HAIR DNA EXTRACTION USING MODIFIED CHELEX METHOD FOR FORENSIC HUMAN IDENTIFICATION

WONG WAI LUN

UNIVERSITI SAINS MALAYSIA

2025

HAIR DNA EXTRACTION USING MODIFIED CHELEX METHOD FOR FORENSIC HUMAN IDENTIFICATION

by

WONG WAI LUN

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

February 2025

CERTIFICATE

This is to certify that the dissertation entitled "HAIR DNA EXTRACTION USING MODIFIED CHELEX METHOD FOR FORENSIC HUMAN IDENTIFICATION" is the bonafide record of research work done by Wong Wai Lun (matric number: 158806) during the period from October 2024 to February 2025 under my supervision. I have read this dissertation and that in my opinion it confirms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science (Honours) (Forensic Science).

Supervisor,

Dr. Nur Waliyuddin Hanis bin Zainal Abidin,

Lecturer of Health Science,

Universiti Sains Malaysia,

Health Campus,

16150 Kubang Kerian,

Kelantan, Malaysia.

Tel: 09-7677611

Date: 27 February 2025

DECLARATION

I hereby declare that this dissertation is the result of my investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

Wong Wai Lun

Date: 27 February 2025

ACKNOWLEDGEMENT

I would like to take this opportunity to express my heartfelt gratitude to everyone who has contributed directly or indirectly to this study.

First and foremost, my deepest appreciation goes to my supervisor, Dr. Nur Waliyuddin Hanis bin Zainal Abidin, for giving me the privilege to work under his research group. I am sincerely grateful for his continuous support, professional guidance, and unwavering encouragement throughout my study.

I would also like to extend my utmost gratitude to the dedicated staff members from various laboratories at the School of Health Sciences, including the Forensic Science Laboratory (Pn. Hafizah Harun, Pn. Hasnita, Pn. Syazwani, and En. Baharuddin), the Molecular Biology Laboratory (Pn. Wan Razlin), the Immunology Laboratory (Pn. Wan Norhasikin), and the Human Identification/DNA Unit (HID) (Pn. Nur Azeelah, En. Mohd Lukman, En. Kamarulazwan, and En. Ahmad Auzan). Their invaluable assistance, guidance, and willingness to help throughout the course of this study are deeply appreciated.

To my research colleagues, Nayomi and Wendy, thank you for your insightful comments, suggestions, and unwavering support. Special thanks also to the members of Room 111 (Amar, Afiq, Zawawis, and Zulkhairie), Room 236 (Chia Sheng, Jia Quan, and Ricxley) of Nurani Putera, and Room 101 (Zhi Lin, Pei Yee, Ethel, Su Peng, and Nayomi) of DM 6 for their generosity, cooperation, and valuable time during the sample collection process, as well as for contributing their samples to this project. Their generosity and involvement provided the foundation for the continuation of my research, without which this study would not have been possible.

Finally, I am especially grateful to my family for their endless love, encouragement, and unwavering support. Their belief in me has been my greatest source of strength throughout this journey, and I could not have reached this point without them.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

mL	Milliliter
µL	Microliter
µM	Micromolar
pg	Picogram
°C	Degree Celsius
bp	Base pairs
ng	Nanogram
M	Molarity (Moles per Liter)
mM	Millimolar
g/mol	Grams per mole
rpm	Revolutions per minute
x g	Relative centrifugal force
nm	Nanometer
min	Minutes
L	Liter
g	Gram
ng/µL	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
$CaCl_2 \cdot 2H_2O$	Calcium Chloride Dihydrate
CODIS	Combined DNA Index System
D-loop	Displacement Loop in mitochondrial DNA
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
dsDNA	Double-Stranded DNA
dNTP	Deoxynucleotide Triphosphates
EBH	Extraction Buffer for Hair
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethidium Bromide
HCl	Hydrochloric Acid
HB	Hair with Bulb
HID	Human Identification/DNA Unit
HXB	Hair without Bulb
HV1	Hypervariable Region 1
HV2	Hypervariable Region 2
JEPeM	Human Research Ethics Committee of Universiti Sains Malaysia
KAPs	Keratin-Associated Proteins
LCN	Low Copy Number
$MgCl_2$	Magnesium Chloride
MPS	Massively Parallel Sequencing
mtDNA	Mitochondria Deoxyribonucleic Acid
Na_2EDTA	Disodium Ethylenediaminetetraacetic Acid
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCBI	National Center for Biotechnology Information

NGS	Next-Generation Sequencing
nuDNA	Nuclear Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
Pfu	Pyrococcus furiosus (DNA polymerase source)
RH	Hair with Unknown Bulb Status
RNA	Ribonucleic Acid
SDS	Sodium Dodecyl Sulfate
SNP	Single Nucleotide Polymorphisms
STR	Short Tandem Repeat
Taq	Thermus aquaticus (polymerase source)
Ta	Annealing temperature
TBE	Tris-Borate-EDTA Buffer
Tm	Melting Temperature
Tris-HCl	Tris Hydrochloride
UV	Ultraviolet Light
ssDNA	Single-Stranded DNA

