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Application Note

Reliable extraction and long-term preservation of DNA from biological samples stored on Ahlstrom GenSaver™ 2.0 and GenSaver™ Color 2.0

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This study was designed to investigate the extraction yield and quality of human genomic DNA from blood and saliva samples stored at ambient temperature on Ahlstrom collection cards. The results demonstrated a very good preservation of human genomic DNA after 20 years of ambient storage on Ahlstrom cards, highlighted by:

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

Introduction

GenSaver™ and **GenSaver™ Color** cards are designed for collection, transport and storage of DNA from biospecimen at ambient temperature. These cards are made of pure absorbent fibers according to highest standards in manufacturing and are impregnated with a patented chemical formula that stabilizes nucleic acids at ambient temperature for 20 years.

GenSaver™ 2.0 and **GenSaver™ Color 2.0** cards offer additional features in order to allow direct DNA amplification from a paper punch and prevent the growth of microorganisms during the ambient storage.

GenSaver™ and **GenSaver™ 2.0** are specially designed for forensic and biobanking applications and are available in a variety of standard formats with 1, 2, or 4 sample collection areas per card. White paper is for blood collection and pink paper for buccal cells, saliva and urine samples. These cards are also available in customized designs according to the needs of the customers.

Materials and methods

Samples collection

Buccal 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 20 years, using accelerated ageing testing conditions.

Human genomic DNA (hcDNA) extraction and amplification

A total of three punchs of 3 mm were 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. hcDNA was extracted 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.

PCR was performed in 96 well plates on a 7500 Real Time PCR System using two different amplification kits, GlobalFiler™ Express PCR Amplification Kit from Applied Biosystems and PowerPlex® Fusion 6C System from Promega, according to the manufacturer's instructions. 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.

DNA analyses were performed by IGNA (Nantes Atlantic Genetic Institute) in Nantes, France. ISO/ICE 17025:2005, accreditation n°8-2555.

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Short tandem repeat analysis

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 HID-Ion AmpliSeq Identity Panel kit (Life Technologies). Clonal amplification of the libraries was performed using Ion PGMTM Hi-Q™ OT2 kit and sequencing of the amplicon libraries was carried out on the Ion Torrent Personal Genome Machine (PGM) system, using Ion PGM™ Hi-Q™ Sequencing kit, all Life Technologies. SNPs data analysis was performed using the Torrent Server of Life Technologies.

Results

Sensitivity study

A total of 40 samples were tested with Ahlstrom specimen collection cards. Results are summarized in Table 1.

Ahlstrom 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 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

hcDNA extraction yield

The data of Figure 1 and 2 show reproducible high extraction yields of human genomic DNA from blood and saliva samples collected on Ahlstrom cards. Moreover, these data demonstrate a very good preservation of DNA for at least 20 years at ambient temperature.

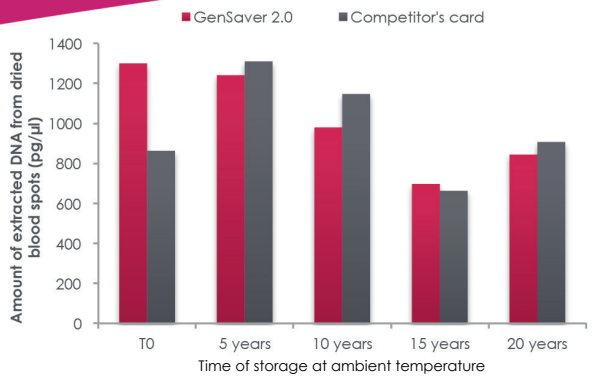

Figure 1. Amount of human genomic DNA extracted from dried blood spots stored on GenSaver™ 2.0 cards at ambient temperature

