

**COMPARATIVE EFFICIENCY BETWEEN DIFFERENT
SWAB TYPES FOR TOUCH DNA RECOVERY FROM
DIFFERENT SURFACES**

ALYA AMIRAH BINTI SIRAJUDIN

UNIVERSITI SAINS MALAYSIA

2025

CERTIFICATE

This is to certify that the dissertation entitled “Comparative Efficiency Between Different Swab Types for Touch DNA Recovery from Different Surfaces” is the bonafide record of research work carried out by Alya Amirah Binti Sirajudin (Matric No.: 164477) during the period from October 2025 to February 2026 under my supervision. I have read this dissertation, and, in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, for submission in partial fulfilment of the requirements for the degree of Bachelor of Science (Honours) (Forensic Science).

Supervisor,

Dr. Nur Walyuddin Hanis bin Zainal Abidin

Lecturer of Health Science,

Universiti Sains Malaysia,

Health Campus,

16150 Kubang Kerian,

Kelantan, Malaysia

Tel:

Date: 30 January 2026

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I further declare that this dissertation has not been previously or concurrently submitted, in whole or in part, for any other degree at Universiti Sains Malaysia or at any other institution. I also grant Universiti Sains Malaysia the right to use this dissertation for purposes of teaching, research, and promotion.

Alya Amirah Binti Sirajudin

Date: 30 January 2026

ACKNOWLEDGMENT

I would like to take this opportunity to express my sincere appreciation to everyone who has contributed, directly or indirectly, to the completion of this final year project.

First and foremost, I would like to extend my deepest gratitude to my supervisor, Dr. Nur Waliyuddin Hanis bin Zainal Abidin, for granting me the invaluable opportunity to undertake this research under his supervision. His expertise, patience, and insightful guidance throughout this study were instrumental in helping me navigate challenges and deepen my understanding of the subject. His encouragement and constructive feedback made this research journey both meaningful and rewarding.

I would also like to express my heartfelt appreciation to Puan Nur Azeelah Abdullah and Encik Muhammad Ismail for their patience, dedication, and willingness to guide me in utilizing the resources of the Human Identification Unit/DNA (HID) laboratory. Their understanding, support, and guidance made my time in the laboratory a positive and enriching experience. I am also grateful to the forensic laboratory staff for their valuable insights during material preparation, which helped minimize errors and improve the quality of my laboratory work.

My sincere appreciation goes to all participants, especially my friends and course mates, for their cooperation, encouragement, and willingness to be involved in this study. Their support and contributions played an important role in the successful completion of this research.

I am deeply grateful to my family for their unconditional love, patience, and constant encouragement behind the scenes. Their belief in me provided strength during moments of doubt and motivation during times of exhaustion.

Last but not least, I want to thank me for believing in me, I want to thank me for doing all this hard work, I want to thank me for having no days off, I want to thank me for never quitting, I want to thank me for always being a giver, and trying to give more than I receive. I want to thank me for trying to do more right than wrong, I want to thank me for just being me at all times.

TABLE OF CONTENTS

CERTIFICATE	ii
DECLARATION	iii
ACKNOWLEDGMENT	iv
LIST OF TABLES.....	ix
LIST OF FIGURES	x
LIST OF SYMBOLS.....	xv
LIST OF ABBREVIATIONS	xvi
LIST OF APPENDICES.....	xviii
ABSTRAK	xix
ABSTRACT	xxi
CHAPTER 1	1
INTRODUCTION.....	1
1.1 Background of Study	1
1.2 Problem Statement.....	5
1.3 Research Question(s)	6
1.4 Objective	7
1.4.1 General Objective.....	7
1.4.2 Specific Objective	7
1.5 Significance of Study.....	7
1.6 Scope of Study	9
CHAPTER 2	11
LITERATURE REVIEW.....	11
2.1 Genetics	11
2.1.1 Molecular Genetic	13
2.1.2 Forensic Genetic	17
2.2 Touch DNA as Trace Evidence	23
2.2.1 Definition of Touch DNA	23
2.2.2 Shedding Mechanism and Variability	23
2.2.3 Factor Influenced Touch DNA Recovery	25
2.2.4 DNA Extraction.....	26

2.2.5 DNA Collection.....	30
2.2.6 Touch DNA Recovery	34
2.3 Surface Characteristics and DNA Recovery	36
2.3.1 Plastic Surfaces	36
2.3.2 Glass Surfaces	37
2.3.3 Ceramic Surfaces	37
2.3.4 Influence of Texture, Porosity and Electrostatic Properties	38
2.4 Analysis of Touch DNA	40
2.4.1 Mitochondria DNA gene as amplification target	40
2.4.2 Application of PCR Amplification	42
2.4.3 Identification of Individual	46
CHAPTER 3	49
METHODOLOGY	49
3.1 General Laboratory Safety in DNA Analysis	49
3.2 Laboratory Facilities and Workstations	49
3.3 Ethical Approval.....	50
3.4 Materials	50
3.4.1 Chemical and Reagents	50
3.4.2 Instruments and Apparatus	50
3.4.3 Chemical and Reagent Preparation	50
3.5 Sample Collection.....	53
3.6 Sample Preparation	54
3.6.1 Microscopic Examination	54
3.6.2 Surface Preparation	55
3.6.3 Object used to represent surfaces	55
3.6.4 Touch DNA deposition.....	58
3.6.5 Swabbing Procedure	58
3.7 Genomic DNA Extraction and Purification	59
3.8 Genomic DNA Quantitation	61
3.9 Polymerase Chain Reaction	62
3.9.1 PCR Optimization	63
3.9.2 PCR Amplification	64
3.10 Agarose Gel Electrophoresis of Amplified PCR Products.....	65
3.11 Statistical Analysis.....	66

CHAPTER 4	68
RESULT	68
4.1 Sample Collection.....	68
4.2 Microscopic Observation of Swab Materials and Surface Types	71
4.2.1 Examination of Swab	72
4.3 Genomic DNA Extraction and Purification	75
4.3.1 Agarose Gel Electrophoresis of Extracted Products	75
4.3.2 qPCR Quantification of Extracted Products	77
4.3.3 Statistical Analysis of DNA Quantification	82
4.4 PCR Amplification	86
4.5 Agarose Gel Electrophoresis of Amplified PCR Products	89
4.5.1 mtDNA 310bp Primer	89
4.5.2 mtDNA 176 bp Primer	95
4.6 Statistical Analysis of Amplifiable DNA.....	102
4.6.1 mtDNA 310bp	103
4.6.2 mtDNA 176bp	104
CHAPTER 5	105
DISCUSSION	105
5.1 Sample Collection.....	105
5.2 Microscopic Characteristics of Swab Types	107
5.2.1 Cotton swab.....	107
5.2.2 Nylon-Flocked swab	107
5.2.3 Dacron swab.....	108
5.3 Interpretation of Gel Image of Extracted Product	108
5.4 Genomic DNA Quantitation using qPCR	110
5.4.1 Interpretation of Ct values and DNA quantity	110
5.4.2 Standard curve performance and assay reliability	111
5.4.3 Interpretation of degradation index (DI)	112
5.4.4 Interpretation of Large and Small Autosomal Target Differences	114
5.5 Statistical Analysis of DNA Quantification	117
5.5.1 Interpretation of the F-Statistic and Associated Parameters	117
5.5.2 Interpretation of Estimated Marginal Means and Confidence Intervals	118
5.5.3 Interpretation of Graphical Outputs	118
5.5.4 Interpretation of Residuals	119
5.6 Polymerase Chain Reaction	120

