# Discrimination the Gender in the Criminal Evidence at Crime Scene

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## Abstract

**Background:** An ideal method for gender identification would be accurate, simple, and cheap,enabling its use in most laboratories. In addition, it should also be able as much as possible to be used for all animals as well as tissues and/or cells. For chromosomal analysis, we need viable cells that are able to divide, and if this is not possible, these methods cannot be used. On the other hand, genetic methods are reliable and do not need living cells, and it is easy to obtain DNA for these studies even in very ancient and nonviable tissues. These methods are therefore the most accepted ones The current study aimed to Determine the gender of a given DNA samples by designed vaulable specific-PCR tool. **Methodology:** Thirty eight ( ninteen from each male and female) human blood samples were collected using EDTA-Na2 tubes for direct DNA extraction after taken the agreement of the volunteers to give the blood sample . via expert .The blood sample mixed well by rotation of EDTA tube. two sets of species specific primer pairs targeting *Homo sapiens* (*AMLE X and AMLE Y*) genes. were designed and checked. PCR and sequencing were performed and sequences were analyzed and register in GenBank. **Results:** the results revealed that, the amplicon of *AMLE X* gene of Homo sapiens were 1164bp and *AMLE Y* were 744bp **Conclusion:** The current study conclude that, validity and accuracy of designed 12S rRNA species specific primer pairs for human and nonhuman animal blood typing as a forensic tool and there is no intraspecies cross amplification.

***Keywords:*** *Homo sapiens, AMLE X and AMLE Y.*

## Introduction

Analysis of the gender chromosomes is important in the determination of gender and instantly excludes 50% of the population. The human gene usually analyzed to determine gender is the Amelogenin (AMEL) locus, after its amplification using PCR DNA fragments of different lengths can be generated. Amelogenin is involved in the formation of enamel, which is the hard, white material that forms the protective outer layer of each tooth[1]. Enamel is composed mainly of mineral-containing crystals. These microscopic crystals are arranged in bundles that give enamel its strength and durability[2]. Studies suggest that lesser amounts of amelogenin may also be present in tissues other than developing tooth enamel. For example, amelogenin has been found in certain bone, bone marrow, and brain cells. The function of amelogenin in these tissues is unknown. One copy of the amelogenin gene is located on each of the sex chromosomes (the X and Y chromosomes) [3]. The sequence on the X chromosome is shorter by 6 bp compared to the allele on the Y chromosome (male gender) [4]. Polymerase Chain Reaction (PCR) analysis that target regions of Amelogenin gene have become the method of choice for gender determination of biological samples [5]. The aim of this study is to Determine the gender of a given DNA samples by designed vaulable specific-PCR tool.

## Methodology

### Sampling:

Thirty eight ( ninteen from each male and female) human blood samples were collected using EDTA-Na2 tubes for direct DNA extraction after taken the agreement of the volunteers to give the blood sample . via expert .The blood sample mixed well by rotation of EDTA [6].

**DNA Extraction Polymerase Chain Reaction:**

G-spinTM Total DNA Extraction Kit (was used to extract DNA from blood according to the manufacturer's protocol instructions (Intronbio/Korea). All extracted DNA samples were submitted for Nanodrop to ensure adequate purity and concentration required for PCR. Setting done by selection dsDNA measuring mode for 2 µl of sample. Blanking were performed using 2 µl microliter of elution buffer and then measuring the DNA samples concentration and purity[7].

The sequence of Homosapiens ( *AMLE X , AMLE Y*) were taken from NCBI data base (AMLE X Sequence ID: AY040206.1; AMLE Y Sequence ID: NG_ 008011.1) were used to design primer pairs using online Primer 3 software [8,9]. The specific primer pairs: AMLEX-F: CTGGAGGGTCTTGCCTGAAG , AMLEX –R: GATGTACCCAAAGGGGTGGG( 1164 bp) ; AMLE Y –F: CCAGGACAGCTAGGTTCAAGTT, AMLEY –R: CACCTTTCCATAAATAGCTTTGTCA( 744 bp) . The specificity of designed primer pairs were checked using online NCBI/primer blast[9,10]. The PCR conditions were calculated using online Protocol Optimize writer software. The conditions were illustrated in table (1).

