Comparative Proteomics of Swine Tissue

2DLC/MS of Tissue Digestion

Preliminary Protein Analysis

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Briefing

- Treating and monitoring diseased tissue
- Sharing swine tissue for continued protein characterization

- They provide tissue
- We provide preparation, analysis

Win-Win

The Goal

Find proteins to monitor disease treatment

- Find many proteins
  - Efficient protein extraction
  - Good sensitivity (low protein concentration)
- Specific Subsets of proteins
  - Growth Factors
  - Mitochondrial
  - Anti Apoptosis
  - Glycolosis
- Consistency

Constraints

- Tissue amount
- Time and temperature sensitive samples
- Ease of analysis

Homogenization Tools

- Rotary homogenizer

Suited for larger amounts of tissue (grams)
Consistent volume loss for tissue
Single sample at a time

- Hand-Pestle

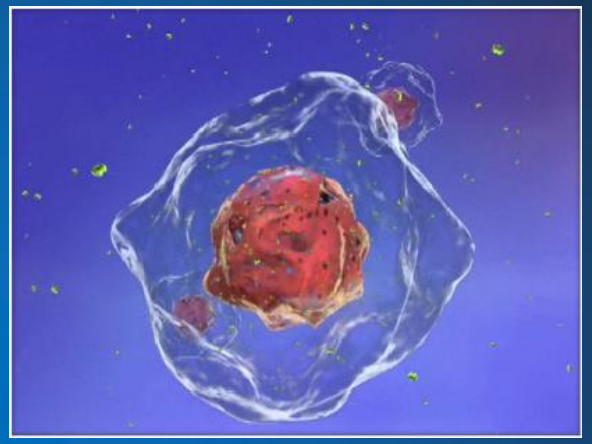
Smaller tissue amounts
Low volume loss
Inconsistent sample processing
Single sample at a time

- Pressure Cycling Technology (PCT)

Smaller tissue amounts
Low volume loss
Consistent sample processing
Multiple samples (up to 48)

PCT Technology Overview

- In closed container, PCT applies and releases pressure on the sample from ambient to 35,000 psi in user customizable intervals
- PCT uses the hydrostatic compression differential of various cellular components to disrupt the cell with gentle yet highly powerful forces



Intact Cell

Cell Lysis with PCT

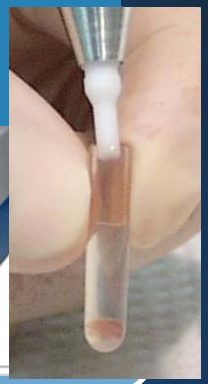
Image courtesy of: http://www.vet.purdue.edu/cpr/secondaryinjury3.html

Sample Preparation Procedure

HEPES Buffer extraction

Group A: Normal tissue, separate study
Group B: Diseased tissue
Group C: Diseased tissue with vehicle control
Group D: Treated diseased tissue
Group E: Normal tissue
N=3 each

es at 35,000 psi)

