

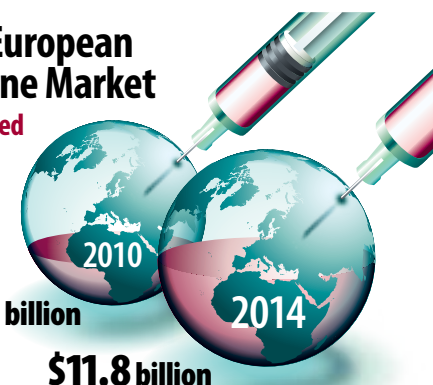
GEN

Genetic Engineering & Biotechnology News

The European Vaccine Market

Projected Total Sales

Source: Kalorama Information



\$7.9 billion

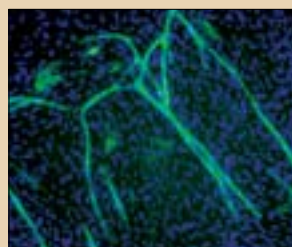
\$11.8 billion

Biotechnology from bench to business

Volume 31, Number 1 January 1, 2011

For more information see page 16

OMICS Drug Discovery Translational Medicine Bioprocessing Biobusiness



Enabling Tools Extend Range of HCA

Improved speed and depth bring the best of both worlds to screening. **p. 18**



Single-Use Systems Become the Norm

Disposables trade their limited-utility image for a new rep as versatile and economical tools. **p. 30**

> Bullet-Train Biotech and Reality

As DNA sequencing takes off, the community must determine the best ways to translate it to patient care. **p. 6**

> Forestalling Nano-technology Litigation

Knowing how to reduce risks is vital to avoiding legal trouble in this still-emerging field. **p. 8**

> European Vaccine Market Growing Briskly

Economic hardship may have reared its ugly head elsewhere, but not in this sector. **p. 16**

> Power in Numbers for Cancer Biomarkers

Effective therapies should target molecular signatures with multiple variants—once they are uncovered. **p. 40**

Adapting Protein Expression for HT



VTU Technology reports that its promoter library allows for tunable optimum protein expression.

The Best Genes, Well-Tailored Systems, and Ideal Growth Conditions Essential to Process

Josh P. Roberts

The three most important things when expressing protein for high-throughput applications are: optimize, optimize, optimize. Put the best genes you can into your expression system, use the most fitting expression systems, and make sure the growth conditions are suited to achieve the most from those systems.

Whether it's drug screening, crystallography, NMR, mass spectrometry, or binding and toxicity studies, the earliest decisions about choice of template and how to express it can have ripple effects throughout a project, points out Frank Schäfer, Ph.D., associate director of R&D, head of DNA and protein sciences at

See Protein Expression on page 34

Novel Methods Expedite Nucleic Acid Sample Prep

Technologies Focused at Both the Research and Clinical Markets Stir Excitement in the Field

K. John Morrow Jr., Ph.D.

Recent developments in genomic identification using microarray technologies are infiltrating the fields of forensics, diagnostics, and sample identification. They include a combination of new hardware designs and innovative approaches to sample processing. In addition, microfluidics and miniaturization are emerging as key components of powerful new multiplexing platforms. The upcoming "Pittcon" meeting will cover all of the latest advances.

A critical first step in genomic profiling is the preparation of high-quality DNA. Aurora Biomed's (www.aurorabiomed.com) product line automates genomic, proteomic, drug-discovery, and analytical applications. The Versa series comprises a group of liquid handlers ranging in throughput and size. These include the Versa Mini nucleic acid preparation workstation, while the Versa 1100 model with various deck modules is able to

See Sample Prep on page 24



Researchers at Brigham Young University are exploring the integration of sample-processing steps on microfluidic systems.

