OUTSOURCED KINASE PROFILING SERVICES –

adding value to in-house kinase programmes

By Dr John Comley

Biochemical kinase profiling using a large panel of kinases with a broad coverage of the human kinome has become the *de facto* norm within the Pharma industry. The importance of this activity is demonstrated by the large number of service providers offering outsourced kinase profiling services. The conventional biochemical kinase profiling service sector is now a mature marketplace with relatively little new innovation in recent years. It is highly competitive with respect to the size and coverage of kinase panels, speed of turnaround and cost of the service. What differentiates providers are their core assay technologies; their ability to rapidly determine ATP competition, an inhibitor's binding mode and association/dissociation kinetics; how they sell or package selected elements of their entire kinase panel; and those add-on components that aim to make a specific offering standout. In addition to their biochemical offerings, most providers now complement this service by offering cell-based kinase profiling assays where cellular kinase-specific phosphorylation is measured. Assays allow for confirmation of inhibitory activity in a relevant cellular background and profile their selectivity against multiple signalling pathways. In recent years the number of companies offering *in situ* profiling alternatives to conventional biochemical services has increased. Mostly these are based on unique or proprietary technologies enabling the analysis of kinases, kinase networks and the regulation of the kinome in cell lysates, living cells, tissues and even living animals. This includes proteomic scale kinase inhibitor and substrate profiling based on mass spectrometry, protein microarrays and immunoblotting. Adoption of the emerging *in situ* kinase profiling alternatives has, however, been slow and this area still represents something of a niche activity. What is increasingly evident is that the enzymatic diversity available, the technical skills and the scientific expertise residing at fee-for-service providers now exceeds the capabilities of all but the largest in-house profiling operations and can add real value to most Pharma kinase programmes. The rationale for outsourcing kinase profiling has never been greater.

Kinases have a pivotal role in human biology, controlling many cellular processes through protein phosphorylation. Dysregulation of kinase function triggers many pathological conditions such as cancer, inflammatory diseases, neural disorders and metabolism problems such as diabetes. Protein kinases therefore represent one of the most promising classes of drug targets owing to their involvement in such diseases. Around 20 protein kinase inhibitors have been approved for clinical use (including Gleevec®, Iressa® and Tarceva® that are used in treatment of various cancers) and more than 150 more are undergoing clinical trials of which about 25 are in Phase III. Kinases are very similar in their active site architecture, which leads to cross-reactivity among kinase targeted small-molecule inhibitors. The major challenge in this area is therefore to develop a drug that selectively suppresses the activity of one, or at most a few, of the 500 plus protein kinases encoded by the human genome. It is important to determine the affinity and selectivity of potential inhibitors to minimise side-effects caused by off-target inhibition while optimising desired selectivity profiles. Biochemical kinase profiling using a large panel of kinase activity assays with a broad coverage of the human kinome is routinely practised within the Pharma industry for the assessment of selectivity and potential side-effects. More recently, biochemical kinase profiling has been supplemented by cell-based kinase assays and other *in situ* approaches. The list of available functional cellular kinase and cell signalling assays that detect the activation of full-length kinases in whole cells is increasing. Used alone or in combination with biochemical assays, *in situ* cellular approaches allows for kinase binding measurements under conditions where critical protein:protein interactions, differential phosphorylation events, and other activity altering modifications are preserved.

Owing to the technical challenges of assembling, maintaining and performing profiles across the entire kinome (ie the widest possible kinase panel, including lipid kinases) this has become an area where outsourced testing at a fee-for-service provider can efficiently compete with the in-house kinase panel profiling operations. This has resulted in the emergence of many providers offering kinase profiling over the past decade. Although providers seek to differentiate their services principally on the basis of their core assay formats/screening technology and the quality of the data derived, competition has inclined to be most intense around the

Figure 1: Preferred outsourced kinase panel profiling assay format

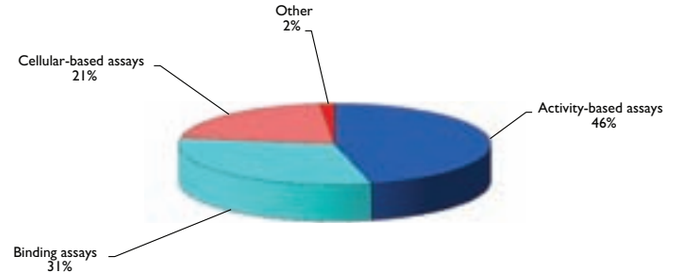


Figure 2: Typical use of outsource kinase profiling (total wells/year)

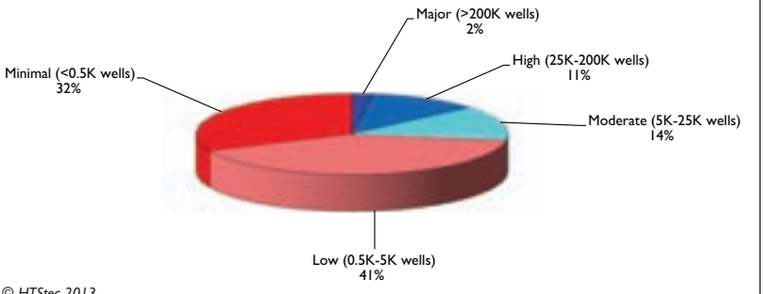


Figure 3: Breakdown of kinase profiling wells outsourced today

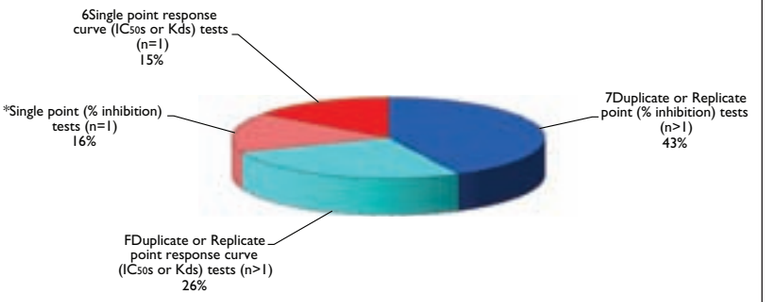


Figure 4: When respondents choose to outsource a broad kinome profile

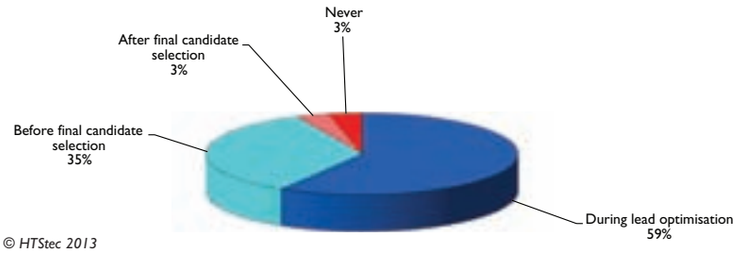


Figure 5: Key criteria in selecting kinase profiling fee-for-service provider

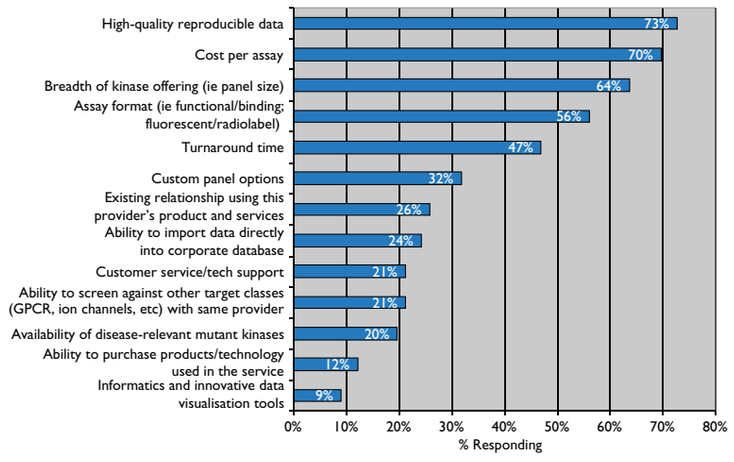
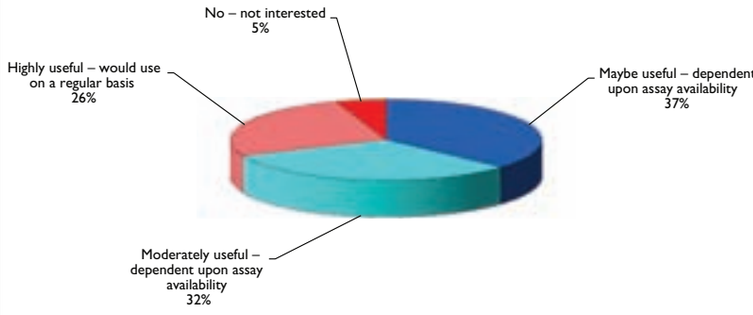


Figure 6: Usefulness of an outsourced panel of individual cell-based kinases



size of kinase panel offered, the turnaround time and ultimately the cost of the profiling service.

