VALIDATION OF BECKMAN COULTER CEQ™ 8000 FOR FORENSIC MITOCHONDRIAL DNA SEQUENCE ANALYSIS

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The technique of mitochondrial DNA (mtDNA) sequence analysis is successfully utilized in forensic casework analysis by several laboratories in the works. Although the techniques are considered the gold standard in mtDNA analysis, they suffer from the following limitations: 1) the ability to distinguish individual sequences with a mixture. 2) the ability to identify the contribution of heteroplasmy at low levels. 3) the level of sensitivity and precision in handling small and degraded amounts of DNA, which is a limiting factor in forensic DNA testing. Most of these problems are probably related to the type of the detection machine and/or software or chemistry used. Therefore, there is a need for an alternative system that will improve and expand on the application of mtDNA sequence analysis in forensic casework analysis.

The Beckman Coulter CEQ™ 8000 is a sequencer that offers the following significant advantages: a) the ability to resolve difficult sequencing regions where other system have failed [Kukanskis *et al.*, BioTechniques 28:630-634, 2000]; b) the capacity to analyze over 700 bases in read-length (Beckman Coulter); c) the potential to scale down reaction volumes to reduce cost/sample [Azadan *et al.,* (2000). BioTechniques 32:24-28]; and d) The CEQ™ 8000 can also be upgraded to a fully automated system for forensic mtDNA sequence analysis.

We have validated the Beckman Coulter 8000 sequencer for forensic casework analysis regarding the following: I) Optimizing conditions important for sequence analysis. II) Determining levels of sensitivity, precision and reproducibility. III) Determining the ability to identify unknown heteroplasmic positions in NIST blind samples. IV) Analysis of non-probative cases. VII) Blind sample testing of previously sequenced CAP proficiency tests. VIII) Analysis of blind samples used by the FBI in their Mito-Search program.

We have found that the system can produce sequences of acceptable quality with as little as 5 ng of amplified DNA. It can reproducibly generate correct sequences from all tested reference samples. The system was also sensitive enough to detect low levels of heteroplasmy (down to 5%; NIST samples). Furthermore, the forensic applicability of the system regarding casework analysis will also be presented. In conclusion, the CEQ™ 8000 Genetic Analysis System is well suited to perform forensic mtDNA sequence analysis.