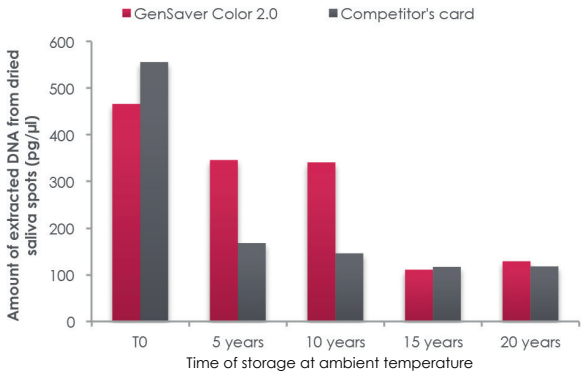

Figure 2. Amount of human genomic DNA extracted from dried saliva spots stored on GenSaver™ Color 2.0 cards at ambient temperature

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 and saliva samples collected on Ahlstrom collection cards and stored for 5,10, 15 and 20 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 saliva samples collected on GenSaver™ Color 2.0 cards. The data of Figure 3, Figure 4 and Figure 5 show that extraction and purification of DNA from Ahlstrom cards provide DNA with sufficient quantity and high quality to support allele calling accuracy as high as 100% in STR analysis, even after 10 years of storage. No sample required a reinjection for 100% accurate allele calls for these periods of time. For 15 years of storage (Figure 6), we can observe partial STR profile with one locus not validated for both cards. This can be explained by the partial degradation of the longer DNA markers under the accelerated ageing conditions. Moreover, NGS data for 15 and 20 years of storage exhibit good results (see Table 3).

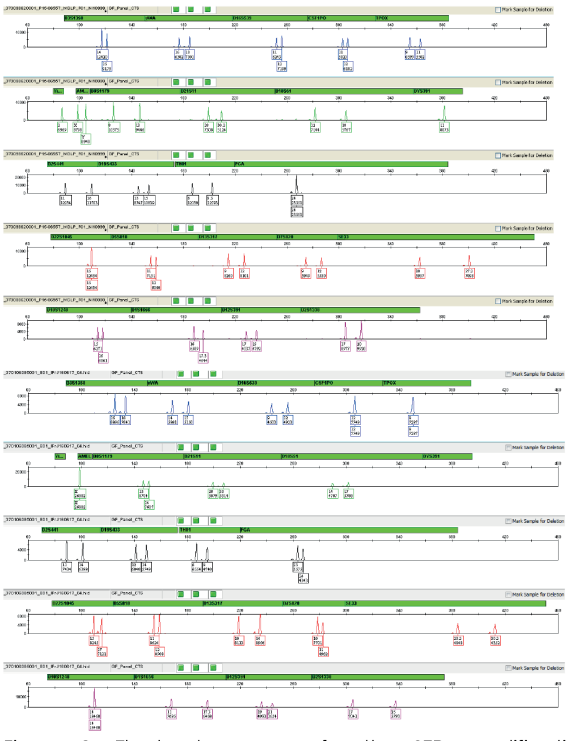

Figure 3. Electrophoregrams for the STR amplification from genomic DNA purified from blood and saliva samples spotted on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards after 24 H drying.

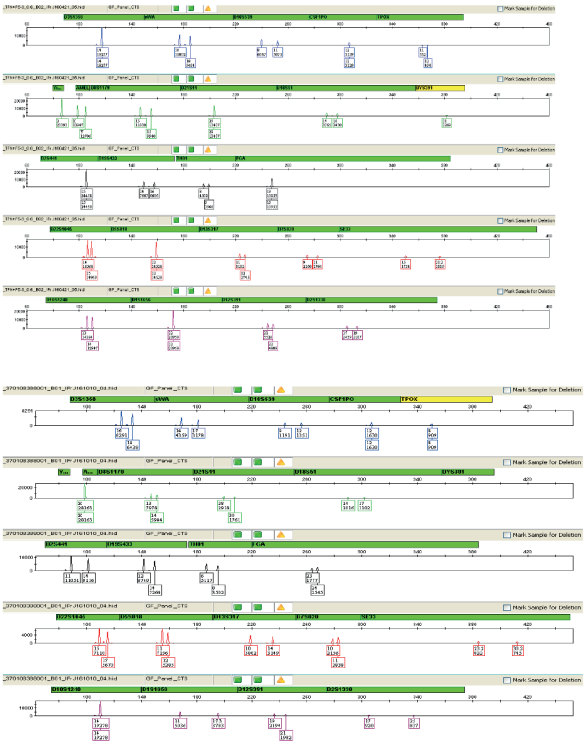

Figure 5. Electrophoregrams for the STR amplification from genomic DNA purified from blood and saliva samples spotted on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards and stored for 10 years at ambient temperature.

