# RECOVERY AND AMPLIFICATION OF TOUCH DNA ON POROUS SURFACES USING MODIFIED CHELEX EXTRACTION METHOD

**NAYOMI KHOO YA MUN**

**UNIVERSITI SAINS MALAYSIA**

**2025**

# RECOVERY AND AMPLIFICATION OF TOUCH DNA ON POROUS SURFACES USING MODIFIED CHELEX EXTRACTION METHOD

**by**

**NAYOMI KHOO YA MUN**

**Thesis submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Forensic Science)**

**UNIVERSITI SAINS MALAYSIA**

**2025**

## DECLARATION

I hereby declare that this dissertation is the result of my own independent research and investigations, except where explicitly stated and duly acknowledged. I further affirm that this work has not been previously submitted, in whole or in part, for any degree or qualification at Universiti Sains Malaysia or any other institution. I also grant Universiti Sains Malaysia the right to utilize this dissertation for purposes of teaching, research, and academic promotion. Additionally, I understand that any form of academic misconduct, such as plagiarism, is a serious offense, and I affirm that this work upholds the principles of academic integrity and originality.

_____

(Nayomi Khoo Ya Mun)

Date: 7th February 2025

## ACKNOWLEDGEMENT

I would like to take this opportunity to extend my deepest gratitude to everyone who has directly or indirectly contributed to the success of this study.

First and foremost, I sincerely thank Universiti Sains Malaysia (USM) and the School of Health Sciences for providing the resources, facilities, and support that made this research possible. The conducive academic environment and technical assistance from the university and faculty were essential to my success. Furthermore, I am deeply grateful to the laboratory staff for their invaluable assistance with equipment, reagents, and technical advice. I would like to specifically thank Puan Hafizah, Encik Baharuddin, Puan Hasnita, Puan Syazwani, and the staff from the HID/DNA Unit, including Puan Nur Azeelah and Encik Muhammad Ismail. Their support was critical to the success of my research, and I truly appreciate their contributions.

I am profoundly thankful to my supervisor, Dr. Nur Waliyuddin Hanis bin Zainal Abidin, for his unwavering support and guidance throughout my project. His dedication, patience, and insightful advice have been instrumental in my progress. Dr. Nur Waliyuddin always made time to address my queries, providing clear instructions and valuable feedback. I am incredibly fortunate to have had him as my supervisor, and I will always be grateful for his mentorship throughout this research journey.

My sincere appreciation goes to my research colleagues, Wai Lun and Wendy, who have been more than just colleagues. They have been pillars of support throughout this project. Their encouragement during challenging moments, especially when my extractions did not yield results, and their willingness to teach me every aspect of the project, have been invaluable without their expertise and support, this project would not have been possible. I am deeply grateful for all that I have learned from them. I would also like to extend my appreciation to

the friends of Room 101 DM6 for contributing their samples to this project. Their generosity provided the foundation for the continuation of my research, and without their participation, this study could not have progressed.

I am especially thankful to my family for their endless love and encouragement. Their unwavering support has been my greatest source of strength throughout this journey.

A special shout-out goes to my amazing friends who have supported me throughout my academic journey. My heartfelt gratitude to Shukur Purba, whose encouragement led me to apply to this university. His belief in my abilities has been instrumental in helping me reach this stage. I am also incredibly thankful to Ke Xin for being a true role model; her determination and academic discipline inspired me to persevere through challenges. Additionally, I deeply appreciate my secondary school friends, Xin Ru and Pei Eng, for their constant emotional and mental support. Their understanding and encouragement have kept me grounded. Lastly, I sincerely thank my work agent, Tracy, for always providing me with a flexible work schedule, allowing me the time to focus on completing my thesis.

Finally, I would like to express my deepest gratitude to my partner. His unconditional love, support, and encouragement have been a true blessing. From taking care of daily tasks to relieving my stress, he has been there for me every step of the way. His belief in me, patience, and ability to make me smile during difficult times have made all the difference. I am forever grateful for everything he has done.

To everyone who has contributed to this project, directly or indirectly, I extend my heartfelt thanks. This thesis would not have been possible without each and every one of you.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF SYMBOLS

| | |
|---|---|
| °C | Degree Celsius |
| µL | Microliter |
| mL | Milliliter |
| g | Gram |
| bp | Base pairs |
| ng/µL | Nanograms per microliter |
| µM | Micromolar |
| rpm | Revolutions per minute |
| % | Percentage |
| µm | Micrometers |
| V | Volts |
| L | Liter(s) |
| 1x, 0.5x, 10x | Dilution Concentration Factors (e.g., Buffer Concentrations) |
| M | Molar (Concentration) |
| pH | Potential of Hydrogen |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| A260/A280 | Absorbance Ratio at 260 nm and 280 nm |
| A260/A230 | Absorbance Ratio at 260 nm and 230 nm |
| BLAST | Basic Local Alignment Search Tool |
| BLASTN | Basic Local Alignment Search Tool for Nucleotides |
| Chelex® | Chelating Resin (Styrene-Divinylbenzene Copolymer with Iminodiacetate Ions) |
| DNA | Deoxyribonucleic Acid |
| dsDNA | Double-Stranded DNA |
| dNTP | Deoxynucleotide Triphosphates |
| EtBr | Ethidium Bromide |
| GC | Guanine-Cytosine |
| HCl | Hydrochloric Acid |
| HID | Human Identification Unit/DNA |
| HV1 | Hypervariable Region 1 |
| HV2 | Hypervariable Region 2 |
| JEPeM | Jawatankuasa Etika Penyelidikan Manusia (Human Research Ethics Committee) |
| L | Ladder (in gel electrophoresis) |
| mg | Milligram |
| $MgCl_2$ | Magnesium Chloride |
| min | Minute |
| mtDNA | Mitochondrial DNA |
| Na2EDTA | Ethylenediaminetetraacetic Acid Disodium Salt |
| NaOH | Sodium Hydroxide |
| NCBI | National Center for Biotechnology Information |
| NGS | Next-Generation Sequencing |
| PBS | Phosphate-Buffered Saline |
| PCR | Polymerase Chain Reaction |
| Pro K | Proteinase K |
| RFLP | Restriction Fragment Length Polymorphism |

| | |
|---|---|
| RNA | Ribonucleic Acid |
| SDS | Sodium Dodecyl Sulfate |
| ssDNA | Single-Stranded DNA |
| STR | Short Tandem Repeat |
| Ta | Annealing temperature |
| Taq | Thermus aquaticus (polymerase source) |
| TBE | Tris-Borate-EDTA Buffer |
| Tm | Melting Temperature |
| UV | Ultraviolet |

