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

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Abstract

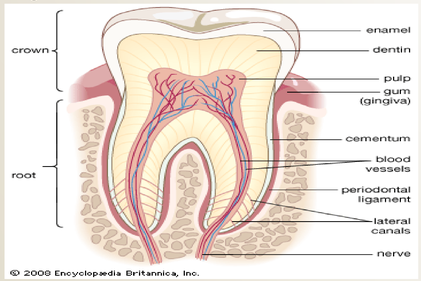
DNA testing is a routine and expected component of mass disaster victim identification. Mass disasters may leave human remains in pieces or burned beyond recognition. The co-mingling of body parts commonly associated with these events often makes an identification without DNA techniques virtually impossible. 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 resource for identifying human remains. As an added hinderance, bones and teeth represent some of the most challenging samples encountered in the laboratory.

Organic and QIAGEN® EZ1® (QIAGEN®, Hilden, Germany) extraction techniques were compared in order to determine if an EZ1® extraction was as reliable as an organic extraction with a 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 yields 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 deoxyribonucleic acid (DNA) becomes an invaluable tool. DNA stores the genetic 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 in 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 nature of the calcium lattice of bone and teeth. This lattice protects the cells that contain DNA (Ye *et al*, 2004). To obtain DNA from a tooth, the tooth must be decalcified and demineralized because the calcium inhibits the release of DNA. Ethylenediaminetetraacetic acid (EDTA) is used to decalcify the tooth to allow for the release of DNA. 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 with calcium rich enamel, which provides protection to the underlying dentin (Encyclopedia Britannica).

Figure 1:



Sample Selection and Processing

Thirteen teeth were obtained from two donors, one female and one male and separated into forty nine samples (n=49). Three teeth were of male origin, while ten teeth were of female origin. 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 seen in 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.

Figure 2:

Root vs. Crown

One male tooth was divided into two samples, a crown and a root. One of the teeth from the female donor was also used in this study as the whole tooth. All three samples were rocked in 10mL of EDTA for forty eight hours. The EDTA was then 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 Marshall University Forensic Science Center (MUFSC) Organic Extraction Protocol for Bone and Teeth.

Hours Rocked in EDTA

One tooth 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 at all. 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 for Bone and Teeth.

Effects of EDTA and Sodium Acetate

The North Louisiana Criminalistics Laboratory (NLCL) demonstrated a significant increase in DNA recovery when sodium acetate was added to the sample in order to decrease the pH (Dukes and others, 2012). This study wanted to replicate NLCL's experiment to determine if MUFSC wanted to modify its procedure manual to add sodium acetate to the incubation prior to an organic extraction in order to increase the yield of DNA. One tooth was divided into four samples. Figure 3 shows which samples were rocked in EDTA for twenty four hours and which ones were not rocked in EDTA at all. It also shows which samples had EDTA added to them during incubation and which samples had 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.

Figure 3:

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

Three crown samples and three root samples, from the male donor, were used. Three whole tooth samples, from the female donor, were used. Crown, root, and whole tooth samples were used for each digestion buffer combination (n=9). The digestion buffer combinations were Buffer ATL, Pro K, and EDTA; Buffer ATL, Pro K, EDTA and DTT; and Buffer ATL, Pro K, and DTT. After all nine (9) 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.

Results and Conclusion

The first study was used to evaluate the number of hours a tooth needed to be rocked in EDTA. The sample that was rocked in EDTA for twenty four hours resulted in the highest yield of amplifiable DNA and produced a full profile. The sample that was rocked in EDTA for forty eight hours did not produce any peaks above the 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.

The second study was used to determine which part of the tooth yielded the most DNA. Three samples, M1a1, M1a2.1 and F2f1, were prepared according to the "Sample Selection: Root versus Crown" section of the materials and methods. Quantitation results show that the crown of the tooth yielded the most DNA, and the root of the tooth yielded the least amount. All three samples produced a full profile.

Results and Conclusion Continued

The third study was used to determine what effects EDTA and sodium acetate had on teeth. Samples that were rocked in EDTA for 24 hours and that had sodium acetate added to them before incubation, yielded the highest amount of amplifiable DNA. Samples F2c1.1 and F2c1.2 resulted in quantitation values of 32.00ng/uL and 22.00ng/uL. All four samples produced full profiles.

The digestion buffer combination study showed that in all incubation procedures the root of the tooth yielded the most DNA . Figures 4, 5, and 6 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.

Figure 4:

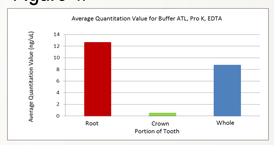


Figure 5:

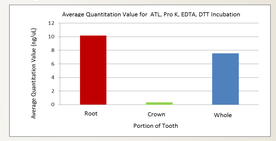
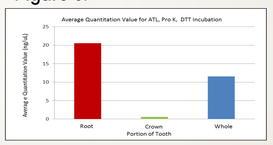


Figure 6:



Overall, organic extractions performed on the teeth, yielded the most DNA. However, the EZ1® extraction showed comparable results to organic extraction. The EZ1® extraction was preferred over organic extraction because fourteen samples can be extracted simultaneously, the extraction had decreased turn-around time (averaging approximately eighteen minutes for the extraction), and samples could be extracted without the use of harsh chemicals such as PCI. Future studies pertaining to this project include developing a protocol for bone and teeth using the Qiagen® Qiacube® and determining an extraction technique for children's teeth.

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Acknowledgments

I thank the MUFSC Laboratory analysts and staff for allowing me to work on this summer project. I thank Jason Chute (MUFSC Technical Leader), Dr. Pamela Staton, (Topic Advisor) and Carly Fannin, my supervisor for helping me design this project and for all of their input on the project. I thank Season Seferyn (Topic Advisor) for assistance with the writing and reviewing of this paper. I thank the National Institute of Justice (Grant Funding #2009-IJ-CX-K111) for their financial support.