



A136 Quality Assessment and Alert Messaging Software for Raw Mitochondrial DNA Sequence Data

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After attending this presentation, attendees will understand the principles associated with expert system software analysis and the progress that has been made for mtDNA sequence data.

This presentation will impact the forensic science community by providing another software tool to assist DNA analysts in processing sequence data.

Expert system software programs and rule firings are not new to the forensic community. In fact, many laboratories have validated an expert system for use with single-source convicted offender data to be uploaded into the national DNA database. Expert systems have demonstrated that they can accurately and rapidly evaluate STR data. Similarly, optimized Filter Metrics have been shown to quickly and accurately assess sequence data and to save time and resources. The analyst can immediately evaluate the data after a run is complete. For example, a sample may merely need to be re-injected or a control failure can be instantly identified and the analyst can take action; a decision can be made immediately by the analyst while the plate is still on the sequencer instead of days after the run when the analyst is analyzing the data.

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 which include STR analysis and mitochondrial DNA (mtDNA) analysis.

By far, mtDNA processing and analysis is more time-consuming than STR analysis. A new software tool, eFAST Software (expert Filter and Assessment of Sequence Trace Software) will be presented, which is designed to automate the assessment of trace files generated by the instrument – minimizing the time needed by the operator to review quality of raw data – and quickly alert the operator of any data that do not meet defined metrics. The eFAST Software calculates the Trace Score, the Contiguous Read Length, and Signal Strength for each trace file as soon as it is generated by the instrument. These Filter Metrics are used to identify “high quality,” “questionable,” and “low quality” sequence trace files. The Filter Metrics are user-defined and can be modified for specific sequence analyses and primers. Additionally, the software will alert an analyst of any control failures as soon as a control run is complete. Negative controls and reagent blank controls are examined for signal and are marked as “questionable” by the software if the signal strength is above a defined threshold. Failed positive controls or failed negative controls on a plate automatically cause all samples in the plate with the same primer to be marked as “fail.”

eFAST Software operates on the same computer running the sequencer's Data Collection software and must be launched prior to processing the samples. “High quality” trace files are copied into one directory and “low quality” or “questionable” trace files can elicit an operator alert (*i.e.*, email sent directly to the operator) for immediate intervention. A graphical user interface (GUI) will allow the operator to examine the data, annotate the data, or forward the data to an appropriate directory. Further, eFAST Software maintains Administrative and User-defined security privileges.

Trace files that pass the quality review are automatically distributed into network directories which reduce the time needed by the analyst to sort and filter the data before it is analyzed. The eFAST Software uses file names to differentiate controls and samples and identify the primers used. When all files in a plate have been processed and distributed, the plate directory on the sequencer is removed.

Quality Assessment, mtDNA Sequences, Filter Metrics