



## LVC/SLIIDR Research Cores

The Louisiana Vaccine Center (LVC) and the South Louisiana Institute for Infectious Disease Research (SLIIDR) is developing and supporting a series of Core Facilities (including Genomics, Proteomics, Protein Purification, Nanotechnology, Vector Development, Molecular Interaction, Imaging) that will serve all LVC/SLIIDR investigators and will also provide new investigators in the field with access to state-of-the-art biotechnology in support of their research. The following is a list of the current status of these Core facilities:

### **1). Genomics Core**

The mission of the Genomics Core is to provide comprehensive genomics, microarray and bioinformatics support to Center investigators through a wide array of state-of-the-art analytical equipment and data analysis capabilities. The Core is housed in the LSUHSC Gene Therapy Program and the adjacent Stanley Scott Cancer Center (CSRB 4<sup>th</sup> and 5<sup>th</sup> floors, LSUHSC). Services include assistance in microarray experimental design and data analysis, protein and nucleotide sequence analysis, functional analysis of high-throughput data, data mining, data organization and repository, whole microbial and mammalian genome mapping, and database development.

Available equipment includes: Affymetrix microarray equipment for RNA profiling, a premier Rosetta bioinformatics package, an Illumina BeadStation® system for analyses of single nucleotide polymorphisms, Illumina robot controls facilitating high volume automated sample processing and state of the art Real Time PCR. It is anticipated that state of the art DNA sequencing services will also be provided by the Genomics Core in the near future. To enhance the capacity for LVC/SLIIDR-related studies, upgrades of the Affymetrix scanner, of two Dual Core Xeon Workstations for Affymetrix users, of Rosetta Resolver bioinformatics software, of the Resolver server and workstations, and also an Illumina BeadXpress Reader have been purchased by the LVC, also facilitating the use of protein and antibody arrays.

The Genomics Core will be managed by Doan Nguyen PhD and Jovanny Zabaletta PhD. LVC will fund a technician dedicated to LVC/SLIIDR activities will facilitate investigator's research in the Genomics Core.

## **2). Proteomics Core**

The Proteomics Core was established at LSUHSC through institutional and State funding (and is moving soon to CSRB 3<sup>rd</sup> floor). The following equipment is available for 2D Gel Electrophoresis analyses: Equipment facilitating traditional Coomassie blue and silver stains includes a BioRad 2-D gel electrophoresis system, BioRad GS800 densitometer, a ProteomeWorks Spot Cutter with PDQuest software for gel imaging, spot picking, analysis, and a Perkin-Elmer Multi-Probe II sample handling workstation. Equipment accommodating fluorescent tags and special purposed stains (phosphorylation, glycosylation): an Amersham IPGPhor and Ettan 2-D gel electrophoresis system, a Typhoon imaging system, and an Ettan handling workstation. A new Dionex nano- to micro-flow LC multiple dimensional high performance liquid chromatograph system can run parallel ion exchange and reversed phase columns for multiple dimensional LC separation to realize off-line mass spectrometry sample preparation. Mass Spectrometry equipment available includes: 1) A ABI 4700 Proteomics Analyzer MALDI TOF-TOF mass spectrometer with Global Proteome Server Explorer workstation (with Mascot) is designed for fast protein identification. Interfacing with off-line Dionex LC allows more efficient in quantization and biomarker discovery. 2) Two ABI classic Voyager DE MALDI -TOF mass spectrometers are used as easy to operate walk-on instruments available on a charge per time basis. These instruments are excellent for MW determination of small proteins/peptides and other biological samples such as oligosaccharides. 3) A Thermo-Fisher LTQ XL Ion Trap Mass Spectrometer with Eksigent 2D nanoLC possesses great sensitivity and can provide LC-based total proteome approach, peptide sequencing, and detecting modifications, such as phosphorylation and acetylation. For data analysis there is an IBM 10-node parallel processor server will run 5-node Sequest and 5-node Mascot software to analyze data from LTQ and 4700, for protein ID search, database blast, and modification determination.

This director of the Proteomics facility is Chau-Wen Chou, PhD. LVC/SLIIDR investigators will also be able to negotiate collaborative projects with the Head of the UNO Analytical Chemistry laboratory who has additional proteomics-related equipment available.

