DOI: https://doi.org/10.5281/zenodo.17164110

Original Research

Evaluation of DNA Yield from Different DNA Extraction Protocols in Various Weights of Tissue Samples

Aparna Sajeevan [a], Muhammad Nasir Ahmed [a], Vina R Vaswani [a], Mohammad Amjad Hussain [b], Sudheer Shenoy P [c]

Abstract

Forensic investigations often rely on successfully extracting DNA from biological samples to identify individuals or establish evidence. The efficiency of DNA extraction protocols can vary significantly depending on the sample type, weight, and extraction method used. This study evaluates the DNA yield and quality from four commonly used DNA extraction protocols across varying weights of tissue samples. The results highlight the importance of selecting an appropriate extraction method and sample weight to optimize DNA yield for forensic applications. The methods regarding yield (according to tissue mass) and purity (A260/280) were compared and it was found that in the present experiment, 50 mg tissue sample is the optimum weight for good yield of DNA at the purity range of 1.8-1.9. This manuscript aims to determine the optimum tissue sample size to provide a good yield of DNA in animal tissue samples using four different DNA extraction protocols.

Keywords: DNA extraction, Quantitative analysis, Qualitative analysis, DNA yield, sample weight

Introduction:

Forensic investigations, a field that applies scientific techniques to analyze evidence from crime scenes, play a crucial role in our society. They often involve the identification of perpetrators or victims through physical or biological traces.[1] DNA analysis, a cornerstone of modern forensics, is a powerful tool that enables the identification of individuals, establishes familial relationships, links suspects to crime scenes, and even exonerates the wrongfully convicted.[2] The reliability of forensic DNA analysis depends on the quality and quantity of DNA extracted from biological samples, underscoring the importance of this work in our society.

DNA extraction is isolating DNA from biological samples, cells, or tissues. This process is critical in forensic contexts because the extracted DNA must be of sufficient quantity and quality for downstream applications such as PCR amplification, sequencing, genotyping, and other profiling techniques. However, the efficiency of DNA extraction can vary depending on the sample type, tissue weight, preservation conditions, and the extraction protocol used. High-quality DNA extraction is essential for accurate analysis and reliable results in forensic applications.[3-6]

One of the key challenges in forensic DNA extraction is that a protocol optimized for one sample type may not apply to another.[7] For instance, a protocol effective for blood samples may not yield high-quality DNA from bone or hair, and the protocol that

[a] Forensic Anthropology Unit, Department of Forensic Medicine and Toxicology, Yenepoya Medical College, [b] Molecular Genetics Division, Yenepoya Research Centre, [c]Stem Cells and Regenerative Medicine Centre, Yenepoya Research Centre, Yenepoya Deemed to be University, Mangalore, Karnataka
Correspondence: Dr. Muhammad Nasir Ahmad
Email: nasirforanth@gmail.com ; nasir@yenepoya.edu.in
Contact: +919406532084

Received on 26.01.2025 | Accepted on 31.03.2025

works well for fresh tissue samples may not be efficient for degraded tissue samples [5]. Additionally, the weight of the tissue sample can significantly influence DNA yield, making it essential to determine the optimal sample size for extraction.[8] The sample size and weight should be carefully considered when estimating DNA quantity and quality without proceeding to PCR. Larger sample weights may yield more DNA but could also introduce inhibitors or degrade DNA quality. Conversely, smaller samples may provide insufficient DNA for accurate quantification.[9] This study explores the relationship between sample weight and DNA yield across different extraction protocols.

The current four DNA extraction methods selected for this study are widely used in forensic investigations. Each method has distinct advantages and limitations depending on the sample type and the level of DNA degradation. The methods are compared to evaluate which produces the highest yield and quality across varying sample weights. Hence, this study aims to determine the ideal weight for tissue samples when only DNA quantity and quality are the focus using different DNA extraction protocols.

