## B5 Efficiency of Human DNA Isolation and Short Tandem Repeat (STR) Profiling From Burnt Teeth

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After attending this presentation, attendees will have deeper insight concerning the efficiency of DNA profiling in burnt teeth.

This presentation will impact the forensic science community by providing a quantitative analysis for obtaining complete or partial DNA profiles from burnt teeth under controlled conditions of time and temperature.

Identification of a deceased individual is important from the relatives' viewpoint and is a vital factor in facilitating criminal investigations, inquests, and other tribunals. Mass disasters, aircraft or car accidents, and accidental deaths frequently involve the presence of fire.

The identification of human remains subjected to incineration depends on the degree of destruction of the remains, which is affected by the intensity and duration of the fire. One of the approaches for identification of burnt remains is genetic analysis. The genetic approach is usually unproblematic in cases of fire victims with conserved internal organs; however, extremely charred bodies frequently render highly degraded DNA, hampering Short Tandem Repeat (STR) analysis. Also, few studies focus on the possibility of amplifying authentic DNA from burnt remains. No consensus has been reached regarding the degree of cremation at which bone will still yield authentic DNA signals. Although previous studies have used similar temperature ranges (between 100ºC and 1,000ºC), the duration of fire exposure differs among these studies. In the studies developed on whole teeth and pulp DNA, the same problem is encountered: the temperatures were similar to those used in the bone studies and the durations of exposure varied. Therefore, more research is needed to evaluate the possibility of extracting DNA from burnt remains.

Since teeth are the hardest tissue of the human body and one of the most abundant types of biological remains available in forensic cases, the present study focused on the evaluation of the efficiency of DNA isolation from burnt teeth and the achievement of obtaining a DNA profile at different conditions of temperature and time exposure.

Twenty-eight healthy erupted third molars, aged 20 to 70 years, were collected from dental clinics. The Smithsonian Institution's ethical committee approved all procedures related to experimentation with human subjects. The teeth were divided into seven groups treated at different temperatures: 100ºC, 200ºC, 300ºC, 400ºC, 500ºC, 600ºC, and 700ºC. The teeth in each group were treated at their assigned temperature for 1 minute, 5 minutes, 10 minutes, and 15 minutes, removing one tooth after each time period. Two non-burnt teeth were used as controls.

Control and burnt teeth were then mechanically ground and submitted to DNA extraction and quantification. Based on the quantification data, it was not possible to obtain DNA from the teeth subjected to 400ºC for both 10 and 15 minutes, 500ºC for 15 minutes, 600ºC for 5 minutes and 700ºC for 5, 10, and 15 minutes. To study the efficiency of obtaining DNA profiles, the following STRs were chosen: D7S820, D13S317, D5S818, CSF1PO, TPOX, TH01, vWA, D16S539, and FES/FPS, along with amelogenin. These regions were analyzed by SYBR® Green Real Time PCR. Each sample was tested in duplicate. The analysis of relative gene expression data was calculated using $2^{\Delta CT}$ method.

Quantitative PCR results were similar for all STRs tested. In the first temperatures and times, 100ºC and 200ºC, one and five minutes, it was possible to get amplification similar to the controls; however, in the majority of STRs, the amplification was very low from 300ºC for one or five minutes onward. This DNA amplification was nearly undetectable, specifically in STRs located in an intron region, like TPOX, CSF1PO, TH01, and vWA. In contrast, the analysis of the amplification of the housekeeping gene used for the Quantitative Polymerase Chain Reaction (QPCR) quantification, Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH), showed that DNA for this region was amplified in all combinations of temperatures and times, finding highest Ct (meaning the least amplification) with the increase in temperatures and times. This indicates that even in burnt teeth subjected to high temperatures, it is possible to amplify DNA, at least housekeeping DNA; however, the data also shows that an STR profile would be difficult to obtain, probably due to the size of these regions which makes them more prone to degradation.

The findings from this research provide a quantitative study for the achievement of obtaining a DNA profile from burnt teeth. Future research may be able to expand on these results, analyzing other potential DNA regions for identification under the same conditions.

**Burnt Teeth, DNA Isolation, STR Profiling**