Developing biomarkers for livestock Science

Ongoing research and future developments

Marinus te Pas

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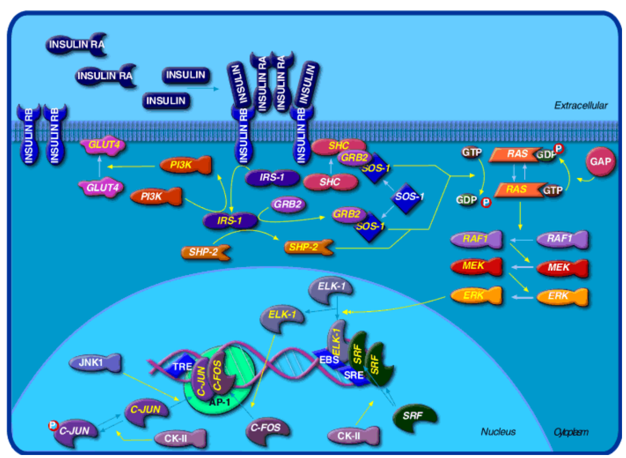
Outline

- Introduction
  - What are biomarkers
  - Why do we need them
- Examples
  - omics levels
- The future
  - Big data
  - Systems biology / Synthetic biology

Introduction: What are biomarkers?

- Biological processes underlie all livestock (production) traits
  - Measure the status of a biological process = know the trait!
- Can be any molecule in a cell
  - No need to know the causal factor for a trait
- Well known example: blood glucose level for diabetes



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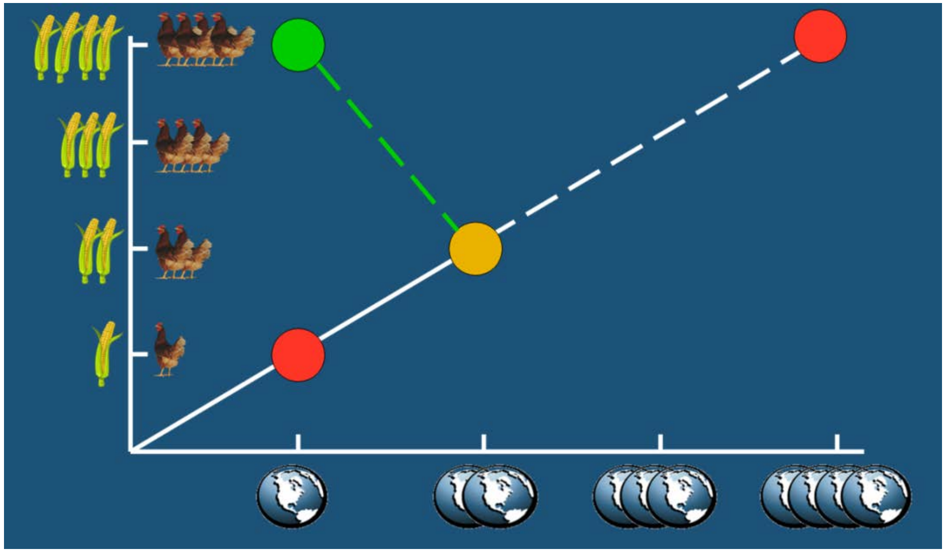
Introduction: Why do we need biomarkers?

- The mission of WageningenUR: Sustainably produce enough high quality food for all people on the planet with an ecological footprint as low as possible



What can the industry do with biomarkers?

- Diagnostic tool
  - What is the biological mechanism underlying a trait?
- Prediction tool
  - What outcome can I expect from an intervention?
- Monitoring tool
  - What is the actual status of a process?

- **Speed up your process, improve your traits**

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Why Biomarkers for meat quality?

- Meat quality has low heritability (h^2=0.1-0.2)
  - Predictive capacity of genetic markers low
- High environmental influence
  - Feed, animal handling (stress), management (housing), ...
- Meat quality can only be measured after 1-several days
- Need to differentiate between retail, processing industry, restaurants, ...

- Biomarkers can do all that and more

Example: Transcriptomics biomarkers for meat quality

- Pork production chain
  - German high quality fresh pork production chain
  - Pietrain based
  - Verification: Yorkshire based chain
  - Biomarker type: RNA expression
  - Availability: Microarray / PCR test

- Biomarkers for traits

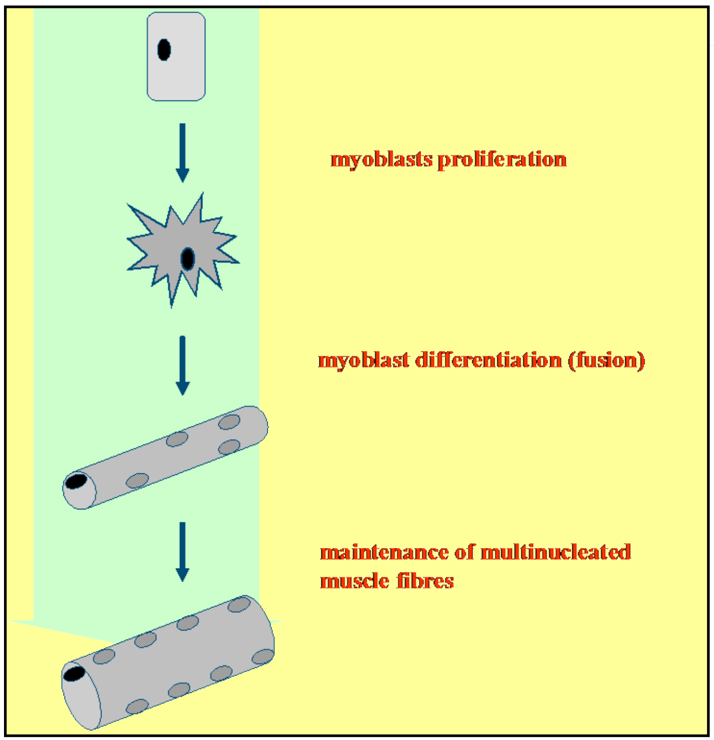
Meat colour	N
• A*	14
• L*	4
• Reflection	10
Drip loss	2
Ultimate pH	6
BFT	4
Carcass weight	4
• Meat thickness	2
• Lean meat %	3

