RAPID SEX-TYPING BY FLUORESCENT BASED PCR OF THE X-Y HOMOLOGOUS AMELOGENIN GENE AND ANALYSIS BY CGE

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Summary
Sexing of human DNA in biological stains can be performed by amplifying a fragment of the X-Y homologous amelogenin gene. We analysed the fluorescent X and Y specific PCR-fragments by capillary gel electrophoresis (CGE) with laser induced fluorescent (LIF) detection. The CGE-procedure was optimised with respect to separation and analysis-time. We validated the CGE-LIF procedure by comparing the obtained XY-sextyping results with the typing results obtained by denaturing Polyacryl-amide gelelectrophoresis with automated detection of the alleles using an ABI 373A automated sequencer. It was found that fluorescence-based PCR together with CGE and LIF detection provided a reliable sex-typing procedure. Besides, extremely fast run times (3 minutes) were possible using replaceable gelmedia and pressure injection.

Introduction

Sex-determination has become a valuable tool in forensic identity testing. A rapid and simple sex determination assay can give valuable information on the origin of biological stains and on the gender of unidentified human remains. Sexing of human DNA in biological stains can be performed by amplifying a fragment of the X-Y homologous amelogenin gene. According to Sullivan et al [1] we used a single pair of primers spanning part of the first intron of the amelogenin gene which generates different length products from the X- (106 bp) and Y-homologues (112 bp). The electrophoretic analysis of the X-Y PCR-products can be automated by the fluorescent tagging of the PCR-products. While conventional gel electrophoresis with automated detection of the alleles has proved to be a reliable technique for many years, the preparation of the gels and of the PCR-products remains labour-intensive and is difficult to fully automate. Capillary gel electrophoresis (CGE) is a new and fast high-resolution tool for the analysis of PCR products[2].
We analysed the fluorescent X and Y specific PCR-fragments by capillary gel electrophoresis (CGE) with laser induced fluorescent (LIF) detection. We validated the CGE-LIF procedure by comparing the obtained XY-sextyping results with the typing results obtained by denaturing Polyacrylamide gelelectrophoresis with automated detection of the alleles using an ABI 373A automated sequencer.

Materials and methods

Amplification conditions and gel-electrophoresis

The amplification reaction parameters of the X-Y homologous amelogenin gene and the sequences of the FAM-labeled forward primer and the unlabeled reverse primer, were according to Sullivan et al [1].

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Gel-electrophoresis and typing of the amplified DNA-samples, using the automated fluorescent detection system on an ABI373A DNA-sequencer was carried out onto standard 6% Polyacrylamide denaturing sequencing gels (12 cm well to read). The length of the amplified fragments were determined from the internal lane standard Genescan-350 ROX (Perkin Elmer). Fragments were sized automatically by the Southern Local method using Genescan PCR Analysis software (Genescan 1.2.2-1, ABI).

Capillary Electrophoresis

Capillary electrophoresis experiments were performed on a P/ACE 2200 capillary electrophoresis instrument from Beckman Instruments (Fullerton, CA, USA). Detection was performed by Laser Induced Fluorescence (LIF) using an Argon Ion laser from Beckman Instruments. Excitation wavelength was 488 nm and emission wavelength was 520 nm. Running temperature was kept at 40 °C. The length of the coated capillary was 27 cm (effective length to detector was 20 cm). The capillary was rinsed with gel buffer for 3 minutes before each injection. Fused silica capillaries with an i.d. of 50 mm were coated with polyacrylamide as described previously [3] .

Linear polyacrylamide gel buffers were prepared by polymerisation of 8% acrylamide in 0.1 M Tris borate (pH 8.3) containing 0.5 % TEMED and 0.08% ammonium persulphate for 24 h at 4°C [4]. Then the buffer was diluted with a concentrated urea solution to a final concentration of 4% polyacrylamide and 7M urea .

