The muscle tissue and its relationship to beef production

Isabelle Cassar-Malek

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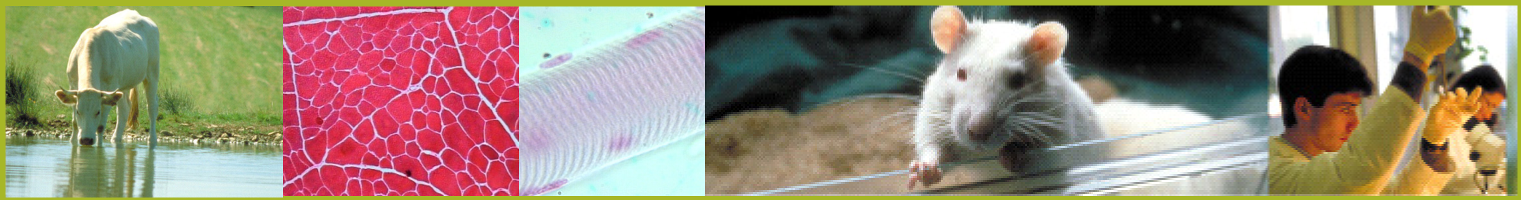

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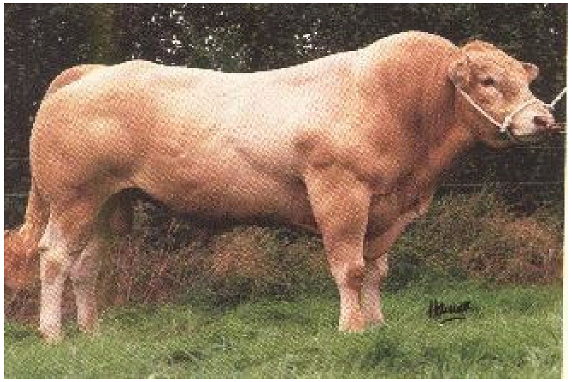
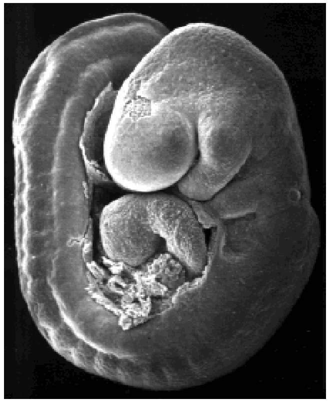
VetAgro Sup

INRA
SCIENCE & IMPACT

Myogenesis of Cattle - Challenges





<u>Beef production</u>
"Construction" of beef quality

<u>A model for humans</u>
(Gibbs and Weinstock , 2002)

Etymology

The word “muscle” comes from the Latin **mus / *musculus*** meaning "little mouse"

Terminology

- **Myo** = muscle

  (myofibril, myoblast, myogenesis, myotome)
- **Sarco** = flesh (sarcolemma)

Muscle tissues

Muscle

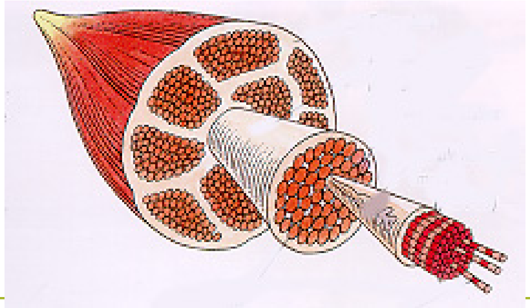
- skeletal (bones attached)
  - From 40 to 50% of body weight
  - Precursor of meat
- cardiac
- smooth (intestinal, endometrial ...)

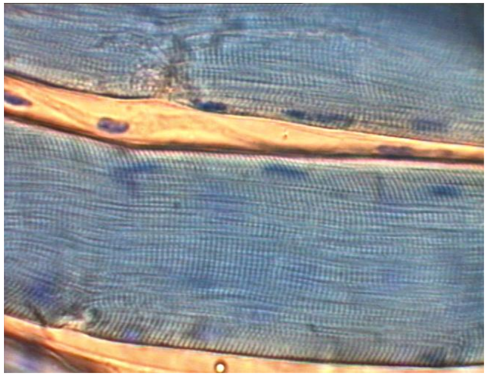
Skeletal muscle

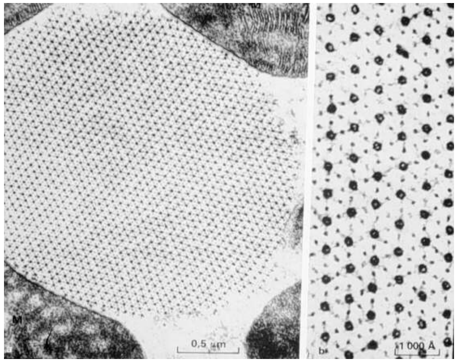
- movement (locomotion, manipulation): voluntary
- posture and body position
- stability of the joints (tendons)

- reserve of proteins
- role in the oxidation of nutrients
- maintenance of body temperature (85% body heat, chill)

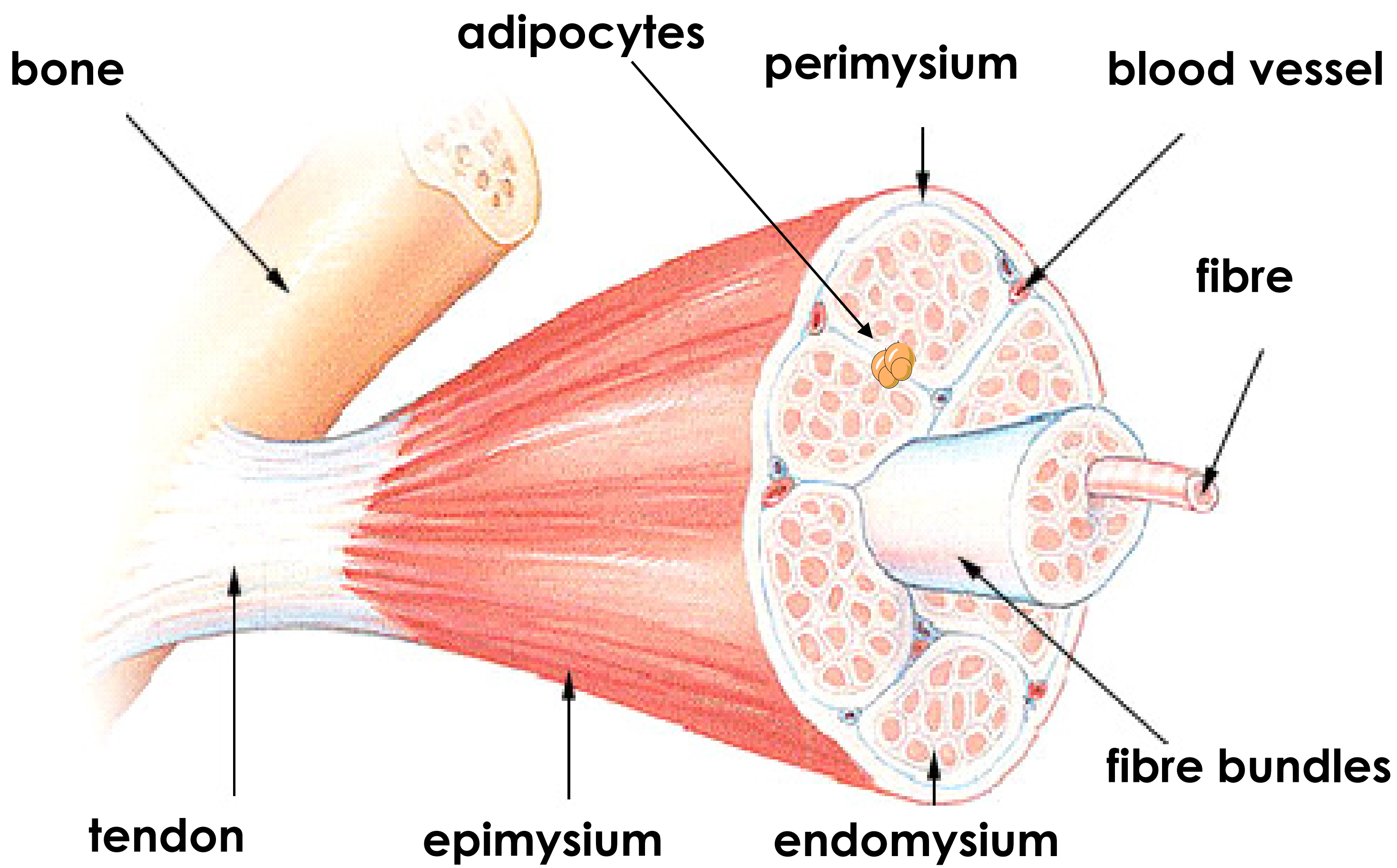
STRUCTURE OF THE SKELETAL MUSCLE



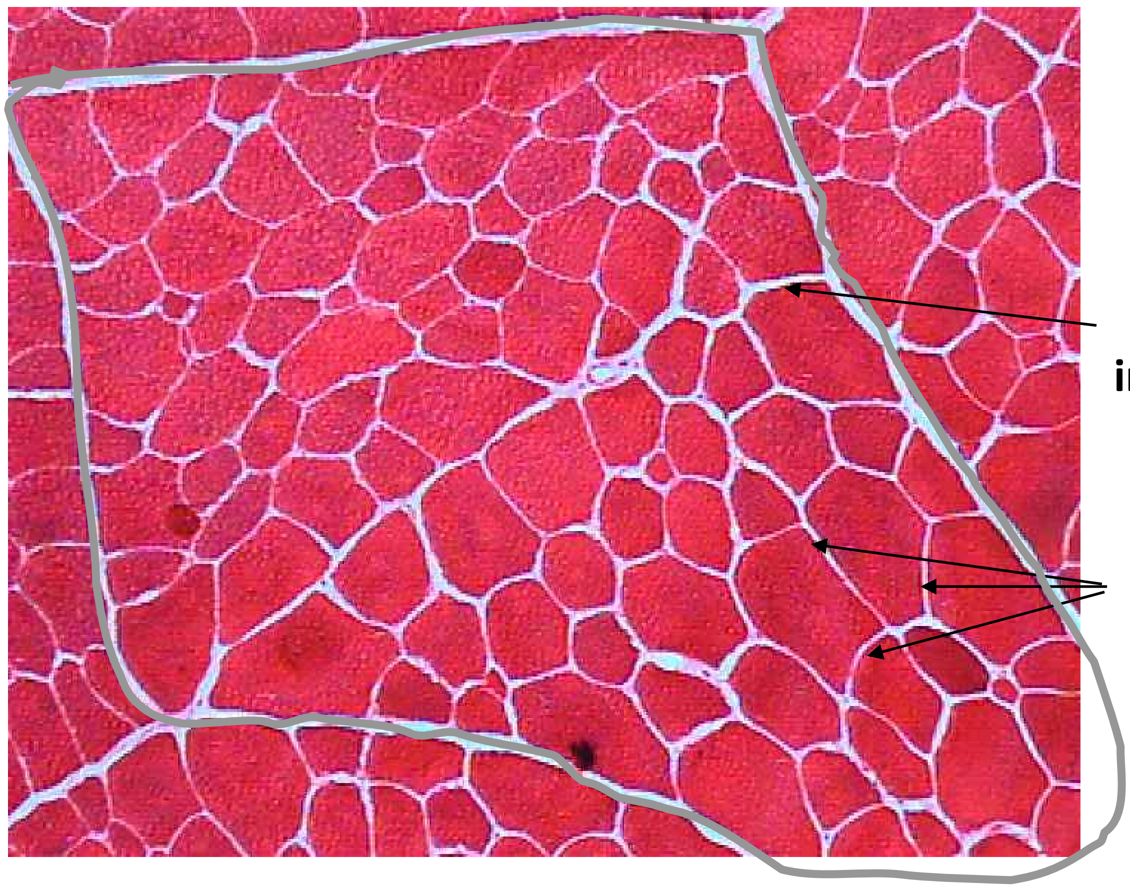




Macroscopic structure



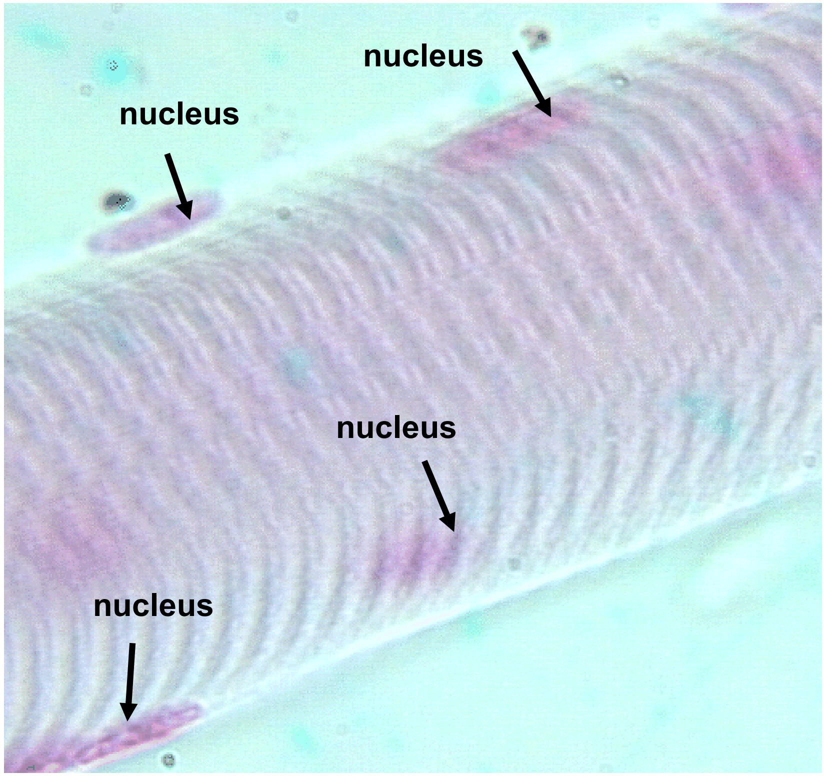
Microscopic structure



Histological section (bovine muscle)

