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ORIGINAL ARTICLE

# A Scoping Review on DNA Extraction Techniques in Hard Tissues

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## ABSTRACT

***Introduction:*** Forensic investigations involving skeletal remains often encounter challenges related to the quality and quantity of extracted DNA. These challenges are influenced by factors such as the postmortem interval, environmental conditions, and the rate of DNA degradation. As the demand for accurate identification in forensic cases grows, researchers and forensic scientists are working to improve the efficiency of DNA extraction methods from skeletal remains to address cases involving unidentified bodies. The goal of this systematic review was to explore and evaluate DNA extraction techniques that are most effective for forensic DNA profiling from hard tissue samples.

***Method:*** The review adhered to PRISMA guidelines for its search strategy, which was implemented using the ScienceDirect database. Initially, 5,526 research articles were identified, and 20 duplicates were removed using CADIMA software. After applying the inclusion and exclusion criteria and screening titles and abstracts, 5,397 articles were excluded. Ultimately, 24 articles were included in the full-text analysis.

***Conclusion:*** This review provides a comprehensive comparison of DNA extraction methods, valuable insights into selecting the most suitable technique for specific forensic applications. It also highlights the importance of standardizing protocols to ensure consistent, reproducible, and high-quality results across different scientific fields.

## KEYWORDS

• DNA Extraction Techniques • Systematic Review • STR Typing • Teeth • Bones

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## INTRODUCTION

Bones are tough tissues in the vertebrate skeleton that have primary roles in providing support, protection, and facilitating movement as levers. The tissue is primarily made up of collagen fibers and mineralized components, which give it its strength and rigidity. Bones are valuable in forensic science due to their durability and ability to store DNA, which can remain intact for extended periods even in adverse environments (Pajnič *et al.* 2016, Caldeira *et al.* 2019, Golob *et al.* 2024). Investigations into skeletal remains frequently encounter challenges with the quality and quantity of DNA extracted, influenced by factors like postmortem interval, environmental conditions, and the rate of DNA deterioration. Nevertheless, these issues do not impede the advancement of obtaining high-quality DNA from these tissues, and other molecular methods have progressed in this area (Chocholova *et al.* 2023, Rancourt *et al.* 2023).

Different techniques have been created to extract DNA from bone. Organic extraction, demineralization, and automated magnetic bead systems are available. This research assesses a new procedure for extracting DNA from bone samples with the EZ2 Connect machine and measures its effectiveness against the standard manual technique (Sutlovic *et al.* 2015). The utilization of modern and traditional organic techniques in extracting DNA from bone fragments has greatly improved the efficiency of recovering DNA from skeletal remains (Vinueza-Espinosa *et al.* 2019, Doniec *et al.* 2024). Automated DNA extraction systems, such as the AutoMate Express, can greatly speed up the process and reduce time, even when working with fully demineralized samples (Pajnič *et al.* 2016). These systems are particularly useful in forensic cases involving skeletonized or decomposed remains (Hazen *et al.* 2013).

In times of catastrophic disasters, damaged bones and teeth are often the only available materials for DNA extraction. The combination of PrepFiler Express BTA and AutoMate Express system can efficiently extract DNA from ancient and modern bones. This enables adjusting elution volumes according to preference, potentially enhancing DNA yield from low-yielding samples. This research investigates how varying elution volumes affect DNA extraction in difficult samples (Caldeira *et al.* 2019). A study found adult permanent teeth yield the highest DNA, while non-adult and deciduous teeth have lower preservation rates. This highlights the importance of using adult permanent teeth for DNA analysis in forensic and archaeological contexts (Leskovar & Pajnič, 2023). Identifying human remains often relies on bones and teeth in advanced decay, which are challenging for DNA extraction due to their mineral content. To address this, the tissues are usually pulverized and processed with lysis buffers containing proteinase K and EDTA to break down the organic structure and remove minerals (Rucinski *et al.* 2012). In conclusion, bones play a crucial role in forensic science due to their durability and ability to store DNA, even in harsh environments. Despite challenges in DNA extraction from skeletal remains, advancements in extraction techniques, such as organic extraction, demineralization, and automated systems, have improved the efficiency and quality of DNA recovery. Automated DNA extraction systems, like the AutoMate Express, offer faster processing, especially for demineralized samples. The study aims to conduct a thorough systematic literature review on DNA extraction methods from hard tissues like bone and teeth.

## METHOD

### Search strategy

The study protocol was conducted in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement of 2020 (Page *et al.* 2021). The search utilized the PICO framework to create search strings, focusing on the following terms:

- Population (P): Bone, teeth, skeleton, osteocytes, or hard tissues
- Intervention (I): Magnetic bead extraction, resin extraction, solid phase, organic extraction, phenol chloroform, automated DNA extraction, or rapid HIT
- Outcome (O): STR typing or microsatellites

The Boolean search string based on the provided information: (Bone OR teeth OR skeleton OR osteocytes OR "hard tissues") AND ("Magnetic bead extraction" OR "resin extraction" OR "solid phase" OR "organic

extraction" OR "phenol chloroform" OR "automated DNA extraction" OR "rapid HIT") AND ("STR typing" OR microsatellites). The research article search was conducted on ScienceDirect (*https://www.sciencedirect.com/*, n.d.). Science Direct is an online platform providing access to a vast collection of scientific research, journals, and books, in the field of science, technology, and medicine. This review includes research articles published between 2014 and 2025 in English, focusing on DNA extraction from hard tissues like bone and teeth. Excluded are review articles, conference proceedings, and case reports.

