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UCLA's Molecular Screening Shared Resource: Enhancing Small Molecule Discovery with Functional Genomics and New Technology

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Abstract: The Molecular Screening Shared Resource (MSSR) offers a comprehensive range of leading-edge high throughput screening (HTS) services including drug discovery, chemical and functional genomics, and novel methods for nano and environmental toxicology. The MSSR is an open access environment with investigators from UCLA as well as from the entire globe. Industrial clients are equally welcome as are non-profit entities. The MSSR is a fee-for-service entity and does not retain intellectual property. In conjunction with the Center for Environmental Implications of Nanotechnology, the MSSR is unique in its dedicated and ongoing efforts towards high throughput toxicity testing of nanomaterials. In addition, the MSSR engages in technology development eliminating bottlenecks from the HTS workflow and enabling novel assays and readouts currently not available.

Robert Damoiseaux

Keywords: Academic drug discovery, high throughput screening, functional genomics, nanomaterials, new technology, RNAi, shRNA, small molecules.

INTRODUCTION TO THE MSSR

The Molecular Screening Shared Resource was founded in 2004 with internal funding from the UCLA David Geffen School of Medicine, Jonsson Comprehensive Cancer Center, and the Departments of Molecular and Medical Pharmacology and Microbiology, Immunology and Molecular Genetics. Its mission was to bring industrial strength drug discovery firepower to the UCLA campus. What started out as a relatively modest operation with one integrated screening system with two plate readers, 30k small molecules and 400 square feet, now occupies over 2000 square feet in the cutting edge California NanoSystems Institute. The MSSR boasts four fully integrated screening systems which include high content screening capabilities, a comprehensive range of functional genomics resources including genome-wide RNAi and cDNA as well as a compound deck of over 200k drug-like small molecules.

A large part of the success of the MSSR lies in its seamless integration with the ULCA campus (see Fig. **1** for an overview). This is exemplified by the partnership with the Jonsson Comprehensive Cancer Center, which is a National Cancer Institute designated Comprehensive Cancer Center and operates one of the largest clinical trial networks globally. The JCCC is also a starting point for global clinical trials that start out locally at UCLA. The JCCC was one of the top recruiting sites in the clinical trials of many drugs that are now common household names such as Herceptin, Avastin, Gleevec, Tarceva, Sprycel and Tykerb. The Clinical Translational Science Institute (CTSI) at UCLA is another important partner of the MSSR. The UCLA CTSI is a dynamic partnership of four institutions—Cedars-Sinai Medical Center, Charles Drew University of Medicine and Science, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, and the UCLA-Westwood. Its mission is to bring UCLA innovations to bear on the greatest health needs of Los Angeles and the nation. CTSI investigators are occupied with a diverse collection of health issues such as cancer, disease prevention, heart disease, HIV, mental health, patient safety, stroke, addiction, and women's health. The UCLA CTSI supports investigators by providing seed grants for e.g. HTS and access to specialized expertise and resources — such as the MSSR. Other CTSI-sponsored resources include access to statisticians, computer databases, clinical tests, next-generation sequencing and other sophisticated, high-tech equipment as well as study volunteers.

Associated with the CTSI is the UC Biomedical Research Acceleration, Integration, and Development (UC BRAID) consortium. UC BRAID is a joint effort of the five UC biomedical campuses to catalyze, accelerate and reduce the barriers for biomedical, clinical and translational research across the UC system by identifying shared challenges and developing system-wide solutions in the form of policy changes, new infrastructure and standardized processes. The MSSR plays an important part in the Drug and Device Development (D4) framework component of the UC BRAID where it serves not only as a drug discovery facility but as well as a resource in the broader drug and device discovery area. Moreover, the D4 component of UC BRAID is tasked with making discoveries and resources accessible to industry and other research organizations.

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Fig. (1). Overview of the main on-campus partners of the MSSR. The MSSR functions in a matrixed environment where the tools, capabilities and resources of the MSSR can be accessed and utilized by all institutes on campus in a direct and horizontal manner. This is especially beneficial for drug discovery projects as the necessary competences and resources, tend to span multiple institutes and departments. The same is true for responding to large grant opportunities such as project program grants. Moreover, the MSSR is bound into the Drug and Device Discovery and Development (D4) alliance which is matrixed across all UC campuses with a medical school.

The MSSR is housed in the California NanoSystems Institute (CNSI) [1], which is an integrated research facility with locations at UCLA and UC Santa Barbara. As a California Institute for Science and Innovation, the CNSI builds on a visionary investment in education of future scientists, research and technological resources by the State of California. Its mission is to encourage collaboration between academia and industry thus enabling the rapid commercialization of discoveries in nanoscience and nanotechnology including the biological and translational aspects of nanoscience. True to this mission, the MSSR is open to scientists from all over the world – industrial, non-profit and academic alike. Research is conducted on a fee-for-service basis and no intellectual property (IP) is retained. This unique position on IP makes the MSSR very accessible to industry and projects can start without lengthy negotiations. The CNSI is also the home to the University of California Center for Environmental Implications of Nanotechnology (UC CEIN) and the MSSR is an integral component of this center [2]. The mission of the UC CEIN is to use a multidisciplinary approach in research, knowledge acquisition, education and outreach to ensure the responsible use and safe implementation of nanotechnology in the environment. The UC CEIN makes use of well-characterized compositional and combinatorial nanomaterial libraries to study their fate and transport in parallel with the materials' bioavailability and potential to engage toxicological pathways in organisms and environmental life forms. Where possible, this exploration involves high throughput screening (HTS), utilizing the MSSR with the goal of understanding structure-activity relationships (SARs). Such SARs can be used for prediction making of primary engineered nanomaterials (ENM) impact on organisms in freshwater, seawater, and terrestrial environments. This will allow the USA and international communities to safely leverage the benefits of nanotechnology to the benefit of the global economy, society and the environment.

COLLABORATIVE WORK MODEL WITH INDUSTRIAL CORE VALUES

What sets the MSSR apart from most industrial HTS facilities is a unique culture of combining the best of industrial-style project management with academic rigor and creativity. The MSSR works closely with each Principle Investigator. In fact, we have adopted a collaborative work

model in which typically one or more members of the Principle Investigator's laboratory work with the MSSR team on a given project. This ensures that a person with detailed knowledge of the biology which stands behind each project is available at all times thereby providing high quality results and value added to the project. In a retrospective analysis, we found that over 95% of our projects were successful in generating hit compounds of the desired activity and we attribute this success rate to our collaborative work model. As this collaborative work model bridges academic and industrial world, it lends itself extremely well towards teaching high throughput screening and automation to the next generation of scientists which, through their work at the MSSR, become intimately involved in the high throughput and drug discovery process. Frequently, their interaction with the MSSR changes entirely their approach to scientific problems and as the students progress in their mastery of HTS and automation, they become more effective, focused and ultimately productive. The collaborative work model also makes the communication with the Principle Investigator more efficient through the direct involvement of the Principle Investigator's team member. If sending a team member is not possible, the MSSR can run HTS campaigns entirely through the effort of the MSSR team.

This collaborative model necessitates the implementation of best industry practices through the entire life cycle of each project including assay development, execution of the HTS campaign, data mining and SAR and specificity analysis. Standard operating procedures (SOP's) are employed that contain not only the workflows necessary for project management but also all of the other pertinent information down to batch information of reagents and consumables. These SOP's are reviewed, revised and refined as the project progresses and our SOP library is an invaluable resource for future researchers who profit from the experience preserved in the MSSR.