**Sequence Analysis**

Trimming of sequences were perform by FinchTV and then submitted to NCBI-BLASTN to see the identity of sequences with reference sequences within NCBI data base.

**Table (1): PCR Condition**

| Primer | Conditions | | | References |
|---|---|---|---|---|
| AMLEX –F<br>AMLEX -R | 1 | 95°C | 2min. | This study |
| | 30 | 95°C | 30sec. | |
| | | 61.3°C | 30sec. | |
| | | 72°C | 2min. | |
| | 1 | 72°C | 5min. | |
| AMLEY –F<br>AMLEY –R | 1 | 95°C | 2min. | This study |
| | 30 | 95°C | 30sec. | |
| | | 60°C | 30sec. | |
| | | 72°C | 2min. | |
| | 1 | 72°C | 5min. | |

## Results and Discussion

The results of PCR reveal that, firstly the primer pairs were specific and non-specific products not appear for all samples. The amplification of male and female Homo sapiens DNA with AMLEY primer pairs gave positive and negative results respectively while The amplification of male and female Homo sapiens DNA with AMLEX primer pairs gave positive results for all samples and this a primary evidence for primer pairs specificity The amplicon of AMLEY gene of Homo sapiens 744bp (Figure 1: A). The amplicon of AMLE X gene of Homosapiens were 1164 bp (Figure 1:B).

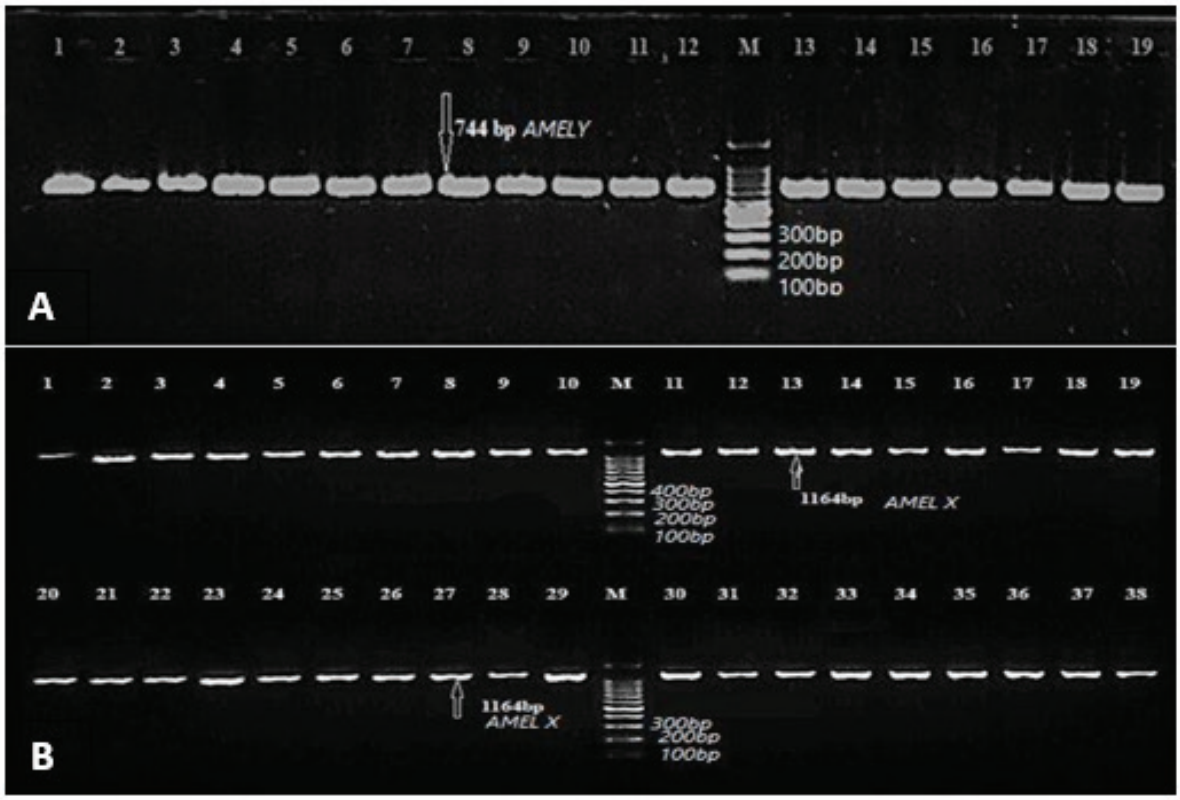


**Figure 1: Agarose gel electrophoresis at 72 volt for 60 minutes 1.5% for A: 744bp amplicon of Homo sapiens AMLE Y gene ; B: 1164bp amplicon of Homosapiens AMLE X gene. M represent 100bp DNA ladder, lane 1-19 represent male samples. lane 20-38 represent female samples .**

