

Identification of trait-associated genes related to meat quality using an RNA-seq analysis approach

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Introduction



Introduction

There is **high variability**
in tenderness within quality
grade classification
(marbling).

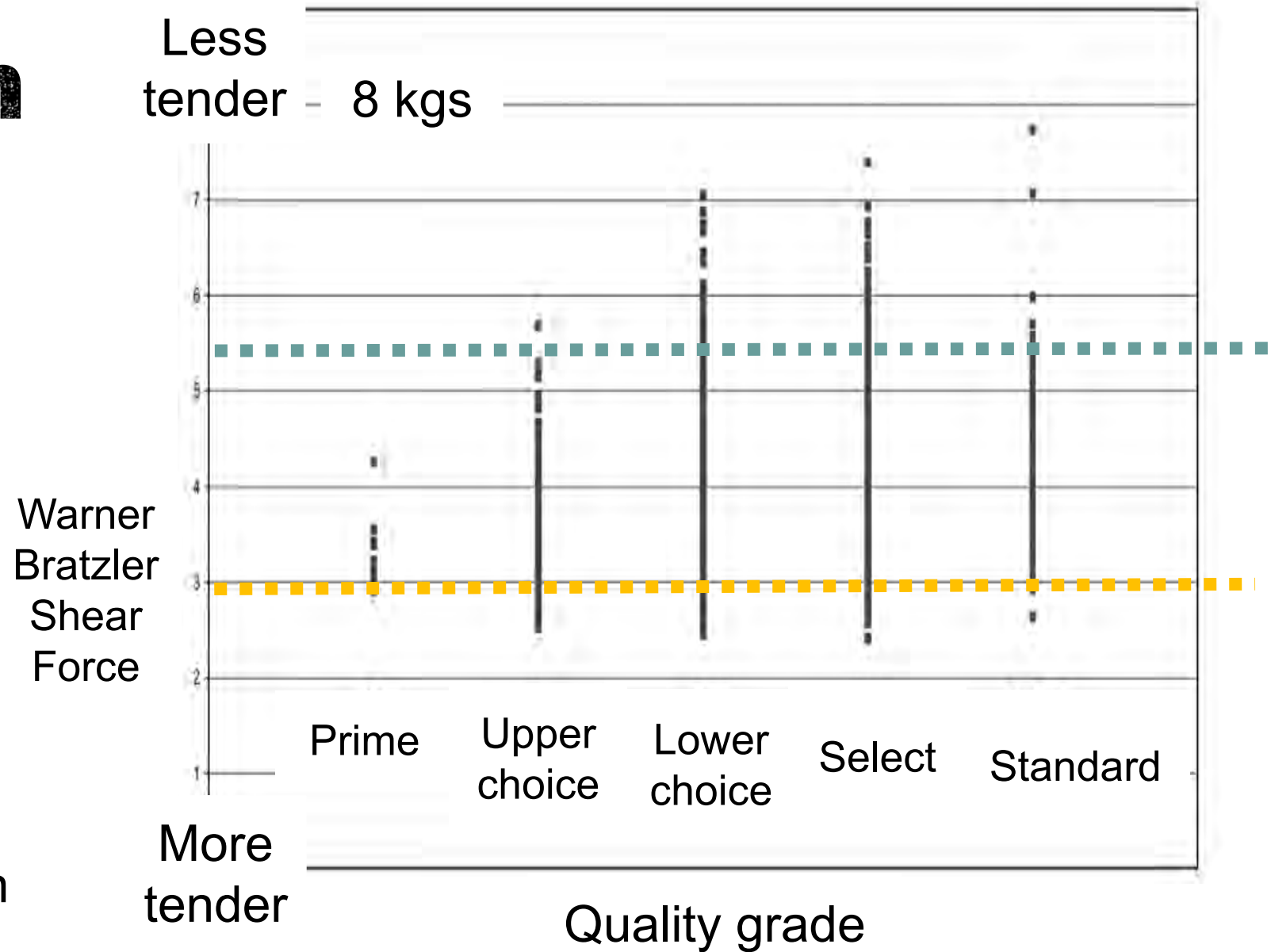
It has been reported that
at least 40% of this variation
is due to **additive genetics**.



Introduction

There is **high variability** in tenderness within quality grade classification (marbling).

It has been reported that at least 40% of this variation is due to **additive genetics**.



Objectives

- 1) To perform a **trait associated (TA) gene expression analysis** using a constructed meat quality index as response
- 2) To assess **differential gene expression (DE)** using WBSF based grouping as predictor
- 3) To perform an **eQTL mapping** for overall gene expression and for DE genes in skeletal muscle



Materials and methods



Population



Population

- Multibreed Angus-Brahman (MAB) UF herd

Based on the Angus composition

1= 100 to 80%

2= 79-65%

3= 62.5% (Brangus)

4= 59 to 40%

5= 39 to 20%

6= 19 to 0%

Phenotypic traits

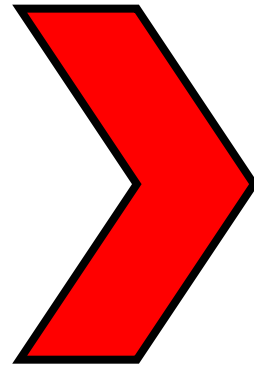
150 steers with meat quality related phenotypes
in *longissimus dorsi* muscle



Meat quality index

Recorded phenotypes:

- WBSF
- Marbling
- Tenderness
- Juiciness
- Connective tissue
- Flavor



Principal
component
analysis



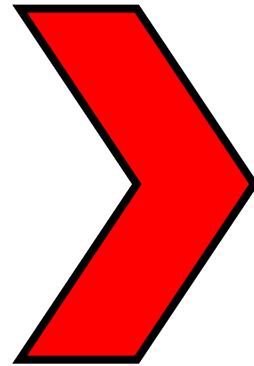
First three PCs
were used



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Principal
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First three PCs
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$$\text{Meat quality index}_i = \sum (\text{PCS}_{ij} * \text{PCW}_j)$$

PCS_{ij} = Principal component score of animal i for the PC_j

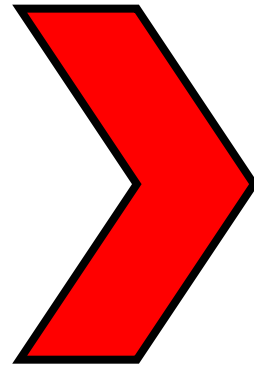
PCW_j = Weight of the PC_j



Meat quality index

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PCS_{ij} = Principal component score of animal i for the PC_j

PCW_j = Weight of the PC_j

Selected animals:
80 individuals
(extremes)



mRNAseq and read mapping

Raw Data

mRNA fragment

Fragment

Paired read

```
@SEQ_ID
GATTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*(((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>CCCCCCC65
```