Materials and Methods:

The muscle tissue samples of *Bubalus Bubalis* were collected procured from a local abattoir in Mangalore and divided into varying weights (25 mg, 50 mg, and 100 mg) and DNA extraction was carried out in triplicate using four different protocols to evaluate the impact of sample weight on DNA yield.

The four DNA isolation protocols were Forensic Protocol;[10,11] Standard Protocol;[12,13] Modified Protocol - I, and Modified Protocol - II.

The study was conducted in India's first Taphonomy Center - Forensic Anthropology Center for Taphonomy (FACT) at the Forensic Anthropology Unit, Department of Forensic Medicine and Toxicology, Yenepoya Medical College, Karnataka.

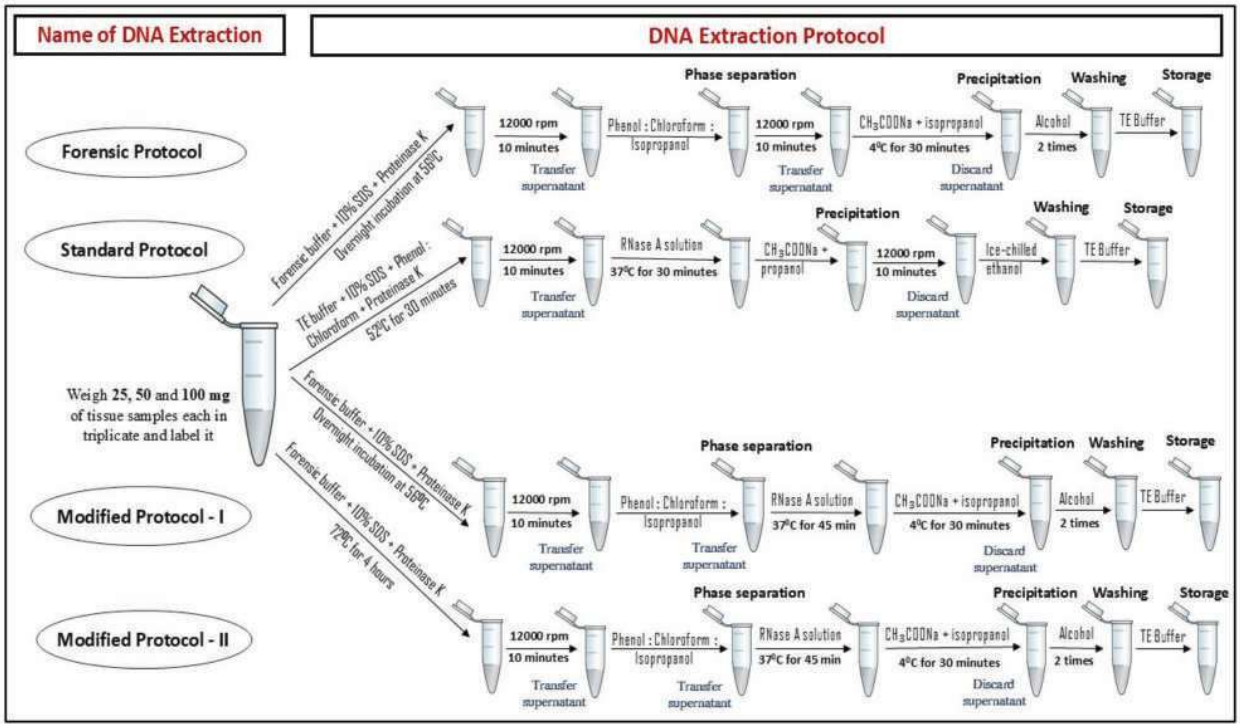

Figure 1: Schematic representation of summarized DNA isolation protocols
(The test tube vector image was adopted from Shutterstock)

Result and Discussion:

The absorbance A260/280 nm ratio, which shows the range of 1.8 to 1.9, is considered standard for DNA purity.[14,15]

The following are the observations of the present study:

- ***DNA Yield (ng/µl):*** In the Forensic Protocol, the DNA yield increased with the increase in sample weight and obtained good purity of DNA.
- ***DNA Quality (A260/280):*** Modified Protocol - I followed by Forensic Protocol provided consistent quality across all sample weights.
- ***Protocol Efficiency:*** The Modified Protocol - I, followed by the Forensic Protocol, was effective but required more time for DNA extraction. The Standard Protocol was faster and more rapid but yielded poor-quality DNA from all the tissue samples.

