

DRUG DISCOVERY

RNA Plays a Role in Neurological Disorders

New Products are Designed for Analysis

Ilene Schneider

At the "34th Annual Society of Neuroscience" meeting, several symposia addressed RNA-based mechanisms involved in neurological disorders. For instance, investigators at several institutions—Rockefeller University (New York City), Emory University (Atlanta), and the University of Pennsylvania (Philadelphia)—reported on their research on identifying the messenger RNA (mRNA)

involved in fragile X syndrome, the most common inherited form of mental retardation.

Other scientists reported on their research on diseases that result from defects in RNA regulation, including several autoimmune diseases and spinal muscular atrophy. Researchers from the University of Utah (Salt Lake City) described RNA editing and RNA interference in the context of neurological disorders.

Automated Electrophoresis

Many of the exhibitors at the conference also were involved in analyzing RNA. **Bio-Rad Laboratories** (www.bio-rad.com) introduced its new Experion™ system, an automated electrophoresis

system that uses **Caliper Life Sciences'** (www.caliperls.com) LabChip® microfluidic technology to speed and automate RNA separation and analysis.

The objective is to achieve "substantial time and labor savings, without sacrificing sensitivity or resolution," explained Gus Salem, Bio-Rad division manager for protein separations.

An Experion system run for 1 to 12 samples typically lasts 30 minutes, compared with 2 or 2 1/2 hours using conventional mini gels, according to Salem.

The automated electrophoresis system enables unattended separation, staining, destaining, imaging, band detection, and data analysis and provides error-free chip priming, minimal hands-on time, low consumption of samples and reagents, and minimal handling of hazardous chemicals. After basic sample preparation, the researcher inserts the primed and loaded chip into the instrument, closes the lid, and waits for the results.

Experion includes an automated priming station, a vortex station to mix reagents, and the electrophoresis station. Three kits are available: standard- and high-sensitivity kits for RNA analysis and one for protein analysis.

The system enables separation profiles of mRNA and total RNA samples down to picogram amounts and quantitates concentrations in nanogram amounts, Salem noted. The ribosomal RNA ratio that is automatically calculated provides additional information on the purity of the total RNA.

"Accurate assessment of RNA

purity is essential to the success of quantitative RT-PCR and microarray experiments," Salem added. "Experion RNA analysis kits offer single-step RNA purity determination."

The kits include 10 or 25 RNA chips, a high-quality gel matrix, fluorescent dye, and an RNA ladder for more uniform peak heights that provide accurate sizing, and quantitation. In addition, there are optimized loading buffers and spin filters for gel preparation.

A protein kit includes 10 or 25 protein chips, electrode cleaner, molecular weight ladder, gel matrix, sample buffer, dye concentrate, and spin filters. Components of all three kits are also available separately.

Experion software provides data analysis and instrument control by means of intuitive control of experiments using workflow icons and pull-down menus. The interface screen displays results in both peak electropherogram and gel views to accommodate a variety of user preferences and requirements.

The software generates sizing and quantitation results. Optional software tools to be introduced in 2005 will support 21 CFR 11 compliance, as well as other regulatory protocols.

Users can access purity, experimental changes, or biological conditions with information automatically calculated in the results table. Automated data capture generates separation results. Data analysis tools, the company explained, help researchers derive additional information from the

data, such as query-based comparisons within the chip that enable statistical analysis of specific samples.

Caliper's LabChip systems reportedly provide automation and throughput, from small benchtop systems employing single-use disposable chips to automated drug discovery screening systems with walkaway automation and chips that perform thousands of experiments.

Other RNA Tools

U.S. Genomics (www.usgenomics.com) developed "single molecule biology" technology for the direct detection of biological molecules including RNA, DNA, and proteins. The platform affords rapid throughput, requires no amplification, and enables low sample volumes, according to Faye A. Boeckman, Ph.D., marketing manager.

The Trilogy instrument family combines microfluidics and optics to achieve rapid single molecule detection, she added. Trilogy focuses multiple lasers in the interrogation zone of the microfluidic channel through which sample flows.

Target molecules of interest are tagged with sequence-specific probes that emit various color fluorescence once excited by the lasers. Because the system allows for color coincidence detection of tags on a target molecule, it can reportedly be used to minimize background and enhance target specificity.

Using high-speed, photon-burst detection of the rapidly flowing sample, Trilogy counts individual target molecules with high sensitivity even in a complex background, according to Dr. Boeckman. The system requires no amplification and provides an integrated software system that enables fast data capture and flexible analysis.

Invitrogen (www.invitrogen.com) exhibited its Stealth™ RNAi and Block-iT™ RNA tools. "RNAi is a fast, effective method for analyzing loss of function," explained Kerry E. Lowrie, Ph.D., business area manager.

