KINASE SCREENING and PROFILING – *spoilt for choice*

Whereas wider coverage of the kinome was the principal focus of kinase profiling a few years ago, today's vendors of kinase screening and profiling products are: 1) increasingly applying automated and industrialised approaches to their fee-for-service offerings; 2) making greater use of time-resolved fluorescence assay formats; 3) pursuing more universal assay approaches; 4) making wider use of assays that measure the accumulation of ADP; 5) placing greater emphasis on screening inhibitors that bind to inactive and low activity kinases; 6) extending the diversity of cellular kinase approaches; 7) developing bench-top turnkey profiling instrument solutions; and 8) becoming increasingly aware of the potential of label-free approaches. Overall, the end user is spoilt for choice with the range of alternative offerings, methodologies and approaches currently available for kinase screening and profiling.

By Dr John Comley

Several years ago in *DDW* we reviewed kinase profiling[1] and highlighted the increasing competition between fee-for-service providers and the marked expansion in the size of kinases panels offered. With the publication of its latest market report on kinases in September 2006 HTStec has re-examined current practices and preferences in kinase screening and profiling, particularly with respect to the need for outsourced services. We now discuss some of this report's key findings and review assay vendor and fee-for-service provider updates on the latest kinase screening and profiling offerings.

Therapeutic focus and most investigated kinases

The therapeutic area making the greatest use of kinase assays today was oncology (**Figure 1**). The second biggest use of kinase assays was made by inflammatory disease. In comparison, all other therapeutic areas (eg diabetes and obesity, autoimmune disease, neurology, cardiovascular and metabolic disease) made less use of kinase assays.

Tyrosine kinases remain the class of kinases most investigated in-house, closely followed by serine and threonine kinases (**Figure 2**). More than three quarters of all respondents were investigating kinases of these classes in-house. In comparison, lipid kinases and other (eg inactive kinases) were only investigated by minority of respondents in-house. The percentage of respondents that investigated these kinase classes at a fee-for service provider (outsourced) followed the same trend but was in all cases a reduced percentage relative to in-house testing.

Figure 1: Therapeutic areas using kinase assays today

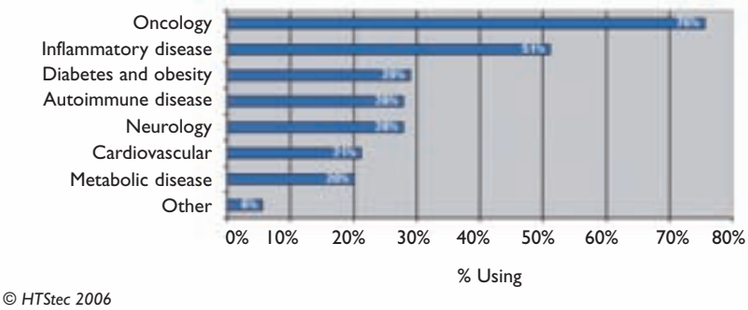


Figure 2: Relative interest in different classes of kinase

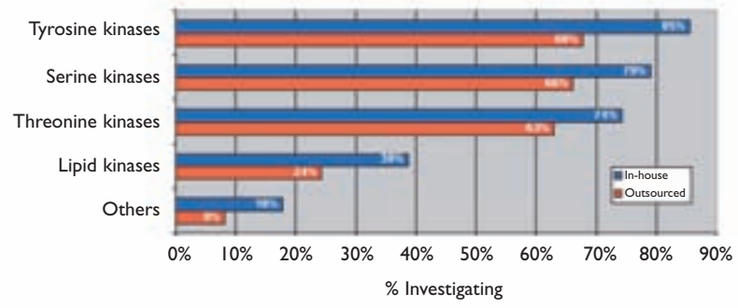
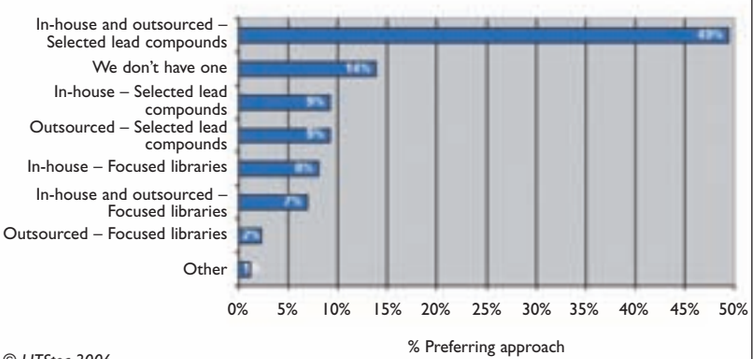


Figure 3: Preferred approach to kinase panel profiling today



Kinase profiling approach

The preferred approach to kinase panel profiling by around half of the respondents surveyed was to use a combination of in-house and outsourced panels for selected lead compounds (**Figure 3**). Less than 10% of respondents preferred to use only in-house or only outsourced profiling for selected lead compounds. Profiling of focused libraries against a kinase panel (in-house or outsourced) was also much less used compared to selected lead compounds.

The number of kinases survey respondents used today in their in-house profiling panels or access in outsourced profiling panels is shown in **Figure 4**. The mean size of the kinase profiling panels being used was 34 kinases in-house, versus 103 kinases outsourced.

Assay technology preferences

Assay technology preferences for kinase panel profiling in-house and outsourced are presented in **Figure 5**. When outsourcing, the majority (41%) of respondents did not have a preferred kinase panel profiling assay technology and were happy to go with CRO's recommendation. Of the named technologies radiometric filter (eg P33 incorporation) was preferred for outsourcing (24% share), followed by TR-FRET (eg LANCE, HTRF, TR-IMAP) (6% share). TR-FRET was the preferred assay technology for in-house profiling, although only around one in four (23%) of respondents choose it. The next preferred in-house technology was FP (Fluorescent Polarisation) with ligand (ie no antibody) (11% share), followed by a number of different technologies each with between 10% and 5% share (radiometric filter, glow luminescence, FlashPlate/Image FlashPlate (radiometric), AlphaScreen and FI (fluorescent intensity) with microfluidic (on chip) separation). In total, survey respondents chose 17 different kinase assay technologies as their preferred technologies and sourced these from 16 different technology suppliers/vendors and 11 outsourced fee-for-service providers. These observations highlight the broad range of technologies that are now available for kinases and large diversity of opinion among end users in this highly competitive marketplace.

Respondent satisfaction with data quality from current kinase screening methods is presented in **Figure 6**. Only a small minority (5% in-house, 11% outsourced) were dissatisfied with the data quality generated by their current kinase screening methods. The remainder are currently satisfied with existing kinase screening methods, although most (56% in-house, 81% outsourced) would be open to better approaches if they were available. On the basis of this response we can conclude that most of the current kinase offerings are adequate for end users needs.

