

Analysis of mitochondrial DNA polymorphisms based on denaturing high-performance liquid chromatography

X.D. Wang, Y.B. Li, J. Wu, Y.P. Hou *

Department of Forensic Genetics, Sichuan University (West China University of Medical Sciences), Chengdu 610041, Sichuan, P.R. China

Abstract. The purpose of this study is to establish a novel method for the detection of polymorphism of mitochondrial DNA (mtDNA) based on denaturing high-performance liquid chromatography (DHPLC) and to explore the new mitochondrial DNA polymorphism in coding region in order to improve the discrimination power of mtDNA typing. We explored the polymorphism of the sequence in the coding region, which covered 1435 bp. Seven pairs of primers for PCR were designed, so that it was nominated as seven loci. To explore the polymorphism of mtDNA, a technique of sample pool was employed for the analysis of DHPLC. All seven loci were analyzed by DHPLC in a Chinese population sample. Our study revealed that there were 53 haplotypes at seven loci in the coding region with covering 1435 bp and the haplotype diversity was 0.8775 in the Chinese population sample. Among these, four loci with higher diversity were proved to be suitable for forensic application and provided new genetic markers for the forensic mtDNA typing. © 2005 Published by Elsevier B.V.

Keywords: Denaturing high-performance liquid chromatography; DHPLC; Mitochondrial DNA; Coding region; Polymorphism

1. Introduction

The recently developed denaturing high-performance liquid chromatography (DHPLC) is possible to be developed into a throughput technique for the large amount of forensic DNA sample analysis [1–6]. The purpose of this work is to establish a new method for mtDNA polymorphism study by DHPLC, and through rapid analysis of the loci in mtDNA coding

* Corresponding author. Tel.: +86 2885501549; fax: +86 28 85501549.

E-mail address: forensic@mail.sc.cninfo.net (Y.P. Hou).

region to discover novel high discriminative polymorphism markers in order to improve the discriminating power of mtDNA analysis system. We hope to carry out rapid forensic analysis in mtDNA coding region by the advantages of high throughput screen competence of DHPLC and to increase the value of mtDNA analysis in forensic application.

2. Material and methods

2.1. Samples and DNA pooling

EDTA anti-coagulation bloods were collected from 120 unrelated Han individuals in Chengdu, China. Genomic DNA was extracted from whole blood by standard phenol/chloroform methods. 120 DNA samples were quantified and randomly distribute to a pool for every 5 samples. Equal volumes of 5 DNA samples were mixed into a tube as a DNA pool; 24 pools were constructed.

2.2. Primer design

7 pairs of primers were designed by Primer3 software to amplify the 1435 bp fragment in coding region of mtDNA. We selected the region between nt6000 and nt10020, which located opposite to the control region in the circle DNA. The length of the amplicons ranged between 250 bp and 350 bp. 4 pairs of primers were designed firstly by Primer3 software; their amplicons were named C, D, E, and F loci, respectively. Another 3 pairs of primers were designed secondly by Primer3 software to link up the three close located D, E, F and loci; their amplicons were named G, H, and I loci, respectively. The overlapping amplicons covered a 1435 bp fragment between nt7840 and nt9131 in coding region of mtDNA.

2.3. PCR reaction condition

Each PCR reaction contained 2–5 ng human genome, $1 \times$ *Taq* buffer, 1.5 mM $MgCl_2$, 200 μ M each dNTP (Pharmacia Biotech, Sweden), 2 U *Taq* polymerase (Promega Corporation, USA), 0.3 μ M each primer, in a total volume of 37.5 μ l. 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 28 cycles of denaturing for 30 s at 94 °C, annealing for 30 s at 58 °C, and extension for 30 s at 72 °C.

2.4. DHPLC analysis

DHPLC were carried out in a WAVE® 3500 nucleic acid fragment system (Transgenomic, USA). WAVEMAKER system was operated according to WAVEMAKER operation manual. All amplicons of the 7 loci from the 24 DNA pools were analyzed at the DHPLC conditions for each locus. The presence of heteroduplex profiles for all loci of 24 DNA pools were observed and judged as polymorphism markers for all loci.

3. Results and discussion

3.1. Homoduplex and heteroduplex profiles of DHPLC

DHPLC profile of single sample for D locus appears a single intense homoduplex peak (Fig. 1). The same like homoduplex profiles were also observed in 19 out of 24 DNA pools. One sample was randomly selected from each of the 19 DNA pools with only

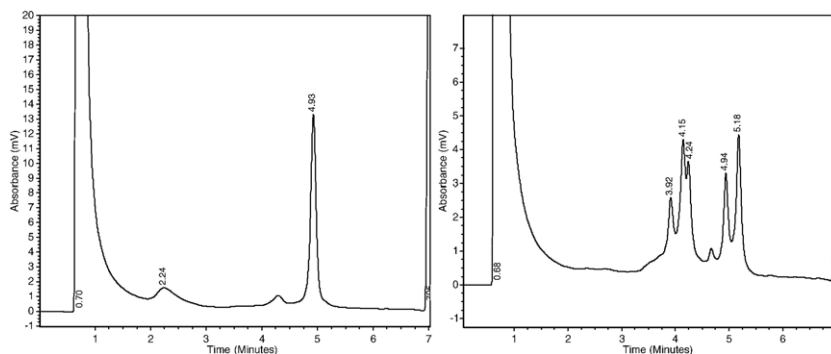


Fig. 1. DHPLC profile of single sample and DNA pool sample for D locus.

homoduplex peaks for D locus. Amplicons of each sample were mixed at equal volume and analyzed at the same condition. The only homoduplex peaks were observed in this mixture suggested that all the samples in these 19 pools (95 samples in total) had same allele at locus D. It is suggested that the majority of the samples contained this allele. Heteroduplex peaks for D locus were observed in 5 out of 24 DNA pools (Fig. 1). The sample with the allele of the highest frequency was randomly selected and amplified. The amplicon was mixed with any one amplicon of the 25 samples in the 5 DNA pools. A total of 9 sample mixtures were observed heteroduplex peaks. The amplicons of the 9 samples were mixed with each other and analyzed in pair-wise. Those with homoduplex peaks indicated the same alleles. Together with the allele of the highest frequency, a total of 6 different alleles were found at locus D in these sample population.

3.2. Haplotype of 7 loci of mtDNA coding region in Chinese population in Chengdu

All of 7 loci in the coding region with covering 1435 bp were analyzed by DHPLC in a Chinese population sample. 53 haplotypes consisting of 7 loci of mtDNA coding region were detected in 120 samples of Chinese Han ethnic group in Chengdu. The diversity of the haplotype was 0.8775. The standard error was 0.0157.

Acknowledgments

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

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