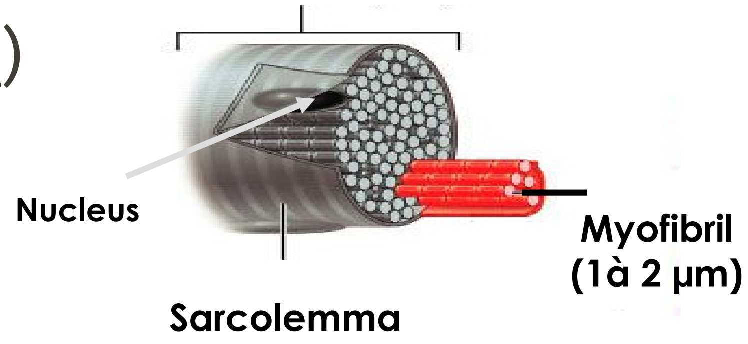
Muscle fibres

- <u>differentiated</u> cells
- From 75 to 90% of muscle volume
- cylindrical
- unbranched
- <u>multinucleated</u>
- length: up to 60 cm
- diameter: 10 to 100 microns
- <u>striated</u>

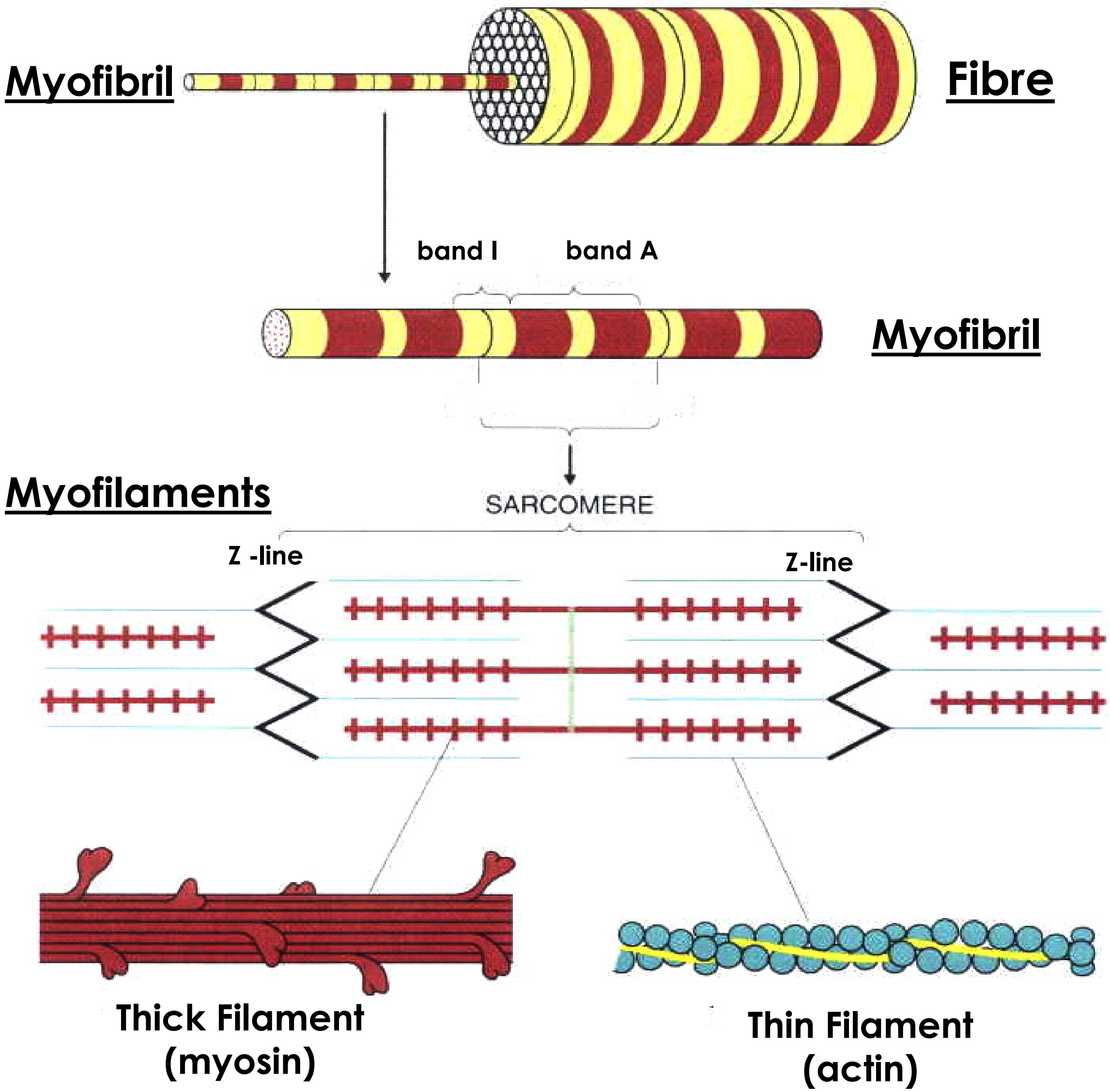


Cellular organisation

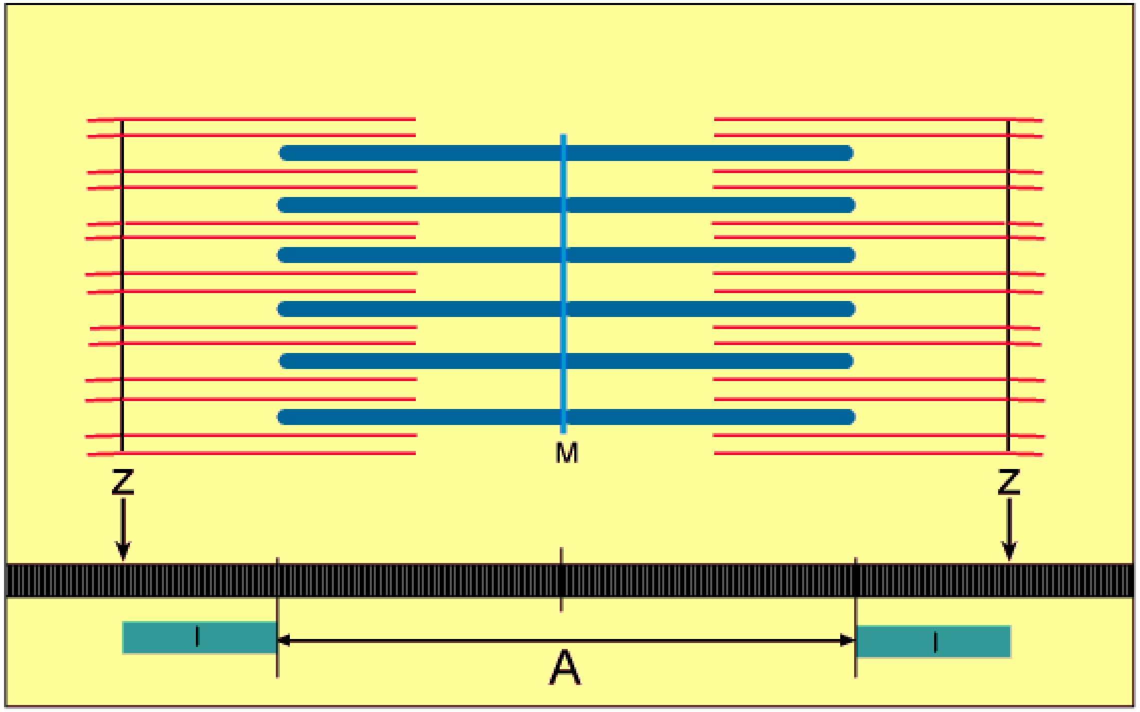
- membrane (sarcolemma)
- cytoplasm (sarcoplasm): incl. glycogen, myoglobin
- post-mitotic nuclei
- mitochondria
- endoplasmic reticulum : differentiated (sarcoplasmic)
- cytoskeleton (myofibrils)



Ultra-structure

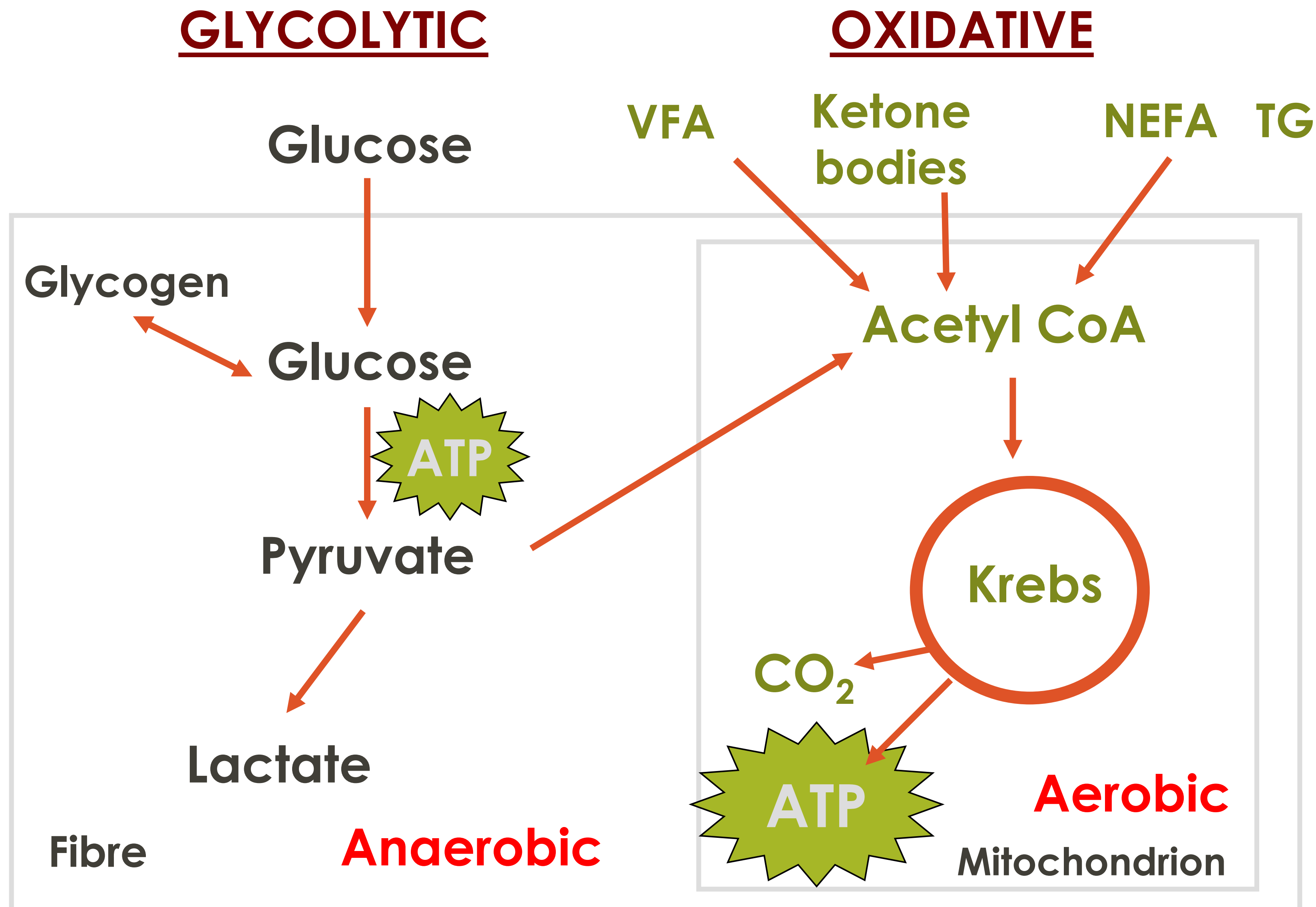


The sarcomer

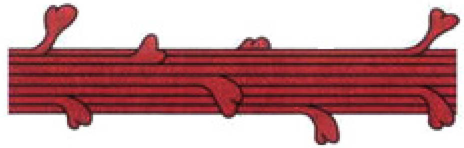


CONTRACTILE AND METABOLIC PROPERTIES

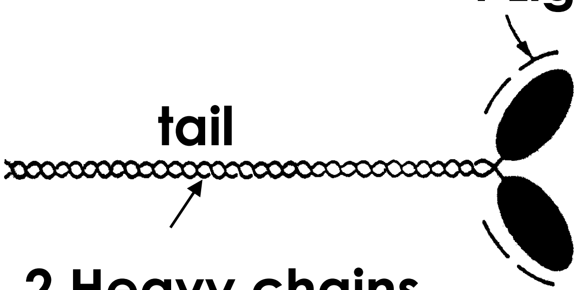
Metabolic pathways



Myosin



Contraction
Anchorage, tension, traction



ATP-ase activity

<u>Several isoforms</u>

- Embryonic : E
- Neonatal or fetal : F
- Slow: I
- Fast: IIa, IIb, IIx

MyHC isoforms

« Adult » isoforms

I (slow)
IIA
IIX (fast)
IIB

Developmental isoforms

Embryonic
Fetal
α-cardiac
Extra occular (exoc)