The systematic review was conducted in CADIMA (*CADIMA*, n.d.), a free web tool facilitating the conduct and assuring for the documentation of systematic reviews, systematic maps and further literature reviews. The identification step of the process involved records (n=5,526) identified from databases such as Science Direct. During the screening phase, duplicate records were removed (n= 20). In the screening step, records (n= 5,397) were excluded after reviewing titles and abstracts. This led to (n=49) reports being sought for retrieval. Upon full-text assessment, (n=25) reports were excluded based on P & O criteria (refers to the Population, and Outcome criteria in the PICO framework (**summarized in Figure 1**). As a result, (n= 24) studies were included in the review.

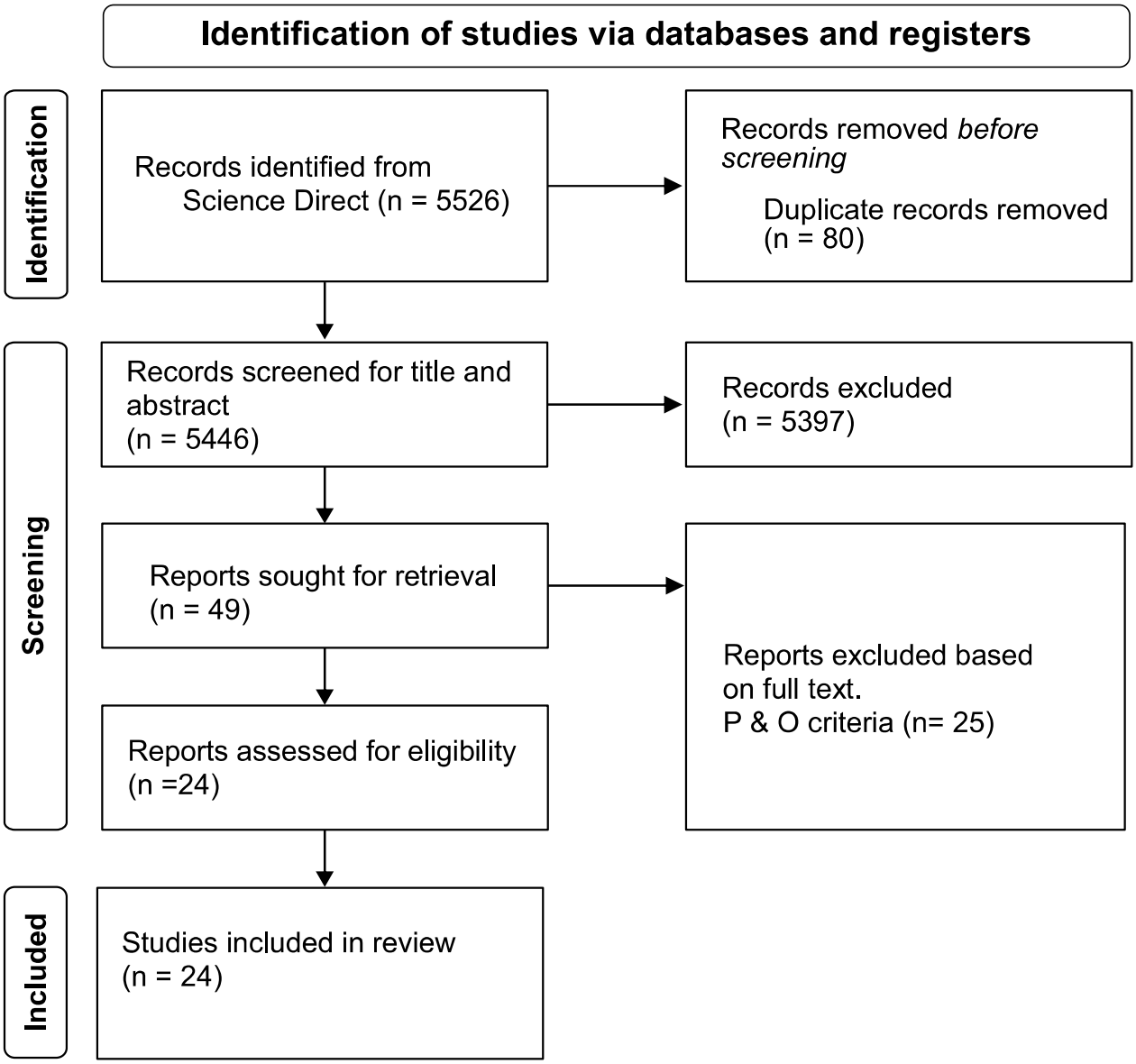


**Figure 1:** Prisma flow diagram summarizing the number of records identified, removed and retrieved for the systematic review

