

Application of proteomics approaches to meat science

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Presentation outline

- Background information
- Examples of application
- Potential meat science application

What is proteomics?

The terms “proteomics” and proteome were coined by Marc Wilkins and colleagues in the early 1990s and mirror the terms “genomics” and genome,” which describes the entire collection of genes in an organism.

D.C. Liebler, 2002

Function of proteomics

Proteomics enables us to do large-scale and systematic characterization of the proteins present in a cell, tissue, or organism rather than focusing on a single protein.

“, the protein complement the genome.”

Proteomics Tools

Database

Software to search and match with database

Mass Spectrometry (MS)

Protein-separation (2D, IEF, CE, LC, HPLC...)

Protein chemistry vs proteomics

- Individual proteins
- Complete sequence analysis
- Emphasis on structure and function
- Structural biology
- Complex mixtures
- Partial sequence analysis
- Emphasis on identification by database matching
- System biology

Liebler, 2002

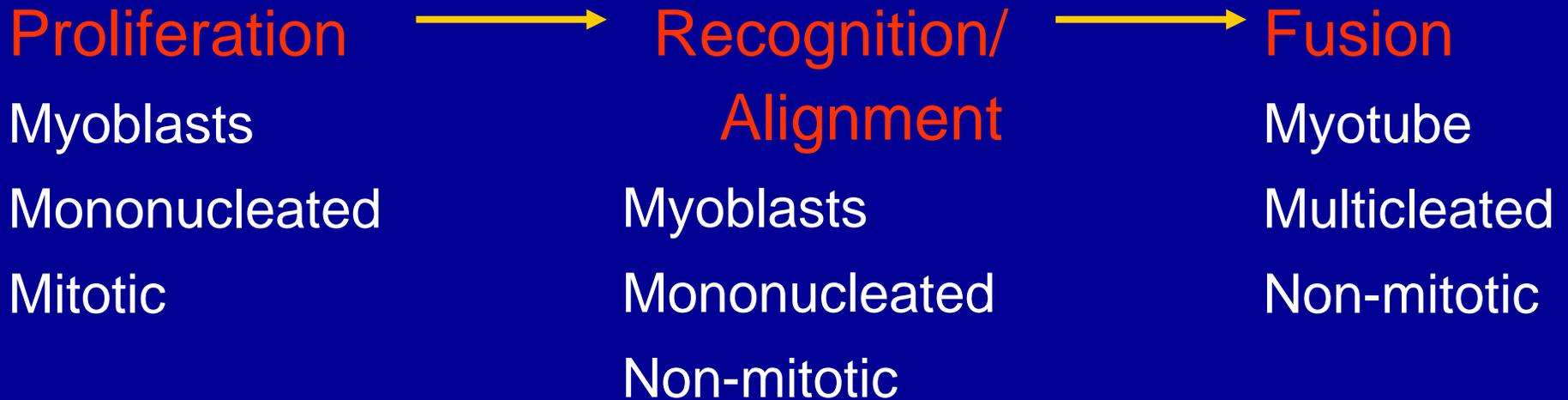
Proteomics applications

- 1) Proteome Mining
- 2) Protein-expression profiling
- 3) Protein-Protein interaction
- 4) Mapping of protein modifications

Proteomics applications

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Prenatal Muscle Growth



Extract proteins at the desired stage



Separate proteins



Excise and digest



Mass Spectrophotometer



Peptide MS data



Protein Identification

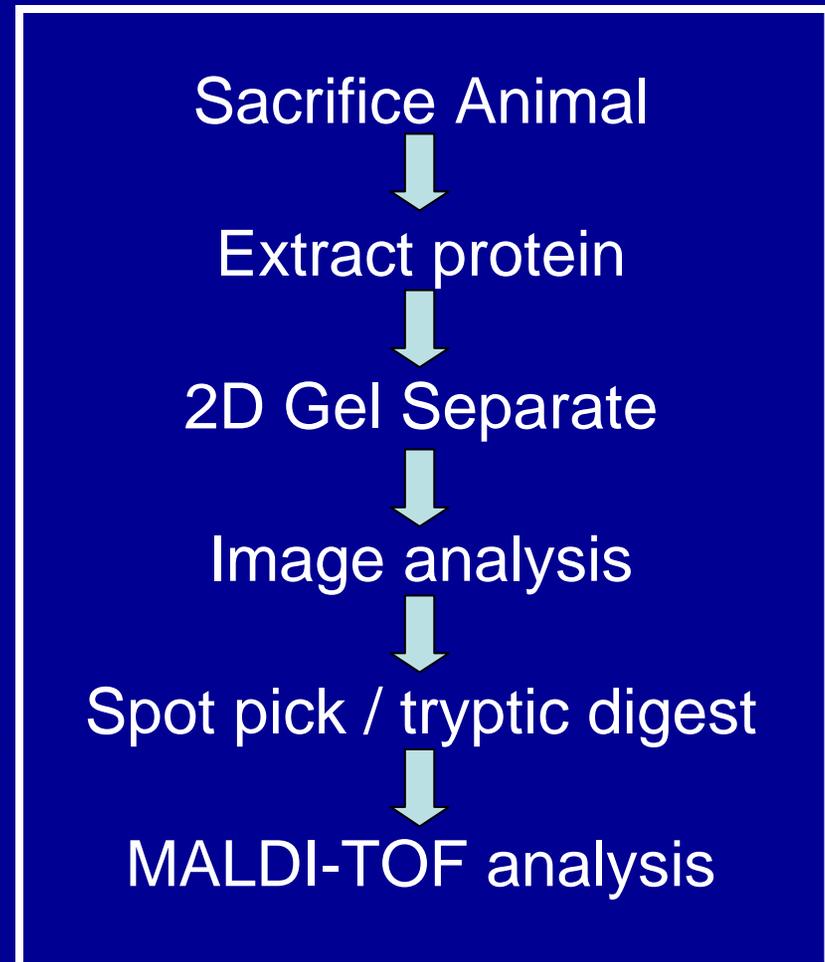
Proteome Mining

Authors: Yan, J.X. *et al.*, 2001.

Proteomics 1:424-434

Title: Separation and identification of rat skeletal muscle proteins using two-dimensional gel electrophoresis and mass-spectrometry.

