J Med Sci 2010;30(4):149-154
http://jms.ndmctsgh.edu.tw/3004149.pdf

Optimal DNA Extraction from Buccal Swab Samples

Tsung-Hsing Cheng[1], Sheng-Pyng Chen[1], Tzu-Chuan Lu[2], Wen-Chi Chen[3], Jenn-Shing Sher[3], and Yi-Shing Shieh[4,5*]

[1]Division of Family Medicine; [2]Department of Surgery; [3]Songshan Armed Force General Hospital, Taipei; [4]Department of Dentistry, Tri-Service General Hospital, National Defense Medical Center, Taipei; [5]School of Dentistry, National Defense Medical Center, Taipei, Taiwan, Republic of China

Background: DNA of high quality and large quantity obtained from buccal cells is important for accurate analysis. The present study is conducted to examine quality and purity of DNA extracted by three commonly used methods and to determine the influence of process time on PCR amplification. **Materials and methods:** The DNA concentration was determined at 260 nm by a spectrophotometer. The ratio of 260/280 was measured to evaluate DNA purity. The collected buccal samples were subjected to DNA extraction immediately or after storage at 4°C for 3 days. The adequacy of the buccal DNA extracts for PCR-based assays was assessed by amplifying three fragments of the specific gene in different sizes (1300 bp, 581 bp, and 300 bp). **Results:** The mean DNA concentration at 260 nm was found to be 1.5, 1.4, and1.1 μg/ml for phenol-chloroform, QIAamp kit, and NaOH methods, respectively. The DNA purity were 2.1, 2.0, and 2.4 for phenol-chloroform, QIAamp kit, and NaOH methods, respectively. The NaOH method yielded the lowest concentration and purity of DNA when compared individually with the other two methods, while there was no statistical difference between the phenol-chloroform and the QIAamp kit methods. Different band patterns were observed in the agarose depending on whether the samples were processed immediately or with delay. Some degree of degradation in the DNA band was noted when there was delay in sample processing. However, whether processed immediately or with delay, the samples can successfully amplify PCR products up to 1300 bps. **Conclusion:** Our results indicated that buccal cell DNA samples can provide precise estimates of human amplifiable DNA. The DNA isolated from buccal cells under appropriate storage can be successfully used in PCR-based assays.

Key words: buccal cells, DNA extraction, oral cavity

INTRODUCTION

Research in molecular biology has increased the need for understanding the genetic basis of diseases which require DNA isolation. The commonly used method in a wide variety of genetic studies is to obtain genomic DNA from nucleated cells of peripheral blood.[1] However, such approach has some disadvantages. For example, it is invasive and confers the most discomfort to study subjects. Urines also provide DNA but with variable yields and contain PCR inhibitor. Buccal cell samples provide an alternative noninvasive and more easily collected source of DNA that is readily accepted by patients.[2] Recent findings also demonstrated that buccal samples stored for a long time yield sufficient dsDNA concentration to achieve high genotyping call rates and concordance with stored blood samples for genotyping study.[3] In addition, for studies of smoking- or betel-related diseases such as oral cancer, DNA isolated from oral buccal cells may also be informative on potential somatic alterations because the oral cavity may have a high exposure of these chemicals and buccal cells may demonstrate early genetic alterations such as methylation of certain genes. Thus, given high-quality collection and storage protocols, it is possible to use stored buccal cell samples for genome-wide association studies.

Many methods for collecting buccal cell samples have been described, including dry procedures using a buccal swab or other implements for scraping the oral mucosa, and wet procedures that involve swishing liquids in the mouth and spitting into a collection vessel. Successful application of samples obtained by both procedures to

Received: November 13, 2009; Revised: April 7, 2010; Accepted: May 5, 2010
*Corresponding author: Yi-Shing Shieh, School of Dentistry, National Defense Medical Center, P.O. Box 90048-503, Taipei, Taiwan, Republic of China. Tel: +886-2-87923148; Fax: +886-2-87919276 or +886-2-87927147; E-mail: ndmcyss@nhri.org.tw

PCR had been reported.[4-7] There are some advantages and limitations associated with each method of collection. The swish method appears to give higher average DNA yield and longer DNA fragments (4, 5). Nevertheless, this procedure requires liquid handling and centrifugation steps during sample processing, which involves more steps, is more cumbersome, and incurs higher cost. On the other hand, dry cheek collection, such as the buccal swab method, involves simple assembly for large-scale mailings, light-weight postage, as well as efficient and cost-effective processing for long-term archiving. Furthermore, buccal swab sample collection is a simple, relatively inexpensive, highly sensitive, risk-free method for screening suspected cells in oral mucosa, which can be easily used in any location, cause minimum trauma and provide an adequate and representative number of epithelial cells.8 Therefore, it is necessary to take advantages of both wet and dry methods to obtain DNA of high quality and large quantity for clinical interests.

