Using DNA Analysis to Further Understand the Origins of Parchment

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Introduction

Parchment is a material made from animal hide with an extensive history of being used for written texts containing historical and cultural information. Unfortunately, individual pages from parchment manuscripts have been removed or lost throughout the years, meaning that many manuscripts are now incomplete. It would be helpful to art conservators to have DNA evidence on the origin animal of the parchment so that the loose pages can be placed back with its original manuscript.

Figure 1: Image of a parchment manuscript. Book of Hours illuminated by Vante di Gabriello di Vante Attavanti. Florence 1480-1490.

Previously, only species have been identified using whole-genome sequencing but this method is expensive and time-consuming. However, using Polymerase Chain Reaction (PCR) to amplify only the D-loop of the mitochondrial DNA (mtDNA) from manuscripts is a more readily available and less expensive approach. The DNA is collected using "eraser crumbs", a non-destructive DNA extraction method, from the follicles on the surface of the parchment. These remnants of DNA can successfully identify the source animal of the parchment. This information, accompanied by stylistic and context clues provided by art conservators, guides the way to rebuilding parchment manuscripts as they were originally intended.

Methods and Materials

I. Species Identification Methods

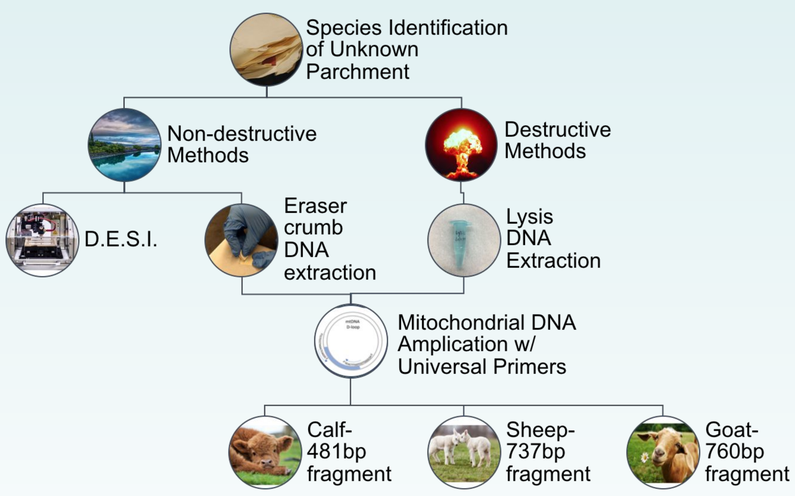

Figure 2: Flow-chart depicting the process used to properly identify the unknown species of a piece of parchment. Using either the eraser crumb extraction or lysis extraction, the mtDNA has to be amplified through PCR using universal primers that work for every species pictured above.

Methods and Materials (cont.)

The degraded DNA that remains within the follicles on the surface of the parchment is extracted using either a destructive or non-destructive method. The D-loop of the mtDNA is then amplified using PCR and universal primers that anneal to the flanking regions of the D-loop of a variety of species' mtDNA. The resulting fragments differ in size depending on which species the parchment is made from - goat, sheep, or calf.

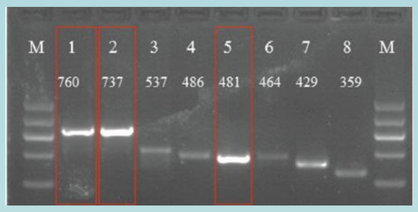

Figure 3: PCR amplification results for eight animal species. Lanes 1-8 represent goat, sheep, deer, buffalo, cattle yak, pig, and camel, respectively.

Fen Guan, et al. Journal of Analytical Methods in Chemistry, vol 2018, Article ID 589-140, 6 pages, 2018.

II. Individual Animals Identification Methods

Short Tandem Repeats (STRs) are small fragments of DNA, that repeat a number of times in a non-coding region of the nuclear DNA at several loci. The combination of multiple STRs in conjunction with the STR specific primers creates an individualized fingerprint for each animal within a single species.

Figure 4: A set of eight different STR loci demonstrating individual differences of repeats within a species. An individual can be identified by the unique combination of their STRs.

