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Amelogenin (genetic marker) role in sex determination of human

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Abstract
The ability to determine the sex of an individual based on DNA evidence can be crucial in instances such as identification of victims of mass disaster, missing persons investigations, and sexual assault cases. The Y chromosome marker Amelogenin is currently in widespread use for determination of chromosomal sex of an unknown DNA donor and differentiating the relative contributions of male and female DNA in a mixed forensic sample. However, many cases of the failure of the Amelogenin marker to correctly determine the sex of DNA donors have been reported, causing the usefulness of the Amelogenin marker in forensics to be questioned. In this paper we review use of Amelogenin as a marker for sex identification in forensics and describe four additional Y chromosome markers, sex-determining region Y (SRY), chromosome or the presence of specific reproductive or secondary sex characteristics.

Keywords: Amelogenin, genetic marker, sex Dtermination, human

Introduction

A molecular marker Amelogenin has an important role in sex determination of human. This gene is located on the X and Y chromosomes of human beings. It is also involved in the production of a protein which develop tooth enamel matrix. By using specific PCR primers, different Amelogenin base pairs fragments can be amplified from the X and Y chromosomes 0f human.Sex determination from the biological specimens gathered from crime scenes plays an important role in criminal investigations [1]. Methods for sex determination using a single plex PCR for a sequence in the SRY gene on the Y chromosome have also been reported [2, 3]. SRY gene has been one of the most common molecular methods for sex identification [4]. However, the SRY gene is only located on Y-chromosome, and it is hard to say whether there is a mistake in the testing procedure or just the individual is female while there is no amplification [5, 6].These methods can indicate a male genotype by the presence of the amplified product from the SRY gene, but cannot accurately indicate a female genotype. Recently, various PCR kits for short tandem repeats have been marketed, and the sex test included in these kits has become a standardized method. Compared with SRY genes in sex identification methods, AMEL gene is more efficient, suitable, and accurate [7].

There are following type of sex markers

Amelogenin, SRY, STS, TSRY. Amelogenin is X linked and is symbolized be AMELX, its alternative symbols are AMG, AIH1 and its location on chromosome X is p22.3-22.1.
While the Amelogenin on Y chromosome is symbolized by AMELY, its alternative symbol is AMGL and its location is on Y chromosome is Locus Yp11. The Amelogenin gene has been most widely studied in humans, where it is a single copy gene, located on the X and Y chromosomes at Xp22.1-Xp22.3 and Yp 11.2 [8]. Other sources of Amelogenin variation arise from the various isoforms of AMELX obtained from alternative splicing of mRNA transcripts
Differences between the X chromosome and Y chromosome versions of the Amelogenin gene (AMELX and AMELY respectively) enable it to be used in sex determination of unknown human samples [9]. However because of AMELY variation among individuals and populations, this method of sex determination is not 100% accurate.

Materials and Methods

Blood sampling was done for human male and female Sex determination by the application of Molecular Sex determination marker Amelogenin. Five samples were taken. DNA was extracted by organic method, following the manufacturer's protocol [10].At -20 °C isolated DNA was stored. DNA was quantified by using Nano Drop 2000c. Gene was amplified on a PCR using sex specific primers. The most commonly used Amelogenin primer sets are those designed by [11].Amelogenin primer sets produced AMELX/AMELY amplicons of 106/112 bp and AMELX/AMELX amplicons of 106/106 bp respectively. The PCR conditions were optimized for the amplification of Amelogenin gene by changing the concentration of deoxynucleotide triphosphate (dNTPs), magnesium chloride (MgCl2) and Taq polymerase. Annealing temperature was also adjusted to achieve amplified gene. The PCR recipe and conditions applied in experimental process to get amplified products are listed in the table .

Table 1

Reagents	Stock conc.	Final conc. In reaction mix	Volume per reaction
Buffer	10X	1X	5µl
dNTPs	2.5mM	0.2mM	4µl
$MgCl_2$	25mM	2mM	4µl
Forward Primer	10µM	0.5µM	2.5µl
Reverse Primer	10µM	0.5µM	2.5µl
Taq DNA polymerase	5U/µl	1.25U	0.3µl
Distilled Water	To make up volume	__	26.7µl
		Total	45µl
Template	10ng/µl	30ng/µl	5µl

PCR using prepared 2x Master Mix

Primers Sequence

Table 2

Name	Sequence	Size
AMEL-Forward	5'-CCCTGGGCTCTGTAAAGAATAGTG-3'	24
AMEL-Reverse	5'-ATCAGAGCTTAAACTGGGAAGCTG-3'	24

The following are the optimized cycling conditions of PCR for amplification of Amelogenin gene. The cyclic conditions for PCR were as per the following: an initial denaturation step at95°C for 4 min followed by 30 cycles of final denaturation at (94°C, 30 sec), annealing (52°C, 30 sec), Extension (72°C, 60 sec) and finial DNA extension step at 72°C for 10 min**.**

