High Throughput Kinase Discovery, Inhibitor Screening and Specificity Analysis using Multi-Array Technology

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Abstract

We developed a high-throughput system for biological discovery and compound screening. A systematic and deterministic approach allowed us to categorize and quantify functional aspects of our library of 22,000 mammalian proteins (based on Unigene) coded by 50,000 individual full-length cDNA clones. Our methodology combined MSD's proprietary Multi-Array™ detection technology with high-throughput cell-free production, labeling, immobilization and purification of proteins. Here we report on several approaches we used to identify and characterize proteins with tyrosine kinase activity. First, we screened a set of 20,000 clones (14,000 unique Unigenes) for proteins with tyrosine kinase activity using a screening assay that simultaneously measured auto-phosphorylation and phosphorylation of a consensus tyrosine kinase substrate. The screen recovered known tyrosine kinases, previously unidentified kinases and, under low stringency conditions, proteins that recruited kinases. Second, we selected a redacted set of clones on the basis of homology to known kinases and demonstrated that we could identify ~ 70% of the expected tyrosine kinases in this redacted set. Third, we used MSD Multi-Array technology to test, in parallel, the specificity of 21 of these well-characterized kinases for a panel of known kinase inhibitors. Finally, we carried out a focused screen of 2,000 compounds for inhibitors of two of the identified kinases. The combination of high-throughput protein expression and Multi-Array technology allows for a unified approach to the discovery of biological targets and the identification of small molecules that regulate their activity.

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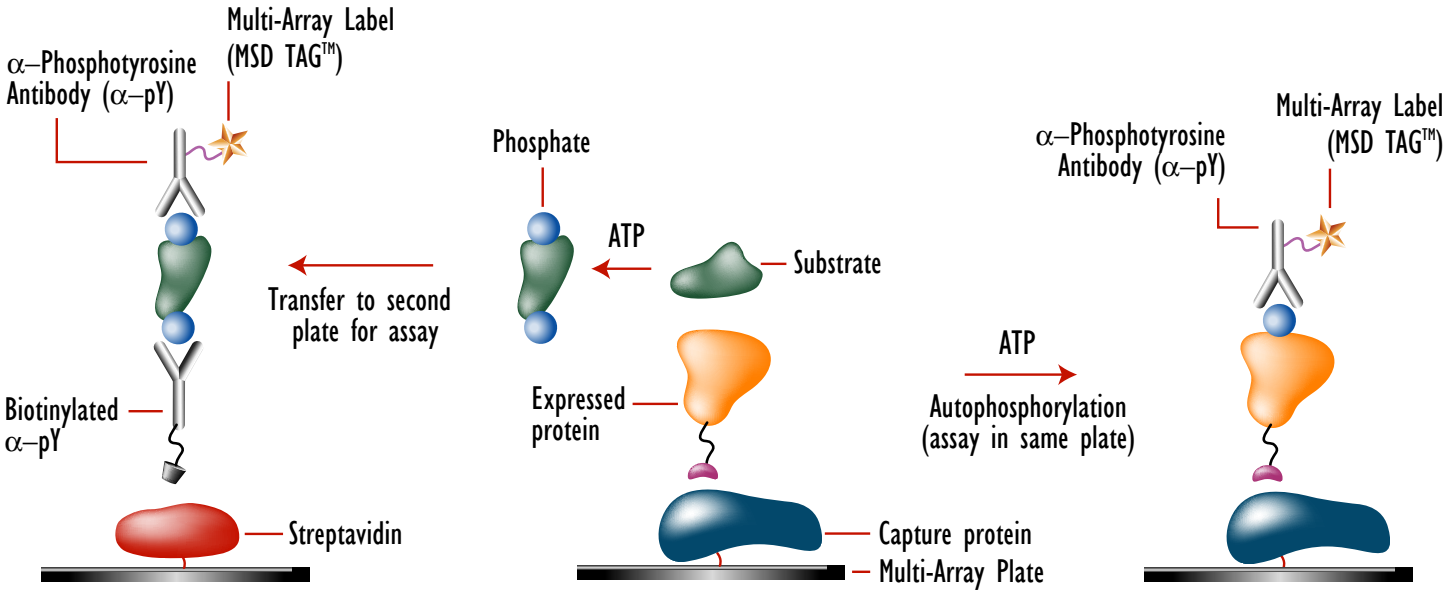
High Throughput In-Vitro Expression Screening for Protein Tyrosine Kinase Activity



Kinase activity assay

Kinase activity was determined by capturing the phosphorylated substrate with an anti-phosphotyrosine (α-pY) antibody and detection using MSD Multi-Array technology.