OVERVIEW OF THE SCREENING OPERATIONS AT THE MSSR

The MSSR supplies all the ingredients necessary for a successful drug discovery campaign. This includes the necessary know-how *via* a team with industrial experience, cutting-edge HTS equipment and the latest chemical and functional genomics libraries. The MSSR accepts HTS projects at all stages - including projects without a HTS-ready assay or projects where the Principle Investigator might have only an idea for an assay. Thus, our work frequently involves assay inception and *de novo* development. Typically, our projects follow the standard workflow outlined in Fig. (**2**). The Principle Investigator who has a novel target will have an initial consultation with the MSSR. The target could be a pathway, protein or phenotype. During this consultation, the type of project (e.g. small molecule drug discovery *vs* functional genomics) and scientific questions such as screening strategy, assay technology and library choices are defined. Moreover, strategies for hit validation including assays with orthogonal readouts and counterscreens have to be thought through. Of course, other issues such as screening cost and cost of consumables are discussed here as well. The MSSR has special rates for many reagents and consumables commonly used in HTS which are passed on directly to the Principle Investigator resulting in substantial savings. After the meeting, the project is then taken to the laboratory where the practical aspects of the HTS campaign are tended to. The first step is the assay development. We follow a standardized methodology for the development and validation of any assay (see Fig. **3** for details) which aids in developing the most relevant and best performing assay for a given project. Especially for cell based assays, a thorough pharmacological validation is necessary to ensure that the assay system reflects the biological system properly - including the involvement of the correct pathways, a suitable hit-rate and desired specificity. Here, access to cDNA and shRNA clones from our functional genomics libraries is extremely helpful. Through the overexpression and knockout of agonists and/or antagonists genes of a given target, one can derive a large degree of certainty in regards to the selectivity of a given cell based assay. The assay development and validation is followed by the execution of the actual HTS campaign. Data is uploaded into our database as the screen continues which allows the Principle Investigator to track the progress. After completion of the screen, the hit compounds or relevant clones from our functional genomics libraries are picked and condensed into small custom libraries. These are then utilized for hit validation in triplicate followed by validation using an orthogonal assay and/or a counterscreen to ensure specificity. At this point compounds are typically ordered in powder for dose-response analysis which we also perform in high throughput. Specificity screens are frequently also performed in dose response to ensure a large enough therapeutic window. The team member of the Principle Investigator participates hands-on in each step of this workflow which enhances confidence in the results and simplifies the transfer of the data back to the Principle Investigator. Performance of a screening campaign also depends strongly on the available infrastructure and libraries, which we will review now in detail.

SCREENING INFRASTRUCTURE

The MSSR has put a special emphasis on the use of fully integrated systems in order to enhance productivity during execution phase. Two Beckman CORE systems with a throughput of over 100,000 samples a day are the foundation of our screening operations. These fully integrated systems consist of high-end multi-mode plate readers addressing all available readouts, liquid handlers, bulk-dispensers, plate washers and automated CO_2-incubators (see Table **1** for a full list of our equipment). The systems are controlled by Beckman-Coulter SAMI scheduling software which allows for 24/7 walk-away automation, data collection and error recovery. In contrast to many HTS systems used in the pharmaceutical industry, our systems are configured for maximal flexibility and accommodate a very diverse set of assay workflows. Cherry picking of hit compounds is also automated on these systems. Recently, we have added two Biocell 1800 systems from Agilent – one of which will serve primarily as a compounding system and the other will serve as a dedicated uHTS system with plate reader based and laser scanning cytometry readouts.

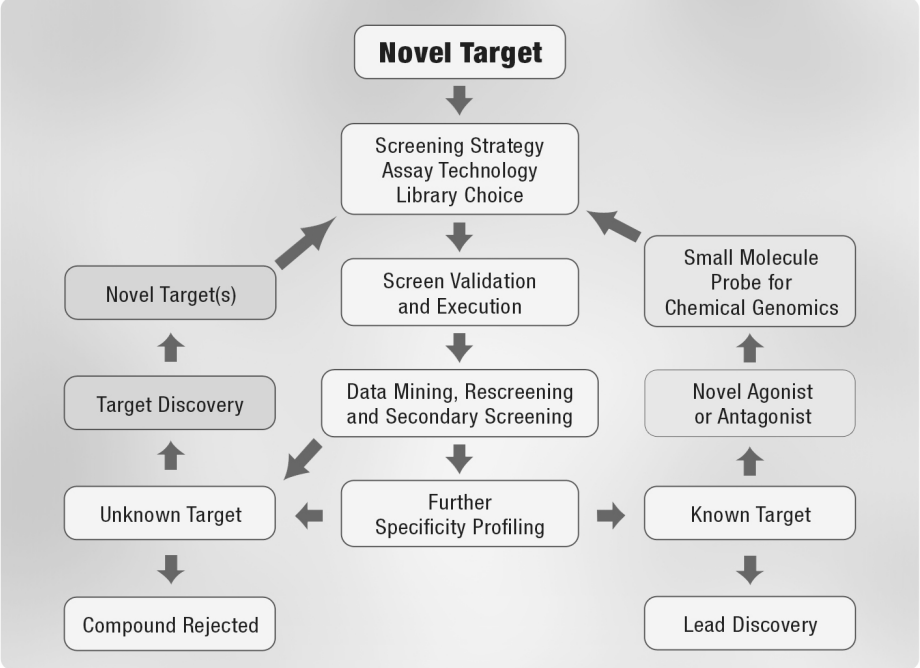

Fig. (2). Typical project workflow at the MSSR. All projects originate with the choice of the target. This choice is pivotal as mistakes here can not be fixed by changing the assay later on. Decisions in regards to screening strategy, assay technology and library choice are very important as well. The validation of the assay has to be performed in the same format and on the same machines which will be utilized for the actual HTS campaign. Data mining is shown here after the screening process. However, data analysis is a process that is parallel to the screening execution thus enabling quality control in real time. The light and dark greyed arms in this diagram are not necessarily part of the traditional pharmaceutical industry workflow. Chemical genomics – i.e. the use of small molecules to inhibit given targets in a cellular context shown in light grey on the right – is a more recent addition to the repertoire of the pharmaceutical industry. The dark grey arm on the left deals with target discovery which is frequently performed using functional genomics such as RNAi and cDNA strategies in conjunction with the compound in question. This figure was adapted from Molecular Screening for Therapeutic Agents, Pharmaceutical Sciences Encyclopedia, Damoiseaux, R., 2011, Wiley and Sons.

DETECTION TECHNOLOGIES

We have a comprehensive range of detection equipment available (see Table **1** for details) which can address any readout from optical density over luminescence to Alpha-screening and time resolved fluorescence. However, about 90% of all screening campaigns at the MSSR are cell based and we see a definite trend towards the usage of high content screening (HCS) as preferred cell based readout prompting us to invest into upgrading our machine park emphasizing HCS. HCS is especially important in functional genomics where frequently only a cellular phenotype of a disease is known and actionable targets have to be found. Here, HCS – in conjunction with our RNAi and cDNA overexpression libraries – has the potential to deliver these targets. Moreover, HCS allows for addressing cell populations and rare cellular events. In terms of HCS detection hardware, the MSSR utilizes the latest hardware in high content screening such as Acumen Explorer and Velos laser scanning and ImageXpress^{XL} brightfield systems. A common bottleneck for HCS screens is the processing of the plates during the screen as well as the evaluation of the images after the screen has been performed. Frequently, these bottlenecks lead to the exclusive use of fixed cell screens for HCS which have to be processed in batch mode in order to enable the computational resources to keep up with the data. At the MSSR we have solved these problems by investing into fully integrated systems allowing for 24/7 walk-away operation on the plate processing side. We invested in industrial strength image processing infrastructure that is based on MetaXpress Power-Core by Molecular Devices. This allows for the parallel processing of image data utilizing multi-core server systems and keeps up with the data generated.

