

## Human identification

# Improve purification turnaround times for challenging forensic samples

### Summary

Here we demonstrate the following:

- An efficient automated purification workflow for challenging forensic samples with the HID NIMBUS Presto System
- Isolation of amplifiable amounts of DNA from limited and challenging forensic sample types using the validated script for the PrepFiler and PrepFiler BTA Automated Forensic DNA Extraction Kits
- Short tandem repeat (STR) profile analysis using the VeriFiler Plus PCR Amplification Kit with improved sensitivity

### Introduction

Extracting DNA from forensic samples and purifying it for analysis can be manually intensive and time-consuming. These processes are especially burdensome with challenging samples like bones, teeth, hair, nails, and touch/trace samples.

A traditional protocol for extracting DNA from bone with full or partial demineralization before DNA purification can take more than a day to complete. Many forensics laboratories have implemented optimized protocols that require less starting material, fewer hands-on steps, and shorter turnaround times. Demineralization is not necessary to process bone samples with the Applied Biosystems™ PrepFiler™ BTA Automated Forensic DNA Extraction Kit, significantly reducing the time needed to extract DNA from bone. While automation options are available, not all offer walk-away convenience or throughput flexibility.

The efficient and automated Applied Biosystems™ HID NIMBUS® Presto System is compatible with a variety of forensic samples, including challenging and compromised specimens. The automated workflow requires little hands-on time and minimal sample handling, which reduces the potential for human error and the risk of contamination. The intuitive software for the HID NIMBUS Presto System enables users with no automation experience to get started quickly.



The HID NIMBUS Presto System is specifically scripted and validated for use with Applied Biosystems™ PrepFiler™ and PrepFiler BTA Automated Forensic DNA Extraction Kits. PrepFiler kits are designed for isolating actionable quantities of high-quality DNA from limited and challenging forensic samples and for efficiently removing PCR inhibitors, which can help streamline data interpretation.

Here we demonstrate how the HID NIMBUS Presto System can be used together with the PrepFiler Automated Forensic DNA Extraction Kits to help improve time-to-results to less than a day for challenging samples.

## Materials and methods

The PrepFiler Automated Forensic DNA Extraction Kit was used to process nail, touch, and hair samples, and the PrepFiler BTA Automated Forensic DNA Extraction Kit was used to process bone and teeth samples (Table 1) on the HID NIMBUS Presto System using the validated script v3.1.5. The Applied Biosystems™ Quantifiler™ Trio DNA Quantification Kit was used to assess the quantity and quality of DNA isolated from each of the 233 samples. The purified DNA was amplified with the Applied Biosystems™ VeriFiler™ Plus PCR Amplification Kit using a 29-cycle amplification protocol and 500 pg DNA per reaction. If less DNA was obtained from a sample, 17.5 µL of the elution volume was added to the PCR reaction. All amplification products were analyzed on the Applied Biosystems™ 3500 Genetic Analyzer.

**Table 1. Sample types and quantities included in the study.**

Sample type	Quantity
Bone*	180
Touch: 6 bottle, 6 cell phone, and 6 keyboard touch samples	18
Hair	12
Tooth	11
Nail	12
<b>Total</b>	<b>233</b>

\* Bone samples had been buried (n = 30), burned (n = 30), or surface decomposed (n = 30), and were analyzed in duplicate.

## Results

Quantifiable results ( $\geq 0.1$  pg/ $\mu$ L DNA) were obtained for 83% of the samples. Amplifiable DNA ( $\geq 1$  pg/ $\mu$ L DNA) was isolated from all touch and nail samples, 91% of the tooth samples, 73% of all bone samples (data not shown), and 50% of the hair samples (Table 2, Figure 1). The DNA obtained from all samples was free of detectable PCR inhibitors. The average  $C_t$  for the internal PCR control (IPC) was 27.5 (data not shown). The degradation indices (DIs) of the samples ranged from 0 (no degradation) to  $>10$  (advanced degradation). Nearly all of the highly degraded samples consisted of bone, with a maximum DI of 53.03. The minimum and maximum DIs are shown in Table 2, and Table 3 summarizes the DIs by sample type.

