DNA SEX TEST: A NEW RAPID AND QUANTITATIVE FORENSIC APPROACH USING AMELOGENIN GENE BASED FLUORESCENT PCR.

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For forensic PCR applications, it is recommended that the investigation of X and Y sequences should be carried out in parallel or simultaneously, and the distinction of male and female DNA cannot be made based solely on the absence of a band [1]. Several PCR-based tests have been developed for gender identification, among them multicopy repeat sequences on the X and Y chromosomes can be amplified together and provide a highly sensitive assay but quantitation of the relative X/Y product is not possible because of significant differences in repeat copy number [2-4]. Alternatively, single copy X-Y homologous regions such as amelogenin offer the advantage of requiring only one pair of primers and both X and Y sequences are of equal copy number [5]. Primers described by Sullivan and coll. flank a 6bp deletion within intron 1 of the X homologue resulting in 106bp and 112bp PCR products from the X and Y chromosomes respectively [6]. Dye labelled PCR products were generated using one primer coupled with fluorescent dye 'FAM' via a 5' aminolinker. These primers allowed DNA samples ranging from 10pg to 100ng to be amplified through 35 cycles comprising 1 minute at 94^0C, 1 minute at 60^0C and 1 minute at 72^0C in a Perkin Elmer 480 thermal cycler. PCR products (20 μl) were visualised simply after electrophoresis in a 4% agarose gel for 90 mins at 100V and by ethidium bromide staining.

With this test we were enabled to sex a total of 50 male and 50 female DNA samples extracted from blood: all gave the expected results of a single band (106bp) in females and a doublet (106 / 112bp) in males. PCR products generated from as little as 1 ng of male and 1 ng of female template were readily visible. Unconfirmed origin DNA, extracted from muscle samples, that had been recovered from the scene of a mass disaster, gave clear-cut results meanwhile control "blind" analysed samples of known sex from the same investigation, were typed correctly. Approximately 20 pg of two severely degraded DNA samples, coming from seventy years buried bone, were amplified through 39 cycles. The products were clearly visible following electrophoresis in an ethidium bromide-stained agarose gel and confirmed conclusions regarding their sex drawn from physical examination of the bones. This also demonstrated the ability of this test to analyse samples of a very degraded nature. Electrophoresis of 1μl in 6%

denaturing acrylamide gel for 4 hrs at 1360 V on a ABI 373A Sequencer in conjunction with 672 software, combined with 2.5μl formamide and 6fmol internal size standard for automatic sizing, generated electrophoretograms in which the DNA segments were depicted as coloured peaks and peak area was automatically estimated as a measure of product yield. Such a high sensitivity and quantitation potential implicit to the test in conjunction with advantages of automated fluorescence dye-detection technology supplied by ABI 373 A GeneSequencer, provided a means to successfully type difficult mixed samples, e.g. detecting trace amounts of male DNA in a vast excess of female DNA template. Clear detection of Y specific product in a particular mixture that contained 100 ng of female DNA and 1 ng of male DNA, was obtained. The expected ratio of peak areas is 201:1 compares with the observed ratio of 228:1. This method could be potentially a useful tool to detect the presence of sperm (or white cells in case of azospermy) on vaginal swabs. Fluorescence detection also allowed to quantitate the relative amplification yelds: using normal males, peak area measurement generated 1:1 ratio of X and Y products while DNA from an XYY individual gave a 1:1.8 ratio and from XXY gave 1.8:1 ratio.

The versatility of this sex test was further demostrated by co-amplifing with 4 STRs loci (HUMTHO1, HUMVW31/A, HUMFES, F13A1) and the HLA-DQA1 locus, thereby providing a combined gender/identity DNA test. Co-amplification with 4 microsatellite with 130-240bp lenght products provides a combined probability of random match between unrelated caucasian of $1.06x10^{-4}$ [7].

A major advantage of the test described in this paper is that amelogenin single copy X-Y homologous regions are detected together, in the same reaction using a single primer pair. Our test greatly simplifies interpretation of results, especially when only the X-specific product has been generated, because this serves as an in-built positive control for the amplification reaction. Interpretation of results is further facilitated by the X and Y specific products being only 6bp different in size: even severely degraded samples can be typed without the risk of amplifying only the smaller of the two alleles. This "allelic drop-out" problem can afflict amplification and typing of loci with wider ranges of allelic sizes such as VNTRs [8]. The test may also have medical application in characterising sex chromsome abnormalities such as Klinefelter' s syndrome (XXY), monitoring sex chromosome dosage in cell lines and typing histopathological samples in which DNA is severely degraded.

