

**Proteolysis (Proteinase K)-PrEP:
Efficient and Rapid Extraction of Genomic DNA from Skeletal Muscle using
The PCT Shredder and Pressure-enhanced Proteolysis with Proteinase K**

Introduction

Genomic DNA extraction from tissues is usually performed by digesting the cellular proteins with Proteinase K, and isolating genomic DNA from the resulting lysate. This lengthy digestion procedure may require several hours (often more than 2-3 hours or even overnight) at 45-55°C [1]. In most genomic DNA extraction protocols, the tissue digestion step is the most time consuming. To accelerate this step and to achieve better digestion of proteins, Pressure BioSciences, Inc. (PBI) has previously developed a method using pressure cycling technology (PCT) to enhance Proteinase K activity. Proteinase K is one of several enzymes enhanced by pressure. Others include trypsin [2], chymotrypsin and pepsin [3, 4], Alcalase, Neutrase, Corolase 7089, Corolase PN-L, and papain [5]. Here we show that DNA extraction from skeletal muscle tissue using the standard Proteinase K method, can be significantly accelerated by first grinding the tissue for ten seconds using the PCT shredder, then subjecting it to Pressure Cycling.

PCT Sample Preparation System (PCT SPS)

The Pressure Cycling Technology Sample Preparation System (PCT SPS) uses rapid cycles of hydrostatic pressure between ambient and ultra high levels to control biomolecular interactions. The PCT SPS can be used to disrupt tissues, cells, and cellular structures to extract proteins and nucleic acids [6]. In addition, the PCT SPS can also be used to accelerate enzymatic reactions such as Proteinase K digestion. The PCT SPS uses a small, semi-automated bench top instrument (Barocycler NEP3229 or the NEP2320) in combination with single-use sample processing containers called FT500 PULSE Tubes (Pressure BioSciences, Inc. South Easton, MA). The combination of PCT and the FT500 employs rapid pressure changes, chemistry, and other biophysical mechanisms to accelerate protein digestion by Proteinase K and other enzymatic reactions.

The PCT Shredder

The *PCT Shredder* is designed to rapidly grind the sample directly in the PULSE Tube, in order to enhance extraction of tough, fibrous and other difficult-to-disrupt biological materials, such as certain plant and animal tissues. Since shredding and PCT are performed in the same tube, loss of sample or the likelihood of cross contamination is significantly reduced when compared to other processing methods. In addition, it has previously been shown that unlike tissue disruption by bead beating, use of *The PCT Shredder* does not result in detectable shearing of genomic DNA.

Enzymatic Digestion and PCT

At certain pressures, PCT alters conformations and interactions of biomolecules by rapidly and repeatedly raising and lowering pressure in the reaction vessel from ambient to high levels (up to 35,000 psi [240 MPa]). High hydrostatic pressure acts on the compressible constituents of the sample resulting in destabilization of secondary structures, but not in the disruption of covalent bonds. The protein unfolding that occurs under high hydrostatic pressure allows better access of proteases to the cellular proteins. Proteinase K is an endopeptidase that cleaves peptide bonds preferentially next to the carboxyl group of N-substituted hydrophobic aliphatic and aromatic amino acids. At the pressures selected for this application, Proteinase K is neither denatured nor inhibited. Thus, PCT accelerates the action of Proteinase K, presumably by unfolding proteins to expose target sites to the enzyme.

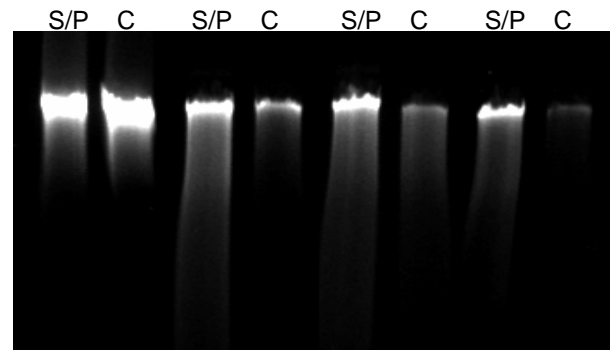


Figure 1

DNA extracted from Rat Skeletal Muscle by PCT Shredder at 20,000 psi (S/P) and control samples extracted without shredding at ambient pressure (C). Samples were digested with Proteinase K at 55° for the indicated time.

Materials and Methods

PCT-Accelerated Proteinase K Digestion of Rat Skeletal Muscle for Genomic DNA Extraction

Rat muscle tissue was processed in a Barocyler NEP3229. Each pressure cycle consisted of 60 seconds at 20,000 psi followed by 5 seconds at atmospheric pressure (tissue was stored at -70° prior to use). A circulating water bath was used to maintain the Barocyler chamber at 55°C.

