



Max Planck Institute  
for Evolutionary Anthropology



# Improving the Recovery of Degraded DNA from Historical Skeletal Remains

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# Disclaimer



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# Talk Overview



- Introduction
  - Degraded DNA and Forensic Science
  - Ancient DNA methods
- Current study
  - Sample overview
  - Extraction method comparison
  - Library Prep comparison
- Conclusions/Outlook



# Historical Skeletal Remains



## Introduction

Degraded DNA

Ancient DNA

Current study

Sample overview

Extraction

Library prep

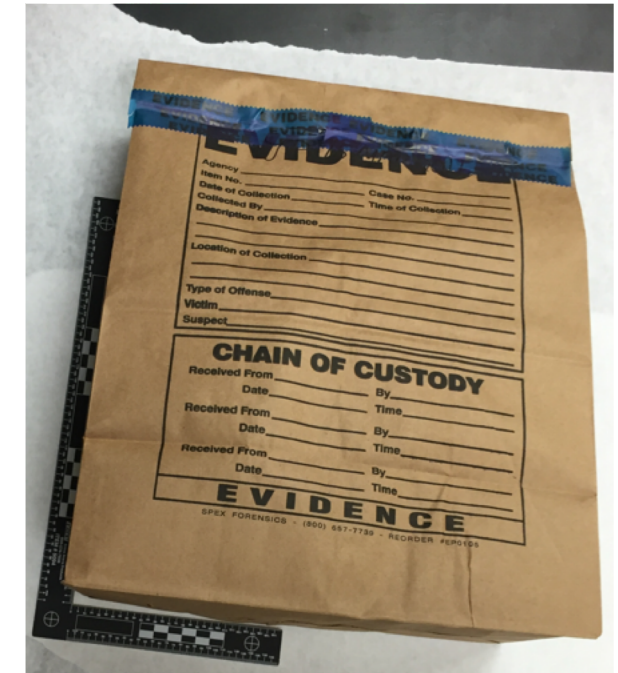
Conclusions/Outlook



Historical cases  
(e.g. Romanovs)



Victims of War



Remains from Cold  
Cases



# Historical Skeletal Remains



## Introduction

Degraded DNA

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Conclusions/Outlook

## Context Clues

- Location
- Clothing

DNA

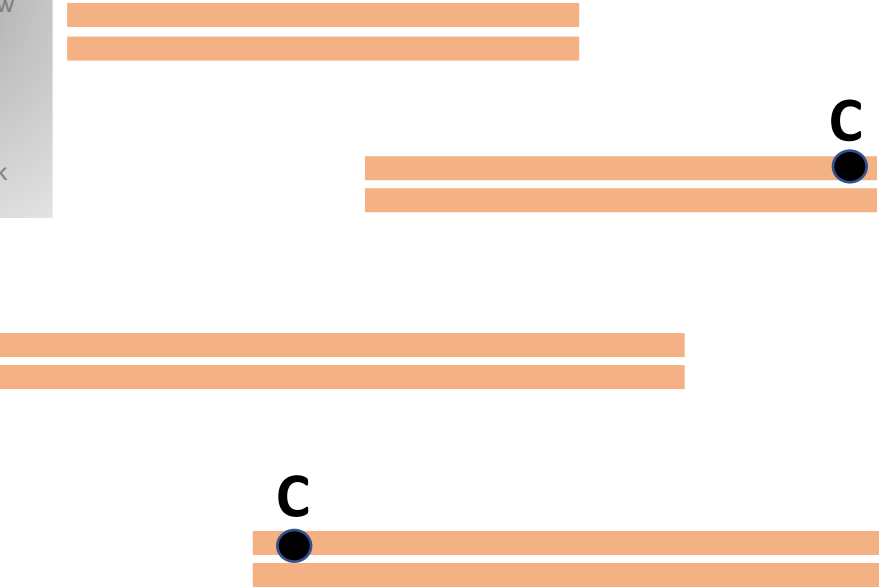
Identification



# Characteristics of Degraded DNA

- Introduction
- Degraded DNA
- Ancient DNA
- Current study
- Sample overview
- Extraction
- Library prep
- Conclusions/Outlook

## Modern DNA



## Degraded DNA



(Briggs et al., 2007; Krause et al., 2010; Sawyer et al., 2012)





