OPTIMIZATION OF A DNA SEQUENCING STRATEGY FOR THE ANALYSIS OF MITOCHONDRIAL DNA HYPERVARIABLE REGIONS I AND II

Mavis Date Chong[1], Cassandra Calloway[2], Sonja Klein[1], Cristian Orrego[1] and Martin Buoncristiani[1]
[1]CA DOJ Jan Bashinski DNA Laboratory, [2] Roche Molecular Systems.

A survey of the methods currently employed by laboratories conducting mitochondrial DNA sequencing analysis for forensic casework would reveal the use of several methods and protocols. Beyond the basic strategy agreed upon by all, that of sequencing the two hypervariable regions (HVI and HVII) of the control region, many variations exist, making the choice of any one approach difficult. It was the purpose of this study to optimize a procedure for routine use with compromised and limited samples, that is robust and efficient, and that results in a minimum of ambiguity with samples displaying heteroplasmy.

Roche Applied Sciences (Indianapolis, IN.) recently introduced the "LINEAR ARRAY™ Mitochondrial DNA HVI/HVII Region-Sequence Typing Kit". This kit utilizes a duplex primer set for the amplification and typing, by strip hybridization, of target sequences for hypervariable regions I and II (HVI/HVII) of the human mitochondrial control region. This duplex primer set was evaluated for amplification and DNA sequencing of the HVI and HVII regions. For comparative purposes, previously described singleplex primer sets of different sequence, which produce similar sized products, were tested. The duplex HVI/HVII amplification was extremely robust**,** effective with highly degraded samples, and more sensitive and specific than the HVI/HVII singleplex amplifications. For example, with 32 amplification cycles, the singleplex primer set required five and one picograms of extracted DNA to yield HVI and HVII products detectable by agarose gel electrophoresis, respectively. In contrast, 0.5 pg of extracted DNA was required to co-synthesize HVI and HVII with the duplex primer set. The duplex amplification yielded target sequence HVI and HVII amplicons only, with no nonspecific products or primer-dimers detected. Furthermore, because HVI and HVII amplicons are co-synthesized in the duplex PCR, this approach is less labor intensive and more amenable to low-copy testing because precious extracted DNA is conserved.

Several sequencing chemistries offered by Applied Biosystems (Foster City, CA.) were also evaluated (BigDye™ ver 1.1, 2.0, and 3.0, and Rhodamine). The ABI Prism® BigDye™ version 1.1 chemistry provided the highest quality sequencing data, with little or no background noise, more uniform peak heights, and the greatest sensitivity. Although ABI recommends that 3-10 ng of PCR product be used per 20 µl of cycle sequencing reaction, interpretable sequence could be obtained with as little as 250 pg of PCR product. In addition, adequate signal and accurate sequence information was obtained when extracted nuclear DNA quantities for duplex PCR ranging from 5 pg to 100 fg were tested. The duplex primer set, in conjunction with the BigDye™ version 1.1 chemistry, in some cases, allowed for the detection of mixtures when the minor component sequence was as low as 10% in electropherograms with no baseline noise. This procedure worked consistently well with DNA extracted from a variety of tissue sources.