In-situ protein expression

cDNA clones

↓

Clones expressed and labeled in 96-well format

↓

Expressed protein immobilized on plate and purified

↓

Kinase phosphorylates consensus substrate or itself (autophosphorylation)

Autophosphorylation Assay

The extent of auto-phosphorylation of the immobilized tyrosine kinase was detected using MSD Multi-Array technology.

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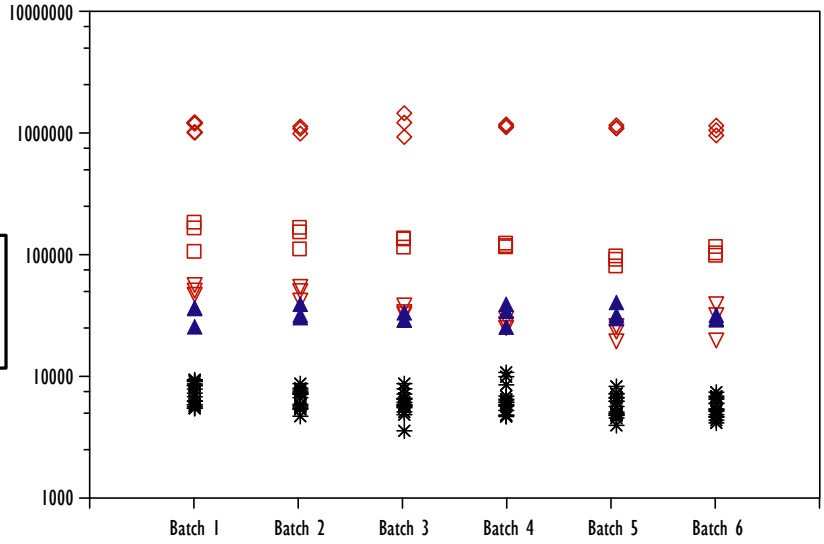
Screening of 20,000 cDNA Clones

Validation

Evenly Spaced Controls through 1 week of screening (10,000 clones)

□ SYK ▽ BTK ◇ FERT2 ✳ Neg. Controls ▲ Kinase Binding Protein (SCAP2)
Kinases



Results

50,000 cDNA clones

↓

20,000 clones expressed and labeled in a 96-well format.

