INTRA-BONE HETEROGENEITY OF RECOVERABLE DNA FROM FRESH, BURIED, AND EXPOSED FEMORA

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ABSTRACT

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DNA recovered from skeletal material is often used to establish positive identification of severely decomposed or fragmented remains. DNA is preferentially sought from long bones like the femur, usually from cortical bone of the midshaft diaphysis. Although this strategy is often successful, the femur has extensive differences in morphology, tissue composition, and points of articulation that may differ in DNA content. In the research presented, intra-femoral heterogeneity was assessed in a proximal/distal manner to determine variation in DNA quantity and quality. Mitochondrial and nuclear DNA yields were compared across nine regions of the diaphysis, and the distal and proximal epiphyses, using fresh bovine and porcine femora and two tissue digestion protocols (non-demineralization and demineralization). In addition, bovine femora were subjected to burial and surface exposure over a six month interval to assess how DNA heterogeneity was affected by environments where forensically relevant remains are often discovered. The epiphyses had significantly more DNA than the metaphyses, which had more than the diaphysis, DNA quality was consistent among all regions tested, and mitochondrial and nuclear DNA had similar regional variation. Bone demineralization resulted in more DNA recovered at the mid-diaphysis, while the non-demineralization protocol did the same for nuclear DNA at the epiphyses. Environmental exposure affected DNA quantity and quality, and burial influenced inter-regional DNA yields over time. These findings indicate that intra-bone DNA heterogeneity can be as important as inter-bone DNA heterogeneity, and should be considered when choosing a sampling location for DNA isolation.

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KEY TO ABBREVIATIONS

AD: Anderson-Darling (statistical test)

AFDIL: Armed Forces DNA Identification Laboratory

BP: Base Pairs (DNA)

CILHI: Central Identification Laboratory in Hawaii

DM: Demineralization Buffer

DNA: Deoxyribonucleic Acid

EDTA: Ethylene Diamine Tetra-acetic Acid

F/R Primer: Forward/Reverse Primer

IGF-1: *Insulin Growth Factor-1* (gene)

IPC: Internal Positive Control

MAD: Median Absolute Deviation

MC1R: *Melanocortin-1-Receptor* (gene)

MSU: Michigan State University

mtDNA: Mitochondrial Deoxyribonucleic Acid

NT: Not Tested

PCR: Polymerase Chain Reaction

qPCR: Quantitative Polymerase Chain Reaction

RB: Reagent Blank

RO: Reverse Osmosis (water)

RPM: Rotations per Minute

SDS: Sodium Dodecyl Sulfate

SLS: Sodium Lauryl Sarcosine

STR: Short Tandem Repeat

SW: Shapiro-Wilk (statistical test)

TE: Tris EDTA

TL: Tissue Lysis Buffer

USDA: United States Department of Agriculture

UV: Ultraviolet

INTRODUCTION

Skeletal material is an invaluable resource for forensic investigators; it can provide information pertaining to a decedent's identity, and may bare indications of trauma that suggest how the individual died. For instance, each year the Armed Forces DNA Identification Laboratory (AFDIL) receives and processes hundreds of bone samples—often degraded and fragmented—derived from civilian and military service members who died during current and past conflicts, with the goal of identifying and repatriating their remains (Edson et al., 2004; Canik, 2013). Furthermore, medical examiners and coroners receive approximately 4,400 unidentified human decedents annually, 1,000 of which are still unidentified after one year, and of those 600 undergo final disposition anonymously (Hickman et al., 2007). The timely identification of human remains can aid law enforcement in their criminal investigation, and in establishing a case against a suspect. Equally important is being able to notify the decedent's family so they can make postmortem arrangements.

Forensic practitioners utilize several techniques to identify skeletonized remains. Forensic anthropologists can develop a biological profile, where the decedent's age, sex, height, and ancestry are estimated based on metric and morphoscopic traits (SWGANTH.org). This is useful in excluding individuals (e.g., missing persons not of the same sex as the decedent), generating leads for law enforcement, and providing tentative identification when used in conjunction with other circumstantial evidence (e.g., a driver's license found with remains). However, to establish positive identification, individualizing information must be obtained. This can be done by comparing postmortem skeletal or dental radiographs with antemortem records

(Murphy et al., 1980; Pretty and Sweet, 2001), and some orthopedic implants have individualizing characteristics (e.g., serial numbers) that can be used to positively identify the recipient (Simpson et al., 2007). Unfortunately, identification of skeletal remains using these methods is not always possible due to a lack of antemortem records, the level of skeletal preservation, or the remains being substantially incomplete or fragmented. Identification then relies upon analyzing DNA from the skeletal material, with the likelihood of establishing identity being increased by assaying bone regions that are rich sources of DNA for recovery.

Environmental Influences on DNA Degradation from Skeletal Material

Environmental exposure and temperature affect both skeletal material and the DNA contained within. Hochmeister et al. (1991) subjected femoral bone to outdoor exposure, outdoor exposure while wrapped in plastic, water immersion, soil burial, and frozen control samples for a three month period. They reported that frozen samples had the highest mean DNA yield (12.6 µg DNA/g bone) followed by outdoor exposure (0.9 µg/g), while bone wrapped in plastic (0.2 µg/g), immersed in water (0.1 µg/g), and buried (0.05 µg/g) had the lowest yields. Only the control and outdoor exposed bones had sufficient high molecular weight DNA for restriction fragment length polymorphism typing, while DNA recovered from bone subjected to all treatments was amplifiable using polymerase chain reaction (PCR). Many authors have shown that bones aged in cooler environments typically contain better preserved DNA than bones from warmer settings (Höss et al., 1996; Burger et al., 1999; Collins et al., 2001; Smith et al., 2003; Fu et al., 2014). Temperature affects DNA degradation and preservation most likely via depurination that is highly temperature dependent (Götherström et al., 2002). Thus, the

environment, and its climate, from which remains are recovered in likely influences the odds of recovering analyzable DNA.

Specific physical and chemical characteristics of soil have also been shown to influence DNA degradation in bone. Groundwater infused soil can increase bone porosity (Hedges and Millard, 1995), resulting in the physical loss of DNA, and aids putrefaction by providing a favorable environment for hydrolytic reactions that cause depurination and subsequent DNA fragmentation (Eglinton et al., 1991). The chemical composition of soil affects bone crystallinity over time through the dissolution and re-precipitation of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) crystals in bone (Hedges and Millard, 1995; Hedges, 2002). This process may release DNA bound to hydroxyapatite, consequently reducing its preservation and potential recovery (Götherström et al., 2002). Furthermore, incorporated soil solutes (e.g., humic substances) can co-extract with DNA, leading to PCR inhibition and limiting subsequent analyses (Kemp et al., 2006; Eilert and Foran, 2009; Hebda, 2013).

Bone dissolution and DNA degradation are also influenced by pH (Hedges and Millard, 1995). Gordon and Buikstra (1981) excavated seven burial sites in Illinois, finding a significant correlation between soil acidity and bone degradation. Crow (2008) experimentally demonstrated that mineral apatite was highly susceptible to dissolution at acidic pH (5.6 – 3.6), and Casallas and Moore (2012) reported near-complete mineral dissolution of bones buried 8 – 10 years in soils at pH 4.2 – 4.8. Hughes et al. (1986) were unable to isolate DNA from peat preserved human remains that were in an aqueous environment of pH 4.5 – 5.5, indicating that low pH contributed to DNA degradation. Likewise, Burger et al. (1999) noted that DNA is better preserved at a slightly alkaline to physiological pH. Though depurination occurs spontaneously at physiological pH (Lindahl, 1993), it is accelerated at low pH and high

temperature (Roger and Hotchkiss, 1961), indicating that acidic and/or warmer climates are associated with increased DNA degradation.

Burial has also been shown to affect DNA yields. Researchers have reported that substantial DNA loss in bone can occur over a period of years (Campos et al., 2012), months (Hochmeister et al., 1991), or less (Hebda and Foran, 2015). How much DNA may be lost in buried bone over even shorter periods (i.e., from days to weeks) is unclear; however, Bär et al. (1988) demonstrated that many soft tissues have pronounced DNA degradation within days after death. The authors proposed that rapid putrefaction led to the initial loss of DNA in their study, as did Campos et al. (2012). Dent et al. (2004) reviewed human decomposition in soil, and noted that putrefaction is normally initiated by autolytic processes and is generally not observed until days after death. Bell et al. (1996) postulated that microbes, from the decedent or present in the environment, can degrade bone within months after death, and Latham and Madonna (2013) discussed how microbial activity can indirectly damage DNA by degrading the bone it is bound to, or directly through enzymatic activity. Environmental exposure, particularly burial, clearly affects DNA degradation, therefore, it is not only important to determine site(s) on bone that are rich sources of DNA, but also evaluate how environment alters DNA quantity and quality at those locations.

The Composition of Bone

Many authors have noted that bone composition facilitates function: the mineral components provide mechanical rigidity and weight bearing strength, while the organic constituents offer elasticity and flexibility (e.g., White and Folkens, 2005; Clarke, 2008). Clarke (2008) reviewed the makeup of bone, characterizing it as a complex tissue composed of 50% –

70% mineral (i.e., hydroxyapatite), 20% – 40% organic material, and less than 10% water and lipids. Hydroxyapatite forms approximately 200 Å long crystalline lattices (Clarke, 2008), and makes up 62% – 66% of a bone's mass (Bigi et al., 1997). Collagen constitutes 90% – 96% of bone's organic material (Rogers et al., 1952), and is primarily comprised of type I collagen, along with small quantities of types III, V, IX, XII, XIV, XIX, and XXI collagen (Clarke, 2008).

Bone develops into two general types: cortical and trabecular (Figure 1). Cortical osseous tissue is solid, dense, and relatively nonporous; conversely, trabecular bone consists of a porous mesh-like network of plates and rods (Clarke, 2008). The adult human skeleton is comprised of approximately 80% cortical and 20% trabecular bone by mass (Eriksen et al., 1994); however, these ratios vary greatly among bones. For example, vertebrae have a 25:75 percent ratio of cortical to trabecular bone, whereas the femoral head is closer to 50:50, and the femoral shaft is almost entirely cortical material (Clarke, 2008).

Bone contains three cell types: osteoblasts, osteocytes, and osteoclasts, which are characterized by their function. Osteoblasts synthesize the collagenous matrix and regulate its mineralization (Anderson, 2003). Fifty to seventy percent of osteoblasts undergo apoptosis after bone tissue deposition (Clarke, 2008), while the remaining cells become osteocytes within the bone's matrix, participating in its maintenance through the exchange of nutrients and wastes (Burger et al., 2003). Cortical bone contains approximately 20,000 osteocytes per cubic millimeter (Martin and Burr, 1989). Osteoclasts are multinucleated cells that participate in osteolysis by binding to the bony matrix, then secreting acid and collagenase to resorb tissue (Vaananen et al., 2000). These three cell types are responsible for bone deposition, maintenance, and resorption that characterize the bone remodeling cycle, and provide a source of DNA for forensic analysis.

The composition of bone also means it is one of the final tissues to decompose, which leads to its forensic importance. Osseous tissue preserves, or at least retains, DNA better than other tissues, where it is believed to act as a physical barrier against the environment (Hochmeister et al., 1991; Collins et al., 2002), and DNA bound to hydroxyapatite is resistant to proteolytic digestion (Brundin et al., 2013). However, bone is a challenging substrate from which to extract and process DNA; many of its components have been shown to inhibit PCR, including collagen (Scholz et al., 1998; Opel et al., 2010), calcium (Bickley et al., 1996; Opel et al., 2010), and potentially hydroxyapatite, which can bind double stranded DNA (Martinson, 1973).

Femoral Bone Anatomy and Forensic Analyses

The femur is the longest, heaviest, and strongest bone in the human body, tasked with supporting and distributing the body's weight (White and Folkens, 2005). Long bones like the femur (Figure 1) contain an elongated hollow shaft of cortical bone (diaphysis) that widens towards the growth plates (metaphyses), with rounded ends made of a robust trabecular meshwork encased in a thin layer of cortical bone (epiphyses) (Gray, 1918; Clarke, 2008). The femur is one of the most commonly utilized skeletal elements in physical/forensic anthropological analyses, and many components of a biological profile have benefited from its study. For instance, an individual's stature can be assessed metrically using the femur's maximum length (Trotter and Gleser, 1952, 1958), and sex can be estimated with about 90% accuracy by measuring the vertical head diameter (Pearson, 1917; Stewart, 1979), and/or midshaft diaphysis circumference (Black, 1978; Di Bennardo and Taylor, 1979). Likewise, investigators exploit predictable patterns in femoral development, such as diaphyseal length and

epiphyseal closure schedules, for sub-adult age estimation (Bass, 1995). In contrast, discriminating among major ancestral groups (e.g., European vs. African) using femoral metric traits has had only limited success (Stewart, 1962; Craig, 1995).

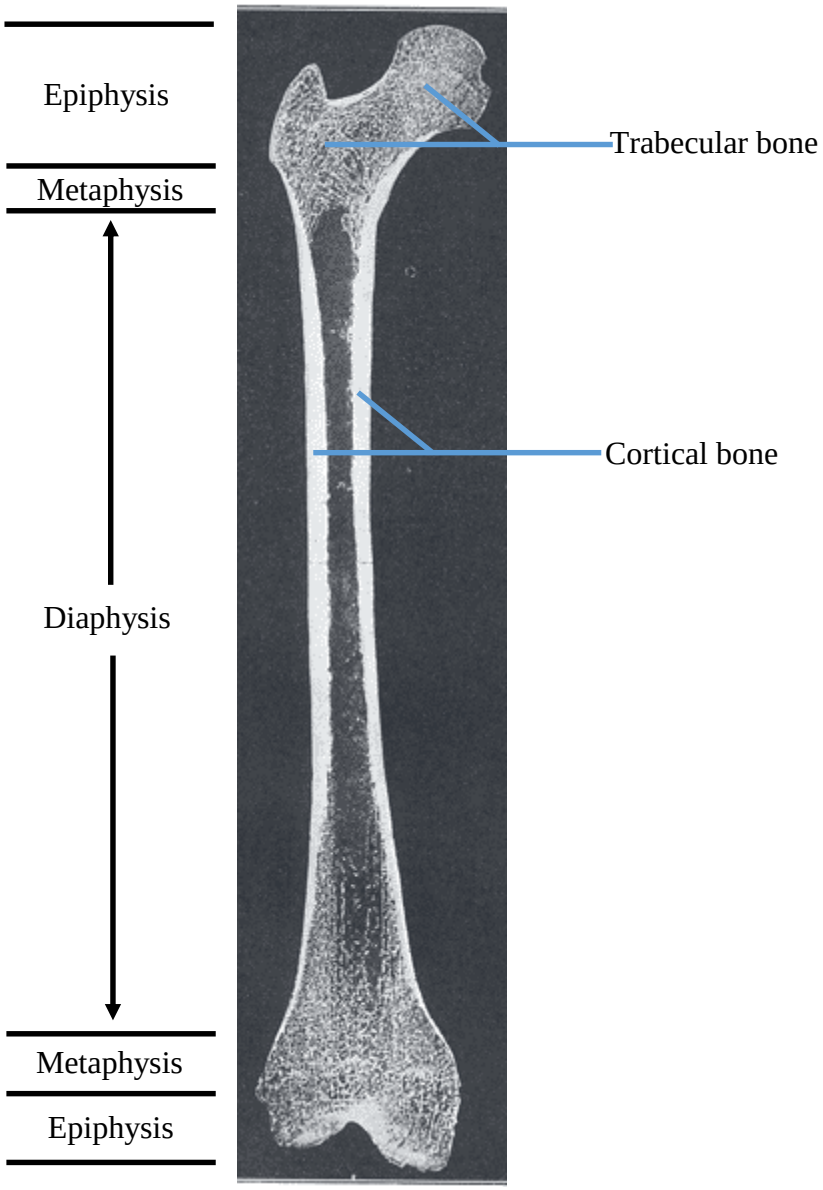

Figure 1: Basic Anatomy of a Human Femur
Sectional view of a human femur showing the diaphysis, metaphyses, and epiphyses, along with the distribution of cortical and trabecular bone. Adapted from Gray (1918). Illustration available at http://www.bartleby.com/107/59.html#i249.

Investigators who recover DNA from skeletal material have relied heavily upon femoral sampling. In the first published forensic case based on skeletal DNA, a femur was used to positively identify a murdered adolescent eight years postmortem (Hagelberg et al., 1991). That same year, the feasibility of obtaining high molecular weight DNA from femoral cortical bone for restriction fragment length polymorphism and PCR based analyses was demonstrated (Hochmeister et al., 1991). Similarly, this element is often used in ancient DNA analysis, such as that recovered from 45,000 year old human remains found in Siberia, isolated from the distal femur (Fu et al., 2014). Approximately 19% of bones tested at AFDIL through 2006 were femora, the highest percentage of any single element (Edson et al., 2004; Leney, 2006). Femora comprised 46% of samples (11356/24656) tested by the International Commission on Missing Persons to identify remains from mass graves sites after the Yugoslavian conflict (Miloš et al., 2007). Overall, DNA from the femur has been commonly utilized in research (e.g., Cattaneo et al., 1995; Misner et al., 2009; Caputo et al., 2013; Fu et al., 2014) as well as forensic casework (e.g., Edson et al., 2004; Leney, 2006; Miloš et al., 2007).

Recovering DNA from Skeletal Material

DNA Yield by Osseous Tissue Type

DNA yield differences between cortical and trabecular bone were established in the early 1990's. Lee et al. (1991) compared bone types from unspecified human remains and found that trabecular bone contained 10 – 20 times more DNA than cortical bone on a per milligram basis when quantified using spectrophotofluorometry. In contrast, in a review of postmortem DNA preservation and recovery, Parsons and Weedn (1996) reported that cortical bone retains DNA better than trabecular bone over long periods, based upon unpublished observations from

AFDIL. Sampling cortical bone became standard operating procedure for AFDIL, which often rejects degraded or ancient remains consisting primarily of trabecular osseous tissue (Edson et al., 2004). The International Society for Forensic Genetics (Prinz et al., 2006) and the International Committee of the Red Cross (2009) advocate sampling from dense cortical bone—preferably from weight bearing long bones—for disaster victim identification.

Recent investigations into DNA quantity and quality between osseous tissue types have resulted in mixed findings. Misner et al. (2009) examined 86 bones derived from 36 skeletons exhumed from the Voegtly Cemetery in Pittsburg, PA to determine if skeletal weathering stages are predictive of DNA quantity and quality. The authors found no correlation between skeletal weathering and recovered DNA; however, osseous tissue type significantly influenced DNA yield and amplification success. Cortical bone of the femur resulted in mtDNA amplicons of 107 bp or larger 79.3% of the time, with a mean yield of 103.2 copies mtDNA/µL, while the rib and pelvis, primarily trabecular bone, had DNA that amplified 63.6% and 36.0% of the time with mean yields of 87.4 and 77.6 copies of mtDNA/µL respectively. Mundorff et al. (2009) reported similar results, where DNA recovered from the femur and tibia led to the identification of the decedent more often than the ribs or pelvis; however, they also noted that commonly overlooked elements such as the patella, tarsals, and foot phalanges were similarly successful. Mundorff and Davoren (2014) followed up by testing every element type from three skeletonized individuals, reporting that elements composed primarily of trabecular bone had better quality and greater quantities of DNA than did the traditionally accepted dense cortical weight bearing long bones. The reason(s) for these discrepancies remain unclear; however, osseous tissue type likely influences the quantity and quality of DNA recovered and should be considered when sampling skeletal remains.

DNA Isolation Methods: Preparing Bone for Digestion and Extraction

Strategies for obtaining DNA from a bone sample can be divided into two general categories: pulverizing bone into a fine powder, or excising a segment and processing it whole. Several techniques are utilized to turn whole bone into powder: freezing the bone—usually in liquid nitrogen—prior to pulverization (Hagelberg and Clegg, 1991; Cattaneo et al., 1995; Hochmeister et al., 1991; Loreille et al., 2007), grinding bone using nonfreezing milling apparatuses (Edson et al., 2004; Loreille et al., 2007; Misner et al., 2009, Adler et al., 2011), and collecting powder produced by repurposed drilling tools (Rennick et al., 2005; Adler et al., 2011; Caputo et al., 2013; Mundorff and Davoren 2014; Hebda and Foran, 2015) are the most common. Loreille et al. (2007) had inconsistent results when comparing DNA yields from bone pulverized in a freezer mill versus a nonfreezing blender cup, concluding that neither method produced substantially higher yields. Adler et al. (2011) compared grinding versus drilling of cortical bone, resulting in 5 – 30 fold more mtDNA from bones that were ground; however, when the drilling speed was reduced from 1,000 to 100 RPM, both drilling and grinding methods produced similar yields. The authors attributed this to high-speed drilling's increased heat output contributing to mtDNA damage, and the use of sharp drill bits that are in good condition has been advocated by some authors to reduce the heat generated by drilling (Matthews and Hirsch, 1972; Adler et al., 2011). Caputo et al. (2013) compared DNA quantity and quality of powdered bone from low-speed drilling and thinly sliced bone segments. The drilling method resulted in 1.5 times more DNA on average; however, the bone slice method produced a greater percentage of full STR profiles (54.8%) compared to drilled bone powder (10%). Drilling was used in this

thesis research because of its ease of use, flexibility, and relatively non-destructive sampling of bone.

Tissue Digestion Methods Used for DNA Isolation

Isolating DNA from bone involves strategies that either do or do not demineralize it prior to DNA extraction. Particulars vary, but digestion buffers generally include a detergent, buffer, chelating agent, and proteinase that are incubated for some amount of time to lyse cells and release DNA (Butler, 2012). The chelating agent commonly used in digestion buffers is ethylene diamine tetra-acetic acid (EDTA), which binds divalent cations. EDTA complexes with the Ca^{2+} in hydroxyapatite, facilitating bone dissolution. Calcium also helps to protect DNA nucleases from proteolytic digestion (Price et al., 1969), thus its removal may be further beneficial during DNA extraction. Furthermore, EDTA binds Mg^{2+}, required by nucleases for DNA hydrolysis (Kunitz, 1950; Price, 1975). Overall, EDTA may have multiple important roles in liberating DNA from bone and preserving it for analyses.

Demineralization protocols differ, but can be classified into procedures where demineralization occurs before the organic components are digested (Hochmeister et al., 1991; Hagelberg and Clegg, 1991), or in a single process where organic and inorganic components are digested simultaneously (Loreille et al., 2007). Hochmeister et al. (1991) incubated bone powder in a 0.5 M EDTA solution—routinely replenishing it over three to five days—followed by three washes in deionized water prior to digestion in extraction buffer and proteinase K. Hagelberg and Clegg (1991) used a similar procedure, decalcifying bone in 0.5 M EDTA for 72 hours preceding incubation in lysis buffer and proteinase K. This method of demineralization was time consuming, DNA was likely lost from the discarded EDTA solution, and some researchers

reported that full demineralization, compared to non-demineralization, reduced DNA yields (Hagelberg and Clegg, 1991; Fisher et al., 1993). Owing to this, AFDIL opted to use a non-demineralizing digestion solution in their casework consisting of Tris, EDTA (not in high enough concentration to appreciably demineralize bone) and sodium dodecyl sulfate (SDS) (Edson et al., 2004). This method was faster; however, according to Loreille et al. (2007), when the supernatant was recovered, undissolved bone containing un-extracted DNA was discarded. This led AFDIL to develop and validate a single step demineralization protocol for casework (Loreille et al., 2007), which was tested side-by-side with samples processed using the casework protocol detailed in Edson et al. (2004). The demineralization protocol resulted in significantly higher DNA yields, improved typing success, and a reduction of starting skeletal material required for analysis. Figure 2 depicts AFDIL's historical success for generating "reportable sequence data" from casework using both their non-demineralization and demineralization protocols through August 2013 (Canik, 2013). Side-by-side comparisons of these data reinforce a conclusion made by Loreille et al. (2007): single step demineralization increases the likelihood of recovering DNA that will produce reportable genetic information.

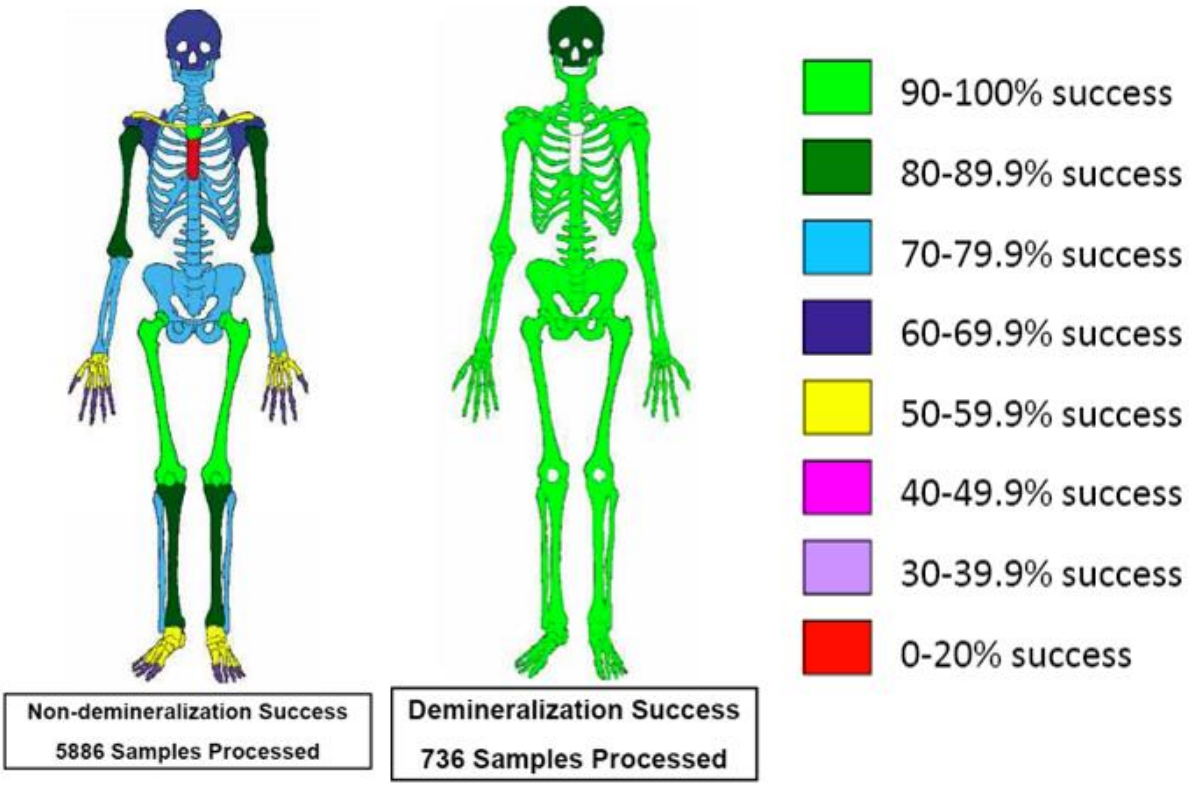

Figure 2: AFDIL's Success in Generating Reportable Sequence Data between Non-Demineralization and Demineralization Protocols by Element Type
Adapted from Canik (2013). Left skeleton illustrates elements processed using AFDIL's non-demineralization protocol and right skeleton using their demineralization protocol. Skeletons are in anatomical position with color coded elements corresponding to their percent success of producing "reportable sequence data". Total sample size per protocol is shown below each illustration.

Inter-Bone Heterogeneity in Recoverable DNA Quantity and Quality

Research primarily from the 2000's established that successful sequencing, short tandem repeat typing, and/or identification from skeletal DNA was influenced by the element used for analysis (Edson et al., 2004; Leney, 2006; Miloš et al., 2007; Mundorff et al., 2009). This included large data sets that covered various postmortem intervals, environmental conditions, and taphonomy. Results from these studies are summarized in Table 1 and graphically depicted in Figure 3. The Central Identification Laboratory and AFDIL reported mtDNA amplification success from skeletal elements derived from thousands of service member's remains spanning

World War II, the Korean War, and the Vietnam War (Edson et al., 2004; Leney, 2006). Miloš et al. (2007) evaluated DNA typing success among elements from more than 25,000 skeletal remains retrieved from mass grave sites in Kosovo, Bosnia, and Herzegovina. Similarly, Mundorff et al. (2009) compared DNA identification success among bones based on over 3,600 skeletal elements from the World Trade Center-Human Remains Database.

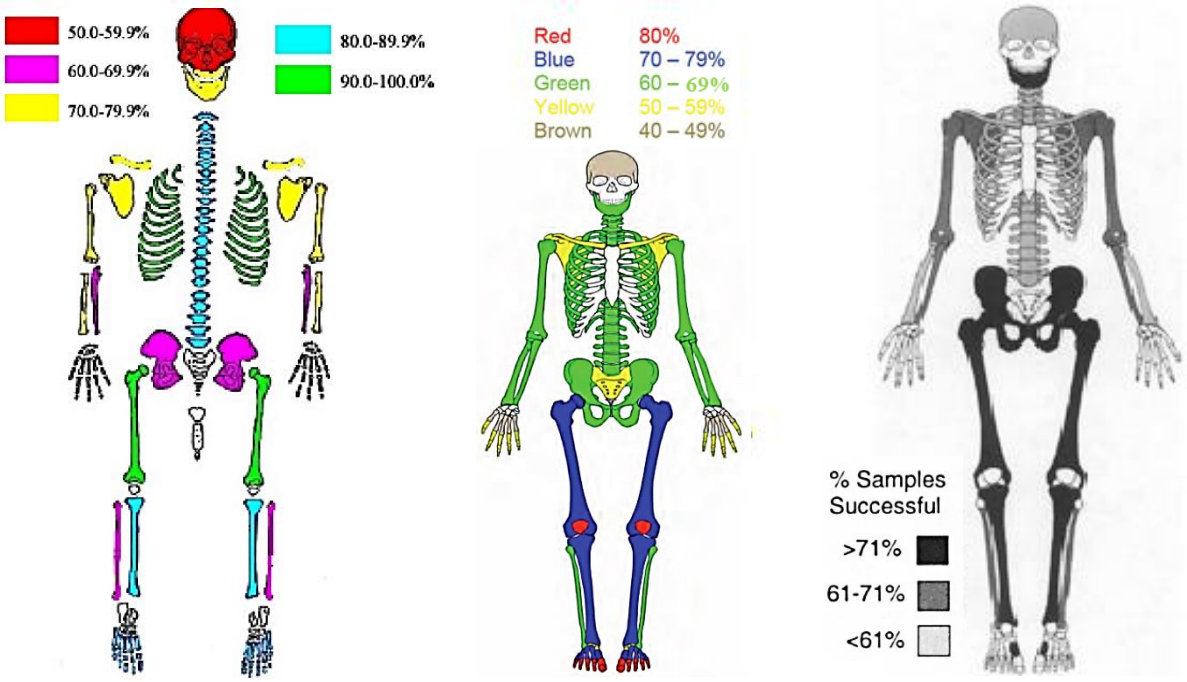

Figure 3: Published Illustrations of Inter-Bone Success of Obtaining Analyzable DNA

Left diagram adapted from Edson et al. (2004), middle from Mundorff et al. (2013) representing data from Mundorff et al. (2009), and right from Leney (2006). Diagrams provide an illustrative representation of the authors' data presented in Table 1. Illustrations are color coded/shaded to correspond with percent success rates listed in each respective legend.

Table 1: Summary of Results from Published Studies Evaluating Inter-Bone Variation of Successfully Obtaining Analyzable DNA

Element sampled is identified in the first column. Subsequent columns represent studies in which success rates were assessed among elements. The first number denotes the percent success and the parenthetical number signifies the sample size for that element. Success was defined in Edson et al. (2004) and Leney (2006) as producing "reportable sequence data". Miloš et al. (2007) defined success as obtaining genetic data from 12 or more loci using a Promega PowerPlex 16 STR kit. Mundorff et al. (2009) defined success as "sample identified through DNA testing". Data from Mundorff et al. (2009) used in this table only incorporate the Complete Elements Database, a subset of the World Trade Center-Human Remains Database. NT = Not Tested CILHI = Central Identification Laboratory in Hawaii

Element Sampled	**% Success of Sampled Element (Sample Size)**			
	Edson et al. 2004 mtDNA	**Leney 2006 mtDNA**	**Miloš et al. 2007 Nuclear**	**Mundorff et al. 2009 mtDNA/Nuclear**
Femur	95 (192)	87 (477)	87 (11356)	71 (143)
Tibia	89 (145)	83 (306)	76 (1329)	70 (125)
Fibula	67 (21)	63 (87)	63 (160)	60 (159)
Metatarsal	81 (21)	74 (23) 1st Metatarsal Only	33 (120)	72 (257)
Innominate	63 (19)	74 (87)	53 (185) Ilium Only	63 (62)
Teeth	80 (184)	72 (442)	83 (6963)	NT
Radius	70 (37)	61 (101)	25 (469)	60 (120)
Ulna	67 (54)	57 (111)	23 (444)	61 (87)
Humerus	79 (149)	71 (339)	46 (2415)	61 (110)
Scapula	79 (19)	69 (39)	57 (35)	54 (92)
Clavicle	77 (30)	58 (56)	26 (128)	54 (97)
Vertebra	86 (14)	59 (21)	62 (146)	61 (72)
Mandible	76 (34)	72 (50)	56 (131)	65 (46)
Skull	51 (76)	47 (197)	41 (757)	47 (494)
Rib	96 (26) Single CILHI Case	63 (43)	NT	64 (1301)
Metacarpal	NT	NT	61 (18)	44 (211)
Patella	NT	NT	NT	80 (83)
Foot Phalanx	NT	NT	NT	80 (25)
Hand Phalanx	NT	NT	NT	57 (83)
Sacrum	NT	NT	NT	59 (27)
Tarsal	NT	NT	NT	51 (37)

These researchers used different methods and objectives, the remains tested had varied postmortem intervals, and they were subjected to different environmental conditions and trauma; however, there were consistent findings among studies. In all instances dense cortical weight bearing long bones like the femur and tibia were more likely to retain DNA that resulted in successful downstream analyses. In contrast, bones with a greater proportion of trabecular tissue (e.g., axial skeleton) were less likely to produce useful genetic information.

These studies shared several limitations however: they were retrospective analyses, remains were subjected to myriad environmental influences, and element types within a single individual were not assessed. Those factors led Mundorff et al. (2013; 2014) to conduct prospective research testing each element type from three individuals that were allowed to naturally decompose lying prone on the ground at the same plot of land in the University of Tennessee Knoxville's "Body Farm". Utilizing the three individuals, along with separate remains from successively longer postmortem intervals, the authors ranked elements based on the quantity and quality of DNA obtained from each. Their results demonstrated a high degree of variability in DNA among elements from a single individual. Counter to some previous findings, small predominately cancellous bones such as the phalanx, patella, and tarsals had higher DNA yields than weight bearing long bones like the femur and tibia. Furthermore, the results remained fairly consistent when the same element types were tested across postmortem intervals ranging from 0 to more than 20 years. However, this research led to several unanswered questions that will need to be resolved in future investigations. First, the remains were skeletonized above ground, and as a result the authors noted that different postmortem conditions (e.g., burial) may produce different outcomes. Also, most elements were tested at a single location to preserve bones for future anthropological investigations; utilizing single site

sampling to represent an entire bone's DNA content assumes homogeneity throughout the bone. Furthermore, bone powder from many elements contained a substantial mixture of cortical and trabecular tissue, confounding contributions by osseous tissue type. Finally, only nuclear DNA was tested, thus it remained unknown if similar results would be obtained based on mtDNA.

Intra-Bone Heterogeneity in DNA Quantity and Quality

While researchers have established that DNA heterogeneity exists among bones, very few have examined variation within a single element. Adler et al. (2011) tested different components of teeth and reported that cementum contained up to five times more mtDNA (copy number per 100 mg substrate) than the more commonly sampled dentin. Yamaguchi and Yamaguchi (1986) measured DNA content in femora from weanling rats, and recovered about twice as much DNA from the epiphyses than the diaphysis. Further rat research by Yamaguchi et al. (2003) demonstrated that twice as much DNA was present in the metaphyses than the diaphysis. However, Yamaguchi et al. (1986 and 2003) reported highly variable DNA yields of the diaphysis between studies, 15 – 20 mg/g in 1986 and 1 – 1.5 mg/g in 2003, making cross study comparisons among the diaphysis, metaphyses, and epiphyses problematic. Finally, Hebda (2013) noted that the locations tested near the metaphyseal end of segmented bovine femora had relatively higher DNA yields when compared to the diaphyseal end, leading to the thought that DNA yields vary along the femur. With the exception of Adler et al. (2011), in which only teeth were tested, all of the researchers' findings were tangential in nature, as their primary emphasis was not in examining intra-element variation, necessitating the need for prospective research to systematically examine potential heterogeneity within a single bone.

Research Goals

The previous research detailed above has shown that DNA quantity and quality recovered from skeletal remains can differ due to many factors. However, there is a paucity of research systematically examining the heterogeneity of recoverable DNA within a single element, and how DNA quantity and quality may change when exposed to common environments where forensically relevant remains often exist. Identifying predictable variation of nuclear and mitochondrial DNA heterogeneity in the femur, if it exists, has the potential to enhance DNA recovery and analysis success.

The research presented here was designed to determine whether DNA is heterogeneously distributed in femora, one of the most commonly utilized elements in historical and forensic analyses. The first goal was to measure DNA quantity and quality throughout fresh femora derived from porcine and bovine model systems, utilizing non-demineralization and demineralization digestion protocols. The second goal was to determine if/how heterogeneity changed when bones were buried or left exposed in an outdoor environment over a six month period. The final goal was to compare intra-femoral variability of DNA quantity and quality to the calcaneus and talus, two tarsals concomitantly processed along with the femur, and characterized by Mundorff and Davoren (2014) as having substantially more DNA than the femur. Overall, the research presented was a systematic analysis of intra-bone DNA heterogeneity in fresh, surface exposed, and buried femora.

MATERIALS AND METHODS

Preparation of Bovine and Porcine Femora and Tarsals

Bovine/Porcine Intra-bone Variation and Tarsal Comparison Experiments

Eight fresh femora and tarsal sets from Holstein dairy cows (*Bos taurus*) and domestic pigs (*Sus scrofa domesticus*) were obtained from the Michigan State University (MSU) Meats Laboratory. A "tarsal set" is operationally defined as the received mass of tissue that contained a talus, calcaneus, and other foot bones articulated with muscle, tendons, and/or ligaments. Femora and tarsal sets were stored in a -80°C freezer if not immediately macerated; they were thawed overnight at room temperature prior to maceration. Soft tissue was removed through maceration in boiling 1% Tergazyme™ (Alconox, White Plains, NY) solution. Maceration took place in a Bayou Classic 42-quart stainless-steal stockpot (Barbour International Inc., Brandon, MS) containing 30 L of reverse osmosis (RO) water (~10 MΩ), and 300 g of Tergazyme™. Solution was preheated to 95°C using three ceramic heating plates set to 300°C. Plates were arranged in a pseudo-trigonal manner for adequate heat and weight distribution of the stockpot and solution. Heating elements were adjusted to maintain boiling temperature. Skeletal material was placed into the stockpot once the solution temperature reached 90°C to 95°C. Bones were macerated until soft tissues were separated: either through boiling or by physically scraping off the gelatinized tissues with a sharp instrument. Bovine bones were macerated one pair at a time (two femora, calcanei, and tali) in two eight-hour intervals, with fresh Tergazyme™ solution used per interval. Bovine femora were rotated longitudinally halfway through each interval. Porcine bones were macerated together in a single seven hour period. Halfway through maceration, they were removed from the stockpot and partially cleaned, then re-immersed for the

remainder of the seven hours. Cleaned bones were assigned an identifier, each replicate consisted of a femur, calcaneus, and talus, and measurements were taken to determine the total diaphyseal length and midshaft circumference of femora (Table 2).

Table 2: Sidedness, Diaphysis Length, and Midshaft Circumference of Bovine and Porcine Femora

Femora were labeled by species and the sequential order processed. Total diaphysis length was measured from the proximal to distal epiphyseal lines, with the range representing differences due to irregular borders. Circumference was measured at the femoral midshaft. *, **, or *** represents femora originating from a single individual.

Bovine

Femur	Sidedness	Diaphysis Length	Circumference at Midshaft
C-01	Right	21.6 – 24.1 cm	15.2 cm
C-02	Right	21.6 – 24.1 cm	15.2 cm
C-03*	Right	26.7 – 30.5 cm	16.3 cm
C-04*	Left	26.7 – 30.5 cm	16.3 cm
C-05**	Right	26.7 – 30.5 cm	16.3 cm
C-06**	Left	26.7 – 30.5 cm	16.3 cm
C-07***	Right	29.2 – 30.5 cm	15.2 cm
C-08***	Left	29.2 – 30.5 cm	15.2 cm

Porcine

Femur	Sidedness	Diaphysis Length	Circumference at Midshaft
P-01	Left	14.0 – 15.2 cm	8.9 cm
P-02	Left	14.0 – 15.2 cm	8.9 cm
P-03	Right	15.2 – 16.5 cm	8.9 cm
P-04	Right	14.0 – 16.5 cm	8.3 cm
P-05	Left	15.2 – 16.5 cm	8.9 cm
P-06	Right	15.2 – 17.8 cm	8.9 cm
P-07	Left	16.5 – 18.4 cm	9.2 cm
P-08	Left	15.2 – 17.1 cm	8.9 cm

Demarcated regions are listed in Table 3 and illustrated in Figure 4 (bovine) and Figure 5 (porcine). Eight equidistant regions—based on the femur's total diaphysis length in Table 2—were marked in pencil starting at the midshaft diaphysis, then extended towards the proximal and distal metaphyses. Midshaft was defined as the central point between the proximal and distal epiphyseal lines. The distal epiphysis, femoral head, and trochanter were individually marked.

Finally, an articulating surface located on the diaphysis's distal posterior aspect was demarcated. Fourteen regions in total were marked and given numbered identifiers: twelve from the femur, and one for the talus and calcaneus respectively. Marked bones were stored at room temperature until tested.

Table 3: Demarcated Regions of the Femur and Tarsals

Region	Location
1	Midshaft Diaphysis
2	Midshaft Diaphysis (Distal to Region 1)
3	Diaphysis (Proximal to Region 1)
4	Diaphysis (Distal to Region 2)
5	Diaphysis (Proximal to Region 3)
6	Diaphysis (Distal to Region 4)
7	Proximal Metaphysis
8	Distal Metaphysis
9	Articulating Surface
10	Distal Epiphysis
11	Femoral Head
12	Trochanter
13	Calcaneus (Tarsal Bone)
14	Talus (Tarsal Bone)

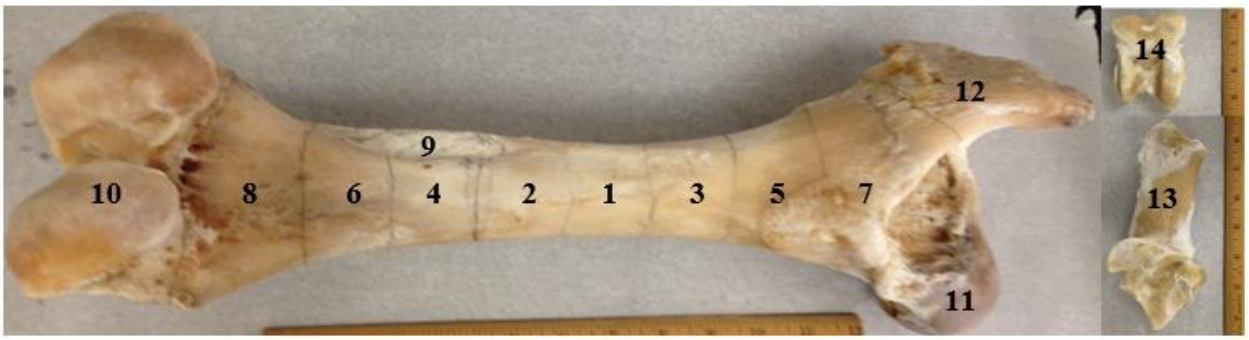

Figure 4: Bovine Femur, Talus, and Calcaneus with Labeled Regions
Femur on the left, talus on the upper right, and calcaneus on the lower right. Numbers correspond to regions in Table 3. Femur and tarsal bones are not to scale.

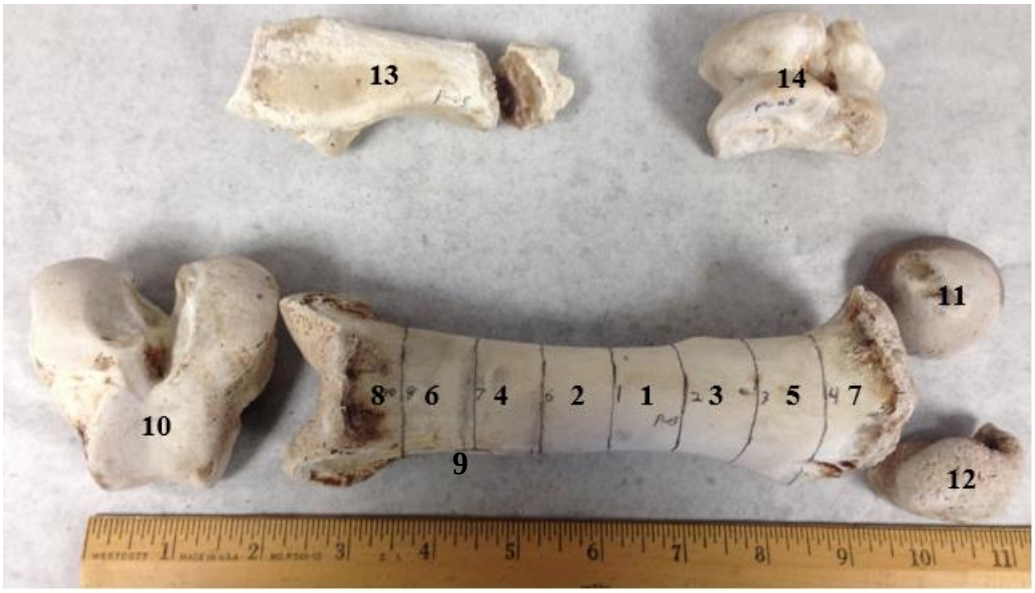

Figure 5: Porcine Femur, Talus, and Calcaneus with Labeled Regions
Immature femur on the bottom with unfused femoral head and trochanter (right side), and unfused distal epiphysis (left side). Talus displayed on the top right, and calcaneus on the top left. Bolded numbers correspond to regions in Table 3. Numbers penciled on femoral diaphysis (visible in picture) were changed to bolded numbers. Region 9 (partially visible in picture) is located on the lateral distal aspect of the femur.

Surface/Burial Comparison Experiment of Bovine Femora and Tarsals

Four fresh femora and tarsal sets from two Holstein dairy cows were obtained from the MSU Meats Laboratory. Regions tested are listed in Table 4, five along the femur, as well as the calcaneus and talus.

Table 4: Regions of Bovine Femora and Tarsals Tested

Identifier	Location	Equivalent Region(s) from Table 3
D	Midshaft Diaphysis	Regions 1 and 2
PM	Proximal Metaphysis	Region 7
DM	Distal Metaphysis	Region 8
F	Femoral Head	Region 11
E	Distal Epiphysis	Region 10
Cal	Calcaneus	Region 13
Tal	Talus	Region 14

Soft tissue/cartilage was mechanically defleshed to expose drilling sites for each region using knives and razorblades. Calcanei were fully, and tali partially, disarticulated from the tarsal set to expose bones for drilling. Regions of bone (Table 4) were initially tested to establish pretreatment DNA yields. Figure 6 depicts the experimental setup of how the bones were exposed. Two femora and tarsal sets—one per individual cow—were labeled and buried in approximately eight inches of black garden soil. The other femora and tarsals were placed on the soil surface above the buried bones. Surface bones were encased by a mesh wire fence weighted down with stones to deter scavenger activity. Bones were located at the geographical coordinates 42° 44′ 43.8″ N 84° 17′ 09.4″ W.

Figure 6: Surface/Burial Setup for Bovine Skeletal Material

Bones were retrieved, tested, and returned at the time points listed in Table 5. Skeletal material was washed onsite using a garden hose to remove excess soil, debris, and insects; then bagged separately and transported to the MSU Forensic Biology Laboratory. There the bones were further cleaned of soil and debris using RO water and a brush, tested, re-bagged, and brought back to the experiment site for reburial/re-placement.

Table 5: Dates Buried/Surface Femora and Tarsals Were Tested

Date Tested	Time Point	Days Exposed/Buried
9/19/2014	Day 0	0
9/26/2014	Week 1	7
10/3/2014	Week 2	14
10/17/2014	Month 1	28
12/13/2014	Month 3	85
3/21/2015	Month 6	177

Drilling Skeletal Material

Bones were drilled inside a Purifier PCR Enclosure (Labconco, Kansas City, MO)—an enclosed ultraviolet (UV) workstation—sanitized with 10% bleach, 70% ethanol, and then the UV lamp was activated for 10 min (unknown J/cm^2) prior to and after drilling. Drill bits, collars, and sleeves were soaked in 10% bleach for 10 min, rinsed with Milli-Q® filtered water (18.2 MΩ), rinsed with 70% ethanol, then UV irradiated in a Spectrolinker™ XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) for 10 min prior to and after use (~2.5 J/cm^2). Other supplies used: including a Dremel 395 MultiPro® rotary device (Robert Bosch Tool Corporation, Mount Prospect, IL), weighing paper (VWR International, Radnor, PA), microspatula, and 2.0 mL microcentrifuge tubes were UV irradiated for 10 min in the Spectrolinker™. Sanitized equipment not in use were stored in a CleanSpot PCR/UV Work Station (Coy Laboratory Products, Grass Lake, MI) with the UV lamp activated (unknown J/cm^2).

Bones containing soil/debris were washed in RO water, then allowed to air dry for at least 10 min. Each bone's outer cortical surface was sanded using a Dremel® number 420 heavy duty cut-off wheel to remove surface contaminants. M35 7/64 inch cobalt drill bits (B&Q International, Eastleigh, England) were used to drill the bone's cortical surface at four locations

per bone/region/segment. Drilling locations were maximally separated, so collected powders would be representative of the bone/region/segment being drilled. Powders produced from the drillings were combined and homogenized using a microspatula. Two milliliter microcentrifuge tubes were weighed on a PB153-S precision balance (Mettler-Toledo, Columbus, OH), then 50 +/- 1 mg of the homogenized powder was added to each tube. Microcentrifuge tubes containing powder were stored at -20°C pending digestion and extraction.

DNA Isolation

Organic extraction coupled with extract concentration using 30K Amicon® Ultra-0.5 mL Centrifugal Filters (Millipore Corporation, Billerica, MA) was utilized to isolate DNA from bone powder; however, two variants were employed depending on the experiment. Method used per experiment is listed in Table 6. Reagent blanks were generated for each extraction. DNA extracts and reagent blanks were stored at -80°C.

Table 6: Organic Extraction Method Used per Experiment

Experiment	Organic Extraction Method
Bovine/Porcine Intra-bone Variation and Tarsal Comparison Experiments	Method One
Surface/Burial Comparison Experiment of Bovine Femora and Tarsals	Method One
Changes in the Recoverable Total DNA of Buried Bovine Bone Segments over a One Month Time Period	Method Two
Changes in the Recoverable Total DNA of Non-Buried Bovine Bone Segments over a One Month Time Period	Method Two
Organic versus SoilMaster™: Total DNA Yield Comparisons Over One Week	Method Two
Mass Difference between Wet and Dry Bone	Not Applicable
Effect of Proteinase K Concentration on Total DNA Yields	Method One
Comparison of Total DNA Yields from Bovine Bones Macerated by MSU Forensic Anthropologist versus MSU Forensic Biologist	Method One

Organic Extraction Method One

Tubes, Amicon® columns, and solutions, except organic solvents and proteinase K, were UV irradiated for 10 min (~2.5 J/cm^2). Two extraction buffers were used side by side: bone powder was digested in a 1:15 ratio, of either tissue lysis buffer (20 mM Tris—pH 7.5; 50 mM EDTA; 0.1% SDS) or demineralization buffer (Loreille et al., 2007) (0.5 M EDTA—pH 8.0; 1% lauryl-sarcosine (SLS)). One percent by total solution volume proteinase K (20 mg/mL) was added to the microcentrifuge tubes containing bone powder and buffer, then vortexed for 15 s and incubated overnight at 56°C on an Orbit™ P2 Digital Shaker (Labnet International, Edison, NJ) set to 250 RPM. The Orbit™ P2 Digital Shaker had a maximum capacity of 16 microcentrifuge tubes. Some extractions were done in batches of 30, in these instances tubes were incubated overnight at 56°C on an model 100 Rocking Platform set to six (VWR International, Radnor, PA).

An equal volume of cold 1:1 phenol chloroform solution was added to the tubes, vortexed for 15 s, and centrifuged for 10 min at maximum speed. Four hundred microliters of the aqueous layers were transferred to 30K Amicon® filters and centrifuged for 10 min at 14,000 × g. An additional 200 µL of the aqueous layers were transferred to the columns and centrifuged for 10 min at 14,000 × g. Flow-through was discarded and the columns were washed with 400 µL of TE (10 mM Tris—pH 7.5, 1 mM EDTA) and centrifuged for 10 min at 14,000 × g. Flow-through was discarded, and the TE wash was repeated. Flow-through was discarded, and 400 µL of low TE (10 mM Tris—pH 7.5; 0.1 mM EDTA) was added to the columns, which were centrifuged for 10 min at 14,000 × g. Columns were inverted into new Amicon® tubes and centrifuged for 2 min at 1,000 × g to collect extracts. Extract volumes were measured and recorded prior to -80°C storage.

Organic Extraction Method Two

Tubes, Amicon® columns, and solutions, except organic solvents and proteinase K, were UV irradiate for 10 min (~2.5 J/cm^2). Five hundred microliters of tissue lysis buffer and 4.5 µL of proteinase K were added to the 2.0 mL microcentrifuge tubes with bone powder, vortexed for 15 s, then incubated overnight at 56°C. An equal volume of cold, Tris-saturated phenol was added to the tubes, vortexed for 15 s, and centrifuged for 5 min at maximum speed. Aqueous layers were transferred to new 2.0 mL microcentrifuge tubes, to which 500 µL of chloroform was added. Tubes were vortexed for 15 s, and centrifuged for 5 min at maximum speed. Aqueous layers were transferred to 30K Amicon® filters and processed as above.

Quantitative PCR Assay for Bovine and Porcine DNA Extracts

Real time PCR amplification was performed on an iCycler thermal cycler, and fluorescence detected using an iQ5 multi-color real-time PCR system (Bio-Rad Laboratories, Hercules, CA). Primer and probe sequences are listed in Table 7. Bovine primers and probes targeting the nuclear *Melanocortin-1-Receptor* (*MC1R*) gene, as well as the Internal Positive Control (IPC) primers, probe, and template were designed by Lindquist et al. (2011). Primers and probe targeting the mitochondrial *ATPase 8* gene were designed by Hebda (2013). Porcine primers and probe targeting the mitochondrial *ATPase*, and nuclear *MC1R* genes were designed using Primer3 software (Rozen and Skaletsky, 2000), based on the *Sus scrofa* complete mtDNA (BLAST Accession NC_00845.1), and *Sus scrofa* mixed breed chromosome six DNA (BLAST Accession NC_010448.3) from the National Center for Biotechnology Information. Primers and probes were ordered from Integrated DNA Technologies (Coralville, IA), Thermo Fisher

Scientific (Waltham, MA), or Sigma-Aldrich (St. Louis, MO). Bovine DNA standards were created via stock bovine muscle digested in tissue lysis buffer, organically extracted (Method Two), and diluted to 200 ng/µL based off parallel qPCR data of bovine standard from Hebda (2013). Porcine DNA standards were created by serial dilution of stock DNA from pig muscle digested in tissue lysis buffer and organically extracted (Method Two), then quantified using a DU-520 UV-Visible Spectrophotometer (Beckman Coulter Inc., Brea, CA). Serial dilutions (1:3) were created using low TE containing 20 µg/mL glycogen, making standard concentrations of 50, 16.67, 5.56, 1.85, 0.62, 0.21, 0.069, and 0.023 ng/µL.

Real-time PCR reactions were set up in 0.2 mL optically clear flat-capped PCR strips (USA Scientific®, Ocala, FL) in a 15 µL volume. Concentrations of ingredients are listed in Table 8. The recipe was based on Lindquist et al. (2011), with modification by Hebda (2013). DNA standards were ran in duplicate. Cycling parameters used are listed in Table 9. Standard curves were generated by iQ™5 Optical System Software, and were used to calculate the DNA concentration (ng/µL) of each extract. DNA concentration was standardized by calculating real-time PCR concentration (ng/µL), multiplied by total extract volume (µL), and divided by the mass of bone powder (mg) to form nanograms DNA recovered per milligram bone powder (ng/mg).

