B113 The Applicability of Microchip Electrophoresis in Developing Methods for Low Copy Number Short Tandem Repeat DNA Profiling

Henrietta Margolis-Nunno, PhD, JD, Ewelina Bajda, BS, Miriam Cohen, BS, and Lawrence Kobilinsky, PhD, John Jay College of Criminal Justice, C.U.N.Y., 445 West 59th Street, New York, NY 10019*

After attending this presentation, attendees will learn about the applic- ability of the Agilent 2100 Bioanalyzer as an instrument that can be used for research and technique development in the analysis of short tandem repeats in low copy number DNA samples commonly found at crime scenes.

This presentation will impact the forensic community and/or humanity by demonstrating how the forensic community performing DNA analysis research may adopt the use of this instrument as a screening tool prior to performing lengthier, laborious, and more expensive techniques.

The Bioanalyzer utilizes microfluidic technology for the separation, sizing and quantitation of DNA fragments by capillary electrophoresis (CE). It is fully automated, uses unlabeled primers, requires less sample volume and has shorter run times than standard CE and gel electrophoresis. This study was performed to evaluate the usefulness of the Bioanalyzer in STR DNA analyses of low copy number (LCN) DNA samples. The Bioanalyzer was first examined for its ability to resolve heterozygous STR alleles and for the reproducibility and accuracy of its sizing calls. Resolution studies, employing commercial STR triplex primers and DNA with known STR alleles, demonstrate that the Bioanalyzer is capable of dis- tinguishing heterozygous STR alleles that are 7-8 base pairs apart as two distinct peaks, and homozygous alleles as a single sharp peak. Heterozygous alleles differing by 4 base pairs are frequently distin- guishable as well. Fragment sizing accuracy and reproducibility studies, using STR fragments of known sizes, and DNA standards and ladders, show that although certain STR loci consistently show relatively larger sizing errors than others, all results are reproducible with low values for coefficient of variation and all are within an error range of 5%. In studies using DNA concentrations that included those found in LCN DNA, Bioanalyzer profiles of STR triplex PCR products show DNA concen- tration dependent differences and a ten-fold increase in sensitivity when the number of PCR cycles is increased from 30 to 34. In addition, the type of stochastic variation that is common to the amplification of LCN DNA is easily visible with the Bioanalyzer; variations in allele or locus drop out and imbalance, and stutter allele drop in were all seen when LCN DNA con- centrations of 25 and 2.5 pg were amplified for 34 cycles. Thus, although

allele sizing is not as precise as with standard capillary electrophoresis (CE), the ease and speed of the Bioanalyzer coupled with its resolution of heterozygous alleles, the reproducibility of its results, and the sensitivity of its profiles to initial DNA concentration and LCN stochastic variation, make it an ideal instrument for preliminary research and technique devel- opment in the analysis of STRs in LCN DNA samples.

Capillary Electrophoresis, Short Tandem Repeats, Low Copy Number

 ** Presenting Author*