# PRESSURE CYCLING TECHNOLOGY (PCT) APPLICATIONS FOR FORENSIC DNA ANALYSIS

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## ABSTRACT

**PURPOSE:** In an effort to increase deoxyribonucleic acid (DNA) recovery from devices used for collecting crime scene biological evidence, such as cotton swabs, samples containing various amounts of purified DNA or epithelial cells were placed onto swabs and processed using Pressure Cycling Technology (PCT). These samples were compared with control samples processed without PCT.

**METHODS:** PCT has yet to be considered for forensic applications but has the potential to enhance current DNA extraction methods by increasing DNA recovery while preserving the quality of the DNA. PCT exposes biological samples to alternating high hydrostatic and ambient pressures, allowing for molecular interactions to be controlled. This results in baroporation and the release of DNA into solution, while generally maintaining the sample's morphological integrity.

**RESULTS:** The data illustrate increased DNA yield in samples following PCT compared with those samples not exposed to pressure technology. These results indicate that PCT is a viable method to enhance DNA recovery from forensic samples. PCT can be used in conjunction with commercially available extraction reagents.

**CONCLUSIONS:** This research study demonstrates the capabilities and potential of PCT applications for forensic DNA analysis of biological evidentiary samples. The impact is that some samples that traditionally yield too little DNA for typing may now be suitable for routine analysis. Thus, more cases may be solved with this combined approach of PCT and DNA extraction.

**KEYWORDS:** pressure cycling technology, DNA extraction, forensic DNA analysis, high hydrostatic pressure

## INTRODUCTION

Most low copy number (LCN) practices focus on "downstream interpretation". However, LCN DNA samples do not yield reproducible and reliable results; thus, we are confronted with the "garbage in, garbage out" problem. One approach to increasing reproducibility of LCN samples is to recover more template DNA for analysis.

In an effort to increase DNA recovery from bones, hair, or from devices used for collecting crime scene biological evidence, such as cotton swabs, samples were processed with Pressure Cycling Technology (PCT). PCT is a novel technology that uses cycles of hydrostatic pressure between atmospheric and ultra-high levels (up to 35,000 psi and greater) to disrupt tissues, cells and cellular structures, releasing their contents into buffers or other solutions within specially designed tubes, Pressure Used to Lyse Samples for Extraction, or PULSE.

The PULSE ram cap can be adjusted to a suitable depth to accommodate the sample volume (200 to 1400µL). Each cap contains two rubber rings to prevent sample leakage or water entry into the tube. The ram compresses the sample at the start of each PCT cycle. When one PCT cycle finishes, the ram partially retracts as pressure is released. The combination of physical movement, rapid pressure changes, reaction chemistry and other bio-physical mechanisms breaks up the cellular structures and releases nucleic acids and other molecules into solution.

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**Contributors**
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## MATERIALS & METHODS

PCT (Pressure BioSciences, South Easton, MA) uses cycles of alternating high hydrostatic and ambient pressures to extract DNA from a variety of sample types, including but not limited to swabs, hairs, soft and hard tissues, and liquid samples. The severe changes in pressure allow for molecular interactions to be controlled and because of baroporation DNA is released into solution while generally maintaining the sample's morphological integrity. The instrument is either manually or computer controlled, is capable of cycling pressure between ambient and 40,000 PSI, and offers a working temperature range of 4°-37°C. The composition of the lysis buffer, pressure cycling parameters, and process temperature can be adjusted for specific applications.

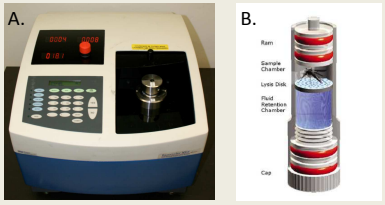



**Figure 1. A) Barocycler® NEP3229**. The Barocycler® NEP3229 (Pressure BioSciences, South Easton, MA) is a commercially available bench top instrument capable of processing up to three 1.5mL samples simultaneously. The instrument can be programmed for a set number of cycles consisting of ultra high amounts of pressure (5 to 35k psi) followed by release of pressure. The amount and duration of the ultra high pressure and the total number of cycles are determined by the user.
**B) PULSE Tubes.** Specially designed single use tubes (**P**ressure **U**sed to **L**yse **S**amples for **E**xtraction) are available with and without lysis discs for sample shredding.

**DNA**: Purified DNA (Quantifiler ® Human DNA Standard) was diluted to a concentration of 1 ng/µl. Cultured corneal epithelial cells were diluted to concentrations of 50 and 200 cells/µl.

**SAMPLES**: DNA was placed on swabs (and dried overnight prior to extraction) or placed directly into the PULSE tube.

**PULSE TUBE TREATMENT**: UltraTrol™ LN (Target Discovery, Inc., Palo Alto, CA) was used as a blocking agent to coat the tubes. A 1:10 dilution of the UltraTrol was placed in the tubes for two minutes of binding (per manufacturer's recommendation).

