

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 Tube 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 stabilizes 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.

Figure 1A 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 are airtight 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-borne 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 the Shredder System, when used in combination with PBI's *ProteoSolve-CE Native* or *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

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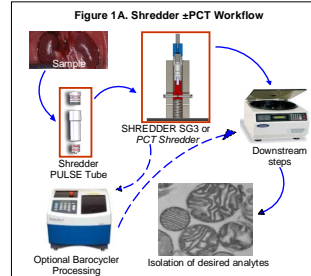
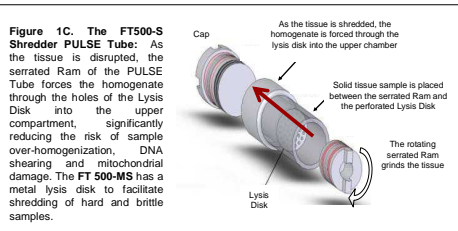


Figure 1B. The SHREDDER SG3 and The PCT Shredder

