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## Extraction and analysis of ancient DNA from human remains from the Polonnaruwa historic site in Sri Lanka

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Ancient DNA (aDNA) refers to DNA that can be recovered and analyzed from museum specimens and fossil remains; archaeological and paleontological findings. The poor long term survival of DNA, low preservation and contamination with modern DNA are some of the identified factors that hinder ancient DNA studies. The preservation of ancient DNA (aDNA) in human bone is reported to be very low in tropical countries like Sri Lanka due to the prevailing climatic and environmental conditions such as high temperature and high humidity. Preservation and survival of mtDNA in older biological samples are high compared to that of nuclear DNA due to a high copy number of mtDNA (1000 – 10,000 copies per cell). Thus, this study focused on extraction and analysis of mtDNA from ancient human bones found in Sri Lanka dated 1000 year BP. A fragment of the first metacarpal bone from the historic skeleton dating back to 1000 year BP, presently displayed at the Polonnaruwa museum and excavated from its historic site was used in this study.

An optimized modified phenol/chloroform extraction method was performed to extract DNA from the bone. The mitochondrial DNA HVS 1 region of extracted mtDNA was amplified by PCR using overlapping 1st round primers and nested primers, respectively generating PCR amplicons of 161 and 228 base pairs, respectively. The second round amplified PCR products were sequenced using both reverse and forward primers. The sequenced products were aligned with the human mitochondrial DNA revised Cambridge Reference Sequence using CLUSTALX option of MEGAVA 4.0 sequence alignment software. The aligned sequence results of ancient bone showed no cross-contaminations or contaminations with modern DNA. In the present study, we have successfully extracted and amplified ancient mtDNA from the human skeletal remains from Polonnaruwa where preservation of ancient DNA is comparatively low. This study optimizes the methodology for mtDNA analysis of the pre-historic human skeletal remains from Sigiriya Potana, Bellan bandi palassa,Fahien lena, Miniethiliya etc.

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