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Multi-substrata analysis on Siberian mummies: A different way for validation in ancient DNA studies?

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Abstract. Ancient DNA results are always submitted to caution due to the technical difficulties induced by the minute amounts, the degraded nature of the template and the high risk of contamination. A list of criteria of validation has been published as a guideline for ancient DNA researchers [[1] A. Cooper, H.N. Poinar, Ancient DNA: do it right or not at all, Science 289 (5482) (2000) 1139.]. In addition to these criteria, the analysis of different substrates, i.e. bone, teeth and hairs of the same individual, could be another way to ensure the reliability of the results. This study presents the data obtained on bones, molar teeth and hairs from two Siberian specimens dated from the 18th century. These two subjects excavated from frozen graves were mummified. Their exceptional state of preservation allowed us to test the amplification of autosomal and Y chromosomal STRs and of the HVI region on the three types of substrate. This approach allowed the identification of artefacts on STR profiles, common when working with Low Copy Number DNA. Indeed, the comparison of the profiles obtained from bones and teeth highlights allelic dropouts and spurious alleles for the bone samples. The possibility to compare results from different substrates, in spite of the limited number of possible cases, represents another, and interesting, criterion to confirm the authenticity of ancient DNA results. © 2006 Published by Elsevier B.V.

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1. Introduction

Due to the technical difficulties induced by the minute amounts, the degraded nature of the templates and the high risk of contamination, the validation of the results in the

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field of ancient DNA became of major interest. The aim of this study was to determine in which way a multiple substrates analysis could be considered as a new criterion of validation. The discovery of two mummies in northeastern Siberia gave us the opportunity to study nuclear and mitochondrial DNA extracted from bones, molar teeth and hair shafts.

2. Materials and method

2.1. Materials

The two individuals were discovered in Yakutia, an autonomous Republic of the Russian Federation, located in the Northeast of Siberia. The extremely harsh and dry climate combined with inhumations directly on permafrost induced good DNA conservation.

The first grave called Munur Urekh (MU) dated from the late 17th century considering the archaeological material associated with the body. The coffin was filled with 5 cm ice and the body was partially frozen.

The Shamanic Tree (ST) grave was dated from the same period. This multiple grave contained four mummified bodies: two females and two males. The samples were taken from the best preserved specimen.

For the two subjects we collected a femur, some hairs and teeth without decay or slit.

2.2. Methods

Two grams of bone powder were extracted by a classic phenol chloroform protocol according to Keyser-Tracqui and Ludes [2].

Tooth samples were deeply decontaminated with DNA away, MilliQ water, absolute alcohol and UV light. The cleaned teeth were pulverised in liquid nitrogen using a 6800 Freezer Mill. DNA extraction was performed with the same protocol as bones.

Sections of 3 cm taken from the root side of 5 hair shafts were extracted as described by Gilbert et al. [3]. For each sample parallel extractions with or without cleaning were tested.

Nuclear DNA was quantified by Real Time PCR with the Quantifiler™ Human DNA Quantification Kit on an ABI PRISM™ 7000 SDS (Applied Biosystems).

Autosomal and Y chromosome STRs were analysed respectively with the AmpFlSTR® Profiler Plus™ Kit (Applied Biosystems) and PowerPlex® Y System (Promega). BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) was used for the sequencing of the HV1 region.

3. Results

As DNA concentrations were all inferior to 0.5 ng μl^{-1} and most of the extracts yielded DNA quantity lower than 100 pg, 34 cycles PCR amplifications were performed [4].

For autosomal STR analyses, tooth extracts gave the best results yielding complete profiles without amplification artefacts (Fig. 1). Moreover, beyond all expectations, we obtained better results with hair than with bone extracts. Indeed, allelic dropouts and spurious alleles were more frequent on the 2 different bone extractions. We have observed the same kind of results for the MU1 individual as well as for Y chromosome STRs.

Sample	Substratum	Allele(s) at marker									
		[1]Amel	D13	D18	D21	D3	D5	D7	D8	FGA	vWA
ST1	Tooth	XY	10/11	13/15	29/29	15/16	10/12	13/16	13/16	21/25	17/19
		XY	10/11	13/15	29/29	15/16	10/12	13/16	13/16	21/25	17/19
	Hair nW[2]	XY	10/11	.	29/29	15/16	10/12	13/16	13/16	21/25	17/19
	Hair W[3]	XY	10/11	**15/15**	29/29	15/16	10/12	13/16	13/16	**25/25**	17/19
	Bone ([4]Ext°1)	XY	**11/11**	**15/15**	29/29	15/16	10/12	**13/13**	**13/13**	21/**24**/25	17/**18**/19
		XY	**11/11**	.	29/29	15/16	10/12	13/16	13/16	**25/25**	17/19
	Bone ([4]Ext°2)	XY	.	.	.	15/16	.	**16/16**	**16/16**	.	.
		XY	.	.	.	.	**11**/12	**16/16**	**16/16**	.	**12/12**
	Consensus	XY	10/11	13/15	29/29	15/16	10/12	13/16	13/16	21/25	17/19

Fig. 1. Results of autosomal STR amplification with the Profiler Plus™ Kit (Applied Biosystems) for Shamanic Tree specimen. Allelic dropouts and spurious alleles are shown in bold font ([1]amelogenin, [2]not washed, [3]washed, [4]extraction).

Mitochondrial DNA sequencing revealed contrasted results. For the ST1 subject, the HV1 sequences obtained for the different substrates presented the same polymorphic positions. But the MU1 sequences highlight well known characteristics for the different substrates. For MU1, heteroplasmies were found on hair and tooth DNA extracts for the 16093 position, known as a mutation hotspot [5], whereas for the bone sample a T/C transition was observed.

4. Conclusion

As far as we know, this work illustrates for the first time the possibility to use hairs as a potential source of nuclear DNA in paleo-anthropological research. Moreover it demonstrates that this approach could represent a new criterion for the authentication of STRs profiles. Further analyses will be realised by this method in order to validate these preliminary results.

Considering mtDNA results, heteroplasmies remain difficult to interpret. This is probably due to the specificities of each substrate and to the sensitivity of the sequencing method. But other techniques, such as PNA probes, could be used to solve this problem.

For anthropological studies it seems obvious that the potential number of cases will be limited due to the rare number of cases with availability of the three types of substrates. Nevertheless this approach could be valuable for forensic identifications of degraded remains.

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