Evaluation of extraction yield and quality of human genomic DNA from buccal cells using GenSwab™ sample collection device

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Introduction

There is a growing trend toward non-invasive, self-administered, remote collection of buccal (cheek) cells, because they are easy to collect, simple to transport at room temperature, and are a rich source of DNA for genetic testing and forensic applications. With Ahlstrom-Munksjö and Gentueri's devices for sample collection and management, samples are preserved by desiccation on a solid support at room temperature, eliminating the requirement of messy and hazardous chemicals to stabilize the sample. Further, desiccation at room temperature simplifies the shipping process because there is no longer a need for expensive shipping materials when transporting liquid samples to the lab.

In the following pages, we describe the use of GenSwab™ device, as a simple yet efficient means to collect, preserve, and transport buccal samples suitable for downstream genomic analyses without compromising DNA yield or integrity.

This study was designed to investigate the extraction yield and quality of human genomic DNA (hgDNA) from buccal cells collected with GenSwab™ sample collection device. The GenSwab™ is designed with a foam swab used to collect buccal cells from the inside of the subject's mouth. The sample is then easily transferred to a treated card, GenSaver™ Color 2.0, that can preserve the sample at room temperature – even for decades. The extracted DNA is subjected to qPCR analysis to determine the yield of DNA and if any PCR inhibitors are present.

Materials and Methods

Collection of Samples

Buccal samples were collected from individuals using GenSwab™ devices according to the instructions below:

1. Place the foam head under the tongue for 20 seconds
2. Rub the foam head against the cheek lining in a broad circular motion for 20 seconds
3. Repeat the same process on the other cheek
4. Unwrap the wrapper and fold the handle so that the foam tip contacts the pink area on the card
5. Fold the card wrapper back over the card and press firmly over the foam swab for 20 seconds to transfer the sample
6. Unwrap the swab cover and remove the handle at the perforation. Discard the swab and reclose the card
7. After collection, cards were placed in GenPouch™ for transportation to the laboratory Eurofins Medigenomix Forensik GmbH which conducted all analyses

Human genomic DNA extraction

Three punches per sample were removed with a disposable 3 mm punch device from the dried matrix spots (Fig 1).

All 3 punches were placed in a clean RNase/DNase-free 1.5 ml tube and incubated in 500 µl ATL-Mix at 56 degrees for at least one hour. The discs were removed from the ATL-buffer and the ATL-buffer was processed for extraction of hgDNA using QIAsymphony SP with the QIAsymphony® DNA Investigator® Kit (Qiagen). The extraction volume was 30 µl. The extraction solution was stored under refrigeration at 5 degrees Celsius during analyses. For each test, the relevant controls were carried out.

Figure 1: Example of sampling from dried matrix spot

Human genomic DNA detection

Quantitative real-time PCR was performed using the Investigator® Quantiplex Pro Kit (Qiagen). The DNA quantitation assay used an internal PCR control (IPC). This IPC was used to assess the levels of amplification inhibition in the samples during PCR. Standard curves for DNA quantitation were prepared using control DNA supplied with the kit. The positive controls were composed as follows: Positive control (IC_Quanti) = DNA Control 007 (from the NGM Detect Kit/ Thermo Fisher Scientific), positive control (KB_AHE1) = a dilution of DNA from the blood that was extracted again simultaneously with the samples. The kit also includes reagents for two different DNA targets (a short fragment 91bp and a longer fragment 353bp), allowing the user to assess degradation of the extracted DNA. The amplification ratio between autosomal short and long fragments summarized in the degradation index provides vital information on DNA quality/ DNA degradation, which can be correlated to performance in later PCR applications.

Results

Human genomic DNA quantitation

DNA extraction and quantitation were evaluated using 11 samples of dried buccal cells collected with the GenSwab™ device. Results are summarized in Table 1 and PCR data such as slope and R^2 are in Table 2.

