International Congress Series 1261 (2004) 322–324

www.ics-elsevier.com

Simultaneous PCR of eight loci for very short Y-STR fragment size

K. Tsukada*, K. Takayanagi, H. Asamura, M. Ota, K. Kobayashi, H. Fukushima

Department of Legal Medicine, Shinshu University School of Medicine, Asahi 3-1-1, Matsumoto, Nagano 390-8621, Japan

Abstract. We selected the DYS19, DYS385, DYS389, DYS390, DYS392, DYS393, DYS483, and amelogenin loci, and designed a new pair of primers to minimize the fragment sizes of these loci as much as possible. As a consequence, these loci were able to detect in the range of 79–259 bp using multiplex PCR amplification. The optimum DNA amount was 100 pg to 10 ng. The haplotype diversity was 0.9979.

Keywords: Y-STR; Degraded DNA; Multiplex PCR; Sexual crime

1. Introduction

Y-STRs are very important tools for identifying perpetrators of sexual crimes, as only males, the most common perpetrators of such crimes, have Y-chromosomes. In a previous paper, we showed the importance of making the STR fragment size small to enable the typing of degraded specimens [1]. In this study, we selected nine loci and designed a new pair of Y-STR primers to minimize the fragment sizes of these loci as much as possible, relative to previously reported fragment sizes. These loci are very popular throughout the world; further, they feature many alleles with little deviation between allele frequencies. In this experiment, we performed multiplex PCR using DNA extracted from mixed stains.

2. Materials and methods

For minimum-sample studies and mixed-sample studies, blood was provided by a healthy male and female. For allele frequency studies, 103 bloodstains from unrelated male individuals were stored at −80 °C. For sex-crime studies, four vaginal stains were stored at −80 °C for 4 years. DNA extraction was performed as described previously [1]. For vaginal stains, the DNA extraction procedure was performed by the one-step method

* Corresponding author. Tel.: +81-263-37-3218; fax: +81-263-37-3084.
E-mail address: tuk-lab@mxl.avis.ne.jp (K. Tsukada).

0531-5131/
doi:10.1016/j.ics.2003.11.015

and the traditional two-step method [2]. Multiplex PCR was carried out using the primers as follows:

DYS19-A VIC-GTGTTTTAGATAGATAGATAGG
DYS19-B GTTTGGGTTAAGGAGAGTGTCAC
DYS385-A 6FAM-TTACATAGTCCTCCTTTCTTTT
DYS385-B GTTTAGAAAGAGGAAAGAGAAAGAAAGGA
DYS389-A PET-CCAACTCTCATCTGTATTATCTATG
DYS389-B GTTTGATGGACTGCTAGATAAATA
DYS390-A 6-FAM-TATATTTTACACATTTTTGGGCC
DYS390-B GTTTGCTATGTGTATACTCAGAAAC
DYS392-A NED-CAAATTTTTTCCTTGTATCACCATTT
DYS392-B GTTTAAACCTACCAATCCCATTCCTT
DYS393-A NED-GTGGTCTTCTACTTGTGTCAATAC [3]
DYS393-B GTTTACTCAAGTCCAAAAAATGAGG [3]
DYS439-A VIC-ACATAGGTGGAGACAGATAGATGAT [4]
DYS439-B GTTTGCCTGGCTTCCAATTCTTTT [4]
Amelogenin-A PET-GGGCTCTGTAAAGAATAGTG
Amelogenin-B GTTTGGTAGGAACTGTAAAT

In order to completely induce adenylation, the 5′ end of each reverse primer was modified [1]. PCR reaction was performed with a total volume of 30 μl, containing 1–5 ng of genomic DNA, × 1 PCR reaction buffer, 0.2 mg/ml of BSA, 0.2 mM of dNTPs, 1.0 u of AmpliTaq Gold polymerase, 2 mM of $MgCl_2$, 0.083 μM DYS19, 0.216 μM DYS385, 0.108 μM DYS389, 0.082 μM DYS390, 0.116 μM DYS392, 0.058 μM DYS393, 0.058 μM DYS439, and 0.1 μM Amelogenin. The cycling parameters were pre-PCR-denaturation at 95 °C for 12 min, followed by 32 cycles of 94 ° C for 45 s, 56 °C for 45 s, 72 °C for 30 s using a TP-3000 (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 LIZ. AMale-Plex and Male-Plex with/without amelogenin loci, respectively. In order to determine the minimum quantity of DNA required to obtain reliable results, we used male DNA sequentially diluted with distilled water. Mixtures of male and female DNA were prepared in ratios from 1:1 (100 pg/100 pg) to 1:50,000 (100 pg/5000 ng).

