

Evaluation of GenSaver™ Color 2.0 for Next Generation Sequencing from buccal samples

Next generation sequencing analyzes were carried out on DNA purified from saliva samples collected on GenSaver™ Color 2.0 cards and stored at ambient temperature for 15 and 20 years.

Materials and methods

Samples collection:

Saliva samples (7µl) were collected on GenSaver™ Color 2.0 according to the manufacturer's instructions.

After collection, cards were air dried for one hour and then stored at specific conditions of temperature and humidity to simulate ageing at ambient temperature for 15 and 20 years.

Amplification:

DNA was amplified by PCR with panel Identity and the kit Precision ID Library from Life Technologies. PCR amplification were done according to supplier's instructions described in Precision ID Panels with Ion S5™ System user guide (PCR in 20µl, 1ng DNA/PCR and 21 cycles). Amplification realized on Veriti™ 96-Well Thermal Cycler from Applied Biosystems.

Libraries preparation:

Libraries were prepared manually with the kit Precision ID Library from Life Technologies according to supplier's instructions described in Precision ID Panels with Ion S5™ System user guide.

Libraries quantification:

Libraries were quantified by real time PCR and TaqMan chemistry with Ion Library TaqMan™ Quantitation kit from Life Technologies on a 7500 Real Time PCR System from Applied Biosystems. Quantifications have been made according to supplier's instructions described in Ion Library TaqMan® Quantitation kit user guide.

Template's preparation:

The preparation of the template and the loading of the chip were carried out on the device Ion Chef from Applied Biosystems with kit Ion S5™ Precision ID Chef & Sequencing according to supplier's instructions described in Precision ID Panels with Ion S5™ System user guide.

Sequencing:

Sequencing were done on Ion S5™ System from Applied Biosystems with kit Ion S5™ Precision ID Chef & Sequencing according to supplier's instructions described in Precision ID Panels with Ion S5™ System user guide.

Sequencing data analysis:

Sequencing data were analyzed with HID SNP Genotyper Plugin and Ion S5™ System-Torrent Suite™ Software 5.2.2 according to the procedure described in HID SNP Genotyper Plugin user guide.

Results:

Next Generation Sequencing Data:

Ahlstrom-Munksjö cards	Years of storage	Bases	≥ Q20	Reads	Mean Read Length
GenSaver™ Color 2.0	5	35 427 393	33 994 181 (96%)	404 997	87 bp
	10	21 049 256	18 712 156 (89%)	236 892	89 bp
	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 1: NGS data for saliva samples collected and stored on GenSaver™ Color 2.0 until 20 years at ambient temperature

Data show that the number of reads is as expected for DNA amount in the PCR reaction (≈1ng) for those samples. The sequences are of good quality since even after 20 years, 94% of the bases have a quality ≥ Q20.

Conclusion:

This study shows that high quality NGS is achievable even after long-term storage of saliva at ambient temperature on GenSaver Color 2.0 cards.