Comparison of aDNA yields from calculus and tooth roots in pre-Columbian skeletal remains

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Introduction

In recent years, dental calculus has emerged as an important source of ancient genetic material (Adler et al. 2013, De la Fuente et al. 2012, Warinner et al. 2014, Warinner et al. 2015). However, calculus has not been extensively used as a source of endogenous host DNA for ancient DNA research with human skeletal remains (Black et al 2011). In this study we compare endogenous DNA yields obtained from extractions performed from both dental calculus and dental tooth roots for three pre-Columbian individuals, originating from three different archaeological sites of the island of Puerto Rico: Paso del Indio (PI 427), Punta Candelero (PC 9) and Tibes (T 4) (Figure 1). We aim to determine whether dental calculus serves as an optimal source of endogenous, ancient DNA when working with poorly preserved remains such as those from sub-tropical Puerto Rican environments. We further assess the effects of physical decontamination procedures on DNA recovery in these samples by comparing yields obtained from dentin tooth root extractions performed with and without mechanical surface decontamination. The results of this study may inform sampling strategies and methodological study design for future ancient DNA research.

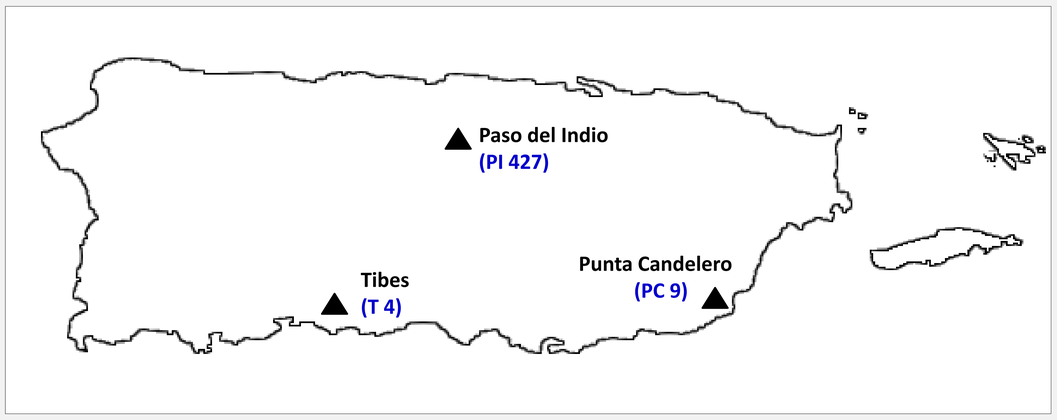


Figure 1. Approximate location of archaeological sites sampled. Sample names in parenthesis. PI 427 was radiocarbon dated to 985 cal AD, T4 was dated to 666 cal AD. PC 9 yielded an indeterminate date.

Materials & Methods

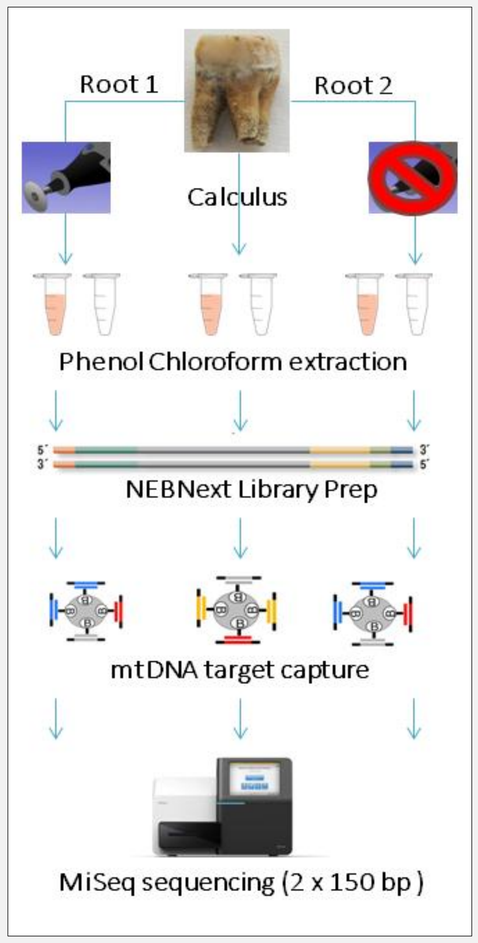


Figure 2. Schematic of methods.

At University of Oklahoma:
One molar with calculus was selected from each individual and wiped with 1% NAOCl solution to eliminate debris and surface dirt. Decalcification followed by Phenol chloroform extractions were performed in three replicates for each molar: (1) tooth root treated with mechanical surface decontamination (Dremel drill), (2) untreated tooth root, (3) calculus. Extraction blanks were used throughout this process. Shotgun libraries were prepared using the NEBNext DNA Library Preparation kit and amplified with Amplitaq Gold (Figure 2). All procedures were performed in clean room facilities following standard contamination control guidelines.

