Shared Resources
## Shared Resources

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These links with access to additional information such as pricing, are available at the Sanford-Burnham Shared Resource site http://www.spbdiscovery.org/technology/sr/Pages/Home.aspx.
Our Mission

Sanford Burnham Prebys Medical Discovery Institute has an extensive Shared Resource system with 22 core facilities. Our primary mission is to provide advanced technology, expertise, and instrumentation to investigators that may not be easily acquired by individual laboratories.

Our cores, staffed by technical experts, offer high-quality interactive services that provide cost-effective sample analysis, 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 advanced instrumentation for independent use. Most cores also offer services for outside non-profit and for-profit investigators.

This brochure provides an overview of the 22 core facilities at the La Jolla site.
Animal Facility

Mary O’Rourke-Braxtan
Facility Director
858-795-5319

Alessandra Sacco, Ph.D.
Scientific Director

The Animal Facility’s mission is to provide a comprehensive animal care and use program for SBP investigators utilizing animals in their cancer research projects. The facility strives to maximize the scientific benefits of animal experimentation while maintaining an emphasis on animal welfare.

The 25,602 sq. ft. AAALAC-accredited vivarium houses mice in ventilated cages and provides husbandry services and complete breeding colony maintenance services with tissue samples for genotyping. The Animal Facility also provides care for a small number of rats and has a separate dedicated BSL2 mouse facility.

Many procedural techniques can be performed by the Procedural Techniques Support and Veterinary Services staff including injections (SQ, IM, IP, IV), tumor measurements and imaging, blood collection, tissue harvest, and surgical pre-and post-op care.

SERVICES

- Husbandry
- Breeding Colony Maintenance
- Veterinary Services
  - Animal transfers and quarantine
  - Animal health screening
- Procedural Techniques Support
  - Blood collection
  - Injections (IP, SQ, Tail Vein, Retro-orbital )
  - Oral gavage
  - Tumor measurements
  - Surgical pre- and post-op care
- Administrative & IACUC support
- Transgenic Services (including CRISPR) available at nearby Core facilities (UCSD, Salk, Scripps)

EQUIPMENT & SUPPORT

- Ventilated mouse cages
- Centralized cage washing facilities
- Autoclaves
- Anesthesia machines
- Procedure rooms
- Biosafety cabinets and changing hoods
- X-Ray Irradiator (cell and animal)
- Motor and sensory equipment
  - Treadmill
  - 5-Station Rota-Rod
  - Water maze with video camera
  - Hot plate analgesia meter
  - Stereotactic microinjection station
The facility provides *Drosophila melanogaster* and *Caenorhabditis elegans* as model systems. A researcher interested in finding out if their gene or protein of interest has a phenotype can utilize the Facility to discuss their ideas and how to design the experiments. The facility also provides training in how to manipulate the organisms and use the equipment. Staff also provides technician assistance.

**SERVICES**
- Provides *D. melanogaster* and *C. elegans* as model systems
- Consultation and assistance for design of experiments
- Training (organisms handling & equipment)
- Technician assistance
- Ordering of flies and worms (RNAi-lines, mutants etc)
- Provides long time storage of flies and worms

**EQUIPMENT**
- Dissecting Microscope Leica MZ16F
- Apotome Optical Sectioning
- Time lapse Zeiss Imager M1

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**Model Organisms**

**Marco Tamayo**  
Facility Manager  
858-646-3100 x4186

**Rolf Bodmer, Ph.D.**  
Scientific Director

The Tumor Analysis service provides expertise in analysis of animal models of human cancers.

Investigators have access to a wide variety of human cancer cell lines for non-invasive xenograft studies and primary human xenograft models as well as transgenic mouse solid tumors and leukemia models.

**SERVICES**
- Formulation advice
- Maximum tolerated dose
- Patient-derived xenograft (PDX) model support
- Analysis of human tumors in immune deficient mice
- Analysis of mouse tumors in immune competent hosts
- Derive 2D and 3D cell cultures from xenograft tumors
- Tumor and cell line resources
- Technical Training in Animal Tumor methods

**EQUIPMENT**
- Bionano Saphyr Genome Mapping
- Qubit Fluorometer (Life Technologies)
The Cell Imaging Facility broadly supports research programs by providing access to sophisticated microscopes for digital imaging. The facility offers expertise, training, and assistance in advanced biological microscopic imaging techniques and use of complex image processing software. The facility provides well-maintained, aligned, and calibrated microscopic equipment, as well as troubleshooting for equipment and experimental problems.

