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Evaluation of Nine Non-CODIS MiniSTR Loci to aid Analysis of Degraded DNA

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= Abstract =

분해된 DNA검체 분석을 위한 9개의 Non-CODIS MiniSTR 유전좌의 유용성 평가

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For highly degraded DNA samples of forensic casework, new miniSTR PCR systems have been developed to supplement the current CODIS STRs. In the present study, we established the three miniplexes for nine miniSTRs (NC01: D10S1248, D14S1434 and D22S1045; NC02: D1S1677, D2S441 and D4S2364; and NC03: D3S3053, D6S474 and D20S482) which had been previously suggested by Butler group (NIST, Gaitherburg, MD, USA). To evaluate the usefulness of the nine miniSTRs in analysis of degraded DNA, the sensitivity and efficacy of the three miniplexes were determined and then compared with those of the BigMini STR system which consists of six CODIS miniSTRs (TH01, CSF1PO, FGA, TPOX, D7S820, and D21S11). The three miniplexes gave better results in both the sensitivity test and efficiency test in comparison with BigMini. In the sensitivity test using serially diluted standard DNA, most loci in the three miniplexes showed reliable results for samples containing 50 pg of DNA and some even showed good sensitivity for samples containing 30 pg of DNA. Additionally, the three miniplexes generated useful profiles for both enzymatically degraded DNA and 50-year old skeletal remain samples. Among the nine miniSTRs, D4S2364, D3S3053, D14S1434, and D1S1677 produced the most successful DNA profiles for old skeletal remains. These results suggest that new miniSTRs could be useful supplements to the 13 CODIS STRs for forensic analysis of degraded DNA.

Key Words: miniSTR, D1S1677, D2S441, D3S3053, D4S2364, D6S474, D10S1248, D14S1434, D20S482, D22S1045, Degraded DNAD20S482, D22S1045, Degraded DNA

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Introduction

Short Tandem Repeat (STR) analysis using a multiplex PCR system has become a valuable tool for forensic DNA typing[1-4]. Application of STR multiplexes results in full profiles in the vast majority of high-quality DNA samples. However, DNA samples of forensic casework are usually highly degraded due to diverse environmental factors[5]. These samples cannot be fully analyzed, as the higher molecular weight markers used in common multiplex PCR systems cause loss of signal and allele drop-out[6]. The reduced information of partial STR profiles results in a lower discrimination power and may lead to random matches in the DNA intelligence database. To solve these problems, a new set of STR primers known as a miniplex was developed by moving the primer binding sites as close as possible to the repeat region to reduce the size of PCR products[7-10]. Recently, redesigned primer sets for the Combined DNA Index System (CODIS) core STR loci have also been reported and evaluated[11-13]. A major advantage of these miniSTRs is that database compatibility is maintained with convicted offender samples which have been processed using common, established multiplex systems. However, all of the CODIS loci cannot be reduced into smaller amplicon because of repeat flaking regions that are not amenable to redesign primer and also because some of the CODIS loci have large allele ranges[11]. Therefore, the need for the development of new miniSTR markers has been suggested so that the genotypes of degraded samples may be determined[14-17]. Coble and Butler conducted literature-based research on 920 STR loci and reported two miniplex sets for six STRs (NC01: D10S1248, D14S1434 and D22S1045; and NC02: D1S1677, D2S441 and D4S2364) which were not linked to the CODIS markers and had high heterozygosities and small allele size ranges15). The third miniplex set for three STRs (NC03: D3S3053, D6S474 and D20S482) and six more miniplex sets for 17 additional miniSTR loci can be found at the web page, STRBase (http://www.cstl.nist.gov/biotech/strbase/).

In the present study, we established the three miniplexes (NC01, NC02 and NC03) and examined the sensitivity and efficiency of the three multiplex PCR systems to evaluate their forensic utility.