## **3). Protein Core**

It is the mission of the Protein Core facility (PCF) to support and advance research capabilities at the Center by providing high quality protein and antigen purification support. The PCF is located at Tulane HSC and its major goal is to provide LVC/SLIIDR researchers with purified protein antigen for use in vaccine research. PCF personnel dedicated to LVC/SLIIDR projects will assist researchers in the selection of optimal recombinant expression systems (prokaryotic and eukaryotic), will sub-clone genes of interest into expression vectors, and will optimize protein expression and purification. The core will also assist in generating site-directed mutants of proteins of interest and in scaling up of recombinant protein production. Emphasis is placed on purity, with removal of bacterial endotoxin and contaminating immunogens from protein preparations, while minimizing degradation.

Available equipment includes: a New Brunswick Bioflow 3000 Fermentor with 3-liter and 10-liter vessels, a Microfluidizer cell disruptor and a Virtis lyophilizer, a Beckman DU-64 spectrophotometer, a flow cytometer, Beckman low- and ultra- speed centrifuges and rotors,

autoclaves, gel dryers, a digital imaging system, a Dynatech Microfluor fluorescence plate reader, a Beckman liquid scintillation counter, a Beckman gamma counter, a Fuji phospho imager and software, and confocal and fluorescent microscopes. To facilitate LVC/SLIIDR research, a New Brunswick BioBlo 4500 20-liter bioreactor for large scale purification and a BioRad BioLogic Douflow chromatography system has been purchased by LVC.

The PCF is directed by John Clements PhD and the LVC has also funded technical support dedicated to LVC/SLIIDR research projects.

#### **4). Nanotechnology Core**

It is the mission of the Vaccine Delivery/Nanotechnology Core facility (Tarun Mandal PhD, Core Director) to support and advance vaccine research capacity by providing novel and innovative vaccine delivery formulations. The major goal of the Core, located at Xavier University, is to maintain a state-of-the-art innovative polymeric vaccine delivery research facility in order to support inter-disciplinary research. Core personnel will provide leadership in planning, designing, and implementing innovative nanotechnology and will also assist investigators in conducting pre-formulation and formulation studies of any potential novel vaccine delivery system for preclinical and NDA studies (New Drug Application following USFDA guidelines). Nano-delivery technology will be developed and/or adapted, in collaboration with LVC/SLIIDR researchers, to address the special requirements of either systemic or mucosal (ie. intranasal, pulmonary, oral, or intra-vaginal) particle-mediated delivery of peptides, proteins and/or recombinant DNA vaccines in preclinical and, ultimately, clinical studies. Targeted particle- or lipid-mediated delivery either of proteins via novel routes (eg. transcutaneous) or of alternative recombinant vaccine vectors is already under development in the Core and this technology will also be made available to other LVC/SLIIDR investigators.

Currently, the NIH-funded nanotechnology research laboratory is equipped with R&D-scale pharmaceutical formulation equipment, with research staff who have developed unique skills in micro-encapsulation for controlled release. Available equipment includes: a Scanning Electron Microscope, a Fluid Bed Coating machine, a Super Critical Fluid (SCF) particle preparation equipment, a high pressure homogenizer to prepare lipid nanoparticles, a particle size analyzer, a zeta sizer, and an automated dissolution apparatus. Other Core equipment includes a preparatory ultracentrifuge, an analytical ultracentrifuge, a fluorescence spectrometer, and a fluorescent microscope. Specifically for vaccine-related research, a Laboratory Mini Spray Drier (forms nanoparticles ideal for pulmonary vaccine delivery) and a Waters Acquity SQD LC/MS System (facilitates measurement of small quantities of vaccine material and their preparation for delivery) will be purchased by the Center. LVC has also funded a Core research technician dedicated for LVC/SLIIDR projects.

#### **5). Vector Development Core**

The Vaccine Technology/Vector Core will greatly facilitate co-operative LVC/SLIIDR research through the design, engineering, preparation and purification of new recombinant vaccine vectors and novel vector technology. The Vector Core is based at LSUHSC in the Gene Therapy Program. Core services include construction of new recombinant vectors, and large-scale preparation of recombinant vectors for use, quality control. The Core maintains an extensive

inventory of plasmids and cell lines that are useful in the development of recombinant vectors. Several vaccine vector systems are currently available through the Core: DNA vaccines, replication-defective adenoviral vectors, lentiviral vectors, and vectors based on oncogenic retroviruses including mouse stem cells virus (MSCV). More recent additions include vectors based on poxviruses (vaccinia or fowlpox) and adeno-associated virus (AAV). Current serotypes of the latter include AAV1, 2, 5, 7, 8, 9 and AAVrh10.

The Core is already equipped for manufacture of the above mentioned vector systems through LSUHSC, Gene Therapy Consortium and NIH funding. Robert Kutner is Core Manager. An electroporator device for *in vivo* inoculation of DNA vaccines has been purchased by LVC to support pre-clinical LVC/SLIIDR studies.