TEM and SEM are available at the nearby Salk Biophotonics facility.

SERVICES

- Wide-field microscopy, phase and differential interference (Nomarski) contrasts, multi-spectral epi-fluorescence microscopy
- Single and multi-photon laser point scanning confocal microscopy
- High-speed spinning disk confocal microscopy.
- Time-lapse imaging in CO₂ and temperature-controlled environment.
- Recording dynamics of single molecule interactions within single cells. Foster Resonance Energy Transfer (FRET), Fluorescence Recovery After Photobleaching (FRAP), calcium flux imaging and Total Internal Reflection (TIRF).
- 3D and 4D Image rendering and morphometric analysis.
- ETraining and consultation

EQUIPMENT

- Nikon N-SIM super resolution/A1ER confocal system
- Three confocal systems:
  1. Zeiss LSM 710 NLO multiphoton laser point scanning confocal microscope
  2. Olympus FluoView-1000; laser point scanning confocal microscope
  3. Yokogawa Spinning Disk Laser confocal microscope
- Six wide-field fluorescence microscopes with cooled CCD cameras, some with automated XYZ stage, microinjection attachment, CO₂/Temp. controlled chambers
- EVOS FL Auto Imaging system
- Multiple image processing software packages
- Electron Microscopy services available through the Salk Biophotonics Core.
Histopathology

Guillermina Garcia
Facility Manager
858-646-3100 x3552

Brook Emerling, Ph.D.
Scientific Director

The Histopathology core facility provides standard and customized histology services, expertise in pathology and tissue microarray analysis, and routine and special histological stains. The core also provides immunohistochemistry and immunofluorescence capabilities, and powerful analysis tools. The facility maintains and provides training for several pieces of common use equipment: paraffin microtome, cryostat, and cytospin. Training is also provided for basic histological techniques.

Before starting a project, please contact us so that we can help you plan the best course of action. Drop into the lab, call, or email for more information.

SERVICES

- Traditional Histology: Conventional and research-specific custom sectioning and staining.
- Immunohistochemistry: Development of custom protocols (overlay assays, competition assays).
- Laser Capture Micro Dissection (MMI CellCut): Sample Preparation for DNA and RNA extraction, training and assistance.
- Digital Pathology: Electronic data acquisition, data analysis, web based data sharing and archiving of histology results. Network of consulting pathologists.
- Custom Image Analysis and development of novel algorithm-based scoring methods to quantify immunohistochemical and histological parameters.
- Assistance with all aspects of tissue acquisition.

EQUIPMENT

- Aperio Scanscope AT2 and FL systems
- Leica CM 3050 cryostat
- Leica RM 2125 paraffin microtome
- Leica BOND-RX automated system for IHC/ISH
- Leica Autostainer ST5010 for H&E
- Shandon Cytospin 3
- Sakura Tissue Tek vacuum infiltration tissue processor
- Leica EG 1160 paraffin embedding station
- MMI Cell Cut Laser Microdissection system
Flow Cytometry

The facility provides access to high-speed cell sorting and analytical flow cytometry in two locations on the Sanford-Burnham campus. Trained investigators have 24-hour access to a variety of analytical flow cytometers available for independent use. Core staff provide technical expertise, hardware and software training, operate the facility’s cell sorters and are available to assist with analysis experiments for those who prefer to have their samples run by an expert cytometrist.

Scientists planning a flow cytometry experiment are encouraged to consult facility staff for assistance with protocols, fluorochrome selection or other aspects of experiment design.

