STR GENOTYPING OF HIGHLY DEGRADED DNA: REPRODUCIBILITY AND METHODICAL OPTIMIZATION

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The reproducibility of STR-genotype analysis of highly degraded or "ancient DNA" can be reduced due to the generation of artifacts during the PCR process. This holds especially true if ancient DNA extracts contain low quantities of severely degraded DNA. Low target quantities may lead to "allelic dropout", resulting in false-homozygous genotyping of a sample[1]. Due to a parallel intake of co-extracted polymerase inhibiting substances, the amount of extract added to a PCR-reaction is limited. One possibility to overcome obstacles resulting from allelic dropout is to analyse five amplification products per sample and locus investigated, reducing the probability of false-homozygous typing to a minimum of 0,008[2]. An artifact intrinsic to the amplification of repetitive sequences is the generation of so-called shadow bands[3]. In ancient DNA amplifications, shadow bands can exceed the peak height or band intensity of the allele product, resulting in seemingly different genotypes for products of the same sample[2].

Both allelic dropout and the degree of the generation of shadow bands are related to quantity and quality of extracted DNA[1, 4]. Consequently, an important strategy to increase the reproducibility of STR-genotyping of samples containing degraded DNA is the optimisation of DNA extraction.

The material chosen for this investigation consisted of archaeological bone samples of different age and environmental sources containing DNA with different degrees of degradation[5]. Starting from a standard DNA extraction protocol[6], systematic variation of parameters resulted in optimised parameters for the materials investigated. The resulting protocols were tested by re-extraction of this material, comparing the results of optimised protocols with the standard protocol[7, 8].

This investigation permitted the development of optimized protocols for the extraction of DNA with three different degrees of degradation and a consensus protocol designed for DNA extraction from skeletal material containing DNA in a wide range of preservation states[5, 7, 8].

[1]Kimpton C, Fisher D, Watson S, Adams M, Urquhard A, Lygo J, Gill P (1994) Evaluation of an automated DNA profiling system employing multiplex amplification of four tetrameric STR loci. Int J Leg Med 106: 302-311
[2]Schmerer WM, Hummel S, Herrman B (1997) Reproduzierbarkeit von aDNA-typing. Anthrop Anz 55(2): 199-206
[3]Weber JL, May PE (1989) Abundant class of human polymorphisms wich can be typed using the polymerase chain reaction. Am J Hum Genet 44: 388-396
4 Murray V, Monchawin C, England PR (1993) The determination of the sequences present in the shadow bands of a dinucleotide repeat PCR. Nucleic Acids Res 21(10): 2395-2398
[5] Schmerer WM, Hummel S, Herrmann B (1999) Optimized DNA extraction to improve reproducibility of short tandem repeat genotyping with highly degraded DNA as target. Electrophoresis 20: 1712-1716
[6]Lassen C, Hummel S, Herrmann B (1997) PCR based sex identification of ancient human bones by amplification of x- and y-chromosomal sequences: a comparison. Ancient Biomolecules 1: 25-33
[7] Schmerer WM (2000) Optimierung der STR-Genotypenanalyse an Extrakten alter DNA aus bodengelagertem menschlichen Skelettmaterial. Cuvillier, Göttingen
[8] Schmerer WM, Hummel S, Herrmann B (2000) STR-genotyping of archaeological human bone: Experimental design to improve reproducibility by optimisation of DNA extraction. Anthrop Anz 58(1): 29-35