5.6.1 Selection of primer	120
5.7 Interpretation of Gel Image of Amplified PCR Product	124
5.8 Statistical Analysis of Amplifiable DNA.....	127
5.8.1 mtDNA 310bp Amplification	127
5.8.2 mtDNA 176bp Amplification	128
5.8.3 Surface Type on Touch DNA Amplification	129
CHAPTER 6	131
CONCLUSION AND FUTURE RECOMMENDATIONS	131
6.1 Conclusion.....	131
6.2 Recommendations for Future Research.....	134
REFERENCES.....	135

LIST OF TABLES

Table 4.1:Structured sampling design showing participant codes (P01–P24), sample codes, swab types (cotton, dacron, nylon flocked), and surface types (plastic, ceramic, and glass) used for Touch DNA collection in this study.....	68
Table 4. 2:Quantitative PCR (qPCR) results showing cycle threshold (Ct) values, DNA quantity, and degradation index for Touch DNA samples extracted from cotton, dacron and nylon-flocked swabs.	78
Table 4. 3: Optimized PCR cycling conditions for mtDNA 310 bp primer using MyTaq™ Red Mix.	87
Table 4. 4 :Optimized PCR cycling conditions for mtDNA 176 bp primer using MyTaq™ Red Mix.	88
Table 4. 5: Summary of PCR amplification success using mtDNA 310 bp primer	89
Table 4. 6 : Summary of PCR amplification success using mtDNA 310 bp primer	95

LIST OF FIGURES

Figure 2.1: Structural organization of DNA, showing the nucleotide components (phosphate, sugar, and nitrogenous base), complementary base pairing (A–T and C–G), the sugar–phosphate backbone, and the resulting double-helix structure.....	15
Figure 2.2: Schematic representation of the polymerase chain reaction (PCR) process, illustrating successive cycles of DNA denaturation, primer annealing, and extension by DNA polymerase leading to exponential amplification of the target DNA sequence.....	44
Figure 3.1: Transparent plastic container used to represent a plastic surface for touch DNA collection.	56
Figure 3.2: Ceramic cup used to represent a ceramic surface for touch DNA collection.	57
Figure 3.3: Double-walled glass cup used to represent a glass surface for touch DNA collection.	57
Figure 4.1: Light microscopic image of a cotton swab, showing twisted and loosely arranged natural fibers with irregular orientation (Zeiss Axiostar Plus, 100× magnification; 10× objective × 10× eyepiece).....	72
Figure 4.2: Light microscopic image of a nylon-flocked swab, showing straight, uniform synthetic fibers with a consistent orientation (Zeiss Axiostar Plus, 100× magnification; 10× objective × 10× eyepiece).....	73
Figure 4.3: Light microscopic image of a dacron swab, showing straight, smooth synthetic fibers with a compact and orderly arrangement (Zeiss Axiostar Plus, 100× magnification; 10× objective × 10× eyepiece).	74
Figure 4.4 Agarose gel electrophoresis of genomic DNA extracted from Touch DNA samples collected using cotton and nylon-flocked swabs. A HindIII-digested	

DNA ladder was used as a molecular size marker to assess DNA integrity and fragment size distribution.....	76
Figure 4.5:Agarose gel electrophoresis of genomic DNA extracted from Touch DNA samples collected using dacron swabs. A HindIII-digested DNA ladder was used as a molecular size marker to assess DNA integrity and fragment size distribution.	76
Figure 4.6:Univariate analysis of DNA yield showing estimated marginal means for swab type across different surfaces (plastic, ceramic, and glass).	83
Figure 4.7:Interaction plot showing mean DNA yield across surface types (plastic, ceramic, and glass) for different swab types (cotton, dacron, and nylon-flocked).	83
Figure 4.8:Bar chart illustrating mean DNA yield across surface types (plastic, ceramic, and glass) for different swab types (cotton, dacron, and nylon-flocked).	84
Figure 4.9:Histogram of standardized residuals with a normal distribution curve, used to assess the normality assumption for DNA yield in the univariate analysis. ..	84
Figure 4.10:Boxplots of DNA yield for each swab type (cotton, dacron, and nylon-flocked) across surface types (plastic, ceramic, and glass), illustrating the distribution and variability of DNA yield within each swab–surface combination.....	85
Figure 4.11: 2% Agarose gel image showing the amplification of the known positive DNA template for mtDNA 310 bp primer optimization using MyTaq™ Red Mix.	87
Figure 4.12: 2% Agarose gel image showing the amplification of the known positive DNA template for mtDNA 176 bp primer optimization using MyTaq™ Red Mix.	88

Figure 4.13: 2% Agarose gel image showing amplification of extracted Touch DNA (sample 1 -11) from different surfaces (plastic, ceramic and glass) using cotton swab mtDNA 310bp and MyTaq TM Red Mix.	91
Figure 4.14: 2% Agarose gel image showing amplification of extracted Touch DNA (sample 12 -24) from different surfaces (plastic, ceramic and glass) using cotton swab and mtDNA 310bp and MyTaq TM Red Mix.....	91
Figure 4.15: 2% Agarose gel image showing amplification of extracted Touch DNA (sample 1 -11) from different surfaces (plastic, ceramic and glass) using dacron swab and mtDNA 310bp and MyTaq TM Red Mix.....	92
Figure 4.16: 2% Agarose gel image showing amplification of extracted Touch DNA (sample 12 -24) from different surfaces (plastic, ceramic and glass) using dacron swab and mtDNA 310bp and MyTaq TM Red Mix.....	93
Figure 4.17: 2% Agarose gel image showing amplification of extracted Touch DNA (sample 1 -11) from different surfaces (plastic, ceramic and glass) using nylon swab and mtDNA 310bp and MyTaq TM Red Mix.....	94
Figure 4.18: 2% Agarose gel image showing amplification of extracted Touch DNA (sample 12 -24) from different surfaces (plastic, ceramic and glass) using nylon swab and mtDNA 310bp and MyTaq TM Red Mix.....	94
Figure 4.19: 2% Agarose gel image showing amplification of extracted Touch DNA by cotton swab (sample 1 -12) from different surfaces (plastic, ceramic and glass) using mtDNA 176bp and MyTaq TM Red Mix.....	97
Figure 4.20: 2% Agarose gel image showing amplification of extracted Touch DNA by cotton swab (sample 13 -24) from different surfaces (plastic, ceramic and glass) using mtDNA 176bp and MyTaq TM Red Mix.....	97