HTStec's recent Kinase Profiling Trends 2013 survey and report[1] represents our latest re-examination of this marketplace after a break in market surveillance of nearly three years. This gap came about mainly due to perceived decline in importance placed on kinase profiling. The latest report update documents current practices and preferences in kinase selectivity profiling, and seeks to understand future user requirements, particularly with respect to the cost and use of outsourced services. In this article we review some of the report's findings on outsourcing together with the kinase services offerings of a number of fee-for-service providers and technology developers.

Current status of kinase profiling

A median of 20%-40% of all kinase profiling activities were outsourced today (2013) by survey respondents. The preferred outsourced kinase panel profiling assay format wanted by 46% of survey respondents was activity assays. This was followed by binding assays (31% preferring), then cellular-based assays (21% preferring) and other format (2% preferring) (**Figure 1**).

Most (41%) outsourcing involves a relatively low number of wells per year (0.5-5K). A further 32% involves minimal number of wells per year (<0.5K). That leaves 27% of outsourcing involving moderate to major number of wells per year (from 5K to >200K) (**Figure 2**).

The majority (43% using) of survey respondents' outsourced kinase profiling wells were duplicate or replicate point (% inhibition) tests (n>1). This was followed by duplicate or replicate point response curve (IC50s or Kds) tests (n>1) (27% using). Only around 15% used either single point (% inhibition) tests (n=1) or single point response curve (IC50s or Kds) tests (n=1) (**Figure 3**).

The typical median size of kinase assay panels being used for outsourced profiling evaluations today (2013) were 26 to 50 SAR for lead optimisation and 201 to 250 for the final decision stage. The preferred median size of a kinase profiling panel to be offered by an outsourced provider was 301-400 kinases. The median number of disease-relevant kinases survey respondents want to see represented in an outsourced panel was 31-50 kinases.

The majority (59%) of survey respondents would choose to do a broad kinome profile during lead optimisation. A further 34% would do it before final candidate selection. A small number (3% in each case) would never choose it or do it after final candidate selection (**Figure 4**).

Figure 7: Interest in phosphoproteome analysis technologies

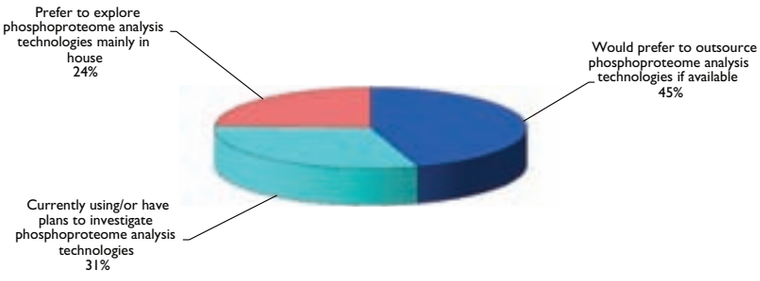


Figure 8: Where kinase service providers should prioritise their efforts going forward

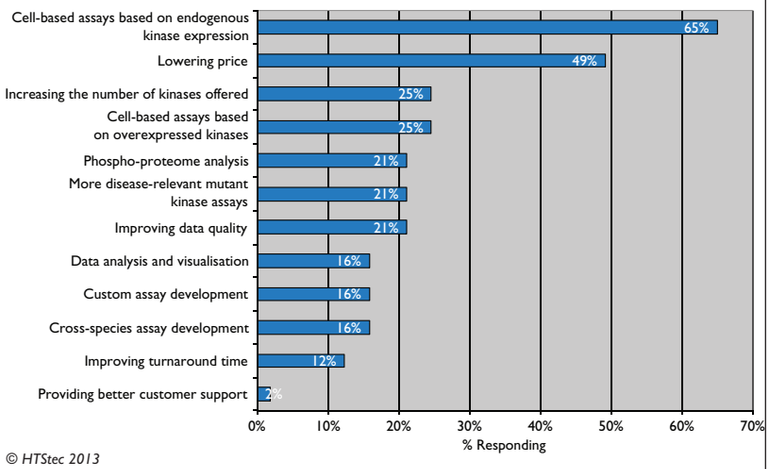
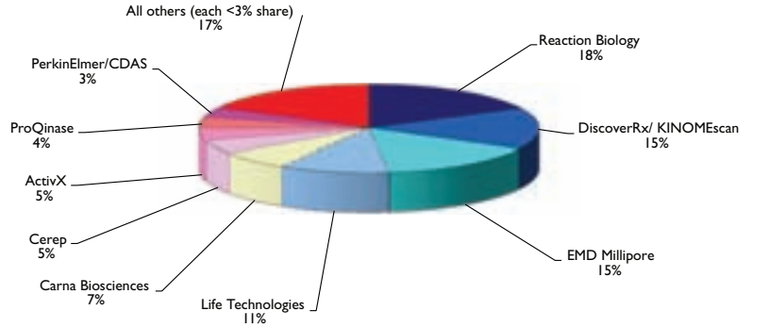


Figure 9: Approximate share of outsourced kinase profiling market today



Selecting a fee-for-service provider

High-quality reproducible data was ranked by survey respondents as the key decision-making criteria when selecting any outsourced kinase profiling fee-for-service provider. This was followed by cost per assay; breadth of kinase offering (ie panel size); assay format (ie functional/binding; fluorescent/radiolabel) and then turnaround time. Ranked least important when selecting an outsourced kinase profiling fee-for-service provider was informatics and innovative data visualisation tools and the ability to purchase products/technology used in the service (**Figure 5**).

Future profiling requirements

Most (37%) of survey respondents thought that an outsourced panel of individual cell-based kinase assays may be useful (ie dependent upon assay availability). A further 32% thought individual cell-based kinase assays would be moderately useful (ie dependent upon assay availability), and 26% highly useful (ie would use on a regular basis) (**Figure 6**).

Most (45%) survey respondents would prefer to outsource phosphoproteome analysis technologies if available, with 24% preferring to explore (but not actually doing it today) phosphoproteome analysis technologies mainly in-house. That left only 31% who are currently using/or have actual plans to investigate phosphoproteome analysis technologies today (**Figure 7**).

Cell-based assays based on endogenous kinase expression (ie more physiological relevant) was ranked by survey respondents as the area where kinase service providers should prioritise their efforts going forward. This was followed by cell-based assays based on overexpressed kinases, improving data quality and then phospho-proteome analysis and custom assay development. Ranked least important was providing better customer support (**Figure 8**).

Key providers of outsourced kinase profiling services

Figure 9 shows the approximate share of outsourced kinase profiling market today. The market is split between at least 15 fee-for-service providers. No single vendor predominates or has the majority of the business, although the offerings from Reaction Biology, DiscoverRx, EMD-Millipore, Life Technologies and Carna Biosciences all have the significant share. In reality each of the key providers tends to address a niche customer segment (eg Pharma, Biotech, Academic Research or geographic locality).

Service provider updates

The following are service provider-contributed updates that highlight details of each vendor's current and planned kinase profiling offering:

The **ActiveX Biosciences** (www.activex.com) KiNativ platform utilises a desthiobiotin-tagged acyl phosphate ATP or ADP probe to covalently modify native kinases from any species. Upon probe modification, samples are digested with trypsin and the probe-labelled peptides captured and analysed by mass spectrometry, leading to both the identification and quantitation of probe-labelled peptides. The method can be used to profile inhibitors by measuring the ability of inhibitors to compete with probe-labelling. In the simplest approach, lysates are treated with inhibitor and probe, allowing for the determination of the potency and selectivity of kinase inhibitors. Alternatively, inhibitors can be profiled in living cells. After compound treatment, cells are harvested, lysed, probe-labelled and processed for mass spectrometry. This approach can uniquely correlate target engagement (fraction of kinase inhibited) with cellular efficacy. Such an approach can be invaluable in confirming the mechanism of action of a compound in a cell-based assay and establishing parameters required in effective compounds. In addition to profiling inhibitors in cells, inhibitors can also be profiled in live animal studies, including those in humans. In this case, at various times after treatment, target tissues are collected, lysed, probe-labelled and analysed by mass spectrometry. In addition to confirming target engagement during the course of the animal study, the approach also allows for the determination of the pharmacodynamics of the inhibitor, active metabolite(s) and metabolite(s) with additional off-targets. By virtue of having utility in any organism with a completed genome, and applicability in all stages of drug discovery, KiNativ is a uniquely powerful drug-discovery tool (**Figure 10**).