mRNAseq and read mapping

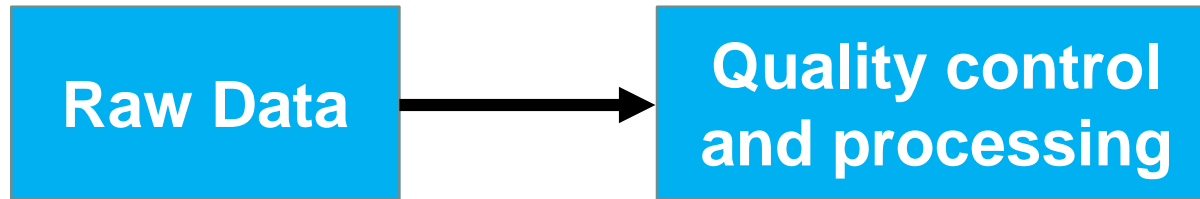
Raw Data



Quality control
and processing



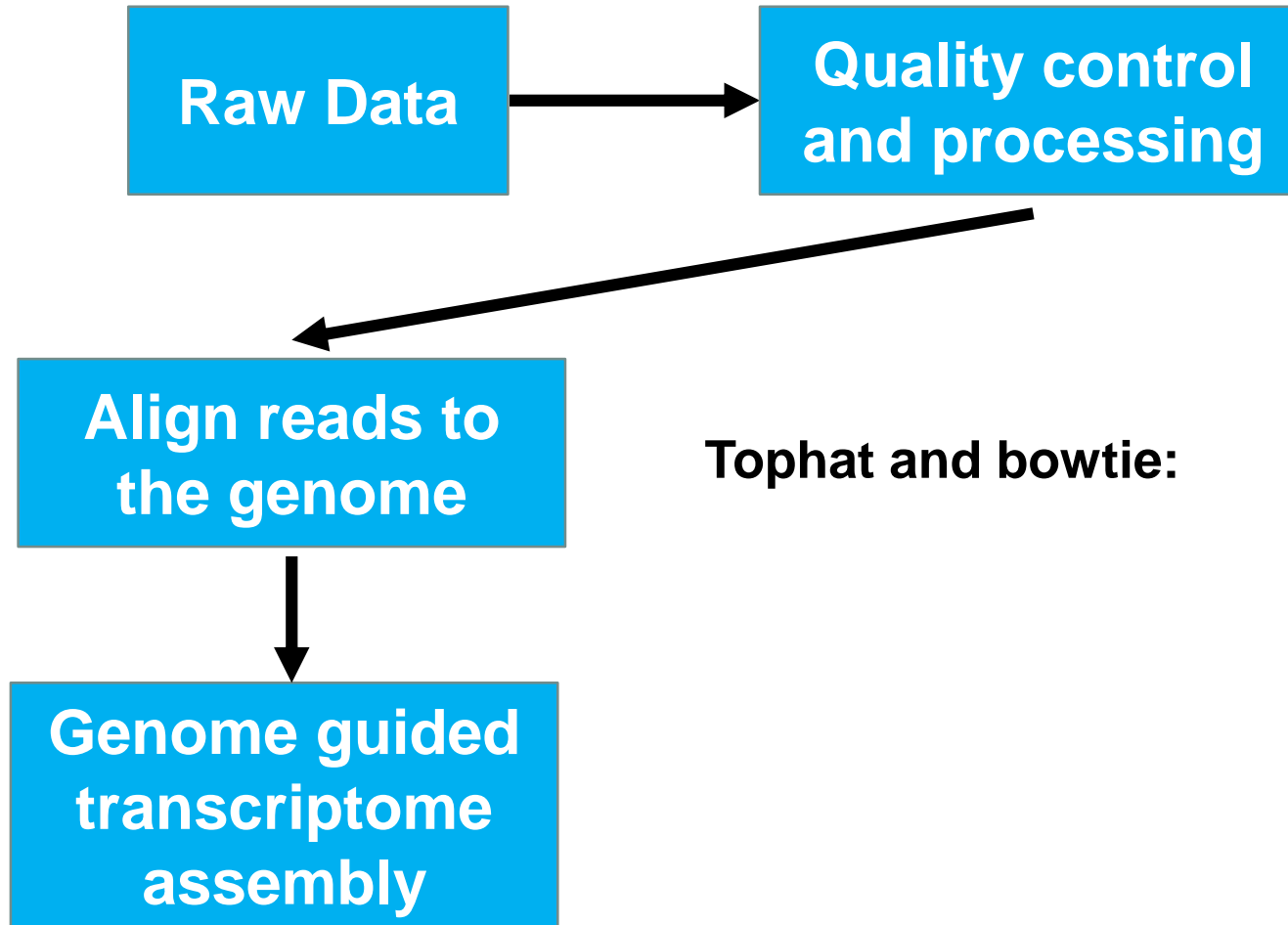
mRNAseq and read mapping



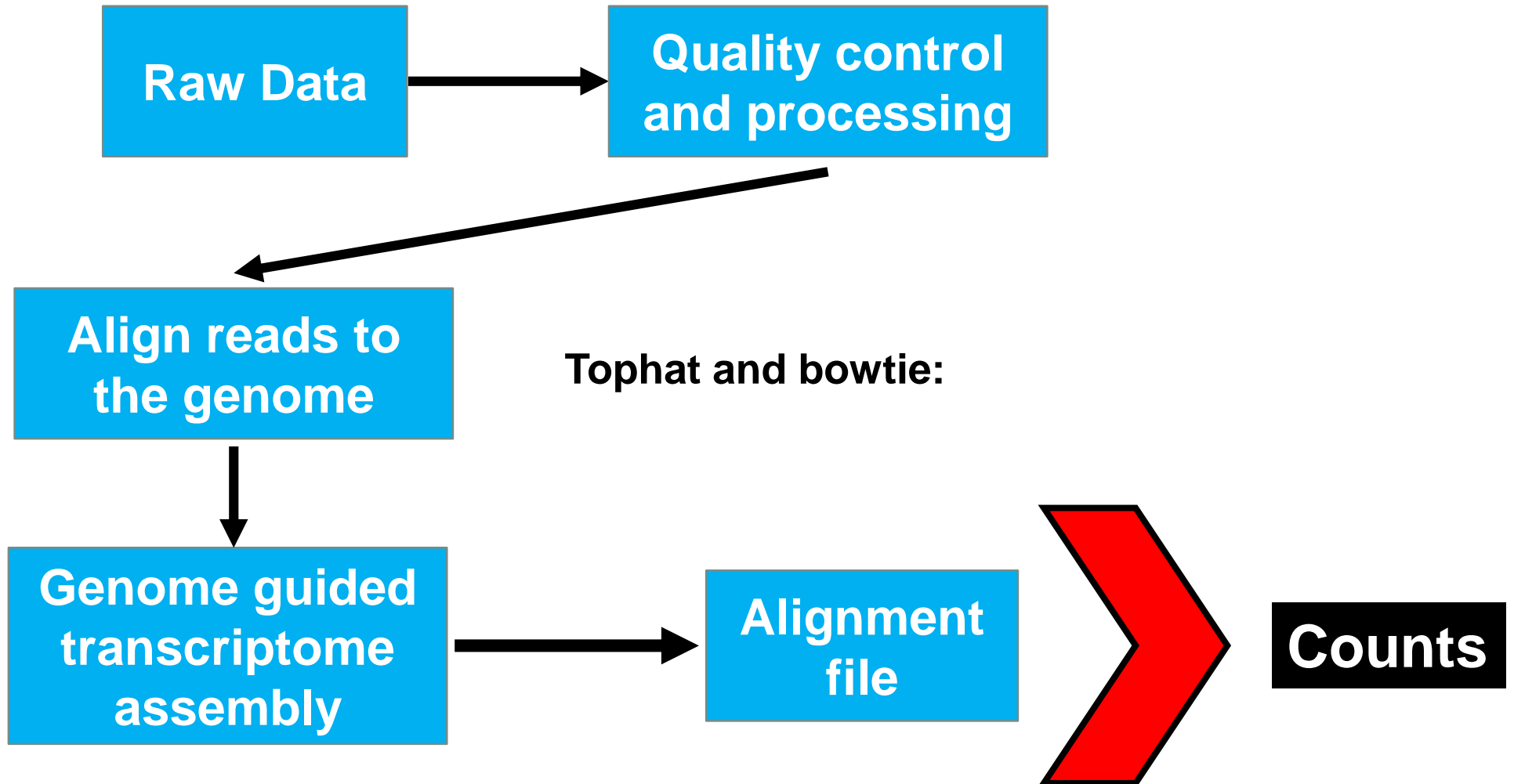
- Base filtering for low sequencing quality
- Read filtering for low sequencing quality
- Single reads screening
- On average, 34.9 million paired reads were uniquely mapped



mRNAseq and read mapping



mRNAseq and read mapping

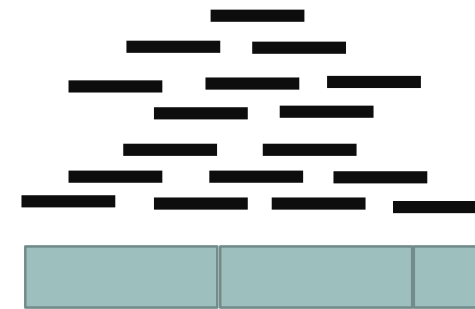


Gene counts

CAMLG	113
CAMP	0
CAMSAP1	429
CAMSAP3	3
CAMTA1	36
CAMTA2	1414
CAND1	102
CAND2	2886
CANT1	118
CAP1	160
CAP2	4326
CAPG	8
CAPN1	669
CAPN10	49
CAPN11	9
CAPN12	0
CAPN13	0
CAPN14	0
CAPN15	197
CAPN2	678
CAPN3	9615
CAPN5	79
CAPN6	99
CAPN7	373
CAPN8	1
CAPNS1	1059
CAPNS2	0
CAPNS3	1120

Gene

Counts



Transcript A

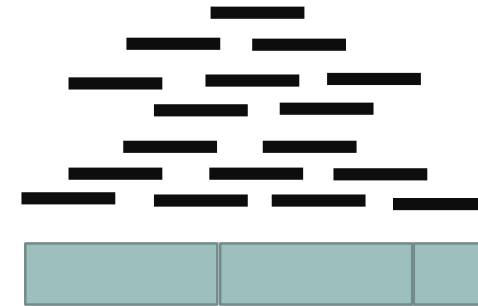


Gene counts

Gene

CAMLG	113
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Counts



Transcript A

Lowly expressed gene were excluded

Total number of included genes: 8,671



Association assay

TA gene expression analysis:

1. Continuous response variable (Meat quality index)
2. Gene counts as a predictor
3. 80 animals
4. Robust regression: less affected by outliers

DE genes

1. Gene counts as response variable
2. WBSF based grouping (tender and tough) as a predictor
3. 40 animals were used, 20 tough and 20 tender
4. Regression using the negative binomial distribution



