

Preservation of DNA on GenSaver™ 2.0 and GenSaver™ Color 2.0 Collection Cards

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Ahlstrom-Munksjö GenSaver™ 2.0 and GenSaver™ Color 2.0 specimen collection cards are designed for collection, transport and long-term preservation of DNA at ambient temperature for high quality STR analysis and NGS data. They allow direct DNA amplification from a paper punch and prevent the growth of microorganisms during the ambient storage. **GenSaver™ 2.0** and **GenSaver™ 2.0 cards** are specially designed for forensic and biobank applications. They are manufactured in accordance to the ISO 18385:2016 standard to avoid any risk of human amplifiable DNA contamination.

Introduction

This study was designed to investigate the extraction yield and quality of human genomic DNA (hgDNA) from blood and saliva samples collected and stored at ambient temperature on Ahlstrom-Munksjö GenSaver™ 2.0 and GenSaver™ Color 2.0 collection cards. The results demonstrated a very good preservation of human genomic DNA stored 22 years at ambient temperature, highlighted by:

- Good extraction yield of DNA
- High-quality STR profiles
- High-quality of extracted DNA leading to relevant NGS data

Materials and methods

Samples collection

Buccal cells and whole human blood samples were collected from individuals on GenSaver™ 2.0 and on GenSaver™ Color 2.0 respectively, based on the manufacturer's recommendation. They were then air dried for 24 hours at ambient temperature.

Samples storage

After drying, the samples were placed in air-permeable envelopes containing a desiccant and stored at ambient temperature protected from moisture and light for 5, 10, 15 and 22 years, using accelerated ageing testing conditions (56°C, HR 10%).

Human genomic DNA extraction and amplification

A 6 mm punch was removed with a disposable punch device from the center of the dried matrix spots and placed in a clean RNase/DNase-free 1.5 ml tube. Extraction of hgDNA was done from the discs using the Crime Prep Adem from Ademtech, according to the

manufacturer's instructions. Crime Prep Adem-kit is specifically designed for forensic DNA laboratories for casework samples. The kit maximizes quantity and quality of recovered DNA.

Real time PCR was performed in 96 well plates on a 7500 Real Time PCR System using Quantifiler™ Trio DNA Quantitation.

The DNA quantitation assay used an internal PCR control (IPC) assay consisting of two primers for amplification of the IPC template DNA and one TaqMan MGB probe labeled with VIC™ dye for detecting the amplified IPC DNA. This IPC was used to assess the levels of amplification inhibition in the samples during qPCR. Standard curves for DNA quantitation were prepared using control DNA supplied with the kit.

Short tandem repeat analysis

STR analyses were generated using extracted DNA. GlobalFiler™ Express PCR Amplification Kit from Applied Biosystems were used according to the manufacturer's instructions

Detection of amplified fragments was performed using the Applied Biosystems® 3500xL Genetic Analyzer and the analysis was performed with GeneMapper® ID-X Software, Version 1.4. Analysis was conducted using a threshold of 200 RFU, and data were evaluated for First Pass Success Rate (full profile obtained from one amplification and one CE injection) and Intra-locus Balance.

Next Generation Sequencing

After DNA extraction, DNA amplification and creation of the amplicon library were performed using the Precision ID Library kit and Identity Panel (Life Technologies). Quantification of the libraries was performed using Ion Library TaqMan™ Quantitation and the analysis was performed on a 7500 Real Time PCR System. Sequencing of the amplicon libraries was carried out on Ion 5S™



System from Applied Biosystems with kit Ion S5™ Precision ID Chef & Sequencing. SNPs data analysis was performed using HID SND Gentyper Plugin and Ion S5™ System – Torrent Suite™ software 5.2.2.

Results

Sensitivity study

A total of 40 samples were tested with Ahlstrom-Munksjö specimen collection cards. Conditions and results are summarized in Table 1.

Ahlstrom-Munksjö Card	Number of samples	PCR cycles	Number of Full Profile	First Pass Success Rate
GenSaver™ 2.0	20	27	20/20	100%
GenSaver™ Color 2.0	20	27	20/20	100%

Table 1

A number of 27 cycles was selected as the optimum cycle number, as it produced an excellent first pass success rate while minimizing partial profiles. Under this condition, all the samples collected on Ahlstrom-Munksjö cards and analyzed with a peak amplitude threshold of 200 RFU produced full profiles. No contamination was observed on any of the cards tested.

