

PBI Provides Corporate Update

Pressure BioSciences, Inc. Provides Corporate Update

South Easton, MA, February 12, 2010 – Pressure BioSciences, Inc. (NASDAQ: PBIO) (“PBI” and the “Company”) today provided an update on various corporate activities, including (1) the national recognition of Pacific Northwest National Laboratory (PNNL) for its collaboration with PBI on the development of a rapid and reproducible method to prepare proteins for analysis, (2) the presentation of data that show a notable improvement in the yields of DNA from challenging forensic samples using the Company’s patented pressure cycling technology (“PCT”), and (3) the presentation of data highlighting significant advantages of PCT over competitive methods in research studies involving human proteins.

1) Recognition of the PNNL – PBI Collaboration. The rapid and accurate analysis of proteins allows for a better understanding of living systems, and for the intricate workings of these systems in health and disease. Continued improvement in this understanding is essential to the development of better diagnostics, disease prevention strategies, vaccines, and life-altering drugs. During 2009, PNNL and PBI scientists collaborated on the development of a PCT-based laboratory process to significantly reduce the time (from hours to minutes) to prepare proteins for scientific analysis, with the goal to offer a faster path to discovery. The Federal Laboratory Consortium recognized PNNL with a 2010 Excellence in Technology Transfer award for this collaborative work with PBI.

2) Presentation of Forensic Data. Pam Marshall, M.S. of the University of North Texas (UNT) recently delivered a presentation at the Winter Meeting of the Association of Forensic DNA Analysts and Administrators in Austin, Texas. Ms. Marshall’s work is a continuation of the study that was presented by Dr. Suzanne Gonzalez of the UNT Center for Human Identification in October 2009, at the annual International Symposium on Human Identification. The UNT data indicate that (a) PCT can be a viable alternative method for the extraction of DNA from forensic samples, (b) PCT can be used with current, commercially available extraction reagents, (c) PCT may result in increased amounts of DNA for analysis from difficult forensic samples, and (d) the potential benefits of using PCT include increased DNA yield, reduced processing time, cost reduction, and the elimination of hazardous reagents.

3) Presentation of Protein Data. Gary Smejkal, Affiliate Assistant Professor at the University of New Hampshire, delivered an oral presentation and two scientific posters at the recent Annual Meeting of the American Electrophoresis Society (AES) highlighting advantages of PCT. Data generated by Mr. Smejkal and colleagues indicated that the amount of protein extracted from the nitrogen-fixing actinobacteria *Frankia* with PCT was significantly greater than that with the standard French press technique. After extraction, the proteins were analyzed by 2-dimensional gel electrophoresis (2DGE).

Application Note

Extraction of Proteins from 30 Million Year Old Amber Using *The PCT Shredder* and Pressure Cycling Technology (PCT)

Gary Smejkal

Affiliate Assistant Professor
University of New Hampshire

Introduction

The mass extinction of the dinosaurs, marked by the Cretaceous-Tertiary boundary, pales in magnitude compared to other lesser known mass extinction events, such as the Permian-Triassic boundary. As over 99% of all of the species that ever lived are now extinct, our understanding of biological processes has been limited by what we have learned from the fewer than 1% of species that have survived more than five major mass extinction events. Recently, collagen peptides were reportedly recovered from mineralized skeletal elements of *Tyrannosaurus rex* and *Brachylophosaurus canadensis* [1, 2], indicating that proteins could be preserved over geological time spans.

Michael Crichton’s novel *Jurassic Park* proposed the recovery of dinosaur DNA from the alimentary tracts of hemophagous insects preserved for millions of years in amber. Though Crichton’s work was purely fictional, amber inclusions have shown remarkable preservation of organisms, at least at the tissue and cellular levels. Reptilian blood cells have actually been identified from partially digested blood meals in parasitic insects encapsulated in Cretaceous amber [3].



Figure 1. Preservation of fine structure in amber, including individual ommatidia of the compound eye of a formicine identified in Oligo-Miocene amber 20-30 million years old. Magnification bar equals 0.6 mm. (Courtesy of P.G. Righetti, Politecnico di Milano, Italy and G.B. Smejkal, Harvard University.)

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CALENDAR OF EVENTS

AMERICAN ACADEMY OF FORENSIC SCIENCES 62ND ANNUAL MEETING	US HUPO 6TH ANNUAL CONFERENCE
Seattle, WA	Denver, CO
February 22-27, 2010	March 7-10, 2010

Press Release: Continued

Mr. Smejkal said: "Sample preparation is critical to the success of 2DGE. As much as 90% of failures in 2DGE are related to poor sample preparation. PBI's PCT technology and their IEF reagent gave us consistent, reproducible results. Additionally, PCT extracts proteins from *Frankia* vesicles, which the French press and other currently available methods are unable to do. Consequently, PCT will allow scientists to characterize the protein constituency of these important *Frankia* organelles, which has been impossible to do until now."