Over the past 12 years the 170 scientists of **BioFocus** have gained a strong reputation and track record of success in kinase drug discovery. The company has developed and validated more than 100 screening assays encompassing serine/threonine, tyrosine and lipid kinase targets, progressed more than 50 high-throughput screening programmes on behalf of clients and undertaken more than 20 integrated drug discovery programmes (see http://www.biofocus.com/publications-posters-and-patents/publications.htm). More than three million compounds have been evaluated in formats including HTRF, fluorescence and radiochemical detection and profiled in mobility shift assays and surface plasmon resonance. Hit finding in novel chemical space can be supplemented by virtual screening approaches to intelligently select and bias screening decks, or by biophysical assessment of binding, eg for fragment screening (**Figure 11**). BioFocus is also an industry leading expert in library design and has designed and synthesised more than 10,000 kinase focused compounds under the SoftFocus® brand (with new kinase focused libraries currently available only under a subscription agreement). Libraries have been created with a focus upon hinge binding (ATP-competitive), DFG-out (non-ATP competitive) and invariant lysine binding

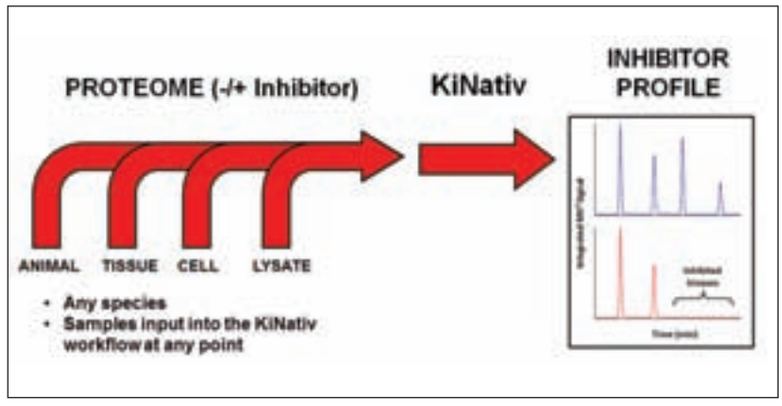


Figure 10: Schematic showing the ActiveX's KiNativ approach to kinase profiling. KiNativ *in situ* profiling enables analysis of kinases and quantitation of their inhibition, from any species and supports drug discovery and development from target discovery through clinical trials

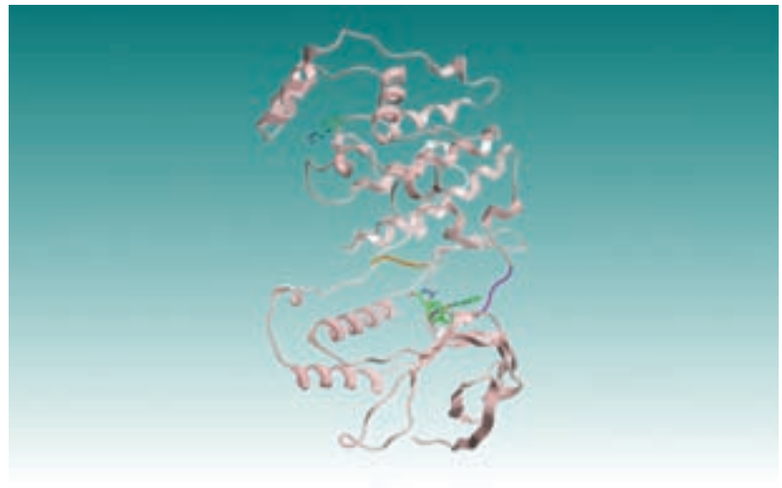


Figure 11: Overlay of fragments binding to p38α; the hinge region is coloured pink and the activation loop orange; four fragments are bound in a diversity of binding modes, one completely allosteric

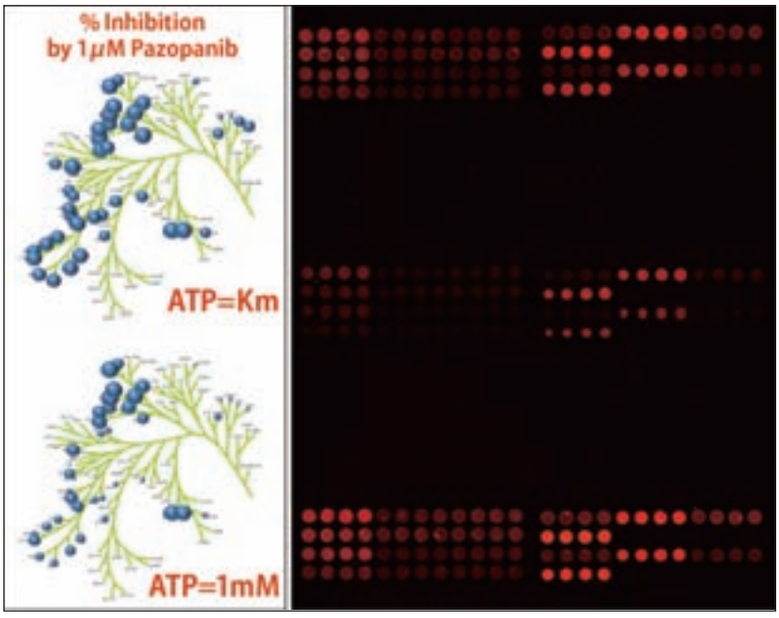


Figure 12: Carna Bioscience's profiling technologies. Left: Inhibition profile of 1uM Pazopanib on a tyrosine kinase panel, in the presence of ATP at Km (top) and 1mM (bottom). The differences might indicate the competition by ATP. The area size of circle is proportional to extent (%) of inhibition. Right: A RPPA array with cell lysate spots immunostained against phosphorylated proteins. Currently Carna's RPPA can quantify 180 phosphoproteins in ~100 lysate samples with one array

(non-ATP-competitive) modes. The combination of biology, including a range of biophysical techniques and crystallography, with medicinal chemistry and library design expertise has resulted in multiple kinase inhibitors in clinical development for therapeutic areas including malaria and rheumatoid arthritis. In addition to this, and demonstrating the strength of the design process, 14 SoftFocus® kinase compounds have been published as co-crystal structures by leading academic and industry laboratories.

Carna Biosciences (**www.carnabio.com**) has been the leading kinase company for 10 years. Its biochemical profiling service utilises >300 in-house manufactured high quality kinases. The main platform Mobility Shift Assay (PerkinElmer) has advantage in detecting both the phosphorylated and non-phosphorylated substrates, ensuring reliable results. Carna's assay panel is customisable to fit client's budget and needs, and also pre-selected panels, QuickScout™, are available. One of the panels covers the MAPK cascades, which evaluate compounds over the entire cascades for on- and off-target effects. The regular biochemical assays are performed with Km concentration of ATP, empirically determined for each kinase in-house, to maximise sensitivity. Carna also offers assays with physiologically relevant high (1mM) ATP to predict *in vivo* inhibition profile. By comparing the results from Km and 1mM ATP assays, clients can obtain insight on the compound's mode of inhibition (eg ATP-competitive or not). With its partners including NTRC, Carna also offers cell-based kinase profiling services for better understanding of *in vivo* behaviour of compounds. Advanced Cellular Dynamics provides the world's largest panel of tyrosine kinase assays, and Cell Assay Innovations provides customisable assays for both serine/threonine and tyrosine kinases. Carna has recently launched another cell-based profiling service, reverse phase protein array (RPPA). RPPA is a proteome technology that can quantify hundreds of phosphorylated targets in cells by immunostaining cell lysates spotted as high density arrays. With one of the world's largest phospho-target panel, Carna's RPPA provides a proteome-wide snapshot of kinase activities and the effect of compounds on it in the cells (**Figure 12**).

