OPTIMIZATION OF DOUBLE LYSIS PROTOCOL FOR THE EXTRACTION OF GENOMIC DNA FROM WHOLE MAMMALIAN BLOOD TISSUE

[1]Tawakalt A.Fagbayi, [2]Olaide O. Olajide, [3]Abisola N. Yusuf-Awari, [4]Voke Toye, &[5]Rex L. Hanior
[1,2,3,5]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos Nigeria
[4]Biologix Support Services, Anthony Village, Lagos, Nigeria

ABSTRACT

Extraction of genomic DNA from mammalian blood is an important approach in clinical and molecular biology studies, thus the need for an effective and affordable protocol cannot be overemphasised. This study was designed to optimize a double lysis DNA extraction protocol using whole mammalian blood tissue. The Double lysis method was subjected to different experimental conditions- optimization protocols to examine its effectiveness and efficiency in extracting and purifying high quantity and quality genomic DNA from whole blood samples compared to a commercial kit (Qiagen kit). The Double lysis optimization protocols were carried out with proteinase K at uniform temperature of 56 °C and without proteinase K at 60 °C in varying incubation periods (10, 90, 150 and 180 minutes respectively). The Qiagen extraction was done following manufacture's guide. Purity check and quantification were done using spectrometry methods and agarose gel electrophoresis. Results revealed that heating for longer period does not have significant effect on the yield and quality of DNA. Comparison of the extraction methods shows that the kit (Qiagen) method produced higher quality and quantity of DNA isolated from whole blood samples compared to the Double Lysis method. The double lysis method also produced DNA of high quality (evident with the clear distinct bands) and quantity in some of the experimental conditions. Therefore, suggesting for more experimentation for the optimization of protocols to improve the quality and quantity of genomic DNA in the Double lysis method.

Key words: DNA, Extraction, Optimization, Qiagen kit, Double lysis.

INTRODUCTION

Deoxyribonucleic acid (DNA) is a complex molecule that contains all of the information necessary to build and maintain an organism's life. DNA is made up of three constituents: a phosphate group, a sugar group and a nitrogenous base -adenine (A), thymine (T), guanine (G) and cytosine (C). These aggregate to form a single unit called nucleotide. To form a strand of DNA, the nucleotides are linked into chains, with the phosphate and sugar groups alternating (Motamedi *et al*, 2011).

DNA extraction is a routine but essential procedure in clinical diagnostics, forensic science, and molecular biology laboratories. Purified genomic DNA is required for downstream applications such as polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) analysis, genotyping, sequencing, and epigenetic studies (Tan & Yiap, 2009; You *et al.,* 2025). DNA extraction enables the identification of genetic disorders and disease-associated mutations, while in forensic science it facilitates human identification and biological evidence analysis (Schweighardt *et al.,* 2015). Furthermore, DNA extraction is useful to create genetically engineered organisms that can produce beneficial products such as insulin, antibiotics, and hormones. The first DNA extraction in history was performed in 1869 by Friedrich Miescher (Dahm 2005). Today, there are different available protocols for DNA extraction. Most DNA extraction protocols consist of two parts: a) cell lysis and solubiliztion of the DNA. b) enzymatic or chemical methods to remove contaminating proteins, RNA, or macromolecules. These protocols are different in cost of consumables, laboratory facilities, the quality and quantity of DNA obtained toxicity and time demands (Boom *et al,* 1990 and Carpi *et al,* 2011).

DNA extraction methods from human/mammalian whole blood samples include salting out methods, organic solvent/chaotropes methods (include phenol-chloroform method and its modifications), glass milk/silica resin methods, anion exchange methods and magnetic beads methods. In addition, some commercial kits are available for this purpose (Na *et al.,* 2025 Miskimen *et al*., 2023). While commercial kits for DNA extraction are simple, rapid and safe but these kits are very expensive with low yield, so it is not suitable for DNA extraction in large scale (Schweighardt *et al*, 2015). Recent studies have demonstrated that various extraction methods yield different quality and quantity of DNA, highlighting the need for protocol optimization based on specific research requirements (Andrews *et al.,* 2022; Molbert *et al.,* 2023; Samsonova *et al.,* 2024). Additionally, advances in magnetic nanoparticle-based extraction methods have shown promise for rapid, cost-effective, and organic-free DNA isolation (Nguyen *et al.,* 2022).

