

2019 NBCEC's 'Brown Bagger' Series

Genetics of meat
quality in *Bos indicus*
influenced cattle



UF | IFAS
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Meat Quality

- Top priority for beef industry
 - Great power to influence demand
 - Can be improved
- Very important for *B. indicus* crosses
 - Routinely penalized for relatively **low marbling** score.
 - Routinely penalized for **perceived** inadequate **tenderness**

Meat Quality




USDA grading system



Based on marbling
and maturity

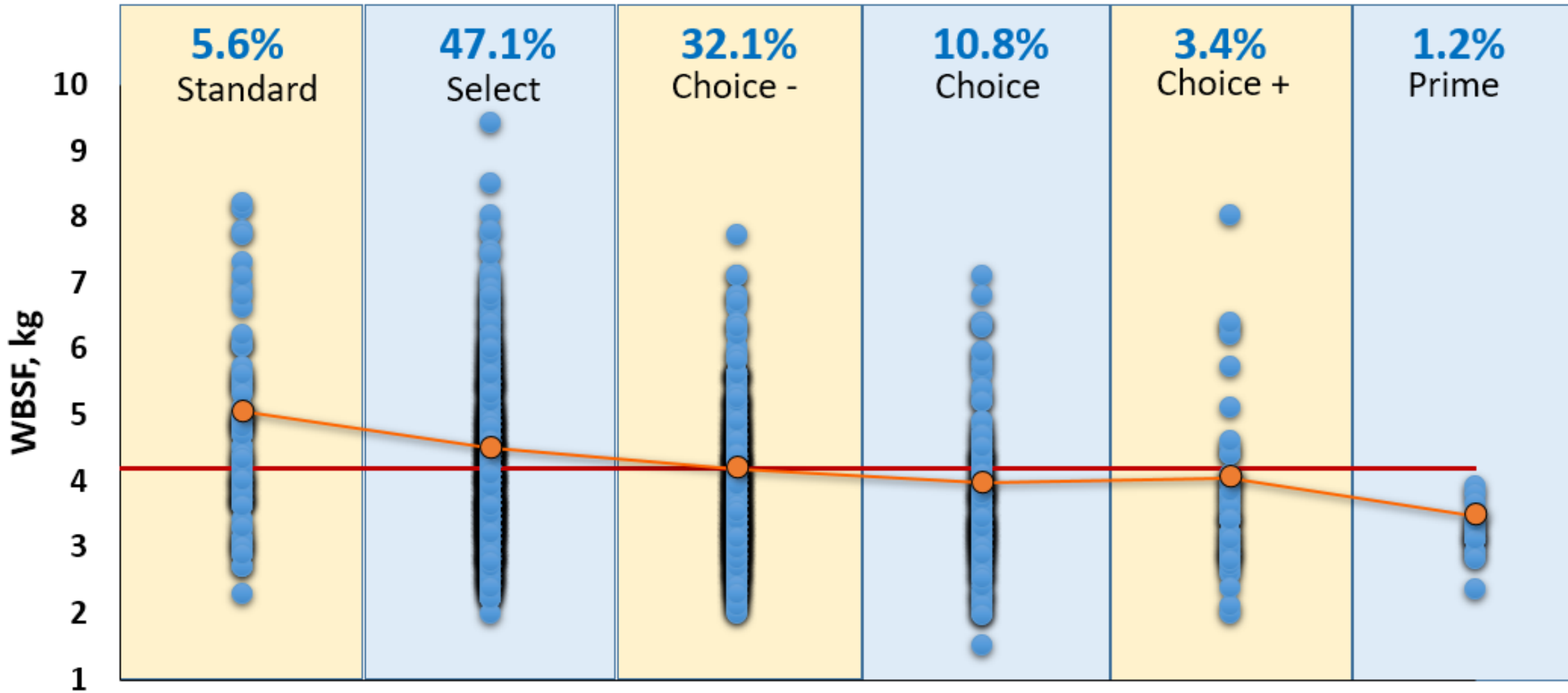


Limited in predicting
eating quality



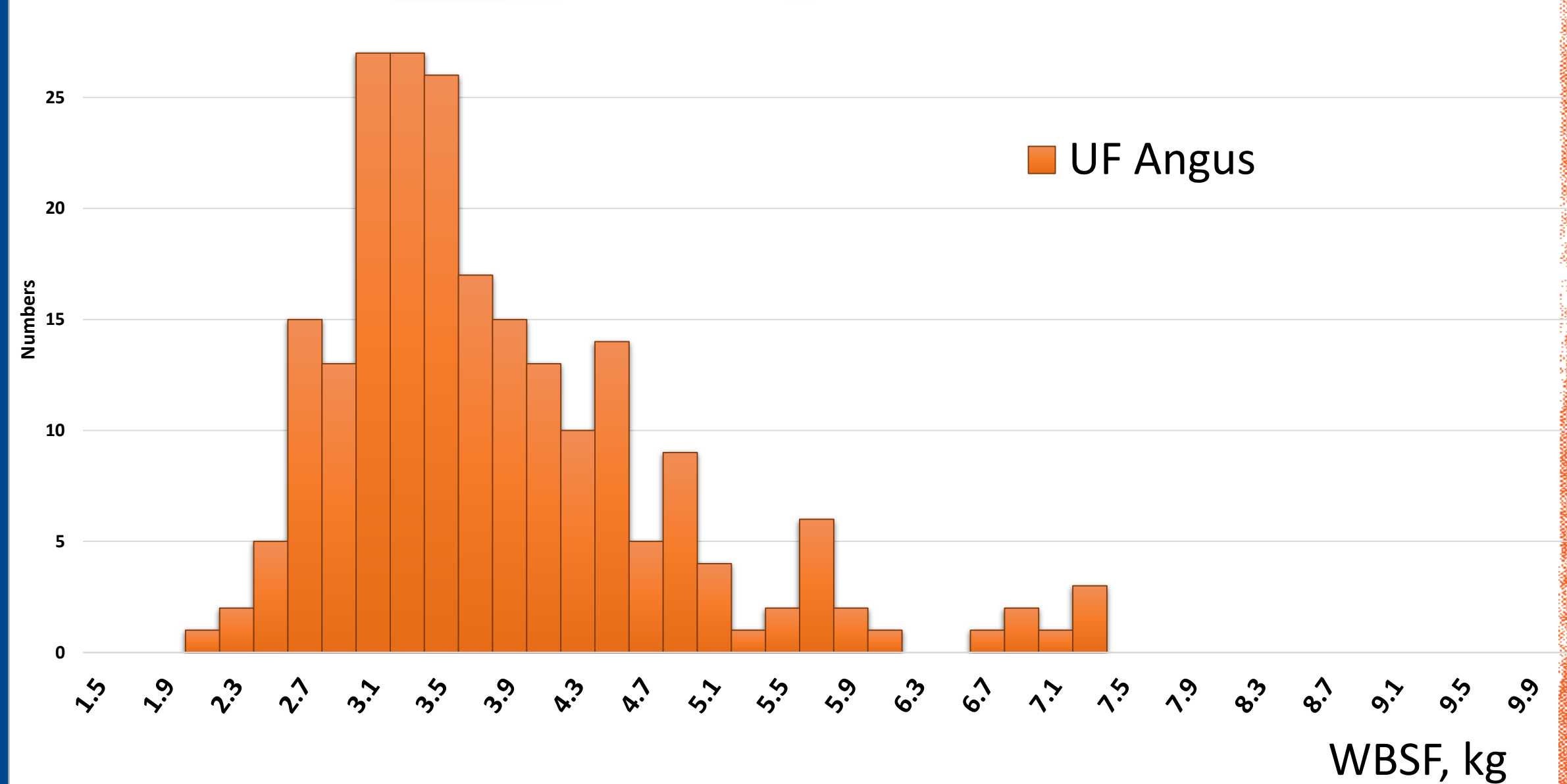
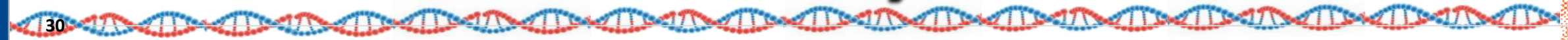
Tenderness

Tenderness by USDA Quality Grade

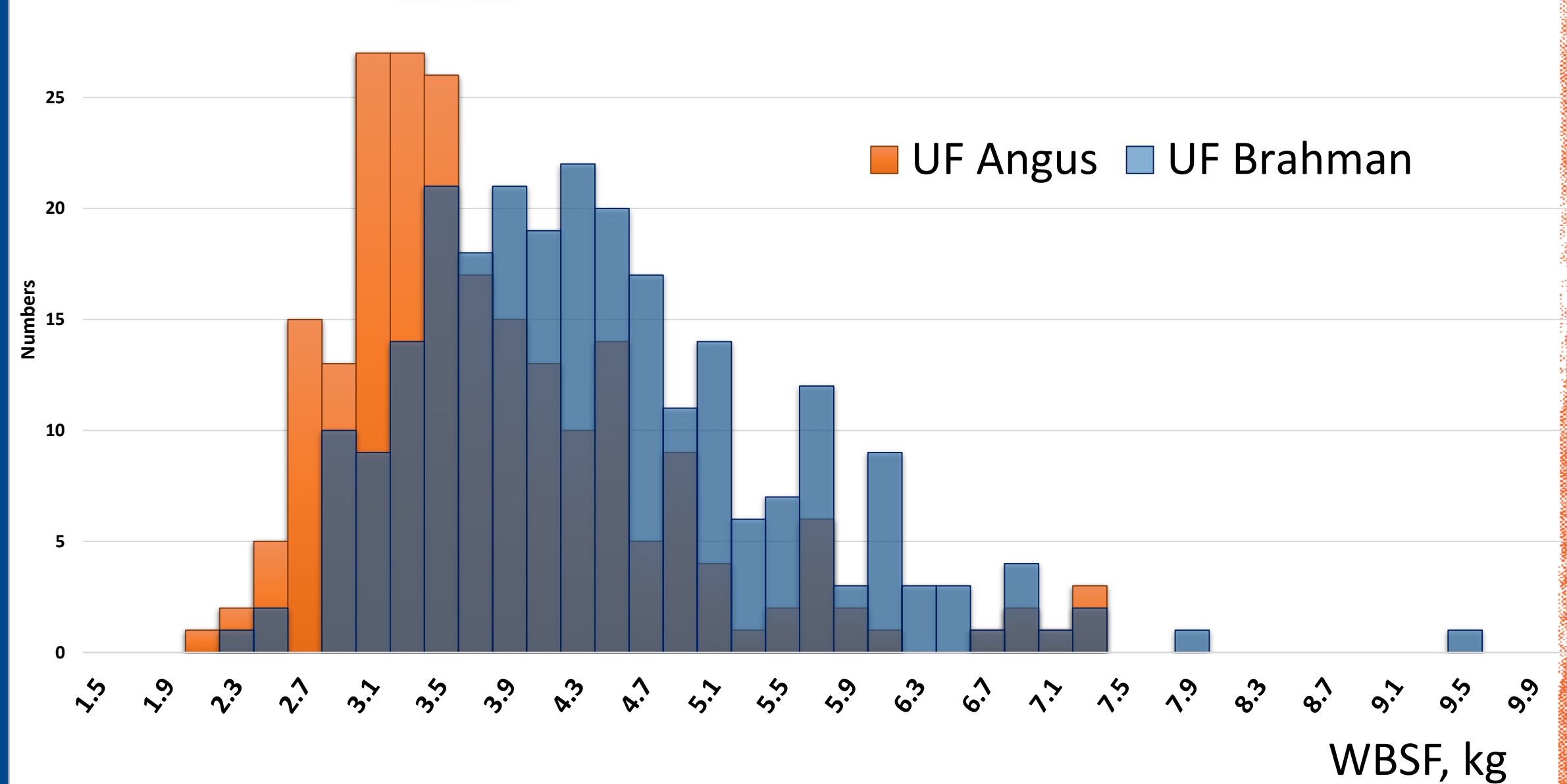
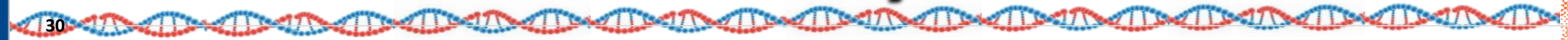


