expanding the profile of KINASE PANELS

With little sign of a decline in Pharma interest in kinase targets, greater emphasis is now being directed towards kinase selectivity profiling. A recent industry market survey suggests that although the market for kinase primary screening reagents is estimated to grow at an annual rate of 15%, that for outsourced kinase profiling is expected to reach 49% by 2005. Increasing competition among fee-for-service providers has seen a marked expansion in the number of kinases offered in profiling panels and greater flexibility in terms of user defined assay parameters. This review takes a look at some of the latest panel offerings and highlights some new tools that will bring enhanced support to the identification of kinase substrates, kinase assay development and kinase profiling in-house. The expectations of scientists engaged in kinase panel profiling are still, however, largely unmet with most seeking still wider coverage of the kinome and a single generic assay format for all kinases.

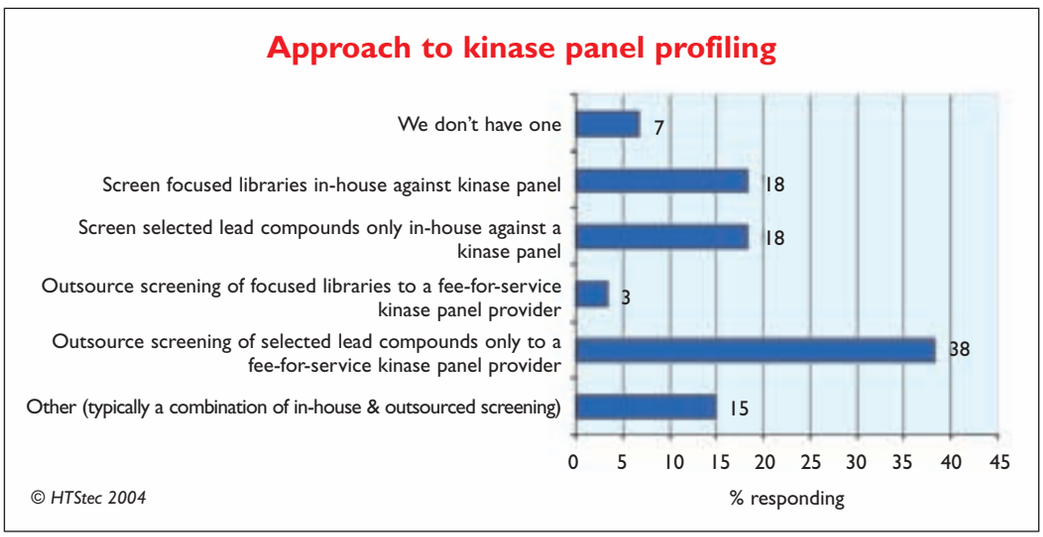
By Dr John Comley

Awareness in the breadth and diversity of kinase targets was enhanced when it was revealed that 518 kinases are encoded in the human genome (the human kinase super family is now commonly referred to as the kinome)[1]. Nearly all aspects of cell life are controlled by reversible phosphorylation of proteins mediated by protein kinases and abnormal phosphorylation is a cause or effect of many diseases. As a consequence many pharma and biotech companies continue to undertake primary screening against kinase targets believed to be clinically important. A significant step in the optimisation of a kinase lead is the determination of the comparative selectivity of those leads against other kinase enzymes. This so-called kinase profile is typically performed against a panel of kinase enzymes, most often drawn from closely-related members of the kinase super family with occasional representation from other families. This approach facilitates the development of inhibitors with enhanced potency against the selected kinase(s) targets. In addition, it may result in the identification of possible novel selectivity against unexpected kinase targets. As more pharma focus on the screening of related target families in parallel across therapeutic area boundaries, greater importance is being given to the early determination of selectivity profiling. This has led to a rapid increase in the number of companies now offering outsourced kinase profiling on a fee-for-service basis and in the availability of new tools to assist kinase profiling in-house. In this article we review some the main findings of a recent market study on kinase panel profiling, compare offerings in the ever expanding outsourced profiling sector and discuss some of the latest kinase profiling tools.