PCR samples were mixed with formamide to a final concentration of 50% formamide. Samples were heated for 3 minutes at 95°C and snap cooled in water-ice for 3 minutes. After this denaturing step samples were placed in the CE instrument and analysed. Sample introduction took place by pressure injection of 40 seconds. Separation voltage was 16.2 kV (600V/cm) applied in the reversed mode (cathode on the injection side).

Results

Gel-electrophoresis with automated fluorescence analysis

Correct XY-typing results were obtained from all male and female DNA-samples. Using the internal lane standard Genescan-350 ROX the X-specific fragment was sized to an average product length of 108.56 bp with an accuracy of 0.111 bp (1SD) and the Y-specific fragment was sized to an average product length of 114.34 (±0.107) bp. Amplification of minimal diluted DNA-samples showed that PCR-products generated from as little as 120 pg of genomic template-DNA could still be detected with this technology.

Capillary gel electrophoresis

Figure 1 shows two electropherograms representing the PCR products from male (XY) and female (XX) genomic DNA samples. Good resolution is achieved within three minutes between the X and Y specific fragments. The PCR products were sized using the FAM labelled 50-500 DNA sizer from Pharmacia as internal standard. The X - and Y specific fragments were detected very close to their theoretical value (106.2 and 111.8 bp resp.). Approximately 6 nl of the sample is actually injected into the capillary. This very low amount of DNA can be detected with a signal to noise ratio wide above the detection limit.

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Discussion

It was found that fluorescence based PCR together with CGE and LIF detection provides a reliable and rapid sex typing procedure. Good agreement in typing 100 samples was obtained between CGE and conventional slab gel electrophoresis. The big advantage of CGE over slab gel electrophoresis is the rapid analysis. Nine samples can be analysed in one hour. The ABI 373A sequencer can handle as much as 36 samples in 5 hours (including gel pouring, separation and analysis). However CGE is a ready to use technique that produces results in 10 minutes. This is of great advantage in those cases where a limited amount of samples have to be analysed in the shortest possible period of time. However, inherent to CGE is the low sample amount which can be loaded into the capillary. This may result in lower sensitivity.

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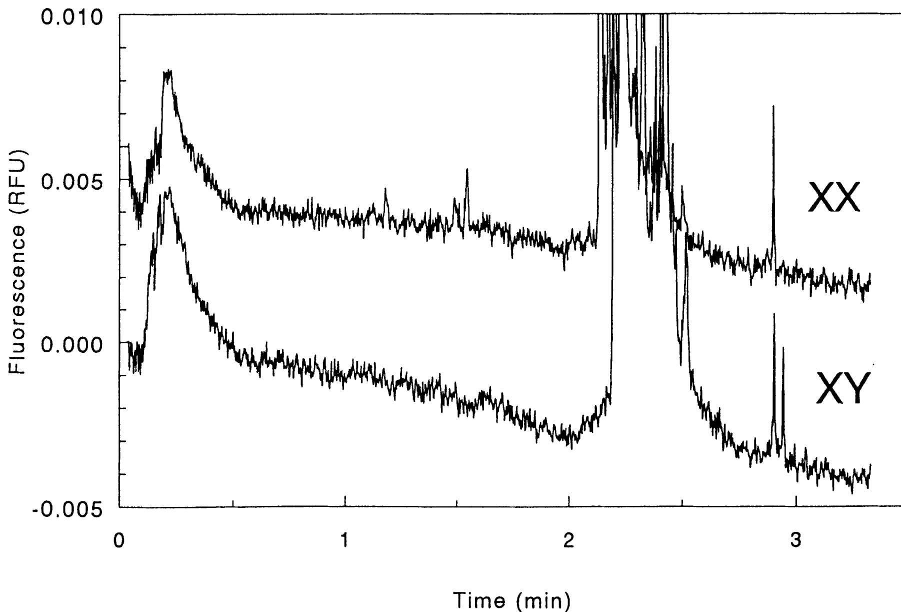

Fig 1 Separation of the XX and XY specific DNA-fragments with CGE. Injection: 40 sec by pressure; Voltage: 16.2 kV; Capillary: 50 μm 20/27 cm

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