"RNAi-specific inhibition of gene expression is mediated by a short double-stranded RNA called short interfering RNA or siRNA."

Cleaving the target message for degradation results in loss of protein and its function, according to Dr. Lowrie. Enabling researchers to obtain information about gene function quickly, RNAi works in any cell or organism.

Critical RNAi success factors for experimental design include efficacy, specificity, and delivery, Dr. Lowrie said. "Stealth RNAi—a synthetic, chemically modified double-stranded RNA that pro-

VEC TECHNOLOGIES, INC.
THE VASCULAR ENDOTHELIAL CELL COMPANY™
 One University Place, Roseland, New York 12144-3476
 518 257 2010 • Fax: 518 257 2012 • Email: vec@vec.com
 www.vec.com

DESIGNATED provider for NCI awardees

HUMAN ENDOTHELIAL CELLS	BOVINE ENDOTHELIAL CELLS
DERMAL MICROVESSEL	LUNG MICROVESSEL
INTERNAL MAMMARY ARTERY	ADRENAL MICROVESSEL
SAPHENOUS VEIN	RETINAL MICROVESSEL
UMBILICAL VEIN	SKELETAL MUSCLE MICROVESSEL
UMBILICAL ARTERY	PULMONARY VEIN
PERIODONTAL VEIN	ADRENAL
	KIDNEY GLOMERULAR MICROVESSEL

HUMAN ENDOTHELIAL CELLS	ENDOTHELIAL CELL MEDIUM
AORTA	MCDB 131 COMPLETE MEDIUM
LUNG MICROVESSEL	
HEART MICROVESSEL	

HUMAN AORTA SMOOTH MUSCLE CELLS	ENDOTHELIAL CELL GROWTH FACTOR
HUMAN DERMAL FIBROBLASTS	ENDO GROW™

INTRODUCING
 HUMAN HEART MICROVESSEL ENDOTHELIAL CELLS
 Available Internationally

Circle 67 on Reader Service Card



MicroCal

Real binding affinities
 All sizes from protons to proteins
 Selective and cross-reactive binding
 Mechanism of Action
 All without immobilization or labeling

more than just binding affinities

discover the power of ultrasensitive calorimetry at:
www.microcalorimetry.com/binding

Circle 56 on Reader Service Card

vides immediate, specific gene inhibition without nonspecific responses—provides highly effective knockdown, increased specificity, minimized toxicity effects, and enhanced stability in serum.”

Ambion (www.ambion.com) exhibited products for analyzing and processing micro RNAs (miRNAs) and small-interfering RNAs (siRNAs). “Micro RNAs are small regulators with global impact,” according to Scott Mogull, marketing manager.

The miRNAs are evolutionarily conserved, small noncoding RNA molecules that regulate gene expression by binding to and modulating the translation of specific mRNA. They are said to be critical in the development of organisms, differentially expressed in tissues, involved in viral infection processes, and related to oncogenesis genes.

There are reported links between miRNAs and brain development, chronic lymphocytic leukemia, and colonic adenocarcinoma. Ambion's mirVana™ products are used to isolate and quantify miRNAs in tissue and cell samples. They have been used to analyze a variety of normal and abnormal tissue samples, including human brain subregions.

Routinely used to establish and confirm gene function in mammalian cells, siRNAs can identify genes involved in cellular pathways and processes. Ambion can measure how siRNA concentration, siRNA pooling, cell type, and time between transfection and analysis influence the final screening data.

The results provide a framework for setting up functional screens with siRNA libraries and show how to use the approach to correlate genes with cellular functions.

Using siRNAs targeting kinases, GPCRs, phosphatases, transcription factors, and proteases, Ambion identified genes involved in apoptosis, cell proliferation, interferon response, and cell cycle from both whole cell assays and high-content screening assays.

In addition, Ambion developed two siRNA delivery procedures to optimize transfection and electroporation. The first method, which involves simultaneously plating and transfecting cells to reduce

time and improve efficiency, facilitates high throughput applications such as library screening and long-term knockdown.

The second method, which was developed for primary, neuronal and suspension cells, overcomes problems with cell viability that are inherent in mammalian cell electroporation.

siRNA Technology

Upstate Cell Signaling Solutions (www.upstate.com) also exhibited siRNA technology and applications. The introduction of long double-stranded RNA (dsRNA) into mammalian cells can activate the interferon