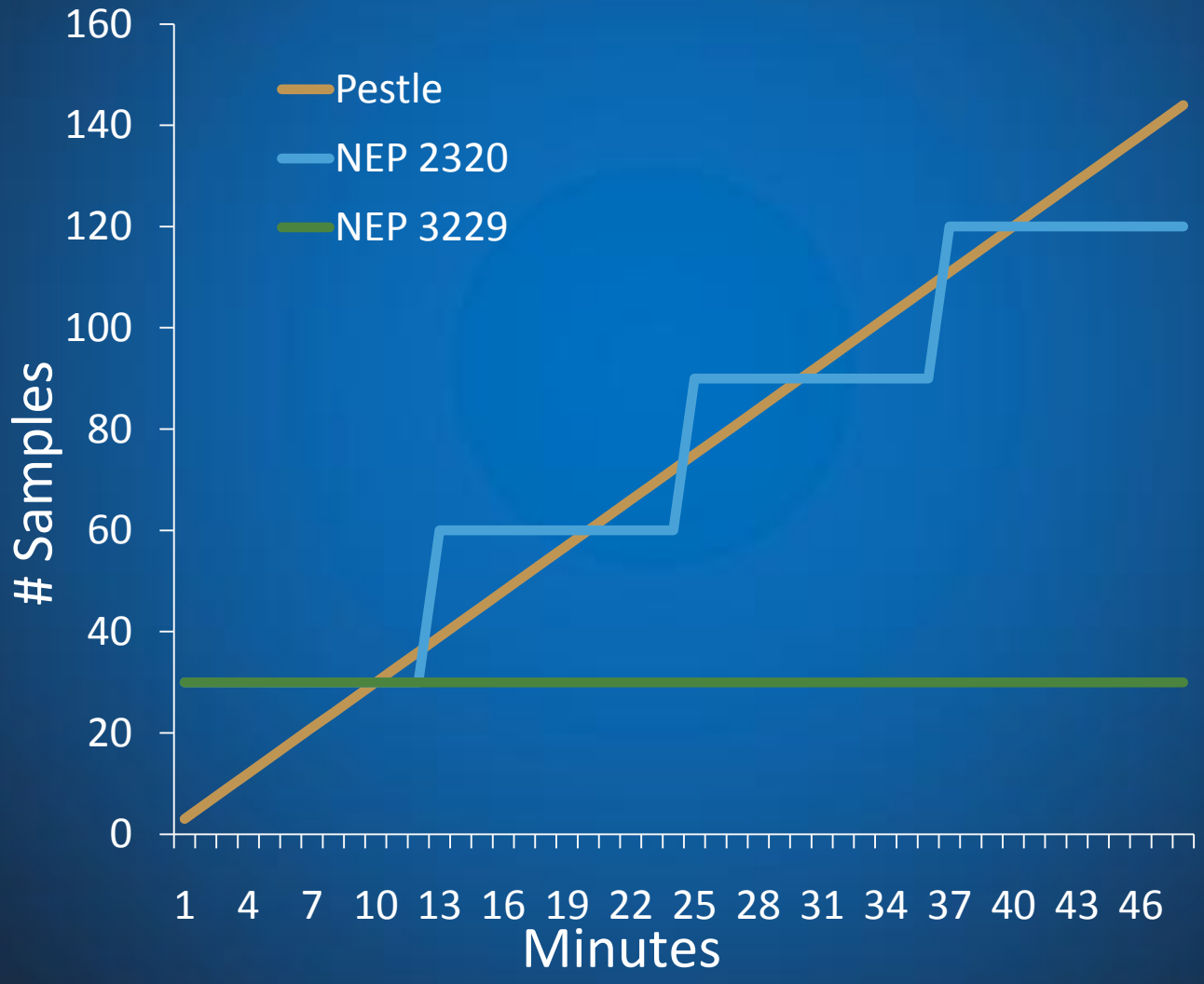


Digest with Trypsin

Inject onto LC/MS

Homogenization Time

Barocycler use is more efficient at more than 10 samples



Sample Preparation Procedure

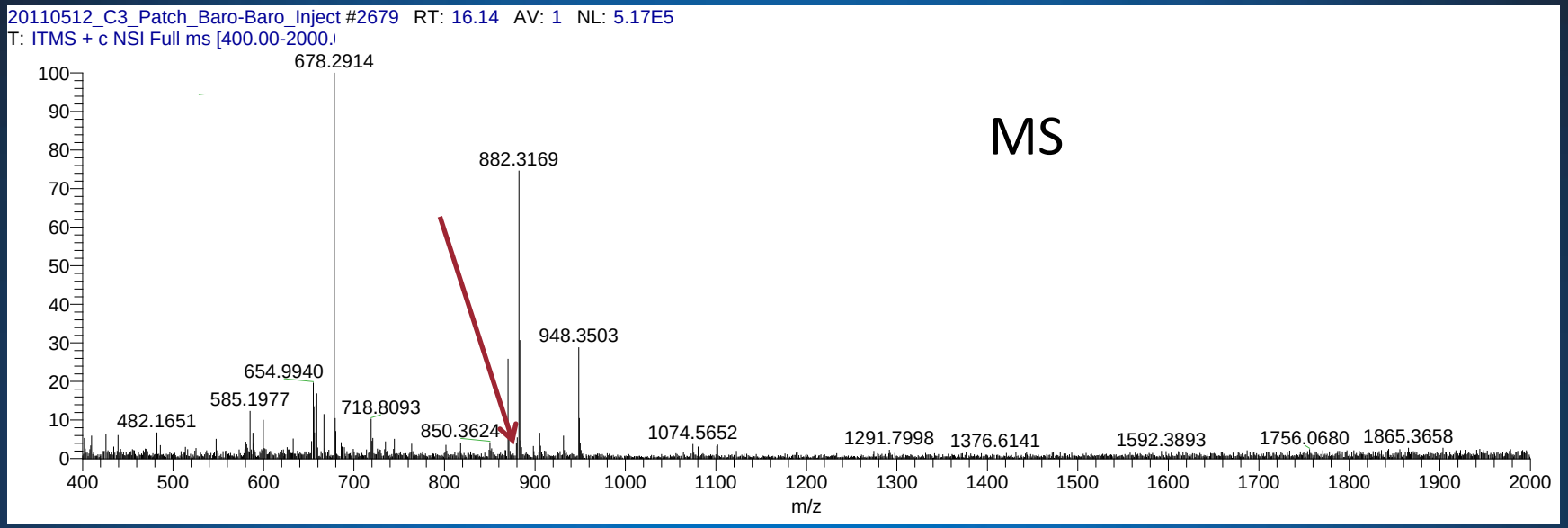
Homogenize Tissue

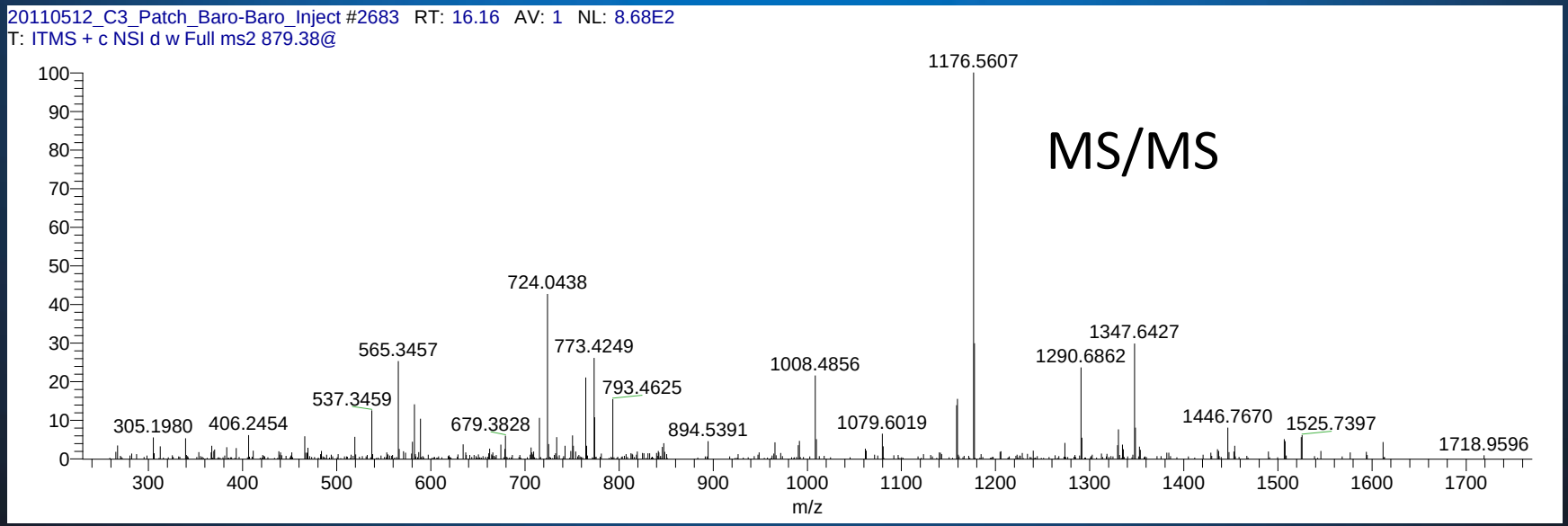
Extract Proteins

Reduced and Alkylated
Digested with Trypsin, ProteaseMax
Overnight at 37°C or
Barocycler PCT (30 cycles at 20,000 psi)

2 Dimensional LC/MS
4 Step strong cation exchange (SCX)
40 minute reverse phase gradient
Thermo LTQ:
Data Dependant Top 5 analysis
Dynamic Exclusion

Example Spectrum





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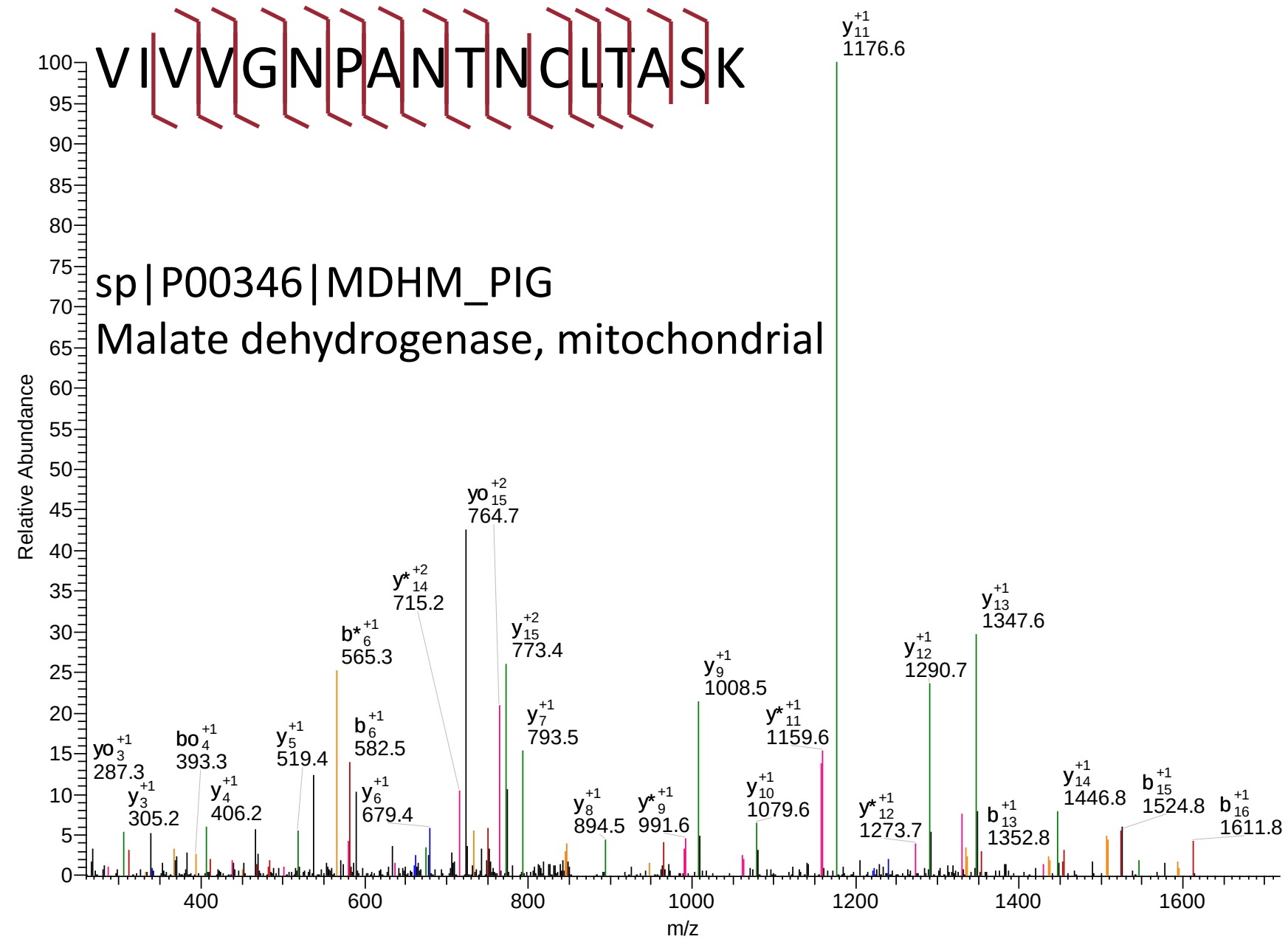