Approach to kinase profiling

A recent global market survey of pharma and biotechs (HTStec's Kinase Panel Profiling Trends 2004) showed that the preferred approach to kinase panel profiling chosen by the majority (38%) of survey respondents was to outsource the

Figure 1



screening of selected lead compounds to a fee-for-service kinase panel provider (**Figure 1**). This approach was particularly favoured by small pharma and biotechs which often lack the resources to set up their own kinase profiling panels. In-house profiling activities were split equally (18% respondents to each) between screening selected lead compounds or screening focused libraries against a kinase panel, with big pharma showing a slight preference to screen focused libraries against a kinase panel. Overall there was minimal interest (4%) in outsourcing the screening of focused libraries to a fee-for-service kinase panel provider, although perhaps Upstate's newly launched KinaseProfiler™ – FP (described in **Table 3**) may impact on this activity. A proportion (15%) of all labs surveyed indicated an interest in alternative hybrid approaches, including a mixture of various in-house and outsourced strategies.

Preferred assay technology

As can be seen in **Table 1** no one assay detection technology stands out clearly as being preferred by the majority of respondents for in-house kinase

Table 1: Assay detection technology used in kinase profiling

PREFERRED ASSAY DETECTION TECHNOLOGY (% RESPONDING)[1]	IN-HOUSE	OUTSOURCED
SPA (Radiometric)	6	11
LEADseeker™ (Radiometric)	6	2
FlashPlate® (Radiometric)	13	2
Image FlashPlate (Radiometric)	0	2
Radiometric Filter (eg ^{33}P incorporation)	11	26
Other Radiometric Assays	2	0
FI (Fluorescent Intensity, includes fluorescent quench & microfluidic-chip assays based of fluorescent labelled peptidic substrates)	6	4
FP (Fluorescent Polarisation)/Antibody	13	8
FP (Fluorescent Polarisation)/Ligand (ie NO antibody)	7	4
FRET (Fluorescent Resonance Energy Transfer)	4	0
TR-FRET (Time Resolved FRET eg LANCE™ or HTRF®)	13	2
TRF (Time Resolved Fluorescence eg DELFIA®)	0	0
FLT (Fluorescence Lifetime)	0	0
AlphaScreen™	7	0
ECL (ElectroChemiLuminescence)	2	0
Glow Luminescence	2	0
Other (includes ELISA)	9	0
We don't have one – go with CRO's recommendation	n/a	40

