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

Judit Olasz[1a], Verena Seidenberg[2], Susanne Hummel[2], Zoltán Szentirmay[1], György Szabados[3], Béla Melegh[4], Miklós Kásler[1]

[1]National Institute of Oncology, [a]Department of Pathogenetics, Ráth Gy. u. 7-9, 1122 Budapest, Hungary; [2]Historic Anthropology and Human Ecology, Johann-Friederich Blumenbach-Institute of Zoology and Anthropology, University of Göttingen, Bürgerstr. 50, 37073 Göttingen, Germany; [3]King St. Stephen Museum, Fő u. 6, 8000 Székesfehérvár, Hungary; [4]Department of Medical Genetics, University of Pécs, Szigeti u. 12, 7624 Pécs, Hungary

Corresponding author: olasz@oncol.hu

ESM 2 Bone samples used for DNA extraction in the different laboratories

Table 1 DNA extractions of the samples – Budapest laboratory.

Person	Sample ID	Source	Extraction no.	Outcome
	BF1	Femur	Extraction 1	successful
	BF2	Femur	Extraction 2	successful
	BF3	Femur	Extraction 3	successful
	BV1	Vertebra	Extraction 4	successful
Béla III	BV2	Vertebra	Extraction 5	weak[a]
	BMT1	Metatarsal	Extraction 6	weak[a]
	BMT2	Metatarsal	Extraction 7	successful
	BT1	Tarsal	Extraction 8	successful
	BT2	Tarsal	Extraction 9	successful
	BT3[b]	Tarsal	Extraction10	only mitochondrial results
	AAC1	Rib	Extraction 1	successful
Anna of Antioch	AAC2	Rib	Extraction 2	successful
	AAC3	Rib	Extraction 3	successful
	I3GC1	Rib	Extraction 1	successful
I/3G	I3GC2	Rib	Extraction 2	successful
	I3GC3	Rib	Extraction 3	successful
	I3GC4	Rib	Extraction 4	successful
	I4HMT1	Metatarsal	Extraction1	successful
	I4HMT2	Metatarsal	Extraction 2	successful
I/4H	I4HMT3	Metatarsal	Extraction 3	successful
	I4HT1	Tarsal	Extraction 4	successful
	I4HT2	Tarsal	Extraction 5	successful
	II52C1	Rib	Extraction 1	weak[a]
	II52C2	Rib	Extraction 2	weak[a]
	II52C3	Rib	Extraction 3	weak[a]
	II52C4	Rib	Extraction 4	successful
II/52	II52T1	Tarsal	Extraction 5	successful
	II52T2	Tarsal	Extraction 6	successful
	II52T3	Tarsal	Extraction 7	successful
	II52T4	Tarsal	Extraction 8	successful
	II52T5	Tarsal	Extraction 9	successful
	II52T6[b]	Tarsal	Extraction 10	only mitochondrial results
	II52F2[c]	Femur	Extraction 11	only mitochondrial results
	II53C1	Rib	Extraction 1	successful
	II53C2	Rib	Extraction 2	successful
II/53	II53C3	Rib	Extraction 3	successful
	II53C4	Rib	Extraction 4	successful
	II53V1	Vertebra	Extraction 5	successful
	II53V2	Vertebra	Extraction 6	successful
	II54C1	Rib	Extraction 1	successful
II/54	II54C2	Rib	Extraction 2	successful
	II54C3	Rib	Extraction 3	successful
	II55C1	Rib	Extraction 1	successful
II/55	II55C2	Rib	Extraction 2	successful
	II55C3	Rib	Extraction 3	successful
	II109C1	Rib	Extraction 1	successful
II/109	II109C2	Rib	Extraction 2	successful
	Mock1		Extraction 1	negative
	Mock2		Extraction 2	negative
	Mock3		Extraction 3	negative
	Mock4		Extraction 4	negative
	Mock5		Extraction 5	negative
	Mock6		Extraction 6	negative
	Mock7		Extraction 7	negative

[a] we assessed the extracts „weak" when less than the half of the expected markers were called.
[b] the bone powder was treated with additional 0.5% NaOCl for 7 minutes, used only for mitochondrial DNA analysis.
[c] the bone powder was treated with additional 0.8% NaOCl for 10 minutes, used only for mitochondrial DNA analysis

Table 2 DNA extractions of the samples – Göttingen laboratory.