The salting out methods for DNA isolation involves the addition of salts then precipitation of the DNA from the protein in subsequent step with an alcohol (isopropanol or ethanol) followed by pelleting by spinning with a centrifuge and the supernatant removed. Usually, the salting out methods is laborious and time consuming but this method is safer than phenol- chloroform method and cheaper than commercial kits (Carpi *et al,* 2011 and Chacon-Cortes and Griffiths 2014).

Recent optimization studies have shown that salting out protocols can produce high molecular weight DNA suitable for next-generation sequencing applications when properly optimized (Daniels *et al*., 2023; Weerakkody & Witharana, 2024). Weerakkody and Witharana (2024) demonstrated an innovative modified salting-out method using laundry detergent that achieved high yield and quality of genomic DNA extraction from whole blood, offering a cost-effective alternative to expensive commercial kits.

The organic solvent (Phenol-chloroform) method has been in common use and has its advantages and disadvantages. It is known for relatively pure DNA yields with high molecular weight, the DNA extracted is double stranded and thus good for RFLP (restriction fragment length polymorphism). But this method is time consuming, requires the sample to be transferred to multiple tubes-increasing contamination risk and involves use of toxic chemicals (Sambrook *et al*.,2001; Mekonnen *et al*.,2024). In this research, two DNA extraction protocols were compared from mammalian blood samples based on quality and quantity of the extracted DNA. These protocols include; the double lysis (salting-out method) and a commercial kit (Qiagen kit).

MATERIALS AND METHODS

Human Sample Collection

About 4 ml blood sample was obtained from three voluntary individuals into EDTA vacutainer tubes via venipuncture. The blood was transported in a mini cooler and then stored in a refrigerator at 4 ºC until extraction in accordance with standard sample preservation practices (You *et al.,* 2025).

Animalsamplecollection

Blood sample of about 1 ml was obtained from rat and mice (mammals) into EDTA vacutainer tubes. The blood was then stored in a refrigerator at 4 ºC until extraction**.**

Genomic DNA isolation using the double lysis protocol

Sample coding:

Sample I.D	Extraction Protocol
1P,2P,3P,4P,5P	Double lysis extraction protocol with proteinase k and heating for 10minutes
1S,2S,3S,4S,5S and1Q,2Q,3Q,4Q,5Q	Double lysis extraction protocol with proteinase k and heating for 90minutes eluted with two different buffers
1Z,2Z,3Z,4Z,5Z	Double lysis extraction with proteinase k and heating for 150 minutes
1,2,3,4,5,	Double lysis extraction protocol without proteinase k and heating for 180minutes
1K,2K,3K,4K,5K	Kit (Qiagen) extraction protocol

The number 1-3 represent the human blood sample. Number 4 represent the rat sample while number 5 represent the mouse sample.

The double lysis protocol was optimized at different experimental conditions as follows;

Optimization Protocol 1- Isolation with Proteinase K with Short Term Heating

Twenty microlitres (20 µl) of Proteinase K were pipetted into a 1.5 ml microtube. Two hundred microliters (200 µl) of the blood sample were then added into the microtube followed by the addition of 200µl of lysis buffer 1 (10 mM Tris HCl, 1 mM EDTA and 2 % SDS) and lysis buffer 2 (10 mM Tris HCl, 1 mM EDTA and 0.2% Triton X-100) and then was vortexed for 15 seconds to homogenize. The samples were heated at 56 ºc for 10 minutes. Two hundred microliter (200 µl) of ice-cold absolute ethanol (98-100 %) was added to the lysate and pulse vortexed to mix. The samples were centrifuged to collect loses samples on the microtube lid. After centrifuging, the lysate /mixture was pipetted into the spin column and again centrifuged at 11,000 rpm for one and a half minutes. The remaining lysate/mixture was centrifuged for another one and a half minute till the spin column was dry. Five hundred microliter (500 µl) of wash buffer 1 was pipetted in to the spin column and again centrifuged at 11,000 rpm for one and a half minutes. Then 50 0µl of wash buffer 2 were also pipetted into the spin column and centrifuged at 11,000 rpm for one and a half minutes. The spin column was spin dried at 13,000 rpm for three minutes to dry up any remaining ethanol in the membrane. The spin column was placed in a 1.5 ml centrifuge tube. Then 30 µl of T.E buffer/ elution buffer was added and the sample was incubated at room temperature for five minutes. Centrifugation at maximum speed was done. Another 30 µl elution buffer was added into the spin column and incubated at room temperature for 5 minutes. Centrifugation at maximum speed was done. Eluted DNA was stored at 4 ºC.

