SUPPLEMENTARY MATERIAL

Sample preparation, DNA isolation and amplification protocol used in **"Complete mitochondrial genome of a woolly mammoth (*Mammuthus primigenius*) from Maly Lyakhovsky Island (New Siberian Islands, Russia) and its phylogenetic assessment"** Igor V. Kornienko, Tatiana G. Faleeva, Nataliay V. Oreshkova, Semyon E. Grigoriev, Lena V. Grigoreva, Evgeniy P. Simonov, Anna I. Kolesnikova, Yuliya A. Putintseva and Konstantin V. Krutovsky

Sample preparation before DNA isolation

Bones

The bone fragments were placed in separate containers and washed with tap water for 10 minutes, then the upper layer of compact bone was removed by a portable dental drill and small bone fragments (50 × 50 mm) were obtained with help of oscillating saw. These bone fragments were cleaned with "Alaminol" washing solution diluted 1:10 with tap water, followed by treatment with 7 mM NaOCl. Each bone fragment was then washed with sterile deionized water. At the next step the fragments were placed into sterile Petri dishes and dried under ultraviolet radiation (wavelength 254 nm, 40 W) for 15 min from each side of the bone. Then the bone fragments were left in open Petri dishes for one day at room temperature to dry completely. Retsch MM200 Mixer Mill was used to grinding the bone samples into homogenized powder. The bone powders were transferred to separate sterile 15 ml tubes and stored at -70 ° C.

Muscle tissue

Fragments of muscle tissue about 5 × 5 mm in size were cut from the deep layers of soft tissues of the trunk using a sterile scalpel and scissors. They were placed into sterile 2 ml tubes and cut into small fragments.

DNA isolation

We used the following DNA extraction protocols and methods:

1. PrepFiler BTA Forensic DNA Extraction Kit (Applied Biosystems) according to manufacturer's instructions.
2. DNA IQ System (Promega) according to manufacturer's instructions.
3. Phenol-chloroform extraction with modifications (see below).

About 150 mg of muscle tissue were placed into 1.5 ml tubes with 550 µl lysing buffer (10 mM Tris-HCl, pH 7.5; 10 mM Na_2EDTA; 50 mM NaCl; 2% SDS) with 15 µl proteinase K (20 mg/ml), and 10 µl of 1 M DDT. For decalcification of bone powder, 10 ml 0.5 M EDTA were added to 0.5 g of the bone powder, mixed thoroughly for 1 min, centrifuged at 10,000 g for 3 min, and discarded supernatant. Then, 4.5 ml of lysing buffer (10 mM Tris-HCl, pH 7.5; 10 mM Na_2EDTA; 50 mM NaCl; 2% SDS) with 200 µl of proteinase K (20 mg/ml) and 200 µl of 1 M DDT were added.

All samples were mixed thoroughly and incubated at +56 °C for one hour, and at +37 °C for 24 hours. Then samples were centrifuged for 5 min at 12,000 g, and supernatant was transferred to new sterile tubes, followed by addition of equal volume of phenol/chloroform/isoamyl alcohol mix (25:24:1) and vortexed. This mixture was centrifuged for 5 min at 12000 g, and upper water phase was transferred to new tubes. The phenol/chloroform/isoamyl alcohol extraction was repeated twice. At the following step, an equal volume of n-butanol was added to the water solution of DNA, vortexed shortly, and centrifuged for 5 min at 12000 g. Upper phase of n-butanol was removed, leaving water solution of DNA, which was cleaned and concentrated by AmiconUltra-4 30K centrifugal filter units. The final volume of all DNA extracts was 50 µl.

The negative control was used with every DNA extraction method.

