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Yoshiaki Shigeta[*] Yuji Yamamoto[†] Yusuke Doi[‡]
Satoru Miyaishi[**] Hideo Ishizu[††]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,

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Abstract

We describe a modified method for typing a polymorphic microsatellite D12S391 locus by PCR using a newly designed primer pair. This primer pair produces shorter D12S391 amplified fragments (104-156 bp) than the primer pair originally described by Lareu et al. (209-261 bp). The detection system for the D12S391 locus using the new primer pair and capillary electrophoresis (CE) analysis was evaluated using various forensic samples. The typing results from 70 DNA samples using the new primer pair and the original primer pair were completely identical. One hundred twenty-five amplified fragments from D12S391 alleles were sized correctly within +/- 0.25 bp of the D12S391 allelic ladder. A rare allele, 19.3, previously found only in Caucasians, was found for the first time in a Japanese subject, and it was clearly distinguished from allele 20 by the CE analysis. This detection system was sensitive and could detect D12S391 types from 16 pg of genomic DNA, and from a minor component at a ratio of 1:10 in mixed samples. This system was more useful for the analysis of degraded DNA than was the method using the original primer pair, and could detect D12S391 types from bloodstains that had been stored for 26 years. In addition, the specificity of the method was demonstrated using nonhuman DNA.

KEYWORDS: short tandem repeats, D12S391, forensic application, capillary electrophoresis

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Original Article

Evaluation of a Method for Typing the Microsatellite D12S391 Locus Using a New Primer Pair and Capillary Electrophoresis

Yoshiaki Shigeta, Yuji Yamamoto*, Yusuke Doi, Satoru Miyaishi, and Hideo Ishizu

Department of Legal Medicine, Legal Medicine and Bioethics, Social and Environmental Sciences, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan

We describe a modified method for typing a polymorphic microsatellite D12S391 locus by PCR using a newly designed primer pair. This primer pair produces shorter D12S391 amplified fragments (104-156 bp) than the primer pair originally described by Lareu *et al.* (209-261 bp). The detection system for the D12S391 locus using the new primer pair and capillary electrophoresis (CE) analysis was evaluated using various forensic samples. The typing results from 70 DNA samples using the new primer pair and the original primer pair were completely identical. One hundred twenty-five amplified fragments from D12S391 alleles were sized correctly within ± 0.25 bp of the D12S391 allelic ladder. A rare allele, 19.3, previously found only in Caucasians, was found for the first time in a Japanese subject, and it was clearly distinguished from allele 20 by the CE analysis. This detection system was sensitive and could detect D12S391 types from 16 pg of genomic DNA, and from a minor component at a ratio of 1:10 in mixed samples. This system was more useful for the analysis of degraded DNA than was the method using the original primer pair, and could detect D12S391 types from bloodstains that had been stored for 26 years. In addition, the specificity of the method was demonstrated using nonhuman DNA.

Key words: short tandem repeats, D12S391, forensic application, capillary electrophoresis

For DNA typing from forensic biological evidence, in which the DNA is often damaged, the detection of short tandem repeats (STRs) using polymerase chain reaction (PCR) is effective, since the fragments to be amplified are short [1-4]. STR typing methods generally use product separation by slab gel electrophoresis with detection by silver staining or labeling with fluorescent dyes. Recently, capillary electrophoresis (CE) analysis, which provides rapidity, high resolution and precision, has been shown to be useful for the separation of amplified fragments [5-7]. We previously reported the polymorphism of the D12S391 microsatellite locus and the allele frequency in a Japanese population sample [8]. This locus showed a compound STR consisting of a 4-base repetition based on (AGAT)*n*(AGAC)*m*(AGAT)*l*. Fourteen different alleles, which were designated allele 15 to allele 28, ranging in size from 209 bp to 261 bp, were detected. In the present study, we modified the PCR reverse primer in order to obtain shorter amplified fragments (104-156 bp). In order to test this new primer pair in the examination of forensic biological evidence, we have evaluated it for accuracy, sensitivity, reproducibility, species specificity and for applications in mixed samples, degraded samples and aged bloodstains, using

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*Corresponding author. Phone: +81-86-235-7195; Fax: +81-86-235-7201
E-mail: y-yamamo@md.okayama-u.ac.jp (Y. Yamamoto)

fluorescence-based CE analysis.

Materials and Methods

Samples for examination and DNA extraction. Blood samples were obtained from 70 unrelated Japanese donors. Semen, buccal swabs and hair roots were obtained from volunteers of known D12S391 types. Samples of 10 organs or tissues, *i.e.*, the cerebrum, heart, lung, spleen, liver, kidney, pancreas, muscle, skin, and blood, were obtained from 2 autopsied bodies. Bloodstains were prepared on cotton cloth by spotting 10 μl aliquots of a blood sample. For the study of aged bloodstains, three 2 cm^2 pieces of bloodstained cloth preserved in the dark at room temperature for 26 years were used. The blood or muscles from 12 kinds of animals, *i.e.*, gorilla, chimpanzee, crab-eating monkey, pig, goat, sheep, cow, horse, rabbit, dog, cat, chicken, pigeon, rat, and mouse, were collected. DNA was obtained by standard proteinase K/SDS lysis followed by phenol/chloroform extraction and ethanol precipitation. For DNA extraction from semen, dithiothreitol was added to the lysis buffer. DNA quantification was carried out using a DU-640 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA).

