# INCREASING THE POWER OF GENOMICS TO IMPACT MEAT QUALITY

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RMC 2014

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# Overview

- Human health
- Product labeling
- Can we change the nutrient composition of meat?
  - Overview
  - Genetic Correlations
  - Whole genome selection
  - Nutrient Composition Results
- Conclusions

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# Meat Quality

- Quantitative
  - Shear force, Ether extract, Nutrient content (fatty acids, minerals, vitamins), color
- Subjective
  - Flavor, juiciness, tenderness

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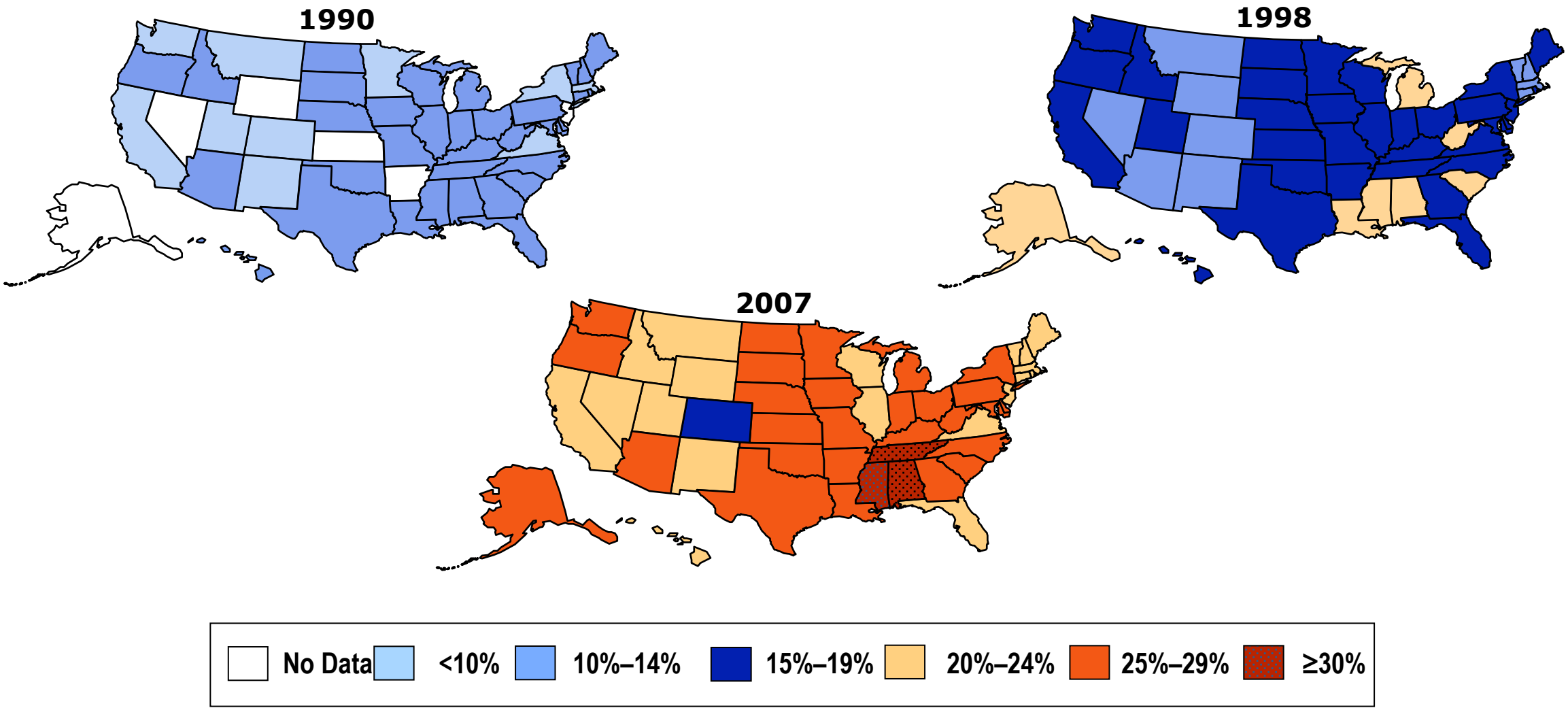
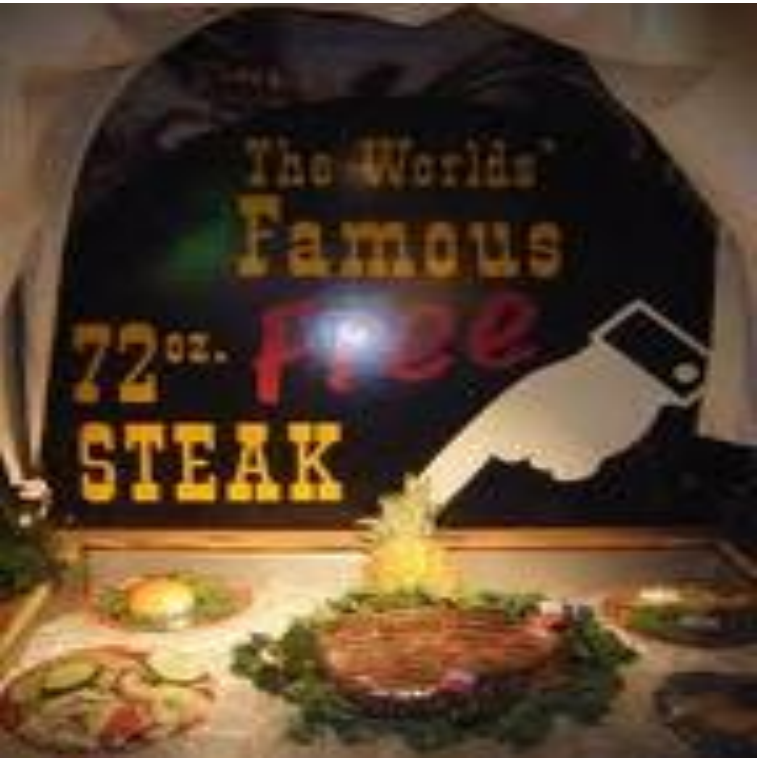
# Obesity Trends* Among U.S. Adults

## BRFSS, 1990, 1998, 2007

**(*BMI ≥30, or about 30 lbs. overweight for 5’4” person)**






Google Maps:
72 ounce steak
7,346 results

If a few of us eat too much, is the logical response to eat zero??

BMC Cardiovasc Disord. 2014 May 31;14(1):70. [Epub ahead of print]

## The impact of omega-3 polyunsaturated fatty acid supplementation on the incidence of cardiovascular events and complications in peripheral arterial disease: a systematic review and meta-analysis.

Enns JE, Yeganeh A, Zarychanski R, Abou-Setta AM, Friesen C, Zahradka P, Taylor CG.

**CONCLUSIONS:** Our results showed that insufficient evidence exists to suggest a beneficial effect of omega-3 PUFA supplementation in adults with peripheral arterial disease with regard to cardiovascular events and other serious clinical outcomes.

Ann Intern Med. 2014 Mar 18;160(6):398-406. doi: 10.7326/M13-1788.

## Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis.

Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, Franco OH, Butterworth AS, Forouhi NG, Thompson SG, Khaw KT, Mozaffarian D, Danesh J, Di Angelantonio E.

**CONCLUSION:** Current evidence does not clearly support cardiovascular guidelines that encourage high consumption of polyunsaturated fatty acids and low consumption of total saturated fats.

BMJ Open. 2014 Apr 19;4(4):e004487. doi: 10.1136/bmjopen-2013-004487.

## Dietary fatty acids in the secondary prevention of coronary heart disease: a systematic review, meta-analysis and meta-regression.

Schwingshackl L[1], Hoffmann G.

**CONCLUSIONS:** The present systematic review provides no evidence (moderate quality evidence) for the beneficial effects of reduced/modified fat diets in the secondary prevention of coronary heart disease. Recommending higher intakes of polyunsaturated fatty acids in replacement of saturated fatty acids was not associated with risk reduction.

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Ann Nutr Metab. 2011;59(2-4):176-86. doi: 10.1159/000334071. Epub 2011 Dec 2.

# Effects of monounsaturated fatty acids on cardiovascular risk factors: a systematic review and meta-analysis.

Schwingshackl L[1], Strasser B, Hoffmann G.

Therefore,

MUFA might represent a useful tool in the design of dietary regimens for obesity and cardiovascular disease.

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# Relative Priority of Consumption

**Essential: High Priority** → **Non-essential: Low Priority (avoid)**

## Omega-3 PUFA

Some food sources are fatty fish and shellfish, flaxseed, walnuts, and canola oil.

- Eicosapentaenoic acid (EPA)-fish
- Docosahexaenoic acid (DHA)-fish
- α-Linolenic acid (ALA)-flax seed, walnuts
- Stearidonic acid (STD)-canola, fish
- Eicosatrienoic acid (ETE)- seafood
- Eicosatetraenoic acid (ETA)- seafood
- Docosapentaenoic acid (DPA)-seafood

## Omega-6 PUFA

Some food sources are nuts, seeds and vegetable oils such as sunflower, safflower, corn and soybean oils.

- Linoleic acid-seeds
- Dihomo-gamma- linolenic acid- metabolic product
- Arachidonic acid-metabolic product

## Mono-unsaturated FA

Some food sources are canola oil and olive oil.

- Oleic acid-olive, canola oil
- Palmitoleic acid-vegetable oil

## Saturated FA

Some sources are animal-based foods such as cream, butter, cheese, and fatty meats.

- Lauric acid-coconut, palm oil
- Myristic acid-dairy fat
- Palmitic acid-meat
- Stearic acid-meat

## Trans-Saturated FA

Some food sources are margarine, fried foods, baked goods and processed foods.

- Elaidic acid-preservative
- Partially hyrdogenated plant and vegetable oils

**Most Beneficial**

▲Cardioprotective

▲Does not promote obesity

**Most Harmful**

**Promotes:** ▼Obesity

▼ Dyslipidemia

▼Atherosclerosis

©American Heart Association

# Product Branding

## February 2011

The AHA put its HeartCheck symbol on three cuts of lean beef:

- Boneless Top Sirloin Petite Roast (select grade)
- Top Sirloin Filet (select grade)
- Top Sirloin Kabob (select grade)

Total fat <5 g/serving

Sat fat <2 g/serving

Cholesterol <95 mg/serving

# FDA Approved Food Labels

## Nutrition Facts

Serving Size 1 cup (228g)
Servings Per Container 2

**Amount Per Serving**

**Calories** 260 Calories from Fat 120

| | % Daily Value* |
|---|---|
| **Total Fat** 13g | **20%** |
| Saturated Fat 5g | **25%** |
| *Trans* Fat 2g | |
| **Cholesterol** 30mg | **10%** |
| **Sodium** 660mg | **28%** |
| **Total Carbohydrate** 31g | **10%** |
| Dietary Fiber 0g | **0%** |
| Sugars 5g | |
| **Protein** 5g | |

Vitamin A 4% • Vitamin C 2%
Calcium 15% • Iron 4%

* Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs:

| | Calories: | 2,000 | 2,500 |
|---|---|---|---|
| Total Fat | Less than | 65g | 80g |
| Sat Fat | Less than | 20g | 25g |
| Cholesterol | Less than | 300mg | 300mg |
| Sodium | Less than | 2,400mg | 2,400mg |
| Total Carbohydrate | | 300g | 375g |
| Dietary Fiber | | 25g | 30g |

Calories per gram:
Fat 9 * Carbohydrate 4 * Protein 4

## Nutrition Facts

Serving Size 1 Tbsp (14g)
Servings Per Container 64

Amount Per Serving

**Calories** 130 Calories from Fat 130

| | % Daily Value |
|---|---|
| **Total Fat** 14g | **22%** |
| Saturated Fat 2g | **10%** |
| *Trans* Fat 2g | |
| Polyunsaturated Fat 4g | |
| Monounsaturated Fat 6g | |
| **Sodium** 0mg | **0%** |
| **Total Carbohydrate** 0g | **0%** |
| **Protein** 0g | |

Not a significant source of cholesterol, dietary fiber, sugars, vitamin A, vitamin C, calcium, and iron.

* Percent Daily Values are based on a 2,000 calorie diet.

# On-going lines of research

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Ponsuksili *et al. BMC Genomics* 2013, **14**:533
http://www.biomedcentral.com/1471-2164/14/533

BMC Genomics

RESEARCH ARTICLE Open Access

# Correlated mRNAs and miRNAs from co-expression and regulatory networks affect porcine muscle and finally meat properties

Siriluck Ponsuksili[1], Yang Du[2], Frieder Hadlich[1], Puntita Siengdee[1], Eduard Murani[2], Manfred Schwerin[1] and Klaus Wimmers[2*]

**Whole-genome association study of fatty acid composition in a diverse range of beef cattle breeds**

M. J. Kelly, R. K. Tume, M. Fortes and J. M. Thompson

*J ANIM SCI* 2014, 92:1895-1901.
doi: 10.2527/jas.2013-6901

Ponsuksili *et al. BMC Genomics* 2013, **14**:533
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**Genome-wide association study for intramuscular fat deposition an composition in Nellore cattle**

*BMC Genetics* 2014, **15**:39 doi:10.1186/1471-2156-15-39

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OPEN ACCESS Freely available online PLOS | ONE

# Genome-Wide Association Studies for Growth and Meat Production Traits in Sheep

**Li Zhang[1]*, Jiasen Liu[1], Fuping Zhao[1], Hangxing Ren[1,2], Lingyang Xu[1], Jian Lu[1], Shifang Zhang[1], Xiaoning Zhang[1], Caihong Wei[1], Guobin Lu[1], Youmin Zheng[1,3], Lixin Du[1]***

1 Animal Genetics and Breeding Department, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, 2 Animal Genetics and Breeding Department, Chongqing Academy of Animal Sciences, Chongqing, China, 3 General Office, National Animal Husbandry Service, Beijing, China

Sun *et al. BMC Genomics* 2013, **14**:458
http://www.biomedcentral.com/1471-2164/14/458

BMC Genomics

RESEARCH ARTICLE Open Access

# The identification of 14 new genes for meat quality traits in chicken using a genome-wide association study

**Genome-wide association study for intramuscular fatty acid composition in an Iberian × Landrace cross**

Y. Ramayo-Caldas, A. Mercadé, A. Castelló, B. Yang, C. Rodríguez, E. Alves, I. Díaz, N. Ibáñez-Escriche, J.L. Noguera, M. Pérez-Enciso, A.I. Fernández and J.M. Folch

*J ANIM SCI* 2012, 90:2883-2893.
doi: 10.2527/jas.2011-4900 originally published online July 10, 2012

Ramayo-Caldas *et al. BMC Genomics* 2014, **15**:232
http://www.biomedcentral.com/1471-2164/15/232

BMC Genomics

RESEARCH ARTICLE Open Access

# From SNP co-association to RNA co-expression: Novel insights into gene networks for intramuscular fatty acid composition in porcine

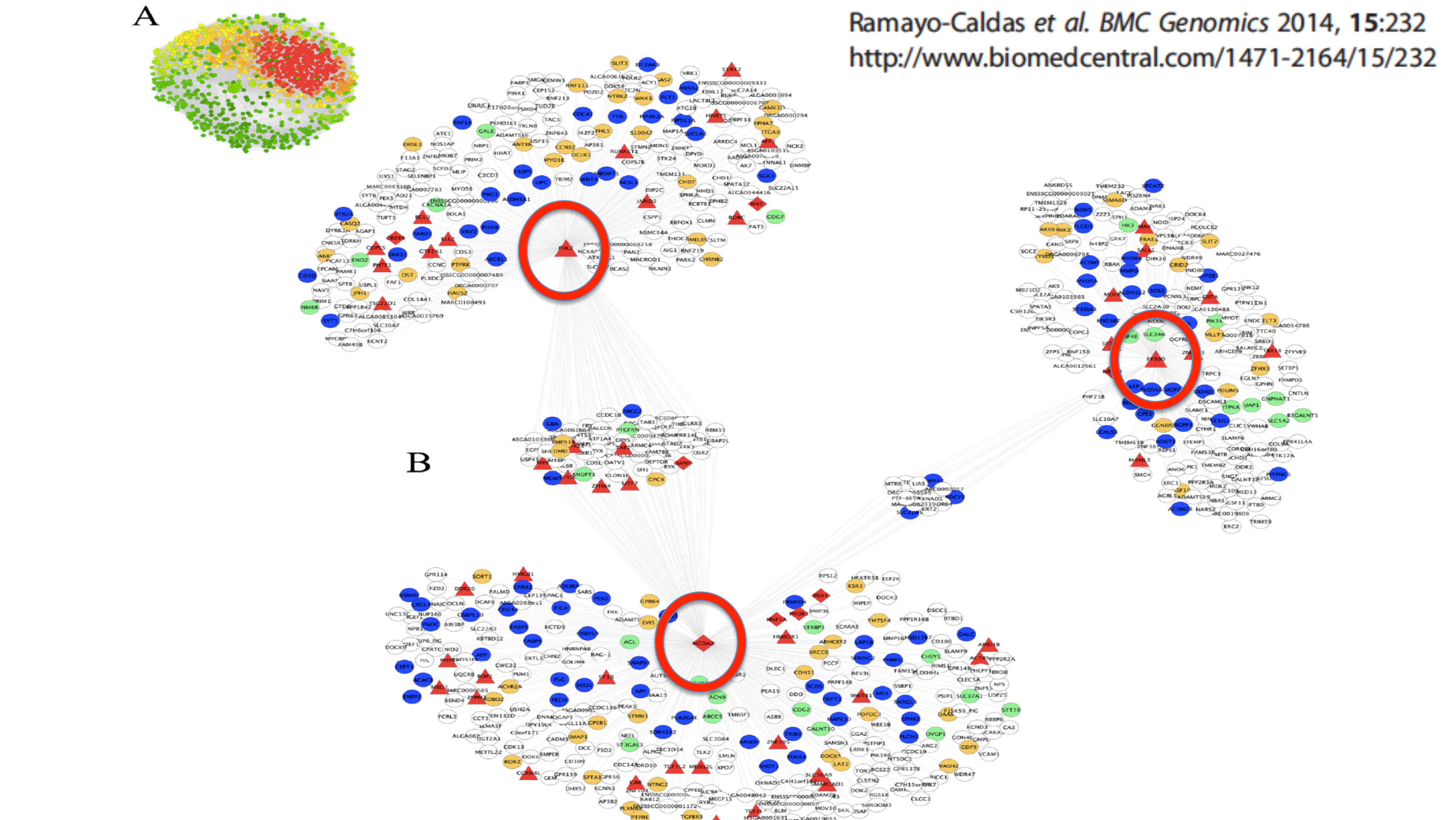
Yuliaxis Ramayo-Caldas[1,2*], Maria Ballester[1,2], Marina RS Fortes[3], Anna Esteve-Codina[2], Anna Castelló[1,2], Jose L Noguera[4], Ana I Fernández[5], Miguel Pérez-Enciso[1,6], Antonio Reverter[7] and Josep M Folch[1,2*]