LIST OF APPENDICES

PENGEKSTRAKAN DNA RAMBUT MENGGUNAKAN KAEDAH CHELEX YANG DIUBAH SUAI UNTUK PENGENALPASTIAN MANUSIA DALAM FORENSIK

ABSTRAK

Analisis Asid Deoksiribonukleik (DNA) adalah asas penting dalam sains forensik, memudahkan pengecaman manusia dalam siasatan jenayah. Dalam kalangan bukti biologi, rambut sering ditemui tetapi menghadirkan cabaran unik dalam pengekstrakan DNA disebabkan oleh struktur protein yang kaya dan kandungan DNA yang secara semula jadi rendah. Kajian ini memberi tumpuan kepada pengoptimuman protokol pengekstrakan dan penggandaan DNA daripada sampel rambut untuk meningkatkan kegunaannya dalam bidang forensik. Sampel rambut dikategorikan kepada tiga kumpulan: dengan bulb, tanpa bulb, dan status bulb yang tidak diketahui. Pengekstrakan DNA genomik dilakukan menggunakan kaedah resin Chelex® 100, yang diperbaiki dengan penambahan *Extraction Buffer for Hair* (EBH) dan Dithiothreitol (DTT) bagi meningkatkan kecekapan pemulihan DNA. Analisis kuantitatif terhadap DNA yang diekstrak menunjukkan variasi antara kategori, dengan hasil tertinggi diperoleh daripada sampel yang mengandungi bulb.

Bagi menilai kebolehgunaan DNA yang diekstrak untuk aplikasi forensik, penggandaan menggunakan *polymerase chain reaction* (PCR) yang menyasarkan kawasan DNA mitokondria (mtDNA) berukuran 176 bp dan 310 bp telah dijalankan. Suhu penyatuan (*annealing temperatures*) dan syarat kitaran telah dioptimumkan untuk *Herculase II Fusion DNA polymerase*. Elektrophoresis gel agarosa mengesahkan penggandaan DNA yang berjaya daripada sampel DNA yang telah dipurifikasi, dengan jalur (*bands*) yang jelas sepadan dengan kawasan mtDNA yang disasarkan.

Hasil kajian ini menunjukkan potensi mtDNA yang diekstrak daripada rambut untuk pengecaman manusia, terutamanya dalam situasi di mana bukti biologi tradisional tidak tersedia. Walaupun sampel dengan bulb menghasilkan kepekatan DNA yang lebih tinggi, penggandaan yang berjaya juga dicapai pada sampel tanpa bulb dan dengan status bulb yang tidak diketahui, menunjukkan kebolehsuaian pendekatan ini. Kajian masa depan perlu meneroka faktor-faktor yang mempengaruhi hasil DNA, seperti fasa pertumbuhan rambut, serta mengintegrasikan analisis DNA nuklear untuk meningkatkan spesifikasi pengecaman individu. Kajian ini memperkasa sains forensik dengan memperbaiki teknik pemulihan dan penggandaan DNA daripada rambut, sekaligus meningkatkan kebolehpercayaan bukti rambut dalam penyiasatan jenayah.

HAIR DNA EXTRACTION USING MODIFIED CHELEX METHOD FOR FORENSIC HUMAN IDENTIFICATION

ABSTRACT

Deoxyribonucleic acid (DNA) analysis is a cornerstone in forensic science, facilitating human identification in criminal investigations. Among biological evidence, hair is frequently encountered but presents unique challenges for DNA extraction due to its protein-rich structure and inherently low DNA content. This study focused on optimizing DNA extraction and amplification protocols for hair samples to enhance their forensic utility. Hair samples were categorized into three groups: with bulbs, without bulbs, and with unknown bulb status. Genomic DNA extraction was carried out using the Chelex® 100 resin method, further refined with the addition of Extraction Buffer for Hair (EBH) and Dithiothreitol (DTT) to improve recovery efficiency. Quantitative analysis of extracted DNA showed variability across categories, with the highest yields obtained from samples containing bulbs.

To assess the forensic applicability of the extracted DNA, polymerase chain reaction (PCR) amplification targeting mitochondrial DNA (mtDNA) regions of 176 bp and 310 bp was performed. Optimized annealing temperatures and cycling conditions was established using Herculase II Fusion DNA polymerase. Agarose gel electrophoresis confirmed successful amplification of purified DNA samples, with distinct bands corresponding to the targeted mtDNA regions.

The findings highlight the utility of mtDNA extracted from hair for human identification, especially in situations where traditional biological evidence is unavailable. Although samples with bulbs yielded higher DNA concentrations, successful amplification was also achieved in samples without bulbs and with

unknown bulb status, demonstrating the versatility of this approach. Future investigations should explore factors influencing DNA yield, such as hair growth phases, and incorporate nuclear DNA analysis to enhance individual specificity. This study advances forensic science by refining hair DNA recovery and amplification techniques, advancing the reliability of hair evidence in criminal investigations.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Deoxyribonucleic acid (DNA) analysis plays a critical role in forensic science, providing an essential tool for accurate individual identification in criminal investigations. Forensic DNA analysis involves extracting, purifying, and interpreting genetic material from biological samples associated with suspects, victims, or crime scenes. This method is invaluable for identifying individuals in criminal cases, especially when other evidence is absent. In particular, "trace DNA" – DNA found in minute quantities – has become a key component in forensic identification, despite the challenges posed by its low concentration. These trace amounts of DNA often fall below the recommended thresholds for reliable detection and interpretation, making it difficult to achieve consistent results (R. A. van Oorschot et al., 2010). Hair, which frequently serves as evidence in criminal investigations, is an ideal candidate for trace DNA analysis, but its effective use is hindered by difficulties in extracting DNA from hair itself directly.

