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TECHNICAL NOTE

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A new "miniSTR-multiplex" displaying reduced amplicon lengths for the analysis of degraded DNA

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Abstract A multiplex PCR was designed for the loci D2S1338, D16S539, D18S51, TH01 and FGA using redesigned primers in order to reduce the lengths of the amplification products compared to the designs used in commercially available multiplex PCR kits, also including amelogenin. The new PCR primers were used to amplify highly degraded DNA from casework samples, which had shown no or only poor results for these loci in previous analyses with standard primer sets. The application of the new miniSTR-multiplex resulted in an increased overall typing success rate for degraded DNA samples. In a concordance study between the conventional and the newly designed primers, no genotype differences were revealed in 124 randomly selected individuals.

Keywords D2S1338 · D16S539 · D18S51 · TH01 · FGA · Degraded DNA · STRs · Casework · Forensic

Introduction

Multiplex PCR systems based on short tandem repeats (STRs) have proved to be a highly useful tool in forensic applications, as the simultaneous amplification of up to 16 loci offers an outstanding discrimination power with low DNA sample consumption. This has been achieved by taking advantage of fluorescence technology and spatial separation of the individual PCR amplified STR loci in a length range of approximately 100–400 base pairs (bp). Application of such STR multiplexes results in full profiles in the vast majority of high-quality DNA samples. However, problematic samples containing only degraded DNA—which in practice often comprise only minute amounts of DNA [1, 2]—cannot be fully analysed, as the higher molecular weight markers (above 250 bp) fail to bring unambiguous results or drop out completely [3]. DNA degradation has to be assumed and dealt with when samples have been exposed to light, humidity, elevated temperatures and bacterial and fungal contamination. It has been demonstrated that DNA artificially degraded by sonication and DNase I treatment leads to relatively stable fragment lengths up to 200 bp, whereas longer DNA fragments could not be amplified in general, resulting in partial STR profiles [3, 4].

The reduced information content of partial STR profiles results in a lower discrimination power and may lead to random matches in DNA intelligence databases. Consequently, it has become an obvious concept to reduce amplicon lengths of degradation-sensitive loci in order to gain the required discrimination power with alternative assays [5–12]. In this study, we applied this concept to a selection of degradation-sensitive and/or weakly amplifying loci from the SGMplus kit (Applied Biosystems, AB, Foster City, CA), i.e. D2S1338, D16S539, D18S51, TH01 and FGA, which are among the core STR loci in national DNA intelligence databases of various countries [13] and therefore are of particular relevance for the discrimination power of STR profiles stored and searched in these databases.

Materials and methods

The STR loci D2S1338, D16S539, D18S51, TH01, FGA and amelogenin were amplified from buccal scrape samples (Chelex extraction, [14]) and casework samples (phenol/chloroform extraction as described in [15]) in a total reaction volume of 25 or 50 µl (10 and 20 µl DNA, respectively) including 1×PCR Buffer II, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 250 µg BSA (Sigma, Munich, Germany), 1 U AmpliTaq Gold polymerase (AB) and 100 nM

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Table 1 Primer sequences, fluorescence labels and fragment lengths of the size-reduced loci included in the miniSTR-multiplex

Locus	MiniSTR primers (5′→3′)	Dye	Product length (bp)	LR[a] (bp)	Ref.
TH01mini/f	CCTGTTCCTCCCTTATTTCCC	FAM	56–84	109	[11]
TH01mini/r	GGGAACACAGACTCCATGGTG		alleles 4–13.3		
Amelo/f	CCCTGGGCTCTGTAAAGAATAGTG	FAM	104, 110		[16]
Amelo/r	ATCAGAGCTTAAACTGGGAAGCTG				
D2mini/f	CAGTGGATTTGGAAACAGAAATG		110–168	177	[6]
D2mini/r	TCAGTAAGTTAAAGGATTGCAGG	FAM	alleles 15–28		
D18S51mini/f	TGAGTGACAAATTGAGACCTT	TET	109–189	156	[5]
D18S51mini/r	GTCTTACAATAACAGTTGCTACTATT		alleles 7–27		
D16S539mini/f	ATACAGACAGACAGACAGGTG	HEX	79–120	153	[5]
D16S539mini/r	GCATGTATCTATCATCCATCTCT		alleles 5–15		
FGAmini/f	GGCATATTTACAAGCTAGTTTCT	HEX	123–191	90	BKA[b]
FGAmini/r	ATTTGTCTGTAATTGCCAGC		alleles 17–33.2		

[a]*LR* Length reduction compared to the estimated fragment length obtained with the SGMplus kit (AB)
[b]Primer sequence information was kindly provided by the Bundeskriminalamt Wiesbaden

primers TH01mini and Amelo, 160 nM primers D2mini, 200 nM primers D18S51mini, 80 nM primers D16S539mini and 300 nM primers FGAmini. Primer sequences are given in Table 1.