Table 7: Primer and Probe Sequences for qPCR

Primer Name	Sequence	Amplicon Length
F Cow *ATPase 8*	5′-CAA AAC ACC CCT TGA GAA ACA-3′	88 bp
R Cow *ATPase 8*	5′-AGG GTT ACG AGA GGG AGA CC-3′	
Cow *ATPase 8* probe	5′-6FAM-CCT CTT TTA TTA CCC CTG TAA TTT T-BHQ1-3′	
F Cow *MC1R*	5′-AAT AAA TCA TAA ACC AGC CTG CTC TTC ATC AC-3′	77 bp
R Cow *MC1R*	5′-AAT AAA TCA TAA AGC TAT GAA GAG GCC AAC GA-3′	
Cow *MC1R* probe	5′-6FAM-CAC AAG GTC ATC CTG CTG TGC C-MGBNFQ-3′	
F Pig *ATPase*	5′-AGC TCT GAT CCA AGC TTA TGT GT-3′	83 bp
R Pig *ATPase*	5′-GCA TGT GTT TGG TGG GTC A-3′	
Pig *ATPase* probe	5′-6FAM-TGC TAG TAA GCT TAT ACC TAC ACG ACA-BHQ1-3′	
F Pig *MC1R*	5′-GCC CGG TTC CTA CGT G-3′	82 bp
R Pig *MC1R*	5′-AGA GGG TCC AGC GTC CAT A-3′	
Pig *MC1R* probe	5′-6FAM-CGG GCC GGA CAT CTC TGA-BHQ1-3′	
F IPC	5′-AAG CGT GAT ATT GCT CTT TCG TAT AG-3′	77 bp
R IPC	5′-ACA TAG CGA CAG ATT ACA ACA TTA GTA TTG-3′	
IPC probe	5′-VIC-TAC CAT GGC AAT GCT-MGBNFQ-3′	
IPC template	5′-AAG CGT GAT ATT GCT CTT TCG TAT AGT TAC CAT GGC AAT GCT TAG AAC AAT ACT AAT GTT GTA ATC TGT CGC TAT GT-3′	

Table 8: Real-time PCR Reaction Recipe and Concentrations

Ingredient	Volume (WC) added per 15 µL Reaction	Working Concentration (WC)	Final Concentration
iQ™ Supermix (Bio-Rad Laboratories)	7.5 µL	2×	1×
Forward Primer (*ATPase* or *MC1R*)	0.9 µL	10 µM	600 nM
Reverse Primer (*ATPase* or *MC1R*)	0.9 µL	10 µM	600 nM
Target Probe (*ATPase* or *MC1R*)	0.25 µL	15 µM	250 nM
Forward Primer IPC	0.75 µL	20 µM	1 µM
Reverse Primer IPC	0.75 µL	20 µM	1 µM
IPC Probe	0.25 µL	15 µM	250 nM
IPC Template DNA	1.0 µL	1:1 billion dilution of 100 µM	1:66.7 billion dilution of 100 µM
Taq DNA Polymerase (Syzygy)	0.125 µL	5 U/µL	0.625 Units
Milli-Q® Water	1.325 µL	-	-
DNA extract / Standard DNA	1.2 µL	-	-

Table 9: qPCR Thermal Cycler Parameters

Temperature	Time	Cycles
95°C	3 min	1
95°C	15 s	50
60°C	1 min	

Qualitative PCR Assay for Bovine and Porcine DNA Extracts

Primers that generate a ~200 bp amplicon (181 – 257 bps), ~400 bp amplicon (390 – 457 bps), ~600 bp amplicon (599 – 642 bps), and ~1,000 bp amplicon (989 – 1017 bps) were utilized to evaluate DNA quality of recovered bovine and porcine mitochondrial and nuclear DNAs. Primers, sequences, and amplicon sizes are summarized in Table 10. Bovine and porcine primers were designed using Primer3 targeting bovine *ATPase* (BLAST Accession NC_006853), porcine *ATPase* (BLAST Accession NC_00845.1), and bovine *MC1R* (BLAST Accession

AC_000175) gene sequences. Porcine nuclear DNA primers targeting the *Insulin Growth Factor-1* gene (*IGF-1*) were designed by Michaud and Foran (2011).

MtDNA quality testing began with the ~1,000 bp amplicon. Failed amplification resulted in successive testing of primer sets that produce smaller amplicons until amplification occurred. Nuclear DNA amplification began at the ~400 bp target, and was tested stepwise until successful amplification was no longer attained, or achieved when the ~400 bp target failed to amplify.

Ten microliter PCR reactions consisted of: 1 µL of GeneAMP 10× PCR Buffer II (Applied Biosystems, Carlsbad, CA), 1 µL of 25 mM $MgCl_2$ (Applied Biosystems), 1 µL of 20 µM F and R primer, 1 µL of 2 mM deoxynucleotide 5′-triphosphates, 0.2 µL of AmpliTaq Gold® DNA Polymerase (5 U/µL, Applied Biosystems), 4 µL of Milli-Q® filtered water, and 1 µL of template DNA diluted to ~1 ng/µL based on quantification assay data. PCR cycling parameters are summarized in Table 11.

Amplified products were assessed via agarose gel electrophoresis. Five microliters of PCR products were separated on a 1% agarose gel stained in ethidium bromide (Sigma-Aldrich). Gels were photographed using an Olympus C-4000 Zoom digital camera (Olympus, Center Valley, PA). Digital photographs were labeled using Adobe Photoshop™ 7 software and printed using an UP-D895 thermal printer on UPP-110S thermal print media (Sony Corporation, Tokyo, Japan). Digital photographs were saved prior to and after modification with Photoshop™.

Table 10: Primer Sequences for Qualitative PCR Assay

Primer Name	Sequence	Amplicon Length
F Cow *MC1R* 200 bp	5′-CAA GGA CTT CAT GAC CAG CA-3′	200 bp
R Cow *MC1R* 200 bp	5′-TAC TGC TGC ACT GCT TCC TG-3′	
F Cow *MC1R* 400 bp	5′-CTG CTG GGT TCC CTT AAC TG-3′	410 bp
R Cow *MC1R* 400 bp	5′-ATG GAG ATG TAG CGG TCC AC-3′	
F Cow *MC1R* 600 bp	5′-GTG GAC CGC TAC ATC TCC AT-3′	599 bp
R Cow *MC1R* 600 bp	5′-CCT CTT TGT CAA GGG ACT GC-3′	
F Cow *MC1R* 1 kb	5′-CTG CTG GGT TCC CTT AAC TG-3′	989 bp
R Cow *MC1R* 1 kb	5′-CCT CTT TGT CAA GGG ACT GC-3′	
F Cow *ATPase* 200 bp	5′-TCG CTT TGT AAC CCT CCA AC-3′	201 bp
R Cow *ATPase* 200 bp	5′-GGG ATG GCT ATG CCT AGG TT-3′	
F Cow *ATPase* 400 bp	5′-CGA CAA AGC TGA CCC ATA CA-3′	390 bp
R Cow *ATPase* 400 bp	5′-CTG GGA TTG CGT CTG TTT TT-3′	
F Cow *ATPase* 600 bp	5′-CTG TGA GCA GGA GCC GTA AT-3′	607 bp
R Cow *ATPase* 600 bp	5′-ATT CCA TAA CGG AGG CCT TT-3′	
F Cow *ATPase* 1 kb	5′-CCC GCC ATC ATC TTA ATT CT-3′	994 bp
R Cow *ATPase* 1 kb	5′-TGG TGT GAA TGA ATG GGG TA-3′	
IGF-1 Forward	5′-AAT CAT TTG CCC CTC AAG TG-3′	N/A
IGF-1 R257	5′-TGA CCC CCT CAT CCT AGT TG-3′	257 bp
IGF-1 R457	5′-GGC AGG AAG ACA CAC ACA TC-3′	457 bp
IGF-1 R642	5′-TCT CTC CCT CTT CTG GCA AA-3′	642 bp
F Pig *ATPase*	5′-TGG ATC AAA CCA CAG CTT CA-3′	N/A
R Pig *ATPase* 200 bp	5′-TGT GGA TGT ATC TAG TTG TGG CAT A-3′	181 bp
R Pig *ATPase* 400 bp	5′-TGG GAA TAG TAA GCT TGG GAA T-3′	414 bp
R Pig *ATPase* 600 bp	5′-TGG TGG GTG TGA ATG AGT GT-3′	604 bp
R Pig *ATPase* 1 kb	5′-CAT GTG TTT GGT GGG TCA TT-3′	1017 bp

Table 11: PCR Thermal Cycler Parameters

Annealing temperature varied among primer sets. Bovine *ATPase 8* and *MC1R* primers used 58°C, porcine *ATPase* used 60°C, and *IGF-1* used 56°C.

Temperature	Time	Cycles
94° C	10 min	1
94° C	30 s	38
56°C – 60°C	1 min	
72° C	45 s	
72° C	5 min	1
4° C	Infinite	Hold

Ancillary Experiments

Changes in the Recoverable Total DNA of Buried Bovine Bone Segments over a One Month Time Period

Two fresh femora from four year old Holstein dairy cows were obtained from the MSU Meats Laboratory, and segmented using an electric butchers saw at their facility. Two segment types were derived from femoral diaphysis: one was quartered longitudinally and cut into ~1 in segments (type A), while the other was hemisected longitudinally and cut into ~3 in long segments (type B). Bone segments were stored at -80°C prior to burial. The 3 in segments underwent a weekly cycle of exhumation, testing, and reburial, while the 1 in segments were exhumed, tested, and stored at -20°C. Exterior soft tissue was excised/scraped off using a razorblade, and marrow was removed. Segments were buried in 8 – 12 in of soil near Giltner Hall on the MSU campus. Photographs of the burial site's location are shown in Figures 7 and 8.

Four bone segments were drilled per time point. Burial and retrieval dates are shown in Table 12. Segments were cleaned using RO water and a brush to remove soil and debris prior to drilling. Bone powders from drillings were digested in tissue lysis buffer, organically extracted and quantified. DNA extracts were stored at -80°C.

Figure 7: Burial Site for Bovine Bone Segments
Giltner Hall's south side and parking lot, red box oriented to picnic area (Left).
Path leading to picnic area, red box oriented towards burial site (Middle).
Undisturbed burial site for bone segments within picnic area (Right).

Figure 8: Giltner Hall Burial Site with Bovine Diaphysis Segments
Buried bone segments that were exhumed, tested, and reburied on a weekly basis (left).
Partitioned burial site containing bone segments exhumed, tested, and then stored (right).
Burial sites between segment types were adjacent to one another.

Table 12: Burial Dates of Bovine Femora Segments
Segments identified by burial time (0D, 2D, 4D, 1W, 11D, 2W, 3W, 4W) and by replicate (1 – 4), where D = day(s), W = week(s). Segments denoted with (A) were retrieved, drilled, and stored at -20°C. Segments denoted with (B) were cyclically retrieved, drilled, and reburied.

Segment Identifier	Date Buried	Date Retrieved	Number of Days Buried
0D-1 – 4 (A)	Not Buried	9/19/2013	0
2D-1 – 4 (A)	9/19/2013	9/21/2013	2
4D-1 – 4 (A)	9/19/2013	9/23/2013	4
1W-1 – 4 (A) & (B)	9/19/2013	9/26/2013	7
11D-1 – 4 (A)	9/19/2013	9/30/2013	11
2W-1 – 4 (A) & (B)	9/19/2013	10/3/2013	14
3W-1 – 4 (A) & (B)	9/19/2013	10/10/2013	21
4W-1 – 4 (A) & (B)	9/19/2013	10/17/2013	28

Changes in the Recoverable Total DNA of Non-Buried Bovine Bone Segments over a One Month Time Period

Four fresh hemisected segments of bovine femoral diaphysis were retrieved from -80°C storage. External soft tissue was excised using a razorblade, and marrow removed. Bones were placed in labeled weigh boats, and placed in vacant office room. Bone segments were drilled for bone powder on the dates listed in Table 13. Powder from drillings was collected, digested in tissue lysis buffer, organically extracted, and quantified. DNA extracts were stored at -80°C.

Table 13: Exposure Dates of Bovine Femora Segments
Segments identified by time exposed (0D, 2D, 4D, 1W, 10D, 2W, 3W, 4W) and by replicate (1–4), where D = day(s), W = week(s).

Segment Identifier	Dates Tested & Returned	Days Exposed	Segment Identifier	Dates Tested & Returned	Days Exposed
0D-1	11/5/2013	0	10D-1	11/15/2013	10
0D-2			10D-2		
0D-3			10D-3		
0D-4			10D-4		
2D-1	11/7/2013	2	2W-1	11/19/2013	14
2D-2			2W-2		
2D-3			2W-3		
2D-4			2W-4		
4D-1	11/9/2013	4	3W-1	11/26/2013	21
4D-2			3W-2		
4D-3			3W-3		
4D-4			3W-4		
1W-1	11/12/2013	7	4W-1	12/3/2013	28
1W-2			4W-2		
1W-3			4W-3		
1W-4			4W-4		

Organic versus SoilMaster™: Total *DNA Yield Comparisons Over One Week*

A segment of fresh bovine diaphysis was taken from -80°C storage, defleshed, and tested at 0, 2, 5, and 7 days. The diaphysis segment was stored in a vacant office for the one week experiment period. Two hundred milligrams of powdered bone, taken at each time point, was homogenized and split between two extraction methods: organic and SoilMaster™. Organic extractions were conducted using Method Two. SoilMaster™ extractions followed manufacturer's protocol in conjunction with modifications used in Hebda (2013), with one alteration: after soil lysis buffer incubation, the microcentrifuge tube was centrifuged for 2 min at 10,000 × g, followed by transfer of all supernatant to a new tube. DNA extracts were quantified then stored at -80°C.

Mass Difference between Wet and Dry Bone

A segment of bovine femoral diaphysis was weighed over time to compare mass deference between hydrated versus dehydrated bone. External soft tissue was excised using a razorblade, and marrow removed. Approximately 500 milligrams of bone powder was evenly distributed among four storage containers tested in duplicate, along with the remaining bone segment, and are listed in Table 14. Materials were stored in the CleanSpot PCR/UV Work Station for the duration of the experiment. Microcentrifuge tubes and weigh boats were weighed prior to and after the addition of bone powder, and subsequently weighed along with the remaining bone segment for a two week period at time points listed in Table 15.

Table 14: Environmental Conditions Influence on Bone Mass Change

Treatment	Description
1	Closed 1.5 mL microcentrifuge tube
2	Open 1.5 mL microcentrifuge tube
3	Powder in condensed pile in weigh boat
4	Powder spread along surface of weigh boat
5	Diaphysis segment minus extracted powder

Table 15: Time Points Tested for Bone Mass Change

Time points listen in first and third columns. "1 – 5" are treatments weighed at given time point and correspond to Table 14.

Time Point	Treatment Weighed	Time Point	Treatment Weighed
0 min	1 – 5	23.5 hours	1 – 5
30 min	1 – 5	48.5 hours	1 – 5
1 hour	1 – 5	75 hours	1 – 5
2 hours	1 – 5	100 hours	1 – 5
3 hours	1 – 5	175 hours	5
4 hours	1 – 5	250 hours	5
5 hours	1 – 5	325 hours	5
6 hours	1 – 5		

Effect of Proteinase K Concentration on Total DNA Yields

External soft tissue was excised using a razorblade, and morrow removed from a segment of fresh bovine diaphysis retrieved from -80°C storage. Approximately one gram of bone powder was generated by drilling, homogenized, and then 50 mg +/- 1 mg aliquots of powder were digested using demineralization or tissue lysis buffer with 0%, 0.5%, 1%, or 2% by total solution volume proteinase K (20 mg/mL) added. DNA was organically extracted and nuclear and mitochondrial DNAs were quantified in duplicate.

Comparison of Total DNA Yields from Bovine Bones Macerated by MSU Forensic Anthropologist versus MSU Forensic Biologist

C-01 and C-02 were immature bones based on incomplete fusion of the epiphyses; however, they were also processed by the MSU Forensic Anthropology Laboratory. C-03 – C-08 had completely fused epiphyses and were processed by the MSU Forensic Biology Laboratory. Assessing how DNA yields were affected by methodology, a pair of bovine femora and tarsals from a Holstein steer were obtained from the MSU Meats Laboratory. One femur and tarsal set was given to the MSU Forensic Anthropology Laboratory for maceration using their standard protocols. The other femur and tarsal set was macerated at the MSU Forensic Biology Laboratory using boiling 1% Tergazyme™ in two eight-hour intervals. Bone powder (50 mg +/- 1 mg) was recovered from post-macerated femora by drilling at the midshaft diaphysis, distal epiphysis, femoral head, and the calcanei, and tali. Powders were digested in tissue lysis and demineralization buffers, organically extracted, then mitochondrial and nuclear DNAs were quantified.

Statistical Analysis of Bovine/Porcine Intra-bone Variation and Tarsal Comparison Experiments

Statistical analyses were performed using XLSTAT version 2014.2.01 (Addinsoft, New York, NY). Anderson-Darling and Shapiro-Wilk tests were used to determine normality. Kruskal-Wallis was conducted to determine analysis of variance followed by Dunn's procedure (two-tailed) for post hoc multiple pairwise comparisons of non-combined mitochondrial and nuclear DNA quantification data. Combined femoral quantification data were analyzed using Kruskal-Wallis followed by a Bonferroni adjusted Dunn's procedure (two-tailed). Regions were combined based on common features: Regions 1 – 6 (diaphysis), Regions 7 – 9 (metaphyses and articulating surface), Regions 10 – 12 (epiphyses), and Regions 13 – 14 (tarsals). Individual pairwise comparisons between combined femoral regions and tarsals, and differences in DNA quantity and quality between digestion buffers were done using Mann-Whitney U. DNA quality was ranked prior to utilizing Mann-Whitney U based on amplicon generated: 4 for ~1,000 bp, 3 for ~600 bp, 2 for ~400 bp, 1 for ~200 bp, and 0 for no amplification. Statistical significance was determined for all tests at $\alpha < 0.05$.

RESULTS

Qualitative Observations from Processing Porcine and Bovine Femora and Tarsals

Post maceration, differences in age were detectable among bones. Bovine C-01 and C-02 femora were immature, based on incomplete fusion of the distal epiphysis and non-fused femoral head and trochanter. C-03 – C-08 were older, determined by the complete fusion of the distal and proximal epiphyses. Porcine P-01 – P-08 were immature, containing non-fused distal and proximal epiphyses. As bones from both species were stored, their exterior surface became greasy, beginning at the epiphyses/metaphyses and migrating towards the midshaft diaphysis.

Drilling porcine bones was easier than their bovine counterparts, which were harder and required greater force to acquire the necessary powder. Conversely, drilling the porcine epiphyses required a soft touch to obtain powder from the thin layer of cortical bone. This was achieved by taking the tip of the drill bit (while the device was on) and lightly touching the bone's surface, then moving the drill back and forth to "shave" off the cortical layer. Qualitatively, there were often region dependent differences in color and consistency of the bone powder. Regions 1 – 6 and 9, in both species, generally produced a dry fine grain white powder. Porcine Regions 7, 8, and 10 – 12 often produced a darker brown powder (not due to thermal damage). Bovine bone powder from Regions 7, 8, and 10 – 12 had a white to yellowish coloration with larger grains and appeared somewhat greasy. Regions 13 and 14 had an intermediate appearance to Regions 1 – 6 and 9, and that of Regions 7, 8, and 10 – 12.

Environmental exposure affected characteristics of the bones both visually and physically. Soft tissue and cartilage on non-buried bone dehydrated and hardened around the element and persisted beyond the six month experiment window. Soft tissue on buried bone

putrefied rapidly during the first month, and largely vanished by three months. Exposed and buried bones had insect activity present during the first month of environmental treatment. Decomposition progressed enough by week 4 to determine that replicates A2/B2 were immature bones based on incomplete fusion of the epiphyses. Buried bones had a brownish discoloration that stained their surface after a few weeks. Environment also influenced the "softness" of bones. Drilling non-buried bone was similar to fresh bone, while buried bone was easier to drill after one week, which was maintained throughout.

Figure 9 depicts differences between digestion buffers using bone powder from an epiphysis. Bone powder remained in tissue lysis buffer, collecting at the bottom of the aqueous/organic mixture. Conversely, demineralization buffer dissolved the bone completely. Bone powder from the epiphyses and metaphyses often formed a white interface between the aqueous and organic phases, whereas the diaphysis did not. This interphase occurred using both buffers; however, this was more pronounced in tissue lysis extractions.

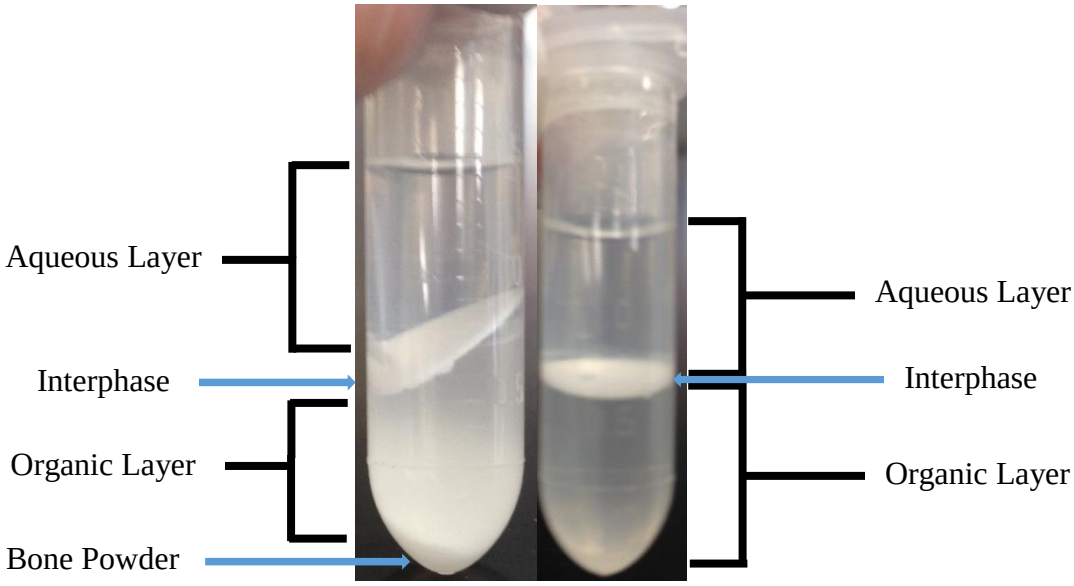

Figure 9: Organic Extraction of Epiphyseal Bone Powder
Picture on the left is an example of bone powder digested in tissue lysis buffer; note the undigested powder at the bottom of the microcentrifuge tube, and the interface between layers. Picture on the right is from the same stock of bone powder digested in demineralization buffer, it contains a less defined interface and no bone powder.

Inter-Bone and Intra-Bone Variation of Recoverable Total DNA in Fresh Porcine and Bovine Femora and Tarsals

Normality Testing of Porcine and Bovine Bones

DNA quantity data from some regions of fresh porcine and bovine bone did not follow a normal distribution. Porcine quantification data had mitochondrial and nuclear DNA yields that were not normally distributed, though nuclear DNA had more, and data from DNAs isolated in tissue lysis buffer were more likely than demineralization buffer to be non-normally distributed. Individual p-values from normality testing of DNA quantification data derived from porcine bones, per region and digestion buffer, are listed in Appendix A1.

Bovine DNA quantity data were not normally distributed in about half the regions for both mitochondrial and nuclear DNA using both buffers. There were more non-normally

distributed regions of nuclear than mitochondrial DNA data. Nuclear DNA data were more likely to be non-normally distributed at the epiphyses, while mtDNA data were at the diaphysis. Combined regional quantification data were not normally distributed in all but one instance, but only from the Anderson-Darling test: mtDNA from the tarsals digested in tissue lysis buffer ($p = 0.069$).

C-01 and C-02 contributed substantially to these non-normal distributions; when they were removed from analysis only mtDNA recovered from Region 6 ($p < 0.04$) and nuclear DNA from Region 4 ($p < 0.04$) digested in demineralization buffer were not normally distributed. Combined mtDNA yields from the diaphysis digested in both buffers ($p < 0.04$), and DNA from the epiphyses digested in demineralization buffer ($p < 0.03$) were not normal. MtDNA from the metaphyses and articulating surface digested in tissue lysis buffer had inconsistent significance between Anderson-Darling ($p = 0.060$) and Shapiro-Wilk ($p = 0.019$) tests. Individual p-values from normality testing of DNA quantification data derived from bovine bones, per region and digestion buffer, are listed in Appendix A2 (with C-01 and C-02) and A3 (without C-01 and C-02).

Quantification of Total DNA from Fresh Porcine Femora and Tarsals

Median mtDNA yields from porcine bone powder digested in tissue lysis (Table 16) and demineralization buffer (Table 17) were lowest in Regions 1 – 4, with higher quantities along regions of the diaphysis closer to the proximal and distal metaphyses. The exception to this was Region 9, which had the highest mtDNA yields from the diaphysis. Regions 10 – 12 had higher mtDNA yields than Regions 1 – 6, 13, and 14. Regions 13 and 14 had similar yields as Regions 7 – 9. These results are graphically depicted in Figure 10. Combined regional data for mtDNA

yields from bone digested in tissue lysis and demineralization buffers, listed in Table 18 and graphically depicted in Figure 11 were highest at the epiphyses, followed by intermediate yields from the tarsals, metaphyses and articulating surface, and the diaphysis had the lowest yields. Individual values for these data, including normalization parameters and total mtDNA recovered, are available in Appendix B1 – B16. Efficiencies of qPCR ranged from 93.6 to 100.0 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.976 to 0.994. No PCR inhibition was detected via the IPC.

Table 16: mtDNA Yields from Fresh Porcine Bones Digested in Tissue Lysis Buffer

Regions and concomitant locations correspond to Table 3. P-(01 – 08) are biological replicates. MtDNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements. NT = Not Tested

Region	Location on Element	mtDNA Yield (ng/mg)									
		P-01	P-02	P-03	P-04	P-05	P-06	P-07	P-08	Median	MAD
1	Midshaft Diaphysis	5.31	11.98	16.96	6.66	7.39	16.85	10.25	18.62	11.12	5.09
2	Midshaft Diaphysis	3.74	5.06	NT*	5.30	20.55	13.09	11.52	16.05	8.41	6.34
3	Proximal Diaphysis	9.55	5.81	25.93	7.60	10.12	14.94	16.49	15.81	12.53	3.62
4	Distal Diaphysis	4.45	5.60	15.41	10.66	9.05	12.45	8.05	14.07	9.86	3.40
5	Proximal Diaphysis	18.35	9.63	40.84	35.94	40.37	29.07	41.35	28.39	32.50	8.10
6	Distal Diaphysis	21.13	6.90	25.13	17.74	16.87	23.59	19.61	23.14	20.37	2.99
7	Proximal Metaphysis	31.02	29.26	73.14	51.82	52.69	40.88	44.89	28.27	42.89	10.83
8	Distal Metaphysis	31.14	25.54	43.75	45.85	37.95	43.70	39.36	33.26	38.66	5.25
9	Articulating Surface	59.76	32.35	72.24	38.66	36.21	54.62	46.53	54.55	50.54	10.55
10	Distal Epiphysis	323.92	146.67	175.70	163.77	205.20	156.82	110.17	110.71	160.30	30.15
11	Femoral Head	175.06	85.60	98.67	91.27	171.20	86.59	104.39	86.94	94.97	8.88
12	Trochanter	172.04	89.80	179.59	89.85	202.88	157.48	198.45	151.96	164.76	24.26
13	Calcaneus	43.44	39.10	55.77	38.88	73.71	32.47	53.04	36.66	41.27	6.70
14	Talus	82.60	51.52	67.76	51.54	53.25	57.60	69.41	44.31	55.43	7.51

* Microcentrifuge Tube for P-03 Region 2 digested in tissue lysis buffer ruptured during incubation and the supernatant was lost and as a result not tested.

Table 17: mtDNA Yields from Fresh Porcine Bones Digested in Demineralization Buffer

Regions and concomitant locations correspond to Table 3. P-(01 – 08) are biological replicates. MtDNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements.

Region	Location on Element	mtDNA Yield (ng/mg)									
		P-01	P-02	P-03	P-04	P-05	P-06	P-07	P-08	Median	MAD
1	Midshaft Diaphysis	13.00	16.35	23.69	11.51	18.35	17.84	17.12	23.89	17.48	2.80
2	Midshaft Diaphysis	10.53	14.99	22.73	13.73	26.62	22.13	13.26	25.16	18.56	5.06
3	Proximal Diaphysis	14.27	8.73	18.18	12.77	12.54	19.14	18.92	20.73	16.23	3.19
4	Distal Diaphysis	12.98	9.57	20.69	14.50	15.44	23.24	19.98	18.63	17.04	3.30
5	Proximal Diaphysis	22.47	12.28	46.87	27.40	48.31	29.38	32.52	23.60	28.39	5.36
6	Distal Diaphysis	18.61	10.53	19.08	14.53	19.15	24.86	21.49	19.11	19.10	1.44
7	Proximal Metaphysis	36.51	36.00	79.82	33.56	50.19	44.95	53.41	25.69	40.73	8.32
8	Distal Metaphysis	35.22	27.27	53.68	37.17	43.89	35.96	44.60	27.09	36.56	7.68
9	Articulating Surface	73.80	46.96	49.29	37.48	54.25	61.94	57.07	68.33	55.66	7.54
10	Distal Epiphysis	195.14	97.61	122.45	111.18	439.92	134.64	104.00	111.47	116.96	15.32
11	Femoral Head	242.18	131.82	141.80	84.48	150.54	90.24	72.49	103.57	117.69	30.15
12	Trochanter	216.86	209.04	115.05	60.11	398.08	70.22	179.41	90.72	147.23	65.72
13	Calcaneus	50.64	43.17	57.44	45.66	69.71	35.26	45.28	35.39	45.47	7.63
14	Talus	67.43	53.05	64.20	38.38	59.79	36.26	60.41	27.58	56.42	9.39

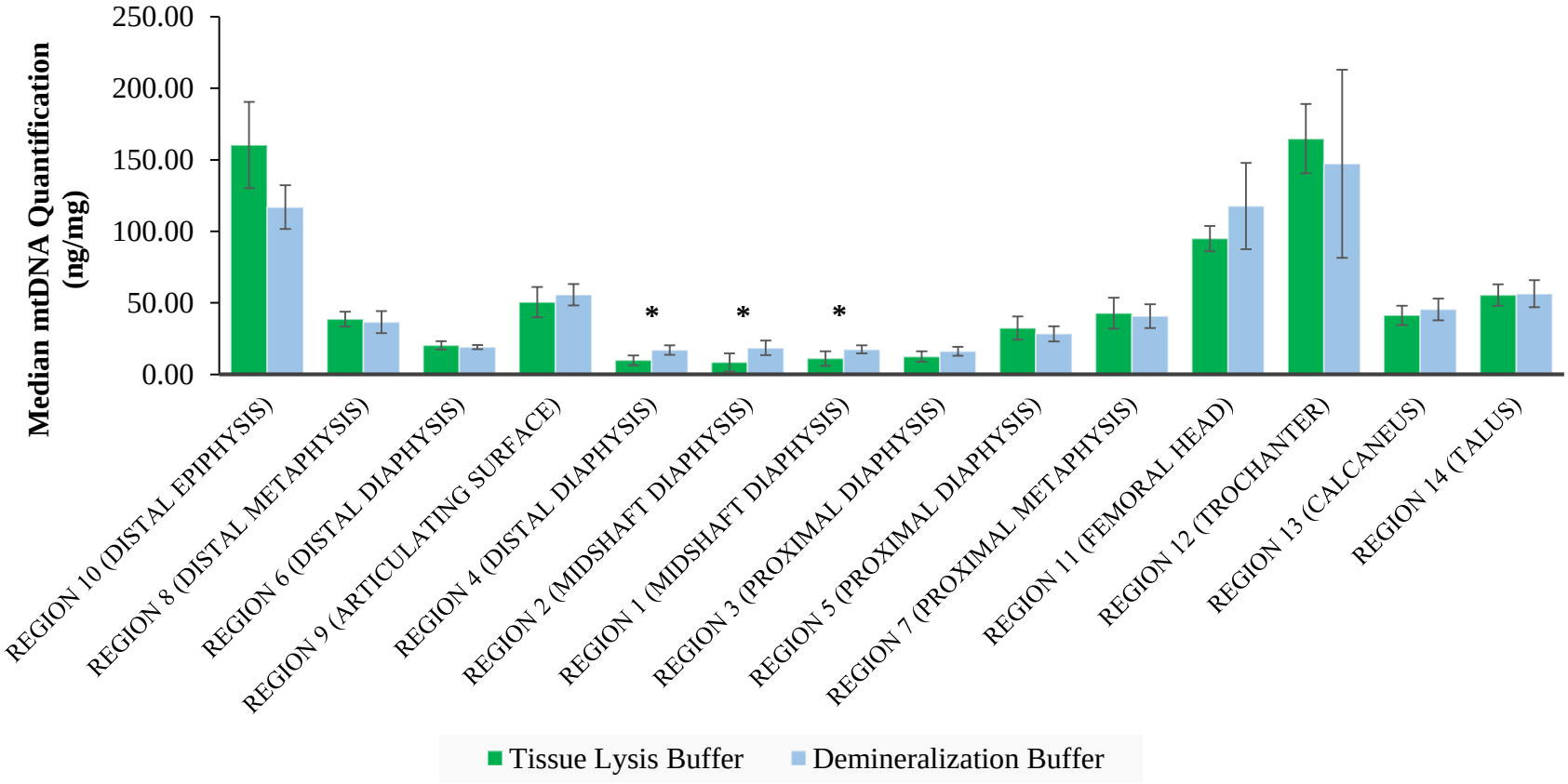

Figure 10: Median mtDNA Yields from Fresh Porcine Bones
The x-axis lists the regions tested, while the y-axis is the median mtDNA yields in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Asterisk indicates significant differences between buffers.

Table 18: Combined mtDNA Yields from Fresh Porcine Bones Digested in Tissue Lysis and Demineralization Buffers

Combined regions listed in first column and correspond to Table 3. Regions combined from P-(01 – 08). MtDNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

TL = tissue lysis buffer DM = demineralization buffer

Combined Regions	Median		MAD	
	TL	DM	TL	DM
1 – 6	14.94	18.78	5.89	4.49
7 – 9	42.29	44.78	9.73	8.86
10 – 12	154.39	118.75	44.14	30.15
13 – 14	52.29	48.15	11.02	11.76

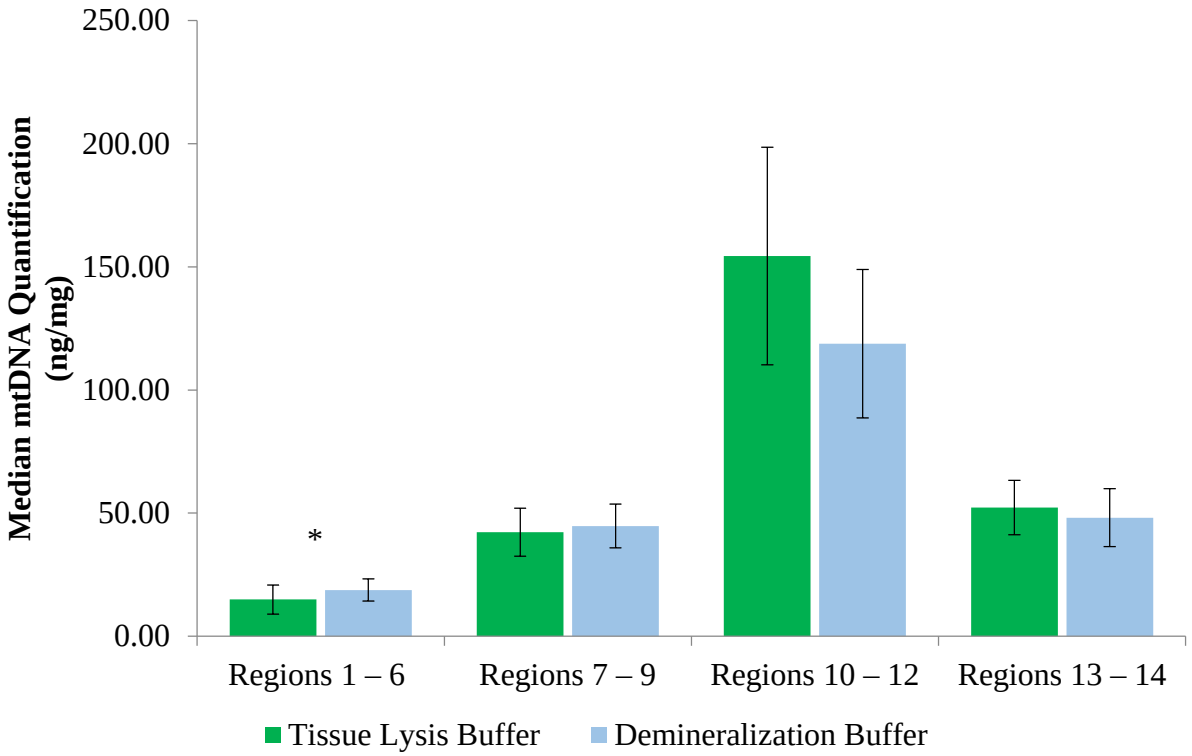

Figure 11: Combined Median mtDNA Yields from Fresh Porcine Bones

The x-axis lists the regions tested, while the y-axis is the median mtDNA yields in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals. Asterisk indicates significant differences between buffers.

Median mtDNA yields differed significantly among regions of porcine bone digested in tissue lysis and demineralization buffers ($p < 0.0001$). P-values for multiple pairwise comparisons are listed in Appendix A4 and A5. Pairwise comparisons of mtDNA yields derived from bone digested in tissue lysis and demineralization buffers are depicted in Figures 12 and 13 respectively. Regions 10 – 12 had significantly more mtDNA than Regions 1 – 6, 7, and 8 ($p = 0.044 – 0.0001$). Region 9 had significantly more mtDNA than Regions 1 – 4, and 6 ($p = 0.025 – 0.0001$), and significantly less than Regions 10 and 12 ($p = 0.035, 0.041$); however, the latter result was inconsistent between buffers. Regions 10 ($p = 0.127$) and 12 ($p = 0.159$) were not significantly different from Region 9 with bone powder digested in demineralization buffer. Regions 13 and 14 had significantly more mtDNA than Regions 1 – 4 and 6 ($p = 0.041 – 0.0001$). Region 13 had significantly less mtDNA than Regions 10 and 12 ($p = 0.049 – 0.021$). MtDNA recovered from Regions 13 and 14 were not significantly different from each other ($p = 0.461, 0.817$), or among Regions 7 – 9 ($p = 0.203 – 0.846$). Significantly more mtDNA was recovered from Regions 1, 2, and 4 digested in demineralization buffer based on individual pairwise comparisons between buffers ($p = 0.050, 0.040$, and 0.007).

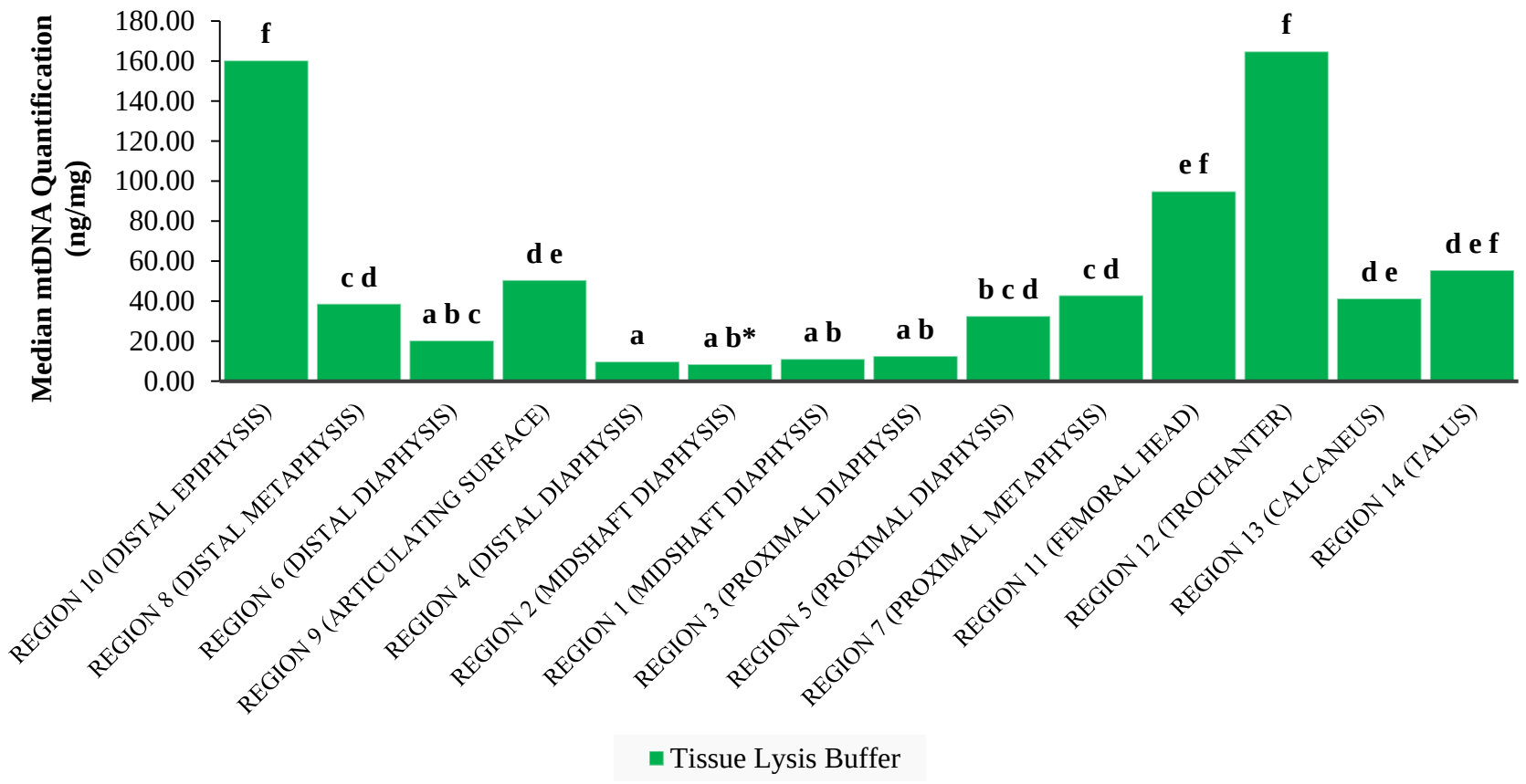

Figure 12: Pairwise Comparisons of mtDNA Yields from Fresh Porcine Bones Digested in Tissue Lysis Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median mtDNA yields normalized in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did. *Region 2 (n = 7)

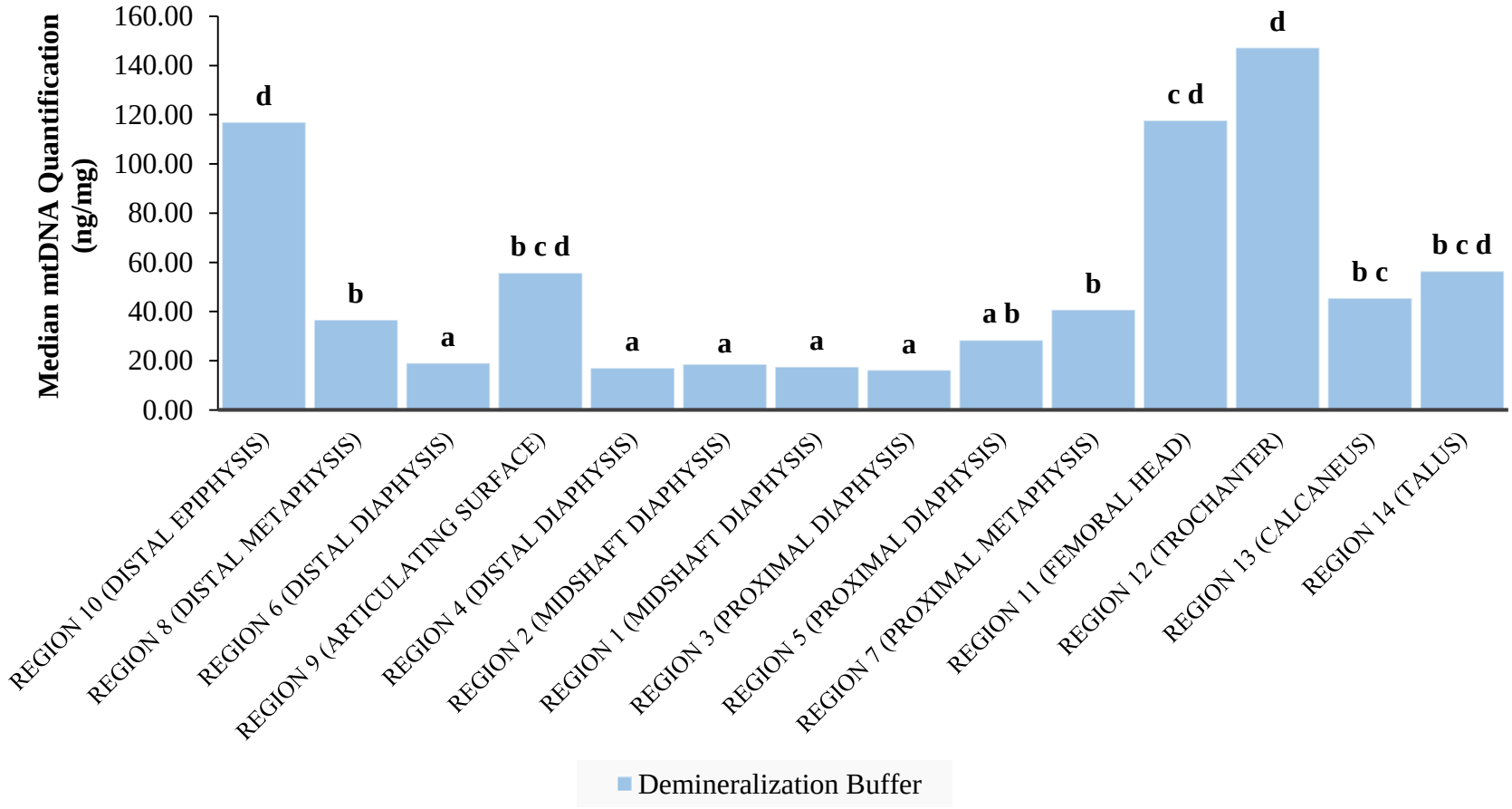

Figure 13: Pairwise Comparisons of mtDNA Yields from Fresh Porcine Bones Digested in Demineralization Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median mtDNA yields normalized in ng per mg bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did.

Combined regional porcine mtDNA yields differed significantly among the femoral diaphysis, metaphyses and articulating surface, and epiphyses digested in both buffers ($p < 0.0001$). P-values for multiple pairwise comparisons of the femur and individual pairwise comparison between tarsals and femora are listed in Appendix A6. Pairwise comparisons of combined mtDNA yields derived from bone digested in tissue lysis and demineralization buffers are depicted in Figures 14 and 15 respectively. Combined regions were significantly different at a Bonferroni corrected significance level of $\alpha = 0.0167$. The epiphyses had significantly higher mtDNA yields than the diaphysis ($p < 0.0001$), and metaphyses and articulating surface ($p = 0.002 – 0.001$) digested in both buffers. The metaphyses and articulating surface had significantly higher mtDNA yields than the diaphysis ($p < 0.0001$). The tarsals had significantly higher yields than the diaphysis ($p < 0.0001$), and significantly lower than the epiphyses ($p < 0.0001$). Comparisons between the tarsals and metaphyses and articulating surface were inconsistent between buffers, where the tarsals had significantly higher mtDNA yields when digested in tissue lysis buffer ($p = 0.041$), but not in demineralization buffer ($p = 0.420$). Significantly more mtDNA was recovered from diaphysis digested in demineralization buffer versus tissue lysis buffer ($p = 0.008$). There was no significant difference between buffers for the metaphyses and articulating surface ($p = 0.533$), epiphyses ($p = 0.675$), or tarsals ($p = 0.491$).

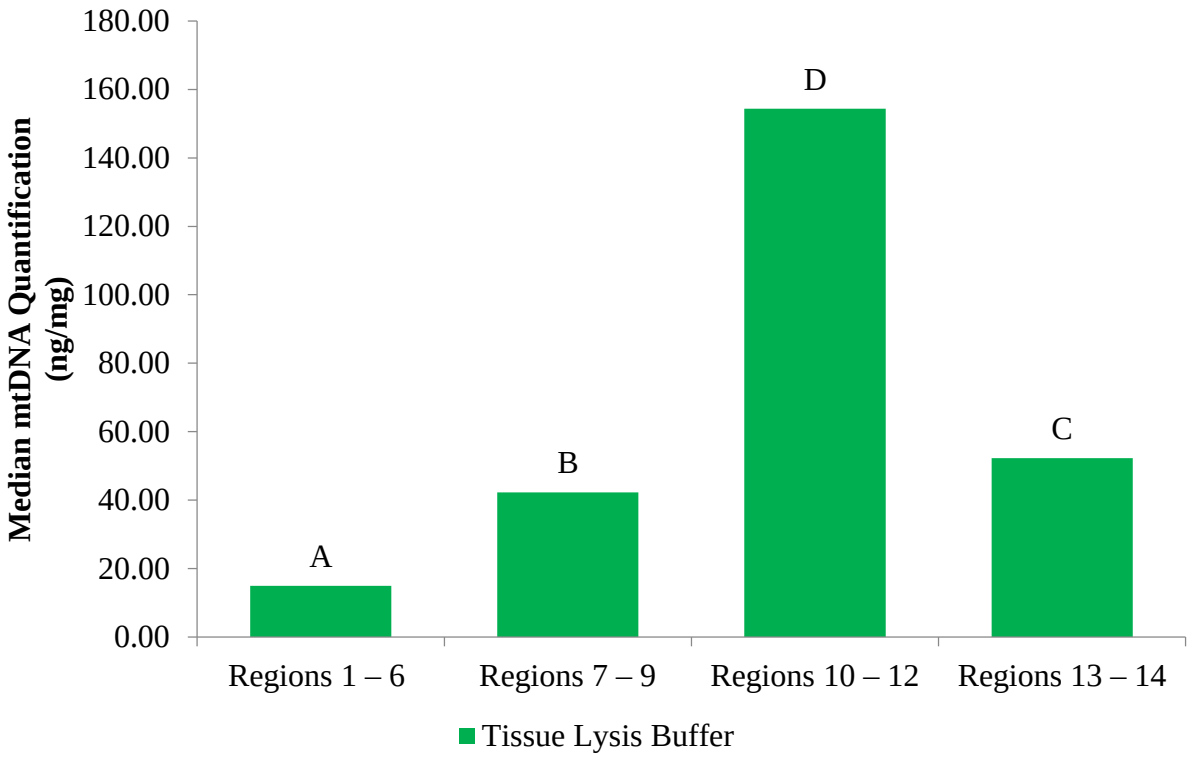

Figure 14: Pairwise Comparisons of Combined mtDNA Yields from Fresh Porcine Bones Digested in Tissue Lysis Buffer

The x-axis lists the regions tested, while the y-axis is the combined mtDNA yields normalized in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

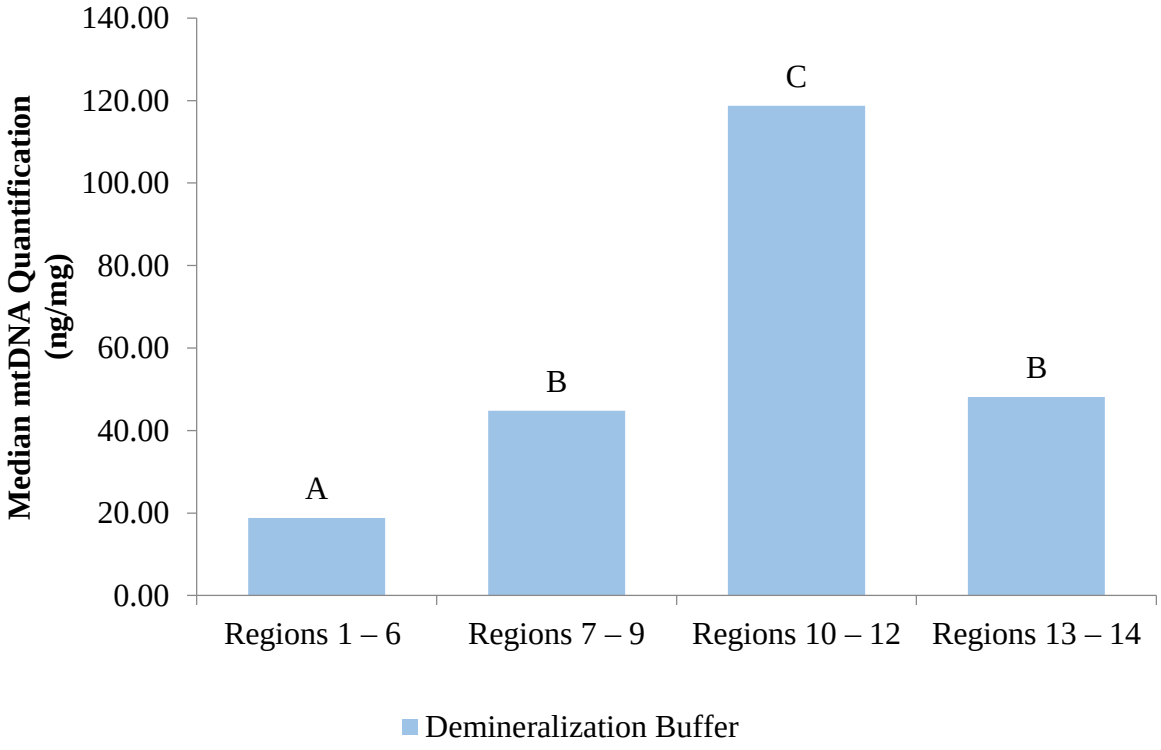

Figure 15: Pairwise Comparisons of Combined mtDNA Yields from Fresh Porcine Bones Digested in Demineralization Buffer

The x-axis lists the regions tested, while the y-axis is the median mtDNA yields normalized in ng per mg bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

Nuclear DNA yields from porcine bone digested in tissue lysis (Table 19) and demineralization buffer (Table 20) were lowest in Regions 1 – 4, with increased DNA along regions of the diaphysis closer to the proximal and distal metaphyses. The exception to this was Region 9, which had yields higher than the diaphysis and similar to the metaphyses. Regions 10 – 12 had higher yields than Regions 1 – 9, 13, and 14. Regions 13 and 14 had similar yields as Regions 7 – 9. Tissue lysis and demineralization buffers had similar variation in quantity among regions; however, bone digested in demineralization buffer had higher DNA yields from Regions 1 – 4, while Regions 9 – 12 had higher yields from bone digested in tissue lysis buffer. Figure 16 graphically depicts median nuclear DNA yields for both buffers. Combined nuclear DNA yields from bone digested in tissue lysis and demineralization buffers are listed in Table 21 and graphically depicted in Figure 17. The epiphyses had the highest DNA yields, followed by intermediate yields from the metaphyses and articulating surface as well as the tarsals, and the diaphysis had the lowest. Individual values for these data, including normalization parameters and total DNA recovered are available in Appendix B17 – B32. Efficiencies of qPCR ranged from 89.9 to 100.9 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.982 to 0.993. No PCR inhibition was detected via the IPC.

Table 19: Nuclear DNA Yields from Fresh Porcine Bones Digested in Tissue Lysis Buffer

Regions and concomitant locations correspond to Table 3. P-(01 – 08) are biological replicates. Nuclear DNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements.

NT = Not Tested

Region	Location on Element	Nuclear DNA Yield (ng/mg)									
		P-01	P-02	P-03	P-04	P-05	P-06	P-07	P-08	Median	MAD
1	Midshaft Diaphysis	40.06	10.71	6.26	2.44	6.27	6.86	7.86	13.62	7.36	2.22
2	Midshaft Diaphysis	34.34	6.92	NT*	1.60	20.72	3.22	13.52	10.98	10.98	8.57
3	Proximal Diaphysis	76.41	19.58	19.14	2.01	8.80	11.17	20.01	13.07	16.10	4.42
4	Distal Diaphysis	47.94	13.29	8.88	3.06	12.65	3.12	4.43	8.51	8.70	4.43
5	Proximal Diaphysis	187.65	45.47	302.45	73.53	184.25	91.00	484.30	214.37	185.95	103.68
6	Distal Diaphysis	189.04	57.24	124.02	69.43	51.97	99.11	242.21	161.57	111.56	52.16
7	Proximal Metaphysis	384.06	225.84	720.00	237.18	132.55	110.32	672.35	163.44	231.51	110.07
8	Distal Metaphysis	236.88	161.28	325.65	71.50	71.50	96.63	348.92	225.71	193.50	109.43
9	Articulating Surface	552.24	256.47	554.69	253.80	263.65	375.33	694.30	584.08	463.79	160.22
10	Distal Epiphysis	151.67	1482.35	3192.00	2389.71	1033.60	1859.59	2203.47	2571.43	2031.53	544.54
11	Femoral Head	1129.41	464.00	1690.39	2841.18	872.00	978.82	835.10	1028.78	1003.80	150.25
12	Trochanter	1312.40	1338.78	3193.47	2204.90	1913.60	1973.16	1720.82	2713.06	1943.38	433.06
13	Calcaneus	397.88	345.44	389.74	301.18	552.00	161.47	366.18	405.84	377.96	30.20
14	Talus	571.90	528.80	608.63	421.80	365.59	384.00	416.47	440.22	431.01	56.22

* See Table 16

Table 20: Nuclear DNA Yields from Fresh Porcine Bones Digested in Demineralization Buffer

Regions and concomitant locations correspond to Table 3. P-(01 – 08) are biological replicates. Nuclear DNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements.

