Building a functional annotation of the equine genome

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Advancements in Equine Genomics

- High quality reference genome (EquCab3 – Kalbflesich et al. 2018)
- Many single-marker traits have been elucidated
  - Color
  - Disease
- Variants contributing significant variation to complex traits also identified
  - Size
  - Speed

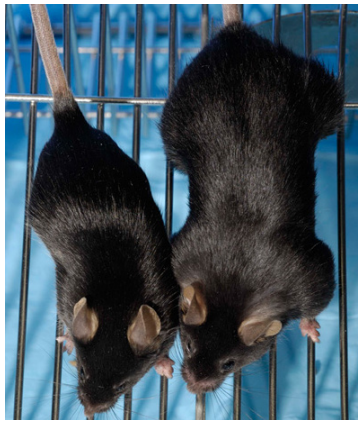
The Story of Speed in the Thoroughbred

- An intronic SNP in the gene myostatin associated with best racing distance (Hill et al. 2010)

- Fast adoption and continued use of the genetic test for this marker by racehorse breeders/trainers

Speed in the Thoroughbred

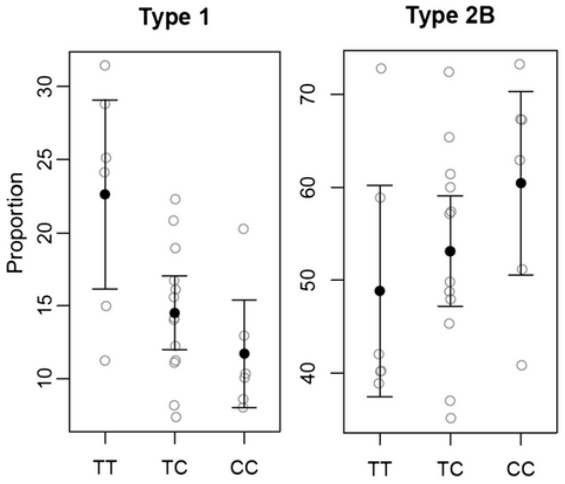
- Question: What does this intron change to alter speed?



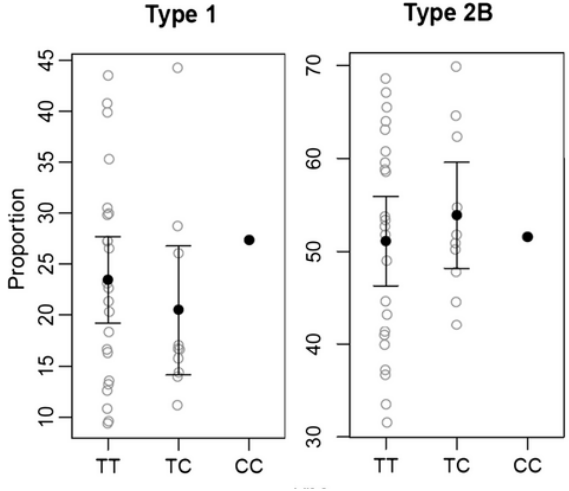
Myostatin is a negative regulator of muscle growth and differentiation

Association with Fiber Type

- Horses with the “short distance” SNP have more fast-twitch muscle fibers
- That association doesn’t hold true across breeds



Thoroughbred

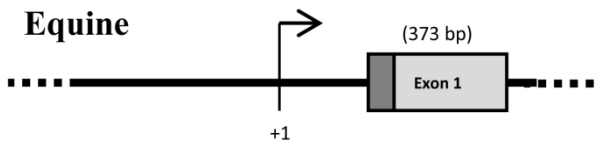


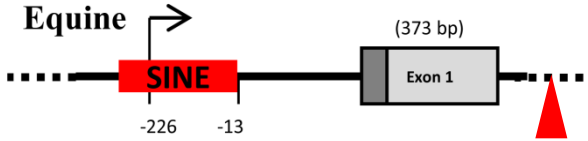
Belgian

Petersen et al. 2014
Anim Genet

But, why?

- In Thoroughbreds, the SNP is in high Linkage Disequilibrium (LD) with an insertion in the gene promoter
- Santagostino et al. (2015) showed gene expression was altered by the insertion
- The intronic SNP had no impact in Rooney et al. (2018) while the insertion did





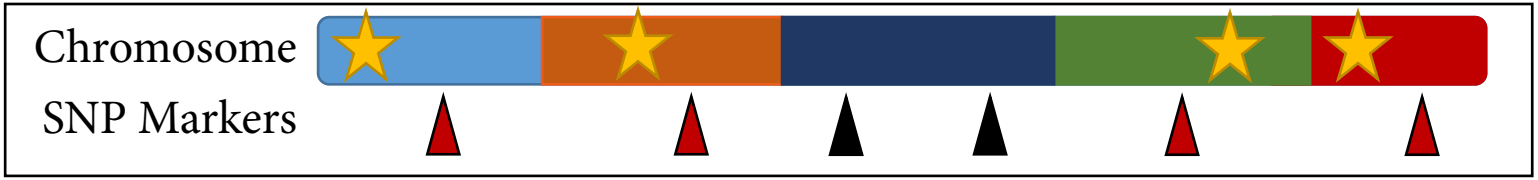
Importance of Linkage Disequilibrium

- The "speed gene" test **IS** predictive in Thoroughbreds
- In other breeds, the SNP is not always predictive of the insertion → the test does not hold true
    - LD in Thoroughbred (r^2) = 0.93
    - LD in Quarter Horse (r^2) = 0.41
    - LD in many other breeds (r^2) = 0

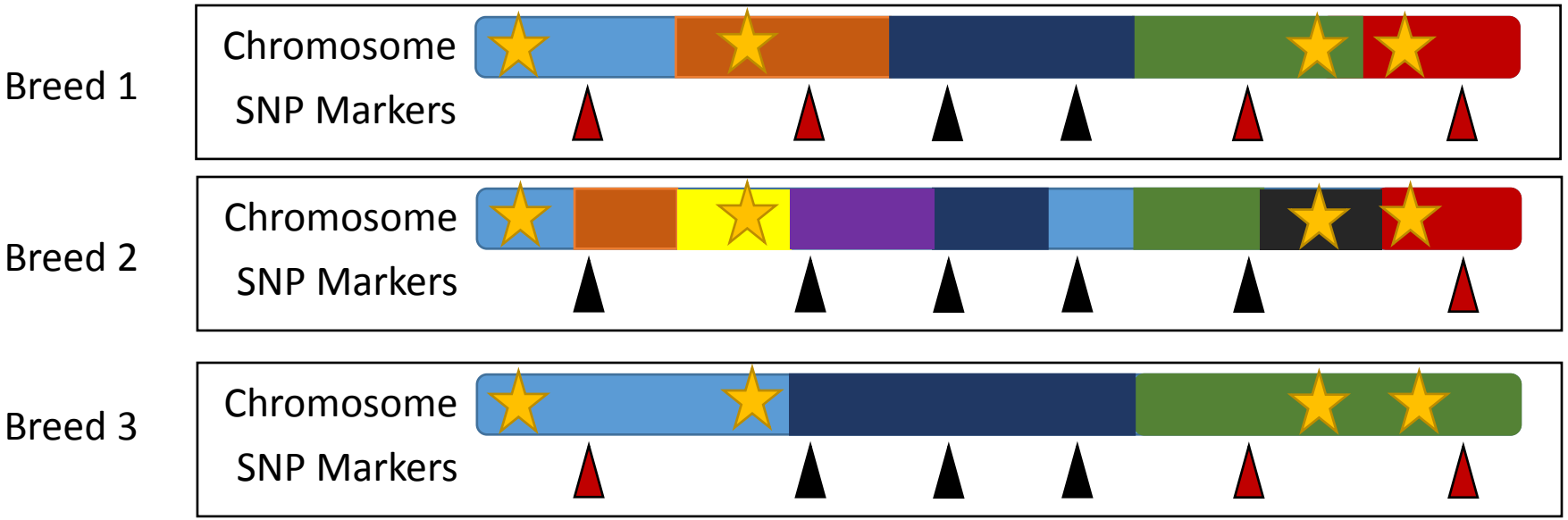
Petersen et al. 2014
Anim Genet

Genomic Prediction and Linkage Disequilibrium

- Genomic prediction relies on linkage disequilibrium, the ability of a single marker to explain variation in the region

