Int J Legal Med (2008) 122:43–49
DOI 10.1007/s00414-007-0193-3

ORIGINAL ARTICLE

Evaluation of miniY-STR multiplex PCR systems for extended 16 Y-STR loci

H. Asamura · S. Fujimori · M. Ota · T. Oki · H. Fukushima

Received: 23 April 2007 / Accepted: 14 August 2007 / Published online: 26 September 2007

Abstract We developed three short amplicon Y-chromosomal short tandem repeat (miniY-STR) polymerase chain reaction multiplex systems for 16 Y-STR loci (DYS441, DYS446, DYS462, DYS481, DYS485, DYS495, DYS505, DYS510, DYS511, DYS549, DYS 575, DYS578, DYS593, DYS618, DYS638, and DYS643), using newly designed primer sets. In an assay of 238 Japanese males using the three miniY-STR systems, amplification product lengths ranged from 91 to 151 bp for all 16 Y-STR loci. We identified 212 different haplotypes among the 238 individuals, finding haplotype diversity and discrimination capacity of 0.9974 and 0.8908, respectively. An assay of degraded DNA samples using the three miniY-STR multiplex systems, including artificially degraded samples and degraded forensic casework samples, proved remarkably effective. In conclusion, analyses of miniY-STR multiplex systems will play an important role in forensic applications involving degraded DNA samples for which genotyping using only commercial kits is ill-suited.

Keywords MiniY-STR · Multiplex · Degraded DNA

Electronic supplementary material The online version of this article (doi:10.1007/s00414-007-0193-3) contains supplementary material, which is available to authorized users.

H. Asamura (✉) · S. Fujimori · M. Ota · T. Oki · H. Fukushima
Department of Legal Medicine,
Shinshu University School of Medicine,
Asahi 3-1-1,
Matsumoto, Nagano 390-8621, Japan
e-mail: asamura@sch.md.shinshu-u.ac.jp

Introduction

Analysis of short tandem repeat (STR) markers currently represents the most useful instrument in the field of forensic genetics. The problem with which forensic scientists have recently been confronted involves the technical difficulty of analyzing degraded DNA samples. In recent years, several papers have demonstrated that short amplicon STR (miniSTR) represents one of the most useful tools for analyzing degraded DNA samples [1–14]. On the other hand, given Y-STRs role as a characteristic feature of haploid paternal inheritance without recombination, analysis of Y-STR plays a major role in male personal identification, deficiency paternity cases including male relatives, and male profiling for sexual assaults involving male–female stain mixtures. A multicenter study was performed in 1997 to characterize 13 polymorphic Y-STR loci [15], while in the USA, 11 polymorphic Y-STR loci have been recommended as core loci by the Scientific Working Group on DNA Analysis Methods Y-STR Subcommittee (http://www.yhrd.org/index.html). Up to now, reports on large volumes of population genetic data from a wide range of ethnic groups for the Y-STR loci have focused on the core loci [16–19], while several papers have reported polymorphic Y-STR loci other than the core loci [20–22].

In an earlier report, we described the development of two short amplicon Y-STR (miniY-STR) systems for eight Y-STR loci, demonstrating that analyses of miniY-STR systems are highly useful in forensic applications involving degraded DNA samples [13]. In the present study, we attempted to develop further miniY-STR multiplex systems for analyzing degraded DNA using newly designed primer sets for three multiplex polymerase chain reaction (PCR) systems for typing 16 polymorphic Y-STR loci (DYS441,

DYS446, DYS462, DYS481, DYS485, DYS495, DYS505, DYS510, DYS511, DYS549, DYS 575, DYS578, DYS593, DYS618, DYS638, and DYS643).

Materials and methods

DNA extraction

Blood and buccal swabs were obtained from 238 unrelated, adult male Japanese individuals corresponding to 42 sons (42 family trios). Fathers of the children were included among 238 individuals, while paternity relationships of father–son pairs were confirmed by genotyping of the mother in addition to the father and son for autosomal STRs using the AmpflSTR identifiler kit (Applied Biosystems, Foster City, CA, USA). DNA was extracted using the nucleic acid isolation system quick Gene-800 (FUJIFILM, Tokyo, Japan) in accordance with the operating manual. Extracted DNA solutions were diluted with distilled water, and final concentrations of DNA solutions used in the assay were 1 ng/μl.

