B23 Repair of Damaged DNA Using Commercially Available Enzymes

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After attending this presentation, attendees will learn about various commercial DNA polymerases and their use in repairing damaged DNA to restore the ability to type the sample for genetic profile.

This presentation will impact the forensic community by providing knowledge regarding damaged DNA repair.

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 (Chung et al., 2004). 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 two weeks, one month, two months, three months, six months, and nine 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. Y family polymerases have the capability to carryout translesion synthesis. During the process, a polymerase incorporates nucleotides opposite a damaged DNA template, thus bypassing lesions that would otherwise impede synthesis (Ballantyne, 2006). DPo4 is a thermal stable member of the Y family polymerases and has been chosen as the candidate for this evaluation work. DPo4 can bypass several lesions like thymine-thymine dimers, cisplatin adducts and N-acetyl-2- aminofluorne adducts (Boudsocq et al. 2001, Ballantyne, 2006).

Preliminary data indicate that alleles that were not detected in the 6 month UV exposed and sunlight exposed samples were recovered using a process of pre-incubation 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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References:

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Damaged DNA, DNA Repair, Allelic Dropout

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