[1] *data from HTStec's Kinase Panel Profiling Trends Report 2004*

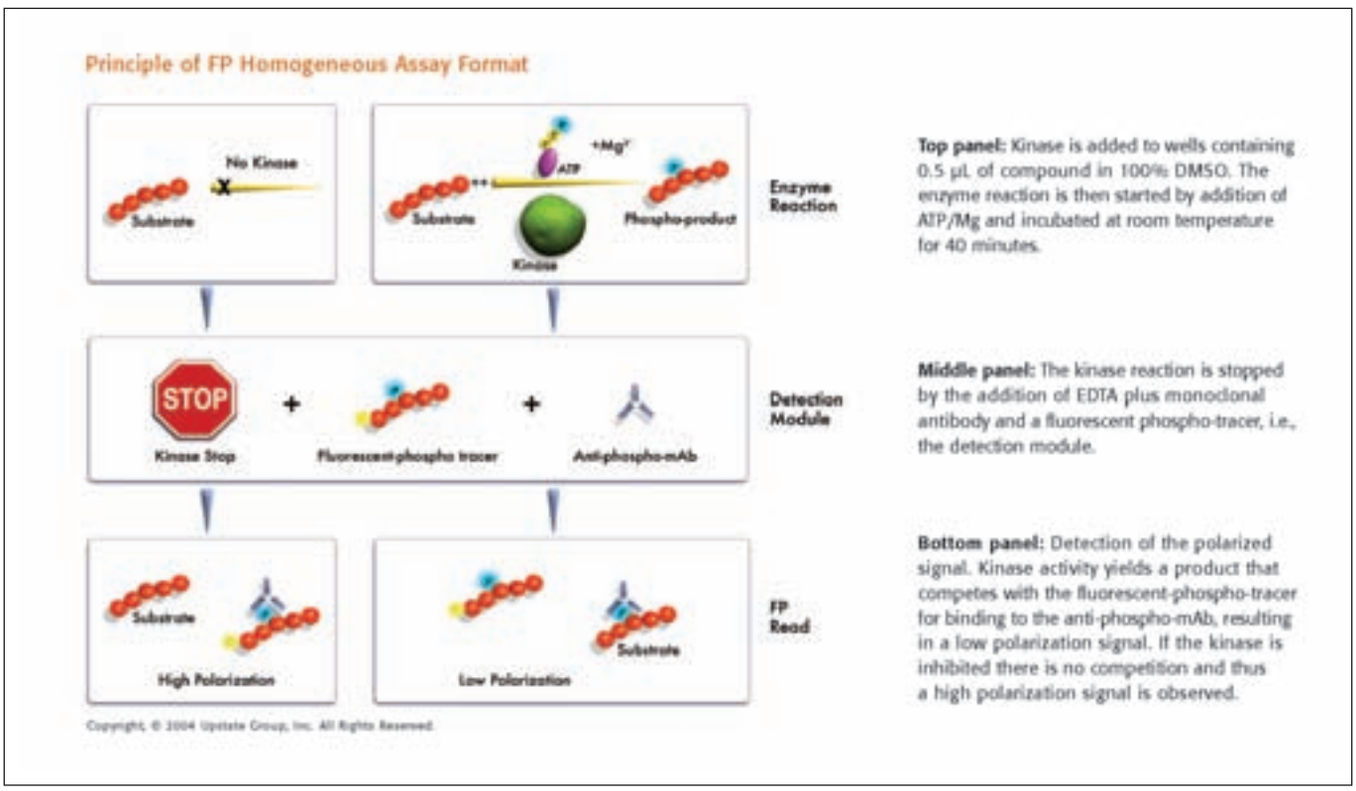


Figure 2

panel profiling, although most interest was shown in Fluorescence Polarisation (FP, with an antibody) (**Figure 2**), Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET, eg HTRF® or LANCE™) and FlashPlate® (radiometric). In contrast, most respondents (40%) did not have a preference for assay technology used in outsourced profiling and are happy to go with the CRO's recommendation, ie express their preference through the selection of the CRO, as most only offer one or at best two core assay technologies. Of the named assay technologies radiometric filter binding (eg ^{33}P incorporation) was the preferred (26%) outsourced assay technology among respondents. This

