Evaluation of Digestion Buffers and Extraction Techniques for the Recovery of DNA from Teeth

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Abstract:

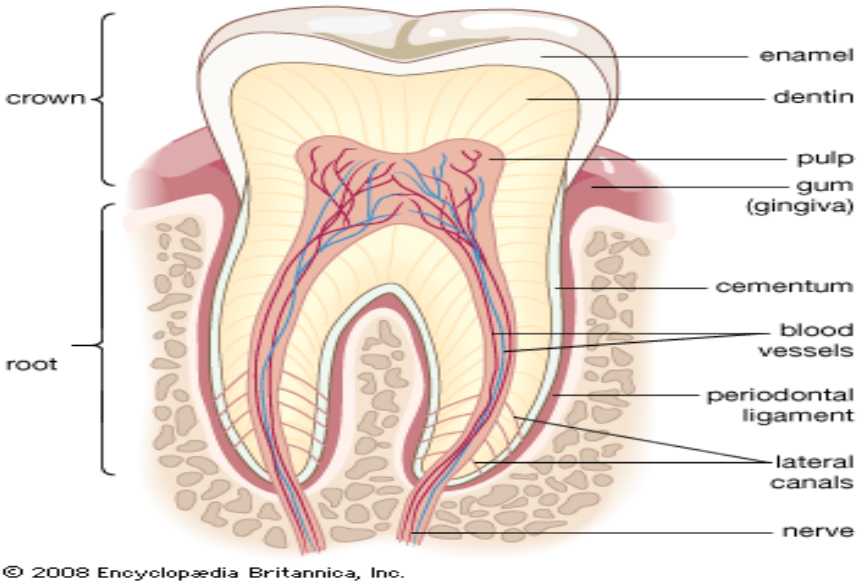
DNA testing is a routine and expected component of disaster victim identification. As mass disasters may leave human remains in pieces or burned beyond recognition, the co-mingling of body parts commonly associated with these events often makes an identification without DNA techniques virtually impossible. Whether the incident involves a fire, airplane crash, terrorist act, or mass grave site, it is likely that significant damage will have occurred to the biological samples and hence the DNA molecules. Extreme environmental conditions are known to negatively impact the quality of recovered remains where DNA may be so degraded that no, or only partial, DNA profiles result. Under such circumstances, analysis of bones or teeth may be the only recourse for identifying human remains although bones and teeth decidedly represent some of the most challenging samples encountered in the laboratory.

Organic and QIAGEN® EZ1® DNA Investigator Kit (QIAGEN®, Hilden, Germany) extraction techniques were compared in order to determine if an EZ1® extraction was as reliable as an organic extraction and decreased turn-around time. An examination of the section of the tooth, the crown, root, or whole tooth, was performed to ascertain which region of the tooth provided the highest quality and quantity of DNA. Reagent combinations were also tested in order to optimize the identification of human remains. Even though the organic extraction yielded the most DNA, extraction using the QIAGEN® EZ1® DNA Investigator Kit showed comparable yield results with an improved turn-around time and exposure to less caustic chemicals.

Introduction:

When a body cannot be identified visually or through dental records, utilizing deoxyribonucleaic acid (DNA) becomes an invaluable tool. DNA stores the information that gets passed on from generation to generation (Butler, 2010). Biological fluids such as blood and saliva are commonly collected for DNA analysis because of the ease of being obtained in a non-invasive manner. Teeth can be an important source for DNA when biological fluids are not available. In fact, teeth and bone were essential to identifying casualties associated with the tsunami that hit the Indian Ocean in 2004, and the terrorist attacks of September 11, 2001 (Ruckinski *et al*, 2011). This is due to the protective bone and enamel, in the case of teeth, that serve to protect DNA from degradation (Ye *et al*, 2004).

Figure 1: Anatomy of a Tooth



The anatomy of a tooth is pictured in Figure 1. There are two main sections of the tooth: the crown and the root. The crown is covered in enamel. That enamel is made up of calcium which provides protection to the underlying dentin (Encyclopedia Britannica). Calcium in the

tooth is considered a PCR inhibitor (Eilert and others, 2009). The root is protected by the bone, but when a tooth is removed, the root no longer has the bone's protection and is more prone to degradation. As a result of these physical differences, the part of tooth sampled was also examined to determine if taking a sample from a certain section of the tooth would yield more DNA. It was hypothesized that because the root is not covered in enamel, and doesn't have the extra layer of calcium, a PCR inhibitor, like the crown does, it would have a larger amount of amplifiable DNA.

To obtain DNA from a tooth, the tooth must be decalcified and demineralized. Seventy percent of the tooth is calcium, which can inhibit the release of DNA. For decalcification, ethylenediaminetetraacetic acid (EDTA) is used (Loreille and others, 2007). According to the Bone Sample Extraction Worksheet from the Analytical Procedures Manual (APM) of the Marshall University Forensic Science Center (MUFSC), the sample is rocked in EDTA for forty eight hours. This current method is time consuming. This portion of the study was performed to determine the number of days a tooth needed to be rocked in EDTA to produce the optimal amount of amplifiable DNA. It was the aim of this study to evaluate a method that would decrease turn-around time.

The North Louisiana Criminalistics Laboratory (NLCL) performed a study that examined different digestion buffers on bone to determine which would yield the most DNA. When using only EDTA (pH 8.0), the recovery of DNA was very low. The NLCL theorized that the slightly basic pH could negatively affect the DNA yield. As a result, they decided to decrease the pH by adding sodium acetate (pH 5.0) to the digestion buffer. The recovery of DNA was increased by 94% when 20uL of sodium acetate was added to the buffer (Dukes and others, 2012). Part of this experiment was to mirror NLCL's experiment to determine if MUFSC wanted to alter its

procedure manual to add sodium acetate to the incubation prior to an organic extraction in order to increase the yield of DNA.