The secondary and confirmatory assay for specificity of primer pairs used in study is sequences of PCR products. four amplicons from each were sent to South Korea for sequencing using Sanger technique (Macrogen/Korea). The retrieved sequences firstly must be trimmed to remove unwanted sequences before submitting them for BLASTN. The trimming performed by Bioedit to get the finally processed sequences. Abbreviation of AMLE Y sequences were used as IraqiAMELY-1 to IraqiAMELY-4 . Same abbreviation will used as isolates name when submitting to GenBank. The identity percentage were illustrated in Table (2). The high percentage of identity of sequences confirm the specificity and validity of specific primer pairs used in current study.

**Table (2): Identity of blasted samples (IraqiAMELY-1 to IraqiAMELY-4) with reference sequences of highest identity percentage**

| Isolate | Sequence ID | Identities | Gaps | Strand |
|---|---|---|---|---|
| IraqAMELY-1 | NG_008011.1 | 453/453(100%) | 0/453(0%) | Plus/Plus |
| IraqAMELY-2 | NG_008011.1 | 446/446(100%) | 0/446(0%) | Plus/Plus |
| IraqAMELY-3 | NG_008011.1 | 450/450(100%) | 0/450(0%) | Plus/Plus |
| IraqAMELY-4 | NG_008011.1 | 446/446(100%) | 0/446(0%) | Plus/Plus |

Concern the abbreviation of AMLE X sequences be IraqiAMELX-1 to IraqiAMELX-4. The identity percentage results were illustrated in Table (3). The high percentage of identity of sequences confirm the specificity and validity of specific primer pairs used in current study.

**Table (3): Identity of blasted samples (IraqiAMELX-1 to IraqiAMELX-4) with reference sequences of highest identity percentage**

| Isolate | Sequence ID | Identities | Gaps | Strand |
|---|---|---|---|---|
| IraqAMELX-1 | NG_012040.1 | 391/391(100%) | 0/391(0%) | Plus/Plus |
| IraqAMELX-2 | NG_012040.1 | 409/409(100%) | 0/409(0%) | Plus/Plus |
| IraqAMELX-3 | NG_012040.1 | 733/733(100%) | 0/733(0%) | Plus/Plus |
| IraqAMELX-4 | NG_008011.1 | 630/630(100%) | 0/630(0%) | Plus/Plus |

The results showed no cross-reactivity of designed primer pairs and the PCR assay based on the designed primer pairs will be simple, fast, sensitive, specific, and cost-effective .

Genetic sex determination methods are not related to subjective physical examination, are accurate, require small samples, and do not necessitate the evaluation of a specific tissue, and any organ can be used. Their applicability depends on the specific methods. Successful assays are simple, need small amount of tissue, and are accurate during the entire pregnancy. Measuring the activity of X chromosome linked enzymes or RNA-based PCRs is complicated by the presence of some gene products only at certain developmental stages[11]. However, this problem is not present when the test is based on DNA. The amelogenin gene which is found on both X and Y chromosomes is in common use for sex discrimination in forensic medicine. A 6 bp deletion in intron 1 on chromosome X compared to the Y chromosome can be detected by using a pair of PCR primers. It can be used in various tissues including long-lasting remnant tissues like dental pulp [12].

## Conclusion

The current study concludes Sensitivity, specificity of the designed specific primer pairs ; Applicability of the designed primer pairs in forensics to investigate blood sample or evidence belonging for detection of gender .

**Ethical Clearance:** The project plan displayed on the scientific committee and scientific ethical committee of the department of Biology-college of science at university of Babylon and get approval

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**Conflict of Interest:** There is no conflict of interest

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