Int J Legal Med (2006) 120: 174–181
DOI 10.1007/s00414-005-0074-6

TECHNICAL NOTE

H. Asamura · H. Sakai · K. Kobayashi ·
M. Ota · H. Fukushima

MiniX-STR multiplex system population study in Japan and application to degraded DNA analysis

Received: 28 August 2005 / Accepted: 6 December 2005 / Published online: 11 February 2006

Abstract We sought to evaluate a more effective system for analyzing X-chromosomal short tandem repeats (X-STRs) in highly degraded DNA. To generate smaller amplicon lengths, we designed new polymerase chain reaction (PCR) primers for DXS7423, DXS6789, DXS101, GATA31E08, DXS8378, DXS7133, DXS7424, and GATA165B12 at X-linked short tandem repeat (STR) loci, devising two miniX-multiplex PCR systems. Among 333 Japanese individuals, these X-linked loci were detected in amplification products ranging in length from 76 to 169 bp, and statistical analyses of the eight loci indicated a high usefulness for the Japanese forensic practice. Results of tests on highly degraded DNA indicated the miniX-STR multiplex strategies to be an effective system for analyzing degraded DNA. We conclude that analysis by the current miniX-STR multiplex systems offers high effectiveness for personal identification from degraded DNA samples.

Keywords X chromosome · Short tandem repeat (STR) · Smaller amplicon lengths · Multiplex systems · Degraded DNA

Introduction

Autosomal short tandem repeat (STR) markers constitute an established and highly effective tool for determining personal identity [1, 2]. Recent forensic interest has focused on gonosomal polymorphisms. A father's Y chromosome is transmitted to male offspring, while his X chromosome is transmitted to female offspring, making analyses of the gonosomal polymorphisms highly useful for special cases of kinship testing. The past few years have seen the gathering of significant volumes of Y-chromosomal STR loci and population genetic data from a wide range of ethnic groups data that are now routinely applied in forensic practices [3, 4]. However, population genetic data for X-chromosomal STR (X-STR) are available for only a handful of ethnic groups, although many X-STR loci have been reported [5–15]. On the other hand, certain forensic cases involving molecular postmortem identification sometimes require analysis of highly degraded samples, with DNA fragmentation and the presence of polymerase chain reaction (PCR) inhibitors. Several previous papers have discussed the usefulness of smaller PCR products for highly degraded samples [16–21]. In this study, we attempted to confirm a more effective system of X-STR analysis for highly degraded DNA. We will describe two miniX-STR multiplex systems that incorporate newly designed PCR primers, wherein the amplicon lengths are made as short as possible, and investigated Japanese population data for the eight X-STR loci using these systems. Additionally, we tested highly degraded DNA to determine whether these multiplex systems were effective in analyzing degraded DNA.

H. Asamura (✉) · H. Sakai · K. Kobayashi ·
M. Ota · 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
Tel.: +81-263-373218
Fax: +81-263-373084

Materials and methods

Fresh blood samples were collected from 333 unrelated, healthy, adult Japanese individuals (195 males and 138 females) after obtaining informed consent. DNA was extracted from whole blood using the Nucleic Acid Isolation System Quick Gene-800 (FUJIFILM, Tokyo, Japan).

Two multiplex PCRs were performed with the newly designed primer sets, except for a forward primer of DXS7133 (only this primer sequence was identical to the primer used in the study reported in a previous paper [8]). Table 1 lists the various primer sequences and concentrations used. PCR reactions were performed in a total volume of 10 μl containing 100 pg of genomic DNA, 1× GeneAmp PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate (dNTP, GeneAmp dNTP MIX),

Table 1 Primer sequences used in this study

X-STR locus	Primer sequences		Dye label	Primer concentration (μM)
	Forward primer	Reverse primer		
Multiplex 1				
DXS7423	5′-AGATTTCCTCCCCATCCATC-3′	5′-GTTGTCACACAAATAAATGAATxGAGT-3′	FAM	0.7
DXS6789	5′-CCTCGTGATCATGTAAGTTGG-3′	5′-GCAGAACCAATAGGAGATAGATGGT-3′	VIC	0.9
DXS101	5′-TCTCCCTTCAAAAACAAAGATAA-3′	5′-GTGCATATTCTGCGCATGT-3′	NED	1.3
GATA31E08	5′-CAGAGCTGGTGATGATAGATGA-3′	5′-GCTCACTTTTATGTGTGTATGTATCTCC-3′	PET	1.3
Multiplex 2				
DXS8378	5′-GCTCCTGGCAGGTCACTATC-3′	5′-GCGACAAGAGCGAAACTCCA-3′	FAM	0.9
DXS7133	5′-AGCTTCCTTAGATGGCATTCA-3′	5′-GTTTTTAACGGTGTTCATGCTT-3′	VIC	0.7
DXS7424	5′-AAAACAGGAAGACCCCATC-3′	5′-GGCTAAGAAGAATCCCGCACA-3′	NED	0.7
GATA165B12	5′-TCATCAATCATCTATCCGTAT ATCA-3′	5′-GAAGTTGACTGTGATTCCTGGTTT-3′	PET	1.1

