SANFORD-BURNHAM CHEMICAL LIBRARY SCREENING SHARED RESOURCE

A Core shared through the C3 collaboration

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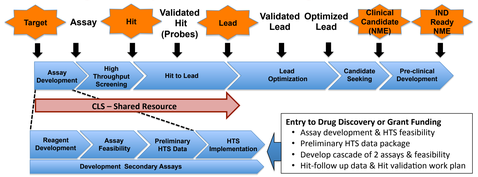
OVERVIEW

The Chemical Library Screening (CLS) resource provides Cancer Center scientists with the ability to develop and conduct large-scale chemical library screens for the identification of selective probes and early leads for drug discovery. High-throughput screening-based integrated chemical probe and lead discovery is not widely available at academic institutions; thus, this resource provides cancer researchers with rare access to technology, expertise and infrastructure resources to develop novel means for characterizing cellular targets. The resource also incorporates Medicinal Chemistry for follow-up and optimization of screening leads.

Drug Discovery and Development: Stages and Milestones

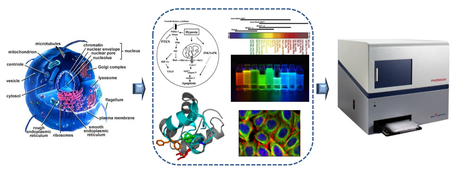


CLS consists of 4 specialized but highly integrated core facilities: ***High Throughput Assay Development*** (HT-AD), ***Compound Management and HT Screening*** (HTS), ***High Content Screening*** (HCS), and ***Medicinal Chemistry*** (Med Chem).

SERVICES

High Throughput Assay Development (HT-AD):
- Design and development of HTS assays. relying on the properties of studied biological systems, available technological tools and specific goals of the project.
- Feasibility studies, assay miniaturization.
- Support hit validation and optimization studies (secondary assay, SAR & MOA studies).

High Content Screening (HCS):
- Help conceptualize image-based assays.
- Optimize assay biology, develop algorithms and read-outs, perform assay miniaturization / validation, execute screen.
- Perform image data management, HC data analysis and visualization.

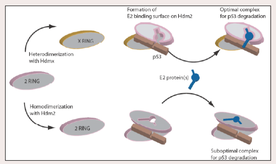


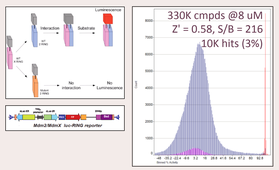
High Throughput Screening (HTS):
- Compound libraries design and support.
- Acquisition and storage of small-molecule compound collections.
- Advice and help with selecting HTS compound collections; preparation of assay-ready compound plates for HTS users.
- Instrumentation support (maintenance, users training).
- High throughput screening (miniaturization, automation, data analysis & hit identification).

Medicinal Chemistry (MedChem):
- General synthesis of small molecule compounds.
- Synthesis of analytical tools for development of HTS/HCS assays
- Medicinal chemistry (synthesis of reference compounds, structure based drug design, lead optimization).
- Analytical chemistry (NMR, LC/MS).

Example - SBMRI – Salk Institute Collaboration:
SMALL MOLECULE INHIBITORS OF MDM2 / MDMX INTERACTION [PI: Geoffrey M. Wahl / Salk Institute]

Mdm2 and MdmX are members of the RING domain E3 ubiquitin ligase family of proteins. Although Mdmx has no intrinsic ubiquitin ligase activity, it could form heterodimers with Mdm2 enhancing its activity. One of the critical substrates of Mdm2/MdmX is p53 tumor suppressor, that undergoes degradation upon ubiquitination. A wild type p53 is retained in ~50% of human tumors, many of which overexpress Mdm2 and/or MdmX. Thus, disruption of Mdm2/MdmX hetero-oligomerization appears as attractive strategy of p53 reactivation of p53.
Cell-bases assay with split-Firefly luciferase fused to RING domains of Mdm2 and MdmX in Saos2 cell was established in 1536-well plates and utilized for primary HTS against MLPCN compound library. Hits were profiled in counter-screen assays monitoring spectral interference, cytotoxicity and disruption of RING-domain of BRCA1 and BARD1 interaction.





Mdm2/MdmX Split-Fluc reporter assay
330,000 cmpds @8 uM ➡ 10,058 hits

Mdm2/MdmX Split-Fluc reporter assay
10,058 hits ➡ 6,226 conf. hits (62%)

Spectral Interference and Cytotoxicity
6,246 hits ➡ 883 specific hits (14%)

883 specific hits ➡ 704 DPI liquids (80% availability)

Mdm2/MdmX Split-Fluc assay

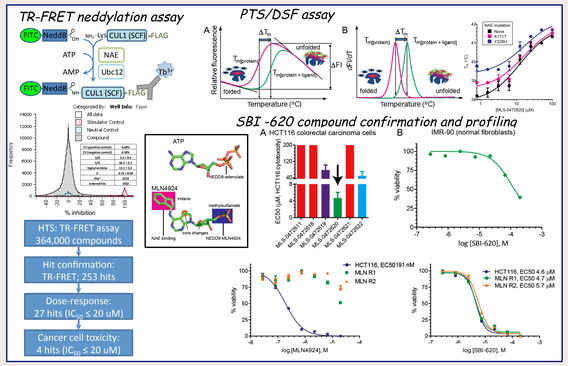
BRCA1/BARD1 Spilt-Fluc Assay

Compound ID	Structure	Mdm2/MdmX IC_{50} [uM]	Cytotox IC_{50} [uM]	BRCA1/BARD1 IC_{50} [uM]
MLS-0215195		0.004	2.4	0.004

PubChem BioAssay AID 504668 (http://www.ncbi.nlm.nih.gov/pcassay).