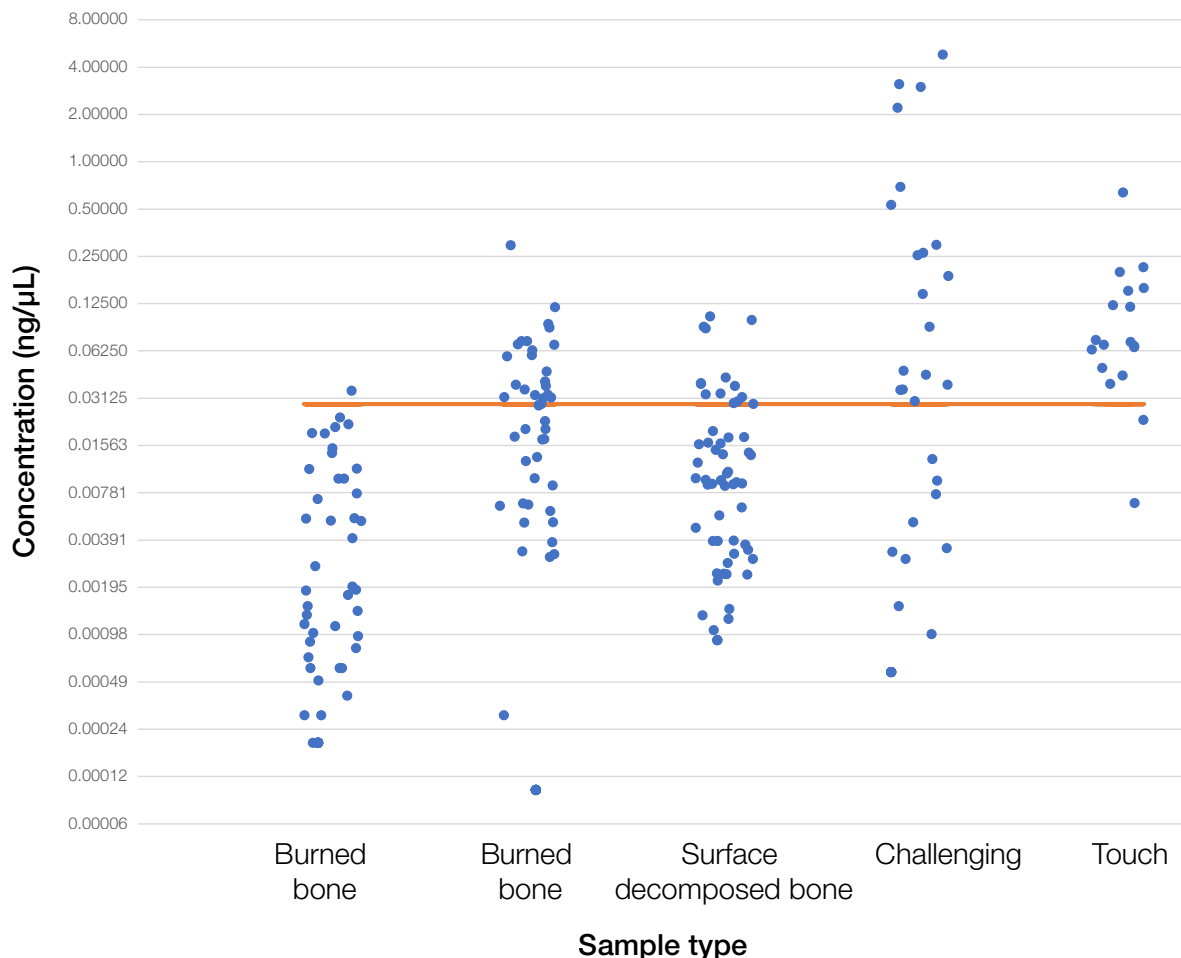
**Table 2. Percentage of samples with amplifiable DNA ( $\geq 1$  pg/ $\mu$ L) and their degradation indices.**

