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## Genetic investigation of modern burned corpses

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**Abstract.** Burning of corpses is a well-known and widely spread funeral procedure that has been performed for a long time in many cultures. Nowadays more and more corpses are burned in cremations and buried in urns, often for practical and financial reasons. Usually cremation takes place at temperatures of over 1000 °C for more than 70 min, destroying the corpse and only leaving severely burned teeth and some fragments of larger bones. In some scientific, criminal or civil cases even after cremation, there is the need of genetic investigations for identification or paternity testing. Here we present a systematic genetic investigation of 10 corpses that were burned in a crematory. After DNA extraction of the remains, the presence of human nuclear and mitochondrial DNA was tested by a highly sensitive Duplex PCR and quantified via real time PCR; genetic typing was done using the AmpFISTR Identifier kit. © 2003 Elsevier B.V. All rights reserved.

*Keywords:* Cremation; Ancient bones; AmpFISTR identifier; Mitochondrial DNA

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### 1. Introduction

Typing of short tandem repeats (STR, “genetic fingerprinting”) is the method of choice when analyzing DNA extracted from human bones or teeth (modern or ancient), traces with minute amounts of DNA or simply highly degraded tissues for identification purposes [1–3]. In forensic casework, the genetic typing of deceased individuals is routinely done, usually to confirm identity or for posthumous paternity testing. In cases of soil burial, the body can easily be unearthed for investigation, while STR typing after cremation is of questionable value. Due to development of genetic analysis, e.g. typing of short tandem repeats or sequencing of mitochondrial DNA (mtDNA), biological material (i.e. biopsies, blood samples etc.) taken for medical investigation can successfully be analyzed even after long time storage or formalin treatment. Especially popular is the use of commercially available multiplex kits which amplify up to 15 loci plus the sex determining Amelogenin gene in one reaction mix [4,5]. The aim of this work was to systematically investigate whether human remains can be reliably and reproducibly individualized by STR typing after cremation.

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## 2. Materials and methods

Ten cadavers autopsied at the Institute of Legal Medicine were chosen and a buccal swab was taken. After cremation, the remains were routinely ground by crematory staff. DNA was extracted from each swab and the respective minced bones (0.5 g each) using a slightly modified Invisorb Forensic kit (Invitek, Berlin, Germany). Extracted DNA was eluted in 70  $\mu$ l buffer. Genetic typing of the swabs was done using the AmpFISTRIdentifiler kit (Applied Biosystems, Weiterstadt, Germany). To test DNA quality and quantity, DNA extracts from cremation remains were first separated on ethidium bromide containing agarose gels. Then 1, 2.5 and 5  $\mu$ l of each sample were subjected to a real time PCR amplifying a 94-bp fragment of nuclear specific DNA (a part of the telomerase gene) followed by a Duplex PCR simultaneously amplifying a nuclear specific 164-bp fragment and a mitochondrial specific 260-bp fragment. This PCR turned out to be a suitable pretest prior to STR typing [own unpublished results]. Capillary electrophoresis was done on an AbiPrism 310 Genetic Analyzer with 1  $\mu$ l PCR product, 11.8  $\mu$ l formamide and 0.2  $\mu$ l ROX500 or LIZ500 standard per sample (all Applied Biosystems). Afterwards, STR typing of the cremation samples was done using the Identifiler kit which amplifies 15 STR loci plus the Amelogenin specific gender determination fragments simultaneously [6]. Amplification and detection of PCR products was done according to the manufacturer's recommendations with 32 instead of 28 cycles. Every extraction was done twice, every PCR amplification three times independently.

## 3. Results and discussion

Separation of 10  $\mu$ l of DNA containing extract on ethidium bromide agarose gels yielded no signals, implying a DNA concentration below 0.1 ng/ $\mu$ l. To test for the presence of any human DNA, every sample was subjected to a real time PCR amplifying a 98-bp nuclear specific fragment. After 40 cycles, 7 out of 10 samples clearly showed specific signals. Comparison with a standard (10-fold dilutions from 10 pg to 100 ng) indicated a DNA concentration of 100 fg to 50 pg/ $\mu$ l (approx. less than 1 to 10 molecules when assuming 6.6 pg DNA/cell). The other three samples showed an even lower DNA concentration making a reliable quantification difficult or impossible. The subsequently employed Duplex PCR yielded reproducible results and very well correlated with the STR typing results in 7 out of 10 samples: Samples which showed strong signals in the Duplex PCR led to the greatest number of detectable STRs. After reproducible STR amplification, the obtained genetic patterns were compared with the alleles from the corresponding persons (=DNA from buccal swabs taken prior to cremation), and to exclude contaminations, with the STR patterns from every person working in the lab. The genetic profiles amplified out of cremation remains neither corresponded to the buccal swabs of the respective cadaver nor did the alleles match the lab personnel. Some samples were clearly contaminated: in two cases, male signals were detected in samples derived from a woman, and in three instances, more than two alleles were amplified per locus.

## 4. Conclusion

This study shows that even though the extraction and subsequent STR typing of DNA from the ashes of a cremated person is possible and in some cases does yield reproducible

results, a reliable post-cremation identification seems to be unlikely. The genetic profiles obtained from the remains after the burning of a corpse did in no case match the alleles from buccal swabs taken prior to cremation and have to be regarded as contaminations. Since the profiles did also not match those of the lab personnel, the remains are probably contaminated during post-cremation processing (i.e. handling of the ashes, grinding of bones, etc.). As a consequence, STR typing of cremation remains has to be considered unreliable and not suitable for forensic purposes (e.g. identification, paternity testing).

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