The features of a new kinase screening platform of greatest interest to respondents are ranked in

Figure 7. A non-radioactive method was ranked as the feature of most interest, closely followed by lower cost per data point than radioactive assays; cell-based assay format and then label-free technology. A microfluidic (on chip) format was ranked the least important feature, possibly reflecting the fact that chip formats (eg CaliperLS Labchips) have gained greatest acceptance for mobility shift separations of product from substrate, for kinase assays performed off chip. Interestingly, with respect to label-free assays 18% of respondents said they have implemented them for kinase profiling and a further 30% stated they are actively investigating them. Currently during the lead optimisation of kinase inhibitors, low throughput fluidic chip-based label-free methods (eg Biacore) are widely used in kinetic analysis (ie to determine on and off-rates). However, it seems probable that higher throughput plate-based label-free systems (eg Corning EPIC) will have a future role in screening low activity or inactive kinase forms in an area where current catalytic assay formats offer very little. Another application area for label-free is the detection of cell interactions between antibodies or ligands and screening potential therapeutic Abs to surface markers and ranking them in the context of kinases receptor TKs. Label-free methods may also facilitate to screening potential structural constructs in proof of folding and crystallography trials, if an interaction with a

Figure 4: Size of kinase panel used in 2006

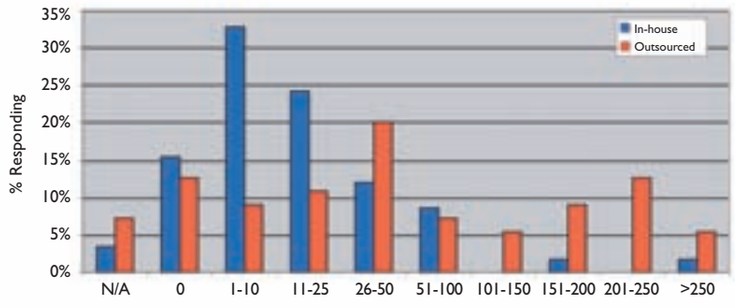


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Figure 5: Preferred assay technology for kinase panel profiling

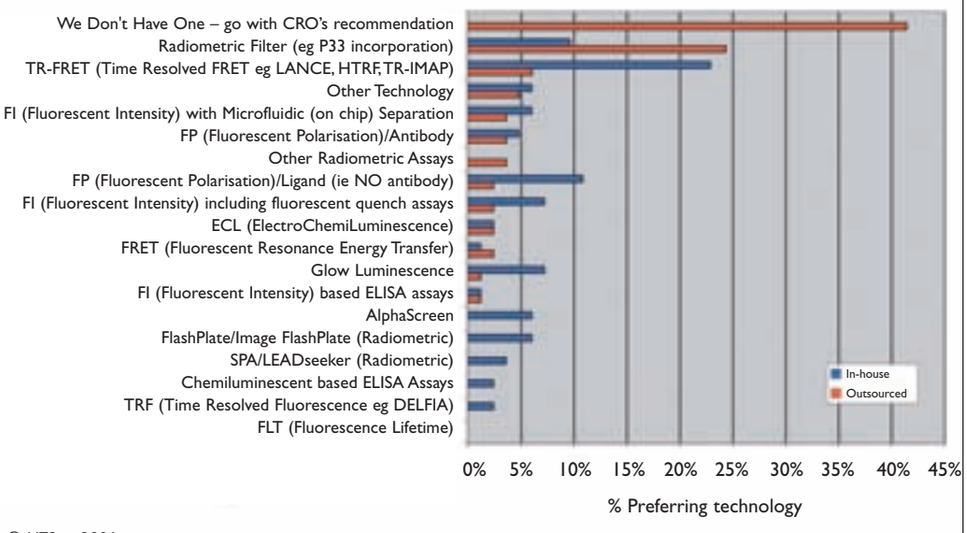

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Figure 6: Satisfaction with data quality from current kinase screening methods

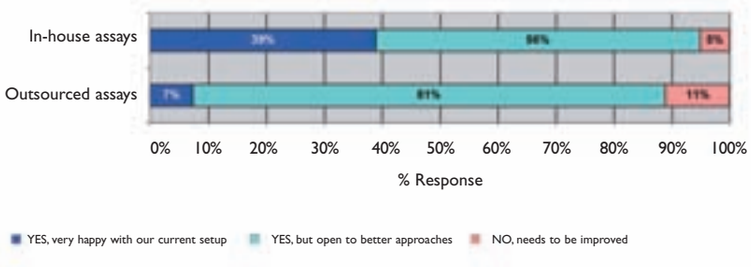

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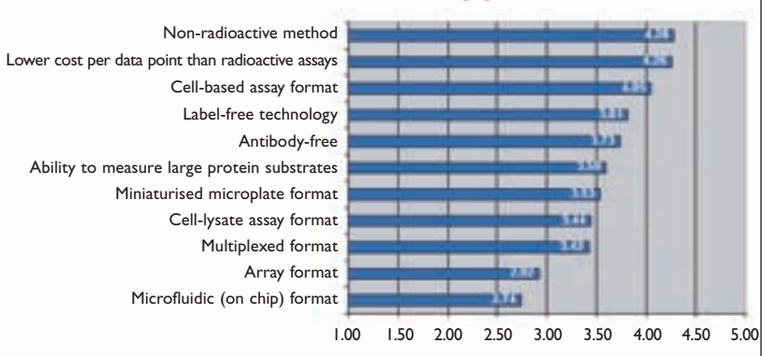
Table 1: Pharma/biotech kinase profiling statistics[1]

IN HOUSE PROFILING	2004	2006
Estimated Market Size[2]	$12.2M	$16.6M
Average Cost/DP	$0.84	$4.09
No. DP Generated/Lab	262,000	151,000
No. FTE/Lab	2.9	1.9
% Singlet DP Profiled	47%	20%
% Duplicate DP Profiled	15%	25%
% Full Dose Response DP Profiled	38%	55%
OUTSOURCED PROFILING	**2004**	**2006**
Estimated Market Size[3]	$16.3M	$37.8M
Average Cost/DP	$17.85	$18.88
No. DP Outsourced	23,000	56,000
% Singlet DP Profiled	2%	26%
% Duplicate DP Profiled	88%	47%
% Full Dose Response DP Profiled	10%	27%

[1] all data from HTStec's published Trends Reports
[2] refers to total pharma/biotech spend in house on reagents for profiling
[3] refers to total pharma/biotech spend on fee-for-service profiling

compound is observed. Clearly label-free is an area of growing importance and market share in kinase screening and profiling.

Figure 7: Most important features of a new kinase screening platform



Changing dynamic of kinase profiling marketplace

In **Table 1** we compare some of the kinase profiling statistics which were estimated in HTStec's 2004 and 2006 market surveys. In-house profiling has shown a decline in the total number of data points generated and the number of FTEs (full time equivalents) that support it, with a shift from % singlet data points to more full dose-response and duplicate data points. In addition, the average cost paid per single data point generated has increased four fold. Over the same period the total number of data points profiled using outsourcing has more than doubled, with increases in the % singlet and % full dose-response data points. The average cost paid per single data point outsourced appears to have changed little. In 2004 around 10X more data points were profiled in-house than outsourced, in 2006 this has decreased to less than 3X more in-house. As a consequence of this shifting dynamic, the 2006 market for outsourced profiling has doubled to $37.8 million since 2004. In contrast, the

2006 market for the primary screening of kinases (ie reagent proportion only) was estimated to be around $80 million.

In **Table 2** the principal vendors and fee-for-service providers serving the kinase screening and profiling market place are summarised. Companies are divided into the following three categories based on their kinase offering: 1) assay technology, enzymes, substrates, reagents and/or kits; 2) outsourced assay services; and 3) turnkey instrument platforms. The following are brief updates on these vendor's kinase product offerings.

Amphora Discovery Services (www.amphoracorp.com) utilises an automated and industrialised approach to kinase profiling and screening, based on the Caliper LabChip® technology, proprietary informatics and a broad, physiologically relevant, peptide library. With all of its assays running on a single platform and designed to be as comparable

Table 2: Vendors and fee-for-service providers serving kinase screening and profiling marketplace*

VENDOR/PROVIDER	WEBSITE	ASSAY TECHNOLOGY/ ENZYMES/SUBSTRATES/ REAGENTS/KITS	OUTSOURCED ASSAY SERVICES	TURNKEY INSTRUMENT PLATFORMS
Amphora Discovery Services	www.amphoradiscovery.com		✓	
BellBrook Labs	www.bellbrooklabs.com	✓		
Biofocus DPI	www.glpg.com		✓	
Caliper Life Sciences	www.caliperls.com	✓	✓	✓
Carna Biosciences	www.carnabio.com	✓	✓	
Cerep	www.cerep.com		✓	
Cisbio International	www.htrf.com	✓		
DiscoveRx	www.discoverx.com	✓		
GE Healthcare	www.gelifesciences.com/kinases	✓		
Invitrogen	www.invitrogen.com	✓	✓	
MDS Pharma Services	www.mdsps.com		✓	
Millipore	www.millipore.com	✓	✓	
Molecular Devices	www.moleculardevices.com	✓		
PerkinElmer	www.perkinelmer.com	✓		
Promega	www.promega.com	✓		
ProQinase	www.proqinase.com		✓	
SpinX	www.spinx-technologies.com			✓
TTP LabTech	www.ttplabtech.com			✓

