GENOMIC ANALYSES FOR PALATABILITY OF BEEF

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Outline

1 Research program overview

2 Genomic analyses for palatability

- 2.1 Genome-wide association (GWAS)
- 2.2 Genomic Prediction
- 2.3 GWAS signals → underlying biology

Research program focus

- Genetic control of complex traits
  - Major advances in animal genomics
  - Improve animal production efficiency & enhance animal products for human heath

Healthfulness Project: development of genetic tools to improve **nutritional** and **health** value of beef.

Sustainable small ruminant production through selection for **resistance to internal parasites**

Genomic tools to improve **meat quality** traits in Angus-Brahman cattle.

Genomics of **resilience** in sheep to climatic stressors

Mapping QTL for **breeding out of season** and **milk production** in sheep

"Healthfulness Project"

- **Project Goal:**
  - **Assess the natural variation in healthfulness traits**
  - **Identify genetic mechanism controlling these traits**
  - **Develop tools to select for:**
    - **More nutritious beef**
    - **Tasty beef**
    - **Improved production**

Genetic evaluation for nutrient composition of beef could result in development of **gEBV** to aid selection for:

- **favorable fatty-acid** profile
- **lower** levels of **cholesterol** and **saturated** fat
- **higher** concentrations of **minerals** and **vitamins**

Beef Healthfulness Project

- **3 Angus herds (n = 2,285): Iowa, Oklahoma, California**
- **Harvest: October 2007 – May 2008**

All animals genotyped with the Illumina Bovine **50K SNP** Bead Chip

- **Growth**
  - Birth, weaning, yearling, slaughter weights
- **Carcass**
  - Hot carcass weight, dressing %, ribeye area, back fat thickness, yield grade, quality grade, KPH
- **Meat Quality**
  - WBSF, Sensory panels (Juiciness, Tenderness, Connective tissue & Flavor: beef, painty/fishy, livery/metallic), TBARS
- **Nutritional value and Healthfulness**
  - Fatty acids, main FA groups (SFA, MUFA, PUFA, n3, n6), AI – Triacylglycerol, Phospholipid, Composite
  - Cholesterol, sphingolipids, creatine, creatinine, carnitine, carnosine, anserine
  - Minerals: iron, sodium, magnesium, manganese, zinc, phosphorus, potassium, calcium, copper
  - Vitamins: E, B6

5 generation pedigree (n = 5,907)

What do consumers want?

- **The National Beef Quality Audit**
  - Seedstock Producers, Cow-Calf Producers, Stockers/ Backgrounders, Feedlot Operators, Packers, Purveyors, Retailers, & Restauranteurs
  - "**Eating satisfaction**" - only quality category for which the packers, food service buyers, and retailers are willing to pay a premium
- **NBQA Strategy Workshop**
  - Discuss the implications of research for the U.S. beef industry, provides the beef industry with a blueprint for the next five years
  - Top 10 Quality Challenges:
    1. Low overall uniformity & consistency of cattle, carcasses, cuts
    2. Inappropriate carcass size & weight
    3. Inadequate tenderness of beef
    4. Insufficient marbling

National Beef Quality Audit

Quality Challenges

Ranked according to priority, 1991 to 2011

NATIONAL BEEF
2011 NBQA
EXECUTIVE SUMMARY
QUALITY AUDIT

1991	1995	2000	2005	2011
External Fat	Overall Uniformity	Overall Uniformity	Traceability	Food Safety
Seam Fat	Overall Palatability	Carcass Weights	Overall Uniformity	**Eating Satisfaction**
Overall Palatability	Marbling	**Tenderness**	Instrument grading	How and where cattle were raised
Tenderness	**Tenderness**	Marbling	Market Signals	Weight and Size
Overall Cutability	External and Seam Fat	Reduced quality due to implants	Segmentation	Lean, Fat and Bone
Marbling	Cut weights	External Fat	Carcass Weights	Cattle Genetics

National Beef Quality Audit – 2011

Specific recommendations to reduce barriers and improve beef profitability

- Increase funding to improve **eating satisfaction**
- Use genetics to optimize cutability and **palatability**
- **Fit Cattle with Customer Expectations**
  - Assist producers with use of selection and management techniques to produce cattle that fit customer expectations and other value-determining attributes

Selection / Prediction challenges

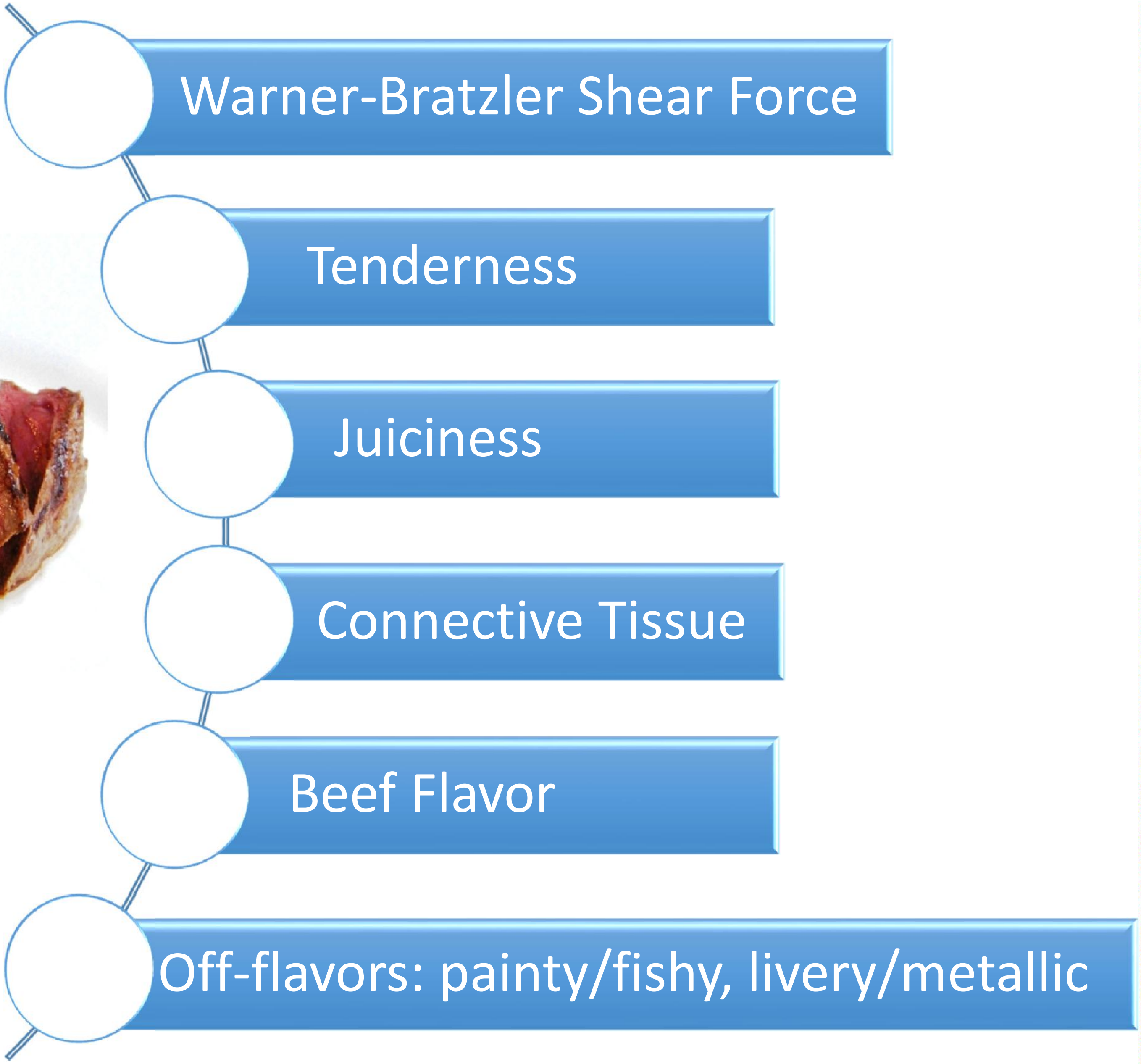
- Trait measured after slaughter, expensive to measure routinely, low consistency
- Currently USDA grading system (marbling and maturity) is used to predict palatability of beef
  - Changes in consumer preference
  - Limitation in the ability to predict eating quality
  - Limited consumer understanding of the system

Higher quality grade = more tender and palatable meat

- Programs to **improve** eating quality
- Ability to better **predict** the eating quality level for market purposes

Increase consumer's confidence that quality expectations are met

Palatability – eating satisfaction

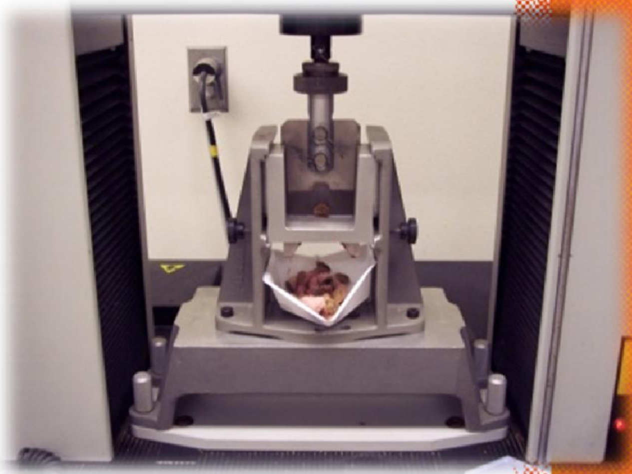


- Warner-Bratzler Shear Force
- Tenderness
- Juiciness
- Connective Tissue
- Beef Flavor
- Off-flavors: painty/fishy, livery/metallic

Sample Collection and Preparation

- Rib sections:
  - Transported to ISU Meat Laboratory, or shipped from California to OSU
  - Fabricated into steaks
  - Frozen at 14d postmortem

Warner-Bratzler Shear Force (WBSF)



- Broiled on impingement oven at 200°C to internal temperature of 68°C
- Cooled at 4°C for 18-24 h
- **Six** 1.27 cm cores removed and sheared
- Average peak load (**kg**) was analyzed

Sensory Panels

- Cooked similarly to steaks for WBS
- Sessions conducted once or twice per day
- 12 samples served randomly to panelists
- Served to eight member trained panel

Average score of all panelists

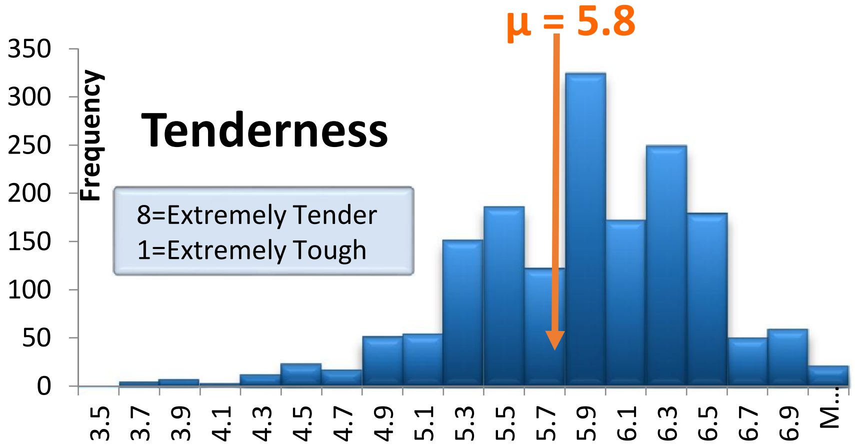
Each steak

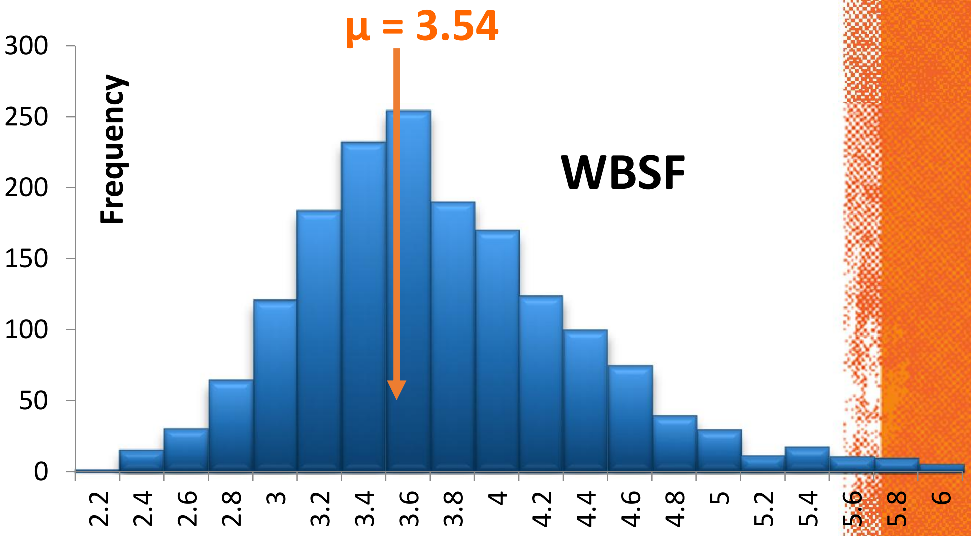
8 trained panelists

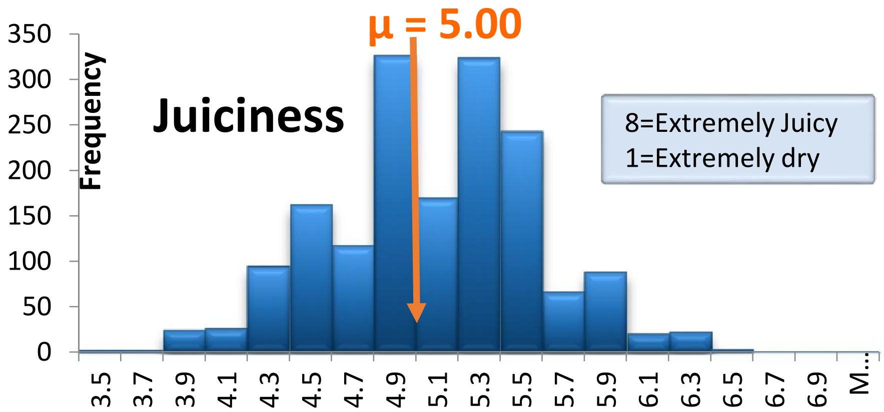
Sensory Panel Evaluation

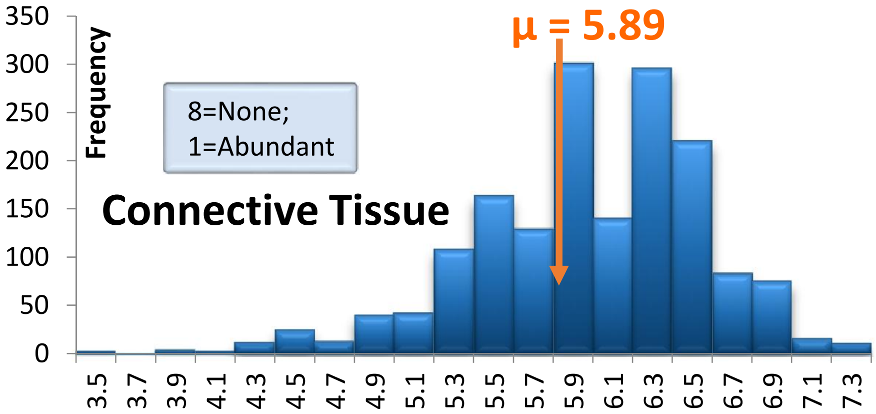
- Evaluated juiciness, tenderness, flavor
  - **Juiciness** – 8 point scale (1 = extremely dry and 8 = extremely juicy)
  - **Tenderness** – 8 point scale (1 = extremely tough and 8 = extremely tender)
  - **Connective Tissue** – 8 point scale (1 = abundant and 8 = none)
  - **Beef**, Painty/Fishy, and Livery/Metallic Flavors – 3 point scale (1 = not detectable, 2 = slightly detectable, and 3 = strong)