# Forensic DNA Typing with Degraded DNA



Introduction

Degraded DNA

Ancient DNA

Current study

Sample overview

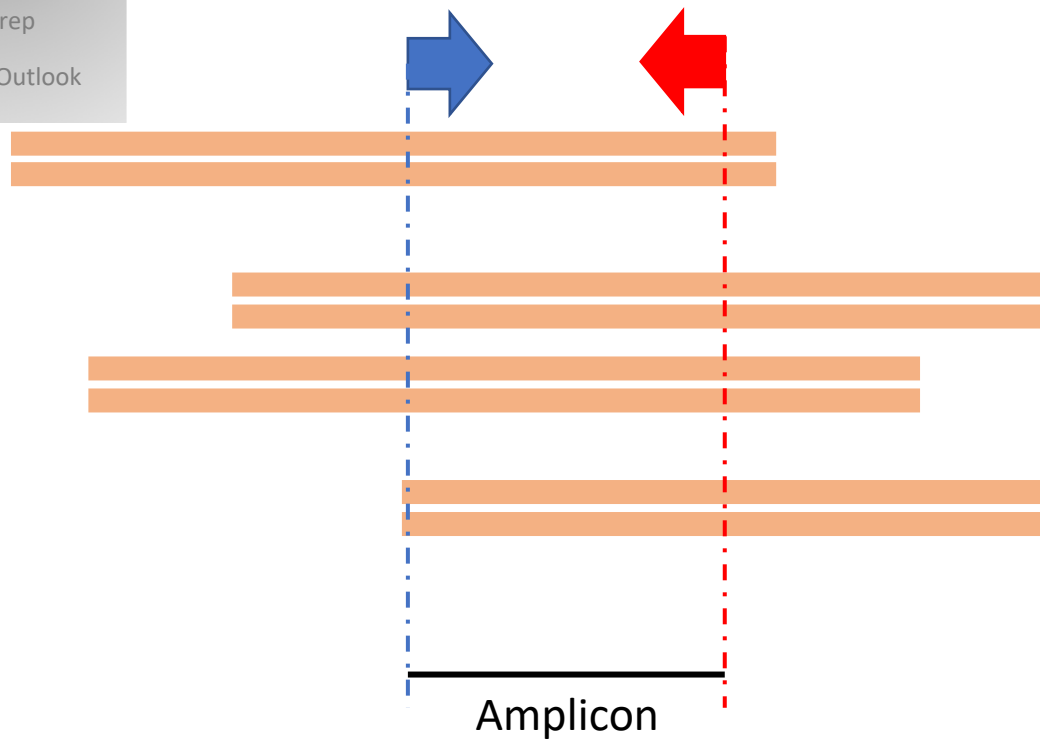
Extraction

Library prep

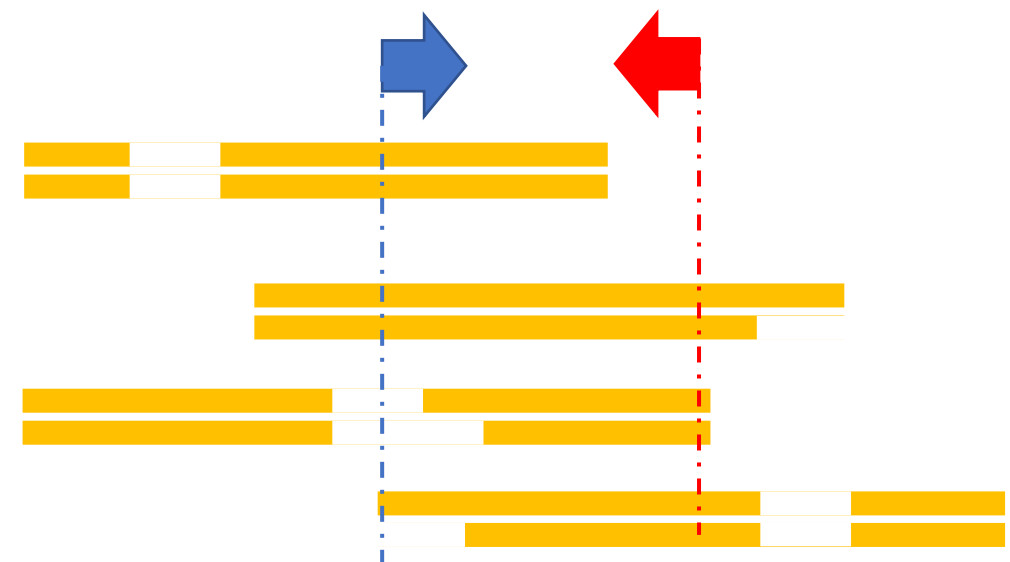
Conclusions/Outlook

## Modern DNA

Target specific loci



## Degraded DNA





# Forensic DNA Typing with Degraded DNA



Introduction

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Sample overview

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Library prep

Conclusions/Outlook

## Troubleshooting

- Increase input amount  
→ Mitochondrial DNA



## Introduction

Degraded DNA

Ancient DNA

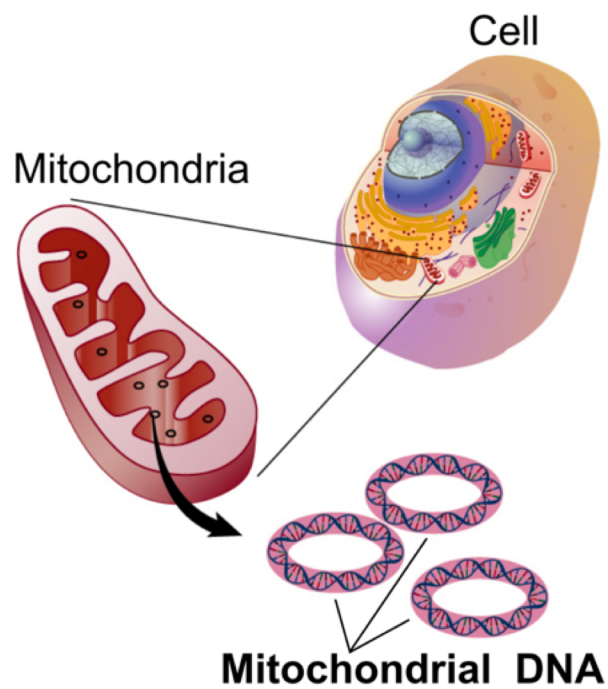
Current study

Sample overview

Extraction

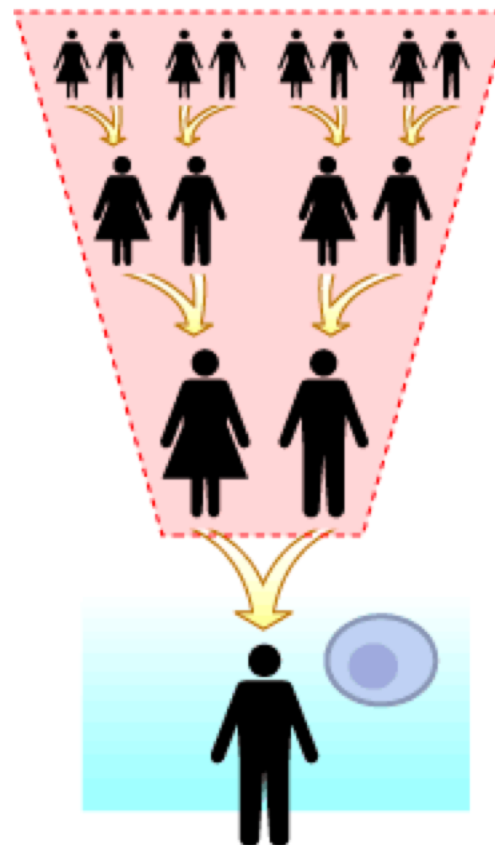
Library prep

Conclusions/Outlook



→ 100s to 1,000s copies per cell

Nuclear DNA is inherited from all ancestors.



Mitochondrial DNA is inherited from a single lineage.

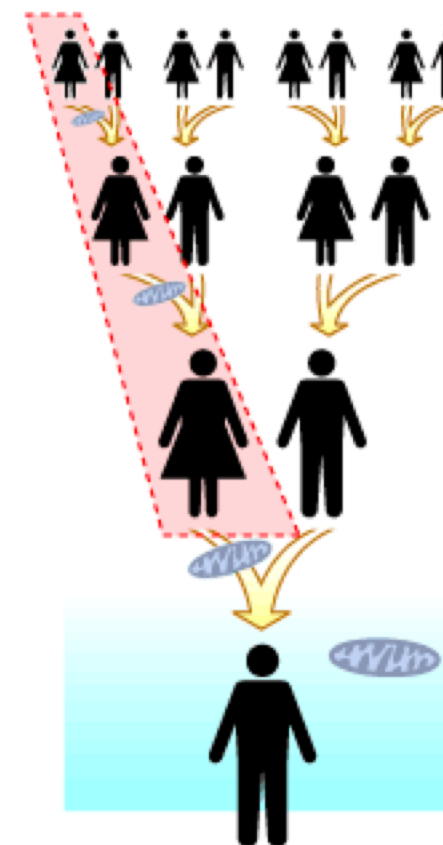


Image credits: National Human Genome Research and University of California Museum of Paleontology





# Forensic DNA Typing with Degraded DNA



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## Troubleshooting

- Increase input amount
- Decrease amplicon target size  
→ Next Generation Sequencing/Massively Parallel Sequencing (NGS/MPS)



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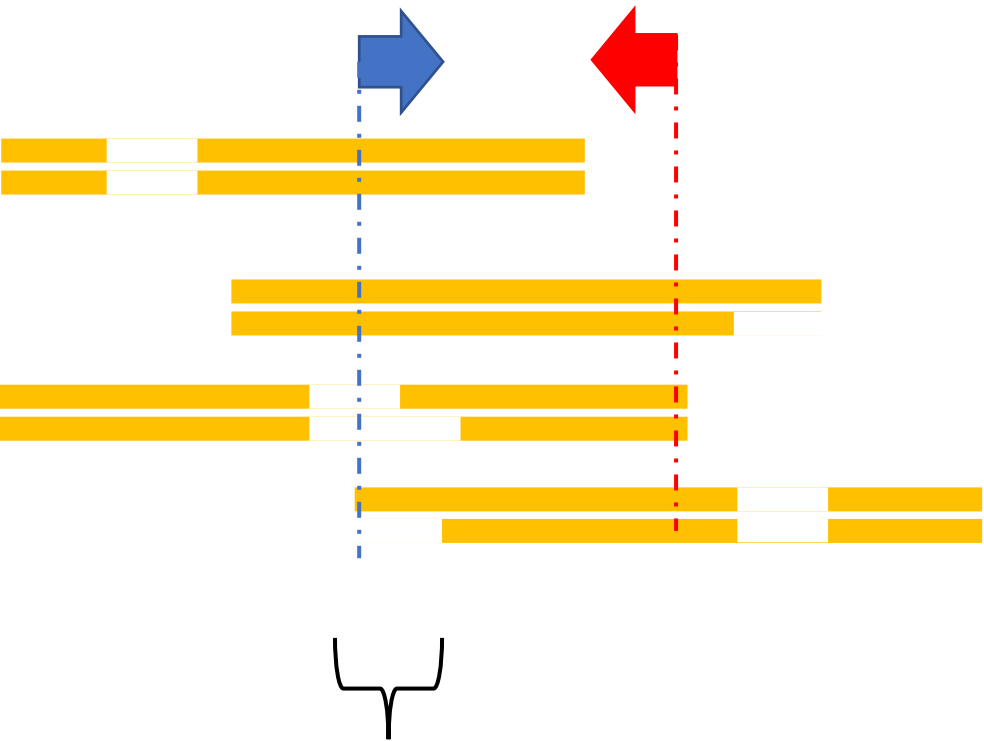
Sample overview

