



SHARED RESOURCES

Overview

Sanford Burnham Prebys has an extensive Shared Resource system. Their primary mission is to provide advanced technology, expertise, and instrumentation to investigators that may not be easily acquired by individual laboratories. The cores, staffed by technical experts, offer high quality interactive services that provide not only cost-effective sample analysis, but also assistance in experimental design, data analysis, and grant or manuscript preparation. Many of the cores offer a choice of full service, or investigator training on their advanced instrumentation for independent use. Most cores facilities can also provide expert services for outside non-profit and for-profit investigators.

Animal Resources

Animal Facility – A 24,000 sq ft AAALAC accredited facility that houses over 9,000 cages of mice in ventilated racks, providing full husbandry with breeding, weaning, and tail samples. The facility also provides care for a small number of rats, and has separate BSL2 mouse facilities. Many procedures can be performed by facility staff, including injections (SC, IP, IV), tumor measurements, blood collections, and surgical assistance. The creation of knockout or transgenic mice, as well as re-derivation and cryopreservation are being done under special agreement with the Transgenic Cores at the UCSD Moores Cancer Center and at the Salk Institute.

Animal Imaging and Analysis – Live animal imaging tools include an IVIS Spectrum for bioluminescence and fluorescence imaging, a LiCor Imager Pearl Impulse System for *in vivo* near infra-red fluorescence, high frequency ultrasound (VisualSonics Vevo 770), and X-ray (Trident). PET/SPECT/CT and MRI imaging capabilities are available at the nearby Sanford Consortium for Regenerative Medicine building. A variety of widely used tumor cell lines labeled with luciferase are available for xenograft tumor growth and metastasis studies. Analytical services support complete blood cell counts (CBC) and analysis of serum components revealing metabolic or organ stress, from small samples of mouse blood. The **Tumor Analysis** service assists with *in vivo* tumor studies, injections or surgeries for cell introduction, imaging-based tumor measurements, anti-tumor compound dosing/efficacy testing, and derivation of cell cultures from patient-derived xenograft tumors.

Model Organisms – Infrastructure for the maintenance and analysis of *Drosophila* and *C. elegans*. The core provides advanced microscopy tools such as a Leica MZ16F fluorescence dissecting microscope, a Zeiss M1 time-lapse microscope, and an Apotome Optical Sectioning microscope, as well as microinjection tools.

Cell Analysis and Histopathology

Cell Imaging – The core houses a wide variety of microscopes, and provides investigator training and use of the instruments, as well as full-service imaging. Facility microscopes include a Zeiss LSM-980 super resolution AiryScan, a Nikon N-SIM super resolution/A1ER confocal microscope, six advanced fluorescence microscopes, three additional confocal systems (a multiphoton Zeiss LSM-710 NLO system, an Olympus FluoView 1000, and a Yokagawa Spinning Disk system). Sophisticated systems for FRET, Calcium imaging, and laser TIRF are also available. The microscopes are integrated with advanced image capture systems and analytical software, and a number are equipped with environmental chambers for extended live cell confocal analysis. TEM, SEM and Lightsheet are available at the nearby Salk Biophotonics facility.

Histopathology – The facility generates slides with frozen or fixed tissue sections and processes them with various stains and antibodies, also providing expertise in pathology and tissue microarray analysis. Leica equipment (ST5010 and BOND-RX) support automated H&E and IHC staining. Leica/Aperio ScanScope AT2 and FL systems enable high resolution scanning, quantitative and morphometric analysis, digital archiving, and electronic distribution and access of standard and fluorescently stained histology slides. A Laser capture microscope system (MMI CellCut) facilitates isolation and molecular analysis of specific tissues and individual cells.

Flow Cytometry – The facility has five analytical flow cytometers (BD LSRFortessa and Fortessa X20, Acea NovoCyte 3000) analyzing up to 18 colors and recently added a Cytex Aurora spectral flow cytometer capable of analyzing over 40 colors. There are two advanced BD FACS Aria cell sorters with up to 16-color capability – both housed in biosafety enclosures, as is the Fortessa X20 analyzer. The Core also has an imaging flow cytometer (Amnis ImageStreamX MarkII) with up to 10 colors. Cell sorting is performed by expert Core staff and on a limited basis by trained investigators, with analytical cytometry available either as a service or for use 24/7 by trained investigators. FlowJo analysis software is available on workstations in the core and through an institutional site license.

