International Journal of Forensic Science and Legal Medicine
Vol. 4, Issue-1, January-June 2024

THE QUANTITATIVE ASSESSMENT OF THE GENOME FOR FORENSIC ANALYSIS ON CIGARETTE BUTTS AT DIFFERENT TIME INTERVALS USING QPCR ANALYSIS

Shimpi R Sameer[1], Anita Yadav[2], Tanushree Dheer[3], Rajesh Kumar[4]*

[1, 2 & 3] Forensic Science, School of Sciences, Sanjeev Agrawal Global Educational (SAGE) University, Bhopal, Madhya Pradesh - 462022
[4]Forensic Medicine & Toxicology, All India Institute of Medical Sciences, Deoghar, Jharkhand – 814152

ABSTRACT

Cigarette butts are valuable forensic evidence, but their quality and quantity can be affected by environmental factors and deposition time. This thesis aims to enhance the accuracy and reliability of DNA analysis using quantitative polymerase chain reaction (qPCR) analysis. Controlled experiments are conducted using simulated cigarette butt samples under different environmental conditions and time intervals. The results reveal variations in DNA degradation patterns over different time intervals, allowing for precise determinations of DNA quantity and comparisons between samples. The findings provide insights into the stability and degradation rates of DNA on cigarette butts, enabling forensic investigators to determine the optimal time frame for successful DNA analysis. This study has significant implications for forensic scientists, investigators, and legal professionals involved in criminal cases involving cigarette butt evidence. Further research is needed to explore additional factors influencing DNA degradation and refine q-PCR methodologies for forensic DNA analysis on cigarette butt evidence.

Keywords: DNA, cigarette, Research, analysis, sample, forensic, time intervals, butt, evidence, degradation, etc.

INTRODUCTION

Forensic science is increasingly utilizing DNA molecular biology techniques for diagnostic purposes, particularly in high-resolution personal identity testing. Over the past 20–25 years [1–3], researchers have developed accurate DNA typing tools, enabling the identification of one or a few origins for evidence samples. Technological advances and validation requirements contribute to their high accuracy [4].69 member countries have national DNA databases with 35 million profiles, according to an INTERPOL review in 2017. In police investigations, 84 of the nations used mitochondrial DNA and Y-chromosome STR analysis. The review covered autosomal STRs, Y-STRs, SNPs, InDel markers, body fluid identification, and genomic platforms[5]. The field of forensic science has matured, and advancements in technology are expected to be

***Corresponding Author Email:** drrajeshkumar.aiims@gmail.com

recognised more modestly. Academics initially used molecular biology for assessing evidence, and researchers now use PCR-based methods and automated fluorescence detection technologies [6-10] instead of Southern blot-based hybridization techniques[11-13]. DNA databases in forensic and criminal investigations improve resistance to change as they standardise microsatellite or short tandem repeat loci[14-15]. These databases are essential for investigating criminal activity and ensuring the accuracy of evidence.

Cigarette butts (CBs) are a significant waste material, with 5.7 trillion cigarettes consumed globally in 2016. 97% of cigarette filters are made of cellulose acetate, a naturally occurring modified polymer [16]. Australia consumes 20 billion filtered cigarettes annually, with 7 billion being thrown away into the environment [17]. China is the world's largest cigarette consumer, consuming 41.9% of all cigarettes. Between 2015 and 2025, Nigeria and Indonesia are expected to increase their smokers by 7 million and 24 million, respectively. Cigarette smoke contains over 4000 carcinogenic chemical components, including formaldehyde, argon, carbon monoxide, nitrogen oxides, ammonia, benzene, hydrogen cyanide, acetone, acetaldehyde, and pyridines. These components are formed during the burning or distillation of tobacco [18–19]. Cigarettes are manufactured using cellulose acetate filters [20], which partially or completely retain particle smoke components. However, poor biodegradability and the maximum 18-month disintegration period pose serious issues for proper waste disposal. Forensic molecular research, developed by Sir Alex Jefreys in 1985, uses DNA molecular understanding from biology and medicine to identify victims and offenders. This method is useful for investigating the victim's or offender's body and crime scene as evidence during a forensic medical examination. Common specimens used in DNA research include blood, sperm patches, vaginal swabs, buccal swabs, and bone. Forensic molecular techniques can help identify suspects by examining the victim's body and crime scene. Mucosal epithelial lip and saliva contain DNA from cigarettes, potentially enabling DNA profiling. This study suggests using cigarette butts for forensic identification, as DNA profiling has not been widely used in Indonesia until recently. The findings could provide insight into the potential of utilizing cigarette butts for forensic identification.