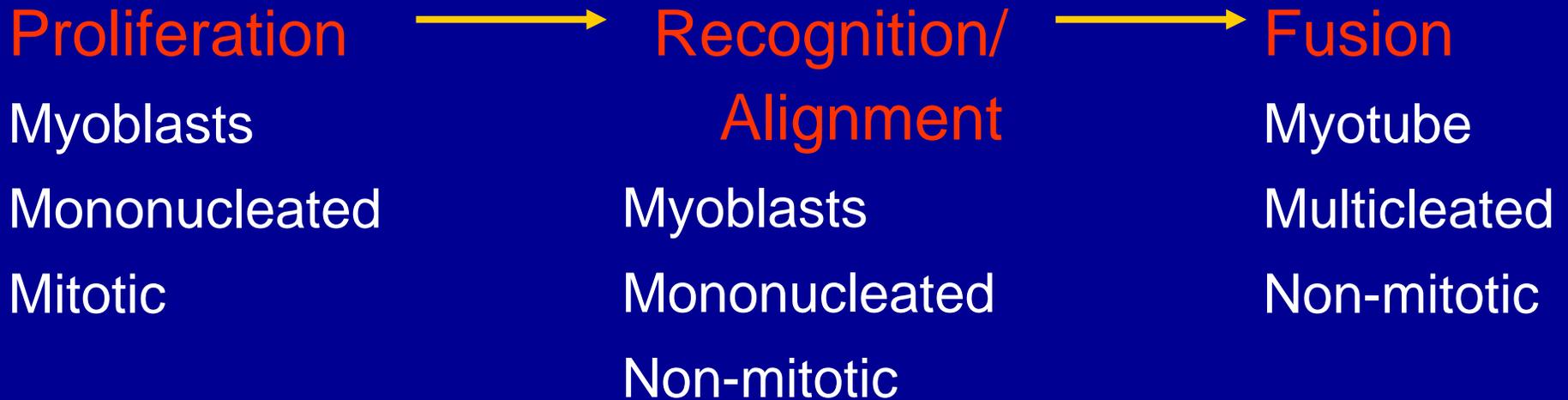
Results: Authors seek to build proteome database. More than 600 protein spots were detected and 74 protein spots identified by MALDI-TOF



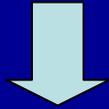
Proteomics applications

- 1) Proteome Mining
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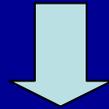
Prenatal Muscle Growth