# Meat Quality Traits in QTLdb

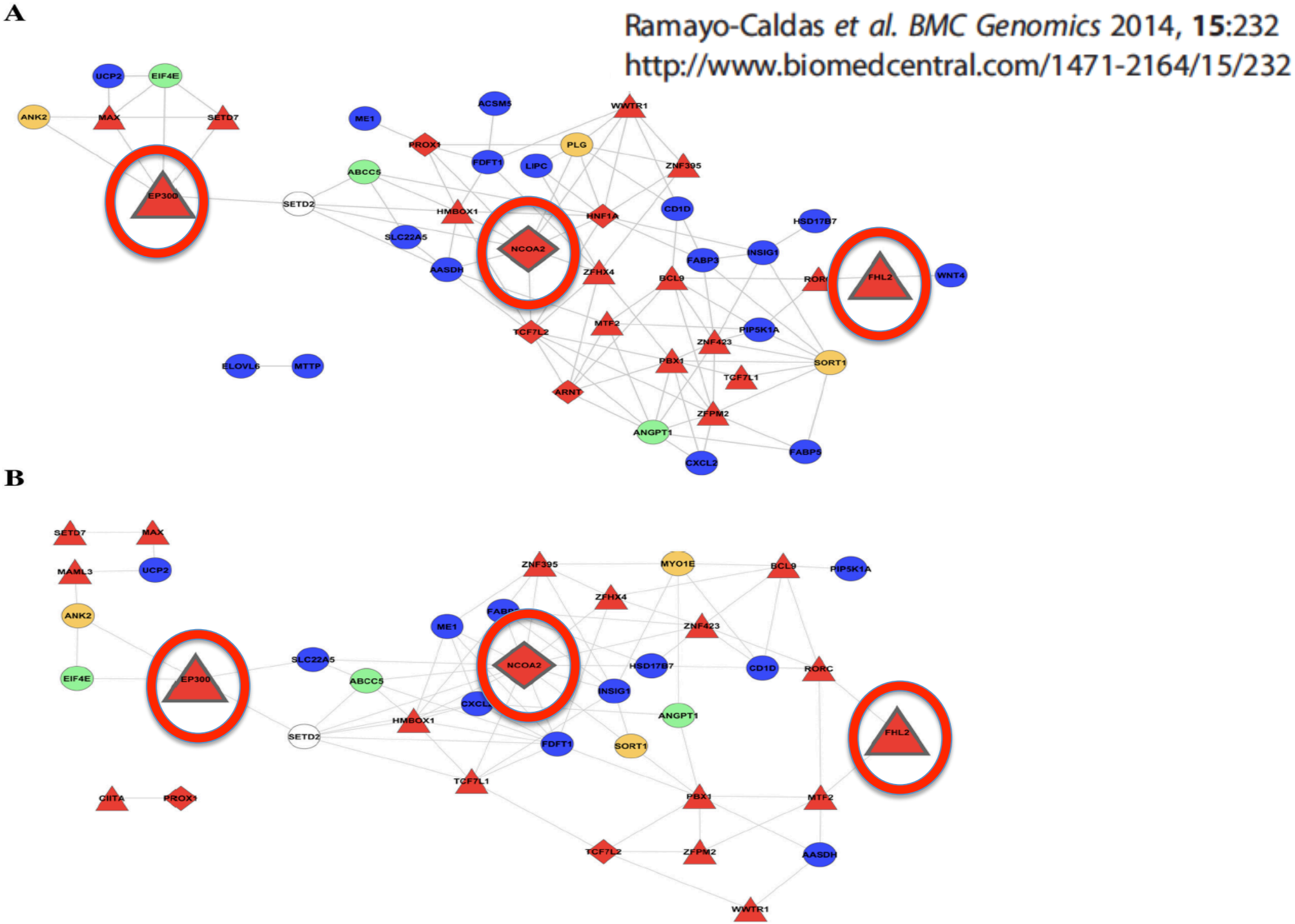
- Fatty acid content
  - Arachidic acid content { FA-C20:0 }
  - Arachidonic acid content { FA-C20:4 }
  - Atherogenic index { AI }
  - C14 index { C14IND }
  - C16 index { C16IND }
  - C18 index { C18IND }
  - C22:1 fatty acid content { FA-C22:1 }
  - Capric acid content { FA-C10:0 }
  - Cis-10-Heptadecenoic acid content { FA-C17:1 }
  - Cis-10-Pentadecenoic acid content { FA-C15:1 }
  - Cis-11,14-Eicosadienoic acid content { FA-C20:2 }
  - Cis-12-C18:1 fatty acid content { FA-C18:1n6 }
  - Cis-Vaccenic acid content { FA-C18:1n7 }
  - Conjugated linoleic acid content { CLAC18:2 }
  - Desaturation index { DSIND }
  - Dihomo-gamma-linolenic acid content { FA-C20:3 }
  - Docosahexaenoic acid content { FA-C22:6 }
  - Docosapentaenoic acid content { FA-C22:5 }
  - Docosatetraenoic acid content { FA-C22:4 }
  - Eicosapentaenoic acid content { FA-C20:5 }
  - Fat melting point { FMP }
  - Lauric acid content { FA-C12:0 }
  - Lignoceric acid content { FA-C24:0 }
  - Linoleic acid content { FA-C18:2 }
  - Linoleic acid to linolenic acid ratio { LAALAR }
  - Linolenic acid content { FA-C18:3 }
  - Long-chain fatty acid content { LCFA }
  - Margaric acid content { FA-C17:0 }
  - Medium-chain fatty acid content { MCFA }
  - Monounsaturated fatty acid content { MUFA }
  - Monounsaturated to saturated fatty acid ratio { RMUSFA }
  - Myristic acid content { FA-C14:0 }
  - Myristic and palmitic acid ratio { FAMPR }
  - Myristoleic acid content { FA-C14:1 }
  - Oleic acid content { FA-C18:1 }
  - Oleic/stearic acid ratio { C18R }
  - Omega-3 to omega-6 fatty acid ratio { N3N6R }
  - Omega-3 unsaturated fatty acid content { FA-n3 }
  - Omega-6 polyunsaturated fatty acid content { FA-n6 }
  - Omega-6 to omega-3 fatty acid ratio { N6N3R }
  - Palmitic acid content { FA-C16:0 }
  - Palmitic aldehyde content { ALD-C16:0 }
  - Palmitoleic acid content { FA-C16:1 }
  - Palmitoleic/palmitic acid ratio { C16R }
  - Pentadecylic acid content { FA-C15:0 }
  - Polyunsaturated fatty acid content { PUFA }
  - Polyunsaturated to saturated fatty acid ratio { RPUSFA }
  - Saturated fatty acid content { SFA }
  - Stearic acid content { FA-C18:0 }
  - Stearic aldehyde content { ALD-C18:0 }
  - Total fatty acid content { TOTFA }
  - Trans-12-C18:1 fatty acid content { FA-C18:1n6 }
  - Trans-15-C18:1 fatty acid content { FA-C18:1n3 }
  - Trans-6/9-C18:1 fatty acid content { FA-C18:1n9-1 }
  - Trans-Vaccenic acid content { FA-C18:1t }
  - Tridecylic acid content { FA-C13:0 }
  - Unsaturated fatty acid content { PERUFA }
  - Unsaturated to saturated fatty acid ratio { RUSFA }

- Sensory characteristics
  - Abnormal flavor intensity { ABFLAV }
  - Abnormal odor intensity { ABODOR }
  - Adhesion { ADHES }
  - Beef flavor intensity { BEEFFLAV }
  - Beef odor intensity { BEEFOD }
  - Connective tissue amount { CONNTISS }
  - Cooking loss { COOKL }
  - Drip loss { DRIPL }
  - Fat luster { FLST }
  - Fat thickness { FATTH }
  - Juiciness { JUICE }
  - Meat firmness { FIRM }
  - Meat flavor score { FLAVS }
  - Muscle compression { COMP }
  - Myofibrillar fragmentation index { MFI }
  - Overall impression, sensory panel { OVIM }
  - Shear force { SF }
  - Tenderness score { TEND }
  - Texture { TEXT }
  - Water holding capacity { WHC }

Ramayo-Caldas *et al. BMC Genomics* 2014, **15**:232
http://www.biomedcentral.com/1471-2164/15/232



Ramayo-Caldas *et al. BMC Genomics* 2014, **15**:232
http://www.biomedcentral.com/1471-2164/15/232



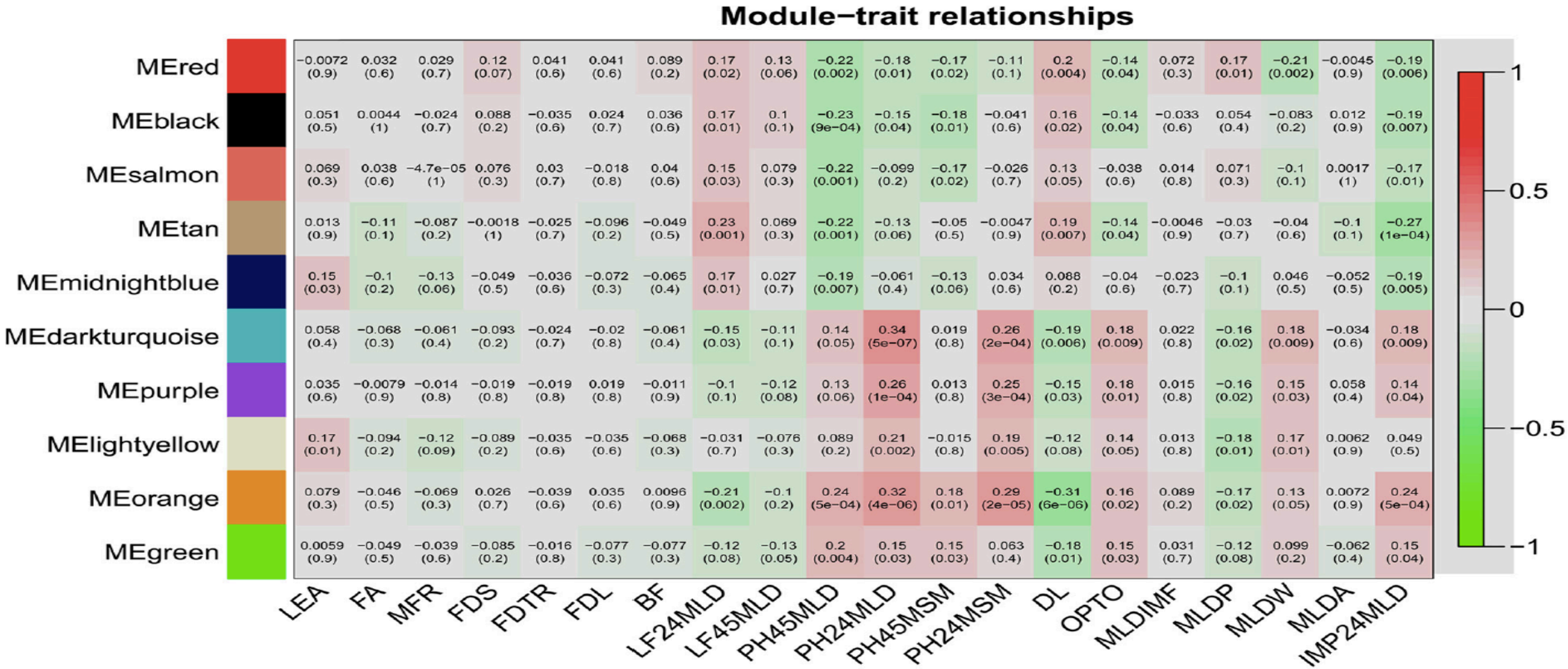



**Figure 1 Correlation matrix of module eigengene values obtained for mRNAs and phenotypes.** Weighted gene co-expression network analysis (WGCNA) groups genes into modules based on patterns of gene co-expression. Each of the modules was labelled with a unique color as an identifier. Twenty-two modules were identified; each module eigengene was tested for correlation with meat and carcass traits. Within each cell, upper values are correlation coefficients between module eigengene and the traits; lower values are the corresponding p-values.

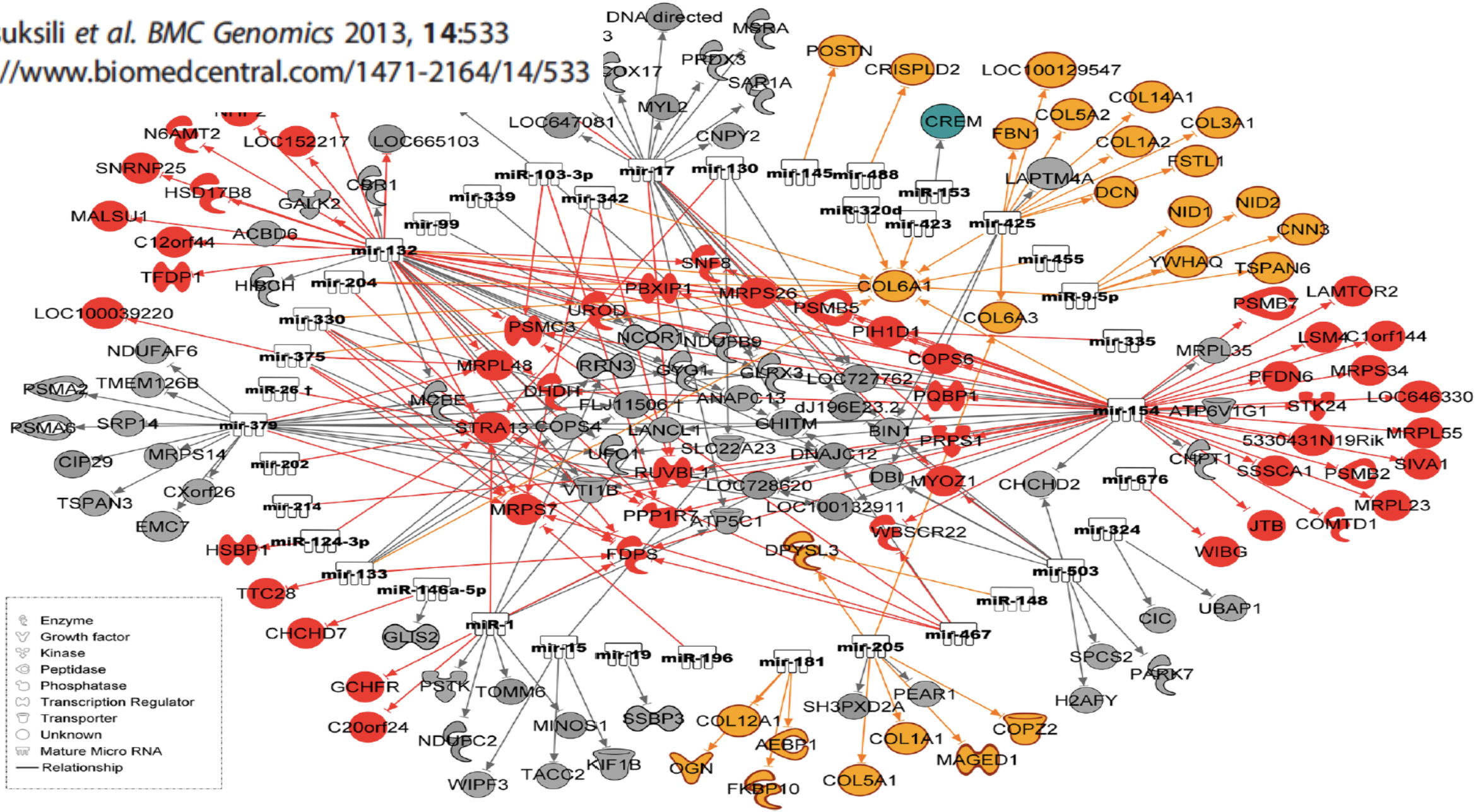



**Figure 3 Regulatory network of negatively-correlated mRNAs and miRNAs.** Genes in modules dark-turquoise, red, black, and tan that were significantly associated with meat quality and were negatively correlated with various miRNAs as indicated by the arrows. Colors of symbols of mRNA encoded proteins indicate the assignment to the respective module (grey = black).

