Basic Medical Research

# DNA quality from buccal swabs in neonates: comparison of different storage time

Klara Yuliarti[1,2,3], Muchtaruddin Mansyur[4], Ina Susianti Timan[3,5], Yulia Ariani[3,6], Ernawati[7], I Gusti Lanang Sidiartha[8], Nadhifa Tazkia Ramadhani[3], Nurul Muhammad Prakoso[3], Damayanti Rusli Sjarif[2,3]



pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.oa.236899
**Med J Indones. 2023;32:7–12**

**Received:** April 04, 2023
**Accepted:** June 19, 2023
**Published online:** July 11, 2023

**Authors' affiliations:**
*[1]Doctoral Program in Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [2]Department of Pediatrics, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [3]Human Genetic Research Center, Indonesian Medical Education and Research Institute (IMERI), Universitas Indonesia, Jakarta, Indonesia, [4]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [5]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [6]Department of Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, [7]Regional Health Laboratory DKI Jakarta, Indonesia, [8]Department of Pediatrics, Faculty of Medicine, Universitas Udayana, Bali, Indonesia*

**Corresponding author:**
Damayanti Rusli Sjarif
Department of Pediatrics, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jalan Diponegoro No. 71, Salemba, Central Jakarta 10430, DKI Jakarta,Indonesia
Tel/Fax: +62-21-29189160
**E-mail:** ukk.npm.idai@gmail.com

**ABSTRACT**

**BACKGROUND** Genomic medicine has great potential for diagnoses, disease prediction, and targeted treatment. Buccal swabs are a suitable non-invasive method for neonates to obtain DNA samples. Due to Indonesia's geographical conditions, samples require a prolonged time to reach the genetic laboratory. This study aimed to compare the DNA quality of buccal swabs in neonates between immediate and after-storage extraction.

**METHODS** This study was part of a study about the profile of human milk oligosaccharide and *FUT2* genotype in Indonesian mother-infant dyads consisting of 20 neonates. 1 swab stick for each participant was taken using a standardized buccal swabbing protocol and divided into 2 isovolume aliquots, which were grouped into the immediate (extraction was performed within 3 days after sampling) and storage groups (extraction was performed on the 14$^{th}$ day after storage in 4°C). DNA yield and purity $A_{260/280}$ ratio were measured by spectrophotometry. The PCR amplification and Sanger sequencing were performed to validate the DNA isolate quality for downstream application.

**RESULTS** The DNA yield for the immediate group was similar compared with the storage group (9.50 [4.89] *versus* 9.10 [5.05] µg), $p = 0.659$, as well as DNA purity $A_{260/280}$ (1.58 [0.24] *versus* 1.56 [0.28]), $p = 0.785$. PCR and sequencing of FUT2 results also showed similar quality between both groups.

**CONCLUSIONS** The similar DNA quality and sequencing results between immediate and storage extraction confirmed that buccal swabs could be stored for 2 weeks, allowing ample time for sample shipping from remote areas to the laboratory.

**KEYWORDS** buccal, DNA, isolation, quality, swabs

Understanding the genetic basis of disease has substantial healthcare benefits. Exploring single-nucleotide variations to explain human variations in metabolism, physiology, and disease risk factors is another cutting-edge development in the genomic medicine era. Obtaining genetic material for analysis is thus essential and has broad implications for understanding disease pathogenesis, establishing a diagnosis for complicated cases, and designing individualized therapies.[1]

Buccal swabs are a suitable non-invasive method for obtaining DNA samples from neonates. However, the literature regarding DNA quality from buccal swabs has yielded various results. Siswanto et al[2] reported

much lower DNA concentration in buccal swab than in the whole blood of premature infants. In contrast, Said et al[3] obtained a significantly higher DNA yield from buccal epithelial cells than that from the whole blood of premature infants. Moreover, buccal swabs pose additional concerns regarding storage time and temperature. As Indonesia has numerous islands, DNA samples often require a prolonged time to reach the genetic laboratory. Various studies have recommended different storage duration and temperature for buccal swabs. Navarro et al[4] mentioned that buccal swabs could be stored for up to 2 weeks at 4°C before processing without a noticeable loss in DNA yield or quality. Rogers et al[5] kept buccal swabs at –80°C before extraction but did not compare the storage time. Grujičić et al[6] compared three groups of different storage time from buccal swabs kept at room temperature. Therefore, this study aimed to compare the DNA quality between immediate and after-storage extractions and to validate the DNA isolate with Sanger sequencing between the immediate extraction (within 3 days) and 2-week-storage extraction groups. This result will help justify the storage of buccal swabs from remote areas before shipment to a genetic laboratory.

