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Abstract

Tough tissues such as muscle and lung generally require 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 or homogenization with a Dounce or rotor-stator homogenizers are some of the classical methods that can be used for the disruption of tissues prior to protein extraction. However, these manual methods are inherently labor-intensive, time consuming and prone to sample-to-sample variability and cross-contamination. In addition, these traditional methods, while effective, can not be easily adapted for use with hard samples such as blood vessels and connective tissue, or for more gentle extraction, as is required for the isolation of intact organelles. Here we demonstrate that a wide variety of sample types were efficiently disrupted using The PCT Shredder or The Shredder SG3, for diverse applications including proteomic profiling, isolation of nucleic acids and even extraction of intact mitochondria. We applied the methodology to a diverse range of sample types, including elastic tissues such as lung and muscle, tough samples such as leaves, hard samples such as seeds, as well as hard-to-disrupt organisms such as *C. elegans* and arthropods. In some protocols the shredder-disrupted samples were subjected to additional extraction using pressure cycling technology (PCT). Using this adaptable new methodology, 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 spinach leaves, apple seeds and whole ticks, as well as RNA from frozen rat lung tissue. In addition, we demonstrate that the Shredder can be used for initial tissue disruption for isolation of intact and functional mitochondria from fresh skeletal muscle, suggesting that brief disruption in the Shredder is compatible with isolation of other intact organelles.

Introduction

The PCT Shredder and the Shredder SG3

The PCT Shredder allows the user to rapidly grind samples directly in 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 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 2 PULSE Tube configurations for the Shredder. The all-plastic design (FT 500-S, Figure 1C) works better with fibrous or sticky samples such as muscle and lung tissue. The FT 500-SM configuration incorporates a perforated metal disk into the PULSE Tube design that improves shredding of hard or brittle samples such as cartilage, seeds and grains.

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, e.g. 10,000 - 20,000 psi, PCT can gently lyse cells and release their intracellular contents, including intact organelles [3]. PCT is also used to accelerate enzymatic activity by a wide range of enzymes including trypsin for proteomic studies [4] and Proteinase K for DNA isolation.

Results and Conclusions

We demonstrate convenient extraction of proteins, RNA, DNA and intact mitochondria from a variety of tough-to-disrupt sample types, including muscle (Fig. 7), cartilage (Fig. 6), tumor (Fig. 4) and lung tissues (Fig 10), *C. elegans* (Fig. 3), whole ticks (Fig. 9), as well as leaves (Fig 8) and grains (Fig. 2). Sample disruption in either type of Shredder can be combined with pressure cycling (Fig 5), but can also be used as a standalone technology for rapid single-tube sample grinding.

Sample disruption by shredding is gentle enough for recovery of intact and functional mitochondria, but can also be used for efficient extraction of intact genomic DNA, as well as proteins from a wide variety of sample types. Traditional manual methods for extraction from solid tissues are time consuming and often prone to cross-contamination due to the need to wash and re-use equipment such as mortars and pestles and other non-disposable homogenizers. In addition, most manual methods rely heavily on operator experience and skill to prevent undesirable side-effects like DNA shearing and mitochondrial damage.

The two configurations of the Shredder PULSE Tube (with and without the metal disk) can be used to optimize extraction from different sample types. The all-plastic FT 500-S works best with fibrous and sticky samples, such as muscle tissue. The FT 500-SM with the metal insert works best with hard but non-sticky samples, such as cartilage and plant seeds/grains.

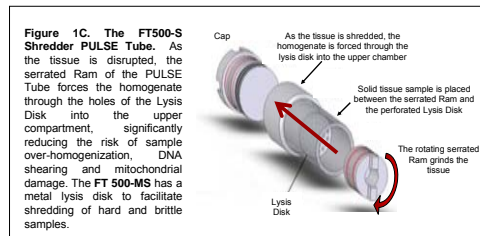


Figure 1C. The FT500-S Shredder PULSE Tube. As the tissue is disrupted, the serrated Ram of the PULSE Tube forces the homogenate through the holes of the Lysis Disk into the upper compartment, significantly reducing the risk of sample over-homogenization, DNA shearing and mitochondrial damage. The FT 500-SM has a metal lysis disk to facilitate shredding of hard and brittle samples.