Figure 3

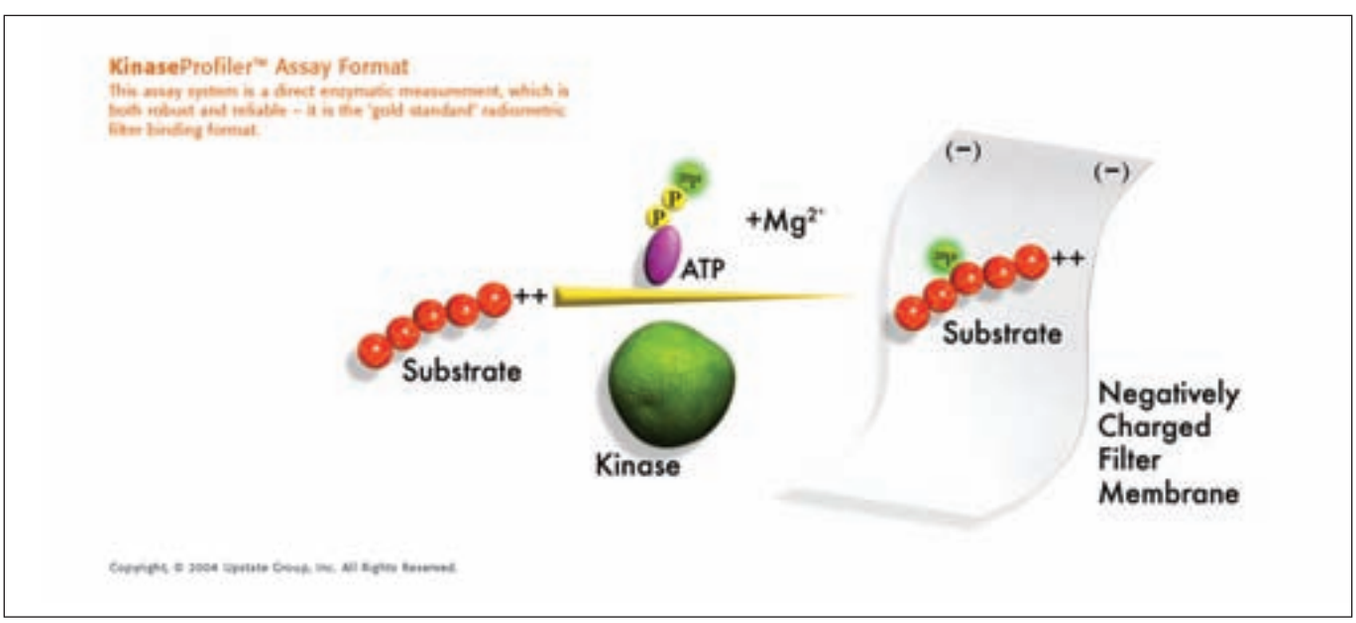


Table 2: The kinase profiling market

PHARMA KINASE PROFILING STATISTIC[1]	IN HOUSE	OUTSOURCED
Market size 2004 ($m)	$12.2m[2]	$16.3m[3]
CAGR (% market 2004)	34%	49%
Average price paid per profiling data point	$0.84	$17.85
Average number of profiling data points per lab/year	262,000	23,000
% singlet data points profiled	47%	2%
% duplicate data points profiled	15%	88%
% full dose response data points profiled	38%	10%

1 data from HTStec's Kinase Panel Profiling Trends Report 2004
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

assay system, which is used in Upstate's KinaseProfiler™ (**Figure 3**) and by ProQinase is considered to be the 'gold standard', although owing to its reliance on radioactivity many labs are restricted from using this approach in-house today.

Kinase profiling market opportunity

In **Table 2** we list some of the kinase profiling statistics which were estimated in HTStec's recent market survey. The overall market for outsourced profiling ($16.3 million in 2004) is not particularly large considering the level of current vendor interest, however strong market growth is predicted with an annual growth rate of 49% in 2005. In contrast, the market for the primary screening of kinases (reagent proportion only) was estimated to have reached $73.9 million with an annual growth rate of 15% in 2005. The average price paid per outsourced profiling data point ($17.95) is low compared to the starting point prices advertised by vendors for a limited number of data points. On average about 10x more data points are screened in-house versus outsourced for kinase panel profiling. Most of the profiling data points outsourced (88%) are duplicates, while in-house most of the profiled data points are singlet (47%) or full dose response curves (38%).

Fee-for-service kinase profiling offerings

Seven companies currently offering outsourced kinase profiling services are listed in **Table 3** together with details of their product offerings. It is evident that this is a constantly changing field, with all vendors predicting rapid expansion in the size of their kinase panel and increasing representation across all sub families (branches) of the kinome. Upstate estimates its panel will increase at the rate of about eight kinases per month. Overall coverage as a percentage of the total kinome (518 kinases) is still relatively poor. **Figure 4** illustrates Upstate's current coverage of about one quarter of the kinome pie and its split into