Palatability statistics









Trait	N	Mean ± SD
WBSF	2,076	3.54 ± 0.77
Tenderness	1,591	5.80 ± 0.59
Juiciness	1,591	5.00 ± 0.49
Connective Tissue	1,591	5.89 ± 0.59
Beef Flavor	1,591	2.50 ± 0.23
Painty	1,591	1.13 ± 0.17
Livery	1,591	1.10 ± 0.12

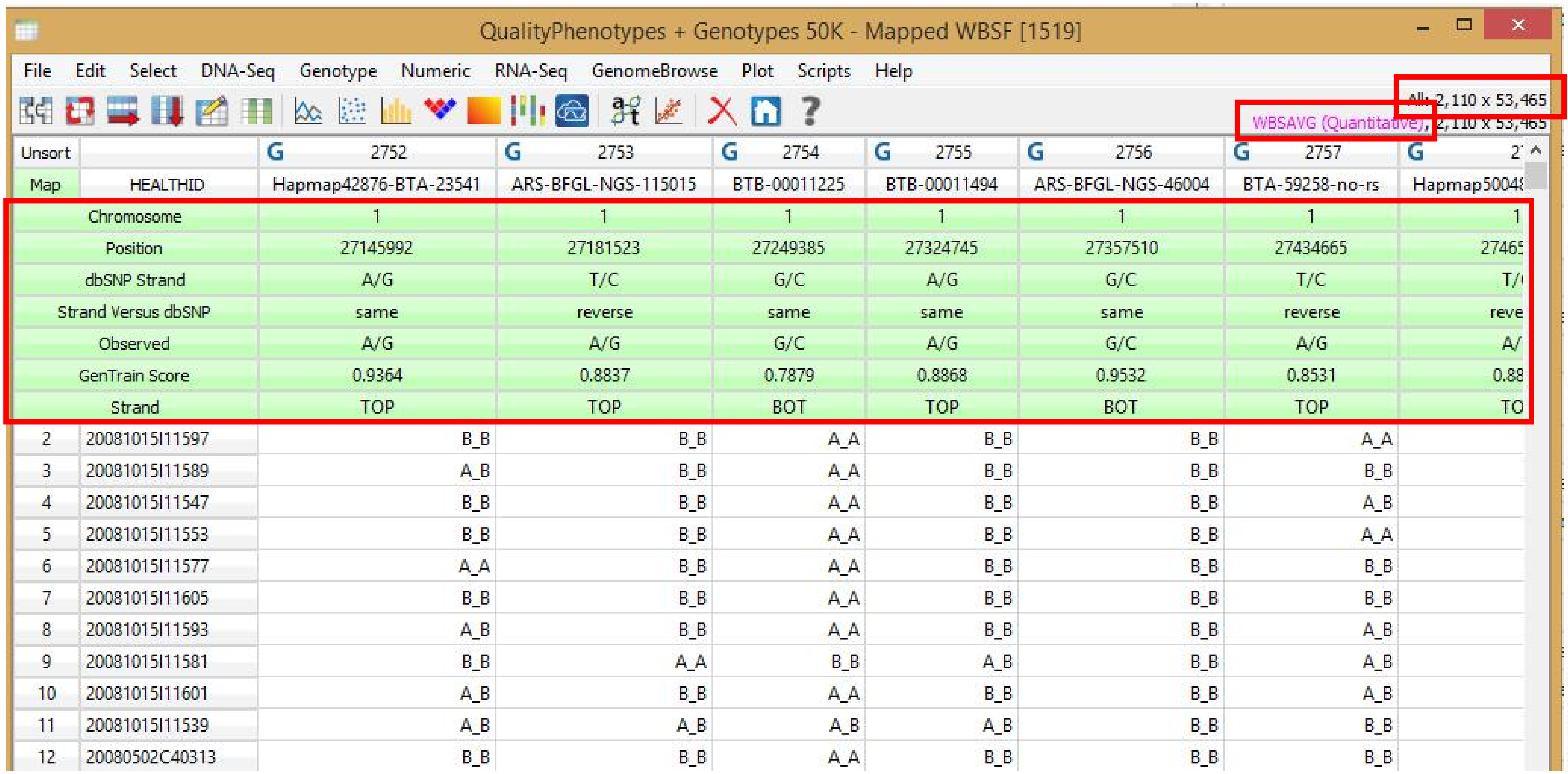
Palatability of Beef - goals

1. Genome-wide association (GWAS)
2. Genomic Prediction
3. GWAS signals → underlying biology

Genome-wide association

- **Identify SNPs and chromosomal regions associated with palatability traits**
  - **WBSF**
  - **Tenderness (sensory panel)**
  - **Juiciness (sensory panel)**
  - **Connective tissue (sensory panel)**
  - **Flavor (sensory panel)**
- **SVS (SNP & Variation Suite) v8.3.4 (Golden Helix)**
  - Mixed Model GWAS using single locus models (**EMMAX**) and multi-locus models (**MLMM**)

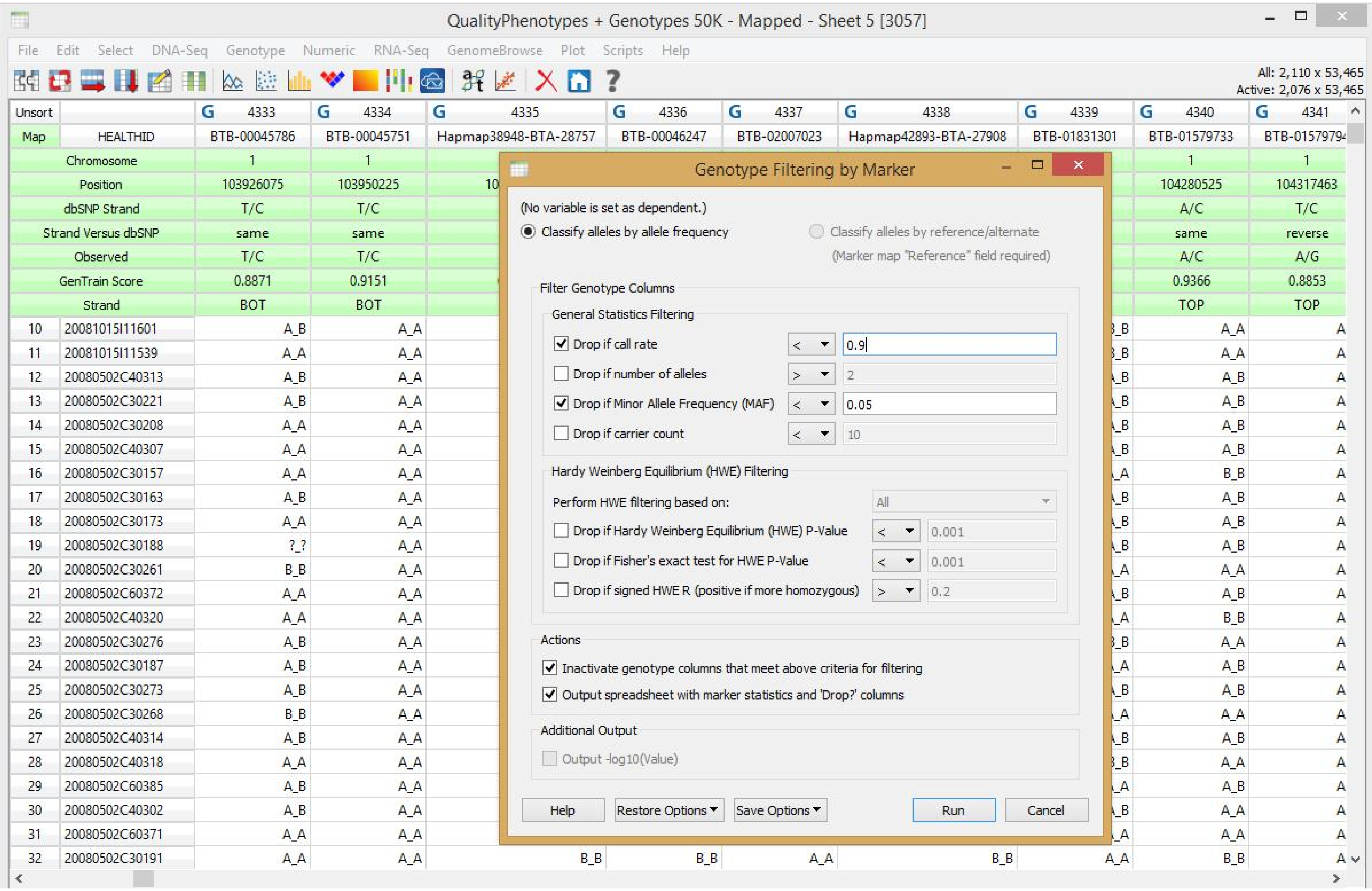
GWAS in SVS – initial steps



All: 2,110 x 53,465
WBSAVG (Quantitative), 2,110 x 53,465

Unsort		G 2752	G 2753	G 2754	G 2755	G 2756	G 2757	G 2
Map	HEALTHID	Hapmap42876-BTA-23541	ARS-BFGL-NGS-115015	BTB-00011225	BTB-00011494	ARS-BFGL-NGS-46004	BTA-59258-no-rs	Hapmap5004
Chromosome		1	1	1	1	1	1	1
Position		27145992	27181523	27249385	27324745	27357510	27434665	27465
dbSNP Strand		A/G	T/C	G/C	A/G	G/C	T/C	T/
Strand Versus dbSNP		same	reverse	same	same	same	reverse	reve
Observed		A/G	A/G	G/C	A/G	G/C	A/G	A/
GenTrain Score		0.9364	0.8837	0.7879	0.8868	0.9532	0.8531	0.88
Strand		TOP	TOP	BOT	TOP	BOT	TOP	TO
2	20081015I11597	B_B	B_B	A_A	B_B	B_B	A_A	
3	20081015I11589	A_B	B_B	A_A	B_B	B_B	B_B	
4	20081015I11547	B_B	B_B	A_A	B_B	B_B	A_B	
5	20081015I11553	B_B	B_B	A_A	B_B	B_B	A_A	
6	20081015I11577	A_A	B_B	A_A	B_B	B_B	B_B	
7	20081015I11605	B_B	B_B	A_A	B_B	B_B	B_B	
8	20081015I11593	A_B	B_B	A_A	B_B	B_B	A_B	
9	20081015I11581	B_B	A_A	B_B	A_B	B_B	A_B	
10	20081015I11601	A_B	B_B	A_A	B_B	B_B	A_B	
11	20081015I11539	A_B	A_B	A_B	A_B	B_B	B_B	
12	20080502C40313	B_B	B_B	A_A	B_B	B_B	B_B	

GWAS in SVS – genotype filtering



All: 2,110 x 53,465
Active: 2,076 x 53,465

Unsort		4333	4334	4340
Map	HEALTHID	BTB-00045786	BTB-00045751	BTB-01579733
Chromosome		1	1	1
Position		103926075	103950225	104280525
dbSNP Strand		T/C	T/C	A/C
Strand Versus dbSNP		same	same	same
Observed		T/C	T/C	A/C
GenTrain Score		0.8871	0.9151	0.9366
Strand		BOT	BOT	TOP
10	20081015I11601	A_B	A_A	A_A
11	20081015I11539	A_A	A_A	A_A
12	20080502C40313	A_B	A_A	A_B
13	20080502C30221	A_B	A_A	A_B
14	20080502C30208	A_A	A_A	A_B
15	20080502C40307	A_A	A_A	A_B
16	20080502C30157	A_A	A_A	B_B
17	20080502C30163	A_B	A_A	A_B
18	20080502C30173	A_A	A_A	A_B
19	20080502C30188	?_?	A_A	A_B
20	20080502C30261	B_B	A_A	A_A
21	20080502C60372	A_A	A_A	A_B
22	20080502C40320	A_A	A_A	B_B
23	20080502C30276	A_B	A_A	A_A
24	20080502C30187	A_B	A_A	A_B
25	20080502C30273	A_B	A_A	A_B
26	20080502C30268	B_B	A_A	A_A
27	20080502C40314	A_B	A_A	A_B
28	20080502C40318	A_A	A_A	A_A
29	20080502C60385	A_B	A_A	A_B
30	20080502C40302	A_B	A_A	A_A
31	20080502C60371	A_A	A_A	A_A
32	20080502C30191	A_A	A_A	B_B

Genotype Filtering by Marker

(No variable is set as dependent.)