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Conclusions/Outlook

# Degraded DNA



- Limits to decreasing amplicon size
- Potentially lose information

Lost information



# Forensic DNA Typing with Degraded DNA



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## Troubleshooting

- Increase input amount
- Decrease amplicon target size
- Identify new methods for working with degraded DNA
  - MPS (AFMES-AFDIL)
  - Ancient DNA



# Ancient DNA

## Introduction

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Extraction

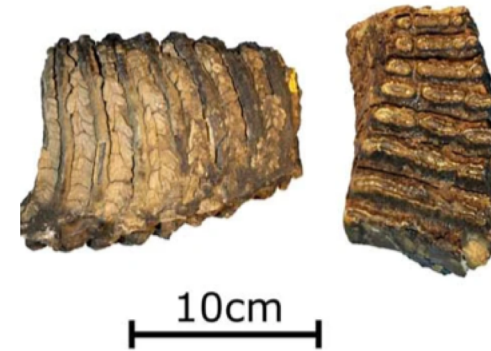
Library prep

Conclusions/Outlook



**First Neandertal DNA**

(Krings et al., 1997)



**Oldest DNA sequences:  
Mammoth (>1 million years old)**

(Valk et al., 2021)



**First Denisovan DNA**

(Krause et al., 2010)



**Hominin DNA from sediments**

(Slon et al., 2017)





# Ancient Sediment DNA: Reconstructing Occupational History (mtDNA)



Introduction

Degraded DNA

Ancient DNA

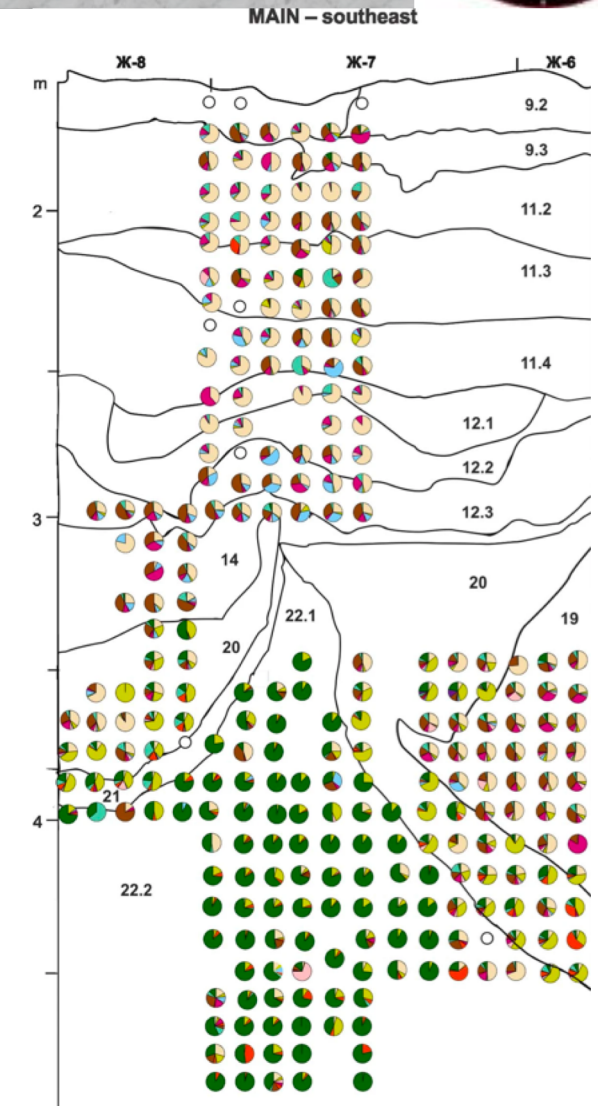
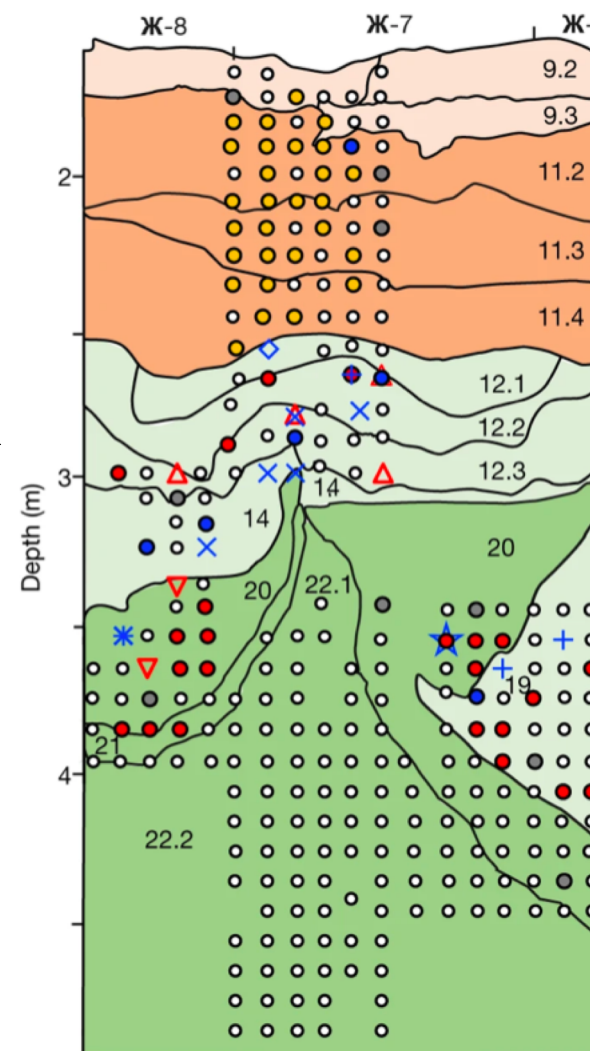
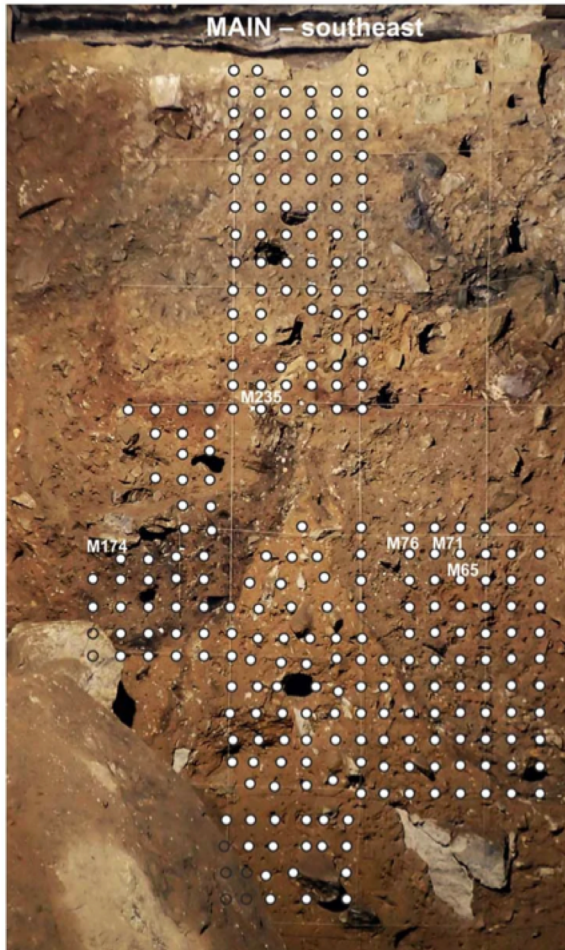
Current study

