B43 A Comparison of Short Tandem Repeat (STR) Allelic Recovery Post-Ultraviolet (UV) Damage Utilizing the PreCR® Repair Method: Singleplex Versus Multiplex Polymerase Chain Reaction (PCR) Amplification

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Learning Overview: After attending this presentation, attendees will gain an understanding of how UV, hydrolytic, and oxidative damage affects the Deoxyribonucleic Acid (DNA) sequence and therefore the STR profile, how the PreCR® Repair Mix functions to repair those damages, and whether or not singleplex amplification may be more efficient in STR allele recovery than the multiplex method.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a better understanding of the nature of the multiplex amplification method and how it relates to UV DNA damage and the PreCR® repair process.

DNA is subject to many environmental factors that cause various types of deleterious damage to the molecule, including oxidative, hydrolytic, and UV light-induced.[1] This is a major issue in forensic DNA profiling as forensic samples are often found in a damaged or degraded state. The damage inflicted on biological samples results in a degraded DNA template to be used in PCR amplification and ultimately causes the loss of STR peak signals in forensic DNA profiling.[2] The PreCR® Repair Mix by New England BioLabs® is composed of enzymes used in the cellular mechanisms of DNA repair.[3] Studies have shown the ability of this mix to repair most types of DNA damage prior to PCR amplification of the core STR loci.[4] However, this repair process does not always result in complete STR profile recovery. Standard STR analysis kits are designed as multiplex PCR amplification reactions, consisting of forward and reverse primers for multiple STR loci. The purpose of this study was to determine whether or not singleplex amplification reactions post-UV damage repaired with PreCR® would result in greater STR allele recovery with the idea that singleplex reactions would allow for less competition between individual STR loci primers and primer binding sites, allowing for more efficient amplification.

As larger STR markers tend to be more susceptible to environmental damages, as observed by the "ski slope effect," this study focused on three STR loci of differing lengths, all with simple, tetrameric repeats for the singleplex amplifications: TH01, D18S51, and CSF1PO.[5,6] Primer sequences for these loci were obtained from STRbase and all forward primers were developed to include a 6-FAM fluorescent tag.[6] Multiplex and singleplex amplifications were performed in triplicate under three different conditions; No Damage, No Repair (NDNR), Damage, No Repair (DNR), and Damage, Repair (DR). Additionally, two damaging events were performed as the consistency of UV damage to a particular sequence is unknown.[2]

Many observations were made with the data obtained in this study. These include the interpretations of any differences in peak height percent recovery post-PreCR® repair within each locus between multiplex and singleplex amplifications, any consistencies or inconsistencies between the two damage events, any allele size shift, alterations in peak height ratios, or introduction or disappearance of non-specific peaks, and differences in peak height percent recoveries post-repair between shorter and longer loci and homozygous and heterozygous loci. Further studies that focus on more STR loci, both heterozygous and homozygous, as well as a larger sample size and more damage events are warranted.

Reference(s):

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DNA Damage, DNA Repair, STR Profiling

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