UF MAB
(N = 1,366)

Variation in WBSF – by breed



Variation in WBSF – by breed



Meat Quality



USDA grading system

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Tenderness

Genomic Tests

Developed on
B. Taurus data

Limited prediction in
B. Indicus -influenced

Need to be
breed/pop. specific

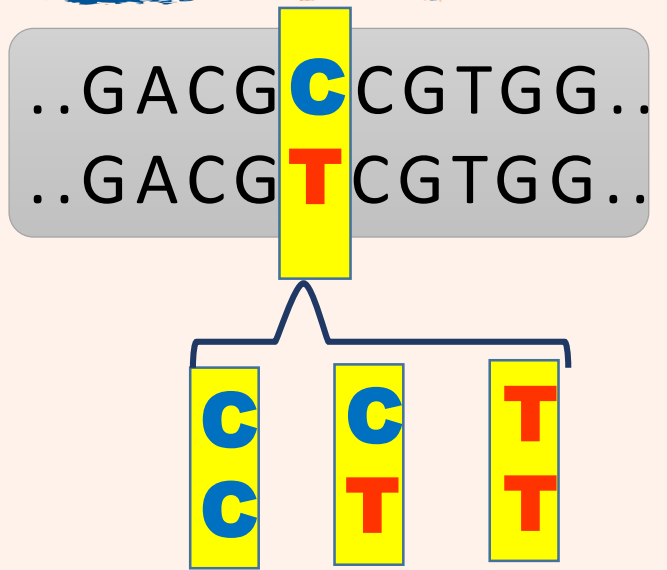
A decorative border consisting of a repeating DNA double helix pattern in red and blue, framing the central text area.

Genetic tests Tenderness

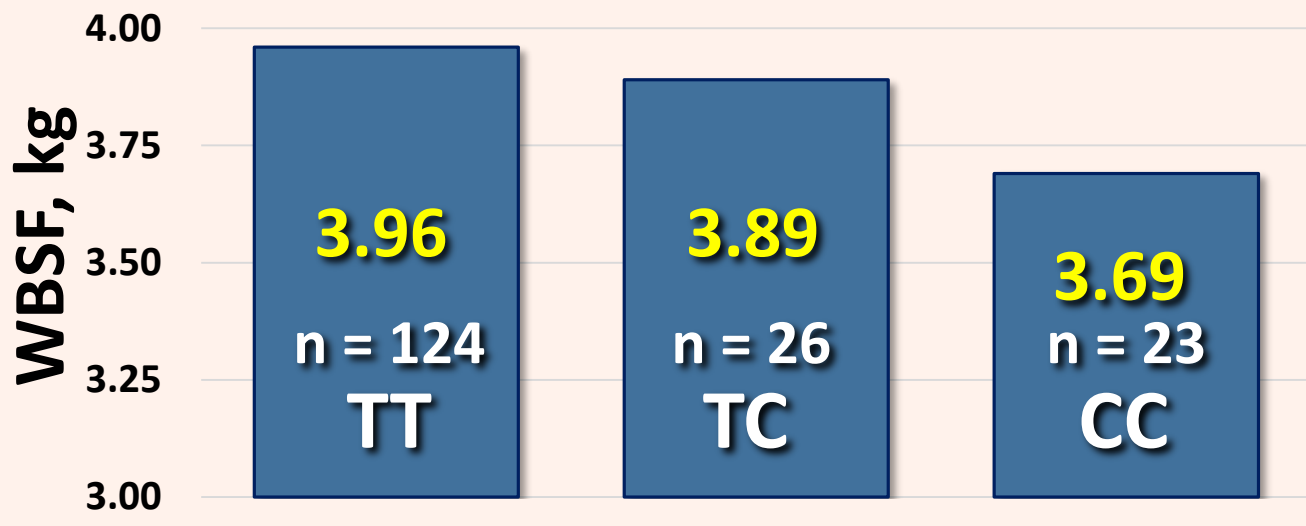
Genomics Tests

UF Angus, n = 153

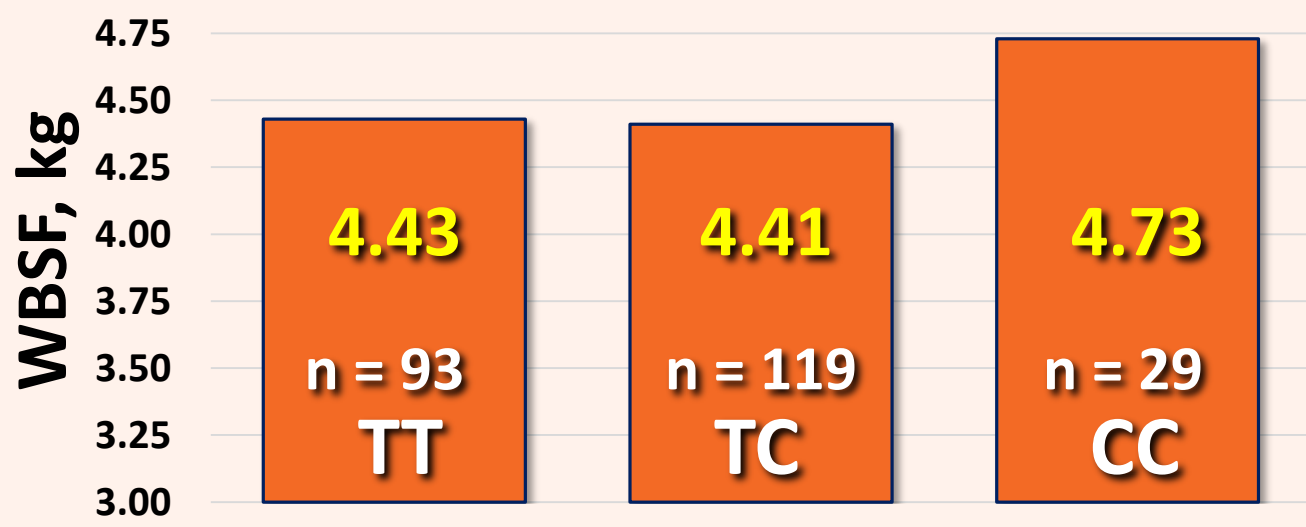
SNP – genetic marker



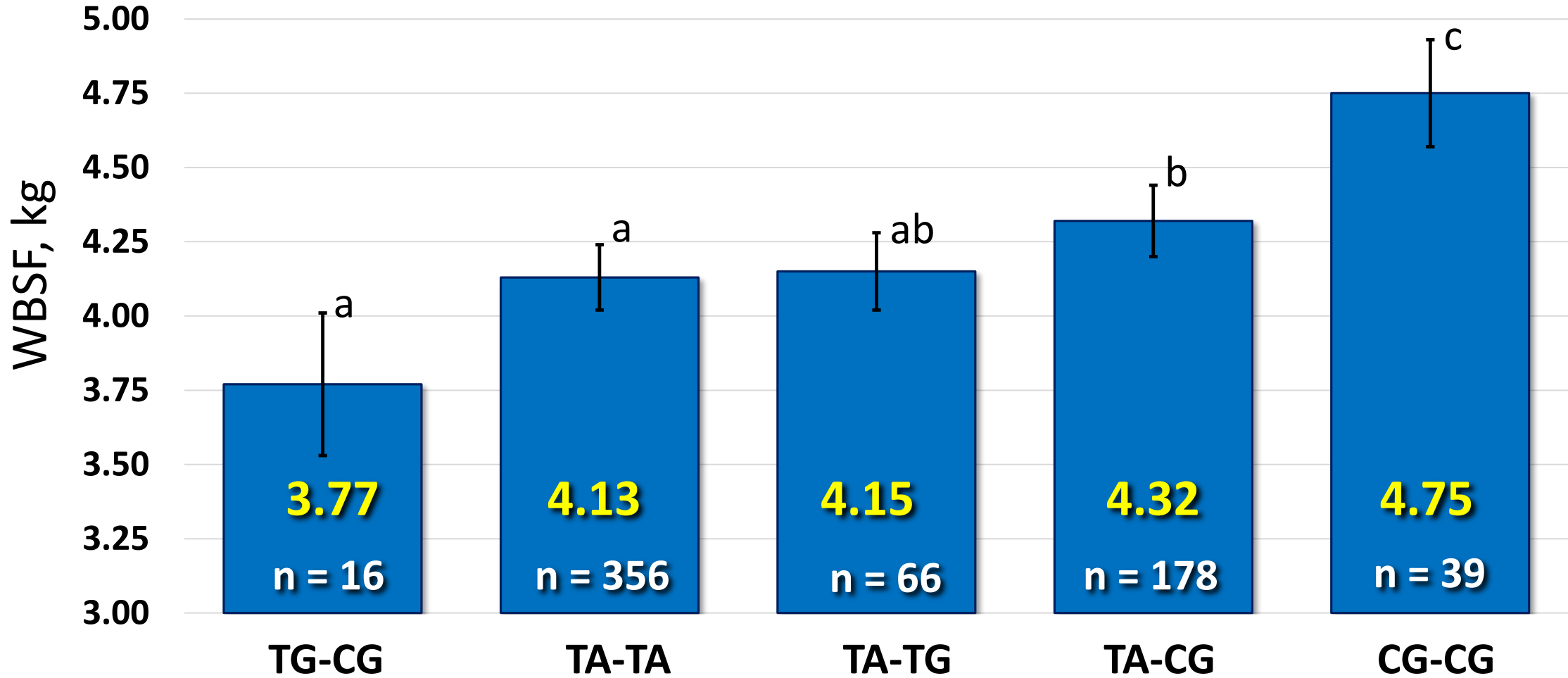
3 possible genotypes



UF Brahman, n = 241



Combination of markers in calpastatin



Meat Quality



USDA grading system

Genomic Tests

Goal: Develop genomic tools to select for superior meat quality in *Bos Indicus* influenced populations.

eating quality

Tenderness

B. Indicus -influenced

Need to be
breed/pop. specific

A decorative border consisting of a repeating DNA double helix pattern in red and blue, framing the central text. The background is white with a blue rectangular area containing the text.

