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Overview and results of PhenoFinlait, a large scale project for milk fat and protein composition analysis

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PhénoFinlait

Background

Expectations of consumers evolve:
Improve the nutritional value of bovine milk for human health

↓

PhenoFinLait: a multispecies consortium gathering research and industry for fine milk composition improvement (cattle, sheep, goat)

↓

Fine milk composition in Fatty Acids (FA) and proteins

Central idea : MIR phenotyping

Milk analysis laboratories perform >25 million milk analyses to predict fat and protein contents

Goal : extract more information from these spectrum data

$\Rightarrow$ **Predict FA and protein on a routine basis and at a low cost**

An example of high throughput phenotyping
Many animals
A large range of traits

The different steps of the project

- **Develop a reference method for proteins**
- **Develop prediction equations for fatty acids and proteins from MIR spectra**
- **Collect on farm data, in addition to conventional performance recording**
  - **MIR Spectra**
  - **Blood and milk samples**
  - **Survey on production and feeding system**
- **Genotype**
- **Analyze the results**

Reference method for protein

(Miranda, Martin)

Requirements :

- High resolution, reliable, robust
- Identify and quantify the main milk proteins
- Distinguish the different isoforms
  - Genetic Variants, splicing variants
  - Post-traductionnal modifications (glycosylation-phosphorylation)
  - Products of proteolysis
- Possibly applicable to medium-large scale analysis

Technological choice :

RP-HPLC coupled to Mass Spec (LC-MS)

Ultimate 3000 (Dionex)

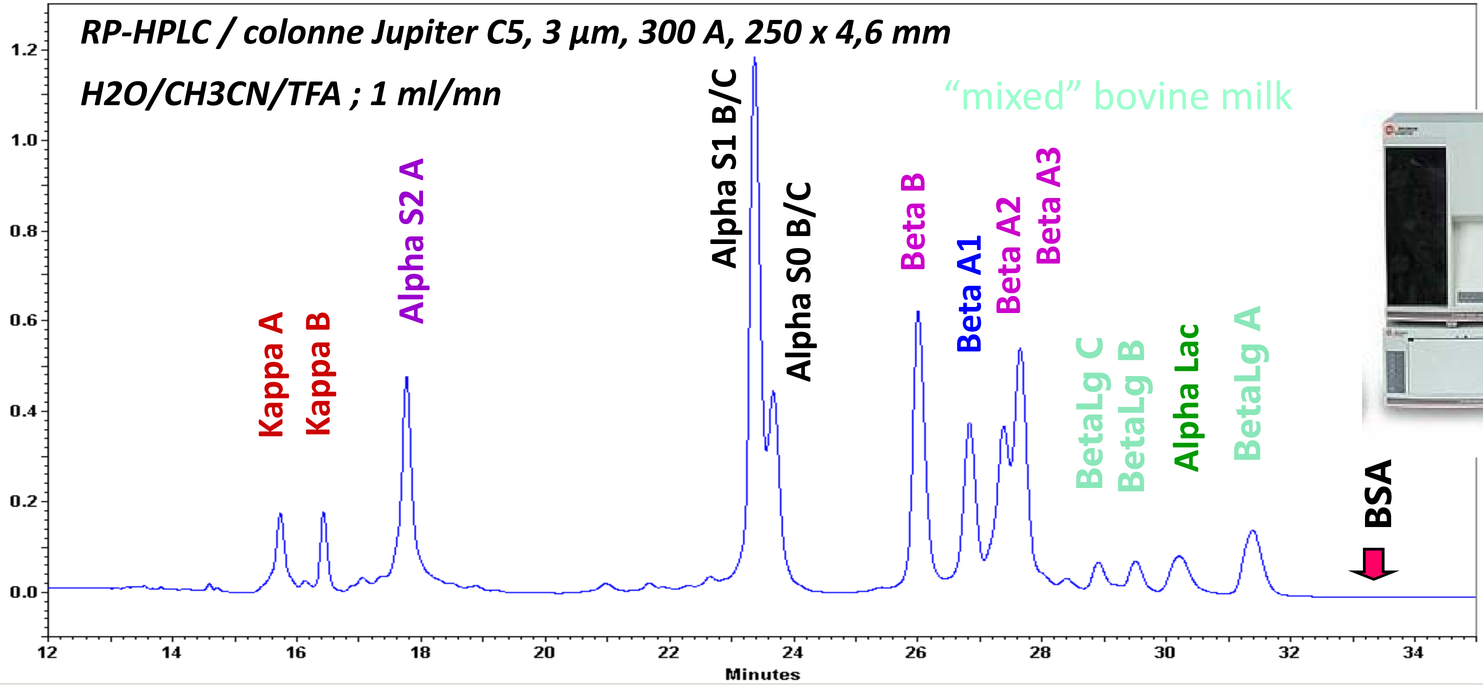
MicroTOf focus (Bruker)

A French dairy industry R&D program on thin milk composition **PhénoFinlait**

Quantitative analysis

Major Lactoproteins

RP-HPLC : quantification by integration of peaks at 214 nm (in % of the total area of peaks)



RP-HPLC / colonne Jupiter C5, 3 µm, 300 A, 250 x 4,6 mm

H2O/CH3CN/TFA ; 1 ml/mn

“mixed” bovine milk

Gold system BECKMAN

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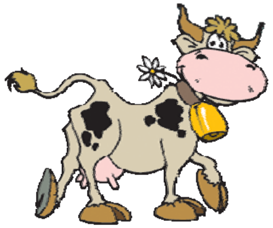
Qualitative Analysis :

- ➢Identification by comparison of observed masses to theoretical masses
- ➢ Creation of a theoretical mass data base in bovine, sheep and goat

Ultimate 3000 (Dionex)

MicroTOf focus (Bruker)