Hair is one of the most commonly found biological materials at crime scenes, especially in violent crimes, where it can be easily transferred between individuals or onto surfaces. The resilience of hair to environmental stressors such as moisture, heat, and ultraviolet light enhances its role as a valuable forensic tool. Hair often remains intact for extended periods, allowing it to preserve potential DNA evidence. However, DNA extraction from hair presents significant challenges. Hair consists of three primary layers: the outer cuticle, the middle cortex, and the innermost medulla. These layers are rich in structural proteins, particularly keratins and keratin-associated

proteins (KAPs), which confer stability and protect hair from environmental degradation (Langbein and Schweizer, 2005). While this durability is beneficial for preserving hair, it complicates DNA extraction, as these proteins resist enzymatic breakdown and hinder the isolation of viable DNA. The stability of keratin in particular can interfere with enzymatic processes, making it challenging to isolate DNA without contamination or inhibition.

In forensic analysis, mitochondrial DNA (mtDNA) is commonly used because it is more abundant in hair 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 (Lutz et al., 1996). On the other hand, autosomal DNA is primarily found in the hair root or bulb, and its presence in hair shafts is limited. This scarcity makes the extraction of sufficient autosomal DNA from hair shafts more challenging, often resulting in unsuccessful or unreliable amplification in polymerase chain reaction (PCR) testing.

One of the primary difficulties in forensic DNA analysis of hair is the lack of standardized, reliable protocols for efficient DNA isolation. The naturally low DNA yield, combined with the protein-rich matrix of the hair sample, complicates the extraction process and can lead to contamination or inhibition during amplification. Despite the exploration of various extraction and amplification techniques, consistent success in obtaining viable DNA from hair has not yet been achieved. As a result, the reliable extraction of DNA from hair sample remains a critical and unresolved issue in forensic science.

Numerous studies, including those by Almeida et al. (2011) and Suenaga and Nakamura (2005), have explored various DNA extraction methods for hair, utilizing

both traditional and modern protocols with differing levels of success. While commercial kits, such as the PrepFiler BTA™ used alongside the AutoMate Express™, have demonstrated potential, inconsistencies in DNA yields – particularly between different hair segments, underscore the need for a universally reliable extraction method tailored to hair samples frequently found at crime scenes. This gap highlights a persistent challenge in forensic DNA analysis, reinforcing the need for more efficient and cost-effective protocols that can consistently recover viable DNA from hairs.

Among existing methods, the Chelex® 100 resin method is particularly promising due to its simplicity, cost-effectiveness, and ability to minimize contamination through its single-tube protocol. Although this method has demonstrated effectiveness in extracting DNA from various biological materials, its use for hair samples remains relatively underexplored. Optimizing the Chelex® 100 resin method could improve the efficiency and reliability of DNA recovery from hair, thereby enhancing its forensic applications.

This study aims to evaluate the effectiveness of the Chelex® 100 resin method for DNA extraction from hair samples, with a focus on optimizing its protocol to maximize DNA yield and quality. By improving the extraction process specifically for hair samples, this research seeks to enhance the forensic utility of hair as a source of evidence, particularly in scenarios where conventional biological samples like blood or saliva are unavailable. The findings are expected to contribute to the reliability of forensic investigations by establishing hair as a more dependable resource for human identification.

1.2 Problem Statement

Human hair is one of the commonly encountered forms of forensic evidence at crime scenes, particularly in violent incidents or cases where other biological samples are unavailable. Despite its prevalence, reliably extracting DNA from hair presents considerable challenges, especially when dealing with hair samples that lack roots or bulbs - the primary sources of DNA. The absence of standardized and universally accepted protocols for extracting sufficient DNA from hair has further compounded these challenges. While prior studies, such as those by Almeida et al. (2011), have explored the use of commercial kits like PrepFiler BTA™ with AutoMate Express™ for hair shafts, and Hue and Linh (2013) have focused on traditional manual methods including phenol and salting-out extraction for rooted hair. Despite these efforts, a straightforward, cost-effective, and efficient DNA extraction protocol specifically optimized for hair samples remains unavailable. Moreover, studies by Brandhagen et al. (2018) and Hue and Linh (2013) utilized approximately 5–6.5 cm of rootless hair and a total of 5 hair roots per extraction, respectively, highlighting the substantial sample requirements, which can be problematic in forensic evidence collection

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

This study aims to address these challenges by systematically evaluating the Chelex method, a simple and cost-effective approach for DNA extraction from hair samples of varying amounts (from minimal to sufficient strands) and with differing bulb statuses. The research focuses on determining the method's effectiveness in yielding high-quality DNA suitable for amplification using PCR with human-specific primers targeting mitochondrial DNA. By optimizing this protocol, the study aspires to enhance forensic methodologies, providing investigators with a reliable and cost-effective solution for extracting usable DNA from hair evidence. Such advancements would be particularly impactful in cases where traditional biological samples, such as blood or saliva, are unavailable, thereby expanding the scope of human identification in forensic investigations.

1.3 Research Questions

i. Can DNA be efficiently extracted from random hair samples, including those with bulb, without bulb, and of uncertain bulb status, using the Chelex® 100 resin method?

ii. What is the success rate of mtDNA amplification from hair samples using mtDNA human-specific primers, and how does the quality of the extracted DNA influence amplification outcomes?

1.4 Objectives

1.4.1 General Objective

To evaluate the feasibility and reliability of using hair samples as a source of DNA for forensic identification.

