

**COMPARATIVE ANALYSIS OF DNA EXTRACTION METHODS IN THE
RECOVERY OF HUMAN DNA FROM EARPHONES**

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COMPARATIVE ANALYSIS OF DNA EXTRACTION METHODS IN THE
RECOVERY OF HUMAN DNA FROM EARPHONES

By

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

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DECLARATION

I hereby declare that the work presented in this dissertation, entitled “Comparative analysis of DNA extraction methods in the recovery of human DNA from earphones” submitted in partial fulfilment of the requirements for the Bachelor of Science in Forensic Science at Universiti Sains Malaysia, is an original piece of research that is a result of my own investigations, conducted under the guidance of Dr. Nur Waliyuddin Hanis bin Zainal Abidin. The information derived from the literature has been duly acknowledged in the text, and a list of references has been provided. I also declare that it has not been previously or concurrently submitted as a whole for any degrees at Universiti Sains Malaysia or any other institution.

Student,

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Date: 1st of February 2026

CERTIFICATE

This is to certify that the dissertation entitled “Comparative analysis of DNA extraction methods in the recovery of human DNA from earphones” is the bona fide work of Leeanne Yong Luo Ying (163569), carried out under my guidance and supervision during the period of October 2025 to January 2026. I have read this dissertation, and in my point of view, it conforms to acceptable standards of scholarly presentation and is fully adequate. This dissertation is submitted to the Universiti Sains Malaysia in partial fulfillment of the requirement for the Bachelor of Science in Forensic Science.

Supervisor,

(DR. NUR WALIYUDDIN HANIS BIN ZAINAL ABIDDIN)

Date: 03/02/2026

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LIST OF ABBREVIATIONS

A230	Absorbance at 230 nm
A260	Absorbance at 260 nm
A280	Absorbance at 280 nm
D-Loop	Displacement Loop in mitochondrial DNA
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
LCN	Low copy number
mtDNA	Mitochondria deoxyribonucleic acid
nuDNA	Nuclear deoxyribonucleic acid
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
SPSS	Statistical Package for the Social Sciences
ssDNA	Single-stranded DNA
STR	Short tandem repeat
TBE	Tris-borate-EDTA

LIST OF SYMBOLS

bp	Base pair
°C	Degrees Celsius
g	Gram
Mg ²⁺	Magnesium ion
μL	Microliter
μM	Micromolar
mL	Milliliter
M	Molar
n	Sample size
ng	Nanogram
nm	Nanometre
%	Percentage
®	Registered trademark
™	Trademark
V	Volt

ANALISIS PERBANDINGAN ANTARA KAEDAH PENGEKSTRAKAN DNA MANUSIA DARIPADA FON TELINGA.

ABSTRAK

Peranti peribadi seperti fon telinga berpotensi menjadi sumber DNA dalam penyiasatan forensik, terutamanya apabila bukti biologi adalah terhad. Walaupun beberapa kajian melaporkan kejayaan pengekstrakan DNA daripada sampel fon telinga, kaedah pengekstrakan yang paling optimum masih belum dikenal pasti. Oleh itu, kajian ini bertujuan membandingkan tiga kaedah pengekstrakan DNA, iaitu kit komersial berasaskan silika, Chelex-100 dan fenol-kloroform, bagi mengenal pasti kaedah paling berkesan untuk mendapatkan DNA manusia daripada fon telinga. Sebanyak 66 sampel dianalisis, dengan 22 sampel diekstrak menggunakan setiap kaedah. Hasil dan ketulen DNA dinilai menggunakan DeNovix DS-11, diikuti dengan PCR menggunakan primer autosomal hGH dan primer DNA mitokondria bagi menilai integriti DNA. Tiga sampel daripada setiap kaedah turut dianalisis menggunakan qPCR masa nyata untuk mengukur DNA yang boleh diamplifikasikan.

Kaedah fenol-kloroform menghasilkan kepekatan DNA tertinggi dan tidak konsisten melalui pengukuran spektrofotometri tetapi menunjukkan hasil terendah dalam qPCR, mencadangkan kemungkinan pencemaran dan anggaran berlebihan. Kaedah berasaskan silika menghasilkan kepekatan terendah melalui spektrofotometri, kepekatan sederhana melalui qPCR, dan merupakan satu-satunya kaedah yang menunjukkan penguatan autosomal yang berjaya. Kaedah Chelex menunjukkan kepekatan kedua tertinggi melalui spektrofotometri dan tertinggi melalui qPCR, serta kadar kejayaan penguatan DNA mitokondria yang paling konsisten. Secara keseluruhan, tiada satu kaedah pengekstrakan yang unggul dari segi kuantiti dan kualiti DNA secara

serentak; namun, kaedah Chelex menunjukkan prestasi paling baik dari segi hasil dan kebolehamplifikasian DNA.

COMPARATIVE ANALYSIS OF DNA EXTRACTION METHODS FOR HUMAN DNA RECOVERY FROM EARPHONES.

