Pressure Cycling Technology (PCT) Reduces Effects of Inhibitors of the PCR

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Abstract

A common problem in the analysis of forensic human DNA evidence, or for that matter any nucleic acid analysis, is the presence of contaminants or inhibitors. Contaminants may co-purify with the DNA and inhibit downstream PCR, or they may present samples effectively as containing fewer templates than exist in the PCR, even when the actual amount of DNA is adequate. Typically, these challenged samples exhibit allele imbalance, allele dropout and sequence specific inhibition which can lead to interpretational difficulties. Lessening the effects of inhibitors may increase the effective yield of challenged low template copy samples. High pressure may alter some inhibitors and render them less effective at reducing the yield of PCR products. In an attempt to enhance the amplicon yield of inhibited DNA samples, pressure cycling technology (PCT) was applied to DNA exposed to various concentrations of hematin (0, 1.25, 2.5, 5, and 7 µM) and humic acid (0, 1.25, 2.5, 5, and 7 ng/µl). The effect of high pressure on the inhibitors and, subsequently, the PCR process was assessed by measuring DNA quantity by qPCR and evaluating STR typing results. The results support that pressure cycling technology reduces inhibitory effects and, thus, in effect enhances yield of amplified products of both hematin and humic acid contaminated samples. Based on the results obtained in this study, this method can improve the ability to type challenged or inhibited DNA samples.

Materials and Methods

DNA

Experiments were performed using the Quantifiler® Human DNA Quantification Kit (Life Technologies) DNA Standard (Raji cell line; 200 ng/µL purified DNA). The standard was diluted to a final concentration of 1 ng/µl for each experimental sample.

PRESSURE CYCLING TECHNOLOGY

Samples undergoing PCT were placed in either FT500-ND PULSE Tubes or MicroTubes. Samples were transferred to the Barocycler® NEP3229 and subjected to 30 cycles of alternating pressures consisting of 35kpsi for 20 seconds and ambient pressure for 10 seconds. Non-pressure treated controls (NPC) controls also were prepared.

QUANTIFICATION

Quantity of DNA was determined using the Quantifiler® Human DNA Quantification Kit on the ABI 7500 Real-Time PCR System (Life Technologies).

BONE SAMPLES

Five human bones were obtained and prepared for DNA extraction. The bones were cleaned and air dried overnight. Then the bones were crushed to powder using a 6750 Freezer/Mill (SPEX SamplePrep L.L.C.), filled with liquid nitrogen, using a protocol of a 10 minute re-chill followed by 5 minutes of grind time at 15 impacts per second. Approximately 0.2 g of bone powder was placed in a PULSE™ Tube. 1 ml of extraction buffer containing 0.5 M EDTA pH 8.0 (Invitrogen Corporation), 1% sodium lauroyl sarcosinate (sarkosyl, n-lauroyl sarcosine; Sigma), and 100 µg/ml Proteinase K (Invitrogen Corporation) were added to each sample and vortexed. Both pressure treated and NPC samples were placed into PULSE Tubes. For three of the bones, the NPC sample contained 0.5 g bone powder. The bones were incubated at 56°C with constant agitation for either two hours or overnight. Following incubation, samples were subjected to either PCT or no pressure. All bone samples were processed using a hi-flow silica column extraction.

AMPLIFICATION AND STR TYPING

Approximately one nanogram of DNA for each extract was amplified using the reagents contained in the AmpF*l*STR® Identifiler® PCR Amplification Kit (Life Technologies) according to the manufacturer's recommendations. A subset of experiments was performed using the reagents contained in AmpF*l*STR® Identifiler® Plus PCR Amplification Kit (Life Technologies) according to the manufacturer's recommendations. PCR products were separated and detected on an ABI 3130xl Genetic Analyzer (Life Technologies) following the manufacturer's recommendations. The detection and interpretation thresholds both were set at 50 relative fluorescence units (RFU).

Results

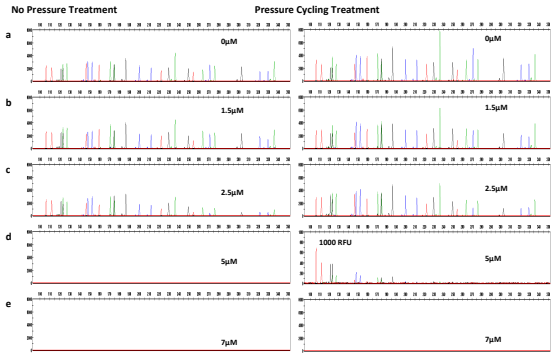

Figure 1 Effect of PCT on Hematin Inhibition

Various concentrations of hematin (0, 1.5, 2.5, 5, and 7 µM) were added to 100 µl DNA (1 ng/µl), placed in MicroTubes and were subjected to either barocycling (30 Cycles [20s at 35kpsi and 10s at ambient psi]) or no pressure. 1 µl of each sample subsequently was amplified and typed for STRs. Samples were run in replicates of five. Non-pressure cycled samples are on the left with pressure cycled samples on the right and concentration of hematin is as follows: (**a**) 0 µM, (**b**) 1.5 µM, (**c**) 2.5 µM, (**d**) 5 µM, and (**e**) 7 µM. Pressure samples were compared with non-pressured controls.