## METHODS

This research was a part of the main study aiming to learn the profile of human milk oligosaccharides and *FUT2* genotype in Indonesian mother-infant dyads. The primary study recruited 120 mother-newborn dyads from Bunda Mother and Child Hospital, Jakarta, Indonesia. However, the participants of this study were a subset of the last 20 participants. All participants provided informed consent. The DNA extraction, polymerase chain reaction (PCR), and sequencing were conducted at the Human Genetic Research Center, Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, Universitas Indonesia, from December 2021 to August 2022. This study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: KET-838/UN2.F1/ETIK/PPM.00.02/2021).

### Sample collection and storage

Samples were collected by rubbing the inside of the right and left cheeks 10 times each without touching the tongue or lips, as described in the kit manual. The ORAgene® OraCollect OCR-100 (DNA Genotek, USA)[7] used in this study is designed for DNA collection in pediatric patients and is painless because of its soft sponge-tip properties. The swab was directly placed into the 500 µl preservation reagent in the kit and shaken vigorously 10 times.[7] One swab stick was taken for each participant. The swabbing was performed by a trained physician who collected numerous samples for newborns in the main study. The 500 µl-raw samples were divided into two aliquots (250 µl each) for each participant. One aliquot was assigned to the immediate group (extracted within 3 days after sampling) and the other to the storage group (extracted on the 14$^{th}$ day after sampling) which was kept in 4°C refrigerator.

### DNA extraction from buccal swabs

Raw samples (250 µl) were put into a 1.5 ml microtube. As much as 20 µl of PrepIT.L2P reagent (DNA Genotek) was added to the sample. The microtubes were vortexed for 10 sec to ensure that the reagent and raw samples were homogenized. The samples were incubated for 10 min in grated ice cubes and then centrifuged (Eppendorf 5415R, Germany) for 5 min with 10,000 × *g* (room temperature); the supernatant was transferred to a fresh, sterilized microcentrifuge tube. As much as 400 µl absolute ethanol (Merck, Germany) was added and mixed thoroughly by inverting the tubes 10 times to precipitate the DNA. The samples were incubated at room temperature (24–26°C) for 10 min and then centrifuged (Eppendorf 5415R) at 10,000 × *g* (room temperature) again for 2 min. The upper aqueous layer was discarded, and the pellet was stored in microtubes. After decanting the supernatant, 250 µl of 70% ethanol (Merck) was added carefully, and the sample was incubated for 1 min. After 1 min of incubation, the solution was removed. Note that no solution should remain in the microtubes. The pellet was then resuspended in 80 µl 10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA) (TE) buffer, and frozen at –20°C or –80°C for further use.

### Concentration and purity determination

DNA quality was determined by DNA yield (concentration) and purity. A quantitative spectrophotometric assay of DNA was performed using a Varioskan microplate reader (Thermo Fisher Scientific, USA) to measure the DNA yield and purity.[8] Absorbance was measured at wavelengths of 260 and 280 ($A_{260}$ and $A_{280}$, respectively) nm. The absorbance quotient ratio ($OD_{260}/OD_{280}$) was used to express DNA

purity. An absorbance quotient ratio between 1.8 and 2.0 was considered good for purified DNA. A ratio of <1.8 indicated protein contamination, whereas a ratio of >2.0 indicated RNA contamination.[9]

**PCR and Sanger sequencing**

PCR for the *FUT2* gene was performed using a primer pair from Lefebvre et al[10] and its sequences as follows: forward 5'ACACACCCACACTATGCCTG'3 and reverse 5'AAGAGAGATGGGTCCTGCTC'3. MyTaq HS Redmix (Meridian Bioscience, USA)[11] was used as a PCR mix. The PCR program consisted of pre-denaturation at 95°C for 1 min, denaturation at 95°C for 15 sec, annealing at 65°C for 15 sec, extension at 72°C for 10 sec, repeated for 35× cycles, and completed by a final extension at 72°C for 10 min.[11] Agarose gel electrophoresis was performed to verify the quality of the PCR products. The PCR products were separated on a 0.8% agarose gel at 100 volts for 45 min. The gels were visualized using a gel documentation system (Accuris Instruments, USA).[12]