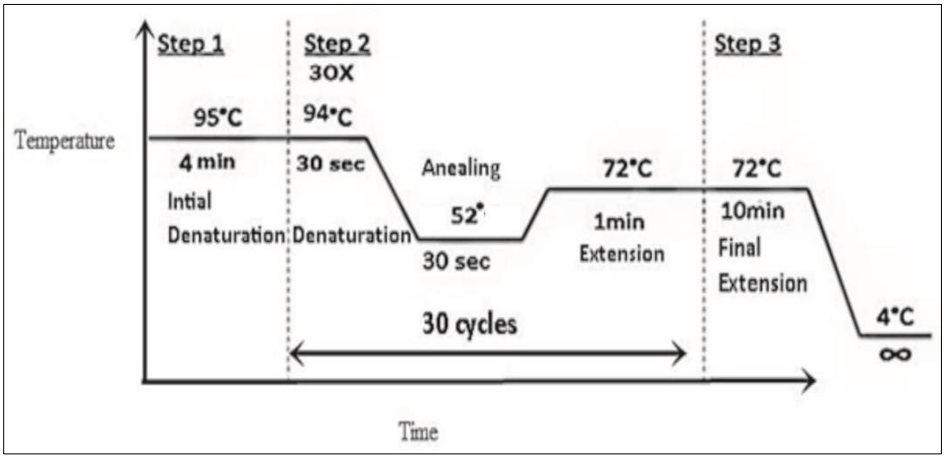

Fig 1

Figure Touch Down-PCR Profile

The PCR products were run on 2% agarose gel for 30 min at 110 V by electrophoresis in TAE buffer and staining the gel with ethidium bromide. Bands were visualized through UV light in Gel Doc. Polyacrylamide Gel Electrophoresis (PAGE) isused to separating fragments of less than 500 bp.

Results

All samples were successfully intensified. PCR products on gel revealed the presence of a single band for female and double bands for male. Six samples were taken, one was male and five were females.

Result of Agarose Gel Electrophoresis

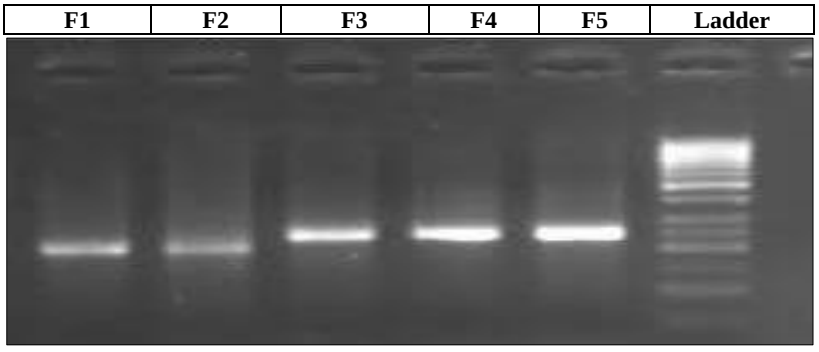

Fig 2: Genomic DNA on agarose Gel

Result of Polyacrylamide Gel Electrophoresis

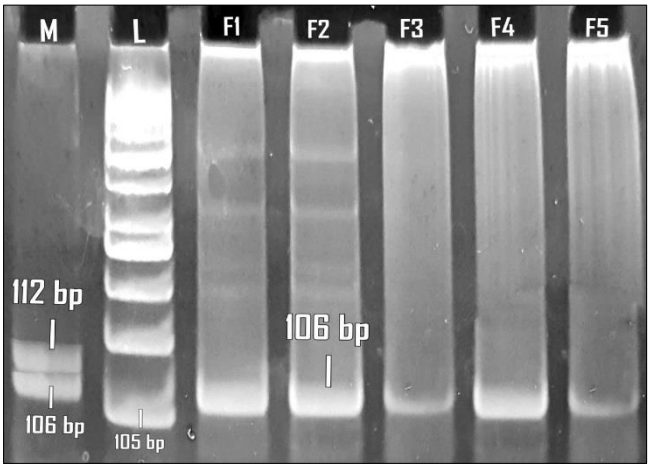

Fig 3: Bands of PCR product on polyacrylamide Gel which showing one DNA band for Female human sample and two bands for Human male sample

Discussion

In one study in Spain, the amelogenin sex determination test using AMELX (977bps) and AMELY (790bps) bands was performed for 1224 individuals of known gender with a 99.84% (1222/1224) accuracy rate. Another study in India, however, found 5 of its 270 men studied (1.85%) possessed an AMELY deletion, terming them "deleted-amelogenin males. In response the authors suggested that while the amelogenin sex test may be accurate in general, other Y chromosome markers such as SRY, STR, can be used for less ambiguous gender identification.[12].

In our study it was observed that a multiplex method for sex determination that includes amelogenin X and Y and two sequences of SRY along with homologous sequences on chromosomes X and 7. In the male control DNA both STS and STSP1 were amplified, resulting in two clear peaks, while in the female control DNA only STS was amplified.

Conclusions

During this study we learnt about many new and innovative techniques like DNA Extraction, Agarose Gel electrophoresis, Nanodrop, Polymerase Chain Reaction and Polyacrylamide Gel Electrophoresis, which will be helpful in future for doing any diagnostic and molecular work.

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