Phenotypic Level

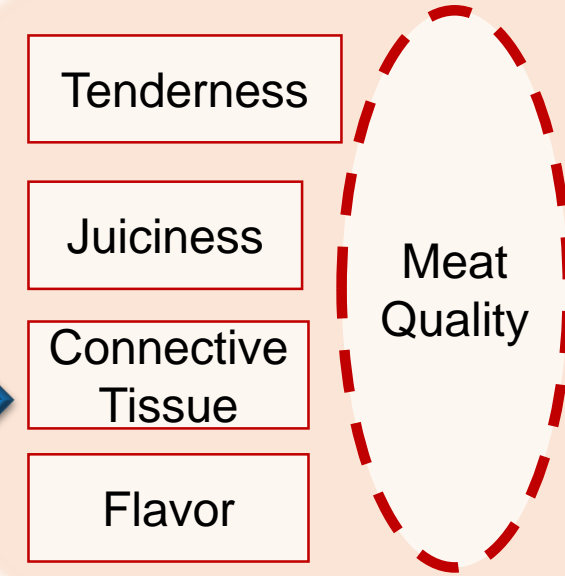
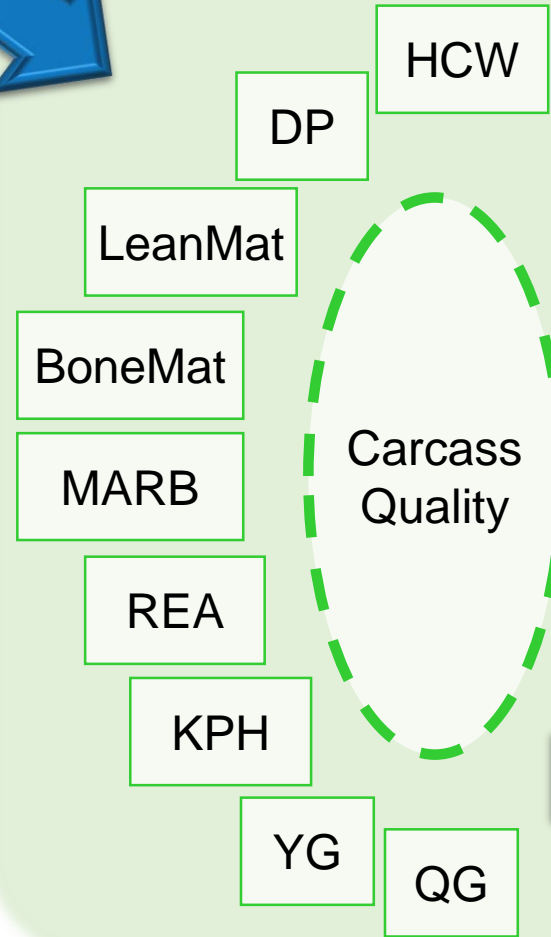
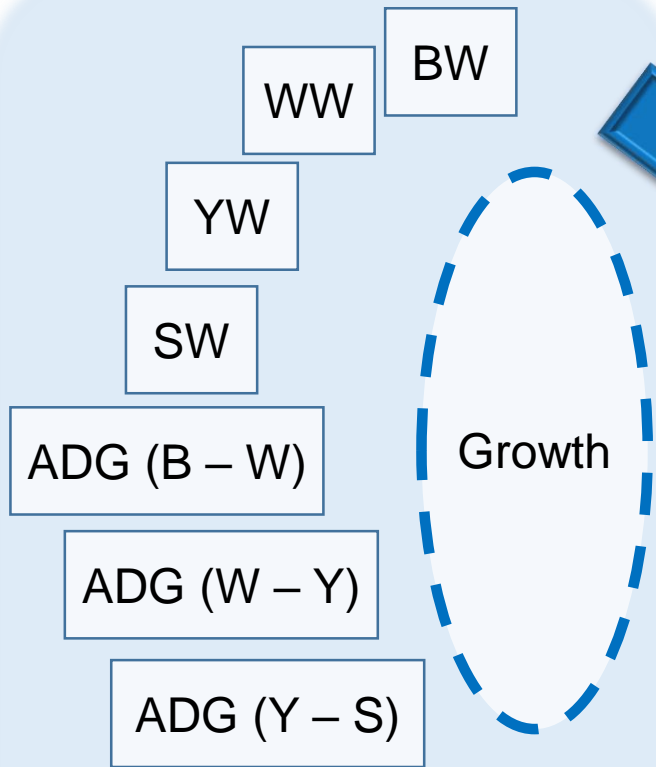
How do we define meat quality?



- Marbling
- Warner-Bratzler Shear Force
- Tenderness (sensory panel)
- Juiciness (sensory panel)
- Connective Tissue (sensory panel)
- Beef Flavor (sensory panel)
- Off-flavors (painty/fishy, livery/metallic)

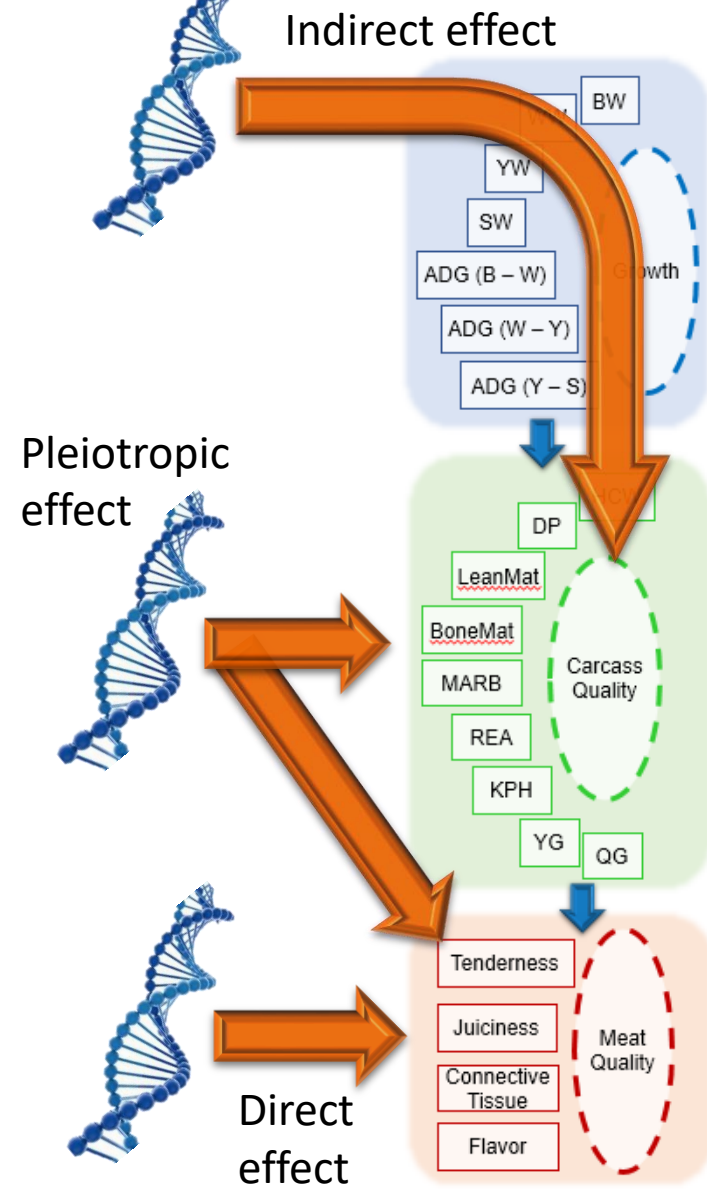
Structural equation modeling

- Derive a network of unobserved latent variables
- Latent variable:
 - Not directly measurable
 - Derived from multiple observable phenotypes



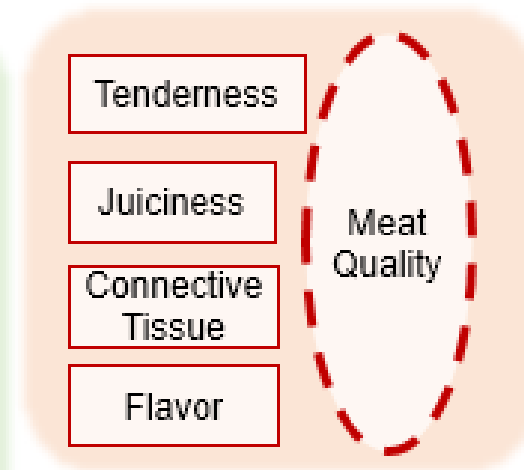
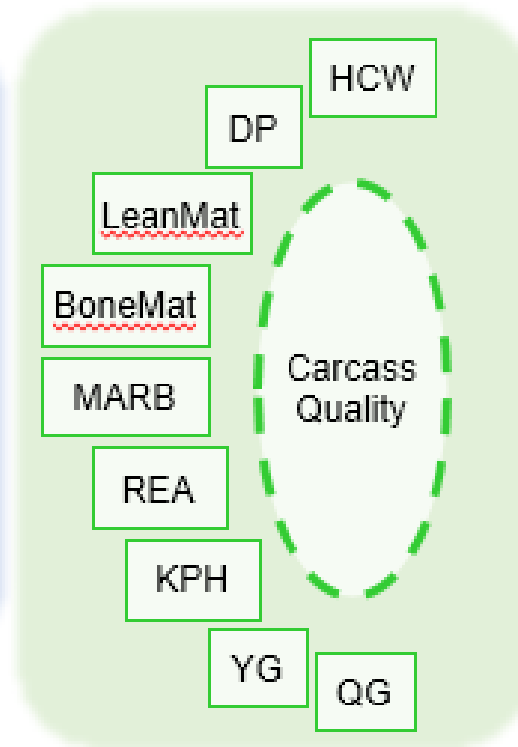
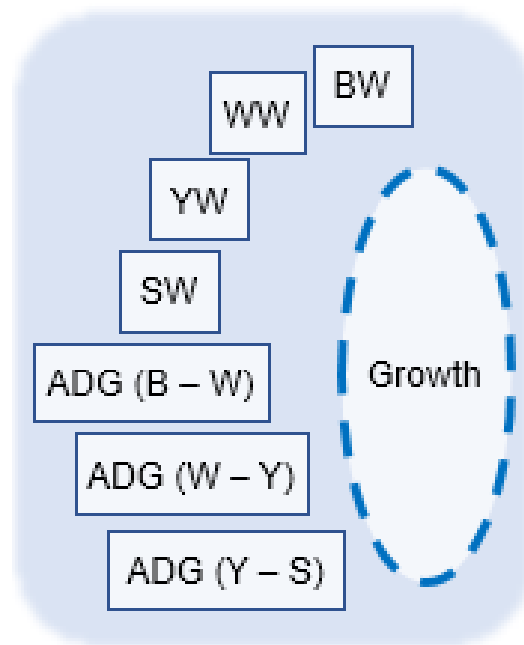
Structural Equation (SE) Modeling

