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TECHNICAL NOTE

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Evaluation of new primers for CSF1PO

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Abstract We describe new primers for the detection of the STR polymorphism at the CSF1PO locus. These primers have been designed to produce shorter amplicons (150–182 bp) than the primers in standard use (295–327 bp). The reliability of the new primers for CSF1PO typing has been demonstrated by testing on known samples and by sequence analysis. These primers are superior to the original primers with regard to electrophoretic resolution and utility for typing of severely degraded DNA.

Key words CSF1PO · STR polymorphism · Forensic identification · Degraded DNA

Introduction

The tetranucleotide tandem repeat polymorphism at the CSF1PO locus (5q33.3-q34) is one of a number of short tandem repeat (STR) polymorphisms under consideration as a core marker for forensic identification purposes [1, 4–8]. The CSF1PO typing primers described by Hammond et al. [4] (and used in the GenePrint STR System typing kit marketed by Promega, Madison Wisc. [8]) yield amplicons in the size range 295–327 bp. Because fragments in this size range are less well resolved than fragments of smaller size and because smaller fragments are better amplified from samples containing severely degraded DNA [3, 5, 11, 15], we have investigated the use of redesigned CSF1PO primers giving smaller PCR products(150–182 bp). The use of these primers results in improved electrophoretic resolution of allelic fragments and enhances typing of degraded samples.

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Materials and methods

DNA extraction

DNA extracted from blood samples was used for reference typing purposes. Aged bloodstains, 16 years old, were from a reference collection maintained at the National Research Institute of Police Science. DNA was extracted from whole blood and aged bloodstain samples by using a standard phenol-chloroform method [13]. The concentration and quality of extracted DNA was determined by UV absorbance using spectrophotometric readings at 260 and 280 nm and by yield gel electrophoresis, respectively [13].

Primer design

Prospective primer sequences were generated from the CSF1PO gene sequence (GenBank accession number X14720) using the program Designer PCR (Research Genetics, USA). The primer pair selected for this study was:

CSF-3F, 5′ GTTGCTAACCACCCTGTGTCTC 3′ (GenBank sequence 11874–11895)

CSF-3R, 5′ TTCCTGTGTCAGACCCTGTTC 3′ (GenBank sequence 12043–12023)

The Tm values calculated for these primers are 58.1°C and 58.6°C respectively. This primer set was expected to yield products in the size range 150–182 bp.

PCR conditions and detection of amplified products

PCR amplifications were performed on a Perkin Elmer 480 thermocycler. Reference samples were typed for CSF1PO using a commercial kit (GenePrint STR Systems, Promega, USA) according to the manufacturer's instructions.

Amplifications using the CSF-3 primers were done in 25 μL reaction mixes containing 5 ng template, 0.5 μM of each primer, 0.25 Unit Taq polymerase (AmpliTaq, Perkin Elmer, USA), 1 × Taq polymerase buffer (50 mM KCl, 10 mM Tris-HCl pH9.0, 1.5 mM $MgCl_2$, 0.1% Triton X-100), and 200 μM each of dATP, dCTP, dGTP and dTTP. The best combination of amplification efficiency and specificity was obtained using a basic PCR protocol similar to that described by Puers et al. [12] with initial denaturation at 96°C for 2 min, then 10 cycles of denaturation 94°C 1 min, annealing 58°C 1 min, extension 70°C 1.5 min, followed by 20 cycles of denaturation 90°C 1 min, annealing 58°C 1 min, extension 70°C 1.5 min, and ending with a terminal elongation at 70°C for 5 min. Raising the annealing temperature to 60°C and 64°C resulted in

the appearance of stronger stutter bands, i.e. N+4 and N-4 bands. Accordingly, 58° C was chosen as the standard annealing temperature.

Amplified DNA products were separated by electrophoresis through a 0.4 mm-thick, 30 cm long, 6% denaturing polyacrylamide gel (C = 5%) in 1 × TBE buffer containing 7 M urea. Samples of 2.5 µl amplified DNA were mixed with 2.5 µl of 2 × loading solution (10 mM NaOH, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol FF; Promega, USA) and denatured at 95° C at 2 min. Electrophoresis was conducted at 35 W for approximate 1.5 h. The PCR products were stained by SYBR-GREEN (Molecular Probes, USA) and the bands were detected on a FluorImager (Molecular Dynamics, USA).

Sequence analysis

CSF1PO amplicons were sequenced using cycle sequencing (fmol DNA Cycle Sequencing System, Promega, USA). The purification of PCR products was performed by filtration though Ultrafree-MC 100,000 NMWL filter cups (Millipore, USA). Primers were end-labeled with ^{32}P using T4 polynucleotide kinase; procedures otherwise followed manufacturer's instructions. Sequence determination entailed electrophoretic separation on 5.5% acrylamide gels containing 7 M urea followed by autoradiography using standard protocols [13].

Results and discussion

Typing reliability

The accuracy of genetic typing using the CSF-3 primers was tested by typing 40 samples previously typed using the GenePrint kit. An allelic ladder based on the CSF-3 primers was constructed by amplifying a mixture of DNA samples of known type. K562 DNA (CSF1PO type 9,10) was used as a common reference for comparing the amplicons of the two primer sets. The typings using the CSF-3 and conventional primers were completely concordant. Figure 1 shows the relative positions and overall appearance of the allelic bands using the GenePrint kit and CSF-3 primers. Note that the samples amplified using GenePrint kit were loaded 1 h before the CSF-3 primer samples; the samples amplified with the GenePrint kit were not adequately resolved if they were loaded at the same time as the CSF-3 sample set. It is clear that even with an extra hour separation time, the larger amplicons are less well resolved than the CSF-3 primer samples.

