

**Pressure BioSciences Scientists Win  
Outstanding Research Article Award from  
The Journal of Biomolecular Techniques (JBT)**

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**Tissue Fractionation by Hydrostatic  
Pressure Cycling Technology: The Unified  
Sample Preparation Technique for  
Systems Biology Studies**

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Major bottlenecks in systems biology studies arise from limitations of current sample preparation techniques. Multiple mutually exclusive sample preparation methods, which are often required to extract distinct classes of molecules from cells and tissues, are incompatible with studies of precious or very limited samples. Moreover, the strong detergents and chaotropic agents commonly required to solubilize sample constituents often interfere with subsequent separation and analysis. Here we describe a rapid, detergent-free sample preparation technique that allows efficient concurrent isolation and fractionation of protein, DNA, RNA, and lipids from biological samples, eliminating the need for multiple replicates. The method relies on a synergistic combination of physical disruption of the cellular material by hydrostatic pressure (pressure cycling technology) and novel extraction conditions to dissolve and partition distinct classes of molecules into separate fractions. We demonstrate parallel recovery of proteins, lipids, and intact DNA and RNA, from animal cells and tissues, for proteomic, lipidomic, and genomic analyses. The protein extracts require minimal cleanup and are compatible with 1D and 2D PAGE, liquid chromatography coupled with tandem mass spectrometry, and Western blotting. The lipid fractions have been profiled by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry without further processing. The isolated DNA and RNA were shown to be intact by agarose gel visualization, and the presence of intact mRNA was confirmed by real time reverse transcription polymerase chain reaction. Analysis and comparison of samples extracted using this method and a more traditional extraction technique revealed several protein species preferentially extracted by the new method.

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**PBI Scientists Win Prestigious Award  
at ABRF 2009**

**The Journal of Biomolecular  
Techniques (JBT) Award**

The JBT Award recognizes the most outstanding research article published during the last year in the journal. This year's JBT Award recipient is:

**Tissue Fractionation by Hydrostatic  
Pressure Cycling Technology: The Unified  
Sample Preparation Technique for  
Systems Biology Studies**

The award was accepted by Dr. Vera Gross, Sr. Scientist (PBI)

**Proteins, Lipids, DNA & RNA**

From

**The Same Sample**

Using Pressure Cycling Technology (PCT)

**ProteoSolve-SB**  
A Pressure Enhanced Systems Biology Kit

- Detergent-Free Extraction
- Automated Bench-top Instrument
- Process Organelles & Membranes
- Process Cells & Tissues
- Improve Reproducibility
- Increase Protein Recovery
- Identify Novel Proteins
- Direct Lipid Profiling
- Isolate DNA and RNA
- Discover Biomarkers