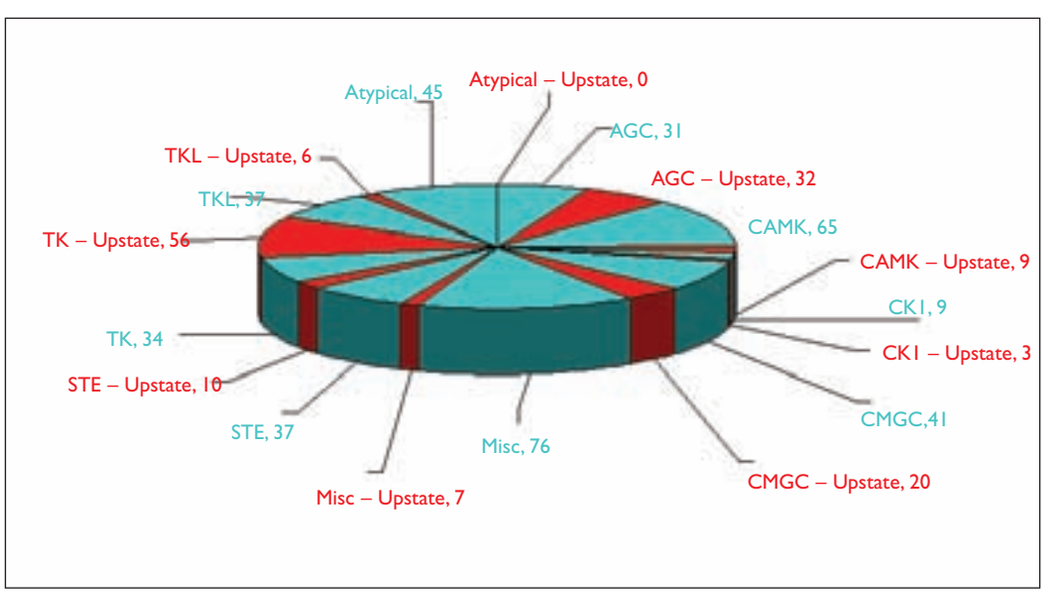


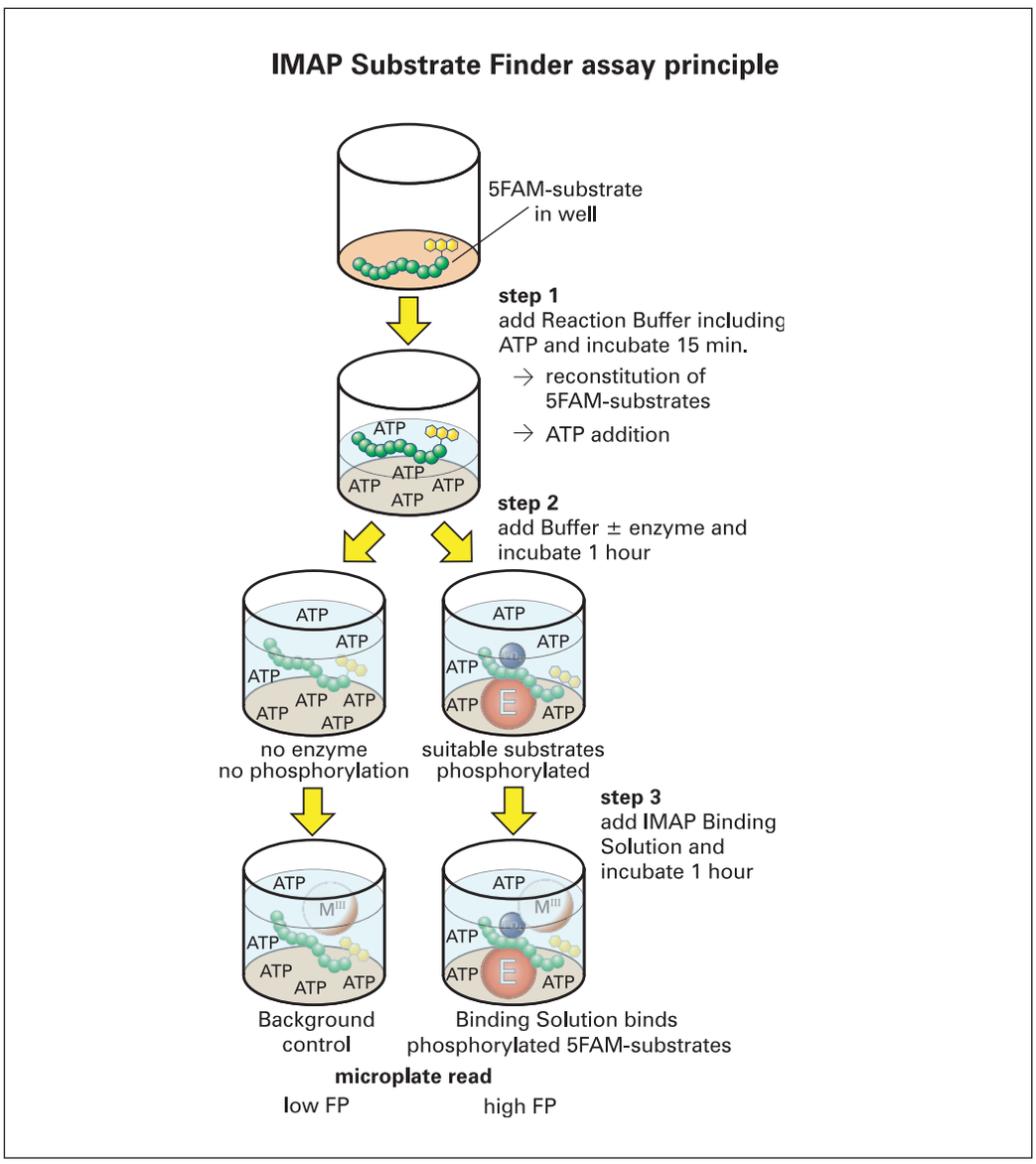
Figure 4
The entire kinase super family[1] represented by a pie chart (518 kinases). Each family is shown by the number of kinases that Upstate has so far developed assays for (in red) and the remaining number of family members (in green). Upstate's coverage is AGC – 51%; CAMK – 12%; CK1 – 25%; CMGC – 33%; Misc – 8%; STE – 21%; TK – 62%; TKL – 14% and Atypical – 0%

