Original Article

Reliability Of Various Sampling Techniques For Genomic DNA Isolation: A Comparative Literature Search

Ashwin Mathew George[1] , Sumathi Felicita[2] , Swapna Sreenivasagan[3], Priyadarshini Vijayashree[4], Remmiya Mary Varghese[5]

[1]**Corresponding Author ,**Professor,Department of Orthodontics and Dentofacial Orthopaedics,Saveetha Dental College and hospitals,SIMATS Deemed University Chennai – 600 077
[2]Professor, Department of Orthodontics and Dentofacial Orthopaedics
Saveetha Dental College and hospitals SIMATS Deemed University Chennai – 600 077
[3]Assistant Professor
Department of Orthodontics and Dentofacial Orthopaedics Saveetha Dental College and hospitals
SIMATS Deemed University Chennai – 600 077
[4]Professor Department of Orthodontics and Dentofacial Orthopaedics Saveetha Dental College and hospitals SIMATS Deemed University Chennai – 600 077
[5]Associate Professor Department of Orthodontics and Dentofacial Orthopaedics Saveetha Dental College and hospitals SIMATS Deemed University Chennai – 600 077
DOI: 10.47750/pnr.2022.13.S04.236

Abstract

Genetic studies are one of the gold standards to back up data on prevalence, survey, and genetic association with common diseases and disorders. The extraction of optimal quantities of DNA samples forms an integral part of genomic studies. This literature search gives an insight into the various methods available for DNA isolation. Further, a comparative evaluation was done to validate which would be the optimal method of sample collection, based on factors such as degree of invasiveness, the quantity of sample required, good storage capability, and cost-effectiveness. Genetic studies that are conducted on a huge population involve a large number of samples from the participants are often needed when conducting genetic studies and this validates a need for many factors considered when selecting the optimal source of sample collection. . The different methods of sample collection compared were a) Venous blood, b) Saliva, c) Buccal Swabs, d)Buccal cell swabs on FTA cards, e) Hair and Urine Samples. A comparison of the various methods would be discussed and the advantages and disadvantages of the various method would be systematically analyzed to give the clinician/researcher a better insight to choose the right method of sample collection based on the facilities available for DNA isolation.

Introduction

Genetic studies have become an important prerequisite in establishing proper insight into the cause and degree of various developmental disorders. Isolated Deoxyribonucleic acid (DNA) samples are needed for genetic study and other epidemiological procedures. Extraction and purification are also essential to determine the unique characteristics of DNA, including its size, shape, and function. Although various methods have been proved to be successful in collecting samples for DNA isolation, the crucial factor is the adequate quantity and quality of DNA that can be extracted from a particular sample. Isolation of DNA was first done by the Swiss DNA physician, Friedrich Miescher, in 1869. DNA isolation is an extraction process of DNA from other cellular components achieved through various

methods of sampling such as blood, saliva, buccal swabs, hair follicle samples, and urine. The dependence of optimum methods of DNA collection is based on the source, age, and size of the sample.

Following some of the above-mentioned common procedures, DNA extraction methods achieve effective cell disruption, denaturation of nucleoprotein complexes, inactivation of nucleases and other enzymes, separation of biological and chemical contaminants, and finally DNA precipitation.[1]The conventional and proven method to obtain genomic DNA is from the nucleated cells of peripheral blood. This venous blood produces an adequate quantity and quality of DNA. Various other methods of DNA isolation include saliva, buccal swabs from the oral cavity, and samples of hair with follicles and urine.[2] The criteria for selecting an appropriate method of Sample selection to isolate DNA based on its reliability include-a)Degree of Invasiveness, b)Lower Sample Volume requirement, c)Cost-effective procedure, d)Long-term archiving and e) Suitability of Self collection.[3] Good sample selection is the primary requisite for achieving the optimum quality of DNA. This literature review aims at giving an insight into the various techniques of sample collection to determine what would be the ideal choice of sampling.

The various methods for extracting DNA remained complex, labour-intensive, and time-consuming and provided only small quantities of DNA. Amongst these procedures, there are protocols that range from basic to sophisticated ones. The steps that are involved in DNA extraction and purification from the rest of the cell include lysis, precipitation, wash, and resuspension. Quantification of the DNA is done using the PCR technique. With the advancement of scientific technology, the extraction of DNA has become much easier and also the quantity of DNA extracted is greater with modern technologies, especially with the availability of commercially kits that help in the automation of the process. Such changes have both sped up production and increased the yield of DNA, thus simplifying a relatively complex procedure.

Comparison and validity of different techniques for collection of samples for DNA isolation.