The NLCL also examined three different digestion buffer combinations for an extraction performed on the QIAGEN® BioRobot EZ1® instrument to determine which buffer combination yielded the most DNA. For this project, the three different digestion buffer combinations chosen had the best average yield of DNA in the NLCL study (Dukes and others, 2012). Those reagent combinations were QIAGEN® Buffer ATL Tissue Lysis Buffer (Buffer ATL), EDTA and sodium acetate, Buffer ATL, EDTA, Dithiothreitol (DTT) and sodium acetate, and Buffer ATL, DTT, and sodium acetate. In addition, a crown, root and a whole tooth were incubated in each buffer combination. The QIAGEN® EZ1® DNA Investigator Kit extractions were analyzed to determine if the yield was comparable to organic extractions. If so, a protocol would be developed for DNA extraction from teeth utilizing the EZ1®or the current organic procedure would be modified.

Based on previous studies, it would be hypothesized that using the root of the tooth and the organic extraction combination would have the highest yield of DNA. According to the NLCL's previous study, the combination of reagents that would yield the largest amount of amplifiable DNA would be Buffer ATL, sodium acetate, and DTT (Dukes and others, 2012). These combinations will be evaluated based on their quantitation results and the corresponding electropherograms.

Materials and Methods:

Figure 2: Pouring Nitrogen Into the SPEX Sample Prep LLC® 6770 Freezer Mill

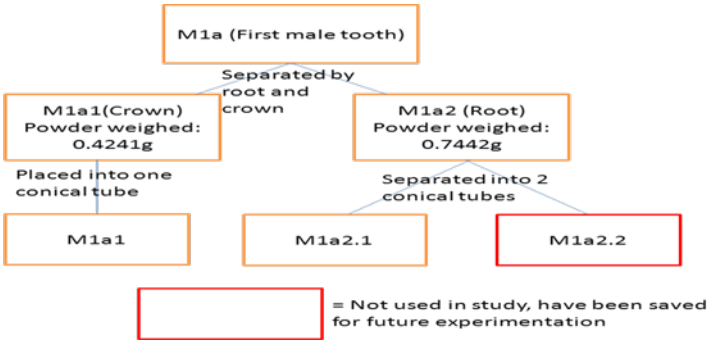
Sample Selection and Processing

In all, thirteen teeth were obtained from two donors, one female and one male and separated into forty nine samples (n=49). Three teeth came from the male and ten teeth came from the female. All teeth were photographed and separated into labeled envelopes. The teeth from the male donor were broken by a hammer and pliers to separate the root and crown. All teeth were then crushed in a SPEX Sample Prep LLC® 6770 Freezer Mill (SPEX, Metuchen, NJ) with liquid nitrogen (See Figure 2). Each tooth was crushed for approximately eighteen minutes. The pulverized teeth were weighed and placed into 15mL conical tubes. The weights ranged from 0.3-0.5g of sample in each tube.

Sample Selection- Root versus Crown

Figure 3 depicts how the samples were separated. Samples M1a1 and M1a2.1 were used in this study. One of the teeth from the female donor (F2f1) was also used in this study as the whole tooth. All three samples were soaked in 10mL of EDTA for forty eight hours. After the teeth were rocked in EDTA for forty eight hours, the EDTA was removed; the samples were incubated at 56°C in 500uL of Stain Extraction Buffer (SEB), 20uL of Proteinase K (Pro K), and 40uL of 1M DTT for six hours while being vortexed every hour. After the six hour incubation, an additional 20uL of Proteinase K and 40uL of 1M DTT were added to the samples and they were incubated at 56°C overnight. The samples were organically extracted based on the MUFSC Organic Extraction Protocol for Bone and Teeth.

Figure 3: Sample Division for the Tooth used for the Root versus Crown Study

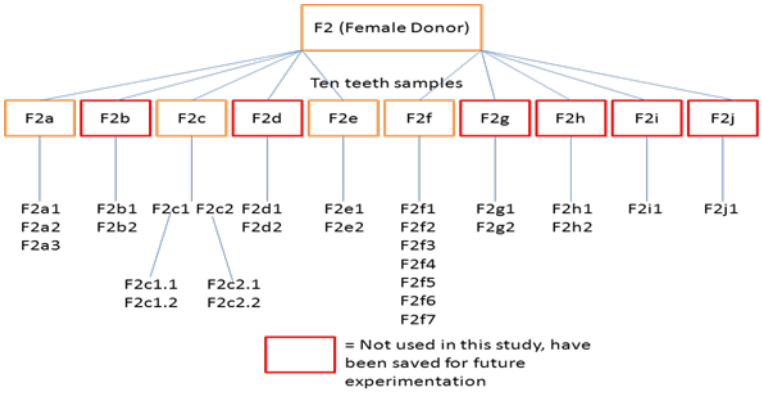


Sample Extraction-Rocked In EDTA

Figure 4 summarizes how the ten teeth from the female donor were separated after being pulverized. Tooth F2a was separated into three samples (F2a1, F2a2, and F2a3). F2a1 was rocked in EDTA for forty eight hours, F2a2 was rocked in EDTA for twenty four hours and the F2a3 was not rocked in EDTA. After the first two samples had been rocked in EDTA for each of their respective time periods, the EDTA was removed. All three samples were then incubated at

56°C in 500uL of Stain Extraction Buffer (SEB), 20uL of Proteinase K, and 40uL of 1M DTT for six hours while being vortexed every hour. After the six hour incubation, an additional 20uL of Pro K and 40uL of 1M DTT were added to the samples and they were incubated at 56°C overnight. The samples were organically extracted based on the MUFSC DNA Organic Extraction Protocol of Bone and Teeth.