Amplification conditions. The new PCR primer pair used for this study was:

forward primer: 5'-AACAGGATCAATGGATGCAT-3'

reverse primer: 5'-CCTCTAATAAATCCCCTCTC-3'

The forward primer was the same as that described by Lareu *et al.* [9]. The reverse primer was newly designed based on the D12S391 sequence deposited in GenBank (accession number G08921). The forward primer was labeled at the 5'-end with HEX (Applied Biosystems, Foster City, CA, USA). The amplification mixture consisted of 1 to 10 ng of genomic DNA, each primer at 0.1 μM, each dNTP at 0.2 mM, 0.02 U/μl AmpliTaq Gold DNA polymerase (Applied Biosystems), 10 mM Tris-HCl pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin and 0.2 μg/μl BSA in a 10 μl final reaction volume. The amplification parameters for this system were: after pre-denaturation at 95 °C for 10 min, denaturation at 94 °C for 45 sec, annealing at 54 °C for 60 sec, extension at 72 °C for 60 sec for 32 cycles, and a final post-extension incubation at 72 °C for 60 min in a GeneAmp 2400 thermal cycler (Applied Biosystems).

Detection of amplified fragments. Capillary electrophresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) was employed for the detection of amplified D12S391 fragments. To 2 μl of the amplified product, 0.63 μl of a carboxy-x-rhodamine (CXR) fluorescent ladder (Promega, Madison, WI, USA) as an internal size standard and 8 μl of deionized formamide were added. The mixture was denatured at 95 °C for 2 min, then cooled in an ice-water bath. Amplified products were injected for 2 sec at 15 kV into a 47 cm capillary filled with POP4 polymer (Applied Biosystems). Separations were run at 15 kV for 22 min at 60 °C. The result was analyzed with 310 GeneScan software version 2.1 (Applied Biosystems). Sizing precision studies were conducted by analyzing a homemade allelic ladder consisting of 14 sequenced alleles (15–28 repeats). The allelic ladder was made by mixing reamplified PCR products from 14 sequenced alleles, namely alleles 15 to 28.

Sequence analysis of the PCR products was performed using forward and reverse primers as described in a previous report [8].

Sensitivity studies. The genomic DNA samples used were a K562 Control DNA (Promega) that was homozygous for allele 23 and 2 other samples that were heterozygous for alleles 16 and 17, and alleles 21 and 26, respectively. Amplification of D12S391 alleles was performed from serial dilutions (1 ng to 8 pg) of these samples.

Mixture studies. DNA samples from a pair of individuals with alleles 21/26 and 16/17 (model A) were mixed at the ratios 1:1, 1:3, 1:5, 1:10, 1:20, 1:30, 1:40, and 1:50, holding the total amount of genome DNA constant at 1 ng. Mixed DNA samples from another pair of individuals with alleles 18/25 and 15/21 (model B) were prepared in the same manner.

Heat degradation studies. Bloodstains on the cotton cloths (alleles 18/20) were artificially degraded by heating them at 150 °C for 0–10 h. The genomic DNA extracted from the bloodstains at each time point was analyzed by agarose gel electrophoresis, and aliquots containing one-tenth of the extract were amplified using the new primer pair and the original primer pair [9].

Results

D12S391 typing and characteristics of the new primer pair. The detection system with PCR was able to perform D12S391 typing using this new

primer pair and CE analysis. Fig. 1 shows an electrophoretogram displaying an allelic ladder containing a total of 14 sequenced alleles. The typing results of the D12S391 locus, from allele 15 to 25 and allele 27, obtained from 70 DNA samples using the new primer pair were completely identical with those obtained using the original primer pair. The corresponding deviations in sizing for 125 amplified D12S391 fragments except for alleles 25 to 28 were determined to be < 0.25 bp for each corresponding allele in the allelic ladder (Fig. 2). The maximum value of one standard deviation was 0.081 bp, and the minimum value was 0.025 bp in each of the examined alleles. In this study, we observed an amplified fragment that is approximately 1 base pair shorter than that of allele 20. The fragment was identified by sequence analysis as allele 19.3 having a $(AGAT)_9$ $(GAT)_1$ $(AGAT)_3$ $(AGAC)_6$ $(AGAT)_1$ repeat structure. This allele 19.3 could easily be distinguished from allele 20 using CE analysis (Fig. 3). In the CE analysis of this locus, a stutter peak was observed as a weak peak one repeat unit smaller in size than its associated allele (Fig. 4). When the stutter percentages were calculated by dividing the height of a stutter peak by the height of its associated peak in 99 amplified D12S391 fragments, the longer alleles had a higher stutter percentage than the shorter alleles (Fig. 5). This percentage ranged from