1.4.2 Specific Objectives

i. To optimize the Chelex® 100 resin DNA extraction method for random hair samples with varying bulb statuses.

ii. To assess the quality and amplification success of mtDNA extracted from hair using mtDNA human-specific primers.

1.5 Significance of Study

Hair serves as critical forensic evidence often found at crime scenes involving physical contact or assault, where it can play a pivotal role in suspect identification in criminal investigations. This study is significant as it explores the potential of using human hair, as a reliable DNA source for forensic purposes However, it can be challenging to extract DNA due to the lack of cellular material in many hair samples. By optimizing the Chelex® 100 resin method for DNA extraction from hair, this research aims to enhance the forensic value of hair evidence, especially in situations where other biological samples are inadequate. Ultimately, this study seeks to improve the accuracy and reliability of forensic investigations, contributing to more resilient and effective investigative processes.

By amplifying trace DNA extracted from hair using Polymerase Chain Reaction (PCR) techniques, this study aims to access the reliability of DNA profiling using hair sample, particularly in cases where traditional biological samples like blood or saliva are unavailable. The research holds potential to expand the range of usable biological evidence in forensic science, increasing the chances of successful human identification, especially in cases involving minimal or degraded samples.

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 scalp hair, which are often overlooked due to their typically lower DNA yields and potential degradation. Expanding the types of evidence available could be pivotal in identifying suspects or victims, particularly when other biological materials are absent. By improving the reliability of hair DNA analysis, this research will provide law enforcement with more robust, scientifically reliable evidence, ultimately leading to more accurate identifications and potentially higher conviction rates in criminal investigations.

1.6 Scope of Study

The scope of this study, titled "Hair DNA Extraction using Modified Chelex Method for Forensic Human Identification," focuses on evaluating the potential of human scalp hair as a reliable DNA source for forensic identification. The research specifically investigates the application of the Chelex® 100 resin DNA extraction method for hair samples with varying bulb statuses, including hairs with bulbs, without bulbs, and with unknown bulb statuses. To reflect real-life forensic scenarios, hair samples were collected randomly, and the study examines their variability to enhance practical relevance. The study involves optimizing the Chelex® 100 resin protocol by incorporating Dithiothreitol (DTT) and Extraction Buffer for Hair (EBH) to improve DNA recovery from hair strands, particularly in challenging samples. Amplification of mitochondrial DNA (mtDNA) is performed using Polymerase Chain Reaction (PCR) with human-specific primers designed to target short amplicons of 176 bp and 310 bp. The PCR products are then analyzed through agarose gel electrophoresis to assess the presence, quality, and reliability of amplified DNA.

The analysis includes a comparative evaluation of DNA yield and amplification success across different hair sample categories to identify optimal conditions for trace DNA recovery and amplification. By focusing on hair as a source of trace DNA, the study aims to address critical challenges in forensic science, especially in cases where conventional biological samples like blood or saliva are unavailable or degraded. The findings are expected to expand the utility of hair evidence in forensic investigations by providing insights into efficient and cost-effective DNA extraction methods tailored to hair samples. However, the study is limited to mitochondrial DNA as the primary target, and nuclear DNA, which offers greater individuality is not extensively explored. In addition, variability in DNA yield due to factors such as hair growth phase, environmental exposure, and inherent characteristics of the samples is acknowledged. Overall, this study seeks to contribute to advancements in forensic human identification by improving the reliability and applicability of hair-derived DNA.

CHAPTER 2

LITERATURE REVIEW

2.1 Genetics

Genetics, the study of heredity and gene function, forms the basis for understanding biological processes and their applications in fields such as medicine, agriculture, and forensics. Historically, genetics emerged with Gregor Mendel's discovery of inheritance principles in the 19th century, which established that traits are passed from one generation to the next via discrete units now known as genes (Kulkarni, 2015). Mendel’s experiments with pea plants laid the foundation for classical genetics, defining the basic laws of inheritance. Although the term "gene" was not coined until 1909 by Johannsen, Mendel's work remains central to understanding heredity. In 1910, Morgan demonstrated that genes reside on chromosomes, further advancing the field (Pearson, 2006).

As molecular biology advanced, genes were identified as specific DNA sequences encoding proteins or functional RNA, with regulatory regions that influence their expression and interaction within the genome (Kulkarni, 2015); 1994)Pearson, 2006). The structure of DNA, described by Watson and Crick in 1953, provided the framework for modern molecular genetics, revealing DNA's role in storing and transmitting genetic information. A gene, once considered a simple unit of inheritance, is now recognized as a complex entity with overlapping sequences, regulatory functions, and intricate transcriptional activities. For example, alternative splicing allows a single gene to produce multiple proteins, adding to the complexity of defining a gene (Pearson, 2006). Moreover, RNA has emerged as a crucial player, not merely as an intermediary

in protein synthesis but also as a regulator of gene expression and a potential hereditary mechanism in certain contexts (Pearson, 2006).

According to publication of Laurentin Táriba (2023b), modern genetics encompasses multiple subfields, including molecular genetics, population genetics, and classical genetics. Molecular genetics focuses on the DNA sequence, its replication, and its expression, facilitated by advances in sequencing technologies and recombinant DNA methodologies. This branch has illuminated the molecular underpinnings of genetic diseases, including disorders such as cystic fibrosis and sickle cell anemia. Meanwhile, population genetics investigates genetic variation within populations, providing insights into evolutionary processes and the molecular basis of diversity. These advancements in understanding the molecular and population-level intricacies of genetics underscore the dynamic nature of genetic information and its interplay with cellular processes.