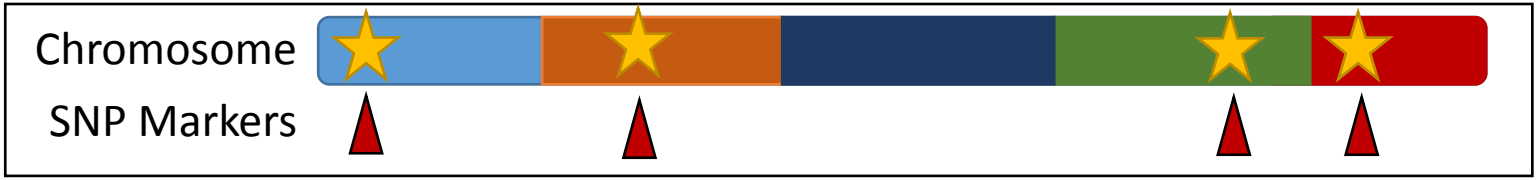


Linkage disequilibrium is Not Constant Across Breeds



"When molecular breeding values were evaluated in breeds that were not in the training set, estimated genetic correlations clustered around zero." Kachman et al. 2013 GSE

Understanding Functional Variation is Key



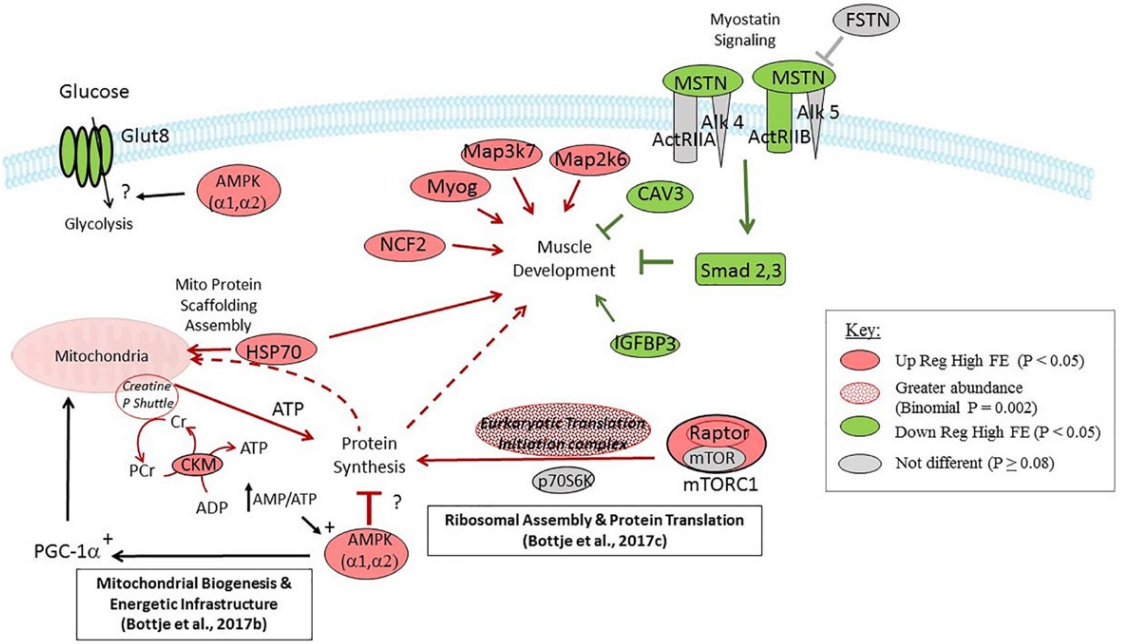
Take Home Message from the Speed Gene Story

1. Important phenotypes can be attributed to changes in gene regulation
2. Genomic variation alters mechanisms of genome regulation
3. Relationships among genomic variants (and their consequence) is not always consistent from one population to the next

If we can assay the true functional variant(s), we do a better job predicting phenotype

The Challenge

- Most important traits are complex
  - Interaction of many loci and the environment
  - Identification of variants associated with these traits has been relatively unproductive



Lassiter et al. 2019 Front Physiol

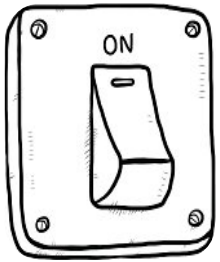
Complex **Disease** Traits in the Horse

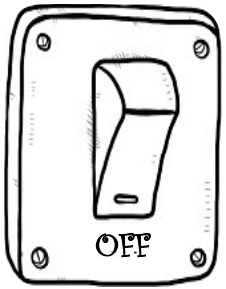
- Atrial fibrillation
- Body size
- Bone fracture
- Brachygnathia
- Chronic progressive lymphedema
- Common variable immunodeficiency
- Corneal stromal loss
- Cribbing
- Cryptorchidism
- Degenerative joint disease
- Guttural pouch tympany
- Insect bite hypersensitivity
- Recurrent laryngeal neuropathy
- Metabolic syndrome
- Myofibrillar myopathy
- Navicular disease
- Neuroaxonal dystrophy
- Osteochondrosis
- Polysaccharide storage myopathy (II)
- Recurrent airway obstruction
- Recurrent exertional rhabdomyolysis
- Recurrent uveitis
- Sarcoid
- Shivers
- Squamous cell carcinoma
- Swayback

What Are We Missing?

- Nearly 90% of QTL are in non-coding regions (Hindorff et al. 2009 PNAS)
  - ~21,000 protein coding genes
  - 450,000+ regulatory elements (Birney et al. 2007 Nature)
- How the genome is being regulated depends on sex, age, activity, health status, environment and varies from tissue to tissue

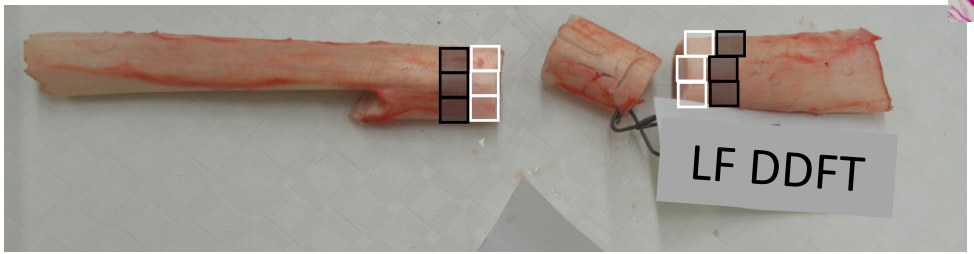
Genome Regulation = on/off Switches

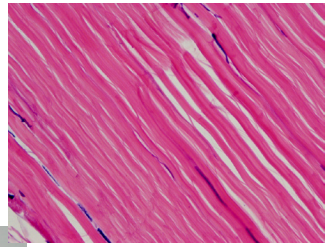




Goal: Build a reference annotation of genome regulation in the horse

- Healthy, adult Thoroughbred mares
- Careful, comprehensive phenotype collection