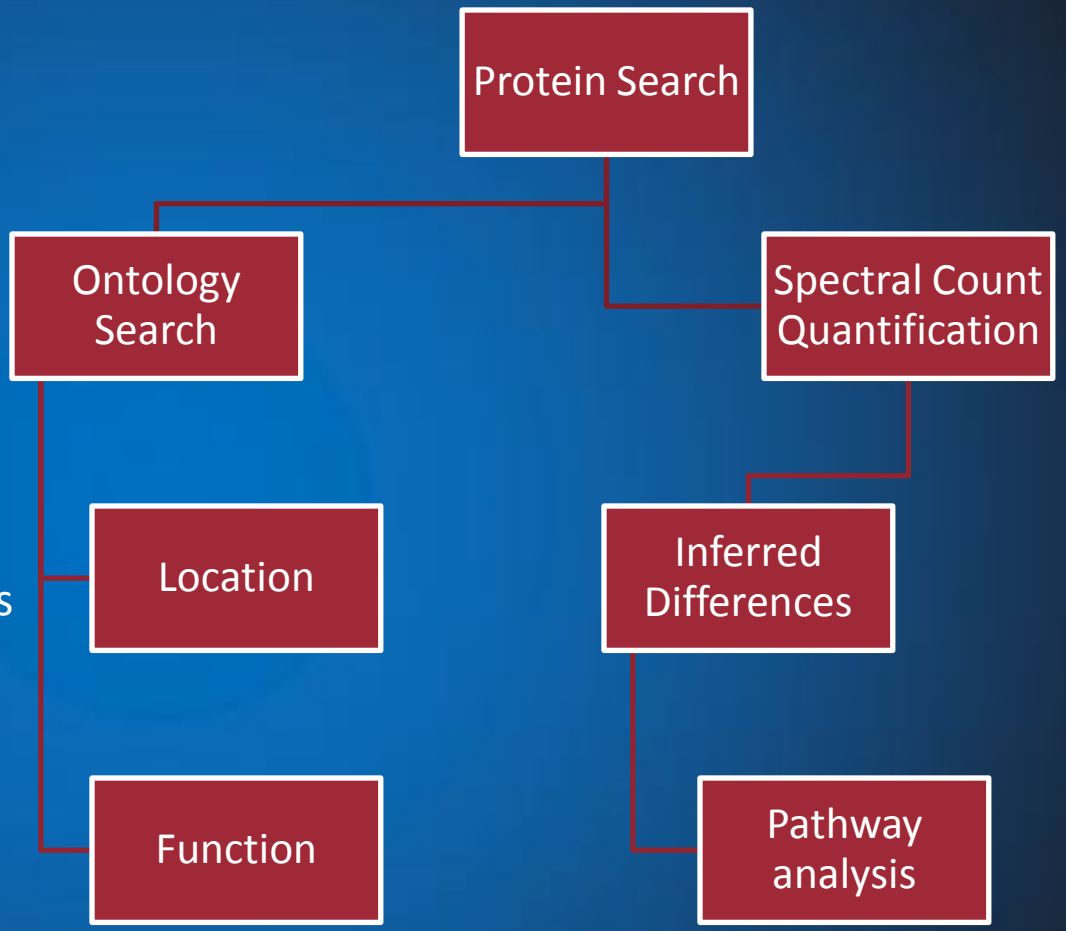
Sequest Database Search

147,157 spectra identified as peptides

	Scan(s)	Reference	MH+	ΔM	z	Peptide	P (pep)	XC	ΔCn
7	20110519_C4_Patch_	sp|Q71FK5|ACTB_CAVPO Actin, cytoplasmic 1 OS=Cavia porcellus	1198.52	0.09	2	K.DSYVGDEAQSK.R	2.2e-007	3.087	0.461
21	20110519_C4_Patch_	sp|P00346|MDHM_PIG Malate dehydrogenase, mitochondrial OS=Sus	1073.58	0.11	2	R.IQEAGTEVVK.A	8.6e-004	2.326	0.370
28	20110519_C4_Patch_	sp|Q767M3|SYVM_PIG Valyl-tRNA synthetase, mitochondrial OS=Su	1625.79	0.15	2	R.YVEAAWYPWWVR.E	4.0e-004	1.404	0.051
33	20110519_C4_Patch_	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1	1471.67	0.12	2	K.YIC@ENQDTISTK.L	5.1e-007	3.590	0.630
34	20110519_C4_Patch_	sp|P00761|TRYP_PIG Trypsin OS=Sus scrofa PE=1 SV=1	1045.56	0.05	2	K.LSSPATLNSR.V	1.2e-005	1.947	0.528
37	20110519_C4_Patch_	sp|P19620|ANXA2_PIG Annexin A2 OS=Sus scrofa GN=ANXA2 PE=	1222.59	0.00	2	K.TPAQYDASELK.A	2.7e-006	3.719	0.629
43	20110519_C4_Patch_	sp|P02543|REVERSED - VIME_PIG Vimentin (Fragments) OS=Sus scr	1146.56	0.01	2	K.MNQIEDQLR.G	3.4e-004	1.823	0.136
45	20110519_C4_Patch_	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1	1414.65	0.02	2	K.YICENQDTISTK.L	2.8e-009	3.210	0.685
47	20110519_C4_Patch_	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1	910.45	0.10	2	K.ADFTEISK.I	5.3e-006	2.705	0.497
56	20110519_C4_Patch_	sp|P67937|TPM4_PIG Tropomyosin alpha-4 chain OS=Sus scrofa GN	1614.77	-0.06	2	K.IDALQDQADEAERA...	1.8e-009	3.759	0.589
or	sp|Q5XLD3|KCRM_PIG Creatine kinase M-type OS=Sus scrofa GN=CKM	1083.54	0.03	2	K.TDLNHENLK.G			8.1
or	sp|P01965|HBA_PIG Hemoglobin subunit alpha OS=Sus scrofa GN=HBA	1422.71	0.03	2	K.VGGQAGAHGAEALER.M			1.1
or	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1 SV	1471.67	0.16	2	K.YIC@ENQDTISTK.L			3.6
or	sp|P02189|MYG_PIG Myoglobin OS=Sus scrofa GN=MB PE=1 SV=2	1488.65	0.06	2	K.HPGDFGADAQGAMSK.A			3.1
or	sp|Q5XLD3|KCRM_PIG Creatine kinase M-type OS=Sus scrofa GN=CKM	1180.54	0.14	2	K.AEEEYPDLSK.H			1.9
or	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1 SV	910.45	0.06	2	K.ADFTEISK.I			5.4
110	20110519_C4_Patch_	sp|P02067|HBB_PIG Hemoglobin subunit beta OS=Sus scrofa GN=H	1314.66	0.11	2	K.VNVDEVGGEALGR.L	6.9e-008	3.320	0.503
121	20110519_C4_Patch_	sp|Q767L7|TBB5_PIG Tubulin beta chain OS=Sus scrofa GN=TUBB P	1446.69	0.10	2	K.EVDEQMLNVQNK.N	1.2e-004	3.458	0.425
127	20110519_C4_Patch_	sp|P19619|ANXA1_PIG Annexin A1 OS=Sus scrofa GN=ANXA1 PE=	1064.49	0.18	2	R.SEIDMNDIK.A	2.6e-007	2.429	0.499
130	20110519_C4_Patch_	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1	910.45	-0.02	2	K.ADFTEISK.I	2.0e-005	2.445	0.446
137	20110519_C4_Patch_	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1	910.45	-0.02	2	K.ADFTEISK.I	1.0e-004	1.994	0.497
139	20110519_C4_Patch_	sp|P02189|MYG_PIG Myoglobin OS=Sus scrofa GN=MB PE=1 SV=2	1592.84	0.11	3	K.VEADVAGHGQEVLIR.L	2.4e-011	3.529	0.538
141	20110519_C4_Patch_	sp|P02189|MYG_PIG Myoglobin OS=Sus scrofa GN=MB PE=1 SV=2	1592.84	0.09	2	K.VEADVAGHGQEVLIR.L	1.6e-009	3.837	0.536
142	20110519_C4_Patch_	sp|Q19AZ8|THRB_PIG Prothrombin OS=Sus scrofa GN=F2 PE=2 SV	898.54	-0.86	2	R.VTAELIPR.S	7.4e-005	2.579	0.336
144	20110519_C4_Patch_	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1	1072.57	0.09	2	K.FQPLVDEPK.N	4.4e-004	2.289	0.319
148	20110519_C4_Patch_	sp|P16293|FA9_PIG Coagulation factor IX (Fragment) OS=Sus scrof	2166.02	0.11	2	K.ITVVAGEYNTEETEPTEQ	3.1e-013	4.837	0.716
150	20110519_C4_Patch_	sp|P02067|HBB_PIG Hemoglobin subunit beta OS=Sus scrofa GN=H	1314.66	-0.93	2	K.VNVDEVGGEALGR.L	1.3e-004	2.741	0.138
151	20110519_C4_Patch_	sp|P16293|FA9_PIG Coagulation factor IX (Fragment) OS=Sus scrof	2166.02	0.03	2	K.ITVVAGEYNTEETEPTEQ	9.6e-004	2.369	0.563
155	20110519_C4_Patch_	sp|P26234|VINC_PIG Vinculin OS=Sus scrofa GN=VCL PE=1 SV=4	1314.74	0.18	2	K.QVATALQNLQTK.T	1.1e-006	2.998	0.269
158	20110519_C4_Patch_	sp|P08835|ALBU_PIG Serum albumin OS=Sus scrofa GN=ALB PE=1	1072.57	0.06	2	K.FQPLVDEPK.N	2.0e-007	1.664	0.433

