Evaluation of long-term storage effects on buccal cell DNA from untreated cards for STR profiling

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Abstract. The success of DNA profiling using long-term stored samples depends on the amount and quality of their recovered DNA templates. Physical and biochemical factors such as microbial activity, humidity and temperature contribute to DNA degradation. In this study, we used genomic DNA extracted from buccal cell samples that were stored for more than 4 years on Bode Buccal DNA Collector™ cards (Bode Technology, Virginia, USA) for typing 27 and 24 short tandem repeat (STR) loci using Powerplex® Fusion 6C system (Promega Corporation, Madison, USA) and Globalfiler™ Express kit (Thermo Fisher Scientific, USA), respectively. Our results demonstrated that the Buccal DNA Collector can be used as a collection medium for buccal swab samples that are not immediately analyzed or those that need to be retrospectively analyzed. There is a sign of DNA degradation which might well be expected because buccal cell samples were deposited on untreated filter paper and have been stored for a long period (> four years) at room temperature. However, STR allele calls were obtained from most of the buccal cell samples, especially when typed using the Powerplex® Fusion 6C system kit.
1. Introduction

A buccal swab is a common sampling procedure used to obtain genetic materials for medical research, genetic testing and DNA profiling. It is superior to other sampling protocols (e.g. arterial, venepuncture and finger prick for blood sampling and biopsy for tissues sampling) and offers a non-invasive and rapid collection of biological samples by non-medical practitioners [1-3]. Buccal cells can be collected by swabbing the inner surface of the cheek using cotton, paper or simply by spitting directly to a storage medium such as treated or non-treated cards [4-5]. It is thus a simple procedure with almost negligible medical complications [4].

Ideally, buccal swab samples need to be properly dried at room temperature or in a controlled environment or directly analyzed soon after they were collected. This is because the quantity and yield of DNA extracted from swab samples will be reduced without proper handling and storage [6-9]. However, swab samples may sometimes need to be stored for a longer period due to the high demand for laboratory testing, lack of analysts and equipment and insufficient funds for purchasing reagents and consumables. Several studies have demonstrated the long-term storage effects of buccal cell DNA collected using cotton swabs [10-12]. There is only one study on the recovery of buccal cell DNA that was stored on untreated cards (i.e. Buccal DNA Collector) at room temperature for 2 years [13]. Their study showed that the extracted DNA samples can reliably be used as templates for simultaneously genotyping of multiple (19-13 loci) short tandem repeat (STR) loci.

The objective of this study is to evaluate the long-term storage effects of buccal cell DNA that were stored for more than 4 years on Bode Buccal DNA Collector™ cards (Bode Technology, Virginia, USA) for typing of 27 and 24 STR loci using Powerplex® Fusion 6C system (Promega Corporation, Madison, USA) and GlobalFiler™ Express kit (Thermo Fisher Scientific, USA), respectively [14-17]. These samples were submitted to the DNA Databank Laboratory, Criminal Investigation Department, Royal Malaysian Police and were collected from either suspect, detainees, drug dependents, convicted or volunteers (collectively referred to as reference samples) for STR profiling. However, none of these samples had been subjected to DNA profiling earlier due to several constraints mentioned earlier.

2. Materials and Methods

This work was conducted as part of ISO/IEC 17025:2005 quality assurance assessment for DNA Databank Division (D13), Royal Malaysia Police Forensic Laboratory (RMPFL) Cheras Selangor, Malaysia. Experimental works were carried out under the Malaysian DNA Identification Act 2009 and DNA Identification Regulations Act 2012 and were approved by the Forensic DNA Databank Laboratory (D13), Criminal Investigation Department Royal Malaysia Police (RMP).

2.1. Sample preparation

This survey involved thirty-nine buccal swab samples (S01-S39) collected in 2015. These samples were taken by gentle scrape on the inner surface of the cheek using Bode Buccal DNA Collector™ (Bode Technology, Virginia, USA) filter paper. The filter paper was then allowed to dry and stored at room temperature.