Region	Location on Element	Nuclear DNA Yield (ng/mg)									
		P-01	P-02	P-03	P-04	P-05	P-06	P-07	P-08	Median	MAD
1	Midshaft Diaphysis	145.50	148.96	130.21	82.60	113.53	86.20	165.00	126.46	128.33	18.90
2	Midshaft Diaphysis	142.29	95.33	161.41	124.87	131.45	88.94	119.08	128.70	126.79	11.61
3	Proximal Diaphysis	149.06	105.85	115.71	98.61	124.39	84.70	163.71	108.31	112.01	12.89
4	Distal Diaphysis	150.45	111.98	176.16	126.72	116.20	91.84	161.40	118.80	122.76	19.24
5	Proximal Diaphysis	225.88	114.29	319.00	157.04	254.04	106.41	225.76	161.15	193.46	48.50
6	Distal Diaphysis	136.53	110.00	171.60	130.00	120.50	110.88	250.29	131.29	130.65	14.96
7	Proximal Metaphysis	281.10	224.12	520.01	261.00	252.71	184.44	361.80	131.39	256.86	52.58
8	Distal Metaphysis	289.52	262.09	245.16	202.04	277.54	153.18	343.53	182.86	253.62	43.74
9	Articulating Surface	486.00	325.10	238.76	257.29	207.08	167.29	348.58	182.39	248.03	71.36
10	Distal Epiphysis	1029.02	968.63	783.67	1050.00	479.47	508.86	702.00	629.06	742.84	229.88
11	Femoral Head	1156.76	951.60	852.24	772.20	712.92	577.16	624.98	789.80	781.00	113.63
12	Trochanter	1207.84	850.20	1209.50	1147.14	864.00	561.16	931.70	519.84	897.85	279.64
13	Calcaneus	457.14	295.10	482.99	423.03	323.43	218.10	335.57	274.90	329.50	74.07
14	Talus	432.27	513.59	416.40	352.78	491.14	219.26	457.14	315.06	424.33	69.18

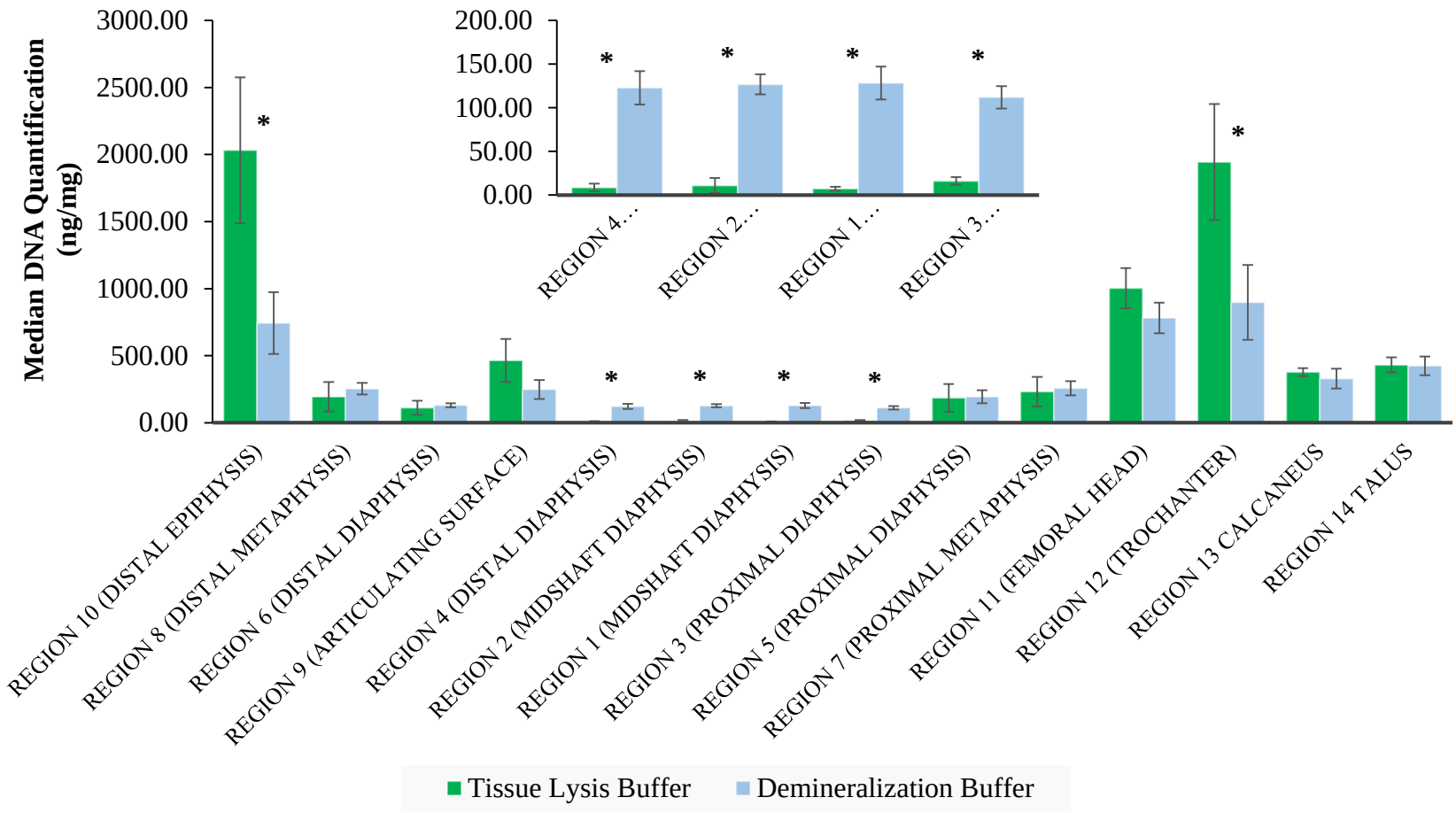

Figure 16: Median Nuclear DNA Yields from Fresh Porcine Bones
The x-axis lists the regions tested, while the y-axis is the median nuclear DNA yields in ng per mg of bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Centered is a nested graph for increased resolution of Regions 1 – 4. Asterisk indicates significant differences between buffers.

Table 21: Combined Nuclear DNA Yields from Fresh Porcine Bones Digested in Tissue Lysis and Demineralization Buffers

Combined regions listed in first column and correspond to Table 3. Regions are combined from P-(01 – 08). DNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

TL = tissue lysis buffer DM = demineralization buffer

Combined Regions	Median		MAD	
	TL	DM	TL	DM
1 – 6	19.14	127.71	16.02	21.27
7 – 9	255.14	255.00	121.39	61.53
10 – 12	1705.60	820.00	674.42	192.98
13 – 14	401.86	384.59	37.32	72.55

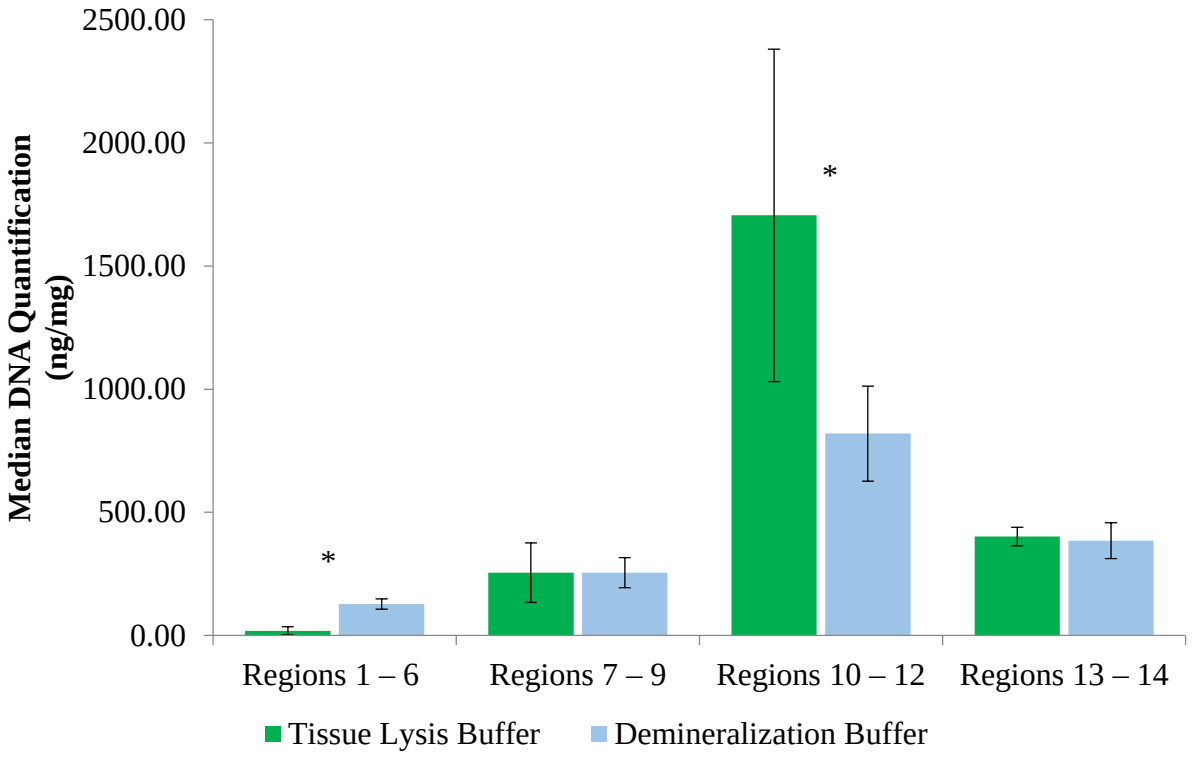

Figure 17: Combined Median Nuclear DNA Yields from Fresh Porcine Bones

The x-axis lists the regions tested, while the y-axis is the median nuclear DNA yields in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals. Asterisk indicates significant differences between buffers.

Median porcine nuclear DNA yields differed significantly among regions of porcine bone digested in tissue lysis and demineralization buffers ($p < 0.0001$). P-values for multiple pairwise comparisons are listed in Appendix A7 and A8. Pairwise comparisons of nuclear DNA yields derived from bone powder digested in tissue lysis and demineralization buffers are depicted in Figures 18 and 19 respectively. Regions 10 – 12 had significantly higher nuclear DNA yields than Regions 1 – 6 ($p = 0.007 – 0.0001$), and Regions 7 and 8 ($p = 0.049 – 0.001$). Region 9 had significantly higher yields than Regions 1 – 4 ($p = 0.022 – 0.0001$), and significantly lower than Regions 10, 11, and 12 ($p = 0.027$, 0.020, and 0.012); however, the latter result was only present in demineralization extracts. Regions 10, 11, and 12 were not significantly different from Region 9 ($p = 0.167$, 0.214, 0.068) with bone digested in tissue lysis buffer. Regions 13 and 14 had significantly higher DNA yields than Regions 1 – 4 ($p = 0.004 – 0.0001$). Region 13 had significantly lower DNA yields than Region 12 ($p = 0.030$) for bone digested in tissue lysis buffer. DNA yields from Regions 13 and 14 were not significantly different from each other ($p = 0.571$), and neither differed significantly when compared to Regions 7 – 12 ($p = 0.062 – 0.709$). Utilizing individual pairwise comparisons between buffers, significantly more DNA was recovered from Regions 1 – 4 digested in demineralization buffer ($p = 0.0003 – 0.0001$), and significantly more DNA was recovered from Regions 10 and 12 digested in tissue lysis buffer ($p = 0.015$, 0.0001).

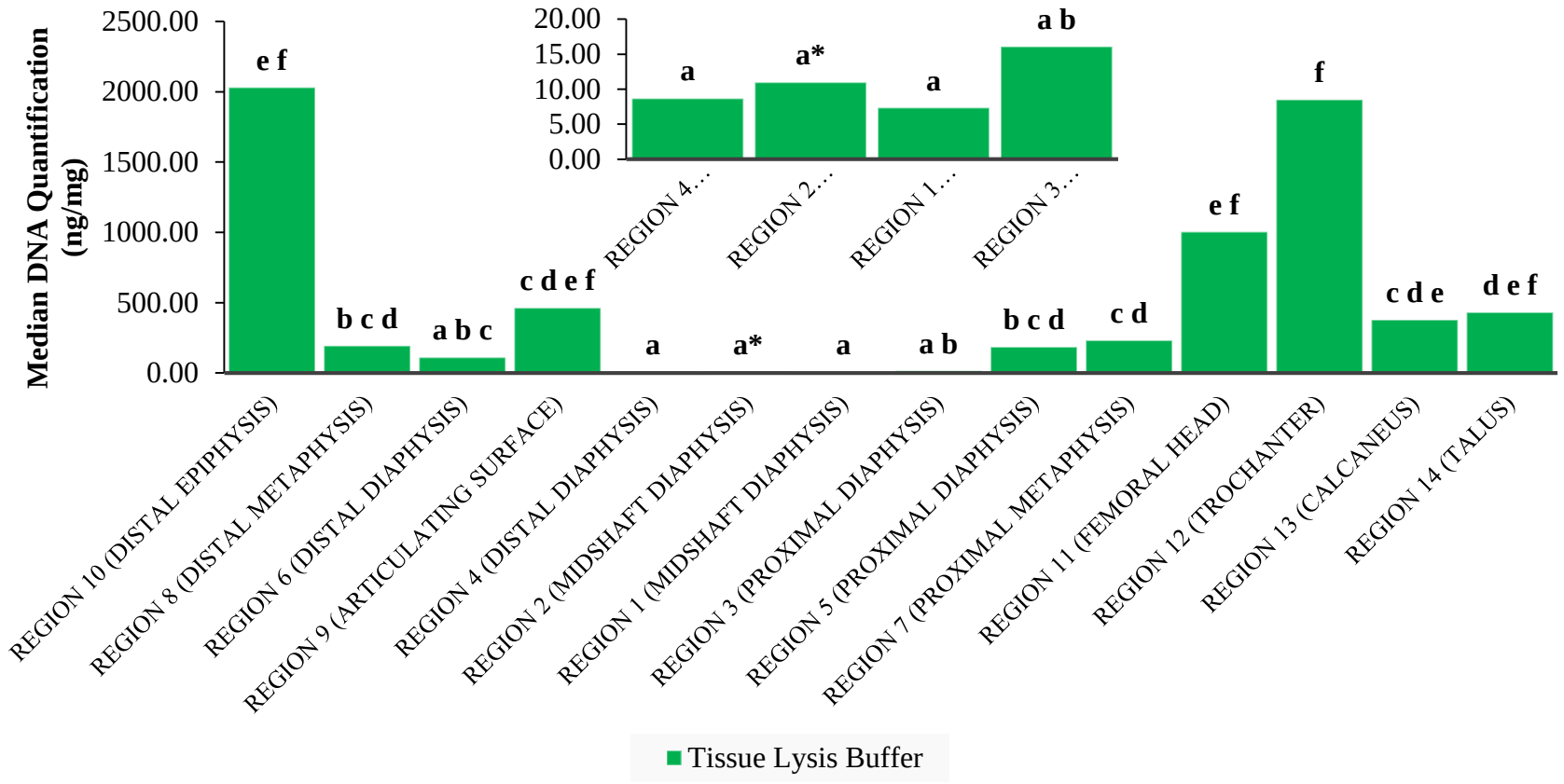

Figure 18: Pairwise Comparisons of Nuclear DNA Yields from Fresh Porcine Bones Digested in Tissue Lysis Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median nuclear DNA yield normalized in ng per mg of bone powder. Green bars represent bone powder digested in tissue lysis buffer. Centered is a nested graph for increased resolution of Regions 1 – 4. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did.
* Region 2 (n = 7)

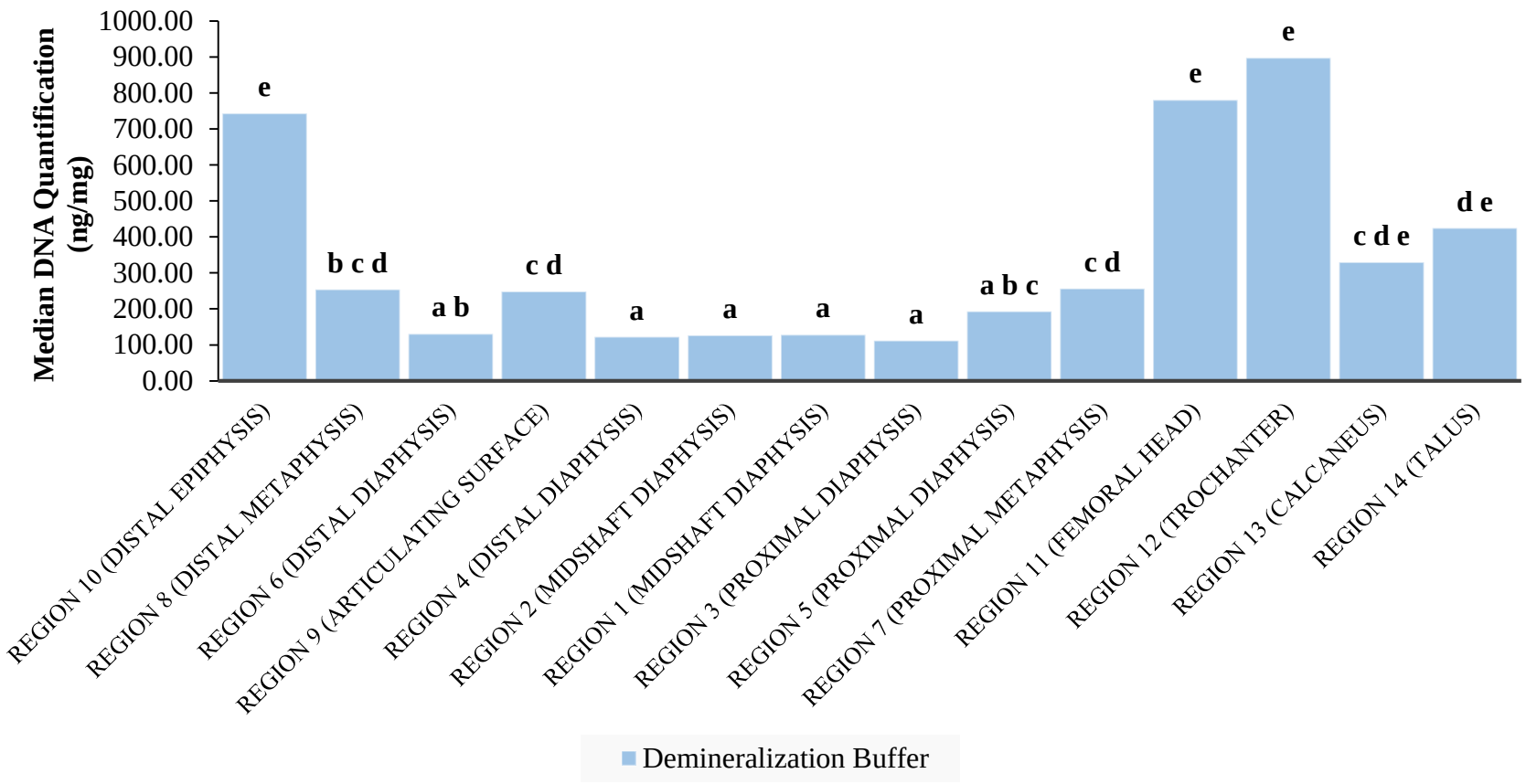

Figure 19: Pairwise Comparisons of Nuclear DNA Yields from Fresh Porcine Bones Digested in Demineralization Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median nuclear DNA yield normalized in ng per mg of bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did.

Combined porcine nuclear DNA yields differed significantly among the femoral diaphysis, metaphyses and articulating surface, and epiphyses digested in both buffers ($p < 0.0001$). P-values for multiple pairwise comparisons of the femur, and individual pairwise comparisons between the tarsals and femora are listed in Appendix A6. Pairwise comparisons of combined nuclear DNA yields derived from bone digested in tissue lysis and demineralization buffers are depicted in Figures 20 and 21 respectively. Combined regions were significantly different at a Bonferroni corrected significance level of $\alpha = 0.0167$. The epiphyses had significantly higher DNA yields, for both buffers, than the diaphysis ($p < 0.0001$), and the metaphyses and articulating surface ($p = 0.001$). The metaphyses and articulating surface had significantly higher yields than the diaphysis ($p < 0.0001$) digested in both buffers. The tarsals had significantly higher DNA yields than the diaphysis ($p < 0.0001$), and the metaphyses and articulating surface ($p = 0.035 – 0.002$), but significantly lower than the epiphyses ($p < 0.0001$) for bone powder digested with both buffers. Comparing yields between buffers, significantly more nuclear DNA was recovered from the diaphysis digested in demineralization buffer ($p < 0.0001$), and the epiphyses digested in tissue lysis buffer ($p < 0.0001$). There was no significant difference in DNA yields between buffers for the metaphyses and articulating surface ($p = 0.688$), or the tarsals ($p = 0.376$).

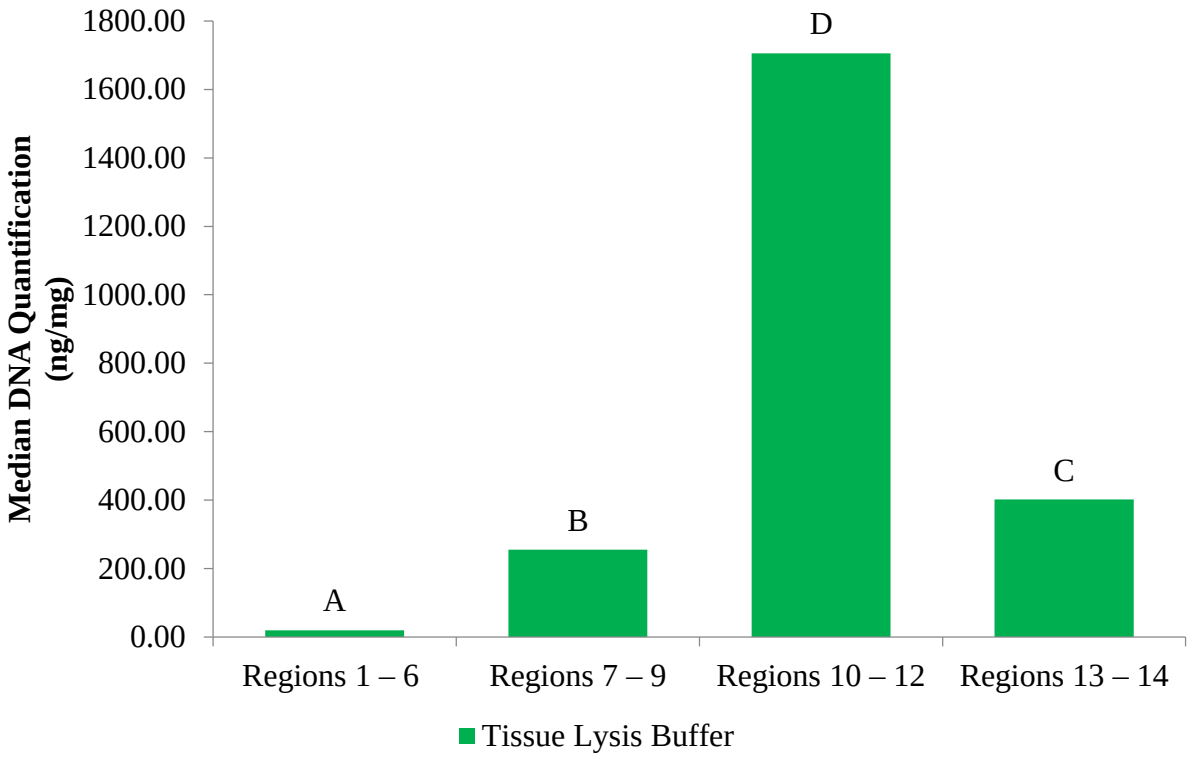

Figure 20: Pairwise Comparisons of Combined Nuclear DNA Yields from Fresh Porcine Bones Digested in Tissue Lysis Buffer

The x-axis lists the regions tested, while the y-axis is the median DNA yields normalized in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

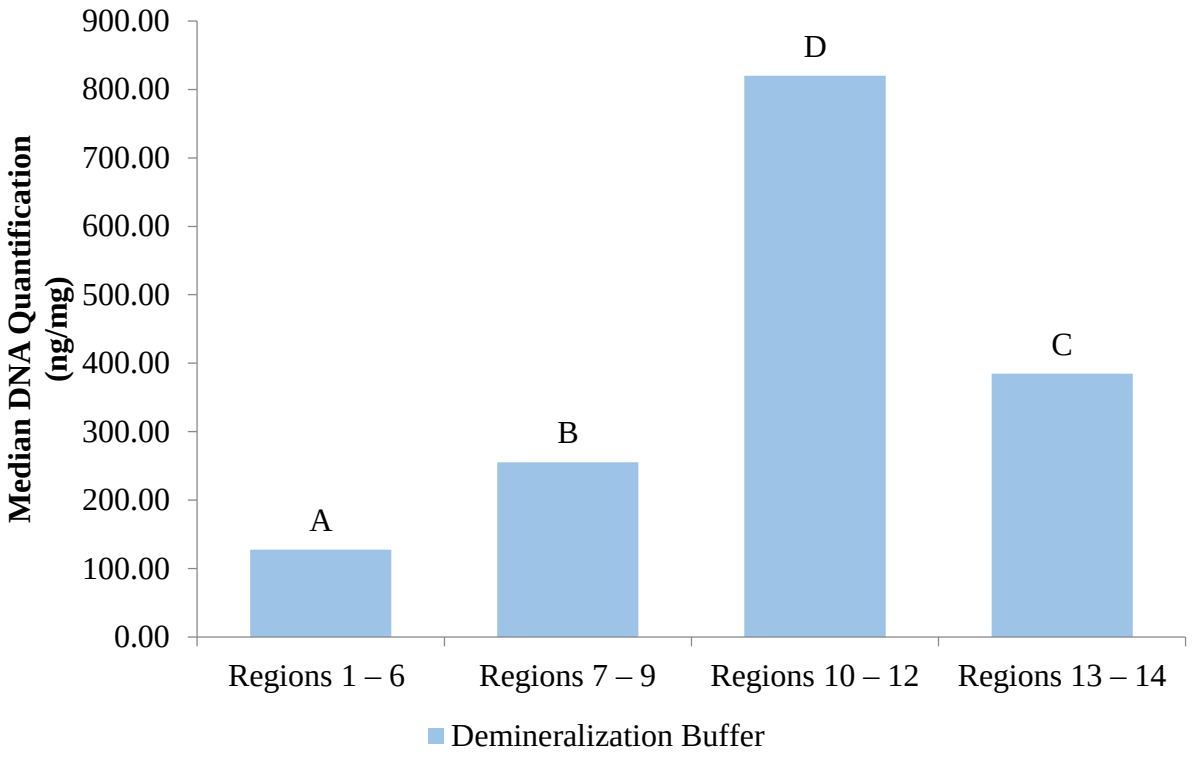

Figure 21: Pairwise Comparisons of Combined Nuclear DNA Yields from Fresh Porcine Bones Digested in Demineralization Buffer
The x-axis lists the regions tested, while the y-axis is the median DNA yields normalized in ng per mg bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

Quality of Total DNA Recovered from Fresh Porcine Femora and Tarsals

Mitochondrial and nuclear DNA PCR amplification results are individually listed in Appendix B33 – B36. MtDNA isolated from bone digested in tissue lysis and demineralization buffers produced the 1017 bp amplicon, the largest tested, for all regions across all eight replicates. Nuclear DNA isolated from bone digested in tissue lysis and demineralization buffers produced the 642 bp amplicon, the largest tested, in all but nine instances. Extracts that failed to amplify the 642 bp amplicon, amplified the 457 bp amplicon. Seven of nine extracts that failed to produce the 642 bp amplicon were from P-01: Regions 5, 6, and 12 from bone digested in

tissue lysis buffer, and Regions 2, 6, 7, and 12 from bone digested in demineralization buffer. P-08 Region 3 digested in demineralization buffer and P-03 Region 7 digested in tissue lysis buffer also failed to produce the 642 bp amplicon. Digestion buffers did not significantly affect the quality of the mitochondrial or nuclear DNA recovered from porcine bone ($p > 0.05$). Reagent blanks and negative controls had no amplification.

Quantification of Total DNA from Fresh Bovine Femora and Tarsals

Median bovine mtDNA yields from bone powder digested in tissue lysis buffer (Table 22) and demineralization buffer (Table 23) were lowest at Regions 1 – 4, with increasing quantities along regions of the diaphysis closer to the proximal and distal epiphyses. The exception to this was Region 9 that had the highest yields from the diaphysis. Regions 10 – 12 had higher yields than Regions 1 – 9, 13, and 14. Regions 13 and 14 had slightly higher yields than Regions 7 – 9. Figure 22 graphically depicts median mtDNA yields for both buffers. Combined mtDNA yields from bone powder digested in tissue lysis and demineralization buffers, listed in Table 24 and graphically depicted in Figure 23 were highest at the epiphyses, followed by intermediate yields from metaphyses and articulating surface as well as the tarsals, and the diaphysis had the lowest yields. Individual values for these data, including normalization parameters and total mtDNA recovered are available in Appendix C1 – C16. Efficiencies of qPCR ranged from 83.9 to 98.5 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.974 to 0.995. No PCR inhibition was detected via the IPC.

Table 22: mtDNA Yields from Fresh Bovine Bones Digested in Tissue Lysis Buffer

Regions and concomitant locations correspond to Table 3. C-(01 – 08) are biological replicates. MtDNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements.

Region	Location on Element	mtDNA Yield (ng/mg)									
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08	Median	MAD
1	Midshaft Diaphysis	7.59	11.38	1.80	3.63	2.90	1.82	2.68	2.24	2.79	0.91
2	Midshaft Diaphysis	5.30	16.35	2.37	1.44	3.86	3.48	4.63	2.66	3.67	1.16
3	Proximal Diaphysis	8.30	22.84	2.36	2.58	4.01	1.92	5.60	3.64	3.83	1.62
4	Distal Diaphysis	7.63	11.69	2.73	2.15	5.88	4.33	4.53	2.20	4.43	1.96
5	Proximal Diaphysis	5.92	16.04	1.89	2.40	4.83	4.27	4.59	4.04	4.43	0.94
6	Distal Diaphysis	5.78	11.54	2.60	3.04	5.72	5.55	6.29	2.94	5.63	1.63
7	Proximal Metaphysis	13.42	43.20	4.81	3.29	8.34	7.04	20.19	11.72	10.03	4.30
8	Distal Metaphysis	14.56	38.27	3.11	2.71	8.82	13.67	5.65	10.26	9.54	4.58
9	Articulating Surface	28.53	52.56	5.93	7.68	27.78	11.94	11.36	12.84	12.39	5.59
10	Distal Epiphysis	62.40	102.90	12.89	20.36	23.02	37.41	28.64	38.45	33.03	11.34
11	Femoral Head	50.89	85.96	11.51	9.48	26.59	26.22	6.82	24.36	25.29	14.79
12	Trochanter	28.77	56.23	11.40	5.23	39.71	23.84	30.35	24.94	26.85	8.18
13	Calcaneus	17.69	39.37	2.89	2.99	30.68	19.72	17.91	11.26	17.80	9.71
14	Talus	57.71	59.77	3.73	2.29	27.34	23.57	14.99	9.52	19.28	12.66

Table 23: mtDNA Yields from Fresh Bovine Bones Digested in Demineralization Buffer

Regions and concomitant locations correspond to Table 3. C-(01 – 08) are biological replicates. MtDNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements.

Region	Location on Element	mtDNA Yield (ng/mg)									
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08	Median	MAD
1	Midshaft Diaphysis	18.63	18.54	3.31	2.01	5.70	4.34	4.22	2.44	4.28	1.63
2	Midshaft Diaphysis	11.17	18.88	2.22	2.98	7.63	4.34	3.65	3.29	3.99	1.39
3	Proximal Diaphysis	13.08	19.96	2.62	3.16	6.23	2.64	5.82	4.75	5.29	2.39
4	Distal Diaphysis	10.82	15.89	2.85	3.15	4.83	6.29	3.09	2.72	3.99	1.21
5	Proximal Diaphysis	12.31	22.74	2.51	3.38	11.14	10.74	7.39	4.58	9.26	3.86
6	Distal Diaphysis	12.24	16.28	2.93	2.98	6.42	13.44	2.96	5.16	5.79	2.85
7	Proximal Metaphysis	19.56	49.40	4.10	4.30	15.41	13.16	20.80	13.96	14.68	5.50
8	Distal Metaphysis	31.56	46.05	4.08	2.89	12.66	18.90	7.04	10.70	11.68	7.41
9	Articulating Surface	38.88	47.11	4.59	10.54	20.86	18.37	14.27	10.53	16.32	5.78
10	Distal Epiphysis	78.24	156.16	13.16	18.78	25.37	30.60	26.02	35.31	28.31	8.27
11	Femoral Head	84.00	75.92	11.89	14.47	31.06	42.38	23.56	30.03	30.55	13.96
12	Trochanter	40.35	45.60	11.04	5.18	24.35	30.86	25.93	18.31	25.14	10.47
13	Calcaneus	26.39	41.29	3.19	3.09	18.06	16.65	13.20	13.61	15.13	7.10
14	Talus	55.23	52.87	3.31	2.34	15.47	17.97	8.91	11.99	13.73	7.62

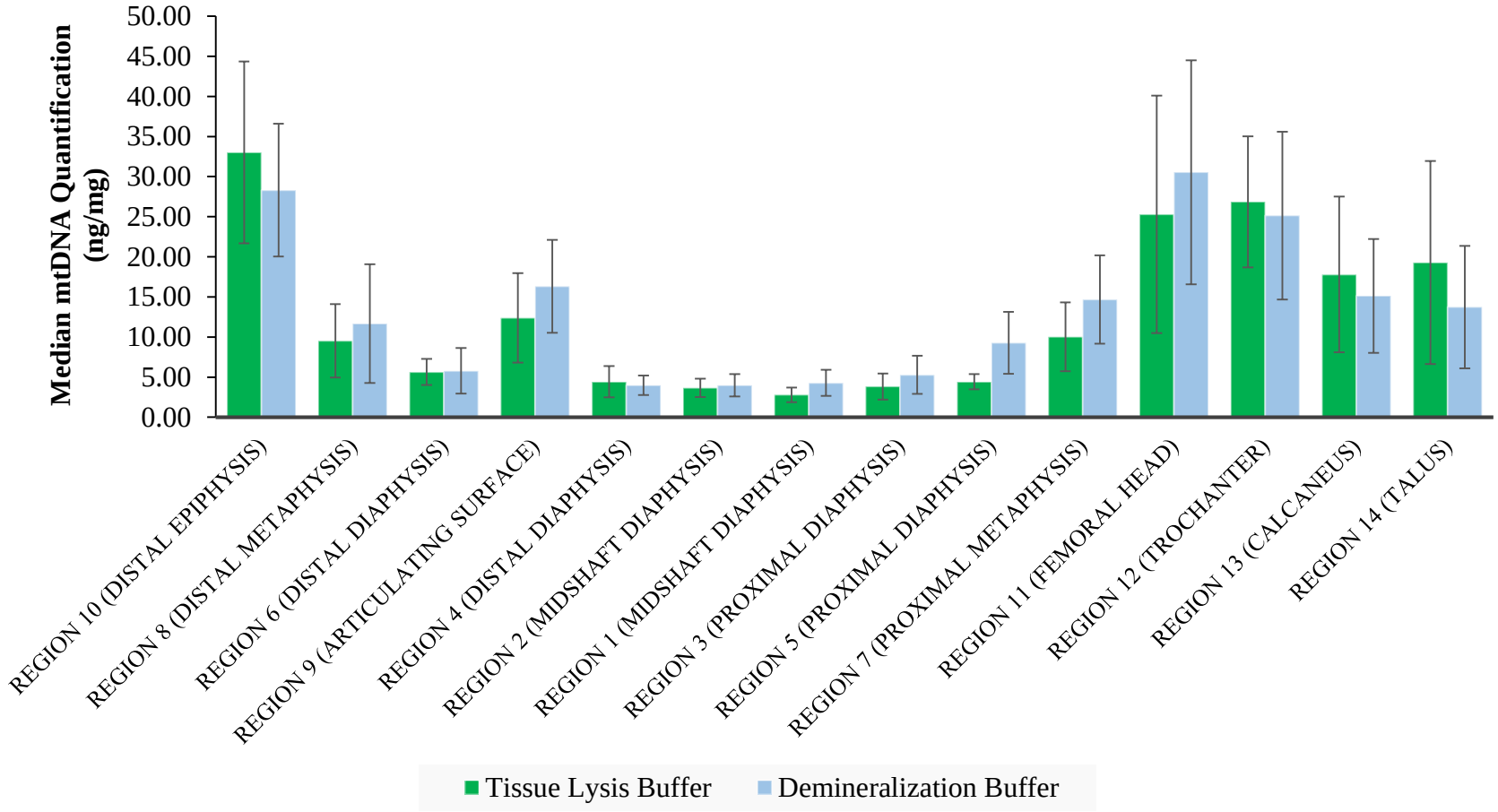

Figure 22: Median mtDNA Yields from Fresh Bovine Bones
The x-axis lists the regions tested, while the y-axis is the median mtDNA yields in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Asterisk indicates significant differences between buffers.

Table 24: Combined mtDNA Yields from Fresh Bovine Bones Digested in Tissue Lysis and Demineralization Buffers

Combined regions listed in first column and correspond to Table 3. Regions combined from C-(01 – 08). MtDNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

TL = tissue lysis buffer DM = demineralization buffer

Combined Regions	Median		MAD	
	TL	DM	TL	DM
1 – 6	4.02	4.79	1.64	2.01
7 – 9	11.54	14.11	5.06	6.72
10 – 12	26.40	28.03	12.68	11.02
13 – 14	17.80	14.54	11.21	8.43

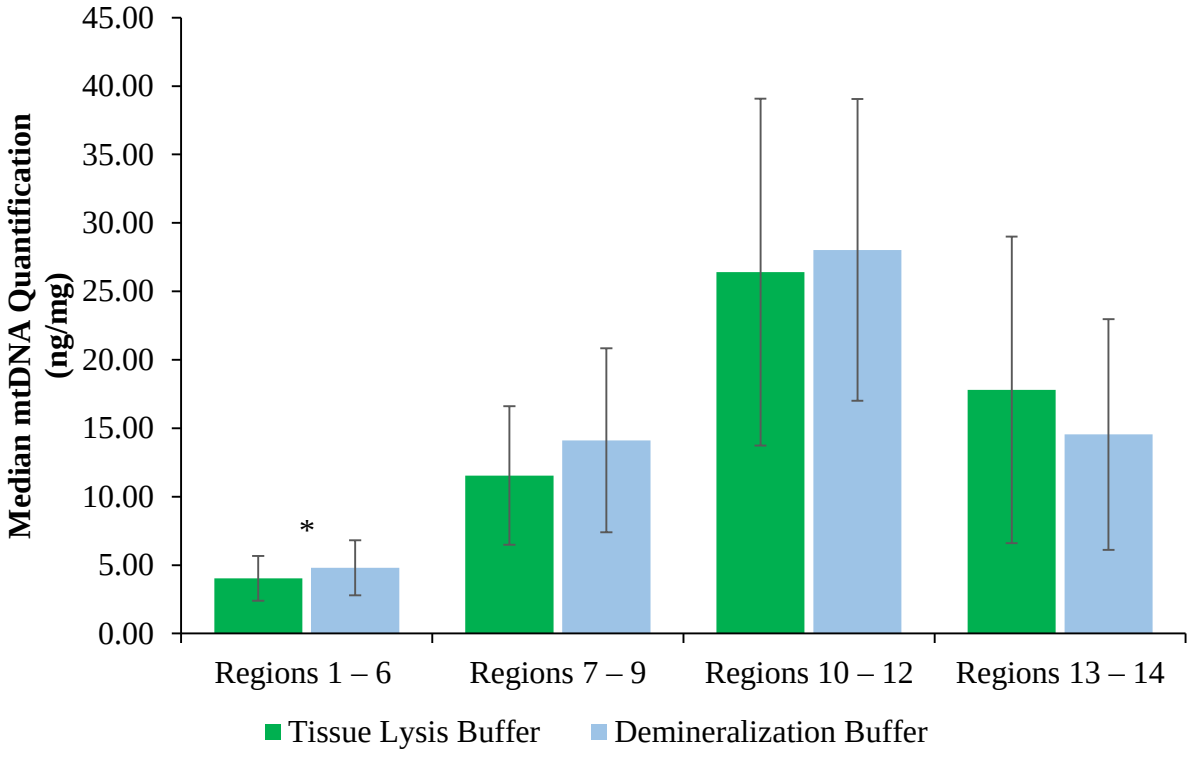

Figure 23: Combined Median mtDNA Yields from Fresh Bovine Bones

The x-axis lists the regions tested, while the y-axis is the median mtDNA yields in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals. Asterisk indicates significant differences between buffers.

Median bovine mtDNA yields differed significantly among regions of bone digested in tissue lysis and demineralization buffers ($p < 0.0001$). P-values for multiple pairwise comparisons are listed in Appendix A9 and A10. Pairwise comparisons of mtDNA yields from bone digested in tissue lysis and demineralization buffers are depicted in Figures 24 and 25 respectively. Regions 10 – 12 had significantly higher mtDNA yields than Regions 1 – 6 ($p = 0.007 – 0.0001$); however, were not significantly different from Region 9 ($p = 0.644 – 0.171$). Region 9 had significantly higher mtDNA yields than Regions 1 – 4 ($p = 0.036 – 0.002$). Region 8 had significantly lower mtDNA yields than Region 10 ($p = 0.038, 0.019$), but was not significantly different from Region 11 ($p = 0.109, 0.051$) or Region 12 ($p = 0.075, 0.183$). Region 7 had significantly higher mtDNA yields than Region 1 ($p = 0.018, 0.031$). Regions 13 and 14 had inconsistent results between buffers. Regions 13 and 14 digested in tissue lysis buffer had significantly higher mtDNA yields than Regions 1 – 5 ($p = 0.044 – 0.004$); however, these were not statistically significant in bone digested in demineralization buffer ($p = 0.067 – 0.239$). MtDNA yields from Regions 13 and 14 were not significantly different from each other ($p = 0.841, 0.869$), and neither were significantly different from Regions 7 – 12 ($p = 0.896 – 0.070$). There were no significant differences between buffers based on individual pairwise comparisons ($p = 0.234 – 0.959$).

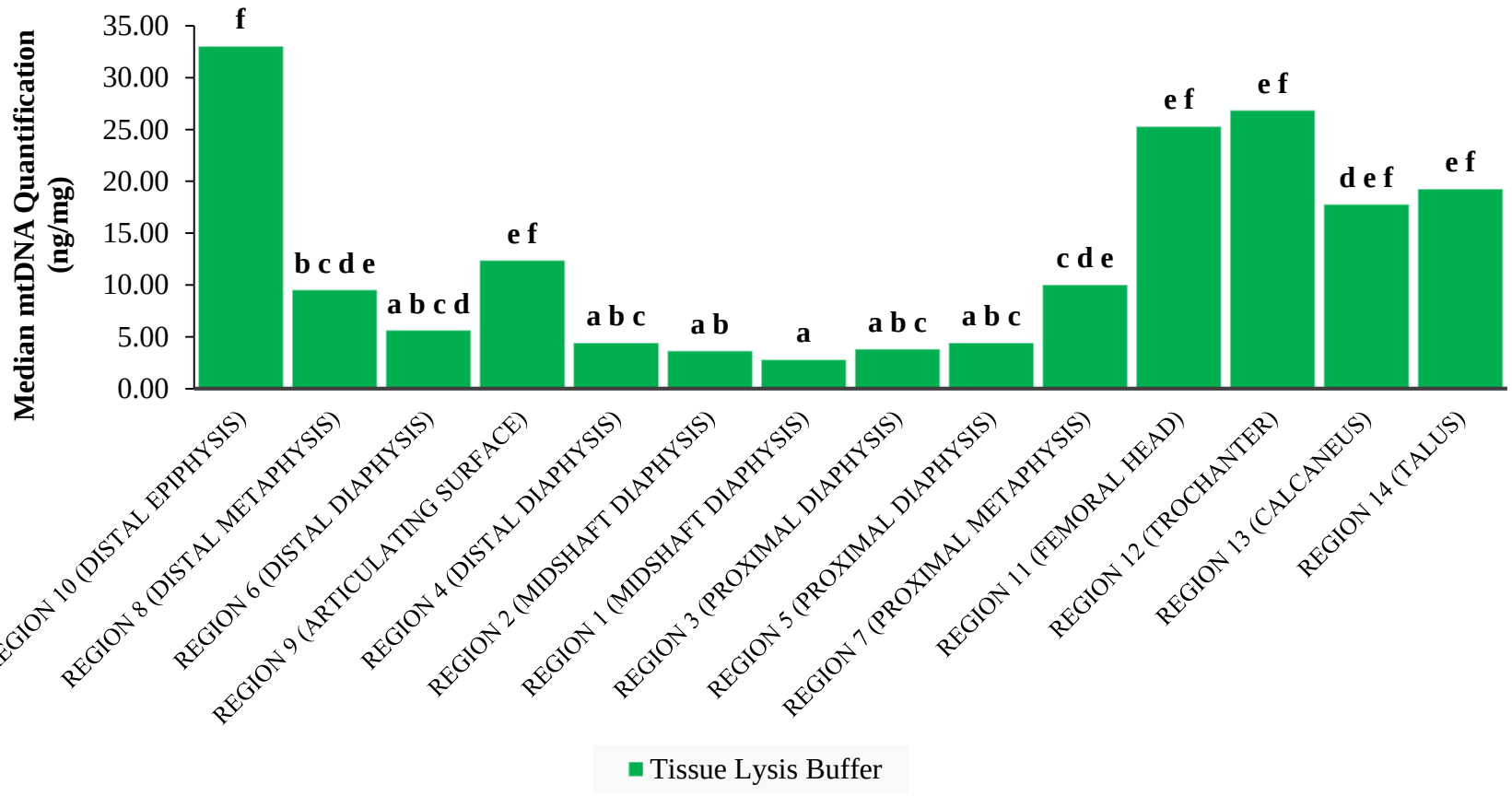

Figure 24: Pairwise Comparisons of mtDNA Yields from Fresh Bovine Bones Digested in Tissue Lysis Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median mtDNA yield normalized in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did.

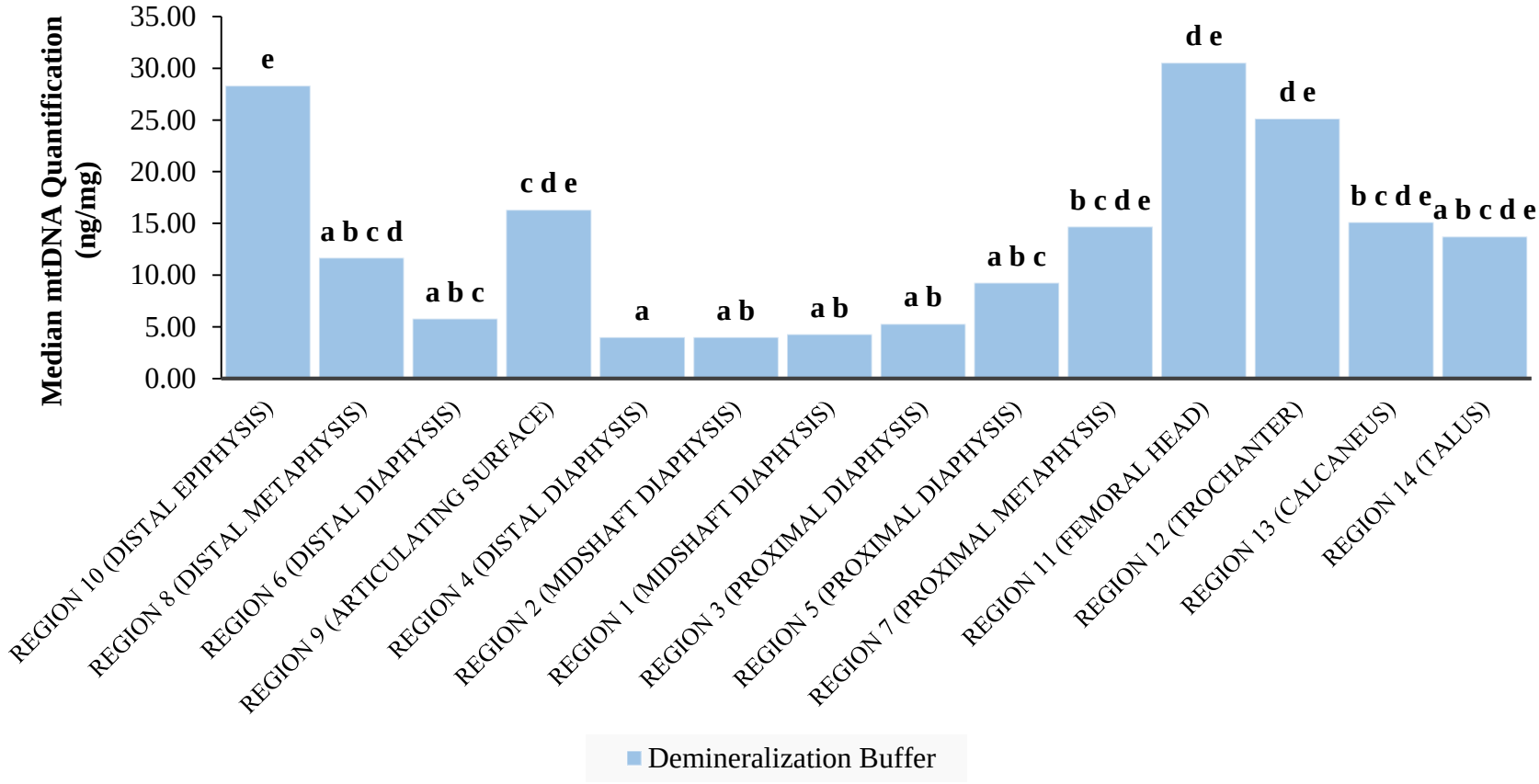

Figure 25: Pairwise Comparisons of mtDNA Yields from Fresh Bovine Bones Digested in Demineralization Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median mtDNA yield normalized in ng per mg bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did.

Combined bovine mtDNA yields differed significantly among the femoral diaphysis, metaphyses and articulating surface, and epiphyses digested in both buffers ($p < 0.0001$). P-values for multiple pairwise comparisons of the femur and individual pairwise comparisons between tarsals and femora are listed in Appendix A6. Pairwise comparisons of combined mtDNA yields derived from bone powder digested in tissue lysis and demineralization buffers are depicted in Figures 26 and 27 respectively. Combined regions were significantly different at a Bonferroni corrected significance level of $\alpha = 0.0167$. The epiphyses had significantly higher mtDNA yields than the diaphysis ($p < 0.0001$) digested in both buffers. Comparisons between the epiphyses and the metaphyses and articulating surface were inconsistent between buffers, where the epiphyses had significantly higher yields from bone digested in demineralization buffer ($p = 0.0004$), but were not significantly different when digested in tissue lysis buffer ($p = 0.020$). The metaphyses and articulating surface had significantly higher yields than the diaphysis for both buffers ($p < 0.0001$). The tarsals had significantly higher mtDNA yields than the diaphysis digested in both buffers ($p = 0.006 – 0.0001$), and significantly lower yields than the epiphyses digested in demineralization buffer ($p = 0.012$). MtDNA yields from the tarsals were not significantly different from the metaphyses and articulating surface digested in both buffers ($p = 0.838 – 0.345$), or the epiphyses digested in tissue lysis buffer ($p = 0.070$). The diaphysis digested in demineralization buffer had significantly higher mtDNA yields than tissue lysis buffer ($p = 0.037$); however, there were no differences between buffers for the metaphyses and articulating surface ($p = 0.310$), the epiphyses ($p = 0.690$), or the tarsals ($p = 0.696$).

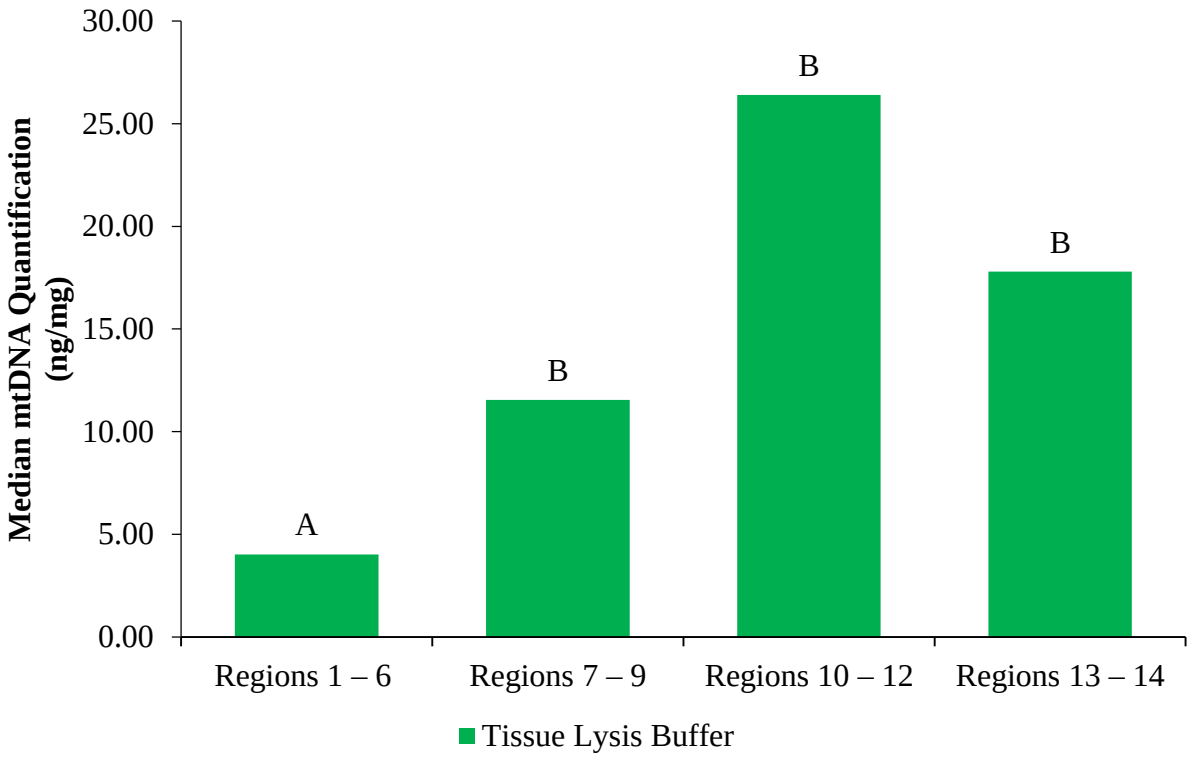

Figure 26: Pairwise Comparisons of Combined mtDNA Yields from Fresh Bovine Bones Digested in Tissue Lysis Buffer

The x-axis lists the regions tested, while the y-axis is the median mtDNA yields normalized in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

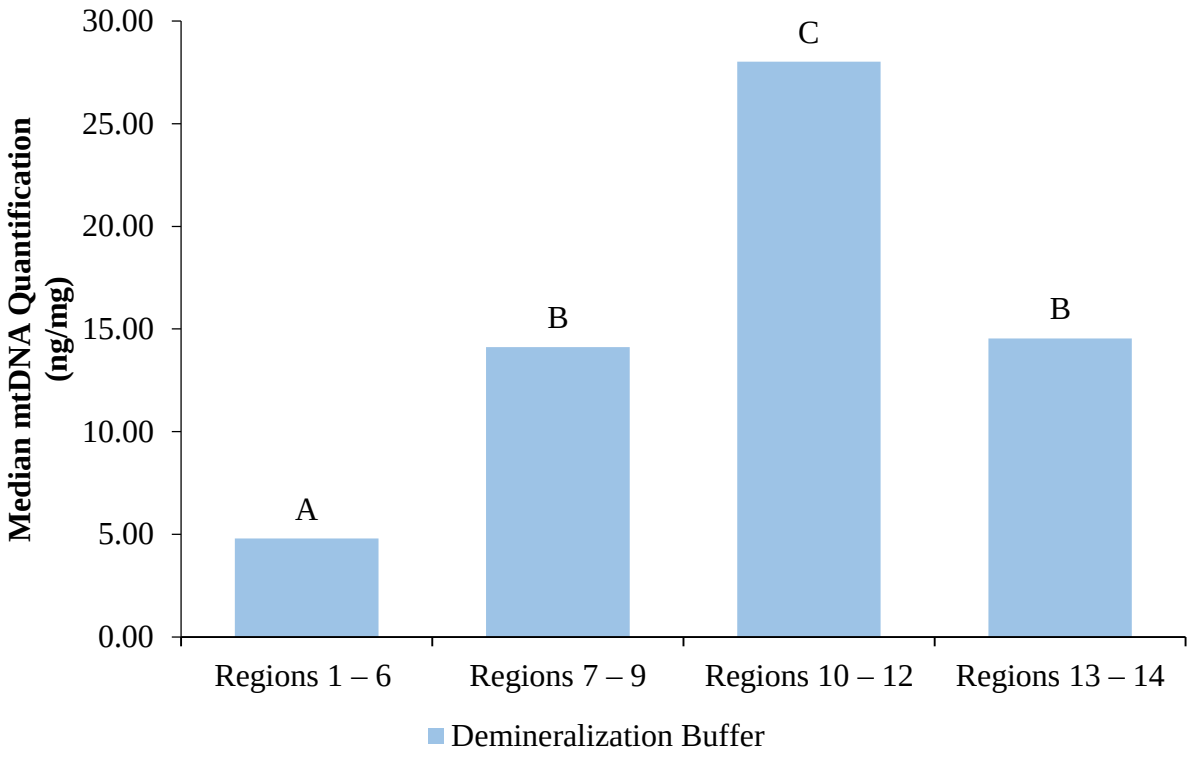

Figure 27: Pairwise Comparisons of Combined mtDNA Yields from Fresh Bovine Bones Digested in Demineralization Buffer

The x-axis lists the regions tested, while the y-axis is the median mtDNA yields normalized in ng per mg bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in mtDNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

Nuclear bovine DNA yields from extracts digested in tissue lysis buffer (Table 25) and demineralization buffer (Table 26) were lowest in Regions 1 – 6, with increased DNA yields at the metaphyses. The exception was Region 9 that had marginally higher yields than Regions 1 – 6, and similar to Regions 7 and 8. Regions 10 – 12 had higher yields than Regions 1 – 9, 13 and 14. Regions 13 and 14 had similar yields to Regions 7 – 9. Both buffers had similar overall variation in quantity; however, demineralization buffer extracts had higher yields at Regions 1 – 4 while Regions 9 – 14 had higher yields from tissue lysis buffer extracts. Combined nuclear DNA yields from bone digested in tissue lysis and demineralization buffers are listed in Table 27 and graphically depicted in Figure 29. The highest yields were at the epiphyses, followed by the metaphyses and articulating surface as well as the tarsals, with the diaphysis having the lowest yields. Individual values for these data, including normalization parameters and total DNA recovered are available in Appendix C17 – C32 (even numbers). Efficiencies of qPCR ranged from 88.4 to 105.6 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.977 to 0.992. No PCR inhibition was detected via the IPC.

