Establishment of an open access compound management facility in Australia to stimulate applied, basic and translational biomedical research

The paradigm for probing biological processes and drug discovery via small molecules has changed significantly over the past 10-15 years. The previous protocol in which 10s-100s of compounds were tested in animal models has been replaced by one where 100,000s-1,000,000s of compounds are routinely screened against a specific target or cellular assay. Big pharma, biotechs and the USA's publicly funded NIH[1] all enjoy the combined benefits of automated compound storage and high throughput screening (HTS) to identify molecules that interact with proteins, receptors, DNA and RNA. One could view this relationship metaphorically as a marriage between chemistry through compound libraries and biology via HTS.

By David Camp

Excellent HTS facilities have already been set up throughout Australia. Until now, however, no dedicated compound management facility has been available to augment this investment. This lack of critical infrastructure is currently being addressed via establishment of the Queensland Compound Library (QCL) at the Eskitis Institute following funding from the Queensland Government and Griffith University.

The guiding principle of the QCL is to facilitate collaboration between Australian chemists and biologists, and add value to the already excellent basic medical research, synthetic organic chemistry and natural product expertise in the region. Chemists can deposit compounds into a central repository thereby allowing biologists access to a unique suite of molecules in screen ready microplates. The consolidation of Australian chemistry at a central repository will result in a greater coverage of chemistry space than any single collection in the country, public or private, currently achieves. Chemists will be able to store potentially valuable collections under optimal conditions with vastly increased opportunities to have their compounds tested for biological activity against an increased number of targets.

Of great importance is the fact that the QCL does not lay claim to any intellectual property (IP) owned or generated by users of the facility. A unique IP model that lies somewhere between the propriety nature of industry and the NIH policy of placing data in the public domain[2] was

developed for the current Australian situation. The QCL model allows synergies to develop and mature into projects that are prosecuted in a way best suited to the collaboration. Thus, molecules submitted by chemists may be tested to interrogate biological function or form the basis of a drug discovery programme.

The facility

When choosing an automated platform that will be supporting a large and diverse customer base, careful attention must be taken to ensure it meets the needs of customers. The system must:

- Ensure sample integrity and accessibility.
- Provide a robust and reliable solution (high uptime).
- Have the appropriate capacity and throughput with possible upgrade paths.

After an extensive evaluation of the available alternatives, a system configuration composed of three main building blocks was chosen. These chief components (**Figure 1**) will be described along with the features that make certain the above criteria are met.

Microtube store

TTP LabTech's[3] comPOUND® sample store holds up to 100,000 microtubes in an inert, dry and dark environment at temperatures down to -20°C. comPOUND® is a modular system and multiple stores can be linked in parallel. Sample tubes can be cherry-picked from all linked stores at the same time, allowing throughputs to increase as a library grows. comPOUND's rapid pneumatic delivery system allows tubes to be sent to a comPANION™ remote unit located up to 15m away from the main store – in this case within the envelope of the BioCel®.

Requested samples are individually cherry picked from the store, obviating unnecessary thaw cycles intrinsic to plate-based systems. Moreover, racks of retrieved tubes can be arrayed in user-specified formats to simplify subsequent plate creation. Each tube carries an individual 2D barcode which is read on the way into and out of the store, making it virtually impossible to obtain the wrong sample. Every microtube used in the QCL has a unique 14 x 14 alphanumeric 2D barcode. This matrix affords 3,600,000,000,000,000 individual barcodes, ie 3.6 quadrillion unique permutations. ABgene[4], suppliers of microtube labware used in the QCL, warrant no two will have the same code.

Compounds submitted by chemists are initially transferred to a microtube and then dissolved in dry DMSO. The microtubes are then deposited into the store for curation under a dry nitrogen atmosphere for up to 5-6 years. The format free environment facilitates cherry picking of individual microtubes for reformatting into microplates. Microtube subsets for retest or counter screens are accessed as easily as the entire set is for a primary screening campaign.

Microtubes are retrieved and collated by the comPANION™ at the rate of approximately 96 tubes every eight minutes. This is more than adequate for a compound collection of 200,000 samples and will provide overnight cherry picking capability for follow-up screening. Higher rates are obviously necessary for industry if more than 1,000,000 samples are screened to progress a project at the same rate as a smaller collection. Here, dedicated buildings containing several high speed pick-and-place robotics are employed. This is clearly outside the scope of Australian publicly funded budgets and current collection numbers. QCL's equipment is, however, scalable and can easily be expanded to double capacity and accommodate a further 200,000-400,000 microtubes when the need arises.

