

The Forensic Applicability of DNA Extraction Methods from Compromised Skeletal Remains:
A Comparative Literature Synthesis

Submitted by

Sydney Tengler

Biochemistry, with minors in Anthropology and Criminal Justice

To

The Honors College

Oakland University

In partial fulfillment of the
requirement to graduate from

The Honors College

Mentor: Holly Yatros, Professor of Anthropology: MSc., MS

Department of Anthropology

Oakland University

April 01, 2022

Table of Contents

Abstract.....	3
Introduction.....	4
Materials and methods.....	7
Review Methodology / Source Selection.....	7
DNA Extraction Techniques.....	8
Sample Grouping.....	9
Results and Discussion.....	10
Standard DNA Extraction Efficiency.....	11
Application to Compromised Skeletal Remains.....	15
Forensic Applicability.....	19
Conclusion.....	22
Acknowledgments.....	23
References.....	24
Appendix.....	28

Abstract

Forensic anthropologists are tasked with analyzing skeletal remains in a variety of conditions. The goal of this project was to explore how chemical DNA extraction techniques can be used to aid in forensic anthropological investigations where the skeletal remains are too compromised to rely on traditional observational methods. The types of compromised bones examined are weathered, aged, heat-damaged, fragmented, and commingled. Various DNA extraction methods (phenol-chloroform, CTAB + isoamyl alcohol, total demineralization, and QIAamp DNA mini Kit) from skeletal remains were analyzed to determine how effective and feasible they are in a forensic investigation and how the methods can be furthered to assist in investigation regardless of the state of the remains. This included identifying aspects from each method to be applied or focused on for a future universal DNA extraction model. Forensic cases, especially those which contain compromised remains, will benefit from this project as the new approach will allow for evidence to be obtained from any bones despite the condition of the remains; this could also apply to ancestral remains found at historical sites. The results demonstrated that total demineralization and the QIAamp DNA Mini Kit show the most potential in contributing to a future universal method, as long as there is sufficient funding, established protocols, and up to date training in forensic laboratories.

Introduction

Forensic anthropology is an area of study that analyzes human skeletal remains for legal and archaeological purposes. Gross anatomical features of skeletal remains, which are the skeletal structures visible by the naked eye, are used to build a biological profile to assist in human identification, including, but not limited to, the following: the estimation of age, sex, height, ancestry, trauma, and the time and manner of death. Bones are composed of an inorganic extracellular matrix largely consisting of hydroxyapatite, a calcium phosphate, which surrounds the cellular components of skeletal cells called osteocytes, osteoclasts, and osteoblasts. The hydroxyapatite enforces the hard structure of bone which allows bones to be a long-lasting DNA source in decayed remains, but also limiting the extraction of the DNA located inside the cellular components of bones (Loreille et al., 2007). Typically, DNA extraction is most favorable using teeth or the diaphyses (shafts) of long bones of the skeleton, such as the femur, tibia, and fibula (Booncharoen et al., 2021).

However, in cases where the skeletal remains have been severely damaged, such as being burnt, weathered, or fragmented, the key gross anatomical skeletal features used for identification can no longer be observed, and the ideal DNA extraction conditions are lost. Certain criminal cases and natural or human-made disasters often result in these types of compromised bones. Mass disasters may result in commingled skeletal remains where multiple sets of remains have been mixed together, making disaster victim identification (DVI) difficult. Commingled, fragmented, weathered, and heat-damaged remains all present unique challenges in the field of forensic anthropology; subjective analyses in these situations are time consuming, require specialized equipment and/or a high level of expertise, which prolongs or even prevents an identification entirely (Ubelaker, 2018).

Due to these complications, researchers have focused on the development of alternative methods to obtain information from compromised remains for victim identification, but the area of forensics still lacks a standard protocol in these cases. For fragmented and commingled remains, the osteometric comparison is an objective method that utilizes statistical and computational models to separate the fragments based on the calculated size and shape relationships (Byrd et al., 2018). However, this method loses its efficacy if there is extensive damage, or if the commingled remains are of similar build, and other means of identification are necessary.

Various DNA extraction methods from skeletal tissues provide an alternative to such damage and have merit in the separation of remains, as well as general identification (Yukseloglu et al., 2019). Phenol-chloride extraction, an organic DNA extraction method, has been a common approach to DNA extraction from skeletal remains; modifications of this classic organic method resulted in other approaches to DNA extraction, such as the CTAB + Isoamyl alcohol extraction. Continuous studies in forensics have been expanding DNA extraction approaches, including those such as the total demineralization (TD) method and using silica-column based extraction, such as the QIAamp DNA Mini Kit. Many DNA isolation methods use a standard set of short tandem repeats (STRs) found in osteocyte DNA for analysis (Lantham et al., 2018). STRs support DNA assurance and integrity and are useful to make distinctions between DNA of non-related individuals. A full STR profile requires a minimum of 13 STR loci for a positive identification, but fewer than that can still contribute to a forensic investigation as a partial profile, and in combination with other evidence, can also lead to an identification. Similarly, either nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) can be obtained from skeletal remains and used in DNA analysis for identification (Schwark et al., 2011). This project