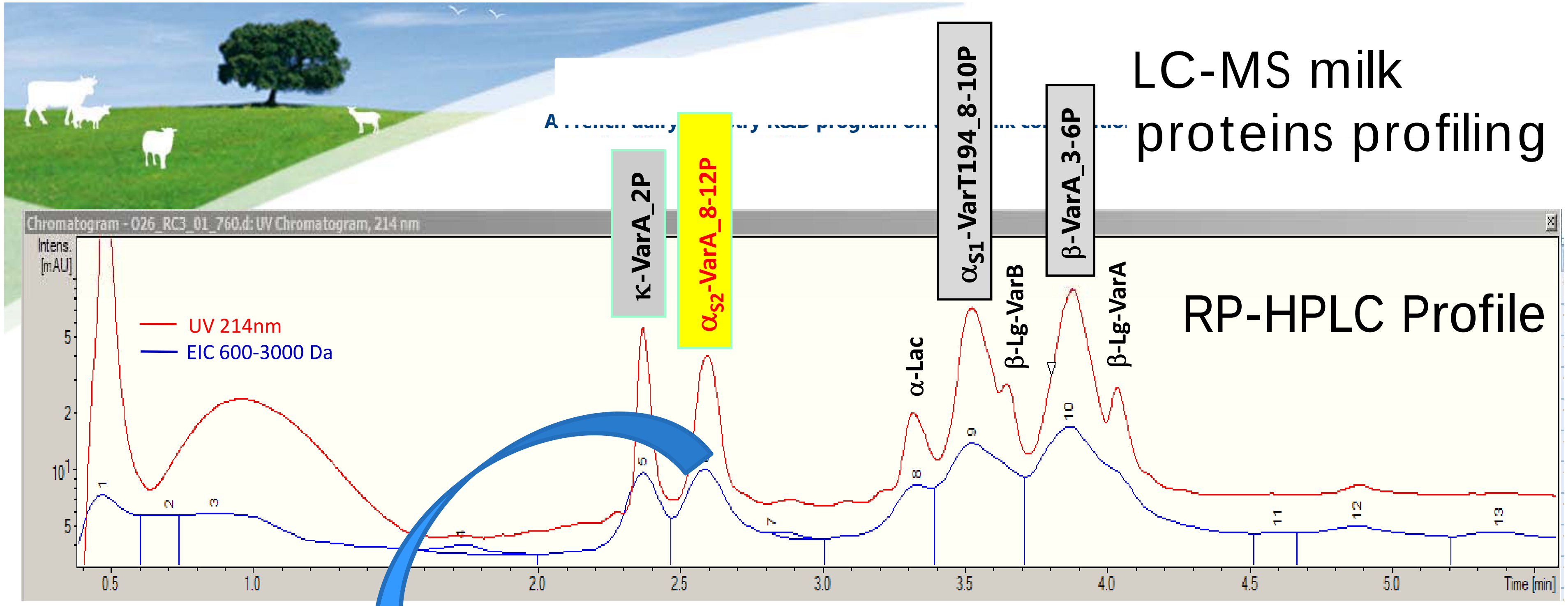


Bovine Database : 3000 masses

✓9 proteins : caseins (κ, α_{s1}, α_{s2}, β), β-Lg, α-la, SA, lactoferrin and lactoperoxydase
✓Known genetic variants
✓phosphorylation and glycosylation isoforms
✓Main proteolysis products

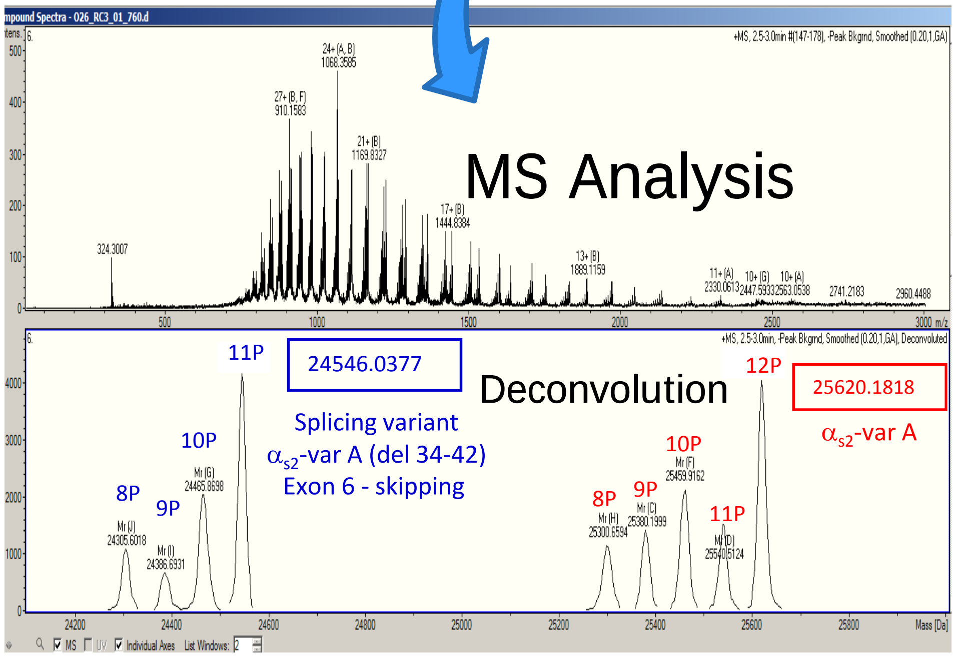
LC-MS milk proteins profiling

RP-HPLC Profile



MS Analysis

Deconvolution



Bovine milk: 25 identified molecules

5 isoforms κ-Cn (1 to 3 glycosylations)

2 isoforms κ-Cn (phosphorylation)

5 isoforms α_{s2}-Cn (phosphorylation)

2 isoforms α_{s1} et β-Cn (phosphorylation)

1 splicing variant α_{s1}-Cn (ΔQ78)

2 genetic variants β-Lactoglobulin

1 genetic variant α-Lactalbumin

5 fragments β-Cn

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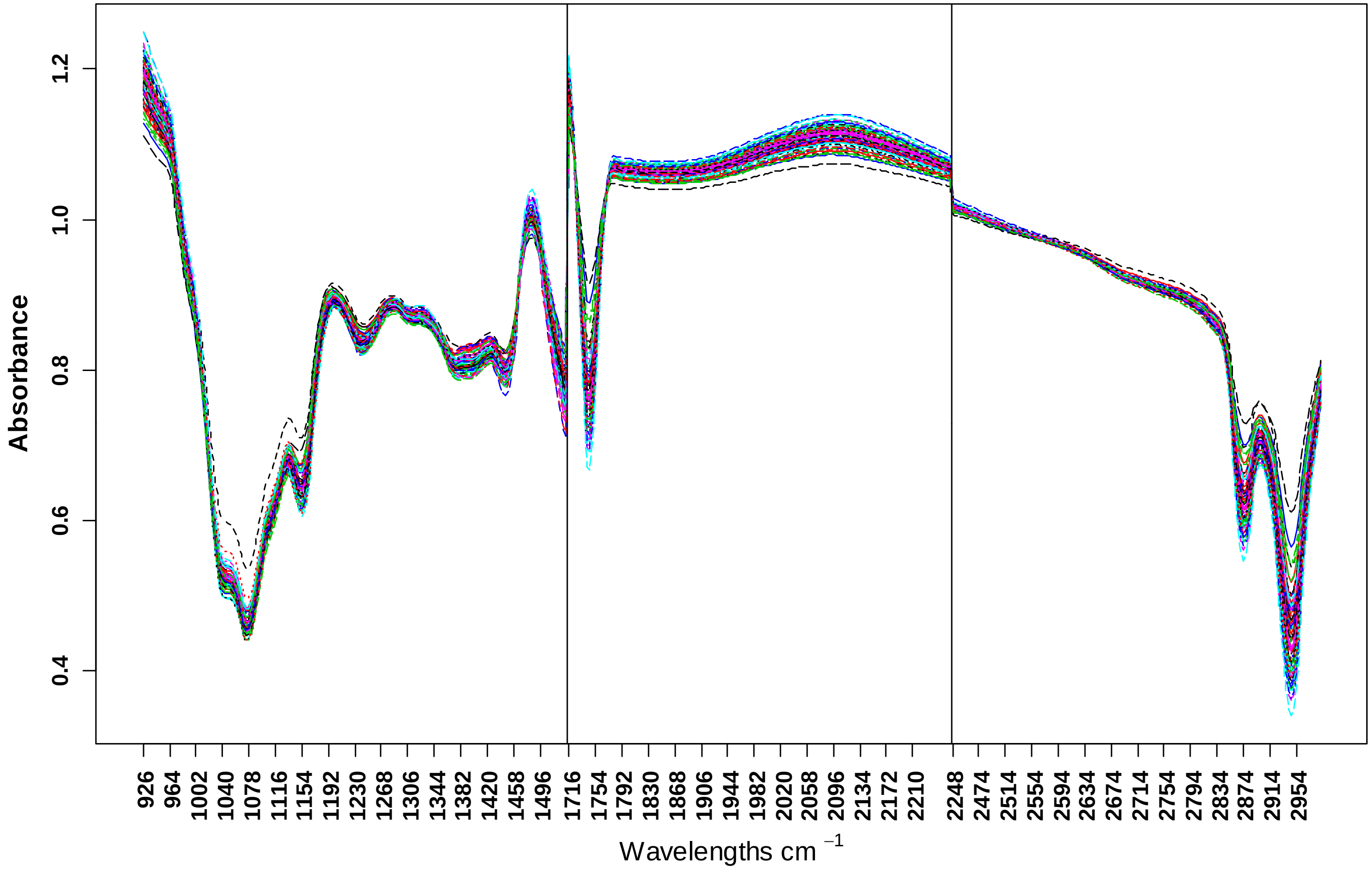
Milk composition prediction from MIR spectra

