

STUDIES ON THE APPLICATION OF SHORTER STR AMPLICONS (MINIPLEXES) IN THE ANALYSIS OF DEGRADED DNA

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Highly fragmented DNA in ancient or degraded forensic samples presents a challenge for current commercial STR typing kits. The longest amplicons in PowerPlex[®] 16 from Promega and in AmpFLSTR[®] Identifiler[™] from Applied Biosystems exceed 350 bp while the average lengths of degraded DNA length may be well under these sizes. A series of redesigned primer kits with shorter amplicons have been recently developed to address this issue (1). These primer sets, known as Miniplexes, typically contain 3-6 multiplexed STR loci with the primer binding sites located as close as possible to the polymorphic region. Five different miniplexes cover the entire set of 13 CODIS STRs. In this study we discuss results obtained in examining the effect of template size on the efficiency of amplification using these new primers sets. Data was obtained by digesting a series of blood samples with DNase I as well as through the examination of a series of simulated forensic samples obtained from collaborative arrangements with the Anthropology Departments of Ohio University and the University of Tennessee Forensic Anthropology Center.

The initial results demonstrated that all Miniplex primer sets performed satisfactorily on fragmented DNA template with the exception of one particular locus, Penta E, which was affected by primer binding site mutations at low concentrations. A full concordance study is currently being undertaken to examine 500+ samples in order to address other potential primer binding site mutations. The results of this study will also be discussed in the presentation.

A study performed to examine amplification efficiency and peak height ratios demonstrated that the Miniplexes successfully amplified DNA at concentrations well below recommended values for commercial systems. However, we found that amplification efficiency was affected when more than 3 loci were included in the multiplex reaction. Typical results showed sensitivities below 100 pg of template using peak height ratios of 60% as an objective criterion. Several loci performed equally well at 60 pg. For DNA extracts from environmentally exposed bone samples, the Miniplex sets yielded higher allele peaks than a commercial kits and in one case provided a full STR profile where only partial sets had been obtained previously.

(1) Butler, J; Shen, Y.; McCord, B. The development of reduced size STR amplicons as tools for the analysis of degraded DNA, *J. Forensic Sciences*, 2003, in press.