

UAB CCC Mass Spectrometry & Proteomics (MSP) Shared Facility

Director: James A. Mobley, Ph.D., Web-site: <http://www.uab.edu/proteomics/bmsf>



1. Organization & Goal

The UAB CCC Mass Spectrometry/Proteomics Shared Facility (MSP-SF) offers access to capabilities that are not available in individual laboratories.

- The facility is organized into five modules
- (1) Administrative & Laboratory Management
 - (2) Experimental Design, Sample Preparation & Separations
 - (3) Discovery & Targeted MS/ Proteomics Analysis
 - (4) Statistics, Bioinformatics, & Reporting
 - (5) Manuscripts, Grants, & Education.

Each module is supported by highly trained scientists who are experts in mass spectrometry, bioanalytical chemistry, statistics, systems biology, and information handling.

The goal of the MSP Shared Facility is to provide state-of-the-art capabilities in mass spectrometry, proteomics, and bioanalytic technologies to support the research needs of UAB internal and external researchers.

2. The Shared Facility

Research Team:

- James Mobley, Ph.D. (Director)
- Brandon Young, B.S. (Lab Manager)
- Kyoko Kojima, Ph.D. (Applications Scientist)
- Gregory Bowersock, B.S. (IT Specialist)

Instrumentation:

- Thermo Fisher Orbitrap Velos



- Agilent 6530 QTOF MS
- Agilent 7700 ICP-MS
- Bruker Ultraflex III MALDI ToF ToF
- Thermo Finnigan LTQ XL nano ESI/CID/ ETD
- Thermo Finnigan LTQ XL HESI/CID
- GE Ettan™ Spot Picker

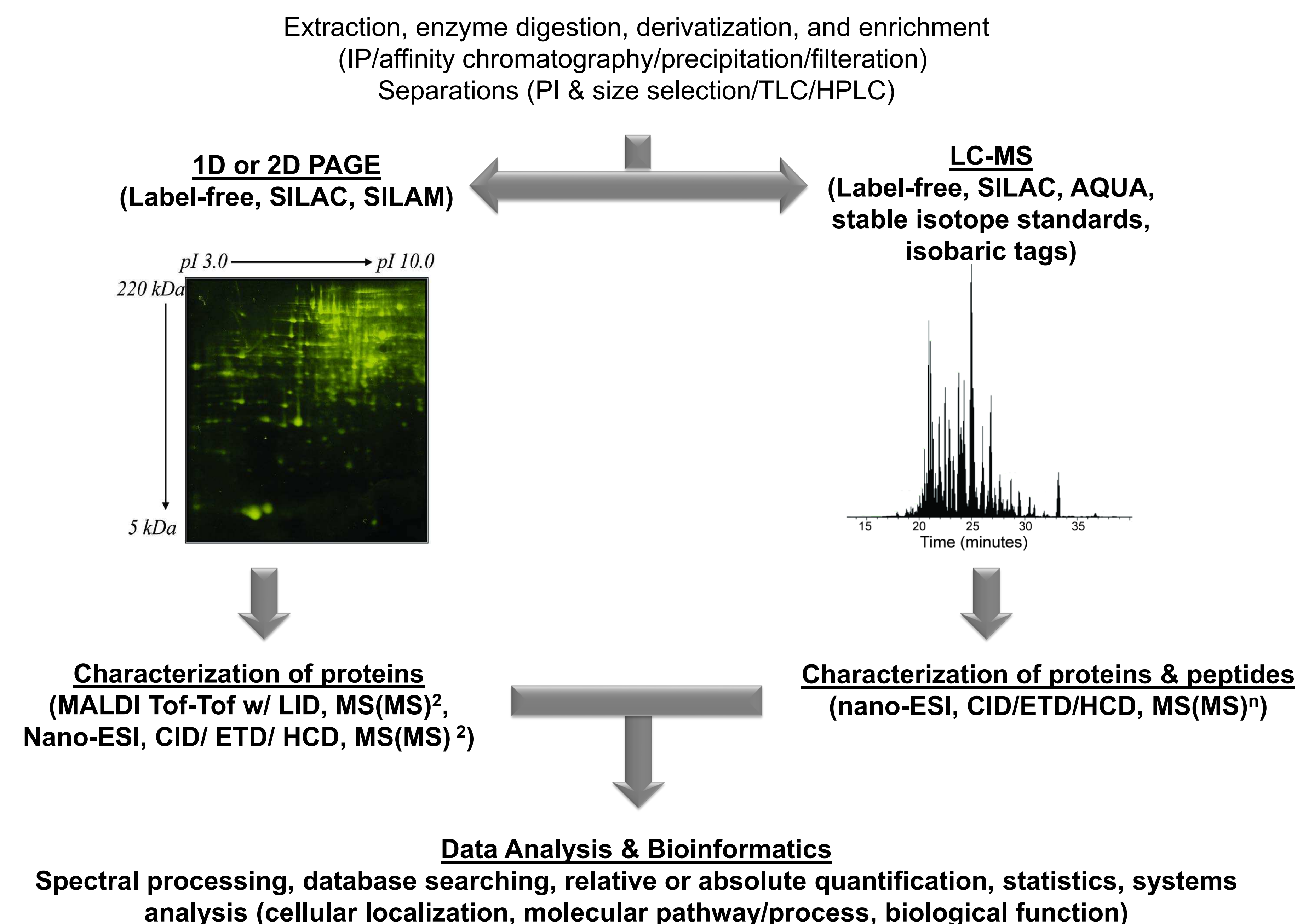
Computing (The primary fileserver is dynamically linked to a 1000 node Cheaha Cluster through the UAB IT Research Computing Group):