Sample overview

Extraction

Library prep

Conclusions/Outlook



Zavala et al., Nature 2021



Introduction

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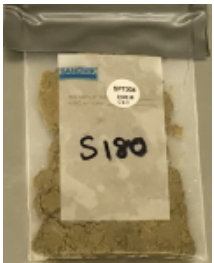
# Secret to Ancient DNA Success

## MPS + methods optimized for recovering and retaining degraded DNA

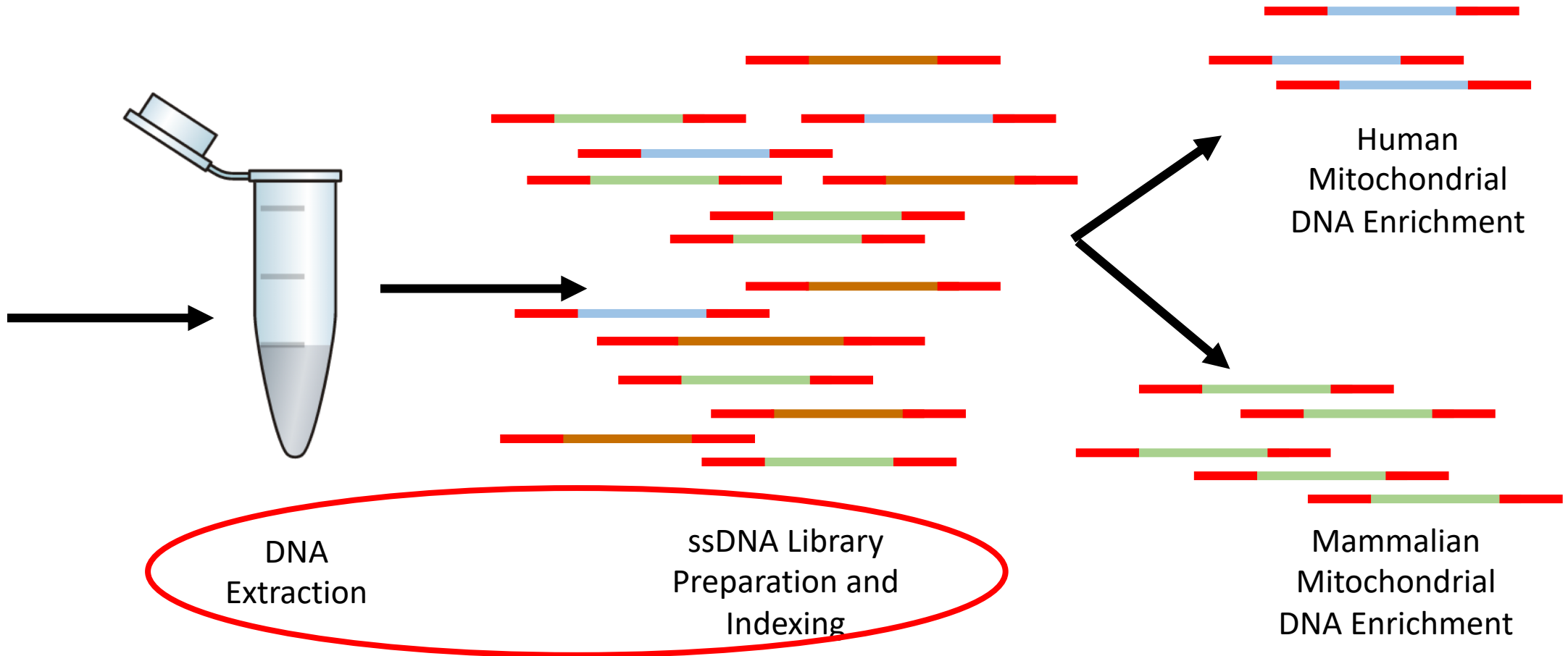


# Ancient DNA Workflow

- Introduction
- Degraded DNA
- Ancient DNA
- Current study
- Sample overview
- Extraction
- Library prep
- Conclusions/Outlook



Sampling  
~50mg







# DNA Extraction



## Introduction

Degraded DNA

Ancient DNA

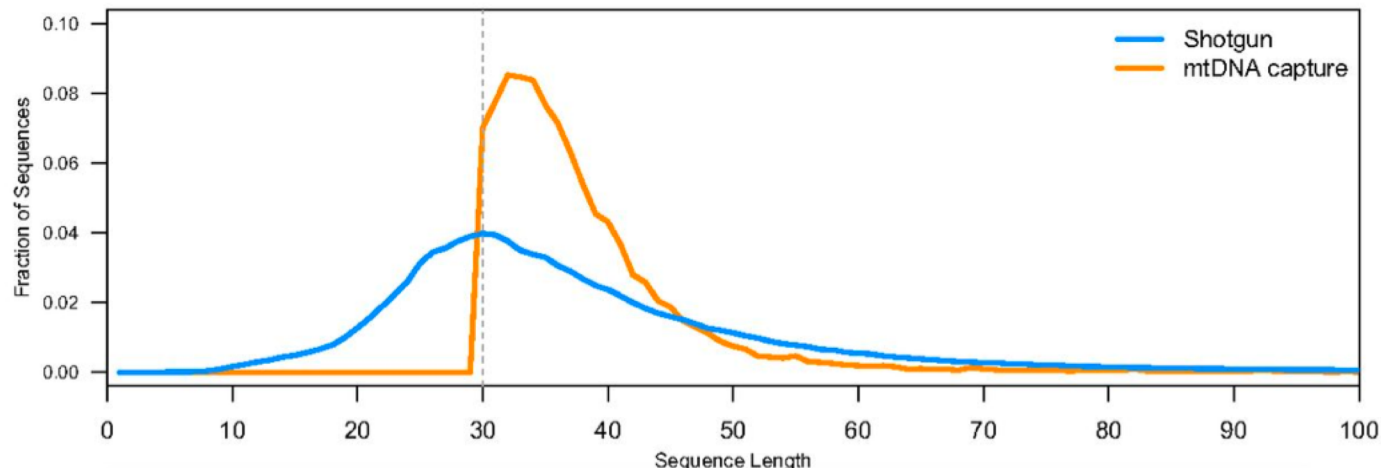
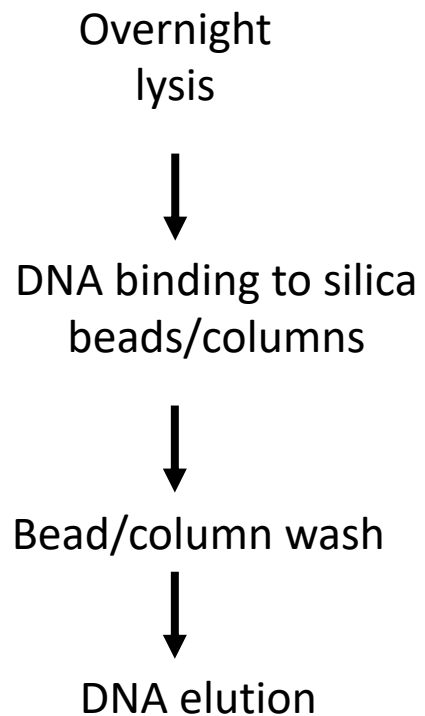
Current study

Sample overview

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Successful Recovery of Short DNA Fragments