Dr. Nate Lawrence, Vice President of Marketing for Pressure BioSciences, commented: "We believe that PNNL has one of the most advanced mass spectrometry programs in the world. Our collaboration with them has helped us to develop new and improved pressure-based instruments, consumables, and processes for the mass spectrometry field, which is an area of primary focus for PBI in 2010. Sales of PCT Systems and consumables in the mass spec field are a key emerging area for PBI, and the successful completion of on-going discussions with potential mass spec strategic partners has the potential to increase these sales even further."

Dr. Lawrence continued: "Our collaborators at UNT continue to generate and present what we believe to be strong, confirmatory data on the significant advantages of PCT in forensic sample analysis, a second area of primary focus for PBI in 2010. We have seen a significant increase in interest in PCT from the forensics field since the UNT collaboration began in mid-2009, and have recently installed several PCT Systems in important forensics laboratories. We believe that as the advantages of incorporating PCT into the forensics lab workflow become better understood and more widely known, that the sales of PCT Systems in the forensics area will increase measurably. We also believe that these data and our UNT collaboration will help complete on-going discussions with potential forensics strategic partners."

Forward Looking Statements

Statements contained in this press release regarding the Company's intentions, hopes, beliefs, expectations, or predictions of the future are "forward-looking" statements within the meaning of the Private Securities Litigation Reform Act of 1995. Such forward looking statements include statements regarding the use, capabilities, and benefits of the Company's Pressure Cycling Technology Sample Preparation System (PCT SPS) for the extraction of DNA and proteins from challenging forensic samples, and from the model organism *Frankia*; that PCT offers advantages in the extraction of DNA and proteins over conventional extraction procedures, including benefits in quality, speed, cost, and safety; the potential for PCT to be a valuable tool for DNA typing; that the data generated by PNNL, UNT and UNH is both significant and compelling; that the analysis of proteins will lead to better diagnostics, therapies, strategies, and drugs; that the Company's collaborations will lead to new and improved pressure-based instruments, consumables and processes; and that the Company's collaborations and the dissemination of PCT data will result in increased sales of PCT products and advance the Company's discussions with potential strategic partners. These statements are based upon the Company's current expectations, forecasts, and assumptions that are subject to risks, uncertainties, and other factors that could cause actual outcomes and results to differ materially from those indicated by these forward-looking statements. These risks, uncertainties, and other factors include, but are not limited to: possible difficulties or delays in the implementation of the Company's strategies that may adversely affect the Company's continued commercialization of PCT and its PCT-dependent products; changes in customer's needs and technological innovations; other forensic, mass spec, and proteomic scientists may not achieve the same results with PCT reported by the scientists at UNT, PNNL, and UNH; and due to unforeseen costs or delays, the Company may require additional working capital to fund its operations before the beginning of 2011 because the Company's sales force may not be successful in selling the Company's PCT product line because scientists may not perceive the advantages of PCT over other sample preparation methods, including in the investigative forensics and mass spectrometry areas; and due to unforeseen costs or delays, the Company may require additional working capital to fund its operations before the beginning of 2011. Additional risks and uncertainties that could cause actual results to differ materially from those indicated by these forward-looking statements are discussed under the heading "Risk Factors" in the Company's Annual Report on Form 10-K for the year ended December 31, 2008, and other reports filed by the Company from time to time with the SEC. The Company undertakes no obligation to update any of the information included in this release, except as otherwise required by law.

Application Note: Continued

In its unfossilized form, the resins from which amber is derived contain diterpenoids which can rapidly dehydrate a "trapped" specimen, a prerequisite for preservation, and possesses anti-microbial properties that inhibit the usual decomposition processes. Such conditions have allowed DNA to be isolated from a 30 million year old fossil termite *Mastotermes electrodominicus* preserved in amber [4]. Similarly, scientists have isolated amino acids, peptides and proteins from organisms in amber.

Bada et al., [6] have isolated amino acids from 40-100 million year old insects in amber, representing the oldest unaltered amino acids reported to date. Compared to other fossils, the rate of amino acid racemization was retarded by four orders of magnitude in amber inclusions. Here we report a method of extracting of protein from yeast inclusions in amber which was developed by Smejkal et al. [7] using *The PCT Shredder* and pressure cycling technology (PCT).

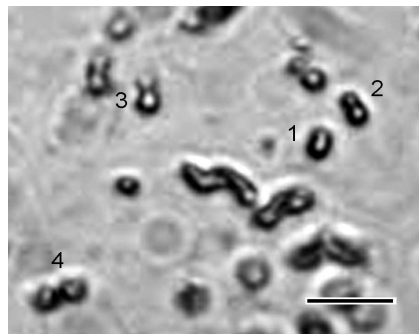


Figure 2. Budding yeast cells in Dominican Republic amber 20-30 million years old. Growth stages are visible: (1) single cell; (4) progeny cell (nearly equal in size to its parent); and intermediary stages (2, 3). Magnification bar equals 0.006 mm. (Courtesy of G.O. Poinar, Oregon State University.)

