



SHARED RESOURCES

Overview

Sanford Burnham Prebys in La Jolla 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. If you have access to Sanford Burnham Prebys intranet, detailed information on each core is available there. From outside the institute, summary and contact information for each core is accessible at: sbpdiscovery.org/shared-resources

Animal Resources

Animal Facility – A 24,000 sq ft AAALAC accredited facility that houses over 10,000 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 at the UCSD Moores Cancer Center transgenic core and at the Salk Institute Transgenic Core.

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 (Faxitron MX-20). 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.

Animal Imaging and Analysis – 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 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 and SEM 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. 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, with analytical cytometry available either as a service or for use by trained investigators.

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 – The facility has a Titan Krios Cryo-EM with a Gatan K3 imager supporting single particle analysis, in a purpose-built facility, as well as a Tecnai T12 and sample prep facilities. An experienced Cryo-EM Core manager was recently hired to run the facility.

X-ray Crystallography – The facility is equipped with a Rigaku FR-E SuperBright X-ray generator and two independent detectors. High throughput crystallization studies are supported by a Phoenix microdrop liquid handling system and Formulatrix incubators with automated image analysis for detecting crystal growth.

NMR – The facility has two 600 MHz Bruker instruments equipped with cryoprobes – one with an automated sample changer. There are also two Bruker 500MHz instruments - one equipped with a sample changer and a probe for solution analysis, the other a wide bore probe for solid-state analysis. A highly automated JEOL 400 MHz NMR with a sample changer supports small molecule analysis.

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).

Genomics Technologies

Next Gen Sequencing – The facility provides library preparation, then high throughput (next-gen) DNA sequencing using an Illumina NextSeq 500. Automated samples preparation is carried out with an Eppendorf Epimotion. The Core supports transcriptome and exome analysis, ChIP-seq, and a variety of other approaches. Single cell sequencing analysis is

now offered using the Illumina/Bio-Rad ddSEQ and the 10X Chromium systems for library preparation. RNA quality analysis is performed utilizing Bioanalyzer, NanoDrop, and Qubit instruments. Spatial Transcriptomics is supported by a Nanostring GeoMx, with the upcoming introduction (mid-2022) of true single transcriptomics with the Nanostring CosMX instrument. STR-based cell line authentication and mycoplasma testing services are also provided.

Functional Genomics – The facility provides transfection testing, assay development, siRNA libraries, and high throughput screening service for RNAi and CRISPR-based analysis. The core has a comprehensive human genome-wide (18,301 genes) modified Dharmacon ONTARGET-Plus siRNA library, in both pooled format and 4 individual siRNAs per target, with focused siRNA sub-libraries targeting human Kinases, Proteases, Ubiquitination-associated genes, and Cancer signaling. Mid-scale shRNA library screening is supported, and the Core can also screen miRNA function with libraries of genome-wide (2,565 mirVana) miRNA mimics and (1,972 miRCURY LNA) antagonists. CRISPR-CAS9 based screening (global pooled or targeted libraries) and custom engineered cell lines are also available. The Core leverages the extensive assay development and screening capabilities of the Prebys Center.

Viral Vectors – Starting with vector plasmid DNA, the core provides custom packaging, concentration, and titration of lentiviral particles. Several ready-to-use lentiviral supernatants are available to fluorescently mark cells. The facility also provides vector backbones and assistance in creating effective constructs.

Proteomics and Metabolomics

Proteomics facility – This facility provides protein identification from IP enriched material, or complex samples. Additionally, analysis of post-translational modifications, peptide mapping and comparative proteomics using mass tagging or label-free analysis can be performed. Following initial digestion and sample preparation utilizing a robotic Bravo AssayMap system, samples are separated by 1D or 2D UHPLC (EASY nLC 1200, and 2D NanoAcquity) and then analyzed by mass spectrometry. Core mass spectrometry instruments include two state-of-the-art Thermo Orbitrap Fusion Lumos Tribrid systems, an Orbitrap Velos Elite, Thermo Q-Exactive Plus, and a Thermo Quantiva. These instruments enable analysis targeted or complex (whole proteome) samples as well as phosphoproteomics and global characterization of other post-translational modifications, with Top-down analysis under development. 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, 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 core provides a cutting-edge computational and systems biology support, specializing in omics data analysis, multi-omics data integration, network and pathway analysis, and machine learning. The Bioinformatics Core has built automated computational pipelines using state-of-art software packages to QC, align, summarize, statistically analyze, and visualize NGS data sets. Analysis includes multi-omics data integration, customized pathway and network analysis, and hypothesis driven in-silico drug discovery. The Core has a dedicated High Performance Cluster with 100 cores, 512 GB memory, and 96 TB Network Attached Storage to enable computation intensive analysis and full data back-up. The Bioinformatics Core has licensed commercial software packages for advanced genomic studies including Omicsoft ArrayStudio, Ingenuity Pathway Analysis, and Metacore. Regular trainings and tutorials are given on using these software and other publicly available bioinformatics software and databases. A growing area of focus is systems biology integrating analysis 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 (the 320K Sanford Burnham Prebys and the 350K NIH collections, as well as smaller collections of drug-like, bioactive, and other focused libraries), along with the robotics, compounds, instrumentation, and expertise to perform the screens, typically in 1536 or 384 well format. The core is equipped with 3 integrated robotic HTS systems, their centerpiece is a High Res Biosolutions POD system with a Staubli arm equipped for integrated biochemical or cell-based screening. Core robotics can screen up to 350,000 wells per day. Major equipment in the core includes plate readers such as the PE ViewLux, Hamamatsu FDSS 7000, PE Envision, and BMG Pherastar, liquid handling workstations include two Labcyte Echo acoustic pipettors (including one that is integrated into a stand-alone LabCyte Access robotic workstation) and 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, screening, and data analysis for high content screens, where the readout is typically images from high-throughput microscopy. The Core's flagship instrument is a PerkinElmer Opera Phenix with confocal imaging and an integrated robotic plate loader. The facility also has a PE Evotec Opera, an open frame Vala IC-200 equipped for live cell screening, and a Cytellect Celigo Cytometer.

Cell-based Disease Modeling and Screening – This facility supports development of cell-based disease models for screening and testing small molecules and drugs, and specializes in culture, scaling, and directed differentiation of human embryonic and induced pluripotent stem cells. Resources and expertise are provided to facilitate development of stem cell-based assays and patient-specific disease models in screenable formats, supporting HTS/HCS analysis.

Cheminformatics – The Cheminformatics facility provides informatics support for analyzing screening data, SAR follow-up of hits, and *in silico* screening through docking and other informatics approaches. The core also supports compound registration, inventory, HTS data collection and analysis, using the Cheminnovation CBIS software. This constantly updated database from reputable commercial compound vendors currently contains over 12 million compounds which properties can be filtered with user-built settings.

Medicinal Chemistry – 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 a full suite of tier 1 and 2 absorption, distribution, metabolism, and excretion (ADME) assays and routine *in vivo* PK assays.