GEN
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Biotechnology from bench to business
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Increasing Yield in Nucleic Acid Sample Prep

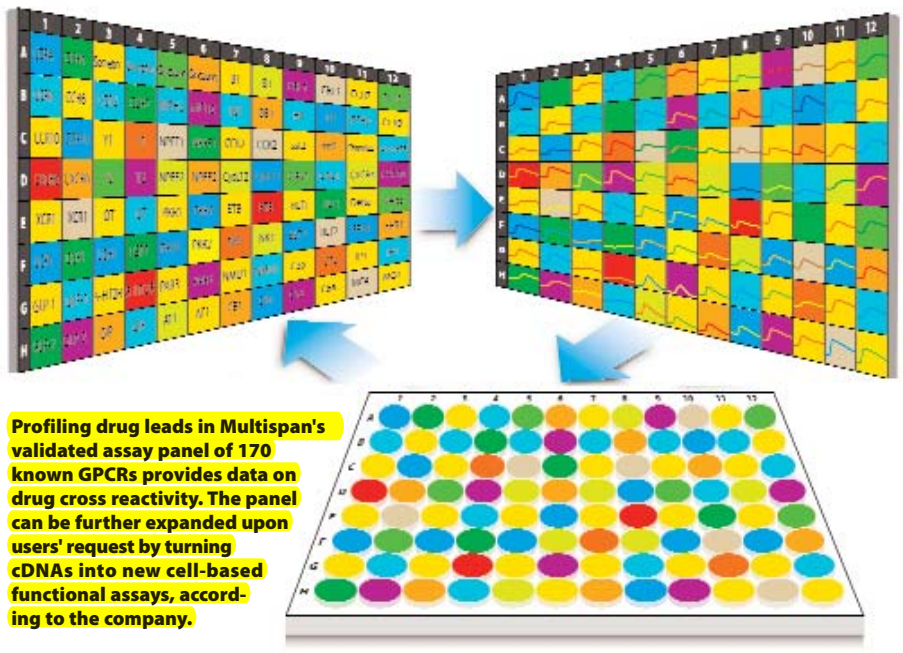
Lab veterans share their tools and techniques to improve DNA extraction.

Creating a Life Science Cluster in Florida

The state has invested a lot of money and effort into luring industry. Will it be successful?

Mary Ann Liebert, Inc. publishers

Cell-Based Assay R&D Role Expands



Profiling drug leads in Multispan's validated assay panel of 170 known GPCRs provides data on drug cross reactivity. The panel can be further expanded upon users' request by turning cDNAs into new cell-based functional assays, according to the company.

Automation, Label-Free Tools, and Other Innovations Create Better Screening

Kathy Liszewski

Cell-based assays have become a quick, lower-cost means of testing drug candidates for toxic effects in early discovery work. Informa Life Sciences' upcoming "Cell-Based Assays" meeting will highlight some advances that are driving the field such as improved screening technologies, noninvasive monitoring, and the increasingly important incorporation of automation.

More than 50% of currently marketed drugs target GPCRs. The multitalented GPCRs affect a host of cellular processes that impact us from development to death. "GPCRs are the most validated class of druggable targets because of their involvement in critical metabolic and dis-

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Downstream Bottlenecks: Are They Myth or Reality?

Throughput Isn't Where It Should Be, and There Is Some Disagreement as to Why

Gail Dutton

Like the story of the blind men describing an elephant, the answer to whether a downstream bioprocessing bottleneck exists is largely one of perception.

"The situation is even worse than it was two years ago," according to Uwe Gottschalk, Ph.D., vp of purification technology, a business unit of Sartorius Stedim Biotech (www.sartorius.com). Ann O'Hara, GM of life science services at GE Healthcare Life Sciences (www.gelifesciences.com), counters that the presence or lack of a bottleneck "is a matter of optimization. The solutions are here."

"The issue is partly a matter of optimization and partly the nature of operations," elaborates Daniel Van Plew, vp and GM of industrial operations and product supply at Regeneron Pharmaceuticals (www.regeneron.com).