ABSTRACT

Personal devices like earphones can be significant in forensic investigations as a source of DNA, especially when biological evidence is lacking. Although several studies have demonstrated successful DNA extraction and profiling from earphone samples, the most suitable DNA extraction method remains undescribed. DNA extraction is one of the most crucial steps in DNA analysis. Therefore, this study aimed to compare three different extraction methods, including silica-based commercial kit, Chelex-100 and phenol-chloroform method, to identify the most efficient method in recovering human DNA from earphones. A total of 66 samples were analysed, with 22 samples extracted using each method. DNA yield and purity were assessed using DeNovix DS-11, followed by PCR amplification using hGH autosomal primer and mitochondrial primer to assess DNA integrity. 3 samples from each method were further subjected to Real-time qPCR for quantification of amplifiable DNA.

The findings demonstrated that phenol-chloroform yielded the highest and fluctuating DNA concentrations, as measured by DeNovix DS-11; however, the lowest yield when measured by qPCR, indicating contamination and overestimation by the spectrophotometer. The silica-based method yielded the lowest concentrations in spectrophotometry measurement, and moderate concentrations in the qPCR, but was the only method with successful autosomal amplification. The Chelex method displayed the second-highest concentration, but the highest concentration in qPCR. Chelex also demonstrated the greatest amplification success rate using mtDNA. These findings indicate that no single extraction method can excel in both quantity and quality

parameters simultaneously. However, the Chelex method was found to recover DNA that performed better than the other methods, in terms of yield and amplifiability.

CHAPTER 1: INTRODUCTION

1.1 Background of study

1.1.1 Forensic genetics

Deoxyribonucleic acid or commonly known as DNA is the molecule that carries hereditary material in humans and almost all other organisms. Nearly all cells in our body contain DNA that is unique to each individual. The chance of two unrelated individuals sharing the same DNA sequence is 1 in 594.1 trillion, making it a reliable tool in forensic science. From the early Restriction Fragment Length Polymorphism (RFLP) analysis to modern Short Tandem Repeats (STR) analysis and Next Generation Sequencing (NGS), DNA technologies have revolutionised forensic science. Forensic DNA analysis is now the standard practice used in investigating crime scenes, determining paternity and facilitating victim identification (Alketbi, 2023).

Commonly, DNA used in forensic identification of individuals is sourced from various types of traditional biological materials, including bodily fluids, hair, and tissues. Bodily fluids that are rich in DNA and commonly processed in forensic laboratories include semen, blood, saliva, and urine. Other than that, items such as firearms, clothing, household items, and everyday objects can also be utilised as sources of DNA in forensic investigations (Adamowicz, et al., 2015). The advances in forensic DNA analysis methods have allowed for the recovery of genetic material from trace amounts left on these items, expanding the range of potential evidentiary items. DNA can even be successfully recovered from various personal objects, such as toothbrushes, underwear, and cosmetic applicators (Torres-Pérez, et al., 2022).

1.1.2 Trace DNA

The Locard's exchange principle dictates that every contact leaves a trace. Every time two objects come into contact, an exchange of material will inevitably occur. Trace DNA is a form of evidence that exemplifies this principle, where minute biological traces such as human skin cells, saliva, or sweat can be deposited onto surfaces or objects through handling and touching. As personal objects are routinely handled and repeatedly come into contact with the skin of the owner, genetic material is often transferred onto them. This transfer of trace DNA can be useful in linking suspects to a crime or victim, which can further enhance criminal investigations. The sensitivity of modern DNA tools has allowed investigators to obtain a complete profile from as little as 100 picograms of DNA (Wickenheiser, 2002), transforming personal items such as toothbrushes or cosmetics into critical evidence that can provide a reliable source of DNA.

1.1.3 Earphones as forensic evidence

Earphones exemplify a common everyday item that many people wear and handle daily. They are compact audio devices that are worn either over or in the ear to allow private listening of audio. These devices tend to accumulate earwax, sebum, skin cells and sweat due to prolonged contact with the skin and ear canal. A 2024 survey in Japan revealed that 31% of young adults who own earphones or earbuds use them every day (Chhatwal, 2024). This highlights the integration of the device into daily activities and the increasing dependency on it. Hence, it has high potential of being present at a crime scene, such as the device that was last used by the perpetrator or victim. In cases where reference samples are difficult to obtain, or where biological evidence is lacking, personal objects like earphones can be used to obtain a DNA sample.

The typical genotyping workflow in forensic DNA analysis comprises activities such as evidence examination, body fluid identification, DNA extraction, assessment, amplification of target loci, detection of amplified products, analysis of data, and generation of a report. Among these, the DNA extraction step heavily impacts the success of obtaining a high-quality genotype profile, which is the ultimate goal. Good quality DNA leads to better amplification and detection of the amplified product. Therefore, this research aims to evaluate different DNA extraction methods, including Phenol-chloroform, Chelex, and Silica-based commercial kit, in recovering DNA from in-ear earphones to evaluate their performance and identify the most suitable method for downstream application (PCR).

1.2 Problem statements

Earphones are widely used electrical devices that many people own and utilise daily. Anyone who owns a mobile device is most likely to have their own pair of earphones. Hence, it can easily be present in a crime scene, potentially as a device that has been used by either the perpetrator or victim. Since the hands-free hearing device is required to be in direct contact with the inner ear in order to transmit sounds, it has the possibility of providing trace DNA source when there is an absence of other conventional biological evidence at a crime scene.

