Building a uHTS laboratory

communications from the frontier

Through sheer commercial need to conduct HTS reliably, rapidly and economically, science and technology have partnered to move laboratories from semi-automated craft guilds to industrialised uHTS research operations. To the benefit of both the pharmaceutical and HTS technology industries, today's researcher, tasked with setting up or revamping an HTS laboratory, is faced with a variety of choices from a wide variety of established vendors.

By Dr Peter Hodder

The benefit of high-throughput screening (HTS) as a drug discovery tool has been demonstrated, and leads propagating from HTS laboratories have successfully enriched clinical research programmes[1]. As compound screening libraries have grown larger and the number of targets to be screened has increased, so has the desire to achieve faster throughputs during HTS campaigns. This need has spurred pharmaceutical companies to incorporate 'ultra' high throughput screening (uHTS) technologies that enable more rapid screening of compound libraries, in excess of 100,000 samples per day. Interestingly, other non-traditional drug discovery operations have been affected by the success of big pharma uHTS operations: uHTS strategies and technologies have been adopted not only by most major pharmaceutical companies, but also in several biotechnology and academic settings[2,3].

Although the success of uHTS is dependent on many factors, one that is obvious is the careful selection of an appropriate robotic screening platform. Unfortunately to the uninitiated, this is only one step of a process; equally important is the consideration of lab infrastructure and processes coordinated with the uHTS campaign. For example, a particular platform may generate assay results at the rate of >100,000/day during the course of a robotic campaign. However, if a massive 'off-line' cell or compound plating effort was required prior to the robotic screening campaign, this must also be considered in throughput calculations.

Recently the author of this article had the opportunity to create a uHTS laboratory at the Scripps Research Institute (TSRI) in Palm Beach County, Florida. Using this experience as a guide, the purpose of this article is to summarise the 'state-of-the art' in uHTS operations, and elucidate important supporting automation that is necessary to sustain lab productivity. In some cases, shortcomings of current technologies or areas of further development are discussed.

Miniaturisation of uHTS assays

One decision facing the uHTS lab director is whether campaigns should be performed in a miniaturised format, the 1536-well format being the most popular. Indeed, uHTS campaigns

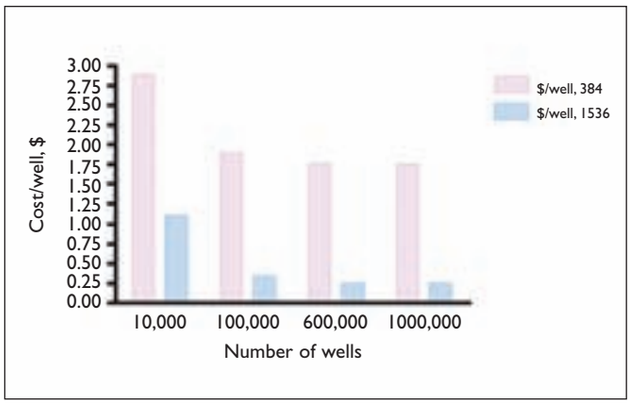

Figure 1a
Robotic consumables, plate and reagent costs associated with a typical cell-based screening campaign versus the number of wells screened. 1536 (6µL assay volume) and 384-well (40µL assay volume) formats compared. As larger compound collections are screened, the cost per well approaches the cost of the assay reagents

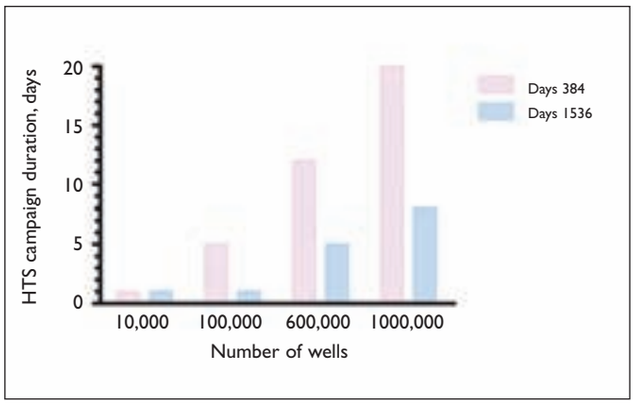

Figure 1b
The length of the screening campaign for the assay described in (a) for a typical 384-well format platform and the uHTS platform described in Figure 2

performed in the 1536-well format are more economical and rapid than their 384-well format counterparts, and as a result less FTE labour is required to achieve comparable productivity (**Figure 1**). It is important to note that miniaturisation is not required to achieve uHTS conditions: Throughputs of greater than >100,000 samples per day can be achieved with automation that specialises in 384-well format, and for some campaigns it is possible to reduce assay volumes down to 10-20 µL/well[4]. Although 1536-well format assays can be performed in 2 to 10 µL/well assay volumes, the incremental cost savings vis-à-vis 384-well format assays may be less important to some lab managers.