PCR and sequencing

Amplification of DNA was carried out in GeneAmp PCR System 9700 (Applied Biosystems, USA) using a set of primers covering whole mitochondrial genome and specifically designed for this study (Table S1). PCR was performed in a 50 µl volume each containing 5 µl DNA, 5 µl of 10× amplification buffer, 5 µl of 25 mM $MgCl_2$, 4 µl of 2.5 mM dNTP mix, 2 µl of BSA (3.2 mg/ml), 2.5 U of AmpliTaq DNA Polymerase (Applied Biosystems), and 2 µl of each primer (1 OD). An initial denaturation step was set for 2 min at 95°C, followed by 35 cycles of 30 s at 95°C, annealing at primer-specific temperature (see Table S1) for 20 s, 2.5 min extension at 72°C, and a final extension of 10 min at 72°C. The PCR products were cleaned with GeneJET PCR Purification spin columns (Thermo Fisher Scientific). Sequencing was performed on an ABI 3130XL automatic sequencer (Applied Biosystems / Thermo Fisher Scientific) using the Big-Dye®Terminator 3.1 kit (Applied Biosystems / Thermo Fisher Scientific) and the same primers that were used for amplification.

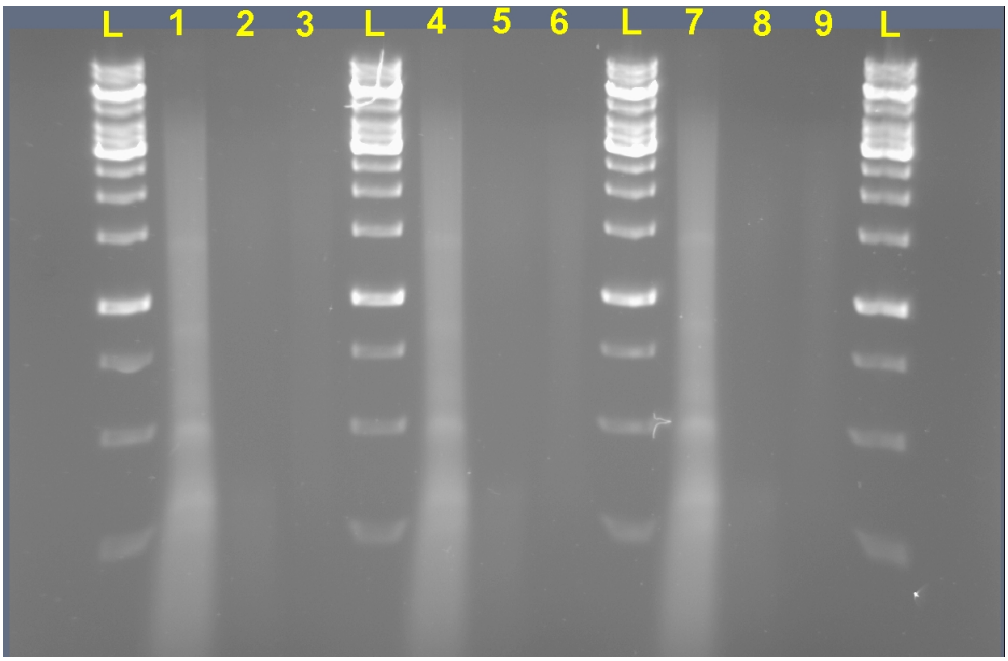

Figure S1. Agarose gel electrophoresis (1%) of total DNA extracted from trunk muscle tissue of woolly mammoth by different methods: phenol/chloroform (1, 4, 7), PrepFiler BTA Forensic DNA Extraction Kit (2, 5, 8), DNA IQ System (3, 6, 9). GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA) was used as size standard (L).