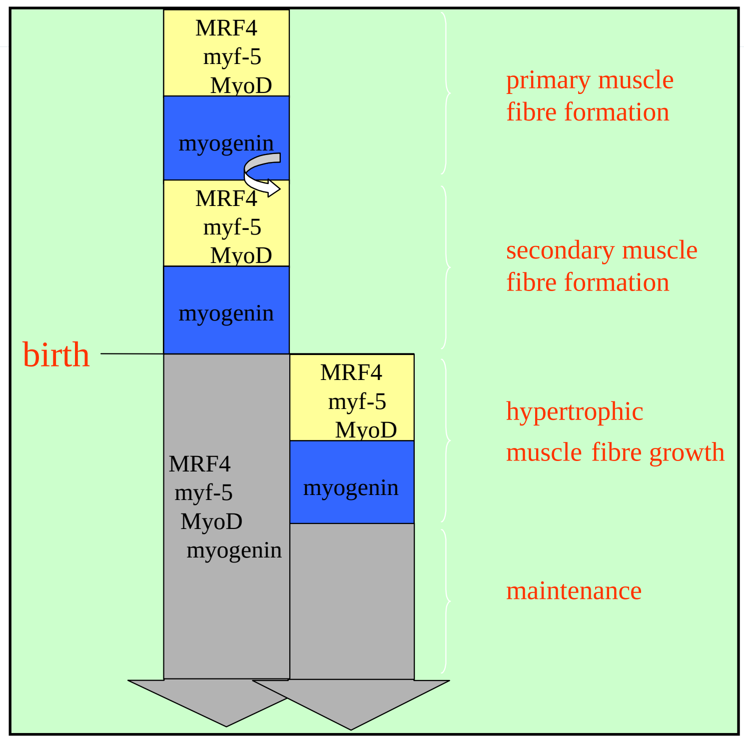
Biological Mechanism: Prenatal events that determine the post mortem meat quality

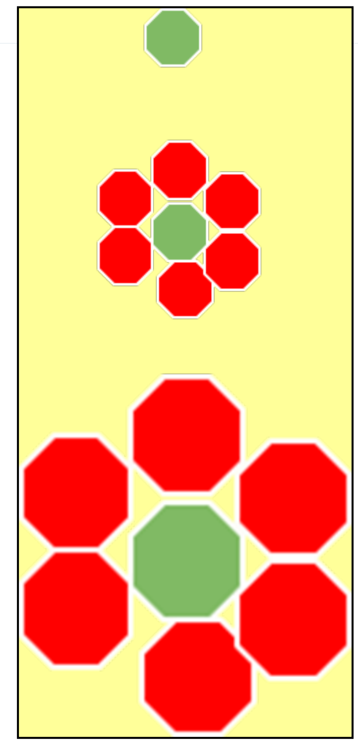
- Muscle fiber development is an exclusive prenatal event
- The number of muscle fibers is determined prenatal
- The number of muscle fibers relates to the thickness of the fibers at slaughter
- Thicker muscle fibers usually relates to more pale and exudative meat

Background: Muscle (fiber) development and growth

(Muscle development of livestock animals, eds. Te Pas, Everts, Haagsman)







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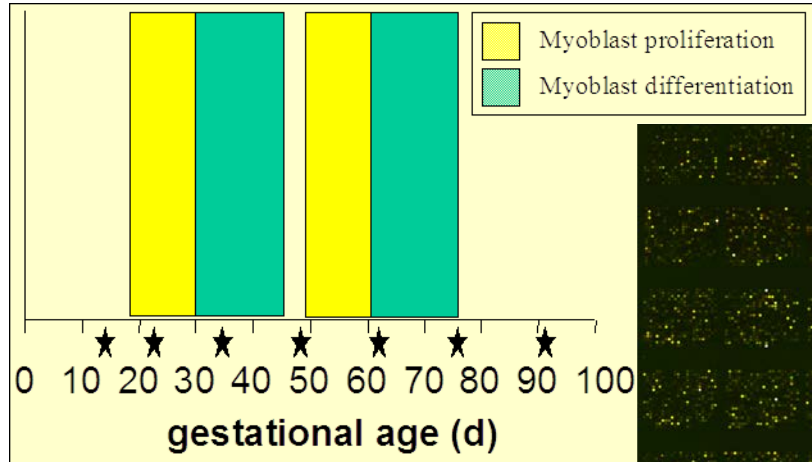
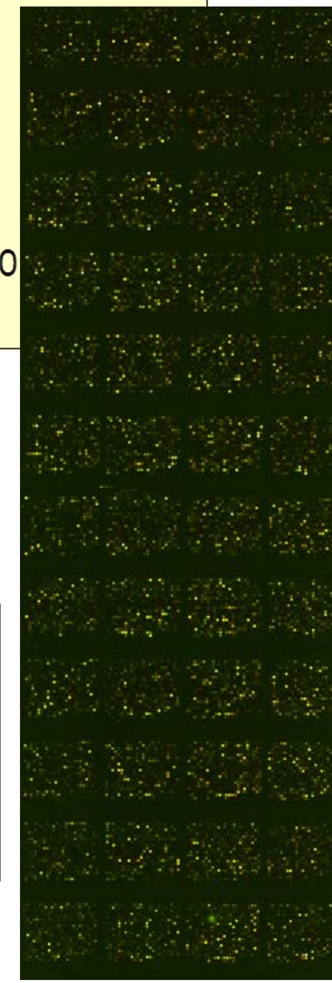
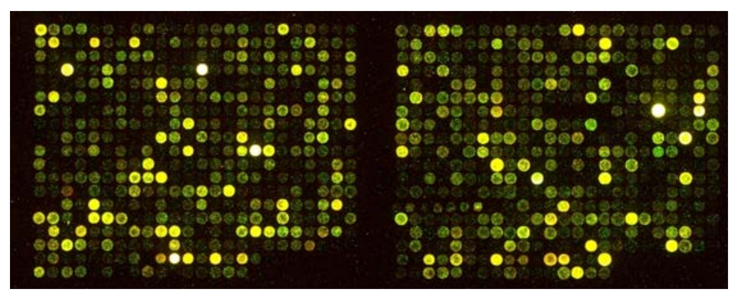
Experimental design

