# A Novel Method for the Extraction of Mitochondrial DNA from Human Hair, Skin, and Blood Stain

Forensic samples are often limited in their quantity; in addition, the quality of their biomolecules may also be in relatively poor condition. If there is insufficient material for nuclear DNA analysis, mitochondrial DNA (mtDNA) can provide great sensitivity and valuable information from samples such as hair, skin, or blood stains, by taking advantage of the fact that cells may contain thousands of copies mtDNA, while somatic cells typically contain two copies of nuclear DNA. [1]

In an effort to increase the safety, speed, simplicity, and efficiency of mtDNA extraction from forensic specimens, Pressure BioSciences, Inc. (PBI) has developed a novel extraction system based on a new, patented process called Pressure Cycling Technology (PCT). This PCT Sample Preparation System (PCT SPS) eliminates the requirement for the use of harsh chemicals and time consuming processes to extract and purify mtDNA from a number of samples. Furthermore, mtDNA may be released from the specimen in the single-use container in which the sample was collected, transported, and stored (PULSE Tube). In addition, mtDNA released by PCT from hair, skin, or blood can be amplified directly by PCR without the need for additional purification. Consequently, the PCT SPS offers a safer, more rapid, simpler, and more efficient method for extracting mtDNA.

## Pressure Cycling Technology (PCT)

PCT uses alternating cycles of high and low pressures to induce cell lysis. Cell suspensions or tissues, such as bone, are placed in specially designed, single-use processing containers (PULSE Tubes) and are then subjected to alternating cycles of high (up to 35,000 PSI) and ambient pressures in a pressure-generating instrument (Barocycler). Maximum and minimum pressures, the time at each pressure level, and the number of cycles is defined using a programmable logic controller. The reaction chamber of the Barocycler instrument is temperature controlled using a peripheral circulating water bath. Safety features in the PCT System design significantly reduce risk of exposure to the researcher to pathogens [2].

## Methods

All samples were obtained fresh from living human donors. Hair samples with roots were pulled from arms or legs using nucleic acid-free tweezers. Skin samples were collected using ~ 1 x 4 $cm^2$ of freshly opened Scotch Tape (3M Company, St. Paul, MN). Fresh whole blood was used in preparing the blood stain cloth. Dried blood stained cloth and fiber were processed in the tests.

The hair and skin on the Scotch Tape, or the blood stained cloth or fiber samples, were loaded in PULSE Tubes with 1.3-1.4 mL of TP buffer (100 mM Tris, 0.15 M KCl, pH 8.0) or RNase/DNase-free water. Samples were subjected to PCT processing for 5-10 cycles of pressure between ambient and 35 kpsi at 4°C. Dwell time at each pressure level was 20 seconds. Controls samples were incubated in TP buffer for 5 minutes, but were not subjected to PCT.

Ten (10) µL of sample were amplified by PCR (95°C for 5 min; 30 cycles of 94°, 58°, 70°C each for 1 min; and 70°C for 10 min) using primers specific for human mtDNA. DNA yields were determined semi-quantitatively by integrating the PCR product peaks obtained using the 2100 BioAnalyzer (Agilent, Palo Alto, CA). Real-time PCR was also employed to verify the presence of mtDNA.

Forensic samples are diverse and include specimens such as a single hair, minute amounts of skin cells, and blood stained cloth or a single cloth fiber. The features of the PCT SPS, which include a collection, transport, and processing container (PULSE Tube) and an automated pressure-generating instrument (Barocycler), offer a significant opportunity to improve the processing of these types of forensic specimens.

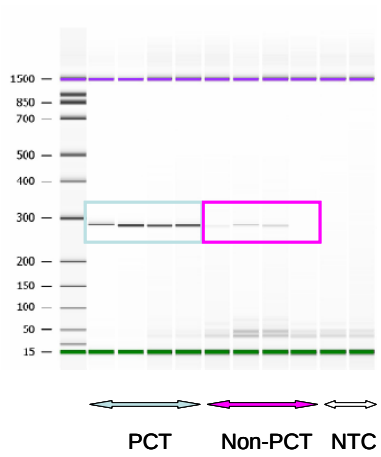


**Figure 1**. mtDNA PCR products from single human hair samples with PCT (10 cycles) or Non-PCT treated control samples and PCR no-template-controls (NTC).