Primer design, multiplex PCR amplification, and electrophoresis

Using Primer3 program for designing PCR primers (http://primer3.sourceforge.net/), we designed new primer sets for 16 Y-STR loci to generate amplicons of less than 150 bp by setting each primer binding site closer to the repeat region (Table S1). Each primer was checked for potential structures of the self dimmer using the AutoDimer software (http://www.cstl.nist.gov/biotech/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm). All reverse primers included seven bases to promote adenylation (tailed primer; Applied Biosystems). Three multiplex PCR systems were performed with fluorescent dye-labeled primer sets. PCR reactions were carried out in a total volume of 10 μl containing 1 ng of genomic DNA, 1× GeneAmp PCR buffer, 200 μM of each deoxyribonucleotide triphosphate (dNTP, GeneAmp dNTP MIX), 1.5 mM $MgCl_2$, 1.25 U of AmpliTaq Gold DNA polymerase (Applied Biosystems), and each primer set, of which, the concentrations were shown in Table S1. A GeneAmp 9700 (Applied Biosystems) in the 9600 mode was used to perform thermal cycling as follows: 95°C for 11 min; 30 cycles of 94°C for 1 min, 56°C for 1 min in MiniY-6plex (58°C for 1 min in MiniY-5plex1, 60°C for 1 min in MiniY-5plex2), 72°C for 1 min, and a final extension at 70°C for 60 min. Of the PCR product, 1 μl was mixed with 15 μl Hi-Di formamide (Applied Biosystems) and 0.2 μl of the GeneScan-500LIZ size standard (Applied Biosystems) and separated with an ABI 3100-Avant Genetic Analyzer (Applied Biosystems) using POP-4 polymer on a 36-cm capillary array. Electrophoresis was performed at injections of 3 kV for 3 s followed by 15 kV for 20 min with an oven temperature of 60°C, while dye sets were set up under the G5 (6FAM, VIC, NED, PET, and LIZ).

Genotyping and allelic ladders

Genotyping were performed with the GeneMapper ID v3.2 Software (Applied Biosystems) and allelic ladders created with a combination of products of varying lengths at each locus. All alleles containing allelic ladders were sequenced to calibrate the number of repeats (allele number). Non-labeled primer sets described in the Genome Database (GDB; http://www.gdb.org) were used for sequencing, except for DYS 481 where the primer binding sites of the reference primer set shown in the GDB were close to the repeat region. The primer set for DYS481 sequencing was newly designed: 5′-GGGCTCTGTGTTTCTGTGAGA-3′ and 5′-ACAGCT CACCAGAAGGTTGC-3′. Each product was obtained through 34 PCR cycles with annealing temperature at 55°C, followed by purification with a Microcon YM-100 centrifugal filter unit (Millipore, Bedford, MA, USA). Sequencing reactions were performed with the same primers using the BigDye v1.1 ready reaction kit (Applied Biosystems). Electrophoresis was performed using an ABI 3100-Avant genetic analyzer, while sequencing was determined with version 5.1.1 of the sequencing analysis software (Applied Biosystems).

Sensitivity study

We determined the minimum quantity of DNA required to acquire reliable results with the present miniY-STR multiplex PCR systems using commercially available 007 DNA (Applied Biosystems) sequentially diluted with distilled water as template DNA. The quantity of DNA mixed with the PCR mixture was 10, 20, 30, 40, 50, 75, and 100 pg. This test was performed five times with the 007 DNA.

Female/male mixture study

Mixture samples of female DNA and male DNA were artificially mixed at various ratios (female/male=500:1, 100:1, 20:1, 5:1, and 1:1). The amount of male DNA was kept constant at 100 pg, while amounts of female DNA were varied (50, 10, 2 ng, 500, and 100 pg). This mixture test was performed three times with different artificial mixture samples.

Statistical calculations

The allele frequency at each locus was determined by direct counting. Gene diversities were given as $(n/(n-1))(1-(\Sigma_{Pi})^2)$ where n=total sample number and, Pi=allele frequency [23]. Haplotype diversity was calculated by the same formula wherein haplotype frequency was substituted for allele frequency [24]. Discrimination capacity was determined by the expressed value for (observed haplotype number /total sample number) [25].