X-STR X-chromosomal short tandem repeat

each primer set, and 1 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). One multiplex (miniX-multiplex 1) PCR mixture contained 0.7 μM primer sets of DXS7423 (fluorescent dye labeled with 6FAM), 0.9 μM DXS6789 (VIC), 1.3 μM DXS101 (NED), and 1.3 μM GATA31E08 (PET). The other multiplex (miniX-multiplex 2) PCR mixture contained 0.9 μM primer sets of DXS8378 (6FAM), 0.7 μM DXS7133 (VIC), 0.7 μM DXS7424 (NED), and 1.1 μM GATA165B12 (PET). PCR was performed using a GeneAmp 9,700 (Applied Biosystems) in 9600 mode. The cycling programs consisted of pre-denaturation at 95°C for 11 min, followed by 28 cycles of denaturing at 94°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 45 s and a final extension at 60°C for 30 min.

Electrophoresis was performed using an ABI 310 Genetic Analyzer (Applied Biosystems) in which 1 μl of multiplex PCR product was mixed with 24.5 μl Hi-Di formamide and 0.5 μl of the GeneScan-500LIZ size standard. Samples were injected for 3 s at 15,000 V and separated at 60°C for 20 min. GeneMapper ID v3.2 Software was used to analyze the data. Allelic ladders were devised using a combination of products of varying lengths among male samples at each locus. All the ladder alleles for each locus were sequenced to calibrate the number of repeats (allele number). PCR was performed separately by each nonlabeled primer set (Table 2) as described in previous papers [5, 7–9, 15]. The BigDye v1.1 Ready Reaction Kit was used for the sequencing reactions with the same primers as above following purification with a Microcon YM-100 centrifugal filter unit (Millipore, Bedford, MA). Finally, the products were sequenced with sequencing analysis software 5.2 using ABI 310 Genetic Analyzer. Using these allelic ladders, we analyzed genotypes on two commercially available cell lines, K562 (Promega, Madison, WI) and 9947A (Applied Biosystems). To determine the minimum quantity of DNA required to obtain reliable results with the miniX-STR multiplex strategies, we used 9947A DNA (Applied Biosystems) sequentially diluted with distilled water (5, 20, 50, and 100 pg/μl and 1 ng/μl). The final concentrations of template DNA in the assay were 5, 10, 20, 30, 40, 50, 75, 100, and 500 pg and 1, 5, and 10 ng.

Based on combined male and female data, we calculated the allele frequency at each locus and polymorphism information content (PIC) [22]. Using female data, we also determined the Hardy–Weinberg equilibrium by an exact test [23], observed heterozygosity (OH), and expected

Table 2 Unlabeled primer sets for sequencing study

X STR locus	Primer sequences		Reference
	Forward primer	Reverse primer	
DXS7423	5′-GTCTTCCTGTCATCTCCCAAC-3′	5′-TAGCTTAGCGCCTGGCACATA-3′	[8]
DXS101	5′-ACTCTAAATCAGTCCAAATATCT-3′	5′-AAATCACTCCATGGCACATGTAT-3′	[7]
GATA31E08	5′-AGGGGAGAAGGCTAGAATGA-3′	5′-CAGCTGACAGAGCACAGAGA-3′	[15]
DXS8378	5′-CACAGGAGGTTTGACCTGTT-3′	5′-AACTGAGATGGTGCCACTGA-3′	[8]
DXS7133	5′-AGCTTCCTTAGATGGCATTCA-3′	5′-TACTTGGTGGGAGGAATAG-3′	[8]
DXS7424	5′-CTGCTTGAGTCCAGGAATTCAA-3′	5′-GAACACGCACATTTGAGAACATA-3′	[9]
GATA165B12	5′-TATGTATCATCAATCATCTATCCG-3′	5′-TTAAAATCATTTTCACTGTGTATGC-3′	[15]

probability of exclusion (PE) [24]. The average power of discrimination (PD) [24] was calculated from male and female data.