METHODOLOGY

Chemicals : Sol. B, 20% SDS, Proteinase K, Phenol-chloroform-Isoamyl alcohol mixture (25:24:1), 5M Sodium Acetate (pH 5.2), Chilled Isopropanol (IPA), 70% ethanol, Nuclease free water, Primers, dNTPs + Buffer etc.

Instruments: Micro-centrifuge, Autoclave, Refrigerator, Nanodrop Machine, Gel-Electrophoresis, PCR Machine, RT-PCR Machine, Cigarette butts collection, Scissor, Forceps, Spatula, Gloves, Petri dish, Pipette / Tips, Centrifuge Tubes, Tube stand / Holder, Eppendorf tubes 2 ml etc.

*Sample Collection***:** 30 cigarette butt samples were collected for 10 days, aseptically in separate autoclaved bags. After incubation, DNA extraction was performed with samples numbered 1-3 and 27-30. The butts were processed chronologically, with 1-3 samples processed first and 27-30 processed last.

Sample Pretreatment

PROCEDURE

DNA Extraction From Cigarette Butts

The cigarette butt was analyzed by cutting it into 2-3 pieces and adding 1.5ml of Solu. B, 75 µl of 20% SDS, and 10 µl of Proteinase K to the cut cloth. The mixture was incubated overnight, and then the liquid was squeezed out. Next, 250 µl of Sodium Acetate and 500 µl of PCI mixture were added, and the solution was centrifuged for 15 minutes. The upper layer containing DNA was transferred to a fresh tube. The DNA was precipitated with 500 µl of chilled IPA and incubated at -20°C overnight. Afterward, the sample was centrifuged for 10 minutes, and the pellet was washed with 70% ethanol and air-dried. Finally, the DNA was dissolved in 12 µl of nuclease-free water and visualized under UV in agarose gel electrophoresis.

Agarose Gel Electrophoresis

1. 1X TAE buffer was prepared by diluting 10X TAE buffer for gel formation. A mixture of 300 mg agarose and 30 ml 1X TAE buffer was made in a flask. The flask was heated, and the agarose was dissolved using gloves. After dissolving, the agarose solution was allowed to cool before sealing a gel-casting tray. To the cooled agarose solution, 0.5 µl of ethidium bromide was added.

The agarose was poured to a depth of 1 cm and left to solidify. The biohazard tape was then removed. Next, the cooled agarose gel was used to add 500 µl of DNA sample, 2.5 µl of loading dye, and 1 µl of ladder, water, and loading dye. A tank was filled with TAE buffer, and the gel was placed in it. Electrophoresis was run at 80V for an hour until the front dye reached the gel's bottom. The electrodes were checked for bubbling, and the gel was photographed under UV transillumination.

DNA quantification

Quantification was done using Qubit 3.0 fluorometer kit and its instruction.

2. Set up the assay tubes for the standards and one assay tube for each user samples.
3. Prepare the working solution by diluting the Qubit reagent 1:200 in Qubit buffer
4. Prepare the assay tube and standard tubes according to the table given below.
5. The tubes for 2-3 sec and incubate 2 min at Room temperature (RT)

Insert the tubes in Qubit Fluorometer and take readings. The readings would be in ng/ µl.

Table 1: Working Setup for DNA quantification

Components	Standard Assay Tubes (µl)	Sample assay tubes (µl)
Working solution (from step 2)	190	199
Standard (from kit)	10	-
Vol. of sample	-	1
Total volume in each tube	200	200

Q-PCR

Preparation of master mix for q-PCR

Reaction mixture is prepared by adding all the components in one tube except sample and dividing the content equally in all wells and then adding sample in last. Reaction mixture was prepared for the total volume of 10µl.

Table 2: Reaction mixture for q-PCR

S/No.	Content	Volume (µl)
1	2X qPCR Mix	5
2	Fw Primer (GAPDH)	0.5
3	Rv Primer (GAPDH)	0.5
4	RNAse free water	3.8
5	Sample (DNA)	0.2

Running the q-PCR

The 2-step q-PCR was operated in which first step was hot start which was carried out at 95^0C for 5 min, then the amplification step which was divided in two stages first being denaturation which was carried at 95^0C for 10 sec, annealing of primer and extension at 60^0C for 20 sec. The amplification cycle was carried out for 45 cycles leading to lasts stage of PCR which was melting.

