

Original Paper

A Study on the DNA Profiling from Buccal Samples on Bode Buccal® DNA Collector after Long-Term Storage

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Abstract

Bode Buccal® Collector are used for sampling collection and fit the purpose for DNA Databank. After 10 years of storage, the DNA collected with Bode still could produce full DNA profiles with the right analysis method and strategy. Factors such as humidity, sampling technique and storage play important roles in maintaining the quality of the DNA collected.

Keywords

Bode buccal® DNA collector, long-term storage, buccal samples

1. Introduction

Buccal samples are widely used as tools for collecting human DNA because they can be obtained non-invasively without any technicalities required (Muniz, McCauley, Pak, Lasarev, & Kisby, 2011). Rapid DNA profiling using buccal samples can be carried out by using direct amplification method to save on time consumption, technical inquiries and human labour. Therefore, buccal samples fit the requirements needed for DNA database purpose. However, a study conducted by Hashom, et al., 2020 revealed that after four years of samples collected, the DNA on Bode Buccal® DNA Collector have shown a slight degradation based on the quality of samples obtained.

Thus, it is suggested that the samples should be stored in a suitable storage condition to maximize the chance to obtain full DNA profile the moment biological samples arrive at the laboratory especially when DNA analysis is not immediately performed (Corradini, Alu, Galinier, & Silingardi, 2019).

One of the main concerns in the forensic DNA laboratory is to ensure the stability of sample overtime. This study evaluates the performance of the Bode Buccal® DNA Collector after long-term storage by calculating the percentage of the loci obtained and the quality of the profiles. This study also offers alternative for the laboratory to prioritise which samples to analyse first.

2. Method

2.1 Collection of Buccal samples

A total of 2,000 buccal samples were involved in this study. The buccal samples were taken during the year of collection date started from the year of 2013 until 2022 as tabulated in Table 1. The Bode Buccal® DNA Collector (Bode Cellmark Forensics Inc., Lorton, VA, USA) was used to swab inner cheek 8 times. The consent from the sample contributors were obtained. After the sample was taken, the buccal DNA collector was placed inside a paper pouch with desiccant and then stored at room temperature (~25-27°C) in storage room until it was analysed in 2022.

2.2 Analysis of Buccal samples

2 μ l Prep-n-Go™ Buffer was dispensed into Applied Biosystems™ (ABI) MicroAmp™ Optical 96-Well Reaction Plate (Thermo Fisher Scientific Inc, Waltham, MA, USA). One disc (1 x 1.2 mm diameter) from the collector was punched using BSD600 Duet Punching System (BSD Robotics, Brisbane, Australia) into the 96-well reaction plate.

PCR reaction setup and thermal cycling were performed according to the manufacturer's recommendations (Globalfiler Express PCR Amplification Kit User Guide Rev. G, n.d.). 14.5 μ l master mix of the Globalfiler Express PCR Amplification Kit (Thermo Fisher Scientific Inc, Waltham, MA, USA) was added into the 96-well reaction plate. The samples were amplified at 27 cycles using ABI GeneAmp® PCR System 9700 Thermal Cyclers (Thermo Fisher Scientific Inc, Waltham, MA, USA). Electrophoresis using ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific Inc, Waltham, MA, USA) with the recommended method and parameters by the manufacturer. Data analysis using ABI GeneMapper® ID-X Software v1.4 with the default setting for Globalfiler Express PCR Amplification Kit and peak amplitude threshold for all the dye channels were set at 150 relative fluorescence unit (RFU).

Table 1. The Total Number of Samples according to the Year of Samples were Collected

NO.	YEAR OF THE SAMPLES WERE TAKEN	TOTAL OF SAMPLES
1	2013	200
2	2014	200
3	2015	200
4	2016	200

5	2017	200
6	2018	200
7	2019	200
8	2020	200
9	2021	200
10	2022	200
TOTAL		2,000

3. Result

STR data from the samples were carefully evaluated for the presence of STR artifacts. No contamination was observed in any negative PCR control throughout the study. This study demonstrates the percentage of DNA profiling in obtaining the full DNA profile from the Bode Buccal® DNA Collector after long-term storage. The results in this study were divided into years of the samples were taken (Table 2).

Table 2. Percentage of DNA Profiling Results in Long-term Storage

YEAR OF THE SAMPLES WERE TAKEN	PERCENT OF SAMPLES OBTAINING THE FULL DNA PROFILE	PERCENT OF SAMPLES OBTAINING THE PARTIAL DNA PROFILE
2013	24%	76%
2014	32%	68%
2015	15%	85%
2016	62%	38%
2017	63.5%	36.5%
2018	64%	36%
2019	91.5%	8.5%
2020	96.5%	3.5%
2021	91%	9%
2022	88.5%	11.5%

Based on the results obtained (Table 2), samples from year 2019 until 2022 show variability in the percentages of obtaining the full DNA profiles especially samples in year 2022. From year 2013 to 2014, the percentage of obtaining full DNA profiles increase despite there were slight degradation over a year.