# Revolutionary Advancements

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Bovine
- 786K
- 648K
- 77K
- 58K
- 20K
- 9K

Sheep
- 600K
- 50K

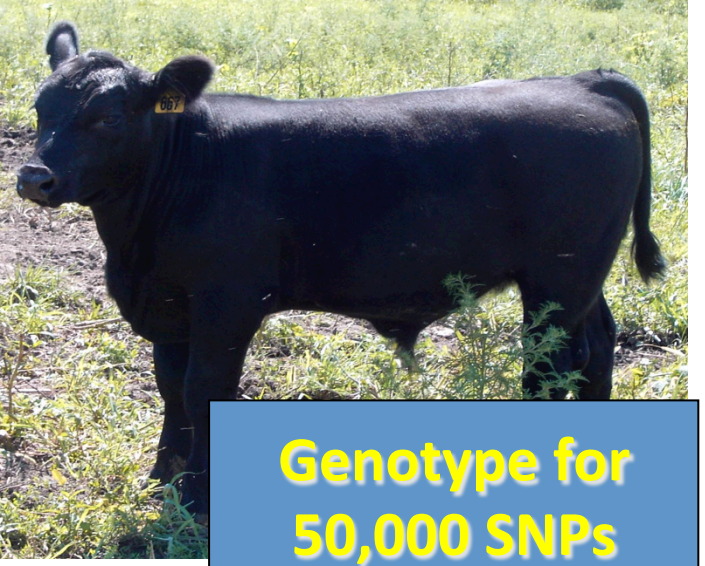
Chicken
- 90K

Porcine
- 70K
- 60K
- 8K

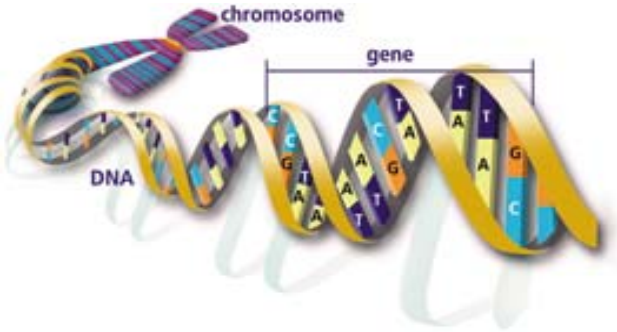
Goat
- 50K

Water Buffalo
- 90K

# Genomic Selection

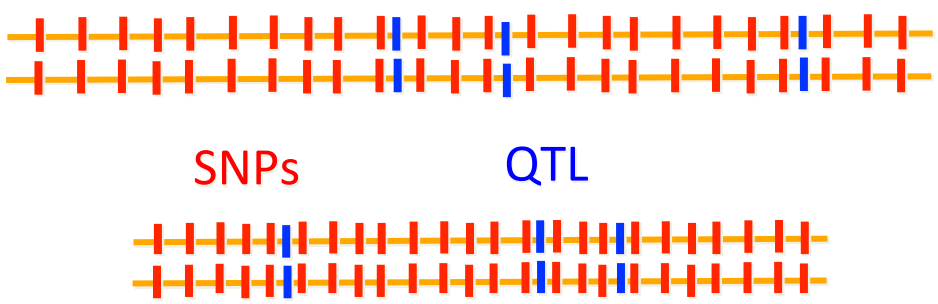


Genotype for
50,000 SNPs



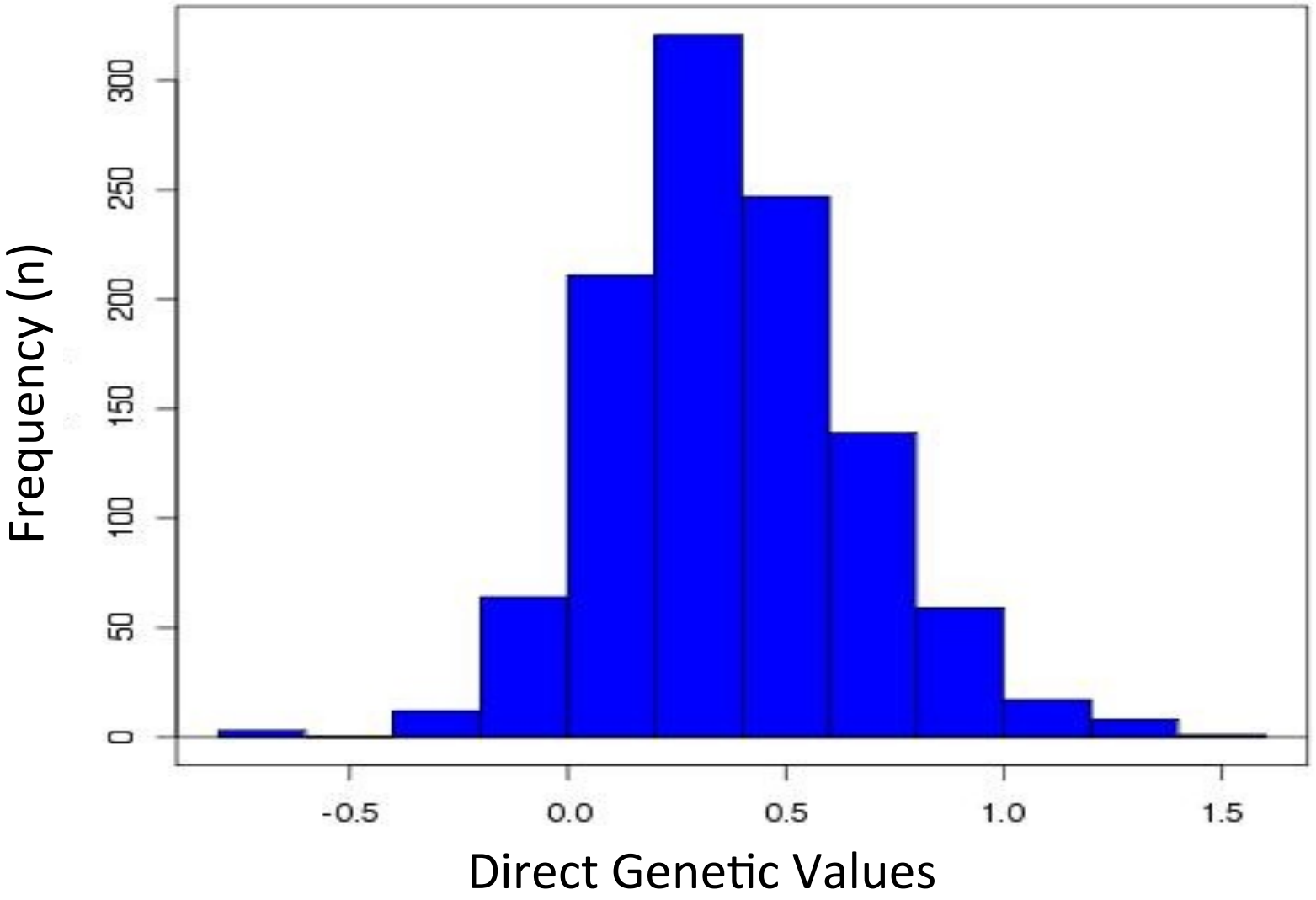
Collect Phenotype
(or estimated merit)
On 1,000 or so animals

Meuwissen et al. 2001




Estimate SNP-based
allelic, haplotypic
or substitution effects

# Direct Genetic Values



# Breeds, Numbers and Coverage

| Breed/Cross | Number | Fold Coverage |
|---|---|---|
| Holstein (Black and White) | 288 | 11.7 |
| Simmental (Dual and Beef) | 216 | 11.1 |
| Angus (Black and Red) | 138 | 9.4 |
| Jersey | 61 | 10.8 |
| Brown Swiss | 59 | 13.6 |
| Gelbvieh | 34 | 12.4 |
| Charolais | 33 | 8.7 |
| Hereford | 31 | 12.1 |
| Limousin | 31 | 7.5 |
| Guelph Composite | 30 | 7.9 |
| Beef Booster | 29 | 12.2 |
| Alberta Composite | 28 | 12.5 |
| Montbeliarde | 28 | 10.5 |
| AyrshireFinnish | 25 | 13.8 |
| Normande | 24 | 11.5 |
| Holstein (Red and White) | 23 | 7.9 |
| Swedish Red | 16 | 10.6 |
| Danish Red | 15 | 12.2 |
| Other Crosses | 11 | 8.5 |
| Belgian Blue | 10 | 11.0 |
| Piedmontese | 5 | 6.9 |
| Eringer | 2 | 17.6 |
| Galloway | 2 | 11.2 |
| Unknown | 2 | 13.0 |
| Scottish Highland | 2 | 15.4 |
| Pezzata Rossa Italiana | 1 | 28.0 |
| Romagnola | 1 | 11.9 |
| Salers | 1 | 6.8 |
| Tyrolean Grey | 1 | 13.1 |
| **Total/mean across breeds** | **1147** | **11.7** |
| **means across animals** | | **11** |

**541 bulls had 800k**

Concordance

All preBeagle 95.5%

All postBeagle 98.4%

Run3 postBeagle 98.8%

**244 parent/offspring pairs**

Opposing Homozygotes

All preBeagle 0.28%

All postBeagle 0.22%

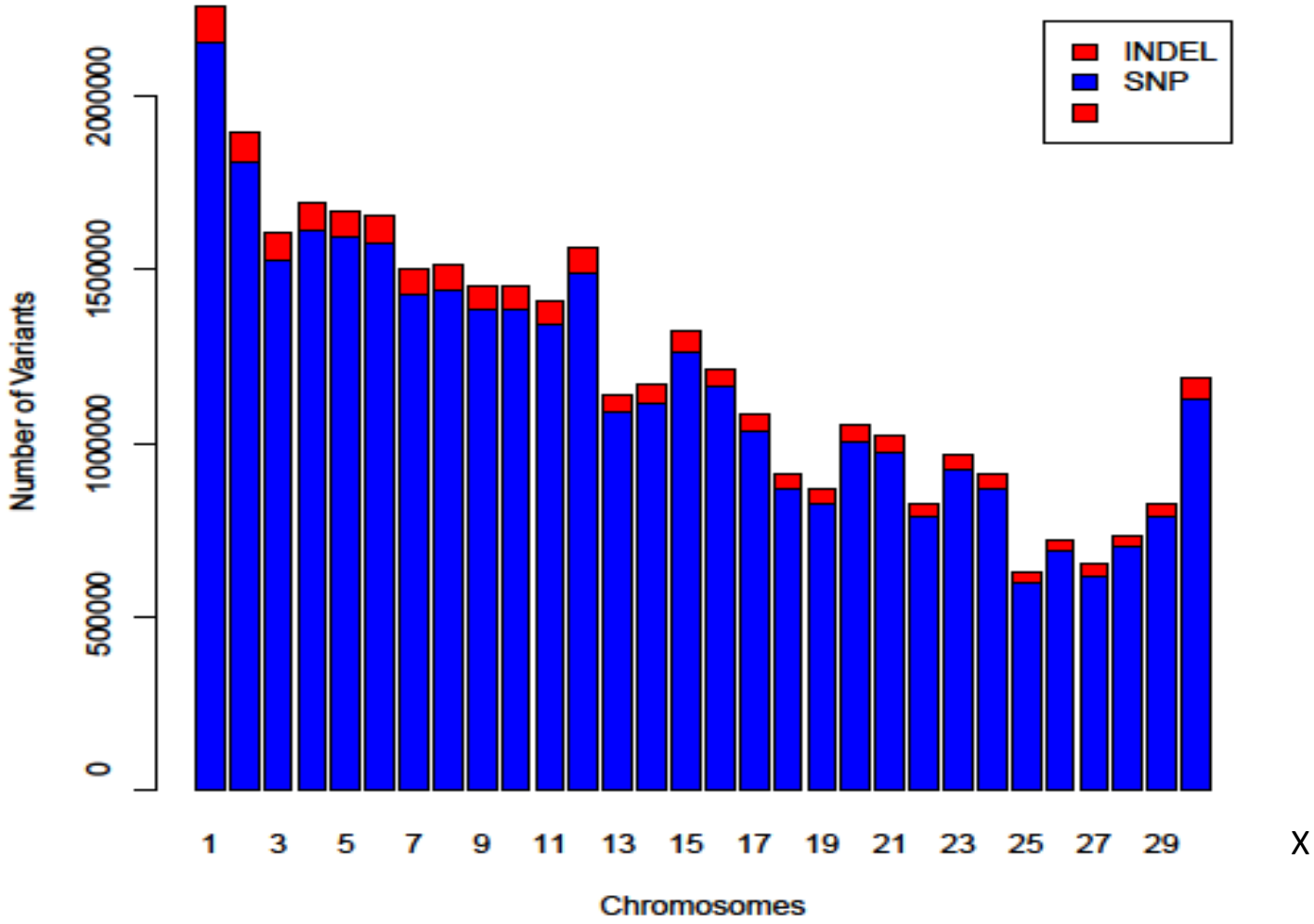
Run3 postBeagle 0.4%

Department of Environment and Primary Industries

biosciences research

# Results – 1000Bulls 4.0

- **36.9 million filtered variants**
  - ~ 27 breeds
  - 35.2 million SNP
  - 1.7 million INDEL
- **Run 3.0**
  - 15 breeds
  - 30.8 million variants
- **Run 2.0**
  - 4 breeds,
  - 28 million variants



# Deriving Gene Networks Underlying Fatty Acid Composition of the Triacylglycerol Lipid Fraction in Angus Longissimus Muscle

Justin W Buchanan, James E koltes, James M Reecy, Dorian J Garrick, Raluca G Mateescu

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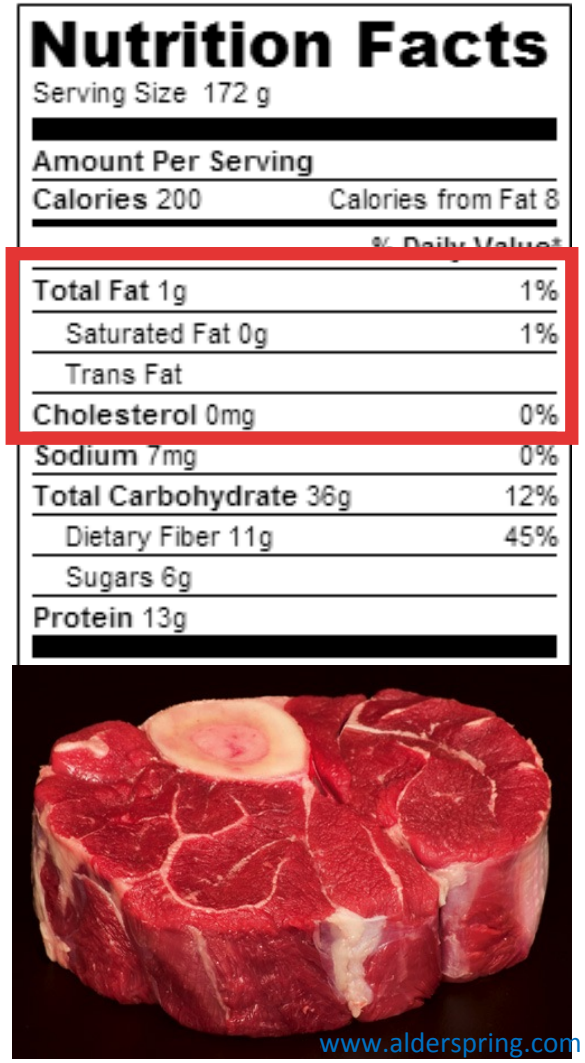
# Introduction

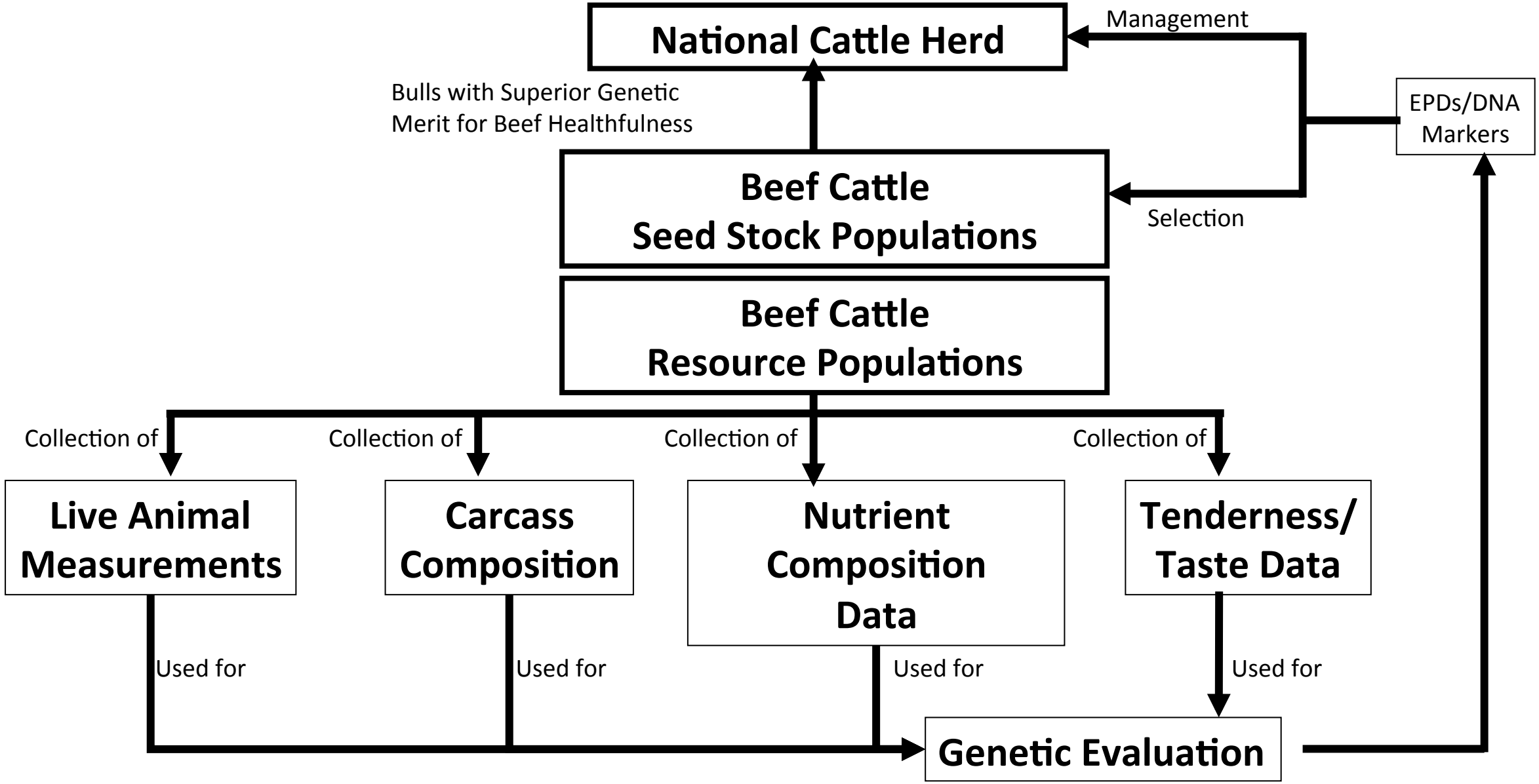
- Complex trait analysis
  - Marker information + performance and pedigree
- Interest in predictions with health related traits[1]
- Fatty acid content is important to consumers[2]




1. Zhang et al., 2008, Abe et al., 2009, Uemoto, 2010, Li et al., 2012, Daley et al., 2010
2. Lusk & Parker 2009

# Hypothesis

Illustrating interactions among significant markers across all sub-phenotypes will identify functional markers contributing the largest variation to the overall fatty acid profile in beef


National Cattle Herd
Management
Bulls with Superior Genetic
Merit for Beef Healthfulness
EPDs/DNA
Markers
Beef Cattle
Seed Stock Populations
Selection
Beef Cattle
Resource Populations
Collection of
Collection of
Collection of
Collection of
Live Animal
Measurements
Carcass
Composition
Nutrient
Composition
Data
Tenderness/
Taste Data
Used for
Used for
Used for
Used for
Genetic Evaluation

# Correlation between Fatty Acids and Meat Quality

**Table 7.** Pearson correlations between fatty acid composition and marbling score, Warner-Bratzler Shear force (WBSF), trained sensory traits, and thiobarbituric acid reactive substances (TBARS) of beef LM[1] (n = 1,715).