Materials and Methods

1. PCR condition and electrophoresis of the three miniplexes and BigMini set

Three miniplexes were performed with the same primer sets as in Coble and Butler[15] and in a recent presentation by Hill et al. (Hill CR, Coble MD, Butler JM (2006) Characterization of 26 New miniSTR loci, 17th International Symposium on Human Identification. Poster 44, Nashville, TN, USA), but changes were made to the fluorescence dye label. The forward primers for D10S1248, D4S2364 and D20S482 were labeled with 6FAM, D14S1434, D2S441 and D3S3053 with HEX, and D22S1045, D1S1677 and D6S474 with NED. Each PCR multiplex was performed in 10.0 μl of reaction mixture containing 0.5 - 1.0 ng of template DNA, 1.0 μl of 10 X Gold ST*R buffer (Promega, Madison, WI, USA), 1.0 U AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA, USA) and appropriate concentrations of primers. Primer concentrations were adjusted empirically to balance peak heights to be between 1.00 and 1.30 μm. Thermal cycling was conducted on a PTC-200 DNA engine (MJ Research, Waltham, MA, USA) using the following conditions: 95℃ for 11 min, 30 cycles of 96℃ for 1min, 55℃ for 1 min, 72℃ for 1 min, and a final extension at 60℃ for 45 min. The PCR condition used for BigMini (TH01,

CSF1PO, FGA, TPOX, D7S820, and D21S11) was the same as Butler et al.11) and it differed from that of the three miniplexes in fact that 1.5 U AmpliTaq Gold® DNA polymerase (Applied Biosystems) was used. The PCR products were mixed with GeneScan-400HD (ROX) Size Standard (Applied Biosystems) and analyzed by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and GeneScan software 3.1 (Applied Biosystems). Genetic characteristics of the nine miniSTRs were summarized in Table 1.

2. Construction of allelic ladders and Genotyper macros

Allelic ladders of NC01 and NC02 were kindly provided by Coble (NIST), and these were recreated using a modified version of Coble and Butler 's protocol with the primers of the present study[15]. After a 1:300 dilution of the allelic ladder provided by Coble, amplification for each triplex was carried out using the primer mix of the present study according to the protocol above with 72℃ extension time increased to 2 min during each cycle for 20 cycles. The final extension was also lengthened to 240 min since there were more PCR products to create. The allelic ladder for NC03 was created using a combination of individual templates, which represent the range of alleles observed in the Korean population (unpublished data). At least two different homozygote samples in the observed size for each miniSTR locus were sequenced to calibrate the repeat number. Genotyper macros were constructed for each of the miniplex combinations to perform allelic designation with new allelic ladders using Genotyper 2.5 software (Applied Biosystems).

3. PCR amplification with serially diluted DNA

A commercial standard 9948 male DNA (Promega) was serially diluted from a concentration of 1 ng/㎕ to 5 pg/㎕ for the sensitivity study. Ten replicates were tested for each concentration of DNA with appropriate amplification cycles: 29 cycles for 1 ng and 500 pg of template DNA, 31 for 300 and 100, 33 for 50 and 30, and 35 for 10 and 5. The sensitivity of the three miniplexes was tested in comparison with BigMini11). Genotype results at each dilution were then compared to the" correct "genotype which was determined using 1 ng of standard DNA. Following the design of Coble and Butler ' s study[15], genotype results that were above the interpretational threshold of 100 relative fluorescent units (RFUs) on the GeneScan analysis

Table 1. Genetic characteristics of nine miniSTR loci examined in the present study

Locus	GenBank accession No.	GenBank allele	9948 DNA allele	Allele spread[a]	Allele size range (bp)	Repeat motif
NC01						
D10S1248	AL391869	13	12, 15	11 - 17	95 - 119	$[GGAA]_n$
D14S1434	AL121612	13	13, 14	9 - 15	73 - 97	$[CTGT]_m[CTAT]_n$
D22S1045	AL022314	17	16, 18	11 - 19	88 - 112	$[ATT]_nACT[ATT]_2$
NC02						
D1S1677	AL513307	15	13, 14	10 - 17	84 - 112	$[TTCC]_n$
D2S441	AC079112	12	11, 12	8 - 15	77 - 105	$[TCTA]_n$
D4S2364	AC022317	9	9, 10	7 - 11	71 - 87	$[GAAT][GGAT][GAAT]_n$
NC03						
D3S3053	AC069259	9	9, 12	7 - 13	87 - 111	$[TATC]_n$
D6S474	AL357514	17	17, 17	13 - 19	110 - 134	$[AGAT]_m[GATA]_n$
D20S482	AL121781	14	13, 14	10 - 17	93 - 121	$[AGAT]_n$

[a] A population study in Koreans (unpublished data)

were determined as the following: the correct genotype, partial, incorrect or failure.