↓

Immobilized on plate

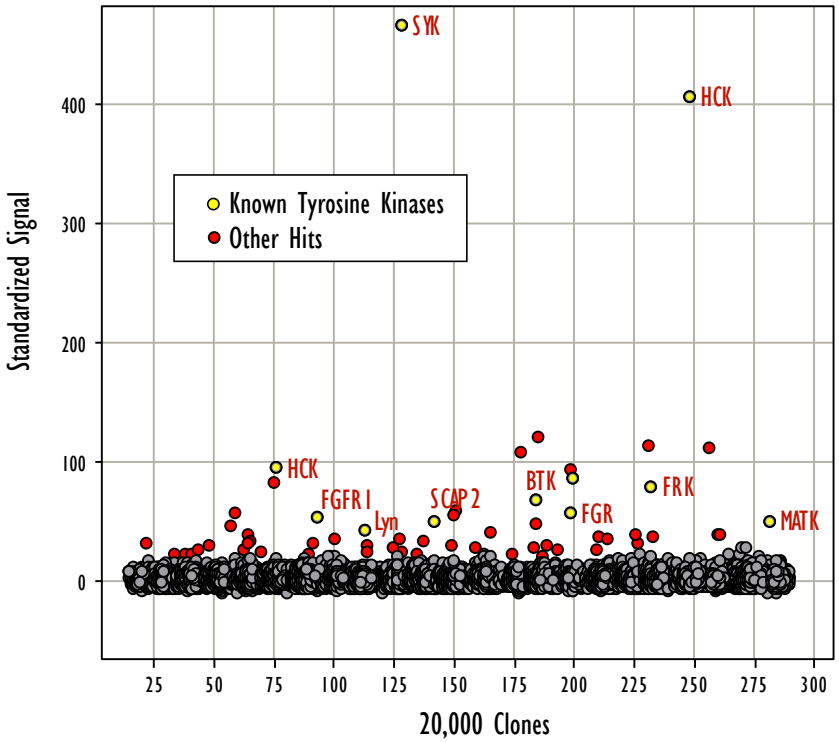
↓

Tyrosine Kinase assay on Sector HTS™ Reader

↓

Identified Kinases and Kinase binding protein

Entire Process <1 month



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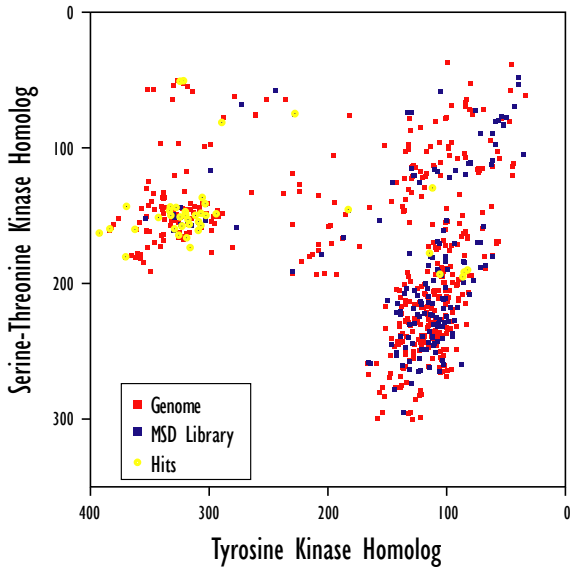
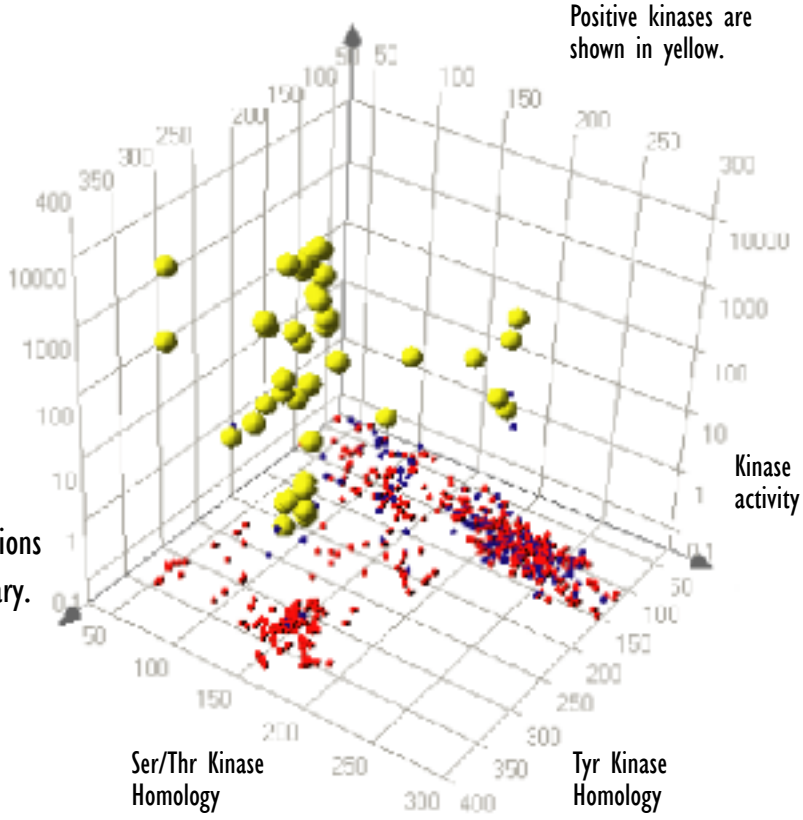
Kinase Activity from Our Library

Demonstration of tyrosine kinase specificity of our assay across the kinase genome



Kinases in the genome and kinases in our library by gene similarity score. The overlap in the two distributions demonstrates the high degree of coverage of our library. Putative kinases scored as having high homology to known tyrosine kinases and confirmed as such in our activity-based assays.