Even though there are studies that have demonstrated that DNA can be extracted from earphones (Seo, et al., 2002; Yudianto, et al., 2017; Yudianto & Nzilibili, 2019), the efficiency of the different extraction methods for materials on earphones has yet been systematically compared. Most studies that evaluate the effectiveness of DNA extraction methods focus more on body fluids, with limited published data regarding trace DNA (Wood, et al., 2017; Alketbi, 2018). This lack of comparative evaluation represents a significant gap in forensic applications. Analysing trace DNA can be

challenging since the amount of DNA obtained is easily influenced by factors such as the extraction methods. Therefore, by conducting a systematic comparison of extraction methods, the performance of each method can be evaluated, which may hopefully contribute to the development of standardised protocols for the analysis of DNA traces on earphones.

1.3 Research questions

- i. Which extraction method provides the best quality of extracted DNA from earphone swabs?
- ii. Which DNA extraction method results in the highest human DNA yield?

1.4 Objectives

General objective:

To identify the most efficient DNA extraction method to recover human DNA from earphones.

Specific objectives:

- i. To assess the quantity and quality of DNA extracted from different methods, including silica-based commercial kit, Chelex, and phenol-chloroform, using spectrophotometry techniques.
- ii. To assess the amplifiability of DNA extracted from each method based on Polymerase Chain Reaction.

1.5 Significance of study

By systematically comparing DNA yield, purity, and amplification success across different extraction techniques, this research aims to provide valuable insights into which method suits samples such as earphones. The findings are expected to improve forensic investigators' ability to recover and analyse touch DNA in cases involving personal belongings, thereby strengthening the investigative process in criminal and missing person cases. Ultimately, this study supports the advancement of forensic protocols and contributes to the broader understanding of trace evidence applications in modern forensic science.

CHAPTER 2: LITERATURE REVIEW

2.1 DNA extraction

One of the most crucial part in DNA analysis is the extraction step (Xavier, et al., 2021). In a forensic context, an effective extraction method is essential to yield sufficient and high-quality DNA that is suitable for downstream applications such as PCR, Short-Tandem Repeat (STR) profiling, and sequencing (Chacon-Cortes & Griffiths, 2014). DNA extraction involves the isolation of DNA from proteins, cell membranes and other cellular material in a sample using physical and/or chemical techniques.

In 1869, Friedrich Miescher conducted the earliest DNA extraction during his study of the cellular function of leucocytes. During his experiments, Miescher noticed a substance precipitated out with acid and redissolved with alkali, unlike other proteins. Upon this discovery, he named the cellular material as ‘nuclei’, a part of the cell which was not very well known at that time. Miescher’s work marked the first chemical isolation of DNA, establishing it as a distinct substance within cells (Dahm, 2005; Chacon-Cortes & Griffiths, 2014).

The typical DNA extraction process used today shares three similar stages. First is the lysis of cell membranes and nuclei to release DNA into aqueous solution. Second is the enzymatic digestion and/or denaturation of proteins to isolate unwanted materials such as DNA-associated proteins, cellular proteins, and other cellular debris. Third is the separation of DNA from the other cellular components, usually through filtration, centrifugation, or bead-based methods (Wai, et al., 2020).

Lysis of cells can be achieved mechanically using physical methods such as grinding with a pestle and mortar, blending, freeze-thawing, or bead-beating. Another

way to break up the cells is by enzymatically. This involves incubating the sample with enzymes, typically Proteinase K, that hydrolyse the cell wall and cell membrane components (Lever, et al., 2015). Chemical lysis, on the other hand, involves using detergent to separate the lipid membrane and solubilise the cell membranes. Enzymes and detergents are often used in combination to target cell surface and cytosolic components. Subsequently, a highly concentrated salt solution is added to precipitate the DNA and separate it from other cellular debris, followed by purification through ethanol washes (Chacon-Cortes & Griffiths, 2014).

DNA isolated from various biological samples can be utilized for a wide range of downstream applications. This mainly includes DNA sequencing, polymerase chain reaction (PCR), quantitative PCR (qPCR), southern blotting, random amplification of polymorphic DNA (RAPD), preparation for genomic libraries, as well as amplified fragment length polymorphism (AFLP), and restriction fragment length polymorphism (RFLP) (Dhaliwal, 2013). On top of that list is short tandem repeat (STR) analysis, single nucleotide polymorphism (SNP) typing, along with massively parallel sequencing (MPS) or next-generation sequencing (NGS) (Alketbi, 2023). These downstream applications are crucial in areas such as medicine, genetics, and forensic science. They enable the diagnosis of genetic diseases and pharmacogenomics in medicine, as well as the identification of a person's carrier status for a particular disease. Some examples of diseases diagnosed by gene sequencing include Down's syndrome, Huntington's chorea, sickle-cell anemia, and hemophilia (Gautam, 2022). Another significant application is DNA fingerprint, which is important for the resolution of complex crimes and paternity tests in forensic investigations (Dairawan & Shetty, 2020).

2.2 Types of DNA extraction methods

Various extraction methods have been formulated for the extraction of genomic DNA from human specimens. However, with the diverse types of samples and biological evidence, there is no single extraction method that is optimal for all sample types (Lever, et al., 2015) (Wai, et al., 2020). The selection of the method is greatly dependent on the biological substance concerned. Currently, the validated methods for DNA extraction most widely used in forensic laboratories can be classified into three groups based on their purification strategies: organic (phenol–chloroform) extraction, solid-phase DNA extraction methods (silica-based), and ionic chelating resins (Chelex) (Alonso, 2013) (Bukyya, et al., 2021).