Methods: TA gene expression

Y = Meat quality index

Low index

High index



Methods: TA gene expression

Y = Meat quality index

Low index

1.15

Tougher

Dryer

More connective tissue

Less marbling

High index

3.35

More tender

More juicy

Less connective tissue

More marbling



Methods: TA gene expression

Y = Meat quality index

Low index

1.15

Tougher

Dryer

More connective tissue

Less marbling

High index

3.35

More tender

More juicy

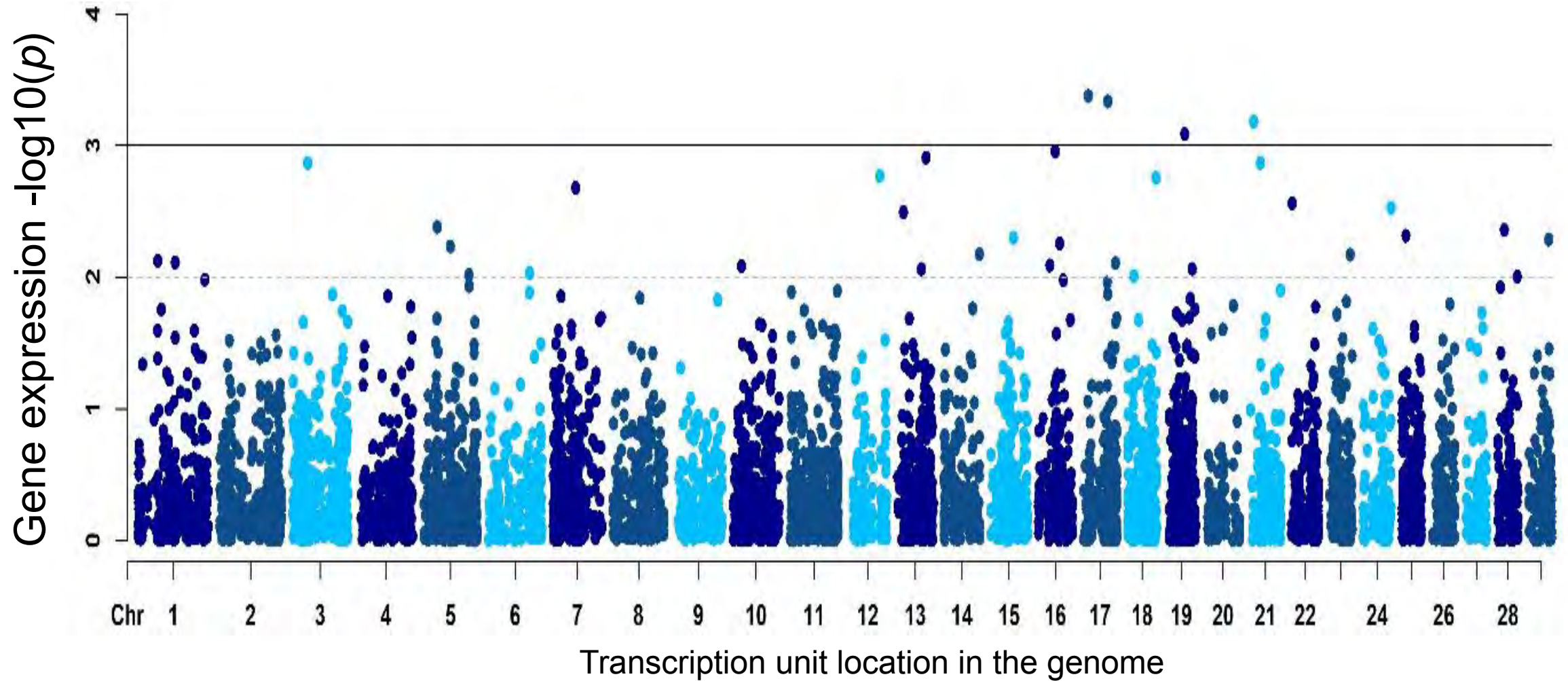
Less connective tissue

More marbling

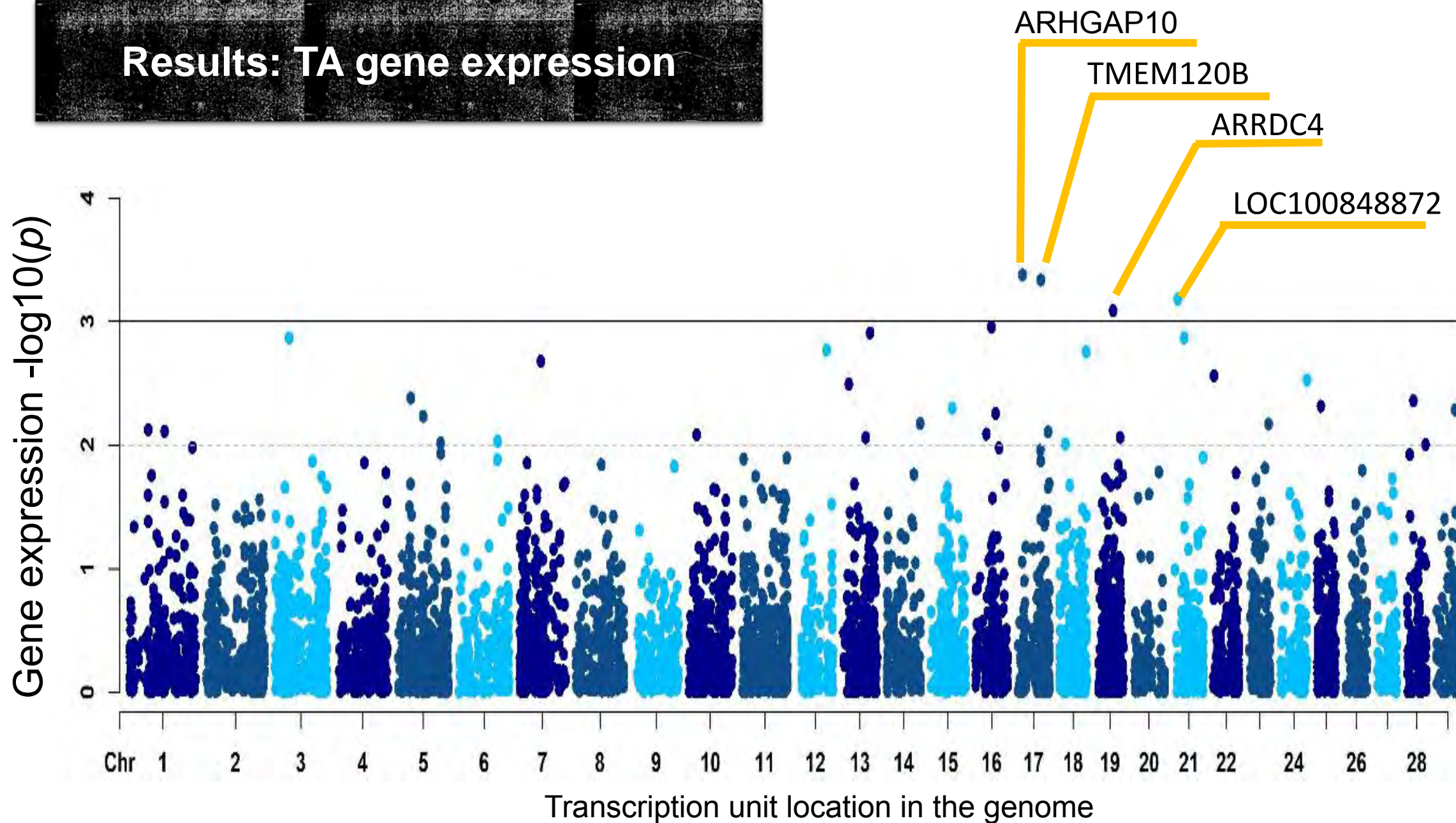
$$\text{MeatQualityIndex}_i = \mu + \beta_0 + \beta_1 * \text{year}_{1i} + \beta_2 * \text{PC}_{2i} + \beta_3 * \text{geneCounts}_{3i} + e_i$$



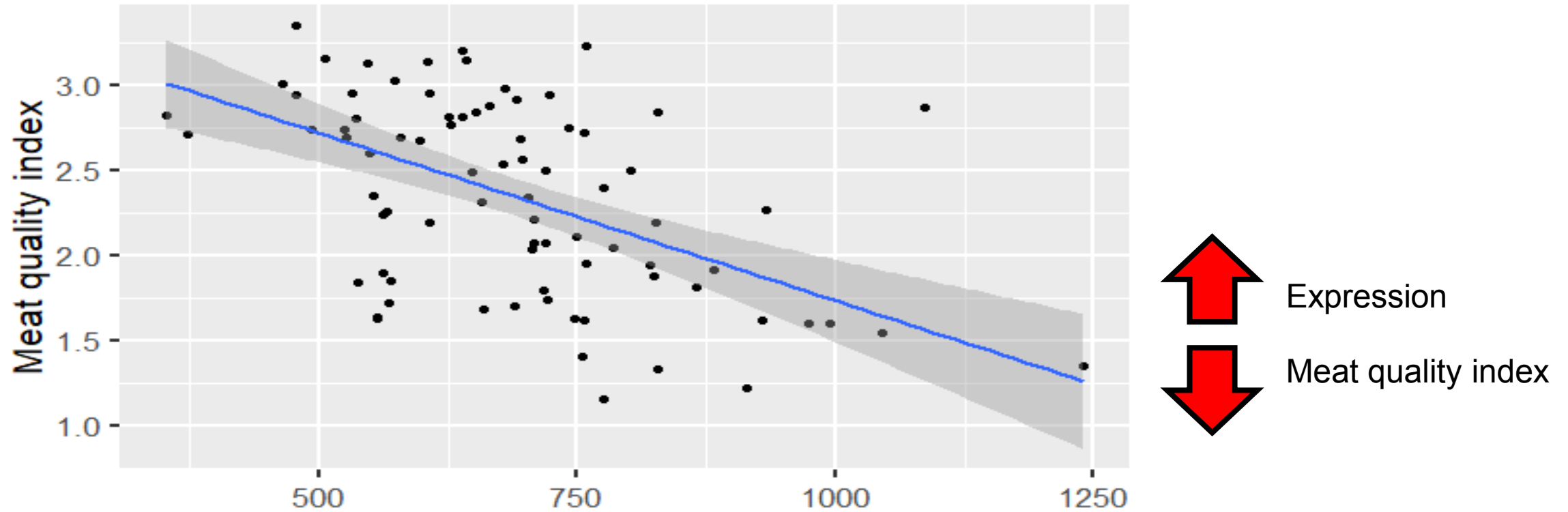
Results: TA gene expression



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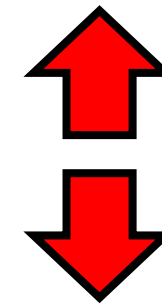
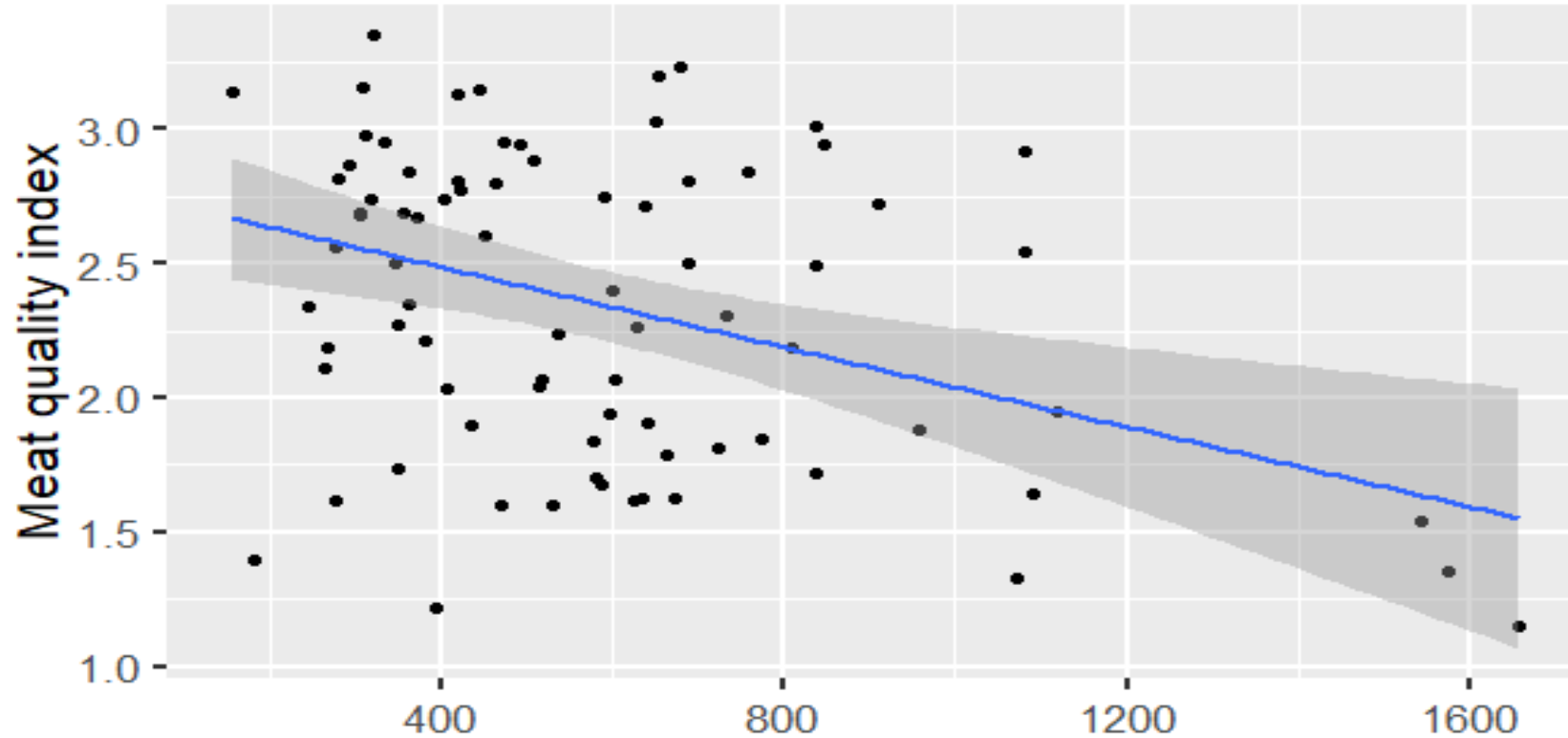


ARHGAP10 gene



This gene controls the Arp2/3 complex and **F-actin dynamics at the Golgi complex** by regulating the activity of the small GTPase Cdc42

