B4 An Assessment of the PreCR® Repair Mix as a Viable Repair Method for Soil-Degraded DNA

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After attending this presentation, attendees will understand some principles of DNA degradation, how the PreCR® repair mix functions, and the ability to repair DNA samples that have been exposed to degradation by substances in soil, such as humic acid.

This presentation will impact the forensic science community by providing an analysis approach for DNA profiling of buried bloodstained evidence that optimizes the recovery of a DNA profile in order to help provide investigative leads.

The ability to detect a DNA profile from a piece of evidence, such as clothing, can be crucial in a forensic investigation. In many cases, evidence is found outdoors, possibly buried. Buried evidence containing human DNA, such as bloodstains, can pose a challenge for profiling due to accelerated degradation processes in soil by its many components. Humic Acid (HA) in soil has been determined to be a contributor of accelerated DNA degradation, as well as a Polymerase Chain Reaction (PCR) inhibitor. Therefore, the goal of this study was to determine if the PreCR® repair mix can be used for the repair of soil-degraded DNA, potentially aiding with casework by establishing or providing potential suspect and/or victim leads in violent crimes in which evidence containing human blood was buried.

Following informed consent, venous blood was obtained from a single-source volunteer in sterile Vacutainer® EDTA tubes. Then, 50µL and 150µL aliquots of blood were deposited in quadruplicate on 2"x2" polyester swatches, for a total of 30 stained fabric swatches, and dried beneath a hood for 24 hours. Once dried, 18 of the stained swatches and 6 non-stained swatches were buried 1.5" deep in a ten-gallon fish tank containing approximately 3" of soil. Six remaining bloodstained swatches were left in petri dishes on the bench. Over the course of four weeks, four stained samples from each blood amount, in addition to two non-stained swatches that were used as negative controls, and two bloodstained swatches from the table were extracted with the QIAmp DNA Mini® Kit, quantified with the Investigator Quantiplex® Kit, then amplified and profiled using the Powerplex Fusion® Kit with GeneMarker® HID software to assess the level of degradation in the donor's original profile. At the time of each extraction, soil moisture, pH, and room temperature and humidity were monitored in an attempt to keep as many variables as possible to a minimum. Portions of the degraded samples from each blood-stained group that had already been quantified were repaired using the PreCR® repair mix prior to Fusion® amplification. Generated profiles aided in determining the extent of repair. The results of each extraction group were evaluated based on the extent of allelic dropout/recovery and corresponding peak height ratios.

After one week buried in soil, the samples with 50µL of blood showed little (one to two alleles) to no allelic activity. Every PreCR®-treated sample for week one showed a recovery of two or more alleles out of a total of 43 alleles in the known person's profile. Weeks two through four with 50µL of blood did not show any DNA prior to being treated with PreCR® for any sample and zero out of nine bloodstained samples did not show any improvement when treated with PreCR®. Studies are currently being conducted following the above-mentioned protocol with larger quantities of blood to examine repair possibilities when larger amounts of DNA are present, in addition to assessing direct HA inhibition with various amounts of HA to study the direct effect of HA on DNA analysis and repair.

This research has highlighted the challenges that come with severely degraded human DNA, but also brings attention to the possibility of repairing soil-degraded DNA from blood-stained fabric using commercially available methods, such as PreCR®. Additional studies will need to be conducted prior to any laboratory casework application. It is hoped that extensions of this work will enable the forensic science community the ability to explore DNA repair.

DNA Repair, STR Analysis, Humic Acid

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