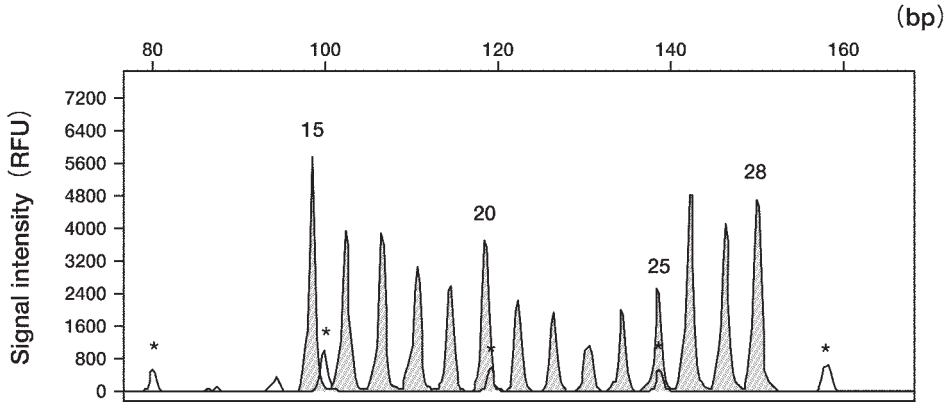

Fig. 1 Electrophoretograms of the D12S391 allelic ladder containing a total of 14 alleles analyzed using ABI 310 Genetic analyzer. "RFU" represents "relative fluorescence units". Peaks marked with asterisks are those of the CXR fluorescent ladder, used as the internal size standard.

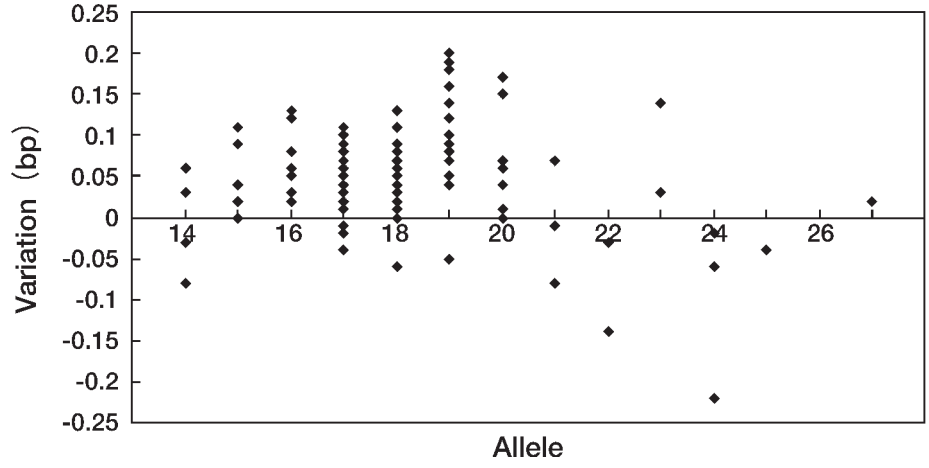

Fig. 2 Results of sizing precision experiments in the present D12S391 typing. The X-axis represents the number of repeats of alleles contained in the allelic ladder. Dots represent the corresponding deviation in sizing obtained for 125 typed alleles.

3.77% for allele 14 to 17.07% for allele 27.

Sensitivity and reproducibility. The amplified fragments from samples containing 16 pg of DNA were clearly typable in K562 control DNA and in a sample with alleles 16/17, with peak heights of approximately 100 relative fluorescence units (RFU) or greater. Moreover, the amplified fragments from a sample with alleles 21/26 and containing 63 pg of DNA were also clearly typable. When a peak height value of 300RFU was used as the threshold, the typing limits were 63 pg for the K562 control DNA, 63 pg for the sample with alleles 16/17 and 250 pg for the sample with alleles 21/26 of DNA, respectively, in these samples. DNA extracted from the semen, buccal swabs, and hair roots of 2 volunteers were examined, and the same D12S391 types were obtained from each sample for the same individual. For DNA extracted from autopsied bodies, the D12S391 types obtained from the cerebrum, heart, lung, spleen, liver, kidney, pancreas, muscle, skin, and blood were identical for the same individual.