Two gene clusters

Chr. 7

— α — β=I — slow

Chr. 12

— emb – IIA – IIX – IIB – Fet – exoc —

Example for pig muscle

for a review: Schiaffino and Reggiani, 2011

Fibre types

CLASSIFICATION	I	IIA	IIX
Speed of contraction	Slow	Fast	Fast
Metabolism	Oxidative	Oxido-glycolytic	Glycolytic
Fatigue resistance	High	High	Low
Glycogen Content	Low	High	High
Lipid Content	High	High	Low
Vascularization	High	High	Low

I $\rightleftarrows$ I/IIA $\rightleftarrows$ IIA $\rightleftarrows$ IIA/IIX $\rightleftarrows$ IIX

Hybrid

Hybrid

Fibres types / physical activity

Sprint ⇨

- Fast IIX fibres
- using glycogen

Jogging, ski, distance runner ⇨

- Slow type I fibres
- using lipids

Alternating sprint/endurance ⇨

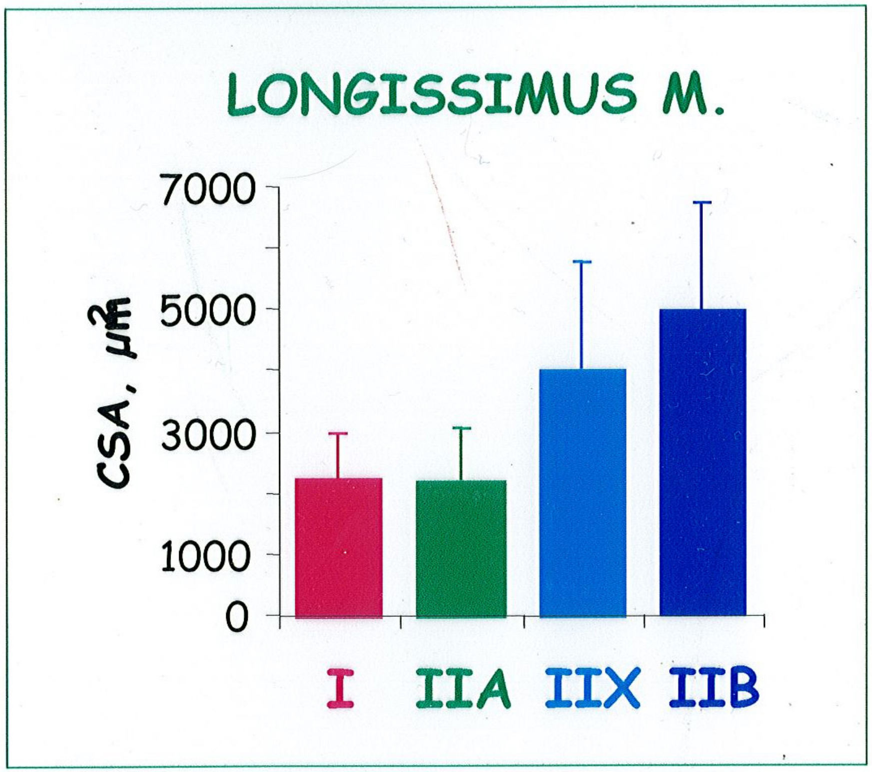
- Fast oxido-glycolytic IIA fibres
- Using both carbohydrates and lipids
- Adaptated to rythm changes

Classification according to fibre type

Type	I	IIA	IIX	
« Mean value »	20	25	55	
m. Diaphragma	55	45	0	(red)
m. Longissimus thoracis	25	25	50	
m. Semitendinosus	15	25	60	(white)

In cattle, according to Totland et al. (1991), Picard et al (2002)

Cross section area of the fibres

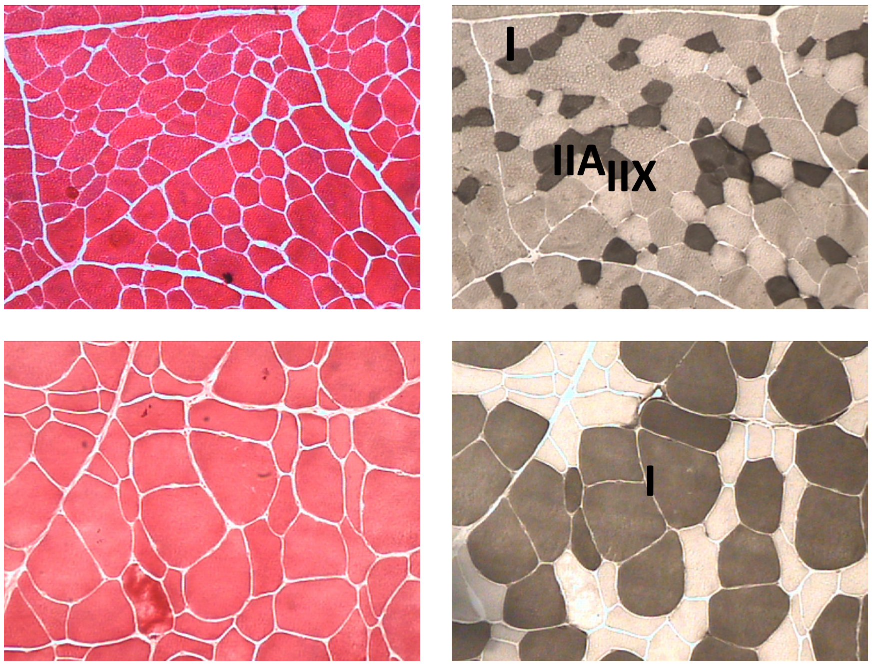


Mean cross section area of fibres

Azorubine **ATPase**

TB

RA



In cattle

TB: m. *triceps femoris*

Ra: m. *rectus abdominis*

Mean area of type I fibres

TB	RA	signif.
1725	3957 µm²	***

TB: I<IIA<IIX

RA: I>IIA>IIX

Oury et al., 2009

CLASSIFICATION OF MUSCLE FIBRES

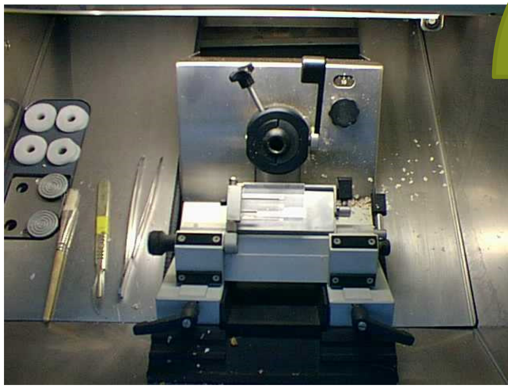
Methods

DIFFERENT METHODS BASED ON

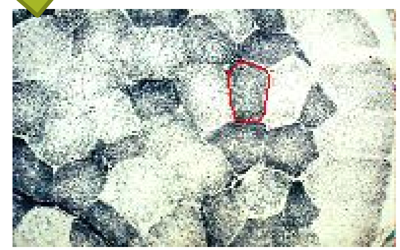
- **functional tests (speed of contraction)**
- **metabolic criteria (the type of energy metabolism)**
    - **speed of energy utilization during contraction**
    - **main source of energy**

1- *In situ* techniques

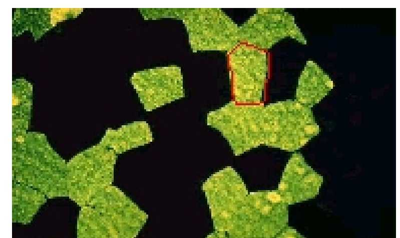
tissues → section



Detection



enzymatic
(SDH activity)



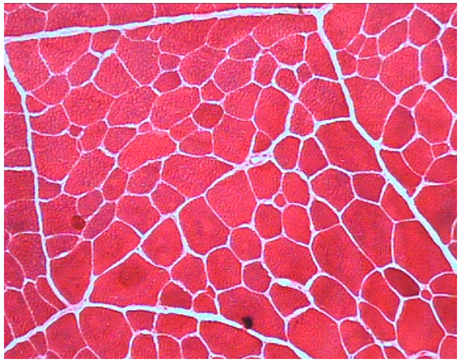
immunology
(slow MyHC)