Preservation of hcDNA

Extraction of hcDNA yield

The data of Figure 1 and 2 show reproducible high extraction yields of human genomic DNA from blood and saliva samples collected on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards. Moreover, these data demonstrate a very good preservation of DNA for at least 22 years at ambient temperature.

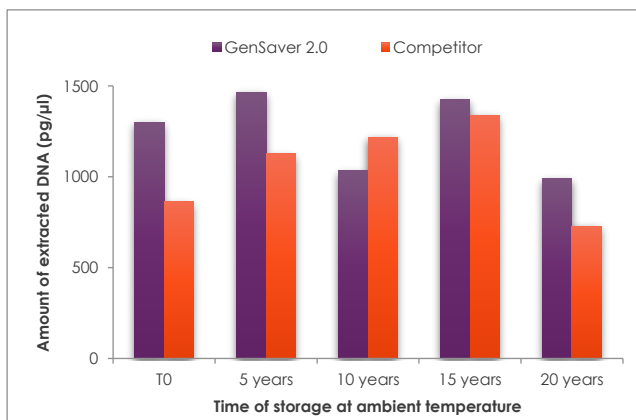


Figure 1. Amount of human genomic DNA extracted from dried blood spots stored on GenSaver™ 2.0 cards at ambient temperature

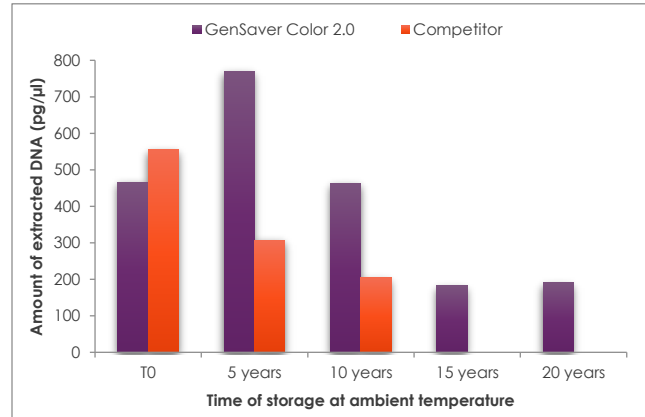


Figure 2. Amount of human genomic DNA extracted from dried saliva spots stored on GenSaver™ Color 2.0 cards at ambient temperature

Higher DNA yields were obtained from blood samples than from saliva samples, probably due to aggregation of cells on the fiber-based material, as regularly reported in the literature. No inhibition of the IPC of each sample was observed during qPCR.

High quality STR profiles

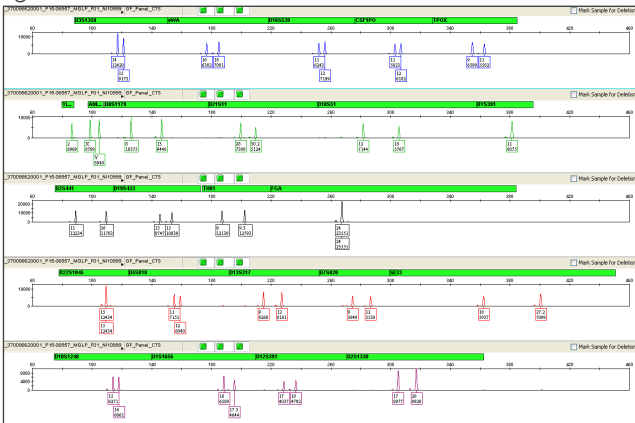
Short Tandem Repeat analysis

STR data were generated to determine the accuracy of allele calls for genomic DNA extracted from blood samples and saliva samples collected on Ahlstrom-Munksjö collection cards and stored for 5, 10, 15 and 22 years at ambient temperature, protected from light. For each period of storage, STR analysis were run for three blood samples collected on GenSaver™ 2.0 cards and three buccal cells samples collected on GenSaver™ Color 2.0 cards.