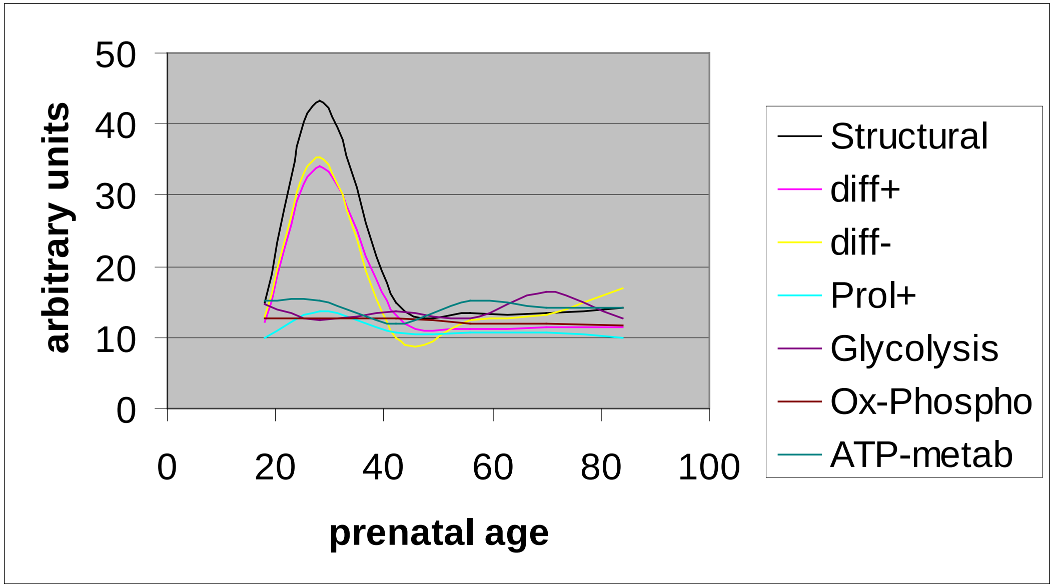
- Slaughter pregnant sows at days of gestation:
  - 14, 21, 35, 49, 63, 77, 91
- Use microarrays to find genetic factors involved