PCR was performed on a Gene Amp PCR System 9600 (Perkin Elmer, Norwalk, CT) comprising 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and final incubation at 60°C for 45 min following initial denaturation at 95°C for 11 min. Of the amplification products, 1-µl aliquots were combined with 20 µl deionized formamide including 1-µl internal lane standard (Genescan-500 TAMRA, AB), heat-denatured at 95°C for 2 min, snap-cooled on ice and subjected to electrophoresis on an ABI PRISM 3100 Genetic Analyzer using POP 6 and default conditions. Data were analysed using GeneScan Analysis (versions 2.1 and 3.7) and Genotyper (version 3.6) software (both AB).

DNase I digestion

A total of 1.2 µg human genome DNA (100 µg/µl) was digested at 37°C with 2.4 U Turbo DNase (Ambion, Austin, TX) in a final volume of 120 µl. At predefined time

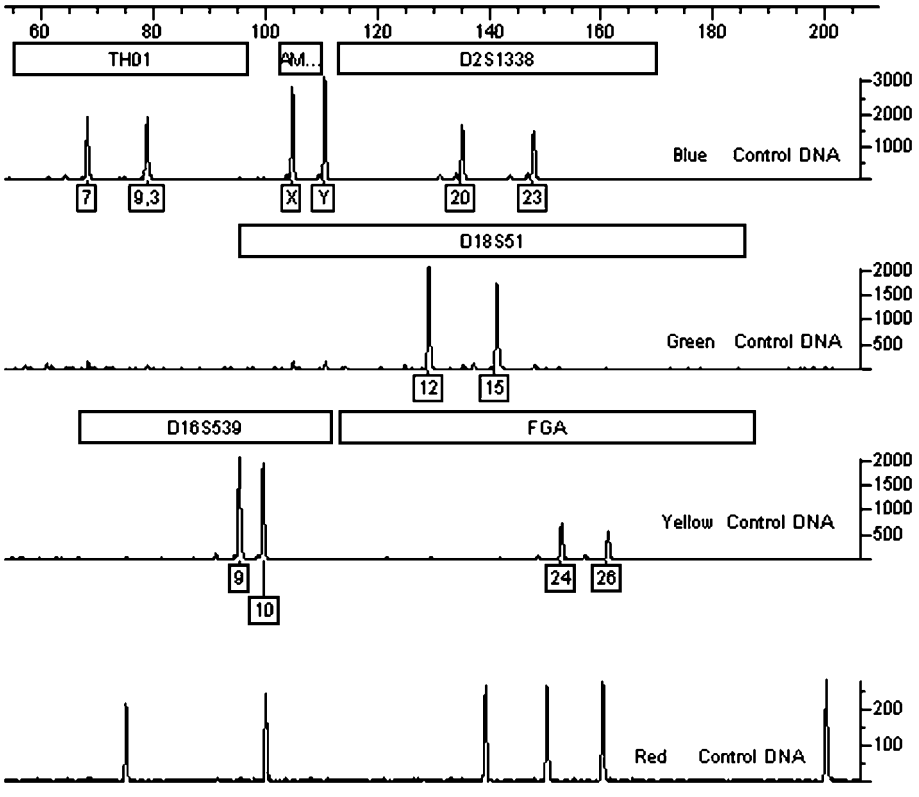

Fig. 1 Electropherogram of the miniSTR-multiplex (Control DNA 007, AB)

Table 2 Amplification results of artificially degraded DNA samples (DNase I treatment)

DNase incubation (min)	TH01mini	Amelo	D2mini	D18mini	D16mini	FGAmini
0	+	+	+	+	+	+
0.5	+	+	+	+	+	+
1	+	+	+	++	+	+
2	+	+	++	++	++	+
3	++	++	++	n.i.	n.i.	n.i.
4	−	−	−	−	−	−
64	−	−	−	−	−	−

+ successful amplification (peaks >100 RFU for heterozygote and >200 RFU for homozygote loci), *n.i.* results not clearly interpretable, − no result, ++ gain of information (no result in this locus with the SGMplus kit)

points (0, 0.5, 1, 2, 3, 4, 8, 16, 32 and 64 min), 10-µl aliquots were removed and added to 490-µl preheated (95°C) buffer (TLE + 20 µg/ml glycogen) to quench the enzymatic reaction. Heat inactivation of DNase I was performed for 10 min at 95°C.