** Companies whose offerings are discussed in this article*

as possible, data quality is very high. In addition to providing standard screening and profiling, Amphora also offers custom assay development, hit-to-lead optimisation services and access to libraries through agreements with key chemistry suppliers. Furthermore, Amphora's integrated technology platform and high data quality lends itself to the development of mineable databases. By combining focused customer or Amphora accessed compound libraries and the capacity for rapid, orthogonal screening, Amphora is able to build unique databases that can be mined at the primary screening stage for structural selectivity patterns (SAR). Based on these patterns, hit series are selected and followed up with potency, aqueous solubility and enzymology studies to provide a complete overview of a library's relevance to multiple kinase targets. The end result is a fully mineable, customer-unique database that enables chemists to make rapid structural class selections, based on selectivity and true potency, at a very early stage in the discovery process. This approach leads the trend for increased data density and makes it possible to merge the kinase screening and profiling processes (**Figure 8**).

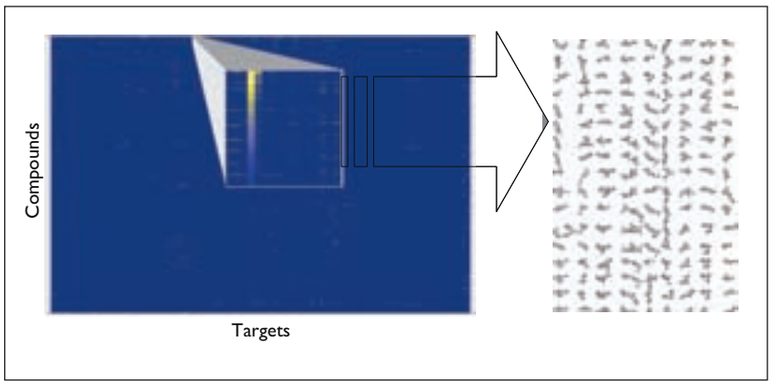


Figure 8
Amphora heat-map generated form a customer-unique database and merged with structures provide a powerful tool for enhanced selectivity profiling

BellBrook Labs' (www.bellbrooklabs.com) Transcreener™ kinase assay uses homogeneous immunodetection of ADP to enable robust detection of kinase initial velocity over a broad range of starting ATP concentrations. Because it detects the invariant product of a phosphorylation reaction, it can be used for lipid and carbohydrate kinases as well as protein kinases. Moreover, some of the more challenging kinase screening methods such as measuring native protein phosphorylation, autophosphorylation and ATPase activity are straightforward using ADP detection. Transcreener is the only truly generic kinase assay method that does not depend on coupling enzymes for signal production; instead it utilises highly selective antibodies that are able to distinguish between nucleotides on the basis of a single phosphate group. The first generation assay uses fluorescence polarisation as a detection mode with a far red tracer to avoid interference from light scattering and fluorescent compounds. More recently, the assay was formatted for time resolved fluorescence resonance energy transfer (TR-FRET) incorporating Invitrogen's (Carlsbad, CA) proprietary LanthaScreen™ lanthanide chelate-antennae complex technology for efficient energy capture (**Figure 9**). The FRET donor – a terbium chelate complex – is covalently attached to BellBrook's proprietary ADP-specific monoclonal antibody; the acceptor is a fluorescein-ADP tracer. This format provides a simple two component detection module resulting in lower reagent costs compared with other TR-FRET detection modules requiring three or more binding components. The most advantageous feature of the TR-FRET format is that the assay signal is delayed relative to the prompt fluorescence from small organic fluors that are typically the source of assay interference.

Biofocus DPI (www.glpg.com) the service division of Galapagos, has two assay development and screening groups, located in Basel, Switzerland and Cambridge, UK. These screening groups have extensive experience in employing high-throughput screening to find small molecules which modulate the activity of a wide range of target classes (including, proteases, kinases, GPCRs, ion channels, phosphatases and nuclear hormone receptors) using biochemical assays and cellular model-systems.

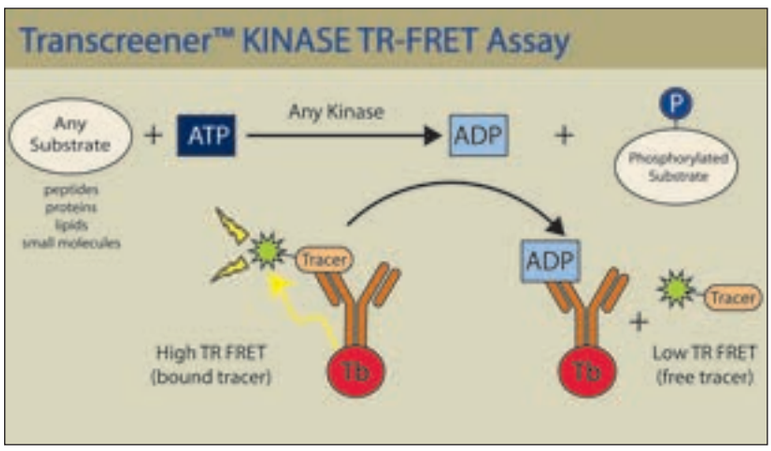


Figure 9: BellBrook Labs is redefining the universal HTS kinase assay using nucleotide detection. The newest assay offering is the Transcreener kinase TR-FRET assay. Transcreener is the only truly generic kinase assay method that does not depend on coupling enzymes for signal production. Because it detects the invariant product of a phosphorylation reaction, it can be used for lipid and carbohydrate kinases as well as protein kinases

Figure 10
Caliper Life Sciences' DeskTop Profiler

Biofocus DPI has a breadth of experience in the kinase area, with >100 successful screening campaigns performed. The company has a proven track record for identifying hit compounds using high-quality, robust assays in a range of formats, including FlashPlate, FilterPlate, SPA, FP, and FRET. Supporting the state-of-the-art screening technologies is access to BioFocus DPI's compound libraries comprising rationally designed, chemogenomic, hit-finding kinase libraries (SoftFocus®), and a large collection of 700,000 diverse compounds. To increase capabilities in the kinase area further, BioFocus DPI has a commercial agreement with Carna Biosciences (Japan) which allows access to a wide range of high-quality existing and bespoke kinase enzymes for hit-finding and profiling.

Caliper Life Sciences (www.caliperls.com), through its Discovery Alliances & Services group (NovaScreen Biosciences, acquired in 2005 and Xenogen Biosciences, acquired in 2006), offers outsourced kinase assays using the Caliper LabChip® platform. Its current assay portfolio consists of 100 kinases, with a planned expansion to more than 200 assays in 2007. In addition to protein kinases, it offers sphingosine 1 & 2 lipid kinases and is currently developing PI3 kinase assays using the LabChip technology platform. Because the LabChip directly detects substrate and product, and does not require the use of antibodies or radiometric filtration, this platform is optimal for lipid kinases. In early 2007, the Discovery Alliances & Services group of Caliper will be launching its rapid KinaseAdvisor profile. This diverse panel of assays consists of 48 kinases with a "less than five day" turnaround for a complete report. The KinaseAdvisor utilises the DeskTop Profiler, Caliper's complete solution for 'in-the-lab' kinase profiling, which consists of a new benchtop microfluidic reader (**Figure 10**) and ProfilerPro kinase profiling kits. ProfilerPro kits consist of 48 kinases in two 384 well microplates along with matching ATP/substrate plates. You simply defrost the plates, mix the contents, stop the reaction and read. Unlike typical outsourced kinase profiling which has lead times of several weeks, ProfilerPro allows researchers to obtain data faster than ever before. The Discovery Alliances & Services group has expanded Caliper's kinase profiling by enabling customers to study kinase inhibitors in anticancer cell proliferation panels (with many cell lines selected from the NCI 60 panel). It can also perform *in vivo* efficacy studies with subcutaneous and orthotopic xenograft oncology models using the Xenogen IVIS bioluminescent detection platform. Additionally, the Discovery Alliances & Services group has access to all of Caliper Life Sciences high-throughput automation technology, which has allowed it to help several large pharmaceutical companies with their kinase HTS projects in 2006. These screens ranged in size from 10,000 to 500,000 compounds.