● Classify alleles by allele frequency
○ Classify alleles by reference/alternate (Marker map "Reference" field required)

Filter Genotype Columns

General Statistics Filtering

- ☑ Drop if call rate < 0.9
- ☐ Drop if number of alleles > 2
- ☑ Drop if Minor Allele Frequency (MAF) < 0.05
- ☐ Drop if carrier count < 10

Hardy Weinberg Equilibrium (HWE) Filtering

Perform HWE filtering based on: All

- ☐ Drop if Hardy Weinberg Equilibrium (HWE) P-Value < 0.001
- ☐ Drop if Fisher's exact test for HWE P-Value < 0.001
- ☐ Drop if signed HWE R (positive if more homozygous) > 0.2

Actions

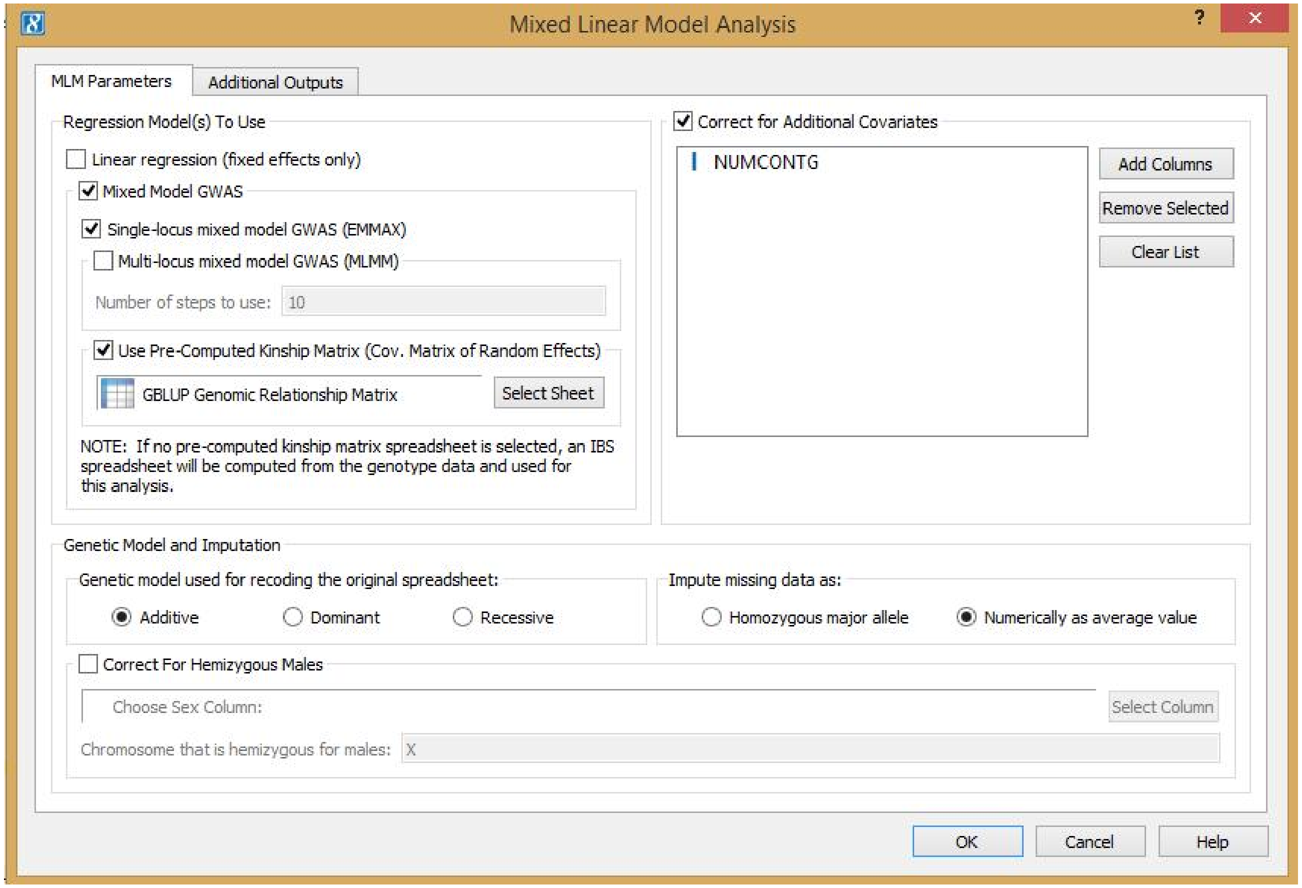
- ☑ Inactivate genotype columns that meet above criteria for filtering
- ☑ Output spreadsheet with marker statistics and 'Drop?' columns

Additional Output

- ☐ Output -log10(Value)

GWAS for WBSF - SVS

- Mixed Model GWAS using a single locus (**EMMAX**) and multi-locus models (**MLMM**)
  - Genomic relationship matrix
  - Contemporary groups



Mixed Linear Model Analysis

MLM Parameters | Additional Outputs

Regression Model(s) To Use

☐ Linear regression (fixed effects only)

☑ Mixed Model GWAS

☑ Single-locus mixed model GWAS (EMMAX)

☐ Multi-locus mixed model GWAS (MLMM)

Number of steps to use: 10

☑ Use Pre-Computed Kinship Matrix (Cov. Matrix of Random Effects)

GBLUP Genomic Relationship Matrix

NOTE: If no pre-computed kinship matrix spreadsheet is selected, an IBS spreadsheet will be computed from the genotype data and used for this analysis.

☑ Correct for Additional Covariates

NUMCONTG

Genetic Model and Imputation

Genetic model used for recoding the original spreadsheet:

● Additive ○ Dominant ○ Recessive

Impute missing data as:

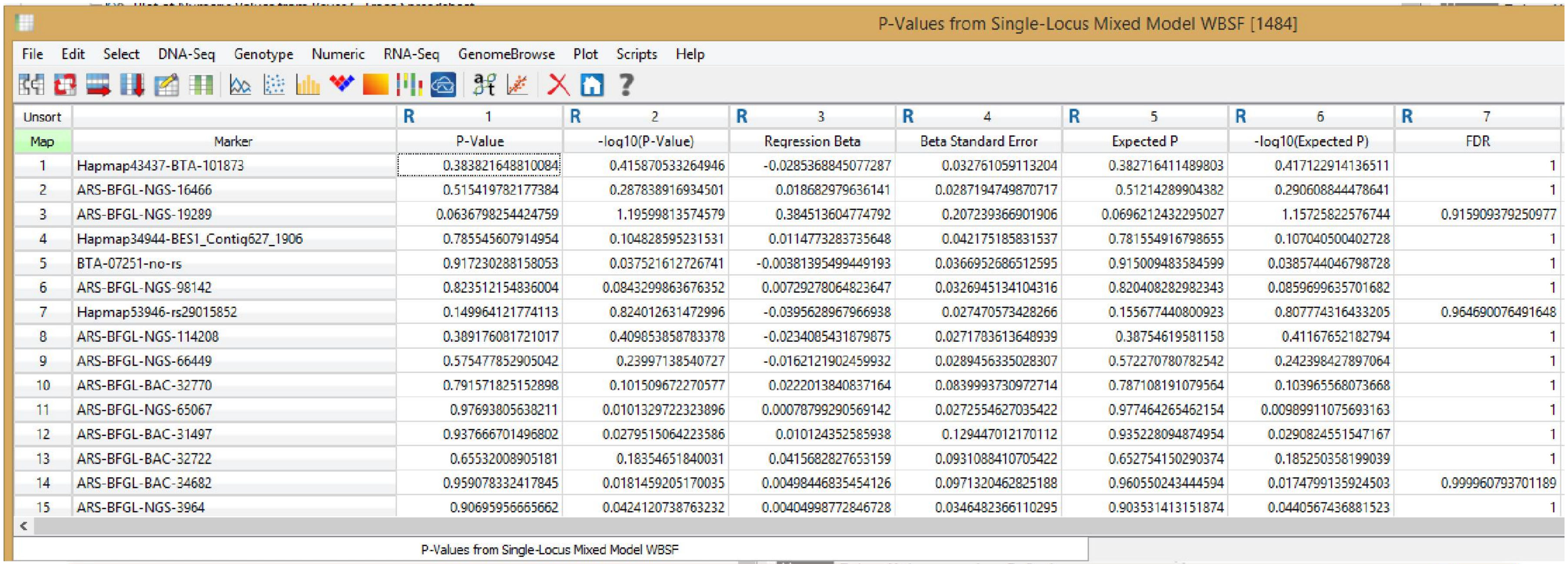
○ Homozygous major allele ● Numerically as average value

☐ Correct For Hemizygous Males

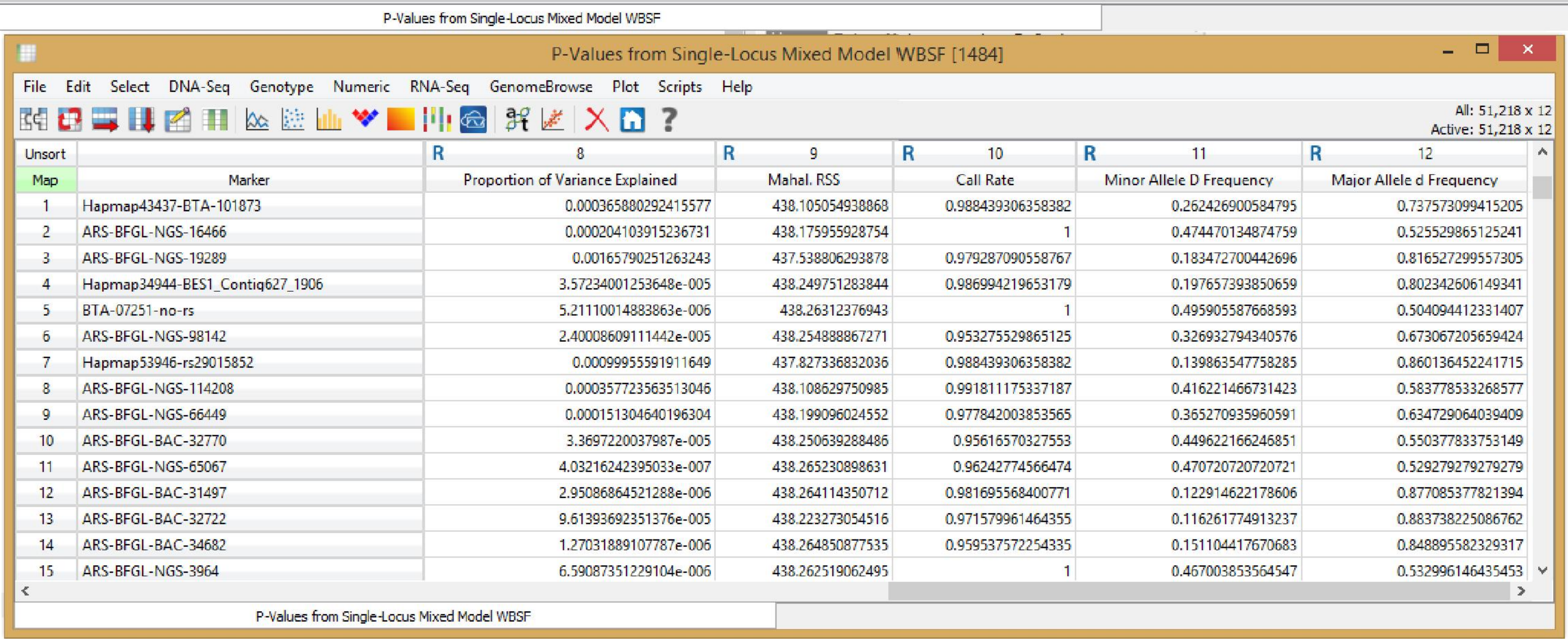
Choose Sex Column:

Chromosome that is hemizygous for males: X

GWAS for WBSF – SVS - output



Unsort		R 1	R 2	R 3	R 4	R 5	R 6	R 7
Map	Marker	P-Value	-log10(P-Value)	Regression Beta	Beta Standard Error	Expected P	-log10(Expected P)	FDR
1	Hapmap43437-BTA-101873	0.383821648810084	0.415870533264946	-0.0285368845077287	0.032761059113204	0.382716411489803	0.417122914136511	1
2	ARS-BFGL-NGS-16466	0.515419782177384	0.287838916934501	0.018682979636141	0.0287194749870717	0.51214289904382	0.290608844478641	1
3	ARS-BFGL-NGS-19289	0.0636798254424759	1.19599813574579	0.384513604774792	0.207239366901906	0.0696212432295027	1.15725822576744	0.915909379250977
4	Hapmap34944-BES1_Contig627_1906	0.785545607914954	0.104828595231531	0.0114773283735648	0.042175185831537	0.781554916798655	0.107040500402728	1
5	BTA-07251-no-rs	0.917230288158053	0.037521612726741	-0.00381395499449193	0.0366952686512595	0.915009483584599	0.0385744046798728	1
6	ARS-BFGL-NGS-98142	0.823512154836004	0.0843299863676352	0.00729278064823647	0.0326945134104316	0.820408282982343	0.0859699635701682	1
7	Hapmap53946-rs29015852	0.149964121774113	0.824012631472996	-0.0395628967966938	0.027470573428266	0.155677440800923	0.807774316433205	0.964690076491648
8	ARS-BFGL-NGS-114208	0.389176081721017	0.409853858783378	-0.0234085431879875	0.0271783613648939	0.38754619581158	0.41167652182794	1
9	ARS-BFGL-NGS-66449	0.575477852905042	0.23997138540727	-0.0162121902459932	0.0289456335028307	0.572270780782542	0.242398427897064	1
10	ARS-BFGL-BAC-32770	0.791571825152898	0.101509672270577	0.0222013840837164	0.0839993730972714	0.787108191079564	0.103965568073668	1
11	ARS-BFGL-NGS-65067	0.97693805638211	0.0101329722323896	0.00078799290569142	0.0272554627035422	0.977464265462154	0.00989911075693163	1
12	ARS-BFGL-BAC-31497	0.937666701496802	0.0279515064223586	0.010124352585938	0.129447012170112	0.935228094874954	0.0290824551547167	1
13	ARS-BFGL-BAC-32722	0.65532008905181	0.18354651840031	0.0415682827653159	0.0931088410705422	0.652754150290374	0.185250358199039	1
14	ARS-BFGL-BAC-34682	0.959078332417845	0.0181459205170035	0.00498446835454126	0.0971320462825188	0.960550243444594	0.0174799135924503	0.999960793701189
15	ARS-BFGL-NGS-3964	0.90695956665662	0.0424120738763232	0.00404998772846728	0.0346482366110295	0.903531413151874	0.0440567436881523	1