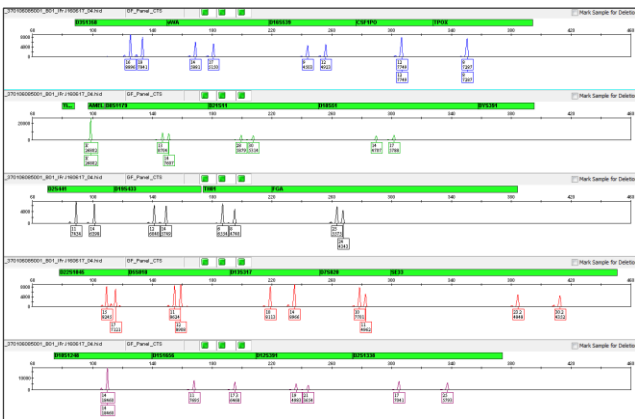
The data of Figures 3, 4, 5 and 6 show that extraction and purification of DNA from GenSaver™ 2.0 and GenSaver™ Color 2.0 cards provide DNA with sufficient quantity and high quality to support good allele calling accuracy. No sample required a reinjection for 100% accurate allele calls.



Figure 3.

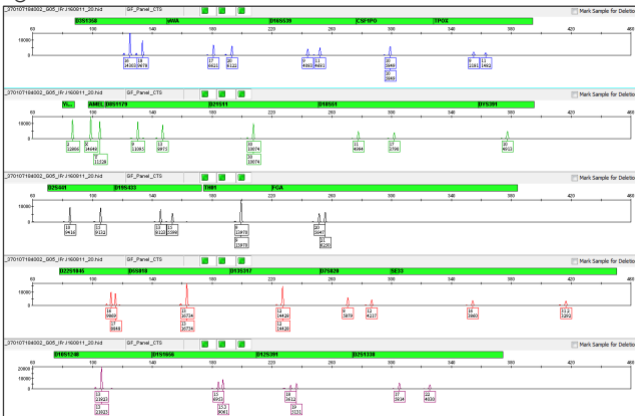


Electropherogram for the STR amplification from genomic DNA purified from blood samples spotted on GenSaver™ 2.0 after 24 H drying.

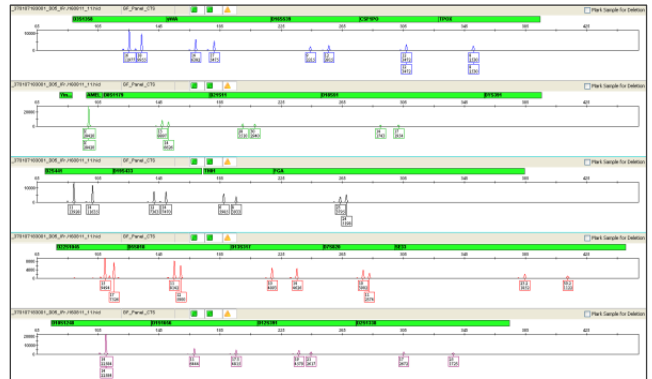


Electropherogram for the STR amplification from genomic DNA purified from buccal cells samples spotted on GenSaver™ Color 2.0 cards after 24 H drying.

Figure 4.

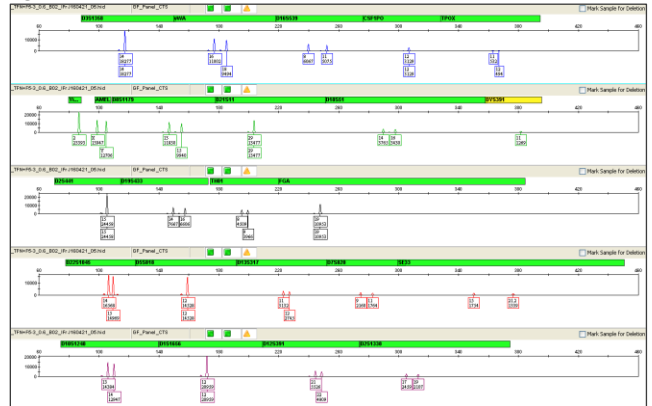


Electropherogram for the STR amplification from genomic DNA purified from blood samples spotted on GenSaver™ 2.0 and stored for 5 years at ambient temperature.