Figure 4: The Female Teeth Divisions



Sample Extraction: Effects of EDTA and Sodium Acetate

Sample F2c was divided into four samples, F2c1.1, F2c1.2, F2c2.2, and F2c2.2 (n=4). F2c1.1 and F2c1.2 were rocked in EDTA for twenty four hours and F2c2.1 and F2c2.2 were not rocked in EDTA. Table 1 shows which samples were rocked, which samples had 750uL of EDTA to them during incubation and which samples had 30uL of sodium acetate added to them during incubation. All four samples' digestion buffers contained SEB, Pro K, and DTT. All four samples were incubated for twenty four hours at 56°C. The samples were organically extracted based on the MUFSC Organic Extraction Protocol for Bone and Teeth.

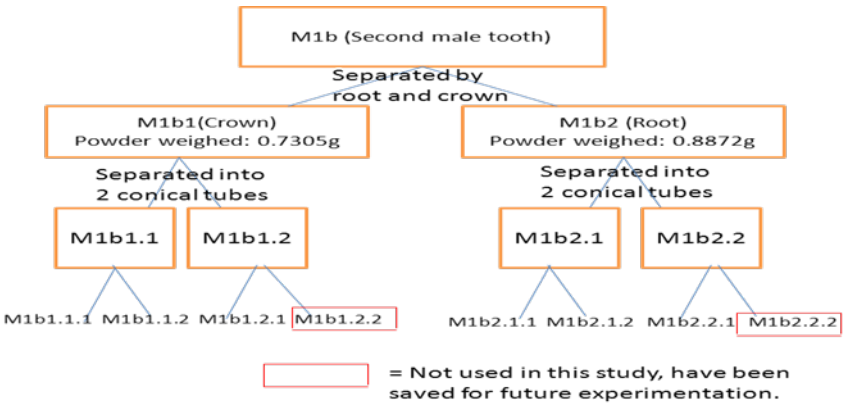
Table 1: Sample Selection for EDTA and Sodium Acetate in Digestion Buffers

Tooth ID	Hours Rocked in EDTA	750uL EDTA added to Non-Rocked Samples	30uL Sodium Acetate added to Digestion Buffer
F2c1.1	24	N/A	Yes
F2c1.2	24	N/A	Yes
F2c2.1	0	Yes	No
F2c2.2	0	Yes	Yes

EZ1® Extraction

Figure 5 depicts how the second male tooth was separated for the EZ1® study. M1b1.1.1, M1b1.1.2, and M1b1.2.1 were the three crown samples utilized. The three root samples that were used were M1b2.1.1, M1b2.1.2, and M1b2.2.1. Three whole tooth samples from the female donor were used, F2f2.1, F2f2.2, and F2f4.1. Crown, root, and whole tooth samples were used for each digestion buffer combination (n=9). After all nine samples had been incubated at 56°C for twenty four hours, 250ul aliquots of each sample were made (n=36) and 1ul of carrier RNA, 30ul of sodium acetate and 50ul of Buffer MTL were added to the samples. All the samples were placed on the QIAGEN® EZ1® Advanced XL Instrument. The instrument had the ability to extract fourteen samples per run and each run lasted approximately eighteen minutes.

Figure 5: Sample Division for the Tooth used for the EZ1® Study



Quantitation

All DNA samples were quantified in a 20uL reaction using the Plexor® HY System (Promega® Corporation, Madison, WI) and Applied Biosystems® Prism 7500 SDS Instrument (Life Technologies™, Foster City, CA). Two columns of standards were prepared according to the MUFSC Analytical Procedures Manual.

Amplification

The protocols for the Promega® PowerPlex® 16 HS Amplification Kit (Promega® Corporation, Madison, WI) and the Applied Biosystems® GeneAmp® PCR System 9700 (Life Technologies™, Foster City, CA) were followed per manufacturer's guidelines. All DNA samples were amplified in a 25uL reaction. The amplification target for each sample was different and can be seen in Table 2 and Table 3. Table 2 lists the sample amplification targets that were organically extracted whereas Table 3 lists the sample amplification targets that were extracted using the EZ1®At MUFSC, the target range for amplification using the PowerPlex® 16 HS system is 0.25-0.75ng. However, it is not uncommon to exceed this range when working with bones and/or teeth in order to obtain a full profile, therefore, a amplification target of 1.25ng was also evaluated.

Table 2: The Amplification Target for Each Sample Organically Extracted

Tooth ID	Quant value (ng/uL)	Pre Amp Action	Amp Load 1 (uL)	Target 1 (ng)	Amp Load 2 (uL)	Target 2 (ng)	Amp Load 3 (uL)	Target 3 (ng)
M1a1.1	1.90	Dilute	1.00	0.50	2.00	1.00	N/A	N/A
M1a2.1.1	0.37	Neat	1.00	0.37	2.00	0.74	N/A	N/A
F2f1	0.72	Concentrate	1.00	0.72	N/A	N/A	N/A	N/A
F2a3.c	0.00	Concentrate	5.00	0.00	10.00	0.00	17.50	0.00
F2a3.d	0.00	Dilute	1.00	0.00	5.00	0.00	10.00	0.00
F2a2.1	6.70	Dilute	1.00	0.50	2.00	1.00	N/A	N/A
F2a1.1	0.01	Concentrate	10.00	0.33	17.50	0.58	N/A	N/A
F2e2.1	0.83	Neat	1.00	0.50	1.50	1.25	N/A	N/A
F2e2.2	15.00	Dilute	1.00	0.50	2.50	1.25	N/A	N/A
F2c1.1	32.00	Dilute	1.00	0.50	2.50	1.25	N/A	N/A
F2c1.2	22.00	Dilute	1.00	0.50	N/A	N/A	N/A	N/A
F2c2.1	3.90	Dilute	1.00	0.50	2.50	1.25	N/A	N/A
F2c2.2	4.10	Dilute	1.00	0.50	2.50	1.25	N/A	N/A

Grey = Root versus Crown Study
Red = Hours Rocked in EDTA Study
Green = Effects of EDTA and Sodium Acetate