Figure 4.21: 2% Agarose gel image showing amplification of extracted Touch DNA by dacron swab (sample 1 -12) from different surfaces (plastic, ceramic and glass) using mtDNA 176bp and MyTaq™ Red Mix.....	98
Figure 4.22 :2% Agarose gel image showing amplification of extracted Touch DNA by dacron swab (sample 12 -24) from different surfaces (plastic, ceramic and glass) using mtDNA 176bp and MyTaq™ Red Mix.....	99
Figure 4.23: 2% Agarose gel image showing amplification of extracted Touch DNA by Nylon Flocked swab (sample 1 -12) from different surfaces (plastic, ceramic and glass) using mtDNA 176bp and MyTaq™ Red Mix.	100
Figure 4.24 :2% Agarose gel image showing amplification of extracted Touch DNA by Nylon Flocked swab (sample 12 -24) from different surfaces (plastic, ceramic and glass) using mtDNA 176bp and MyTaq™ Red Mix.	101
Figure 5.1: BLAST alignment result of forward (10342-F) and reverse (10517-R) primer with Homo sapiens isolate Nvh1641 mitochondrial genome (Accession ID; MG660787.1).The forward primer aligns to positions 10,342-10,361 on the plus strand with a perfect identity (20/20, 100%) and E-value of 1.3.The reverse primer aligns to positions 10,496-10,517 on the complementary minus strand with perfect identity (22/22,100%) and lower E-value of 0.084, indicating a highly significant match. Both primers confirm specificity to the targeted mitochondrial region specific for human (BLASTN Program,Core nucleotide BLAST database,NCBI)	121
Figure 5.2: BLAST alignment results for the forward primer (10291-F) and reverse primer (10556-R) with the mitochondrial genome of Homo sapiens isolate Nvh1641 (Accession ID: MG660787.1). The forward primer aligns to positions 10,269–10,288 on the plus strand with 100% identity (20/20) and an E-value of 1.3. The reverse primer aligns to positions 10,556–10,578 on the minus strand with 100% identity (23/23) and a highly significant E-value of 0.021. Both primers exhibit perfect alignment with their respective	

regions, confirming their specificity to the targeted mitochondrial sequence specific for human (BLASTN Program, Core nucleotide BLAST database, NCBI)..... 122

Figure 5.3: Suggested annealing temperature determined by software based on the selected DNA polymerase, the mtDNA 310 bp primer sequences, and the primer concentration used (Thermo Fisher Scientific™ Tm calculator). . 123

Figure 5.4: Suggested annealing temperature determined by software based on the selected DNA polymerase, the mtDNA 176 bp primer sequences, and the primer concentration used (Thermo Fisher Scientific™ Tm calculator). . 124

LIST OF SYMBOLS

mL	Milliliter
μ L	Microliter
μ M	Micromolar
pg	Picogram
$^{\circ}$ C	Degree Celsius
bp	Base pairs
ng	Nanogram
M	Molarity (moles per liter)
mM	Millimolar
g/mol	Grams per mole
rpm	Revolutions per minute
$\times$ g	Relative centrifugal force
nm	Nanometer
min	Minutes
L	Liter
g	Gram
ng/ μ L	Nanograms per microliter
V	Voltage
pH	Potential of hydrogen

LIST OF ABBREVIATIONS

A230	Absorbance at 230 nm
A260	Absorbance at 260 nm
A280	Absorbance at 280 nm
BLAST	Basic Local Alignment Search Tool
BLASTN	Basic Local Alignment Search Tool for Nucleotides
CaCl ₂ ·2H ₂ O	Calcium Chloride Dihydrate
CODIS	Combined DNA Index System
Ct	Cycle Threshold
D-loop	Displacement Loop in mitochondrial DNA
DNA	Deoxyribonucleic Acid
DI	Degradation Index
dsDNA	Double-Stranded DNA
dNTP	Deoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethidium Bromide
F	F-statistics
HCl	Hydrochloric Acid
HID	Human Identification/DNA Unit
HV1	Hypervariable Region 1
HV2	Hypervariable Region 2
IPC	Internal Positive Control
JEPeM	Human Research Ethics Committee of Universiti Sains Malaysia
LCN	Low Copy Number
MgCl ₂	Magnesium Chloride

MPS	Massively Parallel Sequencing
mtDNA	Mitochondrial Deoxyribonucleic Acid
Na ₂ EDTA	Disodium Ethylenediaminetetraacetic Acid
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCBI	National Center for Biotechnology Information
NGS	Next-Generation Sequencing
nuDNA	Nuclear Deoxyribonucleic Acid
ANOVA	Analysis of Variance
PCR	Polymerase Chain Reaction
Pfu	<i>Pyrococcus furiosus</i> (DNA polymerase source)
qPCR	<i>Quantitative Polymerase Chain Reaction</i>
RNA	Ribonucleic Acid
SDS	Sodium Dodecyl Sulfate
SNP	Single Nucleotide Polymorphisms
STR	Short Tandem Repeat
Taq	<i>Thermus aquaticus</i> (DNA polymerase source)
Ta	Annealing temperature
TBE	Tris-Borate-EDTA Buffer
T _m	Melting Temperature
Tris-HCl	Tris Hydrochloride
UV	Ultraviolet Light
ssDNA	Single-Stranded DNA

LIST OF APPENDICES

APPENDIX A	APPROVAL LETTER FOR ETHICAL CLEARANCE OF THE RESEARCH
APPENDIX B	CONSENT FORM FOR PARTICIPANTS
APPENDIX C	LIST OF CHEMICALS, REAGENTS, COMMERCIAL KITS AND CONSUMABLES USED
APPENDIX D	LIST OF INSTRUMENTS AND APPARATUS USED
APPENDIX E	STANDARD CURVE

ABSTRAK

Analisis asid deoksiribonukleik (DNA) memainkan peranan penting dalam sains forensik, khususnya bagi pengenalpastian individu dalam siasatan jenayah. Apabila bukti biologi yang jelas seperti darah atau air liur tidak ditemui di tempat kejadian, DNA sentuhan boleh menjadi sumber alternatif maklumat genetik. Namun begitu, analisis DNA sentuhan sering berhadapan dengan pelbagai kekangan, termasuk kuantiti DNA yang sangat rendah, pemindahan yang tidak seragam, serta keberkesanan pemulihan yang berbeza bergantung kepada jenis permukaan.