| Fatty acid | Marbling score | WBSF | Initial juiciness | Sustained juiciness | Initial tenderness | Overall tenderness | Connective tissue | Beef flavor | Painty/ fishy flavor | Livery/ metallic flavor | TBARS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C14:0 | **0.12** | **-0.06** | -0.02 | -0.02 | 0.04 | **0.07** | **0.07** | **0.06** | **-0.07** | -0.01 | 0.04 |
| C16:0 | **0.09** | -0.01 | 0.04 | **0.05** | -0.01 | 0.00 | -0.02 | **0.07** | 0.00 | -0.03 | 0.04 |
| C16:1 | **0.15** | **-0.09** | **0.10** | **0.09** | **0.06** | **0.06** | 0.04 | **0.10** | -0.04 | 0.00 | **-0.05** |
| C18:0 | **-0.15** | **0.09** | **0.08** | **0.06** | **-0.09** | **-0.12** | **-0.17** | 0.03 | **0.16** | -0.02 | **-0.06** |
| C18:1[2] | **0.10** | **-0.08** | **0.05** | **0.07** | **0.08** | **0.10** | **0.10** | 0.04 | **-0.11** | 0.00 | **0.09** |
| C18:2 | **-0.41** | **0.15** | **-0.12** | **-0.15** | **-0.19** | **-0.21** | **-0.20** | **-0.08** | **0.12** | 0.01 | **-0.05** |
| C18:3[3] | 0.04 | 0.03 | **-0.11** | **-0.08** | **-0.06** | -0.02 | -0.01 | -0.01 | **-0.10** | **-0.06** | **0.19** |
| C18:3[4] | 0.02 | -0.03 | **-0.16** | **-0.15** | -0.01 | 0.03 | **0.07** | -0.01 | **-0.07** | **-0.05** | **0.18** |
| SFA | -0.02 | 0.01 | 0.03 | 0.02 | **-0.05** | -0.04 | **-0.07** | **0.06** | 0.05 | -0.03 | 0.01 |
| MUFA | **0.16** | **-0.11** | **0.13** | **0.13** | **0.08** | **0.10** | **0.07** | **0.14** | -0.04 | 0.00 | **-0.07** |
| PUFA | **-0.38** | **0.13** | **-0.18** | **-0.19** | **-0.18** | **-0.18** | **-0.15** | **-0.08** | -0.05 | 0.00 | 0.01 |
| PUFA:SFA | **-0.34** | **0.11** | **-0.15** | **-0.16** | **-0.16** | **-0.15** | **-0.14** | **-0.07** | 0.02 | 0.01 | 0.00 |
| Σ n-3 fatty acids | **-0.07** | 0.02 | **-0.12** | **-0.11** | **-0.05** | -0.03 | 0.00 | **-0.06** | **-0.07** | -0.03 | **0.07** |
| Σ n-6 fatty acids | **-0.43** | **0.15** | **-0.15** | **-0.17** | **-0.19** | **-0.21** | **-0.20** | **-0.07** | **0.10** | 0.02 | -0.03 |
| n-3:n-6 ratio | 0.01 | 0.01 | -0.03 | -0.02 | -0.03 | -0.01 | 0.00 | -0.03 | -0.04 | -0.01 | **0.05** |

[1]Significant correlations are shown in bold ($P < 0.05$).
[2]C18:1 = *cis*-9 C18:1 + *cis*-11 C18:1 + *cis*-12 C18:1 + *cis*-13 C18:1 + *trans*-6/9 C18:1 + *trans*-10/11 C18:1 + *trans*-12 C18:1 + *trans*-15 C18:1.
[3]n-3 fatty acids.
[4]n-6 fatty acids.

Garmyn et al., 2010 *JAS*

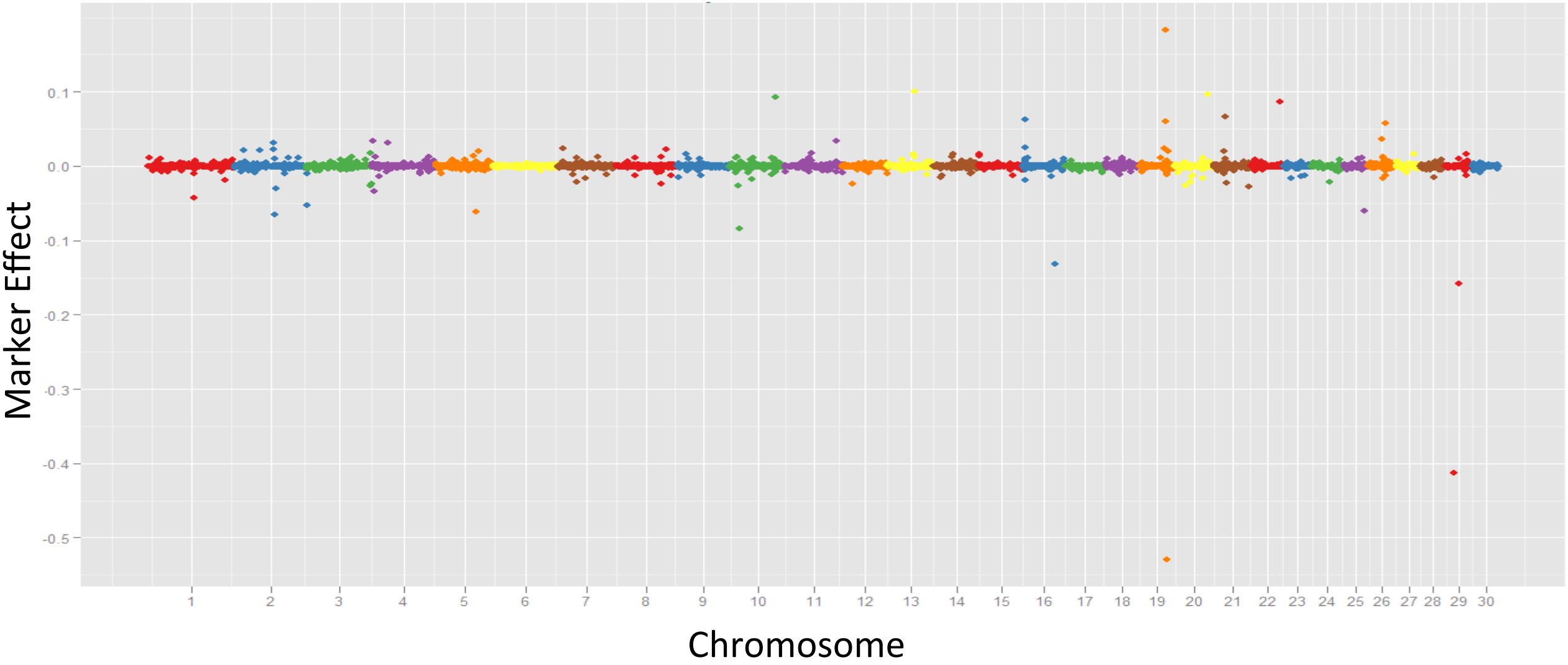
# Results – Lipid Fraction $h^2$

| | Triacylglycerol | | Phospholipid | |
|---|---|---|---|---|
| Trait | $\sigma^2_a$ | $h^2 \pm SE$ | $\sigma^2_a$ | $h^2 \pm SE$ |
| 14:0 | 0.130 | 0.587 ± 0.083 | 0.000 | 0.000 ± 0.030 |
| 16:0 | 1.443 | 0.537 ± 0.085 | 1.366 | 0.138 ± 0.070 |
| 16:1 | 0.147 | 0.509 ± 0.079 | 0.000 | 0.000 ± 0.044 |
| 18:0 | 0.595 | 0.437 ± 0.080 | 0.081 | 0.018 ± 0.038 |
| 18:1 cis-9 | 2.082 | 0.335 ± 0.076 | 2.371 | 0.069 ± 0.049 |
| 18:2 | 0.063 | 0.373 ± 0.074 | 2.490 | 0.082 ± 0.047 |
| SFA | 2.558 | 0.495 ± 0.085 | 0.392 | 0.030 ± 0.040 |
| MUFA | 2.267 | 0.461 ± 0.082 | 0.773 | 0.020 ± 0.038 |
| PUFA | 0.070 | 0.151 ± 0.052 | 0.035 | 0.001 ± 0.033 |
| PUFA, n-3 | 0.004 | 0.040 ±0 .034 | 0.000 | 0.000 ± 0.030 |
| PUFA, n-6 | 0.065 | 0.269 ± 0.067 | 0.918 | 0.019 ± 0.036 |

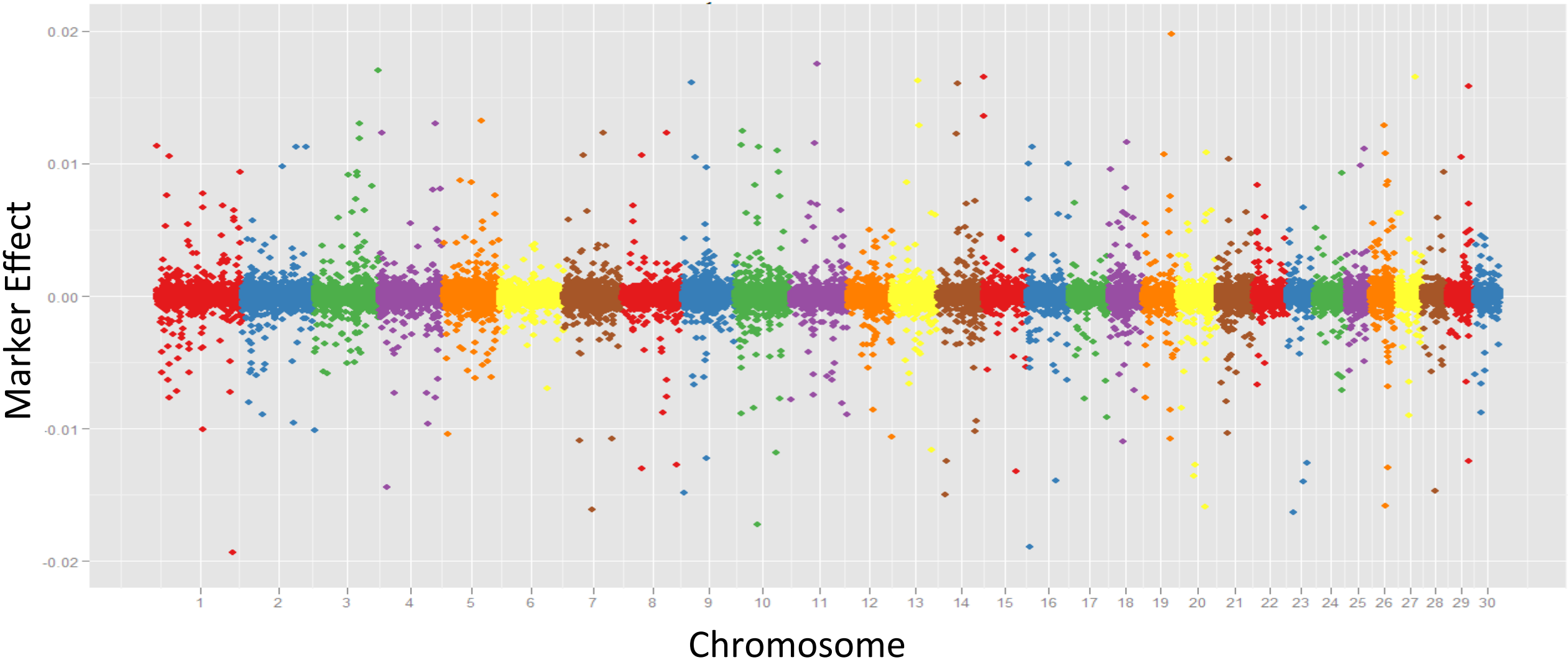
# Results – Genetic Correlations (TAG)

| Trait | SFA | MUFA | PUFA | REA | MARB | YG | EFAT | WBSF |
|---|---|---|---|---|---|---|---|---|
| SFA | | -0.99 | -0.49 | -0.04 | -0.11 | 0.31 | -0.03 | -0.30 |
| MUFA | -0.95 | | 0.36 | 0.05 | 0.12 | -0.37 | 0.03 | 0.24 |
| PUFA | -0.26 | -0.06 | | 0.11 | -0.20 | 0.02 | -0.16 | 0.27 |
| REA[3] | -0.07 | 0.05 | 0.05 | | 0.05 | -0.71 | -0.07 | -0.09 |
| MARB[4] | -0.05 | 0.06 | -0.07 | 0.00 | | -0.26 | 0.98 | -0.50 |
| YG[5] | 0.09 | 0.13 | -0.06 | -0.49 | 0.16 | | -0.24 | 0.14 |
| EFAT[6] | 0.01 | 0.15 | -0.08 | -0.07 | 0.75 | 0.25 | | -0.32 |
| WBSF[7] | -0.05 | 0.18 | 0.03 | 0.04 | -0.23 | 0.00 | -0.19 | |

