PBI Pressure BioSciences Inc.

# PCT NEWS

NASDAQ: PBIO

*Discovery Starts With Sample Preparation™*

**Volume 4, Issue 12** **December 2010**

# Genetic Engineering and Biotechnology News (GEN) Co-Sponsors Round Table Discussion on Novel Bioremediation Techniques

## Genetic Engineering and Biotechnology News (GEN) Co-Sponsors Round Table Discussion on Novel Bioremediation Techniques

**Press Release** Source: Pressure BioSciences, Inc.

On Tuesday December 7, 2010, 9:40 am EST
NEW ROCHELLE, N.Y., Dec. 7, 2010 (GLOBE NEWSWIRE) -- *Genetic Engineering and Biotechnology News* (*GEN*) recently co-sponsored a roundtable discussion on new ways to use biological solutions to assist in environment clean-up after manmade or natural disasters. "Gulf Oil Spill: Using Modern-day Biology to Assess the Environmental Impact and to Help in Remediation" was also sponsored by the Venture Development Center (VDC) at The University of Massachusetts Boston, where the discussions took place. Part I of the roundtable appears on the Video Section of the GEN website (http://www.genengnews.com/video-channel) and a link to the entire roundtable presentation is also included on the GEN website.

"The Gulf Oil Spill catastrophe was a clear wake-up call regarding the critical need for faster, more efficient, and more environmentally-friendly clean-up solutions," said John Sterling, Editor in Chief of *GEN*, who served as co-moderator of the roundtable along with William Brah, Assistant Vice Provost for Research and Executive Director of the VDC. "*GEN* was honored to work with such a prestigious roundtable panel," added Sterling.

The panel members included John Farrington, UMass Dartmouth & Woods Hole Oceanographic Institute (Emeritus); Olivia Mason, Lawrence Berkeley National Laboratory; Doug Bartlett, Scripps Oceanographic Institute; Juanita Urban-Rich, UMASS Boston; and Richard T. Schumacher, Pressure BioSciences, Inc. (Nasdaq:PBIO - News).

During the roundtable, scientists from academia and industry discussed the effectiveness of biological methods and tools that could help improve the understanding of the marine environment, assess the impact that oil spills of any magnitude have on this complex yet delicate ecosystem, and help monitor the effectiveness and even be part of the clean-up procedure during remediation.

The entire video can also be found on the VDC webpage (www.umb.edu/vdc) and on the PBI website (www.pressurebiosciences.com) or the PBI IR-Newsroom (click here or paste the following URL in your browser: http://bit.ly/gqHnRL).

*Genetic Engineering and Biotechnology News* (www.genengnews.com), which is published 21 times a year by Mary Ann Liebert, Inc., is the most widely read biotechnology news magazine worldwide. It includes articles on Drug Discovery, Bioprocessing, OMICS, Biobusiness, and Clinical Research and Diagnostics.

## A Round Table Discussion

### Gulf Oil Spill: Using Modern-day Biology to Assess the Environmental Impact and to Help in Remediation

### Links to Video

**Part 1** **Part 2**

## Pressure BioSciences, Inc. Announces the Release of Key Products Targeted Primarily at the Drug Discovery and Development Market

South Easton, MA, December 13, 2010 – Pressure BioSciences, Inc. (NASDAQ: PBIO) ("PBI" and the "Company") today announced the release for sale of three novel products targeted primarily for the drug discovery and development market. The new products were released at the 50th Annual Meeting of the American Society for Cell Biology ("ASCB") in Philadelphia, which began on Sunday December 12, 2010.

The ASCB is comprised mostly of biologists studying the cell – the fundamental unit of life. Approximately 10,000 attendees from academia, government, the biotechnology industry, and pharmaceutical companies are expected at the Annual Meeting. The Company believes its new products will be well received by the scientists attending the Annual Meeting, particularly those working in the drug discovery and development area.

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## CALENDAR OF PBI EVENTS

| PLANT AND ANIMAL GENOME X1X | MASS SPECTROMETRY APPLICATIONS TO THE CLINICAL LAB (MSACL) |
|---|---|
| JANUARY 15-19 2011 | FEBRUARY 5-9, 2011 |
| SAN DIEGO, CA | SAN DIEGO, CA |

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## Pressure BioSciences, Inc. Announces the Release of Key Products Targeted Primarily at the Drug Discovery and Development Market: Continued from Page 1

Two of the Company's new products are focused on the isolation of mitochondria from solid tissues – skeletal muscle and lung ("mitochondrial kits"). The third new product is a small, portable instrument ("*The* SHREDDER SG3") developed in conjunction with the mitochondrial kits, to allow for a safe, rapid, efficient, and standardized method to isolate mitochondria from human and animal cells. *The* SHREDDER SG3 uses a similar, proprietary consumable as the Company's pressure cycling technology ("PCT") platform. The Company believes that in addition to the use of this instrument with the new mitochondrial kits, *The* SHREDDER SG3 can also be used for a multitude of other sample preparation processes by an estimated 80,000 research laboratories and 450,000 research scientists working in the biological sciences worldwide.