Carna Biosciences (www.carnabio.com) has released its renewed kinase profiling service in which it offers a new assay platform called 'mobility shift assay'. This new profiling service offers customers more robust and reliable test data than ever. The service is low-cost, has a high throughput capability and uses optimised substrates and highly active kinases. So far 150 kinases have been tested and confirmed as available on this platform. In addition, this year Carna is planning to add at least 50 more kinases to the list. Also, 70 other types of kinases are continuously available on its present platforms, such as ELISA and IMAP. Therefore, more than 220 kinases will be available on this new profiling service. All of the kinases used on Carna's profiling service are produced in-house and are advantaged in their high-accuracy and high-quality (without any contaminated kinase derived from host cell).

Cerep's (www.cerep.com) kinase platform has been developed by turning basic research into concrete industrial applications. A comprehensive and coherent hit-to-lead platform was developed in order to meet the exacting requirements needed for the identification and characterisation of the activity and selectivity of kinase inhibitors early in the drug discovery process. Cerep's strategy has been to expand its kinase assays to cover the kinome in terms of diversity. This ever-expanding range of

kinase assays is backed by industrial concepts: constant quality high throughput profiling, homogeneous and robust data delivery, turnaround time and cost reducing. It has allowed Cerep to offer an Express Kinase panel with 45 kinases profiled in one day. Another new offering last year and continuing in 2007 is Cerep's 'stand alone' assay development service. Using this customer-oriented-development service, clients can implement their proprietary assays or have a specific test developed exclusively. With this service, clients can consolidate assay development and screening without cutting quality. Cerep's kinase assays will continue to grow this year and it plans to evolve its profiling offers and implement various critical signalling pathways. A panel of cellular kinase assays will also be developed in 2007 using various cellular models for hit confirmation and pathway profiling.

The growing interest in screening and profiling kinases has prompted the development of many new assay technologies. **Cisbio**'s (www.htrf.com) universal HTRF® KinEASE™ platform developed in partnership with Millipore, uses universal biotinylated substrates with just one monoclonal antibody, labelled with europium cryptate (EuK) for a sensitive, specific detection of the phospho-peptide. Now, the initial HTRF® KinEASE™ assay for screening serine/threonine kinases (STKs) has been extended to include tyrosine kinases (TKs). The KinEASE TK uses a choice of two substrates containing a single phosphorylation site recognised by a Eu(K) labelled anti phospho-tyrosine antibody. After the kinase reaction, the addition of the antibody and streptavidin XL665 to capture the biotinylated substrate allows FRET to occur. This signal increases proportionally with kinase activity and the amount of the kinase added. KinEASE TK has already been validated on more than 50 tyrosine kinases and the optimal substrate determined for each. This single assay system allows even more tyrosine kinases to be screened. A TK reaction buffer, especially optimised for optimal signal to background and suitable for any tyrosine kinases, will be associated with other reagents in a kit. **Figure 11** shows excellent signal to background ratios obtained with a panel of tyrosine kinases (cytosolic and receptor) using the optimal substrate. In the next development step, a HTRF® KinEASE™ platform currently allowing more than 150 serine/threonine or tyrosine kinases to be screened, will be extended to a new kinase family, the MAP kinases.

DiscoveRx (www.discoverx.com) offers kinase binding assays for kinases with little or no activity,

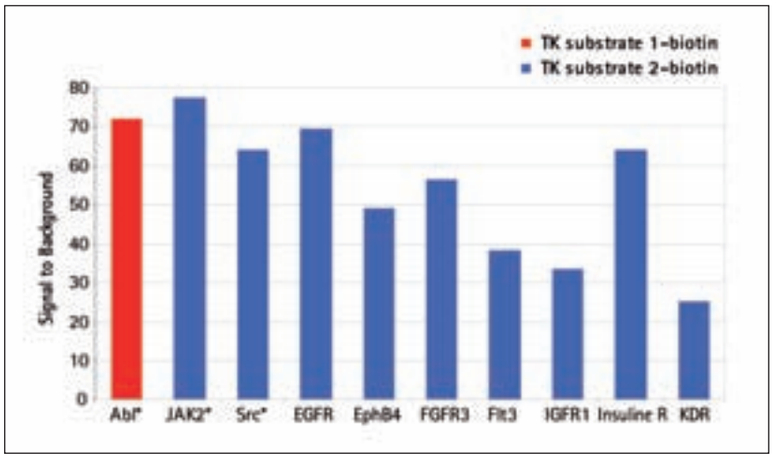
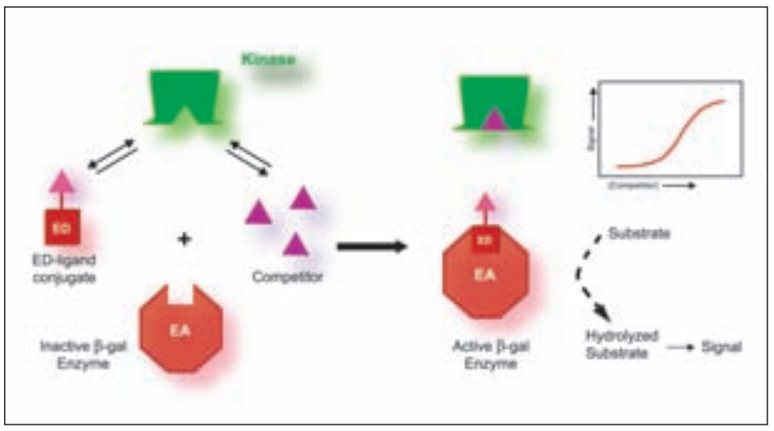


Figure 11: Signal to background obtained with Cisbio's HTRF® KinEASE™ Tyr using a panel of 10 tyrosine kinase/substrate pairs. The kinase concentration (50ng/well), the substrate (1µM), the streptavidin XL665 (62.5nM), the ready to use TK antibody-Eu(K) and incubation time (30min) were all kept constant. Each assay was performed in 50µL for the kinase reaction and 50µL added for the detection giving a final volume of 100µL. Straightforward miniaturisation reduces the final volume to <4µL by simply keeping each reagent's final concentration constant

or where the substrate is unavailable. With this new assay technology, direct or indirect binding of the inhibitor to the kinase rather than functional activity is measured. Currently there is a growing need to screen for inhibitors that bind to inactive and low activity kinases due to the potential for augmented kinase inhibitor selectivity at pathologically important mutant forms of the enzyme. DiscoveRx's kinase binding assay platform utilises EFC technology to homogeneously measure binding of inhibitors to the ATP binding site. In the assay, the ED-inhibitor conjugate and compounds compete for binding to the ATP binding site. If the conjugate is not bound to the kinase, it is free to recombine with EA (inactive EFC detection enzyme), resulting in an active ß-gal enzyme, which