Sample Name	CT Short	Quantity Short (pg/µl)	CT Large	Quantity Large (pg/µl)
GenSwab™ Lot 1006-1-030122AL1	23.46	2080.91	23.13	1710.29
GenSwab™ Lot 1006-2-030122AL2	22.87	3112.55	22.55	2572.4
GenSwab™ Lot 1006-3-030122AL3	22.63	1848.75	23.32	1501.83
GenSwab™ Lot 1006-4-030122AL4	23.87	1571.29	23.51	1310.96
GenSwab™ Lot 1006-5-030122AL5	24.26	1203.38	23.89	1007.08
GenSwab™ Lot 1005-1-20220201AL1	23.47	2072.8	23.09	1765.94
GenSwab™ Lot 1005-2-20220201AL2	23.48	2048.58	23.34	1477.44
GenSwab™ Lot 1005-3-20220201AL3	24.31	1170.13	23.87	1019.37
GenSwab™ Lot 1007-1-20220201AL4	22.96	2927.17	22.45	2746.5
GenSwab™ Lot 1007-2-20220201AL5	24.24	1225.6	23.76	1104.15
GenSwab™ Lot 1007-3-20220201AL6	24.3	1174.26	23.85	1031.57
Negative control	Undetermined	X	undetermined	X
Positive control (KB_AHE1)	25.53	508.06	24.91	492.68
Positive control (IC_Quanti)	28.53	66.2	27.72	69.03

Table 1: Cycle Threshold (CT) and amount of extracted DNA from dried buccal cells samples collected with GenSwab™

	Autosomal			Degradation		
Sample Name	Slope	R^2	PCR efficiency	Slope	R^2	PCR efficiency
GenSwab™ Lot 1006-1-to 1007-3	-3.387	0.993	97.366	-3.289	0.999	101.414

Table 2: Slope, R^2 and PCR efficiency

Results show that extraction from dried buccal cells collected with GenSwab™ device produces DNA yields sufficient for successful qPCR and sequencing analysis.

Degradation Index

The degradation index in Table 3 is the ratio between autosomal short and long fragments. The theoretical value 1 would mean that identical amounts of both PCR products were detected, and no degradation occurred. This is important to assess, as quantification of DNA does not reflect the degree of degradation. High amounts of DNA can also stem from a high quantity of very short DNA fragments which may not be very useful for sequencing applications like WGS and NGS.

Sample Name	Quantity Short (pg/µl)	Quantity Large (pg/µl)	Degradation index
GenSwab™ Lot 1006-1-030122AL1	2080.91	1710.29	1.2
GenSwab™ Lot 1006-2-030122AL2	3112.55	2572.4	1.2
GenSwab™ Lot 1006-3-030122AL3	1848.75	1501.83	1.2
GenSwab™ Lot 1006-4-030122AL4	1571.29	1310.96	1.2
GenSwab™ Lot 1006-5-030122AL5	1203.38	1007.08	1.2
GenSwab™ Lot 1005-1-20220201AL1	2072.8	1765.94	1.2
GenSwab™ Lot 1005-2-20220201AL2	2048.58	1477.44	1.4
GenSwab™ Lot 1005-3-20220201AL3	1170.13	1019.37	1.1
GenSwab™ Lot 1007-1-20220201AL4	2927.17	2746.5	1.1
GenSwab™ Lot 1007-2-20220201AL5	1225.6	1104.15	1.1
GenSwab™ Lot 1007-3-20220201AL6	1174.26	1031.57	1.1
Negative control	X	X	x
Positive control (KB_AHE1)	508.06	492.68	1.0
Positive control (IC_Quanti)	66.2	69.03	1.0

Table 3: Degradation indices of extracted DNA from dried buccal cells samples collected with GenSwab™

Inhibition effect

The Investigator® Quantiplex Pro Kit contains a balanced internal amplification control that is used to check successful amplification and identify the presence of PCR inhibitors. This heterologous amplification system is detected as a 434 bp internal positive control (IPC) in the JOE™ dye channel on an Applied Biosystems real time PCR instrument. IPC results (CT values) for GenSwab™ -batch 1006 are summarized in Table 4, and Figure 1 shows the IPC amplification curve of the tested samples and the negative control sample. All samples have the same CT values as the IPC of the negative control which contains only water.
Therefore, no inhibition was observed.