Spectrum from 75 cow milk samples (UE INRA Mirecourt + Domaine du Pin)
MilkoScan FT6000 (Foss Electric, Hillerod, Denmark)
LILANO (Milk recording laboratory)

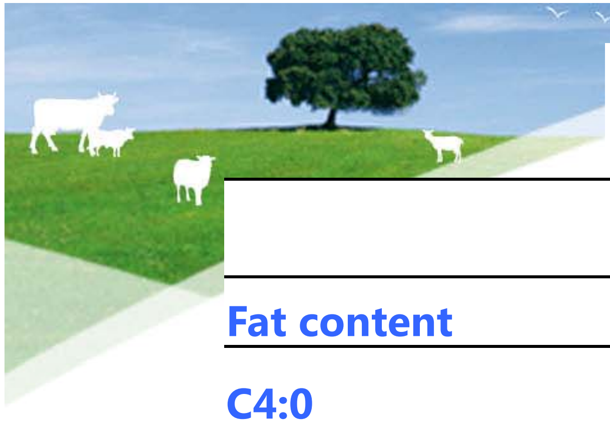


Reference samples

- Selected for their large variability
- Gas chromatography for fatty acids, RP-HPLC + LC-MS for proteins

- 450 bovine samples
- 200 ovine samples
- 300 goat samples

- PLS Analysis with variable selection with a genetic algorithm (Ferrand et al)



Prediction results (cattle)

	Mean	Std Dev	Relative Error (%)	R^2
Fat content	3,816	0,637	0,32	1,00
C4:0	0,149	0,025	5,71	0,88
C6:0	0,087	0,015	3,97	0,95
C8:0	0,050	0,010	5,00	0,94
C10:0	0,111	0,029	6,92	0,93
C12:0	0,126	0,037	11,12	0,86
C14:0	0,435	0,088	6,10	0,91
C16:0	1,271	0,282	6,41	0,92
C18:0	0,342	0,099	12,58	0,81
Total 18:1	0,780	0,203	6,70	0,93
Saturated	2,766	0,510	2,09	0,99
Monoinsatured	0,889	0,220	5,80	0,95
Polyinsatured	0,107	0,019	8,06	0,80
Omega 3	0,029	0,010	16,24	0,77
Omega 6	0,075	0,016	11,23	0,72

Data collection

(Fall 2009 – Winter 2010)

- Farms and animals

Species	Bovine	Ovine	Goat
Breeds	Holstein, Normande, Montbéliarde	Lacaune, Manech	Alpine, Saanen
Farms	1 000	160	204
Surveys	7 900	960	830
Females with spectra	88 000	20 000	70 000
Spectra	425 000	119 000	274 000
Femelles sampled	12 000	3 400	4 300

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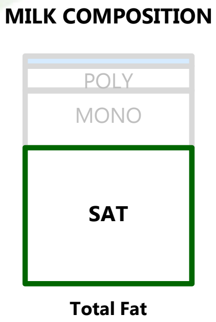
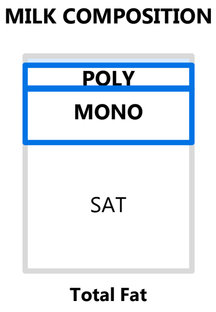
Test-Day heritability estimates



h^2= 0.18 to 0.39

1

g/100g milk	MO	NO	HO
C14:0	0.35	0.38	0.29
C16:0	0.32	0.34	0.28
C18:0	0.20	0.18	0.21
Total SAT	0.32	0.34	0.28



h^2= 0.10 to 0.24

2

g/100g milk	MO	NO	HO
C18:1c9	0.13	0.16	0.11
C18:2c9t11	0.17	0.14	0.11
MONO	0.13	0.18	0.15
POLY	0.21	0.24	0.22

h^2 SAT > h^2 UNSAT

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Correlations between Fatty Acids (expressed in % of milk)

	Milk Yield	Total Fat
SAT	-0.15 to -0.40	+0.77 to +0.95
UNSAT	-0.20 to -0.53	+0.42 to +0.51

	SAT	UNSAT
SAT	0.19 to 0.95	
UNSAT	0.16 to 0.78	0 to 0.69

Profiles in % fat: some results

h^2 **higher** when expressed in **milk** than in fat

Normande	g/100g fat	g/100g milk
C14:1cis9	0.19	**0.34**
C18:2t9cis12	0.10	**0.15**
Total SAT	0.22	**0.38**

Genetic correlations:

Montbeliarde	g/100g fat	g/100g milk
C14:0 / **C18:1**	-0.75	0.24
milk yield / C18:1	0.00	-0.53

Increase SAT = decrease UNSAT in fat and increase UNSAT in milk

Increase milk = no consequence for UNSAT in fat and
decrease UNSAT in milk

QTL Detection in cattle

- 2,867 Holstein, 1,924 Montbeliarde, 2,448 Normande in L1-L2
- Bovine 50K Beadchip® (500 more LD genotypes to come)
- 36,913 to 39,683 SNP according to the breed

- LDLA Meuwissen et Goddard (2001)

- Genome scan with a sliding haplotype of 6 markers

Traits

- FA expressed in g/100g total fat
- All traits obtained from MIR spectra and prediction equations

Total SAT
C4:0
C6:0
C8:0
C10:0
C12:0
C14:0
C15:0
C16:0
C17:0
C18:0
C20:0

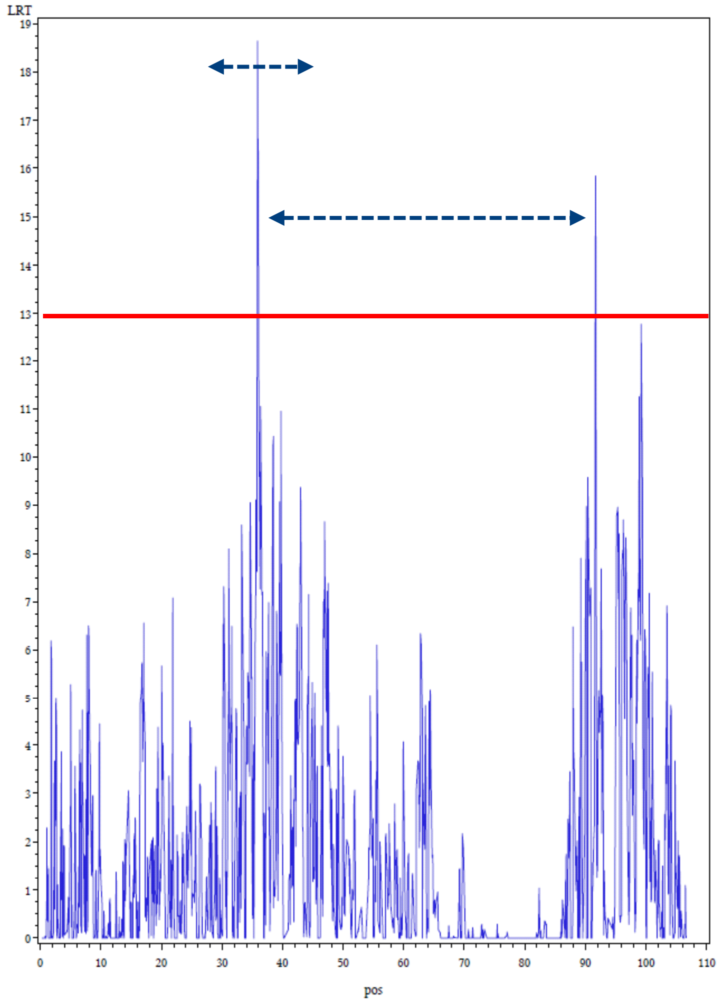
Total UNSAT	
Total MONO	**Total POLY**
C14:1cis9	C18:2c9c12
C16:1cis9	C18:2cis9t11 (CLA)
C18:1cis9	TotC18:2
C18:1cis11	
C18:1t11t10	
C18:1cis12	
TotC18:1	

omega3
omega6
omega7
Desaturation index
index14
index16
index18

Index14 = C14:1cis9/(C14:0+C14:1cis9)