Kajian ini dijalankan untuk menilai keberkesanan beberapa jenis swab dan bahan permukaan dalam pemulihan serta penguatan DNA sentuhan bagi meningkatkan kebolegunaannya dalam aplikasi forensik. DNA sentuhan diperoleh melalui sentuhan semula jadi bagi meniru keadaan sebenar di tempat kejadian. Tiga jenis swab, iaitu kapas, dakron dan nilon-berflok, digunakan untuk mengumpul sampel daripada permukaan kaca, seramik dan plastik. Pengekstrakan DNA dilakukan menggunakan kaedah berasaskan silika yang sesuai untuk sampel DNA bertemplat rendah. Analisis qPCR menunjukkan hasil DNA yang rendah dan tidak konsisten bagi semua kombinasi swab dan permukaan, tanpa perbezaan yang signifikan antara jenis swab.

Keupayaan penguatan DNA dinilai menggunakan PCR yang mensasarkan fragmen DNA mitokondria bersaiz 176 bp dan 310 bp. Hasil electrophoresis gel agarose menunjukkan kadar kejayaan penguatan yang lebih tinggi bagi fragmen 176 bp berbanding fragmen 310 bp, tanpa pengaruh ketara daripada jenis permukaan. Secara keseluruhan, kajian ini menunjukkan bahawa saiz amplicon memainkan peranan utama

dalam kejayaan analisis DNA sentuhan, dan penggunaan sasaran mtDNA yang lebih pendek dapat meningkatkan kebolehpercayaan analisis forensik bagi sampel bertemplat rendah.

ABSTRACT

Deoxyribonucleic acid (DNA) analysis is a fundamental tool in forensic science, enabling human identification in criminal investigations. In cases where visible biological evidence such as blood or saliva is absent, touch DNA has emerged as an alternative source of genetic material. However, touch DNA presents significant analytical challenges due to its low quantity, variable transfer, and inconsistent recovery from different surfaces. This study focused on evaluating the efficiency of different swab types and surface materials in the recovery and amplification of touch DNA to improve its forensic applicability.

Touch DNA samples were deposited through natural handling to simulate realistic forensic scenarios. Three swab types (cotton, dacron, and nylon-flocked) were used to collect samples from glass, ceramic, and plastic surfaces. DNA extraction was performed using a silica-based method optimized for low-template samples. Quantitative polymerase chain reaction (qPCR) analysis revealed low and variable DNA yields across all swab–surface combinations, with no statistically significant differences observed between swab types.

To assess amplification feasibility, polymerase chain reaction (PCR) targeting mitochondrial DNA (mtDNA) regions of 176 bp and 310 bp was conducted. Agarose gel electrophoresis demonstrated a markedly higher amplification success for the 176 bp mtDNA fragment compared to the 310 bp fragment across all conditions. Surface type did not show a consistent effect on amplification outcomes.

These findings indicate that amplicon size is a critical determinant of successful touch DNA analysis. The use of short mtDNA targets enhances amplification reliability, supporting the forensic utility of touch DNA in cases involving low-template biological evidence.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Deoxyribonucleic acid (DNA) is a fundamental biological molecule that carries the genetic information necessary for the growth, functioning, and identity of living organisms (National Human Genome Research Institute, 2025). In forensic science, DNA has long been recognized as one of the most powerful forms of forensic evidence due to its ability to associate individuals with criminal events, victims, or physical items encountered at crime scenes. Traditional forensic DNA analysis has relied largely on biological fluids such as blood, semen, and saliva, which typically contain ample quantities of nucleated cells (Haddrill, 2021). However, technological advances in polymerase chain reaction (PCR) have enabled the analysis of extremely small quantities of DNA, expanding forensic capability to include trace or low-template DNA (Oorschot, Kaye N Ballantyne, & Mitchell).

Trace DNA refers to minute quantities of genetic material that may not be visible to the naked eye. This category includes touch DNA, shed skin cells, and other microscopic biological residues. A major subset of trace DNA is Touch DNA (Burrill, Daniel, & Frascione, 2018). Touch DNA is defined as DNA derived from small numbers of epithelial cells deposited when an individual comes into contact with an object or surface through handling, gripping, or touching. These cells primarily originate from the stratum corneum, the outermost epidermal layer, which continuously sheds keratinized

cells as part of normal skin turnover (Burrill, Daniel, & Frascione, 2018). Because touch DNA often consists of fewer than 10–20 cells, it is considered a low-template biological sample and presents significant analytical challenges in forensic science.

Despite these challenges, touch DNA is forensically valuable because it can link individuals to objects when no visible biological evidence is present. It has been successfully used in cases involving weapons, vehicle interiors, window surfaces, ceramic tiles, mobile phones, and plastic packaging which are objects frequently encountered at crime scenes (Dutta, 2024). The genetic information recovered from touch DNA typically targets autosomal DNA, which contains short tandem repeats (STRs) inherited from both parents (Panneerchelvam & Norazmi, 2023). Autosomal STR profiling remains the gold standard in forensic identification due to its high discrimination power, reproducibility, and well-established databases. Therefore, even minimal quantities of autosomal DNA from touch samples can provide strong evidential value when successfully recovered and amplified (Panneerchelvam & Norazmi, 2023).

In forensic analysis, mitochondrial DNA (mtDNA) is commonly used because it is more abundant in skin cells, making it easier to extract and analyze. mtDNA is particularly useful when autosomal DNA is not available or is present in trace amounts, as it provides valuable genetic information even from degraded samples (Amorim, Fernandes, & Taveira, 2019). On the other hand, autosomal DNA recovered from touch DNA samples is often present in extremely low quantities because it originates from a very small number of shed epithelial cells that are deposited during brief or light contact. These minimal cell numbers, combined with possible environmental degradation, make

autosomal DNA extraction more challenging and frequently result in weak, partial, or unsuccessful PCR amplification.

While forensic DNA workflows often aim to generate full autosomal STR profiles, the primary challenge in touch DNA analysis lies earlier, at the stage of DNA recovery and successful PCR amplification. If insufficient DNA is collected from a surface, the sample may fail to amplify entirely, regardless of downstream procedures. Thus, for low-template samples, the fundamental question is not merely whether an STR profile can be obtained, but whether amplification itself can occur.

Furthermore, the successful recovery of DNA at the crime scene depends heavily on two critical variables which includes the type of swab used for collection and the nature of the sampled surface (Singh, Alketbi, & Sharma, 2025). Past studies have shown that cotton swabs often trap epithelial cells within their densely woven fibers, reducing the amount of DNA released during extraction (Conte, Morgan, & Tracey L. Johnson, 2025). In contrast, synthetic swabs such as dacron, and more recently nylon-flocked swabs, have shown improved performance due to their optimized fiber structure and enhanced elution efficiency (Bruijns, Tiggelaar, & Gardeniers,, The Extraction and Recovery Efficiency of Pure DNA for Different Types of Swabs, 2018) . Despite these observations, many previous studies lack systematic, controlled comparisons across different swab types and surface materials.