2.2. STR amplification using PowerPlex® Fusion 6C kit (PPF6C) and fragment separation

Buccal cell samples on Bode Buccal DNA Collector™ were punched (1.2mm diameter) using BSD600 DUET (Microelectronic Systems Pty Ltd, Australia) and loaded into 96 well plates for STR amplification. Then, 10 μl of PunchSolution™ Reagent (Promega Corporation, Madison, WI) was added into each well [18]. The plate was then incubated for 30 minutes at 70°C. The treated samples were then mixed with PCR reaction mixtures included in the PPF6C kit (Promega Corporation, Madison, USA) and amplification reactions were performed on a GeneAmp® PCR System 9700 Thermal Cycler (Life Technologies, Foster City, CA) with a gold-plated sample block and max ramp speed mode set as described in the PPF6C Technical Manual [18] with slight modifications to the thermal cycling parameters; 96°C for 1 min; 25 cycles for 96°C for 10 s and 60°C for 1 min, followed by a 60°C final extension for 10 min. The 2800M Control DNA (Promega Corporation, Madison, USA) and PCR reaction mix without DNA template were also added in each PCR run which acts as quality control and negative control, respectively. The STR specific amplicons, allelic ladder and internal Size Standard dye of 600 LIZ™ v2 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) were fractionated using capillary electrophoresis in an ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer’s guidelines.
and as described earlier [14-16]. GeneMapper® ID-X software version 1.4 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used for determining STR allele calls.

2.3. STR amplification using Globalfiler™ Express kit (GFE) and fragment separation
One punch of 1.2mm diameter per sample was treated with 3 μl of Prep-n-Go™ Lysis Buffer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at room temperature [19]. To the lysate was then added reaction mixtures included in the GFE kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and PCR was performed on a GeneAmp® PCR System 9700 Thermal Cycler (Life Technologies, Foster City, CA) with a gold-plated sample block and max ramp speed mode. Quality assurance and negative control were set-ups as described in sub-section 2.2. The PCR conditions were as follows; 95°C for 1 min; 27 cycles for 94°C for 3s and 60°C for the 30s, followed by a 60°C final extension for 8 min and final hold at 4°C. STR amplification product separation and determination of STR allele calls were as described in sub-section 2.2.

2.4. STR data analysis
Analytical threshold (AT) and stochastic threshold (ST) for PPF6C were set at 80 RFU and 180 RFU while the AT and ST for GFE were set at 150 RFU and 300 RFU, respectively. The AT and ST data from Penta D, Penta E, DYS576, and DYS570 in PPF6C panel and AT and ST data for Y-indel from the GFE panel were excluded because only overlap AT and ST data from both STR kits were compared.

3. Results and Discussion
Good preservation of genetic materials is crucial for genomic analysis. In forensic work, collected biological specimens may be re-analyzed when a new molecular method is made available for DNA profiling or new leads are forwarded for unsolved crime cases (commonly known as cold cases). Therefore, any collected biological evidence should be properly preserved as a storage medium, duration and condition influence the success of DNA profiling of old samples [20-22].

Here we report the effects of long term storage of 39 buccal swab samples collected using Bode Buccal DNA Collector™ (Bode Technology, Virginia, USA). The samples were kept at room temperature for over 4 years and their quality for DNA profiling was assessed by direct amplification using the Powerplex® Fusion 6C system (Promega Corporation, Madison, USA) and Globalfiler™ Express kit (Thermo Fisher Scientific, USA). A full STR profile was obtained for 30 samples and 22 samples using, PPF6C and GFE kits, respectively. Among these, full STR profiles were generated for 20 samples using both kits and were labeled as S01-S20. Partial STR profiles obtained either using PPF6C or GFE kits were labeled as S21-S39.

Overall, higher numbers of STR allele calls were obtained using PPF6C than the GFE kit, including those samples that were partially amplified. These observations are correlated with average peak height (APH; Table 1a and 1b) and average peak height ratio (PHR: Figures 1a and 1b) which are higher for the PPF6C kit.