Analysis of degraded DNA

To assess the effectiveness of the present miniY-STR multiplex PCR systems in genotyping degraded DNA, we tested artificially degraded DNA digested with DNase and forensic casework samples with poor quality DNA. An artificially degraded DNA sample series was prepared as described earlier [13, 26]: 11.2 μg of male genome DNA was mixed with 10× DNase I reaction buffer (Invitrogen, Carlsbad, CA, USA) and sterile water at a total volume of 110 μl. Of the reaction mixture, 10 μl was removed as control DNA undigested by DNase, and 2.5 U of DNase I (Invitrogen) was added to the remaining 100 μl reaction mixture. Then, 10 μl aliquots were removed from the 100 μl mixture at 2, 5, 10, 20, 30, 45, 60, 90, 120, and 180 min. The 10 μl aliquots removed were mixed with 2 μl of 25 mM EDTA at 65°C for 20 min. The control DNA was mixed with EDTA in the same manner and 2 μl of the total volume of 12 μl including EDTA was used for gel electrophoresis to check DNA fragmentation. Sterile water 840 μl was added to each of the remaining 10 μl aliquots, 1 μl of the 850 μl aliquots was used as template DNA to analyze the current miniY-STR multiplex PCR systems and the AmpflSTR Yfiler kit (Applied Biosystems).

We tested 50 forensic casework samples using the AmpflSTR Yfiler kit. Among the 50 DNA samples, samples for which genotyping were unsuccessful in more than 6 of the 16 Y-STR loci were defined as poor-quality DNA samples. These degraded DNA samples included each part of the filter paper from four different cigarettes, three swabs from PC keyboards, three swabs from plastic bottles, and 20 skeletal remains in various states of decomposition (from 2 to 14 years old). DNA was extracted from the swab samples using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) based on the buccal swab spin protocol specified in the kit handbook. DNA was extracted from the skeletal remains using SDS-proteinase K treatment followed by phenol/chloroform extraction, as

Table 1 Information on the miniY-STR loci and results of allele typing of standard cell line DNA

Mini Y-STR locus sequence motifs[a]		Product length (bp)	Allele range	Cell line DNA		Size reduction[b] (bp)	Position	
				9948	007		(Mb)[c]	
MiniY-6plex								
DYS578	Pf-N_3-$[AAAT]_n$-N_{31}-Pr	104–124	7–12	9	9	58	20.900	(q11.222)
DYS638	Pf-N_{22}-$[TTTA]_n$-N_{36}-Pr	140–148	10–12	11	13	118	16.083	(q11.221)
DYS643	Pf-$[CTTTT]_n$-N_{21}-Pr	102–137	8–15	11	11	28	15.864	(q11.221)
DYS441	Pf-N_7-$[TTCC]_{n-2}$-(T)-N_{-7}-Pr	91–119	13–20	18	15	267	13.420	(q11.21)
DYS575	Pf-N_{43}-$[AAAT]_n$-N_{10}-Pr	138–146	10–12	10	10	83	7.479	(p11.2)
DYS618	Pf-N_4-$[TAT]_n$-N_{51}-Pr	127–136	11–14	12	12	59	21.698	(q11.223)
MiniY-5plex1								
DYS549	Pf-N_7-$[GATA]_n$-N_{22}-Pr	115–135	10–15	13	13	113	19.908	(q11.222)
DYS485	Pf-N_{-1}-(TA)-$[TTA]_{n-1}$-N_{20}-Pr	100–121	11–18	15	15	163	20.487	(q11.222)
DYS462	Pf-N_6-$[GTAT]_{n-1}$-(GTA)-N_{-1}-Pr	92-104	11-14	11	11	90	19.705	(q11.222)
DYS593	Pf-$[AAAAC/AAAAT]_n$-Pr	117-132	15-18	15	15	105	17.023	(q11.221)
DYS505	Pf-N_{-7}-(T)-$[TCCT]_{n-2}$-N_{24}-Pr	97-125	8-15	12	12	61	3.684	(p11.2)
MiniY-5plex2								
DYS446	Pf-N_{-2}-(TCT)-$[TCTCT]_{n-2}$-(TCTC)-N_{-1}-Pr	96-151	11-22	12	14	197	3.174	(p11.2)
DYS495	Pf-N_{18}-$[AAT]_{n-2}$-(A)-N_{-5}-Pr	96-114	13-19	16	16	110	13.449	(q11.21)
DYS511	Pf-N_{42}-$[GATA]_n$-N_5-Pr	134-146	9-12	11	10	88	15.743	(q11.221)
DYS481	Pf-N_8-$[CTT]_n$-N_3-Pr	108-141	19-30	24	22	11	8.469	(p11.2)
DYS510	Pf-N_5-$[TAGA]_n$-Pr	110-130	15-20	17	17	135	15.738	(q11.221)