Individual Calf Identification with STRs Primers

Primer set (Locus)	Repeat Sequence	Amplicon Length	Calf 1 Repeats *	Calf 2 Repeats *
BM1818	$(TG)_n$	253-277bp	136	122
BM2113	$(CA)_n$	124-146bp	62	70
CSSM66	$(AC)_n$	177-203np	91	98
ETH3	$(GT)_n AC(GT)_6$	100-128bp	53	62
ILSTS006	$(GT)_n$	279-297bp	140	147
TGLA122	$(AC)_n(AT)_n$	136-182bp	71	75
HAUT27	$(AC)_n$	137-155bp	76	66
TGLA227	$(TG)_n$	76-104bp	43	49

*NOTE: The repeat values for calf 1 and calf 2 are not representative of the real samples, they are meant to illustrate the difference in repeats between individuals of the same species. Van de Goor LHP, Koskinen MT, van Haeringen WA: Population studies of 16 bovine STR loci for forensic purposes. INT J Legal Med. 2011, 125: 111-119.10.1007/s00414-009-0353-8

Results

I. Species Identification Results

A. Calf DNA Extraction with Lysis Solution

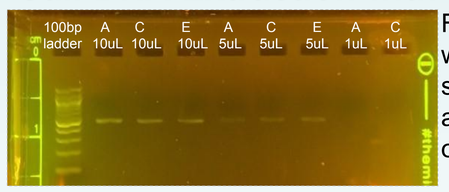

Figure 5A: Parchment was destroyed by lysis solution. The fragments are at the expected size, of ~500bp, for calf DNA.

B. Calf DNA Extraction with Eraser Crumbs

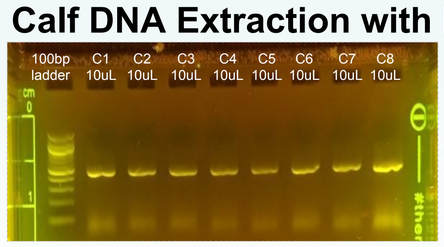

Figure 5B: Nested PCR results with calf DNA isolated using DNeasy column kit and a temperature gradient for annealing step. The fragments are at the expected ~500bp.

Results (cont.)

C. Desorption Electrospray Ionization Results

Figure 6A: DESI image of calf parchment.

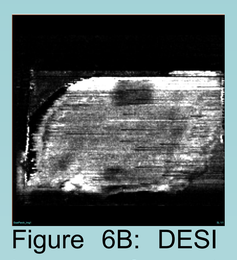

Figure 6B: DESI image of goat parchment.

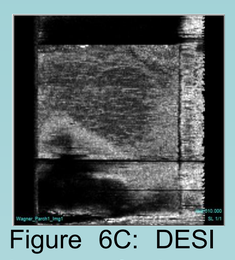

Figure 6C: DESI image of sheep parchment.

II. Individual Animals Identification Results

To test the identification of individual animals through STRs, calf thymus DNA, not DNA extracted from calf parchment, was used.

STR 1824

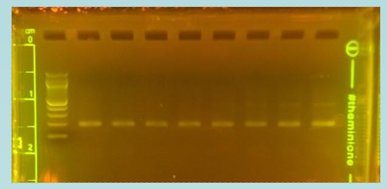

Figure 7A: Fragments expected at 176-188bp, and observed at ~200bp. PCR with annealing temperature gradient.

STR 53

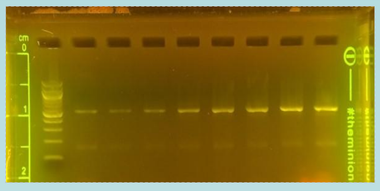

Figure 7B: Fragments expected at 152-187bp, but observed bands at ~150bp and ~500bp. PCR with annealing temperature gradient.

Conclusion

Enough calf DNA was extracted from parchment to successfully identify the species using the eraser crumb technique. However, the same methods did not work on the sheep and goat parchment. Even though sufficient DNA was extracted from goat and sheep parchment, the amplified fragments were not at the correct size so species identification was not possible. Further research should focus on altering the PCR conditions to properly amplify the D-loop of sheep and goat DNA samples. Also, the STRs were successful when using calf thymus DNA, but now they must be tested using DNA extracted directly from calf parchment and then the remaining species.

References

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