Using DNA Analysis For Species Identification Of Animal Parchment

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INTRODUCTION

In the field of art conservation chemists, biologists, engineers, and experts in the humanities collaborate to preserve artwork, documents, artifacts, and other items of cultural heritage. The purpose of this research was to ascertain the provenance of one category of such artifacts: ancient manuscripts written on animal parchment. By extracting DNA from this material and identifying the species from which they were made, historians and conservators can better understand the cultural context of parchment manuscripts.

Project Goals

- ❖ Extract DNA from parchment
- ❖ Use extracted DNA as a template for Polymerase Chain Reaction (PCR)
- ❖ Sequence products for species identification

What is Parchment?

- Parchment, or highly stretched and cleaned animal skin, was the predecessor to paper and the most readily available medium for written works prior to the modern printing press.
- Parchment was typically made with the skin of a calf (*bos taurus),* goat (*capra hircus),* or sheep *(ovis aries)*.
- Some of the ancient world's most treasured religious, social, and political knowledge has been preserved on parchment throughout millennia.

Fig. A: Monk making parchment, 12th C.

Fig. B: *Evangeliorum Fragmenta,* 9th Century A.D. (Held by Abigail Quandt, senior parchment conservator at the Walters Art Museum.

Fig. C: The Dead Sea Scrolls, ~150 B.C. (Source)

How Does This Research Contribute to Art Conservation?

Fig. C: Geographic origins of livestock used for parchment-making

Genomic analysis of parchment is crucial in providing insights into the provenance of documents.

Species identification can provide information on the general location and time of the assembly of the tome

- Genomic identification also serves as a personal identifier for the document. If pages are missing and found later, we can use sequencing to match estranged pieces of the script.

METHODS

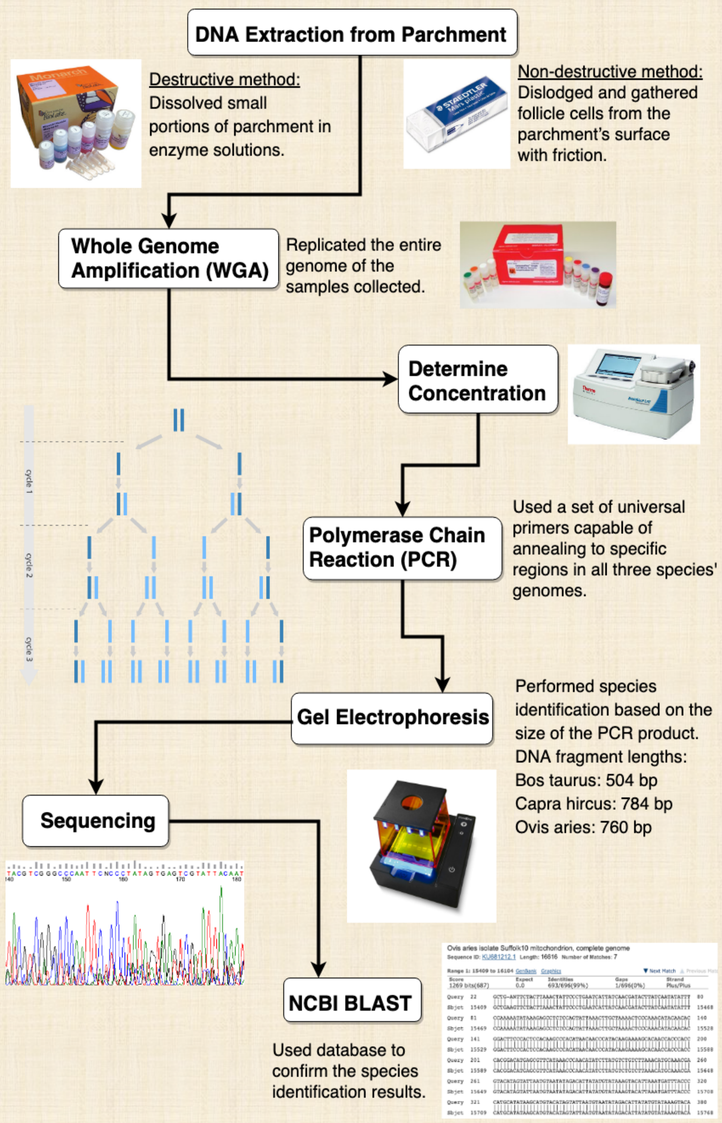

Fig. D: Sequence of techniques utilized

RESULTS

Attempts to amplify DNA segments directly from parchment were unsuccessful. Trace amounts of bovine genomic DNA from BSA* were discovered in the commercial enzymatic reaction mix.

*BSA is a protein and a common ingredient in biotechnical products as an aid for enzymatic reactions.