Protocols for DNA extraction include the use of chemicals such as phenol-chloroform, NaOH, or already prepared kits containing all the implements and a manual with step-by-step instruction for extracting DNA. However, due to practical and methodological reasons, it is necessary to compare the yield and purity of DNA obtained by different methods. Determining the optimal conditions was important to us because we intended to use a buccal swab protocol to collect buccal cell samples through regular screening procedure for suspected oral lesions. We conducted this study to determine how to maximize the amount and quality of hDNA that could be collected from buccal cells using a buccal swab collection protocol. In addition, we determine whether sample processing alters the quality of PCR-base applications.

METHODS

Sample collection and processing

In this study, 15 healthy volunteers were recruited (age range, 22-35). The subjects abstained from smoking, drinking, and/or eating for 45 min before sample collection. Participants were asked to rinse their mouth with tap water 30 s before sampling. Collection of DNA using cotton swabs was performed. For each individual, both sides of buccal mucosa were twirled by swab for 15 seconds and a total of 30 samples were collected. The samples from the same individual were separated into different groups. All samples were divided into three groups, with each group containing 10 buccal samples of DNA extracted using phenol-chloroform, NaOH, and the commercial kit. Among the volunteers, 10 had their samples collected again one week later. A total of 20 samples were obtained and divided into two groups to test the effect of sample processing on DNA quality. Two sample processing conditions, with and without delay, were examined. Sample processing with no delay refers to immediate DNA extraction after sample collection while that with delay denotes DNA extraction after 3 day-storage at 4°C.

DNA extraction

NaOH protocol. The protocol was carried as described previously.[9] Briefly, the swabs were separated from the sticks and the brush section of the swab was placed inside a 1.5-mL microcentrifuge tube, and 300 microliters of NaOH 50 mM were added to each tube. The tubes were closed and vortexed for 10 sec. They were placed in a thermomixer for 5 min at 95°C. Then, the swabs were removed and discarded. Thirty microliters of 1 M Tris HCl, pH = 8.0 were added to each tube. The tubes were centrifuged at 13,000 rpm for 2 min. The supernatant (DNA) in each tube was used for analysis.

Phenol-chloroform protocol. This method was modified according to a previous report.[10] Briefly, to digest the tissue sample, the pellet was resuspended in 200 μl of digestion buffer (100 mM Tris-Cl (pH 8.0), 5 mM EDTA (pH 8.0), 1% SDS) containing freshly thawed proteinase K (500 μg/ml). The sample was then incubated at 55°C in a water bath overnight. After incubation, DNA was isolated with an equal volume of Tris-saturated phenol-chloroform-isoamylalcohol solution (25:24:1) and precipitated with two volumes of ice-cold absolute ethanol. The sample was placed in the freezer (-20°C) for at least 1 h and then centrifuged at 14,000 rpm for 20 min. The supernatant was carefully removed without touching the pellet or the area where the pellet was expected to be. The pellet was washed by adding 1 ml of 70% ethanol and then centrifuged for 15 min at 14,000 rpm. The supernatant was then carefully removed, and washed again with absolute ethanol. The sample was centrifuged again for 15 min at 14,000 rpm. Finally, the DNA was resuspended in 100 μl of distilled water and stored at -20°C.

Commercial kit. This protocol was carried out according to a previous report and the manufacturer instructions (QIAamp DNA blood MiniKit, Quagen).[2] Before extraction, the brush handle was removed. The period of incubation with protease was increased to 30 min. After incubation, the brush was transferred to a 1000 μl sterile Eppendorf tip, and centrifuged in a sterile 15-ml conical polypropylene tube to increase recovery of DNA from

Table 1 Total DNA yields and quality by methods of DNA extraction

		Spectrophotometry			
		Total DNA yield (ug/ml)		A260 : A280	
Methods	N	Mean±SD	Range	Mean±SD	Range
Ph/Ch	10	1.5±0.3	1.0-1.9	2.1±0.2	1.8-2.5
Kit	10	1.4±0.3	1.0-1.8	2.0±0.2	1.8-2.4
NaOH	10	1.1±0.2	0.8-1.5	2.4±0.2	2.1-2.6

Ph/Ch, phenol/chloroform method; Kit, QIAamp kit; NaOH, NaOH method for DNA extraction

the spin columns. The final volume was 150 μl.