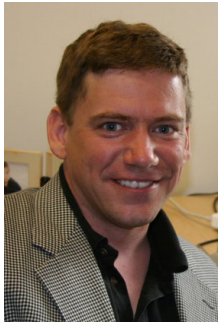




Equine FAANG Project (2016-current)

- Biobank of 86 tissues, 2 cell lines, 5 fluids from each mare (Burns et al. 2018 Anim Genet)
- Whole-genome sequence (30X)
- RNA-sequence of 50* tissues
  (*42 funded by the "Adopt-A-Tissue" program)
  - Poly-A
  - smRNA
- Data available at:
  https://www.ebi.ac.uk/ena/data/view/PRJEB26787

http://server1.intrepidbio.com/FeatureBrowser/customlist/record?listid=7919259427



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SEARCH

- GENES
- POLYMORPHISMS
- GENOMIC REGION
- SAMPLES

FAANG First Round Mapped Sequences

List Identifier:7919259427

DESCRIPTION OF DATASET					
FAANG First Round Mapped Sequences			View All in IGV		
BAM FORMATTED FILES ASSOCIATED WITH SET					
7918965177	EquCab2.0	WGS Sequencing data for 686521	View in IGV	Click to Load to UCSC	Drag to other Apps
7918965188	EquCab2.0	WGS Sequencing data for 683610	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707335	EquCab2.0	FAANG RNA-Seq data for:686521_Adipose_Loin	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707346	EquCab2.0	FAANG RNA-Seq data for:686521_Lamina	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707357	EquCab2.0	FAANG RNA-Seq data for:686521_Left_Ventricle	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707368	EquCab2.0	FAANG RNA-Seq data for:686521_Liver	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707379	EquCab2.0	FAANG RNA-Seq data for:686521_Longissimus	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707390	EquCab2.0	FAANG RNA-Seq data for:686521_Lung	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707401	EquCab2.0	FAANG RNA-Seq data for:686521_Ovary	View in IGV	Click to Load to UCSC	Drag to other Apps
7918707412	EquCab2.0	FAANG RNA-Seq data for:686521_Parietal_Cortex	View in IGV	Click to Load to UCSC	Drag to other Apps

Equine Community Effort

- Over $60,000 from individual labs for RNA-seq

- Characterize keratinocytes

- Cell culture

- RRBS

INSTYTUT ZOOTECHNIKI
PAŃSTWOWY INSTYTUT BADAWCZY
NATIONAL RESEARCH INSTITUTE
OF ANIMAL PRODUCTION

- Karyotyping

- Centromere Mapping

UNIVERSITY OF Nebraska Lincoln

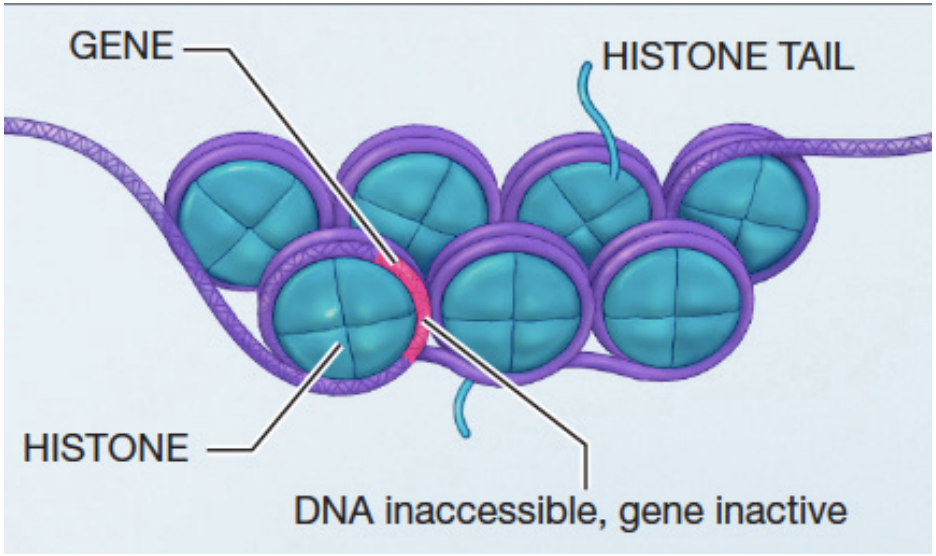
- Characterize microbiome

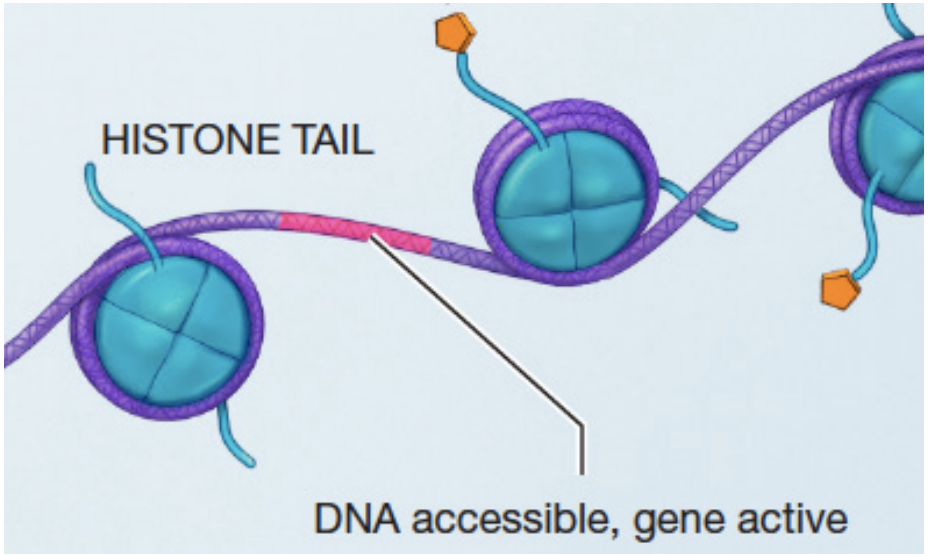
Assays of Genome Regulation – Histone Modification

- Nine prioritized tissues

Laminae	Liver
Lung	Ovary
Adipose	Cerebral cortex
Heart (left ventricle)	Spleen
Longissimus dorsi (skeletal muscle)	

Histone Modifications

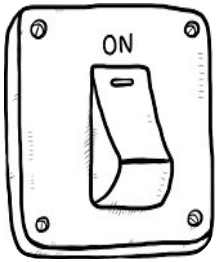


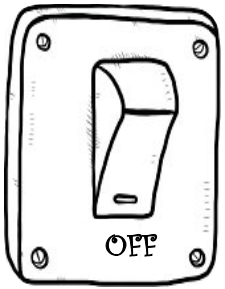


Figures: National Institutes of Health via Wikimedia

Histone Modification

- Sample processing optimized for each tissue
  - Homogenization, fixation, shearing, antibodies
- Sequenced (50bp SE)
  - Activating marks: 35-50 million reads
    H3K4me1, H3K4me3, H3K27ac
  - Repressive mark: 55-80 million reads
    H3K27me3





