Optimization of DNA Extraction Buffer Used in Conjunction with DNA IQ™

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Forensic casework samples often contain low levels of biological material (low template) and are frequently deposited on difficult substrates. Challenging samples such as these have received a great deal of attention, in both research efforts and the court room. These samples, which are commonly derived from "touch" evidence, typically require optimized methods for efficient recovery of biological material. For this study, proteinase-K extraction buffers used in conjunction with the DNA IQ™ System on an automated platform were experimented upon, with the main focus on varying concentrations of sodium dodecyl sulfate (SDS).

Automation is now extensively used in forensic laboratories; however a means for more efficient DNA extraction is desired since problematic samples constitute a large percentage of all casework samples. Organic extraction continues to be considered the "gold standard", as it is capable of isolating DNA from difficult samples. However, this method is time-consuming and non-automatable. At the Virginia Department of Forensic Science (VDFS), Promega's DNA IQ™ system is utilized in conjunction with a robotic platform for the automated extraction of DNA from forensic samples. However, it has been observed that low template samples and samples deposited on various substrates can sometimes prove problematic and produce lower than expected DNA yields using this automated approach. Thus, keeping in mind the high performance ability of the organic extraction method, a buffer similar to the stain extraction buffer used with organic extraction procedures is being evaluated. In particular, proteinase K buffers were tested for the effectiveness of the anionic SDS detergent compared to the anionic sarkosyl detergent; sarkosyl has traditionally been utilized since it is miscible with the DNA IQ™ Lysis buffer.

Although SDS has been demonstrated to be superior to other detergents in extraction buffers, use of 1% SDS (the concentration used in stain extraction buffer) with the automated DNA IQ™ method at VDFS has resulted in an inconsistent performance, particularly with undiluted, low template samples. This is likely due to precipitation of guanidinium salt from the DNA IQ™ Lysis buffer, since concentrations at or above 1% are known to precipitate the salt. Thus, we experimented with lower concentrations of SDS in order to overcome this problem, as well as an extra step of plate heating during the robotic extraction process.

Experimentation with whole blood on various substrates has been successful, where nearly every sample incubated with an SDS-containing buffer yielded higher concentrations of DNA and higher average peak heights. Occasionally, the DNA concentrations obtained suggested a full profile should be generated, yet an incomplete profile resulted during STR analysis, suggesting that precipitation of the guanidinium salt or possible PCR inhibition continued to be a problem. Studies are ongoing utilizing mock casework samples in order to resolve the problem of precipitation or inhibition with the goal of ascertaining if indeed replacing the sarkosyl with SDS is possible, and if it produces a more reliable yield of DNA from low template and problematic samples.