Data Analysis Workflow

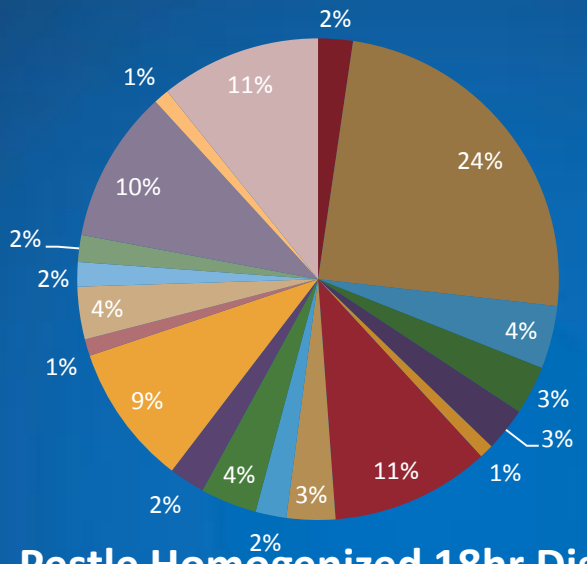
1. Find proteins of interest
2. Compare different sample tissue
3. Find up/down-regulated proteins
4. Determine important pathways



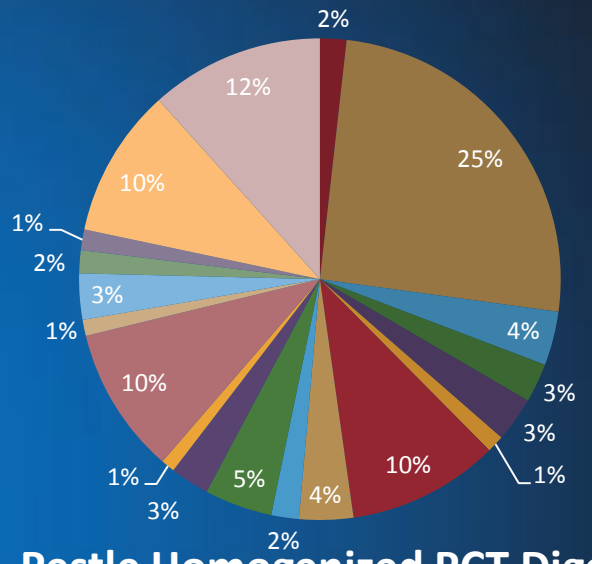
Gene Ontology: Protein Location

- unannotated
- cytoplasm
- cytoskeleton
- cytosol
- endoplasmic reticulum
- endoplasmic reticulum membrane
- extracellular region
- extracellular space
- Golgi apparatus
- integral to membrane
- intracellular
- membrane
- microsome
- mitochondrial inner membrane
- mitochondrial matrix
- mitochondrial outer membrane
- mitochondrion
- myosin complex
- nucleus

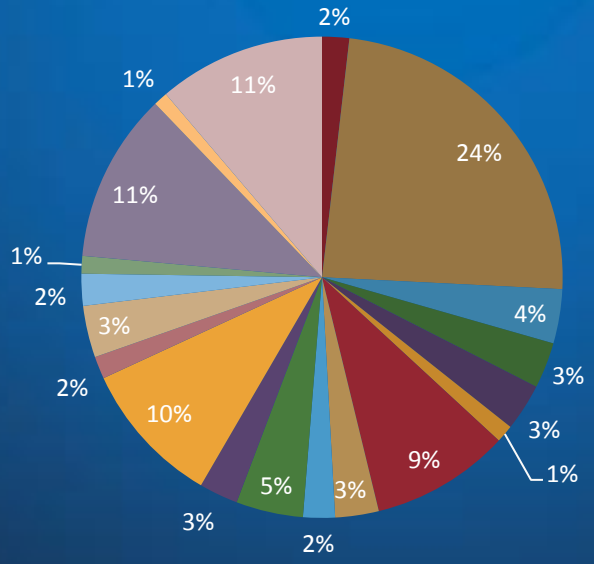
PCT Homogenized 18hr Digest



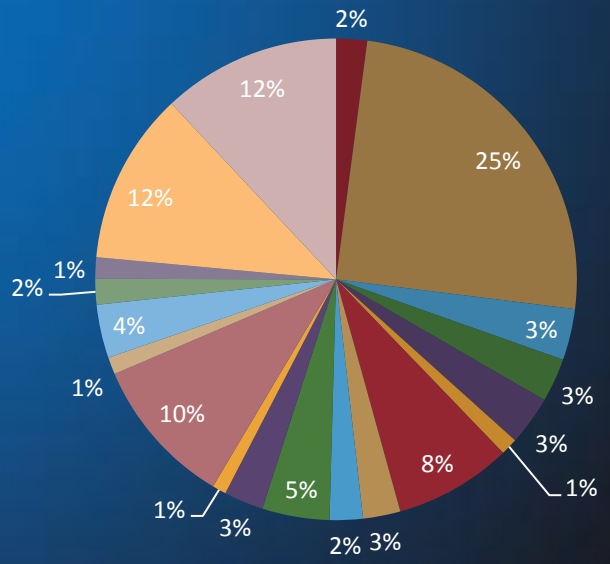
PCT Homogenized PCT Digest



Pestle Homogenized 18hr Digest



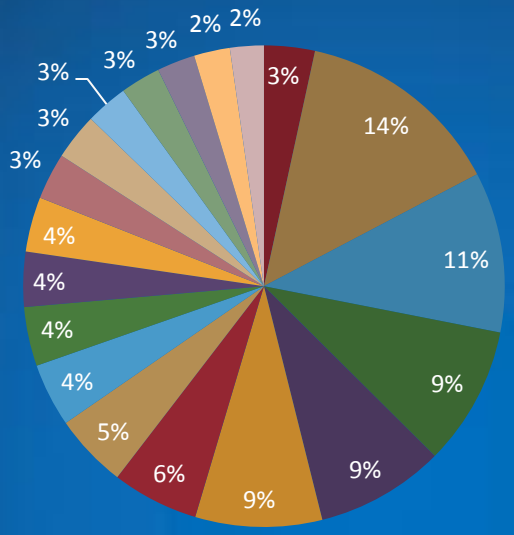
Pestle Homogenized PCT Digest



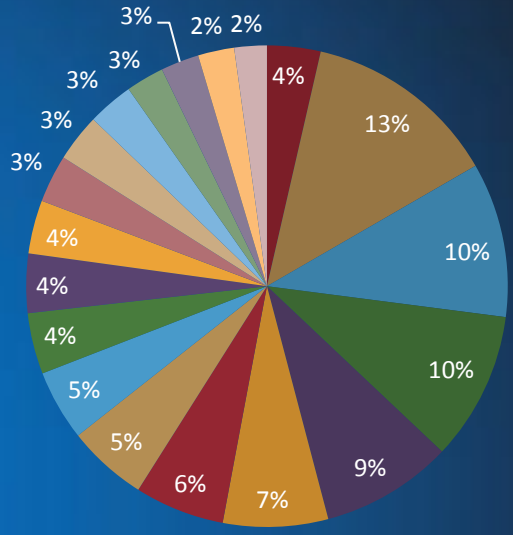
Gene Ontology: Function

- unannotated
- ATP binding
- nucleotide binding
- oxidoreductase activity
- metal ion binding
- hydrolase activity
- catalytic activity
- transferase activity
- actin binding
- GTP binding
- binding
- calcium ion binding
- kinase activity
- peptidase activity
- protein serine/threonine kinase activity
- heme binding