Table 25: Nuclear DNA Yields from Fresh Bovine Bones Digested in Tissue Lysis Buffer

Regions and concomitant locations correspond to Table 3. C-(01 – 08) are biological replicates. Nuclear DNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements.

Region	Location on Element	Nuclear DNA Yield (ng/mg)									
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08	Median	MAD
1	Midshaft Diaphysis	180.00	33.44	39.85	60.55	27.78	16.46	65.54	57.60	48.72	16.05
2	Midshaft Diaphysis	193.00	147.06	91.80	50.98	50.49	46.31	93.60	84.36	88.08	37.60
3	Proximal Diaphysis	160.35	87.55	77.40	52.80	28.84	7.89	87.58	68.12	72.76	17.39
4	Distal Diaphysis	170.45	90.61	100.44	79.38	40.26	57.35	89.29	76.19	84.33	12.12
5	Proximal Diaphysis	164.40	154.67	95.70	71.65	30.49	50.40	88.33	88.71	88.52	27.50
6	Distal Diaphysis	92.35	104.00	113.88	105.50	73.60	116.33	83.53	104.00	104.00	10.76
7	Proximal Metaphysis	350.20	678.40	234.24	155.52	93.60	83.05	223.47	186.30	204.89	80.33
8	Distal Metaphysis	289.88	295.04	194.40	123.53	90.00	144.24	137.25	165.87	155.06	35.44
9	Articulating Surface	251.02	852.00	211.53	198.45	214.40	259.80	164.16	152.82	212.96	42.45
10	Distal Epiphysis	720.00	1913.73	375.65	352.06	246.96	269.36	285.20	257.04	318.63	59.30
11	Femoral Head	462.29	1167.06	324.49	207.60	275.29	239.40	205.29	220.40	257.35	50.90
12	Trochanter	830.20	1051.57	379.00	220.20	307.92	285.36	376.88	256.24	342.40	71.60
13	Calcaneus	279.56	679.59	214.80	156.12	255.36	275.28	233.20	190.49	244.28	33.14
14	Talus	656.47	1109.80	171.60	130.41	275.55	275.00	268.24	171.05	271.62	100.30

Table 26: Nuclear DNA Yields from Fresh Bovine Bones Digested in Demineralization Buffer

Regions and concomitant locations correspond to Table 3. C-(01 – 08) are biological replicates. Nuclear DNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 12 reside on the femur; the calcaneus and talus (Regions 13 and 14) were tested as whole elements.

Region	Location on Element	Nuclear DNA Yield (ng/mg)									
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08	Median	MAD
1	Midshaft Diaphysis	224.00	297.60	81.18	52.51	93.38	137.67	85.28	63.15	89.33	31.50
2	Midshaft Diaphysis	220.92	244.71	89.41	95.40	143.29	111.51	92.08	57.71	103.46	26.94
3	Proximal Diaphysis	210.00	296.25	87.35	99.33	106.29	113.06	92.56	77.38	102.81	12.85
4	Distal Diaphysis	231.76	197.78	114.29	110.35	108.00	113.37	74.12	60.35	111.86	20.80
5	Proximal Diaphysis	183.67	328.28	94.12	97.73	131.20	100.65	94.22	55.02	99.19	18.54
6	Distal Diaphysis	216.73	264.29	116.00	122.82	106.80	168.00	73.76	74.36	119.41	45.35
7	Proximal Metaphysis	279.76	328.64	153.44	128.18	159.22	104.92	190.93	144.53	156.33	31.38
8	Distal Metaphysis	274.08	359.60	122.25	100.90	146.16	187.80	86.06	114.24	134.21	40.73
9	Articulating Surface	346.82	120.14	158.04	165.18	182.82	168.56	154.86	106.53	161.61	14.08
10	Distal Epiphysis	541.18	915.20	228.39	266.40	223.02	216.33	288.32	245.30	255.85	32.65
11	Femoral Head	816.00	1059.18	249.27	226.12	244.35	206.98	200.34	174.04	235.24	31.57
12	Trochanter	395.29	882.00	241.43	113.65	240.84	205.32	259.29	101.78	241.13	81.65
13	Calcaneus	218.57	752.94	136.32	97.34	231.14	210.54	174.59	115.73	192.57	47.41
14	Talus	555.47	1047.76	116.47	78.51	189.41	191.63	137.47	107.46	163.44	51.48

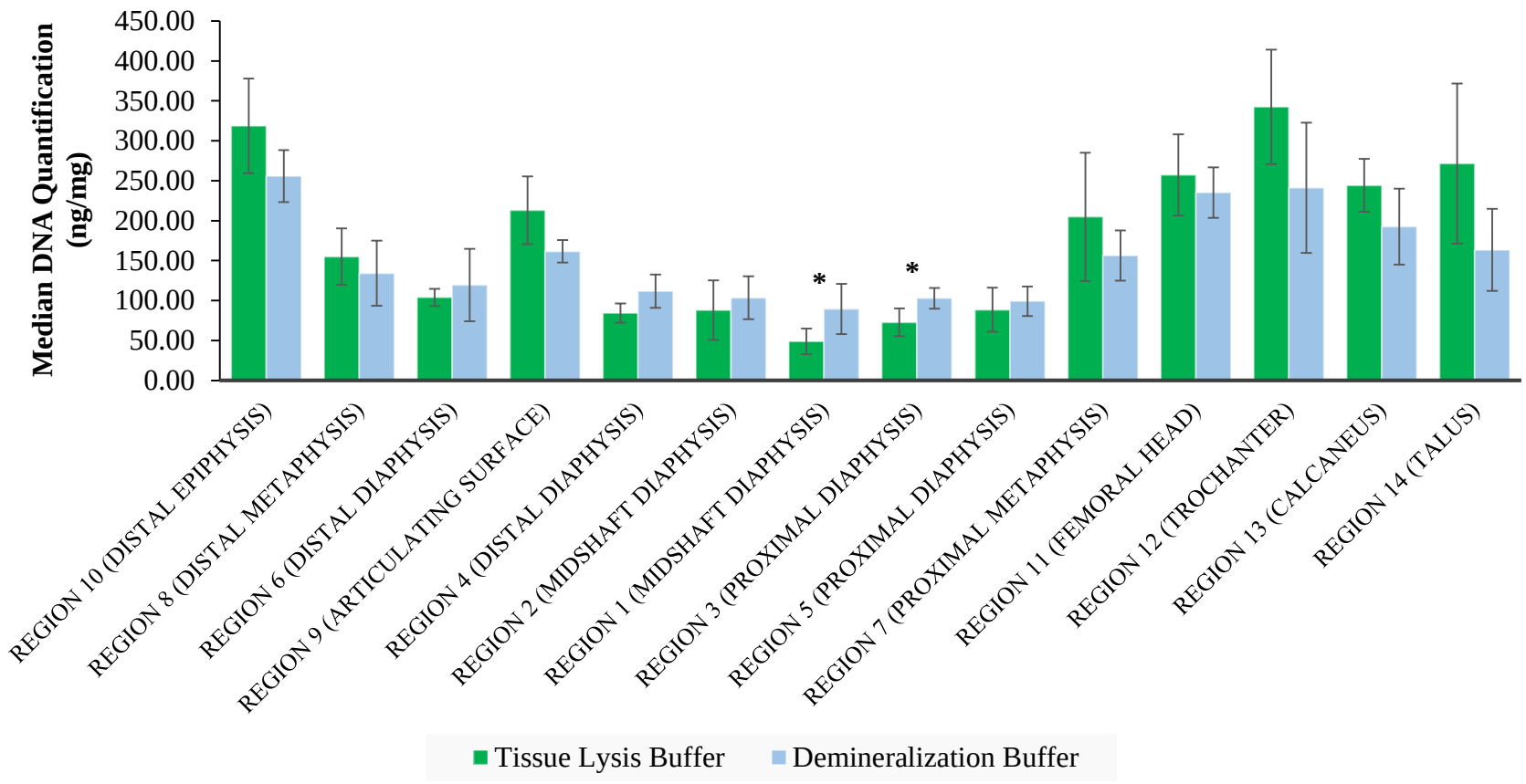

Figure 28: Median Nuclear DNA Yields from Fresh Bovine Bones
The x-axis lists the regions tested, while the y-axis is the median nuclear DNA yield in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Asterisk indicates significant differences between buffers.

Table 27: Combined Nuclear DNA Yields from Fresh Bovine Bones Digested in Tissue Lysis and Demineralization Buffers

Combined regions listed in first column and correspond to Table 3. Regions combined from C-(01 – 08). DNA quantities are reported in ng per mg bone powder. Median was used to characterize central tendency and median absolute deviation (MAD) for variation in data. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

TL = tissue lysis buffer DM = demineralization buffer

Combined Regions	Median		MAD	
	TL	DM	TL	DM
1 – 6	83.94	107.40	24.87	28.12
7 – 9	196.43	156.45	53.39	34.34
10 – 12	296.64	242.89	76.34	36.74
13 – 14	261.80	182.00	59.16	57.33

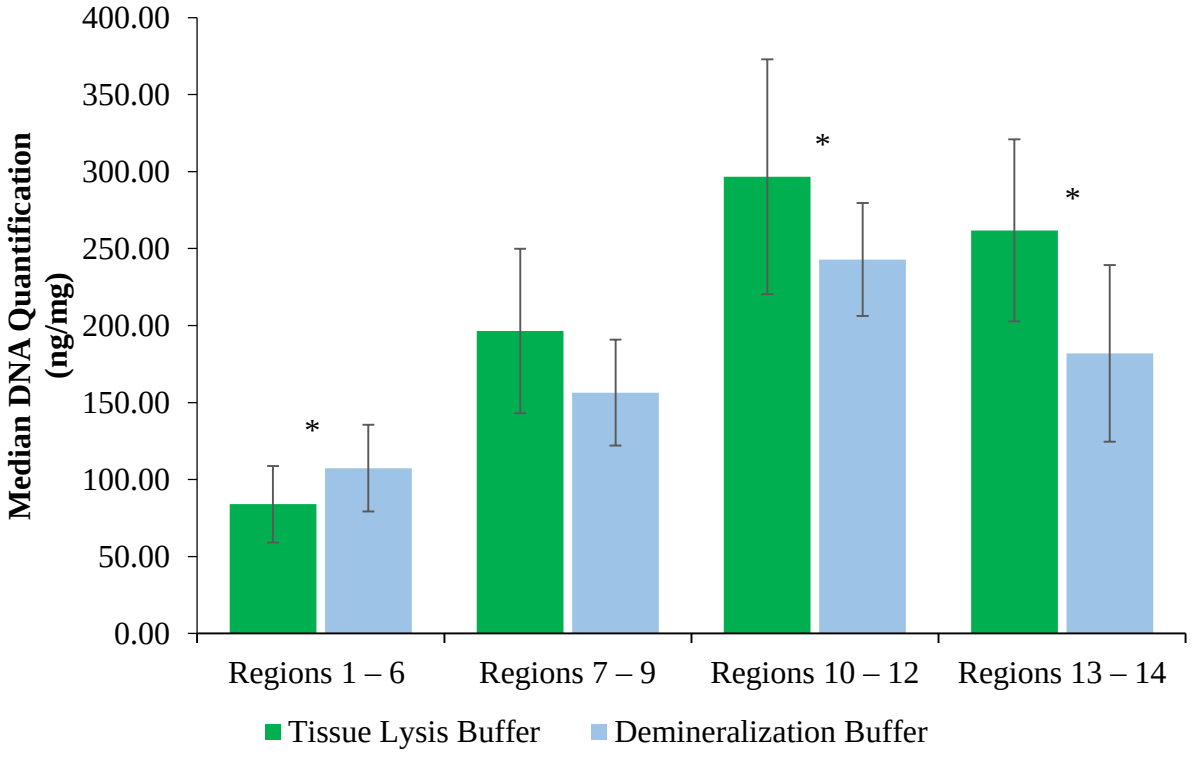

Figure 29: Combined Median Nuclear DNA Yields from Fresh Bovine Bones

The x-axis lists the regions tested, while the y-axis is the median DNA yields in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer, and blue bars bone powder digested in demineralization buffer. Error bars represent median absolute deviation. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals. Asterisk indicates significant differences between buffers.

Median bovine nuclear DNA yields differed significantly among regions of bone digested in tissue lysis and demineralization buffers ($p < 0.0001$). P-values from multiple pairwise comparisons are available in Appendix A11 and A12. Pairwise comparisons of nuclear DNA yields from bone digested in tissue lysis and demineralization buffers are depicted in Figures 30 and 31 respectively. Regions 10 – 12 had significantly higher DNA yields than Regions 1 – 6 ($p = 0.013 – 0.0001$), except for Region 12 digested in demineralization buffer was not significantly different from Region 6 ($p = 0.052$). DNA yields among Regions 1 – 9, 13, and 14 digested in demineralization buffer were not significantly different ($p = 0.994 – 0.064$).

Bone powder digested in tissue lysis buffer had a greater number of regions that differed significantly. Region 7 had significantly more DNA than Regions 1 – 4 ($p = 0.029 – 0.002$). Region 8 had significantly more DNA than Region 1 ($p = 0.006$) and Region 3 ($p = 0.016$). Region 9 had significantly more DNA than Regions 1 – 6 ($p = 0.026 – 0.0001$), but not Region 7 ($p = 0.675$) or Region 8 ($p = 0.446$). Similarly, Regions 13 and 14 had significantly more DNA than Regions 1 – 6 ($p = 0.003 – 0.0001$), but not significant to Regions 7 – 12 or to each other ($p = 0.920 – 0.271$). Significantly more nuclear DNA was recovered from Region 1 ($p = 0.021$) and Region 3 ($p = 0.028$) digested in demineralization buffer than tissue lysis buffer, other regions were not statistically different between buffers ($p = 0.846 – 0.104$).

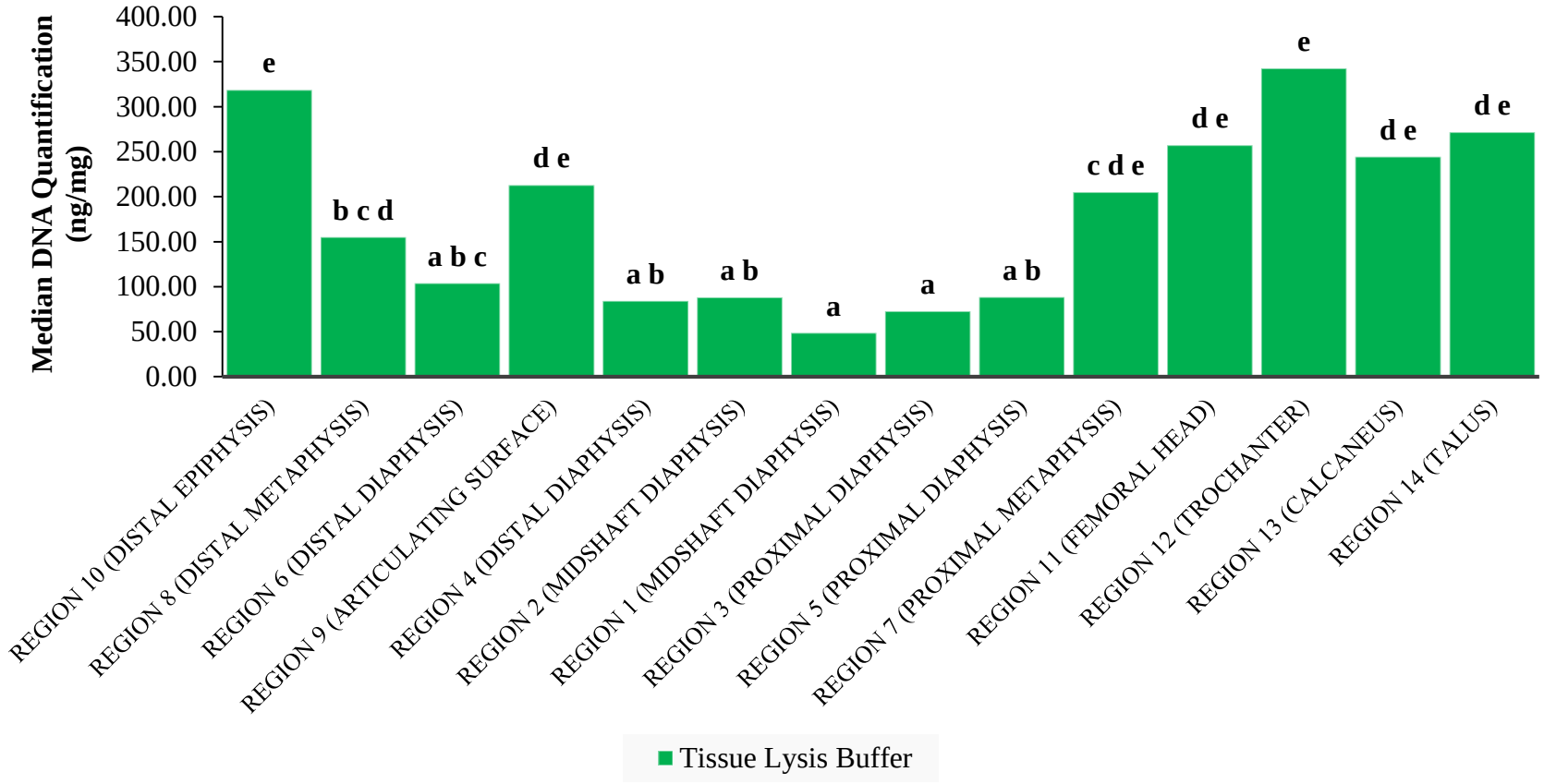

Figure 30: Pairwise Comparisons of Nuclear DNA Yields from Fresh Bovine Bones Digested in Tissue Lysis Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median nuclear DNA yield normalized in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did.

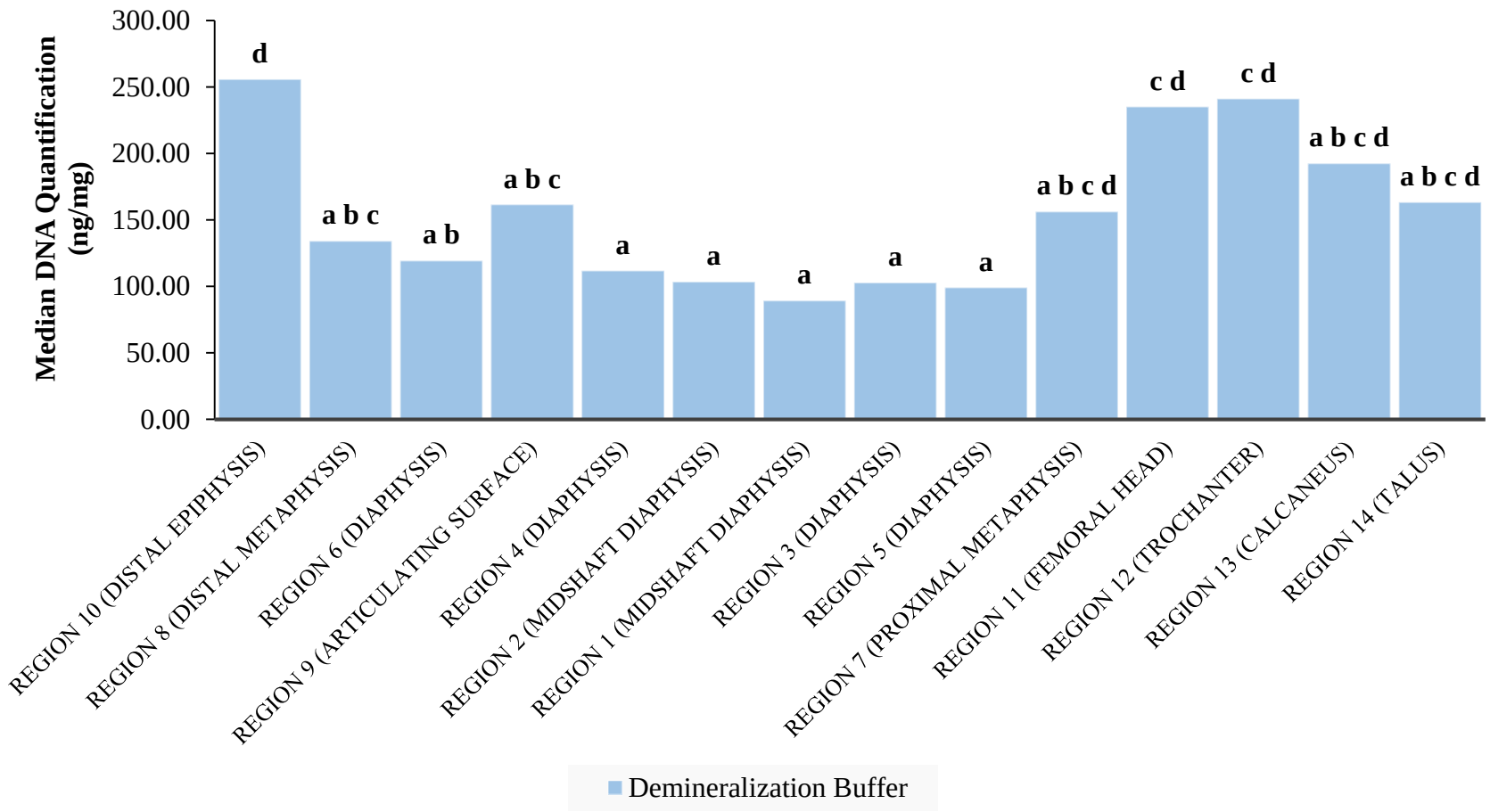

Figure 31: Pairwise Comparisons of Nuclear DNA Yields from Fresh Bovine Bones Digested in Demineralization Buffer
The x-axis lists the regions tested (n = 8), while the y-axis is the median nuclear DNA yield normalized in ng per mg bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did.

Combined bovine nuclear DNA yields differed significantly among the femoral diaphysis, the metaphyses and articulating surface, and the epiphyses digested in both buffers ($p < 0.0001$). P-values for multiple pairwise comparisons of the femur and individual pairwise comparison between tarsals and femora are available in Appendix A6. Pairwise comparisons of combined nuclear DNA yields derived from bone digested in tissue lysis and demineralization buffers are depicted in Figures 32 and 33 respectively. Combined regions were significantly different at a Bonferroni corrected significance level of $\alpha = 0.0167$. The epiphyses had significantly higher DNA yields, for both buffers, than the diaphysis ($p < 0.0001$), and the metaphyses and articulating surface ($p = 0.0158 – 0.008$). The metaphyses and articulating surface had significantly higher yields than the diaphysis ($p = 0.011 – 0.0001$) digested in both buffers. The tarsals had significantly higher yields than the diaphysis ($p = 0.008 – 0.0001$), were not significantly different from the metaphyses and articulating surface ($p = 0.576 – 0.079$), and were significantly lower than the epiphyses ($p = 0.041 – 0.014$) for bone powder digested in both buffers. Comparing yields between buffers, significantly more nuclear DNA was recovered from the diaphysis digested in demineralization buffer ($p < 0.0001$), and the epiphyses and tarsals digested in tissue lysis buffer respectively ($p = 0.046, 0.047$). There was no significant difference between buffers for the metaphyses and articulating surface ($p = 0.153$).

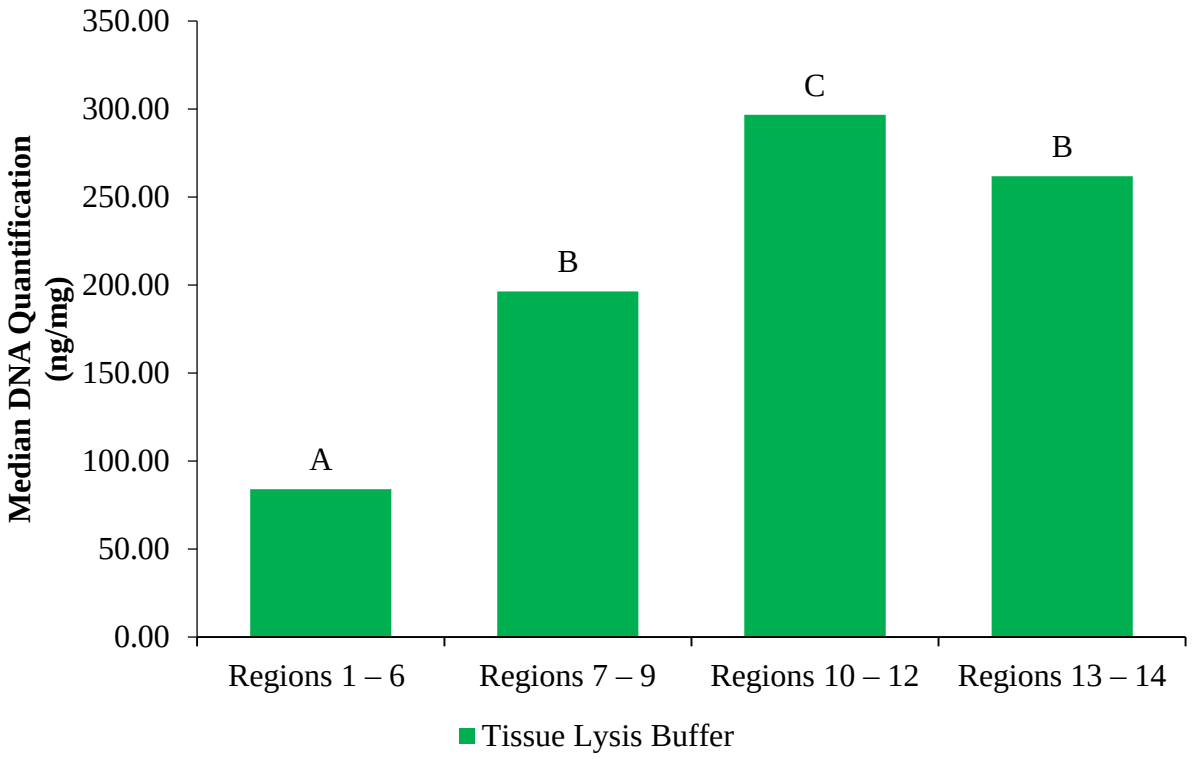

Figure 32: Pairwise Comparisons of Combined Nuclear DNA Yields from Fresh Bovine Bones Digested in Tissue Lysis Buffer

The x-axis lists the regions tested, while the y-axis is the median DNA yields normalized in ng per mg bone powder. Green bars represent bone powder digested in tissue lysis buffer. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

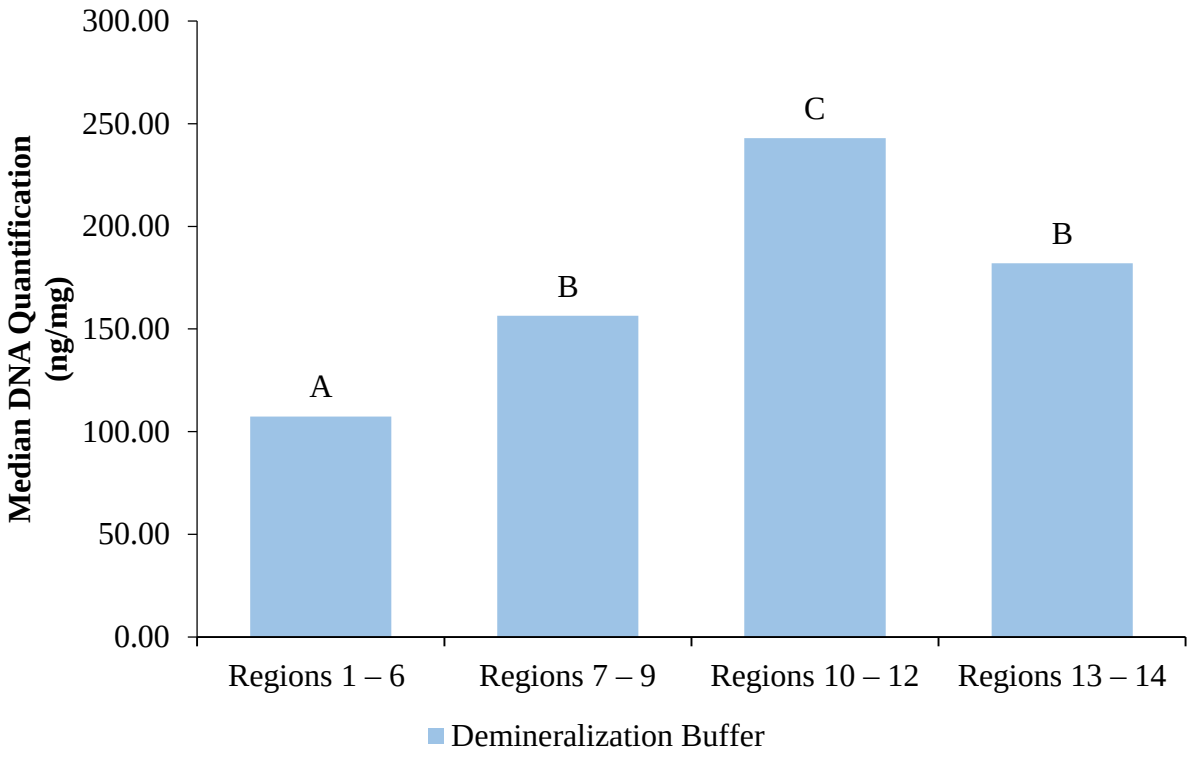

Figure 33: Pairwise Comparisons of Combined Nuclear DNA Yields from Fresh Bovine Bones Digested in Demineralization Buffer

The x-axis lists the regions tested, while the y-axis is the median DNA yields normalized in ng per mg bone powder. Blue bars represent bone powder digested in demineralization buffer. Regions that have the same letter did not differ significantly in DNA yields, while regions with different letters did. Regions 1 – 6 represent the diaphysis, 7 – 9 the metaphyses and articulating surface, 10 – 12 the epiphyses, and 13 – 14 the tarsals.

Quality of Total DNA Recovered from Fresh Bovine Femora and Tarsals

MtDNA PCR amplification results are individually listed in Appendix C33 and C34, and are listed by the maximum amplicon generated for each region per digestion buffer in Table 28. The vast majority of regions for each replicate produced the 994 bp mtDNA amplicon from bone digested in tissue lysis buffer (78.6%) and demineralization buffer (85.7%). Remaining mtDNA extracts derived from digestion in demineralization buffer amplified the 607 bp amplicon (14.3%). Remaining tissue lysis buffer digested extracts produced the 607 bp amplicon (16.1%), except six extracts that amplified the 390 bp amplicon (5.3%). Eighteen of twenty-four tissue lysis buffer, and eleven of sixteen demineralization buffer extracts that were unable to produce the 994 bp mtDNA amplicon, including the six extracts that produced the 390 bp amplicon, came from C-03 and C-04, bones derived from a single cow. Region 2 did not generate the 994 bp amplicon in half of the extracts tested. The remaining extracts that did not produce the 994 bp amplicon were sporadically distributed among the other regions. Overall, there was more variability in mtDNA amplicon size recovered from the diaphysis, particularly regions 1, 2, and 9, than the epiphyses or tarsals. Demineralized bone powder had better quality mtDNA than powder digested in tissue lysis buffer ($p = 0.011$). Reagent blanks for C-01 and C-02 amplified the 994 bp amplicon in both buffers; remaining reagent blanks and negative controls had no amplification.

Table 28: PCR Amplification Results for Mitochondrial DNA Recovered from Fresh Bovine Bones

Regions and concomitant locations coincide with Table 3. The largest amplicon size successfully produced per region (n = 16) are listed under their respective columns.
TL = tissue lysis buffer (n = 8) DM = demineralization buffer (n = 8)

Region	Location on Element	Mitochondrial DNA (*ATPase*)								
		201 bp		390 bp		607 bp		994 bp		Total
		TL	DM	TL	DM	TL	DM	TL	DM	
1	Midshaft Diaphysis	0	0	0	0	2	2	6	6	16
2	Midshaft Diaphysis	0	0	0	0	5	3	3	5	16
3	Proximal Diaphysis	0	0	1	0	0	1	7	7	16
4	Distal Diaphysis	0	0	1	0	1	1	6	7	16
5	Proximal Diaphysis	0	0	0	0	1	1	7	7	16
6	Distal Diaphysis	0	0	1	0	1	3	6	5	16
7	Proximal Metaphysis	0	0	1	0	1	0	6	8	16
8	Distal Metaphysis	0	0	1	0	0	0	7	8	16
9	Articulating Surface	0	0	0	0	3	2	5	6	16
10	Distal Epiphysis	0	0	1	0	1	1	6	7	16
11	Femoral Head	0	0	0	0	0	0	8	8	16
12	Trochanter	0	0	0	0	1	1	7	7	16
13	Calcaneus	0	0	0	0	1	1	7	7	16
14	Talus	0	0	0	0	1	0	7	8	16
Total		0	0	6	0	18	16	88	96	224

Nuclear DNA PCR amplification results are individually listed in Appendix C35 and C36, and are listed by the maximum amplicon generated for each region per digestion buffer in Table 29. Nuclear DNA extracts from bone powder digested in tissue lysis and demineralization buffers amplified the 410 bp amplicon 79.5% and 92.9% of the time respectively. There were two extracts, C-01 Region 3 digested in demineralization buffer and C-08 Region 3 digested in tissue lysis buffer, which generated the 599 bp amplicon; remaining extracts that did not amplify the 410 bp amplicon produced the 200 bp amplicon. Similar to mtDNA, demineralized bone powder had better quality nuclear DNA than bone digested in tissue lysis buffer ($p = 0.004$). More variability in amplicon size was present at the diaphysis than the epiphyses or tarsals. Reagent blanks C-02 and C-05 for tissue lysis buffer amplified 410 bps, while remaining reagent blanks and negative controls had no amplification.

Table 29: PCR Amplification Results for Nuclear DNA Recovered from Fresh Bovine Bones

Regions and concomitant locations coincide with Table 3. The largest amplicon size successfully produced per region (n = 16) are listed under their respective columns.
TL = tissue lysis buffer (n = 8) DM = demineralization buffer (n = 8)

Region	Location on Element	Nuclear DNA (*MC1R*)								Total
		200 bp		410 bp		599 bp		989 bp		
		TL	DM	TL	DM	TL	DM	TL	DM	
1	Midshaft Diaphysis	1	0	7	8	0	0	0	0	16
2	Midshaft Diaphysis	3	0	5	8	0	0	0	0	16
3	Proximal Diaphysis	2	2	5	5	1	1	0	0	16
4	Distal Diaphysis	3	0	5	8	0	0	0	0	16
5	Proximal Diaphysis	3	0	5	8	0	0	0	0	16
6	Distal Diaphysis	1	1	7	7	0	0	0	0	16
7	Proximal Metaphysis	2	0	6	8	0	0	0	0	16
8	Distal Metaphysis	2	0	6	8	0	0	0	0	16
9	Articulating Surface	0	1	8	7	0	0	0	0	16
10	Distal Epiphysis	1	0	7	8	0	0	0	0	16
11	Femoral Head	0	0	8	8	0	0	0	0	16
12	Trochanter	0	0	8	8	0	0	0	0	16
13	Calcaneus	2	2	6	6	0	0	0	0	16
14	Talus	2	1	6	7	0	0	0	0	16
Total		22	7	89	104	1	1	0	0	224

Surface/Burial Comparison Experiment of Bovine Femora and Tarsals

Total DNA Quantification and Quality for Surface Exposed Bovine Bones

Mean mtDNA yields from surface exposed bovine femora and tarsals over a six month period are listed in Table 30 and graphically depicted in Figure 34. MtDNA yields declined over the six month period; however, variation among the five regions of the femur, the calcaneus, and the talus remained consistent throughout the six months. The femoral head and distal epiphysis had the highest mtDNA yields (~16 ng/mg) followed by the calcaneus and talus (~11 ng/mg), with the diaphysis and the proximal and distal metaphyses having the lowest yields (~4 ng/mg) at six months. Individual values for these data, including normalization parameters and total mtDNA recovered are available in Appendix D1 – D6, and final normalized quantifications for mtDNA recovered at each region and time point are listed in Appendix D7. Efficiencies of

qPCR ranged from 92.1 to 100.4 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.993 to 0.996.

There was no relationship between the quantity and quality of recoverable mtDNA, nor was quality affected over the six months based on amplification results. The 994 bp amplicon was successfully produced from the seven regions at each time point. The week one reagent blank amplified 994 bps, while remaining reagent blanks and all negative controls had no amplification. Individual PCR amplification results are listed in Appendix D8.

Real time PCR inhibition occurred (detected by IPC) in the femoral head week two replicate A1 and week four replicate A2, distal epiphysis week four replicates A1 and A2, calcaneus week two and week four replicate A2, and talus week four replicates A1 and A2 as well as month six replicate A2. Inhibited extracts were diluted and re-quantified to determine uninhibited yields listed in Appendix D7, and subsequently used in Table 30 and Figure 34.

Table 30: mtDNA Quantification from Surface Exposed Bovine Bones
Region bone was drilled from listed in first column. Mean mtDNA quantities (n = 2), day zero (n = 4), are reported as ng per mg bone powder. Cells highlighted in yellow had at least one replicate with inhibition.

Region	Mean mtDNA (ng/mg)					
	Day 0	Week 1	Week 2	Week 4	Month 3	Month 6
Diaphysis	24.29	1.70	9.58	11.72	4.91	3.03
Proximal Metaphysis	16.79	6.02	12.34	14.59	5.60	3.98
Distal Metaphysis	25.73	8.55	10.13	15.67	4.91	4.58
Femoral Head	56.37	42.55	38.58	38.77	32.80	16.70
Distal Epiphysis	64.05	39.85	28.39	34.45	16.28	16.06
Calcaneus	30.91	20.41	21.27	19.01	13.60	11.08
Talus	41.57	24.15	38.62	11.01	9.78	10.93

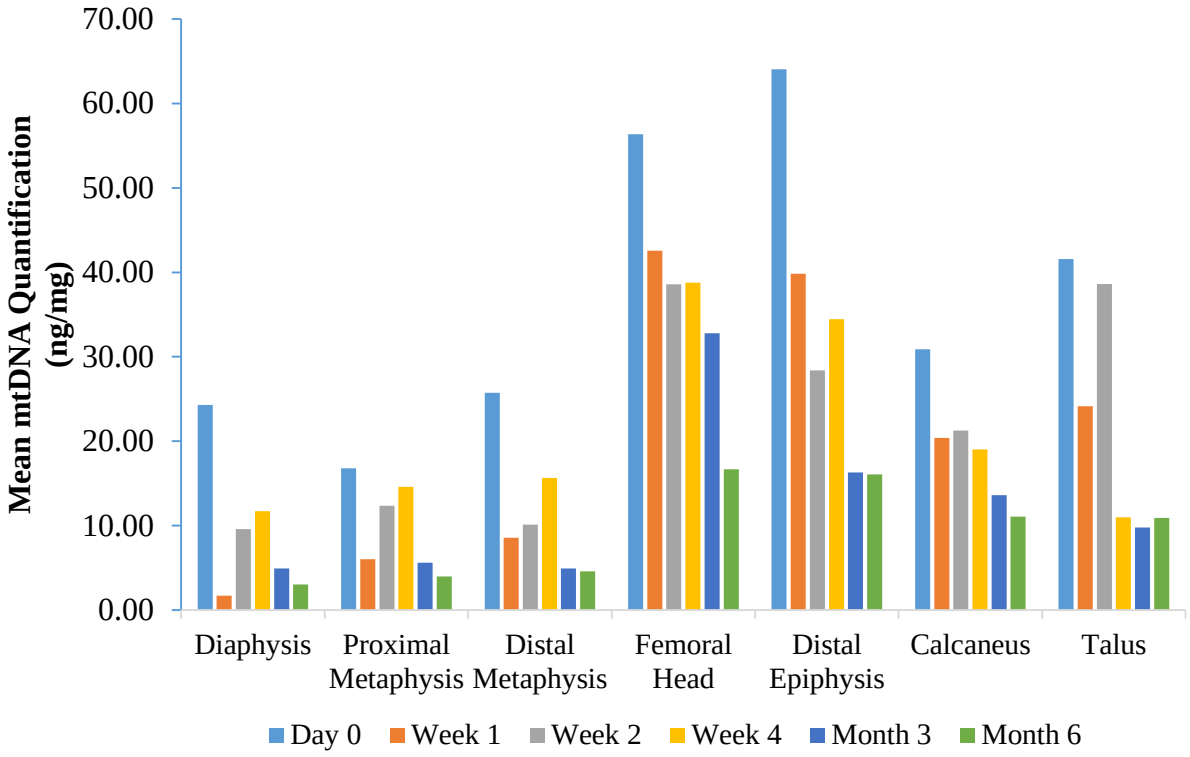

Figure 34: Mean mtDNA Yields from Bovine Bones Exposed for Six Months
The x-axis lists the regions tested, while the y-axis is the mean mtDNA yield from surface exposed bovine bones in ng per mg bone powder. Legend is color coded to its corresponding bar representing a specific time point. Day zero time point had a sample size of four (buried and surface exposed data were combined). Remaining time points had a sample size of two.

Mean nuclear DNA yields from surface exposed bovine femora and tarsals over a six month period are listed in Table 31 and graphically depicted in Figure 35. Mean nuclear DNA yields varied substantially throughout the six months. Week two, week four, and month three extracts often contained higher DNA yields than extracts from the initial time point. Extracts derived from replicate A2 generally had higher DNA quantification than A1 extracts (Appendix D9). The femoral head had higher mean yield at month six (385.65 ng/mg) than day zero (281.53 ng/mg). The other regions had lower mean month six yields than day zero. Mean DNA yield variation among the five regions of the femur, the calcaneus, and the talus remained generally consistent over the six month interval. The femoral head had the highest yields

(385.65 ng/mg), followed by the talus (231.18 ng/mg), distal metaphysis (217.14 ng/mg), and calcaneus (202.92 ng/mg); with the distal epiphysis (169.43 ng/mg), proximal metaphysis (155.11 ng/mg), and diaphysis (130.86 ng/mg) having the lowest yields. Individual values for these data, including normalization parameters and total DNA recovered are available in Appendix D10 – D15, and final normalized quantifications for DNA recovered at each region and time point are listed in Appendix D9. Efficiencies of qPCR ranged from 99.4 to 105.3 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.981 to 1.000.

Table 31: Nuclear DNA Quantification from Surface Exposed Bovine Bones
Region bone was drilled from listed in first column. Mean nuclear DNA quantities (n = 2), day zero (n = 4), are reported as ng per mg bone powder. Cells highlighted in yellow had at least one replicate with inhibition.

Region	Mean Nuclear DNA (ng/mg)					
	Day 0	Week 1	Week 2	Week 4	Month 3	Month 6
Diaphysis	204.48	56.20	156.35	165.36	166.18	130.86
Proximal Metaphysis	203.96	123.21	216.04	221.06	255.54	155.11
Distal Metaphysis	245.80	225.07	181.54	265.19	299.31	217.14
Femoral Head	281.53	313.12	613.03	456.54	399.34	385.65
Distal Epiphysis	305.99	250.64	274.93	367.94	370.98	169.43
Calcaneus	228.49	222.74	383.83	361.20	385.86	202.92
Talus	287.32	229.90	386.38	345.66	279.38	231.18

There was no relationship between the quantity and quality of recoverable nuclear DNA, nor was quality affected over the six months based on amplification results. The 410 bp amplicon was successfully produced from the seven regions at each time point. Reagent blanks and negative controls had no amplification. Individual PCR amplification results are listed in Appendix D16. Real time PCR inhibition occurred in week four and month six replicate A2 from the femoral head and talus, and week four replicate A2 distal epiphysis and calcaneus.

Inhibited extracts were diluted and re-quantified to determine uninhibited yields listed in Appendix D9, and subsequently used in Table 31 and Figure 35.

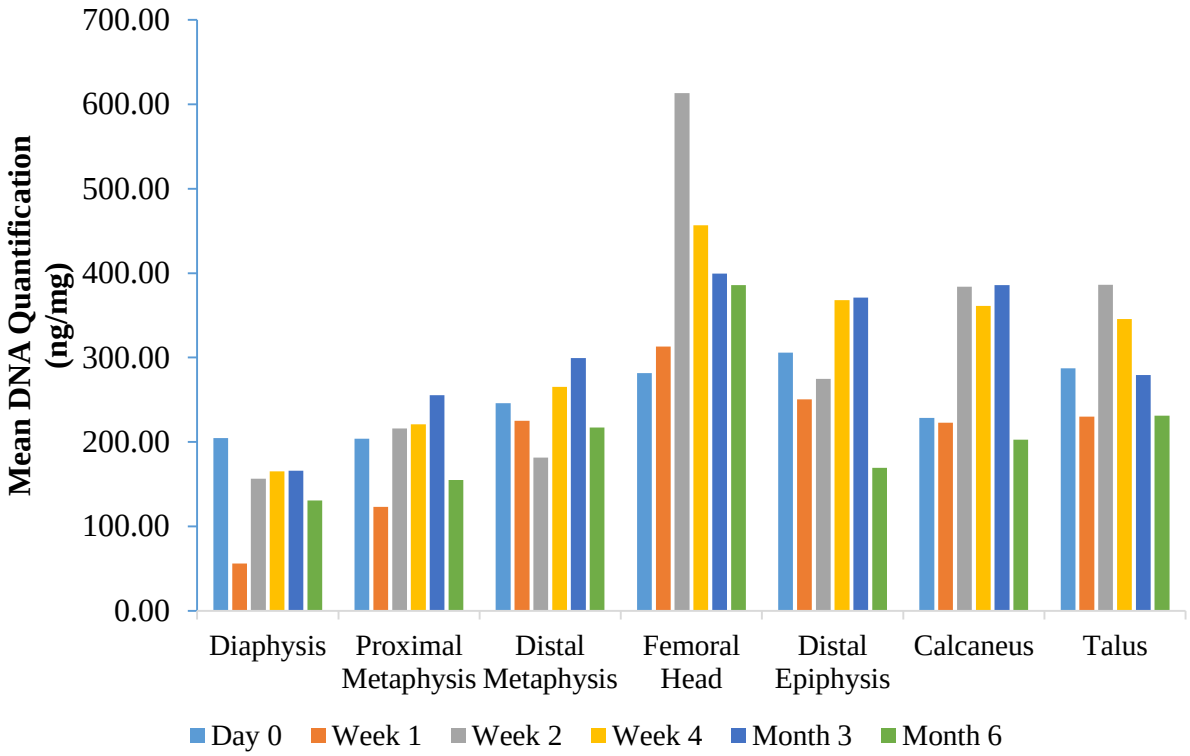

Figure 35: Mean Nuclear DNA Yields from Bovine Bones Exposed for Six Months
The x-axis lists the regions tested, while the y-axis is the mean nuclear DNA yield from bovine bones in ng per mg bone powder. Legend is color coded to its corresponding bar and represents the specific time point listed. Day zero time point had a sample size of four (buried and surface exposed data were combined). Remaining time points had a sample size of two.

Total DNA Quantification and Quality for Buried Bovine Bones

Mean mtDNA yields from buried bovine femora and tarsals over a six month period are listed in Table 32 and graphically depicted in Figures 36 and 37. Mean mtDNA yields from each region decreased by 90% or more during the first week of burial. Additionally, variation among regions present at day zero decreased by month six, except the talus, which maintained a yield of greater than 1 ng/mg at six months. Individual values for these data, including normalization

parameters and total mtDNA recovered, are available in Appendix D17 – D22, and final normalized quantifications for mtDNA recovered at each region and time point are listed in Appendix D23. Efficiencies of qPCR ranged from 92.1 to 100.4 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.993 to 0.996.

There was no relationship between the quantity and quality of recoverable mtDNA, nor was quality affected over the six months based on amplification results. The 994 bp amplicon was successfully produced from the seven regions at each time point. The day zero reagent blank produced the 994 bp amplicon, and remaining reagent blanks and negative controls had no amplification. Individual PCR amplification results are listed in Appendix D24.

Real time PCR inhibition occurred in week two replicate B2 distal epiphysis and calcaneus, week four replicate B1 distal metaphysis distal epiphysis calcaneus and talus, and month six replicate B2 femoral head. Inhibited extracts were diluted then re-quantified to determine uninhibited yields listed in Appendix D23, and subsequently used in Table 32 and Figures 36 and 37.

Table 32: mtDNA Quantification from Buried Bovine Bones

Region bone was drilled from listed in first column. Mean mtDNA quantities (n = 2), day zero (n = 4), are reported as ng per mg bone powder. Cells highlighted in yellow had at least one replicate with inhibition.

Region	Mean mtDNA (ng/mg)					
	Day 0	Week 1	Week 2	Week 4	Month 3	Month 6
Diaphysis	24.29	0.69	0.47	0.40	0.58	0.46
Proximal Metaphysis	16.79	0.86	0.60	0.51	0.34	0.86
Distal Metaphysis	25.73	0.62	0.47	0.88	0.16	0.18
Femoral Head	56.37	3.37	2.97	1.85	0.61	0.93
Distal Epiphysis	64.05	1.55	0.82	0.77	0.50	0.64
Calcaneus	30.91	2.43	0.99	0.72	0.46	0.70
Talus	41.57	1.73	1.77	0.94	1.24	1.52

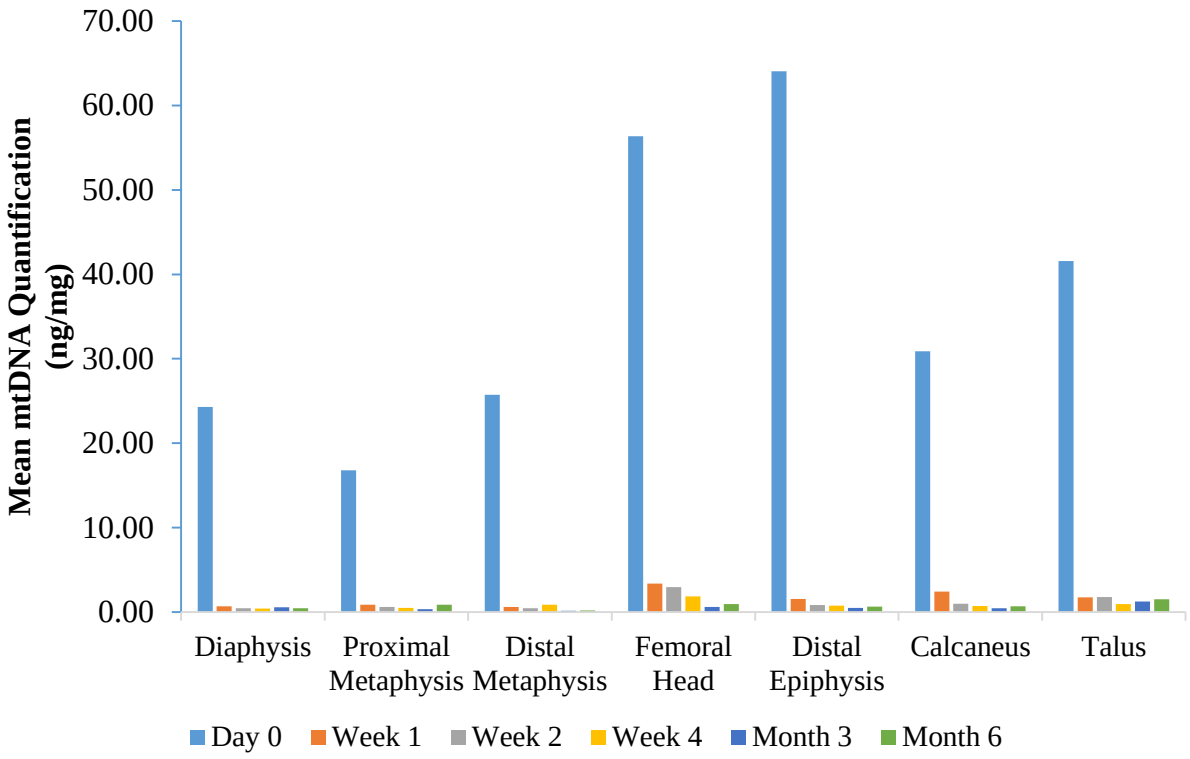

Figure 36: Mean mtDNA Yields from Bovine Bones Buried for Six Months with Day Zero Time Point

The x-axis lists the regions tested, while the y-axis is the mean mtDNA yield from bovine bones in ng per mg bone powder. Legend is color coded to its corresponding bar and represents the specific time point. Day zero time point had a sample size of four (buried and surface exposed data were combined). Remaining time points had a sample size of two.

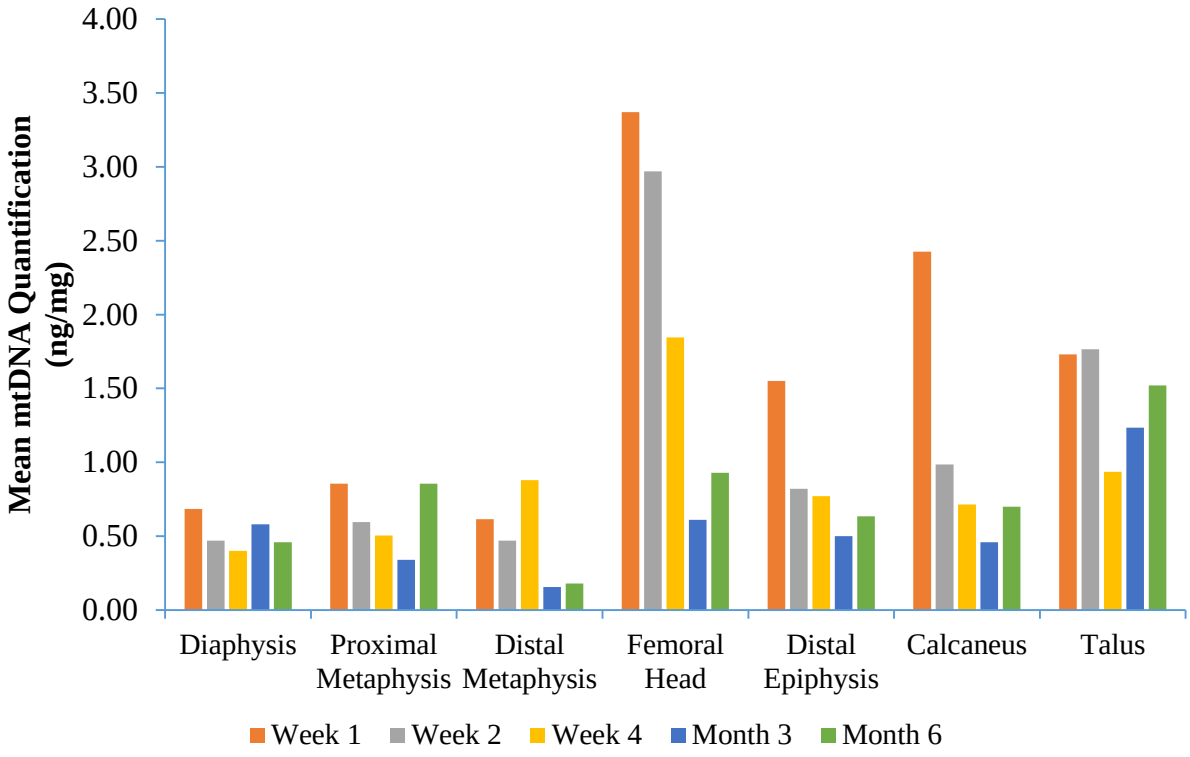

Figure 37: Mean mtDNA Yields from Bovine Bones Buried for Six Months without Day Zero Time Point
The x-axis lists the regions tested, while the y-axis is the mean mtDNA yield from bovine bones in ng per mg bone powder. Legend is color coded to its corresponding bar and represents the specific time point. Each region had a sample size of two for each time point.

Mean nuclear DNA yields from buried bovine bones over a six month period are listed in Table 33 and graphically depicted in Figures 38 and 39. Mean nuclear DNA yields decreased by 85% or more during the first week of burial. The diaphysis and metaphyses had the largest decrease in yields from day zero to week one, then remained relatively stable for the remainder of the six months. Mean yields from the distal epiphysis, femoral head, calcaneus, and talus decreased further from week one to week four, and increased from week four to month six. Overall, at six months the femoral head and talus had the highest nuclear DNA yields (~16 ng/mg), followed by the distal epiphysis and calcaneus (~7 ng/mg), and the lowest yields were from the diaphysis and metaphyses (~1 ng/mg). Individual values for these data, including

normalization parameters and total DNA recovered are available in Appendix D25 – D30, and final normalized quantifications for DNA recovered at each region and time point are listed in Appendix D31. Efficiencies of qPCR ranged from 99.4 to 105.3 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.981 to 1.000.

