Potentially Ancient DNA Extracted from Viking Hall Soils in Stoð, East Iceland

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Introduction

Ancient DNA extraction from soil or from other environmental elements is a relatively new field and, when sequenced, has proven to be a powerful tool to study archaeological sites where little or no solid remains are found[1,2,3]. In Stöðvarfjörður, Iceland, two Viking halls that date to the 9th and 10th centuries are being excavated and studied. These halls may be the oldest settlement yet discovered in Iceland. No bone remains have yet been found. This situation presents an exciting opportunity to examine aDNA taken from soil to learn about Icelandic settlers, their livestock, their diets and genealogy. To our knowledge, this is the first attempt at extracting aDNA from Icelandic soils.

Figure 1. Aerial view of the Viking Hall. Note the "ship" shape of the outer walls. We took a total of 18 samples, 3 of which we analyzed in this project. Both outside the house (the control area) and longfire locations have a control sample taken a few inches above the floor and an experimental sample taken at the floor level. The paving slab location only has an experimental sample without a control, since there was no soil on top of it (**Figure 2 C**).

Figure 2. Collection of samples. In figure a & b, the control is taken from the top hole while the sample is at the bottom. ~2 inches of the outermost dirt layer was removed, and 2-3 inches of dirt from behind the exposed layer was taken as the sample. A) Students clean sampling tools with bleach in the outside the house location. B) The dark color of the floor layer indicates a longfire. This area is believed to be close to the entrance of the hall. C) Sample being collected from under a floor paving slab. The slab uncovered very dark soil, and a deeper paving slab of an older building could be felt under the current soil layer.

Soil Characteristics

Table 1. pH, Nitrogen (N), Phosphorus (P) and Potassium (K) values of soil samples. These values are consistent with Brown Andisols seen in this part of Iceland[4].

Sample Name	Acronym	pH	N	P	K
Longfire 2 Sample	L2S	6	Depleted	Deficient	Depleted
Longfire 2 Control	L2C	6.5	Depleted	Adequate	Depleted
Outside House 1 Sample	OH1S	6	Depleted	Deficient	Depleted
Outside House 1 Control	OH1C	5.5	Depleted	Deficient	Depleted
Paving Slab 2 Sample	PS2S	6	Depleted	Sufficient	Depleted

Extraction Method

- All soil samples were handled inside an ancient DNA lab: masks, hairnets, double gloves and hazmat suits were worn to avoid contaminating the samples
- 1-3 g of sediment was added to 3mL of 0.5M sodium phosphate buffer pH 7.0
- 170 µg of Proteinase K was added to the samples
- Two different homogonizing tools were used:
  - Vortex 2x20 seconds at full speed
  - FastPrep-24 2x20 seconds at 4.0 m/s
- Samples were purified once with ultraPure 25:24:1 Phenol:Chloroform:Isoamylalcohol
- Samples were cleaned and concentrated with a Qiagen MinElute kit
- Contentration was determined via a Qubit dsDNA High Sensitivity Assay Kit and 0.8% Agarose Gel
- Samples were shotgun sequenced at BYU on an Illumina HiSeq 2500.

Figure 3. Students working inside the ancient DNA lab.

Extraction Results

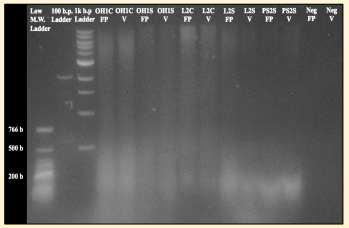

Figure 4. 2 uL of extracted DNA was run on a 0.8% agarose gel for 1 hour. "V" indicates Vortex, "FP" indicates FastPrep-24.

Table 2. DNA concentration in soil samples that were extracted with the phosphate method. "FP" indicates Fastprep and "V" indicates vortex.