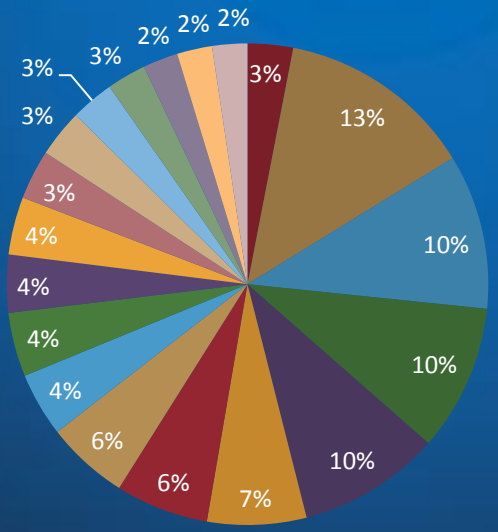
PCT Homogenized 18hr Digest



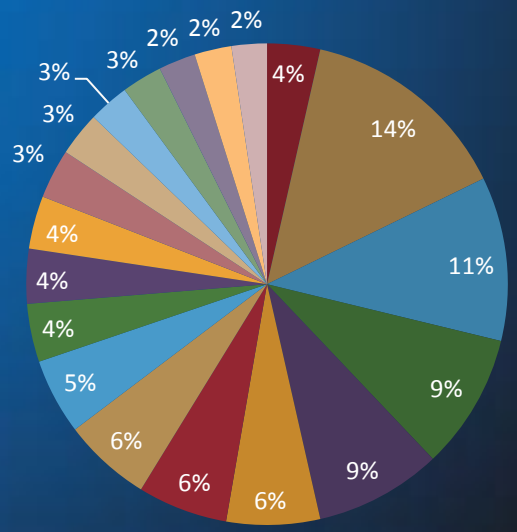
PCT Homogenized PCT Digest



Pestle Homogenized 18hr Digest



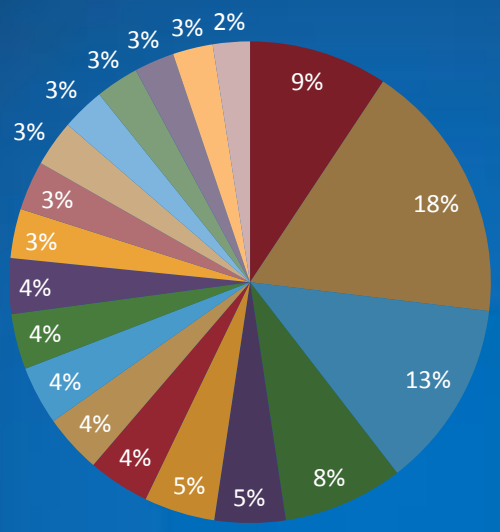
Pestle Homogenized PCT Digest



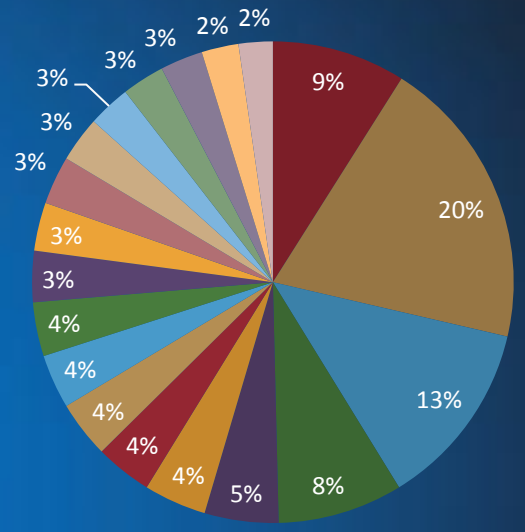
Gene Ontology: Process

- Unannotated
- oxidation-reduction process
- transport
- metabolic process
- phosphorylation
- proteolysis
- cell adhesion
- tricarboxylic acid cycle
- glycolysis
- translation
- signal transduction
- electron transport chain
- response to oxidative stress
- protein phosphorylation
- negative regulation of apoptosis
- ion transport
- carbohydrate metabolic process
- lipid metabolic process
- oxygen transport