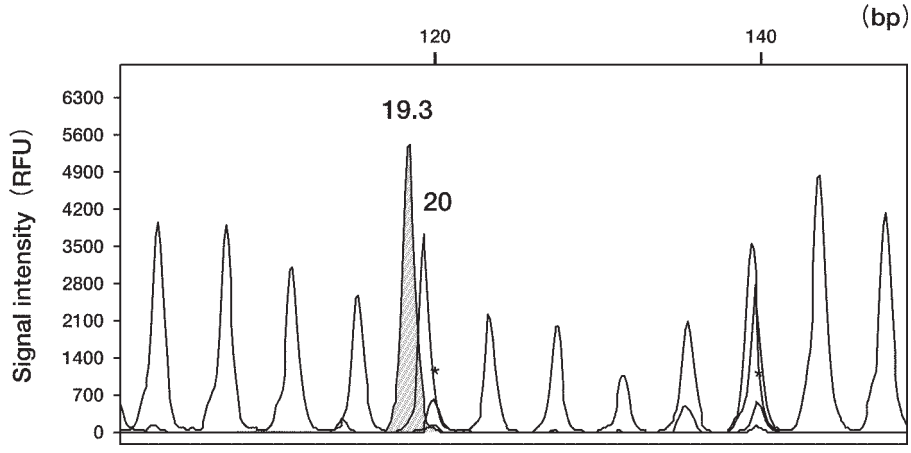

Fig. 3 Separation of alleles 19.3 and 20, which differ in size by only 1 nucleotide. A sample (gray) with allele 19.3 and an overlapping allelic ladder are displayed in the same window. "RFU" represents "relative fluorescence units". Asterisks indicate the CXR fluorescent ladder.

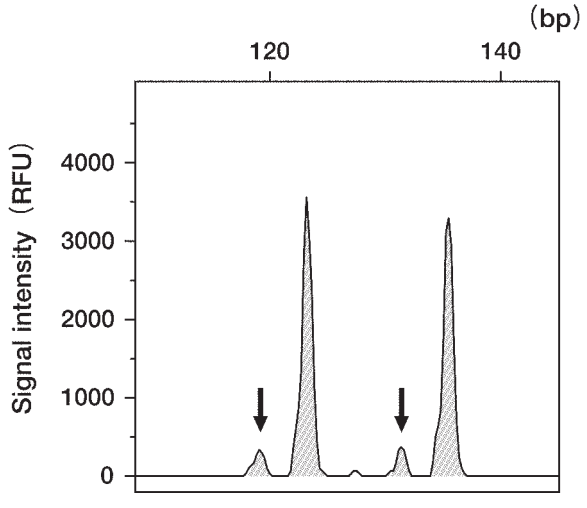

Fig. 4 Stutter peaks observed in the CE analysis of the D12S391 locus from a DNA sample with alleles 21/24. Arrows indicate stutter peaks. "RFU" represents "relative fluorescence units".

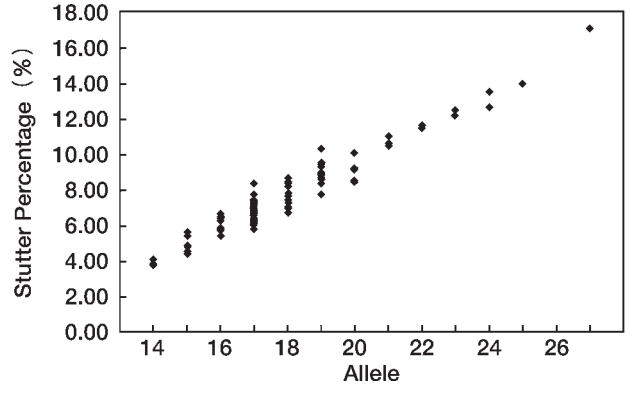

Fig. 5 Stutter percentages determined for 125 amplified fragments of the D12S391 locus. The X-axis represents the number of repeats of alleles contained in the allelic ladder, and the Y-axis represents the percentage of stutter.

Mixture studies. Using a threshold of 100RFU, the limits of detection for mixtures of samples were 1:30 (when the minor component was 16/17) or 1:10 (for minor component 21/26) in model A and 1:20 (for minor component 15/21) or 1:10 (for minor component 18/25) in model B (Fig. 6). Using a threshold of 300RFU, the limits of detection were all 1:5 in model A and model B.

Heat degradation studies. Using a threshold of 100RFU, typing was successful for samples heated for up to 6 h (Fig. 7B), although agarose gel electrophoresis showed highly advanced degradation of the sample DNA (Fig. 7A). Using a threshold of 300RFU, typing was successful for up to 4 h of degradation. On the other hand, typing with the original primer pair was successful only up to 3 h of degradation (Fig. 7 C), even with a threshold of 100RFU.

Aged bloodstains. One one-hundredth of the genomic DNA extracted from 2 cm^2 bloodstains that had been stored for 26 years in the dark was used for the amplification. Two fragment peaks considered to indicate heterozygosity were seen in the amplified products from each of 3 bloodstains: 15/18 from sample A, 20/21 from sample B, and 18/20 from sample C (Fig. 8).