## LIST OF APPENDICES

**PEMULIHAN DAN AMPLIFIKASI DNA SENTUHAN DARIPADA PERMUKAAN POROS DENGAN MENGGUNAKAN KAEDAH PENGEKSTRAKAN CHELEX TERUBAH SUAI**

## ABSTRAK

DNA sentuhan, iaitu bahan genetik yang ditinggalkan melalui sentuhan harian, memainkan peranan penting dalam sains forensik dengan meninggalkan bukti kritikal untuk menghubungkan individu dengan tempat kejadian. Walau bagaimanapun, permukaan poros menimbulkan cabaran yang besar dalam pemulihan DNA sentuhan disebabkan keupayaannya untuk menyerap dan menyebabkan DNA terdegradasi. Kajian ini bertujuan untuk mengoptimumkan kaedah pengumpulan, pengekstrakan, dan amplifikasi DNA sentuhan daripada sepuluh jenis permukaan poros yang sering ditemui bagi menambahbaik protokol analisis forensik.

Dalam kajian ini, fabrik, kayu, kertas, pemegang raket, pemegang basikal, langsir, tikar, beg, meja, dan tuala telah digunakan sebagai sumber sampel DNA sentuhan. Proses pengumpulan sampel menggunakan teknik sapuan berganda, di mana sapuan basah diikuti oleh sapuan kering, untuk memaksimumkan pemulihan DNA. Kaedah pengekstrakan Chelex® 100 digunakan kerana keberkesanannya dalam menghilangkan bahan penghalang sambil mengekalkan integriti DNA. Kuantifikasi DNA dilakukan dengan menggunakan Spektrofotometer DeNovix® DS-11 untuk menilai kuantiti dan ketulenan DNA. Proses amplifikasi tindak balas rantai polimerase (PCR) menfokuskan dua kawasan DNA mitokondria (mtDNA) bersaiz 176 pasangan bes (bp) dan 310 bp, diikuti dengan analisis menggunakan elektroforesis gel agarosa untuk menilai kehadiran DNA manusia.

Hasil kajian menunjukkan variasi ketara dalam keberkesanan pemulihan dan amplifikasi DNA daripada permukaan yang diuji. Langsir, pemegang beg, dan pemegang raket

menunjukkan kejayaan amplifikasi mtDNA untuk sasaran 176 bp, manakala kawasan mtDNA bersaiz 310 bp hanya berjaya dikesan daripada langsir. Permukaan seperti kertas dan kayu, yang mempunyai tahap porositi yang tinggi dan mengandungi sebatian perencat, menunjukkan cabaran terbesar dalam proses pemulihan dan pengesanan DNA. Penemuan ini menekankan peranan penting sifat permukaan, kepentingan pemilihan amplikon yang lebih pendek untuk sampel yang terdegradasi, serta keperluan modifikasi protokol mengikut jenis permukaan tertentu.

Kajian ini memberikan pandangan tentang pengoptimuman proses pemulihan dan pengesanan DNA sentuhan daripada permukaan poros, dengan penekanan kepada nilai mtDNA sebagai sasaran untuk sampel yang terdegradasi atau rendah hasilnya. Dengan memperbaiki kaedah pengekstrakan dan penguatan, kajian ini menyumbang kepada pembangunan protokol forensik yang lebih dipercayai dan berkesan, bagi meningkatkan kebolehgunaan DNA sentuhan dalam penyiasatan jenayah.

# RECOVERY AND AMPLIFICATION OF TOUCH DNA ON POROUS SURFACES USING MODIFIED CHELEX EXTRACTION METHOD

## ABSTRACT

Touch DNA, the trace genetic material deposited through casual contact, plays a critical role in forensic science by providing critical evidence to link individuals to crime scenes. However, porous surfaces pose significant challenges for touch DNA recovery due to their ability to absorb and degrade DNA. This study aimed to optimize the collection, extraction, and amplification of touch DNA from ten commonly encountered porous surfaces to address challenges and enhance forensic protocols.

In this research, fabric, wood, paper, racket grips, bicycle grips, curtain, floor mat, bag handle, table, and towel have been chosen as the source of touch DNA samples. The double-swabbing method, involving a moistened swab followed by a dry swab, was employed for sample collection to maximize DNA recovery. The Chelex® 100 extraction method was utilized for its efficiency in removing inhibitors while preserving DNA integrity. Further purification step was conducted using silica-based method from a commercial kit. DNA quantification was conducted using the DeNovix® DS-11 Spectrophotometer to assess yield and purity. Polymerase chain reaction (PCR) amplification targeted mitochondrial DNA (mtDNA) regions of 176 bp and 310 bp, followed by analysis using agarose gel electrophoresis to evaluate the success of DNA amplification.

The results revealed significant variability in DNA recovery and amplification across the tested surfaces. The curtain, bag handle, bicycle grip and racket grip demonstrated successful amplification for the mtDNA of 176 bp target, while the 310 bp fragment was only amplified from the curtain sample. This could be due to the higher DNA recovery from this

surface, reduced degradation, or fewer inhibitory compounds interfering with PCR amplification, unlike other tested surfaces. Surfaces such as paper and wood, with high porosity and inhibitory compounds, presented the greatest challenges for DNA recovery and amplification. The findings highlight the critical role of surface properties, the importance of shorter amplicon targets for degraded samples, and the need for optimized protocols tailored to specific substrates.

This study highlighted the insights into the optimization of touch DNA recovery and amplification from porous surfaces, emphasizing the value of mtDNA as a target for degraded or low-yield samples. By improving extraction and amplification methodologies, the research contributes to the development of more reliable and effective forensic protocols, enhancing the utility of touch DNA in criminal investigations.