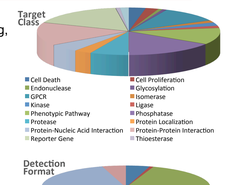
Example – Functional Biochemical Assay:
IDENTIFICATION OF CULLIN NEDDYLATION INHIBITORS [PI: Matt Petroski / SBMRI]

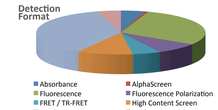
Cullin-RING ubiquitin ligases (CRLs) are responsible for ~20% of all proteasomal protein degradation and linked to multiple diseases, e.g. cancer. CRLs are activated by covalent modification with the ubiquitin-like protein NEDD8; their activity is essential for cancer cell survival. NEDD8-activating enzyme E1 inhibitor MLN4924 selectively induces cancer cell death by triggering S-phase DNA re-replication and inhibits tumor growth in xenograft studies, validating E1 as a drug target. MLN4924 is undergoing clinical trials and shows promising results. Recent studies by PI lab show that cancer cells acquire resistance to MLN4924 suggesting a need for E1 inhibitors with different mode of action. We designed a biochemical CRL neddylation assay and utilized to screen MLPCN library. A biophysical protein thermal shift (PTS)/differential scanning fluorimetry (DSF) assay was developed and utilized for hit confirmation and profiling. Identified compound demonstrates binding to MLN-resistant E1 variants and potent cytotoxicity in MLN-resistant cells.



Broad Expertise in HTS Assays

- Cell-based phenotypic or functional (plate reader or imaging): cell migration, chemotaxis, ECM-protein binding, cell retraction, invadopodia formation, phagocytosis, calcium-/voltage-kinetics
- Cell-based signaling (plate reader or imaging): GPCRs, NFkB, kinases
- Biochemical
- Protein thermal shift





- 2005 – 2013 NIH MLPCN Center:
  - **138 completed MLSMR library screens**
  - 50% biochemical, 38% cell-based pathway , 12% image-based high content screens
  - 52 probes identified
  - ➲ Accumulation of expertise for diverse range of assays

EQUIPMENT

- The core robotic system: plate hotels, multimode readers and a liquid handling robot. Multiple readouts available (FP, FI, Time TRF, Delfia, FRET, BRET, TR-FRET, Lance, LanthaScreen, HTRF, Bio- and Chemi-Luminescence, Absorbance and AlphaScreen). Two plate washers enable development of heterogeneous ELISA-type assays.
- Integrated robotics system HighRes Biosolutions capable of 1536 well plate screening with 200,000 wells/day throughput.
- Hamamatsu FDSS7000 fast-kinetics system.
- Three HCS imaging systems (Brooks Celigo, Vala Sciences IC200, Perkin Elmer Opera QEHS system) enabling the following imaging modalities: live cell imaging (time-lapse & kinetic mode), wide-field and spinning-disc-confocal fluorescence, brightfield, 3D z-stacks, whole well imaging, 3.5x to 60x objectives with 0.12 to 1.2 NA (low to high optical resolution)
- Advanced HCS image analysis and image-data management infrastructure.
- Chemical synthesis equipment including fully automated microfluidic and microwave synthesizers.
- Analytical chemistry systems including 2 LCMS instruments and a bioanalytical triple-quadropole mass analyzer.

Example - Phenotypic Image-based High Content Screen:
PHENOTYPIC SCREEN FOR ACTIVATORS OF PML-ONCOGENIC DOMAINS [PI: John Reed / SBMRI]

Promyelocytic leukemia protein (PML) is a tumor suppressor involved in the regulation of various cellular processes, including cell cycle, DNA repair, and p53-dependent and p53-independent proapoptotic pathways. PML function is correlated with its localization to discrete nuclear speckles termed PML oncogenic domains (PODs, also called PML nuclear bodies). POD formation is associated with pro-apoptotic and anti-viral states. Dissolution of PODs is induced by oncogenes and viral gene products, and is associated with an anti-apoptotic state. Among the stimuli that induce POD-localization are interferons and arsenicals, agents currently used in the treatment of cancer and viral disease. POD formation may be used as a new therapeutic strategy for cancer. While arsenic trioxide and its derivatives are established activators of POD formation, these agents suffer from several limitations (non-selectivity results in numerous off-target effects, not all patients respond to treatment, high relapse potential, undesired pharmaceutical, pharmacokinetic and toxicological properties). The aim of the project was to identify and optimize non-arsenical chemical inducers of PODs that can restore the PML tumor suppressor pathway.

