Examining Various Methods to Extract and Amplify Degraded DNA

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After attending this presentation, attendees will have a better understanding of options available to improve profile results from forensic samples containing degraded DNA. This presentation will impact the forensic science community by providing results from an in-depth study of various DNA extraction methods and commercially available amplification chemistries for improved recovery and profile generation from degraded DNA.

In forensic casework, degraded DNA is encountered more frequently now that technologies have improved and allowed for increased detection of low-level DNA from decades-old cases. It can be difficult to generate full short tandem repeat (STR) profiles from these cases due to DNA degradation. Traditionally, mitochondrial DNA (mtDNA) testing has been more common in cases of highly degraded DNA, especially when the evidence is a bone or rootless hair. However, mtDNA testing is a more lengthy and costly process and few labs have the capabilities to analyze mtDNA. For these reasons it is important to examine whether nuclear DNA results can be improved by using these new methods.

In this study, hair (roots and shafts) were collected from multiple volunteer donors and extracted via four different methods: the QIAGEN® QIAamp® DNA Micro Kit, the InnoGenomics® Hair Extraction Kit, the MicroGEM forensicGEM™ Universal kit and Protocol C from the Brandhagen et al. paper on nuclear DNA in hair shafts[1]. Samples were then quantified using Applied Biosystems Quantifiler™ Trio to determine quantity and level of degradation of DNA. Four different amplification kits were chosen to evaluate the DNA profiling method: Promega® PowerPlex® Fusion, Applied Biosystems AmpFLSTR™ MiniFiler™, InnoGenomics InnoTyper® 21, and QIAGEN Investigator® DIPplex. InnoTyper® 21 and Investigator® DIPplex are not STR kits and were chosen due to their differences and possible improvements over the current STR systems. Both InnoTyper and DIPplex are biallelic and test for retrotransposon insertion polymorphisms (RIPs) and insertion/deletion polymorphisms (INDELs), respectively. The amplicon sizes in these two kits are smaller than the typical STR amplicons, increasing the chance that the primer target region will remain intact within degraded DNA and generate a profile where a traditional STR kit fails to do so. In addition to testing the kits' abilities to amplify the hair shafts and roots, other casework type samples were tested (bone, aged, inhibited and degraded samples).

The sensitivity study in all four amplification kits resulted in optimal profiles with 0.25 to 0.5 ng of DNA. In general, the hair roots had sufficient quantity and performed well in all amplification kits. While an improved quantity of DNA was obtained from hair shafts extracted with the Brandhagen et. al. protocol over the other extraction methods, the amount of DNA was less than 5pg/µl and resulted in limited profile data with all amplification kits, including those with smaller amplicon size. However, the increased DNA yield will likely increase success rates from hair shafts for mtDNA testing. When sufficient DNA from degraded samples is available, the shorter amplicon kits have the potential to generate more complete profile results than STR kits, increasing the statistical power of degraded DNA evidence.

Reference(s):

1. Michael D. Brandhagen, Odile Loreille, and Jodi A. Irwin, "Fragmented Nuclear DNA is the Predominant Genetic Material in Human Hair Shafts," *Genes* 9, (2018): 640, https://doi.org/10.3390/genes9120640.

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