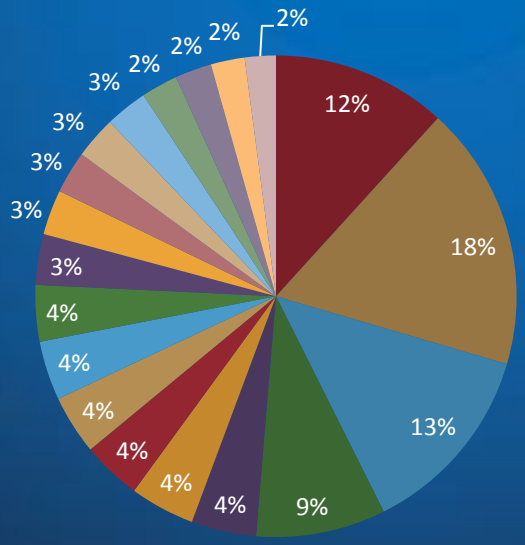
PCT Homogenized 18hr Digest



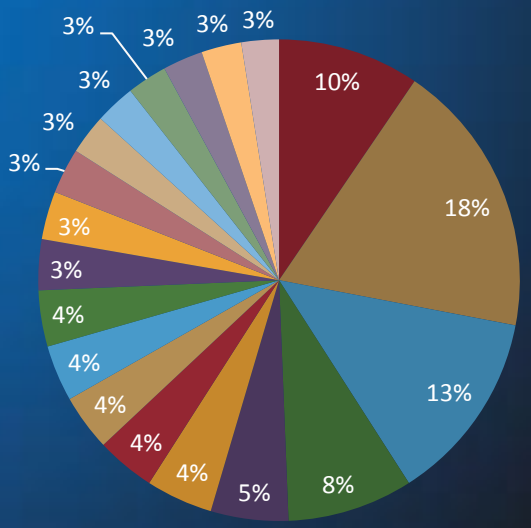
PCT Homogenized PCT Digest



Pestle Homogenized 18hr Digest



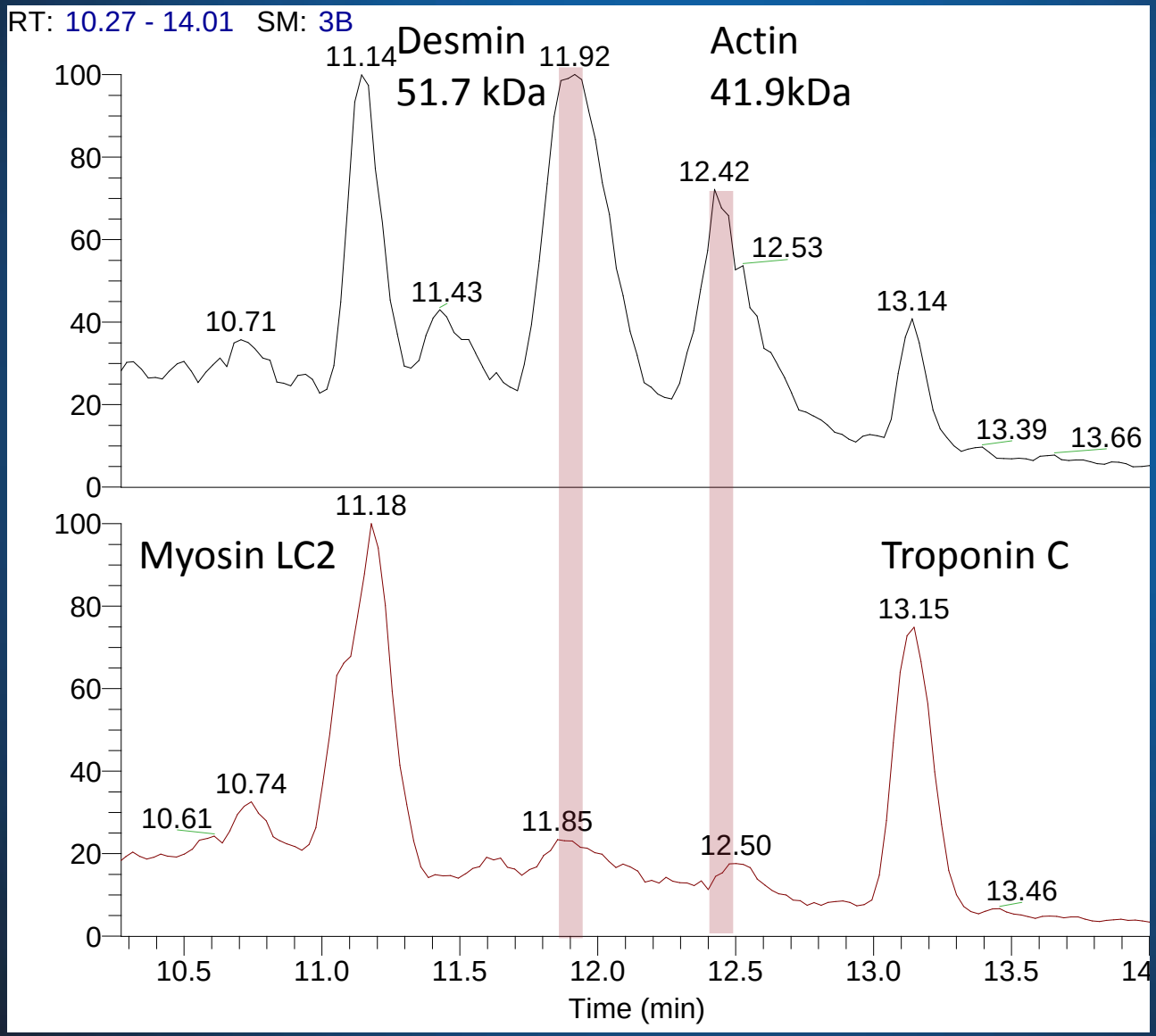
Pestle Homogenized PCT Digest



1mg Tissue: Secondary Extraction

Acid extracted myofilament proteins

Desmin and Actin abundance is reduced in PCT Homogenized samples



Pestle Homogenate

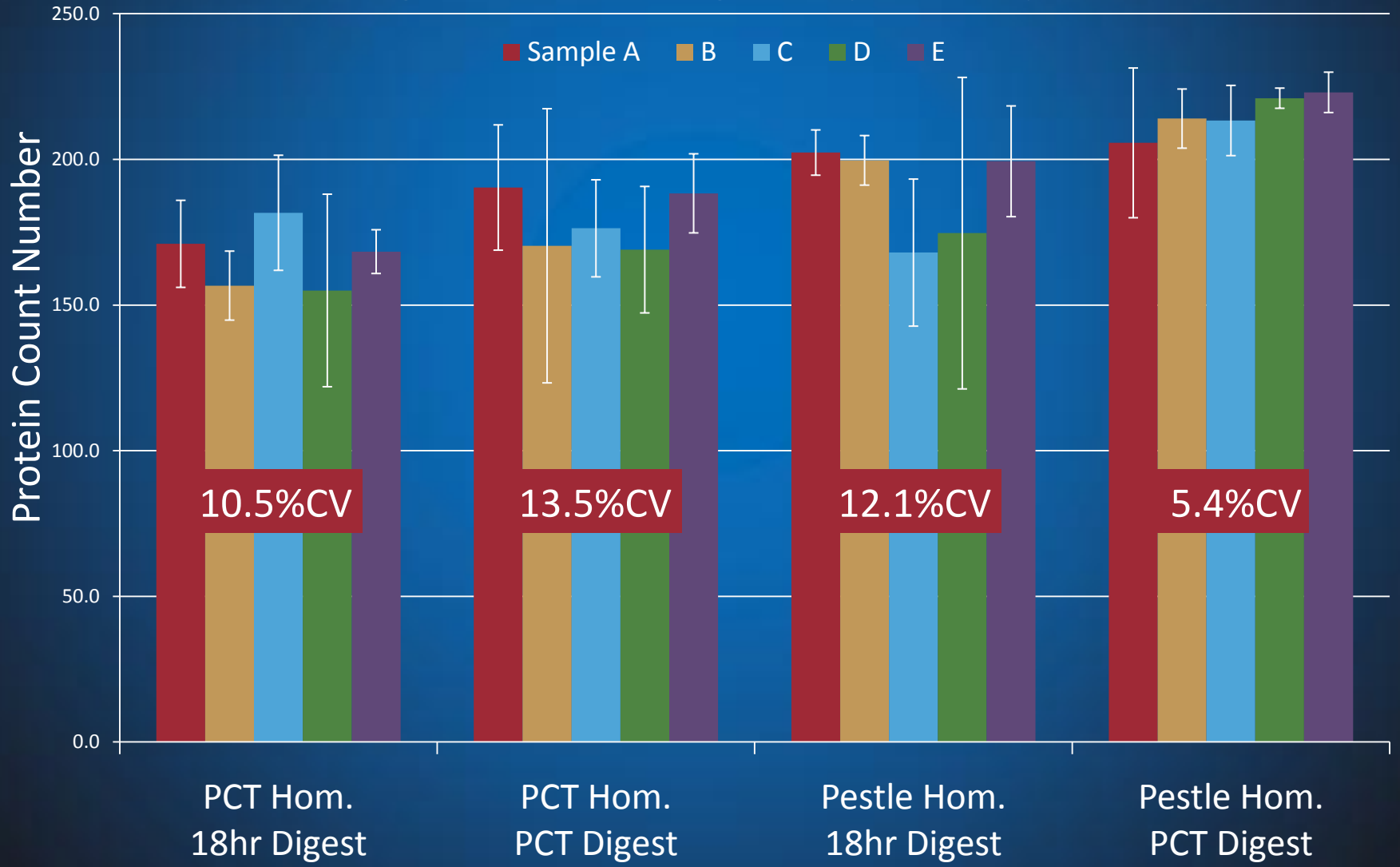
PCT Homogenate