# Myristic Acid (C14:0)

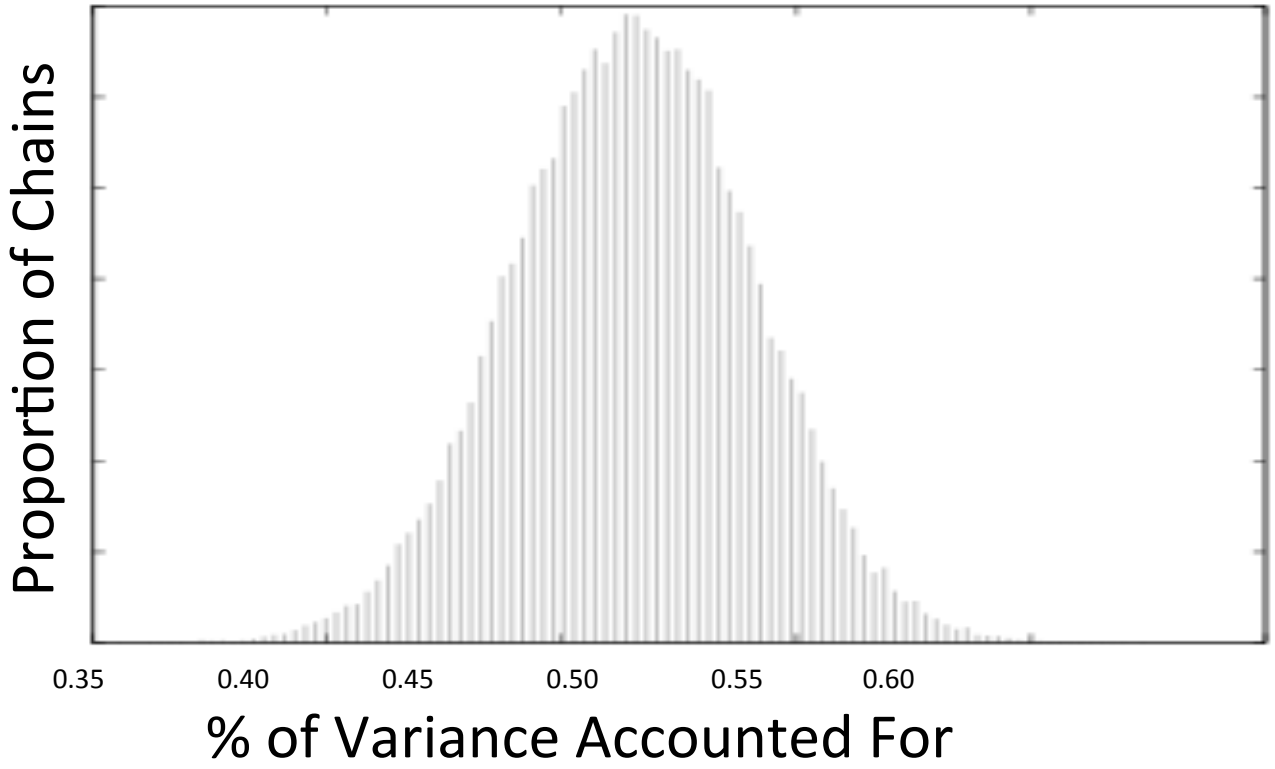


# Myristic Acid (C14:0)

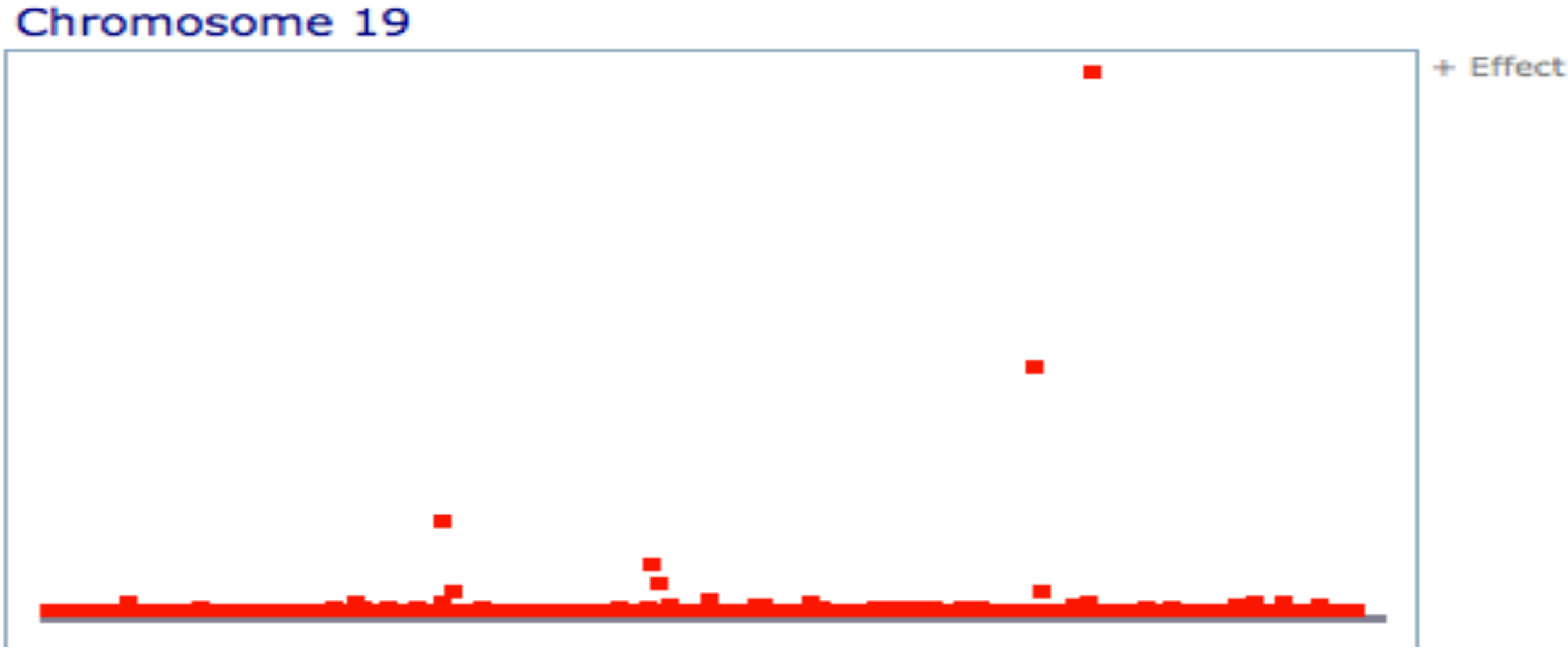


# Myristic Acid (C14:0)

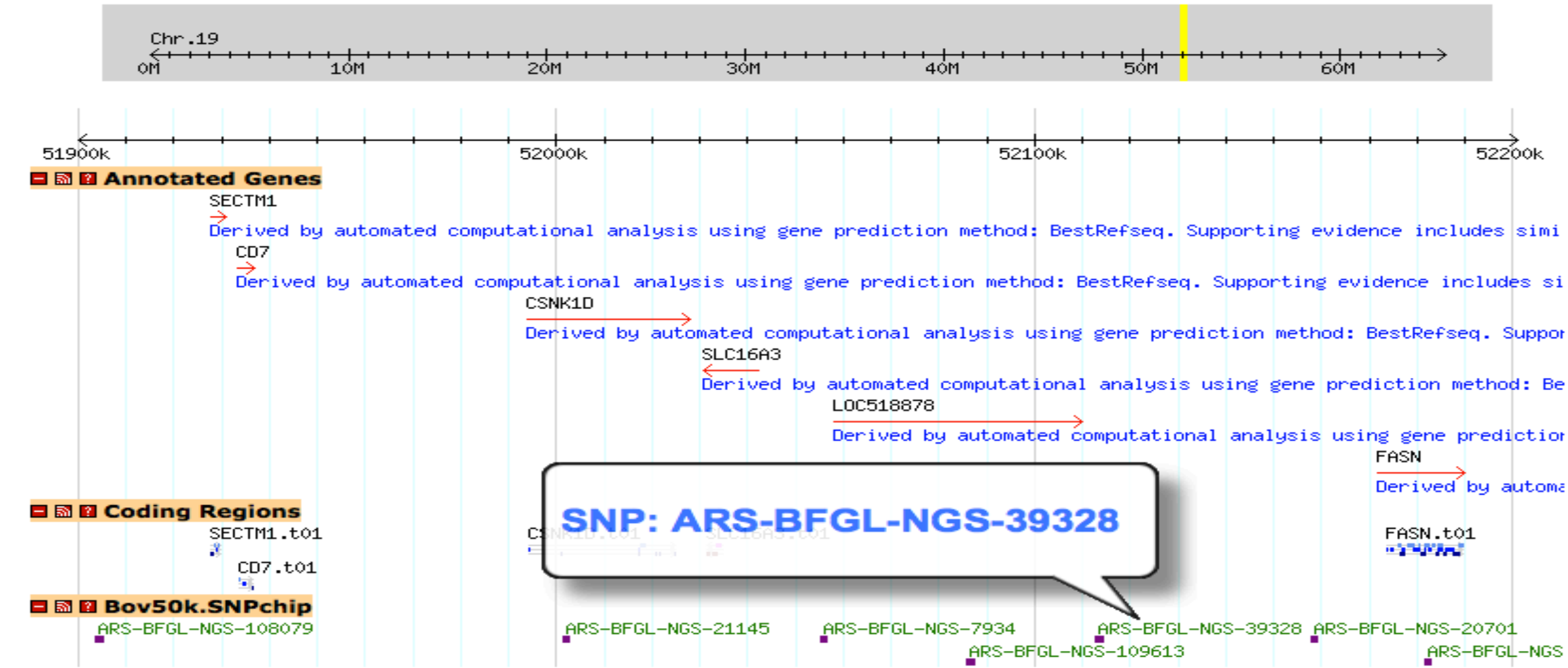
- Pedigree Heritability = 0.63 ± 0.09
- Marker Heritability = 45.1%
- 54 Markers



# Myristic Acid (C14:0)



# Myristic Acid (C14:0)



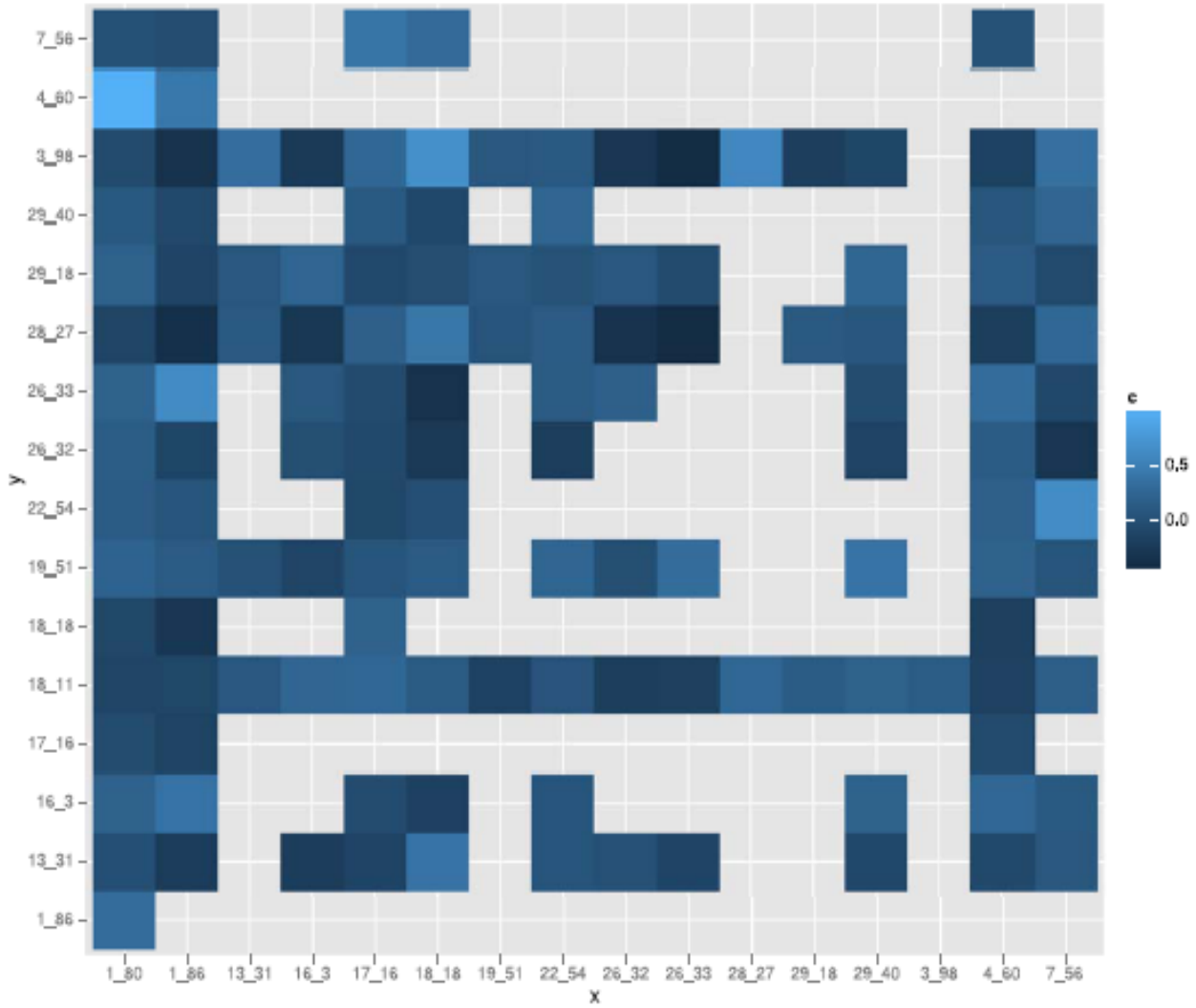
# Fatty Acid Synthase

| Lipid | AA | AG | GG |
|---|---|---|---|
| 14:0 | $2.85^{a}$ | $2.68^{b}$ | $2.45^{c}$ |
| 16:0 | $26.48^{a}$ | $26.91^{b}$ | $25.67^{b}$ |
| 18:1 | $40.38^{b}$ | $40.98^{b}$ | $42.27^{a}$ |
| SFA | $43.82^{a}$ | $43.04^{b}$ | $42.82^{b}$ |
| MUFA | $44.96^{b}$ | $45.39^{a,b}$ | $46.49^{a}$ |
| 16:0/14:0 | $9.52^{b}$ | $9.87^{b}$ | $11.38^{a}$ |
| HI | $1.50^{b}$ | 1.57 | $1.63^{a}$ |

Zhang et al., 2008; Animal Genetics 39(1):62-70.

# Results – Genomic Window Correlations

- Correlations among the top 17 windows explaining 90% of the genetic variation (posterior prob. P > 0.5) in C16:0 were obtained using PCIT.
- Correlations were generated in PCIT using 1 Mb window breeding values obtained from GenSel across all 39 individual fatty acid phenotypes.

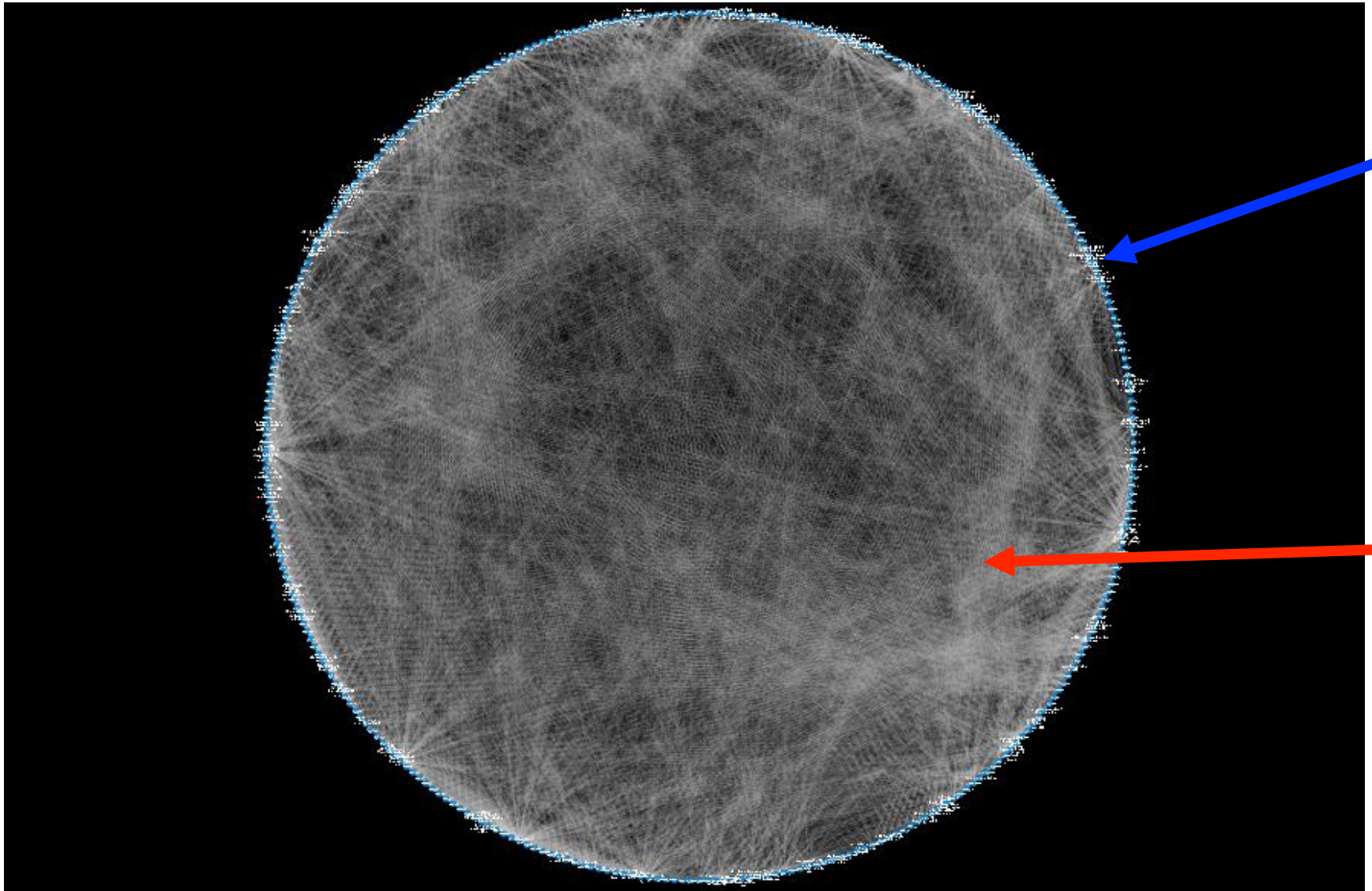


# Results - GWAS

- **Reference phenotype - 16:0**
  - Identified 52 1Mb windows explaining 95% of genetic variation (P > 0.25)
    - Top 5 - 260 SNP's
- 16:0 ➡ 260 SNP's ➡ 38 remaining phenotypes

# Results – Network Visualization

- 260 connected SNP's

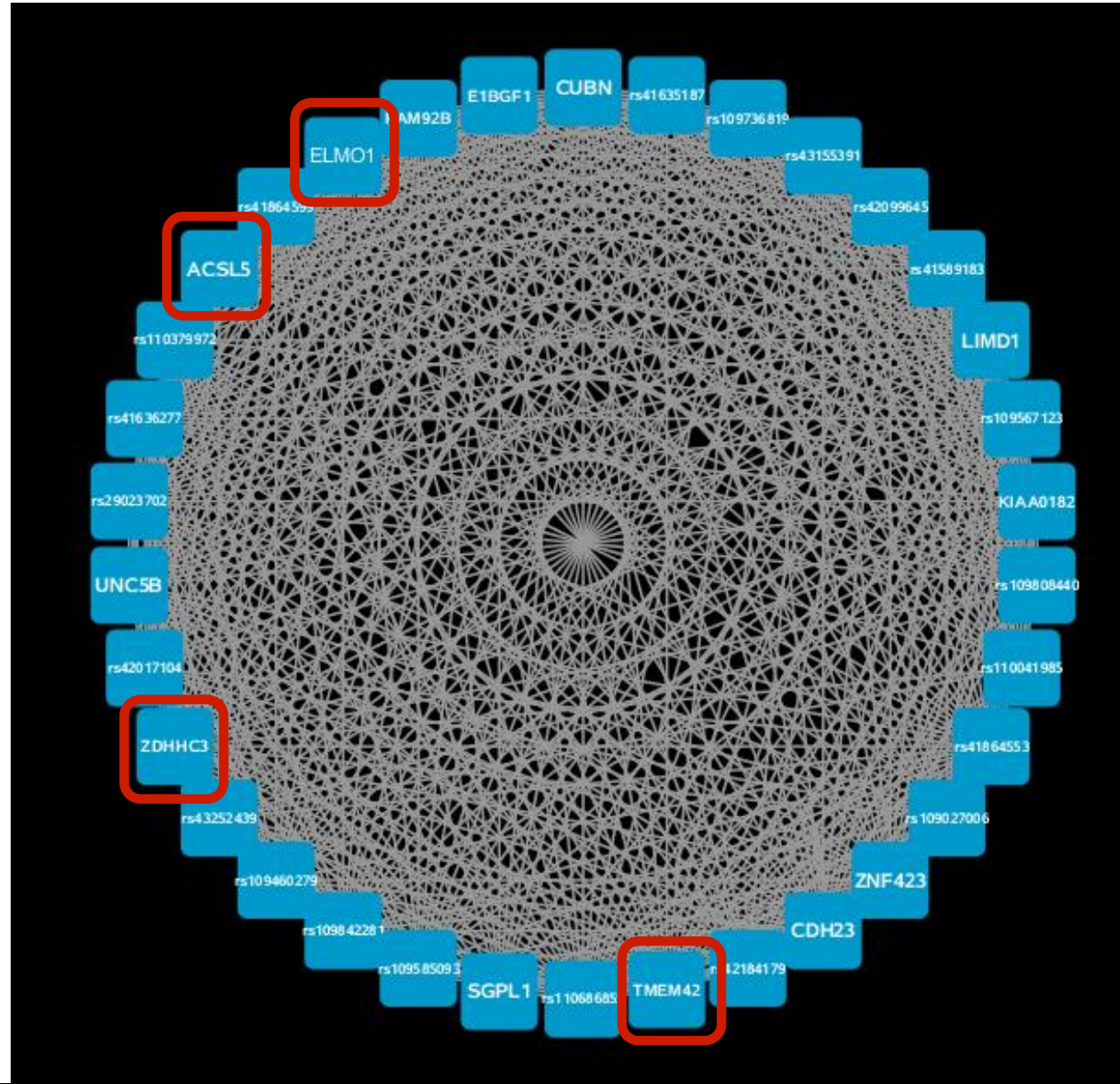


Nodes indicate SNP's

Edges indicate correlations

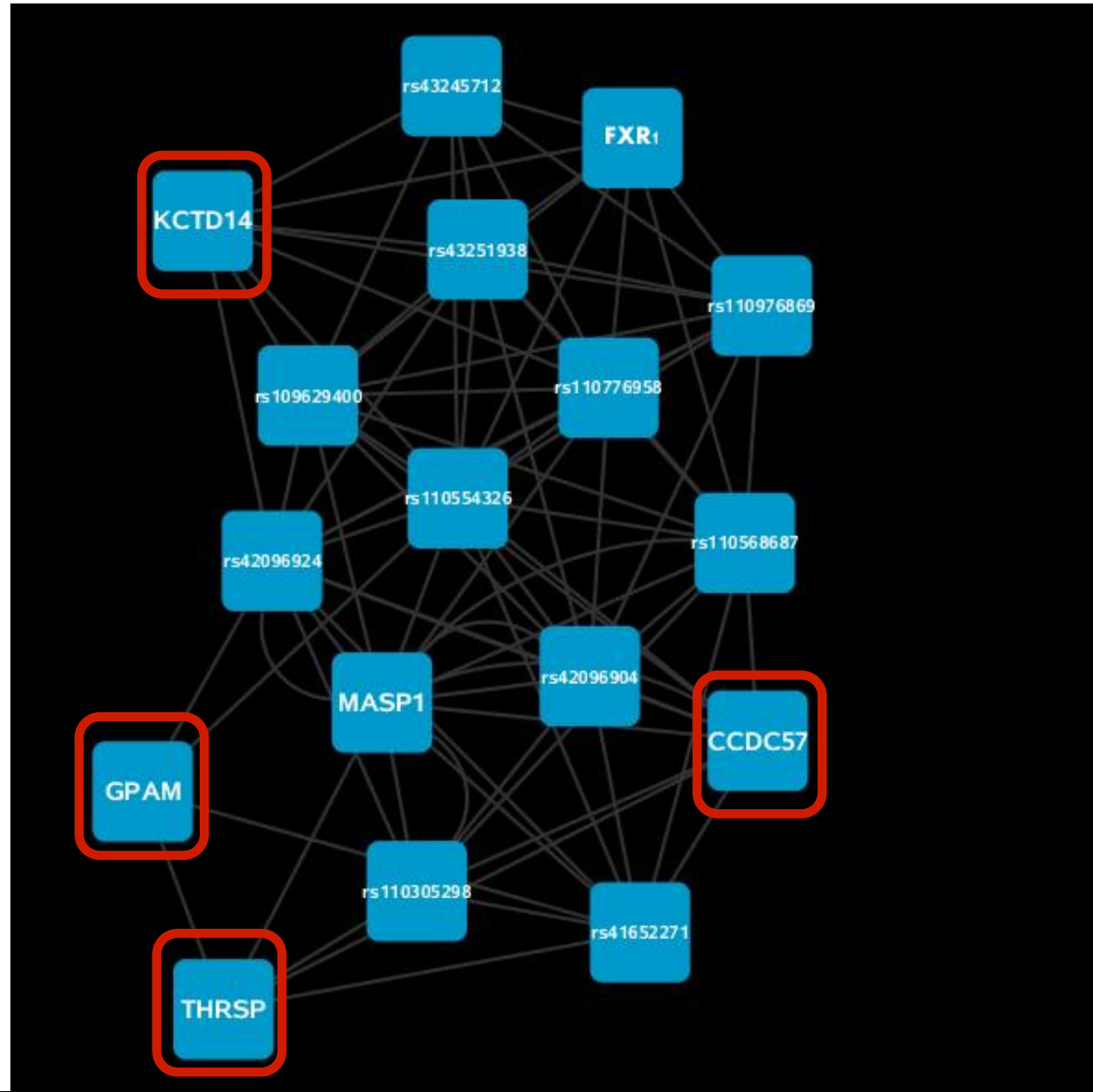
# Results – Clustered Network Scoring

- ACSL5 – *triacylglycerol synthesis*
- ELMO1 – *lipid elongation*
- ZDHHC3 – *broad palmitotransferase*
- TMEM42 – *lipid membrane transport*