Until recently, equipment that specialised in the 1536-well plate format was scarce. At the turn of this century, the majority of uHTS campaigns performed in 1536-well format used equipment and facilities that were originally designed for processing 384-well format plates. This often resulted in data that was subject to artifacts, and propagated the belief that the advantages of miniaturised uHTS campaigns came at the cost of lower data quality. Although the belief was well founded, the introduction of more reliable liquid handling and detection technologies for 1536-well format plates has largely diminished this concern[5]. With proper scientific expertise, comparable results are obtained between the formats, even in challenging assays.

If a lab director is saddled with a legacy of older but functional and reliable HTS equipment and competent personnel, it may be unnecessary to upgrade to a miniaturised format. However, shrinking consumable, reagent and personnel budgets may predicate reorganising a lab to perform miniaturised uHTS. Additionally, labs that employ scarce biologicals may also be attracted to the economy of the miniaturised format, even if their compound library is relatively small.

uHTS automation

Several vendors sell fully-automated HTS robotic platforms (**Table 1**). Although a smaller group of these vendors have established themselves as experts at manufacturing 1536-well format uHTS platforms, almost all others are capable of constructing a uHTS capable platform at a customer's behest. The majority of vendors specialise in bespoke automation platforms that shuttle plates to peripheral liquid handlers and detectors made by the vendor itself, or integrated third party equipment specified by the customer. A minority has dedicated engineers that design and manufacture bespoke equipment to a customer's specification. Currently, some vendors of specialised 1536 automation prefer to base pricing on the value to the customer rather than the cost required to produce the automation itself, but in general the capital outlay required for a uHTS platform is within the grasp of most departmental budgets.

The goal of a uHTS robotic platform is obvious, but automation employed to achieve this goal varies from vendor to vendor. Most vendors have incorporated anthropomorphic robotic arms to move microtiter plates around the platform (**Figure 2a**); less common but equally up to the task are

platforms that incorporate SCARA, gantry and track-based technologies. This presents an important caveat to the customer who is deciding which particular platform to purchase, especially if there is no substantial difference in price. Ultimately, vendors should be chosen that can demonstrate they have the competence to build a platform that balances flexibility and reliability to the satisfaction of the customer. Once specifications are agreed upon, testing cycles and milestone payments can be determined to the satisfaction of both parties. Vendors who do not agree to such an arrangement should be more closely investigated. Another practice to be wary of is the peddling of 'vapour-ware', ie hardware with which the vendor has no experience. In the cases of manufacturing a completely customised platform or dealing with a newly established vendor, this cannot be avoided, and care must be taken to select the right partner before commissioning the project.

Difficult assay protocols such as SPA bead dispensing, plate washing (**Figure 2c**) and FLIPR assays[6] that once remained in the domain of 384-well format have now been successfully

Table 1: Vendors of fully automated, integrated HTS and uHTS platforms

VENDOR	SPECIALTIES	WEBSITE
Aurora Discovery	3456-well plate technology; OEM of liquid handling and detection equipment	http://www.auroradiscovery.com/
Beckman	Scalable automation offerings; OEM of liquid handling and detection equipment	http://www.beckman.com
Caliper	Scalable automation offerings; OEM of liquid handling and detection equipment	http://www.caliperls.com
Cybio	Track-based screening systems; OEM of liquid handling and detection equipment	http://www.cybio-ag.com
Evotec	2080-well plate technology; OEM of bespoke liquid handling and detection equipment	http://www.evotec-technologies.com
GNF Systems	Industrialised 1536-well format uHTS systems; OEM and bespoke equipment manufacture	http://www.gnfsystems.com/
High Resolution Engineering	Custom systems; Third-party integrations	http://www.hireseng.com
Kalypsys	Industrialised 1536-well format uHTS systems; bespoke equipment manufacture	http://www.kalypsys.com/
Protedyne	Small footprint, gantry-based systems; OEM of liquid handling equipment	http://www.protedyne.com
RTS Life Sciences	OEM and bespoke equipment manufacture, uHTS systems	http://www.rts-group.com/
SSI Robotics	Diverse HTS automation applications; bespoke equipment manufacture	http://www.ssirobotics.com
The Automation Partnership	Industrialised uHTS systems, OEM and bespoke equipment manufacture	http://www.automationpartnership.com/
Thermo CRS	Integration of third party hardware, uHTS systems	http://www.thermo.com/
Velocity 11	Small footprint systems and liquid handling equipment	http://www.velocity11.com