Degraded DNA was tested to determine whether the miniX-STR multiplex strategies were effective in analyzing degraded DNA. To avoid typing errors caused by allele dropout in the multiplex strategies, only male samples were selected. The DNA was extracted using SDS-proteinase K treatment followed by phenol/chloroform extraction from a variety of old materials, which included organs of some corpses in advanced states of decomposition, blood stains stored at room temperature for long periods, and a significant number of hard tissue samples (bone and tooth) in various states of decomposition. To single out degraded DNA, the abovementioned samples were analyzed using the AmpflSTR Identifiler kit (Applied Biosystems), a commercial kit. Analyses using the AmpflSTR Identifiler kit were carried out according to the protocol in the user's manual, except for the number of PCR cycles (changed to 30 cycles). Using the results of the analyses, samples for which allele typing was unsuccessful in more than 6 loci among the 15 loci (leaving out the amelogenin locus) were selected as degraded DNA samples. We analyzed these degraded DNA samples using the miniX-STR multiplex strategies. The protocol was the same as mentioned above except for the number of PCR cycles (changed to 30 cycles).

Results and discussion

A combination of X-STR loci and autosomal STRs is highly desirable for kinship testing. In particular, X-STR markers can be highly informative in certain special cases of kinship testing, such as father–daughter or paternal grandmother–granddaughter relationships. On the other hand, molecular postmortem identification sometimes involves highly degraded materials. In this study, we attempted to confirm a more effective analysis system of X-STRs with highly degraded DNA.

One of the most effective methods for improving molecular analysis of highly degraded DNA is by reducing the size of the PCR products. Several previous studies have confirmed that smaller autosomal amplicons lead to more successful analysis of degraded DNA [16–21]. We attempted to apply this method to X-STR analyses. We selected the eight X-STR loci DXS7423, DXS6789, DXS101, GATA31E08, DXS8378, DXS7133, DXS7424, and GATA165B12. X-STR loci were classified into four linkage groups in a previous paper [10]. DXS8378 on Xp22 was identified as belonging to linkage group 1, while DXS6789, DXS101, DXS7424, and DXS7133 were identified as being part of linkage group 2. DXS7423 belonged to linkage group 4. Although further research is required to classify GATA31E08 on Xq27 (140 Mbp, 154.29 cM) and GATA165B12 on Xq24 (121 Mbp, 133.2 cM) into their precise linkage group(s), the X-STR loci in the present study included representatives from each linkage group except for linkage group 3, which included only the HPRTB locus (Fig. 1). The new primer sets were designed to produce amplicons that were as small as possible, including the addition of a guanine to the 5′ end of each reverse primer to promote adenylation. All typing with the two multiplex systems was successful for all eight X-STR loci. An example for the systems is shown in Fig. 2. Although stutter peaks in the loci were identified, in all the loci, except for DXS7424, they were very small (5–10%). The DXS7424 locus stutter peak was indicated to be 10–20%, and small artifact peaks were detected; however, disturbance of its allele typing proved impossible. Allele types of two commercially available cell lines, K562 (showing a diploid state only in the DXS101 locus) and 9947A, are shown in Table 3. As a result of analysis of the sensitivity range of the DNA detection limit, allele typing in all eight loci was successful using 20 pg or more of template DNA [peak highs in all cases exceeded 100 relative fluorescence units (RFU)]. In the analysis using