# Results – Clustered Network Scoring

- GPAM - *Synthesis of glycerolipids*
- THRSP[1] – *Thyroid responsive hormone*
- CCD57[2] – *adipose morphology*
- KCTD14 – *potassium channel activation*

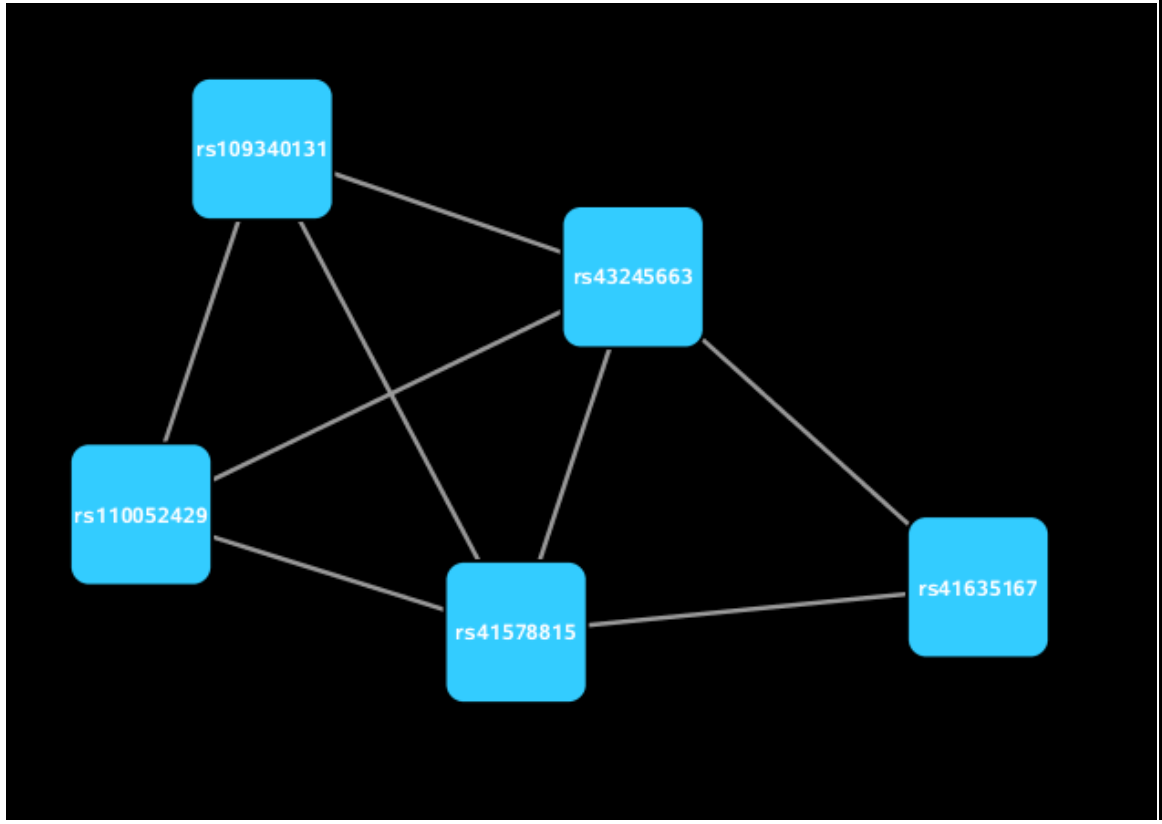


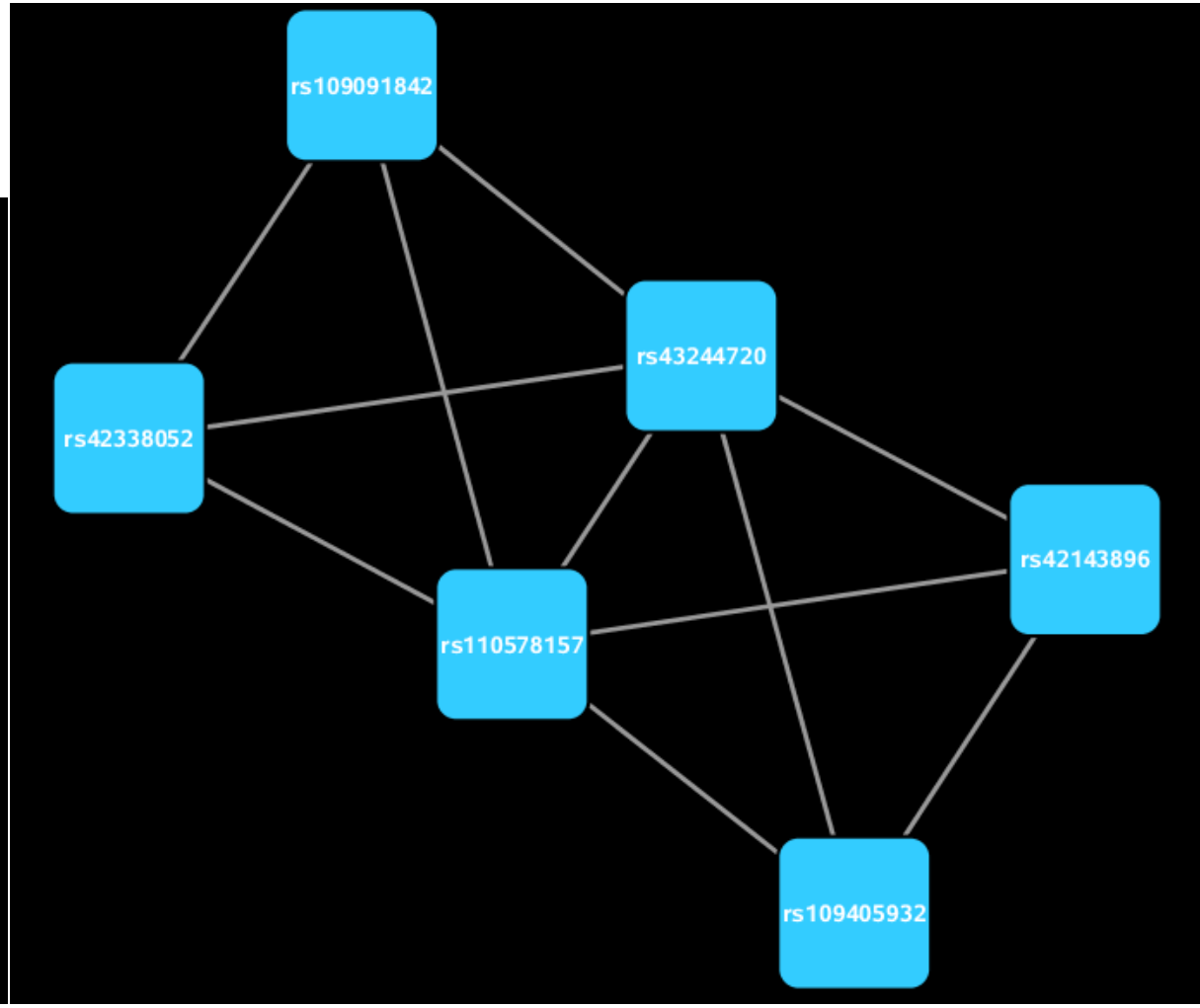

1) Kinlw et al.: 1995. J. Biol. Chem. 270, 16615-16618.

2) Gerdin: 2010. Acta Opthal. 88(s246).

# Results – Clustered Network Scoring

- Unknown SNP clustering across TAG.



# Conclusions

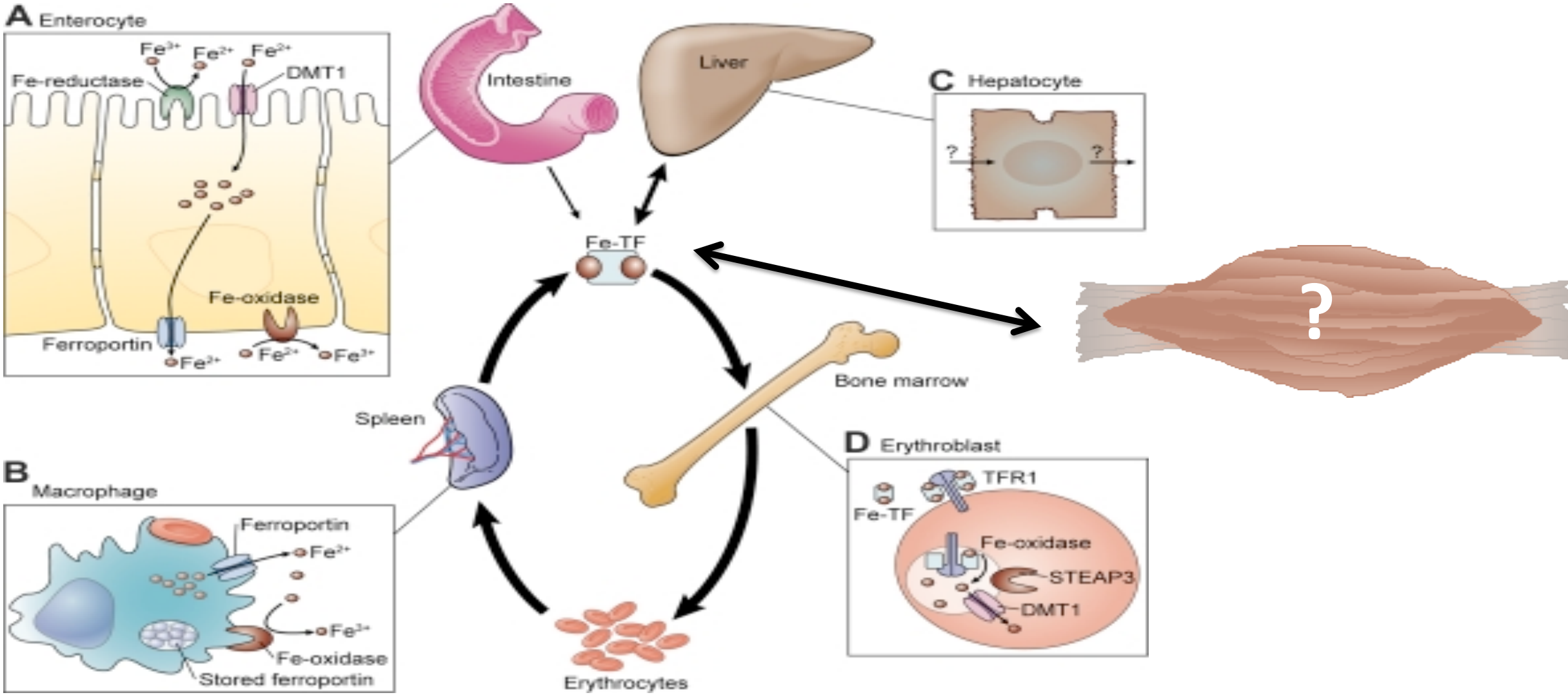
- Approximately 30 clusters of SNPs acting in concert
- Clusters of markers
  - De-novo fatty acid synthesis
- Generate a list of enriched markers and pathway
  - DAVID, GSEA, etc.

# A Systems-Genetics Analysis of Bovine Skeletal Muscle Iron Content

**James Reecy**

**Iowa State University**

# Iron regulation Background




Modified from Andrews 2008 *Blood* 112: 219

# Why study iron in beef?

- Importance to human health: nutritional requirement
- Beef is an excellent source of dietary iron
- Relationships to meat quality traits are known

# Correlation between Minerals and Meat Quality

**Table 6.** Pearson correlations between mineral concentrations and marbling score, Warner-Bratzler Shear force (WBSF), trained sensory traits, and thiobarbituric acid reactive substances (TBARS) of beef LM[1] (n = 1,715).

| Mineral | Marbling score | WBSF | Initial juiciness | Sustained juiciness | Initial tenderness | Overall tenderness | Connective tissue | Beef flavor | Painty/ fishy flavor | Livery/ metallic flavor | TBARS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Calcium | -0.01 | **-0.06** | 0.03 | 0.01 | 0.03 | 0.04 | **0.07** | 0.02 | -0.04 | **0.05** | **-0.08** |
| Copper | 0.02 | **-0.06** | **0.14** | **0.11** | **0.06** | 0.04 | 0.03 | **0.13** | 0.02 | **0.09** | **-0.22** |
| Iron | **0.06** | -0.03 | **0.14** | **0.11** | **0.06** | 0.04 | 0.03 | **0.13** | 0.02 | **0.09** | **-0.23** |
| Magnesium | **-0.05** | **-0.07** | **0.13** | **0.06** | **0.06** | 0.01 | 0.01 | **0.11** | **0.09** | **0.09** | **-0.46** |
| Manganese | **0.13** | **-0.06** | **0.05** | 0.04 | **0.07** | **0.06** | 0.04 | **0.05** | 0.04 | 0.01 | **-0.16** |
| Phosphorus | **-0.06** | **-0.09** | **0.09** | 0.02 | **0.07** | 0.03 | 0.05 | **0.10** | 0.04 | **0.09** | **-0.40** |
| Potassium | -0.03 | **-0.14** | **0.06** | -0.02 | **0.10** | **0.05** | **0.06** | **0.14** | **0.06** | **0.07** | **-0.36** |
| Sodium | 0.04 | **-0.14** | **0.15** | **0.06** | **0.16** | **0.12** | **0.10** | **0.13** | **0.07** | **0.13** | **-0.32** |
| Zinc | -0.03 | 0.02 | **0.08** | 0.04 | 0.02 | -0.01 | -0.01 | **0.07** | 0.02 | 0.04 | **-0.07** |

[1]Significant correlations are shown in bold ($P < 0.05$).

Garmyn et al., 2010 *JAS*

# Mineral Composition

| Mineral | ug/gram | Pedigree Heritability | Marker Heritability |
|---|---|---|---|
| Iron | 14.4 | 0.54 ± 0.09 | 0.407 |
| Zinc | 38.9 | 0.09 ± 0.04 | 0.180 |
| Magnesium | 254.6 | 0.06 ± 0.04 | 0.157 |
| Manganese | 0.07 | 0.01 ± 0.03 | 0.218 |
| Phosphorus | 1965.9 | 0.03 ± 0.03 | 0.032 |
| Sodium | 489.4 | 0.18 ± 0.06 | 0.00056 |

# Genetic Markers associated with Total Iron

**In collaboration with JR Tait**

**Bayes Cpi, estimate scale factor (pi ~ 0.993; marker $h^2$ ~ 0.44)**

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

doi:10.1111/j.1365-2052.2011.02224.x

# Genetic polymorphisms in bovine *transferrin receptor 2* (*TFR2*) and *solute carrier family 40* (*iron-regulated transporter*), *member 1* (*SLC40A1*) genes and their association with beef iron content

Q. Duan*, R. G. Tait Jr†, M. S. Mayes†, D. J. Garrick†, Q. Liu*, A. L. Van Eenennaam‡, R. G. Mateescu§, D. L. Van Overbeke§, A. J. Garmyn§, D. C. Beitz*,† and J. M. Reecy†

*Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011, USA. †Department of Animal Science, Iowa State University, Ames, IA 50011, USA. ‡Department of Animal Science, University of California, Davis, CA 95616, USA. §Department of Animal Science, Oklahoma State University, Stillwater, OK 74078, USA

# Examples of candidate causal mutations

**Under GWAS peaks**

- Chr1: TFRC- frame-shift mutation
- Chr2: SLC40A1- non-synonymous mutation
- Chr17: SMAD1- multiple non-synonymous mutations
- Chr24: SMAD4- non-synonymous mutation
- Chr25: HMOX2- non-synonymous mutation

**Interesting Biological Candidate SNPs**

- IREB2- early stop codon (chr21)
- HFE2- multiple non-synonymous mutations (chr3)
- BMP6- early stop codon (chr23)
- STAT3- multiple non-synonymous mutations (chr 19)