Example of Sequence Analysis

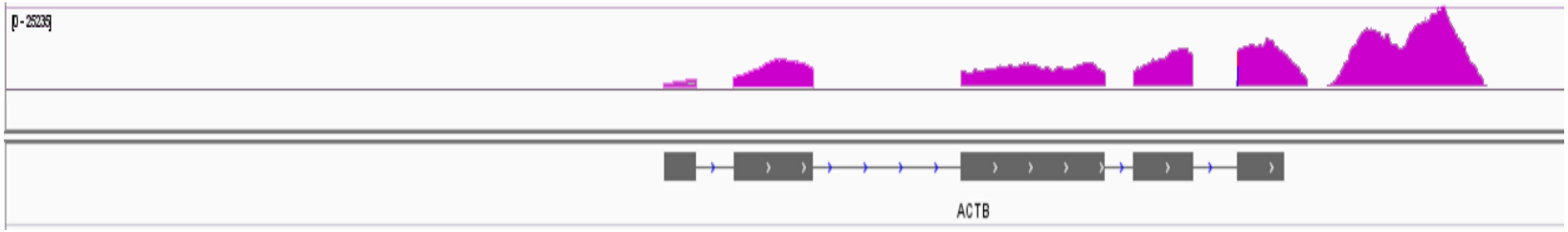
Gene Model

Beta Actin – Commonly expressed gene

Example of Outcome

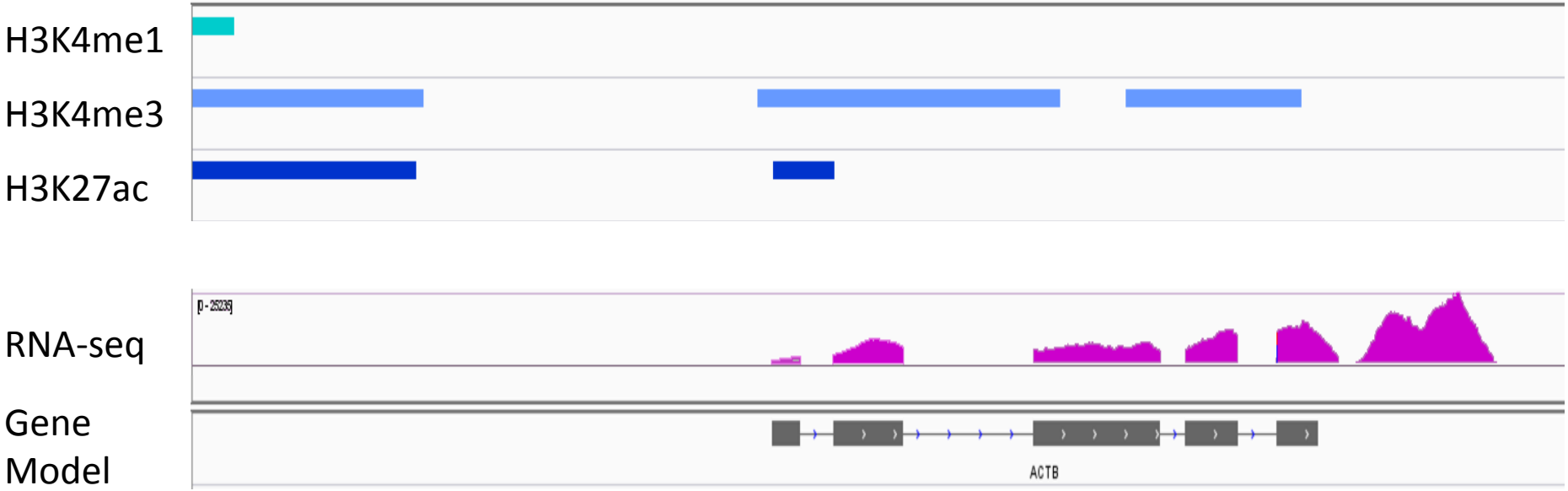
RNA-seq

Gene Model



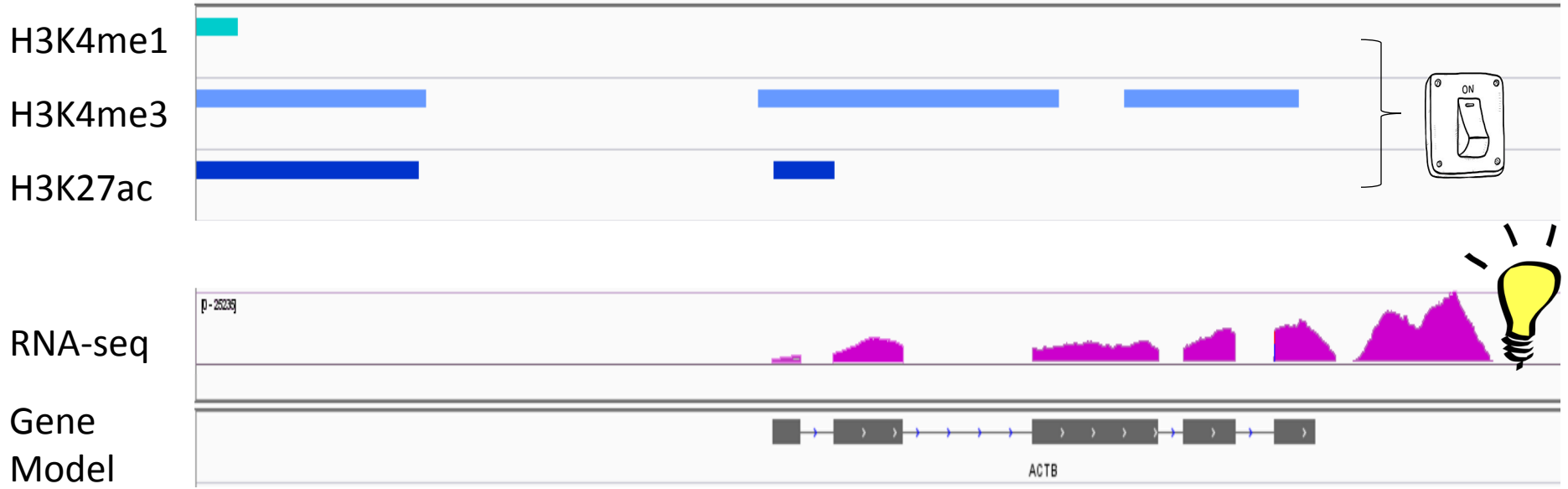
Beta Actin – Commonly expressed gene

Example of Outcome



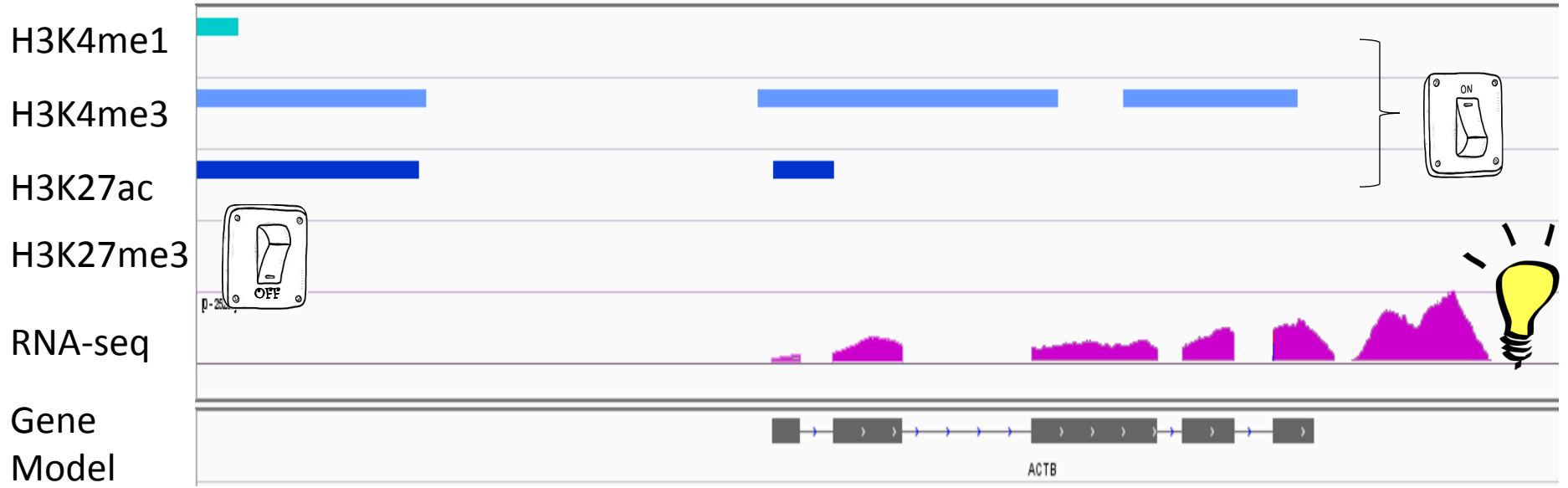
Beta Actin – Commonly expressed gene

Example of Outcome