Sample	Concentration (µg/µl)
L2S - FP	13.6
L2S - V	3.08
L2C - FP	44.6
L2C - V	34.8
OH1S - FP	28.8
OH1S - V	26.0
OH1C - FP	68.0
OH1C - V	61.4
PS2S – FP	1.63
PS2S - V	5.46
Negative – FP	0.202
Negative – V	0.116
Average Yield - FP	**26.1**
Average Yield - V	**23.9**

Analysis of DNA Damage

- After sequencing, adapters were removed, R1 and R2 reads joined, and basic quality score filtering was performed. Low complexity reads, as defined by the entropy of the bases, were filtered out, along with PCR duplicates.
- Alignment was performed using NCBI's mitochondrial DNA TaxId databases, with settings that returned the two best alignments while accommodating damage found at the ends of the strands.
- Aligned reads were extracted from the sequence alignment maps and analyzed with PMD Tools[5] (post-mortem damage) (**Figures 5 and 6**). Between 3-9% of the reads from the subset of each of our sample spots remain after applying a PMD filter of >5 **(Table 3).**
- This analysis places the age of our DNA between measurements of about "...~15–20% of sequences from Neandertals [sic] and Neolithic Scandinavians but only ~0.01–0.02% from any present day individual and 0.27% from the 100-y-old remains of the Australian individual..5"

Table 3. Summary of the PMD analysis of our samples. We selected specific species that settlers would have cultivated or consumed that are not necessarily native to Iceland. PMD = post-mortem damage.

	Longfire 2						Outside House 1						Negative Control	
	Control			Sample			Control			Sample				
Species	Read Count	PMD >5	%	Read Count	PMD >5	%	Read Count	PMD >5	%	Read Count	PMD >5	%	Read Count	PMD >5
Entire Sample	91,296	3,148	3	112,318	4,437	4	78,044	3,451	4	53,008	2,249	4	15,893	1,005
Cranberry	306	23	8	305	29	10	216	17	8	169	16	9	20	1
Wild soybean	1,788	103	6	2,017	126	6	1,275	106	8	1,000	65	7	102	6
Henbane	37	1	3	57	2	4	36	1	3	31	0	0	5	0
Sorghum	99	6	6	144	6	4	161	10	6	31	1	3	2	0
Wild rice	433	45	10	559	71	13	414	51	12	273	49	18	11	3
Human	2	0		8	0		7	0		2	0		14	0
Sheep	1	0		0	0		1	0		0	0		0	0
Atlantic salmon	0	0		0	0		1	0		0	0		0	0

Results

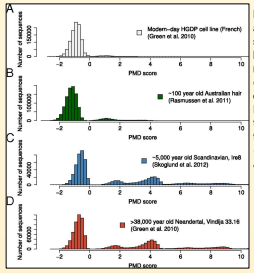

Figure 5. PMDS distribution in ancient and present day human sequences. (*A*) A present day human. (*B*) Hundred-year-old remains of an Australian individual. (*C*) Five thousand-year-old remains of a Scandinavian individual. (*D*) A Pleistocene Neandertal. Note that the *x* axes in *C* and *D* are truncated, and PMD scores reach ~26. Figure taken from Skoglund *et al.*[5]

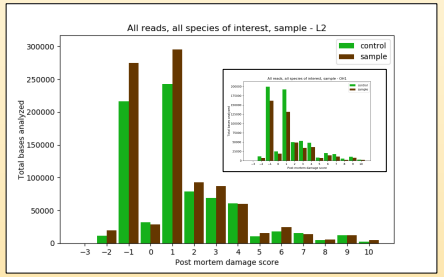

Figure 6. DNA damage estimation in all reads of the species of interest (see Table 3) in the Longhouse Sample and Control – FP and the Outside the House Sample and Control – FP (inset). Compare with **Figure 5**. The damage estimates put our DNA samples at approximately 1200 years of age.

Assuming that we are looking for approximately 1200 year old fragments, our results align with the observation that the overall rate of accumulated PMD is roughly proportional to the age of the endogenous mitochondrial DNA[5].

Next Steps

We need to test DNA extracted from soils that are from the top of the soil horizon at Stoð, to see if the damage observed in our samples is truly indicative of age, or if the properties of the Icelandic soil itself (**Table 1,** volcanic Brown Andisol[4]) damage DNA.

References

1. Slon, Viviane, et al. "*Neandertal and Denisovan DNA from Pleistocene sediments.*" Science 356.6338 (2017): 605-608.
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3. Pedersen, Mikkel Winther, et al. "*Ancient and modern environmental DNA.*" Phil. Trans. R. Soc. B 370.1660 (2015): 20130383.
4. Arnalds, Ólafur. *The soils of Iceland*. Springer, 2015.
5. Skoglund, Pontus, et al. "*Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal.*" Proceedings of the National Academy of Sciences 111.6 (2014): 2229-2234.

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