# CHAPTER 1
# INTRODUCTION

## 1.1 Background of study

The integration of DNA analysis into forensic science has revolutionized criminal investigations, providing a powerful tool for linking individuals to crime scenes with a high degree of certainty (Butler, 2015; Roland A.H. van Oorschot et al., 2019). DNA profiling, which relies on the unique genetic makeup of individuals, has become one of the most reliable forms of evidence in criminal cases, often playing a critical role in forensic identification (Goray et al., 2010). While conventional biological samples such as blood, saliva, and hair are typically abundant and straightforward sources of DNA, there are many situations where such samples are not readily available (Ladd et al., 1999). In these scenarios, investigators often rely on touch DNA, the minute traces of genetic material left behind when an individual comes into contact with an object or surface (van Oorschot & Jones, 1997).

Touch DNA, primarily deposited through skin cells, has become an invaluable resource in forensic investigations, particularly in cases where more prominent biological evidence, such as blood or saliva, is absent (Verdon et al., 2014). Despite its potential, the recovery of touch DNA is fraught with challenges. These traces typically contain low quantities of DNA, making them susceptible to degradation and contamination (Pang & Cheung, 2007). The recovery becomes even more complex when dealing with porous surfaces, such as wood, fabric, paper, and bicycle grips. These materials absorb and trap DNA within their fibers and pores, significantly reducing the yield and complicating the extraction process (van Oorschot & Jones, 1997). Forensic scenarios, such as recovering DNA from personal items like fabric-based furniture or paper documents, require optimized techniques to ensure the successful collection and amplification of touch DNA for profiling purposes (Butler, 2015).

Existing DNA extraction methods, often designed for non-porous surfaces like glass or metal, are not equally effective on porous materials (van Oorschot et al., 2019). The lack of standardized protocols for recovering touch DNA from porous surfaces further compounds the problem, leading to inconsistent results and hindering the ability to reliably link individuals to crime scenes (Verdon et al., 2014). The physical and chemical properties of porous materials, including the presence of inhibitory compounds in wood or residues on fabric, exacerbate these challenges by interfering with DNA extraction and amplification processes (Goray et al., 2010a). Moreover, the small quantities of DNA typically present in touch DNA samples require optimized polymerase chain reaction (PCR) protocols to ensure successful amplification (Ladd et al., 1999; van Oorschot et al., 2019).

To address these challenges, this study focuses on the Chelex® 100 DNA extraction method, which has demonstrated efficiency in removing inhibitors while preserving DNA integrity (Goray et al., 2010b; Walsh et al., 2013). The study evaluates its efficacy in recovering touch DNA from a range of porous surfaces commonly encountered in forensic investigations, including paper, fabric, porous table surfaces, racket grips, bicycle grips, floor mats, towels, curtains, bag handles, and wood. The primary objective is to optimize the collection, extraction, and amplification of touch DNA from these surfaces to establish a reliable and standardized protocol for forensic applications.

The significance of this research lies in its potential to enhance the recovery and analysis of touch DNA from challenging porous surfaces. By refining extraction and amplification methods, this study aims to address the inconsistencies associated with porous materials and improve the reliability of forensic. Click or tap here to enter text.These advancements will contribute to more effective identification of suspects, even in cases where only trace amounts of DNA are available. The findings of this study will strengthen forensic

science's ability to solve crimes with greater precision and reliability, ensuring that touch DNA evidence can be utilized to its fullest potential in criminal investigations.Click or tap here to enter text.

### 1.2 Problem Statement

In forensic science, touch DNA has emerged as a critical tool for linking individuals to crime scenes, particularly in cases where more obvious biological evidence, such as blood or saliva, is absent (Butler, 2015; Roland A. H. van Oorschot and Jones, 1997). This minute genetic material, deposited through casual contact, holds significant potential for suspect identification. However, the recovery and analysis of touch DNA from porous surfaces such as paper, fabric, wood, and bicycle grips remain challenging due to the ability of these materials to absorb and trap DNA within their fibers and pores (Pang & Cheung, 2007; Verdon et al., 2014). This not only reduces DNA yield but also exposes it to degradation, further complicating recovery efforts(Goray et al., 2010b).

Current DNA extraction techniques, including the widely used Chelex method, often yield inconsistent results when applied to porous substrates, as the physical and chemical properties of these surfaces can inhibit efficient DNA recovery (Walsh et al., 2013). For instance, compounds in wood or residues on fabric can interfere with DNA extraction and amplification, hindering the ability to generate reliable forensic profiles (Farash et al., 2018). Furthermore, the lack of standardized protocols tailored specifically for porous materials exacerbates the variability in results, making it difficult to establish consistent and reproducible forensic procedures.

Mitochondrial DNA (mtDNA) presents a promising target for forensic analysis due to its higher copy number and resilience compared to nuclear DNA (Parsons et al., 2007). However, even mtDNA amplification is often impeded by low DNA concentrations and the

presence of inhibitors in porous samples (Farash et al., 2018). This highlights the need to apply both DNA extraction and amplification methods to address these challenges effectively.

This study addresses these gaps by focusing on the optimization of touch DNA recovery, extraction, and amplification from various porous surfaces commonly encountered in forensic investigations. By refining existing protocols and targeting mtDNA regions, this research aims to enhance the reliability of forensic analyses and improve the ability to associate evidence with potential contributors through touch DNA.

## 1.3 Research Questions

1. What is the DNA yield and quality from various porous surfaces using standard vs modified Chelex extraction techniques?
2. Can mitochondrial DNA be successfully amplified from touch DNA recovered from porous surfaces?

## 1.4 Objectives

### 1.4.1 General Objectives

To evaluate the usefulness of touch DNA from porous surfaces for forensic identification.

### 1.4.2 Specific Objectives

1. To extract and quantify DNA from various porous surfaces using Chelex® 100 resin.
2. To assess the quality of extracted DNA from porous surfaces using PCR amplification of human mtDNA regions.