## RESULTS

DNA was successfully extracted from all samples. The DNA yield for the immediate group was similar from that of the storage group (9.50 [4.89] *versus* 9.10 [5.05] µg, respectively; $p = 0.659$). The DNA purity $A_{260/280}$ ratio was also similar between the immediate and storage groups (1.58 [0.24] *versus* 1.56 [0.28], respectively; $p = 0.785$). A comparison between several studies comparing DNA yield at different storage time and temperature is detailed in Table 1. DNA purity showed no difference between the groups (Table 2).

To evaluate the impact of slight differences in the DNA yield and purity between the immediate and storage groups on the downstream process, PCR and Sanger sequencing results were compared to capture the coding region of the *FUT2* gene. Visualization of the PCR products indicated the successful amplification of specific fragments with a size of 1,238 bp, as shown in Figure 1. In addition to successful target amplification, we did not observe the significant appearance of multiple bands, dimers, smears, and off-target fragments. This suggests that even at a purity lower than the recommended value, the presence of chemical contaminants did not significantly interrupt fragment amplification.

Sanger sequencing was performed on each participant to validate whether the DNA quality was sufficient for downstream applications. Sample

**Table 1.** Comparison of DNA yield from buccal swabs based on storage time and temperature

| References | Population (n) | Storage temperature | DNA yield (ng/µl) | | |
|---|---|---|---|---|---|
| | | | Immediate extraction | Storage group | |
| | | | Day 0–3 | Day 7 | Day 14 |
| This study, mean (SD) | Neonates (20) | 4°C | 9.50 (4.89) | NA | 9.10 (5.05) |
| Mulot et al,[13] mean (range) | Adult (20) | −20°C | 3.4 (0.4–8.5) | 3.5 (0.9–9.0) | NA |
| Grujičić et al,[6] mean (range) | Adult (7) | 4°C or −20°C | 3.91 (1.54–8.3) | 3.98 (1.25–8.1) | 4.29 (0.87–7.9) |
| Ghatak et al,[9] mean (SD) | Adult (5) | 4°C or −20°C | 3.55 (0.60) | 2.53 (0.31) | NA |

NA=not available; SD=standard deviation

**Table 2.** Comparison of DNA purity ($A_{260/280}$) from buccal swabs based on storage time

| References | Population (n) | DNA purity ($A_{260/280}$), mean (SD) | | |
|---|---|---|---|---|
| | | Immediate extraction | Storage group | |
| | | Day 0–3 | Day 7 | Day 14 |
| This study | Neonates (20) | 1.58 (0.24) | NA | 1.56 (0.28) |
| Mulot et al[13] | Adult (20) | NA | 1.6 (0.2) | NA |
| Grujičić et al,[6] mean (SD), range | Adult (7) | 1.68 (0.15), 1.45–1.88 | 1.64 (0.16), 1.37–1.88 | 1.67 (0.19), 1.32–1.88 |
| Ghatak et al[9] | Adult (5) | 1.60 (0.05) | 1.70 (0.09) | NA |

NA=not available; SD=standard deviation

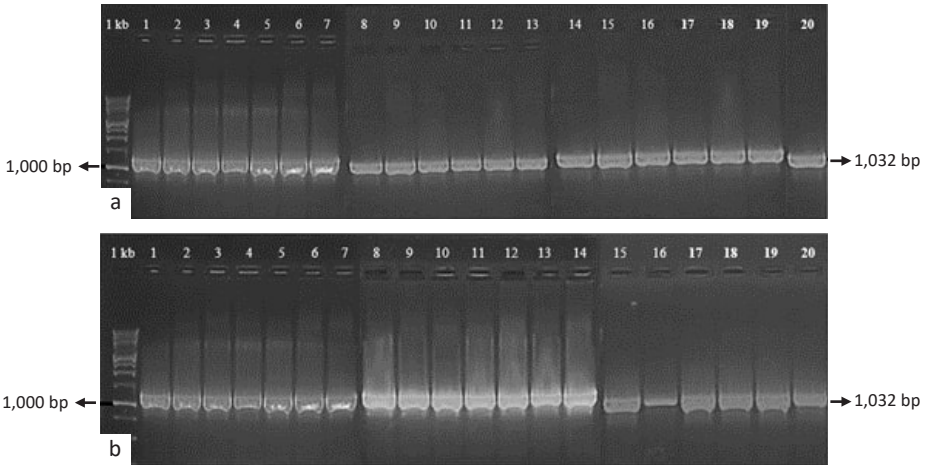


**Figure 1.** Comparison of polymerase chain reaction (PCR) products extracted (a) within 3 days and (b) on day 14 after sampling