Kinase screening and study has been an essential experimental field for **Cisbio** Bioassays HTRF (www.htrf.com), not only from the HTS prospective, but also for downstream processes such as secondary screening and lead optimisation. Biochemical formats and semi-universal ones such as HTRF KinEASE have progressively been supplemented with cell-based solutions covering the main pathways studies along the drug discovery phases. In terms of assay development outsourcing, proximity, communication and execution turnaround are fundamental criteria, especially when a large number of assays need to be optimised, eg elaboration of a profiling platform. Over the year, Cisbio has been a proactive partner in this field, in particular by investing in local organisations and resources – Europe, USA and China – which respond efficiently to researchers' needs. Being homogeneous, robust, and requiring only basic reagent labelling (eg antibodies), HTRF technology enables in addition particularly fast assay development processes. This organisation and technological benefits have allowed the development of a full kinase turnkey profiling platform for a CRO in replacement of radioactivity-based assessment, or to develop on demand for Cisbio's pharma customers, a growing number of cell-based kinase assays to substitute to more conventional heterogenous and time-consuming assays.

The **DiscoveRx** (www.discoverx.com) KINOMEscan screening platform employs a proprietary

active site-directed competition binding assay to quantitatively measure interactions between test compounds and kinases across a growing panel of more than 460 assays, including eight new assays added this year. Several high profile Nature Biotechnology papers have established KINOMEscan as the best-in-class platform for measuring kinase inhibitor potency and selectivity. However, kinase inhibitor drug discovery requires the optimisation of several other drug-like properties such as cellular potency, pharmacokinetics and pharmacodynamics, and activity against clinically relevant mutant kinases. Several of these properties are affected by an inhibitor's binding mode and association/dissociation kinetics – two parameters that have been traditionally problematic to measure routinely during lead optimisation. KINOMEscan has recently introduced scanMODE™ and scanKINETIC™ – biochemical tools that classify inhibitor binding mode (Type I or Type II) and association/dissociation kinetics, respectively. scanMODE™ consists of activated/unactivated kinase assay pairs that biochemically classify inhibitors as having Type I or Type II binding modes. scanMODE™ thus provides structural insights without the requirement for co-crystal structures – a feature that enables rapid, real time structural feedback during lead selection and optimisation. scanKINETIC™ enables the classification of inhibitors as having rapid, slow, or irreversible binding kinetics, which is essential for robust inhibitor characterisation and to resolve potentially conflicting potency data measured in biochemical, cellular and *in vivo* assay models. For example, target inhibition is sometimes maintained for several hours, even after the inhibitor has been 'washed out' from cellular assays. In the absence of kinetic data, these pharmacology results can be difficult to interpret, particularly when multiple inhibitors are being compared. scanKINETIC™ is a semi-quantitative, broadly applicable tool that is currently available for more than 225 kinases and is the largest commercially available panel for this application (**Figure 13**).

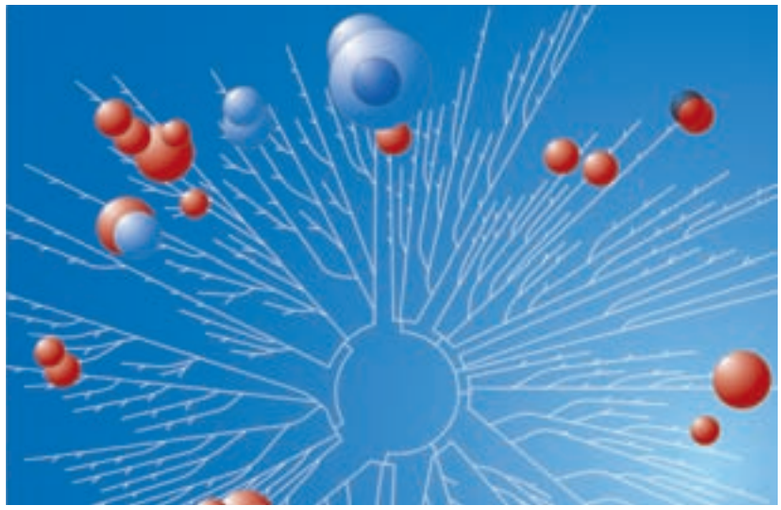


Figure 13: The TREEspot™ phylogenetic tree is an artistic representation of the human kinome dendrogram used by DiscoveRx to describe the relationships between the members of this protein class and a key component to its feature rich compound visualisation tool

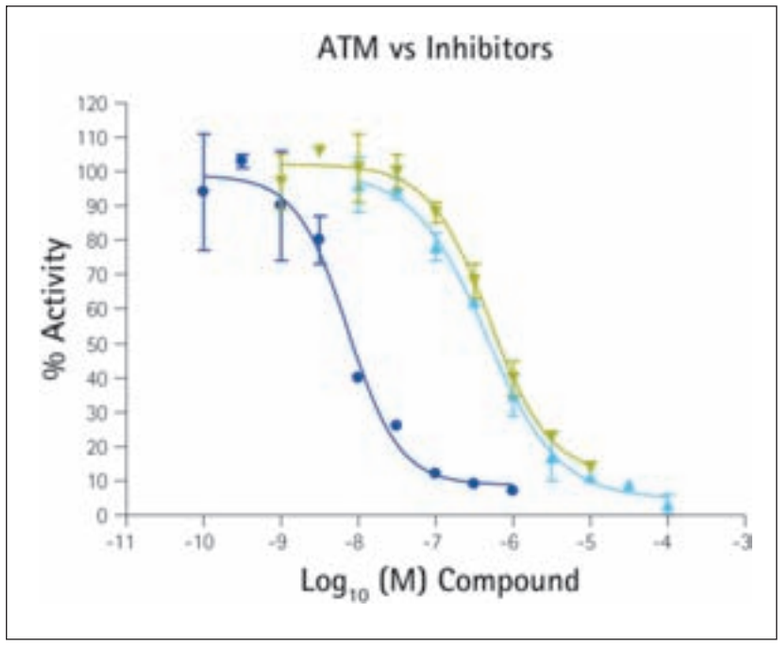


Figure 14: Dose responses for three inhibitors were measured in an ATM activity assay by the EMD Millipore kinase profiling service. Green: PIK-90. Cyan: PI-103. Blue: KU-55933

EMD Millipore's (**www.millipore.com/KinaseProfiler**) kinase profiling service is focused on addressing the needs specific to pharmaceutical industry researchers in new and unique ways. Last year it introduced a one-week turnaround time option which guarantees data delivery to the researcher within five business days of the project start. EMD Millipore introduced this option with industry in mind, where adherence to project timelines directly relates to cost savings. It claims to be the fastest provider and the researcher is not charged for any data that are delivered late. This is one of the new and unique ways in which EMD Millipore is trying to distinguish itself from the competition. A second area it has invested in is biomanufacturing capabilities for challenging proteins. A prime example is its ataxia telangiectasia

Figure 15: Snapshots of a semi-automated kinase profiling lab at Eurofins Cerep/Panlabs

mutated (ATM) kinase, the DNA damage response regulator. Due to the protein size, 352 kDa, the ATM field has struggled to generate pure and active enzyme. Through the technical breakthroughs made by its protein scientists and assay developers, in August EMD Millipore introduced ATM into its profiling service panel and offers the accompanying pure protein for in-house testing. EMD Millipore is now the only provider of a catalytic assay for ATM kinase. These are just two examples of its focus on unmet needs of pharma industry researchers: it aims to continue to invest in science and technology to develop high-value kinase targets and provide a five-day data turnaround that researchers can consistently rely on (**Figure 14**).

Eurofins Pharma Discovery Services (www.eurofins.com/pharma-services/pharma-discovery-services.aspx) was established in March 2013 as a result of the merger of Eurofins Panlabs and Eurofins Cerep, and offers a wide range of kinase assays from biochemical kinase to cellular kinase and binding kinase assays. The majority of Eurofins biochemical kinase assays use activated kinases. They are usually full length kinase or cytoplasmic

domain of RTK. Assays are designed to be as close as possible to ATP and substrate Km. The technology used to measure substrate phosphorylation is TR-FRET (HTRF® or LANCE®). Eurofins cellular kinase assays are implemented to complement the biochemical assay platform. These assays allow for confirmation of inhibitory activity in a relevant cellular background and profile their selectivity against multiple signalling pathways. Eurofins binding kinase assays identify type II inhibitors on unphosphorylated kinases, to compare compounds activity on unphosphorylated and phophorylated kinases and to discriminate between ATP and non-ATP competitive inhibitors. In addition, Eurofins is now using an ADP readout to develop kinase assays on intractable substrates (for which no antibody exists) which allows customers to profile kinase activity on different natural substrates in order to identify substrate selective inhibitors. Besides offering different kinase assay types, Eurofins offers kinase profile panels including: ExpresS Profile (44 kinase), Fastkinase (106 kinase), Taiwan 32P Assays (145 kinase) and Comprehensive Profile (212 kinase). In addition, Eurofins can create custom profiles tailored to a specific drug discovery programme (**Figure 15**).

