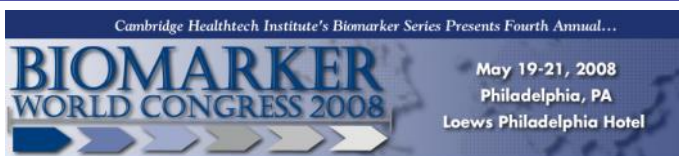


Prestigious Independent Researchers Underscore the
Advantages of Pressure Cycling Technology for Sample Preparation at
CHI's BioMarker World Congress & G.O.T. Summit



Paul H. Pevsner, M.D.

*Research Associate Professor of Pharmacology,
New York University School of Medicine*

**Imaging MALDI vs. Histologic Imaging of Cancer
of the Colon: A New Diagnostic Paradigm**

Monday, May 19, 2:00 to 2:30pm

Abstract:

Imaging of colorectal carcinoma biopsies by histology and imaging MALDI revealed abnormal chemical margins beyond the traditional margins identified by histopathology. The tumor biomarkers in the chemical margins were confirmed by protein extraction with a Barocycler, and mass spectrometry. The same proteins were found in the histologic tumor and in the normal histologic margins beyond the tumor. This new chemical margin may represent a genetic field defect and potential zone of new malignancy. The findings and implications for colorectal tumor diagnosis and staging will be presented.

Application Focus

***Gene Expression Analysis by Microarray
Using RNA Extracted by
Pressure Cycling Technology (PCT)***

(Continued on Page 2)



Dr. Alexander Ivanov

*Research Scientist, Director, Harvard NIEHS Center for
Environmental Health Proteomics Facility,
Harvard School of Public Health*

**Comprehensive Analysis of White Fat Adipose
Tissue Using Detergent-Free Protein Extraction
by Pressure Cycling and High Resolution Tandem
Mass Spectrometry**

Tuesday, May 20, 11:00am

Abstract:

Fat adipose tissue plays a key role in energy metabolism, lipid synthesis and secretion of signaling proteins linked to obesity, insulin resistance, inflammation and other physiological complications. Efficient proteomic analysis of adipose tissue is highly valuable for studies of these diseases. Fat adipose tissue contains up to 80-90% lipids, which makes conventional detergent-based protein solubilization and extraction methods inefficient. This study was enabled by the use of alternating hydrostatic pressure and specialized organic solvents for disruption of cells, micelles and membrane fragments and efficient protein recovery from lipid-rich adipose tissue followed by 1D- and 2D-SDS-PAGE and protein identification by liquid chromatography and high performance tandem mass spectrometry.

CALENDAR OF EVENTS

BIOMARKER WORLD CONGRESS 2008
PHILADELPHIA, PA
MAY 19-21, 2008

G.O.T. SUMMIT
BOSTON, MA
MAY 19-21, 2008

2008 NEW ENGLAND MANUFACTURERS ASSOCIATION
LONGWOOD BIOTECH EXHIBIT
LONGWOOD GALLERIA, BOSTON, MA
MAY 22, 10AM TO 3:00PM

ASM 108TH GENERAL MEETING
BOSTON, MA
JUNE 1-5, 2008

ASM 108TH GENERAL MEETING
DENVER, CO
JUNE 1-5, 2008

ADA 68TH ANNUAL SCIENTIFIC SESSIONS
SAN FRANCISCO, CA
JUNE 6-10, 2008

BIO 2008
SAN DIEGO
JUNE 17-20, 2008

Dr. Alexander Lazarev

Vice President, R&D, Pressure BioSciences, Inc.

CHICAGO BIOMEDICAL CONSORTIUM

THE UNIVERSITY OF CHICAGO
UNIVERSITY OF ILLINOIS AT CHICAGO
NORTHWESTERN UNIVERSITY



The CBC Course in Proteomics and Informatics

College of Medicine Research Building
Room 7175 909 South Wolcott Avenue,
Chicago, IL 60612

May 15, 2008, 11:40 A.M. – 12:00 P.M.

Proteomic Applications of Hydrostatic Pressure

Abstract:

The ability for high hydrostatic pressure to be used as a thermodynamic parameter has been known for several centuries. However, its practical applications in chemistry, and in particular the life sciences - have only begun to be understood in the past decade or so. Unlike temperature, high pressure is considerably more difficult to generate and contain, thus making research studies in this area very difficult. To that end, however, recent breakthroughs in material science have enabled the ability to precisely control high hydrostatic pressure on the laboratory bench, which has led to significant increase in understanding the potential benefits of high pressure in life science research.