Optimization Protocol 2- Isolation with

Proteinase K with Long Term Heating

Twenty microliters (20 µl) of Proteinase K were pipetted into a 1.5 ml microtube. Two hundred microliters (200 µl) of the blood sample were added into the microtube. Then 200 µl of lysis buffer 1 (10 mM Tris HCl, 1 mM EDTA and 2 % SDS) and lysis buffer 2 (10 mM Tris HCl, 1 mM EDTA and 0.2% Triton X-100) were added and vortexed for 15seconds to homogenize. The samples were heated at 56 ºc for 90minutes. Two hundred microliter (200 µl) of ice-cold absolute ethanol (98-100 %) was added to the sample and again pulse vortexed to mix. The samples were centrifuge (Eppendorf) to bring down the samples from the cap into the tubes. The lysate /mixture were pipetted into the spin column and centrifuge at 11,000 rpm for one and a half minutes. The remaining lysate/mixture was centrifuge for another one and a half minute till the spin column was dry. Five hundred microliter (500 µl) of wash buffer 1 was pipetted into the spin column and centrifuged at 11,000 rpm for one and a half minutes. Then 500 µl of wash buffer 2 was also pipetted into the spin column and centrifuged at 10-12rpm for one and a half minutes. The spin column was spanned dry at 13,000 rpm for three minutes to dry up any remaining ethanol in the membrane. The spin column was placed in a 1.5 ml centrifuge tube. Then 30 µl of T.E buffer/ elution buffer was added and the sample was incubated at room temperature for five minutes. Centrifugation at maximum speed was done. Another 30 µl elution buffer was added into the spin column and incubated at room temperature for 5minutes. Centrifugation at maximum speed was done. Eluted DNA was stored at 4 ºC.

Optimization Protocol 3- Isolation with Proteinase K with Long Term Heating

Twenty microliters of Proteinase K were pipetted into a 1.5 ml microtube. Two hundred microliters (200 µl) of blood sample were added into the micro-tube. Then 200 µl of lysis buffer 1 (10 mM Tris HCl, 1 mM EDTA and 2 % SDS) and lysis buffer 2 (10 mM Tris HCl, 1 mM EDTA and 0.2% Triton X-100) was added and vortexed for 15seconds to homogenize. The samples were heated at 56 ºc for 150 minutes. Two hundred (200 µl) of ice-cold absolute ethanol (98-100 %) was added and the sample again pulse vortexed to mix. The samples were centrifuge (Eppendorf) to bring down the samples from the cap into the tubes. The lysate /mixture were pipetted into the spin column and centrifuge at 11,000 rpm for one and a half minutes. The remaining lysate/mixture was centrifuge for another one and a half minute till the spin column was dry. Five hundred (500 µl) of wash buffer 1 was pipetted into the spin column and centrifuged at 11,000 rpm for one and a half minutes. Then 500 µl of wash buffer 2 was also pipetted into the spin column and centrifuge at 11,000 rpm for one and a half minutes. The spin column was spanned dry at 13,000 rpm for three minutes to dry up any remaining ethanol in the membrane. The spin column was placed in a 1.5 ml centrifuge tube. Then 30 µl of T.E buffer/ elution buffer was added and the sample was incubated at room temperature for five minutes. Centrifugation at maximum speed was done. Another 30 µl elution buffer was added into the spin column and incubated at room temperature for 5 minutes. Centrifugation at maximum speed was done. Eluted DNA was stored at 4 ºC.

Optimization Protocol 4- Isolation without Proteinase K with Long Term Heating

Two hundred microliters (200 µl) of blood sample were added into a microtube. Then 200µl of lysis buffer 1 (10 mM Tris HCl, 1 mM EDTA and 2 % SDS) and lysis buffer 2 (10 mM Tris HCl, 1 mM EDTA and 0.2% Triton X-100) was added and vortexed for 15 seconds to homogenize. The samples were heated at 60 ºc for 180 minutes. Two hundred microliters (200 µl) of ice-cold absolute ethanol (98-100 %) were added and the sample pulse vortex to mix. The samples were centrifuge (Eppendorf) to bring down the samples from the cap into the tubes. The lysate/mixture were pipetted into the spin column and centrifuge at 11,000 rpm for one and a half minutes. The remaining lysate/mixture was centrifuge for another one and a half minute till the spin column was dry.