2.2.1 Phenol-chloroform extraction

The organic extraction method is a conventional and well-established technique that involves the use of organic solvents, typically phenol and chloroform. The core principle behind this method is the separation of DNA, RNA, and protein based on their differential solubilities in different immiscible liquids (Gautam, 2022). In this widely used method, the cells are first lysed with a lysis buffer containing detergents such as sodium dodecyl sulphate (SDS), Tris-Cl, and Ethylene diamine tetraacetic acid (EDTA), followed by centrifugation to remove cell debris. Then, a protease is usually added to denature proteins, and phenol-chloroform-isoamyl alcohol solvent is added in the ratio of 25:24:1 for the denaturation and precipitation of proteins (Gupta, 2019).

SDS and phenol enable efficient protein denaturation, while isoamyl alcohol prevents emulsion and facilitates DNA precipitation. The solution is mixed either by vortexing or gentle shaking to form a biphasic emulsion that forms upon centrifugation. The upper aqueous phase with the DNA, is kept, while the lower organic phase,

containing the proteins, is removed. Careful pipetting is important in this step to prevent the transfer of the aqueous phase along with the contamination collected in the interphase (Mülhardt & Beese, 2007). This step is usually repeated 2-3 times before the solvent is precipitated using ethanol or isopropanol to obtain purified DNA. The DNA pellet is centrifuged and dried. The dried pellet is resuspended in TE buffer or sterile distilled water (Dairawan & Shetty, 2020) (Bukyya, et al., 2021).

The phenol-chloroform method that was introduced in 1998 by Barker et al is now considered the gold standard for DNA extraction (Elkins, 2013). This method is relatively inexpensive and is known to provide high yield with longer fragment size (Dairawan & Shetty, 2020) (Molbert, et al., 2023) (Christian , et al., 2023). However, the use of toxic and corrosive organic solvents in this technique becomes its major drawback. Phenol can cause burns on the skin when in contact and get absorbed into the system, affecting the nervous system, liver, and kidneys. Not only that, vapour from phenol can irritate the eyes and respiratory tract, causing nausea and vomiting (Mülhardt & Beese, 2007). Hence, proper personal protective equipment and use of a biohazard hood are required.

2.2.2 Silica-based extraction

The purification of DNA utilising the liquid/solid-phase approach dates back to 1979, when Vogelstein and Gillespie employed silica in a powdered glass form in their protocol to isolate DNA fragments previously separated by agarose gel electrophoresis. Since then, solid-phase purification has been used in most of the commercial extraction kits available in the market (Chacon-Cortes & Griffiths, 2014). Solid-phase extractions are based on the absorption of DNA molecules onto solid matrices, commonly embedded in beads or columns, in the presence of chaotropic salts (Vutukuru, et al., 2025). The underlying principles that form the absorption mechanism include the

following: the interaction of hydrogens with a hydrophilic matrix under chaotropic conditions, the ionic exchange under aqueous conditions facilitated by an anion exchanger, and the affinity and size exclusion mechanisms (Tan & Yiap, 2009).

Silica-based extraction is a common method that applies the solid phase principle, which utilises silica matrices as the solid support. This approach was initially described by Boom et al in 1990. Silica matrices have unique properties for DNA binding. The silica particles are positively charged, causing the negatively charged DNA backbone to have a high affinity towards them. The binding of the DNA and silica beads is facilitated by high salt conditions and pH. Sodium acts as the cation bridge that attracts the negatively charged oxygen in the phosphate backbone of nucleic acids towards silica surfaces. The sodium cations break the hydrogen bonds between water molecules and silica, facilitating DNA absorption (Vutukuru, et al., 2025). Contaminants are then removed with extensive washing, followed by the addition of TE buffer or distilled water to elute the DNA molecules (Tan & Yiap, 2009).

Moreover, most solid-phase techniques use a spin column to bind nucleic acid under centrifugal force. This method is able to purify nucleic acids more rapidly compared to conventional methods. Columns require conditioning using a buffer at a particular pH to transform the surface into a particular chemical form. After that, it usually follows a series of steps that comprise a cell lysis using a lysis buffer, the DNA absorption to the column that is facilitated by a binding solution, washing of DNA using a wash buffer, and the final elution step using TE buffer or distilled water. Generally, the lysis or binding buffer would contain a chaotropic salt, while the wash buffer would contain high concentrations of ethanol or isopropanol (Tan & Yiap, 2009). Any residues from these extraction buffers could significantly hinder downstream analyses (Jue, et al., 2020).

Commercially available kits using silica-based approach includes those manufactured by QIAGEN Pty Ltd, Victoria, Australia (QIAamp[®]), Promega Corporation, Fitchburg, WI, USA (Wizard[®]), Apical Scientific, Malaysia (1st BASE PrimeWay), and many more. Overall, silica-based commercial column kits are recognised for delivering high-quality DNA and having standardised protocols. They are non-toxic and user-friendly; however, they typically require multiple labour-intensive steps, which can lead to DNA loss. The various stages involved also elevate the risk of introducing contaminants (Walsh, et al., 2013). Additionally, these kits can be quite costly. (Jung, et al., 2025) (Aidar & Line, 2007) (Dilley, et al., 2021).