## RESULT AND DISCUSSION

DNA extraction from bone samples is influenced by the choice of extraction method, sample preparation, and the condition of the skeletal remains (Table 1). Phenol: Chloroform Organic Extraction has been widely used due to its ability to recover high DNA yields, particularly in degraded samples (Marshall *et al.* 2014, Calacal *et al.* 2021, Kuś *et al.* 2016, Haarkötter *et al.* 2023). In Marshall *et al.* (2014), human bone samples were cleaned with bleach, ethanol, and air-dried before being pulverized using a Freezer/Mill. Phenol-Chloroform extraction recovered an average of 103.9 ng of DNA, identifying up to 15 STR alleles. Similarly, Kuś et al. (2016) compared three methods on bone fragments from forensic cases and WWII remains, finding that Phenol-Chloroform yielded the highest DNA recovery and allele detection compared to the PrepFiler® kit and QIAamp® DNA Investigator Kit, which performed the worst. Studies on burned bone samples by Zgonjanin et al., 2015 also confirmed that Phenol-Chloroform extraction provided measurable DNA (0.69–12.11 ng/µL), with better STR recovery using AmpF'STR® Identifiler and Yfiler kits. Iyavoo and Goodwin (2017) found that non-decalcified bones (fresh to 1 year old) yielded higher DNA (92.38 ng/µl) than decalcified bones (54.38 ng/µl). Sahib Zar et al. (2015) analyzed 27 degraded DNA samples from human bones aged 100 to 1000 years. The samples were cleaned, treated, and macerated before DNA extraction using a silica-column-based method. Both AmpFlSTR Identifiler and MiniFiler kits successfully typed DNA, with the MiniFiler kit providing more informative profiles and recovering alleles missed by Identifiler.

Silica-based column extractions, including Hi-Flow®, ChargeSwitch®, DNA IQ™, and DNeasy®, have also been employed, particularly for well-preserved remains. In Marshall *et al.* (2014), Hi-Flow® silica-column extraction produced an average DNA yield of 111.8 ng, slightly higher than the organic method (103.9 ng), with STR allele detection of up to 16 alleles. Iyavoo & Goodwin (2022) tested ChargeSwitch®, DNA IQ™, and DNeasy® on pig bones as a forensic model, showing that Phenol-Chloroform extraction outperformed silica-based methods, but all methods successfully amplified STRs with fragment sizes of 70–384 bp and no inhibition. In Vinueza-Espinosa et al. (2019), ancient human bones from the 5th–11th century were processed using Non-Column Silica-Based (NCSi), Silica-HE Spin Columns (SiHEC), and Phenol-Chloroform. The NCSi and SiHEC methods provided the highest mtDNA yields (15.72 ng/µL and 14.32 ng/µL, respectively), whereas Phenol-Chloroform yielded the lowest (1.40 ng/µL).

To enhance DNA recovery, some studies implemented demineralization (EDTA treatment) before extraction. Pajnič *et al.* (2016) analyzed 49 skeletons, treating bone samples with 0.5M EDTA before using the PrepFiler™ BTA Kit. This approach significantly improved allele recovery (64%-71%) compared to non-demineralized samples (34%-58%), particularly in older remains. Similarly, Desmyter *et al.* (2017) tested Phenol + M16 and M16 alone on bones exposed to environmental conditions. The Phenol + M16 method resulted in significantly higher STR recovery (88%-100% for samples submerged in seawater for 16 years, 57%-100% for buried samples, and 71%-100% for air-exposed remains), whereas the M16 method alone showed poor DNA recovery (<3.2 pg/µL and low STR confirmation rates of 0%-9%). Chong *et al.* (2023) also demonstrated that demineralization followed by PrepFiler Express BTA extraction resulted in higher DNA concentrations (355.7 ng for 50 mg of bone powder) compared to the AutoMate Express™ method (300.8 ng for 50 mg), although both produced complete STR profiles.

In forensic applications, PrepFiler® BTA Kit is a preferred method due to its high STR recovery. Hasap et al. (2019) extracted DNA from fresh tibia and forensic casework bones using PrepFiler® BTA and a Modified PCI-Silica Method. Fresh samples had significantly higher DNA yields with PrepFiler® (59.5 ng/µL) compared to PCI-Silica (0.620 ng/µL), and forensic casework samples showed a similar trend (0.230 ng/µL vs. 0.010 ng/µL).