focuses on the extraction of nDNA. The analysis of STR profiles and DNA extraction are both impacted by the condition of the skeletal remains, and one of the main concerns affecting the yield of STRs and DNA are PCR inhibitors. PCR (polymerase chain reaction) is a technique used to amplify DNA sequences, creating copies of a specific section of targeted DNA for detailed study and analysis; PCR inhibitors limit, sometimes completely preventing, the DNA from being successfully amplified (Uzair et al., 2017). When this occurs, the time, materials, and sample that went into the extraction process have been wasted and the analysis is inconclusive.

While there are several methods for DNA extraction and analysis, most depend on the specific conditions of the skeletal remains. This brings forth the idea of developing a more universal method of DNA extraction from any type of skeletal remains regardless of the condition of the bones. A universal method would be applicable across a wide variety of compromised bones and could prevent wasting time, money, resources, and most importantly samples when there is limited availability. Having a universal standard of extraction to use with laid out exceptions would be a benefit to the field of forensics and worth developing. This project aims to analyze how applications, modifications, and/or combinations of chemical techniques can be incorporated into forensic anthropology to aid in identification from compromised skeletal material. This extends to identifying key factors necessary for developing such a potential universal DNA extraction method by comparing and analyzing the DNA extraction methods previously mentioned: PCE, CTAB + Isoamyl Alcohol, TD, and the QIAamp DNA Mini Kit.

In doing so, overarching challenges in the forensic sciences must also be addressed, including funding, time, and labor required for each specific method. Specifying these conditions are essential to fully understand how feasible each method is in an actual forensic investigation

and/or court trial. Similarly, the lack of national guidelines and general inorganization in the field of forensics also are potential issues that need to be taken into consideration to ensure these methods are being used to their full extent. None of the DNA extraction methods, let alone any method or process used in forensics, can be used to its full potential with these faults in the current field of forensics.

Materials and Methods

Review Methodology / Source Selection

For this literature synthesis, various search engine databases were explored to find English language sources published from 2004 to present day relevant to the topic of DNA extraction and compromised skeletal remains. This range was determined specifically to examine and compare how different DNA extraction methods have been studied and modified over time, and how they could be further applied to a universal extraction method; sources regarding the general field of forensics and its history and challenges also fell within this range. All sources originated from either peer reviewed articles in officially recognized scientific journals, books published by those with reliable credentials in the field of forensics or related study, or from manufacturer published documents.

The scope of these sources was set to include any skeletal research studies that referenced DNA extraction (PCE, CTAB + isoamyl alcohol, QIAamp, and/or TD) with a focus on aged, weathered, heat damaged, fragmented or commingled remains, as being examined in this paper. Keeping a broad scope added value to this literature synthesis as it allowed for a wide variety of factors to be investigated which is essential when looking for aspects to be addressed for a potential universal DNA extraction method. This would be harder to achieve with a narrow

scope focusing on just comparing a couple specific extraction methods or types of compromised bone. While this paper is not inclusive of all extraction methods or all possible damage to bones, the comparison drawn from this compilation of sources covers the most common and readily available methods that can be built upon when developing a potential universal method.

However, because the scope is so broad, it must be acknowledged that there are different skeletal sample selection and methodologies are used in each study, which limits the results of this literature synthesis.

DNA Extraction Techniques

As this paper is a compilation of various research studies, the exact procedures vary from study to study; while there are differences in the extraction details for each specific study, the general procedure remains constant based on the DNA extraction type being focused on. These differences extend to variation in bone sample selection and treatment prior to details of the actual extraction. Common sterilization of samples include the physical removal of soft tissue and contaminants via brushing, washing with diluted sodium hypochlorite (bleach) solutions, rinsing with distilled deionized water, and UV irradiation exposure to remove contaminants that would hinder the results (Ye et al., 2004). The bone powder necessary for extraction was obtained by some form of drilling into the bone samples. The basic procedures for each extraction type are described in brief below.

Phenol-chloroform extraction is a classic technique that extracts DNA from whole bone powder. Sodium dodecylsulfate (SDS) and proteinase K are utilized to degrade proteins and non-nucleic acid cellular components, followed the addition of a phenol:chloroform:isoamyl alcohol mixture (25:24:1) and centrifuged to isolate the double stranded DNA in an aqueous phase. This

is an organic method that utilizes the hydrophilic properties of nucleic acids found in DNA in the isolation and extraction process (Ye et al., 2004).