In year 2015, only 15% of the samples produced full DNA profiles. From 2016 onwards, the percentages of full DNA profile obtained was increasing showing that the Bode buccal collector is still able to hold the DNA sample with slight degradation. All samples in this study, were presumed to be slightly degraded and the PCR cycle used was 27. However, for such degraded samples as in Figure 2 and 3, using higher cycle number such as 28 cycles could be implemented to obtain full DNA.

4. Discussion

One of the factors that might contribute for variability in the percentages of obtaining the full DNA profiles is the amount of individual's buccal or epithelial cells deposited on the collection paper (Corradini, Alu, Galinier, & Silingardi, 2019). On average, humans shed approximately 400,000 epithelial cells a day (Menchhoff, et al., 2020). When buccal sample is properly transferred onto the Bode Buccal® DNA Collector and then correctly stored according to the manufacturer's instructions, it is possible to obtain full DNA profile. In fact, the sampling techniques might have affected the amount of cells deposited as reported by Hashom, et al., 2020. These reasons have alerted the department to improve the storage facilities and training for new staff in charged for sample collection.

The percentage of obtaining full DNA profiles increase despite slightly degradation were observed for year 2013 to 2014 samples. This might be caused by the efficient sampling technique by experienced officer in charged for sample collection. Only 15% of the samples for year 2015 produced full DNA profiles which might due to the increasing humidity and temperature in the storage room since Malaysia had been reported to experience equinox in 2015 (Irwan, et al., 2019). The climate had a slight change and the temperature in Peninsular Malaysia also increased. As a result, the samples collected on Bode was degraded faster than usual. Nonetheless, these findings showed that proper sampling technique and storage condition is important in order to reduce the degradation of the sample. These results could help the analysts to carry out sample selection based on a few information such as year and localities of the samples were taken. The analysts were able to expect the outcome of DNA profiles based on the strategy of the analysis and samples that need to be prioritised for the DNA Databank purpose (Hashom, et al., 2019). This action will reduce time consumption and expenditure for DNA analysis. Furthermore, Bode Buccal® DNA Collectors are suitable for direct amplification method and reliable for DNA recovery from buccal samples for up to 4 years after collection.