Image analysis

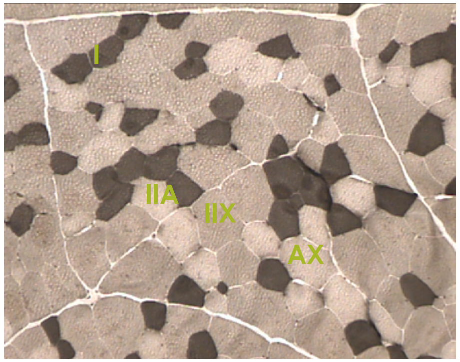
Histochemistry for fibre typing

Detection of three pure fibres and hybrid fibres in cattle muscle

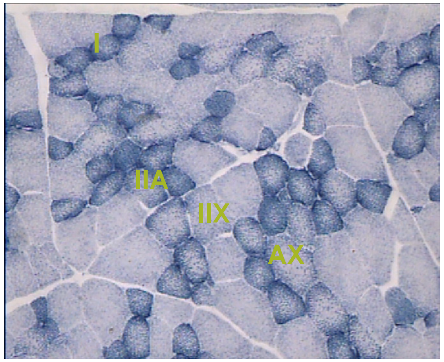
Azorubine



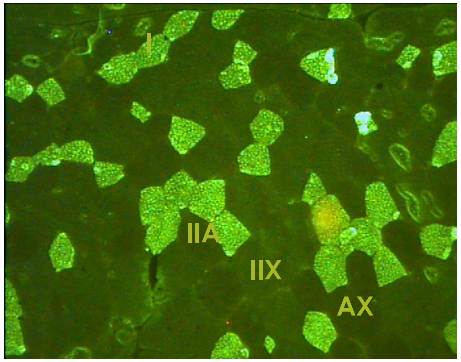
ATPase



SDH

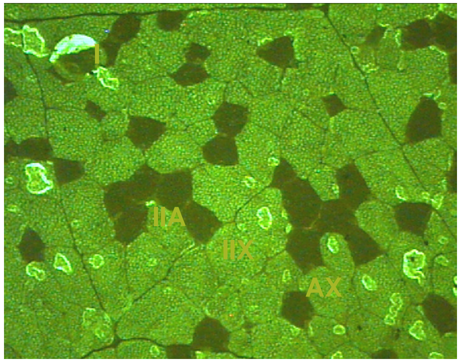


slow MyHC



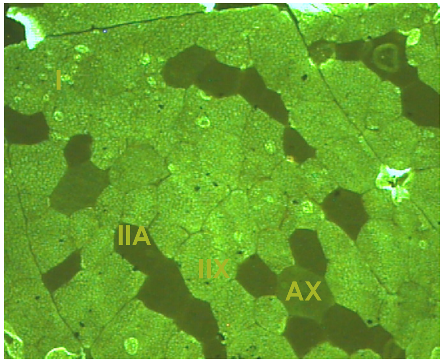
5B9 Alexis

fast MyHC



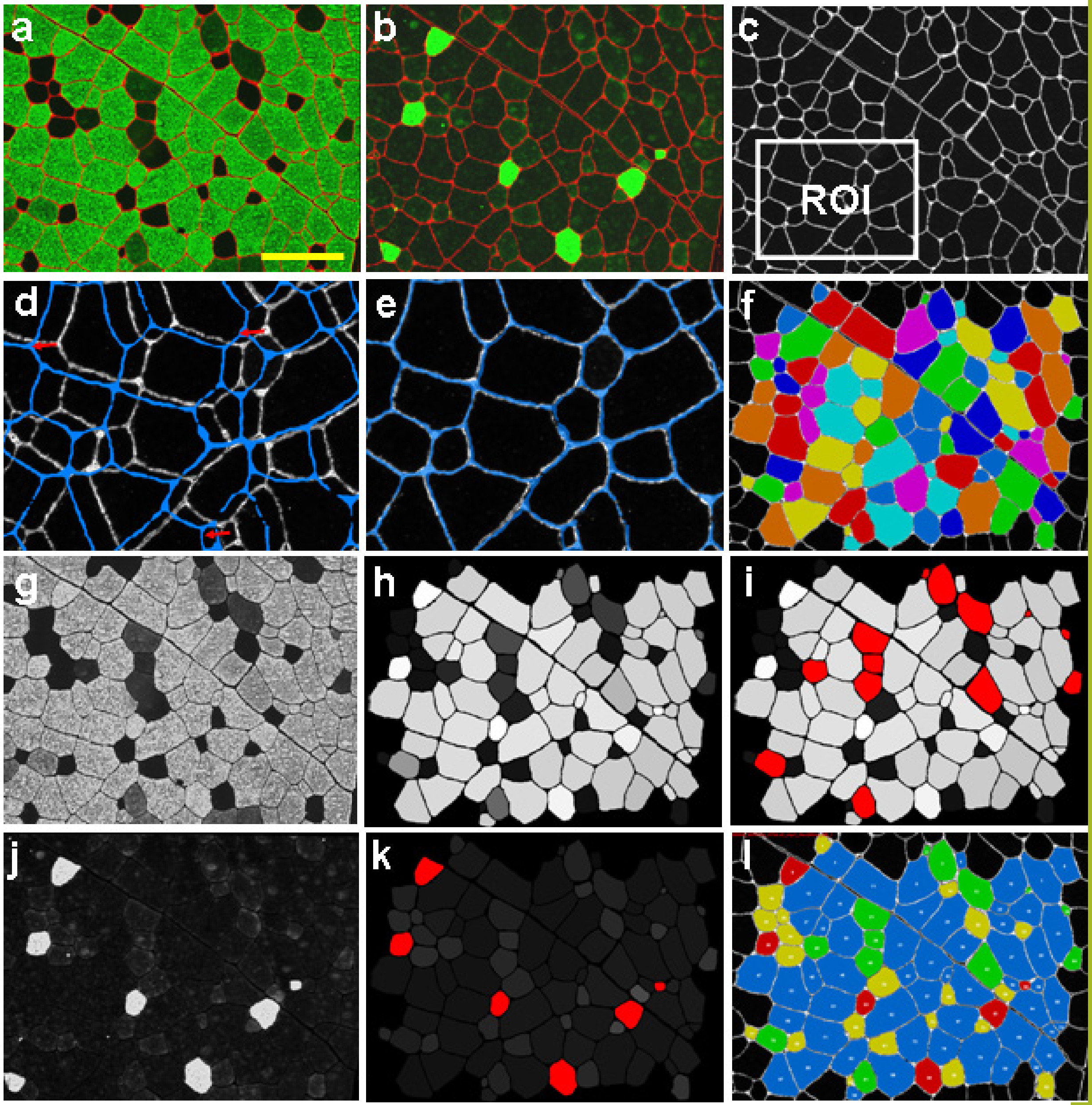
S5 15F4 (Agrobio)

MyCH I + IIx



S5 8H2 (Agrobio)

Image analysis



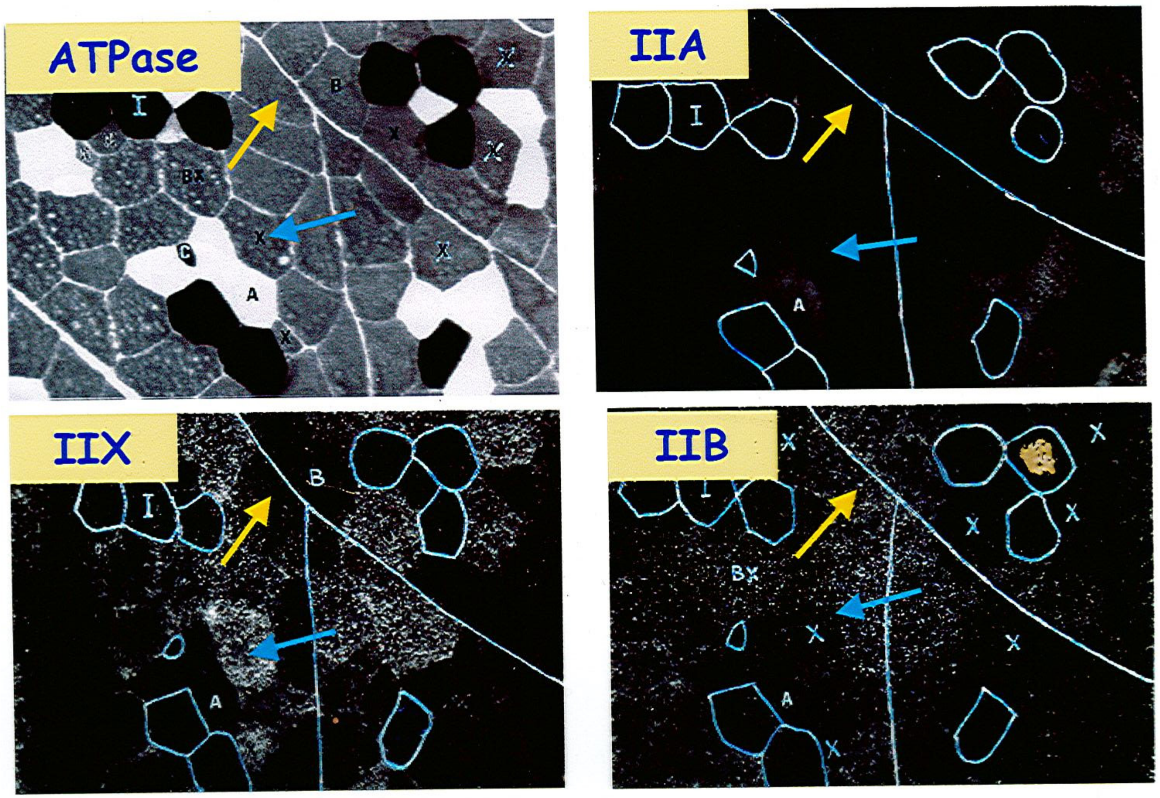
on high number of fibres

- % of each fibre type
- area
- % of area

Meunier et al., 2010, Histochem Cell Biol

MyHC *in situ* hybridization

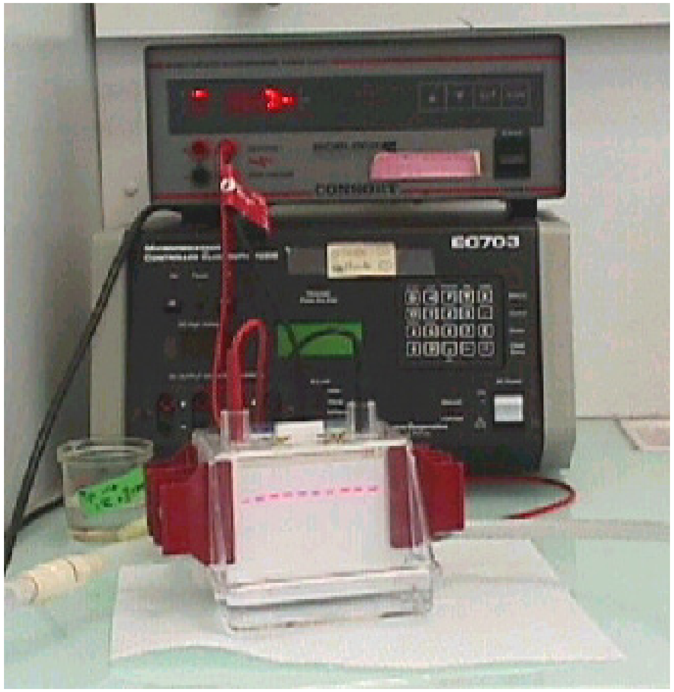
in pig *longissimus* muscle (100 kg BW)



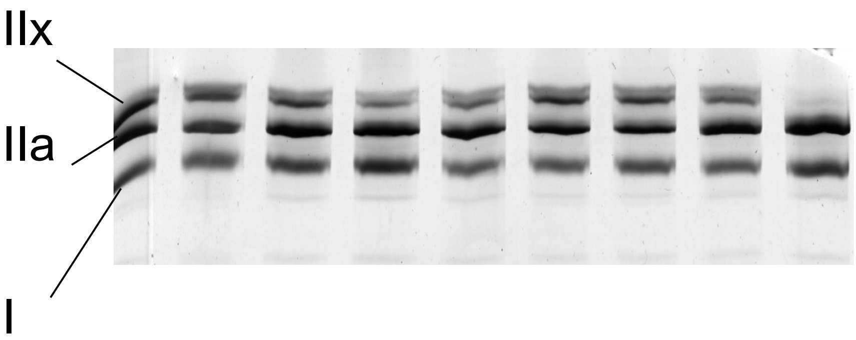
Lefaucheur et al. (1998)

2- From muscle homogenates

Electrophoretic separation of MyHC isoforms



SDS PAGE



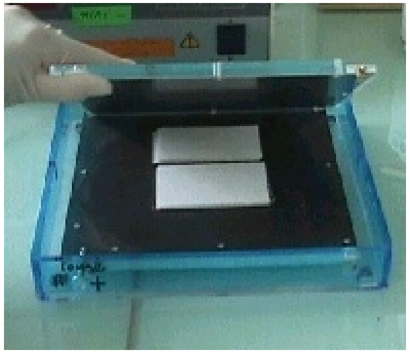
Talmadge and Roy (1995) modified by Picard et al. (2011)