Five hundred microliters (500 µl) of wash buffer 1 was pipetted into the spin column and centrifuged at 11,000 rpm for one and a half minutes. Then 500 µl of wash buffer 2 was also pipetted into the spin column and centrifuge at 11,000 rpm for one and a half minutes. The spin column was spanned dry at 13,000 rpm for three minutes to dry up any remaining ethanol in the membrane. The spin column was placed in a 1.5ul centrifuge tube. Then 30 µl of T.E buffer (10 mM Tris HCl, 1 mM EDTA pH 7.4)/ elution buffer was added and the sample was incubated at room temperature for 5 minutes. Centrifugation at maximum speed was done. Another 30 µl elution buffer was added into the spin column and incubated at room temperature for 5 minutes. Centrifugation at maximum speed was done. Eluted DNA was stored at 4 ºC.

Genomic DNA Isolation Using kit

The isolation of Genomic DNA using the Qiagen kit was done according to the manufacturer's protocols. Twenty microliters (20 µl) of QIAGEN proteinase K was pipetted into the bottom of a 1.5 ml micro centrifuge tube. Then 200 µl of blood sample were added to the micro centrifuge tube. Two hundred (200 µl) of buffer AL was added to the sample and vortexed for 15 seconds to mix to ensure efficient lysis. The sample was incubated at 56 ºC for 10minutes and centrifuge briefly to remove drops from the inside of the lid. Two hundred microliter (200 µl) of ice-cold absolute ethanol (98-100 %) was added to the sample and mixed again by pulse-vortexing for 15 seconds and then centrifuge to remove drops from the inside of the lid. The mixture was carefully applied to the QIAamp Mini spin column without wetting the rim. The cap was closed to avoid aerosol formation and centrifuged at 8,000 rpm for 1 minute. The QIAamp Mini spin column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded. If the lysate has not completely passed through the column after centrifugation, centrifugation was done again at higher speed until the QIAamp Mini spin column was empty. The QIAamp Mini spin column was carefully opened and 500 µl buffer AW1 was added without wetting the rim. The cap was closed and centrifuge at 11,000 rpm for 1minute. The QIAamp Mini spin column was placed in a clean 2 ml collection tube and the collection tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened again and 500 µl AW2 was added without wetting the rim. The cap was closed and centrifuge at full speed for 3 minutes. The QIAamp Mini spin column was then placed in a clean 1.5 ml micro centrifuge tube and the collection tube containing the filtrate was discarded. The QIAamp Mini spin column was carefully opened and 60 µl of buffer AE was added. The sample was incubated at room temperature for 10 minutes and then centrifuge at 8,000 rpm for 1 minute. Eluted DNA was stored at 4 ºC.

Genomic DNA Quantification and Quality Assessment

Quantification of DNA Using Spectrophotometry

The Genomic DNA concentrations (ng/µl) and purity and the optical density (OD) ratio A260/280 were calculated by measuring the OD at wavelength of 260 nm and 280 nm using a spectrophotometer. Purity of DNA samples is assessed in a 260/280 absorbance ratio, and the values in the range 1.8-2.0 are generally considered acceptable (Gallagher & Desjardins, 2007). Any value lesser then 1.8 or higher than 2.0 is said to have a ribonucleic acid (RNA) contamination. The spectrophotometer was turned on and allowed to warm up for some minutes before running samples. A 5 0µl T.E buffer (10 mM Tris HCl, 1 mM EDTA pH 7.4) was loaded into a clean cuvette and use to blank the spectrophotometer making sure it is set to zero. When using the cuvette, it was made sure to avoid touching the sides that the light will pass through. After blanking, the cuvette is removed and a 5 µl of the DNA sample is added into the cuvette- mixing the two solutions without bubbles. The cuvette was placed back into the spectrophotometer measuring the absorbance of the sample. Different pipettes are used for each sample to prevent cross contamination.

Quality Check using Agarose Gel Electrophoresis

Running of 1% Agarose Gel Electrophoresis

A 0.5g of agarose was measured using an analytical weighing balance and mixed with 50ml Tris Borate EDTA (TBE) buffer in a microwavable flask. The solution was microwaved until the agarose was completely dissolved. After cooling at room temperature, a red safe dye was added to the solution and the agarose was poured into a gel tray fitted with well comb. The agarose was allowed at room temperature until it was completely solidified.

The solidified agarose was placed into an agarose gel tank and filled with TBE buffer until the gel was immersed. The DNA samples mixed with red safe dye were carefully loaded into the wells of the gel. The gel was run at 70 millivolts for 30minutes and a trans-illuminator was used to visualize the DNA fragments.

