

# PhD Research Proposal

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## **TITLE :**

Mitochondrial DNA Analysis from Dental Pulp in Highly Degraded Remains: A Forensic Odontology Approach to Human Identification

## **1. Introduction**

Identifying human remains is a crucial part of forensic investigations, especially in cases involving natural disasters, fires, or long-term decomposition. In such scenarios, traditional methods like fingerprinting or facial recognition often fail due to the extent of tissue degradation. Teeth, however, are incredibly resilient. Their hard structure helps protect the inner tissues—especially the dental pulp—from harsh environmental conditions, making them one of the most reliable sources for postmortem DNA.

While nuclear DNA has been the gold standard in forensic identification, it doesn't always hold up well in degraded samples. That's where mitochondrial DNA (mtDNA) comes into play. Unlike nuclear DNA, mtDNA is present in hundreds to thousands of copies per cell, making it more likely to survive extreme environmental conditions. This is especially important when working with severely damaged or aged remains where extracting nuclear DNA is difficult or even impossible.

This study focuses on the potential of mtDNA extracted from dental pulp in such compromised situations. The goal is to evaluate how effective mtDNA can be in identifying individuals when other options are no longer viable. By refining techniques to recover and analyze mtDNA from dental pulp, we aim to contribute a reliable and scientifically sound method to the field of forensic odontology, enhancing identification efforts in even the most challenging cases

## **2. Objectives**

- To extract mitochondrial DNA from dental pulp of human remains exposed to various environmental degradation conditions.

- To evaluate the quality and quantity of mtDNA in comparison to nuclear DNA.
- To assess the success rate of mtDNA sequencing from degraded dental pulp.
- To develop a standardized protocol for mtDNA extraction from teeth in forensic contexts.

### **3. Research Questions**

1. Can mitochondrial DNA be reliably extracted from dental pulp in degraded human remains?
2. How does the degradation environment (soil, water, heat, time) affect the yield and quality of mtDNA?
3. How does mtDNA extraction from dental pulp compare to other tissue sources under similar conditions?

### **4. Hypothesis**

Mitochondrial DNA extracted from dental pulp remains viable and sequenceable even in highly degraded remains, making it a dependable source for forensic human identification.

### **5. Methodology**

#### 5.1 Sample Collection:

- Use of 100 extracted human teeth (premolars/molars), sourced ethically and anonymized.
- Teeth subjected to simulated decomposition: burial (soil), immersion (water), burning (thermal exposure), and control (fresh).

#### 5.2 DNA Extraction:

- Mechanical and chemical access to pulp chamber.
- mtDNA extracted using silica-column based kits optimized for degraded samples.

#### 5.3 Quantification and Amplification:

- Quantify using qPCR targeting mtDNA D-loop region.
- PCR amplification of hypervariable regions (HVR I and II).

#### 5.4 Sequencing:

- Sanger or Next-Generation Sequencing (NGS) depending on DNA quality.

#### 5.5 Comparative Analysis:

- Compare mtDNA yield and sequence success with nuclear DNA from same samples.

### **6. Expected Outcomes**

- High success rate of mtDNA extraction and sequencing from dental pulp in degraded samples.
- A robust, standardized protocol for forensic mitochondrial DNA analysis from teeth.
- Contributions to forensic human identification, especially in mass disasters or old remains.

### **7. Significance of Study**

This research addresses critical gaps in postmortem DNA recovery. By establishing teeth as a reliable source of mitochondrial DNA, especially in compromised forensic scenarios, the study can aid law enforcement and disaster victim identification (DVI) teams.

### **8. References**

1. Amorim A, Fernandes T, Taveira N. Mitochondrial DNA in human identification: a review. *PeerJ*. 2019 Aug 13;7:e7314. doi: 10.7717/peerj.7314. PMID: 31428537; PMCID: PMC6697116.
2. Butler JM. DNA extraction from forensic samples using chelex. *Cold Spring Harb Protoc*. 2009 Jun;2009(6):pdb.prot5229. doi: 10.1101/pdb.prot5229. PMID: 20147187.
3. Higgins D, Austin JJ. Teeth as a source of DNA for forensic identification of human remains: a review. *Sci Justice*. 2013 Dec;53(4):433-41. doi: 10.1016/j.scijus.2013.06.001. Epub 2013 Jul 2. PMID: 24188345.
4. Lee, Steven & Shewale, Jaiprakash. (2017). DNA Extraction Methods in Forensic Analysis. *Encyclopedia of Analytical Chemistry* (pp.1–18)Edition: 2017Chapter: DNA Extraction Methods in Forensic Analysis

5. Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. *Forensic Sci Int Genet.* 2007 Jun;1(2):191-5. doi: 10.1016/j.fsigen.2007.02.006. Epub 2007 Mar 12. PMID: 19083754.
6. Malaver PC, Yunis JJ. Different dental tissues as source of DNA for human identification in forensic cases. *Croat Med J.* 2003 Jun;44(3):306-9. PMID: 12808723.
7. Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, Scheithauer R, Schmitter H, Schneider PM; International Society for Forensic Genetics. DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). *Forensic Sci Int Genet.* 2007 Mar;1(1):3-12. doi: 10.1016/j.fsigen.2006.10.003. Epub 2006 Nov 28. PMID: 19083722.
8. Rajni Trivedi, Prabal Chattopadhyay, Vijendra K Kashyap A New Improved Method for Extraction of DNA From Teeth for the Analysis of Hypervariable Loci July 2002 *The American journal of forensic medicine and pathology: official publication of the National Association of Medical Examiners* 23(2):191-6 DOI:10.1097/00000433-200206000-00016
9. Sakari SL, Jimson S, Masthan KM, Jacobina J. Role of DNA profiling in forensic odontology. *J Pharm Bioallied Sci.* 2015 Apr;7(Suppl 1):S138-41. doi: 10.4103/0975-7406.155863. PMID: 26015692; PMCID: PMC4439652.
10. Sweet D. Forensic dental identification. *Forensic Sci Int.* 2010 Sep 10;201(1-3):3-4. doi: 10.1016/j.forsciint.2010.02.030. Epub 2010 Mar 20. PMID: 20304570.
11. Wilson MR, Polanskey D, Butler J, DiZinno JA, Replogle J, Budowle B. Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts. *Biotechniques.* 1995 Apr;18(4):662-9. PMID: 7598901.
12. Yamada Y, Ohira H, Iwase H, Takatori T, Nagao M, Ohtani S. Sequencing mitochondrial DNA from a tooth and application to forensic odontology. *J Forensic Odontostomatol.* 1997 Jun;15(1):13-6. PMID: 9497750.