The PBI Shredder System:
A Novel Approach for Affordable and Efficient Sample Preparation

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Abstract

The extraction of proteins from tough tissues, such as muscle and lung, generally requires extensive mechanical disruption, or chemical or enzymatic treatment of the samples, to recover and adequately analyze their proteome. Mortar and pestle grinding, pulverization in liquid nitrogen, homogenization with a Dounce, or rotor-stator homogenizers are some of the classical methods used. However, these manual methods are inherently labor-intensive, time consuming, and prone to sample-to-sample variability and to cross-contamination. In addition, these traditional methods, while effective, can not be easily adapted for use with difficult samples such as blood vessels and connective tissue, nor for more gentle extraction, as is required for the isolation of intact organelles.

Here we report on a wide variety of sample types that were efficiently disrupted using the PBI Shredder System (*The PCT Shredder* or *The* SHREDDER SG3) for such diverse applications as proteomic profiling, isolation of nucleic acids, and even extraction of intact mitochondria. We applied the Shredder System methodology to a wide range of sample types: elastic tissues such as lung and muscle; tough samples such as leaves; hard samples such as seeds; hard-to-disrupt organisms such as *C. elegans;* and arthropods. In some protocols, Shredder-disrupted samples were subjected to additional processing using pressure cycling technology (PCT) to maximize yield.

Using this novel Shredder System, we were able to successfully isolate protein from rat skeletal muscle and human ovarian tumor tissue, as well as from *C. elegans* and dry rice grains. We were also able to obtain good yields of DNA from apple seeds and whole ticks, as well as DNA greater than 140 kb in length from spinach leaves.

Additionally, we have shown that the PBI Shredder System can be used for rapid and efficient extraction of RNA from frozen rat lung tissue. The System was also used to isolate intact and functional mitochondria from fresh skeletal muscle.

Introduction

The PCT Shredder and the Shredder SG3

The PCT Shredder allows the user to rapidly grind samples directly in specially designed Shredder PULSE Tubes. The extraction of proteins, DNA, RNA, lipids, organelles, and small molecules from tissues and organisms is often enhanced by the synergistic effects of Shredding and PCT. However, for some samples, shredding alone provides adequate extraction.

The new SHREDDER SG3 differs from the original PCT Shredder (Figure 1B) by the addition of a three position force setting lever that enables the operator to select and apply reproducible force to the sample during the shredding process and eliminates the need for the operator to press down on the SG3 Driver for long periods, when processing multiple samples.

Shredder PULSE Tube Configurations

There are two PULSE Tubes configurations for use in either Shredder. The FT500-PS all-plastic design (Figure 1C) is employed for fibrous or sticky samples such as muscle and lung tissue. The FT500-PMS configuration incorporates a perforated metal disk in the PULSE Tube design that improves shredding of hard or brittle samples such as cartilage, seeds and grains. For samples where disruption with the Shredder is followed by pressure cycling, the FT500-S or FT500-MS PULSE Tubes (rated for high pressure use) are required.

Pressure Cycling Technology (PCT)

PCT destabilizes molecular interactions by rapidly and repeatedly raising and lowering pressure in the reaction vessel from ambient to high pressures (up to 45,000 psi [310 MPa, 3000 Atm]). At pressures in the 35,000-45,000 psi range, PCT can be used to disrupt cellular structures in order to release proteins, DNA, RNA and other analytes [1, 2]. At lower pressures (10,000 - 20,000 psi) PCT can gently lyse cells and release their intracellular contents, including intact mitochondria [3]. PCT may also be used to accelerate enzymatic activity of a wide range of enzymes including trypsin for proteomic studies [4] and Proteinase K for DNA isolation.

Results and Conclusions

The PBI Shredder System was used to extract DNA, RNA, protein and intact mitochondria from a variety of tough-to-disrupt sample types. Traditional manual methods for extraction from solid tissues are time consuming, and they are often prone to cross-contamination from washing and re-using non-disposable preparation equipment.

Figure1A depicts typical work flows using either *The* SHREDDER SG3 or the *PCT Shredder*. Either shredder may be used alone or in combination with Pressure Cycling Technology (PCT) to maximize yield (**Figure 1B**). The design and operation of the Shredder PULSE Tube is Illustrated in **Figure 1C**. The (FT500-S and FT500-MS) Shredder PULSE Tubes a are disposable tubes which can be subjected to pressure of greater than 35,000 psi in a Barocycler, and are compatible with either shredder.