**BAROCYCLING**: 30 Cycles [20s at 35k psi and 10s at ambient psi]

**EXTRACTION**: Maxwell 16® (Trace sample on swab protocol) and QIAamp® DNA Mini Kit

**QUANTIFICATION**: Quantifiler® Human DNA Quantification Kit (reduced volume protocol) using ABI 7500 Real Time PCR System

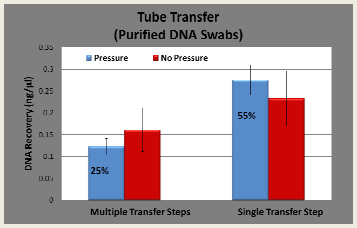



**Figure 2.** Multiple transfer steps were compared with a single transfer step during the pressure cycling procedure in order to determine the effect on DNA recovery. Swabs were created using 50 µl (500 pg/µl) purified DNA and dried overnight prior to extraction. For the single transfer step, the sample is maintained in the PULSE tube through the entire process versus the multiple transfer steps where the incubation and lysis steps are completed in a 1.5ml microcentrifuge tube prior to being added to the PULSE tube. Pressured samples were compared with non-pressured samples.

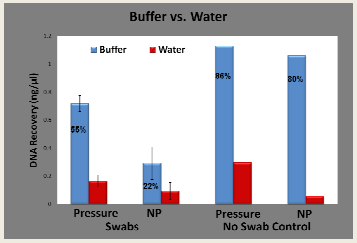



**Figure 3.** Buffer was compared with water in order to determine if buffer use during pressure cycling results in loss of DNA. For swabs and the no swab controls, 50µl (1 ng/µl) of cultured epithelial cells were used (swabs were dried overnight prior to analysis). Pressure samples were compared with non-pressured samples and swabs were compared with the no swab controls.

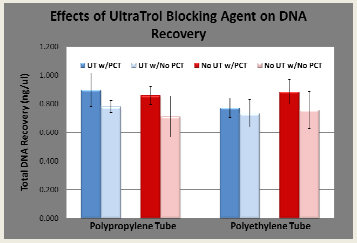



**Figure 4**. The effects of UltraTrol, a blocking agent, on PULSE tube DNA recovery were compared with untreated PULSE tubes. A 1:10 dilution of UltraTrol was placed into the tubes for a total binding time of two minutes. Following a 24-hour drying period, 150µl (1 ng/µl) of purified DNA was added directly to the tubes. This experiment also examined two different types of PULSE tubes, polypropylene and polyethylene in order to determine binding efficiency of the PULSE tubes. Half of the treated and untreated samples were exposed to pressure while half of the samples were not subjected to pressure.

## RESULTS

Experiments to date have evaluated purified DNA and cellular DNA, DNA input quantity, type of buffer, buffer volume, extraction method, tube siliconization (and other blocking agents), addition of BSA and SDS for tube coating, and loss of DNA during tube transfer.

Data suggest maintaining the sample within the PULSE tube throughout the experiment reduces DNA loss during tube transfer events. In an experiment designed to determine if buffers under pressure interfere with or lead to a loss of DNA yield, buffer was compared with water. Results demonstrate increased recovery with buffer and increased recovery in pressured samples compared with non-pressured samples.

The use of a blocking agent, UltraTrol ™ LN (UT), had no statistically significant effect on DNA recovery (in either pressured or non-pressured samples).

Results illustrate a trend towards increased DNA yield in samples, in purified and cellular DNA, following PCT compared with samples not exposed to pressure technology. The trend is more pronounced when smaller DNA quantities are tested.

***Data not shown:*** Results demonstrated an increase in DNA recovery when a 1:2 volume of incubation to lysis buffer was used. While no significant difference was observed in siliconized vs. non-siliconized tubes, or with use of SDS, data suggest that coating the tubes with BSA increases DNA recovery .

The results indicate that PCT is a viable method to enhance DNA recovery from forensic samples. PCT can be used in conjunction with commercially available extraction reagents.

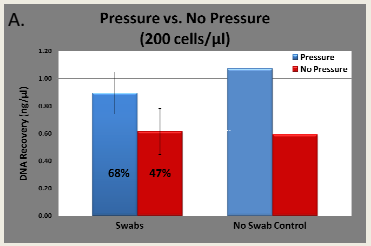
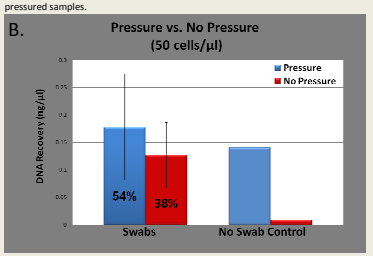



**Figure 5.** Samples exposed to pressure cycling technology were compared with non-PCT samples in order to determine the effect on DNA recovery. Swabs were created using two different concentrations of cultured cells: **Figure A)** 50 µl (200 cells/µl) and **Figure B)** 50 µl (50 cells/µl) and dried overnight prior to extraction. For the no swab control, 50 µl (200 cells/µl) or 50 µl (50 cells/µl) of cultured cells was added directly to the PULSE tube. Pressured samples were compared with non-pressured samples.




## CONCLUSIONS

The data illustrate increased DNA yield in samples following PCT compared with those samples not exposed to pressure technology. These results indicate that PCT is a viable method to enhance DNA recovery from forensic samples. PCT can be used in conjunction with commercially available extraction reagents.

Future directions of this research include studies using white blood cells, additional extraction methods, alternative collection devices, and blocking agents (for PULSE tube coating).

This research study demonstrates the capabilities and potential of PCT applications for forensic DNA analysis of biological evidentiary samples. The impact is that some samples that traditionally yield too little DNA for typing may now be suitable for routine analysis. Thus, more cases may be solved with this combined approach of PCT and DNA extraction.

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