RESULTS

Isolated DNA quality

The quality of genomic DNA for four different experimental conditions in the Double Lysis method and the Kit (Qiagen) were determined. As shown in plate 1 and 2 the kit (Qiagen) extraction method showed a clear band in the agarose gel electrophoresis analysis. In the double lysis method not all the experimental conditions experimented showed clear DNA band. The heating for 180minutes without proteinase K and the heating for 150minutes with proteinase K did not show any DNA band in the agarose gel electrophoresis analysis. In the isolation with proteinase K and heating for 10minutes only rat blood samples (4p) show clear band in the agarose gel electrophoresis analysis while in the heating for 90 minutes with proteinase K, the first DNA elution shows a clear DNA band in only one human blood and rat blood (3s and 4s) and in the second elution only rat and the mouse blood (4q and 5q) show clear band of DNA in the agarose gel electrophoresis analysis.

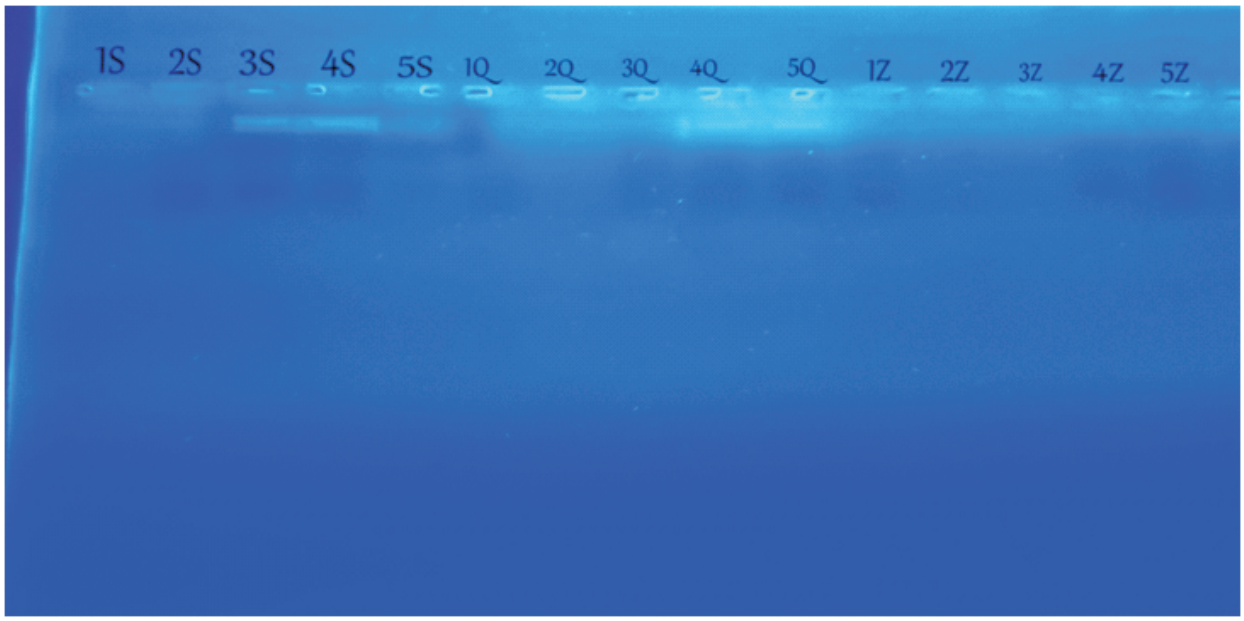
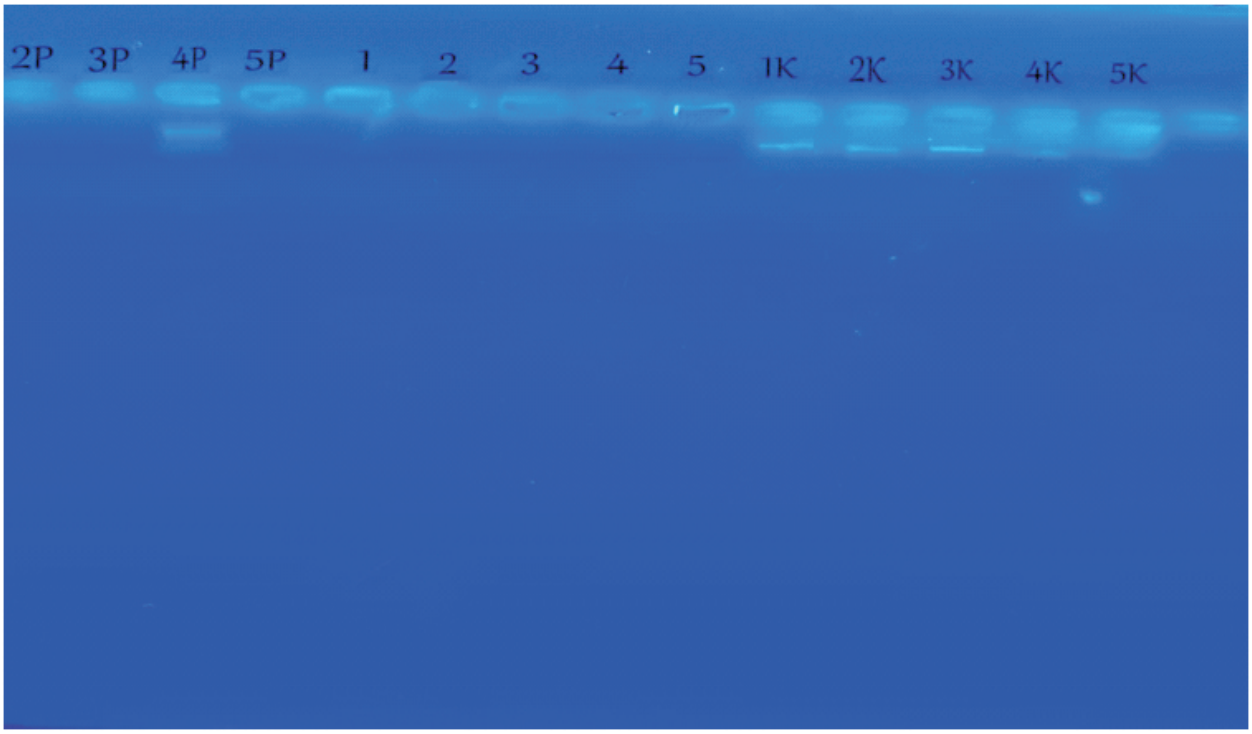