Total demineralization extraction, also referred to as complete demineralization or full demineralization, uses chemical compounds called chelating agents that react with any metal ion to form water-soluble complexes that bind to minerals in the bone, such as calcium and magnesium, including hydroxyapatite (Booncharoen et al., 2021). This breaks down the inorganic mineral structure of the bone and allows access to the DNA in the bone matrix. EDTA is the most common chelating agent used in this method, while EGTA and CDTA are less frequently used agents (Booncharoen et al., 2021).

The CTAB + Isoamyl Alcohol method adds a CTAB (cetyltrimethylammonium bromide) buffer solution to the bone powder in a mortar, then pestled until thoroughly mixed and left at room temperature overnight. Afterwards, the mixture is heated and vortexed for the DNA to be released from the bone tissue. It is then centrifuged, mixed with an equal volume of chloroform:isoamyl alcohol mixture, and centrifuged again (Ye et al., 2004). The resulting aqueous phase contains unpurified DNA.

The QIAamp DNA Mini Kit approach is a commercially available Silica-column based DNA extraction method. The extraction technique follows the Qiagen manufacturer's instructions; however, users are able to alter the instructions as well, although it is not recognized as an official procedure by the manufacturer (Qiagen, 2016).

Sample Grouping

Each research study compiled in this paper had their own unique set of samples, most being ethically sourced human skeletal remains, although few used porcine (pig) or bovine (cow)

skeletal remains, which are easier to access and have a similar bone composition to humans. This paper groups the types of compromised bone into three categories: heat-damaged, aged or weathered, and fragmented or commingled. Any skeletal remains that have been exposed to heat to the extent that the condition of the bones have been impacted is considered to be heat damaged remains, which can vary greatly depending on the degrees of heat damage, as seen in *Figure 1* of the Appendix (Fernández-Jalvo et al., 2018); this paper focuses on bones exposed to temperatures of 150°C or higher. Aged bones and weathered bones are grouped together and refers to any skeletal remains that were not immediately recovered and were left exposed to the natural elements. For this project, fragmented remains refer to skeletal remains broken up to the extent that the gross anatomical features or the sections of bone favorable for DNA extraction are not present or easily identifiable. Fragmented and commingled remains are often related to each other, and both occur in the aftermath of mass disasters, so they are often grouped together in this paper.

Results and Discussion

To best understand how effective and efficient each DNA extraction method is, the strengths and limitations of each individual method must be identified and compared to the other extraction techniques. Similarly, each method must be evaluated in regard to both the ideal / standard set of fresh skeletal remains before being applied to each type of compromised bone category. This allows a more thorough sample selection and analysis closer to what could be encountered when using a true universal DNA extraction technique. It also provides a more well-rounded approach to isolating the factors to be focused on or improved upon for the potential universal method.

Standard DNA Extraction Efficiency

PCE is one of the most popular techniques used in modern day forensics. This method has been found to effectively extract DNA from non-compromised remains through its removal of protein and lipid contaminants to yield isolated DNA extracts. However, this becomes limited if the remains have been exposed to natural elements for too long, or even simply buried, as the remains come in contact with hydrophilic compounds that cannot be removed by this method, as well as possible PCR inhibitors like humic acid (Marshall et al., 2014).

In addition, there are also some concerns regarding the PCE procedure itself. While it is a relatively simple procedure, it includes multiple transfer steps between different tubes, which increases the chance of cross-contamination, sample loss, and other mishandling errors. This is especially problematic when there is limited sample to work with, which is often the case for compromised remains and DVI. Another concern is that both phenol and chloroform, while easily accessible, are hazardous chemicals that require to be handled in a fume hood (Köchler et al., 2005; Marshall et al., 2014).

The total demineralization extraction technique has also been commonly used in forensic laboratories. It has been found that TD of fresh bone and teeth yielded successful DNA extraction, especially using EDTA as a chelating agent, which created a push to research this method on compromised remains as well (Duijs et al., 2020). Originally, there was uncertainty around the impact of EDTA solution pH, EDTA concentration, incubation temperature, incubation time, and volume of EDTA solution on the efficiency of the method. Later studies suggested that EDTA concentration had a significant impact on successful DNA extraction, with best results at 0.5 M EDTA, while the other factors did not have a major influence (Balayan et al.

2015; Booncharoen, 2021). There is a potential risk of over-incubation, in which the DNA will become degraded causing PCR and STR typing to fail yet results about the optimal incubation time are inconclusive. Some studies still suggest incubation time should last between 24 hour and 15 days (Hasan et al. 2014; Jakubowska et al., 2012); others still claim it does not have an impact and could be set to as low as 6 hours (Balayan et al. 2015; Booncharoen, 2021). As for the studies that suggest EDTA pH could influence DNA yield, results found that alkaline EDTA of pH 7 or greater is more effective than acidic EDTA pH below 7 (Sales et al., 2018). Since studies still are not certain whether these factors have a significant influence on DNA yield, it is safer to assume they do until further studies reach a more conclusive answer. Hence, the total demineralization extraction method appears to be optimized using 0.5 M EDTA, a mild alkaline pH 10, and an incubation time best suited for the specific study situation.