4. PCR amplification with artificially degraded DNA

Degraded DNA was prepared by digesting 3.0 μg of blood DNA in 0.02 units/μl DNase I (NEB, Ipswich, MA, UK) for the time periods of 5, 10, 20, 30, and 40 min, and DNA fragmentation was confirmed by agarose gel electrophoresis. The three miniplexes and BigMini amplifications were carried out under the same condition as described above, and the efficiency was tested in comparison with BigMini.

5. PCR amplification with DNA extracted from old skeletal remains

The efficiency of the three miniplexes was tested using 30 skeletal remains of the Korean War (1950-1953) victims. Sample preparation and DNA extraction were carried out according to the method by Lee et al.[18]. The extraction procedure was carried out for each skeletal remain sample at least twice. The three miniplexes and BigMini amplifications were carried out under the same conditions as described above with the following differences: 2.0 U and 3.0 U of AmpliTaq Gold® DNA polymerase (Applied Biosystems) for the three miniplexes and BigMini, respectively, 20.0 μl of final volume, and 35 amplification cycles. The efficiency of the three miniplexes was tested in comparison with BigMini. The genotype results were decided by obtaining consensus allelic scores from twice-performed analyses using two independent DNA extracts. Genotype results that were above the interpretational threshold of 100 RFUs and identical between two independent DNA extracts were determined as the" consensus "genotype.

Results and Discussion

1. Sensitivity test

To evaluate the PCR sensitivity of the three miniplexes, tests were conducted using 10 replicates of DNA in various concentrations, and the

Table 2. Success rates of the genotyping at 15 STRs using NC01, NC02, NC03, and BigMini with 30 DNAs extracted from 50-year old skeletal remains

Locus	Multiplex set	Success rate[a]	Drop-in	Failure
D4S2364	NC02	26 (86.7%)	0 (0.0%)	4 (13.3%)
D3S3053	NC03	24 (80.0%)	2 (6.7%)	4 (13.3%)
D14S1434	NC01	21 (70.0%)	0 (0.0%)	9 (30.0%)
D1S1677	NC02	20 (66.7%)	0 (0.0%)	10 (33.3%)
TPOX	BigMini 1[b]	18 (60.0%)	6 (20.0%)	6 (20.0%)
D2S441	NC02	18 (60.0%)	2 (6.7%)	10 (33.3%)
D20S482	NC03	17 (56.7%)	1 (3.3%)	12 (40.0%)
D10S1248	NC01	15 (50.0%)	0 (0.0%)	15 (50.0%)
D6S474	NC03	15 (50.0%)	0 (0.0%)	15 (50.0%)
TH01	BigMini 1[b]	12 (40.0%)	0 (0.0%)	18 (60.0%)
CSF1PO	BigMini 1[b]	9 (30.0%)	0 (0.0%)	21 (70.0%)
D22S1045	NC01	3 (10.0%)	0 (0.0%)	27 (90.0%)
D7S820	BigMini 2[c]	0 (0.0%)	0 (0.0%)	30 (100.0%)
D21S11	BigMini 2[c]	0 (0.0%)	0 (0.0%)	30 (100.0%)
FGA	BigMini 2[c]	0 (0.0%)	0 (0.0%)	30 (100.0%)

[a] Success profile was decided by obtaining full consensus allelic scores from twice-performed analyses using two
independent DNA extracts.

results were compared with those of BigMini. For all samples containing 100 pg or more of template DNA, NC01, NC02, and NC03 showed correct genotypes, but BigMini showed incorrect genotypes due to allele drop-in in some cases. Correct genotypes were obtained at concentrations as low as 30 pg of DNA for nine, six and eight samples which were tested with NC01, NC02 and NC03, respectively. At 50 pg of DNA, most samples showed correct genotypes. However, there was one sample that showed allele drop-in for NC01 and three samples that showed allele drop-out or drop-in for NC03. As for BigMini, allele drop-out was evident for most of the loci tested with 30 pg of DNA. At 50 pg of DNA, 50% of the samples tested still showed allele drop-out or drop-in.