Table 3: Comparison of fee-for-service kinase panel profiling offerings

COMPANY	WEBSITE	SERVICE (PRODUCT) NAME	NO. KINASES IN PANEL[1]	PLATE FORMATS	ASSAY TECHNOLOGY USED	COMMENTS ON PRODUCT OFFERING
Carna Biosciences	www.carnabio.com	Activity-Based Profiling	Under development	384 well	Plans to offer 62 tyrosine kinases by ELISA, a further 8 serine/threonine kinases will also be available using IMAP™, AlphaScreen™ and TR-FRET platforms	Developing service/drug discovery company focused on kinase-mediated signalling pathways, plans to offers services in kinase profiling and assay development for kinase HTS
Cerep	www.cerep.com	High Throughput Compound Profiling (HTP) High Throughput Compound Screening (HTS)	Under development	96 & 384 well	Plan to offer 52 kinases by the end of 2004 (34 serine/threonine and 18 tyrosine kinases) with the aim to reach 100 kinases by the end of 2005, using a single technology (HTRF®) and a generic assay format for all assays to allow reliable data comparison	Part of Cerep Discovery™, a very broad service offering in most areas of drug discovery
Invitrogen	www.invitrogen.com	SelectScreen™ Kinase Profiling Service	61	384 well	Z'-LYTE™ Kinase Assay Platform – ratiometric FRET assay	Based on Invitrogen's growing library of purified protein kinase targets and its proprietary Z'-LYTE technology, determinations performed at variable ATP concentrations
MDS Pharma Services	www.mdsps.com	FastKinase™ Profiling Service	35	96 & 384 well	Currently EIA based in 96 well plates, but are in the process of switching to FP in 384 well plates	Part of MDS Pharma's wider drug discovery offering, FastKinase is focused on rapid turnaround time (5 business days following compound receipt) with flexible procedures and assay parameters. Cell-based follow-up kinases assays are in development
Novascreen	www.novascreen.com	KinaseAdvisor™	60	384 well & Chip	Assay incubation in plates followed by separation of phosphorylated and unphosphorylated peptides on a microfluidic-chip, based on charge differences	Part of NovaScreen's wider Services offering, with focus on high levels of precision & repeatability enabling better comparison of results within the panel, spanning all 7 groups within the kinome
ProQinase	www.proqinase.com	Compound Selectivity Profiling	80	96 & 384 well	^{33}P PanQinase(r) Activity Assay – radiometric filter binding assay based on ^{33}P incorporation	Exclusive alliance with Cell Signaling Technology to provide HTScan Active Kinases, offers HTS and profiling including fluorescence-based protein kinase assays on request
Upstate	www.upstate.com	KinaseProfiler™	145	96 well	^{33}P Incorporation – direct enzyme measurement, based on the 'gold standard' radiometric filter binding assay	Market leader in cell signaling products and services, offering world's largest and phylogenetically most diverse panel of kinases, and highly flexible approach to assay parameters
		KinaseProfiler™ – FP	80	384 well	Fluorescence Polarisation (with antibody) – Upstate's proprietary FP homogeneous assay format	Service specifically designed for programs requiring higher throughput and larger datasets (eg chemical arrays or focused libraries), optional validation of FP interfering compounds (false positives) in Kinase Profiler™ assay. In addition the same FP reagents are also available for purchase

[1] *numbers are those given by the company at the time the article was submitted to press (September 6, 2004)*

Figure 5



the kinome sub-families. Since Upstate's panel is currently about twice the size of most of its competitors it is apparent that all vendors still have some way to go to enhance their coverage. **Table 3** also highlights that a range of different assay technologies are used in the various vendor offerings, which mirrors the results of HTStec's survey (see above) in terms of the preferred assay technology for kinase profiling. A few comments on the main features and benefits of the different offerings are also included in **Table 3**.

