Chapter 1

Introduction: Cell-Based Assays for High-Throughput Screening

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Summary

Cell-based assays represent approximately half of all high-throughput screens (HTS) currently performed. Here we review the history and status of HTS, and summarize some of the challenges and benefits associated with the use of cell-based assays in HTS, drawing upon themes that will reemerge in subsequent chapters in this book. Approaches for successful experimental design and execution of cell-based HTS are introduced, including strategies for assay development, implementation of primary and secondary screens, and target identification. In doing so, we hope to provide a comprehensive review of the cell-based HTS process and an introduction to the methodologies and techniques described in this book.

Key words: Assay development, Cell-based assay, High-content screening, High-throughput screening, Small-molecule screening, Target identification.

1. High-Throughput Screening: A Brief History

High-throughput screening (HTS) typically refers to a process in which large numbers of chemicals are tested (i.e., screened) with high efficiency to identify biologically active small molecules as candidates for further validation in additional biological or pharmacological experiments. In the pharmaceutical and biotechnology industries, this process normally involves large compound collections (hundreds of thousands to millions of small molecules), industrial-scale robots, and sophisticated automation. Ultimately, the goal of HTS is to generate chemical structures (leads) that will feed into the drug discovery and development pipeline in a therapeutics setting, or that will be used as probes to address biological questions in basic research (tool compounds).

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HTS has seen its emergence and maturation over the past two decades as an early discovery platform in the pharmaceutical industry. In the late 1980s to mid-1990s, significant advances in combinatorial chemistry and genomics helped drive a rapid growth in HTS. The potential to deliver thousands of novel compounds quickly and cheaply generated much optimism about the promise of future drug discovery and helped drive development of HTS technologies to evaluate the large numbers of new compounds available through combinatorial chemistry. Around the same time, rapid progress in genomics revealed many potential new drug targets. A lack of documented "druggability" and structural information for many such novel genomic targets *(1)* led to HTS becoming the method of choice for identification of small-molecule modulators of these targets from within ever-increasing compound collections in the pharmaceutical industry. As a result, the pharmaceutical industry invested large sums of capital to fuel rapid advances in HTS technologies in terms of automation, miniaturization, and assay methodology *(2, 3)*. Efficiency and throughput have been greatly improved. The 384-well plate format is now standard for HTS, with an increasing number of screens using a 1,536-well plate format or even higher well-density format (ultra-HTS or uHTS). Throughputs of ≥100,000 compounds screened per day are routine in lead HTS practitioner laboratories, where up to 50 HTS campaigns are being run each year *(2, 4)*. Return on these investments is evidenced by the increasing numbers of leads, clinical candidates, and marketed drugs arising from HTS. For example, Virumune, an anti-HIV drug, was a direct result of early HTS efforts *(5, 6)*. Additionally, advances in HTS technologies have been of benefit to other fields. Approaches developed for small-molecule screening have also been applied to identification and validation of specific gene functions among collections of cDNAs *(7, 8)* and RNA interference (RNAi) reagents *(9)*.

In recent years, interest in HTS among academic researchers has increased dramatically. There are now in existence many academic screening centers, to which many of the principles and lessons learned within the drug-discovery community are applied (a comprehensive database of academic screening centers and their capabilities is hosted by the Society for Biomolecular Sciences, http://www.sbsonline.org/). Impressive progress has been made in terms of automation, throughput, and improved screen paradigms *(10)*. In addition to providing HTS resources to the academic community for identification of both probes and leads for drug discovery, many of these centers promote open-source data sharing of small-molecule screening data. For example, *ChemBank* (http://chembank.broad.harvard.edu), from the National Cancer Institute's Initiative for Chemical Genetics *(11)*, and PubChem (http://pubchem.ncbi.nlm.nih.gov), from

the National Institutes of Health's Molecular Libraries Roadmap, make freely available data regarding small molecules and small-molecule screens, with the aim of facilitating both basic and applied research in the global scientific community.

