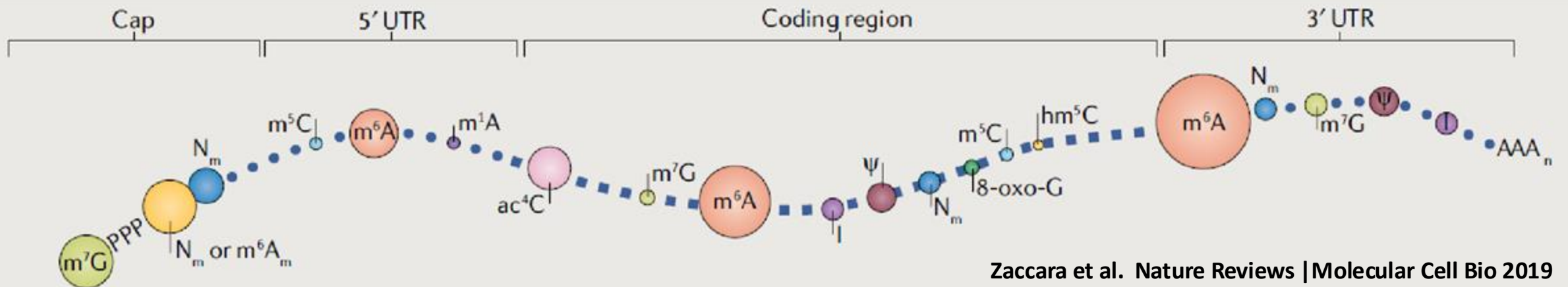


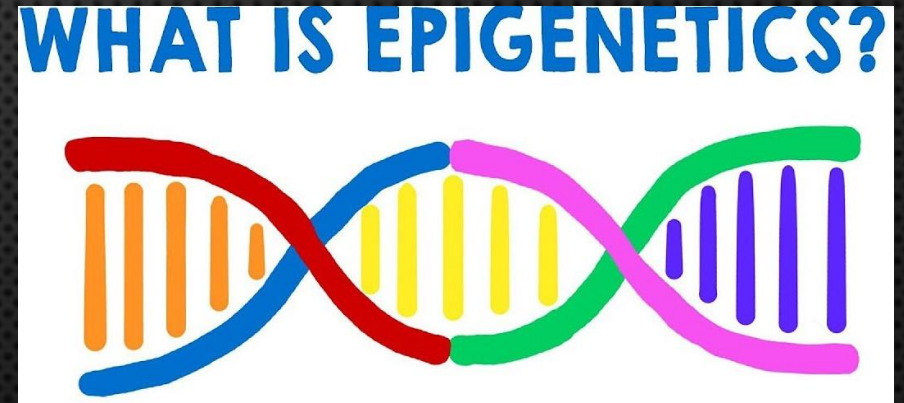
EXAMINING THE POTENTIAL INFLUENCE OF EPIGENETIC EFFECTS ON GENETIC EVALUATIONS



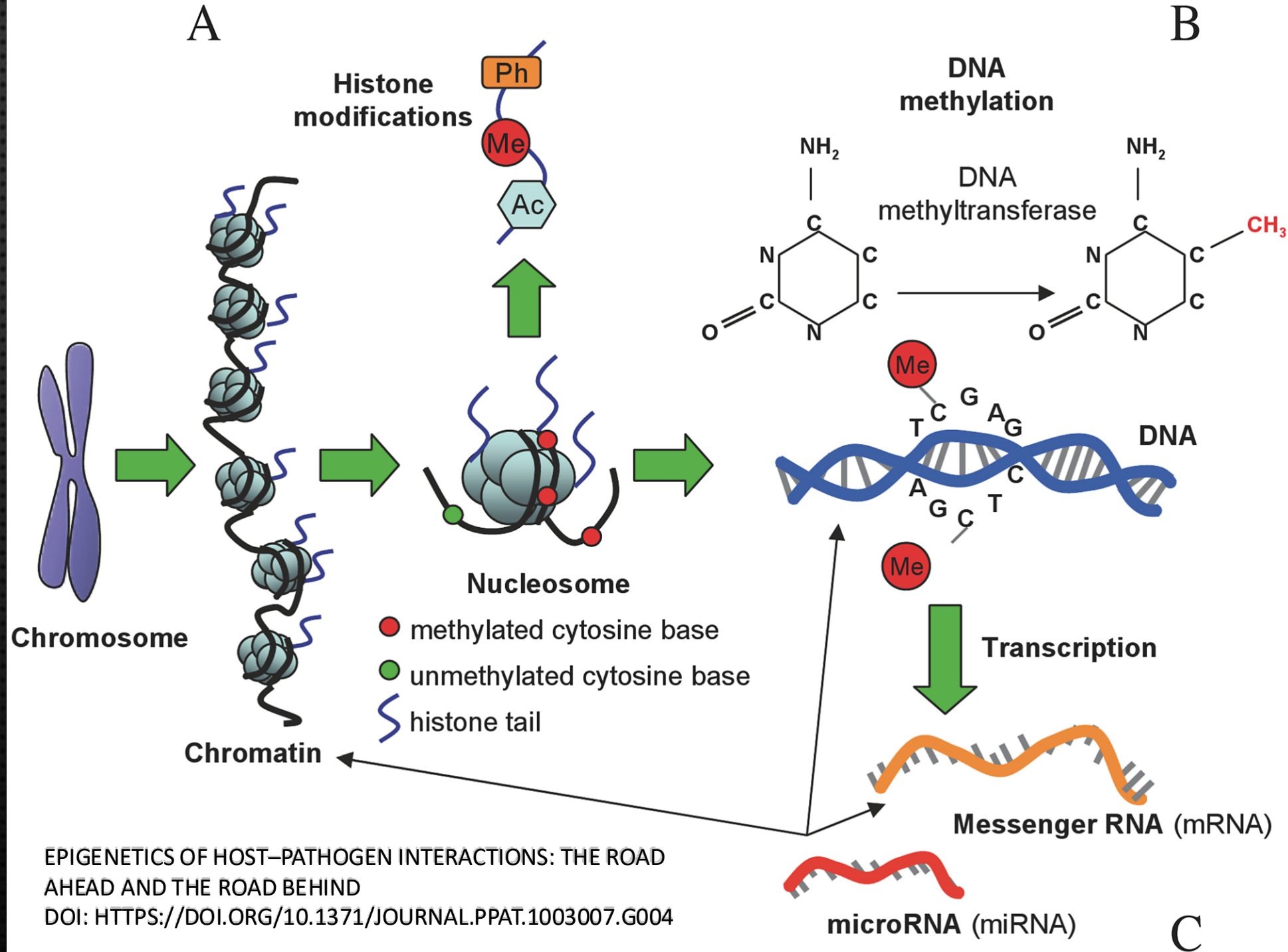
DR. STEPHANIE MCKAY
UNIVERSITY OF MISSOURI

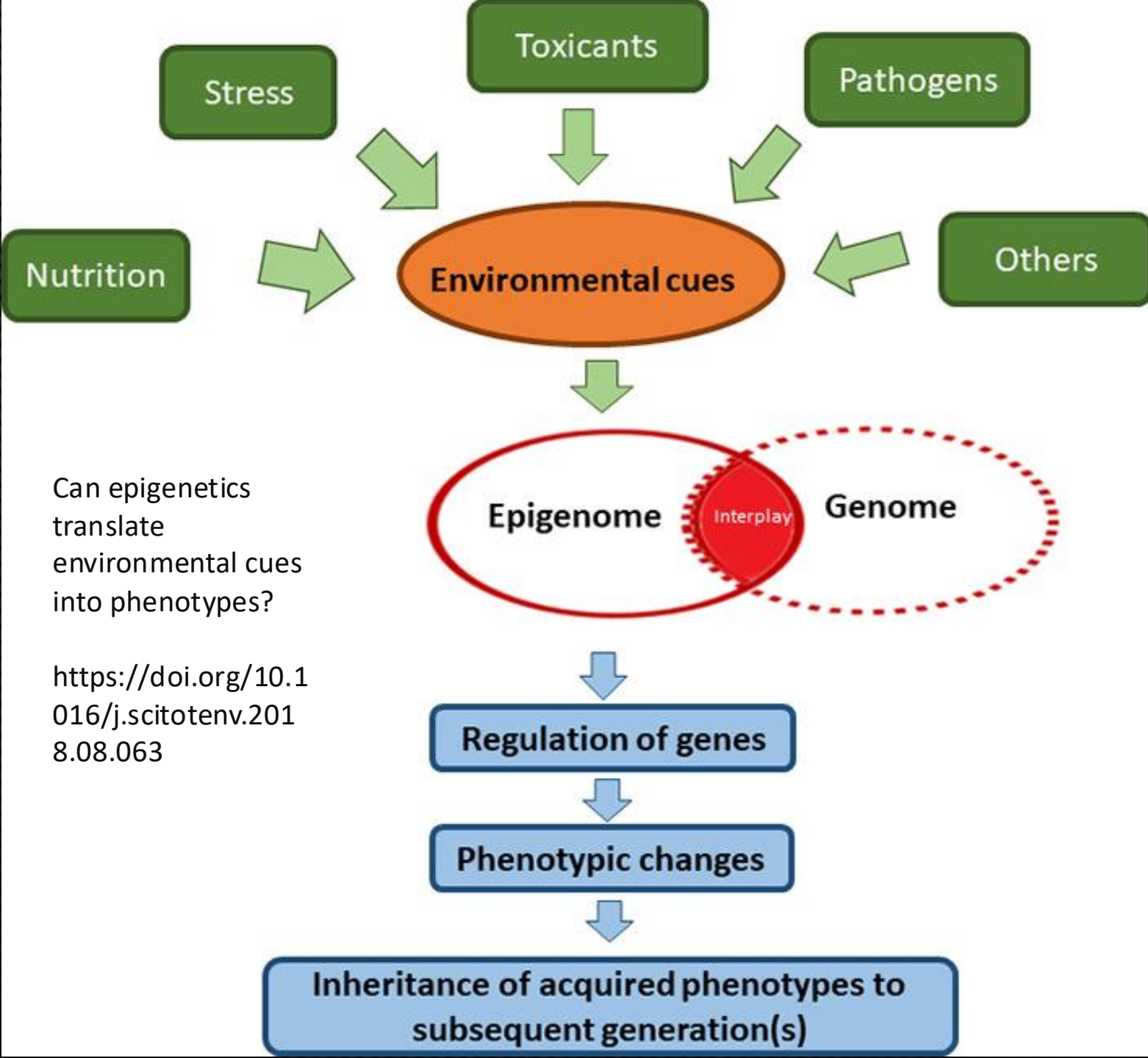
EPIGENETIC MODIFICATIONS

- “An epigenetic trait has been defined as a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence” - a definition that was formulated in 2008 at a Cold Spring Harbor meeting (Berger et al., 2009).
- Modifications of DNA base pairs that do not change the DNA/RNA sequence
- Heritable changes in gene expression and subsequent phenotypic variation
- Phenotypes are influenced because of differential access of transcriptional/translational machinery to DNA/RNA