Western-blot

Electrophoresis

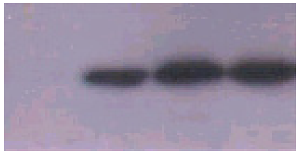
proteins

Transfert



Immuno-detection

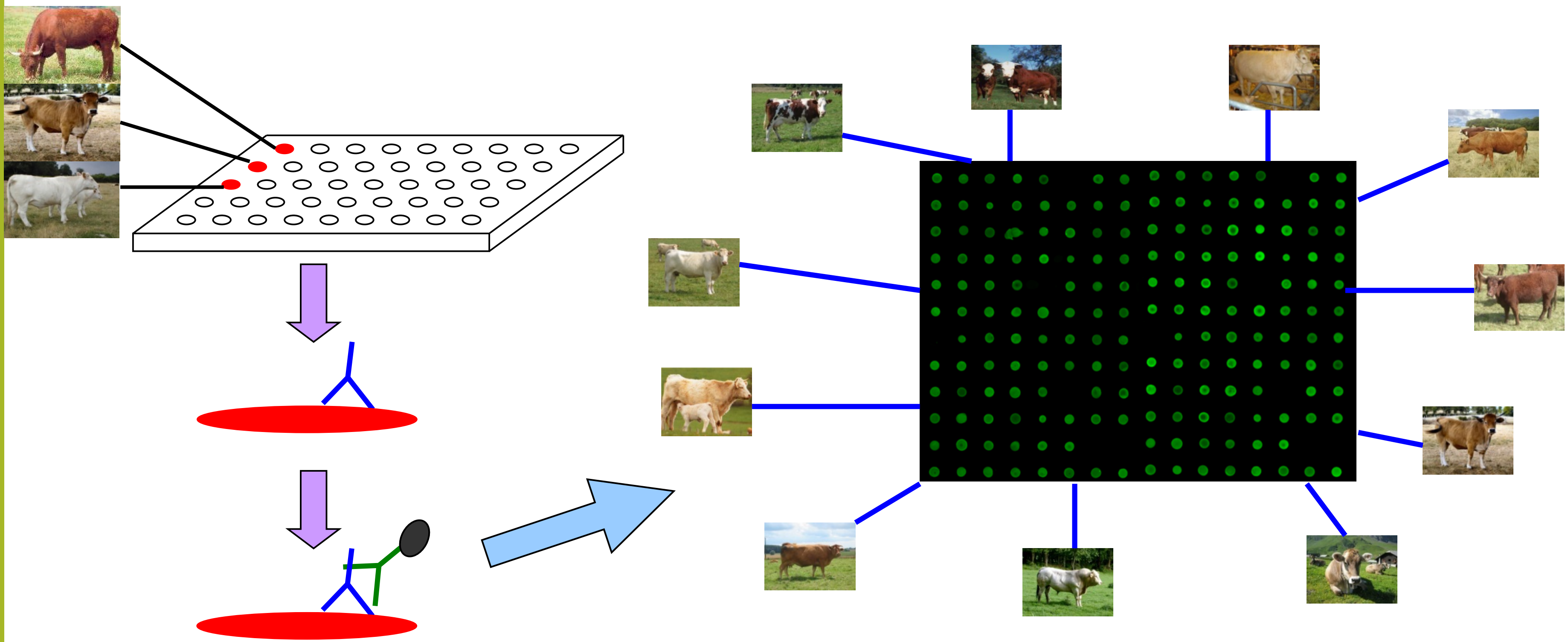
Bovine myoblast culture



desmin

D4 D6 D8 D10

High-throughput protein analysis

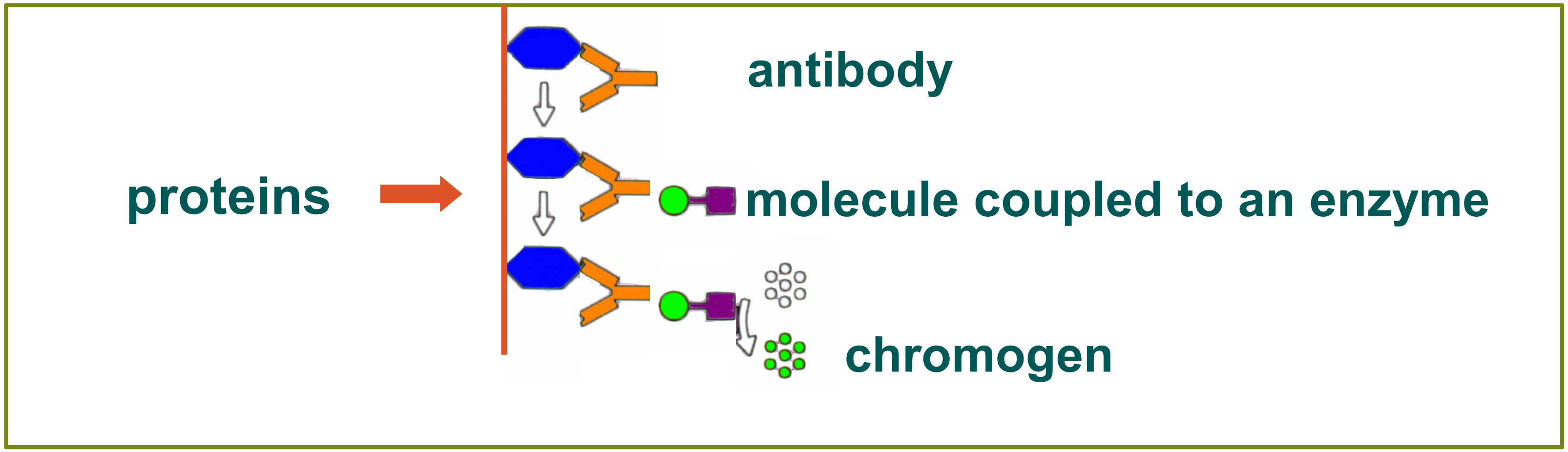


Dot-blot technology: analyse up to 96 samples simultaneously

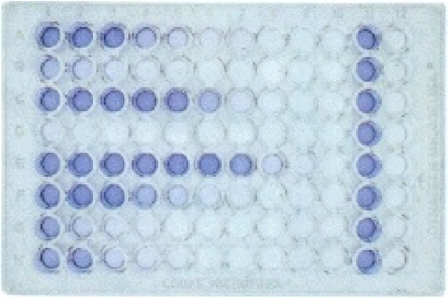
simplification of the western blot method

Guillemin et al. 2009.

ELISA assay

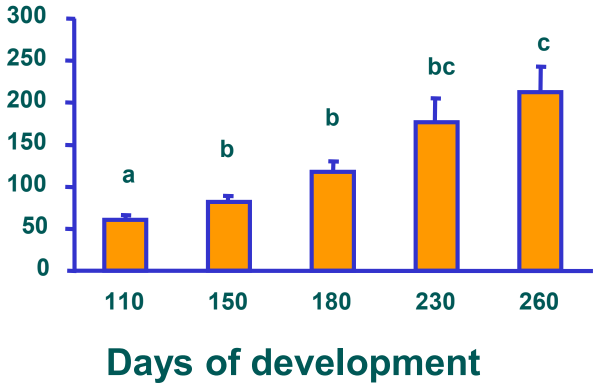


Detection



Optical density

FABP content



Proteomics

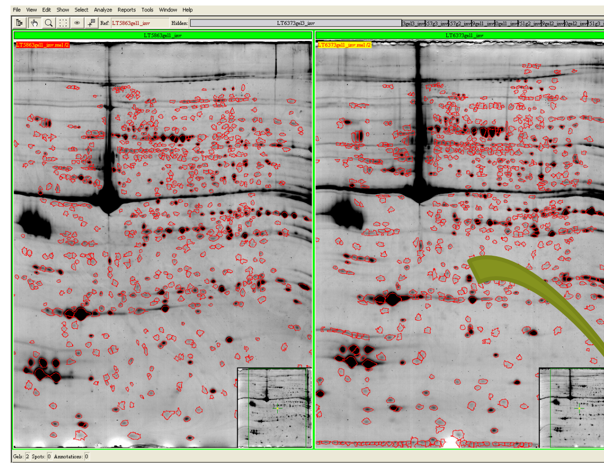
Gel-based proteomics: 2DE

1st D: pI

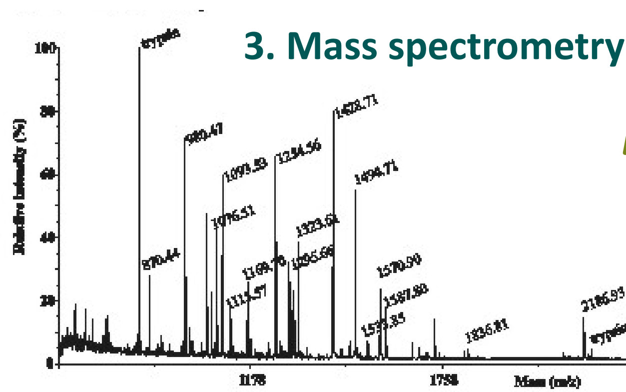
2nd D : PM

1. Separation

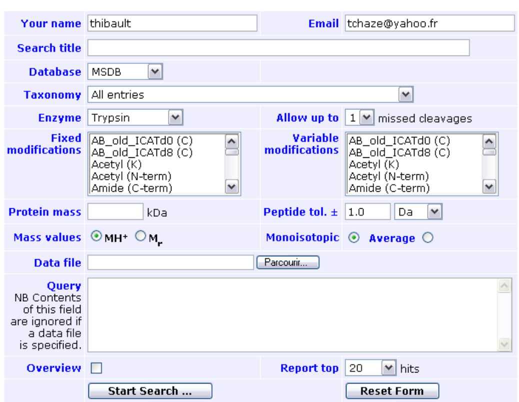
2. Image analysis



3. Mass spectrometry



4. Protein identification (databases)



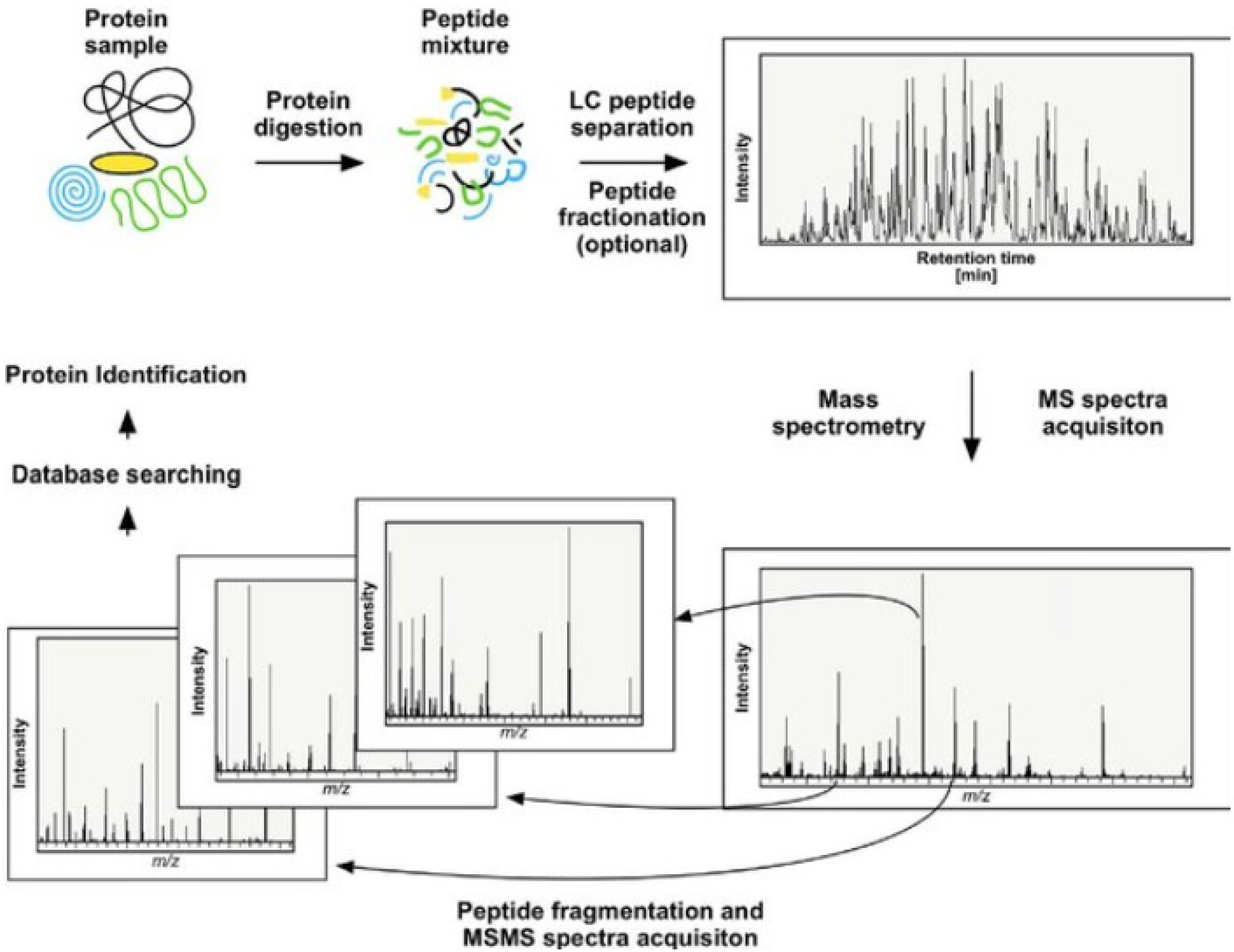
MASCOT Peptide Mass Fingerprint

Your name	thibault	Email	tchaze@yahoo.fr
Search title			
Database	MSDB		
Taxonomy	All entries		
Enzyme	Trypsin	Allow up to	1 missed cleavages
Fixed modifications	AB_old_ICATd0 (C) AB_old_ICATd8 (C) Acetyl (K) Acetyl (N-term) Amide (C-term)	Variable modifications	AB_old_ICATd0 (C) AB_old_ICATd8 (C) Acetyl (K) Acetyl (N-term) Amide (C-term)
Protein mass	kDa	Peptide tol. ±	1.0 Da
Mass values	⦿ MH+ ○ M_r	Monoisotopic	⦿ Average ○
Data file	Parcourir...		
Query NB Contents of this field are ignored if a data file is specified.			
Overview	☐	Report top	20 hits
Start Search ...		Reset Form	

Bouley et al. (2003)