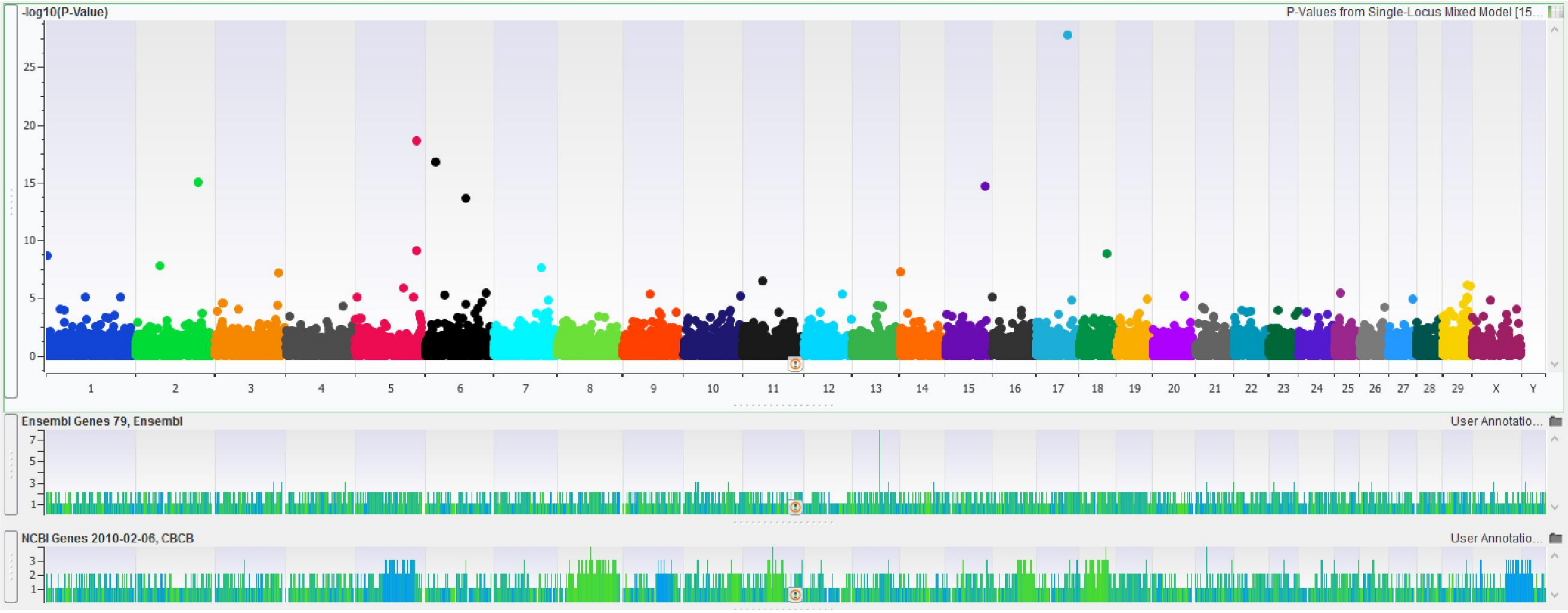


Unsort		R 8	R 9	R 10	R 11	R 12
Map	Marker	Proportion of Variance Explained	Mahal. RSS	Call Rate	Minor Allele D Frequency	Major Allele d Frequency
1	Hapmap43437-BTA-101873	0.000365880292415577	438.105054938868	0.988439306358382	0.262426900584795	0.737573099415205
2	ARS-BFGL-NGS-16466	0.000204103915236731	438.175955928754	1	0.474470134874759	0.525529865125241
3	ARS-BFGL-NGS-19289	0.00165790251263243	437.538806293878	0.979287090558767	0.183472700442696	0.816527299557305
4	Hapmap34944-BES1_Contig627_1906	3.57234001253648e-005	438.249751283844	0.986994219653179	0.197657393850659	0.802342606149341
5	BTA-07251-no-rs	5.21110014883863e-006	438.26312376943	1	0.495905587668593	0.504094412331407
6	ARS-BFGL-NGS-98142	2.40008609111442e-005	438.254888867271	0.953275529865125	0.326932794340576	0.673067205659424
7	Hapmap53946-rs29015852	0.00099955591911649	437.827336832036	0.988439306358382	0.139863547758285	0.860136452241715
8	ARS-BFGL-NGS-114208	0.000357723563513046	438.108629750985	0.991811175337187	0.416221466731423	0.583778533268577
9	ARS-BFGL-NGS-66449	0.000151304640196304	438.199096024552	0.977842003853565	0.365270935960591	0.634729064039409
10	ARS-BFGL-BAC-32770	3.3697220037987e-005	438.250639288486	0.95616570327553	0.449622166246851	0.550377833753149
11	ARS-BFGL-NGS-65067	4.03216242395033e-007	438.265230898631	0.96242774566474	0.470720720720721	0.529279279279279
12	ARS-BFGL-BAC-31497	2.95086864521288e-006	438.264114350712	0.981695568400771	0.122914622178606	0.877085377821394
13	ARS-BFGL-BAC-32722	9.61393692351376e-005	438.22327305451 6	0.971579961464355	0.116261774913237	0.883738225086762
14	ARS-BFGL-BAC-34682	1.27031889107787e-006	438.264850877535	0.959537572254335	0.151104417670683	0.848895582329317
15	ARS-BFGL-NGS-3964	6.59087351229104e-006	438.262519062495	1	0.467003853564547	0.532996146435453

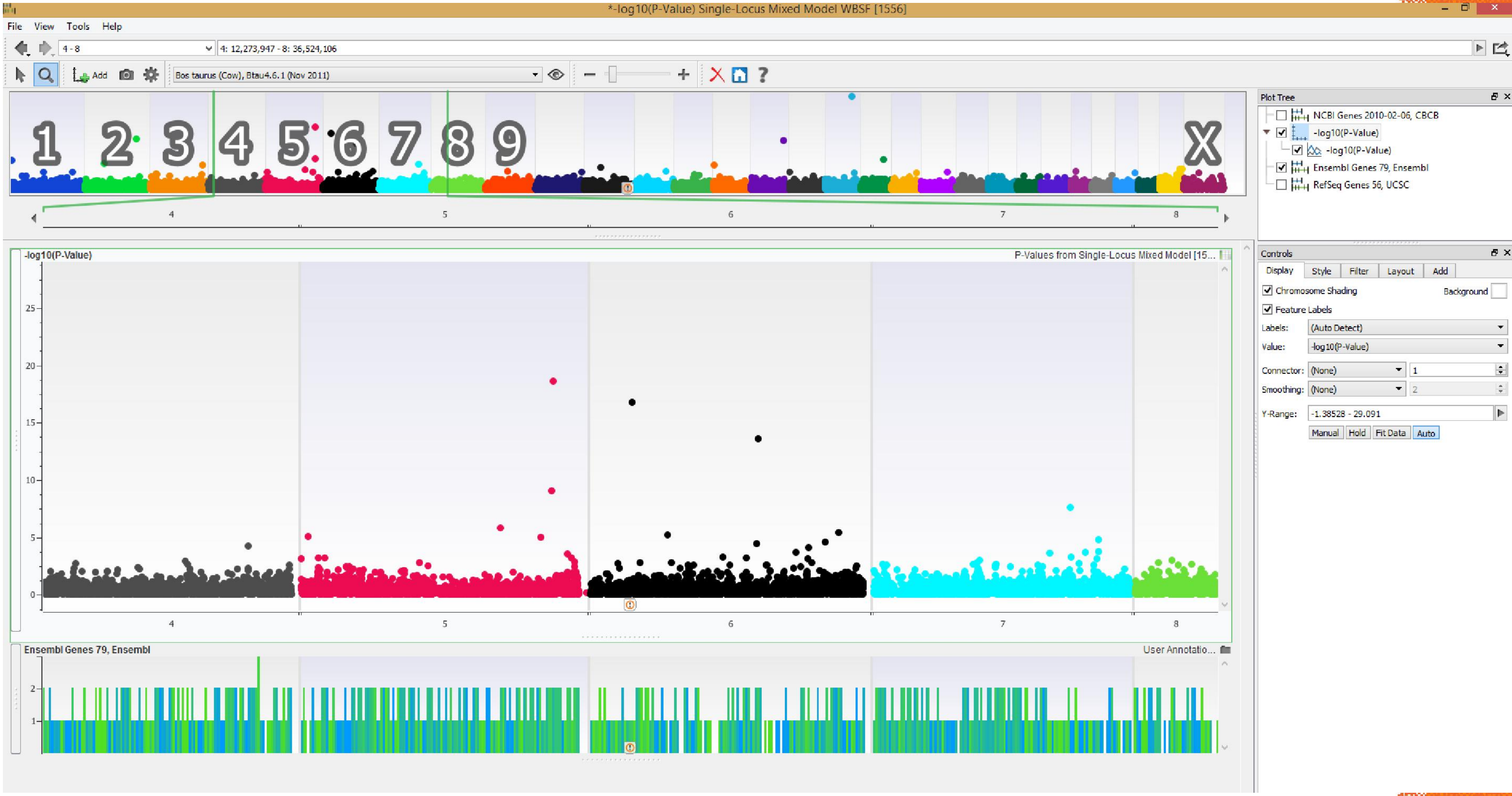
GWAS for WBSF - SVS

- Mixed Model GWAS using a single locus (**EMMAX**)
  - Genomic relationship matrix
  - Contemporary groups

Samples scanned: 2,076
Markers scanned: 51,218
Markers analyzed: 51,141
Pseudo-heritability: 0.37



Exploring data - GenomeBrowser


*-log10(P-Value) Single-Locus Mixed Model WBSF [1556]

4 - 8

4: 12,273,947 - 8: 36,524,106

Bos taurus (Cow), Btau4.6.1 (Nov 2011)

Plot Tree
- ☐ NCBI Genes 2010-02-06, CBCB
- ☑ -log10(P-Value)
  - ☑ -log10(P-Value)
- ☑ Ensembl Genes 79, Ensembl
- ☐ RefSeq Genes 56, UCSC

Controls

☑ Chromosome Shading

☑ Feature Labels

Labels: (Auto Detect)

Value: -log10(P-Value)

Connector: (None) 1

Smoothing: (None) 2

Y-Range: -1.38528 - 29.091

-log10(P-Value)

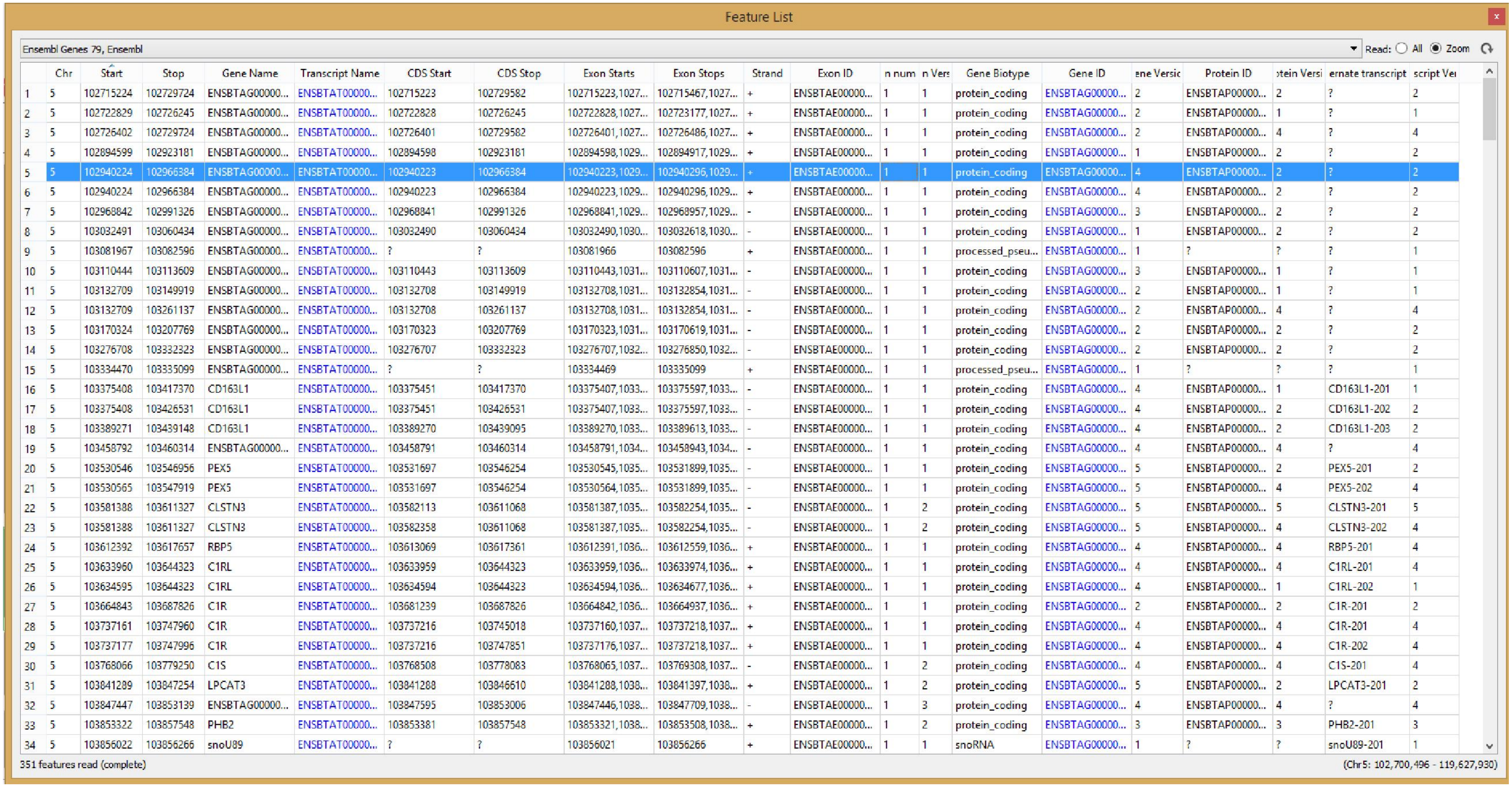
P-Values from Single-Locus Mixed Model [15...

Ensembl Genes 79, Ensembl

User Annotatio...