Commonly Studied Epigenetic Modifications In Livestock





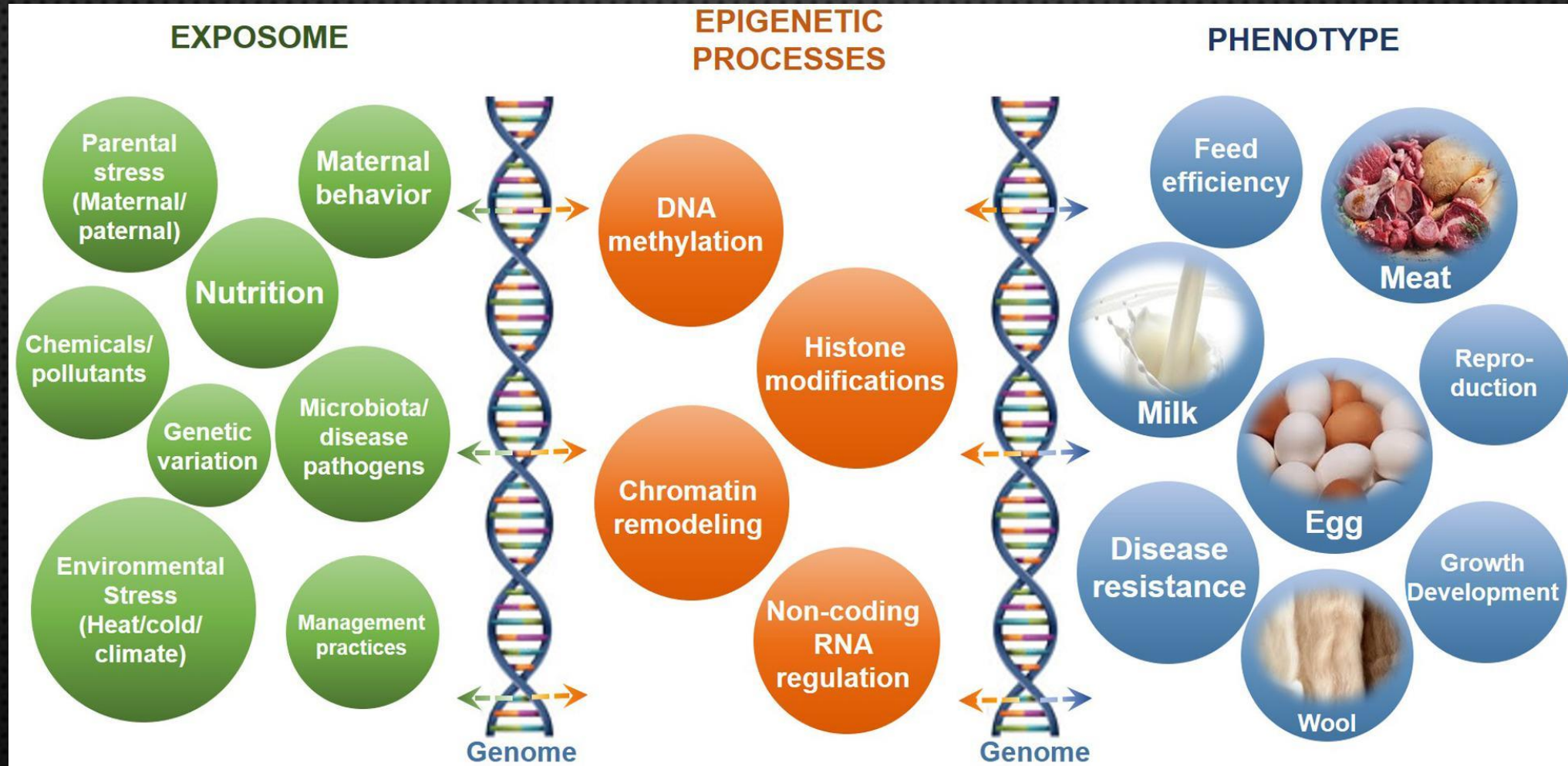
Can epigenetics translate environmental cues into phenotypes?

<https://doi.org/10.1016/j.scitotenv.2018.08.063>

WHAT FACILITATES EPIGENETIC MODIFICATIONS ?

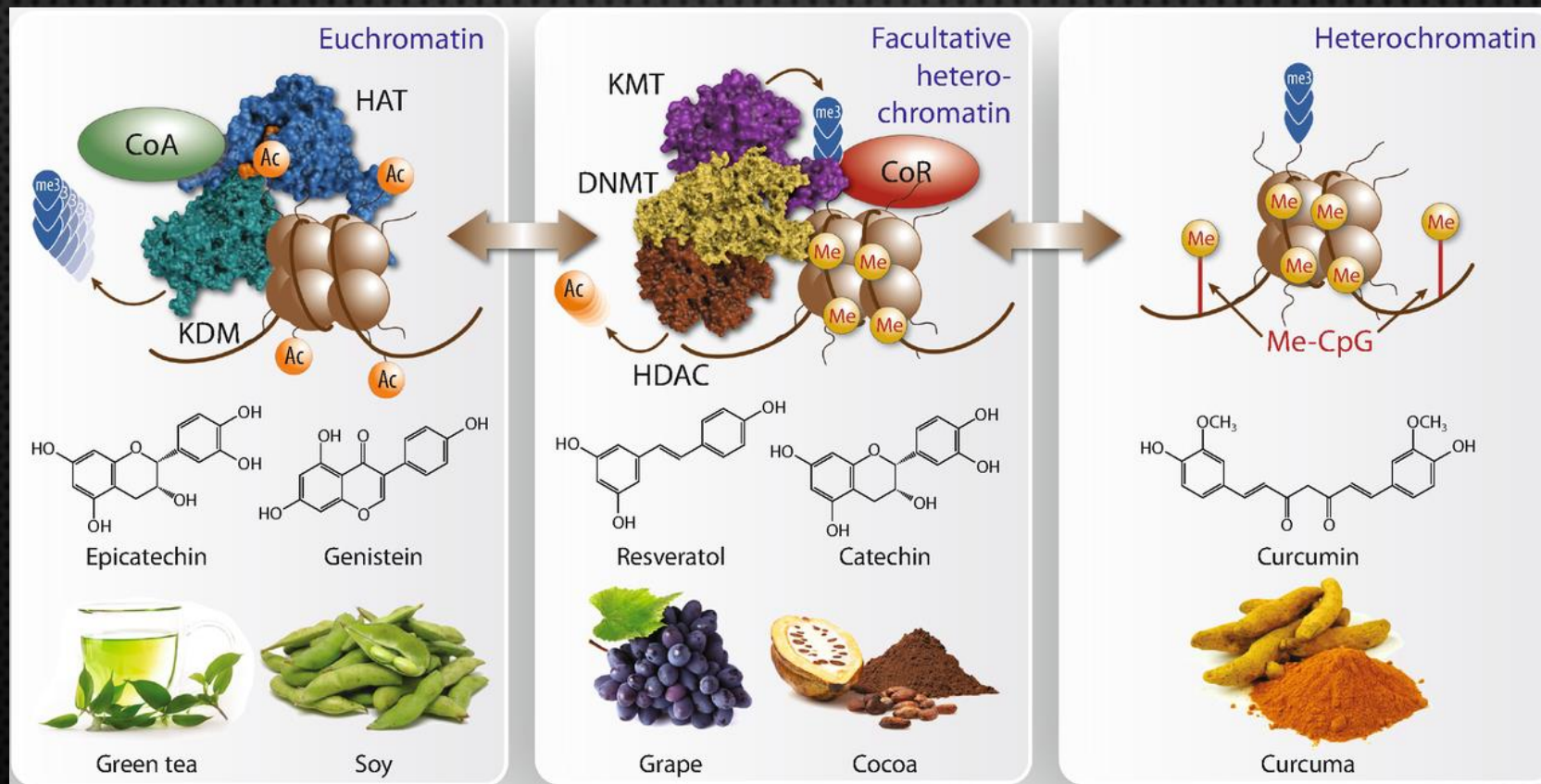
ENVIRONMENT

$$V_P = V_G + V_E$$



NUTRITIONAL EPIGENETICS

- "Is interested in the way in which food affects patterns of gene regulation. It is a resolutely molecular science focused on how the molecules in food interact, via metabolic systems, with the molecules that attach to DNA and control levels of gene expression in the body."
- Food as exposure: Nutritional epigenetics and the new metabolism PMID: 23227106