Yoav Altman
Facility Director
858-646-3106

Ani Deshpande, Ph.D.
Scientific Supervisor

SERVICES

- High speed cell sorting performed by facility personnel
  - Single-cell (clone) sorting into 96 or 384-well plates
  - Simultaneous sorting of up to 4 populations
- Analytical flow cytometry: do-it-yourself 24hr/day, or assisted by appointment
- Imaging Flow Cytometry operated by core staff or do-it-yourself
- Hardware and software training
- Consultation
  - Experiment design
  - Data analysis and interpretation
  - Pre-publication manuscript review

EQUIPMENT

- Analyzers & Sorters
  - Amnis ImageStreamX MarkII imaging flow cytometer, 12 channels, 3 lasers, 3 magnifications & plate loader
  - ACEA Novocyte 3000, 3 lasers, 13-color analyzer with 96-well plate loader
  - BD LSR Fortessa X20, 5 lasers, 18-color analyzer with HTS plate loader for 96 or 384-well plates
  - BD LSR Fortessa 4-laser, 14-color analyzer w/HTS
  - BD FACSCanto 6-color & BD Calibur 4-color Analyzers
  - EMD Millipore Muse Cell 2-color Analyzer
  - BD FACSAria IIu 16-color and BD FACSAriaIIu 15-color high-speed cell sorters in biosafety enclosures
- Countess Automated Cell Counter
- Computer Workstations and data analysis software (FlowJo, ModFit LT, FCS Express, IDEAS)
The Institutional Stem Cell Core has been discontinued, with the functions of the former Core now split into two separate operations:

- **Shared Laboratory**: A well-equipped shared laboratory dedicated to the culture and analysis of stem cells is available to Sanford-Burnham investigators. This shared stem cell lab is managed by Chun-Teng Huang, who also manages the adjacent Viral Vector Core.

- **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 the Snyder lab. For information regarding iPSC projects, contact Evan Snyder.

**EQUIPMENT**

- XVivo Hypoxia Culturing System
- Leica MZ6 Dissecting Stereomicroscopes
- Leica M165 FC Fluorescent Dissecting Stereomicroscopes
- Leica DMI 4000B Inverted Fluorescent Microscope
- Olympus IX71 Inverted Fluorescent Microscope.
- MVE 1830 Eterne Cryostorage System
- Multiple Tissue Culture Hoods and Incubators
The facility provides high-throughput 3rd generation DNA sequencing on the Illumina NextSeq 500. The NextSeq 500 can sequence 20 RNA-seq, or ChIP-seq libraries per day, offers rapid turnaround, with read lengths up to 300 bp, sequencing run times ranging from 14-29 hours, and projects from 25-120 Gb of sequence. Supported sequencing applications include whole transcriptome, ATAC-seq and whole human exome sequencing.

The core also supports single cell applications and is equipped with both a10x Genomics Chromium and BioRad ddSeq single cell isolation systems.

The core provides complete genomic service, from user submitted DNA or RNA to final analyzed data, and all quality checks, library preparation, sequencing, and data analysis are performed by core staff. Additional advanced bioinformatic support is also available.

The core also supports analysis using the Nanostring nCounter, providing sample processing and training. The nCounter is a simple, cost-effective way to profile hundreds of mRNAs, microRNAs, non-coding RNAs, or DNA targets simultaneously with high sensitivity and precision.

**SERVICES**
- NextSeq sequencing
- Single-Cell Sequencing
- Human cell line authentication
- Quality analysis of starting RNA or DNA
- Library Preparation
- Sequencing of samples
- Basic next-generation sequencing bioinformatic analysis
- Advanced bioinformatic analysis

**EQUIPMENT**
- Illumina NextSeq 500 sequencer
- Eppendorf epMotion 7075 Liquid Handling system
- BioRad ddSeq single cell system
- BioRad ZOE fluorescent cell imager
- 10x Genomics Chromium Single-Cell prep system

**Additional analytical equipment:**
- Nanostring nCounter
- 2100 Bioanalyzer (Agilent)
- Qubit Fluorometer (Life Technologies)
- Nanodrop Spectrophotometer

**Shared Instruments**
- 1 Roche LC480 (96 or 384-wells) & 4 LC96 QPCR
- ABI7900HT QPCR (96 & 384-wells)
Functional Genomics

Chun-Teng Huang
Facility Assistant Manager
858-646-31003 x4353

Ani Deshpande, Ph.D.
Scientific Director

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**SERVICES**

- Consultation: provide expert advice on all steps in a functional genomics experiment, from initial design to execution of high throughput projects
- Transfection testing: evaluate cell line transfectability through a battery of transfection reagents in parallel, both for transfection efficiency and impact on viability
- Assay development: cellular assays are taken from the researcher, tested under siRNA transfection or lentiviral transduction conditions, and brought to a 384-well format
- Screen execution: siRNA, lentiviral shRNA, miRNA and ORF collections are available for screening
- Data interpretation (statistics, network analysis)
- Hit reconfirmation and further validation