Proteomics

Shotgun: nano LC MS/MS



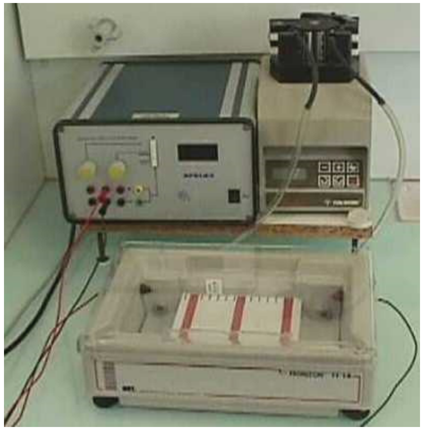
From Vojtech Tambor

Gene expression

Northern-blotting to detect specific RNA molecules among a mixture of RNA

Electrophoresis

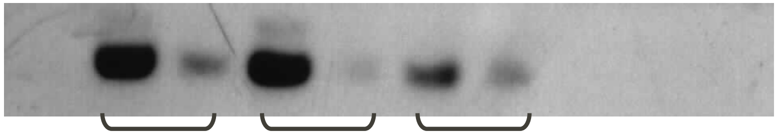
RNA



Transfert

Hybridization

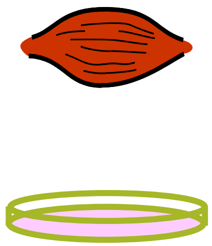
GLUT-4



Heart Ma TAd liver kidney

35

qRT-PCR



RNA → cDNA

Reverse Transcriptase

RT

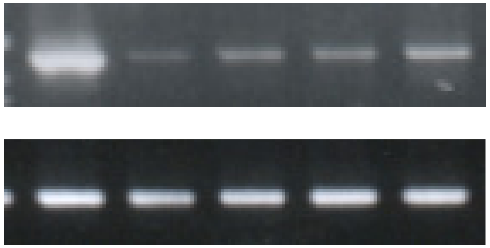
PCR

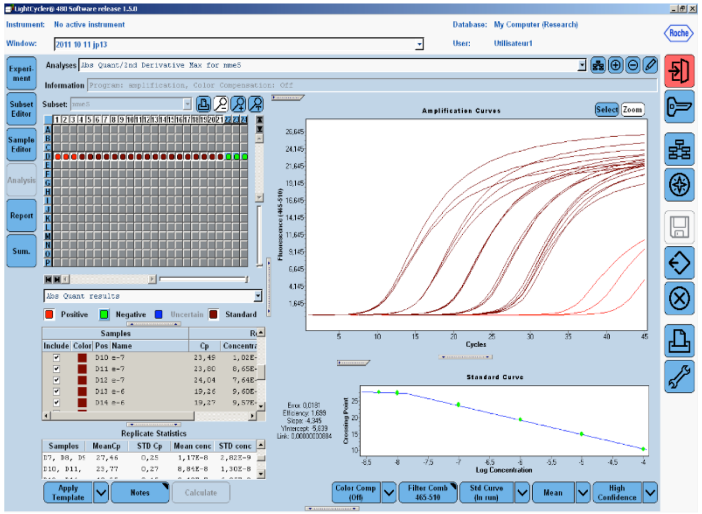
Foetus age (days)

110 180 230 260 15d

myostatin

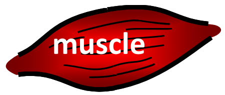
cyclophilin





Transcriptomics

SAMPLE 1



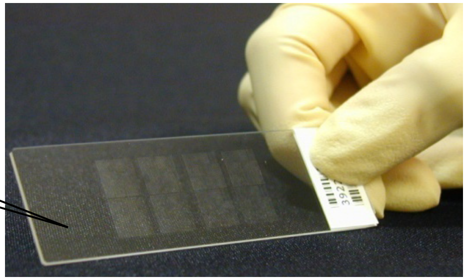
SAMPLE 2

Contractile and metabolic type

RNA extraction
Labelling

targets*

DNA chip



probes

Hybridization

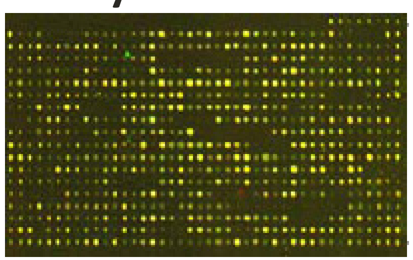


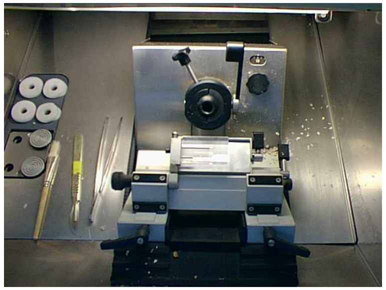
Image analysis

Biostatistics

Molecular signatures
Differential expression

Choosing the most accurate technique

- Reveal the contractile type by immuno-histochemistry using anti-MyHC antibodies and the SDH activity on serial sections (Picard and al., 1998): distinguish the hybrid fibres, and get information on the cross section area of each fibre type
- Not relevant for high throughput phenotyping (large numbers of animals)
- Detect contractile type using electrophoresis of MyHC and metabolic type by assaying metabolic enzymes



IIB OR NOT IIB?

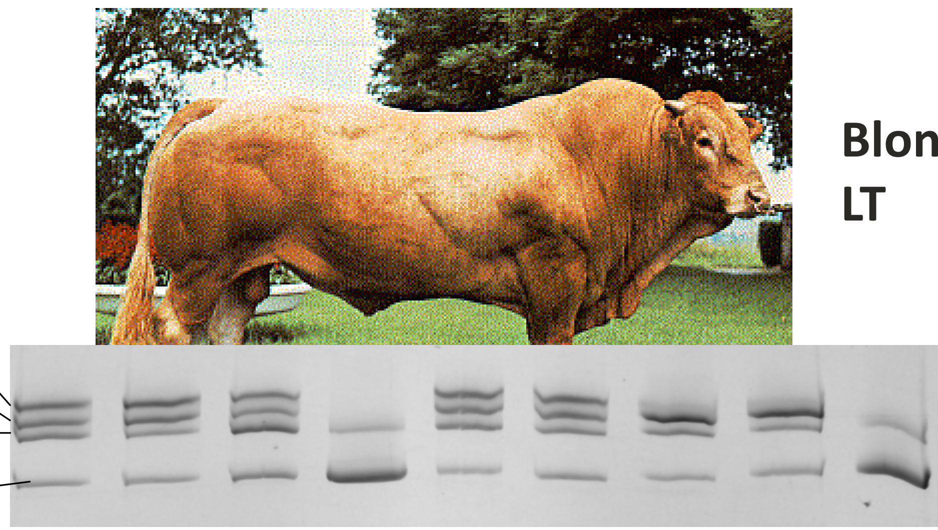
Myosin IIB

MyHC-IIb is the predominant motor protein in most skeletal muscles of rats and mice

The mRNA for this isoform is only expressed in a very small subset of specialized muscles in adult large mammals, including humans.

Is IIb MyHC expressed in cattle?

- **For many authors, IIb MyHC (MYH4, BTA 19) would not be expressed in cattle muscles, but extraoccular muscle:**



Blonde d'Aquitaine bull
LT

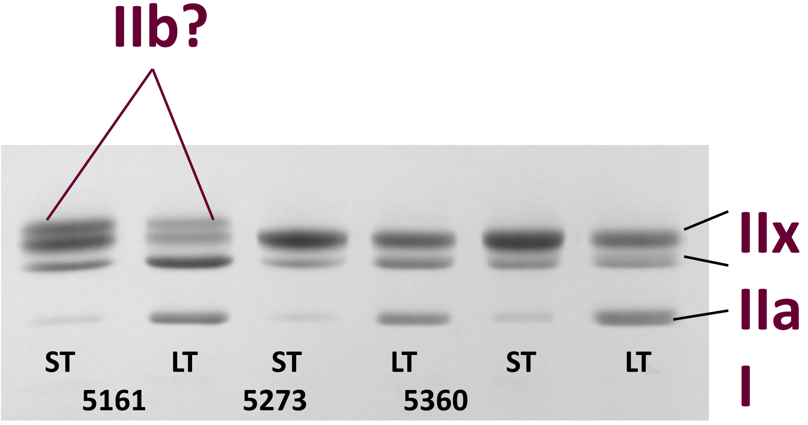
MyHC IIb?
MyHC IIx
MyHC IIa
MyHC I

Picard and Cassar-Malek, 2009

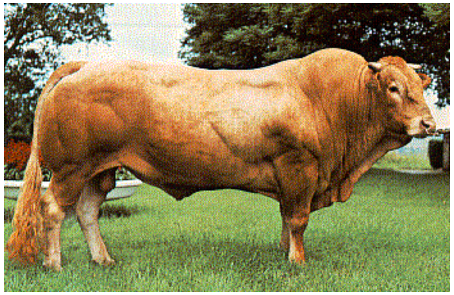
A fourth MyHC isoform in cattle

- A particular MyHC in Blonde d'Aquitaine bulls with common ancestor

SDS-PAGE
5-8% gradient

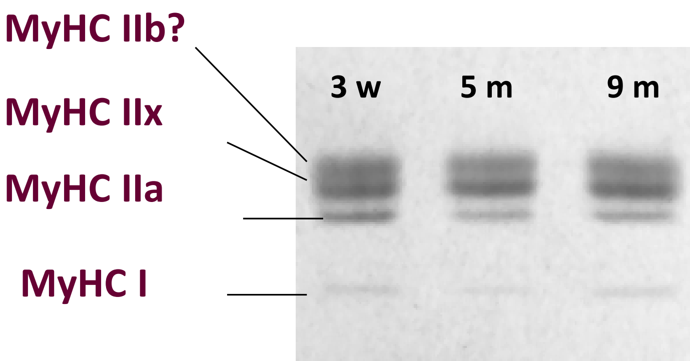


IIb?	<u>35%</u>	<u>18%</u>
IIx	41%	23%
IIa	20%	37%
I	4%	22%

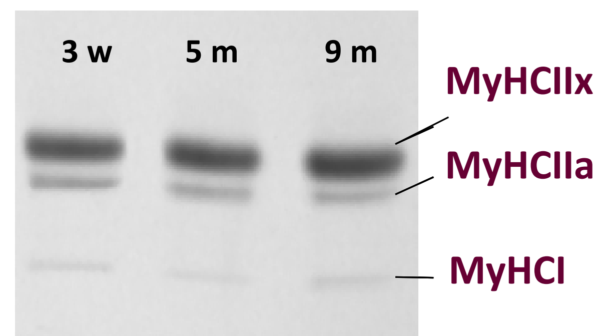


A MyHC expressed in post-natal muscle

- This MyHC is detected at all studied ages
- Onset as soon as fœtal life?



Bull with this MyHC

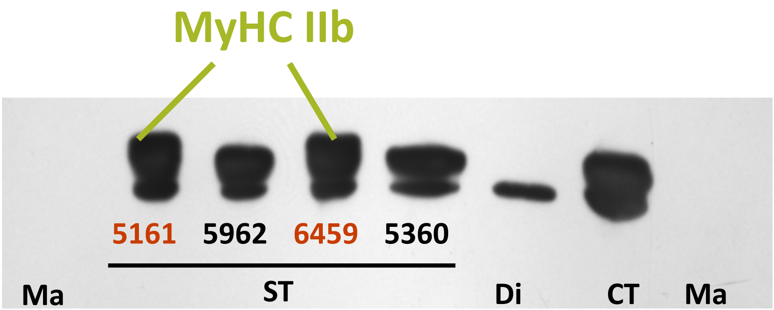


Control

Identification

Immuno-detection

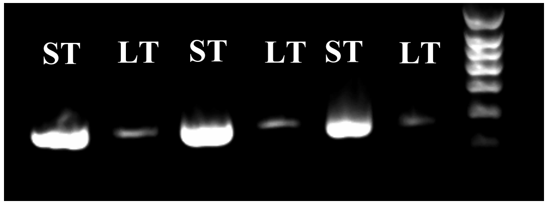
with an antibody specific of the fast MyHC (IIa, IIx, IIb)



Ma : *masseter*, slow (I),
Di: *diaphragma* (I +IIa),
CT: *cutaneus trunci* (IIa+ IIx)

RT-PCR

Primers designed in the 5'-UTR of the MyHC IIb transcript



Amplification of a cDNA fragment and sequencing

This isoform is the IIb MyHC (encoded by MYH4)

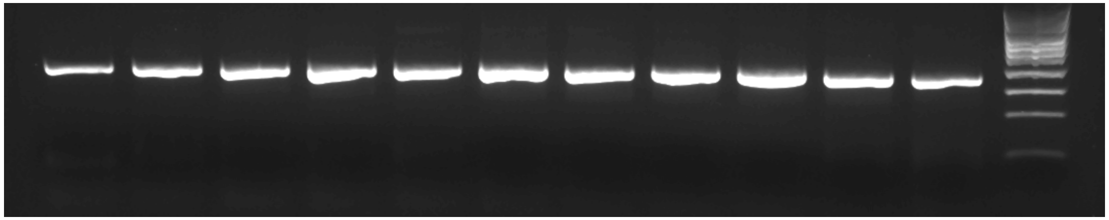
Picard and Cassar-Malek, 2009

Abundance of the transcript

- Specific regulation of MYH4 expression in cattle?

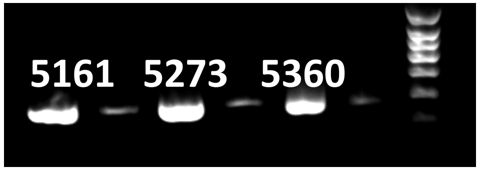
RT-PCR using primers for 5'-UTR MyHC IIb (Chikuni et al, 2004)

AMPLIFICATION
~300 pb in ST



Transcripts observed in all 11 young bulls but the protein was present in 2 only (ST BA)

Charolais

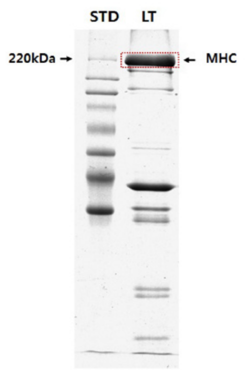


ST LT ST LT ST LT

B. Aquitaine

What's next?