Figure 12
DiscoveRx assay principle



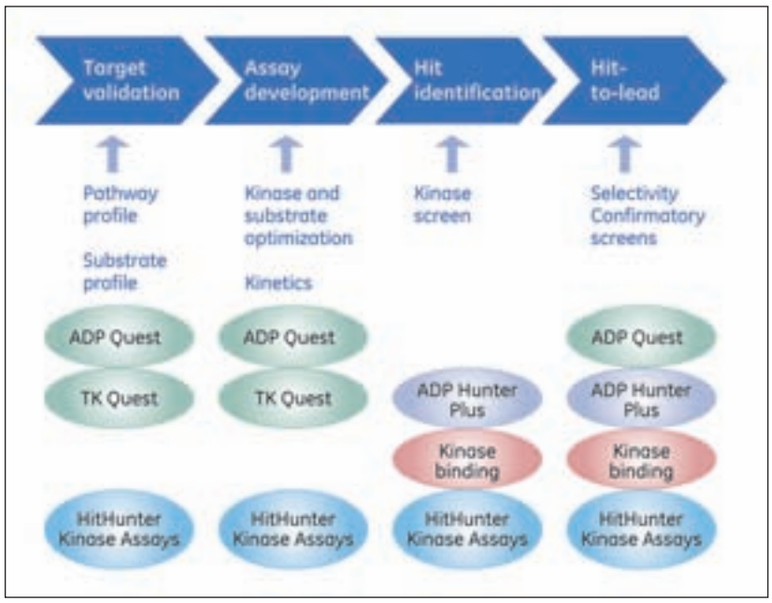
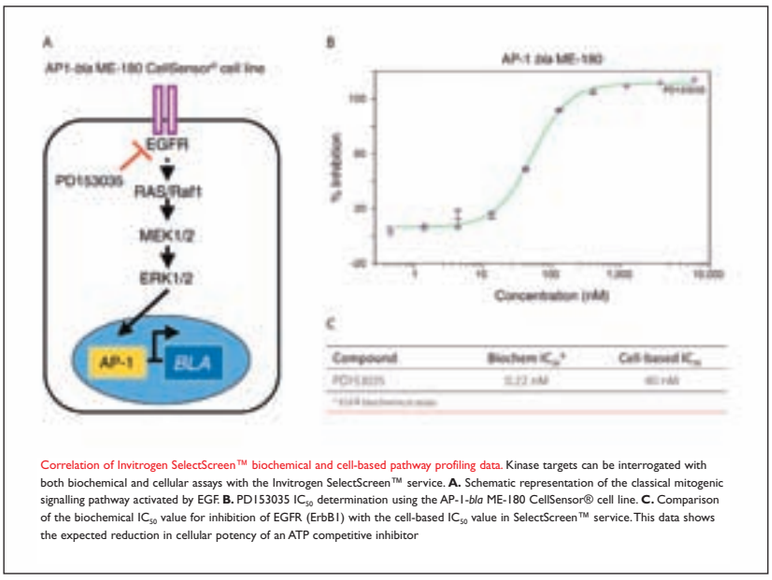


Figure 13: GE Healthcare's range of assay technologies address all phase of kinase-targeted therapeutic campaigns

Figure 14
Invitrogen SelectScreen kinase profiling service

hydrolyses the luminescent substrate. The signal produced by active EFC enzyme is proportional to the amount of compound bound to your kinase. The HitHunter Kinase Binding Assay platform currently includes four ready-to-use kits (p38alpha, PKC/CAMKII, c-Kit/ABL/SRC and LCK) as well as a profiling service that will demonstrate binding characteristics of your kinase and identify the best ED-inhibitor conjugate for screening. If a customer provides DiscoveRx with a sample of their kinase of interest, they will run it against a panel of ED-ligand conjugates and send customers the kinase binding data, enabling them to select the optimal ligand to perform a screen using DiscoveRx's novel kinase binding assay technology (**Figure 12**).



Correlation of Invitrogen SelectScreen™ biochemical and cell-based pathway profiling data. Kinase targets can be interrogated with both biochemical and cellular assays with the Invitrogen SelectScreen™ service. **A.** Schematic representation of the classical mitogenic signalling pathway activated by EGF. **B.** PD153035 IC_{50} determination using the AP-1-*bla* ME-180 CellSensor® cell line. **C.** Comparison of the biochemical IC_{50} value for inhibition of EGFR (ErbB1) with the cell-based IC_{50} value in SelectScreen™ service. This data shows the expected reduction in cellular potency of an ATP competitive inhibitor

GE Healthcare (www.gelifesciences.com/kinases) offers the choice of radioactive or non-radioactive assay formats for researchers involved in kinase screening and profiling. Products include SPA beads – recognised as the gold standard for radiometric kinase screening, and the HitHunter™ and ADP accumulation range of kinase assays. HitHunter and ADP accumulation kinase assays offer homogeneous chemiluminescence and fluorescence based solutions for all phases of kinase-targeted therapeutic campaigns from target validation and assay development to hit identification and lead optimisation. A choice of assay technologies means specific, generic and inactive kinase targets can be evaluated for activity, potency and selectivity. HitHunter Enzyme Fragment Complementation Kinase Assays are antibody based assays with high specificity for the kinase reaction product. Standard kinase reaction conditions facilitate the assessment of functional activity in a broad range of kinases while the enzymatically amplified signal has minimal background and does not produce artifacts caused by non-specific binding. When little or no information is known about the kinase substrate and its associated antibody, HitHunter Kinase Binding Assays can be used to measure and characterise the interaction of inhibitors with an inactive kinase in a competitive binding assay format without the need for a peptide substrate, antibody or ATP. The chemiluminescent signal produced is unaffected by naturally fluorescing compounds and can be read on imagers or PMT-based plate readers. ADP Quest™, ADP Quest HS and ADP Hunter™ Plus assays measure the accumulation of ADP, a product of kinase enzyme activity. Unlike alternative generic approaches that monitor the depletion of ATP from a kinase reaction, these assays follow the product of the reaction in a convenient fluorescence based gain-of-signal format (**Figure 13**).

Invitrogen's (www.invitrogen.com) SelectScreen™ Kinase Profiling Service was launched in 2004 as part of the company's major expansion of its kinase biology platform. The screening service has grown dramatically since launch and Invitrogen now offers partners the ability to screen lead compounds, SAR series and focused library collections against a panel of >220 kinases. The foundation of the service is Invitrogen's industry leading library of purified protein kinase targets and its robust

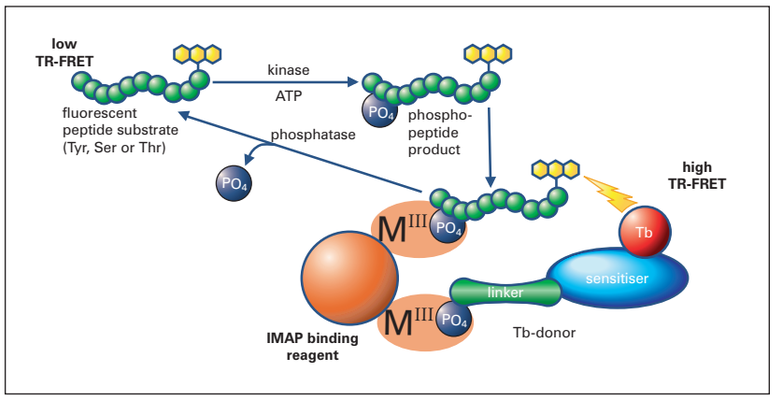


Figure 15: Molecular Devices IMAP TR-FRET assay for kinases. When fluorescent substrates become phosphorylated products, they can bind to the trivalent metals on the IMAP binding reagent that also bears a terbium donor. Energy transfer occurs between donor and fluorophore on the product

Z'-LYTE™ kinase assay platform. The current collection of kinase targets has been assembled based on therapeutic relevance, phylogenetic diversity and representation of key signalling pathways. All the reagents utilised in the service are manufactured according to strict quality guidelines within Invitrogen's Discovery Sciences Unit in Madison, Wisconsin, USA. In addition to ensuring the utmost integrity in assay reagents, Invitrogen has invested significantly in people, process and automation to execute screening campaigns. To keep up with the rapid demand for screening services, Invitrogen opened a second screening facility in Scotland in July 2006. Now clients have access to identical services at both sites and engage in real-time communications with Invitrogen's global screening leaders. Invitrogen has also recently launched a cellular pathway screening service, SelectScreen™ Cell-based Pathway Profiling Service, to enable clients to interrogate kinase targets in their native cellular environment as well as assessing the effect of compounds across signalling pathway space. In 2007, Invitrogen plans to expand the breadth of molecular and cellular assays on to its SelectScreen™ platform, as well as building out screening services for other major target classes (**Figure 14**).

