EVALUATION OF MICROCAPILLARY ELECTROPHORESIS FOR mtDNA QUANTITATION

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Mitochondrial DNA (mtDNA) sequence analysis is a labor intensive process consisting of several steps, which include DNA extraction, amplification, post-amplification purification and quantitation, cycle-sequencing and resolution of products. One focus of this study is to find methods that will increase sample throughput. One potential option is to quantitate mtDNA PCR products using micro-capillary electrophoresis (CE). Currently, post-amplification quantitation is performed using conventional CE (Beckman CE) and takes approximately 8-10 minutes per sample. In contrast, the Agilent 2100 Bioanalyzer microCE, in conjunction with the Agilent DNA 1000 kit, produces results in less than 3 minutes per sample. This study has focused on determining the applicability of the Agilent 2100 Bioanalyzer for forensic use.

The sensitivity and reproducibility of the Agilent 2100 Bioanalyzer and the Beckman CE were evaluated in a side by side study. Preliminary studies indicate that the Agilent 2100 Bioanalyzer can detect DNA concentrations of as little as 0.5 ng/µl with high reproducibility. Additional studies are underway to further evaluate sensitivity. Data will be presented from analysis of evidentiary-type samples such as buccal swabs, blood, hair and bone. In summary, initial experiments indicate that the reproducibility and sensitivity of the Bioanalyzer are comparable to the Beckman CE. The Bioanalyzer has the potential to provide significant savings of labor and time in post amplification quantitation.