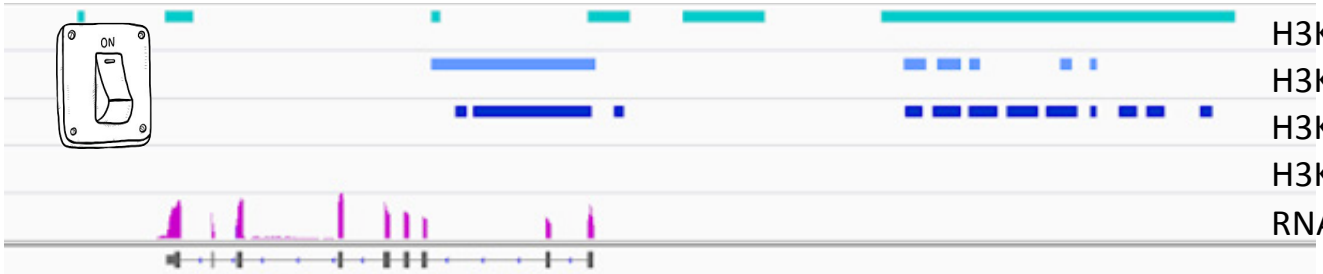
Beta Actin – Commonly expressed gene

Example of Outcome



Beta Actin – Commonly expressed gene

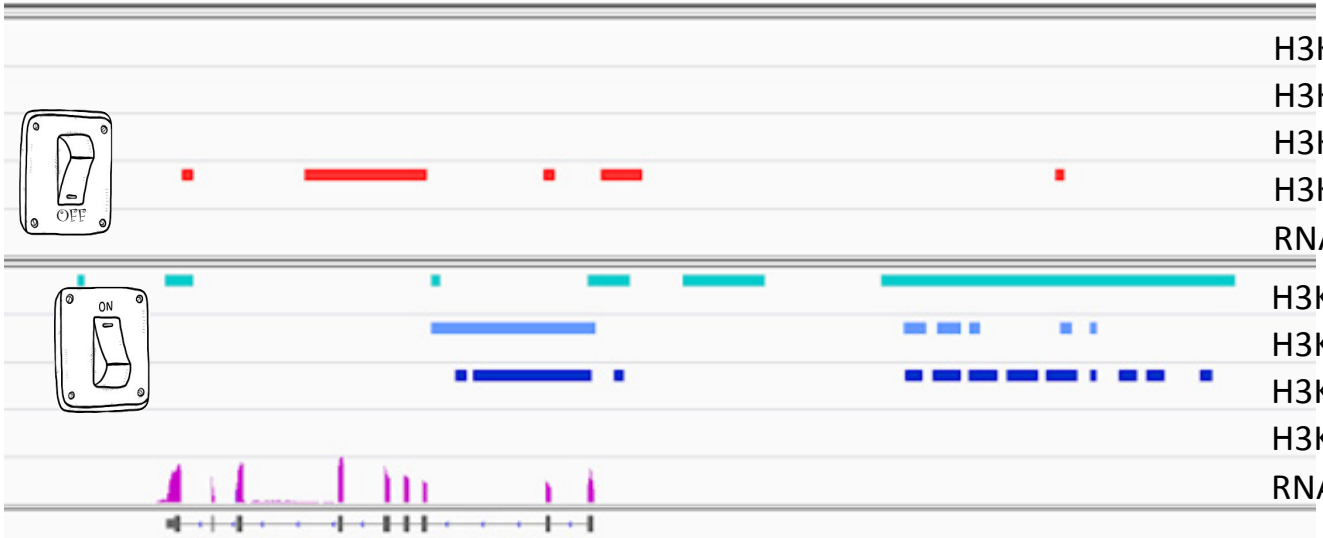
Liver



CYP2E1– Active in liver metabolism

Adipose

Liver

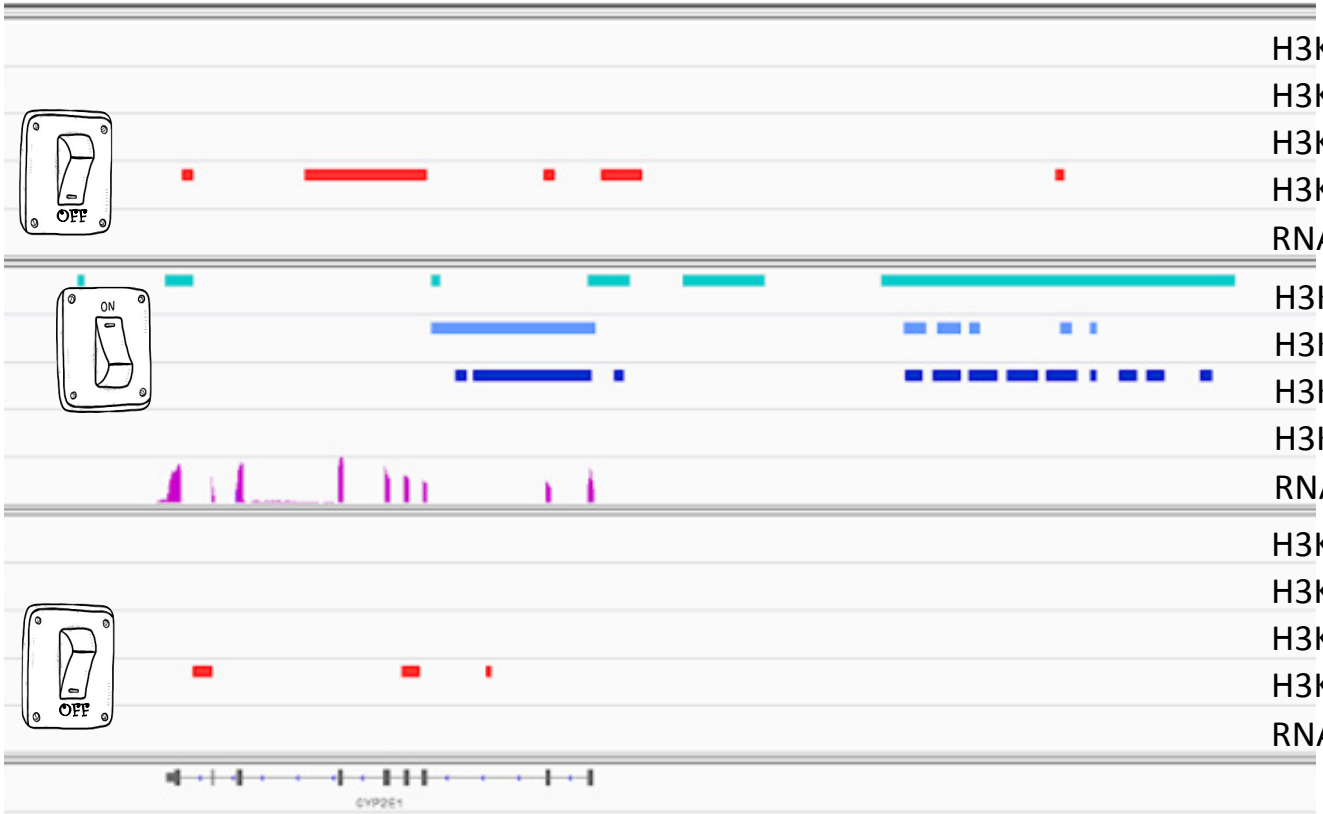


CYP2E1– Active in liver metabolism

Adipose

Liver

Skeletal Muscle



CYP2E1– Active in liver metabolism

Observed Peaks (thousands)