Sample Name	CT IPC
GenSwab™ Lot 1006-1-030122AL1	21.82
GenSwab™ Lot 1006-2-030122AL2	21.58
GenSwab™ Lot 1006-3-030122AL3	21.84
GenSwab™ Lot 1006-4-030122AL4	21.93
GenSwab™ Lot 1006-5-030122AL5	21.90
Negative control	21.90
Positive control (Quanti)	21.85
Positive control (KB_AHE1)	21.95

Table 4 CT values for Internal PCR Control

IPC are all valid (21<CT<22), and the CT values of the IPC are homogeneous for all samples. Compared to the negative control (CT IPC=21,90) which contains only water, the average CT of the IPC for the 5 replicates from GenSwab™ devices (Average CT: 21,81) deviates by -0.09, which is not a significant variation. Therefore, no inhibition of the IPC of each tested sample was observed during qPCR.

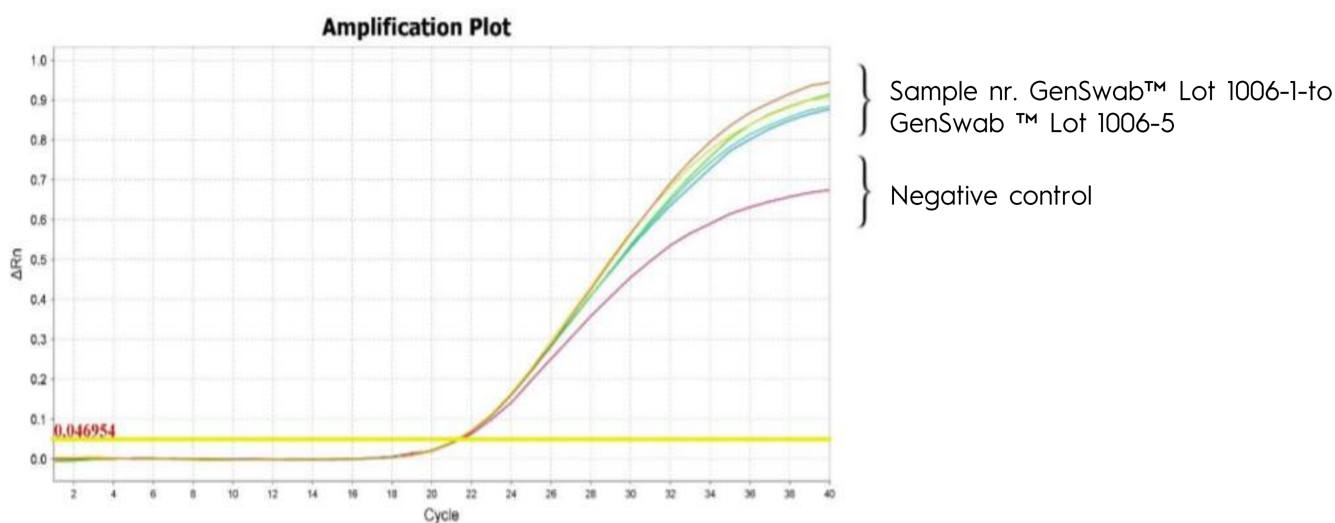

Figure 2: IPC amplification curves of tested samples and negative control

Conclusions

GenSwab™ devices are shown to be a suitable device for collection of buccal cells and should be functionally applicable to downstream methods for hgDNA analysis, including qPCR, Short Tandem Repeats (STR), and Next Generation Sequencing.
The results demonstrate substantial extraction yields and high quality hgDNA from desiccated buccal cells collected with the GenSwab™ device. The quality of DNA is highlighted by minimal degradation and the absence of PCR inhibitors.
The GenSwab™ device holds great promise for removing known barriers to remote sample collection because it is easy for users to collect samples; simple and inexpensive to ship samples to the lab; and eliminates requirement for trained medical professionals to collect samples.

For more information: diagnostics@ahlstrom-munksjo.com, info@gentueri.com

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