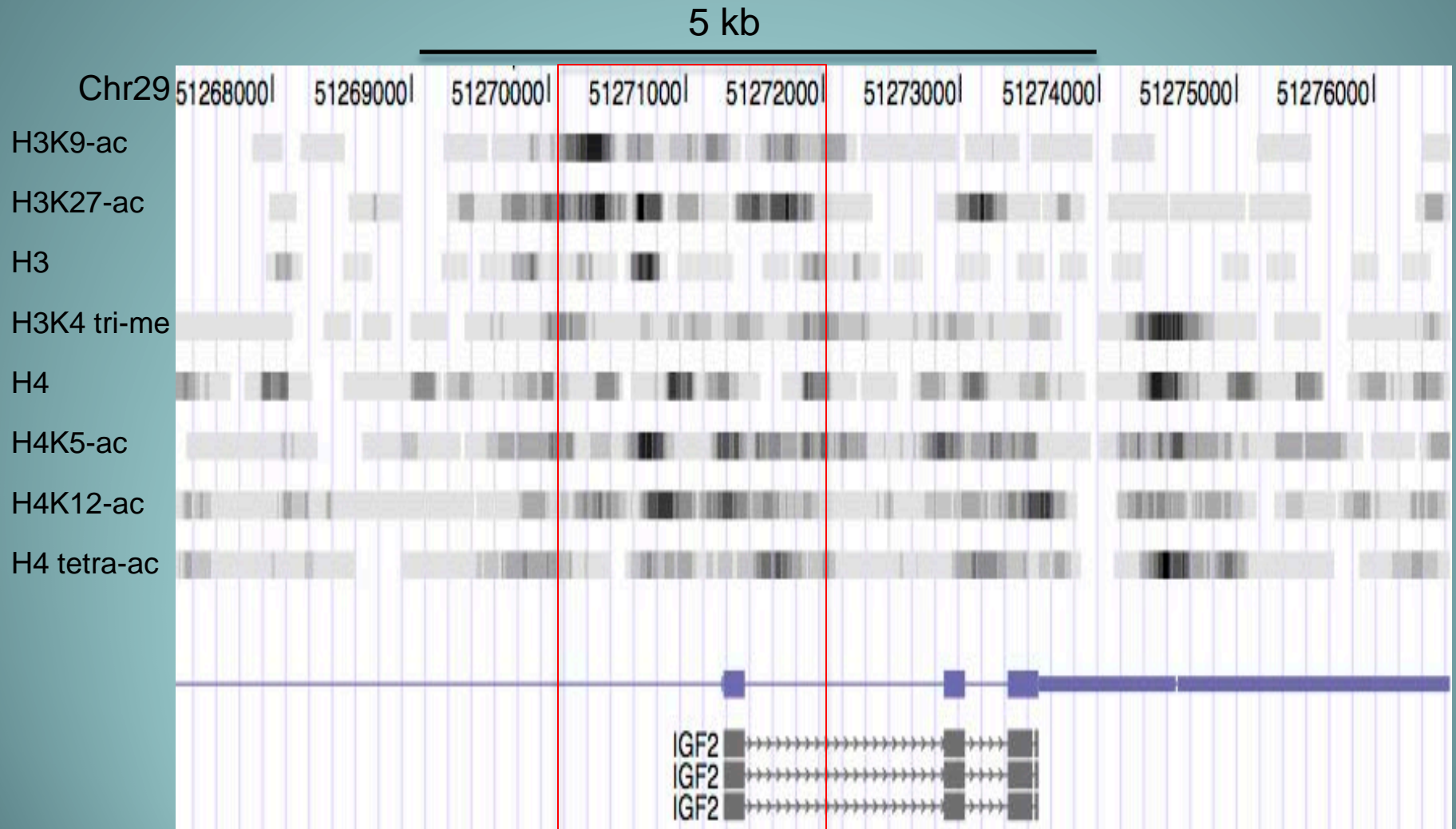
■ No peak detected ■ H3 ■ Acetyl-H3H9 ■ Acetyl-H3H27