Sample type	Samples with amplifiable DNA (%)	Degradation index	
		Minimum	Maximum
<b>Bone</b>			
Buried	52	1.74	53.03
Burned	73	1.43	22.63
Surface decomposed	95	1.25	23.45
<b>Touch</b>			
Bottle	100	1.55	3.42
Cell phone	100	1.41	2.33
Keyboard	100	1.36	3.46
<b>Challenging</b>			
Hair	50	0.75	1.57
Tooth	91	1.09	4.21
Nail	100	1.12	10.20

**Table 3. DI distribution by sample type.**

Sample type	Degradation index (DI)			
	0–4.99	5–10	$>10$	NA*
<b>Bone</b>				
Buried	9	5	14	3
Burned	41	2	1	0
Surface decomposed	51	1	4	1
<b>Touch</b>				
Bottle	6	0	0	0
Cell phone	6	0	0	0
Keyboard	6	0	0	0
<b>Challenging</b>				
Hair	6	0	0	0
Tooth	10	0	0	0
Nail	9	2	1	0

\* Not applicable due to undetermined large autosomal target.



**Figure 1. DNA quantitation results.** The graph does not show the quantity of DNA obtained from two nail samples that yielded >20 ng/μL.

The distribution of DNA yields is shown by sample type in Figure 1. About one third (32.2%) of the samples yielded  $\geq 28.6$  pg/μL DNA (orange line on graph), so the recommended 0.5 ng DNA per sample could be used for amplification. Full profiles were still obtained from samples that yielded as little as 3 pg/μL DNA (Figure 2), for which 52 pg DNA per sample was used for amplification.

**Conclusions**

The HID NIMBUS Presto System and the PrepFiler Automated Forensic DNA Extraction Kits enabled high-quality DNA to be extracted from challenging samples, including bone, hair, tooth, nail, and touch. When these are used in conjunction with the Quantifiler Trio DNA Quantification Kit and the VeriFiler Plus PCR Amplification Kit, informed quantitative and qualitative decisions can be made early in the workflow to increase the chances of allele recovery from compromised samples.

Minimal hands-on time and the easy-to-use HID NIMBUS Presto software can enable a forensic analyst to start a purification run in minutes and focus on completing more complex tasks. The guidance mode leads the user step by step through the setup process, so no automation expertise is needed to operate the HID NIMBUS Presto System. The flexible sample capacity of the HID NIMBUS Presto System allows a user to run anywhere between 1 and 96 samples with the same amount of effort. The HID NIMBUS Presto System can help you obtain quality results quickly and with confidence.