2. Assay Types: Cell-Based vs. Biochemical Screens

Assays developed for HTS can be divided very broadly into two categories: biochemical assays and cell-based assays. Biochemical assays are target based and historically have been the mainstay of HTS campaigns in the pharmaceutical industry. Such in vitro assays include assessment of enzymatic activity (e.g., for kinases *(12)*, proteases *(13)*, or transferases *(14)*), receptor–ligand binding (e.g., for G-protein coupled receptors (GPCRs) *(15)*, ion channels *(16)*, or nuclear receptors *(17)*), or protein–protein interactions *(18)*. Biochemical assays are often direct and specific to the target of interest and can be miniaturized readily with less variability owing to the homogeneous nature of reactions. However, not all targets can be purified or prepared in a manner suitable for biochemical measurement. Additionally, activity of a small molecule in a reconstituted in vitro assay does not always translate into the same activity in a cellular context, because of issues including membrane permeability, off-target effects, and cytotoxicity.

In recent years, cell-based assays have emerged as a more physiological alternative to assays involving purified proteins. In contrast to biochemical target-based assays, cell-based assays often assume no a priori knowledge of a *direct* molecular target. Instead, many cell-based assays aim to identify modulators of a *pathway* of interest in the more physiological environment of a cell, complete with intact regulatory networks and feedback control mechanisms *(19)*. Examples of cell-based assays include functional assays (e.g., second messenger mobilization after GPCR activation *(20, 21)*), reporter gene assays *(22, 23)*, and phenotypic assays for cellular processes (e.g., cell migration *(24)* or cytokinesis *(25)*). In such assays, entire pathways of interest can be interrogated, providing the opportunity for multiple potential intervention points, as opposed to a single predefined step with the biochemical approach. This approach not only expands the repertoire of targets, but also provides additional chemical structures as plausible starting points for lead identification (for example, *see (26)*). Moreover, cell-based assays allow for the selection of compounds that can cross cellular membranes and can also provide indications of acute cytotoxicity as an early alert for later-stage lead/probe-discovery efforts. Today, most currently available

instrumentation in tissue culture, including automated cell culture (such as Select™, The Automation Partnership) and liquid handling, in conjunction with careful assay development, allows execution of high-quality cell-based screens. Cell-based HTS in 384, 1,536, and even 3,456-well plate format has been reported *(27–29)*.

Whole organism-based screens provide an additional level of physiological relevance beyond cell-based assays. Unicellular model organisms (e.g., yeast, bacteria) are readily adapted to grow in microtiter plates; Brown and colleagues and Balgi and Roberge provide comprehensive examples of microbial assays in Chaps. 2 and 9, respectively. Multicellular organisms, with intact cell-to-cell communication and three-dimensional tissue organization, represent additional challenges for adaptation to HTS. However, several model metazoan systems have been successfully adapted for use in small-molecule screens. Examples using zebrafish, worms, and plants in HTS are provided in Chaps. 4–6.

3. Experimental Design and Planning

Several important factors need to be considered when planning a small-molecule screen. The first step is to identify a screening strategy that maintains the appropriate biological context while balancing feasibility in terms of reagent availability and adaptation to automation. For cell-based assays, these considerations includes choice of biological system (primary cell, native or engineered cell line, or model organism), choice of assay approach (functional, reporter-gene, or phenotypic), and assay readout (uniform well readout or high content). Further considerations include follow-up biological experiments (counter screens, secondary assays, target identification, and in vivo validation) and determination of data-analytical strategies for interpretation of data arising from assay development, HTS, and follow-up assays (reviewed in more detail in Chap. 14). In Chap. 3, Mayer and colleagues provide an example of the use of primary mammalian cells for HTS, in the context of the ERK signaling pathway, and use of an engineered cell line with a fluorescent image-based readout is described by An in Chap. 7.

