An Investigation on the Efficacy and Limitations of STR Multiplex Systems in Low Copy Number (LCN) DNA Analysis

Suzanne Shaffer, John Planz, and Arthur Eisenberg

Complete short tandem repeat (STR) genetic profiles can be obtained from samples containing as little as 100 pg of DNA using standard amplification and analysis parameters. Several studies have shown that partial or complete profiles from LCN samples (<100 pg) can be generated by increasing the number of PCR cycles; however, the STR profiles that are generated are often non-reproducible and re-testing is not always feasible due to exhaustion of the limited evidentiary sample. In addition, complications such as increased risk of contamination, allelic drop-in or drop-out, locus drop-out, heterozygote peak imbalance and increased stutter have been reported. A Bayesian statistical model has been previously proposed in order to calculate likelihood ratios of LCN profiles which account for spurious bands/peaks, allele dropout, and stutter. Several individuals have argued that the degree of stutter cannot be predicted in LCN DNA samples, since stutter peaks can be greater than those of the true allele. LCN analysis requires extensive validation by laboratories since the conditions required to generate an STR profile fall outside those developed by multiplex kit manufacturers. The aim of this study is to demonstrate the reliability and reproducibility of LCN DNA profiles based on empirical data. We have begun pilot testing of variable LCN DNA amounts performed under differing PCR and electrophoretic parameters. DNA was isolated from 3 males and 3 females using the DNA IQ™ System (Promega Corporation, Madison, WI). DNA quantities were determined using Quantifiler™ Human DNA Quantification Kit (Applied Biosystems, Foster City, CA). Various LCN amounts of DNA ranging from 3 to100 pg were examined. Each LCN sample dilution was amplified using the PowerPlex® 16 (Promega Corporation, Madison, WI) multiplex STR system for a total of 10 amplifications in order to determine if profile variations are due to the PCR amplification process, or are a result of pipetting errors. PCR was performed on LCN samples at 30, 32 and 34 cycles. All electropherograms were generated using ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) and GeneScan software. Results and conclusions in progress.