2.2.3 Chelex-100 resin

Chelex-100 resin is an ion exchange resin composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups. It is a chelating reagent with a high affinity towards polyvalent metal ions such as magnesium (Mg²⁺). Chelex exhibits high selectivity for divalent ions, enabling the formation of stronger bonds compared to ordinary ion exchangers. The initial protocol for this method was first described by Walsh et al in 1991, using blood as a DNA source (Chacon-Cortes & Griffiths, 2014). Walsh et al also reported the successful extraction of DNA from forensic samples such as bloodstains, seminal stains, saliva, hair and post-coital samples using Chelex-100 (Sweet, et al., 1996). Since then, various modifications have been developed to extract DNA from different types of samples, including dried blood spots (Simon, et al., 2020) and ancient skeletal remains (Schmerer, 2021).

The extraction process involves boiling the sample in a 5-20% suspension of Chelex solution to release the DNA, typically performed in a single tube. For forensic protocols, proteinase K is often added in addition to the Chelex solution, incubated at 56 °C for 20 minutes and at 100 °C for 8 minutes, to lyse the cell membranes as well

as denature proteins. After vortexing and centrifugation, single-stranded DNA will be obtained and remain suspended in the supernatant. It was postulated that boiling the sample in the presence of Chelex prevents the degradation of DNA by DNases that remain after boiling (Lee & Shewale, 2017). DNases are inactivated and the DNA is protected by removing the Mg^{2+} from the reaction.

The Chelex method enables a rapid, simple and inexpensive DNA extraction method, without the use of toxic organic solvents and multiple transfer tubes (Sweet, et al., 1996). Hence, it can be an advantageous method when handling samples containing minute quantities of DNA due to its reduced risk of contamination (Schmerer, 2021). However, the major concern with this method is the lack of a purification step to remove haemoglobin, protein, or other impurities from the extract, which might inhibit downstream applications. Moreover, the DNA obtained from the extraction is single-stranded and therefore is more susceptible to hydrodynamic or enzymatic degradation (Simon, et al., 2020).

2.3 Trace evidence

DNA analysis on samples found at the crime scene has become a common practice in linking suspects to the crime during forensic investigations. Scientific advancements, such as the discovery of PCR-based methodologies, enabled DNA recovery and analysis of very minute and degraded biological samples, and not only of bodily fluids, but also of DNA transferred through brief physical contact with an object (Wickenheiser, 2002) (Burrill, et al., 2019). In 1997, van Oorschot and Jones successfully obtained DNA profiles from hands and handled objects (van Oorschot & Jones, 1997). This finding expanded possibilities and resulted in the recovery and typing of DNA from a wider range of exhibits, hence aiding in the investigation of a

greater range of offences and the dissolution of many difficult cases, such as theft, homicide, armed robbery, assaults, and sex offences (van Oorschot, et al., 2010).

Commonly, conventional biological substances that are forensically relevant, such as blood and semen, may not be encountered at crime scenes; however, touched objects offer a broader potential for uncovering the offender's DNA profile. Trace or touch DNA is known as the minute quantities of DNA transferred onto surfaces or items through skin contact (Wickenheiser, 2002). It is also described as biological samples containing very limited or low quantities of DNA, specifically less than 100 picograms (Gill, 2001) (van Oorschot, et al., 2010). It may be transferred via other means as well, not just by hand, as DNA-bearing cells may originate from multiple parts of the body (Alketbi, et al., 2025). There is no exact tissue of origin specified in the definition of trace DNA, as it's usually recovered speculatively without any visual detection of a body fluid or stain (Burrill, et al., 2019).

Currently, trace DNA deposits are proposed to contain fingerprint secretions, cellular DNA (nucleated/anucleate), and cell-free DNA (cfDNA) (Arsenault, et al., 2024). The main sources originate from the shed skin cells on the skin and hands, with the average human being shedding approximately 400,000 skin cells daily. Skin cells are nucleated, and each human cell has about 5 picograms of nuclear DNA (Wickenheiser, 2002). The DNA recovered from touched items from washed hands was substantially lower than from unwashed hands, further supporting shed skin cells as a viable source of trace DNA (Burrill, et al., 2019). Aside from cells originating from hands, the hands may instead act as carriers, accumulating exogenous cells from other regions of the body. This includes saliva from the mouth, sebum from sebaceous skin like the face and nose, and epithelial cells from the eyes. Hence, acts such as rubbing

the face, nose, and mouth, or chewing fingernails, can deposit more cells onto the hands (Wickenheiser, 2002).

The deposition of trace DNA is influenced by a number of factors, including the pressure of contact, the shedder status of the handler and substrate properties. One study demonstrated that increased pressure of finger contact resulted in higher levels of DNA recovery (Tobias, et al., 2017). Individuals who are “good shedders” tend to shed skin cells at a greater rate, compared to “bad shedders”, hence generating more DNA to be deposited (Alketbi, 2018). Additionally, it was reported that men tend to shed more than women (Fonneløp, et al., 2017). Subsequently, the success of touch DNA recovery is also hugely dependent on the type of substrate surface. According to Kisilevsky and Wickenheiser (1999), shed skin cells adhere more readily to porous surfaces, like wood, than to non-porous surfaces like glass, though recovery from rough, porous surfaces can be more difficult. Aside from the three variables, the methods employed for collection and extraction also significantly influence trace DNA recovery (Alketbi, et al., 2025).