Until recently, vendors focused on making larger chromatography columns as a way to handle upstream productivity increases.
Regeneron

Manufacturers generally seem to favor the optimization argument. Whatever it's called, though, everybody agrees that throughput isn't what it should be. Managers and scientists within the biotech community

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Sticky ends

Affiris is integrating **Definiens'** Enterprise Image Intelligence™ Suite into its Alzheimer's disease vaccination program... **GE Healthcare**, which acquired exclusive rights to the disposable aseptic connector developed by **BioQuate**, launched range of sterile, genderless connectors as part of its ReadyToProcess™ portfolio of bioprocessing systems... **genOway** signed 5-year master service contract with **Boehringer Ingelheim Pharmaceuticals** under which genOway will provide the firm with customized transgenic rat lines... **Bioreliance** established a commercial office in Tokyo... **Vogelbusch** says it applied for patent on fermentation process that boosts efficiency of bioethanol production from raw materials containing hemicellulose... **MethylGene** inked worldwide research collaboration and license agreement with **Otsuka Pharmaceutical** for development of small molecule, kinase inhibitors for local delivery and treatment of ocular diseases excluding cancer... **Aida Pharmaceuticals** acquired the **Jiangsu Institute of Microbiology** in China... **BioForce Nanosciences** introduced a surface-patterning service utilizing its Nano eNabler™ and Nano eNabler CB™ molecular printers.

Cell-Based Assays

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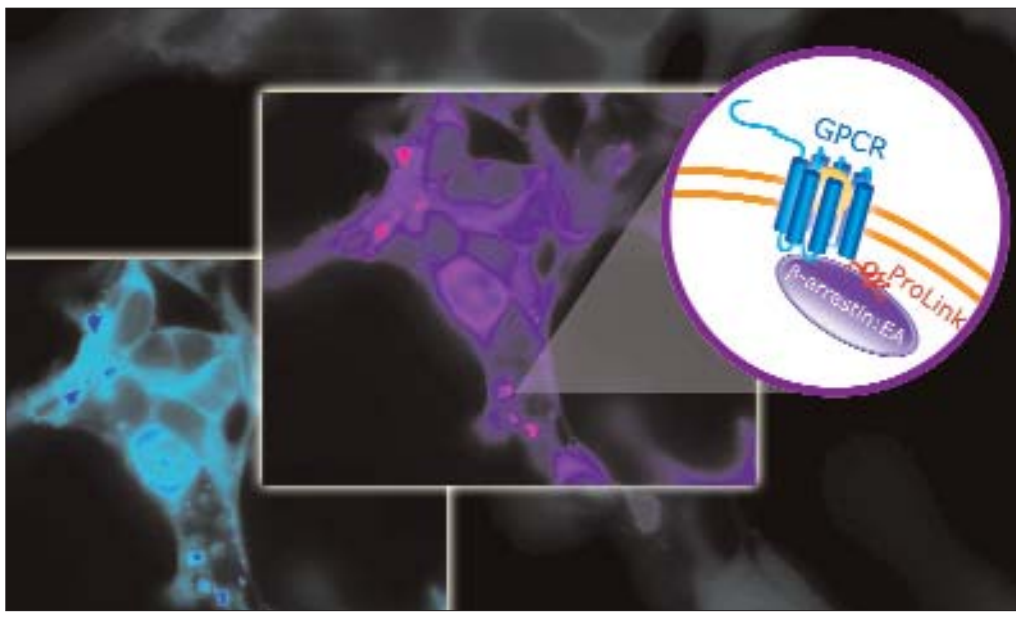
ease pathways as well as the success of such drugs on the market," notes Helena Mancebo, Ph.D., president and CEO, of Multispan (www.multispaninc.com).

GPCR Drug Discovery

Multispan uses its technology to efficiently express active GPCRs in mammalian cells, reports Dr. Mancebo. "These complex proteins are typically difficult to express in cells and to purify in active states. This has created a bottleneck especially for generating antibodies against GPCRs.

"We have been able to express more than one million GPCR molecules on mammalian cells in various cell lines for that purpose. The robustness of this expression technology especially for traditionally difficult GPCRs is now also allowing cell-based assays that were not possible before."

