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Short communication

Identification of more sequence variations in the D8S1179 locus

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Abstract

Routine STR-typing of 10,293 buccal swabs using different multiplex kits presented discordant D8S1179 profiles in four cases. Sequencing analysis identified a G-to-A transition upstream to the repeat, and an A-to-T transversion and a G-to-A transition downstream to the repeat. In the fourth case a four-base pair deletion downstream resulted in altered genotypes using different primer pairs. Current searching algorithms of the German DNA database are not capable of matching profiles that are divergent in only one STR-locus. Thus, to accommodate matching requirements and to avoid errors in individual genetic characterization for D8S1179, as described here, it is suggested that alternative primer pairs be used for routine genotyping as a matter of course.

Keywords: D8S1179; STR; Sequence data; Mutation; Null allele

1. Introduction

D8S1179 (CHLC 1995, GenBank accession number G08710) is one of the microsatellites recommended for STR-typing by the *European Network of Forensic Science Institutes* (ENFSI) and included in the German national DNA database. A variety of commercially available kits are used to identify human remains, forensic specimens and in paternity investigations. However, multiplex kits provided by various manufacturers mostly contain different primer sets. Potentially, differences in the primer binding regions could adversely affect efficient primer–template interaction and may reduce the product yield leading to allelic dropout [1–6]. For the locus D8S1179 the occurrence of null alleles has already been described when using multiplex kits from Promega Corporation and Applied Biosystems [7–11].

The goal of our studies presented here is the identification of the underlying mechanism of four discordant profiles found in samples typed with the Mentype® Nonaplex Twin kit supplied by Biotype AG, Germany.

2. Material and methods

Genetic characterization of 10,293 individuals for the German database was carried out using buccal cell swabs and semi-automated DNA extraction operating on a Qiagen Bio Robot 9604. At least 90% of the tested persons were Caucasians living in Germany. Amplification of D8S1179 was performed using the commercially available multiplex PCR kits Nonaplex I and II according to the manufacturer's instructions (Mentype® Twin, Biotype AG, Dresden, Germany). To quote the manufacturer's information, primer pairs of these multiplexes are shifted at least six base pairs

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Table 1
Amplification results of the variant allele compared to the common allele using different multiplex kits

Variant allele	Nonaplex I	Nonaplex II	SGM	PP16
13V	Full amplification	Reduced yield	Full amplification	No product
10V	No product	Full amplification	Reduced yield	Full amplification
16V	No product	Full amplification	No product	Full amplification
12V	Heterozygous (11/12)	Homozygous (12)	Heterozygous (11/12)	Heterozygous (11/12)

to detect variations in the primer binding regions. Samples with varying profiles were subsequently amplified using the PowerPlex® 16-kit (Promega Corporation, Madison, WI) and the AMPFlSTR® SGM plus™ kit (Applied Biosystems, Foster City, CA). Fragment analysis was performed on an ABI PRISM® 3100 Genetic Analyzer using Genotyper software, Version 3.6 NT (PE Applied Biosystems, Foster City, CA). In addition, verification of sample fragment length was achieved by comparison with fragments of the corresponding allelic ladder supplied by the manufacturers. For sequencing analysis a new primer pair localized largely outside the common amplicon was designed according to the ENSEMBL sequence for D8S1179 (ID 8.125862668-125864848). The direct Taq-cycle-sequencing method was performed as previously described [12].

3. Results and discussion

In routine testing of 10,293 buccal swabs using the Nonaplex I and II twin system, discordant D8S1179 profiles were observed in four cases. Table 1 lists the amplification results of the variant alleles compared to the common alleles using different multiplex kits. Sequencing analysis of the four cases with presumable variations identified four different variations in the repeat flanking region (Table 2 and Fig. 1). The variant alleles showed the reported repeat structures [13], except allele 13 V which exhibited only a simple [TCTA] repeat motive. We identified three single base pair variations responsible for the discrepant amplification yield using different primer pairs. There was a G-to-A transition upstream to the repeat. Downstream to the repeat we found two mutations: an A-to-T transversion and a G-to-A transition.

A deletion of four base pairs downstream of the repeat structure caused a genotype discrepancy (11/12 with Nonaplex I, 12/12 with Nonaplex II) in the fourth sample. Therefore, a conversion of the $[TCTA]_{12}$ allele to an amplified fragment length equivalent to allele 11 could be observed. The primer binding regions for the Nonaplex twin system are unknown, but the amplicon for D8S1179 is shorter in Nonaplex II. Thus, two different explanations have to be discussed: (1) the described deletion of four base pairs prevents completely the annealing of the reverse primer in Nonaplex II, resulting in a null allele (genotype 12/0); (2) another mechanism assumes that the reverse primer binding region is positioned between the variable repeats and the deletion. Consequently, the four-base pair deleted allele $[TCTA]_{12}$ is detected with a fragment length of common allele 12 showing genotype 12/12 with Nonaplex II. The analysis of peak height balance obtained with the Nonaplex II kit for the D8S1179 locus in this sample, seems to indicate an amplification of two alleles, making the latter explanation the more likely one and therefore preferred by the author.

The variation in the downstream II region (G-to-A transition, allele 16V) has already been reported by other authors. It was detected in [8,7,9,10] using the multiplex kits from Applied Biosystems. Clayton et al. [11] also found a null allele in the D8S1179 locus in a population from China, which might have this same mutation. The mutation seems to be associated with long D8S1179 alleles 15–18. This observation could be confirmed by one out of the four samples described in the present study, which is also of Asian origin (Vietnam), and also observed in allele 16, as in most of the reported cases.

Multiplex kits often recommend annealing temperatures that are not optimal for all of the single systems. In some cases owing to suboptimal temperature and also depending on the position in the primer, a single base pair variation in the primer binding region may result in a partial amplification of the allele. Retyping of samples with imbalanced peak heights at reduced annealing temperature can result in equal quantities of both allele amplifications [6]. Pötter [14] presented a new triplex PCR which includes the D8S1179 locus. The primer binding regions are outside the variant

Table 2
Sequencing results of the four variant alleles: repeat structure and variations in the primer binding regions

Variant allele	Repeat structure	Variation	Region	Origin
13V	$[TCTA]_{13}$	G-to-A	Upstream	Ukraine
10V	$[TCTA]_{10}$	A-to-T	Downstream II	Germany
16V	$[TCTA]_2$ $[TCTG]_1$ $[TCTA]_{13}$	G-to-A	Downstream III	Vietnam
12V	$[TCTA]_{12}$	[TAAA] deletion	Downstream I	Germany