Figure 1A. Shredder ±PCT Workflow

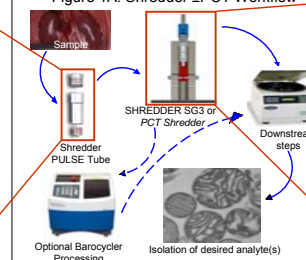
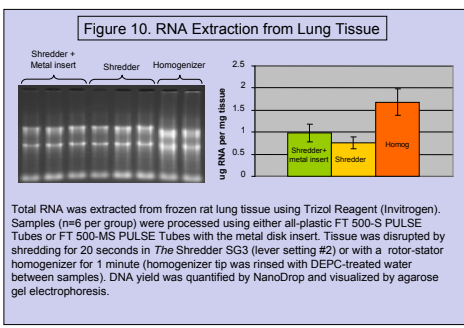
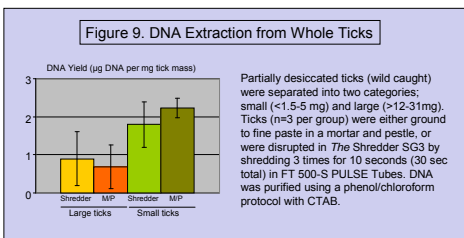
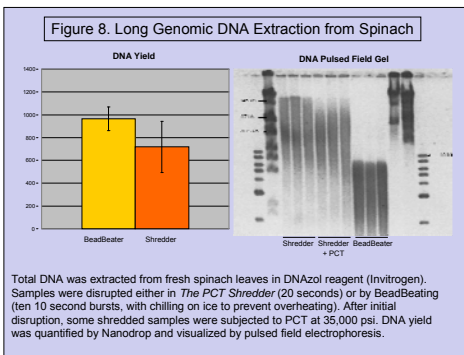
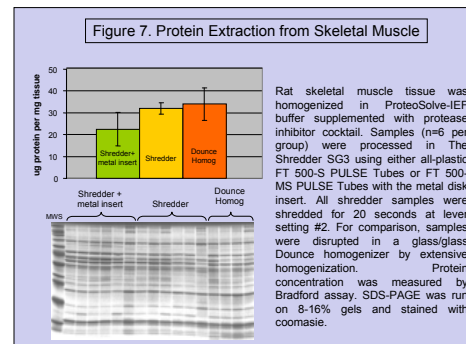
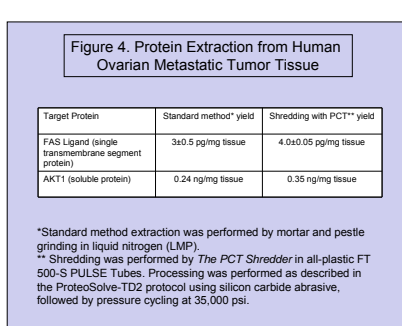
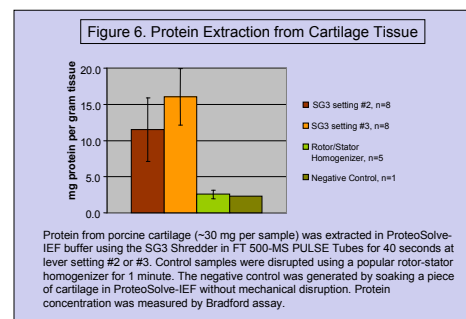
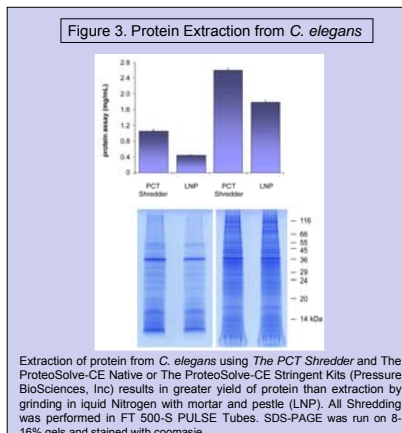
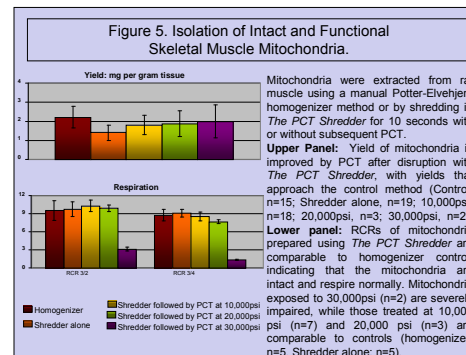
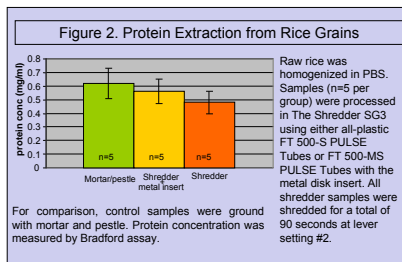


Figure 1B. The SHREDDER SG3 and The PCT Shredder



References

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