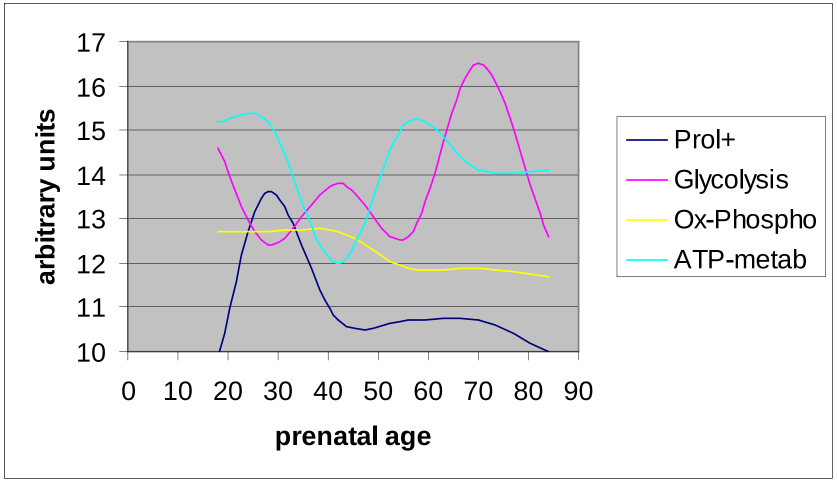




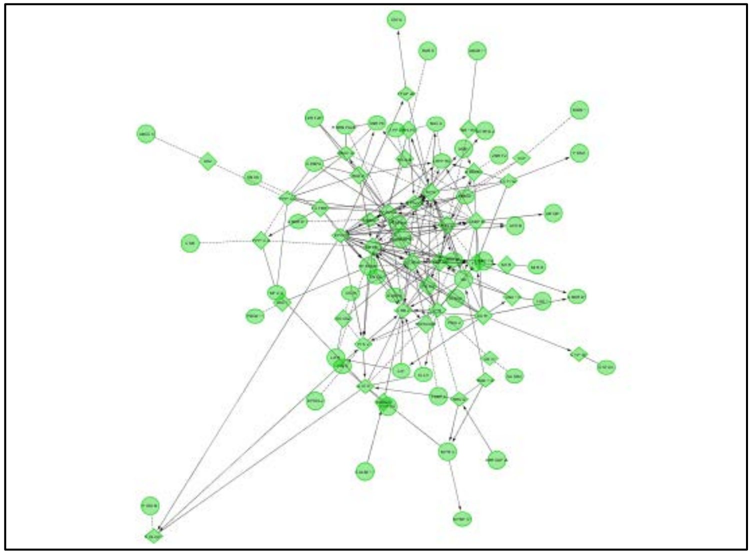


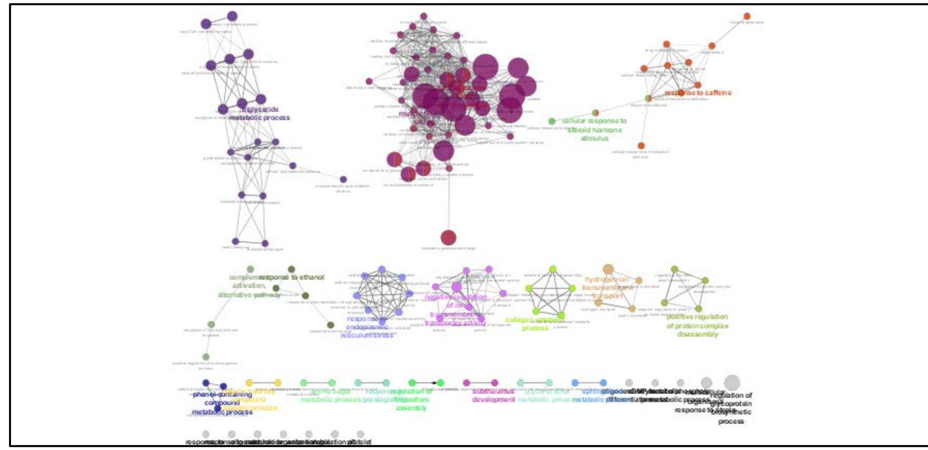
Pig muscle fiber development – Results

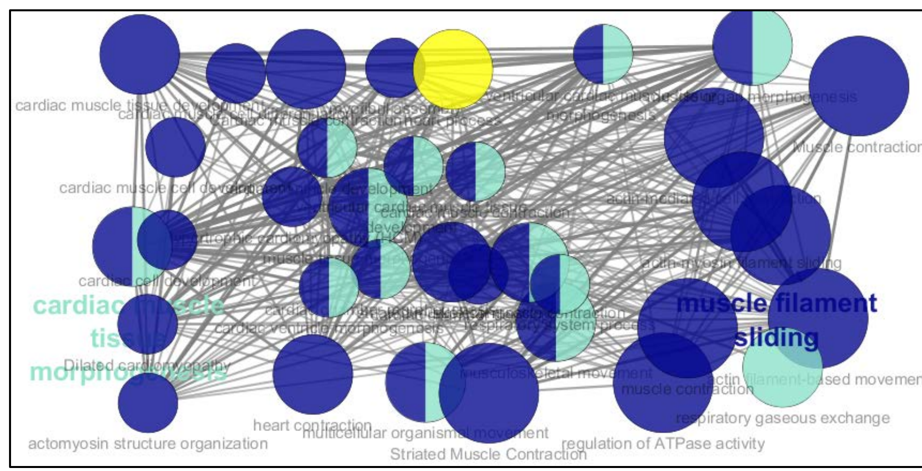




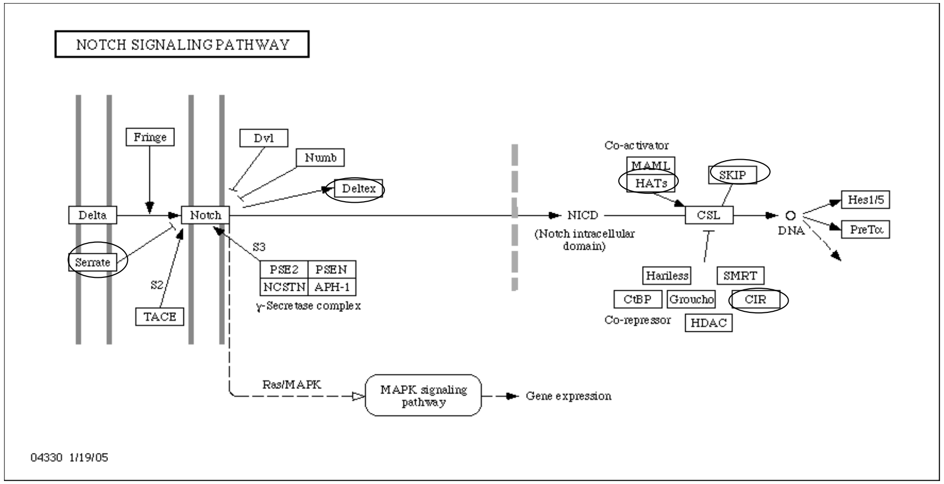
Genes, networks, pathways, networks of pathways

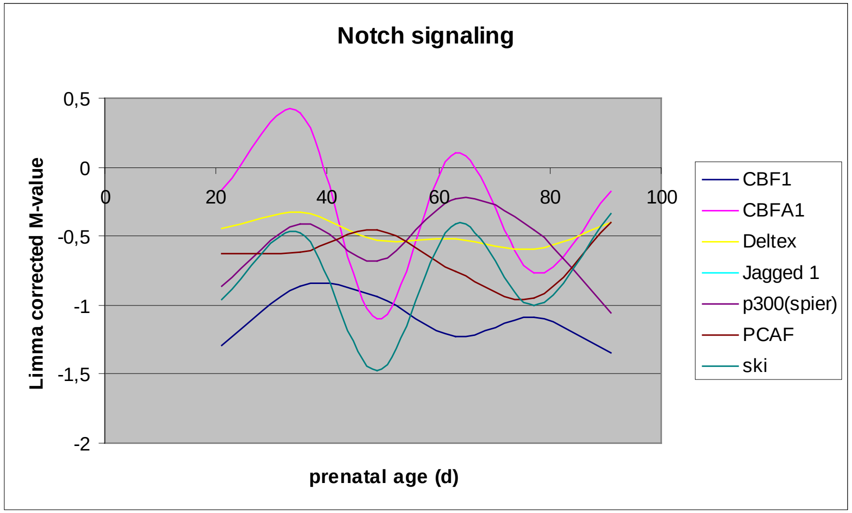






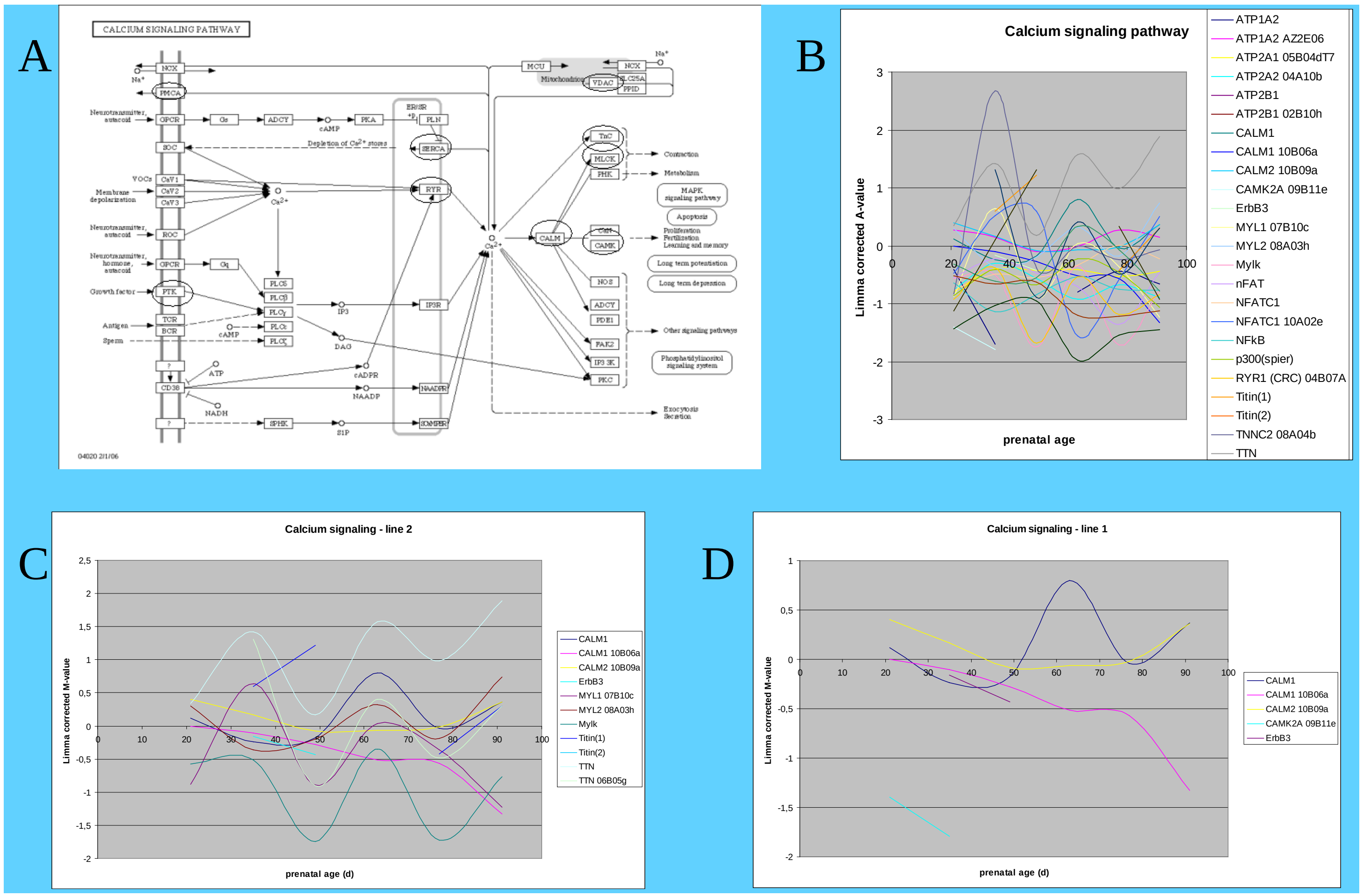
Bioinformatics – Results 1
A simple pathway without subpathways



