

**ENHANCED PCR: AN APPROACH FOR MITOCHONDRIAL DNA ANALYSIS  
OF AMPLIFIED DNA SAMPLES FROM FORENSIC CASEWORK**

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Mitochondrial (mt) DNA sequencing has gained importance in forensic analysis in the past few years. In certain cases the sample is enough only for single extraction and amplification. The most common observation is poor STR amplification because of either degraded DNA and/or presence of very low amount of DNA. In order to increase the sensitivity and obtain acceptable results, we have explored the approach of re-amplification of STR PCR product for mtDNA sequence analysis.

PCR product obtained by amplification using the Profiler Plus™ kit and the recommended 28 cycles PCR program was purified either by Centricon 100 concentrators or Qiagen columns. This step enables removal of PCR reagents and other impurities (from the first amplification) present in the product. The purified amplicon, thus obtained, was used as a template for a second amplification using mtDNA HV1 and HV2 primers. The results demonstrate that concordant and accurate sequence results were obtained by using either extracted DNA or purified STR amplified DNA. It is important to note that the fluorescent tags present in the amplified product from Profiler Plus™ did not interfere with the mtDNA sequencing.

In conclusion, it is possible to increase the sensitivity and obtain acceptable sequencing results from degraded or low level samples using the enhanced PCR approach. The mtDNA sequencing results obtained after the second amplification were of very high quality meeting the acceptability criteria for forensic analysis. The methodology, comparative data and validation of the developed protocol for use in forensic casework will be discussed, including reproducibility, sensitivity, and non-probative casework analysis.