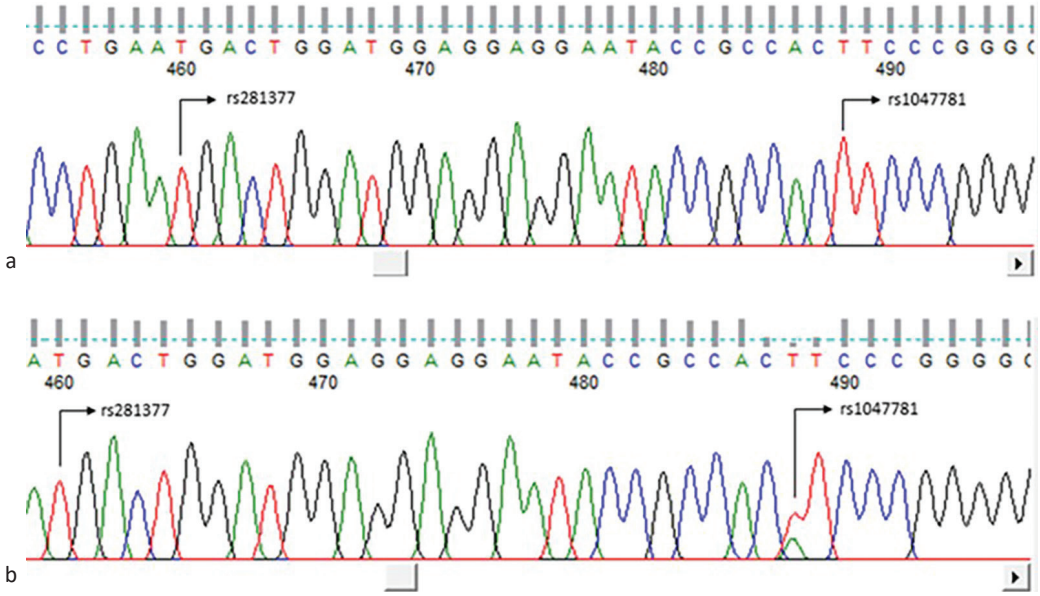


**Figure 2.** Sanger sequencing results for (a) participant 2: DNA extraction within 3 days and (b) participant 5: DNA extraction after 2 weeks in a 4°C refrigerator

aliquot were randomly assigned to each participant for sequencing. Sequencing was performed using both forward and reverse primers to increase confidence in base calling. High-quality sequencing results were obtained for both groups (Figure 2). No significant noise was observed in the baseline sequence. The average quality value was >20 for a single base and >10 for a mixed base.

## DISCUSSION

In this study, the DNA yield and purity $A_{260/280}$ were similar between the immediate and storage groups. This result aligns with three previous studies that compared the impact of different storage time and temperature on DNA quality from buccal swabs. Grujičić et al[6] showed no significant differences in the yield and purity of isolated DNA between three different storage times (day 0, day 7, and day 14). Mulot et al[13] also obtained insignificant results for the DNA yield of cytobrush buccal samples stored at room temperature for 2, 5, and 7 days. Ghatak et al[9] also found no difference in the DNA yield between immediate processing and 1-week storage at 4°C and –20°C. However, these studies were performed in adults, whereas the present study was the first to be conducted in neonates.