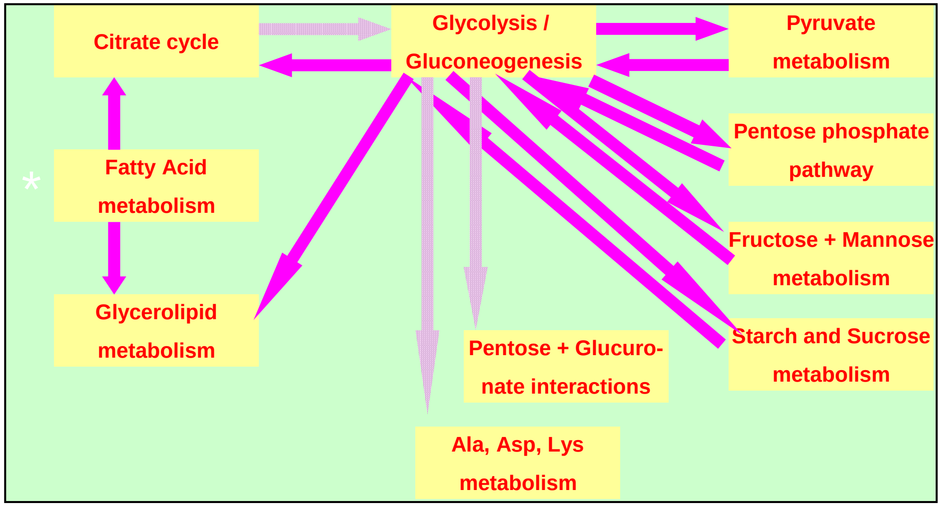


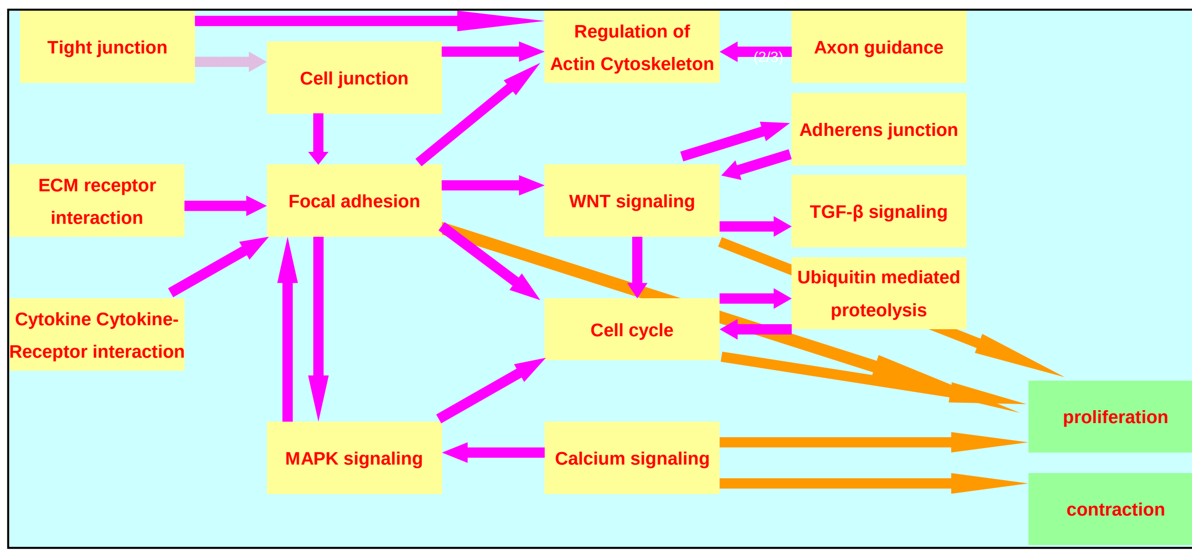
Bioinformatics – Results 2

A more complex pathway



Bioinformatics: From pathways to networks



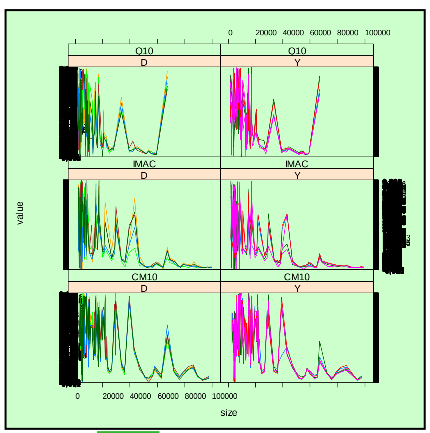


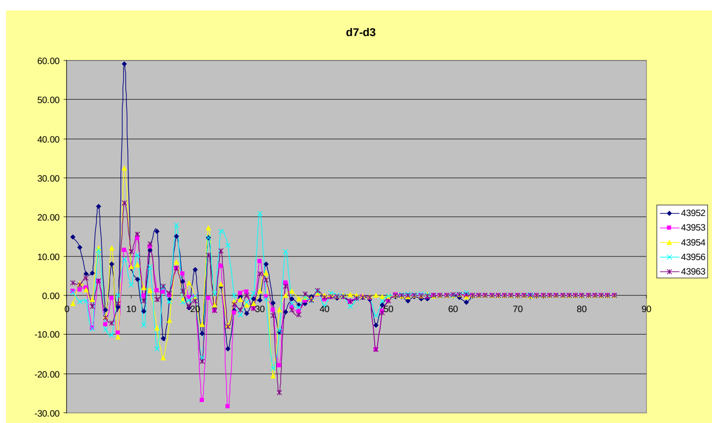
Example Proteomics

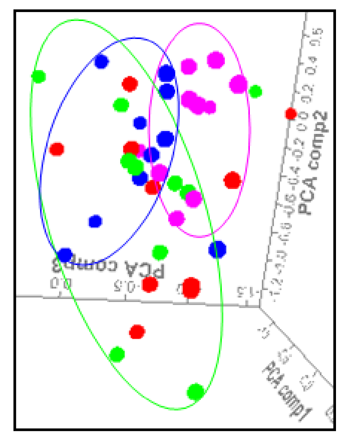
- 150 LW x Duroc
  - Longissimus
  - Sows and castrates
  - Meat quality measurements
- Proteomics
  - SELDI-TOF
    - M/z ratio profiles
    - Association studies
    - Analysis of optimum predictive set of peaks
  - FTMS
    - Identification of proteins and Bioinformatics