Sample	Skeletal element	Extract number	Extraction method[c]	Lab abbreviation
Béla III.	Metatarsal	Ex 1	QiaVac MinElute Standard	HU 3B Mt Ex 1
	Metatarsal	Ex 2	EZ1	HU 3B Mt Ex 2
	Metatarsal	Ex 3	QiaVac MinElute Short	HU 3B Mt Ex 3
	Metatarsal	Ex 4	QiaVac MinElute Organic	HU 3B Mt Ex 4
	Metatarsal	Ex 5	QiaVac MinElute Short	HU 3B Mt Ex 5
	Metatarsal	Ex 6	QiaVac MinElute Organic	HU 3B Mt Ex 6
	Tarsal	Ex 7	QiaVac MinElute Organic	HU 3B Ta Ex 7
Anna of Antioch	Rib	Ex 1	QiaVac MinElute Standard	HU AA Co Ex 1
	Rib	Ex 2	EZ1	HU AA Co Ex 2
	Rib	Ex 3	QiaVac MinElute Short	HU AA Co Ex 3
	Rib	Ex 4	QiaVac MinElute Organic	HU AA Co Ex 4
	Rib	Ex 5	QiaVac MinElute Short	HU AA Co Ex 5
	Vertebra	Ex 6	QiaVac MinElute Organic	HU AA Ve Ex 6
	Femur	Ex 8	QiaVac MinELute Organic	HU AA Fe Ex 8
II/52 (Unmarked) 3. skeleton	Tarsal	Ex 1	QiaVac MinElute Standard	HU 52 Ta Ex 1
	Tarsal	Ex 2	EZ1	HU 52 Ta Ex 2
	Tarsal	Ex 3	QiaVac MinElute Short	HU 52 Ta Ex 3
	Tarsal	Ex 4	QiaVac MinElute Organic	HU 52 Ta Ex 4
	Femur	Ex 5	QiaVac MinElute Organic	HU 52 Fe Ex 5
	Tarsal 2	Ex 6	QiaVac MinElute Organic	HU 52 Ta2 Ex 6
Fetus	Vertebra	Ex 1	QiaVac MinElute Standard	HU FS Ve Ex 1
II/53 → 7. skeleton	Sternum	Ex 1	QiaVac MinElute Standard	HU 53 St Ex 1
	Sternum	Ex 2	EZ1	HU 53 St Ex 2
	Sternum	Ex 3	QiaVac MinElute Short	HU 53 St Ex 3
	Sternum	Ex 4	QiaVac MinElute Organic	HU 53 St Ex 4
	Rib	Ex 5	QiaVac MinElute Organic	HU 53 Co Ex 5
II/109 → 8. skeleton	Rib	Ex 1	QiaVac MinElute Standard	HU 109 Co Ex 1
	Rib	Ex 2	EZ1	HU 109 Co Ex 2
	Rib	Ex 3	QiaVac MinElute Short	HU 109 Co Ex 3
	Rib	Ex 4	QiaVac MinElute Organic	HU 109 Co Ex 4
	Vertebra	Ex 5	QiaVac MinElute Organic	HU 109 Ve Ex 5
II/54 → 9. skeleton	Rib	Ex 1	QiaVac MinElute Standard	HU 54 Co Ex 1
	Rib	Ex 2	EZ1	HU 54 Co Ex 2
	Rib	Ex 3	QiaVac MinElute Short	HU 54 Co Ex 3
	Rib	Ex 4	QiaVac MinElute Organic	HU 54 Co Ex 4
	Femur	Ex 5	QiaVac MinElute Organic	HU 54 Fe Ex 5
II/55 → 10. skeleton	Rib	Ex 1	QiaVac MinElute Standard	HU 55 Co Ex 1
	Rib	Ex 2	EZ1	HU 55 Co Ex 2
	Rib	Ex 3	QiaVac MinElute Short	HU 55 Co Ex 3
	Rib	Ex 4	QiaVac MinElute Organic	HU 55 Co Ex 4
	Rib	Ex 5	QiaVac MinElute Short	HU 55 Co Ex 5
	Rib	Ex 6	QiaVac MinElute Organic	HU 55 Co Ex 6
	Femur	Ex 7	QiaVac MinElute Organic	HU 55 Fe Ex 7
I/3 G → 5. skeleton	Tarsal	Ex 1	QiaVac MinElute Standard	HU 3G Ta Ex 1
	Tarsal	Ex 2	EZ1	HU 3G Ta Ex 2
	Tarsal	Ex 3	QiaVac MinElute Short	HU 3G Ta Ex 3
	Rib	Ex 4	QiaVac MinElute Organic	HU 3G Co Ex 4
	Femur	Ex 5	QiaVac MinElute Organic	HU 3G Fe Ex 5
I/4 H → 6. skeleton	Metatarsal	Ex 1	QiaVac MinElute Standard	HU 4H Mt Ex 1
	Metatarsal	Ex 2	EZ1	HU 4H Mt Ex 2
	Metatarsal	Ex 3	QiaVac MinElute Short	HU 4H Mt Ex 3
	Metatarsal	Ex 4	QiaVac MinElute Organic	HU 4H Mt Ex 4
	Tarsal	Ex 5	QiaVac MinElute Organic	HU 4H Ta Ex 5

[c] in the cases of Béla III and Anna of Antioch the methods "*QiaVac MinElute Standard*" and "*EZ1*" resulted in not successfully amplifiable DNA extracts (HU 3B Mt Ex 1-2 and HU AA Co Ex 1-2). Therefore, new extraction methods "QiaVac MinElute Short" and "QiaVac MinElute Organic" were developed (see ESM2). All other DNA extracts revealed successful amplification.