TMEM120B gene



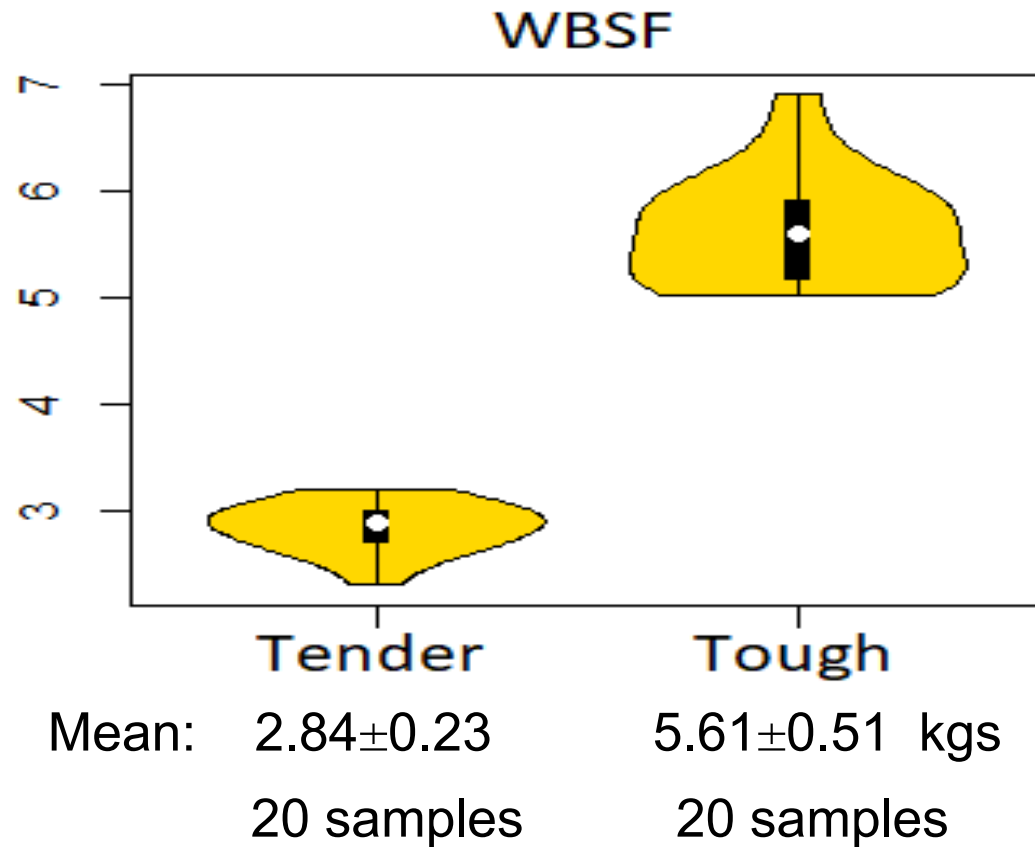
Expression

Meat quality index

TMEM120 proteins are **expressed preferentially during adipocyte differentiation**

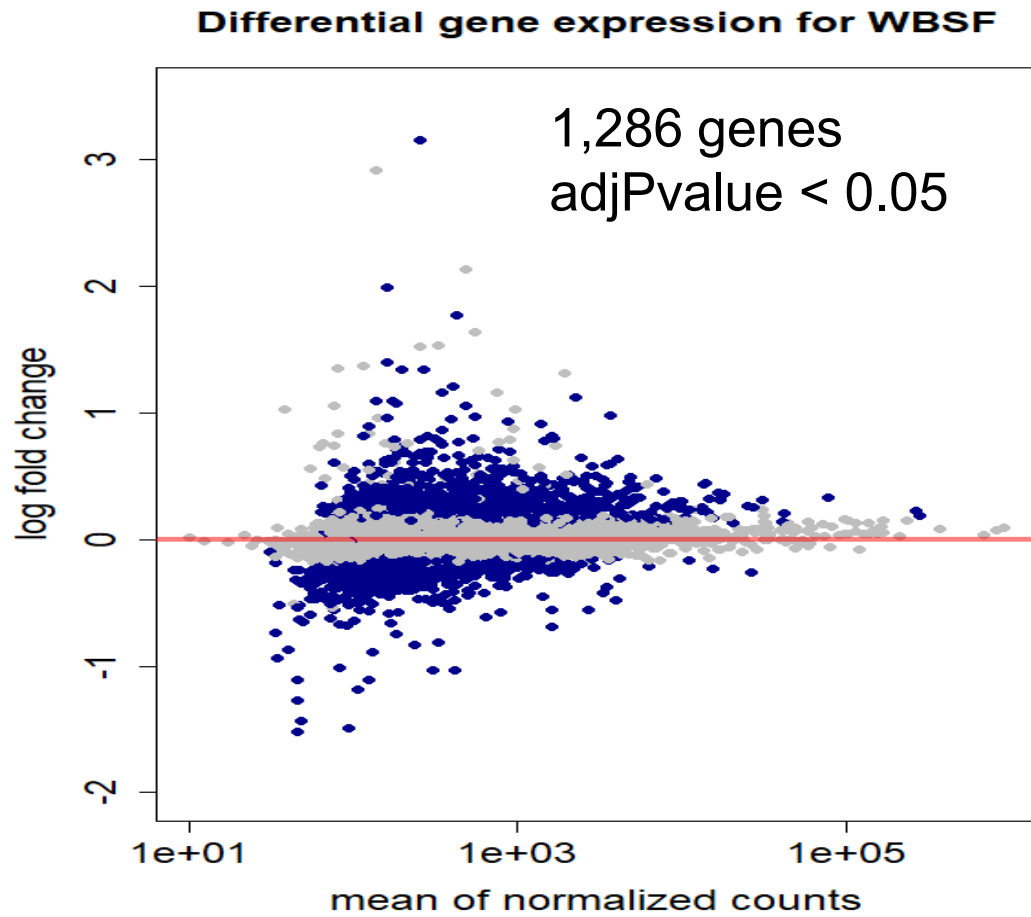


Methods: DE genes



$$\text{GeneCounts}_i = \mu + \beta_0 + \beta_1 * \text{year}_{1i} + \beta_2 * \text{BreedGroup}_{3i} + \beta_3 * \text{WBSFGroup}_{3i} + e_i$$

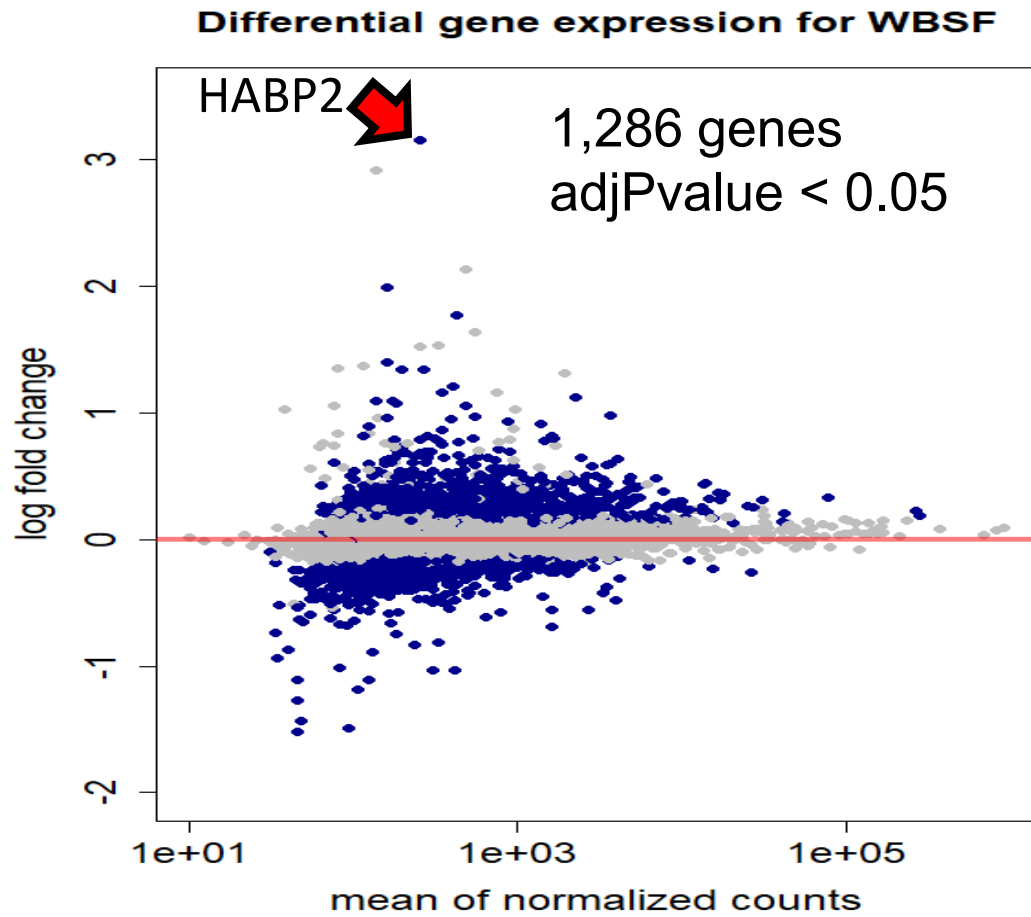
Results: DE genes



Comparison: tough vs tender



Results: DE genes

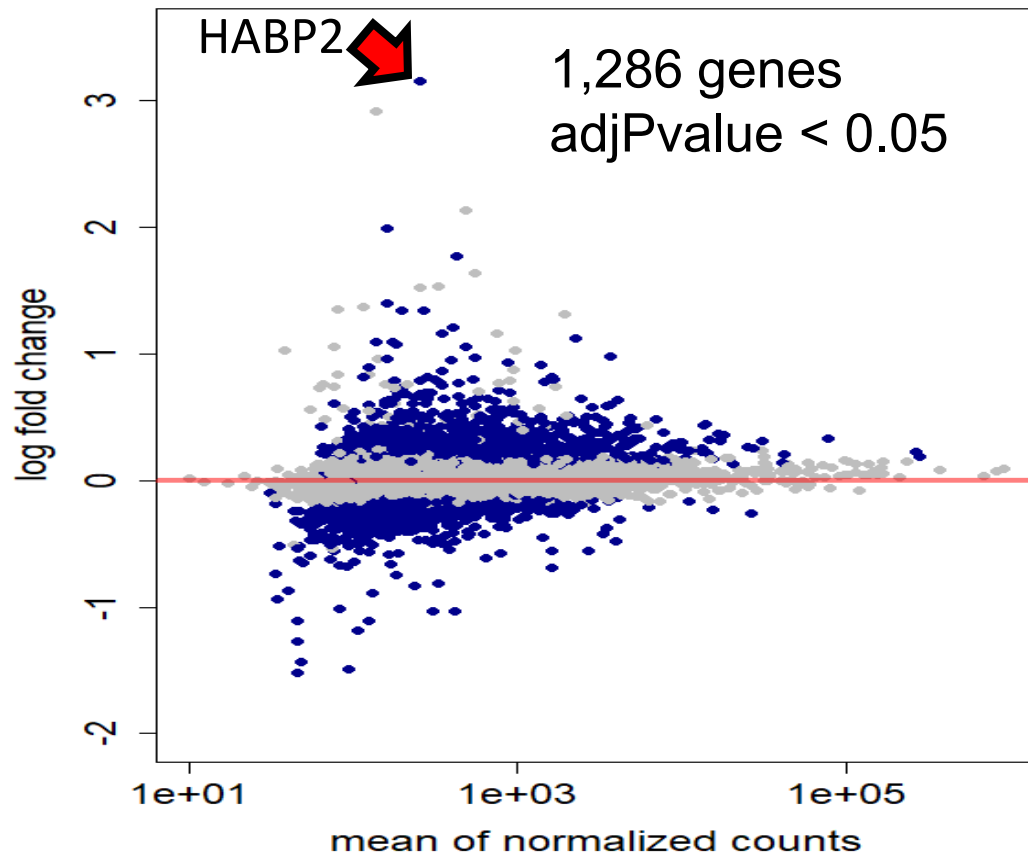


Comparison: tough vs tender



Results: DE genes

Differential gene expression for WBSF



Comparison: tough vs tender

Gene enrichment analysis

GO term	pValue	n.anno	Definition
GO:0045333	4.24E-07	42	Cellular respiration
GO:0015980	6.45E-06	50	Energy derivation by oxidation of organic compounds
GO:0044429	7.38E-06	146	Mitochondrial part
GO:0005739	1.33E-05	211	Mitochondrion
GO:0006091	2.33E-05	66	Generation of precursor metabolites and energy
GO:0070469	5.20E-05	30	Respiratory chain
GO:0017144	2.93E-04	70	Drug metabolic process
GO:0055114	3.76E-04	120	Oxidation-reduction process
GO:0005759	4.51E-04	69	Mitochondrial matrix
GO:1990204	4.73E-04	33	Oxidoreductase complex
GO:0044281	6.42E-04	152	Small molecule metabolic process
GO:0022904	7.22E-04	20	Respiratory electron transport chain
GO:0005740	7.67E-04	105	Mitochondrial envelope
GO:0031966	9.35E-04	101	Mitochondrial membrane
GO:0044455	1.31E-03	40	Mitochondrial membrane part