A variety of detection methods can be used for cell-based assays. These break down broadly into uniform well measurements (one measurement per well, representing a population average) and high-content measurements (multiple measurements per well, often representing subpopulations of cells, or even subcellular features). Uniform well measurements can be obtained using fluorescence, luminescence, and spectrophotometric

methods. Among them, fluorescent detection is arguably the most widely used approach owing to its high sensitivity, the diverse selection of fluorophores available, ease of operation, and various readout modes, such as fluorescence intensity, fluorescence polarization, fluorescence resonance energy transfer (FRET), fluorescence lifetime, time-resolved fluorescence, and combinations of these techniques, such as time-resolved fluorescence polarization. In Chap. 7, An provides two experimental protocols for fluorescence-based assays.

High-content screening (HCS) refers to any technique or process in which multiple measurements are obtained from a single well. Compared with single-parameter assays, HCS provides richer contextual and concurrent information that helps better illustrate both the behavior and the mechanism of action of small molecules and genetic manipulations. The most popular HCS format is image-based screening using an automated microscope (for examples of HCS imaging methods, *see (30)*), and to some researchers HCS is synonymous with image-based screening. In this textbook, however, the broader definition of HCS is used. In Chap. 12, Edwards and colleagues describe an application of high-throughput flow cytometry to identify selective ligands for two related G-protein coupled receptors.

4. Assay Development and Readiness for HTS

Assay development refers to the process in which potential approaches for measuring a particular target or biological process are evaluated; the best approach is further optimized in terms of throughput, cost, sensitivity, and signal dynamic range and variation, and adapted to the instrumentation of the screening facility. Assay development is critical for successful HTS, not only as applied to the primary screen but also for medium-throughput follow-up assays (secondary assays, selectivity assays, and other compound-profiling assays) that use HTS instrumentation to analyze hundreds to a few thousands of compounds. Given the costly nature of HTS campaigns (estimates for fully-loaded screening costs range from $0.50 to $1.00 per well, or $250,000–$500,000 for a 500,000 compound screen), it is worthwhile to spend time in advance to ensure that high-quality data of biological relevance are produced from HTS.

Because HTS tests hundreds of thousands to millions of compounds at once, it often requires additional considerations than development of a bench-top assay. One of the first tasks is to identify optimal conditions using the prototype assay. A full range of experimental conditions needs to be evaluated to determine

the best signal-to-background levels. For cell-based assays, optimization includes, but is not limited to, titration of cell density, titration of assay reagent(s), determination of optimal concentrations of modulator(s) (for small-molecule modifier screens *(31)*), and determination of incubation time with compounds. Positive controls are best included and titrated to provide diagnostic information for each experiment. Additionally, the stability of both assay reagents and readout signals should be evaluated over the time course of an assay with the eventual HTS process in mind. For example, time lapse between the first and last well on a plate, as well as the first and last plate of a batch, needs to be taken into consideration when assessing reagent and signal stability. Sensitivity to compound solvent (usually dimethylsulfoxide (DMSO)) should also be determined for cell-based assays. We recommend that a titration of up to 1% DMSO be performed.

Another early task is miniaturization. If we use 384-well plates as an example, typical reaction volumes are 25–50 μL per well. The goal at this stage is to maintain robust signal detection and acceptable signal-to-background ratios despite considerable reduction of reaction volume, reagent amounts, and cell numbers. There is no absolutely correct order of condition optimization and miniaturization, as long as the final assay is robust in 384-well plate format. In general, however, proceeding in ascending order of parameter contribution to assay noise will make assay development results easier to interpret. Yet another goal is to assess the liquid-handling compatibility with the assay. While most current liquid-handling equipment interfaces well with the 384-well plate format, accuracy and precision when dispensing low volumes of liquid (1–5 μL) can be a concern and should be tested. Instrument settings, such as volume, speed, and height and position of the pins/tips, need to be adjusted to achieve proper distribution and aspiration of the reagents/solutions while avoiding splashing or damaging monolayers of adherent cells by mechanical force.