Rules to define a QTL

C4:0 – BTA 11 - MO

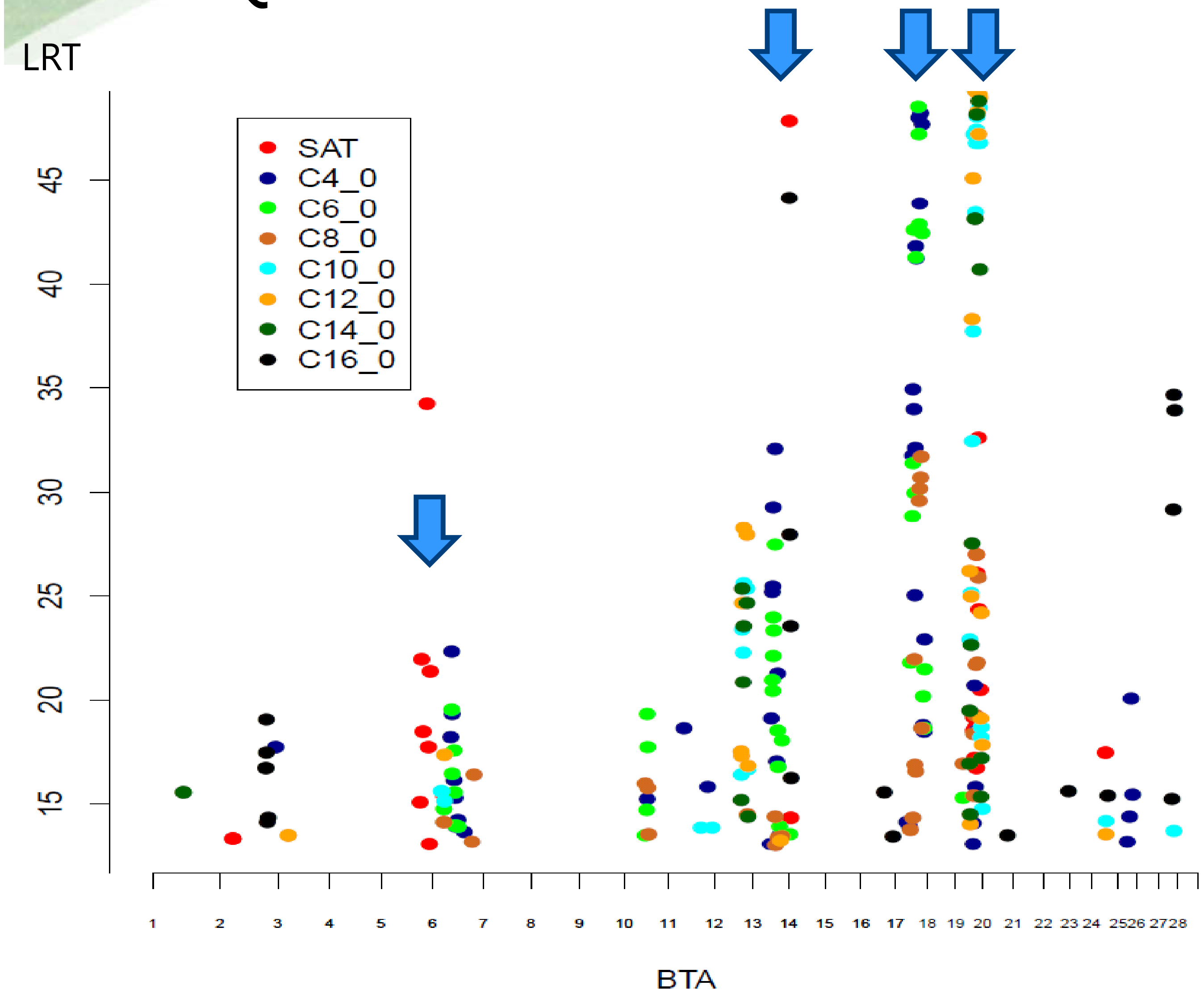


For following plots,

- LRT > 13
- Maximum in each 2 Mb interval
- Distance between 2 QTL > **10** Mb

Short and Medium SAT

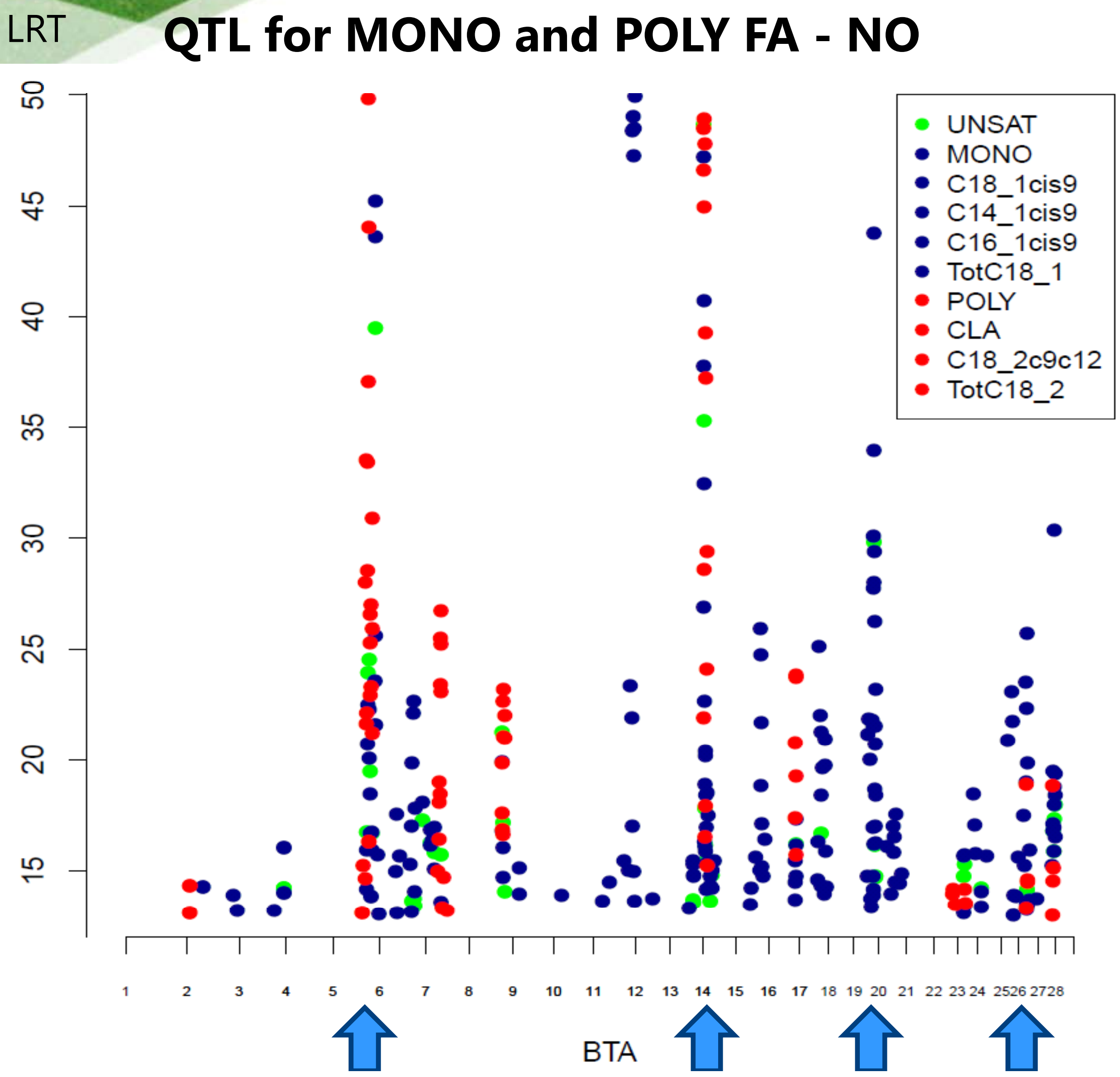
QTL for SAT FA - MO



Nb QTL	Total 3 breeds
C4:0	**36**
C6:0	**29**
C8:0	**30**
C10:0	**28**
C12:0	**20**
C14:0	**13**
C16:0	**23**