Sticky ends

▶ **Thermo Fisher Scientific** shelled out \$2.1 billion to take over Dionex... ▶ The **Presidential Commission for the Study of Bioethical Issues** released its first review of synthetic biology... ▶ **Sigma Life Science** generated knockin rats using its CompoZr[®] Zinc Finger Nuclease technology... ▶ **WaferGen** received a \$7 million investment and inked a research and equipment deal with **IBBL**... ▶ **Luc Montagnier** of HIV research fame became a professor at Shanghai Jiao Tong University in China... ▶ **Precision BioSciences** won a \$3 million NIH grant to enhance its meganuclease platform... ▶ **Stason** will use **Lonza's** gene-expression system with its TNT antibody platform... ▶ Personalized medicine entrepreneur **Patrick F. Terry** is launching and leading a global pricing & market access practice at **Scientia Advisors**... ▶ **ISCA** will use **Cartagenia's** Bench software to automate data acquisition for its CNV Atlas initiative... ▶ **Eli Lilly, Genentech,** and **Human Genome Sciences** entered separate discovery agreements with **Adimab**.

Sample Prep Continued from page 1

fully automate a range of liquid-handling applications, according to Sikander Gill, Ph.D., research scientist.

Dr. Gill describes the isolation of DNA from blood samples using Aurora's high-throughput Magnetic Binding Blood DNA Kit in combination with its Versa automated liquid handlers. This magnetic bead-based genomic DNA system can be used with a range of fluid samples, he says. This method allows the extraction of genomic DNA without the use of centrifugation. As the Versa platforms are designed for use with 96-well plates, they can perform multiplexing protocols, helping hold reagent expenditures to a minimum.

Dr. Gill cautions that some tissues are not appropriate for processing with the Aurora platforms. For example, paraffin-embedded tissues, plant materials with hard shells, and yeast cells with recalcitrant cell walls are challenging. However, Dr. Gill notes that there are kits available for processing these tissues. There are other accessories, such as the Bead Beater (a mechanical disruption apparatus using glass or zirconia-silicate beads), that avoid degradation of nucleic acids while effectively preparing the tissues for analysis.

"Our processing technologies avoid the use of centrifugation, as this adds cumbersome and expensive steps to nucleic acid preparation," Dr. Gill adds. "We also offer column-separation approaches as well as liquid-liquid (aqueous-organic) extraction procedures for preparation of nucleic acids and other cellular components."

Forensics

Zygem (www.zygem.com) is developing an integrated microfluidics system for forensic analysis, using the enzymes it has long been known for," according to James Landers, Ph.D., professor of biochemistry at the University of Virginia and also CSO of the company. Dr. Landers is investigating short tandem repeats using Zygem's microfluidics technology and hardware from Lockheed Martin.

Dr. Landers says that the most important feature of the system is its rapid turnaround time. The company has worked with Lockheed Martin to speed the analytical process to as little as 60 minutes, and Dr. Landers believes that this time frame can be abbreviated further. The partners have concentrated on the three key processes—DNA separation, amplification, and detection—miniaturizing them, reducing the required steps and time frame, and increasing their sensitivity and robustness.

The preparation chemistry takes advantage of an EA1 metalloproteinase from the company's extremophile collection, which can degrade tissues in buffer conditions compatible with PCR and is inactivated by a simple temperature shift. As a result of the temperature characteristics of EA1, DNA

extractions can be carried out at an elevated temperature in a single closed tube or as part of an integrated microfluidics system, improving its efficiency. Because EA1 is inactivated above 95°C, raising the temperature stops the reaction, leaving PCR-ready DNA available for downstream processing.

Just the PCR amplification process alone is ordinarily a three and a half hour process when using conventional technology. The Zygem approach bundles up all three steps in a monolithic format, expediting the time required, according to Dr. Landers. Using special patented polymers for the separation component, Dr. Landers and his colleagues are able to achieve a 30-minute separation in 400 seconds, while still maintaining single-base resolution. In this fashion they are able to progressively drive down the turnaround time.

The microfluidics device, which is the

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Zygem is developing an integrated microfluidics system for forensic analysis. This system, which is based on the enzymes that drive the company's business, will reportedly feature rapid turnaround time.

News GENOMICS & PROTEOMICS

Collectis to Leverage Evrogen's Fluorescent Proteins

Collectis bioresearch (www.collectis-bioresearch.com) entered a nonexclusive agreement with Evrogen (www.evrogen.com) of Moscow for the latter's fluorescent proteins. The license allows Collectis to incorporate these proteins into its own products and will expand the company's offering of genome-engineering tools.