Sample Size and Weight: For DNA quantification and quality estimation (without PCR), tissue weights from 50 mg were optimal, yielding good purity of DNA.

In the present study, Modified Protocol - I followed by Forensic Protocol emerged as the most effective and efficient method for high-quality DNA extraction for all the weights of tissue samples, offering a good balance of yield and purity, whereas Standard Protocol and Modified Protocol-II have not resulted in good purity and yield of DNA. While using another DNA extraction method that we modified, the first protocol (The Modified Protocol - II) was inefficient in terms of optimum yield of DNA this may be due to various factors, including processing the DNA extraction such as lysis or over lysis of tissue, RNAse treatment etc.

The qualitative analysis was carried out using Gel Electrophoresis in which Forensic Protocol and Modified Protocol - I have

DNA extraction method	Weight of tissue	Yield (ng/µl)	A260/280
Forensic Protocol	25 mg	478.29	2.05
	50 mg	**1877.82**	**1.98**
	100 mg	2235.53	1.86
Standard Protocol	25 mg	406.40	2.66
	50 mg	214.27	1.15
	100 mg	284.51	2.32
Modified Protocol - I	25 mg	319.39	1.92
	50 mg	**200.41**	**1.89**
	100 mg	2059.13	1.80
Modified Protocol - II	25 mg	113.77	1.30
	50 mg	41.01	1.45
	100 mg	**195.55**	**1.00**

Table 1: The yield and average values of the A260/280 ratio for tested DNA extraction methods for different weights of the tissue

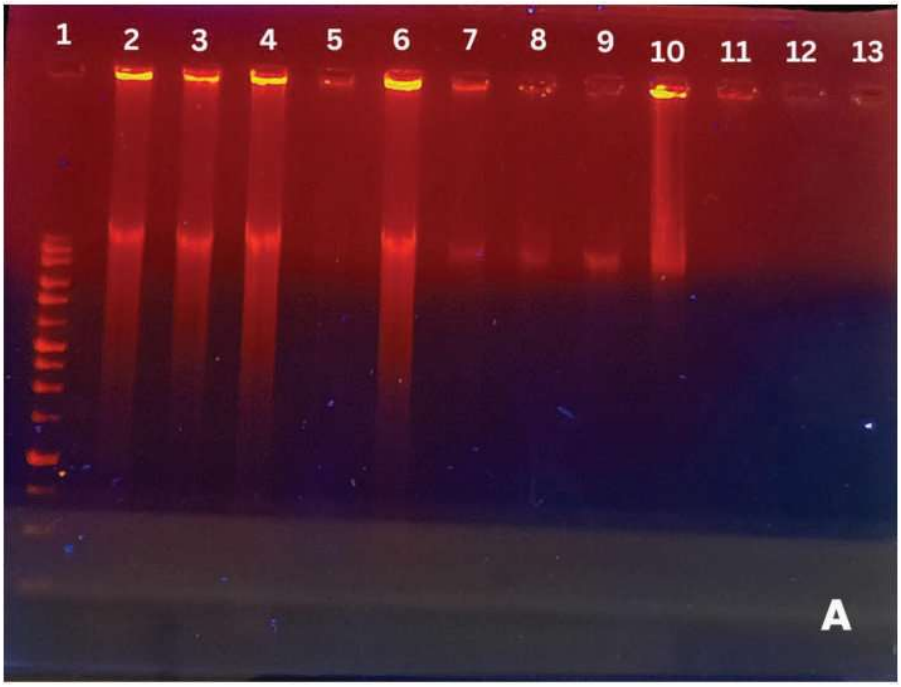

Figure 2 : DNA bands visualized under UV for 25 mg tissue sample
[Well 1: DNA Ladder; Well 2-4: Forensic Protocol; Well 5-7: Modified Protocol I; Well 8-10: Modified Protocol II; Well 11-13: Standard Protocol]