Stem Cells – The Institutional Stem Cell Core has been discontinued. There is now a well-equipped **shared stem cell laboratory** dedicated to the culture and analysis of stem cells by SBP investigators. The facility has an advanced BioSperix Xvivo hypoxia workstation with four independent chambers with a work area for cells and microscopy under hypoxia. **iPSCs:** The generation and characterization of induced Pluripotent Stem Cells (iPSCs) is now being performed on a collaborative basis for both internal and external investigators with Evan Snyder's lab.

Structural Biology

Cryo-EM – This purpose-built facility provides training or full service, and has a Titan Krios cryo-EM with a Gatan K3 imager supporting single particle analysis, as well as a Tecnai T12 TEM and a Vitrobot Mark IV for sample prep.

Protein Analysis – The core focuses on analytical services for proteins by Isothermal Titration Calorimetry, (TA Affinity and MicroCal ITC200 instruments), MicroScale Thermophoresis (Monolith NT.115), Differential Scanning Calorimetry (DSC), fluorescence spectroscopy, and analytical ultracentrifugation (AUC).

The infrastructure for X-Ray crystallography and NMR are no longer part of the Cancer Center cores, but are available as institutional cores.

Genomics Technologies

Next Gen Sequencing – The facility provides library preparation and then high throughput (next-gen) DNA sequencing using an Element AVITI sequencer. RNA quality analysis is performed utilizing Agilent TapeStation, NanoDrop, and Qubit instruments, with automated sample preparation using an Eppendorf Epimotion.

The Core supports transcriptome and exome analysis, ChIP-seq, and a variety of other NGS approaches. Single cell sequencing analysis is offered using the 10X Chromium X system for library preparation. The Core is increasingly focused on spatial transcriptomics, utilizing a Nanostring GeoMx, as well as the new Nanostring CosMX instrument, which provides true single transcriptomics. STR-based cell line authentication and mycoplasma testing services are also provided.

Functional Genomics – The Core provides the infrastructure for cell-based gain-of-function (ORF, CRISPRa) and loss-of-function (siRNA, miRNA mimics, shRNA, CRISPRko, CRISPRi) libraries screening services, starting with reporter knock-in cell line engineering, assay development and carrying all the way through verification of identified targets. Assorted genome-wide and pathway-specific libraries with choice of arrayed or pooled formats are ready-to-screen, with the core's current focus on pooled and arrayed CRISPR libraries. The core is equipped with high-throughput automation capable of performing DNA purification, plasmid quantification, viral vector production, clonal line creation, and many other custom assays in 96- and 384-well platforms.

Viral Vectors – The facility provides custom packaging, concentration, and titration of lentiviral particles, as well as retrovirus, lentivirus, AAV, and VSV-based vectors. Several ready-to-use lentiviral supernatants are available to fluorescently mark cells. Vector backbones and assistance in creating effective constructs are also provided.

Proteomics and Metabolomics

Proteomics facility – The facility provides services ranging from protein identification from IP enriched material, to global analysis of proteins and their post-translational modifications. The Core supports comparative proteomics using mass tagging (e.g., TMT) or label-free analysis. Following initial digestion and sample preparation, typically utilizing a robotic Bravo AssayMap system, samples are separated by 1D or 2D UHPCL (EASY nLC 1200, and 2D NanoAcquity) and then analyzed by mass spectrometry. Advanced mass spectrometry instruments include two Thermo Orbitrap Fusion Lumos Tribrid systems, a Thermo Q-Exactive Plus, and a Thermo Quantiva. Global serum proteomic analysis (thousands of proteins) is now supported with the Seer Proteograph system. Proteomics data analysis is via a pipeline including open-source tools for protein identification, as well as statistical and functional analyses of large proteomic datasets.

Cancer Metabolism – The scientific focus of the core is to investigate the role of metabolism in cancer on both the cellular and organismal level, combining *in vitro* and *in vivo* analysis. To that effect, the facility provides measurement of metabolites in cells, tissue samples, plasma and media. A GC-MS is utilized for broad metabolic flux analysis using stable isotope labeling. A YSI 2950 Analyzer allows for focused analysis of specific metabolites, and a SeahorseXFp (8 well) and Seahorse XFe96 (96 well) for real-time measurement of mitochondrial respiration and glycolysis. Lastly, a Shimadzu HPLC is available for the measurement of metabolites not generally accessible by GC/MS.