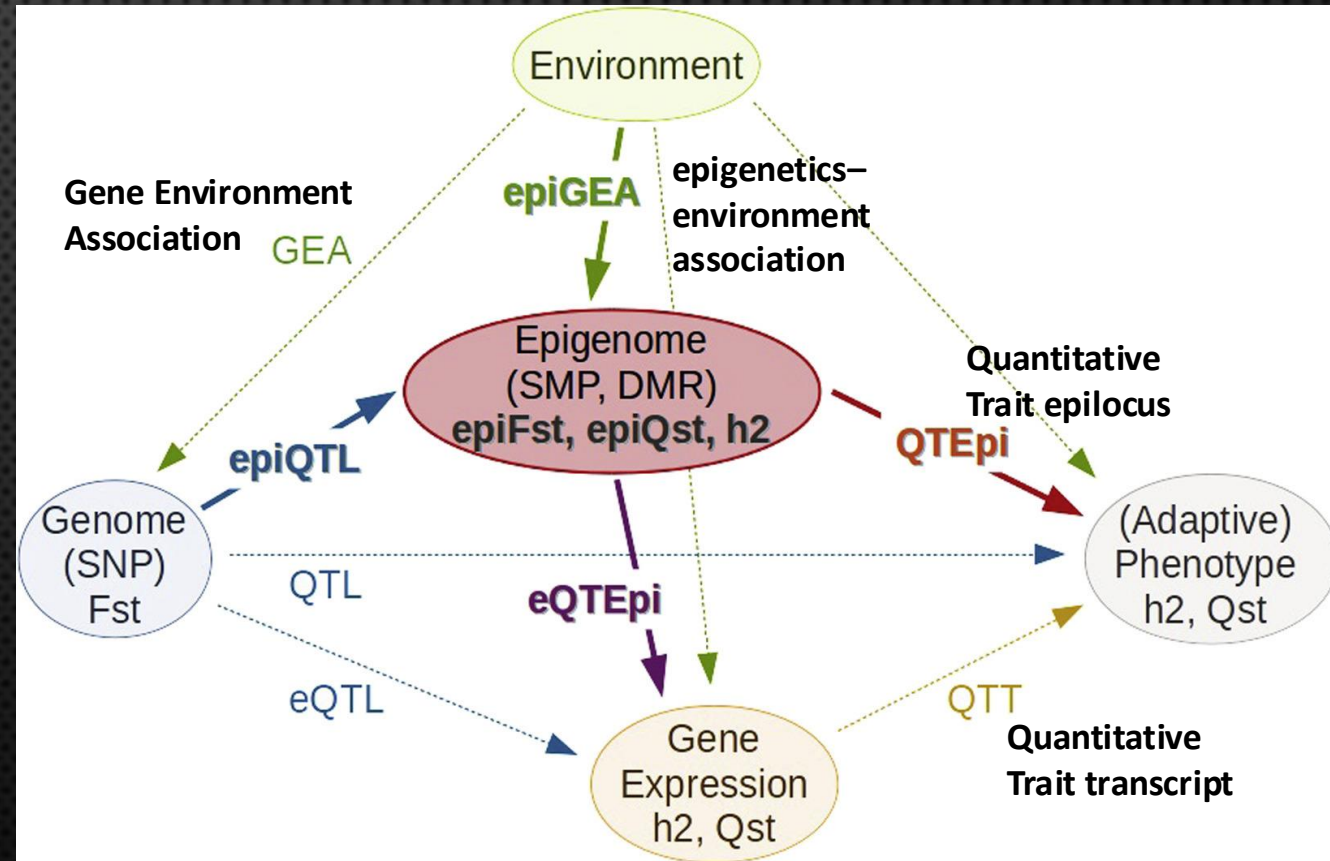
Dietary change	Epigenetic effects	Outcome	Species	Reference
Vitamin B ₁₂ , folate and methionine deficient	DNA methylation	Hypomethylation in methyl-deficient diet	Sheep	Sinclair et al., 2007
Betaine supplementation	DNA methylation	Increased global measures of DNA methylation	Chicks	Hu et al., 2015
Betaine supplementation	DNA methylation, Histone modification	<i>PEPCK1</i> hypomethylated and enriched for H3K27me3. <i>PEPCK2</i> and <i>FBP1</i> hypermethylated and enriched for H3K4me3	Neonatal Piglets	Cai et al., 2014
Zinc supplementation	DNA methylation	Hypomethylation and increased expression of gene <i>A20</i>	Hens	Li et al., 2015
Variable zinc levels	DNA methylation	Hypermethylation in regulatory regions of gene <i>ZIP4</i>	Piglets	Karweina et al., 2015
Restrictive feeding	Histone modification	Increased global measures of H3K9 acetylation and reduction in H3K9me3 in IUGR pigs subjected to restrictive feeding	Pigs	Nebendahl et al., 2016
Folic acid supplementation	DNA methylation	Hypermethylation in IUGR piglets with folic acid supplementation compared to piglets fed control diet	Pigs	Jing-bo et al., 2013
Maternal protein insufficiency	DNA methylation	Hypomethylation in <i>POMC</i> and <i>GR</i> in the sheep brain	Sheep	Begum et al., 2012
Methylating micronutrients	DNA methylation	<i>IYD</i> gene differentially methylated between groups of pigs with extreme obesity related phenotypes	Pigs	Braunschweig et al., 2012

Nutritional Influence on Epigenetic Marks and Effect on Livestock Production

<https://doi.org/10.3389/fgene.2016.00182>

WHAT WE'RE TRYING TO WORK TOWARDS

- Differentially Methylated Regions
 - Can act as epigenetic QTL
- Single Methylation Polymorphisms
 - Can be used as epigenetic markers
 - Assess the role of epigenetics
 - Population structure epifst
 - Phenotypic variation epiqtl
- Use of epigenetic components in selection



WHAT'S STOPPING US FROM DOING QUANTITATIVE EPIGENETICS NOW?

Knowledge:

- Annotating Epigenetic Modifications for each economically important trait and their respective tissues

Resources:

- Lacking an economically feasible means to generate high throughput epigenetic genotypes in livestock

KNOWLEDGE

Current Knowledge:

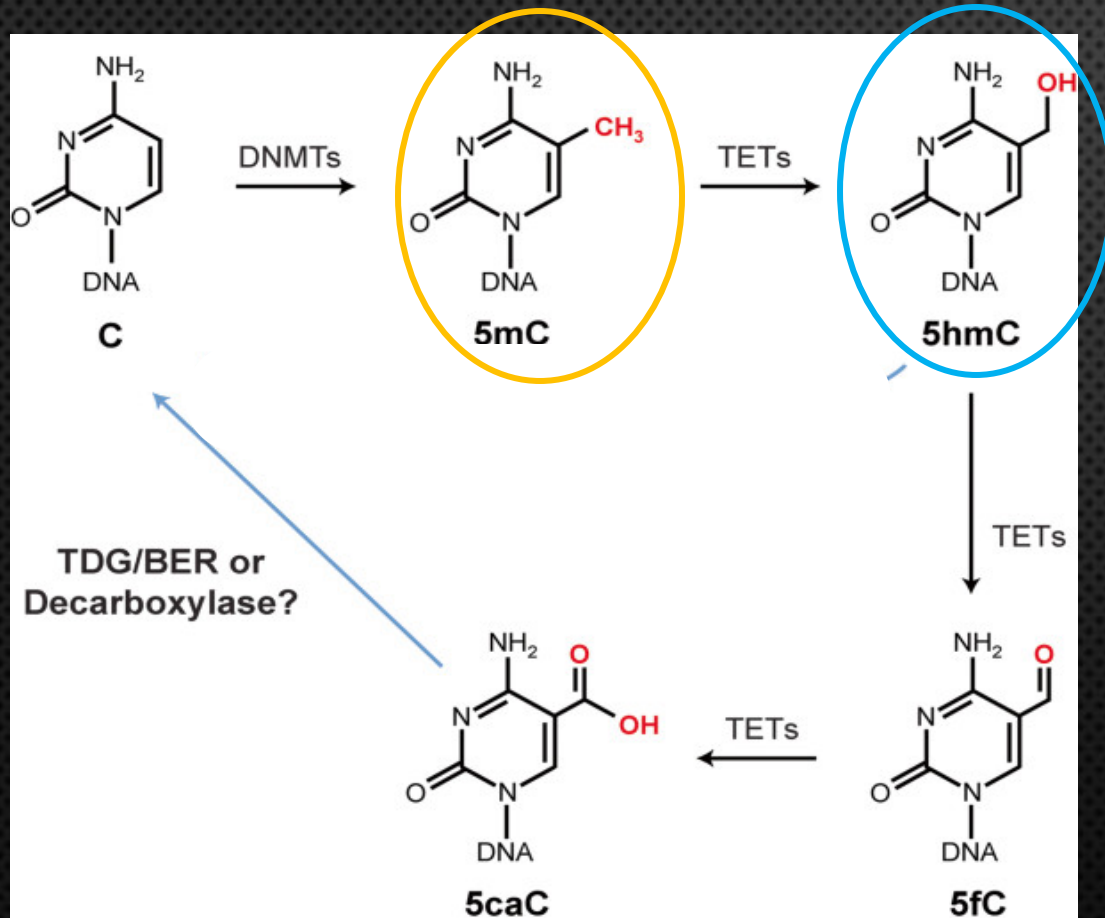
- Easily find differentially methylated regions in any tissue for two phenotypically diverse groups
- DNA methylation can be conserved
 - Pattern and percentage
 - Comparative Epigenomics
- RNA Methylation has more of an impact on gene expression than DNA methylation

What are we working on?