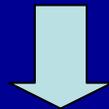
Myoblast



Extract protein



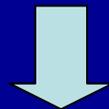
2D Separation



Scan Gel

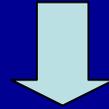


Overlay and compare
images using a computer

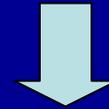


Identify protein
differences

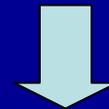
Myotube



Extract protein



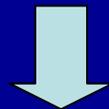
2D Separation



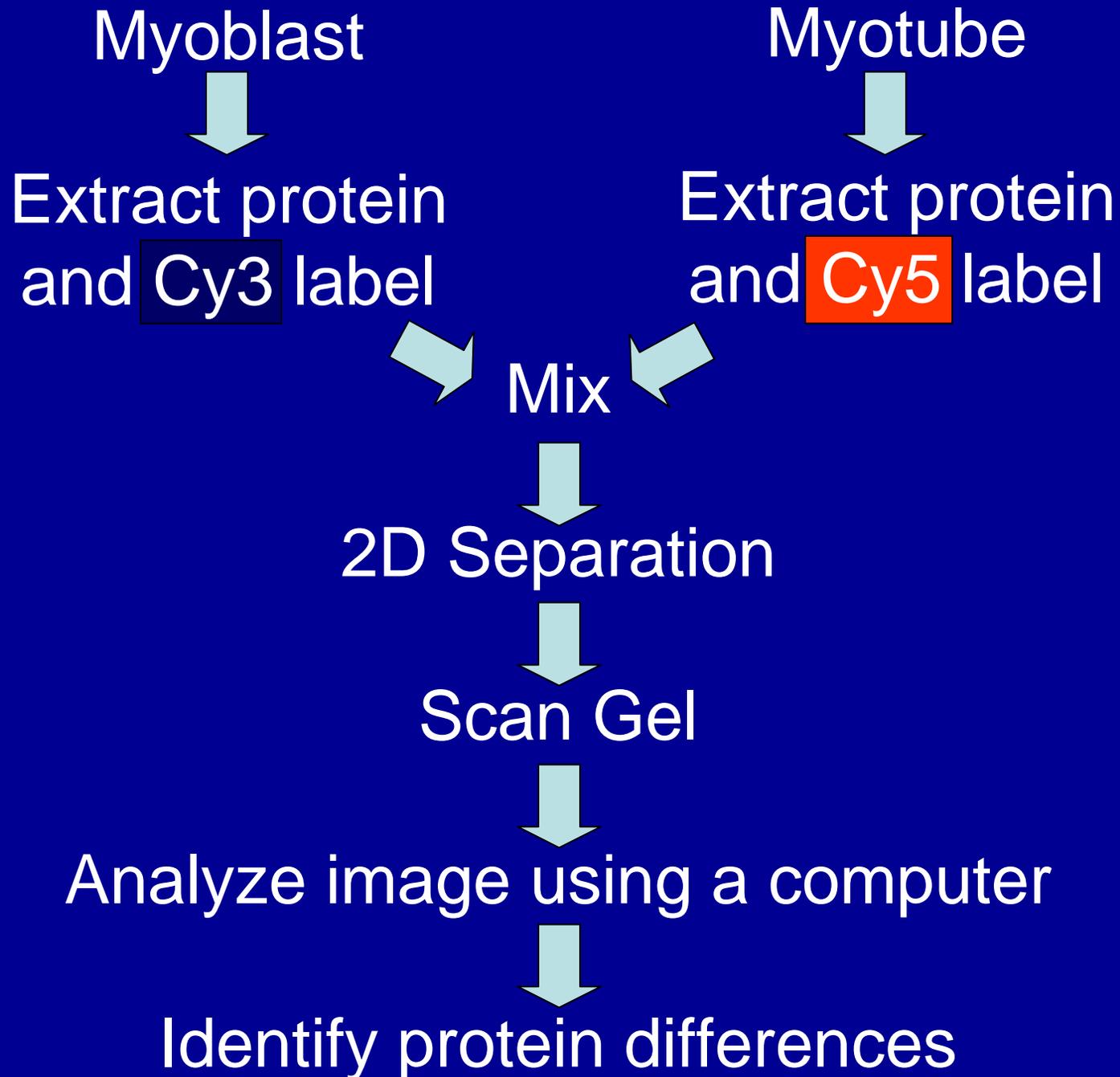
Scan Gel



Overlay and compare
images using a computer



Identify protein
differences

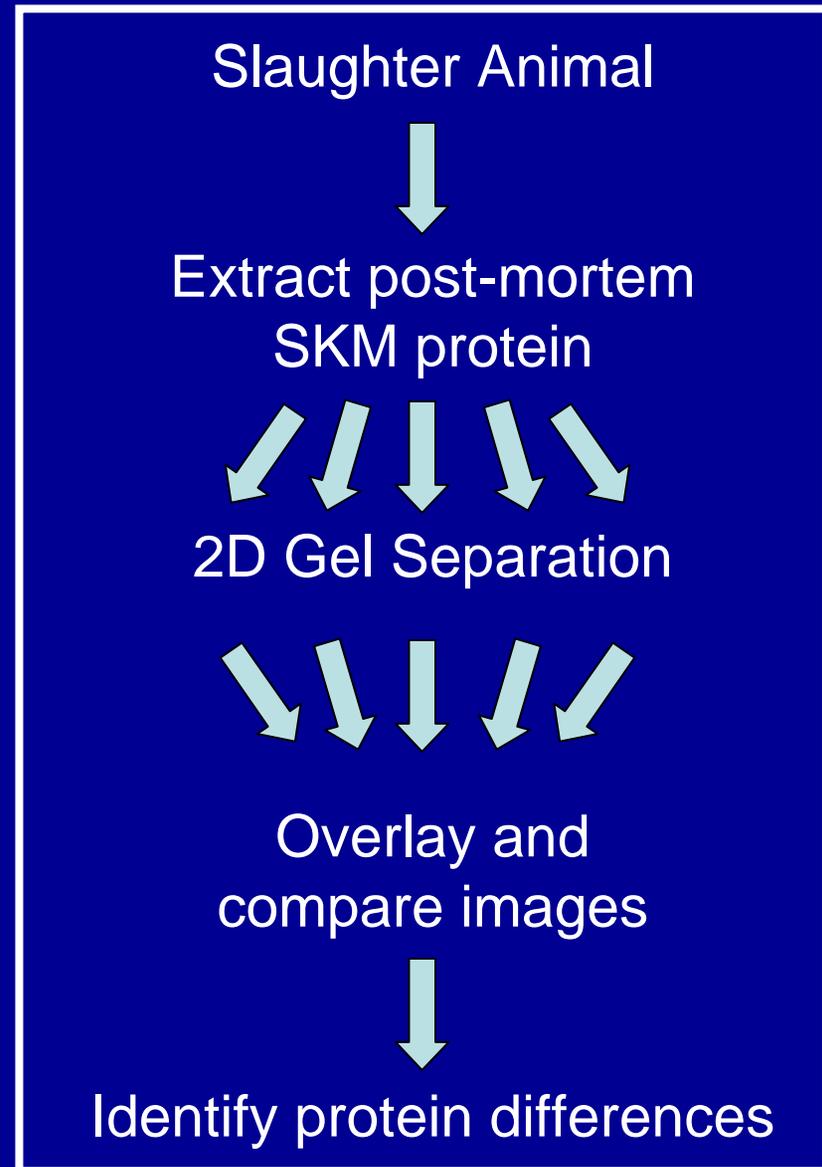


Protein-expression profiling

Authors: Lametsch, R., & Bendixen, E. 2001.
J. Agric. Food. Chem.
49:4531-4537

Title: Proteome analysis applied to meat science: characterizing post-mortem changes (0, 4, 24 & 48 h) in porcine muscle.

Results: They were able to resolve @1000 individual muscle proteins in every 2D gel display. Identified 15 notable and consistent post-mortem changes.



Proteomics applications

- 1) Proteome Mining
- 2) Protein-expression profiling
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A calpain Affinity column



Extract muscle proteins



Pass muscle protein through calpain affinity column



Elute bound proteins and run SDS-PAGE



Determine the identity of bound proteins as with proteome mining

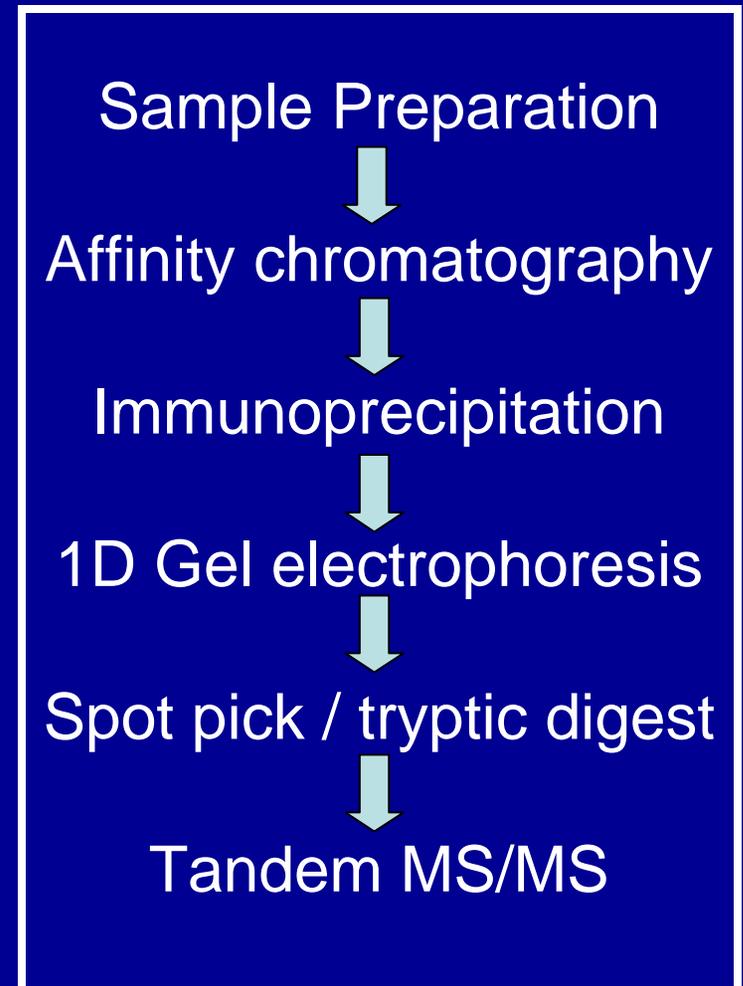
Protein-protein interactions

Authors: Honey, S., et al., 2001.

Nucleic. Acids. Res. **29**:E24

Title: A novel multiple affinity purification tag and its use in identification of proteins associated with a cyclin-CDK complex.

Results: Identified 23 proteins that co-purified with cyclin-CDK complex.