Methods: eQTL analysis

- Genome wide association that uses **gene expression as response variable**
- Goal: to identify genomic regions with **control** on gene expression

Input:

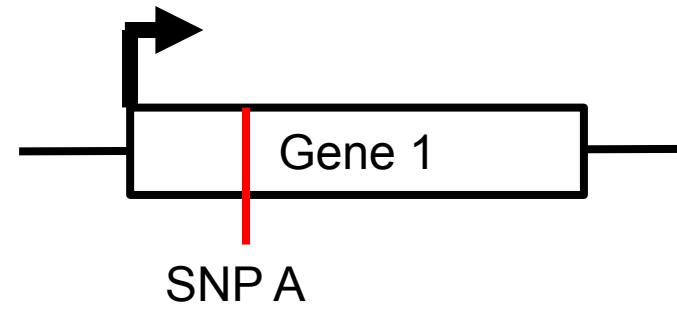
8,671 genes expressed in skeletal muscle, 1,286 of which were DE

115,287 polymorphic markers



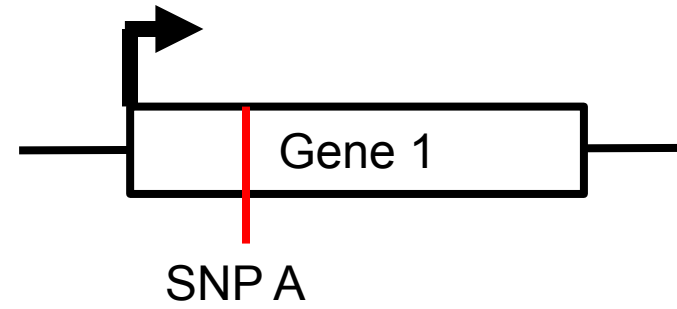
Results: eQTL analysis

Cis-eQTL

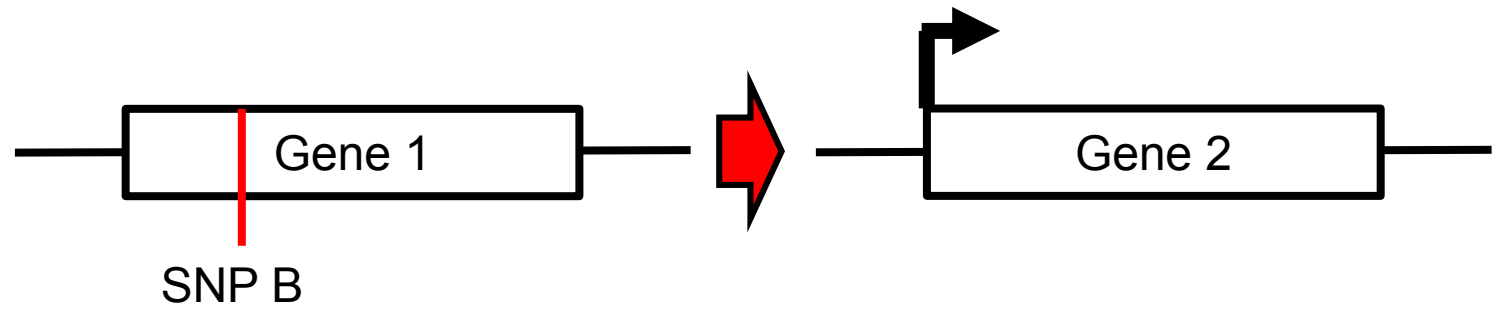


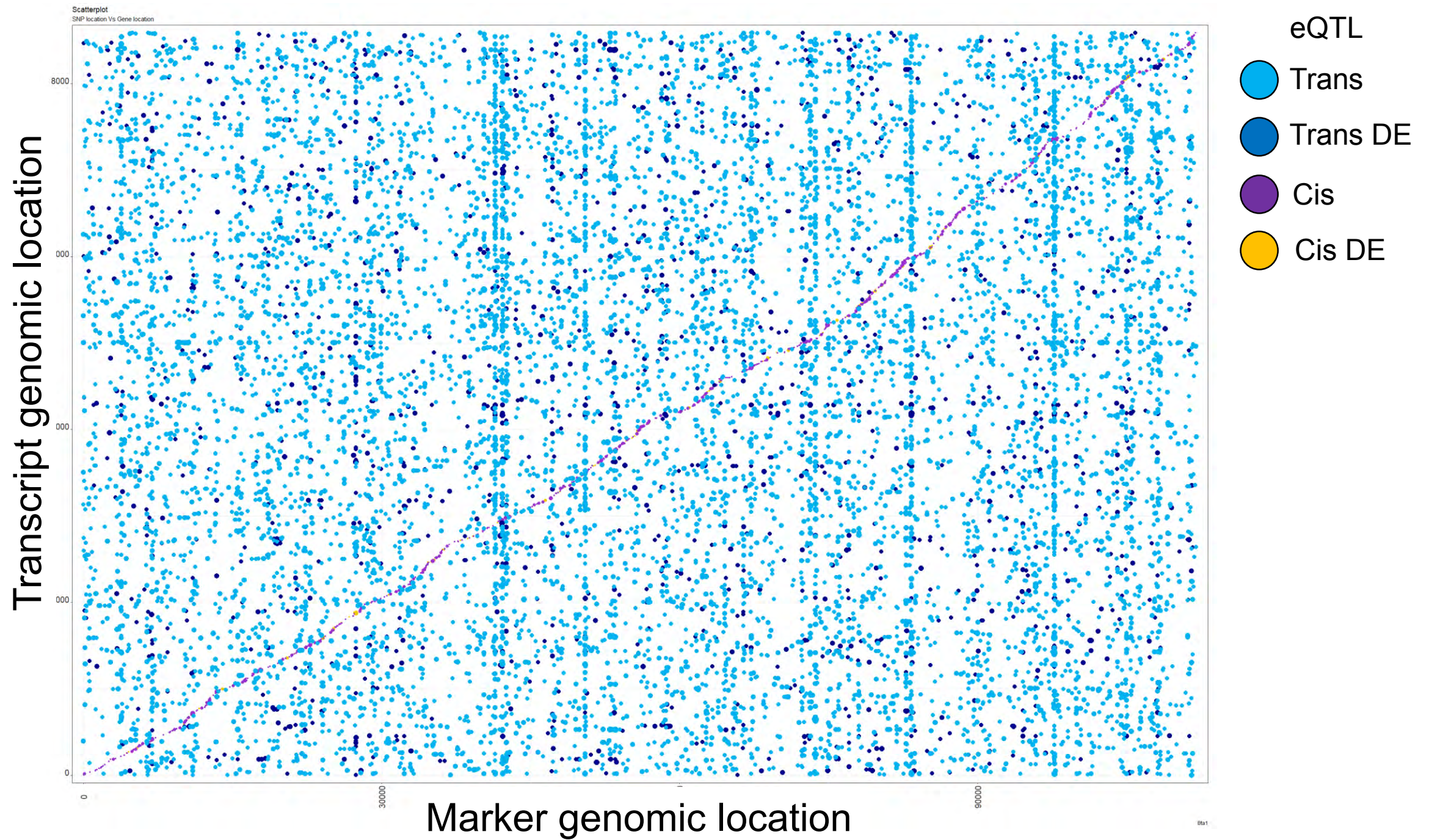
Results: eQTL analysis

Cis-eQTL

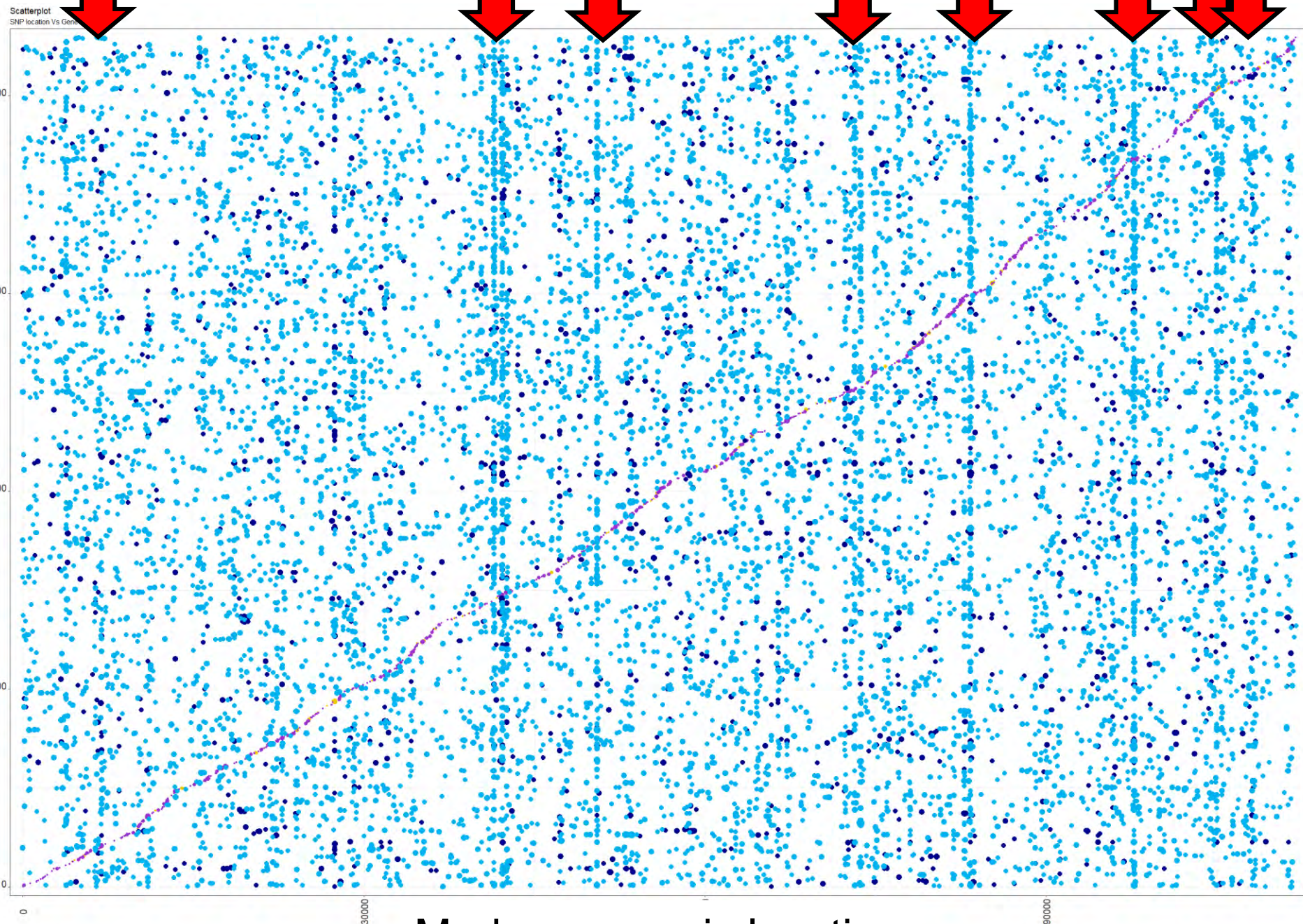


Trans-eQTL





Transcript genomic location



Marker genomic location

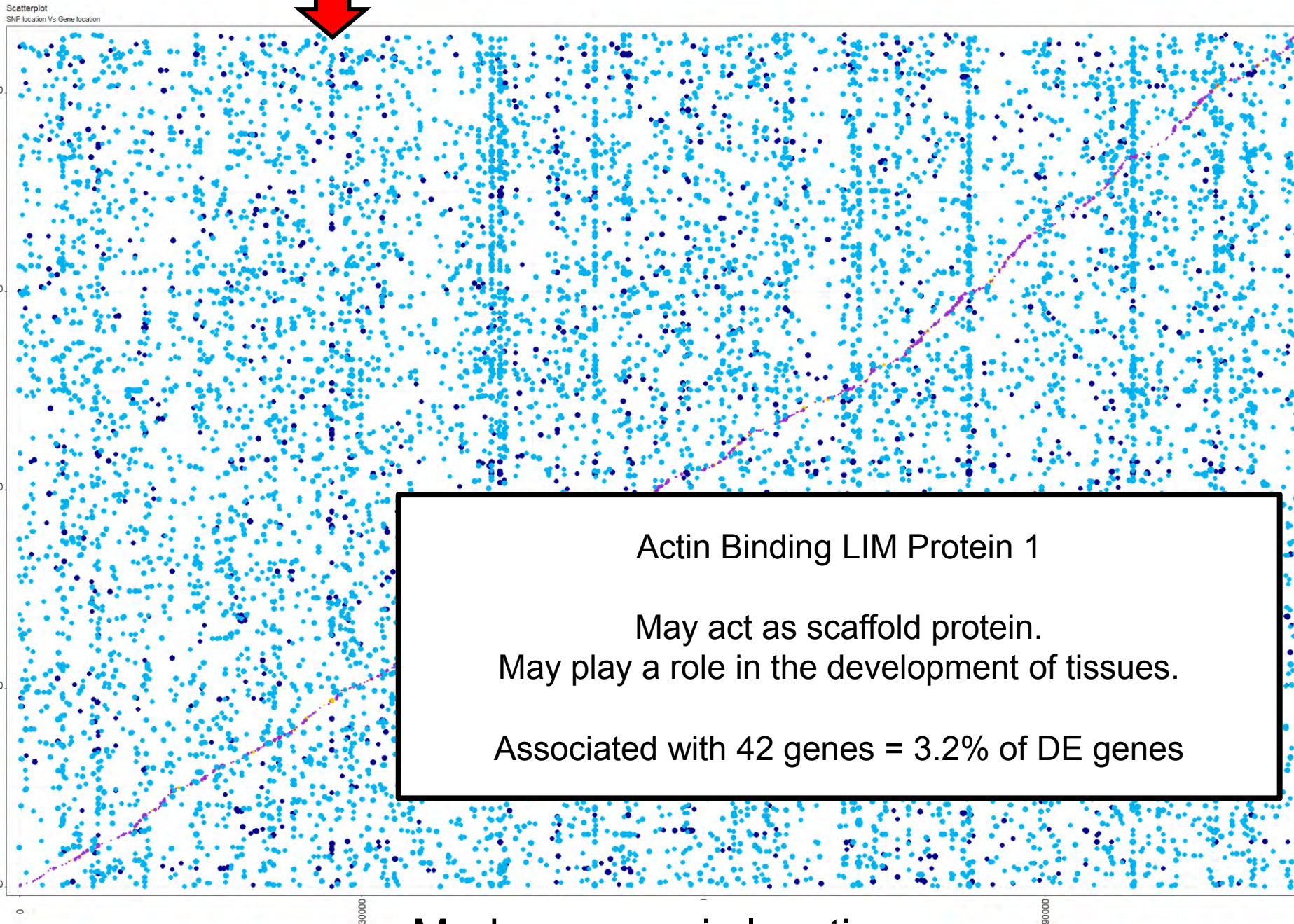
eQTL

- Trans
- Trans DE
- Cis
- Cis DE

Hot spot



Transcript genomic location



Actin Binding LIM Protein 1