A total of 84 DNA extracts were tested over the six months: 32% amplified the 599 bp amplicon (27/84), 51% amplified the 410 bp amplicon (43/84), 8% amplified the 200 bp amplicon (7/84), and 8% failed to amplify (7/84). DNA extracts that failed to amplify had PCR inhibition during qPCR except week four replicate B1 distal metaphysis. Extracts where the 200 bp amplicon was produced came primarily from week two replicate B2 and week four replicate B1, with two exceptions: week one replicate B1 diaphysis and calcaneus. Twenty five of twenty seven extracts that produced the 599 bp amplicon came from bones that were buried for at least a week. Three were from week one, six from week two, two from week four, ten from month three, and four from month six sporadically distributed among the seven regions. Reagent blanks and negative controls had no amplification. Individual PCR amplification results are listed in Appendix D32. Inhibited extracts were diluted and re-quantified to determine uninhibited yields listed in Appendix D31, and subsequently used in Table 33 and Figures 38 and 39.

Table 33: Nuclear DNA Quantification from Buried Bovine Bones

Region bone was drilled from listed in first column. Mean nuclear DNA quantities (n = 2), day zero (n = 4), are reported as ng per mg bone powder. Cells highlighted in yellow had at least one replicate with inhibition.

Region	Mean mtDNA (ng/mg)					
	Day 0	Week 1	Week 2	Week 4	Month 3	Month 6
Diaphysis	204.48	3.88	1.48	2.05	1.31	1.71
Proximal Metaphysis	203.96	0.88	0.72	0.80	0.43	0.93
Distal Metaphysis	245.80	0.48	0.49	0.71	0.21	0.67
Femoral Head	281.53	35.19	17.76	5.43	8.19	16.48
Distal Epiphysis	305.99	7.27	14.00	4.23	7.74	7.58
Calcaneus	228.48	34.38	6.83	0.70	2.70	7.66
Talus	287.32	25.74	11.93	2.18	8.11	16.19

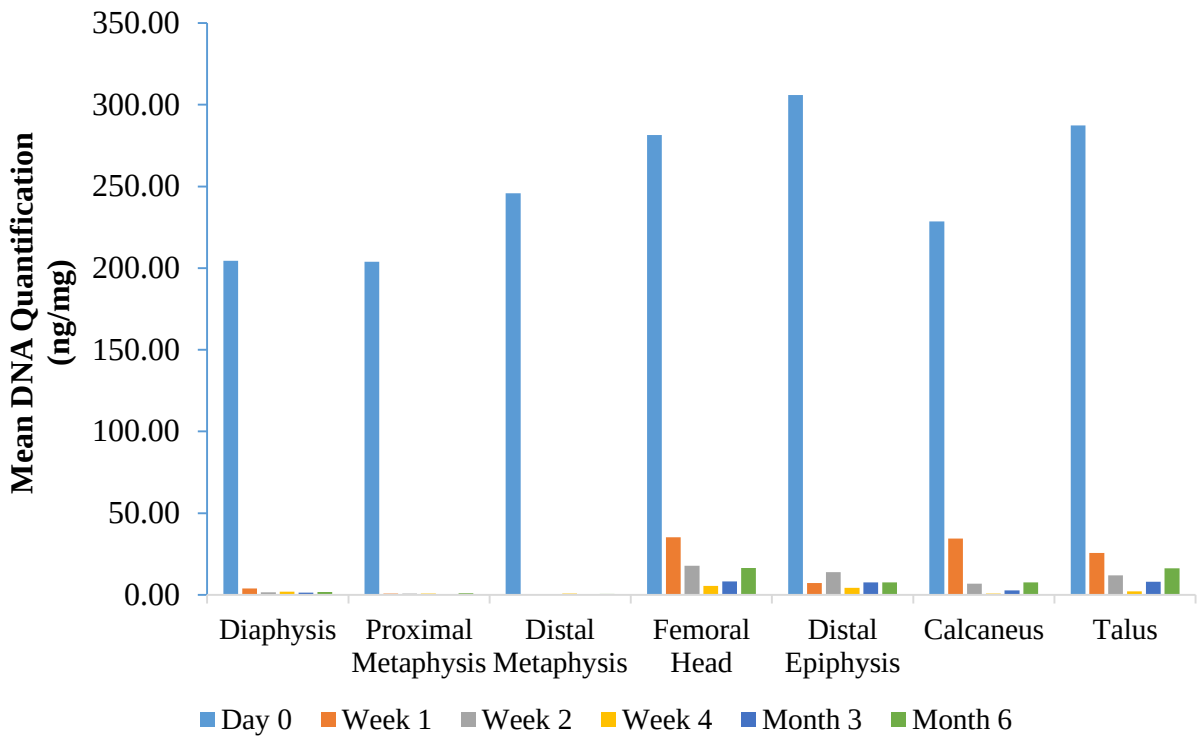

Figure 38: Mean Nuclear DNA Yields from Bovine Bones Buried for Six Months with Day Zero Time Point

The x-axis lists the regions tested, while the y-axis is the mean nuclear DNA yield from bovine bones in ng per mg bone powder. Legend is color coded to its corresponding bar and represents the specific time point. Day zero time point had a sample size of four (buried and surface exposed data were combined). Remaining time points had a sample size of two.

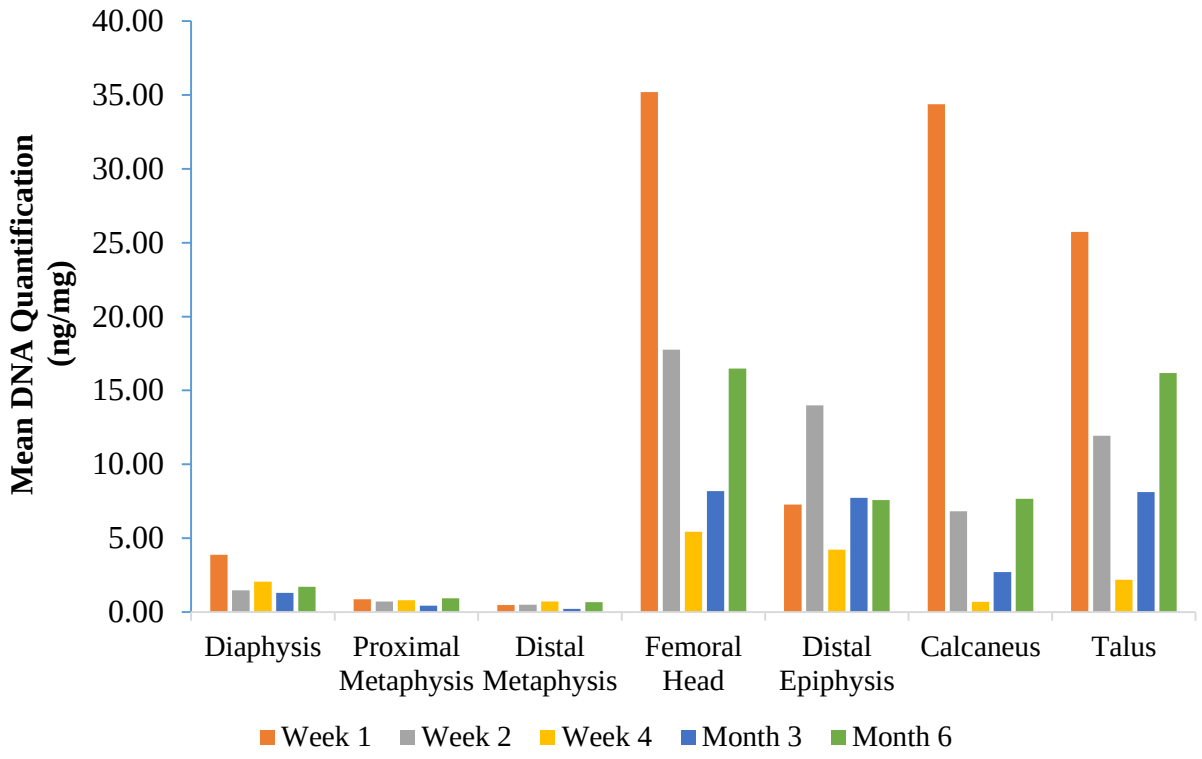

Figure 39: Mean Nuclear DNA Yields from Bovine Bones Buried for Six Months without Day Zero Time Point
The x-axis lists the regions tested, while the y-axis is the mean nuclear DNA yield from bovine bones in ng per mg bone powder. Legend is color coded to its corresponding bar and represents the specific time point. Each region had a sample size of two for each time point.

Ancillary Experiments

Changes in the Recoverable Total DNA of Buried Bovine Bone Segments over a One Month Time Period

The mitochondrial and nuclear DNA quantification results for buried bovine femoral diaphysis are summarized in Tables 34 and 35 respectively. Mean mtDNA yields decreased as burial length increased. Mean day zero yield of 0.43 ng/mg was obtained from the larger segments (type B) that were buried, tested, and reburied on a weekly basis under the assumption similar yields would be obtained from the smaller fragments (type A); however, mean day two (2.16 ng/mg) and day four (1.12 ng/mg) yields from type A were substantially higher than day

zero type B (0.15 ng/mg). Mean mtDNA yields rapidly decreased over the first eleven days, not including mean day zero, then stabilized until the end of the four weeks. Segment types A and B had similar decreases in mtDNA yields over time, and at concomitant time points, both segment types had similar yields at week one (0.32 ng/mg, 0.33 ng/mg), week two (0.05 ng/mg, 0.02 ng/mg), week three (0.03 ng/mg, 0.01 ng/mg), and week four (0.02 ng/mg, 0.02 ng/mg). Retrieving, testing, and reburying segments to be retested at later time points did not affect mean mtDNA recovery when compared to segments that were retrieved and tested once. Individual values for these data, including normalization parameters and total mtDNA recovered, are available in Appendix E1 (type A) and E2 (type B). Efficiencies of qPCR ranged from 100.6 to 104.2 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.998 to 0.999.

Mean nuclear DNA yields of segment type B decreased as burial length increased. Mean day zero yield of 25.77 ng/mg was similar to week one (27.83 ng/mg), but decreased at week two (0.59 ng/mg), and remained around the same through week four (0.69 ng/mg). Day two mean DNA yield of 93.18 ng/mg (type A) was higher than day zero (type B); however, segment type A yields decreased at week one (8.47 ng/mg) and week two (0.50 ng/mg), remaining about the same at week three (0.25 ng/mg) and week four (0.22 ng/mg). Mean nuclear DNA yields between segment types were similar at concurrent time points, except week one segment type A had a lower mean (8.47 ng/mg) than type B (27.83 ng/mg). Retrieving, testing, and reburying segments to be retested later did not appreciably affect mean nuclear DNA recovery when compared to segments that were retrieved and tested once. Individual values for these data, including normalization parameters and total mtDNA recovered are available in Appendix E3

(type A) and E4 (type B). Efficiencies of qPCR ranged from 174.3 to 993.2 as calculated from the slopes of the standard curves, with R^2-values ranging from 0.998 to 0.999.

Table 34: mtDNA Yields of Buried Bovine Femoral Diaphysis
Bone segment identifiers are listed in the first and fourth column. "0D, 2D, 4D, 1W, 11D, 2W, 3W, and 4W" indicate length of burial in days (D) or weeks (W), and "1 – 4" denote biological replicates of femoral diaphysis. "A" represents segments that were retrieved, tested, and stored at -20°C, while "B" indicates segments that were cyclically retrieved, tested, and reburied. MtDNA quantities are normalized in ng per mg of bone powder. Mean mtDNA quantities for each length of burial are bolded. NT = Not Tested

Segment Identifier	A	B	Segment Identifier	A	B
0D-1	NT	0.15	11D-1	0.06	NT
0D-2	NT	0.82	11D-2	0.02	NT
0D-3	NT	NT*	11D-3	0.04	NT
0D-4	NT	0.36	11D-4	0.02	NT
0D Mean	**NT**	**0.44**	**11D Mean**	**0.03**	**NT**
2D-1	1.27	NT	2W-1	0.01	0.01
2D-2	2.24	NT	2W-2	0.09	0.02
2D-3	2.38	NT	2W-3	0.05	0.03
2D-4	2.74	NT	2W-4	0.03	0.02
2D Mean	**2.16**	**NT**	**2W Mean**	**0.05**	**0.02**
4D-1	1.35	NT	3W-1	0.02	0.01
4D-2	1.09	NT	3W-2	0.02	0.01
4D-3	0.97	NT	3W-3	0.05	0.02
4D-4	1.09	NT	3W-4	0.01	0.01
4D Mean	**1.12**	**NT**	**3W Mean**	**0.03**	**0.01**
1W-1	0.19	0.30	4W-1	0.02	0.01
1W-2	0.03	0.29	4W-2	0.01	0.02
1W-3	0.99	0.23	4W-3	0.02	0.04
1W-4	0.08	0.48	4W-4	0.02	0.02
1W Mean	**0.32**	**0.33**	**4W Mean**	**0.02**	**0.02**

*0D-3 (B) failed to produce a testable extract due to Amicon® column damage.

Table 35: Nuclear DNA Yields of Buried Bovine Femoral Diaphysis
Bone segment identifiers are listed in the first and fourth column. "0D, 2D, 4D, 1W, 11D, 2W, 3W, and 4W" indicate length of burial in days (D) or weeks (W), and "1 – 4" denote biological replicates of femoral diaphysis. "A" represents segments that were retrieved, tested, and stored at -20°C, while "B" indicates segments that were cyclically retrieved, tested, and reburied. Nuclear DNA quantities are in ng per mg of bone powder. Mean DNA quantities for each length of burial are bolded. NT = Not Tested

Segment Identifier	A	B	Segment Identifier	A	B
0D-1	NT	0.14	11D-1	0.14	NT
0D-2	NT	72.50	11D-2	0.02	NT
0D-3	NT	NT*	11D-3	0.76	NT
0D-4	NT	4.66	11D-4	0.22	NT
0D Mean	**NT**	**25.77**	**11D Mean**	**0.29**	**NT**
2D-1	39.81	NT	2W-1	0.32	0.006
2D-2	36.01	NT	2W-2	1.11	0.48
2D-3	62.53	NT	2W-3	0.40	1.38
2D-4	234.37	NT	2W-4	0.17	0.49
2D Mean	**93.18**	**NT**	**2W Mean**	**0.50**	**0.59**
4D-1	2.51	NT	3W-1	0.00	1.40
4D-2	77.78	NT	3W-2	0.002	0.48
4D-3	99.48	NT	3W-3	0.94	0.59
4D-4	75.71	NT	3W-4	0.04	0.04
4D Mean	**75.75**	**NT**	**3W Mean**	**0.25**	**0.63**
1W-1	0.24	7.22	4W-1	0.60	0.001
1W-2	19.32	10.98	4W-2	0.04	0.09
1W-3	13.22	13.09	4W-3	0.20	2.16
1W-4	1.11	80.03	4W-4	0.02	0.52
1W Mean	**8.47**	**27.83**	**4W Mean**	**0.22**	**0.69**

*0D-3 (B) failed to produce a testable extract due to Amicon® column damage

Changes in the Recoverable Total DNA of Non-Buried Bovine Bone Segments over a One Month Period

The mitochondrial and nuclear DNA quantification results for non-buried bovine femoral diaphysis are summarized in Tables 36 and 37 respectively. Overall, mean mtDNA yields did not appreciably change from the initial time point and week four; however, there were

substantial increases in yields during the first week of exposure. Mean mtDNA yield at day zero (1.94 ng/mg) was lower than day two (4.17 ng.mg), day four (4.39 ng/mg), and week one (3.12 ng/mg). MtDNA quantification at day ten (1.32 ng/mg), week two (1.55 ng.mg), week three (1.61 ng/mg), and week four (1.90 ng/mg) had similar yields as day zero. Individual values for these data, including normalization parameters and total mtDNA recovered, are available in Appendix E5. Efficiency of qPCR was 105.5 as calculated from the slope of the standard curve, with a R^2-value of 0.998.

Mean nuclear DNA yields changed between the initial time point and one month. DNA yield from day zero (85.82 ng/mg) was lower than day two (235.13 ng/mg), day four (146.47 ng/mg), and week one (153.03 ng/mg). The initial time point was similar to means from day ten (82.35 ng/mg) and week two (88.70 ng/mg); however, means increased at week three (108.83 ng/mg) and week four (152.23 ng/mg). Individual values for these data, including normalization parameters and total DNA recovered, are available in Appendix E6. Efficiency of qPCR was 181.0 as calculated from the slope of the standard curve, with a R^2-value of 0.999.

There was a difference in total DNA yields between buried and non-buried bone. Non-buried bones did not show an appreciable difference in DNA yields over the one month time period, except for the increase during the first week that disappeared by the second. Buried bones on the other hand showed a rapid decline in DNA yields over the same period.

Table 36: mtDNA Yields of Non-Buried Bovine Femoral Diaphysis

Bone segment identifiers are listed in the first and third column. "0D, 2D, 4D, 1W, 10D, 2W, 3W, and 4W" indicate length of exposure in days (D) or weeks (W), and "1 – 4" denote biological replicates of femoral diaphysis tested at each time point. MtDNA quantities are in ng per mg of bone powder. Mean mtDNA quantities for each length of exposure are bolded.

Segment Identifier	Quantification	Segment Identifier	Quantification
0D-1	3.20	10D-1	1.68
0D-2	1.75	10D-2	0.91
0D-3	0.97	10D-3	1.26
0D-4	1.85	10D-4	1.43
0D Mean	**1.94**	**10D Mean**	**1.32**
2D-1	5.64	2W-1	1.53
2D-2	3.34	2W-2	1.66
2D-3	3.39	2W-3	1.82
2D-4	4.32	2W-4	1.19
2D Mean	**4.17**	**2W Mean**	**1.55**
4D-1	5.56	3W-1	2.92
4D-2	3.43	3W-2	0.64
4D-3	4.67	3W-3	1.45
4D-4	3.79	3W-4	1.45
4D Mean	**4.39**	**3W Mean**	**1.61**
1W-1	3.36	4W-1	2.70
1W-2	2.18	4W-2	1.23
1W-3	3.70	4W-3	1.92
1W-4	3.23	4W-4	1.74
1W Mean	**3.12**	**4W Mean**	**1.90**

Table 37: Nuclear DNA Yields of Non-Buried Bovine Femoral Diaphysis
Bone segment identifiers are listed in the first and third column. "0D, 2D, 4D, 1W, 10D, 2W, 3W, and 4W" indicate length of exposure in days (D) or weeks (W), and "1 – 4" denote biological replicates of femoral diaphysis tested at each time point. Nuclear DNA quantities are in ng per mg of bone powder. Mean DNA quantities for each length of exposure are bolded.

Segment Identifier	Quantification	Segment Identifier	Quantification
0D-1	107.52	10D-1	74.62
0D-2	77.84	10D-2	71.61
0D-3	68.99	10D-3	91.51
0D-4	88.94	10D-4	91.66
0D Mean	**85.82**	**10D Mean**	**82.35**
2D-1	288.60	2W-1	54.29
2D-2	110.84	2W-2	106.80
2D-3	192.29	2W-3	110.73
2D-4	348.23	2W-4	82.98
2D Mean	**235.13**	**2W Mean**	**88.70**
4D-1	211.64	3W-1	192.68
4D-2	113.68	3W-2	51.50
4D-3	138.00	3W-3	91.96
4D-4	122.55	3W-4	99.20
4D Mean	**146.47**	**3W Mean**	**108.83**
1W-1	227.57	4W-1	231.16
1W-2	141.69	4W-2	89.14
1W-3	127.57	4W-3	161.67
1W-4	115.27	4W-4	126.93
1W Mean	**153.03**	**4W Mean**	**152.23**

Organic versus SoilMaster™: Total DNA Yield Comparisons Over One Week

Table 38 summarizes nuclear and mitochondrial DNA yields from bone powder extracted utilizing either organic or SoilMaster™. Overall, SoilMaster™ led to higher DNA yields than organic extraction. Furthermore, organically extracted nuclear DNA (0.63 ng/mg) and mtDNA (0.19 ng/mg) yields at day zero were lower than day two nuclear (12.70 ng/mg) and mitochondrial DNA (0.46 ng/mg). SoilMaster™ extraction of bone led to lower day zero yields of nuclear DNA (22.52 ng/mg) than day two (30.50 ng/mg); however, day zero SoilMaster™

mtDNA extract had the highest yield (2.38 ng/mg) and the day seven extract had the lowest (0.42 ng/mg). MtDNA efficiency of qPCR was 96.0 as calculated from the slope of the standard curve, with a R^2-value of 0.999. Nuclear DNA efficiency of qPCR was 132.0 as calculated from the slope of the standard curve, with a R^2-value of 0.987. Individual values for these data, including normalization parameters and total DNA recovered are available in Appendix E7.

Table 38: Total DNA Yield Comparison between Organic and SoilMaster™ Extractions
Normalized values for nuclear and mitochondrial DNA are reported as ng per mg of bone powder.

mtDNA (ng/mg)			Nuclear DNA (ng/mg)		
Time Point	**Organic**	**SoilMaster™**	**Time Point**	**Organic**	**SoilMaster™**
Day 0	0.19	2.38	Day 0	0.63	22.52
Day 2	0.46	1.80	Day 2	12.70	30.50
Day 5	0.22	1.25	Day 5	11.00	21.16
Day 7	0.12	0.42	Day 7	4.64	13.28

Mass Difference between Wet and Dry Bone

Table 39 depicts changes in bone powder and whole bone mases subjected to storage treatments over time. Bone powder in closed microcentrifuge tubes retained its mass over a one week period. Bone powder in open microcentrifuge tubes or weigh boats resulted in a loss of mass, with mass stabilization occurring after several days. The bone segment continually lost mass for the span of the experiment: 4% mass was lost in the first week, and another 1% the following week. Individual values for the change in mass over time are listed in Appendix E8.

Table 39: Mass Differences of Dehydrated Bone Powder and Bone Segment
Bone powder subjected to each treatment was weighed over a one week period. Bone segment was weighed over a two week period. "Δ Mass" indicates the overall change in mass (in milligrams). "% Diff." indicates the percent difference between starting and ending masses of bone powder/segment. N/A = Not Applicable

	Mass of Bone Powder/Bone Segment (mg)								
Condition	1 Closed Tube		2 Open Tube		3 Piled Powder in Weigh Boat		4 Powder Spread in Weigh Boat		5 Bone Segment
Trial	1	2	1	2	1	2	1	2	1
Δ Mass (mg)	0	0	-2	-2	0	-2	-1	-2	-2,669
% Diff.	0	0	4	4	0	4	2	4	5
Mean % Diff.	0		4		2		3		N/A

Effect of Proteinase K Concentration on Total DNA Yields

Nuclear and mitochondrial DNA yields from bovine diaphysis digested in tissue lysis and demineralization buffers with varied volumes of proteinase K are listed in Table 40. Proteinase K increased nuclear and mitochondrial DNA yields from bone powder digested in both buffers; however, increasing the amount proteinase K did not have an appreciable effect on DNA yields for these samples. MtDNA efficiency of qPCR was 84.9 as calculated from the slope of the standard curve, with a R^2-value of 0.993. Nuclear DNA efficiency of qPCR was 195.0 as calculated from the slope of the standard curve, with a R^2-value of 0.990. Individual values for these data, including normalization parameters and total mtDNA and DNA recovered are available in Appendix E9 and E10 respectively.

Table 40: Proteinase K Concentration's Effect on Total DNA Yields
Proteinase K was added to digestion buffer by percent total reaction volume. Trials were qPCR technical replicates. Mean DNA yields for each volume of proteinase K are bolded.

	mtDNA (ng/mg)							
	Tissue Lysis Buffer				**Demineralization Buffer**			
Proteinase K (20 mg/mL)	0%	0.5%	1%	2%	0%	0.5%	1%	2%
Trial 1	5.17	9.43	7.90	9.92	5.33	11.20	10.4	12.18
Trial 2	4.50	8.79	9.88	9.43	5.56	10.96	12.42	10.26
Mean	**4.84**	**9.11**	**8.89**	**9.67**	**5.45**	**11.08**	**11.43**	**11.22**
	Nuclear DNA (ng/mg)							
	Tissue Lysis Buffer				**Demineralization Buffer**			
Proteinase K (20 mg/mL)	0%	0.5%	1%	2%	0%	0.5%	1%	2%
Trial 1	284.67	533.96	503.84	679.59	202.20	295.10	261.60	333.00
Trial 2	259.22	668.78	769.60	648.98	253.80	336.73	416.40	229.20
Mean	**271.95**	**601.37**	**636.22**	**664.29**	**228.00**	**315.92**	**339.00**	**281.10**

Comparison of Total DNA Yields from Bovine Bones Macerated by MSU Forensic Anthropologist versus MSU Forensic Biologist

Nuclear and mitochondrial DNA quantification from bovine bones that were macerated at both sites are listed in Table 41. Elements processed at the MSU Forensic Biology Laboratory resulted in higher total DNA yields utilizing both tissue lysis and demineralization buffers. MtDNA efficiency of qPCR was 99.5 as calculated from the slope of the standard curve, with a R^2-value of 0.997. Nuclear DNA efficiency of qPCR was 99.7 as calculated from the slope of the standard curve, with a R^2-value of 0.985. Individual values for these data, including normalization parameters and total mtDNA and nuclear DNA recovered are available in Appendix E11 and E12 respectively.

Table 41: Total DNA Quantification Comparisons between Bovine Bones Macerated by MSU Forensic Anthropologist and the MSU Forensic Biologist

Region tested is in the first column. DNA/mtDNA quantification is reported in nanograms per mg bone.

FA = DNA derived from an element macerated by MSU Forensic Anthropology

FB = DNA was derived from an element macerated by MSU Forensic Biology.

Region	Mitochondrial DNA Yields (ng/mg)				Nuclear DNA Yields (ng/mg)			
	Tissue Lysis Buffer		Demineralization Buffer		Tissue Lysis Buffer		Demineralization Buffer	
	FA	FB	FA	FB	FA	FB	FA	FB
Diaphysis	2.30	6.40	5.33	10.71	80.07	120.00	184.59	236.08
Distal Epiphysis	31.73	55.12	19.65	45.08	411.22	495.56	244.52	318.24
Femoral Head	26.74	51.06	20.55	36.76	570.72	611.14	299.20	340.00
Calcaneus	4.24	41.29	12.13	24.21	128.12	647.45	220.54	257.40
Talus	6.47	16.00	8.30	14.00	192.71	390.00	261.52	298.29

DISCUSSION

Establishing positive identification of human remains has vital importance to the legal system, family and friends of the decedent, and society in general. DNA identification from skeletal material can be very effective even when remains are highly decomposed, largely incomplete, substantially damaged and/or fragmented, or other forms of positive identification are unavailable. Maximizing DNA recovery from skeletal material relies on how bone is handled from discovery to testing, the element assayed, and the processes used to isolate DNA from it. Many studies have been undertaken to compare how skeletal elements vary in recoverable DNA and successful downstream analyses; however, little research has focused on differences in DNA quantity and quality within a single element. Therefore, a systematic investigation of intra-femoral heterogeneity of DNA content was conducted. The first step in this process was to establish DNA heterogeneity in fresh bone, while the second was to determine how it was affected by burial and outdoor exposure, environments in which forensically relevant remains are commonly found. An ancillary goal was to compare DNA content within the femur to the calcaneus and talus, two tarsals that Mundorff and Davoren (2014) found harbor more DNA.

The most interesting finding of the research presented here was that DNA quantity varied significantly along the femur, and the distribution of DNA was highly similar between porcine and bovine bones, wherein the epiphyses harbored more DNA than the metaphyses, and both had more than the diaphysis. This was also consistent with results from previous studies that utilized weanling rat femora (Yamaguchi and Yamaguchi 1986; Yamaguchi et al., 2003), indicating that common factors are likely influencing DNA heterogeneity. DNA quantity may vary between the

diaphysis and epiphyses because they originate as separate bones. The femur is often thought of in its mature form, as a single element; however, it is the sum total of primary and secondary ossification centers fused together. Epiphyseal closure schedules have been well characterized in domesticated animals (Silver, 1963) and humans (Gardner and Gray, 1970), where the femur's primary ossification center begins at the mid-diaphysis and grows longitudinally to fuse with the distal and proximal epiphyses. The femoral head and trochanter (i.e., proximal epiphysis) of porcine and bovine femora fuse to the diaphysis at 3 – 3.5 years of age, while the distal epiphysis fuses to the diaphysis at 3 – 3.5 years in porcines and 3.5 – 4 years in bovines (Silver, 1963). DNA quantity differed among femoral ossification centers in this study, which is consistent with Mundorff and Davoren's (2014) finding that bones from the same body varied greatly in DNA yield. If bone development affects DNA quantity among unlike elements (e.g., femur versus cranium), then separate ossifications centers of the same bone will likely vary in quantity as well. Comparing DNA yields from primary and secondary ossification sites from other long bones, or elements like vertebrae that have a different morphology, could provide more evidence that ossification centers affect DNA heterogeneity within a single element.

Animals used throughout this research varied in antemortem age, likely influencing DNA heterogeneity. Longitudinal growth of the femoral diaphysis takes place at the epiphyseal plates and between the epiphyses and articular cartilage (Bisgard and Bisgard, 1935). The growing ends of the diaphysis (i.e., metaphyses) and the epiphyses may harbor more cells, resulting in a richer source of DNA. Bovine femora C-01 and C-02 came from cows slaughtered around 36 months of age, while C-03 – C-08 originated from dairy cows 48+ months old (Ryan Varner, MSU Meats Laboratory, personal communication). This age difference likely led to the higher DNA yields from the younger bovine specimens. Porcine femora also came from younger

animals, as the pigs were slaughtered at 3 – 7 months old. The disparity in bovine and porcine femoral development might have contributed to the more pronounced difference in DNA yields at the metaphyses and epiphyses in porcine bone. Old age could affect DNA quantity as well; Atkinson et al. (1962) characterized changes in human femoral density with age, and reported that it decreased as the age of the individual increased and that the metaphyses decreased in density at a faster rate than the diaphysis in individuals over fifty. Additionally, emerging evidence links femoral osteocyte lacunar density—a proxy measure of osteocyte density—with age. In a poster presentation, Hunter and Agnew (2014) described the osteocytic lacunae density in 20 cross sections of midshaft diaphysis from deceased human males ranging in ages from 29 to 79 years with no known skeletal pathology. The authors reported a significantly higher density in males aged 29 – 59 than those aged 60 – 79, as well as a significant negative correlation between age and density. Once an individual becomes physically mature, cell density (and presumably DNA yield) in bone could decline. This postulate is consistent with the current research where younger specimens had more DNA than their older counterparts.

Another potential cause of intra-femoral DNA heterogeneity involves bone remodeling. Regions of the femur with high DNA yields, like the femoral head and distal epiphysis, may undergo more remodeling because they distribute static body weight and facilitate locomotion (Carter, 1984). Similarly, the trochanter and Region 9 are points of muscle attachment that undergo stress during muscle contraction, and thus might require more remodeling. Ruff et al. (2006) reviewed bone functional adaptation, and concluded that bone cells respond to local mechanical stress by adjusting the bone's structure to compensate for an individual's activity. Furthermore, bone remodeling involves the continuous removal and replacement of old osseous tissue to maintain bone strength and mineral homeostasis (Clarke, 2008). Although most

remodeling sites are thought to arise spontaneously (Clarke, 2008), Mashiba et al. (2001) showed that generating micro-damage in dog radii increased remodeling at damaged sites, and in a review of bone remodeling mechanisms Burr (2002) concluded that about 30% of it occurs in a targeted manner. Leney (2006) postulated that bone remodeling of the femur and tibia was why those bones were more likely to contain mtDNA that led to reportable sequence data. The author also proposed that the mandible was a good source of mtDNA because remodeling at points of muscle attachment related to mastication increased bone density. A similar hypothesis could be extended to partially explain why the patella, carpals, and tarsals were found to be a rich source of DNA in previous research (Mundorff et al., 2009; Mundorff and Davoren, 2014), in which entire elements that undergo more remodeling may also contain more cells. Overall, separate ossification sites, antemortem age, and remodeling of the femur, as well as factors not yet considered, likely influenced DNA yield throughout the present research.

The above-mentioned results came from fresh (macerated) bone, which would not be recovered during a forensic investigation. Therefore, non-macerated bovine femora and tarsals buried or left on the soil's surface were used to simulate common environments in which remains are found. The burial and surface exposure of bone led to several interesting findings throughout this research. For instance, many regions of the bones exposed on the soil surface (most notably from the epiphyses and tarsals) had higher nuclear DNA yields at the week two, four, and month three time points than the initial time point. The reason for this is unclear; however, environmental exposure may have softened the bone, making it easier to drill and caused less DNA degradation (discussed below). In contrast, decreased DNA yields at the six-month time point likely resulted from sustained environmental exposure. Why nuclear DNA yields increased more at certain time points than mtDNA did is currently unknown, but the

increase might have been a result of nuclear DNA being more sensitive to damage during drilling, something to keep in mind for future investigations.

Researchers have shown that burying bone can have a drastic effect on DNA quantity over time, but never in such a short period as demonstrated in the current research. Campos et al. (2012) reported that mtDNA quantity was reduced by more than 90% in buried bovine bone compared to control bone at one year. Hebda and Foran (2015) established that nuclear and mitochondrial DNA from bovine femoral diaphyses declined substantially between one week and one month of burial; however, pre-burial yields were not assessed, so the level of DNA loss during the first week was unclear. Based on the present study, the rapid decline in DNA quantity likely occurs within days or weeks of burial, although the exact cause of this is unclear. Soil pH was approximately 5.5 at the burial site, which is considered moderately acidic (Horneck et al., 2011). Lange (2008) determined that pH was the only chemical factor to correlate with skeletal weathering, in which lower pHs (~5.5) were associated with higher weathering stages of skeletons recovered from the Voegtly cemetery. Acidic soil is known to deleteriously affect DNA and bone (Turner-Walker, 2008; Latham and Madonna, 2013); however, the amount of time it takes to degrade DNA to the extent observed in the present research is unclear, and degradation seems unlikely to occur over the course of only a few weeks. Campos et al. (2012) proposed that skeletal DNA quantity declines due to the rapid putrefaction of osteoblasts, osteoclasts, bone-lining cells, and blood cells. Additionally, microbial attack on bone can influence DNA degradation (Latham and Madonna, 2013), and Bell et al. (1996) reported that postmortem alteration of bone, visualized through scanning electron microscopy, was likely due to microbial attack and can occur in as little as three months, the shortest postmortem interval tested. In the current research, burial may have facilitated an anaerobic environment that

allowed the bones to putrefy rapidly, aiding in the steep decline of DNA by one week. Additionally, microbes intrinsic to bone and/or soil could have contributed to DNA loss by degrading both bone and DNA. Further research will be required to determine the cause(s) of short-term DNA loss resulting from burial, paying close attention to bone tissue decomposition, microbial communities, pH, temperature, and soil chemistry.

Another interesting finding from the present study was that DNA quantity in buried femora declined faster at the epiphyses than the diaphysis over six months, which brought DNA yields among regions closer over time. However, it is noteworthy that throughout the period the epiphyses still had as much or more DNA as the diaphysis. Femoral epiphyses contain less cortical bone than the mid-diaphysis (Clarke, 2008), potentially making them more susceptible to factors that deleteriously affect DNA, resulting in differential rates of DNA loss among bone regions. The extension of burial interval from months to years or decades might invert regional DNA variation if dense cortical bone can better withstand environmental attack over time. This could help explain why weight bearing long bones were more likely to contain analyzable DNA when extended postmortem periods were a factor (Edson et al., 2004; Leney, 2006; Miloš et al., 2007), while researchers using non-buried remains with shorter postmortem intervals reported that elements like the carpals, tarsals, and patella had better quantity and quality DNA than long bones (Mundorff et al., 2009; Mundorff and Davoren, 2014).

An interesting, albeit unexpected, result in the current research was that 27 of 84 DNA extracts from bovine buried bones generated the 599 bp nuclear DNA amplicon, a much higher percentage than from surface exposed (0 of 84) or macerated (2 of 224) samples. DNAs that were able to amplify out to 599 bps primarily came from week two (6 of 27) and month three (10 of 27) time points, in which an interaction between burial and drilling might have influenced the

results. Starting at week one, the bones were noticeable softer and easier to drill. Burial likely led to collagen and hydroxyapatite breakdown, weakening bone structure and reducing DNA damage during drilling. After the three month time point when amplification of the 599 bp amplicon was most successful, environmental degradation of DNA could have reduced DNA quality. Finally, several DNAs tested from the week four time point exhibited PCR inhibition (discussed below), potentially lowering the frequency of generating the 599 bp amplicon.

A final interesting finding from the environmental exposure study was that buried and surface exposed bones that were only eight vertical inches apart had drastic differences in total DNA yields, showing that local environment can have a profound effect on DNA. DNA yields of buried bones plummeted over a short period whereas surface exposed elements' did not, yet in many instances nuclear DNA quality was higher in buried bones than in surface exposed samples (as noted above). This discrepancy could have resulted from an interaction between environment and DNA amplification methodology. For example, DNA degradation in buried bone may have been largely complete; however, a small amount of high quality DNA was protected within hydroxyapatite, allowing the 599bp fragment to amplify. This would also be true for surface exposed bone, but the higher overall DNA quantity in them indicates that less complete degradation took place. The bovine DNA quantitative assay used in this research was based on ~80 bp amplicons. If the majority of DNA from surface exposed bones was degraded below 600 bp but not below 80 bp, its abundance might swamp out any high molecular weight DNA bound to hydroxyapatite, resulting in the observation that DNA from surface exposed bones had lower quality than buried ones. Creating real-time PCR amplicons of increasing size (e.g., 100 – 600 bps) and testing DNA extracts from buried and surface exposed bones with them would help determine how degraded the DNAs actually are. If DNA bound within hydroxyapatite and

subsequently released through demineralization is less degraded, it would provide further evidence that demineralization of bone leads to higher quality DNA for analysis, an obvious boon for forensic casework and research alike.

The research on environmental exposure and burial of bone was designed to generate baseline knowledge of how intra-femoral DNA heterogeneity identified in macerated bone differs under more forensically relevant conditions; however, it had several limitations. First, low sample size per treatment (n = 2) made statistical analysis impractical. Second, an age discrepancy between replicates was a potential confound: one set came from an adult, the other a juvenile. Of course in a real world scenario the age of the decedent would not be controlled for, so future investigations that utilize different age groups may be helpful in determining how environment influences DNA yield over time. Third, the fact that environmentally exposed bones were not macerated limited drilling to areas that were amenable to mechanical defleshing. For instance, articulating surfaces often had tendon or ligament attached that could not be completely removed, and areas of bone with rough surfaces retained soft tissue in the crevices, even after vigorous defleshing. Sampling at these locations while other tissues are present would have resulted in soft tissue comingled with bone powder, likely creating a substantial confound, whereas macerating bones prior to environmental exposure would have allowed for more locations to sample. Finally, these bones were exposed to a single environment, a mid-Michigan garden near deciduous woods; how intra-femoral DNA heterogeneity is influenced by different soil types (e.g., sand, silt, clay), climates (e.g., dry vs. humid), or environments (e.g., marsh, desert) remains unanswered.

Substantial inter-replicate variation in DNA quantity was evident throughout this research, which, combined with low sample size (n = 8), contributed to some non-parametric

results, thus all data sets were treated as such. While not ideal, normality tests like Shapiro-Wilk can assess non-normal distributions with sample sizes of around ten (Shapiro et al., 1968), and were employed in this research because normality could not be visually determined (e.g., via histograms or boxplots). Inter-replicate variance was more than 100% in some instances, which is consistent with previous quantitative DNA research on ancient seal ribs (Barta et al., 2014) and skeletonized human remains (Amory et al., 2011; Mundorff and Davoren, 2014). Bovine and porcine bones came from livestock raised for human consumption, therefore individuals of each species lived in a similar habitat, shared the same diet, were brought to slaughter around the same age, and were monitored for disease and other pathologies as required by the USDA, meaning that environment, age, and disease were largely controlled for. The exception was the younger bovine replicates C-01 and C-02, which confounded bovine DNA quantitation data and contributed to overall variance. The removal of C-01 and C-02 data decreased variance and resulted in a normal distribution in all but two samples; however, all data were included for analysis so as to not further limit sample size. Furthermore, the non-parametric data sets for porcine DNA were mostly confined to nuclear DNA (5/7) and more specifically to regions of the mid-diaphysis (3/7). Replicate P-01 DNA yields were much higher at the mid-diaphysis and P-04 yields were lower, otherwise outliers that potentially contributed to non-normally distributed data sets were random.

Another possible cause of inter-replicate variation involved a limitation of the real-time PCR technique used for quantifying DNA. DNA concentrations double every cycle under optimal conditions during the exponential phase of PCR, when quantity measurements take place. Therefore, a deviation of just one PCR cycle between replicates causes a twofold difference in DNA concentration estimates. Further, technical replicates were not conducted in

the current research, so qPCR precision was not assessed. Running the same sample in duplicate or more is common practice (Kline et al., 2005; Nielsen et al., 2008), and would likely help determine if outliers in quantification data resulted from qPCR error. The identification of outliers followed by re-quantification of those samples, and/or the inclusion of technical replicates, could reduce non-parametric data sets in future research.

Inherent biological differences in femora may have affected inter-replicate DNA yields from both species; however, with the exception of C-01 and C-02 having overall higher yields, there was no noticeable pattern based upon the region of bone assayed. For instance, DNA yields from a diaphysis were not consistently more or less variable than the corresponding epiphyses. Interestingly, DNA yields often differed substantially between the left and right femora of the same individual, although not in a consistent manner. Though great care was taken to obtain bone powder from equivalent sites among replicates, the identical spatial distribution of osteocytes and DNA bound to hydroxyapatite between paired femora is highly unlikely, potentially affecting inter-replicate yields. Furthermore, DNA quantity probably varies at locations adjacent to one another. Hebda and Foran (2015) discovered that when DNA was isolated from bone drillings 1 – 2 cm apart, highly variable amounts of DNA were often obtained. They found that homogenizing the bone powder prior to distribution among extraction techniques resulted in more uniform DNA yields, a strategy adopted for inter-regional comparisons in the current research.

DNA quantity likely differs throughout an element; however, DNA yield heterogeneity in this study was only assayed in a proximal/distal orientation (diaphysis to epiphysis); thus, potential medial/lateral intra-regional DNA variation was not considered, and its detection was prevented methodologically because bone powder was purposefully homogenized among drilling

sites. Hunter and Agnew (2014) reported that human medial and lateral femoral midshaft diaphysis had higher osteocyte lacunae density than the anterior or posterior aspects; however, these differences were not significant. In contrast, Carter et al. (2013) characterized the mean osteocyte lacunar density of human proximal femoral diaphyses, and found that the medial/lateral portions had a significantly greater density (up to 30%) than the anterior/posterior aspects. The authors postulated that the regional heterogeneity in osteocyte density within the femoral cortex was caused by its growth/development and remodeling, similar to reasons proposed for intra-femoral DNA heterogeneity in the current research. Similarly, DNA yield might vary at adjacent locations due to discrete pockets of bone remodeling. Beyond this, it is important to note that a 30% difference in osteocyte lacunae density (and presumably in DNA yield), though found to be significant by Carter et al. (2013), equates to one-third of a PCR cycle, which may not be detectable through the real-time assay used in this research. Investigations into DNA heterogeneity at adjacent locations, and assaying DNA quantity in a medial/lateral orientation, are ongoing in our laboratory, and should give a more complete picture of how DNA is distributed throughout the femur.

Increasing DNA yield is irrelevant if the DNA recovered is of insufficient quality for analysis. DNA quality assessment in this research produced unexpected results in both macerated and environmentally exposed bone. For example, one might expect to obtain similar nuclear and mitochondrial DNA quality from macerated bone; however, that was not the case. A couple of factors may have influenced why mtDNA generated larger amplicons than nuclear DNA, as well as the higher quality nuclear DNA recovered from porcine versus bovine bone. Foran (2006) demonstrated that nuclear DNA degraded faster than mtDNA in whole tissues, but when cells were homogenized, mtDNA degradation was similar to nuclear DNA, indicating that

mitochondria provide better physical protection from DNA degradation relative to the nuclear envelope. Furthermore, a single cell can harbor hundreds to thousands of mitochondria, each containing 2 – 7 mtDNA molecules (Robin and Wong 1988; Iborra et al., 2004), with estimated 500 – 10,000 copies per cell (Satoh and Kuroiwa, 1991; Iborra et al., 2004). It is possible that hydroxyapatite or some other aspect of bone preserves a fraction of the mtDNA during the maceration process, increasing the odds of generating larger amplicons. MtDNA amplification in the present study was potentially more successful due to these factors, either singly or in combination.

Maceration in boiling detergent may have itself influenced DNA quantity and quality, as preliminary research on maceration times at the MSU Forensic Laboratory has shown that bovine femoral segments lose DNA as maceration time increases. Furthermore, DNA yields in the current research were approximately two-fold higher at most locations in non-macerated versus macerated bone. This differed from results of Steadman et al. (2006), who compared common maceration techniques' effects on total DNA recovery utilizing porcine ribs by sampling approximately 0.7 g of trabecular bone. The authors found that the detergent/carbonate maceration technique—similar to what was used in the present study—resulted in higher DNA yields (1.60 µg/µL) than mechanical defleshing (0.82 µg/µL). This discrepancy potentially stemmed from DNA degradation in Steadman et al.'s (2006) defleshed bones, where a figure of DNA yield gels showed less high molecular weight DNA present in mechanically defleshed than detergent macerated samples. How much time passed between mechanically defleshing the ribs and DNA isolation was not reported, but if the bones were not sampled immediately, bacteria and nucleases could have resulted in DNA degradation. In contrast, the boiling detergent method would denature nucleases and kill bacteria, better preserving DNA. Since very little lag time

(hours) occurred between mechanically defleshing elements and starting DNA isolation procedures in the research presented here, this may have resulted in higher DNA yields from non-macerated bone.

The duration of maceration could have also influenced DNA quality in this research; however, this was only detected in nuclear DNA, as the largest mtDNA amplicon almost always amplified. Porcine bones were smaller and contained less soft tissue, requiring half the maceration time as bovine bones, which might have led to better nuclear DNA quality from porcine samples. Steadman et al. (2006) associated maceration time in aqueous solution with nuclear DNA degradation, because techniques that took the longest to macerate bone (room temperature water, 0.3% hydrogen peroxide, 10% bleach (sodium hypochlorite concentration unknown), and EDTA/papain) resulted in low-quality DNA. However, bleach degrades DNA, likely contributing to the low DNA quality for that method, and the rest of the techniques took weeks to remove soft tissue from bone, potentially leading to degraded DNA. Maceration could also affect mitochondrial and nuclear DNA degradation differently. Steadman et al. (2006) reported that mtDNA amplification was successful with any of the maceration techniques used; nuclear DNA amplification was not. Similarly, Rennick et al. (2005) macerated skeletal remains for 4 hours in either boiling water, 3% bleach (~0.2% sodium hypochlorite), or 1% 1:1 detergent/carbonate. Nuclear DNA amplification was unsuccessful following these treatments; however, mtDNA amplified for all, and the detergent/carbonate method resulted in higher quality mtDNA than the other techniques. These findings are consistent with the current research; larger amplicons were generated from mtDNA than nuclear DNA in both species, indicating that nuclear DNA may have been more susceptible to degradation during maceration. Steadman et al. (2006) demonstrated that techniques employing high temperatures (boiling) decreased

maceration time from weeks to hours, and increased DNA recovery, but there is a paucity of research to determine if DNA quantity and/or quality decline during maceration using a single technique, or to what extent this causes a disparity between nuclear and mitochondrial DNA recovery.

As noted, drilling could also have influenced DNA quantity and quality throughout this research. The harder portions of the diaphysis were more difficult to drill and took longer to produce bone powder than the metaphyses and epiphyses, potentially affecting DNA recovery in both species. Furthermore, bovine bones were harder to drill than their porcine counterparts, likely resulting from the fact that bovine cortical bone in denser than porcine bone (Aerssens et al., 1998). As described in the Introduction, Adler et al. (2011) reported that increased drilling speed (100 RPM to 1,000 RPM) reduced mtDNA quantity. Throughout the research presented here, drilling speed was set to ~5,000 RPM, the tool's lowest setting. The thermal and mechanical stresses drilling imparts on bone may have contributed to more DNA loss at the diaphysis than the metaphyses and epiphyses. Furthermore, drilling the harder bovine bones might have increased nuclear DNA degradation, resulting in smaller amplicons generated from bovine DNA. MtDNA would likely be damaged as well; however, as discussed above, it is better protected and present in higher copy number than nuclear DNA, either of which could have resulted in less detected degradation. Drilling as a method to obtain bone powder for DNA isolation is fairly common (e.g., Bille et al., 2004; Rennick et al., 2005; Adler et al., 2011; Caputo et al., 2013; Mundorff et al., 2013; Hebda and Foran, 2015), therefore a systematic investigation of how drilling speed and pressure affect DNA quantity and quality from bone would be useful to forensic and archeological investigators.

Interesting comparisons can be made between this study and the research done by Mundorff and Davoren (2014) to assess inter- versus intra-elemental variation in DNA quantity and quality. Those authors skeletonized three human males (47, 50, and 60 years old at the time of death who died within one year of each other), tested 55 elements from each cadaver, and ranked them from greatest to least based on the quantity and quality of nuclear DNA recovered. The talus, calcaneus, the femur all produced full STR profiles, and ranked 5th, 15th, and 49th in DNA quantification respectively. According to their associated National Institute of Justice Technical Report (Mundorff et al., 2013), the authors sampled locations on each element so as to minimize interference for future research by avoiding common points where metric and visual traits are tested. Long bones were sampled "at about 2/3rd the length" along the diaphysis, just above or below the midshaft. Short bones (e.g., tarsals) were sampled at locations with "adequate surface area" while avoiding "important morphological features". The authors stated that a single site was sufficient to collect the 200 mg of bone powder in most cases. This strategy assumes homogeneity of DNA content throughout a bone, which the current research clearly demonstrates is inaccurate. In spite of this, the research presented here supports a couple of Mundorff and Davoren's (2014) findings. First, DNA quality was similar among all three elements in both studies. Second, the talus and calcaneus had higher DNA quantity than the femoral diaphysis, based on the sampling location reported in Mundorff et al. (2013). However, the research presented here demonstrates that when other locations of the femur were assayed, it contained relatively more, similar, or less DNA than the tarsals. The fact that femoral DNA heterogeneity affected rank order among elements reinforces the importance of considering its influence on DNA recovery in future investigations.

Another interesting result in the current research was that the demineralization method resulted in higher DNA yields from the femoral mid-diaphyses, while the non-demineralization method led to more DNA from the epiphyses. Full demineralization of bone has been shown to enhance DNA quantity and quality (Loreille et al., 2007; Seo et al., 2010; Amory et al., 2011); however, the sections of bone used in those studies generally originated from long bone diaphysis. Thirty-nine of forty samples used by Amory et al. (2011) were cut from the diaphysis of a femur, tibia, ulna, or radius, the exception being one mandible fragment (also dense cortical bone). Seo et al. (2010) utilized the diaphyses from eight femora and two tibiae. Loreille et al. (2007) assayed 10 samples, nine of which originated from unspecified long bones or femora, and one vertebra (Kimberly Andreaggi, personal communication). These authors postulated that full demineralization increased the recovery of analyzable DNA bound in crystal aggregates deep within bone matrix, which was based on Salamon et al. (2005) demonstrating that DNA was present in such aggregates of modern and fossilized bovine, porcine, and human bone. The aggregates made up to 50% of the mineral phase by weight, and protected DNA in the presence of harsh chemical treatment (e.g., sodium hypochlorite). Furthermore, they proposed that when these aggregates were dissolved by demineralization, quality DNA was released for analysis. The increase in DNA quantity and quality when demineralization was used in the present study, particularly at the mid-diaphysis, may have resulted from the liberation of DNA trapped within such mineral aggregates.

Another possible influence on nuclear DNA yields was an interaction between detergent and proteinase K in the tissue lysis buffer. Both SDS and SLS have a twelve carbon hydrophobic tail and a hydrophilic anionic head; however, they differ in polar head groups: SDS contains a sulfate while SLS has a carboxylate attached by an amide linkage (reviewed by Privé,

2007). SDS denatures most proteins through the disruption of noncovalent bonds within and between them by binding in a 1:2 ratio, adding a net negative charge to the protein (reviewed by Johnson, 2013). SLS acts in a similar manner, but tends to bind proteins more weakly and disassociates from them more easily than SDS; SLS is often used when permanent denaturation is undesired (Burgess and Deutscher, 2009). Hilz et al. (1975) demonstrated that SDS and proteinase K have an additive effect when degrading proteins. The authors postulated that SDS breaks native protein structure, increasing the number of accessible peptide bonds for proteinase K to cleave. However, proteinase K was also sensitive to SDS concentration: at 0.1% concentration enzyme activity dropped to 86%, and at a 1% concentration activity decreased to 5%. How SLS concentration affected proteinase K activity in the current research is unclear, but similarities between the detergents indicate that it is possible 1% SLS reduced proteinase K's activity in demineralization buffer relative to 0.1% SDS in tissue lysis buffer.

Proteinase K may have also been affected by EDTA concentration. Researchers have proposed that proteinase K has two calcium binding sites, altering the enzyme's structure when occupied. Bajorath et al. (1988) used EDTA to decalcify proteinase K, which caused an 80% reduction in enzymatic activity. Conversely, Müller et al. (1994) suggested that the major role of calcium is to stabilize proteinase K's native conformation, and the loss of calcium does not result in a substantial reduction in enzymatic activity, but instead causes irreversible precipitation of the enzyme, reducing its effective concentration. The demineralization buffer used in the present study had 0.5 M EDTA, which could sequester calcium from proteinase K and reduce overall efficiency. Researchers that compared demineralization to non-demineralization protocols (Loreille et al., 2007; Seo et al., 2010; Amory et al., 2011) used about three times more proteinase K than what was applied in the current research. Increasing proteinase K

concentration for future investigations might compensate for any loss of enzymatic activity resulting from EDTA.

Though speculative, the lower detergent and EDTA concentrations in tissue lysis buffer, compared to demineralization buffer, potentially increased DNA yields at the epiphyses. However, this assumes that epiphyseal cortical bone contains less nuclear DNA bound to hydroxyapatite than the diaphysis. Demineralization under this context would result in decreased nuclear DNA recovery at the epiphyses relative to the midshaft diaphysis. Why mtDNA quantity was not similarly affected remains unclear. Investigating the use of other detergents in demineralization buffer, and adjusting detergent and proteinase K concentrations to determine how they influence DNA recovery from diaphyseal versus epiphyseal bone, may help resolve why each digestion buffer worked better when applied to different regions of the same bone.

PCR inhibition was not detected during DNA amplification from macerated bone; however, it did occur during amplification of 17 of 168 DNAs extracted from surface exposed and buried bones. Sixteen of those DNA isolates came from the epiphyses or tarsals, fourteen of which were from week two or four time points. DNAs tested from the midshaft diaphysis were not inhibited throughout any portion of this research. The cause of this sporadic PCR inhibition is not clear; however, collagen in bone is a known inhibitor (Scholz et al., 1998), which is primarily eliminated postmortem by bacterial collagenases and gas-gangrene bacteria like *Clostridium* (Janaway, 1997; Dent et al., 2004). In the non-macerated bone, collagen might have naturally degraded into soluble peptides that co-extracted at the week two and four time points, but was not as abundant at subsequent time points due to environmental degradation.

There were several instances of PCR inhibition during quality assessment that were not detected during qPCR, wherein amplification did not occur until DNA extracts were diluted five to tenfold. The quantification assay used throughout this research contained iQ™ Supermix that contains "proprietary enhancers and stabilizers" (Bio-Rad, 2015), which are designed to alleviate PCR inhibition during quantitation. In contrast, the PCR cocktail used for the quality assay did not include adjuvants. Bovine serum albumin (BSA) can effectively alleviate PCR inhibition associated with humic substances present in soil (Kreader, 1996) and collagen from bone (Hebda, 2013); however, because DNAs were of bovine origin, BSA was not added to avoid potential DNA contamination. Chemical adjuvants like dimethyl sulfoxide were not tested, although they may alleviate inhibition in some instances. Dilution worked well to overcome inhibition during mtDNA amplification, but not as well for nuclear DNA when initial quantities were low. If the inclusion of BSA with the quality assay does not introduce DNA contamination, then using it would likely improve the assay by reducing future occurrences of inhibition, increasing the likelihood of amplifying samples with low DNA quantity. Alternatively, if serum albumins from other species (e.g., sheep) are effective as adjuvants, then they could be used instead of BSA when bovine DNA is tested.

Overall, this body of research demonstrated that recoverable nuclear and mitochondrial DNA varied depending on the femoral location assayed, and that DNA yields can differ as much or more within an element as among elements. DNA quantity along the femur was consistent in both model systems; however, an important question for forensic applications still needs to be addressed: does this apply to humans? The femur supports static body weight, facilitates locomotion, and has a diverse morphology, osseous tissue distribution, and an abundance of both articulating and non-articulating surfaces. The femur's physical complexity along with its

history of being preferentially selected for DNA testing in research and casework made it the ideal element to establish that DNA is heterogeneously distributed in bone. The causes of DNA yield heterogeneity remain hypothetical at this juncture, but it likely results from a complex combination of the factors discussed above, and others yet to be considered. Specific morphological features of bone may turn out to be better indicators of DNA yield than which element is assayed; however, it seems unlikely that a single element, or location within one, will be ideal for best recovering DNA under all circumstances. Investigators determining optimal bones for DNA isolation should consider, when possible, a complex set of variables such as morphology, density, antemortem age, and the environment in which skeletal material resides.