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**EQUIPMENT and RESOURCES**

- Benchcell work station with Bravo liquid handling platform (Agilent), Wellmate liquid dispenser (Matrix Technologies)
- STAR liquid handling station (Hamilton)
- Micro-plate reader Analyst HT, (Molecular Devices); Envision, (Perkin Elmer)
- High-throughput microscopes (IC100, Beckman-Coulter; INCell 1000, GE)
- Tissue culture facility
- Genome-wide siRNA libraries (Dharmacon OTP) focused libraries, miR agonists and antagonists, cDNA expression
- Developing CRISPR-CAS9 technologies for cell line editing and screening

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Functional Genomics provides the capabilities for RNAi screening, from initial feasibility assessment, all the way through to verification of identified targets. It also serves as repository for reagents and assays, expertise and other technical information, and works towards technical improvements when necessary.

The facility develops image-based cellular assays and adapts them to HTS-siRNA screening in conjunction with the High Content Screening Core and collaborates with the Bioinformatics core facility to aid the screening follow-up process.

The Core’s primary focus has been loss-of-function screening via RNAi (siRNA and shRNA), but gain-of-function screening capabilities (cDNA and miR over-expression) are also supported.
The viral vector core facility at Sanford Burnham Prebys Medical Discovery Institute develops state-of-the-art viral vector-based gene delivery technology. Its portfolio ranges from lentivirus, retrovirus, adenovirus, AAV, VSV, sindbis virus, and Zika virus products as well as customized “The Works” viral vector construction and swapping service package. There are a variety of ready-to-transduce viral vector collections including constitutively expressed or inducible fluorescent proteins for nuclear or membrane localization and bioluminescence reporters for 3D drug screening. The viral core has integrated CRISPRs/Cas technology in the plasmid-free viral vector platform, providing scientists with choices of combined technologies to manipulate genes using CRISPR gene disruption, editing, inhibition, and activation. In addition, we extend technical expertise in nanoparticle research including exosome purification, analysis, and engineering. More importantly, the core actively participates and supports translational research to fight human diseases.
The NMR facility offers capability for NMR studies on proteins, peptides, small molecules and carbohydrates in solution or in solid state. The facility provides the infrastructure for NMR data collection, as well as expertise in data collection and analysis. The core also provides consultation with investigators on the feasibility of NMR for structural studies of protein, as well as help in obtaining binding information by multi-dimensional NMR techniques.

The Crystallography facility is primarily an infrastructure core utilized by experts to produce and analyze crystals. High throughput liquid drop dispensing capabilities coupled with imaging incubators provide the ability to more rapidly identify crystallization conditions, and two independent detectors enhance X-ray analysis.

SERVICES
- **NMR:**
  - Spectrometers maintained for investigator usage
  - Collecting and processing NMR data according to users specifications
  - Training of users for basic NMR experiments
  - Troubleshooting if problems arise
  - Consultation: feasibility studies, optimization.
- **Crystallography:**
  - Crystal structure of small molecules, macromolecules, protein-chemical ligand complexes
  - High throughput capabilities

EQUIPMENT
- 600 MHz Bruker Avance I & III solution, $^1$H/$^{13}$C/$^{15}$N $^1$H-detect cryoprobe: structural & ligand biding studies
- BACS120 auto sample changer on Avance III
- 500 MHz Bruker Avance I, $^1$H/$^{13}$C/$^{15}$N $^1$H-magic angle spinning probe: structural studies of membrane proteins in lipids & insoluble proteins
- 400MHz JEOL JNM-ECS spectrometer, with a 5 mm proton/multi-frequency auto-tune probe, and an auto sample changer – highly automated for chemistry analysis
- Phoenix microdrop liquid handling system and Formulatrix (4° and 22°) imaging incubators (for automated crystal detection).
- Rigaku FR-E SuperBright X-ray generator and two independent detectors.
The Protein Analysis Core provides a variety of analytical services focused on biophysical characterization of structural and functional properties of proteins in solution, under native, non-denaturing conditions. The core performs quality control of protein samples (folding, stability, aggregation) and measure molecular weight of proteins, protein complexes, oligomers and assemblies. It also can characterize protein conformation and shape in solution; determine oligomeric state of protein (including stoichiometry and Kd for self-association) as well as measure protein binding to proteins, peptides, small molecules, compounds, metal ions, lipids, carbohydrates, nucleotides and other ligands (including determination of equilibrium (Kd) and kinetic rate (k_{on}, k_{off}) constants, stoichiometry, binding enthalpy and entropy).
Proteomics