Evotec (www.evotec.com) offers unique proteomics services to address key issues in drug and biomarker discovery. We continuously advance our capabilities in mass spectrometry-based proteomics to ensure unrivalled comprehensiveness and data quality when analysing cells, animal models and patient samples. KinAffinity® is Evotec's hit-to-lead compatible approach to profile drug candidates against endogenously expressed, full-length proteins in the presence of cellular co-factors and native complex partners – a major advantage over traditional biochemical panel screening using only recombinantly expressed, purified proteins or protein domains. Evotec's platform enables rapid target profiling of kinase inhibitors in cell and tissue samples. Unlike traditional biochemical kinase panel screening, the inhibitor's target affinities are determined simultaneously for a large number of native kinases within their physiological cellular environment. KinAffinity® identifies target interactions possibly requiring additional co-factors or the formation of multi-component complexes, thereby complementing traditional biochemical assays that use only recombinant proteins.The platform can determine a target's Kd value to a free compound without needing to immobilise the inhibitor. It employs a ready-to-use affinity matrix comprising well-characterised broad-spectrum kinase inhibitors to enrich the subproteome of endogenously expressed kinases of cells or tissues (**Figure 16**).

Kinexus (www.kinexus.ca) offers a variety of kinase profiling services designed to measure the effects compounds and drugs have on kinase networks within living cells (*in vivo*) or individually in enzymatic assays (*in vitro*). The Kinex™ Antibody Microarray and Kinetworks™ Immunoblotting Screening services evaluate how specific compounds, hormones or other treatments affect the regulation of the kineome within

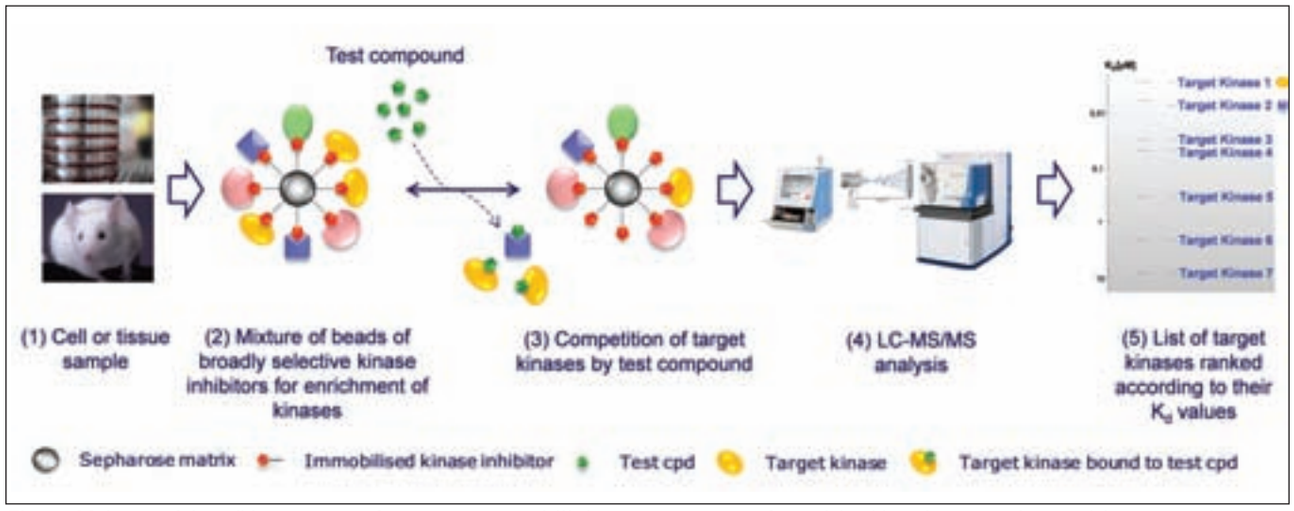


Figure 16: Lysate of the cell line or tissue of interest is prepared (1). A matrix comprising of immobilised broad-spectrum kinase inhibitors is used to enrich the expressed kinome of the cell or tissue (2). Competition assays with the free test compound (3) and quantitative mass spectrometry analysis (4) reveal the compound's affinity profile, ranking all protein kinase targets according to their Kd values to the free compound (5)

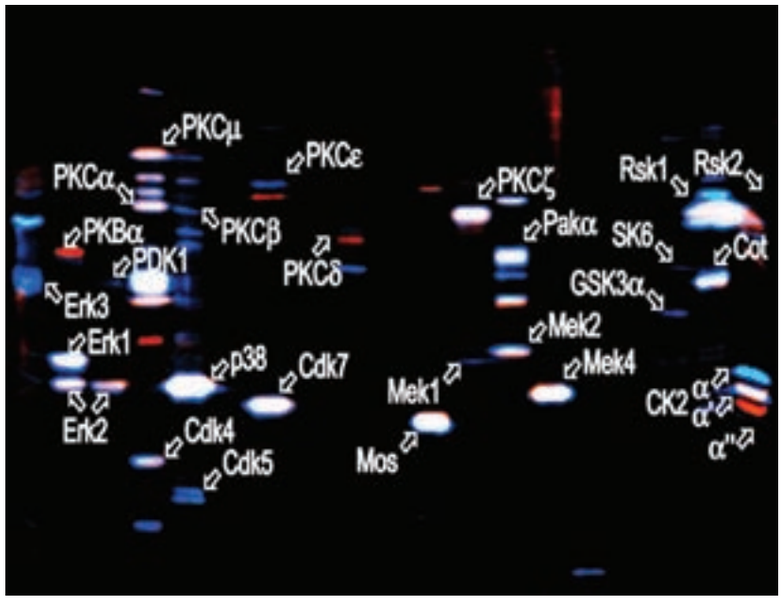


Figure 17: Overlaid images of two Kinexus Kinetworks™ Protein Kinase (KPKS 1.2) screen multi-immunoblots of mouse 32D myeloid cells treated with and without interleukin 3. Migration positions of detected target protein kinases are shown. Increased protein expression with IL3 treatment appears red, decreased expression shows blue and unchanged proteins appear white

living cells or tissues. The microarray (KAM-850) service utilises 850 antibodies of which 466 target the expression levels and phosphorylation states of protein kinases. The other 384 antibodies monitor potential changes in protein phosphatases and regulatory subunits of protein kinases and other cell signalling proteins. The immunoblotting services quantify the expression levels of 76 protein kinases (KPKS 1.2) or 40 phosphorylation sites in protein kinases (KPSS 11.0) or substrates of kinases (KPSS 12.1). These *in vivo* services also provide detailed information on the upstream and downstream regulators that are targeted and affected by protein kinases in response to various treatments. The Inhibitor Compound Profiling (KICP) and the Substrate Profiling (KSPS) services measure the enzymatic activities of more than 400 active recombinant protein or lipid kinases with compounds and protein/peptide substrates *in vitro*. With the KICP service, varying concentrations of compounds or levels of ATP can be used to measure the inhibitory effects against a range of kinases. The DrugKiNET database (www.drugkinet.ca) is a free resource available to query for the prediction, identification and characterisation of known inhibitors of protein kinases. With the KSPS service, any protein or peptide can be measured for its ability to act as a substrate for specific kinases. The PhosphoNET database (www.phosphonet.ca) provides experimental and predictive information on the phosphorylation of 21,000 human proteins at 950,000 phosphosites by 500 protein kinases (**Figure 17**).