CONCLUSION

Previous researchers have demonstrated that DNA yields vary between osseous tissue types and among different bones. The research conducted here was used to characterize DNA heterogeneity in femora, one of the most commonly utilized skeletal elements in research and forensic casework. Femoral DNA quantity has a predictable proximal/distal heterogeneity that varies quite substantially, demonstrating that location on a bone can be just as important as which element is analyzed, a critical consideration for future investigations that focus on optimizing DNA recovery from bone. Furthermore, nuclear and mitochondrial DNA follow similar distributions of heterogeneity; therefore, the same location on an element can be utilized regardless of which DNA type is assayed. Demineralization and non-demineralization techniques can significantly enhance (or reduce) DNA recovery; however, they do so at different locations. Though more research is needed to understand such phenomena, these results exemplify the importance of not relying on a single digestion technique for DNA isolation. Finally, the environment and duration of exposure can drastically alter DNA quantity, quality, and heterogeneity of skeletal material, potentially influencing which bone (or location on it) should be assayed to optimize DNA recovery. Overall, the research presented here clearly demonstrates that DNA is heterogeneously distributed in bone, DNA quantity can vary more within an element than among them, and that environmental conditions (e.g., burial) affect DNA quantity and quality substantially over time. Knowledge about all these factors will ultimately improve practices for recovering DNA in both research and forensic laboratories.

APPENDICES

APPENDIX A: NORMALITY TEST AND PAIRWISE COMPARISON TABLES FOR PORCINE AND BOVINE QUANTIFICATION DATA

Table A1: p-Values from Normality Testing of Fresh Porcine Bone's Regional mtDNA and Nuclear DNA Variation
Region number is listed in the first column, and correspond to Table 3. Bolded values represent regions that did not follow a normal distribution ($\alpha = 0.05$). AD = Anderson-Darling test, SW = Shapiro-Wilk test

Region	Mitochondrial DNA				Nuclear DNA			
	Tissue Lysis		Demineralization		Tissue Lysis		Demineralization	
	AD	SW	AD	SW	AD	SW	AD	SW
1	0.481	0.435	0.468	0.468	**0.008**	**0.006**	0.651	0.625
2	0.477	0.470	0.234	0.269	0.423	0.378	0.687	0.808
3	0.352	0.348	0.373	0.450	**0.004**	**0.003**	0.534	0.640
4	0.935	0.911	0.896	0.934	**0.005**	**0.003**	0.567	0.719
5	0.532	0.499	0.452	0.534	0.090	0.078	0.613	0.637
6	0.138	0.109	0.489	0.238	0.333	0.302	**0.012**	**0.008**
7	0.279	0.205	0.263	0.269	0.073	0.060	0.315	0.377
8	0.912	0.913	0.590	0.607	0.410	0.353	0.913	0.956
9	0.464	0.479	0.982	0.995	0.161	0.145	0.380	0.320
10	0.091	0.072	**< 0.001**	**0.001**	0.567	0.538	0.481	0.347
11	**0.006**	**0.005**	0.187	0.145	**0.005**	**0.005**	0.735	0.725
12	0.251	0.200	0.204	0.149	0.719	0.689	0.332	0.245
13	0.070	0.069	0.455	0.420	0.509	0.778	0.607	0.663
14	0.178	0.247	0.252	0.280	0.263	0.305	0.651	0.635
Pooled Regions								
1 – 6	**0.002**	**0.001**	**0.0004**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
7 – 9	0.242	0.118	0.316	0.243	**0.018**	**0.019**	0.063	**0.030**
10 – 12	**0.022**	**0.004**	**< 0.0001**	**< 0.0001**	0.866	0.785	0.585	0.297
13 and 14	0.507	0.542	0.628	0.604	0.173	0.346	0.389	0.323

Table A2: p-Values from Normality Testing of Fresh Bovine Bone's Regional mtDNA and Nuclear DNA Variation (with C-01 and C-02)

Region number is listed in the first column, and correspond to Table 3. Bolded values represent regions that did not follow a normal distribution ($\alpha = 0.05$). AD = Anderson-Darling test, SW = Shapiro-Wilk test

Region	Mitochondrial DNA				Nuclear DNA			
	Tissue Lysis		Demineralization		Tissue Lysis		Demineralization	
	AD	SW	AD	SW	AD	SW	AD	SW
1	**0.010**	**0.009**	**0.003**	**0.003**	**0.009**	**0.006**	0.051	0.053
2	**0.002**	**0.001**	**0.028**	**0.022**	0.144	0.131	0.097	0.127
3	**0.001**	**0.002**	**0.024**	**0.019**	0.502	0.611	**0.006**	**0.006**
4	0.232	0.169	**0.022**	**0.017**	0.144	0.177	0.070	0.114
5	**0.003**	**0.002**	0.290	0.235	0.393	0.465	**0.014**	**0.014**
6	0.072	0.062	0.098	0.084	0.424	0.464	0.301	0.277
7	**0.029**	**0.018**	**0.044**	**0.032**	**0.048**	**0.032**	0.104	0.131
8	**0.027**	**0.014**	0.152	0.133	0.178	0.190	0.098	0.094
9	0.054	**0.048**	0.140	0.171	**< 0.001**	**< 0.001**	**0.008**	**0.006**
10	0.082	0.074	**0.004**	**0.003**	**< 0.001**	**< 0.001**	**0.002**	**0.001**
11	0.062	0.052	0.129	0.126	**0.001**	**< 0.001**	**0.001**	**0.001**
12	0.716	0.842	0.952	0.946	**0.009**	**0.011**	**0.010**	**0.007**
13	0.552	0.549	0.356	0.348	**0.002**	**0.001**	**0.001**	**< 0.001**
14	0.142	0.109	**0.021**	**0.020**	**0.007**	**0.006**	**0.003**	**0.002**
Pooled Regions								
1 – 6	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.021**	**0.028**	**< 0.0001**	**< 0.0001**
7 – 9	**< 0.0001**	**0.0003**	**0.001**	**0.002**	**< 0.0001**	**< 0.0001**	**0.0004**	**0.001**
10 – 12	**0.004**	**0.003**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
13 and 14	0.069	**0.034**	**0.005**	**0.006**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

Table A3: p-Values from Normality Testing of Fresh Bovine Bone's Regional mtDNA and Nuclear DNA Variation (without C-01 and C-02)

Region number is listed in the first column, and correspond to Table 3. Bolded values represent regions that did not follow a normal distribution ($\alpha = 0.05$). AD = Anderson-Darling test, SW = Shapiro-Wilk test

Region	Mitochondrial DNA				Nuclear DNA			
	Tissue Lysis		Demineralization		Tissue Lysis		Demineralization	
	AD	SW	AD	SW	AD	SW	AD	SW
1	0.592	0.553	0.799	0.835	0.465	0.465	0.438	0.515
2	0.932	0.982	0.102	0.118	0.060	0.053	0.503	0.763
3	0.529	0.572	0.240	0.195	0.701	0.709	0.963	0.988
4	0.341	0.340	0.051	0.054	0.789	0.856	**0.032**	**0.032**
5	0.151	0.167	0.370	0.313	0.304	0.326	0.119	0.300
6	0.084	0.092	**0.032**	**0.022**	0.281	0.300	0.455	0.461
7	0.328	0.340	0.305	0.363	0.494	0.408	0.889	0.983
8	0.673	0.636	0.753	0.730	0.992	0.995	0.595	0.668
9	0.058	0.065	0.776	0.857	0.636	0.746	0.095	0.129
10	0.691	0.671	0.873	0.937	0.191	0.202	0.496	0.493
11	0.094	0.086	0.725	0.764	0.262	0.239	0.765	0.749
12	0.780	0.872	0.744	0.780	0.561	0.533	0.103	0.101
13	0.573	0.559	0.122	0.117	0.946	0.969	0.721	0.686
14	0.584	0.525	0.674	0.610	0.075	0.080	0.450	0.448
Pooled Regions								
1 – 6	**0.036**	**0.038**	**< 0.0001**	**< 0.0001**	0.612	0.594	0.690	0.533
7 – 9	0.060	**0.019**	0.287	0.172	0.895	0.814	0.514	0.499
10 – 12	0.334	0.314	0.687	0.898	0.214	0.118	**0.021**	**0.023**
13 and 14	0.397	0.264	0.071	0.053	0.170	0.129	0.453	0.553

Table A4: p-Values from Pairwise Comparisons of Median mtDNA Yields Derived from Porcine Bones Digested in Tissue Lysis Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in red indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.845	0.913	0.792	0.089	0.410	**0.008**	**0.020**	**0.002**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.004**	**< 0.0001**
2	0.845	1	0.764	0.952	0.066	0.322	**0.005**	**0.014**	**0.002**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.003**	**< 0.0001**
3	0.913	0.764	1	0.709	0.111	0.475	**0.010**	**0.026**	**0.003**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.006**	**< 0.0001**
4	0.792	0.952	0.709	1	**0.049**	0.277	**0.003**	**0.009**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.002**	**< 0.0001**
5	0.089	0.066	0.111	**0.049**	1	0.380	0.332	0.529	0.174	**0.001**	**0.003**	**0.001**	0.244	0.057
6	0.410	0.322	0.475	0.277	0.380	1	0.065	0.132	**0.025**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.041**	**0.005**
7	**0.008**	**0.005**	**0.010**	**0.003**	0.332	0.065	1	0.733	0.698	**0.012**	**0.044**	**0.015**	0.846	0.351
8	**0.020**	**0.014**	**0.026**	**0.009**	0.529	0.132	0.733	1	0.465	**0.004**	**0.019**	**0.006**	0.592	0.203
9	**0.002**	**0.002**	**0.003**	**0.001**	0.174	**0.025**	0.698	0.465	1	**0.035**	0.105	**0.041**	0.846	0.587
10	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.012**	**0.004**	**0.035**	1	0.625	0.944	**0.021**	0.117
11	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.003**	**< 0.0001**	**0.044**	**0.019**	0.105	0.625	1	0.675	0.069	0.280
12	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.015**	**0.006**	**0.041**	0.944	0.675	1	**0.025**	0.134
13	**0.004**	**0.003**	**0.006**	**0.002**	0.244	**0.041**	0.846	0.592	0.846	**0.021**	0.069	**0.025**	1	0.461
14	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.057	**0.005**	0.351	0.203	0.587	0.117	0.280	0.134	0.461	1

Table A5: p-Values from Pairwise Comparisons of Median mtDNA Yields Derived from Porcine Bones Digested in Demineralization Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in red indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.847	0.770	0.945	0.144	0.847	**0.009**	**0.027**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.005**	**0.002**
2	0.847	1	0.628	0.794	0.204	1	**0.016**	**0.044**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.008**	**0.004**
3	0.770	0.628	1	0.823	0.079	0.628	**0.004**	**0.012**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.002**	**0.001**
4	0.945	0.794	0.823	1	0.126	0.794	**0.008**	**0.023**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.004**	**0.002**
5	0.144	0.204	0.079	**0.126**	1	0.204	0.255	0.455	0.053	**0.001**	**0.001**	**0.001**	0.171	0.109
6	0.847	1	0.628	0.794	0.204	1	**0.016**	**0.044**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.008**	**0.004**
7	**0.009**	**0.016**	**0.004**	**0.008**	0.255	**0.016**	1	0.695	0.428	**0.020**	**0.031**	**0.028**	0.817	0.644
8	**0.027**	**0.044**	**0.012**	**0.023**	0.455	**0.044**	0.695	1	0.236	**0.007**	**0.011**	**0.009**	0.533	0.393
9	**0.001**	**0.001**	**< 0.0001**	**0.001**	0.053	**0.001**	0.428	0.236	1	0.127	0.173	0.159	0.574	0.741
10	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.020**	**0.007**	0.127	1	0.872	0.908	**0.037**	0.064
11	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.031**	**0.011**	0.173	0.872	1	0.963	0.054	0.090
12	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.028**	**0.009**	0.159	0.908	0.963	1	**0.049**	0.082
13	**0.005**	**0.008**	**0.002**	**0.004**	0.171	**0.008**	0.817	0.533	0.574	**0.037**	0.054	**0.049**	1	0.817
14	**0.002**	**0.004**	**0.001**	**0.002**	0.109	**0.004**	0.644	0.393	0.741	0.064	0.090	0.082	0.817	1

Table A6: p-Values from Multiple and Individual Pairwise Comparisons of Pooled Median mtDNA and DNA Yields Derived from Fresh Porcine and Bovine Bones Digested in Tissue Lysis and Demineralization Buffer

Pooled region identifiers are listed in the first column, and correspond to identifiers in Table 3. Multiple pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed) with Bonferroni corrected significance ($\alpha = 0.0167$). Bolded values indicate a significant difference between regions compared.

Pooled Regions	**Multiple**						**Individual**	
Porcine	**1 – 6**		**7 -- 9**		**10 – 12**		**13 – 14**	
mtDNA	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**
1 – 6	1	1	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
7 -- 9	**< 0.0001**	**< 0.0001**	1	1	**0.001**	**0.002**	**0.041**	0.420
10 -- 12	**< 0.0001**	**< 0.0001**	**0.001**	**0.002**	1	1	**< 0.0001**	**< 0.0001**
Nuclear DNA	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**
1 – 6	1	1	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
7 – 9	**< 0.0001**	**< 0.0001**	1	1	**0.001**	**0.001**	**0.035**	**0.002**
10 – 12	**< 0.0001**	**< 0.0001**	**0.001**	**0.001**	1	1	**< 0.0001**	**< 0.0001**
Pooled Regions	**Multiple**						**Individual**	
Bovine	**1 – 6**		**7 -- 9**		**10 – 12**		**13 – 14**	
mtDNA	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**
1 – 6	1	1	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.0001**	**0.006**
7 -- 9	**< 0.0001**	**< 0.0001**	1	1	0.020	**0.0004**	0.345	0.838
10 -- 12	**< 0.0001**	**< 0.0001**	0.020	**0.0004**	1	1	0.070	**0.012**
Nuclear DNA	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**	**TL**	**DM**
1 – 6	1	1	**< 0.0001**	**0.011**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.008**
7 – 9	**< 0.0001**	**0.011**	1	1	**0.0158**	**0.008**	0.079	0.576
10 – 12	**< 0.0001**	**< 0.0001**	**0.0158**	**0.008**	1	1	**0.041**	**0.014**

Table A7: p-Values from Pairwise Comparisons of Median Nuclear DNA Yields Derived from Porcine Bones Digested in Tissue Lysis Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in green indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.893	0.658	0.969	**0.023**	0.073	**0.003**	**0.026**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**
2	0.893	1	0.770	0.922	**0.040**	0.110	**0.006**	**0.044**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.002**	**< 0.0001**
3	0.658	0.770	1	0.686	0.068	0.177	**0.011**	0.074	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.004**	**0.001**
4	0.969	0.922	0.686	1	**0.026**	0.079	**0.003**	**0.029**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**
5	**0.023**	**0.040**	0.068	**0.026**	1	0.636	0.457	0.969	0.151	**0.005**	**0.007**	**0.001**	0.277	0.098
6	0.073	0.110	0.177	0.079	0.636	1	0.235	0.664	0.056	**0.001**	**0.002**	**< 0.0001**	0.118	**0.033**
7	**0.003**	**0.006**	**0.011**	**0.003**	0.475	0.235	1	0.451	0.470	**0.035**	**0.049**	**0.011**	0.709	0.347
8	**0.026**	**0.044**	0.074	**0.029**	0.969	0.664	0.451	1	0.140	**0.004**	**0.007**	**0.001**	0.260	0.090
9	**< 0.0001**	**0.001**	**0.001**	**< 0.0001**	0.969	0.056	0.470	0.140	1	0.167	0.214	0.068	0.727	0.828
10	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.005**	**0.001**	**0.035**	**0.004**	0.167	1	0.889	0.658	0.083	0.244
11	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.007**	**0.002**	**0.049**	**0.007**	0.214	0.889	1	0.560	0.111	0.305
12	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.001**	**0.001**	0.068	0.658	0.560	1	**0.030**	0.108
13	**0.001**	**0.002**	**0.004**	**0.001**	0.277	0.118	0.709	0.260	0.727	0.083	0.111	**0.030**	1	0.571
14	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	0.098	**0.033**	0.347	0.090	0.828	0.244	0.305	0.108	0.571	1

Table A8: p-Values from Pairwise Comparisons of Median Nuclear DNA Yields Derived from Porcine Bones Digested in Demineralization Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in green indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.994	0.776	0.872	0.201	0.678	**0.016**	**0.027**	**0.014**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.002**	**0.001**
2	0.994	1	0.770	0.878	0.204	0.683	**0.016**	**0.027**	**0.015**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.002**	**0.001**
3	0.776	0.770	1	0.655	0.118	0.484	**0.007**	**0.012**	**0.006**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**
4	0.872	0.878	0.655	1	0.264	0.799	**0.025**	**0.040**	**0.022**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.003**	**0.001**
5	0.201	0.204	0.118	0.264	1	0.389	0.258	0.348	0.242	**0.001**	**< 0.0001**	**< 0.0001**	0.070	**0.035**
6	0.678	0.683	0.484	0.799	0.389	1	**0.046**	0.072	**0.042**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.007**	**0.003**
7	**0.016**	**0.016**	**0.007**	**0.025**	0.258	**0.046**	1	0.847	0.969	**0.025**	**0.018**	**0.011**	0.496	0.326
8	**0.027**	**0.027**	**0.012**	**0.040**	0.348	0.072	0.847	1	0.817	**0.015**	**0.010**	**0.006**	0.382	0.240
9	**0.014**	**0.015**	**0.006**	**0.022**	0.242	**0.042**	0.969	0.817	1	**0.027**	**0.020**	**0.012**	0.520	0.346
10	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.025**	**0.015**	**0.027**	1	0.902	0.764	0.117	0.205
11	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.018**	**0.010**	**0.020**	0.902	1	0.859	0.091	0.165
12	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.011**	**0.006**	**0.012**	0.764	0.859	1	0.062	0.117
13	**0.002**	**0.002**	**0.001**	**0.003**	0.070	**0.007**	0.496	0.382	0.520	0.117	0.091	0.062	1	0.764
14	**0.001**	**0.001**	**< 0.0001**	**0.001**	**0.035**	**0.003**	0.326	0.240	0.346	0.205	0.165	0.117	0.764	1

Table A9: p-Values from Pairwise Comparisons of Median mtDNA Yields Derived from Bovine Bones Digested in Tissue Lysis Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in blue indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.376	0.553	0.559	0.498	0.376	**0.018**	**0.045**	**0.002**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.007**	**0.004**
2	0.723	1	0.811	0.817	0.746	0.595	**0.045**	0.099	**0.005**	**< 0.0001**	**0.001**	**0.001**	**0.019**	**0.011**
3	0.553	0.811	1	0.994	0.933	0.770	0.077	0.159	**0.011**	**< 0.0001**	**0.003**	**0.001**	**0.036**	**0.021**
4	0.559	0.817	0.811	1	0.926	0.764	0.075	0.157	**0.011**	**< 0.0001**	**0.003**	**0.001**	**0.035**	**0.021**
5	0.498	0.746	0.933	0.926	1	0.835	0.092	0.185	**0.014**	**< 0.0001**	**0.003**	**0.002**	**0.044**	**0.027**
6	0.376	0.595	0.770	0.764	0.835	1	0.139	0.264	**0.024**	**0.001**	**0.007**	**0.004**	0.070	**0.045**
7	**0.018**	**0.045**	0.077	0.075	0.092	0.139	1	0.099	0.437	**0.048**	0.215	0.157	0.741	0.595
8	**0.045**	0.099	0.159	0.157	0.185	0.264	0.717	1	0.255	**0.019**	0.109	0.075	0.488	0.372
9	**0.002**	**0.005**	**0.011**	**0.011**	**0.014**	**0.024**	0.437	0.255	1	0.230	0.644	0.523	0.655	0.805
10	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.001**	**0.048**	**0.019**	0.230	1	0.460	0.574	0.099	0.148
11	**< 0.0001**	**0.001**	**0.003**	**0.003**	**0.003**	**0.007**	0.215	0.109	0.644	0.460	1	0.859	0.364	0.479
12	**< 0.0001**	**0.001**	**0.001**	**0.001**	**0.002**	**0.004**	0.157	0.075	0.523	0.574	0.859	1	0.278	0.376
13	**0.007**	**0.019**	**0.036**	**0.035**	**0.044**	0.070	0.741	0.488	0.655	0.099	0.364	0.278	1	0.841
14	**0.004**	**0.011**	**0.021**	**0.021**	**0.027**	**0.045**	0.595	0.372	0.805	0.148	0.479	0.376	0.841	1

Table A10: p-Values from Pairwise Comparisons of Median mtDNA Yields Derived from Bovine Bones Digested in Demineralization Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in blue indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.945	0.951	0.811	0.585	0.836	**0.031**	0.144	**0.031**	**< 0.0001**	**0.001**	**0.005**	0.085	0.111
2	0.945	1	0.986	0.866	0.538	0.782	0.051	0.126	**0.026**	**< 0.0001**	**< 0.0001**	**0.004**	0.073	0.096
3	0.951	0.896	1	0.764	0.628	0.884	0.069	0.161	**0.036**	**0.001**	**0.001**	**0.006**	0.096	0.126
4	0.811	0.866	0.764	1	0.432	0.655	**0.034**	0.089	**0.016**	**< 0.0001**	**< 0.0001**	**0.002**	**0.050**	0.067
5	0.585	0.538	0.628	0.432	1	0.735	0.183	0.360	0.106	**0.003**	**0.004**	**0.025**	0.239	0.295
6	0.835	0.782	0.884	0.655	0.735	1	0.095	0.210	0.051	**0.001**	**0.001**	**0.010**	0.129	0.166
7	0.060	0.051	0.069	**0.034**	0.183	0.095	1	0.678	0.776	0.098	0.124	0.360	0.878	0.776
8	0.144	0.126	0.161	0.089	0.360	0.210	0.678	1	0.484	**0.038**	0.051	0.183	0.794	0.896
9	**0.031**	**0.026**	**0.036**	**0.016**	0.106	0.051	0.776	0.484	1	0.171	0.210	0.528	0.661	0.569
10	**< 0.0001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.003**	**0.001**	0.098	**0.038**	0.171	1	0.908	0.460	0.070	0.052
11	**0.001**	**< 0.0001**	**0.001**	**< 0.0001**	**0.004**	**0.001**	0.124	0.051	0.210	0.908	1	0.533	0.090	0.068
12	**0.005**	**0.004**	**0.006**	**0.002**	**0.025**	**0.010**	0.360	0.187	0.528	0.070	0.533	1	0.285	0.230
13	0.085	0.073	0.096	**0.050**	0.239	0.129	0.878	0.794	0.661	0.070	0.090	0.285	1	0.896
14	0.111	0.096	0.126	0.067	0.295	0.166	0.776	0.896	0.569	0.052	0.068	0.230	0.869	1

Table A11: p-Values from Pairwise Comparisons of Median Nuclear DNA Yields Derived from Bovine Bones Digested in Tissue Lysis Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in purple indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.378	0.758	0.441	0.393	0.378	**0.002**	**0.006**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
2	0.378	1	0.566	0.911	0.979	0.709	**0.029**	0.065	**0.009**	**< 0.0001**	**0.001**	**< 0.0001**	**0.003**	**0.002**
3	0.758	0.566	1	0.644	0.585	0.344	**0.006**	**0.016**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
4	0.441	0.911	0.644	1	0.933	0.628	**0.022**	0.051	**0.007**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.002**	**0.002**
5	0.393	0.979	0.585	0.933	1	0.689	**0.027**	0.061	**0.008**	**< 0.0001**	**0.001**	**< 0.0001**	**0.003**	**0.002**
6	0.210	0.709	0.344	0.628	0.689	1	0.070	0.141	**0.026**	**< 0.0001**	**0.003**	**< 0.0001**	**0.002**	**0.002**
7	**0.002**	**0.029**	**0.006**	**0.022**	**0.027**	0.070	1	0.732	0.675	0.083	0.228	0.075	0.448	0.391
8	**0.006**	0.065	**0.016**	0.051	0.061	0.141	0.732	1	0.446	**0.038**	0.122	**0.034**	0.271	0.230
9	**< 0.0001**	**0.009**	**0.001**	**0.007**	**0.008**	**0.026**	0.675	0.446	1	0.188	0.432	0.173	0.735	0.661
10	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.083	**0.038**	0.188	1	0.595	0.963	0.328	0.380
11	**< 0.0001**	**0.001**	**< 0.0001**	**< 0.0001**	**0.001**	**0.003**	0.228	0.122	0.432	0.595	1	0.564	0.655	0.729
12	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.075	**0.034**	0.173	0.963	0.564	1	0.306	0.356
13	**< 0.0001**	**0.003**	**< 0.0001**	**0.002**	**0.003**	**0.002**	0.448	0.271	0.735	0.328	0.655	0.306	1	0.920
14	**< 0.0001**	**0.002**	**< 0.0001**	**0.002**	**0.002**	**0.002**	0.391	0.230	0.661	0.380	0.729	0.356	0.920	1

Table A12: p-Values from Pairwise Comparisons of Median Nuclear DNA Yields Derived from Bovine Bones Digested in Demineralization Buffer

Region identifiers are listed in the first column and row, and correspond to identifiers in Table 3. Pairwise comparisons were generated using XLSTAT 2014.2.01 using Dunn's procedure (two-tailed). Significance was determined at $\alpha = 0.05$. Bolded values indicate a significant difference between regions compared. P-values in purple indicate disagreement in significance between tissue lysis and demineralization buffer.

Region	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.805	0.859	0.805	0.853	0.513	0.079	0.210	0.133	**< 0.0001**	**0.002**	**0.009**	0.064	0.106
2	0.805	1	0.945	1	0.951	0.683	0.131	0.313	0.210	**0.001**	**0.004**	**0.019**	0.108	0.171
3	0.859	0.945	1	0.945	0.994	0.633	0.115	0.285	0.185	**0.001**	**0.003**	**0.016**	0.093	0.150
4	0.805	1	0.945	1	0.951	0.683	0.131	0.313	0.210	**0.001**	**0.004**	**0.019**	0.108	0.171
5	0.853	0.951	0.994	0.951	1	0.639	0.116	0.285	0.188	**0.001**	**0.003**	**0.016**	0.095	0.152
6	0.513	0.683	0.633	0.683	0.639	1	0.271	0.548	0.397	**0.004**	**0.013**	0.052	0.230	0.336
7	0.079	0.131	0.115	0.131	0.116	0.271	1	0.617	0.799	0.078	0.164	0.401	0.920	0.890
8	0.210	0.313	0.285	0.313	0.285	0.548	0.617	1	0.805	**0.024**	0.058	0.180	0.548	0.717
9	0.133	0.210	0.185	0.210	0.188	0.397	0.799	0.805	1	**0.044**	0.099	0.274	0.723	0.908
10	**< 0.0001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.004**	0.078	**0.024**	**0.044**	1	0.712	0.356	0.096	0.057
11	**0.002**	**0.004**	**0.003**	**0.004**	**0.003**	**0.013**	0.164	0.058	0.099	0.712	1	0.579	0.196	0.126
12	**0.009**	**0.019**	**0.016**	**0.019**	**0.016**	0.052	0.401	0.180	0.274	0.356	0.579	1	0.460	0.328
13	0.064	0.108	0.093	0.108	0.095	0.230	0.920	0.548	0.723	0.096	0.196	0.460	1	0.811
14	0.106	0.171	0.150	0.171	0.152	0.336	0.890	0.717	0.908	0.057	0.126	0.328	0.811	1

APPENDIX B: MITOCHONDRIAL AND NUCLEAR DNA QUANTIFICATION DATA AND INDIVIDUAL PCR AMPLIFICATION RESULTS FROM FRESH PORCINE FEMORA AND TARSALS

Table B1: mtDNA Quantification of P-01 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-01		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	28.0	9.30	5.31	260.40
2	Midshaft Diaphysis (Distal to Region 1)	50	29.0	6.45	3.74	187.05
3	Diaphysis (Proximal to Region 1)	49	26.0	18.00	9.55	468.00
4	Diaphysis (Distal to Region 2)	50	26.0	8.56	4.45	222.56
5	Diaphysis (Proximal to Region 3)	51	30.0	31.20	18.35	936.00
6	Diaphysis (Distal to Region 4)	49	29.5	35.10	21.13	1035.45
7	Proximal Metaphysis	49	27.0	56.30	31.02	1520.10
8	Distal Metaphysis	50	28.0	55.60	31.14	1556.80
9	Articulating Surface	49	30.0	97.60	59.76	2928.00
10	Distal Epiphysis	51	35.0	472.00	323.92	16520.00
11	Femoral Head	51	36.0	248.00	175.06	8928.00
12	Trochanter	50	34.0	253.00	172.04	8602.00
13	Calcaneus	50	29.0	74.90	43.44	2172.10
14	Talus	50	35.0	118.00	82.60	4130.00
RB	Reagent Blank	-	29.5	0.02	-	0.59

Table B2: mtDNA Quantification of P-01 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-01		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	25.5	26.00	13.00	663.00
2	Midshaft Diaphysis (Distal to Region 1)	51	29.5	18.20	10.53	536.90
3	Diaphysis (Proximal to Region 1)	20	29.0	24.60	14.27	713.40
4	Diaphysis (Distal to Region 2)	50	29.5	22.00	12.98	649.00
5	Diaphysis (Proximal to Region 3)	51	30.0	38.20	22.47	1146.00
6	Diaphysis (Distal to Region 4)	49	30.0	30.40	18.61	912.00
7	Proximal Metaphysis	51	28.0	66.50	36.51	1862.00
8	Distal Metaphysis	50	28.0	62.90	35.22	1761.20
9	Articulating Surface	50	30.0	123.00	73.80	3690.00
10	Distal Epiphysis	51	32.0	311.00	195.14	9952.00
11	Femoral Head	51	34.5	358.00	242.18	12351.00
12	Trochanter	51	35.0	316.00	216.86	11060.00
13	Calcaneus	49	35.0	70.90	50.64	2481.50
14	Talus	49	29.5	112.00	67.43	3304.00
RB	Reagent Blank	-	29.0	0.00	-	0.10

Table B3: mtDNA Quantification of P-02 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-02		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	26.0	23.50	11.98	611.00
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	9.22	5.06	258.16
3	Diaphysis (Proximal to Region 1)	50	30.5	9.53	5.81	290.67
4	Diaphysis (Distal to Region 2)	49	30.0	9.15	5.60	274.50
5	Diaphysis (Proximal to Region 3)	50	29.0	16.60	9.63	481.40
6	Diaphysis (Distal to Region 4)	49	27.5	12.30	6.90	338.25
7	Proximal Metaphysis	51	26.0	57.40	29.26	1492.40
8	Distal Metaphysis	50	28.0	45.60	25.54	1276.80
9	Articulating Surface	51	30.0	55.00	32.35	1650.00
10	Distal Epiphysis	51	40.0	187.00	146.67	7480.00
11	Femoral Head	50	40.0	107.00	85.60	4280.00
12	Trochanter	49	40.0	110.00	89.80	4400.00
13	Calcaneus	50	34.0	57.50	39.10	1955.00
14	Talus	50	40.0	64.40	51.52	2576.00
RB	Reagent Blank	-	29.0	0.00	-	0.09

Table B4: mtDNA Quantification of P-02 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-02		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	28.0	29.20	16.35	817.60
2	Midshaft Diaphysis (Distal to Region 1)	49	27.0	27.20	14.99	734.40
3	Diaphysis (Proximal to Region 1)	51	30.5	14.60	8.73	445.30
4	Diaphysis (Distal to Region 2)	49	29.5	15.90	9.57	469.05
5	Diaphysis (Proximal to Region 3)	51	29.0	21.60	12.28	626.40
6	Diaphysis (Distal to Region 4)	51	30.0	17.90	10.53	537.00
7	Proximal Metaphysis	51	30.0	61.20	36.00	1836.00
8	Distal Metaphysis	51	28.5	48.80	27.27	1390.80
9	Articulating Surface	49	30.0	76.70	46.96	2301.00
10	Distal Epiphysis	51	38.0	131.00	97.61	4978.00
11	Femoral Head	50	39.0	169.00	131.82	6591.00
12	Trochanter	50	39.0	268.00	209.04	10452.00
13	Calcaneus	51	35.0	62.90	43.17	2201.50
14	Talus	50	38.5	68.90	53.05	2652.65
RB	Reagent Blank	-	28.0	0.00	-	0.00

Table B5: mtDNA Quantification of P-03 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. ND = No Data

P-03		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	31.0	27.90	16.96	864.90
2	Midshaft Diaphysis (Distal to Region 1)	51	0.0	ND	ND	ND
3	Diaphysis (Proximal to Region 1)	50	29.0	44.70	25.93	1296.30
4	Diaphysis (Distal to Region 2)	51	30.0	26.20	15.41	786.00
5	Diaphysis (Proximal to Region 3)	49	30.0	66.70	40.84	2001.00
6	Diaphysis (Distal to Region 4)	51	27.5	46.60	25.13	1281.50
7	Proximal Metaphysis	49	30.0	128.00	73.14	3584.00
8	Distal Metaphysis	49	27.0	79.40	43.75	2143.80
9	Articulating Surface	49	30.0	118.00	72.24	3540.00
10	Distal Epiphysis	50	35.0	251.00	175.70	8785.00
11	Femoral Head	51	37.0	136.00	98.67	5032.00
12	Trochanter	49	32.0	275.00	179.59	8800.00
13	Calcaneus	51	31.5	90.30	55.77	2844.45
14	Talus	51	32.0	108.00	67.76	3456.00
RB	Reagent Blank	-	25.0	0.06	-	1.50

Table B6: mtDNA Quantification of P-03 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-03		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	28.5	42.40	23.69	1208.40
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	41.40	22.73	1159.20
3	Diaphysis (Proximal to Region 1)	49	27.0	33.00	18.18	891.00
4	Diaphysis (Distal to Region 2)	49	26.0	39.00	20.69	1014.00
5	Diaphysis (Proximal to Region 3)	49	29.0	79.20	46.87	2296.80
6	Diaphysis (Distal to Region 4)	50	26.0	36.70	19.08	954.20
7	Proximal Metaphysis	51	29.5	138.00	79.82	4071.00
8	Distal Metaphysis	49	30.0	99.40	53.68	2683.80
9	Articulating Surface	51	27.0	93.10	49.29	2513.70
10	Distal Epiphysis	49	30.0	200.00	122.45	6000.00
11	Femoral Head	49	36.0	193.00	141.80	6948.00
12	Trochanter	50	29.5	195.00	115.05	5752.50
13	Calcaneus	51	29.5	99.30	57.44	2929.35
14	Talus	50	30.0	107.00	64.20	3210.00
RB	Reagent Blank	-	27.0	0.00	-	0.00

Table B7: mtDNA Quantification of P-04 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-04		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	28.0	11.90	6.66	333.20
2	Midshaft Diaphysis (Distal to Region 1)	50	25.0	10.60	5.30	265.00
3	Diaphysis (Proximal to Region 1)	50	29.0	13.10	7.60	379.90
4	Diaphysis (Distal to Region 2)	52	28.0	19.80	10.66	554.40
5	Diaphysis (Proximal to Region 3)	51	30.0	61.10	35.94	1833.00
6	Diaphysis (Distal to Region 4)	49	27.0	32.20	17.74	869.40
7	Proximal Metaphysis	51	31.5	83.90	51.82	2642.85
8	Distal Metaphysis	49	24.5	91.70	45.85	2246.65
9	Articulating Surface	50	27.0	71.60	38.66	1933.20
10	Distal Epiphysis	51	32.5	257.00	163.77	8352.50
11	Femoral Head	51	35.0	133.00	91.27	4655.00
12	Trochanter	51	32.5	141.00	89.85	4582.50
13	Calcaneus	51	30.0	66.10	38.88	1983.00
14	Talus	50	30.0	85.90	51.54	2577.00
RB	Reagent Blank	-	23.5	0.00	-	0.00

Table B8: mtDNA Quantification of P-04 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-04		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.5	19.50	11.51	575.25
2	Midshaft Diaphysis (Distal to Region 1)	51	23.5	29.80	13.73	700.30
3	Diaphysis (Proximal to Region 1)	50	28.5	22.40	12.77	638.40
4	Diaphysis (Distal to Region 2)	50	24.0	30.20	14.50	724.80
5	Diaphysis (Proximal to Region 3)	50	26.0	52.70	27.40	1370.20
6	Diaphysis (Distal to Region 4)	51	26.0	28.50	14.53	741.00
7	Proximal Metaphysis	49	29.0	56.70	33.56	1644.30
8	Distal Metaphysis	51	28.0	67.70	37.17	1895.60
9	Articulating Surface	51	27.0	70.80	37.48	1911.60
10	Distal Epiphysis	51	35.0	162.00	111.18	5670.00
11	Femoral Head	50	33.0	128.00	84.48	4224.00
12	Trochanter	49	38.5	76.50	60.11	2945.25
13	Calcaneus	51	28.5	81.70	45.66	2328.45
14	Talus	50	31.0	61.90	38.38	1918.90
RB	Reagent Blank	-	26.0	0.00	-	0.00

Table B9: mtDNA Quantification of P-05 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-05		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	28.0	13.20	7.39	369.60
2	Midshaft Diaphysis (Distal to Region 1)	49	27.0	37.30	20.55	1007.10
3	Diaphysis (Proximal to Region 1)	50	26.5	19.10	10.12	506.15
4	Diaphysis (Distal to Region 2)	51	27.0	17.10	9.05	461.70
5	Diaphysis (Proximal to Region 3)	50	27.5	73.40	40.37	2018.50
6	Diaphysis (Distal to Region 4)	49	26.5	31.20	16.87	826.80
7	Proximal Metaphysis	50	27.5	95.80	52.69	2634.50
8	Distal Metaphysis	51	25.5	75.90	37.95	1935.45
9	Articulating Surface	51	27.0	68.40	36.21	1846.80
10	Distal Epiphysis	50	38.0	270.00	205.20	10260.00
11	Femoral Head	50	40.0	214.00	171.20	8560.00
12	Trochanter	50	32.0	317.00	202.88	10144.00
13	Calcaneus	49	28.0	129.00	73.71	3612.00
14	Talus	51	33.0	82.30	53.25	2715.90
RB	Reagent Blank	-	23.5	0.00	-	0.00

Table B10: mtDNA Quantification of P-05 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-05		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	30.0	31.20	18.35	936.00
2	Midshaft Diaphysis (Distal to Region 1)	50	27.5	48.40	26.62	1331.00
3	Diaphysis (Proximal to Region 1)	51	26.0	24.60	12.54	639.60
4	Diaphysis (Distal to Region 2)	49	26.0	29.10	15.44	756.60
5	Diaphysis (Proximal to Region 3)	50	29.0	83.30	48.31	2415.70
6	Diaphysis (Distal to Region 4)	50	25.0	38.30	19.15	957.50
7	Proximal Metaphysis	49	29.0	84.80	50.19	2459.20
8	Distal Metaphysis	49	29.5	72.90	43.89	2150.55
9	Articulating Surface	50	31.0	87.50	54.25	2712.50
10	Distal Epiphysis	49	34.0	634.00	439.92	21556.00
11	Femoral Head	50	39.0	193.00	150.54	7527.00
12	Trochanter	50	32.0	622.00	398.08	19904.00
13	Calcaneus	49	28.0	122.00	69.71	3416.00
14	Talus	49	31.5	93.00	59.79	2929.50
RB	Reagent Blank	-	26.0	0.00	-	0.00

Table B11: mtDNA Quantification of P-06 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-06		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	32.0	25.80	16.85	825.60
2	Midshaft Diaphysis (Distal to Region 1)	51	25.0	26.70	13.09	667.50
3	Diaphysis (Proximal to Region 1)	50	25.5	29.30	14.94	747.15
4	Diaphysis (Distal to Region 2)	49	27.0	22.60	12.45	610.20
5	Diaphysis (Proximal to Region 3)	50	26.0	55.90	29.07	1453.40
6	Diaphysis (Distal to Region 4)	50	26.5	44.50	23.59	1179.25
7	Proximal Metaphysis	50	28.0	73.00	40.88	2044.00
8	Distal Metaphysis	51	28.0	79.60	43.70	2228.80
9	Articulating Surface	49	26.5	101.00	54.62	2676.50
10	Distal Epiphysis	49	34.0	226.00	156.82	7684.00
11	Femoral Head	51	32.0	138.00	86.59	4416.00
12	Trochanter	49	30.5	253.00	157.48	7716.50
13	Calcaneus	51	30.5	54.30	32.47	1656.15
14	Talus	50	32.0	90.00	57.60	2880.00
RB	Reagent Blank	-	28.0	0.00	-	0.00

Table B12: mtDNA Quantification of P-06 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-06		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	28.0	32.50	17.84	910.00
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	40.30	22.13	1128.40
3	Diaphysis (Proximal to Region 1)	50	27.5	34.80	19.14	957.00
4	Diaphysis (Distal to Region 2)	50	28.0	41.50	23.24	1162.00
5	Diaphysis (Proximal to Region 3)	51	27.0	55.50	29.38	1498.50
6	Diaphysis (Distal to Region 4)	50	28.0	44.40	24.86	1243.20
7	Proximal Metaphysis	50	29.0	77.50	44.95	2247.50
8	Distal Metaphysis	50	33.0	65.50	35.96	1834.00
9	Articulating Surface	51	27.0	117.00	61.94	3159.00
10	Distal Epiphysis	50	33.0	204.00	134.64	6732.00
11	Femoral Head	49	33.0	134.00	90.24	4422.00
12	Trochanter	49	31.0	111.00	70.22	3441.00
13	Calcaneus	51	24.5	73.40	35.26	1798.30
14	Talus	51	31.5	58.70	36.26	1849.05
RB	Reagent Blank	-	28.0	0.00	-	0.00

Table B13: mtDNA Quantification of P-07 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-07		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	25.5	19.70	10.25	502.35
2	Midshaft Diaphysis (Distal to Region 1)	49	26.5	21.30	11.52	564.45
3	Diaphysis (Proximal to Region 1)	51	28.5	29.50	16.49	840.75
4	Diaphysis (Distal to Region 2)	50	27.0	14.90	8.05	402.30
5	Diaphysis (Proximal to Region 3)	50	29.0	71.30	41.35	2067.70
6	Diaphysis (Distal to Region 4)	50	26.5	37.00	19.61	980.50
7	Proximal Metaphysis	51	27.0	84.80	44.89	2289.60
8	Distal Metaphysis	50	26.0	75.70	39.36	1968.20
9	Articulating Surface	50	26.5	87.80	46.53	2326.70
10	Distal Epiphysis	49	30.5	177.00	110.39	5398.50
11	Femoral Head	49	33.0	155.00	104.39	5115.00
12	Trochanter	49	34.0	286.00	198.45	9724.00
13	Calcaneus	50	25.5	104.00	53.04	2652.00
14	Talus	51	30.0	118.00	69.41	3540.00
RB	Reagent Blank	-	27.0	0.00	-	0.00

Table B14: mtDNA Quantification of P-07 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-07		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	27.5	30.50	17.12	838.75
2	Midshaft Diaphysis (Distal to Region 1)	50	26.0	25.50	13.26	663.00
3	Diaphysis (Proximal to Region 1)	50	25.5	37.10	18.92	946.05
4	Diaphysis (Distal to Region 2)	50	30.0	33.30	19.98	999.00
5	Diaphysis (Proximal to Region 3)	51	28.5	58.20	32.52	1658.70
6	Diaphysis (Distal to Region 4)	49	28.0	37.60	21.49	1052.80
7	Proximal Metaphysis	50	27.0	98.90	53.41	2670.30
8	Distal Metaphysis	49	31.0	70.50	44.60	2185.50
9	Articulating Surface	49	29.5	94.80	57.07	2796.60
10	Distal Epiphysis	50	32.5	160.00	104.00	5200.00
11	Femoral Head	49	32.0	111.00	72.49	3552.00
12	Trochanter	50	38.5	233.00	179.41	8970.50
13	Calcaneus	49	29.0	76.50	45.28	2218.50
14	Talus	49	32.0	92.50	60.41	2960.00
RB	Reagent Blank	-	29.0	0.00	-	0.00

Table B15: mtDNA Quantification of P-08 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-08		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	26.0	35.80	18.62	930.80
2	Midshaft Diaphysis (Distal to Region 1)	49	23.0	34.20	16.05	786.60
3	Diaphysis (Proximal to Region 1)	51	28.0	28.80	15.81	806.40
4	Diaphysis (Distal to Region 2)	51	26.0	27.60	14.07	717.60
5	Diaphysis (Proximal to Region 3)	49	26.0	53.50	28.39	1391.00
6	Diaphysis (Distal to Region 4)	49	29.0	39.10	23.14	1133.90
7	Proximal Metaphysis	49	28.5	48.60	28.27	1391.00
8	Distal Metaphysis	49	28.0	58.20	33.26	1629.60
9	Articulating Surface	49	27.0	99.00	54.55	2673.00
10	Distal Epiphysis	49	35.0	156.38	111.70	5473.30
11	Femoral Head	49	35.5	120.00	86.94	4260.00
12	Trochanter	49	34.0	219.00	151.96	7446.00
13	Calcaneus	49	30.5	58.90	36.66	1796.45
14	Talus	50	29.0	76.40	44.31	2215.60
RB	Reagent Blank	-	27.0	0.00	-	0.00

Table B16: mtDNA Quantification of P-08 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

P-08		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	25.5	45.90	23.89	1170.45
2	Midshaft Diaphysis (Distal to Region 1)	50	32.5	38.70	25.16	1257.75
3	Diaphysis (Proximal to Region 1)	49	30.5	33.30	20.73	1015.65
4	Diaphysis (Distal to Region 2)	50	27.0	34.50	18.63	931.50
5	Diaphysis (Proximal to Region 3)	50	27.5	42.90	23.60	1179.75
6	Diaphysis (Distal to Region 4)	51	27.0	36.10	19.11	974.70
7	Proximal Metaphysis	49	29.0	43.40	25.69	1258.60
8	Distal Metaphysis	49	28.0	47.40	27.09	1327.20
9	Articulating Surface	49	27.0	124.00	68.33	3348.00
10	Distal Epiphysis	50	35.5	157.00	111.47	5573.50
11	Femoral Head	51	38.0	139.00	103.57	5282.00
12	Trochanter	50	36.0	126.00	90.72	4536.00
13	Calcaneus	49	30.0	57.80	35.39	1734.00
14	Talus	49	31.0	43.60	27.58	1351.60
RB	Reagent Blank	-	29.0	0.00	-	0.00

Table B17: Nuclear DNA Quantification of P-01 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-01		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	28.0	70.10	40.06	1962.80
2	Midshaft Diaphysis (Distal to Region 1)	50	29.0	59.20	34.34	1716.80
3	Diaphysis (Proximal to Region 1)	49	26.0	144.00	76.41	3744.00
4	Diaphysis (Distal to Region 2)	50	26.0	92.20	47.94	2397.20
5	Diaphysis (Proximal to Region 3)	51	30.0	319.00	187.65	9570.00
6	Diaphysis (Distal to Region 4)	49	29.5	314.00	189.04	9263.00
7	Proximal Metaphysis	49	27.0	697.00	384.06	18819.00
8	Distal Metaphysis	50	28.0	423.00	236.88	11844.00
9	Articulating Surface	49	30.0	902.00	552.24	27060.00
10	Distal Epiphysis	51	35.0	221.00	151.67	7735.00
11	Femoral Head	51	36.0	1600.00	1129.41	57600.00
12	Trochanter	50	34.0	1930.00	1312.40	65620.00
13	Calcaneus	50	29.0	686.00	397.88	19894.00
14	Talus	50	35.0	817.00	571.90	28595.00
RB	Reagent Blank	-	29.5	0.00	-	0.00

Table B18: Nuclear DNA Quantification of P-01 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-01		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	25.5	291.00	145.50	7420.50
2	Midshaft Diaphysis (Distal to Region 1)	51	29.5	246.00	142.29	7257.00
3	Diaphysis (Proximal to Region 1)	20	29.0	257.00	149.06	7453.00
4	Diaphysis (Distal to Region 2)	50	29.5	255.00	150.45	7522.50
5	Diaphysis (Proximal to Region 3)	51	30.0	384.00	225.88	11520.00
6	Diaphysis (Distal to Region 4)	49	30.0	223.00	136.53	6690.00
7	Proximal Metaphysis	51	28.0	512.00	281.10	14336.00
8	Distal Metaphysis	50	28.0	517.00	289.52	14476.00
9	Articulating Surface	50	30.0	810.00	486.00	24300.00
10	Distal Epiphysis	51	32.0	1640.00	1029.02	52480.00
11	Femoral Head	51	34.5	1710.00	1156.76	58995.00
12	Trochanter	51	35.0	1760.00	1207.84	61600.00
13	Calcaneus	49	35.0	640.00	457.14	22400.00
14	Talus	49	29.5	718.00	432.27	21181.00
RB	Reagent Blank	-	29.0	0.43	-	12.47

Table B19: Nuclear DNA Quantification of P-02 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-02		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	26.0	21.00	10.71	546.00
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	12.60	6.92	352.80
3	Diaphysis (Proximal to Region 1)	50	30.5	32.10	19.58	979.05
4	Diaphysis (Distal to Region 2)	49	30.0	21.70	13.29	651.00
5	Diaphysis (Proximal to Region 3)	50	29.0	78.40	45.47	2273.60
6	Diaphysis (Distal to Region 4)	49	27.5	102.00	57.24	2805.00
7	Proximal Metaphysis	51	26.0	443.00	225.84	11518.00
8	Distal Metaphysis	50	28.0	288.00	161.28	8064.00
9	Articulating Surface	51	30.0	436.00	256.47	13080.00
10	Distal Epiphysis	51	40.0	1890.00	1482.35	75600.00
11	Femoral Head	50	40.0	580.00	464.00	23200.00
12	Trochanter	49	40.0	1640.00	1338.78	65600.00
13	Calcaneus	50	34.0	508.00	345.44	17272.00
14	Talus	50	40.0	661.00	528.80	26440.00
RB	Reagent Blank	-	29.0	0.25	-	7.25

Table B20: Nuclear DNA Quantification of P-02 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-02		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	28.0	266.00	148.96	7448.00
2	Midshaft Diaphysis (Distal to Region 1)	49	27.0	173.00	95.33	4671.00
3	Diaphysis (Proximal to Region 1)	51	30.5	177.00	105.85	5398.50
4	Diaphysis (Distal to Region 2)	49	29.5	186.00	111.98	5487.00
5	Diaphysis (Proximal to Region 3)	51	29.0	201.00	114.29	5829.00
6	Diaphysis (Distal to Region 4)	51	30.0	187.00	110.00	5610.00
7	Proximal Metaphysis	51	30.0	381.00	224.12	11430.00
8	Distal Metaphysis	51	28.5	469.00	262.09	13366.50
9	Articulating Surface	49	30.0	531.00	325.10	15930.00
10	Distal Epiphysis	51	38.0	1300.00	968.63	49400.00
11	Femoral Head	50	39.0	1220.00	951.60	47580.00
12	Trochanter	50	39.0	1090.00	850.20	42510.00
13	Calcaneus	51	35.0	430.00	295.10	15050.00
14	Talus	50	38.5	667.00	513.59	25679.50
RB	Reagent Blank	-	28.0	0.35	-	9.80

Table B21: Nuclear DNA Quantification of P-03 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder. ND = No Data

P-03		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	31.0	10.30	6.26	319.30
2	Midshaft Diaphysis (Distal to Region 1)	51	0.0	ND	ND	ND
3	Diaphysis (Proximal to Region 1)	50	29.0	33.00	19.14	957.00
4	Diaphysis (Distal to Region 2)	51	30.0	15.10	8.88	453.00
5	Diaphysis (Proximal to Region 3)	49	30.0	494.00	302.45	14820.00
6	Diaphysis (Distal to Region 4)	51	27.5	230.00	124.02	6325.00
7	Proximal Metaphysis	49	30.0	1260.00	720.00	35280.00
8	Distal Metaphysis	49	27.0	591.00	325.65	15957.00
9	Articulating Surface	49	30.0	906.00	554.69	27180.00
10	Distal Epiphysis	50	35.0	4560.00	3192.00	159600.00
11	Femoral Head	51	37.0	2330.00	1690.39	86210.00
12	Trochanter	49	32.0	4890.00	3193.47	156480.00
13	Calcaneus	51	31.5	631.00	389.74	19876.50
14	Talus	51	32.0	970.00	608.63	31040.00
RB	Reagent Blank	-	25.0	0.29	-	7.25

Table B22: Nuclear DNA Quantification of P-03 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-03		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	28.5	233.00	130.21	6640.50
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	294.00	161.41	8232.00
3	Diaphysis (Proximal to Region 1)	49	27.0	210.00	115.71	5670.00
4	Diaphysis (Distal to Region 2)	49	26.0	332.00	176.16	8632.00
5	Diaphysis (Proximal to Region 3)	49	29.0	539.00	319.00	15631.00
6	Diaphysis (Distal to Region 4)	50	26.0	330.00	171.60	8580.00
7	Proximal Metaphysis	51	29.5	899.00	520.01	26520.50
8	Distal Metaphysis	49	30.0	454.00	245.16	12258.00
9	Articulating Surface	51	27.0	451.00	238.76	12177.00
10	Distal Epiphysis	49	30.0	1280.00	783.67	38400.00
11	Femoral Head	49	36.0	1160.00	852.24	41760.00
12	Trochanter	50	29.5	2050.00	1209.50	60475.00
13	Calcaneus	51	29.5	835.00	482.99	24632.50
14	Talus	50	30.0	694.00	416.40	20820.00
RB	Reagent Blank	-	27.0	0.14	-	3.89

Table B23: Nuclear DNA Quantification of P-04 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-04		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	28.0	4.32	2.44	122.08
2	Midshaft Diaphysis (Distal to Region 1)	50	25.0	3.20	1.60	80.00
3	Diaphysis (Proximal to Region 1)	50	29.0	3.46	2.01	100.34
4	Diaphysis (Distal to Region 2)	52	28.0	5.68	3.06	159.04
5	Diaphysis (Proximal to Region 3)	51	30.0	125.00	73.53	3750.00
6	Diaphysis (Distal to Region 4)	49	27.0	126.00	69.43	3402.00
7	Proximal Metaphysis	51	31.5	384.00	237.18	12096.00
8	Distal Metaphysis	49	24.5	143.00	71.50	3503.50
9	Articulating Surface	50	27.0	470.00	253.80	12690.00
10	Distal Epiphysis	51	32.5	3750.00	2389.71	121875.00
11	Femoral Head	51	35.0	4140.00	2841.18	144900.00
12	Trochanter	51	32.5	3460.00	2204.90	112450.00
13	Calcaneus	51	30.0	512.00	301.18	15360.00
14	Talus	50	30.0	703.00	421.80	21090.00
RB	Reagent Blank	-	23.5	0.26	-	6.11

Table B24: Nuclear DNA Quantification of P-04 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-04		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.5	140.00	82.60	4130.00
2	Midshaft Diaphysis (Distal to Region 1)	51	23.5	271.00	124.87	6368.50
3	Diaphysis (Proximal to Region 1)	50	28.5	173.00	98.61	4930.50
4	Diaphysis (Distal to Region 2)	50	24.0	264.00	126.72	6336.00
5	Diaphysis (Proximal to Region 3)	50	26.0	302.00	157.04	7852.00
6	Diaphysis (Distal to Region 4)	51	26.0	255.00	130.00	6630.00
7	Proximal Metaphysis	49	29.0	441.00	261.00	12789.00
8	Distal Metaphysis	51	28.0	368.00	202.04	10304.00
9	Articulating Surface	51	27.0	486.00	257.29	13122.00
10	Distal Epiphysis	51	35.0	1530.00	1050.00	53550.00
11	Femoral Head	50	33.0	1170.00	772.20	38610.00
12	Trochanter	49	38.5	1460.00	1147.14	56210.00
13	Calcaneus	51	28.5	757.00	423.03	21574.50
14	Talus	50	31.0	569.00	352.78	17639.00
RB	Reagent Blank	-	26.0	0.08	-	2.03

Table B25: Nuclear DNA Quantification of P-05 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-05		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	28.0	11.20	6.27	313.60
2	Midshaft Diaphysis (Distal to Region 1)	49	27.0	37.60	20.72	1015.20
3	Diaphysis (Proximal to Region 1)	50	26.5	16.60	8.80	439.90
4	Diaphysis (Distal to Region 2)	51	27.0	23.90	12.65	645.30
5	Diaphysis (Proximal to Region 3)	50	27.5	335.00	184.25	9212.50
6	Diaphysis (Distal to Region 4)	49	26.5	96.10	51.97	2546.65
7	Proximal Metaphysis	50	27.5	241.00	132.55	6627.50
8	Distal Metaphysis	51	25.5	143.00	71.50	3646.50
9	Articulating Surface	51	27.0	498.00	263.65	13446.00
10	Distal Epiphysis	50	38.0	1360.00	1033.60	51680.00
11	Femoral Head	50	40.0	1090.00	872.00	43600.00
12	Trochanter	50	32.0	2990.00	1913.60	95680.00
13	Calcaneus	49	28.0	966.00	552.00	27048.00
14	Talus	51	33.0	565.00	365.59	18645.00
RB	Reagent Blank	-	23.5	0.21	-	4.94

Table B26: Nuclear DNA Quantification of P-05 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-05		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	30.0	193.00	113.53	5790.00
2	Midshaft Diaphysis (Distal to Region 1)	50	27.5	239.00	131.45	6572.50
3	Diaphysis (Proximal to Region 1)	51	26.0	244.00	124.39	6344.00
4	Diaphysis (Distal to Region 2)	49	26.0	219.00	116.20	5694.00
5	Diaphysis (Proximal to Region 3)	50	29.0	438.00	254.04	12702.00
6	Diaphysis (Distal to Region 4)	50	25.0	241.00	120.50	6025.00
7	Proximal Metaphysis	49	29.0	427.00	252.71	12383.00
8	Distal Metaphysis	49	29.5	461.00	277.54	13599.50
9	Articulating Surface	50	31.0	334.00	207.08	10354.00
10	Distal Epiphysis	49	34.0	691.00	479.47	23494.00
11	Femoral Head	50	39.0	914.00	712.92	35646.00
12	Trochanter	50	32.0	1350.00	864.00	43200.00
13	Calcaneus	49	28.0	566.00	323.43	15848.00
14	Talus	49	31.5	764.00	491.14	24066.00
RB	Reagent Blank	-	26.0	0.14	-	3.61

Table B27: Nuclear DNA Quantification of P-06 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-06		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	32.0	10.50	6.86	336.00
2	Midshaft Diaphysis (Distal to Region 1)	51	25.0	6.57	3.22	164.25
3	Diaphysis (Proximal to Region 1)	50	25.5	21.90	11.17	558.45
4	Diaphysis (Distal to Region 2)	49	27.0	5.66	3.12	152.82
5	Diaphysis (Proximal to Region 3)	50	26.0	175.00	91.00	4550.00
6	Diaphysis (Distal to Region 4)	50	26.5	187.00	99.11	4955.50
7	Proximal Metaphysis	50	28.0	197.00	110.32	5516.00
8	Distal Metaphysis	51	28.0	176.00	96.63	4928.00
9	Articulating Surface	49	26.5	694.00	375.33	18391.00
10	Distal Epiphysis	49	34.0	2680.00	1859.59	91120.00
11	Femoral Head	51	32.0	1560.00	978.82	49920.00
12	Trochanter	49	30.5	3170.00	1973.16	96685.00
13	Calcaneus	51	30.5	270.00	161.47	8235.00
14	Talus	50	32.0	600.00	384.00	19200.00
RB	Reagent Blank	-	28.0	0.18	-	5.04

Table B28: Nuclear DNA Quantification of P-06 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-06		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	28.0	157.00	86.20	4396.00
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	162.00	88.94	4536.00
3	Diaphysis (Proximal to Region 1)	50	27.5	154.00	84.70	4235.00
4	Diaphysis (Distal to Region 2)	50	28.0	164.00	91.84	4592.00
5	Diaphysis (Proximal to Region 3)	51	27.0	201.00	106.41	5427.00
6	Diaphysis (Distal to Region 4)	50	28.0	198.00	110.88	5544.00
7	Proximal Metaphysis	50	29.0	318.00	184.44	9222.00
8	Distal Metaphysis	50	33.0	279.00	153.18	7812.00
9	Articulating Surface	51	27.0	316.00	167.29	8532.00
10	Distal Epiphysis	50	33.0	771.00	508.86	25443.00
11	Femoral Head	49	33.0	857.00	577.16	28281.00
12	Trochanter	49	31.0	887.00	561.16	27497.00
13	Calcaneus	51	24.5	454.00	218.10	11123.00
14	Talus	51	31.5	355.00	219.26	11182.50
RB	Reagent Blank	-	28.0	0.04	-	0.99

Table B29: Nuclear DNA Quantification of P-07 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-07		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	25.5	15.10	7.86	385.05
2	Midshaft Diaphysis (Distal to Region 1)	49	26.5	25.00	13.52	662.50
3	Diaphysis (Proximal to Region 1)	51	28.5	35.80	20.01	1020.30
4	Diaphysis (Distal to Region 2)	50	27.0	1270.00	672.35	221.67
5	Diaphysis (Proximal to Region 3)	50	29.0	835.00	484.30	24215.00
6	Diaphysis (Distal to Region 4)	50	26.5	457.00	242.21	12110.50
7	Proximal Metaphysis	51	27.0	1270.00	672.35	34290.00
8	Distal Metaphysis	50	26.0	671.00	348.92	17446.00
9	Articulating Surface	50	26.5	1310.00	694.30	34715.00
10	Distal Epiphysis	49	30.5	3540.00	2203.47	107970.00
11	Femoral Head	49	33.0	1240.00	835.10	40920.00
12	Trochanter	49	34.0	2480.00	1720.82	84320.00
13	Calcaneus	50	25.5	718.00	366.18	18309.00
14	Talus	51	30.0	718.00	366.18	21240.00
RB	Reagent Blank	-	27.0	0.04	-	1.08

Table B30: Nuclear DNA Quantification of P-07 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-07		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	27.5	294.00	165.00	8085.00
2	Midshaft Diaphysis (Distal to Region 1)	50	26.0	229.00	119.08	5954.00
3	Diaphysis (Proximal to Region 1)	50	25.5	321.00	163.71	8185.50
4	Diaphysis (Distal to Region 2)	50	30.0	269.00	161.40	8070.00
5	Diaphysis (Proximal to Region 3)	51	28.5	404.00	225.76	11514.00
6	Diaphysis (Distal to Region 4)	49	28.0	438.00	250.29	12264.00
7	Proximal Metaphysis	50	27.0	670.00	361.80	18090.00
8	Distal Metaphysis	49	31.0	543.00	343.53	16833.00
9	Articulating Surface	49	29.5	579.00	348.58	17080.50
10	Distal Epiphysis	50	32.5	1080.00	702.00	35100.00
11	Femoral Head	49	32.0	957.00	624.98	30624.00
12	Trochanter	50	38.5	1210.00	931.70	46585.00
13	Calcaneus	49	29.0	567.00	335.57	16443.00
14	Talus	49	32.0	700.00	457.14	22400.00
RB	Reagent Blank	-	29.0	0.05	-	1.45

Table B31: Nuclear DNA Quantification of P-08 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-08		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	26.0	26.20	13.62	681.20
2	Midshaft Diaphysis (Distal to Region 1)	49	23.0	23.40	10.98	538.20
3	Diaphysis (Proximal to Region 1)	51	28.0	23.80	13.07	666.40
4	Diaphysis (Distal to Region 2)	51	26.0	16.70	8.51	434.20
5	Diaphysis (Proximal to Region 3)	49	26.0	404.00	214.37	10504.00
6	Diaphysis (Distal to Region 4)	49	29.0	273.00	161.57	7917.00
7	Proximal Metaphysis	49	28.5	281.00	163.44	8008.50
8	Distal Metaphysis	49	28.0	395.00	225.71	11060.00
9	Articulating Surface	49	27.0	1060.00	584.08	28620.00
10	Distal Epiphysis	49	35.0	3600.00	2571.43	126000.00
11	Femoral Head	49	35.5	1420.00	1028.78	50410.00
12	Trochanter	49	34.0	3910.00	2713.06	132940.00
13	Calcaneus	49	30.5	652.00	405.84	19886.00
14	Talus	50	29.0	759.00	440.22	22011.00
RB	Reagent Blank	-	27.0	0.06	-	1.62

Table B32: Nuclear DNA Quantification of P-08 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng of per mg of bone powder.