These genotype results were also calculated to percentage value at each locus and displayed in a graph (Fig. 1). The sensitivity of D22S1045 in NC01 and D1S1677 in NC02 was parallel to that of TPOX in BigMini, and D22S1045 and D1S1677 showed the highest sensitivity and correct genotypes for concentrations as low as 30

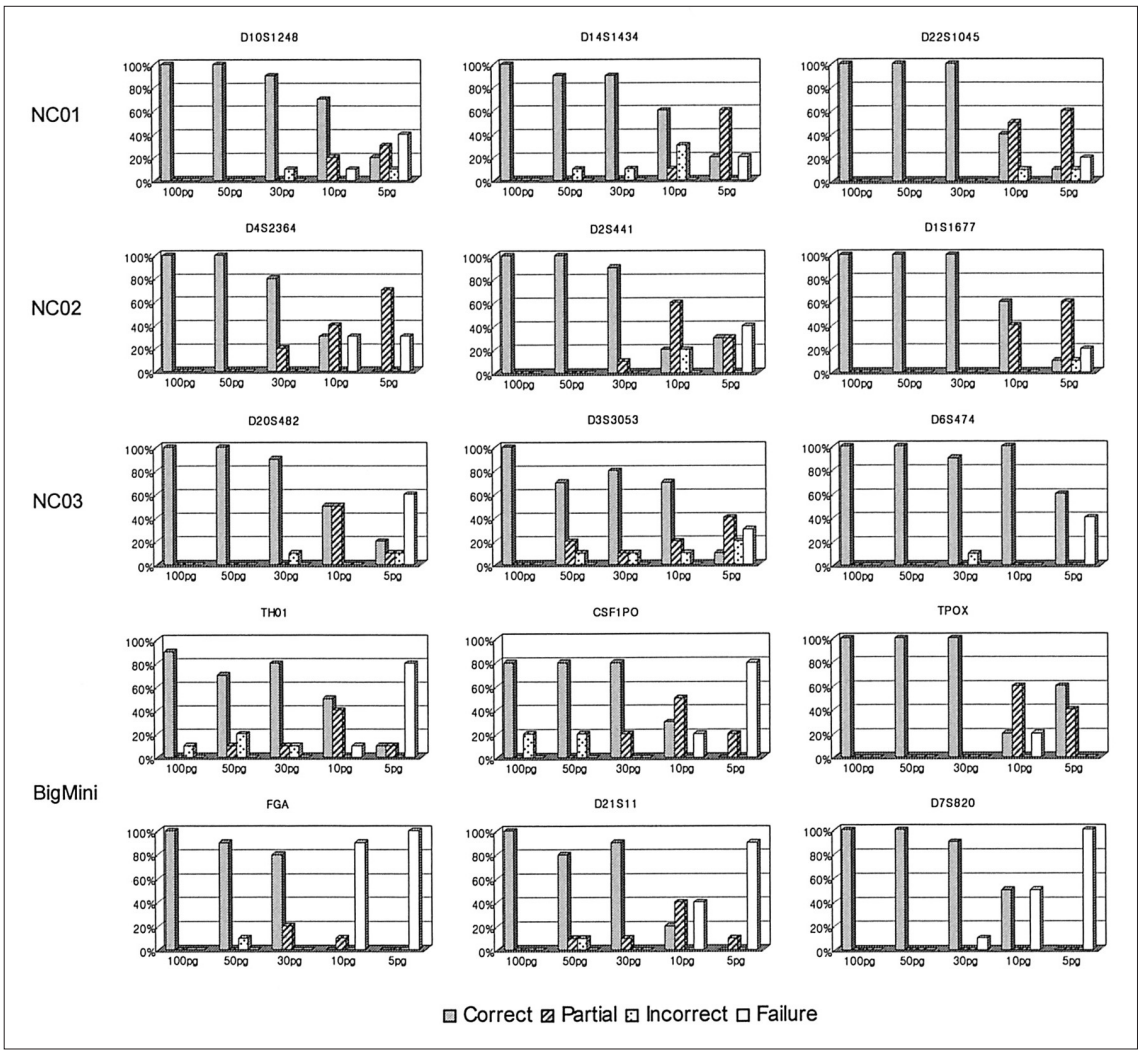

Fig. 1. Sensitivity test of 15 STRs on NC01, NC02, NC03, and BigMini using serially diluted 9948 DNA templates: 1000, 500, 300, 100, 50, 30, 10, and 5 pg.