Kim (2014) by using electrophoresis and nano LC-MS/MS of MyHCs did not observed unique peptides of MyHC IIb in Hanwoo Steer LT muscle



+

→

MyHC1-IIx (n=14)
MyHC- IIa (n=8)
MyHC-I (n=21)
MyHC- IIb (n=0)

However this has to be tested on our samples!

Conclusion

IN CATTLE

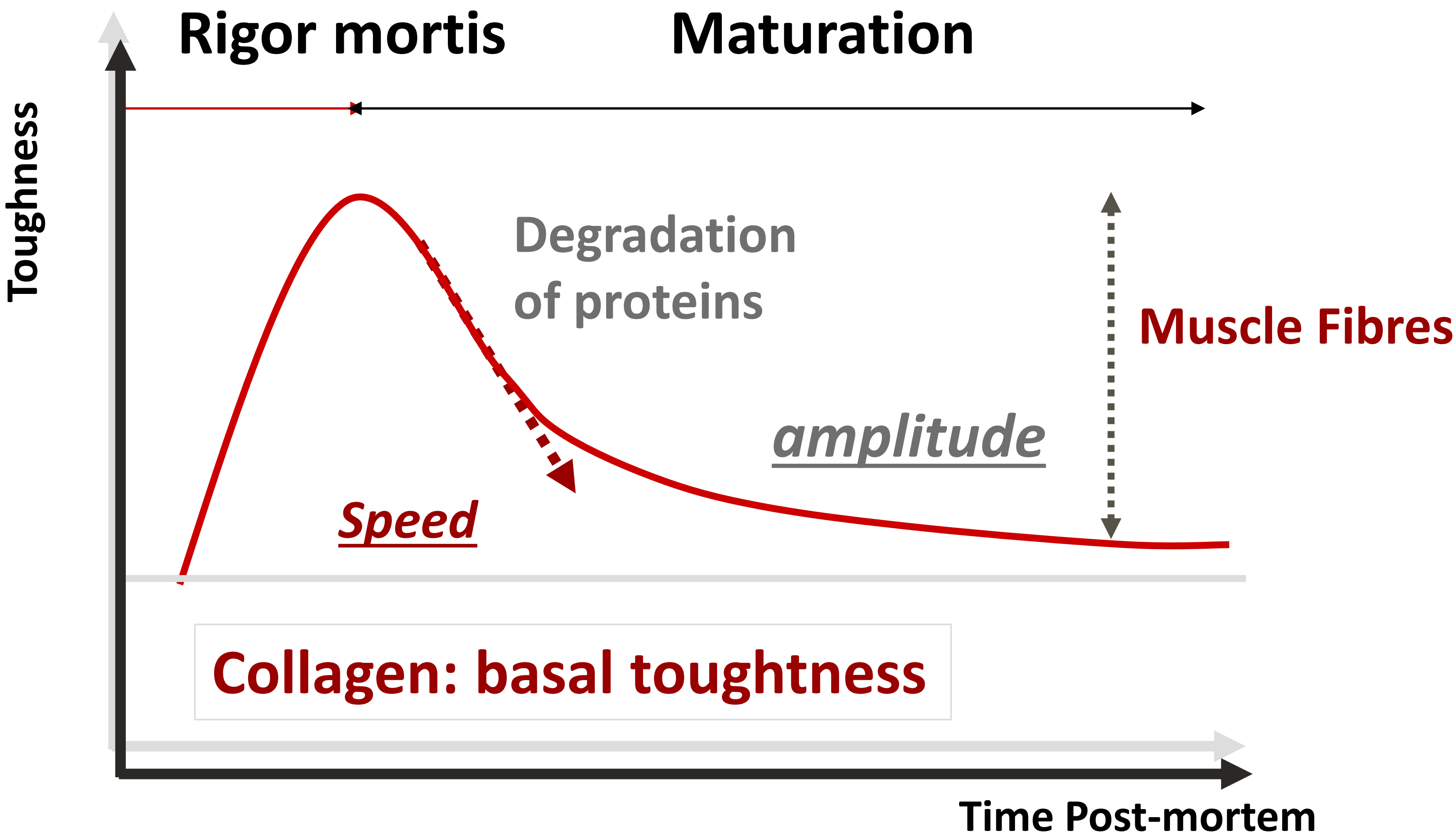
- **Three types of fibres I, IIA, IIX**
- **and a fourth one IIB in some french bovines**
- **with a variable frequency between breeds**
- **6% in Charolais, 35% in Blonde d’Aquitaine, 45% in Limousin**