An advantage this TD technique has is that it tends to preserve the DNA by inhibiting the enzyme deoxyribonuclease (DNases), which degrade DNA by catalyzing and breaking down the phosphodiester backbone of DNA (Loreille et al. 2007). Similarly, calcium has been found to be a PCR inhibitor, so as the demineralization process removes calcium it should improve the downstream process and overall analysis (Mckinnon & Higgins., 2021). However, like PCE, demineralization processes may still coextract PCR inhibitors like humic acid if exposed to soil, so these inhibitors will have to try to be removed if present (Duijs et al., 2020).

The QIAamp DNA Mini Kit is one of many DNA kits provided by Qiagen, less commonly used in forensic investigations, but is commercially available. This technique utilizes silica columns in the extraction process to reversibly bind to DNA, isolating it from nucleotides, proteins, and salts. (Marshall, 2014). Studies have shown DNA extraction from various skeletal remains and conditions is often successful with the QIAamp approach, but Hashiyada's 2009

study showed that STR profiling becomes even more especially difficult in these situations, often being completely unsuccessful (Hashiyada et al., 2009). This suggests that the STR profiling depends on the conditions and quality of skeletal remains, though DNA extraction itself can be successful regardless.

The method is also able to handle more large-scale samples of bone powder if available and yield a higher quantity of DNA (Hashiyada et al., 2009). The silica-based column, common in many other DNA kits by Qiagen such as the QIAquick™ method, excludes many PCR inhibitors as well (Ye et al., 2004). Many of these silica-based techniques that use modern hard tissue DNA protocols do not have a demineralization step as there is generally enough sufficient DNA present that is not restricted by the hydroxyapatite (Mckinnon & Higgins, 2021). However, this is not always the case, especially when the remains are compromised. In these cases, studies have shown that adding a demineralization step to silica based techniques is beneficial and can significantly increase DNA yield (Vanek et al., 2011; Loreille et al. 2007). Qiagen has posted various user-developed protocols, not officialized or optimized by Qiagen, but are modified based on the official manufacture procedures to fit specific situations if needed (Qiagen). This suggests that the QIAamp DNA Mini Kit and other silica-based techniques are versatile and have potential for future modification and improvement.

The CTAB + Isoamyl alcohol approach is a nondestructive method that has also been shown to produce high quantities of DNA. The cetyltrimethylammonium bromide compound is a cationic detergent that effectively breaks down cell membranes and walls and leaves proteins and neutral polysaccharides in solution; this makes it ideal for DNA extraction from hard skeletal tissue (Ye et al., 2004) However, this method does not extract PCR inhibitors during its process, so a separate process has to be used in tandem with CTAB to remove the inhibitors and purify

the DNA. This is usually done by adding a silica-based purification step, such as the Qiagen QIAquick™ PCR Purification Kit, or by diluting the DNA sample as a whole, thereby diluting the PCR inhibitors as well (Ye et al., 2004). The silica-based purification is preferred as diluting the sample may not be ideal or even feasible if there are limited amounts of sample, especially in the case of compromised remains. This CTAB method has been found to yield successful STR profiles (Ye et al., 2004).

Based on extraction from a standard set of remains in good condition, PCE, TD, QIAamp, and the CTAB + Isoamyl alcohol methods are all able to successfully extract DNA in specific situations. Likewise, each method had benefits and limitations. Both TD and PCE were able to obtain DNA and partial or full STR profiles yet were unable to fully remove the PCR inhibitors. Results from the Jakubowska et al. 2012 study found that the partial inhibition was greater in TD extracts than PCE extracts, at 33% and 17% respectively (Jakubowska et al., 2012). Many studies show that higher amounts of extracted DNA were obtained from TD in comparison to PCE though (Jakubowska et al., 2012; Mckinnon & Higgins, 2021). The idea that PCE is more efficient in removing PCR inhibitors, but may yield lower of amounts of DNA recovered, while chelex methods (those that use chelating agents) such as TD are able to yield higher DNA amounts, but be less efficient in removing the PCR inhibitors is further supported in Vanek et al (Vanek et al., 2019). This is where silica-based extraction techniques, such as the QIAamp DNA Mini Kit by Qiagen, have a distinct advantage as PCR amplification is more successful since most of the PCR inhibitors are removed during the extraction process. Some reports have shown that the QIAamp DNA Mini kit and other silica-based techniques have potential in surpassing TD and/or PCE methods in part due to this advantage (Vanek et al., 2011).