- The **SE** modeling analysis + **GWAS** = powerful approach to uncover:
 - genomic regions **directly** controlling variation in latent variables
 - genomic regions **indirectly** controlling variation in latent variables
 - genomic regions with **pleiotropic** effect responsible for the observed genetic correlations



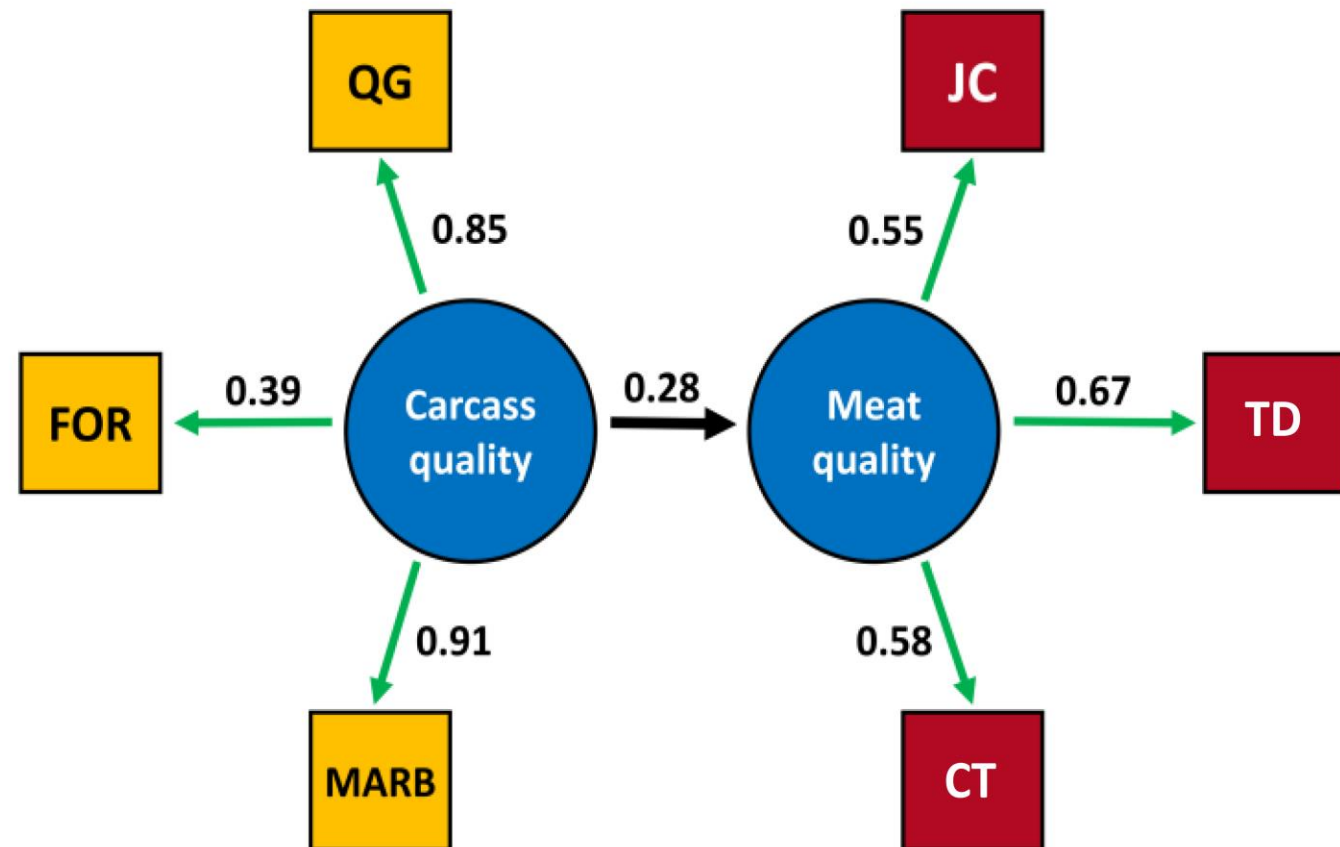
Population

- *B. Indicus* influenced: **UF** Angus x Brahman (Multibreed) Herd
 - Animals from 100%Angus to 100% Brahman (6 breed groups)
 - **726** steers (2007 – 2015) with **250K** genotypes
 - Used **22** observed phenotypes



Final model for structural equation

- The final structural model: **carcass** quality (**independent** latent variable) and **meat** quality (**dependent** latent variable)
- **Carcass quality** was measured by FOR, marbling and QG
- **Meat quality** was measured by connective tissue, juiciness and tenderness
- **Growth** did not fit the model



A decorative border consisting of a repeating DNA double helix pattern in red and blue, framing the central content.

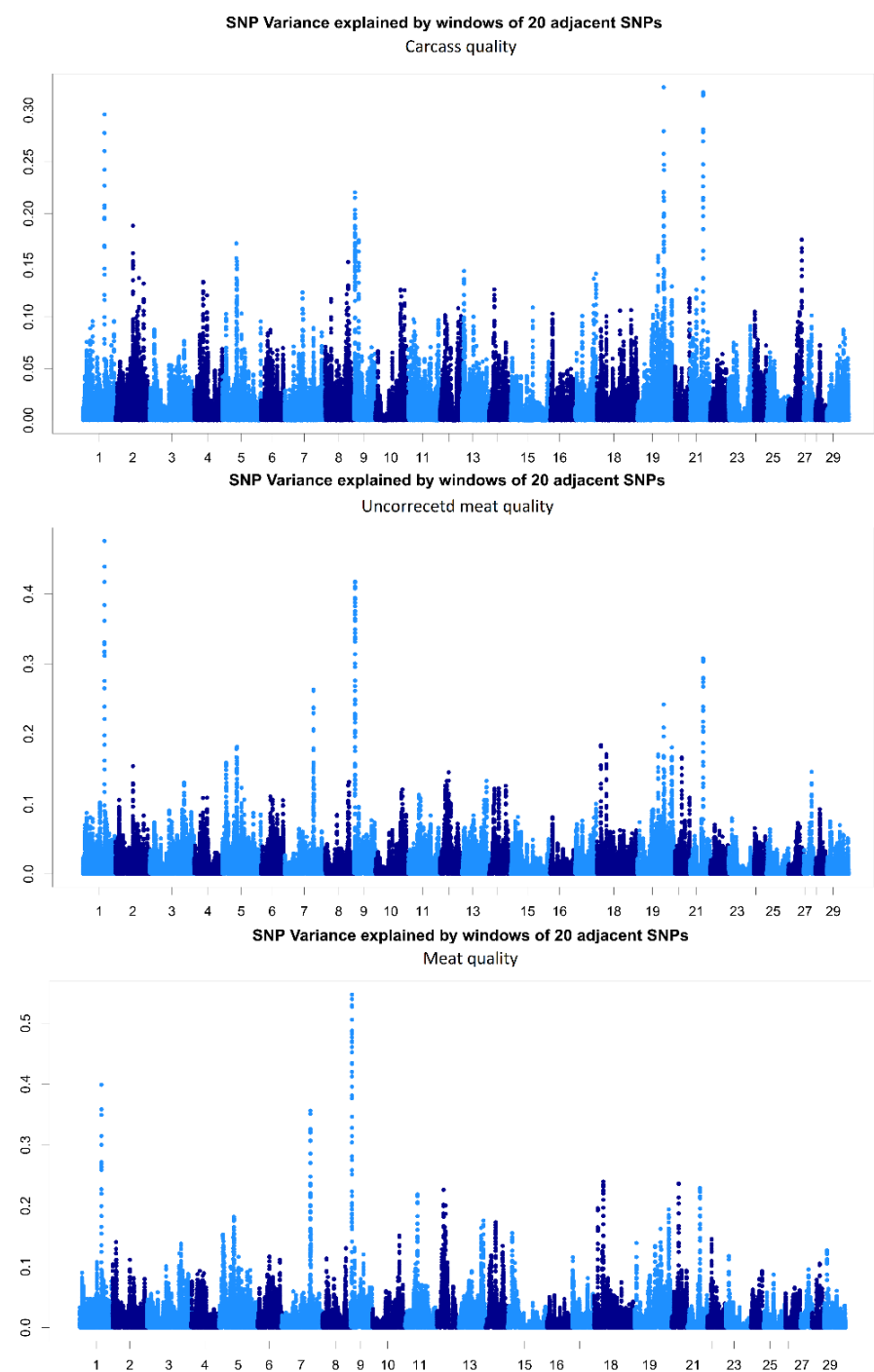
DNA Level

GWAS

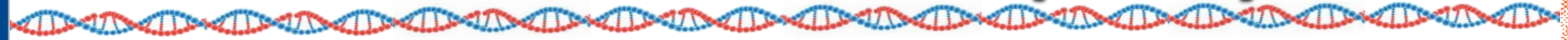


- **571** associated genomic regions (643 genes)
 - **Carcass quality**: 159 regions / 179 genes)
 - **Meat quality**: 242 regions / 266 genes
 - **Carcass and meat** quality: 106 regions / 114 genes
 - **Indirect** effect on **meat** quality: 64 regions / 84 genes

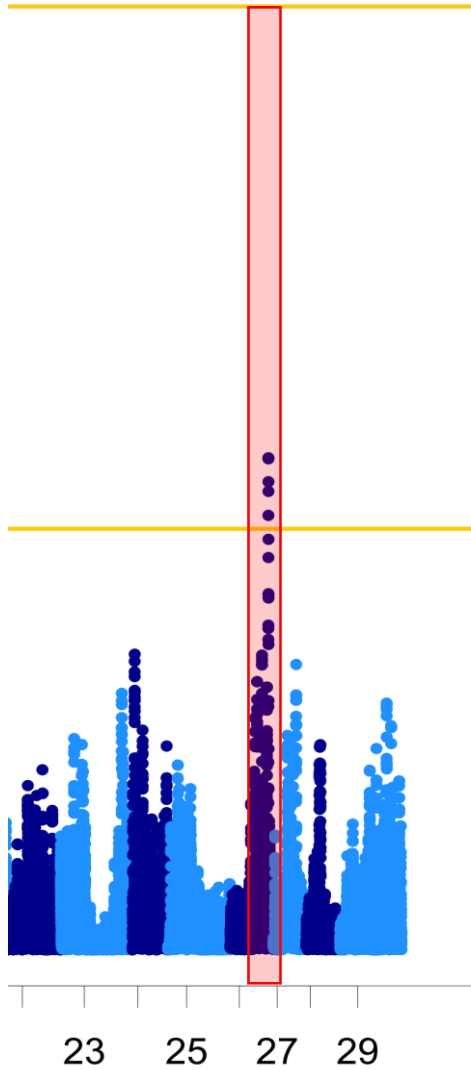
ssGBLUP (BLUPf90), MAF>0.05, SNP calling rate > 0.9, 112,267 SNPs, window size = 20 SNPs