The applications of genetics span diverse fields. In medicine, clinical genetics applies Mendelian principles to identify inherited disorders, tracing their molecular origins to mutant alleles (Pagon, 2013). Advances in molecular biology have enabled the development of gene therapies and personalized medicine strategies, revolutionizing the treatment of genetic disorders. In agriculture, genetic principles have been employed for centuries in selective breeding programs. Today, molecular tools facilitate the creation of genetically modified organisms (GMOs), enhancing crop yields and resilience by introducing desirable traits from other species (Laurentin Táriba, 2023a). Additionally, population genetics aids conservation biology by informing breeding programs for endangered species and understanding the genetic structure of populations (Kulkarni, 2015).

The complexities of defining a gene have evolved with discoveries in genetic regulation. RNA molecules, once thought to be passive intermediates, are now recognized as active participants in gene expression and regulation. The discovery of phenomena such as alternative splicing, where one gene can produce multiple proteins, and the regulatory roles of non-coding RNAs, has enriched the field. These advancements highlight the sophisticated mechanisms governing genetic activity and present both opportunities and challenges in genetic research (Pearson, 2006).

This foundational understanding of genetics, encompassing both its classical and molecular dimensions, serves as a basis for this final year project, which explores the potential of hair DNA for human identification. By leveraging advancements in DNA extraction and amplification, the study aims to address the unique challenges of working with hair samples as a source of genetic evidence. This integration highlights the significance of genetic tools in criminal investigations, particularly in DNA profiling, which bridges the fields of genetics and forensic science to identify individuals with unparalleled precision.

2.1.1 Molecular Genetics

Molecular genetics is a vital area of genetics that focuses on understanding the chemical and physical nature of genes and how they regulate development, growth, and physiological functions. It plays a crucial role in linking genotype to phenotype by explaining the processes through which genetic information translates into biological traits (Corvin and Gill, 2012).

DNA, located within chromosomes, is composed of a sugar-phosphate backbone and four nitrogenous bases: adenine (A), guanine (G), cytosine (C), and

thymine (T). These bases pair specifically (A with T and G with C) through hydrogen bonds, creating complementary DNA strands. This complementarity is essential for DNA replication, a highly precise process carried out by DNA polymerase enzymes, which add nucleotides to the 3’ end of a new DNA strand. However, errors known as mutations can occur during replication, either spontaneously or due to external factors such as radiation or chemical mutagens. These mutations can disrupt genetic function and lead to observable changes in an organism’s traits (Corvin and Gill, 2012).

The Central Dogma of Biology outlines the transfer of genetic information from DNA to RNA and subsequently to proteins. This process begins with transcription, where DNA is converted into messenger RNA (mRNA), which then carries the genetic codes to ribosomes. At the ribosomes, translation occurs, converting the mRNA sequence into amino acid sequences of proteins with the help of transfer RNAs (tRNAs). The genetic code, a universal system of three-nucleotide sequences, facilitates this conversion from nucleic acids to proteins (Saier, 2019). The proteins produced through this process perform essential cellular roles, including enzymatic functions, structural support, and gene expression regulation. This flow of information illustrates the direct connection between a gene's sequence and the observable characteristics of an organism (Natasha, 2009).

Despite the simplicity of the Central Dogma, certain exceptions exist, such as reverse transcription. In this process, RNA is converted back into DNA, which occurs naturally in retroviruses and during telomerase activity in eukaryotic cells. Telomerase is particularly important for maintaining the ends of chromosomes, called telomeres, and preserving genomic stability during cell division (Natasha, 2009; Corvin and Gill, 2012).

Molecular genetics offers insights into cellular mechanisms and sheds light on how genetic mutations and environmental factors interact to influence traits. It also plays a key role in identifying genetic causes of various conditions, including psychiatric disorders, by connecting mutations to biochemical pathways and phenotypic outcomes. This relationship highlights the dynamic interplay between genetic makeup and the environment in shaping an organism's characteristics (Natasha, 2009).

2.1.2 Forensic Genetics

Forensic genetics has significantly advanced over the years, becoming a vital tool in modern forensic science with diverse applications, including criminal investigations, paternity testing, disaster victim identification, and anthropological research. Its development began with foundational discoveries like Landsteiner's ABO blood group system in 1900 and Locard's exchange principle in 1910, which emphasized the transfer of materials during contact, laying the groundwork for the field. The breakthrough discovery of DNA's double-helical structure in 1953 further enabled molecular-level analyses that revolutionized forensic capabilities (C. Li, 2018; Kowalczyk et al., 2018).

Central to forensic genetics is the ability to create DNA profiles unique to each individual, primarily using genetic markers such as Short Tandem Repeats (STRs). Located in non-coding regions of DNA, STRs are highly polymorphic and reproducible, making them gold standard for distinguishing individuals with remarkable precision and linking biological evidence to individuals in criminal investigations (Kulkarni, 2015; Kowalczyk et al., 2018). Not just that, multiplex STR kits, capable of analysing up to 24 loci, have greatly enhanced the discrimination power and efficiency of DNA profiling (Schneider, 2007). Other markers, including single nucleotide polymorphisms

(SNPs) and mitochondrial DNA (mtDNA), have expanded the field's utility. SNPs, while less polymorphic than STRs, are important for analyzing degraded samples and provide insights into ancestry and phenotypic traits like skin, hair, and eye color due to their low mutation rate and ability to analyse shorter DNA fragments (C. Li, 2018; Morling, 2004). Meanwhile, mtDNA is highly valuable for analysing maternal lineage and is often utilized for degraded samples like bones or hair shafts. It serves as a stable genetic source that remains resistant to environmental degradation, making it particularly useful when nuclear DNA is insufficient (Schneider, 2007; Morling, 2004).