Reproducibility is another critical factor. Well-to-well, plate-to-plate, day-to-day, and batch-to-batch (protein or cells) variations should be tested using positive (when a positive control is available) and DMSO-only controls. At the Broad Institute HTS facility, we recommend using a 384-well compound plate comprising half positive control (e.g., columns 1–12) wells and half DMSO-containing wells (e.g., columns 13–24) to evaluate reproducibility. This compound plate can then be pin-transferred into replicate assay plates on two consecutive days to evaluate well-to-well, plate-to-plate, and day-to-day variations of the assay. Alternatively, dose responses of positive controls can be evaluated on a day-to-day and batch-to-batch basis.

The most widely accepted measurement of assay quality and readiness is the Z' factor *(32)*. This metric quantifies the separation of a positive activity (sample) and background control in the absence of intervention of test compounds. It is determined as follows:

$$Z' = 1 - \frac{3 \times (\sigma_s + \sigma_c)}{|\mu_s - \mu_c|}$$

where σ_s and σ_c are the standard deviations of the sample and the control, respectively, and μ_s and μ_c are the means of sample and control, respectively. $Z' \geq 0.5$ indicates an excellent assay. An assay with $0 < Z' < 0.5$ is considered marginal; it may be suitable for HTS, but further optimization is often required. Assays with $Z' \leq 0$ are not suitable for HTS. Because Z' is based on standard deviations, large numbers of replicates are needed to compute meaningful Z' values. At the Broad Institute HTS facility, a minimum of 96 wells of a 384-well plate are used for Z' calculations. After the assay has achieved an acceptable Z' value, a pilot screen of approximately 2,000–10,000 compounds can be run to validate the high-throughput assay.

5. Execution of HTS and Beyond

The execution phase of HTS also requires careful planning. Screeners need to ensure that they have a sufficient and timely supply of target materials (proteins, cells, membrane preparations), consumables (plates, tips), and reagents, not only for the primary screen but also for retesting, EC_{50} determinations. and secondary assays. At the Broad Institute, primary screens are typically performed in duplicate, and IC_{50} or EC_{50} information is obtained using a duplicate, eight-point dose–response scheme at the retesting stage. Analysis, interpretation, and mining of HTS data represent other critical components for successful screens. Appropriate strategies for data analysis and interpretation should be considered in advance for all stages of the HTS process. In Chap. 14, Josiah discusses parameters used to design plate maps and controls and describes how to use the information obtained from those controls to assess screen performance. Specific image-based HCS analysis considerations are highlighted in Chaps. 11 and 15.

6. Target Identification

Once interesting "hits" are identified in a phenotypic cell-based assay, one frequently asked question is what are the protein targets? For cell-based assays in which a target of interest is overexpressed, e.g., receptor function assays, the attribution of "hit"

activity to the overexpressed target is easier to ascertain, and the remaining question is whether there are any off-target events that lead to the same phenotype. On the other hand, cell-based assays that screen for a phenotype (e.g., cell cycle arrest or reporter activity) instead of a target mean that many intervention points or protein candidates could conceivably produce the same phenotype. In this latter scenario, target identification becomes a more pressing issue in order to understand the mechanism of action of the "hits," and to facilitate the downstream discovery process.

There have been many successful examples of identification of the protein target(s) of bioactive small molecules. Among the most ground breaking are the discovery of (1) immunophilins as targets of a group of naturally occurring immunosuppressants: cyclophilin A as the target of cyclosporine A (CsA) *(33–36)*, and FKBP12 as the target of FK506 and rapamycin *(37–39)*; and (2) the cloning and identification of the first histone deacetylase (HDAC) as the target of trapoxin, a small molecule that increases cellular histone acetylation levels and causes cell cycle arrest *(40)*. The immunophilin discovery provided profound insight into mechanisms of action of widely used clinical immunosuppressants and the signaling cascade in T-cell signal transduction, while the isolation and cloning of the first HDAC catalyzed much of the subsequent research into chromatin and its function as a key regulatory element.

