

## **Nondestructive DNA extraction method for mitochondrial DNA analyses of museum specimens**

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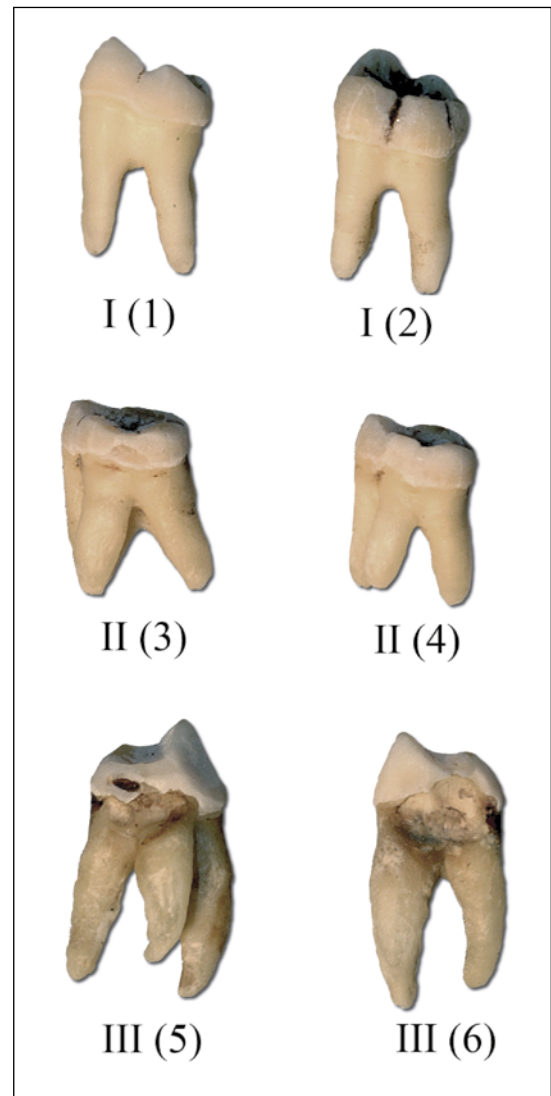
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Museum specimens have provided the material for a large proportion of ancient DNA studies conducted during the last 20 years. However, a major drawback of the genetic analyses is that the specimens investigated are usually damaged, as parts of skin, bone, or a tooth have to be removed for DNA extraction. To get around these limitations, we have developed a nondestructive extraction method for bone, tooth, and skin samples. We found that it is possible to amplify mitochondrial DNA (mtDNA) sequences up to at least 414 bp long from samples up to 164 years old. Using this method, almost 90% (37 of 42) of the investigated samples yielded amplifiable mtDNA. Moreover, we found that repeated extractions of the same samples allowed amplifications of the same length for all samples at least three times and for some samples up to at least five times. Thus this method opens up the possibility to repeatedly use museum collections for mtDNA analyses without damaging the specimens and thus without reducing the value of irreplaceable collections for morphological analyses.



**Table S1. Catalogue Numbers of the Hyena Specimens Used**

| Cat. No. | Species                   | Museum                        |
|----------|---------------------------|-------------------------------|
| 41225    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 46123    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82399    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82400    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82404    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82412    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82423    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82432    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82454    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82455    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82467    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82477    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82482    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82533    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 82537    | <i>Crocuta crocuta</i>    | Museum f. Naturkunde Berlin   |
| 6061     | <i>Crocuta crocuta</i>    | Naturhistorisches Museum Wien |
| 7397     | <i>Crocuta crocuta</i>    | Naturhistorisches Museum Wien |
| 11086    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 14823    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 14825    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 16689    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 42760    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 45066    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 82292    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 82314    | <i>Hyaena hyaena</i>      | Museum f. Naturkunde Berlin   |
| 1151     | <i>Hyaena hyaena</i>      | Naturhistorisches Museum Wien |
| 1756     | <i>Hyaena hyaena</i>      | Naturhistorisches Museum Wien |
| 11597    | <i>Hyaena hyaena</i>      | Naturhistorisches Museum Wien |
| 12073    | <i>Hyaena hyaena</i>      | Naturhistorisches Museum Wien |
| 21496    | <i>Hyaena hyaena</i>      | Naturhistorisches Museum Wien |
| 904      | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 6089     | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 16808    | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 16809    | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 16810    | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 16811    | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 18041    | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 23426    | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 82383    | <i>Parahyaena brunnea</i> | Museum f. Naturkunde Berlin   |
| 21494    | <i>Parahyaena brunnea</i> | Naturhistorisches Museum Wien |



**Figure S2. Photograph of six chimpanzee teeth of three individuals (I–III) after five sequential extractions.** After each extraction, the teeth were soaked in double-distilled water to remove any traces of guanidinium-thiocyanate (GuSCN) buffer and then dried to mimic the process of extraction by independent researchers as exactly as possible.

