GENETIC IDENTIFICATION OF HIGHLY DEGRADED DNA BY CAPTURE-BASED MASSIVELY PARALLEL NEXT GENERATION SEQUENCING

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Mitochondrial (mt)DNA typing found its niche in forensic genetics due to its elevated copy number relative to nuclear DNA, which - in practical terms - increases the sensitivity and analysis success rates in challenging forensic samples. Previous studies indicate that mtDNA also seems less vulnerable to degradation effects compared to nuclear DNA, which is why its analysis is more promising to be successful in severely damaged or environmentally challenged samples.

Traditionally, mtDNA has been directly Sanger-type sequenced form PCR products and detected via (capillary) gel electrophoresis. There are various amplification strategies involving numerous different primers that have been developed in the past in response to the lack of commercially available amplification and sequencing kits. It has been demonstrated that inappropriate amplification strategies had contributed to artifactual sequencing data (phantom mutations) that hamper the reliability of mtDNA data developed not only in the forensic context but also in other fields of genetic research.

With the emergence of Massively Parallel (Next Generation) Sequencing (MPS) techniques standardized PCR amplification kits become commercially available for both nuclear and mitochondrial DNA markers, which will likely result in higher quality of mtDNA data if interpreted correctly. Another benefit of MPS is the increased amount of amplicons that can be sequenced and differentiated in a single MPS run, which allows for an increased number of mtDNA segments to be analyzed (simultaneously) from a single sample. It has been shown that this enables full mitogenomes to be sequenced even from specimens that contain only minute amounts of mtDNA, such as hair shafts. These amplification strategies are based on two (or more) independent PCR multiplex reactions with more than 80 amplicons each that are subsequently pooled and sequenced in a single run. Average amplicon sizes range between 150 and 200 nucleotides and allow for successful analysis of degraded DNA as present in ancient specimens.

In contrast to standard techniques, MPS technology allows for sequencing of (mt)DNA segments that are below the typical fragment sizes applied to electrophoretic separation. This would particularly be useful for the analysis of highly degraded (mt)DNA that lacks detectable quantities above 100 nucleotides. These and smaller fragments typically cannot be amplified with the classical PCR approach but need alternative assays. It has been demonstrated that Primer Extension Capture (PEC) MPS is one of the most promising library generation techniques with respect to forensic genetic applications. PEC-based libraries have been shown to be more specific and sensitive for capturing target-specific (mt)DNA sequences compared to other approaches (e.g. shot gun sequencing) and resulted in forensically useful data in specimens that were too degraded for PCR-based approaches.