ISSN: 2768-0126

Volume 1 Issue 2
www.escientificlibrary.com

ES Journal of Dental Sciences

Reduced volume for direct PCR amplification of buccal samples on Bode Technology™ Buccal DNA Collector™

Research article

S.I. Afifah*, T.Ngat-Ngoh, A. R. Mahaya, A. M. Rashid, N.M.L. Hazirah, R.M.D. Hanim and A.P. Victor

DNA Databank Division, Criminal Investigation Department, Royal Malaysia Police, Malaysia

Received: Jan 28, 2020; **Accepted:** Feb 27, 2020; **Published:** Mar 04, 2020

***Corresponding author:** Siti Afifah Ismail, DNA Databank Division, Criminal Investigation Department, Royal Malaysia Police, Malaysia

Abstract

Bode Technology™ Buccal DNA Collector™ designed to be easy to use and to store the sample. The main objective of this evaluation is to optimize a reduced volume PCR amplification for STR analysis of reference buccal samples collected on Bode Technology™ Buccal DNA Collector™. A total number of 21 buccal samples collected on Bode Technology™ Buccal DNA Collector™ were used for this evaluation. A 1.2 mm disc was punched from the samples into a 96-well plate using BSD600-Duet Punching System. The samples were amplified using 14.5 µl, 10 µl and 8 µl volume of Globalfiler™ Express amplification mix at 25 cycles with a GeneAmp PCR System 9700. The electrphoresis of PCR products was carried out on ABI 3500xL Genetic Analyzer. An electrophoresis master mix was prepared for each sample using 9.5 µl of Hi-Di™ formamide and 0.5 µlGeneScan™ 600 LIZ® size standard. After that, 1 µl of PCR product was used from each sample. Data analysis using GeneMapper*ID-X* Software v1.4. Correct profiles were observed for all samples. One sample produced no profile when using 8 µL of amplification master mix. No partial profiles were observed when amplified with 8 µL, 10 µL and 14.5 µL of the amplification mix. All amplification mix volumes showed off-scale results for several samples. Reducing the amplification mix volume to 8 µl for PCR amplification using Globalfiler™ Express assay, resulted in a first-pass success rate of 95.2%. This was slightly lower than 10 µl and the full volume amplification mix. First-pass success rate with 10 µl of amplification mix for direct amplification achieved 100%. The volume using 10 µl amplification mix was found to be reliable to generate full profile. This approach can reduce the cost of DNA profiling of buccal samples on Buccal DNA Collector™ using Globalfiler™ Express assay.

Keywords:

Direct PCR amplification,buccal samples, Buccal DNA Collector.

Introduction

Direct PCR amplification is common method used for analysing DNA database samples or also known as reference samples. This method only requires simple steps and takes less than 2 hours to get a DNA profile from one sample [1]. Buccal or blood samples are types of sample that will be collected as reference sample especially for DNA database. Bode Technology™ Buccal DNA Collector™ designed to be easy to use and to store the sample because

it only involves one step of sample transfer. After that, the DNA sample on the collection paper of the Buccal DNA Collector™ ready for analysis. The main objective of this evaluation is to optimize a reduced volume PCR amplification for STR analysis of buccal samples collected on the Buccal DNA Collector™. Indirectly, this approach can reduce the cost of DNA profiling of buccal samples on Buccal DNA Collector™using Globalfiler™ Express assay [2].

Materials and methods

A total of 21 buccal samples on Bode Technology™ Buccal DNA Collector™ were used in this evaluation. 2 µL of Prep-n-Go™ Buffer was added to the samples and to the negative control. A 1.2 mm disc was punched from the samples into a 96-well plate using BSD600-Duet Punching System for three times (for three different volumes of amplification mix). Three cleaning punches were punched between each sample. The amplification mix with Master Mix, Primer Set, and PCR Enhancer was prepared as per the manufacturer protocol [3]. Volumes of 8 µL, 10 µL and 14.5 µL (full volume) of the amplification mix were dispensed to each well containing a sample or control.The samples were amplified for 25 cycles with a GeneAmp PCR System 9700. The electrphoresis of PCR products was carried out on ABI 3500xL Genetic Analyzer. A master mix was prepared for each sample using 9.5 µl of Hi-Di™ formamide and 0.5 µlGeneScan™ 600 LIZ® size standard. After that, 1 µl of PCR product was used from each sample. Data analysis using GeneMapper*ID-X* Software v1.4.

Results

Correct profiles were observed for all samples. One sample produced no profile when using 8 µL of amplification master mix. . No partial profiles were observed when amplified with 8 µL, 10 µL and 14.5 µL of the amplification mix (Table 1). All amplification mix volumes showed off-scale results for severalsamples. For example, BUB1 showed off-scale result when using 10 µL of amplification mix but showed on-scale results when using 8 µL and 14.5 µL of amplification mix which indicating some variability in the DNA content within the individual 1.2 mm punches (Table 2).

