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SHORT COMMUNICATION

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New sensitive amplification primers for the STR locus D2S1338 for degraded casework DNA

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Abstract PCR primers for the locus D2S1338 were redesigned in order to reduce the length of the amplification product compared with the conventional design. The new amplification primers were applied to highly degraded casework samples, which gave no or only weak results for D2 in previous analyses. The application of the new primers resulted in an increased overall typing success rate. In a concordance study between the conventional and the newly designed primers, no genotype differences were revealed in 503 randomly selected individuals. This suggests robust amplification conditions with respect to mutation-based allele drop-out.

Keywords D2S1338 · Degraded DNA · Short STRs · Casework · Forensic

Introduction

Forensic studies involving the length reduction of PCR-amplified DNA fragments have been described for autosomal STR loci [1, 2, 3, 4, 5, 6], X and Y-chromosomal markers [4, 7, 8, 9, 10, 11] and for the mtDNA control region [12]. The authors generally observed an increased sensitivity and increased stability accompanied by a higher rate of success when amplifying degraded DNA with the newly designed primers. Similarly, the demand for decreased amplicon sizes has been reported for alternative detection methods, such as mass spectrometry applications [6, 13].

In the current study we present a new primer design for the locus D2S1338, which is a standard STR locus in national DNA intelligence databases of various countries [14]. The marker can be typed using commercially available STR multiplex amplification kits (e.g. AmpFlSTR SGM Plus,

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Table 1 Summary of degraded casework samples which did not result in successful amplification using D2S1338 as high molecular weight (HMW) marker. The alternative D2 primer design as low molecular weight (LMW) marker resulted in successful analysis in most cases

Casework samples	D2S1338 result as HMW marker	D2S1338 result as LMW marker
Swab beverage carton	–	++
Swab juice carton	–	++
Swab cup	–	++
Swab can	–	++
Swab can	–	++
Swab glass	–	++
Swab glass	n.i.	++
Swab glass bottle	–	++
Swab chocolate bar	–	++
Swab clock	n.i.	++
Swab tapes	–	++
Swab belt	–	++
Swab faeces	–	++
Cutting shoe	–	++
Cutting glove	–	+
Cutting glove	n.i.	++
Cutting toilette paper (faeces)	–	+
Cutting slip	–	++
Cigarette end	n.i.	++
Cigarette end	–	++
Cigarette end	n.i.	++
Cigarette end	n.i.	++
Cigarette end	–	+
Cigarette end	–	++
Cigarette end	n.i.	+
Cigarette end	–	++
Cigarette end	n.i.	++
Scrape blood-trace	n.i.	+
Fingernail scraping	n.i.	++

n.i. non-interpretable result.
– no result.
+ <100 RFU (heterozygotes), <200 RFU(homozygotes).
++ >200 RFU.