Surface characteristics also play an essential role in determining DNA recovery efficiency. Common crime scene substrates such as plastic, ceramic, and glass differ in porosity, texture, and electrostatic properties, which influence how cells adhere and how easily they can be collected. Smooth, non-porous materials like plastic and glass are

known to retain fewer epithelial cells, whereas ceramic may exhibit microtextural variations that affect cell adherence (Conte, Morgan, & Tracey L. Johnson, 2025). Yet, existing literature remains inconsistent regarding which swab–surface combinations yield the highest recovery, and few studies directly compare the interaction between swab type and substrate under standardized experimental conditions.

The analytical challenges associated with touch DNA extend into the laboratory extraction phase. Various extraction methods have been developed to maximize DNA recovery from low-template samples. silica-based extraction is widely recognized as an effective method for low-template DNA because silica has strong binding affinity for nucleic acids and removes potential inhibitors (Swargiary & Pandey, Touch DNA Recovery from Non- porous Surfaces, 2025). However, even with an optimal extraction method, amplification failure may occur if the initial recovery from the surface is insufficient. Therefore, understanding how swab type and surface material influence amplification success is crucial for improving touch DNA protocols.

Given these considerations, the present research aims to systematically evaluate the recovery efficiency of cotton, dacron, and nylon-flocked swabs across three commonly encountered forensic surfaces focusing on plastic, ceramic, and glass. By focusing specifically on PCR amplification outcome rather than full STR profiling, this research provides practical and evidence-based insight into the initial feasibility of touch DNA analysis and offers recommendations for improving collection strategies in forensic investigations.

1.2 Problem Statement

Forensic investigators rely heavily on DNA evidence to identify individual and link them to crime scene. The success of DNA profiling depends on how efficiently investigators recover the genetic material from surfaces at crime scene (Bukyya, et al., 2021). Swabbing remains the most widely used collection method because it is practical and simple. However, the type of swab and the surface materials strongly affect the amount of DNA recovered (Alketbi, Sharma, & Singh, 2025). Numerous studies have established that swabs are among the most widely used collection tools, with cotton, rayon, and nylon flocked swabs being the most common. Research to date has demonstrated that swab type influences DNA yield, with flocked swabs generally outperforming traditional cotton in terms of collection and release efficiency (Conte J. M., 2025). Similarly, the nature of the surface whether smooth, rough, porous, or non-porous also plays a significant role in determining how much DNA can be recovered.

Mitochondria DNA (mtDNA) extraction is relatively more straightforward due to its higher abundance within cells, making it the preferred choice in many forensic applications (Lutz, Weisser, & Pollak, 1996). However, extracting autosomal DNA from Touch DNA samples is significantly more difficult due to its limited presence, often resulting in inconsistent yields and lower success rates in PCR amplification (Suenaga & Nakamura, 2005). Even with mtDNA, the quality and consistency of DNA extracted from Touch DNA can vary, affecting its reliability in forensic investigations.

Although past work has compared the performance of swabs, much of the literature has either focused on a single type of substrate or tested swabs under controlled laboratory conditions that do not fully reflect real casework variability. In addition, most published studies highlight the superiority of nylon flocked swabs but provide limited comparative data across multiple swab types when applied to different surfaces such as glass, ceramic, and plastic materials that are frequently encountered in forensic investigations (Conte J. M., 2025).

This creates a knowledge gap as forensic practitioners still lack clear, evidence-based guidance on which swab type is most efficient for DNA recovery when dealing with commonly encountered surfaces in casework. Without this information, there is a risk of suboptimal evidence collection, leading to reduced DNA yields, partial genetic profiles, or even inconclusive results in court. Therefore, this study aims to address the gap by systematically comparing the efficiency of different swab types on selected surfaces. By doing so, the research seeks to generate practical insights that can strengthen forensic sampling protocols and improve the reliability of DNA evidence used in criminal justice.

1.3 Research Question(s)

1. How do cotton, dacron, and nylon-flocked swabs differ in their efficiency of collecting Touch DNA from glass, ceramic, and plastic surfaces?
2. Does the mtDNA extracted from each swab-surface combination exhibit sufficient quality to achieve successful PCR amplification when using mtDNA human-specific primers?

1.4 Objective

1.4.1 General Objective

To evaluate and compare the efficiency of different swab types in recovering touch DNA from common surfaces based on PCR amplification success, and to determine which swab surface combinations provide sufficient amplifiable DNA for forensic identification.

1.4.2 Specific Objective

1. To compare the DNA collection efficiency of three swab types specifically cotton, dacron, and nylon-flocked when used on different surfaces which is glass, ceramic, plastic.
2. To evaluate whether the quality and amplification success of mtDNA extracted from each swab-surface combination using mtDNA human-specific primers of different amplicon length.

1.5 Significance of Study

Touch DNA has become increasingly relevant in modern forensic investigations, particularly in cases where visible biological evidence is absent. However, its successful analysis is often hindered by low DNA quantity, variable shedding rates, and suboptimal evidence collection techniques. This study is significant because it directly addresses one of the most fundamental challenges in touch DNA analysis which focused on identifying the most effective swab and surface combination for recovering amplifiable DNA.

By systematically comparing cotton, dacron, and nylon-flocked swabs across three commonly encountered surfaces which is glass, ceramic, and plastic, this research provides empirical data that can guide forensic practitioners in selecting the most appropriate collection method. Since PCR amplification success depends heavily on the amount and quality of DNA recovered during swabbing, understanding which combinations yield the greatest recovery has direct implications for casework accuracy and reliability.

The findings of this study could enable law enforcement agencies, such as the Royal Malaysia Police, to make use of a broader range of biological samples, including touch DNA, which is often overlooked due to its typically low DNA yield and the challenges associated with its recovery. Expanding the types of evidence considered during investigations could be pivotal in identifying suspects or victims, particularly when visible biological materials like blood, saliva, or semen are absent. By improving the reliability of touch DNA collection and subsequent amplification, this research will provide law enforcement with more robust and scientifically defensible evidence, ultimately leading to more accurate identifications and potentially higher success rates in criminal investigations.

1.6 Scope of Study

The scope of this study, titled “Comparative Efficiency between Different Swab Types for Touch DNA Recovery from Different Surfaces,” focused on evaluating the potential of Touch DNA as a reliable biological source for forensic identification. The research specifically investigates the application of three commonly used swab types which is cotton, dacron and nylon-flocked swab in collecting touch DNA from three frequently encountered forensic surfaces which is glass, ceramic and plastic. To reflect real-life forensic scenarios, touch DNA was deposited onto these surfaces through normal handling to simulate natural shedding variation, acknowledging the inherent inconsistency in trace DNA transfer. The study employs a silica-based DNA extraction method, which is widely recognized for its high binding affinity and suitability for low-template samples. Following extraction, the DNA yield and purity are quantified to determine the effectiveness of each swab–surface combination.

The study then assesses amplification success using Polymerase Chain Reaction (PCR) with human specific primers designed to target short amplicons of 310 bp and 176bp fragment, which serves as an indicator of the presence and integrity of mitochondria DNA (mtDNA) in low-template samples. Following amplification, PCR products are analyzed through agarose gel electrophoresis to evaluate the presence, clarity, and reliability of the resulting DNA bands. This analytical approach allows the study to determine whether the recovered touch DNA is of sufficient quality and quantity to support successful amplification, thereby identifying the most effective swab and surface combination for practical forensic applications.