See Award  
Winning  
JBT  
Publication

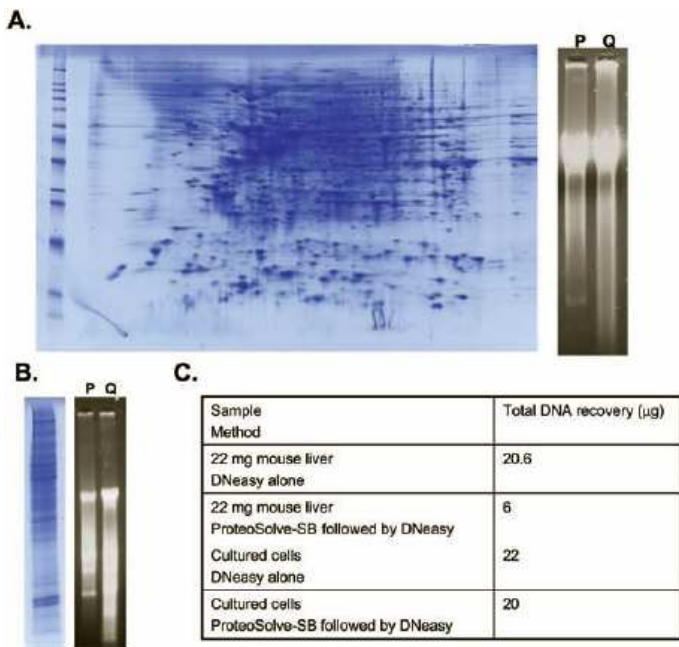


**CALENDAR OF EVENTS  
ASMS  
PHILADELPHIA, PA  
MAY 31 – JUNE 4, 2009**

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## Tissue Fractionation by Hydrostatic Pressure Cycling Technology: The Unified Sample Preparation Technique for Systems Biology Studies

Figure 6 (Page 195)



**A:** DNA and protein recovery from the cell culture. One aliquot of cells was processed with PCT and ProteoSolve-SB (P). DNA was extracted from the solid phase using the DNeasy kit. The control aliquot of cells was processed directly with the Qiagen DNeasy kit according to manufacturer's instructions (Q). No intact protein was recovered from the control sample due to extensive proteinase K digestion of the proteins. DNA quantification by Qubit assay indicates that the yield of DNA from the new method is comparable to the yield of DNA after extensive proteinase K digestion.

**B:** DNA and protein recovery from mouse liver with PCT and ProteoSolve-SB (P). DNA was extracted from the solid phase using the DNeasy kit. Control tissue was processed directly with the DNeasy kit according to manufacturer's instructions (Q). No protein was recovered from the control sample due to extensive proteinase K digestion of the tissue. DNA quantification by Qubit assay indicates that the new method yields about 30% of DNA that can be recovered after extensive tissue digestion.

**C:** Table of DNA recovery from liver tissue and cell culture.

## Poster Presented at ABRF 2009 February 7-10, 2009, Memphis, TN

### Searching for efficient and high-throughput alternatives for essential sample preparation techniques in mass spectrometry-based functional proteomics

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#### Abstract

Good control, efficiency, and reproducibility of protein extraction from cells and tissues are essential aspects for diverse biological and basic research applications. Effective and specific enzymatic digestion of proteins prior to mass spectrometry (MS) analysis is one of the fundamental techniques most commonly used in any proteomics laboratory. However, neither routine procedures for cell and tissue lysis nor for in-solution and in-gel protein digestion have been significantly altered in common practice for over a decade. Here we have tested and optimized several alternative techniques for preparing lysates of mammalian cells, as well as in-solution and in-gel enzymatic digestion compatible with downstream qualitative and quantitative MS-based proteomics applications.

#### Results

Examination of a range of experimental conditions demonstrates their roles in efficiency, selectivity, and throughput of proteolytic digestion, as well as to optimize conventional digestion protocols. Specifically, we demonstrated: (1.) addition of methanol or urea improves the peptide identification rate; (2.) addition of Lys-C prior to addition of trypsin improves the specificity of digestion; (3.) reducing agent TCEP outperforms DTT in improving the peptide identification rate; (4.) extension of digestion protocols from 12- to 24-hour long incubation with trypsin at atmospheric pressure and 37°C did not result in evident gain in the peptide identification for the mixture of protein standards but improved reproducibility in the identification outcome; (5.) two hour-long digestion with PCT assistance was found as an appropriate compromise between throughput and efficiency of digestion.

Application of PCT resulted in significant improvement of throughput and reproducibility of sample preparation for proteomic analyses in both enzymatic protein digestion and lysis of cultured cells. Superior extraction rate for cytosolic, nuclear, ribosomal, and membrane-associated proteins, as well as for proteins related to vital GO processes (RNA splicing, chromatin assembly, organelle organization among others), were observed in PCT-assisted and organic solvent-assisted sample preparation. Further optimization of sample recovery in PCT-assisted digestion protocols is currently conducted using newly designed sample tubes with lower surface to volume ratio. The combination of PCT and solvent assistance will be applied to further improve cell lysis efficiency.