Developing effective assays in HTS

It is not often that members of the major pharmaceutical companies get together to discuss actual data from their work in drug discovery, so the IBC meeting *Real Case Studies on Developing Effective Assays*, which took place in Philadelphia, USA on 30–31 October 1997, provided a welcome change. Furthermore, many of the presenters were prepared to talk about some of the problems they had encountered in the design and implementation of assays for high-throughput screening (HTS).

Importing new technologies

A presymposium workshop addressed the introduction of new technology into the HTS field.

Assay miniaturization

Smaller, faster, cheaper. This was one of the main themes of both the symposium and the workshop. The adaptation of fluorescence-based assays to microvolume assays was introduced by Dr Jonathan Burbaum (Pharmacopeia, Cranbury, NY, USA). Two case studies were presented in which data from 1536-well assays was shown to closely match the 96-well data. Evaporation from 1536-well plates was not a significant problem provided that plates were not left uncovered for more than 20 min.

With small-volume wells, liquid handling becomes difficult. Dr Don Rose (Cartesian Engineering, Durham, NC, USA) displayed the Nanojet 3000 Aspirate and Dispense system in the exhibitors hall. The development of this tool was discussed during the workshop, and a poster was also presented by MicroFab Technologies (Plano, TX, USA), who specialize in inkjet microdispensing.

Detection of the signal from such small-volume fluorescent assays was addressed by Dr Al Kolb (Packard, Meriden, CT, USA). The presentation focused on the acquisition and adaptation of technology from other fields of science to make sensitive detectors for the next generation of assays.

Novel assay techniques

The use of nucleic acid structures for probing and screening applications was introduced by Dr Seth Stern (University of Massachusetts Medical Center, MA, USA). He discussed many of the standard uses of chemically modified nucleic acid structures to identify *cis*-binding elements. He also discussed the issues of using a structurally diverse library of RNA to bind to non-nucleic acid target molecules, thereby identifying motifs that might be required in a lead compound.

Cell-based assay systems using reporter gene constructs were discussed

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by Dr Shannon Beard (Xenometrix, Boulder, CO, USA). In collaboration with Oncogene Science (Uniondale, NY, USA), Xenometrix have developed a large range of reporter gene constructs in several cell types to investigate compound efficacy and toxicity. Dr Jason Ruans (Glaxo Wellcome, Research Triangle Park, NC, USA) described a novel cell-based system that uses a lawn of cells to rapidly screen a 442,000-peptide library in a 7-transmembrane (7TM) receptor assay. Because this assay did not conform to a standard 'well-based' system, development of automated image analysis software was necessary.

Dr Maura Kibbey (IGEN, Gaithersburg, MD, USA) reported on electrochemiluminescence technology for sensitive non-radiolabeled assays, explaining their flow cell system and presenting some data from binding assays. A 96-well format version of the flow cell is under development to conform to current automation requirements.

Case studies

Almost all of the presentations focused on the problems that have been encountered with specific assays and the technology that has been employed to overcome them. It was clear that the level of automation varies between companies and that solutions to some problems did not necessarily require expensive equipment purchases.

In a presentation entitled 'Changing the Wheel When the Car is Still Moving', Dr John Houston outlined Glaxo Wellcome's (Stevenage, Herts, UK) strategy for rapidly improving the drug discovery process. Many implications can arise from simple decisions. When Glaxo Wellcome decided to automate and optimize HTS, they had to find money, space, personnel and the right equipment. Once introduced, it became clear that, for the scale of screening that was required, 96-well assays would not be cost effective. But should the conversion to new assay formats be evolutionary or revolutionary? For fluorescence assays, the switch to 1536-well assays could be made relatively easily, and supporting data from a human factor VIIa fluorescence assay were presented. The conclusion was that, over the next five years, the HTS assays would be scaled from 96-well to 386-well formats and then to 1536 wells.

The use of luminescence in assays as an alternative to radioactivity was presented by Dr Chris Roelant (Radioactive Tracer Replacement Technologies, Leuven, Belgium). The developmental strategy for several assays was presented with comparisons of 384-well and 96-well formats. The scaling down of luminescent assays seems to be relatively straightforward. Luminescence was also the theme of the presentation by Dr Michelle Palmer (Tropix, Bedford, MA, USA). They introduced some of the reagents that they supply for use in capture-based and cell-based assays. Kinase assays and protease assays were discussed, and the sensitivity of the Tropix chemiluminescence systems was emphasized.

'Doing cell-based assays is a bit like eating broccoli, you don't want to do it but you know you have to' – Dr Richard Harrison of RPR

Transient transfections were used by Novartis (Basel, Switzerland) in one of their reporter gene assays. Dr Guenther Scheel described the major problem with this technique, which was to obtain a consistent high signal from the transfected cells. Electroporation proved to be the most efficient method of transfection and, after optimization, a single electroporation using 0.1 mg of DNA produces enough transfected cells for 100,000 wells. Dr Richard Harrison (Rhône-Poulenc Rorer, Collegeville, PA, USA) presented work on an assay that uses the secreted embryonic alkaline phosphatase gene as a reporter, linked to a heat-shock promoter. The initial problems were poor induction of the reporter gene and high levels of phosphatase activity in the control cells. Automation, switching cells lines and coating the plates with poly-lysine overcame these problems.

Dr John Haley (Oncogene Science, Uniondale, NY, USA) gave a presentation on the use of YAC-based vectors in screening. Induction of γ-globin gene expression occurs in response to the binding of *cis*-acting elements some distance from the gene. Reporter genes were spliced into YACs carrying the long-range promoter elements and were used for screening. This approach maintained the structural integrity of the DNA, and thus the binding sites for the *cis* elements, and by comparison with a plasmid-based reporter construct showed that more 'hits' were identified using the YAC system.