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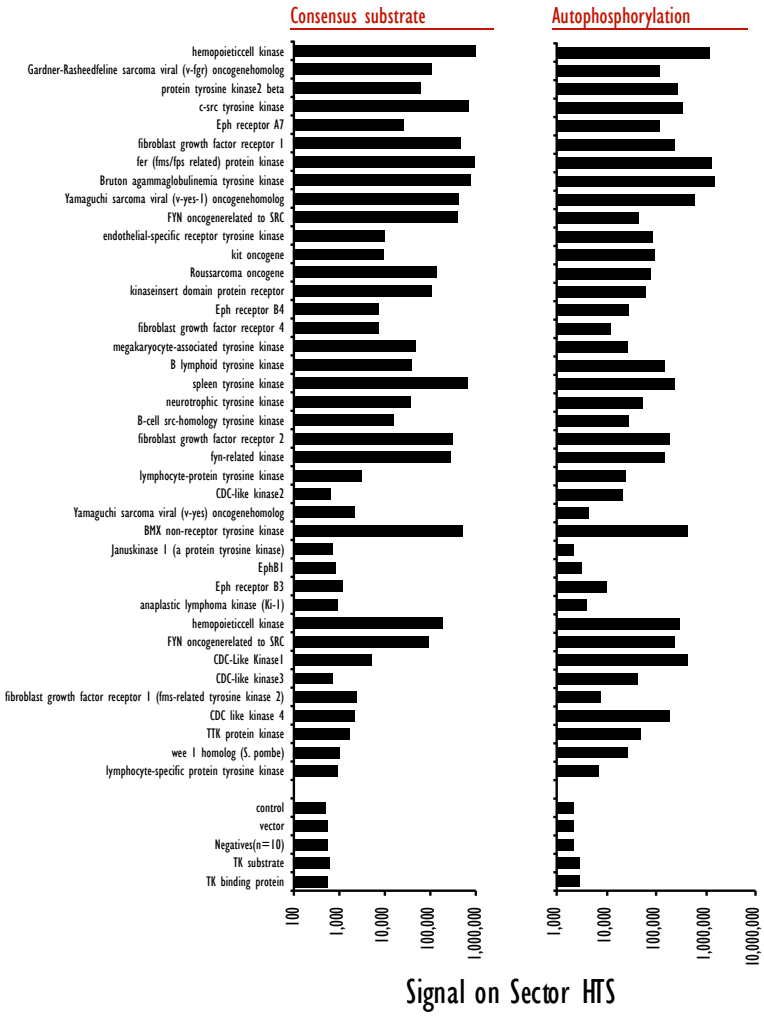
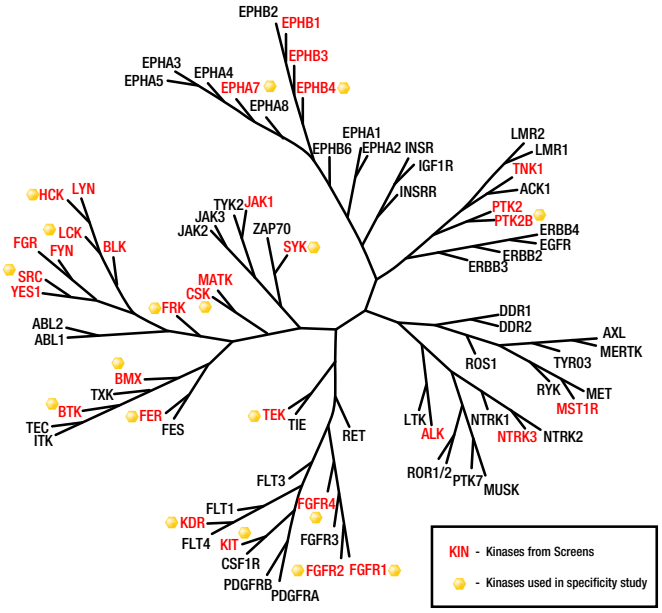
Protein Tyrosine Kinases Active from High Throughput Expressed Clones