pg of DNA. The sensitivity of D10S1248, D4S2364, D2S441, D20S482 and D6S474 in the three miniplexes corresponded to that of D7S820 in BigMini, all of which started to show allele drop-out or drop-in for samples containing 30 pg of template DNA. The sensitivity of D14S1434 in NC01 and D3S3053 in NC03 corresponded to FGA and D21S11 in BigMini, all of which started to show allele drop-out or drop-in for samples containing 50 pg of template DNA. TH01 and CSF1PO in BigMini displayed the lowest sensitivity, starting to show allele drop-out even for samples containing 100 pg of template DNA. According to the above results, sensitivity of most STRs in NC01, NC02 and NC03 was better than that of six STRs in BigMini, and among the STRs in the three miniplexes, D22S1045 and D1S1677 showed the highest

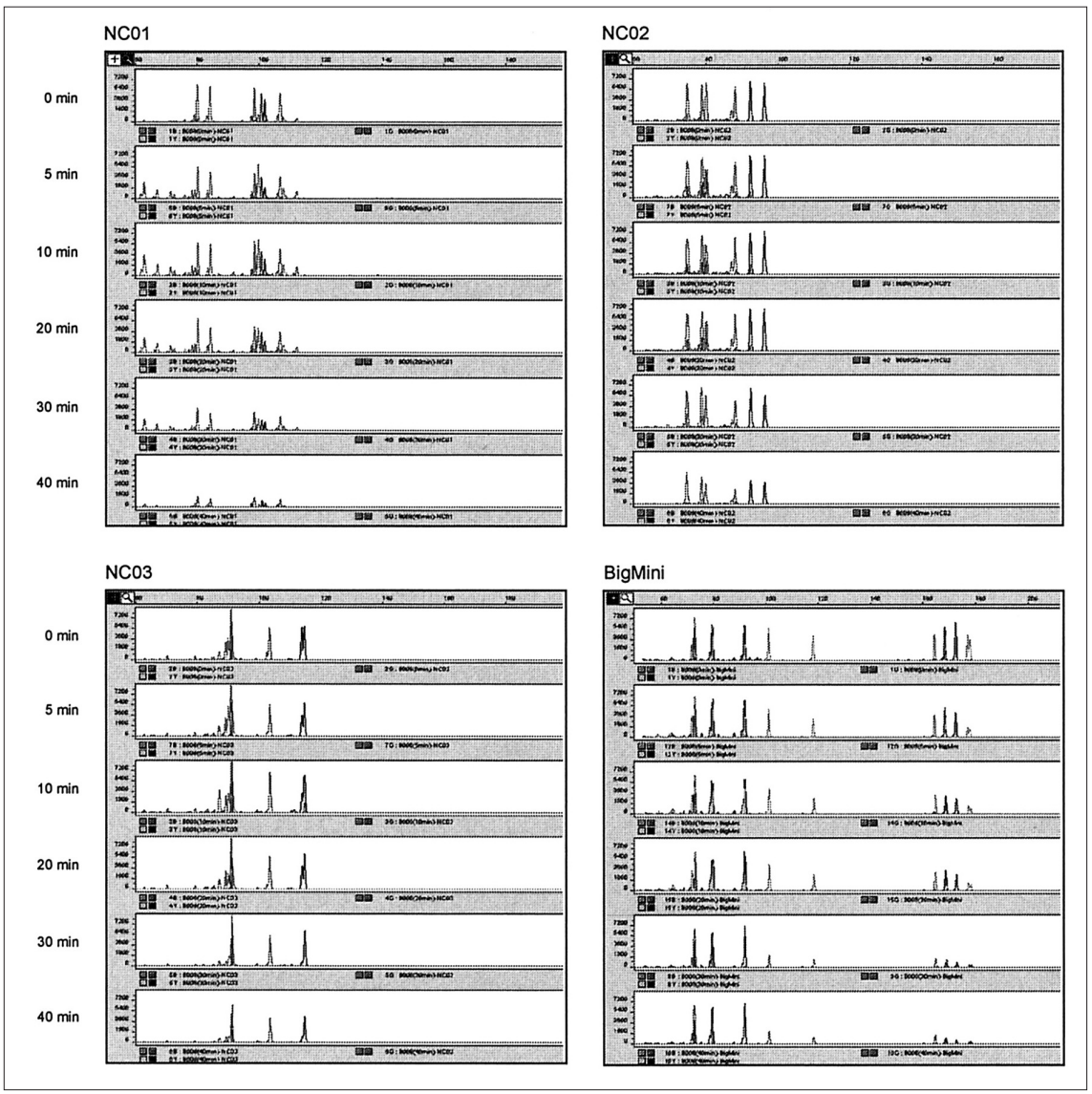

Fig. 2. Efficiency test using DNAs digested with DNase I for various time periods: 0, 5, 10, 20, 30, and 40 minutes, respectively.

sensitivity at low copy number DNA (LCN) templates.

2. Efficiency test

To test the efficiency of the three miniplexes for degraded DNA, PCR amplification was performed using blood DNA samples which were incubated with DNase I for several time periods, and the results were compared with those for BigMini (Fig. 2). For each time period, every locus of NC01, NC02, NC03 and BigMini was successfully genotyped. However, template size had an effect to some degree on amplification efficiency for artificially degraded DNA containing a mixture of fragments of different lengths, and accordingly, three high molecular weight loci in BigMini (FGA, D21S11 and D7S820), which had an allele size range of 125 to 281 bp, began to show signal decrease after 30 min of DNase I treatment. On the other hand, NC02 and NC03, which had an allele size range of 75 to 134 bp, did not show signal decrease even after 40 min of DNase I treatment.

The forensic usefulness of the three miniplexes was also evaluated on 50-year old skeletal remain samples, and the results were compared with those for BigMini. From a total of 30 skeletal remain samples tested with NC01, NC02 and NC03, only one did not produce consensus profile in any locus, and 18 samples showed consensus profiles at five or more of STR loci. As for BigMini, six samples did not produce any consensus profiles in any locus, but these samples showed consensus profiles in one or more loci in NC01, NC02 and NC03. In comparison with