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Ref.: (please refer to)  
Prof.Bri./Dr.Ho. Sp1230/5

### Preliminary Certificate of Analysis


**Subject:** L. v. Beethoven  
**Evidence Submitted:** by Paul Kaufmann on 27 June, 2005  
**Item:** a 850 mL Rubbermaid box, labelled with 'Larger Fragment'  
 Inside there was a Tearzone SAFEGUARD Specimen Bag containing a bone fragment (app. (max.) 5 x (max.) 7,5 x (max.) 1,0 cm in size), apparently the fragment no. 1 from the book of Bankl & Jesserer ('Die Krankheiten Ludwig van Beethovens', 1987, Publisher Wilhelm Maudrich, Vienna); here labelled as item 2.

### Results.

DNA was isolated from 0.3 g of bone powder from the skull fragment and characterized first through a PCR-based detection and quantification method for human nuclear DNA (Quantifiler, ABI, Foster City, CA). No human nuclear DNA could be detected using the Quantifiler approach.

Then a segment of the hypervariable region 2 (HV2) of the mitochondrial DNA (mtDNA) was amplified by PCR and sequenced on both strands. The following differences compared to the forensic reference sequence, the Anderson sequence (a.k.a. Cambridge Reference Sequence, CRS), were noted at HV2 (nucleotide positions (np) 201 – 368):

np	263	315.1
CRS	A	
2. bone	G	C

  
 Dr. rer. nat. C. Hohoff

  
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