SMALL MOLECULE LIBRARIES

Our compound libraries consist of over 200k molecules which are split into 4 segments: pharmacological validation and repurposing libraries (Biomol, Prestwick and Microsource spectrum and NIH clinical collection), targeted libraries, lead-like libraries, diverse libraries (UCLA) and diverse sets of smart libraries (see Fig. **4**). All of our compounds are at least 90% pure, typically better. On average, we find 95% of the hit compounds can be resupplied as powder for follow up. With the exception of our diverse library which was a pre-plated set, all of our sets are custom sets and are not likely to be found in another screening facility. We have applied extensive filtering against liabilities such as reactive groups, aggregators etc. [3]. The drug-likeness and usefulness of the pharmacological validation and repurposing libraries is well established. In this context, it is interesting to note that the NIH clinical collection serves to fill gaps in this area that were previously not addressed; hence we included this library in our deck. The targeted library set is a set of 8k compounds which were subjected to high-throughput docking to kinases, proteases,

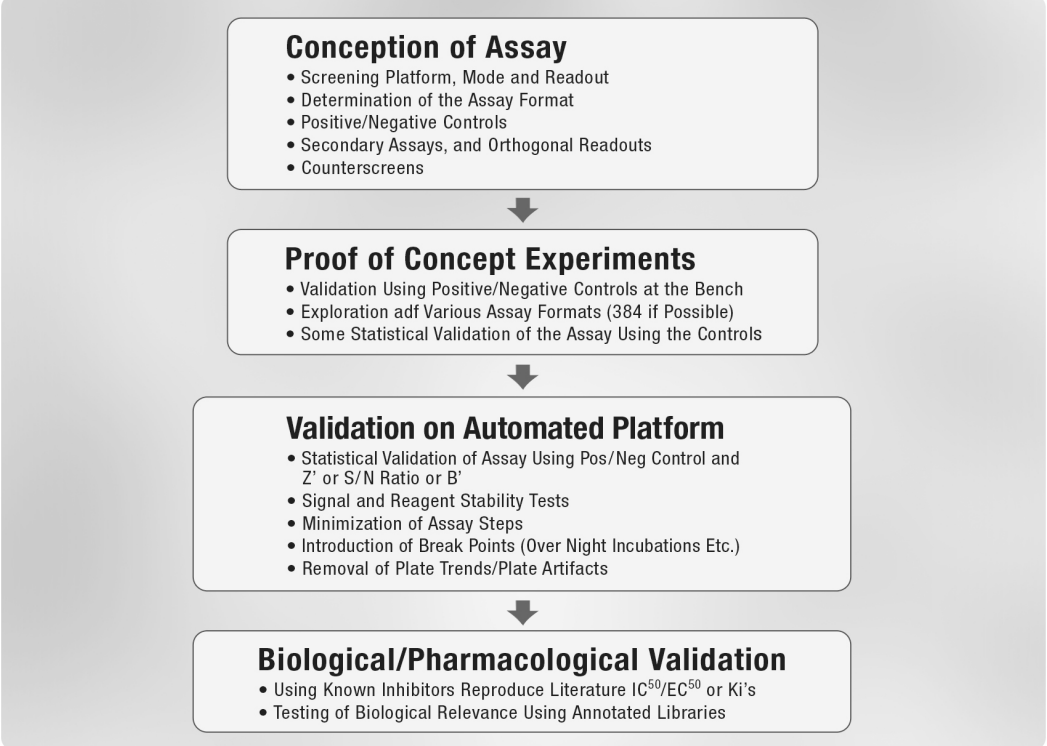

Fig. (3). Detailed view of the assay development workflow. Many details have to be thought about as the performance of a HTS campaign is completely dependent on the performance of a given strategy. Importantly, the conception of the assay has to take into consideration the constraints of the HTS environment. Even under the best circumstances, there will be hundreds to thoughsands of hits, depending on the compound deck size screened. This necessitates that all follow up assays should be automatable as well. This figure was adapted from Molecular Screening for Therapeutic Agents, Pharmaceutical Sciences Encyclopedia, Damoiseaux, R., 2011, Wiley and Sons.

ion channels and GPCR's and which are included in this set based on their predicted ability to bind to these high-value targets. The drug-likeness of this set is excellent, compounds from this set obey the rule of 5 and many of them even the more stringent rule of 4 [4]. The lead-like library is a set of 20k compounds which obeys the more stringent rule of 4 rather than the more permissive rule of 5. This library was selected from a set of about 250k compounds to yield compounds which have more favorable properties for subsequent medicinal chemistry optimization since the average molecule from this set is smaller, has fewer h-acceptor or donor sites and a better logP. It addresses the problem that during chemical optimization the compounds typically get heavier and less drug-like, leading to more potential for ADME-problems in subsequent stages. The diverse library is the DiverSet E from Chembridge, which is a well established 30k compound set that contains a lot of interesting structures and has generated many interesting hits in our hands. This set contains a vast structural diversity which has been selected from over 500k compounds. Another 50k custom diverse set from Life Chemicals was selected from over 600k compounds. Our diverse sets of smart libraries were selected from large compounds sets of 600k and 250k compounds. We utilized large computer clusters (250 nodes and more) at the CNSI to filter the sets for compounds which were drug like and did not have any other liabilities, fewer than 8 rotatable bonds and so on. The resulting compound set was then broken into clusters of similar structures which in turn was Monte-Carlo-sampled in order to include a representative subset. This was a challenging process which took about 3 weeks computing time on the cluster. The resulting compound libraries have excellent properties (i.e. obeys at least the rule of 5) and they are proprietary to the MSSR. We manage our compound libraries with great care. All working copies are stored in desiccators which we have found to give us the best compound stability, since water is the biggest enemy of compound library integrity. Our compound repository is housed under desiccated conditions at +4°C and additional copies are stored sealed at -80°C in our freezer farm for future use.

FUNCTIONAL GENOMICS LIBRARIES

In addition to drug discovery and chemical genomics, the MSSR provides functional genomics libraries using inhibitory RNA (RNAi) for gene knock out (KO) and the complementary cDNA overexpression methodologies (see Fig. **4** for an overview) [5, 6]. It is the wide range of functional genomics library resources that makes the MSSR unique in the functional genomics realm. These libraries have many uses. We talked already about the use of e.g. cDNA and shRNA reagents for assay validation. The other main application for functional genomics libraries is target discovery and validation. This application is especially important in the context of a known a mode of action mandated by the FDA for each new chemical entity. One might question the use especially of RNAi for target validation given that off-target effects can and do occur

Table 1. List of leading edge HTS equipment available for use at the MSSR.

HTS Infrastructure of the University of California, Los Angeles' Molecular Screening Shared Resource	
Equipmlent Type:	**Equipment Manufacturer**
Plate Reader	
Acquest	Molecular Devices
FlexStation	Molecular Devices
Victor3V with Stackers	Perkin Elmer
Topcount with 6 PMT's and Stackers	Perkin Elmer
Fusion Alpha With Stackers	Perkin Elmer
Fusion	Perkin Elmer
High Content Screening	
Image Xpress^{micro}	Molecular Devices
Image Xpress^{XL}	Molecular Devices
Velos Laser Scanning Cytometer	Molecular Devices
Acumen Explorer	TTP Labtech
Bulk Liquid Handlers	
ELx405 Plate Washer	BioTek
ELx406SUB3LB Dispenser/Washer (96-1536 Well Plates)	BioTek
Multidrop (4)	Therme
Multidrop with Stacker	Titer Tek
Liquid Handlers	
Biomek FX®	Beckman Coulter
Bravo (2)	Agilent
Vprep (3)	Agilent
Genesis 150	Tecan
Genesis 200	Tecan
Precision 2000	BioTek
Biomek 2000 (2)	Beckman Coulter
Robotics	
BioCel 1800 (2)	Agilent
VP 6-Axis Robotic Arm	Denso
ORCA Robotic Arm with 3m Rail (2)	Beckman Coulter
Twister® II Plate Loaders with Stacks (2)	Zymark
Miscellaneous	
PlateLoc (2)	Agilent
PlatePierce	Agilent
Vspin	Agilent
Rotating Plate Hotel for 200 Plates (2)	Beckman Coulter
Cytomat 6000 Automated Incubator for 189 Plates	Heraeus
Cytomat 6001 Automated Incubator for 189 Plates	Heraeus
Cytimat 2C Automated Incubator for 42 Plates	Heraeus
Lidding Station (2)	Beckman Coulter
BenchCel Robot	Agilent
STX Automated Incubator for 220 Plates (2)	Liconix