Evrogen fluorescent proteins range in color from blue to far-red. They can be used for multicolor labeling to observe different cellular events in a particular cell or a cell population.

Ingenuity Systems and CLC bio to Synergize Analysis Products

Ingenuity Systems (www.ingenuity.com) and CLC bio (www.clcbio.com) joined forces to increase synergy between their software solutions. They hope to provide researchers performing both array and next-generation sequencing with the ability to more easily exchange data between CLC bio's genomic analysis products and Ingenuity's pathway analysis software (IPA). The first milestone of the partnership is focused upon streamlined workflows between CLC bio's CLC Genomics Server, CLC Genomics Workbench, and Ingenuity's IPA.

The CLC Genomics Workbench facilitates the analysis and visualization of next-generation sequencing data and helps streamline users' workflow. The CLC Genomics Server is a high-throughput sequencing-focused three-tier solution, offering bioinformatics computing

on a server-architecture located centrally in the client's organization. Ingenuity's IPA software aids in the modeling, analysis, and understanding of complex biological and chemical systems. It integrates data from a variety of experimental platforms and provides insight into the molecular and chemical interactions, cellular phenotypes, and disease processes of a system.

UCB to Utilize Activiomics' Platform in Inflammatory Disease Research

The University of California, Berkeley will employ Activiomics' (www.activiomics.com) Tiquas (targeted in-depth quantification of cell signaling) phosphoproteomics platform to elucidate signaling mechanisms of its therapeutic antibodies. Tiquas quantifies global kinase activity without the need for labeling or antibody isolation. It profiles and cross-compares phosphopeptides, ensuring accurate and reproducible data, Activiomics points out.

The technology works by using a protease to break down a cell or tissue extract into peptide fragments. Phosphopeptide enrichment, mass spectrometry, and Tiquas software allow thousands of phosphopeptides to be quantified, the firm adds.

U.S. Army Awards CHLA \$1.05M for Next-Gen Sequencing

Researchers at the Children's Hospital of Los Angeles (CHLA) have been awarded \$1.05 million by the U.S. Army to establish a core genome-profiling facility

focused on carrying out whole-genome sequencing to investigate the genetic basis of childhood diseases including cancer. CHLA claims the new unit will be the first of its kind to use next-generation sequencing specifically to study diseases in the young.

"With this award we will develop the infrastructure to create a center for precision medicine to enable genomic profiling of life-threatening disease in children and young adults," comments Timothy J. Triche, M.D., who is the recipient of the award. Dr. Triche is director of the Center for Personalized Medicine at CHLA.

PacBio Teams with Harvard to Sequence Haitian Cholera Strain

Scientists from Pacific Biosciences (www.pacificbiosciences.com) and Harvard Medical School have employed single-molecule, real-time (SMRT™) DNA sequencing technology to characterize the pathogen responsible for the recent cholera epidemic in Haiti. Published online in the *New England Journal of Medicine*, the results provide a whole-genome sequence analysis and genetic profile of the Haitian *Vibrio cholerae* outbreak strain.

The analysis confirms that the cholera pathogen now present in Haiti is closely related to the El Tor O1 variant from South Asia. Given that the existence of this strain has never been documented in the Caribbean region or throughout Latin America, the evidence suggests that the Haitian epidemic began as a result of the introduction of a new strain from a distant geographic source. n

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Sample Prep

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size of a microscope slide, is processed by an instrument about the size of a computer tower. Dr. Landers and his collaborators at Lockheed Martin look forward to a miniaturized system, the size of a laptop, and eventually to a handheld instrument for use in the field. However, at this point the platform is designed for a laboratory environment, so the size of the instrument is not a pressing concern.

It is not clear at this time where the technology will fit into the overall picture of forensic investigation. There is no question that the microfluidics analysis of short-term DNA repeats as a means of identification can speed processing and decrease the backlog of cases in forensic labs around the world. The unknown is whether it would be of value at a crime scene.