— UNSAT FA

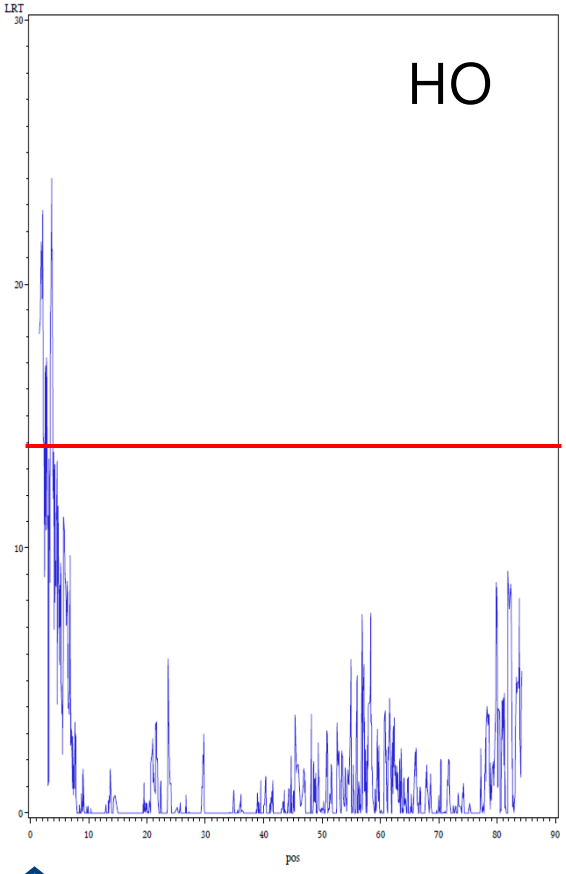
QTL for MONO and POLY FA - NO



Nb QTL	Total 3 breeds
omega3	**24**
omega6	**19**
C14:1cis9	**28**
C16:1cis9	**29**
C18:1cis9	**28**
CLA	**17**
Tot_C18:1	**24**
C18:2cis9cis12	**12**

