



Translational Proteomics Center

Facility Guidelines

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Brief description of the facility

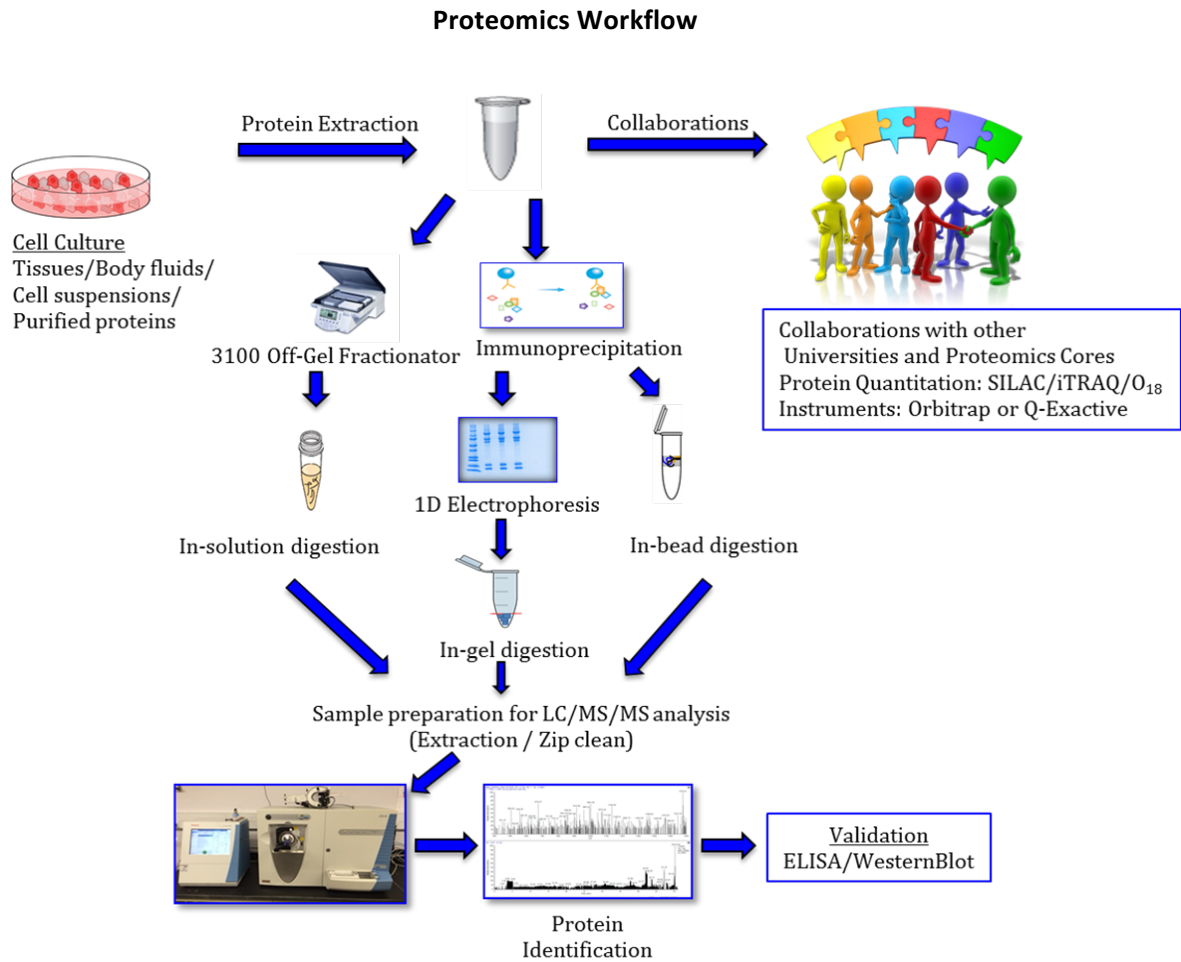
The Translational Proteomics Center is now in its second 5-year cycle (2011-2016) of the RCMI Program. The major purpose of this center is to establish an integrated proteomics platform for biomarker discovery and characterization of proteins to interface between basic and clinical research conducted in Puerto Rico in the areas of diagnosis and therapeutic monitoring of human diseases. The establishment of this facility has enabled biomedical scientists in Puerto Rico to expand translational research areas to benefit patient care in the Hispanic community by fostering multidisciplinary collaborations. The Translational Proteomics Center provides training, resources, and tools for new and established investigators seeking to use innovative approaches of proteomics to their research, as well as to undergraduate and graduate students in the biomedical field. The Center has been successful in fostering collaborations and partnerships among researchers aiming to reduce health disparities, including HIV and other infectious diseases, cancer, and neurological/neurodegenerative diseases as well as basic research in support of these fields. The specific aims of this activity for the 2011-2016 cycle including 2016-2017 no-cost extension are the following:

1) To expand our *research core capabilities* in Clinical and Basic Science Proteomics from the present number of methodologies by expanding national and international collaborations and by including Bioinformatics in Proteomics Research.