"We can envision a time in the future where our instrumentation could be used for onsite identification of a suspect," Dr. Landers suggests. However, there is no consensus today on whether such technology would be welcome in a chaotic venue where DNA from many individuals would be dispersed. "Only when the technology is available will we know how these approaches will fit into the needs of crime-scene investigators."

"Portability, speed, and cost of analysis will be welcomed by the genetic analysis community, but there are issues concerning the use of this technology in the field," Joan Bienvenue, Ph.D., a forensic scientist and program manager at Lockheed Martin, explains. "While you can find people on both sides of the fence, forensic typing for criminal purposes is presently carried out in incredibly well-controlled laboratory situations. The policy changes that would be required for use in the field are not trivial."

"Even in a very well-organized forensic laboratory, there are three to four instruments that are used, in an analysis that can take ten to twelve hours to process a DNA sample," explains Paul Kinnon, CEO. "So the first step will be to put our system into use, and as the technology evolves it will migrate into other functions. This will enable investigators to move forward efficiently and get on with their jobs."

The multiplexability of the technology opens up a wide range of possibilities for other applications. "With the system's rapid turnaround, it would be possible to run a molecular diagnostics panel on a patient and discuss the results with him in less than an hour, rather than sending the patient home and doing the consult days later," says Dr. Landers. "This could have a significant effect on management of patient anxiety while at the same time providing an opportunity for timely clinical intervention."

Large RNA Molecules

Three-dimensional structural studies of large RNAs (>50 nucleotides) are a particularly difficult task, notes Michael Summers, Ph.D., Howard Hughes professor at the University of Maryland, Baltimore. "Historically, x-ray crystallography has been the

approach of choice for investigating the 3-D structure of biomolecules, but RNAs do not yield easily to this technology. Large RNAs are negatively charged on their exterior and tend to be heterogeneous in terms of their conformation, all of which means they don't form good crystals."

RNAs are quite a different target as compared to protein molecules, which are much more easily analyzed through crystallographic techniques. In national data banks there are many more protein structures available than large RNA structures. To expand this slim catalog, one could employ nuclear magnetic resonance for the analysis, but this is also a problematic approach for the large RNAs.

While proteins contain approximately 20 amino acids, there are only four basic nucleotide building blocks in RNA. This means that while the spectral NMR signals are well defined for protein molecules, they



According to Aurora Biomed, its Versa 1100 automated, multichannel, liquid-handling system maximizes accuracy, precision, and throughput while minimizing time and consumable costs. It also offers flexibility in volume range, liquid-handling modules, deck modules, and labware adapters for custom applications used in specific protocols.

crowd together for the RNA molecules, with much overlapping, making the data difficult to interpret.

In nucleic acid NMR investigations, one must rely on aromatic signals for information since the repeated ribose molecule is too homogeneous. Because of the reliance on these ring structures, the use of C^{13} isotopes is not effective. For this reason, Dr. Summers' group turned to another strategy.

"We rely on an older technique, that is, using 2-D spectra with deuterated nucleotides," continues Dr. Summers. "We can deuterate specific nucleotides to improve our resolution. NMR is very good for obtaining local structural information regarding the location of hydrogen atoms. This works well with proteins, but with nucleic acids it is more difficult, since the hydrogen atoms are sequestered in the middle of the helices and not easily resolvable. So we combine the high-resolution NMR with low-resolution global structural data from cryoelectron tomography."

Dr. Summers is particularly concerned

with how viral genomes assemble in the cell, and for addressing the replication cycle of HIV, a knowledge of the 3-D relationships of the RNA and protein molecules is essential. Dr. Summers argues that the regulation of the assembly process is guided by a specific, highly conserved region within the RNA genome. Antiretroviral drug design depends on a detailed understanding of the 3-D structure of the HIV RNA and protein molecules.

"At present, we don't know how the assembly process works at the atomic level. However, we are aware that all of the regulation of replication is guided by genes in the 5' untranslated portion of the genome," Dr. Summers adds.

It is known that during the process of retrovirus assembly, RNA plays many different roles, as it must be spliced and packaged in a variety of ways in order to produce functional viral particles. Dr. Summers' investigations are focused on basic science at this point, but

it is clear that in the future these findings will contribute to new therapeutics for the control of RNA viral-based diseases.

