B84 Comparison of DNA Polymerase Products for Use in Forensic mtDNA Identifications

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After attending this presentation, attendees will learn the practical value of different DNA polymerase products in the processing of degraded skeletal remains for mtDNA analysis.

This presentation will impact the forensic community and/or humanity by demonstrating the improvement of amplification specificity, sensitivity and fidelity for processing of degraded skeletal remains for mtDNA analysis.

This presentation will describe the assessment of several commer- cially available DNA polymerase products for use in forensic mitochon- drial DNA (mtDNA) applications. Attendees will be aware of which DNA polymerase products offer properties that can improve the fidelity, sensitivity and robustness of mtDNA amplification in the forensic DNA laboratory.

The mitochondrial DNA section of the Armed Forces DNA Identification Laboratory (AFDIL) is charged with assisting the Joint POW/MIA Command, Central Identification Laboratory (JPAC-CIL) in the identification of the remains of US service members lost in previous military conflicts (*i.e.*, Southeast Asia, Korea, and World War II) through the application of mtDNA sequencing. Identifications are achieved by compiling physical, anthropological, dental, circumstantial, and mtDNA evidence; however, some rely solely on mtDNA sequence information. A significant proportion of the samples that AFDIL processes are considered challenged or highly degraded due to the age, nature, and exposure condi- tions of the recovered remains. The degraded nature of the DNA extracted from these samples makes them susceptible to "*Taq* errors" or the *Taq*- mediated insertion of a non-authentic base during amplification.

Recent discoveries and advancements in the engineering of DNA polymerases have resulted in the commercial availability of enzymes or enzyme blends with properties favorable to the forensic mtDNA investi- gator. These properties include increased thermostability, sensitivity, pro- cessivity and fidelity compared to *Taq* DNA polymerase. An improved DNA polymerase has the potential to allow an investigator to glean more informative sequence data from a limited quantity of probative evidence by reducing or eliminating Taq errors, reading through problematic poly- cytosine stretches, and producing stronger amplicons from low quality DNA extracts.

AFDIL reviewed the amplification abilities of several commercially available DNA polymerase products. The three selected products include the Expand High Fidelity PCR System (Roche Applied Science, Mannheim, Germany), Accuprime™ *Taq* DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA), Phusion™ High Fidelity DNA Polymerase (Finnzymes, Espoo, Finland) and GoTaq® Green Master Mix (Promega Corporation, Madison, WI). The Roche and Invitrogen enzymes kits offer increased fidelity, primarily by the addition of a proofreading enzyme to the normal *Taq*-polymerase mediated amplification reaction. Phusion™ DNA polymerase is a unique Pyrococcus-like enzyme that has both 5'-3' DNA polymerase and 3'-5' exonuclease (proofreading) activities. GoTaq® Green Master Mix contains only native Taq DNA polymerase without any special proofreading abilities. Instead it offers greater streamlining of the amplifi- cation process by containing everything needed for amplification (except DNA template and primers) as well as agarose-gel loading dyes and a density increasing compound that allow for direct loading of amplification product on agarose gels.

The abilities of the tested DNA polymerase products to amplify low quality, low quantity and known error prone DNA extracts is compared to that of AmpliTaq Gold® (Applied Biosystems, Foster City, CA), a chemi- cally inactivated, hot-start DNA polymerase with no proofreading ability. AmpliTaq Gold is the DNA polymerase product currently employed by AFDIL. The selected DNA polymerase systems were evaluated for sensi- tivity, robustness, and ease-of-use. The results of these comparisons are presented.

The views expressed herein are those of the authors and not necessarily those of the Armed Forces Institute of Pathology, the U.S. Army Surgeon General, nor the US Department of Defense. Mention or discussion of any specific product does not connote endorsement of said product.

DNA Polymerase, mtDNA, Comparison

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