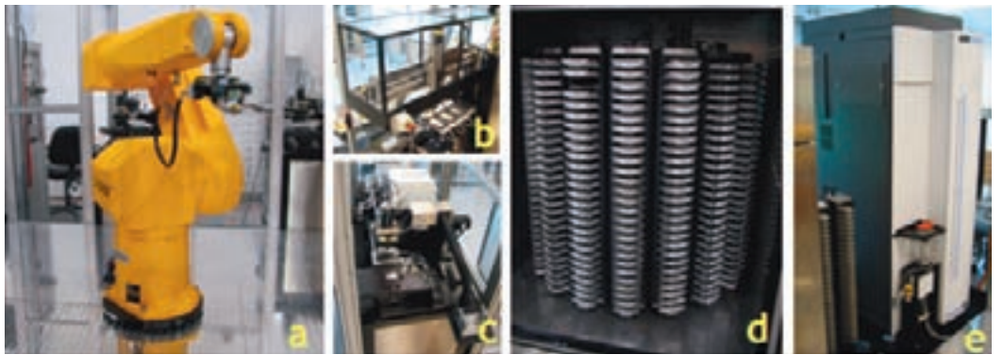

Figure 2
a Scripps Florida's uHTS platform. The entire robot occupies a footprint of 220ft^2 **b** A 1536-pintool is used to transfer test compounds rapidly from compound plates to assay plates. **c** two liquid handlers capable of dispensing up to 32 different reagents and 1536-well plate washing capability are integrated on to the platform. **d** Three incubators, each capable of holding ~700,000 samples in 1536-well format are used to store assay and compound plates. **e** an imaging plate reader performs absorbance, luminescence and fluorescence (with time resolution) measurements on 1536-well plates. Not shown is a compound management robot capable of storing ~700,000 samples in 384-well format for cherry picking efforts; it occupies a footprint of 198ft^2

miniaturised and automated. Some popular protocols remain refractory, due to engineering and software challenges. Examples are radioactive filter binding assays, which at the time of writing have just been successfully miniaturised to 384-well format and gene-expression assays that require modification of protocol steps based upon intermediate data. Although high-content screening (HCS) assays can be miniaturised, it is difficult to achieve uHTS on a platform that integrates HCS instrumentation. This is due to the time required to focus, acquire and analyse the well images produced by the HCS instrument. Although solutions to these problems, especially in the case of HCS instrumentation, are on the horizon, laboratories whose livelihood depends upon execution of these assays should take a 'wait-and-see' policy to miniaturisation and uHTS.

Facilities

The importance of facilities requirements have long been acknowledged in laboratories that specialise in 1536-well format assays; sophisti-

Figure 3
Scripps Florida's uHTS automation room. Assay development, compound management and uHTS operations are housed in a 1,800ft^2 class 10,000 clean room

cated environmental controls are critical to prevent evaporation of assay reagents from microtiter plates and promote test compound stability. Borrowing technologies used for semiconductor-processing clean rooms, a modern uHTS facility incorporates an HVAC system that maintains temperature, humidity and particulate concentrations within 10% of desired set points (**Figure 3**).

The facility requirements for uHTS laboratories have increased with the demand to automate a wider variety of assays. It is now increasingly popular to automate assays that are either sensitive to environmental contamination or employ reagents that are potentially hazardous to the laboratory technician. Therefore, it is important to consider potential facility requirements even if assays are only in 96 or 384-well format.

There have been three major approaches to address the more complex facilities requirements listed above. The first and most obvious has been to dedicate an automation platform to a particular assay format, and govern its safe operation by drafting standard operating procedures. This has been particularly effective for platforms that execute radioactivity-based assays, since a large body of literature exists to effectively guide the safe use of radioactive materials. In cases where relatively non-hazardous assay reagents (eg, a BSL-1 mammalian cell line, RNAi sample, PCR reaction components) need to be protected from airborne contamination, a popular and economical secondary approach is to place the relevant automation in an environmentally controlled enclosure (**Table 2**). With significant but straightforward modifications, the same enclosures can contain potentially hazardous particulate or remove toxic solvent fumes. A minor drawback of these enclosures is that access to the robotic platform is severely limited, hampering maintenance and troubleshooting efforts. A third approach is to locate the automation within a room specialised for the assay performed. This is especially popular for infectious disease research. Although costly, this approach maximises automation productivity and guarantees the highest level of safety.