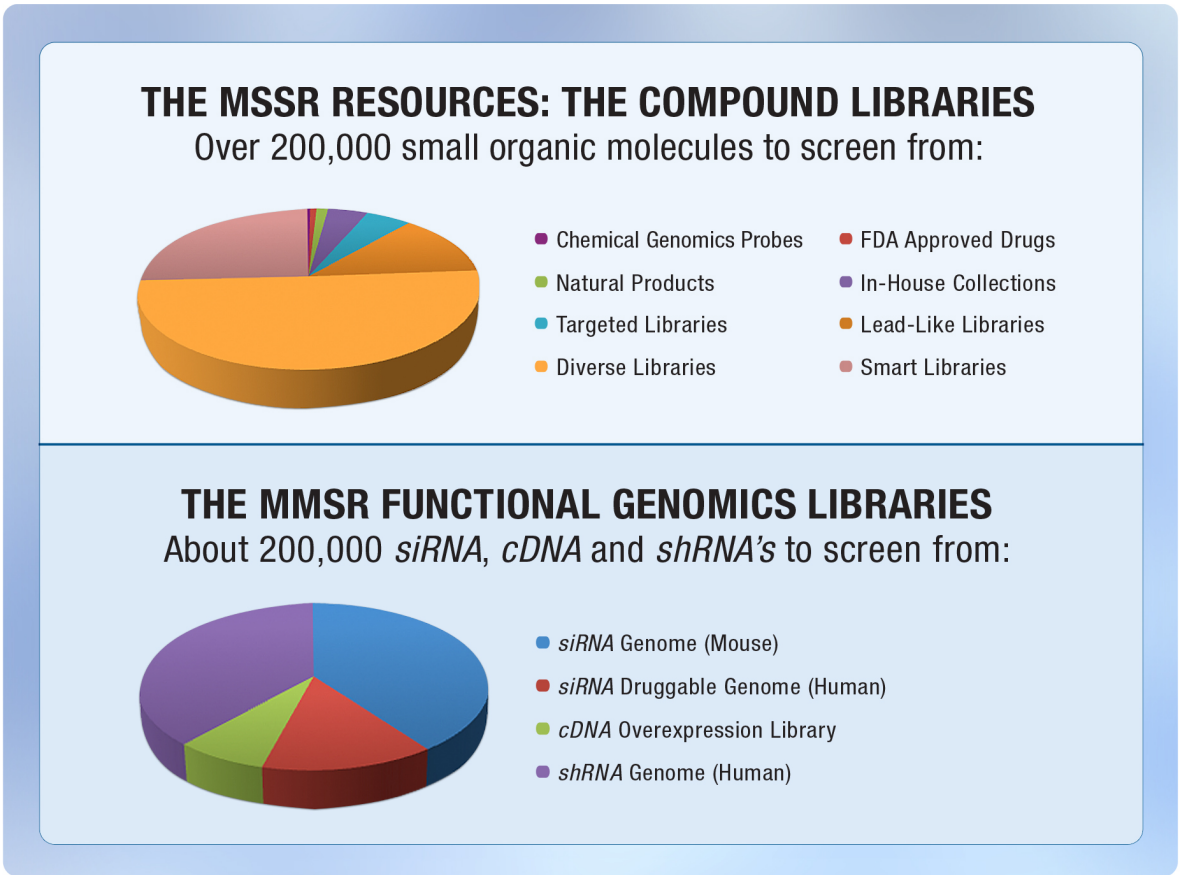

Fig. (4). Graphical representation of the small molecular and functional genomics libraries at the MSSR. On average, about 95% of the small molecules can be re-ordered off the shelf as powder for IC_{50} determination. The MSSR maintains glycerol stocks for the cDNA and shRNA libraries and larger siRNA quantities for the siRNA druggable genomes for rescreening purposes. The cDNA set was compiled at the MSSR from a set of NIH MGC plates and is not commercially available.

frequently with this type of technology. However, the use of chemical genomics libraries for target discovery is not without problems neither. Frequently, the promiscuity of the compounds in chemical genomics libraries can result in a lack of confidence in the targets identified by chemical genomics as well. Kinases are a prime example. cDNA in contrast does not have this shortcoming as no off-target effects are known. The confidence of a target found by RNAi can be augmented by complementation with a cDNA of the same target but from a different species which is not subject to knockdown. Thus, cDNA overexpression and RNAi are very complementary technologies [5].

The MSSR offers three commercially available siRNA libraries covering the human and mouse druggable and the entire mouse genome. Kinases and GPCR's are available as sub-libraries with 4 siRNA constructs per gene. This allows for advanced analysis methodologies such as redundant siRNA analysis (RSA), enabling the elimination of outliers and siRNA's which might be off-target. Our arrayed shRNA libraries contain over 60k clones targeting about 18k genes with an emphasis on coverage of desirable target classes such as kinases, proteases, phosphatases, GPCRs and ion channels. In addition to the traditional target classes we offer various custom libraries such as a validated cancer specific shRNA set of about 150 targets from the NCI and a cancer specific set of over 2k clones covering 500 cancer related genes selected by the Sanger Institute. Other sublibraries cover transcription factors, ubiquitinization and de-ubiquitinization pathways, apoptosis etc. These libraries are proprietary to the MSSR as they were custom selected and arrayed at the MSSR. The capability of customizing shRNA libraries enables the MSSR as well to tailor shRNA libraries to the needs of a particular Principle Investigator. The production of shRNA lentiviral particles requires special precautions and equipment. We utilize an integrated system consisting of a Velocity 11 Benchcell plate handling robot with Vprep liquid hander, PlateLoc plate sealer and Vcode barcoder housed in a custom HEPA-filtered biosafety cabinet for aliquoting our shRNA particles into assay plates once the libraries are in lentiviral form. The MSSR offers a mixed mouse and human cDNA genome wide collection for screening. The library covers about 16k full length cDNA clones custom arrayed at the MSSR and sublibraries customized to particular projects are available as well. Such a cDNA overexpression library allows as well for gain-of-function (GOF) strategies which are complementary to loss-of-function RNAi strategies. The MSSR has as well arrayed genome wide knock-out or deletion libraries of the model system *E. coli* and *S. Cervisiae* [7] available. These libraries contain all non-essential genes and are especially useful for nano-toxicology studies as they offer insight into the mechanism of nano-toxicity.

DECENTRALIZED DATA ANALYSIS

Data analysis is a vital process of HTS – it is where actives are segregated from inactives, plate effects can be discovered through QC and removed and compounds selected for follow-up. Our collaborative model for our drug development and HTS campaigns demands an IT infrastructure which is able to support not only an ever-growing number of users who are distributed globally, but is also flexible enough to allow for access to the data by collaborators who might not have any cheminformatics experience or infrastructure. Moreover, the data must only be accessible to the collaborator it is intended for and safe from unauthorized access as the integrity of the data of our clients and their confidentiality is of utmost importance. Our solution to this problem was to work with Collaborative Drug Discovery (CDD). Their concept is a cloud based cheminformatics solution with backup server locations on two continents. CDD is based on the concept of "Vaults" which stores data securely and privately and is only visible to the Vault user(s). One of the added benefits for us was that CDD enables user to pick and chose which data and compounds they might want to share securely with other CDD users. CDD offers all data mining tools needed for HTS such as quality control using Z' for each plate, z score for hit identification, automated IC_{50} calculations, similarity searches for structure activity relationship (SAR) analysis *via* e.g. Tanimoto algorithms etc. The MSSR staff mentors and teaches the users the ins and outs of data analysis which is as well important in order to equip the next generation of scientists with data analysis knowledge. For more complicated data analysis our users can take advantage of the UCLA Department of Biostatistics which is a 5-minute walk from the MSSR and also operates as a weekly "walk in clinic" where our users have direct and immediate access to a biostatistician. A good example of the incorporation of statisticians is the generation of predictive models of toxicity for nano-materials using self-organizing maps [8].

Moreover, a "CDD Public" mode enables our users to publish their data to the entire community if and when they desire, which is very useful to satisfy the NIH "open access" requirements. At the same time, we utilize internally an Accelrys based, industrial strength cheminformatics package to manage our compound collections, parity check our collections for duplicate compounds etc.