Alex Rosa Campos, Ph.D.
Facility Director
858-646-3100 x4180

Elena Pasquale, Ph.D.
Scientific Director

The Facility provides state-of-the-art mass spectrometry (MS)-based proteomic services for identification of proteins and their post-translational modifications, and quantitative and differential proteomic profiling of proteome samples. Facility staff regularly identifies, validates, develops, and implements new methodologies for identification, quantification and characterization of biologically important proteins. Such approaches can support a mechanistic understanding of cancer and other life threatening diseases, as well as identify new therapeutic targets and diagnostic biomarkers.

The Proteomics Facility also supports users with grant writing proteomics software applications, data analysis, and experimental design of proteomic studies. The facility is continuously expanding its capabilities in large-scale differential proteomics analysis and in protein post-translational modifications for discovery of potential biomarkers and mapping protein profiles.

SERVICES

- Protein/Peptide Identification
- Identification and Localization of Post-Translational Modifications (e.g., phosphorylation, ubiquitinylation, acetylation, methylation, nitrosylation)
- Quantitative comparison of protein abundances in complex mixtures using label-free or label-based techniques (e.g., SILAC, TMT)
- Identification of protein-protein interactions by Affinity Purification followed by Mass Spectrometry (APMS) analysis
- Targeted protein identification and quantification

EQUIPMENT

- Thermo Orbitrap Fusion Lumos with ETD coupled to 2D NanoAcquity
- Thermo Orbitrap Fusion Lumos coupled to NanoEASY 1200
- Thermo Quantiva Triple Quadrupole coupled to NanoEASY 1200
- Thermo Q-Exactive Plus coupled to 2D NanoAcquity
- Thermo Orbitrap Elite coupled to NanoEASY 1200
- Bruker Daltronics AutoflexII MALDI TOF/TOF
- Agilent AssayMap BRAVO Platform for automated protein sample preparation
- Michrom MDLC pump and fraction collector
The Cancer Metabolism Core provides expert advice and analytical services for research in metabolism, both in cancer and other systems. The principal methodology used is gas chromatography-mass spectrometry (GC-MS), for the sensitive quantification of amino acids/ TCA cycle and glycolysis metabolites, fatty acids/cholesterol, short-chain fatty acids, sugars or sugar-phosphates. Other metabolites that are amenable to GC-MS analysis may also be measurable, in consultation with the Facility Director. We also are specialists in stable isotope-based metabolic tracer (13C, 15N, 2H) methods, which allow determination of cellular metabolic fluxes. Metabolites in cells, tissue samples, plasma/ serum and media can be assayed by GC-MS.

In addition, the YSI analyzer allows a rapid and inexpensive analysis of metabolic function in cells via comparative glucose, lactate and glutamine measurement, and relative oxidative and glycolytic activity of cells can be measured using Seahorse analyzers.

SERVICES
- GC-MS-based quantification or stable-isotope-labeling analysis of metabolites including amino acids, keto acids, fatty acids, cholesterol, short-chain fatty acids, sugars, sugar phosphates.
- Rapid measurement of major metabolites (glucose, glutamine, lactate, glutamate) in medium samples using the YSI 2950 analyzer.
- GC-MS and YSI analyses are mostly full service, while user training on the Seahorse analyzer is provided for SBP users.

EQUIPMENT
- GCMS-QP2010 Plus for metabolite quantification and metabolic flux analysis. Includes negative chemical ionization option for highly sensitive detection.
- YSI 2950 metabolite analyzer, to measure glucose, glutamine, lactate, and glutamate in media samples in 96-well format.
- Seahorse XFp to measure the two major energy producing pathways of the cell – mitochondrial respiration and glycolysis - in real-time, with automated injection of metabolic substrates or inhibitors.
- Shimadzu Prominence HPLC for bioenergetics and other small molecules analyses.
The Bioinformatics Core provides a cutting-edge computational and systems biology support to the institute. We specialized in omics data analysis, multi-omics data integration, network and pathway analysis, and machine learning.

