A11 Restoration of Partial Short Tandem Repeat Profiles Resulting From DNA Lesions Induced by Bleach and Hydrogen Peroxide Treatments

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After attending this presentation, attendees will become familiar with the degradation of the Short Tandem Repeat (STR) profiles caused by treatment of DNA with either bleach or hydrogen peroxide (H_2O_2) and the improvement of the profiles after treatment of the extract with a commercial kit containing a suite of enzymes designed to restore PCR amplification of damaged DNA to its normal level.

This presentation will impact the forensic science community by providing the analyst with the awareness that DNA from evidence that is either refractory in PCR amplification or that yields partial STR profiles may be restored either to yield full profiles or to increase its value as probative evidence.

Biological evidence collected at a crime scene or clothing from a victim may yield probative information about an incident. However, the evidence may have been exposed to the environment, or stains may have intentionally been treated with cleansing agents which typically contain bleach or H_2O_2. The DNA present in the evidence may be adversely affected by such environmental or chemical influences. Ultraviolet light, heat, humidity and freeze/thaw cycling are familiar environmental effects that are known to produce double-strand breaks, single-strand nicks, modified bases, and loss of nucleotide bases. Similarly, DNA lesions may be produced by bleach and H_2O_2. The lesions may prevent procession of DNA polymerase during the polymerase chain reaction (PCR), inhibiting the amplification of STR regions, and lead to full or partial loss of the DNA profile.

Living cells possess several DNA repair mechanisms to correct lesions produced during growth and exposure to various endogenous events and exogenous agents. The repair pathways use enzymes such as glycosylase to excise modified or mismatched bases, endonucleases to remove abasic residues, DNA polymerase to fill in the gaps, and DNA ligase to seal nicks. However, since evidence is composed of dead cells, these repair pathways no longer function. To repair damaged DNA from skeletal remains, forensic anthropologists resorted to using in-house formulations of several enzymes, and this work finally led to the development of a DNA repair kit by suppliers of molecular biology products.

In the work reported here, a commercial DNA repair kit was evaluated for the repair of damaged DNA presenting partial STR profiles. Either bleach or H_2O_2 was applied to HL-60 cell line DNA, or blood and semen stains on cotton sheeting and non-porous surfaces using standard concentrations. The sterilization efficacy of the oxidizing agents on bacteria was examined in parallel experiments. Initially, DNA damage (oxidation) was assessed using a real-time PCR quantification assay. An increase in cycle threshold value for treated DNA compared to untreated DNA indicated DNA damage. Next, the DNA was amplified to obtain STR profiles. Samples with partial STR profiles were chosen to test the DNA repair kit and optimize the protocol with respect to reaction time, temperature, and enzyme mix volume.

Treatment with bleach or H_2O_2 led to full or partial STR profile loss, depending on the severity of the process. Incubation at 37° C with the repair enzymes resulted in allele recovery from the partially damaged DNA within three hours, but the best recovery often required an overnight time period. Generally, treatment with the enzyme mix resulted in increased peak heights for most all of the alleles, particularly for the larger STR loci that had peak heights below the detection (50 rfu) and/or stochastic thresholds (200 rfu) before repair. No allele drop-ins were observed. Currently, the emphasis is on optimizing the reaction conditions to reduce the time required to achieve full profile restoration from partially damaged DNA.

The use of the commercial repair kit may provide a means to obtain a full STR profile from environmentally exposed evidence or from evidence obtained after oxidizing treatments with bleach or H_2O_2 that would otherwise be refractory or result in partial profiles. The repair does not involve multiple, time-consuming steps. It can be easily incorporated into the current STR analysis procedure and should work on a liquid-handling robotic system.

Bleach and Peroxide, STR Analysis, DNA Repair

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