

## Pressure Cycling Technology (PCT) US HUPO 2008, "Proteomics & Beyond" – Part II

### Poster Profile

#### Unified Sample Preparation Approach Using Hydrostatic Pressure Cycling: Simultaneous Isolation of Proteins, Nucleic Acids and Lipids from a Single Sample

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#### **Abstract:**

Systems biology studies are gaining momentum driven by success in genomics, transcription profiling, proteomics, and rapidly emerging metabolomic technologies. While powerful and sensitive methods are available for the analysis of nucleic acids, proteins and small molecules, major bottlenecks arise from the limitations of current sample preparation techniques. We have developed a *detergent-free* sample preparation approach which allows *concurrent* isolation and fractionation of protein, DNA, RNA and lipids from cells and tissues. This novel method relies on a synergistic combination of cell disruption by alternating hydrostatic pressure (Pressure Cycling Technology, or PCT) and optimized reagents that dissolve and partition distinct classes of molecules into separate fractions. Previously we reported the performance of the PCT-driven detergent-free protein isolation system (1), which laid the foundation for the development of the ProteoSolveLRS reagent kit. In the current study we present the expansion of the ProteoSolveLRS strategy for rapid simultaneous isolation of DNA, RNA, proteins and lipids from individual samples. Gel electrophoresis and real-time RT-PCR confirm that nearly quantitative recovery of intact genomic DNA and high yields of intact RNA are obtained using this novel technique. Additionally, high yields of proteins and lipids are obtained from the same sample for proteomic analysis. The results have been highly reproducible and several protein species more efficiently extracted by the new method have been identified by in-gel tryptic digestion and LC-MS/MS. We also report direct analysis of the lipid fractions using MALDI-TOF mass spectrometry.

### Application Focus

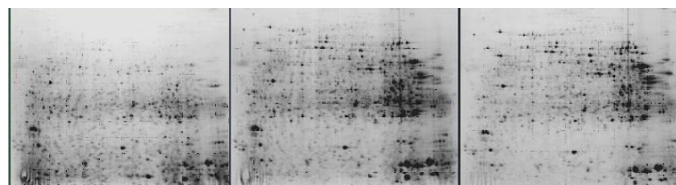
#### Increased Protein Yields from Murine Liver Tissues Using PCT

Sequencing of the the human genome revealed approximately 30,000 genes. However, the number of genes is significantly lower than the prior estimate of 80,000-140,000 [1]. The actual number of genes, alone, is too small to account for the complexity of humans. The explanation for the complexity is in the dynamic nature of proteins that make up the proteome. This is attributable to the alternate splicing of RNA and post-transcriptional modifications of proteins, like glycosylation or phosphorylation. Researchers are now focusing their efforts on developing a better understanding of the human proteome. It is expected that the elucidation of the proteome will lead to better diagnosis and treatment of disease through the identification of clinically important biomarkers. The quality of proteins extracted during initial sample preparation is critical to the success of downstream separation techniques and detection technologies. Here, we present a comparison of Pressure Cycling Technology (PCT) to conventional physical methods (sonication, homogenization, and pulverization) for the extraction of proteins from murine liver tissue. In addition, to illustrate the reproducibility of the PCT method and to demonstrate efficient processing of extremely small amounts of tissue, submilligram quantities of rat liver tissue were processed. Following extraction, total protein concentrations were determined by quantitative protein assays, and samples were analyzed by one- and two-dimensional gel electrophoresis (1DGE and 2DGE, respectively).

Sonicator  
1,739 spots

PCT  
2,126

Dounce  
1,853 spots



**Figure 1.** 2DGE of mouse liver lysates illustrating the loss of higher MW proteins that occurs with sonication. Overall protein spot detection was significantly higher in PCT processed samples. PCT was performed for 10 pressure cycles; each cycle consisted of 20 seconds at 35,000 PSI followed by 20 seconds at ambient pressure. IPGs were pH 4.5-6.5.

### CALENDAR OF EVENTS

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

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

## Poster Profile (Cont.)

### Results and Discussion

Pressure mediated extraction in combination with the unique chemistry of the ProteoSolveLRS kit has been demonstrated to be an efficient method to extract proteins from lipid-rich samples such as adipose and brain tissue (1). Here we expand the utility of this sample preparation system, by demonstrating that four major components of biological samples; proteins, lipids, RNA and DNA, can all be easily and efficiently isolated from a single sample of cultured cells or tissue. In addition we confirm that the RNA recovery is comparable to, or better than, that achieved using three currently available kits and reagents.