Table 3. Steps in q-PCR

S/No.	Content	Temp (^{o}C)	Duration (sec)
1	Pre-denaturation	95	3 min
2	Denaturation	95	10
3	Annealing & Extension	60	20
4	Melting	---	---

RESULTS AND DISCUSSIONS

2D GEL ELECTROPHORESIS RESULTS

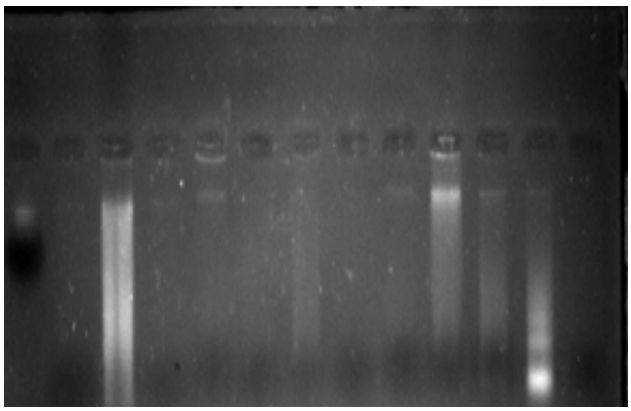


Figure 1: Gel image of DNA extracted from cigarette butt

phenol chloroform method is cost effective and efficient method to extract DNA from cigarette butts from the gel bands it can also be inferred that

by increasing the time interval of incubation, less amount of DNA is being recovered but still is enough for further investigating analysis.

In this study ,use of Qubit fluorometer 3.0 for the quantification of DNA extracted from cigarette butts.

Table 4 : DNA quantification

Average	15.65 ng/µl
Mean	11.6 ng/µl
Median	91 ng/µl
Mode	4.49 ng/µl
Range(Minimum-Maximum	1-92 ng/µl

Table 5 : CT value and fold change of GAPDH

Data	Average (Mean)	Median	Range	Mode
Fold Change	~77.08	78.25	10	78.5
CT Value	~16.87	17.07	1.98	No mode

The quantification analysis revealed that the recovery of DNA from cigarette butts decreased with an increasing duration of incubation, as shown in Table 4. Despite this decline in DNA quantity, the extracted DNA remained viable for further forensic investigations. To assess sample integrity, GAPDH, a widely expressed housekeeping gene, was used in qPCR studies. Higher fold changes in gene expression indicated better sample integrity. These findings underscore the importance of handling and storing forensic samples carefully to preserve DNA quality. Future research should focus on optimizing methods for enhancing DNA recovery and preservation to ensure reliable forensic results.

Q-PCR

q-PCR-based tests evaluate DNA quantity and quality, particularly in biological samples made from small amounts. This method helps assess the number of amplifiable target sequences in the sample. Target DNA is identified through specific amplification using DNA-binding fluorescent dyes or sequence-specific probes. QPCR can determine the quantity of target DNA by comparing a reference sample's threshold cycle to the target DNA sequence. Extracted genomic DNA integrity was quantified using q-PCR, showing remarkable amplification and a considerable CT value.

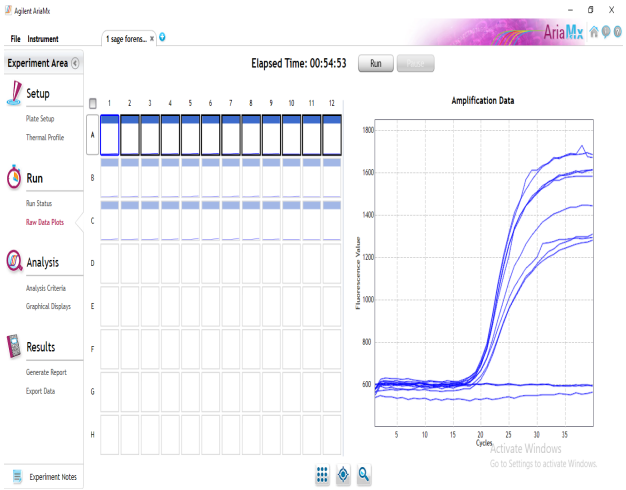


Figure 2 : Raw data plot of qPCR

CONCLUSION

The study aimed to assess the phenol-chloroform method's ability to extract DNA from cigarette butt. Results showed successful recovery, emphasising the importance of timing extraction after crimes. The research suggests dealing with material promptly for optimal recovery rates. The obtained DNA is functional and can be used for future investigative purposes.