May act as scaffold protein.
May play a role in the development of tissues.

Associated with 42 genes = 3.2% of DE genes

eQTL

- Trans
- Trans DE
- Cis
- Cis DE

Hot spot



Marker genomic location

Conclusions



Conclusions

❖ TA gene expression analysis:

The **expression of 4 genes** were determined as associated with meat quality index in the present population

❖ DE gene analysis:

A total number of **1,286 genes were DE** in the tough vs tender comparison

❖ eQTL mapping:

Eight eQTL hot spots for global expression were identified and **one hot spot for DE genes** was found

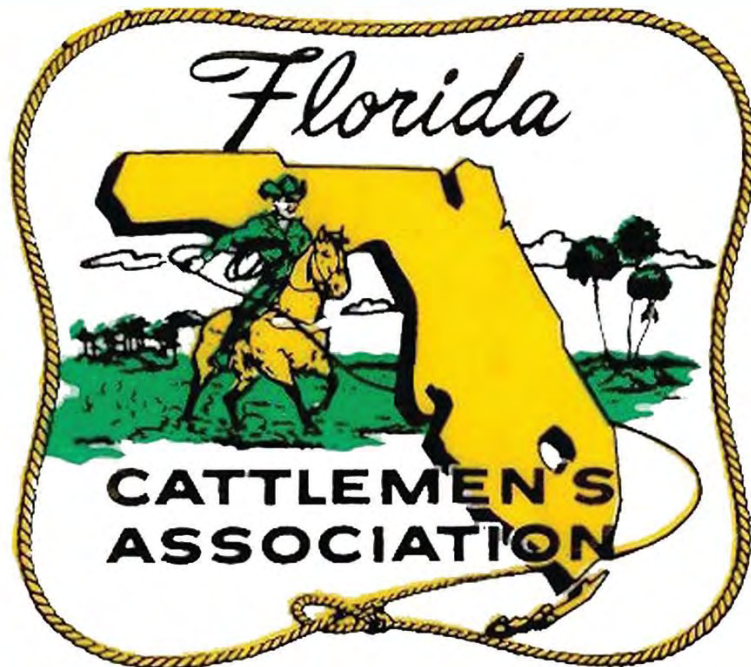


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Eduardo Rodriguez

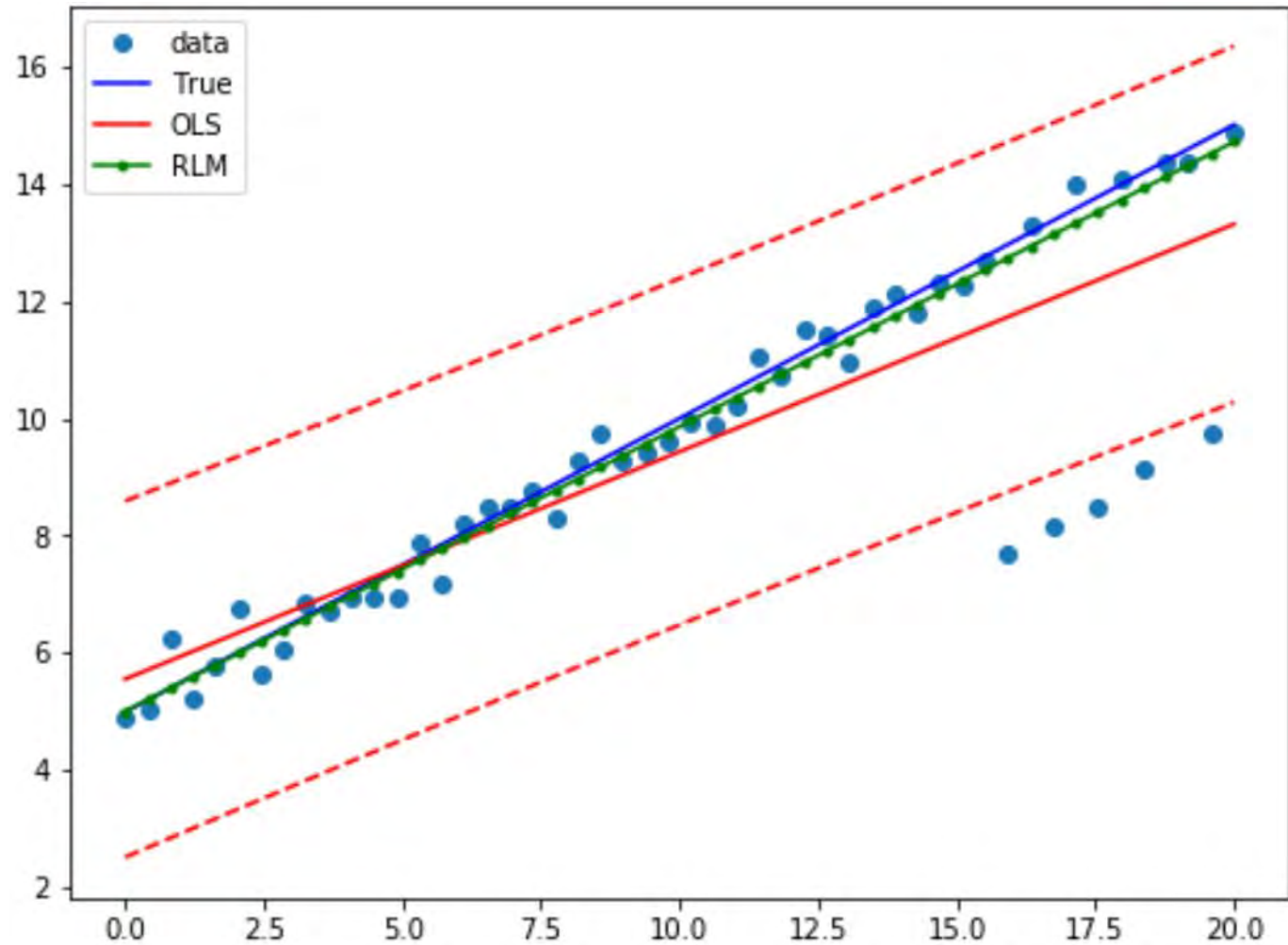


Thanks.