It is imperative to maintain a low humidity environment because of the potential for precipitation and degradation of samples due to ingress of adventitious water[5,6]. At best, the presence of water can precipitate compounds but, in the worse case scenario, promote degradation; especially if a strong acid, such as trifluoroacetic acid, was used to cleave a molecule from a solid support and/or purify the compound prior to curation[7]. Precipitated compounds may be rescued by forcing them back into solution, typically through ultrasonic techniques[7]. Degraded compounds, on the other hand, are discarded.

A direct consequence of precipitation is that chemistry may not be screened at the expected concentration resulting in a higher rate of false negatives[8]. Compound management groups have recognised this issue over the past five years and responded by ensuring all curation and liquid handling manipulations are carried out under an inert atmosphere (nitrogen) or low relative humidity (10%). Analytical equipment such as an Echo™ 550[9] acoustic auditor resident within the BioCel® can determine the extent of water uptake in microplates (a microtube variant – the Echo™ 380 is under development by Labcyte) while LC-UV-ELSD-(CLND)-MS-(NMR)[10] techniques scan for potential degradation products[7].

Figure 1
The automated compound management facility being established at the Eskitis Institute. The TTP Labtech comPOUND® microtube stores are located at the rear left, TekCel's Active Sample Manager (ASM) and serverST microtitre plate store/server to the right and Velocity 11's BioCel® sample processing robot at the front. *Image courtesy of Velocity 11, TTP Labtech and TekCel*

Reformatting and replication robot

A Velocity 11 BioCel® 1800[11] lies at the heart of the compound library and undertakes all reformatting and replicating tasks. Reformatting is where an aliquot is taken from a particular storage format, such as microtubes, and transferred to another format, like a 384 well microplate. Microplates can also be reformatted in a process known as quadranting. Here, 96 or 384 well plates may be reformatted into higher density 384 (4x96) or 1536 (4x384) well plates respectively. Replication, as the name suggests, is a direct transfer of an aliquot held in one format to a destination in the same format, eg the preparation of daughter plates from mother plates where both would be the same plate density and layout.

The Velocity11 BioCel® has a proven track record in the field of compound handling. The use of high quality devices placed around a very high-speed three-axis radial robot allows for rapid and secure processing of the tube racks and plates supplied from the storage units. Two VPrep® precision liquid handlers allow for simultaneous processing of both septum-sealed tubes and microplates. A Labcyte Echo®550 acoustic dispenser provides non-contact, very low volume (nL) dispensing capability. Because liquid is transferred through an acoustic mechanism, consumables are not required for operation, making the Echo 550 an extremely attractive alternative to syringe based technologies that may use tips to transfer liquid.

Nanolitre dispensing is becoming increasingly more common as assays are miniaturised ever further into low volume 384 well plates and 1536 well plates. Some cell based assays like those run using FLIPR® technology also benefit from acoustic droplet ejection, by reducing the volume of DMSO present, a causative agent for cell death, as low as possible. The Echo® 550 can also deliver minuscule volumes of concentrated solution (up to 10µM) rather than larger volumes of more dilute solutions typically used to probe molecular targets with other technologies.

To complement the environmental control in the storage units the BioCel® is equipped with inert gas shelves on the liquid handler and a PlateLoc® thermal plate sealer with integrated inert gas purging to allow plates to be sealed with a protective inert gas layer. Other equipment on the BioCel includes a VSpin® centrifuge, VCode® print and apply station, VStack® labware stackers and 1D and 2D barcode scanners.

Plate store

Microtubes are typically reformatted into microplates, the labware of choice for the majority of biological assays. The first microplate to be produced from microtubes is often referred to as the 'mother' plate. From this plate, numerous copies, (otherwise referred to as 'daughter' or 'assay' plates depending on the circumstance), can be produced rapidly and efficiently. In some cases, a mother plate may be diluted and the resulting set referred to as a 'working' plate to distinguish it

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9 Labcyte, URL http://www.labcyte.com.
10 Abbreviations – LC: liquid chromatography; UV: ultraviolet; ELSD: evaporative light scattering detection; CLND: chemiluminescent nitrogen detection; MS: mass spectrometry; NMR: nuclear magnetic resonance (spectroscopy).
11 Velocity 11, URL http://www.velocity11.com.

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from a daughter plate that will ultimately be used in a bioassay. Replication, or stamping, of mother into daughter plates facilitates rapid delivery to screening groups by the compound library. Mother plates and working plates are maintained in the same dry nitrogen atmosphere as the microtubes.