## **6. Immunology Core**

The Immunology Core will serve LVC/SLIIDR investigators in the measurement of immune responses in vaccine-related studies and data analysis. The Core is located at LSUHSC (CSR 4<sup>th</sup> floor) in space near all other LSU-based core facilities described in this proposal. State-of-the-art equipment includes: a FACS Aria (9-parameter analysis/sorting), a FACS LSRII (18-parameter analysis), a FACS Calibur (4-parameter analysis), a Gel Logic Imaging system for Western Blot, a Dynatech ELISA system and reader, a BioRad multiplex system for multiple parameter (cytokine/chemokine) analysis in small fluid samples, a Tri-Carb Liquid Scintillation counter, a Perkin-Elmer Top Count Gamma Scintillation counter, and an Olympus Fluorescence Microscope station. The Core is directed by Ping Zhang MD and has a fully qualified FACS operator (Constance Porretta MS). Full FACS acquisition services will be provided to LVC/SLIIDR researchers. An LVC-funded research technician will be dedicated to support Center vaccine research and assist with data analysis

## **7. Molecular Interaction Core**

This core is located at the Research Institute for Children (Children's Hospital Campus) and utilizes Biacore technology ([www.biacore.com](http://www.biacore.com)) to study intermolecular interactions in real time. Using the principle of surface plasmon resonance, this technique can be used to determine association and dissociation kinetics, and perform concentration analyses. This technology has proven extremely useful in determining affinities of monoclonal antibodies and of polyclonal antisera. Because antibody affinity is often a correlate of protective activity, this presents a method for analyzing the quality of the antibody response elicited by experimental vaccines. Other uses for this technique include defining interactions between peptides and other low molecular weight ligands and larger molecules including antibodies, receptors, and enzymes. More details about this methodology may be obtained on the company's website.

The Molecular Interaction Core is directed by Seth Pincus MD. LVC will support a research technician position in the Core to assist LVC/SLIIDR investigators. After consultation with the Core, the investigator will provide paired antibody/antigen, receptor/ligand, or enzyme/substrate, which the core scientists will then study.

## **8). Imaging Core**

The mission of the Imaging Core is to provide histology, microscopy and imaging services to support and advance LVC/SLIIDR research activities. The Core is located in LSUHSC Gene Therapy Program (CSRB 5<sup>th</sup> floor) adjacent to the Antigen Discovery Core (Genomics/Proteomics) and offers expert advice on experimental design and interpretation of results. Available equipment includes: a Leica CM3050S Cryostat with CryoJane Module, a Shandon Hypercenter XP Paraffin Processor, a Shandon Histocentre Paraffin Embedding Station, a Shandon Finesse ParaffinMicrotome, a Leica DMRXA deconvolution microscopy system, a BioRad Radiance 2100 Laser Scanning Confocal System, a Nikon E600 brightfield / epifluorescence microscopy system, and a Leica Mz75 brightfield / epifluorescence stereomicroscopy system. The Core has also recently purchased a state-of-the-art P.A.L.M. Laser Microdissection System capable of dissecting minute fragments of target tissue from sections for detailed histological, immunological or genomic analyses. This offers significant advantages to Center researchers for gene-based and phenotypic analyses of minute regions of tissue (as few as 10 cells) in studies of host/pathogen interaction. A Xenogen IVIS® SPECTRUM imaging system is also now available, allowing real-time imaging to non-invasively monitor and record cellular and genetic activity within a living organism with attendant advantages of high throughput (of mice) and high-sensitivity in vivo imaging of both fluorescence and bioluminescence with resolution (at 3.9cm field) to 20 microns. This equipment will facilitate in vivo localization and tracking of labeled cellular and genetic material within an organism in studies of disease pathogenesis and host response. In addition, the Gene Therapy Program has recently purchased an Olympus Fluoview FV1000 multiphoton laser scanning microscope equipped with a Mai Tai Titanium:Sapphire laser to allow fluorescence imaging deep within specimens. Utilizing nanosecond-pulsed infrared illumination, the Fluoview MPE (multiphoton excitation) is able to image up to 1mm into a specimen thanks to the scatter-free penetration of infrared light and long working distance objectives. The system's software modules are easy to use and include flexibility in scanning modes and applications including time-course, FRET, FRAP, photo-stimulation, 3D rendering, spectral un-mixing (up to 2nm), co-localization, and multi-point observation.

The Imaging Core is managed by Luis Marrero. Technical support for LVC/SLIIDR research activities will be available in the Core.