REFERENCES

1. Budowle, B. and A.J. Eisenberg. 2007. Forensic Genetics, p.501-517. In D.L.Rimoin, J.M.Connor, R.E. Pyeritz, and B.R. Korf (Eds.), Emery and Rimoin's Principles and Practice of Medical Genetics, 5th ed. Vol. 1. Elsevier, Philadelphia.
2. Budowle, B., J.V. Planz, R. Campbell, and A.J. Eisenberg. 2005. Molecular diagnostic applications in forensic science, p. 267-280. In G.Patrinos and W. Ansorge (Eds.), Molecular Diagnostics. Elsevier, Amsterdam.
3. Budowle,B.andA. van Daal. 2008. Forensically relevant SNP classes. Bio Techniques 44:603- 610.
4. Budowle, B., H.A. Deadman, R.S. Murch, and F.S. Baechtel. 1988. An introduction to the methods of DNA analysis under investigation in the FBI Laboratory. Crime Lab. Dig. 15:8-21.
5. F.-X. Laurent, L. Pene, Identification Sciences: DNA and biological evidence, in: M.M. Houck (Ed.), Proceedings of the 18th INTERPOL International Forensic Science Managers Symposium, 2016, pp. 697e710. Held in Lyon, France, October 11-13, 2016
6. Budowle, B., J.A. Smith, T. Moretti, and J. DiZinno. 2000. DNA Typing Protocols: Molecular Biology and Forensic Analysis. Eaton Publishing Company/Biotechniques Books, Natick, MA.
7. Budowle,B.,R.Chakraborty,A.M.Giusti,A.J. Eisenberg, andR.C.Allen. 1991. Analysis of the VNTR locus D1S80 by the PCR followed by highresolution

PAGE. Am. J. Hum. Genet. 48:137- 144.
8. Collins,P.J.,L.K. Hennessy, C.S.Leibelt,R.K. Roby,D.J.Reeder, andP.A.Foxall. 2004. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFlSTRIndentifiler PCR Amplification Kit. J. Forensic Sci. 49:1265-277.
9. Green,R.L.,I.C.Roinestad,C.Boland, andL.K. Hennessy. 2005. Developmental validation of the Quantifiler Real-Time PCR Kits for the quantification of human nuclear DNA samples. J. Forensic Sci. 50:809-825.
10. Moretti, T.R., A.L. Baumstark, D.A. Defenbaugh, K.M. Keys, and B. Budowle. 2001. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. J. Forensic Sci. 46:647-660.
11. Budowle, B. and F.S. Baechtel. 1990. Modifications to improve the effectiveness of restriction fragment length polymorphism typing. Appl. Theor. Electrophor. 1:181-187.
12. Gill, P., A.J. Jeffreys, and D.J. Werrett. 1985. Forensic application of DNA fingerprints. Nature 318:577-579.
13. Jeffreys, A.J., V. Wilson, and S.L. Thein. 1985. Hypervariable minisatellite regions in human DNA. Nature 314:67-73.
14. Budowle, B., T.R. Moretti, S.J. Niezgoda, and B.L.Brown. 1998. CODIS and PCR-based short tandem repeat loci: law enforcement tools, pp. 73-88. Proceedings from the Second European Symposium on Human Identification 1998. Promega Corporation, Madison.
15. Martin,P.D.,H.Schmitter, andP.M.Schneider. 2001. A brief history of the formation of DNA databases in forensic science within Europe. Forensic Sci. Int. 119:225-231.
16. Puls, J., Wilson, S.A., Hölter, D., 2011. Degradation of cellulose acetate-based materials: a review. J. Polym. Environ. 19 (1), 152–165.
17. Wallbank, L.A., MacKenzie, R., Beggs, P.J., 2017. Environmental impacts of tobacco product waste: international and Australian policy responses. Ambio 46 (3), 361–370.
18. Hoffmann, D., Hoffmann, I., El-Bayoumy, K., 2001. The less harmful cigarette: a controversial issue. A tribute to Ernst L. Wynder. Chem. Res. Toxicol. 14 (7), 767–790.
19. Martin, T., 2018. Cigarette Tar and How It Can Hurt You, viewed 1 April 2018
20. Hoffmann, D.H.I., 1997. The changing cigarette, 1950–1995. J. Toxicol. Environ. Health Part A 50 (4), 307–364.

*****