Differences in swab procedures, swab kits, and raw sample volumes might explain the variation

in the DNA yield. Livy et al[14] obtained a higher DNA yield of 21.03 (17.1), compared with the yield in this study, primarily due to a larger raw sample volume of 400 µl, whereas this study used 250 µl. Ghatak et al[9] used one cotton swab and put the sample in 500 µl of self-prepared fluid consisting of 10 mM Tris (pH 8.0) + 10 mM EDTA + 2% sodium dodecyl sulfate. Despite using a larger raw sample volume, Ghatak et al[9] achieved a lower DNA yield than the present study. This difference could be attributed to the swabbing technique used. In contrast to the swabbing technique used by Ghatak et al[9] in which the participants swabbed themselves, swabbing in this study was conducted by a trained physician following a standardized protocol, as described in the kit manual. In the storage time aspect, immediate extraction in Ghatak et al's[9] study resulted in a higher average yield of 3.55 (0.60) µg than samples that went to storage before processing (2.53 [0.31] µg). This was probably due to DNA degradation by the bacteria and nucleases in the buccal epithelial cell samples.[4] Another study by Said et al[3] in 85 premature infants implied that the average DNA yield from buccal swabs was 25.5 µg (range 8.95 to 42.1 µg), much higher than the present study that obtained a DNA yield of 9.50 µg for immediate extraction and 9.10 µg for after-storage extraction. This was probably because Said et al[3] used two cotton swab sticks (cytology brushes) with 900 µl cell lysis fluid, whereas this study used one cotton swab with 500 µl preservation reagent, as included in the ORAgene® OraCollect OCR-100 (DNA Genotek) swab kit. Interestingly, significantly higher DNA yields were obtained from buccal swabs than from blood.

All studies revealed DNA purity below the recommended value, between 1.8 and 2.0. Livy et al,[14] who did not compare storage time, also obtained low $A_{260/280}$ purity (1.33 [0.32]) from adult buccal swabs. Although the present study showed lower DNA purity, the sequencing results were of high quality (Figure 2). Good sequencing results despite lower than recommended DNA purity was also demonstrated by Grujičić et al.[6]

According to previous studies, the recommended $A_{260/280}$ nucleic acid purity is in the 1.8–2.0 ratio. A lower $A_{260/280}$ purity ratio may indicate the presence of protein contaminants that may inhibit downstream applications.[15] The difference in DNA $A_{260/280}$ purity result might be due to the DNA extraction method. We used conventional methods to extract DNA, whereas Livy et al[14] used QIAamp DNA Blood Kits (Qiagen, Netherlands). The reagents used in this study consisted of PrepIT.L2P DNA reagent (DNA Genotek) for sample preparation, ethanol 100% for precipitation, ethanol 70% as a wash buffer, and TE solution as a DNA elution buffer. The QIAamp DNA Blood Kit (Qiagen) included a lysis buffer, wash buffers 1 and 2, and an elution buffer. Using a column during DNA extraction might increase the DNA purity since the column captures DNA better and leaves the impurities in the flow-through.[16] In addition to that, adding the wash buffer twice also improves the DNA $A_{260/280}$ purity.[17] To prevent food contamination to purity, the participants were asked to rinse their mouths thoroughly and refrain from eating for around 45 min. In this study, infants were required not to be breastfed for at least 45 min before swabbing; therefore, the possibility of breast milk contamination was considered very low. The PCR product from a buccal swab was sequenced to validate whether the DNA isolate could be used for genetic analysis. The sequencing quality was at a high confidence level for variant analysis, represented by two common single-nucleotide variants of rs281377 and rs1047781 in the *FUT2* gene, which were superimposed on both DNA sample groups.[18,19]

In conclusion, buccal swabs could be stored for a certain period without affecting DNA quality, which benefits health services and neonatology research. Buccal swabs have great potential as a non-invasive sampling method for genetic analyses in neonates.

**Conflict of Interest**

The authors affirm no conflict of interest in this study.

**Acknowledgment**

We would like to thank the patients and family for their participation in this research. We thank the hospital staff in Bunda Mother and Child Hospital, Menteng, Central Jakarta for providing help for this research. We also thank dr. Valensia Vivian as the field research asisstant.

**Funding Sources**

This research is supported by the Indonesian Ministry of Education, Culture, Research, and Technology through the PDUPT research grant number NKB-1051/UN2.RST/HKP.05.00/2022.

## REFERENCES

1. Van-Wieren-de-Wijer DB, Maitland-van-der-Zee AH, de Boer A, Belitser SV, Kroon AA, de Leeuw PW, et al. Determinants of DNA yield and purity collected with buccal cell samples. Eur J Epidemiol. 2009;24(11):677–82.
2. Siswanto JE, Berlian T, Putricahya E, Panggalo LV, Yuniani L. [DNA isolation in blood and buccal swab samples in retinopathy of prematurity patients: comparison of the concentration and purity index]. Sari Pediatri. 2016;18(4):270–7. Indonesian.

