DNA EXTRACTION FROM HAIR USING PRESSURE CYCLING TECHNOLOGY

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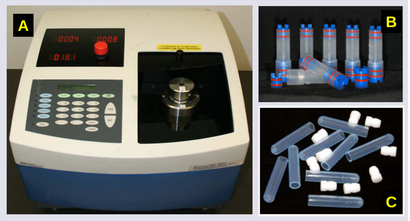
Introduction

Current methods for extracting DNA from hair shafts involve extensive sample preparation and processing, and often require the use of hazardous chemicals. Even though most hair extraction methods include a post extraction clean-up or concentration step, PCR inhibitors may still be present and interfere with downstream sample processing and analyses. A technology novel to the forensic community, i.e., pressure cycling**,** has the potential to enhance current DNA extraction methods by increasing DNA recovery while preserving the quality of the DNA.

Pressure Cycling Technology

Pressure Cycling Technology (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 result in baroporation and the release of DNA into solution while generally maintaining the sample's morphological integrity.

Figure 1. A) Barocycler® NEP3229. The Barocycler® NEP3229 (Pressure BioSciences, South Easton, MA) is a benchtop instrument capable of processing up to 48 samples simultaneously. The instrument can be programmed for a set number of cycles consisting of ultrahigh amounts of pressure (5 to 35kpsi) followed by release of pressure. The amount and duration of the ultrahigh pressure and the total number of cycles are determined by the user. **B) PULSE Tubes.** Specially designed tubes (**P**ressure **U**sed to **L**yse **S**amples for **E**xtraction) are available with and without lysis discs for sample shredding. **C) PCT MicroTubes™.** The PCT MicroTubes™ are designed for reduced-volume reactions and allow the user to process up to 48 samples in a single barocycler run. PCT MicroCaps are used with the MicroTubes to control the reaction volume, which can vary between 50, 100 and 150µL.



Samples to be extracted are placed in appropriate tubes. 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 instrument can be programmed for a set number of cycles consisting of ultrahigh pressure (5 to 35kpsi) followed by release of pressure. The amount and duration of the ultrahigh pressure and the total number of cycles are controlled by the user. 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.

Study Design

Hairs from 20 individuals were collected and the shafts were cut 2cm from the root into 2cm fragments. Each fragment was cleaned and sonicated prior to being extracted using one of six methods (Table 1). Samples undergoing PCT were placed into FT500-ND PULSE tubes or MicroTubes™ with 100µL caps and subjected to 30 cycles of 20sec at 35kpsi and 10sec at ambient pressure. Following extraction, the samples were amplified for the HV1 and HV2 regions of the mtDNA D-loop. PCR product of selected samples was quantified on the Agilent 2100 Bioanalyzer using the DNA 1000 LabChip® Kit (Agilent Technologies, Santa Clara, CA). All samples were sequenced using BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems™, Foster City, CA).

Table 1. Extraction Methods

Organic	No Pressure	2-hour incubation in 200µL Stain Extraction Buffer with ProK, PCIA extraction and MicroCon® YM-100 DNA concentration
	PCT	200µL reaction volume, PCT performed after incubation period
	miPCT	100µL reaction volume, miPCT performed after incubation period
DNA IQ™	No Pressure	1-hour incubation following DNA IQ™ Casework Sample Kit Protocol for the Maxwell 16® instrument (Promega, Madison, WI)
	PCT	200µL reaction volume, PCT performed after incubation period
	miPCT	100µL reaction volume, miPCT performed after incubation period

Results

Amplified product was quantified for three hairs across all six methods. The average mtDNA PCR product yield was higher for the full-reaction PCT method (200µL) for both organic and DNA IQ™ hair extraction methods. The highest PCR product yields were obtained by extracting DNA using the DNA IQ™ Casework Sample Kit for Maxwell 16® with PCT after the incubation step. The half-volume reactions were the least successful approaches based on average mtDNA PCR product yield (Figure 2).

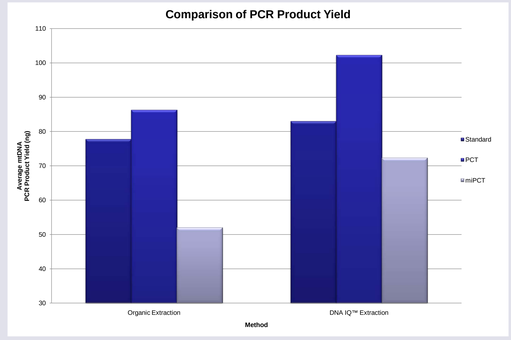


Figure 2. Three hairs were used to determine the average the amount of mtDNA recovered for each extraction method using Agilent quantification of the HV1 region (25µL volume). For each extraction type (organic of DNA IQ™) the methods were classified as either the standard procedure, the procedure with PCT at 200µL, or the procedure with miPCT at 100µL.

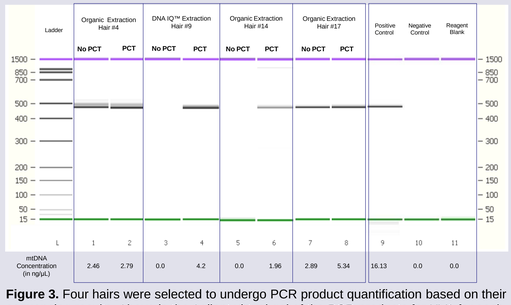


Figure 3. Four hairs were selected to undergo PCR product quantification based on their sequencing results. Above is the Agilent virtual gel of the PCR products for HV1 for each sample extracted by two methods, one of which uses PCT and one of which does not. Below the gel is the calculated concentration of PCR product for each sample.