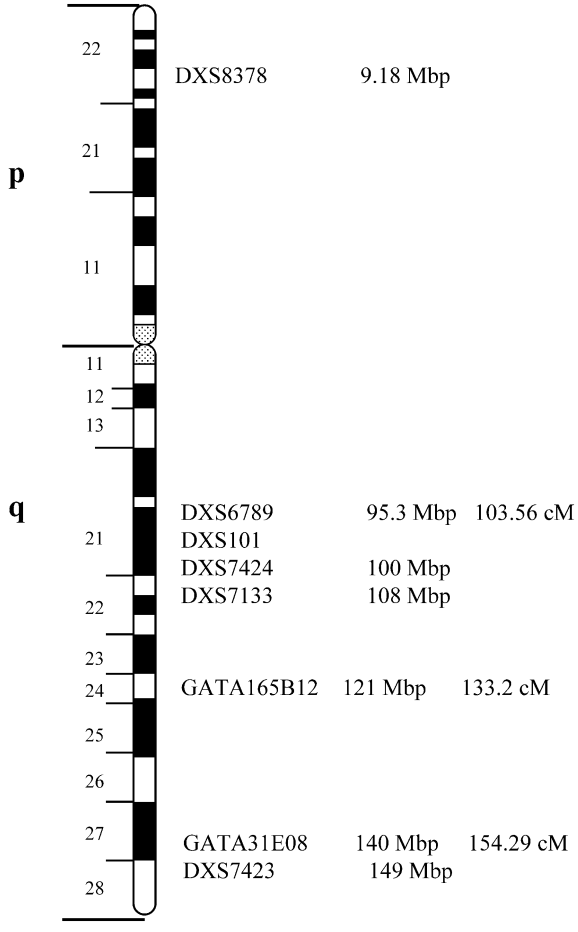


Fig. 1 Positions of eight X-chromosomal short tandem repeat (X-STR) loci on chromosome X ideogram. Detail positions were obtained in mega base pairs (http://www.ncbi.nlm.nih.gov) and centimorgans (http://www.marshfieldclinic.org/reseach/genetics/)

10 pg of template DNA, allele dropout was detected in DXS7423 and GATA165B12 loci, while allele typing was successful in the other loci. To avoid mistyping due to allele dropout, we set the low template level within a range from 20 to 30 pg. In the group using 1 ng or more of template DNA, although allele typing was successful, target peaks were too high, and broad peaks and nonspecific peaks were also observed. The amplification products for these X-linked loci from 333 Japanese individuals ranged in length from 76 to 169 bp. The size reductions compared with the amplicon lengths of each reference allele ranged from 19 bp in DXS7133 to 127 bp in GATA31E08 (Table 3).

Table 4 shows the observed allele frequencies and forensic statistical parameters for the loci determined following an investigation of genetic data for the Japanese population. The genotype frequency distributions for female data showed no significant deviation from the Hardy–Weinberg equilibrium by an exact test, except for DXS6789 (P=0.008). Heterozygosity values ranged from 0.493 (DXS7133) to 0.812 (DXS101), while the PE ranged from 0.279 (DXS7133) to 0.659 (DXS101). In all loci, allele frequencies did not differ significantly between males and females, and the PIC ranged from 0.414 (DXS7133) to 0.777 (DXS101). The PD for male data ranged from 0.471 (DXS7133) to 0.805 (DXS6789), while the PD for females ranged from 0.666 (DXS7133) to 0.937 (DXS101). Of the eight loci, DXS101 showed the highest heterozygosity, PIC, PD for female data, and PE. A combination of analyses of the eight X-STR loci appears to hold significant promise for Japanese forensic practice. A comparison of these allele frequencies from the Japanese population to foreign population data showed no significant divergences from corresponding data for the populations of Korean and Taiwan [13, 15].

