



### **B101 New Tools for Mitochondrial DNA Sequencing and Analysis at the University of North Texas Center for Human Identification Laboratory**

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After attending this presentation, attendees will understand the strategy for mitochondrial DNA sequencing in use at the University of North Texas Center for Human Identification.

This presentation will impact the forensic community by demonstrating new techniques and strategies to improve sequence quality, increase efficiency, and reduce costs.

The University of North Texas Center for Human Identification (UNTCHI) is one of three laboratories in the United States funded by the National Institute of Justice explicitly for the identification of missing persons and is a partner in the National DNA Index System (NDIS) database for missing persons. The UNTCHI has established a multi-faceted approach to the identification of unknown human remains for missing person and cold case investigations by integrating both forensic anthropology and odontology with state-of-the-art DNA testing methods. The services offered by this program are available to criminal investigators nationwide to help identify missing persons and skeletal remains and provide guidance and kits for the collection of appropriate family reference samples. As of May 2007, 43 states have submitted approximately 1,000 human remains and 2,000 reference samples which have resulted in over 100 identifications.

Both mitochondrial DNA analysis and nuclear DNA analysis are evaluated since the DNA from many of the recovered remains is highly degraded and/or since family reference samples are often limited. Substantial effort is attempted at UNTCHI to incorporate new techniques that improve quality, increase efficiency, and reduce costs. The bottlenecks in sample processing are regularly assessed. Since this program began, the UNTCHI has validated and implemented new kit assays, upgraded instrumentation and software, and adapted robotics. With the success of individual cases and the appreciation of the program by investigators, medical examiners, and families, there is an anticipation of an influx of human remains and reference samples in the very near future. The UNTCHI has positioned itself with ample staff and high throughput capabilities to meet the future demands.

The focus of this paper is to share the many changes and improvements made at UNTCHI for mitochondrial DNA testing and describe how each process is being integrated into the case workflow. Much progress in the mitochondrial DNA process has been made and published in forensic sciences in the areas of extraction, amplification, use of robotics, sequencing, quantitation, and quality control measures. The UNTCHI staff has adopted many of these procedures, made modifications to other procedures, conducted comparison studies, and developed new techniques.

During this presentation, the authors will share results in conventional extraction procedure of skeletal remains as compared to the demineralization procedure published by Loreille et al. The success of robotics and the high throughput extraction process (Plopper, FJ et al.) and sequencing (Roby, RK et al.) implemented for the family reference samples will be reported. For sequencing of smaller amplicons, the presentation will also include a comparison of a new cycle sequencing strategy to replace dRhodamine Terminator Cycle Sequencing Kits (Applied Biosystems, Foster City, CA). This procedure consists of a reduced reaction of BigDye® Terminator v. 1.1 Cycle Sequencing Kit (Applied Biosystems) by adding a sequence enhancing and dilution buffer followed by a simple bead purification method to remove unincorporated BigDye® terminators. Lastly, the authors will share the validation performed of new versions of analysis software and present the use of a software tool to quickly assess sequence quality.

**Mitochondrial DNA, Missing Persons, High Throughput**