Pressure Cycling Technology (PCT): Potential Applications in Forensics

Pressure BioSciences, Inc with Research from Harris County Institute of Forensic Sciences,
University of North Texas, and Florida International University

Recent emergence of new technologies has enabled forensics scientists to significantly improve analysis of DNA samples. These technologies have been shown to enhance DNA testing in several forensically-important evidentiary materials, including sexual assault items, bone, and touch samples. However, new methods of sample extraction have not kept pace with the advances of the analytical methods, subsequently limiting their full capabilities. Here we report advances in the use of pressure cycling technology (PCT) by several PBI collaborators. Their experiments show that PCT can improve either the yield or quality of DNA from poor quality bone samples, as well as shorten the time-to-result for identification from typical bone samples. Other studies will be described that demonstrate that PCT can improve the yield of DNA from low copy number touch samples, and thus, improve the potential for identification. We also report on a new method, in development, that uses PCT to selectively enrich for DNA extracted from sperm collected in rape cases. This method could help relieve the back-log of rape kit samples and could lead to better and more rapid identification of perpetrators.

PULSE Tubes

Barocycler

PCT Sample Preparation System

The Pressure Cycling Technology Sample Preparation System ("PCT SPS") employs rapid cycles of hydrostatic pressure, between ambient and ultra high levels, to precisely control biomolecular interactions. The PCT SPS can be used to accelerate enzymatic reactions, such as protein digestion with trypsin and other proteolytic enzymes, to prepare samples for analysis by mass spectrometry. In addition, the PCT SPS can be used to disrupt tissues and cells to extract cellular structures and components such as proteins, lipids, nucleic acids, and small molecules for further analysis. The PCT SPS is comprised of a Barocycler (a small, semi-automated bench-top instrument used to generate high hydrostatic pressure) and specially-designed, high pressure tolerant, single-use processing tubes (e.g., PULSE Tubes and PCT MicroTubes). Temperature can be controlled during the PCT-based sample preparation process by an external circulating water bath, which can be an important addition to the Pressure Cycling Technology Sample Preparation System

Harris County Institute of Forensic Sciences

Evaluation of Pressure Cycling Treatment Using a Barocycler® NEP3229 for Extraction of Low-Template Forensic DNA Samples

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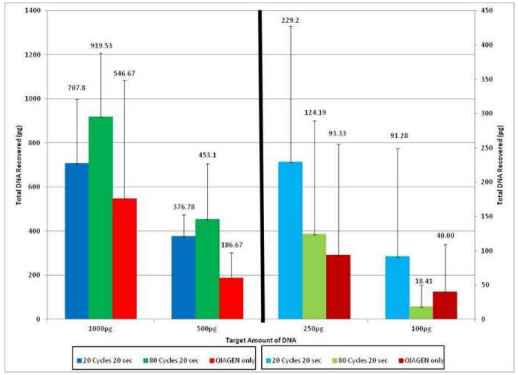

Samples were prepared by adding 1000pg, 500pg, 250pg, and 100pg of DNA from a calibrated solution of diluted human saliva to one-half of a cotton swab and drying overnight. Incubation was performed in PULSE tubes at 56° C in the Barocycler® NEP3229 chamber and, under varying conditions including 20, 40, 60, or 80 pressure cycles alternating between 20, 40, 60, or 80 seconds at 35k psi and 10 seconds at ambient pressure. The Figure below examines only the parameters that obtained the highest DNA yields at the different target amounts compared to the QIAgen extraction. These results show that pressure cycling treatment reproducibly increased the total amount of DNA recovered from a sample by 128% or more at quantities up to 500ng; where a doubling of recovered DNA would be most beneficial.

UNT HEALTH SCIENCE CENTER

Application of Pressure Cycling Technology (PCT) Reduces the Impact of PCR Inhibitors

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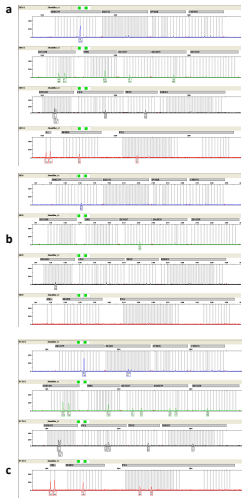

Effect of PCT on Human Bone Processing

Electropherograms representing a human bone sample processed with and without PCT. Two different quantities of bone powder were used for the NPC sample (0.2 g and 0.5 g) while 0.2 g of bone powder was used for the pressure treated sample. The bones were incubated in PULSE Tubes with extraction buffer at 56ºC overnight. Following incubation, the pressure treated sample was subjected to barocycling (30 Cycles [20s at 35kpsi and 10s at ambient psi]). Subsequently, DNA was extracted from all samples using hi-flow silica column extraction. The first elution of each sample was amplified (all samples yielded less than 0.1 ng/µl, 10 µl were amplified) and typed for STRs using the AmpFlSTR® Identifiler® Plus PCR Amplification Kit. Non pressure treated sample derived from approximately (**a**) 0.5 g bone powder and (**b**) 0.2 g bone powder. Pressure-treated sample derived from approximately (**c**) 0.2 g bone powder.

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Differential Lysis of Sperm and Epithelial Cells

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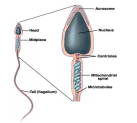
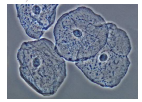
Sperm and epithelial cells should respond differently to pressure cycling based on their different composition.

Epithelial cells are larger, with more diffuse structures. They should be more distorted by pressure, and thus more sensitive to its effects.

Sperm DNA is associated with protamines, proteins with a high cysteine content, crosslinked with disulfide bridges– dense packing of DNA (12-18% cysteine).

Epithelial cell nuclei are surrounded by histone proteins. These are not as cross linked as protamines – less dense packing (0.2% cysteine).





Epithelial cell