The analysis includes a comparative evaluation of DNA yield and amplification success across different swab–surface combinations to identify the most effective conditions for recovering trace amounts of touch DNA. By focusing on touch DNA as a source of low-template biological material, the study addresses critical challenges in forensic science, particularly in cases where conventional biological evidence such as blood or saliva is absent, invisible, or highly degraded. The findings are expected to expand the practical utility of touch DNA in forensic investigations by providing insights into efficient and cost-effective DNA collection methods tailored to commonly encountered surfaces. However, the study is limited to the use of mitochondrial DNA as the primary target, and nuclear DNA, which offers greater individuality is not extensively explored. In addition, natural variability in DNA deposition due to factors such as handling pressure, individual shedding differences, and surface characteristics is acknowledged. Overall, this study seeks to contribute to advancements in forensic human identification by improving the reliability, consistency, and applicability of touch DNA recovery in real-world casework.

CHAPTER 2

LITERATURE REVIEW

2.1 Genetics

Genetics is the branch of biology concerned with the study of heredity, genes, and the mechanisms by which traits are transmitted from one generation to the next. It provides the foundation for understanding biological variation and the molecular processes that underlie health, disease, evolution, and identity (Durmaz, et al., 2015). The historical development of genetics began with Gregor Mendel's pioneering work in the 19th century, where his experiments on pea plants led to the formulation of the basic laws of inheritance (Stenseth, Andersson, & Hoekstra, 2022). Although Mendel's findings were not recognized until the early 20th century, they established the concept that traits are controlled by discrete hereditary units. This idea paved the way for classical genetics, and later advancements by Thomas Hunt Morgan in 1910 confirmed that these hereditary units, now called genes, reside on chromosomes, linking cytology and heredity (Pearson, 2006).

The field progressed rapidly with the discovery of the structure of DNA by Watson and Crick in 1953. Their model of the double helix revealed how genetic information is stored, replicated, and transmitted from one cell to the next (Cobb, 2013). As molecular biology expanded, genes came to be defined not only as sequences that encode proteins but also as complex structures that include regulatory regions responsible for controlling gene expression (Hill, Zande, & Wittkopp, Molecular and evolutionary processes generating variation in gene expression, 2021). Modern genetics recognizes that a single

gene can give rise to multiple protein products through alternative splicing and that non-coding RNAs play important roles in regulating gene activity. These discoveries have shifted genetics from a simple trait-based discipline to a multifaceted field exploring interactions between DNA, RNA, proteins, and environmental factors.

Contemporary genetics now encompasses classical genetics, molecular genetics, population genetics, and genomics. Classical genetics continues to explain observable inheritance patterns, while molecular genetics focuses on DNA structure and function with the support of technologies such as PCR, sequencing, and recombinant DNA methods. Population genetics investigates genetic variation within and between populations to reveal evolutionary trends, and genomics examines entire sets of genes and their interactions, offering insights into complex traits and diseases (Amorim A. , 2013). Collectively, these subfields highlight the dynamic and interconnected nature of genetic information.

The applications of genetics extend across medicine, agriculture, conservation, and forensic science. In medicine, genetic knowledge underpins the diagnosis of inherited disorders, the development of gene therapies, and personalized treatment approaches tailored to an individual's genetic profile. In agriculture, genetic principles guide selective breeding and the creation of genetically modified crops designed for improved yield, resistance, and nutritional value (Shan, 2023). Conservation biology relies on genetic data to protect endangered species and maintain biodiversity (Kardos, 2021). In forensic science, the use of genetic analysis has transformed human identification, enabling highly accurate DNA profiling from even minute biological traces.

This foundation in genetics is directly relevant to the present study, which investigates touch DNA including trace biological material left behind through the shedding of skin cells. Touch DNA typically contains very low amounts of nuclear DNA, making autosomal profiling difficult or incomplete. However, mitochondrial DNA (mtDNA), which is more abundant in cells and more resilient to degradation, often remains detectable even when sample quantity is limited. The principles of molecular and forensic genetics therefore underpin the methods used to extract, amplify, and evaluate touch DNA. An understanding of gene structure, DNA composition, and genetic inheritance is essential in determining how effectively different swab types and surface materials facilitate the recovery of amplifiable mtDNA for forensic identification. This integration illustrates the crucial role of genetics in advancing forensic methodologies and enhancing the reliability of trace DNA analysis.

2.1.1 Molecular Genetic

Molecular genetics is a core domain within genetics that focuses on the chemical and physical basis of heredity by examining genes as molecular entities and investigating how they function within cells to regulate development, growth, and physiological processes (Schleif, 2020). By studying genes at the DNA and RNA level, molecular genetics provides the mechanistic bridge between genotype and phenotype, explaining how differences in genetic sequences can produce observable biological variation through changes in gene expression and cellular pathways. This linkage is essential in both biological research and applied fields such as medicine and forensic science, where the

interpretation of genetic material depends on understanding how nucleic acids are structured, maintained, and processed.

At the molecular level, DNA serves as the primary hereditary molecule and is organized into chromosomes within the nucleus (in eukaryotes). The DNA molecule consists of a sugar–phosphate backbone linked to nitrogenous bases which consist of adenine (A), thymine (T), guanine (G), and cytosine (C) (Alberts , et al., 2002). The stability and informational function of DNA depend heavily on base pairing, where adenine pairs specifically with thymine and guanine pairs with cytosine through hydrogen bonds, creating two complementary antiparallel strands. This complementarity is not merely structural, it forms the basis of DNA replication, enabling genetic information to be copied with high fidelity prior to cell division. Replication proceeds through the coordinated action of enzymes, particularly DNA polymerases, which synthesize new DNA by adding nucleotides to the 3' end of a growing strand. The directionality of synthesis and strand complementarity ensure that each original strand can serve as a template for the formation of a new complementary strand, preserving genetic continuity across generations of cells (Alberts , et al., 2002).

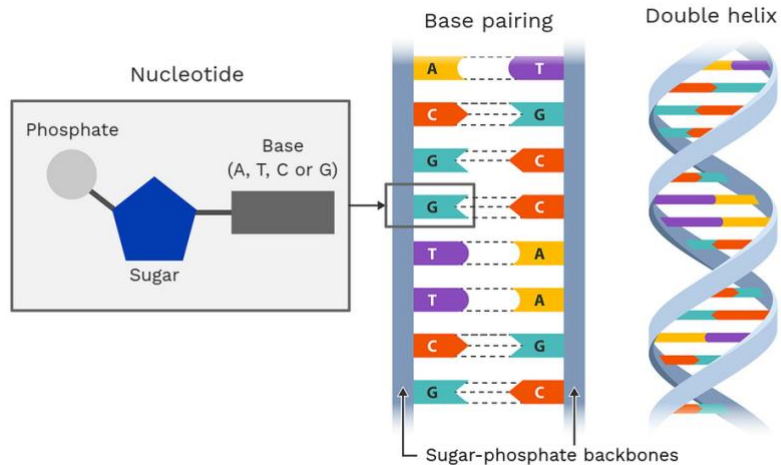


Figure 2.1 : Structural organization of DNA, showing the nucleotide components (phosphate, sugar, and nitrogenous base), complementary base pairing (A–T and C–G), the sugar–phosphate backbone, and the resulting double-helix structure (Labster, 2026).