Table 3: The Amplification Target for Each Sample Extracted by the EZ1®

Tooth ID	Quant value (ng/uL)	Pre Amp Action	Amp Load 1 (uL)	Target 1 (ng)	Amp Load 2 (uL)	Target 2 (ng)
M1b2.1.1d	15.00	Dilute	1.00	0.50	N/A	N/A
M1b2.1.1c	14.00	Dilute	1.00	0.50	N/A	N/A
M1b2.1.1b	12.00	Dilute	1.00	0.50	N/A	N/A
M1b2.1.1e	12.00	Dilute	1.00	0.50	N/A	N/A
M1b2.1.1a	10.00	Dilute	1.00	0.50	2.50	1.25
F2f2.1b	10.00	Dilute	1.00	0.50	N/A	N/A
F2f2.1e	8.60	Dilute	1.00	0.50	N/A	N/A
F2f2.1c	8.40	Dilute	1.00	0.50	N/A	N/A
F2f2.1a	8.30	Dilute	1.00	0.50	2.50	1.25
F2f2.1d	8.30	Dilute	1.00	0.50	N/A	N/A
M1b1.1.1a	0.57	Neat	1.00	0.50	2.20	1.25
M1b1.1.1c	0.46	Neat	1.00	0.46	N/A	N/A
M1b1.1.1b	0.44	Neat	1.00	0.44	N/A	N/A
M1b1.1.1d	0.38	Neat	2.00	0.76	N/A	N/A
M1b1.1.1e	0.36	Neat	2.00	0.72	N/A	N/A
M1b2.1.2a	14.00	Dilute	1.00	0.50	2.50	1.25
M1b2.1.2d	11.00	Dilute	1.00	0.50	N/A	N/A
M1b2.1.2e	9.80	Dilute	1.00	0.50	N/A	N/A
M1b2.1.2b	9.20	Dilute	1.00	0.50	N/A	N/A
M1b2.1.2c	6.90	Dilute	1.00	0.50	N/A	N/A
F2f2.2c	8.30	Dilute	1.00	0.50	N/A	N/A
F2f2.2a	7.70	Dilute	1.00	0.50	2.50	1.25
F2f2.2d	7.70	Dilute	1.00	0.50	N/A	N/A
F2f2.2b	7.40	Dilute	1.00	0.50	N/A	N/A
F2f2.2e	6.50	Dilute	1.00	0.50	N/A	N/A
M1b1.1.2a	0.36	Neat	2.00	0.50	3.50	1.25
M1b1.1.2e	0.35	Neat	2.00	0.70	N/A	N/A
M1b1.1.2c	0.33	Neat	2.00	0.66	N/A	N/A
M1b1.1.2b	0.31	Neat	2.00	0.62	N/A	N/A
M1b1.1.2d	0.30	Neat	2.00	0.60	N/A	N/A
M1b2.2.1a	22.00	Dilute	1.00	0.50	2.50	1.25
M1b2.2.1b	19.00	Dilute	1.00	0.50	N/A	N/A
F2f4.1a	12.00	Dilute	1.00	0.50	2.50	1.25
F2f4.1b	11.00	Dilute	1.00	0.50	N/A	N/A
M1b1.2.1a	0.57	Neat	1.00	0.57	2.20	1.25
M1b1.2.1b	0.49	Neat	1.00	0.49	N/A	N/A

Yellow = Buffer ATL, Pro K, EDTA Incubation
Purple = Buffer ATL, Pro K, EDTA, DTT Incubation
Blue = Buffer ATL, Pro K, DTT Incubation

Sample Electrophoresis and Data Analysis

Capillary electrophoresis was performed on Applied Biosystems® 3130 Genetic Analyzer (Life Technologies, Foster City, CA) and Applied Biosystems® 3130 XL (Life Technologies™, Foster City, CA) according to the manufacturer's user's manual. A master mix was made consisting of Formamide and ILS 600. Once 10uL of master mix had been dispensed into the appropriate wells of the 96-well plate, 1.0uL of each sample was added to those wells. The amplification positive and negative were placed in their respective wells. A run negative, consisting of solely master mix, and an allelic ladder were added into their designated wells (See Figure 6 for sample setup). After the samples had been run, they were analyzed using the GeneMapper™ ID v3.2.1 software with parameters with a calling threshold set at 50 Relative Fluorescence Units (RFUs). A twenty percent filter was used for every sample analyzed. All samples were analyzed according to the MUFSC Analytical Procedures Manual.

Figure 6: Example of Plate Set Up from EZ1® Extraction Study

	1	2	3	4	5	6	7	8	9	10	11	12
A	F2e2.1-0.5	F2c2.1-0.5	M1b1.1.1d	F2f2.1a-0.5	RB5c	M1b1.1.2e	F2f2.2b	RB6d	F2f4.1b	M1b2.1.2a-1.25	F2c1.1-1.25	FORMAMIDE
B	RB3	RB9	M1b1.1.1e	F2f2.1b	RB5d	M1b2.1.2a-0.5	F2f2.2c	RB6e	RB7a	F2f2.2a-1.25	F2c2.1-1.25	FORMAMIDE
C	F2e2.2-0.5	F2c2.2-0.5	M1b2.1.1a-0.5	F2f2.1c	RB5e	M1b2.1.2b	F2f2.2d	M1b1.2.1a-0.5	RB7b	M1b1.2.1a-1.25	F2c2.2-1.25	FORMAMIDE
D	RB4	RB10	M1b2.1.1b	F2f2.1d	M1b1.1.2a-0.5	M1b2.1.2c	F2f2.2e	M1b1.2.1b	M1b1.1.1a-1.25	M1b2.2.1a-1.25	POS1	FORMAMIDE
E	F2c1.1-0.5	M1b1.1.1a-0.5	M1b2.1.1c	F2f2.1e	M1b1.1.2b	M1b2.1.2d	RB6a	M1b2.2.1a-0.5	M1b2.1.1a-1.25	F2f4.1a-1.25	NEG1	FORMAMIDE
F	F2c1.2	M1b1.1.1b	M1b2.1.1d	RB5a	M1b1.1.2c	M1b2.1.2e	RB6b	M1b2.2.1b	F2f2.1a-1.25	F2e2.1-1.25	FORMAMIDE	FORMAMIDE
G	RB8	M1b1.1.1c	M1b2.1.1e	RB5b	M1b1.1.2d	F2f2.2a-0.5	RB6c	F2f4.1a-0.5	M1b1.1.2a-1.25	F2e2.2-1.25	FORMAMIDE	FORMAMIDE
H	PXHSLADDER	RN1	PXHS LADDER	RN2	PXHS LADDER	RN3	PXHS LADDER	RN4	PXHS LADDER	RN5	PXHS LADDER	FORMAMIDE