*All genes listed have documented roles in iron homeostasis

# Measuring Gene Expression with RNA-sequencing (RNA-seq) technology

**Illumina HiSeq**

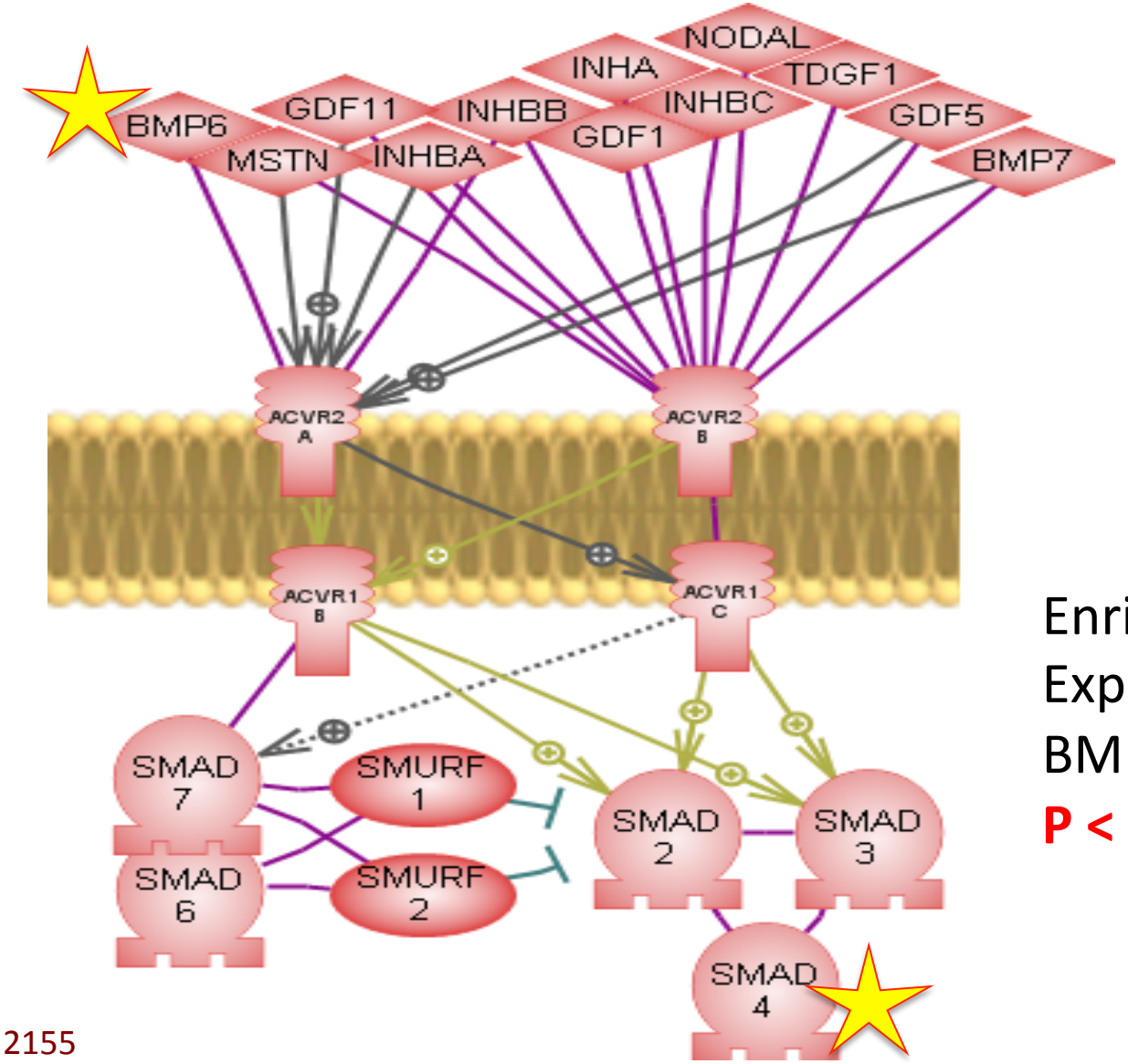
- 10 extreme total iron samples
- ISU Angus breeding project
- Samples were multiplexed
- TopHat* used to align to UMD3.1 reference genome
- Quantification with HTSeq^

*Langmead et al., 2009 *Genome Biol.* 10: R25
^Trapnell et al., 2009 *Nature Biotech*. 28: 511

# Differentially expressed genes

N = 3010 differentially expressed genes at $q < 0.05$ !!!

# Gleaning information from RNA-seq
# A proof of principal analysis



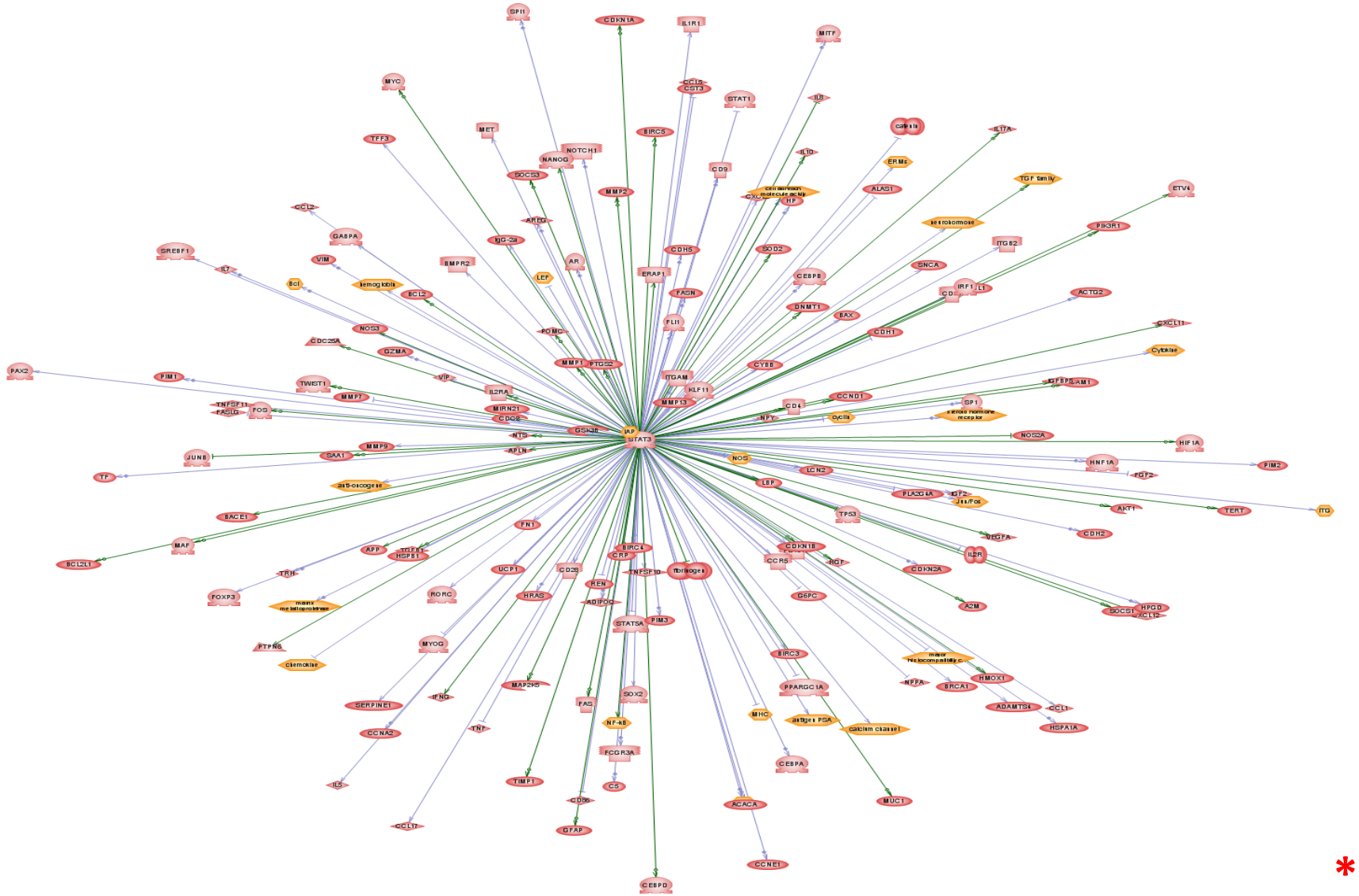

Enrichment of differentially Expressed genes in the BMP6 pathway

**$P < 6.38E^{-8}$**

**Pathway Studio software**

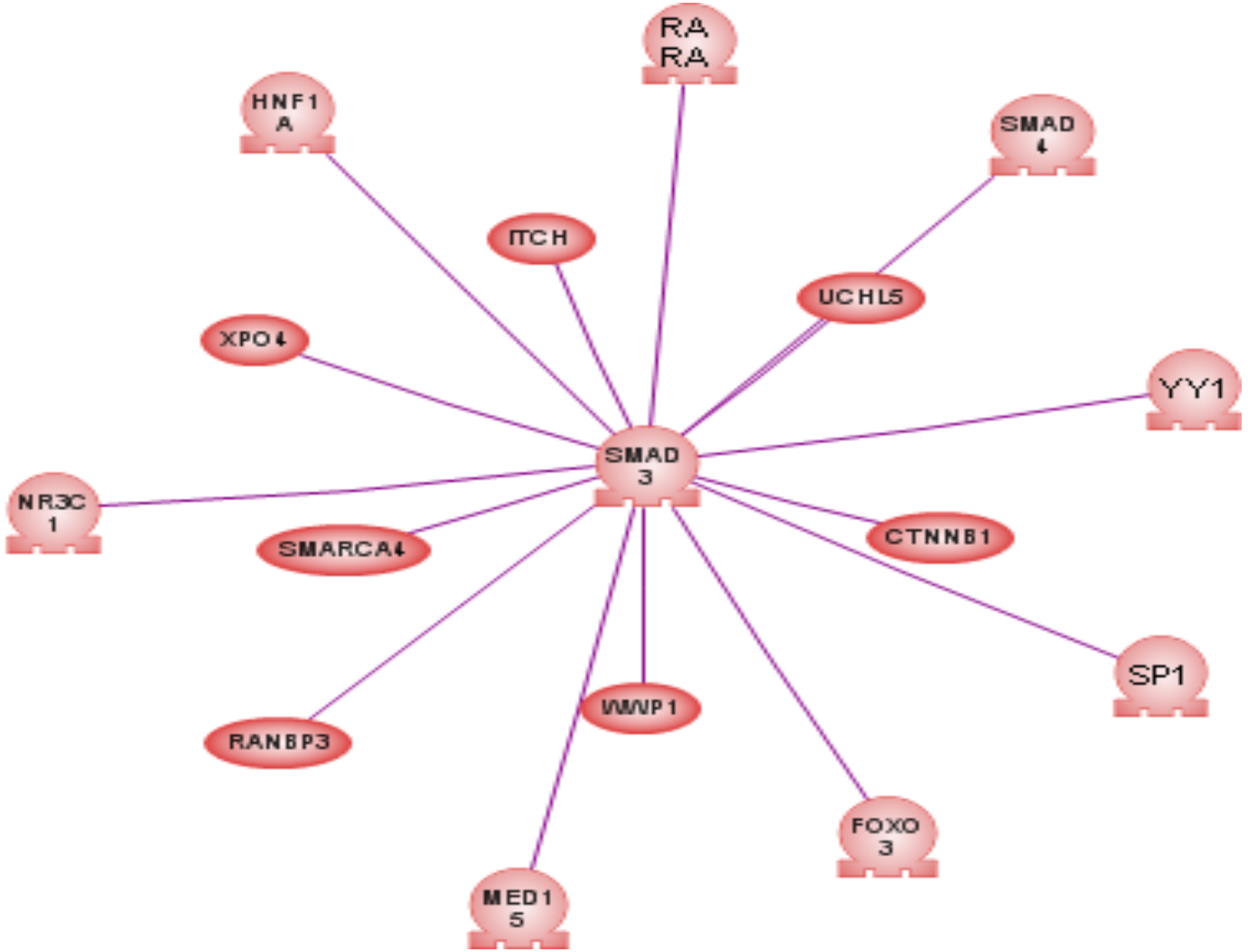
Nikitin et al., 2003 *Bioinformatics* 19: 2155

# Enrichment of a known regulator: STAT3*




*P < $9E^{-44}$

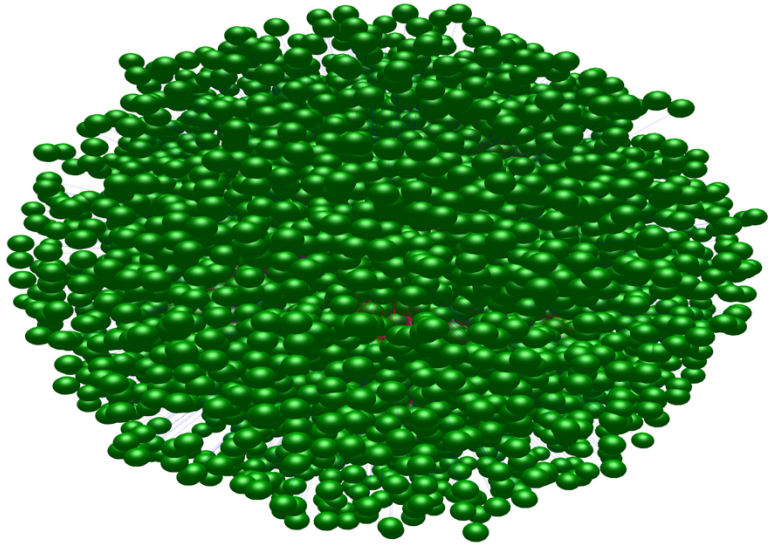
# A potential novel regulator: SMAD3*




*P < 3.07E-33

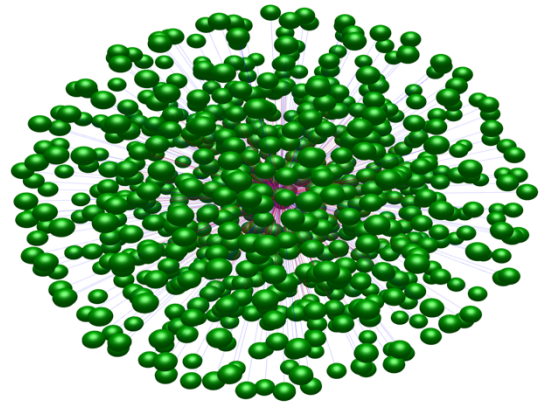
# PCIT- differential “hubbing”

**High Iron group**



- **>7000** correlates with CNOT7

**Low Iron group**



- **<700** correlates with CNOT7

Figure by Biolayout Express 3D: Theocharidis et al., 2009. Nat Prot. 4: 1535-1550.

# Network analysis #2: PCIT[+]

**P**artial **C**orrelation and **I**nformation **T**heory Approaches

**Candidate = CNOT7**

- Greatest amount of differential partial correlation
  - >**7000** correlated transcripts ($p < 0.05$)- HIGH iron
  - **<700** correlated transcripts ($p < 0.05$)- LOW iron
- CNOT7 is DE ($q < 1 * E^{-16}$)
- CNOT7 contains non-synonymous, splice-site, 5' and 3' UTR SNPs
- Regulates BMP family members via interaction with Tob*
  - BMP2 repression
  - BMP2 signals Hemojuvelin to regulate Hepcidin[+]

[+]Watson-Haigh et al., 2009 Bioinformatics 26: 411

*Washio-Oikawa et al., 2007 JBMR 22: 1217

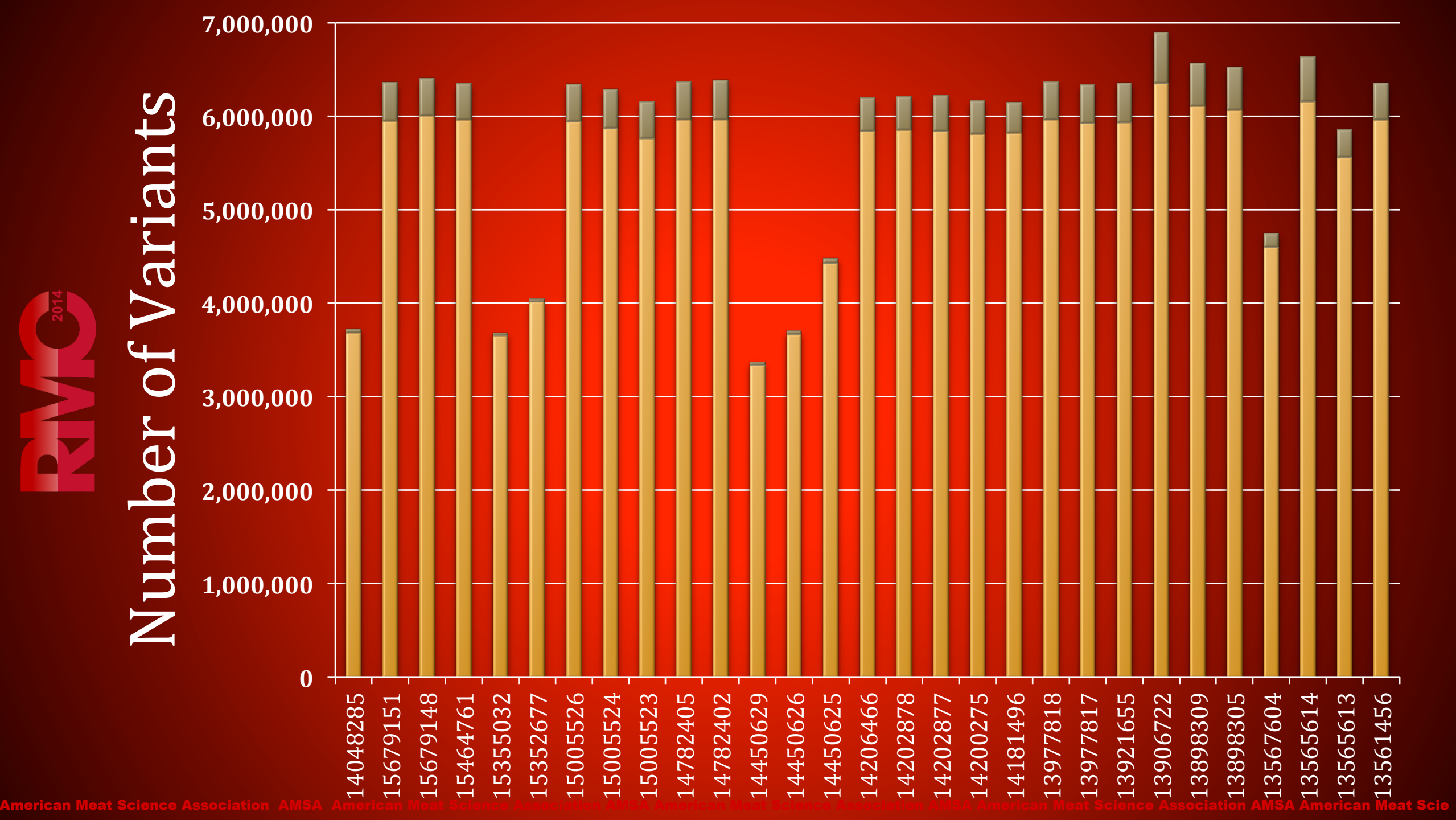
[+]Truksa et al., 2009 Blood 113: 688; Lin et al., 2007 Blood 110: 2187

# Summary & Implications

- Bovine skeletal muscle total iron content may be a good model to better understand iron homeostasis in the context of hemochromatosis and anemia in humans
- It is unknown if high iron content results in pathologies similar to hemochromatosis or not
- Beef cattle can be selected for altered iron content

