Pressure Cycling Technology (PCT) Applications for DNA Extractions from Challenging Forensic Samples

Suzanne Gonzalez, Elizabeth Feller, Dixie Peters, Bruce Budowle, and Arthur Eisenberg
University of North Texas Health Science Center, Institute of Investigative Genetics, UNT Center for Human Identification, 3500 Camp Bowie Blvd., Fort Worth, TX 76107

Introduction

Skeletal remains, teeth, hair, and fingernails are samples that often contain very limited quantities of DNA and incur DNA damage and degradation due to environmental, bacterial, and post-mortem insults. Several components of bone, hair, and teeth also act as potent inhibitors of PCR, including collagen, calcium ions, melanin, and humic acids found in soil. In order to effectively extract the available genetic material, matrix-embedded cells must be released and PCR inhibitors efficiently removed.

Pressure Cycling Technology (PCT) uses rapid cycles of hydrostatic pressure between ambient and ultra high levels to control biomolecular interactions. During exposures to multiple cycles of pressure, biomolecules such as nucleic acids, proteins, lipids, and small molecules can be extracted into a lysis buffer from cells and tissue, allowing for a high degree of precision, reproducibility, convenience, speed, and safety. This novel extraction method has the potential to increase the amount of template DNA recovered for PCR from aged, degraded, damaged, inhibited, or otherwise compromised forensic samples.

PCT extractions were used in conjunction with the Maxwell® 16 Instrument (Promega Corporation, Madison, WI), a magnetic-particle-handling device, which uses the paramagnetic-based DNA IQ™ Resin (Promega Corporation, Madison, WI) to bind DNA. In order to maximize DNA recovery, the resin bound to DNA is taken through a series of purification steps in cartridges prefilled with reagents. The instrument can process up to 16 samples in approximately 30 minutes. This method, if successful, would eliminate the use of organic solvents, which are known teratogens and suspected

carcinogens with mutagenic effects. This semi-automatable extraction technique reduces the opportunity for sample contamination by limiting the number of manipulation steps and may diminish the loss of DNA due to repeated extractions and transfers.

Materials and Methods

DNA Extractions

Duplicate bone samples were processed using 200mg of bone powder and were digested at 56°C for 1 hour in Bone Incubation Buffer (Promega Corporation, Madison, WI) with Proteinase K (18mg/ml). Remaining bone powder was removed by centrifugation and samples were transferred to a DNA IQ™ Casework Sample Kit for Maxwell® 16 (Promega Corporation, Madison, WI) for purification.

Hairs from 20 individuals were collected and the shafts were cut 2cm from the root into 2cm fragments. Each fragment was cleaned by sonicating for 20 minutes in a 5% (w/v) Terg-a-zyme™ (Alconox Inc., White Plains, NY) solution. Single 2cm cuttings from each individual were extracted using a standard organic procedure or pre-processed using the Tissue and Hair Extraction Kit (Promega Corporation, Madison, WI) followed by extraction and purification using the DNA IQ™ Casework Sample Kit for Maxwell® 16.

Barocycling

Duplicate samples were compared by processing with and without PCT. Samples undergoing PCT were placed into FT500-ND PULSE tubes (Pressure BioSciences Inc., South Easton, MA) immediately following incubation. Samples were transferred to the Barocycler® NEP3229 (Pressure BioSciences Inc., South Easton, MA) and subjected to 30 cycles of alternating pressures consisting of 35kpsi for 20sec and at ambient pressure for 10sec.

STR Analysis

Genomic DNA was quantified using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster City, CA). Autosomal STRs were typed using AmpFℓSTR® MiniFiler® (Applied Biosystems, Foster City, CA) or PowerPlex®16 HS (Promega

Corporation, Madison, WI) PCR Amplification Kits following manufacturer's recommendations (10μL and 17.5μL extract input, respectively). DNA fragments were separated by capillary electrophoresis on the 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA). Genotype data was analyzed using GeneMapper® *ID* v3.2 software (Applied Biosystems, Foster City, CA).

mtDNA Sequencing

Hair samples were amplified for the HV1 and HV2 regions of the mtDNA D-loop. PCR product 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) and purified using the BigDye XTerminator® Purification Kit (Applied Biosystems, Foster City, CA). Samples were subjected to electrophoresis on a 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA) and data collected using Sequencing Analysis Software v5.3.1 with KB™ Basecaller v1.4 (Applied Biosystems, Foster City, CA). Mitochondrial haplotype data were analyzed using Gene Codes Sequencher™ 4.7 software (Gene Codes Corporation, Ann Arbor, MI).