As for the CTAB method, the STR obtained from this method, according to Ye et al., were much greater than the STR profiles obtained from the PCE method (Ye et al., 2004). There is limited information regarding how CTAB compares to the TD or Silica methods, which will require future testing. However, CTAB is often improved when in combination with another method, including Qiagen purification steps (Ye et al., 2004). Similarly, total demineralization combined with a silica-based DNA purification process also resulted in improved DNA yield and STR profiles (Booncharoen et al. 2021). This idea is furthered by many studies reporting that DNA yields from silica based extraction processes with an additional demineralization step were significantly higher than relied on the DNA extraction alone (Loreille et al., 2007; Vanek et al., 2011).

Application to Compromised Skeletal Remains

Aged and weathered skeletal remains encounter many issues that make DNA extraction more difficult, most notably being the increased exposure to contaminants (including PCR inhibitors) and degradation of DNA. With aged bones, TD tends to be more successful than PCE once again, providing fuller STR profiles and a significantly better DNA average yield when compared to the PCE method (Jakubowska et al., 2012). This was found to be an effective method in studies with samples ranging from less than 1 year to around 7 years, as well as samples over 20 years old (Booncharoen et al., 2021). However, it is possible that the efficacy of TD may decrease as significant increments of time pass (approximately 50+ years), as shown in Jakubowska et al., where DNA extraction from a 62-year-old sample via total demineralization was unsuccessful (Jakubowska et al., 2012). A different study on ancient bones suggested that prolonged demineralization is essential for DNA extraction as tissues are highly mineralized with

little to no DNA outside of the hydroxyapatite (Mckinnon & Higgins, 2021). This indicates that TD incubation time may actually impact the efficiency of TD if degraded remains are involved, whereas the impact of this factor was inclusive regarding extraction from remains in good condition. This data suggests that there may be a post-mortem window that is optimized for demineralization both prior to and after full mineralization of the skeletal remains, but further studies are required to be conclusive. In other words, there may be some factor, or a specific set of factors, that only hinders the demineralization process after a certain post-mortem interval, but becomes ineffective once the bones have fully mineralized. If further studies support this finding, the identification of this factor would be crucial for its elimination and advancement to a more universal technique, even if it is just a universal adaptation of the total demineralization technique.

As for DNA extraction of weathered and aged remains via QIAamp, extraction did not appear to be significantly impacted by the condition of the remains, including the postmortem interval or environmental exposure; the skeletal samples ranged from days old to a few years old, and the death scenes included buildings, bodies of water, ditches, and wooded areas, and yet DNA was successfully yielded from all these samples (Hashiyada et al., 2009). As determined previously for QIAamp extraction from ideal remains, STR profiles were still unsuccessful in these degraded remains despite also having successful rates of extraction. In comparison to the organic PCE method of degraded remains, QIAamp and other silica-based methods resulted in higher DNA yields, as seen in the extraction of ideal remains as well (Vinueza-Espinosa et al., 2019). This is likely due to the increased amount of PCR inhibitors present from environmental exposure to the skeletal remains for extended periods of time which are removed in the QIAamp process, but not the PCE. Similarly, the CTAB method is also more effective than PCE in these

situations; one study found that the CTAB produced results between five and ten times higher than the concentration obtained by using the PCE method (Ye et al., 2004). Therefore, organic methods such as PCE tend to lose their efficiency the longer the remains are exposed to the weather until the presence of PCR inhibitors and their effects have reached the maximum threshold.

As for heat damaged or burnt skeletal remains, DNA extraction and profiling is challenging as the organic matrix of the bone is destroyed in an early phase in the burning process, destroying DNA along with it. Burnt bone is believed to share similar physical changes to the bone as ancient samples do, which would suggest the TD, CTAB, and QIAamp methods as potential effective techniques (Mckinnon & Higgins, 2021). Without standard procedures for heat damaged remains, demineralization processes are only used based on when the individual forensic worker deems it necessary; this leads to inconsistent results, which can evolve into a larger issue when there are limited samples and/or there is a push to get quick results (Mckinnon & Higgins, 2021). However, demineralization has been shown to improve DNA yield of burnt bones despite having limited DNA available from extensive damage; this seems to be the effect of demineralization on all variations of heat-damaged bones (Mckinnon & Higgins, 2021).

One study found the CTAB method was also effective on damaged remains (soaked, buried, or burned), and adding this purification step after the CTAB extraction method caused improved DNA yield and successful STR profiling (Ye et al., 2004). This method is still restricted if there is a long postmortem interval or extensive damage. In such cases, silica-based purification can be applied to the CTAB method or operate as a standalone protocol itself to obtain higher quality and quantity of DNA yield (Ye et al., 2004).