OTHER UCLA CORES

No HTS laboratory lives in a vacuum. Our work is directly dependent on the availability of a multitude of other services upstream and downstream of the early drug discovery process. Moreover, the efficiency of bringing the fruits of our work into the clinic or marketplace – and thus the benefit society reaps - ultimately depends on how well the MSSR is integrated with other cores relevant to drug discovery, development and ultimately licensing. UCLA offers more than 160 fee-for-service cores ranging from FACS sorting over stem cell cores all the way to GLP certified virus production facilities for clinical trials.

We are extremely fortunate to have a very strong chemistry department with multiple members of faculty engaging in the development of novel libraries and medicinal chemistry. In fact, we have a strong medicinal chemistry core where our hits can be prioritized according to their suitability to be turned to leads and PK/ADME properties can be adjusted and potency and selectivity addressed. We also collaborate with the Specialized Chemistry Center of the University of Kansas, which is part of the Probe Production Centers Network (MLPCN) supported by the NIH. The necessary measurments of e.g. pharmacokinitics (PK) and toxicity (TOX) in a good laboratory practice (GLP) validated environment can be performed through the UCLA department of laboratory animals (DLAM). This resource was utilized already to gain full FDA approval for two positron-emission probes. The CTSI is currently setting up specialized phase I clinical trial facilities in addition to the already existing facilities mentioned above on campus. Last but not least, our Office of Intellectual Property (OIP) has collaborated with the Anderson Business School at UCLA to set up a streamlined pipeline for licensing the resulting IP. The formation of spin-off companies is as well encouraged and space in one of the incubators on campus (e.g. in the CNSI) can be applied for. The ultimate goal is rapid commercialization of the discoveries.

NEW TECHNOLOGY, IP AND ACADEMIC-INDUSTRIAL COLLABORATIONS

New technology that is spun off into companies plays an important role in the UCLA drug discovery ecosystem. For example, the spinoff company Librede, Inc. is working closely with the MSSR on the development of next generation ion channel screening modalities [9]. Librede was founded by UCLA researchers and the inventors of this technology. Ion channels make up a very large percentage of all FDA approved drug targets and are involved in a wide range of debilitating diseases such as Alzheimer's disease, epilepsy, diabetes and contain important anti-targets, e.g. hERG, making it as well interesting to perform full deck screens from a compound library design point of view. Unfortunately, assaying ion channels is both lower throughput and expensive. Ion channels must be incorporated into a cell membrane to allow measurement of their ionic transport and functionality. While ion channels can be measured using a multitude of paradigms, electrophysiological measurements are the gold standard. Automated patch clamp instruments have been developed, but even the most recently developed automated patch clamp instruments are still over an order of magnitude lower throughput than conventional drug screening using e.g. cell based assays and also require expensive instrumentation, specialized cell lines, and expensive consumables. Thus, for existing methods of ion channel screening, there is a large gap in information quality, throughput, and cost. To this end, Librede Inc. is developing an alternative cell-free technology for ion channel screening using self-organizing, artificial cell membranes. With the support of the MSSR, Librede obtained multiple SBIR grants totaling over $1 million and is currently developing a prototype of a fully integrated ion channel screening system utilizing their patent pending formulation of cell-free artificial membranes.

Another example of technology development which enhances screening capabilities is the development of an automated zebrafish arrayer in conjunction with the UC-CEIN. We succeeded in producing a fully functional prototype which we currently use to support our nano-toxicity and small molecule zebrafish screening operations. Zebrafish has emerged as one of the most exciting *in vivo* screening tools for drug/compound efficacy or toxicity evaluations [10, 11]. Its high fecundity, embryo transparency and fast development make it an excellent model for HTS. However, the conventional screening process involves steps that limit its capability to reach high throughput level: for example, the manual process of selecting and dispensing healthy embryos into multiwell plates is such a rate limiting step. We solved this problem by developing an automatic embryo dispensing system. By integrating a robotic arm with a vision recognition system and a liquid dispensing system, we were able to fully automate the zebrafish arraying process. In short, the robotic arm, carrying the vision recognition system, locates healthy embryos in Petri dishes and sends coordinates back to a processing unit. This allows the robotic arm, carrying the liquid dispensing system, to pick and place the selected embryos in each well of a multiwell plate (see as well Fig. **5** for details). For safety, the whole system was mounted on an industrial grade vibration isolation table preventing oscillation during operation. An enclosure with safety switch and emergency stops were also installed to minimize risk of operating such a powerful system. The other bottleneck in the zebrafish screening workflow is the visual examination of embryos through dissecting microscopes. In order to boost this process, we implemented a high content imaging device (ImageXpress^{Micro}) to perform automatic phase-contrast bright-field imaging. Instead of manually going through embryos one by one, the high content imaging device can capture high-resolution microscopic images of 96 embryos within 5 minutes. The integration of the state-of-the-art robotic platform for embryo arraying and high content imaging platform with image acquisition allowed us to achieve high throughput screening using zebrafish embryos and have successfully applied this system to the toxicity screening of engineered nanomaterials. We have found laser scanning cytometry as well extremely useful for zebrafish screening [12].

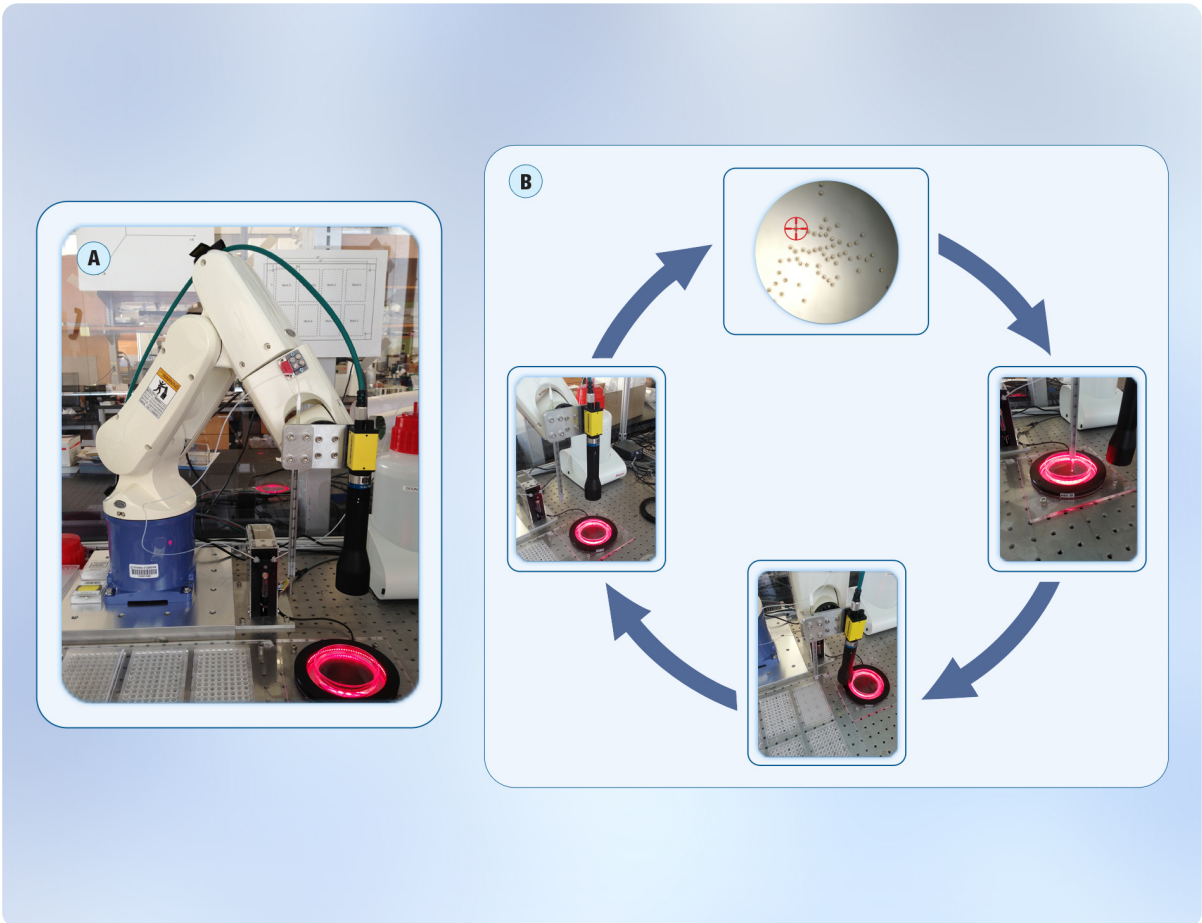

Fig. (5). Image and function of a zebrafish arrayer. (**A**): Image of the zebrafish arrayer. The system consists of a robotic arm with a precision of 10 micron in X,Y,Z axis direction which allows for precise picking of fertilized zebrafish embryos without damaging them, a camera and a pipette integrated with an automated liquid handling device. (**B**): Function of the zebrafish arrayer. Depicted on the left side is the the arm bringing the camera over the petri dish containing the zebrafish embryos. On top: The software evaluates the image taken by the camera and selects an embryo. The system calculates the XZY coordinates from the image. Right: The arm moves the pipette on top of the embryo and activates the liquid handler to suck up the embryo. Bottom: The arm moves over to a 384 well plate and dispenses the emryo into the plate.