Concentration and purity determination

Spectrophotometric determination of the amount and purity of DNA was conducted. Readings of the absorbance were taken at wavelengths of 260 and 280 nm. The reading at 260 nm was employed to calculate concentration (yield). The ratio of the readings at 260 and 280 nm (OD260/OD280) provides an estimate of DNA purity.

DNA integrity

The integrity of genomic DNA was assessed by resolving DNA extracts on a 1% agarose gel by electrophoresis, followed by visualization with eithidium bormide staining. Each DNA sample was graded according to the electrophoretic migration of sample DNA in comparison to a known molecular weight marker.

PCR-based assays

The adequacy of the buccal DNA extracts for PCR-based assays was assessed by amplifying three fragments of the glutamine-fructose-6-phosphate transaminase 1 in different sizes (GFPT1; 300 bp, 581 bp, and 1300 bp) as described by Daniel et al.[11] The PCR primers used were 300 bp, sense 5'- CACTGTTTGCTTCAGCTATGC -3' and antisense 5'- CTGGGGTCTTTTGAGGTCAC -3'; 581 bp, sense 5'- CACTGTTTGCTTCAGCTATGC -3' and antisense 5'- AGGAACTTTAAAGCATGACAATC -3'; 1300 bp; sense 5'-TTGGTTTTTCTTAGCAAATTCCTT-3', antisense 5'-AATTGTTCCGTCAAAATGCC-3'. PCR reactions were run with PCR Master Mix (Promega), which comprised 30 cycles of 94°C×30 s, 55°C×30 s, and 72°C×1 min, followed by 5 min at 72°C. PCR products were analyzed by 1.0% agarose gel electrophoresis, visualized with ethidium bromide, and then photographed. Images were saved as TIFF files and then analyzed with ImageJ (http://rsb.info.nih.gov/ij/). Signal intensities of the PCR data were quantified from TIFF images using ImageJ. Each PCR experiment was repeated at least three times, and representative results are shown. Means and standard deviations (SDs) were calculated from the signal intensities of all examined samples in each group.

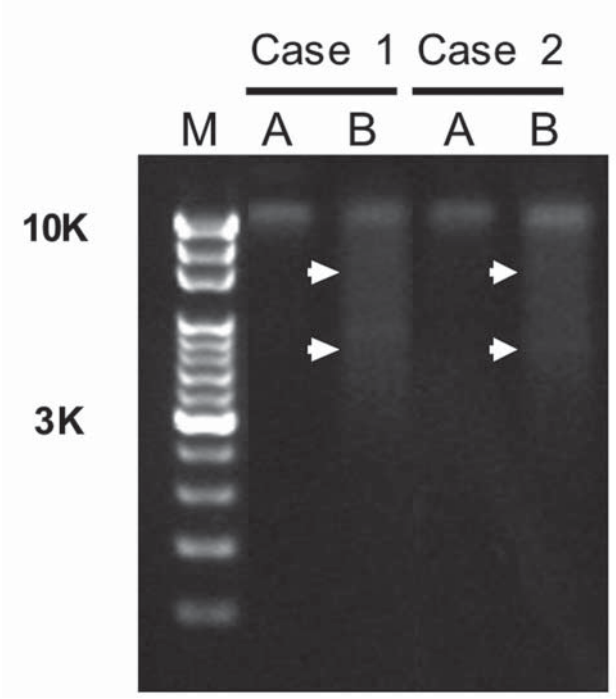

Fig. 1 Process effect on the quality of DNA evaluated by agarose gel electrophoresis. When the samples were processed immediately after collection, the DNA bands were strong and clear. When the samples were stored 3 days at 4°C, the quality of DNA decreased and the bands showed some degree of degradation (arrow indicated). (A, DNA extraction was performed within 2 hr; B, DNA extraction was performed after stored 3 days at 4°C; M, marker.)

Statistics

Data were analyzed using SPSS 13.0. Differences in the mean of DNA concentration and purity were tested using the *t* test. The *p* values were recorded accordingly with $p < 0.05$ considered significant.