# Next-Generation Genomic Selection Advancements

American Meat Science Association AMSA American Meat Science Association AMSA American Meat Science Association AMSA American Meat Science Association AMSA American Meat Scie


RMC 2014
Number of Variants
7,000,000
6,000,000
5,000,000
4,000,000
3,000,000
2,000,000
1,000,000
0
14048285
15679151
15679148
15464761
15355032
15352677
15005526
15005524
15005523
14782405
14782402
14450629
14450626
14450625
14206466
14202878
14202877
14200275
14181496
13977818
13977817
13921655
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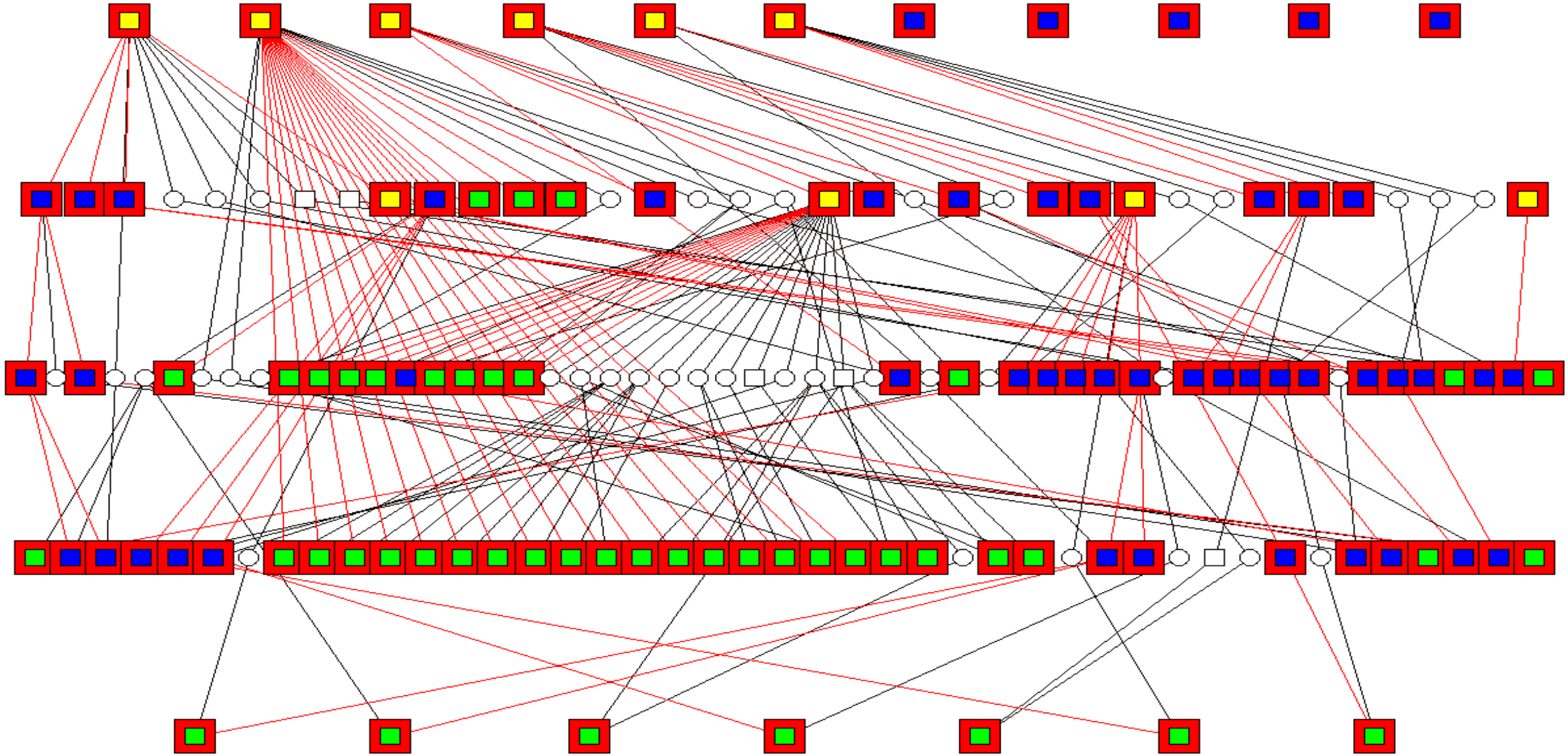
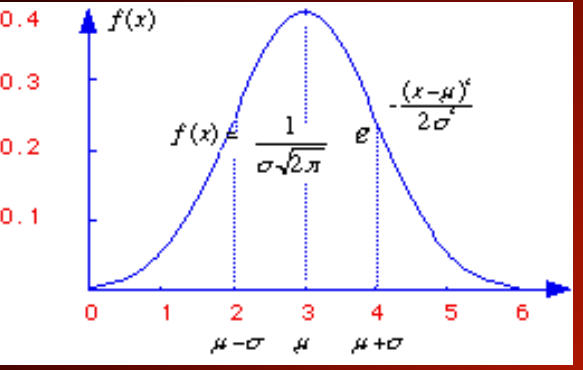
American Meat Science Association AMSA American Meat Science Association AMSA American Meat Science Association AMSA American Meat Science Association AMSA American Meat Scie

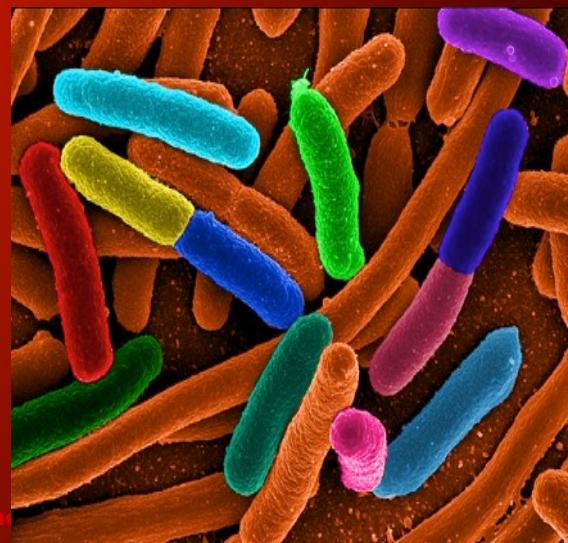
# Angus Pedigree





# The Metagenome and Variation in Growth and Carcass Traits

James Reecy, Mary Sue Mayes, Luke Kramer, Alysta Markey, Eric Fritz-Waters, James Koltes



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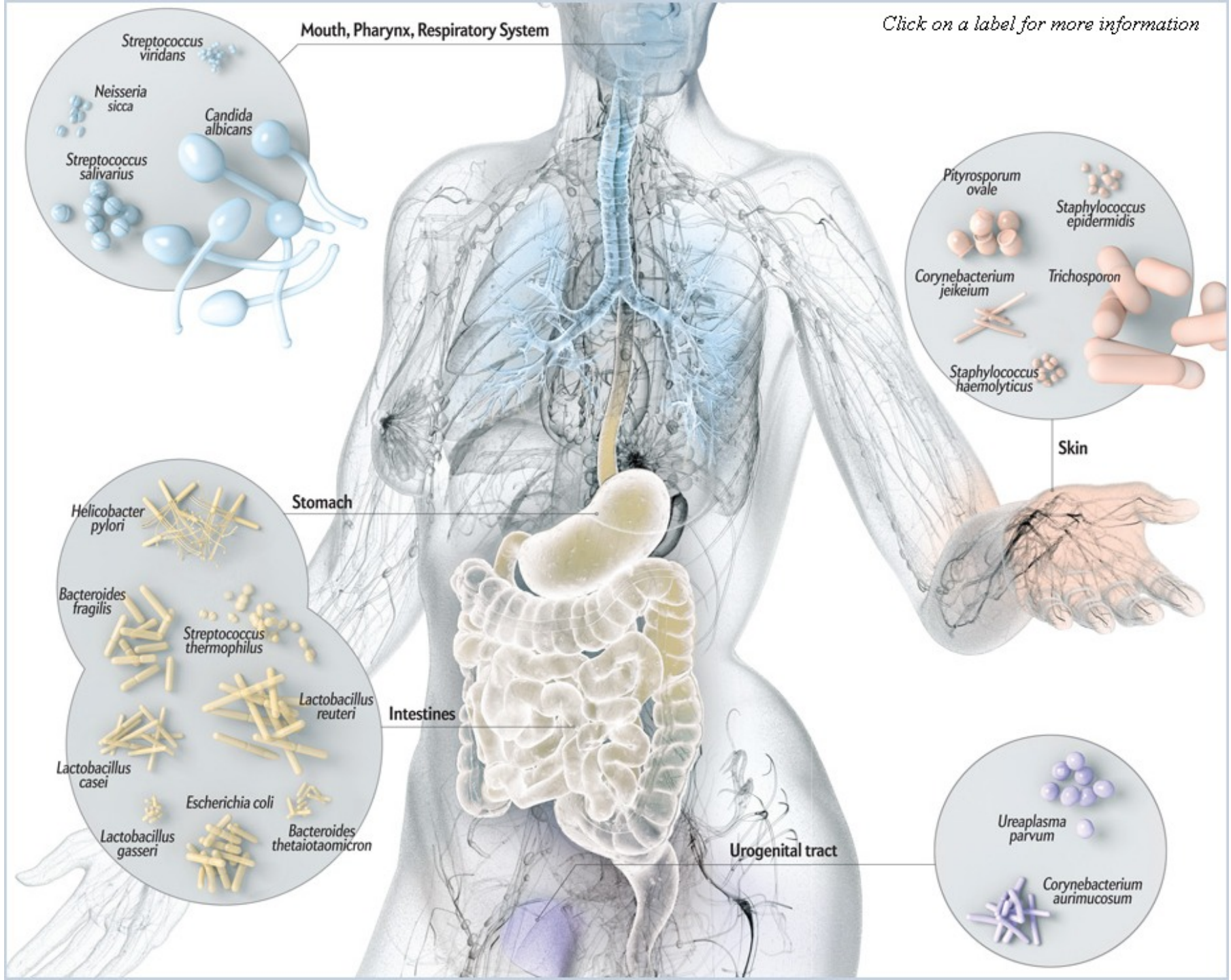
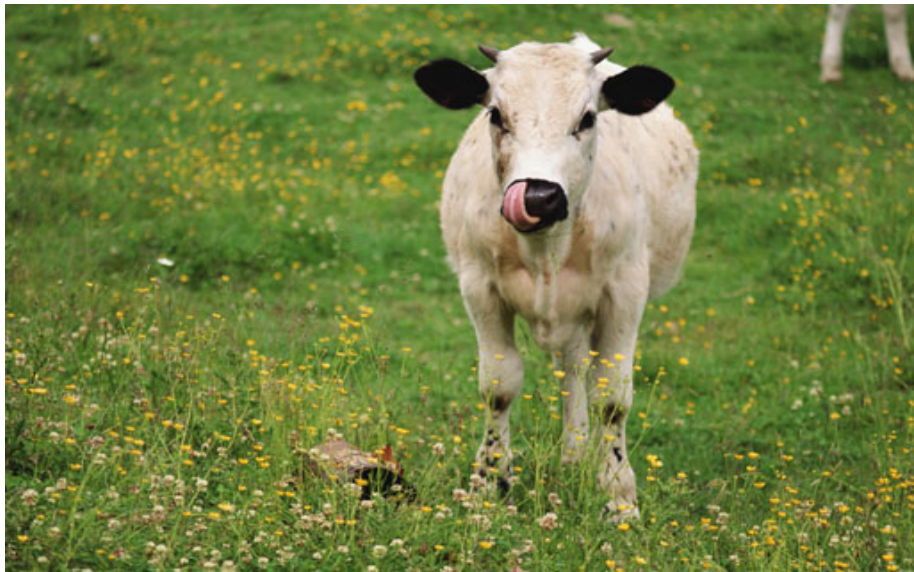
Micro-organisms




Are Every Where

Environment

Phenotype



# Number of Entities Within Rank Associated with Phenotypic Traits

| Trait | Class | Order | Family | Genus | Species |
|---|---|---|---|---|---|
| Birth Wt. | 0 | 0 | 1 | 9 | 39 |
| Weaning Wt. | 1 | 6 | 7 | 12 | 61 |
| Yearling Wt. | 2 | 3 | 7 | 27 | 38 |
| Slaughter Wt. | 1 | 1 | 1 | 16 | 39 |
| Hot carcass Wt. | 3 | 1 | 1 | 11 | 48 |
| Backfat | 1 | 1 | 2 | 23 | 61 |
| KPH | 2 | 2 | 3 | 14 | 41 |
| Ribeye Area | 2 | 2 | 6 | 15 | 52 |
| Marbling score | 2 | 3 | 4 | 18 | 76 |
| Quality grade | 1 | 5 | 3 | 18 | 59 |

[a] Kidney, pelvic and heart fat percentage

# Species Associated with Traits

| | HCW | KPH | Marbling | QualityGrade | REA |
|---|---|---|---|---|---|
| species_Enterobacteria_phage_K1F | 0 | 0 | 0 | 0 | 0 |
| species_Enterobacteria_phage_EcoDS1 | 0.2177328 | 0.0035365 | 0 | 0 | 0 |
| species_Mycoplasma_wenyonii | 0.3044042 | 0.0190094 | 0 | 0 | 2.59E-07 |
| species_Salmonella_phage_Vi_II-E1 | 7.10E-12 | 0 | 3.95E-12 | 0 | 2.02E-06 |
| species_Actinobacillus_pleuropneumoniae | 0.8850868 | 0.2747797 | 0.0002149 | 0.000613897 | 0.7603428 |
| species_Sutterella_wadsworthensis | 0.062737 | 0.0013486 | 0.0002831 | 6.81E-05 | 0.7920538 |
| species_Enterobacteria_phage_JK06 | 0.8896157 | 0.8560171 | 0.0003223 | 4.79E-05 | 0.7673534 |
| species_Bifidobacterium_gallicum | 0.0087116 | 0.0901811 | 0.0005233 | 0.000165915 | 2.02E-06 |
| species_Pseudoalteromonas_atlantica | 0.1706983 | 0.1533438 | 0.0008369 | 0.003229353 | 0.4451454 |
| species_Nitrosococcus_watsonii | 0.0079335 | 0.7771776 | 0.0010064 | 0.002482783 | 0.0992206 |
| species_Arcobacter_sp._L | 0.8991421 | 0.2821882 | 0.0021566 | 0.008066617 | 0.7005278 |
| species_Morganella_morganii | 0.2120593 | 0.0211809 | 0.0026023 | 0.004604519 | 0.6615708 |
| species_Alteromonas_sp._SN2 | 0.0851897 | 0.0015287 | 0.0034674 | 0.020249766 | 0.7299276 |
| species_Vibrio_campbellii | 0.5472983 | 0.0035365 | 0.0039138 | 0.01070613 | 0.2031598 |
| species_Pectobacterium_wasabiae | 0.3547648 | 0.0404012 | 0.0039138 | 0.007177128 | 0.2203204 |
| species_Neisseria_lactamica | 0.8868203 | 0.1019574 | 0.0040832 | 0.00452896 | 0.5016017 |
| species_Hyphomicrobium_sp._MC1 | 0.8896157 | 0.6798214 | 0.0054018 | 0.004604519 | 0.8587245 |
| species_Trypanosoma_brucei | 0.518487 | 0.6007111 | 0.0065696 | 0.000295125 | 0.7337219 |

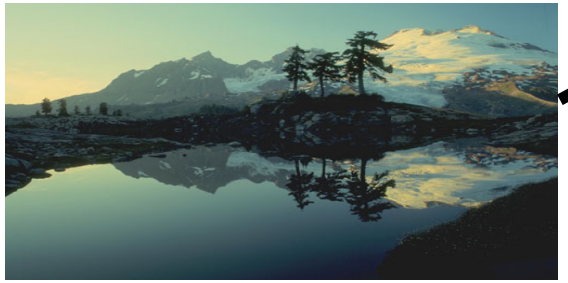
# Conclusions

- Small amounts of host DNA are present in feces relative to metagenomic DNA
- The environment has a tremendous impact on the metagenome
- The number of Taxa associated with a given trait varies across taxonomic rank
  - Species > Genus > Family > Order > Class

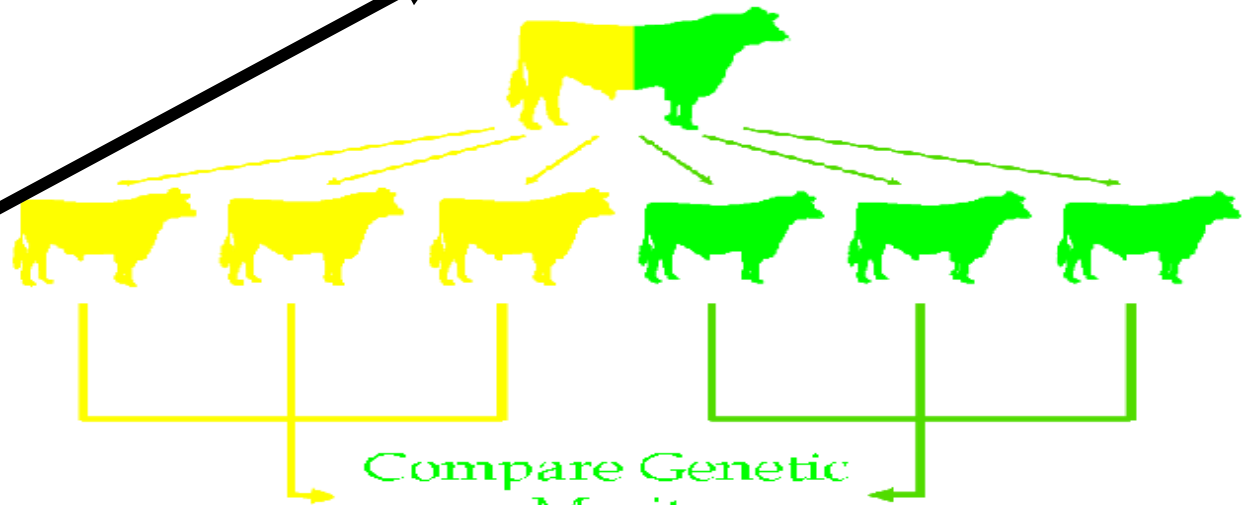
# Development of molecular tools in cattle

Genotype

Environment



Phenotype




Genomic markers

Epigenetic markers

Expression markers

Proteomic markers

Metabolomic markers

Metagenomic markers

?

# Meat Quality

- Carcass traits
  - Marbling score, IMF, Quality grade,
- Nutrients
  - Fatty acids, bioactive compounds, minerals
- Eating experience
  - Flavor, tenderness, juiciness
- Meat
  - Color, water holding capacity

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