Rapid extraction methods have been tested as an alternative to traditional protocols. Phua *et al.* (2019) developed a Rapid Extraction Method using SLS, EDTA, Proteinase K, and DTT incubation for 2 hours at 56°C, which was compared to a Total Demineralization (TD) protocol. Although the TD method produced higher DNA concentrations (66.33

ng/µL for tibia vs. 54.59 ng/µL using the rapid method), the new approach offered a faster workflow while still recovering amplifiable DNA. Di Stefano B et al. (2024) used a manual decalcification process with Na2EDTA and Maxwell® FSC DNA IQ™ for extraction. They found petrous bone yielded the highest DNA (440 pg/µL, 100% STR success), while the right femur (5.9 pg/µL, 62.5% STR success) and left femur (1.2 pg/µL, 28.5% STR success) showed lower yields. Metacarpal and tooth had moderate to low DNA and STR success. Zar et al. (2013) analyzed 24 human skeletal remains (200-500 years old) from mass graves in Pakistan. The bones were cleaned, fragmented, and stored at -20°C before DNA extraction using the QIAamp Blood Maxi column and QIAvac system. Real-time PCR detected DNA in 17 samples, with 7 samples showing no DNA due to degradation. Most degraded samples had <10 pg/µL, while others ranged from 1-69 pg/µL. Femur (171 pg/µL) and tibia (117.5 pg/µL) had the highest yields, while radius, metacarpal, and fibula had lower or undetectable DNA. Ramírez *et al.* (2023) applied manual scraping, UV exposure, and pulverization with a Dremel drill, followed by extraction using organic solvents, a commercial kit, and demineralization.

The effect of environmental damage on DNA extraction has also been explored. DNA analysis in burnt bones is challenging due to degradation at high temperatures. Studies show amplification failure at temperatures as low as 200°C, though shorter DNA targets are more resistant. Some success was achieved using specialized PCR systems, but results are inconsistent due to uneven burn damage (Imaizumi, 2015). Charred bone studies (Grela et al., 2021) found that nuclear DNA (nDNA) concentration decreased significantly with increased burning duration. QIAamp® had the highest nDNA yield in bone at 15 minutes of burning (31.01 ng/µL), while PrepFiler® peaked at 29.80 ng/µL. Phenol-Chloroform extraction recovered DNA only up to 5 minutes of burning, after which it failed. In cases where bones were buried for decades, Duijs & Sijen (2020) showed that Maxwell FSC DNA IQ extraction yielded 2.85–25.00 ng DNA/g, while full demineralization yielded lower amounts (0.30–1.74 ng DNA/g).

Overall, Phenol: Chloroform remains the most effective method for high DNA yield, especially in degraded samples, but automated methods like Maxwell® and AutoMate Express™ provide improved efficiency and contamination control. Demineralization significantly enhances STR recovery, particularly in aged and buried samples, while PrepFiler® BTA is a preferred forensic method due to its high-quality DNA recovery. Sample condition plays a crucial role, with petrous and femur bones yielding the highest DNA concentrations, whereas smaller bones, ribs, and charred remains generally provide lower yields.

**Table 1:** Summarize the key points of the literatures included in the study

| Author and year | Sample & sample size | Pretreatment | DNA extraction method type | Extraction technique | Results and STR Loci identified |
|---|---|---|---|---|---|
| Marshall *et al.* 2014 [15] | 10 human bone samples (anonymized) | The bones were cleaned with 50% bleach, rinsed, soaked in ethanol, air-dried, and pulverized in a Freezer/Mill before being pooled for extraction. | Manual | 1. Hi-Flow® silica-column extraction<br>2. Phenol: Chloroform Organic Extraction | DNA Concentration & Allele Detection (PowerPlex® ESI 17 Pro System):<br>• Hi-Flow® extraction: 111.8 ng (0.79–900.28 ng), 16 alleles (3–29)<br>• Organic extraction: 103.9 ng (0.54–854.67 ng), 15 alleles (1–29) |
| Calacal *et al.* 2021 [16] | 16 femur samples: 5 from untreated cadavers, 7 from embalmed remains, and 4 from disaster victims. Blood samples collected within 9 days postmortem. | Femur samples were cleaned, sonicated in 5% Terg-a-Zyme®, washed, dried, and pulverized in a cryogenic grinder. Blood samples on FTA™ cards were processed within 1–7 days post-collection. | Manual/ Automated | 1. Phenol: Chloroform Organic Extraction<br>2. DNA IQ™ (manual workflow)<br>3. Maxwell® 16 LEV (automated workflow) | More bone powder increased DNA concentration, but yield per mass remained stable. PowerPlex® Fusion System (Promega) system Allele recovery was consistent across different weights (87–90%). |