- Primary fileserver (30TB, 32GB RAM, 2-AMD 3.1GHz 8 Core processors)
- Unix application server powered by an Oracle Database (64GB RAM, 2-AMD 2.6GHz 12 Core processors)
- Primary domain controller for our network (2GB RAM, 1-Intel 2.0GHz Dual Core processor)
- Secondary fileserver (4GB RAM, 2-Intel 2.0GHz Dual Core Processors)
- Application server (32GB RAM, 2-AMD 2.2GHz 12 Core processors)
- Tape backup server (4GB RAM, 2-Intel 2.0GHz Dual Core Processors)
- Genedata application server (8GB RAM, 2-Intel 1.6GHz Quad Core processors)
- Virtual server/ backup domain controller and an LTO-3 Tape Library with 14 slots

Software/ Licenses:

- SCAFFOLD & MASCOT (Search Engines)
- Nonlinear SameSpots (2D-PAGE analysis)
- Genedata Expressionist, Refiner MS & Analyst (Peptidomics Discovery)
- Premier Biosoft ProteoIQ (All-In-One Proteomics Analysis Tool)
- ProteomeSoftware Scaffold PTM Q*/S (All-In-One Proteomics Analysis Tool)
- Mascot Distiller Q* (Quantitative & De Novo Sequencing Proteomics Analysis Tool)
- Thomson Reuters Metacore (Pathway Analysis)
- Qiagen Ingenuity IPA