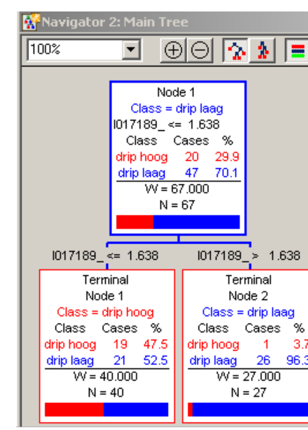
Biomarker analysis

- Associations Protein peak heights – meat quality traits
  - Long list, but...
- Predictive test development
  - PSLR: find combinations of peaks with highest predictive capacity for meat quality traits
  - Calculated mean, minimal, and maximal predictive values









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Biomarkers: Predictive capacity and protein numbers

Trait	Mean	s.d.	Min	Max
Drip loss	0.481	0.148	0.181	0.800
Fat34FOM	0.466	0.161	0.140	0.720
Loin34FOM	0.202	0.232	-0.276	0.678
IMF	0.285	0.176	-0.088	0.593
Fatthickham	0.374	0.168	0.064	0.609
NPPCmarbling	0.292	0.194	-0.181	0.728
NPPCcolor	0.164	0.148	-0.117	0.511
Ultimate pH LD	0.382	0.221	-0.152	0.695
Ultimate pH SM	0.515	0.160	0.069	0.841

Trait	Selected proteins	
	N	**Proteins**
Drip loss	5	C06624_7, C08453_2, I05698_9, Q01350_8, Q02622_1
Fat34FOM	5	C03140_9, C06624_7, I03904_9, I04417_7, I06617_2
Loin34FOM	3	C03140_9, C08453_2, I06617_2
IMF	1	I04417_7
Fatthickham	3	C08453_2, I04417_7, I06617_2
NPPCmarbling	7	C03140_9, C03905_2, C06110_0, C06624_7, C08453_2, I03904_9, I04417_7
NPPCcolor	5	C04419_7, C05001_4, C06624_7, C08453_2, C010260_
Ultimate pH LD	9	C04419_7, C05702_9, C06624_7, C08453_2, I03904_9, I04417_7, I06617_2, Q01350_8, Q02622_1
Ultimate pH SM	20	C03140_9, C03612_5, C03905_2, C03975_1, C04419_7, C04988_6, C05620_4, C05702_9, C06110_0, C06624_7, C08453_2, C08478_3, C010260_, I03904_9, I04417_7, I06617_2, I08447_3, Q01288_2, Q01350_8, Q02622_1

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Proteomic relations between 2 traits

- Meat quality traits are related
- Biomarkers for drip loss and ultimate pH share a number of proteins / biological mechanisms

	Drip loss				Ultimate pH			
	High		Low		High		Low	
Proteins	Up	Down	Up	Down	Up	Down	Up	Down
Antichymotrypsin (SERPINA3N)	x						x	
Calsequestrin		x		x				
F1RK48 (unknown)	x				x			
F1SUE1 - OGN (Osteoglycin)	x			x	x			
Haptoglobin				x	x			x
Isocitrate dehydrogenase	x		x				x	
Lactate dehydrogenase	x		x					
Pyruvate kinase			x		x			

The Biology underlying the Biomarkers

	Drip loss				Ultimate pH			
	High		Low		High		Low	
Biological activities	Up	Down	Up	Down	Up	Down	Up	Down
Energy metabolism	4	2	3	3	2	1	3	1
Protein degradation	1	3		1	1	1	1	
ECM	1			1	1			1
Signal transduction	1	2		4	2			1
Chaperonin (structural)	1							
Muscle structural protein		3		1	2	1		
Calcium metabolism		2		3	2			1
Apoptosis		1		1		1		1
Nucleotide metabolism		1		1	2			
Muscle mass determination				1				1
Anti-oxidant				2		1		
HSP	1	2		1				

Proteomic biomarkers for Reproduction management in dairy cattle

- Required for continued productivity
- Detection of oestrus necessary, but increasingly difficult
- Detection of early pregnancy could help

- Present situation:
  - Earliest reliable detection of pregnancy at day 35 after insemination
  - Earliest re-insemination at day 21 after previous insemination
  - Re-insemination of pregnant animal has risk of loosing embryo

What a new test should offer

- Pregnancy detection before day 21 after previous insemination
- Preferably in easy to collect biological samples
- High reliability
- Preferably on-site / in-line

Experimental design

- 30 pregnant cows
- 30 non-pregnant cows
  - Pregnancy detected at day 35: PAG and trans-rectal ultrasonography
- Milk samples at day 19 after insemination
- Proteomics and Progesterone measurement

- If Progesterone < 5 ng/ml: not pregnant
- If Progesterone > 5 ng/ml: no pregnancy status detection possible
- PAG test showed no results at day 19
- Therefore: additional markers necessary
  - Proteomics

Our Biomarker

No. of Components	Components	Mean Sensitivity	Mean Specificity	Mean Correctly classified
1	Progesterone	0.8	0.67	0.73
2	MFGM660; Progesterone	0.82	0.8	0.81
3	MS147; MS9; Progesterone	0.86	0.85	0.85
4	MS147; MS9; MFGM713; Progesterone	0.92	0.93	0.92
5	MS147; MS9; MFGM661; MFGM197; Progesterone	0.96	0.93	0.94
6	MS147; MS9; MFGM661; MFGM197; MS92; Progesterone	**0.96**	**0.96**	**0.96**

Patent pending

The determination of pregnancy

- The combination of all components is required for pregnancy detection
- The relative abundances of the proteins determine the detection of pregnancy

Y = (6163.87 + (-247.94*CSN3) + (-969.05*P4HB) + (100.93*RhoB) + (100.38*ALOX12) + (-17.25*CTSZ) + (15.49*Progesterone)

If Y>0, than the cow is pregnant.