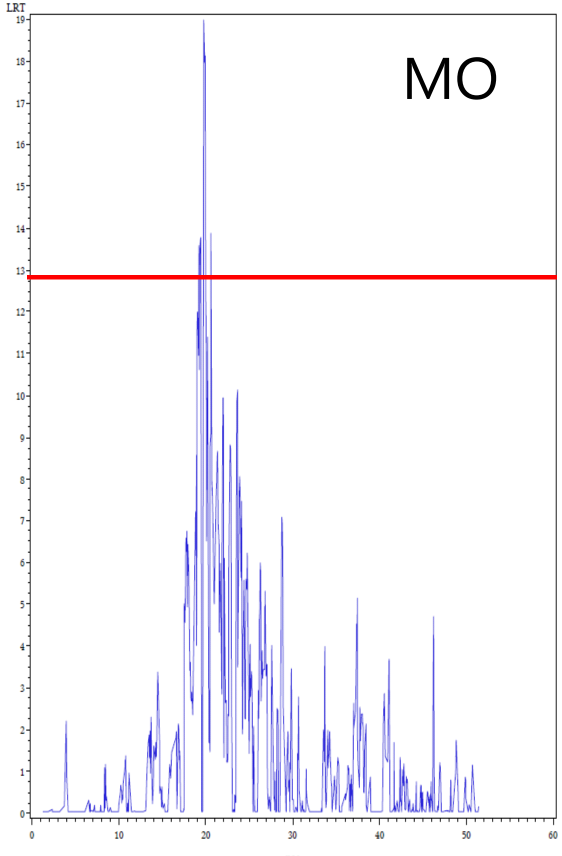
Known regions

BTA 14 - C8:0



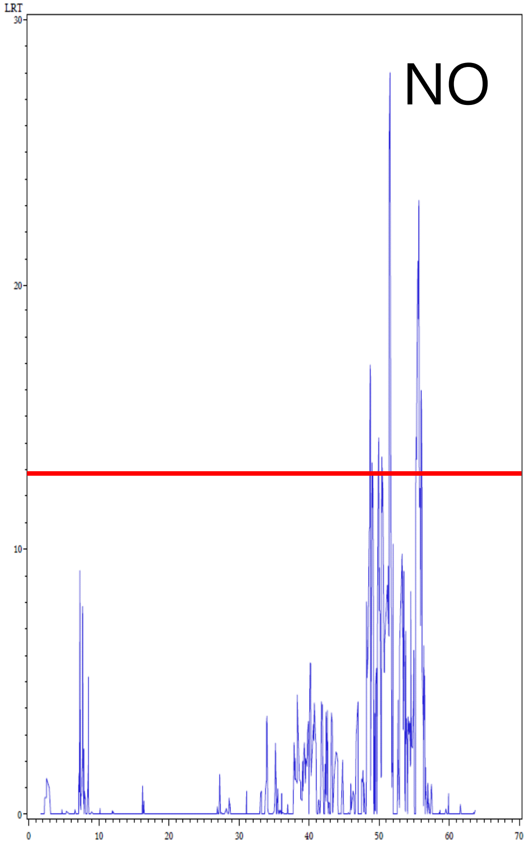
DGAT1

BTA 26 - C14:1cis9



SCD1

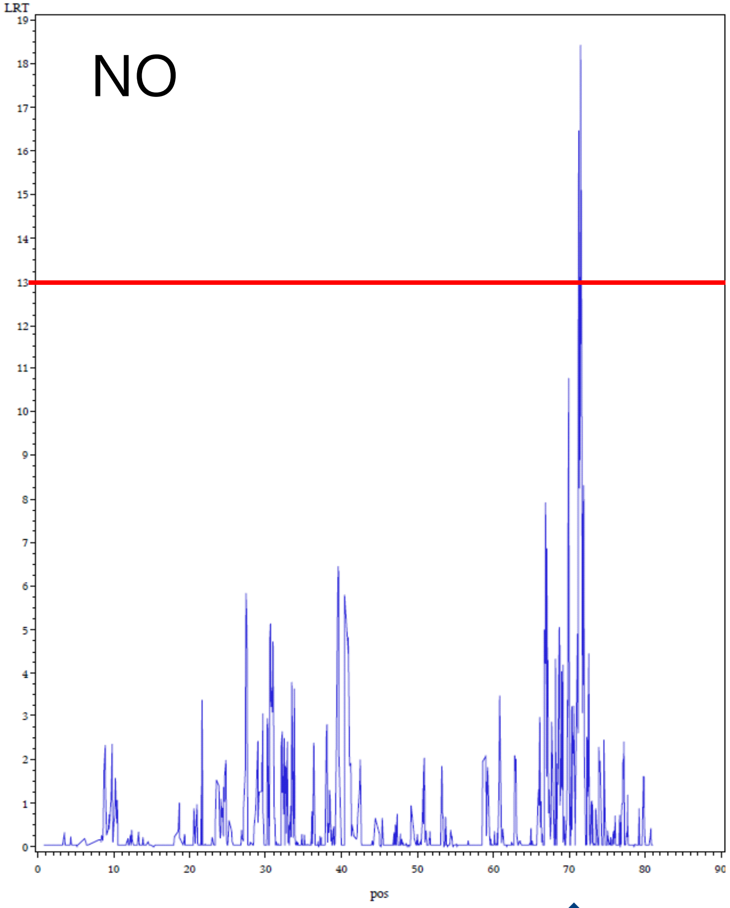
BTA 19 - Tot C18:1



FASN

Focus on some regions

BTA 16 – Omega3



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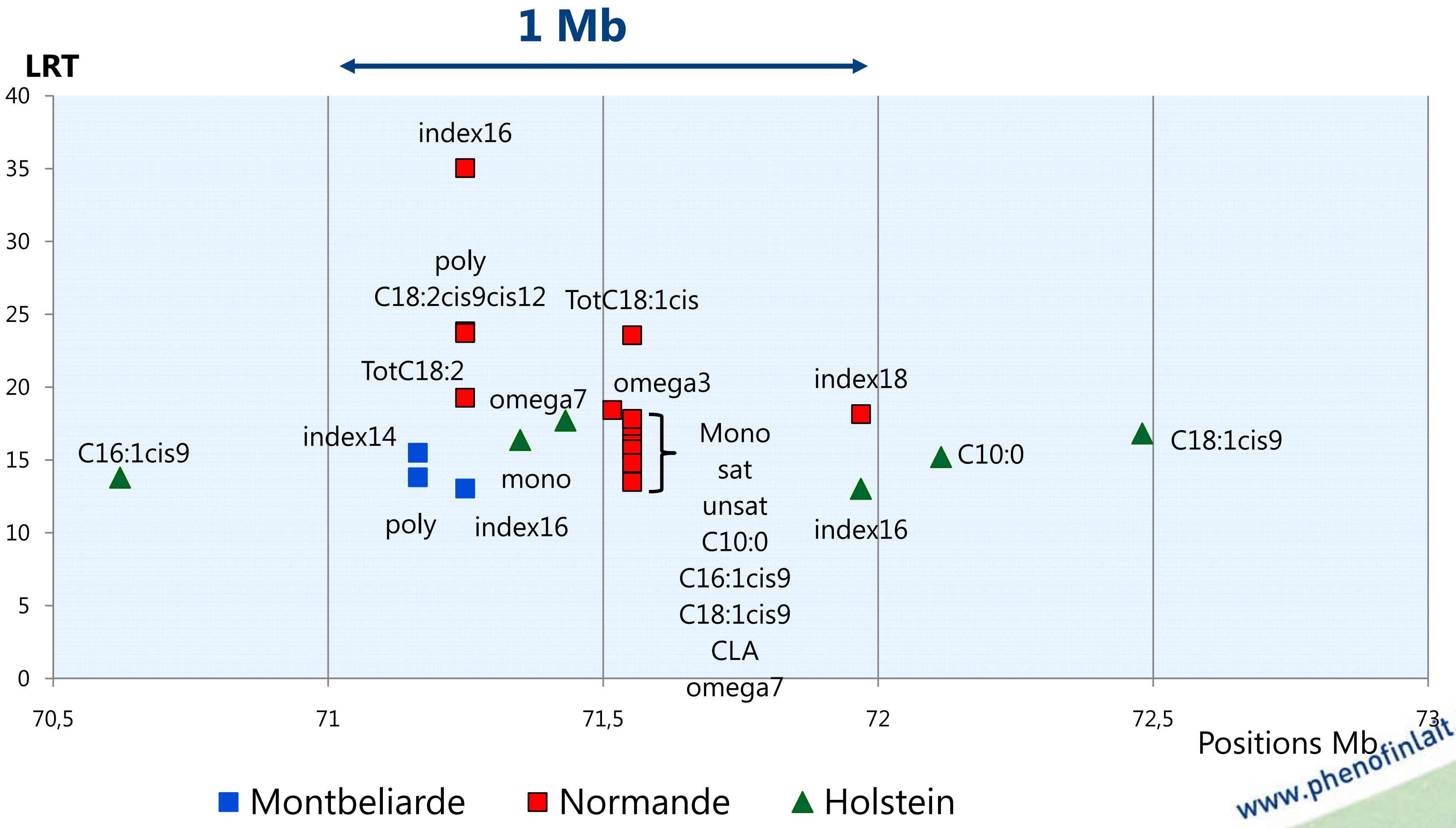
Fine localisation

BTA 16

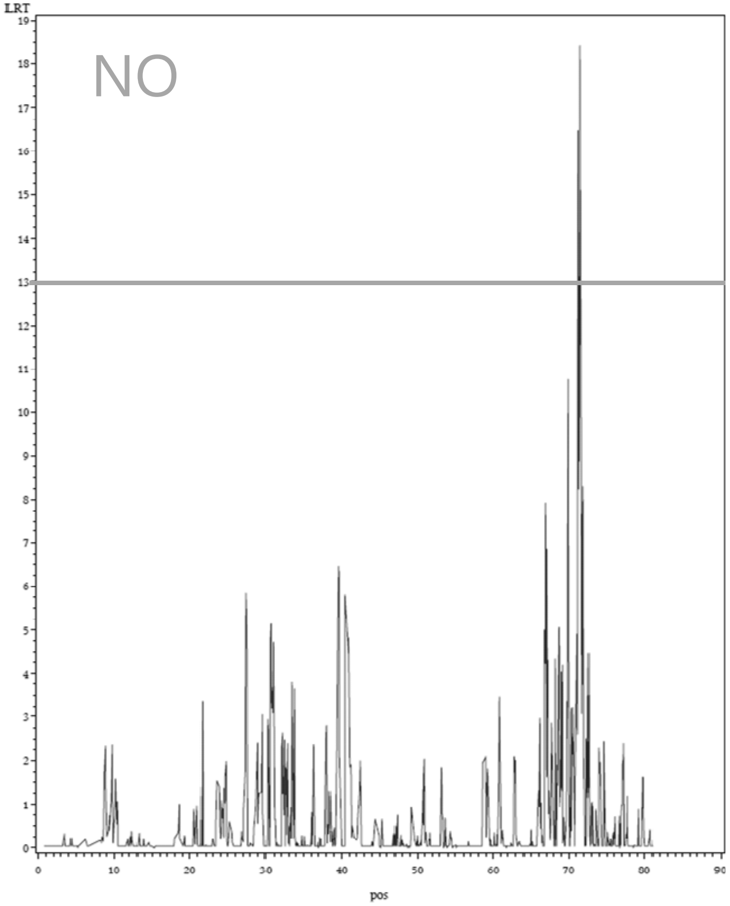
QTL for 17 traits detected in the 70.6 – 72.5 region