Compound management technologies

Maintaining a screening library remains one of the most labour intensive and expensive aspects of any uHTS operation. In most operations, the fate of compounds must be managed not only within the screening laboratory, but also

among external collaborators. In the case of therapeutic group collaborators, custom automation may not only be required to rapidly locate and retrieve the requested samples, but also to provide them in an 'assay ready' format, eg dilutions of compounds in a 384-well plate. Medicinal chemistry support adds additional requirements, including determination of sample purity or structure identification, and also the process of registration and incorporation of newly-synthesised samples into the compound collection.

Compound management technologies can be divided into three major categories: the consumables necessary for physical storage of the samples, the hardware and software that interfaces with these consumables, and instrumentation necessary to determine the purity and structure of the compounds themselves. Although an effective compound management operation does not necessarily require full automation, several well-established automation vendors have now specialised in providing modular, scalable compound management systems with integrated sample tracking databases (**Table 3**). Throughput standards have not been formally developed for compound management automation but most systems are capable of processing samples at the rate of 104 samples/day. Although this is slow when compared to the throughput of even an HTS platform, it is usually sufficient to support cherry-picking efforts in a uHTS laboratory.

The most popular consumable used in compound management automation is the 'tube rack'. Similar in footprint to a microtiter plate, the typical tube rack consists of separable, individually barcoded compound vials to which powdered or solvated sample is added. The 96-well format tube rack remains a popular format, most likely due to its ease of manipulation by both manual and automated means, and its ability to store relatively large sample volumes (~1.5 mL). For operations that do not require large volumes of sample or lack storage space, 384-tube racks have been developed. However, the advantages of this format are offset by the inability of long term compound storage once the tube seal is compromised.

Currently, no 1536-tube racks are available. However, some companies have devised technical innovations to store and access compounds in 1536-well microtiter plates. In these cases, a specialised lid is used to seal the microtiter plate and minimise solvent evaporation/hydration during long term storage; the lid is removed by custom automation when it is necessary to access the contents of the plate, and replaced for long term storage. Although samples within the plate will eventually succumb to degradation, this is balanced by the fact that a relatively small amount of sample is sacrificed, especially for the 1536-well format, and the lids themselves are reusable. In many operations, the reusable-lid technology is popular among robotics operators, since little or no manual labour is spent unsealing or resealing compound plates between HTS campaigns. As mentioned above, another attractive feature is the small amount of space occupied by a library in 1536-well format: in one instance, more than 700,000 samples can be stored in an online incubator (**Figure 2d**).

Table 2: Vendors of automation enclosures and clean-room technologies

VENDOR	WEBSITE
Airfiltronix	http://www.airfiltronix.com/
Bigneat	http://bigneat.com/
Terra Universal	http://www.terrauniversal.com
Flow Sciences	http://www.flowsciences.com/
Hemco	http://www.hemcocorp.com

Several compound vendors and brokers can solvate, reformat and store compound libraries on a fee-for-service basis. Costs for services vary widely based on the number of copies made, but in all cases are significantly cheaper than the capital equipment and FTE costs associated with compound reformatting. Currently, this presents attractive outsourcing options for companies that lack the physical space or budget to set up a compound management group. Outsourcing of compound management operations will continue to grow, perhaps even attracting companies that must downsize their internal operations. Most compound vendors are able to resupply previously purchased compounds to customers at fixed costs. Although less prevalent, it is not hard to imagine that several companies will also offer 'cherry-picking' services for a customer's stored compound library in the near future.

In hit-to-lead efforts, analytical instrumentation is essential for quality control of compounds produced by medicinal chemistry efforts. Identical instrumentation can be used to assess the quality of screening libraries[7]. Although NMR methods can

Table 3: Vendors of compound management automation, software or related consumables