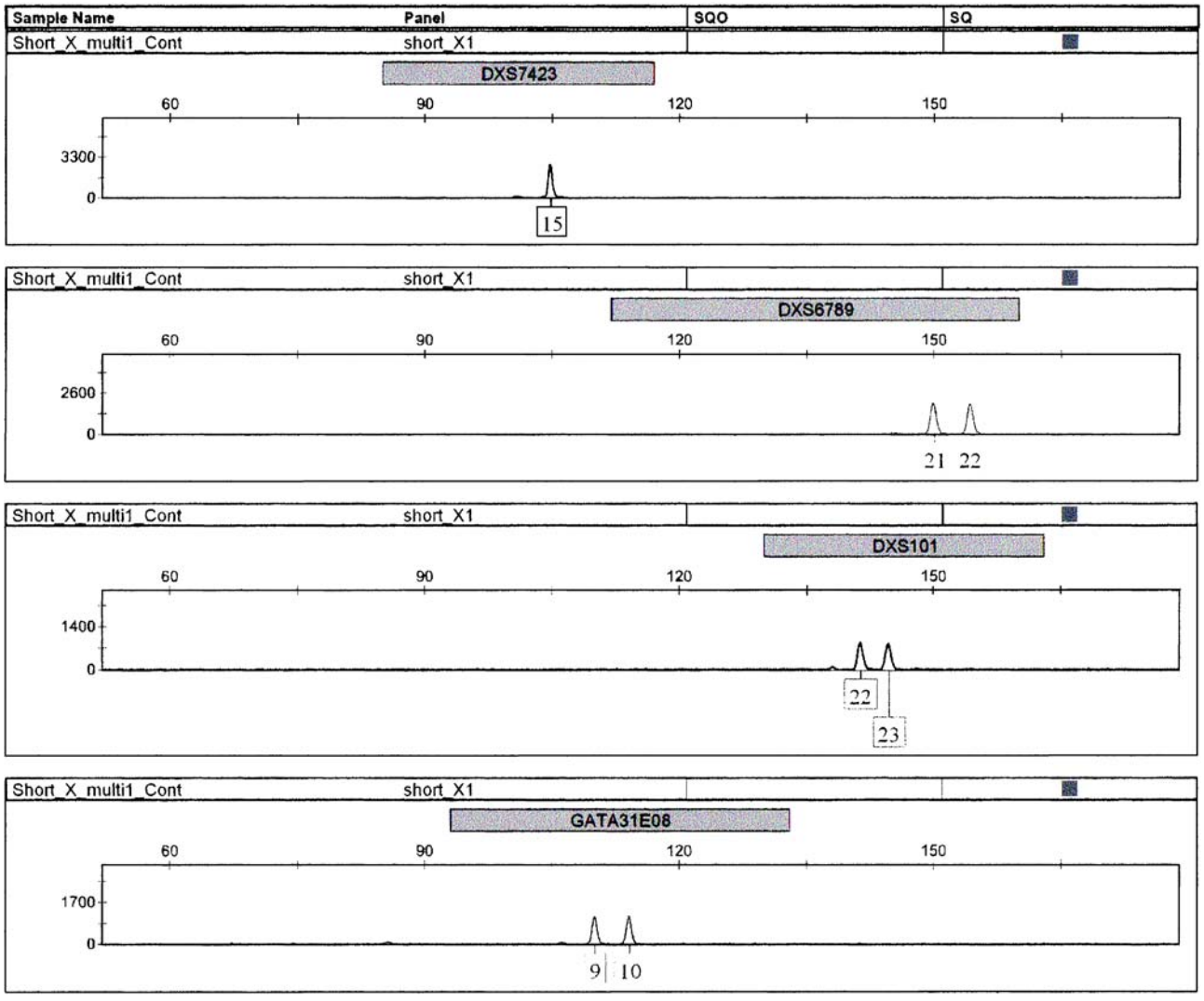
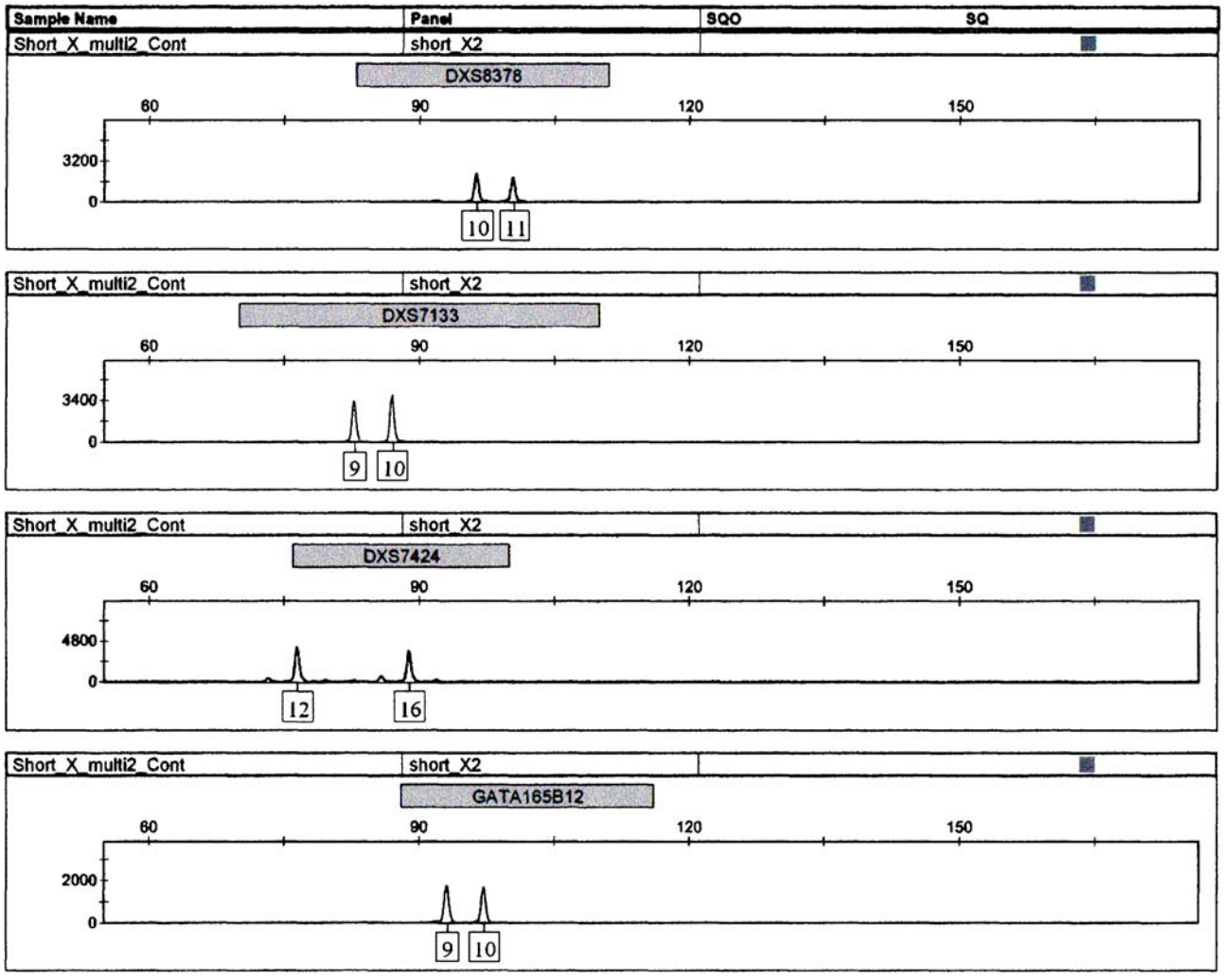