While there have been many examples of ad hoc successes in target identification for bioactive small molecules, the field is still struggling to find a systematic, case-independent, broadly applicable approach with a high success rate *(41)*. One of the major challenges is to devise methods through which investigators can reliably identify not only highly abundant proteins as targets for high-affinity bioactive small molecules, which happened to be the case for many of the historic successes, but also lower-abundance proteins for medium-affinity bioactive small molecules. While there may not be a panacea in target identification for all bioactive small molecules, a number of recent studies show progress towards a systematic approach, as described below.

The affinity-based approach is the most direct way to identify proteins that bind bioactive small molecules. This approach usually involves chemical modification of the small molecule of interest (bait compound) for attachment through a linker to a solid support (for pull-down-type experiments) or by introducing to it a reactive group that can be activated to bind its protein target (e.g., aryl azide for photo-affinity labeling *(42)*). Usually the bait compound also has a detection tag such as ^{125}I, ^{3}H, or biotin, or functionality that can react specifically with other chemical groups on detection agents, e.g., terminal acetylenes that react with azide-modified rhodamine by the copper(I)-catalyzed 1,2,3-triazole formation click chemistry *(43, 44)*. One common requirement of these methods is the understanding of the structure–activity relationship (SAR) of the bioactive small molecule,

such that modification does not interfere with the known activity of the bioactive small molecule. New ways to create high-affinity probes from low-affinity small-molecule probes without tedious chemistry–biology iterations have also been described which involve fusion of two modest small-molecule probes *(45–47)*.

A promising proteomics-based technique for detecting specific bioactive small molecule-interacting proteins via the affinity approach is stable isotope labeling by amino acids in cell culture (SILAC) *(41, 48)*. SILAC starts by labeling proteins from cells grown separately in heavy amino acid-supplemented medium and in light (normal) amino acid-supplemented medium. The heavy protein preparation and the light protein preparation are made from these otherwise identical culture conditions and then subject to chromatography through a bioactive small-molecule affinity matrix and a control compound affinity matrix, respectively. Eluents of both are then mixed and examined by mass spectrometry for ratio of abundance. For proteins specifically retained by the bioactive small-molecule matrix, there should be more heavy signal relative to light signal, whereas for nonspecific binders to the matrix the heavy:light ratio should be close to unity. Better sensitivity and higher throughput are two advantages of this ratiometric, mass spectrometry-based approach as opposed to traditional gel-based approaches.

Other complementary, systematic schemes to the affinity-based approach in target identification include genetics and function/phenotype association investigations. Genome-wide heterozygote hypersensitivity to small molecules in yeast has been used to suggest drug targets *(49–51)*, and the same principle has been applied to mammalian cells in gene-dosage screens *(52)*. A yeast three-hybrid system has also been reported, where the reporter activity is controlled by potential protein targets that interact with the bioactive small molecule in question *(53)*. The advent of genome-wide knockdown by RNAi should facilitate these systematic genetic approaches and provide a wealth of phenotypes that can be compared with those achieved by bioactive small molecules. With increased realization in the academic and pharmaceutical community that target identification represents a critical challenge, and with the increased activities to address the issues associated, it is likely that additional innovative, systematic approaches will be reported in the near future.

7. Summary

HTS has matured over the past two decades into an indispensable part of drug discovery and basic research in both the pharmaceutical industry and academia. Cell-based assays, a focus of this

textbook with comprehensive chapters describing topics from screening methodology to data analysis, account for approximately half of all HTS campaigns and present exciting potential for breakthroughs in both our understanding of biology and expansion of our arsenal of small-molecule tools and drugs. Granted, there are challenges, such as systematic target identification following certain cell-based screens. However, we hope that the detailed techniques and methodologies provided in this book will enable researchers to practice the protocols in a HTS setting, and that such practice will lead to new innovations that fulfill the promise of cell-based assays.