Proteomics applications

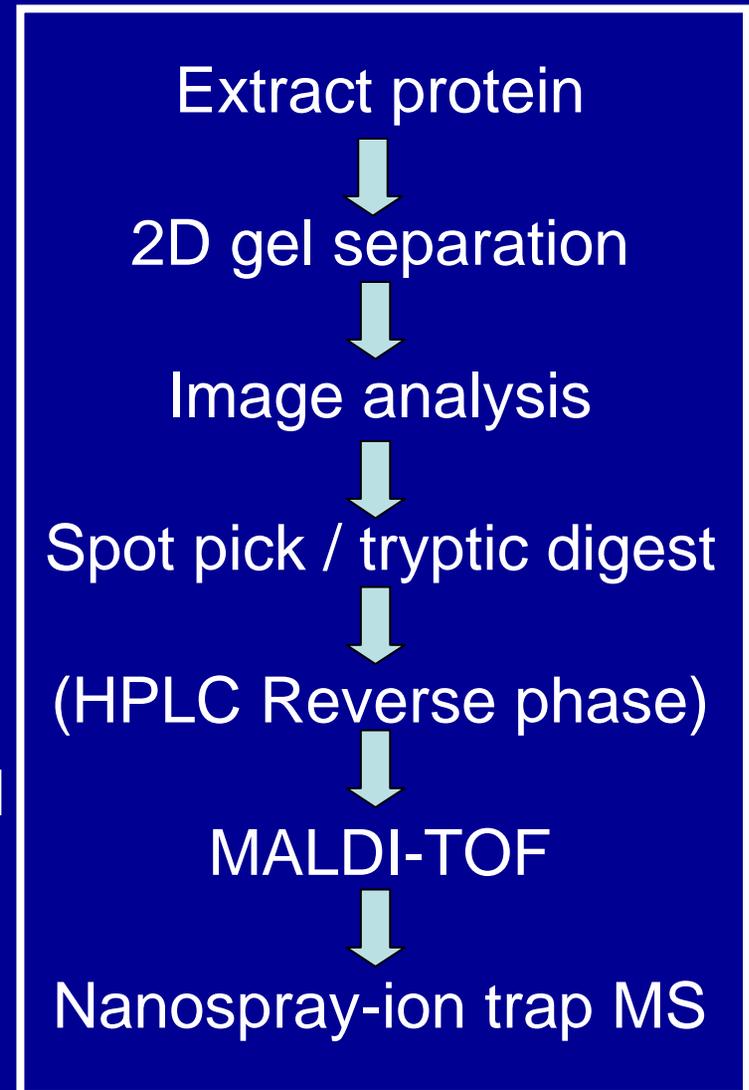
- 1) Proteome Mining
- 2) Protein-expression profiling
- 3) Protein-Protein interaction
- 4) Mapping of protein modifications

Mapping of protein modifications

Authors: González, B., *et al.*, 2002.
Eur.J.Physiol. 443:377-386

Title: Protein variants of skeletal muscle regulatory myosin light chain isoforms: prevalence in mammals, generation and transitions during muscle remodeling.

Results: Demonstrated that the slow and fast rMLC isoforms are diversified in a manner that results in triad pattern of variants (same molecular weight but different PIs).



Application to Meat Science

- Past history

Application to Meat Science

Meat Scientists have always taken advantage of new scientific tools and will do the same with proteomics to:

- 1) Confirm existing hypothesis
- 2) Refute current proposals
- 3) Make new discoveries

Application to Meat Science

Past history:

Proteolysis is an important factor in the development of postmortem changes, including tenderness.

Hoagland et al., 1917

Application to Meat Science

It took decades and application of numerous new scientific tools to demonstrate the critical role that proteolysis plays in the postmortem changes and meat tenderness.

Application to Meat Science

Meat scientists were looking for:

- a) Increase in free amino acids content
- b) Increase in free -terminal groups
- c) Increase in NPN content
- d) All are results of complete protein digestion

Application to Meat Science

Microscopy

SDS-PAGE

Polyclonal antibodies

Monoclonal antibodies

Western blotting

Column chromatography

Application to Meat Science

Minor degradation in key myofibrillar and cytoskeletal proteins by μ -calpain is the mechanism by which meat tenderness is improved during postmortem storage.

Why use Proteomics in Meat Science?

Genomics has not revealed a consistent genetic marker for meat quality.

Using Proteomics and genomics strategies will improve our chances of finding markers

Identification animals that
excel in pork carcass and
meat quality traits

F₂ Duroc X Landrace pork quality
QTL population

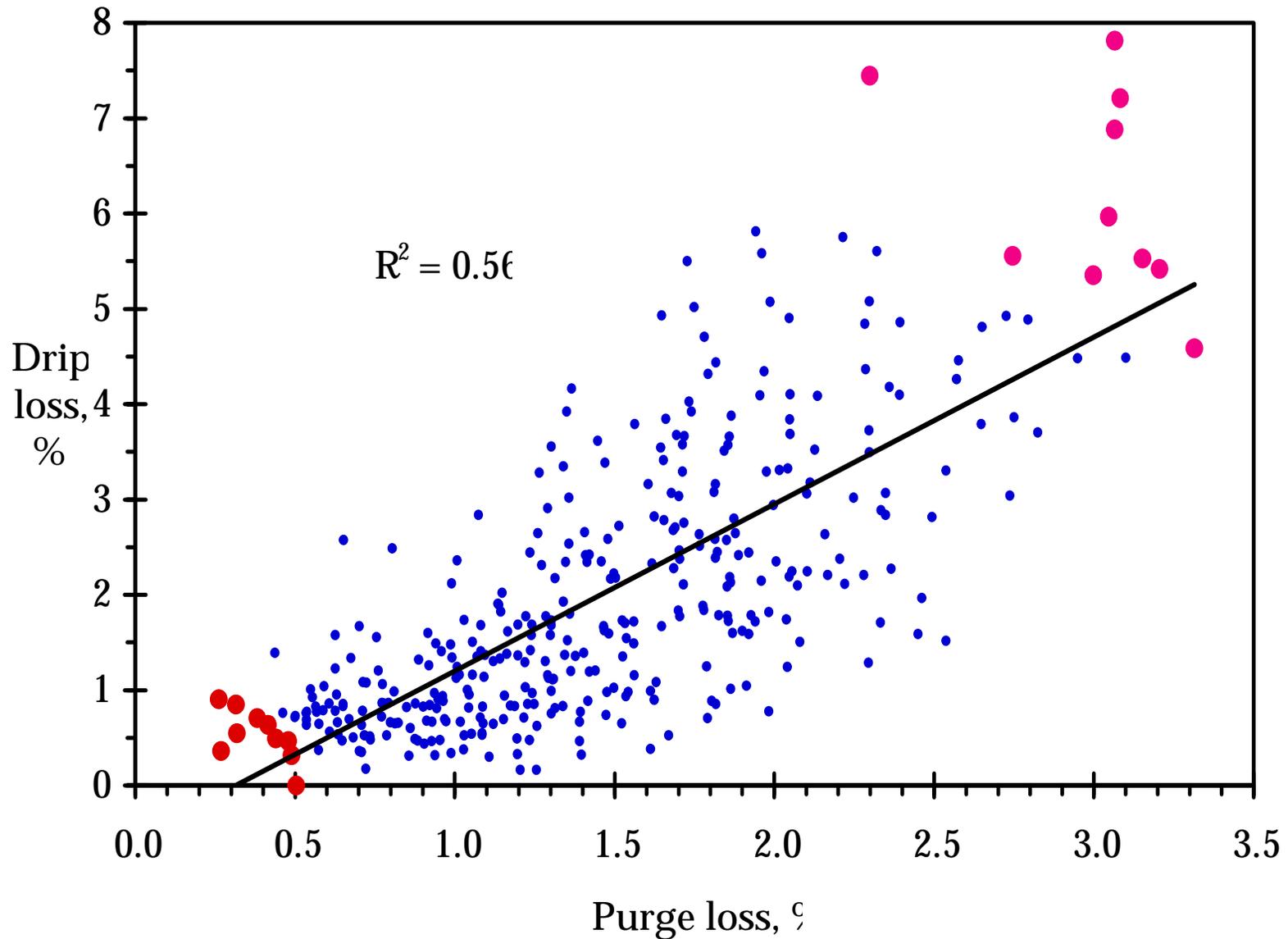
Comparative Expression Profiling

- Our strategy is to contrast animals with measurable quality differences and investigate differential protein expression with the ultimate goal of developing a rapid assay to identify animals/carcasses that excel in the desired traits

