



## Criminalistics Section – 2004

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### **B142 Mitochondrial DNA Analysis by Pyrosequencing**

*Susan Hastings, MS\*, University of Central Florida, National Center for Forensic Science, 12354 Research Parkway, Room 225, Orlando, FL 32826; Jack Ballantyne, PhD, University of Central Florida, PO Box 162367, Orlando, FL 32816-2367*

The attendee will be introduced to mitochondrial DNA analysis by pyrosequencing.

This presentation will introduce the community to mitochondrial DNA analysis using a novel sequencing technology.

Mitochondrial DNA (mtDNA) analysis is often used in forensic cases in which traditional nuclear DNA testing is, or is likely to be, unsuccessful. Such cases typically involve compromised, or difficult-to-analyze specimens such as hair or bone. Currently, mtDNA analysis proceeds by standard sequencing of DNA isolated from such specimens. Although current methodology is robust, its principal shortcoming is that it is labor intensive and time consuming. This diminishes the efficacy of mtDNA analysis in the investigative process and discourages its more widespread use within the criminal justice system. We have evaluated a new sequencing technology, pyrosequencing, for its potential applicability to forensic casework.

Pyrosequencing uses the basic biochemistry of polymerase mediated DNA chain elongation to determine the order of the bases. When a deoxynucleotide triphosphate (dNTP) is added to the growing DNA strand, a pyrophosphate (PP<sub>i</sub>) is released. A sulfurylase utilizes this molecule to generate ATP, which in turn fuels a luciferase reaction. Luciferin is converted to oxyluciferin, generating visible light proportional to the quantity of ATP. This light is detected by a CCD camera and translated into a peak on the resulting program.

We have evaluated the performance of pyrosequencing with regard to sensitivity, specificity, and compromised template DNA (including mixtures). The advantages of this technology include vastly improved timeliness of analysis (hours rather than days), a requirement of only femtogram quantities of template DNA, and the potential for higher mtDNA throughput by automation. This work has resulted in a set of assays and associated standard operating procedures that should aid in the transfer of the technology to operational casework and provide an impetus for more widespread use of mtDNA testing for those cases requiring it. These will be discussed in detail.

#### **Mitochondrial DNA, DNA Sequencing, Pyrosequencing**