***table cont...***

| Author and year | Sample & sample size | Pretreatment | DNA extraction method type | Extraction technique | Results and STR Loci identified |
|---|---|---|---|---|---|
| Zgonjanin *et al*; 2015 [17] | 5 DNA extracts from femur samples of burned body | Bones cleaned, washed, air-dried, and then ground into a fine powder using an MM 301 mill. | Manual | 1. Phenol: Chloroform Organic Extraction | The DNA quantity ranged from 0.69 to 12.11 ng/µl. The efficiency of STR typing varied as follows:<br>• AmpF'STR® Identifiler: 10–16/16<br>• AmpF'STR® NGMTM: 13–16/16<br>• AmpF'STR® Yfiler®: 17/17, but only for a few samples. |
| Kuś et al. 2016 [18] | Bone fragments were collected from autopsies, criminal cases, and exhumed World War II soldier remains. | Bone samples cut 2x2, cleaned with a surgical blade, washed, UV-irradiated, and then ground using a Freezer Mill. | Manual/ Automated | 1. Phenol: Chloroform Organic Extraction<br>2. PrepFiler® Forensic DNA Extraction Kit<br>3. QIAamp®DNA Investigator Kit | • DNA Extraction Efficiency: Organic (phenol/chloroform) yielded the highest DNA recovery, followed by PrepFiler®, while QIAamp® performed the worst.<br>• Older Case Samples: Organic extraction remained the most effective, with PrepFiler® showing moderate efficiency.<br>• Allele Recovery: The organic method identified the most alleles, PrepFiler® detected fewer, and QIAamp® had the lowest allele recovery. |
| Pajnič *et al*. 2016 [1] | 49 skeletons (25 from World War II and 24 from contemporary forensic cases) | Bone samples were cleaned with a rotary tool and treated with detergent, water, and ethanol. Tooth samples were similarly cleaned and irradiated with UV light. | Manual | PrepFiler™ BTA Kit: DNA extracted using 0.5M EDTA (Promega) for decalcification or demineralization. | Demineralization improved allele recovery (64%-71%) over non-demineralization methods (34%-58%) across aged and contemporary samples using STR kits (Identifiler, PowerPlex 16, ESX 17, Essplex Plus, NGM). |
| Desmyter *et al.* 2017 [19] | 28 bone fragments and teeth samples | - Bone fragments were cleaned, decontaminated, and cut into 0.3–2 g pieces. After a bleach rinse, they were decalcified in 0.5M EDTA at 56°C for three | Manual | Phenol + M16:<br>• Decalcified in 0.5 M EDTA, then degraded with Proteinase K in lysis buffer A.<br>• Organic extraction followed by volume reduction with an Amicon filter.<br>M16:<br>• Similar decalcification and degradation as Phenol + M16.<br>• Direct volume reduction using an Amicon filter. | The results show differences in DNA concentration and STR profile (ESIPro1 and ESX1 Kits (Promega) kits recovery.<br>• Sea Water (16 years): The Phenol + *M16* method yielded high STR recovery (88%-100%) with DNA concentrations up to 12 pg/ml, while the *M16* method showed poor recovery (<3.2 pg/ml, 0%-9% allele confirmation).<br>• Buried Samples (1–30+ years): The Phenol + *M16* method provided high STR profile recovery (57%-100%), with DNA concentrations ranging from <2 to 170 pg/ml. In contrast, the *M16* method resulted in low or no STR recovery (<3.2 pg/ml, 0%-12%).<br>• Air (Forest/Room Temperature, 5–15 years): Phenol + *M16* yielded high STR confirmation (71%-100%), but DNA concentration remained low (<2 pg/ml in some cases). The M16 method showed lower allele recovery (0%-74%).<br>• Water Exposure (0.5 years): High DNA concentrations (41-559 pg/ml) were observed with *Phenol + M16*, leading to 100% STR recovery. The *M16* method was not tested for these samples. |