Dabney et al., PNAS 2013



# Single Stranded DNA Library Prep

## Introduction

Degraded DNA

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Conclusions/Outlook

## Protocol step

3.1.1

3.1.2

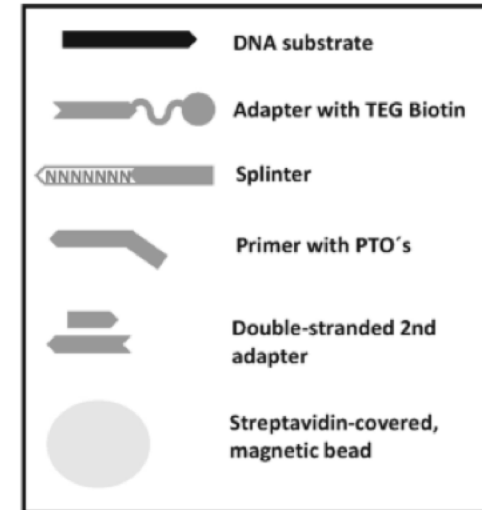
3.2.2

3.2.3

3.3.2

3.4.2

3.4.4



Conversion of all Available DNA Fragments into Library Molecules

Gansauge and Meyer 2013



# Hybridization Capture

## Introduction

Degraded DNA

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DNA library

Target human DNA

microbial DNA

Non-target human DNA

Target Regions of Human DNA

Enrichment of Target DNA

(Slide credit: V. Slon)



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Conclusions/Outlook

Ancient DNA →

Human Evolution  
Population Studies

Forensic DNA →

Identification/  
Individualization



Can methods from ancient DNA improve success rates for identification with historical remains?



# Armed Forces DNA Identification Lab (AFMES-AFDIL)



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Conclusions/Outlook

- Works on the identification of American soldiers
- Identifications from Korean War → available methods insufficient
- Validated a NGS/MPS protocol for degraded mtDNA in 2015
- First 18 months tested 153 samples from the Korean War:
  - 37 teeth: 57% success rate
  - 37 femurs: 48% success rate
  - 49 tibia: 47% success rate
  - 30 humerus: 30% success rate

**Success = Typed mitochondrial genome**

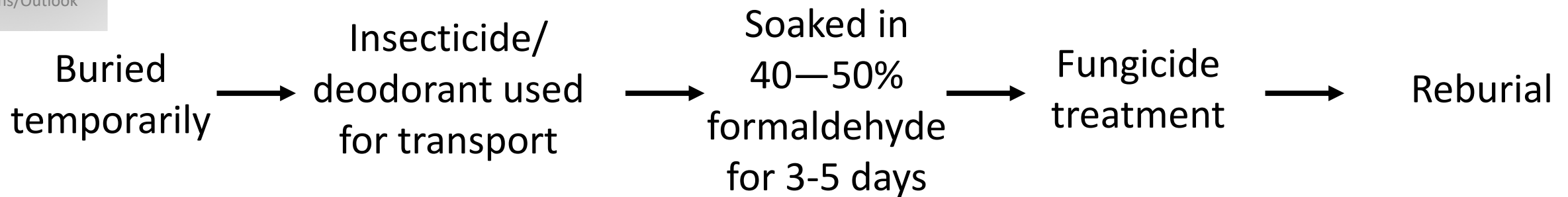
**44% overall success rate**





# Unidentified Historical Remains

15 previously tested long bones from unidentified soldiers  
from WWII and the Korean War



5 that are consistently successful

5 that sometimes successful

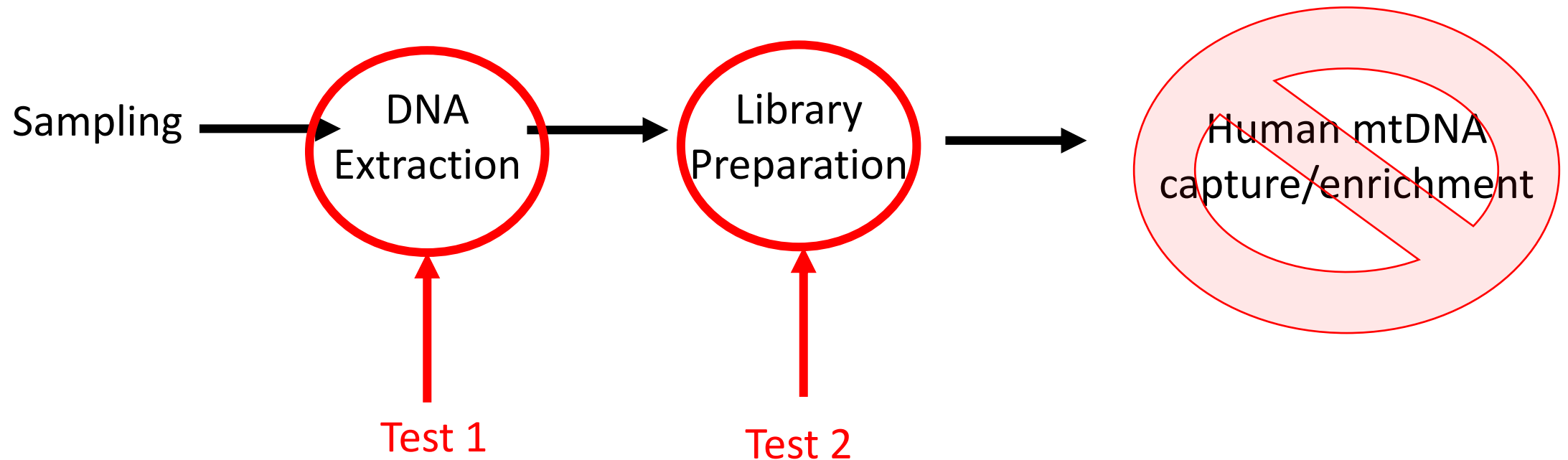
5 that consistently fail





# Project Outline

- Introduction
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- Ancient DNA
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# Test 1: Different Extraction Protocols

Introduction

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## Forensic Extraction Protocols

- PCIA + Min-Elute purification (P.M.)
- PCIA + **FFPE step** + Min-Elute purification (P.F.M)
  - For formalin fixed paraffin-embedded samples
- PCIA + **UDG treatment** + Min Elute purification (P.U.M)
  - Repairs base modification damage patterns on DNA

## Ancient DNA Extraction Protocol

- Dabney (37C incubation) (aDNA37)
  - Pellet re-extraction @56C + Dabney (aDNA56)
- } Same bone pellet

