Evaluation of Mitochondrial DNA Amplification Employing the FASTTYPE™ mtDNA Amplification System

Pamela Pogue, Amy Smuts, Rita Chen and John V. Planz
BioSynthesis, Inc., Lewisville, TX

Advancements in sequencing technology have increased the feasibility of using mitochondrial DNA (mtDNA) sequence data for the purpose of human identification and other forensic applications. At present, laboratories conducting sequence analysis must independently obtain amplification and sequencing primers, PCR reaction buffers, and a thermostable polymerase to conduct testing. To enhance quality control of our DNA sequencing facility at BioSynthesis, we have developed a fully validated kit for use in mtDNA sequence analysis. The system is subdivided to provide individual reactions for the HV1 and HV2 control regions based on the amplification primers commonly in use by laboratories sequencing these regions. Individual reaction mixes consisting of primers, dNTPs, and buffers are prealiquoted and lyophilized in color-coded 200µl amplification tubes. Reaction setup involves adding template DNA, water and *Taq*, and placing tubes in a thermocycler. The resulting amplicons are then purified in the conventional manner. The system also includes a set of individual sequencing primers that are lyophilized and can be reconstituted to the correct concentration for sequencing reactions. The system was evaluated with the use of *Taq* polymerase from several sources, as well as Ampli*Taq* Gold. The system provides a mechanism to increase uniformity and quality assurance in mtDNA typing while reducing the possibilities of reagent contamination.