3. Sample Preparation & MS Analysis Options



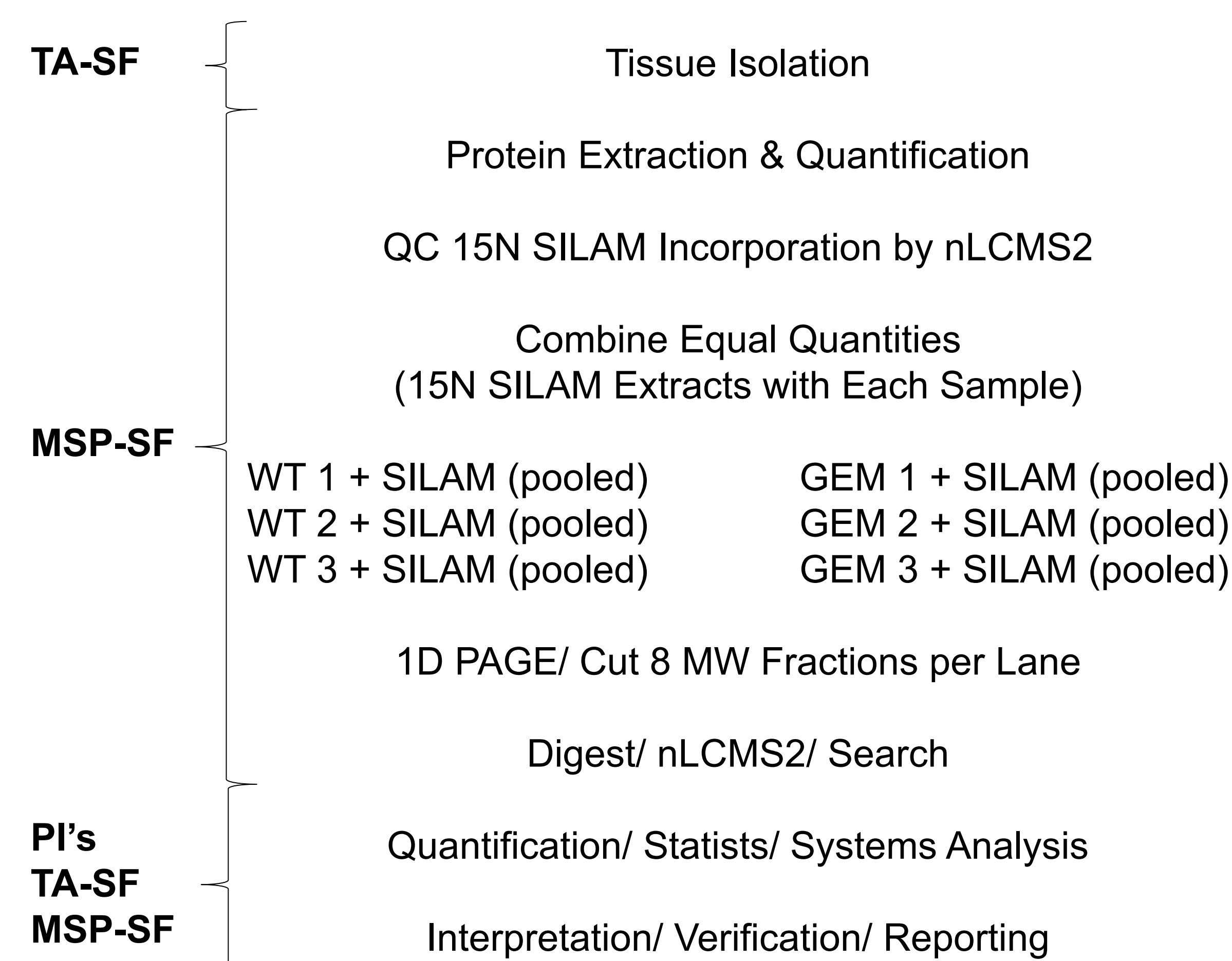
4. New Applications/ Services Recently Added

Latest Research Addition (Stable Isotope Labeled Amino Acids in Mice [SILAM] is now provided to the Cancer Center members through a joint collaboration between the MSP-SF and the the Transgenic Animal Shared Facility (TA-SF):

We have joined the Transgenic Animal Shared Facility TA-SF to offer a Stable Isotope Labeled Amino Acids in Mice [SILAM] workflow combined with SILAM biomaterials as controls to all CCC members in addition to the UAB research community. Bio-specimens first obtained from the C57BL/6J (control) animals will be utilized as global internal standards for MS driven -omics studies.

This is a highly unique opportunity that allows investigators to gain access to otherwise cost inhibitory uniformly 15N-labeled proteins, glycans, and metabolites from tissues and biological fluids. These bio-specimens are now accessible for all CCC members. For the workflow example below we extracted proteins from specific tissue of 15N-enriched heavy labeled animals (n=3), pooled them and combined equal quantities of protein with similarly prepared extracts from individual GEM mice (n=3) vs. WT mice (n=3) in order to globally assess changes between the two groups.