Genome browser view of genomic data of ORC1L gene region in chromosome 3



Dispersed epigenetic 'domains' close to TSS of IGF2



Motif discovery: Histone H3, acetyl-H3K9 and acetyl-H3K27 have different binding preferences

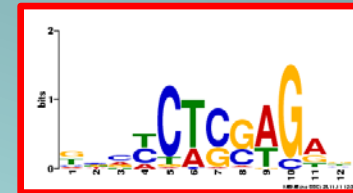
H3



H3K9

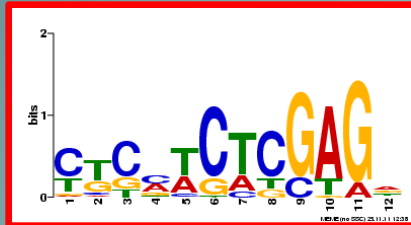


H3K27

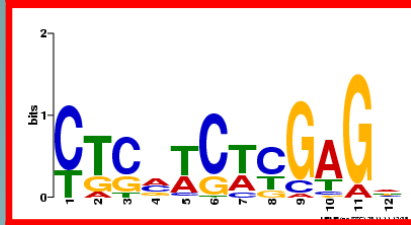


A high degree of conservation in histone binding: consensus sequencing of motifs

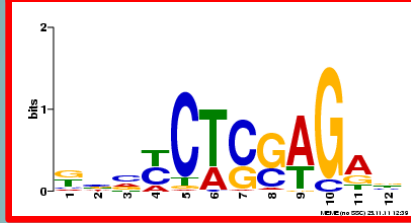
H₃



H₃K₉



H₃K₂₇



Degenerate consensus
sequence

X = A or C

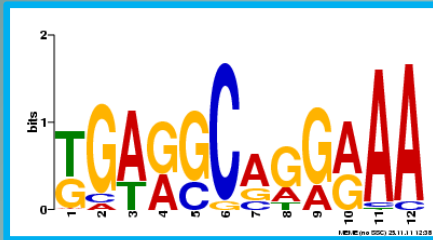
Z = A,C,G,T

H ₃	T	T	C	A	T	C	T	C	G	A	G	T
	C	T	C	A	T	C	T	C	C	A	G	G
	T	T	C	C	A	C	T	C	G	A	G	A
	C	G	G	C	T	G	A	T	G	T	G	C
H ₃ K ₉	T	T	C	A	T	C	T	C	G	A	G	T
	C	T	C	A	A	C	T	G	G	A	G	A
	T	G	C	C	T	C	T	C	G	A	G	A
	C	G	G	C	T	G	A	T	G	T	G	C
H ₃ K ₂₇	G	C	C	T	C	T	C	G	A	G	A	T
	T	C	C	A	C	T	C	G	A	G	A	T
	T	C	A	T	C	T	C	C	A	G	G	G
	T	G	A	T	G	T	G	G	A	C	A	G

T C X T C T C G A G Z

A	0	0	6	3	0	2	0	0	10	0	6
C	0	9	6	0	9	0	8	2	0	1	2
G	4	3	0	0	3	0	2	10	0	11	2
T	8	0	0	9	0	10	1	0	2	0	2

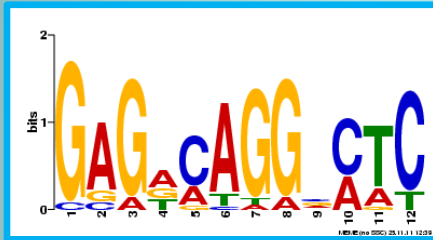
H₃



H₃K₉



H₃K₂₇



H₃

H₃K₉

H₃K₂₇

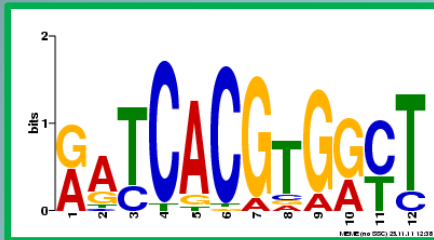
T	G	A	G	G	C	A	G	G	A	A	A
T	G	A	G	G	C	A	G	G	A	A	A
G	A	G	A	C	A	G	G	C	C	T	C