P-08		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	25.5	243.00	126.46	6196.50
2	Midshaft Diaphysis (Distal to Region 1)	50	32.5	198.00	128.70	6435.00
3	Diaphysis (Proximal to Region 1)	49	30.5	174.00	108.31	5307.00
4	Diaphysis (Distal to Region 2)	50	27.0	220.00	118.80	5940.00
5	Diaphysis (Proximal to Region 3)	50	27.5	293.00	161.15	8057.50
6	Diaphysis (Distal to Region 4)	51	27.0	248.00	131.29	6696.00
7	Proximal Metaphysis	49	29.0	222.00	131.39	6438.00
8	Distal Metaphysis	49	28.0	320.00	182.86	8960.00
9	Articulating Surface	49	27.0	331.00	182.39	8937.00
10	Distal Epiphysis	50	35.5	886.00	629.06	31453.00
11	Femoral Head	51	38.0	1060.00	789.80	40280.00
12	Trochanter	50	36.0	722.00	519.84	25992.00
13	Calcaneus	49	30.0	449.00	274.90	13470.00
14	Talus	49	31.0	498.00	315.06	15438.00
RB	Reagent Blank	-	29.0	0.03	-	0.74

Table B33: Porcine Mitochondrial DNA PCR Amplification Chart for Quality Assay (Tissue Lysis Buffer)

Region and location correspond to Table 3. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 181 bp amplicon, 2 = 414 bp amplicon, 3 = 604 bp amplicon, 4 = 1017 bp amplicon.

NT = Not Tested NA = No Amplification

Region	Location	Mitochondrial DNA (*ATPase*)							
		P-01	**P-02**	**P-03**	**P-04**	**P-05**	**P-06**	**P-07**	**P-08**
1	Midshaft Diaphysis	4	4	4	4	4	4	4	4
2	Midshaft Diaphysis (Distal to Region 1)	4	4	NT	4	4	4	4	4
3	Diaphysis (Proximal to Region 1)	4	4	4	4	4	4	4	4
4	Diaphysis (Distal to Region 2)	4	4	4	4	4	4	4	4
5	Diaphysis (Proximal to Region 3)	4	4	4	4	4	4	4	4
6	Diaphysis (Distal to Region 4)	4	4	4	4	4	4	4	4
7	Proximal Metaphysis	4	4	4	4	4	4	4	4
8	Distal Metaphysis	4	4	4	4	4	4	4	4
9	Articulating Surface	4	4	4	4	4	4	4	4
10	Distal Epiphysis	4	4	4	4	4	4	4	4
11	Femoral Head	4	4	4	4	4	4	4	4
12	Trochanter	4	4	4	4	4	4	4	4
13	Calcaneus	4	4	4	4	4	4	4	4
14	Talus	4	4	4	4	4	4	4	4
RB	Reagent Blank	NA	NA	NA	NA	NA	NA	NA	NA

Table B34: Porcine Mitochondrial DNA PCR Amplification Chart for Quality Assay (Demineralization Buffer)

Region and location correspond to Table 3. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 181 bp amplicon, 2 = 414 bp amplicon, 3 = 604 bp amplicon, 4 = 1017 bp amplicon.

NA = No Amplification

Region	Location	Mitochondrial DNA (*ATPase*)							
		P-01	**P-02**	**P-03**	**P-04**	**P-05**	**P-06**	**P-07**	**P-08**
1	Midshaft Diaphysis	4	4	4	4	4	4	4	4
2	Midshaft Diaphysis (Distal to Region 1)	4	4	4	4	4	4	4	4
3	Diaphysis (Proximal to Region 1)	4	4	4	4	4	4	4	4
4	Diaphysis (Distal to Region 2)	4	4	4	4	4	4	4	4
5	Diaphysis (Proximal to Region 3)	4	4	4	4	4	4	4	4
6	Diaphysis (Distal to Region 4)	4	4	4	4	4	4	4	4
7	Proximal Metaphysis	4	4	4	4	4	4	4	4
8	Distal Metaphysis	4	4	4	4	4	4	4	4
9	Articulating Surface	4	4	4	4	4	4	4	4
10	Distal Epiphysis	4	4	4	4	4	4	4	4
11	Femoral Head	4	4	4	4	4	4	4	4
12	Trochanter	4	4	4	4	4	4	4	4
13	Calcaneus	4	4	4	4	4	4	4	4
14	Talus	4	4	4	4	4	4	4	4
RB	Reagent Blank	NA	NA	NA	NA	NA	NA	NA	NA

Table B35: Porcine Nuclear DNA PCR Amplification Chart for Quality Assay (Tissue Lysis Buffer)

Region and location correspond to Table 3. Values (1 – 3) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 257 bp amplicon, 2 = 457 bp amplicon, 3 = 642 bp amplicon.

NT = Not Tested NA = No Amplification

Region	Location	Nuclear DNA (*IGF-1*)							
		P-01	**P-02**	**P-03**	**P-04**	**P-05**	**P-06**	**P-07**	**P-08**
1	Midshaft Diaphysis	3	3	3	3	3	3	3	3
2	Midshaft Diaphysis (Distal to Region 1)	3	3	NT	3	3	3	3	3
3	Diaphysis (Proximal to Region 1)	3	3	3	3	3	3	3	3
4	Diaphysis (Distal to Region 2)	3	3	3	3	3	3	3	3
5	Diaphysis (Proximal to Region 3)	2	3	3	3	3	3	3	3
6	Diaphysis (Distal to Region 4)	2	3	3	3	3	3	3	3
7	Proximal Metaphysis	3	3	2	3	3	3	3	3
8	Distal Metaphysis	3	3	3	3	3	3	3	3
9	Articulating Surface	3	3	3	3	3	3	3	3
10	Distal Epiphysis	3	3	3	3	3	3	3	3
11	Femoral Head	3	3	3	3	3	3	3	3
12	Trochanter	2	3	3	3	3	3	3	3
13	Calcaneus	3	3	3	3	3	3	3	3
14	Talus	3	3	3	3	3	3	3	3
RB	Reagent Blank	NA	NA	NA	NA	NA	NA	NA	NA

Table B36: Porcine Nuclear DNA PCR Amplification Chart for Quality Assay (Demineralization Buffer)

Region and location correspond to Table 3. Values (1 – 3) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 257 bp amplicon, 2 = 457 bp amplicon, 3 = 642 bp amplicon.

NA = No Amplification.

Region	Location	Nuclear DNA (*IGF-1*)							
		P-01	**P-02**	**P-03**	**P-04**	**P-05**	**P-06**	**P-07**	**P-08**
1	Midshaft Diaphysis	3	3	3	3	3	3	3	3
2	Midshaft Diaphysis (Distal to Region 1)	2	3	3	3	3	3	3	3
3	Diaphysis (Proximal to Region 1)	3	3	3	3	3	3	3	2
4	Diaphysis (Distal to Region 2)	3	3	3	3	3	3	3	3
5	Diaphysis (Proximal to Region 3)	3	3	3	3	3	3	3	3
6	Diaphysis (Distal to Region 4)	2	3	3	3	3	3	3	3
7	Proximal Metaphysis	2	3	3	3	3	3	3	3
8	Distal Metaphysis	3	3	3	3	3	3	3	3
9	Articulating Surface	3	3	3	3	3	3	3	3
10	Distal Epiphysis	3	3	3	3	3	3	3	3
11	Femoral Head	3	3	3	3	3	3	3	3
12	Trochanter	2	3	3	3	3	3	3	3
13	Calcaneus	3	3	3	3	3	3	3	3
14	Talus	3	3	3	3	3	3	3	3
RB	Reagent Blank	NA	NA	NA	NA	NA	NA	NA	NA

APPENDIX C: MITOCHONDRIAL AND NUCLEAR DNA QUANTIFICATION DATA AND INDIVIDUAL PCR AMPLIFICATION RESULTS FROM FRESH BOVINE FEMORA AND TARSALS

Table C1: mtDNA Quantification of C-01 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-01		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	30.0	12.90	7.59	387.00
2	Midshaft Diaphysis (Distal to Region 1)	50	25.0	10.60	5.30	265.00
3	Diaphysis (Proximal to Region 1)	51	29.0	14.60	8.30	423.30
4	Diaphysis (Distal to Region 2)	49	29.0	12.90	7.63	373.87
5	Diaphysis (Proximal to Region 3)	50	30.0	9.86	5.92	296.00
6	Diaphysis (Distal to Region 4)	51	30.0	9.83	5.78	294.78
7	Proximal Metaphysis	49	26.0	25.30	13.42	657.58
8	Distal Metaphysis	50	30.0	22.50	14.56	742.56
9	Articulating Surface	49	30.0	46.60	28.53	1397.97
10	Distal Epiphysis	50	30.0	104.00	62.40	3120.00
11	Femoral Head	51	29.0	89.50	50.89	2595.39
12	Trochanter	51	29.0	50.60	28.77	1467.27
13	Calcaneus	50	29.0	30.50	17.69	884.50
14	Talus	51	27.0	109.00	57.71	2943.21
RB	Reagent Blank	-	26.0	0.06	-	1.56

Table C2: mtDNA Quantification of C-01 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-01		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	28.0	32.60	18.63	912.87
2	Midshaft Diaphysis (Distal to Region 1)	49	25.0	21.90	11.17	547.33
3	Diaphysis (Proximal to Region 1)	50	30.0	21.80	13.08	654.00
4	Diaphysis (Distal to Region 2)	51	30.0	18.40	10.82	551.82
5	Diaphysis (Proximal to Region 3)	49	30.0	20.10	12.31	603.19
6	Diaphysis (Distal to Region 4)	49	30.0	20.00	12.24	599.76
7	Proximal Metaphysis	51	29.0	34.40	19.56	997.56
8	Distal Metaphysis	51	29.0	55.50	31.56	1609.56
9	Articulating Surface	49	29.0	65.70	38.88	1905.12
10	Distal Epiphysis	51	30.0	133.00	78.24	3990.24
11	Femoral Head	50	30.0	140.00	84.00	4200.00
12	Trochanter	51	30.0	68.60	40.35	2057.85
13	Calcaneus	49	30.0	43.10	26.39	1293.11
14	Talus	49	31.0	87.30	55.23	2706.27
RB	Reagent Blank	-	30.0	0.06	-	1.80

Table C3: mtDNA Quantification of C-02 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-02		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	34.0	16.40	11.38	557.60
2	Midshaft Diaphysis (Distal to Region 1)	51	30.0	27.80	16.35	834.00
3	Diaphysis (Proximal to Region 1)	49	30.0	37.30	22.84	1119.00
4	Diaphysis (Distal to Region 2)	49	30.0	19.10	11.69	573.00
5	Diaphysis (Proximal to Region 3)	51	29.0	28.20	16.04	817.80
6	Diaphysis (Distal to Region 4)	49	28.0	20.20	11.54	565.60
7	Proximal Metaphysis	50	32.0	67.50	43.20	2160.00
8	Distal Metaphysis	50	32.0	164.00	102.90	1913.60
9	Articulating Surface	50	30.0	87.60	52.56	2628.00
10	Distal Epiphysis	51	32.0	164.00	102.90	5248.00
11	Femoral Head	51	32.0	137.00	85.96	4384.00
12	Trochanter	51	31.0	92.50	56.23	2867.50
13	Calcaneus	49	30.0	64.30	39.37	1929.00
14	Talus	50	31.0	96.40	59.77	2988.40
RB	Reagent Blank	-	30.0	0.04	-	1.20

Table C4: mtDNA Quantification of C-02 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-02		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	30.0	30.90	18.54	927.00
2	Midshaft Diaphysis (Distal to Region 1)	51	30.0	32.10	18.88	963.00
3	Diaphysis (Proximal to Region 1)	51	29.0	35.10	19.96	1017.90
4	Diaphysis (Distal to Region 2)	50	29.0	27.40	15.89	764.60
5	Diaphysis (Proximal to Region 3)	50	29.0	39.20	22.74	1136.80
6	Diaphysis (Distal to Region 4)	49	25.0	31.90	16.28	797.50
7	Proximal Metaphysis	50	26.0	95.00	49.40	2470.00
8	Distal Metaphysis	50	29.0	79.40	46.05	2302.60
9	Articulating Surface	49	29.0	79.60	47.11	2308.40
10	Distal Epiphysis	50	32.0	244.00	156.16	7808.00
11	Femoral Head	49	30.0	124.00	75.92	3720.00
12	Trochanter	50	30.0	76.00	45.60	2280.00
13	Calcaneus	51	30.0	70.20	41.29	2106.00
14	Talus	49	34.0	76.20	52.87	2590.80
RB	Reagent Blank	-	29.0	0.04	-	1.16

Table C5: mtDNA Quantification of C-03 Extracts Digested in Tissue Lysis Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-03		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.0	3.10	1.80	89.90
2	Midshaft Diaphysis (Distal to Region 1)	49	26.0	4.46	2.37	115.96
3	Diaphysis (Proximal to Region 1)	50	30.0	3.94	2.36	118.20
4	Diaphysis (Distal to Region 2)	50	27.0	5.06	2.73	136.62
5	Diaphysis (Proximal to Region 3)	50	29.0	3.26	1.89	94.54
6	Diaphysis (Distal to Region 4)	49	30.0	4.25	2.60	127.50
7	Proximal Metaphysis	50	32.0	7.52	4.81	240.64
8	Distal Metaphysis	50	30.0	5.18	3.11	155.40
9	Articulating Surface	51	31.0	9.75	5.93	302.25
10	Distal Epiphysis	51	31.0	21.20	12.89	657.20
11	Femoral Head	49	30.0	18.80	11.51	564.00
12	Trochanter	50	25.0	22.80	11.40	570.00
13	Calcaneus	50	30.0	4.81	2.89	144.30
14	Talus	50	30.0	6.22	3.73	186.60
RB	Reagent Blank	-	26.0	0.00	-	0.00

Table C6: mtDNA Quantification of C-03 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-03		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	30.0	5.62	3.31	168.60
2	Midshaft Diaphysis (Distal to Region 1)	51	30.0	3.78	2.22	113.40
3	Diaphysis (Proximal to Region 1)	51	27.0	4.83	2.51	133.38
4	Diaphysis (Distal to Region 2)	49	32.0	4.36	2.85	139.52
5	Diaphysis (Proximal to Region 3)	50	26.0	4.83	2.51	125.58
6	Diaphysis (Distal to Region 4)	50	29.0	5.05	2.93	146.45
7	Proximal Metaphysis	50	28.0	7.33	4.10	205.24
8	Distal Metaphysis	51	29.0	7.18	4.08	208.22
9	Articulating Surface	51	26.0	9.01	4.59	234.26
10	Distal Epiphysis	49	31.0	20.80	13.16	644.80
11	Femoral Head	49	31.0	18.80	11.89	582.80
12	Trochanter	49	26.0	20.80	11.04	540.80
13	Calcaneus	50	32.0	4.98	3.19	159.36
14	Talus	51	30.0	5.63	3.31	168.90
RB	Reagent Blank	-	28.0	0.01	-	0.28

Table C7: mtDNA Quantification of C-04 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-04		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	32.0	5.79	3.63	185.28
2	Midshaft Diaphysis (Distal to Region 1)	50	27.0	2.67	1.44	72.09
3	Diaphysis (Proximal to Region 1)	50	30.0	4.30	2.58	129.00
4	Diaphysis (Distal to Region 2)	50	27.0	3.99	2.15	107.73
5	Diaphysis (Proximal to Region 3)	51	29.0	4.22	2.40	122.38
6	Diaphysis (Distal to Region 4)	50	25.0	6.07	3.04	151.75
7	Proximal Metaphysis	50	27.0	6.09	3.29	164.43
8	Distal Metaphysis	51	30.0	4.61	2.71	138.30
9	Articulating Surface	51	29.0	13.50	7.68	391.50
10	Distal Epiphysis	50	29.0	35.10	20.36	1017.90
11	Femoral Head	50	30.0	15.80	9.48	474.00
12	Trochanter	50	30.0	8.71	5.23	261.30
13	Calcaneus	49	30.0	4.88	2.99	146.40
14	Talus	49	30.0	3.74	2.29	112.20
RB	Reagent Blank	-	28.0	0.03	-	0.84

Table C8: mtDNA Quantification of C-04 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-04		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	28.0	3.51	2.01	98.28
2	Midshaft Diaphysis (Distal to Region 1)	50	30.0	4.97	2.98	149.10
3	Diaphysis (Proximal to Region 1)	49	31.0	5.00	3.16	155.00
4	Diaphysis (Distal to Region 2)	51	28.0	5.74	3.15	160.72
5	Diaphysis (Proximal to Region 3)	51	28.0	6.16	3.38	172.48
6	Diaphysis (Distal to Region 4)	51	29.0	5.24	2.98	151.96
7	Proximal Metaphysis	50	29.0	7.41	4.30	214.89
8	Distal Metaphysis	51	31.0	4.76	2.89	147.56
9	Articulating Surface	51	27.0	19.90	10.54	537.30
10	Distal Epiphysis	50	30.0	31.30	18.78	939.00
11	Femoral Head	51	31.0	23.80	14.47	737.80
12	Trochanter	51	28.0	9.44	5.18	264.32
13	Calcaneus	50	31.0	4.99	3.09	154.69
14	Talus	51	26.0	4.59	2.34	119.34
RB	Reagent Blank	-	29.0	0.00	-	0.00

Table C9: mtDNA Quantification of C-05 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-05		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	30.0	4.84	2.90	145.20
2	Midshaft Diaphysis (Distal to Region 1)	50	25.5	7.56	3.86	192.78
3	Diaphysis (Proximal to Region 1)	50	27.0	7.43	4.01	200.61
4	Diaphysis (Distal to Region 2)	50	37.0	7.95	5.88	294.15
5	Diaphysis (Proximal to Region 3)	51	23.0	10.70	4.83	246.10
6	Diaphysis (Distal to Region 4)	50	37.0	8.94	5.72	286.08
7	Proximal Metaphysis	50	30.0	13.90	8.34	417.00
8	Distal Metaphysis	49	30.0	14.40	8.82	432.00
9	Articulating Surface	50	32.0	43.40	27.78	1388.80
10	Distal Epiphysis	50	28.0	41.10	23.02	1150.80
11	Femoral Head	51	30.0	45.20	26.59	1356.00
12	Trochanter	51	26.0	77.90	39.71	2025.40
13	Calcaneus	51	30.5	51.30	30.68	1564.65
14	Talus	50	27.5	49.70	27.34	1366.75
RB	Reagent Blank	-	28.0	0.02	-	0.56

Table C10: mtDNA Quantification of C-05 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-05		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.0	9.83	5.70	285.07
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	13.90	7.63	389.20
3	Diaphysis (Proximal to Region 1)	49	28.0	10.90	6.23	305.20
4	Diaphysis (Distal to Region 2)	49	31.5	7.52	4.83	236.88
5	Diaphysis (Proximal to Region 3)	50	32.0	17.40	11.14	556.80
6	Diaphysis (Distal to Region 4)	50	30.0	10.70	6.42	321.00
7	Proximal Metaphysis	51	29.0	27.10	15.41	785.90
8	Distal Metaphysis	50	28.0	22.60	12.66	632.80
9	Articulating Surface	51	28.0	38.00	20.86	1064.00
10	Distal Epiphysis	50	29.5	43.00	25.37	1268.50
11	Femoral Head	51	31.0	51.10	31.06	1584.10
12	Trochanter	50	27.0	45.10	24.35	1217.70
13	Calcaneus	51	28.0	32.90	18.06	921.20
14	Talus	51	30.0	26.30	15.47	789.00
RB	Reagent Blank	-	26.0	0.05	-	1.30

Table C11: mtDNA Quantification of C-06 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-06		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	27.0	3.43	1.82	92.61
2	Midshaft Diaphysis (Distal to Region 1)	50	31.0	20.40	3.48	174.22
3	Diaphysis (Proximal to Region 1)	49	28.0	3.36	1.92	94.08
4	Diaphysis (Distal to Region 2)	49	28.5	7.44	4.33	212.04
5	Diaphysis (Proximal to Region 3)	50	30.0	7.12	4.27	213.60
6	Diaphysis (Distal to Region 4)	49	30.0	9.06	5.55	271.80
7	Proximal Metaphysis	50	27.5	12.80	7.04	352.00
8	Distal Metaphysis	49	28.5	23.50	13.67	669.75
9	Articulating Surface	50	30.0	19.90	18.47	597.00
10	Distal Epiphysis	50	28.0	66.80	37.41	1870.40
11	Femoral Head	50	28.5	46.00	26.22	1311.00
12	Trochanter	50	29.0	41.10	23.84	1191.90
13	Calcaneus	50	31.0	31.80	19.72	985.80
14	Talus	49	27.5	42.00	23.57	1155.00
RB	Reagent Blank	-	29.0	0.06	-	1.74

Table C12: mtDNA Quantification of C-06 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-06		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	29.5	7.51	4.34	221.55
2	Midshaft Diaphysis (Distal to Region 1)	50	29.5	7.35	4.34	216.83
3	Diaphysis (Proximal to Region 1)	51	31.0	4.34	2.64	134.54
4	Diaphysis (Distal to Region 2)	49	27.5	11.20	6.29	308.00
5	Diaphysis (Proximal to Region 3)	50	30.5	17.60	10.74	536.80
6	Diaphysis (Distal to Region 4)	50	28.0	24.00	13.44	672.00
7	Proximal Metaphysis	50	21.5	30.60	13.16	657.90
8	Distal Metaphysis	50	30.0	31.50	18.90	945.00
9	Articulating Surface	50	28.0	32.80	18.37	918.40
10	Distal Epiphysis	51	29.5	52.90	30.60	1560.55
11	Femoral Head	51	28.0	77.20	42.38	2161.60
12	Trochanter	50	29.0	53.20	30.86	1542.80
13	Calcaneus	50	29.0	28.70	16.65	832.30
14	Talus	61	29.0	31.60	17.97	916.40
RB	Reagent Blank	-	27.5	0.01	-	0.28

Table C13: mtDNA Quantification of C-07 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-07		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.0	4.62	2.68	133.98
2	Midshaft Diaphysis (Distal to Region 1)	50	30.0	7.71	4.63	231.30
3	Diaphysis (Proximal to Region 1)	50	29.0	9.65	5.60	279.85
4	Diaphysis (Distal to Region 2)	49	25.0	8.87	4.53	221.75
5	Diaphysis (Proximal to Region 3)	51	26.5	8.84	4.59	234.26
6	Diaphysis (Distal to Region 4)	51	30.0	10.70	6.29	321.00
7	Proximal Metaphysis	51	29.0	35.50	20.19	1029.50
8	Distal Metaphysis	51	28.0	10.30	5.65	288.40
9	Articulating Surface	51	28.0	20.70	11.36	579.60
10	Distal Epiphysis	50	31.0	46.20	28.64	1432.20
11	Femoral Head	51	30.0	11.60	6.82	348.00
12	Trochanter	50	28.0	54.20	30.35	1517.60
13	Calcaneus	50	26.5	33.80	17.91	895.70
14	Talus	49	31.0	23.70	14.99	734.70
RB	Reagent Blank	-	28.0	0.02	-	0.56

Table C14: mtDNA Quantification of C-07 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-07		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	30.5	6.78	4.22	206.79
2	Midshaft Diaphysis (Distal to Region 1)	49	32.0	5.59	2.65	178.88
3	Diaphysis (Proximal to Region 1)	50	26.0	11.20	5.82	291.20
4	Diaphysis (Distal to Region 2)	51	30.0	5.26	3.09	157.80
5	Diaphysis (Proximal to Region 3)	49	28.5	12.70	7.39	361.95
6	Diaphysis (Distal to Region 4)	51	28.5	5.29	2.96	150.77
7	Proximal Metaphysis	50	30.5	34.10	20.80	1040.05
8	Distal Metaphysis	51	28.5	12.60	7.04	359.10
9	Articulating Surface	50	29.0	24.60	14.27	713.40
10	Distal Epiphysis	50	26.5	49.10	26.02	1301.15
11	Femoral Head	51	30.5	39.40	23.56	1201.70
12	Trochanter	51	28.5	46.40	25.93	1322.40
13	Calcaneus	49	29.0	22.30	13.20	646.70
14	Talus	50	29.5	15.10	8.91	445.45
RB	Reagent Blank	-	28.0	0.01	-	0.28

Table C15: mtDNA Quantification of C-08 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-08		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	30.0	3.73	2.24	111.90
2	Midshaft Diaphysis (Distal to Region 1)	50	28.5	4.67	2.66	133.10
3	Diaphysis (Proximal to Region 1)	50	26.0	7.00	3.64	182.00
4	Diaphysis (Distal to Region 2)	49	28.5	3.79	2.20	108.02
5	Diaphysis (Proximal to Region 3)	51	29.0	7.10	4.04	205.90
6	Diaphysis (Distal to Region 4)	50	25.0	5.87	2.94	146.75
7	Proximal Metaphysis	50	27.0	21.70	11.72	585.90
8	Distal Metaphysis	50	28.5	18.00	10.26	513.00
9	Articulating Surface	49	26.0	24.20	12.84	629.20
10	Distal Epiphysis	50	27.0	71.20	38.45	1922.40
11	Femoral Head	50	29.0	42.00	24.36	1218.00
12	Trochanter	51	27.0	47.10	24.94	1271.70
13	Calcaneus	51	29.0	19.80	11.26	574.20
14	Talus	50	27.5	17.30	9.52	475.75
RB	Reagent Blank	-	28.0	0.01	-	0.28

Table C16: mtDNA Quantification of C-08 Extracts Digested in Demineralization Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

C-08		Mitochondrial DNA (*ATPase*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	28.5	4.36	2.44	124.26
2	Midshaft Diaphysis (Distal to Region 1)	49	28.0	5.75	3.29	161.00
3	Diaphysis (Proximal to Region 1)	50	26.5	8.97	4.75	237.71
4	Diaphysis (Distal to Region 2)	51	28.5	4.87	2.72	138.80
5	Diaphysis (Proximal to Region 3)	49	28.5	7.88	4.58	224.58
6	Diaphysis (Distal to Region 4)	50	26.0	9.92	5.16	257.92
7	Proximal Metaphysis	51	31.5	22.60	13.96	711.90
8	Distal Metaphysis	50	28.0	19.10	10.70	534.80
9	Articulating Surface	49	29.0	17.80	10.53	516.20
10	Distal Epiphysis	50	27.5	64.20	35.31	1765.50
11	Femoral Head	51	28.0	54.70	30.03	1531.60
12	Trochanter	51	29.0	32.20	18.31	933.80
13	Calcaneus	51	26.0	26.70	13.61	694.20
14	Talus	50	27.0	22.20	11.99	599.40
RB	Reagent Blank	-	26.5	0.00	-	0.00

Table C17: Nuclear DNA Quantification of C-01 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-01		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	30.0	306.00	180.00	9180.00
2	Midshaft Diaphysis (Distal to Region 1)	50	25.0	386.00	193.00	9650.00
3	Diaphysis (Proximal to Region 1)	51	29.0	282.00	160.35	8178.00
4	Diaphysis (Distal to Region 2)	49	29.0	288.00	170.45	8352.00
5	Diaphysis (Proximal to Region 3)	50	30.0	274.00	164.40	8220.00
6	Diaphysis (Distal to Region 4)	51	30.0	157.00	92.35	4710.00
7	Proximal Metaphysis	49	26.0	660.00	350.20	17160.00
8	Distal Metaphysis	50	30.0	448.00	289.88	14784.00
9	Articulating Surface	49	30.0	410.00	251.02	12300.00
10	Distal Epiphysis	50	30.0	1200.00	720.00	36000.00
11	Femoral Head	51	29.0	813.00	462.29	23577.00
12	Trochanter	51	29.0	1460.00	830.20	42340.00
13	Calcaneus	50	29.0	482.00	279.56	13978.00
14	Talus	51	27.0	1240.00	656.47	33480.00
RB	Reagent Blank	-	26.0	0.29	-	7.54

Table C18: Nuclear DNA Quantification of C-01 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-01		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	28.0	392.00	224.00	10976.00
2	Midshaft Diaphysis (Distal to Region 1)	49	25.0	433.00	220.92	10825.00
3	Diaphysis (Proximal to Region 1)	50	30.0	350.00	210.00	10500.00
4	Diaphysis (Distal to Region 2)	51	30.0	394.00	231.76	11820.00
5	Diaphysis (Proximal to Region 3)	49	30.0	300.00	183.67	9000.00
6	Diaphysis (Distal to Region 4)	49	30.0	354.00	216.73	10620.00
7	Proximal Metaphysis	51	29.0	492.00	279.76	14268.00
8	Distal Metaphysis	51	29.0	482.00	274.08	13978.00
9	Articulating Surface	49	29.0	586.00	346.82	16994.00
10	Distal Epiphysis	51	30.0	920.00	541.18	27600.00
11	Femoral Head	50	30.0	1360.00	816.00	40800.00
12	Trochanter	51	30.0	672.00	395.29	20160.00
13	Calcaneus	49	30.0	357.00	218.57	10710.00
14	Talus	49	31.0	878.00	555.47	27218.00
RB	Reagent Blank	-	30.0	0.31	-	9.30

Table C19: Nuclear DNA Quantification of C-02 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-02		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	34.0	48.20	33.44	1638.80
2	Midshaft Diaphysis (Distal to Region 1)	51	30.0	250.00	147.06	7500.00
3	Diaphysis (Proximal to Region 1)	49	30.0	143.00	87.55	4290.00
4	Diaphysis (Distal to Region 2)	49	30.0	148.00	90.61	4440.00
5	Diaphysis (Proximal to Region 3)	51	29.0	272.00	154.67	7888.00
6	Diaphysis (Distal to Region 4)	49	28.0	182.00	104.00	5096.00
7	Proximal Metaphysis	50	32.0	1060.00	678.40	33920.00
8	Distal Metaphysis	50	32.0	461.00	295.04	14752.00
9	Articulating Surface	50	30.0	1420.00	852.00	42600.00
10	Distal Epiphysis	51	32.0	3050.00	1913.73	97600.00
11	Femoral Head	51	32.0	1860.00	1167.06	59520.00
12	Trochanter	51	31.0	1730.00	1051.57	53630.00
13	Calcaneus	49	30.0	1110.00	679.59	33300.00
14	Talus	50	31.0	1790.00	1109.80	55490.00
RB	Reagent Blank	-	30.0	0.00	-	0.00

Table C20: Nuclear DNA Quantification of C-02 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-02		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	30.0	496.00	297.60	14880.00
2	Midshaft Diaphysis (Distal to Region 1)	51	30.0	416.00	244.71	12480.00
3	Diaphysis (Proximal to Region 1)	51	29.0	521.00	296.25	15109.00
4	Diaphysis (Distal to Region 2)	50	29.0	341.00	197.78	9889.00
5	Diaphysis (Proximal to Region 3)	50	29.0	566.00	328.28	16414.00
6	Diaphysis (Distal to Region 4)	49	25.0	518.00	264.29	12950.00
7	Proximal Metaphysis	50	26.0	632.00	328.64	16432.00
8	Distal Metaphysis	50	29.0	620.00	359.60	17980.00
9	Articulating Surface	49	29.0	203.00	120.14	5887.00
10	Distal Epiphysis	50	32.0	1430.00	915.20	45760.00
11	Femoral Head	49	30.0	1730.00	1059.18	51900.00
12	Trochanter	50	30.0	1470.00	882.00	44100.00
13	Calcaneus	51	30.0	1280.00	752.94	38400.00
14	Talus	49	34.0	1510.00	1047.76	51340.00
RB	Reagent Blank	-	29.0	1.51	-	43.79

Table C21: Nuclear DNA Quantification of C-03 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-03		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.0	68.70	39.85	1992.30
2	Midshaft Diaphysis (Distal to Region 1)	49	26.0	173.00	91.80	4498.00
3	Diaphysis (Proximal to Region 1)	50	30.0	129.00	77.40	3870.00
4	Diaphysis (Distal to Region 2)	50	27.0	186.00	100.44	5022.00
5	Diaphysis (Proximal to Region 3)	50	29.0	165.00	95.70	4785.00
6	Diaphysis (Distal to Region 4)	49	30.0	186.00	113.88	5580.00
7	Proximal Metaphysis	50	32.0	366.00	234.24	11712.00
8	Distal Metaphysis	50	30.0	324.00	194.40	9720.00
9	Articulating Surface	51	31.0	348.00	211.53	10788.00
10	Distal Epiphysis	51	31.0	618.00	375.65	19158.00
11	Femoral Head	49	30.0	530.00	324.49	15900.00
12	Trochanter	50	25.0	758.00	379.00	18950.00
13	Calcaneus	50	30.0	358.00	214.80	10740.00
14	Talus	50	30.0	286.00	171.60	8580.00
RB	Reagent Blank	-	26.0	0.02	-	0.52

Table C22: Nuclear DNA Quantification of C-03 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-03		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	30.0	138.00	81.18	4140.00
2	Midshaft Diaphysis (Distal to Region 1)	51	30.0	152.00	89.41	4560.00
3	Diaphysis (Proximal to Region 1)	51	27.0	165.00	87.35	4455.00
4	Diaphysis (Distal to Region 2)	49	32.0	175.00	114.29	5600.00
5	Diaphysis (Proximal to Region 3)	50	26.0	181.00	94.12	4706.00
6	Diaphysis (Distal to Region 4)	50	29.0	200.00	116.00	5800.00
7	Proximal Metaphysis	50	28.0	274.00	153.44	7672.00
8	Distal Metaphysis	51	29.0	215.00	122.25	6235.00
9	Articulating Surface	51	26.0	310.00	158.04	8060.00
10	Distal Epiphysis	49	31.0	361.00	228.39	11191.00
11	Femoral Head	49	31.0	394.00	249.27	12214.00
12	Trochanter	49	26.0	455.00	241.43	11830.00
13	Calcaneus	50	32.0	213.00	136.32	6816.00
14	Talus	51	30.0	198.00	116.47	5940.00
RB	Reagent Blank	-	28.0	2.09	-	58.52

Table C23: Nuclear DNA Quantification of C-04 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-04		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	32.0	96.50	60.55	3088.00
2	Midshaft Diaphysis (Distal to Region 1)	50	27.0	94.40	50.98	2548.80
3	Diaphysis (Proximal to Region 1)	50	30.0	88.00	52.80	2640.00
4	Diaphysis (Distal to Region 2)	50	27.0	147.00	79.38	3969.00
5	Diaphysis (Proximal to Region 3)	51	29.0	126.00	71.65	3654.00
6	Diaphysis (Distal to Region 4)	50	25.0	211.00	105.50	5275.00
7	Proximal Metaphysis	50	27.0	288.00	155.52	7776.00
8	Distal Metaphysis	51	30.0	210.00	123.53	6300.00
9	Articulating Surface	51	29.0	349.00	198.45	10121.00
10	Distal Epiphysis	50	29.0	607.00	352.06	17603.00
11	Femoral Head	50	30.0	346.00	207.60	10380.00
12	Trochanter	50	30.0	367.00	220.20	11010.00
13	Calcaneus	49	30.0	255.00	156.12	7650.00
14	Talus	49	30.0	213.00	130.41	6390.00
RB	Reagent Blank	-	28.0	3.16	-	88.48

Table C24: Nuclear DNA Quantification of C-04 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-04		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	28.0	91.90	52.51	2573.20
2	Midshaft Diaphysis (Distal to Region 1)	50	30.0	159.00	95.40	4770.00
3	Diaphysis (Proximal to Region 1)	49	31.0	157.00	99.33	4876.00
4	Diaphysis (Distal to Region 2)	51	28.0	201.00	110.35	5628.00
5	Diaphysis (Proximal to Region 3)	51	28.0	178.00	97.73	4984.00
6	Diaphysis (Distal to Region 4)	51	29.0	216.00	122.82	6264.00
7	Proximal Metaphysis	50	29.0	221.00	128.18	6409.00
8	Distal Metaphysis	51	31.0	166.00	100.90	5146.00
9	Articulating Surface	51	27.0	312.00	165.18	8424.00
10	Distal Epiphysis	50	30.0	444.00	266.40	13320.00
11	Femoral Head	51	31.0	372.00	226.12	11532.00
12	Trochanter	51	28.0	207.00	113.65	5796.00
13	Calcaneus	50	31.0	157.00	97.34	4867.00
14	Talus	51	26.0	154.00	78.51	4004.00
RB	Reagent Blank	-	29.0	0.23	-	6.67

Table C25: Nuclear DNA Quantification of C-05 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-05		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	30.0	46.30	27.78	1389.00
2	Midshaft Diaphysis (Distal to Region 1)	50	25.5	99.00	50.49	2524.50
3	Diaphysis (Proximal to Region 1)	50	27.0	67.60	30.49	1441.80
4	Diaphysis (Distal to Region 2)	50	37.0	54.40	40.26	2012.80
5	Diaphysis (Proximal to Region 3)	51	23.0	67.60	30.49	1554.80
6	Diaphysis (Distal to Region 4)	50	37.0	115.00	73.60	3680.00
7	Proximal Metaphysis	50	30.0	156.00	93.60	4680.00
8	Distal Metaphysis	49	30.0	147.00	90.00	4410.00
9	Articulating Surface	50	32.0	335.00	214.40	10720.00
10	Distal Epiphysis	50	28.0	441.00	246.96	12348.00
11	Femoral Head	51	30.0	468.00	275.29	14040.00
12	Trochanter	51	26.0	604.00	307.92	15704.00
13	Calcaneus	51	30.5	427.00	255.36	13023.00
14	Talus	50	27.5	501.00	275.55	13777.50
RB	Reagent Blank	-	28.0	0.88	-	24.64

Table C26: Nuclear DNA Quantification of C-05 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-05		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.0	161.00	93.38	4669.00
2	Midshaft Diaphysis (Distal to Region 1)	51	28.0	261.00	143.29	7308.00
3	Diaphysis (Proximal to Region 1)	49	28.0	186.00	106.29	5208.00
4	Diaphysis (Distal to Region 2)	49	31.5	168.00	108.00	5292.00
5	Diaphysis (Proximal to Region 3)	50	32.0	205.00	131.20	6560.00
6	Diaphysis (Distal to Region 4)	50	30.0	178.00	106.80	5340.00
7	Proximal Metaphysis	51	29.0	280.00	159.22	8120.00
8	Distal Metaphysis	50	28.0	261.00	146.16	7308.00
9	Articulating Surface	51	28.0	333.00	182.82	9324.00
10	Distal Epiphysis	50	29.5	378.00	223.02	11151.00
11	Femoral Head	51	31.0	402.00	244.35	12462.00
12	Trochanter	50	27.0	446.00	240.35	12042.00
13	Calcaneus	51	28.0	421.00	231.14	11788.00
14	Talus	51	30.0	322.00	189.41	9660.00
RB	Reagent Blank	-	26.0	0.73	-	18.98

Table C27: Nuclear DNA Quantification of C-06 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-06		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	27.0	31.10	16.46	839.70
2	Midshaft Diaphysis (Distal to Region 1)	50	31.0	74.70	46.31	2315.70
3	Diaphysis (Proximal to Region 1)	49	28.0	13.80	7.89	386.40
4	Diaphysis (Distal to Region 2)	49	28.5	98.60	57.35	2810.10
5	Diaphysis (Proximal to Region 3)	50	30.0	84.00	50.40	2520.00
6	Diaphysis (Distal to Region 4)	49	30.0	190.00	116.33	5700.00
7	Proximal Metaphysis	50	27.5	151.00	83.05	4152.50
8	Distal Metaphysis	49	28.5	248.00	144.24	7068.00
9	Articulating Surface	50	30.0	433.00	259.80	12990.00
10	Distal Epiphysis	50	28.0	481.00	269.36	13468.00
11	Femoral Head	50	28.5	420.00	239.40	11970.00
12	Trochanter	50	29.0	492.00	285.36	14268.00
13	Calcaneus	50	31.0	444.00	275.28	13764.00
14	Talus	49	27.5	490.00	275.00	13475.00
RB	Reagent Blank	-	29.0	0.27	-	7.69

Table C28: Nuclear DNA Quantification of C-06 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-06		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	29.5	238.00	137.67	7021.00
2	Midshaft Diaphysis (Distal to Region 1)	50	29.5	189.00	111.51	5575.50
3	Diaphysis (Proximal to Region 1)	51	31.0	186.00	113.06	5766.00
4	Diaphysis (Distal to Region 2)	49	27.5	202.00	113.37	5555.00
5	Diaphysis (Proximal to Region 3)	50	30.5	165.00	100.65	5032.50
6	Diaphysis (Distal to Region 4)	50	28.0	300.00	168.00	8400.00
7	Proximal Metaphysis	50	21.5	244.00	104.92	5246.00
8	Distal Metaphysis	50	30.0	313.00	187.80	9390.00
9	Articulating Surface	50	28.0	301.00	168.56	8428.00
10	Distal Epiphysis	51	29.5	374.00	216.33	11033.00
11	Femoral Head	51	28.0	377.00	206.98	10556.00
12	Trochanter	50	29.0	354.00	205.32	10266.00
13	Calcaneus	50	29.0	363.00	210.54	10527.00
14	Talus	61	29.0	337.00	191.63	9773.00
RB	Reagent Blank	-	27.5	0.04	-	1.02

Table C29: Nuclear DNA Quantification of C-07 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-07		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	29.0	113.00	65.54	3277.00
2	Midshaft Diaphysis (Distal to Region 1)	50	30.0	156.00	93.60	4680.00
3	Diaphysis (Proximal to Region 1)	50	29.0	151.00	87.58	4379.00
4	Diaphysis (Distal to Region 2)	49	25.0	175.00	89.29	4375.00
5	Diaphysis (Proximal to Region 3)	51	26.5	170.00	88.33	4505.00
6	Diaphysis (Distal to Region 4)	51	30.0	142.00	83.53	4260.00
7	Proximal Metaphysis	51	29.0	393.00	223.47	11397.00
8	Distal Metaphysis	51	28.0	250.00	137.25	7000.00
9	Articulating Surface	51	28.0	299.00	164.16	8372.00
10	Distal Epiphysis	50	31.0	460.00	285.20	14260.00
11	Femoral Head	51	30.0	349.00	205.29	10470.00
12	Trochanter	50	28.0	673.00	376.88	18844.00
13	Calcaneus	50	26.5	440.00	233.20	11660.00
14	Talus	49	31.0	424.00	268.24	13144.00
RB	Reagent Blank	-	28.0	0.70	-	19.60

Table C30: Nuclear DNA Quantification of C-07 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-07		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	49	30.5	137.00	85.28	4178.50
2	Midshaft Diaphysis (Distal to Region 1)	49	32.0	141.00	92.08	4512.00
3	Diaphysis (Proximal to Region 1)	50	26.0	178.00	92.56	4628.00
4	Diaphysis (Distal to Region 2)	51	30.0	126.00	74.12	3780.00
5	Diaphysis (Proximal to Region 3)	49	28.5	162.00	94.22	4617.00
6	Diaphysis (Distal to Region 4)	51	28.5	132.00	73.76	3762.00
7	Proximal Metaphysis	50	30.5	313.00	190.93	9546.50
8	Distal Metaphysis	51	28.5	154.00	86.06	4389.00
9	Articulating Surface	50	29.0	267.00	154.86	7743.00
10	Distal Epiphysis	50	26.5	544.00	288.32	14416.00
11	Femoral Head	51	30.5	335.00	200.34	10217.50
12	Trochanter	51	28.5	464.00	259.29	13224.00
13	Calcaneus	49	29.0	295.00	174.59	8555.00
14	Talus	50	29.5	233.00	137.47	6873.50
RB	Reagent Blank	-	28.0	0.63	-	17.64

Table C31: Nuclear DNA Quantification of C-08 Extracts Digested in Tissue Lysis Buffer
Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-08		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	50	30.0	96.00	57.60	2880.00
2	Midshaft Diaphysis (Distal to Region 1)	50	28.5	148.00	84.36	4218.00
3	Diaphysis (Proximal to Region 1)	50	26.0	131.00	68.12	3406.00
4	Diaphysis (Distal to Region 2)	49	28.5	131.00	76.19	3733.50
5	Diaphysis (Proximal to Region 3)	51	29.0	156.00	88.71	4524.00
6	Diaphysis (Distal to Region 4)	50	25.0	208.00	104.00	5200.00
7	Proximal Metaphysis	50	27.0	345.00	186.30	9315.00
8	Distal Metaphysis	50	28.5	291.00	165.87	8293.50
9	Articulating Surface	49	26.0	288.00	152.82	7488.00
10	Distal Epiphysis	50	27.0	476.00	257.04	12852.00
11	Femoral Head	50	29.0	380.00	220.40	11020.00
12	Trochanter	51	27.0	484.00	256.24	13068.00
13	Calcaneus	51	29.0	335.00	190.49	9715.00
14	Talus	50	27.5	311.00	171.05	8552.50
RB	Reagent Blank	-	28.0	0.96	-	26.88

Table C32: Nuclear DNA Quantification of C-08 Extracts Digested in Demineralization Buffer

Region and location correspond with Table 3. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

C-08		Nuclear DNA (*MC1R*)				
Region	**Location**	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
1	Midshaft Diaphysis	51	28.5	113.00	63.15	3220.50
2	Midshaft Diaphysis (Distal to Region 1)	49	28.0	101.00	57.71	2828.00
3	Diaphysis (Proximal to Region 1)	50	26.5	146.00	77.38	3869.00
4	Diaphysis (Distal to Region 2)	51	28.5	108.00	60.35	3078.00
5	Diaphysis (Proximal to Region 3)	49	28.5	94.60	55.02	2696.10
6	Diaphysis (Distal to Region 4)	50	26.0	143.00	74.36	3718.00
7	Proximal Metaphysis	51	31.5	234.00	144.53	7371.00
8	Distal Metaphysis	50	28.0	204.00	114.24	5712.00
9	Articulating Surface	49	29.0	180.00	106.53	5220.00
10	Distal Epiphysis	50	27.5	446.00	245.30	12265.00
11	Femoral Head	51	28.0	317.00	174.04	8876.00
12	Trochanter	51	29.0	179.00	101.78	5191.00
13	Calcaneus	51	26.0	227.00	115.73	5902.00
14	Talus	50	27.0	199.00	107.46	5373.00
RB	Reagent Blank	-	26.5	0.00	-	0.00

Table C33: Bovine Mitochondrial DNA PCR Amplification Chart for Quality Assay (Tissue Lysis Buffer)

Region and location correspond to Table 3. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 201 bp amplicon, 2 = 390 bp amplicon, 3 = 607 bp amplicon, 4 = 994 bp amplicon.

NA = No Amplification

Region	Location	Mitochondrial DNA (*ATPase*)							
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08
1	Midshaft Diaphysis	4	4	3	3	4	4	4	4
2	Midshaft Diaphysis (Distal to Region 1)	4	4	3	3	3	3	3	4
3	Diaphysis (Proximal to Region 1)	4	4	2	4	4	4	4	4
4	Diaphysis (Distal to Region 2)	4	4	2	4	4	3	4	4
5	Diaphysis (Proximal to Region 3)	4	4	3	4	4	4	4	4
6	Diaphysis (Distal to Region 4)	4	4	2	4	3	4	4	4
7	Proximal Metaphysis	4	4	3	2	4	4	4	4
8	Distal Metaphysis	4	4	2	4	4	4	4	4
9	Articulating Surface	4	4	3	3	4	3	4	4
10	Distal Epiphysis	4	4	3	2	4	4	4	4
11	Femoral Head	4	4	4	4	4	4	4	4
12	Trochanter	4	4	4	3	4	4	4	4
13	Calcaneus	4	4	3	4	4	4	4	4
14	Talus	4	4	3	4	4	4	4	4
RB	Reagent Blank	4	4	NA	NA	NA	NA	NA	NA

Table C34: Bovine Mitochondrial DNA PCR Amplification Chart for Quality Assay (Demineralization Buffer)

Region and location correspond to Table 3. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 201 bp amplicon, 2 = 390 bp amplicon, 3 = 607 bp amplicon, 4 = 994 bp amplicon.

NA = No Amplification

Region	Location	Mitochondrial DNA (*ATPase*)							
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08
1	Midshaft Diaphysis	4	4	4	4	3	3	4	4
2	Midshaft Diaphysis (Distal to Region 1)	4	4	3	3	4	3	4	4
3	Diaphysis (Proximal to Region 1)	4	4	4	3	4	4	4	4
4	Diaphysis (Distal to Region 2)	4	4	3	4	4	4	4	4
5	Diaphysis (Proximal to Region 3)	4	4	4	3	4	4	4	4
6	Diaphysis (Distal to Region 4)	4	4	3	3	3	4	4	4
7	Proximal Metaphysis	4	4	4	4	4	4	4	4
8	Distal Metaphysis	4	4	4	4	4	4	4	4
9	Articulating Surface	4	4	4	3	4	4	4	3
10	Distal Epiphysis	4	4	3	4	4	4	4	4
11	Femoral Head	4	4	4	4	4	4	4	4
12	Trochanter	4	4	3	4	4	4	4	4
13	Calcaneus	4	4	3	4	4	4	4	4
14	Talus	4	4	4	4	4	4	4	4
RB	Reagent Blank	4	4	NA	NA	NA	NA	NA	NA

Table C35: Bovine Nuclear DNA PCR Amplification Chart for Quality Assay (Tissue Lysis Buffer)

Region and location correspond to Table 3. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 200 bp amplicon, 2 = 410 bp amplicon, 3 = 599 bp amplicon, 4 = 989 bp amplicon.

NA = No Amplification

Region	Location	Nuclear DNA (*MC1R*)							
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08
1	Midshaft Diaphysis	2	2	2	1	2	2	2	2
2	Midshaft Diaphysis (Distal to Region 1)	2	2	1	2	1	1	2	2
3	Diaphysis (Proximal to Region 1)	2	2	2	1	2	1	2	3
4	Diaphysis (Distal to Region 2)	2	2	1	2	1	1	2	2
5	Diaphysis (Proximal to Region 3)	2	2	2	1	1	1	2	2
6	Diaphysis (Distal to Region 4)	2	2	1	2	2	2	2	2
7	Proximal Metaphysis	2	2	1	1	2	2	2	2
8	Distal Metaphysis	2	2	1	1	2	2	2	2
9	Articulating Surface	2	2	2	2	2	2	2	2
10	Distal Epiphysis	2	2	1	2	2	2	2	2
11	Femoral Head	2	2	2	2	2	2	2	2
12	Trochanter	2	2	2	2	2	2	2	2
13	Calcaneus	2	2	1	1	2	2	2	2
14	Talus	2	1	2	2	2	2	1	2
RB	Reagent Blank	NA	2	NA	NA	2	NA	NA	NA

Table C36: Bovine Nuclear DNA PCR Amplification Chart for Quality Assay (Demineralization Buffer)

Region and location correspond to Table 3. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 200 bp amplicon, 2 = 410 bp amplicon, 3 = 599 bp amplicon, 4 = 989 bp amplicon.