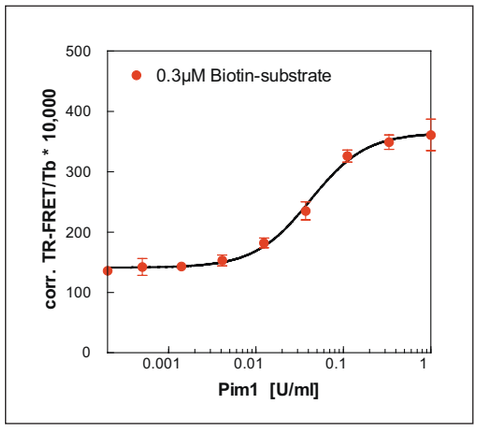


Figure 16
Molecular Devices IMAP TR-FRET using streptavidin for detection. Enzyme assays for Pim1 used 300nM PKC pseudosubstrate-derived peptide (biotin-ERMRPRKRQGSVRRRV-NH2) and 100µM ATP, reaction time 1h at RT. FAM-Streptavidin (Anaspec) 30nM

Last year, **Molecular Devices** (www.moleculardevices.com) introduced a new detection mode, TR-FRET, to the IMAP platform for kinases, phosphodiesterases and phosphatases. IMAP TR-FRET utilises all of the current components of the IMAP FP system plus a terbium (Tb) labelled TR-FRET donor ('Tb Donor') tightly associated with the IMAP binding entity (**Figure 15**). When a fluorescent-labelled substrate gets phosphorylated, it can bind in proximity to the Tb donor, enabling energy transfer. Both fluorescein (FAM) and TAMRA are suitable fluorescent labels. IMAP TR-FRET combines the advantages of IMAP FP (no antibodies, homogeneous format, easy assay development, scalability, high throughput) with those of TR-FRET mode (broad range of substrate size and concentration, low background due to time resolved mode). IMAP TR-FRET extends the range of molecular weights for kinase substrates from small peptides up to large proteins. For example, MEK1 can be assayed using inactive ERK2 labelled with fluorescein as substrate. Fluorescent-labelled streptavidin can now be used to detect phosphorylation of biotinylated peptide substrates (**Figure 16**). The latter makes IMAP TR-FRET compatible with existing biotinylated substrate libraries for substrate identification. IMAP TR-FRET further supports the expansion of the IMAP technology for non-protein kinases like glucose kinase and sphingosine kinases, emerging drug targets for which IMAP is one of very few HTS-friendly technologies.

Phosphodiesterases (PDEs) are another hard-to-screen target class enabled through IMAP. The recent availability of recombinant PDEs (EMD Biosciences) combined with IMAP's high assay fidelity and throughput should accelerate drug discovery in this area.

MDS Pharma Services (www.mdsps.com) provides a comprehensive line of kinase assay services to address the *in vitro* and *in vivo* aspects of drug discovery screening and profiling. MDS offers a wide selection of kinases (greater than 170) in radiometric, fluorescent and cell-based formats. Clients may select from its radiometric 'gold standard' assays (currently 155 kinase assays) with a turnaround time of two weeks or its FastKinase™ fluorescence

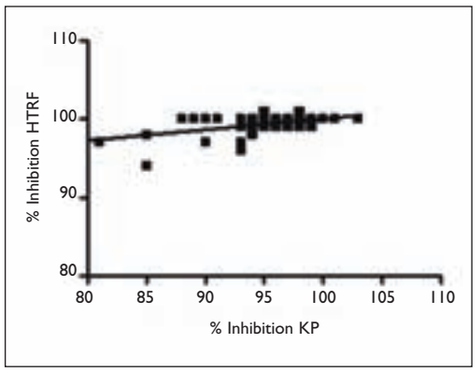


Figure 17: Kinase activity was measured at 10µM ATP and 1µM staurosporine. Each data point represents 1 of 60 kinases tested in the Millipore's KinaseProfiler radiometric (KP) and HTRF formats. Significance between the 2 data sets was $p < 0.0001$

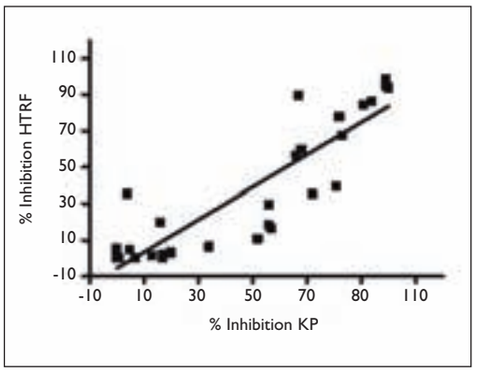
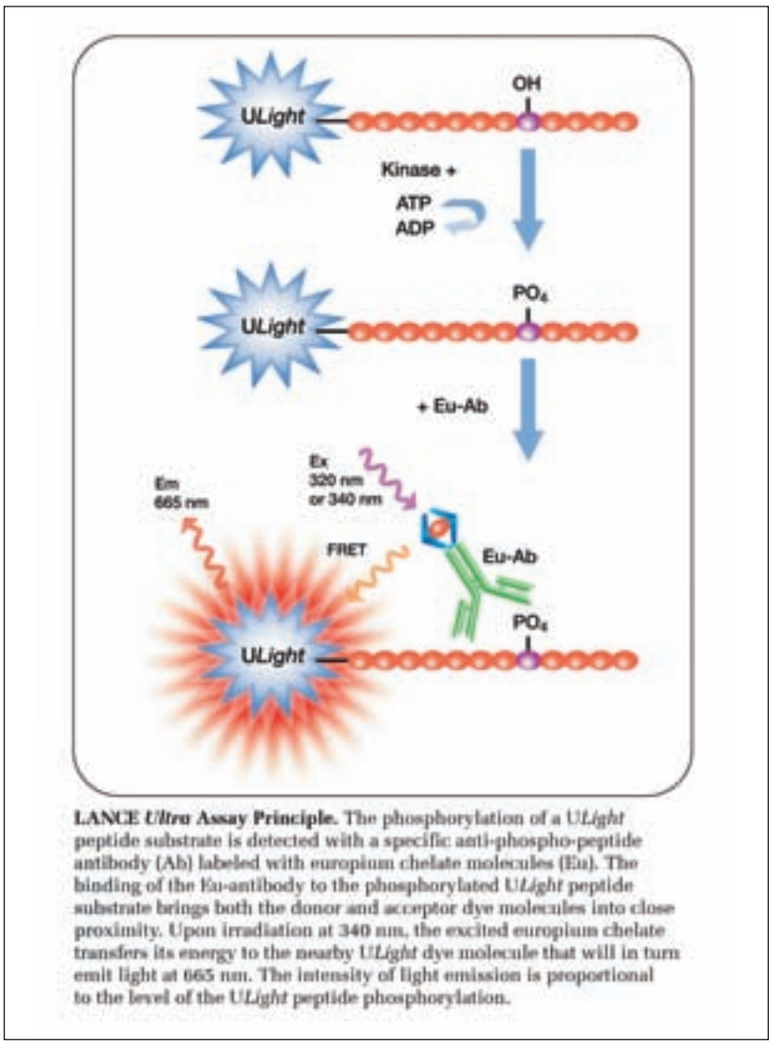


Figure 18: BRSK2 activity was measured at 10µM ATP each data points represents the % inhibition obtained against 1 of 30 inhibitors tested in the Millipore's KinaseProfiler radiometric (KP) and HTRF formats. Significance between the 2 data sets was $p < 0.0001$

polarisation based platform of 94 kinases. FastKinase™ enables the rapid profiling of larger numbers of compounds and guarantees a five-day turnaround time with the flexibility of single concentration testing (in duplicate) or upfront IC_{50} determination. In addition, PathTrak™ cellular solutions assays enable clients to predict kinase efficacy and selectivity in the native cellular environment. PathTrak™ detects phosphorylation states of endogenuos intracellular proteins without the need for over-expression. The assays measure changes in phospho-protein levels due to compound mediated inhibition of kinase activity in signalling pathways. MDS Pharma Services is working on expanding its kinase testing services to include two new FastKinase™ assay packages (tyrosine kinase specific and kinome diversity panels), additional PathTrak™ assays, multiplexed kinase testing and high-content analysis.