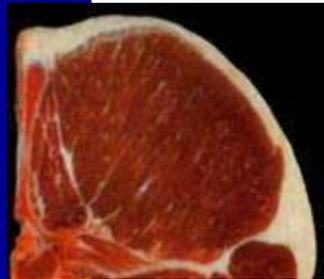
To identify proteins affecting a given trait, we will compare the proteome of samples that differ extremely in the trait of interest. To increase the chance of success, we will, when feasible, use multiple measures of the same trait to select the samples to be compared.

Another approaches, probably unique to our lab is pooling of sample. We will take sample from multiple individual to reduce or eliminate anima-to-animal variation.

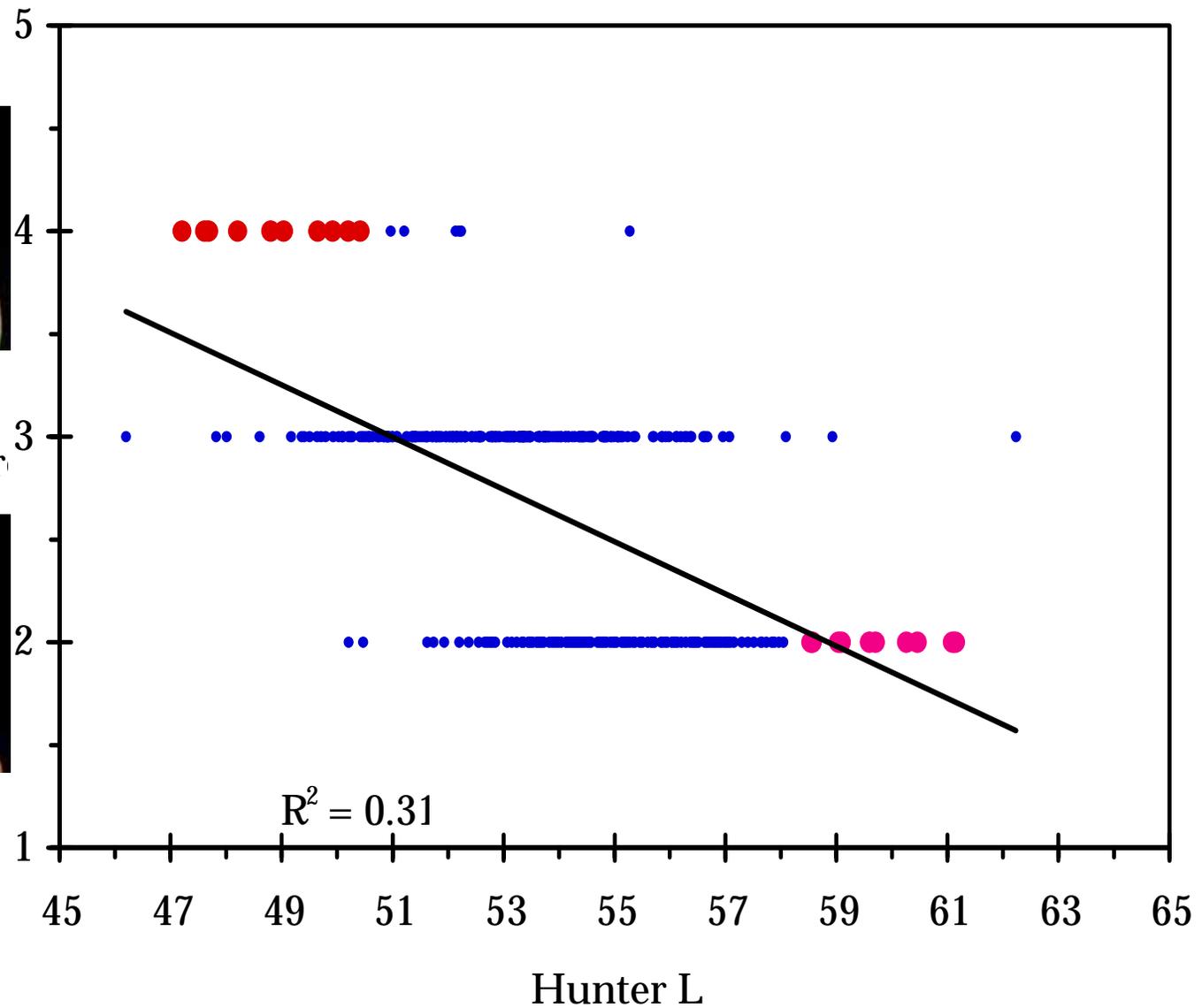
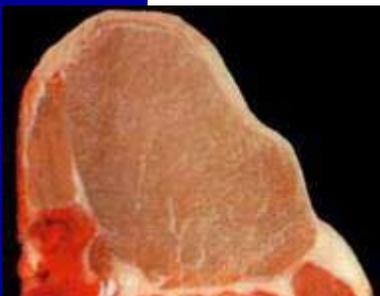
Selection of samples for pooling



