



### A134 Sex Estimation Based on Analysis of the Enamel Proteome

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After attending this presentation, attendees will better understand the use of the tooth enamel proteome in human sex estimation that could be applied to a broad range of samples.

This presentation will impact the forensic science community by offering an alternate approach to estimate sex in a statistically rigorous and scientifically quantifiable manner. This approach can be applied to deciduous teeth and to teeth from degraded skeletal remains.

Sex estimation is necessary to place skeletal remains in forensic context. The current field of forensic anthropology relies on two basic methods to estimate sex in skeletal remains: analysis of sex-specific osteological markers and detection of DNA markers specific to the X- and Y-chromosomes; however, sexually dimorphic osteological markers may be missing when not developed in sub-adult skeletons or degraded by environmental processes. Detection of DNA markers from X- and Y-chromosomes is more direct and predictive; however, the DNA backbone contains phosphodiester bonds and can easily degrade below the point at which it can be amplified. Therefore, this analysis is not often available.

Amelogenin genes on the X- and Y-chromosomes are expressed as protein in teeth and play a major role in the biosynthesis of enamel. Protein is more stable than DNA and can persist in skeletal elements well after DNA degrades. Enamel is also the most robust and archaeologically persistent tissue in the body. Detection of peptides unique to the Y-chromosome form of amelogenin protein (AMELY\_Human) in the enamel proteome is an unambiguous signal for the presence of Y-chromosome in the sample.

In this study, archaeological and modern teeth samples were processed. The enamel was demineralized in 1.2M hydrochloric acid, pH neutralized, alkylated, and treated with trypsin and mass-spectrometry compatible detergent. The resulting data collected from the mass spectrometer was analyzed using PEAKS™ analytical software.

This study measured the peptide ion signals that were specific to the AMELX\_HUMAN and AMELY\_HUMAN protein. After being normalized for enamel mass, the specific ion signals were plotted onto a Cartesian graph and a calibration curve was established. The curve revealed a clear male cluster, a female cluster, and a cluster of male false negatives. The range of values ranged widely across different samples, up to three orders of magnitude. Nevertheless, the male values for AMELY\_HUMAN protein had a linear correlation with AMELX\_HUMAN values, the R squared value was 0.88 ( $p > 0.001$ ) and a co-efficient of 0.17. To validate the calibration curve, this study processed 1,000-year-old archaeological deciduous teeth and was able to see clustering with male samples. Detection of the Y-chromosome AMELY\_HUMAN is an unambiguous indicator of male sex; however, the lack of detectable AMELY\_HUMAN protein could be either a male false negative or due to female sex. The likelihood of false negative assignment will reduce as the AMELX\_HUMAN signal increases. This approach has the potential to evaluate sex estimation in a statistically rigorous and a scientifically quantifiable manner. It also has the potential to be applied to skeletons with no osteological markers for sex, such as degraded or juvenile skeletal assemblages.

#### Sex Estimation, Enamel, Proteomics