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The biology of the Biomarker

Protein fraction	Protein name	Embryo	Placenta	Mammary gland / lactation
MS147	Kappa-casein (CSN3)	+/-	-	+
MFGM661	Rho-related GTP-binding protein (RhoB)	+/-	+	+
MS9	Protein disulfide-isomerase (P4HB)	+	+	-
MFGM197	Arachidonate 12-lipoxygenase, 12S-type (ALOX12)	+	-	-
MS92	Cathepsin Z (CTSZ)	+	+	-
MFGM713	Osteoclast-stimulating factor 1 (OSTF1)	+	+	-

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Example Metabolomics: Dairy cattle – diet and milk composition

- Milk composition is important for uses
- Diet influences milk composition
  - Directly: feed components in milk
  - Via cow metabolism
  - Via metabolism gut microbiota
- Metabolomics
  - Measures metabolite composition in milk
  - NMR / GCMS / LCMS

Experimental design

- Mid lactation dairy cows fed 2 diets for 10 weeks
  - Control diet = standard diet
  - Experimental diet = control diet + PUFA
- Collect milk after 10 weeks feeding
- Fatty acid composition was published before
- Did the diet also change the polar metabolite composition?
  - Must be via metabolism: cow or microbiota
  - Unknown mechanism

Results summary

Polar metabolites	N
Identified	49
Association with diet	14
Association with DGAT1 genotype	8
Interaction: diet-DGAT1	15

- NMR
- Animal-specific reactions to the diet: may be DGAT1 genotype-related

Acetate	Hippurate
Acetoacetate	Inositol myo- (putative)
Acetylcarnitine and butyrate (probable)	Lactate
Acetylcarnitine and isovalerylcarnitine (probable)	Lactose
Aconitate	Lactose (probable)
Alanine	L-Choline; Phosphate-choline; Gpcholine
Ascorbate	Lysine
Aspartate	Maleate
Betaine	Malonate (putative)
BHBA	Nacetylmannosamine (probable) or neuraminate
Butyrate	Orotate
Carnitine	Oxaloacetate
Carnitine acyl-	Oxoglutarate
Choline (glycero)phosphoryl-	Pantothenate
Citrate	Proline
Creatine-phosphate	Pyruvate
Creatinine	Serine phospho-
Formate	sialolactose or lactose
Fumarate	Succinate
Galactose	UDP
Galactose-1-phosphate	Uridine
Gluconolactone	Uridine conjugate
Glucose	Valine
Glutamate	Xanthine
Glycerol phospho-	Xylose

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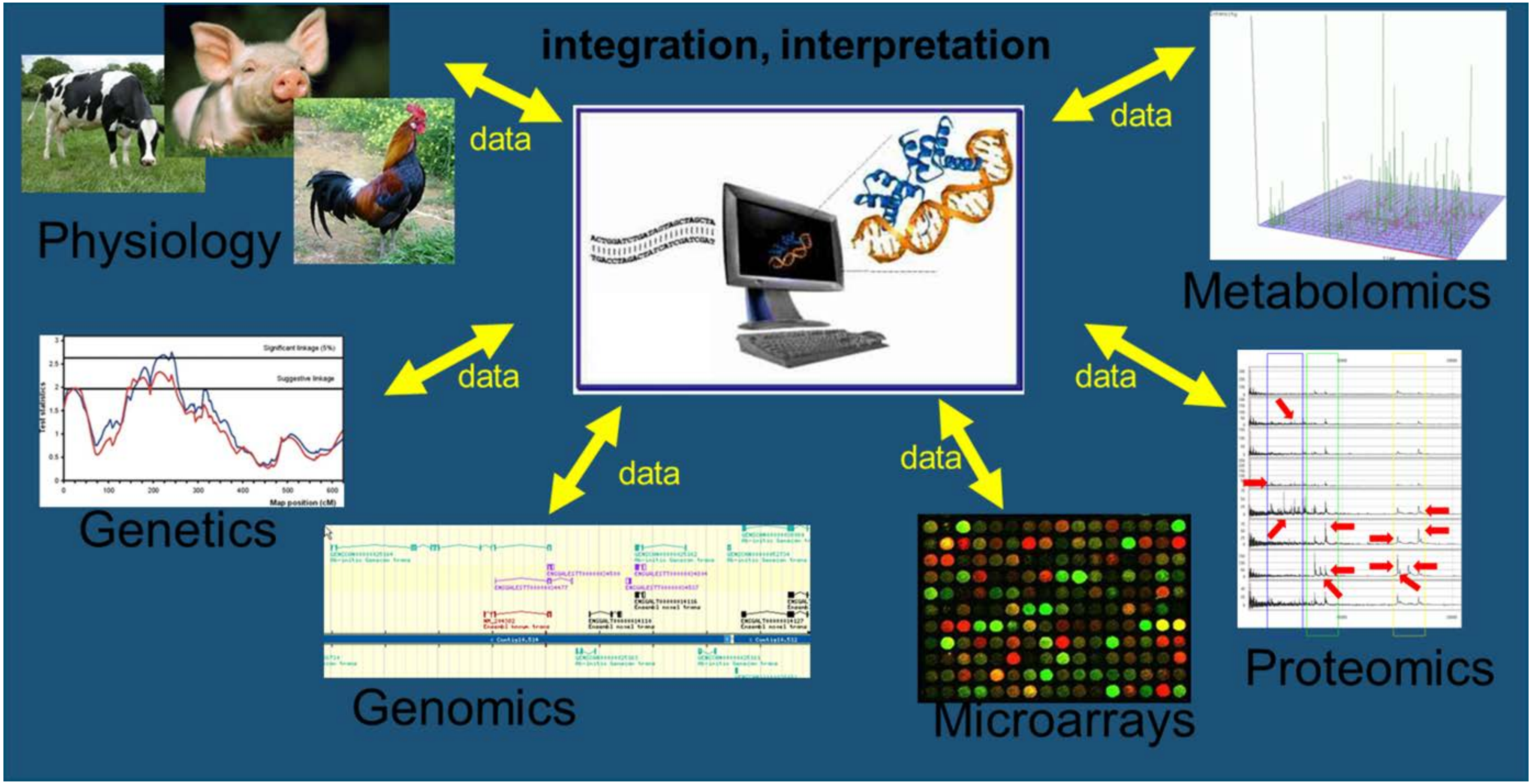
The future