Results and Discussion:

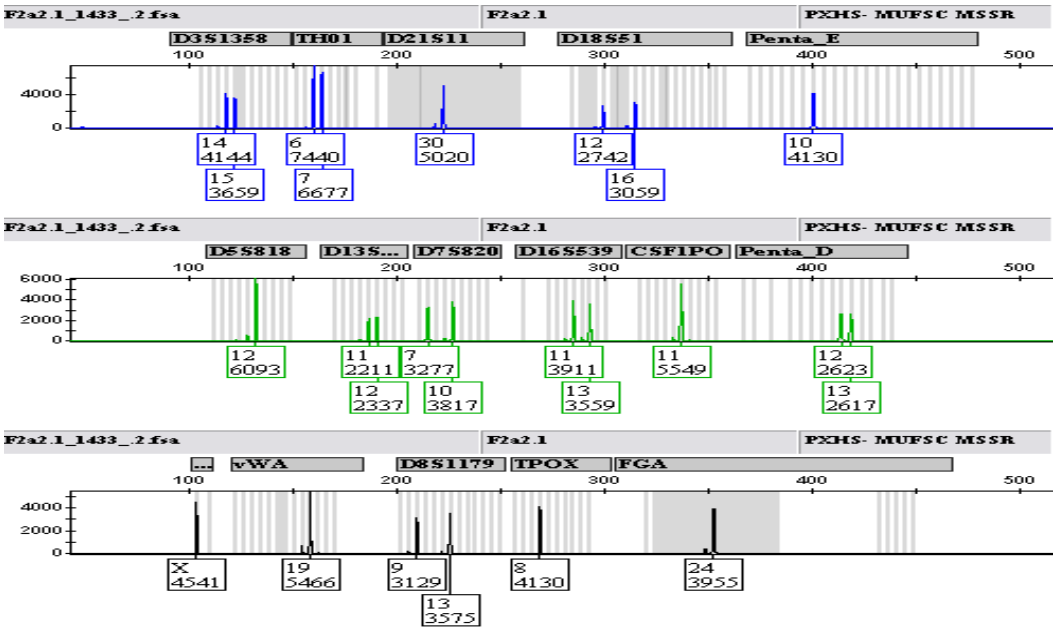
EDTA Study

The quantitation results for the three samples utilized in this study are in Table 4. Sample F2a2 was rocked in EDTA for twenty four hours and resulted in the highest yield of amplifiable DNA. That sample produced a full profile which can be seen in Figure 7. The sample that was rocked in EDTA for forty eight hours did not produce any peaks above calling threshold set by MUFSC. The sample that was not rocked, thus had no presence of EDTA, produced a full profile with some peak heights lower than the sample that was rocked in EDTA for 24 hours.

Table 4: Quantitation Values from the EDTA Study

Tooth ID	Hours Rocked with EDTA	Quant Value (ng/uL)
F2a3	48	0.00
F2a2	24	6.70
F2a1	0	0.013

Figure 7: Profile of F2a2 (Rocked in EDTA for 24 Hours)



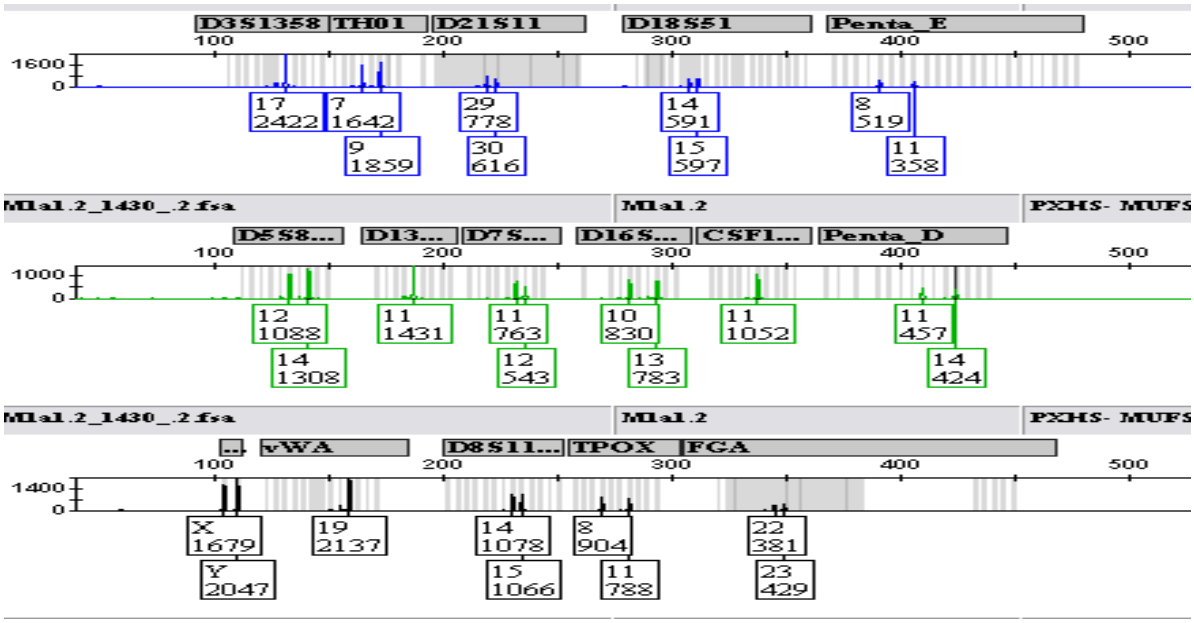
Regions of the Tooth Study

The three samples, M1a1, M1a2.1 and F2f1, were prepared according to the "Sample Selection: Root versus Crown" section of the materials and methods. The quantitation results (Table 5) show that the crown of the tooth yielded the most DNA, and the root of the tooth yielded the least amount. The crown of the tooth produced a full profile which can be seen in Figure 8. The other two samples, M1a2.1 and F2f1, also resulted in full profiles.