Plate 1: Agarose Gel Electrophoresis of Genomic DNA Samples Isolated from Mammalian Blood Using Double Lysis method



Agarose Gel Electrophoresis of Genomic DNA samples Isolated from Mammalian Blood Using Kit and Double Lysis method.

Quantification of Isolated DNA

The yield and purity range of genomic DNA from the two methods were determined (Table 2), the DNA concentrations was used to compare the yield. As shown in table 2, the Kit (Qiagen) extraction method produced the highest concentration on average compared to the two other conditions experimented on from the Double lysis method. The average concentration for the Kit (Qiagen) method, the 10minutes heating with proteinase K and the 18 0minutes heating without proteinase K of the Double lysis method are 740 ng/µl, 527µg/ml and 610µg/ml respectively.

Table 2: Quantity Check Result of Genomic DNA from Double Lysis and the QIAGEN Kit Protocol.

Sample	DNA Concentration (ng/µl)	Absorbance ratio 260/280	260/230	230	260	280	340	Molecular weight
1k	735	1.47	0.68	0.249	0.169		0.028	186
2k	690	0.115					0.099	363
3k	785	1.38	0.53	0.628	0.330	0.239	0.025	204
4k	765	1.57	0.67	0.27	0.185		0.021	209
5k	725	0.118					0.103	410
1p	570	1.53	0.62	0.24	0.182		0.283	926
2p	515	0.112					0.03	137
3p	590	1.45	0.43	0.833	0.373		0.572	406
4p	380	0.258					0.054	402
5p	580	1.14	0.43	1.937	0.842		0.564	1001
1	740	0.738					0.019	49
2	570	1.03	0.32	0.396	0.123		0.237	460
3	515	0.121					0.649	684
4	625	1.18	0.88	1.068	0.812		0.126	268
5	600	0.587					0.123	263
		0.76	0.22	1.638	0.365			
		0.478						
		1.16	0.84	1.085	0.910			
		0.786						
		1.48	0.18	0.248	0.044			
		0.030						
		1.14	0.25	1.689	0.418			
		0.365						
		1.03	0.69	0.903	0.622			
		0.602						
		1.25	0.36	0.675	0.244			
		0.194						
		1.20	0.55	0.432	0.239			
		0.199						