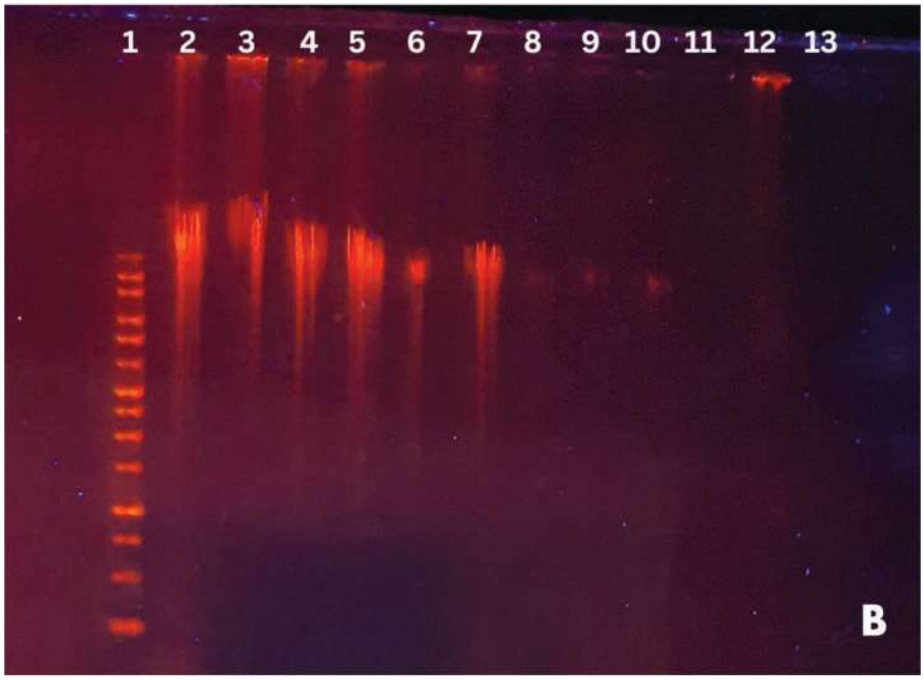

Figure 3 : DNA bands visualized under UV for 50 mg tissue sample
[Well 1: DNA Ladder; Well 2-4: Forensic Protocol; Well 5-7: Modified Protocol I; Well 8-10: Modified Protocol II; Well 11-13: Standard Protocol]