## 1.5 Significance of Study

This study holds significant importance in the field of forensic science, particularly in the analysis of touch DNA. Touch DNA, comprising minute genetic traces left behind through casual contact with surfaces, often provides critical evidence in criminal investigations where more prominent biological samples, such as blood or saliva, are absent (Butler, 2015; Roland A. H. van Oorschot and Jones, 1997). However, recovering touch DNA from porous surfaces, such as wood, fabric, paper, and similar materials, poses significant challenges. These surfaces absorb and trap DNA within their fibers and pores, making it difficult to extract and analyze sufficient quantities for reliable forensic profiling (Pang & Cheung, 2007; Verdon et al., 2014). Addressing these challenges by developing effective recovery and amplification techniques bridges a critical gap in forensic investigation methods. For example, in a burglary case where a suspect may have only touched a wooden door or a fabric-covered chair, the ability to recover and analyze DNA from these surfaces could be crucial for linking the suspect to the scene of the crime (Goray et al., 2010).

This study focuses on applying the Chelex® 100 DNA extraction method to recover viable DNA from a range of porous surfaces, including paper, fabric, porous table surfaces, racket grips, bicycle grips, floor mats, towels, curtains, bag handles, and wood. By evaluating the efficacy of the Chelex extraction method, this research seeks to establish a reliable and standardized protocol for recovering and amplifying DNA from challenging surfaces commonly encountered in forensic investigations (Walsh et al., 2013). The findings are expected to contribute to the advancement of standardized methodologies in forensic science, ensuring greater consistency and reliability in touch DNA recovery. A standardized approach is particularly valuable in legal contexts, where the admissibility and reliability of forensic evidence can significantly influence judicial outcomes (Butler, 2015; Verdon et al., 2014). Enhanced protocols for touch DNA analysis will strengthen the evidence used to corroborate

or refute a suspect's presence at a crime scene, thereby supporting the integrity of legal proceedings.

Additionally, this study's findings have the potential to inspire future research and innovation in forensic DNA analysis. As porous materials are highly variable in their physical and chemical properties, the challenges associated with touch DNA recovery remain complex and multifaceted. The insights gained from this study may encourage further exploration into novel DNA recovery methods, the use of polymerases better suited for challenging forensic samples, and improved extraction protocols tailored to specific substrates.(Farash et al., 2018). Advancements in these areas could enhance the capability of forensic science to address increasingly complex cases involving degraded or trace DNA samples (Butler, 2015).

By optimizing the recovery and amplification of touch DNA from porous surfaces, this research contributes to the ongoing effort to refine forensic methodologies. It underscores the importance of improving touch DNA protocols, ensuring accurate and efficient forensic identification, even in cases where DNA evidence is minimal or compromised (van Oorschot et al., 2019a; Verdon et al., 2014). Ultimately, the study aims to strengthen the role of forensic science in criminal investigations, providing more reliable tools for solving crimes and delivering justice.

### 1.6 Scope of Study

This study focuses on optimizing the recovery, extraction, and amplification of touch DNA from various porous surfaces frequently encountered in forensic investigations. Porous materials such as paper, fabric, porous table surfaces, racket grips, bicycle grips, floor mats, towels, curtains, bag handles, and wood were selected for analysis due to their prevalence in real-world scenarios and their challenging nature for DNA recovery (Pang & Cheung, 2007; Verdon et al., 2014). These surfaces are often found in environments like households,

workplaces, and crime scenes, making them highly relevant to forensic applications. By narrowing the focus to porous materials, this study aims to address the specific challenges associated with DNA retention, degradation, and extraction from substrates that tend to absorb and trap biological material within their fibers and pores (Farash et al., 2018).

The scope encompasses the use of the double-swabbing method for sample collection. This technique involves a moistened swab to loosen and collect DNA, followed by a dry swab to recover residual material. This approach was chosen for its effectiveness in maximizing DNA recovery from porous surfaces (Goray et al., 2010b; Pang & Cheung, 2007). The extracted DNA was processed using the Chelex® 100 extraction method, a cost-effective and widely used technique known for its ability to isolate DNA while removing inhibitors that can interfere with downstream analyses (Walsh et al., 2013). The study evaluates the suitability of this method for recovering touch DNA from challenging porous substrates.

Quantification of DNA yield and purity was conducted using the DeNovix® DS-11 Spectrophotometer, providing insights into the effectiveness of the extraction process. This step ensures that sufficient and high-quality DNA is available for amplification. The study specifically targeted mitochondrial DNA (mtDNA) regions for polymerase chain reaction (PCR) amplification, focusing on two amplicon sizes, such as 176 bp and 310 bp. mtDNA was selected due to its high copy number and resilience, making it an ideal target for degraded or low-yield samples typically encountered in forensic investigations (Parson & Bandelt, 2007). The amplified products were analyzed using agarose gel electrophoresis to confirm successful amplification and evaluate the influence of surface properties on the process.

The study's scope is limited to porous surfaces, ensuring a focused and in-depth understanding of their unique challenges in forensic DNA recovery and amplification. While nuclear DNA analysis was not included, the research emphasizes the forensic relevance of

mtDNA analysis as a reliable alternative for cases involving degraded or trace amounts of DNA (Parsons et al., 2007). Additionally, the study explores the impact of surface properties, environmental factors, and inhibitors on DNA recovery and amplification, offering practical insights for improving forensic protocols (Farash et al., 2018).

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 Forensic DNA Analysis

Forensic DNA analysis has transformed criminal investigations since its inception in 1987, offering an unparalleled ability to link individuals to crime scenes through unique genetic profiles. This revolutionary tool leverages the stable and inheritable nature of DNA, enabling the identification of individuals with high accuracy even in the absence of traditional biological evidence. Initially reliant on restriction fragment length polymorphism (RFLP), the field has evolved to adopt polymerase chain reaction (PCR) methods, which are more sensitive and efficient. PCR-based techniques such as short tandem repeat (STR) profiling have become the cornerstone of forensic DNA analysis, as they allow for the successful analysis of degraded and minute samples often encountered in forensic contexts (Haddrill, 2021; McCord et al., 2019).