Kinase activity measured using radiolabelled ATP, as used in **Millipore**'s (www.millipore.com) KinaseProfiler™ continues to be the gold standard to which non-radiometric formats are compared. However, rapid and cost-effective medium or large-scale profiling with this method is not always practical. HTRF™ is the most widely used present-day kinase screening technology in biotech and pharmaceutical companies and avoids difficulties associated with waste management of a radioactive assay format. To address the need to profile large compound libraries and chemical arrays in a commonly used format, Millipore is introducing a new service: KinaseProfiler HTRF. The service is based on assays developed in partnership with Cisbio using one monoclonal antibody for serine/threonine kinases (STKs) and one for tyrosine kinases (TKs). To date Millipore's KinaseProfiler HTRF development team has validated assays for more than 80 STKs and 30 TKs. Cisbio has demonstrated activity for an additional 24 STKs and 30 TKs that are currently in development to support screening services available to Millipore's clients. **Figure 17** shows the inhibition data generated by 60 kinases in both the radiometric and HTRF formats at 10mM ATP and 1mM staurosporine. **Figure 18** shows the inhibition data of both formats in the presence of 30 kinase inhibitors. Both graphs demonstrate the consistency between the two assay formats. Combined with follow-up IC_{50} determinations performed in the radiometric assay, customers will have access to a comprehensive solution for their large-scale kinase profiling needs. The new HTRF-based KinaseProfiler service will be available for general access early this year.

PerkinElmer (www.perkinelmer.com) has recently introduced LANCE® Ultra, enhanced LANCE TR-FRET assays which utilise ULight™, a new light-insensitive, small molecular weight red-shifted emission acceptor dye. ULight provides the ability to directly label small molecules such as peptides and cAMP. New products include both specific and generic kinase substrates and antibodies directly labelled with the ULight acceptor dye, and an array of Europium chelate donor dye labelled anti-phospho-antibodies (**Figure 19**). PerkinElmer has also introduced a new collection of SureFire™ cell-based kinase assays in collaboration with TGR BioSciences of Thebarton, Australia. These assays are based upon HTS-proven AlphaScreen® technology, enabling them



LANCE *Ultra* Assay Principle. The phosphorylation of a U*Light* peptide substrate is detected with a specific anti-phospho-peptide antibody (Ab) labeled with europium chelate molecules (Eu). The binding of the Eu-antibody to the phosphorylated U*Light* peptide substrate brings both the donor and acceptor dye molecules into close proximity. Upon irradiation at 340 nm, the excited europium chelate transfers its energy to the nearby U*Light* dye molecule that will in turn emit light at 665 nm. The intensity of light emission is proportional to the level of the U*Light* peptide phosphorylation.

Figure 19: PerkinElmer LANCE Ultra assay principle

to work with very large molecules such as full length endogenous proteins expressed at physiologically natural levels. AlphaScreen SureFire assays are suited for screening a wide range of target kinases and detecting activated, phosphorylated protein kinases in whole cells. Using AlphaScreen SureFire technology, researchers can interrogate a diverse range of targets such as GPCRs, RTKs, growth factor and cytokine receptors and receptors involved in the inflammatory and stress response. Fully validated kits are available for ERK 1/2 and MEK 1 in the ras/raf pathway, AKT and p70S6K in the PI3K pathway, and JNK, p38MAPK and STAT-3. Additional PerkinElmer product introductions include a new glutathione-coated donor bead. Utilised in conjunction with AlphaScreen acceptor bead offerings, researchers can screen full-length substrates expressed as GST fusions without further need for chemical modification such as biotinylation. The new donor bead type greatly reduces the possibility of biotin mimetic compound interference.

Drug screening against kinase targets is a difficult task that typically involves several different assays depending on the kinases being screened. Having a single detection platform to screen all kinases simplifies the screening process significantly. **Promega**'s (www.promega.com) Kinase-Glo® Plus Luminescent Kinase Assay can be used to screen virtually any kinase/kinase substrate combination. This homogeneous assay is performed in a single well of a 96-, 384- or 1536-well plate by adding a volume of reagent equal to the volume of a completed kinase reaction and measuring luminescence. The luminescent format is preferred over

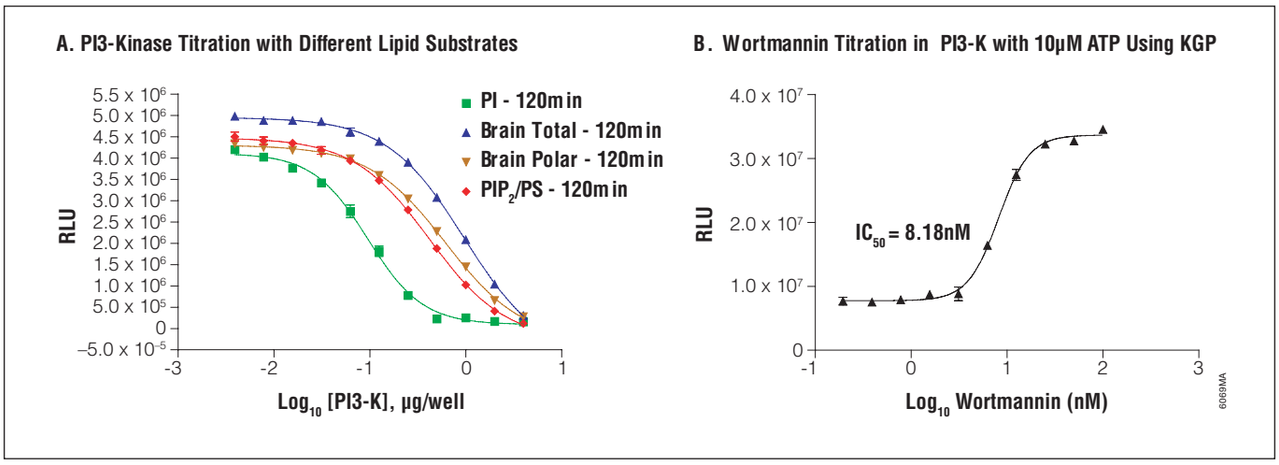


Figure 20: Panel A shows IC_{50} data for PI3-kinase (PI3-K), a lipid kinase, generated using Promega's Kinase-Glo Plus with a variety of lipid substrates. Using phosphoinositide as a substrate (Panel B), the PI3-K inhibitor Wortmannin was used to generate an IC_{50} curve

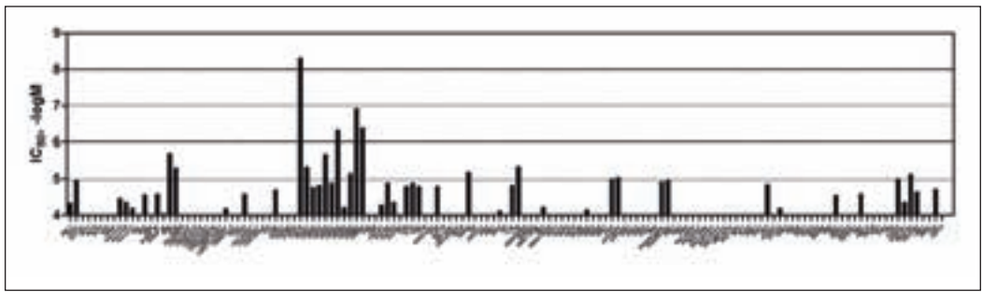


Figure 21: IC_{50} profile of a kinase inhibitor with 141 different recombinant protein kinases. The data were generated with ProQinase's FreeSelect service, which is optimally designed for single compound IC_{50}-profiling

fluorescence because compound autofluorescence is no longer an issue. Kinase-Glo Plus sensitively monitors the depletion of ATP in a kinase reaction. The luminescence-based assay format offers excellent signal-to-background ratios, and routinely delivers Z' factor values >0.7. The assay can be performed at ATP concentrations up to 100µM. Custom formulations that are linear up to 500µM ATP are also available. The Kinase-Glo Plus Assay can easily be used to generate dose curves without the need to specifically label peptides or use radioisotopes (**Figure 20**).