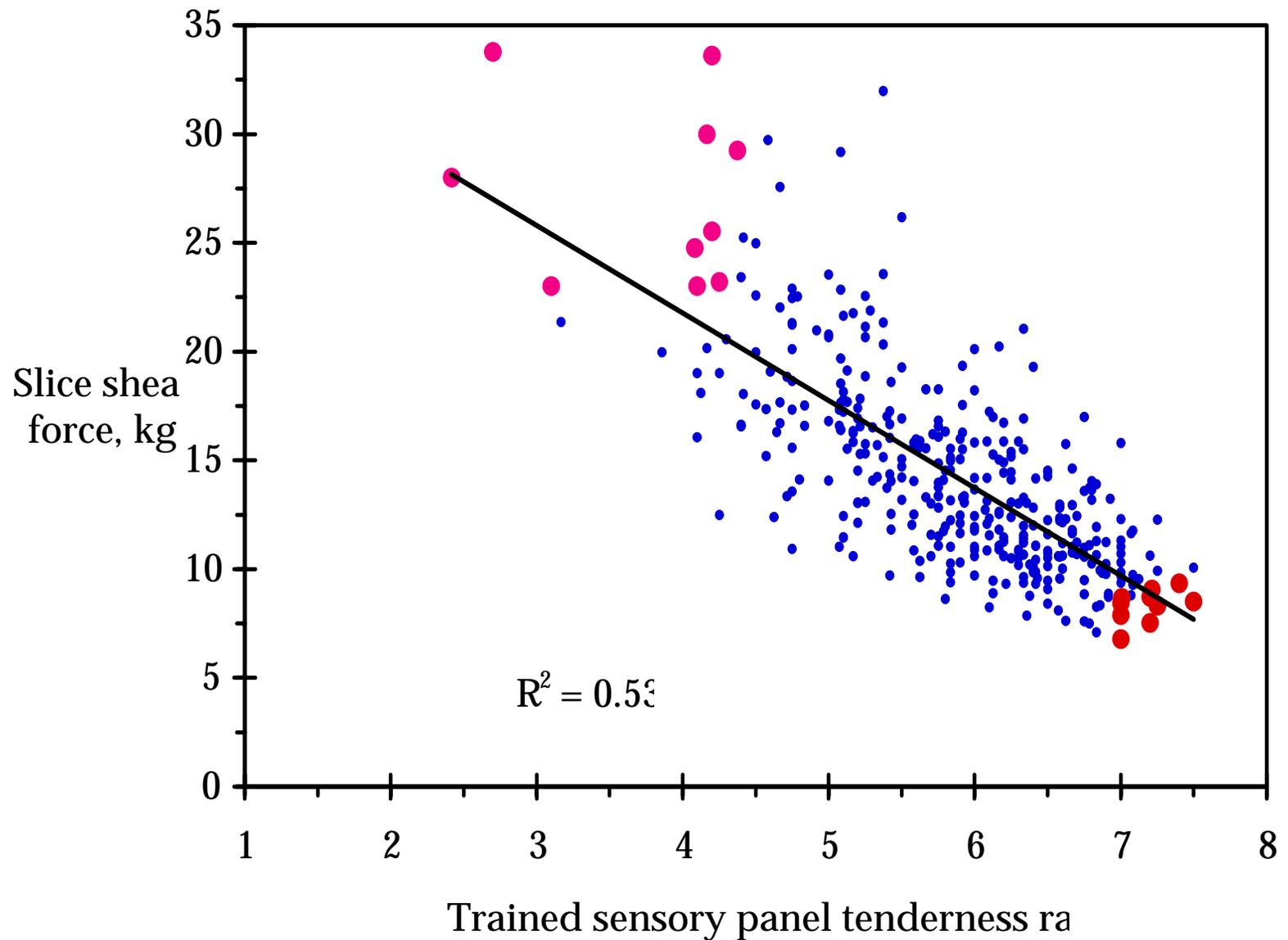
Selection of samples for pooling



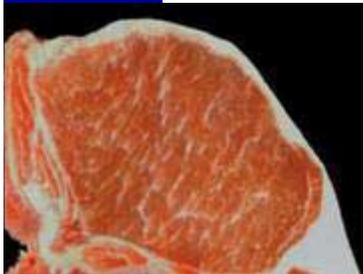
NPPC
color score



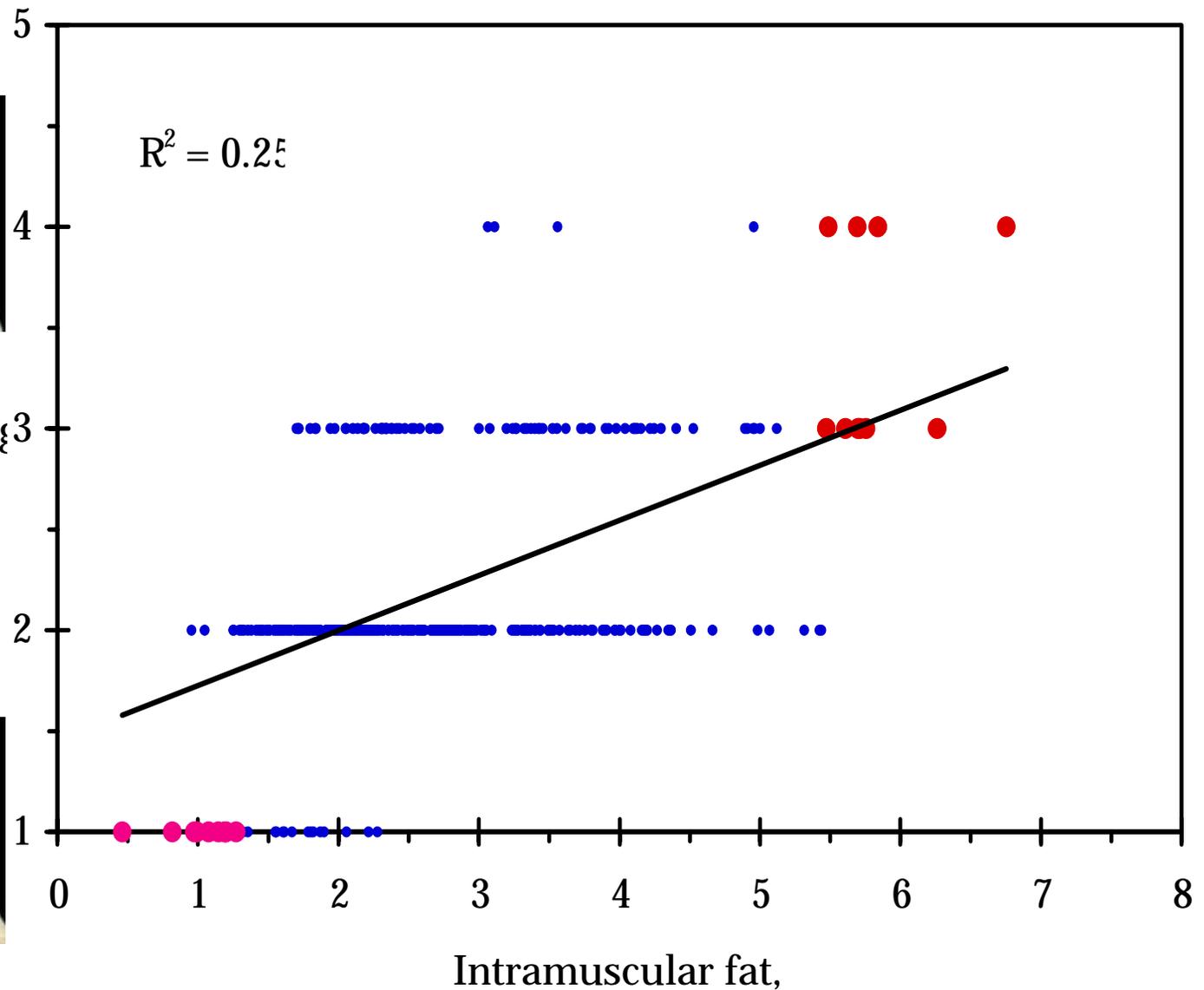
Selection of samples for pooling



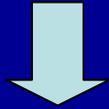
Selection of samples for pooling



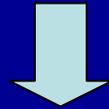
NPPC
marbling
score



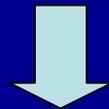
Low Quality



Extract protein



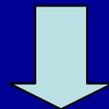
2D Separation



Scan Gel

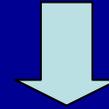


Overlay and compare
images using a computer

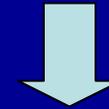


Identify protein
differences

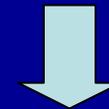
High Quality



Extract protein



2D Separation



Scan Gel



Low Quality



Extract protein
and **Cy3** label

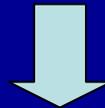
High Quality



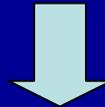
Extract protein
and **Cy5** label



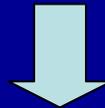
Mix



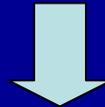
2D Separation



Scan Gel



Analyze image using a computer



Identify protein differences

If I were a graduate student interested in application of proteomics

- I would identify a faculty/postdoc/graduate student who emphasizes proteomics approaches and work with them to learn the techniques and then apply these methods and approaches to my problems in meats science.

WWW based proteomic resources

ExPASy Molecular Biology Server

<http://www.expasy.ch/>

Databases

Tools and Software Packages

Protocols

[ExPASy Home page](#)[Site Map](#)[Search ExPASy](#)[Contact us](#)Hosted by [NCSC US](#) Mirror sites: [Canada](#) [China](#) [Korea](#) [Switzerland](#) [Taiwan](#)

SWISS-2DPAGE

Two-dimensional polyacrylamide gel electrophoresis database

SWISS-2DPAGE contains data on proteins identified on various 2-D PAGE and SDS-PAGE reference maps. You can locate these proteins on the 2-D PAGE maps or display the region of a 2-D PAGE map where one might expect to find a protein from SWISS-PROT [[More details](#) / [References](#) / [Disclaimer](#)].

Release 15.2, November 2001 and updates up to 22-May-2002 (contains 861 entries in 33 reference maps from human, mouse, *Arabidopsis thaliana*, *Dictyostelium discoideum*, *Escherichia coli* and *Saccharomyces cerevisiae*).

[\[Search\]](#) [\[Documents\]](#) [\[Services\]](#) [\[Software\]](#) [\[Related servers\]](#) [\[Other databases\]](#) [\[Job openings\]](#)

Access to SWISS-2DPAGE

- [by description](#) (any word in the DE, OS, OG, GN and ID lines)
- [by accession number](#) (AC lines)