DISCUSSION

This study demonstrates that genomic DNA extraction efficiency from mammalian blood is strongly influenced by incubation duration, enzymatic digestion, and overall protocol design. The consistently superior DNA quality obtained using the Qiagen kit, evidenced by clearer and more distinct electrophoretic bands, aligns with both earlier and recent reports emphasizing the reliability and reproducibility of silica column–based commercial extraction systems (Lee *et al.*, 2010; Na *et al*., 2025). However, despite producing highly pure DNA, the relatively low DNA yield observed in this study supports ongoing concerns regarding the cost-effectiveness and scalability of commercial kits for large-sample or resource-limited studies, a limitation also noted in recent comparative evaluations (Psifidi *et al.*, 2015; Vásquez Bonilla *et al.*, 2024).

In contrast, the double lysis method demonstrated variable performance across experimental conditions, producing detectable DNA bands only under selected protocols. This variability is consistent with earlier investigations by Lahiri *et al.* (1992), who compared multiple solution-based DNA extraction methods from whole blood and reported marked differences in DNA yield, purity, and integrity as visualized by gel electrophoresis. Similar observations were reported by Gustincich et al. (1991) and Olerup and Zetterquist (1992), where CTAB and salting-out based methods yielded DNA of differing purity and concentration, highlighting the sensitivity of in-house protocols to procedural conditions.

The protocol-specific outcomes observed in this study, where certain double lysis conditions yielded clear DNA bands while others failed, mirror findings from Psifidi *et al.* (2015), who demonstrated that modified in-house and commercial protocols often outperform standard methods in terms of DNA purity and concentration, albeit with variability in total yield. More recent studies further reinforce that targeted optimization of low-cost extraction workflows, including controlled incubation times and enzymatic treatment, can significantly improve DNA recovery and quality (Neal *et al.*, 2024; Vásquez Bonilla *et al.*, 2024). Collectively, these findings suggest that while commercial kits remain the gold standard for DNA purity, optimized low-cost methods such as double lysis protocols hold promise as viable alternatives when carefully refined for specific sample types and laboratory constraints.

CONCLUSION

Two extraction methods were examined for effectiveness and efficiency in extracting and purifying high quantity and quality genomic DNA from whole blood samples. Four protocols were optimized in the Double Lysis Optimization methods and it can be seen that heating for a longer period of time does not have any effect on the extraction process since no DNA band was produced of the long-term heating on the agarose gel electrophoresis analysis and the yield from the spectrophotometry result are relatively low.Comparison of the extraction methods shows that the Kit (Qiagen) method has the highest quality and quantity DNA isolated from whole blood samples compared to the Double Lysis method. Therefore, more optimization still needs to be done to improve the quality and quantity of the genomic DNA in the double lysis method.

AUTHORS' CONTRIBUTIONS

TAF – Conceptualisation, Methodology, Formal analysi, Supervision, Project Administration, Writing – Review & Editing, Resources.**OOO** – Investigation, Formal analysis, Visualisation, Writing – Original Draft, Resources. **ANY** – Investigation, Writing – Review & Editing. **VT** – Investigation, Formal analysis, Resources. **RLH** – Investigation, Formal analysis, Ethical approval acquisition, Resources. **JOA** – Investigation, Writing – Review & Editing.

FUNDING

This research was fully financed by the authors. No external funding or grant support was received for this study.

CONFLICT OF INTEREST

The authors declare no conflict or competing of interest.

REFERENCES

Andrews, S., Molbert, M., & Samsonova, M. (2022).Comparative evaluation of DNA extraction methods for molecular applications.***Molecular Genetics and Genomics***, 297(5), 1235-1247.

Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M. and vander Noordaa, J. (1990).Rapid and simple method for purification of nucleic acids.*Journal of ClinicalMicrobiology*.**28**(3): 495-503.

Carpi, F. M., Pietro, F. D., Vincenzetti, S., Mignini, F. and Napolioni, V. (2011). Human DNA extraction methods: patents and applications. *Recent Pathology DNA Gene Sequence*. **5**(1):1-7.

Chacon-Cortes, D. and Griffiths, L. R. (2014). Methods for extracting genomic DNA from whole blood samples: current perspectives. *Journal of Biorepository Science and Applied Medicine*.**2:**1-9.

Dahm, R. (2005). Friedrich Miescher and the discovery of DNA.*Developmemtal Biology*.**278**(2): 274-288.