Results and discussion

By evaluation of 708 partial SGMplus profiles obtained from degraded DNA casework samples, we identified the relative proportion of dropped out loci. These casework samples had been analysed using standard 50-µl SGMplus assays under default conditions (except for using 30 PCR cycles). As expected, higher molecular weight STR loci (average 300 bp) such as D18S51, D2S1338 and FGA frequently failed to amplify. In 80, 69 and 45% of the cases, the loci D18S51, D2S1338 and FGA, respectively, showed no results (threshold was defined as 100 RFU for heterozygote and 200 RFU for homozygote alleles). D16S539 and D21S11 (average 230 bp) failed in about 30% of the investigated cases. Interestingly, TH01 albeit only 165–204 bp in length had a drop-out rate of 17%, which may be due to a generally poor amplification efficiency of this marker in the SGMplus multiplex. The amplification of the remaining loci (between 106 and 209 bp) failed in less than 10% of the cases only.

We designed a "miniSTR-multiplex" containing the STR loci TH01, D2S1338, D18S51, D16S539 and FGA displaying reduced fragment lengths and amelogenin [16]. The new amplification primers (miniSTR primers) for the STR marker TH01, D2S1338, D18S51, D16S539 and FGA resulted in a reduction of the fragment length of 109, 177, 156, 153 and 162 bp, respectively, (see Fig. 1) compared with the conventional SGMplus design.

A sensitivity study of the miniSTR-multiplex demonstrated that unambiguous and reproducible results were obtained with 100 pg of genomic DNA (Control DNA 007, AB) over an annealing temperature range of 54–60°C. Interpretable STR profiles could still be obtained with 50 pg of template DNA; however, in some cases, a strong peak imbalance and allele drop-out were observed.

An experiment on artificially degraded DNA showed that DNase I treatment for 2 min resulted in loss of the higher molecular weight SGMplus loci. These loci were successfully amplified with the new miniSTR primers. Even after 3-min DNase I treatment, at least a partial profile of these could be obtained (Table 2).

The forensic usefulness of the new primers was evaluated on 35 casework samples which showed loss of loci due to DNA degradation as demonstrated in previous analyses using the SGMplus kit. An example (swab faeces) is shown in Fig. 2a and b. Generally, amplification with the new miniSTR-multiplex gave useful profiles in all samples tested. In 28 samples (80%), all six loci were successfully typed. Five in five samples (14.3%) and in two samples three out of six loci (5.7%) clear results were obtained. These samples showed distinctive signals in all loci; however, some were below the threshold defined in this study (100 RFU for heterozygote and 200 RFU for homozygote alleles) and were therefore not called.

Table 3 and Fig. 3 summarize the results of both, preceding SGMplus analyses and subsequent miniSTR-multiplex amplification of degraded DNA casework samples. In all of these cases, the application of the miniSTR-multiplex increased the overall number of successfully analysed loci, thus increasing the discrimination power of the STR profiles.

In a concordant study, buccal scrape samples from 124 randomly selected individuals from the Austrian DNA intelligence database [17] were amplified using the miniSTR-multiplex. Comparing the results with those obtained by conventional analysis (SGMPlus kit, AB) revealed no differences between the genotypes obtained. A database search in Ensembl Genome Browser (release 30.35c) for known SNPs in the miniSTR primer binding sequences revealed only one SNP in the D2mini-R primer binding site (rs10208428; A/G; validation status unknown). Analysis of another 503 randomly selected samples from the Austrian DNA intelligence database using the D2mini primer could again not reveal any genotype differences [6]. It has to be taken into consideration, however, that the alternative primer designs might lead to the appearance of null alleles (primer-binding site mutations) in rare events. These also occur with the different commercial amplification kits at a low frequency for the relevant European populations and need to be addressed when comparing STR profiles derived from different amplifi-