Full sequence was obtained for HV1 and HV2 for 17 of 20 individuals for all 6 methods tested. Hairs from individuals 9 and 14 were the most challenging samples. The hairs from these two individuals were both very fine; past studies have shown that the diameter of a hair shaft is correlated with quantity of mtDNA present. Full sequence was only obtained for hair #14 using the organic extraction with PCT. DNA from hair #9 was successfully extracted using the organic extraction method with PCT, the DNA IQ™ Casework Sample Kit for Maxwell 16® with PCT, and the reduced-volume DNA IQ™ Casework Sample Kit for Maxwell 16® with PCT. Only one hair sample that underwent PCT resulted in incomplete sequence (hair #2). The only method that resulted in complete sequencing for all 20 individuals was the organic extraction with PCT. In some cases, the sequence data obtained for PCT were of greater quality than the sequence data of hairs from the same individual that did not undergo PCT (Figures 4, 5, and 6).

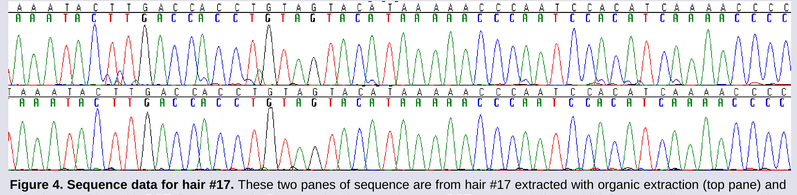


Figure 4. Sequence data for hair #17. These two panes of sequence are from hair #17 extracted with organic extraction (top pane) and organic extraction with PCT (bottom pane). Samples that have undergone PCT treatment often exhibit better quality sequence with a lower baseline, as shown above.

Results

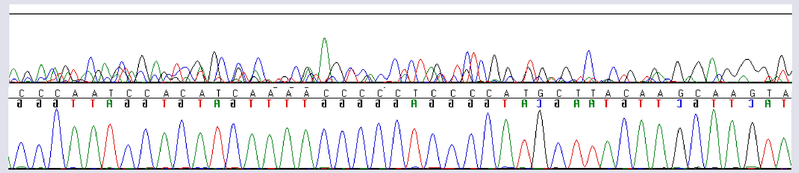


Figure 5. Sequence data from hair #14. Complete sequence from hair #14 was only obtained using organic extraction method with PCT (bottom pane). For comparison, the top pane is sequence from hair #14 with organic extraction without PCT.

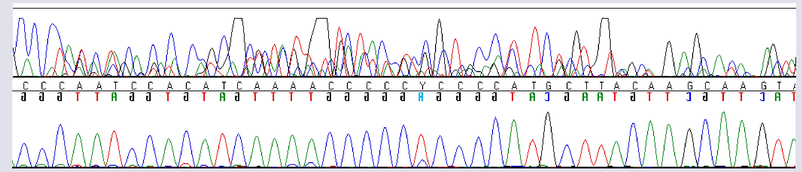


Figure 6. Sequence data from hair #9. DNA from hair #9 was successfully extracted with three PCT methods. The top pane of sequence is hair #9 with the traditional DNA IQ™ extraction without pressure treatment. The bottom pane of sequence is hair #9 with the DNA IQ™ extraction and PCT.

Discussion

The results of this study indicate that PCT enhances mtDNA recovery from hairs when combined with current established extraction methods. The hairs used originate from individuals which span the spectrum of hair thickness, color and texture, allowing for an overall evaluation of how these extraction methods would perform in routine forensic hair analysis. Due to the ProK digestion steps in each procedure, the hair shaft is broken down before PCT processing. This increases the surface area resulting in a more efficient enzymatic reaction and delivery of ultrahigh pressure during PCT cycling. The method that combined the DNA IQ™ Casework Sample Kit for Maxwell 16® and PCT at full-volume reactions performed the best, and would be a practical tool for extracting DNA from hairs without having to use an organic extraction using hazardous phenol-based reagents.

Because of the moderate success of the method which used half-volume reactions, this modification should be further investigated. Even with a lower performance, these extraction techniques were still able to yield more than 2.0ng/µL of mtDNA PCR product on average from a single hair sample . These results show that the miPCT methods can still be considered viable alternatives.

One consideration for the use of PCT is that the pressure being delivered will disrupt biomolecular interactions of any and all specimens in the PULSE tube. This may serve as a physical approach to releasing DNA from any bound PCR inhibitors, as opposed to the traditional enzymatic digestion or biochemical disruption of PCR inhibitors binding to DNA. By using PCT, PCR inhibitors might be more efficiently removed from extracts as well as isolated for further study.

Conclusions

Overall, PCT technology is a promising tool that can be used for DNA extraction. Using PCT in conjunction with the DNA IQ™ Casework Sample Kit for Maxwell 16® provided the best extraction results for mtDNA from hair shafts. Through more thorough testing, PCT may emerge as a safe and time-saving alternative to organic extractions for hairs and other biological materials.

Contributors
Special thanks to Matt Potter and Nate Lawrence at Pressure BioSciences, Inc. for providing the instrument and supplies; Cami Green, Kimberly Huston, and Erin McCombs at Promega for providing reagents.
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