B117 Biochemical Repair and Lesion Bypass of Damaged DNA

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After attending this presentation, attendees will be introduced to strategies for the repair of damaged DNA templates derived from forensi- cally relevant samples.

This presentation will impact the forensic community and/or humanity by demonstrating that it is possible to biochemically repair DNA damage introduced by a variety of insults, leading to the recovery of a profile from previously intractable samples.

The use of DNA typing techniques has revolutionized the forensic analysis of biological evidence. DNA typing now plays a critical role within the criminal justice system. Numerous individuals have been con- victed and falsely accused individuals exonerated based on DNA evidence, and increasing use is being made of databases of DNA profiles for criminal intelligence information. However, one of the limiting factors of the tech- nology is that DNA extracted from forensic samples is often so damaged that is not possible to obtain a genetic profile from it due to the presence DNA polymerase stalling lesions, including single base modifications, adducts, and breaks, both single and double stranded. To date, there has been no reliable method for the repair of such damage to facilitate the recovery of a genotype from intractable samples. The development of repair methods able to do just this has been the focus of this research.

Forensic samples are subject to a myriad of insults including heat, light, humidity, and microorganism growth, which can induce a variety of types of damage. Therefore, a single repair strategy is not sufficient. Previous work has shown that one of the primary causes of the inability to type damaged DNA is the presence of strand breaks. To deal with single strand breaks and gaps, a modified base excision repair (BER) strategy has been developed in which a cocktail of repair enzymes is allowed to react with damaged DNA. Such a strategy is able to manage the modified, non- extensible or ligatable ends found in damaged DNA, fill in short gaps, and ligate the finished product. However, due to the presence of other base modifications and adducts, this is not sufficient for the recovery of a profile from many forensic samples.

Coupling BER gap repair with another strategy – direct bypass of lesions – has proved successful for the repair of damaged DNA templates. A new class of translesion DNA polymerases has recently been discovered. These distributive enzymes are thermostable and, due to relaxed constraints at their active sites, can form non-canonical base pairs, allowing the bypass of lesions that would typically stall a DNA polymerase. Adjusting the PCR conditions, including buffer constituents and cycling conditions, has allowed the inclusion of these polymerases in a PCR reaction with a standard processive thermostable DNA polymerase. The resulting blend of enzymes facilitates the bypass of DNA damage as well as the accurate amplification of desired genetic loci.

***in Vitro* DNA Repair, Translesion Polymerases, Base Excision Repair**

 ** Presenting Author*