The company is collaborating with Promega (www.promega.com) and Molecular Devices (www.moleculardevices.com) to develop an array of applications for Multispan's GPCR cell-based assays. "We have produced a comprehensive panel of functional assays for more than 160 GPCRs using Molecular Devices' GPCR assay instruments and reagents. We are now developing new applications of the assays in collaboration with Promega. These assays will be valuable for clients involved in high-throughput screening and lead optimization."



DiscoveRx has developed a GPCR screening platform without forced coupling.

An advantage of such profiling experiments is the ability to identify off-target effects at an early stage of drug development, according to Dr. Mancebo. "Another benefit is for screening GPCR antibodies. We profiled a panel of 60 GPCRs to evaluate the specificity of antibodies developed against chemokine and other receptors. This represents another significant development in the field."

Protein-Protein Interactions

DiscoveRx (www.discoverx.com) has developed a new family of cell-based assays for the detection of protein-protein interactions using an enzyme fragment complementation (EFC) assay. This system uses an enzyme acceptor (EA), an inactive a-galactosidase enzyme that binds to its enzyme donor (ED) forming an active enzyme that will hydrolyze a substrate and provide a signal. PathHunter™ a-Arrestin high-throughput screening assays monitor GPCR activation following ligand stimulation without the need for an imaging instrument, fluorescent protein tag, or radioactivity, points out Keith Olson, Ph.D., vp, R&D.

This instrumentation provides several advantages for faster and more efficient screening, adds Dr. Olson. "The major enabling feature of this new approach is that we have developed a straightforward, HTS-friendly system for monitoring protein-protein interaction. This has already rescued a number of targets for us that were not amenable to our traditional approaches and provides the added value of potentially revealing novel pharmacophores related to protein-protein interaction sites."

DiscoveRx has applied this technology to a number of orphan GPCR targets. "This approach provides the added benefit of reporting at the outset of the assay whether or not the GPCR shows any endogenous interaction with arrestin," Dr. Olson reports. "Most targets do, and in many cases there is enough constitutive

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interaction with arrestin that it affords the chance to screen for both agonist and inverse agonists using our cell lines.

"Orphan GPCRs, once validated, can often represent a vast untapped target and an opportunity to generate intellectual property. Orphans are difficult targets in general, but our assay format reduces the risk to some extent since arrestin is upstream of the second messenger or reporter gene methods. This allows a campaign to be run with just one assay, even in cases where nothing is known about the G-protein coupling.

"The fact that we have a panel of over 65 orphan GPCR-expressing cells also presents the opportunity to profile existing compounds or other ligands in either de-orphanization or drug-repositioning applications," remarks Dr. Olson.

"The PathHunter technology in its first iteration was designed for high-affinity binding of the two components of a-galactosidase," Dr. Olson explains. "As we refined our approach to GPCRs through analysis of arrestin recruitment, we found that we needed to modify EFC to be more amenable to protein-protein interaction detection. We modified the smaller component of our EFC system, the ED, generating a new complementation peptide called ProLink.

"This new peptide had a low affinity for EA but can still complement effectively as long as the two EFC components are brought together by other interacting proteins. This new tag provides a generic, reversible system for monitoring protein-protein interactions in cells with the advantage of an enzyme-amplification step to boost the assay signal compared to traditional approaches like BRET or FRET."

Acea Bioscience's RC-CES is a multicomponent system consisting of electronic plates (E-Plate) for culturing cells, a reader that interfaces with the E-Plate, an electronic analyzer, and a computer. The 6x96 Mult-E-Plate system can accomodate up to six 96-well E-Plates.

Screening Ion Channels

Ion channels play critical roles in processes that range from nervous-system signaling to muscle contraction and cell growth. Because of their global expression and importance they are sought after therapeutic targets.

"Electrophysiological assays provide one of the most direct and accurate ways to characterize ion-channel activity," notes Birgit T. Priest, Ph.D., senior research fellow, Merck Research Laboratories (www.merck.com). "They measure current or a voltage difference between two electrodes. Typically, one of the electrodes is in contact with the cytoplasm inside the cell, whereas the other electrode is placed in the solution surrounding the cell.