	Adipose	Brain	Heart	Laminae	Liver	Lung	Ovary	Muscle	Spleen
H3K4me1	107.3	95.9	121.7	114.7	116.8	93.0	103.0	95.8	73.5
H3K4me3	26.9	27.1	26.5	29.4	28.5	28.5	28.4	28.1	28.7
H3K27ac	79.6	78.8	68.7	82.4	87.6	69.1	64.3	76.5	51.4
H3K27me3	25.2	24.2	68.1	37.4	63.9	30.2	40.8	42.6	5.8

Proportion of Genome Covered

Mark	Percent (across 9 tissues)
H3K4me1	2.8 – 5.1
H3K4me3	1.4 – 1.7
H3K27ac	1.8 – 3.3
H3K27me3	0.6 – 1.3

Tissue-specific Elements

Tissues Elements	Adipose	Brain	Heart	Lamina	Liver	Lung	Muscle	Ovary	Total
Active enhancers	9,584	12,151	8,250	16,668	17,203	3,450	10,385	9,454	87,145
Active promoters	1,584	3,043	1,479	2,735	4,488	1,341	2,760	1,854	19,284

Application – Understanding Genome Function

- Not all transcripts are defined
  - Human genome – 202,762 transcripts annotated
  - Horse – 56,546 (up from 29,196 in prior genome)
  - Cattle – 43,947
  - Pig – 49,448
- FAANG is helping to characterize regions of genome transcription on a tissue-specific basis AND associate gene transcription with regulatory elements

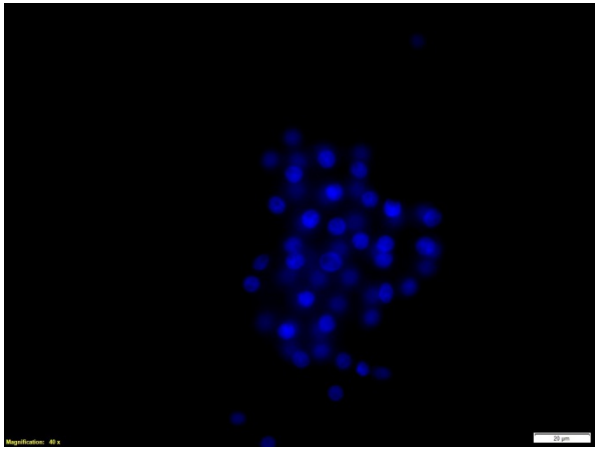
Assay for Open Chromatin

- ATAC-Seq

Image: https://www.activemotif.com/catalog/1233/atac-seq-service

Methods: Fresh vs Frozen Samples ATAC-seq

- Standard protocol – isolate cell nuclei immediately after harvest
- Completed trials isolating nuclei from frozen tissue



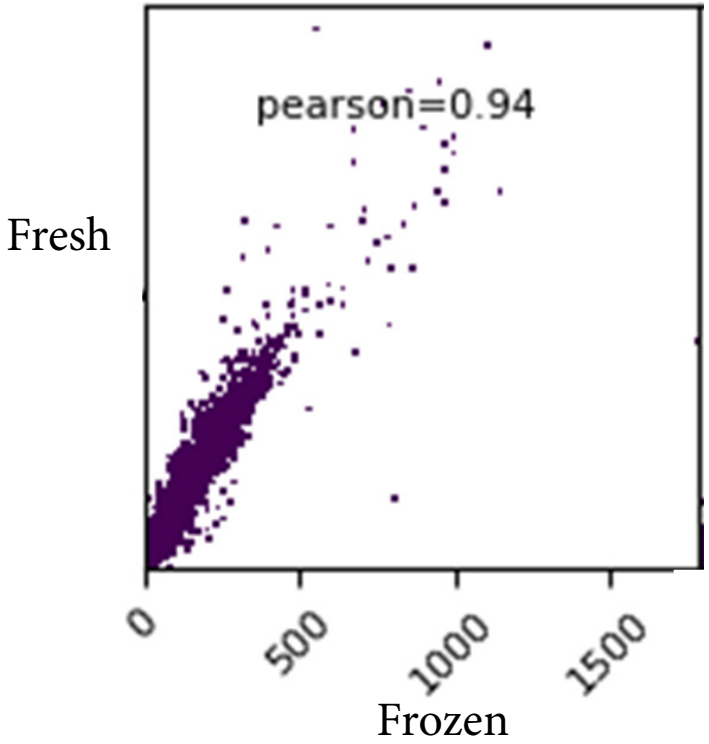
ATAC-seq with Fresh vs Frozen Tissue (Liver)

	Frozen	Fresh
# of Peaks	70,375	74,399
Ave width (bp)	651	599
RiP	10,597,576	11,182,311
FRiP	25.14%	31.84%

Peak similarity
(Chikina and Troyanskaya 2012)

0.726

Genomic Coverage



ChRO-Seq*

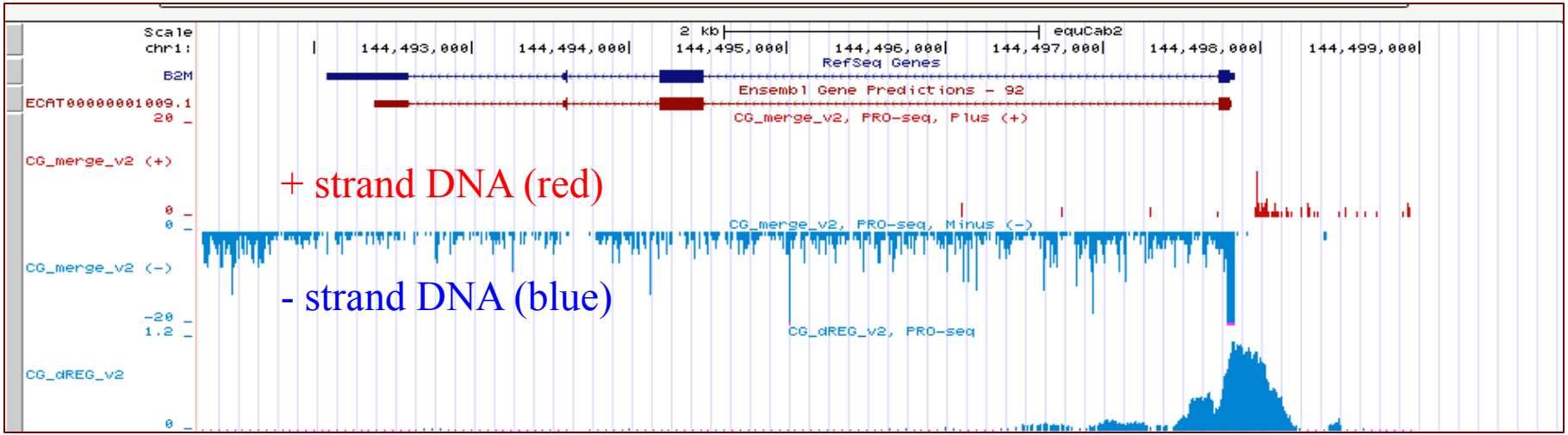
- Chromatin run-on and sequencing
- Creates data similar to that from
  - RNA-seq
  - ChIP-seq
  - ATAC-seq