Commingled or fragmented remains are commonly found in disaster scenarios, and often experience an additional type of trauma as well, whether it be burnt, weathered, or another type of damage. Regardless, the impact of the DNA extraction on these commingled or fragmented remains will depend on the other trauma present on the bones, as described previously for the aged, weathered, and heat damaged remains. For example, burnt fragmented remains should use a DNA extraction favorable for heat-damaged remains, weathered commingled remains should use an extraction method favorable for weathered remains, and so on. For commingled remains though, extraction of DNA may be used to make distinctions between multiple individuals, in which STRs play a crucial role, so the CTAB method would not be ideal.

Occasionally, fragmented skeletal remains present a unique challenge, in which the fragmented remains may not include the sections of the skeleton that are ideal for DNA extraction. This challenge stems from the difficulty in identifying which bones the fragments belong to; the difficulty to do this increases significantly if there are commingled remains involved in the case as well since there would be not only the task of identifying which type of bone it is, but also which set of remains it belongs to. In some cases, this is essentially impossible to accomplish if there are simply too many fragmented or commingled remains involved. Without being able to distinguish which fragments belong to the favorable sections of bones for DNA extraction, forensic scientists face a conundrum: is it worth it to try to extract DNA from any viable fragments with a blind hope some may produce successful results? If the number and/or identification of victims is already known (and is a relatively low number) and the DNA extraction is being performed simply for matching and confirmation purposes, then it is potentially worth it. However, if the number and identities of the victims are unknown or if the remains are severely fragmented, this can easily become an extremely costly and time consuming

process with the risk of unsuccessful results. Adding another form of trauma to the remains, such as heat-damaged or weathered, only adds on to the challenges the scientists are already facing.

These results suggest that total demineralization and the QIAamp DNA Mini Kit have merit in future universal method applications as it was found to have success across all the examined types of compromised remains. In addition, studies comparing TD to the other extraction types, TD often produced higher DNA yields and STR profiles (Jakubowska et al., 2021). Even though the DNA yield and STR profiles obtained via the QIAamp DNA Mini Kit were not always as high as those from TD, it was the only method that resulted in significant PCR inhibitor removal desirable in an ideal extraction method. This is due to its silica-based extraction properties, which would be beneficial to incorporate in a universal method as well. The PCR and the CTAB methods, although successful under certain conditions, faced significant restrictions depending on the compromised conditions of the remains and thus are not as ideal for a universal method.

Forensic Applicability

None of these extractions matter if they cannot feasibly be applied in an actual forensic investigation. Once again, ease and access to the method and its materials must be taken into consideration, along with the costs and labor required for the extraction method. PCE, despite being straightforward and cost effective, still uses health hazardous chemicals and is labor heavy with multiple sample handling transfer steps that should be avoided if possible (Marshall et al., 2014). Although this PCE method works for fresh remains in good conditions, it is limited if degradation is present, and yet it is often still used, partially out of reluctance to change from the way things have always been done (Airlie et al., 2021). This reluctance has been seen in the

study of forensics as a whole and needs to be addressed in order to keep developing objective methods that can be holistic, multidisciplinary, and collaborative (Airlie et al., 2021).

Cost can also be an issue in laboratories. The QIAamp DNA Mini Kit is commercially available and relatively inexpensive at approximately \$200 for 50 preparation kits (Qiagen), making it easily accessible and cost efficient. This can quickly amount to great expenses, though, in cases of mass disasters where the vast amount of skeletal remains may end up being cost prohibitive. In comparison, everything needed for the TD and CTAB methods are less accessible and cost efficient. This is where funding, governmental or public, can make a significant difference. Programs such as The Research and Evaluation for the Testing and Interpretation of Physical Evidence in Publicly Funded Forensic Laboratories (Public Labs) program directs projects to fund research for development of efficient, accurate, reliable, and cost effective methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes (National Institute of Justice, 2019), which would apply to these DNA extraction methods from skeletal remains. While this specific program requires the applicants to be publicly funded forensic laboratories at any level and only provides a relatively small amount of funding, the concept behind it would greatly benefit the field of forensics as it continues to grow, especially with sufficient funding for both the governmental and public funded laboratories. In addition, since the results obtained from extraction may be used as evidence in criminal cases, potentially even requiring a forensic scientist to be an expert witness in court, it is important to maintain consistency when carrying out extraction procedures. This is where the field of forensics would greatly benefit by establishing clear DNA extraction protocols for the common types of extraction. Established national protocols or guidelines would ensure the DNA

extractions are all held to the same requirements and expectations in all laboratories and forensic investigations.