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References

1. Hopkins, A. L. and Groom, C. R. (2002) The druggable genome. *Nat. Rev. Drug Discov.* 1, 727–30.
2. Beggs, M. (2000) HTS-where next. *Drug Discov. World* winter, 25–30.
3. Hertzberg, R. P. and Pope, A. J. (2000) High-throughput screening: New technology for the 21st century. *Curr. Opin. Chem. Biol.* 4, 445–451.
4. Liu, B., Li, S., and Hu, J. (2004) Technological advances in high-throughput screening. *Am. J. Pharmacogenomics* 4, 263–276.
5. Grozinger, K., Proudfoot, J., and Hargrave, K. (2006) Discovery and development of nevirapine. In Chorghade, M. S. (ed.) Drug Discovery and Development. Wiley-VCH, Weinheim, Germany, pp. 353–363.
6. Kell, D. (1999) Screensavers: Trends in high-throughput analysis. *Trends Biotech.* 17, 89.
7. Zitzler, J., Link, D., Schafer, R., Liebetrau, W., Kazinski, M., Bonin-Debs, A., et al. (2004) High-throughput functional genomics identifies genes that ameliorate toxicity due to oxidative stress in neuronal HT-22 cells: GFPT2 protects cells against peroxide. *Mol. Cell. Proteomics* 3, 834–840.
8. Korherr, C., Gille, H., Schafer, R., Koenig-Hoffmann, K., Dixelius, J., Egland, K. A., et al. (2006) Identification of proangiogenic genes and pathways by high-throughput functional genomics: TBK1 and the IRF3 pathway. *Proc. Natl. Acad. Sci. U.S.A.* 103, 4240–4245.
9. Moffat, J., Grueneberg, D. A., Yang, X., Kim, S. Y., Kloepfer, A. M., Hinkle, G., et al. (2006) A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. *Cell* 124, 1283–1298.
10. Inglese, J., Auld, D. S., Jadhav, A., Johnson, R. L., Simeonov, A., Yasgar, A. et al. (2006) Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc. Natl. Acad. Sci. U.S.A.* 103, 11473–11478.