...all in one assay

*Chu et al. 2018, Nature Genetics

Reading ChRO-seq Traces

Equine Beta 2 Microglobulin

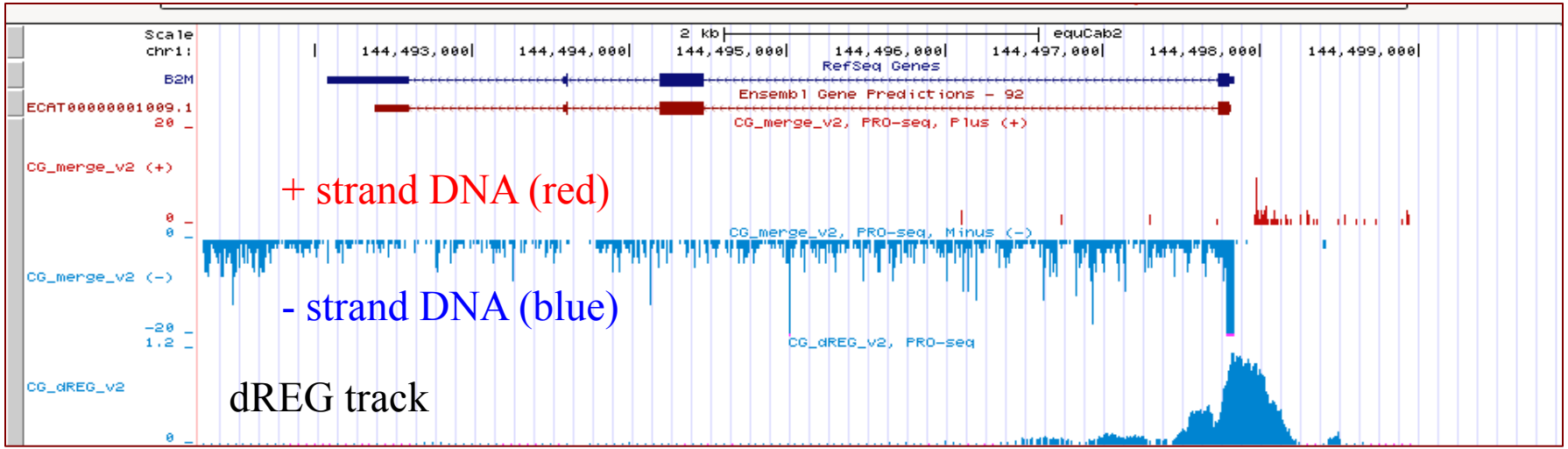


Unprocessed, nascent RNA transcript

Direction of Transcription

Reading ChRO-seq Traces

Equine Beta 2 Microglobulin

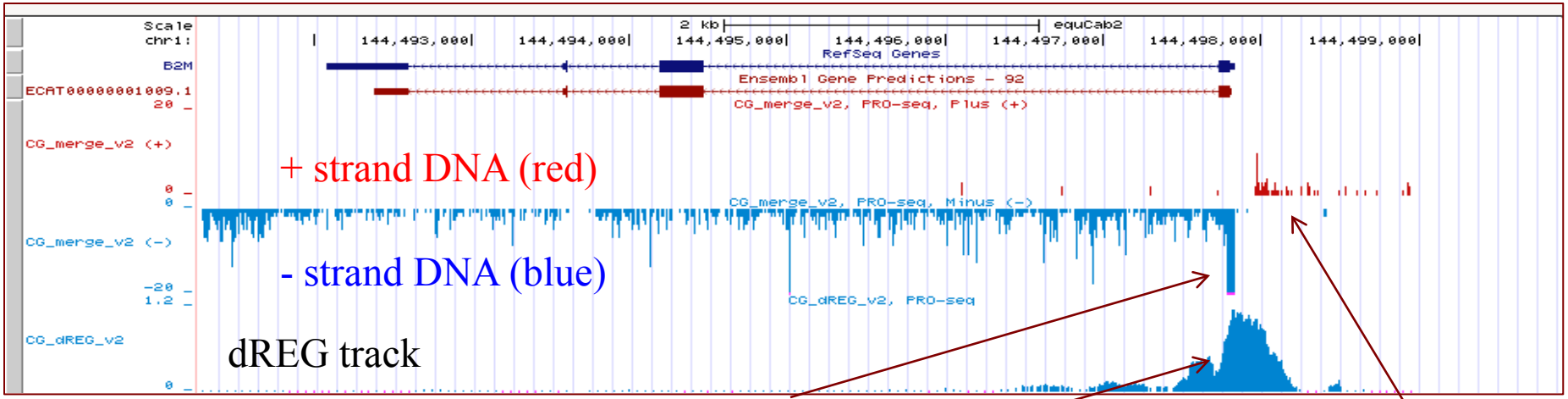


dREG = Detection of Regulatory Elements
Machine Learning Imputation, Danko lab

Direction of Transcription

Reading ChRO-seq Traces

Equine Beta 2 Microglobulin



Transcriptional Start Site (TSS)

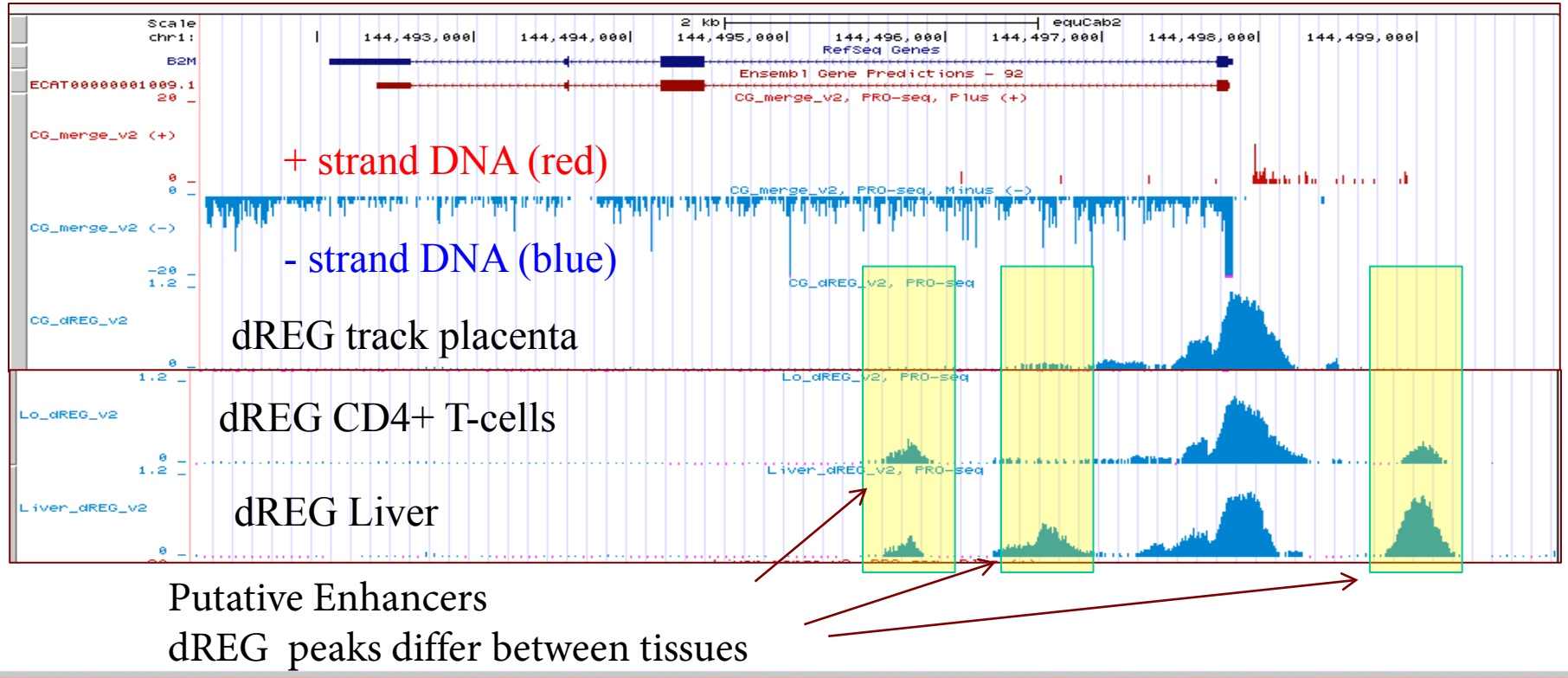
Regulatory Sites (dREG peaks)