***table cont...***

| Author and year | Sample & sample size | Pretreatment | DNA extraction method type | Extraction technique | Results and STR Loci identified |
|---|---|---|---|---|---|
| Iyavoo & Goodwi 2022 [20] | Pig bone samples (femur and rib) were used as a model for human bones, recovered from animals exposed for 0, 3, and 12 months at the TRACES facility (UK | Soft tissue, marrow, and algae were removed, then bones were soaked in 5% sodium hypochlorite, rinsed, and air-dried. Bone portions weighing 1–2 g were pulverized using liquid nitrogen. | Manual | 1. ChargeSwitch® gDNA Plant Kit (Thermo Fisher Scientific)<br>2. DNA IQ™ System Kit (Promega)<br>3. DNeasy® Blood & Tissue Kit (Qiagen)<br>4. PrepFiler® BTA Forensic DNA Extraction Kit (Thermo Fisher Scientific)<br>5. Phenol: Chloroform Organic Extraction | • The phenol-chloroform-isoamyl alcohol method yielded the highest DNA amounts, followed by ChargeSwitch® gDNA Plant Kit, DNeasy® Blood & Tissue Kit, PrepFiler® BTA Forensic DNA Extraction Kit, and DNA IQ™ System Kit. Results for the 0-month and 3-month timepoints were similar.<br>• Amplification was successful for all extracts, with fragment sizes ranging from 70 bp to 384 bp, and no inhibition was observed. |
| Zar *et al.* 2013 [21] | 24 human skeletal remains, aged 200-500 years, were collected from mass graves in Pakistan | The bone samples were fragmented, cleaned with UV light, 10% bleach, and 95% ethanol, then grounded using a SPEX 6750 Freezer/ Miland stored at -20°C for DNA extraction. | Automatic | DNA was extracted using the QIAamp Blood Maxi column and QIAvac system (Qiagen, Germany). | Real-time PCR showed DNA in 17 samples, with 7 samples showing no DNA, likely due to degradation. Most degraded samples had <10 pg/µL, while some ranged from 1-69 pg/µL, and a few had >100 pg/µL<br>Femur (171 pg/µL) and tibia (117.5 pg/µL) had the highest DNA yield, followed by humerus, skull, and ulna (~104–112.5 pg/µL). Radius, metacarpal, and fibula showed lower yields (≤22.5 pg/µL), with some samples undetectable. |
| Hong *et al.* 2017 [22] | 70 skeletal samples | Skeletal surfaces were cleaned with dental tools to remove contamination, then demineralized in 0.5 M EDTA at room temperature for two weeks. | Manual/ Automated | 1. PerkinElmer Chemagic MSM I automated system<br>2. Phenol: Chloroform Organic Extraction | • Organic method: 0.63 ng DNA per gram of bone<br>• PerkinElmer Chemagic MSM I method: 0.65 ng DNA per gram of bone<br>• Automated methods showed slightly better autosomal STR genotyping (PowerPlex® Fusion System) success than organic methods.<br>• For Y chromosomal STR genotyping, automated methods performed better than organic methods. |
| Caldeira *et al.* 2019 [2] | 11 bone samples and one tooth sample | Samples were cleaned and processed using mechanical and chemical methods. Samples were then frozen in liquid nitrogen using a SPEX Sample Prep Freezer/Mill 6770 | Automatic | DNA extraction used the PrepFiler Express BTA™ kit on the AutoMate Express™ system, with a standard protocol and a modified version incorporating triple reagents. | The modified protocol resulted in higher DNA recovery compared to the normal method. |
| Vinueza-Espinosa *et al;* 2019 [6] | Five individuals from the 5th–11th century Casseres site (Catalonia, Spain) were sampled, collecting petrous bone, tooth pulp and cementum, rib, and two upper limb bones (radius, ulna, metacarpal, or phalange) per individual. | Samples were cleaned with a dental instrument, yielding 120–800 mg of bone powder, with 30–200 mg used for DNA extraction. | Manual | 1. Non-Columns Silica-Based (NCSi).<br>2. Silica-HE Spin Columns-Based (SiHEC)<br>3. Silica-XS Spin Columns-Based (SiXSC)<br>4. Phenol-Chloroform (P-Chl) | • Highest mtDNA Yield: NCSi (15.72 ng/µl) and SiHEC (14.32 ng/µl).<br>• Best Bone Sources: Petrous (18.12 ng/µl) and pulp cavity (12.80 ng/µl).<br>• Lowest & No Amplification: Rib (2.29 ng/µl) and Phenol-Chloroform (1.40 ng/µl).<br>• Sequencing Success: High-quality sequences obtained from NCSi and SiHEC. |