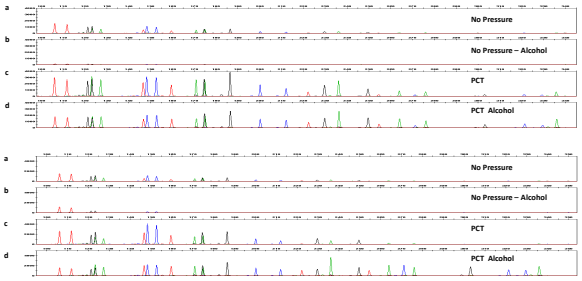

Figure 3 Effect of Ethyl Alcohol Addition on Hematin and Humic Acid Inhibition

Top Panel: 2.5 µM/µl of hematin were added to 100 µl DNA (1 ng/µl), placed in MicroTubes and were subjected either to barocycling (30 Cycles [20s at 35kpsi and 10s at ambient psi]) or no pressure. Comparison samples for pressure and no pressure groups were created with the addition of EtOH at a final concentration of 10%. 1 µl of each sample subsequently was amplified typed for STRs. Samples were run in replicates of three. Non-pressure cycled samples containing 1 ng/µl control DNA and 2.5 µM/µl hematin, without (**a**) and with (**b**) addition of 10% Ethyl Alcohol. PCT samples containing 1 ng/µl DNA and 2.5 µM/µl hematin, without (**c**) and with (**d**) addition of 10% Ethyl Alcohol are displayed.

Bottom Panel: 2.5 ng/µl of humic acid were added to 100 µl DNA (1 ng/µl), placed in MicroTubes and were subjected to either barocycling (30 Cycles [20s at 35kpsi and 10s at ambient psi]) or no pressure. Comparison samples for pressure and no pressure groups were created with the addition of EtOH at a final concentration of 10%. 1 µl of each sample subsequently was amplified and typed for STRs. Samples were run in replicates of three. Non-pressure cycled samples containing 1 ng/µl DNA and 2.5 ng/µl humic acid, without (**a**) and with (**b**) addition of 10% Ethyl Alcohol are displayed. Pressure cycled samples containing 1 ng/µl DNA and 2.5 ng/µl humic acid exposed to PCT without (**c**) and with (**d**) addition of 10% Ethyl Alcohol are displayed.

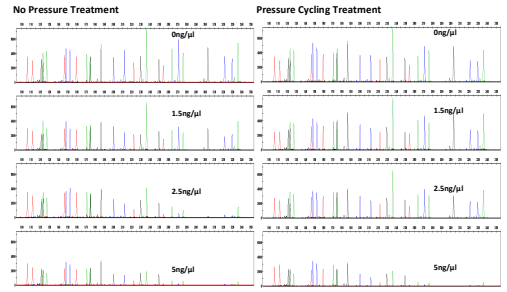

Figure 2 Effect of PCT on Humic Acid Inhibition

Various concentrations of humic acid (0, 1.5, 2.5, and 5 ng/µl) were added to 100 µl DNA (1 ng/µl), placed in MicroTubes and were subjected to either barocycling (30 Cycles [20s at 35kpsi and 10s at ambient psi]) or no pressure. 1 µl of each sample subsequently was amplified and typed for STRs. Samples were run in replicates of five. Non-pressure cycled samples are on the left with pressure cycled samples on the right and concentrations of humic acid are as follows: (**a**) 0 ng/µl, (**b**) 1.5 ng/µl, (**c**) 2.5 ng/µl, and (**d**) 5 ng/µl. Pressure samples were compared with non-pressured controls.

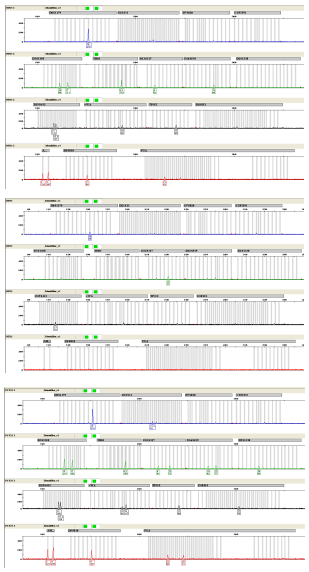

Figure 4 Effect of PCT on Human Bone Processing

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 56°C 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 AmpF*l*STR® 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.

Conclusions and Future Directions

This study shows the potential of enhanced PCR efficiency for samples containing an inhibitor when PCT treated compared with those samples not exposed to PCT. This research study suggests that PCT potentially has applications for forensic DNA analysis of certain challenged forensic DNA samples by reducing the effects of inhibitors known to be present in some bone samples. Future research will focus on elucidating the mechanism(s) that overcomes the effect of inhibition.

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