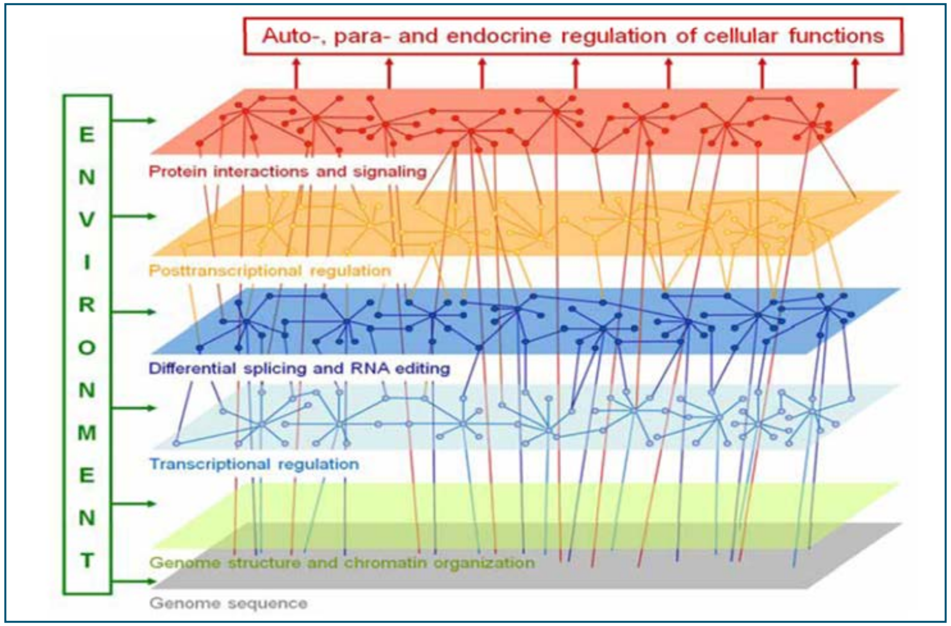
- Biology is integration of all levels
  - DNA
  - Epigenetic modifications
  - Expression (transcriptomics / proteomics)
  - Metabolism (=function)
  - Phenotypes (phenomics)
- To understand life (traits) we need to include all levels:
  - Integration!!

Integration at ABGC



Integration is biology

- Interactions between and within levels
- Influence of the environment on genome / genetic functioning
- Traits are the end products of the entire chain



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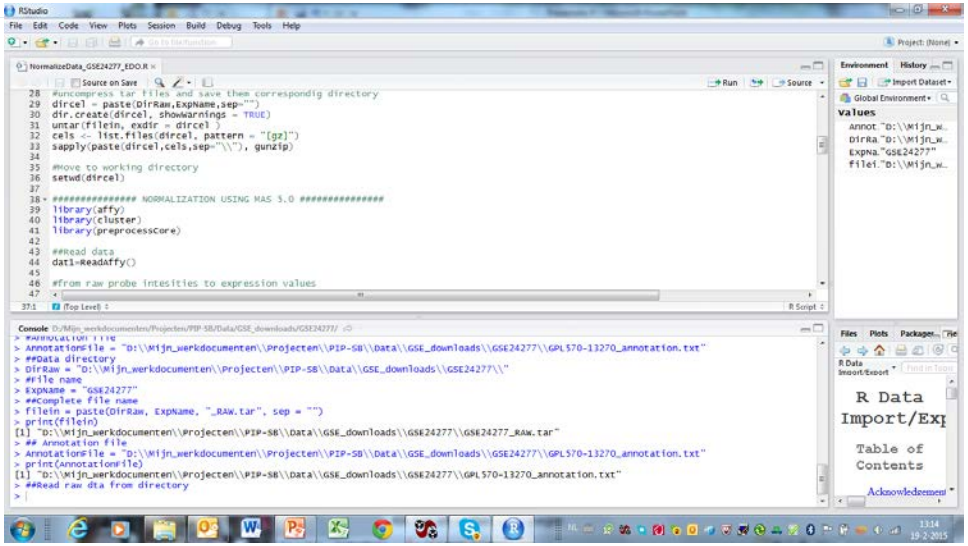
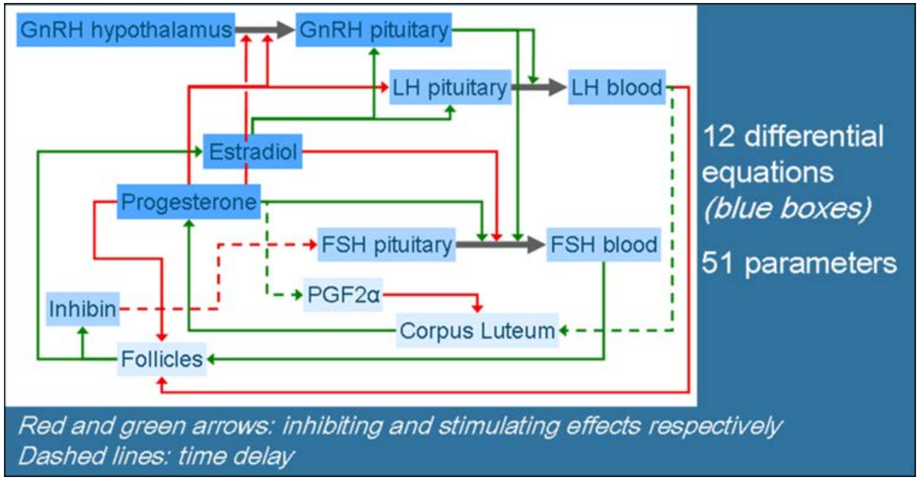
Big data: The future now!

- High throughput analyses
- Many data on all biological levels
- Consequence: large data sets (up to exabytes (10^{18} and higher...!)
    - Storage
    - Handling
    - Understanding




```
## Set directory
rm(list=ls())
setwd("D:/Mijn_werkdocumenten/Projecten/PIP-SB/Data/GSE_downloads/GSE24277");
#Load library\
library(GEOquery)
#Annotation file
AnnotationFile = "D:\\Mijn_werkdocumenten\\Projecten\\PIP-SB\\Data\\GSE_downloads\\GSE24277\\GPL570-13270_annotation.txt"
##Data directory
DirRaw = "D:\\Mijn_werkdocumenten\\Projecten\\PIP-SB\\Data\\GSE_downloads\\GSE24277\\"
#File name
ExpName = "GSE24277"
##Complete file name
filein = paste(DirRaw, ExpName, "_RAW.tar", sep = "")
print(filein)
## Annotation file
AnnotationFile = "D:\\Mijn_werkdocumenten\\Projecten\\PIP-SB\\Data\\GSE_downloads\\GSE24277\\GPL570-13270_annotation.txt"
print(AnnotationFile)
##Read raw dta from directory
#uncompress tar files and save them correspondig directory
dircel = paste(DirRaw,ExpName,sep="")
dir.create(dircel, showWarnings = TRUE)
untar(filein, exdir = dircel)
cels <- list.files(dircel, pattern = "[gz]")
sapply(paste(dircel,cels,sep="\\"), gunzip)
```

System biology -> Synthetic biology
The future?



- A systems biology mathematical model for dairy cattle reproduction
- ....
- Modify biological pathways and networks to improve biology? (of our traits)

Thank you for your attention

Questions?

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