RESULTS

DNA concentration and purity

The means and standard deviations of DNA yield were 1.5±0.3, 1.4±0.3, and 1.1±0.2 for phenol-chloroform, QIAamp kit, and NaOH methods, respectively. No statistically significant difference regarding

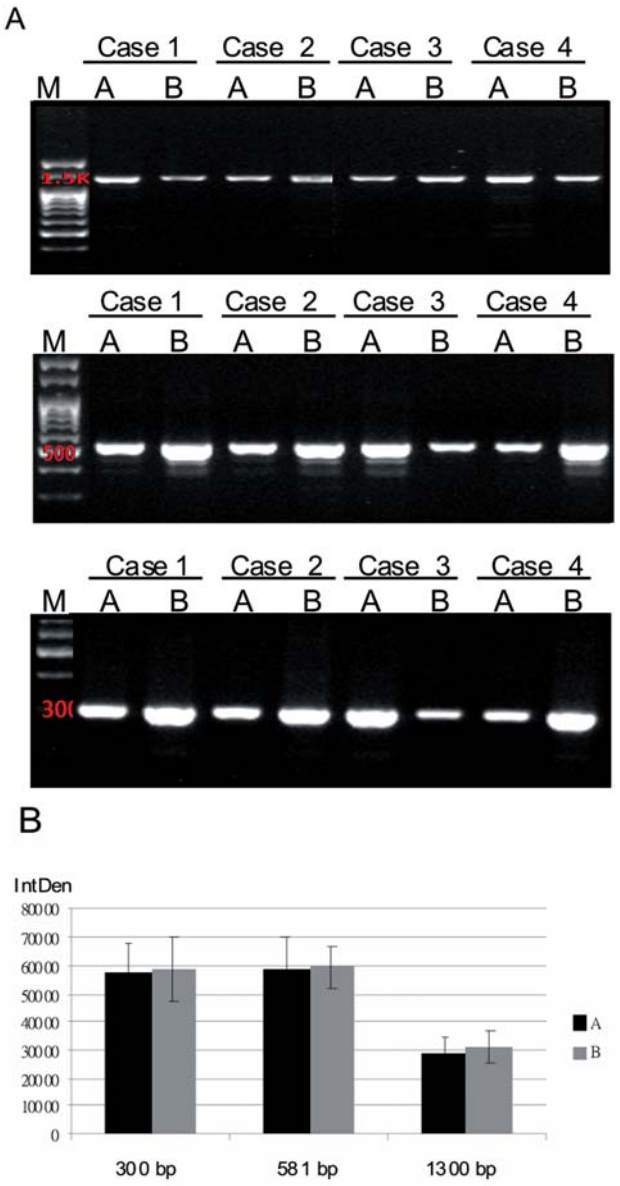

Fig. 2 (A). PCR amplification GFPT1 gene with different length of product (1300 bp, 581 bp, and 300 bp). The PCR reactions were 100% successful for DNA from both conditions. (B). Quantification of PCR product is described in "Materials and Methods". Column, mean; bar, SD. (A, DNA extraction was performed within 2 hr; B, DNA extraction was performed after stored 3 days at 4°C; M, marker.)

DNA concentration between the phenol-chloroform and QIAamp kit methods was found (p = 0.415). However, these two methods yielded significant higher DNA concentration than the NaOH method. Similarly, the DNA purity extracted by the NaOH method, as assayed by the A260:A280 ratio, was the lowest (P = 0.028 *and* P< 0.0001), while there was no statistically significant difference in purity of DNA extracted by the phenol-chloroform and the QIAamp kit methods (P = 0.483).

Table 2 The total DNA yields and quality by immediate or delay processing

		Spectrophotometry			
		Total DNA yield (ug/ml)		A260 : A280	
Process tim	N	Mean±SD	Range	Mean±SD	Range
Immediate	10	1.6±0.2	1.2-2.0	2.0±0.2	1.8-2.4
Delay	10	1.5±0.1	1.3-1.7	2.0±0.2	1.8-2.5

Immediate, DNA extraction immediately after sample collection; delay, DNA extraction was performed 3 days after sample collection.

Effect of sample processing on DNA integrity

For DNA extracted under immediate and or delay process, different band patterns were observed in the agarose gel. As shown in Figure 1, no degradation traces were observed in the DNA sample processed immediately after buccal cell sample collection. However, samples processed with delay showed bands with some degree of DNA degradation. Table 2 displays the means and standard deviations of DNA yield and purity. As can be seen, there was no statistical difference in yield and purity between DNA extracted immediately or with delay.