Figures 2 and 3 show the effectiveness of the Shredder System for extracting relatively long DNA (>140 kb) from spinach leaves and apple seeds when compared to extraction by bead beating. While **Figure 4** shows that the Shredder is also capable of extracting protein from plant tissue.

Figure 5 shows that whole ticks can be shredded using Shredder PULSE Tubes. Using the System, researchers can safely shred the ticks without the need for sharp blades or scalpels. In addition, the closed tube reduces the exposure of the researcher to potential tick-harbored pathogens. The System is ideal for collection, transport, storage, and processing of small quantities of plant tissue for genomic and proteomic studies.

Figures 6 and 7 demonstrate that the PBI Shredder System is capable of processing solid tissue, such as muscle or cartilage, for the preparation of proteins or other analytes.

Figure 8 shows that that the Shredder System, when used in combination with PBI's *ProteoSolve-CE Native* or the *ProteoSolve-CE Stringent* Kits, is capable of disrupting the tough cuticle of the nematode *C. elegans* for protein preparations that seek to maintain protein structure or to enrich for total proteins for further analyses.

Although the PBI Shredder System is capable of shredding tough samples such as muscle, cartilage and seeds, it is also gentle enough for recovery of intact and functional mitochondria (**Figure 9**).

The PBI Shredder System has proven to be a versatile tool for sample preparation and is capable of processing a wide variety of samples efficiently, safely, and inexpensively.

References

[1] Gross *et al.* (2008) Biotechniques 45(1):99-100
[2] Schumacher *et al.* (2002). Am. Laboratory 34, 38-43.
[3] Gross *et al.* (2011) Anal. Biochem. 418:213-223.
[4] Lopez-Ferrer, *et al.*, (2008). J. Proteome Res., 7(8):3276-81

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, DNA shearing and mitochondrial damage. The **FT 500-MS** has a metal lysis disk to facilitate shredding of hard and brittle samples.

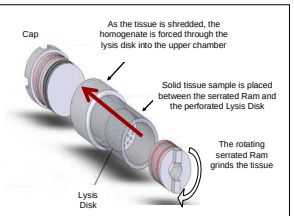


Figure 1A. Shredder ±PCT Workflow

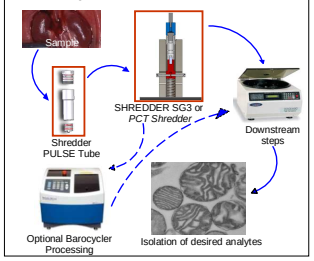
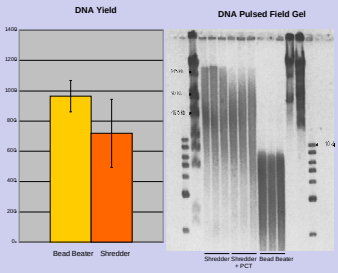


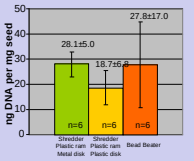
Figure 1B. *The* SHREDDER SG3 and The PCT Shredder

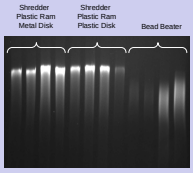
Figure 2. Long Genomic DNA Extraction from Spinach



Total DNA was extracted from fresh spinach leaves in DNAzol reagent (Invitrogen). Samples were disrupted either in *The PCT Shredder* (20 seconds) or by bead beating (ten 10 s bursts, with chilling on ice to prevent overheating). After initial disruption, some shredded samples were also subjected to PCT at 35,000 psi. DNA length was estimated by comparison to a DNA standard in a Pulsed Field gel. DNA yield was determined by Nanodrop.

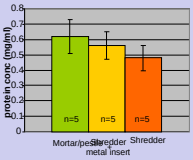
Figure 3. DNA Extraction from Whole Apple seeds





Total DNA was extracted from apple seeds in Qiagen buffer AP1 supplemented with RNase. One intact apple seed was used for each sample. Shredding was carried out for 30 seconds at ambient temperature in either all-plastic FT 500-S PULSE Tubes or FT 500-MS PULSE Tubes with the metal disk insert. Controls were disrupted in a BioSpec Mini-beadbeater-1 for 30 seconds. DNA yield was quantified by Nanodrop and visualized by agarose gel electrophoresis.

