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The Body in the Ditch: Alternative Funerary Practices on the Northern Frontier of the Roman Empire?

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Supplementary Material

DNA Extraction from the Vindolanda North Field skeleton

Sample Preparation

A section of the bone, approx. 3cm x 2.3cm, was removed immediately adjacent to the area where a sample had previously been removed. All surfaces were sanded, cleaned and UV treated to remove any exogenous DNA prior to pulverisation using a freezer mill. A total of 6.9g of powdered sample was collected.

Extraction

Extraction protocol followed:

Rohland, N. & Hofreiter, M. (2007) Ancient DNA extraction from bones and teeth. Nature Protocols Volume 2(7) p.1756

Two independent extractions of the femur sample (3g each) were carried out sequentially following the above protocol. All necessary anti-contamination measures were taken throughout examination and processing.

Quantification

The nuclear DNA present in the two femur extracts was quantified by real-time PCR using the Quantifiler Trio kit (Applied Biosystems). This kit targets three multi-copy markers, a large autosomal fragment (214bp), a small autosomal fragment (80bp) and a Y chromosome fragment (75bp). If DNA is present but degraded we would expect to see the small fragment amplified but little or no amplification of the large fragment. There is also an internal positive control (IPC) with a known amplification range so that we can determine if inhibitors may be present in the sample.

Results

The results show that nuclear DNA is present in both of the femur DNA extracts and that it has originated from a male individual. There is a higher quantity of the small target present than the large target which is what we would expect to see for a degraded sample.

Sample	Large target (ng/µl)	Small target (ng/µl)	Sex	IPC*
Femur extract 1	0.004	0.035	Male	Y
Femur extract 2	0.008	0.071	Male	Y

*Internal positive control amplified within expected range with no inhibition observed

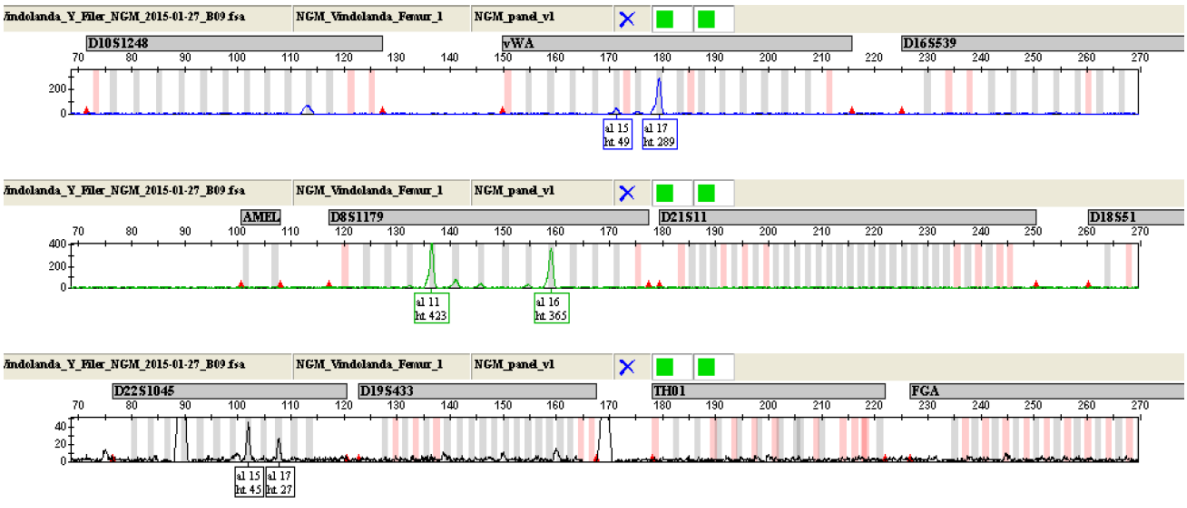
Amplification

The AmpFℓSTR® NGM™ PCR Amplification Kit (AB), which is a forensic DNA profiling kit, was used to amplify both of the femur DNA extracts. This kit contains 15 STR markers and Amelogenin as a sex marker.
Alleles were amplified at four loci in both extracts. Six out of the seven alleles were present in both profiles, confirming that these are from the same sample and resulting in the following consensus profile:

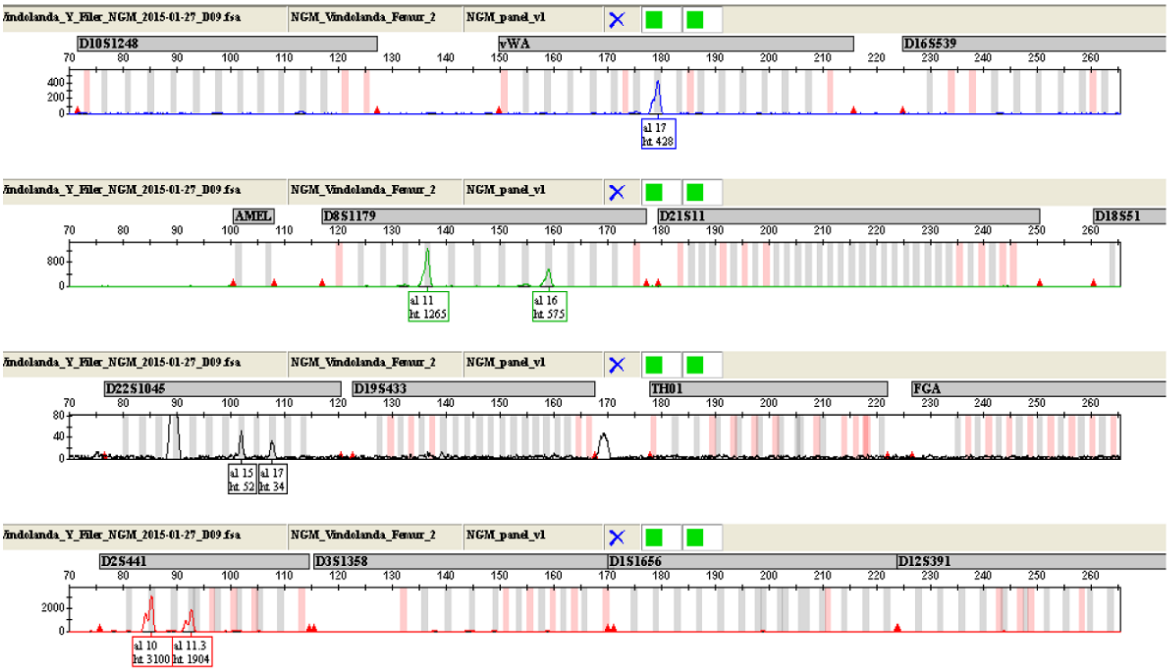
Locus	Femur
vWA	17
D8S1179	11,16
D22S1045	15,17
D2S441	10,11.3

The profiles appear to be single source and did not originate from the operator and also, as expected for a degraded sample, no alleles were present for any loci over 200bp. All this information together suggests that the DNA profile obtained from the bone sample is authentic.

NGM DNA profile Femur extract 1



NGM DNA profile Femur extract 2



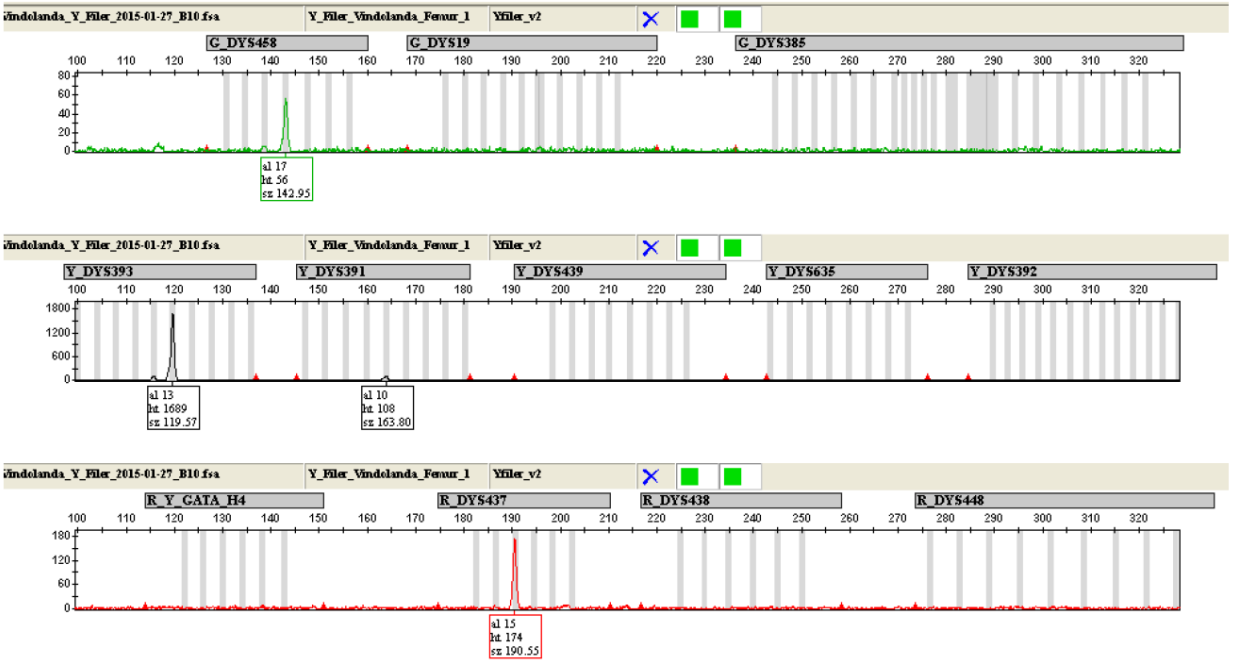
As no sex marker was amplified for either of the femur samples a further STR test was carried out using the AmpFℓSTR® YFiler™ PCR Amplification Kit (AB), which is a kit that targets 17 STR markers on the Y chromosome.

As can been seen from the profiles below, 4 loci were amplified for Femur extract 1 and 3 loci for extract 2, resulting in the following consensus profile:

Locus	Femur
Y_DYS393	13
Y_DYS391	10
R_DYS437	15

The Y Filer results confirm that the bone sample is male and again, that the two femur extracts are from the same sample and are single source.

Y Filer DNA profile Femur extract 1



Y Filer DNA profile Femur extract 2