Exploring data - GenomeBrowser

Exploring data - GenomeBrowser

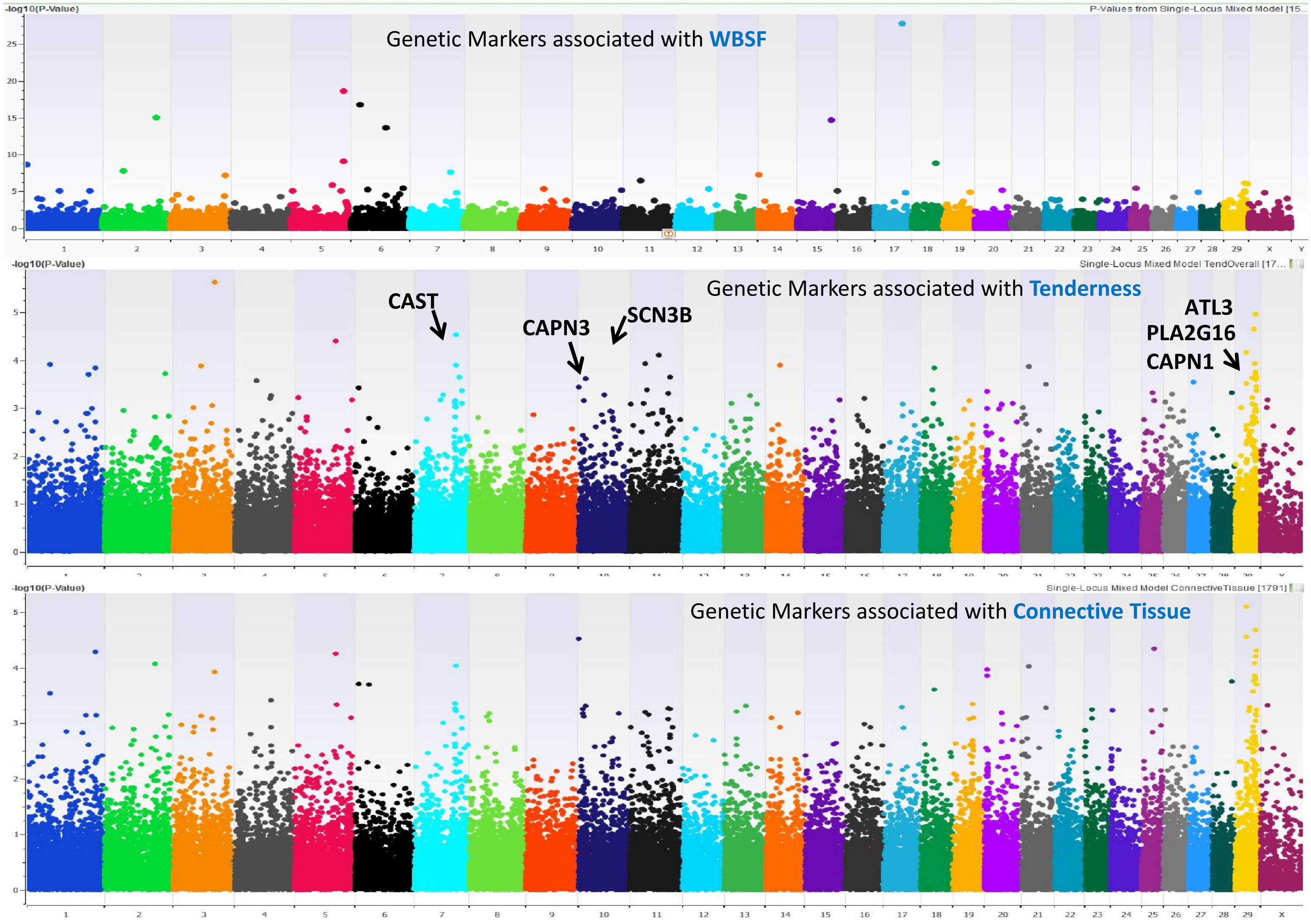


	Chr	Start	Stop	Gene Name	Transcript Name	CDS Start	CDS Stop	Exon Starts	Exon Stops	Strand	Exon ID	n num	n Vers	Gene Biotype	Gene ID	ene Versic	Protein ID	tein Versi	ernate transcript	script Ver
1	5	102715224	102729724	ENSBTAG00000...	ENSBTAT00000...	102715223	102729582	102715223,1027...	102715467,1027...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	2	?	2
2	5	102722829	102726245	ENSBTAG00000...	ENSBTAT00000...	102722828	102726245	102722828,1027...	102723177,1027...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	1	?	1
3	5	102726402	102729724	ENSBTAG00000...	ENSBTAT00000...	102726401	102729582	102726401,1027...	102726486,1027...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	4	?	4
4	5	102894599	102923181	ENSBTAG00000...	ENSBTAT00000...	102894598	102923181	102894598,1029...	102894917,1029...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	1	ENSBTAP00000...	2	?	2
5	5	102940224	102966384	ENSBTAG00000...	ENSBTAT00000...	102940223	102966384	102940223,1029...	102940296,1029...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	2	?	2
6	5	102940224	102966384	ENSBTAG00000...	ENSBTAT00000...	102940223	102966384	102940223,1029...	102940296,1029...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	2	?	2
7	5	102968842	102991326	ENSBTAG00000...	ENSBTAT00000...	102968841	102991326	102968841,1029...	102968957,1029...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	3	ENSBTAP00000...	2	?	2
8	5	103032491	103060434	ENSBTAG00000...	ENSBTAT00000...	103032490	103060434	103032490,1030...	103032618,1030...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	1	ENSBTAP00000...	2	?	2
9	5	103081967	103082596	ENSBTAG00000...	ENSBTAT00000...	?	?	103081966	103082596	+	ENSBTAE00000...	1	1	processed_pseu...	ENSBTAG00000...	1	?	?	?	1
10	5	103110444	103113609	ENSBTAG00000...	ENSBTAT00000...	103110443	103113609	103110443,1031...	103110607,1031...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	3	ENSBTAP00000...	1	?	1
11	5	103132709	103149919	ENSBTAG00000...	ENSBTAT00000...	103132708	103149919	103132708,1031...	103132854,1031...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	1	?	1
12	5	103132709	103261137	ENSBTAG00000...	ENSBTAT00000...	103132708	103261137	103132708,1031...	103132854,1031...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	4	?	4
13	5	103170324	103207769	ENSBTAG00000...	ENSBTAT00000...	103170323	103207769	103170323,1031...	103170619,1031...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	2	?	2
14	5	103276708	103332323	ENSBTAG00000...	ENSBTAT00000...	103276707	103332323	103276707,1032...	103276850,1032...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	2	?	2
15	5	103334470	103335099	ENSBTAG00000...	ENSBTAT00000...	?	?	103334469	103335099	+	ENSBTAE00000...	1	1	processed_pseu...	ENSBTAG00000...	1	?	?	?	1
16	5	103375408	103417370	CD163L1	ENSBTAT00000...	103375451	103417370	103375407,1033...	103375597,1033...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	1	CD163L1-201	1
17	5	103375408	103426531	CD163L1	ENSBTAT00000...	103375451	103426531	103375407,1033...	103375597,1033...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	2	CD163L1-202	2
18	5	103389271	103439148	CD163L1	ENSBTAT00000...	103389270	103439095	103389270,1033...	103389613,1033...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	2	CD163L1-203	2
19	5	103458792	103460314	ENSBTAG00000...	ENSBTAT00000...	103458791	103460314	103458791,1034...	103458943,1034...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	4	?	4
20	5	103530546	103546956	PEX5	ENSBTAT00000...	103531697	103546254	103530545,1035...	103531899,1035...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	5	ENSBTAP00000...	2	PEX5-201	2
21	5	103530565	103547919	PEX5	ENSBTAT00000...	103531697	103546254	103530564,1035...	103531899,1035...	-	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	5	ENSBTAP00000...	4	PEX5-202	4
22	5	103581388	103611327	CLSTN3	ENSBTAT00000...	103582113	103611068	103581387,1035...	103582254,1035...	-	ENSBTAE00000...	1	2	protein_coding	ENSBTAG00000...	5	ENSBTAP00000...	5	CLSTN3-201	5
23	5	103581388	103611327	CLSTN3	ENSBTAT00000...	103582358	103611068	103581387,1035...	103582254,1035...	-	ENSBTAE00000...	1	2	protein_coding	ENSBTAG00000...	5	ENSBTAP00000...	4	CLSTN3-202	4
24	5	103612392	103617657	RBP5	ENSBTAT00000...	103613069	103617361	103612391,1036...	103612559,1036...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	4	RBP5-201	4
25	5	103633960	103644323	C1RL	ENSBTAT00000...	103633959	103644323	103633959,1036...	103633974,1036...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	4	C1RL-201	4
26	5	103634595	103644323	C1RL	ENSBTAT00000...	103634594	103644323	103634594,1036...	103634677,1036...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	1	C1RL-202	1
27	5	103664843	103687826	C1R	ENSBTAT00000...	103681239	103687826	103664842,1036...	103664937,1036...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	2	ENSBTAP00000...	2	C1R-201	2
28	5	103737161	103747960	C1R	ENSBTAT00000...	103737216	103745018	103737160,1037...	103737218,1037...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	4	C1R-201	4
29	5	103737177	103747996	C1R	ENSBTAT00000...	103737216	103747851	103737176,1037...	103737218,1037...	+	ENSBTAE00000...	1	1	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	4	C1R-202	4
30	5	103768066	103779250	C1S	ENSBTAT00000...	103768508	103778083	103768065,1037...	103769308,1037...	-	ENSBTAE00000...	1	2	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	4	C1S-201	4
31	5	103841289	103847254	LPCAT3	ENSBTAT00000...	103841288	103846610	103841288,1038...	103841397,1038...	+	ENSBTAE00000...	1	2	protein_coding	ENSBTAG00000...	5	ENSBTAP00000...	2	LPCAT3-201	2
32	5	103847447	103853139	ENSBTAG00000...	ENSBTAT00000...	103847595	103853006	103847446,1038...	103847709,1038...	-	ENSBTAE00000...	1	3	protein_coding	ENSBTAG00000...	4	ENSBTAP00000...	4	?	4
33	5	103853322	103857548	PHB2	ENSBTAT00000...	103853381	103857548	103853321,1038...	103853508,1038...	+	ENSBTAE00000...	1	2	protein_coding	ENSBTAG00000...	3	ENSBTAP00000...	3	PHB2-201	3
34	5	103856022	103856266	snoU89	ENSBTAT00000...	?	?	103856021	103856266	+	ENSBTAE00000...	1	1	snoRNA	ENSBTAG00000...	1	?	?	snoU89-201	1

351 features read (complete)

(Chr5: 102,700,496 - 119,627,930)

GWAS on other “tenderness” traits

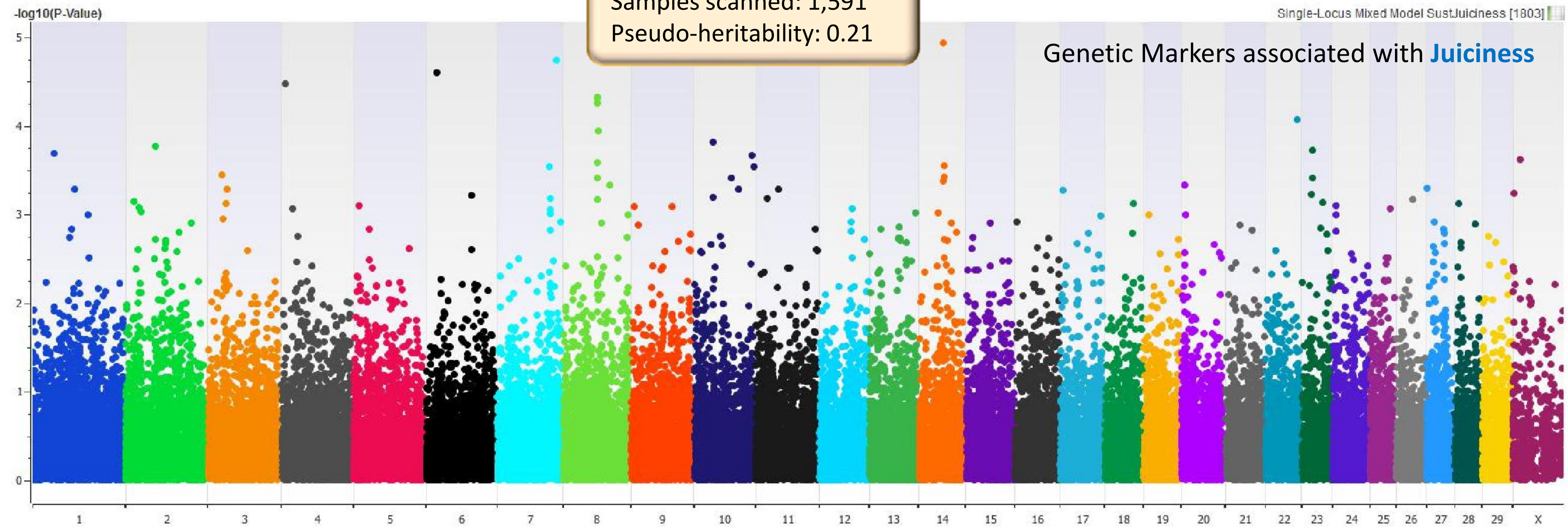

Genetic Markers associated with **WBSF**

Genetic Markers associated with **Tenderness**

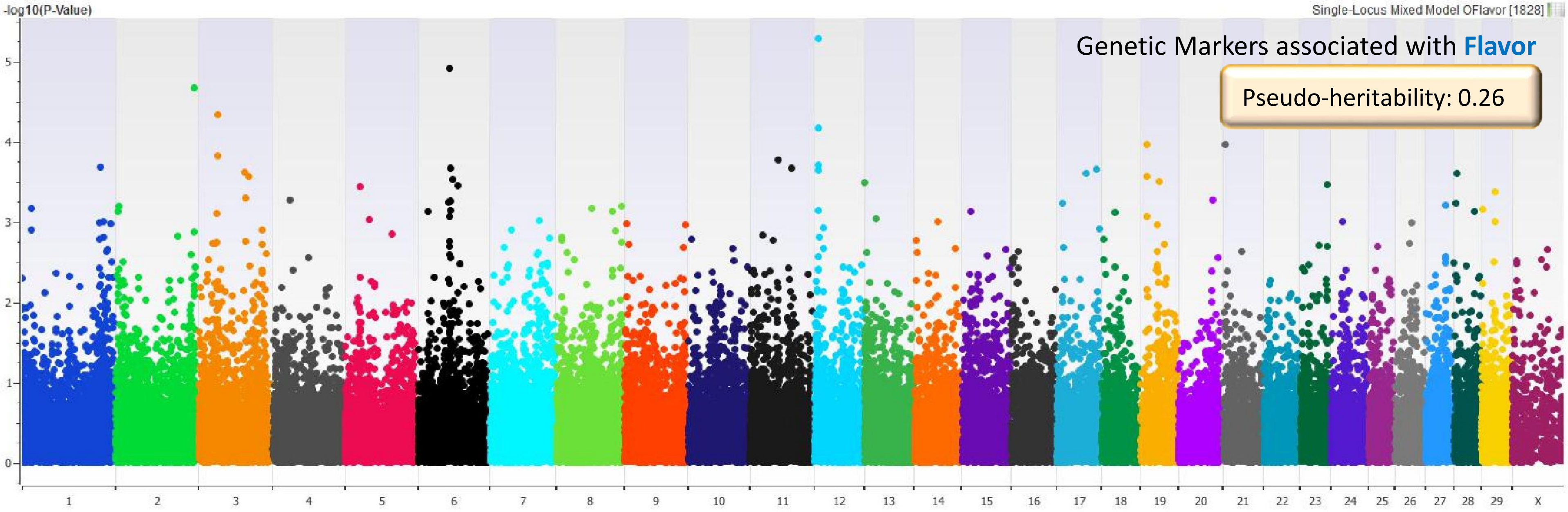
Genetic Markers associated with **Connective Tissue**