**Mitochondria and Mitochondrial Kits.** Mitochondria are small structures found inside most human and animal cells. They play a major role in generating the energy required to power most cell processes and are involved in other important cell functions. Mitochondria have been implicated in several human diseases, including heart disease, stroke, Parkinson's disease, cancer, and other mitochondrial diseases. Mitochondria might also play a role in aging. PBI's newly released mitochondrial kits contain all of the chemical ingredients necessary for a scientist to extract mitochondria from skeletal muscle and lung tissue for subsequent analysis.

***The* SHREDDER SG3.** ***The* SHREDDER SG3** is small, portable mechanical system that can be used to break apart tough, fibrous, and other difficult-to-disrupt tissues, such as skeletal muscle and lung. ***The* SHREDDER SG3** can also be used to break apart tough organisms, such as ticks and nematodes (worms). Although tough enough to do the foregoing, it is also gentle enough to break apart cells to release mitochondria for subsequent study. ***The* SHREDDER SG3** uses proprietary consumable processing containers that can be subsequently placed inside the Company's PCT-based instruments for further processing.

Dr. Vera Gross, Senior Research Scientist at PBI, and an expert in cell biology, said: "The extraction of mitochondria from solid tissue, such as lung and skeletal muscle, is currently done using labor-intensive methods that require extensive operator experience, usually resulting in high variability among researchers. Our new mitochondrial kits, when paired with ***The* SHREDDER SG3** offer researchers a semi-automated, rapid, and reproducible method that we believe will generate high quality mitochondria preparations for analysis."

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## Simple Protocols for Isolation of Intact Mitochondria Using *The* SHREDDER SG3 and PBI Mitochondria Isolation Kits

**Pressure BioSciences, Inc.**
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### Introduction

Isolation of intact mitochondria from human and animal tissue is crucial for studies that focus on the elucidation of their function and dysfunction in conditions such as aging, diabetes and cancer. As potential drug targets, high quality functional mitochondrial isolates are important for drug screening studies [1]. Mitochondria isolation from solid tissue is usually carried out using labor-intensive homogenizer-based methods [2] that require extensive operator experience. Here we describe a semi-automated method to release mitochondria from solid fibrous tissues, such as muscle and lung, using *The PCT Shredder* or *The* SHREDDER SG3 for tissue homogenization. This method can be combined with a subsequent pressure cycling technology (PCT) step (Figure 1A) to further increase yield.

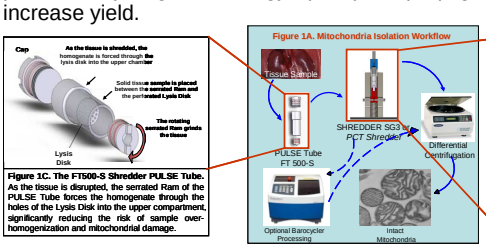


Figure 1C. The FT500-S Shredder PULSE Tube. As the tissue is disrupted, the serrated Ram of the PULSE Tube forces the homogenate through the holes of the Lysis Disk into the upper compartment, significantly reducing the risk of sample over-homogenization and mitochondrial damage.

Figure 1B. *The* SHREDDER SG3

### Results and Discussion

Here we demonstrate convenient extraction of intact mitochondria from skeletal muscle and lung tissue, using the new **PBI Mitochondria Isolation Kits** with ***The* SHREDDER SG3** (or *The PCT Shredder*) and optional subsequent pressure cycling for increased yield. **The PBI Mitochondria Isolation Kit: Rat Muscle** was used to isolate intact and functional mitochondria from freshly harvested rat skeletal muscle tissue.

Protein assays of the mitochondria-enriched preparations demonstrate that sample processing by PCT after initial disruption with ***The* SHREDDER SG3** or *The PCT Shredder*, releases more mitochondria than shredding alone and that this yield approaches that obtained using the more traditional and labor-intensive manual homogenizer method (Figure 2, Upper panel). The results also demonstrate that for isolation of muscle mitochondria, a pressure of 10,000 psi is sufficient to increase yield and that little additional yield is recovered at higher pressures. No significant difference was observed in the respiratory control ratios (RCRs) of muscle mitochondria extracted by the standard manual homogenizer method, compared to those processed using the new kit. Respiration assays of mitochondria extracted with and without pressure cycling (Figure 2, Lower panel) confirm that muscle mitochondria exposed to hydrostatic pressure of 10,000 psi for five cycles, exhibit normal respiration kinetics. As expected, mitochondria exposed to 30,000 psi exhibited very low RCR values.