New kinase profiling tools

One of the main bottlenecks in the development of *in vitro* kinase assays is the identification of suitable peptide substrates for new kinases and their subsequent optimisation for HTS performance.

IMAP is a proprietary technology from Molecular Devices (www.moldev.com) that uses the specific binding of trivalent metal complexes to phosphate groups and fluorescence polarisation readout. In a microwell assay format, fluorescently-labelled peptides are phosphorylated in a kinase reaction. Addition of the IMAP Binding System stops the kinase reaction and specifically binds the phosphorylated substrates. The binding causes a change in the motion of the peptide, and results in an increase in the observed fluorescence polarisation. The IMAP™ Substrate Finder plate from Molecular Devices is a new IMAP platform assay development tool that significantly accelerates the identification of substrates for kinases. This plate enables the researcher to identify suitable substrates for a kinase

of interest in a few hours using an 'HTS friendly' assay platform. In a 384-well plate it has arrayed 59 fluorescein-labelled peptides, including controls, in quadruplicates in a ready-to-use concentration. The researcher adds ATP and +/- enzyme and incubates for up to an hour. The assay is completed by adding the IMAP binding solution and reading fluorescence polarisation (see **Figure 5**). The peptide substrates in this first plate target the CamK/AGC family of kinases. Further plates will include substrates for Tyr kinases and other Ser/Thr kinases from different parts of the kinome. The IMAP substrate plates also have other applications in the IMAP assay development process, these include: 1) profiling of substrate specifics of kinases; 2) comparison of substrate specifics of different mutants of one kinase; 3) finding substrate pairs that would be suitable for multiplexing with IMAP; 4) testing a compound against an entire set of substrate/kinase pairs; and 5) evaluating enzyme purity by comparing phosphorylation patterns. IMAP has now solved many of the early issues by including a binding buffer which gets around the tendency of acidic peptides to bind independently of phosphorylation, while also increasing maximum tolerated concentrations of ATP. Although IMAP is primarily used with peptide substrates, in some cases fluorescent-labelled proteins (eg Histone H1) have been shown to be useful.

Caliper LS LabChip 3000

Jerini Peptide Technologies (JPT, www.jerini.com/peptide) has developed peptide microarrays containing either annotated peptides derived from human phosphorylation sites or randomly generated peptides for the identification of kinase substrates and profiling kinase substrate specifics. Currently JPT offers a ready-to-use set of 720 biotinylated peptides from human phosphorylation sites for kinase profiling. JPT also recently established a collaboration with PamGene (www.pamgene.com) to develop a new technology for kinase studies. Using PamGene's 96-well three-dimensional microarray platform and JPT's kinase peptide substrates, it will be possible to generate kinetic read-out of multiple kinase activities in one experiment, or analyse multiple conditions on a single plate, eg compound dose-response curves. The high content and high quality kinetic data is designed to provide significant benefits to signal transduction-focused lead optimisation.

Cell Signaling Technology (www.cellsignal.com) offers a tyrosine kinase substrate screening kit that includes 95 different non-phospho-peptides as kinase substrates. Also included are biotinylated phospho- and non-phospho-peptides of identical sequence to serve as positive and negative controls respectively. Potential substrate phosphorylation is detected by a phospho-tyrosine specific antibody provided in the kit. The kit can be used in the development of kinase HTS assays, based on AlphaScreen™, DELFIA®, LANCE™ and HTRF® assay technologies. Cisbio (www.htrf-assays.com) is promoting HTRF® assay development technology as a reference solution for kinase screening through its recent agreements with Cell Signaling Technology and Upstate. Cell Signaling Technology will supply a select group of native antibodies and substrates that Cisbio will label and market for use in conjunction with HTRF®, Upstate will use HTRF® for its PI-3 kinase assay. Cerep also plans to make increasing use of HTRF® in its newly revamped kinase panel offering.