11. Tolliday, N., Clemons, P. A., Ferraiolo, P., Koehler, A. N., Lewis, T. A., Schreiber, S. L., et al. (2006) Small molecules, big players: The national cancer institute';s initiative for chemical genetics. *Cancer Res.* 66, 8935–8942.
12. Burns, S., Travers, J., Collins, I., Rowlands, M. G., Newbatt, Y., Thompson, N., et al. (2006) Identification of small-molecule inhibitors of protein kinase B (PKB/AKT) in an alphascreenTM high-throughput screen. *J. Biomol. Screen.* 11, 822–827.
13. Sudo, K., Yamaji, K., Kawamura, K., Nishijima, T., Kojima, N., Aibe, K., et al. (2005) High-throughput screening of low molecular weight NS3-NS4A protease inhibitors using a fluorescence resonance energy transfer substrate. *Antivir. Chem. Chemother.* 16, 385–392.
14. Swaney, S., McCroskey, M., Shinabarger, D., Wang, Z., Turner, B. A., and Parker, C. N. (2006) Characterization of a high-throughput screening assay for inhibitors of elongation factor P and ribosomal peptidyl transferase activity. *J. Biomol. Screen.* 11, 736–742.
15. Allen, M., Reeves, J., and Mellor, G. (2000) High throughput fluorescence polarization: A homogeneous alternative to radioligand binding for cell surface receptors. *J. Biomol. Screen.* 5, 63–69.
16. Xu, J., Wang, X., Ensign, B., Li, M., Wu, L., Guia, A., et al. (2001) Ion-channel assay technologies: Quo vadis? *Drug Discov. Today* 6, 1278–1287.
17. Parker, G. J., Law, T. L., Lenoch, F. J., and Bolger, R. E. (2000) Development of high throughput screening assays using fluorescence polarization: nuclear receptor-ligand-binding and kinase/phosphatase assays. *J. Biomol. Screen.* 5, 77–88.
18. Kenny, C. H., Ding, W., Kelleher, K., Benard, S., Dushin, E. G., Sutherland, A. G., et al. (2003) Development of a fluorescence polarization assay to screen for inhibitors of the FtsZ/ZipA interaction. *Anal. Biochem.* 323, 224–233.
19. Clemons, P. A. (2004) Complex phenotypic assays in high-throughput screening. *Curr. Opin. Chem. Biol.* 8, 334–338.
20. Chambers, C., Smith, F., Williams, C., Marcos, S., Zhen, S., Hayter, P., et al. (2003) Measuring intracellular calcium fluxes in high throughput mode. *Comb. Chem. High Throughput Screen* 6, 355–362.
21. Kariv, I., Stevens, M. E., Behrens, D. L., and Oldenburg, K. R. (1999) High throughput quantitation of cAMP production mediated by activation of seven transmembrane domain receptors. *J. Biomol. Screen.* 4, 27–32.
22. Li, X., Shen, F., Zhang, Y., Zhu, J., Huang, L., and Shi, Q. (2007) Functional characterization of cell lines for high-throughput screening of human neuromedin U receptor subtype 2 specific agonists using a luciferase reporter gene assay. *Eur. J. Pharm. Biopharm.* 67, 284–292.
23. Beck, V., Pfitscher, A., and Jungbauer, A. (2005) GFP-reporter for a high throughput assay to monitor estrogenic compounds. *J. Biochem. Biophys. Methods* 64, 19–37.
24. Yarrow, J. C., Totsukawa, G., Charras, G. T., and Mitchison, T. J. (2005) Screening for cell migration inhibitors via automated microscopy reveals a Rho-kinase inhibitor. *Chem. Biol.* 12, 385–395.
25. Eggert, U. S., Kiger, A. A., Richter, C., Perlman, Z. E., Perrimon, N., Mitchison, T. J., et al. (2004) Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets. *PLoS Biol.* 2, 2135–1243.
26. Krejci, P., Pejchalova, K., and Wilcox, W. R. (2007) Simple, mammalian cell-based assay for identification of inhibitors of the Erk MAP kinase pathway. *Invest. New Drugs* 25, 391–394.
27. Bradley, J., Gill, J., Bertelli, F., Letafat, S., Corbau, R., Hayter, P., et al. (2004) Development and automation of a 384-well cell fusion assay to identify inhibitors of CCR5/CD4-mediated HIV virus entry. *J. Biomol. Screen.* 9, 516–524.
28. Wunder, F., Stasch, J. P., Hutter, J., Alonso-Alija, C., Huser, J., and Lohrmann, E. (2005) A cell-based cGMP assay useful for ultra-high-throughput screening and identification of modulators of the nitric oxide/cGMP pathway. *Anal. Biochem.* 339, 104–112.
29. Brandish, P. E., Chiu, C. S., Schneeweis, J., Brandon, N. J., Leech, C. L., Kornienko, O., et al. (2006) A cell-based ultra-high-throughput screening assay for identifying inhibitors of D-amino acid oxidase. *J. Biomol. Screen.* 11, 481–487.
31. Koeller, K. M., Haggarty, S. J., Perkins, B. D., Leykin, I., Wong, J.-C., Kao, M. C. J., et al. (2003). Chemical genetic modifier screens: Small molecule trichostatin suppressors as probes of acetylation in transcription, cell cycle progression, and stability of the cytoskeleton. *Chem. Biol.* 10, 397–410.
32. Zhang, J.-H., Chung, T. D. Y., and Oldenburg, K. R. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* 4, 67–73.