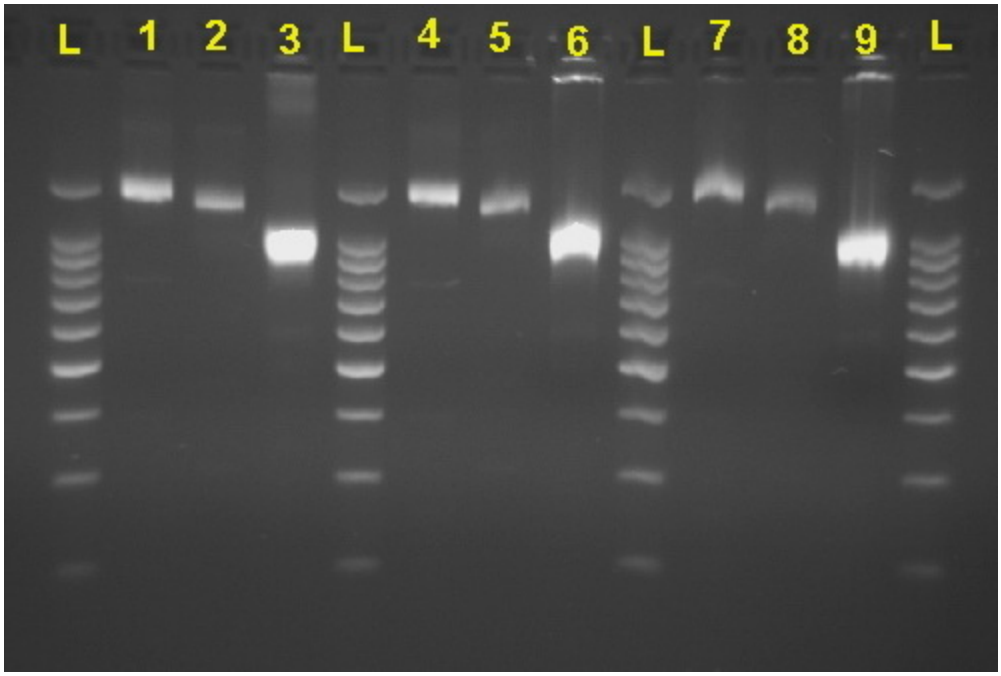

Figure S2. Agarose gel electrophoresis (2%) of PCR products obtained using DNA of woolly mammoth: 1, 4, 7 – 1474 bp long fragment amplified using the PCR primers 15377F and 16789R; 2, 5, 8 – 1338 bp long fragment amplified using the PCR primers 15513F and 16789R); 3, 6, 9 – 988 bp long fragment amplified using the PCR primers 15863F and 16789R); L – 100 bp DNA ladder.

Table S1. Primers used to amplify and sequence mitochondrial genome of *Mammuthus primigenius* from Maly Lyakhovsky island

Pair number	Primer name*	Primer sequence (5'-3')	T_A, °C
1	327F	GGCCATACGATTAGTCCAAA	53
	1214R	TTTCATCATTCCCTTGCGGTA	
2	1101F	CTAAATCTAGCCCTACCAACC	53
	1900R	TTTATGTTTGCCGAGTTCC	
3	1589F	ATTAATTTCATAGTTGGCTTGG	53
	2521R	ACTATAGATAGAAACCGACCTG	
4	2344F	TCAACGGAACAAGTTACCCTA	54
	3322R	TTAAATCAAATGGAGCTCGG	
5	3108F	CAAATTCAAAATACGCCCTC	53
	3981R	TCCTATTCAGGCTGTTAGTCA	
6	3934F	TAACAACACTACTCGCAGGA	53
	5084R	AGCCCTCCAATCTAGAAGG	
7	5029F	AAGATATCACCTTACATCAACTG	53
	6271R	GACTTTTACGCCAGTTGGAA	
8	5328F	CCTATGTTTGCTAACCGCTGA	54
	6271R	GACTTTTACGCCAGTTGGAA	
9	6154F	GGGCTATAATATCAATTGGCT	53
	6997R	AATTTACGGGATTTAAACCTG	
10	6810F	TGACTCAACGGATGTCCAC	53
	7599R	CAGATTTCGGAACATTGACCA	
11	7335F	CTGGAGCTATGAATATACTGA	52
	8216R	TTGCTATGCTTAAGTTTACGGTT	
12	8021F	TCCAAATCGCCTAATTACCAAC	53
	8821R	GTAAACCTTCTTGCACGACA	
13	8672F	GCCCTACTTATAACATCTGG	53
	9651R	AGTAATGCGATTTCTAGGTCA	
14	9314F	GCTGCTTGATATTGACATTTCG	52
	10128R	TTTTGTACGTAGTCTAGTCCG	
15	10020F	CAATATAAACTTCACGTTGTCT	53
	10991R	TTCATATCTGTTTGTCGCAAG	
16	10834F	CAATCGCTGGATCTATAGTGT	53
	11719R	ACTTTTATCTGGAGTTGCAC	
17	11442F	AAACCTTCATTTACCCGAGA	53
	12263R	GCCTATATCACCGATACGAT	
18	12137F	GCGCTAATAACCTACTACAAC	54
	13104R	TGCTGCCTAGGATTAATCGT	
19	12997F	GCAAGTTACAGTACCCGAA	53
	13846R	ATACTATTGCTATGGCTACTGA	