Quantifying Proteins

Spectral Counting is used to quantify proteins

1 spectral count = 1 MS/MS spectrum related to a specific protein

Pestle Homogenate and PCT Digest may be an improvement

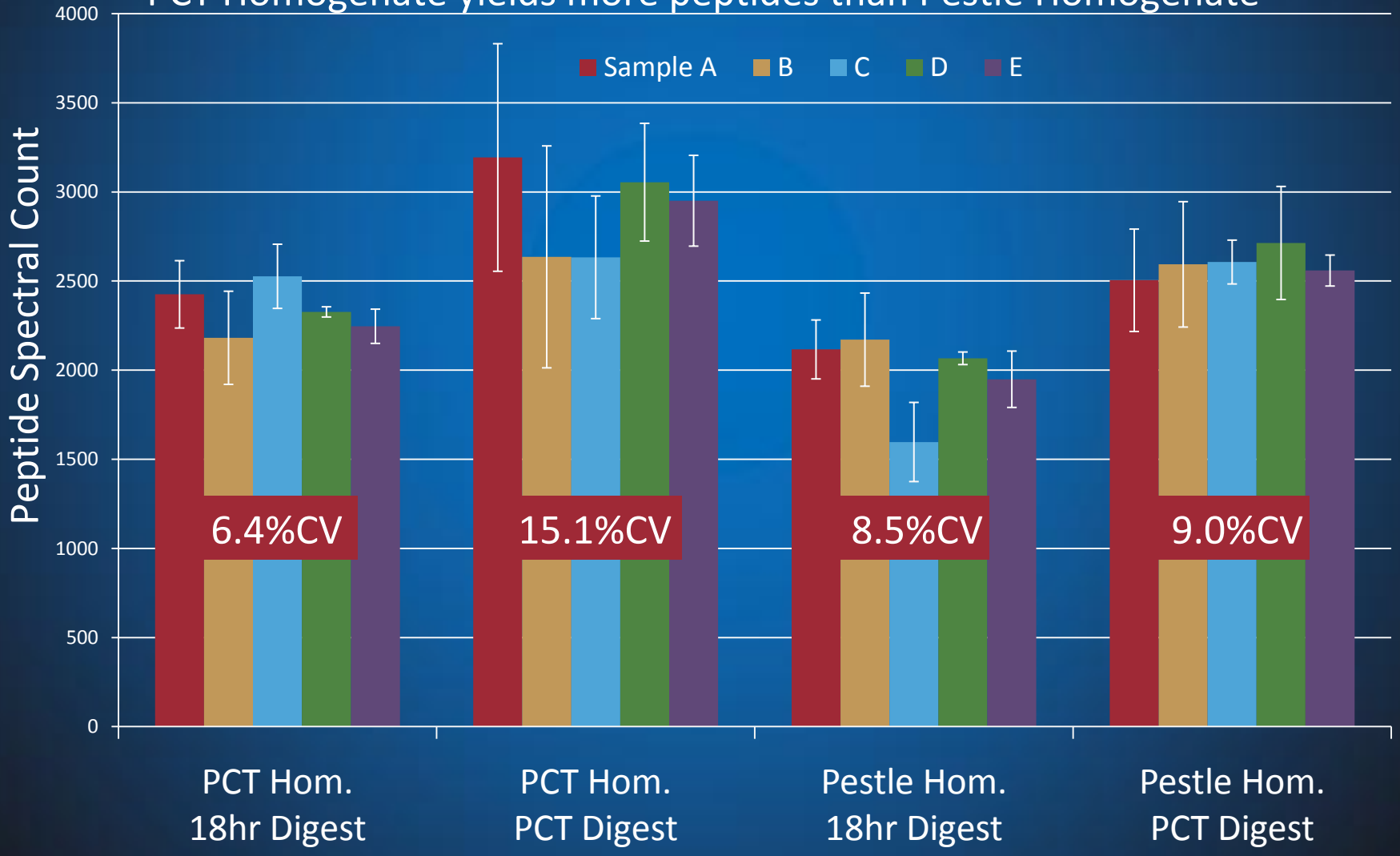


Quantified Peptides

Spectral Counting is used to quantify proteins

1 spectral count = 1 MS/MS spectrum related to a specific protein

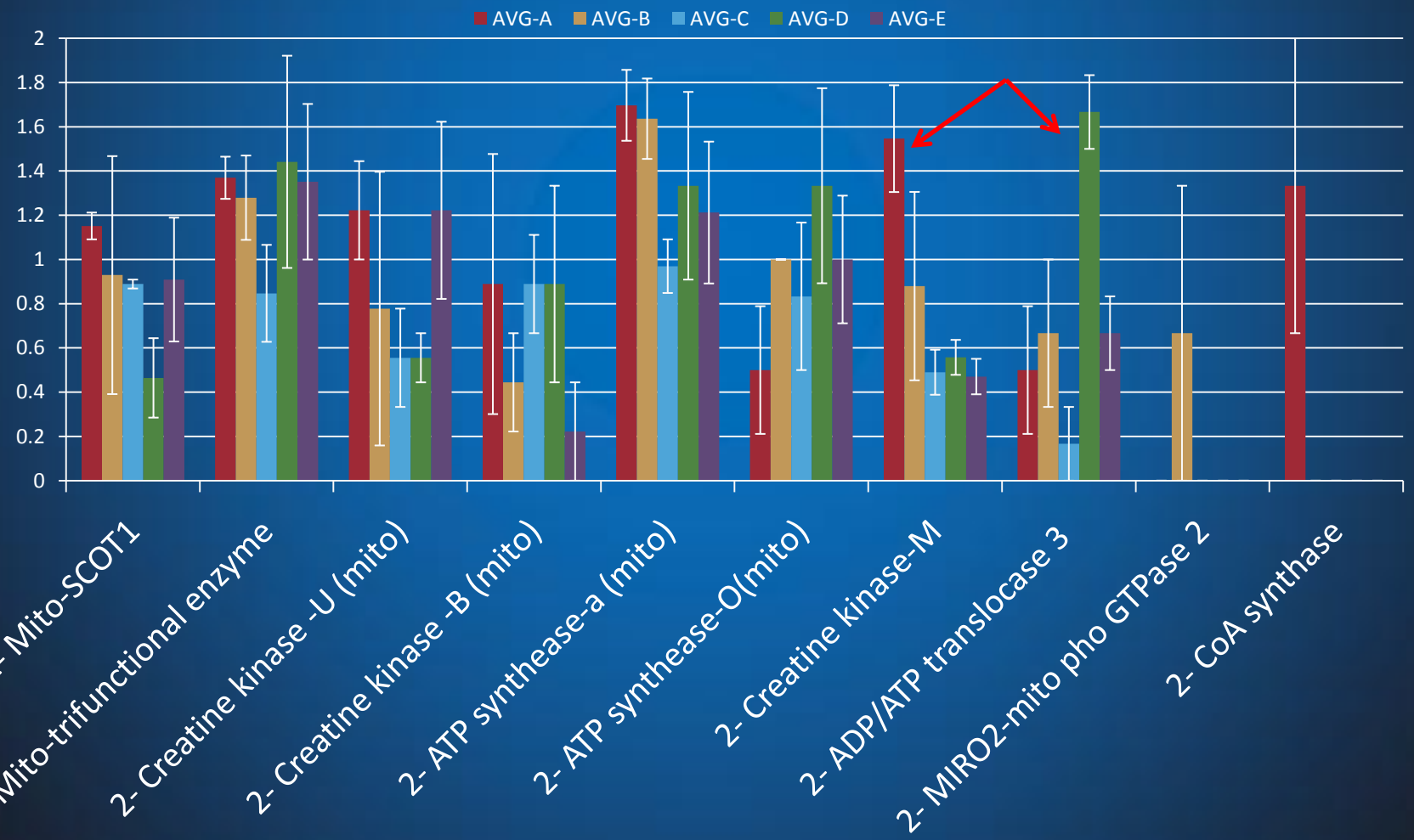
PCT Homogenate yields more peptides than Pestle Homogenate