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Dr. Nathan Lawrence, Vice President of Marketing for PBI, commented: "The study of mitochondria has gained much importance over the past few years as the association between mitochondrial dysfunction and significant human diseases has been shown. Data from more than 50 mitochondria studies are slated for presentation over the four days of the ASCB Annual Meeting. We are therefore pleased to introduce our new mitochondrial kits and *The* SHREDDER SG3 to this specific group, as we believe these novel products offer many advantages to current methods for the extraction of mitochondria from skeletal muscle and lung, including convenience, reproducibility, and cost savings. As we enter this new and exciting sample preparation market, we expect these newly-released products to generate revenue, increase interest and sales in our core PCT-based products, and open new doors for PBI during 2011 and beyond."

**Forward Looking Statements**

Statements contained in this press release regarding PBI'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 belief that the Company's new products will be well received by attendees at the Annual Meeting of the ASCB; that PBI's new mitochondrial kits contain all necessary chemical ingredients to extract mitochondria for scientific study; the capabilities of the Company's mitochondrial kits and *The* SHREDDER SG3; that *The* SHREDDER SG3 can break apart tough samples but is gentle enough to release mitochondria from cells, in a rapid, reproducible, semi-automated fashion; that *The* SHREDDER SG3 can be used for a multitude of other sample preparation processes; that there are an estimated 80,000 research laboratories and 450,000 research scientists worldwide that undertake such other sample preparation processes; that the study of mitochondria has gained importance over the past few years; the role of mitochondria in cell functions, human diseases and aging; that the combination of the new mitochondrial kits and *The* SHREDDER SG3 offer many advantages over current methods to extract mitochondria; and that these new products will generate revenue, increase interest and sales in PCT-based products, and open new doors for PBI in 2011. 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, including its PCT-based mitochondrial kits and *The* SHREDDER SG3; changes in customer's needs and technological innovations; other scientists may not achieve the same results with the mitochondrial kits and *The* SHREDDER SG3 reported by PBI scientists; and the Company's sales force may not be successful in selling the Company's PCT product line, including the new mitochondrial kits and *The* SHREDDER SG3, because scientists may not perceive the advantages of PCT over other sample preparation methods for mitochondrial extraction. Further, the Company expects that it will need additional capital to fund its continuing operations beyond the first quarter 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 PBI's Annual Report on Form 10-K for the year ended December 31, 2009, and other reports filed by PBI from time to time with the SEC. PBI undertakes no obligation to update any of the information included in this release, except as otherwise required by law.

Visit us at our website:
http://www.pressurebiosciences.com
or the PBI-IR Newsroom:
http://360.cx/gEPvQB

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## Simple Protocols for Isolation of Intact Mitochondria Using *The* SHREDDER SG3 and PBI Mitochondria Isolation Kits: Continued from Page 2

It is likely that such high pressure results in isolation of fragments, rather than intact mitochondria. However, the mitochondria exposed to 20,000 psi appear almost as robust as controls, indicating that the range of pressures compatible with isolation of functional mitochondria, is wide. Electron micrographs (Figure 3) demonstrate the comparable morphologies of mitochondria extracted using the control manual method and those isolated using the new **Muscle with** pressure cycling at 10,000 psi.

The PBI Mitochondria Isolation Kit: Rat Muscle was used to isolate intact mitochondria from frozen/thawed rat lung tissue for proteomic analyses. Two dimensional PAGE (Figure 4) and SDS-PAGE (Figure 5A) demonstrate that the protein profiles of control mitochondria samples isolated by manual homogenization, are essentially the same as the test samples prepared using The PBI Mitochondria Isolation Kit: Rat Muscle with *The* SHREDDER SG3 (or *The PCT Shredder*) with or without subsequent pressure cycling. Western blots confirm that the lung mitochondria preparations are enriched for protein markers for all three mitochondrial compartments, providing further evidence that the mitochondria are intact (Figure 5A). The yield of lung mitochondria was significantly increased when shredding was followed by PCT treatment for 5 cycles at 20,000 psi (Figure 5B), and additional cycles of pressure (20,000 psi for 15 cycles) did not significantly improve yield.

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