PCR-amplification and detection of the human D1S80 VNTR locus

Amplification conditions, population genetics and application in forensic analysis

Ate D. Kloosterman¹, Bruce Budowle², and Petra Daselaar¹

¹Gerechtelijk Laboratorium van het Ministerie van Justitie, Volmerlaan 17, 2288 GD Rijswijk, The Netherlands
²Forensic Science Research and Training Center, Laboratory Division, Federal Bureau of Investigation Academy, Quantico, VA, USA

Received July 1, 1992 / Received in revised form November 9, 1992

Summary. A series of experiments has been performed to evaluate amplification and typing of the D1S80 VNTR locus. The validation study that has been carried out showed that correct D1S80 typing results can be obtained when a defined amplification protocol and a high-resolution polyacrylamide gel electrophoresis method are used. The use of the Chelex extraction protocol has substantially reduced the processing time. DNA-extraction, amplification and subsequent typing can be performed in one day. The discrimination power of this locus is 0.94 in a Dutch Caucasian population sample. The system is extremely sensitive: 0.1ng of genomic DNA gave a correct typing result. The test could also detect the correct genotypes in mixed samples containing DNA from different individuals. Even if the major type was in a 20-fold excess, the minority type could still be amplified and typed correctly. We have found no deviation from Hardy-Weinberg equilibrium in a Dutch Caucasian population sample. Evidence for the somatic stability of this locus was obtained from a set of experiments where we compared DNA-profiles from corresponding blood, semen and saliva samples. The results of this study suggest that in the near future analysis of the D1S80 locus by DNA-amplification can be applied in actual forensic case work.

Key words: AMPFLP – D1S80 – Polymerase Chain Reaction (PCR) – Population Genetics – Forensic DNA typing

Introduction

Highly polymorphic Variable Number of Tandem Repeats (VNTR) loci can be used for identity testing [1]. When an appropriate restriction enzyme is chosen for the digestion of genomic DNA these length polymorphisms are usually detected by Southern blot analysis. This conventional typing procedure for forensic samples such as hair roots, bloodstains, semen and saliva stains is often limited by the inherent sensitivity of the Southern

Correspondence to: A. D. Kloosterman
from different individuals was also evaluated.

pared with population genetic studies from Finland [9], a Caucasian population sample of 150 individuals and compared with data from the relevant population(s) [5, 9].

allele frequency data from the relevant population(s) [5, 18, 19, 20].

The characteristics of the D1S80 VNTR-locus are given in Table 1.

The Polymerase Chain Reaction (PCR) enables the typing of known polymorphic regions with much smaller amounts of human DNA [3], even if the DNA is degraded to some extent. Using appropriate PCR-primers which flank the tandem repeats and thermostable Taq DNA polymerase, typing of VNTR loci can be accomplished on ethidium bromide-stained agarose gels and on ethidium-bromide or silver stained polyacrylamide gels. It is possible to amplify VNTR alleles of up to 5 kbp in length [4], but in general VNTR loci with alleles of 2 kbp or less give more reliable PCR results. PCR amplification of many polymorphic VNTR regions have been described so far [5, 18, 19, 20].

Here, we describe the application of the DNA amplification technique for the detection of the D1S80 locus [5] using a high resolution, horizontal PAGE technique and subsequent silver staining [6, 7]. The characteristics of the D1S80 VNTR-locus are given in Table 1.

In this paper we describe several validation studies for this PCR-based identification system for the forensic practice.

1. The forensic validation of any new genetic marker in identity testing requires the collection of genotype and allele frequency data from the relevant population(s) [8]. To this end these were determined in a Dutch Caucasian population sample of 150 individuals and compared with population genetic studies from Finland [9], USA [9] and Germany [10].

2. The sensitivity of the system was determined with regard to the minimal amount of DNA-template. The ability of the test to detect the correct genotypes in mixed samples containing genomic DNA originating from different individuals was also evaluated.

Materials and methods

DNA extraction and quantification. DNA for PCR analysis was isolated from blood, semen and saliva samples by Chelex extraction using previously described procedures [11, 12]. Chelex-extracted DNA from each sample was quantified using the slot blot procedure described by Waye et al. [13]. DNA-extracts were normalized to a concentration of 1 ng DNA per µl.

Primer synthesis. The primer sequences for the D1S80-locus (Kasai et al. [5], Budowle et al. [6] and Sajantila et al. [9]) were:

primer 1: 5'GAA ACT GGC CTC CAA ACA CTG CCC GCC GC-3'

primer 2: 5'-GTC TTG TTG GAG ATG CAC GTG CCC CTT GC-3'

Oligonucleotides were obtained from Pharmacia (the Netherlands) or from Operon (CA, USA).

PCR reaction parameters. Reaction mixtures (total volume 50 µl) consisted of

- 5 µl 10 × PCR buffer (GeneAmp, Perkin Elmer Cetus, containing 500 mM KC1; 100 mM Tris-HCl pH 8.3; 15 mM MgCl2 and 0.01% [w/v] gelatin).
- 4 µl 2.5 mM dNTPs;
- 1 µl 12.5 µM 3' primer;
- 1 µl 12.5 µM 5' primer;
- 2.5 Units AmpliTaq DNA Polymerase (Perkin Elmer Cetus).
- 10 ng genomic DNA
- sterile H2O added to a final volume of 50 µl.

A 2.5 mM dNTP solution was prepared by mixing equal volumes of 10 mM-solutions of each of dATP, dCTP, dGTP and dTTP (GeneAmp dNTPs, Perkin Elmer Cetus).

No paraffin oil was layered over the reaction mixtures. The tubes were placed in the GeneAmp PCR System 9600 from Perkin Elmer Cetus for amplification.

Temperature cycling conditions for the D1S80-locus were as follows:

- denaturation 10 s, 95°C; annealing 10 s, 67°C; extension 30 s, 70°C; 29 cycles; final extension 5 min, 70°C.

Size fractionation of DNA amplification products. PCR products were separated by electrophoresis in a discontinuous buffer system using ultrathin (400 µm) polyacrylamide gels (7.5% T, 2% C) with piperazine diacrylamide as the crosslinker [6, 7]. Gels contained...