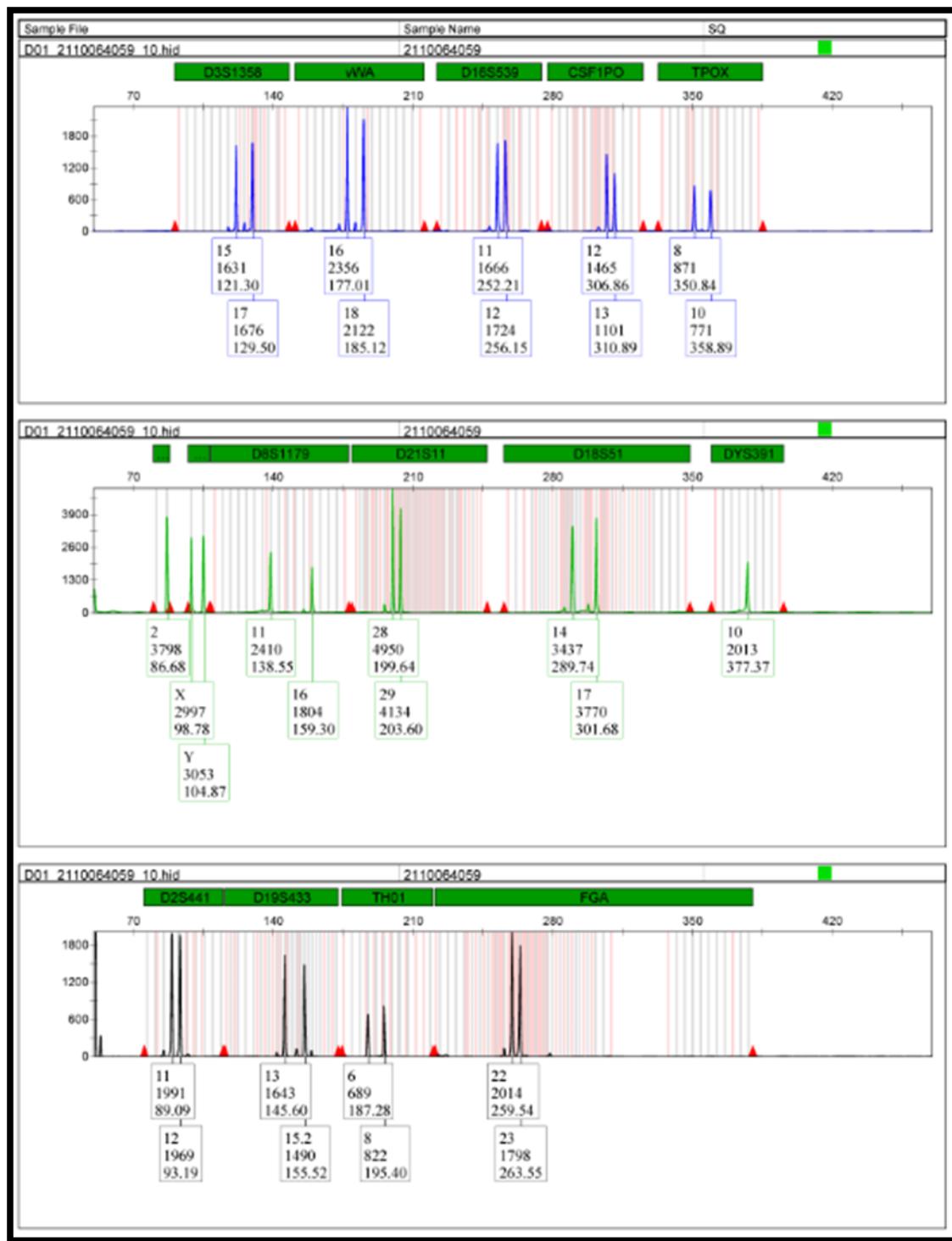


Figure 1. Electropherogram of Full DNA Profile Generated from Sample Taken on the Year of 2022