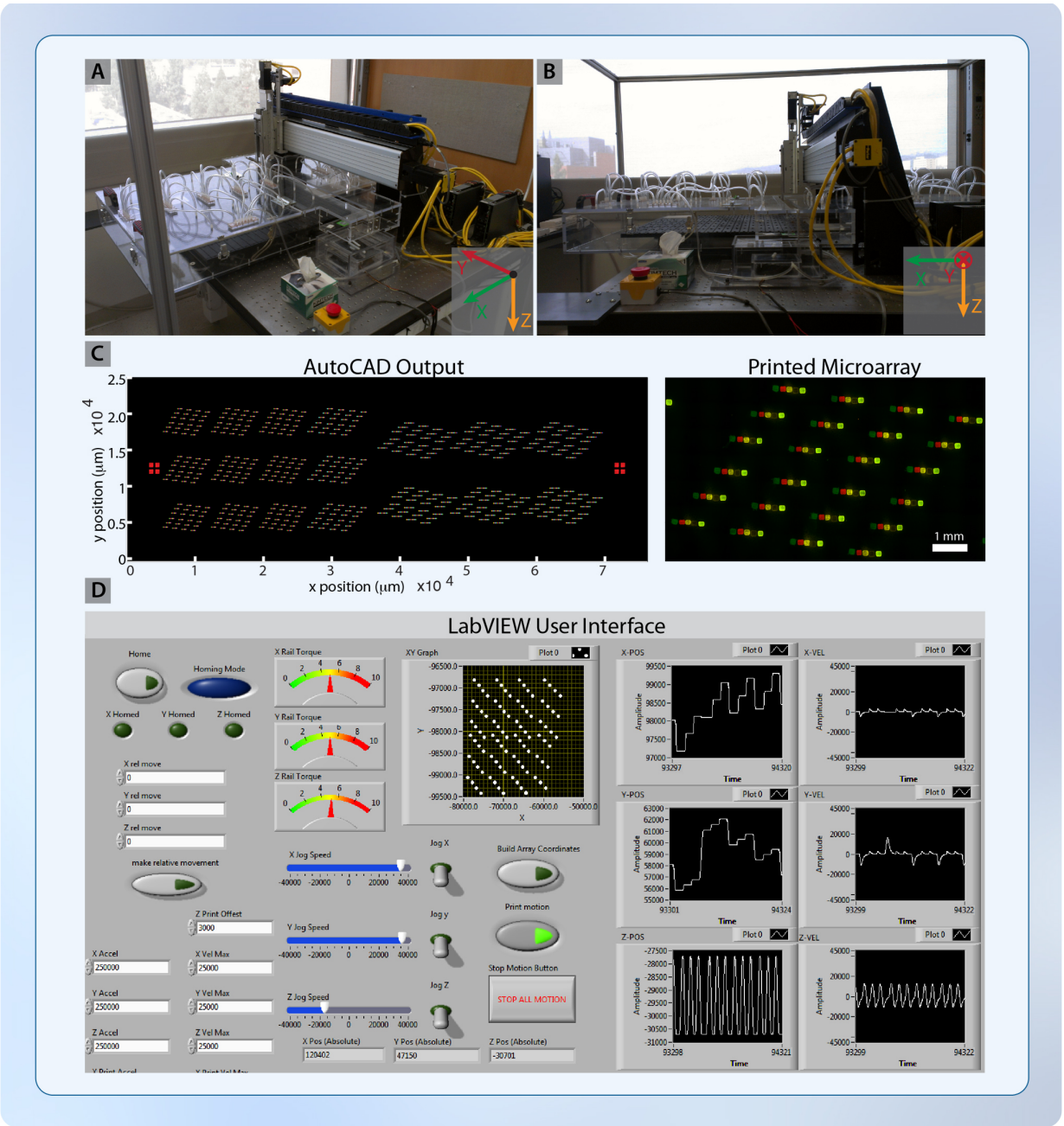

Fig. (6). (**A**) Iso-lateral view of the 3-axis robot. Acrylic housing and inlet tubes serve as an environmental regulation chamber for humidity control. (**B**) Side view of the printer. (**C**) Both the Matlab output of an array to be printed, generated using an AutoCAD template, as well as the corresponding print generated using the code. Colors correspond to different proteins printed, conjugated using Alexa-fluor 488 (green) 568 (red) and 647 (~~Cy5~~). (**D**) Snapshot of the LabVIEW user interface during a print run. Jog functionalities are used when not in a print run to align all the stations and save to memory. During a print run, the position and velocity are monitored in real time, as well as the torque on each axis. Each time the robot makes the dipping motion for a print, a white dot appears on the x-y grid, indicating the progress of the print in real time as well.

My closing example deals with the development of next generation screening systems. Microfluidics have enabled the generation of data on a never before seen scale. Currently, microfluidics are heavily employed in e.g. next generation sequencing which is actually unthinkable without microfluidics [13]. However, high throughput screening has yet to take full advantage of this novel paradigm. A barrier between high throughput screening and microfluidics is cheap, quick and reliable sample handling. For example, the printing of small molecule arrays on e.g. glass slides which can later be integrated into microfluidic devices is surprisingly difficult. This is due to the fact that each microfluidic device has its own location of channels requiring complete flexibility in array layout and location. Moreover, the polymers microfluidic devices are made of, frequently shrink during polymerization thus complicating the alignment of device and array on glass – especially when high density is desired. We solved some of these problems

by developing our own custom micro-arraying platform which is geared towards integration with microfluidics (see Fig. **6**). In short, microarray printing is accomplished using an in-house custom built, 3-axis robot, controlling a 20-pin print head with full automation using in-house Matlab and LabVIEW software. The hardware is based on the original micro-arrayer design of deRisi: Print head motion is actuated using three orthogonal linear motion stages utilizing servos and ball screws, driven by three TQ-10 Parker amplifiers. However, the control of the 3-axis print head motion is accomplished using an updated Galil DMC-1832 PCI card and our custom LabVIEW software. Print maps and patterns are generated in the industry standard AutoCad, and directly converted to Galil DMC code and tool paths using our Matlab scripts. Combining these two functionalities allows for on-demand printing of any pattern that can be drawn in AutoCAD, allowing for integration of printed microarrays with microfluidic channels. We have also built easy scaling functionality into the printing software that can accommodate shrinkage of microfluidic features. Print solutions are prepared in 384 microtiter plate format, where each well corresponds to a separate layer in the AutoCAD template. Our printing software allows the user to take any CAD file drawn as circles and produce an exact or scaled printed array of that file using our software. We plan to make this software open source and accessible on our website, both the individual components (LabVIEW and Matlab) and a wrapped version currently being written in Python.