2.3.1 Trace DNA collection

In criminal cases, the sampling techniques are very important to collect the best evidence. Various techniques can be employed for the collection of touch DNA traces, such as swabbing, adhesive tapes, or cutting out the area of interest (Alketbi, 2018). Swabbing is the most versatile and widely used method for collecting trace samples. They are either applied dry or moistened, where water is the main choice for moistening agent, though some laboratories use 0.01% sodium dodecyl sulphate or isopropanol (van Oorschot, et al., 2010). The wet-dry double-swab technique, introduced in 1997, is the common method performed for the collection of trace material. It involves applying a moist swab onto surfaces to rehydrate dried epithelial cells, causing them to

adhere to the swab better, followed by a second dry swab to collect the moisture (Tozzo, et al., 2021; Abdullah, et al., 2023). Though under non-optimal experimental conditions, the double-swab method was found to yield less DNA than other methods and also damages the surfaces of items (Plaza, et al., 2016).

The double swabbing method for DNA recovery has evolved to several alterations, including wet-moist double swabbing, wet-wet double swabbing, and moist-dry single swabbing. The wet-moist technique, which involves applying a wet swab, followed by a moist swab with a smaller volume of moistening agent, has been used for the DNA recovery from clothing and fabric (Abdullah, et al., 2023). The wet-wet double swabbing involves applying two wet swabs back to back. Hedman et al (2020) discovered that a wet second swab yielded more DNA than a dry second swab when examined on both absorbing and non-absorbing surfaces. Further, the moist-dry single swab involves applying one side of the swab that is moistened with water onto the substrate, followed by the dried side. This method has been used to recover DNA from firearms (Abdullah, et al., 2023). On the contrary, tape lifting has been reported to produce a higher yield of trace DNA than swabbing (Tozzo, et al., 2021; Kanokwongnuwut, et al., 2021; Burmuzoska, et al., 2022); though they can be tedious due to their stickiness, and multiple strips of tape may pose downstream processing difficulties (van Oorschot, et al., 2010).

2.3.2 Trace DNA extraction

Touch DNA is one of the frequently encountered sources of DNA during casework. It has been recognised as a potential template source for DNA profiling for decades. However, trace forensic samples can be challenging because they contain small amounts of cellular material and tend to have poor STR profiling success (Kanokwongnuwut, et al., 2021). The extraction method employed for evidence

samples is critical, as a better quality and higher quantity of DNA increases the likelihood of obtaining successful DNA profiles.

Silica-based solid-phase extractions are dominantly preferred in forensic laboratories as they are most efficient at recovering high amounts of pure DNA (Dilley, et al., 2021). In a study evaluating extraction methods on 76 simulated touch DNA, it was discovered that three extraction kits (QIAamp® DNA Investigator Kit, QIAasymphony® DNA Investigator® Kit, and DNA IQ™) yielded extracts with higher DNA concentration and amplification success rate, compared to Chelex-100 (Ip, et al., 2015). However, recently it was revealed that the QIAamp® DNA Investigator kit, a common silica column commercial kit, demonstrated poor extraction efficiency on low concentration samples, likely attributed by the multiple wash stages and tube transfers involved (Dilley, et al., 2021).

Chelex-100 resin is also commonly employed in forensic laboratories to extract DNA from a wide range of evidence samples (Lee & Shewale, 2017). A study conducted by Jung et al concluded that the Chelex method is suitable for handling partially contaminated or degraded remains (Jung, et al., 1991). In addition, it was reported that the Chelex-100 resin outperformed the organic method using phenol-chloroform in recovering DNA from saliva stains (Sweet, et al., 1996). The major drawback, however, is the contaminated extract obtained when using Chelex resin. Overall, there is still a shortage of published data examining different efficiencies of extraction methods on trace DNA, with most studies primarily focusing on body fluids (Alketbi, 2018).

2.4 DNA recovery from personal items

Although traditional known reference samples like bloodstains or buccal swabs are ideal for identifying victim remains in mass disasters or missing person cases, they

are often not accessible to generate a DNA profile for the victims of these types of events (Adamowicz, et al., 2015). In cases where there are few or no living biological relatives who can donate reference samples, personal belongings from the victim can be utilised to obtain the DNA for comparison. There are numerous studies demonstrating personal, wearable items as a potential source for obtaining DNA for human identification.

For example, several studies have demonstrated that toothbrushes are an excellent source of DNA. Tanaka et al. (2000) successfully identified two victims and one suspect using toothbrushes obtained in two actual deaths. In a comparison involving undergarments and toothbrushes, Abidiel et al. (2022) discovered that undergarments recovered a greater amount of DNA, while toothbrushes recovered more DNA profiles. Additionally, in a study examining cosmetic applicators, 14 out of 76 samples produced full STR profiles, with makeup sponges being the most efficient for DNA typing (Adamowicz, et al., 2015). According to a review on items processed by the Dubai Police Force, wearable items such as jewellery and glasses demonstrated the highest success rate for DNA profiling (Alketbi, et al., 2025). However, these types of evidence are prone to containing PCR inhibitors, such as toothpaste in toothbrushes and dye in makeup applicators, which remain a constant challenge.