33. Harding, M. W., Handschumacher, R. E., and Speicher, D. W. (1986) Isolation and amino acid sequence of cyclophilin. *J. Biol. Chem*. 261, 8547–8555.

34. Handschumacher, R. E., Harding, M. W., Rice, J., Drugge, R. J., and Speicher, D. W. (1984) Cyclophilin: A specific cytosolic binding protein for cyclosporin A. *Science* 226, 544–547.

35. Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T., and Schmid, F. X. (1989) Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 337, 476–478.

36. Takahashi, N., Hayano, T., and Suzuki, M. (1989) Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337, 473–475.

37. Lane, W. S., Galat, A., Harding, M. W., and Schreiber, S. L. (1991) Complete amino acid sequence of the FK506 and rapamycin binding protein, FKBP, isolated from calf thymus. *J. Protein. Chem*. 10, 151–160.

38. Harding, M. W., Galat, A., Uehling, D. E., and Schreiber, S. L. (1989) A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 341, 758–760.

39. Siekierka, J. J., Hung, S. H. Y., Poe, M., Lin, C. S., and Sigal, N. H. (1989) A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. *Nature* 341, 755–757.

40. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* 272, 408–411.

41. Burdine, L. and Kodadek, T. (2004) Target identification in chemical genetics: The (often) missing link. *Chem. Biol.* 11, 593–597.

42. Colca, J. R. and Harrigan, G. G. (2004) Photo-affinity labeling strategies in identifying the protein ligands of bioactive small molecules: Examples of targeted synthesis of drug analog photoprobes. *Comb. Chem. High Throughput Screen*. 7, 699–704.

43. Kolb, H. C. and Sharpless, K. B. (2003) The growing impact of click chemistry on drug discovery. *Drug Discov. Today* 8, 1128–1137.

44. Speers, A. E. and Cravatt, B. F. (2004) Profiling enzyme activities *in vivo* using click chemistry methods. *Chem. Biol.* 11, 535–546.

45. Maly, D. J., Choong, I. C., and Ellman, J. A. (2000) Combinatorial target-guided ligand assembly: Identification of potent subtype-selective c-Src inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* 97, 2419–2424.

46. Sem, D. S., Bertolaet, B., Baker, B., Chang, E., Costache, A. D., Coutts, S., et al. (2004) Systems-based design of bi-ligand inhibitors of oxidoreductases: Filling the chemical proteomic toolbox. *Chem. Biol.* 11, 185–194.

47. Profit, A. A., Lee, T. R., and Lawrence, D. S. (1999) Bivalent inhibitors of protein tyrosine kinases. *J. Am. Chem. Soc.* 121, 280–283.

48. Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., et al. (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol. Cell. Proteomics* 1, 376–386.

49. Lum, P. Y., Armour, C. D., Stepaniants, S. B., Cavet, G., Wolf, M. K., Butler, J. S., et al. (2004) Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. *Cell* 116, 121–137.

50. Giaever, G., Flaherty, P., Kumm, J., Proctor, M., Nislow, C., Jarmaillo, D. F., et al. (2004) Chemogenomic profiling: Identifying the functional interactions of small molecules in yeast. *Proc. Natl. Acad. Sci. U.S.A.* 101, 793–798.

51. Perlstein, E. O., Ruderfer, D. M., Roberts, D. C., Schreiber, S. L., Kruglyak, L. (2007) Genetic basis of individual differences in the response to small-molecule drugs in yeast. *Nat. Genet.* 39, 496–502.

52. Luesch, H. (2006) Towards high-throughput characterization of small molecule mechanisms of action. *Mol. BioSyst.* 2, 609–620.

53. Kley, N. (2004) Chemical dimerizers and three-hybrid systems: Scanning the proteome for targets of organic small molecules. *Chem. Biol.* 11, 599.