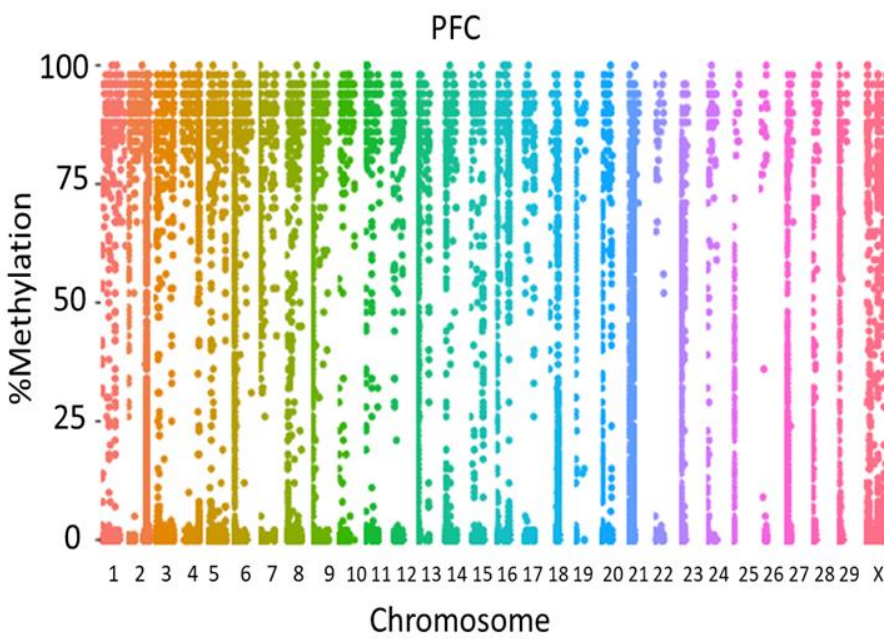
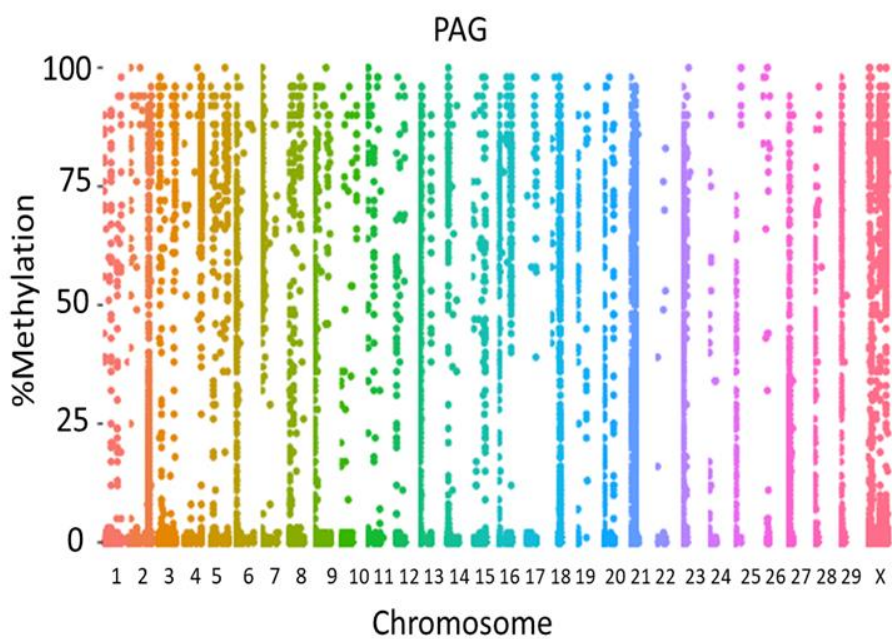
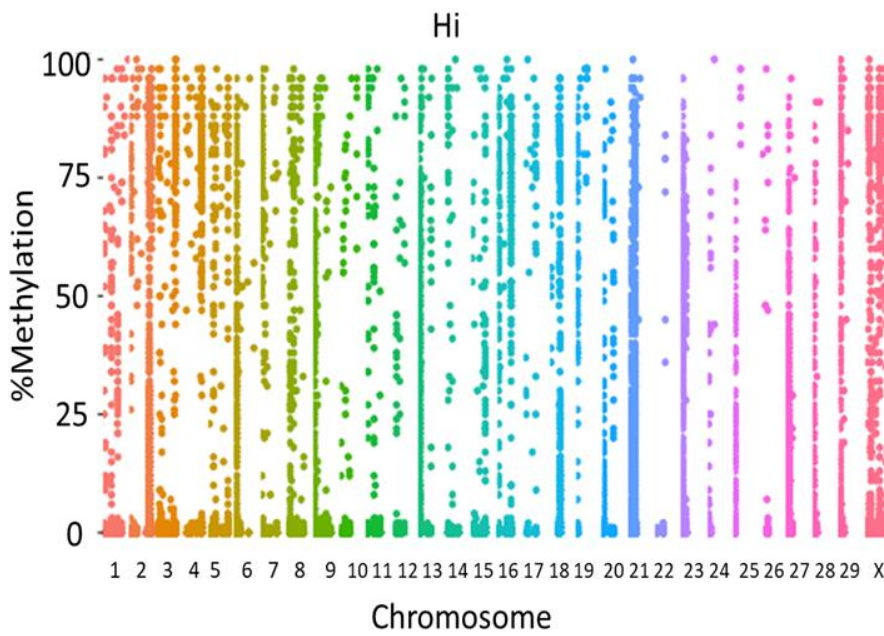
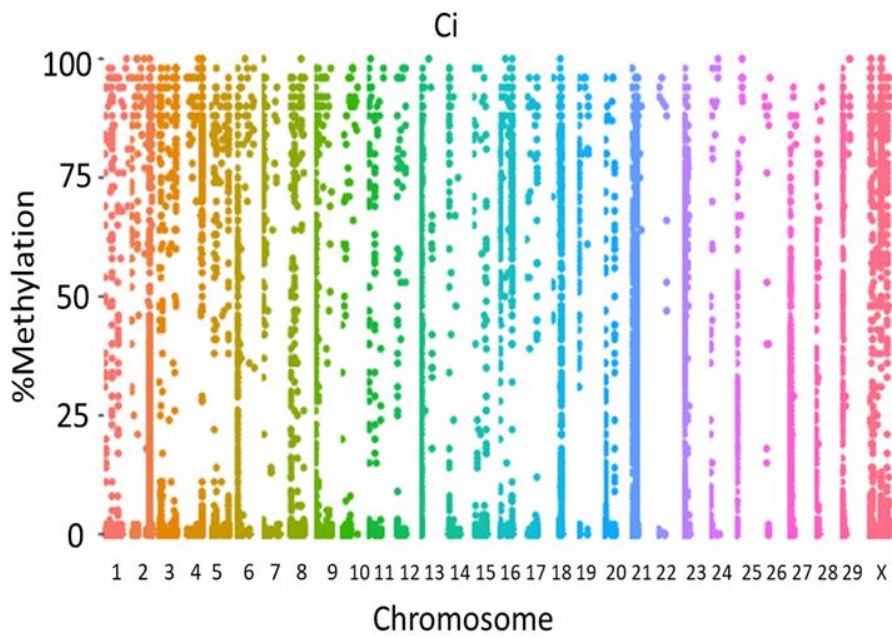
- More Resources
 - Pan-Epigenome
 - Pan-Genome



