International Congress Series 1288 (2006) 837–839

www.ics-elsevier.com

The risk of incorrect typing of D1S80 by unstable minisatellite

R. Kobayashi [a,b], N. Iizuka [b,c], Y. Itoh [b,*]

[a] *Depatment of Microbiology, Tokyo Medical University, Tokyo, Japan*
[b] *Department of Forensic Medicine, Juntendo University School of Medicine, 2-1-1Hongo bunkyo-ku Tokyo 113-8421, Japan*
[c] *Medico-Legal Section, Criminal Investigation Laboratory, Metropolitan Police Department, Tokyo, Japan*

Abstract. The D1S80 locus is very useful for personal identification in Japan. To analyze PCR amplification products at the D1S80 locus, DIG-labeled primer was used for PCR amplifications. After electrophoresis, the PCR products were transferred to a nylon membrane and detected with alkaline phosphatase-labeled anti-DIG antibody (AP-DIG Ab). Numerous extra bands were detected on the membranes, indicating that PCR amplification products at the D1S80 locus contain many extra products which cause the undesirable bands to appear during D1S80 typing. To obtain a correct genotype, it was necessary to perform Southern blotting using an oligonucleotide that includes an internal sequence of the amplification products as a probe. © 2005 Published by Elsevier B.V.

Keywords: DNA; PCR; D1S80; Minisatellite; Typing

1. Introduction

The minisatellite locus D1S80 (location; 1p35–p36), GenBank sequence accession no. D28507), is a variable number of tandem repeat (VNTR) locus with a 16 bp repeat size [1]. With alleles defined by the number of repeat units, the D1S80 locus is highly polymorphic in Japan [2]. However, it is well known that extra bands frequently appear during typing [3]. In this paper, we demonstrate that PCR amplification products at the D1S80 locus have numerous extra bands which may cause incorrect genotypes to be

* Corresponding author. Tel.: +81 3 5802 1051; fax: +81 3 5802 1050.
E-mail address: yitoh@med.juntendo.ac.jp (Y. Itoh).

0531-5131/ © 2005 Published by Elsevier B.V.
doi:10.1016/j.ics.2005.08.030

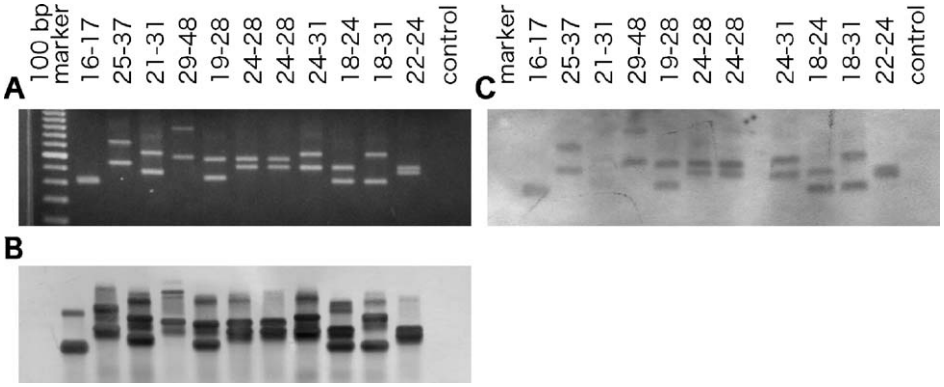

Fig. 1. Comparison of D1S80 typing results by three different methods: (A) EtBr-staining. (B) DIG-detection. (C) Southern blotting.

obtained and that Southern blotting using an internal sequence as a probe is very helpful in determining D1S80 genotypes.

2. Materials and methods

The primer (MCT118F) was labeled with DIG-11-dUTP (Roche, USA) according to the manufacturer's instructions (DIG-MCT118F). The probe (MCT118P: 5′–CTG CGT GTG AAT GAC CCA GGA GCG TAT C–3′) was designed and also labeled with DIG-11-dUTP (DIG-MCT118P). PCR amplification was performed as described by Kasai et al. [4]. After electrophoresis of PCR amplification products with DIG-MCT118F and MCT118R using 2% agarose gel, DNA fragments were stained by ethidium bromide (EtBr-staining). Then, these DNA fragments were transferred to a nylon membrane and detected using AP-DIG Ab and NBT/BCIP (DIG-detection). The PCR amplification products with unlabeled primers were also transferred to a nylon membrane, hybridized with DIG-MCT118P and detected with AP-DIG Ab and NBT/BCIP (Southern blotting).

3. Results and discussion

PCR amplification products at the D1S80 locus were analyzed using a DIG-labeled primer. Although only real bands of the products appeared under UV light after ethidium bromide staining (Fig. 1A), numerous bands were detected when using the DIG-labeled primer (Fig. 1B). This finding indicates that extra bands are produced under the regular PCR conditions and can be visualized when using the AP-DIG Ab and NBT/BCIP detection. These extra bands may be detected with ethidium bromide staining if additional amplification cycles are performed. This may cause incorrect genotypes to be obtained. However, Southern blotting using an internal sequence as a probe could isolate and detect the real bands (Fig. 1C). This finding indicates that Southern blotting may be very helpful in determining D1S80 genotypes.

References

[1] Y. Nakamura, et al., Isolation and mapping of a polymorphic DNA sequence (pMCT118) on chromosome 1p (D1S80), Nucleic Acids Res. 16 (1988) 9369.

[2] K. Kasai, et al., DNA typing of MCT118 locus from human bloodstains and body fluid stains by PCR amplification (in Japanese), Rep. NRIPS. 45 (1992) 24–35.
[3] G.T. Duncan, et al., Microvariation at the human D1S80 locus, Int. J. Leg. Med. 110 (1997) 150–154.
[4] K. Kasai, et al., Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science, J. Forensic Sci. 35 (1990) 1196–1200.