Technological innovations have further propelled the field. Polymerase Chain Reaction (PCR), a cornerstone of forensic genetics, allows the amplification of minute DNA samples, even from challenging or trace evidence like hair. This method is foundational for STR and SNP profiling, and advanced protocols like direct PCR reduce processing time and minimize DNA loss during extraction (Kowalczyk et al., 2018; Morling, 2004). Advanced techniques, such as massively parallel sequencing (MPS), a next-generation sequencing (NGS)-based technology, allow for the simultaneous analysis of multiple genetic markers, including STRs and SNPs. This approach improves the resolution of complex DNA mixtures and offers deeper insights into genetic variations, making it highly effective for complex kinship identification (C. Li, 2018; Kowalczyk et al., 2018). Low Copy Number (LCN) DNA analysis is another innovation that facilitates the study of trace DNA but requires stringent contamination controls due to its sensitivity (Morling, 2004). Together, these techniques ensure that even minimal or degraded samples can yield reliable genetic profiles, enhancing the ability to identify suspects and exonerate the innocent.

Forensic genetics plays a central role in criminal investigations, where DNA evidence is crucial for linking biological traces from crime scenes to suspects or database profiles. STR-based DNA profiling has become the standard method in many jurisdictions, reinforced by robust statistical analyses to assess the weight of evidence (Kowalczyk et al., 2018; Morling, 2004). Beyond criminal cases, forensic genetics is also vital in mass disaster scenarios and missing person investigations. In such contexts, mtDNA and SNP analysis are essential for identifying human remains, particularly when traditional STR profiling is ineffective due to sample degradation (Schneider, 2007). Additionally, forensic DNA phenotyping, which predicts physical traits from genetic material, provides investigative leads, though its predictive power and ethical implications remain subjects of debate (Morling, 2004). To ensure accuracy and reliability, international guidelines and standards, such as ISO 17025 for laboratory accreditation, have been implemented (Morling, 2004). These standards emphasize rigorous quality control, proficiency testing, and population-specific allele frequency databases such as Combined DNA Index System (CODIS) to support the statistical interpretation of DNA evidence (Schneider, 2007; Kowalczyk et al., 2018).

However, with great power come ethical and legal challenges. Privacy concerns regarding the retention of genetic profiles in databases and the potential misuse of phenotypic predictions highlight the need for careful governance. The increasing use of DNA to predict traits raises concerns about biases and misinterpretations, necessitating balanced oversight and public trust (Morling, 2004); Kowalczyk et al., 2018).

The integration of advanced technologies, such as metagenomics for analyzing microbial communities on evidence, offers significant potential to advance forensic genetics. This approach can be applied to critical areas of forensic identification,

including individual identification, determining the origin of biological stains at crime scenes, and detecting drug abuse (C. Li, 2018). As forensic genetics continues to drive scientific progress, it will remain focused on leveraging technological innovations while upholding ethical principles to promote justice and societal well-being.

2.1.2(a) 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 (R. A. van Oorschot et al., 2010). Its forensic applications became prominent in the late 1990s when advancements in Polymerase Chain Reaction (PCR) enabled DNA profiling from low-template samples (R. A. H. van Oorschot et al., 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 et al., 2024). However, concerns about contamination, secondary transfer, and ethical implications have necessitated stringent handling and interpretation protocols (R. A. van Oorschot et al., 2010; Raymond et al., 2009).

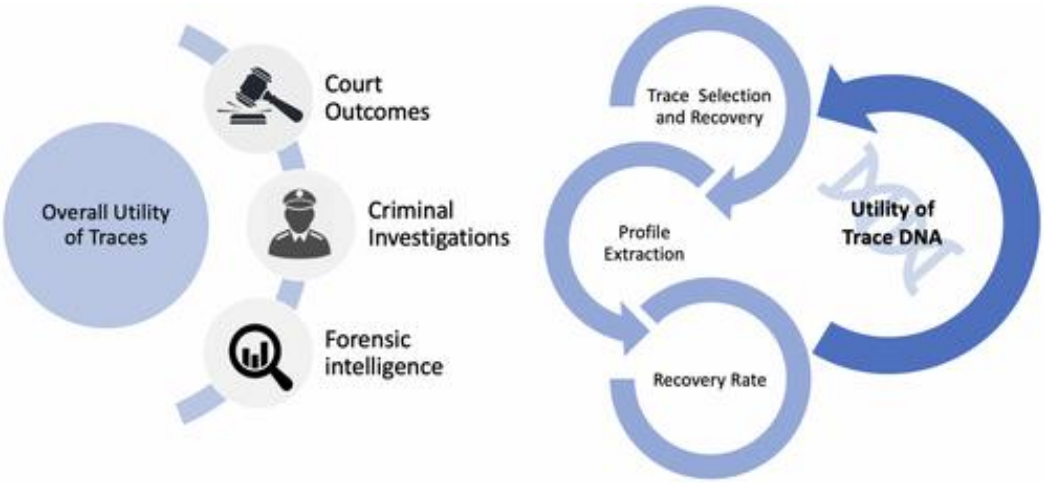

Figure 2.1: Summary of utility of trace evidence and significance of trace DNA (Hoffmann et al., 2024).