FUTURE OUTLOOK

The MSSR worked and published in many disease areas such as oncology, infectious diseases, cardiovascular, metabolic and muscoskeletal diseases (see Table **2** for an overview of published screens) [5, 6, 10-12, 14-32] as well as nano-toxicology [10, 11, 33-36]. While the main focus of the MSSR remains small molecule HTS, we have added considerably to our service menu since our inception. This is partly driven by our understanding that the post-genomic era has led to a dramatic increase in potential drug targets and highlighted the need to pick and chose targets for drug discovery wisely. It is here that functional genomics will have a role to play in the years to come. As we build our infrastructure and functional genomics libraries to be able to produce the tools necessary to deconvolute complex diseases and advance basic science, we put special emphasis on the development of new technology and the build-out of our machine park. In addition, we plan on enhancing our library in size and diversity to cover more chemical space for our clients. Last but not least, we work to integrate small molecule screening and new technology such as microfluidics in order to reach new levels of throughput and realize greater cost savings for our customers. Ultimately, we envision making HTS available to every scientist as a research tool.

CONFLICT OF INTEREST

The author confirms that this manuscript has no conflict of interest.

Table 2. Overview of published screens run at the MSSR. Due to confidentiality, we cannot disclose unpublished projects or projects run with biotechnology or pharmaceutical company partners.

Target Types Screened by the Molecular Screening Shared Resource
Amino-Acylase-3
Angiogenesis
Anthrax Toxin
Bacterial Toxicity of nano-Polycations
Bacterial Toxicity of nano-Silver
Biofilms
CYP1A1
Cytotoxicity of nano-Zinc
Development of Malaria Diagnostic Assays
Dychenne Muscular Dystrophy
Embryonic Stem Cells
Enveloped Viruses
Glioblastoma
Hepcidin Antagonists
Herpesvirus Reactivation
HIV Vpr
Ion Channels
Leprosy
Michondrial Transport
Nano-Phototoxicity
Pancreatic Cancer
PPAR Gamma
Prostate Cancer
Psd-95
Radioprotectants
Read-Through Compounds for Non-Sense Mutations
SortA
Splicing Modulators
Syndecan-1 Mediated Nanotoxicity
TIM22
Toxicity of nano-Metal and Metaloxides
Ubiquiting Ligases
Zebrafishtoxicity of Nanomaterials
ZHE1

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REFERENCES

[1] Weiss, P. CNSI website. WWW.CNSI.UCLA.EDU (9-11-2013),

[2] Thomas, C. R.; George, S.; Horst, A. M.; Ji, Z.; Miller, R. J.; Peralta-Videa, J. R.; Xia, T.; Pokhrel, S.; Madler, L.; Gardea-Torresdey, J. L.; Holden, P. A.; Keller, A. A.; Lenihan, H. S.; Nel, A. E.; Zink, J. I. Nanomaterials in the environment: from materials to high-throughput screening to organisms. *ACS Nano.,* **2011**, *5*(1), 13-20.

[3] Chuprina, A.; Lukin, O.; Damoiseaux, R.; Buzko, A.; Shivanyuk, A. Drug- and lead-likeness, target class, and molecular diversity analysis of 7.9 million commercially available organic compounds provided by 29 suppliers. *J. Chem. Inf. Model.,* **2010**, *50*(4), 470-479.

[4] Petit, J.; Meurice, N.; Kaiser, C.; Maggiora, G. Softening the Rule of Five--where to draw the line? *Bioorg. Med. Chem.,* **2012**, *20*(18), 5343-5351.

[5] Solaimani, P.; Damoiseaux, R.; Hankinson, O. Genome-wide RNAi high-throughput screen identifies proteins necessary for the AHR-dependent induction of CYP1A1 by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. Sci.,* **2013**, *136*(1), 107-119.

[6] Zheng, S.; Damoiseaux, R.; Chen, L.; Black, D. L. A broadly applicable high-throughput screening strategy identifies new regulators of Dlg4 (Psd-95) alternative splicing. *Genome Res.,* **2013**, *23*(6), 998-1007.

[7] Ivask, A.; Suarez, E.; Patel, T.; Boren, D.; Ji, Z.; Holden, P.; Telesca, D.; Damoiseaux, R.; Bradley, K. A.; Godwin, H. Genome-wide bacterial toxicity screening uncovers the mechanisms of toxicity of a cationic polystyrene nanomaterial. *Environ. Sci. Technol.,* **2011**, *46*(4), 2398-2405.

[8] Rallo, R.; France, B.; Liu, R.; Nair, S.; George, S.; Damoiseaux, R.; Giralt, F.; Nel, A.; Bradley, K.; Cohen, Y. Self-organizing map analysis of toxicity-related cell signaling pathways for metal and metal oxide nanoparticles. *Environ. Sci. Technol.,* **2011**, *45*(4), 1695-1702.

[9] Poulos, J. L.; Jeon, T. J.; Damoiseaux, R.; Gillespie, E. J.; Bradley, K. A.; Schmidt, J. J. Ion channel and toxin measurement using a high throughput lipid membrane platform. *Biosens. Bioelectron.,* **2009**, *24*(6), 1806-1810.

[10] Lin, S.; Zhao, Y.; Ji, Z.; Ear, J.; Chang, C. H.; Zhang, H.; Low-Kam, C.; Yamada, K.; Meng, H.; Wang, X.; Liu, R.; Pokhrel, S.; Madler, L.; Damoiseaux, R.; Xia, T.; Godwin, H. A.; Nel, A. E. Zebrafish high-thoughput screening to study the impact of dissolvable metal oxide nanoparticles on the hatching enzyme, ZHE1. *Small,* **2013**, *9*(9-10), 1776-1785.

[11] Lin, S.; Zhao, Y.; Ji, Z.; Ear, J.; Chang, C. H.; Zhang, H.; Low-Kam, C.; Yamada, K.; Meng, H.; Wang, X.; Liu, R.; Pokhrel, S.; Madler, L.; Damoiseaux, R.; Xia, T.; Godwin, H. A.; Nel, A. E. Metal oxides: zebrafish high-throughput screening to study the impact of dissolvable metal oxide nanoparticles on the hatching enzyme, ZHE1 (Small 9-10/2013). *Small,* **2013**, *9*(9-10), 1775-1785.

[12] George, S.; Xia, T.; Rallo, R.; Zhao, Y.; Ji, Z.; Lin, S.; Wang, X.; Zhang, H.; France, B.; Schoenfeld, D.; Damoiseaux, R.; Liu, R.; Bradley, K. A.; Cohen, Y.; Nel, A. E. Use of a high-throughput screening approach coupled with *in vivo* zebrafish embryo screening to develop hazard ranking for engineered nanomaterials. *ACS Nano,* **2011**, *5*(3), 1805-1817.

[13] Liu, P.; Mathies, R. A. Integrated microfluidic systems for high-performance genetic analysis. *Trends Biotechnol.,* **2009**, *27*(10), 572-581.

[14] Sanchez, A. M.; Thomas, D.; Gillespie, E. J.; Damoiseaux, R.; Rogers, J.; Saxe, J. P.; Huang, J.; Manchester, M.; Bradley, K. A. Amiodarone and bepridil inhibit anthrax toxin entry into host cells. *Antimicrob. Agents Chemother.,* **2007**, *51*(7), 2403-2411.

[15] Kamata, M.; Wu, R. P.; An, D. S.; Saxe, J. P.; Damoiseaux, R.; Phelps, M. E.; Huang, J.; Chen, I. S. Cell-based chemical genetic screen identifies damnacanthal as an inhibitor of HIV-1 Vpr induced cell death. *Biochem. Biophys. Res. Commun.,* **2006**, *348*(3), 1101-1106.

[16] Aghajan, M.; Jonai, N.; Flick, K.; Fu, F.; Luo, M.; Cai, X.; Ouni, I.; Pierce, N.; Tang, X.; Lomenick, B.; Damoiseaux, R.; Hao, R.; Del Moral, P. M.; Verma, R.; Li, Y.; Li, C.; Houk, K. N.; Jung, M. E.; Zheng, N.; Huang, L.; Deshaies, R. J.; Kaiser, P.; Huang, J. Chemical genetics screen for enhancers of rapamycin identifies a specific inhibitor of an SCF family E3 ubiquitin ligase. *Nat. Biotechnol.,* **2010**, *28*(7), 738-742.

