A28 Optimization and Application of DNA Repair Enzymes to Damaged DNA for Short Tandem Repeat DNA Analysis

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After attending this presentation, attendees will be familiar with environmental and chemical agents causing DNA damage, the need for methods to repair such damage, and the importance and impact of a successful repair method for Short Tandem Repeat (STR) DNA analysis of damaged samples.

This presentation will impact the forensic science community as it discusses a method that may augment traditional STR analysis by restoring previously unobtainable and incomplete STR profiles.

In forensic investigations, DNA analysis plays a major role in human identification. However, DNA evidence collected from a crime scene may have been damaged from exposure to environmental and/or chemical stresses. UV radiation, heat, humidity, and oxidation have been shown to damage DNA, generating double-strand breaks, single-strand nicks, and/or modified bases. Such damage may prevent procession of DNA polymerase during Polymerase Chain Reaction (PCR), inhibiting STR amplification, and potentially resulting in full or partial loss of the DNA profile.

In living cells, excision repair pathways can correct lesions in DNA caused by either endogenous processes or exogenous agents. These repair mechanisms include enzymes such as glycosylase to excise modified or mismatched bases, endonuclease to remove abasic sites, DNA polymerase to fill in gaps, and DNA ligase to seal nicks in the DNA.

DNA evidence recovered from a crime scene, however, does not have the protection and benefit of these cellular processes. Thus, previous studies have investigated using and applying mixtures of these enzymes to repair damaged DNA in vitro.

In this study, a commercially available DNA repair kit was evaluated for the repair of damaged DNA samples showing partial STR profiles. HL-60 cell line DNA was damaged artificially with UVC radiation, heat/acid, and oxidation treatments. Damage was assessed initially using a human DNA real-time PCR quantification assay. An increase in cycle threshold value for treated DNA compared to untreated DNA indicated DNA damage. STRs from the artificially damaged DNA were amplified, separated using capillary electrophoresis, and analyzed. Damaged DNA samples that produced partial STR profiles were chosen to test and optimize the DNA repair kit.

The initial results indicated that using the repair kit following a protocol developed by the Bureau of Alcohol, Tobacco, Firearms and Explosives and Armed Forces DNA Identification Laboratories for forensic-like samples was more effective in repairing damaged DNA samples than the manufacturer's standard protocol. This modified protocol was optimized for DNA repair with respect to repair reaction time, temperature, and repair mix volume. The optimized repair protocol was then applied to DNA samples showing partial STR profiles that had been extracted from UVC- and environmentally-exposed bloodstains.

The results indicated that when using the modified and optimized protocol, treatment of damaged DNA with the repair enzyme mixture led to an overall increase in average peak heights for STR analysis. Particularly for the larger STR loci, repair successfully recovered alleles that had previously shown peak heights below the detection (50 rfu) and/or stochastic thresholds (200 rfu).

The modified repair method may provide a means to obtain a full STR profile from environmentally and/or chemically damaged DNA that would otherwise be refractory without multiple, time-consuming treatments. In addition, the modified method only requires a few additional steps that could easily be incorporated into the current STR analysis procedure.

Damaged Samples, DNA Repair, STR Analysis

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