Published in final edited form as:

Future Med Chem. 2011 July ; 3(9): 1101–1110. doi:10.4155/fmc.11.84.

The University of Kansas High-Throughput Screening Laboratory. Part II: enabling collaborative drug-discovery partnerships through cutting-edge screening technology

Peter R McDonald¹, Anuradha Roy¹, and Rathnam Chaguturu^{1,†}

¹High-Throughput Screening Core Facility, Structural Biology Center, University of Kansas, Lawrence, KS 66047, USA

Abstract

The University of Kansas High-Throughput Screening (KU HTS) core is a state-of-the-art drug-discovery facility with an entrepreneurial open-service policy, which provides centralized resources supporting public- and private-sector research initiatives. The KU HTS core was established in 2002 at the University of Kansas with support from an NIH grant and the state of Kansas. It collaborates with investigators from national and international academic, nonprofit and pharmaceutical organizations in executing HTS-ready assay development and screening of chemical libraries for target validation, probe selection, hit identification and lead optimization. This is part two of a contribution from the KU HTS laboratory.

In 2008, the University of Kansas High-Throughput Screening (KU HTS) core was reborn in a brand new 4500-square foot laboratory housed in the Structural Biology Center, a new addition to KU. KU reinvigorated the HTS core infrastructure by recruiting a new director and a team of highly skilled scientific research and project managers with both industrial and academic screening experience, and a host of cutting edge screening resources. Since the goal from the start was drug discovery and development, the KU HTS core was structured and designed to optimize that process using the pharmaceutical industry's best practices. It comprises a 14-member team covering the research, administration and information technology aspects of the screening core. In addition to the KU HTS staff, director and project managers, the KU HTS core benefits from an in-house informatician, biostatistician and cheminformatician. Several program assistants and undergraduate interns also provide invaluable support to the KU HTS core. The team has extensive background in HTS-related fields including HTS, high-content screening (HCS) and small interfering RNA (siRNA) screening, as well as experience in clinical and environmental sample testing, immunoassays, flow cytometry, cell proliferation assays, neurophysiology, ion channels, G-protein coupled receptors (GPCRs), gene expression, protein purification and post-transcriptional control. Our efforts are founded on the latest advances in the scientific understanding of cell-signaling processes and disease states. Our research is supported by cutting-edge technology and laboratory automation, including an impressive collection of

© 2011 Future Science Ltd

[†]Author for correspondence: Tel.: +1 785 864 1717, Fax: +1 785 864 8141, rathnam@ku.edu.

Financial & competing interests disclosure

The University of Kansas High-Throughput Screening Laboratory is a University of Kansas Institute for Advancing Medical Innovations and Cancer Center Shared Resource, and is funded in part by the National Institutes of Health/NCRR Centers of Biomedical Research Excellence grants P20 RR015563 and P30 RR030926 (principal investigator, Barbara Timmermann). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

over 11 liquid handling robotic platforms and 17 detection systems, several novel label-free technological platforms, automated acoustic nanoliter dispensing, confocal automated microscopy for HCS, an ever expanding collection of more than 180,000 small molecules and plant extracts, a modest siRNA library, sophisticated compound-storage and retrieval system, and an in-house laboratory-information and data-management system.

Fostering collaborations for drug discovery

The screening projects undertaken/executed by the KU-HTS core are obtained through collaborative partnerships with both the academic institutions within and outside the state of Kansas, and the industry (Box 1) [1]. The project entrepreneurship is supported by strong project management and direction, to expand the application of HTS and HCS for disease-related drug discovery, both internally and externally, to enable new drug treatments and therapies (Box 2). These collaborations require effective communication to translate the basic research of principal investigators into effective disease targets for screening, by means of either assay development by KU HTS, or assay transfer and miniaturization. A regular role of the KU HTS core is to provide pilot data for grant applications, as well as collaborating with principal investigators to submit R03 or R21 grant applications through the NIH's Molecular Libraries Probe Production Centers Network program [101]. For these grant applications, many principal investigators need assistance in developing and validating assays, to provide pilot screening data demonstrating assay readiness for grants. Other collaborations have led to NIH Fast Track submissions for screening at Molecular Libraries Probe Production Centers Network screening centers.