A different set of problems, described by Dr Kurt Hertogs, were encountered by the clinical scientists from Virco NV (Edegem, Belgium), who automated an assay to investigate the drug-resistant state of HIV patients. HTS is normally applied to test the effect of thousands of compounds on one cell type, conversely, Virco have only 12 compounds but thousands of different viruses. Initially, they devised a procedure to generate recombinant virus stocks from 200 µl samples of patient plasma in only 7–10 days. These could then be used for drug-resistance studies. Automation of the entire procedure was useful because it offered improved data reliability, safety for workers, data tracking and quality control.

Proximity assays

The final day of the symposium was dedicated to the use of proximity assays. Surprisingly, only one speaker, Dr Kerry Koller (Affymax Research Institute, Palo Alto, CA, USA), focused on the use of Amersham International's SPA system, although there were two posters presented on SPA and Cytostar-based assays. Koller detailed the development of a high-level expression system for the production and purification of tagged novel recombinant 7TM receptors. FACS analysis was used to identify the highly expressing cell lines where no receptor antibody or radioligand was available. Membrane preparations were

then used as a source of receptor for the SPA assay.

The use of proximity assays with natural product libraries was discussed by Dr Julian Abery (Xenova, Slough, UK). Assays were required to be tolerant to a range of physiochemical conditions because the compounds were not all in the same medium. Dual-label lanthanide time-resolved fluorescence (TRF) and homogeneous TRF were the two case studies presented. TRF uses the delayed emission of a fluorescence signal from fluors with long half-lifes, so that endogenous fluorescence from assay components with very short half-lifes does not interfere with the signal from labeled assay components. In the first assay, a capture system was set up to identify inhibitors of the tumor necrosis factor (TNF)-α/TNF-receptor interaction. Different labels were applied to each of the two assay components to reduce the possibility of false positives. The drawback of the capture assay is a requirement for several washing steps. The second assay was a homogeneous assay and again used two labels. The TNF-α and its receptor were labeled with terbium and rhodamine, respectively. When the TNF-α bound to the receptor and the terbium was excited, the energy was transferred to the rhodamine because of its close proximity. Both assay systems proved very robust and were stable in a wide variety of pH and media.

Dr Claudio Mapelli (Bristol-Myers Squibb, Princeton, NJ, USA), Dr Christine Pernelle (Rhône-Poulenc Rorer, Vitry sur Seine Cedex, France) and Dr Jeffrey Hermes (Merck & Co., Rahway, NJ, USA) all presented data on the use of Packard's HTRF (homogeneous time resolved fluorescence) system. The assays discussed included protease assays, SRC family tyrosine kinase assays, receptor autophosphorylation and receptor–ligand interactions. In all cases, the HTRF technique was compared with other forms of proximity assays. The system uses chemistry developed by CIS-Bio (Bagnols sur Ceze Cedex, France) – a caged europium cryptate is used as the long half-life donor, and the acceptor molecule is a modified APC protein from algae. The donor and acceptor are used to label the two components of the assay; if they are <90 Å apart when excited, the donor will transfer the energy to the acceptor. Europium cryptate is excited at 337 nm and emits at 620 nm, while the acceptor emits at 665 nm. The advantage of the Packard system is that by simultaneous detection of fluorescence emissions at both 620 nm and 665 nm, there is an internal control for inner filter effects. Colored compounds or high turbidity in the medium will affect both the 620 nm and 665 nm readings but, by using a ratio of the two emissions, the inner filter effects can be minimized. True hit compounds that prevent the association of the two labeled molecules will result in a decrease of the 665 nm signal but not of the 620 nm signal. The speakers concurred that the HTRF technology outperformed other radioactive proximity assays. The only disadvantage seems to be the availability of labeled assay components, although custom labeling can be arranged and the number and range of labeled generic reagents is being increased by Cis-Bio.

An emerging assay technology applicable for secondary screening was described by Dr Alex Harris (Chiron Technologies, Emeryville, CA, USA). It involves computer algorithms devised to design bDNA probes to enable the amplification of a chemiluminescent signal from RNA molecules in cells. Already available are probes for interferon γ, interleukin 2 (IL-2), IL-4, IL-6, IL-10, insulin, TNF-α, c-fos, HSP70 (70-kDa heat shock protein), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), β-actin and luciferase, which allow for the detection of RNA at very low levels from only 50–100 cells. Quantification of the amplified signal enables differences in the gene expression between cells to be measured. The second part of the presentation focused on the use of affinity selection/mass spectrometry and its uses for screening combinatorial mixtures of compounds. Up to 500 compounds are mixed with a purified target protein, the mixture is then passed through a high-speed size exclusion column and unbound compounds are separated. Reverse-phase HPLC separates the bound compounds, which are then passed into a mass spectrometer for analysis. With up to 20 mass spectrometry runs a day, the throughput can be 10,000 compounds.

Overview

It was helpful to hear presentations on the new technology available for HTS, but more interesting were the problems that assay designers were faced with and the solutions that have been implemented. For those who missed this symposium, the IBC are planing to do it all again next year.

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In short...

DeveloGen, a new German company based in Göttingen have attracted $3 million from private investors to target control genes in the fight against diabetes and obesity. The scientific founders are Prof. P. Gruss, Prof. H. Jäckle (both from the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) and Prof. W. Driever (University of Freiburg, Germany). DeveloGen aim to tackle diabetes and obesity using the genetics of developmental biology. 'The company promises to be one of the highest valued start ups on the German biotechnology scene so far', says DeveloGen's CEO Herbert Stadler.