Tyrosine Kinase Family Tree



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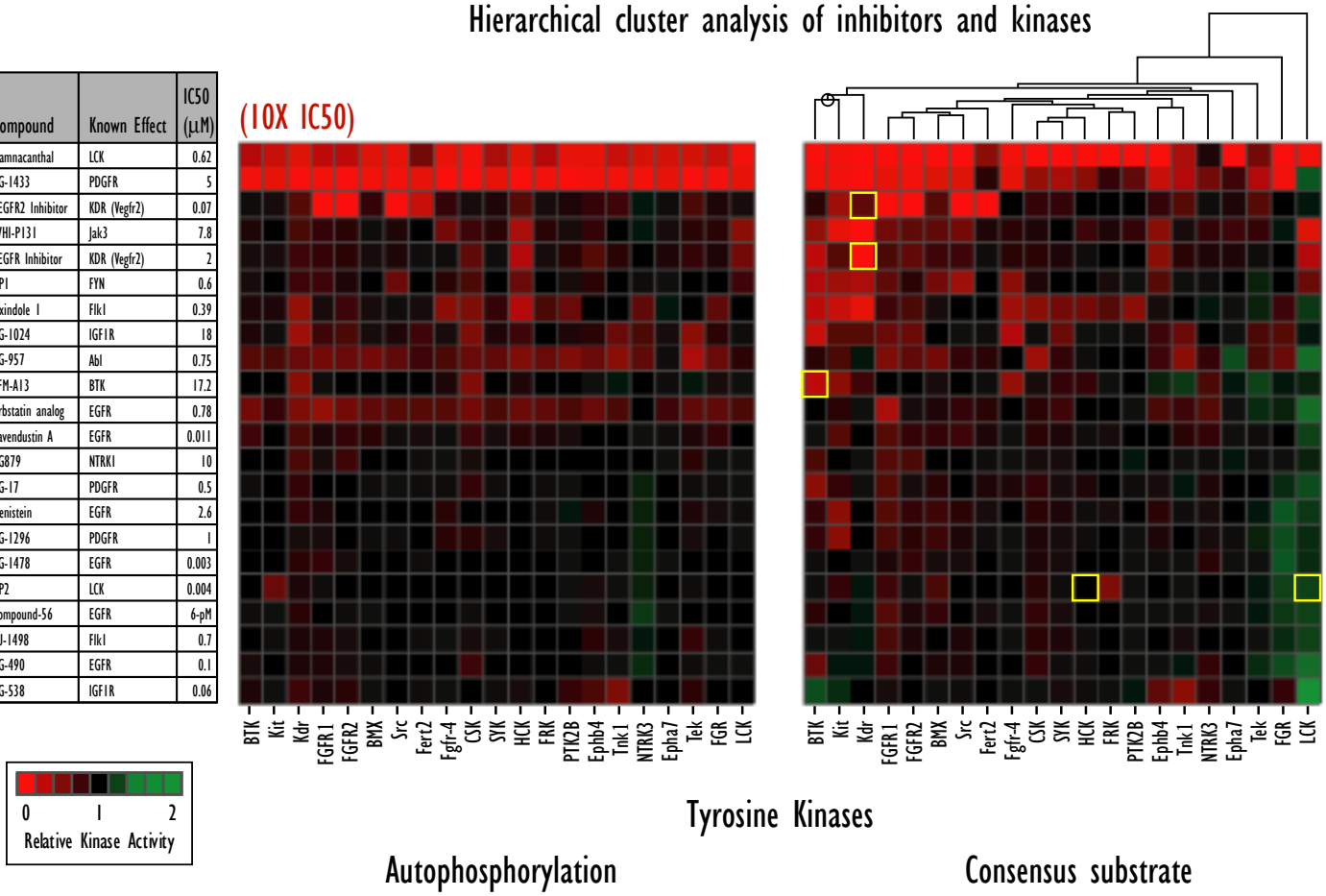
Multi-Array Tyrosine Kinase Specificity of Inhibitors Towards In-Vitro Tyrosine Kinase Activity (Autophosphorylation and Substrate)

Compound	Known Effect	IC50 (µM)
Damnacanthal	LCK	0.62
AG-1433	PDGFR	5
VEGFR2 Inhibitor	KDR (Vegfr2)	0.07
WHI-P131	Jak3	7.8
VEGFR Inhibitor	KDR (Vegfr2)	2
PP1	FYN	0.6
Oxindole I	Flk1	0.39
AG-1024	IGF1R	18
AG-957	Abl	0.75
LFM-A13	BTK	17.2
Erbstatin analog	EGFR	0.78
Lavendustin A	EGFR	0.011
AG879	NTRK1	10
AG-17	PDGFR	0.5
Genistein	EGFR	2.6
AG-1296	PDGFR	1
AG-1478	EGFR	0.003
PP2	LCK	0.004
Compound-56	EGFR	6-pM
SU-1498	Flk1	0.7
AG-490	EGFR	0.1
AG-538	IGF1R	0.06



A survey of known inhibitors reveals patterns of their actions on a set of tyrosine kinases, allowing classification of these kinases into groups reflecting their lineage. The individual drug profiles are useful supplements to general studies of the physical organic basis of chemical phenotypes.

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From Biological Discovery to Compound Discovery

Rapid progression to titratable inhibitors for chemical genomic and other types of study. The biological screening format used previously is also employed here to screen compounds.

