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Proteomics Researchers Wait as FLEXGene Readies Cloning Consortium

HAVING RAISED $500,000 and spent almost one year developing a protocol for building a library of sequence-verified, expression-ready clones of human genes, the FLEXGene Consortium seems poised to make protoemics researchers' lives a whole lot easier. After all, who wouldn't want to shave a month or two off the time it takes to clone a gene for expression, and still feel confident the protein is exactly what you think it is?

Josh LaBaer, the driving force behind the project and the director of the Institute of Proteomics at Harvard Medical School, is working busily to make that happen. He and the other members of the consortium's scientific advisory panel — financial contributors whom he declines to name — are in the midst of completing experiments to test various commercial and academic gene expression technologies, and at the same time completing the business model for the non-profit consortium before heading out to solicit donations.

But still this is just the beginning. Although most researchers would agree on the need for a comprehensive repository of full-length cDNAs, the cost of carrying it out — most notably with respect to verifying that each cDNA has the correct sequence—is somewhat daunting. (LaBaer's estimates hover around $53 million.) Furthermore, the logistics of the operation are somewhat complicated, given the challenge of continued on page 5

Starting from Scratch, Lynx Therapeutics Aims To Make 2D Electrophoresis Fast and Fluid

PROMISING to alleviate some of the shortcomings of 2D gels, Lynx Therapeutics is developing a two-dimensional microchannel electrophoresis system that may reverse — quite literally — a few conventions in the process.

The system, called Protein ProFiler, is still in development, but Lynx has already been drumming up interest at meetings, including this year's HUPO conference in San Diego. It aims to improve reproducibility, protein recovery, resolution, sensitivity, and signal dynamic range for differential protein expression studies. "We are very excited by the prospect of this system [eventually] replacing all 2D electrophoresis," said John Wiktorowicz, director of proteomics at Lynx and a former group leader for bioseparation at Applied Biosystems. But as of now, no data continued on page 6

News SPOTS

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NHLBI PROTEOMICS

JOHN WIKTOROWICZ JOINS PROTEOMICS GROUP IN GALVESTON

In July the University of Texas Medical Branch recruited John Wiktorowicz, PhD, Associate Professor, to be the Assistant Director of the UTMB Biomolecular Resource Facility and the Director of the Proteomics Core.

John previously was Director of Proteomics at Lynx Therapeutics where he directed the development of ProFiler™, an all-liquid 2D electrophoresis system. Prior to his six years at Lynx, he was the R & D Section Leader/Senior Staff Scientist at Applied Biosystems.

In announcing the recruitment, Dr. Alex Kurosky, Principal Investigator of the UTMB Proteomics Center, said, "John Wiktorowicz adds a considerable amount of proteomics expertise to our center." More information about the UTMB Proteomics Center is available at http://www.bioinfo.utmb.edu/proteomics/NHLBI.

SYMPOSIUM AT BOSTON UNIVERSITY'S CARDIOVASCULAR PROTEOMICS CENTER HIGHLIGHTS OXIDATIVE POST-TRANSLATIONAL MODIFICATIONS IN THE CARDIOVASCULAR SYSTEM

The Cardiovascular Proteomics Center of Boston University School of Medicine hosted a symposium Oct. 6-8, 2004, on "Oxidative Post-Translational Modifications in the Cardiovascular System," a new field integrating physiology, pathology, biomedical science, oxidative chemistry, and bioanalysis in the study of reactive oxygen and nitrogen species in the cardiovascular system. The conference was the first of a series planned at the Boston Center, whose focus is this very theme.

Scientists from the United States, United Kingdom, Spain, Portugal, Japan, and Australia discussed multiple oxidant modifications and multiple oxidant species and debated the use of proteomics and protein chemistry instrumentation to 'dive deep' or 'screen' for oxidative modifications. They noted that with new mass spectral methods for detecting protein modifications, the ability to screen broadly for multiple modifications will not be as onerous as it has been in the past, when investigation was limited by the availability of specific reagents.

Conference investigators also opened discussion on the criteria that make for a physiologically relevant oxidative post-translational modification, one that is responsible for functional effects or for the progression of disease.

The three-day format featured oral presentations, posters, and an educational session with nomination of best student/trainee presentations. Amanda Isom, of the University of Alabama; Tyler Heibeck, of Boston University; and Matthew West, University of Louisville, were cited for excellence.