On-Chip Biomarker Extraction

"While nucleic acid biomarkers are useful for determining the type and stage of cancers, their quantification requires a series of isolation and amplification steps that can take considerable time," says Adam Woolley, Ph.D., professor of chemistry and biochemistry at Brigham Young University. "To overcome these drawbacks, we have explored the integration of sample-processing steps on microfluidic systems."

It is generally accepted that cancer screening that takes advantage of the simultaneous quantitation of multiple biomarkers will yield a more sensitive and accurate profiling of malignancies and a concomitant improvement in cancer mortality and morbidity statistics.

For example, while the prostate specific antigen (PSA) is perhaps the most widely adopted test for the early detection of can-

cer, as a single marker it has a high rate of both false positives and false negatives. Moreover, the PSA test provides no information on the aggressiveness of a tumor, so the result may be that the patient is subjected to costly and unnecessary therapies that offer no benefits, while potentially compromising quality of life.

Most biomarkers are detected using ELISAs, whose performance could be enhanced through extensive validation and quality control. But this would require a multiplexing system, and Dr. Woolley argues that microfluidic format could provide higher speed and lower reagent consumption. "Many process steps, including sample desalting, labeling, and extraction have been successfully performed in microchip systems. The simultaneous monitoring of multiple biomarkers has the potential to greatly improve the quality of the diagnosis."

Dr. Woolley believes that although the 96-well immunoassay technology is effective for analyzing large numbers of samples, it is an inefficient approach to smaller volumes, which can be monitored much more economically using the microfluidics approach.

His team has constructed microfluidic devices employing poly(methyl methacrylate) with monolithic columns for immunoaffinity extraction. Using a laser-induced fluorescence detection system, they have quantified a feto protein, a biomarker for liver cancer, down to 1 ng/mL levels in serum samples.

They have now applied the solid-phase extractors for sequence-specific nucleic acid biomarker determination. Monolithic columns were made using glycidyl methacrylate (GMA) and ethylene glycol dimethacrylate. The reactive GMA epoxy groups were employed for the functionalization of the monolith with amine-terminated DNA probes complementary to the target nucleic acid fragment.

Using these columns, they extracted and eluted fluorescently labeled 20-mer target single-stranded DNA fragments in as little as 15 minutes, according to Dr. Woolley. "We tested a number of approaches for eluting the fragments," he continues, "and settled on dilute (1.5–7.0 molar) urea as the best option. We are extending our investigations to urine as a noninvasive approach to cancer biomarker detection."

Benefits Accrued

In an era in which cost cutting is all the rage, these new technologies are to be particularly welcomed. While the saying "time is money" lacks originality, it is particularly relevant here, as these technologies are focused at both the clinical and research markets. Pressure from federal healthcare regulation and the demands of the consumer market makes rapid and economic nucleic acid preparation especially alluring.

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SRM Atlas, an informatics resource, facilitates setup of SRM/MRM quantification assays of targeted proteins by providing access to these parameters. Created by ordering more than 150,000 synthetic peptides based on 20,300 proteins, the resource generated high-resolution accurate mass spectra on Q-TOF LC/MS systems to create MS/MS profiles for peptide library searching and fragmentation data.

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- High-throughput automated sample prep for large-scale biomarker validation
- Introduction of specificity, sensitivity, robustness, and reproducibility into biomarker validation studies
- SISCAPA technology for enhancing peptide enrichment
- Multiplexed protein assays using triple quad LC/MS
- Workflows and instruments for achieving high sensitivity

Who Should Attend

- Scientists involved in drug discovery and development as well as biomarker discovery and validation
- Researchers applying biomarker research to molecular diagnostics, therapy monitoring, or personalized medicine
- Proteomic scientists
- Genomic researchers interested in qualifying protein levels
- Mass spectroscopists working in life science laboratories

Panelists Include

Robert Moritz, Ph.D., Professor and Director of Proteomics, Institute for Systems Biology
Leigh Anderson, Ph.D., Founder and Chief Executive Officer, Plasma Proteome Institute
Christine Miller, Senior Application Scientist, Agilent Technologies

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