Table 5: Quantitation Values of the Different Regions of the Tooth Organically Extracted

Tooth ID	Tooth Region	Quant Value (ng/uL)
M1a1	Crown	1.9
M1a2.1	Root	0.37
F2f1	Whole	0.72

Figure 8: Profile of M1a1 (Crown of a Tooth)



Effects of EDTA and Sodium Acetate in the Digestion Buffers Study

Samples F2c1.1 and F2c1.2 yielded the highest amount of amplifiable DNA. These samples rocked in EDTA for 24 hours and had sodium acetate added to them before incubation. The quantitation values that were generated from this portion of the study are in Table 6. Samples F2c1.1 and F2c1.2 resulted in quantitation values of 32.00ng/uL and 22.00ng/uL respectively. All four samples produced full profiles that were comparable to Figure 7.

Table 6: Quantitation Values of Samples Affected by EDTA and Sodium Acetate

Teeth ID	Hours Rocked with EDTA	EDTA added (if not rocked)	Sodium Acetate added	Quant Value (ng/uL)
F2c1.1	24	N/A	Yes	32.00
F2c1.2	24	N/A	Yes	22.00
F2c2.1	0	Yes	No	3.90
F2c2.2	0	Yes	Yes	4.10

Digestion Buffers for EZ1® Extractions

The reagent buffer combination study showed that in all incubation procedures the root of the tooth yielded the most DNA which can be seen in Table 7. Figures 9, 10, and 11 show the average quantitation value for each region of the tooth per digestion buffer combination. The digestion buffer combination that had the highest DNA yield consisted of Buffer ATL, Pro K, and DTT.

Table 7: Quantitation Values for the Digestion Buffers Used for Each Portion of the Tooth

Tooth ID	Type	EZ1 Protocol Performed with EZ1	Quant Value (ng/uL)
M1b2.1.1d	Root	ATL, Pro K, EDTA	15.00
M1b2.1.1c	Root	ATL, Pro K, EDTA	14.00
M1b2.1.1b	Root	ATL, Pro K, EDTA	12.00
M1b2.1.1e	Root	ATL, Pro K, EDTA	12.00
M1b2.1.1a	Root	ATL, Pro K, EDTA	10.00
F2f2.1b	Whole	ATL, Pro K, EDTA	10.00
F2f2.1e	Whole	ATL, Pro K, EDTA	8.60
F2f2.1c	Whole	ATL, Pro K, EDTA	8.40
F2f2.1a	Whole	ATL, Pro K, EDTA	8.30
F2f2.1d	Whole	ATL, Pro K, EDTA	8.30
M1b1.1.1a	Crown	ATL, Pro K, EDTA	0.57
M1b1.1.1c	Crown	ATL, Pro K, EDTA	0.46
M1b1.1.1b	Crown	ATL, Pro K, EDTA	0.44
M1b1.1.1d	Crown	ATL, Pro K, EDTA	0.38
M1b1.1.1e	Crown	ATL, Pro K, EDTA	0.36
M1b2.1.2a	Root	ATL, Pro K, EDTA, DTT	14.00
M1b2.1.2d	Root	ATL, Pro K, EDTA, DTT	11.00
M1b2.1.2e	Root	ATL, Pro K, EDTA, DTT	9.80
M1b2.1.2b	Root	ATL, Pro K, EDTA, DTT	9.20
M1b2.1.2c	Root	ATL, Pro K, EDTA, DTT	6.90
F2f2.2c	Whole	ATL, Pro K, EDTA, DTT	8.30
F2f2.2a	Whole	ATL, Pro K, EDTA, DTT	7.70
F2f2.2d	Whole	ATL, Pro K, EDTA, DTT	7.70
F2f2.2b	Whole	ATL, Pro K, EDTA, DTT	7.40
F2f2.2e	Whole	ATL, Pro K, EDTA, DTT	6.50
M1b1.1.2a	Crown	ATL, Pro K, EDTA, DTT	0.36
M1b1.1.2e	Crown	ATL, Pro K, EDTA, DTT	0.35
M1b1.1.2c	Crown	ATL, Pro K, EDTA, DTT	0.33
M1b1.1.2b	Crown	ATL, Pro K, EDTA, DTT	0.31
M1b1.1.2d	Crown	ATL, Pro K, EDTA, DTT	0.30
M1b2.2.1a	Root	ATL, Pro K,DTT	22.00
M1b2.2.1b	Root	ATL, Pro K,DTT	19.00
F2f4.1a	Whole	ATL, Pro K,DTT	12.00
F2f4.1b	Whole	ATL, Pro K,DTT	11.00
M1b1.2.1a	Crown	ATL, Pro K,DTT	0.57
M1b1.2.1b	Crown	ATL, Pro K,DTT	0.49

Yellow = Buffer ATL, Pro K, EDTA Incubation
Purple = Buffer ATL, Pro K, EDTA, DTT Incubation
Blue = Buffer ATL, Pro K, DTT Incubation

Figure 9: Average quantitation value of the different regions of the tooth for the digestion buffer combination of Buffer ATL, Pro K, and EDTA.