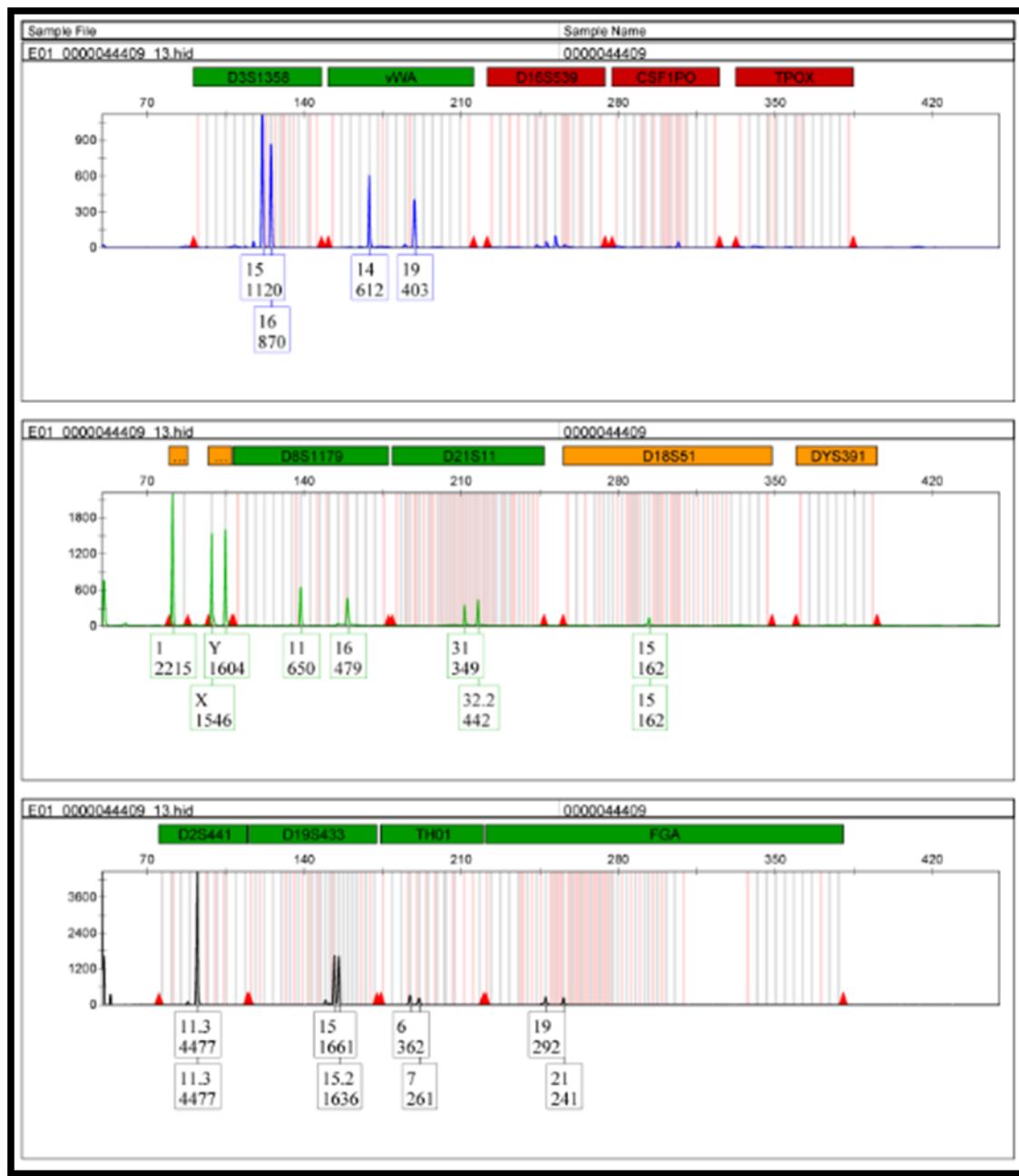


Figure 2. Electropherogram of Partial DNA Profile Generated from Sample Taken on the Year of 2013

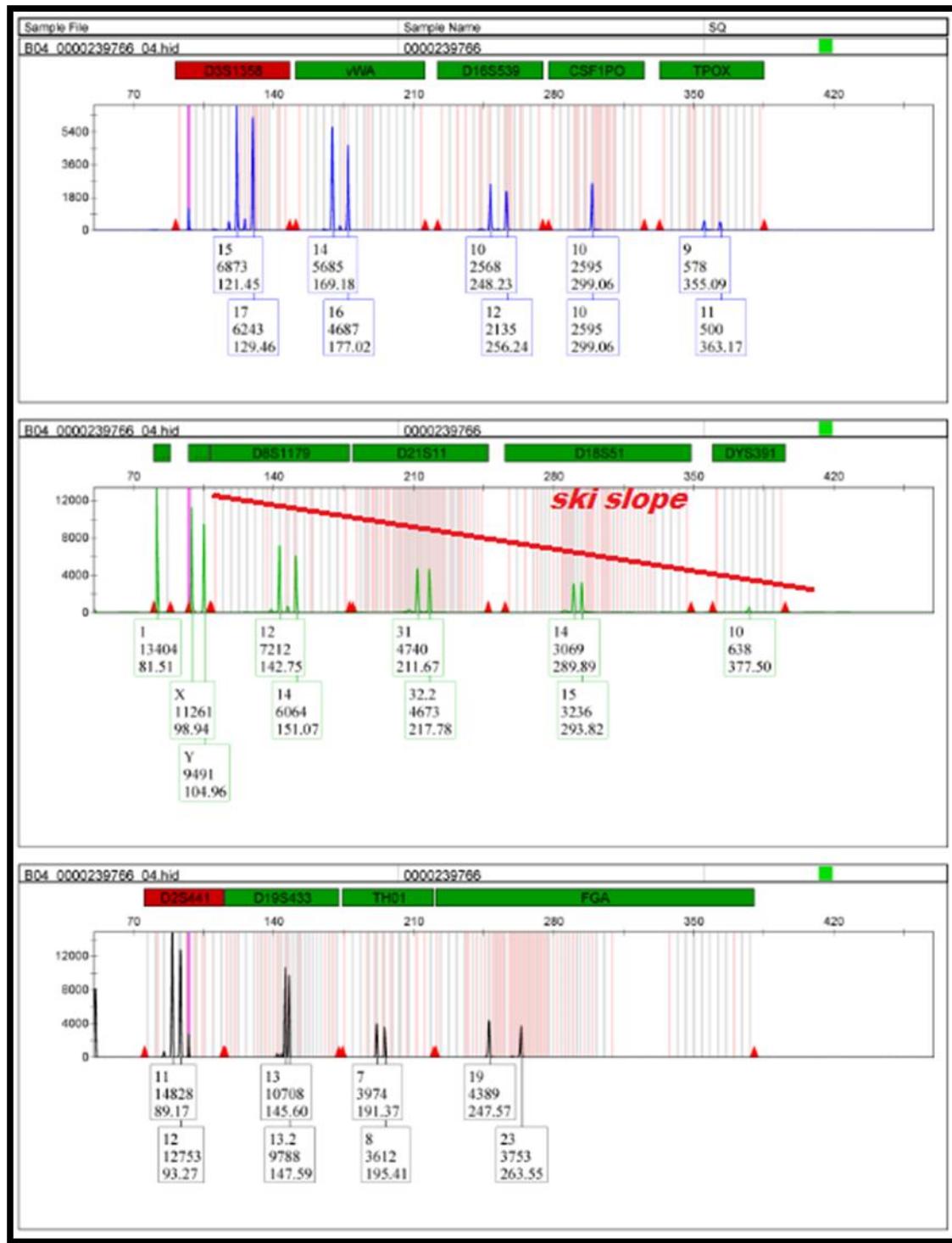


Figure 3. Electropherogram of Full DNA Profile Generated from Sample Taken on the Year of 2017 which Demonstrated Ski-slope Effect