"This electrode configuration allows the operator to control the voltage across the cell membrane and measure currents that flow across the cell membrane. Drugs can be applied to the solution bathing the cells, and their effect on the currents and thus the ion channels can be determined."

Recently developed automated electrophysiology platforms are greatly enhancing the throughput of electrophysiology assays, positioning them to play a more central role in ion-channel drug discovery, says Dr. Priest.

"Traditional manual electrophysiology assays provide high-quality, detailed information about the characteristics of an ion channel under investigation, and the effects of test substances. These assays, however, have an extremely low throughput of typically two to three compounds per week per assay and require specially trained personnel.

"Automated electrophysiology technologies can significantly increase the throughput to the point where it may be possible to screen small libraries or support a medicinal chemistry effort." Automated electrophysiology instruments are commercially available from several companies, including

Molecular Devices' IonWorks™ HT, IonWorks Quattro, and PatchXpress™ instruments, the QPatch™ instrument from Sophion (www.sophion.dk), and the Port-a-Patch™ and Patchliner™ from Nanion (www.nanion.de).

Label-Free Detection

Label-free detection technologies are becoming increasingly popular because they permit the noninvasive monitoring of live cells in real time. "We use cell-sensor impedance technology as a label-free means to monitor live cells," reports Anker Jon Hansen, Ph.D., principal scientist at Novo Nordisk (www.novonordisk.com). "Measuring electrical impedance is a relatively new method to gauge cell-adhesive properties."

Dr. Hansen applies the technology to study how NK cells kill target cells. "Most in vitro assays of cell-mediated killing are based on quantifying cytoplasmic constituents using radioactive labels or naturally occurring compounds that are released and measured over time. Real-time monitoring of target-cell status based on electrical impedance measurements can give much quicker and more comprehensive information."

Dr. Hansen performs impedance measurements using Acea Biosciences' (www.aceabio.com) tools. He says that there are several advantages to this technology. "This is a noninvasive way to monitor cell behavior from the beginning to the end of the entire experiment. It monitors the kinetics of cell attachment, spreading, and death, for example."

Another advantage is the ability to better distinguish off-target effects. "You can measure short-term as well as long-term effects, which helps rule out off-target effects such as toxicity to cells. Also, the impedance readout can help determine the optimal time points to measure particular responses."

Acea Biosciences' microelectrode arrays are fabricated either on glass slides or microporous membranes integrated into wells of microtiter plates to provide for real-time cell electrical sensing (RT-CES™). The instrumentation displays electrode-impedance cell index values that can be used to measure cell viability, number, morphology, and adhesion degree in many cell-based assays, Dr. Hansen adds.

Advances in Automation

The process of performing cell-based assays involves plating cells, equilibrating them to culture conditions, adding the compounds, performing the assay, and finally reading the results. This can be a slow and expensive process that produces variable results especially when done manually, according to Ali Griffen, Ph.D., associate team leader, cancer bioscience at AstraZeneca (www.astrazeneca.com).

"Automation can greatly improve reproducibility. This saves time and money. With recent progress in automating cell-based assays, the field is now moving to using them earlier in drug discovery. Previously cell-based assays were done at later stages of drug discovery because they were labor intensive and low throughput."

Technical advances are changing that paradigm, explains Dr. Griffen. "For example, there have been improvements in equipment such as plate washers that more gently wash cells. Additionally, assays themselves have changed. The use of reporter assays in engineered cell lines that overexpress the protein studied provide more robust signals. Another advance is the ability to integrate different types of equipment from liquid handling to high-content readers."

Not all problems have been solved, cautions Dr. Griffen. "Researchers would like to further miniaturize assays but face the problem of generating enough signal for accurately detecting changes. Also, the challenge remains to automate and enhance throughput for relevant 3-D assays such as those involving tube formation. Until recently, these were typically done in 24-well plates that were read manually.

"Some assays are just not simple out-of-the-box types of assays. Often you need sophisticated imaging and high-content analysis to make sense of and verify your data," Dr. Griffen says.

Ultimately, as cell-based screens continue to take precedence over biochemical assays, most expect advances in automation will continue to drive and enable further streamlining of the drug discovery process. GEN