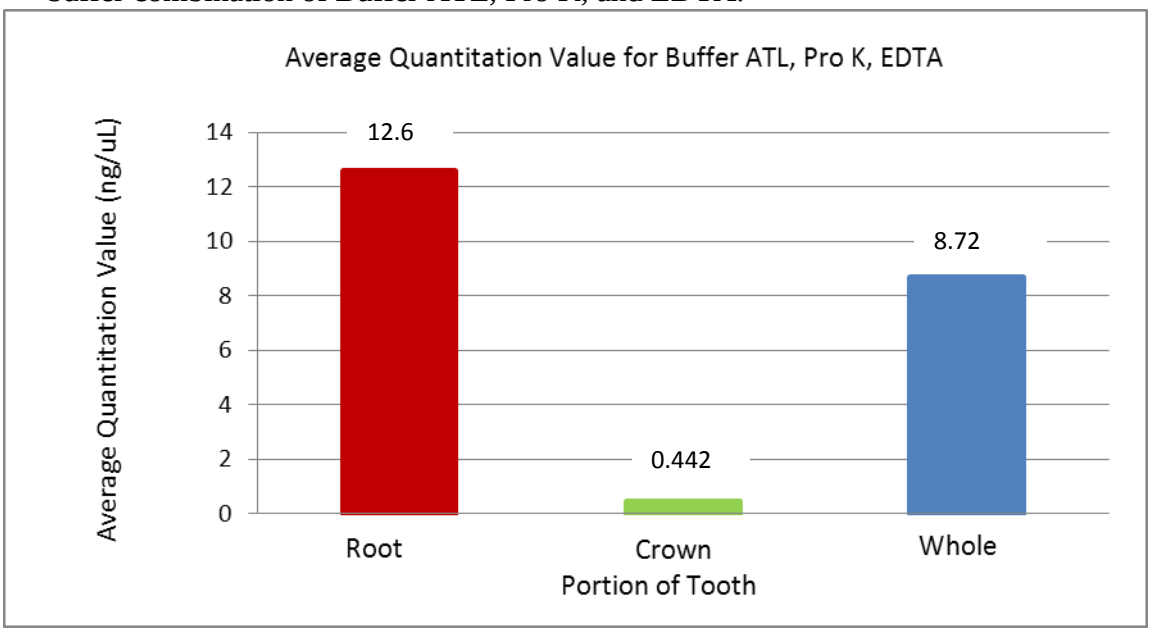


Figure 10: Average quantitation value of the different regions of the tooth for the digestion buffer combination of Buffer ATL, Pro K, EDTA, and DTT.

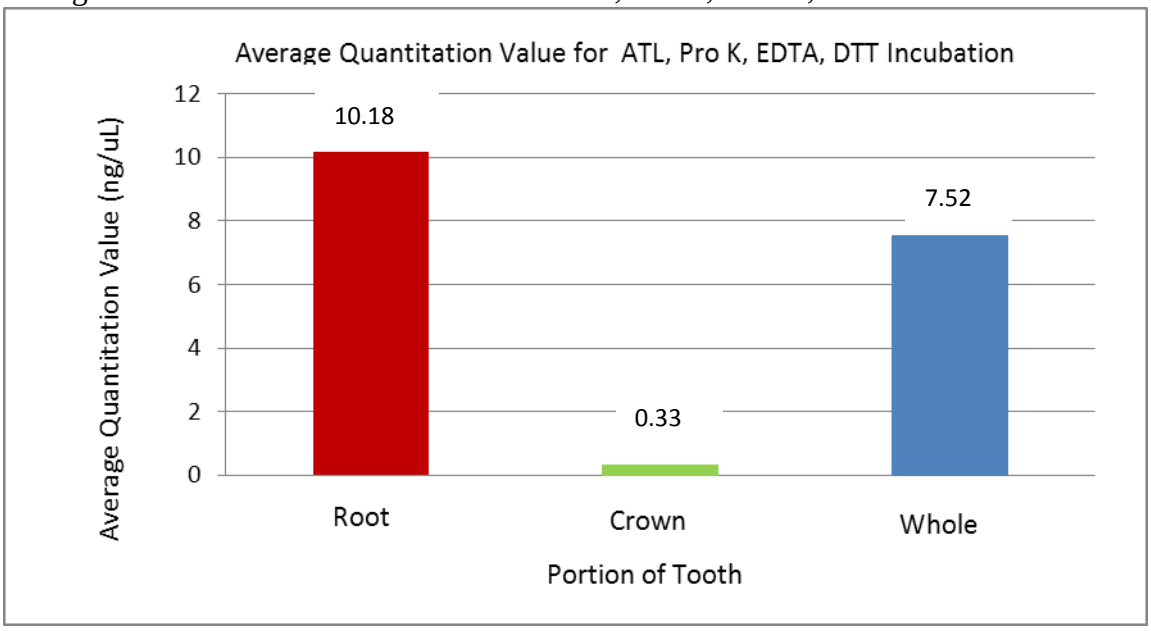
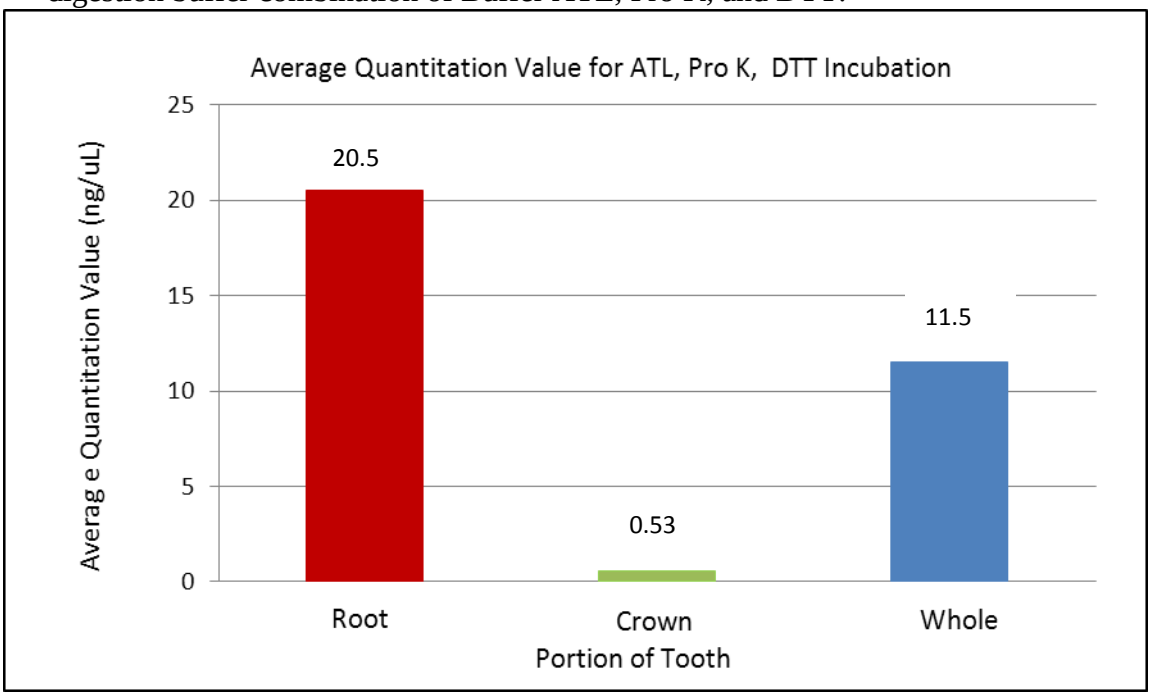