Materials and Methods

Amber triturates were extracted in 125 mM Tris-HCl pH 6.8 containing 2% SDS, 5 mM tributylphosphine, 20 mM aminoethylbenzene sulfonyl fluoride, 10 mM EDTA and 25 mM phenylacetylthiazolium bromide by using a Shredder PULSE Tube specially fitted with a stainless steel serrated ram insert in stead of the commercially available serrated plastic ram. The samples were subjected to PCT (pressurized for 100 X 100 second cycles at 35,000 psi maximum pressure in the Barocycler NEP 3229). Each Shredder PULSE Tube was coupled to the insert of a sterile Ultrafree CL centrifugal filter (Millipore Corporation, Danvers, MA) and evacuated by centrifugation at 1000 RCF for one minute. Filtrates were applied directly to 8-16% PAGE (BioRad, Hercules, CA). Gels were stained using the mass spectrometry compatible SilverQuest Silver Stain Kit (Invitrogen, Carlsbad, CA). Following trypsin digestion, the resulting proteins were analyzed by mass spectrometry.

Application Note: Continued

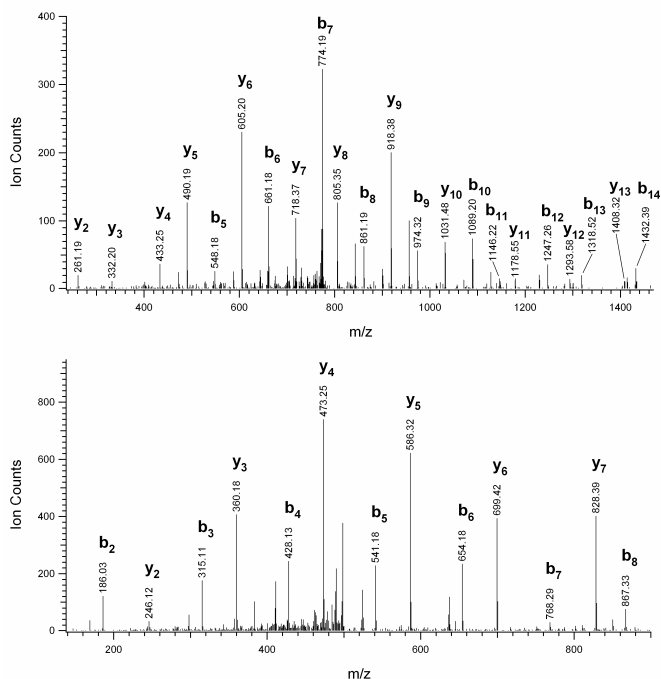


Figure 3. Peptides with sequence homology to *Saccharomyces enolase 1* (top) and *alcohol dehydrogenase* (bottom) isolated from 40 million year old amber inclusions. (Courtesy of F. Chu, University of New Hampshire.)

Results and Discussion

Proteins isolated from the amber failed to penetrate a 4% polyacrylamide gel and accumulated as a single band on the surface of the gel, suggesting an extraordinary high molecular mass, possibly due to extensive crosslinking (a second band which penetrated the gel was identified as keratin). Thus, PAGE may prove to be an effective means of (i) concentrating trace proteins from paleontological samples, while (ii) removing substances that could otherwise interfere with later analysis by mass spectrometry, and (iii) also removing contemporary contaminants which have much lower molecular mass.

Trypsin digests of putative protein extracted from amber were analyzed by mass spectrometry. Through this method, 86 peptides with sequence homology to 20 *Saccharomyces* proteins were identified. Although we cannot yet rigorously prove that these proteins are derived from ancient yeast, the high degree of crosslinking of these proteins suggests that these are of prehistoric origin rather than the result of contemporary contamination.

Peptide sequences of the top five extracted proteins were further evaluated to verify species assignment. For example, enolase 1 sequences from the yeasts *S. cerevisiae* and the tree *Hevea brasiliensis* were compared.

Application Note: Continued

Although seven of the eleven identified peptides showed conserved regions with high sequence similarity, none of the peptides have identical sequence between these two species.

Over the course of millions of years, there is endless opportunity for the modification or even the complete destruction of proteins. However, for living organisms that become trapped in terpenous resins, subsequent dehydration could curtail indigenous proteolysis as well as the non-enzymatic hydrolysis of proteins. Because the amber environment is water-free, the hydrolysis of peptide bonds is inhibited. As a result, putative proteins extracted from amber may provide a reservoir of proteins for analysis which may reveal a wealth of information and insight into evolution. *The PCT Shredder* and pressure cycling technology (PCT) combine to provide an effective method for extracting proteins from organisms preserved in amber.

References

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