Effect of sample processing on PCR-based assay

To evaluate the suitability of DNA extracted from buccal cells, gene fragments of different sizes were amplified. The 300 bp fragment of the GFPT1 gene, 581 bp fragment of the GSTM1 gene, and 1300 bp fragment of the GSTP1 gene were successfully amplified from all samples whether processed immediately or with delay (Fig. 2).

DISCUSSION

Obtaining human DNA of high concentrations from buccal cell samples is critical for the optimal use of DNA, especially for samples with low yields, and for the successful use of techniques that require narrow ranges of DNA concentration, such as the determination of methylation or microsatellite repeats. The advantages of using buccal cell DNA for clinical studies include: 1) easy and noninvasive method for obtaining samples; 2) only simple implements, such as cytologic brush, required, and 3) reliable test results for screening. Previous reports had successfully applied buccal cell DNA for

clinical and biologic uses.[4,12-15] For example, Christofolini et al. studied the blood and buccal cell DNA for *FMR1* mutation using PCR and showed buccal cell DNA successfully yielded PCR produces, and the results were in full agreement with blood sample findings.[15] For research purposes, it is of importance that PCR can amplify specific gene fragments from target DNA samples. According to our results, the yield and purity of DNA extracted using phenol-chloroform and commercial methods are better than those using NaOH. The kit method provides a more feasible approach and achieves DNA extraction of better quality. However, the phenol-chloroform method is less expensive and less time-consuming. With cost consideration disregarded, the commercial kit is time-saving and better for DNA extraction in laboratory studies.

The quality of DNA extracted from buccal cells may be related to the subsequent PCR. Long-term storage may lead to degradation of human DNA. Generally, the laboratory is not always available for sample processing immediately after collection in a field study. Therefore, sample storage is frequently necessary. However, the time of collection and storage duration influence the quality of DNA extracted.[16] A decrease in DNA quality was observed when the material was not immediately placed in cell lysis buffer for processing. Degradation of DNA bands was observed in the specimens processed with delay.[16] King et al. used PCR primers for amplification of short (0.3 kb), intermediate (1.1 kb), and long (7.8 kb) gene fragments to examine the quality of DNA from buccal swab collection. The PCR reactions were 100% successful for the short or intermediate DNA fragments. However, PCRs failed to produce long fragment products.[2] These studies attributed degradation of DNA to the time delay in storage or processing. Although there was some degree of DNA degradation in samples stored under cooling condition for 3 days, our study showed no obvious difference in PCR amplification of DNA extracted from buccal cells immediately after collection or after 3-day storage in a freezer at -20°C. Furthermore, it is estimated that for the vast majority of polymorphisms (99%), PCR amplification reactions do not require DNA fragments longer than 1 kb. Therefore, with cooling storage, genomic DNA collected from buccal samples could be widely applied in epidemiological studies.

Our result was consistent with other reports that no obvious difference was observed for PCR amplification in DNA extracted from buccal cells immediately after collection or after storage in a freezer at -80°C for up to 10 weeks.[10] Feigelson et al.[17] reported that buccal cell samples collected with mouthwash held for 10 and 30 days at room temperature had significantly less human DNA than those processed after 1 day (P = 0.01). However, 1-week storage at room temperature did not affect yields of DNA extraction or amplification of PCR in their study. Lum and Le Marchand[18] and Le Marchand et al.[5] came to the same conclusion. Garcia-Closas et al.[6] noted that storage of unprocessed samples at -80°C for up to 1 year would not significantly reduce human DNA yields. Harty et al.[19] reported that PCR amplification was successful in all samples regardless of storage duration, though long storage may reduce the DNA yield. Therefore, it may be another advantage for large-scale studies that the collected buccal cells can be stored at room temperature or in a freezer for a short period of time before DNA extraction. Degradation of DNA from the buccal swab occurred after storage was confirmed by the present findings. However, we also observed that degradation could be minimized by immediately sample processing.

In conclusion, both simple phenol-chloroform method and commercial kit are considered better methods for DNA extraction from buccal swab samples. The DNA isolated from buccal cells under appropriate storage can be successfully employed to perform PCR-based assays. However, important questions remain with regard to the yield and quality of human DNA that can be obtained from different DNA extraction methods.