VENDOR	PREDOMINANT TECHNOLOGIES	FORMATS	WEBSITE
Abgene	Consumables; Benchtop Hardware	96, 384	http://www.abgene.com/
ASDI Biosciences	Compound Reformatting and Storage Services	96, 384, 1536	http://www.asdibiosciences.com
Aurora Discovery	Integrated Hardware and Software; Consumables	3456	http://www.auroradiscovery.com/
Biostorage Technologies	Compound Storage Services	Any	http://www.biostoragetech.com/
Discovery Partners	Compound Reformatting and Storage Services	96, 384, 1536	http://www.discoverypartners.com
GNF	Integrated Hardware and Software; Consumables	384, 1536	http://web.gnf.org/
Kalypsys	Integrated Hardware and Software; Consumables	384, 1536	http://www.kalypsys.com/
Matrix	Consumables; Benchtop Hardware	96, 384	http://www.matrixtechcorp.com/
REMP	Integrated Hardware and Software; Consumables	96, 384	http://www.remp.com/
RTS Life Sciences	Integrated Hardware and Software	96, 384	http://www.rts-group.com/
Spectrum BioScience	Compound Reformatting and Storage Services	96, 384	http://www.spectrumbio.com
Tekcel	Integrated Hardware and Software; Consumables	96, 384	http://www.tekcel.com/
The Automation Partnership	Integrated Hardware and Software; Consumables	96, 384	http://www.automationpartnership.com/
Titian Software	Software	Any	http://www.titian.co.uk/
Tomtec	Benchtop Hardware; Consumables	96, 384	http://www.tomtec.com/
TTP Labtech	Integrated Hardware and Software	96	http://www.ttplabtech.com

be employed, currently liquid chromatography-mass spectrometry (LC-MS) platforms remain the gold standard for determining compound purity and mass of screening collections[8]. Regardless, both methods consume significant amounts of sample for analysis, and suffer tremendously from throughput concerns, maximally at 103 analyses/day.

The development of LC-MS analytical techniques and technologies to increase sample throughput has been hampered by the inherent serial nature of LC-MS sampling and the time required to interpret resulting chromatograms and spectra. Significant developments have been made to increase sample throughput (parallel LC columns, incorporation of MUX technology) and automate compilation and interpretation of results. However, LC-MS technology still lacks innovations necessary to fully integrate into a uHTS effort.

Database technologies

Although several pharmaceutical companies have invented and still maintain proprietary in-house databases for the management of HTS data, this is due largely to the fact they required information management long before any commercial solutions were available. Nowadays, several vendors have turnkey HTS databases which obviate the need and expense of developing an in-house solution. Most of these vendors offer sophisticated products that support assay registration, compound registration and management, plate barcode tracking, data review and analysis, report generation and SAR tools. If customisation is required, vendors can develop specific applications, or write bridging software code that allows a competitor's software to interface with their product.

Because of the nature of the software development process, and the production of ever-faster computing technology, it is a relatively straight-

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forward process to tailor commercially available HTS database software to the needs of a particular operation and keep the software and hardware on the 'cutting edge'. In addition, all competitive databases have incorporated the data storage and visualisation needs of scientists downstream of the HTS process, allowing one product to be used across the drug discovery organisation. Areas of future database technology development will most likely focus on the facilitation of interdisciplinary analyses on functional genomic, proteomic, DMPK/clinical and HTS data, simplified presentation of compound quality control information, and summarisation and indexing of HCS data.

Future directions

One important and overlooked benefit of miniaturisation is the effect it has on uHTS laboratory operations. Most tangible are the reductions in space required for consumables storage and the amount of effort involved marshalling the resources necessary to implement and sustain a uHTS campaign. Since a proportionally smaller amount of time is spent staging the smaller quantities of microtiter plates and reagents, logistics become simplified for the lab manager. Productive facilities with both uHTS and compound management capabilities can be contained in smaller spaces, and staffed with fewer people.

With the plethora of standardised technologies that perform uHTS operations in 1536-well format, and the increasing accessibility of technologies, screening labs in the future will undoubtedly be very different. As costs for uHTS systems decrease, smaller drug-discovery companies and academic research labs with sufficient know-how will be able to leap-frog the expense and effort associated with equipping 384-well format operations. Instead they will be able to partner directly with experienced automation providers and consultants to set up turnkey 1536-well uHTS operations. Once set up, researchers will be able to bring in custom compound, protein or nucleic-acid based samples from a variety of commercially available and well-characterised libraries. For these operations and their smaller cash coffers, a 'Goldilocks' incentive will be created: combined with scientific expertise, screening operations that require the smallest footprint and least expensive operating costs will prove successful and be the sources of future therapeutics. DDW

Dr Peter Hodder *currently heads the Lead Identification Department at Scripps in Palm Beach County, Florida. This centralised ultra-high throughput screening (uHTS) laboratory supports Scripps' intramural drug discovery efforts, as well as NIH-funded Molecular Library Screening Network goals and collaborative efforts with external partners. The department also manages Scripps' 600,000-member small-molecule HTS library. Dr Hodder heads a consulting service for researchers who wish to set up HTS and uHTS laboratories.*