Box 1

University of Kansas High-Throughput Screening collaborations, both active and prospective

The University of Kansas High-Throughput Screening Laboratory core has an even division of internal and external collaborations, past present and prospective. Through these collaborations, the core provides not only screening services but also consultation including project discussions, letters of support, grant application write-ups, pilot data generation and project execution. These collaborations are with:

- Abbott
- Caravan Ingredients
- Dartmouth
- Edenspace
- Heartland Plant Innovations
- Jubilant
- Kansas State University
- LIMR Chemical Genomics Center
- Myelin Repair Foundation
- Northwestern University
- Queen's Med Center
- Sigma Aldrich Fine Chemicals
- Stowers Institute for Medical Research

- Temple University
- Tulane University
- University of Kansas, Lawrence
- University of Kansas Medical Center
- University of Missouri Kansas City
- University of Nebraska
- University of Texas at Dallas
- Wake Forest University
- Washington University
- XenoTech

Box 2

Sampling of research and disease areas addressed by University of Kansas High-Throughput Screening Laboratory

- Anti-infectives
- Antioxidants
- Cancer
- Cardiac muscle
- Diabetes
- HIV infection
- Myelin repair
- Sickle cell anemia
- Stem cell differentiation
- Stem cell growth
- Wound healing

Medicinal chemistry

The University of Kansas is one of the very few institutions where the HTS center co-exists with several medicinal chemistry support laboratories. The medicinal chemists actively participate in selecting and synthesizing novel compounds for the HTS compound collection. The KU HTS compound collection includes more than 3000 compounds synthesized by Jeff Aube's Center for Chemical Methodologies and Library Development (CMLD), which is located in the same building as the KU HTS laboratory. The laboratory of Barbara Timmerman has deposited over a thousand fractionated natural products in the KU HTS laboratory's growing natural product compound collection. The chemists also provide compound analytical services to the HTS laboratory, and are involved in the synthesis of material for assay set-up and optimization, and also in the scale-up synthesis for enabling a primary HTS campaign. For example, synthesis of fluorescent dyes for enzymatic assays or compound(s) required for sustaining basic cell growth in culture. In some instances, the chemists also weigh into the structure and use of a compound for positive-control selection.

After the identification of primary screen actives, the medicinal chemists support the HTS laboratory and its clients with advice on hit triage, selection of scaffolds for further study, *in silico* compound mining and molecular modeling. Thus, the close interaction of the KU HTS laboratory with the medicinal chemistry department at all stages of a high-throughput assay development is a unique feature for an academic drug-discovery infrastructure.

Chemical-library management

The compound library at the KU HTS core includes over 180,000 diverse compounds, a growing collection of over 1000 natural products, and a siRNA library of 2157 siRNA duplexes for use in target identification and validation. The size of commercially available chemical libraries has grown collectively to over 11 million compounds, with considerable overlap between the libraries, from dozens of commercial sources [2]. The KU HTS collection of small organic molecules was carefully selected from several of these commercial vendors, targeting successful identification of lead compounds for use in screening projects. In addition to using the Lipinski rule in the selection process, the HTS core also drew upon the vast drug-discovery experience of professors in the Medicinal Chemistry department at KU in selecting these compounds. This library of compounds is optimized for structural diversity and drug-like properties. The largest compound sets in the library include a ChemBridge Library and a ChemDiv Library, which are both diverse library sets. Recent additions include new plant extracts, both from within and outside of the USA, and a collection of US FDA-approved drugs, internationally approved drugs, and acquisition of the NIH clinical collections. The FDA-approved drug collection is a rich source of therapeutic potential, and provides ample opportunity for repurposing small molecules for new disease applications [102]. The NIH clinical collections represent 727 compounds that were assembled by the NIH through the Molecular Libraries Roadmap Initiative as part of its mission to enable the use of compound screens in biomedical research [103]. Similar to the FDA collection and the international drug collection, the NIH clinical collection provides the researcher with data from their use in human clinical trials. These new clinically tested libraries are highly drug-like and have known information on their safety provides, providing invaluable starting points for postscreening medicinal chemistry optimization and application to new disease targets. The KU HTS library also includes a very valuable collection of more than 15,000 unique natural compounds, including a set of information-rich molecules developed years ago through the intuition and experience of world-class medicinal chemist Joseph Burckhalter. The Burckhalter compounds were recently discovered and donated by his family, to honor the late professor Burckhalter, who was an inductee in the National Inventors Hall of Fame [3].