DNA Methylation and Demethylation



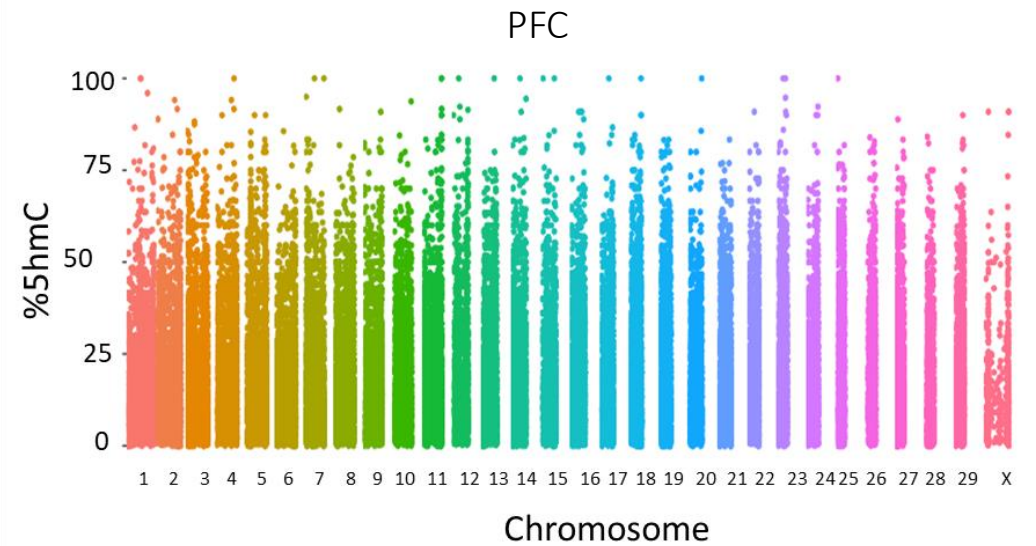
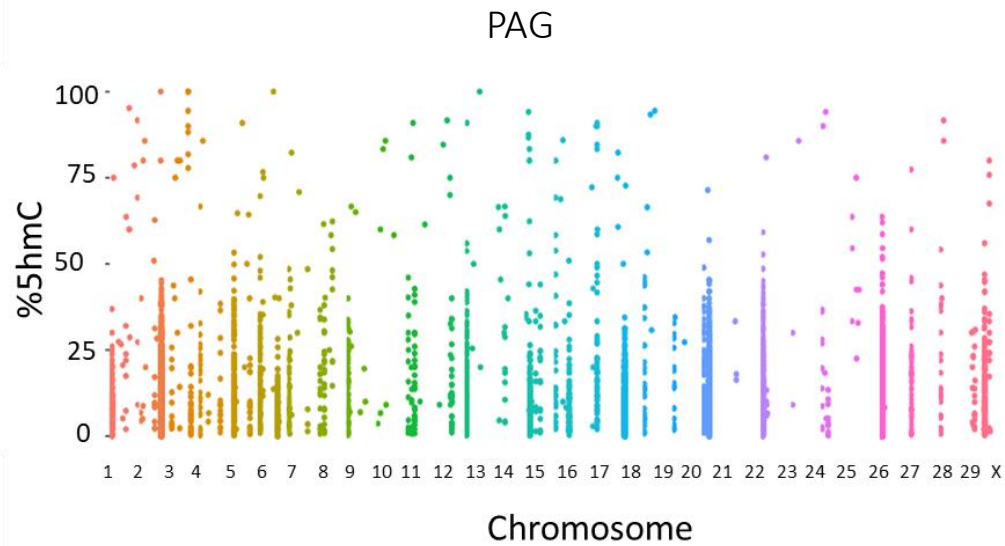
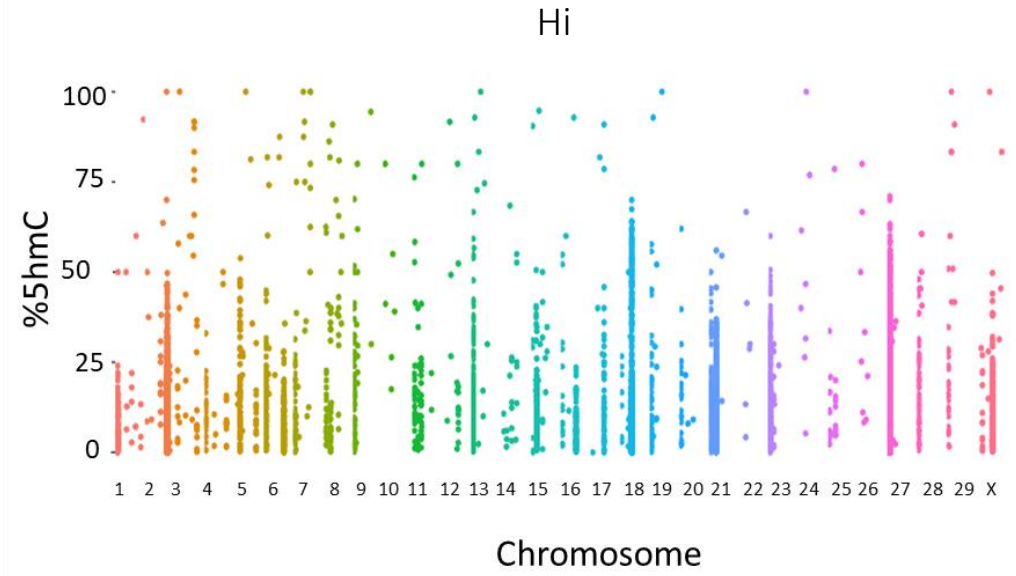
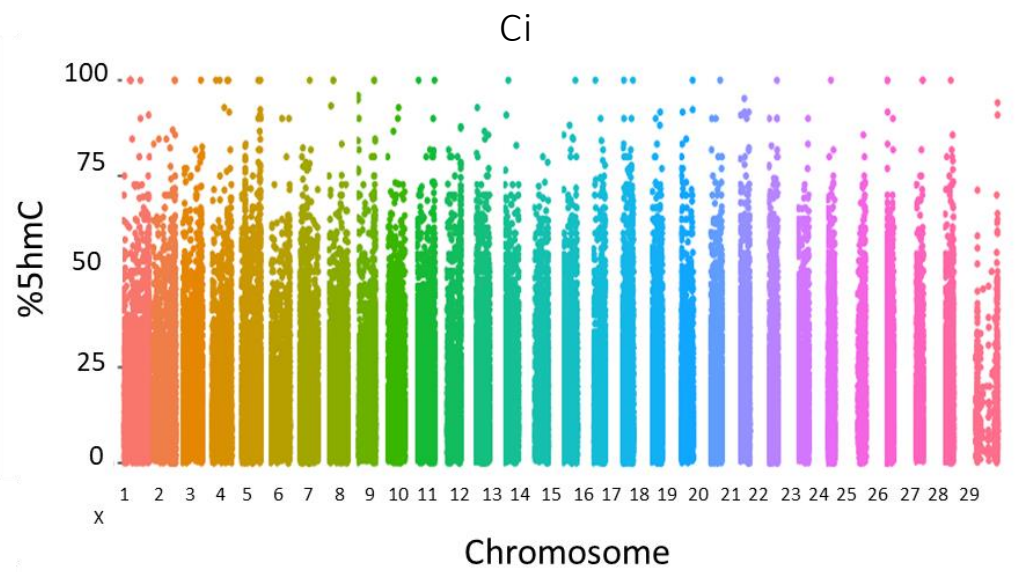
- 5-mC and 5-hmC can be inherited or be added during animal's lifetime
- 5-methylcytosine often prevents transcription from initiating at the modified site
- 5-hydroxymethylcytosine often permits transcription
- 5-hmC levels are higher in central nervous system tissues than any other tissue in the body in mammals



NEUROEPIGENOMICS: 5-METHYLCYTOSINE

- Methylation % is variable across the genome for each tissue
- Methylation does not seem to be localized to specific locations
- 5mC is fairly consistent across tissue types until you get to BTA21 – BTA29

