Advances in high throughput screening

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The 2nd conference on *Advances in High Throughput Screening* (21–22 January 2004, The Hatton, London, UK) was organized by the SMI group. The conference comprised of two days of lectures and networking opportunities. Topics of the conference included identifying the challenges faced in the use of HTS and how these challenges can be resolved, evaluations of the methods currently used in HTS and newly developed technologies, which could be applicable to the sector.

Challenges facing HTS

Lorenz Mayr (Technology Programme Head, Lead Discovery Centre, Novartis; http://www.novartis.com) gave a comprehensive review of the challenges faced by HTS groups, and the way Novartis has invested to counter these. These challenges include the need for increased productivity in the pharmaceutical industry, as illustrated by the falling number of NMEs over the past few years. This is coupled with the increased regulatory pressure, which means that drugs have less time free of any generic competition. The HTS sector has responded to combat this trend by increasing both the number of targets screened and the number of compounds screened per target, and a variety of strategies have been developed in the various pharmas to meet the resulting challenge.

Mayr stated that only 40% of HTS campaigns generate new lead material and in his opinion it seems clear that the HTS groups can still increase productivity. At Novartis miniaturized screening using high-density plates with new readout technologies, such as confocal and lifetime measurements (through the Evotec collaboration) are applied for faster screening and generating extra value from the readouts. Additionally they have developed the speedscreen technology, an affinity based method, where the target protein is incubated with a mixture of compounds, separated by size exclusion chromatography and further analyzed by MS to determine compound identity.

At Novartis an important step forward has been the creation of Technology centres, where tool production, assay development, screening and other hit-to-lead steps are located in one group, enabling better communication and an overall increase in productivity.

Current methods used in HTS

Several speakers elaborated on the technologies and the different screening strategies used to overcome the challenges stated in Mayr's presentation.

Dominique Besson (Global head of HTS Logistics, Serono Pharmaceutical Research Institute (http://www.serono.com; Europe's largest Biotechnology company), discussed the strategy followed in its HTS group, which supports both small molecule and protein therapeutics research lines in parallel. Serono has invested in equipment and technology with a high degree of flexibility. The HTS groups screen small focused subsets and, because of this small file size, the usual format for screening is 384-well plates. As reagent sourcing had become a problem for kinase targets, Caliper lab-on-a-chip technology is applied for kinase assays and this enables around 30,000 samples per week to be screened in far less reagent volume. Serono also entered a collaboration with Graffinity to allow access to their affinity based screening technology, which is a label free technology detecting molecular binding events.

Everard Pap (Head of Assay Technologies, Aventis; http://www.aventis.com) illustrated the Aventis 'Filtering and Learning loop' process. This process involves a feedback step where results from confirmed active structures guide the development of new libraries. This iterative process combined with the application of focused libraries, has increased the number of actives per thousand compounds screened from under 1 to 2.2. However, this method has not been successful for all target classes.

Technology used by Aventis includes the Caliper lab-on-a-chip technology, an antibody free method, which is used in kinase assays and shows a low level of compound interference frequently observed with other fluorescence based methods. Biotrove's living chip technology is used by Aventis for both biochemical and cellular assays. This impressive technology uses a plate containing 24576 'reaction cylinders', essentially open-ended tubes. Each of these cylinders has a hydrophilic interior, which captures the reagents and is loaded with samples from the chemical library. Additional arrays are loaded with enzyme, substrates and any additional assay components before the assay is assembled by stacking the arrays, which enables the assay components to mix. As the loading of arrays with assay reagents can be achieved by dipping the plate into a

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solution, liquid handling is simplified and costly robotics are avoided for these steps. Using fluorescence readouts, imaging is used to generate the results. The compound arrays can be frozen and prepared independently. A limitation remains that the technology is unsuitable for absorbance assays.

Jörg Hüser (Director HTS Technologies, Bayer Healthcare; http://www.bayer.com) talked about the methods developed to cope with Bayer's compound collection, which has grown to around 1.2 million in size after collaborations with Arqule, Comgenex and Evotec OAI. Another collaboration with Millennium will generate new targets over the next 5 years. The adopted strategy has been to invest in workstations (such as the Cybi-screen) and robotic platforms (for example CRS robotics). The estimated throughput from these systems is in the region of 120,000 to 240,000 compounds per day. This throughput has been obtained by the use of high-density well plates particularly the 1536 well format. Currently they have assay platforms for all major target classes, and use both biochemical and cell based assays.

Bayer also considered productivity of the materials management group, which is interlinked with the screening group. To that end, they have commenced reformatting of the whole compound library into a 1536 well format and 85% of the compound file are currently in this format. This has enabled a higher number of cherry picks per day.

Improving data quality

The data obtained from HTS is continually being enhanced, to lever more value from the different types of assays, for example, through confocal and high content readouts. However, there are other methods to improve the quality and Swen Reinmann, (Business Development Manager, Genedata; http://www.genedata.com) introduced the Genedata screener package, highlighting the fact that it is easy to miss patterns in plates across a whole screen.

For IC_{50} analysis, most data fitting tools only work on 70–80% of the data but the remaining 20–30% of curves, which have to be fitted manually, are in fact the most time consuming. The Genedata software has the ability to map trends and distributions on entire plate runs and highlight any patterns. This algorithm can then generate an 'improved' hit list, which should include the removal of false positives and the identification of false negatives. Reinmann gave an example of a screen where the repeat rate was improved from 13% to 34%, by the removal of false positives.

Reinmann also outlined the dose-response module, which automatically masks outliers and redraws the calculated fits. The module is robust enough to determine if the best fit is a straight line and not a curve for a dose response removing the highly time consuming step of invaliding incorrect fits. This technology is currently undergoing evaluation at multiple pharmaceutical companies.

New technologies for HTS

Sherri Mills (Group leader, Drug Discovery Systems, Pierce Biotechnology; http://www.piercebiotech.com/) outlined Pierce Biotechnology's product IQ™. IQ is a detection platform used for kinase, phosphatase and protease assays. It uses an iron compound in solution that acts as a quencher of fluorescent signals when it binds to, for example, a phosphorylated peptide. It can also be used in protease assays and has been applied successfully in 1536-well plates. The method is antibody free and can be used with any peptide substrate with a wide range of fluorescent dyes and with DMSO concentrations up to 15%.

Duncan McBranch (Founder and Chief Operating Officer, QTL Biosystems; http://www.qtlbio.com), introduced their lightspeed™ assay format. Using polymer superquenching to detect binding events it has been shown to be applicable for kinase, protease and DNA assays. Advantages of this type of format are that again it is antibody free and the assay format is quite simple with only a few process steps required, which improves the usage on robotic systems

Developments and challenges

The overall general theme from all HTS representatives was that developments in technology and organisation have had to occur in parallel to process large compound libraries successfully in a timely manner. However, there still remain significant challenges ahead in fully integrating the novel technologies so that they become fully mainstream.