Table 1a. Average peak height (RFU) of 20 samples with full STR profiles obtained using PP6CF and GFE kits.

| Sample ID | Average peak height (RFU) |
|-----------|--------------------------|
|           | PP6CF        | GFE         |
| S01       | 5759         | 5417        |
| S02       | 2683         | 4805        |
| S03       | 4950         | 5094        |
| S04       | 2937         | 3104        |
| S05       | 3816         | 3522        |
| S06       | 8531         | 5288        |
| S07       | 9761         | 9516        |
| S08       | 11200        | 5867        |
| S09       | 5248         | 6356        |
| S10       | 6434         | 8180        |
| S11       | 4645         | 6881        |
| S12       | 3303         | 5056        |
Table 1b. Average peak height (RFU) of 19 samples with partial STR profiles obtained using PP6CF and GFE kits.

| Sample ID | Average peak height (RFU) |
|-----------|---------------------------|
|           | PP6CF | GFE  |
| S21       | 3109  | 4675 |
| S22       | 2092  | 1571 |
| S23       | 1234  | 1566 |
| S24       | 3183  | 9306 |
| S25       | 8815  | 8048 |
| S26       | 1720  | 3866 |
| S27       | 2209  | 934  |
| S28       | 3829  | 1068 |
| S29       | 4378  | 5064 |
| S30       | 2408  | 1764 |
| S31       | 12546 | 5634 |
| S32       | 3997  | 2590 |
| S33       | 5449  | 2606 |
| S34       | 3248  | 789  |
| S35       | 3739  | 1975 |
| S36       | 3299  | 789  |
| S37       | 4891  | 2824 |
| S38       | 3160  | 2975 |
| S39       | 3444  | 5602 |
| Mean (x̅) | 4039  | 3350 |

Figure 1a. Average peak height ratio (PHR) of 20 samples with full STR profiles (23 loci) obtained using PP6CF and GFE kits. x̅; the mean value of average PHR.
Five samples showed discordant allele calls between PPF6C and GFE kits; sample S23 at locus CSF1PO and samples S25, S27, S28 and S29 at locus TPOX. The CSF1PO locus for sample S23 was called as homozygous (allele 11 with 366 RFU) using GFE, but allele 10 and 11 with 411 RFU and 355 RFU (respectively) were called using the PPF6C kit. Sample S25, S27, S28 and S29 were found to be heterozygous and homozygous using PPF6C and GFE kits, respectively. These discordant results might be due to primer mismatch that causes the null allele or allele dropout that has been previously reported for the GFE kit [23-27]. Another possibility is that both CSF1PO and TPOX are larger-size STR sequences (300bp-4500bp) and any alleles from these loci are possibly the first to drop out when amplifying degraded DNA samples [7].

![Figure 1b. Average peak height ratio (PHR) of 19 samples with partial STR profiles obtained using PPF6C and GFE kits.](image)

It is highly recommended to swab the filter paper more than twice to get a sufficient amount of DNA (i.e. buccal cells) for downstream analysis. Besides, a larger amount of DNA was found at the filter tip than the area near the handle of the Buccal DNA Collector [13]. In this study, the samples were randomly punched from Buccal DNA Collector and it is unlikely that the number of swipes and punch area contribute systematically to a higher number of STR allele calls for PPF6C than those generated using GFE. The PPF6C allows maximum discrimination with higher numbers of loci and works well with low amounts of DNA and inhibitor-laden samples [28]. Our results thus further support the general reliability of PPF6C kits for STR genotyping of long term stored buccal swab specimens.

Overall, our results demonstrated that the Buccal DNA Collector can be used as a collection medium for the long storage of genetic materials. In particular, full STR profiles were obtained for many samples using both kits and variations in the number of STR allele calls between kits might be due to primer design and sample degradation as discussed above. The latter is evident by the failure to obtain STR allele calls for CSF1PO, TPOX, and D22S1045, for which PCR products often are larger than for other STR loci. The Bode Buccal DNA Collector™ also has several other advantages from an operational perspective as it cheaply and samples can be stored at room temperature. In the long run, the use of the Buccal DNA Collector is a DNA storage medium that can save cost (purchasing of freezer and bill for power consumption) and laboratory space.

4. Conclusion
Overall, our study demonstrated that Bode Buccal DNA Collector™ can be used as a sampling method and storage medium for the long term preservation of genetic materials. There few signs of slight DNA degradation which is might well expect because buccal cell samples were deposited on untreated filter
paper and stored for a long period at room temperature. However, STR allele calls were obtained from most of the buccal cell samples, especially when typed using the PPF6C kit.

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