NA = No Amplification

Region	Location	Nuclear DNA (*MC1R*)							
		C-01	C-02	C-03	C-04	C-05	C-06	C-07	C-08
1	Midshaft Diaphysis	2	2	2	2	2	2	2	2
2	Midshaft Diaphysis (Distal to Region 1)	2	2	2	2	2	2	2	2
3	Diaphysis (Proximal to Region 1)	3	2	1	2	2	1	2	2
4	Diaphysis (Distal to Region 2)	2	2	2	2	2	2	2	2
5	Diaphysis (Proximal to Region 3)	2	2	2	2	2	2	2	2
6	Diaphysis (Distal to Region 4)	2	2	1	2	2	2	2	2
7	Proximal Metaphysis	2	2	2	2	2	2	2	2
8	Distal Metaphysis	2	2	2	2	2	2	2	2
9	Articulating Surface	2	2	2	1	2	2	2	2
10	Distal Epiphysis	2	2	2	2	2	2	2	2
11	Femoral Head	2	2	2	2	2	2	2	2
12	Trochanter	2	2	2	2	2	2	2	2
13	Calcaneus	2	2	2	1	2	2	1	2
14	Talus	2	1	2	2	2	2	2	2
RB	Reagent Blank	NA	NA	NA	NA	NA	NA	NA	NA

APPENDIX D: MITOCHONDRIAL AND NUCLEAR DNA QUANTIFICATION DATA AND INDIVIDUAL PCR AMPLIFICATION RESULTS FROM SURFACE EXPOSED AND BURIED BOVINE FEMORA AND TARSALS

Table D1: mtDNA Quantification of Day 0 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Day 0		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	29.5	37.00	21.40	631.36
	2	49	30.0	56.30	34.47	1034.08
Proximal Metaphysis	1	51	31.0	27.80	16.90	523.84
	2	50	30.0	20.40	12.24	367.20
Distal Metaphysis	1	51	31.5	12.20	7.54	237.36
	2	51	32.0	32.40	20.33	650.54
Femoral Head	1	51	31.0	101.00	61.39	1903.00
	2	51	32.0	118.00	74.04	2369.25
Distal Epiphysis	1	50	31.5	110.00	69.30	2182.95
	2	50	33.0	128.00	84.48	2787.84
Calcaneus	1	51	27.5	76.40	41.20	1132.89
	2	51	30.0	50.90	29.94	898.24
Talus	1	51	34.0	66.40	44.27	1505.07
	2	50	29.0	68.50	39.73	1152.17
Reagent Blank		-	28.0	0.003	-	0.084

Table D2: mtDNA Quantification of Week 1 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Week 1		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	49	27.0	4.66	2.57	69.33
	2	51	27.0	1.55	0.82	22.16
Proximal Metaphysis	1	51	28.0	10.20	5.60	156.80
	2	50	27.0	11.90	6.43	173.50
Distal Metaphysis	1	51	26.0	5.37	2.74	71.18
	2	49	25.5	27.60	14.36	366.26
Femoral Head	1	51	30.0	35.20	20.71	621.18
	2	50	29.0	111.00	64.38	1867.02
Distal Epiphysis	1	51	30.5	54.00	32.29	984.97
	2	51	30.0	80.60	47.41	1422.35
Calcaneus	1	49	27.0	12.30	6.78	182.99
	2	50	31.0	54.90	34.04	1055.18
Talus	1	50	30.0	34.90	20.94	628.20
	2	51	30.0	46.50	27.35	820.59
Reagent Blank		-	28.0	0.004	-	0.112

Table D3: mtDNA Quantification of Week 2 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Week 2		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	29.0	13.00	7.54	218.66
	2	50	26.5	21.90	11.61	307.59
Proximal Metaphysis	1	51	29.0	19.00	10.80	313.31
	2	49	30.5	22.30	13.88	423.26
Distal Metaphysis	1	51	28.5	10.50	5.87	167.23
	2	50	31.0	23.20	14.38	445.90
Femoral Head	1	51	30.5	51.00	30.50	930.25
	2	50	32.0	72.90	46.66	1492.99
Distal Epiphysis	1	50	30.0	42.50	25.50	765.00
	2	50	32.5	48.10	31.27	1016.11
Calcaneus	1	50	32.0	33.50	21.44	686.08
	2	49	32.5	31.80	21.09	685.48
Talus	1	49	33.5	59.00	40.34	1351.28
	2	51	30.5	61.70	36.90	1125.42
Reagent Blank		-	27.0	0.00	-	0.00

Table D4: mtDNA Quantification of Week 4 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "**" next to value represents quantification obtained using a 1:10 dilution of extract.

Week 4		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	27.5	18.00	9.71	266.91
	2	50	30.5	22.50	13.73	418.61
Proximal Metaphysis	1	49	32.0	14.10	9.21	294.66
	2	50	31.0	32.20	19.96	618.88
Distal Metaphysis	1	50	30.0	15.40	9.24	277.20
	2	49	29.5	36.70	22.09	651.80
Femoral Head	1	51	31.5	65.80	40.64	1280.20
	2	50	31.5	5.85**	3.69	116.09
Distal Epiphysis	1	51	28.0	39.50	21.69	607.22
	2	51	27.5	4.72**	2.55	69.99
Calcaneus	1	51	29.5	32.30	18.68	551.16
	2	50	31.0	31.20	19.34	599.66
Talus	1	49	28.0	27.30	15.60	436.80
	2	51	27.5	11.90	6.42	176.46
Reagent Blank		-	29.5	0.00	-	0.00

Table D5: mtDNA Quantification of Month 3 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Month 3		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	29.5	9.26	5.36	158.01
	2	51	26.5	8.59	4.46	118.28
Proximal Metaphysis	1	51	29.5	4.60	2.66	78.49
	2	50	29.0	14.70	8.53	247.25
Distal Metaphysis	1	50	26.5	7.80	4.13	109.55
	2	50	31.0	9.17	5.69	176.25
Femoral Head	1	51	27.5	48.50	26.15	719.18
	2	50	29.0	68.00	39.44	1143.76
Distal Epiphysis	1	49	29.0	17.10	10.12	293.49
	2	50	25.5	44.00	22.44	572.22
Calcaneus	1	51	28.0	28.30	15.54	435.04
	2	50	29.0	20.10	11.66	338.08
Talus	1	50	31.0	17.90	11.10	344.04
	2	50	30.0	14.10	8.46	253.80
Reagent Blank		-	29.0	0.00	-	0.00

Table D6: mtDNA Quantification of Month 6 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "**" next to value represents quantification obtained using a 1:10 dilution of extract.

Month 6		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	25.0	7.18	3.59	89.75
	2	49	26.5	4.57	2.47	65.50
Proximal Metaphysis	1	49	27.0	6.10	3.36	90.75
	2	50	28.0	8.21	4.60	128.73
Distal Metaphysis	1	50	31.0	6.98	4.33	134.16
	2	50	29.0	8.32	4.83	139.94
Femoral Head	1	51	31.5	41.30	25.51	803.53
	2	51	28.5	14.10	7.88	224.56
Distal Epiphysis	1	51	26.5	34.50	17.93	475.05
	2	51	30.0	24.10	14.18	425.29
Calcaneus	1	49	30.5	11.10	6.91	210.73
	2	50	27.5	27.70	15.24	418.96
Talus	1	49	30.0	32.10	19.65	589.59
	2	49	29.0	0.37**	0.22	6.26
Reagent Blank		-	29.0	0.00	-	0.00

Table D7: mtDNA Normalized Quantification of Surface Exposed Bovine Skeletal Material

Region bone powder was collected from in first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "A1 and A2" were replicate femora, calcanei, and tali exposed during the course of this experiment. Cells highlighted in yellow represent extracts that had PCR inhibition. Values with "**" were diluted by a factor of 10 to alleviate inhibition, listed value represents mtDNA quantification multiplied by dilution factor.

Surface	Mitochondrial DNA (ng/mg) (*ATPase*)											
Region	Day 0		Week 1		Week 2		Week 4		Month 3		Month 6	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Diaphysis	21.40	34.47	2.57	0.82	7.54	11.61	9.71	13.73	5.36	4.46	3.59	2.47
Proximal Metaphysis	16.90	12.24	5.60	6.43	10.80	13.88	9.21	19.96	2.66	8.53	3.36	4.60
Distal Metaphysis	7.54	20.33	2.74	14.36	5.87	14.38	9.24	22.09	4.13	5.69	4.33	4.83
Femoral Head	61.39	74.04	20.71	64.38	30.50	46.66	40.64	36.90**	26.15	39.44	25.51	7.88
Distal Epiphysis	69.30	84.48	32.29	47.41	25.50	31.27	21.69	47.20**	10.12	22.44	17.93	14.18
Calcaneus	41.20	29.94	6.78	34.04	21.44	21.09	18.68	19.34	15.54	11.66	6.91	15.24
Talus	44.27	39.73	20.94	27.35	40.34	36.90	15.60	6.42	11.10	8.46	19.65	2.20**

Table D8: Bovine Mitochondrial DNA PCR Amplification Chart for Quality Assay (Surface Exposed Skeletal Material)

Region listed in first column. "A1 and A2" refer to replicate number. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 201 bp amplicon, 2 = 390 bp amplicon, 3 = 607 bp amplicon, 4 = 994 bp amplicon.

NA = No Amplification

Surface	**Mitochondrial DNA (*ATPase*) Amplicon Length**											
Region	**Day 0**		**Week 1**		**Week 2**		**Week 4**		**Month 3**		**Month 6**	
	A1	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**
Diaphysis	4	4	4	4	4	4	4	4	4	4	4	4
Proximal Metaphysis	4	4	4	4	4	4	4	4	4	4	4	4
Distal Metaphysis	4	4	4	4	4	4	4	4	4	4	4	4
Femoral Head	4	4	4	4	4	4	4	4	4	4	4	4
Distal Epiphysis	4	4	4	4	4	4	4	4	4	4	4	4
Calcaneus	4	4	4	4	4	4	4	4	4	4	4	4
Talus	4	4	4	4	4	4	4	4	4	4	4	4
Reagent Blank	NA		4		NA		NA		NA		NA	

Table D9: Nuclear DNA Normalized Quantification of Surface Exposed Bovine Skeletal Material

Region bone powder was collected from in first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "A1 and A2" were replicate femora, calcanei, and tali exposed during the course of this experiment. Cells highlighted in yellow represent extracts that had PCR inhibition. Values with "*" or "**" were diluted by a factor of 5 or 10 respectively to alleviate inhibition, listed value represents DNA quantification multiplied by dilution factor.

Surface	Nuclear DNA (ng/mg) (*MC1R*)											
Region	Day 0		Week 1		Week 2		Week 4		Month 3		Month 6	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Diaphysis	192.04	208.16	63.37	49.02	142.68	170.02	152.60	178.12	202.45	129.90	139.50	122.22
Proximal Metaphysis	147.71	193.20	76.31	170.10	170.02	262.05	148.24	293.88	175.26	335.82	138.86	171.36
Distal Metaphysis	218.65	158.12	48.38	401.76	126.85	236.22	184.80	345.57	279.31	319.30	258.54	175.74
Femoral Head	160.47	375.84	278.24	348.00	413.25	812.80	273.62	639.45*	352.65	446.02	282.88	488.41
Distal Epiphysis	158.76	413.82	257.75	243.53	211.20	338.65	322.27	413.60**	257.45	484.50	222.39	116.47
Calcaneus	216.23	206.47	150.98	294.50	248.32	519.34	211.13	511.27**	282.20	489.52	126.98	278.85
Talus	364.67	287.10	259.80	200.00	296.71	476.04	181.71	509.60**	358.36	200.40	287.76	174.60**

Table D10: Nuclear DNA Quantification of Day 0 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Day 0		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	29.5	332.00	192.04	5665.16
	2	49	30.0	340.00	208.16	6244.90
Proximal Metaphysis	1	51	31.0	243.00	147.71	4578.88
	2	50	30.0	322.00	193.20	5796.00
Distal Metaphysis	1	51	31.5	354.00	218.65	6887.38
	2	51	32.0	252.00	158.12	5059.76
Femoral Head	1	51	31.0	264.00	160.47	4974.59
	2	51	32.0	599.00	375.84	12026.98
Distal Epiphysis	1	50	31.5	252.00	158.76	5000.94
	2	50	33.0	627.00	413.82	13656.06
Calcaneus	1	51	27.5	401.00	216.23	5946.20
	2	51	30.0	351.00	206.47	6194.12
Talus	1	51	34.0	547.00	364.67	12398.67
	2	50	29.0	495.00	287.10	8325.90
Reagent Blank		-	28.0	0.02	-	0.56

Table D11: Nuclear DNA Quantification of Week 1 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Week 1		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	49	27.0	115.00	63.37	1710.92
	2	51	27.0	92.60	49.02	1323.64
Proximal Metaphysis	1	51	28.0	139.00	76.31	2136.78
	2	50	27.0	315.00	170.10	4592.70
Distal Metaphysis	1	51	26.0	94.90	48.38	1257.89
	2	49	25.5	772.00	401.76	10244.76
Femoral Head	1	51	30.0	473.00	278.24	8347.06
	2	50	29.0	600.00	348.00	10092.00
Distal Epiphysis	1	51	30.5	431.00	257.75	7861.52
	2	51	30.0	414.00	243.53	7305.88
Calcaneus	1	49	27.0	274.00	150.98	4076.45
	2	50	31.0	475.00	294.50	9129.50
Talus	1	50	30.0	433.00	259.80	7794.00
	2	51	30.0	340.00	200.00	6000.00
Reagent Blank		-	28.0	0.08	-	2.24

Table D12: Nuclear DNA Quantification of Week 2 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Week 2		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	29.0	246.00	142.68	4137.72
	2	50	26.5	343.00	181.79	4817.44
Proximal Metaphysis	1	51	29.0	299.00	170.02	4930.57
	2	49	30.5	421.00	262.05	7992.56
Distal Metaphysis	1	51	28.5	227.00	126.85	3615.31
	2	50	31.0	381.00	236.22	7322.82
Femoral Head	1	51	30.5	691.00	413.25	12603.98
	2	50	32.0	1270.00	812.80	26009.60
Distal Epiphysis	1	50	30.0	352.00	211.20	6336.00
	2	50	32.5	521.00	338.65	11006.13
Calcaneus	1	50	32.0	388.00	248.32	7946.24
	2	49	32.5	783.00	519.34	16878.44
Talus	1	49	33.5	434.00	296.71	9939.93
	2	51	30.5	796.00	476.04	14519.20
Reagent Blank		-	27.0	1.13	-	30.51

Table D13: Nuclear DNA Quantification of Week 4 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "* and **" next to value represents quantification obtained using a 1:5 or 1:10 dilution of extract respectively.

Week 4		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	27.5	283.00	152.60	4196.45
	2	50	30.5	292.00	178.12	5432.66
Proximal Metaphysis	1	49	32.0	227.00	148.24	4743.84
	2	50	31.0	474.00	293.88	9110.28
Distal Metaphysis	1	50	30.0	308.00	184.80	5544.00
	2	49	29.5	574.00	345.57	10194.36
Femoral Head	1	51	31.5	443.00	273.62	8618.96
	2	50	31.5	203.00*	127.89	4028.54
Distal Epiphysis	1	51	28.0	587.00	322.27	9023.69
	2	51	27.5	76.70**	41.36	1137.34
Calcaneus	1	51	29.5	365.00	211.13	6228.26
	2	50	31.0	82.70**	51.27	1589.49
Talus	1	49	28.0	318.00	181.71	5088.00
	2	51	27.5	94.50**	50.96	1401.29
Reagent Blank		-	29.5	1.43	-	42.19

Table D14: Nuclear DNA Quantification of Month 3 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Month 3		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	29.5	350.00	202.45	5972.30
	2	51	26.5	250.00	129.90	3442.40
Proximal Metaphysis	1	51	29.5	303.00	175.26	5170.31
	2	50	29.0	579.00	335.82	9738.78
Distal Metaphysis	1	50	26.5	527.00	279.31	7401.72
	2	50	31.0	515.00	319.30	9898.30
Femoral Head	1	51	27.5	654.00	352.65	9697.79
	2	50	29.0	769.00	446.02	12934.58
Distal Epiphysis	1	49	29.0	435.00	257.45	7466.02
	2	50	25.5	950.00	484.50	12354.75
Calcaneus	1	51	28.0	514.00	282.20	7901.49
	2	50	29.0	844.00	489.52	14196.08
Talus	1	50	31.0	578.00	358.36	11109.16
	2	50	30.0	334.00	200.40	6012.00
Reagent Blank		-	29.0	0.00	-	0.00

Table D15: Nuclear DNA Quantification of Month 6 Extracts from Surface Exposed Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "**" next to value represents quantification obtained using a 1:10 dilution of extract.

Month 6		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	25.0	279.00	139.50	3487.50
	2	49	26.5	226.00	122.22	3238.95
Proximal Metaphysis	1	49	27.0	252.00	138.86	3749.14
	2	50	28.0	306.00	171.36	4798.08
Distal Metaphysis	1	50	31.0	417.00	258.54	8014.74
	2	50	29.0	303.00	175.74	5096.46
Femoral Head	1	51	31.5	458.00	282.88	8910.79
	2	51	28.5	874.00	488.41	13919.74
Distal Epiphysis	1	51	26.5	428.00	222.39	5893.39
	2	51	30.0	198.00	116.47	3494.12
Calcaneus	1	49	30.5	204.00	126.98	3872.88
	2	50	27.5	507.00	278.85	7668.38
Talus	1	49	30.0	470.00	287.76	8632.65
	2	49	29.0	29.50**	17.46	506.32
Reagent Blank		-	29.0	0.00	-	0.00

Table D16: Bovine Nuclear DNA PCR Amplification Chart for Quality Assay (Surface Exposed Skeletal Material)

Region listed in first column. "A1 and A2" refer to replicate number. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 200 bp amplicon, 2 = 410 bp amplicon, 3 = 599 bp amplicon, 4 = 989 bp amplicon, NA = No Amplification.

Surface	**Nuclear DNA (*MC1R*) Amplicon Length**											
Region	**Day 0**		**Week 1**		**Week 2**		**Week 4**		**Month 3**		**Month 6**	
	A1	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**	**A1**	**A2**
Diaphysis	2	2	2	2	2	2	2	2	2	2	2	2
Proximal Metaphysis	2	2	2	2	2	2	2	2	2	2	2	2
Distal Metaphysis	2	2	2	2	2	2	2	2	2	2	2	2
Femoral Head	2	2	2	2	2	2	2	2	2	2	2	2
Distal Epiphysis	2	2	2	2	2	2	2	2	2	2	2	2
Calcaneus	2	2	2	2	2	2	2	2	2	2	2	2
Talus	2	2	2	2	2	2	2	2	2	2	2	2
Reagent Blank	NA		NA		NA		NA		NA		NA	

Table D17: mtDNA Quantification of Day 0 Extracts from Buried Bovine Skeletal Material
Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Day 0		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	49	32.0	12.30	8.03	257.04
	2	50	30.0	55.40	33.24	997.20
Proximal Metaphysis	1	50	31.0	21.30	13.21	409.39
	2	50	31.0	40.00	24.80	768.80
Distal Metaphysis	1	51	32.0	46.80	29.36	939.67
	2	50	30.5	74.90	45.69	1393.51
Femoral Head	1	50	27.0	29.90	16.15	435.94
	2	50	33.0	112.00	73.92	2439.36
Distal Epiphysis	1	51	31.0	41.10	24.98	774.45
	2	50	32.0	121.00	77.44	2478.08
Calcaneus	1	49	31.0	41.70	26.38	817.83
	2	50	31.0	42.10	26.10	809.16
Talus	1	50	34.0	56.60	38.49	1308.59
	2	49	33.0	65.00	43.78	1444.59
Reagent Blank		-	27.0	0.004	-	0.108

Table D18: mtDNA Quantification of Week 1 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Week 1		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	29.5	1.30	0.77	22.63
	2	51	29.5	1.03	0.60	17.58
Proximal Metaphysis	1	51	32.0	1.71	1.07	34.33
	2	51	30.0	1.09	0.64	19.24
Distal Metaphysis	1	51	27.0	0.80	0.43	11.49
	2	50	31.5	1.27	0.80	25.20
Femoral Head	1	51	29.0	6.29	3.58	103.72
	2	51	31.0	5.20	3.16	97.98
Distal Epiphysis	1	51	30.5	3.37	2.02	61.47
	2	51	26.0	2.12	1.08	28.10
Calcaneus	1	49	28.0	4.81	2.75	76.96
	2	51	33.5	3.20	2.10	70.42
Talus	1	50	29.5	2.99	1.76	52.04
	2	51	31.0	2.79	1.70	52.57
Reagent Blank		-	24.0	0.00	-	0.00

Table D19: mtDNA Quantification of Week 2 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "**" next to value represents quantification obtained using a 1:10 dilution of extract.

Week 2		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	28.0	0.72	0.40	11.31
	2	50	30.5	0.88	0.54	16.39
Proximal Metaphysis	1	51	30.5	0.63	0.38	11.47
	2	51	31.0	1.33	0.81	25.06
Distal Metaphysis	1	49	27.5	0.27	0.15	4.18
	2	50	30.0	1.32	0.79	23.76
Femoral Head	1	51	34.0	1.90	1.27	43.07
	2	49	30.5	7.50	4.67	142.39
Distal Epiphysis	1	51	30.0	1.60	0.94	28.24
	2	50	30.0	0.12**	0.07	2.11
Calcaneus	1	49	28.5	2.19	1.27	36.30
	2	51	30.0	0.11**	0.07	1.96
Talus	1	51	29.0	3.24	1.84	53.43
	2	50	30.0	2.82	1.69	50.76
Reagent Blank		-	30.0	0.001	-	0.03

Table D20: mtDNA Quantification of Week 4 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "* and **" next to value represents quantification obtained using a 1:5 or 1:10 dilution of extract respectively.

Week 4		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	29.5	0.69	0.40	11.77
	2	49	32.0	0.62	0.40	15.12
Proximal Metaphysis	1	49	28.0	0.95	0.54	15.15
	2	51	32.0	0.75	0.47	15.12
Distal Metaphysis	1	49	30.0	0.002**	0.001	0.036
	2	51	31.5	2.79	1.72	54.28
Femoral Head	1	50	30.5	2.66	1.62	49.49
	2	50	27.5	3.77	2.07	57.02
Distal Epiphysis	1	50	27.0	0.07*	0.04	0.96
	2	50	29.0	2.31	1.34	38.85
Calcaneus	1	49	32.0	0.19*	0.12	3.93
	2	51	30.5	1.38	0.83	25.17
Talus	1	49	32.5	0.20*	0.13	4.27
	2	51	30.5	2.04	1.22	37.21
Reagent Blank		-	27.5	0.00	-	0.00

Table D21: mtDNA Quantification of Month 3 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Month 3		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	30.0	1.11	0.65	19.59
	2	49	31.0	0.81	0.51	15.85
Proximal Metaphysis	1	51	28.0	0.50	0.28	7.70
	2	50	31.0	0.64	0.40	12.26
Distal Metaphysis	1	51	31.0	0.19	0.11	3.52
	2	50	29.0	0.35	0.20	5.94
Femoral Head	1	51	31.0	1.15	0.70	21.67
	2	51	29.0	0.92	0.52	15.11
Distal Epiphysis	1	50	31.0	1.06	0.66	20.37
	2	51	30.0	0.58	0.34	10.20
Calcaneus	1	49	26.0	0.92	0.49	12.69
	2	51	28.5	0.77	0.43	12.33
Talus	1	50	29.5	2.46	1.45	42.82
	2	51	29.5	1.76	1.02	30.03
Reagent Blank		-	30.0	0.00	-	0.00

Table D22: mtDNA Quantification of Month 6 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Month 6		**Mitochondrial DNA (*ATPase*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	32.0	0.95	0.61	19.42
	2	50	27.0	0.58	0.31	8.49
Proximal Metaphysis	1	49	30.0	0.52	0.32	9.51
	2	51	28.5	2.49	1.39	39.66
Distal Metaphysis	1	50	27.0	0.45	0.24	6.59
	2	49	27.0	0.21	0.12	3.14
Femoral Head	1	51	31.0	1.29	0.78	24.31
	2	50	28.0	1.92	1.08	30.11
Distal Epiphysis	1	51	30.0	1.06	0.62	18.71
	2	49	30.0	1.06	0.65	19.47
Calcaneus	1	50	27.5	1.52	0.84	22.99
	2	49	29.0	0.94	0.56	16.10
Talus	1	50	27.5	3.80	2.09	57.48
	2	51	29.5	1.64	0.95	27.98
Reagent Blank		-	29.0	0.00	-	0.00

Table D23: mtDNA Normalized Quantification of Buried Bovine Skeletal Material

Region bone powder was collected from in first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "B1 and B2" were replicate femora, calcanei, and tali buried during the course of this experiment. Cells highlighted in yellow represent extracts that had PCR inhibition. Values with "*" or "**" were diluted by a factor of 5 or 10 respectively to alleviate inhibition, listed value represents DNA quantification multiplied by dilution factor.

Burial	**Mitochondrial DNA (ng/mg) (*ATPase*)**											
Region	**Day 0**		**Week 1**		**Week 2**		**Week 4**		**Month 3**		**Month 6**	
	B1	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**
Diaphysis	8.03	33.24	0.77	0.60	0.40	0.54	0.40	0.40	0.65	0.51	0.61	0.31
Proximal Metaphysis	13.21	24.80	1.07	0.64	0.38	0.81	0.54	0.47	0.28	0.40	0.32	1.39
Distal Metaphysis	29.36	45.69	0.43	0.80	0.15	0.79	0.01**	1.72	0.11	0.20	0.24	0.12
Femoral Head	16.15	73.92	3.58	3.16	1.27	4.67	1.62	2.07	0.70	0.52	1.08	0.78
Distal Epiphysis	24.98	77.44	2.02	1.08	0.94	0.70**	0.20*	1.34	0.66	0.34	0.62	0.65
Calcaneus	26.38	26.10	2.75	2.10	1.27	0.70**	0.60*	0.83	0.49	0.43	0.84	0.56
Talus	38.49	43.78	1.76	1.70	1.84	1.69	0.65*	1.22	1.45	1.02	2.09	0.95

Table D24: Bovine Mitochondrial DNA PCR Amplification Chart for Quality Assay (Buried Skeletal Material)

Region listed in first column. "B1 and B2" refer to replicate number. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 201 bp amplicon, 2 = 390 bp amplicon, 3 = 607 bp amplicon, 4 = 994 bp amplicon, NA = No Amplification.

Surface	**Mitochondrial DNA (*ATPase*) Amplicon Length**											
Region	**Day 0**		**Week 1**		**Week 2**		**Week 4**		**Month 3**		**Month 6**	
	B1	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**
Diaphysis	4	4	4	4	4	4	4	4	4	4	4	4
Proximal Metaphysis	4	4	4	4	4	4	4	4	4	4	4	4
Distal Metaphysis	4	4	4	4	4	4	4	4	4	4	4	4
Femoral Head	4	4	4	4	4	4	4	4	4	4	4	4
Distal Epiphysis	4	4	4	4	4	4	4	4	4	4	4	4
Calcaneus	4	4	4	4	4	4	4	4	4	4	4	4
Talus	4	4	4	4	4	4	4	4	4	4	4	4
Reagent Blank	4		NA		NA		NA		NA		NA	

Table D25: Nuclear DNA Quantification of Day 0 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Day 0		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	49	32.0	240.00	156.73	5015.51
	2	50	30.0	435.00	261.00	7830.00
Proximal Metaphysis	1	50	31.0	312.00	193.44	5996.64
	2	50	31.0	454.00	281.48	8725.88
Distal Metaphysis	1	51	32.0	494.00	309.96	9918.75
	2	50	30.5	486.00	296.46	9042.03
Femoral Head	1	50	27.0	288.00	155.52	4199.04
	2	50	33.0	658.00	434.28	14331.24
Distal Epiphysis	1	51	31.0	403.00	244.96	7593.78
	2	50	32.0	635.00	406.40	13004.80
Calcaneus	1	49	31.0	359.00	227.12	7040.80
	2	50	31.0	426.00	264.12	8187.72
Talus	1	50	34.0	280.00	190.40	6473.60
	2	49	33.0	456.00	307.10	10134.37
Reagent Blank		-	27.0	0.15	-	4.05

Table D26: Nuclear DNA Quantification of Week 1 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Week 1		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	29.5	9.00	5.31	156.65
	2	51	29.5	4.22	2.44	72.01
Proximal Metaphysis	1	51	32.0	2.55	1.60	51.20
	2	51	30.0	0.26	0.15	4.50
Distal Metaphysis	1	51	27.0	1.67	0.88	23.87
	2	50	31.5	0.11	0.07	2.26
Femoral Head	1	51	29.0	46.60	26.50	768.44
	2	51	31.0	72.20	43.89	1360.47
Distal Epiphysis	1	51	30.5	18.30	10.94	333.80
	2	51	26.0	7.07	3.60	93.71
Calcaneus	1	49	28.0	70.90	40.51	1134.40
	2	51	33.5	43.00	28.25	946.21
Talus	1	50	29.5	27.90	16.46	485.60
	2	51	31.0	57.60	35.01	1085.36
Reagent Blank		-	24.0	0.01	-	0.24

Table D27: Nuclear DNA Quantification of Week 2 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "**" next to value represents quantification obtained using a 1:10 dilution of extract.

Week 2		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	28.0	1.37	0.77	21.48
	2	50	30.5	3.60	2.20	66.98
Proximal Metaphysis	1	51	30.5	0.90	0.54	16.34
	2	51	31.0	1.49	0.91	28.08
Distal Metaphysis	1	49	27.5	1.30	0.73	20.06
	2	50	30.0	0.43	0.26	7.70
Femoral Head	1	51	34.0	12.30	8.20	278.80
	2	49	30.5	43.90	27.33	833.43
Distal Epiphysis	1	51	30.0	13.30	7.82	234.71
	2	50	30.0	3.61**	2.17	64.98
Calcaneus	1	49	28.5	14.90	8.67	246.99
	2	51	30.0	0.85**	0.50	15.05
Talus	1	51	29.0	21.60	12.28	356.19
	2	50	30.0	19.30	11.58	347.40
Reagent Blank		-	30.0	0.09	-	2.70

Table D28: Nuclear DNA Quantification of Week 4 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Week 4		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	29.5	4.09	2.37	69.79
	2	49	32.0	2.67	1.74	55.80
Proximal Metaphysis	1	49	28.0	1.55	0.89	24.80
	2	51	32.0	1.15	0.72	23.09
Distal Metaphysis	1	49	30.0	2.01	1.23	36.92
	2	51	31.5	0.31	0.19	5.99
Femoral Head	1	50	30.5	7.63	4.65	141.96
	2	50	27.5	11.30	6.22	170.91
Distal Epiphysis	1	50	27.0	0.00**	0.00	0.00
	2	50	29.0	14.60	8.47	245.57
Calcaneus	1	49	32.0	0.00**	0.00	0.00
	2	51	30.5	2.34	1.40	42.68
Talus	1	49	32.5	0.16**	0.10	3.36
	2	51	30.5	5.62	3.36	102.51
Reagent Blank		-	27.5	0.04	-	1.10

Table D29: Nuclear DNA Quantification of Month 3 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number.

Month 3		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	51	30.0	2.40	1.41	42.35
	2	49	31.0	1.90	1.20	37.26
Proximal Metaphysis	1	51	28.0	1.21	0.66	18.60
	2	50	31.0	0.31	0.19	5.94
Distal Metaphysis	1	51	31.0	0.62	0.38	11.68
	2	50	29.0	0.08	0.05	1.34
Femoral Head	1	51	31.0	9.75	5.93	183.72
	2	51	29.0	18.40	10.46	303.42
Distal Epiphysis	1	50	31.0	5.71	3.54	109.75
	2	51	30.0	20.30	11.94	358.24
Calcaneus	1	49	26.0	5.45	2.89	75.19
	2	51	28.5	4.50	2.51	71.67
Talus	1	50	29.5	17.40	10.27	302.85
	2	51	29.5	10.30	5.96	175.76
Reagent Blank		-	30.0	0.00	-	0.00

Table D30: Nuclear DNA Quantification of Month 6 Extracts from Buried Bovine Skeletal Material

Region listed in the first column. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder. "1" and "2" next to region denotes replicate number. "*" next to value represents quantification obtained using a 1:5 dilution of extract.

Month 6		**Nuclear DNA (*MC1R*)**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	1	50	32.0	2.00	1.28	40.96
	2	50	27.0	3.95	2.13	57.59
Proximal Metaphysis	1	49	30.0	0.43	0.26	7.81
	2	51	28.5	2.87	1.60	45.71
Distal Metaphysis	1	50	27.0	1.56	0.84	22.74
	2	49	27.0	0.89	0.49	13.26
Femoral Head	1	51	31.0	19.60	11.91	369.33
	2	50	28.0	7.51*	4.21	117.91
Distal Epiphysis	1	51	30.0	6.84	4.02	120.71
	2	49	30.0	18.20	11.14	334.29
Calcaneus	1	50	27.5	23.10	12.71	349.39
	2	49	29.0	4.42	2.62	75.86
Talus	1	50	27.5	39.20	21.56	592.90
	2	51	29.5	18.70	10.82	319.09
Reagent Blank		-	29.0	0.00	-	0.00

Table D31: Nuclear DNA Normalized Quantification of Buried Bovine Skeletal Material

Region bone powder was collected from in first column. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "B1 and B2" were replicate femora, calcanei, and tali buried during the course of this experiment. Cells highlighted in yellow represent extracts that had PCR inhibition. Values with "*" or "**" were diluted by a factor of 5 or 10 respectively to alleviate inhibition, listed value represents DNA quantification multiplied by dilution factor.

Burial	**Nuclear DNA (ng/mg) (*MC1R*)**											
Region	**Day 0**		**Week 1**		**Week 2**		**Week 4**		**Month 3**		**Month 6**	
	B1	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**
Diaphysis	156.73	261.00	5.31	2.44	0.77	2.20	2.37	1.74	1.41	1.20	1.28	2.13
Proximal Metaphysis	193.44	281.48	1.60	0.15	0.54	0.91	0.89	0.72	0.66	0.19	0.26	1.60
Distal Metaphysis	309.96	296.46	0.88	0.07	0.73	0.26	1.23	0.19	0.38	0.05	0.84	0.49
Femoral Head	155.52	434.28	26.50	43.89	8.20	27.33	4.65	6.22	5.93	10.46	11.91	21.05**
Distal Epiphysis	244.96	406.40	10.94	3.60	7.82	21.70**	0.00**	8.47	3.54	11.94	4.02	11.14
Calcaneus	227.12	264.12	40.51	28.25	8.67	5.00**	0.00**	1.40	2.89	2.51	12.71	2.62
Talus	287.10	307.10	16.46	35.01	12.28	11.58	1.00*	3.36	10.27	5.96	21.56	10.82

Table D32: Bovine Nuclear DNA PCR Amplification Chart for Quality Assay (Buried Skeletal Material)

Region listed in first column. "B1 and B2" refer to replicate number. Values (1 – 4) correspond to the length of PCR amplicon successfully generated for each region per replicate: 1 = 200 bp amplicon, 2 = 410 bp amplicon, 3 = 599 bp amplicon, 4 = 989 bp amplicon, NA = No Amplification.

Surface	**Nuclear DNA (*MC1R*) Amplicon Length**											
Region	**Day 0**		**Week 1**		**Week 2**		**Week 4**		**Month 3**		**Month 6**	
	B1	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**	**B1**	**B2**
Diaphysis	2	2	1	2	3	2	1	2	3	3	3	2
Proximal Metaphysis	2	2	2	2	3	2	1	2	3	3	3	2
Distal Metaphysis	2	2	3	2	2	1	NA	2	2	3	2	2
Femoral Head	2	2	3	2	3	1	1	2	3	2	2	NA
Distal Epiphysis	2	2	3	2	3	NA	NA	3	3	3	2	2
Calcaneus	2	3	1	2	3	NA	NA	2	3	3	3	2
Talus	3	2	2	2	3	2	NA	3	2	2	3	2
Reagent Blank	NA		NA		NA		NA		NA		NA	

APPENDIX E: ANCILLARY EXPERIMENT DATA

Changes in the Recoverable Total DNA of Buried Bovine Bone Segments over a One Month Time Period

Table E1: mtDNA Quantification of Buried Femoral Diaphysis (Segment Type A)
Bone segment identifiers are listed in the first column. "0D, 2D, 4D, 1W, 11D, 2W, 3W, and 4W" indicate length of burial in days (D) or weeks (W), and "1 – 4" denote each biological replicate of femoral diaphysis. "Type A" represents segments that were retrieved, tested, and stored at -20°C. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

Mitochondrial DNA (*ATPase*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
2D-1	57	32.6	2.22	1.27	72.39
2D-2	52	30.6	3.81	2.24	116.48
2D-3	50	32.0	3.72	2.38	119.00
2D-4	49	26.4	5.08	2.74	134.26
4D-1	50	31.8	2.12	1.35	67.50
4D-2	54	30.0	1.96	1.09	58.86
4D-3	51	30.2	1.63	0.97	49.47
4D-4	52	31.0	1.89	1.09	56.68
1W-1	51	33.0	0.30	0.19	9.69
1W-2	51	28.4	0.06	0.03	1.53
1W-3	50	28.0	1.77	0.99	49.50
1W-4	52	27.0	0.15	0.08	4.16
11D-1	50	30.0	0.10	0.06	3.00
11D-2	49	28.2	0.03	0.02	0.98
11D-3	49	28.2	0.06	0.04	1.96
11D-4	50	29.0	0.03	0.02	1.00
2W-1	50	15.0	0.04	0.01	0.50
2W-2	49	30.0	0.15	0.09	4.41
2W-3	51	23.2	0.12	0.05	2.55
2W-4	49	26.0	0.05	0.03	1.47
3W-1	50	30.8	0.02	0.02	1.00
3W-2	50	28.6	0.04	0.02	1.00
3W-3	50	29.2	0.09	0.05	2.50
3W-4	51	24.2	0.03	0.01	0.51
4W-1	51	24.8	0.03	0.02	1.02
4W-2	50	26.2	0.02	0.01	0.50
4W-3	51	29.4	0.03	0.02	1.02
4W-4	50	30.4	0.04	0.02	1.00

Table E2: mtDNA Quantification of Buried Femoral Diaphysis (Segment Type B)
Bone segment identifiers are listed in the first column. "0D, 2D, 4D, 1W, 11D, 2W, 3W, and 4W" indicate length of burial in days (D) or weeks (W), and "1 – 4" denote each biological replicate of femoral diaphysis. "Type B" represents segments that were cyclically retrieved, tested, and reburied. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder. "*" indicates failed extract due to Amicon® column damage.

Mitochondrial DNA (*ATPase*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
0D-1	51	30.2	0.26	0.15	7.65
0D-2	50	31.8	1.29	0.82	41.00
0D-3	50	6.0*	1.44	0.17	8.50
0D-4	50	31.0	0.59	0.36	18.00
1W-1	53	25.0	0.61	0.29	15.37
1W-2	50	26.4	0.58	0.31	15.50
1W-3	55	25.2	0.50	0.23	12.65
1W-4	51	26.0	0.94	0.48	24.48
2W-1	49	27.4	0.02	0.01	0.49
2W-2	51	26.0	0.04	0.02	1.02
2W-3	49	26.2	0.05	0.03	1.47
2W-4	50	24.6	0.04	0.02	1.00
3W-1	50	27.6	0.01	0.01	0.50
3W-2	49	28.6	0.03	0.01	0.49
3W-3	51	29.0	0.04	0.02	1.02
3W-4	51	26.2	0.02	0.01	0.51
4W-1	49	27.6	0.02	0.01	0.49
4W-2	50	24.8	0.04	0.02	1.00
4W-3	50	27.4	0.07	0.04	2.00
4W-4	51	28.8	0.04	0.02	1.02

Table E3: Nuclear DNA Quantification of Buried Femoral Diaphysis (Segment Type A)
Bone segment identifiers are listed in the first column. "0D, 2D, 4D, 1W, 11D, 2W, 3W, and 4W" indicate length of burial in days (D) or weeks (W), and "1 – 4" denote each biological replicate of femoral diaphysis. "Type A" represents segments that were retrieved, tested, and stored at -20°C. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

Nuclear DNA (*MC1R*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
2D-1	57	32.6	69.60	39.81	2269.17
2D-2	52	30.6	61.20	36.01	1872.52
2D-3	50	32.0	97.70	62.53	3126.50
2D-4	49	26.4	435.00	234.37	11484.13
4D-1	50	31.8	3.94	2.51	125.50
4D-2	54	30.0	140.00	77.78	4200.12
4D-3	51	30.2	168.00	99.48	5073.48
4D-4	52	31.0	127.00	75.71	3936.92
1W-1	51	33.0	0.37	0.24	12.24
1W-2	51	28.4	34.70	19.32	985.32
1W-3	50	28.0	23.60	13.22	661.00
1W-4	52	27.0	2.14	1.11	57.72
11D-1	50	30.0	0.23	0.14	7.00
11D-2	49	28.2	0.04	0.02	0.98
11D-3	49	28.2	1.32	0.76	37.24
11D-4	50	29.0	0.38	0.22	11.00
2W-1	50	15.0	1.06	0.32	16.00
2W-2	49	30.0	1.81	1.11	54.39
2W-3	51	23.2	0.89	0.40	20.40
2W-4	49	26.0	0.33	0.17	8.33
3W-1	50	30.8	0.00	0.00	0.00
3W-2	50	28.6	0.005	0.002	0.10
3W-3	50	29.2	1.61	0.94	47.00
3W-4	51	24.2	0.94	0.04	2.04
4W-1	51	24.8	1.24	0.60	30.60
4W-2	50	26.2	0.07	0.04	2.00
4W-3	51	29.4	0.36	0.20	10.20
4W-4	50	30.4	0.03	0.02	1.00

Table E4: Nuclear DNA Quantification of Buried Femoral Diaphysis (Segment Type B)
Bone segment identifiers are listed in the first column. "0D, 2D, 4D, 1W, 11D, 2W, 3W, and 4W" indicate length of burial in days (D) or weeks (W), and "1 – 4" denote each biological replicate of femoral diaphysis. "Type B" represents segments that were cyclically retrieved, tested, and reburied. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

Nuclear DNA (*MC1R*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
0D-1	51	30.2	0.24	0.14	12.24
0D-2	50	31.8	114.00	72.50	5700.00
0D-3	50	6.0*	0.00*	0.00	0.00
0D-4	50	31.0	7.51	4.66	375.50
1W-1	53	25.0	15.30	7.22	810.90
1W-2	50	26.4	20.80	10.98	1040.00
1W-3	55	25.2	26.50	13.09	1457.50
1W-4	51	26.0	158.00	80.03	8058.00
2W-1	49	27.4	0.01	0.006	0.49
2W-2	51	26.0	0.94	0.48	47.94
2W-3	49	26.2	2.59	1.38	126.91
2W-4	50	24.6	1.00	0.49	50.00
3W-1	50	27.6	2.53	1.40	126.50
3W-2	49	28.6	0.82	0.48	40.18
3W-3	51	29.0	1.03	0.59	52.53
3W-4	51	26.2	0.08	0.04	4.08
4W-1	49	27.6	0.002	0.001	0.10
4W-2	50	24.8	0.18	0.09	9.00
4W-3	50	27.4	3.74	2.16	187.00
4W-4	51	28.8	0.85	0.52	43.35

Changes in the Recoverable Total DNA of Non-Buried Bovine Bone Segments over a One Month Time Period

Table E5: mtDNA Quantification of Non-Buried Bovine Femoral Diaphysis
Bone segment identifiers are listed in the first column. "0D, 2D, 4D, 1W, 10D, 2W, 3W, and 4W" indicate length of exposure in days (D) or weeks (W), and "1 – 4" denote each biological replicate of femoral diaphysis. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

Mitochondrial DNA (*ATPase*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
0D-1	50	24.0	6.67	3.20	160.00
0D-2	50	28.0	3.12	1.75	87.50
0D-3	50	30.8	1.58	0.97	48.50
0D-4	51	28.0	3.38	1.85	94.35
2D-1	50	30.0	9.40	5.64	282.00
2D-2	49	29.2	5.61	3.34	163.66
2D-3	51	33.8	5.11	3.39	172.89
2D-4	50	31.6	6.83	4.32	216.00
4D-1	50	26.0	10.7	5.56	278.00
4D-2	50	29.0	6.09	3.43	171.50
4D-3	50	25.0	9.33	4.67	233.50
4D-4	51	25.0	7.74	3.79	193.29
1W-1	49	27.0	6.10	3.36	164.64
1W-2	49	26.2	4.07	2.18	106.82
1W-3	50	26.8	6.91	3.70	185.00
1W-4	51	28.4	5.80	3.23	164.73
10D-1	51	28.4	3.02	1.68	85.68
10D-2	49	24.2	1.85	0.91	44.59
10D-3	50	24.6	2.56	1.26	63.00
10D-4	51	29.4	2.48	1.43	72.93
2W-1	50	23.2	3.30	1.53	76.50
2W-2	49	27.4	2.96	1.66	81.34
2W-3	51	30.2	3.07	1.82	92.82
2W-4	51	23.0	2.64	1.19	60.69
3W-1	50	30.2	4.83	2.92	146.00
3W-2	50	25.0	1.27	0.64	32.00
3W-3	51	26.2	2.82	1.45	73.95
3W-4	51	27.2	2.73	1.45	73.95
4W-1	51	29.4	4.68	2.70	137.70
4W-2	49	28.0	2.16	1.23	60.27
4W-3	49	32.6	2.90	1.92	94.08
4W-4	49	28.4	3.01	1.74	85.26
RB	-	27.0	0.005	-	0.135

Table E6: Nuclear DNA Quantification of Non-Buried Bovine Femoral Diaphysis
Bone segment identifiers are listed in the first column. "0D, 2D, 4D, 1W, 10D, 2W, 3W, and 4W" indicate length of exposure in days (D) or weeks (W), and "1 – 4" denote each biological replicate of femoral diaphysis. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

Nuclear DNA (*MC1R*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
0D-1	50	24.0	224.00	107.52	5376.00
0D-2	50	28.0	139.00	77.84	3892.00
0D-3	50	30.8	112.00	68.99	3449.50
0D-4	51	28.0	162.00	88.94	4535.94
2D-1	50	30.0	481.00	288.60	14430.00
2D-2	49	29.2	186.00	110.84	5431.16
2D-3	51	33.8	291.00	192.29	9806.79
2D-4	50	31.6	551.00	348.23	17411.50
4D-1	50	26.0	407.00	211.64	10582.00
4D-2	50	29.0	196.00	113.68	5684.00
4D-3	50	25.0	276.00	138.00	6900.00
4D-4	51	25.0	250.00	122.55	6250.05
1W-1	49	27.0	413.00	227.57	11150.93
1W-2	49	26.2	265.00	141.69	6942.81
1W-3	50	26.8	238.00	127.57	6378.50
1W-4	51	28.4	207.00	115.27	5878.77
10D-1	51	28.4	134.00	74.62	3805.62
10D-2	49	24.2	145.00	71.61	3508.89
10D-3	50	24.6	186.00	91.51	4575.50
10D-4	51	29.4	159.00	91.66	4674.66
2W-1	50	23.2	117.00	54.29	2714.50
2W-2	49	27.4	191.00	106.80	5233.20
2W-3	51	30.2	187.00	110.73	5647.23
2W-4	51	23.0	184.00	82.98	4231.98
3W-1	50	30.2	319.00	192.68	9634.00
3W-2	50	25.0	103.00	51.50	2575.00
3W-3	51	26.2	179.00	91.96	4689.96
3W-4	51	27.2	186.00	99.20	5059.20
4W-1	51	29.4	401.00	231.16	11789.16
4W-2	49	28.0	156.00	89.14	4367.86
4W-3	49	32.6	243.00	161.67	7921.83
4W-4	49	28.4	219.00	126.93	6219.57
RB	-	27.0	0.27	-	7.29

Organic versus SoilMaster™: Total DNA Yield Comparisons Over One Week

Table E7: Total Quantification of Non-Buried Bovine Femoral Diaphysis Extracted Utilizing Organic and SoilMaster™.
Bone segment identifiers are listed in the first column. "0D, 2D, 5D, and 7D" indicate length of exposure in days (D), "O" = Organic Extraction, "S" = SoilMaster™ kit Extraction. Extract volume and milligrams of bone powder was considered in order to normalize DNA yields. Normalized values are reported as ng per mg of bone powder.

Mitochondrial DNA (*ATPase*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
O-0D	100	100.0	0.19	0.19	19.00
O-2D	101	101.0	0.46	0.46	46.46
O-5D	99	99.0	0.22	0.22	21.78
O-7D	99	99.0	0.12	0.12	11.88
S-0D	101	25.0	9.62	2.38	240.38
S-2D	100	25.0	7.19	1.80	180.00
S-5D	101	25.0	5.05	1.25	126.25
S-7D	99	25.0	1.64	0.42	41.58
Nuclear DNA (*MC1R*)					
Segment Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
O-0D	100	100.0	0.63	0.63	63.00
O-2D	101	101.0	12.70	12.70	1282.70
O-5D	99	99.0	11.00	11.00	1089.00
O-7D	99	99.0	4.64	4.64	459.36
S-0D	101	25.0	91.00	22.52	2274.52
S-2D	100	25.0	122.00	30.50	3050.00
S-5D	101	25.0	85.80	21.16	2137.16
S-7D	99	25.0	52.60	13.28	1314.72

Mass Difference between Wet and Dry Bone

Table E8: Mass of Drying Bone over Time in Four Containers plus Whole Bone
Drying time in hours listed in first column. Mass of bone powder in milligrams are listed in subsequent columns corresponding to treatment and time point. "1 and 2" are replicates.
NT = Not Tested

Drying Time (Hours)	Mass of Bone Powder (mg) per Treatment								
	Open Microcentrifuge Tube		Closed Microcentrifuge Tube		Weigh Boat (Bone Powder Pile)		Weigh Boat (Bone Powder Spread Out)		Whole Bone
	1	2	1	2	1	2	1	2	1
0	51	50	50	50	51	51	50	51	51,286
0.5	50	49	51	50	49	52	50	49	51,120
1	50	49	50	50	50	52	50	49	51,027
2	50	49	51	50	50	52	50	48	50.880
3	50	49	51	50	50	52	50	49	50,784
4	50	49	50	50	50	52	50	49	50,702
5	50	49	50	50	50	52	50	49	50,632
6	50	49	51	50	50	52	50	49	50,569
23.5	49	49	51	50	50	51	49	49	49,974
48.5	49	49	51	50	50	51	49	50	49,519
75	49	48	51	50	49	51	49	49	49,302
100	49	48	50	50	49	51	49	49	49,154
175	NT	NT	NT	NT	NT	NT	NT	NT	48,953
250	NT	NT	NT	NT	NT	NT	NT	NT	48,706
325	NT	NT	NT	NT	NT	NT	NT	NT	48,617

Effect of Proteinase K Concentration on Total DNA Yields

Table E9: mtDNA Quantification of Bovine Bones Digested with Varied Concentrations of Proteinase K

Bone identifier listed in the first column. TL = tissue lysis buffer, DM = demineralization buffer, RB = reagent blank. "0, 0.5, 1, and 2" represents percent by total solution volume of 20 mg/mL proteinase K added. "(1) and (2)" represent technical replicates per treatment. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

Mitochondrial DNA (*ATPase*)					
Bone Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
TL 0% (1)	49	29.0	8.74	5.17	253.46
TL 0% (2)	49	29.0	7.60	4.50	220.50
TL 0.5% (1)	49	31.0	14.90	9.43	462.07
TL 0.5% (2)	49	31.0	13.90	8.79	430.71
TL 1% (1)	50	26.0	15.20	7.90	395.00
TL 1% (2)	50	26.0	19.00	9.88	494.00
TL 2% (1)	49	30.0	16.20	9.92	486.08
TL 2% (2)	49	30.0	15.40	9.43	462.07
RB TL	-	30.0	0.001	-	0.03
DM 0% (1)	50	30.0	8.89	5.33	266.50
DM 0% (2)	50	30.0	9.26	5.56	278.00
DM 0.5% (1)	49	30.0	18.30	11.20	548.80
DM 0.5% (2)	49	30.0	17.90	10.96	537.04
DM 1% (1)	50	30.0	17.40	10.40	520.00
DM 1% (2)	50	30.0	20.70	12.42	621.00
DM 2% (1)	50	30.0	20.30	12.18	596.82
DM 2% (2)	50	30.0	17.10	10.26	513.00
RB DM	-	30.0	0.005	-	0.15

Table E10: Nuclear DNA Quantification of Bovine Bones Digested with Varied Concentrations of Proteinase K

Bone identifier listed in the first column. TL = tissue lysis buffer, DM = demineralization buffer, RB = reagent blank. "0, 0.5, 1, and 2" represents percent by total solution volume of 20 mg/mL proteinase K added. "(1) and (2)" represent technical replicates per treatment. Extract volume and milligrams of bone powder was considered in order to normalize nuclear DNA yields. Normalized values are reported as ng per mg of bone powder.

Nuclear DNA (*MC1R*)					
Bone Identifier	**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
TL 0% (1)	49	29.0	481.00	284.67	13948.83
TL 0% (2)	49	29.0	438.00	259.22	12701.78
TL 0.5% (1)	49	31.0	844.00	533.96	26164.04
TL 0.5% (2)	49	31.0	1130.00	668.78	32770.22
TL 1% (1)	50	26.0	967.00	503.84	25192.00
TL 1% (2)	50	26.0	1480.00	769.60	38480.00
TL 2% (1)	49	30.0	1110.00	679.59	33299.91
TL 2% (2)	49	30.0	1060.00	648.98	31800.02
RB TL	-	30.0	0.00	-	0.00
DM 0% (1)	50	30.0	337.00	202.20	10110.00
DM 0% (2)	50	30.0	423.00	253.80	12690.00
DM 0.5% (1)	49	30.0	482.00	295.10	14459.90
DM 0.5% (2)	49	30.0	550.00	336.73	16499.77
DM 1% (1)	50	30.0	436.00	261.60	13080.00
DM 1% (2)	50	30.0	694.00	416.40	20820.00
DM 2% (1)	49	30.0	555.00	333.00	16317.00
DM 2% (2)	49	30.0	382.00	229.20	11230.80
RB DM	-	30.0	0.001	-	0.03

Comparison of Total DNA Yields from Bovine Bones Macerated by MSU Forensic Anthropologist versus MSU Forensic Biologist

Table E11: Mitochondrial DNA Quantification of Bovine Bones Macerated by MSU Forensic Biology/Anthropology Laboratories

Region tested is in the first column. FA = element macerated by MSU Forensic Anthropology. FB = element macerated by MSU Forensic Biology. Extract volume and milligrams of bone powder was considered in order to normalize mtDNA yields. Normalized values are reported as ng per mg of bone powder.

mtDNA		**Tissue Lysis Buffer**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	FA	50	25.5	4.51	2.30	115.01
	FB	49	28.0	11.20	6.40	313.60
Femoral Head	FA	50	29.0	46.10	26.74	1336.90
	FB	49	31.0	80.70	51.06	2501.70
Distal Epiphysis	FA	50	29.0	54.70	31.73	1586.30
	FB	50	26.0	106.00	55.12	2756.00
Calcaneus	FA	51	27.0	8.00	4.23	216.00
	FB	51	26.0	81.00	41.29	2106.00
Talus	FA	51	26.0	12.70	6.47	330.20
	FB	50	25.0	32.00	16.00	800.00
Reagent Blank		-	24.0	0.00	-	0.00
mtDNA		**Demineralization Buffer**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	FA	49	22.5	11.60	5.33	261.00
	FB	51	28.0	28.90	10.71	564.00
Femoral Head	FA	49	27.0	37.30	20.55	1007.10
	FB	51	30.0	62.50	36.76	1875.00
Distal Epiphysis	FA	51	24.5	40.90	19.65	1002.05
	FB	50	26.0	86.70	45.08	2254.20
Calcaneus	FA	51	27.5	22.50	12.13	618.75
	FB	50	22.5	53.80	24.21	1210.50
Talus	FA	51	27.5	15.40	8.30	423.50
	FB	49	28.0	24.50	14.00	392.00
Reagent Blank		-	24.0	0.00	-	0.00

Table E12: Nuclear DNA Quantification of Bovine Bones Macerated by MSU Forensic Biology/Anthropology Laboratories

Region tested is in the first column. FA = element macerated by MSU Forensic Anthropology. FB = element macerated by MSU Forensic Biology. Extract volume and milligrams of bone powder was considered in order to normalize nuclear DNA yields. Normalized values are reported as ng per mg of bone powder.

Nuclear DNA		**Tissue Lysis Buffer**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	FA	50	25.5	157.00	80.07	4003.50
	FB	49	28.0	210.00	120.00	5880.00
Femoral Head	FA	50	29.0	984.00	570.72	28536.00
	FB	49	31.0	966.00	611.14	29946.00
Distal Epiphysis	FA	50	29.0	709.00	411.22	20561.00
	FB	50	26.0	953.00	495.56	24778.00
Calcaneus	FA	51	27.0	242.00	128.12	6534.00
	FB	51	26.0	1270.00	647.45	33020.00
Talus	FA	51	26.0	378.00	192.71	9828.00
	FB	50	25.0	780.00	390.00	19500.00
Reagent Blank		-	24.0	0.00	-	0.00
Nuclear DNA		**Demineralization Buffer**				
Region		**Bone Mass (mg)**	**Extract Volume (µL)**	**Quantification (ng/µL)**	**Normalized DNA (ng/mg)**	**Total DNA Recovered (ng)**
Diaphysis	FA	49	22.5	402.00	184.59	9045.00
	FB	51	28.0	430.00	236.08	12040.00
Femoral Head	FA	49	27.0	543.00	299.20	14661.00
	FB	51	30.0	578.00	340.00	17340.00
Distal Epiphysis	FA	51	24.5	509.00	244.52	12470.50
	FB	50	26.0	612.00	318.24	15912.00
Calcaneus	FA	51	27.5	409.00	220.54	11247.50
	FB	50	22.5	572.00	257.40	12870.00
Talus	FA	51	27.5	485.00	261.52	13337.50
	FB	49	28.0	522.00	298.29	8352.00
Reagent Blank		-	24.0	0.00	-	0.00

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