2) To increase *research productivity* of investigators using Proteomics that would lead to improved diagnosis and therapeutic monitoring in health disparities including HIV and other infectious diseases, cancer, neurodegenerative, and cardiovascular illnesses by providing a training program in proteomics to local basic, and clinical researchers, post-docs, graduate students and the industry.

Proteomics is a rapidly evolving field in biomedical research that takes advantage of top notch biochemical and molecular technologies. The Translational Proteomics Center provides access to training, resources, and consults regarding in sample extraction, separation, fractionation, purification, differential expression analysis, protein profiling, protein identification, and quantitative proteomics.

Services



The proteomics platforms we offer at the RCMC Translational Proteomics Center are shown above (Click on the diagram to view a larger version). Several components are integrated such as fingerprinting, fractionation, profiling, protein identification and data analysis. Not all experiments require the use of all components. Researchers are encouraged to discuss their approach and ask for collaboration letters to the Key Director when writing proposals for research funding, and include the core fees (Page 8) in their budgets.

Methodologies Available at the RCMI Translational Proteomics Center

Protein extraction and purification. Although we do not perform protein extraction and purification protocols in-house, we provide training and suggestions regarding protein extraction and preparation from experimental samples (tissue culture, cells suspensions, purified and recombinant proteins in solution) or biological specimens (different types of tissue, blood, urine, cerebrospinal fluid) according to the proteomic approach that will be used by the PI. Please consult to the Key Director for specifics and recommendations prior to protein extraction.

Protein separation and fractionation. Sample separation techniques such as one- (1D) and two- (2D) dimensional gel electrophoresis, and off-gel fractionation are available in the Center. Further protein separations with differential expression analysis can be accomplished by 2D-differential in gel electrophoresis (DIGE).

Immunoprecipitation and in bead digestion for studies of protein interactions. Although we do not perform in house protein purification as described above, we offer the service of immunoprecipitation of your protein of interest and evaluate possible protein-protein interactions. Immunoprecipitation is a method that enables the purification of a protein using an antibody for the protein of interest or a to investigate about the protein interacting partners.

Protein identification. A top notch Easy-nano LC 1000 couple to LTQ XL mass spectrometer from Thermo Scientific is available and configured for maximum coverage in protein identification. It can also be configured for better detection of post-translational modifications and other experiments of interest. Nanoflow LC is optimized for separating biomolecules such proteins and peptides at ultrahigh pressures. LC-MS mass spectrometer delivers high MS high performance to generate extensive structural information for routine proteomics and metabolism applications.

Quantitative Proteomics. Proteomics is a science that encompasses much more than just identifying proteins in a sample. The proteome is dynamic and constantly changing to adapt to new environments and thus, differences in abundance of certain proteins at a given time can give insights into what changes are occurring at a molecular level that may explain diseases and symptoms. In quantitative proteomics, differentially labeled samples are combined and analyzed together using a mass spectrometer. The differences in the peak intensities of the labeled pooled samples or isotope pairs accurately reflect difference in the abundance of the corresponding proteins at a given time point. The RCMI Translational Proteomics Center has incorporated methods for sample preparation, and

corresponding analysis software, such as Isobaric Tag for Relative and Absolute Quantitation (iTRAQ), Isotope-Coded Affinity Tags (ICAT), Stable Isotope Labeling with Amino Acids in Cell Culture (SILAC), and O¹⁸ labeling. We are currently in the process of incorporating a relatively new approach to quantitation using label free methods. However, collaboration with other Mass Spectrometry facilities containing High throughput Mass Spectrometers (Orbitrap, Q-TOF) and Bioinformatics support is recommended for completion of the quantitative proteomics methods.

Post-translational modification analysis. Protein phosphorylation/dephosphorylation is the major regulatory post-translational protein modification in eukaryotic cells. In our interest to provide methods to study post-translational modifications we have incorporated phosphoproteomics analysis by mass spectrometry combined with metal oxide-based phosphoenrichment.

