B76 Where, Oh Where, Has the DNA Gone?

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Learning Overview: The goal of this presentation is to provide attendees with a better understanding of the potential for DNA loss from degraded and low concentration sources when utilizing commonly used silica-based extraction methods.

Impact on the Forensic Science Community: Findings of this study will impact the forensic science community in the development of more efficient DNA extraction protocols that specifically retain DNA from degraded and low copy number sources.

The behavior of aged, degraded, and/or low copy DNA during routine laboratory methods is still poorly understood. Previous research on this topic is sparse, but has demonstrated that ample amounts of DNA are inadvertently lost during extraction and purification. These observations suggest that there may actually be more DNA in compromised sources than is commonly recognized. Notably, some DNA extraction and purification methods cause DNA eluates to become lower in copy number than expected, or than would be useful for downstream applications. Considering the case of silica-based extractions, DNA can be lost via two mechanisms. First, it is possible that not all the source DNA binds to the silica column (i.e., DNA could be found in the flow through). Second, it is possible that DNA is not efficiently eluted off of the silica (i.e., DNA could be retained on the silica column).

In this study, DNA standards of known concentration were subjected to the QIAGEN® DNeasy® Blood and Tissue Kit (a silica-based extraction) to identify: (1) how much DNA has not bound to the silica, and (2) how much DNA has not been eluted off the silica column. In order to do so, DNA standards were created using genomic porcine DNA obtained from Zyagen. “Intact DNA” was represented by Standard 1Z, with a peak fragment length of 20k bp, at a concentration of ~100ng/µl. To represent “degraded DNA,” standard 3Z was sheared by sonication to a peak fragment length of roughly 300bp. Low copy variants of each of these standards were created through dilution of standards to <1ng/µl (Standard 2Z and 4Z).

High concentration standards 1Z and 3Z were extracted using the DNeasy® kit. After following the provided extraction protocol, each spin column was moved into a new collection tube and another 200µl of buffer AE was used to elute the DNA that potentially remained in the column. For Standards 1Z and 3Z, this was repeated until the column was eluted a total of ten times in ten separate collection tubes.

To determine if DNA fails to bind onto the columns, the flow through from the purification steps of the initial extraction was passed across a new spin column, washed, and eluted with AE buffer. DNA remaining on this column was eluted into new collection tubes a total of ten times. This was repeated eight more times, resulting in a total of total of 100 eluates from the original DNA. Low copy standards 2Z and 4Z were similarly tested, but silica columns were eluted five times, and four new silica columns were used to attempt to capture DNA from the four flow throughs (totaling 25 tubes). The DNA that was contained in these tubes was quantified using a Qubit® Fluorometer three times. The eluates were run on a Agilent® Fragment Analyzer to determine the size of the DNA fragments that were recovered in each step.

Molecules from all standards were recovered during this process, but detectable amounts varied by method used. For example, standard 1Z eluates had detectable amounts of DNA in 40 eluates (Qubit®) versus 56 eluates using the Fragment Analyzer. However, some general trends were detected. First, molecules were detected in the flow through of all standards, especially those at higher concentrations. Second, the larger fragments (over 4,000bp in length) were recovered at a higher frequency in initial elutions in a new spin column. Thirdly, smaller fragment standard (~300bp in length) eluates were not detectable using the Qubit®. In total, this suggests that modifications to the extraction protocol may be needed to recover adequate DNA from low concentration, damaged sources.

Silica-Based, DNA Loss, DNA Extraction

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