***table cont...***

| Author and year | Sample & sample size | Pretreatment | DNA extraction method type | Extraction technique | Results and STR Loci identified |
|---|---|---|---|---|---|
| Hasap *et al.* 2019 [23] | 5 fresh tibia and 18 casework bones were obtained from Prince of Songkla University. | - | Automated/ Manual | 1. PrepFiler® BTA Kit<br>2. Modified PCI-Silica Method | • Fresh Samples: Median DNA concentration was 59.5 ng/µl (PrepFiler® BTA) vs. 0.620 ng/µl (modified PCI-silica).<br>• STR Typing (Casework): 30 alleles (PrepFiler® BTA) vs. 8 alleles (modified PCI-silica). |
| Phua *et al.* 2019 [24] | Fresh femur and tibia (n = 5 each) | - | Manual | 1. DNA Extraction (Rapid Method): Bone powder incubated with SLS, EDTA, proteinase K, DTT at 56°C for 2 hours, then centrifuged and diluted 1:50 with water.<br>2. Comparison Method: DNA also extracted using total demineralization protocol (TD). | DNA Concentration Results:<br>• The developed method showed moderate DNA yields from femur and tibia, with femur (0.5g) yielding the highest median concentration of 54.59 ng/µL and tibia (0.5g) showing 51.02 ng/µL.<br>• The total demineralization (TD) method provided higher DNA concentrations, with tibia (0.5g) yielding 66.33 ng/µL and femur (0.5g) at 64.54 ng/µL. |
| Duijs & Sijen, 2020 [25] | Bone specimens (femur), buried for 4–44 years and stored at -80°C, were used. | They were cleaned by scraping flesh and dirt, then ground into powder using a cryogenic grinder for six minutes at 15 cps with liquid nitrogen. | Automatic | Maxwell FSC DNA IQ DNA extraction protocol<br>With and without demineralization | Maxwell extraction yields ranged from 2.85 to 25.00 ng DNA/g, while full demineralization yielded 0.30–1.74 ng DNA/g.<br>Incubation time effects:<br>• 2-minute: Higher DNA (0.025-0.044 ng/mL), better allele counts (50.5-51), and peak heights (652-823 RFU).<br>• 2.5-hour: Lower DNA (0.005-0.037 ng/mL), fewer alleles (25.5-50.8), and lower peak heights (239-1016 RFU). Shorter incubation times provided better yields.<br>Grinding time effects:<br>• 4 minutes: Very low DNA yield (0.08-0.15 ng DNA/g), no peaks.<br>• 8 minutes: Higher yield (2.23-5.54 ng DNA/g) and more peaks (24.1-28.3).<br>In forensic cases:<br>• Full Demineralization: Yields 0.01-35.20 ng DNA/g, with 3 full profiles.<br>• Maxwell: Yields 0.30-42.13 ng DNA/g, with 4 full profiles, including Y-markers. Maxwell generally yielded more DNA but fewer full profiles. |
| Grela *et al.* 2021 [26] | Canine bones and teeth were charred at 400°C for 5–60 minutes. | Samples were cleaned by grinding (Extol, Czech Republic), rinsed, UV-irradiated, and dried. | Manual | 1. PrepFiler® Forensic DNA Extraction Kit<br>2. QIAamp® DNA Investigator Kit<br>3. Phenol: Chloroform Organic Extraction<br>4. Syngen DNA Mini Kit | Nuclear DNA (nDNA) concentration decreased with longer burning durations:<br>• QIAamp®: Highest nDNA in bone at 15 min (31.01 ng/µL); limited recovery beyond 30 min.<br>• PrepFiler®: Peak nDNA in bone at 15 min (29.80 ng/µL); tooth nDNA peaked at 10 min (2.81 ng/µL).<br>• Organic: Bone nDNA highest at 5 min (18.34 ng/µL), failed after 15 min; tooth nDNA peaked at 10 min (1.55 ng/µL).<br>After 30 min, nDNA was undetectable across all methods. |

*table cont...*

| Author and year | Sample & sample size | Pretreatment | DNA extraction method type | Extraction technique | Results and STR Loci identified |
|---|---|---|---|---|---|
| Calacal *et al.* 2015 [27] | Six bone types (femur, rib, clavicle, vertebra, patella, metatarsal) collected from human cadevers | Bone samples were de-fleshed, air-dried, sanded, cut, washed, dried at 56°C, and pulverized. | Manual | 1 Phenol: Chloroform Organic Extraction with Microcon YM-100 concentrators | • Bone samples yielded sufficient DNA without decalcification. Most (95%) had >1 ng per 0.1 g. Above-ground remains had lower yields, while buried remains showed more inhibitors from soil and microbes.<br>• Bone samples yielded >1 ng DNA per 0.1 g, enabling complete STR profiles. Increasing template DNA from 0.1 to 0.5 ng improved allele recovery by 10–50%. |
| Iyavoo and Goodwin 2017 [28] | Bones (fresh to 1 year old) | Bones cleaned, soaked in 5% bleach for 15 minutes, rinsed, and dried. 1–2 g of bone was pulverized. Decalcification performed using 0.5 M EDTA. | Manual | phenol-chloroform extraction | Non-decalcified bones had higher DNA yields (92.38 ng/µl) than decalcified ones (54.38 ng/µl). Amplification showed lower peak heights for decalcified samples, aligning with DNA quantification |
| Rancourt *et al.* 2023 [5] | 20 dried fetal pig bones. Teeth from a juvenile pig, including 4 incisors (rootless) and 2 molars (with roots). | Bones were scrubbed, rinsed, and incubated in 5% Tergazyme® for 45 minutes to remove exogenous DNA, then dried. Teeth were rinsed, incubated overnight in Tergazyme®, and soft tissue scraped off. After a second incubation, teeth were rinsed and dried. | Manual/ Automated | 1. PrepFiler Express BTA™ Forensic DNA Extraction Kit (Thermo Fisher Scientific)<br>2. AutoMate Express™ Forensic DNA Extraction System (Thermo Fisher Scientific) | • DNA Yield for Bones: 173 to 4614 ng, highest with no demineralization, frozen. Significant difference in demineralization.<br>• Purity for Bones: Ratios from 0.02 to 0.71, significant difference in demineralization.<br>• DNA Yield for Teeth: 85.0 to 1398.9 ng, highest with no demineralization. Significant difference in demineralization.<br>• Purity for Teeth: Ratios from 0.04 to 0.57, no significant difference. |
| Chong *et al.* 2023 [29] | 20 mg Femur bone QC1, 100 mg Femur bone QC2 50 mg Environmentally challenged bones | Bone was cleaned, sanded, sonicated, rinsed, and dried. Portions were hammered into chips for STR typing and pulverized into powder for DNA extraction and processing using various methods. | Manual/ Automated | 1. Demineralization DNA extraction<br>2. AutoMate Express DNA extraction The PrepFiler Express BTA Forensic DNA Extraction kit (Thermo Fisher Scientific) | Both Demineralization and AutoMate Express methods consistently detected all 20 CODIS loci across different bone powder amounts (50, 25, 10, and 5 mg). Demineralization generally yielded higher concentrations of DNA (355.7 ng for 50 mg) compared to AutoMate Express (300.8 ng for 50 mg). Despite yield differences, both methods produced complete DNA profiles. |
| Haarkötter *et al.* 2023 [30] | Human Humerus (n=1), Ulna (n=1), Tibia (n=1), Femur (n=10), Petrous (n=25) | - | Manual/ Automated | 1. Phenol: Chloroform Organic Extraction (Ph-Chl)<br>2. InnoXtract™ Bone (InnoGenomics, New Orleans, LA, USA)<br>3. AutoMate Express™ Nucleic Acid Extraction System<br>4. PrepFiler™ BTA Forensic DNA Extraction kit (ThermoFisher Scientific, Waltham, MA, USA) | Ph-Ch method extracted DNA detected the highest number of alleles (29 alleles), followed by PrepFiler™ BTA (25 alleles) and InnoXtract™ detected the fewest alleles (13 alleles). |