Pf Forward primer, *Pr* reverse primer, $N_{\text{negative value}}$ negative value indicates the number of bases at the 3′ end of the primer included repeat region

[a] Sequence motif of amplified PCR product of allele (n); (n) indicates the number of repeats.

[b] Size reduction in comparison with reference primers presented in the Genome Database (http://www.gdb.org)

[c] Each chromosome position was determined by a BLAST (NCBI Homo sapiens Build 35.1).

described in a previous paper [27]. Analysis using the miniY-STR multiplex PCR systems was performed in the same manner as described above.

Results and discussion

Designed primer and performance of the three miniY-STR PCR systems

We selected the 16 polymorphic Y-STR loci DYS441, DYS446, DYS462, DYS481, DYS485, DYS495, DYS505, DYS510, DYS511, DYS549, DYS575, DYS578, DYS593, DYS618, DYS638, and DYS643 whose positions are widely dispersed over the Y chromosome (Table 1). DYS446, DYS481, DYS505, and DYS575 are located on Yp11.2; DYS441 and DYS495 on Yq11.21; DYS510, DYS511, DYS593, DYS638, and DYS643 on Yq11.221; DYS462, DYS485, DYS549, and DYS578 on Yq11.222; and DYS618 on Yq11.223. Primer sets for these 16 Y-STR loci were newly designed to generate amplicons of less than 150 bp by setting each primer binding site closer to the repeat region. Table 1 shows the sequence motifs for all 16 Y-STR loci. In DYS446, bases at the 3′ end of both the forward and reverse primers belong to the repeat region. In DYS485 and DYS505, bases at the 3′ end of the forward primer belong to the repeat region, while in DYS441, DYS462, and DYS495, bases at the 3′ end of the reverse primer belong to the repeat region. In DYS593, the repeat region adjoins both the forward and reverse primers. In DYS643, no base lies between the forward primer and repeat region. In DYS510, no base lies between the reverse primer and repeat region. Genotyping of 238 Japanese male individuals and the commercial 9948 (Promega, Madison, WI, USA) and 007 (Applied Biosystems) cell line DNA using the three miniY-STR multiplex PCR systems was successful for all 16 loci. Based on this result, we assumed that no mutations occurred in the primer binding sites for the newly designed primers. Figure S1 shows the results of the control DNA 9948 (Promega). While minor broad artifact peaks and stutter peaks were detected in several loci, these peaks had no effect on allele typing. The broad peaks were considered to be residual dye molecules or dye blobs as described in a previous paper [5], and these peaks could be physically removed through a spin column. Detected in DYS481, the highest stutter peak ranged from 15–20% in data for 238 analyses. In sensitivity studies, allele typing using 20 pg template DNA was successful with peak heights of more than 100 relative fluorescence units (RFU) in 15 Y-STR loci, except for DYS549. In DYS549, a peak of greater than 100 RFU was detected with 30 pg template DNA. In 12 loci other than DYS441, DYS549, DYS485, and DYS481, allele typing using only

Table 2 The result of genotyping using the three miniY-STR systems and a commercial kit for artificially fragmented DNA

Enzyme reaction time	MiniY-6plex							MiniY-5plex1					MiniY-5plex2					AmpflSTR Yfiler
	DYS578	DYS638	DYS643	DYS441	DYS575	DYS618	DYS549	DYS485	DYS462	DYS593	DYS505	DYS446	DYS495	DYS511	DYS481	DYS510		
0 min	**[a]	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		16/16[b]
2.5 min	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		14/16
5 min	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		8/16
10 min	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		5/16
15 min	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		2/16
20 min	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		2/16
25 min	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		1/16
40 min	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			0/16
60 min	*	*	*	*	*		*	*	*	*	*	*	*	*	*			0/16
90 min	*	*	*	*			*	*	*	*	*	*	*	*				0/16
180 min	*	*	*	*			*	*	*	*	*	*	*					0/16

[a] Asterisk mark indicates successful genotyping in this locus with more than 100 RFU of peak height.
[b] Number of loci typed successfully in the 16 loci using the AmpflSTR Yfiler kit.