Forensic DNA analysis is particularly valuable in scenarios involving touch DNA, where trace amounts of genetic material are deposited on surfaces through contact. Recent advancements in DNA extraction, quantification, and amplification have enabled the recovery and analysis of DNA from challenging substrates, including porous materials commonly found at crime scenes (Haddrill, 2021; McCord et al., 2019). Furthermore, the use of mitochondrial DNA (mtDNA) has addressed limitations associated with highly degraded samples or low DNA yields. With its higher copy number per cell compared to nuclear DNA, mtDNA provides a reliable alternative for forensic identification, particularly for substrates like fabric, wood, and paper.

DNA profiling primarily focuses on analyzing highly variable regions of the genome, such as Short Tandem Repeats (STRs) and mitochondrial DNA (mtDNA) (Jobling & Gill, 2004). STR analysis remains the gold standard for forensic identification due to its high

discriminatory power, while mtDNA is especially advantageous for degraded samples and trace evidence, such as touch DNA and hair shafts (Parsons et al., 2007). These techniques ensure accurate identification even in cases involving minimal biological material.

Innovations in forensic DNA technologies, such as high-fidelity polymerases and Next-Generation Sequencing (NGS), have further enhanced the sensitivity, specificity, and resolution of forensic DNA analysis. These advancements allow forensic scientists to analyze degraded, low-yield, or complex DNA mixtures, solving cold cases and identifying victims of mass disasters with greater precision (Hofreiter et al., 2015). Such innovations are directly applicable to touch DNA analysis, particularly for challenging surfaces like porous materials, making them highly relevant to the scope of this study.

## 2.2 Touch DNA

### 2.2.1 Definition and mechanism

Touch DNA refers to minute amounts of genetic material deposited on surfaces through direct or indirect contact (Sessa et al., 2019)**.** It primarily originates from epithelial cells shed during touch and contains trace genetic information that is often invisible to the naked eye. The transfer of DNA depends on several factors, including the duration and pressure of contact, as well as individual biological traits like the rate of skin cell shedding and DNA deposition (Alketbi & Goodwin, 2019)**.** Environmental and substrate-specific factors, such as the porosity of the material or external contaminants, also influence the recovery and quality of touch DNA, making its analysis more complex (Alketbi, 2023).

### 2.2.2 Significance of touch DNA in cases lacking visible biological samples

Touch DNA plays a crucial role in forensic investigations, particularly in cases where traditional biological evidence, such as blood, saliva, or hair, is absent. The ability to retrieve

genetic profiles from trace evidence allows investigators to establish critical links between individuals, crime scenes, and objects. For instance, porous surfaces like paper or fabric can retain trace DNA that serves as key evidence in cases where no other biological material is available. The utility of touch DNA in solving high-profile cases and reopening cold cases demonstrates its potential to support forensic identification (McCord et al., 2019). However, maximizing the reliability of touch DNA analysis requires the use of advanced collection techniques and high-sensitivity analytical methods.

### 2.2.3 Challenges associated with touch DNA

Despite its forensic relevance, touch DNA recovery presents numerous challenges due to the small quantities of DNA typically deposited and its high susceptibility to contamination. Environmental factors, such as heat, humidity, and ultraviolet light, degrade DNA over time, further complicating recovery efforts. Porous surfaces absorb and trap genetic material within their fibers, making extraction and analysis significantly more difficult (Alketbi & Goodwin, 2019). Additionally, improper collection techniques can lead to cross-contamination or loss of valuable evidence. These challenges highlight the importance of optimized collection strategies, such as the double-swabbing technique, and advanced DNA analysis technologies, such as high-sensitivity PCR, to ensure reliable results. Developing substrate-specific protocols is essential to address these limitations and improve the forensic utility of touch DNA.

## 2.3 Porous vs. Non-Porous Surfaces in DNA Recovery

### 2.3.1 Differences in DNA retention and recovery between porous and non-porous surfaces

Porous and non-porous surfaces exhibit distinct characteristics in DNA retention and transfer. On porous surfaces, DNA can become trapped within the spaces or fibers of the material, making it more resistant to external removal. In contrast, non-porous surfaces tend to retain DNA on their surface, where it is more susceptible to being wiped away or transferred

due to contact with other objects. It is generally assumed that porous surfaces may yield better results in DNA recovery compared to non-porous surfaces (Wickenheiser, 2010).

DNA retention and recovery differ significantly between porous and non-porous surfaces due to their distinct physical properties. Non-porous surfaces, such as glass, metal, and plastic, allow DNA to remain on the surface without significant absorption, facilitating easier collection and higher recovery rates. DNA on these surfaces is more accessible to common forensic collection techniques, such as swabbing or tape-lifting (Alketbi & Goodwin, 2019)**.** Conversely, porous surfaces, including fabric, wood, and paper, absorb and trap DNA within their intricate structures. This embedded DNA is less accessible and often results in lower recovery yields, which complicates forensic analysis. These differences are particularly significant in crime scenes where varied surfaces may influence the quality and quantity of DNA evidence.

### 2.3.2 Characteristics of porous surfaces that complicate DNA extraction

Porous surfaces present unique challenges in forensic DNA recovery due to their ability to absorb liquids and biological material into their internal structures. Once absorbed, DNA can become embedded within the substrate, making it less accessible to standard collection methods such as swabbing. Additionally, porous materials like wood and paper often contain inhibitory compounds, which interfere with DNA extraction and amplification processes (Farash et al., 2018)**.** Environmental factors, including exposure to humidity, ultraviolet light, and heat, further contribute to DNA degradation on porous surfaces, reducing its usability for forensic profiling. These challenges underscore the need for optimized techniques to enhance DNA recovery from such materials.

### 2.3.3 Examples of porous and non-porous surfaces in forensic contexts

Non-porous surfaces, such as glass, metal, and ceramic, are frequently encountered in forensic investigations. These surfaces, found in items like windows, utensils, and tools, are preferred for DNA recovery due to their low absorption rates and easier collection methods. In contrast, porous surfaces, such as fabric (e.g., clothing or towels), wood (e.g., furniture or doors), and paper (e.g., documents or packaging materials), present greater challenges. Porous materials absorb DNA, complicating the extraction process and often yielding lower DNA concentrations. Advances in collection methods, such as the double-swabbing technique and the use of water-soluble tapes, have been developed to address these challenges effectively (Pang & Cheung, 2007). These methods have proven particularly valuable in improving DNA recovery from porous surfaces frequently encountered in forensic casework.