Fig. 2 Electropherograms of the two miniX-STR multiplex systems in a control female sample. **a** X-multiplex 1 with the loci of DXS7423 (FAM), DXS6789 (VIC), DXS101 (NED), and GATA31E08 (PET). **b** X-multiplex 2 with loci of DXS8378 (FAM), DXS7133 (VIC), DXS7424 (NED), and GATA165B12 (PET)

Fig. 2 (continued)



Highly degraded DNA was tested to determine whether the miniX-STR multiplex strategies were effective in analyzing of degraded DNA. Samples, selected degraded DNA, were from three organs (liver, skin, and muscle) of a corpse in a state of advanced decomposition, three blood stains stored at room temperature for more than 27 years, and 14 bone samples and nine teeth samples in various states of decomposition. Table 5 shows the number of loci

Table 3 Information on the X-STR loci used in this study and a result of allele typing of standard cell line DNA

X-STR locus	Product length (bp)	Allele range	Cell line DNA		Size reduction against amplicon of reference (bp)	Reference
			K562	9947A		
Multiplex 1						
DXS7423	99–115	12–16	17	14, 15	76	[8]
DXS6789	122–162	14–24	21	21, 22	32	[5]
DXS101	142–169	21–30	23, 24	24, 26	58	[7]
GATA31E08	101–133	7–15	11	11, 11	127	[15]
Multiplex 2						
DXS8378	95–111	9–13	10	10, 11	19	[8]
DXS7133	76–100	6–12	10	9, 10	26	[8]
DXS7424	79–100	11–18	17	14, 16	74	[9]
GATA165B12	90–110	8–13	10	9, 11	35	[15]

Table 4 Observed allele frequencies and forensic efficiency parameters for the eight X-STR loci in the Japanese population

Allele	Multiplex-1				Multiplex-2			
	DXS7423	DXS6789	DXS101	GATA31E08	DXS8378	DXS7133	DXS7424	GATA165B12
6						0.002		
7				0.111		0.002		
8				0.028				0.002
9				0.176	0.015	0.675		0.261
10				0.251	0.562	0.253		0.501
11				0.318	0.285	0.064	0.004	0.196
12	0.004			0.106	0.113	0.004	0.021	0.038
13				0.004	0.025		0.053	0.002
14	0.285	0.006		0.004			0.108	
15	0.622	0.134		0.002			0.353	
16	0.089	0.327					0.395	
17		0.047					0.047	
18							0.019	
19		0.021						
20		0.130						
21		0.234	0.002					
22		0.068	0.040					
23		0.025	0.119					
24		0.008	0.304					
25			0.217					
26			0.176					
27			0.091					
28			0.036					
29			0.013					
30			0.002					
OH	0.543	0.688	0.812	0.804	0.674	0.493	0.580	0.630
P^a	0.235	0.008	0.376	0.472	0.318	0.247	0.111	0.890
PIC	0.454	0.769	0.777	0.748	0.527	0.414	0.653	0.581
PE	0.321	0.631	0.659	0.619	0.389	0.279	0.492	0.430
PD_f	0.712	0.927	0.937	0.921	0.776	0.666	0.850	0.809
PD_m	0.508	0.805	0.798	0.776	0.574	0.471	0.720	0.643