In vitro profiling of drug candidates is a substantial part of all drug development projects. Compounds at late stages of pre-clinical development especially require extensive profiling against growing panels of targets and counter targets since the selectivity profile of a lead compound is an important parameter for decision processes regarding further actions leading to clinical trials. Moreover, broad profiling of lead compounds allows the identification of unknown targets and therefore sometimes may open insight on the molecular mechanism of a drug effect. **ProQinase**'s (www.proqinase.com) novel *in vitro* kinase profiling service FreeSelect is optimally designed for IC_{50} characterisation of a single test compound. This service allows the customer to obtain broad IC_{50} data on a growing set of currently 170 different recombinant protein kinases (**Figure 21**). Using a novel modular set-up, a robotic system performs highly reproducible IC_{50} measurements over a concentration range of 12 half-logarithmic dilutions of a single compound against 32 kinases in parallel. The technology is based on a radiometric kinase assay, which is still the gold standard in kinase assay technology. This method is based on the use of 33P-ATP in combination with scintillant-coated microtiter plates. In contrast to other *in vitro* profiling technologies that require different assay conditions for different kinases, FreeSelect allows IC_{50} measurement under identical assay conditions for all kinases.

SpinX Technologies (www.spinx-technologies.com) has developed a bench-top instrument (SpinX Lab) integrating liquid handling and fluorescent detection to perform assays in nanolitre volumes (**Figure 22**). The system uses disposable microfluidic cards which are combined into a 384-well microplate format so that standard liquid handlers can be used to load the different assay components. Each assay component is loaded on to a single well in concentrated stock solution, preserving compound and reagent integrity. These wells act as input reservoirs leading into a network of microfluidic structures where, according to the user-defined protocol, the different assay components are brought together to perform dilutions, set up the biochemical reactions, and read the result. The system is presently being validated for Early Access sites using a variety of homogeneous enzymatic assays with fluorescent readout. For kinase profiling, it offers the unique advantage that any compound need only be loaded

Figure 22
SpinX Technologies' bench top instrument the SpinX Lab

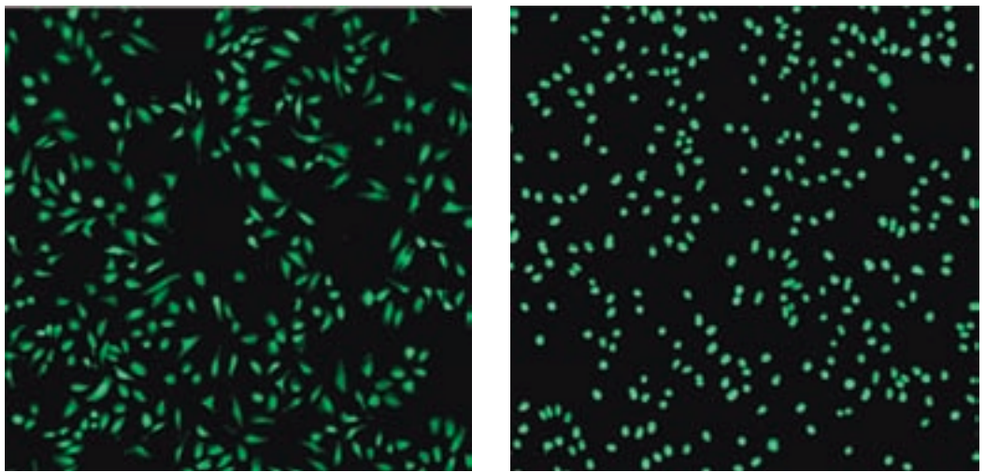


Figure 23
Virtual well view in TTP LabTech's Acumen eX3 software showing translocation of a GFP-tagged protein kinase from cytoplasm to nucleus. A: (left panel) non-translocated control; B: (right panel) translocated

into a single well from which serial dilution in 100% DMSO is performed in the microfluidic structures, with the resulting solutions distributed to different assays in parallel, improving ease of use and precision. Current developments on the SpinX system address two emerging trends in the kinase profiling market: the growing importance of time-resolved fluorescence as a preferred assay format and an increasing use of Ki determinations. To address the former, SpinX is developing a time-resolved fluorescence module to complement its prompt fluorescence readout. For the latter, the technology is uniquely suited to integrating all assay preparation steps required to enable automated Ki as well as mode-of-action studies.

Cell-based high-content assays are most widely applied for secondary profiling of kinase activity after the majority of compounds have been dismissed. Their application in HTS poses significant challenges mainly due to the low throughput of existing technologies. For high-content kinase assays there is also the added complication that fixation protocols required for immunodetection are multi-step and time consuming. Laser-scanning fluorescence microplate cytometers, such as **TTP LabTech**'s (www.ttplabtech.com) the Acumen® eX3 combine the object-recognition capabilities of microscope-based CCD imagers with the fast read speeds of bulk fluorescence readers. This instrument has been applied to the primary screening of kinases and their role in the regulation of the cell cycle. Where anti-phosphokinase antibodies are employed, protein kinase activation can be detected using single colour protocols by the emergence of fluorescence staining in cells[2]. In contrast, methods using anti-kinase antibodies or GFP-tagged kinases report activation by quantifying the translocation of enzyme within each cell, most commonly from cytoplasm to nucleus (**Figure 23**). The whole well scanning capability of microplate cytometers allows high content analysis of every cell in every well at high throughput. This has many benefits for kinase profiling including overcoming the problems of variable stimulation and random cell distribution often observed following immunostaining. Additionally, whole well scanning enables normalisation of biological responses to total cell number, offering a simple toxicity or proliferation readout with every test. Additionally, the introduction of multilaser microplate cytometers further extends the range of fluorescent probes and proteins that can be combined in multicolour, multiplexed assay protocols for kinase profiling.

Summary

Whereas wider coverage of the kinome in terms of diversity was the principal focus of kinase profilers a few years ago[1], the following trends have now assumed amplified significance among vendor's product offerings:

1 Increasing use by fee-for-service providers of automated and industrialised approaches to kinase profiling and screening, offering partners comprehensive solutions ranging from a few lead compounds to large-scale kinase profiling, all with enhanced turnaround times.

2 Growing importance of time-resolved fluorescence as a preferred assay format.

3 Wider pursuit of universal assay approaches that extend the breadth of single assay systems, allowing more kinases to be screened under identical assay conditions.

4 Increased availability of assays that measure the accumulation of ADP, a product of kinase enzyme activity (as opposed to methods that focus on the depletion of ATP).
5 Greater emphasis on screening inhibitors that bind to inactive and low activity kinases.
6 Greater diversity of cellular kinase approaches, including compound mediated inhibition of kinase activity in signalling pathways, which enable the prediction of kinase efficacy and selectivity in the native cellular environment.
7 Emergence of several bench-top instrument turnkey solutions based on microfluidic approaches that greatly simplify IC_{50} and Ki determinations.
8 Growing awareness of the potential of label-free approaches to address kinase assay deficiencies.

Overall the end user is spoilt for choice with the range of alternative offerings, methodologies and approaches currently available for kinase screening and profiling. DDW

Dr John Comley *is Managing Director of HTStec Limited an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation and assay reagent technologies) to drug discovery. Since it formation in 2003, HTStec has published 22 market reports on drug discovery technologies and Dr Comley has authored 16 review articles in* Drug Discovery World. *Further information on accessing the market report 'Kinases Screening & Profiling Trends 2006' can be obtained by visiting www.htstec.com or by emailing john.comley@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*

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