## Combined Forensic and Ancient DNA Extraction Protocol

- AFDIL digestion + Dabney purification



# Evaluation: Informative Sequences

Introduction

Degraded DNA

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Current study

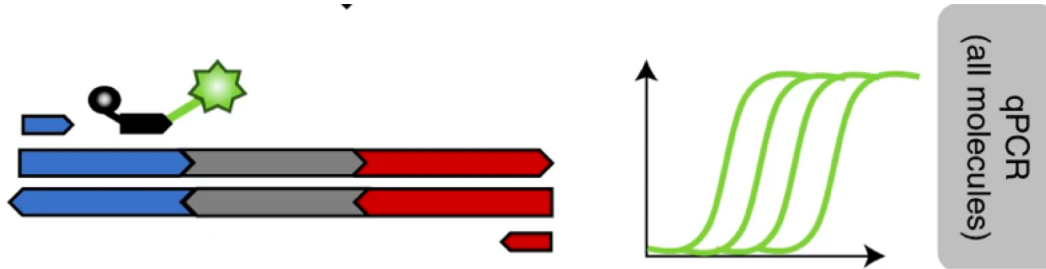
Sample overview

Extraction

Library prep

Conclusions/Outlook

## Quantification of library molecules



Gansauge et al 2020

Number of DNA molecules in the library

X

## Identify Informative sequences from sequencing data

$$\frac{\# \text{ sequences covering target regions}}{\text{total sequences}}$$

Proportion of Informative Sequences

=

Total Informative Sequences



# Test 1: Different Extraction Protocols

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Current study

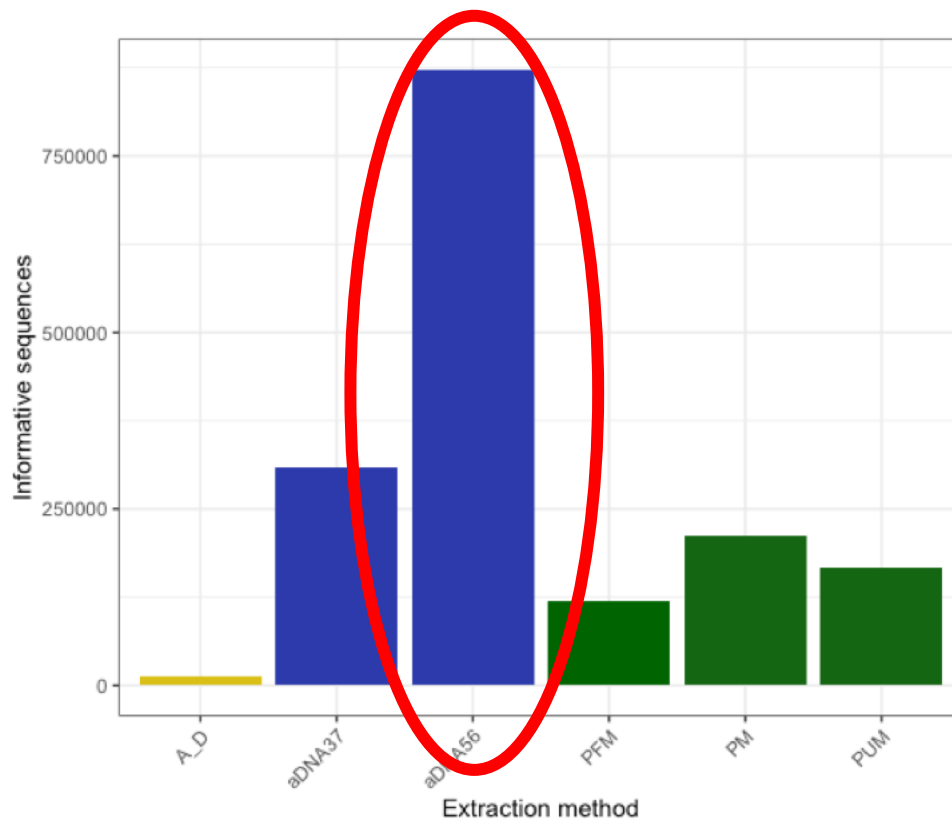
Sample overview

Extraction

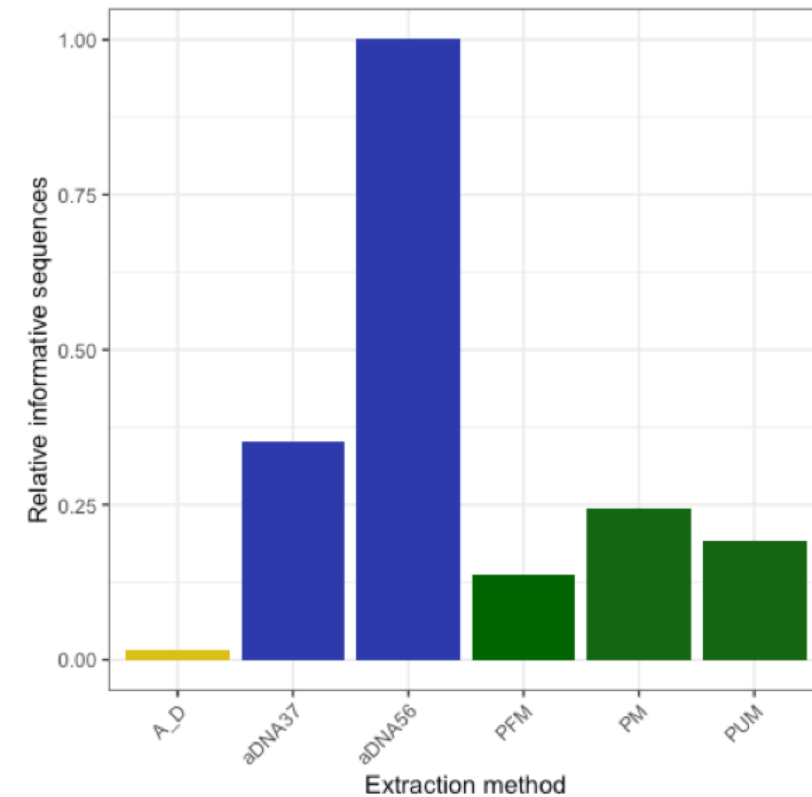