Nonhuman animals. DNA samples from the remaining animals, except for those from a gorilla and a chimpanzee, did not show any detectable amplified fragment. The DNA samples from the gorilla and chimpanzee produced only one peak each. The peak observed in the gorilla sample corresponded to a size within the allelic ladder. Sequence analysis of the amplified fragments showed that the gorilla fragment had the same size as human allele 23 (136 bp), but a different repeat motif, $(ATAT)_1$ $(AGGT)_4$ $(AGAT)_{11}$ $(AGAY)_1$ $(AGAC)_6$. The sequence in the chimpanzee showed a $(AGAT)_3$ $(AGAC)_7$ repeat motif with a size of 84 bp, which was shorter than any known fragment size in humans.

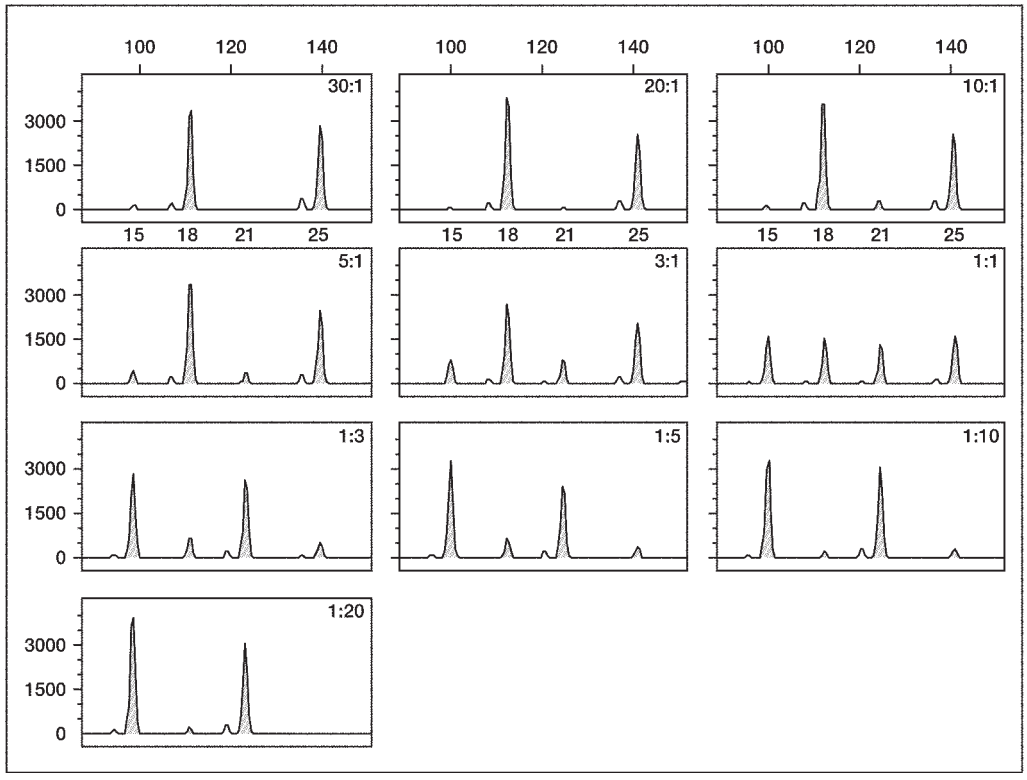

Fig. 6 D12S391 typing of mixtures of DNA samples in model B. Two DNA samples, alleles 18/25 and alleles 15/21, were mixed together in different ratios, 30:1—1:20, prior to amplification. The X-axes indicate the base pair size of the amplified fragments, and the Y-axes indicate relative fluorescence units (RFU).

Discussion

In PCR detection of STRs, the amplification of shorter fragments is more likely to be successful than that of longer fragments in the degraded forensic DNA samples. To detect the D12S391 locus, Ricci *et al.* [10] constructed and evaluated a primer pair that produced shorter fragments (125–173 bp, alleles 14–26) than the original primer pair described by Lareu *et al.* (209–253 bp, alleles 15–26)[9]. The results for the detection of the STR in forensic casework samples using their primer pair were little better in terms of sensitivity than those using the original primer pair. Our new primer pair was designed to improve forensic practice by amplityping fragments that are shorter (104–156 bp, alleles 15–28) than that amplified by the primer pair of Ricci *et al.* [10]. PCR using this new primer pair successfully amplified the D12S391 fragments. For 70 DNA samples, the typing results obtained using the new primer pair and those obtained using the original primer pair were in complete

