B75 The Influence of DNA Extraction Methods on the Quantity and Quality of Retained Genetic Material

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Learning Overview: After attending this presentation, attendees will better understand how to characterize the influence of various DNA extraction methods on the quantity (i.e., concentration) and quality (i.e., strand length) of resulting DNA eluates.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by illustrating how better documentation of the influence of DNA extraction and purification on the quantity and quality of DNA eluates will provide critical information for forensic and ancient DNA researchers to consider before choosing an extraction method. Moreover, the findings of this study should direct efforts toward improving extraction yields and decreasing damage during DNA extraction and purification.

DNA recovered from forensic and ancient DNA sources is generally expected to be in low copy numbers and degraded with regard to strand length. However, considering the following equation, it is important to realize that the exact amount of recoverable DNA from any source is unknown: net yield of DNA=original amount–loss in sampling–loss in extraction/purification–loss due to amplification bias (e.g., due to Polymerase Chain Reaction [PCR] inhibitors).

In most cases, these variables are unknown or poorly documented. This is also true regarding the degree of fragmentation caused by extraction and purification methods. Thus, it would be useful to have some estimation of these variables prior to initiating the study of compromised materials. In this study, the objectives included estimating: (1) the amount of DNA lost during DNA extraction and purification; and (2) with regard to strand length, the degree to which molecules are damaged as a result of the extraction method.

Four DNA standards were created from freshly extracted pig (*Sus scrofa*) liver DNA: (1) standard 1 was ~9,000–12,000bp at 90ng/µl (i.e., representing "intact" genomic DNA); (2) standard 2 was ~9,000–12,000bp at 1ng/µl (i.e., representing the conditions of touch DNA); (3) standard 3 was ~200–400bp at 100ng/µl (probably an unrealistic state, but interesting to test, nevertheless); and (4) standard 4 was ~200–400bp at 0.8ng/µl (i.e., representing forensic or ancient DNA). Ten aliquots of each of these standards were then subjected to 14 commercial extraction methods, many of which are marketed as suitable for forensic and ancient DNA applications (QIAGEN® DNA Investigator, QIAGEN® DNA Micro Kit, MinElute® PCR Purification, QIAGEN® DNeasy® Blood and Tissue Kit, Invitrogen™ Charge Switch, MP Biomedicals™ Geneclean Kit for Ancient DNA [Dehybernation A and B], Promega® DNA IQ, Thermo Fisher PrepFiler™, Bio-Rad™ InstaGene Matrix, TaKaRa NucleoSpin® gDNA Cleanup XP, NucleoSpin® DNA Trace, and TaKaRa NucleoSpin® Trace 8). These standards were also subjected to seven published ancient DNA extraction methods. "DNA in" of the standards was compared to "DNA out" using an Agilent® Fragment Analyzer that is capable of simultaneously measuring DNA quantity (i.e., concentration) and quality (i.e., DNA strand length).

Data regarding both DNA loss and fragmentation were obtained. Observations were made of both DNA loss and fragmentation. Some kits performed very well, whereas others were associated with tremendous loss and/or fragmentation. This indicates that DNA recovered from compromised sources may, in fact, be more concentrated and intact than is generally recognized. It also demonstrates that many extraction methods used to study degraded DNA are far from optimal, and that research should be focused on improving their efficacies.

Low Copy Number, Standards, Fragment Analyzer