ALLELE DROP-OUT AT LOCI D13S317 AND CD4 ASSOCIATED WITH VARIATIONS IN PRIMER SEQUENCES

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With the typing of large series of DNA samples, one could expect to find very low frequency variants of the 'normal' sequence. The majority of such polymorphisms would probably pass unobserved, except when occurring in primer sequences where they could alter the affinity of the primer for the template. Such variations could affect the quantitative yield of the PCR product and in the worst scenario, abolish the amplification. We believe sequence variation is the major cause of what is called allele 'drop-out'.

We accidentally found complete allele drop-out for STR locus D13S317. A very rare allele 7 was found in two brothers of Asian origin and we decided to characterize this allele more in detail by sequencing. To our surprise, allele 7 was not amplified when other primer pairs were used. This phenomenon was also observed using STR kits form different commercial suppliers. Cloning and sequencing finally revealed a 4-nucleotide deletion in the primer of the drop-out allele in combination with a 4-nucleotide insertion in the non-repeated sequence of the STR.

During a paternity test including locus CD4, we found a paternal mutation (1 exclusion for 9 loci tested). In a second case, complete absence of paternal alleles was found for the same locus. When typing of this locus was repeated, sometimes a faint band corresponding to allele 5 was observed. Sequencing of cloned alleles revealed a variant allele 5 sequence presenting a nucleotide substitution in the 3' penultimate position of the forward primer. This variation, with a 1 nucleotide difference in primer sequence, was reported before and amplification yield in function of amplification conditions was studies in detail (Watanabe et al., J Forensic Sci 1988; 43: 733-737). PCR product yield with variation in the penultimate of ultimate 3'-nucleotide of a primer sequence is largely depending on the nature of the (mis-)paired bases and on the experimental conditions, which could explain the problem to reproduce results.

In conclusion, several thousand chromosomes are actually typed by a large number of laboratories and polymorphisms at very low frequency will be found in sequences corresponding to primer sequences. These polymorphisms affect the efficiency of hybridization of primer and template and could results in partial or complete drop-out. Comparative databank searches (e.g. D13S317) should take this possibility in account. In paternity testing (e.g. CD4), it is clear that exclusions should not be based on a single locus and even on two loci is questionable. Since mutations changing the repeat number during meiosis are usually small, the observation of large differences associated with homozygosity (e.g. in paternity testing), is suggestive for allele drop-out.