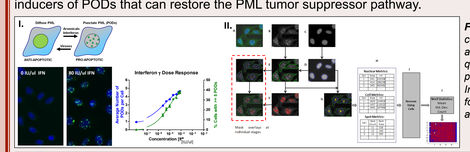


***Figure: I** – Assay concept using PML-antibody to visualize and quantify number of POD-positive nuclear foci; **II** – Image analysis algorithm for POD nuclear foci assay.*

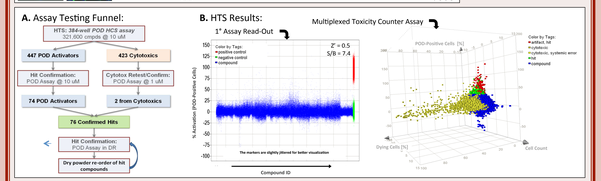


***Figure: A** – Testing funnel with compound results; **B** – HCS data showing 2D scatter plot of primary assay read-out and 3D scatter plot with multiplexed cytotoxicity assay results added.*

Related Publication: Yip KW, et al. Journal of Biomolecular Screening. 2011; 16(2):251-258. PMID: 21233309.

Example - Functional Imaging Assay:
384-WELL FORMAT CELL MIGRATION ASSAY [PI: CLS Core Facility / SBMRI]

One of the hallmarks of numerous types of aggressive cancers is an increase in cell migration activity, which is required to move from the primary tumor to metastatic sites. Discovery of novel anti-metastatic drug leads (e.g. migration inhibitors) relying on signaling pathway target-based approaches has often been hindered by the existence of compensatory or redundant mechanisms in the tumor cells, which allow the cell to maintain its increased migration or invasion activity. Therefore, we optimized and validated a functional cell-based assay to evaluate cell migration in an assay format amenable to medium-high throughput. This allows cancer center investigators to test new drug leads early-on in the drug discovery process and more importantly test combinations of early leads targeting multiple redundant pathways. We tested a commercially available 384-well format cell migration assay plate from Platypus Technologies using an image cytometry detection system with HT1080 cells using several different media conditions and image analysis algorithms. The optimized assay used serum-starved cells to slow cell proliferation and measure the true migration potential of the cells. Best results (Z' > 0.5) were achieved using an image analysis algorithm that was able to detect the true zero timepoint "empty" migration area rather than employ a standardized mask for all wells. The assay was validated using a small bio-active library.

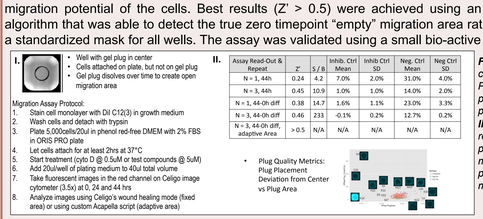
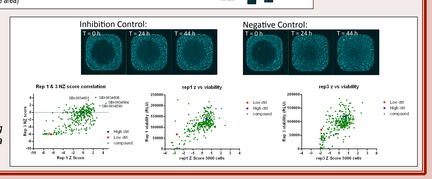


***Figure:** Assay results from small pilot screen. Top: example images of inhibition and vehicle (negative) control wells at 0, 24, and 44 hour time points. Bottom: results of two repeats with left panel showing repeatibility, and middle and right panel showing wound healing vs. viability data for each of the repeats.*

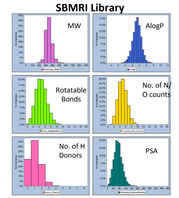


Compound Libraries & Management

- Acces to ~900,000 screening samples

Commercial Small Molecules:	
SBMRI (In House):	325,000
LOPAC:	1,280
ChemBridge:	50,000
MS Spectrum:	2,000
NCI Diversity Sets:	2,869
Current MLSMR (NIH):	365,120
Specialty Collections:	
FDA Approved Drugs:	1,040
EU Approved Drugs:	240
NCI Clinical/Onc collection:	535

Specialty Collections:	
Kinase Inhibitors:	874
Protease Inhibitors:	53
Inhibitor select (EMD):	64
StemSelect (EMD):	303
Natural Products:	
Harbor Branch Peak Frctn*:	7,360
Magellan BioSciences*:	5,120
Nat X:	5,440
National Cancer Institute:	148,258



Cancer Centers Collaborations

- ***The C3 collaborative agreement facilitates scientific interactions between scientists of the 3 NCI-designated cancer centers (UCSD, Salk, SBMRI) by promoting enhanced access to specialized core facilities. To start, each Center has contributed one unique core to the C3 collaboration, with CLS being the Sanford-Burnham core.***
- Sanford-Burnham has performed CLS services for 4 UC San Diego Cancer Center PIs, and and project are under discussion with a number of additional Cancer Center members.

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