[17] Kendall, G. C.; Mokhonova, E. I.; Moran, M.; Sejbuk, N. E.; Wang, D. W.; Silva, O.; Wang, R. T.; Martinez, L.; Lu, Q. L.; Damoiseaux, R.; Spencer, M. J.; Nelson, S. F.; Miceli, M. C. Dantrolene enhances antisense-mediated exon skipping in human and mouse models of duchenne muscular dystrophy. *Sci. Transl. Med.,* **2012**, *4*(164), 164ra160.

[18] Murphy, J. M.; Armijo, A. L.; Nomme, J.; Lee, C. H.; Smith, Q. A.; Li, Z.; Campbell, D. O.; Liao, H. I.; Nathanson, D. A.; Austin, W. R.; Lee, J. T.; Darvish, R.; Wei, L.; Wang, J.; Su, Y.; Damoiseaux, R.; Sadeghi, S.; Phelps, M. E.; Herschman, H. R.; Czernin, J.; Alexandrova, A. N.; Jung, M. E.; Lavie, A.; Radu, C. G. Development of new deoxycytidine kinase inhibitors and noninvasive *in vivo* evaluation using positron emission tomography. *J. Med. Chem.,* **2013**, *56*(17), 6696-6708.

[19] Zhang, H.; Xia, T.; Meng, H.; Xue, M.; George, S.; Ji, Z.; Wang, X.; Liu, R.; Wang, M.; France, B.; Rallo, R.; Damoiseaux, R.; Cohen, Y.; Bradley, K. A.; Zink, J. I.; Nel, A. E. Differential expression of syndecan-1 mediates cationic nanoparticle toxicity in undifferentiated versus differentiated normal human bronchial epithelial cells. *ACS Nano.,* **2011**, *5*(4), 2756-2769.

[20] Suree, N.; Yi, S. W.; Thieu, W.; Marohn, M.; Damoiseaux, R.; Chan, A.; Jung, M. E.; Clubb, R. T. Discovery and structure-activity relationship analysis of Staphylococcus aureus sortase A inhibitors. *Bioorg. Med. Chem.,* **2009**, *17*(20), 7174-7185.

[21] Zuo, J.; Quinn, K. K.; Kye, S.; Cooper, P.; Damoiseaux, R.; Krogstad, P., Fluoxetine is a potent inhibitor of coxsackievirus replication. *Antimicrob. Agents Chemother.,* **2012**, *56*(9), 4838-4844.

[22] Reyes, V. C.; Li, M.; Hoek, E. M.; Mahendra, S.; Damoiseaux, R. Genome-Wide Assessment in Escherichia coli Reveals Time-Dependent Nanotoxicity Paradigms. *ACS Nano.,* **2012**, *6*(11), 9402-9415.

[23] Lin, S.; Zhao, Y.; Xia, T.; Meng, H.; Ji, Z.; Liu, R.; George, S.; Xiong, S.; Wang, X.; Zhang, H.; Pokhrel, S.; Madler, L.; Damoiseaux, R.; Nel, A. E. High content screening in zebrafish speeds up hazard ranking of transition metal oxide nanoparticles. *ACS Nano.,* **2011**, *5*(9), 7284-7295.

[24] Kim, K.; Pollard, J. M.; Norris, A. J.; McDonald, J. T.; Sun, Y.; Micewicz, E.; Pettijohn, K.; Damoiseaux, R.; Iwamoto, K. S.; Sayre, J. W.; Price, B. D.; Gatti, R. A.; McBride, W. H. High-throughput screening identifies two classes of antibiotics as

radioprotectors: tetracyclines and fluoroquinolones. *Clin. Cancer Res.,* **2009**, *15*(23), 7238-7245.

[25] Fung, E.; Sugianto, P.; Hsu, J.; Damoiseaux, R.; Ganz, T.; Nemeth, E. High-throughput screening of small molecules identifies hepcidin antagonists. *Mol. Pharmacol.,* **2013**, *83*(3), 681-690.

[26] Stoilov, P.; Lin, C. H.; Damoiseaux, R.; Nikolic, J.; Black, D. L. A high-throughput screening strategy identifies cardiotonic steroids as alternative splicing modulators. *Proc. Natl. Acad Sci. U S A,* **2008**, *105*(32), 11218-11223.

[27] Tsirulnikov, K.; Abuladze, N.; Bragin, A.; Faull, K.; Cascio, D.; Damoiseaux, R.; Schibler, M. J.; Pushkin, A. Inhibition of aminoacylase 3 protects rat brain cortex neuronal cells from the toxicity of 4-hydroxy-2-nonenal mercapturate and 4-hydroxy-2-nonenal. *Toxicol. Appl. Pharmacol.,* **2012**, *263*(3), 303-314.

[28] Lee, D. J.; Li, H.; Ochoa, M. T.; Tanaka, M.; Carbone, R. J.; Damoiseaux, R.; Burdick, A.; Sarno, E. N.; Rea, T. H.; Modlin, R. L. Integrated pathways for neutrophil recruitment and inflammation in leprosy. *J. Infect. Dis.,* **2010**, *201*(4), 558-569.

[29] Du, L.; Jung, M. E.; Damoiseaux, R.; Completo, G.; Fike, F.; Ku, J. M.; Nahas, S.; Piao, C.; Hu, H.; Gatti, R. A. A new series of small molecular weight compounds induce read through of all three types of nonsense mutations in the ATM gene. *Mol. Ther.,* **2013**, *21*(9), 1653-1660.

[30] Lee, S.; Deng, H.; Yu, F.; Melega, W. P.; Damoiseaux, R.; Bradley, K. A.; Sun, R. Regulation of Kaposi's sarcoma-associated herpesvirus reactivation by dopamine receptor-mediated signaling pathways. *J. Acquir. Immune Defic. Syndr.,* **2008**, *48*(5), 531-540.

[31] Dabir, D. V.; Hasson, S. A.; Setoguchi, K.; Johnson, M. E.; Wongkongkathep, P.; Douglas, C. J.; Zimmerman, J.; Damoiseaux, R.; Teitell, M. A.; Koehler, C. M. A small molecule inhibitor of redox-regulated protein translocation into mitochondria. *Dev. Cell,* **2013**, *25*(1), 81-92.

[32] Hasson, S. A.; Damoiseaux, R.; Glavin, J. D.; Dabir, D. V.; Walker, S. S.; Koehler, C. M. Substrate specificity of the TIM22 mitochondrial import pathway revealed with small molecule inhibitor of protein translocation. *Proc. Natl. Acad Sci. U S A,* **2010**, *107*(21), 9578-9583.

[33] Li, M.; Noriega-Trevino, M. E.; Nino-Martinez, N.; Marambio-Jones, C.; Wang, J.; Damoiseaux, R.; Ruiz, F.; Hoek, E. M. Synergistic bactericidal activity of Ag-TiO(2) nanoparticles in both light and dark conditions. *Environ. Sci. Technol.,* **2011**, *45*(20), 8989-8995.

[34] Zhang, H.; Ji, Z.; Xia, T.; Meng, H.; Low-Kam, C.; Liu, R.; Pokhrel, S.; Lin, S.; Wang, X.; Liao, Y. P.; Wang, M.; Li, L.; Rallo, R.; Damoiseaux, R.; Telesca, D.; Madler, L.; Cohen, Y.; Zink, J. I.; Nel, A. E. Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. *ACS Nano,* **2012**, *6*(5), 4349-4368.

[35] Xiong, S.; George, S.; Ji, Z.; Lin, S.; Yu, H.; Damoiseaux, R.; France, B.; Ng, K. W.; Loo, S. C. Size of TiO(2) nanoparticles influences their phototoxicity: an *in vitro* investigation. *Arch Toxicol.,* **2013**, *87*(1), 99-109.

[36] Xiong, S.; George, S.; Yu, H.; Damoiseaux, R.; France, B.; Ng, K. W.; Loo, J. S. Size influences the cytotoxicity of poly (lactic-co-glycolic acid) (PLGA) and titanium dioxide (TiO(2)) nanoparticles. *Arch. Toxicol.,* 201, *87*(6), 1075-1086.

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