ACKNOWLEDGMENTS

This work was supported by research grants from the DOH98-TD-I-111-TM011-001,NHRI-EX97-9602BC, NSC96-2628-B-016-007-MY3, and DOD98-21-02, Taiwan, Republic of China.

REFERENCES

1. Hansen TV, Simonsen MK, Nielsen FC, Hundrup YA. Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. Cancer Epidemiol Biomarkers Prev. 2007;16:2072-2076.
2. King IB, Satia-Abouta J, Thornquist MD, Bigler J, Patterson RE, Kristal AR, Shattuck AL, Potter JD, White E. Buccal cell DNA yield, quality, and collection costs: comparison of methods for large-scale studies. Cancer Epidemiol Biomarkers Prev. 2002;11:1130-1133.
3. Woo JG, Sun G, Haverbusch M, Indugula S, Martin LJ, Broderick JP, Deka R, Woo D. Quality assessment

of buccal versus blood genomic DNA using the Affymetrix 500 K GeneChip. BMC Genet 2007;8:79.
4. Richards B, Skoletsky J, Shuber AP, Balfour R, Stern RC, Dorkin HL, Parad RB, Witt D, Klinger KW. Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. Hum Mol Genet 1993;2:159-163.
5. Le Marchand L, Lum-Jones A, Saltzman B, Visaya V, Nomura AM, Kolonel LN. Feasibility of collecting buccal cell DNA by mail in a cohort study. Cancer Epidemiol Biomarkers Prev. 2001;1:701-703.
6. García-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, Bender PK, Beck JC, Le Marchand L, Lum A, Alavanja M, Hayes RB, Rutter J, Buetow K, Brinton LA, Rothman N. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev. 2001;10:687-696.
7. Zheng S, Ma X, Buffler PA, Smith MT, Wiencke JK. Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations. Cancer Epidemiol Biomarkers Prev. 2001;10:697-700.
8. Jones AC, Pink FE, Sandow PL, Stewart CM, Migliorati CA, Baughman RA. The Cytobrush Plus cell collector in oral cytology. Oral Surg Oral Med Oral Pathol 1994;77:95-99.
9. Saab YB, Kabbara W, Chbib C, Gard PR. Buccal cell DNA extraction: yield, purity, and cost: a comparison of two methods. Genet Test 2007;11:413-416.
10. Cao W, Hashibe M, Rao JY, Morgenstern H, Zhang ZF. Comparison of methods for DNA extraction from paraffin-embedded tissues and buccal cells. Cancer Detect Prev. 2003;27:397-404.
11. Ng DP, Koh D, Choo SG, Ng V, Fu Q. Effect of storage conditions on the extraction of PCR-quality genomic DNA from saliva. Clin Chim Acta 2004;343:191-194.
12. Thomson DM, Brown NN, Clague AE. Routine use of hair root or buccal swab specimens for PCR analysis: advantages over using blood. Clin Chim Acta 1992;207:169-174.
13. Leonard J, Doot M, Martin C, Airth-Kindree W. Correlation of buccal mucosal transudate collected with a buccal swab and urine levels of cocaine. J Addict Dis 1994;13:27-33.
14. Wijnen PA, Drent M, van Dieijen-Visser MP, Bekers O. Pharmacogenetic testing after a simple DNA isolation method on buccal swab samples. Pharmacogenomics 2009;10:983-987.
15. Christofolini DM, Lipay MV, Ramos MA, Brunoni D, Melaragno MI. Screening for fragile X syndrome among Brazilian mentally retarded male patients using PCR from buccal cell DNA. Genet Mol Res. 2006;5:448-453.
16. Nedel F, André Dde A, de Oliveira IO, Tarquinio SB, Demarco FF. Buccal cells submitted to three different storage conditions before DNA extraction. J Appl Oral Sci 2009;17:113-115.
17. Feigelson HS, Rodriguez C, Robertson AS, Jacobs EJ, Calle EE, Reid YA, Thun MJ. Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. Cancer Epidemiol Biomarkers Prev. 2001;10:1005-1008.
18. Lum A, Le Marchand L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer Epidemiol Biomarkers Prev. 1998;7:719-724.
19. Harty LC, Garcia-Closas M, Rothman N, Reid YA, Tucker MA, Hartge P. Collection of buccal cell DNA using treated cards. Cancer Epidemiol Biomarkers Prev. 2000;9:501-506.