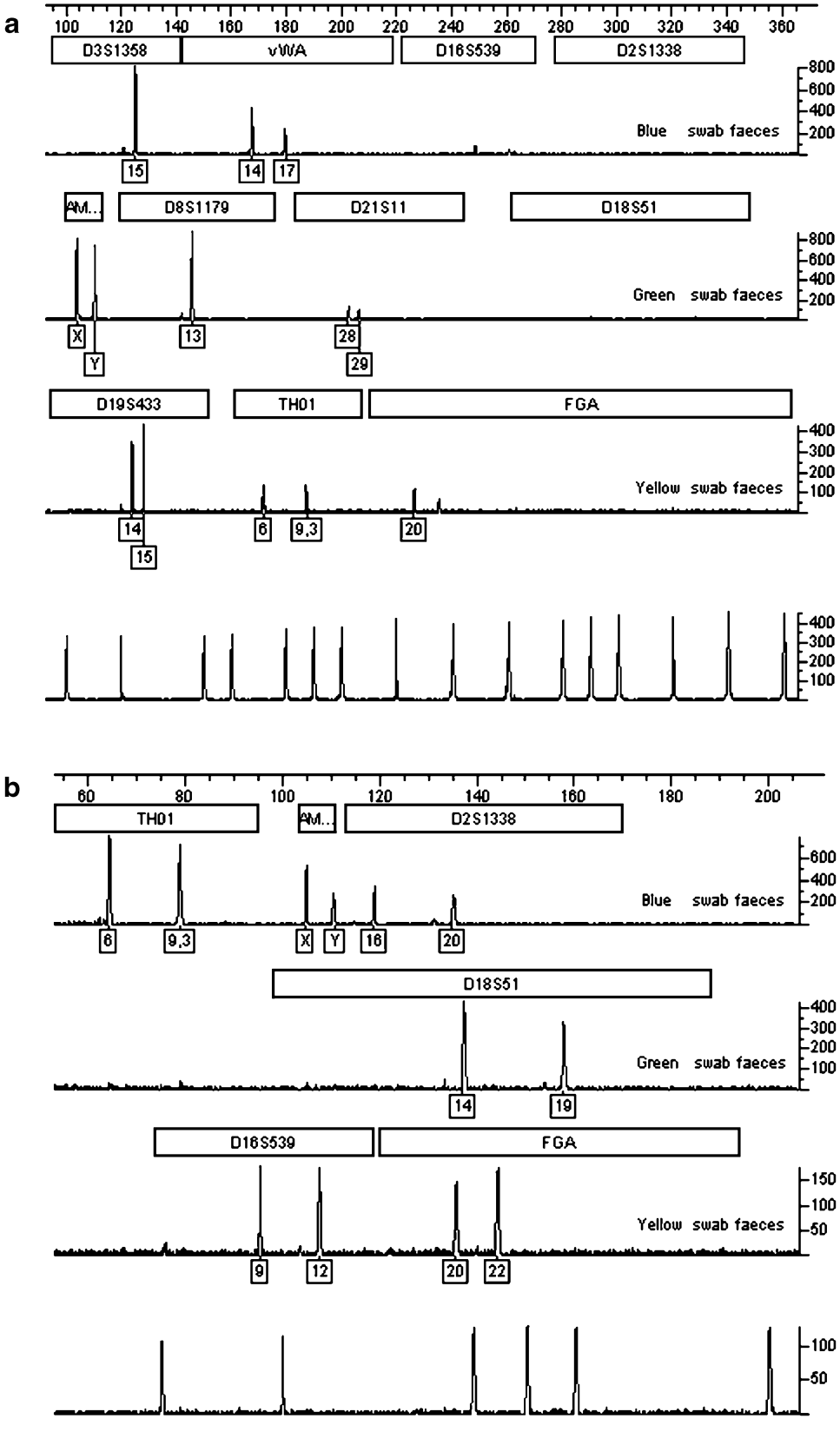

Fig. 2 Results of the amplification of a degraded DNA casework sample (swab from faeces) with **a** the SGMplus kit (50-µl assay with 400 pg DNA) and **b** the miniSTR-multiplex (25-µl assay with 200 pg DNA)

Table 3 Amplification results of degraded casework samples with the SGMplus kit and the miniSTR-multiplex

