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 of the parchment so that the loose pages can be placed back with its original manuscript.

Methods and Materials

I. Species Identification Methods

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.

Methods and Materials (cont.)

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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.

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

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.

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

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

I. Species Identification Results

A. Calf DNA Extraction with Lysis Solution

Figure 7A: Fragments expected at 178-188bp, and observed at ~200bp. PCR with annealing temperature gradient.

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

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

STR 53

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.

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References


References

I. Species Identification Methods

A. Calf DNA Extraction with Lysis Solution

B. Calf DNA Extraction with Eraser Crumbs

II. Individual Animals Identification Results

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.

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