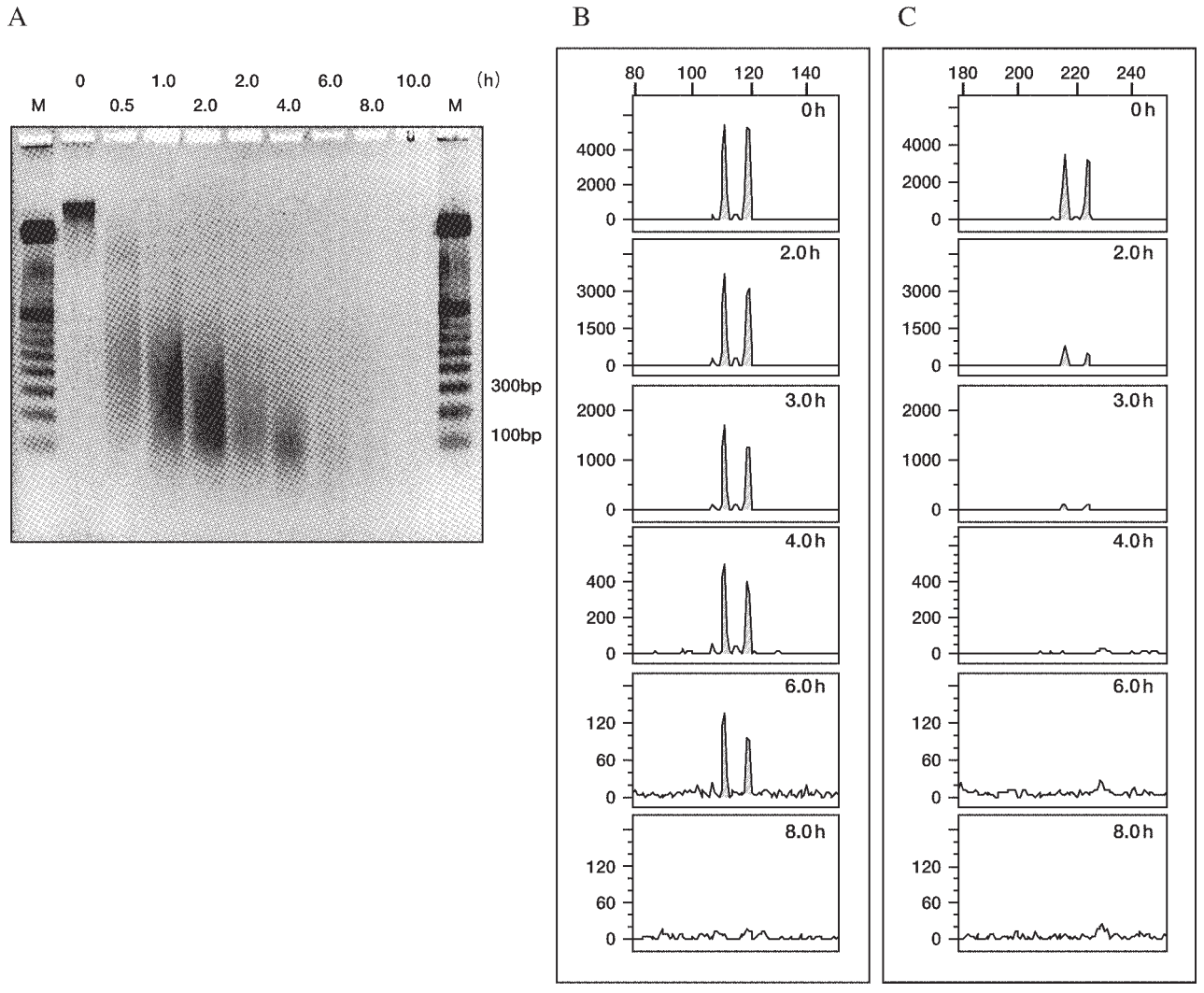

Fig. 7 D12S391 typing of bloodstains with alleles 18/20 heated for various periods at 150 °C. The degradation at each time point was evaluated by electrophoretic analysis (A) using 1.5% agarose for separation and ethidium bromide for detection. DNA from each hour or the 100 base-pair ladder markers (M) were loaded onto each lane. Comparison of electrophoretograms obtained with the new primer pair (B) and those obtained with the original primer pair (C). The X-axes indicate the base pair size of the amplified fragments, and the Y-axes indicate relative fluorescence units (RFU).

agreement. All alleles observed by CE analysis with this new primer pair were sized correctly within ± 0.25 bp of the associated alleles in the allelic ladder. Gill *et al.* [11] proposed that alleles can only be scored if they are within 0.5 bp of a putative allelic ladder. A rare allele, 19.3, with an incomplete repeat, previously detected in Caucasians [12] but not in Japanese subjects, was detected in a Japanese, and this allele could be clearly discriminated from allele 20 in the present study. A stutter peak is generally 4 bases shorter than the main allele peak in a tetranucleotide repeat locus [13,14]. Therefore, in forensic DNA analysis, in which mixed samples are encountered, recognizing the characteristics of the stutter peak is important. Meldgaard *et al.* [15] characterized the stutter peaks in 14 STR loci, and found that the stutter percentage for the D12S391 locus was high compared with that of the other STR loci. It will be necessary to examine forensic samples carefully when analyzing this locus, since it has high stutter peaks in the present study. The detection system using the new primer pair was highly sensitive, with a detection limit of 16 pg, which is roughly equivalent to a few cells. However, we must be careful of allele dropout during PCR amplification from a small quantity of DNA [16].