Trace DNA originates from various biological materials, including skin cells, sweat, saliva, and hair (R. A. H. van Oorschot et al., 2019). These biological materials are deposited on surfaces like weapons, clothing, or furniture, often unintentionally (R. A. van Oorschot et al., 2010; Raymond et al., 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 (R. A. van Oorschot et al., 2010; van Oorschot et al., 2019). Research by R. A. H. van Oorschot et al. (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 (R. A. van Oorschot et al., 2010). Double-swabbing and the use of optimized moistening agents have significantly improved recovery rates from porous and non-porous substrates (Raymond et al., 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 (R. A. van Oorschot et al., 2010).

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 (R. A. H. van Oorschot et al., 2019). Mixture profiles are common, as trace DNA often includes contributions from multiple individuals, making interpretation complex (R. A. van Oorschot et al., 2010). 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 R. A. H. van Oorschot et al. (2019) on indirect transfer mechanisms. Moreover, environmental factors such as heat, humidity, and substrate type can degrade DNA, reducing its recoverability and reliability (R. A. van Oorschot et al., 2010).

The evolution of analytical techniques has addressed some of these challenges. STR profiling and PCR remain foundational in amplifying low-template DNA (R. A. H. van Oorschot et al., 2019). 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 (R. A. van Oorschot et al., 2010).

Trace DNA has been pivotal in solving cold cases, identifying victims in mass disasters, and exonerating the wrongfully convicted (Hoffmann et al., 2024); van Oorschot et al., 2019). Its ability to link suspects to scenes or objects with minimal biological evidence has transformed forensic investigations. For instance, studies by Raymond et al. (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 (R. A. H. van Oorschot et al., 2019). Research on the persistence of trace DNA under various environmental conditions and its transfer mechanisms is vital for enhancing forensic applications (Raymond et al., 2009). In addition, gaps in standardizing collection and analysis methods necessitate ongoing research to ensure trace DNA's continued reliability as a forensic tool (R. A. van Oorschot et al., 2010; Raymond et al., 2009).

2.2 Hair

Hair is a slender, thread-like structure that originates from follicles embedded in the skin and is primarily composed of the protein keratin. Serving various functions such as insulation, protection, and sensory detection, hair is a defining feature of mammals, including humans. It consists of dead keratinized cells formed within hair follicles, which are present across most of the human body, except in areas of glabrous skin. These follicles produce two main types of hair: thick terminal hair and fine vellus hair. While much attention is given to aspects like hair growth, types, and care, hair is also a significant biomaterial. Its composition of protein and DNA makes it particularly valuable in forensic investigations.

2.2.1 Biological Traits

2.2.1(a) Anatomy of Hair

According to studies of Harkey (1993), hair anatomy comprises three main components: the shaft, root, and follicle, each with distinct structures and functions. The hair shaft is the visible part of the hair above the skin and is composed of three layers: the cuticle, cortex, and medulla. The outermost layer, the cuticle, consists of overlapping keratinized cells, serving as a protective barrier against physical and chemical damage. Beneath the cuticle lies the cortex, which forms the bulk of the shaft and contains densely packed keratin fibers and melanin pigments responsible for hair colour. The innermost layer, the medulla, is less dense and may be absent in finer hair. Its role is still not entirely understood but may involve insulating properties.

The root extends below the skin and includes the living part of the hair, anchored within the hair follicle. The root is surrounded by the inner and outer root sheaths,

providing structural support and protection during growth. The base of the root houses the hair bulb, which contains matrix cells responsible for producing the hair shaft. Melanocytes in the bulb contribute to pigmentation by synthesizing melanin (Martel et al., 2024; Harkey, 1993).

The hair follicle is a complex structure embedded within the dermis and sometimes extending into the subcutis. It consists of three segments: the infundibulum, isthmus, and inferior segment. The inferior segment includes the dermal papilla, a key structure containing capillaries that nourish the follicle and regulate hair growth. Surrounding the follicle are associated structures, including sebaceous glands that secrete sebum for lubrication and arrector pili muscles responsible for piloerection (goosebumps). The follicle also plays a critical role in the cyclical nature of hair growth, involving anagen (growth), catagen (regression), telogen (resting), and exogen (shedding) phases (Martel et al., 2024; Harkey, 1993).

The detailed cross-sectional diagram of hair is presented in Figure 2.2, illustrating its complex structure. These components collectively highlight the complicated biology of hair, showcasing its roles in protection, sensory perception, and thermoregulation, while emphasizing its importance in forensic and biological sciences.