BigMini, the three miniplexes gave more useful profiles in most samples tested (Table 2). It may attribute the fact that the genotyping of the three STRs in BigMini (D7S820, D21S11 and FGA), which had a relatively large allele size range of 125 to 281, failed for all 30 samples. Therefore, these results are rather likely due to the allele size difference among STRs in each multiplex. Among 12 other smaller STRs, which had an allele size range of 51 to 134 bp, D4S2364 in NC02 showed the highest success rate (86.7%), and D3S3053, D14S1434 and D1S1677 were next showing relatively high success rates (80.0%, 70.0% and 66.7%, respectively) (Table 2). Among small STR loci in BigMini, TPOX showed the highest success rate (60.0%) but it was lower than those of above four STRs in the miniplexes. For the STR loci with PCR amplicons of 150 bp or less, efficiency of primers rather than amplicon sizes may have an effect on amplification efficiency for degraded DNA samples.

On the other hand, six samples showed allele drop-in with the three miniplexes and/or BigMini. Among these, three samples showed allele drop-in for both the three miniplexes and BigMini, and the other three samples showed allele drop-in only for BigMini. In the three miniplexes, D20S482 showed allele drop-in once while D2S441 and D3S3053 showed allele drop-in twice. In BigMini, only TPOX displayed allele drop-in six times showing the highest rate of allele drop-in (20%) among all 15 loci. Therefore, the evaluation of typing LCN or degraded DNA should include an interpretation strategy for resulting data that is careful with regard to allele drop-out or allele drop-in19, 20).

Conclusion

For genetic analysis of degraded DNA, the nine STRs in the three miniplexes, NC01, NC02 and NC03, proved to be an effective analysis tool as seen from the sensitivity test using diluted DNA and from the efficiency test using enzymatically degraded DNA and naturally degraded DNA. However, although the nine miniSTRs have good characteristics for typing LCN or degraded DNA analysis in comparison with the current CODIS miniSTRs, resulting data should be interpreted carefully all the same with regard to allele

drop-out or allele drop-in. Finally, the application of the new miniSTRs will increase the overall number of successfully analyzed loci in the analysis of degraded DNA, thus increasing the discrimination power of the STR profiles.

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