Resolution of an unidentified human remains case through recognition of Type II DNA damage

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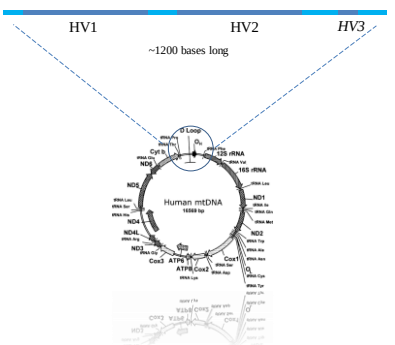
Case details

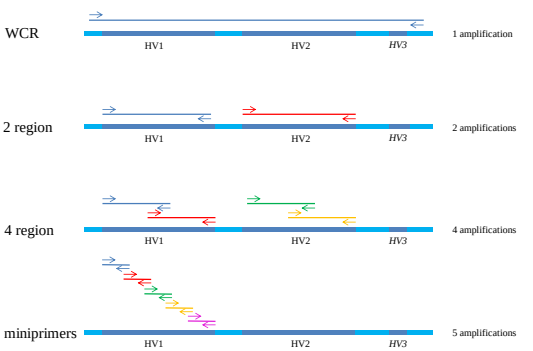
In December 2013, a passerby discovered a human tibia in Penn Treaty Park, Philadelphia, Pennsylvania.

The Philadelphia Medical Examiner's Office requested that the FBI DNA Casework Unit test the bone in order to develop nuclear and mitochondrial DNA (mtDNA) profiles for CODIS.

Mitochondrial control region



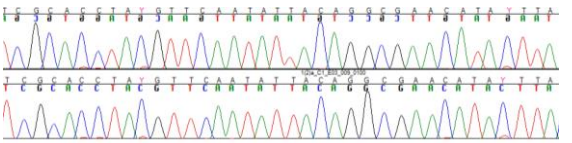
FBI mtDNA amplification strategies



In this case:

Tibia mixture

Due to the presence of mixed bases, the DNA Casework Unit (DCU) reported that the tibia was a mixture for mtDNA and therefore could not be reported.



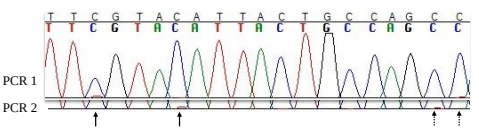
However, it was more complicated than this…

Tibia mixture

While mixed bases were present throughout the sequencing results, two patterns were apparent:

1) Of the >20 mixed positions in HV1/HV2, all were major cytosine (blue) with a minor thymine (red)

2) Upon reamplification, the mixed bases moved to different positions



Tibia mixture…or is it?

1) Would not expect a mixture in a bone.

2) Given a mixture, we would not expect positions of mixture to change after re-amplification.

3) Also, given a mixture, we would not expect all of >20 mixed bases to be 'Y' with C major/T minor.

So we have our doubts, but the SOP states that:

> **A mixture is a sequence with multiple mixed base positions.** Determination of a mixture is dependent on the length of sequence obtained, the expected sequence information, and the site of the mixed base positions. **If any region of a sample is determined to be a mixture, absent primer binding site mutations, the entire extract will be considered a mixture and not used.** If available, the sample will be re-extracted.

Hypothesis:

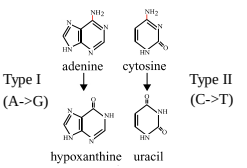
The mixed bases from the tibia are due to type II DNA damage, not a mixture of DNA from multiple individuals.

Type II DNA damage

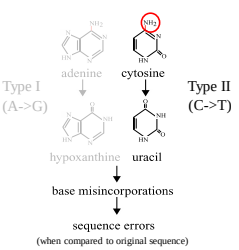
Type II DNA damage is one of two types of deamination observed in degraded DNA.

Climate (hot, humid) and time dependent.

Used by the ancient DNA community to authenticate our results.



Type II DNA damage

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AATCGATTCGACGAATTT
TTAGCTAAGCTGCTTAAA
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AATCGATTCGACGAATTT
TTAGCTAAGCTGCTTAAA

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AATCGATTCGAUGAATTT
TTAGCTAAGCTGCTTAAA

AATCGATTCGACGAATTT
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AATCGATTCGAUGAATTT
TTAGCTAA…

AATCGATTCGACGAATTT
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AATCGATTCGAUGAATTT
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AATCGATTCGAUGAATTT
TTAGCTAA…

