OVERCOMING PCR INHIBITION OF LOW QUANTITY AND LOW QUALITY SAMPLES WITH AMPLIFICATION ENHANCERS

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The use of forensic DNA to solve crimes, sexual assaults, homicides, cold, and missing person cases, as well as exonerate the innocent is well established. Forensic evidence samples such as hair and touch samples may have less than 100 pg of DNA (1,2,8). The low quality and low quantity amounts of DNA are due, in part, to non-optimal conditions including damaged, degraded, low copy number (LCN), and inhibited (humic acid, hematin, indigo dyes) DNA samples. In order for this evidence to have value, the DNA must be amplified so that it can be analyzed for identification (4). Forensic PCR protocols specify, for optimal amplification, 1.0 ng of DNA should be used (7). Yet, the quality and quantity of typical low copy DNA falls below this requirement. LCN or touch samples can have approximately 15 cells or less. The presence of LCN samples could be due to damage or degradation from ancient remains (1,8,9), or trace biological evidence such as fingerprints (3,5). Also, samples may include inhibitors that disrupt the amplification reactions during PCR, resulting in partial profiles or no typing, reducing the probative value of the samples (16).

There are several methods and modifications that are being utilized to analyze LCN and degraded samples during PCR amplification, such as increasing cycle numbers, adding more *Taq polymerase* and BSA, and whole genome amplification; however, the resulting profiles may be difficult to interpret with increased allelic imbalance and stutter product formation (2,8).

A new method to enhance amplification includes the use of a novel reagent, know as PCRboost (Biomatrica, Inc.), that was reported to improve PCR performance five-fold or more on challenging and difficult to amplify samples (18). This project will explore the amplification enhancement of PCRboost on low quantity and low quality DNA samples that are spiked with inhibitors.

This project will explore the sensitivity of control DNA under 1.0 ng with varied concentrations at 1.0 ng, 0.5 ng, 0.25 ng, 0.125ng, 0.0625 ng, and 0.03125 ng with and without PCRboost. Additionally, this project will examine different amplification strategies with and without PCRboost on varying amounts of inhibited DNA samples. It will also look at forensic organic (phenol chloroform) extraction laboratory techniques which may cause inhibition while purifying DNA samples, and strategies to overcome inhibition with and without PCRboost. Also, it will investigate the amplification of damaged, degraded, and low copy number DNA samples with and without PCRboost.

Control DNA at 0.62 ng was spiked in duplicate with 0.4 uL, 0.8 uL, and 1.2 uL of Phenol/Chloroform/Isoamyl Alcolhol 25:24:1 (PhOH). Samples were then quantified with Real-Time PCR (qPCR) using the Quantifiler Kit (Applied Biosysems) (17). PCR amplification was performed using the Identifiler Kit (Applied Biosystems) as per the manufacturers recommendations, and the Identifiler protocol was modified to explore different amplification strategies with and without 2x Taq and BSA, as well as with and without PCRboost. The products were separated and detected using capillary electrophoresis (CE) on the ABI 310 Genetic Analyzer (Applied Biosystems). Analysis of the data was performed by GeneMapperID software, from Applied Biosystems, which assesses both quantitative and qualitative data of all samples.

Enhanced recovery of alleles and increased peak heights was observed with PCRboost on the control DNA spiked with 0.4 uL and 0.8 uL of PhOH.

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