APPLICATION OF MASSIVELY PARALLEL SEQUENCING IN THE ANALYSIS OF DEGRADED BLOOD SAMPLES & DENTAL REMAINS

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Degraded DNA poses a critical challenge for forensic laboratories and is often encountered in crime scene evidence and unidentified remains associated with missing persons cases or mass disaster tragedies. Improvements in upstream extraction and quantification, coupled with massively parallel sequencing (MPS) technology collectively increase the likelihood of obtaining informative nuclear DNA profiles.

Human dental remains can provide a valuable sample source for DNA typing when suitable soft tissue is unavailable. Using traditional methods, teeth samples can be challenging to process, resulting in low quantity and/or quality nuclear DNA and insufficient profiles for comparisons. This study examines performance of a three-part nuclear DNA analysis workflow for teeth samples based on 1) improved dental tissue recovery using the Dental Forensic Kit (DFK^{MR}) (Universidad de los Andes) and DNA extraction with QuickExtract™ FFPE DNA Extraction Kit, 2) quantification with InnoQuant® HY for sensitive assessment of total human and male DNA quantity/quality, and 3) massively parallel sequencing for simultaneous genotyping of 231 short tandem repeat (STR) and single nucleotide polymorphism (SNP) markers with the ForenSeq® DNA Signature Prep Kit. Majority of the amplicons in the ForenSeq kit are shorter in base pair length compared to CE assays, providing a significant advantage when analyzing degraded DNA samples.

Prior to application to teeth samples, an initial sample set of artificially and progressively degraded DNA from blood was evaluated. The degraded blood DNA achieved highly sensitive and informative quantification results, enabling successful first pass genotyping. Twenty-three STR alleles (out of 85) and 70 identity-informative SNP loci (out of 94) were recovered from 2 pg total long target DNA input (0.86 ng total short target input) with a corresponding degradation index of 460 (severely degraded). The three-part workflow was subsequently applied to teeth samples (dental pulp, root cement tissues) with postmortem intervals of the teeth ranging from 8 days to approximately 6 months. Informative SNP and STR DNA profiles were obtained, to include 78 STR alleles and 85 identity-informative SNP loci typed in a 1 month, 4-day PMI root cement sample with 1 pg total long target DNA input and a DI of 76. These data indicate successful performance of the proposed workflow for degraded DNA from blood and teeth samples.