- [by clicking on a spot](#): click on one of our 2-D PAGE or SDS-PAGE reference maps, click on a spot and then get the corresponding information from the SWISS-2DPAGE database.
- [by author](#) (RA lines)
- [by spot serial number](#) (2D and 1D lines)
- [by full text search](#)
- [SRS](#), searching in SWISS-2DPAGE using the Sequence Retrieval System
- [retrieve in a table all the protein entries identified on a given reference map](#)
- [compute estimated location on reference maps for a user-entered sequence](#)

SWISS-2DPAGE documents

- [User manual](#)
- [Release notes](#) (November 12, 2001)
- **Protocols:**
 - [Technical information](#) about 2-D PAGE (IPG's, silver staining, protocols, etc)
 - [High performance 2-D gel comparison](#)
- **Figure captions of SWISS-2DPAGE maps available from publications:**
 - Human [CSF](#), [ELC](#), [HEPG2](#), [HEPG2SP](#), [LIVER](#), [LYMPHOMA](#), [PLASMA](#), [PLATELET](#), [RBC](#), [U937](#), [CEC](#), [KIDNEY](#).
 - [Dictyostelium discoideum](#), [Escherichia coli](#), [Saccharomyces cerevisiae](#).

Services

- [Downloading SWISS-2DPAGE by FTP](#)
- [SWISS-2DSERVICE](#) - Get your 2-D Gels performed according to Swiss standards
- [2-D PAGE training](#) - attend a one week course in Geneva
- [2-D PAGE museum](#) - gels run by trainees during the 2-D PAGE courses

Software

- [Melanie 3](#) - Software package for 2-D PAGE analysis
- [Make2ddb package](#) - A package preparing the data and the programs necessary to build a federated 2-DE database on one's own web site.

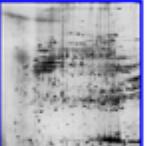
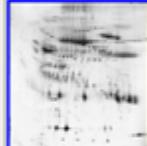
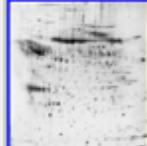
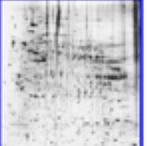
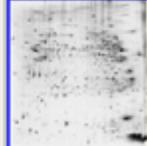
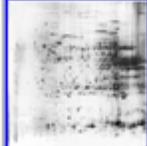
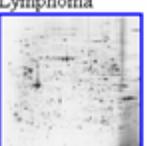
Gateways to other 2-D PAGE related servers and services



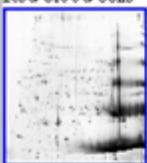
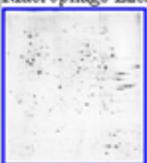
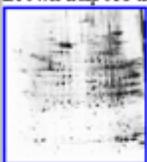
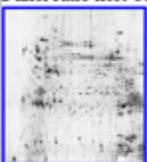
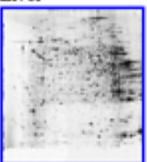
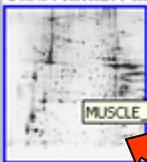
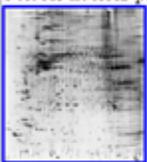
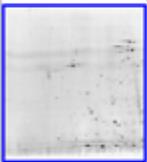
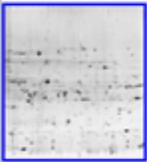
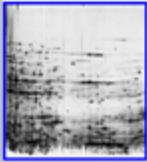
SWISS-2DPAGE Map Selection

SWISS-2DPAGE Map Selection allows you to select a 2-D PAGE map which will be displayed. You will then be requested to click on a spot and will obtain information on the corresponding protein. These SWISS-2DPAGE maps have been analysed and built using the [Melanie](#) software.

