

ProteoSolve_{LRS}

Award Winning ProteoSolve_{LRS} Technology Played a Significant Role in the Identification of Potential Biomarkers in Breast and Colon Cancer Tissue

Richard T. Schumacher,
President and CEO of
Pressure BioSciences, Inc.
Accepts the 2007 Frost & Sullivan
North American Technology
Innovation Award for
ProteoSolve_{LRS} in Orlando, FL.



As announced on November 27, 2007, Frost and Sullivan named Pressure BioSciences the recipient of the 2007 North American Frost and Sullivan Award for Technology Innovation. The Award recognized PBI for the development of a method for the detergent-free extraction of proteins from lipid-rich tissues. Each year, Frost & Sullivan presents the North American Technology Innovation Award to the company that has demonstrated excellence in new products and technologies within their industry.

CALENDAR OF EVENTS

PLANT & ANIMAL GENOMICS XV
SAN DIEGO, CA
JANUARY 12-16, 2008

LABAUTOMATION 2008
PALM SPRINGS, CA
JANUARY 27-29, 2008

Dr. Paul H. Pevsner
New York University School of Medicine
Department of Pharmacology

Presented four posters at the LC-MS Course and Symposium at Robinson College in Cambridge, England

Scientists at New York University (NYU) School of Medicine have reported that their use of the Company's patent-pending, award winning technology (ProteoSolve_{LRS}) was in large part responsible for the identification of potential biomarkers in breast and colon cancer tissue. ProteoSolve_{LRS} is the Company's recently-released method for the detergent-free extraction of proteins from lipid-rich and other tissues. Results of the studies were presented at the LC-MS Course and Symposium, Robinson College, Cambridge, England by Dr. Paul H. Pevsner of the NYU School of Medicine Department of Pharmacology.

Dr. Paul H. Pevsner, principle investigator for the research studies, said: "Our studies showed that the combination of cycled high pressure (PCT) with the ProteoSolve_{LRS} kit, used in conjunction with other instrumentation in our laboratory, allowed us to identify potential biomarkers of breast and colon cancer. This is a significant finding, since these potential biomarkers may prove to be important indicators of disease detection and progression. It is possible that these findings may alter the current paradigm of histopathology tissue diagnosis for tumors. In the future, examination of biopsy tissue may require not only histopathology, but also mass spectrometry for complete diagnosis."

Dr. Pevsner continued: "The combination of PCT and ProteoSolve_{LRS} is particularly suited for the study of the small, often nano-quantities of samples that are usually available for proteomic studies. The combination of this method with mass spectrometry instrumentation may become a method of choice for bio-molecular identification, not just in cancer, but in neurological and coronary diseases as well."

Posters Presented

- **Growth Inhibition of Retinoic Acid Treated MCF-7 Breast Cancer Cells-Identification of Sox 9 and other Proteins**
- **Colorectal Carcinoma - Field Defects in Satellite Tissue**
- **in-Vitro Fertilization Growth Media Proteins in Competent Embryos**
- **Stroke- Prophylactic Estrogen (Estradiol) Therapy**

University of Lyon Uses PCT to Study Legionnaire's Disease

Poster presented at the
Colloque Legionella et Legioneloses, ENS
Sciences, Lyon, France, October 18 & 19 by
Dr. Danièle Atlan

The tough exterior membrane of protozoan organisms, and their ability to form cysts, makes these organisms resilient to lysis. Among these organisms, amoebae belonging to the genus *Acanthamoeba* have been shown to be important for the replication, virulence, and biocide resistance of *Legionella pneumophila*, the causative agent of Legionnaire's disease. Amoebae are considered to be the natural host of *Legionella* and are believed to play a significant role in Legionella infection in humans through inhalation of contaminated aerosols. *L. pneumophila* cells released from amoeba lysis are well-adapted to infect human alveolar macrophages. For these reasons, amoebae - and particularly *Acanthamoeba castellanii* - have become a suitable model to study host-pathogen interactions during *L. pneumophila* infection.

This study focused on the mechanisms that allow the bacterium *Legionella* to become more resistant to biocides after its passage through the amoeba. During infection, *Legionella* need to modulate host functions to their advantage, which involves complex bacterial regulatory networks leading to the up or down regulation of expression of several genes. Several genes may be associated with virulence, however much needs to be learned regarding the molecular mechanisms that allow the intracellular life of *L. pneumophila*. The University of Lyon approach was to compare the expression of several genes of interest between free *L. pneumophila* cells and bacterial cells replicating within the amoeba *A. castellanii*. PCT (Pressure Cycling Technology) was used by the Lyon scientists to achieve the lysis of amoeba cells containing *L. pneumophila*.

The resulting data clearly show that the Barocycler NEP3229 and PCT can successfully disrupt bacterial and amoeba cells. The Barocycler NEP3229 Sample Preparation System showed high efficiency in breaking *A. castellanii* cells. In addition, RT PCR assays subsequently showed other advantages of the PCT procedure, for example:

- Safe breaking of dangerous material (i.e. amoeba containing pathogen Legionella cells)
- Good recovery of RNA, which indicated good lysis of bacterial cells, even those protected within amoeba vacuoles
- Good preservation of RNA integrity when a limited number of cycles (5) were used

In their summary, the Lyon scientists stated that the PCT Sample Preparation System clearly solved the problem of breaking different types of cells (eukaryotic and prokaryotic) in one run without biological risk to the experimenter.

(Adapted from Poster: [Application of Pressure Cycling Technology to RNA Extraction from *Legionella pneumophila* Cells](#))

Application Focus

Electrophoretic Analyses of Proteins and Peptides Isolated From Cortical Bone Using Pressure Cycling Technology (PCT)

Extraction of proteins from extensively calcified osseous tissue, such as cortical bone, has been particularly challenging for traditional methods of sample preparation. However, a comprehensive proteomic analysis of bone is only possible when the total protein constituency is effectively released. The efficiency of sample preparation is therefore a critical component of the analytical process. Historically, extraction of protein from bone required prolonged acid demineralization over several days to enable complete penetration of histochemical reagents to cellular components. Here we (PBI) describe a method for the extraction of protein from ostrich tibia, which was used as a model sample to develop an extraction process that uses pressure cycling technology (PCT) and also which obviates the need for acid demineralization prior to extraction. The ability to extract proteins from bone without prior demineralization offers important advantages in efficient representative extraction of protein, as well as significant time savings during sample preparation.

Results and Discussion

Ostrich bone samples were exposed to different acids to determine whether demineralization prior to PCT would result in increased protein yield. Samples pretreated with HCl yielded more protein than samples pretreated with either formic or acetic acid. However, significant amounts of protein were released during the demineralization process and were therefore potentially lost for analysis (See Figure 1).

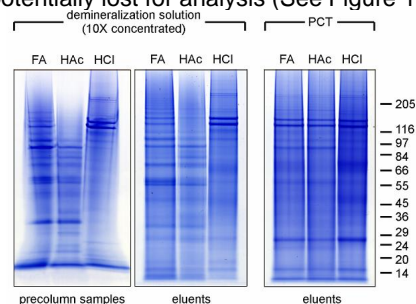


Figure 1. Interference of salts with electrophoresis (left) and their removal using Norgen ProteoSpin columns (middle). Acid solutions were reserved and concentrated 10X to show the loss of proteins resulting from demineralization in formic, acetic, or hydrochloric acid. Proteins extracted by PCT shown on right.

In addition to being faster than traditional processing methods, the PCT Sample Preparation System enables the researcher to extract more protein from cortical bone, which may allow for a more comprehensive analysis of the proteome from bone, a biomaterial that is universally considered difficult to process.