Our compound library collection is currently being imported into a newly installed compound-management system, the Universal Labstore™ from Nexus Biosystems, Inc, funded by a grant from the Kansas Biosciences Authority. The Labstore is a large 590-cubic foot compound storage system, occupying over 73 square feet of floor space. The Labstore is a laboratory system for high-volume storage and retrieval of chemical compounds and biological samples in a cold, nitrogen-purged environment at -20°C and 10% oxygen. Nitrogen is pumped into the system to displace oxygen to reduce humidity and prevent hydration of DMSO samples. The Labstore is composed of robotic systems for internally managing samples, internal imaging devices including both a video camera and a high megapixel camera, a large -20°C freezer storage area, a heated 30°C storage area, computer controllers, user interfaces, external condensers, and sensors and alarms to alert and regulate safe temperatures and oxygen levels. The new high-resolution camera allows capture and instant reading of an entire rack of 384 individual 2D-barcode tubes with a single photo. The Labstore also includes a special module for cherry picking and rearranging 2D-barcode tubes from a 384-tube rack.

The Nexus Labstore stores compounds across 5616 plates in 384-well format, for a maximum capacity of 2,156,544 wells, with the option of quadrupling this storage with the use of 1536-well plates. The Nexus Labstore is very flexible, with the ability to hold plates, tubes and dram vials. Its shelves can hold either 5616 SBS-footprint plates or 74,880 dram vials, or any combination of the two, as the system intelligently sorts and defragments the shelves to maximize storage capacity. A benefit of the system is that its modular nature fits well with our growing needs, as it can be upgraded with new stations, storage units and modules in the future.

The compounds in our small-molecule library are solubilized in DMSO stock solutions. During compound storage, many compounds fall out of solution. Mixing compounds in 384-well plates does not sufficiently solubilize all compounds, and many compounds are heat-sensitive, preventing heat as a means of compound dissolution. To avoid heat and ensure full dissolution of all compounds, the KU HTS core uses adaptive focused acoustics through the Covaris L8 system to mix and dissolve compounds in DMSO, in their original 384-well storage plates. The Covaris L8 is an adaptive focused acoustics instrument that uses high-powered acoustic waves to dissolve the compounds. Compound dissolution ensures maximum compound activity by ensuring homogenous samples in DMSO [4].

Screening-related instrumentation & assays

The HTS laboratory is equipped with robotics for liquid handling, compound management, cell culture, plate readers, microscopes and other instrumentation necessary for HTS and HCS. In addition to the HTS- and HCS-related instrumentation listed in Table 1, the HTS core also includes basic research equipment, such as automated multichannel pipettes, Western blotting systems and other standard laboratory instruments (Table 1). To accommodate the cell-based screens performed at the KU HTS laboratory, the laboratory is fitted with six cell-culture hoods and six cell-culture incubators, including a quarantine hood and quarantine incubator for newly introduced and primary cell lines. Cell lines are purchased new from American Type Culture Collection to confirm validity and prevent contamination. Transfected cell lines and primary cell lines from collaborators are kept under quarantine conditions while awaiting mycoplasma testing, to prevent contamination that could mimic false positive results or infect other cell lines. The automated liquid handling instruments are coupled to plate-loading devices and robotic arms, to load microwell plates. Liquids are transferred through different means depending on the volumes being transferred. Nanoliter volumes, such as compound addition, are transferred using acoustic technology for noncontact dispensing of solvated compound stocks, eliminating carryover or contamination of compounds with each other, and to minimize cost through the complete absence of consumables. More conventional bulk dispensing cartridges, cassettes, disposable tips and stainless steel 384-head syringes are used for larger volumes, such as dispensing cancer cells in suspension across all wells of a screening batch of plates.

