The Proteomics Core Laboratory (PCL) at the Washington University School of Medicine

This facility consists of a 3500 square foot customized proteomics laboratory on the 7th floor of the Southwest Tower at the WU School of Medicine. The laboratory has been designed to optimize throughput according to proteomics workflows from sample preparation through to mass spectrometric and data analysis. With the recent NIH funding of the Proteomics Laboratory as an NCI-designated Proteome Characterization Center (U24 Grant), the laboratory has acquired two next generation mass spectrometers for analysis of tumor tissue and biological fluids. The new instrumentation is available to all ICTS members for global and targeted proteomics analysis in the Translational Science and Clinical Proteomics. With institutional and industry support the laboratory has added also added new instrumentation for deploying micro-scale preparation of samples for proteomics and phosphoproteomics as detailed below. The laboratory is fully equipped with small instrumentation for sample preparation (high-capacity lyophilize, multichannel pipettors, micro- and standing centrifuges, low temperature freezers with an inventory management system)

The Proteomics Core laboratory instruments for automated, microscale preparation of biological fluids and tissue are given below.

Covaris cryoPREP™ Extraction Systems. The PCL is equipped with micro-cryopulverization and tissue homogenization devices for the optimal extraction of proteins from small samples tissue pieces (1x1 mm), and maintainance of the tissue in a frozen state during all manipulations. The t-PREP™ System has been designed as a collaboration between the PCL and Covaris for the purpose of cryopulverization and solubilization of biopsy-size tissue samples using isothermic AFA ultrasonication (Model S220).

Barocycler (NEP3229). The Barocycler is used for high-pressure (35,000, PSI) endoprotease digestion of tumor lysates. Two sample modules are available, a three sample-capacity holder for method development and a 96 well configuration simultaneously using specially designed, single-use containers called PULSE Tubes. The Barocycler NEP3229 has an external chiller hook-up, automatic fill and dispense valves, and an integrated micro-processor with an easy-to-use keypad.

Robotics. The laboratory is equipped with three robotic liquid-handling devices for the solid-phase extraction of peptides, the enrichment of proteins using immobilized tips and the processing of multi-well plates for ELISA assays. The Beckman Biomek NX® Laboratory Automation Workstation is used for automated solid-phase extraction of peptides from endoprotease digests using multiple stationary phases to maximize proteomic coverage. The NXP workstation has multiple configurations, including multichannel (96 and 384), Span-8 and Span-8 with an optional gripper. The multichannel model is available with 96- and 384-channel pipetting heads for rapid plate-to-plate applications and assay miniaturization with high precision in the submicroliter range. The Agilent Bravo Automated Liquid Handling Platform (SRT model) is used primarily for the preparation of samples for high-sensitivity ELISA assays using the ERENNA platform (described below). The Thermo Scientific® Versette is a compact automated liquid handler that is used for throughput mmunoaffinity sample preparation with Mass Spectrometric Immunoassay (MSIA*) pipette tips. It also supports 96/384 plate replication, plate stamping and serial dilution procedures.

The Proteomics Core laboratory instruments for advanced global and targeted proteomics analysis are listed below. All instruments are maintained under service contract and are interfaced to automated nano-liquid chromatographs for 24/7 operation.

AB Sciex TripleTOF 5600 Plus. The mass spectrometer is a hybrid quadrupole-time-of-flight mass spectrometer that is designed for rapid tandem MS acquisition (100 per second) and for selected reaction monitoring with stable isotope dilution. The instrument is fitted with a Digital Picoview Nanospray source (New Objectives, Woburn, MA) and a 10 µm Silica PicoTip emitter (New Objectives, Woburn, MA). The data are acquired using an ion spray voltage of 2.9 kV, curtain gas of 10 PSI, nebulizer gas of 14 PSI, and an interface heater temperature of 175°C. The MS is operated with a resolution of greater than or equal to 30,000 for survey scan. For data-dependent acquisition survey scans are acquired in 250 ms and 100 product ion scans with a dwell time of 10 milliseconds. For targeted, quantitative SRM analysis the dwell time is increased to 50 ms. The instrument is equipped with a 40 GHz multichannel TDC detector with four-anode/ channel detection.
Thermo Scientific Orbitrap-ELITE with ETD. This instrument was installed ~ 1 year ago and provides significant improvements in performance compare to the Orbitrap XL. The instrument has significantly improved acquisition time for tandem mass spectra (~ 10 fold greater) than the XL while maintaining a high resolving power up to 240,000. The instrument is capable of high energy collision dissociation (HCD) and mass analysis in the Orbitrap for high resolution scans for quantification using iTRAQ-type measurements. Electron transfer dissociation (ETD) capabilities are used for the sequencing of large peptides (aa residues > 20) and labile post-translational modifications such as O-linked phosphorylation and O-linked GlcNAc. The instrument is benchmarked with a standard mixture of ten proteins every 8 h.