The sizes of the amplicons obtained with the CSF-3 primers were determined by measuring end-labeled primer extension products against an M13 sequencing ladder, under denaturing gel conditions. Amplicons sized 1–2 bp longer than predicted based on primary sequence data and repeat number determined by conventional typing. For example, the allelic fragment with 12 repeats sized at 170 bp rather than the predicted 169 bp. Size shifts of one base can be attributed to non-templated base addition [2, 9]. This was demonstrated by treating amplicons representing different allelic repeats with Klenow enzyme, the 3′-5′ exonuclease activity of which removes the non-templated added base. Treated samples exhibited a 1 bp size reduction relative to the untreated controls. Any residual difference in the sizes of the CFS1PO amplicon relative to the M13 sequencing ladder may be due to sequence specific mobility shifts [14].

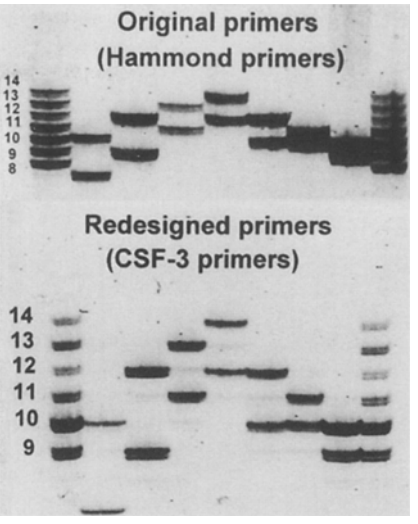

Fig. 1 Comparison of STR patterns obtained with the Promega primers (upper) and the CSF-3 primers (lower). Electrophoretic separation was performed as described in materials and methods. Samples amplified using Promega primers added to the gel 1 h before the CSF-3 primed samples; nevertheless, the CSF-3 primed samples migrate further and are better resolved than the Promega primers samples. The samples in each set are CSF1PO types (from left to right) ladder; 7,10; 9,12; 11,13; 12,14; 10,12; 10,11; K562(9,10); ladder. The ladder used with the Promega primer was supplied with GenePrint kit; the ladder used with the CSF-3 primers was constructed from a mixture of samples

The accuracy of CSF1PO typing using the CSF-3 primers was confirmed by sequence analysis. CSF-3 primed amplicons representing the 9, 10, 11, and 12 alleles (as determined by conventional typing) were sequenced. The sequences obtained were completely concordant with expectation, i.e. the sequence determined by sequence analysis matched the sequence expected based on conventional typing.

Degraded DNA

Because STR amplicons are relatively small in size, usually less than 350 bp, STR typing is particularly useful in the analysis of forensic samples containing severely degraded DNA [3, 5, 11, 15]. To determine whether the smaller CSF-3 amplicon might extend the value of this marker in typing degraded DNA, two sets of experiments were performed. First, we tested DNA artificially degraded by heating at 100° C for 30–150 min [10]. For degraded DNA samples containing very low molecular weight DNA (< 600 base pairs), amplification using the GenePrint kit gave very weak or no PCR products. CSF1PO types could be determined, however, when the CSF-3 primers were used. Second, we tested degraded DNA extracted from five 16-year-old bloodstain samples. Figure 2 shows the results obtained with the two primer sets; 40 ng degraded DNA(determined spectrophotometri-

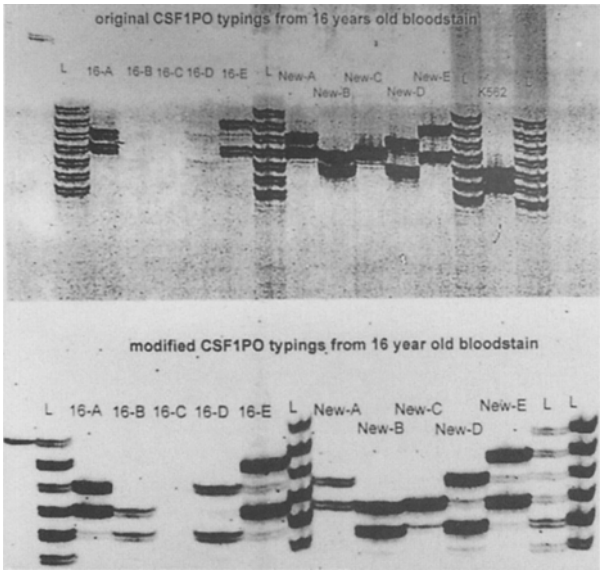

Fig. 2 CSF1PO typing on 16-year-old bloodstain samples using Promega (upper) and CSF-3 (lower) primers. The PCR products from the degraded samples are shown on the left and the PCR products from fresh reference samples from the same individuals are shown on the right

cally) were added to each reaction mix. Samples A and E contained DNA fragments larger than 1 Kb and could be typed with both primer sets. The DNA in samples B, C, and D was more severely degraded with most of the DNA fragments less than 500 bp. With the GenePrint kit, sample D amplified weakly and samples B and C yielded no detectable PCR products. With the CSF-3 primers, samples B and D gave strong PCR products; sample C again failed to amplify. These experiments indicate that the CSF-3 primers may be of value for CSF1PO typing of samples that cannot be typed using the standard primers.

In conclusion, we have demonstrated that the CSF1PO polymorphism can be typed reliably using a PCR primer set that yields shorter amplicons than the standard primers. The shorter PCR products are better resolved on slab gels and can be used to advantage in the typing of highly degraded DNA.

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