IMGC - Wageningen

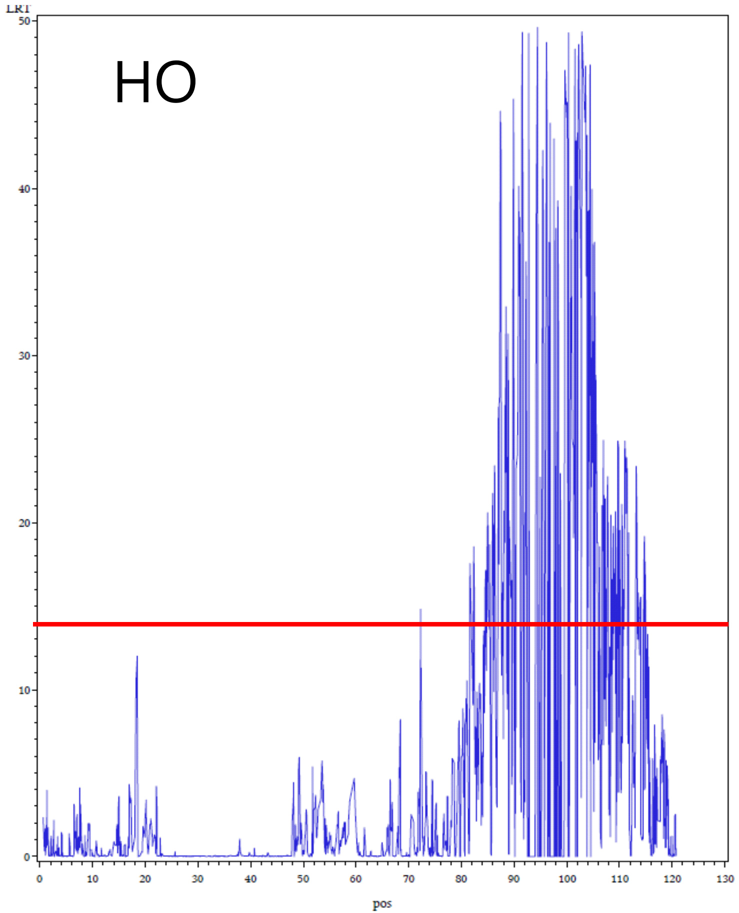
Focus on some regions

BTA 16 – Omega3



Fine localisation

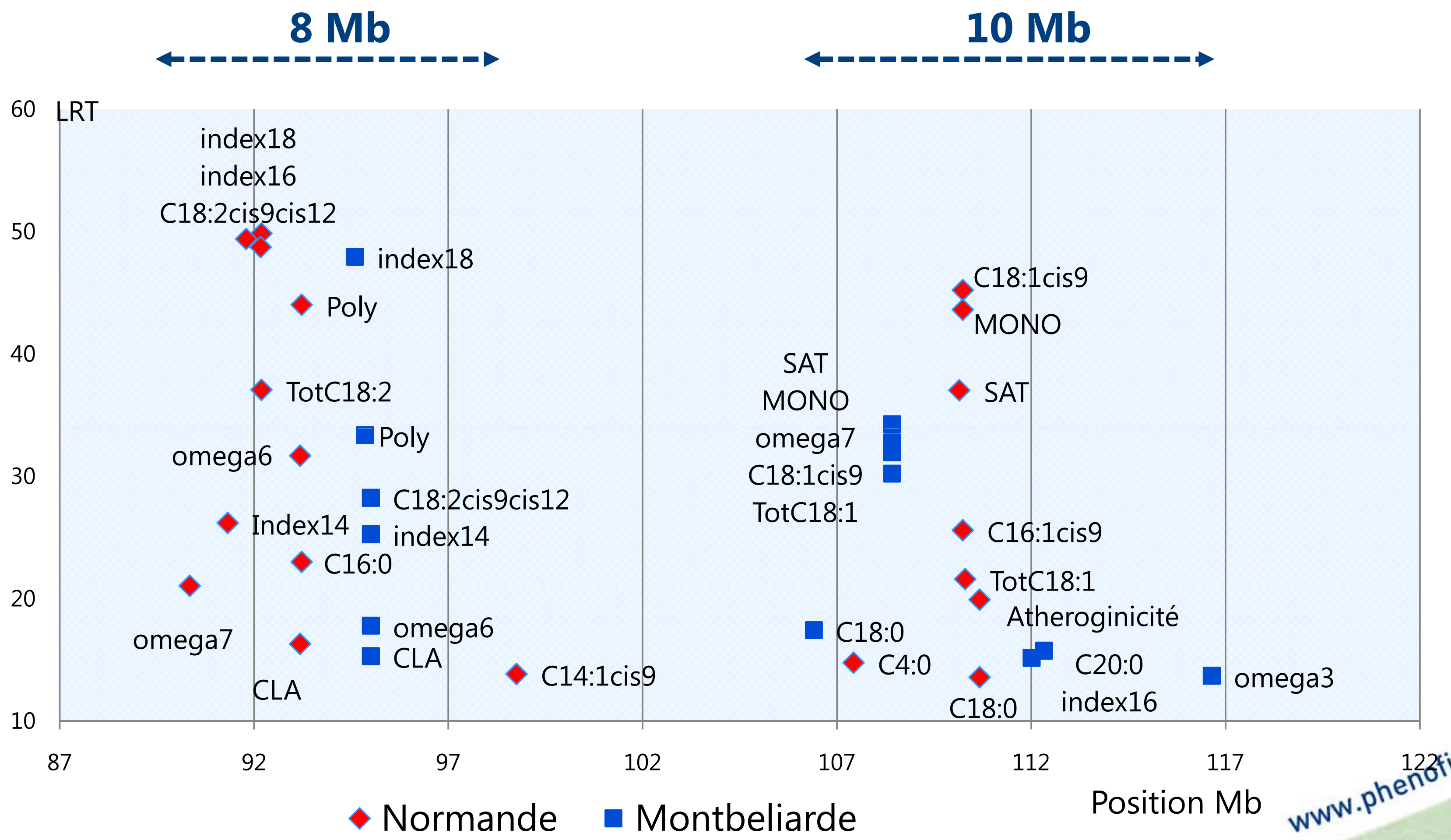
BTA 5 – MONO



Imprecise localisation

BTA 5: MO and NO

QTL for 23 traits detected - Probably 2 regions



IMGC - Wageningen

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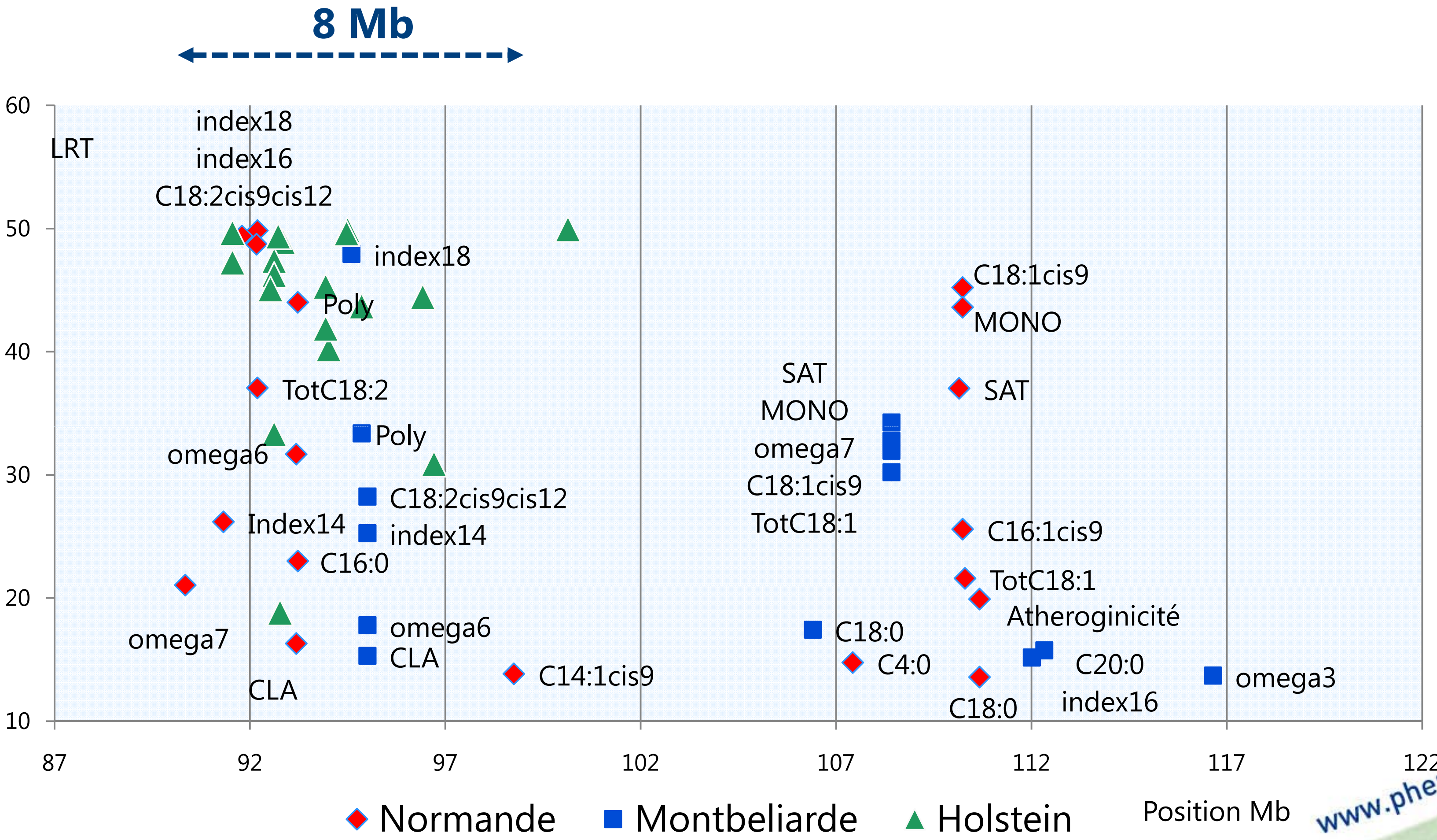
BTA 5: MO, NO and HO

The first region is shared across the three breeds



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Summary of QTLs shared accross breeds

QTLs are assumed to be shared if the distance between peaks **< 2 Mb**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
C4:0		🟨									🟧		🟥				🟥			🟧									
C6:0									🟧				🟨	🟨			🟥		🟡										
C8:0					🟧					🟨			🟥				🟥		🟥				🟧						
C10:0													🟧			🟧			🟥								🟡		
C12:0																			🟥										
C14:0																			🟥										
C16:0														🟡													🟥		
C18:0					🟥														🟨	🟧									
C20:0																			🟧	🟧									
C14:1cis9											🟥												🟡		🟥				
C16:1cis9													🟡		🟧	🟧			🟥										