Most assays performed in the KU HTS core are biochemical and cell-based screening assays, requiring label-based signal detection platforms. The most heavily used readers include a filter-based reader (Perkin Elmer Envision) and a monochromator (Tecan Safire2) for measuring absorbance, fluorescence, luminescence, and AlphaScreen assays. These instruments are used for both kinetic assays and end-point assays, for both biochemical and cell-based assay types. For higher resolution plate and well reading, the high-content BD Pathway platform provides resolution at a subcellular level through automated microscopy and analysis, allowing quantification of protein presence and activity on a cell-by-cell basis, through fluorophores, fluorescent proteins and fluorescently labeled antibodies and stains. HCS is valuable for both primary screening as well as a source of rich information for secondary assays and determining mechanism of action of hit compounds. In addition to

automated microscopy and plate readers, the KU HTS laboratory is also equipped with a range of specialty instruments, including plate delidders, sealers, desalers, acoustic mixers and label-free readers (Table 1).

The KU HTS core recently acquired a Hamamatsu FDSS 7000 functional drug-discovery system, a novel imaging-based plate reader with fluorescent and luminescent capabilities for use in ion channel and GPCR research. Several disease states are related to dysfunctional ion channels and GPCRs, and image-based plate readers have become the industry standard for use in this area of research. Previously, faculty members at the KU Lawrence campus and the Medical Center in Kansas City did not have the ability to conduct cutting-edge GPCR and ion channel research afforded by the FDSS 7000, as no such instrumentation is available anywhere in the state of Kansas, or in the neighboring states of Missouri, Oklahoma and Nebraska. The FDSS 7000 will fill a substantial need of many researchers in the Midwest for high-throughput analysis of GPCRs, calcium immobilization, membrane potential, ion influx, and Aequorin and luciferase assays in mammalian cells.

The majority of compound library screens performed at KU HTS rely on solvated compound dispensing using the Labcyte ECHO 550, an acoustic liquid handler. The ECHO uses acoustic energy to eject compounds from a source plate to the destination assay plate. Acoustic transfer occurs very quickly, allowing an entire 384-well plate to be transferred in 2–4 min (depending on volume), comparable to the time required for a conventional tip-based instrument to load tips, aspirate and dispense. The ECHO allows precise and accurate transfer of volumes as low as 2.5 nanoliters, and larger volumes through increments of 2.5 nanoliters. The airborne transfer of compounds from the source plate to a facing (inverted) destination plate prevents cross-contamination and avoids the problem of liquids sticking to tips. The ECHO allows transfer of any source well to any destination well, so it can easily cherry pick and perform serial dilutions and dose responses quickly, without a need for intermediate plates, and can dispense to any plate format, including 1536- and 3456-well plate formats.

Label-free HTS for orphan targets

Currently, HTS labs in general are focused on label-based technology, and even among the NIH Roadmap Initiative's Molecular Libraries Program institutions, only Johns Hopkins Ion Channel Center has the capacity to make use of high-throughput label-free technology [104]. The use of fluorescence and luminescence labels restricts research to a simplistic biological assessment with just point-of-contact measures and one signaling pathway per ligand–receptor complex. However, therapeutic targets do not function in isolation, but operate in a systems biology context involving a complex set of integrated biochemical pathways. High content partially addresses this need, but at the expense of labels, antibodies and other cellular perturbations or requirements. There is a tendency to use engineered cell lines over the native cells, primarily to overexpress the target of interest and hence boost the signal read-out; but this creates an unnatural stoichiometry between the target under study and the rest of the cellular components. Label-based screening methods are limited by the availability of easily adaptable technology formats to de-orphanize the highly refractory targets, resulting in a huge portion of the genome and therapeutic landscape remaining untouched. The KU HTS laboratory hopes to fill this niche through increased use of label-free screening. One of our projects required label-free screening due to the lack of antibodies and research reagents for the novel protein target. We used a high-throughput 384-well surface plasmon resonance label-free instrument, the AP-3000 from Fujifilm, to screen a 5152-compound library in qHTS mode, identifying over a dozen compounds with > 50% binding to the orphan protein at submicromolar concentrations. In the absence of research on

this novel orphan protein, this screening and compound identification would not have been possible without label-free screening.