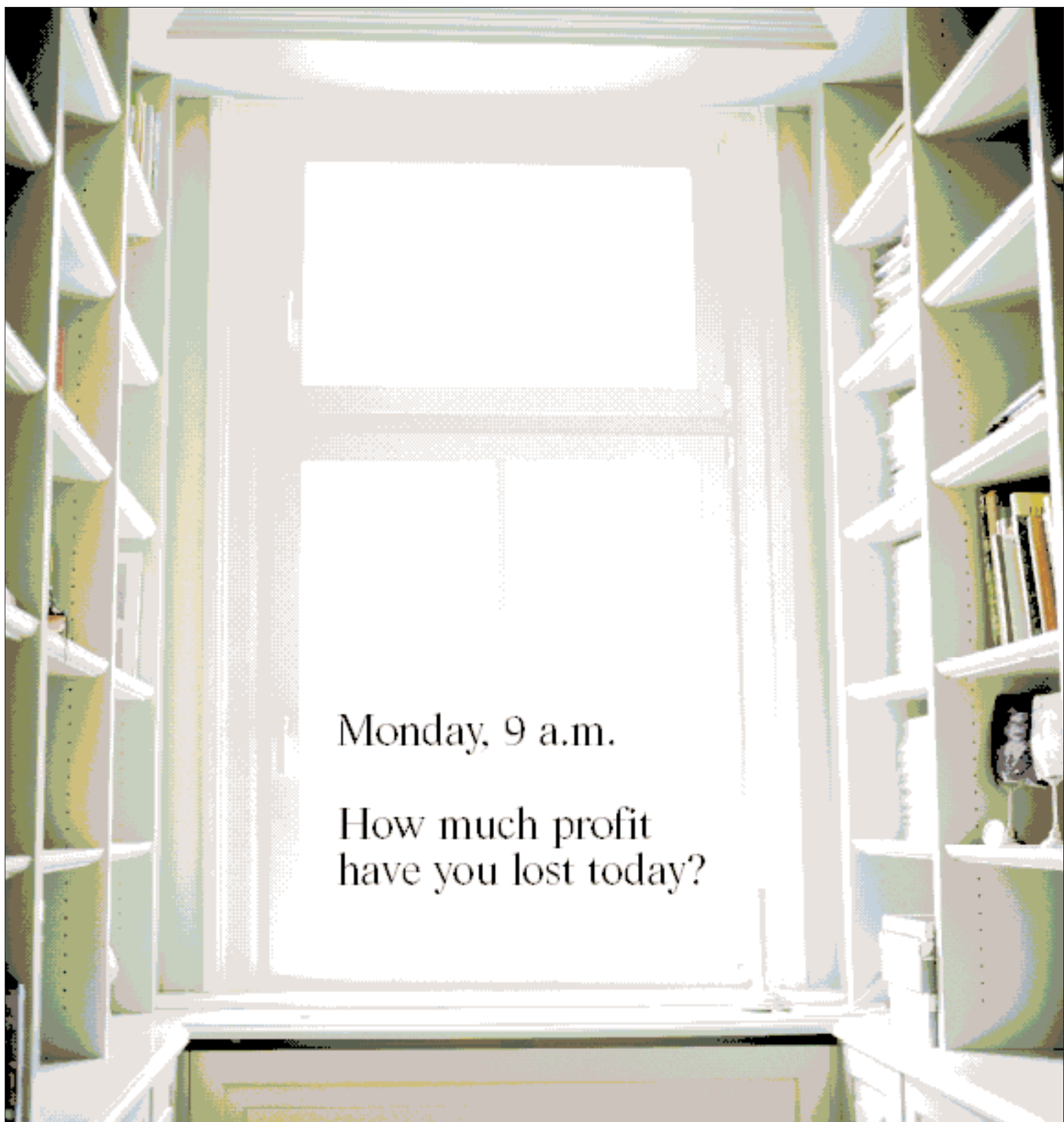
response, causing the cell to shut down its protein translation machinery. By introducing siRNA, the interferon response can be bypassed.

Upstate offers a variety of products, including the siImporter transfection reagent to co-transfect siRNA and plasmids together, customer-configured siRNA antibody kits that pair siRNA with any antibody, pKD siRNA mammalian expression plasmids, and preconfigured siRNA/ siAb assay kits.

By using siImporter, researchers can deliver high-efficiency nucleic acid into cells with little to no toxicity, according to company literature. GEN



Bio-Rad's Experion automated electrophoresis platform.



Monday, 9 a.m.

How much profit
have you lost today?

With the biopharmaceutical industry subject to a world of constantly changing regulations, how can your company stay up-to-the-minute compliant – and still remain competitive? Enter, the BioPharm-Alliance. By conducting careful analyses of how your company addresses the industry's critical success factors – time-to-market, cost-of-goods, production capabilities, and regulatory compliance – the BioPharm-Alliance's team of experts will help your company optimize processes, increase capacity, and lower costs.

See how the BioPharm-Alliance can help your company make every minute count. For more information, call 1-888-BPA-8522, visit www.biopharmalliance.com or e-mail profit@biopharmalliance.com.



Visit
GEN's
Website

Updated Daily

www.genengnews.com