When the typing was carried out for mixed samples containing a total of 1 ng of genomic DNA, the minor component could be detected in mixtures containing at least a 1:10 ratio of that component at a threshold of 100RFU, and thus the quantity of the minor component was about 90 pg. Using a threshold of 300RFU, the minor component could be detected in mixtures containing at least a 1:5 ratio of the minor component, *i.e.*, about 170 pg. Wallin *et al.* [17] examined STR sites using total amounts of 1 ng or 5 ng of genomic DNA from mixed samples, and showed that the detection limits of the minor component were 1:10 in 1 ng and 1:30 in 5 ng. On the other hand, the CE analyzer is such that the longer the injection time of the samples, the higher the fragment peak that was obtained. Thus it is possible to detect weak peaks from a minor component. Therefore, detection using the CE instrument should be useful for mixed samples. However, in mixed samples, peaks from the minor component may be concealed behind the stutter peak. In such a case, since the greatest stutter percentage observed was 17.07% in the present study, a stutter percentage greater than 20% may indicate that the peak at the stutter position is due not only to stutter but also to a minor component.

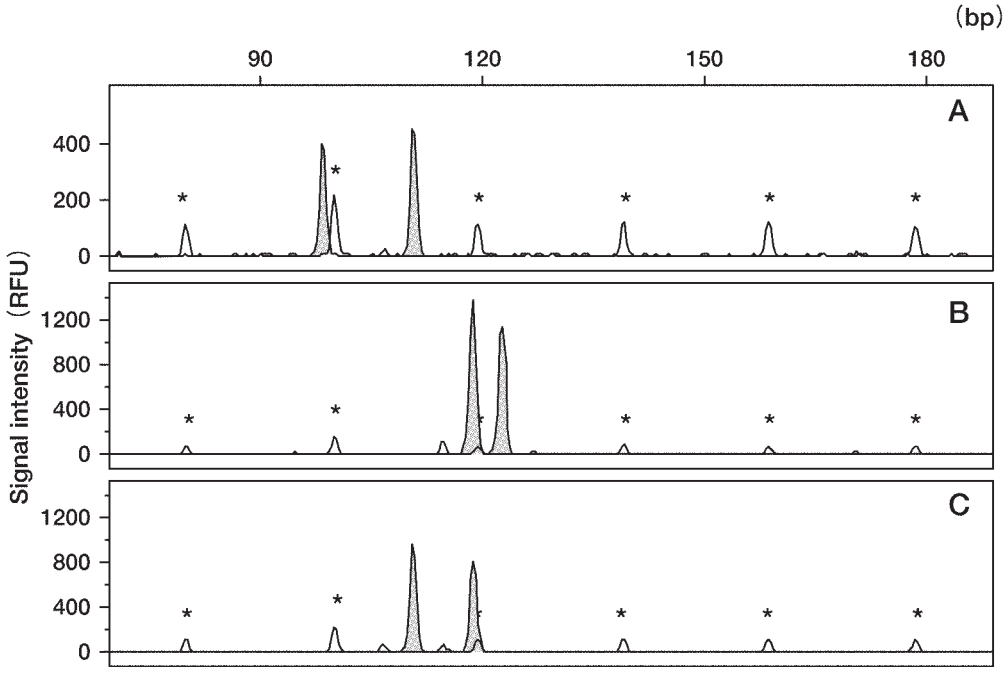

Fig. 8 D12S391 typing of 3 26-year-old bloodstains. "RFU" represents "relative fluorescence units". Asterisks indicate the CXR fluorescent ladder.

Wallin *et al.* [17] reported studies of the detection limit of three STR loci in fragmented genomic DNA with DNase I, which served as a model of degraded samples and environmentally stressed samples. They found that the PCR product yield for the loci was reduced in proportion to the length of the amplified product as the DNA sample became more degraded, and that the amplifiable product size approximately paralleled the extent of genomic DNA degradation shown by agarose gel analysis. We observed similar results in our heat degradation experiments. Since the fragments to be amplified were short, only 104 bp to 156 bp, in our new system, D12S391 typing was possible from considerably damaged DNA. Moreover, D12S391 typing was possible in 26-year-old bloodstains using this system. Thus, the D12S391 typing system using new primer pair seemed to be more useful for forensic samples than the original primer pair, in which the amplified fragment size ranges from 209 bp to 261 bp.

In samples from 12 kinds of animals, only one fragment from a gorilla was within the human allelic ladder, although its basic repetitive structure was different from that of the human. Although the fragment from a chimpanzee had the same basic repetitive structure as that of the human, the number of repeats was smaller than in the human samples. In an investigation of species specificity using 9 STRs that was performed by Crouse *et al.* [18], rhesus, orangutan, chimpanzee and gorilla DNAs showed bands within the human allelic ladder, and different types of bands were seen even in the same species. It thus may be necessary to examine more samples from gorillas, chimpanzees and other primates in order to prove that human and animal samples can be distinguished using this system.