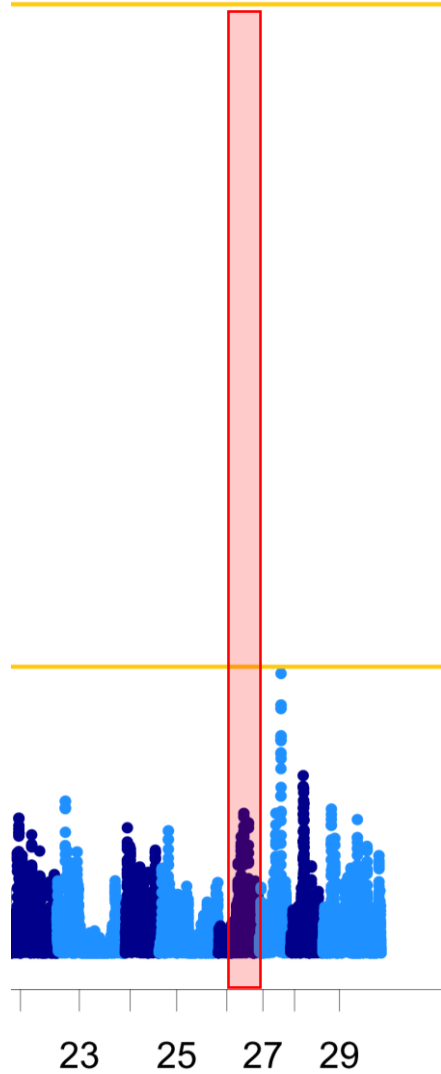
Direct effects on carcass quality



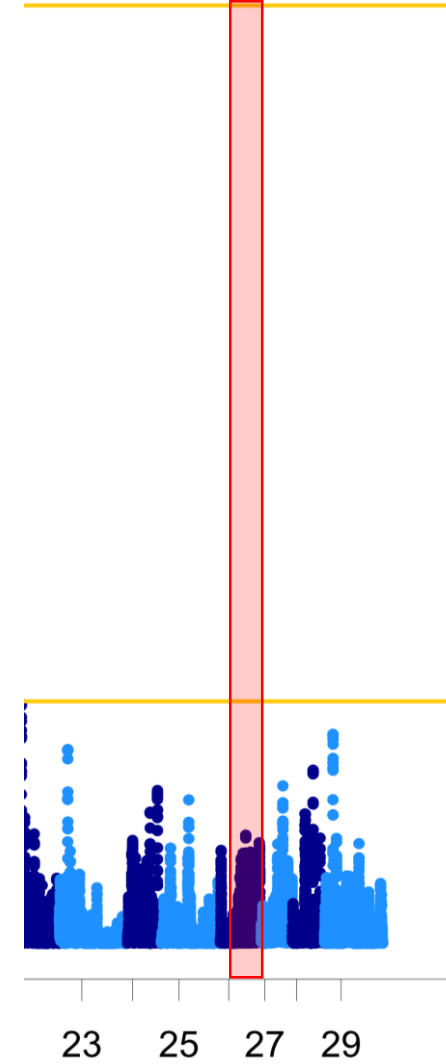
Carcass quality



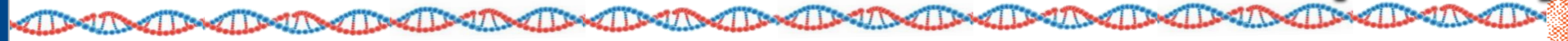
Meat quality



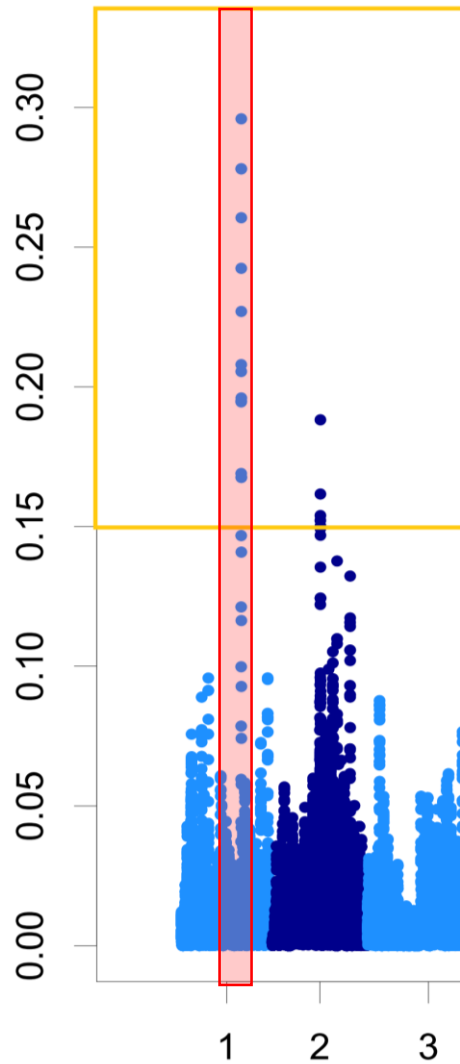
Corrected meat quality



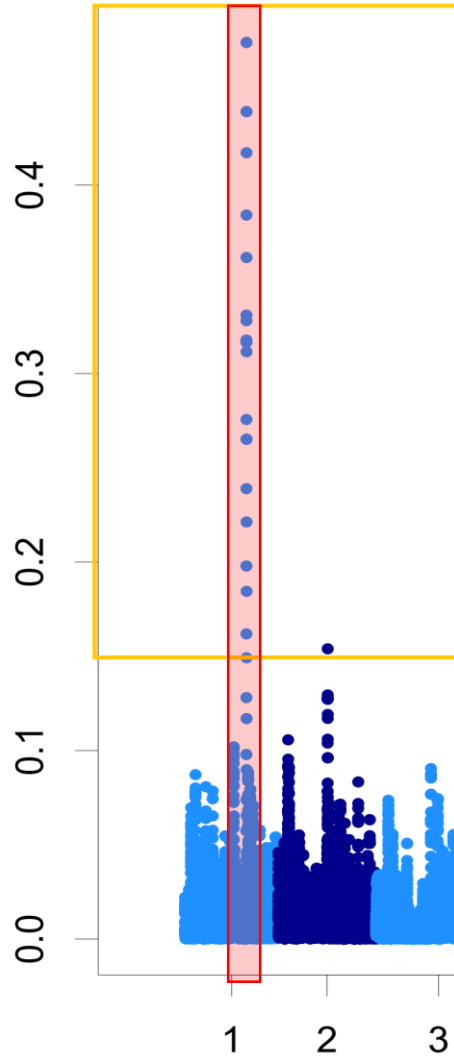
Direct effects on carcass & meat quality



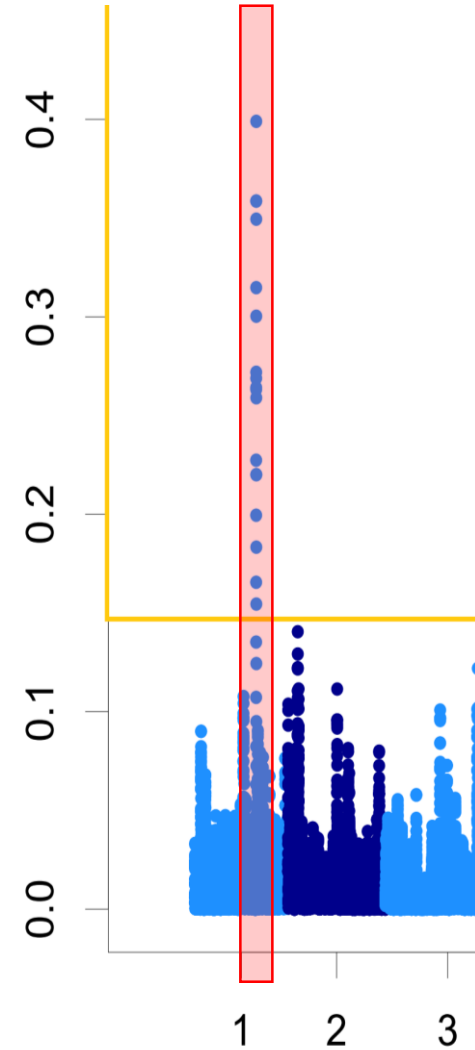
Carcass quality



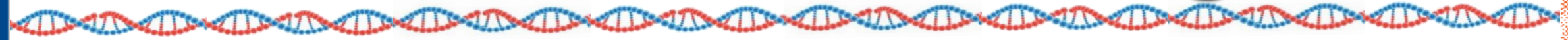
Meat quality



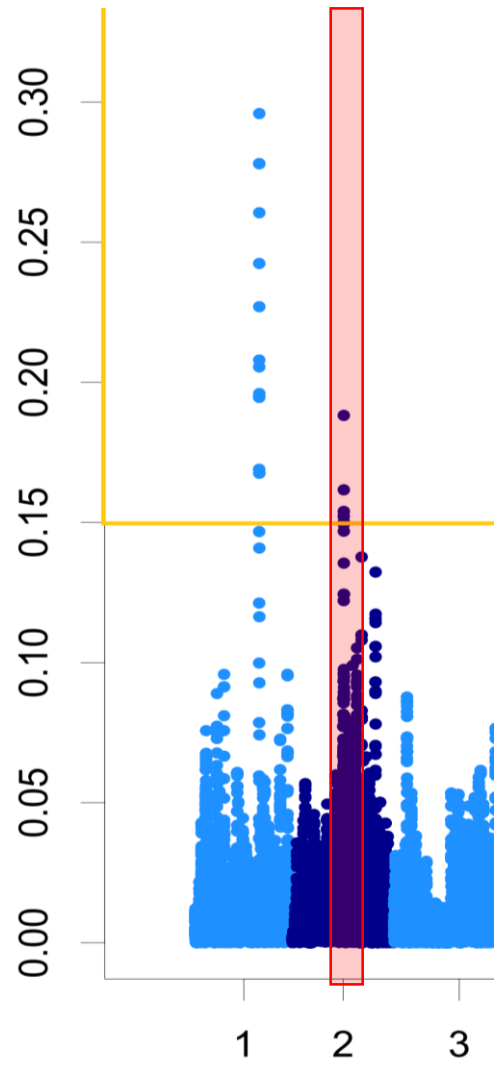
Corrected meat quality



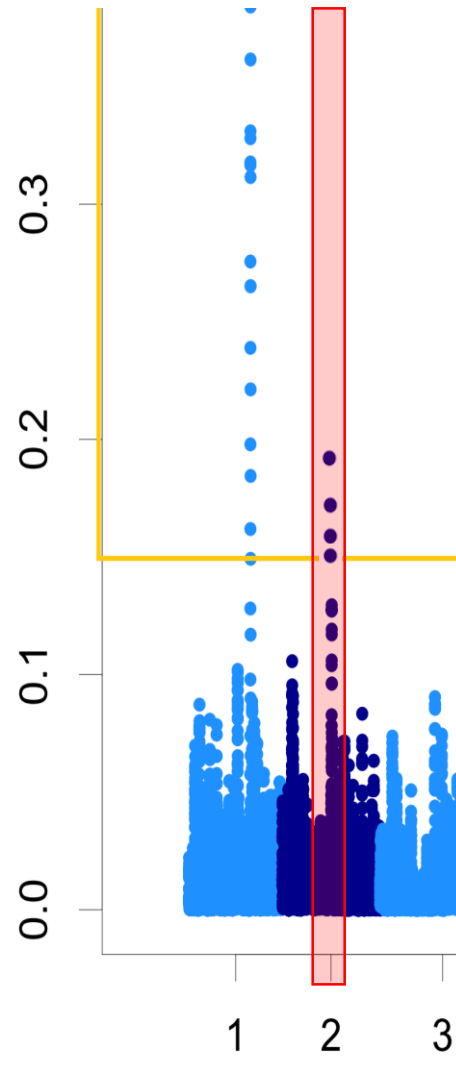
Indirect effects on meat through carcass