Despite the high accuracy of DNA replication, molecular genetics also recognizes that genetic stability is not absolute. Errors can occur during replication, producing mutations that may result in permanent changes in the DNA sequence. Mutations may arise spontaneously through polymerase misincorporation or structural instability, or they may be induced by environmental mutagens such as ionizing radiation, ultraviolet light, or reactive chemicals (Alberts, et al., 1983). Depending on their location and type, mutations may have no apparent effect, alter gene function subtly, or severely disrupt cellular processes.

For example, mutations occurring within coding regions can change amino acid sequences, potentially modifying protein structure and function, while mutations within regulatory regions can alter when and how much a gene is expressed (Hill, Zande, & Wittkopp, 2020). Through these mechanisms, mutations can generate phenotypic variation and, in some contexts, contribute to disease processes. This dual emphasis on stability and

change is a central theme in molecular genetics because it explains both biological continuity and diversity.

The functional consequences of DNA sequences are classically explained through the Central Dogma of molecular biology, which outlines how genetic information flows from DNA to RNA and ultimately to protein. In transcription, segments of DNA are used as templates to synthesize messenger RNA (mRNA), a process regulated by transcription factors and promoter elements that determine which genes are active in specific tissues or at specific developmental stages (Zrimec, Buric, Kokina, Garcia, & Zelezniak, 2021). The resulting mRNA carries genetic instructions from the nucleus in eukaryotes to ribosomes in the cytoplasm, where translation occurs. Translation converts the nucleotide sequence of mRNA into an amino acid sequence by reading the genetic code in triplets called codons. Transfer RNAs (tRNAs), each carrying specific amino acids, match their anticodons to mRNA codons, allowing ribosomes to assemble a polypeptide chain in the correct order (Clancy & Brown, 2021).

This genetic code is often described as universal because the codon-to-amino acid assignments are conserved across most life forms, reinforcing the fundamental unity of biological systems. Proteins produced through translation serve essential cellular roles, including enzymatic catalysis, cell signaling, transport, structural maintenance, and regulation of gene expression itself (Kubyskin, Acevedo-Rocha, & Budisa, 2018). Therefore, the Central Dogma provides a direct conceptual explanation for how a gene's sequence can influence observable traits, supporting the genotype–phenotype relationship that molecular genetics aims to interpret (Ostrander, 2026).

Beyond describing molecular mechanisms, molecular genetics offers a framework for understanding how genetic variation and environmental factors interact to shape biological outcomes. Traits are not determined solely by DNA sequence rather, gene expression is influenced by regulatory networks, epigenetic modifications, and environmental exposures that can alter transcriptional activity and cellular pathways. This interaction is particularly relevant when examining complex conditions such as psychiatric disorders, where genetic susceptibility may contribute to altered biochemical signaling but environmental factors can affect onset, severity, or progression.

By connecting mutations and regulatory variation to molecular pathways and phenotypic effects, molecular genetics supports the identification of genetic contributors to disease and provides insight into how biological systems respond dynamically to internal and external influences. Overall, molecular genetics provides the essential mechanistic foundation for any scientific context that requires DNA interpretation, including the analysis of low-level genetic material in forensic investigations.

2.1.2 Forensic Genetic

Forensic genetics has undergone extensive development and is now an essential discipline within modern forensic science, with wide-ranging applications including criminal investigations, paternity determination, disaster victim identification, and anthropological research. The origins of forensic genetics can be traced to early scientific milestones such as Landsteiner's discovery of the ABO blood group system in 1900 and Locard's Exchange Principle introduced in 1910, which established the concept that

contact between individuals or objects results in the transfer of material. These foundational principles laid the conceptual framework for forensic evidence analysis. A major scientific breakthrough occurred in 1953 with the elucidation of the double-helical structure of DNA, which enabled molecular-level examination and fundamentally transformed forensic identification methods (Li C. , 2018).

A core principle of forensic genetics is the ability to generate individual-specific DNA profiles through the analysis of genetic markers, most notably Short Tandem Repeats (STRs). STRs are highly polymorphic sequences located within non-coding regions of the genome and exhibit strong reproducibility, making them the preferred markers for individualization in forensic investigations. The implementation of multiplex STR systems, which allow multiple loci to be analyzed simultaneously, has significantly enhanced both the efficiency and discriminatory capacity of forensic DNA profiling (Schneider, 2007). Beyond STRs, additional markers such as single nucleotide polymorphisms (SNPs) and mitochondrial DNA (mtDNA) have broadened the scope of forensic genetic analysis. SNPs are particularly useful when analysing degraded DNA due to their short target regions and low mutation rates, and they also provide valuable information related to ancestry and externally visible traits (Li C. , 2018); (Morling, 2004). In contrast, mtDNA analysis is frequently applied to severely degraded samples, including skeletal remains and hair shafts, as its high copy number per cell increases the likelihood of successful profiling when nuclear DNA is limited (Schneider, 2007) (Schneider, 2007).

Advances in forensic genetics have been driven largely by technological innovation, with polymerase chain reaction (PCR) serving as a cornerstone technique that enables the amplification of extremely small quantities of DNA from trace evidence. PCR-

based methodologies underpin STR and SNP profiling and have allowed the reliable examination of samples that were previously unsuitable for forensic analysis. More recent developments, such as massively parallel sequencing (MPS), also known as next-generation sequencing (NGS), facilitate the concurrent analysis of multiple genetic marker types, improving the interpretation of complex mixtures and strengthening kinship analysis (Kowalczyk, Zawadzka, Szewczuk, Gryzinka, & Jakubczak, 2018). Nevertheless, highly sensitive techniques such as low copy number (LCN) DNA analysis demand rigorous contamination prevention measures to minimise analytical artefacts and ensure data reliability (Morling, 2004).

Within forensic casework, DNA evidence plays a pivotal role in linking biological material recovered from crime scenes to suspects or reference profiles. STR-based profiling remains the most widely adopted method across jurisdictions and is supported by statistical evaluation to determine evidential weight. Forensic genetics is equally critical in mass disaster victim identification and missing persons cases, where mtDNA and SNP analyses are often relied upon due to extensive sample degradation (Schneider, 2007). Despite its effectiveness, the expanding use of forensic genetic technologies particularly forensic DNA phenotyping raises ethical and legal concerns related to privacy, data retention, and potential misuse. Consequently, international standards such as ISO/IEC 17025 emphasise laboratory accreditation, quality assurance, and method validation to ensure the ethical and scientifically sound application of forensic genetic evidence (Morling, 2004) .