Examination of the individual fractions after pressure mediated extraction with the ProteoSolveLRS kit has demonstrated the following:

**A)** The lipid fraction contains no additional extractable protein. **B)** The polar solvent phase contains the bulk of the sample proteins, and only traces of RNA and DNA. **C)** The pellet and the interface contain the bulk of the RNA and DNA, with the pellet containing ~70% and the interface containing ~20%. Additionally, preliminary results indicate that the RNA in the pellet/interface fraction is stable for extended periods of time at -20°C.

The new expanded application of the ProteoSolveLRS kit provides efficient simultaneous extraction of proteins, lipids and nucleic acids from samples that are precious or unique, such as human or wild animal biopsy tissue or samples that are difficult to duplicate, such as small cell populations like early stem cell cultures. Another advantage is in more accurate analysis of non-homogenous samples. Since splitting samples for separate protein, lipid and nucleic acid analyses is not necessary, artifacts due to uneven distribution of components in the sample are avoided.

The new method is advantageous not only for small and precious samples, but also for larger samples where a single convenient method for purification of multiple components is desired. Since the ProteoSolveLRS kit is easily scalable for larger samples, it has many advantages over other currently available methods. In many applications, ProteoSolveLRS can be used with sample-to-solvent ratios as high as 300 mg per mL, and possibly higher in some cases. After ProteoSolveLRS extraction of the bulk of the sample's proteins and lipids, the nucleic acid-enriched fraction can be brought up in a relatively small volume of reagent such as Trizol, thus allowing for more efficient RNA precipitation from large samples that contain little RNA (like soil, yogurt, skin, etc).

ProteoSolveLRS is compatible with most common downstream applications. Since proteins can be dried or precipitated out of the detergent-free solvent, no extensive clean-up, washing or concentration of proteins is necessary. Protein pellets can be dissolved directly in a buffer of choice and subjected to further analyses.

The lipid fractions are available for direct profiling by mass spectrometry or for separation and enzymatic digestion for structural analysis.

The DNA/RNA fraction is compatible with many commonly used reagents and kits for isolation of DNA and/or RNA, such as Trizol and the Qiagen DNeasy and RNeasy kits.

### Summary

The combination of sample disruption by PCT and extraction in ProteoSolveLRS can be used to efficiently and easily extract lipids, proteins, RNA and DNA from many types of samples without the need for multiple replicates, tissue homogenizers or sonicators, liquid nitrogen grinding, or other inconvenient and inconsistent sample disruption methods.

## Application Focus (Cont.)

### Increased Protein Yields from Murine Liver Tissues Using PCT

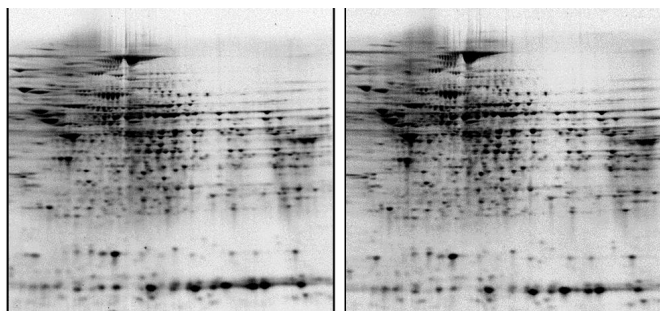
#### Results

Two conventional methods for protein extraction were compared to PCT using murine liver tissues. After samples were processed using either a sonicator, dounce homogenizer, or PCT, the resulting lysates were analyzed for the total number of protein spots on a 2DGE. Lysates derived by sonication yielded 1,739 protein spots, while 1,853 protein spots were detected from samples processed by the ground glass dounce. By contrast, 2DGE of PCT lysates from mouse liver (250 mg) yielded 2,126 protein spots.

As shown in Figure 1, high molecular weight proteins were significantly diminished in lysates produced by probe sonication. This was commensurate with an increase in low molecular weight proteins in the sonication lysates. We hypothesize either that the gradual deterioration of sonicator probes and the release of metal ions into the sample could facilitate reactivation of proteolytic enzymes or that the oxidation of proteins causes degradation of the high molecular weight proteins. While there does not appear to be a similar detectable loss of high molecular proteins derived from the ground glass dounce method, fewer total spots were detected compared to PCT-extracted samples. PCT released more protein from mouse liver, including unique proteins, which were not detectable in sonication or dounce homogenates. [4]

#### PCT Reproducibility and Sensitivity

To demonstrate the reproducibility and sensitivity of the PCT SPS, protein was extracted from relatively small quantities of murine liver. The PCT SPS extracted sufficient amounts of protein from  $0.7 \pm 0.3$  mg liver samples to produce the 2D gels in Figure 2. This amount of tissue is similar to the amount of tissue in a typical needle biopsy.



**Figure 2.** Submilligram quantities ( $0.7 \pm 0.3$  mg) of rat liver tissue processed in standard PULSE Tubes using silanized glass beads for volume displacement.