K-Screen laboratory-information management system

The KU HTS core employs an in-house developed laboratory information management system (LIMS) system, K-Screen, for database management [105]. K-Screen is a comprehensive, extensible, and user-friendly software package for HTS data management, analysis, mining and visualization [5]. K-Screen was developed based on the standard open-source Linux/Apache/MySQL/PHP platform, utilizing a number of open-source software programs (Table 2), and the software itself will soon be released to the public at the K-Screen website [105] as open-source software. K-Screen supports a diverse array of plate readers and has an automated statistical-analysis routine. Once a set of raw data is imported, K-Screen automatically performs statistical analysis and calculates 'z' factors, medians of positive/negative controls and samples. Currently, activities are normalized using four methods: percent of control, normalized percent inhibition, Z score, and plate median normalization. Heat maps of all normalized data are generated. K-Screen fits the dose-response data automatically to four-parameter Sigmoid or Brain-Cousen curves. It also allows manual adjustment of all curve parameters and labeling outliers. HTS applies a normalization method and accepts/rejects plates based on data quality. K-Screen also allows easy searching of PubChem, and is being actively used by the KU HTS laboratory and its clients as their primary LIMS. Further development of the K-Screen HTS LIMS system will continue to support KU HTS operations, and future developments include integration of more data-analysis tools, better integration with PubChem, enhancements to dose-response curve fitting, and plate-based quality-control reporting.

Screening workflow & data handling

The HTS core facility at KU will screen an average of 20 therapeutic targets per year in both small- and large-scale screens, both biochemical and cell-based (Figure 1). Most of the targets pursued by the KU faculty are novel and often where there are no bona fide assays developed. In the absence of validated assays, HTS personnel spend much of their time in developing novel assay-technology platforms.

The primary objective of the HTS process is to find 'active' compounds, usually screening at a single concentration. The objective of subsequent work with 'actives' is to confirm the activity, and to differentiate 'actives' from 'inactives' by employing potency and efficacy threshold values. The compounds that pass through this stage are referred to as 'hits', which then could be developed as molecular probes to investigate mechanistic aspects of the biochemical target site. The goal of HTS data analysis is to discriminate actives from inactives, relate biological similarity to chemical similarity, identify tractable hits from the screen based on medicinal chemists' 'expert' knowledge, and identify multiple series of compounds that make attractive starting points for quantitative structure-activity relationship-based optimization (improvements in the hit quality), and manage the downstream capacity for the available chemistry resources.

In a typical screening project, an incoming assay addressing specific biology is optimized in HTS format for robustness, sensitivity and cost effectiveness, aiming to minimize the processing steps to eliminate variability and false screening hits, based on principles from the NIH Chemical Genomics Center Assay Guidance Manual [106]. For a typical cell-based assay, we create a bank of mycoplasma-free cells of at least 25 cryovials at the same passage number to minimize variability during screening batches, and cell-seeding density will be determined using a growth curve. Biochemical assays require their own steps of assay

development and optimization, such as titrating substrate or enzyme, or determining optimal temperatures or sensitivity of proteins. During assay development, we test the assay using known modulators to confirm the validity of the assay. The individual steps of a new assay are tweaked as necessary to optimize the assay, maximizing signal while reducing noise. A suitable assay will have a high 'Z' factor above 0.70, and a low coefficient of variation. Before a large screen, assay variability will be measured with controls testing plate-to-plate variability, as well as intra- and inter-day variability, across 3 days.

Full compound library screens are not performed until after assay validation using smaller compound libraries. For these validation screens, we use one or several libraries of known modulators and FDA-approved drugs. Our validation libraries include the Microsource Library of pharmacologically characterized compounds, the Prestwick Chemical Library, and compounds synthesized within the Medicinal Chemistry core as well as the CMLD directed by KU's Jeff Aube. The Prestwick Chemical Library consists entirely of marketed drugs, presenting the greatest possible degree of drug-likeness. The Prestwick library was designed to reduce the risk of low-quality hits, using only active compounds that were selected for their high chemical and pharmacological diversity as well as for their known bioavailability and safety in humans [107].