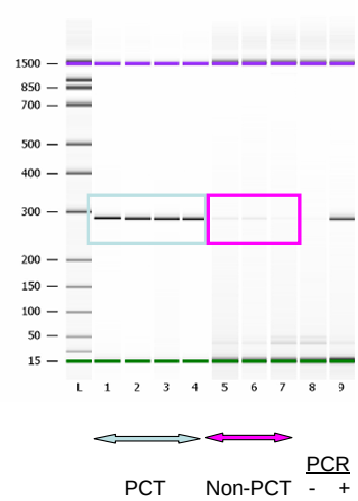


**Figure 2**. mtDNA PCR products from epidermal cells collected on Scotch Tape. PCT (10 cycles, Lanes 1-4) or Non-PCT (Lanes 5-7) treated control samples, no-template (Lane 8) and positive (Lane 9) PCR controls. Note: it is not necessary to remove cells from the tape prior to PCT processing.

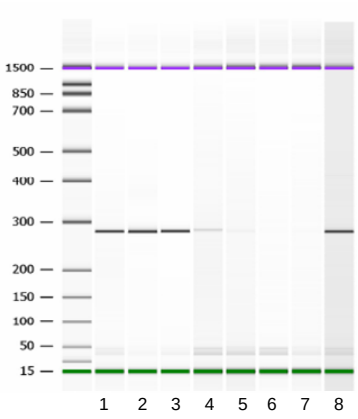


**Figure 3**. mtDNA PCR products from whole blood stained on single fiber. Lanes 1-2, PCT treated (5 cycles); Lane 3, bead beating control using Mini-cell Disrupter (BioSpec Products, Bartlesville, OK); Lane 4, non-PCT or non-BB treated blood-stained fiber control sample; Lane 5, no blood stained cloth control (no mechanical extraction); Lanes 6-7, no-template PCR control; and Lane 8, PCR positive control. Note: it is not necessary to isolate the blood stain from cloth or fiber prior to PCT processing.

## Results and Discussion

Figures 1-3 show the release of mtDNA from a single human hair, skin tape, and blood stained fiber, respectively. In all experiments, mtDNA was amplified in a PCR reaction without carrying out additional purification. These experiments show that after only 5-10 minutes of sample processing by PCT, mtDNA is ready for downstream applications, thus significantly reducing the time to result. Control experiment results from these experiments show that little or no mtDNA is released from any of these samples without the application of PCT.

The PCT SPS offers the forensic scientist the ability to more rapidly extract mtDNA from a variety of samples. Although it is not necessarily technically challenging to analyze mtDNA from hair, skin, or blood as compared to harder tissues, such as bone fragments, the PCT SPS extraction method eliminates the need for multi-step processes, yielding mtDNA ready for PCR amplification in minutes. Besides the advantage of time saving, the PCT SPS also offers other, significant advantages in the field of forensics. The PULSE Tube may be used as the collection and transport device from a crime scene to the forensic laboratory - a feature that is particularly advantageous with minute samples, such as a single hair, skin, or fiber. Further, the PULSE Tube is ideal for helping to maintain an unquestionable chain of custody, since the collected sample can be processed in the collection tube without further manipulations. Subsequently, the PULSE Tube reduces the risk of contaminating the sample, while providing a significant increase in the level of safety for the user. Finally, the Barocycler automates the process of sample preparation, which makes the PCT SPS method easy, fast, efficient and reproducible.

## References

[1] Saks, M.J. and Koehler, J.J. (2005) The Coming Paradigm Shift in Forensic Identification Science. *Science, 309. no. 5736, pp. 892 - 895*

[2] Schumacher RT et al. (2002). *Am. Laboratory*, 34, 38-43



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