- Newly-synthesized PCR primers successfully differentiated between the three species in question.
- Gel Electrophoresis analysis consistently showed a product in the negative control that matched the band for calf.

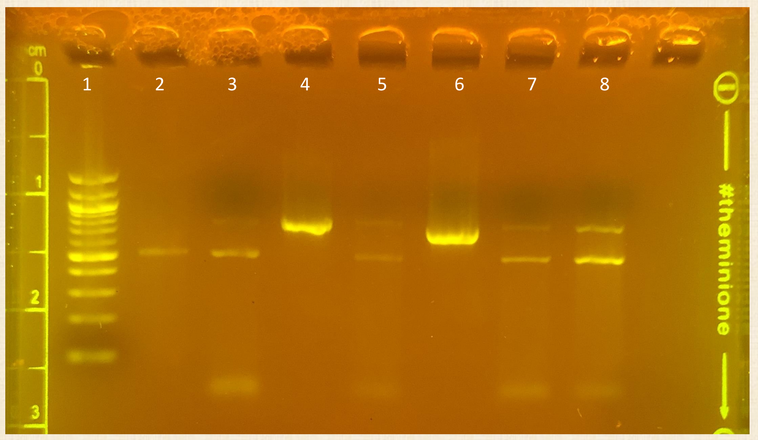

Fig. E: 1.8% Agarose gel; Run Time: 25 min.

1. 100 base pair DNA ladder, 2. *B. taurus*: positive control, 3. Parchment sample: calf, 4. *C. hircus:* positive control, 5. Parchment sample: goat, 6. *O. aries:* positive control, 7. Parchment sample: sheep, 8. Negative control

CONCLUSIONS

Primer Design

- A primer is a short strand of nucleotides and serves as an initiation point for DNA synthesis during PCR. Without a functional primer set, no product can be obtained.
- Discovered a primer pair capable of annealing to the sequences of calf (*bos taurus),* goat (*capra hircus),* and sheep *(ovis aries)* universally

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cow     CATCTGTCCTATACTTTCTCCTCATCCTAGTGCTAATACCAACGGCCGGCACAATCGAAA   156
sheep   ----------------------------AGTCATAATACCAGTAGCTAGCATCATCGAAA    32
goat    CATCTATCATATATTTCCTCATCATTCTAGTAATAATACCAGCAGCTAGCACCATTGAAA   180
                                     ***  ********   **  ***  ** ****

cow     ACAAATTACTAAAATGAAGACAGGTCTTTGTAGTACATCTAATATACTGGTCTTGTAAAC   216
sheep   ACAACCTCCTAAAATGAAGACAAGTCTTTGTAGTACAATCAATATACTGGTCTTGTAAAC    92
goat    ACAACCTTCTAAAATGAAGACAAGTCTTTGTAGTACAATCAATACACTGGTCTTGTAAAC   240
        ****  * ************** **************   **** ***************

cow     CAGAGAAGGAGAACAACTAACCTCCCTAAGACTCAAGGAAGAAACTGCAGTCTCACCATC   276
sheep   CAGAGAAGGAGAACAACCAACCTCCCTAAGACTCAAGGAAGAAGCTATAGCCCCACTATC   152
goat    CAGAAAAGGAGAATAGCCAATCTCCCTAAGACTCAAGGAAGAAGCCATAGCCTCACTATC   300
        **** ******** * * ** ********************** *   ** * *** ***

cow     AACCCCCAAAGCTGAAGTTCTATTTAAACTATTCCCTGAACACTATTAATATAG------   330
sheep   AACACCCAAAGCTGAAGTTCTACTTAAACTATTCCCTGAATCATTATCAACGATACTTAT   212
goat    AGCACCCAAAGCTGAAATTCTATTTAAACTATTCCCTGAACCACTATTAACCACATCTAT   360
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Fig. F: Region of D-loop mitochondrial DNA identical between species

- Providing these universal primer sequences to conservators will be useful in future studies concerning the identification of species from parchment

Revealing Contamination

- Product in the negative control was identified as calf contamination and linked to Bovine Serum Albumin (BSA)
- BSA is a protein derived from cows. It is widely used as an enzyme-stabilizer, anti-adhesion agent, and concentration standard in biological experiments
- If the ingredient is not purified sufficiently, traces of *bos taurus* genomic DNA can be found in products containing BSA
- This interference poses a significant issue to scientists working to distinguish cow from other animal species using PCR

Fig G: Bovine Serum Albumin

Future Directions

- Ultimately, unable to successfully extract DNA from parchment. Aside from contamination from BSA, this may be due to low concentrations of short or damaged fragments of DNA.
- Experiments involving the amplification of the entire genome prior to PCR amplification are underway.
- Additional time is needed to investigate these methods of DNA extraction in hopes of making a significant contribution to the field of art conservation

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