C18:1cis9					🟡									🟥		🟧			🟥				🟧				🟧		
omega3	🟨				🟥	🟨					🟥		🟡	🟥											🟨		🟥		
omega6					🟥						🟥			🟡													🟥		
index14					🟥						🟥			🟥	🟡										🟧		🟥		
index16					🟥						🟨			🟥		🟥			🟥										
index18					🟥									🟥															
CLA					🟥									🟡															
C18:2c9c12					🟥									🟡													🟧		
TotC18:1					🟡									🟡															

🟥 **HO-MO-NO** 🟧 **HO-NO** 🟨 **HO-MO** 🟡 **MO-NO**

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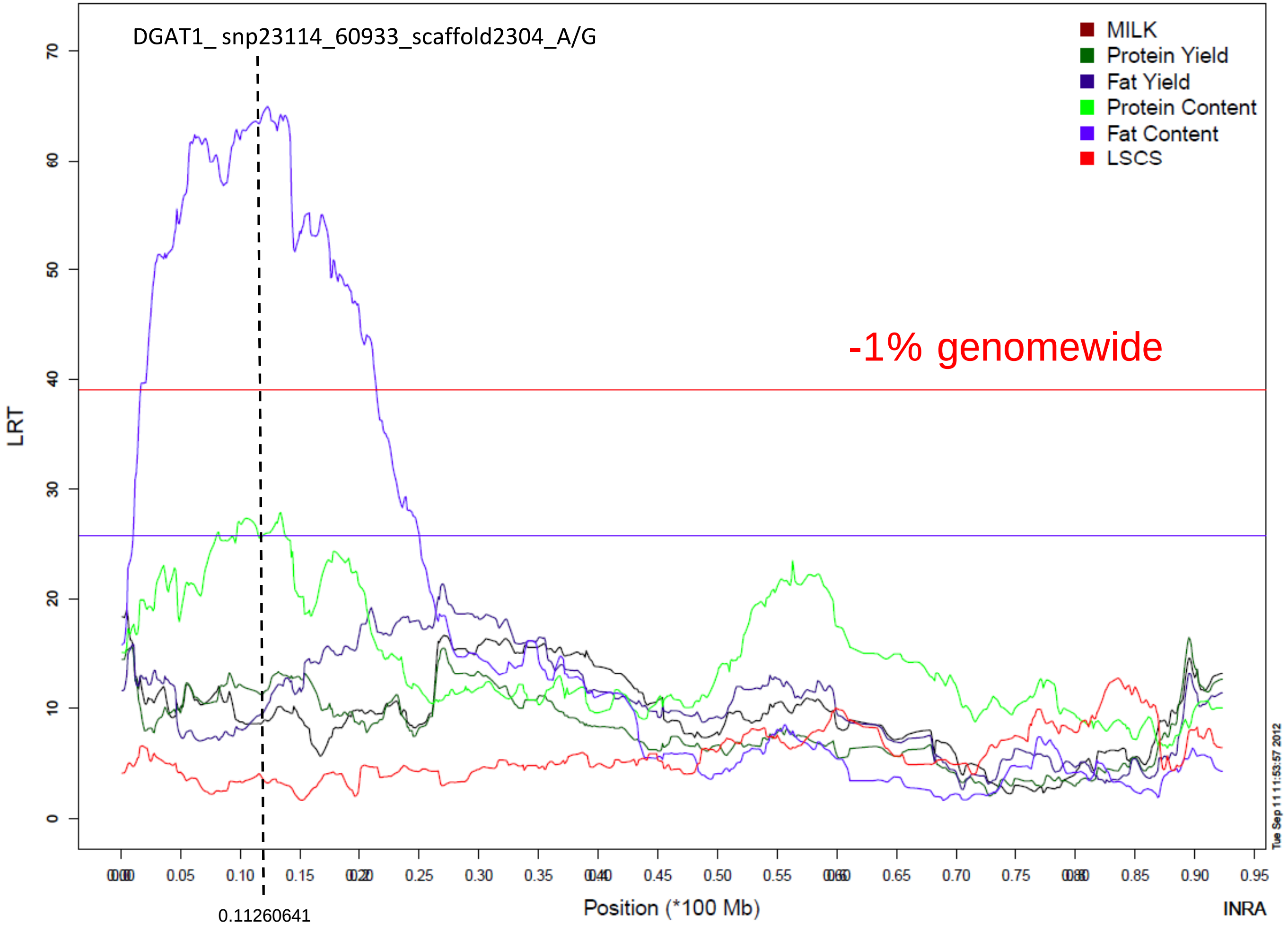
Chromosome 14 - Saanen



Maroteau et al

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Conclusion

- **An ambitious but complex project**
- Not yet completed, many analyses still to carry out
- Very large data base for the future
- A reference population for milk composition, to prepare genomic selection

List of participants

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C Maroteau, O Leray, JM Astruc, G Lagriffoul, I Palhière, K Duhem

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FranceAgriMer

France Génétique Elevage

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