10 pg template DNA proved successful, with peaks of greater than 100 RFU. In addition, allele typing using 10 pg of template DNA was successful in all 16 Y-STR loci with increased PCR cycles (32 cycles). On the other hand, in female/male mixture studies, typing of male DNA was successful for all ratios, except for the genotyping of DYS446. At high ratios of female DNA (500:1 and 100:1), non-specific peaks were detected in NED and PET of MiniY-5plex1 and 6FAM of MiniY-5plex2, while no peaks attributable to female DNA was detected in the others, even with higher female DNA ratios. The peaks in NED and PET of MiniY-5plex1 proved to have no effect on allele typing, as the peaks were always detected outside the range of the actual peaks of DYS462 (NED), DYS593 (NED), and DYS505 (PET). The non-specific peak detected in 6FAM of MiniY-5plex2 was detected within the range of the actual peak of DYS446. As a result of a BLAST search (http://www.neci.nlm.nih.gov/mapview/), the non-specific peak may originate from a similar region on Xq21 of female DNA. Therefore, in the three miniY-STR multiplex PCR systems, analyses for female/male mixtures should be avoided only for DYS446 genotyping.

Japanese population data

In an assay of 238 Japanese male individuals using the three miniY-STR multiplex PCR systems, amplification product lengths without tailed seven bases ranged from 91 bp (DYS441) to 151 bp (DYS446) for all 16 Y-STR loci. The average reduction in size in the 16 loci compared to the reference primer sets indicated in the Genome Database (http://www.gdb.org) was 105.4 bp (Table 1). The number of alleles detected in the 238 samples ranged from 3 (DYS638 and DYS575) to 12 (DYS446 and DYS481). Table S2 gives the observed frequencies and gene diversities. Gene diversity ranged from 0.1506 (DYS575) to 0.8831 (DYS446). In ten Y-STR loci among all 16 loci, gene diversity exceeded more than 0.6. Table S3 shows the gene diversity of 40 Y-STR loci in the Japanese population, including the present miniY-STR16 loci, miniY-STR 8 loci (DYS504, DYS508, DYS522, DYS540, DYS556, DYS570, DYS576, and DYS632) reported in our earlier paper [13], and 16 loci analyzed using the AmpflSTR Yfiler kit in the literature [19]. Among 40 Y-STR loci, 23 have a gene diversity exceeding 0.6. MiniY-STR loci account for 13 of the 23 Y-STR loci, while among 16 Y-STR loci genotyped with the AmpflSTR Yfiler kit, ten loci indicate a gene diversity exceeding 0.6. On the other hand, in the assay of 238 samples, we identified 212 different haplotypes, with haplotype diversity and discrimination capacity of 0.9974 and 0.8908, respectively, of which, 200 haplotypes (84.0%) were detected only once, six haplotypes (2.5%) were detected from two individuals,

Table 3 Results of allele typing using the miniY-STR multiplex PCR systems for degraded DNA samples in forensic casework

Successful results of AmpflSTR Yfiler[a]	Number of samples	MiniY-6plex						MiniY-5plex1						MiniY-5plex2				Successful results of the miniY-STR loci[c]
		DYS578	DYS638	DYS643	DYS441	DYS575	DYS618	DYS549	DYS485	DYS462	DYS593	DYS505	DYS446	DYS495	DYS511	DYS481	DYS510	
10	2	2/2[b]	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	16
9	3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	16
8	4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	16
7	2	2/2	1/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	15.5
6	2	2/2	0/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	1/2	2/2	2/2	2/2	2/2	2/2	2/2	14.5
5	1	1/1	0/1	0/1	1/1	1/1	0/1	1/1	1/1	0/1	0/1	1/1	1/1	1/1	1/1	0/1	0/1	9
4	3	3/3	2/3	2/3	2/3	3/3	0/3	2/3	3/3	2/3	1/3	3/3	3/3	3/3	3/3	0/3	1/3	10.7
3	5	5/5	1/5	4/5	4/5	2/5	3/5	3/5	3/5	4/5	3/5	3/5	5/5	5/5	3/5	3/5	3/5	10.8
2	3	2/3	2/3	2/3	1/3	2/3	0/3	0/3	2/3	1/3	1/3	2/3	2/3	3/3	2/3	0/3	0/3	7.3
0	5	3/5	4/5	1/5	3/5	2/5	1/5	2/5	2/5	0/5	2/5	1/5	2/5	4/5	3/5	2/5	2/5	6.8
Total	30	27/30	19/30	22/30	24/30	23/30	17/30	21/30	24/30	21/30	21/30	19/30	26/30	29/30	25/30	18/30	19/30	