Another variable to consider is the time length it takes to complete the entire DNA extraction process. The DNA extraction itself is one of many factors that contribute to the overall process and analysis; pre-extraction sample preparation and the post-extraction PCR and analysis are other factors, but the extraction itself has the most variability and greatly impacts how long the complete procedure takes. Extraction of DNA from skeletal remains in general is a time consuming and laborious process that takes hours or even days to complete, especially if long incubation periods are recommended such as occasionally in TD (Hasan et al. 2014; Duijs et al., 2020). This becomes even more problematic if mass disasters are involved or if there is a time constraint in a criminal case. It is not uncommon for law enforcement to put pressure on forensic scientists for quick results if their data and analysis is involved in a criminal case (Jeanguenat & Dror, 2017). In these cases where this forensic analysis will be impacting the lives of a person and the outcome in court, it is critical to perform the extraction process with as little human error as possible. If scientists try to speed up the process by using a more time-conservative process rather than the most appropriate one in order to obtain results quickly due to the time pressure, there is a chance for more errors to occur and the quality of the results can be hindered. Consequently, research into automated DNA extraction has been gaining popularity as this has the potential to accelerate the DNA extraction and profiling process, while providing more time to focus on other aspects in the lab and minimizing the chance of subjective bias and human error (Duijs et al., 2020). The development of automated or semi-automated processes would be beneficial for the entire field of forensics, not just for DNA extraction from skeletal remains.

Conclusion

As determined in this literature synthesis, the main factors that influence efficiency of a DNA extraction technique for forensic investigations include DNA yield, PCR inhibition, STR profiling, and the ease and accessibility of the materials and model. This means an ideal DNA extraction should be optimized to have a high DNA yield, removal of contaminants and inhibitors for successful DNA isolation and amplification, use of non-hazardous chemicals, and minimal transfer steps to minimize contamination or loss of sample / DNA. These are essential factors to focus on when developing potential universal DNA extraction models. There has already been much success seen with combining pre-existing DNA extraction techniques, as seen with the demineralization and silica-based purification steps being added onto other methods to increase efficiency. Out of the methods discussed in this paper, total demineralization and the QIAamp DNA Mini Kit show the most promise for future applications in a universal model. Of course, there are many other extraction techniques, as well as types of compromised skeletal remains not mentioned in this paper, so in order for the method to truly be considered as universal as possible, future studies should be conducted to analyze the key factors under those conditions, and the universal method would be adjusted as needed. Laboratory research should also be conducted to confirm these findings, preferably using the same pre-extraction conditions that were not able to be done via a literature synthesis.

The idea of a universal DNA extraction method in the future of forensics, although convenient, is not a necessity. Using individual extraction methods based on the conditions of the remains can still be effective as long as there is adequate funding, clear established national protocols, and continuous training to obtain the best results. With or without a universal method,

if those three conditions can be met for each individual DNA extraction type, the field of forensic anthropology as a whole would become more secure.

Acknowledgements

I would like to thank Oakland University's Chemistry and Anthropology departments, as well as the Honors College, for providing inspiration and assistance throughout the project, with special thanks to Professor Holly Yatros for their mentorship and guidance.

References

- Airlie, M., Robertson, J., Krosch, M. N., & Brooks, E. (2021). Contemporary issues in forensic science—Worldwide survey results. *Forensic Science International*, 320, <https://doi.org/10.1016/j.forsciint.2021.110704>.
- Balayan, A., Kapoor, A., Chaudhary, G., & Raina, A. (2014). Evaluation of techniques for human bone decalcification and amplification using sixteen STR markers. *Egyptian Journal of Forensic Sciences*, 5(1), 30-35. <https://doi.org/10.1016/j.ejfs.2014.05.002>.
- Booncharoen, P., Khacha-ananda, S., Kanchai, C. et al (2021). Factors influencing DNA extraction from human skeletal remains: bone characteristic and total demineralization process. *Egyptian Journal of Forensic Science*, 11 (1). <https://doi.org/10.1186/s41935-021-00216-8>.
- Byrd, J. E., & LeGarde, C. B. (2018). Evaluation of method performance for osteometric sorting of commingled human remains. *Forensic Sciences Research*, 3(4), 343–349. <https://dx.doi.org/10.1080%2F20961790.2018.1535762>.
- Duijs, F. E., & Sijen, T. (2020). A rapid and efficient method for DNA extraction from bone powder, *Forensic Science International: Reports*, 2. <https://doi.org/10.1016/j.fsir.2020.100099>.
- Fernández-Jalvo, Y., Tormo, L., Andrews P., & Marin-Monfort D.M. (2018). Taphonomy of burnt bones from Wonderwerk Cave (South Africa). *Quaternary International*, 495 (1), 19-29.
- Hashiyada, M., Nakayashiki, N., & Funayama, M. (2009). Utility validation of extraction of