Validation Analyses. The Center provides equipment for western blotting, ELISA, fluorometry and luminometry detection technologies for direct protein expression validations. Investigators are responsible for supporting funds for specific antibodies and reagents. The Varioskan Flash spectral scanning multimode reader offers optimal performance and provides flexibility for a variety of different applications with unlimited wavelength selection, up to three onboard dispensers, and unparalleled optical performance. It includes fluorescence intensity, time-resolved fluorescence (TRF), photometric, and optional luminometric detection technologies. It is designed for optimization and analysis of various assays, such as binding assays, ADMETox, molecular biology assays, enzyme kinetic studies, ion-channel and cell signaling assays.

Instrument capabilities

Instrument	Purpose
Easy-nano LC / LTQ XL from Thermo Scientific	Protein identification, quantification & post-translational modifications
1D and 2D gel electrophoresis stations	Fractionation / Separation
Power Pac 3000-Power supply and vertical 1D gel electrophoresis unit	Analyze protein samples
Ettan IPGPhor 3	IEF, DIGE
Ettan DaltTwelve	Vertical Electrophoresis
Ettan Imager	Design to create high quality images of 2-D DIGE gels

Ettan Spot picker	Offers maximum picking accuracy and efficiency for selecting protein spots
DeCyder 6.4	DIGE software
miVac DNA concentrator Integrated System	Used for the removal of aqueous and/or organic solvents
SmartspecPlus Spectrophotometer	Determine protein concentration
Lab Line Maxi Rotator	Rotator
Fisher IsoTemp	Heat block
Sorvall Legend Micro 21R & 17 Centrifuges	Separates substances of different densities in a sample
5510 Branson Sonicator	Sample preparation and for cleaning purposes
Universal Platform & Excella Shaker Incubator	Incubate bacterial culture and temperature dependent incubations
Denver Analytical Balance	Weight small amounts of chemical substances
Off Gel Fractionation	Separate proteins or peptides according to their isoelectric points
Varioskan Flash Spectral Reader	Used to measure fluorescence intensity
Turbo Trans Blot Transfer System	Transferring proteins from gel to membranes
Digital Dry Bath	Temperature sensitive incubations
Labline Max Rotator	Gentle rocking motion applications (staining/destaining gels)
Portable pH Meter	Measure buffers and solutions pH's
NAireWater-Jacketed Incubator	Cell culture growth (Controlled environment)
Bio Rad Gel Doc XR+	Gel documentation system
SHEL Lab Convention Oven	Dry sterilized glassware (5 to 40 Celsius)
Sorvall WX Ultra Series Ultracentrifuge and Rotor	Achieve faster separation rates in a minimum amount of time
Omni Overhead Stirrer and Support Stand	Tissue homogenizer
Shaker incubator	Sample preparation

Sample Requirements

Scientists interested in using the RCMI Translational Proteomics Center are strongly encouraged to consult with our key Director on experimental design, amount of sample required and sample extraction techniques before the initiation of sample preparation and/or experimental approaches as these are crucial steps in any proteomics experiment. Protein samples have to be of good quality and non-degraded for Proteomics experiments as demonstrated by gel electrophoresis. A 1D gel of total protein after extraction and purification is required for all experiments. As a guide, investigators can plan to use at least 25 µg of total protein per sample for 1D electrophoresis, western blotting, ELISA, and SELDI screenings and 300 µg of total protein for electrophoresis, 2D-DIGE experiments, and subsequent MS/MS analysis.

Estimated sample processing time for the two major platforms: 2D-DIGE and MS/MS

We are providing a summary of steps and sample processing times for the two major platforms used in the Center to assist in the scheduling and planning of experiments.

Services Provided and Required Steps	Estimated Amount of Time
<i>Steps for 2D-DIGE experiments- for independent analysis from users</i>	<i>11-12 days total time</i>
1. Sample clean-up	5 hrs
2. Protein quantification	5 hrs
3. Sample labeling	5 hrs
4. Strip Rehydration	Overnight
5. Isoelectric focusing	13 hrs
6. Electrophoresis	24-36 hrs
7. Image acquisition	1 hr per gel
8. Statistical analysis	Up to 6 hrs
9. Spot picking	2 hrs
<i>Note: 2D-DIGE experiments have two parts, analytical gels (steps 1-8) and preparative gels (steps 3-9). These experiments can only be stopped after steps 1 and 5 by storing samples at -80°C</i>	
<i>Steps for MS/MS experiments</i>	<i>10-12 days total time</i>
1. Immuno precipitation	2 days
2. 1D Gel Electrophoresis	3 hrs setup, overnight
3. Gel Staining	2 days
4. Enzymatic digestion	3 hrs setup, and then overnight
5. Peptide extraction	2 days
6. Zip-tip clean-up (optional)	2 days
7. MS/MS	2 days
8. Database search	3 hrs
<i>Note: Not all steps are necessary for all samples, in some cases the estimated time may be less.</i>	

