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AMPLIFICATION OF COMPROMISED DNA USING TRANSLESION POLYMERASES

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With the advent of methods for handling samples containing a limited quantity of template DNA (i.e. miniSTRs, low copy number profiling techniques, whole genome amplification) it is now possible to obtain genetic data from previously intractable samples, which can be of great use in applications such as forensic genetic profiling and in the analysis of ancient DNA. However, in many cases, the template may have been further compromised by damage done to its primary structure. Such lesions can inhibit PCR amplification, resulting in DNA typing failure. Previous work in both the forensic and ancient DNA fields has shown that modified bases are among the most prevalent lesions that can halt polymerase mediated primer extension. The use of translesion polymerases as a method for circumventing this problem is the subject of this report. The translesion polymerase has the ability to add a base opposite a damaged nucleotide, forming a non-Watson/Crick base pair and allowing polymerization to continue. Careful consideration of the structure and function of these specialized polymerases has facilitated the development of a set of modified conditions in which both translesion and replicative polymerases act synergistically in PCR reactions, bypassing certain types of DNA damage such as oxidative modifications or UV-induced lesions. This technique has been adapted for both end-point and real-time PCR applications to allow for genotyping, quantification, pre-amplification, and component analysis. The systems could be applied to the detection a wide variety of sequence polymorphisms in any number of organisms. The combination of these techniques with downstream applications such as miniSTRs or low copy number profiling techniques should provide a powerful tool for the handling of highly compromised samples.