NEUROEPIGENOMICS: 5 - HYDROXYMETHYLCYTOSINE

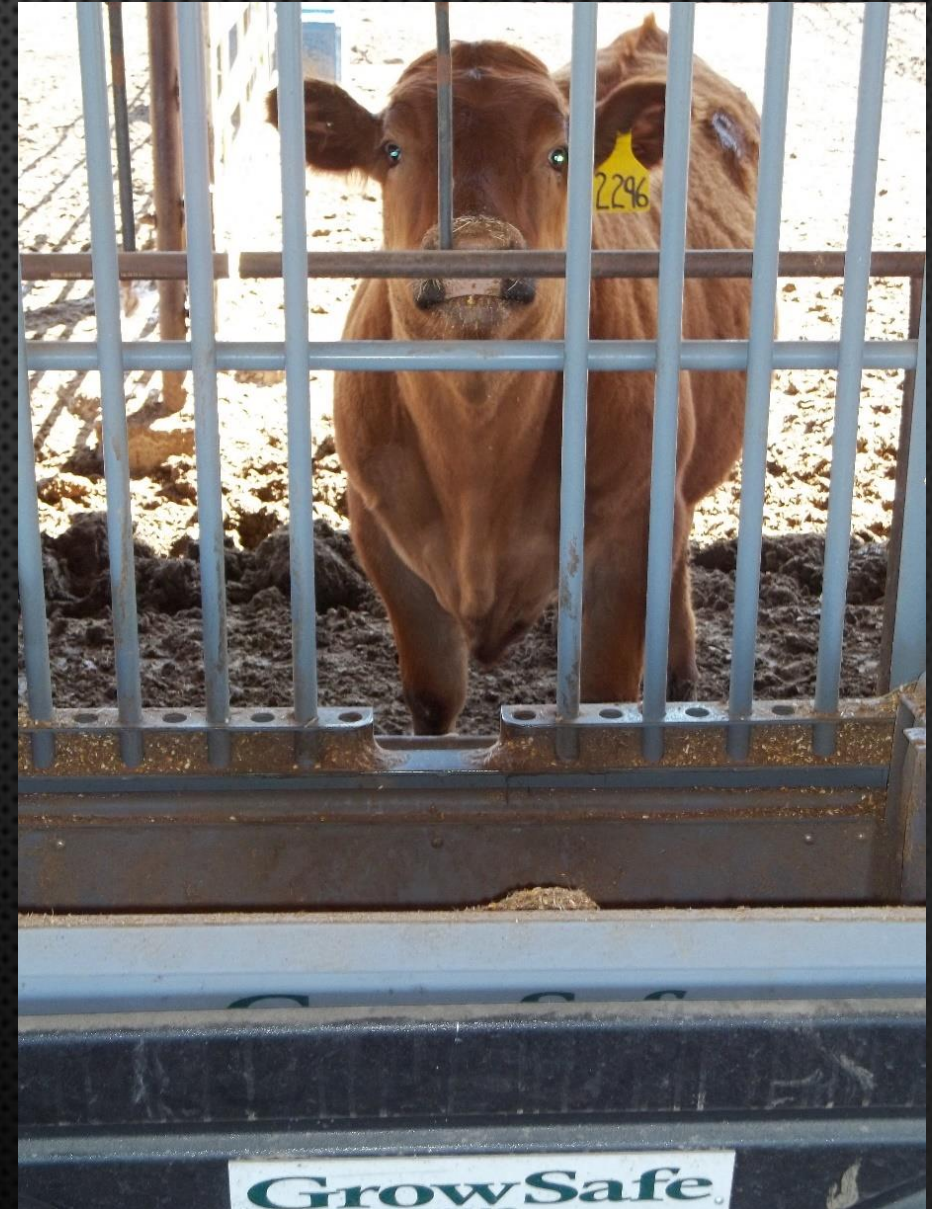


5-mC & 5-hmC SUMMARY

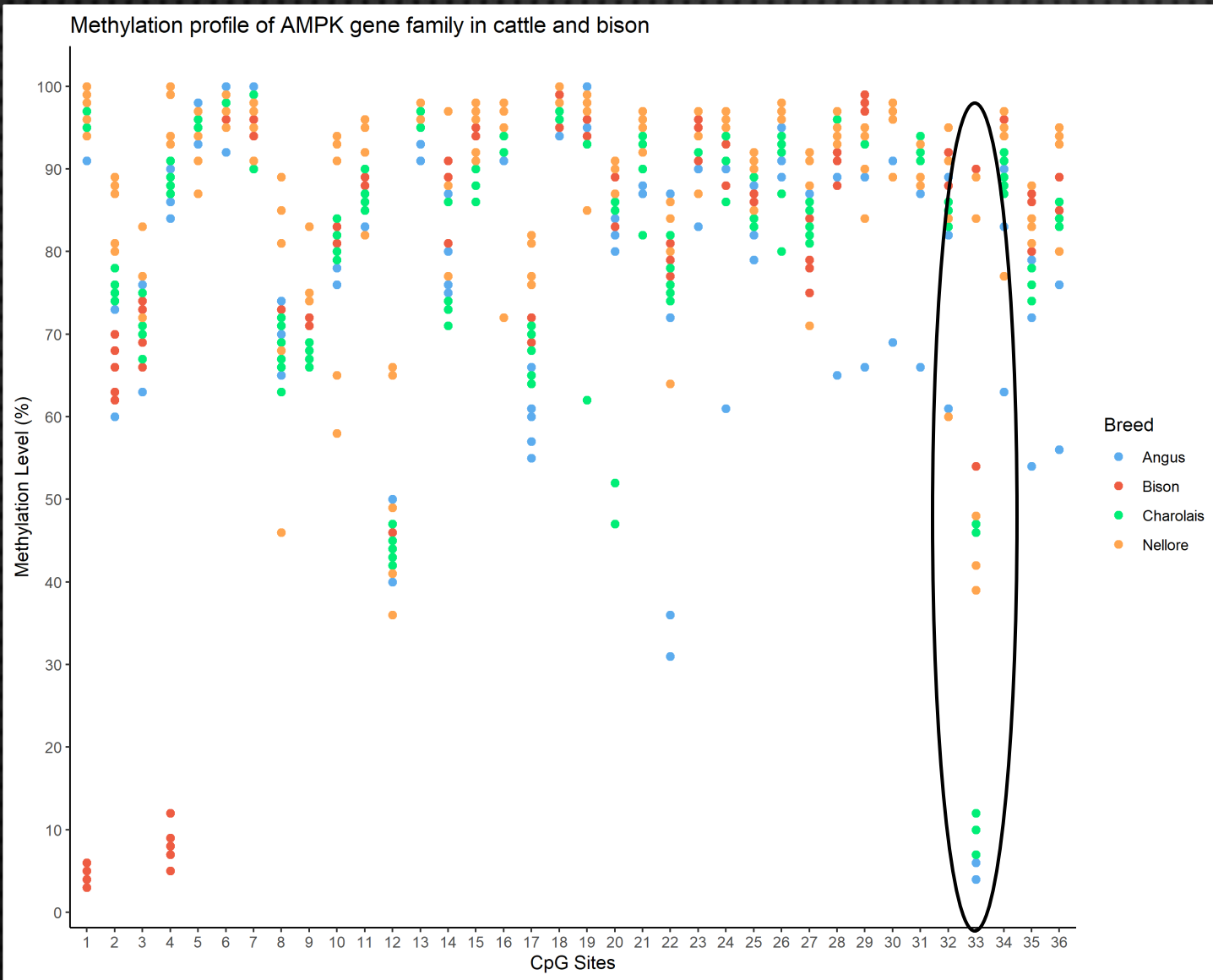
- 5-mC and 5-hmC is found across chromosomes and gene regions in cattle brain tissues
- Differentially Methylated Regions and Differentially Hydroxymethylated Regions were found in each tissue

AND...

- We've done this for multiple traits, tissues, breeds and species
 - Docility, RFI, Johne's and Domestication



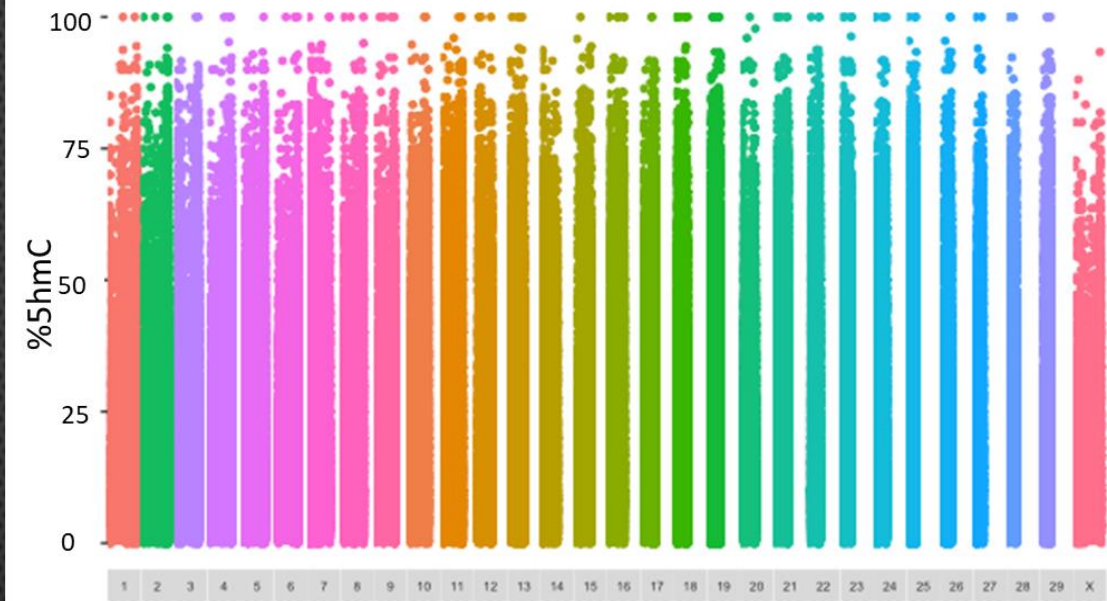
COMPARATIVE EPIGENOMICS - CONSERVATION OF % AND PATTERN OF METHYLATION



- Conservation of percent and pattern of DNA Methylation in 2 genic regions
 - *PRKAA1* (Sites 1-4)
 - *PRKAB1* (Sites 5 – 26)
- Sites 1 and 4 were unmethylated in Bison and found thymine instead of cytosine in the reference genome.
- Site 33 showed the greatest variation

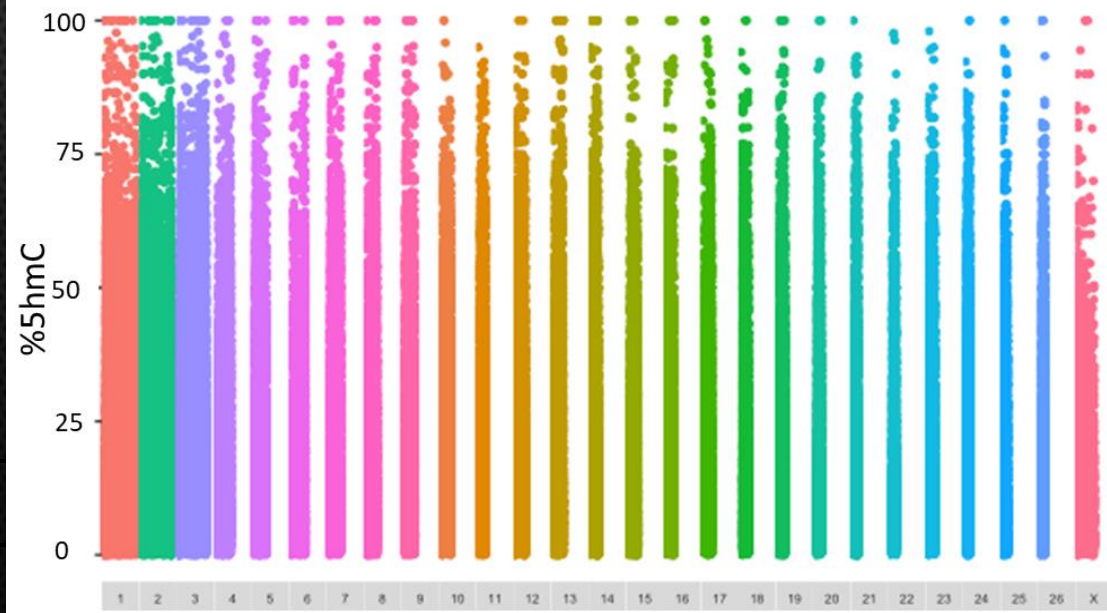
COMPARATIVE 5-hmC IN CATTLE AND SHEEP CEREBELLUM

- Number of 5hmC sites identified:
 - Sheep - 125,951 5-hmC sites
 - Cattle - 317,136 5-hmC sites
- Chromosomes with highest number of 5hmC sites when normalized for chromosome length
 - Sheep – OAR24
 - Cattle – BTA25
- Percent 5hmC:
 - Cattle - 91% of 5-hmC sites were < 50% hydroxymethylated
 - Sheep - 93% of 5-hmC sites were < 50% hydroxymethylated



