Title: Recovering DNA profiles from low quantity and low quality forensic samples

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Abstract:

Successful forensic analysis depends on the ability to identify and individualize biological evidence. Many forensic evidence samples such as hairs, bones, teeth and sexual assault evidence often contain less than 100 pg of DNA (1,2). Low DNA yields may be due to damage or degradation (1,2), small cell numbers found in low copy number (LCN) or 'touch' samples (1-5), oligospermic (6) or aspermic perpetrators (7), or low male DNA from extended interval post-coital samples in sexual assault cases (8). Trace biological evidence, including fingerprints, also provide low yields of DNA (9-11). Degradation is another factor that can contribute to further damage compromised sample types, including those derived from ancient or degraded bones or teeth (12,13). Degradation results in reduction or loss of the structural integrity of cells and proteins, which in turn affects the quantity and quality of recovered nuclear and mitochondrial DNA (mtDNA). Sub-optimal storage can also detrimentally affect sample integrity. Reduction in DNA recovery has been observed with refrigerated liquid DNA extracts and also those exposed to multiple freeze-thaw cycles; the loss may be exacerbated by the use of certain microfuge tubes (14,15). Therefore, the development of optimal methods is critical for successful recovery of DNA profiles from these types of low quality and quantity forensic samples, particularly if downstream analysis or re-testing is necessary.

Low yields or loss of DNA due to these and other factors may preclude or diminish the ability to test LCN crime scene samples using current STR methods, thus mtDNA testing is typically dictated for low quantity samples suffering from advanced states of degradation. Forensic PCR protocols typically specify 1.0 ng of DNA for optimal amplification (16). However, the quantity and quality of template DNA from typical low copy forensic samples falls below this requirement. Furthermore, samples may also contain inhibitors to PCR that co-extract with the DNA, resulting in sub-optimal amplification reactions providing partial profiles or no typing, thereby greatly reducing the probative value of the samples. Modifications to existing amplification and typing protocols (e.g. mini amplicons, whole genome amplification and LCN protocols) to increase the DNA signal are currently being investigated to increase the analytical success rate of challenged samples (17-19). However, complete forensic DNA profiles are not always achieved when the samples are extremely low quantity and quality.

A method was recently reported where inclusion of a novel reagent, PCRboost™ (Biomatrica, Inc.) was able to enhance amplification 5-fold or more of challenging and difficult to amplify samples. ***This study aims to evaluate the use of PCRboost for forensic DNA analysis to enhance amplification and recovery of forensic DNA profiles from low quantity and low quality samples.***

This study will be conducted in three phases: (1) Amplification of control DNA (including 9947a) using serial dilutions down to pg amounts; various formulations of PCRboost will also be evaluated; (2) Amplification of damaged, degraded and low copy DNA samples including non- probative bone and teeth samples; (3) Amplification of DNA containing varying amounts of inhibitors. Other experiments including amplification of mixtures will also be performed. Results from preliminary experiments conducted by members of an inter-laboratory consortium will be presented.

Amplicons from multiplex STR and/or mtDNA amplification will be assessed by capillary electrophoreisis using the Applied Biosystems 310 or 3130 genetic analyzer. Analysis of the data will be

performed using the new GeneMapper ID software from Applied Biosystems. Assessment of qualitative and quantitative data of all samples will be evaluated using GMID software. Analyses of the replicates of each set of data will be performed and statistical analysis will be done to rigorously evaluate and assess any differences between control and test samples with and without PCRboost.

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