2.4.1 DNA recovery from earphones

Analysis by the Dubai Police Force revealed that items subjected to direct and prolonged skin contact exhibit significantly higher probabilities of yielding complete, single-source DNA profiles (Alketbi, et al., 2025). This finding strongly supports the forensic value of earphones as optimal DNA sources, given their intimate and extended contact with the external ear canal. There are two types of glands located on the outer human ear canal: sebaceous glands that generate sebum and modified apocrine glands

that secrete apocrine sweat (Roeser & Ballachanda, 1997). Hence, these substances can be deposited onto earphones when they are inserted into the ear canal. In addition to sweat and sebum, Seo et al (2002) identified the presence of nucleated epithelial cells in materials collected from earphones, suggesting these devices contain trace DNA, as epithelial cells are one of the main components of trace DNA deposit (Burrill, et al., 2019) .

Currently, there is a lack of literature on earphones as a source of DNA for human identification, with only three articles available. Firstly, Seo et al (2002) were able to identify six autosomal STR loci and performed mitochondrial DNA (mtDNA) sequencing of hypervariable regions 1 and 2 (HV-I and HV-II) on DNA extracted from matter adhering to earphones. This study employed the organic phenol-chloroform method, yielding up to 1 ng/ μ L of DNA. In addition, it was discovered that the yield of genomic DNA was attributed to the amount of matter on the earphone, not to the type of earwax (Seo, et al., 2002).

Similarly, Yudianto et al. (2017) also employed mtDNA sequencing on earphone swabs to assess the effects of different exposure durations. The study concluded that earphones can serve as an alternative DNA source for forensic identification, although results show that long exposure does reduce DNA levels. In a subsequent study, Yudianto and Nzilibili (2019) further investigated the recovery of DNA from earphone swabs after prolonged exposure to room temperature, focusing more on the hypervariable regions 1 and 2 (HV-I and HV-II) in the D-loop region of mtDNA. In both of these studies, the DNAzol extraction kit was utilised, and the extracted DNA ranged from 23.2 μ g/mL to 152.9 μ g/mL (Yudianto, et al., 2017; Yudianto & Nzilibili, 2019).

Although the following study did not examine earphones specifically, it investigated earwax, which is the primary component of DNA adhering to earphones from users' ear canals. Recently, Amer et al. (2023), demonstrated successful extraction of DNA from human earwax samples and obtained complete STR profiles. This further validates the potential of earphones as reliable forensic DNA sources. The QIAamp[®] DNA Mini Kit, a silica-based commercial kit, was used in this study and obtained an average of 0.1040 ng/ μ L DNA across 15 samples.

2.5 Real-time PCR

Real-time Polymerase Chain Reaction (real-time PCR) is a variation of the PCR assay which enables the monitoring of the PCR progress in real time. The technique, also known as quantitative PCR, became commercially available when Applied Biosystems (ABI) introduced the 7700 instrument in 1996. PCR itself, which was first introduced by Kerry Mullis in 1983, is a molecular process used to amplify target sequences into millions of copies (Dorak, 2006). PCR-based methods, including real-time PCR, are widely used for the quantitation of low-level DNA samples due to their high sensitivity and simplicity (Kang, et al., 2011). Besides enhanced accuracy, sensitivity, and speed, the key advantage of real-time PCR over conventional PCR is its ability to provide reliable quantification relationship between the initial amount of target sequences and the quantity of amplicon produced in a specific cycle. This feature allows for accurate quantification of the target nucleic acids. The ingredients needed include the template DNA, primers, nucleotides (dNTPs), and thermostable DNA polymerase (Artika, et al., 2022).

CHAPTER 3: METHODOLOGY

3.1 Materials

3.1.1 Chemicals and reagents

All the chemicals, reagents, commercial kits and consumables used in this study were listed in Appendix C.

3.1.2 Instruments and apparatus

All instruments and apparatus used in this study were listed in Appendix D.

3.1.3 Chemical and reagent preparations

3.1.3 (a) 70% Ethanol

To prepare 500 mL of 70% ethanol, 350 mL of absolute ethanol was measured and added to 150 mL of deionised water. The solution was mixed and stored in a 500 mL Schott Duran® bottle.

3.1.3 (b) 1 M Tris Hydrochloride (Tris-HCL) pH 8.0

To prepare 1 M Tris-HCl buffer, 12.11 g of Tris base was weighed and added to a 100 mL Schott Duran® bottle containing 80 mL of deionised water. The pH of the solution was observed using a pH meter and adjusted to pH 8.0 by slowly adding hydrochloric acid (HCl). Once the desired pH was obtained, deionised water was added to adjust the final volume of the solution to 100 mL. The solution was then autoclaved to sterilise and stored at room temperature.

3.1.3 (c) 0.5 M Ethylenediaminetetraacetic Acid (EDTA) pH 8.0

A total of 18.61 g of Ethylenediaminetetraacetic acid (EDTA) disodium salt, dehydrate was added to a 100 mL Schott Duran® bottle containing 80 mL of distilled water. Sodium hydroxide (NaOH) pellets were added to the solution to help dissolve

the salt and adjust the pH. Around 2 g of NaOH pellets were added before the solution obtained a pH 8.0, observed using pH meter. Once the desired pH was obtained, deionised water was added to adjust the final volume of the solution to 100 mL. The solution was then autoclaved to sterilise and stored at room temperature.

3.1.3 (d) Tris-EDTA Buffer (TE Buffer) pH 8.0

To prepare TE buffer, 1 mL of 1 M Tris Hydrochloride (Tris-HCL) and 0.2 mL of 0.5 M Ethylenediaminetetraacetic Acid (EDTA) was added to a 100 mL Schott Duran® bottle. Deionised water was added to adjust the final volume of the solution to 100 mL. The bottle was inverted a few times to mix the solution. The solution was sent to be autoclaved to sterilise before storing at room temperature.