GEM Model/ WT Animals; compared via a universal standard (15N-SILAM)



5. Pricing Information

Service Description	Unit Price (UAB)	Unit Price (External)	Unit
Consultation	\$100	\$133	Hour
Measuring Protein Concentration	\$15	\$20	Each - Sample
Cell/Tissue Homogenization (Frozen)	\$20	\$27	Each - Sample
Cell/Tissue Homogenization (FFPE)	\$80	\$106	Each - Sample
1D Gel Separation	\$15	\$20	Each - Sample
Enzymatic Digestion	\$25	\$33	Each - Sample
Solid Phase Extraction (zip-tip)	\$25	\$33	Each - Sample
Solid Phase Extraction (cap-trap)	\$80	\$106	Each - Sample
Gel Staining (includes imaging gel)	\$30	\$40	Each - Sample
Centrifugal Filtration	\$30	\$40	Each - Sample
Advanced Sample Preparation	\$50	\$67	Hour
MALDI-TOF-MS	\$15	\$20	Each - Sample
MALDI-TOF/TOF-MS/MS	\$50	\$67	Each - Sample
Low/ Medium Resolution nLC-ESI-MS/MS (non-complex sample and qualitative analysis)	\$50	\$67	Each - Sample
High Resolution nLC-ESI-MS/MS (complex sample and/or quantitative analysis)	\$98	\$130	Each - Sample
Bioinformatics (basic)	\$50	\$67	Hour
Bioinformatics (advanced)	\$100	\$133	Hour

Additional details for services and billing:

Advanced Sample Preparation Includes:

- 1) Immuno-enrichment, Immuno-depletion
- 2) Post-translational modification (PTM) enrichments such as glycosylation, carboxylation, phosphorylation, acetylation, etc.
- 3) Cryo-sectioning and histology of frozen tissues
- 4) Chemical labeling/derivatization for quantitative proteomics (ex: TMT 6-plex)
- 5) Preparative or analytical HPLC separations (offline)
- 6) 2D PAGE (small or large format, native or denatured), including automatic spot picking
- 7) Methods development (new and unique applications)

LC-MS/MS:

- 1) High resolution refers to the nanoLC (nLC) LTQ Velos Pro Orbitrap MS (~60-100K resolution)
- 2) Low/ Medium resolution refers to the LC-LTQ-XL MS systems run in triple play mode (~20K resolution), and the QTOF 6530 MS (~30K resolution)

Bioinformatics:

- 1) Basic applications include raw data conversion to MzXML, databases searches, any analysis through the TPP suite, basic PTM analysis with Scaffold with no manual interpretation, and basic quantification using spectral counting.
- 2) Advanced applications include quantification using MASCOT Distiller Q+, Scaffold Q+/S, Refiner MS, or SameSpots, statistical analysis (single or multivariate), Systems Biology/ Pathway Analysis, generating reports including figures and tables, programming time, generation of tailored databases, PTM analysis using Scaffold PTM and Manual Interpretation.

6. Time-Based Workflow & Cost Example

Global discovery: 6 cell pellets (3-untreated controls vs. 3 treated)

Week 1 (sample preparation - \$570)

- Consultation with the Director (1 X \$100/hr, first hour free = \$0)
- Samples are accepted, and service request form is uploaded to LIMS
- Cell lysis (6 X \$20each = \$120)
- Protein quantification (6 X \$15each = \$90)
- 1D PAGE (6X \$15each = \$90)
- Gel staining and imaging (1 X \$30each = \$30)
- Enzymatic digestion (in case of 1 fraction per sample: 6 X \$25each = \$150)

Week 2 (MS analysis, generating final protein list - \$688)

- LC-MS analysis (6 X \$98each = \$588)
- Data extraction and searching (1 X \$50/ hr. = \$50)
- Post LC-MS analysis, peptide grouping/ quantification (1 X \$50/hr. = \$50)

Week 3 (optional; stats & pathway analysis - \$400)

- Statistical analysis, systems biology/ pathway analysis, reporting (estimated 4hr's; 4 X \$100/ hr. = \$400)

Complete data analysis is uploaded to the LIMS within 14-21 days following sample submission, with a total charge of \$1,568.