Optimizing denaturing HPLC as a robust technique for identification of Short Tandem Repeats (STR) in forensic medicine

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Keywords: STR, Identity detection, DHIPLC, Multiplex PCR, Capillary electrophoresis

ABSTRACT

Introduction: Short Tandem Repeats (STRs) are defined as short lengths of 2–7 base pairs spreading through the DNA which due to their highly diverse individually distribution are widely applied in forensic identity detection and other forensic medicine purposes. Burdening considerable costs by the conventional methods such as capillary electrophoresis, we aimed to compare concomitant usage of multiplex PCR and denaturing high-performance liquid chromatography (DHIPLC) as cheap, fast, highly accurate, and more accessible methods, with capillary electrophoresis (CE) to evaluate their potential for early screening of STRs.

Materials and methods: The present study randomly included 200 blood samples from the subjects referred to forensic medicine of Semnan, Iran. According to the size and allele frequency, we selected 8 major STR loci including CSF1PO, VWA, D18S51, TPOX, Amelogenin, FGA, SE33, and Penta D. A quad-STR multiplex PCR was performed for each locus and the PCR products were then analyzed using DHIPLC machine and compared with the basic genetic properties obtained by capillary electrophoresis.

Results: By optimizing the PCR and DHIPLC conditions, our findings suggest this strategy as an effective method for STR detection. The genotypes were determined using size of loci which led to comparable results with capillary electrophoresis confirming an insignificant variation in the detection of TOPX, Amelogenin, CSF1PO, and D18S51 (p = 0.331), but discrepant results for FGA and VWA loci (p = 0.002).

Conclusion: Our study proposed DHIPLC method as an effective screening method to characterize TOPX, Amelogenin, CSF1PO, and D18S51 as frequently used STR loci during identity detection in forensic medicine.

1. Introduction

For decades, DNA sequence analysis for forensic purposes has been widely used for analyzing the criminal cases in which the identity of suspected individuals are under query. This necessitates, therefore, designing and optimizing robust methodologies capable to accurately recognize the particular loci in genomic DNA of individuals with unique population distribution. In this regard, several lines of studies have reported the Short Tandem Repeats (STRs) as small segments of 2–7 or more base pairs in length scattering through the human genomic regions. Due to their higher mutation rate than the other regions of DNA resulting in high genetic diversity among the individuals, several STR loci have been growingly applied in forensic science for the identity detection purposes. These segments possess a high level of polymorphisms which are simply detected using PCR method. Moreover, the short length of these sequences (up to 200bp) provides this possibility to be evaluated using tiny amounts of basal DNA material even from the very low quality samples. These characteristics, thus, has turned them as sensitive and accurate platform in the identity detection.

Since the STR typing offers a cost-effective and non-intensive strategy for genetic diversification of the individuals, it has engrossed a substantial consideration. According to the recent advances in promoting conventional methodologies, as well as building novel techniques, it requires comparative analysis of various techniques which are basically used as routine experimentation or those under evaluation.

Nowadays, a remarkable change has been emerged in using high-throughput DNA experimentation for forensic medicine using high-tech