***table cont...***

| Author and year | Sample & sample size | Pretreatment | DNA extraction method type | Extraction technique | Results and STR Loci identified |
|---|---|---|---|---|---|
| Doniec et al. 2024 [7] | 45 skeletal samples (15 molar/premolar, 15 femur, 15 petrous temporal bones) from WWII victim burial sites, with an estimated PMI of 70–80 years. | Bone samples were sanded, cut, cleaned, and ground into powder. DNA was then extracted automatically from the bone powder using an EZ2 Connect instrument. | Manual/ Automated | 1. Phenol: Chloroform Organic Extraction<br>2. EZ1&2 DNA Investigator Kit (Qiagen Ltd, Hilden, Germany). | The higher bone powder amounts (500 mg and above) yielded better DNA (1.5–5.2 ng/g) and complete STR profiles (95–100% completeness). Smaller amounts (100–400 mg) had lower yields and completeness. |
| Di Stefano et al. 2024 [31] | 27 skeletons exhumed from a Cres Island mass grave | Bones were cleaned, decontaminated, UV-treated, and pulverized at 30 Hz using a MM 400 Ball Mill with liquid nitrogen. Twelve left femurs were processed separately at Lab B with a Bead Beater MillMix 20 before DNA extraction. | Manual | 1. Decalcification of Na2EDTA followed by extraction using Maxwell® FSC DNA IQ™ | Results Summary:<br>• Right Femur: 21.7% >LOD, 3.4% >LLOQ, Average DNA: 5.9 pg/µL, STR success: 62.5%<br>• Left Femur: 25% >LOD, 0% >LLOQ, Average DNA: 1.2 pg/µL, STR success: 28.5%<br>• Tooth: 15.6% >LOD, 0% >LLOQ, Average DNA: 1.0 pg/µL, STR success: 20% |
| Sahib Zar *et al.* 2015 [32] | 27 degraded DNA samples from human bones aged 100 to 1000 years | Samples were cleaned and treated with tools, UV light, bleach, and ethanol. After maceration using liquid nitrogen, the bone powder was stored at -20°C for DNA extraction. | Manual | silica-column-based QIAamp Blood Maxi column (Qiagen) | The study showed that both AmpFlSTR Identifiler and MiniFiler kits successfully typed DNA from 100–1000-year-old remains. The MiniFiler kit provided more informative profiles and recovered alleles missed by Identifiler. |
| Ramírez *et al.* 2023 [33] | Twenty-nine bone samples (~25–29 years old, 1983–1985). | The surface of the samples was scraped to remove exogenous DNA, then exposed to UV light for 15 minutes per side. The samples were pulverized with a Dremel drill at 1000 rpm. | Manual | 1. Organic extraction method (Phenol: Chloroform: Isoamyl Alcohol)<br>2. Commercial kit<br>3. DNA extraction with demineralization | Organic solvents showed DNA concentrations ranging from 0.0039 to 0.26 ng/µL, with inhibitors in 3 samples. The commercial kit had lower DNA yields (0.0011 to 0.08 ng/µL), with no amplification in some cases. Previous demineralization resulted in DNA concentrations of 0.006 to 0.26 ng/µL, with inhibitors in 6 samples. |

## CONCLUSIONS

Overall, the findings highlight that organic extraction remains the gold standard for high-yield DNA recovery, particularly in challenging forensic and archaeological cases. However, automated methods provide efficiency and consistency, making them valuable for forensic laboratories handling large sample volumes. The type of bone, pretreatment strategy, and extraction modifications significantly influence DNA yield, with petrous and femur bones showing the best recovery. Future forensic applications may benefit from hybrid approaches that combine automation with optimized organic extraction techniques to maximize DNA recovery across different sample conditions.

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