## 2.4 Methods for Collecting Touch DNA from Porous Surfaces

### 2.4.1 Overview of touch DNA collection techniques

Touch DNA collection techniques are crucial for recovering minute traces of genetic material deposited on surfaces through contact. Common methods include swabbing, tape-lifting, and scraping. Among these, the double-swabbing technique has proven to be highly effective, particularly for porous and challenging surfaces. This method involves two steps, including a moistened swab is first used to loosen and collect DNA from the substrate, followed by a dry swab to recover any residual material. This approach ensures maximum DNA recovery by addressing both surface-bound and embedded DNA (Alketbi & Goodwin, 2019; Pang & Cheung, 2007).

Other techniques, such as tape-lifting, utilize adhesive tapes to collect DNA from surfaces where swabbing is less effective, such as rough or delicate substrates. However, tape-lifting may not penetrate porous materials, making it less suitable for substrates like wood or

fabric (Farash et al., 2018). Effective collection methods must be adapted to the type of surface to maximize recovery and minimize contamination.

### 2.4.2 Advantages and limitations of the double-swabbing technique for porous surfaces

The double-swabbing technique is widely recognized as one of the most effective methods for collecting touch DNA from porous surfaces. The use of a moist swab helps release DNA trapped within the fibers or structure of porous substrates, while the dry swab efficiently collects the remaining material. This dual approach increases DNA yield and is particularly effective on surfaces like fabric, wood, and paper, which absorb and trap biological material (Alketbi & Goodwin, 2019; Pang & Cheung, 2007).

However, the technique is not without limitations. The moist swab can mobilize inhibitory compounds present in porous materials, such as wood or chemical residues in fabric, which can interfere with subsequent DNA analysis (Farash et al., 2018). Additionally, porous surfaces may absorb moisture from the swab, potentially diluting the DNA and reducing recovery efficiency. Optimizing the swab material and collection solution, such as using water-based solvents or detergents, can help mitigate these challenges (Bruijns et al., 2018).

### 2.4.3 Factors influencing the efficiency of touch DNA collection from challenging substrates

The efficiency of touch DNA collection from porous and complex surfaces is influenced by several key factors that affect DNA transfer, retention, and recovery. One crucial factor is the shedding status of the donor, as individuals vary in their propensity to shed DNA. Some people, classified as "good shedders," tend to deposit more DNA through contact, while others deposit minimal amounts. Activities performed by an individual before DNA deposition, such as touching the face, sweating, or engaging in physical tasks, can significantly influence

the amount of DNA transferred during contact (Alketbi, 2018; Lowe et al., 2002). These behaviours can "load" the fingers with DNA, increasing the likelihood of transfer to objects or surfaces.

Another factor is the surface characteristics of the substrate on which DNA is deposited. Porous surfaces like wood, fabric, and paper absorb and embed DNA into their intricate structures, making recovery more challenging compared to smooth, non-porous surfaces such as glass, metal, or plastic. While porous surfaces tend to retain DNA more persistently under certain conditions, the embedded DNA is harder to recover using standard collection techniques. In contrast, non-porous surfaces allow DNA to remain on the surface, making it more accessible for collection (Pesaresi et al., 2003; Wickenheiser, 2002).

The time between DNA deposition and recovery also plays a significant role in DNA collection efficiency. DNA is most effectively recovered when collected soon after deposition. Prolonged delays expose DNA to environmental factors, leading to degradation and reduced recovery efficiency. Studies have shown that the amount of DNA recovered decreases significantly over time, especially with exposure to heat, humidity, and ultraviolet (UV) light. For example, Fregeau et al. (2010) and Raymond et al. (2016) demonstrated that DNA yields decline substantially as the interval between deposition and collection increases, underscoring the importance of timely recovery in forensic investigations.

Finally, environmental conditions have a profound impact on DNA preservation and recovery. High humidity promotes microbial activity, which accelerates DNA degradation, while UV light induces chemical damage to DNA molecules by causing strand breaks and cross-linking of thymine bases. Proper storage of evidence and immediate DNA collection can mitigate these effects, helping to preserve DNA integrity. Studies have shown that materials

exposed to prolonged environmental stress yield lower DNA quantities and profiles with reduced quality (Goray et al., 2010).

## 2.5 DNA Extraction Techniques for Porous Surfaces

### 2.5.1 Review of common DNA extraction methods

DNA extraction is a vital step in forensic science, aiming to isolate DNA of sufficient quality and quantity for downstream applications such as PCR amplification. Commonly employed methods include phenol-chloroform extraction, silica-based spin columns, magnetic bead technology, and Chelex® 100 resin extraction. The phenol-chloroform method, while effective, has become less favored due to its labor-intensive nature, the toxicity of reagents, and its limited compatibility with automated workflows (Linacre & Templeton, 2014).

Silica-based methods and magnetic bead technologies are widely adopted for their efficiency and ability to produce high-purity DNA. These methods rely on the interaction between DNA's negative charge and positively charged silica or biotin-magnetic complexes, which facilitate the removal of inhibitors and produce high-quality DNA for amplification (Bruijns et al., 2018). However, these techniques are costlier than Chelex extraction and require multiple processing steps that may introduce additional variability (Linacre & Templeton, 2014; Walsh et al., 2013). While silica-based methods excel at removing inhibitors, their higher cost makes them less practical for cases involving low-resource forensic labs or large-scale sample processing.

Chelex® 100 resin, in comparison, offers a cost-effective and simplified extraction approach, which is particularly valuable in forensic investigations involving challenging substrates like porous materials. As discussed in the next section, its simplicity makes it suitable for many forensic scenarios while minimizing the risks of contamination during handling.

