B91 Using the 2100 Bioanalyzer as the Platform in Rapid and Inexpensive PCR-Based STR Genotyping for Discrimination of Biological Specimens Recovered in Transportation Accidents

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After attending this presentation, attendees will understand the application of a rapid genotyping assay with application to small sample sizes that is designed for in house use with the Agilent 2100 Bioanalyzer.

This presentation will impact the forensic community and/or humanity by demonstrating the application of the genotyping assay to a case study for discrimination of small population pools will demonstrate the practical use of the method. Additionally, this provides the forensic sci- entist access to an inexpensive method using universally recognized loci for rapid genotyping.

In fatal accidents, there is the potential for misidentification of samples at an accident site. Results of toxicological or other biochemical testing of samples that are in conflict with the preliminary identification suggest sample misidentification but are not definitive. Genotyping can serve as an additional and independent test for correct sample identifi- cation; however, depending on the method used, genotyping can be expensive and requires instrumentation and software for analysis that is generally available only from external services. This suggests a need for more accessible and inexpensive methods for accurate differentiation of small population pools.

A protocol will be presented describing a qualitative method of geno- typing using the Agilent 2100 Bioanalyzer as the platform for separation of PCR products amplified from STR loci. The presentation will demonstrate the use of primers and PCR conditions from the well established CODIS STR primer sets and information found at STRBase (http://www.cstl.nist.gov/biotech/strbase/) and the novel use of the Bioanalyzer for rapid, inexpensive electrophoresis and analysis of CODIS STR PCR products for genotyping.

Results of a case study will be discussed where toxicological results did not correspond to the stated identification of the specimens. An expensive external service had been used to perform fluorescent-based capillary electrophoresis for genotyping. In-house analysis using inexpensive unlabeled primers for PCR and less expensive instrumentation and software was utilized for electrophoresis and analysis. Initial evaluation of the Bioanalyzer-based method revealed that electrophoresis using the DNA500 chip gave sufficient separation of PCR products from a variety of tissues and blood to discriminate between three control subjects using 8 STR loci and amelogenin, a sex determination locus. Furthermore, the observed presence of homozygous alleles at different loci was sufficient for unambiguous identification of each individual's specimens.

Five samples from a case study were examined using the Bioanalyzer protocol. The results confirmed the toxicological results and provided correct specimen identification. In this study, the presence of homozygous alleles at three loci showed one of the five samples to be unique and dif- ferentiated this sample from the remaining four. Two of the remaining samples were found to be homozygous at identical alleles for two loci. The expected occurrence of homozygosity at these loci in two individuals was determined to be approximately 1%, which in a small population is suffi- cient to suggest that the samples were from the same individual. The overlay of the electropherograms and comparison of products sizes for the PCR products from the remaining loci was sufficient to complete identifi- cation and suggest that the five case study samples were from three indi- viduals, confirming the toxicological analysis.

The protocol presented is inexpensive, rapid, uses techniques and instrumentation readily available in many forensic labs, and does not require specially trained personnel. The analysis was conducted with the Bioanalyzer software and accomplished quickly by determining the presence of homozygous alleles and overlays of size-separated PCR products. The protocol takes advantage of the well-characterized CODIS primer sets such that a large body of literature is available regarding char- acterization and use of the STR loci for genotyping. The application illus- trated here relies on relative comparison of electrophoretically separated products. Therefore, absolute identification of the specific alleles is not nec- essary further adding to analytical simplicity.

DNA Typing, Short Tandem Repeats, 2100 Bioanalyzer

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