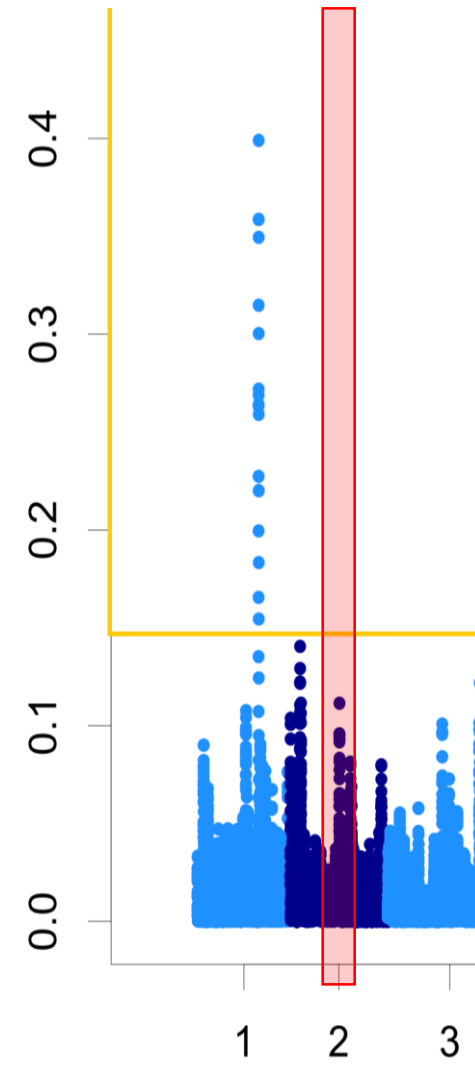
Carcass quality



Meat quality



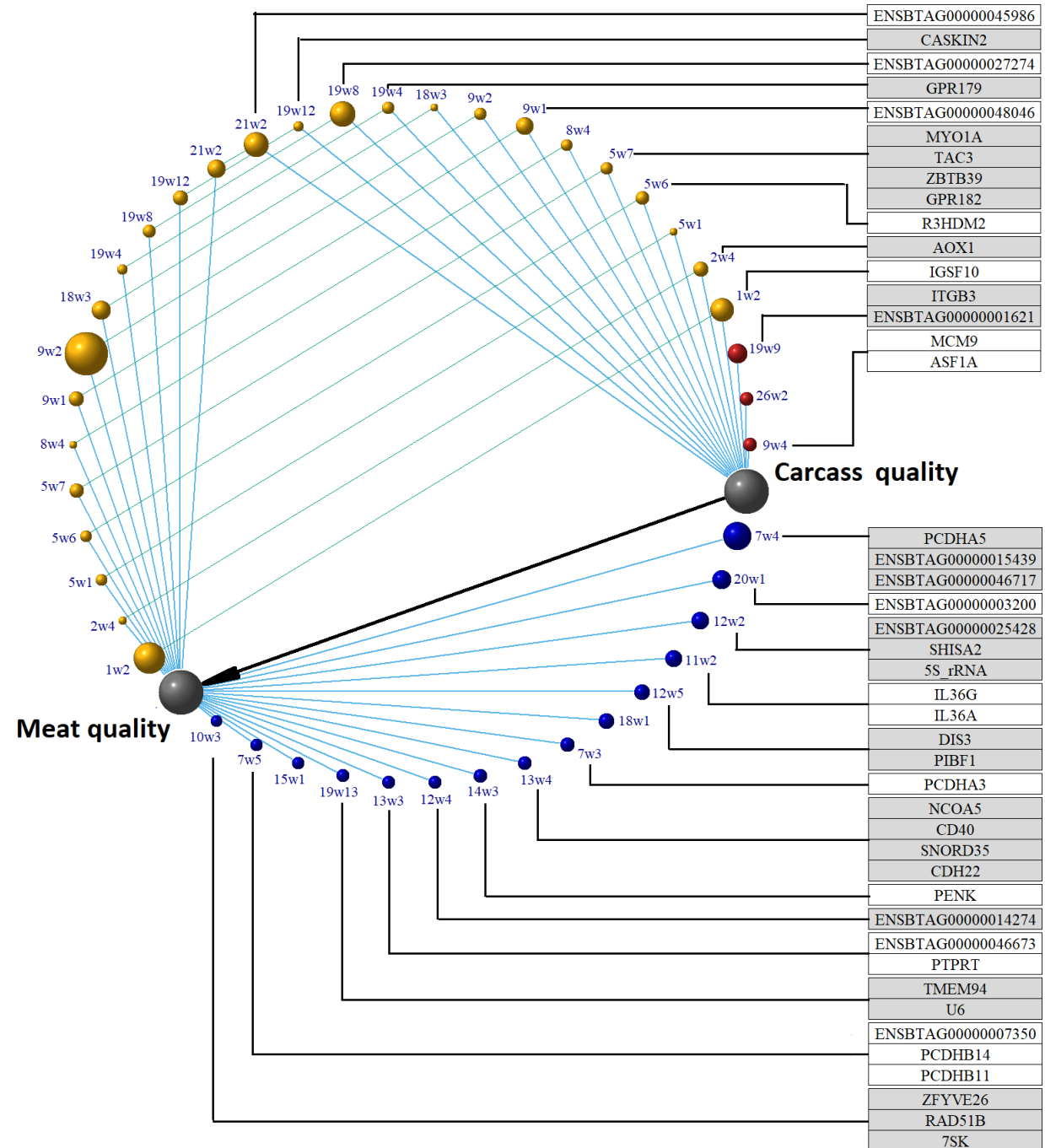
Corrected meat quality



Results SEM



- 31 regions (44 genes) – explaining $> .15 \sigma_A^2$
- Functional Annotation Clustering Analysis
- 3 mechanisms:
 - **Postmortem proteolysis** of structural transmembrane proteins.
 - **Intramuscular fat** composition and deposition.
 - **Cell** differentiation and proliferation.



A decorative border of a DNA double helix structure, with red and blue strands, surrounds the central text. The background is a dark blue rectangle with a white, torn-paper-like border.

RNA Level

Gene expression - eQTL

• Meat quality index:

- PCA on marbling, WBSF, cooking loss, juiciness, tenderness, connective tissue
- Used PC1, PC2 and PC3 to calculate a meat quality index
- Select **80** animals with **low** and **high** meat quality index
- Global gene expression (**RNAseq**)

Goal: Combine gene expression and genotypic information to understand the genetic architecture of meat quality.

Low index

N = 40

Avg = **1.15**

Tougher
Drier
More CT
Less marbling

High index

N = 40

Avg = **3.35**

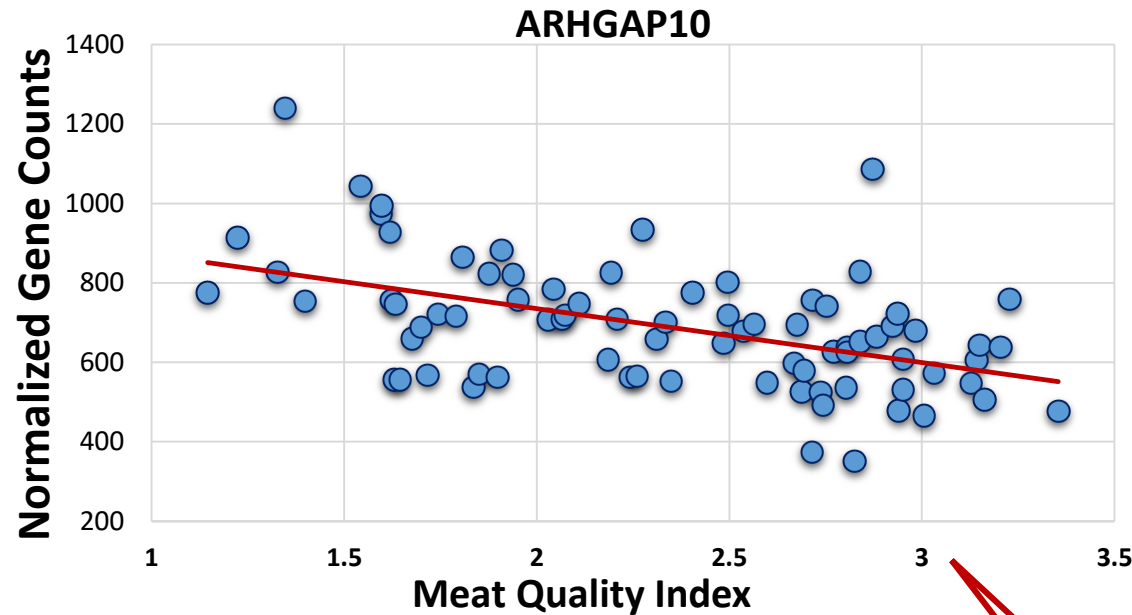
More tender
More juicy
Less CT
More marbling

- Illumina HiSeq 3000 PE100 platform
- Sequencing depth: **30X**
- Raw data: 101 bp **paired-end reads**