Picard et al., 2010

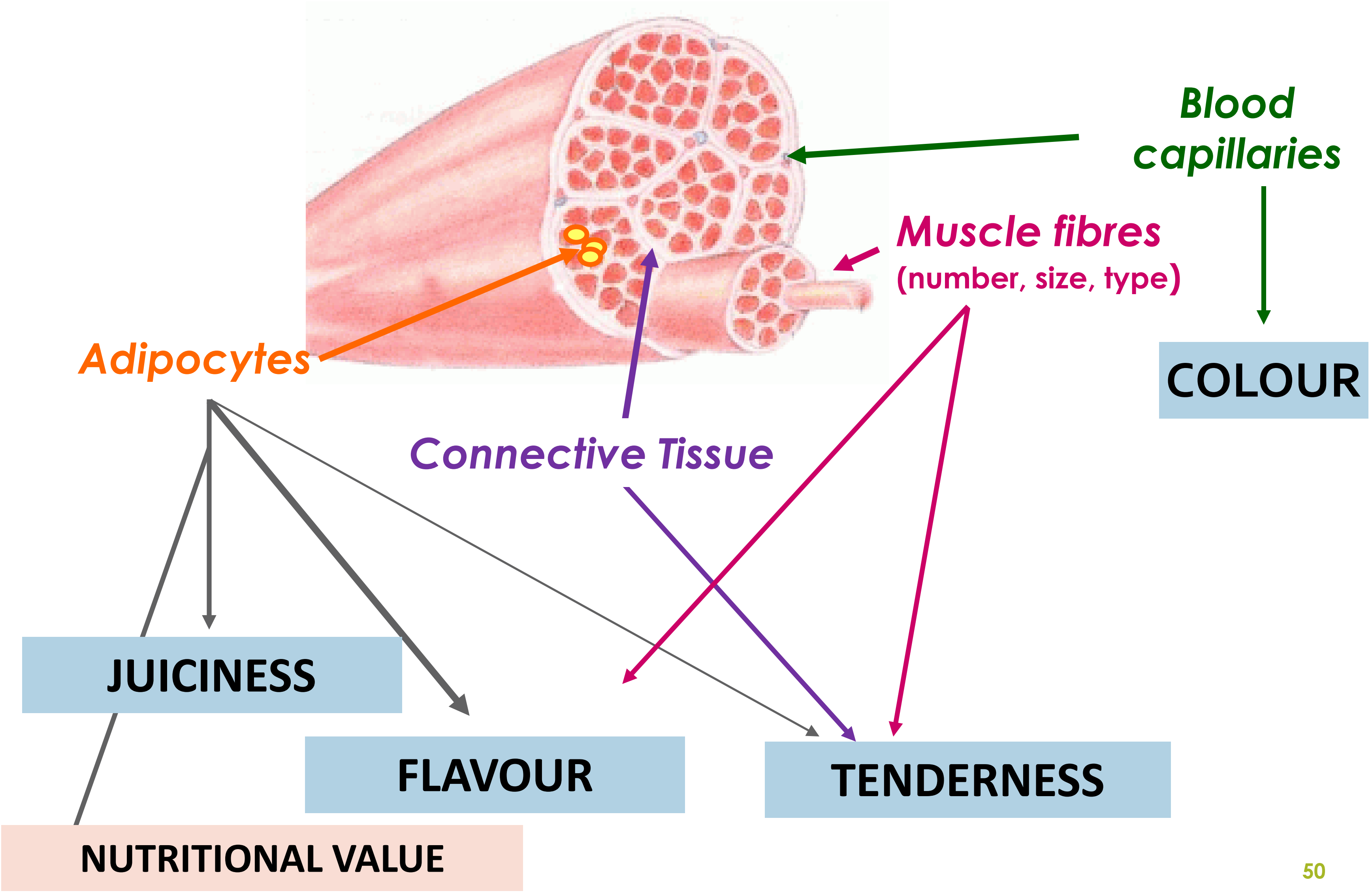
FROM MUSCLE TO MEAT



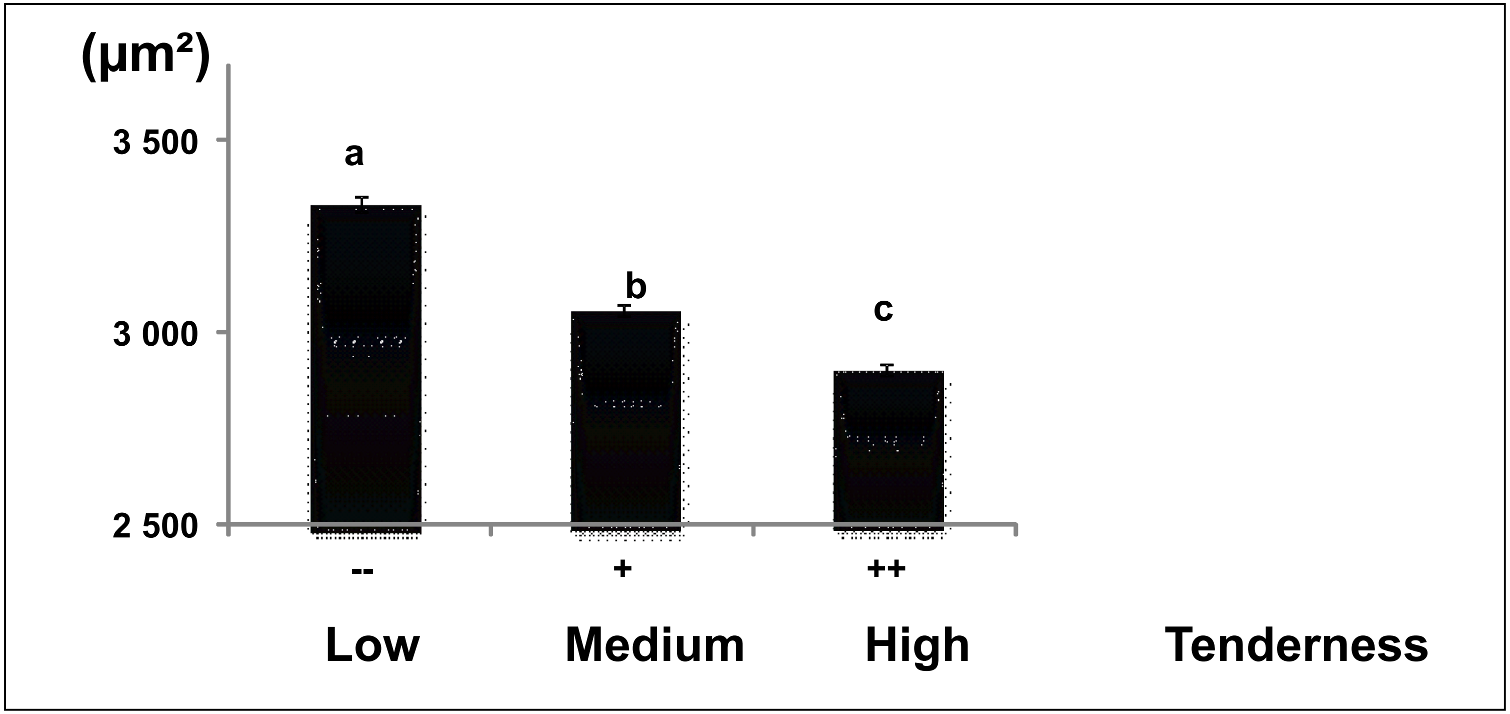
Conversion of muscle into meat



How muscle biochemistry affects Beef quality



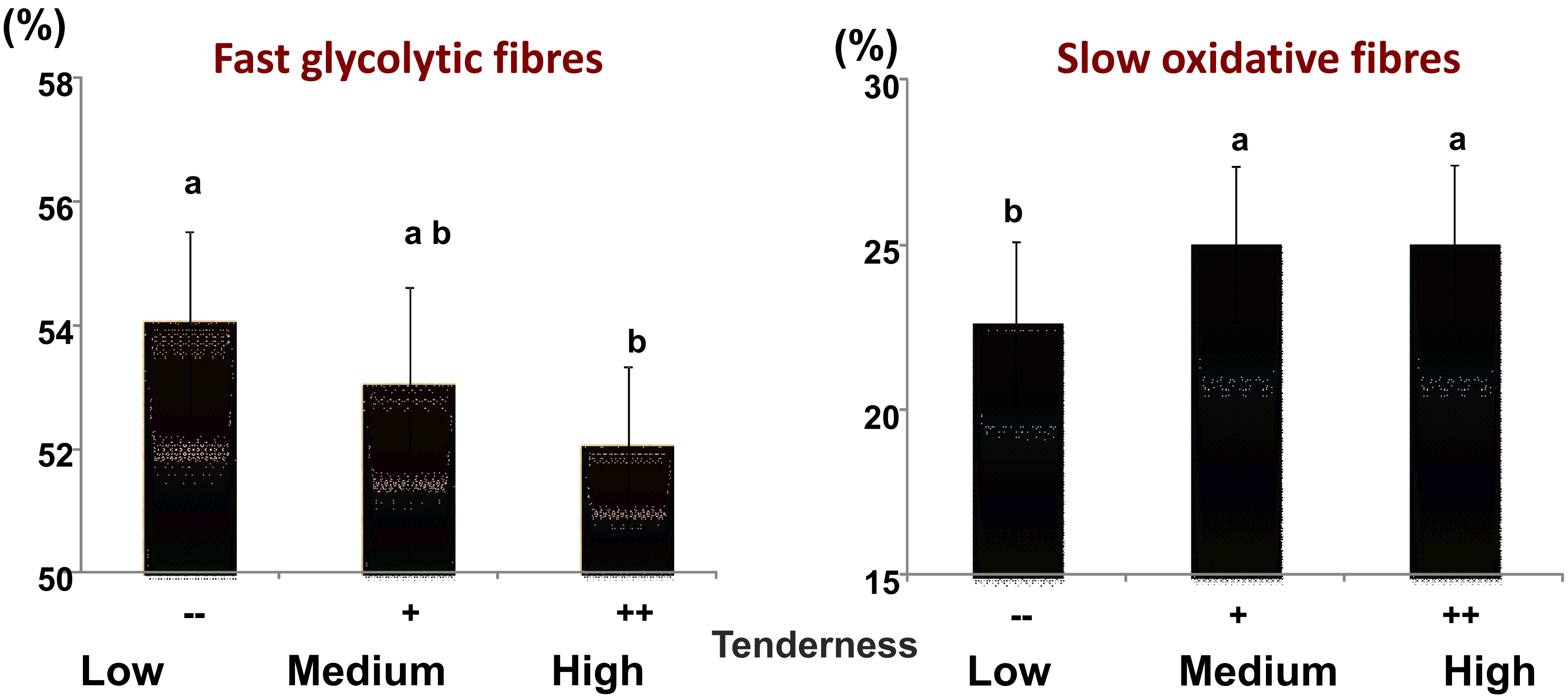
Cross sectional area of fibres/quality



For *Longissimus thoracis* muscle (LT), the tenderest muscles have the lowerest cross sectional area of fibres

Chriki et al., ICoMST 2011

Fibre type/quality



For *LT*, the muscles the less tender have the higher % of IIX fibres and the lower % of slow I fibres

Chriki et al., ICoMST 2011

Muscle properties according to the breed

- Muscle mass
+ collagen
+ intra-muscular fat

+ Muscle mass
- collagen
- intra-muscular fat

+ red
slow
oxydative

+ white
fast
glycolytic

Dairy breeds

Hardy breeds

Beef breeds

Research for the meat sector

- For beef cattle research, a main objective is to control both the development of muscles and qualities of the meat, with specific attention towards tenderness (the top priority quality attribute).
- Variability in beef tenderness originates from genetic polymorphisms and modulation of gene expression according to rearing conditions.
- Beef tenderness is a complex phenotype (post-mortem expression). Identification of relevant markers at the DNA or protein level is ongoing.
- The next challenge is to integrate the knowledge and develop detection tests for desirable animals to ensure proper breeding programmes or management systems.

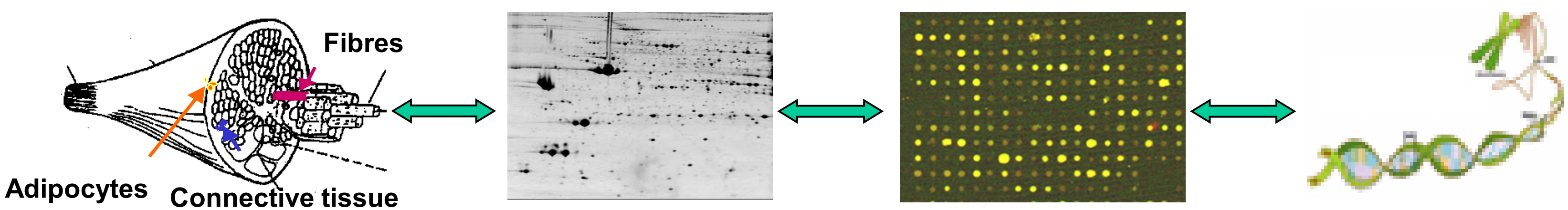
Biomarkers of tenderness

- The relationships between fibres' properties and tenderness is different according to the muscle

Difficult to establish general law

- Muscle characteristics (collagen, fibres, lipids) explain around 30% of tenderness variability

Need to identify other muscle characteristics involved in tenderness by Genomics



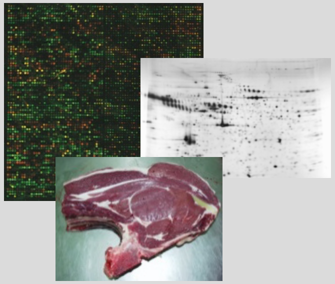
Biochemistry/Histochemistry Proteomics Transcriptomics Genetics

From omics to prediction tools for the Beef sector

Omics signatures

Transcriptome

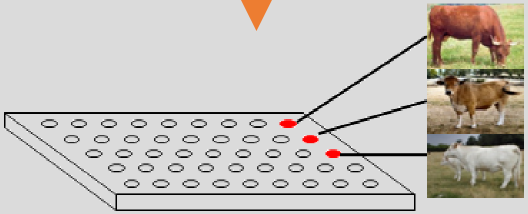
Tenderness



Proteome

List of candidate markers

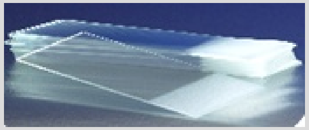
Large-scale VALIDATION



from 2005 to 2012…

from 2008 to 2012…

Development of TOOLS
(DNA chip, protein array)



Inra + French Beef Industry
from 2011

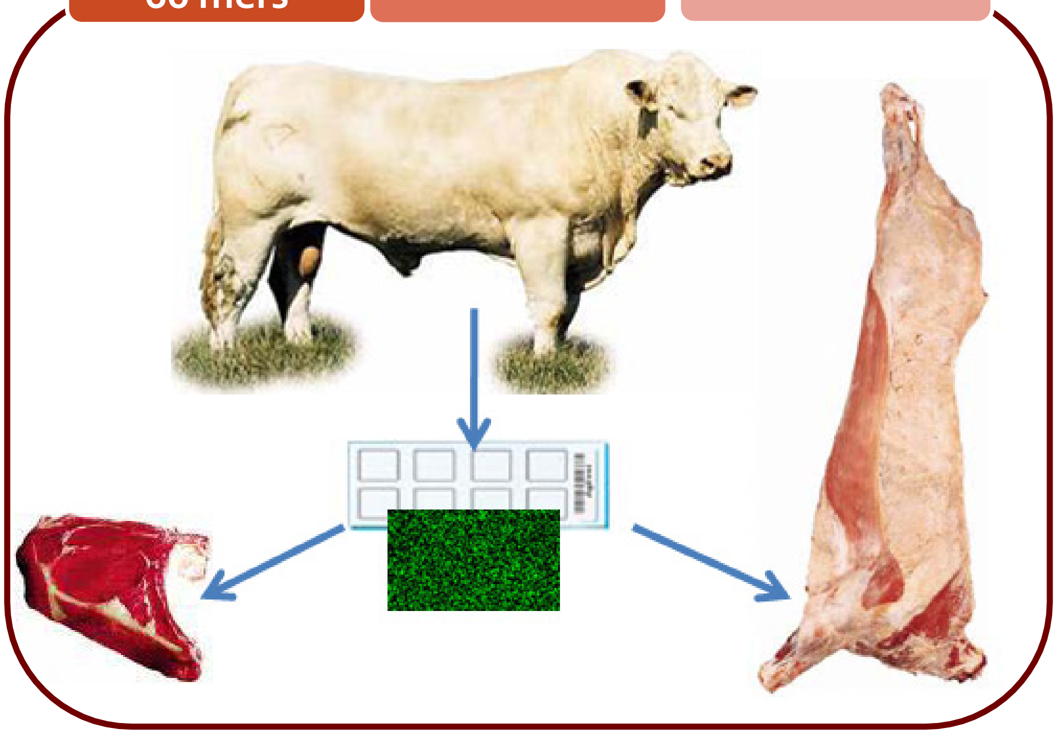
from 2015
Reverse Phase Protein Array
nanotechnologies

Use by the Beef industry

Picard et al, 2015,
Cassar-Malek & Picard, 2016

A “meat quality chip” for Beef prediction

8X15K chip (Agilent) 60 mers | 3000 genes | Several probes /gene

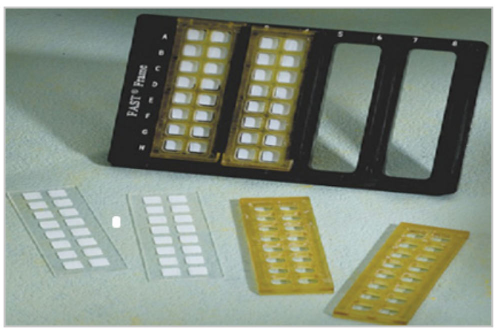


+ **Development of turnkey software**

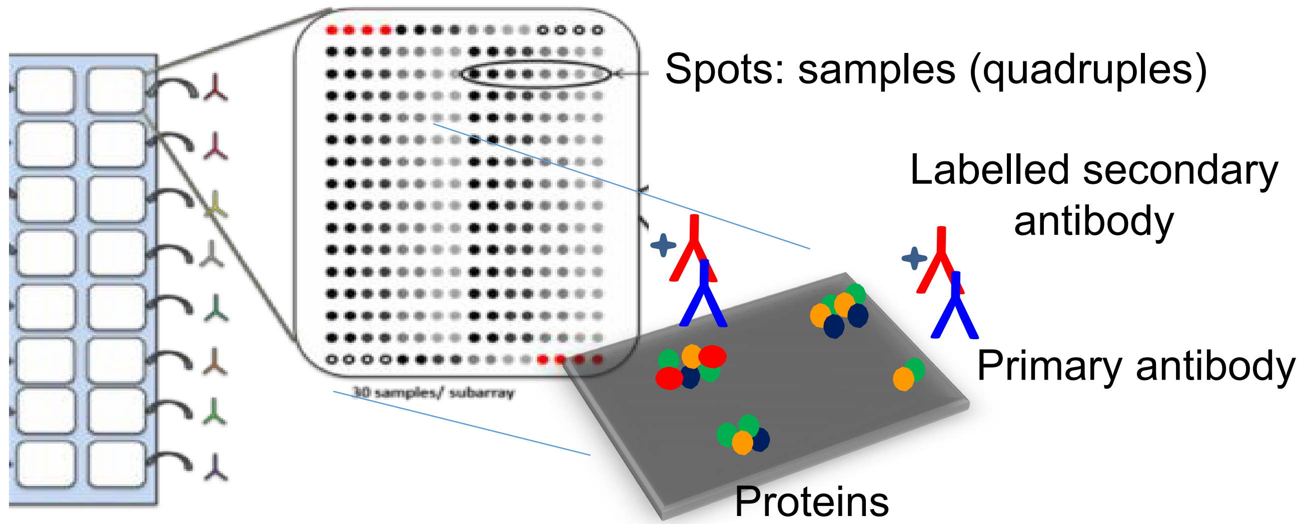
Hocquette et al. 2012. BMC Veterinary Research, 8:135.

Towards high throughput screening of protein biomarkers

Reverse Phase Protein Arrays, a recent methodology



RPPA



Quantification of the abundance of one biomarker in up to 500 samples

On going project + other methods (nano technologies)

Toward less invasive markers

Search for plasma markers

The plasma perfuses all tissues of the body and thus may contain information on physiological mechanisms and performance.

In livestock animals, plasma proteomics is a promising strategy to identify biomarkers of the potential of meat production.

Take-home messages

- The skeletal muscle tissue is a specialized tissue but heterogeneous in structure.
- The muscles are characterized by their contractile and metabolic properties (isoforms of contractile proteins, preferential metabolic pathway).
- They are involved in different types of movements (fast or slow, short or endurance effort).
- The muscle characteristics can explain only one third of variability in Beef quality (tenderness). Biomarkers are identified for development of « diagnostic » tools.