3. Said M, Cappiello C, Devaney JM, Podini D, Beres AL, Vukmanovic S, et al. Genomics in premature infants: a non-invasive strategy to obtain high-quality DNA. Sci Rep. 2014;4:4286.
4. Navarro D, Durán NS, Álvarez R. A method for preserving buccal swabs samples for gDNA integrity. Biotechniques. 2016;61(3):153.
5. Rogers NL, Cole SA, Lan HC, Crossa A, Demerath EW. New saliva DNA collection method compared to buccal cell collection techniques for epidemiological studies. Am J Hum Biol. 2007;19(3):319–26.
6. Grujičić NK, Davidović S, Takić D, Mojsin M, Stevanović M. Direct PCR amplification of the HVSI region in mitochondrial DNA from buccal cell swabs. Arch Biol Sci. 2012;64(3):851–8.
7. DNA Genotek. DNA collection kits for diagnostic: easy and reliable DNA collection with ORAcollect-DNA [Internet]. DNA Genotek [cited 2022 Dec 28]. Available from: https://www.dnagenotek.com/ROW/products/collection-human/oracollect-dna/OCR-100.html.
8. Thermo Fisher Scientific. Microplate readers [Internet]. DNA Genotek [cited 2022 Dec 28]. Available from: https://www.thermofisher.com/id/en/home/life-science/lab-equipment/microplate-instruments/plate-readers.html.
9. Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech. 2013;24(4):224–31.
10. Lefebvre G, Shevlyakova M, Charpagne A, Marquis J, Vogel M, Kirsten T, et al. Time of lactation and maternal fucosyltransferase genetic polymorphisms determine the variability in human milk oligosaccharides. Front Nutr. 2020;7:574459.
11. Meridian Bioscience. MyTaq™ DNA polymerases [Internet]. Meridian Bioscience [cited 2022 Dec 28]. Available from: https://www.bioline.com/mytaq.
12. Wittmeier P, Hummel S. Agarose gel electrophoresis to assess PCR product yield: comparison with spectrophotometry, fluorometry and qPCR. Biotechniques. 2022;72(4):155–8.
13. Mulot C, Stücker I, Clavel J, Beaune P, Loriot MA. Collection of human genomic DNA from buccal cells for genetics studies: comparison between cytobrush, mouthwash, and treated card. J Biomed Biotechnol. 2005;2005(3):291–6.
14. Livy A, Lye S, Jagdish CK, Hanis N, Sharmila V, Ler LW, et al. Evaluation of quality of DNA extracted from buccal swabs for microarray based genotyping. Indian J Clin Biochem. 2012;27(1):28–33.
15. Prakoso N, Priambodo R, Ariani, Y, Hafifah C, Sjarif D. Identification of a novel variant in exon 5 of galactosamine (N-acetyl)-6-sulfatase gene in mucopolysaccharidosis IVA patients in Indonesia. J Nat Sci Biol Med. 2019;10(3):S99–102.
16. Dilhari A, Sampath A, Gunasekara C, Fernando N, Weerasekara D, Sissons C, et al. Evaluation of the impact of six different DNA extraction methods for the representation of the microbial community associated with human chronic wound infections using a gel-based DNA profiling method. AMB Express. 2017;7(1):179.
17. Ghaheri M, Kahrizi D, Yari K, Babaie A, Suthar RS, Kazemi E. A comparative evaluation of four DNA extraction protocols from whole blood sample. Cell Mol Biol (Noisy-le-grand). 2016;62(3):120–4.
18. National Library of Medicine. dbSNP short genetic variations: reference SNP (rs) report rs281377 [Internet]. Bethesda: National Center for Biotechnology Information; 2022 [cited 2023 Mar 9]. Available from: https://www.ncbi.nlm.nih.gov/snp/rs281377.
19. National Library of Medicine. dbSNP short genetic variations: reference SNP (rs) report rs1047781 [Internet]. National Center for Biotechnology Information; 2022 [cited 2023 Mar 9]. Available from: https://www.ncbi.nlm.nih.gov/snp/rs1047781.