Casework samples	SGM+ results[a]	TH01mini	Amelo	D2mini	D18mini	D16mini	FGAmini	MiniSTR results[b]	Benefit[c]	Overall *n* of loci[d]
Swab can	4	++	+	++	++	++	++	6	5	9
Swab glass bottle	8	+	+	++	++	+	++	6	3	11
Swab glass bottle	3	++	+	++	++	++	++	6	5	8
Swab glass bottle	7	++	+	++	++	+	++	6	4	11
Swab glass bottle	10	+	+	++	+	+	+	6	1	11
Swab glass bottle	9	+	+	++	++	+	+	6	2	11
Swab glass bottle	9	+	+	++	++	+	+	6	2	11
Swab plastic bottle	5	++	+	++	++	++	++	6	5	10
Swab glass	5	++	+	n.i.	++	++	++	5	4	9
Swab glass	8	+	+	++	++	+	++	6	3	11
Swab bottle	6	+	+	++	++	++	++	6	4	10
Swab rectum	10	+	+	++	+	+	+	6	1	11
Swab faeces	7	+	+	++	++	++	++	6	4	11
Swab faeces	7	++	+	++	++	++	+	6	4	11
Swab toothbrush	6	+	+	++	++	++	++	6	4	10
Swab collar	10	+	+	+	++	+	+	6	1	11
Swab glove	3	+	+	++	++	++	++	6	4	7
Swab glove	9	+	+	++	++	+	+	6	2	11
Swab glove	5	++	+	++	++	++	++	6	5	10
Swab slip	9	+	+	++	++	+	+	6	2	11
Swab steering wheel	5	++	+	n.i.	++	n.i.	n.i.	3	2	7
Swab steering wheel	5	++	+	++	++	++	++	6	5	10
Swab screwdriver	1	++	+	++	++	++	n.i.	5	4	5
Swab screwdriver	4	++	+	n.i.	++	++	++	5	4	8
Swab screwdriver	7	+	+	++	++	++	++	6	4	11
Swab screwdriver	8	+	+	++	++	+	++	6	3	11
Swab carrier bag	5	++	+	++	++	+	+	6	3	8
Swab blood trace	7	+	+	++	++	++	n.i.	5	3	10
Swab blood trace	10	+	+	+	++	+	+	6	1	11
Cutting stamp	8	+	+	++	++	+	++	6	3	11
Cutting tissue	4	++	+	++	++	++	++	6	5	9
Cigarette end	5	++	+	n.i.	n.i.	++	n.i.	3	2	7
Cigarette end	7	++	+	+	++	+	n.i.	5	2	9
Cigarette end	8	+	+	++	++	+	++	6	3	11
Cigarette end	7	+	+	++	++	++	++	6	4	11

+ Successful amplification, *n.i.* results not clearly interpretable (peaks <100 RFU for heterozygote and <200 RFU for homozygote loci), ++ gain of information [no result in this locus in preceding conventional analysis (SGMplus)]

[a]Number of loci that produced successful results with the SGMplus kit

[b]Number of loci that produced successful results with the miniSTR-multiplex

[c]Number of additional loci gained through the miniSTR-multiplex

[d]Overall number of loci in profile

cation primers. With respect to national DNA intelligence database comparisons, this is most effectively accomplished with appropriate allele matching algorithms.

In conclusion, the miniSTR-multiplex proved a useful additional tool complementing conventional STR analysis.

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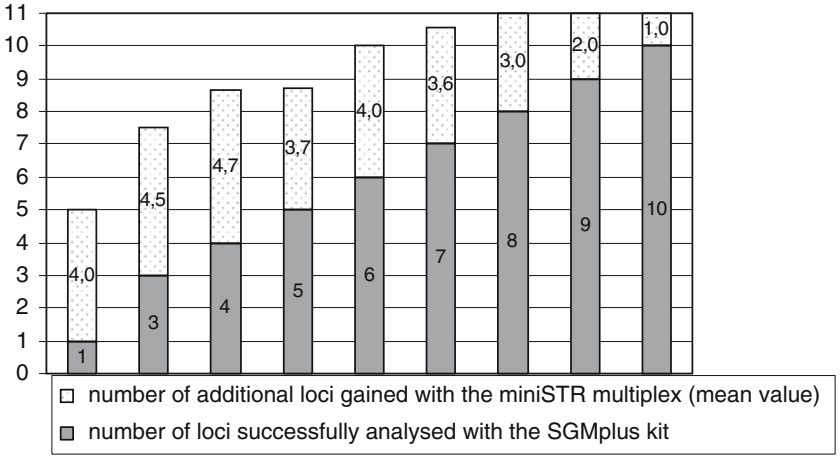

Fig. 3 Schematic representation of the number of successfully amplified loci with the 11 loci SGMplus and the six loci miniSTR-multiplex on 35 degraded DNA samples. The additional results of the miniSTR-multiplex were added to the results of the preceding SGMplus analyses

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