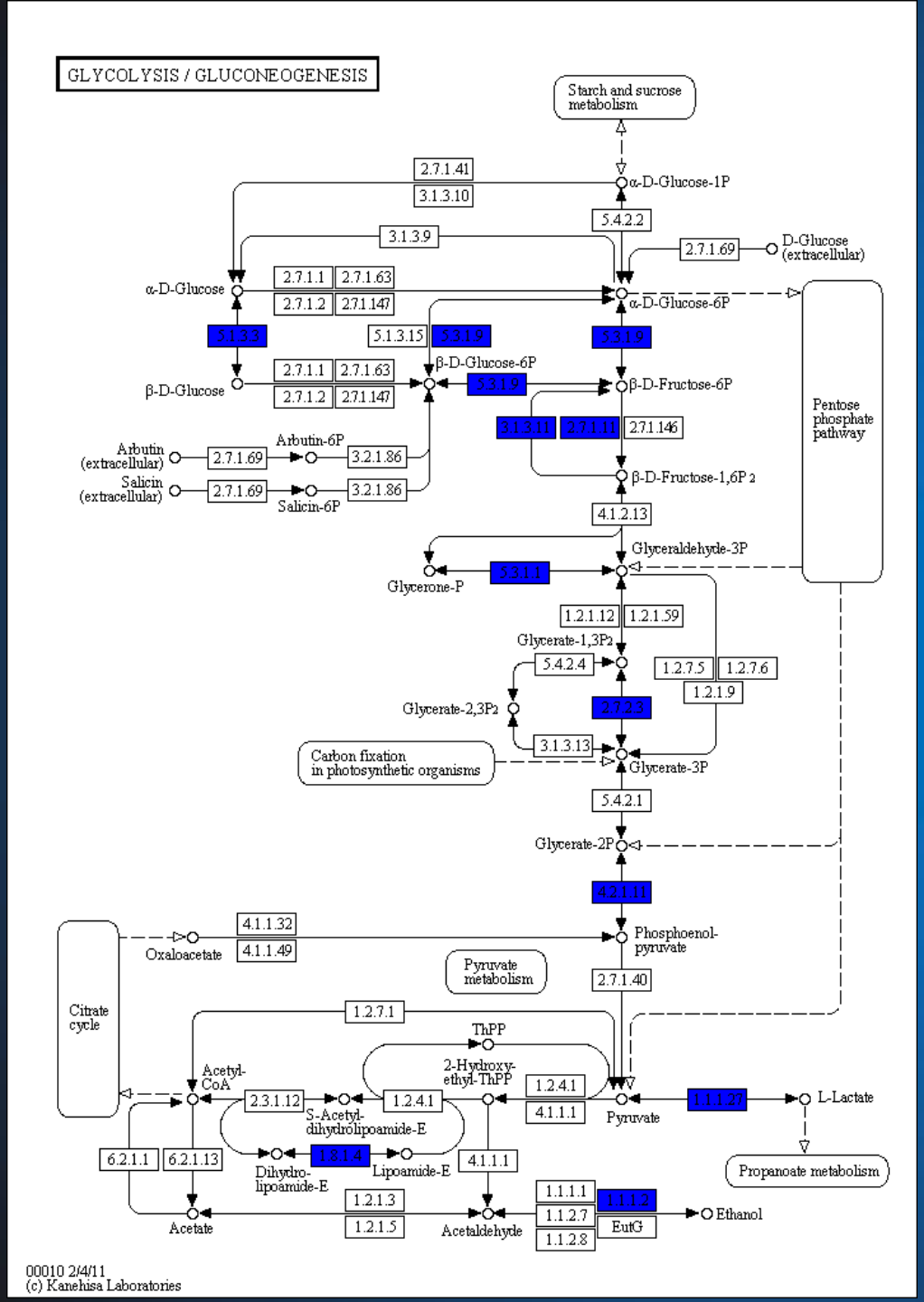
Normalized Protein Comparison

Sample to sample comparison of proteins of interest

Proteins may be up or down-regulated if results are consistent and error is low



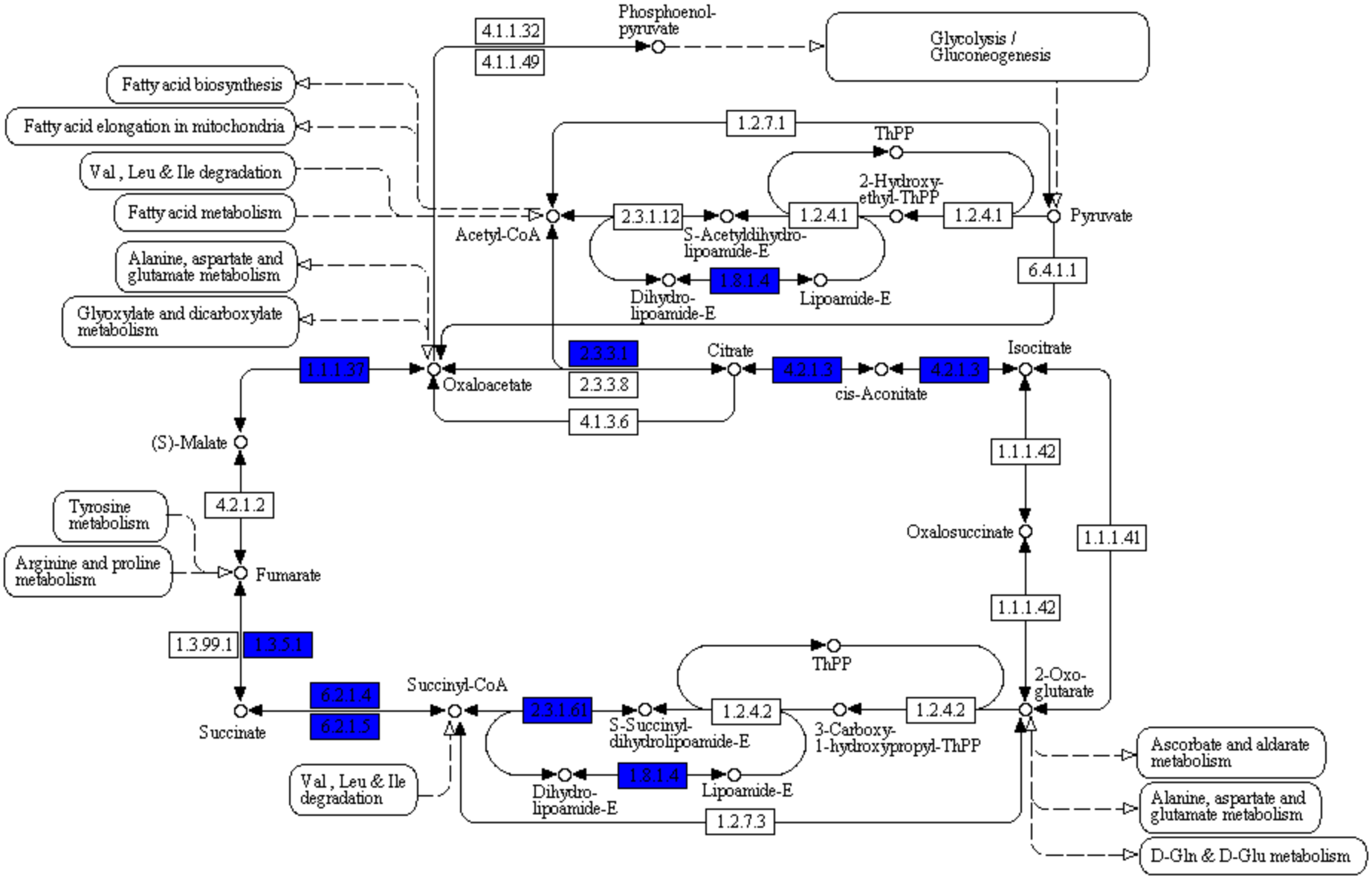
Protein Function Glycolysis Pathway Analysis



TPI1; triosephosphate isomerase 1
PGK2; phosphoglycerate kinase 2
AKR1A1; aldo-keto reductase family 1, member A1 (aldehyde reductase)
FBP1; fructose-1,6-bisphosphatase 1
DLD; dihydrolipoamide dehydrogenase
GPI; glucose-6-phosphate isomerase
GALM; galactose mutarotase (aldose 1-epimerase)
LDHB; lactate dehydrogenase B
PGK1; phosphoglycerate kinase 1
ENO3; enolase 3 (beta, muscle)
PFKM; phosphofructokinase, muscle

http://www.genome.jp/kegg/pathway.html

Protein Function
TCA Pathway Analysis



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(c) Kanehisa Laboratories

Results

- Faster sample preparation is achieved with the Barocycler
  - Efficient sample homogenizations above 10 samples
  - 30 minute digestion is fast enough for same-day LCMS injection

- Protein and peptide yield results are equivalent to traditional methods

- Pathways can be monitored for up/down regulated proteins based on disease state or therapy effect

Future Work

- Continue sample preparation development to reduce variables like analyzed tissue amount, extraction efficiency
- Complete sample analysis (60 tissues) will yield consistent data for protein comparison
- Develop software tools that automate data analysis workflow

Acknowledgements

Pressure Biosciences: Tom Strader and the use of their Barocycler NEP 2320

Human Proteomics Program:
Hard work and long hours put in by Han Zhang, Huseyin Guner and Ying Ge

Thanks for listening!