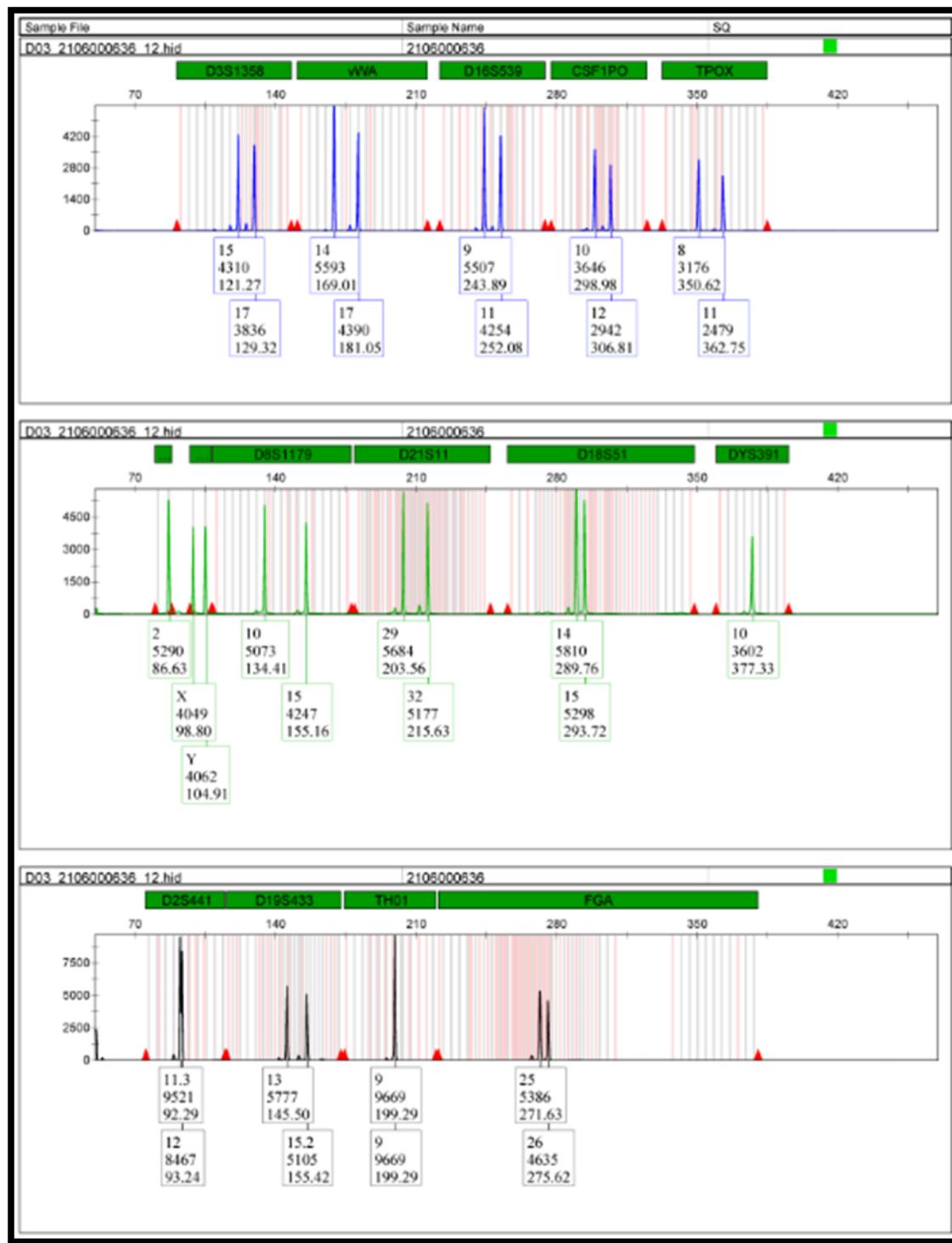


Figure 4. Electropherogram of Full DNA Profile Generated from Sample Taken on the Year of 2021 which not Demonstrated Ski-slope Effect

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