3. Results

This multiplex system was not typed, except for amelogenin locus using female DNA. The gene diversity of DYS19, DYS385, DYS389 I, DYS389 II, DYS390, DYS392, DYS393, and DYS439 loci were 0.6471, 0.9532, 0.6832, 0.7868, 0.7860, 0.6762, 0.5469, and 0.5176, respectively. The computed haplotype diversity was 0.9979. When the ratio of the amount of female DNA was increased and PCR amplification was carried out using AMale-Plex, a non-specific peak was observed. Therefore, PCR reaction was performed using Male-Plex. In both cases, the optimum DNA amount in the range was 100 pg to 10 ng (Fig. 1). In the case of Male-Plex, when 100 pg of male DNA was used in

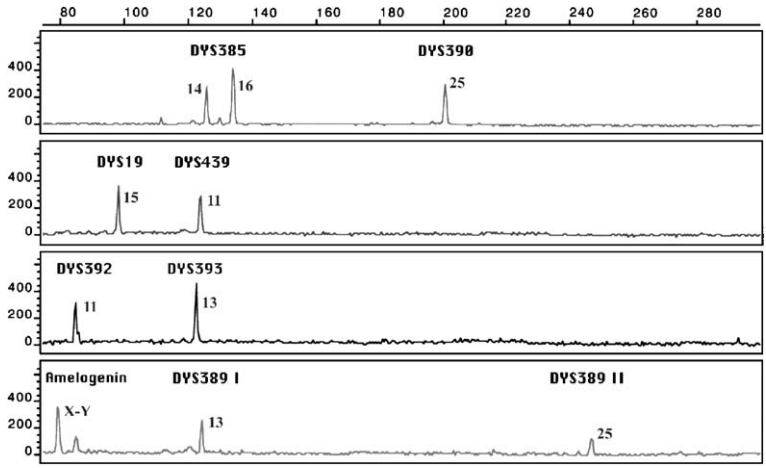

Fig. 1. Electropherogram of AMale-Plex. PCR amplification carried out using 100 pg of male DNA as a template.

1000 ng of female DNA, the allele typing was completely successful. In sex-crime sample studies, all of the vaginal stains were typed as the same alleles by both the one- and two-step extraction procedures.

4. Discussion

It is possible to easily and effectively perform typing using limited samples, as AMale-Plex and Male-Plex were detectable in a DNA amount of 100 pg as a template. In cases in which it is clear that the male DNA is not mixed with female DNA, although the sex of specimens is unknown, PCR reaction can be performed using AMale-Plex. When female DNA amplified by AMale-Plex is used, although all of the Y-loci are not amplified, as the amelogenin locus is amplified by female DNA, the advantage of being able to check whether a PCR reaction is successful is provided. However, in male/female mixed stains, it is necessary to perform a PCR reaction using Male-Plex. In the case of mixed stains, as Male-Plex is able to type a very wide range of mix ratios, we showed that it is not necessary to estimate of amount of DNA extracted by the one-step procedure.

We consider that AMale-Plex and Male-Plex will be effective tools for the allele typing of highly degraded specimens, as a new pair of primers has been designed to minimize the fragment sizes of all loci as much as possible, relative to previously reported fragment sizes.

References

[1] K. Tsukada, et al., Multiplex short tandem repeat typing in degraded samples using newly designed primers for TH01, TPOX, CSF1PO, and vWA loci, Legal Med. 4 (2002) 239–245.

[2] K. Yoshida, et al., The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen, Forensic Sci. Int. 72 (1) (1995) 25–33.

[3] B.M. Dupuy, et al., DXYS267: DYS393 and its X chromosome counterpart, Forensic Sci. Int. 112 (2–3) (2000) 111–121.

[4] C.M. Ruitberg, et al., New primer sets for Y chromosome and CODIS STR loci. (Poster presented at the Eleventh International Symposium on Human Identification, Biloxi, MS), 2000.