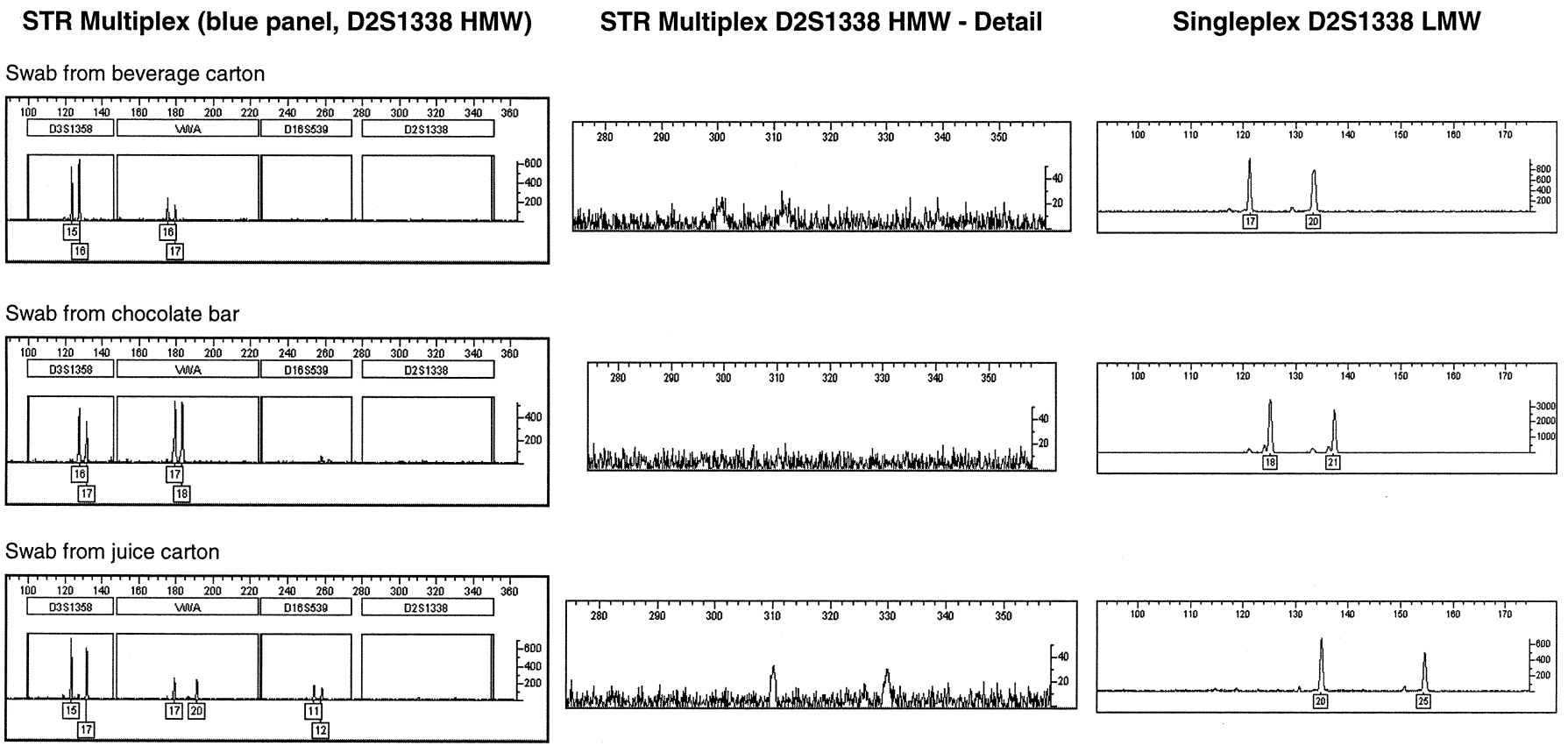

Fig. 1 Examples of SGM plus (ABI) electropherograms of degraded casework samples (FAM-labelled profile only). The left and the middle rows show the results of the amplification with D2S1338 as high molecular weight (HMW) marker (middle row displays D2 in detailed view), the right row depicts the amplification of D2S1338 as low molecular weight marker (LMW) in singleplex format with the newly designed primers

Applied Biosystems, Foster City, CA), in which it is included as a high molecular weight marker (alleles ranging from 289 bp to 341 bp). This is why D2 shows weak amplification when degraded DNA samples have to be analysed, and sometimes fails to give amplification products when highly degraded DNA is investigated. In order to provide an alternative assay for such samples, the D2 PCR primers were shifted closer to the repeat region which resulted in a shortened amplicon. The new primers were tested on samples which showed severe DNA degradation after analysis with commercially available PCR multiplex kits.