Bioinformatics

Bioinformatics – The Bioinformatics Core provides computational and systems biology support, specializing in omics data analysis, multi-omics data integration, network and pathway analysis, statistical analysis, and machine learning. The Core has built automated computational pipelines using state-of-the-art software packages to QC, align, summarize, statistically analyze, and visualize NGS data sets. Analysis includes multi-omics data integration and customized pathway and network analysis. The Core has a dedicated High-Performance Cluster with 100 cores, 512 GB memory, and 96 TB Network Attached Storage to enable computationally intensive analysis, as well as cloud-based resources. The Bioinformatics Core has licensed commercial software packages for advanced genomic studies including Ingenuity Pathway Analysis (IPA) and TRANSFAC. Regular trainings and tutorials are given on using these software packages and other publicly available bioinformatics software and databases. A growing area of focus is systems biology by integrating analyses across multiple data types (data generated in-house or mined), such as genomics, proteomics, and metabolomics, helping to create testable hypotheses and better understand the underlying biology.

Chemical Biology and Drug Discovery

These cores are part of the Conrad Prebys Center for Chemical Genomics (CPCCG)

High Throughput Assay Development – The facility provides assistance in HTS-compatible assay development (384 or 1536 wells), including assay optimization, and miniaturization. The core staff is experienced in a wide variety of assay types for biochemical or cell-based assays, enabling selection and development of assays most suitable to each particular project, with an emphasis on optimal assay sensitivity in identification of hits. The core also provides support for Structure-Activity Relationship (SAR) studies aimed at characterization and optimization of hits obtained in primary screening. Assistance in preparing screening-related grants is also provided.

Chemical Libraries and Screening – The facility provides access to small molecule libraries - over one million total compounds, with large as well as smaller collections of drug-like, bioactive, and other focused libraries. The facility has the robotics, compounds, instrumentation, and expertise to perform the screens, typically in 1,536 or 384 well format. The central automation includes three Beckman Access Workstations coupled to ECHO 655 acoustic dispensers, and carousels suitable for either biochemical or live-cell screening. The core robotics can screen up to 300,000 wells per day. The core has three BMG PHERAstar FSX multimode plate readers, as well as a Hamamatsu FDSS/ μ CELL for kinetic analysis of luminescence or fluorescence. Liquid handling is further supported by two additional standalone Echo dispensers plus six Thermo Multidrop Combi, and two CERTUS FLEX reagent dispensers. Multiple qPCR instruments support thermal shift and transcriptional readout assays. Other automation includes a V11 Bravo as well as the two Beckman Coulter FX core systems. Two BSL-2 labs support tissue culture and cell-based screening in the core.

High Content Screening – The facility supports assay development, image-based screening, image data analysis and mining, and algorithm development for high content screens, where the readout is typically images from high-throughput microscopy in 384- or 1,536 well formats. The Core's flagship instrument is a PerkinElmer Opera Phenix with confocal imaging and an integrated robotic plate loader. The facility also has an open frame Vala IC-200 equipped for live cell screening, and a Cytellect Celigo Cytometer.

Protein Production - The facility supports all aspects of protein production in bacteria, insect cells, or mammalian cells, from vector design to purification of 1-1000 mg of protein by approaches including FPLC or affinity chromatography. The quality and properties of the purified proteins is assessed in the Proteomics and Protein Analysis Cores.

Cell-based Disease Modeling and Screening – The facility supports development of patient cell specific and human induced pluripotent stem cell (hiPSC)-based disease models for drug screening and target identification. Resources and expertise are provided for standard and large-scale cell culture, the creation and directed differentiation of hiPSCs, and the development of stem cell-based assays and patient-specific disease models in screenable formats, supporting HTS/HCS analysis.

Cheminformatics – The Cheminformatics group provides databases and tools for compound registration, inventory management, plate formatting and tracking, data entry and organization, data mining, and reporting. Facility databases include the results from all CPCCG screens, and a searchable catalog of over ten million commercially available compounds. Informatics support is provided for storing and analyzing screening data, identifying the most promising hits, SAR follow-up of hits, and in silico screening through docking and other approaches.

Medicinal Chemistry and DMPK– The core provides researchers with medicinal chemistry expertise for follow-up of screening hits and library design, as well as DMPK assays - both in vitro and in vivo pharmacokinetics. The Core is equipped for synthetic chemistry functions from milligram to multi-gram scale, with a wide array of equipment to synthesize and modify organic molecules for both initial lead generation and subsequent structure-activity relationship (SAR) studies. The DMPK facility is equipped with two triple quad LC-MS/MS systems and runs absorption, distribution, metabolism, and excretion (ADME) assays, as well as routine in vivo PK assays.