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Three points detection of short fragments derived from the amelogenin gene for gender determination—new possibilities for the capillary electrophoresis system

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Abstract. The sex-typing technique described allows detection of conventionally used fragments from first intron, shortened by Alonso to the 62/68 bp (for the X and Y, respectively) and fluorescently labelled. Additionally, two mutation points along the fragments, which differs for the X and Y copies, are detected with minisequencing. The challenge compromises also possibility of single injection analysis of fluorescently labelled fragments and mutation points with the use of the same matrix/filter set. In the present study, the new developed technique for gender-typing was tested on fresh and partially degraded forensic samples.

Keywords: Amelogenin; Sex-typing; Minisequencing

1. Introduction

According to Sasaki and Shimokawa [2], the homology of the X and Y copies of the amelogenin gene is 91%. The sex chromosomes arose 300 millions years ago and now, when comparing human amelogenin gene with its copy on Y chromosome, 19 regions of homology can be seen [1]. Regions carrying one deletion are useful for PCR sex determination methods. Fragments derived from the amelogenin gene (GeneBank acc. no. M55418 for the Amel X and M 55419 for the Amel Y) have been widely used for sex determination in forensic and archaeological samples. The primers which flank a 6-bp deletion in first intron of the X and Y homologous sequences (106 bp for X and 112 bp for Y copies) described by Sullivan et al. [3] are used as a part of commercially systems. The intronic sequences of amelogenin gene consist a panel of different polymorphisms

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X:
CCCTGGGCTCTGTAAAGAATAGTGTGTT**G**A
TTCTT**T**ATCCCA**G**AT**GTTTCTCAAGTGGTCCT**
Y:
CCCTGGGCTCTGTAAAGAATAGTGGGT**G**GATTC
TT**C**ATCCCA**A**AT*AAAGTG***GTTTCTCAAGTGGTCCC**

Fig. 1. The sequences derived from X and Y chromosomes (60/66 bp, respectively). The sequences in bold represent the primers; the differences between X and Y are on bold and big letters; the 6-bp deletion is in cursive.

including ins/del and single nucleotide exchanges allowing various techniques of detection and differentiation of two sequences to be applied. However, the commercial multiplex systems are designed to flank fragments from 100 up to 400 bp and the space between 0 and 100 bp is available for detection.

2. Materials and methods

DNA was extracted from 4 frozen venous blood samples with a standard phenol–chloroform method, from 35 stain and 20 hair samples with chelex method and from 5 bone samples with slightly modified Ivanov et al. protocol.

2.1. DNA amplification

PCR was performed with Qiagen Multiplex PCR Reaction Kit on Biometra thermocycler. Fig. 1 presents the sequences of the primers redesigned by Alonso. The reversal primer was labelled with 6FAM. The reaction was carried out in 10 μl total volume containing 0.4 μM of each primer. The amplification consisted of 95 °C denaturation for 15 min and 95, 60 and 72 °C was repeated for 28 cycles. The amplification has ended after 30 min at72 °C.

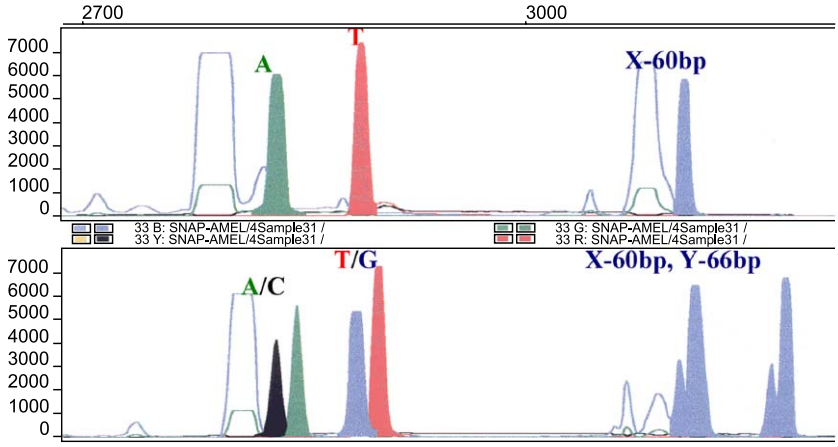

Fig. 2. The results of electrophoresis and detection of two partially degraded DNA samples; a profile of female and male (first and second rows, respectively).

2.2. Minisequencing

Products of the PCR reaction were cleaned from dNTPs and primers with SAP and EXO I (USB, USA). For 5 μl of product, 0.5 units of SAP and 2 units of EXO I were used. Reaction was performed on Biometra at 37 °C for 1 h and 75 °C for 15 min. After cleaning, the products were subjected to minisequencing reaction with an ABI Prism SNaPshot Multiplex Kit of AB Applied Biosystems. The single base extension reaction was conducted in 5 μl total volume containing 0.2 μM of each snap primer. For reaction, the forward (24 bp) and unlabeled reversal (17 bp) primers were used (Fig. 1). After enzymatic cleaning, the products were applied to detection.

2.3. Electrophoresis and detection

The products were analysed on ABI Prism 310 Sequencer with the use of the filter set E or E5 and designed for the SNaPshot chemistry matrix with GeneScan 3.1. The time of retention was 15 min with 4 s of injection.

3. Results

Fig. 2 presents an example of data obtained for two partially degraded samples. The efficiency of typing of DNA extracted from frozen blood and bone samples was 100% and was in agreement with the data obtained using standard commercial kits. Whereas, the efficiencies of DNA-typing from stain samples were 75% and 85% for hair samples.

4. Discussion

The choice of the method usually depends on investigation. Our study showed that it was technically possible to amplify and detect fragments and single base differences in the same reaction/injection. That information could be valuable for further studies. In some cases, the information provided on SNPs is very useful with those on STRs and could give more full information in identification cases.

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