A. Venous blood

Venous blood has been considered the gold standard for DNA isolation with manifold advantages It is a very reliable source and around the 50-100ml volume of blood is required for DNA isolation. DNA is found in White Blood Cells and not in Red Blood cells as they lack nuclei. The common methods of DNA extraction used when obtaining whole blood samples include a solution-based method and solid-phase extraction methods that consist of subcategories such as salting out methods, organic solvent methods, glass milk or silica resin methods. [4]

With technological advancements, rapid and efficient purification of genomic DNA from blood samples can be completed in less than 15 minutes following sample preparation and lysis using Magnetic bead-based technology without the need for hazardous chemicals and centrifugation.

However, the withdrawal of venous blood via phlebotomy using venipuncture is comparatively an invasive procedure and patients are usually apprehensive about the procedure. There is also difficulty to draw blood from individuals with thin veins and also hesitation of physicians/paramedics to draw blood when there are elderly or feeble individuals or young children.[5]

B. Saliva Samples

Saliva samples can be collected by spitting a minimum of 2 mL of saliva without any air bubbles, fasting, and refraining from smoking for up to 30 min before sample collection.[6] The collection of saliva samples can usually be done by self-collection and it is a rather simple and painless process. This makes it a viable option in terms of large-scale genetic studies.[7] One of the drawbacks of saliva samples is that they are often present with bacterial DNA and this is mainly associated with the method of storage. It is recommended that immediately after saliva collection, it

should be frozen at or below -20 ºC. If a freezer is not available, specimens can be stored at 4 ºC to prevent bacterial growth and further degradation of salivary molecules, no longer than 6 hrs duration.

The drawback of bacterial contamination can be overcome by using antibacterial agents and chemicals which prevent the growth of bacteria from the time of collection and DNA purification. [8]

C. Buccal Cell (swabs)

Buccal cell collection can be performed easily by a buccal swab with a cotton swab or by using a mouth-wash procedure. DNA isolation using buccal swabs provides many advantages, such as cost-effective processing, lower sample volume requirement, long-term archiving, and suitability of self-collection. There are two methods of collection from buccal cells for DNA extraction: a) mouthwash rinses with patients asked to swish with undiluted mouthwash and b) Cytobrush sampling of the inner cheeks technique. DNA from buccal cells can be isolated from buccal cells with a rapid method using proteinase K digestion.[9] Buccal samples via mouthwash specimens have proven to be superior to the quality of cytobrush samples in terms of obtaining high molecular weight DNA.[10]. Literature evidence from a study showed that after 5 days of collection from mouthwashes, the quantity of DNA that can be isolated significantly reduces, suggesting a limitation of storage time. Another limitation with Buccal Swab collection is that a lower quantity of DNA is available when used, especially in infants. [11]

D. Buccal cells (FTA Cards)

DNA can also be extracted from a chemically treated filter paper, FTA(Finders Technology Associates) Cards. These cards are used for collection, preservation, and subsequent DNA and RNA isolation. FTA Cards are cotton-based, cellulose-based cards containing chemicals that burst cells, denature proteins, and protect DNA, leaving a sample suitable for molecular identification without the risk of disease contamination. FTA technology can lyse cells on contact, denature proteins, remove contaminants, and protect DNA from degradation. The FTA card can be stored by wrapping it in plastic wrap and leaving it at room temperature for a period longer than 2 months. When stored in a desiccant, it can be stored for a long period. The cost of storage is substantially reduced as it can be stored at room temperature avoiding the need for temperature control. Furthermore, the shipment of samples is simplified as this may be done by traditional mail. [12] However, the downside of using FTA cards apart from the cost factor is that they are easy to contaminate, especially with sensitive projects involving deep sequencing and metagenomics as they are often sensitive to contaminants.

E. **Hair samples and urine samples**:

A single hair follicle can be used to extract a reliable source of DNA if a multiple tubes approach is used.[13] DNA samples are invariably present in all keratinized tissues like hair and nails, but the quantity and length of amplifiable DNA samples vary not only among individuals but also among the different samples. [14] Human DNA is permeable to kidney barriers and DNA molecules are large enough to be analyzed by genetic analysis. Urinary DNA analysis is not restricted by sample volume and is readily performed.[15]

Another important factor that should be taken into consideration is quantifying the concentration of the extracted DNA from different samples collected. The method to identify and compare the concentration of DNA is done by Cq (Quantification Cycle) values using the QRealTimePCR(Quantification Real-Time Polymerase Chain reaction)often referred to as qPCR.) The anticipation of the former's potential to precisely measure target nucleic acids distinguishes qPCR from traditional (legacy) PCR procedures. [16]

qPCR is typically used to determine the absolute amount of a target sequence or to compare the relative amounts of a target sequence between samples. This technique uses a target-specific fluorescent signal emitted during amplification to monitor the target's amplification in real-time. The Cq identifies the fractional number of cycles required for the fluorescence to reach a quantification threshold, and these values are proportional to the starting concentration of each sample target collected. The Cq values are inversely proportional to the amount of target nucleic acid in the collected sample and correlate with the number of target copies. Lower Cq values (typically less than 29 cycles) indicate a high

concentration of the target sequence. Higher Cq values (above 38 cycles) indicate that the target nucleic acid is present in lower concentrations. When the Cq values of blood and saliva were compared, it was discovered that blood samples showed amplification of DNA at a lower CQ value, indicating that the concentration of DNA in blood was higher than in saliva.[17]