Table 1: summarizes the results from the buccal samples

Cycles / Volume	No profiles	Partial profiles	Full, on-scale profiles	Off-scale profiles
25 cycles, 8 µL	4.8% (1 sample)	0%	76.2% (16 samples)	19.0% (4 samples)
25 cycles, 10 µL	0%	0%	71.4% (15 samples)	28.6% (6 samples)
25 cycles, 14.5 µL (full volume)	0%	0%	71.4% (15 samples)	28.6% (6 samples)

Table 2: List of samples (BUB= Buccal sample on Buccal DNA Collector™,* = no profile, ** = Off-scale).

25 cycles, 8 µL	25 cycles, 10 µL	25 cycles, 14.5 µL
BUB1	**BUB1****	BUB1
BUB2	**BUB2****	**BUB2****
BUB3	BUB3	BUB3
BUB4	BUB4	BUB4
BUB5**	**BUB5****	BUB5
BUB6**	**BUB6****	BUB6
BUB7	BUB7	BUB7
BUB8	BUB8	BUB8
BUB9	BUB9	BUB9
BUB10	BUB10	BUB10
BUB11**	**BUB11****	**BUB11****
BUB12	BUB12	BUB12
BUB13**	BUB13	**BUB13****
BUB14	BUB14	BUB14
BUB15	BUB15	BUB15
BUB16	BUB16	**BUB16****
BUB17	BUB17	BUB17
BUB18	**BUB18****	**BUB18****
BUB19	BUB19	BUB19
BUB20	BUB20	**BUB20****
BUB21*	BUB21	BUB21

Discussions

Reducing the amplification mix volume to 8 µl for PCR amplification using Globalfiler™ Express assay, resulted in a first-pass success rate of 95.2%. This was slightly lower than 10 µl and the full volume amplification mix (i.e. 14.5µl). The 1.2 mm disc with 10 µl of amplification mix for direct amplification achieved 100% same as when using full volume of amplification mix.

Figure 1 demonstrates inter-locus peak height balance of the buccal sample heterozygous peak heights for 10 µL of amplification mix. Values were similar across dye channels as size of the amplicon increased. Some exceptions are not unexpected; the samples may contain PCR inhibitors that could suppress peak heights for larger loci, depending on the nature of the inhibition. These samples did not undergo

extraction or a clean-up procedure prior to amplification. Peak height ratio (PHR) data for 10 µL of amplification mix was calculated and is summarised in Figure 2. All peak height ratios for all samples were above 60% and were generally well-balanced.

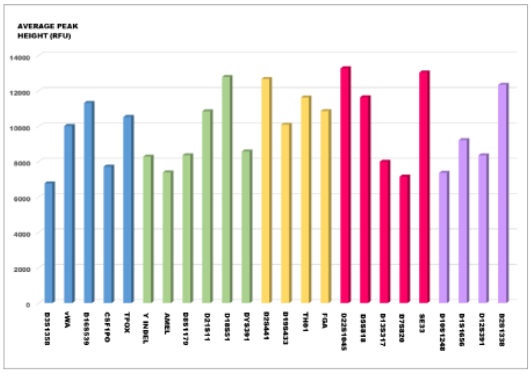

Figure 1: Inter-locus balance by sample for buccal samples amplified for 25 cycles, 10 µL amplification mix.

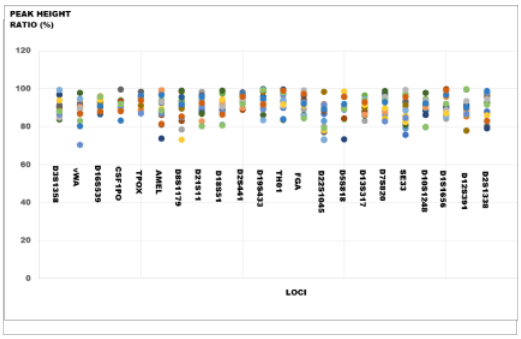

Figure 2: Peak height ratios for buccal samples amplified for25 cycles, 10 µL amplification mix.amplified for 25 cycles, 10 µL amplification mix.

Conclusion

Fully concordant genotypes were obtained from 21 of the buccal samples. The results of this evaluation demonstrated that the 10 µl amplification mix produced results similar to those obtained when samples were amplified with 14.5 µL amplificationmix.The volume using 10 µl amplification mix was found to be reliable to produce full profile.

Acknowledgement

We would like to thank all the officers in DNA Databank Division, Criminal Investigation Department, Royal Malaysia Police for their supports of this project.

References

1. M.P. Garcia-Palacios, J. Martínez-Gómez, C. Gomes, S. Palomo-Díez, E. Arroyo-Pardo, V. Esteban-Ramos, A new strategy for a "direct" amplification of forensic samples, Forensic Science International: Genetics Supplement Series 6 (2017) e560–e561.
2. R. Almheiri, R. Alghafri. Reduced volume for direct PCR amplification of blood reference samples using Identifiler® Direct and Globalfiler™ Express assays,Forensic Science International: Genetics Supplement Series 6 (2017) e340–e341.
3. Technical Note: Alternate GlobalFiler™ Express PCR Amplification Kit Protocol: Bode Buccal DNA Collector™ samples using GeneMapper™ ID-X, Thermo Fisher Scientific Inc., 2016.