At Arizona State University:
Libraries were quantified with KAPA SYBR FAST qPCR kit and amplified for an additional 6-8 cycles with Accuprime *Pfx.* Complete mtDNA enrichment capture was performed as in Maricic et al. (2010). The enriched samples were sequenced on the Illumina MiSeq at DNASU Genomics Core. To ensure replication, each library was captured twice and sequenced three times. FastQ files from each sequencing run were then combined and processed with a bioinformatics pipeline based on Schubert et al. (2014). Damage patterns and rescaling were performed with MapDamage v2.0 (Jónsson et al. 2013). MtDNA haplogroup typing was performed with Haplogrep 2.0 Beta (Kloss-Brandstatter et al. 2011) using both raw and 1x coverage filtered genotype data (>1x).

Results

Out of nine extracts obtained (three sample types per individual) only seven were successfully constructed into libraries (Table 1). Sequence data analysis indicates that DNA yields differed across treatments for each individual. For PI 427 the library made from treated dentin extract contained, on average, more sequence reads mapping to the reference and higher coverage than the library containing calculus extract. The opposite pattern was observed with sample PC 9, where the library created from calculus yielded the highest amount of mapped sequence reads, and the highest coverage. However, due to the unsuccessful library preparation of two of our sample types, we cannot effect a complete comparison across all treatments for either of these individuals. Sample T 4 had low sequence coverage and depth for all treatments and as such provides inconclusive evidence.

We recovered sufficient reads for mtDNA genotyping from three sample types (>60% of the reference was covered). Sequence reads obtained for PC 9 (calculus and untreated dentine) had diagnostic mutations for haplogroup C1b2 (16325C, 16327T, 16519C, 4242T, 11914A). Haplogroup C1 has been found in high frequencies (n=4 out of 8) among other pre-contact samples from Punta Candelero and Paso del Indio in Puerto Rico (Nieves-Colón, unpublished). Sequences obtained for PI 427 (treated) had diagnostic mutations that place it under macro-haplogroup M (489C, 10400T, 14783C). Other mutations identified were not sufficient to place this individual in a derived sub-lineage. These three libraries exhibit a U shaped damage pattern of increased C to T and A to G transitions at the 5' and 3' end of the sequences, respectively, suggestive of ancient DNA preservation. Other libraries yielded insufficient reads for a clear assessment of post-mortem damage (Table 2).

Sample	Tissue	Q30 mapped reads	Mean Coverage	% ref seq covered	mtDNA Haplogroup
PI 427	Treated dentin	3,075	12.5x	99.99	M?
	Untreated dentin	*Unsuccessful library preparation*			
	Calculus	118	0.3x	25.90	Undetermined
PC 9	Treated dentin	*Unsuccessful library preparation*			
	Untreated dentin	336	1.1x	64.4	C1b2
	Calculus	546	1.9x	85.40	C1b2
T 4	Treated dentin	61	0.3x	24.70	Undetermined
	Untreated dentin	65	0.3x	24.71	Undetermined
	Calculus	57	0.3x	23.60	Undetermined

Table 1. Summary statistics per library. Read data was aggregated across three sequencing runs.

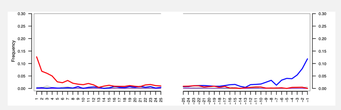
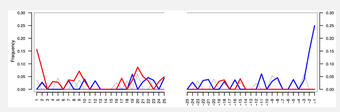
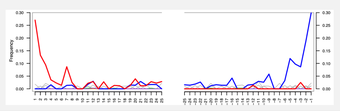
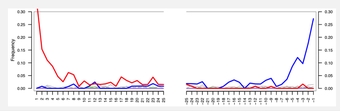
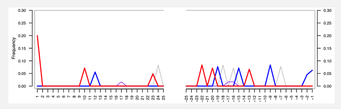
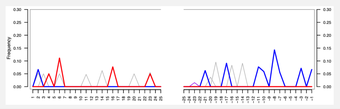
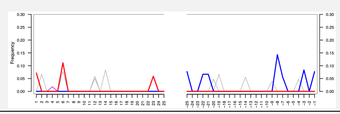
Sample	Treated dentin	Untreated dentin	Calculus
PI 427		N/A	
PC 9	N/A		
T 4			

Table 2. Deamination plots per library. Red = C to T transitions Blue = G to A transitions

Conclusions

Our preliminary results suggest that both calculus and dentin serve as viable sources of endogenous ancient DNA in these samples. Mechanically treated dentin extractions seem to result in higher DNA yields than untreated dentin extractions, but these results may differ depending on overall DNA preservation of individual samples. Further research and wider sampling are necessary to determine what factors may influence the differential preservation of endogenous ancient DNA between dentine and calculus.

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