Material and methods

The STR locus D2S1338 was amplified from buccal scrape samples (Chelex extraction, [15]) and casework samples (phenol/chloroform extraction as described in [16]) in a total reaction volume of 20 µl including 1×PCR buffer II, 1.5 mM $MgCl_2$, 200 µM each dNTP, 2.5 U Amplitaq Gold polymerase (ABI, Foster City, CA), 0.6 µg/µl BSA (Sigma, Munich, Germany) and 0.25 µM of each of the primers D2mini/f 5'–CAGTGGATTTGGAAACAGAAATG–3' and D2mini/r 5'–TCAGTAAGTTAAAGGATTGCAGG–3'. Primers were designed with the aid of the Primer Express software package (ABI) following the manufacturer's recommendations.

PCR was performed on a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT) comprising 30 cycles of 94°C for 45 s, 56°C for 1 min, and 72°C for 1 min following initial denaturation at 95°C for 11 min and final incubation at 72°C for 30 min. Aliquots of 1 µl of the amplification products were combined with 20 µl deionized formamide including 1 µl internal lane standard (Genescan-500 ROX, ABI), heat-denatured at 95°C for 2 min, snap-cooled on ice, and subjected to electrophoresis on an ABI PRISM 310 genetic analyser using POP 4, or on an ABI PRISM 3700 DNA sequencer using POP 6 and default conditions. Data were analysed using GeneScan Analysis (Versions 2.1 and 3.7) and Genotyper (Version 3.6).

Results and discussion

The new amplification primers for the STR marker D2S1338 resulted in a reduction of the fragment length by 176 bp compared with the conventional design (SGM Plus, ABI) so that alleles 15–28 are now located in the range between 114 bp and 166 bp. A sensitivity study demonstrated that the PCR was sensitive down to 50 pg of genomic DNA (Control DNA 007, ABI) and gave reproducible results over an annealing temperature range of 55–57°C.

The forensic applicability of the new primers was tested on 29 casework samples which had shown substantial DNA degradation in a preceding analysis using D2 in a multiplex as high molecular weight marker (289–341 bp). The results indicated complete D2 locus drop-out, strong peak height imbalance within the D2 locus (>50%) or very small non-interpretable peaks (Table 1, examples see Fig. 1). The amplification with the new D2 primers gave usable results in 24 of the 29 samples tested (83%). The remaining five samples (17%) also showed clear D2 peaks, but they were below the threshold which in this study was defined as 100 RFU for heterozygote peaks and 200 RFU for homozygote peaks. The amount of DNA as well as the number of cycles were identical in both studies. In a separate analysis (collaborative exercise of the European DNA Profiling Group EDNAP organized by P.M. Schneider, manuscript in preparation) artificially degraded DNA was investigated. The analysis of the D2 locus only led to results when the new D2 primers were applied, since the extent of degradation did not allow for amplification of larger DNA fragments. When comparing amplification results between D2S1338 in the AmpFlSTR SGM Plus multiplex kit and the newly designed format, it has to be kept in mind that the long amplicon primers in the multiplex protocol are not optimised for degraded samples. As the primer sequences of D2S1338 from the AmpF/STR SGM Plus kit were not available, it was not possible to carry out a study including the long amplicon primers in a singleplex format. Therefore, the increased typing success rate can only be attributed to the combined phenomena of new primer design, the reduced amplicon length and the singleplex amplification format.

In a concordance study buccal scrape samples from 503 randomly selected individuals from the Austrian DNA intelligence database [17] were amplified with the new D2 primers. Comparing the results with those obtained by conventional analysis (AmpF/STR SGM Plus) revealed no differences between the genotypes obtained. This suggests that mutation-based drop-out of D2 alleles is expected to be only rare, if at all, in the Caucasian population.

Conclusions

Careful PCR primer design introducing short amplicons increases the chance of a successful STR analysis in casework samples. In selected cases, in which DNA is heavily degraded, short amplification products are a good alternative to attain information for a required STR marker. In our study a new primer design for the STR locus D2S1338 resulted in successful amplification of severely degraded DNA from case work samples, and proved to be robust against mutation-based amplification drop-out when compared with the results obtained by conventional primers in a multiplex protocol. An application study with multiplex STR loci displaying reduced fragment length in order to increase the discrimination power is in progress.

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