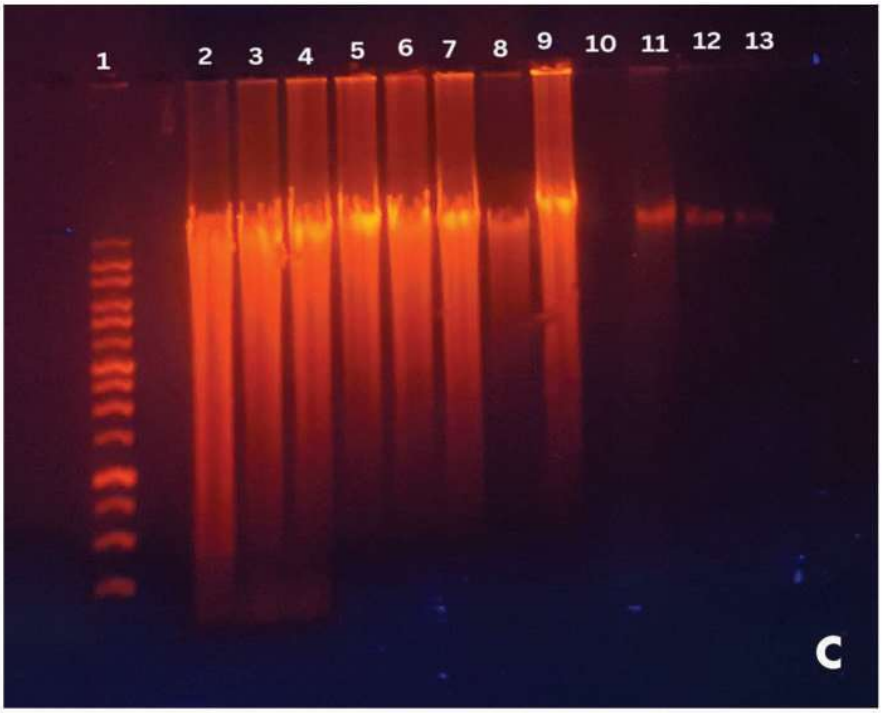

Figure 4 : DNA bands visualized under UV for 100 mg tissue sample
[Well 1: DNA Ladder; Well 2-4: Forensic Protocol; Well 5-7: Modified Protocol I; Well 8-10: Modified Protocol II; Well 11-13: Standard Protocol]

obtained DNA bands when visualised under UV transillumination whereas no visible bands were visualized for the Standard Protocol.

The poor yield was reported by various authors[15-17] in which values of DNA purity were not in the range of 1.8-1.9, indicating the presence of contamination in the DNA extraction. Furthermore, the previous study[18] reported that Standard Protocols may not be free of contamination and toxicity in producing high quantity and quality DNA, which could also be a limitation in this study.

It was reported that animal species and types of tissue samples were used to extract good DNA yield at a purity of 1.89, varying with the weights of the samples used for extraction.[19] A study conducted on mesenteric lymph nodes of pigs to extract optimum DNA yield reported that 600 mg sample weight produced 600.58 ng/µl with a DNA purity of 1.89[14] and contrary to the study[19] found that 20 mg Shark muscle sample yielded 5.15 µg with purity of 1.75. The present study results of DNA concentration and purity were slightly different, which may be due to the tissue and animal type.

Conclusion:

This study highlights the importance of selecting the correct DNA extraction protocol based on the tissue type and the intended forensic application. It demonstrates that the choice of DNA extraction protocol and sample weight significantly impacts DNA yield and quality in forensic contexts. While Modified Protocol—I is robust for obtaining high-quality DNA across various tissue samples, the Forensic Protocol offers a rapid alternative. Standard Protocol, though reliable, is less practical for high-throughput forensic applications.

Our findings suggest that tissue weights of 50 mg are optimal for simple DNA quantification and quality assessment (without PCR). These findings can guide forensic laboratories in selecting the

appropriate DNA extraction method and sample size for non-PCR-based DNA profiling, ensuring that the quality of evidence is not compromised in criminal investigations.

Limitation of the study:

This study is only conducted on *Bubalus Bubalis* samples; further research on different animal tissue samples is suggested to validate the result.

Further research should focus on applying these methods to highly degraded or challenging forensic samples, such as those from older cases or environmental exposures. Additionally, exploring the cost-benefit ratio of each technique in real-world forensic settings could help determine the most practical options for routine casework.

Acknowledgement:

We acknowledge the Department of Forensic Medicine and Toxicology, Forensic Anthropology Centre for Taphonomy (FACT) and Yenepoya deemed to be University, Mangalore

Authorship: All authors contributed critically to the manuscript draft and gave final accordance for publication.

Funding: This research was supported by the intramural seed grant of Yenepoya deemed-to-be University (YU/Seed grant/173/2024) under which the doctoral study of First author is ongoing.

Conflict of Interest Statement: The authors had declared no conflict of interests.