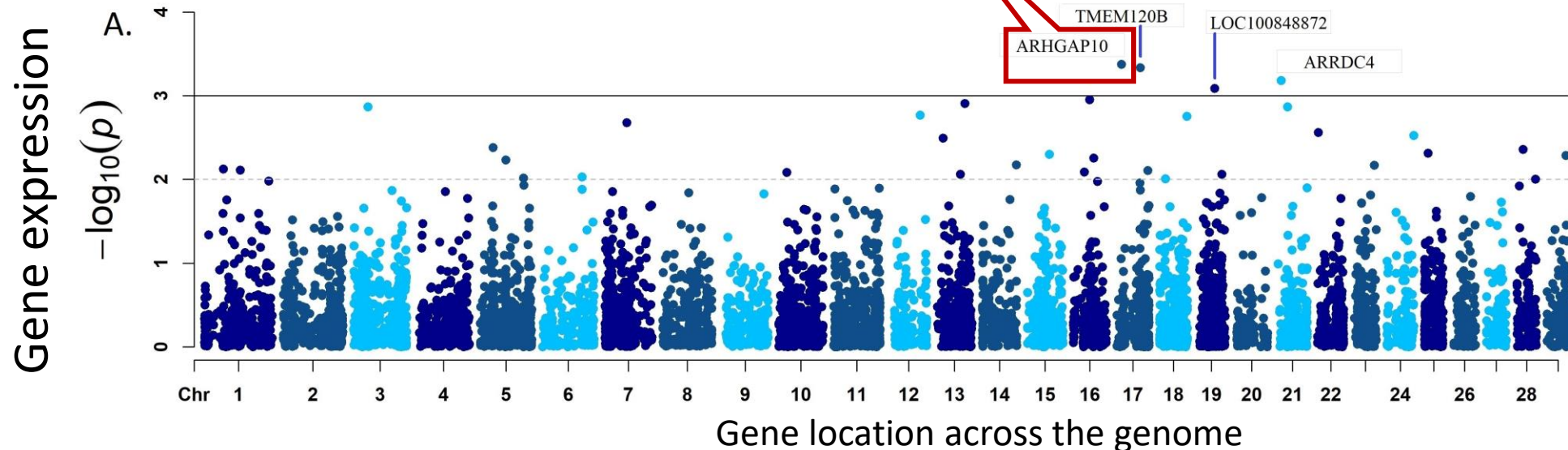
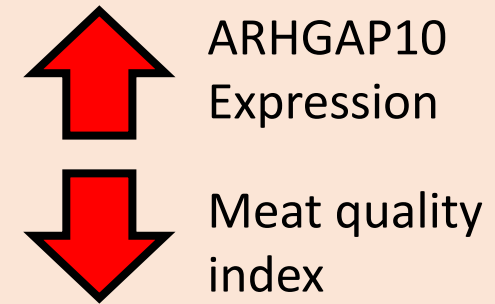
- **QC:** base + read filtering, single reads screening
- Mapping the reads: **Tophat & Bowtie**
(genome guided transcriptome assembly)
- Gene counts (**HTSeq**) and Exon Counts (**DEXSeq**)

Gene Expression Association Analysis

Associated genes = 208
P-value ≤ 0.05
Tested genes = 8,799

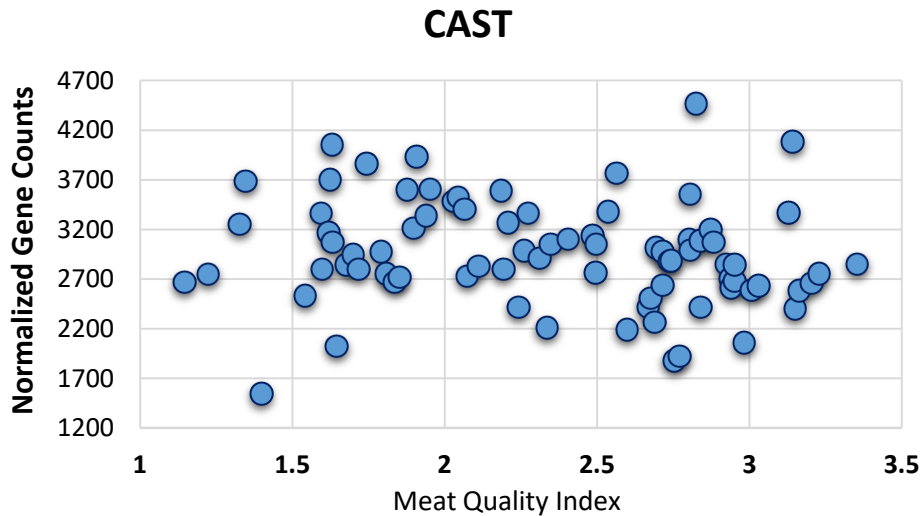


ARHGAP10 - regulates actin cytoskeleton remodeling.
↑ expression, more stable actin cytoskeleton structure.



Map genomic regions for gene expression

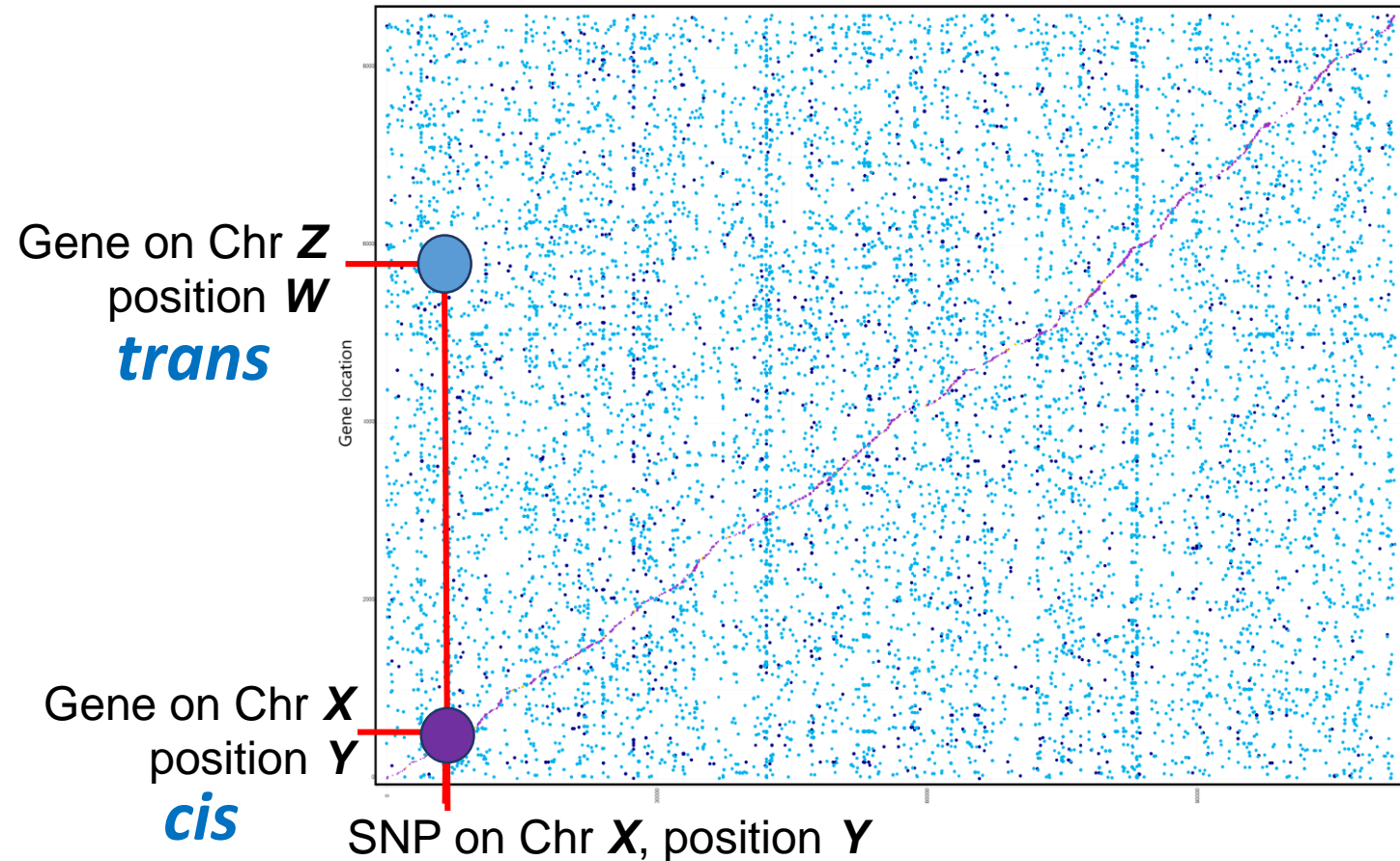
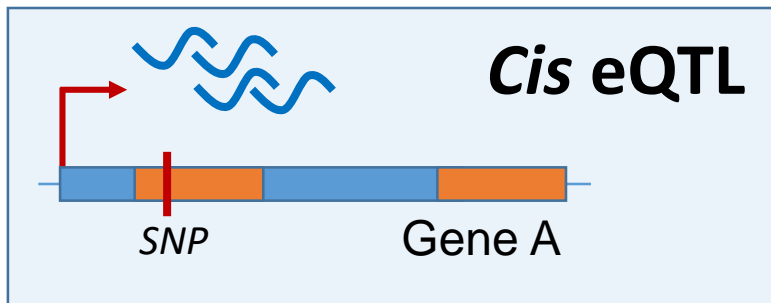
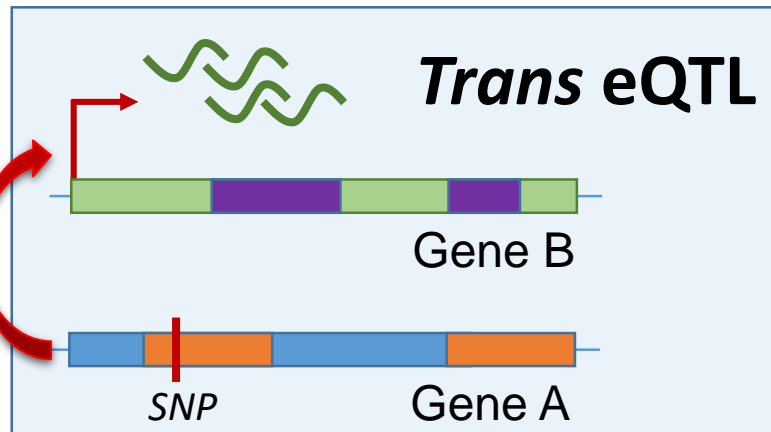
- Use gene expression as a trait, for each gene run a GWAS (250K)
 - 8,588 autosomal genes expressed in skeletal muscle
 - 8,377 identified eQTL (12% *cis* and 88 % *trans*)



- *Matrix eQTL*
- 112,042 *SNPs* and 8,588 *genes*
- Gene *counts* = *response*
- *SNP genotype* and *year* of birth = *fixed* effects
- *PC1* = *covariate* (*pop structure*)