Cattle

Chromosome

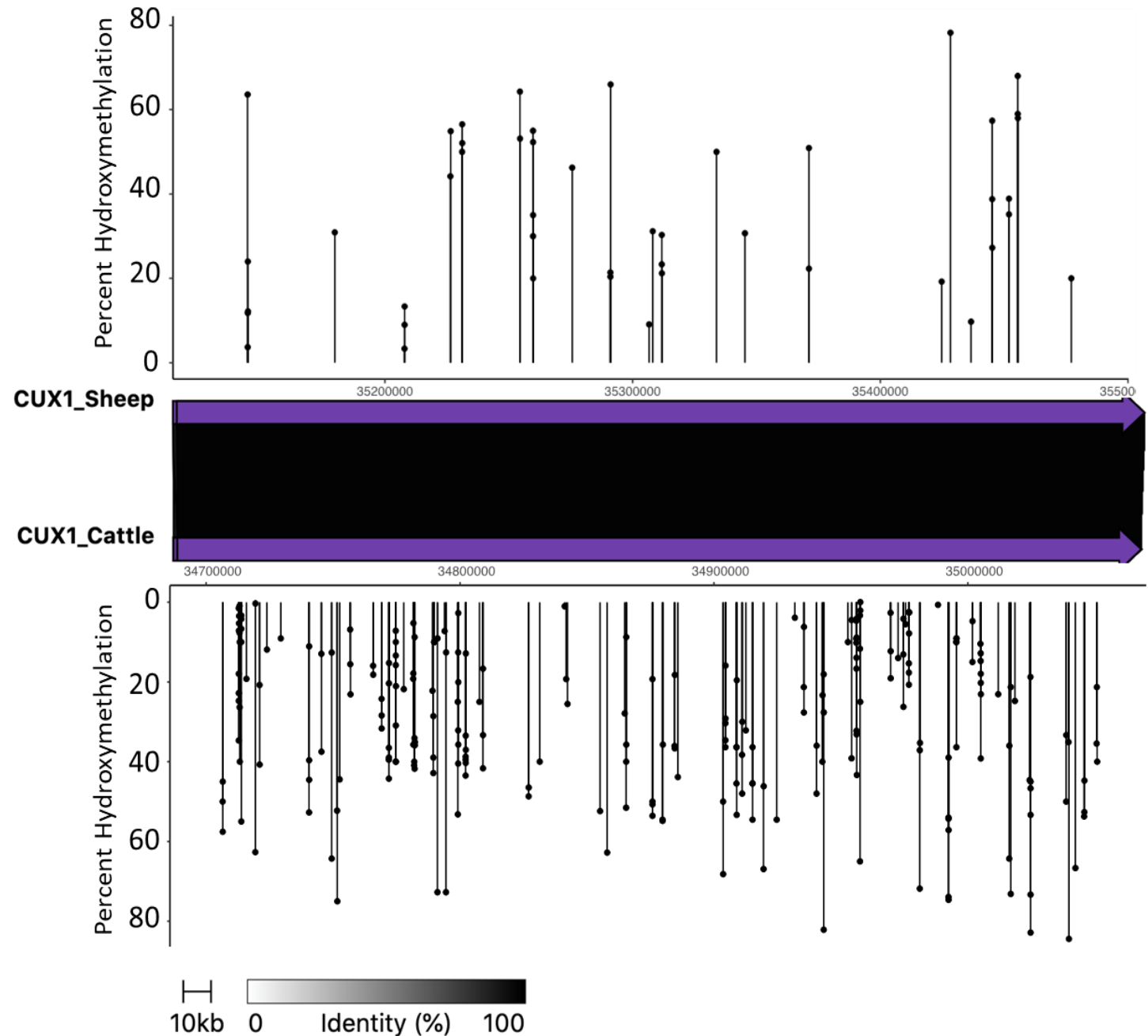


Sheep

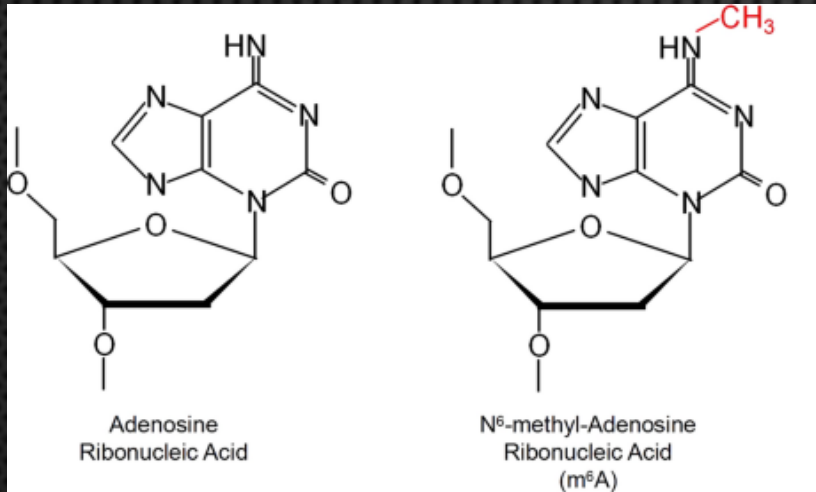
Chromosome

GENE *CUX1* 5-HMC IN CATTLE AND SHEEP

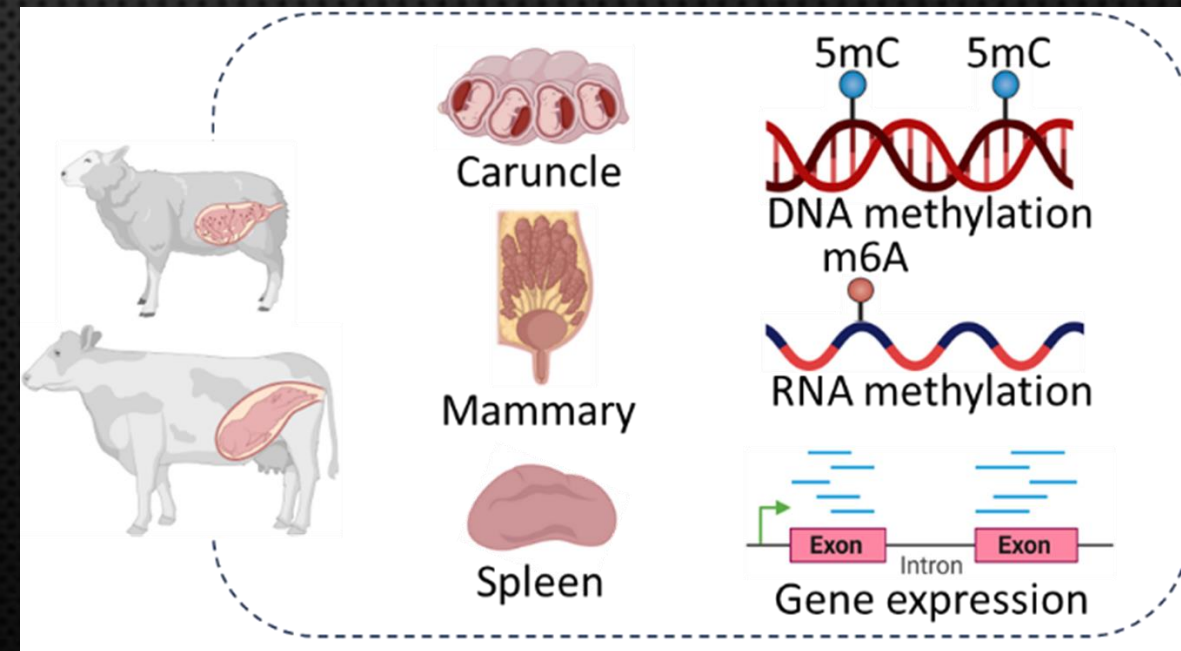
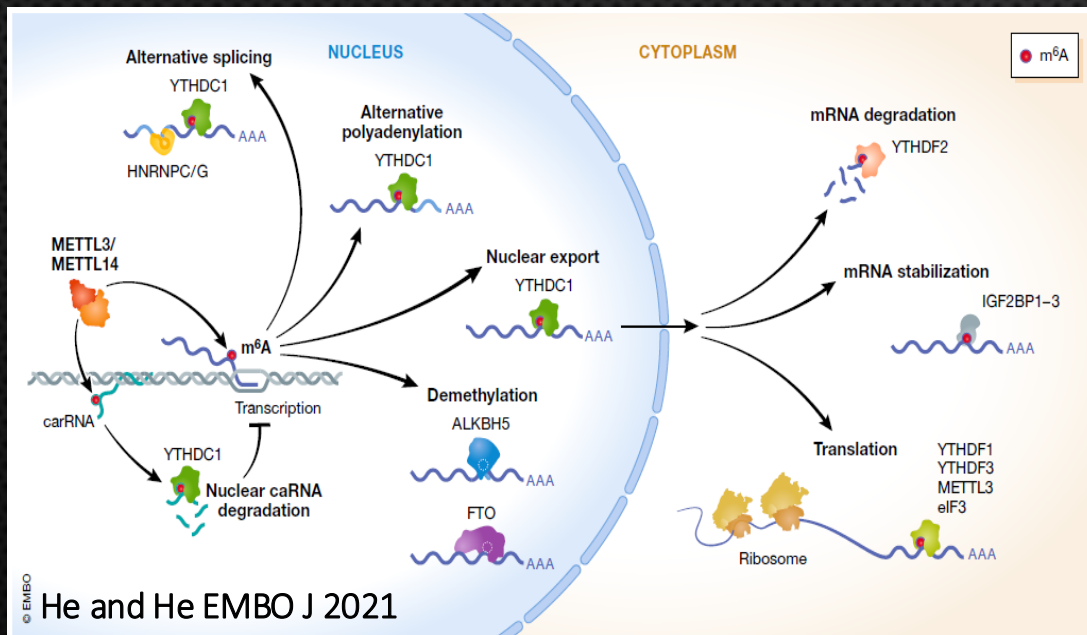
- *CUX1* is highly hydroxymethylated on BTA25 and OAR24
- *CUX1* encodes for a DNA binding protein with suggested functions in:
 - Neuronal cell differentiation
 - Dendrite development and branching
 - Synaptogenesis
 - Cell cycle progression
 - Broad regulation of gene expression

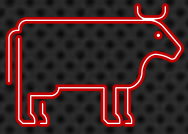


INTERSECTION OF RNA AND DNA METHYLATION

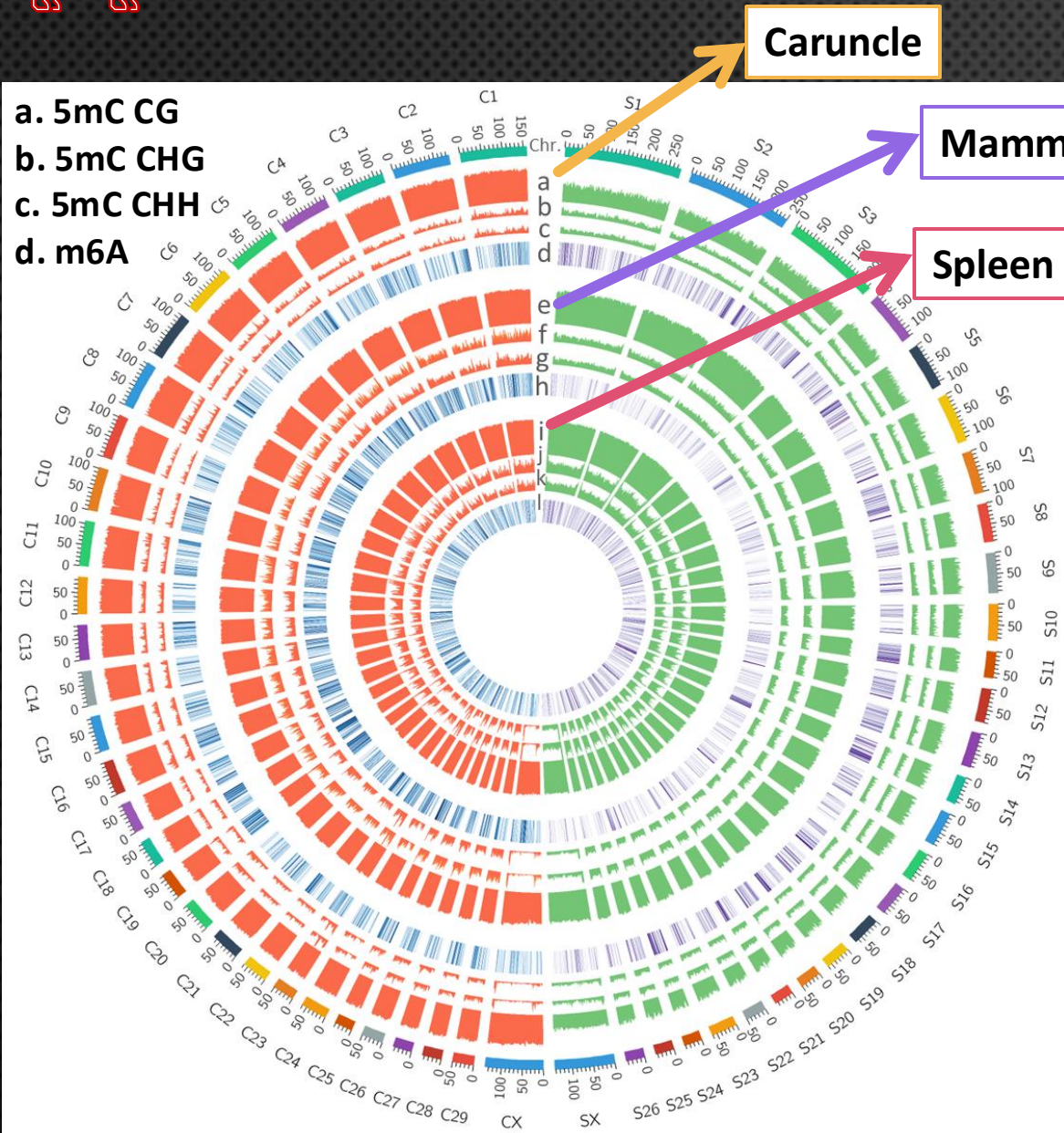
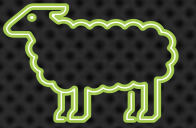


