Barcoding of Degraded DNA from Northwest Coast Objects: A method for fostering reciprocal relationships between museums and communities

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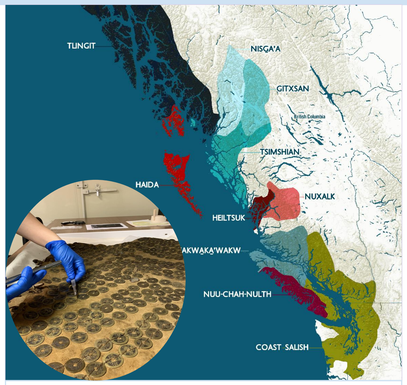


Figure 1. Map of Native American cultures represented in the Hall of PNC. All 31 objects sampled were from these locations. Image shows sampling of Tlingit Armor by Museum Object Conservators.

Introduction

The Hall of the Pacific Northwest Coast (PNC) is the oldest gallery in the American Museum of Natural History (AMNH)[1] and is currently undergoing renovations that aim to include the voices of Indigenous community members from the cultures represented in the hall[1,2]. Several artifacts in the PNC collection are made of unknown animal species dating back to the early 19th Century and contain degraded DNA. A number of these objects have been identified by Indigenous consultants and objects conservators as culturally valuable to identify material composition. Our research utilized extraction techniques for degraded DNA[3] and universal mini barcodes[4,5] to perform DNA barcoding on . Results of this study will be provided to the project's Consulting Curators to help maintain traditions and preserve Indigenous knowledge. This is part of a larger effort by the museum to develop reciprocal relationships with communities represented in the collections[6,7,8].

Methods

Sample Acquisition

11 Artifacts were selected and sampled by Objects Conservators in collaboration with Indigenous Representatives

Extraction & Quantification

DNA extraction followed modified protocol for ancient DNA tissue (Qiagen DNeasy Blood/Tissue Kit)

Amplification & Clean-Up

Performed PCR using ~100bp universal mini-barcodes[5] and species specific primers designed in Geneious. PCR Clean-up conducted with Ampure XP beads

Fragment Analysis

Fragment length was investigated with Gel Electrophoresis and DNA quantified with Qubit

Sanger Sequencing

Amplified fragments were sequenced utilizing capillary electrophoresis in the forward and reverse directions

Analysis

Geneious was used to assemble and compare sequences against the NCBI nucleotide database (Blastn)[9]

Results

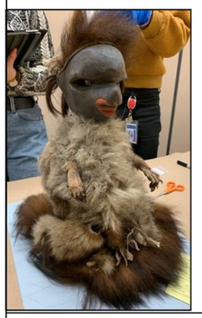
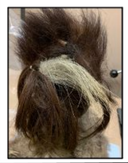
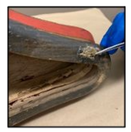
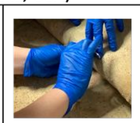
Object & Culture	Material & Description	Primer	Results
 Death Bringer, Nuxalk (*Nuxalkmc),* (British Columbia, Canada)	 white coarse hairs on back of head	Universal Mini barcode[5] (12s region, ~100bp)	**Mountain Goat** (*Oreamnos americanus*, 97.8% identical sites) or **domesticated goat** (*Capra aegagrus,* 95.6% identical sites). Species-specific primer will likely confirm Mountain Goat identification. 
	Mummified skin/claw	Universal Mini barcode[5] (12s region, ~100bp)	**Vancouver Island Marmot** (*Marmota vancouverensis)* with 100% pairwise identity and 100% identical sites. 
 Mask, Tsimshian, (Coastal British Columbia, Canada & Alaska, USA)	 Skin on chin	Universal Mini barcode[5] (12s region, ~100bp)	**Anatidae family** (waterbirds including *ducks, geese* and *swans).* Potential matches: *Anser albifrons* (Greater white-fronted goose), *Branta nigricans* (Black Brant). Input from indigenous leaders and species-specific primer will confirm this identification. 
	 Skin along ears	Universal Mini barcode[5] (12s region, ~100bp)	**Mountain Goat** (*Oreamnos americanus*, 98.9% identical sites) or **domesticated goat** (*Capra aegagrus,* 96.7% identical sites). This was a low-quality extraction and contamination cannot be ruled out. A specific primer could help to confirm. 
 Traditional Armor, Tlingit, Skita Qwan (Alaska, USA)	 Hide	*Alces alces* primer (12s region, ~150bp) & Universal Mini barcode[5] (12s region, ~100bp)	Both hide and sinew are **North American Moose** (*Alces alces*) with 100% pairwise identity and identical sites 
	 Sinew around coins		

Figure 2. Of the 11 objects sampled, high quality DNA that successfully amplified was only collected from 4 objects (3 of which are included here). Blast search results of NCBI nucleotide database[9] was able to identify seven materials from four objects with a high level of confidence. The object, culture, primers used in PCR amplification and the blast results from the assembled sequenced data are shown.

Discussion

- This diverse selection of samples provides promising evidence for successful DNA barcoding using universal mini barcodes typically used for microbiome studies on ancient DNA from museum artifacts with a range of materials (skin, hair, feather, hide, sinew).
- Highly processed materials such as wool and hair without the attached root had lower extraction success. Skin, sinew and hide performed the best.
- Despite less coverage than the specific primer, the universal barcode bound to the 12s region with an overlapping section of ~50-75bp.
- Next steps include utilization of species specific primers to confirm identification of materials in the cases where matches were not 100% confirmed or contamination was a concern.
- Due to the age and small starting material of the samples, many of the DNA extractions were low quality and highly degraded. Further studies will benefit significantly from increased starting material for DNA extractions.
- As genetic methods become more accessible and affordable, genetic identification of cultural materials could be a promising way for cultural institutions to develop reciprocal relationships with communities by answering questions of cultural importance and engaging communities in the scientific process.
- The new addition of a state-of-the-art Ancient Biomolecules lab facility in the Sackler Institute of Comparative Genomics at the AMNH (set to open fall 2021) will greatly improve the quality of studies like this and further collaborations between museum collections and science.

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