DNA profiling of Hungarian King Béla III and other skeletal remains originating from the Royal Basilica of Székesfehérvár

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ESM 3 Materials and methods

DNA extraction – Göttingen

Sample preparation: as described in the main text. In case the "QiaVac MinElute Short" protocol was used the Proteinase K incubation at 37°C was performed for 1 h instead of 18h.

EZ1: Following the 18h-incubation an additional 50 µl of Proteinase K (600 mAnson-U/ml) was added and the samples were rotated for another 2 h at 56°C. Then 50 µl of SDS (10 mg/ml) was added followed by incubation for 5 min at 65°C. The lysate was centrifuged for 3 min at 3,300 g The supernatant was transferred to Amicon Ultra-4 Centrifugal Filter Device 30 K (Millipore) and concentrated to approx. 250 ml by centrifugation at 5,000 g. The remaining lysate was purified with the BioRobot EZ1 using the Trace Protocol on the Forensic Card and the EZ1 DNA Tissue Kit (Qiagen). The elution volume was 50 µl.

QiaVac MinElute Standard: Following the 18h-incubation an additional 50 µl of Proteinase K (600 mAnson-U/ml) was added and the samples were rotated for another 2 h at 56°C. Then 50 µl of SDS (10 mg/ml) was added followed by incubation for 5 min at 65°C. The lysate was centrifuged for 3 min at 3,300 g. The supernatant was mixed with 16 ml of PB buffer (Qiagen) and 100 µl of sodium acetate buffer (3M; pH 5.2), centrifuged for 3 min at 3,300 g and transferred to MinElute columns with large-volume funnels on a QIAvac 24 Plus vacuum system (both Qiagen). The lysate was pulled through by vacuum, followed by three washing steps with 700 µl of PE buffer (Qiagen). The MinElute columns were centrifuged for 1 min at 15,700 g and then dried at room temperature with open lids for 20 minutes. DNA elution was performed three times with 20 µl of warm RNase-free water (Qiagen).

QiaVac MinElute Short: Following the 1h-incubation an additional 50 µl of Proteinase K (600 mAnson-U/ml) was added and the samples were rotated for another hour at 56°C. The lysate was centrifuged for 3 min at 3,300 g. The supernatant was purified on MinElute columns with large-volume funnels using a QIAvac 24 Plus vacuum system (cf. above).