[a] Number of loci typed successfully with the AmpflSTR Yfiler kit.
[b] Number of samples typed successfully for each locus using the miniY-STR multiplex PCR systems.
[c] Mean number of loci typed successfully among all 16 miniY-STR loci using the miniY-STR multiplex PCR systems.

three haplotypes (1.3%) from three individuals, one haplotype (0.4%) from four individuals, one haplotype (0.4%) from five individuals, and one haplotype (0.4%) from eight individuals. Samples showing the same haplotypes in the 16 Y-STRs were analyzed for the further investigation using the previous miniY-STR systems for eight other Y-STR loci [13]. As a result of the assay, 235 haplotypes were identified among 238 samples, and haplotype diversity and discrimination capacity were 0.9994 and 0.9874, respectively (Table S4). In the relationship between investigated father and son cases (42 cases), no mutations were detected in any of the 16 Y-STR loci. Further investigation is needed to obtain a reliable mutation rate.

Analysis of degraded DNA samples

Several commercially available Y-STR multiplex PCR system kits, including the AmpflSTR Yfiler kit (Applied Biosystems) and Power Plex Y system (Promega), have gained widespread use. Although these commercial kits are not necessarily manufactured for the analysis of degraded DNA, analysis of degraded DNA using these kits generally yields moderate results. In general, analyses of degraded DNA sample allele typing tends to encounter difficulties with STR loci exceeding 200 bp due to DNA fragmentation. Our test (involving 50 tested samples) involving the selection of degraded male DNA samples using the AmpflSTR Yfiler kit showed that allele typing encounters problems with longer amplicon loci, such as DYS392 (only 24% were successful in genotyping), DYS448 (32%), DYS19 (36%), DYS439 (48%), DYS389II (52%), and DYS385 (56%), while genotyping tends to be successful for shorter amplicon loci such as DYS393 (88% genotyping success), DYS458 (86%), DYS389I (84%), DYS391 (80%), and DYS456 (80%). Unfortunately, among the 16 loci included in the kit, the majority of these longer amplicon loci for which genotyping tended to be unsuccessful were the loci with higher gene diversity. The development of further miniY-STR PCR systems may play an important role in analyzing degraded DNA samples.

We tested artificially degraded DNA digested with DNase (Table 2) and forensic casework samples with poor quality DNA. In a test of artificially fragmented DNA series using the three miniY-STR multiplex systems, genotyping was successful (>100 RFU) in all 16 loci with DNA digested for less than 25 min. Moreover, 12 loci other than DYS575, DYS618, DYS511, and DYS510 were clearly typed, even with DNA digested for 180 min. On the other hand, in tests of fragmented DNA series using a commercial kit AmpflSTR Yfiler, half of the loci (eight loci) proved unsuccessful with DNA digested for 5 min, and only one locus could be typed with DNA digested for 25 min. No locus was successful in genotyping DNA digested for more than 40 min. A direct relationship between amplification efficiency for physically fragmented DNA and the size of PCR product fragments was equally clear. Moreover, in tests of forensic casework samples with poor quality DNA, miniY-STR systems proved effective in analyzing degraded DNA samples (Table 3). In 22 out of 30 DNA samples defined as poor quality DNA samples, the number of loci typed successfully using a commercial kit AmpflSTR Yfiler ranged from three to ten loci. In tests of these 22 samples using the miniY-STR systems, the number of loci typed successfully exceeded ten loci, on average. Moreover, in 5 of the 30 degraded DNA samples, no locus could be typed using the commercial kit, while the miniY-STR systems were successful for 6.8 loci on average for the five samples. These results, which suggest the usefulness of miniY-STR systems producing short amplicon STR in analyzing degraded DNA samples, are consistent with previous reports on miniSTR.

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