For validation screening, it is very prudent to screen a target against a small library of highly diverse small molecules, such as the Prestwick library, which already has known bioavailability and toxicity data, and importantly, known effects in humans [6]. This allows potential repurposing of compounds, enabling the researcher to pick low-hanging fruit using compounds that have already been tested in clinical trials. Drug repurposing is useful for two core purposes. First, drug repurposing can help identify useful off-target mechanisms of action of the known FDA-approved drugs or other drug-like compounds, allowing the compound to be developed as targeting a different disease. Several past screens by the KU HTS core have screened compounds with known mechanisms of action against different and unique disease targets to identify low-hanging fruit for drug repurposing (Box 3). The second primary application of repurposing compounds is based around their known mechanisms of action. Many drug targets are involved in multiple biological pathways, and, as such, can be repurposed against that same target acting in a different disease or biological process [7]. Compounds that are hits in a screen of a collection of known compounds, such as the Prestwick library, can then be repurposed for new intellectual property and patenting, potentially benefiting from faster transition from hit to lead.

Box 3

University of Kansas High-Throughput Screening Laboratory screening targets

- ABCB6
- Antioxidant response element
- ARMET/ER stress
- ATP synthase
- B-cell lymphoma
- BRCA1&2
- Caspase 8
- Cathepsin
- Friedreich's ataxia

- Gamma globin
- Gap junctional intercellular communication
- GroEL
- HasAp
- Histidine kinase
- HMG CoA synthase
- HPV 16E6
- HSP90
- Mesothelin
- mhtt-CaM
- Micro RNA
- Na-Pi transporter
- Ncb5or modulators
- Nuclear factor of activated T-cells
- Nuclear factor (erythroid-derived 2)-like 2 (Nrf2)
- OATP transporter
- Optiferrin
- Osteosarcoma
- PAK inhibitors
- PchB
- PI3 Kinase
- Prolactin A
- Prolyl hydroxylase
- Protein stabilization
- Pyruvate kinase
- ROS testing
- RXRa receptor
- Sarco/endoplasmic reticulum Ca²⁺-ATPase (SERCA)
- Shigella flexneri
- Synaptogenesis
- Thyroid cancer
- Tuberculosis
- UDPG dehydrogenase
- Wnt-Akt- β catenin

- Wound healing

During full-compound library screens, we test one compound per well in a 384-well microplate format. For validation screens, we screen in a seven-point titration manner known as quantitative HTS, popularized by the NIH Chemical Genomics Center [8]. We occasionally receive novel compounds from combinatorial synthesis through our collaboration with the KU CMLD, and new plant extracts through our collaboration with Barbara Timmermann, Chair of the Department of Medicinal Chemistry at KU.

An example screening batch may require cells being seeded into 384-well plates on day 1, compounds added to cells on day 2, compound exposure for a range of days, typically 4–96 h, followed by addition of a detection reagent on day 4, followed immediately by data acquisition on a plate reader, then data analysis on day 5. Following primary data analysis, the top actives will be cherry picked and retested with the assay in a concentration–response curve, to validate the compound activity and to confirm a dose–responsive pattern, to eliminate false positive hits. Data uploaded into K-Screen are analyzed with dose–response curve-fitting software by the bioinformatician, and compounds are ranked. The cheminformatician will perform cluster analysis and look for patterns or structure–activity relationships between the hits. Cluster analysis of the screening data provides groups of hits that share structural similarity, which will then be useful as starting structural backbones for drug optimization by the KU medicinal chemistry core researchers.

Future perspective

The operations of the KU HTS support drug-discovery efforts by investigators at KU, the University of Kansas Medical Center and external institutions, many of which lack the infrastructure for HTS assay development and screening campaigns for new probe molecule discovery and development [1]. The focus of the KU HTS core has been to enhance the infrastructure of the laboratory to meet the current and expected medical needs of our customers, through improvements in expertise, state-of-the-art equipment, data analysis and focused chemical libraries.