In a study conducted by Yueshan et al, genomic DNA extractions from saliva samples produced an adequate quantity of genomic DNA when using the Affymetrix Drug Metabolism Enzymes and Transporters (DMET) arrays, but when human amplifiable DNA was measured, it was discovered that a large percentage of this DNA was from bacteria or fungi. When compared to blood-derived DNAs, saliva-derived DNAs had a mean of 37.3 per cent human amplifiable DNA, resulting in a significant decrease in genotyping call rate (88.8 per cent) (99.1 per cent). The percentage of human amplifiable DNA correlated with a higher genotyping call rate, and nearly all samples containing more than 31.3 per cent human DNA had a genotyping call rate of at least 96 per cent. SNP genotyping results for saliva-derived DNA (n=39) illustrated a 98.7% concordance when compared with blood DNA. In conclusion, when compared with blood DNA and tested on the DMET array, saliva-derived DNA provided adequate genotyping quality with a significantly lower number of SNP calls. If saliva-derived DNA has more than 31.3 percent human amplifiable DNA, it works very effectively. [18].

Discussion

Genetic studies have gained importance in various fields of diagnostic medicine, and the important progress in diagnostic sciences is the increased sensitivity and specificity of newer diagnostic procedures. The development of newer testing procedures has changed a rather complex procedure of gDNA(Genomic deoxyribonucleic acid) isolation into rather simple procedures within the reach of the researcher and clinician. gDNA is chromosomal DNA, in contrast to extra-chromosomal DNAs like plasmids. The genomic DNA of most organisms is the same in every cell, but only a subset of these genes are expressed to allow for cell activity and differentiation within the body.

The Criteria for sample selection to isolate DNA have many aspects to be analysed such as variation with age, the risk factor involved and the storage potential. Since this literature search indicates that Blood and Saliva seem to be the two viable methods for sample collection, a comprehensive comparison between the methods is discussed based on various criteria:-

Variation with age: As individuals age, there is more peeling of the cells of the oral mucosa and most of these cells are epithelial even though other cells are seen.[6] The DNA sample in saliva originates from the inner mucosal lining and white blood cells. Differences in DNA quantity and quality become a critical factor in achieving the genotyping success rate. Studies have proved that the DNA yield from saliva is comparable to what was isolated from blood, Another aspect that needs to be taken into consideration is the difficulty and lack of cooperation of taking blood samples from young children.

The risk that is of major concern for the sample collection using saliva is the presence of non-human DNA but as long as the extracted DNA in downstream processing has been strictly adhered to and the application is assured for assays designed and targeted for the human-specific genome, this drawback can be easily overcome. The collection of saliva is also preferred as is a less invasive method and also doesn't require medical personnel.[19] The chances of cross-infection with saliva samples should be considered negligible compared to blood samples. Some of the drawbacks with saliva sample collection would be contamination of saliva with food particles and with nicotine from Patients who smoke just before sample collection

Storage: Another important factor for optimum DNA isolation is the storage temperature required after sample collection. For venous blood, studies have shown that the highest mean DNA yields, relative to the control samples, were obtained from blood stored at 4 degrees C. Blood stored at 37 degrees C for > or = 3 days or 23 degrees C for > or = 7 days yielded less ($P < .05$).[20] However, salivary samples stored at 37 degrees for up to 18 months don't interfere with the quality and ability of genetic analysis.[21]

This literature search aimed to evaluate the DNA yield and the quality in terms of the purity of gDNA using various methods of collection. From the findings of articles that are available to us in literature, the quality and quantity of DNA isolation are found to be the best from samples collected from venous blood. [18] The next best method of choice would be samples collected from saliva which proves to have an adequate quantity based on the facts discussed in this review. The most important criteria concerning Saliva collection are its ease of collection and less invasiveness when compared to whole blood collection. Most of the articles point out that when large-scale epidemiological studies are carried out in genetics, DNA isolation from the whole saliva should be considered the best alternative to the collection of blood samples concerning the quantity and quantity of DNA isolation possible. The quantity of total DNA estimated from 2mL blood samples was an average of 28.4 (mu grams), and an average of 10.8 (Mu grams) from 0.5mL of saliva. [19] However, another study conducted by Mee-Lee Looi showed that it was 7.8 µg / 0.5mL saliva and 7.4 ug/0.5mL blood sample. [22]

Among the different methods of Sample collection discussed and the factors that determine their advantages and disadvantages , the author's opinion is that the degree of invasiveness and compliance should be considered as the most important criteria when selecting the optimum method. In a study done by **,** they compared the yield and quantity of DNA isolation for four different sampling methods:- Blood, Saliva, Buccal Cells(Swabs) and Buccal cells (FTA cards) the response rates varied considerably between DNA collection methods.[23] With regard to patient compliance, an average, of 75% of those requested to deliver saliva or buccal cells samples were willing, however, but less than one-third turned up to have their blood sample taken. Therefore the simple, self-administrated sample collection methods, including saliva and buccal cell samples, increase participation rates significantly in particular among elderly participants.