This seminar aims to present an overview of applications of high hydrostatic pressure in bioscience and biotechnology, ranging from analytical sample preparation, separations, pathogen inactivation, vaccine development, and in the control of macromolecular conformation and interactions. Particular emphasis will be given to applications of alternating hydrostatic pressure termed Pressure Cycling Technology (PCT), where hydrostatic pressure repeatedly oscillates between atmospheric and hundreds of megapascals within seconds. The thermodynamic and physiochemical impact of oscillating pressure on cells, tissues, and other complex multi-phasic systems will be discussed. Examples of protein, lipid, DNA, and RNA extracts from several cultured cells and various tissue types will be presented.

Continued from page 1

Application Focus

Gene Expression Analysis by Microarray Using RNA Extracted by Pressure Cycling Technology (PCT)

In molecular tests, mRNA is often converted using reverse transcriptase (RT) *in vitro* to form complementary DNA (cDNA). The resulting cDNA can then be amplified in a polymerase chain reaction (RT-PCR), where it is less prone to degradation and therefore more suitable for analysis. The resulting cDNAs can then be evaluated in hybridization reactions such as in microarrays. However, the first critical step in the preparation of cDNA is the release of mRNA from the cell with minimal perturbation. Unless the mRNA is introduced into the RT reaction in sufficient copy number and high quality, there may be a bias in both the resulting representation and quantification, thus making interpretation by a microarray more difficult.

Application Focus (Cont')

To reduce the likelihood of bias being introduced during sample preparation, PBI has developed techniques based on pressure cycling technology (PCT) to release mRNA from cells and tissues.

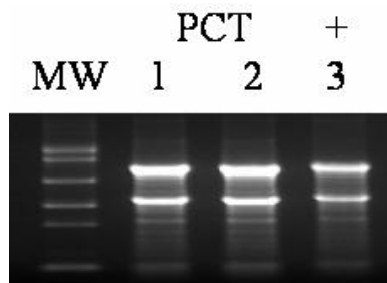


Fig. 1. RNA extracted from rat brain examined by formaldehyde agarose gel electrophoresis. MW depicts molecular weight marker, Lanes 1 and 2 are duplicates of rat brain extracted by PCT, and Lane 3 is rat brain extracted by mortar and pestle

Results and Discussion

Data show that the PCT SPS yields quality and quantities of RNA equal to or better than conventional extraction procedures, such as mortar and pestle grinding. High quality, reproducible total RNA was extracted using the PCT SPS as indicated by the rRNA bands in the formaldehyde agarose gel (See Fig. 1).

Total RNA prepared by the PCT SPS and subsequently purified by the Tri Reagent Protocol was successfully used to prepare a cDNA library. The resulting cDNA was analyzed on microarray assays [2]. In this experiment, cDNA derived from PCT extracted RNA produced a high quality microarray with spots of greater intensity than those produced from RNA extracted by mortar and pestle (See Fig. 2A and 2B).

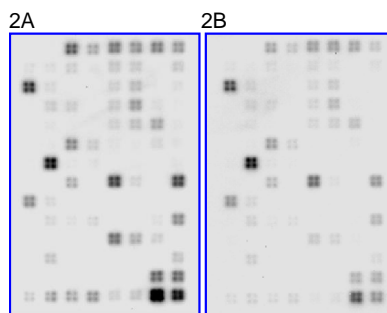


Fig. 2 (A) cDNA microarray analysis derived from RNA extracted by the PCT SPS (B) cDNA microarray analysis derived from RNA extracted by mortar and pestle

In comparison to the mortar/pestle extracted RNA sample (positive control), the PCT extracted RNA was of better quality and produced a superior microarray, as indicated by the relative intensity of spots on the microarray.

The PCT SPS provides a standardized method for preparing mRNA suitable for use with sophisticated analyses, such as microarrays. Data show that the integrity of fragile molecules, such as mRNA, is maintained during extraction by the PCT SPS.

Furthermore, the PCT SPS extraction method is compatible with standard downstream purification processes. RNA extracted by the PCT SPS is suitable for rtPCR and may be coupled with other techniques, such as amplification.