The future goals of the KU HTS involve increased screening technology, libraries and education. Greater application of label-free technology will help the laboratory in deorphanizing refractory targets pursued by our faculty, while minimizing the long and costly assay-development process. Label-free technology would be especially useful as an alternate readout technology for use in the hit-to-lead optimization process [2], and will enable us to work increasingly with primary cell lines, without the need for engineered cell lines. The KU HTS core also plans to strengthen its current chemical library holdings with focused chemical libraries. Strategic libraries for screening will improve our chances of finding valuable hits, which could then be developed into lead molecules with potential for intellectual property rights. Also, the KU HTS core plans to continue to provide educational opportunities for KU faculty and students. This provides opportunities for learning novel technologies in drug-discovery platforms through seminars, workshops, internships and course teaching, while providing professional-development opportunities for the staff of the HTS core. Through these improvements, the KU HTS core facility will continue to expand working with investigators in generating quality data to support highly competitive grant applications for extramural funding.

Bibliography

1. McDonald PR, Roy A, Chaguturu R. The University of Kansas High-Throughput Screening Laboratory. Part I: meeting drug discovery needs in the heartland of America with entrepreneurial flair. *Future Med Chem*. 2011; 3(7):789–795. [PubMed: 21644824]
2. Roy, A.; Taylor, BJ.; McDonald, PR.; Price, AR.; Chaguturu, R. Hit-to-probe-to-lead optimization strategies: a biological perspective to conquer the valley of death. In: Seethala, R.; Zhang, L., editors. *Handbook of Drug Screening*. 2. Informa Healthcare; NY, USA: 2009. p. 21-55.
3. Lazzarino C. Special delivery. *Kansas Alumni*. 2009; (3):66–67.
4. Nixon E, Holland-Crimmin S, Lupotsky B, et al. Applications of adaptive focused acoustics to compound management. *J Biomol Screen*. 2009; 14(5):460–467. [PubMed: 19487768]
5. Tai D, Chaguturu R, Fang J. K-Screen: a free application for high throughput screening data analysis, visualization, and laboratory information management. *Comb Chem High Throughput Screen*. 2011 (In press).
6. Wermuth CG. Selective optimization of side activities: another way for drug discovery. *J Med Chem*. 2004; 47(6):1303–1314. [PubMed: 14998318]
7. Grau D, Phil M, Serbedzija G. Innovative strategies for drug repurposing. *Drug Discov Dev*. May 18, 2005 (5)
8. Inglese J, Auld DS, Jadhav A, et al. Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc Natl Acad Sci USA*. 2006; 103:11473–11478. [PubMed: 16864780]

Websites

101. Molecular Libraries Program Center Network. <http://mli.nih.gov/mli/mlpcn>
102. United States Food and Drug Administration Approved Drug Products. www.accessdata.fda.gov/scripts/cder/drugsatfda
103. The NIH clinical collections. www.nihclinicalcollection.com
104. Molecular Libraries Program Center Capabilities. <http://mli.nih.gov/mli/mlpcn/mlpcn-probe-production-centers/>
105. K-Screen. University of Kansas High Throughput Screening Laboratory; http://kscreen.org/kscreen_wiki/index.php/main_Page
106. Assay Guidance Manual Version 5.0. Eli Lilly and Company and NIH Chemical Genomics Center; 2008. www.ncgc.nih.gov/guidance/manual_toc.html
107. Prestwick Chemical Library®. www.prestwickchemical.com/index.php?pa=26
108. Autodock. <http://autodock.scripps.edu>
109. Cytoscape. www.cytoscape.org
110. EMBOSS. <http://ambermd.org>
111. Genmapp. www.genmapp.org
112. K-Screen. <http://kscreen.org>
113. Modeller. www.salilab.org/modeller
114. OpenBabel. <http://openbabel.org/wiki>
115. R project. www.r-project.org
116. Visual Molecular Dynamics. www.ks.uiuc.edu/Research/vmd
117. Amber. <http://ambermd.org>
118. CLC Genomics Workbench. www.clcbio.com
119. Gene Spring. www.agilent.com/chem/genespring
120. Ingenuity Pathway Analysis. www.ingenuity.com
121. MatLab. www.mathworks.com
122. Molecular Operating Environment. www.chemcomp.com
123. Pilot pipeline. <http://accelrys.com/products/pipeline-pilot>
124. SYBYL. <http://tripos.com>

