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Multiplex-PCR as tool for assessing ancient DNA preservation levels in human remains prior to next-generation sequencing

ANGELA GRAEFEN[1], MARTINA UNTERLÄNDER[1], HERMANN PARZINGER[2], JOACHIM BURGER[1]

[1]*Arbeitsgruppe Palaeogenetics Group, Institute of Anthropology, Mainz University, info@bioarchaeology.de. 2Stiftung Preussischer Kulturbesitz – Prussian Cultural Heritage Foundation, Berlin*

Next-Generation high throughput techniques, which allow an enormous amount of genetic material to be sequenced within a relatively short time, are becoming increasingly popular within the field of palaeogenetics. In recent years, these techniques have enabled large-scale projects such as the sequencing of the entire mitochondrial genomes of the Neanderthal and the woolly mammoth, which would hardly have been possible with standard PCR. However, the high running costs of next-generation technology necessitate a careful sample selection. Whilst DNA damages are continually repaired in the living, healthy organism, various factors such as endogenous processes, environmental influence and soil composition cause increasing degradation of the DNA after death. Due to the high copy number per cell, mitochondrial DNA can often be extracted and sequenced. On the other hand, nuclear DNA with only two copies per cell, or y-chromosomal DNA with only a single copy per cell in the male individual, tends to be much more difficult to amplify successfully. To estimate the quantitative and qualitative aDNA preservation status for specific samples, a multiplex PCR was developed, allowing multiple segments of varying sizes from nuclear and mitochondrial genome to be amplified in one run and thus providing information on the degree of strand damage. This way, the expected success rate of Next-Generation sequencing of a given sample can be determined beforehand, increasing the cost-effectiveness of such methods by only using promising sample material. The multiplex PCR was developed and tested on Iron Age skeletons from East Kazakhstan and the Altai within the scope of the project "Palaeogenetic analyses of economic advances and social mobility within the Eurasian Steppe 3500–300 B.C.".