For Proteinase K treatment, ~75 mg of tissue was cut into small pieces and placed either into a FT 500-SR PULSE Tube for shredding and PCT treatment, or into a centrifuge tube for control incubation at ambient pressure. 1080µL of Qiagen Buffer ATL and 120µL of 15 mg/mL Proteinase K solution were added to controls. 540µL of Qiagen Buffer ATL and 120µL Proteinase K solution were added to each sample to be shredded. Samples were shredded on low speed for 10 seconds, and then an additional 540µL Qiagen Buffer ATL was added. All samples were vortexed thoroughly. Proteinase K treatment was performed at 55°C for all groups.

Total time at pressure was either 10, 15, 20, or 60 minutes (10, 15, 20, or 60 cycles with 1 min at high pressure per cycle). All samples were vortexed before and after PCT treatment. To determine whether tissue dissolution was complete, samples were periodically removed from the Barocyler for observation. At these times all PCT treated samples and all controls were vortexed thoroughly. Complete lysis of all shredded rat skeletal muscle tissue was observed after 60 minutes at 20,000 psi in a 55° Barocyler, while visible pieces of tissue remained in all controls. After Proteinase K treatment, any residual (undissolved) tissue fragments were removed by centrifugation. DNA was isolated from the clarified lysate according to the DNeasy Blood and Tissue kit protocol (Qiagen). DNA recovery was quantified using the Nanodrop (Thermo Scientific). Aliquots from each sample were run on agarose minigels (Lonza) and visualized with ethidium bromide.

Results and Discussion

The results shown in Figures 1 and 2 confirm that at atmospheric pressure (control) DNA recovery from rat skeletal muscle requires at least an hour at 55°C. If the tissue is first gently ground for 10 seconds in *The PCT Shredder*, and then digested under PCT conditions, tissue dissolution occurs much more rapidly as indicated both by visual observation (dissolution of tissue pieces) and by increased DNA recovery at shorter digestion times.

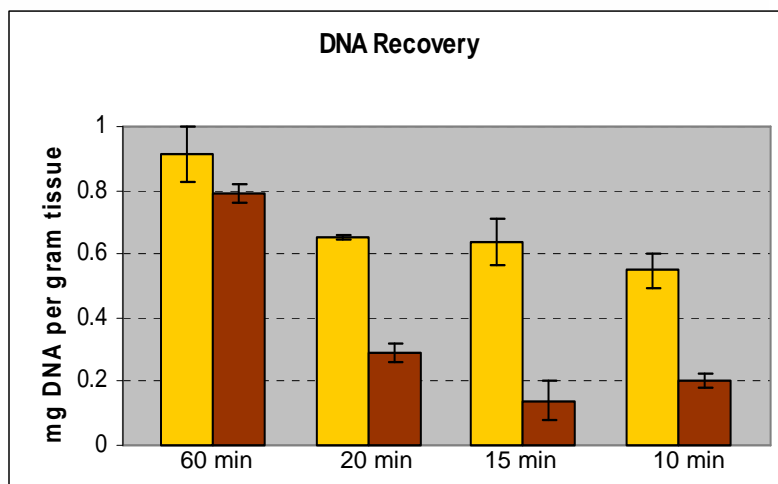


Figure 2

DNA recovery from triplicate samples using *The PCT Shredder* with PCT at 20,000 psi (yellow bars) or control (brown bars). DNA yield is expressed as mg DNA per gram tissue. Error bars indicate standard deviation.

Use of *The PCT Shredder* alone has also been shown to speed tissue dissolution and increase DNA recovery. Using the same method as described above, DNA recovery from shredded samples incubated in FT-500 tubes was compared to control (n=3 for each group). The most pronounced differences were observed at shorter time points. After 20 minute digestion, DNA recovery from the shredded samples was 2.7 fold-higher than control, averaging around 0.8µg per mg tissue. With longer digestion times, the difference became less pronounced. After an hour in Proteinase K, DNA recovery from shredded samples was 1.2 times higher than in controls. Gel electrophoresis demonstrates similar patterns of genomic DNA in samples isolated at 20,000 psi and at atmospheric pressure, supporting the conclusion that the PCT protocol is gentle and does not lead to shearing of the genomic DNA.

These results demonstrate that *The PCT Shredder* is an effective tool for accelerating Proteinase K digestion of fibrous tissue such as skeletal muscle and improving the efficiency of genomic DNA isolation.

References

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