International Congress Series 1288 (2006) 679–681

www.ics-elsevier.com

STR typing with high performance liquid chromatography

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Abstract. The purpose of our work was to study the STR typing method with high performance liquid chromatography and to comprehend the rules of STR typing with HPLC. The HPLC conditions to separate each allele at D10S2325 locus were optimized. STR typing of D10S2325 was carried out by comparing the retention time of the allele ladder on HPLC with that of a sample. Our method was validated by typing same samples with the polyacrylamide gel electrophoresis. The sensitivity of this method and the ability to analyze mixed samples were tested. The results of our study implied that it was important to consider both the conditions of chromatography and the variation of retention time caused by the sample concentration when STR typing was carried out with high performance liquid chromatography. © 2005 Published by Elsevier B.V.

Keywords: High-performance liquid chromatography; HPLC; STR typing; D10S2325; Polymorphism

1. Introduction

Short tandem repeats (STR) loci that are distributed ubiquitously throughout the genome are now routinely used in forensic casework and paternity evaluations. The high performance liquid chromatography (HPLC) technique provides a novel method for forensic STR analysis [1]. HPLC was considered to be cost-effective method with automatic, rapid, sensitive characteristics. HPLC is possible to be developed into a throughput technique for the large amount of forensic DNA sample analysis. The purpose of our work was to study the STR typing method with HPLC and to comprehend the rules of STR typing with HPLC.

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0531-5131/ © 2005 Published by Elsevier B.V.
doi:10.1016/j.ics.2005.10.059

2. Material and methods

2.1. STR locus and primers

The pentanucleotide STR polymorphism D10S2325 [2] was selected for this study. The redesigned primers for length reduction of STR amplicons were employed according to Wiegand et al. [3] and the primer sequences were followed: Primer1: 5′-CTC ACG AAA GAA GCC TTC TGA-3′, Primer2: 5′-ATT CCA GCC TGG GTG ACG GA-3′.

2.2. Samples and extraction

EDTA-blood samples were collected from 20 unrelated Han individuals in Chengdu, China. Genomic DNA was extracted from whole blood by standard phenol/chloroform methods.

2.3. PCR reaction condition

Each PCR reaction contained 2–5 ng human genome, 1 × Taq buffer, 1.5 mM $MgCl_2$, 200 μM each dNTP (Pharmacia Biotech, Sweden), 1.5 U Taq polymerase (Promega Corporation, USA), 0.3 μM each primer, in a total volume of 37.5 μl. PCR amplifications were carried out in a GeneAmp PCR System 9600 (Perkin-Elmer, USA) with pre-denaturing for 2 min at 94 °C, followed by 30 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 64 °C, and extension for 30 s at 72 °C.

2.4. HPLC analysis

HPLC were carried out in a WAVE® 3500 nucleic acid fragment system (Transgenomic, USA). WAVEMAKER system was operated according to WAVEMAKER operation manual. All amplicons were analyzed for D10S2325 locus.

3. Results and discussion

3.1. HPLC profile of Allelic Ladder for D10S2325 locus

HPLC profile of Allelic Ladder for D10S2325 locus showed a total of 14 peaks. It was identical to the result of Allelic Ladder observed in polyacrylamide gel electrophoresis.

3.2. Correcting variation of retention time caused by the sample concentration

It was observed that analyzing STR with HPLC was stable in the same batch of samples but variable among different batch of samples, especially after making up the buffer newly, so that the allele ladder should be

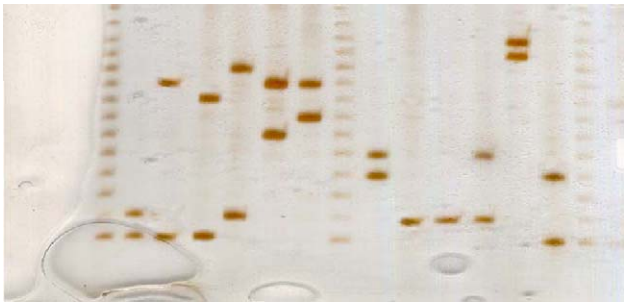

Fig. 1. STR typing by PAGE. The number of samples from 1 to 12 from left to right.

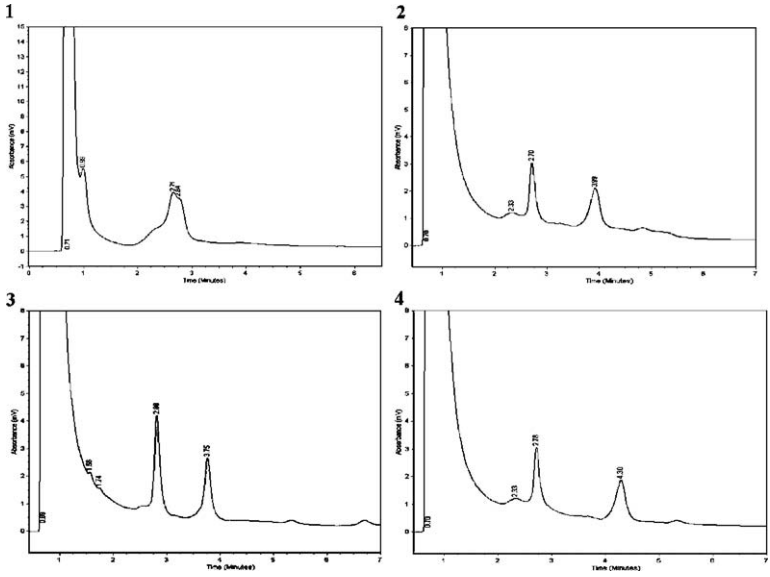

Fig. 2. STR typing with HPLC. The number of samples shown on left in each figure.

compared with sample in the same batch. It was also observed that the retention time was affected regularly by the variation of the sample concentration. The affection was revised by an equation of liner regression.

$$\mathrm{RT}-m/\mathrm{RT}-s=1.004+(\mathrm{PH}-s/\mathrm{PH}-m\times 0.001296)$$

3.3. Comparing STR typing by HPLC with PAGE

The result of typing 20 samples with HPLC was in accordance with that of PAGE (Figs. 1 and 2). HPLC was able to type single sample contained at least 6.25 ng DNA.

In conclusion, the results of our study implied that the method of typing STR with HPLC was successful. It was necessary to consider both the chromatographic conditions and the variation of retention time caused by the sample concentration when we analyzed STR with HPLC.

Acknowledgment

This study was supported by grants (No. 30271446) from the National Nature Science Foundation, China and Chinese Medical Board of New York, USA.

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