

Proteolysis (Proteinase K)-PrEP: Pressure Enhanced Proteinase K Digestion of Tissue for Accelerated Genomic DNA Extraction

Introduction

Genomic DNA extraction from tissues often includes digestion of the cellular proteins with Proteinase K prior to genomic DNA isolation from the resulting tissue lysate. Protein digestion is the most time consuming step in most DNA isolation procedures. This lengthy digestion procedure may require 2-3 hours of incubation at 45-55°C. In fact, some protocols call for as much as an overnight digestion to adequately remove contaminating protein [1]. To accelerate this time consuming step and to achieve better digestion of proteins, Pressure BioSciences, Inc. (PBI) has developed a method using pressure cycling technology (PCT) to enhance protein digestion by Proteinase K. 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 tissue digestion by Proteinase K is accelerated under pressure, resulting in faster genomic DNA isolation both at 55°C and at ambient temperature in PBI's Pressure Cycling Technology Sample Preparation System (PCT SPS).

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 is comprised of a small, semi-automated bench top instrument (Barocycler NEP3229 or NEP2320) and single-use sample processing containers called FT500-ND PULSE Tubes (Pressure BioSciences, Inc., South Easton, MA). Used together, the PULSE Tubes transmit the pressure generated by the Barocycler to the sample, resulting in pressure enhanced proteolysis and accelerated genomic DNA isolation.

Enzymatic Digestion and PCT

At certain pressures, PCT alters conformations and interactions of biomolecules through the rapid and repeated raising and lowering of 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 does not disrupt 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 not significantly denatured nor inhibited. Thus, PCT accelerates the catalytic action of Proteinase K, presumably by unfolding proteins to better expose target sites to the enzyme.

Materials and Methods

Accelerated Proteinase K Digestion of Rat Tissues using PCT

In preparation for the Proteinase K treatment, ~25 mg of frozen rat heart or liver was cut into small pieces and placed either into a PULSE Tube for PCT-treatment or into a centrifuge tube for incubation at ambient pressure. Samples subjected to PCT were processed in a Barocycler NEP3229. Each cycle consisted of 1 minute at 20,000 or 35,000 psi followed by 5 seconds at atmospheric pressure for 60-130 cycles. Tissue digestion and DNA isolation were performed with the DNeasy Blood and Tissue kit (Qiagen) according to manufacturer's instructions. ATL Buffer (360µL) and Proteinase K (40µL at 15mg/mL) were added to each sample and vortexed thoroughly. Proteinase K treatment was performed either at ambient or high pressure and at room temperature or at 55°C. To conduct PCT at elevated temperatures, a circulating water bath was used to maintain the Barocycler chamber at the desired temperature. To determine whether tissue dissolution was complete, samples were periodically removed from the Barocycler for observation. At these times, all PCT samples and all controls were vortexed thoroughly. When PCT was performed at 20,000 psi at 55°C, complete lysis of rat heart muscle tissues was observed after as few as 60 cycles, while visible pieces of undigested tissue remained in all control samples incubated at ambient pressure at

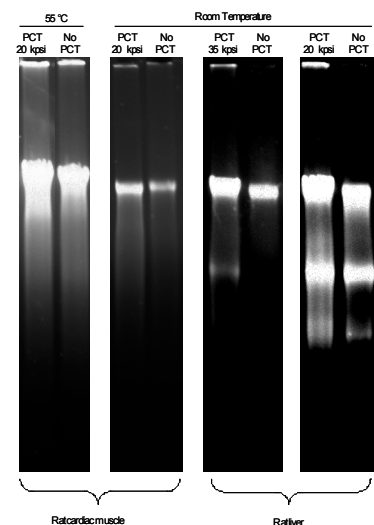


Figure 1. Genomic DNA Extracted from Rat Heart and Liver. Tissues were digested with Proteinase K with pressure cycling at 20 or 35 kpsi and compared to samples treated at atmospheric pressure. Digestion was performed either at ambient temperature or at 55°C.

55°C. After Proteinase K treatment, any residual tissue fragments were removed by centrifugation. DNA was isolated from the lysate according to the DNeasy Blood and Tissue kit protocol (Qiagen). Recovery of DNA was quantified using the Qubit fluorimeter (Invitrogen). Aliquots from each sample were run on agarose minigels (Lonza) and were visualized with ethidium bromide.

Results and Discussion

Results show that PCT enhances Proteinase K activity as indicated by both visual observation (dissolution of tissue pieces) and by increased DNA recovery at shorter digestion times. PCT in combination with Proteinase K resulted in more efficient recovery of DNA. Gel electrophoresis demonstrates similar patterns of genomic DNA in samples isolated at 20 and 35 kpsi and at atmospheric pressure, supporting the conclusion that the PCT protocol is gentle and does not lead to shearing of the genomic DNA (See Figure 1).

Tissue	Time in Minutes	Temp	Pressure	Avg recovery: μ g DNA per mg tissue	% of control*
Liver	130	Ambient	35 kpsi	0.66 (n=2)	228%
		Ambient	Ambient	0.29 (n=2)	
	90	Ambient	35 kpsi	1.09 (n=3)	279%
		Ambient	Ambient	0.39 (n=3)	
	100	Ambient	20 kpsi	1.47 (n=5)	155%
		Ambient	Ambient	0.95 (n=3)	
Heart Muscle	60	Ambient	20 kpsi	0.60 (n=2)	155%
		Ambient	Ambient	0.39 (n=2)	
	120	Ambient	20 kpsi	1.03 (n=2)	154%
		Ambient	Ambient	0.67 (n=2)	
	60	55°C	20 kpsi	3.95 (n=3)	153%
		Ambient	Ambient	2.59 (n=3)	

Table 1. Genomic DNA Extracted from Rat Liver and Heart Muscle After Proteinase K Digestion, as a Function of Time, Temperature and Pressure. *Expressed as increase in DNA recovery (per mg tissue) versus control experiment performed at ambient pressure. Note that while DNA recovery in both control and PCT samples varies from experiment to experiment, the PCT enhanced DNA recovery is always higher than the corresponding control.

Results shown in Table 1 demonstrate that PCT is an effective tool for enhancing the activity of Proteinase K and for improving the efficiency of tissue digestion for genomic DNA isolation. Pressure cycling technology is an attractive option for rapid preparation of genomic DNA from various types of tissues by reducing the time-to-result and by increasing yield.

References

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