In conclusion, this sex test is rapid sensitive and robust: PCR products are readily visible on agarose gel after ethidium bromide staining. Moreover, fluorescent technology allows a quantitative extimation of PCR products and results in useful application in both forensic and medical purposes for characterising mixtures of DNA, co-amplification with STRs and determinig sex chromosome aneuploidy.

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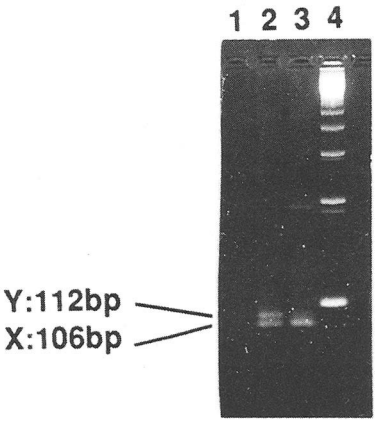
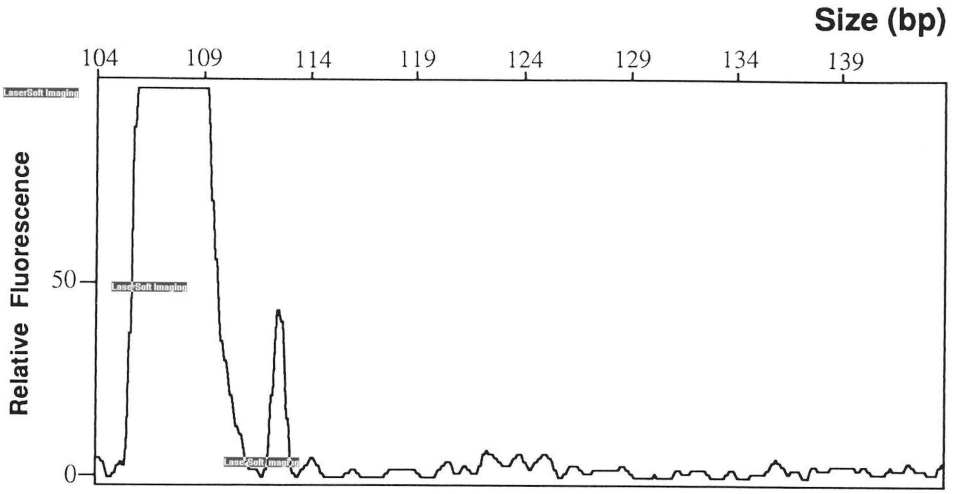


Figure 1. Sexing of seventy years buried bones. Lane 1, negative control water blank; lanes 2 and 3, PCR products from bone specimens, identified as male and female respectively; lane 4, 123 bp ladder size standard.



Peak / Lane	Min.	Size	Peak Height	Peak Area	Scan #
1B, 11	209	107.20	7122	46374	1047
2B, 11	216	112.87	54	203	1084

Figure 2. PCR product electophoretogram of male/female mixed DNA, generated by an Applied Biosystems 373 A GeneSequencer. Peaks from PCR product. The male specific peak (112bp) is clearly distinguishable from the larger female peak (106bp). Size in bp and peak height and area in arbitrary units are determined for each fragment. Time (min) and scan number of band detection from the start of electrophoresis are also given. Male/female DNA template ratio 1/100.

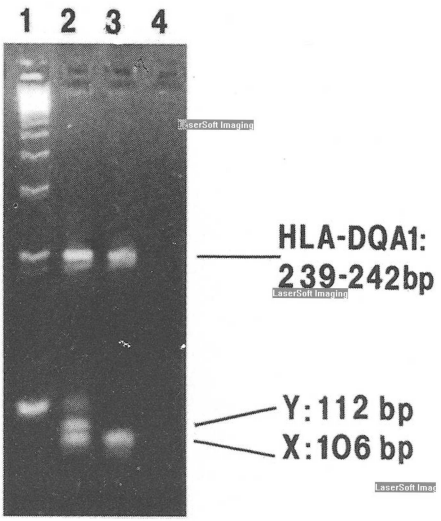


Figure 3. Amelogenin-HLA-DQA1 coamplification product evaluation gel. Lane 1, 123 bp ladder size standard, lanes 2 and 3, DNA samples; lane 4, negative control.