Results and Discussion

Twelve challenging bones were selected for this study. Samples were previously processed at the University of North Texas Center for Human Identification, Fort Worth, TX using **AmpFℓSTR® Profiler Plus® ID and COfiler® PCR Amplification Kit**s (Applied Biosystems, Foster City, CA). Composite profiles consisting of 3 independent amplifications using 32 PCR cycles (manufacturer's recommendations are 28 cycles) were previously obtained, which yielded partial STR profiles for 6 samples and full profiles for 6 samples. Composite DNA profiles (generated by multiple, independent amplifications) and DNA profiles generated by increasing the number of PCR cycles beyond the manufacturer's recommendations are currently prohibited for upload into the National DNA Indexing System (NDIS). Therefore, we sought to determine if PCT added to a bone extraction procedure would yield sufficient quantities of DNA so that standard typing protocols could be used to obtain uploadable results.

Duplicate bone samples were compared with and without the PCT procedure. Quantification results revealed more than a 2-fold increase in average DNA recovery from bone in samples that underwent PCT compared with the no pressure controls (Figure 1). PCT samples amplified with MiniFiler® and PowerPlex® 16 HS (using the manufacturer's recommended cycle numbers) exhibited an average increase in the number of detectable alleles (51.4% and 26.7%, respectively). Of the bones tested, seven of twelve samples processed with PCT met the minimum requirements of 8 reportable loci (excluding amelogenin) for NDIS upload.

Hairs from 20 individuals were compared using PCT in conjunction with the Maxwell or standard organic extraction procedures. Compared with no pressure control samples, the average PCR product yield was approximately 4-fold greater in PCT samples in conjunction with an organic extraction, and 54% greater using the DNA IQ™ Casework Sample Kit for the Maxwell® 16, with the greatest total yields obtained with the latter method (Figure 2). Complete sequence coverage was obtained for HV1 and HV2 mtDNA regions for 97.5% (39/40) of the hair samples processed with PCT and the organic or Maxwell® 16 extractions. In most instances, sequence data obtained for PCT processed samples were of equal or greater quality than the sequence data of hairs from the same individual not treated with pressure.

Conclusions

PCT extraction followed by DNA purification using the DNA IQ™ Casework Sample Kit for the Maxwell® 16 offers a notable enhancement to conventional extraction procedures tested herein. Potential benefits include increased DNA yield, reduced processing time, cost reduction, and the elimination of hazardous organic reagents used in most common extraction techniques for challenged forensic samples. This novel extraction method can increase the amount of DNA recovered for downstream analysis from forensic samples, including aged, degraded, damaged, inhibited, or otherwise challenging samples. PCT has the concomitant benefit of decreasing both labor time and consumables cost required for DNA analysis. Based on these findings, we believe that

PCT should be pursued for further validation as a valuable tool in the repertoire to augment the DNA typing efficacy and workflow.

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References

1. Pressure BioSciences, Inc. AN-1004.v2 - A Novel Method for the Extraction of Mitochondrial DNA from Human Hair, Skin, and Blood Stain - 03/02/06

2. Pressure BioSciences, Inc. AN-1003.v1 - DNA Extraction from Bone Fragments without Pulverization - 03/02/06

3. Tao, Feng, Chunqin Li, Gary Smejkal, Alexander Lazarev, Nathan Lawrence, and Richard T. Schumacher. *Pressure Cycling Technology (PCT) Applications in Extraction of Biomolecules from Challenging Biological Samples*. **High Pressure Bioscience and Biotechnology** 1 (2007): 166-73.

4. Promega Corporation. *Tissue and Hair Extraction Kit (for use with DNA IQ™) Protocol*. Technical Bulletin, TB307. Revised 05-09.