OH Observed heterozygosity, *PIC* Polymorphism information content, *PE* power of exclusion, PD_f power of discrimination in females, PD_m power of discrimination in males

[a] *P* values of the exact tests for Hardy–Weinberg equilibrium

for which typing was successful. Autosomal STR loci detected (excluding the amelogenin locus) using the AmpflSTR Identifiler ranged from 0 to 8. A much higher proportion of homozygote loci was observed in almost all samples. As such, allele dropout was suspected to have occurred in some loci. The samples selected for this test were determined to be typical degraded DNA samples. In the analyses using the miniX-STR multiplex strategy, although in some of the degraded DNA samples small artifact peaks could be detected at off-ladder locations, at least four loci were successful typed in 22 of the 29 samples. In DXS7423, GATA31E08, DXS7133, and DXS 7424, more than 20 samples could be typed successfully, while the number of samples for which typing was successful was less than half in DXS6789 and DXS101. It may be that these two loci have longer amplicons than the other loci, influencing the detection sensitivity. On the whole, these results appear to show that the present miniX-STR multiplex strategy was more successful in the typing of degraded samples.

We conclude that the present miniX-STR multiplex systems offer considerable potential for personal identification from degraded DNA samples.

Table 5 The result of allele typing using the X-STR multiplex systems for degraded DNA samples

Sample name	X-STR loci								Successful results of the X-STR loci	AmpflSTR Identifiler
	DXS7423	DXS6789	DXS101	GATA31E08	DXS8378	DXS7133	DXS7424	GATA165B12		
DegDNAliver	+	+	+	+	+	+	+	+	8[a]	6 (3)[b]
DegDNAskin	+	+	+	+	+	+	+	+	8	6 (2)
DegDNAmuscle	+	−	−	+	+	+	+	+	6	3 (2)
Bloodstain1	+	−	−	−	−	−	−	+	2	0
Bloodstain2	−	−	−	−	−	−	−	+	1	0
Bloodstain3	−	−	−	−	−	−	−	−	0	0
DegDNAbone1	+	+	+	+	+	+	+	+	8	6 (2)
DegDNAbone2	+	+	−	+	+	+	+	+	7	5 (1)
DegDNAbone3	+	−	−	−	−	−	+	+	3	0
DegDNAbone4	+	−	−	+	+	+	+	+	6	4 (1)
DegDNAbone5	+	+	−	+	+	+	+	+	7	5 (2)
DegDNAbone6	−	−	+	+	+	+	−	−	4	5 (0)
DegDNAbone7	−	−	−	+	+	+	+	−	4	5 (0)
DegDNAbone8	+	+	−	+	+	+	+	+	7	6 (2)
DegDNAbone9	−	−	−	+	+	+	+	−	4	2 (1)
DegDNAbone10	+	+	−	+	+	+	+	+	7	5 (0)
DegDNAbone11	+	+	−	+	+	+	+	+	7	8 (1)
DegDNAbone12	+	−	+	−	+	+	+	+	6	3 (0)
DegDNAbone13	−	+	+	+	−	+	−	+	5	8 (2)
DegDNAbone14	+	+	+	+	+	+	+	+	8	8 (4)
DegDNAteeth1	+	+	+	+	+	+	+	+	8	7 (3)
DegDNAteeth2	−	−	+	+	+	−	−	−	3	2 (0)
DegDNAteeth3	+	−	+	+	+	+	+	+	7	7 (1)
DegDNAteeth4	+	+	+	+	−	+	−	−	5	5 (0)
DegDNAteeth5	−	−	−	−	−	−	−	−	0	1 (0)
DegDNAteeth6	+	+	−	+	+	+	+	−	6	4 (0)
DegDNAteeth7	+	−	+	−	−	−	+	−	3	3 (1)
DegDNAteeth8	+	+	−	+	−	−	+	−	4	1 (0)
DegDNAteeth9	+	−	+	+	−	+	+	−	5	3 (0)
Total	21/29	14/29	13/29	22/29	19/29	21/29	21/29	18/29		