50,000 cDNA clones

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Identified Kinases

↓

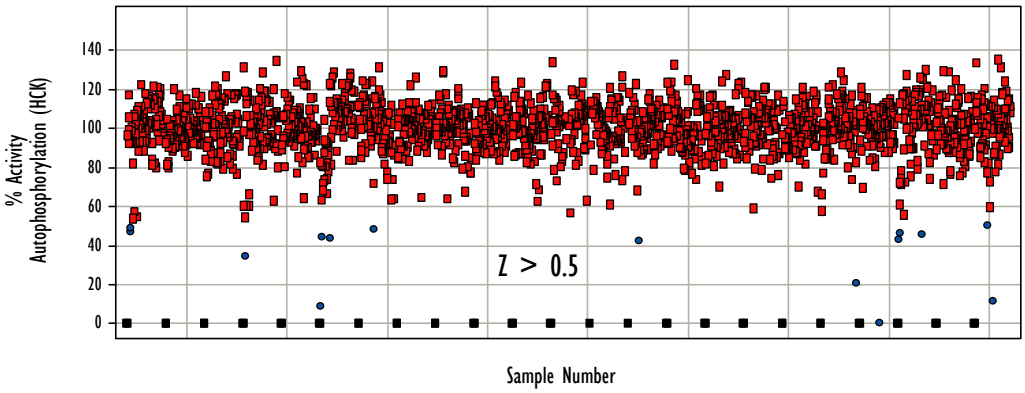
Express and label

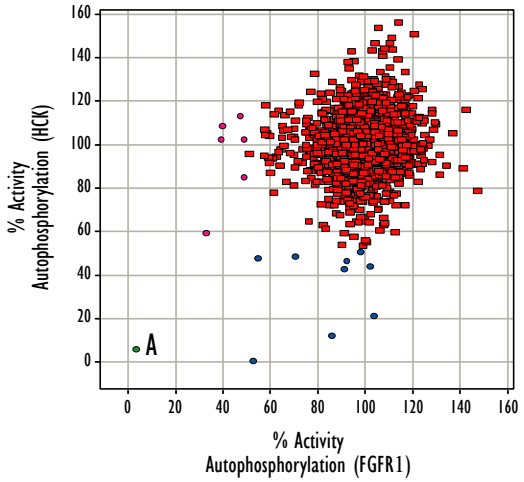
↓

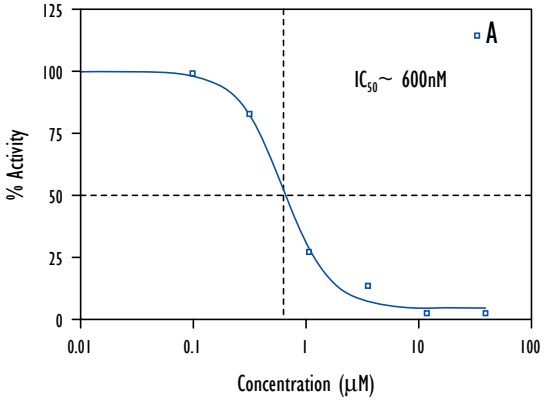
Immobilized on plate

↓

Screen Kinase for Inhibitor







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Summary

The combination of Multi-Array Technology with our unique cDNA library and high throughput methods for protein expression and labeling powerfully augments existing paradigms of drug discovery and development. We showed the application of these techniques to basic discovery, inhibitor screening and specificity analysis of tyrosine kinases and their substrates, demonstrating an effective and available "clone to screen" strategy. We validated this solution in real time, taking an identified clone through a 2,000 focused compound library screen in less than a day.

We offer:

- Discovery solutions on a genome scale for kinases and other activities in cDNA libraries and clone collections. We have genome scale screens for proteins involved in ubiquitinylation, phosphorylation, protein binding, phosphorylated protein binding, and kinase substrate determination.
- Compound specificity analysis for inhibitors in high throughput formats that allow practical profiling and categorization of thousands of lead compounds against panels of targets.
- Clone to screen solutions.
- Custom screening of the MSD clone library comprising 22,000 unique genes among 50,000 members (based on the Unigene classification), largely full length and sequence confirmed. Demonstrated formats include in vitro assays for ubiquitinylation, phosphorylation, protein:protein interactions, phosphorylated protein:protein interactions, kinase substrates discovery, acetylation, proteolysis, and post-translational modification.
- Cell-based assays.

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