In the Bioinformatics Core, we have built automated computational pipelines using state-of-art software packages to QC, align, summarize, statistical analyze, and visualize NGS data sets. We will provide different levels of data analyses based on the complexity of your data sets. This may include multi-omics data integration, customized pathway and network analysis, and hypothesis driven in-silico drug discovery.

Visit our internal website for more details.

http://intranet/researchsupport/sr/bioinformaticsLJ/Pages/Home.aspx
Conrad Prebys Center for Chemical Genomics

**Drug Discovery Resources**

Eduard Sergienko, Ph.D.
Assay Development Facility Director
858-646-3100 x3462

The Assay Development facility provides support in design and development of new and optimization of existing high-throughput screening assays, as well as for Structure-Activity Relationship (SAR) studies of hits obtained in primary screening, and can advise and help in preparation of data packages for screening-related grants.

Ian Pass, Ph.D.
High Throughput Screening Facility Director
858-646-3100 x5453

The High-Throughput Screening core facility provides infrastructure for large chemical library screening of biological targets with diverse screening instrumentation, compound libraries (see below) and expertise on high throughput screens and automation.

Susanne Heynen-Genel, Ph.D.
High Content Screening Facility Director
858-646-3100 x3329

The High-Content Screening core facility provides assay development, screening, and data analysis/mining expertise and services for high content screens using high-throughput microscopy systems. The core can also develop and conduct phenotypic assays, from functional assays, to transcriptional reporter modulation. Additionally, it can combine resources with the and Functional Genomics core for RNAi screening projects.

SERVICES and RESOURCES

- Design and development of cell-based and biochemical assays in diverse plate formats and detection platforms
- Full-scale capabilities and infrastructure providing rapid screening on a broad diversity of assays and detection platforms
- Several fully integrated industrial-scale high-throughput screening (HTS) workstations
- HTS microscopy/HCS and novel algorithm development for image analysis
- Full Hit-to-Lead chemistry and exploratory pharmacology
- Powerful NMR based Chemical Fragment Screening
- Highly integrated informatics infrastructure and efficient data mining capabilities
- Close ties with Protein production facility
- Cell production facility for scale-up tissue culture, including ES and iPSC capabilities
- Project management
- Support of projects performed by either PI laboratory or CPCCG personnel
- Full support of grant applications for available funding mechanisms

For general inquiries:

Michael Jackson, Ph.D.
Senior Vice President, Drug Discovery and Development
858-795-5201
CHEMICAL LIBRARIES

A wide array of compound libraries are available for collaborative projects performed at CPCCG.

- Spectrum Collection: 2,000 compounds of known drugs, Microsource Discovery Systems.
- LOPAC Collection: 1,280 pharmacologically active compounds from Sigma-Aldrich.
- Kinase Inhibitor Collection: 244 known kinase inhibitors compiled from EMD Biosciences.
- US and International Drug Collections: 1,040 drugs in clinical trials in the USA and 240 non US drugs from Microsource Discovery Systems.
- Greenpharm Natural Product Collection: 240 known phytochemicals sold by Prestwick Chemicals.
- Prestwick Chemical Library: 1,200 small molecules, 100% being marketed drugs.
- StemSelect Collection: 303 pharmacologically active, structurally diverse modulators of cell fate from EMD
- Bioactive Lipid Library: 201 bioactive lipids from ENZO Life Sciences.
- Natural Product Library: 502 compounds from ENZO Life Sciences
- REDOX Library: 80 compounds with defined prooxidant or antioxidant activity from ENZO Life Sciences.
- InhibitorSelect Signal Pathway Collection: 64 compounds from EMD, target several signal pathways
- MLSMR Library: About 360,000 compounds, compiled for MLPCN, 95% with molecular weight ≤500Da.
- Sanford-Burnham HTS Library: About 320,000 compounds for general HTS screening selected from a pool of over three million compounds from five major chemical vendors (ChemBridge, Asinex, Enamine, Life Chemicals, and ChemDiv) using cheminformatics selection strategies.