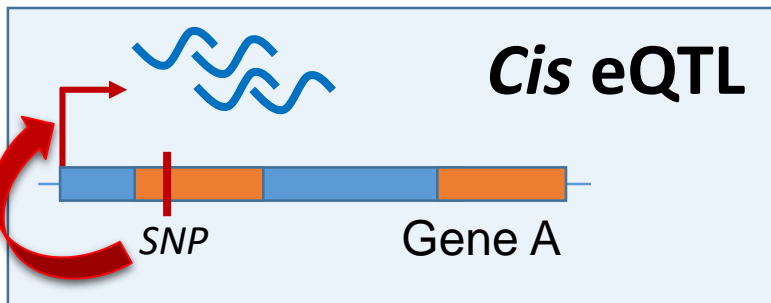
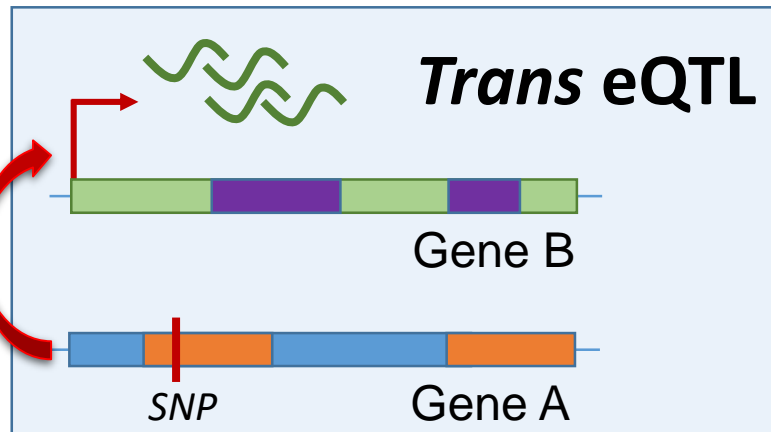
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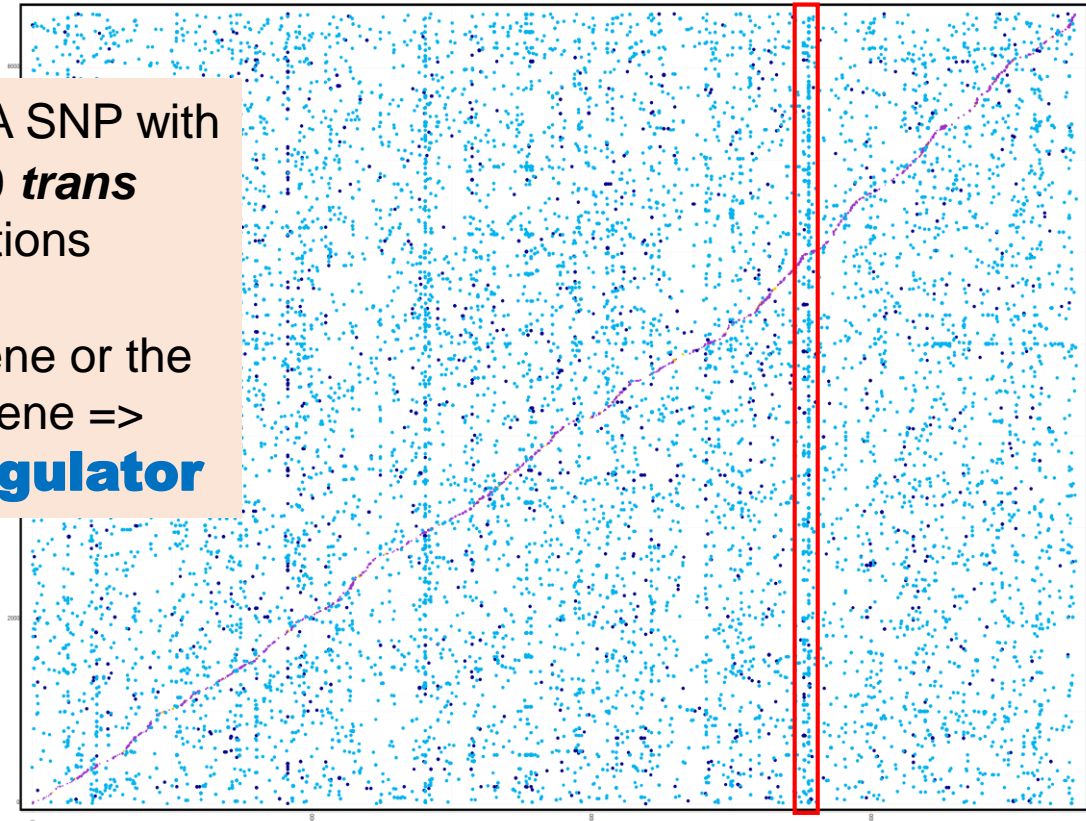


Hot spot: A SNP with at least 20 *trans* associations

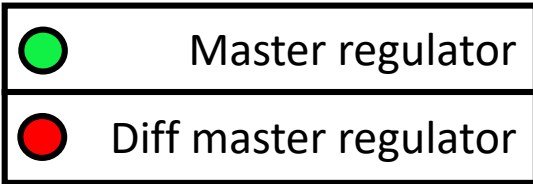
Harboring gene or the adjacent gene =>

Master regulator

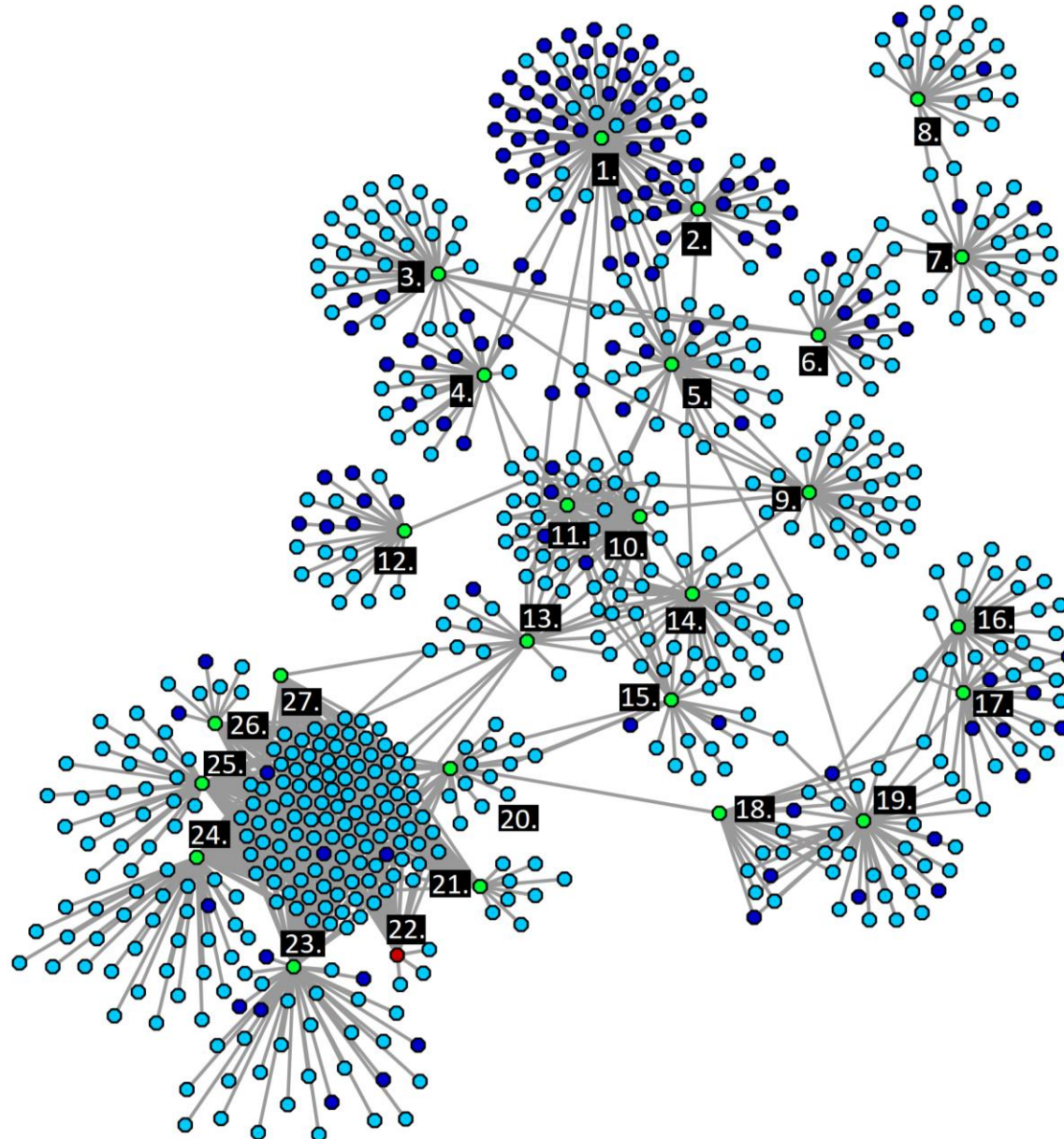
Master regulator



Network of expression master regulators

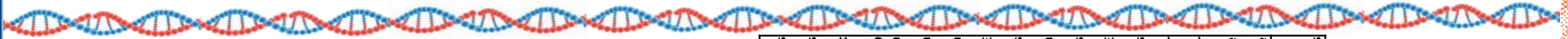


- **27** expression **master regulators**
- Associated with **674** regulated **genes**
- Classified as membrane associated or cytoskeletal proteins, transcription factors and DNA methylases.



1	NTF3
2	PDE8B
3	GAD1
4	ZNF445
5	FAT4
6	PCGF5
7	ALAD
8	ZNF804A
9	ENSBTAG00000035487
10	PAX8
11	PSD4
12	OR4S1
13	RUNX1T1
14	TTC25
15	ENSBTAG00000011638
16	CSAD
17	KRT7
18	MYH14
19	KLK4
20	KCNU1
21	TOR1AIP1
22	KDM4A
23	OR13F1
24	ENSBTAG00000000336
25	TM4SF1
26	SIPA1L2
27	GPR98

Genes identified through different approaches



What genes were identified simultaneously using different approaches?

Gene	SEM Analysis	GWA Analysis	GWA Enrichment	GWA DAVID classification	Gene Expression	Exon Expression	Marbling DE	WBSF DE Isoforms	Marbling DE Isoform	WBSF DE Analysis	Tenderness DE	Tenderness DE Isoforms	eQTL Master Regulator	sQTL Master Regulator	Regulated eQTL gene	Regulated sQTL Gene	# Common Analyses
NTF3		■			■								■				3
GPR98		■			■								■				3
ZNF804A													■	■			2
ALAD													■	■			2
ENSBTAG0000000336													■	■			2
ABLIM1					■	■				■					■		4
ACTN2					■		■			■	■					■	4
ANKRD12					■		■	■		■	■						4
ANKRD23					■		■	■		■	■				■		4
C4A					■	■				■	■						4
EFCAB14	■				■			■		■	■						4
IBTK					■	■				■	■				■	■	4
ITGB6					■					■	■				■	■	5
LOC100852159					■			■	■	■	■						4
NFKB2					■	■				■	■				■	■	4
PARK7			■		■					■	■						4
RTN4	■		■		■					■	■						4
SBNO2					■	■				■	■				■	■	4
STAT5A					■	■				■	■						4
TMEM13					■	■		■		■	■					■	4
TTN					■	■				■	■				■	■	4
USP7					■	■		■		■	■					■	4


Conclusions



- Different types of analyses combined with multiple layers of omics information:
 - At the **phenotypic** level – structural equation modeling
 - At the **DNA** level – GWAS on individual traits and latent variables
 - At the **RNA** level – global gene expression in samples with extreme meat quality
- Provides new insight in the regulatory **network architecture** in LD muscle
- Positional identification of gene expression **master regulators**

Goal: Develop genomic tools to select for superior meat quality in *Bos Indicus* influenced populations.

What's next?

- 
- Gene Network of master regulators:
 - How they affect meat quality
 - How can they be manipulated
 - Re-analyze the GWAS data for parent(breed)-of-origin effects (*Joel Leal Gutierrez*)
 - Large Angus dataset (~2,200) with whole-genome sequence data (*Fernanda Rezende*)
 - Combine and compare our multibreed/Brahman data with Nellore data (*Julio de Carvalho Balieiro*)
 - Multibreed + Brahman whole-genome sequence

Acknowledgements

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- Florida Cattlemen's Association