GWAS on other palatability traits

Samples scanned: 1,591
Pseudo-heritability: 0.21



Genetic Markers associated with **Juiciness**



Genetic Markers associated with **Flavor**

Pseudo-heritability: 0.26

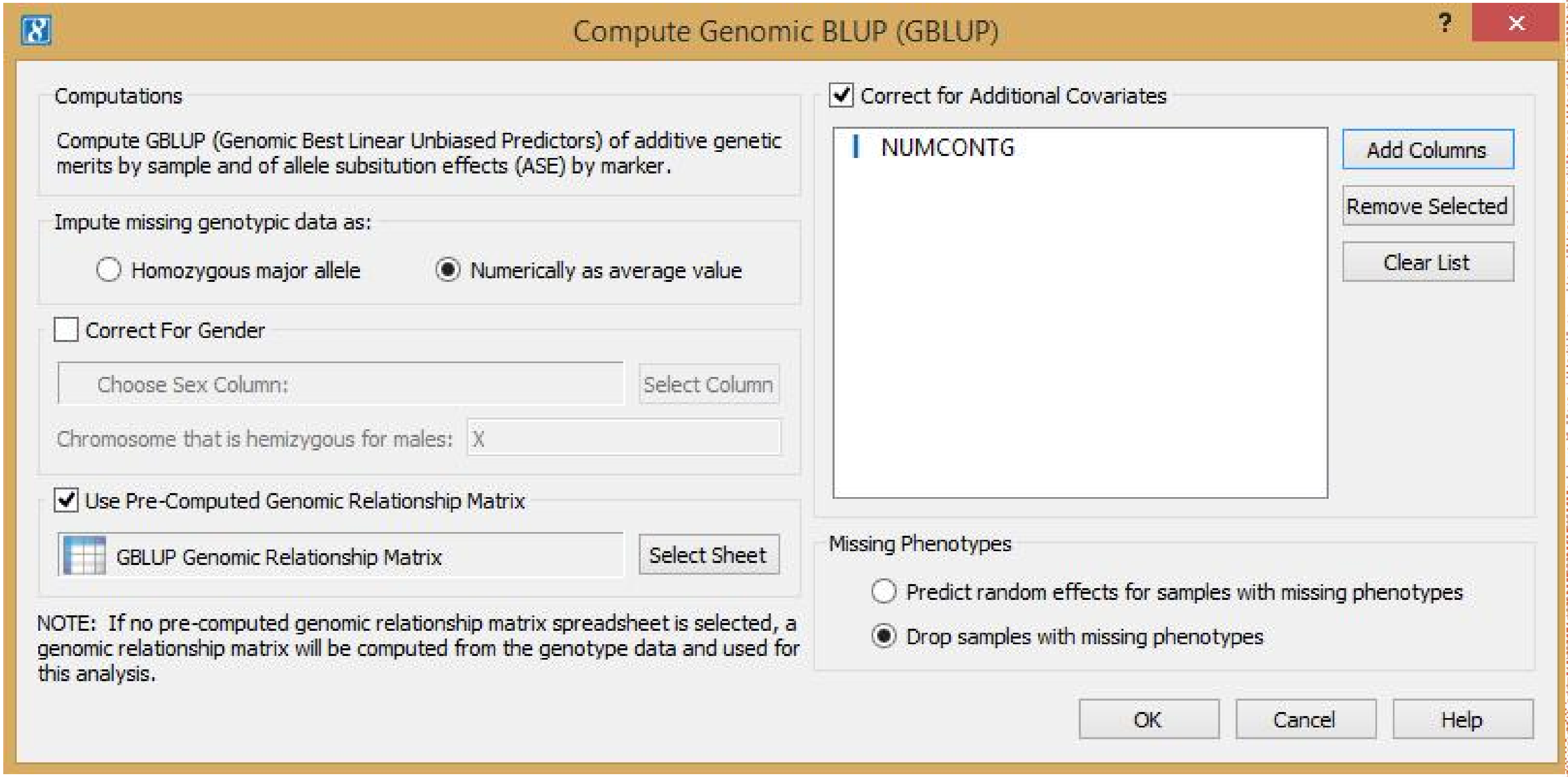
Palatability of Beef - goals

1 Genome-wide association (GWAS)

2 **Genomic Prediction**

3 GWAS signals underlying biology

Genomic Prediction - WBSF



Computations

Compute GBLUP (Genomic Best Linear Unbiased Predictors) of additive genetic merits by sample and of allele subsitution effects (ASE) by marker.

Impute missing genotypic data as:

○ Homozygous major allele ● Numerically as average value

☐ Correct For Gender

Choose Sex Column:

Chromosome that is hemizygous for males: X

☑ Use Pre-Computed Genomic Relationship Matrix

GBLUP Genomic Relationship Matrix

NOTE: If no pre-computed genomic relationship matrix spreadsheet is selected, a genomic relationship matrix will be computed from the genotype data and used for this analysis.

☑ Correct for Additional Covariates

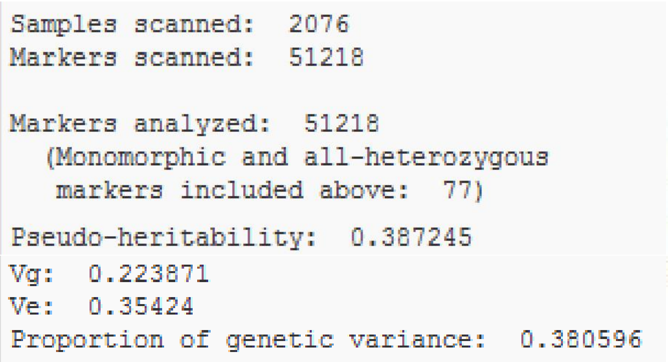
NUMCONTG

Missing Phenotypes

○ Predict random effects for samples with missing phenotypes

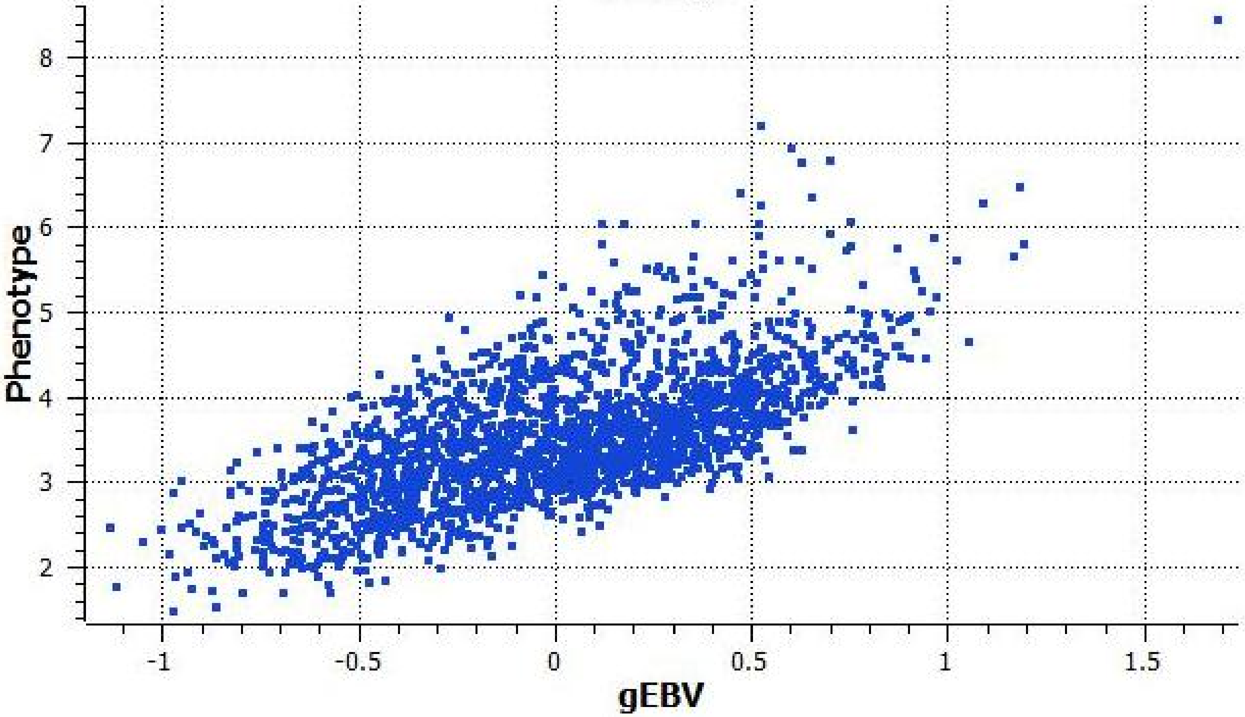
● Drop samples with missing phenotypes

GBLUP Prediction - WBSF



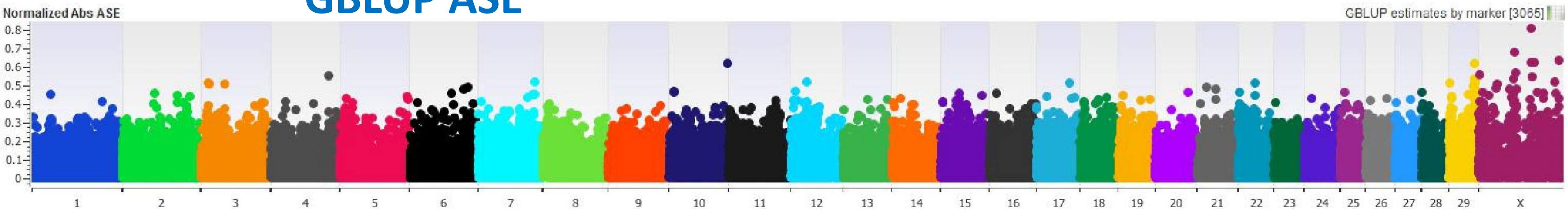
```
Samples scanned:  2076
Markers scanned:  51218

Markers analyzed:  51218
  (Monomorphic and all-heterozygous
   markers included above:  77)
Pseudo-heritability:  0.387245
Vg:  0.223871
Ve:  0.35424
Proportion of genetic variance:  0.380596
```

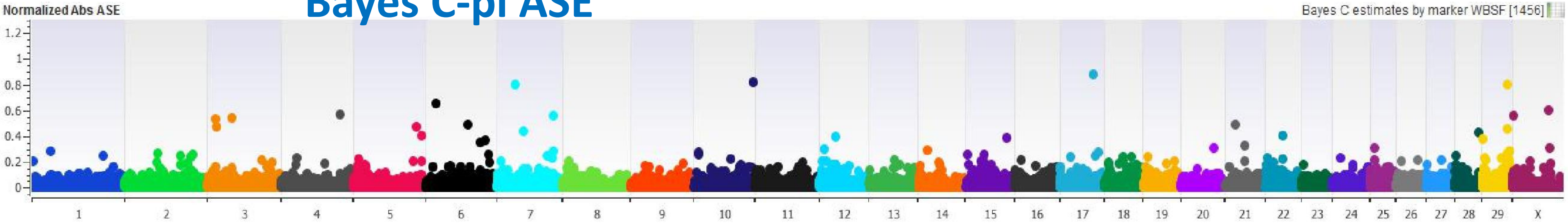


ASE vs GWAS results

GBLUP ASE



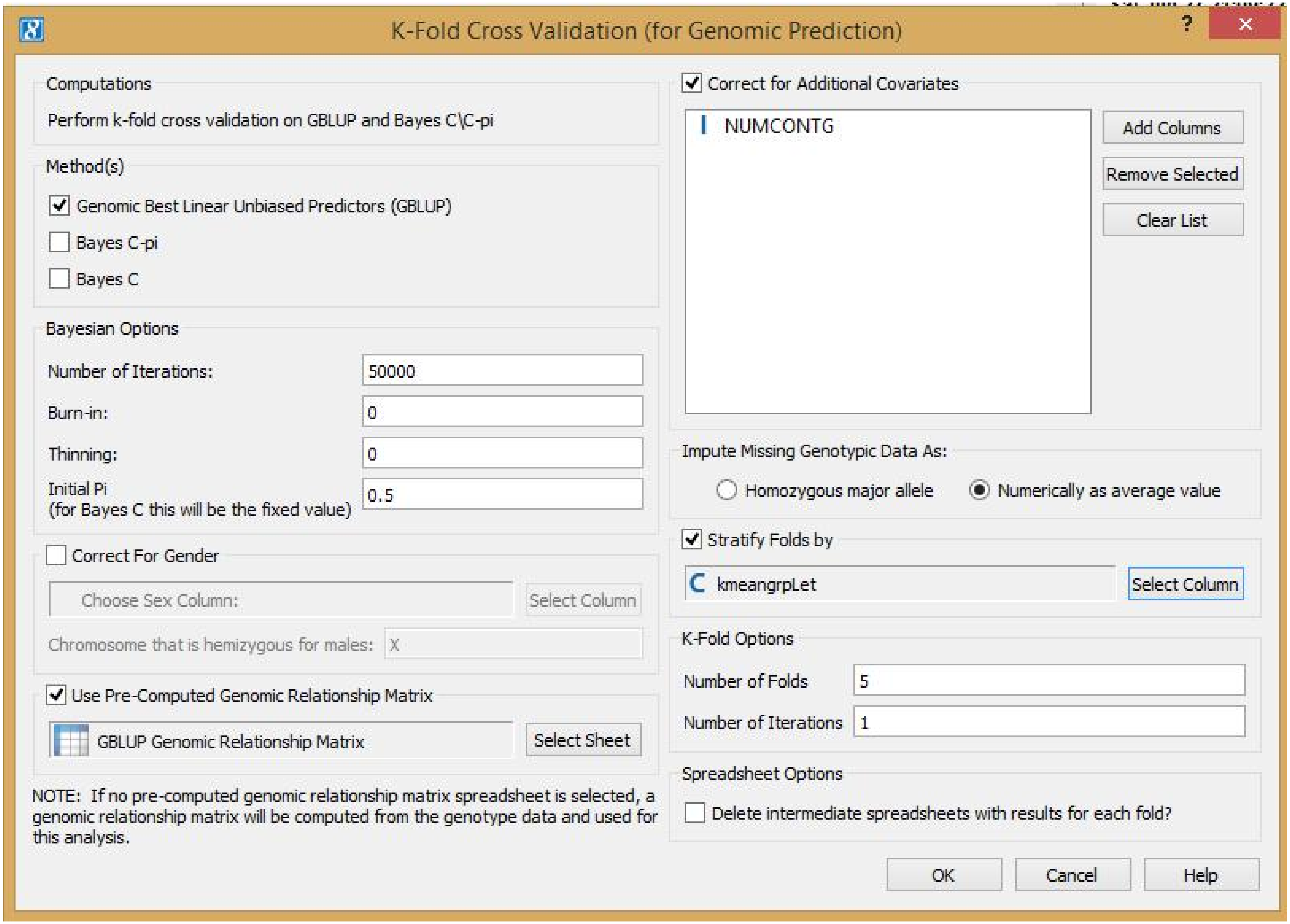
Bayes C-pi ASE



Genomic Prediction

- **Accuracy** of DGV - key to successful application of genomic selection
- Cross validation - assess performance of **prediction**
- SVS: five-fold cross-validation for evaluation of predictive performance of GBLUP/BayesC for **WBSF**

5-fold cross-validation - WBSF



K-Fold Cross Validation (for Genomic Prediction)

Computations

Perform k-fold cross validation on GBLUP and Bayes C\C-pi

Method(s)

☑ Genomic Best Linear Unbiased Predictors (GBLUP)

☐ Bayes C-pi

☐ Bayes C

Bayesian Options

Number of Iterations: 50000

Burn-in: 0

Thinning: 0

Initial Pi (for Bayes C this will be the fixed value) 0.5

☐ Correct For Gender

Choose Sex Column:

Chromosome that is hemizygous for males: X

☑ Use Pre-Computed Genomic Relationship Matrix

GBLUP Genomic Relationship Matrix

NOTE: If no pre-computed genomic relationship matrix spreadsheet is selected, a genomic relationship matrix will be computed from the genotype data and used for this analysis.