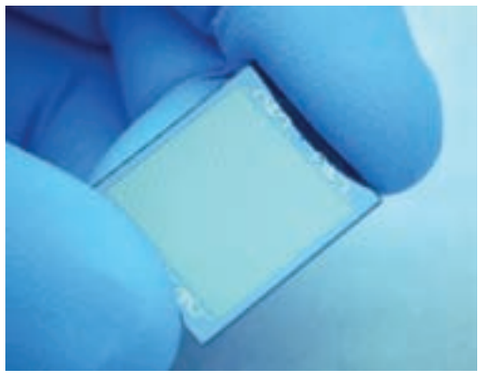


Figure 18: G4.1 Biochip – The PepArray™ technology consists of three components: a novel solution photochemistry that uses photogenerated reagent precursor (PGR-P) and conventional compounds for synthesis; digital photolithography that uses a graphic projector to direct photoreactions on chip; and this microfluidic device that provides picolitre scale reaction chambers (picoarray reactor) to allow parallel chemical and biochemical reactions

LC Sciences (www.lcsciences.com) provides a comprehensive kinase selectivity profiling service utilising high density protein kinase substrate peptide microarrays based on its proprietary PepArray™ technology for proteomic scale kinase substrate profiling, measurement of kinase kinetic activities, and drug discovery research. A proprietary microarray platform and advanced microfluidic technologies enable massive parallel, synthesis of thousands of customer specified peptides on a 1cm^2 biochip. Through the use of these peptides as specific kinase substrates on an addressable peptide microarray, LC Sciences can characterise kinase kinetics and determine relative enzymatic reaction rates for each specific substrate. Profiling kinase selectivity on a high-density peptide microarray offers the opportunity to study tens of thousands of specific substrates in a single experiment. Bulk entry of custom sequences provides the flexibility to synthesise completely customer designed arrays quickly and therefore study the selectivity of a wide range of kinases for, not only literature reported, but also potential novel substrates. Customers can send their active kinase sample along with specific sequences to LC Sciences' labs in Houston, TX. Its service includes all sample prep and handling,

microarray synthesis, all enzymatic reactions, specific detection of phosphorylation, image extraction and data analysis. It sends a fully analysed data report in about two to three weeks and can provide post-experiment technical support to ensure researchers can extract meaningful results from their microarray data (**Figure 18**).

Since 2004, **Life Technologies** (www.lifetechnologies.com/discoveryservices) has completed more than 10,000 kinase profiling projects for drug discovery researchers around the world with a 99.5% on-time delivery rate. Through its SelectScreen® Services, Life Technologies provides screening, profiling and custom assay development services for multiple target classes and has the capacity to manage up to one million compounds for library screening projects with an capability to screen upwards of 200,000 wells per day. Recent infrastructure enhancements have given Life Technologies the ability to offer full lead optimisation services to clients, while accelerating kinase profiling delivery times to an average of 3.6 days. Additionally, Life Technologies has introduced a new real-time data transfer capability that provides clients with data as it becomes available; rather than waiting for full data delivery at the end of the project. This enables medicinal chemists to access data more rapidly to make downstream decisions. A new online ordering system enables scientists to more efficiently place, track and save orders for any SelectScreen® Service. In addition to the 319 biochemical assays available for kinase profiling, Life Technologies also offers cellular profiling and recently added 13 LanthaScreen® cell lines to its cellular profiling services. For a menu of Life Technologies profiling and screening services, go to Lifetech's website (**Figure 19**).

Figure 19: Life Technologies offer screening and profiling through its SelectScreen® Services

Luceome Biotechnologies (www.luceome.com) enables drug discovery by profiling potential inhibitors against protein kinases in cell lysates. Luceome's proprietary technology utilises luciferase fragment complementation, in which re-assembly of a split-luciferase enzyme in cell lysates leads to bioluminescence. In Luceome's KinaseSeeker assay, the reassembly of luciferase fragments is mediated by the interaction of a protein kinase with an active site-directed chemical inducer of dimerisation (CID). Addition of a kinase inhibitor results in the displacement of the probe, resulting in dissociation of the luciferase fragments and a corresponding loss in luminescence. Luminescence readout translates into a highly sensitive and robust assay (Z' = 0.78) with low background and minimal interference from test compounds. KinaseSeeker enables rapid profiling and identification of both active site-directed and allosteric inhibitors. Luceome has assembled a panel of 213 kinases comprised of wild type, drug-resistant and clinically relevant mutants. These are available as multiple options to researchers; a) a complete panel, priced at $2,151, for those seeking a comprehensive profile, b) three preset 50-kinase panels, priced at $505, for those seeking an affordable profile for their compound against different kinase groups and, c) à la carte service, priced at

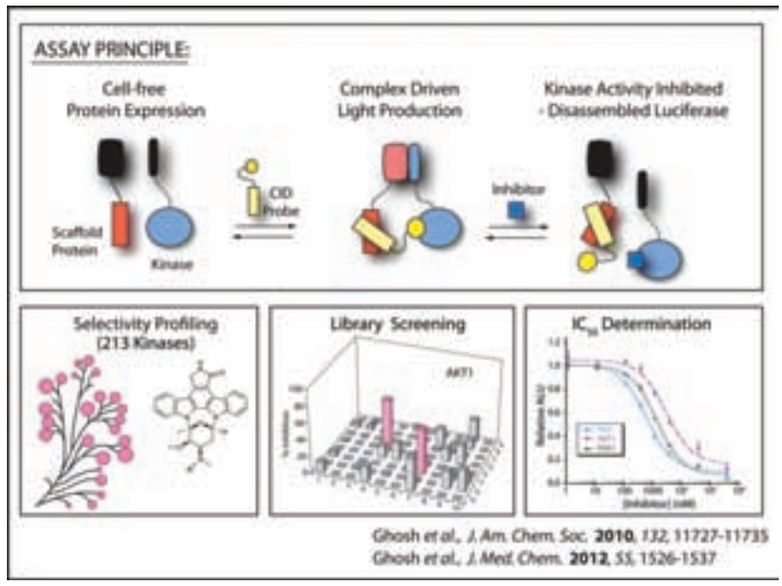


Figure 20: Luceome's KinaseSeeker Assay and available screening options

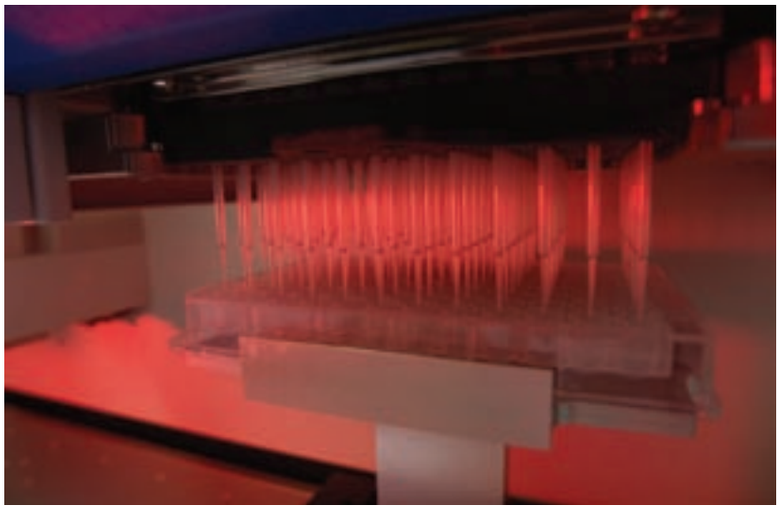


Figure 21: Precision replication of samples on 96-well plates prior to screening against enzyme panels using a PlateMate plus liquid handler at the International Centre for Kinase Profiling in Dundee

$11/kinase, for those that prefer to build their own kinase panel. In addition, Luceome provides a custom assay development service to customers who wish to use the luciferase fragment complementation assay for screening inhibitors in-house (**Figure 20**).

The **MRC Protein Phosphorylation Unit** at Dundee (www.kinase-screen.mrc.ac.uk) pioneered analysis of the selectivity of protein kinase inhibitors, setting up the first service to tackle this problem in 1998. 'Kinase Profiling' proved to be of great help to the pharmaceutical industry, speeding up the development of specific protein kinase inhibitors with therapeutic potential. The International Centre for Kinase Profiling offers a variety of services. MRC-PPU Premier Screen profiles compounds against an extensive panel of 140 enzymes. This screen is undertaken once every three weeks and the data is returned two weeks from the screen start date. MRC-PPU Express Screen screens compounds against a subset of 50 kinases representing all areas of the human kinome. This screen is undertaken once every three weeks and the data is returned one week from the screen start date. A Lipid Kinase Screen launched in July 2012 screens compounds against a panel of 16 lipid kinases and is undertaken once every three weeks with the data returned one week from the screen start date. If follow up data is required on a hit from a previous screen compounds can be submitted to its IC_{50} Determination Service, or to find a substrate for an enzyme we offer a Substrate Screen service. Finally, to determine if a compound is ATP competitive it can screen at fixed concentration against varying ATP concentrations. The International Centre for Kinase Profiling at Dundee offers expertise, experience, efficiency and excellence and is your ideal low-cost partner for profiling services (**Figure 21**).