2.1.2.1 Trace DNA

Trace DNA refers to minute quantities of genetic material left behind through minimal contact or transfer, often containing less than 100 picograms (pg) of DNA (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019). Its forensic applications became prominent in the late 1990s when advancements in Polymerase Chain Reaction (PCR) enabled DNA profiling from low-template samples (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019). As summarized in Figure 2.1, the utility of trace DNA has expanded significantly, playing a crucial role in criminal investigations, intelligence efforts, and proactive policing strategies. Beyond its use for identification, trace DNA contributes to intelligence gathering, connecting events and crime scenes, and generating leads in cold cases (Hoffmann, Meakin, Morelato, & Roux, 2024). However, concerns about contamination, secondary transfer, and ethical implications have necessitated stringent handling and interpretation protocols (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019).

Trace DNA originates from various biological materials, including skin cells, sweat, saliva, and hair (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019). These biological materials are deposited on surfaces like weapons, clothing, or furniture, often unintentionally (Raymond, Oorschot, Gunn, Walsh, & Roux, 2009). Non-visible biological materials such as skin-associated cells or DNA in sweat often contribute to the presence of trace DNA, complicating its source identification (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019). Research by (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019) has shown that non-self-DNA, originating from indirect

transfer, can be identified on items touched by multiple individuals, emphasizing its prevalence and significance in forensic science.

Advancements in collection methods have been crucial in increasing the recovery rates of trace DNA. Techniques such as swabbing, tape lifting, and direct analysis of collected material have demonstrated variable efficiencies depending on the surface and sample type (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019)). Double-swabbing and the use of optimized moistening agents have significantly improved recovery rates from porous and non-porous substrates (Raymond, Oorschot, Gunn, Walsh, & Roux, 2009). Furthermore, emerging technologies like laser microdissection and flow cytometry have enhanced the selective collection of DNA from complex mixtures, paving the way for more precise downstream analysis (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019)

Despite its forensic potential, trace DNA analysis faces significant challenges. The low quantity and quality of DNA in trace samples increase the risk of contamination and stochastic effects during analysis (Conte, Morgan, & Tracey L. Johnson, 2025). Mixture profiles are common, as trace DNA often includes contributions from multiple individuals, making interpretation complex. Secondary transfer which is the movement of DNA from an individual to an object via an intermediary will further complicates interpretation, as demonstrated by studies of (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019) on indirect transfer mechanisms. Moreover, environmental factors such as heat, humidity, and substrate type can degrade DNA, reducing its recoverability and reliability. The evolution of analytical techniques has addressed some of these challenges.

STR profiling and PCR remain foundational in amplifying low-template DNA. Mini-STR kits, designed with shorter amplicons, enhance recovery from degraded samples. Probabilistic genotyping software further assists in interpreting complex profiles, offering statistical likelihoods for DNA contributors (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019). Trace DNA has been pivotal in solving cold cases, identifying victims in mass disasters, and exonerating the wrongfully convicted (Hoffmann, Meakin, Morelato, & Roux, 2024). Its ability to link suspects to scenes or objects with minimal biological evidence has transformed forensic investigations. For instance, studies by (Raymond, Oorschot, Gunn, Walsh, & Roux, 2009) on DNA persistence have demonstrated that trace evidence can remain detectable on surfaces for weeks under optimal conditions, aiding in historical crime scene analyses.

Current limitations in trace DNA recovery and analysis include variability in transfer rates, degradation, and interpretative challenges in mixed profiles. Research on the persistence of trace DNA under various environmental conditions and its transfer mechanisms is vital for enhancing forensic applications (Raymond, Oorschot, Gunn, Walsh, & Roux, 2009). In addition, gaps in standardizing collection and analysis methods necessitate ongoing research to ensure trace DNA's continued reliability as a forensic tool (Van Oorschot, Szkuta, Meankin, Kokshoorn, & Goray, 2019).

2.2 Touch DNA as Trace Evidence

2.2.1 Definition of Touch DNA

Touch DNA refers to the genetic material that is transferred from an individual to an object or surface through physical contact, typically via skin cells shed from the outer layers of the epidermis (Sinha, Yadav, & Bumbrah, 2021). Unlike traditional biological fluids such as blood or saliva, touch DNA is often invisible and present in very small quantities. The DNA recovered may originate from a combination of shed epithelial cells, cellular debris, and extracellular DNA released during normal skin contact.

In forensic contexts, touch DNA is commonly recovered from items that have been handled or worn, including weapons, clothing, door handles, mobile phones, and other frequently touched surfaces (Burril, Daniel, & Frascione, 2019). The amount and quality of touch DNA transferred depend on multiple factors, such as the duration and pressure of contact, the nature of the surface, individual shedding propensity, and subsequent handling or environmental exposure (Swargiary & Pandey, 2025). Due to its low quantity and potential for secondary transfer, touch DNA samples often present significant analytical and interpretational challenges in forensic investigations.

2.2.2 Shedding Mechanism and Variability

The shedding mechanism refers to the biological process by which skin cells and associated DNA are released from the human body and subsequently transferred to external surfaces through physical contact. Human skin undergoes continuous renewal, during which keratinised epithelial cells from the outermost layer of the epidermis

(stratum corneum) are routinely sloughed off (Burril, Daniel, & Frascione, 2019). During handling or contact with objects, these shed skin cells, along with extracellular DNA fragments, may be deposited onto surfaces, forming what is commonly referred to as touch DNA. The transfer of DNA can occur through direct contact or indirectly via secondary or tertiary transfer, further complicating the interpretation of forensic evidence (Burril, Daniel, & Frascione, 2019).

Shedding variability is a well-documented phenomenon in forensic genetics and refers to differences between individuals in the quantity of DNA they deposit upon contact. Individuals are often categorised as high, medium, or low shedders based on the relative amount of DNA they transfer, although this classification is not fixed and may vary over time. Factors influencing shedding variability include physiological differences such as skin condition, perspiration, and sebaceous activity, as well as behavioural factors such as hand washing, frequency of contact, and pressure or friction applied during handling (Tozzo, 2022). Environmental factors, including surface type and cleanliness, also play a significant role in determining the amount of DNA recovered.

Importantly, shedding propensity is not solely dependent on the number of cells transferred but also on the amount of recoverable DNA present within or outside those cells (Woollacott, 2025). Research has demonstrated that extracellular DNA, released through cell lysis or natural skin processes, may contribute substantially to touch DNA profiles (Burril, Daniel, & Frascione, 2019). As a result, an individual who sheds fewer intact cells may still deposit a detectable amount of DNA. This variability presents a major challenge in forensic interpretation, as the presence or absence of an individual's DNA on