How Efficient is Your DNA Extraction Method?

Tips to Maximize Profiling Success

The success of forensic DNA profiling starts with effective extraction and purification of samples to preserve as much DNA as possible while removing inhibitors to PCR. Increasing the quality and quantity of extracted DNA improves the chances of successful downstream PCR and genotyping, which is especially important for crime scene evidence with limited DNA.

Therefore, it makes sense to assess the efficiency of your DNA extraction method. Doing so, however, is not always easy and straightforward. This article shares information to assist forensic laboratories in the DNA extraction method evaluation process.

Comparative Study Data

Comparative studies between new and existing methods are one way to test DNA extraction efficiency. These are often performed prior to method selection and validation; however, due to time and resource limitations, many forensic laboratories find it challenging to assess all available options with a wide range of sample types and conditions.

A large global data set comparing DNA extraction efficiency between numerous laboratories and DNA extraction methods over multiple years would be helpful. Such a data set does, in fact, exist. It comes from the German DNA Profiling Group, or GEDNAP, which has organized DNA proficiency tests for human identification laboratories for over 25 years.

In the last seven years, over 200 labs from 44 countries have participated in GEDNAP semiannual DNA proficiency testing with many opting-in to an optional DNA extraction efficiency module. For each module, participants are provided three identical blood samples and are instructed to perform identical DNA extractions on each. The resulting DNA extracts are submitted to GEDNAP, along with information on the extraction method, chemistry, and elution volume (µL) used.

The Institute for Forensic Molecular Genetics (IFMG) then performs real-time quantitative PCR analysis, in duplicate, for each of the three replicate extracts. Total DNA yield (ng) per replicate is determined and GEDNAP reports the mean yields and standard deviations for all methods tested, including both manual and automated protocols. This provides comparative

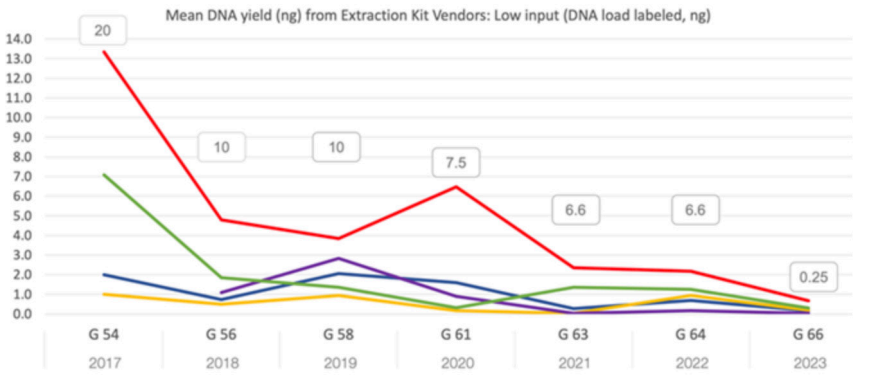

Figure 1. Average DNA yield by manufacturer for low DNA input extraction efficiency modules (between 0.25 and 20 ng DNA input). DNA loads for each module labeled. Red line represents Applied Biosystems PrepFiler Forensic DNA Extraction Kits from Thermo Fisher Scientific. Other manufacturers displayed include Ademtech, Macherey Nagel, QIAGEN, and Promega. Not all manufacturers were represented at each DNA extraction efficiency module.

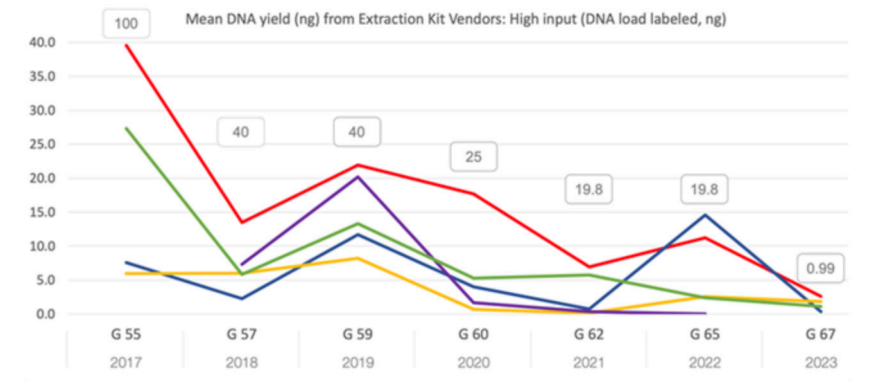

Figure 2. Average DNA yield by manufacturer for high DNA input extraction efficiency modules (between 0.99 and 100 ng DNA input). DNA loads for each module labeled. Red line represents PrepFiler Forensic DNA Extraction Kits from Thermo Fisher Scientific. Other manufacturers displayed include Ademtech, Macherey Nagel, QIAGEN, and Promega. Not all manufacturers were represented at each DNA extraction efficiency module.

study results between all DNA extraction chemistries, methods, and instrumentation used.

Figures 1 and 2 show GEDNAP DNA extraction efficiency module data for 14 consecutive tests (modules G54-G67) analyzed from 2017 to 2023, as shown in a poster presented at the recent 30th Congress of the International Society for Forensic Genetics (ISFG). The graphs display average DNA yield by extraction kit manufacturer separated by low (Figure 1) or high (Figure 2) DNA input amount per proficiency testing year.

Applied Biosystems PrepFiler Forensic DNA Extraction Kit (indicated by the red lines) yielded the highest average DNA quantity, with the majority of PrepFiler data generated using the benchtop Applied Biosystems AutoMate Express Forensic DNA Extraction System or large-format liquid handling robotics.

It is also worth noting that PrepFiler kits displayed the highest comparative efficiency for all modules with the lower input samples (between 0.25 and 20 ng DNA input), which are generally more relevant to forensic DNA labs testing challenging biological evidence. Simply put, if your lab frequently tests samples of limited DNA quantity, the extraction efficiency of your method is even more critical to enabling downstream profiling success.

The Other Side of Efficiency – Hands-on Time

While an extremely important factor, DNA yield is not the only consideration when evaluating a method's overall efficiency. It's also worth thinking about the overall complexity of the method. The more manual hands-on steps required, the more time and labor consumed per test, which is often in short supply in forensic labs. Numerous manual manipulations can also introduce unwanted variation between users and samples.

Therefore, many forensic labs, especially those with higher caseloads, will aim to incorporate automation—either in the form of cartridge-based benchtop systems, or larger format liquid handling robotics. These systems can enable processing a greater number of samples with significantly less hands-on time, while also increasing reproducibility. There are multiple options available, but one automated liquid handling solution is unique in that it enables purification with the PrepFiler extraction chemistry mentioned above and automates additional downstream steps in the forensic workflow.

The Applied Biosystems HID NIMBUS Presto QNA system was designed to enable fast turnaround times and easy, walk-away workflows for DNA purification, quantification setup, and STR amplification setup. The Department of Forensic Science at Sam Houston State University recently evaluated this system, testing a variety of challenging samples including fired cartridge casings, touch,[1] decomposing cadaver,[2] and skeletal.[3] Results obtained with the automated workflow, as reported here were better than or comparable to traditional manual methods, demonstrating consistent and reproducible outcomes from diverse sample types.

These results bolster confidence that it is in fact possible to increase operational efficiency by reducing hands-on time, while maintaining high DNA extraction efficiency to increase DNA profiling success.

[1]Cellphones, keyboards, and water bottles.
[2]Hair, nails, and teeth.
[3]Surface decomposed, burned, and buried remains.