VALIDATION AND COMPARATIVE STUDY OF THE AMPFISTR® IDENTIFILER™ AND THE POWERPLEX® 16 STR MULTIPLEX SYSTEMS FOR FORENSIC CASEWORK USING CAPILLARY ELECTROPHORESIS

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Introduction: The newest form of DNA testing to become commercially available for forensic DNA analysis is STR (short tandem repeat) analysis. STR testing has been performed on DNA isolated from forensic case samples for a variety of reasons. These include: 1) increasing the chance of excluding a falsely-accused individual, 2) determining whether a sample contains a mixture of DNA from more than one individual, 3) assisting in the interpretation of data from samples containing a mixture of DNA and 4) limiting the number of individuals included as possible donors of the DNA obtained from a sample by providing increased statistical frequencies.

The ability to amplify and detect very small amounts of DNA template (typically 1ng) is essential for forensic applications. Research labs focused attention on the development of tetranucleotide repeat loci which display few or no microvariants, minimal stutter bands, and have a relatively low mutation rate. The polymorphisms in the currently developed STR systems result almost exclusively from variation in the number of tetranucleotide repeats present at the locus, and not from insertion or deletion of one or two bases. This allows rapid and precise typing of easily amplified alleles ranging from 100 to 350 bases in length.

The use of these STR multiplex systems provides extremely powerful discrimination. As an example of the enormous discriminating ability of these systems, consider the combined twelve-locus system. The matching probability has been calculated at nearly 1 in 3 trillion. Now the only completely automated system for STR analysis is capillary electrophoresis (CE). A CE system provides the automated electrophoresis, detection and analysis available on semi-automated systems as well as automated gel assembly and sample loading processes. As the name implies, capillary electrophoresis is carried out in a microcapillary tube rather than between glass plates. Once samples, gel polymer and buffer, are loaded onto the instrument, the capillary is filled with gel polymer and sample is loaded automatically. Up to ninety-six samples are automatically loaded and analyzed unattended.

Objective: To evaluate and compare the currently available AmpFlSTR® Identifiler™ and the PowerPlex® 16 fluorescent STR multiplex systems for identity testing in forensic case work using the ABI Prism® 310 genetic Analyzer system.

Nature of Study: Comparative and Validation study

Materials and Methods: DNA was extracted from standard forensic specimens such as blood, semen, hair and saliva and some degraded samples and old stains. Different methods of DNA extraction were also evaluated where DNA from the same individuals was isolated using: phenol/chloroform, chelex, and FTA® paper extraction protocols. Samples were also investigated by diluting known concentrations of DNA. DNA was also extracted from samples taken from some primates and non-primates. The effect of inhibitors as dyes and soil was investigated on some samples. Some forensic cases covering a wide range of evidence type were included in the study. Mixtures with known STR profiles of different levels of DNA concentrations were interpreted both with and without the aid of reference samples. After quantitation of the extracted DNA amplification was carried out for 16 loci (15+ amelogenin). The amplified product was tested on the ABI 310 genetic analyzer and the obtained profiles were interpreted and analyzed.

Results and Conclusion: The results obtained were tabulated and statistically analyzed. It revealed that both systems are very powerful forensic investigation tools as the four and five-color multiplex systems allow for the simultaneous amplification of 16 loci and detection in a single injection using the automated

ABI Prism® 310 Genetic Analyzer capillary electrophoresis system. The combined use of the fluorescent STR multiplex systems and the ABI Prism® 310 Genetic Analyzer offers significant benefits in sensitivity, wider range of samples and a significant reduction in analysis time.