Transcription on non-coding strand

Direction of Transcription

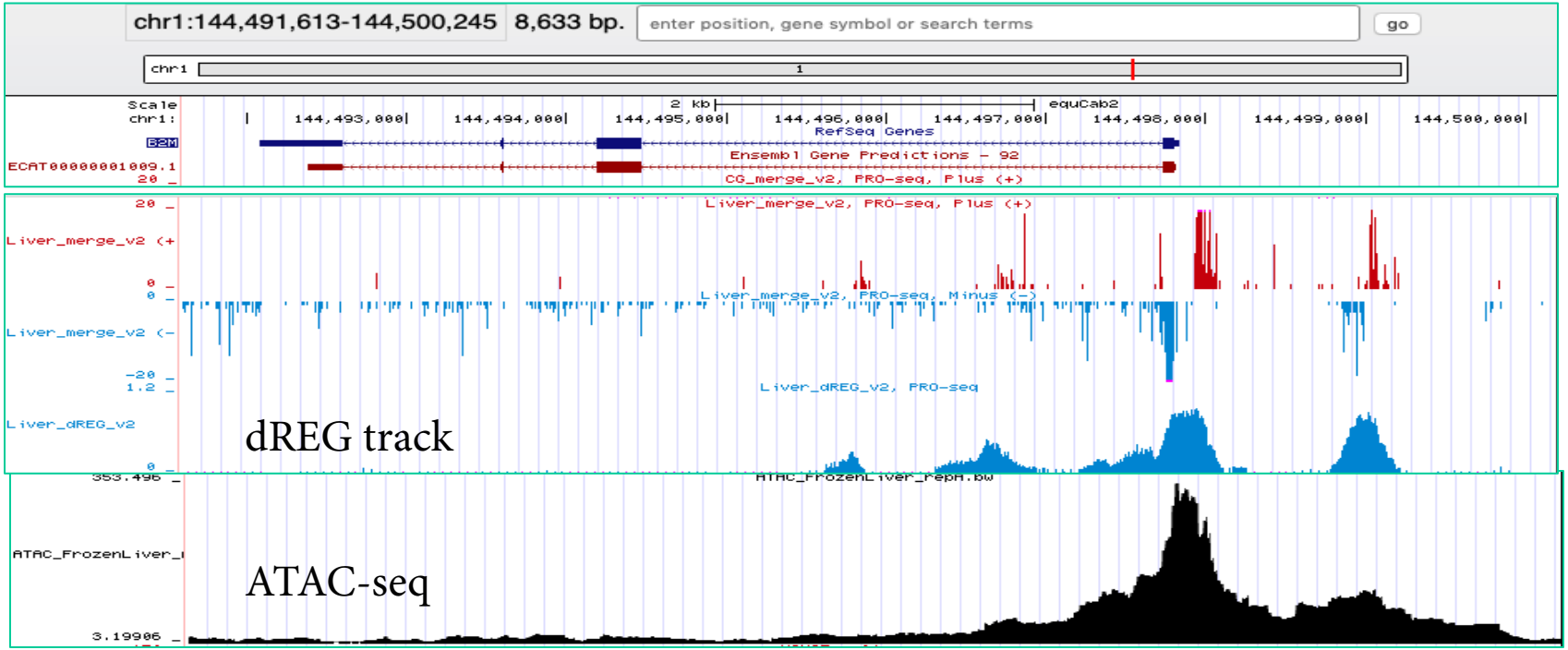
Reading ChRO-seq Traces

Equine Beta 2 Microglobulin



Putative Enhancers
dREG peaks differ between tissues

ChRO-seq vs ATAC-seq (Liver)

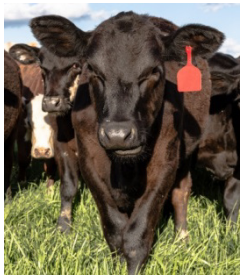


What is Next?

- Stallions
- Developmental stages
- Across-breed studies
- Across-species analyses
- New techniques?

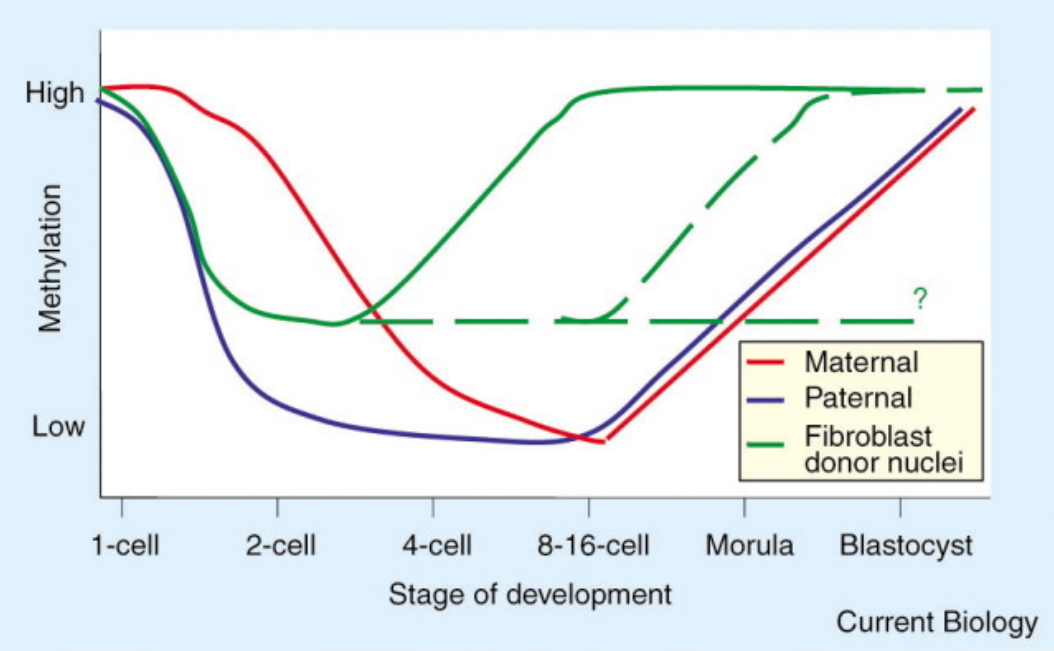
What Does FANNG Mean for Agriculture?

- New baseline for comparison of genome function in disease/treatments
- Personalized Management
- Improved genomic prediction
- Application to ET, ICSI, cloning?



What Does This Mean for Agriculture?

- Application to ET, ICSI, cloning?



Fairburn et al. 2002 Curr Biol

Acknowledgements

- Tissue “Adopters”
- Zhou and Ross labs, UC Davis