Library prep

Conclusions/Outlook

## Relative Informative Sequences



## Shotgun Data





# Test 1: Different Extraction Protocols

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Degraded DNA

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Current study

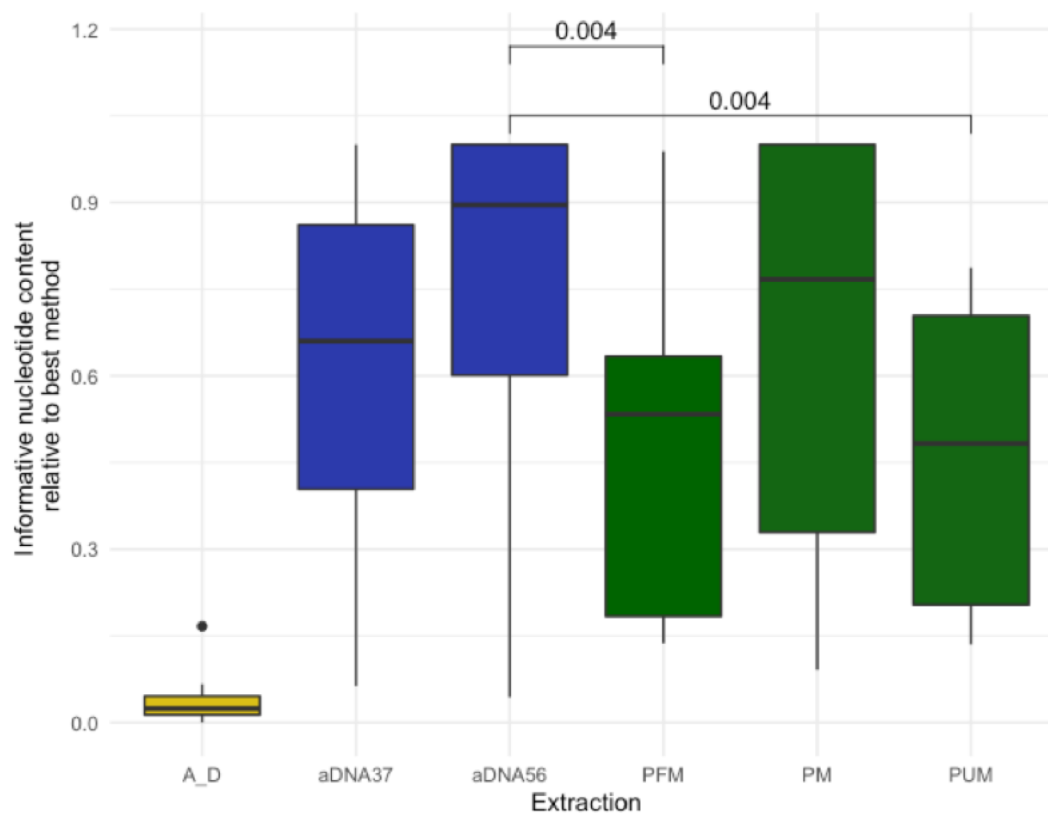
Sample overview

Extraction

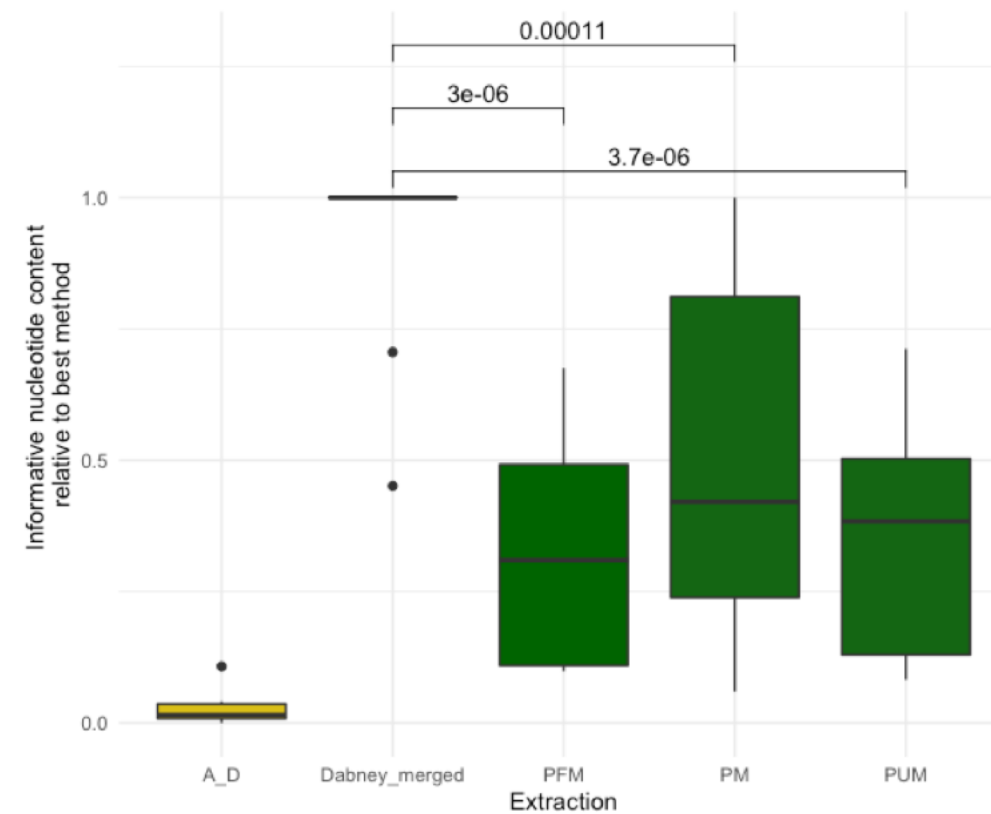
Library prep

Conclusions/Outlook

## Relative Informative Sequences



## Shotgun Data



Comparisons with Wilcoxon Test





# Test 1: Different Extraction Protocols



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Conclusions/Outlook

Ancient DNA extraction protocol  
performed significantly better



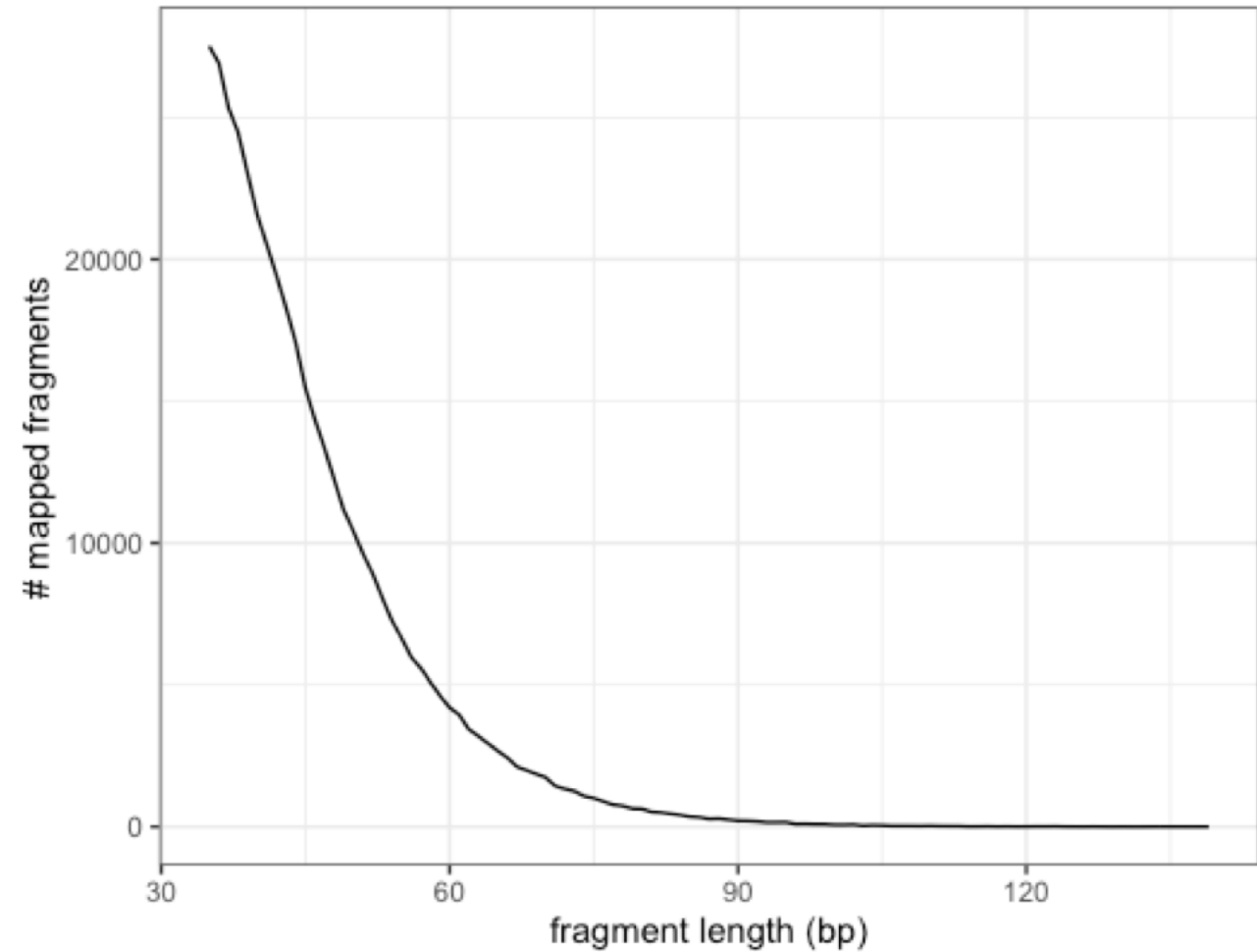
AFMES-AFDIL in the process of validating the  
ancient DNA extraction protocol (56C incubation)  
for their casework