Daniels, B.ℕ., Nurge, J., Sleeper, O., Lee, A., López, C., Christie, M.ℝ., Toonen, R.𝕁., White, C., & Davidson, J.ℳ. (2023). *Genomic DNA extraction optimization and validation for genome sequencing using the marine gastropod Kellet's whelk.PeerJ, 11*, Article e16510

Gustincich, S., Manfioletti, G., Del Sal, G., Schneider, C. and Carninci, P. (1991).A fast method for high-quality genomic DNA extraction from whole human blood.*BioTechniques*.**11** (3): 298-301.

Lahiri, D.K., Bye, S., Nurnberger, J.I., Jr. Hodes, M.E. and Crisp, M. (1992). A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. *Journal of Biochemistry and Biophysics Methods*.**25**(4): 193-205.

Lee, J. H., Park, Y., Choi, J. R., Lee, E. K. and Kim, H. S. (2010).Comparisons of three automated systems for genomic DNA extraction in a clinical diagnostic laboratory.*Yonsei Medical Journal*.**51**(1): 104 -110.

Mekonnen, T., Woldemichael, K., & Abebe, T. (2024).Optimizing phenol-chloroform DNA extraction for clinical samples: Efficiency and safety assessment.*Journal of Molecular Diagnostics*, 26(1), 45-55.

Miller, S. A., Dykes, D. D. and Polesky, H. F. (1998).A simple salting out procedure for extracting DNA from human nucleated cells.*Nucleic Acids Resources*.**16**:12-15.

Moradi, M.T., Yari, K. h. and Khodarahmi, R. (2014). A novel efficient, fast and inexpensive DNA extraction protocol from whole blood applicable for studying drug-DNA interaction. *Journal of Reproductive Pharmaceutical Science*.**3**:80-84.

Motamedi, J., Zebarjadi, A. R, Kahrizi, D. and Salmanian, A. H. (2011).*In vitro* propagation and *Agrobacterium*-mediated transformation of safflower (*Carthamus tinctorius* L.) using a bacterial mutated *aro*A gene. *Austrialian Journal of Crop Science*.**5**(4):479-486.

Na, W., Kim, S., & Lee, H. (2025).Evaluation and optimization of genomic DNA extraction protocols from mammalian blood samples.*Journal of Molecular Biology Methods*, 152, 113254.

Neal, C. J., Zbinden, Z. D., Douglas, M. E., & Douglas, M. R. (2024). *Reducing DNA extraction costs through factorial design for the DNAdvance Kit*. *BMC Research Notes, 17*(1), 397.

Nguyen, L.-T. T., Le, N.-H. T., Ta, H. K. T., & Nguyen, K. D. (2022). Isolation of DNA from Arthrospira platensis and whole blood using magnetic nanoparticles *(Fe_3O_4@OA and Fe_3O_4@OA@SiO_2)*. *Journal of Analytical Science and Technology, 13*(1), Article 12.

Olerup, O. and Zetterquist, H. (1992). HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSCP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. *Tissue Antigens*.**39** (5): 225-235.

Psifidi, A., Dovas, C. I, Bramis, G., Lazou, T., Russel, C. L and Arsenos, G.(2015). Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using blood samples.*PLoS ONE* .**10**(1): 0115960.

Sambrook, J., Russell, D. W. and Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual (3-Volume Set). Cold spring harbor laboratory press, Cold Spring Harbor, New York.

Schweighardt, A. J., Tate, C. M., Scott, K. A., Harper, K. A. and Robertson, J. M. (2015).Evaluation of commercial kits for dual extraction of DNA and RNA from human body fluids.*Journal of Forensic Science*. **1**:157-165.

Tan, S. C., Yiap, B. C. (2009). DNA, RNA, and protein extraction: the past and the present. Journal of Biomedical Biotechnoogy.**2009**:1-10.

Vásquez Bonilla, M. M., Guerrero-Freire, M. S., Ledesma, Y., Laglaguano, J. C., & de Waard, J. H. (2024).A rapid and inexpensive 96-well DNA-extraction method from blood using silicon dioxide powder (Glassmilk).*Biology Methods & Protocols, 9*(1), bpae079.

Weerakkody, R., & Witharana, C. (2024).Cost-effective high-molecular-weight DNA extraction from whole blood using modified salting-out protocols.*Molecular Biology Reports*, 51(2), 1015-1025.

Wink, M. (2009). An introduction to molecular biotechnology: molecular fundamentals, methods and application in modern biotechnology. Wiley-VCH, Weinheim, Germany.