Figure 11: Average quantitation value of the different regions of the tooth for the digestion buffer combination of Buffer ATL, Pro K, and DTT.



Conclusion:

EDTA Study

This purpose of this study was to improve the current protocol of the MUFSC for the extraction of bone and teeth. According to the current protocol, the teeth are rocked in EDTA for at least forty eight hours. Sample F2a3 was rocked for forty eight hours, F2f2 was rocked for twenty four hours and F2a1 was not rocked at all. F2a1, the tooth that was rocked for twenty four hours yielded the most DNA. It would be recommended that as a result of this study that the protocol of MUFSC be modified to rock teeth in EDTA for twenty four hours.

Regions of Tooth Study

This study examined which region of the tooth yielded more DNA. It was hypothesized that the root of the tooth would yield more DNA it did not have the enamel like the crown did which meant that it did not have the extra layer of calcium, a PCR inhibitor. The teeth were

rocked in EDTA for forty eight hours and it was determined that the crown of the tooth yielded more DNA. This is not what was hypothesized; however, the forty eight hours and organically extracted. It is possible that the crown sample was rocked in EDTA long enough to decalcify the tooth allowing for a higher yield of amplifiable DNA.

Effects of DNA and Sodium Acetate in Digestion Buffer Study

According to the study performed by the NLCL, sodium acetate improved DNA yield for EZ1® extraction. This study examined the effects of sodium acetate for incubation prior to organic extraction. The results revealed that the samples that were rocked in EDTA for twenty four hours and had sodium acetate added to them yielded the most DNA. The two samples that were not rocked in EDTA but had EDTA added to them during incubation had a greater DNA yield than the samples from the "EDTA Study". The sample that had the sodium acetate added to it as well, F2c2.2 had a slight increase of DNA yield compared to the sample that only had EDTA added to it during incubation. The DNA yield was not as high for the samples that were not rocked in EDTA but had the EDTA added to them during incubation as when the samples were rocked in EDTA and this might be because the EDTA needs time to decalcify the tooth in order to release the DNA. Based on the results from this study, if an organic extraction is going to be performed on a tooth, it is suggested that the tooth be rocked in EDTA for twenty four hours and that sodium acetate is added to the digestion buffer to decrease the pH.

Digestion Buffers for EZ1® Extraction

Three digestion buffer combinations were examined to determine which one yields more DNA from an EZ1® extraction. The digestion buffer combinations each had a different volume. Therefore, each combination varied in the number of 250uL aliquots. The digestion buffer

combination that yielded the most DNA was Buffer ATL, Pro K, and DTT. The root of the tooth in each of the digestion buffer combinations yielded more DNA than the crown or the whole tooth. The root most likely yielded the most DNA because it is not covered in enamel, a PCR inhibitor like the crown and the teeth were not rocked in EDTA in order to remove that PCR inhibitor.

Organic extraction yielded the most DNA but the samples that were extracted by the EZ1® showed comparable results to the organic extraction. Because of this, laboratories including MUFSC can start using the EZ1® to extract teeth samples. Using the EZ1® instead of performing organic extractions has many advantages. It is less time consuming and decreases turn-around time. The EZ1® extracts fourteen samples in approximately eighteen minutes and does not use harmful chemicals such as Phenol Chloroform Isoamyl Alcohol (PCI) which is used during an organic extraction. It also saves money because PCI must be disposed of properly by utilizing a chemical waste company such as Clean Harbors. An extraction on the EZ1® requires less sample (0.15g-0.25g) than an organic extraction (0.3g-0.5g).

Future studies could include optimizing and creating a protocol for bone samples on the EZ1®. It may be the same or a similar procedure to that of teeth, but would have to be determined through experimentation. This study could go one step further and develop a protocol for teeth and bone on the QIAGEN® QiaCube® (QIAGEN®, Hilden, Germany). A study can be conducted to determine how much DNA the QiaCube® would yield and how much of a sample is needed in order to result in the quantification of an optimal amount of amplifiable DNA. An additional suggestion for future study is to determine what extraction method would be best for children's teeth. Children's teeth do not have roots so it is hypothesized that the teeth would have to be rocked in EDTA to decalcify the tooth and then be organically extracted.

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