The following 2-D PAGE maps are available. Please select one:

	<p>Colorectal epithelia cells</p> 	<p>Cerebrospinal Fluid</p> 	<p>Colorectal adenocarcinoma cell line (DL-1)</p> 
	<p>Erythroleukemia Cell</p> 	<p>HepG2 Secreted Proteins</p> 	<p>HepG2</p> 
	<p>Promyelocytic leukemia cells</p> 	<p>Kidney</p> 	<p>Liver</p> 
<p>HUMAN <i>Homo sapiens</i></p>	<p>Lymphoma</p> 	<p>Soluble nuclear proteins and matrix from liver tissue</p> 	<p>SDS-PAGE of nucleolar proteins from Human HeLa cells ***</p> 



	Red blood cells 	Macrophage Like Cell Line 	
MOUSE <i>Mus musculus</i>	Brown adipose tissue 	Pancreatic islet cells 	Liver 
	Gastrocnemius muscle  MUSCLE_MOUSE	Soluble nuclear proteins and matrix from liver tissue 	White adipose tissue 
	<i>Escherichia coli</i> 	<i>Escherichia coli</i>(4-5) 	<i>Escherichia coli</i>(4.5-5.5) 
ECOLI <i>Escherichia coli</i>	<i>Escherichia coli</i>(5-6) 	<i>Escherichia coli</i>(5.5-6.7) 	<i>Escherichia coli</i>(6-11) 
	<i>Escherichia coli</i>(6-9) 		
	<i>Arabidopsis thaliana</i> 	<i>Dictyostelium discoideum</i> 	<i>Saccharomyces cerevisiae</i> 
OTHER SPECIES			

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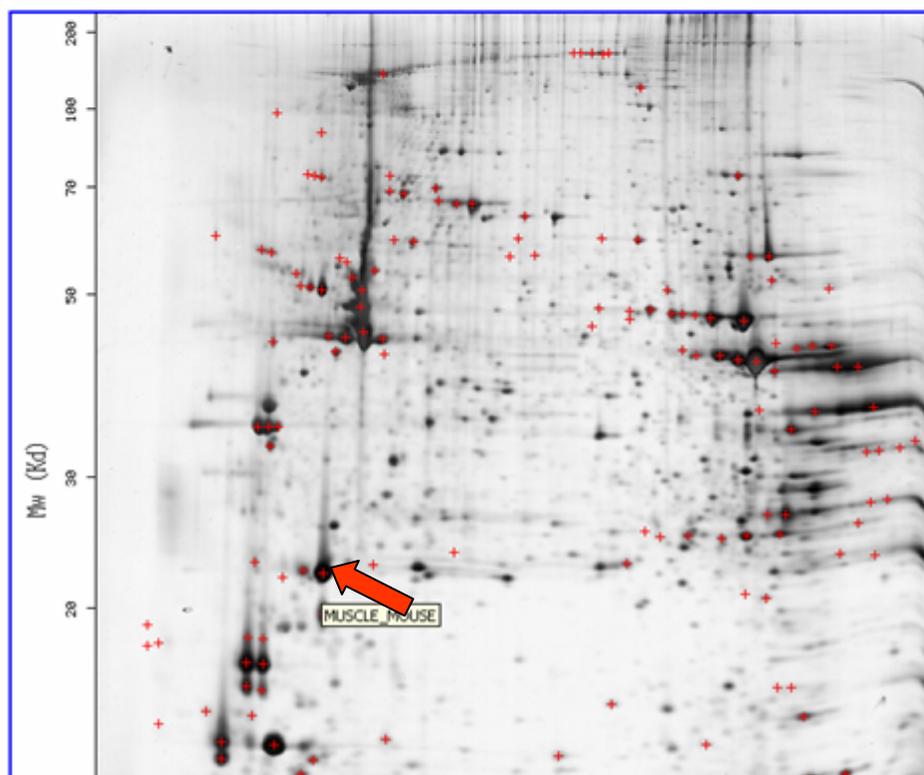
[SWISS-2DPAGE](#)



Map Selection: MUSCLE_MOUSE

Spots corresponding to known proteins are highlighted in red. Please click on one of them or select the same map in another format:

- [MUSCLE_MOUSE large, spots highlighted](#)
- [MUSCLE_MOUSE small, spots not highlighted](#)
- [MUSCLE_MOUSE large, spots not highlighted](#)



[ExPASy Home page](#)[Site Map](#)[Search ExPASy](#)[Contact us](#)[SWISS-2DPAGE](#)**Search**[\[by description\]](#)[\[by accession number\]](#)[\[by clicking on a spot\]](#)[\[by author\]](#)[\[by serial number\]](#)[\[by full text search\]](#)[\[SRS\]](#)**1 protein has been found in the clicked spot (2D-0017LY):****General information about the entry**[View entry in original SWISS-2DPAGE format](#)Entry name **MLE1_MOUSE**Primary accession number **P05977**

Entered in SWISS-2DPAGE in Release 07, September 1998

Last modified in Release 14, October 2001

Name and origin of the protein

Description Myosin light chain 1, skeletal muscle isoform (MLC1F) (A1 catalytic) (Alkali).

Gene name(s) MYL1 OR MYLF

From Mus musculus (Mouse). [TaxID: [10090](#)]

Taxonomy Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.

References

- [1] MAPPING ON GEL.
MEDLINE=96089471; PubMed=8529625; [NCBI, ExPASy, EBI, Israel, Japan]
Corbett J.M., Wheeler C.H., Dunn M.J.;
"Coelctrophoresis of cardiac tissue from human, dog, rat and mouse: towards the establishment of an integrated two-dimensional protein database.";
Electrophoresis 16:1524-1529(1995).
- [2] MAPPING ON GEL.
Sanchez J.-C., Chiappe D., Converset V., Hoogland C., Binz P.-A., Paesano S., Appel R.D., Wang S., Sennitt M., Nolan A., Cawthorne M.A., Hochstrasser D.F.;
"The mouse SWISS-2DPAGE database: a tool for proteomics study of diabetes and obesity.";
Proteomics 1:136-163(2001).

2D PAGE maps for identified proteins[Compute the theoretical pI/Mw](#)[How to interpret a protein map](#)

MAP LOCATIONS:

Journals related to proteomics

Molecular and Cellular Proteomics (Full Text)

<http://www.mcponline.org/>

Electrophoresis (Table of contents)

<http://www.interscience.wiley.com/jpages/0173-0835/>

Applied Genomics and Proteomics (Table of contents)

<http://www.openmindjournals.com/genomics.html>

Proteomics (Table of contents)

<http://www.interscience.wiley.com/jpages/1615-9853/>

Journal of Proteome Research (Table of contents)

<http://pubs.acs.org/journals/jprobs/>

Journal of Mass Spectrometry (Table of contents)

<http://www.interscience.wiley.com/jpages/1076-5174/>

Mass Spectrometry Reviews (Table of contents)

<http://www.interscience.wiley.com/jpages/0277-7037/>

Thank you