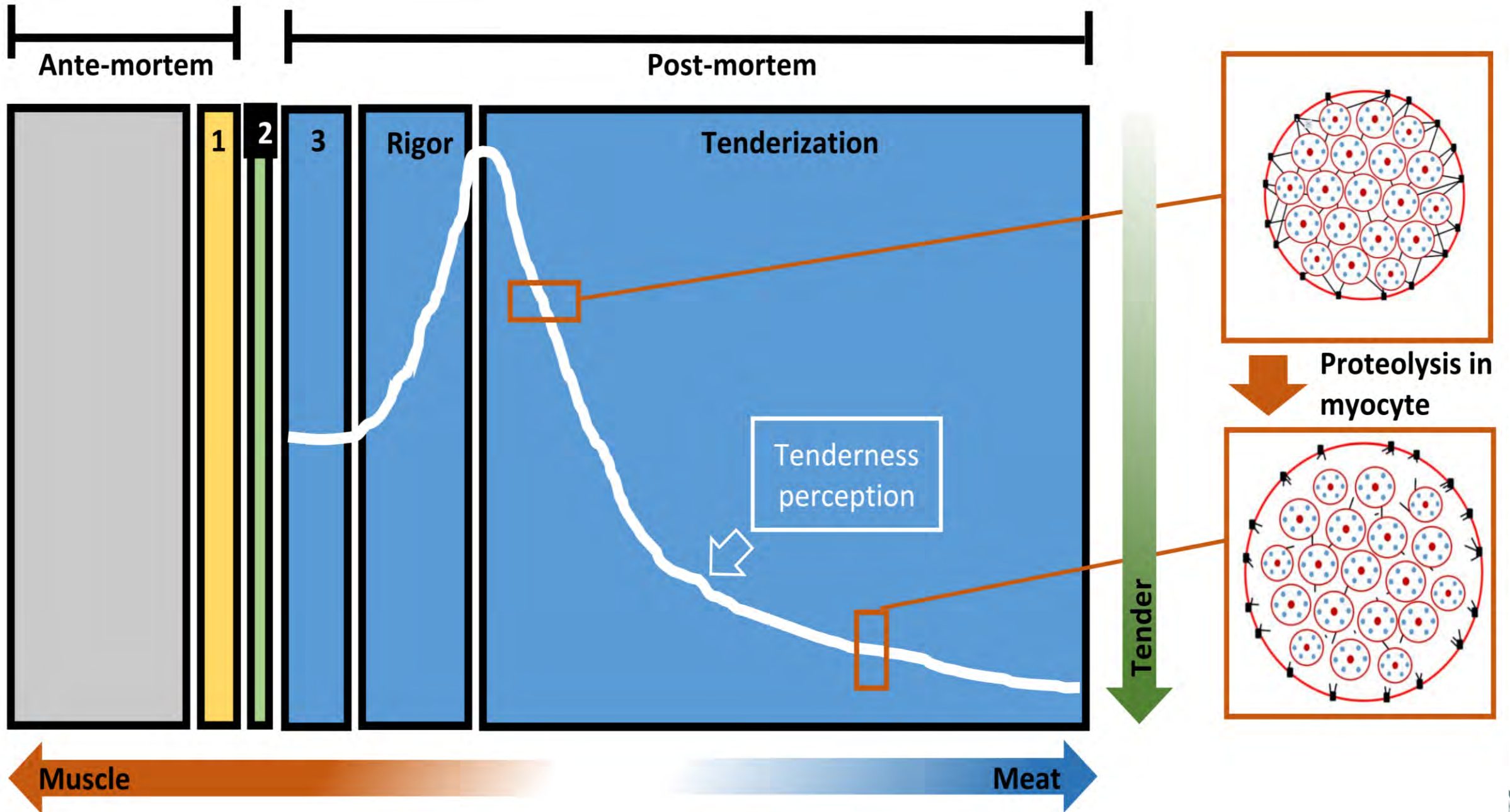
Association analysis

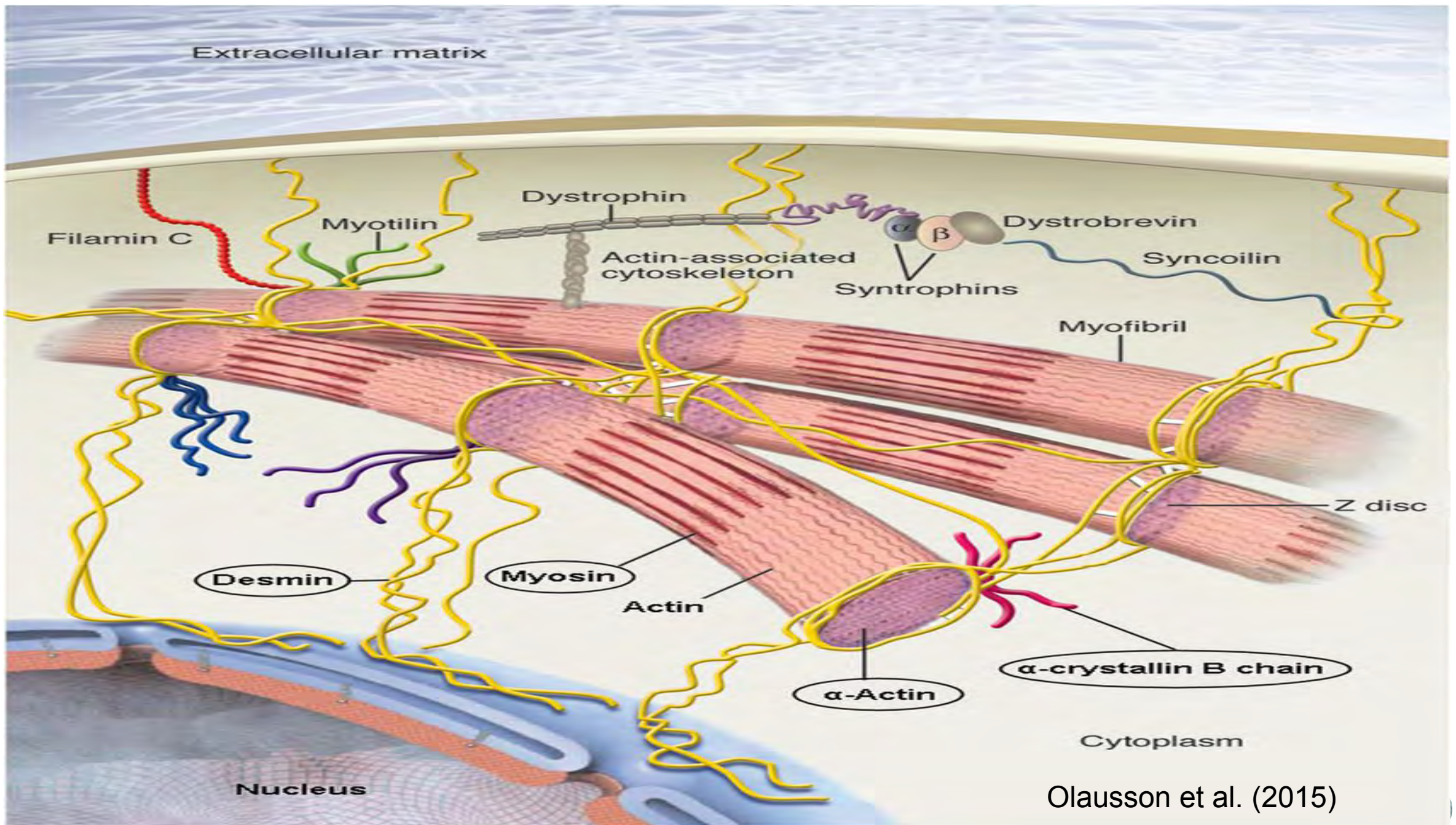
Robust regression:



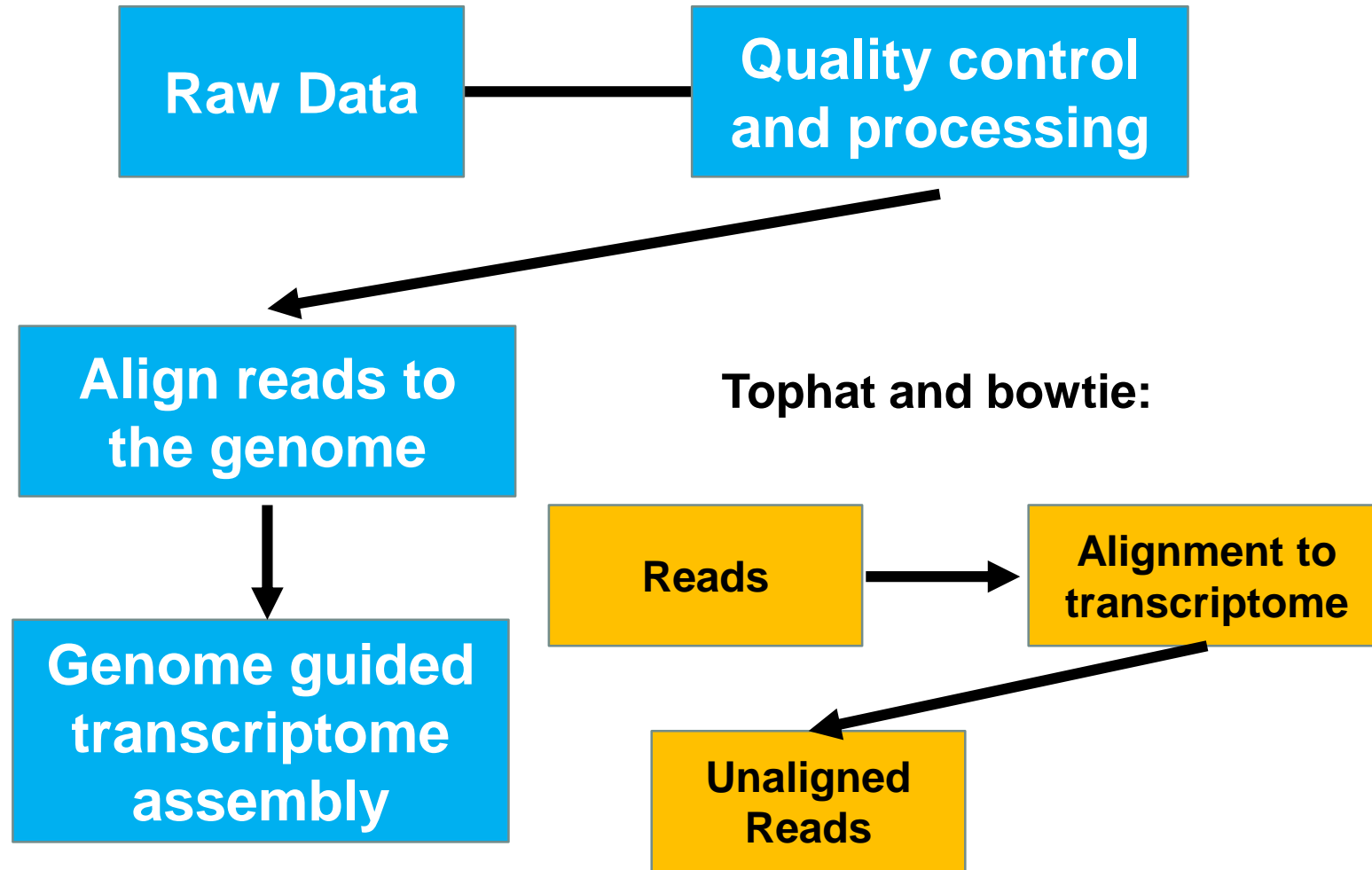
<https://www.statsmodels.org>

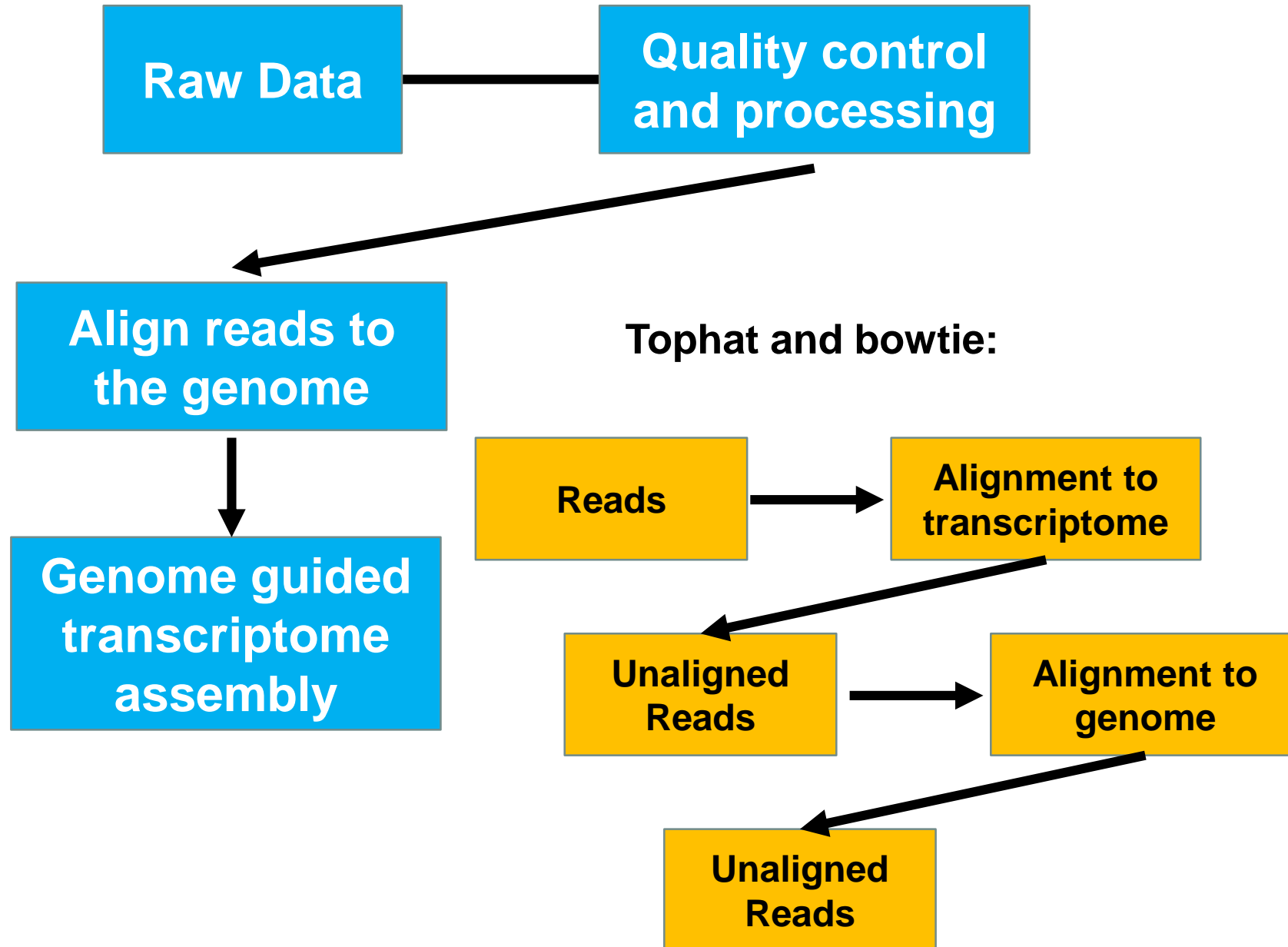


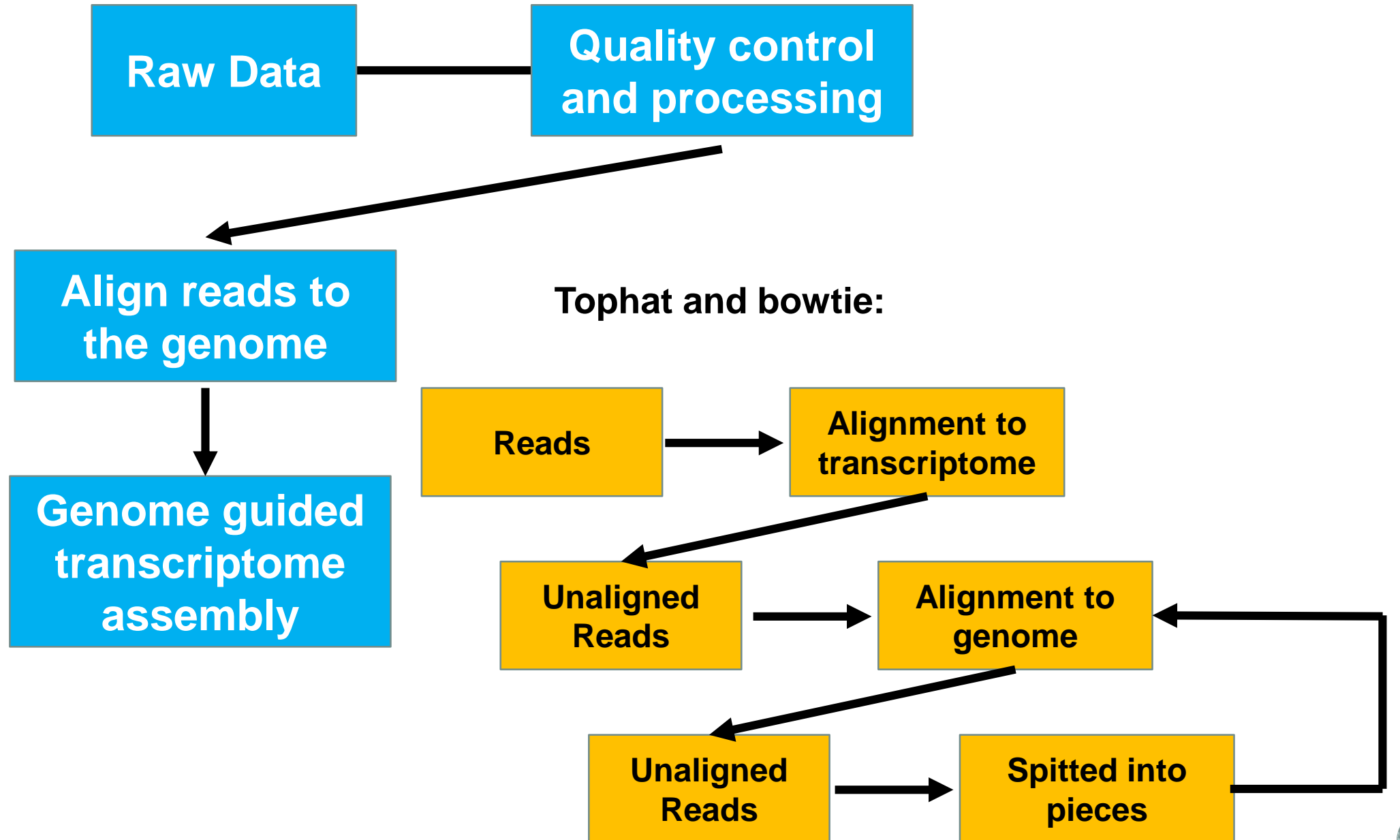


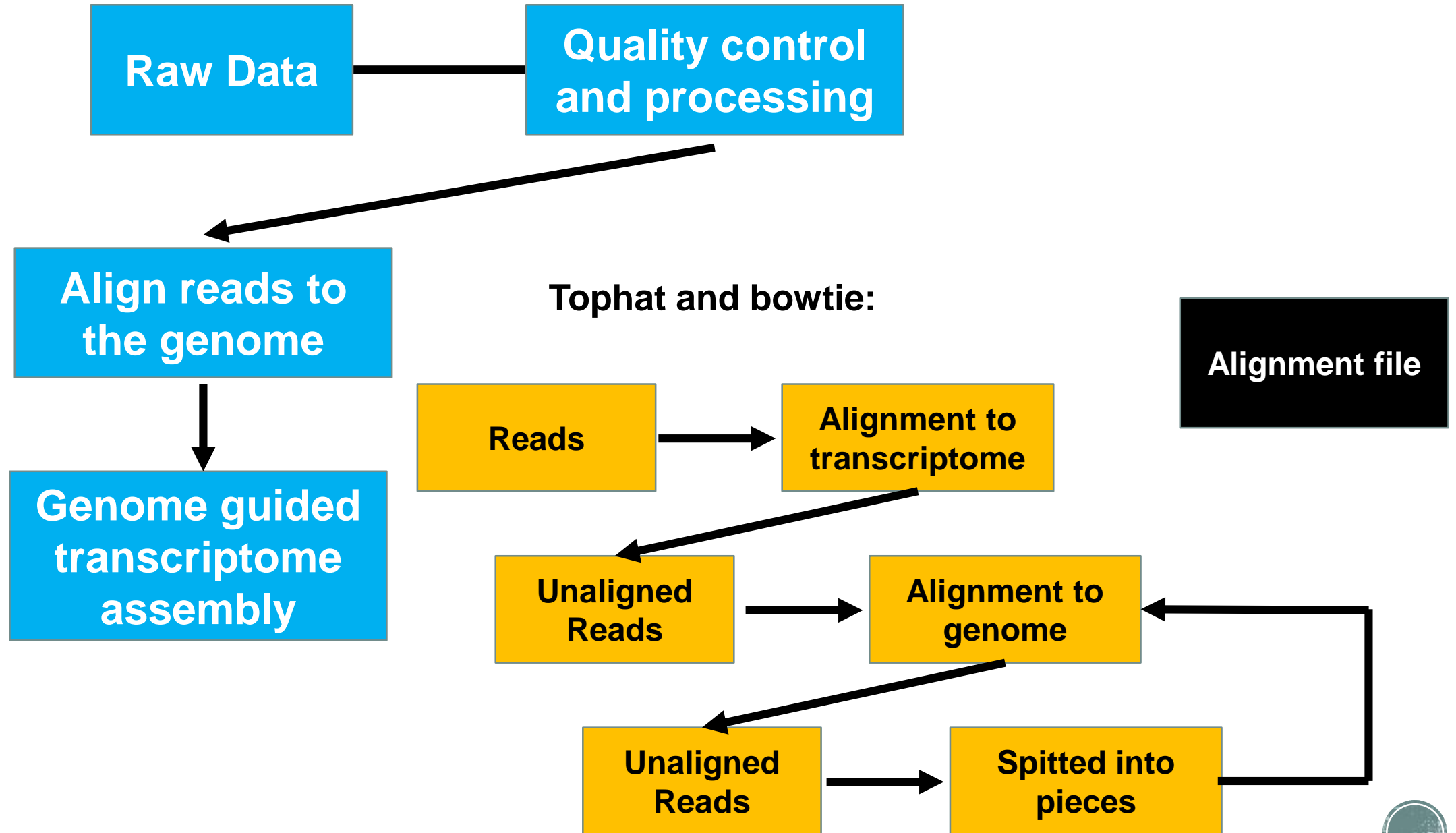


Olausson et al. (2015)









Gene expression

Exon usage

Association by exon