G A G G C A G G A A A

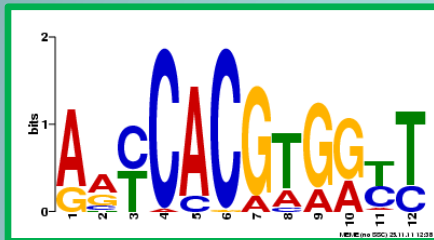
A	0	3	0	1	0	3	0	0	2	2	2
C	0	0	0	0	3	0	0	0	1	1	0
G	3	0	3	2	0	0	3	3	0	0	0
T	0	0	0	0	0	0	0	0	0	0	1

A consensus sequence with CACGTG elements (G-BOX)

H₃



H₃K₉



H₃K₂₇



H₃

H₃K₉

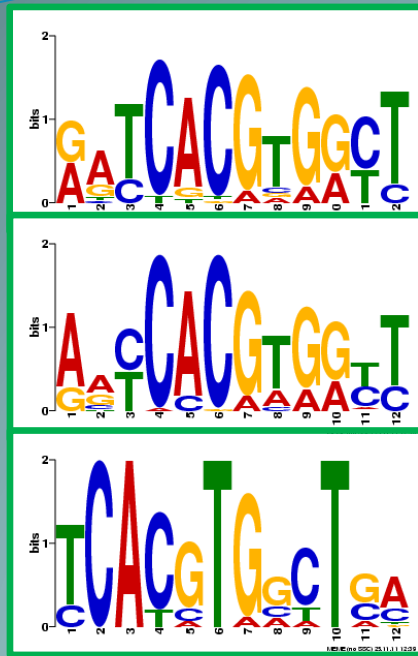
H₃K₂₇

G	A	T	C	A	C	G	T	G	G	C	T
A	A	C	C	A	C	G	T	G	G	T	T
T	C	A	C	G	T	G	G	C	T	G	A

T C A C G T G G C T

A	0	0	3	0	0	0	0	0	0	0
C	1	3	0	3	0	0	0	0	2	0
G	0	0	0	0	3	0	3	3	0	0
T	2	0	0	0	0	3	0	0	1	3

H₃



H₃K₉

H₃K₂₇

Degenerate
consensus
sequence:
Y = A,C,T

H₃

G A T C A C G T G G C T

A A C C A C G T G G C T

A G T C A C G T G A T C

G A T C A C A T G G C T

H₃K₉

A A C C A C G T G G T T

G A T C A C G T G A T C

A A C C C C G A G A T T

H₃K₂₇

T C A C G T G G C T G A

T C A C G T A A C T C A

T C A T C T G C A T G A