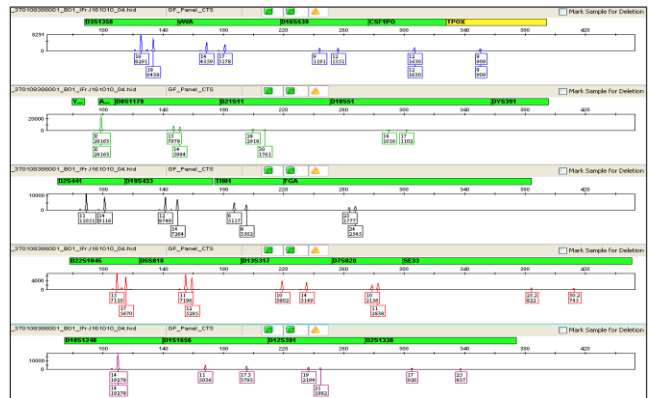


Electropherogram for the STR amplification from genomic DNA purified from buccal cells samples spotted on GenSaver™ Color 2.0 cards and stored for 5 years at ambient temperature.

Figure 5.



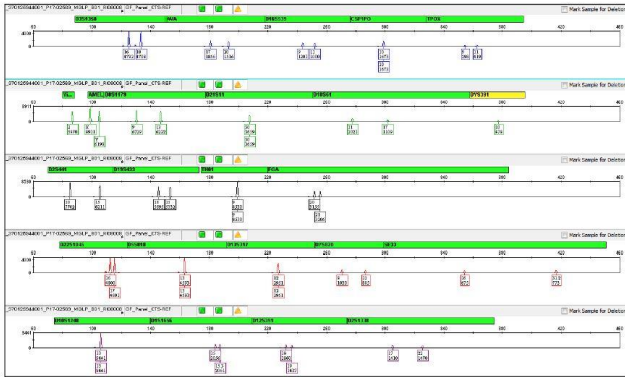
Electropherogram for the STR amplification from genomic DNA purified from blood samples spotted on GenSaver™ 2.0 and stored for 10 years at ambient temperature.



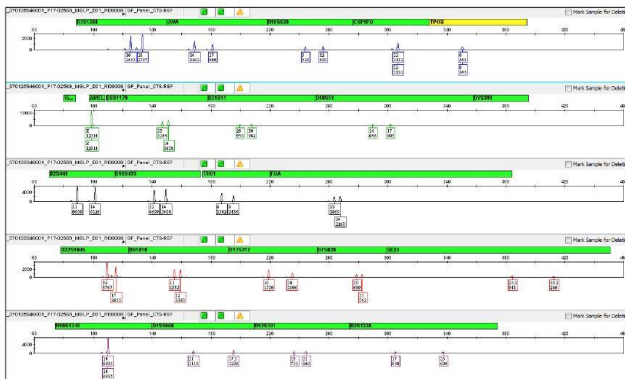
Electropherogram for the STR amplification from genomic DNA purified from buccal cells samples spotted on GenSaver™ Color 2.0 cards and stored for 10 years at ambient temperature.



Figure 6.



Electropherograms for the STR amplification from genomic DNA purified from blood samples spotted on GenSaver™ 2.0 and stored for 20 years at ambient temperature.



Electropherograms for the STR amplification from genomic DNA purified from buccal cells samples spotted on GenSaver™ Color 2.0 cards and stored for 20 years at ambient temperature.

Next Generation Sequencing

NGS data were obtained from DNA purified from blood (GenSaver™ 2.0) or buccal cells (GenSaver™ Color 2.0) stored at ambient temperature for 15 and 20 years (table 3). The high quantity and quality of DNA stored and extracted is correlated with high number of Reads and a Quality test (Q20) value at 95%. This high data quality is consistent and demonstrates that NGS is achievable even after long-term storage of DNA at ambient temperature on Ahlstrom-Muncksjö cards.

AM card	Storage time (years)	Bases	≥ Q20	Reads	Mean Read Length
GenSaver™ 2.0	15	35 899 842	34 420 724 (96%)	439 855	82 bp
	20	37 970 318	35 669 237 (95%)	472 610	80 bp
GenSaver™ Color 2.0	15	25 613 871	24 621 019 (96%)	325 937	79 bp
	20	38 027 859	35 628 198 (94%)	490 048	78 bp

Table 3