

## **AUTOMATION OF DNA PURIFICATION FOR MITOCHONDRIAL DNA ANALYSIS**

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The automation of mitochondrial DNA (mtDNA) purification is a challenging task given that even the most careful manual methods can result in poor DNA yield and contamination since the samples being analyzed can be degraded and of limited quantity. It is with these hurdles in mind that the Virginia Department of Forensic Science has begun evaluation of the Qiagen® EZ1® Advanced Robotic Workstation for the automated extraction of mtDNA from hair shafts and bone samples. The non-grinding enzymatic approach for hair sample preparation and overnight demineralization for bones allows for the use of the DNA Investigator chemistry protocol combined with the EZ1® closed robotic workstation system. Adapting decontamination measures to the existing platform facilitates the efficient extraction and purification for such problematic samples by virtue of its design. Successful automation of these methods improves efficiency and consistency, as well as reducing contamination in the laboratory.

The evaluation of the EZ1® Advanced Robotic Workstation included tests for susceptibility to contamination with liquid blood samples 100 times the concentration of DNA typical to mtDNA casework, as well as a sensitivity series of liquid blood at very low concentrations of DNA. A comparison of mtDNA results from a sensitivity series of hair shaft samples differing in length (2cm to 0.2cm) extracted using the EZ1® and manual organic methods demonstrated concordance in mtDNA sequence and a higher quality of mtDNA product from the same sized hair extracted on the EZ1®. Mock casework type hair samples were extracted using the EZ1® and mtDNA sequences were successfully obtained. A sensitivity series of bone samples differing in weight from 0.2g to 0.05g demonstrated that this isolation method is effective in recovery of DNA in very small bone samples. Non-probative casework bone samples, varying in age of recovery from 8 to 19 years, were extracted using the optimized protocol and demonstrated that the mtDNA sequences are concordant with the results of previous organic method extractions. Bones extracted with this method on the EZ1® demonstrated at least an equivalent and in most amplifications, a higher amount of mtDNA product from the same amount of bone. Evaluation of blanks run in conjunction with the liquid blood samples, the hairs, and bones used in these studies demonstrated that the contamination rate was no higher than that encountered with manual extractions. Additionally, the sequences obtained in the blanks may not have been solely attributed to extraction on the EZ1®.

The Virginia Department of Forensic Science has successfully demonstrated that the Qiagen® EZ1® Advanced Robotic Workstation can be used to obtain quality mtDNA sequences. This was consistent for both hair and bone samples that may be typically encountered in performing forensic casework and identification of human remains. Most importantly it is achieved without being susceptible to contamination between samples during isolation and purification.