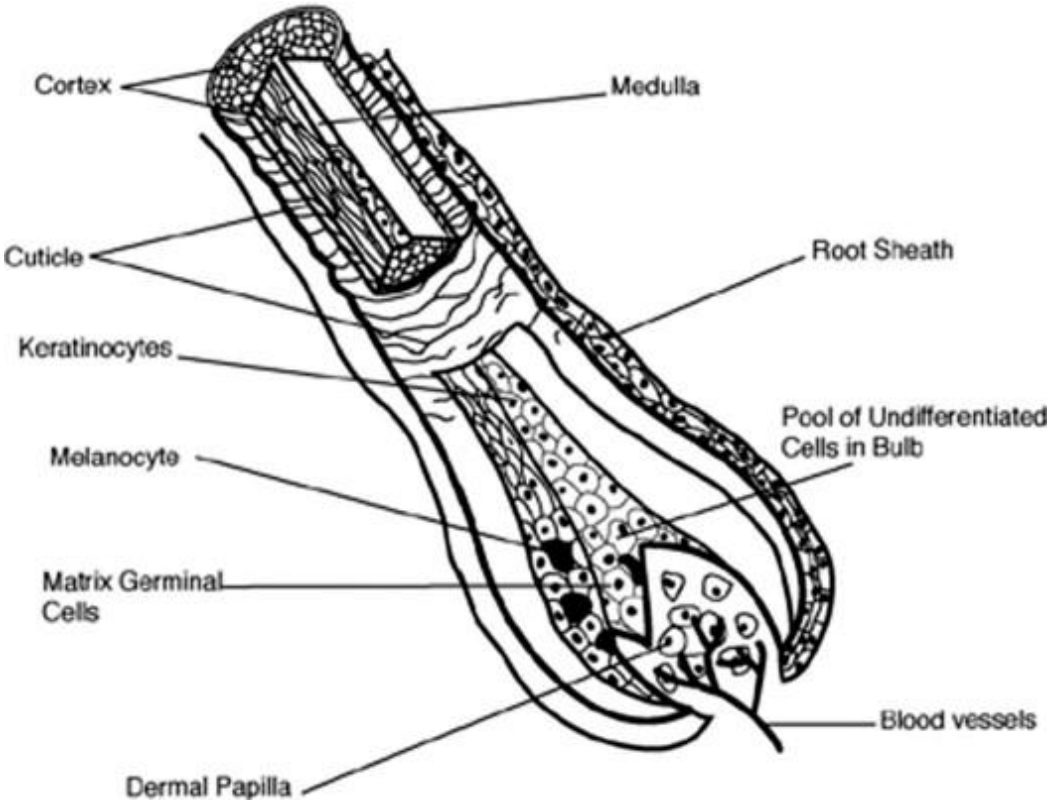

Figure 2.2: Cross-sectional view of hair anatomy and its follicle (Bengtsson et al., 2012).

2.2.1(b) Source of DNA in Hair

Nuclear DNA (nuDNA) and mitochondrial DNA (mtDNA) differ significantly in their location, inheritance, and forensic applications when derived from hair. Nuclear DNA resides in the nucleus of cells and is biparentally inherited, making it unique to an individual except for identical twins. It is predominantly found in the follicular tissue of hair roots, particularly in actively growing anagen-phase hairs, which contain living cells with intact nuclei. These samples can yield sufficient DNA for Short Tandem Repeat (STR) analysis, a highly discriminatory method used in forensic investigations. However, the hair shaft, formed during keratinization, typically lacks nuclear DNA or contains it in highly degraded and fragmented forms, limiting its forensic utility (Linacre and Ottens, 2016; Bengtsson et al., 2012; B. Mahajan, 2019).

In contrast, mitochondrial DNA is abundant in the cytoplasm of cells and is maternally inherited. It is present in high copy numbers, even in the keratinized hair shaft, which makes it particularly valuable in cases where hair lacks a root. mtDNA analysis relies on sequencing specific regions, such as the hypervariable regions, to establish maternal lineage or exclude potential sources (Bengtsson et al., 2012; Gilbert et al., 2004). While mtDNA's lack of individuality compared to nuDNA limits its discriminative power, its stability over time and ability to persist in degraded samples make it indispensable in analyzing ancient or severely compromised specimens (Gilbert et al., 2004; Heywood et al., 2003).

Hair is a significant source of DNA evidence due to its widespread presence at crime scenes and its potential to link individuals to specific locations or activities. The follicle offers high-quality nuclear DNA suitable for precise identification, while the shaft provides mtDNA, which is critical for maternal lineage tracing or identifying

remains when nuclear DNA is unavailable (B. Mahajan, 2019); Higuchi et al., 1988). However, factors such as environmental exposure, hair colouring and the hair's growth phase influence DNA recovery, emphasizing the need for careful sample collection and preservation (Bengtsson et al., 2012); Heywood et al., 2003). These unique attributes underline the versatility and limitations of hair as a source of forensic evidence.

2.2.1(c) Factors Influencing DNA Presence

2.2.1(c)(i) Growth Phase

The growth phase of hair significantly impacts DNA availability, particularly regarding nuclear and mitochondrial DNA. During the anagen phase, hair is actively growing, and the hair bulb is densely packed with germinal matrix cells that contain a high number of mitochondria and nuclear DNA molecules. This phase facilitates the extraction of both mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) because of the abundant soft tissue and cellular material surrounding the root. Consequently, hairs in the anagen phase generally yield the highest success rates for DNA analysis (Roberts and Calloway, 2007; Lawas et al., 2020).

As the hair transitions to the catagen phase, the active growth ceases, and the hair undergoes cellular regression. This stage is characterized by reduced mitotic activity and a shift in the hair follicle's structural dynamics, which diminishes the availability of intact nuclear DNA due to degradation and apoptosis of cells in the follicle matrix (Roberts and Calloway, 2007; Philpott and Kealey, 1994). However, some mtDNA may still be preserved because of its relatively higher stability and abundance within the hair's cellular structures (Liu et al., 2023).