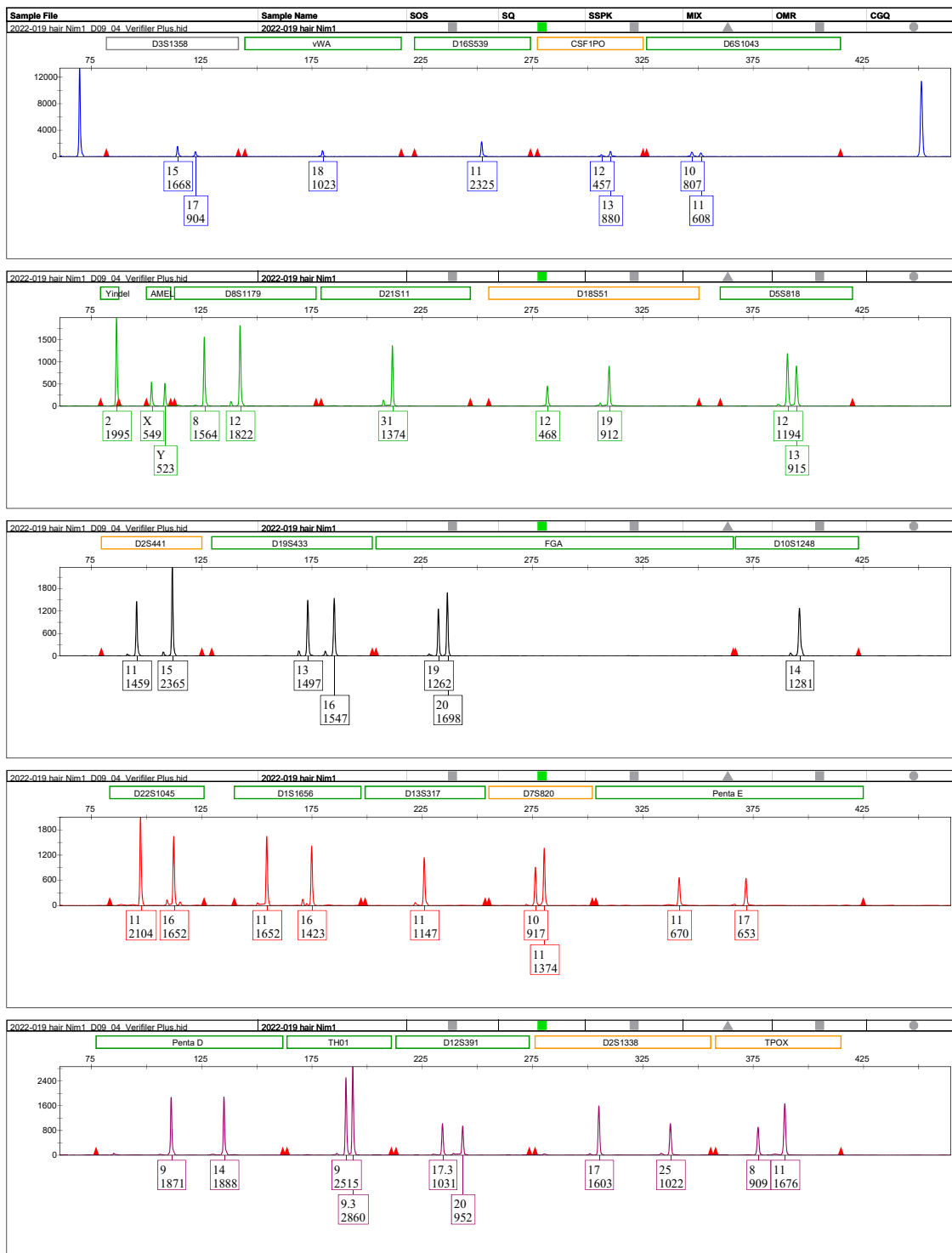


Figure 2. Electropherogram from a hair sample amplified using 52 pg of DNA as input.

### Meet the team

The work described here was performed in the Department of Forensic Science at Sam Houston State University (SHSU) and overseen by Dr. Sheree Hughes, Associate Professor and Chair of Forensic Science at SHSU and Director, Southeast Texas Applied Forensic Science (STAFS) Facility

“One of my favorite features about the HID NIMBUS Presto System is its user-friendly interface and easy setup process. This helps eliminate potential human errors while also saving valuable time when processing a high volume of samples. With the extra time, I can focus on completing downstream analysis.”

—Kayli Carrillo, SHSU doctoral student

“It was such a relief to be able to rely on the HID NIMBUS Presto System when I had a high-quantity, high-priority request for several skeletal extracts. Because it is able to purify 96 samples at a time, processing close to 100 samples required only a single day in the lab rather than the several days I would need to complete this task manually.”

—Jennifer Snedeker, SHSU doctoral student

“There is so much value in being able to customize plastics, sample number, and elution volumes to meet project needs. The flexibility of being able to select my input and output plastic consumables on the HID NIMBUS Presto System helped streamline the DNA analysis workflow for my research project. It simplified the batching process, and I was able to complete more samples in a shorter amount of time.”

—Natalia Czado, SHSU doctoral student

 Learn more at [thermofisher.com/hidnp](https://thermofisher.com/hidnp)

**applied biosystems**