Ethical Considerations: The study was approved by the Institutional Animal Ethics Committee with a reference number of YU/IAEC/P(L)09/2024 dated 21.09.2024

References:

1) Milliet Q, Delémont O, Margot P. A forensic science perspective on the role of images in crime investigation and reconstruction. Science & justice. 2014 Dec 1;54(6):470-80.
2) Faisal SM, Khan AS, Shahid A. The Role of Forensic Evidence in Modern Criminal Investigations. Pakistan Islamicus (An International Journal of Islamic & Social Sciences). 2024 Jan 13;4(01):01-12.
3) Chong KW, Thong Z, Syn CK. Recent trends and developments in forensic DNA extraction. Wiley Interdisciplinary Reviews: Forensic Science. 2021 Mar;3(2):e1395.
4) Lear G, Dickie I, Banks J, Boyer S, Buckley HL, Buckley TR, Cruickshank R, Dopheide A, Handley KM, Hermans S, Kamke J. Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples. New Zealand Journal of Ecology. 2018 Jan 1;42(1):10-50A.
5) Buś MM, Allen M. Collecting and preserving biological samples from challenging environments for DNA analysis. Biopreservation and biobanking. 2014 Feb 1;12(1):17-22.
6) Nagy ZT. A hands-on overview of tissue preservation methods for molecular genetic analyses. Organisms Diversity & Evolution. 2010 Mar;10:91-105.
7) Adamowicz MS, Stasulli DM, Sobestanovich EM, Bille TW. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. PloS one. 2014 Dec 30;9(12):e116351.
8) Bürgmann H, Pesaro M, Widmer F, Zeyer J. A strategy for optimizing quality and quantity of DNA extracted from soil. Journal of microbiological methods. 2001 May 1;45(1):7-20.
9) Ellison SL, English CA, Burns MJ, Keer JT. Routes to improving the reliability of low level DNA analysis using real-time PCR. BMC biotechnology. 2006 Dec;6:1-1.

10) Pawar SG, Harel VS, More BP, Kulkarni KV. DNA Y-STR Typing is an Important Tool to Solve Criminal Cases. International Journal of Biotech Trends and Technology. 2019;9(1):15-21.
11) Bhati P. Significance of autosomal STR Markers Kits to determine sexual assault case involving brother-in-law. Res. J Forensic Sci. 2023 July;11(2):7-14.
12) Gautam A. Phenol-chloroform DNA isolation Method. In DNA and RNA Isolation Techniques for Non-experts 2022 Mar 30 (pp. 33-39). Cham: Springer International Publishing.
13) Barbier FF, Chabikwa TG, Ahsan MU, Cook SE, Powell R, Tanurdzic M, Beveridge CA. A phenol/chloroform-free method to extract nucleic acids from recalcitrant, woody tropical species for gene expression and sequencing. Plant methods. 2019 Dec; 15:1-3.
14) Bo YY, Liang LD, Hua YJ, Zhao Z, Yao MS, Shan LB, Liang CZ. High-purity DNA extraction from animal tissue using picking in the TRIzol-based method. Biotechniques. 2021 Mar 1;70(3):186-90.
15) Psifidi A, Dovas CI, Banos G. A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. Molecular and cellular probes. 2010 Apr 1;24(2):93-8.
16) Manuja A, Manchanda S, Kumar B, Khanna S, Sethi RK. Evaluation of different methods of DNA extraction from semen of buffalo (Bubalus bubalis) bulls. Buffalo Bull. 2010 Jun 1;29(2):109-28.
17) Psifidi A, Dovas CI, Bramis G, Lazou T, Russel CL, Arsenos G, Banos G. 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. 2015 Jan 30;10(1):e0115960.
18) Silva EC, Pelinca MA, Acosta AC, Silva DM, Gomes Filho MA, Guerra MM. Comparative study of DNA extraction methodologies from goat sperm and its effects on polymerase chain reaction analysis. Genet Mol Res. 2014 Aug 7;13(3):6070-8.
19) Peñafiel N, Flores DM, Rivero De Aguilar J, Guayasamin JM, Bonaccorso E. A cost-effective protocol for total DNA isolation from animal tissue. Neotropical Biodiversity. 2019 Jan 1;5(1):69-74.