Pair number	Primer name*	Primer sequence (5’-3’)	T_A, °C
20	13797F	AAATGCACTCAAAACTACAAC	53
	14750R	TTATTTGAGCCTGTTTCGTGA	
21	14468F	CTACCTATACTCGGAAACCTG	53
	15313R	TCATTTATGGCTTACAAGACC	
22	15069F	CTACATACATCTAAACACCGAA	53
	16006R	CGGCCATAGCTGAATCACA	
23	15377F	CGCTATCAATACCCAAAACTGA	55
	15916R	CTTATTTAAGAGGAAAGAG	
24	15513F	GACCATACTATGTATAATC	55
	16378R	CGTATGCGTATGCGTATAC	
25	15863F	TCTTACTTCAGGACCATCT	55
	16789R	CAAATCTAGGAACATGGCTCTT	
26	15377F	CGCTATCAATACCCAAAACTGA	55
	16789R	CAAATCTAGGAACATGGCTCTT	
27	15513F	GACCATACTATGTATAATC	55
	16789R	CAAATCTAGGAACATGGCTCTT	
28	29F	TACTGAAAATACCTAGACGAG	54
	629R	GCTTTACGCCGTATGCTTA	
29	133F	TGAAATCAAGAGTATAAACGGGAG	55
	629R	GCTTTACGCCGTATGCTTA	
30	29F	TACTGAAAATACCTAGACGAG	55
	541R	ACTACTGCTGTTTCCCGTG	
31	1950F	AGCATTACTAGTATTCGAGGCA	54
	2477R	CACGTAGGACTTTAATCGTTG	
32	3663F	TTGATTTCACTAGCATGCATCCCA	54
	4511R	AAATGTAAATGGCTAAGTTCAGGA	
33	4600F	CTAATATGAAACAAAACACCT	52
	5481R	AATAACATTATAGATTTGGTCG	
34	4600F	CTAATATGAAACAAAACACCT	52
	5554R	GTTTCCAAAGCCTCCAAT	
35	11917F	AGAAGCCTATATCTCTAACTGAC	54
	12806R	GTCCTCCTATTTTGCGGAT	
36	13619F	AATCAAAACCCATAGCTATACAAA	53
	14033R	TATTATGAGTGTGCTTTATGTGGT	
37	16591F	AGTTACCCTTGGCTCAATTTC	58
	50R	ACTCGTCTAGGTATTTTCAG	
38	16591F	AGTTACCCTTGGCTCAATTTC	55
	60R	AGTTGGATATACTCGTCTAGG	
39	16550F	GAAGAGATAGTTACATAGTGG	58
	50R	ACTCGTCTAGGTATTTTCAG	

Pair number	Primer name*	Primer sequence (5’-3’)	T_A, °C
40	16550F	GAAGAGATAGTTACATAGTGG	58
	60R	AGTTGGATATACTCGTCTAGG	
41	16142F	AACCTAAATTCCCAGCGTAC	58
	16789R	CAAATCTAGGAACATGGCTCTT	
42	16146F	TAAATTCCCAGCGTACACGC	58
	16789R	CAAATCTAGGAACATGGCTCTT	
43	16142F	AACCTAAATTCCCAGCGTAC	58
	16611R	GAAATTGAGCCAAGGGTAAC	

* The numbers in the primer name represent the corresponding nucleotide positions in the reference mitogenome used to design the primers (Genbank accession number DQ316067).