

# DNA isolation from teeth by organic extraction and identification of sex of the individual by analyzing the AMEL gene marker using PCR

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## Abstract

**Background:** To identify the sex of the deceased individual from dental hard tissue such as enamel and dentine. **Objective:** To isolate the DNA from dental hard tissue (enamel and dentin) from teeth extracted for prophylactic purpose, to assess the quality and purity of DNA and to identify the sex using polymerized chain reactor (PCR). **Materials and Methods:** DNA was extracted following phenol/chloroform (organic) extraction from 20 male and 20 female teeth. The samples that contain the amelogenin gene (*amel*) were amplified by PCR. The products of the PCR were run on agarose gel with ethidium bromide staining on gel documentation system. **Results:** The results on the gel showed the presence of X-specific bands at 212 bp and Y-specific bands at 218 bp. Males were distinguished from females by the presence of two bands whereas female samples showed only one, that is, X-specific band on the gel. The gender from the known samples was determined with complete accuracy, and the results were analyzed statistically by the Chi-square test. **Conclusion:** In our study, the PCR-based method showed 100% specificity and sensitivity.

**Key words:** Amelogenin gene, deoxyribo nucleic acid, polymerized chain reaction

## Introduction

Recognition of importance of human teeth in personal identification has been recognized from time immemorial. Odontological examinations have been a critical determinant in the search of human identity. The availability of complete dental care in the civilized countries of today

in conjunction with maintenance of dental records have served to enhance the specificity of the dental examination as a tool in identification.<sup>[1]</sup> Forensic odontology is of paramount importance from a legal and social viewpoint.<sup>[2]</sup> Sex determination is an integral part of characterization of forensic samples. PCR offers an efficient and sensitive method for sex determination by amplifying a gender-specific sequence. The present study uses an AMEL gene marker that has a different "signature" for male and female, by using AMEL gene base primers in PCR amplification.<sup>[3]</sup>

## Materials and Methods

A cross-sectional study was done on 40 samples (20 males and 20 females) from teeth extracted for therapeutic purpose. Permission to conduct the study was obtained

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from institutional ethical committee (I.E.C) before commencement of the study. Teeth were individually decontaminated with 5% bleach (sodium hypochlorite, NaOCl) for 20 min at room temperature. As, a second decontamination step, each tooth was dried under a 256 nm ultraviolet light source for 20 min at room temperature<sup>[4]</sup> and the following procedures were performed.

- DNA extraction [phenol/chloroform (organic) extraction]
- Amplification of the extracted DNA by PCR
- Product analysis.

### Phenol/chloroform (organic) extraction

Organic extraction yields high quality, double stranded DNA, in new as well as in old/degraded teeth and therefore can be utilized in situations where PCR typing is performed.

- Add 40-60 mg of tooth powder to 500  $\mu$ l of EDTA
- Incubate at 37°C for 3 days
- Add 1 ml of TRIS NaCl EDTA (TAE) buffer with sodium dodecyl sulfate (SDS) and add 10  $\mu$ l of proteinase K and mix by pulse vortexing for 15 seconds
- Incubate at 56°C for 24 hours
- Transfer the entire lysate into 2 ml eppendorf
- 1 ml of lysate is added with 1 ml of phenol chloroform isoamyl alcohol and pulse vortexing is done for 15 seconds
- Incubate for 5 min at room temperature
- Centrifuge at 10,000 rpm for 10 min in refrigerated centrifuge
- Collect the supernatant into a 1.5 ml fresh tube and discard the collection tube containing the flow through.
- Add equal volume of isopropanol
- Incubate for 30 min to 1 h at room temperature
- Centrifuge at 10,000 rpm for 15 min in refrigerated centrifuge
- Discard the supernatant
- Wash the DNA pellet with 70% ethanol twice
- Dissolve the DNA pellet in 20  $\mu$ l nuclease free water (NFW) and store at -4°C until the PCR procedure.

### PCR amplification

PCR is a primer-mediated amplification of specific sequences of DNA, Three basic steps are involved.<sup>[5]</sup>

**Denaturation**—to promote the single stranded DNA formation by heating the template to 94°C for 1 minute.

**Annealing**—the temperature was lowered significantly to promote binding of base pairs of template and the primer, at 54°C for 2 minutes.

**Extension**—the temperature then shifted to optimum temperature for DNA polymerase to synthesize sequences

complimentary to the template using annealed primer as a starting point for extension of a newly synthesized single strand. Extension temperature used was 74°C for 1 minute.

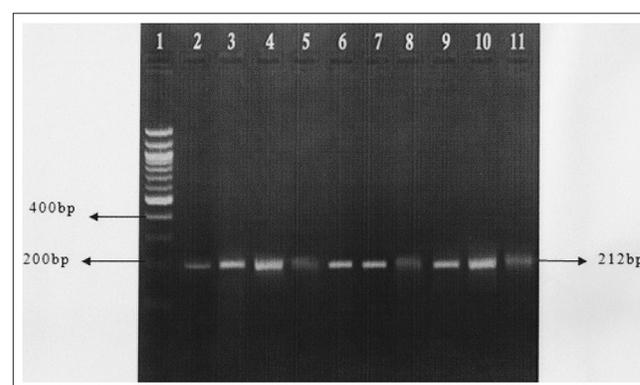
These three steps constitute a single cycle of the reaction, the apparatus was fed with this temperature program after the reaction mixtures were in place and a total of 40 cycles were performed per reaction, with a ramping time of less than a minute between the cycles. Samples were held at a temperature of 4°C.