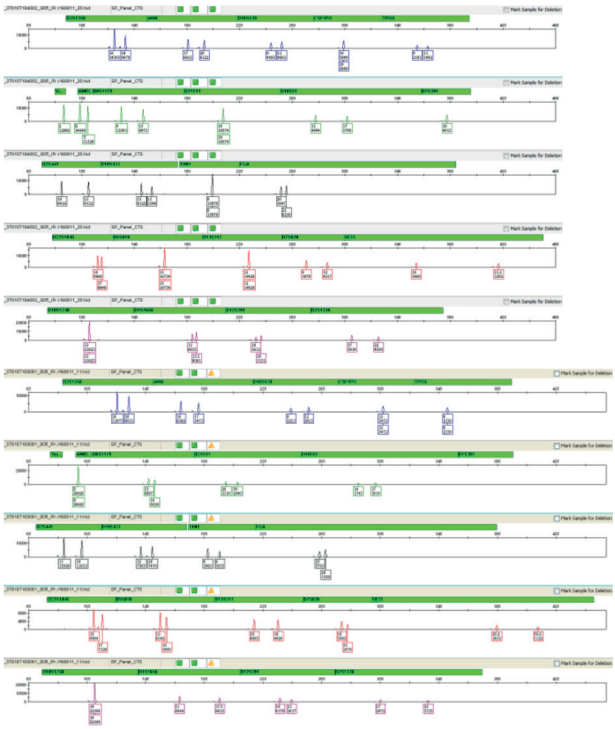

Figure 4. Electrophoregrams for the STR amplification from genomic DNA purified from blood and saliva samples spotted on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards and stored 5 years at ambient temperature.

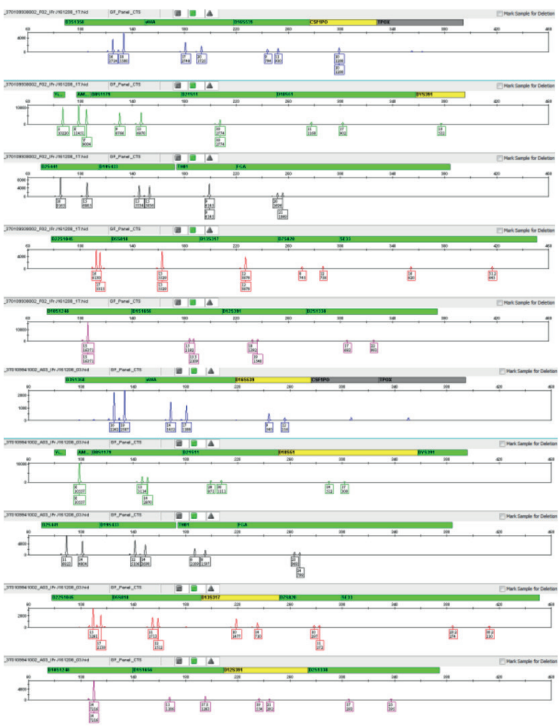

Figure 6. Electrophoregrams for the STR amplification from genomic DNA purified from blood and saliva samples spotted on GenSaver™ 2.0 and GenSaver™ Color 2.0 cards and stored for 15 years at ambient temperature.

No significant difference was observed in the intra-locus alleles, as reported in Table 2.

Ahlstrom card	Storage time	Number of validated loci /24	PHR	SD
GenSaver™ 2.0	T0	24	0.91	0.028
	5 years	24	0.78	0
	10 years	24	0.78	0.007
	15 years	23	0.84	0.014
GenSaver™ Color 2.0	T0	24	0.87	0
	5 years	24	0.77	0.028
	10 years	24	0.78	0.042
	15 years	23	0.75	0.035

Table 2

Next Generation Sequencing

NGS data were obtained from DNA purified from blood (GenSaver™ 2.0) or saliva (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 cards.

Ahlstrom card	Years of storage	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