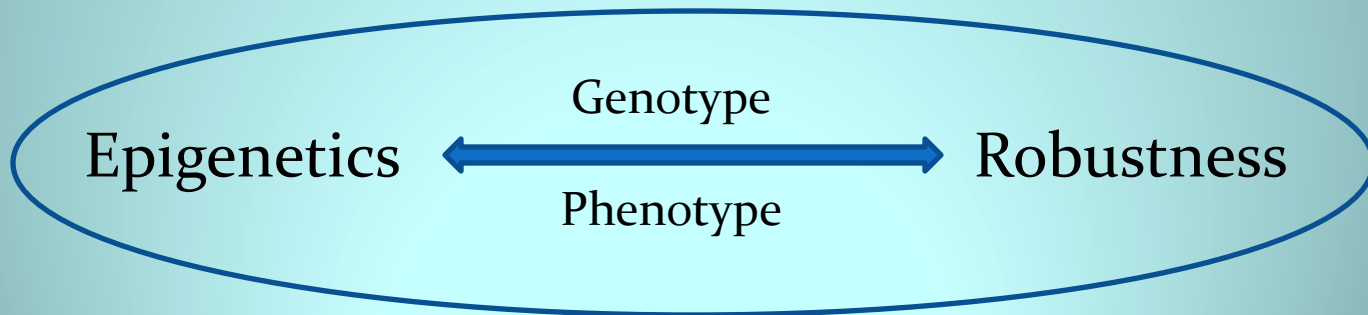
C C A C G T G G T T C C

T C A C G T G G Y T

A	0	0	11	0	1	1	1	4	1	0
C	4	12	1	11	1	0	0	1	5	2
G	0	0	0	0	10	0	11	7	0	0
T	8	0	0	1	0	11	0	0	6	10

Summary:

- 1 Butyrate-induced acetylation in H3K9 and H3H27 change the sequence-based binding preference of H3*
- 2 A high degree of conservation in the histone binding evidently presented*
- 3 Epigenetics is intimately related to robustness as both lie between genotypes and phenotypes*



Acknowledgments:

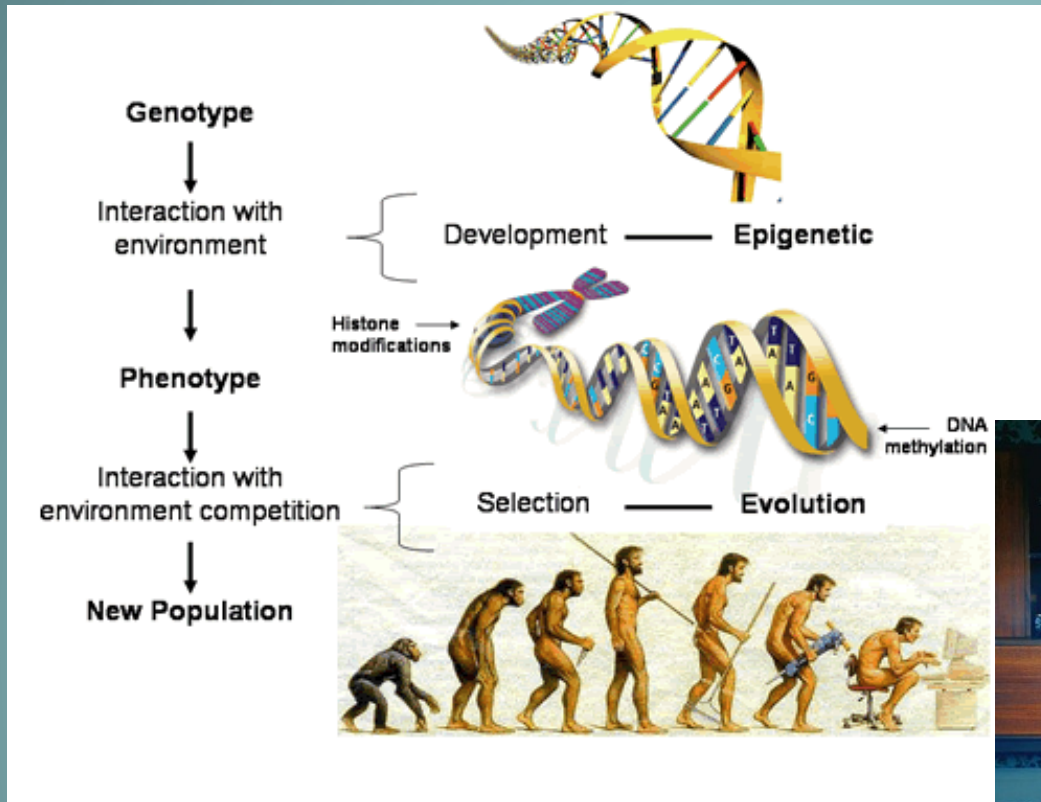
ChIP-seq:

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ARS, USDA

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Dr. Yuan Gao
Dr. Joo Heon Shin
Lieber Institute for Brain Development
Johns Hopkins University

Think epigenetics...



Thanks!