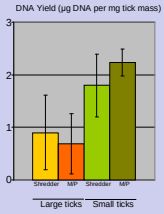
Figure 4. Protein Extraction from Rice Grains



Raw rice was homogenized in PBS. Samples (n=5 per group) were processed with *The* SHREDDER SG3 using either all-plastic FT 500-S PULSE Tubes or FT 500-MS PULSE Tubes with the metal disk insert. All shredder samples were shredded for a total of 90 seconds with a lever setting #2.

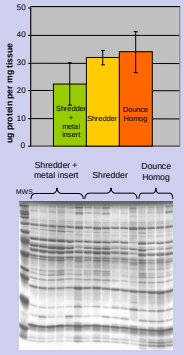
For comparison, control samples were ground with mortar and pestle. Protein concentration was measured by Bradford assay.

Figure 5. DNA Extraction from Whole Ticks



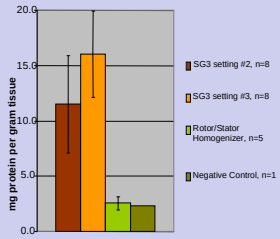
Partially desiccated ticks (wild caught) were separated into two categories; small (<1.5-5 mg) and large (>12-31mg). Ticks (n=3 per group) were either ground to fine paste in a mortar and pestle, or were disrupted in *The* Shredder SG3 by shredding 3 times for 10 s (30 s total) in FT 500-S PULSE Tubes. DNA was purified using a phenol/chloroform protocol with CTAB.

Figure 6. Protein Extraction from Skeletal Muscle



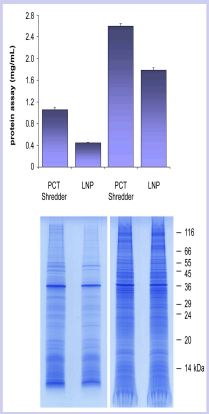
Rat skeletal muscle tissue was homogenized in ProteoSolve-IEF buffer supplemented with protease inhibitor cocktail. Samples (n=6 per group) were processed in The Shredder SG3 using either all-plastic FT 500-S PULSE Tubes or FT 500-MS PULSE Tubes with the metal disk insert. All shredder samples were shredded for 20 seconds at lever setting #2. For comparison, samples were disrupted in a glass/glass Dounce homogenizer by extensive homogenization. Protein concentration was measured by Bradford assay. SDS-PAGE was run on 8-16% gels and stained with Coomasie blue stain.

Figure 7. Protein Extraction from Cartilage Tissue



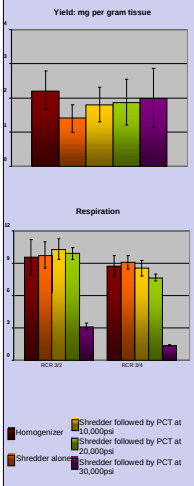
Protein from porcine cartilage (~30 mg per sample) was extracted in ProteoSolve-IEF buffer using the SG3 Shredder in FT 500-MS PULSE Tubes for 40 seconds at lever setting #2 or #3. Control samples were disrupted using a popular rotor-stator homogenizer for 1 minute. The negative control was generated by soaking a piece of cartilage in ProteoSolve-IEF without mechanical disruption. Protein concentration was measured by Bradford assay.

Figure 8. Protein Extraction from *C. elegans*



Extraction of protein from *C. elegans* using *The PCT Shredder* and The ProteoSolve-CE Native or The ProteoSolve-CE Stringent kits from PBI results in greater yield of protein than extraction by grinding in liquid nitrogen with mortar and pestle (LNP). All shredding was performed in FT 500-S PULSE Tubes. SDS-PAGE was run on 8-16% gels and stained with Coomasie blue stain.

Figure 9. Isolation of Intact and Functional Mitochondria from Skeletal Muscle



Mitochondria were extracted from rat muscle using a manual Potter-Elvehjem homogenizer method or by shredding in *The PCT Shredder* for 10 seconds with or without subsequent PCT.

Upper Panel: Yield of mitochondria is improved by PCT after disruption with *The PCT Shredder*, with yields that approach the control method (Control, n=15; Shredder alone, n=19; 10,000psi, n=18; 20,000psi, n=3; 30,000psi, n=2).

Lower panel: RCRs of mitochondria prepared using *The PCT Shredder* are comparable to homogenizer control, indicating that the mitochondria are intact and respire normally. Mitochondria exposed to 30,000psi (n=2) are severely impaired, while those treated at 10,000 psi (n=7) and 20,000 psi (n=3) are comparable to controls (homogenizer;n=5, Shredder alone; n=5).

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