B135 A Review of Fluorescent Artifacts in Genetic Analysis Systems

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After attending this presentation, attendees will have reviewed the artifacts visualized with the use of the AmpFLSTR® PCR Amplification Kits and ABI PRISM® genetic analysis instruments.

This presentation will support the caseworking forensic scientist in further developing interpretation and troubleshooting skills.

Molecular biology techniques using DNA have revolutionized the field of forensic sciences in testing of biological specimens. The forensic community realized the advantages of DNA over traditional serological techniques and first adopted restriction fragment length polymorphism (RFLP) and sequence-specific oligonucleotide (reverse dot blot) assays. Many laboratories adopted silver-stained slab gels with long tandem repeats following those techniques. Then, fluorescent dye technology was introduced. Fluorescent multicolor dye technology allows multiple loci (including loci that have alleles with overlapping size ranges) to be analyzed in a single capillary injection or gel lane. Alleles with overlapping loci are distinguished by labelling locus-specific PCR primers with different colored dyes. Laboratories can now analyze hundreds of loci in a single day using five-dye fluorescent labelling from Applied Biosystems (Foster City, CA). Additionally, the forensic community has adopted the use of multicolor sequencing for mitochondrial DNA analysis.

PCR-based technology, and especially current fluorescent short tandem repeat (STR) analysis, has proven its advantages in the past decade. PCR-based testing oftentimes produces results when the DNA is highly degraded, unlike RFLP testing. PCR-based STR testing produces discrete results. PCR-based testing has much greater sensitivity and the small size of the STR loci improves the chance of obtaining a result, particularly for degraded DNA samples. Additionally, the small size range of STR loci makes them ideal candidates for co-amplification where multiple STR loci or other markers can be profiled in a single amplification. Furthermore, advances in technology have resulted in an increase in sensitivity, allowing the forensic community to obtain results using far less input DNA than in previous years.

Forensic DNA testing for the identification of evidentiary material involved in the resolution of legal disputes is a powerful technique. With any scientific procedure, a quality assurance (QA) program that addresses the techniques is critical. The forensic community has excellent QA programs to assure accurate results and to meet the rigors of the courts. Each method has its own advantages, limitations, and quality assurance and quality control procedures. A good QA program encompasses as many aspects of DNA testing as reasonably possible. Once a sample is amplified for STR analysis using multiplex AmpFLSTR® PCR Amplification Kits (Applied Biosystems) and run on the ABI PRISM® genetic analysis instruments (Applied Biosystems), many steps are performed and an array of reagents and consumable items are used in the final analysis of that sample. Because of this, troubleshooting can be complex.

Inherent with all of these techniques and procedures are artifacts, or anomalies. The presence of artifacts can be attributed to a number of factors including phenomenon innate with the technique, cleaning procedures, and raw material. For example, with RFLP analysis, autoradiographs often show anomalies from static electricity, defective intensifying screens [Benzinger et al. “An illustrated guide to RFLP troubleshooting,” *Journal of Forensic Sciences* 1998; 43(3)665-679], shadow bands, and fingerprints. The results obtained from samples amplified for STR analysis using any one of the numerous fragment analysis systems can exhibit their own anomalies. These anomalies can be reproducible anomalies and non-reproducible anomalies. Examples of these two (2) categories of anomalies will be explored.

Peaks other than the target alleles may be detected on the electropherogram displays. Examples of reproducible anomalies include stutter, incomplete 3’ A nucleotide addition (-A), mixtures, and dye-labelled artifacts. Artifacts can be intermittent and are not always reproducible. In our experience, non-reproducible artifacts can be correlated to sources other than the amplification chemistry or the moiety of the sample. For example, spikes caused by salt accumulation or dried polymer released from a dirty block and traveling through a capillary are examples of nonreproducible artifacts. Another example is an artifact resembling a “stair-step” that may be associated with a need to change the water in the autosampler tray on the ABI PRISM® 310 Genetic Analyzer.

Several artifacts associated with practices using the AmpFLSTR® PCR Amplification Kits and run on the ABI PRISM® genetic analysis instruments will be presented.

Artifacts, Fluorescent, STR

 ** Presenting Author*