The QCL employs the Active Sample Manager, or ASM, from TekCel (now part of Hamilton Life Science Robotics GmbH) for microplate storage and retrieval. The ASM is a compact, modular, localised storage system for secure compound management and, when configured with the serverST, provides storage capacity for up to 2,200 microplates. TekCel's ASM delivers plates at a high-throughput rate of up to 120 per hour. The serverST can then unseal and deliver the picked plates via a handoff arm directly into the work area (the BioCel®) for processing. SealTite® lids facilitate fully automated and repeated sealing and unsealing of microplates, with no heat and adhesives to contaminate compounds in storage. SealTite lids are manufactured from stainless steel with a 100% DMSO-compatible inner liner that conforms to the shape of the microplate wells.

The ASM provides a controlled environment between ambient (room) temperature conditions and -20°C. An inert gas environment is being employed by the QCL to maintain a low humidity within the storage environment. These environmental options afford optimum conditions for compound storage to help ensure sample integrity.

Intended outcomes

Utilisation of a unique national facility

Australian chemists and biologists will, for the first time, be able to deposit and access compounds via a central repository. Two obvious possibilities ensue:
1. Biologists can requisition all, or a subset of, the chemical repository for evaluation against their targets. Because the QCL provides format free storage of microtubes, it can deliver any number of molecules in a wide range of plate types and formats to screening groups. At this point in time, screening would be undertaken at the biologist's institute. The potential value of a chemist's compounds is potentially increased via exposure to a greater range of biological targets than may have previously been possible.

Chemists depositing compounds can pursue independent collaborations. In this scenario, the chemist can actively promote their samples for screening by biologists of their choosing. The automation in the QCL can facilitate this option as the chemist's samples can be cherry picking from the repository and reformatted into the screening layout required by their collaborators.

Synergise biomedical research

A compound library can be populated by a range of chemistry to probe biological space for a number of different objectives. Structurally complex compounds such as some natural products are good starting points for phenotypic targets. Phenotypic screens like those performed in chemical genetics greatly expand the biology space being interrogated[12]. Follow-up efforts to decipher the mode of action are often investigated in a basic research environment[13]. Drug discovery, on the other hand, commences with a much narrower selection of chemical space as exemplified by Lipinski's rules[14] and is typically prosecuted by industry.

Regardless of the molecular make-up of a compound library, screening to unearth a chemical tool to probe biological function or finding a lead for drug development can dovetail into a relatively new discipline aimed at improving the translation of compounds past Phase 1 trials. This new discipline, commonly referred to as translational research, is essentially the progression of basic biomedical research into clinical applications by placing an emphasis on new and safer therapeutics[15].

Whatever the objective, the QCL can efficiently retrieve the desired subset for reformatting into screen friendly microtitre plates. This is a bonus for the screening group as a more pertinent set of compounds is accessed for screening so that reagents and other consumables are conserved. Ultimately, both time and money are saved. The results from screening can inform the chemical community of potentially successful synthetic goals to pursue. New molecules can then enter screening to both complete and also renew the cycle.

Facilitate collaborative research

Biological screening is arguably the strongest mechanism to engage both the biology and chemistry research communities. Screening the QCL's combined collection has enormous potential to negate missed opportunities for both chemists producing novel molecules and biologists in quest of chemical starting points for validated targets. Lead compounds identified following evaluation against a biological target may be progressed as an academic investigation or prosecuted further to deliver a therapeutic, agrochemical or other marketable product. It becomes the remit of the biology and chemistry groups to progress a project in the QCL model, and potential collaborations are negotiated directly between the two groups.

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Enabling value adding and prosecution of a commercial outcome

Australia is strong in terms of the quality of its basic science funded by the Australian Research Council and National Health and Medical Research Council. Its major weaknesses are the lack of mechanisms and strong incentives for translating the innovative discoveries through to commercial outcomes, and the paucity of a culture of entrepreneurial and risk-taking activity which can promote developmental research. To achieve this, inventors are given 100% ownership of the IP they create. No claim to IP arising out of any discovery by the facility is made in this model. This provides a protected IP environment for progression of promising commercial ventures in a timely fashion.

Conclusion

The establishment of the Queensland Compound Library will be a valuable tool in stimulating basic biomedical research and drug discovery in Australia. The combination of a unique IP model, pre-existing, successful HTS infrastructure and a fully integrated compound handling system will create an environment for facilitating collaborations between different groups of scientists.

The integration of environmentally controlled plate and tube storage with sample processing ensures that compound integrity is maintained without compromising flexibility. The modular approach allows future capacity building and throughput increases to be realised with minimal disruption to on-going operations.

A modular system described here provides a practical compound management solution for small biotechnology firms and well-financed academic institutes. Core units can be acquired to initially establish a fully automated facility. The capacity and throughput of the facility can be expanded over time via additional storage and processing equipment. This approach allows an organisation to build up the capability in a stepwise manner without overly impinging on resources required by other programmes.

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