☑ Correct for Additional Covariates

NUMCONTG

Impute Missing Genotypic Data As:

○ Homozygous major allele ● Numerically as average value

☑ Stratify Folds by

kmeangrpLet

K-Fold Options

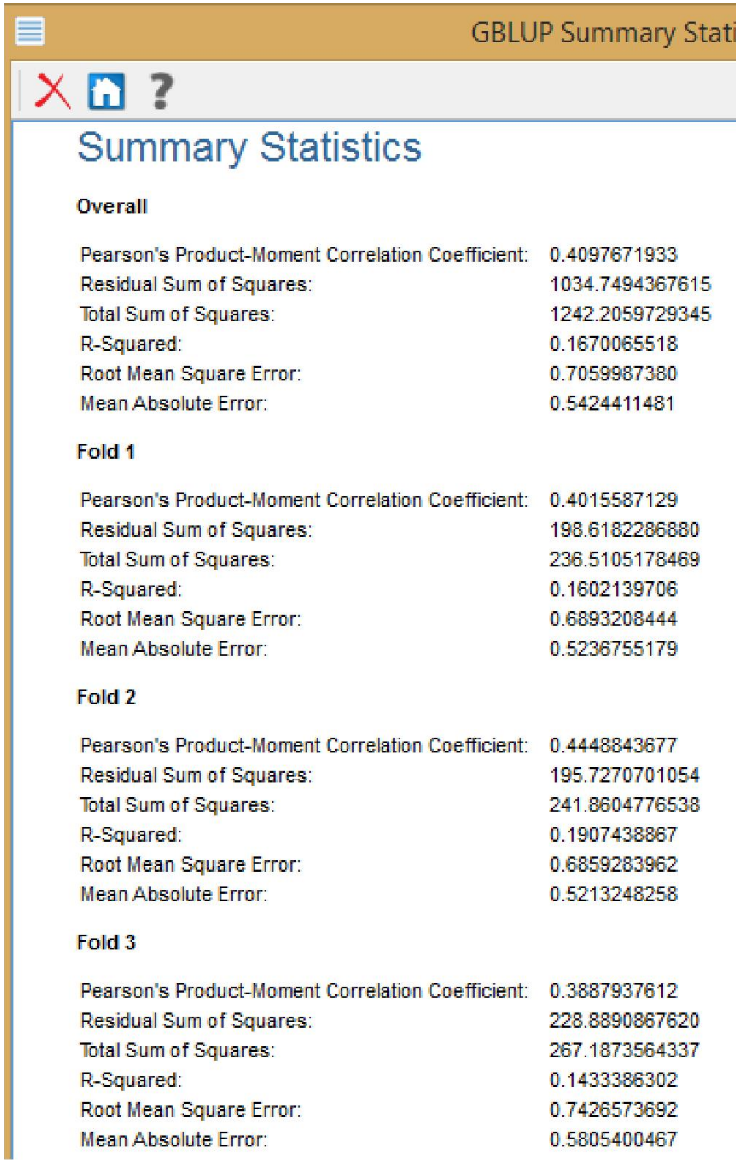
Number of Folds 5

Number of Iterations 1

Spreadsheet Options

☐ Delete intermediate spreadsheets with results for each fold?

Cross-validation - WBSF



Summary Statistics

Overall

Pearson's Product-Moment Correlation Coefficient:	0.4097671933
Residual Sum of Squares:	1034.7494367615
Total Sum of Squares:	1242.2059729345
R-Squared:	0.1670065518
Root Mean Square Error:	0.7059987380
Mean Absolute Error:	0.5424411481

Fold 1

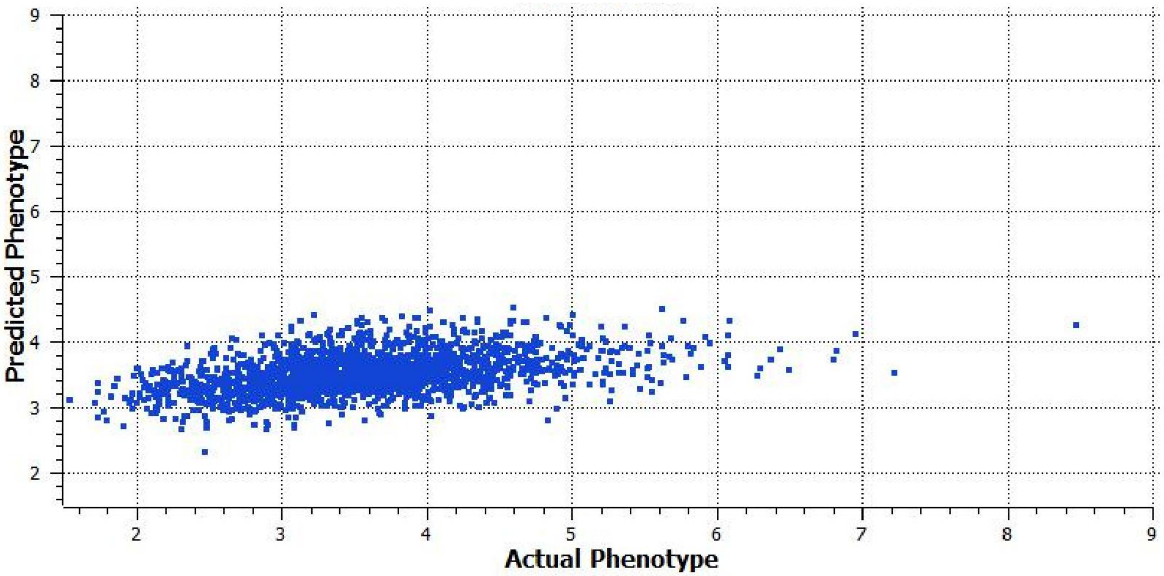
Pearson's Product-Moment Correlation Coefficient:	0.4015587129
Residual Sum of Squares:	198.6182286880
Total Sum of Squares:	236.5105178469
R-Squared:	0.1602139706
Root Mean Square Error:	0.6893208444
Mean Absolute Error:	0.5236755179

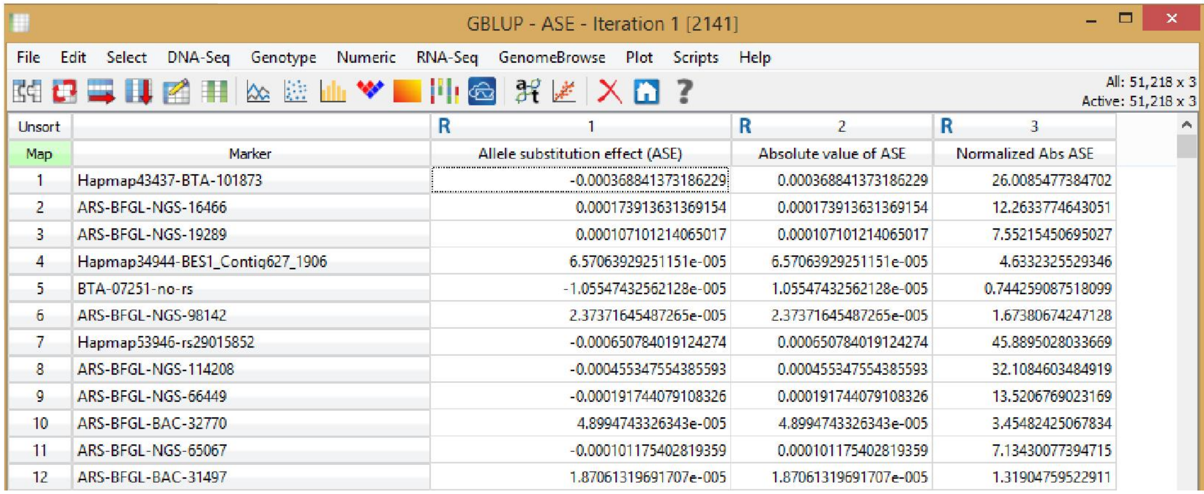
Fold 2

Pearson's Product-Moment Correlation Coefficient:	0.4448843677
Residual Sum of Squares:	195.7270701054
Total Sum of Squares:	241.8604776538
R-Squared:	0.1907438867
Root Mean Square Error:	0.6859283962
Mean Absolute Error:	0.5213248258

Fold 3

Pearson's Product-Moment Correlation Coefficient:	0.3887937612
Residual Sum of Squares:	228.8890867620
Total Sum of Squares:	267.1873564337
R-Squared:	0.1433386302
Root Mean Square Error:	0.7426573692
Mean Absolute Error:	0.5805400467

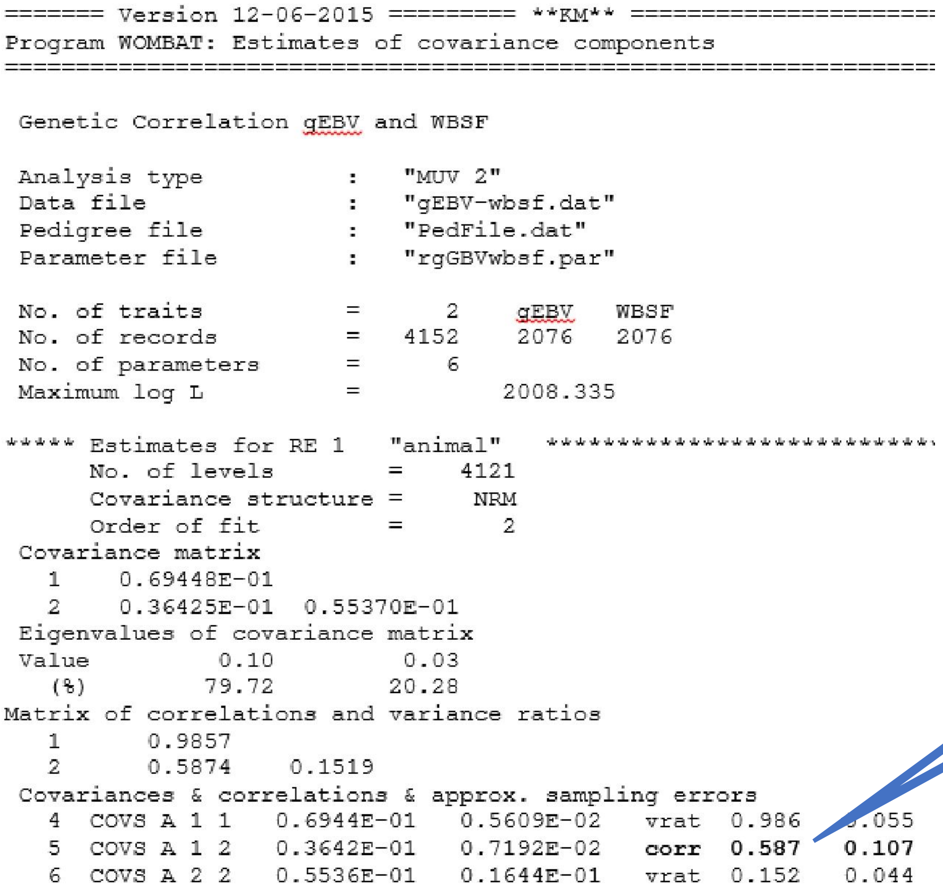




Unsort		R 1	R 2	R 3
Map	Marker	Allele substitution effect (ASE)	Absolute value of ASE	Normalized Abs ASE
1	Hapmap43437-BTA-101873	-0.000368841373186229	0.000368841373186229	26.0085477384702
2	ARS-BFGL-NGS-16466	0.000173913631369154	0.000173913631369154	12.2633774643051
3	ARS-BFGL-NGS-19289	0.000107101214065017	0.000107101214065017	7.55215450695027
4	Hapmap34944-BES1_Contig627_1906	6.57063929251151e-005	6.57063929251151e-005	4.6332325529346
5	BTA-07251-no-rs	-1.05547432562128e-005	1.05547432562128e-005	0.744259087518099
6	ARS-BFGL-NGS-98142	2.37371645487265e-005	2.37371645487265e-005	1.67380674247128
7	Hapmap53946-rs29015852	-0.000650784019124274	0.000650784019124274	45.8895028033669
8	ARS-BFGL-NGS-114208	-0.000455347554385593	0.000455347554385593	32.1084603484919
9	ARS-BFGL-NGS-66449	-0.000191744079108326	0.000191744079108326	13.5206769023169
10	ARS-BFGL-BAC-32770	4.8994743326343e-005	4.8994743326343e-005	3.45482425067834
11	ARS-BFGL-NGS-65067	-0.000101175402819359	0.000101175402819359	7.13430077394715
12	ARS-BFGL-BAC-31497	1.87061319691707e-005	1.87061319691707e-005	1.31904759522911

Accuracy of gEBV

- **Genetic correlation between gEBV and phenotype.**
  - Bivariate animal model in **Wombat**



```
======= Version 12-06-2015 ========= **KM** =====================
Program WOMBAT: Estimates of covariance components
==================================================================

 Genetic Correlation gEBV and WBSF

 Analysis type           :   "MUV 2"
 Data file               :   "gEBV-wbsf.dat"
 Pedigree file           :   "PedFile.dat"
 Parameter file          :   "rgGBVwbsf.par"

 No. of traits           =      2    gEBV   WBSF
 No. of records          =   4152    2076   2076
 No. of parameters       =      6
 Maximum log L           =          2008.335

***** Estimates for RE 1   "animal"   *****************************
      No. of levels          =    4121
      Covariance structure =     NRM
      Order of fit           =       2
 Covariance matrix
   1    0.69448E-01
   2    0.36425E-01  0.55370E-01
 Eigenvalues of covariance matrix
 Value          0.10          0.03
   (%)         79.72         20.28
Matrix of correlations and variance ratios
   1      0.9857
   2      0.5874    0.1519
 Covariances & correlations & approx. sampling errors
   4  COVS A 1 1   0.6944E-01   0.5609E-02   vrat  0.986   0.055
   5  COVS A 1 2   0.3642E-01   0.7192E-02   corr  0.587   0.107
   6  COVS A 2 2   0.5536E-01   0.1644E-01   vrat  0.152   0.044
```

Accuracy:

0.59

Palatability of Beef - goals

1 Genome-wide association (GWAS)

2 Genomic Prediction

3 GWAS signals → underlying biology

Palatability trait

- Warner-Bratzler Shear Force
- Tenderness
- Juiciness
- Connective Tissue
- Beef Flavor
- Off-flavors: painty/fishy, livery/metallic

- **8-10 individual traits (sub-phenotypes):**
  - 50,000 SNP effects for each one on >2,000 animals

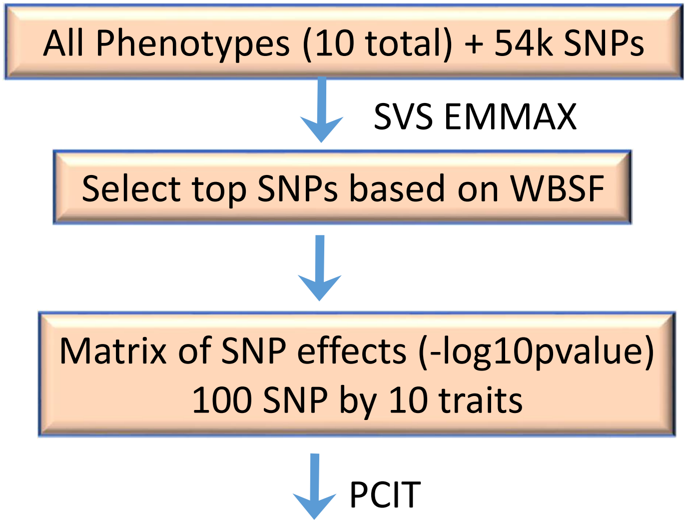
- **Systems biology: integration of data sets**
  - Holistic view of the system – key players can emerge