Thermo Scientific-TSQ Vantage Ultra. This linear triple-quadrupole mass spectrometer is used for the quantification of peptides and modified peptides using stable isotope dilution mass spectrometry with selected reaction monitoring. The instrument is interfaced to a an Accel MS pump with an IonMax Source equipped with a low flow metal needle (Thermo Scientific). Reversed phase separations of peptides are most often performed using a Hypersil Gold 3 µM C18 column (1 x 150 mm) in an aqueous acetonitrile/formic acid solvent system. Pinpoint software is used to build assays and monitor performance during quantitative SRM experiments.

Applied Biosystems QTrap 4000. This linear triple-quadrupole-ion trap mass spectrometer is used for the quantification of peptides and modified peptides using stable isotope dilution mass spectrometry with selected reaction monitoring. The instrument is interfaced. The interface is an ABI microspray source using the 75 µM capillary system.

Thermo Scientific Orbitrap XL. This instrument is primarily used for global proteomics methods development and the quality assessment of synthetic peptides for the development of quantitative targeted assays. The instrument is configured as a linear ion trap with a Thermo-Fisher Orbitrap mass analyzer. The instrument is benchmarked with a standard mixture of ten proteins every 8 h.

Eksigent High-performance nano-Liquid Chromatographs-All high performance mass spectrometers are interfaced with 2DPlus (Eksigent, Dublin, CA) LC's, Nanoflex modules and AS2 autosamplers. Two backup systems are maintained for complete LC-system switch in the event of failure of the LC-MS system to meet benchmark criteria. The 2D LC system are configured to load samples in tandem to minimize loss in MS acquisition time during column equilibrations. The separation columns are CHiPLC configuration (ChromXP C18 (200 µmX15cm; particle size 3 µm, 120 Å) and the aqueous acetonitrile and formic acid (0.1%) are used for high performance peptide separations (half peak widths of ~ 15 s). The samples were loaded (xx - xx micrograms) in a volume of 10 µL at a flow rate of 800 µL/min. All gradient elution of peptides was performed at a flow rate of 800 nL/min. The laboratory has recently purchased an Eksigent Ekspert nanoLC 425 system that will enable us to inject much smaller sample volumes (100 nL instead of our routine 10 µL) and should also increase robustness and LC performance. on the high performance mass spectrometers. This will further improve our ongoing work to conserve sample from biopsies.

Singulex ERENNA. This single-molecule counting (SMC) platform is used for microparticle based immunoassays to provide higher sensitivity and broader dynamic range over plate-ready immunoassay platforms. With the high-precision Erenna Immunoassay System, even the smallest changes in biomarker levels can be measured, allowing researchers to gain unprecedented insights into complex disease biology, drug efficacy and drug safety. The Erenna Immunoassay System is the most sensitive immunoassay instrument available, utilizing patented digital single-molecule detection for high-precision quantification of low-
abundance biomarkers. The instrument is used to develop and deploy immunoassay to compare orthogonal molecular measurement using MS-based methods to ELISA approaches.

Computing Facilities. The PCC is supported by secure network with 12 MS Windows 2003 Servers behind a Linux firewall, and on a direct fiber-optic connection to Washington University School of Medicine backbone. These computers are dedicated to global and targeted proteomics analysis using the following software: i) processing MS instrument files (Matrix Science Ltd. MASCOT Distiller v 2.4.3.3, AB Sciex Protein Pilot v 4.5); searching protein and six-frame translated nucleotide database with MS data (Matrix Science Ltd. MASCOT server v 2.3.02 2 processor system, Sage-N Sorcerer v 4.0.4, Proteome Software Scaffold v 3.6.4, Proteome Software Scaffold PTM v 2.0.0); MS quantification and spectral analysis (Rosetta Elucidator v 3.3.0.0.220, Progenesis v 4.0.4573.30654, AB Sciex PeakView v 1.2, Bioinformatics Solutions, Inc. Peaks v. 6.0, Thermo Pinpoint v 1.2.0) and statistical and protein network analysis software (Ingenuity Pathway Analysis (5-user license, Partek Genomic Suite v.6.10.1020, PNNL DanteR v0.1.1). The proteomics data are stored on a Fujitsu Eternus 400 File server present storage cap 50Tb upgradable to 100Tb and four RAID arrays with double parity support. Incremental backups are made each day and tape backups monthly. The computers are housed in a halogen based fire extinguisher facility.