### 2.5.2 Efficacy of Chelex® 100 for porous materials and its advantages in removing inhibitors

Chelex® 100 is one of the most widely used DNA extraction methods in forensic science, particularly for isolating DNA from complex and inhibitor-rich substrates. The Chelex resin works by chelating divalent metal ions such as magnesium, which are cofactors for DNases. By sequestering these ions, Chelex protects DNA from enzymatic degradation, ensuring higher yields and better preservation of genetic material (Walsh et al., 2013).

For porous materials like wood, fabric, and paper, Chelex has demonstrated efficacy in removing inhibitors commonly present in these substrates, such as lignin in wood or chemical dyes in fabrics (Linacre and Templeton, 2014). These inhibitors can significantly interfere with DNA amplification processes, making the inhibitor-removal capabilities of Chelex critical for successful forensic analysis. Additionally, its simplicity and cost-effectiveness make it a popular choice in low-resource forensic labs, as it minimizes the number of steps involved, thereby reducing the risk of contamination during processing.

However, Chelex is not without its limitations. It can leave residual organic compounds in the extracted DNA, which may interfere with downstream applications like PCR amplification. These limitations can be addressed by implementing additional purification steps post-extraction or optimizing Chelex protocols tailored for porous substrates. For example, combining Chelex with silica-based spin columns may improve DNA recovery and purity from challenging samples (Bruijns et al., 2018).

### 2.5.3 Challenges in isolating DNA from porous substrates like wood and fabric

Porous surfaces like wood and fabric present significant challenges for DNA extraction due to their structural complexity and chemical composition. DNA deposited on porous materials often becomes embedded deep within the substrate, making recovery difficult with standard collection methods like swabbing. Furthermore, porous materials often contain

compounds like lignin in wood or residual dyes in fabrics, which act as inhibitors during the DNA extraction process (Bruijns et al., 2018).

Environmental factors also exacerbate the difficulties associated with porous substrates. Moisture promotes microbial activity, leading to accelerated DNA degradation, while ultraviolet (UV) light causes chemical damage to DNA, such as strand breaks and cross-linking of thymine bases, rendering the DNA unusable for analysis (Linacre and Templeton, 2014). These challenges necessitate the use of advanced techniques, such as pre-treatment with enzymatic solutions or detergents, to loosen embedded DNA before extraction. Optimized Chelex-based protocols, potentially combined with post-extraction purification methods, have shown promise in improving DNA yields from porous materials (Walsh et al., 2013).

While porous substrates pose unique challenges for DNA extraction, methods like Chelex® 100 remain valuable due to their simplicity and cost-effectiveness. Future advancements, such as combining Chelex with other extraction methods, may further improve DNA recovery from challenging forensic samples.

## 2.6 Quantitative and Qualitative DNA Analysis Methods

### 2.6.1 Tools for assessing DNA concentration and purity

Quantifying DNA concentration and assessing its purity are essential steps in forensic and molecular biology applications to ensure successful downstream analyses such as PCR and sequencing. One of the most reliable tools for this purpose is the DeNovix® DS-11 Spectrophotometer, which measures nucleic acid concentrations and purity using UV spectrophotometry. This device allows DNA quantification at a wavelength of 260 nm, which is the absorption maximum for nucleic acids, and assesses purity through full-spectrum analysis. It requires minimal sample volume (0.5–1.0 µL) and provides a broad quantification range, making it suitable for low-yield forensic samples, such as those recovered from touch DNA or degraded materials (Hindash & Hindash, 2022a)**.**

The DS-11 spectrophotometer incorporates advanced technologies like SmartPath™ optical adjustments, which optimize measurements for samples of varying concentrations. This feature ensures accuracy by reducing potential overestimation or underestimation caused by contaminants. Furthermore, the spectrophotometer evaluates sample quality through absorbance ratios (A260/A280 and A260/A230), providing a comprehensive overview of DNA purity (Hindash & Hindash, 2022a). Its ease of use and precise calibration make it a preferred instrument in forensic DNA quantification.

### 2.6.2 Importance of A260/A280 and A260/A230 ratios in determining DNA quality

The purity of DNA is a critical determinant of the success of downstream processes, such as PCR amplification, sequencing, or forensic analysis. Two spectrophotometric absorbance ratios, A260/A280 and A260/A230, are widely used to evaluate DNA quality, as they provide insights into the presence of contaminants that may interfere with these processes.

The A260/A280 ratio measures DNA purity concerning protein contamination. An ideal ratio of approximately 1.8 is indicative of pure DNA, while values below this threshold suggest contamination by proteins, phenol, or other substances. Such contaminants can arise from the DNA extraction process, where residual chemicals, including guanidine or phenol, may remain in the sample. These impurities can lead to inaccurate estimations of DNA concentration and interfere with enzymatic reactions, reducing the efficiency of amplification or other downstream applications. For example, in forensic contexts, where DNA yields from touch samples may already be minimal, maintaining a proper A260/A280 ratio is essential to ensure the integrity of the extracted DNA (Hindash and Hindash, 2022; Wilfinger et al., 1997).

The A260/A230 ratio assesses DNA purity concerning organic contaminants, such as ethanol, salts, and detergents, which are often introduced during DNA extraction and washing steps. A ratio greater than 2.0 is generally considered optimal, reflecting minimal contamination. Lower values, typically below 1.5, indicate significant contamination, which can severely impact DNA amplification efficiency. This ratio is particularly important in forensic DNA analysis, where inhibitors from porous materials (e.g., lignin in wood or dyes in fabrics) are frequently encountered. Abnormal A260/A230 ratios signal the need for additional purification to eliminate inhibitors, ensuring the reliability of downstream forensic applications (Hindash and Hindash, 2022; Wilfinger et al., 1997).

Both the A260/A280 and A260/A230 ratios are invaluable tools in determining the quality of extracted DNA. They allow forensic scientists to assess the need for further washing steps or alternative extraction methods to remove contaminants. By interpreting these ratios alongside DNA concentration data, forensic laboratories can ensure the extracted DNA meets the necessary quality standards for successful analysis and profiling.