- Overarching goal: Elucidate the effect of DNA and RNA methylation on gene expression and investigate the potential contribution of epitranscriptomics towards functional annotation of genomes.





RELATIONSHIP OF DNA/RNA METHYLATION



- RNA methylation:
 - Uneven distribution among chromosomes
 - Enriched at ends of chromosomes
 - Some enrichment in same location among tissues
- Multiple analysis (not shown) demonstrate that RNA methylation has a greater impact on gene expression compared to DNA methylation

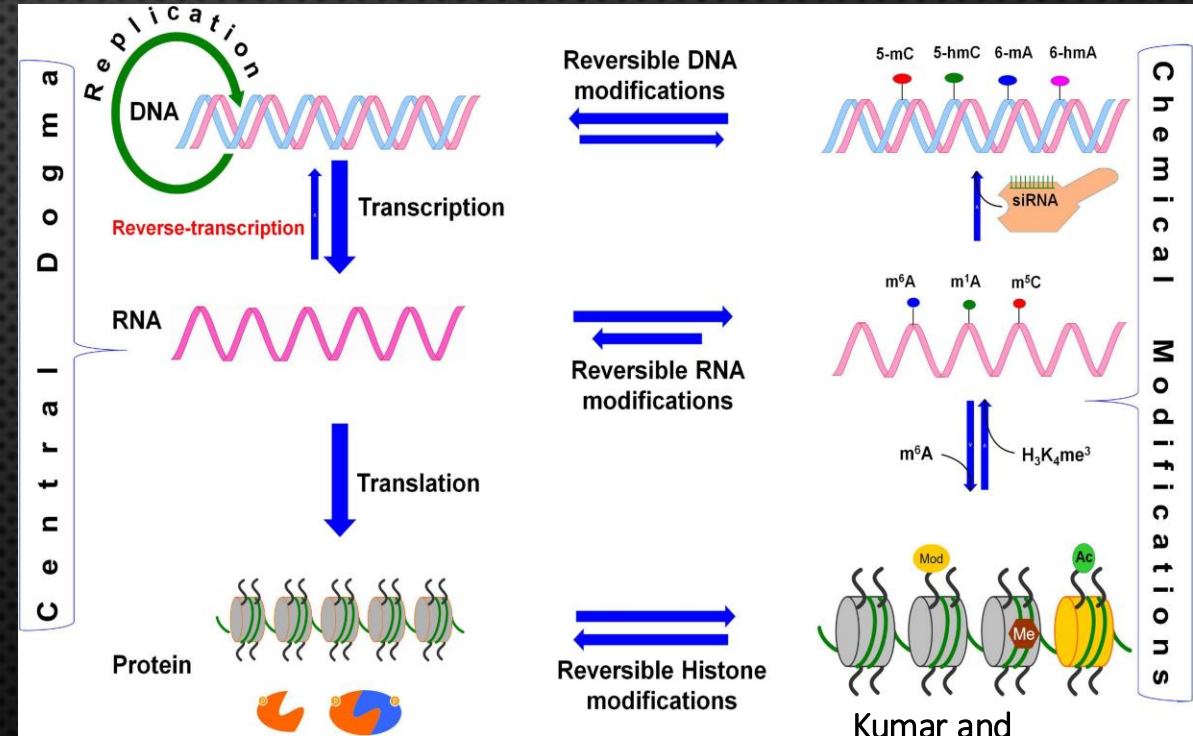
RESOURCES

What do we have now?

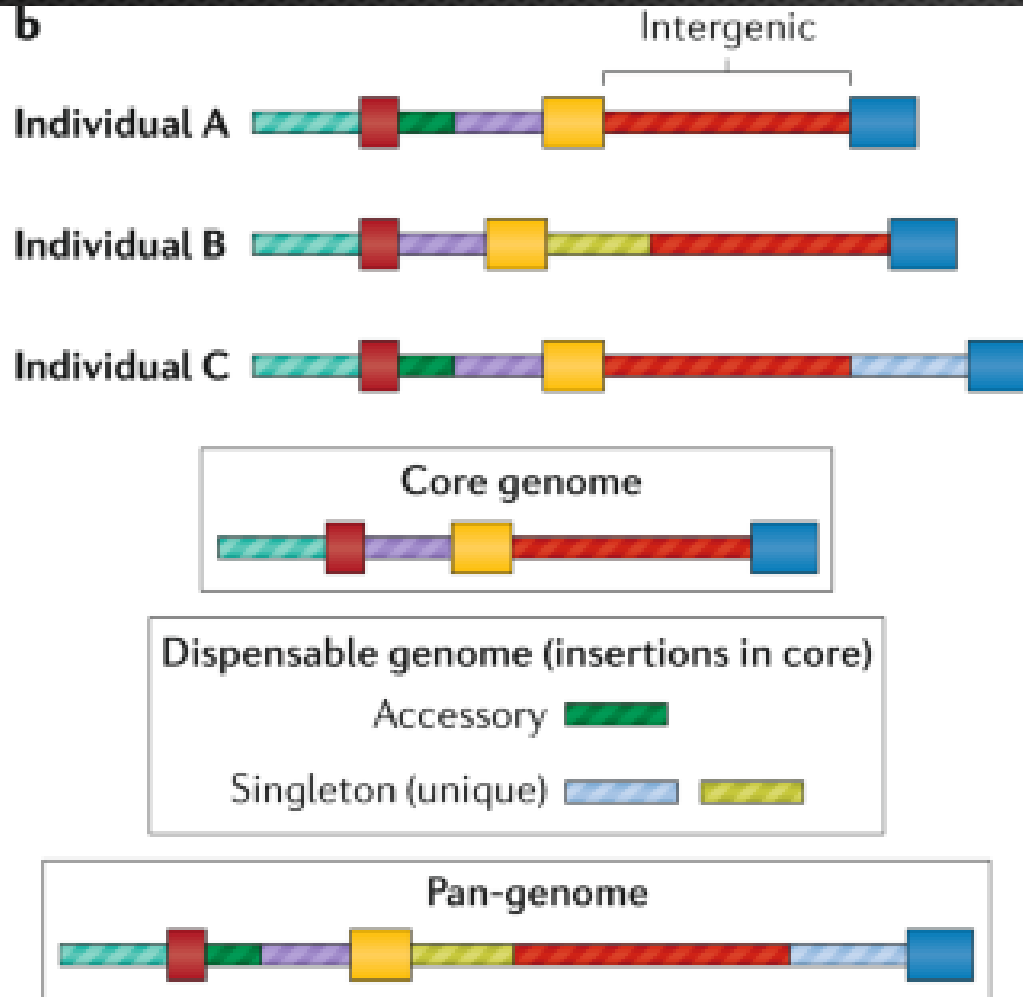
- Sequencing methods that enable single base resolution to detect 5mC and 5hmC
- Pull-down method to enable detection of genomic regions harboring m6A peaks
- Tons of existing sequencing data

What are we working on right now?

- Pan-Genome
- Pan-Epigenome
- Biomodal – new 6 base sequencing methods



PANGENOME vs PAN-EPIGENOME



- Pangenome- whole genome sequences of multiple individuals representing the genetic diversity of a species.
- Core genome - sequences shared between all individuals of the species
- Goal: Uncover and catalog the full extent of genetic variation
- <https://humanpangenome.org/>

PAN-EPIGENOME

- Bovine and Ovine Pan-Epigenome
 - WGBS, Ox-RRBS, ChIP-Seq, ATAC-Seq
 - MeRIP-Seq, WGS, RNA-Seq
- Comprehensive characterization of epigenetic diversity in multiple breeds of cattle and sheep which will serve as the basis for the bovine and ovine pan-epigenome
- Identify epigenetic variation with potential to impact phenotype
- Employ comparative epigenomics to identify consensus epigenomes shared between sheep and cattle



IS ALL HOPE LOST?

- NO
- Selection and Methylation:
 - Inadvertent selection of methylation is probably happening
 - Chris Baes – Resilient Dairy Genome Project



- **What do we need moving forward?**
 - Methylation Chip
 - New RNA sequencing methodologies
 - Sample size acceptance
 - Extending tissue resources associated with economically important traits



THANK YOU!



Dr. Shangqian Xie
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Dr. Darren Hagen



Dr. Rick Funston



Dr. Bob Weaber



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Helen Yampara-Iquise

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