### Product analysis

The products of the polymerase chain were run on an Agarose Gel with ethidium bromide staining on gel documentation system (GDS). The GDS consists of a UV visualizer which is connected to a computer, where the results could be visualized, analyzed and stored.

### Results

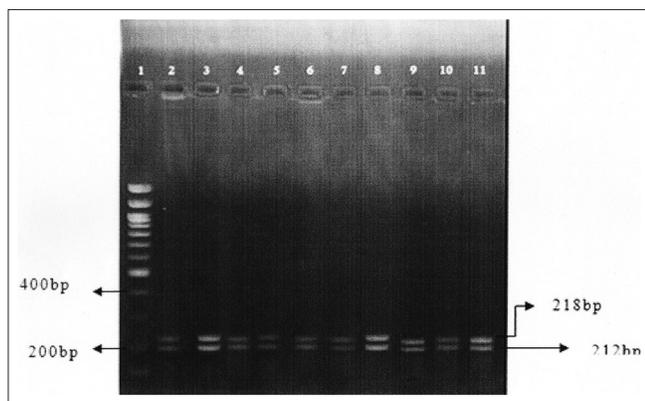
The results on the gel showed the presence of X-specific bands at 212 bp [Figure 1] and Y-specific bands at 218 bp [Figure 2], males were distinguished from females by the presence of two bands whereas female samples showed only one, that is, X-specific band on the gel. The gender from the known samples was determined with complete accuracy, and the results were analyzed statistically by the Chi-square test [Table 1a and b]. DNA from a single tooth yielded an average of 55-86 micrograms quantity and an average purity ranging from 1.5 to 1.8 following standard conditions of extraction and this DNA has proven to be of sufficient quantity and of a good purity to be used as a forensic evidence material.



**Figure 1:** Image showing amplified 212bp (AMEL gene) suggestive of female sample

**Table 1a: Results for XX and XY**

	Observed <i>N</i>	Expected <i>N</i>	Residual
0	20	20.0	0.0
1	20	20.0	0.0
Total	40		



**Figure 2:** Image showing amplified 212bp and 218bp (AMEL gene) suggestive of male sample

**Table 1b: Test statistics**

	XX	XY
Chi-Square	0.000 <sup>a</sup>	0.000 <sup>a</sup>
Df	1	1
Asymp. sig.	1.000	1.000

<sup>a</sup>P=0.000 (Highly significant)

## Discussion

The study was conducted to evaluate and establish teeth and specifically dental hard tissue (enamel and dentin) as an important source of DNA, and to utilize the DNA obtained in gender determination. Although any tissue is amenable to extraction of DNA, dental tissues such as enamel, dentin, pulp and cementum, offers us the advantage of being resistant to physical, environmental degradation and has proved to be a good source of genomic DNA.<sup>[6]</sup> Tooth enamel is a unique entity among all mineralized tissues because of the presence of high mineral content. It is non-collagenous and does not undergo resorption and remodeling. Its formation occurs through a transient collaborating network of enamel matrix proteins that controls hydroxyapatite crystal growth and orientation. Amelogenins constitute about 90% of the total enamel matrix proteins and play a major role in enamel biomineralization and morphological changes in enamel.

Amelogenin has a variety of isoforms present in the enamel matrix that are generated by alternative splicing of primary amelogenin RNA transcript. There are three schools of thought about exhibition of varied isoforms by amelogenin. First is the presence of two distinct sets of genes located in both the sex chromosomes, second is the production of numerous amelogenin mRNAs by alternative splicing and lastly they are proteolytic processed after secretion.<sup>[7]</sup> Majority of the isoforms can coalesce into spherical structures called nanospheres averaging about 20 nm in diameter and containing about 100 amelogenin molecules.<sup>[3]</sup>

Amelogenins have a distinct difference in size and pattern of nucleotide sequence in male and female enamel. This difference between the two enamel phenotypes is a sensitive sex determinant for very minute DNA samples produced from unknown human skeletal/dental remains. The amelogenin gene present on X-chromosome has 106 base pairs in length whereas this gene present on Y-chromosome has 112 base pairs. This information can provide us a strong distinction between male and female amelogenins as well as highlights the fact that females have two identical amelogenin genes present on X-chromosome, whereas males have two different genes, present on both the sex chromosomes.

This difference in male and female genotypes can be utilized as a indispensable tool having good specificity and sensitivity and financially viable for modern forensic science.<sup>[8]</sup>

## Limitations

### Limitations of PCR (Polymerase chain reaction)

PCR is an extremely sensitive technique but is prone to contamination from extraneous DNA, leading to false positive results. Another potential problem is due to cross-contamination between samples. Concentration of Mg is very crucial as low Mg<sup>2+</sup> leads to low yields (or no yield) and high Mg<sup>2+</sup> leads to accumulation of nonspecific products. Non-specific binding of primers and primer-primer dimer formation are other possible reasons for unexpected results. Reagents and equipments are costly, hence can't be afforded by small laboratories.<sup>[9]</sup>

### Limitations of PCI (Phenol chloroform isoamyl alcohol)

The method is time consuming, uses hazardous organic solvents and involves multiple tube transfers that increase the possibility of sample contamination and mislabeling.<sup>[10]</sup>

## Conclusion

Extraction of DNA from teeth samples yields sufficient amounts of good quality DNA useful for PCR-based diagnostic methods. The teeth could serve as a reliable source of DNA for amplification-based forensic methods in sex determination. DNA could be obtained from any tooth, regardless of the age of subject. AMEL gene serves as a good marker for sex determination in the Indian population by using the AMEL gene-based primers in PCR. In our study, the PCR-based method was sensitive and proved to be successful for sex determination with 100% specificity and sensitivity.

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