## 2.7 PCR Amplification in Forensic DNA Analysis

### 2.7.1 Role of PCR in amplifying low quantities of DNA for forensic analysis

Polymerase chain reaction (PCR) is an indispensable tool in forensic DNA analysis, enabling the amplification of minute quantities of DNA to generate sufficient material for profiling and identification. This technique is particularly beneficial in cases where DNA is limited or degraded, such as forensic evidence recovered from porous surfaces. The three key steps of PCR such as denaturation, annealing, and extension, facilitate the exponential replication of specific DNA regions, even when the DNA is fragmented or exists in low quantities, as shown in Figure 2.1 (Broll, H., 2010). The amplification process is guided by sequence-specific primers and thermostable DNA polymerases, such as Taq DNA polymerase, which can withstand high temperatures during denaturation (Walsh et al., 2013; Borah, 2011).

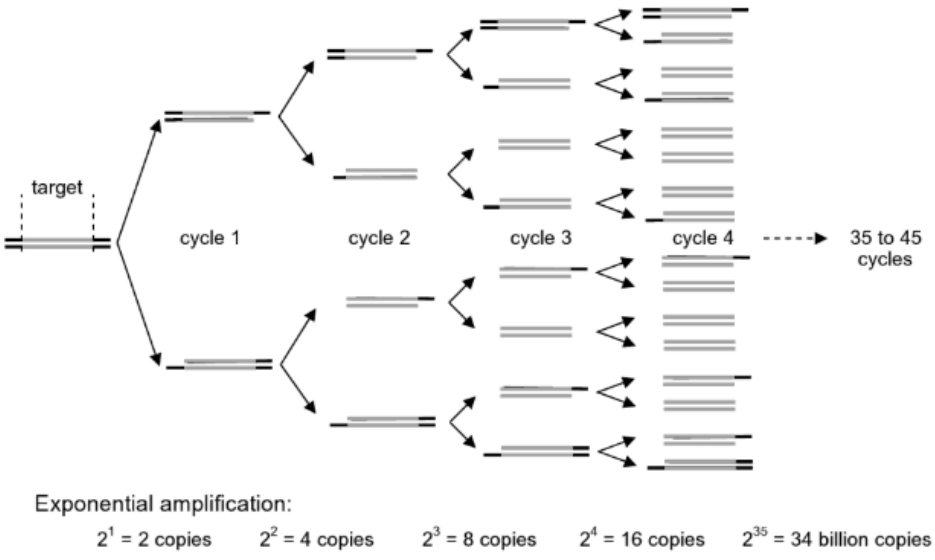


**Figure 2.1:** Theoretical amplification efficiency in PCR. From a theoretical point of view, the amount of target DNA is duplicated in each individual cycle, resulting in 34 billion identical copies of the same DNA after 35 cycles (Broll, H., 2010).

The robustness of PCR allows forensic scientists to recover DNA from trace or touch samples deposited on challenging substrates like wood, fabric, or paper. Even with degraded DNA, the use of short amplicon targets ensures successful amplification of specific DNA regions. This capability has made PCR a vital technique for forensic applications, where the DNA retrieved from crime scenes is often scarce or compromised by environmental conditions. By amplifying these low-template DNA samples, PCR provides critical evidence that can link individuals to crime scenes.

### 2.7.2 Importance of targeting mtDNA for degraded samples due to its high copy number

Mitochondrial DNA (mtDNA) has a unique advantage in forensic science due to its high copy number per cell compared to nuclear DNA (Cavalcanti et al., 2024). While each cell contains only two copies of nuclear DNA, hundreds to thousands of copies of mtDNA are present, making it more likely to recover viable mtDNA even from degraded or minimal samples. This property is particularly advantageous when analyzing touch DNA from porous surfaces, which often yield low quantities of DNA.

In this study, we focus on amplifying specific regions of the mitochondrial genome that are well-suited for analysis in forensic contexts, particularly those that can be effectively amplified from degraded or low-yield samples. These regions were selected based on their robustness in PCR amplification, even under challenging conditions, making them ideal for forensic applications involving touch DNA. By optimizing the amplification of these regions, we aim to enhance the reliability and sensitivity of mtDNA analysis, thereby improving the capacity to link individuals to crime scenes, especially when only trace amounts of DNA are available (McDonald et al., 2024).

In forensic applications, mtDNA is especially valuable when nuclear DNA is unavailable or insufficient, as is often the case with touch DNA from degraded porous materials. By amplifying mtDNA using PCR, forensic experts can generate genetic profiles that may match individuals or their maternal relatives. Since mtDNA is maternally inherited and does not undergo recombination. This capability has proven critical in cases involving cold cases, mass disasters, and unidentified remains (Andréasson et al., 2006; Just et al., 2015).

### 2.7.3 Challenges in PCR amplification from touch DNA on porous surfaces

Touch DNA collected from porous surfaces presents unique challenges for PCR amplification due to the low quantity of DNA and the presence of inhibitors. Porous materials, such as wood, fabric, and paper, often absorb and trap biological material within their fibers or pores, resulting in reduced DNA yields. Additionally, these materials may introduce inhibitory compounds, that interfere with the enzymatic activity of DNA polymerases during PCR amplification (Farash et al., 2018).

The stochastic effects associated with low-template DNA, such as allelic dropout or drop-in, further complicate the generation of complete DNA profiles from touch samples on porous surfaces. These effects are exacerbated by environmental factors, including exposure to heat, humidity, and ultraviolet light, which can degrade DNA over time. Advanced PCR techniques, such as direct PCR and the use of high-fidelity polymerases like Herculase II, have been explored to address these challenges. Direct PCR eliminates the need for extensive DNA extraction, reducing DNA loss and minimizing the impact of inhibitors. Meanwhile, high-fidelity polymerases enhance the accuracy of amplification by reducing error rates, making them suitable for low-quality or degraded DNA samples (Sikorsky et al., 2007; van Oorschot et al., 2019a).

Despite these advancements, the success of PCR amplification from touch DNA on porous surfaces often depends on optimizing collection, extraction, and amplification protocols. Pre-treatment methods to neutralize inhibitors and target shorter DNA fragments have shown promise in improving amplification efficiency. The continued development of these strategies is crucial for addressing the inherent challenges of working with touch DNA on porous materials.