Characterization of the N+3 stutter product in the trinucleotide repeat locus DYS392

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DYS392 is a Y-chromosome trinucleotide short tandem repeat (STR) featured in most Y-STR multiplex assay systems. The PCR amplification of the DYS392 locus typically results in three distinct detectable PCR products: the true allele product (N), a stutter product three bases smaller (N-3) and a reproducible low-level product, three bases larger (N+3). Sequence analysis of the N+3 product indicated that its sequence is one TAT repeat longer and the N-3 product contains one less repeat than the true allele product. Our experiments demonstrated that both N-3 and N+3 stutter percentages increased as (1) the allele number increased, as (2) the magnesium concentration was increased in the reaction or if (3) the amount of input DNA was decreased. Different primer sequences had very small effects on stutter formation. Since both N-3 and N+3 stutter products behave in a similar and reproducible fashion, the rules that apply to the interpretation of N-3 stutter products in STR analysis can be applied to N+3 stutters.