Netherlands Translational Research Center BV (NTRC) (www.ntrc.nl) provides integrated kinase profiling services. Oncolines™ – NTRC's cancer cell line profiling service – are proliferation assays in 44 well-characterised human cancer cell lines. The cell lines are derived from diverse tumour tissue origins and represent the most important genetic driver mutations in human cancer. The Oncolines™ cell lines are cultured under conditions as recommended by the original investigators. NTRC has licensed these cell lines from the American Type Culture Collection (ATCC). After a client submits a compound to NTRC, duplicate dose-response curves are effectively determined. The Oncolines™ study includes free bioinformatics analysis of drug sensitivity markers using waterfall plot and volcano plot analysis. The response time for the entire study is four weeks. In addition, NTRC has set up a database of more than 100 reference compounds, including all marketed kinase inhibitor drugs that were profiled in Oncolines™. This database is an effective toolbox for NTRC's SynergyFinder™ service in which the optimal co-treatment with a client's compound is determined. SynergyFinder™ measures compound synergies and discriminates synergy from additivity. Dose-response matrix screening and determination of the Bliss-score of drug-drug combinations, is followed by curve shift analysis and determination of a Combination Index. Furthermore, NTRC provides the biochemical kinase assay profiling services of Carna Biosciences Inc to its customers in Europe. NTRC also provides Carna's reverse-phase protein array platform, which involves the microarray analysis of 180 disease-relevant phosphoproteins in cells (**Figure 22**).

PerkinElmer's (www.perkinelmer.com) updated RapidKinase Profiling Services offers 216 individual kinases covering the entire human kinome. The most recent development is panel expansion to include 24 human kinases from previous offerings. Assays are performed on the PerkinElmer EZReader utilising ProfilerPro technology where measurement of phosphorylated versus unphosphorylated substrate are separated by charge diffusion

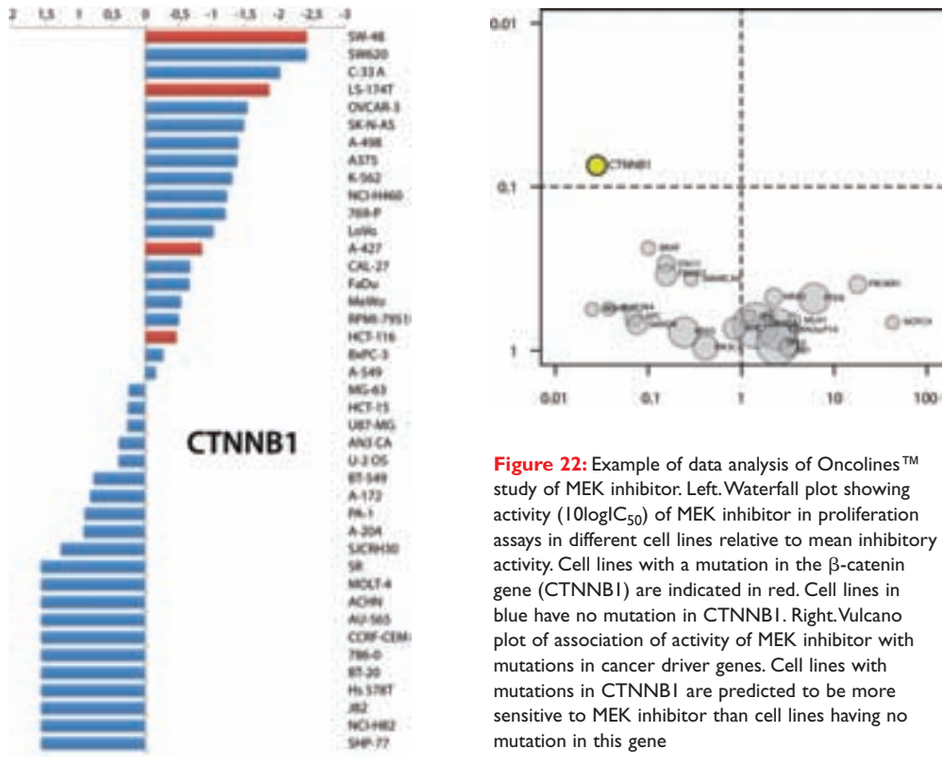


Figure 22: Example of data analysis of Oncolines™ study of MEK inhibitor. Left. Waterfall plot showing activity ($10\log IC_{50}$) of MEK inhibitor in proliferation assays in different cell lines relative to mean inhibitory activity. Cell lines with a mutation in the β-catenin gene (CTNNB1) are indicated in red. Cell lines in blue have no mutation in CTNNB1. Right. Vulcano plot of association of activity of MEK inhibitor with mutations in cancer driver genes. Cell lines with mutations in CTNNB1 are predicted to be more sensitive to MEK inhibitor than cell lines having no mutation in this gene

and detected separately by laser-induced fluorescence. Because no antibodies are used, both protein and lipid kinases can be studied. With PerkinElmer's full flexibility RapidKinase screen, a client can submit compounds for testing any calendar day and choose a combination of any ProfilerPro Kinase plates that are offered. Additionally, PerkinElmer's RapidKinase Whole Panel programme is run on a monthly schedule for routine submissions and rapid turnaround. Options for follow up IC_{50} determination are available at the client's request (**Figure 23**).

Figure 23: PerkinElmer's EZReader utilising ProfilerPro technology

Determination of the inhibitory profiles of kinase inhibitors is crucial to track inhibition of desired and undesired combinations of kinases allowing the development of inhibitors endowed with a best possible ratio between therapeutic efficacy and unwanted side effects. Based on **ProQinase**'s (www.ProQinase.com) biochemical kinase profiling service packages, '300wt ProteinKinaseProfiler' and 'WholePanelProfilerPLUS' – currently comprising a panel of up to 387 recombinant human kinases – the selectivity may be assessed at highly competitive rates while guaranteeing a coverage of the human kinome by more than 60%[2]. As revealed by HTStec's recent Kinase Profiling Trends 2013 report, the most preferred kinase profiling services comprise a set of 300-400 kinase targets including >30 disease-relevant mutants – features that are very well met by ProQinase's profiling packages. Furthermore, it is of utmost importance that screening services offered

generate highly reproducible data. ProQinase has validated the robustness of its kinase activity-based gold-standard radiometric platform. Multiple-rounds of independent testing against 352 protein kinases demonstrated that more than 90% of all single values of five approved kinase inhibitors did not vary by more than 15% of the respective mean value. In addition to large panel biochemical profiling, assays allowing the determination of inhibition of a given target kinase in cells are highly demanded. However, due to the complex nature of cellular systems, the establishment of cellular kinase-specific phosphorylation assays can be challenging and time-consuming. ProQinase does not only offer established cell-based phosphorylation assays for 30 different kinases but also offers its acquired scientific experience in this field for custom-tailored developments of new kinase specific cellular phosphorylation assays (**Figure 24**).

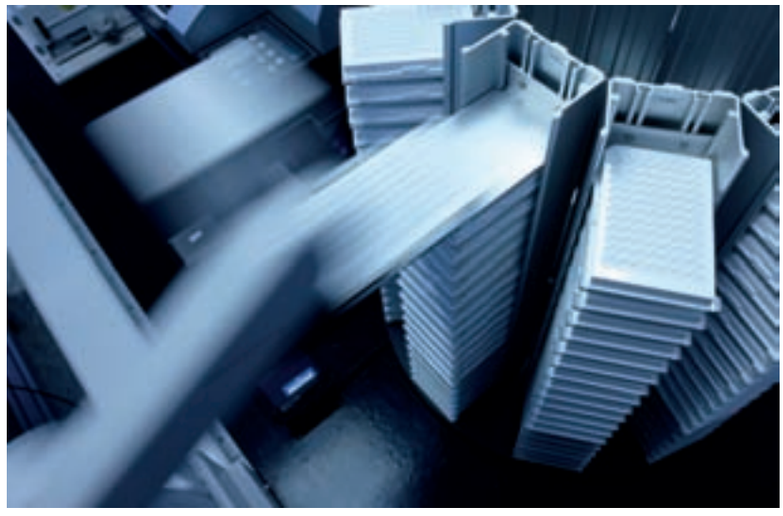


Figure 24: ProQinase kinase profiling service currently offers fully automated processing for 387 different protein kinase activity assays

Reaction Biology Corp (RBC) (www.reactionbiology.com) offers the largest collection of kinases available for activity-based profiling and HTS, including 342 wild type, 85 mutant, 19 atypical and 17 lipid kinases. RBC's new Hotspot Technology allows it to use nanolitre screening techniques to make this p33 gold standard assay format affordable. Its key activities include: HTS, IC_{50} determinations, selectivity profiling, customer-based assay development, chemical library screening/collaboration and cell-based assays. In addition, RBC has discovered a way to offer an inexpensive monthly full panel to its clients; comprised of 457 kinases in total. In today's drug discovery practices, screening a large library of compounds against one kinase in hopes of identifying new hits has been abandoned. A more effective compound centric approach has been adapted, in which screening a small focused compound library against a large panel of kinases in parallel can be a more effective way of identifying potent and selective inhibitors for multiple kinases. By using this approach, along with the largest active kinase panel available to the industry, RBC has successfully identified many new activities from 278 well studies of kinase inhibitors[3]. RBC's full panel was named the most used full panel in the industry by HTStec's Kinase Profiling Trends 2013 Survey. RBC was also given the highest number of positive rankings of any CRO in the kinase profiling industry in the same survey (ranked highest in responsive customer service, good turnaround time and low cost). RBC's Hotspot technology has also been successfully used for a broad offering of epigenetic targets (**Figure 25**).