+ Successful in typing, − unsuccessful in typing

[a]Number of loci typed successfully in the miniX-STR multiplex sytems

[b]Number of loci typed successfully except for amelogenin locus in the AmpflSTR Identifiler. The number in parentheses shows number of loci detected for heterozygosity

References

1. Kinjhodzic R, Kubat M, Skavic J (2004) Bosnian population data for the 15 STR loci in the Power Plex 16 kit. Int J Legal Med 118:119–121
2. Veselinovic I, Kubat M, Furac I, Skavic J, Martinovic Klaric I, Tasic M (2004) Allele frequencies of the 15 AmpflSTR Identifiler loci in the population of Vojvodina Province, Serbia and Montenegro. Int J Legal Med 118:184–186
3. Kwak KD, Jin HJ, Shin DJ et al (2005) Y-chiromosomal STR haplotypes and their applications to forensic and population studies in east Asia. Int J Legal Med 119:195–201
4. Berger B, Lindinger A, Niederstatter H, Grubwieser P, Parson W (2005) Y-STR typing of an Austrian population sample using a 17-loci multiplex PCR assay. Int J Legal Med 119:241–246
5. Hering S, Kuhlisch E, Szibor R (2001) Development of the X-linked tetrameric microsatellite marker HumDXS6789 for forensic purposes. Forensic Sci Int 119:42–46
6. Edelmann J, Hering S, Michael M et al (2001) 16 X-chromosome STR loci frequency data from a German population. Forensic Sci Int 124:215–218
7. Edelmann J, Szibor R (2001) DXS101: a highly polymorphic X-linked STR. Int J Legal Med 114:301–304
8. Edelmann J, Deichsel D, Hering S, Plate I, Szibor R (2002) Sequence variation and allele nomenclature for the X-linked STRs DXS9895, DXS8378, DXS7132, DXS6800, DXS7133, GATA172D05, DXS7423 and DXS8377. Forensic Sci Int 129:99–103
9. Edelmann J, Hering S, Kuhlisch E, Szibor R (2002) Validation of the STR DXS7424 and the linkage situation on the X-chromosome. Forensic Sci Int 125:217–222
10. Szibor R, Krawczak M, Hering S, Edelmann J, Kuhlisch E, Krause D (2003) Use of X-linked markers for forensic purposes. Int J Legal Med 117:67–74
11. Edelmann J, Szibor R (2003) The X-linked STRs DXS7130 and DXS6803. Forensic Sci Int 136:73–75
12. Turrina S, De Leo D (2003) Population data of three X-chromosomal STRs: DXS7132, DXS7133 and GATA172D05 in North Italy. J Forensic Sci 48:1428–1429

13. Chen MY, Pu CE (2004) Population data on the X chromosome short tandem repeat loci DXS10011, DXS101, DXS6789, DXS7132, DXS8377, and DXS9895 in Taiwan. Forensic Sci Int 146:65–67
14. Hering S, Brundirs N, Kuhlisch E et al (2004) DXS10011: studies on structure, allele distribution in three populations and genetic linkage to further q-telomeric chromosome X markers. Int J Legal Med 118:313–319
15. Shin SH, Yu JS, Park SW, Min GS, Chung KW (2005) Genetic analysis of 18 X-linked short tandem repeat markers in Korean population. Forensic Sci Int 147:35–41
16. Hellmann A, Rohleder U, Schmitter H, Witting M (2001) STR typing of human telogen hairs—a new approach. Int J Legal Med 114:269–273
17. Wiegand P, Kleiber M (2001) Less is more—length reduction of STR amplicons using redesigned primers. Int J Legal Med 114:285–287
18. Tsukada K, Takayanagi K, Asamura H, Ota M, Fukushima H (2002) Multiplex short tandem repeat typing in degraded samples using newly designed primers for the TH01, TPOX, CSF1PO, and vWA loci. Leg Med 4:239–245
19. Butler JM, Shen Y, McCord BR (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. J Forensic Sci 48:1054–1064
20. Coble MD, Butler JM (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. J Forensic Sci 50:43–53
21. Asamura H, Uchida R, Takayanagi K, Ota M, Fukushima H (2005) Allele frequencies of the six miniSTR loci in a population from Japan. Int J Legal Med (in press)
22. Botstein D, White RI, Skolnich M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331
23. Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372
24. Desmarais D, Zhong Y, Chakraborty R, Perreault C, Busque L (1998) Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA). J Forensic Sci 43:1046–1049