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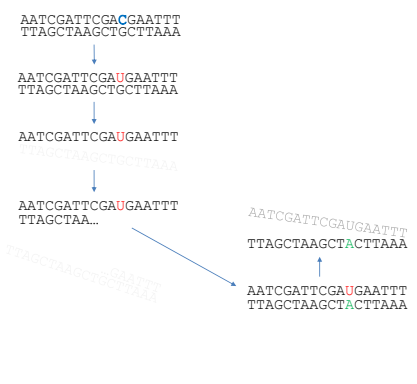
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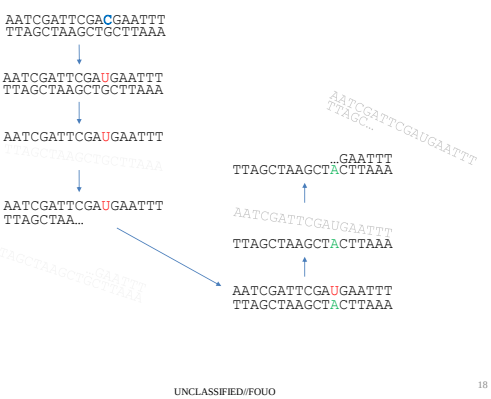
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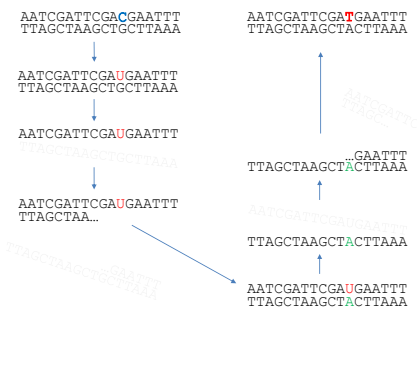
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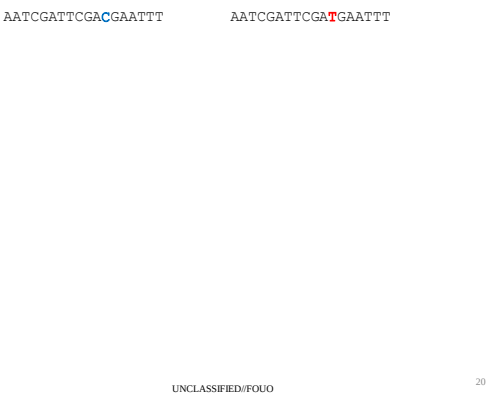
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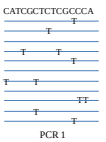
Type II DNA damage

Deamination is a (generally) random process and most of the DNA fragments in a sample will remain unaffected by damage at any given position. That's why:

1) There is a minor 'T' peak under a major 'C.' Most fragments still have a cytosine.

2) The mixed bases change positions upon re-amp. Each amplification starts from different molecules, damaged in different locations.



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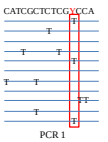

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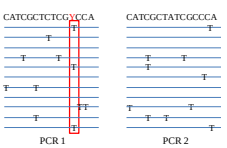

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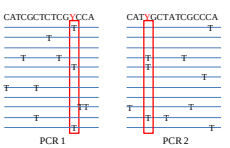
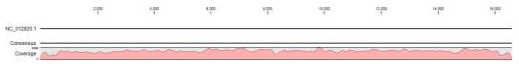
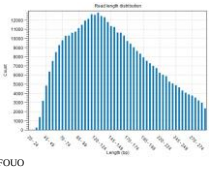


Next Generation Sequencing

In order to investigate the possibility that we were seeing Type II damage, we used a portion of the bone powder retained for methodology and development to generate NGS data, which allows us to examine individual DNA fragments from an extract.

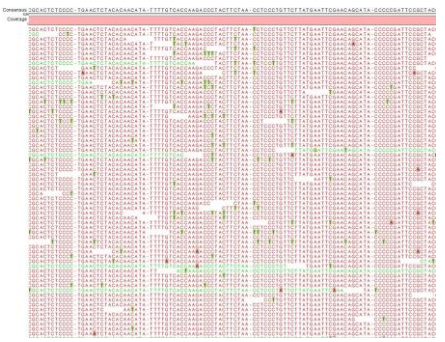
Hybridization capture was used to select for human mtDNA fragments before sequencing. Successfully sequenced whole mitochondrial genome.





Illumina MiSeq statistics:
12,830,544 total reads - 6,415,198 merged
404,771 mapped reads (duplicates removed)
Depth of coverage: 583x to 5296x (avg 3654x)
Mean mapped read length: 150 bases

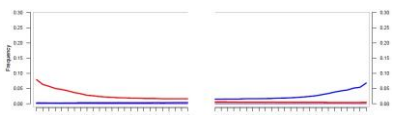
Next Generation Sequencing



Next Generation Sequencing

Deamination affects bases at or near the ends of the DNA strand more often that those internal to the strand.

Using a program called *MapDamage*, we can use this knowledge to verify that the damage is authentic, and not just sequencing error.



base misincorporation 5' end *base misincorporation 3' end*

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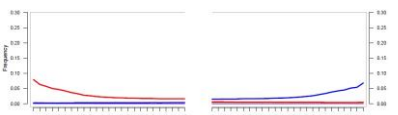

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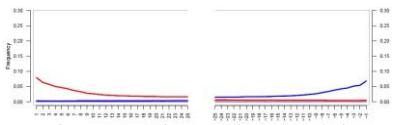

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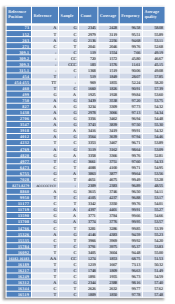

= mtDNA haplogroup B2B3
(Native American)

Carbon dating

Given the moderate level of DNA degradation observed, we sent a small amount of powder to Lawrence Livermore National Lab for carbon-14 dating.

Goal is to evaluate how reliable type II damage is at indicating an extended age in forensic samples – there's a lot of damage, but does it mean anything in a forensic setting?

CAMS #	Sample	$F^{14}C$	Date Range
176108	16-9-2-A	0.9620 ± 0.0037	1486 – 1649
176109	16-9-2-B	0.9576 ± 0.0036	1461 – 1636

Conclusion

The tibia from Penn Treaty Park is approximately **462 years old**, and possesses a **Native American**-derived haplotype.

The mixed bases observed in the casework mtDNA data were due to **type II DNA damage**.

Due to its higher resolution, next generation sequencing allowed us to generate useable data where traditional Sanger sequencing failed. Furthermore, we used **less material** and generated **more sequence**.

Thank you

The Organizers

Odile Loreille
Lara Adams
Jim Blankenship
Phila. ME's office

Jodi Irwin
Lilly Moreno
Tony Onorato
Rebecca Just