**Table S2. Age, Species Affiliation, Catalogue Number, and Amplification Success for 27 Hyena Specimens with Available Sampling Data**

| Age (years)  | Species                   | Cat. No. | PCR Product    |                |                 |
|--|---------------------------|----------|----------------|----------------|-----------------|
|  |                           |          | Long           | Short          | —               |
| 37   | <i>Hyaena hyaena</i>      | 11597    |                | ×              |                 |
| 46   | <i>Hyaena hyaena</i>      | 12073    | ×              |                |                 |
| 70   | <i>Hyaena hyaena</i>      | 45066    |                |                | ×               |
| 75   | <i>Crocuta crocuta</i>    | 46123    |                | ×              |                 |
| 75   | <i>Crocuta crocuta</i>    | 7397     | ×              |                |                 |
| 78   | <i>Crocuta crocuta</i>    | 41225    |                | ×              |                 |
| 78   | <i>Crocuta crocuta</i>    | 6061     | ×              |                |                 |
| 90   | <i>Crocuta crocuta</i>    | 82432    |                | ×              |                 |
| 90   | <i>Crocuta crocuta</i>    | 82455    |                | ×              |                 |
| 90   | <i>Hyaena hyaena</i>      | 16689    |                | ×              |                 |
| 90   | <i>Hyaena hyaena</i>      | 82292    |                | ×              |                 |
| 91   | <i>Crocuta crocuta</i>    | 82454    | ×              |                |                 |
| 91   | <i>Crocuta crocuta</i>    | 82467    |                | ×              |                 |
| 95   | <i>Hyaena hyaena</i>      | 82314    |                | ×              |                 |
| 96   | <i>Crocuta crocuta</i>    | 82477    | ×              |                |                 |
| 97   | <i>Crocuta crocuta</i>    | 82404    |                | ×              |                 |
| 98   | <i>Crocuta crocuta</i>    | 82482    | ×              |                |                 |
| 100  | <i>Parahyaena brunnea</i> | 21494    |                | ×              |                 |
| 101  | <i>Crocuta crocuta</i>    | 82533    | ×              |                |                 |
| 101  | <i>Crocuta crocuta</i>    | 82423    | ×              |                |                 |
| 102  | <i>Crocuta crocuta</i>    | 82537    | ×              |                |                 |
| 103  | <i>Parahyaena brunnea</i> | 82383    | ×              |                |                 |
| 103  | <i>Hyaena hyaena</i>      | 1151     |                | ×              |                 |
| 104  | <i>Crocuta crocuta</i>    | 82400    | ×              |                |                 |
| 105  | <i>Hyaena hyaena</i>      | 1756     |                | ×              |                 |
| 125  | <i>Hyaena hyaena</i>      | 14825    | ×              |                |                 |
| 127  | <i>Hyaena hyaena</i>      | 14823    |                | ×              |                 |
| 144  | <i>Parahyaena brunnea</i> | 904      |                | ×              |                 |
| 161  | <i>Hyaena hyaena</i>      | 21496    |                |                | ×               |
| 164  | <i>Parahyaena brunnea</i> | 6089     |                | ×              |                 |
| <b>Average age in years</b>  |                           |          | <b>93 ± 20</b> | <b>99 ± 29</b> | <b>115 ± 64</b> |
| The definition of long and short for the PCR products is as in Table 2, main text. |                           |          |                |                |                 |

# SHORT TECHNICAL REPORTS

**Table S3. PCR Results for First Three Extractions Using General Primers (12Sa' and 12So)**

| Extraction | Tooth (Individual)                   |                        |                                     |                         |                           |               |
|------------|--------------------------------------|------------------------|-------------------------------------|-------------------------|---------------------------|---------------|
|            | 1 (I)                                | 2 (I)                  | 3 (II)                              | 4 (II)                  | 5 (III)                   | 6 (III)       |
| 1          | <b>4 Pan</b><br>6 Colobus<br>1 human | <b>6 Pan</b><br>2 numt | <b>1 Pan</b><br>7 human<br>1 turtle | <b>8 Pan</b>            | <b>3 Pan</b><br>2 Colobus | <b>9 Pan</b>  |
| 2          | <b>7 Pan</b>                         | <b>11 Pan</b>          | <b>6 Pan</b><br>6 human             | <b>11 Pan</b><br>1 numt | <b>6 Pan</b><br>1 numt    | <b>11 Pan</b> |
| 3          | <b>3 Pan</b><br>1 human<br>3 numt    | <b>7 Pan</b>           | <b>6 Pan</b><br>5 human             | <b>4 Pan</b><br>2 numt  | <b>7 Pan</b>              | <b>8 Pan</b>  |

The numbers give the quantities of clones that were found for a certain sequence. Pan, chimpanzee mitochondrial DNA (mtDNA) sequences; human, human mtDNA sequences; numt, nuclear insertions of mtDNA that originate most likely from chimpanzee nuclear DNA (although human DNA cannot be excluded); Colobus, sequences identical to a monkey species (*colobus* sp.); and turtle, a single clone that was found to be closest in GenBank (2 mismatches) to two turtle species (*Dipsochelys hololissa* and *Aldabrachelys elephantina*) that have identical sequences for this part of the 12S gene.