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REPAIR OF DAMAGED DNA USING COMMERCIALLY AVAILABLE ENZYMES

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Biological stains like blood and semen are frequently exposed to environmental conditions in crime scenes. The DNA in these biological stains is subject to damage in the presence of environmental factors like UV, moisture, heat, chemicals and nucleases from microorganisms. The most common types of DNA damage are DNA breaks—double and single strand breaks due to UV exposure and damage by nucleases. It is often difficult to obtain a complete genetic profile for human identification purposes from highly damaged forensic samples.

The purpose of this research is to evaluate different DNA repair treatments and other strategies for damaged DNA using commercially available polymerases. The first step was to obtain damaged DNA exposed to ambient condition, UV light and sunlight. Blood and semen samples were exposed to these three conditions over a period of 2 weeks, 1 month, 2 months, 3 months, 6 months and 9 months and then collected and stored at -20^{o}C. Quantification of the samples was performed using the qPCR Quantifiler kit (Applied Biosystems). The extent of damage and subsequent repair was assessed by multiplex PCR amplification and analysis of the qualitative and quantitative results of 16 different genetic loci that display a range of sizes in base pairs as separated by capillary electrophoresis (ABI 310 Prism). DNA that displayed damage (as determined by allelic dropout of high molecular weight loci) were treated using different polymerases. Several different treatments were utilized including, Restorase DNA Polymerase (SIGMA-ALDRICH), single and double doses of AmpliTaqGold DNA Polymerase (Applied Biosystems) and Y family polymerases. Preliminary data indicate that alleles that were not detected in the 6 month UV exposed and sunlight exposed samples were recovered using a pre-incubated with Restorase DNA Polymerase before amplification with Taq Polymerase. The results for the different time points of blood and semen using different variables and enzymes will be presented.

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