- genomic DNA from hard tissues, bone and nail, using PrepFiler™ Forensic DNA Extraction Kit. *Forensic Science International: Genetics Supplement Series*, 2(1), 172-3. <https://doi.org/10.1016/j.fsigss.2009.08.042>.
- Jakubowska, J., Maciejewska, A., & Pawłowski, R. (2012). Comparison of three methods of DNA extraction from human bones with different degrees of degradation. *International Journal of Legal Medicine*, 126(1), 173–178. <https://dx.doi.org/10.1007%2Fs00414-011-0590-5>.
- Jeanguenat, A.M. & Dror, I.E. (2017). Human Factors Effecting Forensic Decision Making: Workplace Stress and Well-being. *Journal of Forensic Sciences*, 63(1), 258-261. <https://doi.org/10.1111/1556-4029.13533>.
- Köchler, S., Niederstätter, H., & Parson, W. (2005). DNA Extraction and Quantitation of Forensic Samples Using the Phenol–Chloroform Method and Real-Time PCR. *Methods in molecular biology* (Clifton, N.J.). 297. 13-30. <https://doi.org/10.1385/1-59259-867-6:013>.
- Latham, K. E., & Miller, J. J. (2018). DNA recovery and analysis from skeletal material in modern forensic contexts. *Forensic Sciences Research*, 4(1), 51–59.
- Loreille, O., Diegoli, T., Irwin, Jodi., Coble, M., & Parsons, T. (2007). High efficiency DNA extraction from bone by total demineralization. *Forensic Science international: Genetics*. 1(2). 191-5. <https://doi.org/10.1016/j.fsigen.2007.02.006>.
- Marshall, P. L., Stoljarova, M., Schmedes, S. E., King, J. L., & Budowle, B. (2014). A high volume extraction and purification method for recovering DNA from human bone, *Forensic Science International: Genetics*, 12, 155-160. <https://doi.org/10.1016/j.fsigen.2014.06.011>.
- Mckinnon, M. & Higgins, D. (2021) Comparison of bone demineralisation procedures for DNA

recovery from burned remains, *Forensic Science International: Genetics*, 51,

<https://doi.org/10.1016/j.fsigen.2020.102448>

National Institute of Justice. (2019). *Research and Evaluation in Publicly Funded Forensic Laboratories*. <https://nij.ojp.gov/topics/forensics/research-and-evaluation-publicly-funded-forensic-laboratories#2yh0w>.

QIAGEN®. (2016). QIAamp® DNA Mini and Blood Mini Handbook.

Sales, E., da Silva C.E.R., Letichevsky, S., dos Santos, R., Leitao R., dos Santos, C.T., de Oliveira, L.F., de Avillez, R., Monteiro, M., Costa-Felix, R., Paciornik, S., & dos Anjos., M. (2018). Chemical induced demineralization study in cortical bone. *Journal of Instrumentation*, 13. <http://dx.doi.org/10.1088/1748-0221/13/05/C05010>.

Schwark T, Heinrich A, Preusse-Prange A, & von Wurmb-Schwark N. (2011). Reliable genetic identification of burnt human remains. *Forensic Science International: Genetics*, 5(5), 393-9. <https://doi.org/10.1016/j.fsigen.2010.08.008>.

Ubelaker, D. H. (2018). Recent advances in forensic anthropology. *Forensic Sciences Research*, 3(4), 275–277. <https://dx.doi.org/10.1080%2F20961790.2018.1466384>.

Uzair, A., Rasool, N., & Wasim, M. (2017). Evaluation of different methods for DNA extraction from human burnt bones and the generation of genetic profiles for identification. *Medicine, Science and the Law*, 57(4), 159-166. <https://doi.org/10.1177/0025802417723808>.

Vanek, D., Silerova, M., Urbanova, V., Saskova, L., Dubska, J., & Beran, M. (2011). Genomic DNA extraction protocols for bone samples: The comparison of Qiagen and Zymo Research spin columns. *Forensic Science International: Genetics Supplement Series*, 3(1), e397 - e398. <http://dx.doi.org/10.1016/j.fsigss.2011.09.060>.

Ye, J., Ji, A., Parra, E. J., Zheng, X., Jiang, C., Zhao, X., Hu, L., & Tu, Z. (2004). A Simple and Efficient Method for Extracting DNA From Old and Burned Bone. *Journal of Forensic Sciences*, 49(4), 754-759. <https://doi.org/10.1520/JFS2003275>.

Yukseloglu, E., Dastan, K., Yonar, F., Rayimoglu, G., Karatas, O., Islek, D., & Dogan, M. (2019). The comparison of DNA extraction techniques in human bone and tooth samples exposed to high heat. *Medicine Science / International Medical Journal*, 8(3), 489-95. <https://dx.doi.org/10.5455/medscience.2019.08.9051>.

Appendix

Figure 1



Figure 1 displays the range of discoloration on skeletal remains from different degrees of heat, with sample 1 being slightly burnt, and increasing the temperature all the way up to sample 5 with the calcination of bone (Fernández-Jalvo et al., 2018).