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Multiplex PCR using newly designed primers for very short fragments of TH01, TPOX, CSF1PO, and vWA loci

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Abstract

We performed multiplex PCR for the TH01, TPOX, CSF1PO, and vWA loci using newly designed pairs of primers that yield smaller fragments than previously reported [Int. J. Leg. Med. 114 (2001) 285; Am. J. Hum. Genet. 55 (1994) 175; Int. J. Leg. Med. 106 (1994) 183.] [1–3]. This system required genomic DNA in a range of 50 pg–2 ng, and proved to be sensitive as a typing method. Furthermore, it was possible to determine the allele types even from 18-year-old bloodstains.

Keywords: STR; Multiplex; Degraded DNA; Old bloodstains

1. Introduction

Short tandem repeats (STRs) are very useful tools for identifying individuals in forensic science and criminal investigations. Because the fragment size of STRs is smaller than that of RFLP-VNTR they are suitable for the amplification of DNA extracted from degraded specimens. Multiplex PCR systems feature the advantage of detecting many STR loci simultaneously in a single analysis, but require much more template DNA than monoplex PCR systems. This makes multiplex PCR a highly useful tool in criminal investigations. We encountered some difficulties in the successful amplification of these STRs using highly degraded DNA samples, particularly with a fragment size of greater than 200 bp. We therefore designed new pairs of primers to reduce the PCR fragment sizes. In this study, we

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investigated the usefulness of newly developed multiplex PCR for the TH01, TPOX, CSF1PO, and vWA loci, which are highly polymorphic markers. These loci can be detected in the range of 74–143-bp amplified products. Electrophoresis was performed using an ABI 310 Genetic Analyzer, and alleles ware determined using GeneScan 2.1 software.

2. Materials and methods

2.1. DNA extraction

Five bloodstain samples stored at room temperature for 18 years were used in this study. DNA extraction from bloodstains was performed using SDS-Proteinase K treatment, followed by phenol/chloroform extraction. DNA samples were measured spectrophotometrically (260–280 nm) to determine their concentration and estimate the purity of nucleic acids.

2.2. PCR amplification

Multiplex PCR was carried out using the primers specified in Table 1. The PCR reaction was performed in a total volume of 30 μl, containing 1–10 ng of genomic DNA, 10 mM of Tris–HCl (pH 8.3), 50 mM of KCl, 0.2 mg/ml of bovine serum albumin (BSA), 0.2 mM of dNTPs, 1.5 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA), 2 mM of $MgCl_2$, 0.066 μM of TH01, 0.047 μM of TPOX, 0.11 μM of CSF1PO, and 0.238 μM of vWA primers, respectively. The cycling parameters were pre-PCR denaturation at 95 °C for 2 min, followed by 32 cycles of denaturing at 94 °C for 45 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s using a Takara PCR Thermal Cycler MP (Takara Ohtsu, Japan). A final extension was performed at 72 °C for 10 min. The detection of signals was performed using the ABI Prism 310 Genetic Analyzer. Fragment sizes were determined using the internal standard Genescan 500 (TAMRA, Applied Biosystems).

2.3. Minimum sample studies

In order to determine the minimum quantity of DNA required to obtain reliable results, we used genomic DNA extracted from the cell line K562 (Promega, Madison, WI, USA) and

Table 1
Primers and other details of the STR systems used

Locus	PCR primers (5′ → 3′)	Dye	Product length (bp)
TH01-A	CTGTTCCTCCCTTATTTCCCTC	HEX	74–98
TH01-B	GTGCAGGTCACAGGGAACACAGAC		(5–11 alleles)
TPOX-A	CACTAGCACCCAGAACCATC [4]	HEX	107–135
TPOX-B	CCTTGTCAGCGTTTATTTGCC [4]		(6–13 alleles)
CSF1PO-A	CTGCCTTCATAGATAGAAGATAG	6-FAM	90–122
CSF1PO-B	TTTCCTGTGTCAGACCCTGTTC		(7–15 alleles)
vWA-A	ATAATCAGTATGTGACTTGGAT	TET	99–143
vWA-B	ATGATAAATACATAGGATGGATG		(10–21 alleles)

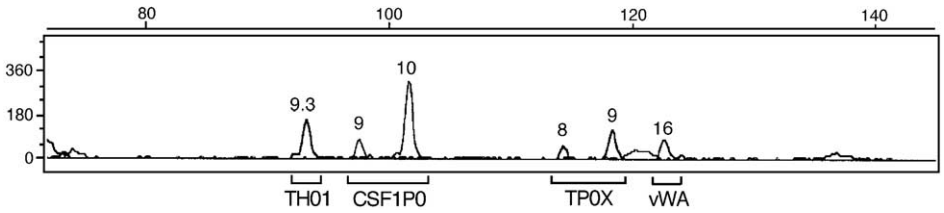

Fig. 1. Electropherogram of amplified DNA from cell line K562.

sequentially diluted with distilled water (10, 100 pg/μl, 1 ng/μl). The final concentrations of template DNA in the assay were 50, 60, 70, 80, 90, 100, 150, 250, 500 pg, 1, 2, 5, and 10 ng.

3. Results

Allele typing was successful in the range of 50 pg–2 ng DNA in a minimum sample study. Fig. 1 shows the typical electropherogram using 50 pg DNA. Broad peaks were observed in the case of DNA usage of 5 ng or more. When 1 ng of extracted DNA from old bloodstains was used as the template for PCR, allele typing was virtually impossible except for sample 2. In addition, when 5 ng of DNA template was used, the signal peaks of four loci were determined with sufficient accuracy to identify allele types, but non-specific signals were also detected . Furthermore, when the amount of template DNA was increased to 10 ng the alleles of four loci were typed easily.

4. Discussion

This newly developed multiplex system is one of the most sensitive systems known, as it was able to determine every allele of four loci, even with 50 pg of template DNA. It is therefore possible to easily and effectively type different alleles of four loci using limited samples. This multiplex PCR system enabled the allele typing of DNA samples from old bloodstains, and proved to be a powerful tool for individual identification in forensic science.

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