5. Promega Corporation. *DNA IQ™ Casework Sample Kit for Maxwell® 16*. Technical Bulletin, TB354. Revised 05-09.

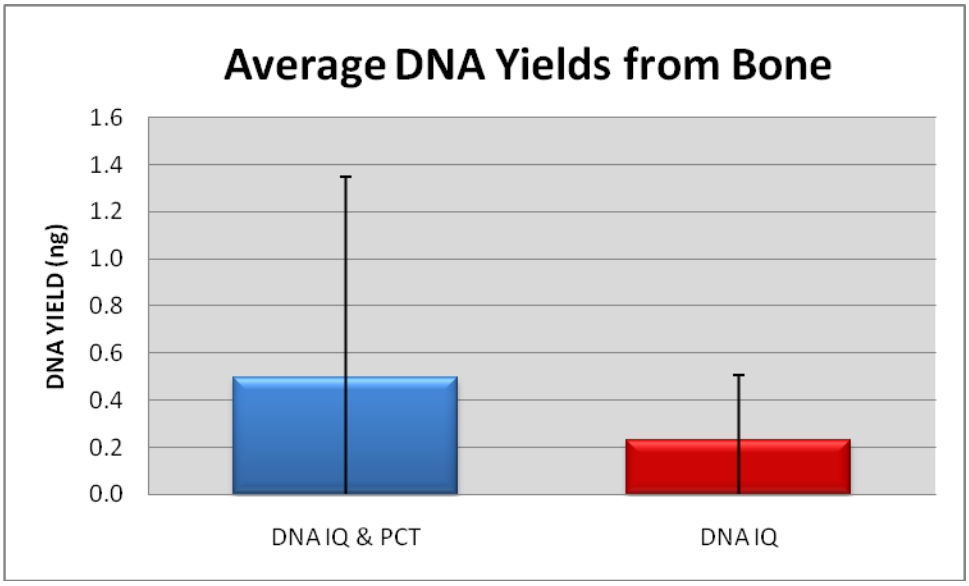

Figure 1. **Average DNA Yields from Bone**. The average DNA yields were calculated for 12 challenging bone samples subjected to Pressure Cycling Technology and DNA extraction/purification using the DNA IQ™ Casework Sample Kit for Maxwell® 16 (DNA IQ & PCT) and compared to average yield of duplicate samples processed with the DNA IQ™ Casework Sample Kit for Maxwell® 16 without PCT (DNA IQ). Samples processed with PCT resulted in at least a two-fold increase in DNA recovery.

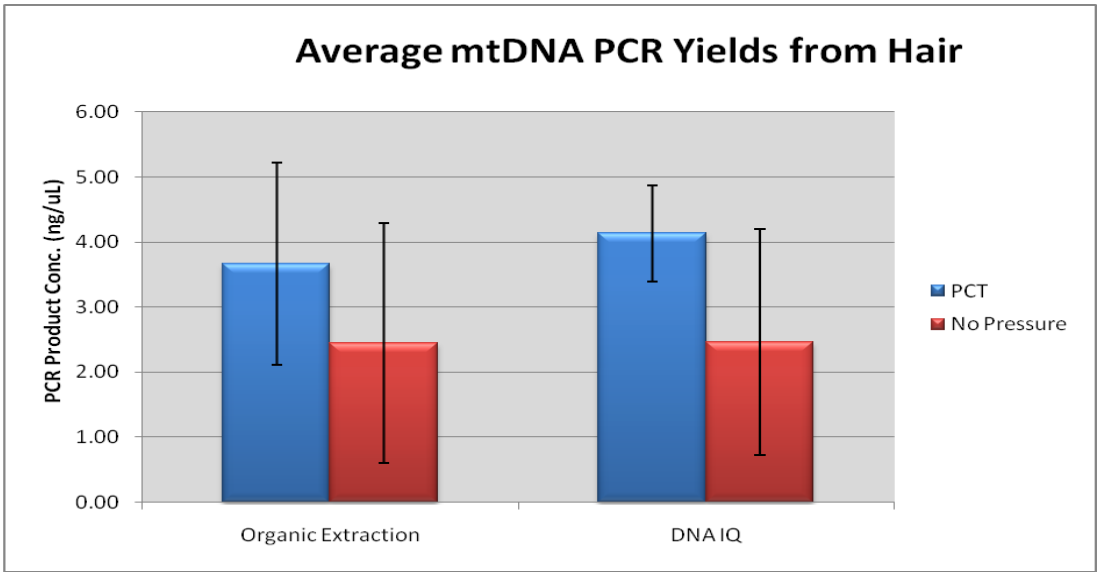

Figure 2. **Average mtDNA PCR Product Yields from Hair**. The average PCR product yields for mitochondrial DNA hypervariable region 1 (HV1) were calculated for single 2cm hair shaft cuttings sampled from 4. Samples were extracted using both an organic method (Organic Extraction) or extracted using the DNA IQ™ Casework Sample Kit for Maxwell® 16 (DNA IQ). Average yields were compared for each method run in combination with Pressure Cycling Technology (PCT) or without PCT (No Pressure). Samples processed with PCT resulted in a 67% increase in amplified product for samples extracted with organic method. An average increase of 59% in product yield was obtained using the DNA IQ™ Casework Sample Kit for Maxwell® 16 and PCT compared to the no pressure control samples.