From the present findings, we concluded that detection of the D12S391 locus using PCR with a newly designed primer pair followed by capillary electrophoresis was useful for forensic specimens, especially for aged or damaged samples, because the PCR-amplified fragment is short, and thus detection from degraded DNA was possible.

References

1. Hagelberg E, Gray IC and Jeffreys AJ: Identification of the skeletal remains of a murder victim by DNA analysis. Nature (1991) **352**, 427–429.
2. Gill P, Kimpton CP and Sullivan K: A rapid polymerase chain reaction method for identifying fixed specimens. Electrophoresis (1992) **13**, 173–175.
3. Jeffreys AJ, Allen MJ, Hagelberg E and Sonnberg A: Identification of the skeletal remains of Josef Mengele by DNA analysis. Forensic Sci Int (1992) **56**, 65–76.
4. Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E and Sullivan K: Identification of the remains of the Romanov family by DNA analysis. Nat Genet (1994) **6**, 130–135.
5. Srinivasan K, Girard JE, Williams P, Roby RK, Weedn VW, Morris SC, Kline MC and Reeder DJ: Electrophoretic separations of polymerase chain reaction-amplified DNA fragments in DNA typing using a capillary electrophoresis-laser induced fluorescence system. J Chromatogr A (1993) **652**, 83–91.
6. Butler JM, McCord BR, Jung JM and Allen RO: Rapid analysis of the short tandem repeat HUMTH01 by capillary electrophoresis. Biotechniques (1994) **17**, 1062–1070.
7. Wang Y, Ju J, Carpenter BA, Atherton JM, Sensabaugh GF and Mathies RA: Rapid sizing of short tandem repeat alleles using capillary array electrophoresis and energy-transfer fluorescent primers. Anal Chem (1995) **67**, 1197–1203.
8. Shigeta Y, Yamamoto Y, Doi Y, Miyaishi S and Ishizu H: Polymorphism of the D12S391 microsatellite in a Japanese population sample. Forensic Sci Int (1999) **102**, 61–66.
9. Lareu MV, Pestoni C, Schürenkamp M, Rand S, Brinkmann B and Carracedo A: A highly variable STR at the D12S391 locus. Int J Legal Med (1996) **109**, 134–138.
10. Ricci U, Giovannucci Uzielli ML and Klintschar M: Modified primers for D12S391 and a modified silver staining technique. Int J Legal Med (1999) **112**, 342–344.
11. Gill P, d'Aloja E, Dupuy B, Eriksen B, Jangblad M, Johnsson V, Kloosterman AD, Kratzer A, Lareu MV, Mevag B, Morling N, Phillips C, Pfitzinger H, Rand S, Sabatier M, Scheithauer R, Schmitter H, Schneider P, Skitsa I and Vide MC: Report of the European DNA Profiling Group (EDNAP)-an investigation of the hypervariable STR loci ACTBP2, APOAII and D11S554 and the compound loci D12S391 and D1S1656. Forensic Sci Int (1998) **98**, 193–200.
12. Phillips CP, Syndercombe CD, Lareu MV, Hasskamp J and Carracedo A: Band shift analysis of three base-pair repeat alleles in the short tandem repeat locus D12S391. Forensic Sci Int (1998) **93**, 79–88.
13. Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES and Adams M: Automated DNA profiling employing multiplex amplification of short tandem repeat loci. PCR Methods and Appl (1993) **3**, 13–22.
14. Sparkes R, Kimpton C, Gilbard S, Carne P, Andersen J, Oldroyd N, Thomas D, Urquhart A and Gill P: The validation of a 7-locus multiplex STR test for use in forensic casework. (II), Artefacts, casework studies and success rates. Int J Legal Med (1996) **109**, 195–204.
15. Meldgaard M and Morling N: Detection and quantitative characterization of artificial extra peaks following polymerase chain reaction amplification of 14 short tandem repeat systems used in forensic investigations. Electrophoresis (1997) **18**, 1928–1935.
16. Lygo JE, Johnson PE, Holdaway DJ, Woodroffe S, Whitaker JP, Clayton TM, Kimpton CP and Gill P: The validation of short tandem repeat (STR) loci for use in forensic casework. Int J Legal Med (1994) **107**, 77–89.
17. Wallin JM, Buoncristiani MR, Lazaruk KD, Fildes N, Holt CL and Walsh PS: TWGDAM validation of the AmpFISTR blue PCR amplification kit for forensic casework analysis. J Forensic Sci (1998) **43**, 854–870.
18. Crouse CA and Schumm J: Investigation of species specificity using nine PCR-based human STR systems. J Forensic Sci (1995) **40**, 952–956.