Biography

Figure 1. High-throughput screening workflow

(A) Cells are seeded by a Thermo Wellmate into 384 well plates under sterile conditions of a laminar flow tissue-culture hood, followed by overnight incubation. (B) Compounds for screening are retrieved from the Labstore™ Nexus automated compound repository. (C) Compounds are dispensed onto cells using noncontact acoustic nanoliter transfer on the Labcyte ECHO. (D) Following exposure to compounds, cells are treated with various detection reagents or stains. (E) For whole-well detection, plates are scanned on plate readers such as the Tecan Safire2 (left) or PerkinElmer Envision (right). (F) High-content screening and analysis is performed for cell-based assays on the BD Pathway 855.

Table 1

Screening related equipment and instrumentation in the University of Kansas High-Throughput Screening Laboratory core[†].

Equipment	Manufacturer
<i>Plate readers</i>	
Envision [®] with stackers	Perkin Elmer
FDSS7000	Hamamatsu
Glomax [®] reader	Promega
Safire2 with Stackers	Tecan
SectorImager 2400	Meso Scale Discov
SpectraMax 340PC 384	Molecular Devices
SpectraMax Gemini XS	Molecular Devices
SpectraMax Plus 384	Molecular Devices
<i>Specialty and label free</i>	
BD Pathway [™] 855 Imager	Becton, Dickinson
Biacore 3000 label free	GE Healthcare
Ti-inverted digital microscope	Nikon
xCELLigence label free	Roche
<i>Bulk liquid handlers</i>	
AquaMax [™] DW4	Molecular Devices
ELx405 CWS Plate Washers (2)	BioTek
Mosquito	TTP Labtech
Multidrops (6)	Thermo
WellMates [®] (2)	Thermo
<i>Liquid handlers</i>	
Biomek FX [®]	Beckman Coulter
Catalyst-5 Express [®]	Thermo
ECHO 550 [®]	Labcyte
Genesis FE2000	Tecan
PlateMate 2×3	Thermo
PlateMate Plus	Thermo
Precision 2000	BioTek
<i>Miscellaneous</i>	
ALPS 300 [™] Automated Sealer	Thermo
ALPS 50 [™] Plate Sealer	Thermo
EasyCyte [™] Plus system	Guava
FPLC for Protein Purification	GE Healthcare
iEMS Microplate Shaker Incubators (3)	Thermo
KinedX plate loader with stackers	Peak Robotics

Equipment	Manufacturer
L8 microplate mixing system	Covaris
Labstore™ US-200	Nexus
Multichannel Verification System	Artel
Plate delidder	Let's Go Robotics
RapidStaks (2)	Thermo
Titramax 1000 Plate Shaker	Heidolph
Twister® II plate loaders with stacks (2)	Zymark
XPeel	Nexus

[†] In addition to basic laboratory equipment and instrumentation, the University of Kansas High-Throughput Screening Laboratory core has a wide variety of screening-related technology, including cutting-edge instrumentation, such as acoustics for 1536-well dispensing and label-free instruments.

Table 2

Informatics software programs frequently used at the University of Kansas.

Name	Primary function	Ref.
<i>Open-source and/or freeware</i>		
AutoDock	Molecular docking toolset	[108]
Cytoscape	Bioinformatics visualization and analysis	[109]
EMBOSS	Protein and DNA sequence analysis	[110]
Genmapp	Genomics visualization and analysis	[111]
K-Screen	Compound management and analysis	[112]
Modeller	3D homology and modeling analysis	[113]
OpenBabel	Molecular modeling toolset	[114]
R	Statistical visualization and analysis	[115]
VMD	Molecular visualization system	[116]
<i>Require a license, commercially available</i>		
Amber	Biomolecular simulation	[117]
CLC Genomics Workbench	Sequencing analysis and visualization	[118]
Gene Spring	Statistical visualization and analysis	[119]
Ingenuity Pathway Analysis	Model/analyze biological systems	[120]
MatLab	Computing environment	[121]
MOE	Molecular Simulation	[122]
Pilot pipeline	Informatics platform	[123]
SYBYL	Molecular design and analysis	[124]