The LabChip 3000 Screening System (www.caliperls.com) provides an easy to use platform for Kinase Selectivity Screening in any drug discovery lab eliminating the need to send lead compounds to outside fee-for-service laboratories. The LabChip 3000 comes with standardised reaction conditions for a broad range of kinases, which allows direct data comparison within a panel. Application notes for more than 32 kinases assays are available today, with more kinases being regularly added to the portfolio. Assay development is simple on the LabChip 3000 system with a common set of buffers being used over a broad range of kinase assays all of which operate in a fixed ATP/Km ratio. Plug and play assay development software is provided to further define the optimal assay conditions for a particular enzyme and substrate. The microfluidic based separation assay has extremely low sensitivity to compound associated false positives and negatives, gives highly reproducible quality data with Z' values >0.95 and compound inhibition of <20% can be identified with high confidence[2]. This potentially allows for SAR generation directly from a primary screen, thereby avoiding the expense of secondary screening reagents and accelerating the overall lead development time.

Figure 6
The SpinX 'brick' containing 16 microfluidic 'tiles' stacked together to present the footprint and pitch of a conventional SBS plate. Each tile corresponds to the equivalent of thousands of pipetting steps to set up hundreds of different assays

SpinX Technologies (www.spinx-technologies.com) intends to bridge the gap between the low cost per data point of in-house profiling and the flexibility of outsourced profiling. To this end, SpinX Technologies is developing a turnkey bench-top automation solution for kinase profiling. The instrument relies on microfluidics to automate all steps involved in optimising and running assays, using 500nL final volumes in a closed system. Unique among microfluidic approaches, one type of chip can be configured via software for any assay protocol under any set of conditions.

All assay components – enzymes, detection reagents, buffers, and the compounds themselves – are dispensed only once into the microfluidic chip, using conventional liquid handling. The system performs all further operations, running unattended through dilution of compounds and reagents, combination of different kinases with different compounds, mixing, incubation for any length of time, and the fluorescence-based readout of the assay. Enabling this degree of flexibility is SpinX's programmable microfluidics, based on a novel valving system. The Virtual Laser Valve (VLV) uses a short pulse of focused laser light to perforate a thin foil separating two microfluidic structures. The two core functions of the VLV are metering of sub-microlitre liquid volumes with high precision and directing liquid volumes from any liquid container on the chip to another liquid container on the chip. By alternating these two functions, any assay protocol can be expressed in a sequence of valving operations, performed while the liquids are present in the microfluidic chip. The user defines the assay strategy and protocol, and dedicated software translates these instructions into the appropriate sequence of valving operations. Compatibility with conventional liquid handling instruments is ensured by SpinX's innovative interface: input wells on the edge of the microfluidic chip with dimensions identical to those of the wells in 1536 plates. Each chip corresponds to one row of a 1536 plate and chips are stacked together to form 'bricks' with the footprint of conventional plates (see **Figure 6**). SpinX Technologies has developed a prototype to validate the system for 500nL kinase assays, with fully integrated readout based on fluorescence polarisation. A bench-top instrument will be commercially available by the summer of 2005. In

References

1 Manning, G et el (2002). The protein kinase complement of the human genome. Science 298:1912-1934.

2 Pommereau, A et al (2004). Two simple and generic antibody-independent kinase assays: comparison of a luminescent and a microfluidic assay format. Journal Of Biomolecular Screening 9(5): 409-416.

the future, the system will be extended to other read-out modalities, as well as to other types of bio-assays, including cell-based assays.

Conclusions

It was clear from HTStec's survey that scientists engaged in kinase panel profiling think there is still room for improvement among the commercial offerings, in particular the following areas were highlighted:

- Wider coverage of kinome, ie panels should have better representation of kinases from the different branches of the kinome map as described by Manning et al (2002)[1].
- Single generic assay format for any kinase that has high sensitivity, is non-radioactive, preferably not requiring specific antibodies ie enabling standardisation with respect to assay conditions (Km of ATP, Km of substrate and same methodology for detection) and fast assay development.
- Better focused libraries to screen multiple kinases in parallel on as many kinases as possible.
- Better commercial substrate libraries for determining suitable ligands.
- A reduction in the price per data point for out-sourced profiling, particularly for dose response curves, and faster turnaround times.
- A turnkey assay system including detection technology, pre-dispensed reagents (enzyme, substrate etc), more user-friendly instrumentation (simple data capture, interpretation and processing) that would be directly applicable to almost any kinase, enabling simple and flexible running of your own kinase panel in-house.
- Assays that relate purified kinase activity with cellular potency. DDW

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. Further information on accessing the market report 'Kinase Panel Profiling Trends 2004' can be obtained by visiting www.htstec.com or e-mail surveys@htstec.com to receive a free copy of the Report's Executive Summary and Table of Contents.*