B181 Analysis of Dye Terminator Sequence Data: Pattern Recognition and Signal Background

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After attending this presentation, attendees will understand procedures and processes for further analysis of sequence data; the attendee can learn other methods in evaluating sequence data.

This presentation will impact the forensic community and/or humanity through additional analysis tools for mitochondrial DNA sequencing results with increased base calling accuracy.

Mitochondrial DNA (mtDNA) sequence analysis is a technique that is well characterized, validated, and useful in the analysis of forensic evidence and identification of human remains. The technique has become more popular as a result of successes in identifying older skeletal remains and hair samples. Forensic laboratories sequence two regions within the D-loop of the mitochondrial DNA genome. These two regions within the D-loop's 1.1 Kb fragment can display multiple variations between individuals. The two variable regions, hypervariable region 1 (HV1) and hypervariable region 2 (HV2), are amplified, detected, and analyzed for forensic identification. It is common practice for forensic laboratories to report HV1 and HV2 sequence information. Unlike STR analysis where discrete alleles according to size are reported, mtDNA analysis reports the observed base sequence. The standard for the forensic community is to report the sequence information as compared to the revised Cambridge Reference Sequence's (rCRS) light strand. The scientists critically analyze each base of the evidentiary specimens and the reference samples in HV1 and HV2 and compare it to the rCRS. As mitochondrial DNA sequencing becomes more commonplace in the forensic laboratories, and as national databases are being used for identification purposes, accurate base calling is critical. Phenomena such as artifacts and noise in sequencing technology should be minimized and easily recognized by the trained analyst. Data quality must be sufficient to unambiguously call the reported base positions. Hence, mtDNA is sequenced in both the forward and reverse directions. An analyst must be experienced in recognizing confirmed quality sequence data, distinguishing single source versus mixed sequence data, and identifying heteroplasmy. The correct interpretation of mtDNA analysis is important.

Patterns in peak heights in local mitochondrial DNA sequencing frames from the D-loop will be presented. These patterns are quite easy to establish and allow for prediction modeling in local sequence frames. Identifying these patterns in local sequence frames from single-source samples can increase base calling accuracy. A single base change can affect the peak heights, or patterns, in these local sequence frames. Patterns in peak heights have previously been characterized (Zakeri et al., 1998) using dRhodamine and Big Dye™ terminator sequencing on a Model 377 DNA Sequencer. In summary, Zakeri et al. demonstrate that the peak height of a base 3' to one or two bases can be predicted using dRhodamine terminators and the peak height of a particular base can be predicted using BigDye™ terminators by knowing the local sequence frame. This study focuses on similar modeling techniques using today's higher throughput multicapillary ABI PRISM 31xx instrumentation, new dye terminator chemistries available today, and mitochondrial DNA sequence data only. This combination of chemistry and instrumentation is commonplace in the forensic mitochondrial DNA sequencing laboratory. Multiple samples have been sequenced on the ABI PRISM 31xx using both dRhodamine dye and BigDye™ v.3.1 terminators. Trends are observed and will be described. These trends can be programmed and adopted in artificial intelligent computerized program systems. Peak patterns, frame patterns, and dye terminator effects can be characterized to improve the base-calling accuracy made by software programs and the forensic DNA analyst.

BigDye® Terminator, dRhodamine Dye Terminators, Sequencing

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