Rules of operation

All users will be required to meet with the Key Activity Coordinator and complete the **Project Registration Form** prior to be included in the Center's research calendar and waiting lists. Users will be placed on a waiting list if instruments are not immediately available. All investigators interested in using our facility must cover the cost for sample preparation and analysis. Universal Safety Precautions apply for the use of these instruments. Additional precautions will be explained in the facility and will be communicated to all users. All projects must have the corresponding up to date Biosafety, IRB, and Animal Use and Care Committee approvals from the appropriate institution prior to starting Proteomics analyses, **please provide copy of the approval letters with the Registration Form.**

As an NIH funded facility, we are required to compile information on our user's progress for external evaluation. The RCMI Translational Proteomics Center is evaluated yearly and its success is evidenced by the number of scholarly publications, presentations, grant submissions and funding, and participation in Seminar series and Journal Club of our users. Principal Investigators are required to certify in written form that they will provide evidence of any report, poster presentation, oral presentation, and manuscript or research proposal which includes data or analysis performed at the Translational Proteomics Center and give proper credit by acknowledging of RCMI Support (G12 MD007600).

Fees for services (Prior consultation with the Key Director is recommended).

ID	Service provided	Proteomics Core Facility Users Fee
1	Immunoprecipitation Procedure** **Investigators must provide Antibody of their preference and Beads costs. IP will be performed in sample set, for a total of 5 samples including controls. For more samples price will vary.	\$ 150
2	One-dimensional SDS PAGE	\$50/gel
3	Gel staining	\$25/gel + Price of Staining Kit
4	Enzymatic digestion (in-gel)	\$20/sample
5	Analysis by LC-MS/MS (Easy nLC-LTQ XL), Protein ID and MW by Sequest Data Search	\$50/sample
6	Protein validation by fluorometry/ELISA	\$50 + Price of ELISA Kit
7	Protein Validation by Western Blot (WB)	\$75 + AB Price
8	Ingenuity Pathway Analysis for Validated Proteins (fee for the service)* *The software is also available for users Free independent analysis	\$100
9	Consulting and Final Report	Free

Reports

The Proteomics core will provide a general report/summary including generic methods to Principal investigators at the end of the study. During the study, PIs are encouraged to be involved in all parts of experiments and analyses; core personnel will provide written communication with news and progress of the study. The general report for DIGE will include a table with spots numbers, p-values, and expression fold changes of all detected spots in a study. Pictures of spots and gels are available upon request and can also be accessed by the PI or graduate student with the DIGE DECYDER Program located in the Proteomics Core. Users are expected to prepare reports, pictures, tables, and graphs for proposals, manuscripts, and progress reports. For MS/MS, a list of identified proteins that pass exclusion criteria with peptide sequences will be provided in a table format with a generic method section to facilitate report/manuscript preparation. Further analysis, preparation of reports/manuscripts, organization and integration of data relative to the system of interest, and any further bioinformatics analysis and validation is done by PI. Dr. Abiel Roche at the RCMI Program can provide help with Bioinformatics analyses. The Key Activity Coordinator is available to collaborate in manuscript review prior to submission. Instrumentation and consultation for protein validation by ELISA and Western blots is also available at the Translational Proteomics Center.

Physical Location

The RCMI Translational Proteomics Center is located at the Comprehensive Cancer Center in Centro Medico Second floor laboratory #5. Visitors and Investigators need special access permits to visit our facilities, please contact Center Staff in advance at **787-772-8300 Ext. 1411**. Visiting hours for the RCMI Translational Proteomics Center are Monday through Friday from 8:30 AM to 4:00 PM. Instruments operate 24 hrs a day, 7 days a week and access can be provided outside visiting hours. Please contact us to schedule an appointment.

Scientists with active funding or in the process of submitting a grant proposal and needing assistance with letter of supports and proteomics experimental design are welcomed. Applications for sample analysis are accepted one month in advance. Instruments are available under the supervision of Carla Salgado and Yadira Cantres in a programmed schedule.

To receive information related to the Center activities and accomplishments, please contact us.

Contact

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Laboratory Location

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Acknowledgment of RCMI Support



THIS IS AN RCMI SUPPORTED FACILITY

Users of the Instruments or Services should acknowledge RCMI support by including the following sentence in their publications:

“Infrastructure support was provided in part by grants from G12MD007600 (National Institute on Minority Health and Health Disparities) from the National Institutes of Health.”

Cooperation in this respect is Vital to the success of the Program

Approval of Guidelines

Approved by: _____/ Loyda M. Melendez, PhD

Date: _____

Approved by: _____/Emma Fernandez, Ph.D.

Date: _____