Conclusion:

The utmost criteria for genomic DNA isolation from different modalities of sample collection apart from the need of being Non-Invasive in nature, is that should be without limitations for quantification and storage. This would enhance and ensure that there is increased participation from the population, especially when an epidemiological long-term study is conducted that may involve children. Blood samples have proven to be a gold standard source of genomic DNA for biomarker genotyping, however, the inference from this literature search indicates that Saliva samples for DNA isolation would be the optimal choice because of the manifold advantages discussed.

References

1. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present. J Biomed Biotechnol 2009; 2009: 574398.

2. 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: 224–231.

3. Vesty A, Biswas K, Taylor MW, et al. Evaluating the Impact of DNA Extraction Method on the Representation of Human Oral Bacterial and Fungal Communities. PLoS One 2017; 12: e0169877.

4. Chacon Cortes DF, Griffiths L. Methods for extracting genomic DNA from whole blood samples: current perspectives. Journal of Biorepository Science for Applied Medicine 2014; 2014: 1–9.

5. Sun F, Reichenberger EJ. Saliva as a source of genomic DNA for genetic studies: review of current methods and applications. Oral Health Dent Manag 2014; 13: 217–222.

6. Gassó P, Pagerols M, Flamarique I, et al. The effect of age on DNA concentration from whole saliva: implications for the standard isolation method. Am J Hum Biol 2014; 26: 859–862.

7. Rogers NL, Cole SA, Lan H-C, et al. New saliva DNA collection method compared to buccal cell collection techniques for epidemiological studies. Am J Hum Biol 2007; 19: 319–326.

8. Rylander-Rudqvist T, Håkansson N, Tybring G, et al. Quality and Quantity of Saliva DNA Obtained from the Self-administrated Oragene Method—A Pilot Study on the Cohort of Swedish Men. Cancer Epidemiol Biomarkers Prev 2006; 15: 1742–1745.

9. 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.

10. García-Closas M, Egan KM, Abruzzo J, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and

mouthwash. Cancer Epidemiol Biomarkers Prev 2001; 10: 687–696.

11. Saftlas AF, Waldschmidt M, Logsden-Sackett N, et al. Optimizing buccal cell DNA yields in mothers and infants for human leukocyte antigen genotyping. Am J Epidemiol 2004; 160: 77–84.

12. Stangegaard M, Børsting C, Ferrero-Miliani L, et al. Evaluation of four automated protocols for extraction of DNA from FTA cards. J Lab Autom 2013; 18: 404–410.

13. Goossens B, Waits LP, Taberlet P. Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. Mol Ecol 1998; 7: 1237–1241.

14. Bengtsson CF, Olsen ME, Brandt LØ, et al. DNA from keratinous tissue. Part I: hair and nail. Ann Anat 2012; 194: 17–25.

15. Botezatu I, Serdyuk O, Potapova G, et al. Genetic analysis of DNA excreted in urine: a new approach for detecting specific genomic DNA sequences from cells dying in an organism. Clin Chem 2000; 46: 1078–1084.

16. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009; 55: 611–622.

17. Arya M, Shergill IS, Williamson M, et al. Basic principles of real-time quantitative PCR. Expert Rev Mol Diagn 2005; 5: 209–219.

18. Hu Y, Ehli EA, Nelson K, et al. Genotyping performance between saliva and blood-derived genomic DNAs on the DMET array: a comparison. PLoS One 2012; 7: e33968.

19. Abraham JE, Maranian MJ, Spiteri I, et al. Saliva samples are a viable alternative to blood samples as a source of DNA for high throughput genotyping. BMC Med Genomics 2012; 5: 19.

20. Cushwa WT, Medrano JF. Effects of blood storage time and temperature on DNA yield and quality. Biotechniques 1993; 14: 204–207.

21. Anthonappa RP, King NM, Rabie ABM. Evaluation of the long-term storage stability of saliva as a source of human DNA. Clin Oral Investig 2013; 17: 1719–1725.

22. Looi M-L, Zakaria H, Osman J, et al. Quantity and quality assessment of DNA extracted from saliva and blood. Clin Lab 2012; 58: 307–312.

23. Hansen TVO, Simonsen MK, Nielsen FC, et al. 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.