A highlight of the inaugural meeting was Medical Grand Rounds given by Jay W. Heinecke of the University of Washington on Friday, Oct. 8. His talk probed the question, "Is Atherosclerosis an Inflammatory Disease?"

The next conference on "Oxidative Post-Translational Modifications in the Cardiovascular System," is planned for 2006.

For more highlights of the recent symposium or information about the Center, its publications and future conference opportunities, visit the Center's website at http://www.bumc.bu.edu/Dept/Home.aspx?DepartmentID=382.

The website features publications produced by center personnel. A recent example, presented at the conference, is:

Sethuraman M, McComb ME, Heibeck T, Costello CE, Cohen RA. (2004) Isotope-coded Affinity Tag Approach to Identify and Quantify Oxidant-sensitive Protein Thiols. *Molecular and Cellular Proteomics* 3: 273-278.

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structure is put in place, LaBaer hopes to start producing clones sometime this year, and in theory scientists should have access to the clones through distributors shortly thereafter.

As far as the final cost, LaBaer had this to say: "Although it is a lot of money, and I don't want to belittle the amount of money because it is substantial, it's a whole lot less than the Human Genome Project — by orders of magnitude."

— JSM

Lynx...

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demonstrating the system's capabilities have been published in a scientific journal, and Lynx is currently seeking collaborators to evaluate the technology.

"We did have the luxury of having a clean sheet of paper" to start, said Wiktorowicz, one of the inventors of the new electrophoresis apparatus, which is protected by two patents. The device itself consists of two 6 x 6 inch sealed glass plates with etched inner surfaces, thus forming a system of intersecting internal channels of 100 microns in diameter. A single channel, filled with a liquid gel of entangled polymers, defines the first electrophoresis dimension, and in contrast to conventional 2D gels it separates proteins largely by size, not charge.

Samples are loaded from an injection complex at the edge of this channel; perpendicular to this and attached to it like teeth to a comb is an array of buffer-filled channels for the second dimension of separation: isoelectric focusing. Molecules covalently bound to the walls of these channels produce stable pH gradients.

Protein samples are fluorescently labeled prior to the run, an approach similar to Amersham Bioscience's two-dimensional gel electrophoresis labeling technique (2D DIGE). Three differently colored labels allow Lynx to run two protein samples simultaneously for differential analysis, as well as a panel of internal peptide standards.

The labeled proteins can be traced during the run and spotted at the end with a CCD camera. After they have reached their final positions in the pH gradient, bands selected for recovery are coupled to beads in the buffer that contain a photoreactive group. When the channel content is pushed out, the bead-coupled proteins are bound to a filter and can be identified by mass spectrometry, or subjected to other procedures like Western blotting.

According to Wiktorowicz, the system has several advantages over conventional 2D gel electrophoresis: separating the proteins in liquid phase instead of a crosslinked gel simplifies their recovery, and using intersecting channels means no movement of equipment is necessary to switch from one dimension to the other.

Heat dissipation in the channels is very efficient, allowing for higher voltages, better resolution and much shorter run times. Using isoelectric focusing as the second separation step instead of the first means that diffusion does not limit the final resolution and proteins will not run off the plate.

Furthermore, the gradient-forming chemicals are currently stable enough to perform about 10 runs, thus increasing reproducibility, Wiktorowicz said. Finally, the linear dynamic range of the fluorescent label can reach up to five orders of magnitude, more than other staining methods, and its sensitivity is 70 times higher — for half-second exposures — than that of silver staining. Since a researcher can monitor the proteins during the run, the switch to the second dimension can be made according to the position of the peptide standards.

However, Wiktorowicz admitted that some of the limitations of 2D gel electrophoresis remain, such as sensitivity to ion concentration and the tendency for some proteins to precipitate at their isoelectric point. But, he said, "everything is labeled, so you can watch what happens."

Peter James, a professor at Lund University in Sweden who has heard Wiktorowicz give presentations on the technology, noted further that the system "suffers all the problems of standard 2D," including problems with high and low pI proteins, large and small proteins, and membrane proteins.

So far Lynx has tested its apparatus mostly with well-characterized 1 kDa peptide standards. To analyze more complex protein samples, Wiktorowicz said, prefractionation is necessary to remove highly abundant proteins.

At the moment, Lynx is looking for biological examples to demonstrate the instrument's capabilities, both in-house and through collaborations. Wiktorowicz declined to give a time-line for commercialization but noted that someone other than Lynx might be selling the instrument. "Our goal is of course to get it out there as soon as we possibly can because we recognize the need for it," he said.

— JK