Gene network theory: use SNP association data to guide the inference of gene regulatory networks

Association Weight Matrix *(Fortes 2010)*

multivariate view of GWAS using PCIT (partial correlation and information theory *Reverter & Chan 2008***)**

Network Analysis (Palatability)



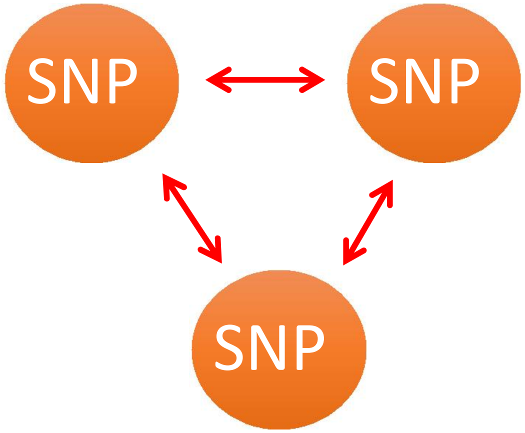
All Phenotypes (10 total) + 54k SNPs

SVS EMMAX

Select top SNPs based on WBSF

Matrix of SNP effects (-log10pvalue)
100 SNP by 10 traits

PCIT

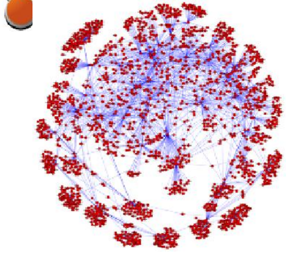


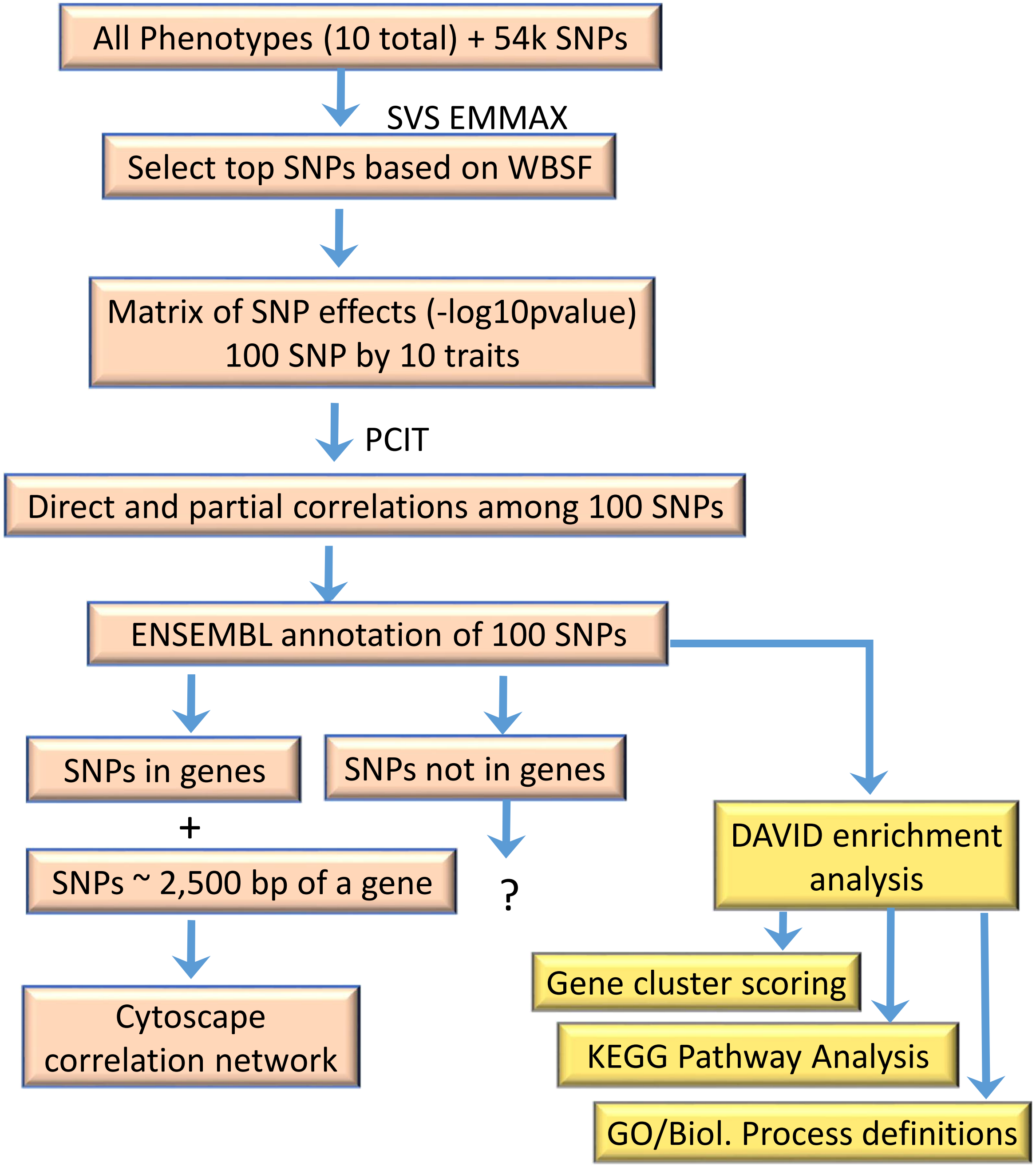
- **Partial Correlation Information Theory** *(Reverter and Chan, 2008)*
- Significant SNPs correlated among multiple sub-phenotypes are important for regulating the overall phenotype
    - Optimized to handle SNPs and genomic windows

HiPerGator
The University of Florida Supercomputer for Research

Network Analysis (Palatability)

- **Networking and Clustering**
  - Direct correlations visualized in **Cytoscape**
  - Clustering analysis - **MCODE** - highly connected genes
  - Enriched **GO** terms were identified using **DAVID** and visualized using **REViGO**





All Phenotypes (10 total) + 54k SNPs

SVS EMMAX

Select top SNPs based on WBSF

Matrix of SNP effects (-log10pvalue)
100 SNP by 10 traits

PCIT

Direct and partial correlations among 100 SNPs

ENSEMBL annotation of 100 SNPs

SNPs in genes

+

SNPs ~ 2,500 bp of a gene

Cytoscape correlation network

SNPs not in genes

?

DAVID enrichment analysis

Gene cluster scoring

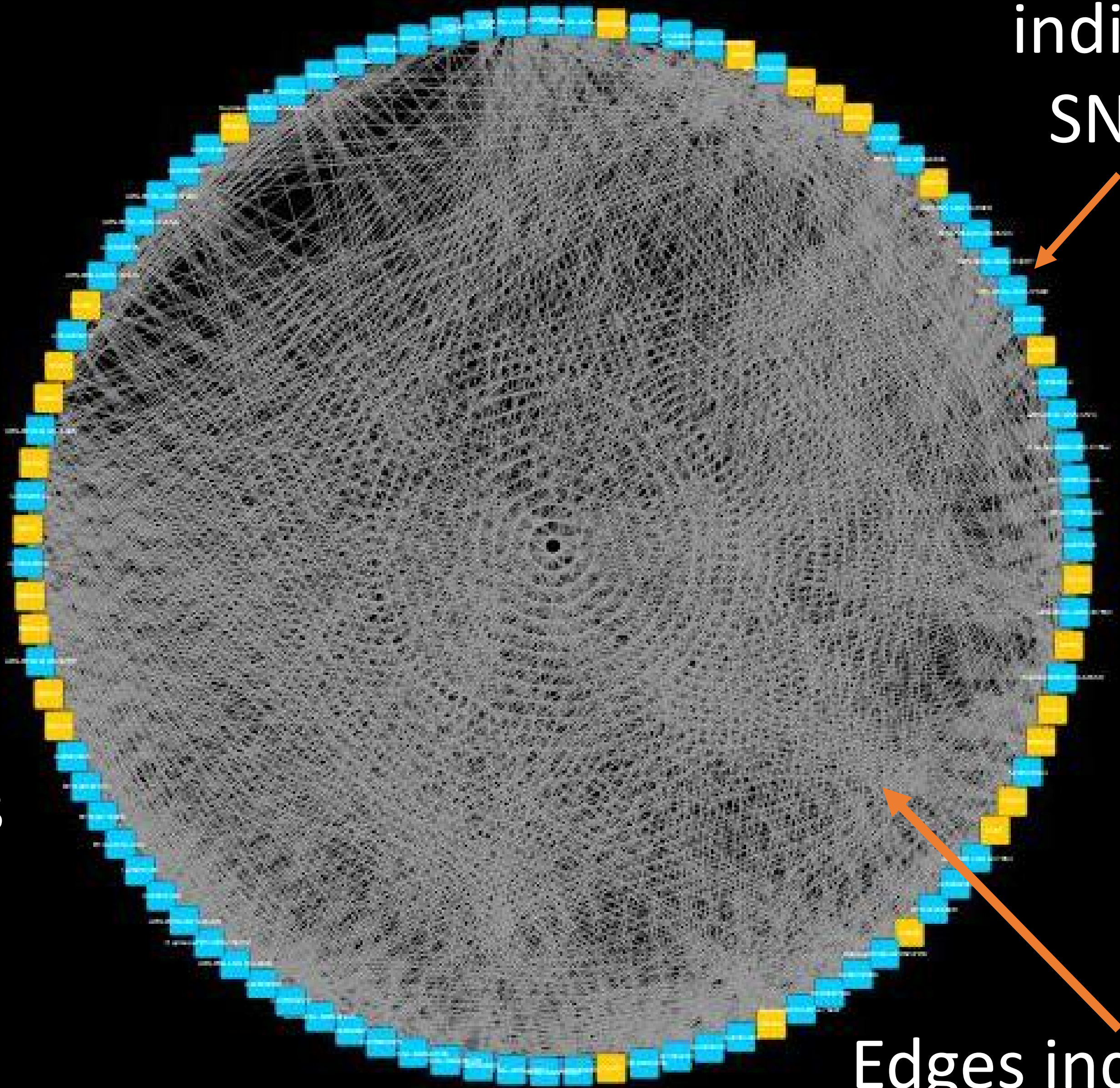
KEGG Pathway Analysis

GO/Biol. Process definitions

Network Visualization

Reference phenotype - **Warner-Bratzler Shear Force** Most significant SNPs

- **99** SNPs



Nodes indicate SNP's

Edges indicate correlations

Network Visualization

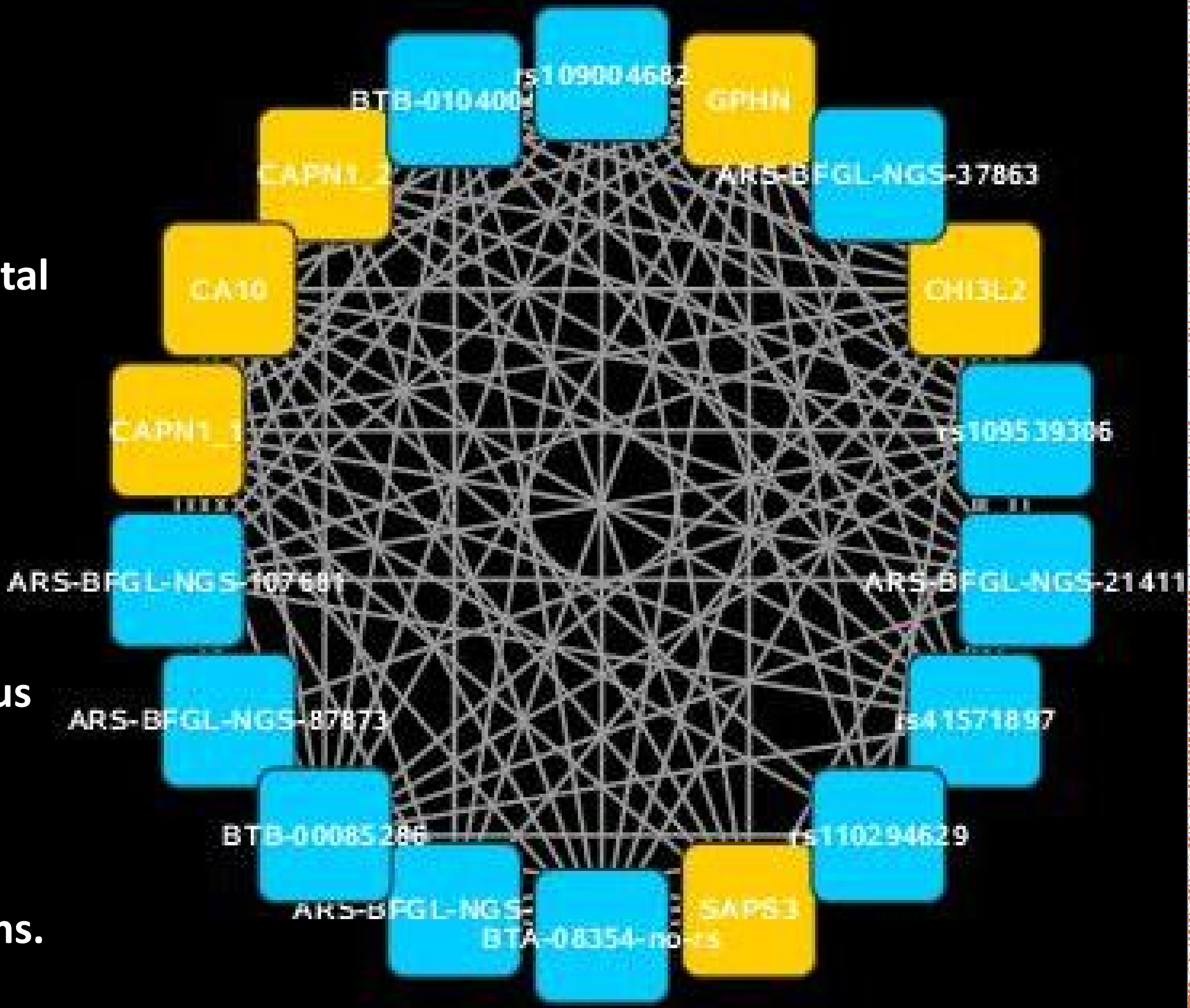
SAPS3: modulates protein phosphatase catalytic subunits

CAPN1: modulates proteolysis of cytoskeletal remodeling and signal transduction.

CHI3L2: Involved in cartilage biogenesis.

CA10: Catalyzes reversible hydration of carbon dioxide in various processes.

GPHN: Involved in membrane protein-cytoskeleton interactions.



Conclusions

- Palatability or eating satisfaction - important for the long-term **sustainability** of beef industry
- Collection of **palatability** phenotypes on large numbers of animals is still problematic
  - Findings focused on QTL detection rather than genomic prediction
- New methods and approaches to move from SNP signals closer to **functional variants**

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Questions?