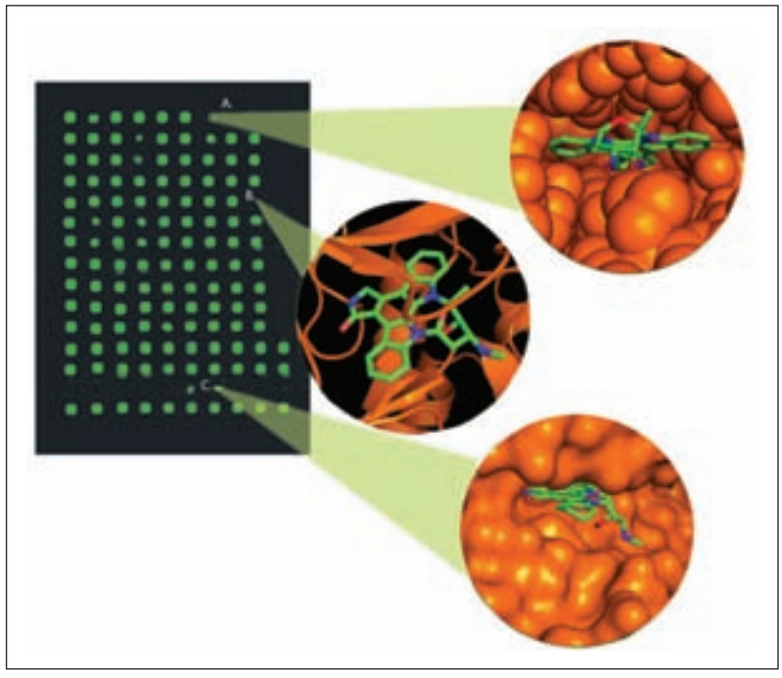


Figure 25: Reaction Biology's Miniaturized HotSpot Technology: All kinase reactions are performed by using the gold standard radioisotope filter binding format, the hot radioisotope signals are transformed to fluorescence for detection. Each fluorescent spot represents a particular enzyme reaction, and the total fluorescent signal of each spot is reflecting the inhibited potency of a particular small molecule. The brighter of a spot, the weaker the inhibition. A complete inhibition will reduce the fluorescent signal to the background level. For example, Spot B is a complete inhibition comparing to Spot A and C, which are partial inhibitions. The three images on the right side are simple demonstrations of the kinase and small molecule interactions

Figure 26: SignalChem's cost-effective approach to kinase profiling discovery in a time-sensitive manner

SignalChem's (www.signalchem.com) compound selectivity profiling service utilises a large and diverse panel of highly active protein kinase, phosphatase, phosphodiesterase and histone deactylase enzyme targets that are produced in its manufacturing facility. SignalChem's active enzyme targets are subjected to rigorous quality control analyses and are extensively assayed against a panel of biologically relevant substrates ensuring that all reactions are performed under optimal assay conditions. SignalChem's profiling service determines the respective inhibitory profile and the putative mechanism of action. All compounds are profiled against a panel of targets either using an individual dose, single or multiple concentrations in order to allow in-depth IC_{50} determinations. In addition, the protein kinase assays can be performed under varying ATP concentrations to evaluate the competitive effects of ATP. The compound selectivity profiling service offered by SignalChem is a very economical and convenient approach to the drug discovery continuum with a two-week turnaround of your specific profiling results. Compounds are screened either with the direct radiometric assay method or the indirect ADP-Glo method and SignalChem maintains all information under the strictest confidentiality. Upon completion, all materials will be either returned to the client or disposed of accordingly. The selectivity profile of any small molecule is of fundamental clinical importance as part of the drug development process. Information obtained from these studies performed by SignalChem will undoubtedly provide a useful insight into the proposed mechanism of action of a given compound as well as leading to the identification of 'off' target effects, thus leading to the selection of better lead candidates (**Figure 26**).

Discussion

As evidenced by the large number of service providers that contributed summaries of their offerings to this article, kinase profiling services is perceived to be a key research area and one where outsourcing can add real value.

All providers which offer conventional biochemical profiling seek to compete on the size and coverage of their kinase panels, speed of turnaround and cost of their service. What differentiates them are their core assay technologies (eg Reaction Biology's radiometric p33 gold standard assay format, DiscoveRx's proprietary active site-directed competition binding assay, PerkinElmer's mobility shift assay of phosphorylated from unphosphorylated substrate, Cisbio's HTRF KinEASE used by multiple providers, Eurofins' use of an ADP readout to develop kinase assays for which no antibody exists, etc); their ability to rapidly determine ATP competition, an inhibitor's binding mode and association/dissociation kinetics; how they sell or package selected elements of their entire kinase panel; and those add-on service components that aim to make a specific offering standout (eg Life Technologies has introduced a new real-time data transfer capability that provides clients with data as it becomes available; EMD Millipore is currently the only provider of a catalytic assay for ATM kinase). In addition to their biochemical offerings, most providers complement this service by offering cell-based kinase profiling assays where cellular kinase-specific phosphorylation is measured. Assays allow for confirmation of inhibitory activity in a relevant cellular background and profile their selectivity against multiple signalling pathways. The numbers of specific cellular kinase assays available is only a small proportion of most providers' full panel and is quite variable between providers, reflecting the complex nature of the cellular systems, and the challenges involved in developing these assays. Some providers have chosen to

focus their cell-based offering on specific targets, eg NTRC's Oncolines™ which is profiling in human cancer cell lines.

What is interesting are the number of newer entrants in the kinase profiling arena now offering *in situ* profiling alternatives to the conventional biochemical services. These are mostly based on unique or proprietary technologies enabling the analysis of kinases in cell lysates, living cells, tissues and even living animals. Two mass spectrometry-based proteomic approaches are described. KiNativ from AxtiveX Biosciences leads to both the identification and quantitation of probe-labelled peptides. The method can be used to profile inhibitors by measuring the ability of inhibitors to compete with probe-labelling. This approach can uniquely correlate target engagement (fraction of kinase inhibited) with cellular efficacy. KinAffinity® from Evotec uses a matrix comprising immobilised broad-spectrum kinase inhibitors to enrich the expressed kinome of the cell or tissue. Competition assays with the free test compound and quantitative mass spectrometry analysis reveal the compound's affinity profile and ranking against all protein kinase targets. Three other approaches use protein microarrays. NTRC provides Carna's reverse-phase protein array platform, a phosphoproteome technology which involves the microarray analysis of 180 disease-relevant phosphoproteins in cells. LC Sciences utilises high density protein kinase substrate peptide microarrays for proteomic scale kinase substrate profiling and measurement of kinase kinetic activities. The Kinexus microarray and immunoblotting screening services evaluate how specific compounds, hormones or other treatments affect kinase networks and the regulation of the kineome within living cells or tissues (*in vivo*) or individually in enzymatic assays (*in vitro*). Finally another approach from Luceome Biotechnologies enables drug discovery by profiling potential inhibitors against protein kinases in cell lysates.

In summary, conventional biochemical kinase profiling is a mature marketplace with relatively little new innovation over recent years. It is a highly competitive marketplace with respect to price and service. Most changes have been in the addition of new kinases to panels, in the refinement of the screening/ordering process, in the expansion of follow-up activities and in bringing on stream new cellular kinase assays. In contrast, adoption of the emerging *in situ* kinase profiling alternatives has been slow and this area still represents something of a niche market. What is increasingly evident is that the enzymatic diversity available, the technical skills (eg in sourcing and maintaining enzymes, and efficiently performing profiles across the entire kinome) and the scientific expertise residing in fee-for-service providers (most of them ex-Pharma employees) now exceeds the capabilities of all but the largest in-house profiling operations and can add real value to most kinase programmes. The rationale for outsourcing kinase profiling has never been greater. DDW

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