PCR OPTIMIZING STRATEGIES: REDUCTION OF THE ACCUMULATION OF STUTTER BANDS IN STR AMPLIFICATIONS OF MODERN AND HIGHLY DEGRADED DNA

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The generation of so called stutter bands or shadow bands - by-products of the PCR amplification differing in length from the original allele by one repeat unit[1] - is a well known problem in connection with the amplification of repetitive DNA like short tandem repeat loci. The occurrence of this artifact can strongly complicate genotype analysis of repetitive sequences, sometimes leading to the necessity to develop guidelines for allele designation. Especially in the amplification of highly degraded or ancient DNA, the generation of these by-products can be intensified[2], resulting in shadow bands that can exceed the peak height or band intensity of the real allele. As "artifact alleles"[3] these intense shadow bands can lead to mistyping of amplification products. Since an amplification product derived from degraded DNA is not necessarily in each case affected, this phenomenon can result in seemingly different genotypes for independent products of the same sample[3]. To improve the reproducibility of amplification results and consequently decrease the probability of mistyping by reducing the generation of this artifact, the optimization of the PCR amplification process itself represents the most important strategy starting from target DNA extracted by the use of optimized protocols[4].

Within this context, an investigation to optimize the amplification of repetitive sequences from non-degraded modern and highly degraded DNA with the aim to analyse and reduce the generation of shadow bands was carried out[5]. In this study the STR locus HUMVWA31/A was employed using the primers published by Kimpton *et al.*[6]. Amplifications of this locus - in comparison to many other STR loci - show a strong tendency for the accumulation of shadow bands[6, 7].

The results of this investigation showed that the generation of shadow bands during the amplification process can be reduced by up to 30% by changing parameters of the denaturation and elongation step, addition of reagents like betaine, betaine and DMSO or BSA, replacement of reaction buffer and polymerase, or adjustment of the A/T.G/C ratio of the dNTP mix according to the sequence amplified[5].

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