3.1.3 (e) 20% Sodium Dodecyl Sulphate (SDS)

To prepare 100 mL of 20% SDS solution, 20 g of SDS powder was weighed and added to a 100 mL Schott Duran® bottle containing 80 mL of deionised water. The mixture was dissolved before deionised water was added to adjust the volume to 100 mL.

3.1.3 (f) 5 M Sodium Chloride (NaCl)

To prepare 100 mL of 5 M sodium chloride (NaCl), 29.2 g of NaCl was weighed and added to a 100 mL Schott Duran® bottle containing 80 mL of deionised water. The mixture was stirred to dissolve the NaCl. The volume was adjusted to 100 mL using deionised water.

3.1.3 (g) Digestion buffer

1 mL of 1 M Tris-HCl, 2 mL of 0.5 M EDTA, 10 mL of 20% SDS, 1 mL of 5 M NaCl, and 86 mL of deionised water is added to a 100 mL Schott Duran® bottle. The bottle is inverted a few times to ensure the mixture is uniform.

3.1.3 (h) 2 M Sodium Acetate (CH₃COONa)

A total of 102.206 g of sodium acetate powder was dissolved in 200 mL of deionised water. The pH was adjusted to 5.2 by adding glacial acetic acid. The final volume was adjusted to 250 mL by adding deionised water. The solution was then autoclaved and stored at room temperature

3.1.3 (i) 10x Tris-Borate-EDTA (TBE Buffer)

To prepare 10x TBE buffer, 108 g of Tris base, 55 g of boric acid, and 9.3 g of ethylenediaminetetraacetic acid (EDTA) disodium salt were weighed and added to a 1000 mL Schott Duran® bottle containing 800 mL of deionised water. The mixture was stirred thoroughly to ensure it is completely dissolved. Deionised water was added to the solution to adjust the volume to 1000 mL.

3.1.3 (j) 0.5x Tris-Borate-EDTA (TBE Buffer)

To prepare 0.5x TBE buffer, 50 mL of the prepared stock 10x TBE buffer is measured and added to 950 mL of deionised water. The solution was mixed and stored in a 1000 mL Schott Duran® bottle.

3.1.3 (k) 5% Chelex ® 100

To prepare 100 mL of 5% Chelex® 100 solution, 5 g of Chelex® 100 resin was weighed and added to a clean 100 mL Falcon tube. Deionised water was added to the solution to adjust the final volume to 100 mL.

3.1.3 (l) Phosphate-buffered saline (PBS)

To prepare 1000 mL of PBS solution, three PBS tablets were added to 1000 mL of deionised water in a Schott Duran® bottle. The bottle was inverted a few times to dissolve the tablets in the solution.

3.2 Ethical Approval

This study involved human DNA samples, hence, it was conducted in full compliance with the institutional policies and ethical guidelines. Before initiating the study, ethical approval was obtained from the Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM-USM), with an assigned study protocol code of USM/JEPeM/KK/25111026 (Appendix A). Consent forms that contain the information of the study such as the procedure, purpose, risks and benefits, are provided to the volunteer participants before sample collection.

3.3 Study Area

The study was carried out in the Health Campus of Universiti Sains Malaysia (USM), Kota Bharu. This study focuses on targeting the students and staff of USM. All the laboratory work has taken place specifically in the Makmal Sain Forensik and Makmal HID of the Health Campus.

3.4 Study Population

Participants were recruited from among the staff and students working and studying in the Health Campus, Universiti Sains Malaysia. Participants were eligible for the study if they fulfilled the following criteria:

- i) Inclusion criteria
 - Participants aged 18 and above
 - Participants who are healthy
 - Participants who regularly use earphones or earbuds
- ii) Exclusion criteria
 - Participants with active ear infections
 - Participants with skin diseases (such as eczema)

3.5 Sampling method

Participant recruitment poster for the sample collection was advertised to all USM students and staff via various platforms, including WhatsApp, Telegram, and social media platforms. Individuals who were interested were screened through a verbal interview to ensure they understood the study and fulfilled the requirements before agreeing to participate. The eligible individuals were asked to fill out an informed consent form as part of the procedure for sampling and then scheduled to meet in the Makmal Sains Forensik.

Participants were required to provide their in-ear earphones that had been recently used for at least 3 days. Each participant was scheduled for three sessions throughout three separate weeks, one sample collection for one extraction method. Each session took no more than 15 minutes, and no damage was done to any devices.

3.6 Sample collection

For sample collection, one cotton swab was used to collect materials from the earphone surfaces. The swabbing technique used was the moist-dry single swab technique, which involves moistening one side of the swab with approximately 6 μ L of deionised water and leaving the other side dry. The moistened side was applied to the earphone surface first, followed by the dry side. The earphone tips and other parts of the earphone that have been in contact with the ear were swabbed thoroughly for at least 15 seconds to collect sufficient materials. After, the swab tips were put into 1.5 mL microcentrifuge tubes for subsequent steps.

As a result, a total of 66 samples were collected, with 22 samples allocated to each extraction method. To minimise inter-individual variability, the same individuals were used across all methods. Samples processed using silica-based commercial kits