# DNA degradation patterns



## DNA fragment size distribution







# DNA damage patterns



Introduction

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Current study

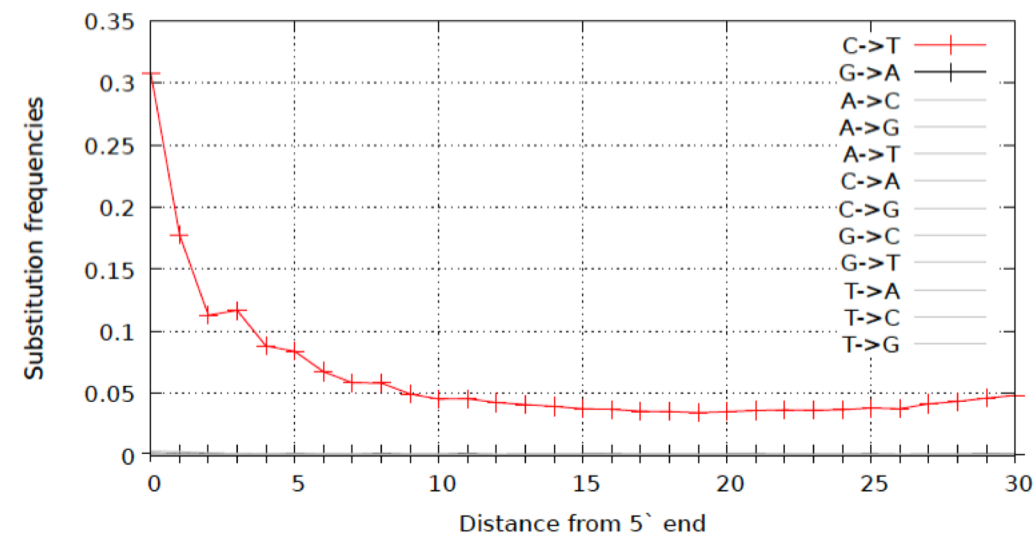
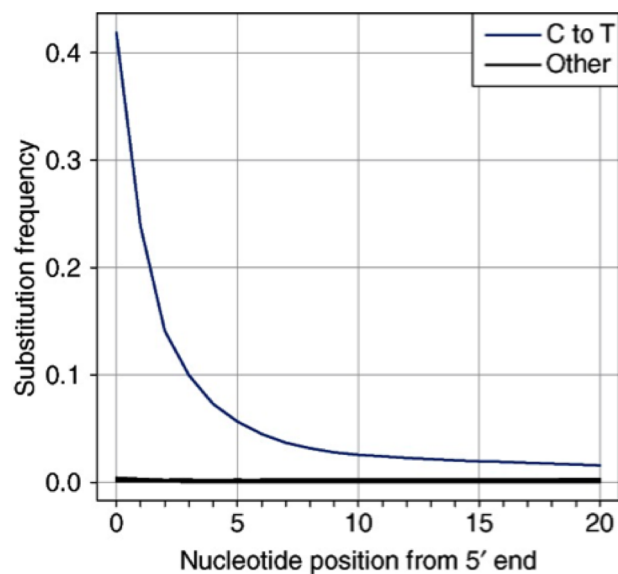
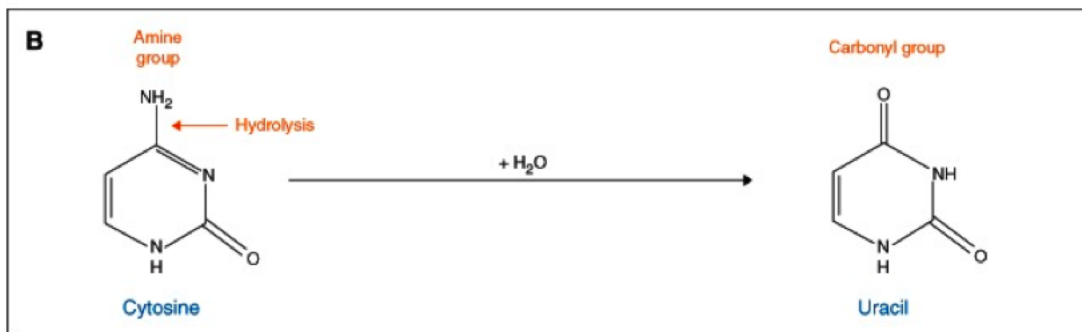
Sample overview

Extraction

Library prep

Conclusions/Outlook

## Deamination



Dabney et al., 2013



# Test 2: Different Library Preparation Protocols



Introduction

Degraded DNA

Ancient DNA

Current study

Sample overview

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Library prep

Conclusions/Outlook

Extract input:  
Ancient DNA extraction  
protocol  
(56C incubation)

KAPA Hyper dsDNA kit

SRSLY ssDNA kit

MPI ssDNA library prep



# Test 2: Different Library Preparation Protocols



Introduction

Degraded DNA

Ancient DNA

Current study

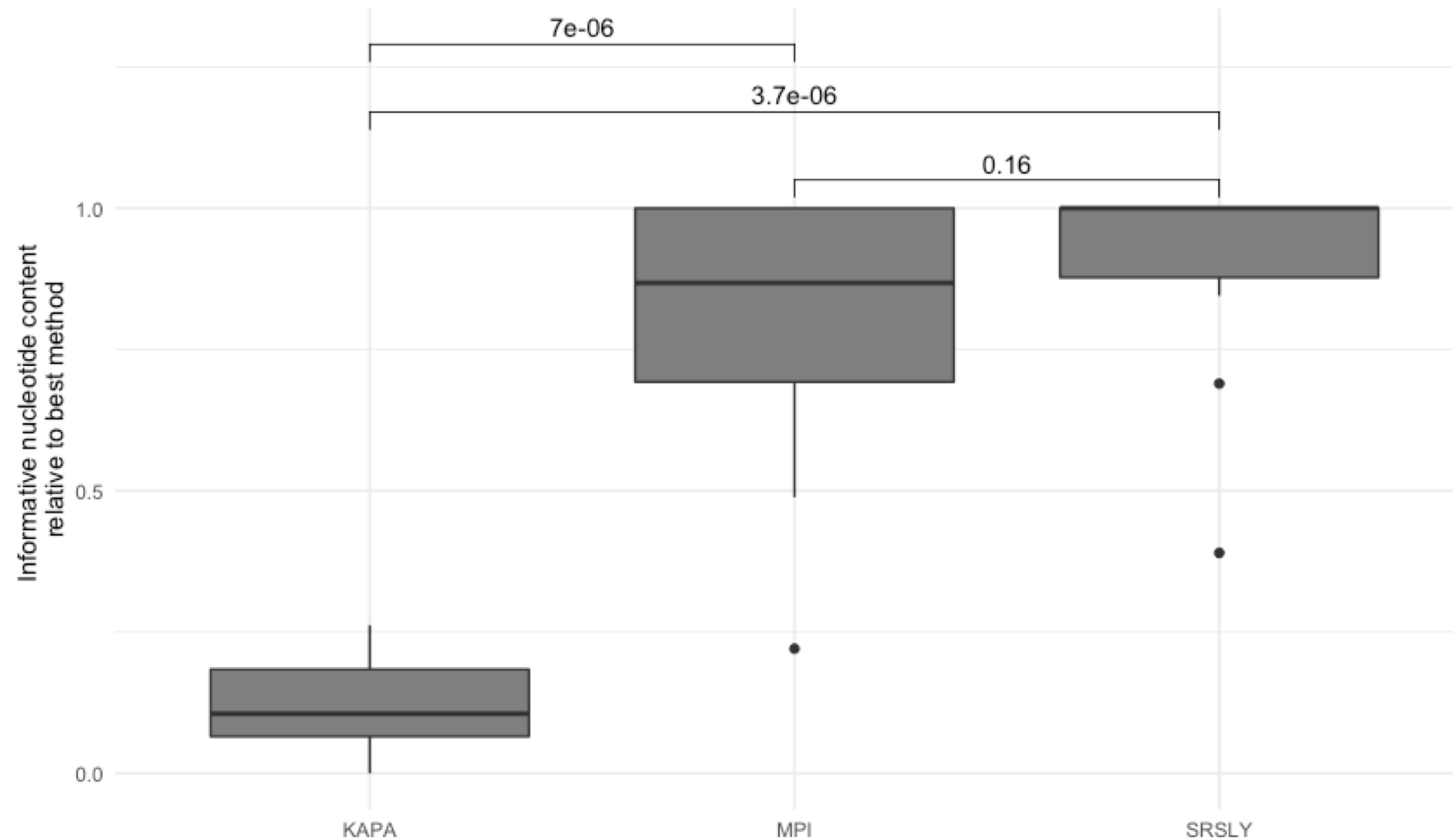
Sample overview

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Conclusions/Outlook

## Relative Informative Sequences



ssDNA library preparation methods recovered significantly more informative sequences



# Conclusions



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Conclusions/Outlook

- The integration of ancient DNA methods can increase the amount of recovered DNA from historical skeletal remains
- Future work
  - Test impact on mtDNA coverage after hybridization capture





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MAX-PLANCK-GESELLSCHAFT

**AFDIL**  
Armed Forces DNA Identification Laboratory





# Questions?

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